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(71) Applicant: **MINERVA BIOTECHNOLOGIES CORPORATION** [US/US]; 40 Bear Hill Rd., Waltham, MA 02451-1002 (US).

(72) Inventors: **BAMDAD, Cynthia**; 40 Bear Hill Rd., Waltham, MA 02451-1002 (US). **SMAGGHE, Benoit**; 40 Bear Hill Rd., Waltham, MA 02451-1002 (US).

(74) Agent: **KIM, Joseph Hyosuk, Ph.D.**; Nixon & Vanderhye P.C., 901 North Glebe Road, 11th Floor, Arlington, VA 22203-1808 (US).

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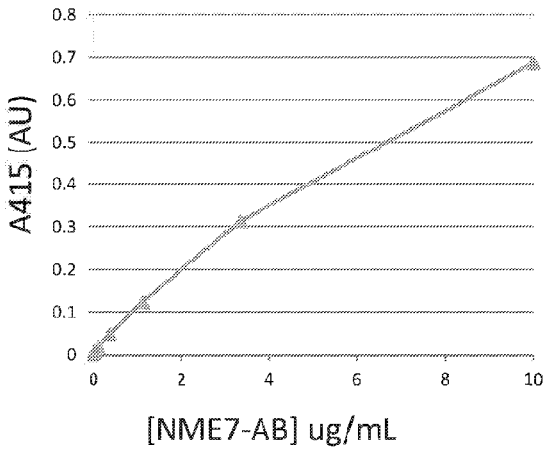
(54) Title: ANTI-NME ANTIBODY AND METHOD OF TREATING CANCER OR CANCER METASTASIS

Fig. 1A



(57) Abstract: The present application discloses anti-NME antibodies and their use in treating or preventing diseases. The present application is directed to a method of treating or preventing cancer in a subject, comprising administering to the subject an antibody made against a member of the NME family. The NME family may be NME7 family.

WO 2020/163325 A1

ANTI-NME ANTIBODY AND METHOD OF TREATING CANCER OR CANCER METASTASIS

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention:

[0002] The present application relates to NME proteins, peptides derived from NME proteins, and antibodies generated from the peptides thereof or antibody or antibody fragments selected by virtue of their ability to bind to said peptides. The present application also relates to treating or preventing diseases associated with the expression of NME in a patient.

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

[0004] NDPK (nucleoside diphosphate protein kinase) proteins are a family of proteins grouped together because they all contain an NDPK domain. The first NME proteins discovered, previously called NM23 proteins, were NM23-H1 and NM23-H2. For decades it was unclear whether they induced differentiation or prevented differentiation of hematopoietic cells. The inventors previously discovered that NM23-H1 prevents differentiation when it is a dimer, which binds to the MUC1* growth factor receptor, but at higher concentrations NM23-H1 becomes a hexamer, which does not bind to MUC1*, and it induces differentiation. NM23 used to be called a metastasis suppressor when it was found that it was under-expressed in some very aggressive cancers. The present inventors previously disclosed that NM23-H1 dimers bind to and dimerize the extracellular domain of the MUC1* growth factor receptor that is over expressed on the vast majority of cancers and such binding promotes the growth of cancer cells. Conversely, at higher concentrations, NM23 forms tetramers and hexamers that do not bind to MUC1* and do not promote tumorigenesis. Very recently more NME family proteins (NME 1-10) have been discovered although until now, their functions have not been elucidated. NME7 is a newly discovered NME family protein, but its NDPK domain has no enzymatic activity, unlike other NME family members. NME7 is either not expressed at all in adult tissues or is expressed at extremely low levels.

SUMMARY OF THE INVENTION

[0005] The present application is directed to a method of treating or preventing cancer in a subject, comprising administering to the subject an antibody made against a member of the NME family. The NME family may be NME7 family. The antibody may bind to NME7. The antibody may bind to $NME7_{AB}$ or $NME7_{AB}$ -like protein. The antibody may bind to NME7-X1. The antibody may inhibit binding between NME7 and its cognate binding partner. The

cognate binding partner may be MUC1*. The cognate binding partner may be PSMGFR portion of the MUC1* extracellular domain. In one aspect, the antibody may be generated or selected for its ability to bind to a peptide selected from those listed in Figures 6-9 (SEQ ID NOS:88 to 145). Preferably, the peptide may be selected from those listed in Figure 9 (SEQ ID NOS:141 to 145).

[0006] The peptide may be highly homologous to, or to which is added or subtracted up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 amino acid residues at the N-terminus or C-terminus, of the peptides listed in Figure 6-9 (SEQ ID NOS:88 to 145). In one aspect, the antibody may be selected for its ability to bind to $NME7_{AB}$ or NME7-X1 but not to NME1. The antibody may be polyclonal, monoclonal, bivalent, monovalent, bispecific, an antibody fragment containing the variable region, or an antibody mimic. The antibody may be human or humanized. The antibody may be a single chain scFv.

[0007] In another aspect, the invention is directed to a method of treating or preventing cancer in a subject, comprising administering to the subject a peptide that is highly homologous or identical to regions of $NME7_{AB}$. The peptide may be at least 80% homologous to one or more of the peptides listed in Figure 6. The peptide may be at least 80% homologous to one or more of the peptides listed in Figure 7. The peptide may be at least 80% homologous to one or more of the peptides listed in Figure 8. The peptide may be at least 80% homologous to one or more of the peptides listed in Figure 9. The peptide may be selected from peptides listed in Figure 6-9 (SEQ ID NOS:88 to 145). The peptide may be selected from those listed in Figure 9 (SEQ ID NOS:141 to 145). Or, the peptide may be highly homologous to, or to which is added or subtracted up to 7 , up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 amino acid residues at the N-terminus or C-terminus, of the peptides listed in Figure 6-9 (SEQ ID NOS:88 to 145). The peptide may be connected to another peptide via a spacer or linker.

[0008] In another aspect, the invention is directed to a chimeric antigen receptor (CAR), for the treatment or prevention of cancer wherein the targeting extracellular portion of the CAR comprises at least a peptide fragment of a member of the NME family. NME family may be NME7 family. The member of the NME7 family may be NME7. Or, the member of the NME7 family may be $NME7_{AB}$ or $NME7_{AB}$ -like protein. The member of the NME7 family may be also NME7-X1. The targeting extracellular portion of the CAR may include a peptide of the peptides listed in Figure 6-9 (SEQ ID NOS:88 to 145). The peptide may be selected from those listed in Figure 9 (SEQ ID NOS:141 to 145). The peptide may include a peptide, which is highly homologous to, or to which is added or subtracted up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 amino acid residues at the N-terminus or C-terminus, of the peptides

listed in Figure 6-9 (SEQ ID NOS:88 to 145). The peptide may be connected to another peptide via a spacer or linker.

[0009] In yet another aspect, the invention is directed to a method of treating or preventing cancer or cancer metastasis, comprising engineering the chimeric antigen receptor according to claim 3, into an immune system cell and administering the cell to a subject in need thereof.

[0010] In another aspect, the invention is directed to a chimeric antigen receptor (CAR), for the treatment or prevention of cancer, wherein the targeting extracellular portion of the chimeric antigen receptor comprises a portion of an antibody that binds to $NME7_{AB}$, $NME7_{AB}$ -like protein or NME7-X1. The portion of the antibody may be a single chain scFv or may be human or humanized.

[0011] In yet another aspect, the invention is directed to a method of vaccinating a person against cancer or metastatic cancer comprising immunizing the person with a peptide fragment of a member of the NME family. The NME family may be NME7 family. The member of the NME7 family may be NME7 or NME7b. The member of the NME7 family may be $NME7_{AB}$ or $NME7_{AB}$ -like protein. The NME7 family may be NME7-X1. The immunizing peptide may be a peptide from the peptides listed in Figure 6-9 (SEQ ID NOS:88 to 145). Preferably, the peptide may be selected from those listed in Figure 9 (SEQ ID NOS:141 to 145). The immunizing peptide may include a peptide, which is highly homologous to, or to which is added or subtracted up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 amino acid residues at the N-terminus or C-terminus, of the peptides listed in Figure 6-9 (SEQ ID NOS:88 to 145). The immunizing peptide may be connected to another peptide via a spacer or linker.

[0012] In yet another aspect, the invention is directed to a method of treating or preventing cancer in a subject, comprising administering to the subject a nucleic acid that inhibits the expression of NME7, NME7b, $NME7_{AB}$ -like protein or NME7-X1. The nucleic acid may be an anti-sense nucleic acid that suppresses expression of NME7, $NME7_{AB}$ -like protein or NME7-X1. The nucleic acid may be an inhibitory RNA, siRNA, RNAi, or shRNA that inhibits expression of NME7, $NME7_{AB}$ -like protein or NME7-X1.

[0013] In another aspect, the invention is directed to a method of treating or preventing cancer in a subject, comprising administering to the subject genetically edited nucleic acids that inhibit expression of NME7, NME7b, $NME7_{AB}$ -like protein or NME7-X1. The genetically edited nucleic acids that inhibit expression of NME7, NME7b, $NME7_{AB}$ -like protein or NME7-X1 may be inserted into cells that may be then administered to the patient. The genetically edited nucleic acids that inhibit expression of NME7, NME7b, $NME7_{AB}$ -like

protein or NME7-X1 may be inserted into cells using a viral vector. The viral vector may be a lentiviral system.

[0014] In another aspect, the invention is directed to a method of growing cancer cells comprising contacting the cells with $NME7_{AB}$, NME7b, $NME7_{AB}$ -like protein or NME7-X1, 2i or 5i. The method may include culturing the cells in a medium that contains $NME7_{AB}$, NME7b, $NME7_{AB}$ -like protein or NME7-X1, 2i or 5i, or growing cells in an animal that expresses human $NME7_{AB}$, NME7b, $NME7_{AB}$ -like protein or NME7-X1, or to which $NME7_{AB}$, NME7b, $NME7_{AB}$ -like protein or NME7-X1 is administered. The cancer cells may be breast, prostate, ovarian, colorectal, pancreatic, liver, melanoma or brain cancer cells. Drug candidates may be tested on the cells. The efficacy of the drugs may be assessed by comparing cancer growth to a no drug control or comparing expression levels of metastatic markers or stem cell markers to a no drug control or comparing the ability of the resultant cells to form tumors in animals from low cell copy number compared to a no drug control and determining the efficacy of a candidate drug for the treatment of cancer or metastasis. The cells may be obtained from a patient being assessed for treatment for cancer and drugs that would be effective for that patient are selected based on results using methods described above. The cells may not be obtained from a patient being assessed for treatment for cancer but drugs that would be effective for that patient are selected based on results using the methods described above.

[0015] In another aspect, the invention is directed to a method of generating antibodies or antibody-like molecules from peptides or peptide mimics having a sequence derived from the sequence of NME. The NME may be NME7. The peptide may be used as an immunogen to generate antibodies or antibody-like molecules. The peptide may be administered to an animal to generate anti-NME7 antibodies. The peptide may be administered to a human to generate anti-NME7 antibodies. The peptide may have a sequence listed in Figure 6-9 (SEQ ID NOS:88 to 145). Preferably, the peptide may be selected from those listed in Figure 9 (SEQ ID NOS:141 to 145). The peptide may include a peptide, which is highly homologous to, or to which is added or subtracted up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 amino acid residues at the N-terminus or C-terminus, of the peptides listed in Figure 6-9 (SEQ ID NOS:88 to 145).

[0016] In another aspect, the invention is directed to a method of detecting presence of cancer or the progression of cancer, comprising the steps of:

[0017] 1) obtaining a sample from a patient having cancer or at risk of developing a cancer;

[0018] 2) subjecting that sample to an assay capable of detecting or measuring levels of a member of the NME7 family, or levels of nucleic acids encoding a member of the NME7 family;

[0019] 3) comparing levels of the measured member of the NME7 family or the member of the NME7 family-encoding nucleic acids in the test sample to levels in control patients or control cells;

[0020] 4) determining that the levels of the member of the NME7 family or nucleic acids encoding the member of the NME7 family are elevated compared to the controls; and

[0021] 5) concluding that the donor of the test sample has cancer or has had a progression of cancer if the control to which the test was compared came from a donor previously diagnosed with a cancer. In this method, the detection of the member of the NME7 family in circulation or in a tissue may be an indicator of cancer in a patient. The member of the NME7 family may be NME7, NME7b, NME7-X1, or $NME7_{AB}$ -like protein.

[0022] In yet another aspect, the invention is directed to a method comprising:

[0023] detecting presence of a member of the NME7 family or MUC1* in a patient; and

[0024] administering anti-NME7 or anti-MUC1* antibody or antibodies to the patient exhibiting the member of the NME7 family or MUC1* expression. The member of the NME7 family may be NME7, NME7b, NME7-X1, or $NME7_{AB}$ -like protein.

[0025] In yet another aspect, the invention is directed to a method for treating or preventing cancer comprising:

[0026] 1) obtaining a sample from a patient suspected of having a cancer or at risk of developing a cancer or at risk of developing a metastatic cancer;

[0027] 2) measuring an amount of the member of an NME7 family or a member of the NME7 family encoding nucleic acid, wherein the measured levels are significantly above those measured in a control sample;

[0028] 3) determining that the patient has a cancer or has developed a more aggressive or a metastatic cancer;

[0029] 4) administering to the patient an effective amount of a therapeutic agent that suppresses expression of the member of the NME7 family, inhibits cleavage of NME7 or inhibits NME7 binding to its targets. The target of the member of the NME7 family may be MUC1*. The target of the member of the NME7 family may be PSMGFR portion of the MUC1* extracellular domain. The member of the NME7 family may be NME7, NME7b, NME7-X1, or $NME7_{AB}$ -like protein.

[0030] In any of the methods above regarding cancer, cancer may include breast, prostate, ovarian, colorectal, pancreatic, liver, melanoma or brain cancer.

[0031] In one aspect, the invention is directed to an NME7 specific antibody or fragment thereof that binds to the NME7 B3 peptide of SEQ ID NO:145 or SEQ ID NO:169. The antibody may be monoclonal antibody or bivalent, monovalent, an Fab, or a single chain variable fragment antibody (scFv). The antibody may be linked to an antibody drug conjugate. The drug may be linked to a toxin or pro-toxin.

[0032] The invention is also directed to an isolated nucleic acid encoding the antibody.

[0033] The invention is also directed to an isolated hybridoma expressing the monoclonal antibody discussed above. The antibody may specifically bind to $NME7_{AB}$ or NME7-X1, but not to NME1. The antibody may disrupt interaction between $NME7_{AB}$ and MUC1* extra cellular domain or between NME7-X1 and MUC1* extra cellular domain. Or, the antibody may disrupt binding between $NME7_{AB}$ and PSMGFR or between NME7-X1 and PSMGFR. Further, the antibody may disrupt binding between $NME7_{AB}$ and N-10 or between NME7-X1 and N-10.

[0034] In another aspect, the antibody may not disrupt interaction between $NME7_{AB}$ and MUC1* extra cellular domain or between NME7-X1 and MUC1* extra cellular domain. $NME7_{AB}$ or NME7-X1 binds to the N-10 peptide (SEQ ID NO:170) but not to a C-10 peptide (SEQ ID NO:171). In particular, the antibody may be 5A1, 4A3 or 5D4.

[0035] The antibody may comprise an amino acid sequence in the heavy chain variable region comprising the following:

[0036] in the CDR1 region YTFTNYGMN (SEQ ID NO:439);

[0037] in the CDR2 region WINTYTGEPTYVDDFKG (SEQ ID NO:440); and

[0038] in the CDR3 region LRGIRPGPLAY (SEQ ID NO:441); and

[0039] an amino acid sequence in the light chain variable region comprising the

[0040] following:

[0041] in the CDR1 region SASSSVSYMN (SEQ ID NO:444);

[0042] in the CDR2 region GISNLAS (SEQ ID NO:445); and

[0043] in the CDR3 region QQRSSYPPT (SEQ ID NO:446).

[0044] In another aspect, the antibody may comprise an amino acid sequence in the heavy chain variable region comprising the following:

[0045] in the CDR1 region NTFTEYTMH (SEQ ID NO:429);

[0046] in the CDR2 region GFNPNNGVTNYNQKFKG (SEQ ID NO:430); and

[0047] in the CDR3 region RYYHSTYVFYFDS (SEQ ID NO:431); and

[0048] an amino acid sequence in the light chain variable region comprising the

[0049] following:

[0050] in the CDR1 region SASQGISNYLN (SEQ ID NO:434);

[0051] in the CDR2 region YTSSLHS (SEQ ID NO:435); and

[0052] in the CDR3 region QQYSKLPYT (SEQ ID NO:436).

[0053] In another aspect, the antibody may comprise an amino acid sequence in the heavy chain variable region comprising the following:

[0054] in the CDR1 region NTFTEYTMH (SEQ ID NO:388);

[0055] in the CDR2 region GFNPNNGVTNYNQKFKG (SEQ ID NO:389); and

[0056] in the CDR3 region RYYHSLYVFYFDY (SEQ ID NO:390); and

[0057] an amino acid sequence in the light chain variable region comprising the

[0058] following:

[0059] in the CDR1 region ITSTDIDDDMN (SEQ ID NO:393);

[0060] in the CDR2 region EGNTLRP (SEQ ID NO:394); and

[0061] in the CDR3 region LQSDNLPLT (SEQ ID NO:395).

[0062] The antibody may be human, humanized or an engineered antibody mimic.

[0063] The antibody may be non-human, such as murine or camelid.

[0064] The invention is also directed to a method of administering to a patient for prevention or treatment of cancer comprising administering to the patient a composition comprising the antibody described above.

[0065] The invention is also directed to a method for preventing or treating cancer metastasis in a patient, comprising administering to the patient a composition comprising the antibody described above.

[0066] The invention is also directed to a method for diagnosing cancer or cancer metastasis comprising contacting a patient specimen and normal specimen with the antibody above, and comparing the results from both specimen, wherein presence of positive binding to the antibody in the patient specimen indicates the presence of cancer or cancer metastasis in the patient. The antibody may be linked to an imaging agent. The patient specimen may be blood, bodily fluid, tissue, circulating cells, *in vitro*, *in vivo*, including intra-operative.

[0067] The invention is also directed to a cell that is engineered to express an anti-$NME7_{AB}$ antibody or fragment thereof. The cell may be an immune cell, such as T cell or NK cell, or a stem or progenitor cell, preferably stem or progenitor cell that is then differentiated to become a T cell.

[0068] The cell may comprise a chimeric antigen receptor (CAR) that recognizes tumor associated antigen. Expression of the anti-NME7 antibody may be inducible. Nucleic acid encoding an anti-$NME7_{AB}$ antibody may be inserted into the Foxp3 enhancer or promoter. The anti-$NME7_{AB}$ antibody may be in an NFAT-inducible system. NFATc1 response element may be inserted upstream of the antibody sequence that is inserted into Foxp3 enhancer or promoter region.

[0069] The anti-$NME7_{AB}$ antibody or fragment thereof may bind to the NME7 B3 peptide, or disrupt binding of $NME7_{AB}$ or NME7-X1 to the PSMGFR peptide of the MUC1* extra cellular domain.

[0070] The CAR may recognize a tumor associated antigen and an anti-NME7 antibody. The tumor associated antigen may be MUC1*.

[0071] The invention is also directed to an anti-cancer vaccine comprising a composition comprising one or more peptides derived from $NME7_{AB}$ listed in Figure 6 – Figure 9 or a peptide having at least 80%, 85%, 90%, 95%, 97% sequence identity thereof as the immunogenicity eliciting portion. The peptide may be a peptide of SEQ ID NOS:141-145 or a peptide having at least 80%, 85%, 90%, 95%, 97% sequence identity thereof. The peptide may be a peptide of SEQ ID NO: 145 or a peptide having at least 80%, 85%, 90%, 95%, 97% sequence identity thereof.

[0072] In another aspect, the invention is directed to a BiTE comprising the above-described antibody.

[0073] In yet another aspect, the invention is directed to a method of generating anti-$NME7_{AB}$ antibodies wherein Cysteine residue in the NME7 B3 peptide is mutated to avoid disulfide bonding.

[0074] In yet another aspect, the invention is directed to a method of generating cells with enhanced metastatic potential comprising culturing the cells with $NME7_{AB}$ or NME7-X1.

[0075] The invention is also directed to a cell that is engineered to express $NME7_{AB}$ or NME7-X1, a transgenic animal that expresses $NME7_{AB}$ or NME7-X1, wherein the $NME7_{AB}$ or NME7-X1 may be human, and also wherein expression of $NME7_{AB}$ or NME7-X1 may be inducible.

BRIEF DESCRIPTION OF THE DRAWINGS

[0076] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0077] 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;

[0078] **Figure 1** shows a graph of HRP signal from ELISA sandwich assay showing NME7-AB dimerizes MUC1* extra cellular domain peptide.

[0079] **Figure 2** is a graph of RT-PCR measurements of gene expression for stem cell markers and cancer stem cell markers for T47D cancer cells after being cultured in traditional media or a media containing NME7, wherein cells that became non-adherent (floaters) were analyzed separate from those that remained adherent.

[0080] **Figure 3** is a graph of RT-PCR measurements of gene expression for a variety of stem and putative cancer stem cell markers for DU145 prostate cancer cells. Cells were cultured either in traditional media or a media containing NME1 dimers ("NM23") or NME7 (NME7-AB). Rho kinase inhibitor was not used because by passage 2, cells remained adherent.

[0081] **Figures 4** is a graph of RT-PCR measurement of the metastatic markers and pluripotent stem cell markers showing that the 2i inhibitors (GSK3-beta and MEK inhibitors), which were previously shown to revert stem cells to a more naïve state, also induce cancer cells to a more metastatic state although not as well as $NME7_{AB}$.

[0082] **Figure 5** is a sequence alignment between human NME1 and human NME7-A or –B domain.

[0083] **Figure 6** lists immunogenic peptides from human NME7 with low sequence identity to NME1 and selected for their ability to generate therapeutic anti-NME7 antibodies for the treatment or prevention of cancers.

[0084] **Figure 7** lists immunogenic peptides from human NME7 that may be important for structural integrity or for binding to MUC1* selected for their ability to generate therapeutic anti-NME7 antibodies for the treatment or prevention of cancers.

[0085] **Figure 8** lists immunogenic peptides from human NME1 that may be important for structural integrity or for binding to MUC1* and selected for their ability to generate therapeutic anti-NME7 antibodies for the treatment or prevention of cancers.

[0086] **Figure 9** lists immunogenic peptides from human NME7 selected for their low sequence identity to NME1 and for their homology to bacterial NME1 proteins that have been implicated in cancers. These peptides are preferred for their ability to generate therapeutic anti-NME7 antibodies for the treatment or prevention of cancers. The peptides shown in this Figure include and added Cysteine covalently bound at the C-terminal end.

[0087] **Figures 10A-10B** show graphs of ELISA assays in which either NME7-AB (Fig. 10A) or NME1 (Fig. 10B) is adsorbed to the plate and anti-NME7 antibodies generated by NME7 peptides A1, A2, B1, B2 and B3 are tested for their ability to bind to NME7 but not to NME1. C20 is an anti-NME1 antibody.

[0088] **Figure 11** shows graphs of ELISA assays in which anti-NME7 antibodies generated are tested for their ability to inhibit binding of NME7-AB to a surface immobilized MUC1* peptide but not inhibit binding of NME1.

[0089] **Figure 12** shows a graph of a cancer cell growth experiment in which breast cancer cells were grown in the presence or absence of NME7 antibodies or short peptides derived from NME7, which were used to generate or select the antibodies. In addition, an antibody generated by immunization with nearly the entire NME7-AB peptide, amino acids 100-376, was shown to inhibit cancer cell growth.

[0090] **Figure 13** shows a graph of a cancer cell growth experiment in which breast cancer cells were grown in the presence or absence of combinations of NME7 antibodies or combinations of the short peptides derived from NME7, which were used to generate or select the antibodies. Both antibodies as well as their immunizing NME7-AB peptides inhibited growth of cancer cells.

[0091] **Figures 14A-14B** show tables of scientist observations when cancer cells were grown in either NME7-AB or 2i inhibitors, which both are able to transform cancer cells to a more metastatic state, and in the presence or absence of NME7 derived peptides A1, A2, B1, B2 and B3. The NME7-AB peptides inhibited the transition of adherent cancer cells to the floater cells, which RT-PCR measurements show have increased expression of metastatic markers, especially CXCR4.

[0092] **Figures 15A-15C** show graphs of RT-PCR measurements of expression of CXCR4 and other metastatic markers in T47D breast cancer cells that were grown in either NME7-AB or 2i inhibitors, each of which transform cancer cells to a more metastatic state, and the inhibitory effect of anti-NME7 antibodies on the metastatic transformation. Fig. 15A shows PCR graph of CXCR4 expression of T47D cancer cells grown in $NME7_{AB}$ or 2i in the presence or absence of anti-NME7 antibodies. Fig. 15B shows a graph of RT-PCR measurements of

CXCR4, CHD1 and SOX2 expression in T47D breast cancer cells that were grown in 2i inhibitors for 72 hours or 144 hours,in the presence of $NME7_{AB}$ immunizing peptides and shows the peptides are themselves inhibitory to the metastatic transformation. Peptides A1, A2 and B1 which were used in the inhibitory Combo 2 and 3 in Fig. 15A are also inhibitory as peptides. Peptide B3 is the most inhibitory and is the immunizing peptide for antibody 61 which was the most inhibitory antibody tested in Fig. 15A. Fig. 15C shows the graph of Fig. 15B with the scale of the Y-axis reduced.

[0093] **Figure 16** shows a table of recorded RNA levels in samples that were used for RT-PCR measurement of CXCR4 in Figure 31 as well as the threshold cycle number for CXCR4 expression as well as for the control housekeeping gene.

[0094] **Figure 17** shows a graph of RT-PCR measurement of the expression of NME7-X1 in a panel of human stem cells and cancer cells.

[0095] **Figure 18** shows a graph of RT-PCR measurement of the expression of NME7, NME7a, NME7b and NME7-X1 in a panel of human stem cells and cancer cells. NME7a is full-length NME7, NME7b is missing a small portion of the DM10 domain, NME7-X1 is missing all of the DM10 domain and a small portion of the N-terminus of the first NDPK A domain. The bar labeled NME7 means that primers were used that detected both NME7a and NME7b.

[0096] **Figures 19A-19F** show photographs of Western blots in which various cancer cell lines are probed for expression of NME7 species using antibodies generated by immunization with NME7 derived peptides. Fig. 19A shows Western blot wherein antibody 52 that binds to the A1 peptide is used to probe a panel of cells for the presence of full-length NME7, $NME7_{AB}$ or NME7-X1. Fig. 19B shows Western blot wherein antibody 56 that binds to the B1 peptide is used to probe a panel of cells for the presence of full-length NME7, $NME7_{AB}$ or NME7-X1. Fig. 19C shows Western blot wherein antibody 61 that binds to the B3 peptide is used to probe a panel of cells for the presence of full-length NME7, $NME7_{AB}$ or NME7-X1. Fig. 19D shows Western blot wherein commercially available polyclonal antibody H278, raised against both the NME7 A and B domain, is used to probe a panel of cells for the presence of NME7. As the figure shows, antibody H278 also recognizes NME1. Fig. 19E shows a gel published on website for commercially available anti-NME7 antibody B9, showing it binds to a species with an apparent molecular weight of full-length NME7. Fig. 19F shows a Western blot in which we used anti-NME7 antibody B9 to probe a gel that was loaded only with NME1. As can be seen in the figure, antibody B9 recognizes NME1 as well as full-length NME7. This is not

surprising because like antibody H278, B9 was raised against both A and B domains of NME7 where the A domain of NME1 is highly homologous to the A domain of $NME7_{AB}$.

[0097] **Figures 20A-20C** show graphs of RT-PCR measurements of metastatic markers in cancer cells after being cultured in a serum-free media containing NME7-AB compared to the standard media. Fig. 20A shows SK-OV3, a MUC1-positive ovarian cancer cell line increased expression of metastatic markers CXCR4, CDH1 aka E-cadherin, SOX2 and NME7-X1; Fig. 20B shows OV-90 a MUC1-negative ovarian cancer cell line increased expression of metastatic markers CXCR4 and NME7-X1; Fig. 20C shows MDA-MB a breast cancer cell line that expresses minimal levels of MUC1 increased expression of metastatic markers CDH1 aka E-cadherin and SOX2.

[0098] **Figures 21A-21F** show photographs of Western blots and description of cancer cell lines analyzed. For Western blots in Fig. 21A and 21B, all cancer samples were normalized such that they were loaded onto gel at a concentration of 40ug/mL. In Fig. 21A, various cancer cell lines are probed for the expression of full-length MUC1 using an anti-tandem repeat monoclonal antibody VU4H5. In Fig. 21B, various cancer cell lines are probed for the expression of cleaved form MUC1* using a polyclonal anti-PSMGFR antibody. Fig. 21C is a description of the cancer cell lines analyzed. Fig. 21D shows that HER2 positive BT474 breast cancer cells, marked "BT474 (parent cells)" express little to no MUC1 or MUC1* until they acquire resistance to Herceptin and other chemotherapy drugs, marked "BTRes1" in figure. Parent cells were made resistant to Herceptin, Taxol, Doxorubicin and cyclophosphamide by culturing the cells in sub-lethal levels of Herceptin. Fig. 21D shows that the expression level of HER2 has not changed but expression of MUC1* has dramatically increased as the cells acquired resistance to Herceptin. Fig. 21E shows a graph of the growth of the parent BT474 cells compared to the drug resistant metastatic cells in response to treatment with Herceptin in the presence or absence of an anti-MUC1* Fab. As can be seen in the figure, the BT474 parent cells show a Herceptin concentration dependent decrease in cell growth, whereas the two Herceptin resistant cell lines, BTRes 1 and BTRes2, show no decrease in cancer cell growth in response to treatment with Herceptin. However, when treated with an anti-MUC1* Fab, the resistant cell lines show a Herceptin concentration dependent decrease in cancer cell growth. Fig. 21F shows a graph of the percent cell death of the parent BT474 cells compared to the drug resistant BTRes1 cells, in response to treatment with Taxol in the presence or absence of an anti-MUC1* Fab.

[0099] **Figures 22A-22E** show photographs of Western blots of a co-immunoprecipitation experiment. T47D breast cancer cell extracts were incubated with an antibody against the

MUC1 cytoplasmic tail, Ab-5, or a control antibody, IgG, and co-immunoprecipitated. The gels were blotted with two different commercially available anti-NME7 antibodies B9 (Fig. 22A) and CF7 (Fig. 22B). Both gels show unique NME7 bands at ~33kDa and ~30kDa. The gels were stripped and re-probed with an antibody against the extracellular domain of MUC1*, anti-PSMGFR (Fig. 22C) and (Fig. 22D), which shows that the NME7 species and MUC1* interact. A recombinant NME7-AB and a recombinant NME7-X1 were mixed together and run on a gel, then probed with an anti-NME7 antibody, showing that the two unique NME7 species that are naturally occurring in breast cancer cells and that interact with MUC1* are an NME7-AB-like species and NME7-X1 (Fig. 22E).

[00100] **Figures 23A-23C** show photographs of Western blots of a co-immunoprecipitation experiment. Human induced pluripotent stem, iPS7, or embryonic stem, HES3, cell extracts were incubated with an antibody against the MUC1 cytoplasmic tail, Ab-5, or a control antibody, IgG, and co-immunoprecipitated. The gel was blotted with a commercially available anti-NME7 antibody B9 (Fig. 23A). Both cell types show unique NME7 bands at ~33kDa and ~30kDa. The gel was stripped and re-probed with an antibody against the extracellular domain of MUC1*, anti-PSMGFR (Fig. 23B), which shows that the NME7 species and MUC1* interact. A recombinant NME7-AB and a recombinant NME7-X1 were mixed together and run on a gel, then probed with an anti-NME7 antibody, showing that the two unique NME7 species that are naturally occurring in breast cancer cells and that interact with MUC1* are an NME7-AB-like species and NME7-X1 (Fig. 23C).

[00101] **Figure 24** shows a graph of an ELISA experiment assaying new anti-NME7 antibodies for their ability to bind to NME7-AB. NME7-AB is known to bind to the extra cellular domain of MUC1*. The surface of the multi-well plate was coated with a recombinant NME7-AB. Anti-NME7-AB antibodies were separately added to wells. Standard washes were performed and visualized by adding an HRP-conjugated secondary antibody. As can be seen, 7 of the 10 new anti-NME7 antibodies bound strongly to NME7-AB.

[00102] **Figure 25** shows a graph of an ELISA experiment assaying new anti-NME7 antibodies for their ability, or preferably inability, to bind to NME1. The surface of the multi-well plate was coated with a recombinant NME1-S120G dimers, which are also known to bind to the MUC1* extra cellular domain. Anti-NME7-AB antibodies were separately added to wells. Standard washes were performed and visualized by adding an HRP-conjugated secondary antibody. As can be seen only one antibody showed just minimal binding to NME1.

[00103] **Figure 26** shows a graph of an ELISA competitive inhibition assay. NME7-AB/anti-NME7 antibody complexes were made before adding to a multi-well plate coated with

MUC1* extra cellular domain peptide, PSMGFR. Recall that NME7-AB has two pseudo-identical domains A and B that are each able to bind to MUC1* extra cellular domain. Antibodies that bind to the NME7 B3 peptide, which is in the B domain, do not bind to the NME7 A domain. Therefore, only partial inhibition of the NME7-AB/MUC1* interaction is expected.

[00104] **Figure 27** shows a graph of an ELISA displacement assay. NME7-AB was first bound to surface-immobilized MUC1* extra cellular domain peptide on the plate, then disrupted by the addition of anti-NME7 antibodies.

[00105] **Figure 28** shows a graph of an ELISA displacement assay. In this case, the multi-well plate was coated with a truncated MUC1* peptide, N-10, which has the 10 N-terminal amino acids missing of the PSMGFR sequence. NME7-AB is known to bind to the N-10 peptide. NME7-AB was bound to surface-immobilized N-10 peptide on the plate, then disrupted by the addition of anti-NME7 antibodies.

[00106] **Figure 29** shows a graph of the amount of RNA present in samples of T47D breast cancer cells were cultured in either their normal recommended media, RMPI, serum-free media containing only NME7-AB as the growth factor at 4nM, which is optimal, or 8nM, or serum-free media containing only NME1 S120G dimers as the growth factor at 8nM; because NME1 is a homodimer and NME7-AB is a monomer comprised of two pseudo-identical domains, 8nM NME1 is the molar equivalent of 4nM NME7-AB. The cancer cells were cultured in the presence or absence of anti-NME7 B3 antibodies. In this experiment, floating cells were separated from the adherent cells and analyzed separately. Significant data argues that the floater cells are the cancer stem cells. An increase or decrease in the amount of RNA in a sample argues that an agent increased or decreased, respectively, the number of cells in a given population that were generated.

[00107] **Figure 30** shows a graph of a PCR measurement of metastatic marker CXCR4 in T47D breast cancer cells that were cultured in either their normal recommended media, RPMI, serum-free media containing only NME7-AB as the growth factor at 4nM, which is optimal, or 8nM, or serum-free media containing only NME1 S120G dimers as the growth factor at 8nM; because NME1 is a homodimer and NME7-AB is a monomer comprised of two pseudo-identical domains, 8nM NME1 is the molar equivalent of 4nM NME7-AB. The cancer cells were cultured in the presence or absence of anti-NME7 B3 antibodies. In this experiment, floating cells were separated from the adherent cells and analyzed separately. Significant data argues that the floater cells are the cancer stem cells. As can be seen in the figure, growth in NME7-AB media increases CXCR4 in the floater population of cells and anti-NME7 B3

antibodies decreased its expression, arguing that anti-NME7 antibodies decreased generation of cancer stem cells.

[00108] **Figure 31** shows a graph of a PCR measurement of stem cell marker and metastatic marker SOX2 in T47D breast cancer cells that were cultured in either their normal recommended media, RMPI, serum-free media containing only NME7-AB as the growth factor at 4nM, which is optimal, or 8nM, or serum-free media containing only NME1 S120G dimers as the growth factor at 8nM; because NME1 is a homodimer and NME7-AB is a monomer comprised of two pseudo-identical domains, 8nM NME1 is the molar equivalent of 4nM NME7-AB. The cancer cells were cultured in the presence or absence of anti-NME7 B3 antibodies. In this experiment, floating cells were separated from the adherent cells and analyzed separately. Significant data argues that the floater cells are the cancer stem cells. As can be seen in the figure, growth in NME7-AB media increases SOX2 expression in the floater population of cells and anti-NME7 B3 antibodies decreased its expression, arguing that anti-NME7 antibodies decreased generation of cancer stem cells.

[00109] **Figure 32** shows a graph of a PCR measurement of stem cell marker and metastatic growth factor receptor MUC1 in T47D breast cancer cells that were cultured in either their normal recommended media, RMPI, serum-free media containing only NME7-AB as the growth factor at 4nM, which is optimal, or 8nM, or serum-free media containing only NME1 S120G dimers as the growth factor at 8nM; because NME1 is a homodimer and NME7-AB is a monomer comprised of two pseudo-identical domains, 8nM NME1 is the molar equivalent of 4nM NME7-AB. The cancer cells were cultured in the presence or absence of anti-NME7 B3 antibodies. In this experiment, floating cells were separated from the adherent cells and analyzed separately. Significant data argues that the floater cells are the cancer stem cells. As can be seen in the figure, growth in NME7-AB media increases MUC1 expression in the floater population of cells and anti-NME7 B3 antibodies decreased its expression, arguing that anti-NME7 antibodies decreased generation of cancer stem cells.

[00110] **Figures 33A – 33B** show IVIS photographs of immune compromised nu/nu mice Day 6 post tail vein injection of cancer cells. Fig. 33A shows IVIS photographs of mice injected with 500,000 T47D-wt breast cancer cells. Fig. 33B shows IVIS photographs of mice injected with 10,000 T47D breast cancer cells that were grown for 10 days in NME7-AB in a minimal media. The floating cells were collected. These floating cells are referred to herein as cancer stem cells, CSCs. As can be seen in the figure, the mice injected with wild type cancer cells show no signs of metastasis. However, the mice injected with 50-times less cells, but cancer stem cells, show that the injected cancer cells are clearly metastasizing.

[00111] **Figures 34A – 34D** show IVIS photographs of immune compromised nu/nu mice Day 10 post tail vein injection of cancer cells. Fig. 34A shows IVIS photographs of mouse injected with 500,000 T47D-wt breast cancer cells. Fig. 34B shows IVIS photographs of mouse injected with 10,000 T47D-CSC (cancer stem cells). Fig. 34C shows IVIS photographs of mouse injected with 10,000 T47D-CSC (cancer stem cells) and injected on Day 7 with anti-NME7 antibody. Fig. 34D shows the hand recording of the IVIS measure of emitted photons. As can be seen in the figure, the mouse chosen for treatment is more metastatic than the comparable T47D-CSC mouse. The efficacy of the first antibody injection may have been blocked by the Day 6 injection of free NME7-AB. Control mouse injected with 500,000 T47D-wt cells shows some weak emission of photons that may be background or surviving cancer cells.

[00112] **Figure 35A-35C** shows IVIS photographs of immune compromised nu/nu mice Day 12 post tail vein injection of cancer cells. Fig. 35A shows IVIS photographs of mouse injected with 500,000 T47D-wt breast cancer cells. Fig. 35B shows that mouse injected with 10,000 T47D-CSC (cancer stem cells) that was not treated with anti-NME7 antibody died from excess tumor burden before IVIS photograph could be taken. Fig. 35C shows IVIS photographs of mouse injected with 10,000 T47D-CSC (cancer stem cells) and injected on Day 7 and Day 10 with anti-NME7 antibody. As can be seen in the figure, the mouse treated with anti-NME7 antibody is clearing away the cancer metastases. Control mouse injected with 500,000 T47D-wt cells shows less photon emissions indicating fewer surviving cancer cells or may be background.

[00113] **Figures 36A-36B** shows IVIS photographs of immune compromised nu/nu mice Day 14 post tail vein injection of cancer cells. Fig. 36A shows IVIS photographs of mouse injected with 500,000 T47D-wt breast cancer cells. Fig. 36B shows IVIS photographs of mouse injected with 10,000 T47D-CSC (cancer stem cells) and injected on Day 7, Day 10, and Day 12 with anti-NME7 antibody. As can be seen in the figure, the mouse treated with anti-NME7 antibody nearly completely free of cancer cell metastases. Control mouse injected with 500,000 T47D-wt cells shows no photon emissions.

[00114] **Figures 37A-37V** shows time course of IVIS photographs of immune compromised nu/nu mice from Day 6 to Day 26 post cancer cell tail vein injection. Figs. 37A, 37C, 37E, 37G, 37I, 37K, 37M and 37O show IVIS photographs of mouse that had been injected Day 0 into the tail vein with 500,000 T47D-wt cells. Figs. 37B, 37D, 37F, 37H, 37J, 37L, 37N and 37P show IVIS photographs of mouse that had been injected Day 0 into the tail vein with 10,000 T47D cancer stem cells, to which anti-NME7 antibody was administered from Day 7

to Day 17, whereupon treatment was suspended, then resumed on Day 21. Figs. 37Q, 37R, 37S, 37T, and 37U show enlarged IVIS photographs of the treated mouse between Day 17, when anti-NME7 antibody treatment was suspended, through Day 21, when antibody treatment was resumed to Day 26. Fig. 37V shows the scale bar of the IVIS measurements. As can be seen in this time course, cancer cells that had been grown in NME7 readily metastasize and such metastasis can be effectively treated, prevented or reversed by treatment with an antibody that binds to NME7.

[00115] **Figure 38A-38C** shows time course of IVIS photographs of immune compromised nu/nu mice from Day 6 to Day 19 post injection with either 500,000 T47D wild type breast cancer cells or 10,000 T47D cancer stem cells. Fig. 38A shows mice that were injected into the tail vein (i.v.). Fig. 38B shows mice that were injected intra-peritonealy (i.p.). Fig. 38C shows mice that were injected sub-cutaneously (s.c.).

[00116] **Figures 39A-39C** shows human lung tissue specimens stained with an anti-NME7 antibody that binds to the B3 peptide. The figure shows lack of NME7 expression on normal tissues, increasing expression of NME7 as tumor grade and metastasis increase.

[00117] **Figures 40A-40C** shows human small intestine tissue specimens stained with an anti-NME7 antibody that binds to the B3 peptide. The figure shows lack of NME7 expression on normal tissues, increasing expression of NME7 as tumor grade and metastasis increase.

[00118] **Figures 41A-41D** show human colon tissue specimens stained with an anti-NME7 antibody that binds to the B3 peptide. The figure shows lack of NME7 expression on normal tissues, increasing expression of NME7 as tumor grade and metastasis increase.

[00119] **Figures 42A-42F** shows photographs of female nu/nu mice weighing approximately 20g each, which were injected into the tail vein with 10,000 Luciferase positive T47D metastatic breast cancer stem cells and treated with the anti-$NME7_{AB}$ antibody 4A3 also known as 8F9A4A3. To image cancer cells, the Luciferase substrate, Luciferin, is intraperitoneally injected 10 minutes before being photographed in IVIS instrument. Figs. 42A-42C show IVIS photographs with animals face down. Fig. 42D-42F show IVIS photographs with animals face up. Figs. 42A and 42D show control animals injected with phosphate buffered saline solution. Figs. 42B and 42E show a prevention model in which animals were injected with anti-$NME7_{AB}$ antibody 4A3 24 hrs before injection of the metastatic cancer cells, then approximately every other day for a total of 12 antibody injections over 22 days. Figs. 42C and 42F show a reversal model in which animals were injected with anti-$NME7_{AB}$ antibody 4A3 24 hrs after injection of the metastatic cancer cells, then approximately every other day for a total of 11 antibody injections over 20 days.

[00120] **Figures 43A-43F** shows photographs of female nu/nu mice weighing approximately 20g each, which were injected into the tail vein with 10,000 Luciferase positive T47D metastatic breast cancer stem cells and treated with the anti-$NME7_{AB}$ antibodies 5A1, also known as 8F9A5A1, or 5D4, also known as 5F3A5D4. To image cancer cells, the Luciferase substrate, Luciferin, is intraperitoneally injected 10 minutes before being photographed in IVIS instrument. Figs. 43A-43C show IVIS photographs with animals face down. Figs. 43D-43F show IVIS photographs with animals face up. Figs. 43A and 43D show control animals injected with phosphate buffered saline solution. Figs. 43B, 43E, 43C and 43F show a prevention model in which animals were injected with anti-$NME7_{AB}$ antibodies 24 hours before injection of the metastatic cancer cells, then approximately every other day for a total of 12 antibody injections over 22 days. Images were taken on Day 27.

[00121] **Figures 44A-44D** shows photographs of female nu/nu mice that on Day 0 were injected into the tail vein with 10,000 Luciferase positive T47D metastatic breast cancer stem cells mixed with $NME7_{AB}$ to a final concentration of 32nM. On Day 1 and Day 2 animals were injected into the tail vein with more 32nM $NME7_{AB}$, which we have shown increases metastases. This is a system to demonstrate reversion of established metastases. On Day 7 animals were treated with individual anti-$NME7_{AB}$ antibodies 8F9A5A1, 8F9A4A3, or 5F3A5D4. Fig. 44A shows control animals injected with phosphate buffered saline solution. Fig. 44B shows animals treated with anti-$NME7_{AB}$ monoclonal antibody 8F9A5A1, also known as 5A1. Fig. 44C shows animals treated with anti-$NME7_{AB}$ monoclonal antibody 8F9A4A3, also known as 4A3. Fig. 44D shows animals treated with anti-$NME7_{AB}$ monoclonal antibody 5F3A5D4, also known as 5D4. Green arrows indicate low antibody dosage (5-7mg/kg) over the indicated period and Red arrows indicate high dosage (15mg/kg). As can be seen in the figure, animals treated with anti-$NME7_{AB}$ antibodies have less metastases than the control animals even though many of the animals in the groups to be treated with antibody have more metastasis before any treatment. Higher concentrations of anti-$NME7_{AB}$ antibody are more effective than low concentrations. For example between Day 11 and Day 17, animals were treated with high dose and most of the treated animals have cleared metastases by about Day 17. However, 1 low dose of antibody resulted in metastasis recurrence. Animals again respond to high dose treatment by Day 32.

[00122] **Figures 45A-45B** shows photographs of female nu/nu mice that on Day 0 were injected sub-cutaneously into the right flank with 10,000 Luciferase positive T47D metastatic breast cancer stem cells, mixed with $NME7_{AB}$ to a final concentration of 32nM, then mixed in a 1:1 vol:vol with Matrigel. Tumor engraftment was allowed to progress Day 0 – Day 6.

Animals were then treated i.v. by tail vein injection with anti-$NME7_{AB}$ antibodies. Control animals were injected with PBS. Fig. 45A shows IVIS photographs of control animals. Fig. 45B shows IVIS photographs of animals injected into tail vein with a cocktail of anti-$NME7_{AB}$ antibodies 5A1, 4A3 and 5D4 to a total concentration of 15mg/kg. Antibodies or PBS were administered 4 times between Day 7 and Day 18. As can be seen in the figure, the anti-$NME7_{AB}$ antibody treated animals show less metastases than the control group. In the treated group, 2 of the 5 animals have primary tumors that are larger than those in the control group. This could be because the anti-$NME7_{AB}$ antibodies prevented the spread of the cancer cells, so they remained concentrated in the primary tumor. In this experiment, PCR analysis showed that after 11 days in culture with $NME7_{AB}$, the T47D breast cancer cells had upregulated CXCR4 by 109-fold, OCT4 by 2-fold, NANOG by 3.5-fold and MUC1 by 2.7-fold.

[00123] **Figures 46A-46Q** shows photographs of female nu/nu mice that on Day 0 were injected sub-cutaneously into the right flank with 10,000 Luciferase positive T47D metastatic breast cancer stem cells, mixed with $NME7_{AB}$ to a final concentration of 32nM, then mixed in a 1:1 vol:vol with Matrigel. Tumor engraftment was allowed to progress Day 0 – Day 6. Animals were then treated i.v., by tail vein injection, with anti-$NME7_{AB}$ antibodies. Control animals were injected with PBS. On Day 38 animals were sacrificed and livers harvested then analyzed by IVIS to detect cancer cells that had metastasized to the liver. Figs. 46A-46B show whole body IVIS photographs of control animals that were injected with only PBS. Figs. 46C-46D show whole body IVIS photographs of control animals that were injected with the anti-$NME7_{AB}$ antibody 5A1. Figs. 46E-46F show whole body IVIS photographs of control animals that were injected with the anti-$NME7_{AB}$ antibody 4A3. Figs. 46G-46H show whole body IVIS photographs of control animals that were injected with the anti-$NME7_{AB}$ antibody 5D4. Figs. 46A, 46C, 46E, and 46G are IVIS photographs taken at Day 7 before any treatment. Figs. 46B, 46D, 46F, and 46H are IVIS photographs taken at Day 31 after anti-$NME7_{AB}$ antibody treatment or mock treatment. As can be seen in the figure, animals in the PBS control group show metastasis (blue dots) in the whole body IVIS photographs, while animals treated with anti-$NME7_{AB}$ antibodies do not. Figs. 46I-46P show photographs and IVIS photographs of livers and lung harvested from animals after sacrifice. Figs. 46I, 46K, 46M, and 46O are regular photographs. Figs. 46J, 46L, 46N, and 46P are IVIS photographs, illuminating the cancer cells that have metastasized there. As can be seen in the figure, the anti-$NME7_{AB}$ antibodies greatly inhibited metastasis to the liver, which is a primary site for breast cancer metastasis. Fig. 46Q is a bar graph of the measured photons emitted and enumerated by IVIS instrument for livers harvested from control animals versus the treated animals.

[00124] **Figures 47A-47F** shows photographs of immunofluorescent experiments in which various cancer cell lines are stained for the presence of $NME7_{AB}$. Fig. 47A shows T47D breast cancer cells stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 47B shows ZR-75-1 breast cancer cells, also known as 1500s, stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 47C shows H1975 non-small cell lung cancer cells stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 47D shows H292 non-small cell lung cancer cells stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 47E shows HPAFII pancreatic cancer cells stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 47F shows DU145 prostate cancer cells stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. As can be seen in the figure, all the cancer cell lines we tested show strong and membranous staining for $NME7_{AB}$. The monoclonal antibody used in these experiments was 5D4. In parallel, $NME7_{AB}$ antibodies 5A1 and 4A3 were used to stain the same cell lines and produced the same results.

[00125] **Figures 48A-48I** shows photographs of immunofluorescent experiments in which various lung cancer cell lines are stained for the presence of $NME7_{AB}$. Figs. 48A-48C shows H1975 non-small cell lung cancer cells, which are an adenocarcinoma, stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 48A is an overlay of DAPI and anti-$NME7_{AB}$ staining. Fig. 48B shows anti-$NME7_{AB}$ staining alone. Fig. 48C is a magnified view of the overlay of DAPI and anti-$NME7_{AB}$ staining. Figs. 48D-48F shows H292 non-small cell lung cancer cells, which are a mucoepidermoid pulmonary carcinoma, stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 48D is an overlay of DAPI and anti-$NME7_{AB}$ staining. Fig. 48E shows anti-$NME7_{AB}$ staining alone. Fig. 48F is a magnified view of the overlay of DAPI and anti-$NME7_{AB}$ staining. Figs. 48G-48I shows H358 non-small cell lung cancer cells, which are a metastatic bronchioalveolar carcinoma, stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 48G is an overlay of DAPI and anti-$NME7_{AB}$ staining. Fig. 48H shows anti-$NME7_{AB}$ staining alone. Fig. 48I is a magnified view of the overlay of DAPI and anti-$NME7_{AB}$ staining.

[00126] **Figure 49A-49I** shows PCR graphs of cancer cell lines, breast T47D, Lung H1975, lung H358 and pancreatic HPAFII before and after culture in $NME7_{AB}$. Fig. 49A measured breast metastatic marker CXCR4. Fig. 49B measured stem cell marker OCT4. Fig. 49C measured metastatic marker ALDH1. Fig. 49D measured stem cell marker SOX2. Fig. 49E measured stem cell marker NANOG. Fig. 49F measured marker CDH1, also known as E-cadherin. Fig. 49G measured metastatic marker CD133. Fig. 49H measured stem cell marker ZEB2. Fig. 49I measured stem, cancer and metastatic marker MUC1. The floater cells, also

known as tumor spheres become able to grow anchorage independently and show markers of metastasis that are more elevated than the adherent cells. Animals injected with cancer stem cells are those injected with the $NME7_{AB}$ grown floater cells. As can be seen in the figure markers of metastasis, stem cell markers, or markers of epithelial to mesenchymal transition (EMT) are elevated after culture in $NME7_{AB}$, indicating a transition to a more metastatic state.

[00127] **Figure 50A-50D** shows IVIS photographs of NSG mice injected into the tail vein with 10,000 cancer cells that were either NCI-H358 parent cells or NCI-H358 cells after 10 days in culture with $NME7_{AB}$. Fig. 50A and 50C show IVIS photographs of the mouse that was injected with the NCI-H358 lung cancer cells that had been grown in $NME7_{AB}$ for 10 days. Fig. 50B and 50D show IVIS photographs of the mouse that was injected with the parental NCI-H358 cells. Fig. 50A and 50B show the IVIS photographs where mice are imaged face down. Fig. 50C and 50D show the IVIS photographs where mice are imaged face up. As can be seen in the figure, the $NME7_{AB}$ grown cells have greatly increased metastatic potential.

[00128] **Figure 51** shows PCR graph of a MUC1 negative prostate cancer line PC3 before and after 2 or 3 passages in culture in either dimeric NM23-H1, also known as NME1, or $NME7_{AB}$. The graph shows the fold difference in markers of stem cells, cancer cells as well as metastatic markers. As can be seen in the figure, repeated culture in NME1 or NME7AB induces upregulation of stem, cancer and metastatic markers but also upregulates expression of MUC1 by 5-8 times.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[00129] **Definitions**

[00130] In the present application, “a” and “an” are used to refer to both single and a plurality of objects.

[00131] As used herein, “about” or “substantially” generally provides a leeway from being limited to an exact number. For example, as used in the context of the length of a polypeptide sequence, “about” or “substantially” indicates that the polypeptide is not to be limited to the recited number of amino acids. A few amino acids add to or subtracted from the N-terminus or C-terminus may be included so long as the functional activity such as its binding activity is present.

[00132] As used herein, administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

[00133] As used herein, "amino acid" and "amino acids" refer to all naturally occurring L-α-amino acids. This definition is meant to include norleucine, ornithine, and homocysteine.

[00134] As used herein, in general, the term "amino acid sequence variant" refers to molecules with some differences in their amino acid sequences as compared to a reference (e.g. native sequence) polypeptide. The amino acid alterations may be substitutions, insertions, deletions or any desired combinations of such changes in a native amino acid sequence.

[00135] Substitutional variants are those that have at least one amino acid residue in a native sequence removed and a different amino acid inserted in its place at the same position. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule.

[00136] Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the invention are proteins or fragments or derivatives thereof which exhibit the same or similar biological activity and derivatives which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, and so on.

[00137] Insertional variants are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a native amino acid sequence. Immediately adjacent to an amino acid means connected to either the α-carboxy or α-amino functional group of the amino acid.

[00138] Deletional variants are those with one or more amino acids in the native amino acid sequence removed. Ordinarily, deletional variants will have one or two amino acids deleted in a particular region of the molecule.

[00139] As used herein, “fragments” or “functional derivatives” refers to biologically active amino acid sequence variants and fragments of the polypeptide of the present invention, as well as covalent modifications, including derivatives obtained by reaction with organic derivatizing agents, post-translational modifications, derivatives with nonproteinaceous polymers, and immunoadhesins.

[00140] As used herein, "carriers" include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the pharmaceutically acceptable carrier is an aqueous pH buffered solution. Examples of pharmaceutically acceptable carriers include without limitation buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN®, polyethylene glycol (PEG), and PLURONICS®.

[00141] As used herein "pharmaceutically acceptable carrier and/or diluent" includes any and all solvents, dispersion media, coatings antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

[00142] It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired.

[00143] The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μg/ml of carrier. In the case of compositions

containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

[00144] As used herein, "vector", "polynucleotide vector", "construct" and "polynucleotide construct" are used interchangeably herein. A polynucleotide vector of this invention may be in any of several forms, including, but not limited to, RNA, DNA, RNA encapsulated in a retroviral coat, DNA encapsulated in an adenovirus coat, DNA packaged in another viral or viral-like form (such as herpes simplex, and adeno- structures, such as polyamides.

[00145] As used herein, "host cell" includes an individual cell or cell culture which can be or has been a recipient of a vector of this invention. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change.

[00146] As used herein, "subject" is a vertebrate, preferably a mammal, more preferably a human.

[00147] As used herein, "mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, and so on. Preferably, the mammal is human.

[00148] As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. "Palliating" a disease means that the extent and/or undesirable clinical manifestations of a disease state are lessened and/or the time course of the progression is slowed or lengthened, as compared to a situation without treatment.

[00149] As used herein, “A1” peptide, “A2” peptide, “B1” peptide, “B2” peptide and “B3” peptide refer to peptides that bind to human $NME7_{AB}$, but not (or significantly less) to human NME1. The peptides used to generate these antibodies are common to both $NME7_{AB}$ and NME7-X1, and are set forth as below.

[00150] A1 is NME7A peptide 1 (A domain): MLSRKEALDFHVDHQS (SEQ ID NO:141)

[00151] A2 is NME7A peptide 2 (A domain): SGVARTDASES (SEQ ID NO:142)

[00152] B1 is NME7B peptide 1 (B domain): DAGFEISAMQMFNMDRVNVE (SEQ ID NO:143)

[00153] B2 is NME7B peptide 2 (B domain): EVYKGVVTEYHDMVTE (SEQ ID NO:144)

[00154] B3 is NME7B peptide 3 (B domain): AIFGKTKIQNAVHCTDLPEDGLLEVQYFF (SEQ ID NO:145)

[00155] Further, for the sake of clarity, NME7A (with capital letter "A") refers to the subunit A portion of NME7. NME7a (with small letter "a") refers to the full-length NME7 that is described elsewhere in this application. And, NME7B (with capital letter "B") refers to the subunit B portion of NME7. NME7b (with small letter "b") refers to a species of NME7 that is partially devoid of the DM10 region, which is described elsewhere in this application.

[00156] 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.

[00157] As used herein, an "effective amount of an agent to inhibit an NME family member protein" refers to the effective amount of the agent in hindering the activating interaction between the NME family member protein and its cognate receptor such as

[00158] As used herein, "NME derived fragment" refers to a peptide sequence that is either a fragment of the NME or is highly homologous to the peptide sequence that is a fragment of the NME.

[00159] As used herein, the "MUC1*" extra cellular domain is defined primarily by the PSMGFR sequence (GTINVHDVETQFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGA (SEQ ID NO:6)). 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.

[00160] As used herein, the term "PSMGFR" is an acronym for Primary Sequence of MUC1 Growth Factor Receptor as set forth as GTINVHDVETQFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGA (SEQ ID NO:6). In this regard, the "N-number" as in "N-10 PSMGFR" or simply "N-10", "N-15 PSMGFR" or simply "N-15", or "N-20 PSMGFR" or simply "N-20" 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" or simply "C-10", "C-15 PSMGFR" or simply "C-15", or "C-20 PSMGFR" or simply "C-20" refers to the number of amino acid residues that have been deleted at the C-terminal end of PSMGFR. A mixture of deletions and additions is also possible. For instance, N+20/C-27 refers to a peptide fragment of wild-type MUC1 in which 20 amino acids are added to the PSMGFR at the N-terminus and 27 amino acids are deleted from the C-terminus.

[00161] 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.

[00162] As used herein, "high homology" is considered to be at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identity in a designated overlapping region between any two polypeptides.

[00163] As used herein, "NME family proteins" or "NME family member proteins", numbered 1-10, are proteins grouped together because they all have at least one NDPK (nucleotide diphosphate kinase) domain. In some cases, the NDPK domain is not functional in terms of being able to catalyze the conversion of ATP to ADP. NME proteins were formerly known as NM23 proteins, numbered H1 and H2. Recently, as many as ten (10) NME family members have been identified. Herein, the terms NM23 and NME are interchangeable. Herein, terms NME1, NME2, NME5, NME6, NME7, NME8 and NME9 are used to refer to the native protein as well as NME variants. In some cases these variants are more soluble, express better in *E. coli* or are more soluble than the native sequence protein. For example, NME7 as used in the specification can mean the native protein or a variant, such as $NME7_{AB}$ that has superior commercial applicability because variations allow high yield expression of the soluble, properly folded protein in *E. coli*. $NME7_{AB}$ consists primarily of the NME7 A and B domains but is devoid of most of the DM10 domain (SEQ ID NO:39), which is at the N-terminus of the

native protein. "NME1" as referred to herein is interchangeable with "NM23-H1". It is also intended that the invention not be limited by the exact sequence of the NME proteins. The mutant NME1-S120G, also called NM23-S120G, are used interchangeably throughout the application. The S120G mutants and the P96S mutant are preferred because of their preference for dimer formation, but may be referred to herein as NM23 dimers, NME1 dimers, or dimeric NME1, or dimeric NM23.

[00164] NME7 as referred to herein is intended to mean native NME7 having a molecular weight of about 42kDa.

[00165] A "family of NME7" refers to full length NME7 as well as naturally occurring or artificially created cleaved form having a molecular weight about 30kDa, 33kDa, or a cleaved form having a molecular weight of about 25kDa, a variant devoid or partially devoid of the DM10 leader sequence (SEQ ID NO:162), which is NME7 amino acids 1-91 of NME7 represented by SEQ ID NO:82 or 147, such as NME7b, NME7-X1, $NME7_{AB}$ or a recombinant NME7 protein, or variants thereof whose sequence may be altered to allow for efficient expression or that increase yield, solubility or other characteristics that make the NME7 more effective or commercially more viable. The "family of NME7" may also include "$NME7_{AB}$ -like" protein, which is a protein in the range of 30 to 33kDa that is expressed in cancer cells.

[00166] As used herein, an "an agent that maintains stem cells in the naïve state or reverts primed stem cells to the naïve state" refers to a protein, small molecule or nucleic acid that alone or in combination maintains stem cells in the naïve state, resembling cells of the inner cell mass of an embryo. Examples include but are not limited to human NME1 dimers, bacterial, fungal, yeast, viral or parasitic NME proteins that have high sequence identity to human NME proteins, especially NME1, NME7, NME7-X1, $NME7_{AB}$, NME6, 2i (Silva J et al, 2008; Hanna et al, 2010), 5i (Theunissen TW et al, 2014), nucleic acids such as siRNA that suppress expression of MBD3, CHD4 (Rais Y1 et al, 2013), BRD4, or JMJD6 (Liu W et al 2013).

[00167] As used herein, the terms "$NME7_{AB}$", "NME7AB" and "NME-AB" are used interchangeably.

[00168] As used herein, an "an agent that promotes pluripotency" or "reverts somatic cells to a stem-like or cancer-like state" refers to a protein, small molecule or nucleic acid that alone or in combination induces expression of or suppresses expression of certain genes such that the genetic signature shifts to one that more closely resembles stem cells or cancer cells. Examples include but are not limited to NME1 dimers, NME7, NME7-X1, $NME7_{AB}$, 2i, 5i, nucleic acids such as siRNA that suppress expression of MBD3, CHD4, BRD4, or JMJD6, microbial NME

proteins that have high sequence homology to human NME1, NME2, NME5, NME6, NME7, NME8, or NME9, preferably with the regions that house NDPK domains.

[00169] As used herein, in reference to an agent being referred to as a "small molecule", it may be a synthetic chemical or chemically based molecule having a molecular weight between 50Da and 2000Da, more preferably between 150 Da and 1000 Da, still more preferably between 200Da and 750Da.

[00170] As used herein, in reference to an agent being referred to as a "natural product", it may be chemical molecule or a biological molecule, so long as the molecule exists in nature.

[00171] As used herein, FGF, FGF-2 or bFGF refer to fibroblast growth factor (Xu RH et al, 2005; Xu C et al, 2005).

[00172] As used herein, "Rho associated kinase inhibitors" may be small molecules, peptides or proteins (Rath N, et al, 2012). Rho kinase inhibitors are abbreviated here and elsewhere as ROCi or ROCKi, or Ri. The use of specific rho kinase inhibitors are meant to be exemplary and can be substituted for any other rho kinase inhibitor.

[00173] As used herein, the term "cancer stem cells" or "tumor initiating cells" refers to cancer cells that express levels of genes that have been linked to a more metastatic state or more aggressive cancers. The terms "cancer stem cells" or "tumor initiating cells" can also refer to cancer cells for which far fewer cells are required to give rise to a tumor when transplanted into an animal. Cancer stem cells and tumor initiating cells are often resistant to chemotherapy drugs.

[00174] As used herein, the terms "stem/cancer", "cancer-like", "stem-like" refers to a state in which cells acquire characteristics of stem cells or cancer cells, share important elements of the gene expression profile of stem cells, cancer cells or cancer stem cells. Stem-like cells may be somatic cells undergoing induction to a less mature state, such as increasing expression of pluripotency genes. Stem-like cells also refers to cells that have undergone some de-differentiation or are in a meta-stable state from which they can alter their terminal differentiation. Cancer like cells may be cancer cells that have not yet been fully characterized but display morphology and characteristics of cancer cells, such as being able to grow anchorage-independently or being able to give rise to a tumor in an animal.

[00175] As used herein, "spacers" or "linkers" of different lengths can be incorporated anywhere in the peptide. Spacer attachment is usually through an amide linkage but other functionalities are possible.

[00176] NME, NME7 and protein family of NME7

[00177] The present inventors discovered that NME7 and NME7-X1 are highly expressed in early human stem cells and also in most cancer cells (Fig. 17, Fig. 18, Fig. 19A-Fig. 19F, Fig. 22, Fig. 23, Fig. 39, Fig. 40, Fig. 41, Fig. 47, Fig. 48. Figure 17 shows a graph of RT-PCR measurement of the expression of NME7-X1 in a panel of human stem cells and cancer cells. **Figure 18** shows a graph of RT-PCR measurement of the expression of NME7, NME7a, NME7b and NME7-X1 in a panel of human stem cells and cancer cells. NME7a is full-length NME7, NME7b is missing a small portion of the DM10 domain, NME7-X1 is missing all of the DM10 domain and a small portion of the N-terminus of the first NDPK A domain. The bar labeled NME7 means that primers were used that detected both NME7a and NME7b. **Figures 19A-19F** show photographs of Western blots in which various cancer cell lines are probed for expression of NME7 species using antibodies generated by immunization with NME7 derived short peptides. Figure 19A shows Western probed with antibody of the invention #52 which binds to NME7 derived peptide A1. Figure 19B shows Western probed with antibody of the invention #56 which binds to NME7 derived peptide B1. Figure 19C shows Western probed with antibody of the invention #61 which binds to NME7 derived peptide B3. **Figures 22A-22E** show photographs of Western blots of a co-immunoprecipitation experiment. T47D breast cancer cell extracts were incubated with an antibody against the MUC1 cytoplasmic tail, Ab-5, or a control antibody, IgG, and co-immunoprecipitated. The gels were blotted with two different commercially available anti-NME7 antibodies B9 (Fig. 22A) and CF7 (Fig. 22B). Both gels show unique NME7 bands at ~33kDa and ~30kDa. The gels were stripped and re-probed with an antibody against the extracellular domain of MUC1*, anti-PSMGFR (Fig. 22C) and (Fig. 22D), which shows that the NME7 species and MUC1* interact. A recombinant NME7-AB and a recombinant NME7-X1 were mixed together and run on a gel, then probed with an anti-NME7 antibody, showing that the two unique NME7 species that are naturally occurring in breast cancer cells and that interact with MUC1* are an NME7-AB-like species and NME7-X1 (Fig. 22E). **Figures 23A-23C** show photographs of Western blots of a co-immunoprecipitation experiment. Human induced pluripotent stem, iPS7, or embryonic stem, HES3, cell extracts were incubated with an antibody against the MUC1 cytoplasmic tail, Ab-5, or a control antibody, IgG, and co-immunoprecipitated. The gel was blotted with a commercially available anti-NME7 antibody B9 (Fig. 23A). Both cell types show unique NME7 bands at ~33kDa and ~30kDa. The gel was stripped and re-probed with an antibody against the extracellular domain of MUC1*, anti-PSMGFR (Fig. 23B), which shows that the NME7 species and MUC1* interact. A recombinant NME7-AB and a recombinant NME7-X1 were mixed together and run on a gel, then probed with an anti-NME7 antibody, showing that

the two unique NME7 species that are naturally occurring in breast cancer cells and that interact with MUC1* are an NME7-AB-like species and NME7-X1 (Fig. 23C). **Figures 39A-39C** shows human lung tissue specimens stained with an anti-NME7 antibody that binds to the B3 peptide. The figure shows lack of NME7 expression on normal tissues, increasing expression of NME7 as tumor grade and metastasis increase. **Figures 40A-40C** shows human small intestine tissue specimens stained with an anti-NME7 antibody that binds to the B3 peptide. The figure shows lack of NME7 expression on normal tissues, increasing expression of NME7 as tumor grade and metastasis increase. **Figures 41A-41D** show human colon tissue specimens stained with an anti-NME7 antibody that binds to the B3 peptide. The figure shows lack of NME7 expression on normal tissues, increasing expression of NME7 as tumor grade and metastasis increase. Figure 47 and Figure 48 show immunofluorescent photographs showing that NME7 is secreted by and binds to an extra cellular receptor of a wide variety of cancer cell lines.

[00178] Further, we demonstrated that like NM23-H1, NME7 binds to and dimerizes the MUC1* growth factor receptor on both stem cells and cancer cells (Fig. 1). Figure 5 shows a sequence alignment of NME1 and NME7 A and B domains.

[00179] The inventors recently discovered that NME7 is a primitive form of NME1 (NM23-H1) that is expressed in very early embryonic stem cells. NME7 is either not expressed at all, or is expressed at extremely low levels, in adult tissues. However, the inventors discovered that NME7 is expressed at high levels in cancerous cells and tissues and at even higher levels in metastatic cancer cells and tissues. A cleaved form of NME7 may be a secreted form allowing it to bind to and activate extracellular receptors. We detect full-length NME7, MW 42kDa, as well as NME7 species that are approximately 33kDa and 30kDa. The 33kDa and 30kDa species are secreted from cancer cells. Western blots detect full-length NME7 in cell lysates, but smaller 30-33kDa NME7 species in their conditioned media. Western blots probed with either an antibody that recognizes NME7 or an antibody that only recognizes the DM10 domain show that the lower molecular weight NME7 species that are secreted into the conditioned media are devoid of the DM10 domain. These data are consistent with the idea that naturally occurring NME7 species are comparable to the recombinant $NME7_{AB}$ we generated as they have nearly the same molecular weight, both are secreted and are both devoid of the 91 amino acids of the DM10 domain which may keep the protein retained within the cell.

[00180] We discovered a new NME7 isoform, NME7-X1, and also discovered that it is over-expressed in stem cells and cancer cells and is particularly over-expressed in prostate cancers (Fig. 17, Fig. 18, Fig. 19, and Fig. 22). NME7-X1, molecular weight ~30kDa, comprises NME7

amino acids 125-376, whereas the recombinant $NME7_{AB}$, molecular weight ~33kDa, that we generated spans amino acids 92-376, so includes 33 more N-terminal amino acids. NME7b spans amino acids 37-376 and is devoid of only 37 amino acids of the DM10 domain is also overexpressed in prostate cancers (Fig. 18). We generated a human recombinant NME7-X1 and show that it is the secreted 30kDa NME7 species in cancer cells that runs just lower than a naturally occurring ~33kDa NME7 species that appears to be a naturally occurring "$NME7_{AB}$ -like" protein that is a cleavage product or alternative isoform.

[00181] We tested a panel of cancer cell lines and found that they express high levels of NME7 and lower molecular weight species that may be truncations similar to $NME7_{AB}$, such as $NME7_{AB}$ -like protein, or alternate isoforms such as NME7-X1.

[00182] Whereas NM23-H1 (aka NME1) has to be a dimer, NME7 is a monomer with two binding sites for MUC1* extracellular domain. We generated a recombinant human NME7 that is devoid of the DM10 domain, which we call $NME7_{AB}$. A sandwich ELISA binding assay that shows that a recombinant $NME7_{AB}$ simultaneously binds to two PSMGFR peptides wherein the extracellular domain of MUC1* is comprised of most or all of the PSMGFR sequence (Fig. 1). In a nanoparticle binding assay, NME7 was also shown to be able to bind to and dimerize the PSMGFR portion of the MUC1* extracellular domain.

[00183] Agents that disable NME7, block its interaction with its binding partners or suppress its expression are potent anti-cancer therapeutics. Such agents may be antibodies, small molecules or nucleic acids. They may act on NME7 directly, on molecules that regulate NME7 expression, or on enzymes that cleave NME7 to cancer-promoting forms.

[00184] We discovered that like NM23-H1 dimers, a recombinant $NME7_{AB}$ monomer was fully able to support pluripotent human stem cell growth in the absence of any other growth factor, cytokine or serum. Competitively inhibiting the interaction between NME7 and MUC1* extracellular domain, comprised essentially of the PSMGFR sequence, induced differentiation of stem cells, showing that it is the interaction of NME7 and MUC1* that promotes stem cell growth and inhibits differentiation.

[00185] Next, we showed that $NME7_{AB}$ alone is also able to fully support human cancer cell growth. $NME7_{AB}$, when added to regular cancer cell growth media, stimulated cancer cell growth and in particular the growth of MUC1-positive and MUC1*-positive cancer cells. Inhibiting the interaction of NME7 with MUC1* inhibited cancer cell growth. Blocking the MUC1* growth factor receptor with an anti-MUC1* Fab potently inhibited cancer cell growth. Similarly, antibodies that bind to NME7 inhibit cancer cell growth. In one example of inhibition of cancer growth by anti-NME7 antibody, the polyclonal antibody was generated

from immunizing an animal with the portion of NME7 that spans amino acids 100-376 (Fig. 12 and Fig. 13). However, we found that antibodies generated from immunizing with shorter peptides from $NME7_{AB}$ or from NME7-X1 also inhibit cancer growth. In particular, they inhibit the growth of MUC1 and MUC1*-positive cancers. Anti-NME7 antibodies of the invention inhibited the formation of the non-adherent "floater" cells that are able to form tumor spheres and which can travel from primary tumor and metastasize (Fig. 14, Fig. 16, Fig, 29). Anti-NME7 antibodies of the invention inhibited the upregulation of metastatic and stem cell markers, now believed to also be characteristic of metastasis (Fig. 15, Fig. 30, Fig. 31, Fig. 32).

[00186] NME7 Causes Cancer Metastasis

[00187] The inventors further discovered that culturing cancer cells in a minimal media containing $NME7_{AB}$ induced a wide variety of cancer cells to become transformed to a more metastatic state. Evidence of this induced metastatic state include a change from adherent cell growth to non-adherent cell growth, aka, "floater" cells and accompanying up-regulation of specific metastatic markers that were especially upregulated in the floating cells. These metastatic markers that are upregulated after culture in $NME7_{AB}$ include but are not limited to CXCR4, CHD1 aka E-cadherin, MUC1, ALDH1, CD44, and pluripotent stem cell markers such as OCT4, SOX2, NANOG, KLF2/4, FOXa2, TBX3, ZEB2 and c-Myc (Fig. 2, Fig. 3, Fig. 20, Fig. 49, Fig. 51). Cancer cells cultured in $NME7_{AB}$ had dramatically higher engraftment rates when xenografted into test animals, which were over 90%. In addition, very low numbers of implanted cancer cells formed tumors in the test animals, which is evidence that $NME7_{AB}$ had transformed them into cancer stem cells also known as metastatic cancer cells. Cancer cells cultured in $NME7_{AB}$ and injected into the tail vein of NOD/SCID/GAMMA mice bearing estrogen release pellets metastasized in animals from low numbers of cells compared to the parent cells, grown in regular media (Fig. 33 - Fig. 38). Because cancer cells make either an NME7 cleavage product or alternative isoform that is essentially equivalent to $NME7_{AB}$, the methods described here are not limited to using $NME7_{AB}$; other NME7 species could work as well. For example, we discovered another NME7 isoform, NME7-X1, is expressed by cancer cells. It is identical to our recombinant $NME7_{AB}$ with the exception that the X1 isoform is missing 33 amino acids from the N-terminus. NME7-X1 is expected to function like $NME7_{AB}$. "$NME7_{AB}$ -like" protein has also been detected in cancer cells as being about 33Da species.

[00188] We note that the inventors' previous work showed that $NME7_{AB}$ alone is able to revert human stem cells to an earlier naïve state. We discovered that culturing cancer cells in the presence of other reagents that make stem cells revert to a more naïve state, makes the cancer cells transform to a more metastatic state. We demonstrated that culturing cancer cells

$NME7_{AB}$ (Fig. 2), or in dimeric NME1 (Fig. 3), or "2i" inhibitors (Fig. 4), are each able to transform regular cancer cells into metastatic cancer cells, which are also called cancer stem cells "CSCs" or tumor initiating cells "TICs". However, $NME7_{AB}$ induced cancer cells to enter a more metastatic state better than NME1, also known as NM23-H1, which was better than 2i.

[00189] 2i is the name given to two biochemical inhibitors that researchers found made human stem cells revert to a more naïve state. 2i are MEK and GSK3-beta inhibitors PD0325901 and CHIR99021, which are added to culture medium to final concentrations of about 1 mM and 3 mM, respectively. $NME7_{AB}$ and NME7-X1 are at a final concentration of about 4nM when added to separate batches of minimal medium to make cancer cells transform to metastatic cells, although lower and higher concentrations also work well in the range of about 1nM to 16nM. Human or bacterial NME1 dimers are used at a final concentration of 4nM to 32nM, with 16nM typically used in these experiments, wherein the human NME bears the S120G mutation. Lower concentrations may be required if using wild type. It is not intended that these exact concentrations are important. It is important that the NME1 proteins are dimers and the range of concentrations over which this happens is in the low nanomolar range although certain mutations allow higher concentrations to remain as dimers. Similarly, the concentrations of NME7 proteins can vary. $NME7_{AB}$ and NME7-X1 are monomers and concentrations used to transform cancer cells to metastatic cells should allow the proteins to remain as monomers.

[00190] In addition to NME7, $NME7_{AB}$, NME7-X1, and the 2i inhibitors MEKi and GSK3i, other reagents and inhibitors have been shown by others to cause stem cells to revert to a more naïve state. These inhibitors, "i's" include JNKi, p38i, PKCi, ROCKi, BMPi, BRAFi, SRCi as well as growth factors activing and LIF (Gafni et al 2013, Chan et al 2013, Valamehr et al 2014, Ware et al 2014, Theunissen et al 2014). These reagents can also be used to make cancer cells progress to a more metastatic state. Cells that have been induced to transform to a more metastatic state using any single factor or combination of the inhibitors or growth factors, that make stem cells revert to a more naïve state, can then be used as discovery tools to identify or test drugs to treat or prevent cancer metastasis.

[00191] Various molecular markers have been proposed as being indicators of metastatic cancer cells. Different cancer types may have different molecules that are up-regulated. For example, the receptor CXCR4 is up-regulated in metastatic breast cancers while E-cadherin, also known as CHD1, is up-regulated more in metastatic prostate cancers. In addition to these specific metastasis markers, typical markers of pluripotency such as OCT4, SOX2, NANOG, and KLF4 are up-regulated as cancers become metastatic. The starting cancer cells and the later

metastatic cancer cells are assayed by PCR to measure expression levels of these genes. We demonstrated that these cancer cells, cultured in agents such as $NME7_{AB}$ that cause them to be transformed to a more metastatic state, as evidenced by increased expression of metastatic markers and pluripotent stem cell markers, function as metastatic cancer cells.

[00192] A functional test of whether or not a population of cancer cells is metastatic is to implant very low numbers, e.g. 200, of the cells in immuno-compromised mice and see if they develop into a tumor. Typically 5-6 million cancer cells are required to form a tumor in an immuno-compromised mouse. We showed that as few as 50 of the NME-induced metastatic cancer cells formed tumors in mice. In addition, mice that were injected throughout the test period with human $NME7_{AB}$, NME1, or NME7-X1 developed remote metastases.

[00193] In one particular experiment, T47D human breast cancer cells were cultured in standard RPMI media for 14 days with media changes every 48 hours and passed by trypsinization when approximately 75% confluent. The cells were then plated into 6-well plates and cultured in minimal stem cell media (see Example 1) that was supplemented with 4nM $NME7_{AB}$ B. Media was changed every 48 hours. By about Day 4, some cells become detached from the surface and float. Media is carefully changed so as to retain the "floaters" as these are the cells that have the highest metastatic potential as evidence by RT-PCR measurement of metastatic markers. On Day 7 or 8, the floaters are harvested and counted. Samples are retained for RT-PCR measurement. The key marker measured is CXCR4, which is up-regulated by 40-200-times after being briefly cultured in $NME7_{AB}$.

[00194] The freshly harvested floater metastatic cells were xenografted into the flank of female nu/nu athymic mice that have been implanted with 90-day slow release estrogen pellets. Floater cells were xenografted with 10,000, 1,000, 100 or 50 cells each. Half of the mice in each group of 6 were also injected daily with 32nM $NME7_{AB}$ near the original implantation site. The parent T47D cells that were cultured in RPMI media without $NME7_{AB}$ were also implanted into mice at 6 million, 10,000 or 100 as controls. Mice implanted with the NME7-induced floater cells developed tumors even when as few as 50 cells were implanted. Mice that were implanted with the floater cells and that received daily injections of $NME7_{AB}$ also developed remote tumors or remote metastases in various organs. 11 out of the 12 mice, or 92%, that were injected with human $NME7_{AB}$ after implantation of the $NME7_{AB}$ cultured cancer cells developed tumors at the injection site. Only 7 out of the 12 mice, or 58%, that were not injected with human $NME7_{AB}$ after implantation developed tumors. 9 out of the 11 mice, or 82%, that exhibited tumors and were injected with human $NME7_{AB}$ developed

multiple tumors remote from the injection site. None of the mice that were not injected with $NME7_{AB}$ developed multiple, visible tumors.

[00195] After sacrifice, RT-PCR and Western blots showed that the remote bumps on the mice injected with $NME7_{AB}$ were indeed human breast tumors. Similar analysis of their organs showed that in addition to remote bumps, mice had randomly metastasized to the liver and lung with human breast cancer characteristic of the human breast cancer cells that were implanted. As expected, only the mice implanted with 6 million cells grew tumors.

[00196] We have demonstrated that human recombinant $NME7_{AB}$ is comparable in size and sequence to NME7-X1 and to a 30-33kDa NME7 cleavage product. We have shown that $NME7_{AB}$ promotes cancerous growth and causes cancer cells to accelerate to the highly metastatic cancer stem cell (CSC) state also called tumor initiating cells (TIC). Therefore, we conclude that NME7-X1 and an NME7 cleavage product that removes the DM10 domain also promote cancerous growth and causes cancer cells to accelerate to the highly metastatic cancer stem cell (CSC) state also called tumor initiating cells (TIC). In one example, $NME7_{AB}$ was added to cancer cells in a serum-free media and in the absence of any other growth factors or cytokines. Within 7-10 days, the cancer cells had reverted to the highly metastatic CSCs/TICs as evidenced by more than 100-fold increase in the expression of molecular markers such as CXCR4, which are indicators of metastatic cancer cells. In one example, T47D breast cancer cells were cultured in either standard RPMI media or in a Minimal Stem Cell Media (Example 1) to which was added recombinant $NME7_{AB}$ to a final concentration of 16nM. After 10 days cells were collected and analyzed by RT-PCR for expression of molecular markers of CSCs which were elevated by 10-200-times (Fig. 2). This is a specific, detailed example of how we transformed one cancer cell type to a more metastatic state. It is not intended that the invention be limited by these details as there are a range of cancer cells that are transformed in this way, a range of reagents that revert stem cells to a more naïve state that also progress cancer cells to a more metastatic state and a range of concentrations over which the added reagents transform the cancer cells. Other types of cancer cells have required longer periods of culture in $NME7_{AB}$ for dramatic upregulation of metastatic markers and ability to form tumors from very low numbers of cancer cells implanted. For example, prostate cancer cells cultured in $NME7_{AB}$, 2i, human NME1 or bacterial NME1 that has high homology to human NME1 or human NME7 showed dramatic increase in metastatic markers after 2-3 passages.

[00197] Metastasis marker CXCR4 is particularly elevated in metastatic breast cancer cells, while CHD1 is particularly elevated in metastatic prostate cancer. Here we show that

pluripotent stem cell markers such as OCT4, SOX2, NANOG, KLF2/4 and TBX3 are also up-regulated when cancer cells transform to more metastatic cells.

[00198] DU145 prostate cancer cells were cultured similarly and those cells cultured in $NME7_{AB}$ also showed dramatic increases in expression of CSC markers (Fig. 3). In prostate cancer cells, CHD1 (aka E-cadherin) and CXCR4 were up-regulated compared to the control cancer cells, which were not grown in $NME7_{AB}$, along with other pluripotent stem cell markers. Figure 20A-20C shows that ovarian cancer cell lines SK-OV3, OV-90 and breast cancer cell line MDA-MB all transitioned from adherent to non-adherent floater cells and increased expression of metastatic markers after 72 or 144 hours in culture with $NME7_{AB}$. Ovarian, prostate, pancreatic cancer cells and melanoma cells were also cultured in $NME7_{AB}$ and were transformed to a more metastatic state after as few as 3 days in culture. Figure 21 shows that breast, ovarian, prostate, pancreatic cancer cells and melanoma cells express MUC1 and MUC1*.

[00199] Here we have shown that $NME7_{AB}$ transforms a wide range of cancer cells to a more metastatic state. We have also shown that cancer cells express a naturally occurring species that is approximately the same molecular weight as recombinant $NME7_{AB}$ 33kDa (Fig. 17, Fig. 18. Fig. 19, and Fig. 22 and is also devoid of the DM10 domain like $NME7_{AB}$ and also express an alternative isoform NME7-X1 30kDa which is the same sequence as $NME7_{AB}$ except is missing 33 amino acids from the N-terminus. A co-immunoprecipitation experiment was performed on T47D breast cancer cells, wherein the cell extracts were incubated with an antibody against the MUC1 cytoplasmic tail, Ab-5, or a control antibody, IgG, and co-immunoprecipitated. The immunoprecipitated species were separated by gel electrophoresis. The gels were blotted with two different commercially available anti-NME7 antibodies. Both gels show unique NME7 bands at ~33kDa and ~30kDa (Fig. 22A - 22B). The gels were stripped and re-probed with an antibody against the extracellular domain of MUC1*, anti-PSMGFR (Fig. 22C- 22D), which shows that the NME7 species and MUC1* interact. A recombinant $NME7_{AB}$ and a recombinant NME7-X1 that we made were mixed together and run on a gel, then probed with an anti-NME7 antibody, showing that the two unique NME7 species that are naturally occurring in breast cancer cells and that interact with MUC1* are an $NME7_{AB}$ -like species and NME7-X1 (Fig. 22E). A similar experiment was carried out in human stem cells. Figure 23A-23C show photographs of Western blots of a co-immunoprecipitation experiment. Human induced pluripotent stem, iPS7, or embryonic stem, HES3, cell extracts were incubated with an antibody against the MUC1 cytoplasmic tail, Ab-5, or a control antibody, IgG, and co-immunoprecipitated. The gel was blotted with a

commercially available anti-NME7 antibody B9 (Fig. 23A). Both cell types show unique NME7 bands at ~33kDa and ~30kDa. The gel was stripped and re-probed with an antibody against the extracellular domain of MUC1*, anti-PSMGFR (Fig. 23B), which shows that the NME7 species and MUC1* interact. A recombinant $NME7_{AB}$ and a recombinant NME7-X1 that we made were mixed together and run on a gel, then probed with an anti-NME7 antibody, showing that the two unique NME7 species that are naturally occurring in breast cancer cells and that interact with MUC1* are an $NME7_{AB}$ -like species and NME7-X1 (Fig. 23C). Because $NME7_{AB}$ is a recombinant protein, we do not know if the naturally occurring species may contain an extra 1-15 additional amino acids or devoid of 1-15 additional amino acids than the recombinant $NME7_{AB}$, yet run with the same apparent molecular weight. By "$NME7_{AB}$ -like", we mean an NME7 species that runs with an apparent molecular weight of approximately 33kDa that is able to function the way the recombinant $NME7_{AB}$ does, in that it is able to stimulate cancer cell growth, induce transition of cancer cells to a more metastatic state and is able to fully support pluripotent growth of human stem cells.

[00200] We conclude that cancer cell lines and cancer cell populations that express NME7 and lower molecular weight NME7 species contain some cancer cells that are CSCs or metastatic cancer cells. These cancers can be made more metastatic or increase the population of cells that are metastatic by culturing the cells in $NME7_{AB}$, NME7-X1 or lower molecular weight NME7 species. Figure 19 shows a Western blot of a panel of cancer cells all expressing NME7 as well as lower molecular weight species $NME7_{AB}$ -like at 33kDa and NME7-X1 at 30kDa. Figure 21 shows that cancer cell lines T47D breast cancer, PC3 and DU145 prostate cancer, BT-474 breast cancer, CHL-1 and A2058 both melanoma cell lines and CAPAN-2 and PANC-1 both pancreatic cell lines all express MUC1 and MUC1*. In Figure 21A, BT474 cells appear not to express MUC1 or MUC1* however, we previously showed (Fessler et al 2009) that when these HER2 positive breast cancer cells become resistant to chemotherapy drugs, i.e. metastatic, they do so by increasing expression of MUC1* (Fig. 21D). Blocking the MUC1* receptor with an anti-MUC1* Fab reversed their resistance to Herceptin (Fig. 21E), Taxol (Fig. 21F) as well as other chemo agents. These cancer types and other cancer types that express NME7 and lower molecular weight NME7 species such as 33kDa, 30kDa can be made more metastatic or increase the population of cells that are metastatic by culturing the cells in $NME7_{AB}$, NME7-X1 or lower molecular weight NME7 species.

[00201] Conversely, the metastatic potential of these and other cancer types that express NME7 and lower molecular weight NME7 species such as 33kDa or 30kDa can be reversed by treating the cells with anti-NME7 antibodies. Anti-NME7 antibodies or antibodies that bind

to $NME7_{AB}$ or NME7-X1 are administered to a patient for the treatment or prevention of cancers including breast, prostate, ovarian, pancreatic and liver cancers. Because we have shown that $NME7_{AB}$ exerts its tumorigenic effects by binding to and activating the MUC1* growth factor receptor, anti-NME7 antibodies will be effective against any MUC1*-positive cancers, which include but are not limited to breast, lung, liver, pancreatic, gastric colorectal, prostate, brain, melanoma, kidney and others. Anti-NME7, anti- $NME7_{AB}$ or anti-NME7-X1 antibodies are administered to patients for the treatment or prevention of cancers that are $NME7_{AB}$, $NME7_{AB}$ -like, or NME7-X1 positive or a MUC1* positive.

[00202] Testing Patient Cancer Cells for Effective Therapies

[00203] $NME7_{AB}$, NME7-X1 as well as 2i and other reagents that revert stem cells to a more naïve state also induce cancer cells to transform to a more metastatic state. After treatment with any one or combination of these reagents, cancer cells have a higher engraftment rate and require up to 100,000-times less cells to cause a tumor to form in a test animal. Therefore, methods described in this disclosure can be used to enable xenografting of a patient's primary tumor cells into a test animal.

[00204] Candidate therapeutic agents can then be tested on the recipient animal. Effective therapeutic agents identified in this way can be used to treat the donor patient or other patients with similar cancers. In one embodiment, a method of identifying effective therapeutics for a particular patient or a particular type of cancer comprises the steps of: 1) cancer cells are obtained from a cell line, a patient or a patient to whom the therapeutic being tested will be administered; 2) cancer cells are cultured in $NME7_{AB}$, NME7-X1, human NME1, bacterial NME1 that has high homology to human NME1 or NME7, 2i, or other reagents shown to revert stem cells to a more naïve state; 3) resultant cancer cells are implanted into a test animal to which human $NME7_{AB}$, NME7-X1, human NME1, bacterial NME1 that has high homology to human NME1 or NME7, 2i, or other reagents shown to revert stem cells to a more naïve state may also be administered or animal is transgenic for human $NME7_{AB}$ or NME7-X1; 4) candidate anti-cancer therapeutic agents are administered to the animal; 5) efficacy of the therapeutic agents are assessed; and 6) effective therapeutic agent is administered to the donor patient or to another patient with similar cancer.

[00205] Anti-NME7 Antibodies

[00206] Anti-NME7 antibodies are potent anti-cancer agents. NME7 is a growth factor that promotes the growth of cancer cells and also promotes their progression to a more metastatic state or a more aggressive state. NME7 and a truncated form of NME7 that is ~ 33 kDa or 30 kDa have been shown to fully support cancer growth even in serum-free media devoid of any

other growth factors or cytokines. In pull-down assays, ELISAs and nanoparticle binding experiments, we have shown that the growth factor receptor MUC1* is a binding partner of NME7 and $NME7_{AB}$. Promotion of this interaction by eliminating all other growth factors or cytokines increased expression of cancer stem cell markers. Blocking this interaction even in the presence of serum, using a polyclonal antibody that specifically binds to NME7 actively killed the cancer cells. Thus, anti-NME7 or anti- $NME7_{AB}$ antibodies are potent anti-cancer agents that can be administered to a patient for the treatment or prevention of cancers. More than 75% of all cancers are MUC1* positive. MUC1* is the transmembrane cleavage product of MUC1 wherein most of the extracellular domain has been shed, leaving a portion of the extracellular domain that contains most of the PSMGFR sequence and may contain 9-20 additional amino acids N-terminal to the boundary of the of the PSMGFR sequence.

[00207] One aspect of the invention is a method of treating or preventing cancer in a subject, comprising administering to the subject an effective amount of an anti-NME7 antibody. In one instance, the anti-NME7 antibody is able to bind to $NME7_{AB}$. In another instance, the anti-NME7 antibody is able to bind to NME7-X1. In yet another instance, the anti-NME7 antibody that is administered to a patient inhibits or prevents its binding to its target in the promotion of cancers. In one case, the target is the extracellular domain of a cleaved MUC1. More specifically, the NME7 target that promotes cancer is the PSMGFR region of the MUC1* extracellular domain. In one aspect, an effective therapeutic agent is one that disrupts or prevents the interaction between an NME7 species and MUC1* extracellular domain, consisting primarily of the PSMGFR portion of MUC1* or the PSMGFR peptide. Agents for the treatment or prevention of cancers are those agents that directly or indirectly inhibit the expression or function of NME7, an $NME7_{AB}$ -like cleavage product or alternative isoform, including NME7-X1. In one case an effective anti-cancer therapeutic agent is one that binds to the NME7 species or disables its tumorigenic activity. An effective therapeutic agent for the treatment or prevention of cancers is an agent that binds to or disables NME7, an $NME7_{AB}$ -like cleavage product or alternative isoform, or NME7-X1. In one aspect, the therapeutic agents that binds to the NME7 species is an antibody. The antibody may be polyclonal, monoclonal, bispecific, bivalent, monovalent, single chain, scFv, or an antibody mimic that may be animal in origin, human-animal chimera, humanized or human. The antibody can be generated by inoculation or immunization with an NME7 species or fragment thereof or selected, for example from a library or a pool of antibodies, for their ability to bind to an NME7 species, including NME7, an $NME7_{AB}$ -like cleavage product or alternative isoform, including NME7-X1.

[00208] Generation of Anti-NME7 Antibodies

[00209] Anti-NME7 antibodies can be generated outside of the patient such as in a host animal or in a patient. Antibodies can be generated by immunization of NME7 or NME7 fragments or selected from a library or pool of antibodies that may be natural, synthetic, whole or antibody fragments based on their ability to bind to desired NME7 species such as $NME7_{AB}$ or NME7-X1. In one aspect, the antibody is generated from immunization with, or selected for its ability to bind to, a peptide selected from those listed in Figure 6-9. In another aspect, the antibody is generated from peptides whose sequences are not identical to those of human NME1 or the antibodies are selected for their ability to bind to NME7 species and their inability to bind to human NME1.

[00210] One method used to identify NME7 or NME7-X1 derived peptides that give rise to antibodies that inhibit cancer growth and inhibit transition to metastasis or peptides that are themselves inhibitory is as follows: 1) protein sequences of human NME1, human NME7, human NME7-X1 and several bacterial or fungal NME proteins that have high sequence homology to either human NME1 or human NME7 are aligned; 2) regions of high sequence homology among all the NMEs are identified; 3) peptide sequences that are unique to NME7 or NME7-X1 but are flanking the regions of high sequence homology are identified. The peptides are then synthesized and used to generate antibodies in a human or host animal. The resultant antibodies are selected for therapeutic use if: 1) they bind to $NME7_{AB}$ or NME7-X1, but not to NME1; 2) have the ability to inhibit cancer growth; 3) have the ability to inhibit the transition of cancer cells to a more metastatic state; or 4) inhibit metastasis in vivo. In some cases, antibodies for therapeutic use are selected for their ability to disrupt binding of $NME7_{AB}$ or NME7-X1 to the MUC1* extra cellular domain, to the PSMGFR peptide or to the N-10 peptide.

[00211] Use of Anti-NME7 Antibody for Treatment of Cancer

[00212] Those antibodies that inhibit cancer growth or transition to a more metastatic state are selected for use as anti-cancer therapeutics and may be administered to a patient for the treatment or prevention of cancers. Selected antibodies may be further optimized for example by engineering or making human chimera antibodies or fully human antibodies. To demonstrate the efficacy of this approach, we selected NME7 peptides from regions of NME7 suspected to be critical to its cancerous function. We then generated antibodies using these peptides and then tested both the resultant antibodies as well as the immunizing peptides for their ability to: a) inhibit cancerous growth; and b) inhibit the induced transition from cancer cells to metastatic cancer cells. NME7 peptides were selected as immunizing agents for

antibody production and as inhibitory agents themselves (Fig. 9, Example 7). Peptides A1 (SEQ ID NO:141), A2 (SEQ ID NO:142), B1 (SEQ ID NO:143), B2 (SEQ ID NO:144) and B3 (SEQ ID NO:145), wherein A refers to the domain from which the peptide is derived, i.e. the NDPK A domain and the B denotes that the peptide is derived from the NDPK B domain (Fig 5). Each peptide was used as an immunogen and injected into 2 rabbits each for production of polyclonal antibodies. The antibodies that were harvested from the blood of the immunized rabbits were purified over a column derivatized with the immunizing peptide. The purified antibodies were then tested for their ability to bind to human NME7. All of the resultant antibodies bound to human NME7 but not human NME1 as desired (Fig. 10A-10B, Example 8). These results show that by choosing peptides whose sequence is found in NME7 but not exactly identical in NME1, antibodies are generated that specifically bind to NME7 but not NME1. Because NME1 has healthy function, it is in most cases desirable to generate antibodies that do not interfere with NME1. The antibodies were also tested for their ability to inhibit the binding of NME7 to a MUC1* extracellular domain peptide. The ELISA experiment shown in Figure 11 shows that the antibodies inhibited the binding of $NME7_{AB}$ to a MUC1* extracellular domain peptide much more than they inhibited binding of NME1. Recall that each of the NME7 A domain and B domain can bind to a PSMGFR peptide. Therefore, complete inhibition of $NME7_{AB}$ binding to a PSMGFR peptide cannot be accomplished with a single antibody or peptide that is derived from just one domain. These antibodies and their respective immunizing peptides also inhibited cancer cell growth (Fig. 12-13). These antibodies also inhibited the formation of non-adherent "floater" cells that result from growing cancer cells in $NME7_{AB}$ (Fig. 14). As can be seen in the figure, the ploYclonal antibody generated by immunization with the B3 peptide reduced the number of metastatic floater cells by 95%, indicating that anti-NME7 antibodies that bind to the B3 peptide are most effective at inhibiting cancer metastasis. Similarly, the antibodies inhibited the expression of metastatic marker CXCR4 (Fig. 15A). Again, the B3 antibodies were most efficient at inhibiting expression of CXCR4; bar labeled NME7 FL (NME7 floater cells) shows 70-fold increase in CXCR4 that B3 antibody 61 decreased to 20-fold (bar labeled NME7+61 FL). In addition, the immunizing peptides themselves inhibited the upregulation of CXCR4 and other metastatic markers when T47D cancer cells were grown in $NME7_{AB}$ or 2i.

[00213] This is but one example of selecting peptides that generate antibodies that inhibit the cancerous function of NME7 and NME7 species. Sequence alignment among human NME1, human NME7, human NME7-X1 and bacterial NME proteins that had high sequence homology to human NME1 or NME7 identified five regions of homology. The fact that

peptides A1, A2, B1, B2 and B3 all generated antibodies that inhibited cancer growth or their transition to a metastatic state means that the five regions from which these peptides were derived are regions of NME7 that are important for its function in the promotion of cancer. Other peptides from these regions will also give rise to anti-NME7 antibodies that will inhibit cancer growth and metastasis and are therefore potent anti-cancer therapeutics. Antibodies generated from peptides A1, A2, B1, B2 and B3 were shown to inhibit cancer growth and inhibited the transition to a more metastatic state. Monoclonal antibodies generated by immunization with the same or similar peptides and subsequent testing of the monoclonals will identify antibodies that, after humanizing or other engineering known to those skilled in the art, would be administered to a patient for the treatment or prevention of cancers.

[00214] In a particular experiment, the antibodies generated by immunization with peptides A1, A2, B1, B2 and B3, as well as the immunizing peptides themselves, were added to cancer cells in culture to see if the addition of the antibodies or the immunizing peptides would inhibit cancer cell growth. At low concentrations and added separately, the antibodies as well as the immunizing peptides inhibited cancer cells growth (Fig. 12 for one example). However, when added at higher concentrations or combined, the antibodies as well as the immunizing peptides robustly inhibited cancer cell growth (Fig. 13). The corresponding human NME7 amino acid numbers of immunizing peptides A1, A2, B1, B2 and B3 are 127-142, 181-191, 263-282, 287-301, 343-371, respectively, from human full-length NME7 having SEQ ID NO:82 or 147.

[00215] To clarify, when residue numbers of NME7 are discussed, they refer to the residue numbers of NME7 as set forth in SEQ ID NO:82 or 147.

[00216] The antibody used in cancer growth inhibition experiments and one of the antibodies shown in Figure 12 was generated by immunizing with NME7 peptide corresponding to amino acids 100-376 of NME7 (SEQ ID NO:82 or 147). To generate higher affinity and specific anti-NME7 antibodies, the following steps are followed: immunize animal with a peptide containing human NME7 amino acids 100-376, then: 1) de-select those antibodies that bind to human NME1; 2) select those antibodies that inhibit $NME7_{AB}$, 2i, or other NME induced transition of cancer cells to a more metastatic state; 3) select those antibodies that inhibit the growth of cancer cells; 4) select those antibodies that inhibit the growth of MUC1* positive cancer cells; 5) select those antibodies that inhibit binding of $NME7_{AB}$ or NME7-X1 to MUC1* extracellular domain, essentially inhibit binding to the PSMGFR peptide; and/or 6) select those antibodies that bind to one or more of the peptides listed in Figure 9 - A1, A2, B1, B2 or B3 peptides.

[00217] Higher affinity monoclonal antibodies or monoclonal antibodies generated from longer peptides may be more effective antibody therapeutics. Alternatively, combinations of ani-NME7, anti- $NME7_{AB}$ or anti-NME7-X1 antibodies are administered to a patient to increase efficacy.

[00218] Anti-NME7 antibodies inhibit the transition of cancer cells to metastatic cancer cells.

[00219] Anti-NME7 antibodies inhibit transition of cancer cells to metastatic cancer cells also called cancer stem cells (CSCs) or tumor initiating cells (TICs). Recall that we have demonstrated that culturing a wide variety of cancer cells in the presence of $NME7_{AB}$ causes them to transition from regular cancer cells to the metastatic CSCs or TICs. Thus, antibodies that bind to NME7, $NME7_{AB}$ or NME7-X1 will inhibit the progression of cancer cells to a more metastatic state.

[00220] Cancer cells transform to a more metastatic state when cultured in the presence of agents that revert stem cells to a more naïve state. We have demonstrated that culturing cancer cells in $NME7_{AB}$, human NME1 dimers, bacterial NME1 dimers or MEK and GSK3-beta inhibitors, called "2i", causes the cells to become more metastatic. As the cells transition to a more metastatic state, they become non-adherent or less adherent and float off of the culture plate. These floating cells, "floaters" were collected separately from those that were adherent and were shown to: a) express much higher levels of metastatic genes; and b) generated tumors when xenografted into mice at very low copy number. RT-PCR measurement of specific metastatic markers such as CXCR4 for breast cancers, CHD1 for prostate cancer, and other pluripotent stem cell markers such as OCT4, SOX2, NANOG, KLF4 and others were dramatically over-expressed in cancer cells that were cultured in $NME7_{AB}$ and most over-expressed in the cells that became non-adherent, called "floaters" here and in figures.

[00221] In one example, $NME7_{AB}$ specific antibodies, generated by immunization with NME7-derived peptides A1, A2, B1, B2 and B3, as well as the immunizing peptides themselves, were added into the media along with either $NME7_{AB}$ or 2i to determine if they inhibited the transformation of regular cancer cells to metastatic cancer stem cells. Antibodies and peptides were separately added along with the agent that causes metastatic transformation; in this case $NME7_{AB}$ or the 2i inhibitors PD0325901 and CHIR99021. $NME7_{AB}$ and 2i were separately used to induce the cancer cells to be transformed to a more aggressive metastatic state. 2i was used so that it could not be argued that the antibodies that were added to the media simply sopped up all of the $NME7_{AB}$ so that the causative agent effectively was not there (Example 10).

[00222] Visual observation was independently recorded by two scientists as the experiment progressed (Fig. 14). The most striking observation was that the antibodies and the peptides dramatically reduced the number of floater cells, which was the first indication that the antibodies and peptides inhibit the transformation to metastatic cancer cells. In particular, cells to which the antibody generated from immunization with the B3 peptide barely generated any floater cells. mRNA was extracted from both the floater cells, the adherent cells and the control cancer cells. The amount of mRNA, which indicates cell viability and growth, was measured. Cells that were treated with antibody had much less mRNA, indicating less live dividing cells (Fig. 16), which confirms that anti- $NME7_{AB}$ antibodies inhibit cancer cell growth as well as their transition to a more metastatic state. RT-PCR was used to measure expression levels of metastatic markers, including CXCR4. Treatment with the anti-NME7 antibodies greatly reduced the amount of metastatic markers, such as CXCR4, indicating that the anti-NME7 antibodies or peptides inhibit the transition to metastatic cancer (Fig. 15A-15C). These results show that antibodies that bind to $NME7_{AB}$ can be administered to a patient for the treatment or prevention of metastatic cancers.

[00223] Peptides derived from $NME7_{AB}$ or NME7-X1 competitively inhibit the binding of intact $NME7_{AB}$ and NME7-X1 and are anti-cancer agents.

[00224] In another aspect of the invention, therapeutic agents for the treatment or prevention of cancers are peptides derived from the NME7 sequence, which are administered to a patient for the treatment or prevention of cancers. In one aspect, the NME7-derived peptides are administered to a patient so that the peptides, which should be shorter than the entire NME7 and unable to confer the oncogenic activity of NME7, bind to the targets of NME7 and competitively inhibit the interaction of intact NME7 with its targets, wherein such interactions promote cancer. Since $NME7_{AB}$ is fully able to confer oncogenic activity, the sequence of $NME7_{AB}$ is preferred as the source for the shorter peptide(s), wherein it must be confirmed that the peptides themselves are not able to promote cancerous growth or other tumorigenic or oncogenic activity. In a preferred embodiment, one or more peptides having the sequence of a portion of $NME7_{AB}$ and being preferably about 12-56 amino acids in length are administered to a patient. To increase half-life, the peptides may be peptide mimics, such as peptides with unnatural backbone or D-form amino acids for L. In yet another case, the anti-cancer therapeutic agent is a peptide or peptide mimic wherein the peptide has a sequence highly homologous to at least a portion of NME7, $NME7_{AB}$, or NME7-X1 or its target the MUC1* extracellular domain, comprising the PSMGFR peptide, also called "FLR" in some cases herein.

[00225] Figure 6 - Figure 9 provide a listing of preferred amino acid sequences that are predicted to inhibit NME7 binding to its cognate target. In a still more preferred embodiment, the peptides that are chosen for administration to a patient suffering from cancer or at risk of developing cancer are chosen because they bind to an NME7 binding partner and they do not themselves confer tumorigenic activity. In a yet more preferred embodiment, the NME7 binding partner is the extracellular domain of MUC1*. In a still more preferred embodiment, the NME7 binding partner is the PSMGFR peptide.

[00226] By the term "conferring tumorigenic activity or oncogenic activity", it is meant that the peptides themselves cannot support or promote the growth of cancers. Another way of testing whether or not a peptide or peptides derived from NME7 can promote tumorigenesis is to test whether or not the peptides can support pluripotent growth of human stem cells. NME proteins and peptides that support pluripotent human stem cell growth also support cancer growth. In yet another method, peptides are de-selected if they can cause somatic cells to revert to a less mature state.

[00227] Fragments of $NME7_{AB}$ inhibit cancer cell growth and the transition of cancer cells to a more metastatic state. As a demonstration, NME7 peptides A1, A2, B1, B2 and B3 added separately (Fig. 12) or in combinations (Fig. 13) inhibit the growth of cancer cells. In addition, NME7 peptides A1, A2, B1, B2 and B3 inhibited the transition of cancer cell to a more metastatic state (Fig. 15).

[00228] Thus, antibodies generated by immunizing with peptides specific to NME7, and specific to $NME7_{AB}$ or NME7-X1 will block the cancerous action of NME7 species and will be potent anti-cancer agents. Similarly, these results show that the peptides specific to NME7, and specific to $NME7_{AB}$ or NME7-X1 will block the cancerous action of NME7 species. In one aspect of the invention, the peptides are chosen from the list shown in Figure 6. In one aspect of the invention the peptides are chosen from the list shown in Figure 7. In one aspect of the invention the peptides are chosen from the list shown in Figure 8. In yet another aspect of the invention the peptides are chosen from the list shown in Figure 9. These antibodies may be generated by immunizing or may be generated or selected by other means, then selected for their ability to bind to NME7, $NME7_{AB}$, NME7-X1 or NME7 derived peptides, including but not limited to NME7 derived peptides A1 (SEQ ID NO: 141), A2 (SEQ ID NO: 142), B1 (SEQ ID NO: 143), B2 (SEQ ID NO: 144) or B3 (SEQ ID NO: 145). Such antibodies may be polyclonal, monoclonal, bispecific, bivalent, monovalent, single chain, scFv, human or humanized or may be an antibody mimic such as protein scaffolds that present recognition regions that bind to a specific target.

[00229] Anti-NME7 antibodies for use in the treatment or prevention of cancers can be generated by standard methods known to those skilled in the art wherein those methods are used to generate antibodies or antibody-like molecules that recognize NME7, $NME7_{AB}$ or a shorter form of $NME7_{AB}$ wherein an additional 10-25 amino acids form the N-terminus are not present. Such antibodies may be human or humanized. Such antibodies may be polyclonal, monoclonal, bispecific, bivalent, monovalent, single chain, scFv, human or humanized or may be an antibody mimic such as protein scaffolds that present recognition regions that bind to a specific target.

[00230] Anti-NME7 antibodies that are generated by immunization with the NME7 derived peptides A1 (SEQ ID NO: 141), A2 (SEQ ID NO: 142), B1 (SEQ ID NO: 143), B2 (SEQ ID NO: 144) or B3 (SEQ ID NO: 145) or antibodies that bind to the A1, A2, B1, B2 or B3 peptides are antibodies that bind to $NME7_{AB}$ and NME7-X1, but resist binding to NME1 which may be required for the function of some healthy cells. Such antibodies inhibit the binding of $NME7_{AB}$ or NME7-X1 to their target receptor, MUC1*. Antibodies that bind to A1, A2, B1, B2 or B3 peptides are antibodies can be administered to a patient diagnosed with or at risk of developing a cancer or metastasis. Such antibodies may be human or humanized. Such antibodies may be polyclonal, monoclonal, bispecific, bivalent, monovalent, single chain, scFv, or may be an antibody mimic such as protein scaffolds that present recognition regions that bind to a specific target.

[00231] Anti-NME7 antibodies that are generated by immunization with the B3 peptide or antibodies that bind to the B3 peptide are especially specific for the recognition of $NME7_{AB}$ and NME7-X1. Such antibodies are also very efficient at inhibiting the binding of $NME7_{AB}$ or NME7-X1 to their target receptor, MUC1*. Antibodies that bind to the B3 peptide are also exceptionally efficient at preventing, inhibiting and reversing cancer or cancer metastases. Such antibodies may be human or humanized. Such antibodies may be polyclonal, monoclonal, bispecific, bivalent, monovalent, single chain, scFv, or may be an antibody mimic such as protein scaffolds that present recognition regions that bind to a specific target.

[00232] Note that the polyclonal antibody #61, which was generated in rabbits immunized with the B3 peptide, inhibited the transformation of cancer cells to cancer stem cells as evidenced by antibody #61 blocking upregulation of metastatic marker CXCR4 (Fig.15).

[00233] The B3 peptide (SEQ ID NO: 145) derived from NME7 has a Cysteine at position 14, which complicates the generation of anti-NME7 antibodies. We mutated Cysteine 14 to Serine to make AIFGKTKIQNAVHSTDLPEDGLLEVQYFF (SEQ ID NO:169) and immunized animals to generate anti-NME7 monoclonal antibodies. The resultant antibodies

bind to the native B3 sequence as well as the B3Cys14Ser peptide. Seven (7) high affinity and specific monoclonal antibodies were generated: 8F9A5A1, 8F9A4A3, 5F3A5D4, 5D9E2B11, 5D9E10E4, 5D9G2C4, and 8H5H5G4. However, sequence alignment showed that there are only three (3) unique sequence antibodies: 8F9A5A1, 8F9A4A3, and 5F3A5D4 as seen below.

HEAVY CHAIN ALIGNMENT

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8F9A5A1H        IQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGEPTYV 60
8F9A4A3H        VQLQQSGPELVKPGASVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
5D9E2B11H       VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
5D9E10E4H       VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
5D9G2C4H        VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
5F3A5D4H        VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
8H5H5G4H        VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
                :** ****:* *** :******:** ***:* *:****: **.*:*:* :*  .*  .*

8F9A5A1H        DDFKGRFAFSLETSATTAYLQINNLKNEDTSTYFCARLR--GIRPGPLAYWGQGTLVTVS 118
8F9A4A3H        QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSLYVEYFDYWGQGTTLTVS 120
5D9E2B11H       QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVEYFDSWGQGTT---- 116
5D9E10E4H       QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVEYFDSWGQGTTLTVS 120
5D9G2C4H        QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVEYFDSWGQGTTLTVS 120
5F3A5D4H        QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVEYFDSWGQGTTLTVS 120
8H5H5G4H        QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVEYFDSWGQGTTLTVS 120
                :.***: ::::::.*::***::::..*..**::.*:***    .     :  *****

8F9A5A1H        A 119 (SEQ ID NO:172)
8F9A4A3H        S 121 (SEQ ID NO:173)
5D9E2B11H       - 116 (SEQ ID NO:174)
5D9E10E4H       S 121 (SEQ ID NO:175)
5D9G2C4H        S 121 (SEQ ID NO:176)
5F3A5D4H        S 121 (SEQ ID NO:177)
8H5H5G4H        S 121 (SEQ ID NO:178)



8F9A4A3H        VQLQQSGPELVKPGASVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
5D9E2B11H       VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
5D9E10E4H       VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
5D9G2C4H        VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
5F3A5D4H        VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
8H5H5G4H        VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYN 60
                ********:*****:****************************************

8F9A4A3H        QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSLYVEYFDYWGQGTTLTVS 120
5D9E2B11H       QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVEYFDSWGQGTT---- 116
5D9E10E4H       QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVEYFDSWGQGTTLTVS 120
5D9G2C4H        QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVEYFDSWGQGTTLTVS 120
5F3A5D4H        QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVEYFDSWGQGTTLTVS 120
8H5H5G4H        QKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVEYFDSWGQGTTLTVS 120
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8F9A4A3H        S 121 (SEQ ID NO:179)
5D9E2B11H       - 116 (SEQ ID NO:180)
5D9E10E4H       S 121 (SEQ ID NO:181)
5D9G2C4H        S 121 (SEQ ID NO:182)
5F3A5D4H        S 121 (SEQ ID NO:183)
8H5H5G4H        S 121 (SEQ ID NO:184)
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LIGHT CHAIN ALIGNMENT

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8F9A4A3L        ETTVTQSPASLSMAIGEKVTIRCITSTDIDDDMNWYQQKPGEPPKLLISEGNTLRPGVPS  60
5D9E2B11L       DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPS  60
5D9E10E4L       DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPS  60
5D9G2C4L        DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPS  60
5F3A5D4L        DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPS  60
8H5H5G4L        DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPS  60
8F9A5A1L        EILLTQSPAIIAASPGEKVTITCSASSSV-SYMNWYQQKPGSSPKIWIYGISNLASGVPA  59
                :  :**: : :: : *::*** * :* .: . :**:****.   *: *   ..*  ***:

8F9A4A3L        RFSSSGYGTDFVFTIENMLSEDVADYYCLQSDNLPLTFGSGTKLEIKR     108 (SEQ ID
NO:185)
5D9E2B11L       RFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR     108 (SEQ ID
NO:186)
5D9E10E4L       RFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR     108 (SEQ ID
NO:187)
5D9G2C4L        RFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR     108 (SEQ ID
NO:188)
5F3A5D4L        RFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR     108 (SEQ ID
NO:189)
8H5H5G4L        RFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR     108 (SEQ ID
NO:190)
8F9A5A1L        RFSGSGSGTSFSFTINSMEAEDVATYYCQQRSSYPPTFGGGTKLEIKR     107 (SEQ ID
NO:191)
                ***.** **.: :**..:  **:* *** * .. * ***.********


5D9E2B11L       DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPS  60
5D9E10E4L       DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPS  60
5D9G2C4L        DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPS  60
5F3A5D4L        DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPS  60
8H5H5G4L        DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPS  60
                ************************************************************

5D9E2B11L       RFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR     108 (SEQ ID
NO:192)
5D9E10E4L       RFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR     108 (SEQ ID
NO:193)
5D9G2C4L        RFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR     108 (SEQ ID
NO:194)
5F3A5D4L        RFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR     108 (SEQ ID
NO:195)
8H5H5G4L        RFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR     108 (SEQ ID
NO:196)
                *********************************************


8F9A4A3L      ETTVTQSPASLSMAIGEKVTIRCITSTDIDDDMNWYQQKPGEPPKLLISEGNTLRPGVPS  60
8F9A5A1L      EILLTQSPAIIAASPGEKVTITCSASSSV-SYMNWYQQKPGSSPKIWIYGISNLASGVPA  59
              *  :***** :: : ****** * :*:.: . *********. **: *   ..*  ***:

8F9A4A3L      RFSSSGYGTDFVFTIENMLSEDVADYYCLQSDNLPLTFGSGTKLEIKR     108 (SEQ ID
NO:197)
8F9A5A1L      RFSGSGSGTSFSFTINSMEAEDVATYYCQQRSSYPPTFGGGTKLEIKR     107 (SEQ ID
NO:198)
              ***.** **.* ***:.* :**** *** * .. * ***.********
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[00234] Monoclonal antibodies 5D9E2B11, 5D9E10E4, 5D9G2C4, and 8H5H5G4 all have the same sequence as 5F3A5D4, also known as 5D4. Here, when we refer to antibody

5F3A5D4, aka 5D4, it is understood that it also applies to 5D9E2B11, 5D9E10E4, 5D9G2C4, and 8H5H5G4. As can be seen in Fig. 24 and Fig. 25 anti-NME7 antibodies 8F9A5A1, 8F9A4A3, and 5F3A5D4 all bind to $NME7_{AB}$ but not to NME1. This is important because the A domain of NME7 has high homology to NME1, which is required for normal cell function. For an anti-cancer therapeutic or an anti-metastasis therapeutic it will be imperative to inhibit $NME7_{AB}$ but not NME1.

[00235] Figure 26, Figure 27 and Figure 28 show that these anti-NME7 antibodies are also able to disrupt the binding of $NME7_{AB}$ to the MUC1* PSMGFR peptide and the N-10 PSMGFR peptide. As can be seen, there is not a total displacement of $NME7_{AB}$ from the MUC1* peptides. However, recall that $NME7_{AB}$ is comprised of an A domain and a B domain, each of which are capable of binding to MUC1*. These antibodies were designed to disrupt binding of the B domain to MUC1*; the A domain of $NME7_{AB}$ would still be able to bind to the MUC1* peptide on the plate surface. For a useful therapeutic, the antibody would only need to disrupt the binding of one domain to MUC1* and in so doing ligand-induced dimerization and activation of MUC1* growth factor receptor would be blocked. Antibodies or antibody mimics that bind to the NME7 B3 peptide or the B3Cys14Ser peptide (SEQ ID NO:169) are antibodies can be administered to a patient diagnosed with or at risk of developing a cancer or metastasis.

[00236] It is well known in the field that it is difficult to make cancer cells metastasize in an animal model. It is estimated that in a human tumor only about 1 in 100,000 or even 1 in 1,000,000 cancer cells is able to break away from the tumor and implant elsewhere to initiate a metastasis [Al-Hajj et al., 2003]. Some researchers report that T47D breast cancer cells injected into an immune compromised mouse will metastasize after about 12 weeks [Harrell et al 2006]. Other researchers report that AsPC-1 pancreatic cancer cells will metastasize after about 4 weeks [Suzuki et al, 2013].

[00237] Here, we show that T47D breast cancer cells grown for 10 days in a serum-free media containing recombinant $NME7_{AB}$ as the only growth factor. It was observed that when grown in $NME7_{AB}$, about 25% of the cancer cells began floating, stopped dividing but were still viable. PCR measurement showed that these "floating" cells greatly upregulated expression of the breast cancer metastatic factor CXCR4.

[00238] In some of the figures presented herein, these floater cells are referred to as cancer stem cells (CSCs). Immune compromised female nu/nu mice were implanted with 90-day release estrogen pellets. Either 500,000 T47D-wt cells or 10,000 T47D-CSCs (cancer stem cells) were injected into the tail vein (i.v.), sub-cutaneously (s.c.), or into the intra-peritoneal

space (i.p.) of the nu/nu mice. These cancer cells were engineered to express Luciferase. To visualize the tumors or cancer cells, animals are injected with Luciferin, then visualized on an IVIS instrument 10 minutes later. As can be seen in the IVIS measurements of Fig. 33A - Fig. 33B, by Day 6 the 500,000 T47D-wt cells injected into the tail vein show no signs of live cancer cells or cancer cell engraftment.

[00239] In stark contrast, the 10,000 T47D-CSC injected into the tail vein have metastasized. Before the Day 6 IVIS measurement, the T47D-CSC mice were injected with 32nM recombinant $NME7_{AB}$. The next day, one of the two CSC mice was injected with a cocktail of anti-NME7 monoclonal antibodies 8F9A5A1, 8F9A4A3, and 5F3A5D4 in a volume of 200uL at a concentration that corresponds to 15 mgs/kg. The nearly coincident injection of $NME7_{AB}$ and anti-NME7 antibody likely nullified the effect of the antibody. Figure 34 shows that by Day 10, the treated mouse is almost entirely metastatic. As can be seen in the figure, the mouse chosen for treatment is more metastatic than the comparable T47D-CSC mouse.

[00240] That animal was again injected with the anti-NME7 antibodies on Day 10. The IVIS measurement of Day 12 (Fig. 35) shows that the antibody treated mouse is beginning to clear the metastases. By Day 14 (Fig. 36) the untreated mouse has died from rampant metastases and the treated mouse has cleared the metastases. Figure 37 shows the time course of IVIS measurements for the mouse injected with 500,000 T47D-wt cells and the mouse injected with T47D-CSCs that received anti-NME7 treatment until Day 17 when antibody treatment was suspended. As can be seen, on Day 17 there remained a small cluster of cancer cells, which by Day 19 had grown larger. By Day 21 the metastases had spread and antibody treatment was resumed. As is shown in the figure, after resumption of anti-NME7 antibody treatment, the animal was cleared of all metastases and shows no signs of ill health.

[00241] Figure 38 shows the IVIS time course for animals that were injected sub-cutaneously or intra-peritoneally. Antibody injections for animals injected with CSCs sub-cutaneously or intra-peritoneally were also injected with anti-NME7 antibodies s.c. or i.p.. In these animals, antibody injections stopped at Day 17 and did not resume. Figure 39 – Figure 40 show that a polyclonal anti-NME7 antibody generated by immunization with the B3 peptide stains advanced cancers and metastatic cancers but not normal tissues or low-grade cancers, where only 1 in 100,000 or 1 in 1,000,000 cancer cells would be a metastatic cancer cells. Taken together, these data show that anti-NME7 antibodies 8F9A5A1, 8F9A4A3, and 5F3A5D4 or 8F9A5A1, or 8F9A4A3, or 5F3A5D4 administered to a patient diagnosed with

or at risk of developing a cancer would prevent, inhibit the formation of, or reverse cancer metastases.

[00242] In addition to treating metastatic animals with a cocktail of anti-NME7AB antibodies, we also administered monoclonal anti-NME7AB antibodies individually and showed they were capable of preventing as well as reversing cancer metastases. In one demonstration, female nu/nu mice weighing approximately 20g each, were implanted with 90-day estrogen release pellets between 8-10 weeks of age. Cancer cells were made metastatic by culturing for 10-15 days in a serum-free media supplemented with growth factor $NME7_{AB}$. Both adherent and floating cells show upregulation of metastatic markers and in animals are able to metastasize within 4-7 days. In this case, the floating cells were harvested on Day 11 of *in vitro* culture and injected into the tail vein of the test animals. To test a prevention model, one group of animals was injected into the tail vein, 24 hours before injection of the metastatic cancer cells, with anti-$NME7_{AB}$ antibody 8F9A4A3 at 15mg/kg and injected thereafter with the same dosage approximately every 48 hours. Fig. 42A - Figure 42F shows photographs of female nu/nu mice, which were injected into the tail vein with 10,000 Luciferase positive T47D metastatic breast cancer stem cells and treated with the anti-$NME7_{AB}$ antibody 4A3 also known as 8F9A4A3. To image cancer cells, the Luciferase substrate, Luciferin, is intraperitoneally injected 10 minutes before being photographed in IVIS instrument. Figure 42A-42C show IVIS photographs with animals face down. Fig. 42D-42F show IVIS photographs with animals face up. Fig. 42A and 42D show control animals injected with phosphate buffered saline solution. Fig. 42B and 42E show a prevention model in which animals were injected with anti-$NME7_{AB}$ antibody 4A3 24 hrs before injection of the metastatic cancer cells, then approximately every other day for a total of 12 antibody injections over 22 days. Fig. 42C and 42F show a reversal model in which animals were injected with anti-$NME7_{AB}$ antibody 4A3 24 hrs after injection of the metastatic cancer cells, then approximately every other day for a total of 11 antibody injections over 20 days. As can be seen in the figure, anti-$NME7_{AB}$ antibody 8F9A4A3 can prevent, as well as reverse an established metastasis.

[00243] Anti-$NME7_{AB}$ antibodies 5A1 and 5D4 were also tested in a metastasis prevention model and shown to greatly inhibit cancer metastasis. Figure 43A-43F shows photographs of female nu/nu mice weighing approximately 20g each, which were injected into the tail vein with 10,000 Luciferase positive T47D metastatic breast cancer stem cells and treated with the anti-$NME7_{AB}$ antibodies 5A1, also known as 8F9A5A1, and 5D4, also known as 5F3A5D4. To image cancer cells, the Luciferase substrate, Luciferin, is intraperitoneally injected 10 minutes before being photographed in IVIS instrument. Fig. 43A-43C show IVIS photographs

with animals face down. Fig. 43D-43F show IVIS photographs with animals face up. Fig. 43A and 43D show control animals injected with phosphate buffered saline solution. Fig. 43B, 43E, 43C and 43F show a prevention model in which animals were injected with anti-$NME7_{AB}$ antibodies, at 15mg/kg 24 hours before injection of the metastatic cancer cells, then approximately every other day for a total of 12 antibody injections over 22 days. Photographs were taken either at Day 24 or at Day 27. Specifically, mouse#1 in the group treated with antibody 5A1 was photographed at Day 27 while mouse #2 and #3 were photographed on Day 24 because animals died on Day 26.

[00244] Anti-$NME7_{AB}$ antibodies 5A1 and 5D4 were also tested in a metastasis reversal model and shown to greatly inhibit established cancer metastases. In this experiment, animals were injected on Day 0 into the tail vein with 10,000 T47D metastatic cancer cells mixed with $NME7_{AB}$ at a final concentration of 32nM. Further, animals were injected twice, Day 3 and Day 4, with more $NME7_{AB}$ which our experiments have shown make the metastasis more difficult to reverse. The first antibody injection was on Day7. Because the degree of metastasis in each test animal is somewhat variable, we wanted to make certain that the apparent clearance of metastatic cancer cells was due to the anti-$NME7_{AB}$ treatment. We therefore treated the animals with alternating high dose and low doses. As can clearly be seen in Figure 44, high dose anti-$NME7_{AB}$ results in clearance of the metastasis, which if not completely eradicated comes back and even increases with lower dose. This experiment shows that all three anti-$NME7_{AB}$ antibodies tested, 5A1, 4A3 and 5D4, which are able to bind to the NME7-B3 peptide, inhibit cancer metastasis in a concentration dependent manner. Figure 44A-44D show photographs of female nu/nu mice that were injected into the tail vein with 10,000 Luciferase positive T47D metastatic breast cancer stem cells mixed with $NME7_{AB}$ at a final concentration of 32nM. Animals were then injected into the tail vein with 32nM $NME7_{AB}$ before being treated with individual anti-$NME7_{AB}$ antibodies. Fig. 44A shows control animals injected with phosphate buffered saline solution. Fig. 44B shows animals treated with anti-$NME7_{AB}$ monoclonal antibody 8F9A5A1. Fig. 44C shows animals treated with anti-$NME7_{AB}$ monoclonal antibody 8F9A4A3. Fig. 44D shows animals treated with anti-$NME7_{AB}$ monoclonal antibody 5F3A5D4. Green arrows indicate low antibody dosage (5-7mg/kg) over the indicated period and Red arrows indicate high dosage (15mg/kg). As can be seen in the figure, the metastasis clears considerably when antibody is administered at 15mg/kg.

[00245] In addition to demonstrating that the anti-$NME7_{AB}$ antibodies of the invention can inhibit metastasis, we tested their effect on metastasis from a primary tumor, which would more closely mimic the physiology of cancer metastasis. We generated T47D metastatic breast

cancer cells, also known as cancer stem cells (CSCs) by culturing the cancer cells in a minimal serum-free media containing $NME7_{AB}$ for 10-15 days. These T47D CSCs were then implanted sub-cutaneously into the right flank of NSG mice into which had been implanted a 90-day estrogen release pellet. The implanted cancer cells were Luciferase positive so that after injection of the Luciferase substrate, Luciferin, the cancer cells emit photons and can be photographed in an IVIS instrument to measure and locate the implanted cancer cells. Figure 45A-45B shows photographs of female nu/nu mice that on Day 0 were injected sub-cutaneously into the right flank with 10,000 Luciferase positive T47D metastatic breast cancer stem cells, mixed with $NME7_{AB}$ to a final concentration of 32nM, then mixed in a 1:1 vol:vol with Matrigel. Tumor engraftment was allowed to progress Day 0 – Day 6. Animals were then treated i.v. by tail vein injection with anti-$NME7_{AB}$ antibodies. Control animals were injected with PBS. Fig. 45A shows IVIS photographs of control animals. Fig. 45B shows IVIS photographs of animals injected into tail vein with a cocktail of anti-$NME7_{AB}$ antibodies 5A1, 4A3 and 5D4 to a total concentration of 15mg/kg. Antibodies or PBS were administered 4 times between Day 7 and Day 18. As can be seen in the figure, the anti-$NME7_{AB}$ antibody treated animals show less metastases (blue dots in whole body) than the control group. In the treated group, 2 of the 5 animals have primary tumors that are larger than those in the control group. This could be because the anti-$NME7_{AB}$ antibodies prevented the spread of the cancer cells, so they remained concentrated in the primary tumor. In this experiment, PCR analysis, performed prior to injection of the cancer cells, showed that after 11 days in culture with $NME7_{AB}$, the T47D breast cancer cells had upregulated CXCR4 by 109-fold, OCT4 by 2-fold, NANOG by 3.5-fold and MUC1 by 2.7-fold.

[00246] In another experiment, we tested the effect of anti-$NME7_{AB}$ antibodies of the invention on metastasis from a primary tumor to organs that breast cancers typically metastasize to. Breast cancers commonly metastasize to liver, lung, bone and brain, in that order. We generated T47D metastatic breast cancer cells by culturing in a minimal serum-free media containing $NME7_{AB}$ for 11 days. These T47D CSCs were then implanted sub-cutaneously into the right flank of NSG mice into which had been implanted a 90-day estrogen release pellet. Figure 46A-46P shows photographs of female nu/nu mice that on Day 0 were injected sub-cutaneously into the right flank with 10,000 Luciferase positive T47D metastatic breast cancer stem cells, mixed with $NME7_{AB}$ to a final concentration of 32nM, then mixed in a 1:1 vol:vol with Matrigel. Tumor engraftment was allowed to progress Day 0 – Day 6. Animals were then treated i.v., by tail vein injection, with anti-$NME7_{AB}$ antibodies. Control animals were injected with PBS. On Day 38 animals were sacrificed and livers harvested then

analyzed by IVIS to detect cancer cells that had metastasized to the liver. Fig. 46A-46B show whole body IVIS photographs of control animals that were injected with only PBS. Fig. 46C-46D show whole body IVIS photographs of control animals that were injected with the anti-$NME7_{AB}$ antibody 5A1. Fig. 46E-46F show whole body IVIS photographs of control animals that were injected with the anti-$NME7_{AB}$ antibody 4A3. Fig. 46G-46H show whole body IVIS photographs of control animals that were injected with the anti-$NME7_{AB}$ antibody 5D4. Fig. 46A, 46C, 46E, and 46G are IVIS photographs taken at Day 7 before any treatment. Fig. 46B, 46D, 46F, and 46H are IVIS photographs taken at Day 31 after anti-$NME7_{AB}$ antibody treatment or mock treatment. As can be seen in the figure, animals in the PBS control group show metastasis (blue dots) in the whole body IVIS photographs, while animals treated with anti-$NME7_{AB}$ antibodies do not. Fig. 46I-46P show photographs and IVIS photographs of livers and lung harvested from animals after sacrifice. Fig. 46I, 46K, 46M, and 46O are regular photographs. Fig. 46J, 46L, 46N, and 46P are IVIS photographs, illuminating the cancer cells that have metastasized there. As can be seen in the figure, the anti-$NME7_{AB}$ antibodies greatly inhibited metastasis to the liver, which is a primary site for breast cancer metastasis. Fig. 46Q is a bar graph of the measured photons emitted and enumerated by IVIS instrument for livers harvested from control animals versus the treated animals. As can be seen in the inserted graph of IVIS measurements, the inhibition of metastasis to the liver follows the rank order of inhibition of metastasis when cells were injected into the tail vein, which also matches the rank order of potency in being able to disrupt the NME7AB-MUC1* interaction.

[00247] We performed immunofluorescent imaging of many cancer cell lines to determine if cultured cancer cell lines express NME7AB. As Figure 47A-47F and Figure 48A-48I clearly show, each MUC1 positive cancer cell line we tested is positive for NME7AB and its binding is membranous, consistent with NME7AB being secreted from cancer cells whereupon it binds to the extra cellular domain of MUC1*. Figure 47A-47F shows photographs of immunofluorescent experiments in which various cancer cell lines are stained for the presence of NME7AB. Fig. 47A shows T47D breast cancer cells stained with varying concentrations of anti-NME7AB antibody 5D4. Fig. 47B shows ZR-75-1 breast cancer cells, also known as 1500s, stained with varying concentrations of anti-NME7AB antibody 5D4. Fig. 47C shows H1975 non-small cell lung cancer cells stained with varying concentrations of anti-NME7AB antibody 5D4. Fig. 47D shows H292 non-small cell lung cancer cells stained with varying concentrations of anti-NME7AB antibody 5D4. Fig. 47E shows HPAFII pancreatic cancer cells stained with varying concentrations of anti-NME7AB antibody 5D4. Fig. 47F shows DU145 prostate cancer cells stained with varying concentrations of anti-NME7AB antibody 5D4. As

can be seen in the figure, all the cancer cell lines we tested show strong and membranous staining for NME7AB. The monoclonal antibody used in these experiments was 5D4. In parallel, NME7AB antibodies 5A1 and 4A3 were used to stain the same cell lines and produced the same results.

[00248] Figure 48A-48I shows photographs of immunofluorescent experiments in which various lung cancer cell lines are stained for the presence of NME7AB. Fig. 48A-48C shows H1975 non-small cell lung cancer cells, which are an adenocarcinoma, stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 48A is an overlay of DAPI and anti-$NME7_{AB}$ staining. Fig. 48B shows anti-$NME7_{AB}$ staining alone. Fig. 48C is a magnified view of the overlay of DAPI and anti-$NME7_{AB}$ staining. Fig. 48D-48F shows H292 non-small cell lung cancer cells, which are a mucoepidermoid pulmonary carcinoma, stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 48D is an overlay of DAPI and anti-$NME7_{AB}$ staining. Fig. 48E shows anti-$NME7_{AB}$ staining alone. Fig. 48F is a magnified view of the overlay of DAPI and anti-$NME7_{AB}$ staining. Fig. 48G-48I shows H358 non-small cell lung cancer cells, which are a metastatic bronchioalveolar carcinoma, stained with varying concentrations of anti-$NME7_{AB}$ antibody 5D4. Fig. 48G is an overlay of DAPI and anti-$NME7_{AB}$ staining. Fig. 48H shows anti-$NME7_{AB}$ staining alone. Fig. 48I is a magnified view of the overlay of DAPI and anti-$NME7_{AB}$ staining.

In addition, culturing these cell lines in a serum-free media containing $NME7_{AB}$ even further increased their expression of stem cell and metastatic markers. In particular, the cells that became non-adherent, referred to here as floaters, have even higher expression of stem cell and metastatic markers than their adherent counterparts. Figure 49A-49I shows PCR graphs of cancer cell lines, breast T47D, Lung H1975, lung H358 and pancreatic HPAFII before and after culture in $NME7_{AB}$. Fig. 49A measured breast metastatic marker CXCR4. Fig. 49B measured stem cell marker OCT4. Fig. 49C measured metastatic marker ALDH1. Fig. 49D measured stem cell marker SOX2. Fig. 49E measured stem cell marker NANOG. Fig. 49F measured marker CDH1, also known as E-cadherin. Fig. 49G measured metastatic marker CD133. Fig. 49H measured stem cell marker ZEB2. Fig. 49I measured stem, cancer and metastatic marker MUC1. The floater cells, also known as tumor spheres become able to grow anchorage independently and show markers of metastasis that are more elevated than the adherent cells. Animals injected with cancer stem cells are those injected with the $NME7_{AB}$ grown floater cells. As can be seen in the figure markers of metastasis, stem cell markers, or markers of epithelial to mesenchymal transition (EMT) are elevated after culture in $NME7_{AB}$, indicating a transition to a more metastatic state. **Figure 50** shows Day 6 IVIS photographs of

NSG mice injected into the tail vein with either 10,000 H358 lung cancer parentcells or H358 cells after 10-12 days in culture with $NME7_{AB}$. As can be seen in the figure, the NCI-H358 lung cancer cells grown in $NME7_{AB}$ have greatly increased metastatic potential compared to the parent cells, which are themselves reportedly metastatic cells. The functional increase in metastasis in 6 days from the NCI-H358 $NME7_{AB}$ metastatic cancer stem cells from just 10,000 cells is consistent with Figure 49, showing that H358 cells greatly increased expression of metastatic markers after culture in $NME7_{AB}$.

Figure 51 shows PCR graph of a MUC1 negative prostate cancer line PC3 before and after 2 or 3 passages in culture in either dimeric NM23-H1, also known as NME1, or $NME7_{AB}$. The graph shows the fold difference in markers of stem cells, cancer cells as well as metastatic markers. As can be seen in the figure, repeated culture in NME1 or $NME7_{AB}$ induces upregulation of stem, cancer and metastatic markers but also upregulates expression of MUC1 by 5-8 times.

[00249] Collectively, these data have demonstrated that an NME7 that is devoid of the DM10 domain is secreted by cancer cells and binds to the extra cellular domain of a MUC1 that is devoid of tandem repeat domain, whereupon the NME7 dimerizes the MUC1* extra cellular domain which results in increased cancer cell growth and an increase in the cancer cells' metastatic potential. It stands to reason that antibodies that disrupt the interaction between $NME7_{AB}$ and MUC1* extra cellular domain would inhibit cancer cell growth and would inhibit cancer metastasis. Here, we have shown that anti-$NME7_{AB}$ antibodies that inhibit interaction between $NME7_{AB}$ and MUC1* extra cellular domain do in fact inhibit cancer cell growth and cancer metastasis. Therefore, it follows that anti-$NME7_{AB}$ antibodies can be administered to a patient, diagnosed with or at risk of developing a cancer or metastasis, for the treatment or prevention of cancers.

[00250] Because NME1 is expressed in the cytoplasm of all cells and can be lethal if knocked out, and importantly the NME1 A domain has high sequence homology to the NME7 A domain, it is critical that anti-$NME7_{AB}$ antibodies for therapeutic use bind to $NME7_{AB}$ or NME7-X1, but not to NME1. In one aspect of the invention antibodies that would be optimal for therapeutic use were selected for their ability to bind to peptides that were unique to $NME7_{AB}$ or NME7-X1 and were not present in the NME1 sequence. Figure 6 – Figure 9 lists $NME7_{AB}$ unique peptides.

[00251] In a preferred embodiment, antibodies suitable for administration to a patient for the treatment or prevention of cancer or cancer metastasis are selected from the group of antibodies that bind to the NME7 B3 peptide. In yet a more preferred embodiment, antibodies

suitable for administration to a patient for the treatment or prevention of cancer or cancer metastasis are selected from the group of antibodies that bind to the NME7 B3 peptide, bind to $NME7_{AB}$ but do not bind to NME1. Examples of antibodies suitable for therapeutic use for the treatment or prevention of cancers or cancer metastasis, which have demonstrated such anti-cancer activity and anti-metastatic activity *in vitro* and *in vivo* here, include anti-NME7 antibodies 5A1, 4A3 and 5D4. These are but examples and other antibodies generated as described here and selected as described here will have the same anti-cancer and anti-metastatic activity. Such antibodies may be full antibodies or fragment thereof, including scFvs or antibody mimics wherein the variable domains of the antibody are incorporated into a protein scaffold that mimic an antibody. The antibodies may be of human or non-human species, including murine, camelid, llama, human or humanized and may be monoclonal, polyclonal, scFvs or fragments thereof.

[00252] Anti-NME7 antibodies for treatment or prevention of cancers or metastases can be used in many different therapeutic formats. For example, any of the antibodies described herein, or a fragment thereof, can be administered to a patient as a stand-alone antibody or antibody fragment, or attached to a toxin such as an antibody drug conjugate (ADC), or incorporated into a bi-specific antibody or incorporated into a BiTE (bispecific T cell engager), or incorporated into a chimeric antigen receptor (CAR) or engineered to be expressed by a cell that also expresses a CAR. The cell may be an immune cell, a T cell, an NK cell or a stem or progenitor cell, which may then be differentiated into a T cell or an NK cell.

[00253] Any of the antibodies described herein, or a fragment thereof, can be used as a diagnostic reagent to probe a bodily fluid, cell, tissue or bodily specimen for the presence of $NME7_{AB}$ or NME7-X1, which would be an indicator of cancer or susceptibility to cancers. Antibodies for diagnostic uses may be connected to an imaging agent, a nucleic acid tag, may be of any species including camelid, and can be used in whole body applications or on a bodily fluid, such as blood, cell, or tissue, in vitro, in vivo or intra-operatively.

[00254] The selection criteria, for therapeutically useful or diagnostically useful anti-NME7 antibodies, depends on the format or modality of the therapeutic or diagnostic into which the antibody will be incorporated. If the antibody or antibody fragment is to be administered to a patient as a stand-alone agent for the treatment or prevention of cancers or cancer metastases, then the antibody is selected for its ability to: i) bind to $NME7_{AB}$ or NME7-X1, but not to NME1; ii) bind to the PSMGFR peptide; iii) bind to the N-10 peptide and iv) disrupt the interaction between $NME7_{AB}$ or NME7-X1 and the MUC1* extra cellular domain or the interaction between $NME7_{AB}$ or NME7-X1 and the N-10 peptide. The antibody may also be

selected for its ability to bind to the NME7 B3 peptide. This therapeutic format also encompasses a cell that has been engineered to express a CAR and a secreted anti-NME7 antibody.

[00255] Other modalities require other selection criteria for anti-NME7 antibodies. If the anti-NME7 antibody is to be incorporated into an ADC, the ADC must be internalized by the target cell to trigger killing of the target cell. Recall that $NME7_{AB}$ or NME7-X1 will be bound to the extra cellular domain of MUC1*. If the antibody disrupts binding of the NME to MUC1* extra cellular domain, then the toxin-conjugated antibody will not be internalized and the cell will not be killed. Similarly, if the anti-NME7 antibody is to be incorporated into a CAR or a BiTE, the interaction between $NME7_{AB}$ or NME7-X1 cannot be disrupted or the immune cell will no longer be able to direct its killing agents to the cancer cell. If the anti-NME7 antibody is to be used as a diagnostic reagent, the interaction between $NME7_{AB}$ or NME7-X1 cannot be disrupted or antibody and associated label will be washed away. Therefore, for ADC, CAR T, or CAR-NK, BiTEs or diagnostic applications, the anti-NME7 antibody is selected for its ability to: i) bind to $NME7_{AB}$ or NME7-X1, but not to NME1; ii) bind to the PSMGFR peptide; iii) bind to the N-10 peptide and iv) bind to $NME7_{AB}$ or NME7-X1 without disrupting the interaction with the MUC1* extra cellular domain or the interaction between $NME7_{AB}$ or NME7-X1 and the N-10 peptide. The antibody may also be selected for its ability to bind to the NME7 B3 peptide.

[00256] In one aspect of the invention, a cell is engineered to express an anti-$NME7_{AB}$ antibody of the invention or fragment thereof. The cell may be an immune cell, such as a T cell or NK cell or it may be a stem or progenitor cell, which may be differentiated into a more mature immune cell such as a T cell or NK cell. In a preferred embodiment, the cell that is engineered to express an anti-$NME7_{AB}$ antibody is also engineered to express a chimeric antigen receptor (CAR). In a preferred embodiment, the CAR recognizes a tumor associated antigen. In a preferred embodiment, the CAR targets MUC1*. In a more preferred embodiment, the CAR is directed to the tumor by anti-MUC1* antibody MNC2. In another aspect of the invention, cell that is engineered to express a CAR is also engineered to inducibly express an anti-NME7 antibody. In one example, the nucleic acid encoding an anti-$NME7_{AB}$ antibody is inserted into the Foxp3 enhancer or promoter. In another example, the anti-$NME7_{AB}$ antibody is in an NFAT-inducible system. In one aspect, the NFAT-inducible system incorporates NFATc1 response elements inserted upstream of an anti-$NME7_{AB}$ antibody sequence. They may be inserted into an IL-2 promoter, a Foxp3 enhancer or promoter or other suitable promoter or enhancer.

[0100] In another aspect of the invention, peptides that are unique to $NME7_{AB}$ or NME7-X1 are incorporated into an entity used to immunize or vaccinate people against cancers or cancer metastases. In a preferred embodiment, the peptide comprises all or part of the NME7 B3 peptide, which may be the NME7 B3 peptide with Cys-14-Ser mutation.

[00257] Another aspect of the invention involves a method of generating anti-$NME7_{AB}$ antibodies in a host animal, where the animal is immunized with the NME7 B3 peptide. In a preferred embodiment, the NME7 B3 peptide has Cysteine 14 mutated to Serine (SEQ ID NO:169) to avoid disulfide bond formation which inhibits NME7 specific antibody generation.

[00258] Another aspect of the invention involves a method of generating cells with enhanced metastatic potential involving culturing the cells with $NME7_{AB}$ or NME7-X1. These cells can then be used in many aspects of drug discovery.

[00259] Another aspect of the invention involves a cell that is engineered to express $NME7_{AB}$ or NME7-X1. The $NME7_{AB}$ or NME7-X1 may be of human sequence. Their expression may be inducible. In one aspect the cell is an egg which is then developed into an animal that may be a transgenic animal able to express human $NME7_{AB}$ or NME7-X1.

[00260] NME7 binds to and dimerizes the extra cellular domain of the MUC1* growth factor receptor. Tissue studies show that MUC1* increases as tumor grade and metastasis increase. Here we show that NME7 expression increases as tumor grade and metastasis increases (Fig. 39 – Fig. 41). Here, we have shown that antibodies that inhibit the interaction of NME7 and MUC1* inhibit tumor growth and metastases.

[00261] Other NME family members may bind to and dimerize the extra cellular domain of the MUC1* growth factor receptor. For example, we have shown that NME1, NME2 and NME6 can exist as dimers and that they bind to and dimerize the MUC1* extra cellular domain. $NME7_{AB}$ and NME7-X1 have two domains that can bind to the MUC1* extra cellular domain so as monomers they dimerize and activate the MUC1* growth factor receptor. We have now shown that anti-NME7 antibodies inhibit cancer and cancer metastases. Similarly, antibodies or antibody mimics that bind to these other NME proteins may be anti-cancer or anti-metastasis therapeutics that can be administered to a patient diagnosed with or at risk of developing a cancer or a metastasis. In one aspect of the invention, antibodies that can be used therapeutically for the treatment of cancers or metastases are antibodies that bind to NME1, NME2, NME3, NME4, NME5, NME6, NME7, NME8, NME9 or NME10. In one aspect of the invention, the therapeutic antibody or antibody mimic inhibits the binding of the NME protein and its cognate growth factor receptor. In one aspect of the invention, the therapeutic antibody or antibody mimic inhibits the interaction of the NME protein with the extra cellular

domain of MUC1*. In another aspect of the invention, the therapeutic antibody or antibody mimic binds to a peptide, derived from NME1, NME2, NME3, NME4, NME5, NME6, NME7, NME8, NME9 or NME10, wherein the peptide is homologous to the NME7 A1, A2, B1, B2 or B3 peptide.

[00262] Below is a sequence alignment that shows a homology and identity alignment between NME7 and other NME family members. The underlined or underlined and bolded sequences correspond to NME7 peptides A1 (SEQ ID NO: 141), A2 (SEQ ID NO: 142), B1 (SEQ ID NO: 143), B2 (SEQ ID NO: 144) and B3 (SEQ ID NO: 145).

nucleoside diphosphate kinase 7 isoform a [Homo sapiens] (Hu_7)
MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKRTKYDNLH
LEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRK**EKTLALIKPDAISKAGEIIEIIN**
KAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDDAI
CEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFFPS
SGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVN
VEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHL
RPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDN

>NME2 Theoretical pI/Mw: 8.52 / 17298.04
MANLERTFIAIKPDGVQRGLVGEIIKRFEQKGFRLVAMKFLRASEEHLKQHYIDLKDRPFFPGLVKYMNSGPVVAMVWEGLNV
VKTGRVMLGETNPADSKPGTIRGDFCIQVGRNIIHGSDSVKSAEKEISLWFKPEELVDYKSCAHDWVYE

```
global/global (N-W) score: 171; 26.5% identity (56.8% similar) in 155 aa
overlap (1-131:1-152)

                  10          20        30        40        50
7A      ----EKTLALIKPDAISKA--GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPF
            :.:.  ::::.....  ::::. ... :: .. .:..  :...  . ..: ..::::
2       MANLERTFIAIKPDGVQRGLVGEIIKRFEQKGFRLVAMKFLRASEEHLKQHYIDLKDRPF
                10        20        30        40        50        60

             60        70        80        90       100       110
7A      FNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAA
        :  :.....::..::     ...   . .:: .: . ..     .::. :  .  ::
2       FPGLVKYMNSGPVVAMVWEGLNVVKTGRVMLGETNPADSKPG---TIRGDFCIQVGRNII
                70        80        90       100          110

           120       130
7A      HGPDSFASAAREMELFF----------------- (SEQ ID NO:199)
        :: ::  :: .:. :.:
2       HGSDSVKSAEKEISLWFKPEELVDYKSCAHDWVYE (SEQ ID NO:200)
        120       130       140       150

global/global (N-W) score: 104; 24.4% identity (51.3% similar) in 156 aa
overlap (1-134:1-152)

                  10        20        30        40        50
7B      NC----TCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVT
              :   .:: .:..::.:.:.  ... :::. ::...  .. .... :   :
2       MANLERTFIAIKPDGVQRGLVGEIIKRFEQKGFRLVAMKFLRASEEHLKQHYIDLKDR-P
                10        20        30        40        50
```

```
          60        70        80        90       100       110
7B      EYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNA
         .  .:  : ::: :::  .  :..:: : . : ..:  ..   :::.:. :     .:
2       FFPGLVKYMNSGPVVAMVWEGLNVVKTGRVMLGETNPADSK---PGTIRGDFCIQVGRNI
       60        70        80        90       100           110

         120       130
7B      VHCTDLPEDGLLEVQYFF------------------ (SEQ ID NO:201)
        .: .:  ...  :.. .:
2       IHGSDSVKSAEKEISLWFKPEELVDYKSCAHDWVYE (SEQ ID NO:202)
         120       130       140       150
```

>NME3 Theoretical pI/Mw: 5.96 / 19088.97
MICLVLTIFANLFPSAYSGVNERTFLAVKPDGVQRRLVGEIVRRFERKGFKLVALKLVQASEELLREHYVELRERPFYSRLVK YMGSGPVVAMVWQGLDVVRASRALIGATDPGDATPGTIRGDFCVEVGKNVIHGSDSVESAQREIALWFREDELLCWEDSAGHW LYE

```
              10          20          30          40          50
7A      EKTLALIKPDAISK--AGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNEL
        :.:.  .:::....  .::::. ... :: .. ::... :..   . .:. . :::...:
3       ERTFLAVKPDGVQRRLVGEIVRRFERKGFKLVALKLVQASEELLREHYVELRERPFYSRL
               30          40          50          60          70          80

        60          70          80          90          100         110
7A      IQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASE-SIRALFGTDGIRNAAHGP
        .... .::..::     :..   . :.: .. :    ::.  .::. : ..  .:. ::
3       VKYMGSGPVVAMVWQGLDVVRASRALIGATDPG----DATPGTIRGDFCVEVGKNVIHGS
               90         100         110           120         130

        120         130
7A      DSFASAAREMELFF (SEQ ID NO:203)
        ::  :: ::. :.:
3       DSVESAQREIALWF (SEQ ID NO:204)
        140         150

                                    10          20          30
7B      N-C--------------------TCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNM
          :                       :   ::: .:.. :.:.:.  ..  ::.. :... .
3       MICLVLTIFANLFPSAYSGVNERTFLAVKPDGVQRRLVGEIVRRFERKGFKLVALKLVQA
               10          20          30          40          50          60

       40          50          60          70          80          90
7B      DRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHL
        ..  ..: : :        :  .:  : ::: :::  :  ..... : . : .::  :
3       SEELLREHY-VELRERPFYSRLVKYMGSGPVVAMVWQGLDVVRASRALIGATDPGDAT--
                  70          80          90         100         110

      100         110         120         130
7B      RPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFF------------------ (SEQ ID NO:205)
         :::.:. :     .:..: .:  :..  :.  .:
3       -PGTIRGDFCVEVGKNVIHGSDSVESAQREIALWFREDELLCWEDSAGHWLYE (SEQ ID NO:206)
         120         130         140         150         160
```

>NME4 Theoretical pI/Mw: 10.30 / 20658.59

MGGLFWRSALRGLRCGPRAPGPSLLVRHGSGGPSWTRERTLVAVKPDGVQRRLVGDVIQRFERRGFTLVGMKMLQAPESVLAE HYQDLRRKPFYPALIRYMSSGPVVAMVWEGYNVVRASRAMIGHTDSAEAAPGTIRGDFSVHISRNVIHASDSVEGAQREIQLW FQSSELVSWADGGQHSSIHPA

29.3% identity (68.4% similar) in 133 aa overlap (1-131:56-185)

```
               10          20        30        40        50
7A      EKTLALIKPDAISK--AGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNEL
        :.:::. .:::....  .:..:. ... ::::.. .:::.  ..   . . : . .::.  :
4       ERTLVAVKPDGVQRRLVGDVIQRFERRGFTLVGMKMLQAPESVLAEHYQDLRRKPFYPAL
           60         70        80        90       100       110

        60        70        80        90       100       110
7A      IQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPD
        :.....::..::     ...   . ..: ..:. :   :  .::. :..   ::. :. :
4       IRYMSSGPVVAMVWEGYNVVRASRAMIGHTDSAEA---APGTIRGDFSVHISRNVIHASD
          120       130       140       150          160       170

      120       130
7A      SFASAAREMELFF (SEQ ID NO:207)
        :  .: ::..:.:
4       SVEGAQREIQLWF (SEQ ID NO:208)
             180
```

28.8% identity (56.8% similar) in 132 aa overlap (3-134:40-167)

```
             10        20        30        40        50        60
7B      TCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMV
        :   ::: .:.. :.: ... ..  :: . .:.:..  .  . : :.  .     :  ..
4       TLVAVKPDGVQRRLVGDVIQRFERRGFTLVGMKMLQAPESVLAEHYQDLRRK-PFYPALI
       40        50        60        70        80        90

             70        80        90       100       110       120
7B      TEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDL
          : ::: :::  .  :.... : . : .:   :    :::.:. :.    .:..: .:
4       RYMSSGPVVAMVWEGYNVVRASRAMIGHTDSAEAA---PGTIRGDFSVHISRNVIHASDS
       100       110       120       130          140       150

            130
7B      PEDGLLEVQYFF (SEQ ID NO:209)
          : .  :.: .:
4       VEGAQREIQLWF (SEQ ID NO:210)
          160
```

>NME5 **Theoretical pI/Mw: 6.08 / 29296.23**

MEISMPPPQIYVEKTLAIIKPDIVDKEEEIQDIILRSGFTIVQRRKLRLSPEQCSNFYVEKYGKMFFPNLTAYMSSGPLVAMI LARHKAISYWLELLGPNNSLVAKETHPDSLRAIYGTDDLRNALHGSNDFAAAEREIRFMFPEVIVEPIPIGQAAKDYLNLHIM PTLLEGLTELCKQKPADPLFWYMCCRREHWTLRSILLVCMSGIRMSLPHCADYCSFVEGFEIWLADWLLKNNPNKPKLCHHPI VEEPY

44.3% identity (74.8% similar) in 131 aa overlap (1-131:13-143)

```
               10        20        30        40        50        60
7A      EKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQ
        :::::.:::: ..:  :: .:: ..:::::. . . :: ..  .:.:.. .. :: .:
5       EKTLAIIKPDIVDKEEEIQDIILRSGFTIVQRRKLRLSPEQCSNFYVEKYGKMFFPNLTA
              20        30        40        50        60        70

               70        80        90       100       110       120
7A      FITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSF
        ....::..:: . :  ::  : .:::: :: ::.    .:.::..::: .::: :: ..:
5       YMSSGPLVAMILARHKAISYWLELLGPNNSLVAKETHPDSLRAIYGTDDLRNALHGSNDF
              80        90       100       110       120       130

               130
7A      ASAAREMELFF (SEQ ID NO:211)
        :.: ::....:
5       AAAEREIRFMF (SEQ ID NO:212)
             140
```

28.0% identity (58.3% similar) in 132 aa overlap (3-134:15-143)

```
              10        20        30        40        50        60
7B      TCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMV
        :  :.::  :..    .:   :  .:: :   . . ..  .  .:: : :      . ...
5       TLAIIKPDIVDKEE--EIQDIILRSGFTIVQRRKLRLSPEQCSNFY-VEKYGKMFFPNLT
            20        30        40        50        60        70

              70        80        90       100       110       120
7B      TEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDL
        . : ::: ::: . ...: . . :. :: .  .:.. .: .:::::.:   ..::.: ..
5       AYMSSGPLVAMILARHKAISYWLELLGPNNSLVAKETHPDSLRAIYGTDDLRNALHGSND
              80        90       100       110       120       130

             130
7B      PEDGLLEVQYFF (SEQ ID NO:213)
           .  :....:
5       FAAAEREIRFMF (SEQ ID NO:214)
              140
```

>NME6 Theoretical pI/Mw: 7.81 / 22003.16

MTQNLGSEMASILRSPQALQLTLALIKPDAVAHPLILEAVHQQILSNKFLIVRMRELLWRKEDCQRFYREHEGRFFYQRLVEF MASGPIRAYILAHKDAIQLWRTLMGPTRVFRARHVAPDSIRGSFGLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEE PQLRCGPVCYSPEGGVHYVAGTGGLGPA

37.6% identity (68.4% similar) in 133 aa overlap (3-131:22-153)

```
             10        20             30        40        50
7A      TLALIKPDAISKAGEIIEIINKA----GFTITKLKMMMLSRKEALDFHVDHQSRPFFNEL
        ::::::::::...   :.: ...      : :.... ..  ...   :. .:..: :...:
6       TLALIKPDAVAHP-LILEAVHQQILSNKFLIVRMRELLWRKEDCQRFYREHEGRFFYQRL
               30         40        50        60        70        80

        60        70        80        90       100       110
7A      IQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPD
        ..:...::: :. . . ::: :. :.::.    ::  : .::::. ::    ::..:: :
6       VEFMASGPIRAYILAHKDAIQLWRTLMGPTRVFRARHVAPDSIRGSFGLTDTRNTTHGSD
                90       100       110       120       130       140

       120       130
7A      SFASAAREMELFF (SEQ ID NO:215)
        : .::.::.  ::
6       SVVSASREIAAFF (SEQ ID NO:216)
                150
```

29.3% identity (57.9% similar) in 133 aa overlap (3-134:22-153)

```
             10         20        30        40        50        60
7B      TCCIVKPHAVSEGL-LGKILMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDM
        :  ..:: ::.. : :  . . : .  : :  :. .   . . ..::. ..:    :. .
6       TLALIKPDAVAHPLILEAVHQQILSNKFLIVRMRELLWRKEDCQRFYREHEGRFF-YQRL
               30        40        50        60        70         80

               70        80        90       100       110       120
7B      VTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTD
        :  : :::  :. . ...: . .: . ::.    :::. : ..:. :: :  .:..: .:
6       VEFMASGPIRAYILAHKDAIQLWRTLMGPTRVFRARHVAPDSIRGSFGLTDTRNTTHGSD
                90       100       110       120       130       140

              130
7B      LPEDGLLEVQYFF (SEQ ID NO:217)
           ..  :.  ::
6       SVVSASREIAAFF (SEQ ID NO:218)
                150
```

>NME8 **Theoretical pI/Mw: 4.90 / 67269.94**

**MASKKREVQLQTVINNQSLWDEMLQNKGLTVIDVYQAWCGPCRAMQPLFRKLKNELNEDEILHFAVAEADNIVTLQPFRDKCE
PVFLFSVNGKIIEKIQGANAPLVNKKVINLIDEERKIAAGEMARPQYPEIPLVDSDSEVSEESPCESVQELYSIAIIKPDAVI
SKKVLEIKRKITKAGFIIEAEHKTVLTEEQVVNFYSRIADQCDFEEFVSFMTSGLSYILVVSQGSKHNPPSEETEPQTDTEPN
ERSEDQPEVEAQVTPGMMKNKQDSLQEYLERQHLAQLCDIEEDAANVAKFMDAFFPDFKKMKSMKLEKTLALLRPNLFHERKD
DVLRIIKDEDFKILEQRQVVLSEKEAQALCKEYENEDYFNKLIENMTSGPSLALVLLRDNGLQYWKQLLGPRTVEEAIEYFPE
SLCAQFAMDSLPVNQLYGSDSLETAEREIQHFFPLQSTLGLIKPHATSEQREQILKIVKEAGFDLTQVKKMFLTPEQIEKIYP
KVTGKDFYKDLLEMLSVGPSMVMILTKWNAVAEWRRLMGPTDPEEAKLLSPDSIRAQFGISKLKNIVHGASNAYEAKEVVNRL
FEDPEEN**

36.1% identity (69.2% similar) in 133 aa overlap (1-131:316-448)

```
               10         20        30        40        50
7A      EKTLALIKPDAIS-KAGEIIEIINKAGFTITKLKMM**MLSRKEALDFHVDHQS**RPFFNELI
        :::::::.:. .  .  ...:::.   : : . ....::.:::  .  ..... .::.::
8       EKTLALLRPNLFHERKDDVLRIIKDEDFKILEQRQVVLSEKEAQALCKEYENEDYFNKLI
          320       330       340       350       360       370

       60        70        80        90       100       110
7A      QFITTGPIIAMEILRDDAICEWKRLLGPAN**SGVARTDASES**IRALFGTDGIR-NAAHGPD
        . .:.:: .:. .:::...  ::.:::: .   :     ::. : :. :..  :  .: :
8       ENMTSGPSLALVLLRDNGLQYWKQLLGPRTVEEAIEYFPESLCAQFAMDSLPVNQLYGSD
          380       390       400       410       420       430

       120       130
7A      SFASAAREMELFF (SEQ ID NO:219)
        :. .: ::.. ::
8       SLETAEREIQHFF (SEQ ID NO:220)
          440
```

Waterman-Eggert score: 269; 85.9 bits; E(1) < 1.1e-21
33.6% identity (72.7% similar) in 128 aa overlap (1-127:451-577)

```
               10         20        30        40        50
7A      EKTLALIKPDAISKAGE-IIEIINKAGFTITKLKMM**MLSRKEALDFHVDHQS**RPFFNELI
        ..::.:::: : :.  : :..:..::: .:..: :.:. ..   ..    .. :...:.
8       QSTLGLIKPHATSEQREQILKIVKEAGFDLTQVKKMFLTPEQIEKIYPKVTGKDFYKDLL
              460       470       480       490       500       510

       60        70        80        90       100       110
7A      QFITTGPIIAMEILRDDAICEWKRLLGPAN**SGVARTDASES**IRALFGTDGIRNAAHGPDS
        .....:: ..: . . .:. ::.::.::..   :.  . .:::: :: . ..: .:: ..
8       EMLSVGPSMVMILTKWNAVAEWRRLMGPTDPEEAKLLSPDSIRAQFGISKLKNIVHGASN
              520       530       540       550       560       570

      120
7A      FASAAREM (SEQ ID NO:221)
         :  :.:.
8       -AYEAKEV (SEQ ID NO:222)
```

Waterman-Eggert score: 119; 40.4 bits; E(1) < 5.3e-08
33.8% identity (73.8% similar) in 65 aa overlap (3-65:156-220)

```
             10          20        30        40        50        60
7A      TLALIKPDAI--SKAGEIIEIINKAGFTITKLKMM**MLSRKEALDFHVDHQS**RPFFNELIQ
        ..:.::::::.  .:. :: . :.:::: :   .  .:.......:.    ..  :.:...
8       SIAIIKPDAVISKKVLEIKRKITKAGFIIEAEHKTVLTEEQVVNFYSRIADQCDFEEFVS
          160       170       180       190       200       210
```

7A FITTG (SEQ ID NO:223)

```
       :.:.:
8      FMTSG (SEQ ID NO:224)
         220


33.6% identity (65.5% similar) in 116 aa overlap (3-118:453-566)

             10        20        30        40        50        60
7B     TCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMV
       :  ..:::::.::    .::  ...:::... .. . .   ..:..:    :    :.:..
8      TLGLIKPHATSEQRE-QILKIVKEAGFDLTQVKKMFLTPEQIEKIYPKVTGK-DFYKDLL
            460       470       480       490       500       510

             70        80        90       100       110
7B     TEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVH (SEQ ID
NO:225)
         .  :: ..: . . ::.  .:.. ::.::: :. : : ..:: :: .:..: ::
8      EMLSVGPSMVMILTKWNAVAEWRRLMGPTDPEEAKLLSPDSIRAQFGISKLKNIVH (SEQ ID
NO:226)
              520       530       540       550       560


 Waterman-Eggert score: 128;  41.3 bits; E(1) <  2.9e-08
23.3% identity (60.3% similar) in 116 aa overlap (20-134:334-448)

      20        30        40        50        60        70
7B     ILMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNN
       .:  :.:  :.:  ...  ... ... . . :..    .. .. .: ::: .:. . ..:
8      VLRIIKDEDFKILEQRQVVLSEKEAQALCKEYENE-DYFNKLIENMTSGPSLALVLLRDN
           340       350       360       370       380       390

      80        90       100       110       120       130
7B     ATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQ-NAVHCTDLPEDGLLEVQYFF (SEQ ID
NO:227)
       . . .... ::   : : .  : .: : :.  ..  : .. .:  : .  :.:.::
8      GLQYWKQLLGPRTVEEAIEYFPESLCAQFAMDSLPVNQLYGSDSLETAEREIQHFF (SEQ ID
NO:228)
            400       410       420       430       440


>--
 Waterman-Eggert score: 76;  26.4 bits; E(1) <  0.00088
23.4% identity (46.8% similar) in 111 aa overlap (6-105:159-268)

         10        20        30        40        50        60
7B     IVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEM
       :.:: ::    . .:   :  ::: : : .   . . .: .::         .....:. :
8      IIKPDAVISKKVLEIKRKITKAGFIIEAEHKTVLTEEQVVNFYSRIADQC-DFEEFVSFM
      160       170       180       190       200       210

         70        80        90                  100
7B     YSGPCVAMEIQQNNATKTFREFCGPA-----------DPEIARHLRPGTLR (SEQ ID NO:229)
        ::    . ..:.. .   :   :             .::.  .. :: ..
8      TSGLSYILVVSQGSKHNPPSEETEPQTDTEPNERSEDQPEVEAQVTPGMMK (SEQ ID NO:230)
       220       230       240       250       260
```

>NME9
MLSSKGLTVVDVYQGWCGPCKPVVSLFQKMRIEVGLDLLHFALAEADRLDVLEKYRGKCE
PTFLFYAIKDEALSDEDECVSHGKNNGEDEDMVSSERTCTLAIIKPDAVAHGKTDEIIMK
IQEAGFEILTNEERTMTEAEVRLFYQHKAGESPSSVRHRNALQCRPWKPGQRRC (SEQ ID NO:231)

41.3% identity (67.4% similar) in 46 aa overlap (3-46:100-145)

```
             10          20        30        40
7A      TLALIKPDAIS--KAGEIIEIINKAGFTITKLKMMMLSRKEALDFH
        :::.:::::..  :. :::  :..::: :   .   ... :.  :.
9       TLAIIKPDAVAHGKTDEIIMKIQEAGFEILTNEERTMTEAEVRLFY
      100       110       120       130       140
```

>--
Waterman-Eggert score: 30; 13.5 bits; E(1) < 0.85
28.6% identity (71.4% similar) in 14 aa overlap (69-82:100-113)

```
        70        80
7A      AMEILRDDAICEWK (SEQ ID NO:232)
        .. :.. ::. . :
9       TLAIIKPDAVAHGK (SEQ ID NO:233)
      100       110
```

>--
Waterman-Eggert score: 29; 13.2 bits; E(1) < 0.91
25.8% identity (74.2% similar) in 31 aa overlap (12-42:121-149)

```
               20        30        40
7A      ISKAGEIIEIINKAGFTITKLKMMMLSRKEA (SEQ ID NO:234)
        :..::  .::...   :.:. .. .. ...:
9       IQEAG--FEILTNEERTMTEAEVRLFYQHKA (SEQ ID NO:235)
                 130       140
```

39.6% identity (69.8% similar) in 53 aa overlap (1-53:98-150)

```
               10        20        30        40        50
7B      NCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKG (SEQ ID NO:236)
        .::  :.:: ::..:   .:.: :..::::: . .  .: ...:. ::.   :
9       TCTLAIIKPDAVAHGKTDEIIMKIQEAGFEILTNEERTMTEAEVRLFYQHKAG (SEQ ID NO:237)
        100       110       120       130       140       150
```

```
>NME10 NP_008846.2 protein XRP2 [Homo sapiens]
MGCFFSKRRKADKESRPENEEERPKQYSWDQREKVDPKDYMFSGLKDETVGRLPGTVAGQQFLIQDCENC
NIYIFDHSATVTIDDCTNCIIFLGPVKGSVFFRNCRDCKCTLACQQFRVRDCRKLEVFLCCATQPIIESS
SNIKFGCFQWYYPELAFQFKDAGLSIFNNTWSNIHDFTPVSGELNWSLLPEDAVVQDYVPIPTTEELKAV
RVSTEANRSIVPISRGQRQKSSDESCLVVLFAGDYTIANARKLIDEMVGKGFFLVQTKEVSMKAEDAQRV
FREKAPDFLPLLNKGPVIALEFNGDGAVEVCQLIVNEIFNGTKMFVSESKETASGDVDSFYNFADIQMGI (SEQ
ID NO:238)

23.5% identity (66.2% similar) in 68 aa overlap (11-78:246-308)

               20        30        40        50        60        70
7A     AISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAM
       .:..: ..:. .   :: ... : . .. ..:   .: ... :   ... ... ::.::.
10     TIANARKLIDEMVGKGFFLVQTKEVSMKAEDAQ--RVFREKAP---DFLPLLNKGPVIAL
         250       260       270         280          290       300


7A     EILRDDAI (SEQ ID NO:239)
       :.  : :.
10     EFNGDGAV (SEQ ID NO:240)


>--
 Waterman-Eggert score: 35;  15.1 bits; E(1) <  0.73
28.9% identity (57.8% similar) in 45 aa overlap (66-108:200-244)

          70         80        90       100
7A     PIIAMEILRDDAIC-EWKRLLGPANSGVARTDASES-IRALFGTD (SEQ ID NO:241)
       :: . : :.   .  : .: . : . :  . ...:: . .::. :
10     PIPTTEELKAVRVSTEANRSIVPISRGQRQKSSDESCLVVLFAGD (SEQ ID NO:242)
     200       210       220       230       240

>--
 Waterman-Eggert score: 33;  14.4 bits; E(1) <  0.87
14.7% identity (52.0% similar) in 75 aa overlap (7-80:35-109)

         10        20        30        40        50        60
7A     IKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQ-FITTG
       . :     .:   : ...   :..  .... . ..   .  ::..   ...  . .:  :
10     VDPKDYMFSGLKDETVGRLPGTVAGQQFLIQDCENCNIYIFDHSATVTIDDCTNCIIFLG
           40        50        60        70        80        90

          70        80
7A     PIIAMEILRDDAICE (SEQ ID NO:243)
       :. .  ..:.   :.
10     PVKGSVFFRNCRDCK (SEQ ID NO:244)
          100

>--
 Waterman-Eggert score: 45;  17.5 bits; E(1) <  0.22
21.6% identity (58.8% similar) in 51 aa overlap (4-50:130-180)

              10        20        30         40        50
7B     CCIVKP--HAVSEGLLGKILMAIRDAGFEI--SAMQMFNMDRVNVEEFYEV (SEQ ID NO:245)
       :: ..:  .. :.  .: .     . .:..  .....::    :...:  :
10     CCATQPIIESSSNIKFGCFQWYYPELAFQFKDAGLSIFNNTWSNIHDFTPV (SEQ ID NO:246)
     130       140       150       160       170       180
```

[00263] As an example, antibodies or antibody mimics that bind to the NME7 homologous peptides ("homologous peptides") in particular homologous to A1, A2, B1, B2 or B3 peptides, may be administered to a patient diagnosed with or at risk of developing a cancer or cancer metastasis.

[00264] **Homologous peptides to A1, A2, B1, B2 or B3 peptides**

[00265] Homologous peptides to A1, A2, B1, B2 or B3 peptides may include without limitation the following:

[00266] NME2A1

[00267] (amino acids)

[00268] RASEEHLKQHYIDLKD (SEQ ID NO:247)

[00269] NME2A2

[00270] (amino acids)

[00271] PADSKPGT (SEQ ID NO:248)

[00272] NME2B1

[00273] (amino acids)

[00274] QKGFRLVAMKFLRASEEHLK (SEQ ID NO:249)

[00275] NME2B2

[00276] (amino acids)

[00277] IDLKDRPFPGLVKY (SEQ ID NO:250)

[00278] NME2B3

[00279] (amino acids)

[00280] GDFCIQVGRNIIHGSDSVKSAEKEISLWF (SEQ ID NO:251)

[00281] NME3A1

[00282] (amino acids)

[00283] QASEELLREHYVELRE (SEQ ID NO:252)

[00284] NME3A1

[00285] (amino acids)

[00286] PGDATPGT (SEQ ID NO:253)

[00287] NME3B1

[00288] (amino acids)

[00289] RKGFKLVALKLVQASEELLR (SEQ ID NO:254)

[00290] NME3B2

[00291] (amino acids)

[00292] VELRERPFYSRLVKY (SEQ ID NO:255)

[00293] NME3B3

[00294] (amino acids)

[00295] GDFCVEVGKNVIHGSDSVESAQREIALWF (SEQ ID NO:256)

[00296] NME4A1

[00297] (amino acids)

[00298] QAPESVLAEHYQDLRR (SEQ ID NO:257)

[00299] NME4A2

[00300] (amino acids)

[00301] SAEAAPGT (SEQ ID NO:258)

[00302] NME4B1

[00303] (amino acids)

[00304] RRGFTLVGMKMLQAPESVLA (SEQ ID NO:259)

[00305] NME4B2

[00306] (amino acids)

[00307] QDLRRKPFYPALIRY (SEQ ID NO:260)

[00308] NME4B3

[00309] (amino acids)

[00310] GDFSVHISRNVIHASDSVEGAQREIQLWF (SEQ ID NO:261)

[00311] NME5A1

[00312] (amino acids)

[00313] RLSPEQCSNFYVEKYG (SEQ ID NO:262)

[00314] NME5A2

[00315] (amino acids)

[00316] SLVAKETHPDS (SEQ ID NO:263)

[00317] NME5B1

[00318] (amino acids)

[00319] RSGFTIVQRRKLRLSPEQCS (SEQ ID NO:264)

[00320] NME5B2

[00321] (amino acids)

[00322] VEKYGKMFFPNLTAY (SEQ ID NO:265)

[00323] NME5B3

[00324] (amino acids)

[00325] AIYGTDDLRNALHGSNDFAAAEREIRFMF (SEQ ID NO:266)

[00327] NME6A1

[00328] (amino acids)

[00329] LWRKEDCQRFYREHEG (SEQ ID NO:267)

[00330] NME6A2

[00331] (amino acids)

[00332] VFRARHVAPDS (SEQ ID NO:268)

[00333] NME6B1

[00334] (amino acids)

[00335] SNKFLIVRMRELLWRKEDCQ (SEQ ID NO:269)

[00336] NME6B2

[00337] (amino acids)

[00338] REHEGRFFYQRLVEF (SEQ ID NO:270)

[00339] NME6B3

[00340] (amino acids)

[00341] GSFGLTDTRNTTHGSDSVVSASREIAAFF (SEQ ID NO:271)

[00342] NME8A1

[00343] (amino acids)

[00344] VLSEKEAQALCKEYEN (SEQ ID NO:272)

[00345] NME8A2

[00346] (amino acids)

[00347] VEEAIEYFPES (SEQ ID NO:273)

[00348] NME8A3

[00349] (amino acids)

[00350] FLTPEQIEKIYPKVTG (SEQ ID NO:274)

[00351] NME8A4

[00352] (amino acids)

[00353] PEEAKLLSPDS (SEQ ID NO:275)

[00354] NME8A5

[00355] (amino acids)

[00356] VLTEEQVVNFYSRIAD (SEQ ID NO:276)

[00357] NME8B1

[00358] (amino acids)

[00359] EAGFDLTQVKKMFLTPEQIE (SEQ ID NO:277)

[00360] NME8B2

[00361] (amino acids)

[00362] PKVTGKDFYKDLLEM (SEQ ID NO:278)

[00363] NME8B3

[00364] (amino acids)

[00365] AQFGISKLKNIVH (SEQ ID NO:279)

[00366] NME8B4

[00367] (amino acids)

[00368] DEDFKILEQRQVVLSEKEAQ (SEQ ID NO:280)

[00369] NME8B5

[00370] (amino acids)

[00371] KEYENEDYFNKLIEN (SEQ ID NO:281)

[00372] NME8B6

[00373] (amino acids)

[00374] AQFAMDSLPVNQLYGSDSLETAEREIQHFF (SEQ ID NO:282)

[00375] NME8B7

[00376] (amino acids)

[00377] KAGFIIEAEHKTVLTEEQVV (SEQ ID NO:283)

[00378] NME8B8

[00379] (amino acids)

[00380] SRIADQCDFEEFVSF (SEQ ID NO:284)

[00381] NME9A1

[00382] (amino acids)

[00383] TMTEAEVRLFY (SEQ ID NO:285)

[00384] NME9B1

[00385] (amino acids)

[00386] EAGFEILTNEERTMTEAEVR (SEQ ID NO:286)

[00387] NME10A1

[00388] (amino acids)

[00389] SMKAEDAQRVFREK (SEQ ID NO:287)

[00390] NME10A2

[00391] (amino acids)

[00392] GQRQKSSDES (SEQ ID NO:288)

[00393] NME10A3

[00394] (amino acids)

[00395] IQDCENCNIYIFDHSA (SEQ ID NO:289)

[00396] NME10B1

[00397] ELAFQFKDAGLSIFNNTWSNIH (SEQ ID NO:290)

[00398] In some cases, peptides derived from other NME proteins can be made more homologous to NME7 A1, A2, B1, B2 or B3 peptides by shifting the frame or extending the NME7 peptides such that the extended peptides are more homologous to the NME7 peptides that gave rise to antibodies that inhibit cancer or cancer metastases. As another example, antibodies or antibody mimics that bind to the NME7 homologous extended peptides ("extended peptides") may be administered to a patient diagnosed with or at risk of developing a cancer or cancer metastasis.

[00399] **Homologous extended peptides to A1, A2, B1, B2 or B3 peptides**

[00400] Homologous peptides to A1, A2, B1, B2 or B3 peptides that are extended peptides may include without limitation the following:

[00401] NME2A1

[00402] (amino acids)

[00403] RASEEHLKQHYIDLKDRPFFPGL (SEQ ID NO:291)

[00404] NME2A2

[00405] (amino acids)

[00406] LGETNPADSKPGTIRGDF (SEQ ID NO:292)

[00407] NME2B1

[00408] (amino acids)

[00409] GLVGEIIKRFEQKGFRLVAMKFLRASEEHLKQHY (SEQ ID NO:293)

[00410] NME2B2

[00411] (amino acids)

[00412] YIDLKDRPFFPGLVKYMNSGPVVAM (SEQ ID NO:294)

[00413] NME2B3

[00414] (amino acids)

[00415] PGTIRGDFCIQVGRNIIHGSDSVKSAEKEISLWF (SEQ ID NO:295)

[00416] NME3A1

[00417] (amino acids)

[00418] LKLVQASEELLREHYVELRERPFYSRL (SEQ ID NO:296)

[00419] NME3A1

[00420] (amino acids)

[00421] LIGATDPGDATPGTIRGDF (SEQ ID NO:297)

[00422] NME3B1

[00423] (amino acids)

[00424] LVGEIVRRFERKGFKLVALKLVQASEELLRE (SEQ ID NO:298)

[00425] NME3B2

[00426] (amino acids)

[00427] EHY-VELRERPFYSRLVKYMGSGPVVAM (SEQ ID NO:299)

[00428] NME3B3

[00429] (amino acids)

[00430] PGTIRGDFCVEVGKNVIHGSDSVESAQREIALWF (SEQ ID NO:300)

[00431] NME4A1

[00432] (amino acids)

[00433] GFTLVGMKMLQAPESVLAEHYQDLRRKPF (SEQ ID NO:301)

[00434] NME4A2

[00435] (amino acids)

[00436] GHTDSAEAAPGTIRGDF (SEQ ID NO:302)

[00437] NME4B1

[00438] (amino acids)

[00439] LVGDVIQRFERRGFTLVGMKMLQAPESVLAEHY (SEQ ID NO:303)

[00440] NME4B2

[00441] (amino acids)

[00442] EHYQDLRRKPFYPALIRYMSSGPVVAM (SEQ ID NO:304)

[00443] NME4B3

[00444] (amino acids)

[00445] PGTIRGDFSVHISRNVIHASDS VEGAQREIQLWF (SEQ ID NO:305)

[00446] NME5A1

[00447] (amino acids)

[00448] GFTIVQRRKLRLSPEQCSNFYVEKYGKMFF (SEQ ID NO:306)

[00449] NME5A2

[00450] (amino acids)

[00451] LLGPNNSLVAKETHPDSLRAIYGTD (SEQ ID NO:307)

[00452] NME5B1

[00453] (amino acids)

[00454] IQDIILRSGFTIVQRRKLRLSPEQCSNFY (SEQ ID NO:308)

[00455] NME5B2

[00456] (amino acids)

[00457] FYVEKYGKMFFPNLTAYMSSGPLVAM (SEQ ID NO:309)

[00458] NME5B3

[00459] (amino acids)

[00460] PDSLRAIYGTDDLRNALHGSNDFAAAEREIRFMF (SEQ ID NO:310)

[00461] NME6A1

[00462] (amino acids)

[00463] FLIVRMRELLWRKEDCQRFYREHEGRFFYQRL (SEQ ID NO:311)

[00464] NME6A2

[00465] (amino acids)

[00466] LMGPTRVFRARHVAPDSIRGSFG (SEQ ID NO:312)

[00467] NME6B1

[00468] (amino acids)

[00469] ILSNKFLIVRMRELLWRKEDCQRFY (SEQ ID NO:313)

[00470] NME6B2

[00471] (amino acids)

[00472] FYREHEGRFFYQRLVEFMASGPIRA (SEQ ID NO:314)

[00473] NME6B3

[00474] (amino acids)

[00475] ARHVAPDSIRGSFGLTDTRNTTHGSDSVVSASREIAAFF (SEQ ID NO:315)

[00476] NME8A1

[00477] (amino acids)

[00478] FKILEQRQVVLSEKEAQALCKEYENEDYFNKLI (SEQ ID NO:316)

[00479] NME8A2

[00480] (amino acids)

[00481] WKQLLGPRTVEEAIEYFPESLCAQFAMD (SEQ ID NO:317)

[00482] NME8A3

[00483] (amino acids)

[00484] AGFDLTQVKKMFLTPEQIEKIYPKVTGKDFYKDL (SEQ ID NO:318)

[00485] NME8A4

[00486] (amino acids)

[00487] EWRRLMGPTDPEEAKLLSPDSIRAQFG (SEQ ID NO:319)

[00488] NME8A5

[00489] (amino acids)

[00490] KAGFIIEAEHKTVLTEEQVVNFYSRIADQCDFEE (SEQ ID NO:320)

[00491] NME8B1

[00492] (amino acids)

[00493] ILKIVKEAGFDLTQVKKMFLTPEQIEKIY (SEQ ID NO:321)

[00494] NME8B2

[00495] (amino acids)

[00496] YPKVTGKDFYKDLLEMLSVGP (SEQ ID NO:322)

[00497] NME8B3

[00498] (amino acids)

[00499] DPEEAKLLSPDSIRAQFGISKLKNIVH (SEQ ID NO:323)

[00500] NME8B4

[00501] (amino acids)

[00502] LRIIKDEDFKILEQRQVVLSEKEAQ (SEQ ID NO:324)

[00503] NME8B5

[00504] (amino acids)

[00505] KEYENE-DYFNKLIENMTSGPSLA (SEQ ID NO:325)

[00506] NME8B6

[00507] (amino acids)

[00508] PESLCAQFAMDSLPVNQLYGSDSLETAEREIQHFF (SEQ ID NO:326)

[00509] NME8B7

[00510] (amino acids)

[00511] IKRKITKAGFIIEAEHKTVLTEEQVVNFY (SEQ ID NO:327)

[00512] NME8B8

[00513] (amino acids)

[00514] FYSRIADQCDFEEFVSFMTSG (SEQ ID NO:328)

[00515] NME9A1

[00516] (amino acids)

[00517] AGFEILTNEERTMTEAEVRLFY (SEQ ID NO:329)

[00518] NME9B1

[00519] (amino acids)

[00520] IIMKIQEAGFEILTNEERTMTEAEVRLFY (SEQ ID NO:330)

[00521] NME10A1

[00522] (amino acids)

[00523] GFFLVQTKEVSMKAEDAQRVFREKAP (SEQ ID NO:331)

[00524] NME10A2

[00525] (amino acids)

[00526] EANRSIVPISRGQRQKSSDESCLVVLFAGD (SEQ ID NO:332)

[00527] NME10A3

[00528] (amino acids)

[00529] IQDCENCNIYIFDHSA (SEQ ID NO:333)

[00530] NME10B1

[00531] ELAFQFKDAGLSIFNNTWSNIHDFTPVDCT (SEQ ID NO:334)

[00532] Some NME proteins exert a function that is necessary for normal cell growth or development. For example NME1 is thought to be required for normal cell function. Other NME proteins have catalytic domains whose function is required in normal cells or tissues. In these cases, therapeutic antibodies can be selected based on their ability to bind to the targeted, cancer associated NME, but not to a non-targeted NME. For example, the anti-NME7 antibodies presented here, 8F9A5A1, 8F9A4A3, and 5F3A5D4, were selected for their ability to bind to $NME7_{AB}$ but not to NME1; they were further selected based on their ability to inhibit cancer and cancer metastases.

[00533] In another aspect of the invention, anti-NME7 antibodies, antibody fragments, for example scFvs, or fragments of antibody mimics are incorporated into chimeric antigen receptors (CARs) which are engineered to be expressed in immune cells. The immune cell can be engineered to express an anti-NME7 CAR, an anti-MUC1* CAR, or both. One of the CARs may be expressed off of an inducible promoter. Alternatively, an immune cell may be engineered to express a CAR such as an anti-MUC1* CAR and an inducible anti-NME7 antibody or antibody fragment. In some instances the inducible promoter may contain NFAT response elements. In one aspect, these engineered species are expressed in T cells, NK cells or dendritic cells. The immune cells may be obtained from the patient or from a donor. In some cases, immune molecules such as MHCs, checkpoint inhibitors or receptors for checkpoint inhibitors are mutated or cut out, for example using CrisPR or CrisPR-like technology. In another aspect, ITAM molecules, Fos, or Jun are mutated or genetically excised via Talens, Sleeping Beauty, CrisPR or CrisPR-like technologies in patient or donor derived immune cells.

[00534] In one aspect of the invention, the anti-NME7 antibodies or antibody mimics for use in CAR T format are chosen from among the group of antibodies or antibody mimics that are specific for NME7 but do not disrupt the binding of NME7 to the extra cellular domain of MUC1*. In this way, the anti-NME7 antibody or antibody mimic that targets the CAR T to the tumor will not simply pluck the ligand from the receptor, whereupon the T cell would be unable

to inject the target cancer cell with Granzyme B. Such antibodies or antibody mimics are generated by immunizing an animal with an NME7 peptide, such as NME7 peptides A1, A2, B1, B2 or B3 or selected by virtue of their ability to bind to NME7 peptides A1, A2, B1, B2 or B3. Antibodies or antibody mimics can be screened for their ability to specifically bind to NME7, but not to NME1 or NME2, and also for their inability to disrupt binding between NME7 and MUC1* extra cellular domain. For example, in an ELISA setup, the PSMGFR peptide is immobilized to the surface. A labeled $NME7_{AB}$ is allowed to bind to the surface immobilized MUC1* extra cellular domain, and detection of the $NME7_{AB}$ label is measured in the presence or absence of the test antibody or antibody mimic. In one aspect of the invention, an antibody that does not diminish binding between $NME7_{AB}$ and surface-immobilized MUC1* extra cellular domain peptide is selected as an antibody that is incorporated into a CAR and engineered to be expressed in an immune cell and then administered to a patient for the treatment or prevention of cancer or cancer metastases.

[00535] In one aspect of the invention, an anti-NME7 antibody or fragment thereof is administered to a patient diagnosed with or at risk of developing a cancer or cancer metastasis. In one aspect, the anti-NME7 antibody or antibody fragment binds to an NME peptide discussed above in particular under sections "**Homologous peptides to A1, A2, B1, B2 or B3 peptides**" and the "**Homologous extended peptides to A1, A2, B1, B2 or B3 peptides**".

[00536] In another aspect, the antibody, antibody fragment or antibody mimic binds to an NME7 derived peptide chosen from among A1, A2, B1, B2 or B3 (SEQ ID NOS: 141-145). In yet another aspect, the antibody, antibody fragment or antibody mimic binds to an NME7 peptide comprising most or all of the B3 peptide. In one aspect of the invention, the anti-NME7 antibody, antibody fragment or antibody mimic comprises sequences derived from the variable domains of anti-NME7 antibodies 8F9A4A3 (SEQ ID NOS:1001-1015), 8F9A5A1 (SEQ ID NOS: 1016 – 1030), or 8H5H5G4 (SEQ ID NOS: 1031 – 1045) shown below.

[00537] **Anti-NME7 B3 peptide monoclonal antibodies**

[00538] 8F9A4A3 Heavy chain variable region sequence mouse

[00539] (DNA)

[00540] Gtccagctgcaacagtctggacctgaactggtgaagcctggggcttcagtgaagatatcctgcaagacttctggaaa cacattcactgaatacaccatgcactgggtgaagcagagccatggaaagagccttgagtggattggaggttttaatcctaacaatggtg ttactaactacaaccagaagttcaagggcaaggccacattgactgtagacaagtcctccagcacagcctacatggagctccgcagcct gacatctgaggattctgcagtctattactgtgcaagacggtactaccatagtctctacgtgttttactttgactactggggccaaggcacca ctctcacagtctcctca (SEQ ID NO:335)

[00541] (amino acids)

[00542] VQLQQSGPELVKPGASVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGF NPNNGVTNYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSLYVF YFDYWGQGTTLTVSS (SEQ ID NO:1001)

[00543] IGHV1-24*01 V-REGION sequence human (closest match hu antibody sequence)

[00544] (DNA)

[00545] Caggtccagctggtacagtctggggctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggtttccg gatacaccctcactgaattatccatgcactgggtgcgacaggctcctggaaaagggcttgagtggatgggaggttttgatcctgaagat ggtgaaacaatctacgcacagaagttccagggcagagtcaccatgaccgaggacacatctacagacacagcctacatggagctgag cagcctgagatctgaggacacggccgtgtattactgtgcaaca (SEQ ID NO:336)

[00546] (amino acids)

[00547] QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEW MGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCAT (SEQ ID NO:1002)

[00548] human (closest match hu antibody sequence)

[00549] IGHJ4*01 J-REGION sequence

[00550] (DNA)

[00551] tactttgactactggggccaaggaaccctggtcaccgtctcctca (SEQ ID NO:337)

[00552] (amino acids)

[00553] YFDYWGQGTLVTVSS (SEQ ID NO:1003)

[00554] humanized heavy chain variable seq (SEQ ID NO: 1001+ SEQ ID NO:1002 + SEQ ID NO:1003)

[00555] humanized 8F9A4A3 Heavy chain variable region sequence

[00556] (DNA)

[00557] caggtccagctggtacagtctggggctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggtttccg gaaacacattcactgaatacaccatgcactgggtgcgacaggctcctggaaaagggcttgagtggatgggaggttttaatcctaacaat ggtgttactaactacaaccagaagttcaagggcagagtcaccatgaccgaggacacatctacagacacagcctacatggagctgagc agcctgagatctgaggacacggccgtgtattactgtgcaagacggtactaccatagtctctacgtgttttactttgactactggggccaag gaaccctggtcaccgtctcctca (SEQ ID NO:338)

[00558] (amino acids)

[00559] QVQLVQSGAEVKKPGASVKVSCKVSGNTFTEYTMHWVRQAPGKGLEW MGGFNPNNGVTNYNQKFKGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCARRYYH SLYVFYFDYWGQGTLVTVSS (SEQ ID NO:1004)

[00560] humanized heavy chain variable seq (codon optimized version of 1004)

[00561] humanized 8F9A4A3 Heavy chain variable region sequence (codon optimized)

[00562] (DNA)

[00563] caggttcagctggttcagtctggcgccgaagtgaagaaacctggcgcctctgtgaaggtgtcctgcaaggtgtccgg aaataccttcaccgagtacaccatgcactgggtccgacaggcccctggcaaaggacttgaatggatgggcggcttcaaccccaacaa cggcgtgaccaactacaaccagaaattcaagggccgcgtgaccatgaccgaggacacaagcacagacaccgcctacatggaactg agcagcctgagaagcgaggacaccgccgtgtactactgcgccagaaggtactaccacagcctgtacgtgttctacttcgactactggg gccagggcaccctggtcacagtttcttct (SEQ ID NO:339)

[00564] (amino acids)

[00565] QVQLVQSGAEVKKPGASVKVSCKVSGNTFTEYTMHWVRQAPGKGLEW MGGFNPNNGVTNYNQKFKGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCARRYYH SLYVFYFDYWGQGTLVTVSS (SEQ ID NO:1005)

[00566] humanized heavy chain variable seq ("modified" SEQ ID NO:1005 sequence, where modified means certain amino acids that are thought to be critical for binding or structure have been reverted to the mouse sequence).

[00567] Modified humanized 8F9A4A3 Heavy chain variable region sequence

[00568] (DNA)

[00569] caggtccagctggtacagtctggggctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggtttccg gaaacacattcactgaatacaccatgcactgggtgcgacaggctcctggaaaagggcttgagtggattggaggttttaatcctaacaat ggtgttactaactacaaccagaagttcaagggcaaagtcaccctgaccgtggacacatctagcagcacagcctacatggagctgagc agcctgagatctgaggacacggccgtgtattactgtgcaagacggtactaccatagtctctacgtgttttactttgactactggggccaag gaaccctggtcaccgtctcctca (SEQ ID NO:340)

[00570] (amino acids)

[00571] QVQLVQSGAEVKKPGASVKVSCKVSGNTFTEYTMHWVRQAPGKGLEWI GGFNPNNGVTNYNQKFKGKVTLTVDTSSSTAYMELSSLRSEDTAVYYCARRYYHSL YVFYFDYWGQGTLVTVSS (SEQ ID NO:1006)

[00572] humanized heavy chain variable seq (SEQ ID NO:1006 codon optimized)

[00573] Modified humanized 8F9A4A3 Heavy chain variable region sequence (codon optimized)

[00574] (DNA)

[00575] caggttcagctggttcagtctggcgccgaagtgaagaaacctggcgcctctgtgaaggtgtcctgcaaggtgtccgg aaataccttcaccgagtacaccatgcactgggtccgacaggcccctggcaaaggactggaatggatcggcggcttcaaccccaaca acggcgtgaccaactacaaccagaaattcaagggcaaagtgaccctgaccgtggacaccagcagcagcacagcctacatggaact gagcagcctgagaagcgaggacaccgccgtgtactactgcgccagaaggtactaccacagcctgtacgtgttctacttcgactactgg ggccagggcaccctggtcacagtttcttct (SEQ ID NO:341)

[00576] (amino acids)

[00577] QVQLVQSGAEVKKPGASVKVSCKVSGNTFTEYTMHWVRQAPGKGLEWI GGFNPNNGVTNYNQKFKGKVTLTVDTSSSTAYMELSSLRSEDTAVYYCARRYYHSL YVFYFDYWGQGTLVTVSS (SEQ ID NO:1007)

[00578] 8F9A4A3 Light chain variable region sequence mouse

[00579] (DNA)

[00580] gaaacaactgtgacccagtctccagcatccctgtccatggctataggagaaaaagtcaccatcagatgcataaccag cactgatattgatgatgatatgaactggtaccagcagaagccaggggaacctcctaagctccttatttcagaaggcaatactcttcgtcct ggagtcccatcccgattctccagcagtggctatggtacagattttgtttttacaattgaaaacatgctctcagaagatgttgcagattactac tgtttgcaaagtgataacttgcctctcacgttcggctcggggacaaagttggaaataaaacgg (SEQ ID NO:342)

[00581] (amino acids)

[00582] ETTVTQSPASLSMAIGEKVTIRCITSTDIDDDMNWYQQKPGEPPKLLISEGN TLRPGVPSRFSSSGYGTDFVFTIENMLSEDVADYYCLQSDNLPLTFGSGTKLEIKR (SEQ ID NO:1008)

[00583] human (closest match hu antibody sequence)

[00584] IGKV5-2*01 V-REGION sequence

[00585] (DNA)

[00586] gaaacgacactcacgcagtctccagcattcatgtcagcgactccaggagacaaagtcaacatctcctgcaaagccag ccaagacattgatgatgatatgaactggtaccaacagaaaccaggagaagctgctattttcattattcaagaagctactactctcgttcct ggaatcccacctcgattcagtggcagcgggtatggaacagattttaccctcacaattaataacatagaatctgaggatgctgcatattact tctgt (SEQ ID NO:343)

[00587] (amino acids)

[00588] ETTLTQSPAFMSATPGDKVNISCKASQDIDDDMNWYQQKPGEAAIFIIQEA TTLVPGIPPRFSGSGYGTDFTLTINNIESEDAAYYFC (SEQ ID NO:1009)

[00589] human (closest match hu antibody sequence)

[00590] IGKJ4*02 J-REGION sequence

[00591] (DNA)

[00592] ctcacgttcggcggagggaccaaggtggagatcaaa (SEQ ID NO:344)

[00593] (amino acids)

[00594] LTFGGGTKVEIK (SEQ ID NO:1010)

[00595] humanized light chain variable seq (SEQ ID NO: 1008 + SEQ ID NO:1009 + SEQ ID NO:1)

[00596] humanized 8F9A4A3 Light chain variable region sequence

[00597] (DNA)

[00598] gaaacgacactcacgcagtctccagcattcatgtcagcgactccaggagacaaagtcaacatctcctgcataaccag cactgatattgatgatgatatgaactggtaccaacagaaaccaggagaagctgctattttcattattcaagaaggcaatactcttcgtcctg gaatcccacctcgattcagtggcagcgggtatggaacagattttaccctcacaattaataacatagaatctgaggatgctgcatattactt ctgtttgcaaagtgataacttgcctctcacgttcggcggagggaccaaggtggagatcaaacgg (SEQ ID NO:345)

[00599] (amino acids)

[00600] ETTLTQSPAFMSATPGDKVNISCITSTDIDDDMNWYQQKPGEAAIFIIQEGN TLRPGIPPRFSGSGYGTDFTLTINNIESEDAAYYFCLQSDNLPLTFGGGTKVEIKR (SEQ ID NO:1011)

[00601] humanized light chain variable seq (codon optimized version of SEQ ID NO:1011)

[00602] humanized 8F9A4A3 Light chain variable region sequence (codon optimized)

[00603] (DNA)

[00604] Gagacaaccctgacacagagccctgccttcatgtctgccacacctggcgacaaagtgaacatcagctgcatcacca gcaccgacatcgacgacgacatgaactggtatcagcagaagcctggcgaggccgccatcttcatcatccaagagggcaacacactg cggcctggcatccctcctagattttctggcagcggctacggcaccgacttcaccctgaccatcaacaacatcgagagcgaggacgcc gcctactacttctgcctgcaaagcgacaacctgcctctgacctttggcggaggcaccaaggtggaaatcaagcgg (SEQ ID NO:346)

[00605] (amino acids)

[00606] ETTLTQSPAFMSATPGDKVNISCITSTDIDDDMNWYQQKPGEAAIFIIQEGN TLRPGIPPRFSGSGYGTDFTLTINNIESEDAAYYFCLQSDNLPLTFGGGTKVEIKR (SEQ ID NO:1012)

[00607] humanized light chain variable seq ("modified" SEQ ID NO:1012 sequence, where modified means certain amino acids that are thought to be critical for binding or structure have been reverted to the mouse sequence).

[00608] Modified humanized 8F9A4A3 Light chain variable region sequence

[00609] (DNA)

[00610] gaaacgacagtgacgcagtctccagcattcatgtcagcgactccaggagacaaagtcaccatctcctgcataaccag cactgatattgatgatgatatgaactggtaccaacagaaaccaggagaagctgctattctgctgattagcgaaggcaatactcttcgtcct ggaatcccacctcgattcagtagcagcgggtatggaacagattttaccctcacaattaataacatagaatctgaggatgctgcatattact tctgtttgcaaagtgataacttgcctctcacgttcggcggagggaccaaggtggagatcaaacgg (SEQ ID NO:347)

[00611] (amino acids)

[00612] ETTVTQSPAFMSATPGDKVTISCITSTDIDDDMNWYQQKPGEAAILLISEG NTLRPGIPPRFSSSGYGTDFTLTINNIESEDAAYYFCLQSDNLPLTFGGGTKVEIKR (SEQ ID NO:1013)

[00613] humanized light chain variable seq (SEQ ID NO:1013 codon optimized)

[00614] Modified humanized 8F9A4A3 Light chain variable region sequence (codon optimized)

[00615] (DNA)

[00616] gagacaaccgtgacacagagccctgccttcatgtctgccacacctggcgacaaagtgaccatcagctgcatcacca gcaccgacatcgacgacgacatgaactggtatcagcagaagcctggcgaggccgccatcctgcttatctctgagggaaacacactgc ggcctggcatccctcctagattttccagcagcggctacggcaccgacttcaccctgaccatcaacaacatcgagagcgaggacgccg cctactacttctgcctgcaaagcgacaacctgcctctgacctttggcggaggcaccaaggtggaaatcaagcgg (SEQ ID NO:348)

[00617] (amino acids)

[00618] ETTVTQSPAFMSATPGDKVTISCITSTDIDDDMNWYQQKPGEAAILLISEG NTLRPGIPPRFSSSGYGTDFTLTINNIESEDAAYYFCLQSDNLPLTFGGGTKVEIKR (SEQ ID NO:1014)

[00619] humanized heavy and light chains joined via a flexible linker.

[00620] Modified humanized 8F9A4A3 sequence (codon optimized)

[00621] (DNA)

[00622] Caggttcagctggttcagtctggcgccgaagtgaagaaacctggcgcctctgtgaaggtgtcctgcaaggtgtccgg aaataccttcaccgagtacaccatgcactgggtccgacaggcccctggcaaaggactggaatggatcggcggcttcaaccccaaca acggcgtgaccaactacaaccagaaattcaagggcaaagtgaccctgaccgtggacaccagcagcagcacagcctacatggaact gagcagcctgagaagcgaggacaccgccgtgtactactgcgccagaaggtactaccacagcctgtacgtgttctacttcgactactgg ggccagggcaccctggtcacagtttcttctggcggtggcggaagcggaggcggtggctccggtggcggaggcagcgaaacgaca gtgacgcagtctccagcattcatgtcagcgactccaggagacaaagtcaccatctcctgcataaccagcactgatattgatgatgatatg aactggtaccaacagaaaccaggagaagctgctattctgctgattagcgaaggcaatactcttcgtcctggaatcccacctcgattcagt agcagcgggtatggaacagattttaccctcacaattaataacatagaatctgaggatgctgcatattacttctgtttgcaaagtgataactt gcctctcacgttcggcggagggaccaaggtggagatcaaacgg (SEQ ID NO:349)

[00623] (amino acids)

[00624] QVQLVQSGAEVKKPGASVKVSCKVSGNTFTEYTMHWVRQAPGKGLEWI GGFNPNNGVTNYNQKFKGKVTLTVDTSSSTAYMELSSLRSEDTAVYYCARRYYHSL YVFYFDYWGQGTLVTVSSGGGGSGGGGSGGGGSETTVTQSPAFMSATPGDKVTISCI TSTDIDDDMNWYQQKPGEAAILLISEGNTLRPGIPPRFSSSGYGTDFTLTINNIESEDA AYYFCLQSDNLPLTFGGGTKVEIKR (SEQ ID NO:1015)

[00625] 8F9A5A1 Heavy chain variable region sequence

[00626] (DNA)

[00627] atccagttggtgcagtctggacctgagctgaagaagcctggagagacagtcaagatctcctgcaaggcttctgggtat accttcacaaactatggaatgaactgggtgaagcaggctccaggaaagggtttaaagtggatgggctggataaacacctacactgga

gagccaacatatgttgatgacttcaagggacggtttgccttctctttggaaacctctgccaccactgcctatttgcagatcaacaacctca aaaatgaggacacgtctacatatttctgtgcaagattgaggggatacgaccgggtcccttggcttactggggccaagggactctggtc actgtctctgca (SEQ ID NO:350)

[00628] (amino acids)

[00629] IQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMG WINTYTGEPTYVDDFKGRFAFSLETSATTAYLQINNLKNEDTSTYFCARLRGIRPGPL AYWGQGTLVTVSA (SEQ ID NO:1016)

[00630] IGHV7-81*01 V-REGION sequence

[00631] (DNA)

[00632] caggtgcagctggtgcagtctggccatgaggtgaagcagcctggggcctcagtgaaggtctcctgcaaggcttctg gttacagtttcaccacctatggtatgaattgggtgccacaggcccctggacaagggcttgagtggatgggatggttcaacacctacact gggaacccaacatatgcccagggcttcacaggacggtttgtcttctccatggacacctctgccagcacagcatacctgcagatcagca gcctaaaggctgaggacatggccatgtattactgtgcgaga (SEQ ID NO:351)

[00633] (amino acids)

[00634] QVQLVQSGHEVKQPGASVKVSCKASGYSFTTYGMNWVPQAPGQGLEW MGWFNTYTGNPTYAQGFTGRFVFSMDTSASTAYLQISSLKAEDMAMYYCAR (SEQ ID NO:1017)

[00635] IGHJ4*03 J-REGION sequence

[00636] (DNA)

[00637] tactttgactactggggccaagggaccctggtcaccgtctcctca (SEQ ID NO:352)

[00638] (amino acids)

[00639] YFDYWGQGTLVTVSS (SEQ ID NO:1018)

[00640] SEQ ID NO:1019

[00641] humanized 8F9A5A1 Heavy chain variable region sequence

[00642] (DNA)

[00643] Caggtgcagctggtgcagtctggccatgaggtgaagcagcctggggcctcagtgaaggtctcctgcaaggcttctg gtatacttcacaaactatggaatgaactgggtgccacaggcccctggacaagggcttgagtggatgggatggataaacacctaca ctggagagccaacatatgttgatgacttcaagggacggtttgtcttctccatggacacctctgccagcacagcatacctgcagatcagc agcctaaaggctgaggacatggccatgtattactgtgcaagattgaggggatacgaccgggtcccttggcttactggggccaaggg accctggtcaccgtctcctca (SEQ ID NO:353)

[00644] (amino acids)

[00645] QVQLVQSGHEVKQPGASVKVSCKASGYTFTNYGMNWVPQAPGQGLEW MGWINTYTGEPTYVDDFKGRFVFSMDTSASTAYLQISSLKAEDMAMYYCARLRGIR PGPLAYWGQGTLVTVSS (SEQ ID NO:1019)

[00646] humanized 8F9A5A1 Heavy chain variable region sequence (codon optimized)

[00647] (DNA)

[00648] caggttcagctggtgcagtctggccacgaagtgaaacagcctggcgcctctgtgaaggtgtcctgtaaagccagcg gctacacctttaccaactacggcatgaactgggtgccccaggctcctggacaaggcttggaatggatgggctggatcaacacctacac cggcgagcctacctacgtggacgacttcaagggcagattcgtgttcagcatggacaccagcgccagcacagcctacctgcagatca gctctctgaaggccgaggatatggccatgtactactgcgccagactgagaggcatcagacctggacctctggcctattggggacagg gcacactggtcacagtgtcctct (SEQ ID NO:354)

[00649] (amino acids)

[00650] QVQLVQSGHEVKQPGASVKVSCKASGYTFTNYGMNWVPQAPGQGLEW MGWINTYTGEPTYVDDFKGRFVFSMDTSASTAYLQISSLKAEDMAMYYCARLRGIR PGPLAYWGQGTLVTVSS (SEQ ID NO:1020)

[00651] Modified humanized 8F9A5A1 Heavy chain variable region sequence

[00652] (DNA)

[00653] cagatccagctggtgcagtctggccccgaggtgaagcagcctggggcctcagtgaaggtctcctgcaaggcttctg ggtataccttcacaaactatggaatgaactgggtgaagcaggcccctggacaagggcttgagtggatgggatggataaacacctaca ctggagagccaacatatgttgatgacttcaagggacggtttgccttctccatggacacctctgccagcacagcatacctgcagatcagc agcctaaaggctgaggacaccgccacctattactgtgcaagattgaggggatacgaccgggtcccttggcttactggggccaaggg accctggtcaccgtctcctca (SEQ ID NO:355)

[00654] (amino acids)

[00655] QIQLVQSGPEVKQPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLEWM GWINTYTGEPTYVDDFKGRFAFSMDTSASTAYLQISSLKAEDTATYYCARLRGIRPG PLAYWGQGTLVTVSS (SEQ ID NO:1021)

[00656] Modified humanized 8F9A5A1 Heavy chain variable region sequence (codon optimized)

[00657] (DNA)

[00658] cagattcagctggtgcagtctggccccgaagtgaaacaacctggcgcctctgtgaaggtgtcctgcaaggccagcg gctacacctttaccaactacggcatgaactgggtcaagcaggcccctggacaaggcctggaatggatgggctggatcaacacctaca ccggcgagcctacctacgtggacgacttcaagggcagattcgccttcagcatggacaccagcgccagcacagcctacctgcagatc agctctctgaaggccgaggacaccgccacctactactgtgccagactgagaggcatcagacccggacctctggcctattggggaca gggaacactggtcaccgtgtcctct (SEQ ID NO:356)

[00659] (amino acids)

[00660] QIQLVQSGPEVKQPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLEWM GWINTYTGEPTYVDDFKGRFAFSMDTSASTAYLQISSLKAEDTATYYCARLRGIRPG PLAYWGQGTLVTVSS (SEQ ID NO:1022)

[00661] 8F9A5A1 Light chain variable region sequence

[00662] (DNA)

[00663] gaaattttgctcacccagtctccagcaatcatagctgcatctcctggggagaaggtcaccatcacctgcagtgccagct caagtgtaagttacatgaactggtaccagcagaaaccaggatcctccccaaaatatggatttatggtatatccaacctggcttctggag ttcctgctcgcttcagtggcagtgggtctgggacatctttctctttcacaatcaacagcatggaggctgaagatgttgccacttattactgtc agcaaaggagtagttacccacccacgttcggaggggggaccaagctggaaataaaacgg (SEQ ID NO:357)

[00664] (amino acids)

[00665] EILLTQSPAIIAASPGEKVTITCSASSSVSYMNWYQQKPGSSPKIWIYGISNL ASGVPARFSGSGSGTSFSFTINSMEAEDVATYYCQQRSSYPPTFGGGTKLEIKR (SEQ ID NO:1023)

[00666] IGKV3D-15*02 V-REGION sequence

[00667] (DNA)

[00668] gaaatagtgatgatgcagtctccagccaccctgtctgtgtctccaggggaaagagccaccctctcctgcagggccag tcagagtgttagcagcaacttagcctggtaccagcagaaacctggccaggctcccaggctcctcatctatggtgcatccaccagggcc actggcatcccagccaggttcagtggcagtgggtctgggacagagttcactctcaccatcagcagcctgcagtctgaagattttgcagt ttattactgtcagcagtataataac (SEQ ID NO:358)

[00669] (amino acids)

[00670] EIVMMQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYG ASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNN (SEQ ID NO:1024)

[00671] IGKJ4*02 J-REGION sequence

[00672] (DNA)

[00673] ctcacgttcggcggagggaccaaggtggagatcaaa (SEQ ID NO:359)

[00674] (amino acids)

[00675] LTFGGGTKVEIK (SEQ ID NO:1025)

[00676] humanized 8F9A5A1 Light chain variable region sequence

[00677] (DNA)

[00678] gaaatagtgatgatgcagtctccagccaccctgtctgtgtctccaggggaaagagccaccctctcctgcagtgccagc tcaagtgtaagttacatgaactggtaccagcagaaacctggccaggctcccaggctcctcatctatggtatatccaacctggcttctggc atcccagccaggttcagtggcagtgggtctgggacagagttcactctcaccatcagcagcctgcagtctgaagattttgcagtttattact gtcagcaaaggagtagttacccacccacgttcggcggagggaccaaggtggagatcaaacgg (SEQ ID NO:360)

[00679] (amino acids)

[00680] EIVMMQSPATLSVSPGERATLSCSASSSVSYMNWYQQKPGQAPRLLIYGIS NLASGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQRSSYPPTFGGGTKVEIKR (SEQ ID NO:1026)

[00681] humanized 8F9A5A1 Light chain variable region sequence (codon optimized)

[00682] (DNA)

[00683] gagatcgtgatgatgcagagccccgccacactgagtgtgtctccaggcgaaagagccacactgtcctgtagcgcca gcagcagcgtgtcctacatgaactggtatcagcagaagcccggacaggcccctagactgctgatctacggcatcagcaatctggcca gcggcatccctgccagattttctggctctggctccggcaccgagttcaccctgacaatctctagcctgcagagcgaggacttcgccgtg tactactgccagcagagaagcagctaccctcctacctttggcggaggcaccaaggtggaaatcaagcgg (SEQ ID NO:361)

[00684] (amino acids)

[00685] EIVMMQSPATLSVSPGERATLSCSASSSVSYMNWYQQKPGQAPRLLIYGIS NLASGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQRSSYPPTFGGGTKVEIKR (SEQ ID NO:1027)

[00686] Modified humanized 8F9A5A1 Light chain variable region sequence

[00687] (DNA)

[00688] gaaatagtgctgacccagtctccagccaccctgtctgtgtctccaggggaaagagccaccctctcctgcagtgccag ctcaagtgtaagttacatgaactggtaccagcagaaacctggccaggctcccaggctctggatctatggtatatccaacctggcttctgg catcccagccaggttcagtggcagtgggtctgggacaagcttcagcctcaccatcagcagcctgcagtctgaagattttgcagtttatta ctgtcagcaaaggagtagttacccacccacgttcggcggagggaccaaggtggagatcaaacgg (SEQ ID NO:362)

[00689] (amino acids)

[00690] EIVLTQSPATLSVSPGERATLSCSASSSVSYMNWYQQKPGQAPRLWIYGIS NLASGIPARFSGSGSGTSFSLTISSLQSEDFAVYYCQQRSSYPPTFGGGTKVEIKR (SEQ ID NO:1028)

[00691] Modified humanized 8F9A5A1 Light chain variable region sequence (codon optimized)

[00692] (DNA)

[00693] gagatcgtgctgacacagtctcccgccacactgagtgtgtctccaggcgaaagagccacactgtcctgtagcgccag cagcagcgtgtcctacatgaactggtatcagcagaagcccggacaggcccctagactgtggatctacggcatcagcaatctggccag cggcatccctgccagattttctggctctggctccggcaccagcttcagcctgacaatcagcagcctgcagagcgaggacttcgccgtg tactactgccagcagagaagcagctaccctcctacctttggcggaggcaccaaggtggaaatcaagcgg (SEQ ID NO:363)

[00694] (amino acids)

[00695] EIVLTQSPATLSVSPGERATLSCSASSSVSYMNWYQQKPGQAPRLWIYGIS NLASGIPARFSGSGSGTSFSLTISSLQSEDFAVYYCQQRSSYPPTFGGGTKVEIKR (SEQ ID NO:1029)

[00696] Modified humanized 8F9A5A1 scFV sequence (codon optimized)

[00697] (DNA)

[00698] Cagattcagctggtgcagtctggccccgaagtgaaacaacctggcgcctctgtgaaggtgtcctgcaaggccagcg gctacacctttaccaactacggcatgaactgggtcaagcaggcccctggacaaggcctggaatggatgggctggatcaacacctaca ccggcgagcctacctacgtggacgacttcaagggcagattcgccttcagcatggacaccagcgccagcacagcctacctgcagatc agctctctgaaggccgaggacaccgccacctactactgtgccagactgagaggcatcagacccggacctctggcctattggggaca gggaacactggtcaccgtgtcctctggcggtggcggaagcggaggcggtggctccggtggcggaggcagcgagatcgtgctgac acagtctcccgccacactgagtgtgtctccaggcgaaagagccacactgtcctgtagcgccagcagcagcgtgtcctacatgaactg gtatcagcagaagcccggacaggcccctagactgtggatctacggcatcagcaatctggccagcggcatccctgccagattttctggc tctggctccggcaccagcttcagcctgacaatcagcagcctgcagagcgaggacttcgccgtgtactactgccagcagagaagcag ctaccctcctacctttggcggaggcaccaaggtggaaatcaagcgg (SEQ ID NO:364)

[00699] (amino acids)

[00700] QIQLVQSGPEVKQPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLEWM GWINTYTGEPTYVDDFKGRFAFSMDTSASTAYLQISSLKAEDTATYYCARLRGIRPG PLAYWGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSVSPGERATLSCSASS SVSYMNWYQQKPGQAPRLWIYGISNLASGIPARFSGSGSGTSFSLTISSLQSEDFAVY YCQQRSSYPPTFGGGTKVEIKR (SEQ ID NO:1030)

[00701] 8H5H5G4 Heavy chain variable region sequence

[00702] (DNA)

[00703] gtccagctgcaacagtctggacctgatctggtgaagcctgggacttcagtgaagatatcctgtaagacttctggaaaca cattcactgaatacaccatgcactgggtgaagcagagccatggaaagagccttgagtggattggaggttttaatcctaacaatggtgtta ctaactacaaccagaagttcaagggcaaggccacattgactgtagacaagtcctccagcacagcctacatggagctccgcagcctga catctgaggattctgcagtctattactgtgcaagacgttactaccatagtacctacgtgttctactttgactcctggggccaaggcaccact ctcacagtctcctca (SEQ ID NO:365)

[00704] (amino acids)

[00705] VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGF NPNNGVTNYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVF YFDSWGQGTTLTVSS (SEQ ID NO:1031)

[00706] IGHV1-24*01 V-REGION sequence

[00707] (DNA)

[00708] caggtccagctggtacagtctggggctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggtttccg gatacaccctcactgaattatccatgcactgggtgcgacaggctcctggaaaagggcttgagtggatgggaggttttgatcctgaagat ggtgaaacaatctacgcacagaagttccagggcagagtcaccatgaccgaggacacatctacagacacagcctacatggagctgag cagcctgagatctgaggacacggccgtgtattactgtgcaaca (SEQ ID NO:366)

[00709] (amino acids)

[00710] QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEW MGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCAT (SEQ ID NO:1032)

[00711] IGHJ4*03 J-REGION sequence

[00712] (DNA)

[00713] tactttgactactggggccaagggaccctggtcaccgtctcctca (SEQ ID NO:367)

[00714] (amino acids)

[00715] YFDYWGQGTLVTVSS (SEQ ID NO:1033)

[00716] Humanized 8H5H5G4 Heavy chain variable region sequence

[00717] (DNA)

[00718] caggtccagctggtacagtctggggctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggtttccg gaaacacattcactgaatacaccatgcacTgggtgcgacaggctcctggaaaagggcttgagtggatgggaggttttaatcctaacaa tggtgttactaactacaaccagaagttcaagggcAgagtcaccatgaccgaggacacatctacagacacagcctacatggagctgag cagcctgagatctgaggacacggccgtgtattactgtGcaagacgttactaccatagtacctacgtgttctactttgactcctggggcca agggaccctggtcaccgtctcctca (SEQ ID NO:368)

[00719] (amino acids)

[00720] QVQLVQSGAEVKKPGASVKVSCKVSGNTFTEYTMHWVRQAPGKGLEW MGGFNPNNGVTNYNQKFKGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCARRYYH STYVFYFDSWGQGTLVTVSS (SEQ ID NO:1034)

[00721] Humanized 8H5H5G4 Heavy chain variable region sequence (codon optimized)

[00722] (DNA)

[00723] caggttcagctggttcagtctggcgccgaagtgaagaaacctggcgcctctgtgaaggtgtcctgcaaggtgtccgg aaataccttcaccgagtacaccatgcactgggtccgacaggcccctggcaaaggacttgaatggatgggcggcttcaaccccaacaa cggcgtgaccaactacaaccagaaattcaagggccgcgtgaccatgaccgaggacacaagcacagacaccgcctacatggaactg agcagcctgagaagcgaggacaccgccgtgtactactgcgccagaaggtactaccacagcacctacgtgttctacttcgacagctgg ggccagggcacactggtcacagtttcttct (SEQ ID NO:369)

[00724] (amino acids)

[00725] QVQLVQSGAEVKKPGASVKVSCKVSGNTFTEYTMHWVRQAPGKGLEW MGGFNPNNGVTNYNQKFKGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCARRYYH STYVFYFDSWGQGTLVTVSS (SEQ ID NO:1035)

[00726] Modified Humanized 8H5H5G4 Heavy chain variable region sequence

[00727] (DNA)

[00728] caggtccagctggtacagtctggggctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggtttccg gaaacacattcactgaatacaccatgcactgggtgcgacaggctcctggaaaagggcttgagtggatcggaggttttaatcctaacaat

ggtgttactaactacaaccagaagttcaagggcaaggtcaccctgaccgtggacacatctagcagcacagcctacatggagctgagc agcctgagatctgaggacacggccgtgtattactgtgcaagacgttactaccatagtacctacgtgttctactttgactcctggggccaa gggaccctggtcaccgtctcctca (SEQ ID NO:370)

[00729] (amino acids)

[00730] QVQLVQSGAEVKKPGASVKVSCKVSGNTFTEYTMHWVRQAPGKGLEWI GGFNPNNGVTNYNQKFKGKVTLTVDTSSSTAYMELSSLRSEDTAVYYCARRYYHST YVFYFDSWGQGTLVTVSS (SEQ ID NO:1036)

[00731] Modified Humanized 8H5H5G4 Heavy chain variable region sequence (codon optimized)

[00732] (DNA)

[00733] caggttcagctggttcagtctggcgccgaagtgaagaaacctggcgcctctgtgaaggtgtcctgcaaggtgtccgg aaataccttcaccgagtacaccatgcactgggtccgacaggcccctggcaaaggactggaatggatcggcggcttcaaccccaaca acggcgtgaccaactacaaccagaaattcaagggcaaagtgaccctgaccgtggacaccagcagcagcacagcctacatggaact gagcagcctgagaagcgaggacaccgccgtgtactactgcgccagaaggtactaccacagcacctacgtgttctacttcgacagctg ggccagggcacactggtcacagtttcttct (SEQ ID NO:371)

[00734] (amino acids)

[00735] QVQLVQSGAEVKKPGASVKVSCKVSGNTFTEYTMHWVRQAPGKGLEWI GGFNPNNGVTNYNQKFKGKVTLTVDTSSSTAYMELSSLRSEDTAVYYCARRYYHST YVFYFDSWGQGTLVTVSS (SEQ ID NO:1037)

[00736] 8H5H5G4 Light chain variable region sequence

[00737] (DNA)

[00738] gatatccagatgacacagactacatcctccctgtctgcctctctgggagacagagtcaccatcagttgcagtgcaagtc agggcattagcaattatttaaactggtttcagcagaaaccagatggaactattaagctcctgatctattacacatcaagtttacattcagga gtcccatcaaggttcagtggcagtgggtctgggacagattattctctcaccatcagtaatgtggaacctgaagatattgccacttactattg tcagcagtatagtaagcttccttacacgttcggaggggggaccaagctggagataaaacgg (SEQ ID NO:372)

[00739] (amino acids)

[00740] DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTS SLHSGVPSRFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR (SEQ ID NO:1038)

[00741] IGKV1-27*01 V-REGION sequence

[00742] (DNA)

[00743] gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccgggcgagt cagggcattagcaattatttagcctggtatcagcagaaaccagggaaagttcctaagctcctgatctatgctgcatccactttgcaatcag

gggtcccatctcggttcagtggcagtggatctgggacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttatt actgtcaaaagtataacagtgcccct (SEQ ID NO:373)

[00744] (amino acids)

[00745] DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAA STLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAP (SEQ ID NO:1039)

[00746] IGKJ4*02 J-REGION sequence

[00747] (DNA)

[00748] ctcacgttcggcggagggaccaaggtggagatcaaa (SEQ ID NO:374)

[00749] (amino acids)

[00750] LTFGGGTKVEIK (SEQ ID NO:1040)

[00751] humanized 8H5H5G4 Light chain variable region sequence

[00752] (DNA)

[00753] gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgcagtgcaagtc agggcattagcaattatttaaacTggtatcagcagaaaccagggaaagttcctaagctcctgatctattacacatcaagtttacattcagg ggtcccatctcggttcagtggcagtggatctgggacagatttcactctcaccatcagcagcctgcagcctgaagatgttgcaacttatta ctgtcagcagtatagtaagcttccttacacgttcggcggagggaccaaggtggagatcaaacgg (SEQ ID NO:375)

[00754] (amino acids)

[00755] DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQKPGKVPKLLIYYT SSLHSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQYSKLPYTFGGGTKVEIKR (SEQ ID NO:1041)

[00756] humanized 8H5H5G4 Light chain variable region sequence (codon optimized)

[00757] (DNA)

[00758] gacatccagatgacacagagccctagcagcctgtctgccagcgtgggagacagagtgaccatcacatgtagcgcca gccagggcatcagcaactacctgaactggtatcagcagaaacccggcaaggtgcccaagctgctgatctactacaccagcagcctg cacagcggcgtgccaagcagattttctggcagcggctctggcaccgacttcaccctgaccatatctagcctgcagcctgaggacgtg gccacctactactgtcagcagtacagcaagctgccctacacctttggcggaggcaccaaggtggaaatcaagcgg (SEQ ID NO:376)

[00759] (amino acids)

[00760] DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQKPGKVPKLLIYYT SSLHSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQYSKLPYTFGGGTKVEIKR (SEQ ID NO:1042)

[00761] Modified humanized 8H5H5G4 Light chain variable region sequence

[00762] (DNA)

[00763] gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgcagtgcaagtc agggcattagcaattatttaaactggtatcagcagaaaccagggaaagttcctaagctcctgatctattacacatcaagtttacattcagg ggtcccatctcggttcagtggcagtggatctgggacagattacactctcaccatcagcagcctgcagcctgaagatgttgcaacttatta ctgtcagcagtatagtaagcttccttacacgttcggcggagggaccaaggtggagatcaaacgg (SEQ ID NO:377)

[00764] (amino acids)

[00765] DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQKPGKVPKLLIYYT SSLHSGVPSRFSGSGSGTDYTLTISSLQPEDVATYYCQQYSKLPYTFGGGTKVEIKR (SEQ ID NO:1043)

[00766] Modified humanized 8H5H5G4 Light chain variable region sequence (codon optimized)

[00767] (DNA)

[00768] gacatccagatgacacagagccctagcagcctgtctgccagcgtgggagacagagtgaccatcacatgtagcgcca gccagggcatcagcaactacctgaactggtatcagcagaaacccggcaaggtgcccaagctgctgatctactacaccagcagcctg cacagcggcgtgccaagcagattttctggcagcggctctggcaccgactacaccctgaccatatctagcctgcagcctgaggacgtg gccacctactactgtcagcagtacagcaagctgccctacacctttggcggaggcaccaaggtggaaatcaagcgg (SEQ ID NO:378)

[00769] (amino acids)

[00770] DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQKPGKVPKLLIYYT SSLHSGVPSRFSGSGSGTDYTLTISSLQPEDVATYYCQQYSKLPYTFGGGTKVEIKR (SEQ ID NO:1044)

[00771] Modified humanized 8H5H5G4 scFV sequence (codon optimized)

[00772] (DNA)

[00773] Caggttcagctggttcagtctggcgccgaagtgaagaaacctggcgcctctgtgaaggtgtcctgcaaggtgtccgg aaataccttcaccgagtacaccatgcactgggtccgacaggcccctggcaaaggactggaatggatcggcggcttcaaccccaaca acggcgtgaccaactacaaccagaaattcaagggcaaagtgaccctgaccgtggacaccagcagcagcacagcctacatggaact gagcagcctgagaagcgaggacaccgccgtgtactactgcgccagaaggtactaccacagcacctacgtgttctacttcgacagctg ggccagggcacactggtcacagtttcttctggcggtggcggaagcggaggcggtggctccggtggcggaggcagcgacatcca gatgacacagagccctagcagcctgtctgccagcgtgggagacagagtgaccatcacatgtagcgccagccagggcatcagcaact acctgaactggtatcagcagaaacccggcaaggtgcccaagctgctgatctactacaccagcagcctgcacagcggcgtgccaagc agattttctggcagcggctctggcaccgactacaccctgaccatatctagcctgcagcctgaggacgtggccacctactactgtcagca gtacagcaagctgccctacacctttggcggaggcaccaaggtggaaatcaagcgg (SEQ ID NO:379)

[00774] (amino acids)

[00775] QVQLVQSGAEVKKPGASVKVSCKVSGNTFTEYTMHWVRQAPGKGLEWI GGFNPNNGVTNYNQKFKGKVTLTVDTSSSTAYMELSSLRSEDTAVYYCARRYYHST

YVFYFDSWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCS ASQGISNYLNWYQQKPGKVPKLLIYYTSSLHSGVPSRFSGSGSGTDYTLTISSLQPED VATYYCQQYSKLPYTFGGGTKVEIKR (SEQ ID NO:1045)

[00776] Human IgG1 heavy chain constant region sequence: (for making full antibody - pair with either kappa or lambda constant region; 2 plasmids, express together)

[00777] (DNA)

[00778] gctagcaccaagggcccatcggtcttcccctggcaccctcctccaagagcacctctgggggcacagcggccctgg gctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccg gctgtcctacagtcctcaggactctactccctcagcagcgtggtgacagtgccctccagcagcttgggcacccagacctacatctgca acgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcacacatgcccaccgtgcc cagcacctgaactcctgggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggt cacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcca agacaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatgg caaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccga gaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctat cccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgagg ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga (SEQ ID NO:380)

[00779] (amino acids)

[00780] ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:1046)

[00781] Human IgG2 heavy chain constant region sequence: (for making full antibody – pair with either kappa or lambda constant region; 2 plasmids, express together)

[00782] (DNA)

[00783] gcctccaccaagggcccatcggtcttcccctggcgccctgctccaggagcacctccgagagcacagccgccctg ggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgctctgaccagcggcgtgcacaccttccca gctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcaacttcggcacccagacctacacctgca acgtagatcacaagcccagcaacaccaaggtggacaagacagttgagcgcaaatgttgtgtcgagtgcccaccgtgcccagcacca cctgtggcaggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacgtgcgtgg

tggtggacgtgagccacgaagaccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagcc acgggaggagcagttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtaca agtgcaaggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaaccacaggt gtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcgaca tcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacacctcccatgctggactccgacggctccttcttc ctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac cactacacgcagaagagcctctccctgtctccgggtaaatag (SEQ ID NO:381)

[00784] (amino acids)

[00785] ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPP CPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:1047)

[00786] Human Kappa light chain constant region sequence:

[00787] (DNA)

[00788] aggacggtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtg cctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagtgtc acagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtc tacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag (SEQ ID NO:382)

[00789] (amino acids)

[00790] RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:1048)

[00791] Human Lambda light chain constant region sequence:

[00792] (DNA)

[00793] ggtcagcccaaggctgcccccctcggtcactctgttcccgccctcctctgaggagcttcaagccaacaaggccacact ggtgtgtctcataagtgacttctacccgggagccgtgacagtggcctggaaggcagatagcagccccgtcaaggcgggagtggaga ccaccacaccctccaaacaaagcaacaacaagtacgcggccagcagctatctgagcctgacgcctgagcagtggaagtcccacag aagctacagctgccaggtcacgcatgaagggagcaccgtggagaagacagtggcccctacagaatgttcatag (SEQ ID NO:383)

[00794] (amino acids)

[00795] GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:1049)

[00796] Human IgG1 Fc region sequence: (to be fused to scFv for homo-dimerizes)

[00797] (DNA)

[00798] gagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggaccgtcagtctt cctcttccccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaaga ccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagc acgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagcc ctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccggg aggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaat gggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggac aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctcc ctgtctccgggtaaatga (SEQ ID NO:384)

[00799] (amino acids)

[00800] EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK* (SEQ ID NO:1050)

[00801] Human IgG2 Fc region sequence:

[00802] (DNA)

[00803] gagcgcaaatgttgtgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttcctcttcccccca aaacccaaggacaccctcatgatctcccggaccccctgaggtcacgtgcgtggtggtggacgtgagccacgaagaccccgaggtcca gttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcagttcaacagcacgttccgtgtgg tcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaacaaaggcctcccagccccc atcgagaaaaccatctccaaaaccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgacca agaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggcagccgga gaacaactacaagaccacacctcccatgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtg gcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggt aaatag (SEQ ID NO:385)

[00804] (amino acids)

[00805] ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSN

KGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK* (SEQ ID NO:1051)

[00806] In another aspect of the invention, an immune cell engineered to express a CAR is administered to a patient diagnosed with or at risk of developing a cancer or cancer metastasis, wherein the immune cell is also engineered to express an anti-NME7 antibody or antibody fragment, which may be expressed off of an inducible promoter. In one aspect, the CAR is guided by an anti-MUC1* antibody fragment. In one case, the CAR is huMNC2-CAR44. In one aspect, the anti-NME7 antibody or antibody fragment binds to an NME peptide listed under sections "**Homologous peptides to A1, A2, B1, B2 or B3 peptides**" and the "**Homologous extended peptides to A1, A2, B1, B2 or B3 peptides**" above. In another aspect, the antibody or antibody fragment binds to an NME7 derived peptide chosen from among A1, A2, B1, B2 or B3 (SEQ ID NOS: 141-145). In yet another aspect, the antibody, antibody fragment or antibody mimic binds to an NME7 peptide comprising the B3 peptide. In one aspect of the invention, the anti-NME7 antibody, antibody fragment or antibody mimic comprises sequences derived from the variable domains of anti-NME7 antibodies 8F9A4A3 (SEQ ID NOS:1001 – 1015), 8F9A5A1 (SEQ ID NOS:1016 – 1030), or 8H5H5G4 (SEQ ID NOS:1031– 1045).

[00807] Such antibodies may be human or humanized. Such antibodies may be polyclonal, monoclonal, bispecific, bivalent, monovalent, single chain, scFv, or may be an antibody mimic such as protein scaffolds that present recognition regions that bind to a specific target. As is appreciated by those skilled in the art, antibodies can be of non-human origin, human or humanized. Methods for humanizing antibodies include fusing all or some of the mouse variable regions to V- and J- regions of a closest match human antibody sequence, for example, as shown in sequences listed as SEQ ID NOS:1001-1045. Full antibodies, rather than single chain constructs, can also be made. For example, the heavy chain variable mouse sequence is fused to human V- and J- regions then fused to the human heavy chain constant regions of IgG1, IgG2 or IgG3. Similarly, the light chain variable mouse sequences are fused to human V- and J- regions then fused to either the human Kappa or Lambda constant regions of IgG1, IgG2 or IgG3. Plasmids are expressed together and associate to form the full antibody (SEQ ID NOS:1047-1051).

[00808] In another aspect of the invention, small molecules are anti-cancer agents that are selected for their ability to inhibit the tumorigenic effects of NME7, $NME7_{AB}$ or NME7-X1. For example, a high throughput screen identifies small molecules that will treat cancer. In a multi-well plate, small molecules are separately added to wells in which cancer cells are

cultured in a medium containing $NME7_{AB}$. If the small molecule diminishes the amount of cells that become floaters and/or reduces the expression of metastatic markers such as CXCR4, CHD1 or pluripotent stem cell markers, then that small molecule is an anti-cancer drug candidate. Another method of identifying small molecules that are anti-cancer agents is to select those small molecules that bind to NME7, $NME7_{AB}$ or NME7-X1 or suppresses expression of the NME7 species. Yet another high throughput screen is to select for small molecules that inhibit the binding of $NME7_{AB}$ to the PSMGFR peptide of the MUC1* extracellular domain and those small molecules will be anti-cancer agents.

[00809] The sequences of $NME7_{AB}$ and NME7-X1 differ only in that NME7-X1 is missing some of the N-terminal sequence that $NME7_{AB}$ has. Experiments show that there is a naturally occurring NME7 species that is nearly identical to $NME7_{AB}$, which we call $NME7_{AB}$ -like species. Antibodies that bind to NME7-X1 may also bind to the naturally occurring species that mimics $NME7_{AB}$, unless there are conformational differences that an antibody can differentiate. Therefore, if it is desired to inhibit NME7-X1 but not $NME7_{AB}$ -like species, or vice versa, siRNA, anti-sense nucleic acids, or genetic editing techniques can be used to inhibit expression of one but not the other.

[00810] In one case, the anti-cancer therapeutic agent is a nucleic acid that directly or indirectly suppresses specific expression of NME7, NME7-X1 or $NME7_{AB}$ -like species. Such nucleic acids can be siRNA, RNAi, anti-sense nucleic acids and the like that directly suppress the NME7 species. In another aspect of the invention, the nucleic acid can indirectly suppress the NME7 species for example by altering the expression of a molecule that regulates it. For example, the super enhancer BRD4 suppresses expression of NME7. Therefore, an effective therapeutic for the treatment or prevention of cancer is an agent that increases expression of BRD4. An effective therapeutic may be an agent that increases expression of BRD4's co-factor, JMJD6.

[00811] Peptides derived from $NME7_{AB}$ or NME7-X1, or the entire protein, are used to generate anti-NME7 or anti-NME7-X1 antibodies in animals that we have demonstrated inhibit cancer growth and inhibit transition of cancer cells to metastatic cancer cells. Similarly, NME7 derived peptides can be administered to a human such that they generate antibodies that treat or prevent cancer or inhibit transition of cancer cells to metastatic cancer cells. NME7 peptides or proteins are administered to a person as a type of vaccine to stimulate the production of anti-NME7, anti- $NME7_{AB}$ or anti-NME7-X1 antibodies in the recipient. The results shown in Figure 12 and Figure 13 indicate that immunizing a person with a collection of peptides derived from NME7, especially in the NME7-X1 or $NME7_{AB}$ sequences may be a more effective

vaccine than immunizing with a single peptide. Said peptides or proteins may further be conjugated to a carrier protein or other adjuvant, known to those skilled in the art to aid in the stimulation of an immune response.

[00812] NME7 peptides that lie outside of the DM10 domain are preferred to generate antibodies for the treatment or prevention of cancer. Peptides that can be administered to a patient for the prevention of cancer or metastasis contain sequences of the peptides listed in Figure 6 – Figure 9. A1, A2, B1, B2 and B3 are examples of peptides that generate antibodies that bind to $NME7_{AB}$ and NME7-X1 and are administered to a patient for the treatment or prevention of cancer. The invention is not limited to peptides of the exact sequence as is naturally occurring in NME7 or NME7-X1. As is known to those skilled in the art, substitution of several amino acids of a peptide sequence can still give rise to antibodies that specifically recognize the natural protein sequence. It is not intended that the invention be limited to the peptides demonstrated herein to inhibit cancer growth or inhibit the transition of regular cancer cells to metastatic cancer cells. The methods used here to identify peptides A1, A2, B1, B2 and B3 can also be used to identify other peptide sequences that could be equally or more effective than the peptides demonstrated here.

[00813] Chimeric antigen receptor molecules comprising portions of human $NME7_{AB}$ or NME7-X1 or comprising an antibody fragment that binds to $NME7_{AB}$ or NME7-X1 are anti-cancer therapeutics and are administered to a patient for the treatment or prevention of cancers or cancer metastases.

[00814] In one instance, the recognition units or variable regions of anti-NME7 antibodies are fused to molecules of T cells using the technology known as CAR (chimeric antigen receptor) technology or CAR T technology. The salient feature of antibodies or fragments thereof that can be used therapeutically to treat or prevent cancers is the identification of antibody-like variable regions that recognize NME7 and prevent its interaction with targets that promote cancers. In one case, the target is the PSMGFR region of MUC1*.

[00815] Antibodies, antibody fragments or single chain antibodies can be engineered into chimeric molecules, including chimeric antigen receptors, also known as CARs, which molecules are then transfected or transduced into an immune system cell, such as a T cell, and administered to a patient. The humanized antibodies or antibody fragments, typically an scFv, comprises much of the extracellular domain of a CAR. The antibody fragment is biochemically fused to immune system signaling molecules, such as CD8 as the transmembrane domain and cytoplasmic signaling motifs such as T cell receptor signaling molecules also called activation domains, or co-stimulatory domains including but not limited to CD3-zeta, CD28, 41bb, OX40.

CARs can be transfected into T cells or other cells, preferably immune system cells and administered to a patient. Here we describe CARs in which the extracellular portion contains an anti-NME7, anti- $NME7_{AB}$ or anti-NME7-X1 antibody, antibody fragment or single chain, scFv antibody fragment. In a preferred embodiment, the antibody or antibody fragment is human or humanized.

[00816] Effective anti-NME7 or anti-NME7-X1 antibodies or fragments will have the ability to bind to native NME7, $NME7_{AB}$ or NME7-X1. In practice, the parent antibody, from which the extracellular domain of the CAR is engineered, is generated by immunizing an animal with an NME7, $NME7_{AB}$ or NME7-X1 derived peptide. In one aspect of the invention, the immunizing peptide is comprised of NME7 amino acids 1-376. In one aspect of the invention, the immunizing peptide is comprised of NME7 amino acids 92-376. In another aspect of the invention, the immunizing peptide is comprised of NME7 amino acids 125-376. In yet another aspect of the invention, the immunizing peptide is made up of sequences listed in Figure 6 – Figure8. In another aspect of the invention, the immunizing peptide is made up of sequences listed in Figure 9. Alternatively, the parent antibody or the antibody fragment is selected from a library or pool of antibodies, which may be natural, synthetic or fragments of either, wherein they are selected for their ability to bind to NME7, $NME7_{AB}$ or NME7-X1, peptides listed in Figure 6 - Figures 8, or peptides listed in Figure 9.

[00817] The targeting portion of a CAR need not be an antibody or antibody fragment. Here we describe a CAR wherein the extracellular domain contains an NME7 fragment. NME7-derived peptide(s) are engineered into a different sort of CAR wherein the targeting portion of the extracellular domain is a protein fragment or peptide rather than an antibody or antibody fragment. The peptide CARs are transfected or transduced into an immune system cell, typically a T cell. The NME7 fragments or NME7 derived peptides are selected for their ability to bind to their cognate binding partners but should not be able to function as intact NME7, $NME7_{AB}$ or NME7-X1 and confer tumorigenic activity. NME7 fragments or NME7 derived peptides are biochemically fused to immune system signaling molecules, such as CD8 as the transmembrane domain and cytoplasmic signaling motifs such as T cell receptor signaling molecules also called activation domains, or co-stimulatory domains including but not limited to CD3-zeta, CD28, 41bb, OX40.

[00818] In one aspect of the invention, the NME7 fragment is most or all of the NME7 NDPK B domain. In another aspect of the invention, the NME7 fragment is an NME7 peptide that contains one or more of the peptide sequences listed in Figure 6 – Figure 9. Experiments indicate that, for strategies that use NME7 or fragments of NME7, $NME7_{AB}$, or NME7-X1 as

the targeting portion of a chimeric antigen receptor (CAR) for engineered immune cell therapeutics, fairly large fragments of $NME7_{AB}$ or NME7-X1 would be more effective than shorter peptides, for example peptides less than 15 amino acids in length. Alternatively, a collection of CARs, each bearing a different $NME7_{AB}$ derived peptide can collectively be transfected or transduced into an immune system cell and administered to a patient for the treatment or prevention of cancers. Experiments shown in Figure 12-Figure 13 support the validity of this approach.

[00819] CARs that contain an NME7 fragment in its extracellular domain are transfected or transduced into an immune system cell, typically a T cell, and administered to a patient for the treatment or prevention of cancers. In one aspect, the cancer is a MUC1*-positive cancer. In another aspect, the cancer is a metastatic cancer.

[00820] Agents that inhibit an enzyme that cleaves NME7 can be used to treat or prevent cancers. Some forms of NME7 are sequestered within the cell and therefore are not secreted from the cell whereupon they can act as growth factors to promote cancers. Full-length NME7 is 42kDa. However, we found that a ~33kDa NME7 species that is devoid of the DM10 domain and appears to be essentially identical to the recombinant $NME7_{AB}$ that we generated, is secreted from cancer cells and stem cells. This ~33 kDa NME7 species and another ~25kDa NME7 species may be cleavage products that would be eliminated by an agent that inhibited cleavage of NME7.

[00821] The detection of elevated levels of NME7, or an ~33kDa NME7 species, which we call $NME7_{AB}$ -like species, or NME7-X1 in a patient sample is diagnostic of the presence of cancer or its progression to a more aggressive or metastatic state. The inventors have discovered that both early stage, naïve stem cells and cancer cells, especially MUC1*-positive cancer cells, express high levels of a ~33kDa NME7 that is devoid of the DM10 domain and NME7-X1.

[00822] NME7-X1 was recently listed in a protein database as being a theoretical alternative isoform of NME7, however, it had never been detected in tissues or cells. We designed primers that differentiate NME7-X1 from NME7 by PCR. The expression levels of human NME7, NME7a, NME7b and NME7-X1 were measured by PCR in a panel of cells that included fibroblast cells, human embryonic stem cells, human iPS cells, T47D human breast cancer cells, DU145 human prostate cancer cells, PC3 human prostate cancer cells, HEK295 human fetal liver cells, and other human stem cell lines. NME7 is expressed at higher levels in cancer cells than in stem cells. Particularly, NME7-X1 is expressed 10-fold higher in prostate cancer cells and 3-fold higher in breast cancer cells, than it is in fibroblast cells or stem cells. NME7-

X1 is expressed ~5-fold higher in HEK293 fetal liver cells than it is in fibroblast cells or stem cells and therefore predicts that NME7-X1 is elevated in liver cancers. NME7b is expressed 17-25-times higher in prostate cancer cells than in stem cells.

[00823] Detection of elevated levels of NME7 species in a patient sample will be indicators that the patient has a cancer or is at risk of developing a cancer. Levels of NME7 species levels can be measured or assessed by PCR, hybridization schemes, cycling probe technologies, FISH, immunocytochemistry, IHC, Western blot, immunoprecipitation, sandwich assays, ELISA assays and the like. The patient sample may be a fluid sample, a blood sample, milk, urine, cells, liquid biopsy, biopsy and the like. In a patient diagnosed with cancer, elevated levels of NME7 species are indicators of increased metastatic potential. Elevated levels of NME7-X1 are indicators of prostate cancer. Antibodies of the invention are used to detect and distinguish NME7 species and are used as a diagnostic tool.

[00824] Because adult cells and tissues do not express significant levels of NME7 or secrete NME7, an effective way to diagnose cancer or to diagnose a more aggressive or metastatic form, or a shift to a more aggressive form, is to measure levels of NME7 in a sample from a patient, from a collection of cells or tissues or from cultured cells, compared to NME7 levels in a healthy sample or compared to levels of NME7 known to exist in healthy adult cells or tissues. Increased levels of NME7 indicate the presence of cancer, the presence of a metastatic cancer or the onset of metastasis. Increased levels of NME7 is also indicative of a MUC1*-positive cancer. The sample assayed for the presence of NME7 may be a collection of cells that may be cultured cell lines or cells from a patient, a bodily fluid, a blood sample, a tissue specimen, or a biopsy specimen. Therefore, a diagnostic assay that will detect the presence of cancer or the progression of cancer, comprises the steps of: 1) obtaining a sample from a patient having cancer or at risk of developing a cancer; 2) subjecting that sample to an assay capable of detecting or measuring levels of NME7, or levels of nucleic acids encoding NME7; 3) comparing levels of the measured NME7 protein or NME7-encoding nucleic acids in the test sample to levels in control patients or control cells; 4) determining that the levels of NME7 or nucleic acids encoding NME7 are elevated compared to the controls; and 5) concluding that the donor of the test sample has cancer or has had a progression of cancer if the control to which the test was compared came from a donor previously diagnosed with a cancer.

[00825] In this assay, the control sample to which the test sample is compared can be non-cancerous cells, cultured cells, a sample from a healthy donor, a non-cancerous sample from the donor, or a sample from the donor of the test sample wherein the control sample was taken from the donor at a previous point in time. The source of such samples may be any specimen

taken from the patient being tested for the presence or progression of cancer, including bodily fluids, cerebrospinal fluid, bone marrow samples, blood, tissues, cells, biopsy tissues or cells, cultured cells derived from a patient's cells and the like. The source of the sample to which the test sample is compared can be bodily fluids, cerebrospinal fluid, bone marrow samples, blood, tissues, cells, biopsy tissues or cells, or cultured cells that may be derived from a healthy donor or the test patient wherein the samples were taken at a previous point in time. The measured levels to which the test sample is compared may be from previously recorded data and compiled into lists for comparison to test samples.

[00826] Theranostics

[00827] Patients diagnosed with elevated levels of NME7 protein or nucleic acids encoding NME7 are then treated with therapeutic agents that suppress expression of NME7, inhibit cleavage of NME7 or inhibit NME7 binding to its targets, wherein such interaction promotes cancers. An important target of NME7 or a cleavage product of NME7, is MUC1*. NME7 binds to and dimerizes the extracellular domain of MUC1*. Therefore, patients diagnosed with elevated levels of NME7 will benefit from treatment with therapeutic agents that inhibit NME7 and/or therapeutic agents that inhibit the dimerization of a cleaved form of MUC1, whose extracellular domain is comprised of some or all of the PSMGFR sequence. Thus assessing suitability of cancer treatments and administration of an effective amount of a therapeutic for the treatment or prevention of cancers would consists of the steps of: 1) obtaining a sample from a patient suspected of having a cancer or at risk of developing a cancer or at risk of developing a metastatic cancer; 2) measuring an amount of NME7 or a cleavage product thereof or an NME7 encoding nucleic acid wherein the measured levels are significantly above those measured in a control sample; 3) determining that the patient has a cancer or has developed a more aggressive or a metastatic cancer; 4) administering to the patient an effective amount of a therapeutic agent that suppresses expression of NME7, inhibits cleavage of NME7 or inhibits NME7 binding to its targets and/or administering to the patient an effective amount of a therapeutic agent that suppresses expression of MUC1, inhibits cleavage of MUC1 to MUC1* or inhibits MUC1* binding to its targets. In a preferred embodiment, the therapeutic agent that inhibits NME7 binding to its targets, inhibits its interaction with MUC1*. In a more preferred embodiments, it inhibits its interaction with the extracellular domain of MUC1* comprised essentially of the PSMGFR sequence. In a preferred embodiment, the therapeutic agent that inhibits MUC1* binding to its targets, inhibits the interaction between MUC1* and NME7. In a more preferred embodiment, the therapeutic agent that inhibits the interaction between

MUC1* and NME7 inhibits the binding of MUC1* to the portion of NME7 that is comprised essentially of the sequence of $NME7_{AB}$.

[00828] Chemically modified peptides

[00829] Polypeptide or antibody therapeutics may suffer from short circulating half-life, and proteolytic degradation and low solubility. To improve the pharmacokinetics and pharmacodynamics properties of the inventive biopharmaceuticals, methods such as manipulation of the amino acid sequence may be made to decrease or increase immunogenicity and decrease proteolytic cleavage; fusion or conjugation of the peptides to immunoglobulins and serum proteins, such as albumin may be made; incorporation into drug delivery vehicles for the biopharmaceuticals such as the inventive peptides and antibodies for protection and slow release may also be made; and conjugating to natural or synthetic polymers are also contemplated. In particular, for synthetic polymer conjugation, pegylation or acylation, such as N-acylation, S-acylation and so forth are also contemplated.

[00830] Nucleic Acid Constructs

[00831] Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the polypeptide. The suitable host cell may be a bacterial cell such as *E. coli*, a yeast cell, such as *Pichia pastoris*, an insect cell, such as *Spodoptera frugiperda*, or a mammalian cell, such as a COS, HEK or CHO cell.

[00832] The present invention also provides for methods of producing the polypeptides of the invention by growing cells of the host-vector system described herein, under conditions permitting production of the polypeptide and recovering the polypeptide so produced. The polypeptides useful for practicing the present invention may be prepared by expression in a prokaryotic or eukaryotic expression system.

[00833] The recombinant gene may be expressed and the polypeptide purified utilizing any number of methods. The gene may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pZErO.

[00834] The polypeptides may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, any number of purification methods may be used, including

but not limited to conventional ion exchange chromatography, affinity chromatography, different sugar chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration.

[00835] When used herein, polypeptide includes functionally equivalent molecules in which amino acid residues are substituted for residues within the sequence resulting in a silent or conservative change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity, which acts as a functional equivalent, resulting in a silent or conservative alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. The potential glycosylation amino acids include serine, threonine, and asparagine. Also included within the scope of the invention are proteins or fragments or derivatives thereof which exhibit the same or similar biological activity and derivatives which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc.

[00836] Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors encoding the polypeptides of the invention using appropriate transcriptional/translational control signals and protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinations (genetic recombination). Expression of nucleic acid sequence encoding the polypeptides of the invention may be regulated by a second nucleic acid sequence so that the polypeptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of the polypeptides described herein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the polypeptide include, but are not limited to the long terminal repeat as described in Squinto et al., (1991, Cell 65:1-20); the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat the promoter contained in the 3'long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:144-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase

promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25), see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), Sendai virus, lenti virus, albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58); alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

[00837] Thus, according to the invention, expression vectors capable of being replicated in a bacterial or eukaryotic host comprising nucleic acids encoding a polypeptide as described herein, are used to transfect the host and thereby direct expression of such nucleic acid to produce polypeptides which may then be recovered in biologically active form. As used herein, a biologically active form includes a form capable of binding to the relevant receptor and causing a differentiated function and/or influencing the phenotype of the cell expressing the receptor.

[00838] Expression vectors containing the nucleic acid inserts can be identified by without limitation, at least three general approaches: (a) DNA-DNA hybridization, (b) presence or

absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of foreign nucleic acids inserted in an expression vector can be detected by DNA-DNA hybridization using probes comprising sequences that are homologous to an inserted nucleic acid sequences. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign nucleic acid sequences in the vector. For example, if an *efl* nucleic acid sequence is inserted within the marker gene sequence of the vector, recombinants containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign nucleic acid product expressed by the recombinant constructs. Such assays can be based, for example, on the physical or functional properties of the nucleic acid product of interest, for example, by binding of a ligand to a receptor or portion thereof which may be tagged with, for example, a detectable antibody or portion thereof or binding to antibodies produced against the protein of interest or a portion thereof.

[00839] The polypeptide, in particular modified of the present invention, may be expressed in the host cells transiently, constitutively or permanently.

[00840] Effective doses useful for treating the diseases or disorders indicated in the present application may be determined using methods known to one skilled in the art (see, for example, Fingl, et al., The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds. Macmillan Publishing Co, New York, pp. 1-46 (1975). Pharmaceutical compositions for use according to the invention include the polypeptides described above in a pharmacologically acceptable liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation prior to administration *in vivo*. For example, the pharmaceutical composition may comprise a polypeptide in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such treatment. The administration route may be any mode of administration known in the art, including but not limited to intravenously, intrathecally, subcutaneously, intrauterinely, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

[00841] Administration may result in the distribution of the active agent of the invention throughout the body or in a localized area. For example, in some conditions, which involve

distant regions of the nervous system, intravenous or intrathecal administration of agent may be desirable. In some situations, an implant containing active agent may be placed in or near the lesioned area. Suitable implants include, but are not limited to, gelfoam, wax, spray, or microparticle-based implants.

[00842] The present invention also provides for pharmaceutical compositions comprising the polypeptides described herein, in a pharmacologically acceptable vehicle. The compositions may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, incuding, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

[00843] **Gene Therapy**

[00844] Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[00845] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[00846] For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

[00847] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

[00848] In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors, or by direct injection of naked DNA, or coating with lipids or cell-surface receptors or transfecting agents,

encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors) and so on. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor. Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

[00849] In a specific embodiment, viral vectors that contain nucleic acid sequences encoding the polypeptide are used. The nucleic acid sequences encoding the polypeptide to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. Lentiviral vectors, such as retroviral vectors, and other vectors such as adenoviral vectors and adeno-associated viruses are examples of viral vectors that may be used. Retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA.

[00850] Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia because they naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. In addition, adeno-associated virus (AAV) has also been proposed for use in gene therapy.

[00851] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[00852] In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion,

chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion and so on. Numerous techniques are known in the art for the introduction of foreign genes into cells and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[00853] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T-lymphocytes, B-lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, and so on.

[00854] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[00855] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding the polypeptide are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention.

[00856] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

[00857] Therapeutic Composition

[00858] The formulation of therapeutic compounds is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pa., USA. For example, from about 0.05 ng to about 20 mg per kilogram of body weight per day may be administered. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. The active compound may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intra nasal,

intra ocular, intradermal or suppository routes or implanting (eg using slow release molecules by the intraperitoneal route or by using cells e.g. monocytes or dendrite cells sensitized *in vitro* and adoptively transferred to the recipient). Depending on the route of administration, the peptide may be required to be coated in a material to protect it from the action of enzymes, acids and other natural conditions which may inactivate said ingredients.

[00859] For example, the low lipophilicity of the peptides will allow them to be destroyed in the gastrointestinal tract by enzymes capable of cleaving peptide bonds and in the stomach by acid hydrolysis. In order to administer peptides by other than parenteral administration, they will be coated by, or administered with, a material to prevent its inactivation. For example, peptides may be administered in an adjuvant, co-administered with enzyme inhibitors or in liposomes. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes.

[00860] The active compounds may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[00861] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, chlorobutanol, phenol, sorbic acid, theomersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the composition of agents delaying absorption, for example, aluminium monostearate and gelatin.

[00862] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterile active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00863] When the peptides are suitably protected as described above, the active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound.

[00864] The tablets, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should

be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

[00865] Delivery Systems

[00866] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis, construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, intra ocular, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[00867] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody or a peptide of the invention, care must be taken to use materials to which the protein does not absorb. In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome. In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose.

[00868] Sequence Listing Free Text

[00869] 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.

[00870] MTPGTQSPFF LLLLLTVLTV VTGSGHASST PGGEKETSAT QRSSVPSSTE KNAVSMTSSV LSSHSPGSGS STTQGQDVTL APATEPASGS AATWGQDVTS VPVTRPALGS TTPPAHDVTS APDNKPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDNRPALGS TAPPVHNVTS ASGSASGSAS TLVHNGTSAR ATTTPASKST PFSIPSHHSD TPTTLASHST KTDASSTHHS SVPPLTSSNH STSPQLSTGV SFFFLSFHIS NLQFNSSLED PSTDYYQELQ RDISEMFLQI YKQGGFLGLS NIKFRPGSVV VQLTLAFREG TINVHDVETQ FNQYKTEAAS RYNLTISDVS VSDVPFPFSA QSGAGVPGWG IALLVLVCVL VALAIVYLIA LAVCQCRRKN YGQLDIFPAR DTYHPMSEYP TYHTHGRYVP PSSTDRSPYE KVSAGNGGSS LSYTNPAVAA ASANL (SEQ ID NO:1) describes full-length MUC1 Receptor (Mucin 1 precursor, Genbank Accession number: P15941).

[00871] MTPGTQSPFFLLLLLTVLT (SEQ ID NO:2)

[00872] MTPGTQSPFFLLLLLTVLT VVTA (SEQ ID NO:3)

[00873] MTPGTQSPFFLLLLLTVLT VVTG (SEQ ID NO:4)

[00874] SEQ ID NOS:2, 3 and 4 describe N-terminal MUC-1 signaling sequence for directing MUC1 receptor and truncated isoforms to cell membrane surface. Up to 3 amino acid residues may be absent at C-terminal end as indicated by variants in SEQ ID NOS:2, 3 and 4.

[00875] GTINVHDVETQFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGAGVPGW GIALLVLVCVLVALAIVYLIALAVCQCRRKNYGQLDIFPARDTYHPMSEYPTYHTHG RYVPPSSTDRSPYEKVSAGNGGSSLSYTNPAVAAASANL (SEQ ID NO:5) describes a truncated MUC1 receptor isoform having nat-PSMGFR at its N-terminus and including the transmembrane and cytoplasmic sequences of a full-length MUC1 receptor.

[00876] GTINVHDVETQFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGA (SEQ ID NO:6) describes the extracellular domain of Native Primary Sequence of the MUC1 Growth Factor Receptor (nat-PSMGFR – an example of "PSMGFR"):

[00877] TINVHDVETQFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGA (SEQ ID NO:7) describes the extracellular domain of Native Primary Sequence of the MUC1 Growth Factor Receptor (nat-PSMGFR – An example of "PSMGFR"), having a single amino acid deletion at the N-terminus of SEQ ID NO:6).

[00878] GTINVHDVETQFNQYKTEAASPYNLTISDVSVSDVPFPFSAQSGA (SEQ ID NO:8) describes the extracellular domain of "SPY" functional variant of the native Primary Sequence of the MUC1 Growth Factor Receptor having enhanced stability (var-PSMGFR – An example of "PSMGFR").

[00879] TINVHDVETQFNQYKTEAASPYNLTISDVSVSDVPFPFSAQSGA (SEQ ID NO:9) describes the extracellular domain of "SPY" functional variant of the native Primary Sequence of the MUC1 Growth Factor Receptor having enhanced stability (var-PSMGFR – An example of "PSMGFR"), having a single amino acid deletion at the C-terminus of SEQ ID NO:8).

[00880] tgtcagtgccgccgaaagaactacgggcagctggacatctttccagcccgggatacctaccatcctatgagcgagta ccccacctaccacacccatgggcgctatgtgccccctagcagtaccgatcgtagcccctatgagaaggtttctgcaggtaacggtggc agcagcctctcttacacaaacccagcagtggcagccgcttctgccaacttg (SEQ ID NO:10) describes MUC1 cytoplasmic domain nucleotide sequence.

[00881] CQCRRKNYGQLDIFPARDTYHPMSEYPTYHTHGRYVPPSSTDRSPYEKVS AGNGGSSLSYTNPAVAAASANL (SEQ ID NO:11) describes MUC1 cytoplasmic domain amino acid sequence.

[00882] gagatcctgagacaatgaatcatagtgaaagattcgttttcattgcagagtggtatgatccaaatgcttcacttcttcgac gttatgagcttttattttacccaggggatggatctgttgaaatgcatgatgtaaagaatcatcgcacctttttaaagcggaccaaatatgata acctgcacttggaagatttatttataggcaacaaagtgaatgtcttttctcgacaactggtattaattgactatggggatcaatatacagctc

gccagctgggcagtaggaaagaaaaaacgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataa acaaagctggatttactataaccaaactcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacc ctttttcaatgagctgatccagtttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactg ctgggacctgcaaactctggagtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcag cgcatggccctgattcttttgcttctgcggccagagaaatggagttgtttttccttcaagtggaggttgtgggccggcaaacactgctaa atttactaattgtacctgttgcattgttaaaccccatgctgtcagtgaaggtatgttgaatacactatattcagtacattttgttaataggagag caatgtttatttttcttgatgtactttatgtatagaaaataa (SEQ ID NO:12) describes NME7 nucleotide sequence (NME7: GENBANK ACCESSION AB209049).

[00883] DPETMNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRT FLKRTKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAI SKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEIL RDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELF FPSSGGCGPANTAKFTNCTCCIVKPHAVSEGMLNTLYSVHFVNRRAMFIFLMYFMY RK (SEQ ID NO:13) describes NME7 amino acid sequence (NME7: GENBANK ACCESSION AB209049).

[00884] atggtgctactgtctactttagggatcgtctttcaaggcgaggggcctcctatctcaagctgtgatacaggaaccatggc caactgtgagcgtaccttcattgcgatcaaaccagatggggtccagcggggtcttgtgggagagattatcaagcgttttgagcagaaag gattccgccttgttggtctgaaattcatgcaagcttccgaagatcttctcaaggaacactacgttgacctgaaggaccgtccattctttgcc ggcctggtgaaatacatgcactcagggccggtagttgccatggtctgggaggggctgaatgtggtgaagacgggccgagtcatgctc ggggagaccaaccctgcagactccaagcctgggaccatccgtggagacttctgcatacaagttggcaggaacattatacatggcagt gattctgtggagagtgcagagaaggagatcggcttgtggtttcaccctgaggaactggtagattacacgagctgtgctcagaactggat ctatgaatga (SEQ ID NO:14) describes NM23-H1 nucleotide sequence (NM23-H1: GENBANK ACCESSION AF487339).

[00885] MVLLSTLGIVFQGEGPPISSCDTGTMANCERTFIAIKPDGVQRGLVGEIIKR FEQKGFRLVGLKFMQASEDLLKEHYVDLKDRPFFAGLVKYMHSGPVVAMVWEGL NVVKTGRVMLGETNPADSKPGTIRGDFCIQVGRNIIHGSDSVESAEKEIGLWFHPEEL VDYTSCAQNWIYE (SEQ ID NO:15) NM23-H1 describes amino acid sequence (NM23-H1: GENBANK ACCESSION AF487339).

[00886] atggtgctactgtctactttagggatcgtctttcaaggcgaggggcctcctatctcaagctgtgatacaggaaccatggc caactgtgagcgtaccttcattgcgatcaaaccagatggggtccagcggggtcttgtgggagagattatcaagcgttttgagcagaaag gattccgccttgttggtctgaaattcatgcaagcttccgaagatcttctcaaggaacactacgttgacctgaaggaccgtccattctttgcc ggcctggtgaaatacatgcactcagggccggtagttgccatggtctgggaggggctgaatgtggtgaagacgggccgagtcatgctc ggggagaccaaccctgcagactccaagcctgggaccatccgtggagacttctgcatacaagttggcaggaacattatacatggcggt gattctgtggagagtgcagagaaggagatcggcttgtggtttcaccctgaggaactggtagattacacgagctgtgctcagaactggat

ctatgaatga (SEQ ID NO:16) describes NM23-H1 S120G mutant nucleotide sequence (NM23-H1: GENBANK ACCESSION AF487339).

[00887] MVLLSTLGIVFQGEGPPISSCDTGTMANCERTFIAIKPDGVQRGLVGEIIKR FEQKGFRLVGLKFMQASEDLLKEHYVDLKDRPFFAGLVKYMHSGPVVAMVWEGL NVVKTGRVMLGETNPADSKPGTIRGDFCIQVGRNIIHGGDSVESAEKEIGLWFHPEEL VDYTSCAQNWIYE (SEQ ID NO:17) describes NM23-H1 S120G mutant amino acid sequence (NM23-H1: GENBANK ACCESSION AF487339).

[00888] atggccaacctggagcgcaccttcatcgccatcaagccggacggcgtgcagcgcggcctggtgggcgagatcatc aagcgcttcgagcagaagggattccgcctcgtggccatgaagttcctccgggcctctgaagaacacctgaagcagcactacattgac ctgaaagaccgaccattcttccctgggctggtgaagtacatgaactcagggccggttgtggccatggtctgggaggggctgaacgtg gtgaagacaggccgagtgatgcttggggagaccaatccagcagattcaaagccaggcaccattcgtggggacttctgcattcaggtt ggcaggaacatcattcatggcagtgattcagtaaaaagtgctgaaaaagaaatcagcctatggtttaagcctgaagaactggttgacta caagtcttgtgctcatgactgggtctatgaataa (SEQ ID NO:18) describes NM23-H2 nucleotide sequence (NM23-H2: GENBANK ACCESSION AK313448).

[00889] MANLERTFIAIKPDGVQRGLVGEIIKRFEQKGFRLVAMKFLRASEEHLKQH YIDLKDRPFFPGLVKYMNSGPVVAMVWEGLNVVKTGRVMLGETNPADSKPGTIRG DFCIQVGRNIIHGSDSVKSAEKEISLWFKPEELVDYKSCAHDWVYE (SEQ ID NO:19) describes NM23-H2 amino acid sequence (NM23-H2: GENBANK ACCESSION AK313448).

[00890] Human NM23-H7-2 sequence optimized for *E. coli* expression:

[00891] (DNA)

[00892] atgcatgacgttaaaaatcaccgtacctttctgaaacgcacgaaatatgataatctgcatctggaagacctgtttattggc aacaaagtcaatgtgttctctcgtcagctggtgctgatcgattatggcgaccagtacaccgcgcgtcaactgggtagtcgcaaagaaaa aacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaaattatcaacaaagcgggtttcaccatcacgaaac tgaaaatgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttcaatgaactgattcaattcatc accacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctgggcccggcaaactcaggtg ttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcacatggtccggactcattcgcat cggcagctcgtgaaatggaactgtttttcccgagctctggcggttgcggtccggcaaacaccgccaaatttaccaattgtacgtgctgta ttgtcaaaccgcacgcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtgatgctggctttgaaatctcggccatgcag atgttcaacatggaccgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggttaccgaatatcacgatatggttacggaaatg tactccggtccgtgcgtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgtgaattctgtggtccggcagatccggaaat cgcacgtcatctgcgtccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaacgctgtgcactgtaccgatctgccggaa gacggtctgctggaagttcaatacttttttcaaaattctggataattga (SEQ ID NO:20)

[00893] (amino acids)

[00894] MHDVKNHRTFLKRTKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTAR QLGSRKEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRP FFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIRNA AHGPDSFASAAREMELFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIR DAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNN ATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILD N- (SEQ ID NO:21)

[00895] Human NME7-A:

[00896] (DNA)

[00897] atggaaaaaacgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataaacaaagct ggatttactataaccaaactcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttcaat gagctgatccagtttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacc tgcaaactctggagtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggc cctgattcttttgcttctgcggccagagaaatggagttgttttttga (SEQ ID NO:22)

[00898] (amino acids)

[00899] MEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQS RPFFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIR NAAHGPDSFASAAREMELFF- (SEQ ID NO:23)

[00900] Human NME7-A1:

[00901] (DNA)

[00902] atggaaaaaacgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataaacaaagct ggatttactataaccaaactcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttcaat gagctgatccagtttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacc tgcaaactctggagtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggc cctgattcttttgcttctgcggccagagaaatggagttgttttttccttcaagtggaggttgtgggccggcaaacactgctaaatttacttga (SEQ ID NO:24)

[00903] (amino acids)

[00904] MEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQS RPFFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIR NAAHGPDSFASAAREMELFFPSSGGCGPANTAKFT- (SEQ ID NO:25)

[00905] Human NME7-A2:

[00906] (DNA)

[00907] atgaatcatagtgaaagattcgttttcattgcagagtggtatgatccaaatgcttcacttcttcgacgttatgagcttttatttt acccaggggatggatctgttgaaatgcatgatgtaaagaatcatcgcacctttttaaagcggaccaaatatgataacctgcacttggaag

atttatttataggcaacaaagtgaatgtcttttctcgacaactggtattaattgactatggggatcaatatacagctcgccagctgggcagta ggaaagaaaaaacgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataaacaaagctggatttact ataaccaaactcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttcaatgagctgat ccagtttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacctgcaaact ctggagtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggccctgattct ttgcttctgcggccagagaaatggagttgtttttttga (SEQ ID NO:26)

[00908] (amino acids)

[00909] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAISKA GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDD AICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFF- (SEQ ID NO:27)

[00910] Human NME7-A3:

[00911] (DNA)

[00912] atgaatcatagtgaaagattcgttttcattgcagagtggtatgatccaaatgcttcacttcttcgacgttatgagcttttatttt acccaggggatggatctgttgaaatgcatgatgtaaagaatcatcgcacctttttaaagcggaccaaatatgataacctgcacttggaag atttatttataggcaacaaagtgaatgtcttttctcgacaactggtattaattgactatggggatcaatatacagctcgccagctgggcagta ggaaagaaaaaacgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataaacaaagctggatttact ataaccaaactcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttcaatgagctgat ccagtttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacctgcaaact ctggagtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggccctgattct ttgcttctgcggccagagaaatggagttgtttttttccttcaagtggaggttgtgggccggcaaacactgctaaatttacttga (SEQ ID NO:28)

[00913] (amino acids)

[00914] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAISKA GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDD AICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFFPSS GGCGPANTAKFT- (SEQ ID NO:29)

[00915] Human NME7-B:

[00916] (DNA)

[00917] atgaattgtacctgttgcattgttaaaccccatgctgtcagtgaaggactgttgggaaagatcctgatggctatccgaga tgcaggttttgaaatctcagctatgcagatgttcaatatggatcgggttaatgttgaggaattctatgaagtttataaaggagtagtgaccg aatatcatgacatggtgacagaaatgtattctggcccttgtgtagcaatggagattcaacagaataatgctacaaagacatttcgagaattt

tgtggacctgctgatcctgaaattgcccggcatttacgccctggaactctcagagcaatctttggtaaaactaagatccagaatgctgttc actgtactgatctgccagaggatggcctattagaggttcaatacttcttctga (SEQ ID NO:30)

[00918] (amino acids)

[00919] MNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFY EVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTL RAIFGKTKIQNAVHCTDLPEDGLLEVQYFF- (SEQ ID NO:31)

[00920] Human NME7-B1:

[00921] (DNA)

[00922] atgaattgtacctgttgcattgttaaaccccatgctgtcagtgaaggactgttgggaaagatcctgatggctatccgaga tgcaggttttgaaatctcagctatgcagatgttcaatatggatcgggttaatgttgaggaattctatgaagtttataaaggagtagtgaccg aatatcatgacatggtgacagaaatgtattctggcccttgtgtagcaatggagattcaacagaataatgctacaaagacatttcgagaattt tgtggacctgctgatcctgaaattgcccggcatttacgccctggaactctcagagcaatctttggtaaaactaagatccagaatgctgttc actgtactgatctgccagaggatggcctattagaggttcaatacttcttcaagatcttggataattagtga (SEQ ID NO:32)

[00923] (amino acids)

[00924] MNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFY EVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTL RAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDN— (SEQ ID NO:33)

[00925] Human NME7-B2:

[00926] (DNA)

[00927] atgccttcaagtggaggttgtgggccggcaaacactgctaaatttactaattgtacctgttgcattgttaaaccccatgct gtcagtgaaggactgttgggaaagatcctgatggctatccgagatgcaggttttgaaatctcagctatgcagatgttcaatatggatcgg gttaatgttgaggaattctatgaagtttataaaggagtagtgaccgaatatcatgacatggtgacagaaatgtattctggcccttgtgtagc aatggagattcaacagaataatgctacaaagacatttcgagaattttgtggacctgctgatcctgaaattgcccggcatttacgccctgga actctcagagcaatctttggtaaaactaagatccagaatgctgttcactgtactgatctgccagaggatggcctattagaggttcaatactt cttctga (SEQ ID NO:34)

[00928] (amino acids)

[00929] MPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAM QMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFC GPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFF- (SEQ ID NO:35)

[00930] Human NME7-B3:

[00931] (DNA)

[00932] atgccttcaagtggaggttgtgggccggcaaacactgctaaatttactaattgtacctgttgcattgttaaaccccatgct gtcagtgaaggactgttgggaaagatcctgatggctatccgagatgcaggttttgaaatctcagctatgcagatgttcaatatggatcgg gttaatgttgaggaattctatgaagtttataaaggagtagtgaccgaatatcatgacatggtgacagaaatgtattctggcccttgtgtagc

aatggagattcaacagaataatgctacaaagacatttcgagaattttgtggacctgctgatcctgaaattgcccggcatttacgccctgga actctcagagcaatctttggtaaaactaagatccagaatgctgttcactgtactgatctgccagaggatggcctattagaggttcaatactt cttcaagatcttggataattagtga (SEQ ID NO:36)

[00933] (amino acids)

[00934] MPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAM QMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFC GPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDN-- (SEQ ID NO:37)

[00935] Human NME7-AB, also known as $NME7_{AB}$:

[00936] (DNA)

[00937] atggaaaaaacgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataaacaaagct ggatttactataaccaaactcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttttcaat gagctgatccagtttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacc tgcaaactctggagtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggc cctgattcttttgcttctgcggccagagaaatggagttgtttttttccttcaagtggaggttgtgggccggcaaacactgctaaatttactaatt gtacctgttgcattgttaaaccccatgctgtcagtgaaggactgttgggaaagatcctgatggctatccgagatgcaggttttgaaatctc agctatgcagatgttcaatatggatcgggttaatgttgaggaattctatgaagtttataaaggagtagtgaccgaatatcatgacatggtga cagaaatgtattctggcccttgtgtagcaatggagattcaacagaataatgctacaaagacatttcgagaattttgtggacctgctgatcct gaaattgcccggcatttacgccctggaactctcagagcaatctttggtaaaactaagatccagaatgctgttcactgtactgatctgccag aggatggcctattagaggttcaatacttcttcaagatcttggataattagtga (SEQ ID NO:38)

[00938] (amino acids)

[00939] MEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQS RPFFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIR NAAHGPDSFASAAREMELFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILM AIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQ NNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFK ILDN-- (SEQ ID NO:39)

[00940] Human NME7-AB1:

[00941] (DNA)

[00942] atggaaaaaacgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataaacaaagct ggatttactataaccaaactcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttttcaat gagctgatccagtttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacc tgcaaactctggagtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggc cctgattcttttgcttctgcggccagagaaatggagttgtttttttccttcaagtggaggttgtgggccggcaaacactgctaaatttactaatt

gtacctgttgcattgttaaaccccatgctgtcagtgaaggactgttgggaaagatcctgatggctatccgagatgcaggttttgaaatctc agctatgcagatgttcaatatggatcgggttaatgttgaggaattctatgaagtttataaaggagtagtgaccgaatatcatgacatggtga cagaaatgtattctggcccttgtgtagcaatggagattcaacagaataatgctacaaagacatttcgagaattttgtggacctgctgatcct gaaattgcccggcatttacgccctggaactctcagagcaatctttggtaaaactaagatccagaatgctgttcactgtactgatctgccag aggatggcctattagaggttcaatacttcttctga (SEQ ID NO:40)

[00943] (amino acids)

[00944] MEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQS RPFFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIR NAAHGPDSFASAAREMELFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILM AIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQ NNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFF- (SEQ ID NO:41)

[00945] Human NME7-A sequence optimized for *E. coli* expression:

[00946] (DNA)

[00947] atggaaaaaacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaaattatcaacaaagcg ggtttcaccatcacgaaactgaaaatgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttca atgaactgattcaattcatcaccacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctgg gcccggcaaactcaggtgttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcacat ggtccggactcattcgcatcggcagctcgtgaaatggaactgtttttctga (SEQ ID NO:42)

[00948] (amino acids)

[00949] MEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQS RPFFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIR NAAHGPDSFASAAREMELFF- (SEQ ID NO:43)

[00950] Human NME7-A1 sequence optimized for *E. coli* expression:

[00951] (DNA)

[00952] atggaaaaaacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaaattatcaacaaagcg ggtttcaccatcacgaaactgaaaatgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttca atgaactgattcaattcatcaccacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctgg gcccggcaaactcaggtgttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcacat ggtccggactcattcgcatcggcagctcgtgaaatggaactgtttttcccgagctctggcggttgcggtccggcaaacaccgccaaatt tacctga (SEQ ID NO:44)

[00953] (amino acids)

[00954] MEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQS RPFFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIR NAAHGPDSFASAAREMELFFPSSGGCGPANTAKFT- (SEQ ID NO:45)

[00955] Human NME7-A2 sequence optimized for *E. coli* expression:

[00956] (DNA)

[00957] atgaatcactccgaacgctttgtttttatcgccgaatggtatgacccgaatgcttccctgctgcgccgctacgaactgct gttttatccgggcgatggtagcgtggaaatgcatgacgttaaaaatcaccgtacctttctgaaacgcacgaaatatgataatctgcatctg gaagacctgtttattggcaacaaagtcaatgtgttctctcgtcagctggtgctgatcgattatggcgaccagtacaccgcgcgtcaactg ggtagtcgcaaagaaaaaacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaaattatcaacaaagcgg gtttcaccatcacgaaactgaaaatgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttcaa tgaactgattcaattcatcaccacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctggg cccggcaaactcaggtgttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcacatg gtccggactcattcgcatcggcagctcgtgaaatggaactgtttttctga (SEQ ID NO:46)

[00958] (amino acids)

[00959] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAISKA GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDD AICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFF- (SEQ ID NO:47)

[00960] Human NME7-A3 sequence optimized for *E. coli* expression:

[00961] (DNA)

[00962] atgaatcactccgaacgctttgtttttatcgccgaatggtatgacccgaatgcttccctgctgcgccgctacgaactgct gttttatccgggcgatggtagcgtggaaatgcatgacgttaaaaatcaccgtacctttctgaaacgcacgaaatatgataatctgcatctg gaagacctgtttattggcaacaaagtcaatgtgttctctcgtcagctggtgctgatcgattatggcgaccagtacaccgcgcgtcaactg ggtagtcgcaaagaaaaaacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaaattatcaacaaagcgg gtttcaccatcacgaaactgaaaatgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttcaa tgaactgattcaattcatcaccacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctggg cccggcaaactcaggtgttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcacatg gtccggactcattcgcatcggcagctcgtgaaatggaactgtttttcccgagctctggcggttgcggtccggcaaacaccgccaaattt acctga (SEQ ID NO:48)

[00963] (amino acids)

[00964] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAISKA GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDD

AICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFFPSS GGCGPANTAKFT- (SEQ ID NO:49)

[00965] Human NME7-B sequence optimized for *E. coli* expression:

[00966] (DNA)

[00967] atgaattgtacgtgctgtattgtcaaaccgcacgcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtg atgctggctttgaaatctcggccatgcagatgttcaacatggaccgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggtta ccgaatatcacgatatggttacggaaatgtactccggtccgtgcgtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgt gaattctgtggtccggcagatccggaaatcgcacgtcatctgcgtccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaa cgctgtgcactgtaccgatctgccggaagacggtctgctggaagttcaatacttttctga (SEQ ID NO:50)

[00968] (amino acids)

[00969] MNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFY EVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTL RAIFGKTKIQNAVHCTDLPEDGLLEVQYFF- (SEQ ID NO:51)

[00970] Human NME7-B1 sequence optimized for *E. coli* expression:

[00971] (DNA)

[00972] atgaattgtacgtgctgtattgtcaaaccgcacgcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtg atgctggctttgaaatctcggccatgcagatgttcaacatggaccgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggtta ccgaatatcacgatatggttacggaaatgtactccggtccgtgcgtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgt gaattctgtggtccggcagatccggaaatcgcacgtcatctgcgtccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaa cgctgtgcactgtaccgatctgccggaagacggtctgctggaagttcaatacttttcaaaattctggataattga (SEQ ID NO:52)

[00973] (amino acids)

[00974] MNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFY EVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTL RAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDN- (SEQ ID NO:53)

[00975] Human NME7-B2 sequence optimized for *E. coli* expression :

[00976] (DNA)

[00977] atgccgagctctggcggttgcggtccggcaaacaccgccaaatttaccaattgtacgtgctgtattgtcaaaccgcac gcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtgatgctggctttgaaatctcggccatgcagatgttcaacatggac cgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggttaccgaatatcacgatatggttacggaaatgtactccggtccgtgc gtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgtgaattctgtggtccggcagatccggaaatcgcacgtcatctgcg tccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaacgctgtgcactgtaccgatctgccggaagacggtctgctggaa gttcaatacttttctga (SEQ ID NO:54)

[00978] (amino acids)

[00979] MPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAM QMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFC GPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFF- (SEQ ID NO:55)

[00980] Human NME7-B3 sequence optimized for *E. coli* expression:

[00981] (DNA)

[00982] atgccgagctctggcggttgcggtccggcaaacaccgccaaatttaccaattgtacgtgctgtattgtcaaaccgcac gcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtgatgctggctttgaaatctcggccatgcagatgttcaacatggac cgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggttaccgaatatcacgatatggttacggaaatgtactccggtccgtgc gtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgtgaattctgtggtccggcagatccggaaatcgcacgtcatctgcg tccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaacgctgtgcactgtaccgatctgccggaagacggtctgctggaa gttcaatacttttttcaaaattctggataattga (SEQ ID NO:56)

[00983] (amino acids)

[00984] MPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAM QMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFC GPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDN- (SEQ ID NO:57)

[00985] Human NME7-AB, also known as $NME7_{AB}$ sequence optimized for *E. coli* expression:

[00986] (DNA)

[00987] atggaaaaaacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaaattatcaacaaagcg ggtttcaccatcacgaaactgaaaatgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttca atgaactgattcaattcatcaccacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctgg gcccggcaaactcaggtgttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcacat ggtccggactcattcgcatcggcagctcgtgaaatggaactgtttttcccgagctctggcggttgcggtccggcaaacaccgccaaatt taccaattgtacgtgctgtattgtcaaaccgcacgcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtgatgctggcttt gaaatctcggccatgcagatgttcaacatggaccgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggttaccgaatatca cgatatggttacggaaatgtactccggtccgtgcgtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgtgaattctgtgg tccggcagatccggaaatcgcacgtcatctgcgtccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaacgctgtgcact gtaccgatctgccggaagacggtctgctggaagttcaatacttttttcaaaattctggataattga (SEQ ID NO:58)

[00988] (amino acids)

[00989] MEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQS RPFFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIR NAAHGPDSFASAAREMELFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILM AIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQ

NNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFK ILDN- (SEQ ID NO:59)

[00990] Human NME7-AB1, also known as $NME7_{AB}1$ sequence optimized for *E. coli* expression:

[00991] (DNA)

[00992] Atggaaaaaacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaaattatcaacaaagc gggtttcaccatcacgaaactgaaaatgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttc aatgaactgattcaattcatcaccacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctg ggcccggcaaactcaggtgttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcaca tggtccggactcattcgcatcggcagctcgtgaaatggaactgtttttcccgagctctggcggttgcggtccggcaaacaccgccaaat ttaccaattgtacgtgctgtattgtcaaaccgcacgcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtgatgctggctt tgaaatctcggccatgcagatgttcaacatggaccgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggttaccgaatatca cgatatggttacggaaatgtactccggtccgtgcgtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgtgaattctgtgg tccggcagatccggaaatcgcacgtcatctgcgtccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaacgctgtgcact gtaccgatctgccggaagacggtctgctggaagttcaatacttttctga (SEQ ID NO:60)

[00993] (amino acids)

[00994] MEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQS RPFFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIR NAAHGPDSFASAAREMELFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILM AIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQ NNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFF- (SEQ ID NO:61)

[00995] Mouse NME6

[00996] (DNA)

[00997] Atgacctccatcttgcgaagtccccaagctcttcagctcacactagccctgatcaagcctgatgcagttgcccaccca ctgatcctggaggctgttcatcagcagattctgagcaacaagttcctcattgtacgaacgagggaactgcagtggaagctggaggact gccggaggttttaccgagagcatgaagggcgtttttctatcagcggctggtggagttcatgacaagtgggccaatccgagcctatatc cttgcccacaaagatgccatccaactttggaggacactgatgggacccaccagagtatttcgagcacgctatatagccccagattcaat tcgtggaagtttgggcctcactgacacccgaaatactacccatggctcagactccgtggtttccgccagcagagagattgcagccttctt ccctgacttcagtgaacagcgctggtatgaggaggaggaaccccagctgcggtgtggtcctgtgcactacagtccagaggaaggtat ccactgtgcagctgaaacaggaggccacaaacaacctaacaaaacctag (SEQ ID NO:62)

[00998] (amino acids)

[00999] MTSILRSPQALQLTLALIKPDAVAHPLILEAVHQQILSNKFLIVRTRELQWK LEDCRRFYREHEGRFFYQRLVEFMTSGPIRAYILAHKDAIQLWRTLMGPTRVFRARY

IAPDSIRGSLGLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEEPQLRCGPVHY SPEEGIHCAAETGGHKQPNKT- (SEQ ID NO:63)

[001000] Human NME6:

[001001] (DNA)

[001002] Atgacccagaatctggggagtgagatggcctcaatcttgcgaagccctcaggctctccagctcactctagccctgat caagcctgacgcagtcgcccatccactgattctggaggctgttcatcagcagattctaagcaacaagttcctgattgtacgaatgagag aactactgtggagaaaggaagattgccagaggttttaccgagagcatgaagggcgttttttctatcagaggctggtggagttcatggcc agcgggccaatccgagcctacatccttgcccacaaggatgccatccagctctggaggacgctcatgggacccaccagagtgttccga gcacgccatgtggccccagattctatccgtgggagtttcggcctcactgacacccgcaacaccacccatggttcggactctgtggtttc agccagcagagagattgcagccttcttccctgacttcagtgaacagcgctggtatgaggaggaagagccccagttgcgctgtggccct gtgtgctatagcccagagggaggtgtccactatgtagctggaacaggaggcctaggaccagcctga (SEQ ID NO:64)

[001003] (amino acids)

[001004] MTQNLGSEMASILRSPQALQLTLALIKPDAVAHPLILEAVHQQILSNKFLIV RMRELLWRKEDCQRFYREHEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTLMG PTRVFRARHVAPDSIRGSFGLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEE PQLRCGPVCYSPEGGVHYVAGTGGLGPA- (SEQ ID NO:65)

[001005] Human NME6 1:

[001006] (DNA)

[001007] Atgacccagaatctggggagtgagatggcctcaatcttgcgaagccctcaggctctccagctcactctagccctgat caagcctgacgcagtcgcccatccactgattctggaggctgttcatcagcagattctaagcaacaagttcctgattgtacgaatgagag aactactgtggagaaaggaagattgccagaggttttaccgagagcatgaagggcgttttttctatcagaggctggtggagttcatggcc agcgggccaatccgagcctacatccttgcccacaaggatgccatccagctctggaggacgctcatgggacccaccagagtgttccga gcacgccatgtggccccagattctatccgtgggagtttcggcctcactgacacccgcaacaccacccatggttcggactctgtggtttc agccagcagagagattgcagccttcttccctgacttcagtgaacagcgctggtatgaggaggaagagccccagttgcgctgtggccct gtgtga (SEQ ID NO:66)

[001008] (amino acids)

[001009] MTQNLGSEMASILRSPQALQLTLALIKPDAVAHPLILEAVHQQILSNKFLIV RMRELLWRKEDCQRFYREHEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTLMG PTRVFRARHVAPDSIRGSFGLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEE PQLRCGPV- (SEQ ID NO:67)

[001010] Human NME6 2:

[001011] (DNA)

[001012] Atgctcactctagccctgatcaagcctgacgcagtcgcccatccactgattctggaggctgttcatcagcagattctaa gcaacaagttcctgattgtacgaatgagagaactactgtggagaaaggaagattgccagaggttttaccgagagcatgaagggcgtttt

ttctatcagaggctggtggagttcatggccagcgggccaatccgagcctacatccttgcccacaaggatgccatccagctctggagga cgctcatgggacccaccagagtgttccgagcacgccatgtggccccagattctatccgtgggagtttcggcctcactgacacccgcaa caccacccatggttcggactctgtggtttcagccagcagagagattgcagccttcttccctgacttcagtgaacagcgctggtatgagg aggaagagccccagttgcgctgtggccctgtgtga (SEQ ID NO:68)

[001013] (amino acids)

[001014] MLTLALIKPDAVAHPLILEAVHQQILSNKFLIVRMRELLWRKEDCQRFYRE HEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTLMGPTRVFRARHVAPDSIRGSF GLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEEPQLRCGPV- (SEQ ID NO:69)

[001015] Human NME6 3:

[001016] (DNA)

[001017] Atgctcactctagccctgatcaagcctgacgcagtcgcccatccactgattctggaggctgttcatcagcagattctaa gcaacaagttcctgattgtacgaatgagagaactactgtggagaaaggaagattgccagaggttttaccgagagcatgaagggcgtttt ttctatcagaggctggtggagttcatggccagcgggccaatccgagcctacatccttgcccacaaggatgccatccagctctggagga cgctcatgggacccaccagagtgttccgagcacgccatgtggccccagattctatccgtgggagtttcggcctcactgacacccgcaa caccacccatggttcggactctgtggtttcagccagcagagagattgcagccttcttccctgacttcagtgaacagcgctggtatgagg aggaagagccccagttgcgctgtggccctgtgtgctatagcccagagggaggtgtccactatgtagctggaacaggaggcctagga ccagcctga (SEQ ID NO:70)

[001018] (amino acids)

[001019] MLTLALIKPDAVAHPLILEAVHQQILSNKFLIVRMRELLWRKEDCQRFYRE HEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTLMGPTRVFRARHVAPDSIRGSF GLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEEPQLRCGPVCYSPEGGVHY VAGTGGLGPA- (SEQ ID NO:71)

[001020] Human NME6 sequence optimized for *E. coli* expression:

[001021] (DNA)

[001022] Atgacgcaaaatctgggctcggaaatggcaagtatcctgcgctccccgcaagcactgcaactgaccctggctctgat caaaccggacgctgttgctcatccgctgattctggaagcggtccaccagcaaattctgagcaacaaatttctgatcgtgcgtatgcgcg aactgctgtggcgtaaagaagattgccagcgtttttatcgcgaacatgaaggccgtttcttttatcaacgcctggttgaattcatggcctct ggtccgattcgcgcatatatcctggctcacaaagatgcgattcagctgtggcgtaccctgatgggtccgacgcgcgtctttcgtgcacgt catgtggcaccggactcaatccgtggctcgttcggtctgaccgatacgcgcaataccacgcacggtagcgactctgttgttagtgcgtc ccgtgaaatcgcggccttttttcccggacttctccgaacagcgttggtacgaagaagaagaaccgcaactgcgctgtggcccggtctgtt attctccggaaggtggtgtccattatgtggcgggcacgggtggtctgggtccggcatga (SEQ ID NO:72)

[001023] (amino acids)

[001024] MTQNLGSEMASILRSPQALQLTLALIKPDAVAHPLILEAVHQQILSNKFLIV RMRELLWRKEDCQRFYREHEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTLMG

PTRVFRARHVAPDSIRGSFGLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEE PQLRCGPVCYSPEGGVHYVAGTGGLGPA- (SEQ ID NO:73)

[001025] Human NME6 1 sequence optimized for *E. coli* expression:

[001026] (DNA)

[001027] Atgacgcaaaatctgggctcggaaatggcaagtatcctgcgctccccgcaagcactgcaactgaccctggctctgat caaaccggacgctgttgctcatccgctgattctggaagcggtccaccagcaaattctgagcaacaaatttctgatcgtgcgtatgcgcg aactgctgtggcgtaaagaagattgccagcgtttttatcgcgaacatgaaggccgtttcttttatcaacgcctggttgaattcatggcctct ggtccgattcgcgcatatatcctggctcacaaagatgcgattcagctgtggcgtaccctgatgggtccgacgcgcgtctttcgtgcacgt catgtggcaccggactcaatccgtggctcgttcggtctgaccgatacgcgcaataccacgcacggtagcgactctgttgttagtgcgtc ccgtgaaatcgcggccttttcccggacttctccgaacagcgttggtacgaagaagaagaaccgcaactgcgctgtggcccggtctga (SEQ ID NO:74)

[001028] (amino acids)

[001029] MTQNLGSEMASILRSPQALQLTLALIKPDAVAHPLILEAVHQQILSNKFLIV RMRELLWRKEDCQRFYREHEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTLMG PTRVFRARHVAPDSIRGSFGLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEE PQLRCGPV- (SEQ ID NO:75)

[001030] Human NME6 2 sequence optimized for *E. coli* expression:

[001031] (DNA)

[001032] Atgctgaccctggctctgatcaaaccggacgctgttgctcatccgctgattctggaagcggtccaccagcaaattctg agcaacaaatttctgatcgtgcgtatgcgcgaactgctgtggcgtaaagaagattgccagcgtttttatcgcgaacatgaaggccgtttct ttatcaacgcctggttgaattcatggcctctggtccgattcgcgcatatatcctggctcacaaagatgcgattcagctgtggcgtaccctg atgggtccgacgcgcgtctttcgtgcacgtcatgtggcaccggactcaatccgtggctcgttcggtctgaccgatacgcgcaataccac gcacggtagcgactctgttgttagtgcgtcccgtgaaatcgcggccttttcccggacttctccgaacagcgttggtacgaagaagaag aaccgcaactgcgctgtggcccggtctga (SEQ ID NO:76)

[001033] (amino acids)

[001034] MLTLALIKPDAVAHPLILEAVHQQILSNKFLIVRMRELLWRKEDCQRFYRE HEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTLMGPTRVFRARHVAPDSIRGSF GLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEEPQLRCGPV- (SEQ ID NO:77)

[001035] Human NME6 3 sequence optimized for *E. coli* expression:

[001036] (DNA)

[001037] Atgctgaccctggctctgatcaaaccggacgctgttgctcatccgctgattctggaagcggtccaccagcaaattctg agcaacaaatttctgatcgtgcgtatgcgcgaactgctgtggcgtaaagaagattgccagcgtttttatcgcgaacatgaaggccgtttct ttatcaacgcctggttgaattcatggcctctggtccgattcgcgcatatatcctggctcacaaagatgcgattcagctgtggcgtaccctg atgggtccgacgcgcgtctttcgtgcacgtcatgtggcaccggactcaatccgtggctcgttcggtctgaccgatacgcgcaataccac

gcacggtagcgactctgttgttagtgcgtcccgtgaaatcgcggccttttcccggacttctccgaacagcgttggtacgaagaagaag aaccgcaactgcgctgtggcccggtctgttattctccggaaggtggtgtccattatgtggcgggcacgggtggtctgggtccggcatg a (SEQ ID NO:78)

[001038] (amino acids)

[001039] MLTLALIKPDAVAHPLILEAVHQQILSNKFLIVRMRELLWRKEDCQRFYRE HEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTLMGPTRVFRARHVAPDSIRGSF GLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEEPQLRCGPVCYSPEGGVHY VAGTGGLGPA- (SEQ ID NO:79)

[001040] OriGene-NME7-1 full length

[001041] (DNA)

[001042] gacgttgtatacgactcctatagggcggccgggaattcgtcgactggatccggtaccgaggagatctgccgccgcg atcgccatgaatcatagtgaaagattcgttttcattgcagagtggtatgatccaaatgcttcacttcttcgacgttatgagcttttatttttaccc aggggatggatctgttgaaatgcatgatgtaaagaatcatcgcacctttttaaagcggaccaaatatgataacctgcacttggaagattta tttataggcaacaaagtgaatgtcttctctcgacaactggtattaattgactatggggatcaatatacagctcgccagctgggcagtagga aagaaaaaacgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataaacaaagctggatttactataa ccaaactcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttcaatgagctgatcca gtttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacctgcaaactctg gagtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggccctgattctttt gcttctgcggccagagaaatggagttgtttttccttcaagtggaggttgtgggccggcaaacactgctaaatttactaattgtacctgttg cattgttaaaccccatgctgtcagtgaaggactgttgggaaagatcctgatggctatccgagatgcaggttttgaaatctcagctatgcag atgttcaatatggatcgggttaatgttgaggaattctatgaagtttataaaggagtagtgaccgaatatcatgacatggtgacagaaatgta ttctggcccttgtgtagcaatggagattcaacagaataatgctacaaagacatttcgagaattttgtggacctgctgatcctgaaattgccc ggcatttacgccctggaactctcagagcaatctttggtaaaactaagatccagaatgctgttcactgtactgatctgccagaggatggcct attagaggttcaatacttcttcaagatcttggataatacgcgtacgcggccgctcgagcagaaactcatctcagaagaggatctggcag caaatgatatcctggattacaaggatgacgacgataaggtttaa (SEQ ID NO:80)

[001043] (amino acids)

[001044] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAISKA GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDD AICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFFPSS GGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNV EEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLR PGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDNTRTRRLEQKLISEEDLAAN DILDYKDDDDKV (SEQ ID NO:81)

[001045] Abnova NME7-1 Full length (amino acids)

[001046] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAISKA GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDD AICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFFPSS GGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNV EEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLR PGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDN (SEQ ID NO:82)

[001047] Abnova Partial NME7-B

[001048] (amino acids)

[001049] DRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREF CGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKIL (SEQ ID NO:83)

[001050] Histidine Tag

[001051] (ctcgag)caccaccaccaccaccactga (SEQ ID NO:84)

[001052] Strept II Tag

[001053] (accggt)tggagccatcctcagttcgaaaagtaatga (SEQ ID NO:85)

[001054] N-10 peptide:

[001055] QFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGA (SEQ ID NO:86)

[001056] C-10 peptide

[001057] GTINVHDVETQFNQYKTEAASRYNLTISDVSVSDV (SEQ ID NO:87)

[001058] LALIKPDA (SEQ ID NO:88)

[001059] MMMLSRKEALDFHVDHQS (SEQ ID NO:89)

[001060] ALDFHVDHQS (SEQ ID NO:90)

[001061] EILRDDAICEWKRL (SEQ ID NO:91)

[001062] FNELIQFITTGP (SEQ ID NO:92)

[001063] RDDAICEW (SEQ ID NO:93)

[001064] SGVARTDASESIRALFGTDGIRNAA (SEQ ID NO:94)

[001065] ELFFPSSGG (SEQ ID NO:95)

[001066] KFTNCTCCIVKPHAVSEGLLGKILMA (SEQ ID NO:96)

[001067] LMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVT (SEQ ID NO:97)

[001068] EFYEVYKGVVTEYHD (SEQ ID NO:98)

[001069] EIQQNNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNA (SEQ ID NO:99)

[001070] YSGPCVAM (SEQ ID NO:100)

[001071] FREFCGP (SEQ ID NO:101)

[001072] VHCTDLPEDGLLEVQYFFKILDN (SEQ ID NO:102)

[001073] IQNAVHCTD (SEQ ID NO:103)

[001074] TDLPEDGLLEVQYFFKILDN (SEQ ID NO:104)

[001075] PEDGLLEVQYFFK (SEQ ID NO:105)

[001076] EIINKAGFTITK (SEQ ID NO:106)

[001077] MLSRKEALDFHVDHQS (SEQ ID NO:107)

[001078] NELIQFITT (SEQ ID NO:108)

[001079] EILRDDAICEWKRL (SEQ ID NO:109)

[001080] SGVARTDASESIRALFGTDGI (SEQ ID NO:110)

[001081] SGVARTDASES (SEQ ID NO:111)

[001082] ALFGTDGI (SEQ ID NO:112)

[001083] NCTCCIVKPHAVSE (SEQ ID NO:113)

[001084] LGKILMAIRDA (SEQ ID NO:114)

[001085] EISAMQMFNMDRVNVE (SEQ ID NO:115)

[001086] EVYKGVVT (SEQ ID NO:116)

[001087] EYHDMVTE (SEQ ID NO:117)

[001088] EFCGPADPEIARHLR (SEQ ID NO:118)

[001089] AIFGKTKIQNAV (SEQ ID NO:119)

[001090] LPEDGLLEVQYFFKILDN (SEQ ID NO:120)

[001091] GPDSFASAAREMELFFP (SEQ ID NO:121)

[001092] Immunizing peptides derived from human NME7

[001093] ICEWKRL (SEQ ID NO:122)

[001094] LGKILMAIRDA (SEQ ID NO:123)

[001095] HAVSEGLLGK (SEQ ID NO:124)

[001096] VTEMYSGP (SEQ ID NO:125)

[001097] NATKTFREF (SEQ ID NO:126)

[001098] AIRDAGFEI (SEQ ID NO:127)

[001099] AICEWKRLLGPAN (SEQ ID NO:128)

[001100] DHQSRPFF (SEQ ID NO:129)

[001101] AICEWKRLLGPAN (SEQ ID NO:130)

[001102] VDHQSRPF (SEQ ID NO:131)

[001103] PDSFAS (SEQ ID NO:132)

[001104] KAGEIIEIINKAGFTITK (SEQ ID NO:133)

[001105] Immunizing peptides derived from human NME1

[001106] MANCERTFIAIKPDGVQRGLVGEIIKRFE (SEQ ID NO:134)

[001107] VDLKDRPF (SEQ ID NO:135)

[001108] HGSDSVESAEKEIGLWF (SEQ ID NO:136)

[001109] ERTFIAIKPDGVQRGLVGEIIKRFE (SEQ ID NO:137)

[001110] VDLKDRPFFAGLVKYMHSGPVVAMVWEGLN (SEQ ID NO:138)

[001111] NIIHGSDSVESAEKEIGLWFHPEELV (SEQ ID NO:139)

[001112] KPDGVQRGLVGEII (SEQ ID NO:140)

[001113] Immunizing peptide derived from human NME7, but which does not bind NME1

[001114] MLSRKEALDFHVDHQS (SEQ ID NO:141) peptide A1

[001115] SGVARTDASES (SEQ ID NO:142) peptide A2

[001116] DAGFEISAMQMFNMDRVNVE (SEQ ID NO:143) peptide B1

[001117] EVYKGVVTEYHDMVTE (SEQ ID NO:144) peptide B2

[001118] AIFGKTKIQNAVHCTDLPEDGLLEVQYFF (SEQ ID NO:145) peptide B3

[001119] Human NME7 a

[001120] (DNA)

[001121] atgaatcatagtgaaagattcgttttcattgcagagtggtatgatccaaatgcttcacttcttcgacgttatgagcttttatttt
acccaggggatggatctgttgaaatgcatgatgtaaagaatcatcgcacctttttaaagcggaccaaatatgataacctgcacttggaag
atttatttataggcaacaaagtgaatgtcttttctcgacaactggtattaattgactatggggatcaatatacagctcgccagctgggcagta
ggaaagaaaaaacgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataaacaaagctggatttact
ataaccaaactcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttcaatgagctgat
ccagtttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacctgcaaact
ctggagtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggccctgattct
tttgcttctgcggccagagaaatggagttgtttttttccttcaagtggaggttgtgggccggcaaacactgctaaatttactaattgtacctgtt
gcattgttaaaccccatgctgtcagtgaaggactgttgggaaagatcctgatggctatccgagatgcaggttttgaaatctcagctatgca
gatgttcaatatggatcgggttaatgttgaggaattctatgaagtttataaaggagtagtgaccgaatatcatgacatggtgacagaaatgt
attctggcccttgtgtagcaatggagattcaacagaataatgctacaaagacatttcgagaattttgtggacctgctgatcctgaaattgcc
cggcatttacgccctggaactctcagagcaatctttggtaaaactaagatccagaatgctgttcactgtactgatctgccagaggatggc
ctattagaggttcaatacttcttcaagatcttggataattag (SEQ ID NO:146)

[001122] (amino acids)

[001123] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAISKA GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDD AICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFFPSS GGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNV EEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLR PGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDN (SEQ ID NO:147)

[001124] Human NME7 b

[001125] (DNA)

[001126] atgcatgatgtaaagaatcatcgcacctttttaaagcggaccaaatatgataacctgcacttggaagatttatttataggc aacaaagtgaatgtcttttctcgacaactggtattaattgactatggggatcaatatacagctcgccagctgggcagtaggaaagaaaaa acgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataaacaaagctggatttactataaccaaactc aaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttttcaatgagctgatccagtttattaca actggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacctgcaaactctggagtggca cgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggccctgattcttttgcttctgcg gccagagaaatggagttgtttttttccttcaagtggaggttgtgggccggcaaacactgctaaatttactaattgtacctgttgcattgttaaa ccccatgctgtcagtgaaggactgttgggaaagatcctgatggctatccgagatgcaggttttgaaatctcagctatgcagatgttcaata tggatcgggttaatgttgaggaattctatgaagtttataaaggagtagtgaccgaatatcatgacatggtgacagaaatgtattctggccct tgtgtagcaatggagattcaacagaataatgctacaaagacatttcgagaattttgtggacctgctgatcctgaaattgcccggcatttac gccctggaactctcagagcaatctttggtaaaactaagatccagaatgctgttcactgtactgatctgccagaggatggcctattagagg ttcaatacttcttcaagatcttggataattag (SEQ ID NO:148)

[001127] (amino acids)

[001128] MHDVKNHRTFLKRTKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTAR QLGSRKEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRP FFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIRNA AHGPDSFASAAREMELFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIR DAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNN ATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILD N (SEQ ID NO:149)

[001129] Human NME7-AB also known as $NME7_{AB}$

[001130] (DNA)

[001131] atggaaaaaacgctagccctaattaaaccagatgcaatatcaaaggctggagaaataattgaaataataaacaaagct ggatttactataaccaaactcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttttcaat gagctgatccagtttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacc

tgcaaactctggagtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggc cctgattcttttgcttctgcggccagagaaatggagttgtttttttccttcaagtggaggttgtgggccggcaaacactgctaaatttactaatt gtacctgttgcattgttaaaccccatgctgtcagtgaaggactgttgggaaagatcctgatggctatccgagatgcaggttttgaaatctc agctatgcagatgttcaatatggatcgggttaatgttgaggaattctatgaagtttataaaggagtagtgaccgaatatcatgacatggtga cagaaatgtattctggcccttgtgtagcaatggagattcaacagaataatgctacaaagacatttcgagaattttgtggacctgctgatcct gaaattgcccggcatttacgccctggaactctcagagcaatctttggtaaaactaagatccagaatgctgttcactgtactgatctgccag aggatggcctattagaggttcaatacttcttcaagatcttggataattag (SEQ ID NO:150)

[001132] (amino acids)

[001133] MEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQS RPFFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIR NAAHGPDSFASAAREMELFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILM AIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQ NNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFK ILDN (SEQ ID NO:151)

[001134] Human NME7-X1

[001135] (DNA)

[001136] atgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttcaatgagctgatccag tttattacaactggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacctgcaaactctgg agtggcacgcacagatgcttctgaaagcattagagccctctttggaacagatggcataagaaatgcagcgcatggccctgattcttttgc ttctgcggccagagaaatggagttgtttttttccttcaagtggaggttgtgggccggcaaacactgctaaatttactaattgtacctgttgcat tgttaaaccccatgctgtcagtgaaggactgttgggaaagatcctgatggctatccgagatgcaggttttgaaatctcagctatgcagat gttcaatatggatcgggttaatgttgaggaattctatgaagtttataaaggagtagtgaccgaatatcatgacatggtgacagaaatgtatt ctggcccttgtgtagcaatggagattcaacagaataatgctacaaagacatttcgagaattttgtggacctgctgatcctgaaattgcccg gcatttacgccctggaactctcagagcaatctttggtaaaactaagatccagaatgctgttcactgtactgatctgccagaggatggccta ttagaggttcaatacttcttcaagatcttggataattag (SEQ ID NO:152)

[001137] (amino acids)

[001138] MMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDDAICEWKRL LGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFFPSSGGCGPANT AKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFYEVYK GVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTLRAIFG KTKIQNAVHCTDLPEDGLLEVQYFFKILDN* (SEQ ID NO:153)

[001139] Human NME7 a (optimized for E coli expression)

[001140] (DNA)

[001141] atgaatcactccgaacgctttgtttttatcgccgaatggtatgacccgaatgcttccctgctgcgccgctacgaactgct gttttatccgggcgatggtagcgtggaaatgcatgacgttaaaaatcaccgtacctttctgaaacgcacgaaatatgataatctgcatctg gaagacctgtttattggcaacaaagtcaatgtgttctctcgtcagctggtgctgatcgattatggcgaccagtacaccgcgcgtcaactg ggtagtcgcaaagaaaaaacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaaattatcaacaaagcgg gtttcaccatcacgaaactgaaaatgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttcaa tgaactgattcaattcatcaccacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctggg cccggcaaactcaggtgttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcacatg gtccggactcattcgcatcggcagctcgtgaaatggaactgtttttcccgagctctggcggttgcggtccggcaaacaccgccaaattt accaattgtacgtgctgtattgtcaaaccgcacgcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtgatgctggcttt gaaatctcggccatgcagatgttcaacatggaccgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggttaccgaatatca cgatatggttacggaaatgtactccggtccgtgcgtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgtgaattctgtgg tccggcagatccggaaatcgcacgtcatctgcgtccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaacgctgtgcact gtaccgatctgccggaagacggtctgctggaagttcaatacttttttcaaaattctggataat (SEQ ID NO:154)

[001142] (amino acids)

[001143] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAISKA GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDD AICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFFPSS GGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNV EEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLR PGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDNTG (SEQ ID NO:155)

[001144] Human NME7 b (optimized for E coli expression)

[001145] (DNA)

[001146] atgcatgacgttaaaaatcaccgtacctttctgaaacgcacgaaatatgataatctgcatctggaagacctgtttattggc aacaaagtcaatgtgttctctcgtcagctggtgctgatcgattatggcgaccagtacaccgcgcgtcaactgggtagtcgcaaagaaaa aacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaaattatcaacaaagcgggtttcaccatcacgaaac tgaaaatgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttcaatgaactgattcaattcatc accacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctgggcccggcaaactcaggtg ttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcacatggtccggactcattcgcat cggcagctcgtgaaatggaactgtttttcccgagctctggcggttgcggtccggcaaacaccgccaaatttaccaattgtacgtgctgta ttgtcaaaccgcacgcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtgatgctggctttgaaatctcggccatgcag atgttcaacatggaccgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggttaccgaatatcacgatatggttacggaaatg tactccggtccgtgcgtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgtgaattctgtggtccggcagatccggaaat

cgcacgtcatctgcgtccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaacgctgtgcactgtaccgatctgccggaa gacggtctgctggaagttcaatacttttttcaaaattctggataat (SEQ ID NO:156)

[001147] (amino acids)

[001148] MHDVKNHRTFLKRTKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTAR QLGSRKEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRP FFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIRNA AHGPDSFASAAREMELFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIR DAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNN ATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILD NTG (SEQ ID NO:157)

[001149] Human NME7-AB also known as $NME7_{AB}$ (optimized for E coli expression)

[001150] (DNA)

[001151] atggaaaaaacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaaattatcaacaaagcg ggtttcaccatcacgaaactgaaaatgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttca atgaactgattcaattcatcaccacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctgg gcccggcaaactcaggtgttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcacat ggtccggactcattcgcatcggcagctcgtgaaatggaactgtttttcccgagctctggcggttgcggtccggcaaacaccgccaaatt taccaattgtacgtgctgtattgtcaaaccgcacgcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtgatgctggcttt gaaatctcggccatgcagatgttcaacatggaccgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggttaccgaatatca cgatatggttacggaaatgtactccggtccgtgcgtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgtgaattctgtgg tccggcagatccggaaatcgcacgtcatctgcgtccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaacgctgtgcact gtaccgatctgccggaagacggtctgctggaagttcaatacttttttcaaaattctggataat (SEQ ID NO:158)

[001152] (amino acids)

[001153] MEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQS RPFFNELIQFITTGPIIAMEILRDDAICEWKRLLGPANSGVARTDASESIRALFGTDGIR NAAHGPDSFASAAREMELFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILM AIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQ NNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFK ILDNTG (SEQ ID NO:159)

[001154] Human NME7-X1 (optimized for E coli expression)

[001155] (DNA)

[001156] atgatgatgctgagccgtaaagaagccctggattttcatgtcgaccaccagtctcgcccgtttttcaatgaactgattcaa ttcatcaccacgggtccgattatcgcaatggaaattctgcgtgatgacgctatctgcgaatggaaacgcctgctgggcccggcaaactc aggtgttgcgcgtaccgatgccagtgaatccattcgcgctctgtttggcaccgatggtatccgtaatgcagcacatggtccggactcatt

cgcatcggcagctcgtgaaatggaactgtttttcccgagctctggcggttgcggtccggcaaacaccgccaaatttaccaattgtacgt gctgtattgtcaaaccgcacgcagtgtcagaaggcctgctgggtaaaattctgatggcaatccgtgatgctggctttgaaatctcggcca tgcagatgttcaacatggaccgcgttaacgtcgaagaattctacgaagtttacaaaggcgtggttaccgaatatcacgatatggttacgg aaatgtactccggtccgtgcgtcgcgatggaaattcagcaaaacaatgccaccaaaacgtttcgtgaattctgtggtccggcagatccg gaaatcgcacgtcatctgcgtccgggtaccctgcgcgcaatttttggtaaaacgaaaatccagaacgctgtgcactgtaccgatctgcc ggaagacggtctgctggaagttcaatacttttttcaaaattctggataat (SEQ ID NO:160)

[001157] (amino acids)

[001158] MMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDDAICEWKRL LGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAREMELFFPSSGGCGPANT AKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVEEFYEVYK GVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLRPGTLRAIFG KTKIQNAVHCTDLPEDGLLEVQYFFKILDNTG (SEQ ID NO:161)

[001159] DM10 domain of NME7

[001160] (amino acids)

[001161] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRK (SEQ ID NO:162)

[001162] a fragment or variation of PSMGFR peptide

[001163] SNIKFRPGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRY (SEQ ID NO:163);

[001164] a fragment or variation of PSMGFR peptide

[001165] SVVVQLTLAFREGTINVHDVETQFNQYKTEAASRY (SEQ ID NO:164);

[001166] a fragment or variation of PSMGFR peptide

[001167] VQLTLAFREGTINVHDVETQFNQY (SEQ ID NO:165);

[001168] a fragment or variation of PSMGFR peptide

[001169] SNIKFRPGSVVVQLTLAFREGTIN (SEQ ID NO:166);

[001170] a fragment or variation of PSMGFR peptide

[001171] SNIKFRPGSVVVQLTLAFREGTINVHDVETQFNQYKTE (SEQ ID NO:167);

[001172] a fragment or variation of PSMGFR peptide

[001173] VQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISDVSVSDVP (SEQ ID NO:168).

[001174] Cys at residue 14 is mutated to Ser of NME7B peptide 3 (B domain):

[001175] AIFGKTKIQNAVHSTDLPEDGLLEVQYFF (SEQ ID NO:169)

[001176] N-10 peptide

[001177] QFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGA (SEQ ID NO:170)

[001178] C-10 peptide

[001179] GTINVHDVETQFNQYKTEAASRYNLTISDVSVSDV (SEQ ID NO:171)

EXAMPLES

[001180] Example 1 - Components of minimal serum-free base ("MM") (500mls)

[001181] 400 ml DME/F12/GlutaMAX I (Invitrogen# 10565-018)

[001182] 100 ml Knockout Serum Replacement (KO-SR, Invitrogen# 10828-028)

[001183] 5 ml 100x MEM Non-essential Amino Acid Solution (Invitrogen# 11140-050)

[001184] 0.9 ml (0.1mM) β-mercaptoethanol (55mM stock, Invitrogen# 21985-023.

[001185] Example 2 - Generation of Protein Constructs

[001186] For generating recombinant NME7, first, constructs were made to make a recombinant NME7 that could be expressed efficiently and in soluble form. The first approach was to make a construct that would encode the native NME7 (a) or an alternative splice variant NME7 (b), which has an N-terminal deletion. In some cases, the constructs carried a histidine tag or a strep tag to aid in purification. NME7-a, full-length NME7 expressed poorly in *E. coli* and NME7-b did not express at all in *E. coli*. However, a novel construct was made in which the DM10 sequence was deleted and the NME7 comprised essentially the NDPK A and B domains having a calculated molecular weight of 33kDa.

[001187] This novel $NME7_{AB}$ expressed very well in *E. coli* and existed as the soluble protein. $NME7_{AB}$ was first purified over an NTA-Ni column and then further purified by size exclusion chromatography (FPLC) over a Sephadex 200 column. Fractions were collected and tested by SDS-PAGE to identify fractions with the highest and purest expression of $NME7_{AB}$. The FPLC trace for the combined fractions that were the most pure were combined. The purified $NME7_{AB}$ protein was then tested and shown to fully support the growth of human stem cells and further reverts them to the most naïve, pre-X-inactivation state. The purified $NME7_{AB}$ was also shown to accelerate the growth of cancer cells.

[001188] Example 3 - ELISA assay showing $NME7_{AB}$ simultaneously binds to two MUC1* extra cellular domain peptides

[001189] Results are shown in Figure 1. The PSMGFR peptide bearing a C-terminal Cysteine (PSMGFR-Cys) was covalently coupled to BSA using Imject Maleimide activated BSA kit (Thermo Fisher). PSMGFR-Cys coupled BSA was diluted to 10ug/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 washed 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 NME7, diluted in PBS-T + 1% BSA, was added at different concentrations. After 1h at RT the plate was washed 3x with PBS-T and anti-NM23-H7 (B-9, Santa Cruz Biotechnology), diluted in PBS-T + 1% BSA, was added at 1/500 dilution. After 1h at RT the plate was washed 3x with PBS-T and goat anti mouse-HRP, diluted in PBS-T + 1% BSA, was added at 1/3333 dilution. After 1h at RT the plate was washed 3x with PBS-T and binding of NME7 was measured at 415nm using ABTS solution (Pierce).

[001190] ELISA MUC1* dimerization: The protocol for NME7 binding was used, and NME7 was used at 11.6ug/mL.

[001191] After 1h at RT the plate was washed 3x with PBS-T and His-Tagged PSMGFR peptide (PSMGFR-His) or biotinylated PSMGFR peptide (PSMGFR-biotin), diluted in PBS-T + 1% BSA, was added at different concentration. After 1h at RT the plate was washed 3x with PBS-T and anti-Histag-HRP (Abcam) or streptavidin-HRP (Pierce), diluted in PBS-T + 1% BSA, was added at a concentration of 1/5000. After 1h at RT the plate was washed 3x with PBS-T and binding of PSMGFR peptide to NME7 already bound to another PSMGFR peptide (which could not signal by anti-His antibody or by streptavidin) coupled BSA was measured at 415nm using a ABTS solution (Pierce).

[001192] Example 4 - Functional testing of human recombinant $NME7_{AB}$

[001193] For testing recombinant $NME7_{AB}$ for ability to maintain pluripotency and inhibit differentiation, a soluble variant of NME7, $NME7_{AB}$, was generated and purified. Human stem cells (iPS cat# SC101a-1, System Biosciences) were grown per the manufacturer's directions in 4ng/ml bFGF over a layer of mouse fibroblast feeder cells for four passages. These source stem cells were then plated into 6-well cell culture plates (Vita™, Thermo Fisher) that had been coated with 12.5 ug/well of a monoclonal anti-MUC1* antibody, MN-C3. Cells were plated at a density of 300,000 cells per well. The base media was Minimal Stem Cell Media consisting of: 400 ml DME/F12/GlutaMAX I (Invitrogen# 10565-018), 100 ml Knockout Serum Replacement (KO-SR, Invitrogen# 10828-028), 5 ml 100x MEM Non-essential Amino Acid Solution (Invitrogen# 11140-050) and 0.9 ml (0.1mM) β-mercaptoethanol (55mM stock, Invitrogen# 21985-023). The base media can be any media. In a preferred embodiment, the base media is free of other growth factors and cytokines. To the base media was added either 8nM of $NME7_{AB}$ or 8nM NM23-H1 refolded and purified as stable dimers. Media was changed every 48 hours and due to accelerated growth, had to be harvested and passaged at Day 3 post-plating. Comparable pluripotent stem cell growth was achieved when stem cells were grown in NM23-H1 dimers or in NME7 monomers.

[001194] NME7 and NM23-H1 (NME1) dimers both grew pluripotently and had no differentiation even when 100% confluent. As can be seen in the photos, NME7 cells grew faster than the cells grown in NM23-H1 dimers. Cell counts at the first harvest verified that culture in NME7 produced 1.4-times more cells than culture in NM23-H1 dimers. ICC staining for the typical pluripotent markers confirmed that $NME7_{AB}$ fully supported human stem cell growth, pluripotency, and resisted differentiation.

[001195] The NME7 species of ~30-33kDa may be an alternative splice isoform or a post translational modification such as cleavage, which may enable secretion from the cell.

[001196] Example 5 - Inducing transition of cancer cells to metastatic cancer cells by culturing cells under conditions that revert stem cells to a more naïve state

[001197] Cancer cells are normally cultured in a serum-containing media such as RPMI. We discovered that culturing cancer cells in the presence of reagents that make stem cells revert to a more naïve state, makes the cancer cells transform to a more metastatic state.

[001198] We demonstrated that $NME7_{AB}$, human NME1 dimers, bacterial NME1 dimers, NME7-X1 and "2i" inhibitors were each able to transform regular cancer cells into metastatic cancer cells, which are also called cancer stem cells "CSCs" or tumor initiating cells "TICs". 2i is the name given to two biochemical inhibitors that researchers found made human stem cells revert to a more naïve state. 2i are MEK and GSK3-beta inhibitors PD0325901 and CHIR99021, which are added to culture medium to final concentrations of about 1mM and 3mM, respectively.

[001199] $NME7_{AB}$ and NME7-X1 are at a final concentration of about 4nM when added to separate batches of minimal medium to make cancer cells transform to metastatic cells, although lower and higher concentrations also work well in the range of about 1nM to 16nM. Human or bacterial NME1 dimers are used at a final concentration of 4nM to 32nM, with 16nM typically used in these experiments, wherein the human NME bears the S120G mutation. Lower concentrations may be required if using wild type. It is not intended that these exact concentrations are important. It is important that the NME1 proteins are dimers and the range of concentrations over which this happens is in the low nanomolar range although certain mutations allow higher concentrations to remain as dimers.

[001200] Similarly, the concentrations of NME7 proteins can vary. $NME7_{AB}$ and NME7-X1 are monomers and concentrations used to transform cancer cells to metastatic cells should allow the proteins to remain as monomers. Various molecular markers have been proposed as being indicators of metastatic cancer cells. Different cancer types may have different molecules that are up-regulated. For example, the receptor CXCR4 is up-regulated in metastatic breast

cancers while E-cadherin, also known as CHD1, is up-regulated more in metastatic prostate cancers.

[001201] In addition to these specific metastasis markers, typical markers of pluripotency such as OCT4, SOX2, NANOG, and KLF4 are up-regulated as cancers become metastatic. The starting cancer cells and the later metastatic cancer cells can be assayed by PCR to measure expression levels of these genes.

[001202] Figure 2 shows a graph of RT-PCR measurements of T47D breast cancer cells that were cultured in a media that contained $NME7_{AB}$. A rho I kinase inhibitor, ROCi, ROCKi or Ri, was added to prevent the transformed cells from floating off the plate. Expression levels of various metastatic markers as well as pluripotent stem cell markers were measured for the parent cells and for the $NME7_{AB}$ cultured cells. The results show that the floater cells express higher amounts of metastatic and pluripotency markers compared to the cells that received ROCi. We reasoned it was because those measurements were the average of cells that did not transform and those that did but the ROCi made them remain adherent. This can clearly be seen in figures wherein "-Ri" means adherent cells that did not receive ROCi and so were not mixed with the highly metastatic cells that float.

[001203] Prostate cancer cells also transitioned to a more metastatic state when cultured in media containing NM23, aka NME1, or $NME7_{AB}$. Here we show that for every cell line tested so far, culture in $NME7_{AB}$, human NME1 dimers, or bacterial NMEs that have high sequence homology to human, induces transition to a more metastatic state.

[001204] Figure 4 shows a graph of RT-PCR measurements of expression levels of metastatic and pluripotency markers for breast cancer cells that are cultured in media containing either 2i inhibitors, $NME7_{AB}$ or both. As can be seen, 2i inhibitors are also able to induce the transition of cancer cells to a more metastatic state. Ovarian cancer cell lines SK-OV3, OV-90, pancreatic cancer cell lines CAPAN-2 and PANC-1, breast cancer cell line MDA-MB all displayed the morphological transition of going from adherent to non-adherent when cultured in $NME7_{AB}$ and or 2i inhibitors.

[001205] Figure 20 shows graphs of RT-PCR measurement of metastatic or pluripotency markers for various cancer cell lines cultured for 72 or 144 hours in $NME7_{AB}$. Figure 20A shows that SK-OV3 cells increase expression of metastatic markers CHD1, SOX2 and NME7-X1 when cultured in $NME7_{AB}$. Figure 20B shows that OV-90 cells increase expression of metastatic markers CXCR4 and NME7-X1 after culture in $NME7_{AB}$.

[001206] Example 6 - Demonstration that cancer cells cultured in NME7 become metastatic

[001207] A functional test of whether or not a population of cancer cells is metastatic is to implant very low numbers, e.g. 200, of the cells in immuno-compromised mice and see if they develop into a tumor. Typically 5-6 million cancer cells are required to form a tumor in an immuno-compromised mouse. We showed that as few as 50 of the NME-induced metastatic cancer cells formed tumors in mice. In addition, mice that were injected throughout the test period with human $NME7_{AB}$, NME1, or NME7-X1 developed remote metastases.

[001208] T47D human breast cancer cells were cultured in standard RPMI media for 14 days with media changes every 48 hours and passed by trypsinization when approximately 75% confluent. The cells were then plated into 6-well plates and cultured in minimal stem cell media (see Example 1) that was supplemented with 4nM $NME7_{AB}$. Media was changed every 48 hours. By about Day 4, some cells become detached from the surface and float. Media is carefully changed so as to retain the "floaters" as these are the cells that have the highest metastatic potential as evidenced by RT-PCR measurement of metastatic markers. On Day 7 or 8, the floaters are harvested and counted. Samples are retained for RT-PCR measurement. The key marker measured is CXCR4 which is up-regulated by 40-200 times after being briefly cultured in $NME7_{AB}$.

[001209] The freshly harvested floater metastatic cells are xenografted into the flank of female nu/nu athymic mice that have been implanted with 90-day slow release estrogen pellets. Floater cells were xenografted as 10,000, 1,000, 100 or 50 cells each. Half of the mice in each group of 6 were also injected daily with 32nM $NME7_{AB}$ near the original implantation site. The parent T47D cells that were cultured in RPMI media without $NME7_{AB}$ were also implanted into mice as 6 million, 10,000 or 100 as controls. Mice implanted with the NME7-induced floater cells developed tumors even when as few as 50 cells were implanted. Mice that were implanted with the floater cells and that received daily injections of $NME7_{AB}$ also developed remote tumors or remote metastases in various organs. 11 out of the 12 mice, or 92%, that were injected with human $NME7_{AB}$ after implantation of the $NME7_{AB}$ cultured cancer cells, developed tumors at the injection site. Only 7 out of the 12 mice, or 58%, that were not injected with human $NME7_{AB}$ after implantation developed tumors. 9 out of the 11 mice, or 82%, that got tumors and were injected with human $NME7_{AB}$ developed multiple tumors remote from the injection site. None of the mice that were not injected with $NME7_{AB}$ developed multiple, visible tumors.

[001210] After sacrifice, RT-PCR and Western blots showed that the remote bumps on the mice injected with $NME7_{AB}$ were indeed human breast tumors. Similar analysis of their organs showed that in addition to remote bumps, mice had randomly metastasized to the liver and lung

with human breast cancer characteristic of the human breast cancer cells that were implanted. As expected, only the mice implanted with 6 million cells grew tumors.

[001211] Several experiments like the one described above were performed with essentially the same results. In each experiment, there were either 24 or 52 mice, including all proper controls.

[001212] Example 7 - Peptides selected because their sequence is unique to NME7, A1, A2, B1, B2 and B3, inhibit the binding of NME7 species to MUC1* extracellular domain peptide.

[001213] NME7 peptides were selected as immunizing agents for antibody production. NME7 peptides A1, A2, B1, B2 and B3 (Fig. 9) were chosen using a process of sequence alignment among human NME1, human NME7 and several bacterial NMEs that were homologous to human NME1 or human NME7. Five regions that had high sequence homology among all were identified. However, to prevent selecting peptides that would give rise to antibodies that would inhibit human NME1 as well as human NME7, we chose NME7 sequences that were adjacent to the homologous regions wherein those peptides had sequences that were different from human NME1. We did ELISA assays to see if the peptides on their own could bind to a synthetic MUC1* peptide on the surface and inhibit the binding of human NME7 or human NME1 to the immobilized peptide (Fig. 11). Figure 11 shows that the peptides inhibited the binding of NME7 and NME1 to the immobilized PSMGFR peptide. Recall that each of the NME7 A domain and B domain can bind to a PSMGFR peptide. Therefore complete inhibition of $NME7_{AB}$ binding to a PSMGFR peptide cannot be accomplished with a single antibody or peptide that is derived from just one domain. This showed that those regions from which the peptides were derived were the regions that interacted with MUC1* and would give rise to antibodies that would bind to those regions of NME7 and inhibit its binding to MUC1* receptor.

[001214] In another experiment, the free peptides A1, A2, B1, B2 and B3 were added to cancer cells in culture that were undergoing transition to a more metastatic state by culturing in either $NME7_{AB}$ or 2i. Figure 14 shows a table of scientist observations when cancer cells are grown in either $NME7_{AB}$ or 2i inhibitors, and shows that the free peptides inhibited the morphological change from adherent cells to floaters, which for breast cancer cells is directly correlated to increased expression of metastatic markers, especially CXCR4. RT-PCR measurements confirm that the $NME7_{AB}$ peptides inhibited the increase in expression of metastasis marker CXCR4.

[001215] Figure 15 shows a graph of RT-PCR measurements of CXCR4 expression in T47D breast cancer cells that were grown in either $NME7_{AB}$ or 2i inhibitors, each of which transform cancer cells to a more metastatic state, and the inhibitory effect of NME7-derived peptides, A1, A2, B1, B2 and B3, on the metastatic transformation. Figure 32 shows a table of recorded RNA levels in samples that were used for RT-PCR measurement of CXCR4 in Figure 15 as well as the threshold cycle number for CXCR4 expression as well as for the control housekeeping gene.

[001216] Example 8 - Anti-NME7 antibodies specifically bind to human NME7 but not to human NME1

[001217] A standard ELISA assay was performed to determine whether or not the NME7 antibodies we generated by immunization with $NME7_{AB}$ peptides A1, A2, B1, B2, and B3 would bind specifically to $NME7_{AB}$, but not to human NME1 as it has healthy functions and it may be detrimental to a human to block it with an antibody. The ELISAs of Fig. 24-25 show that all of the NME7 antibodies that were generated from peptides A1, A2, B1, B2, and B3 bind to human $NME7_{AB}$ (Fig. 24) but not to human NME1 (Fig. 25). The peptides used to generate these antibodies are common to both $NME7_{AB}$ and NME7-X1. This assays show that the antibodies generated from peptides A1, A2, B1, B2, and B3 specifically bind to $NME7_{AB}$ and by extension will bind to NME7-X1.

[001218] **NME7A peptide 1** (A domain): MLSRKEALDFHVDHQS (SEQ ID NO:141)

[001219] **NME7A peptide 2** (A domain)**:** SGVARTDASES (SEQ ID NO:142)

[001220] **NME7B peptide 1** (B domain)**:** DAGFEISAMQMFNMDRVNVE (SEQ ID NO:143)

[001221] **NME7B peptide 2** (B domain)**:** EVYKGVVTEYHDMVTE (SEQ ID NO:144)

[001222] **NME7B peptide 3** (B domain)**:** AIFGKTKIQNAVHCTDLPEDGLLEVQYFF (SEQ ID NO:145)

[001223] Example 9 - Anti-NME7 specific antibodies and the peptides that generated them inhibit cancer cell growth

[001224] Rabbits were immunized with NME7 peptides A1, A2, B1, B2, and B3 and antibodies were generated, collected and purified over a column to which the immunizing peptide had been conjugated. T47D breast cancer cells were plated and cultured according to ATCC protocols in RPMI media supplemented with serum. Antibodies generated from immunization with peptides A1, A2, B1, B2, and B3 were added at the concentrations indicated in Figure 12. Immunizing peptides A1, A2, B1, B2, and B3, and the PSMGFR extracellular domain peptide of MUC1*, "FLR" here, were also added separately to growing T47D breast

cancer cells. Taxol and the E6 anti-MUC1* Fab were added as controls. The graph of Figure 12 shows that the antibodies generated, as well as the free peptides, potently inhibited the growth of the cancer cells. Note the comparison to inhibition using Taxol, which is a chemotherapy agent that kills healthy and cancer cells alike. Also, for comparison, a polyclonal antibody generated using a large stretch of NME7 from amino acid 100 to 376 is shown. Although this antibody is a potent inhibitor of cancer growth it could have non-specific effects since it can bind to NME1 as well as to NME7.

[001225] In a similar experiment, combinations of the antibodies generated from immunization with peptides A1, A2, B1, B2, and B3 as well as the peptides themselves were added to growing cancer cells at the concentrations indicated. The graphs of cell growth shown in Figure 13 show that the combinations of antibodies and peptides potently inhibited the growth of cancer cells. In these two experiments, the cells were MUC1* positive breast cancer cells.

[001226] Example 10 - Anti-NME7 antibodies inhibit the transition of cancer cells to metastatic cancer cells

[001227] Cancer cells transform to a more metastatic state when cultured in the presence of agents that revert stem cells to a more naïve state. We have demonstrated that culturing cancer cells in $NME7_{AB}$, human NME1 dimers, bacterial NME1 dimers or MEK and GSK3-beta inhibitors, called "2i", causes the cells to become more metastatic. As the cells transition to a more metastatic state, they become non-adherent and float off of the culture plate. These floating cells, "floaters" were collected separately from those that were adherent and were shown to: a) express much higher levels of metastatic genes; and b) when xenografted into mice, the floater cells were able to generate tumors when implanted at very low numbers. RT-PCR measurement of specific metastatic markers such as CXCR4 in breast cancers, CHD1 in prostate cancer, and other pluripotent stem cell markers such as OCT4, SOX2, NANOG, KLF4, c-Myc and others were dramatically over-expressed in cancer cells that were cultured in $NME7_{AB}$ and most over-expressed in the cells that became non-adherent, called "floaters" here and in figures.

[001228] Here we show that the NME7-specific antibodies, generated by immunization with NME7-derived peptides A1, A2, B1, B2 and B3, as well as the peptides themselves, inhibit the transition from cancer cell to metastatic cancer cells. In the first of these experiments, the antibodies generated by immunization with A1, A2, B1, B2 and B3 were tested for their ability to inhibit the metastatic transition induced by culture of T47D breast cancer cells in $NME7_{AB}$ or in 2i inhibitors. The most striking observation was that the antibodies and the peptides

dramatically reduced the number of floater cells, which was the first indication that the antibodies and peptides had inhibited the transformation to metastatic cancer cells. In particular, cells to which the antibody generated from immunization with the B3 peptide barely generated any floater cells.

[001229] Figure 14 shows the recorded observations of the percentage of floater cells visible for each antibody relative to the control wells that did not receive any antibody treatment. mRNA was extracted from both the floater cells and the adherent cells. RT-PCR was used to measure expression levels of metastatic markers, including CXCR4. Treatment with the anti-NME7 antibodies greatly reduced the amount of metastatic markers, such as CXCR4, indicating the antibodies inhibited the transition to metastatic cancer. (See Figure 15). Notably, the antibody generated by immunization with peptide B3, aka antibody #61, essentially completely inhibited the transition to a more metastatic state. Figure 15B shows that breast cancer cells that were treated with the $NME7_{AB}$ peptides, A1, A2, B1, B2 and B3, alone were able to potently inhibit the transition to a more metastatic state induced by culturing the cells in a media containing the 2i inhibitors. Peptide B3 was especially effective as was antibody #61 that it generated. Figure 15C shows the same graph but with the Y-axis expanded to show the peptide inhibition of metastatic markers. The amount of mRNA, which indicates cell viability and growth, was measured. Cells that were treated with antibody had much less mRNA, indicating that in addition to inhibiting the transition to a more metastatic state, the anti- $NME7_{AB}$ antibodies inhibited the growth of the cancer cells. Figure 16 shows a table of the amounts of RNA recovered for the inhibition experiment shown in Figure 15A.

[001230] Example 11 - Anti-NME7 antibodies generated with NME7-derived peptides A1, A2, B1, B2 and B3 identify novel NME7 species not detectable using any commercially available antibodies.

[001231] As is known to those skilled in the art, some antibodies recognize a linear portion of the target protein and can be used in Western blot assays while other antibodies recognize a non-linear conformational motif and can be used in pull-down or immunoprecipitation assays. Previous to this application, cleaved NME7 or isoform NME7-X1 was not known to exist. Using antibodies that were commercially available at the time of filing shows that existing antibodies could not specifically detect these important NME7 species. B9 (Santa Cruz Biotechnology) is a monoclonal antibody raised against NME7 amino acids 100-376. Figure 19D-19F shows that it only detects full-length 42kDa NME7. Another commercially available antibody, H278, is a rabbit polyclonal raised against NME7 amino acids 100-376, which includes amino acid sequences that are not unique to NME7. Figure 19D-19F shows that this

antibody also stains NME1, which is 17kDa as well as full-length NME7 and other bands that do not appear to be specific to $NME7_{AB}$.

[001232] NME7 antibodies generated by immunization with $NME7_{AB}$ peptides A1, A2, B1, B2 or B3 identify new NME7 species including the full-length 42kDa protein, a ~33kDa NME7 species that may be a cleavage product or alternative isoform, a ~30kDa NME7 species that may be a cleavage product or alternative isoform, wherein the ~30kDa species appears to be NME7-X1. Figure 19A-C shows that antibodies generated by peptides A1, B1 and B3 identify the secreted forms of NME7, $NME7_{AB}$ and NME7-X1 in a wide range of cancer cell lines, including T47D breast cancer cells, PC3 and DU145 prostate cancer cells, HEK293 fetal liver cells, and leukemia cells IM-9, K562, and MV411.

[001233] Example 12 – Generation of Anti-NME7 antibodies

[001234] A synthetic peptide having the sequence of the B3 region of NME7, AIFGKTKIQNAVHCTDLPEDGLLEVQYFFC, was used to immunize rabbits. Antibodies that resulted from immunization with NME7 peptide B3 inhibited the growth of MUC1* positive cancer cells and also inhibited the formation of cancer stem cells, which are characterized by upregulation of metastatic markers, ability to grow anchorage independently, and are able to form tumors in animals from as few as 200 cells, whereas regular cancer cells typically require implantation of about 4 million cells for tumor engraftment.

[001235] In some cases, the NME7 B3 peptide was made with a C14A or C14V mutation. This sequence more reproducibly generated anti-NME7 antibodies.

[001236] Monoclonal antibodies were generated in mice according to standard methods by immunizing with NME7 B3, B3 with C14A mutation, or B3 with C14V mutation. The antibodies listed were selected because of their ability to bind to NME7, NME7-X1, $NME7_{AB}$, but importantly did not bind to NME1, which is thought to be required for some normal cellular functions. These antibodies also bind to the NME7 derived peptides B3, B3 with C14A mutation, and B3 with C14V mutation.

[001237] Experiments showed that these anti-NME7 antibodies inhibited the binding of NME7 to the MUC1* extra cellular domain, but did not block the binding of NME1 to the MUC1* extra cellular domain peptide. Further, the antibodies inhibited the formation of cancer stem cells.

[001238] Monoclonal antibody 8F9A4A3

[001239] Heavy chain variable region sequence

[001240] H-1,8,9,10,11

[001241] gtccagctgcaacagtctggacctgaactggtgaagcctggggcttcagtgaagatatcctgcaagacttctggaaac acattcactgaatacaccatgcactgggtgaagcagagccatggaaagagccttgagtggattggaggttttaatcctaacaatggtgtt actaactacaaccagaagttcaagggcaaggccacattgactgtagacaagtcctccagcacagcctacatggagctccgcagcctg acatctgaggattctgcagtctattactgtgcaagacggtactaccatagtctctacgtgttttactttgactactggggccaaggcaccac tctcacagtctcctca (SEQ ID NO:386)

[001242] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001243] VQLQQSGPELVKPGASVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGF NPNNGVTNYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSLYVF YFDYWGQGTTLTVSS (SEQ ID NO:387)

[001244] Heavy chain variable region CDR1:

[001245] NTFTEYTMH (SEQ ID NO:388)

[001246] Heavy chain variable region CDR2:

[001247] GFNPNNGVTNYNQKFKG (SEQ ID NO:389)

[001248] Heavy chain variable region CDR3:

[001249] RYYHSLYVFYFDY (SEQ ID NO:390)

[001250] Light chain variable region sequence

[001251] K-3,4,9,10,11

[001252] gaaacaactgtgacccagtctccagcatccctgtccatggctataggagaaaaagtcaccatcagatgcataaccag cactgatattgatgatgatatgaactggtaccagcagaagccaggggaacctcctaagctccttatttcagaaggcaatactcttcgtcct ggagtcccatcccgattctccagcagtggctatggtacagattttgtttttacaattgaaaacatgctctcagaagatgttgcagattactac tgtttgcaaagtgataacttgcctctcacgttcggctcggggacaaagttggaaataaaacgg (SEQ ID NO:391)

[001253] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001254] ETTVTQSPASLSMAIGEKVTIRCITSTDIDDDMNWYQQKPGEPPKLLISEGN TLRPGVPSRFSSSGYGTDFVFTIENMLSEDVADYYCLQSDNLPLTFGSGTKLEIKR (SEQ ID NO:392)

[001255] Light chain variable region CDR 1:

[001256] ITSTDIDDDMN (SEQ ID NO:393)

[001257] Light chain variable region CDR2:

[001258] EGNTLRP (SEQ ID NO:394)

[001259] Light chain variable region CDR3:

[001260] LQSDNLPLT (SEQ ID NO:395)

[001261] **Monoclonal antibody 5D9E2B11**

[001262] Heavy chain variable region sequence

[001263] H-1,4,7,8,12

[001264] gtccagctgcaacagtctggacctgatctggtgaagcctgggacttcagtgaagatatcctgtaagacttctggaaaca cattcactgaatacaccatgcactgggtgaagcagagccatggaaagagccttgagtggattggaggttttaatcctaacaatggtgtta ctaactacaaccagaagttcaagggcaaggccacattgactgtagacaagtcctccagcacagcctacatggagctccgcagcctga catctgaggattctgcagtctattactgtgcaagacgttactaccatagtacctacgtgttctactttgactcctggggccaaggcaccact ctcacagtctcctca (SEQ ID NO:396)

[001265] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001266] VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGF NPNNGVTNYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVF YFDSWGQGTTLTVSS (SEQ ID NO:397)

[001267] Heavy chain variable region CDR1:

[001268] NTFTEYTMH (SEQ ID NO:398)

[001269] Heavy chain variable region CDR2:

[001270] GFNPNNGVTNYNQKFKG (SEQ ID NO:399)

[001271] Heavy chain variable region CDR3:

[001272] RYYHSTYVFYFDS (SEQ ID NO:400)

[001273] Light chain variable region sequence

[001274] K-3,4,5,6,12

[001275] gatatccagatgacacagactacatcctccctgtctgcctctctgggagacagagtcaccatcagttgcagtgcaagtc agggcattagcaattatttaaactggtttcagcagaaaccagatggaactattaagctcctgatctattacacatcaagtttacattcagga gtcccatcaaggttcagtggcagtgggtctgggacagattattctctcaccatcagtaatgtggaacctgaagatattgccacttactattg tcagcagtatagtaagcttccttacacgttcggaggggggaccaagctggagataaaacgg (SEQ ID NO:401)

[001276] Translated protein:

[001277] DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTS SLHSGVPSRFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR (SEQ ID NO:402)

[001278] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001279] DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTS SLHSGVPSRFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR (SEQ ID NO:403)

[001280] Light chain variable region CDR 1:

[001281] SASQGISNYLN (SEQ ID NO:404)

[001282] Light chain variable region CDR2:

[001283] YTSSLHS (SEQ ID NO:405)

[001284] Light chain variable region CDR3:

[001285] QQYSKLPYT (SEQ ID NO:406)

[001286] **Monoclonal antibody 5D9E10E4**

[001287] Heavy chain variable region sequence

[001288] H-2,4,7,10,12

[001289] gtccagctgcaacagtctggacctgatctggtgaagcctgggacttcagtgaagatatcctgtaagacttctggaaaca cattcactgaatacaccatgcactgggtgaagcagagccatggaaagagccttgagtggattggaggttttaatcctaacaatggtgtta ctaactacaaccagaagttcaagggcaaggccacattgactgtagacaagtcctccagcacagcctacatggagctccgcagcctga catctgaggattctgcagtctattactgtgcaagacgttactaccatagtacctacgtgttctactttgactcctggggccaaggcaccact ctcacagtctcctca (SEQ ID NO:407)

[001290] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001291] VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGF NPNNGVTNYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVF YFDSWGQGTTLTVSS (SEQ ID NO:408)

[001292] Heavy chain variable region CDR1:

[001293] NTFTEYTMH (SEQ ID NO:409)

[001294] Heavy chain variable region CDR2:

[001295] GFNPNNGVTNYNQKFKG (SEQ ID NO:410)

[001296] Heavy chain variable region CDR3:

[001297] RYYHSTYVFYFDS (SEQ ID NO:411)

[001298] Light chain variable region sequence

[001299] K-2,6,8,14,15

[001300] gatatccagatgacacagactacatcctccctgtctgcctctctgggagacagagtcaccatcagttgcagtgcaagtc agggcattagcaattatttaaactggtttcagcagaaaccagatggaactattaagctcctgatctattacacatcaagtttacattcagga gtcccatcaaggttcagtggcagtgggtctgggacagattattctctcaccatcagtaatgtggaacctgaagatattgccacttactattg tcagcagtatagtaagcttccttacacgttcggaggggggaccaagctggagataaaacgg (SEQ ID NO:412)

[001301] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001302] DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTS SLHSGVPSRFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR (SEQ ID NO:413)

[001303] Light chain variable region CDR 1:

[001304] SASQGISNYLN (SEQ ID NO:414)

[001305] Light chain variable region CDR2:

[001306] YTSSLHS (SEQ ID NO:415)

[001307] Light chain variable region CDR3:

[001308] QQYSKLPYT (SEQ ID NO:416)

[001309] **Monoclonal antibody 5D9G2C4**

[001310] Heavy chain variable region sequence

[001311] H-4,9,10,11,13

[001312] gtccagctgcaacagtctggacctgatctggtgaagcctgggacttcagtgaagatatcctgtaagacttctggaaaca cattcactgaatacaccatgcactgggtgaagcagagccatggaaagagccttgagtggattggaggttttaatcctaacaatggtgtta ctaactacaaccagaagttcaagggcaaggccacattgactgtagacaagtcctccagcacagcctacatggagctccgcagcctga catctgaggattctgcagtctattactgtgcaagacgttactaccatagtacctacgtgttctactttgactcctggggccaaggcaccact ctcacagtctcctca (SEQ ID NO:417)

[001313] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001314] VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGF NPNNGVTNYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVF YFDSWGQGTTLTVSS (SEQ ID NO:418)

[001315] Heavy chain variable region CDR1:

[001316] NTFTEYTMH (SEQ ID NO:419)

[001317] Heavy chain variable region CDR2:

[001318] GFNPNNGVTNYNQKFKG (SEQ ID NO:420)

[001319] Heavy chain variable region CDR3:

[001320] RYYHSTYVFYFDS (SEQ ID NO:421)

[001321] Light chain variable region sequence

[001322] K-4,6,7,8,10

[001323] gatatccagatgacacagactacatcctccctgtctgcctctctgggagacagagtcaccatcagttgcagtgcaagtc agggcattagcaattatttaaactggtttcagcagaaaccagatggaactattaagctcctgatctattacacatcaagtttacattcagga gtcccatcaaggttcagtggcagtgggtctgggacagattattctctcaccatcagtaatgtggaacctgaagatattgccacttactattg tcagcagtatagtaagcttccttacacgttcggaggggggaccaagctggagataaaacgg (SEQ ID NO:422)

[001324] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001325] DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTSSLHSGVPSRFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR (SEQ ID NO:423)

[001326] Light chain variable region CDR 1:

[001327] SASQGISNYLN (SEQ ID NO:424)

[001328] Light chain variable region CDR2:

[001329] YTSSLHS (SEQ ID NO:425)

[001330] Light chain variable region CDR3:

[001331] QQYSKLPYT (SEQ ID NO:426)

[001332] **Monoclonal antibody 5F3A5D4**

[001333] Heavy chain variable region sequence

[001334] H-2,3,4,13,15

[001335] gtccagctgcaacagtctggacctgatctggtgaagcctgggacttcagtgaagatatcctgtaagacttctggaaacacattcactgaatacaccatgcactgggtgaagcagagccatggaaagagccttgagtggattggaggttttaatcctaacaatggtgttactaactacaaccagaagttcaagggcaaggccacattgactgtagacaagtcctccagcacagcctacatggagctccgcagcctgacatctgaggattctgcagtctattactgtgcaagacgttactaccatagtacctacgtgttctactttgactcctggggccaaggcaccactctcacagtctcctca (SEQ ID NO:427)

[001336] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001337] VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGFNPNNGVTNYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVFYFDSWGQGTTLTVSS (SEQ ID NO:428)

[001338] Heavy chain variable region CDR1:

[001339] NTFTEYTMH (SEQ ID NO:429)

[001340] Heavy chain variable region CDR2:

[001341] GFNPNNGVTNYNQKFKG (SEQ ID NO:430)

[001342] Heavy chain variable region CDR3:

[001343] RYYHSTYVFYFDS (SEQ ID NO:431)

[001344] Light chain variable region sequence

[001345] K-1,2,3,4,9

[001346] gatatccagatgacacagactacatcctccctgtctgcctctctgggagacagagtcaccatcagttgcagtgcaagtcagggcattagcaattatttaaactggtttcagcagaaaccagatggaactattaagctcctgatctattacacatcaagtttacattcagga

gtcccatcaaggttcagtggcagtgggtctgggacagattattctctcaccatcagtaatgtggaacctgaagatattgccacttactattg tcagcagtatagtaagcttccttacacgttcggaggggggaccaagctggagataaaacgg (SEQ ID NO:432)

[001347] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001348] DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTS SLHSGVPSRFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR (SEQ ID NO:433)

[001349] Light chain variable region CDR 1:

[001350] SASQGISNYLN (SEQ ID NO:434)

[001351] Light chain variable region CDR2:

[001352] YTSSLHS (SEQ ID NO:435)

[001353] Light chain variable region CDR3:

[001354] QQYSKLPYT (SEQ ID NO:436)

[001355] Monoclonal antibody 8F9A5A1

[001356] Heavy chain variable region sequence

[001357] H-3,4,6,10,11

[001358] atccagttggtgcagtctggacctgagctgaagaagcctggagagacagtcaagatctcctgcaaggcttctgggtat accttcacaaactatggaatgaactgggtgaagcaggctccaggaaagggtttaaagtggatgggctggataaacacctacactgga gagccaacatatgttgatgacttcaagggacggtttgccttctctttggaaacctctgccaccactgcctatttgcagatcaacaacctca aaaatgaggacacgtctacatatttctgtgcaagattgaggggatacgaccgggtcccttggcttactggggccaagggactctggtc actgtctctgca (SEQ ID NO:437)

[001359] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001360] IQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMG WINTYTGEPTYVDDFKGRFAFSLETSATTAYLQINNLKNEDTSTYFCARLRGIRPGPL AYWGQGTLVTVSA (SEQ ID NO:438)

[001361] Heavy chain variable region CDR1:

[001362] YTFTNYGMN (SEQ ID NO:439)

[001363] Heavy chain variable region CDR2:

[001364] WINTYTGEPTYVDDFKG (SEQ ID NO:440)

[001365] Heavy chain variable region CDR3:

[001366] LRGIRPGPLAY (SEQ ID NO:441)

[001367] Light chain variable region sequence

[001368] K-1,2,3,4,5

[001369] gaaattttgctcacccagtctccagcaatcatagctgcatctcctggggagaaggtcaccatcacctgcagtgccagct caagtgtaagttacatgaactggtaccagcagaaaccaggatcctcccccaaaatatggatttatggtatatccaacctggcttctggag ttcctgctcgcttcagtggcagtgggtctgggacatctttctctttcacaatcaacagcatggaggctgaagatgttgccacttattactgtc agcaaaggagtagttacccacccacgttcggaggggggaccaagctggaaataaaacgg (SEQ ID NO:442)

[001370] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001371] EILLTQSPAIIAASPGEKVTITCSASSSVSYMNWYQQKPGSSPKIWIYGISNL ASGVPARFSGSGSGTSFSFTINSMEAEDVATYYCQQRSSYPPTFGGGTKLEIKR (SEQ ID NO:443)

[001372] Light chain variable region CDR 1:

[001373] SASSSVSYMN (SEQ ID NO:444)

[001374] Light chain variable region CDR2:

[001375] GISNLAS (SEQ ID NO:445)

[001376] Light chain variable region CDR3:

[001377] QQRSSYPPT (SEQ ID NO:446)

[001378] **Monoclonal antibody 8H5H5G4**

[001379] Heavy chain variable region sequence

[001380] H-1,3,5,6,10

[001381] gtccagctgcaacagtctggacctgatctggtgaagcctgggacttcagtgaagatatcctgtaagacttctggaaaca cattcactgaatacaccatgcactgggtgaagcagagccatggaaagagccttgagtggattggaggttttaatcctaacaatggtgtta ctaactacaaccagaagttcaagggcaaggccacattgactgtagacaagtcctccagcacagcctacatggagctccgcagcctga catctgaggattctgcagtctattactgtgcaagacgttactaccatagtacctacgtgttctactttgactcctggggccaaggcaccact ctcacagtctcctca (SEQ ID NO:447)

[001382] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001383] VQLQQSGPDLVKPGTSVKISCKTSGNTFTEYTMHWVKQSHGKSLEWIGGF NPNNGVTNYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRYYHSTYVF YFDSWGQGTTLTVSS (SEQ ID NO:448)

[001384] Heavy chain variable region CDR1:

[001385] NTFTEYTMH (SEQ ID NO:449)

[001386] Heavy chain variable region CDR2:

[001387] GFNPNNGVTNYNQKFKG (SEQ ID NO:450)

[001388] Heavy chain variable region CDR3:

[001389] RYYHSTYVFYFDS (SEQ ID NO:451)

[001390] Light chain variable region sequence

[001391] K-2,5,8,9,15

[001392] gatatccagatgacacagactacatcctccctgtctgcctctctgggagacagagtcaccatcagttgcagtgcaagtc agggcattagcaattatttaaactggtttcagcagaaaccagatggaactattaagctcctgatctattacacatcaagtttacattcagga gtcccatcaaggttcagtggcagtgggtctgggacagattattctctcaccatcagtaatgtggaacctgaagatattgccacttactattg tcagcagtatagtaagcttccttacacgttcggaggggggaccaagctggagataaaacgg (SEQ ID NO:452)

[001393] Translated protein, wherein the underlined sequence is the complementarity determining region (CDR):

[001394] DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWFQQKPDGTIKLLIYYTS SLHSGVPSRFSGSGSGTDYSLTISNVEPEDIATYYCQQYSKLPYTFGGGTKLEIKR (SEQ ID NO:453)

[001395] Light chain variable region CDR 1:

[001396] SASQGISNYLN (SEQ ID NO:454)

[001397] Light chain variable region CDR2:

[001398] YTSSLHS (SEQ ID NO:455)

[001399] Light chain variable region CDR3:

[001400] QQYSKLPYT (SEQ ID NO:456)

[001401] All of the references cited herein are incorporated by reference in their entirety.

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* * * * *

[001402] 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. Such equivalents are intended to be encompassed in the scope of the claims.

What is claimed is:

1. An NME7 specific antibody or fragment thereof that binds to the NME7 B3 peptide of SEQ ID NO:145 or SEQ ID NO:169.

2. The antibody of claim 1, which is a monoclonal antibody.

3. The antibody of claim 1, which is bivalent, monovalent, an Fab, or a single chain variable fragment antibody (scFv).

4. The antibody of claim 1, which is linked to an antibody drug conjugate.

5. The antibody of claim 1, wherein the drug is linked to a toxin or pro-toxin.

6. An isolated nucleic acid encoding the monoclonal antibody according to claim 2.

7. An isolated hybridoma expressing the monoclonal antibody according to claim 2.

8. The antibody of claim 1, which specifically binds to $NME7_{AB}$ or NME7-X1, but not to NME1.

9. The antibody of claim 1 that disrupts interaction between $NME7_{AB}$ and MUC1* extra cellular domain or between NME7-X1 and MUC1* extra cellular domain.

10. The antibody of claim 1 that disrupts binding between $NME7_{AB}$ and PSMGFR or between NME7-X1 and PSMGFR.

11. The antibody of claim 1 that disrupts binding between $NME7_{AB}$ and N-10 or between NME7-X1 and N-10.

12. The antibody of claim 1 that does not disrupt interaction between $NME7_{AB}$ and MUC1* extra cellular domain or between NME7-X1 and MUC1* extra cellular domain, wherein the $NME7_{AB}$ or NME7-X1 binds to the N-10 peptide (SEQ ID NO:170) but not to a C-10 peptide (SEQ ID NO:171).

13. The antibody of claim 2, wherein the antibody is 5A1, 4A3 or 5D4.

14. The antibody of claim 2, comprising an amino acid sequence in the heavy chain variable region comprising the following:

in the CDR1 region YTFTNYGMN (SEQ ID NO:439);

in the CDR2 region WINTYTGEPTYVDDFKG (SEQ ID NO:440); and

in the CDR3 region LRGIRPGPLAY (SEQ ID NO:441); and

an amino acid sequence in the light chain variable region comprising the following:

in the CDR1 region SASSSVSYMN (SEQ ID NO:444);

in the CDR2 region GISNLAS (SEQ ID NO:445); and

in the CDR3 region QQRSSYPPT (SEQ ID NO:446).

15. The antibody of claim 2, comprising an amino acid sequence in the heavy chain variable region comprising the following:

in the CDR1 region NTFTEYTMH (SEQ ID NO:429);

in the CDR2 region GFNPNNGVTNYNQKFKG (SEQ ID NO:430); and

in the CDR3 region RYYHSTYVFYFDS (SEQ ID NO:431); and

an amino acid sequence in the light chain variable region comprising the following:

in the CDR1 region SASQGISNYLN (SEQ ID NO:434);

in the CDR2 region YTSSLHS (SEQ ID NO:435); and

in the CDR3 region QQYSKLPYT (SEQ ID NO:436).

16. The antibody of claim 2, comprising an amino acid sequence in the heavy chain variable region comprising the following:

in the CDR1 region NTFTEYTMH (SEQ ID NO:388);

in the CDR2 region GFNPNNGVTNYNQKFKG (SEQ ID NO:389); and

in the CDR3 region RYYHSLYVFYFDY (SEQ ID NO:390); and

an amino acid sequence in the light chain variable region comprising the following:

in the CDR1 region ITSTDIDDDMN (SEQ ID NO:393);

in the CDR2 region EGNTLRP (SEQ ID NO:394); and

in the CDR3 region LQSDNLPLT (SEQ ID NO:395).

17. The antibody of claim 1, which is human, humanized or an engineered antibody mimic.

18. The antibody of claim 1, which is non-human.

19. The antibody of claim 18, which is murine or camelid.

20. A method of administering to a patient for prevention or treatment of cancer comprising administering to the patient a composition comprising the antibody of claim 1 - claim 16.

21. A method for preventing or treating cancer metastasis in a patient, comprising administering to the patient a composition comprising the antibody of claim 1 – claim 16.

22. A method for diagnosing cancer or cancer metastasis comprising contacting a patient specimen and normal specimen with the antibody of claim 1 - claim 16, and comparing the results from both specimen, wherein presence of positive binding to the antibody in the patient specimen indicates the presence of cancer or cancer metastasis in the patient.

23. The method of claim 22, wherein the antibody is as set forth in claim 12.

24. The method of claim 22, wherein the antibody is linked to an imaging agent.

25. The method of claim 22, wherein the patient specimen is blood, bodily fluid, tissue, circulating cells, *in vitro*, *in vivo*, including intra-operative.

26. A cell that is engineered to express an anti-$NME7_{AB}$ antibody or fragment thereof.

27. The cell of claim 26, wherein the cell is an immune cell.

28. The cell of claim 26, wherein the immune cell is T cell or NK cell.

29. The cell of claim 26, wherein the cell is a stem or progenitor cell.

30. The cell of claim 29, wherein the stem or progenitor cell that is then differentiated to become a T cell.

31. The cell of claim 26, which comprises chimeric antigen receptor (CAR) that recognizes tumor associated antigen.

32. The cell of claim 26, wherein expression of the anti-NME7 antibody is inducible.

33. The cell of claim 26, where nucleic acid encoding an anti-$NME7_{AB}$ antibody is inserted into the Foxp3 enhancer or promoter.

34. The cell of claim 26, wherein an anti-$NME7_{AB}$ antibody is in an NFAT-inducible system.

35. The cell of claim 34, wherein NFATc1 response element, which is inserted upstream of the antibody sequence that is inserted into Foxp3 enhancer or promoter region.

36. The cell of claim 26, wherein the anti-$NME7_{AB}$ antibody or fragment thereof binds to the NME7 B3 peptide, or disrupts binding of $NME7_{AB}$ or NME7-X1 to the PSMGFR peptide of the MUC1* extra cellular domain.

37. The immune cell of claim 27, comprising expresses a CAR that recognizes a tumor associated antigen and an anti-NME7 antibody.

38. The immune cell of claim 37, wherein the tumor associated antigen is MUC1*.

39. An anti-cancer vaccine comprising a composition comprising one or more peptides derived from $NME7_{AB}$ listed in Figure 6 – Figure 9 or a peptide having at least 80%, 85%, 90%, 95%, 97% sequence identity thereof as the immunogenicity eliciting portion.

40. The anti-cancer vaccine of claim 39, wherein the peptide is a peptide of SEQ ID NOS:141-145 or a peptide having at least 80%, 85%, 90%, 95%, 97% sequence identity thereof.

41. The anti-cancer vaccine of claim 40, wherein the peptide is a peptide of SEQ ID NOS: 145 or a peptide having at least 80%, 85%, 90%, 95%, 97% sequence identity thereof.

42. A BiTE comprising the antibody of claim 1 – claim 16.

43. A method of generating anti-$NME7_{AB}$ antibodies wherein Cysteine residue in the NME7 B3 peptide is mutated to avoid disulfide bonding.

44. A method of generating cells with enhanced metastatic potential comprising culturing the cells with $NME7_{AB}$ or NME7-X1.

45. A cell that is engineered to express $NME7_{AB}$ or NME7-X1.

46. A transgenic animal that expresses $NME7_{AB}$ or NME7-X1.

47. The transgenic animal of claim 46, wherein the $NME7_{AB}$ or NME7-X1 is human.

48. The transgenic animal of claim 47, wherein expression of $NME7_{AB}$ or NME7-X1 is inducible.

ELISA shows NME7 Dimerizes MUC1*

MUC1 * extra cellular domain peptide immobilized on plate was bound by NME7 to saturation; a second MUC1 * peptide with a C-terminal His-tag or Biotin tag was added and visualized by HRP labeled antibody to either His-tag or HRP labeled streptavidin

Fig. 1A

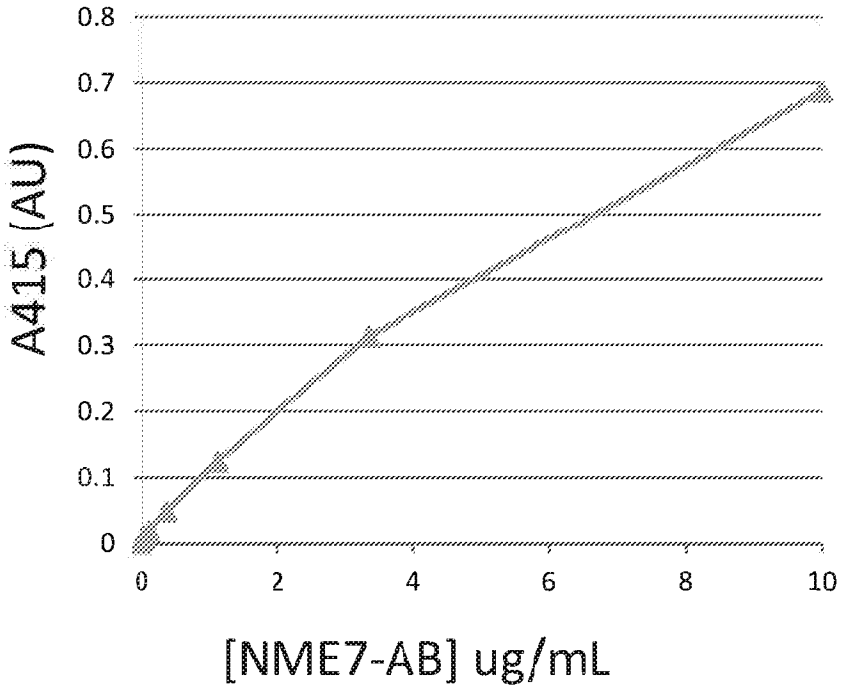
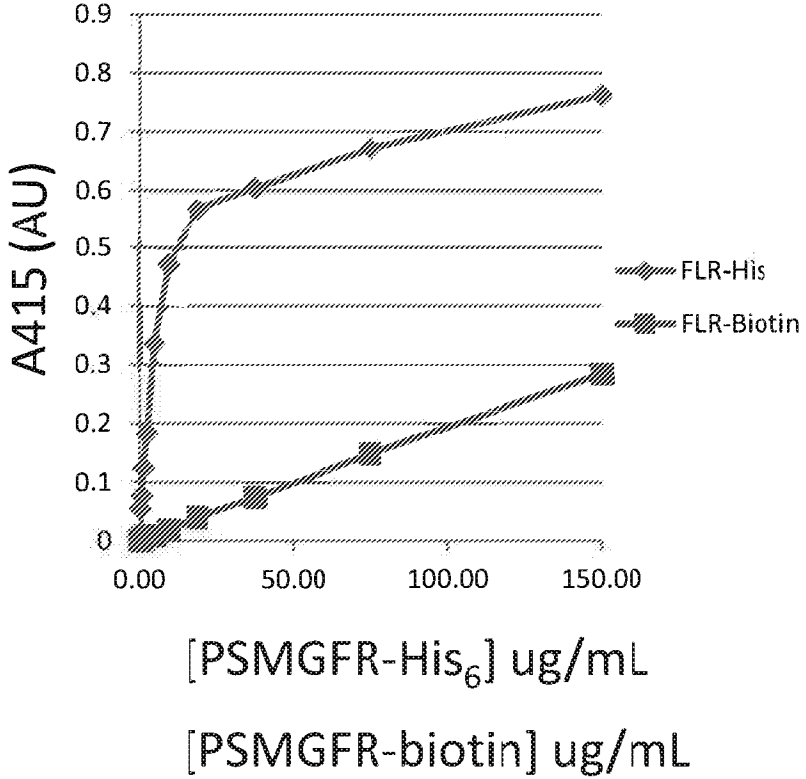


Fig. 1B



Figures 1A - 1B

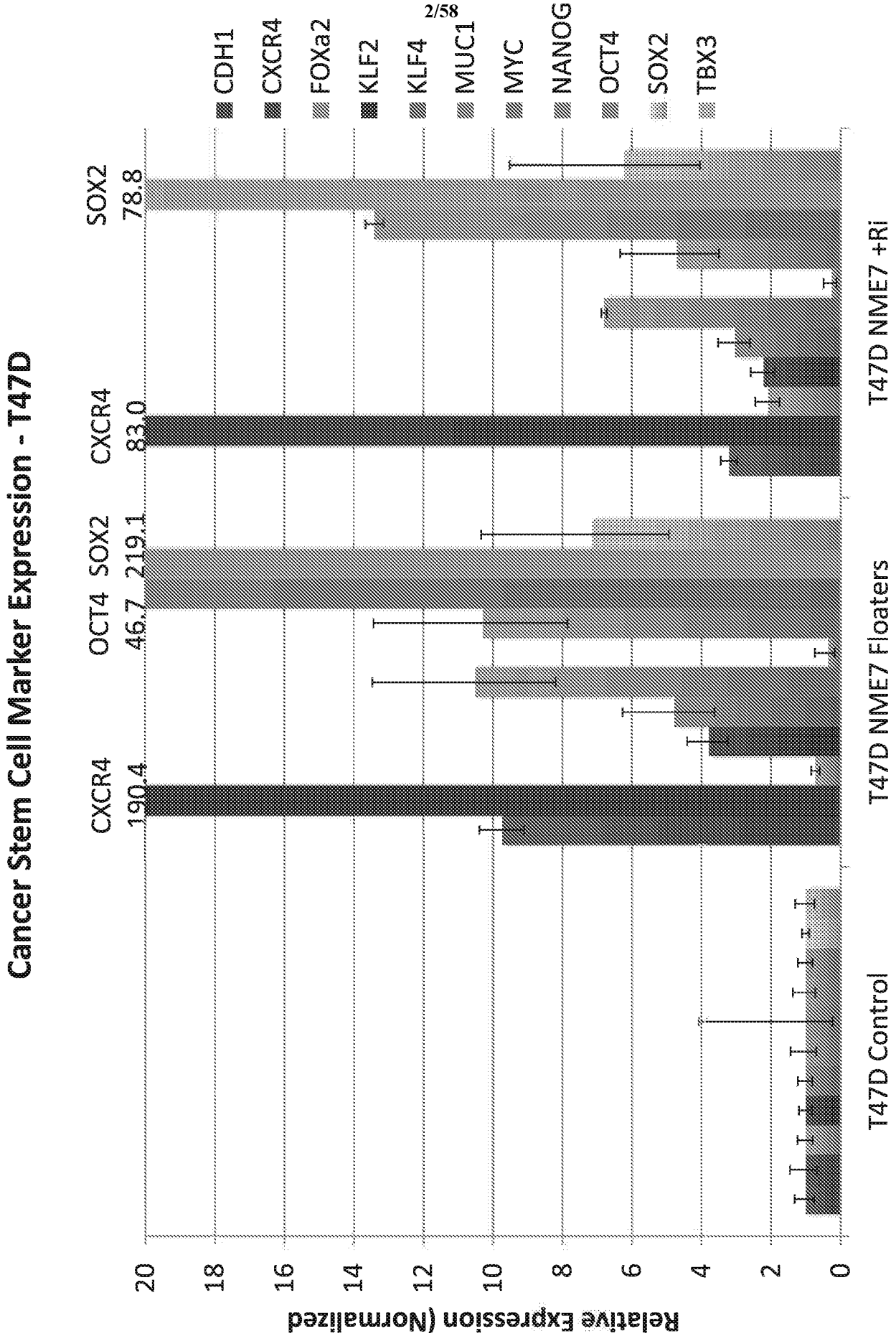

Cancer Stem Cell Marker Expression - T47D
Relative Expression (Normalized)
0
2
4
6
8
10
12
14
16
18
20
CDH1
CXCR4
FOXa2
KLF2
KLF4
MUC1
MYC
NANOG
OCT4
SOX2
TBX3
CXCR4 190.4
OCT4 46.7
SOX2 219.1
CXCR4 83.0
SOX2 78.8
T47D Control
T47D NME7 Floaters
T47D NME7 +Ri

Figure 2

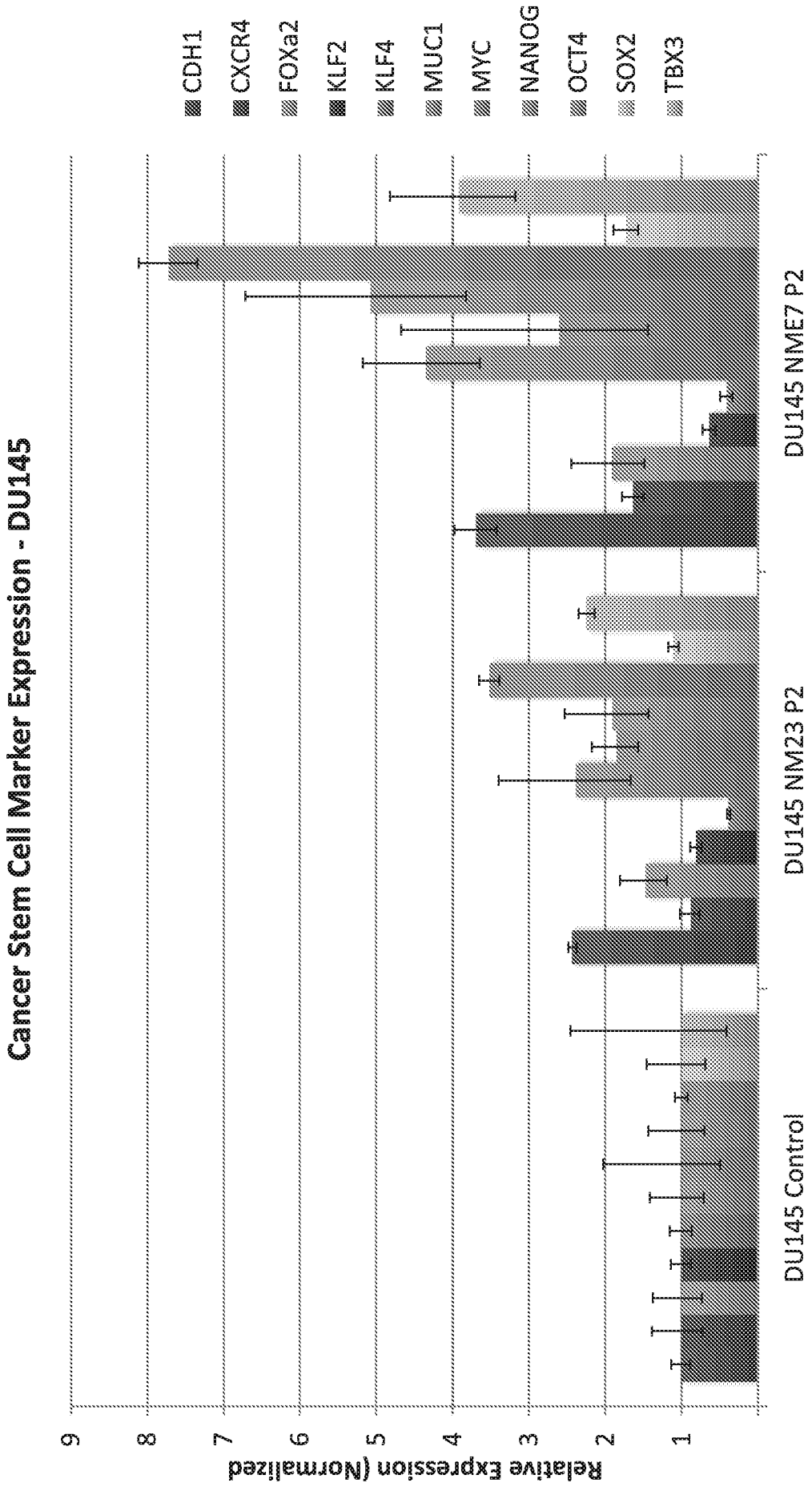

Cancer Stem Cell Marker Expression - DU145
Relative Expression (Normalized)
9
8
7
6
5
4
3
2
1
CDH1
CXCR4
FOXa2
KLF2
KLF4
MUC1
MYC
NANOG
OCT4
SOX2
TBX3
DU145 Control
DU145 NM23 P2
DU145 NME7 P2

Figure 3

Breast cancer cells are progressed to a more metastatic state by culturing in the presence of 2i inhibitors or in NME7-AB

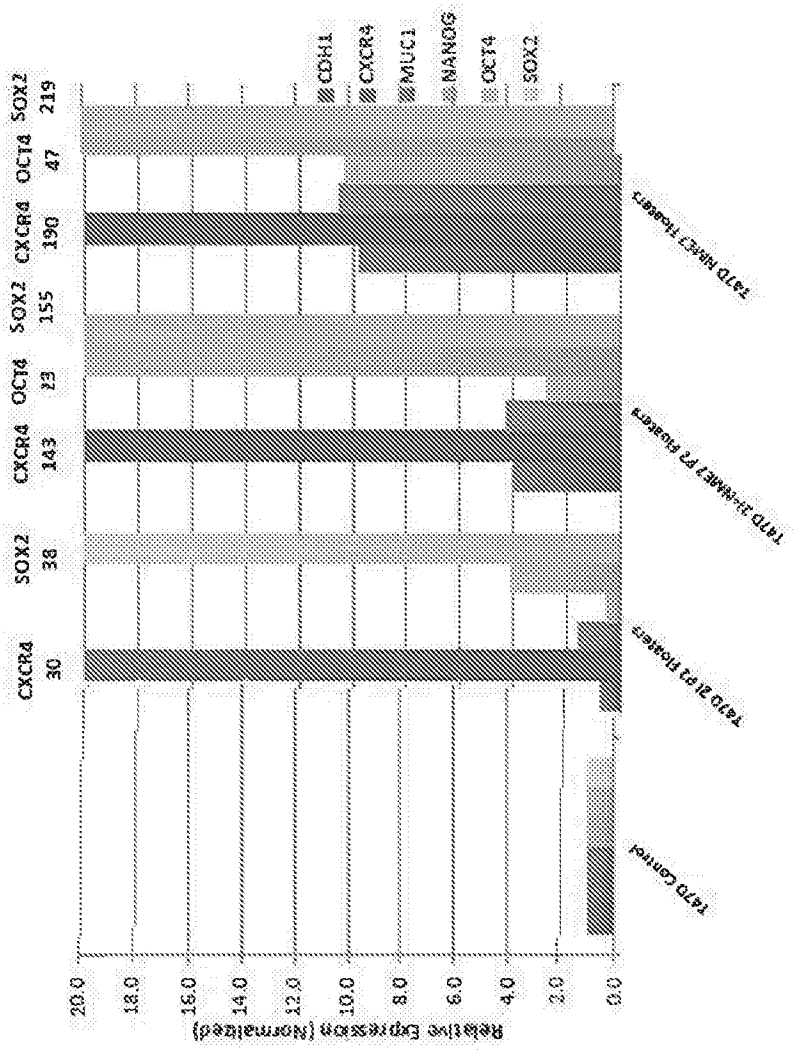


Figure 4

Figure 5
Sequence alignment of human NME1 to human NME7-A and –B domains

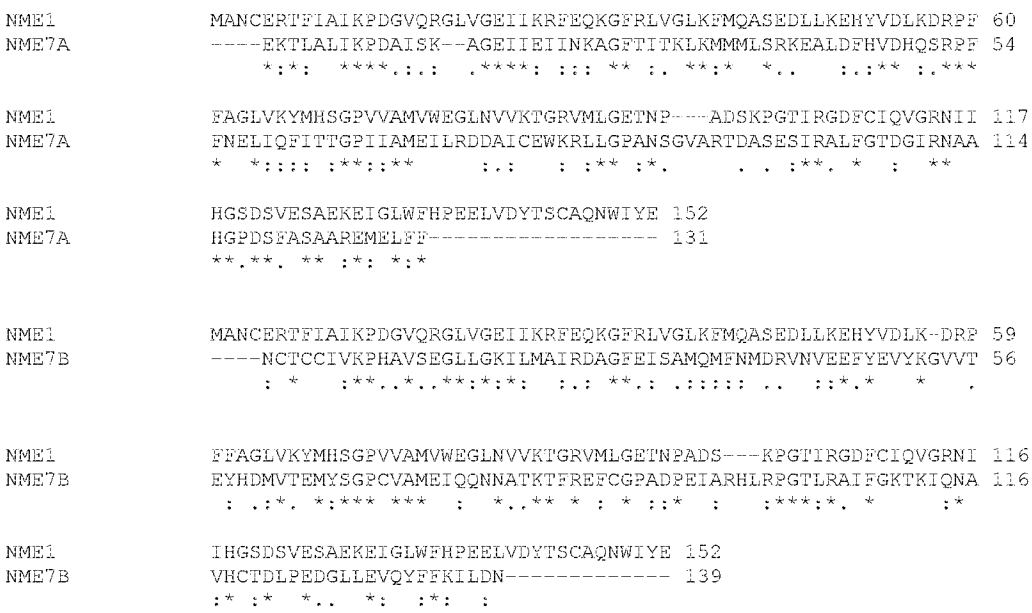


Figure 6

NME7 specific peptides for generating antibodies to inhibit NME7 for the treatment or prevention of cancers.

The following peptide sequences are identified as being immunogenic peptides giving rise to antibodies that target human NME7 but not human NME1. The sequences were chosen for their lack of sequence homology to human NME1.

1. LALIKPDA
2. MMMLSRKEALDFHVDHQS
3. ALDFHVDHQS
4. EILRDDAICEWKRL
5. FNELIQFITTGP
6. RDDAICEW
7. SGVARTDASESIRALFGTDGIRNAA
8. ELFFPSSGG
9. KFTNCTCCIVKPHAVSEGLLGKILMA
10. LMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVT
11. EFYEVYKGVVTEYHD
12. EIQQNNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNA
13. YSGPCVAM
14. FREFCGP
15. VHCTDLPEDGLLEVQYFFKILDN
16. IQNAVHCTD
17. TDLPEDGLLEVQYFFKILDN
18. PEDGLLEVQYFFK
19. EIINKAGFTITK
20. MLSRKEALDFHVDHQS
21. NELIQFITT
22. EILRDDAICEWKRL
23. SGVARTDASESIRALFGTDGI
24. SGVARTDASES

Figure 6 (Continued)

25. ALFGTDGI
26. NCTCCIVKPHAVSE
27. LGKILMAIRDA
28. EISAMQMFNMDRVNVE
29. EVYKGVVT
30. EYHDMVTE
31. EFCGPADPEIARHLR

Figure 7

NME7 specific peptides for generating antibodies to inhibit NME7 for the treatment or prevention of cancers.

The following are preferred as they are likely areas that are important for structural integrity or for binding to the MUC1* peptide. Bivalent antibodies wherein each variable region would bind to each one of a pair are preferred.

35. ICEWKRL
36. LGKILMAIRDA

37. HAVSEGLLGK
38. VTEMYSGP

39. NATKTFREF
40. AIRDAGFEI

41. AICEWKRLLGPAN
42. DHQSRPFF

43. AICEWKRLLGPAN
44. VDHQSRPF
45. PDSFAS
46. KAGEIIEIINKAGFTITK

Figure 8

The following peptide sequences are from human NME1 and were selected for their high homology to human NME7 as well as for their homology to other bacterial NME proteins that are able to mimic its function.

47. MANCERTFIAIKPDGVQRGLVGEIIKRFE
48. VDLKDRPF
49. HGSDSVESAEKEIGLWF

Especially preferred for their high homology to human NME7-A or -B and also to HSP 593 are:

50. ERTFIAIKPDGVQRGLVGEIIKRFE
51. VDLKDRPFFAGLVKYMHSGPVVAMVWEGLN
52. NIIHGSDSVESAEKEIGLWFHPEELV
53. KPDGVQRGLVGEII

Figure 9

NME7-AB specific peptides preferred for generating antibodies for the treatment or prevention of cancer.

NME7A peptide 1
MLSRKEALDFHVDHQS

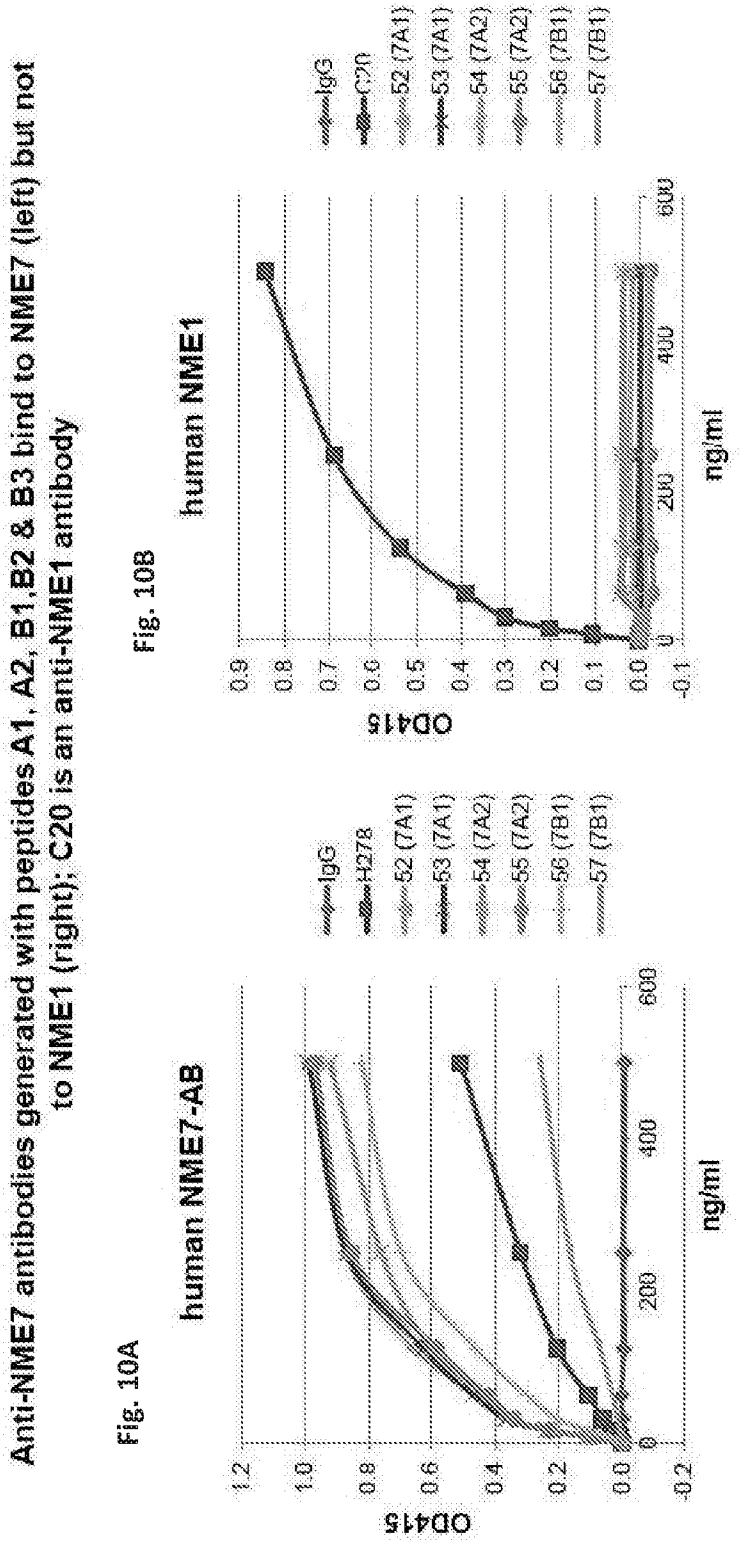
NME7A peptide 2
SGVARTDASES

NME7B peptide 1
DAGFEISAMQMFNMDRVNVE

NME7B peptide 2 EVYKGVVTEYHDMVTE

NME7B peptide 3
AIFGKTKIQNAVHCTDLPEDGLLEVQYFF

NME7B peptide 3 Cys to Ser mutation
AIFGKTKIQNAVHSTDLPEDGLLEVQYFF



Figures 10A-10B

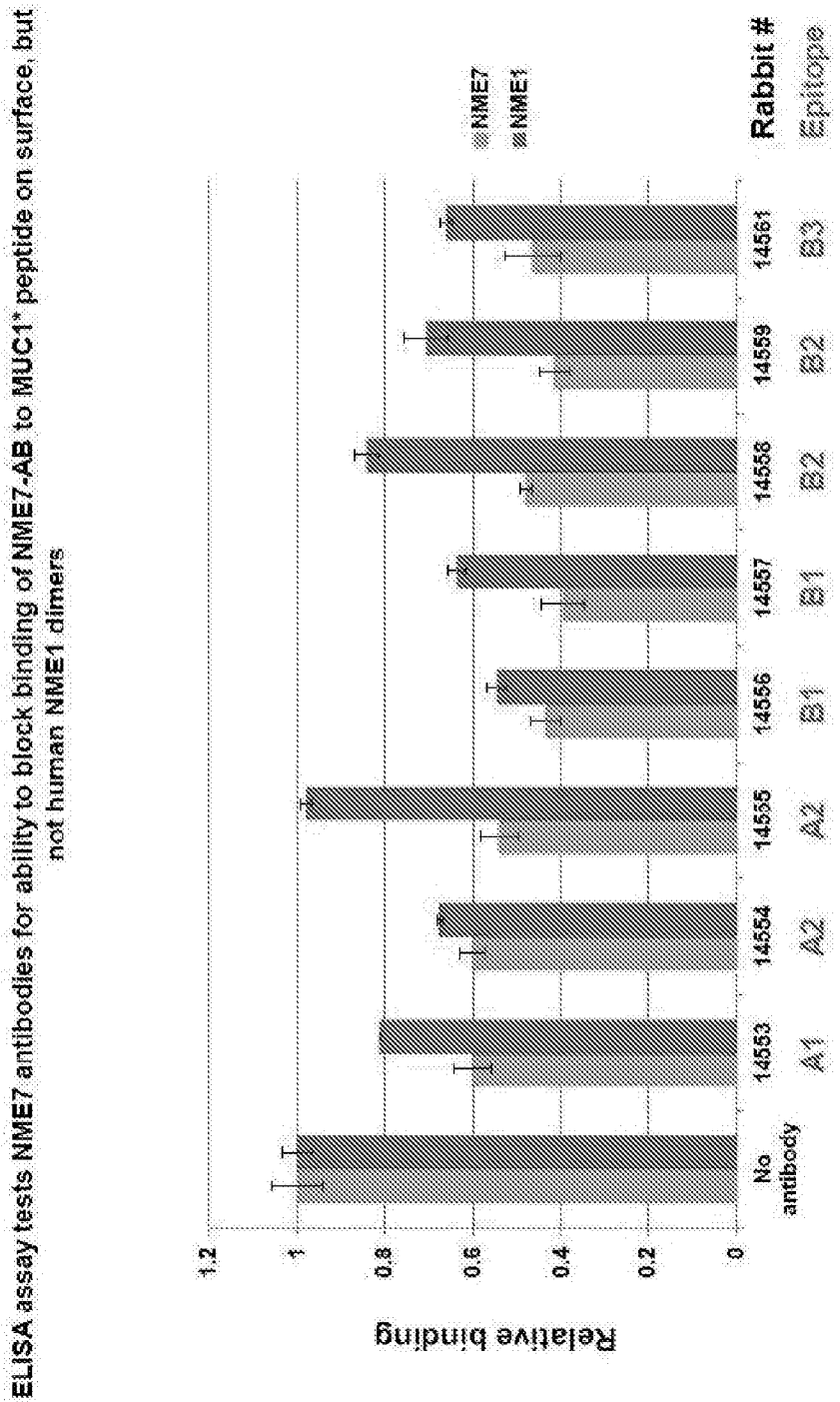

ELISA assay tests NME7 antibodies for ability to block binding of NME7-AB to MUC1* peptide on surface, but not human NME1 dimers
Relative binding
1.2
1
0.8
0.6
0.4
0.2
0
NME7
NME1
No antibody
14553
14554
14555
14556
14557
14556
14559
14561
Rabbit #
A1
A2
A2
B1
B1
B2
B2
B3
Epitope
Antibody Source

Figure 11

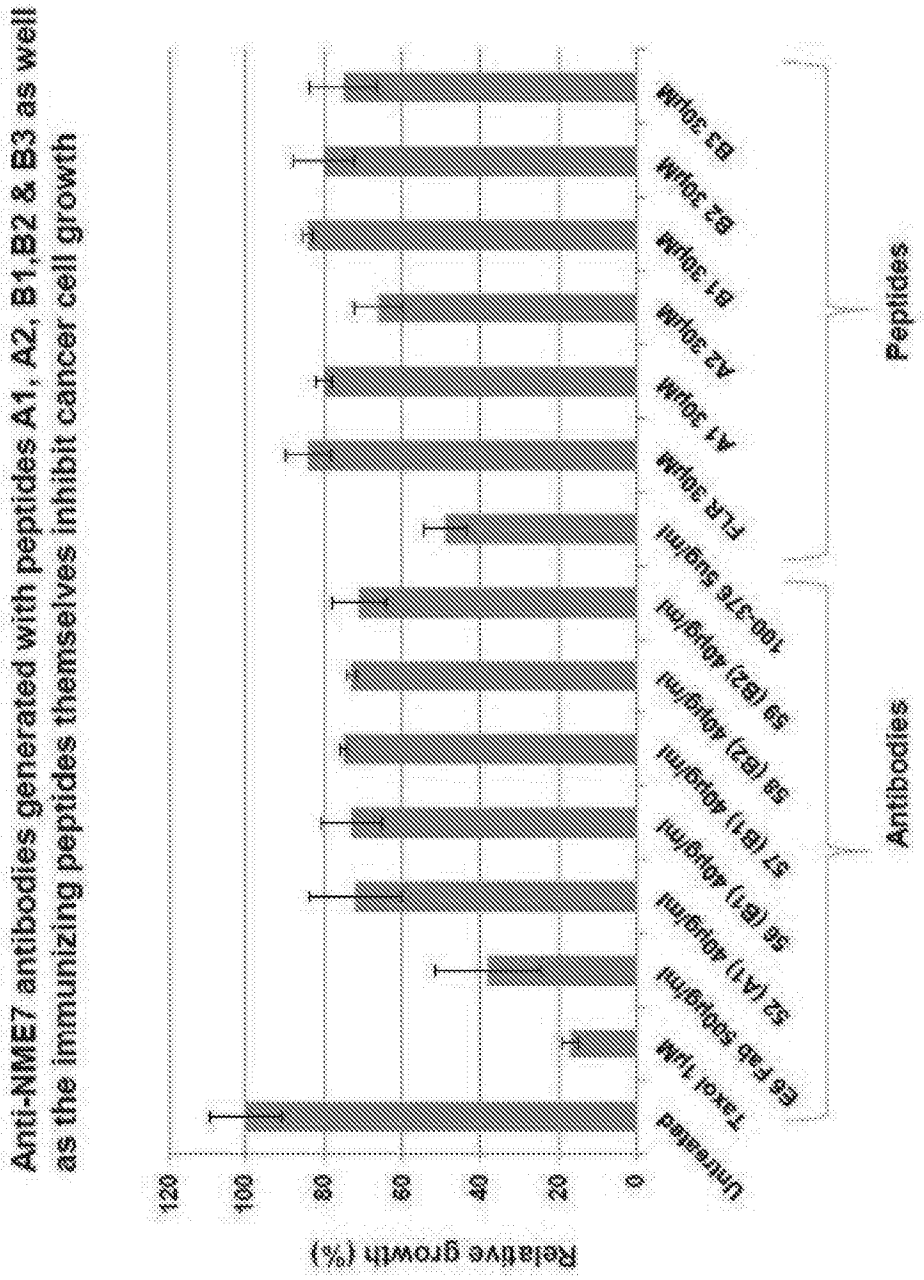

Anti-NME7 antibodies generated with peptides A1, A2, B1,B2 & B3 as well as the immunizing peptides themselves inhibit cancer cell growth
Relative growth (%)
120
100
80
60
40
20
0
Untreated
Taxol 1μM
E6 Fab 60ng/ml
S2 (A1) 40ng/ml
S6 (B1) 40ng/ml
S7 (B1) 40ng/ml
S9 (B2) 40ng/ml
S3 (B2) 40ng/ml
160-376 5ng/ml
FLR 30μM
A1 30μM
A2 30μM
B1 30μM
B2 30μM
B3 30μM
Antibodies
Peptides

Figure 12

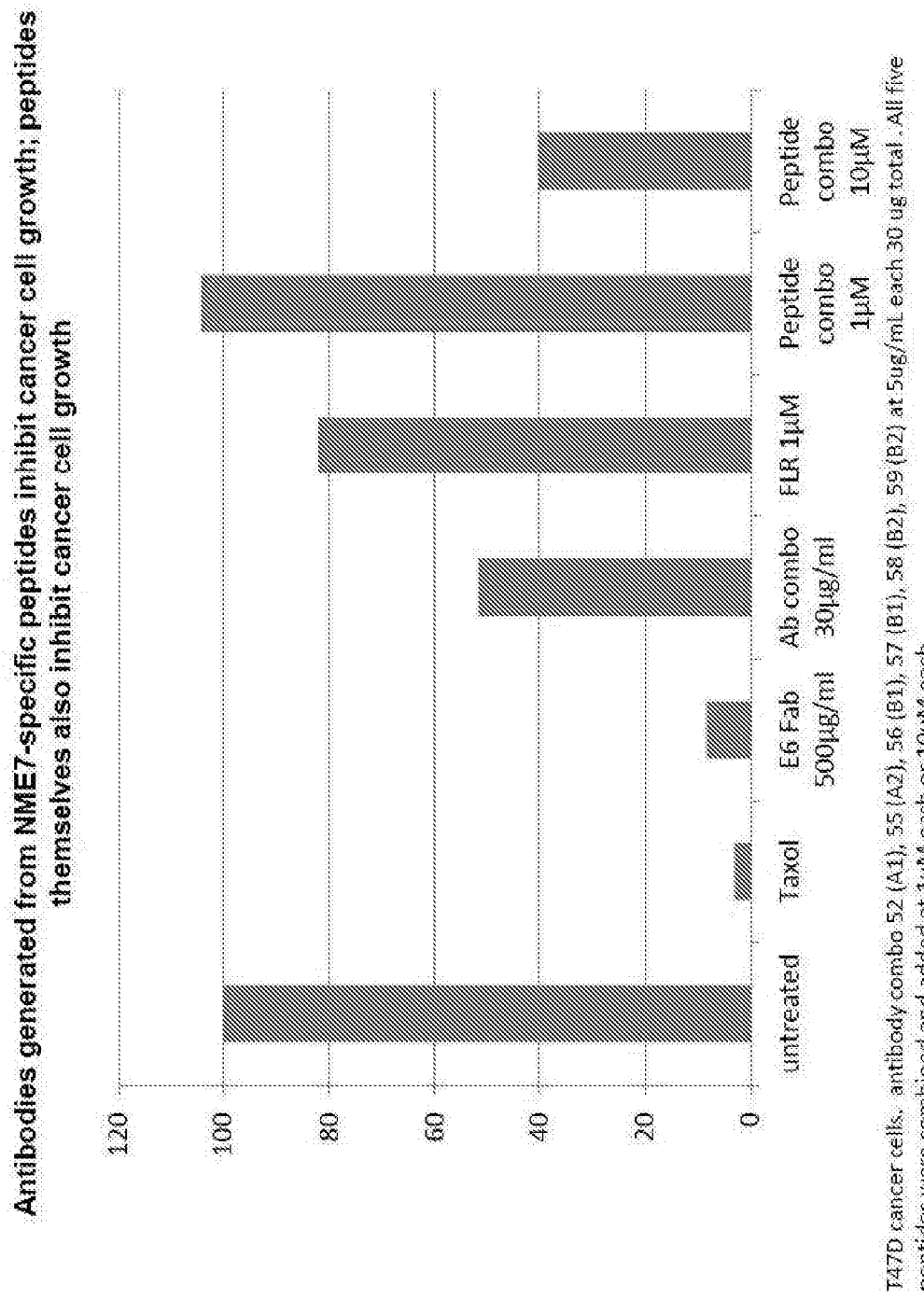

Antibodies generated from NME7-specific peptides inhibit cancer cell growth; peptides themselves also inhibit cancer cell growth
120
100
80
60
40
20
0
untreated
Taxol
E6 Fab 500µg/ml
Ab combo 30µg/ml
FLR 1µM
Peptide combo 1µM
Peptide combo 10µM
T47D cancer cells. antibody combo 52 (A1), 55 (A2), 56 (B1), 57 (B1), 58 (B2), 59 (B2) at 5ug/mL each 30 ug total . All five peptides were combined and added at 1uM each or 10uM each

Figure 13

Treating cancer cells with anti-NME7 antibodies inhibits transition to "floater" cells, which PCR shows have greatly increased expression of metastatic markers such as CXCR4; xenograft experiments show that the floater cells form a tumor at extremely low copy number – 50 – and thus fulfill the requirement for being classified cancer stem cells or metastatic cancer cells.

Fig. 14A

Antibodies	Floater observation
Control IgG	100%
53,55,57 (A1,A2,B1)	70%
53,57 (A1,B1)	50%
61 (B3)	5%

JR observations

Fig. 14B

Antibodies	Floater observation
Control IgG	100%
53,55,57 (A1,A2,B1)	65%
53,57 (A1,B1)	40%
61 (B3)	5%

VH observations

The number of "Floater" cells, which are the ones that have higher expression of metastatic markers and that form tumors in animals at extremely low copy number is typically 20% of the amount of plated cells by Day 7. Here, we define 100% as the number of floater cells that results when a control antibody is added. Other percentages of floater cells is relative to the control in which a control IgG antibody was added.

Figures 14A-14B

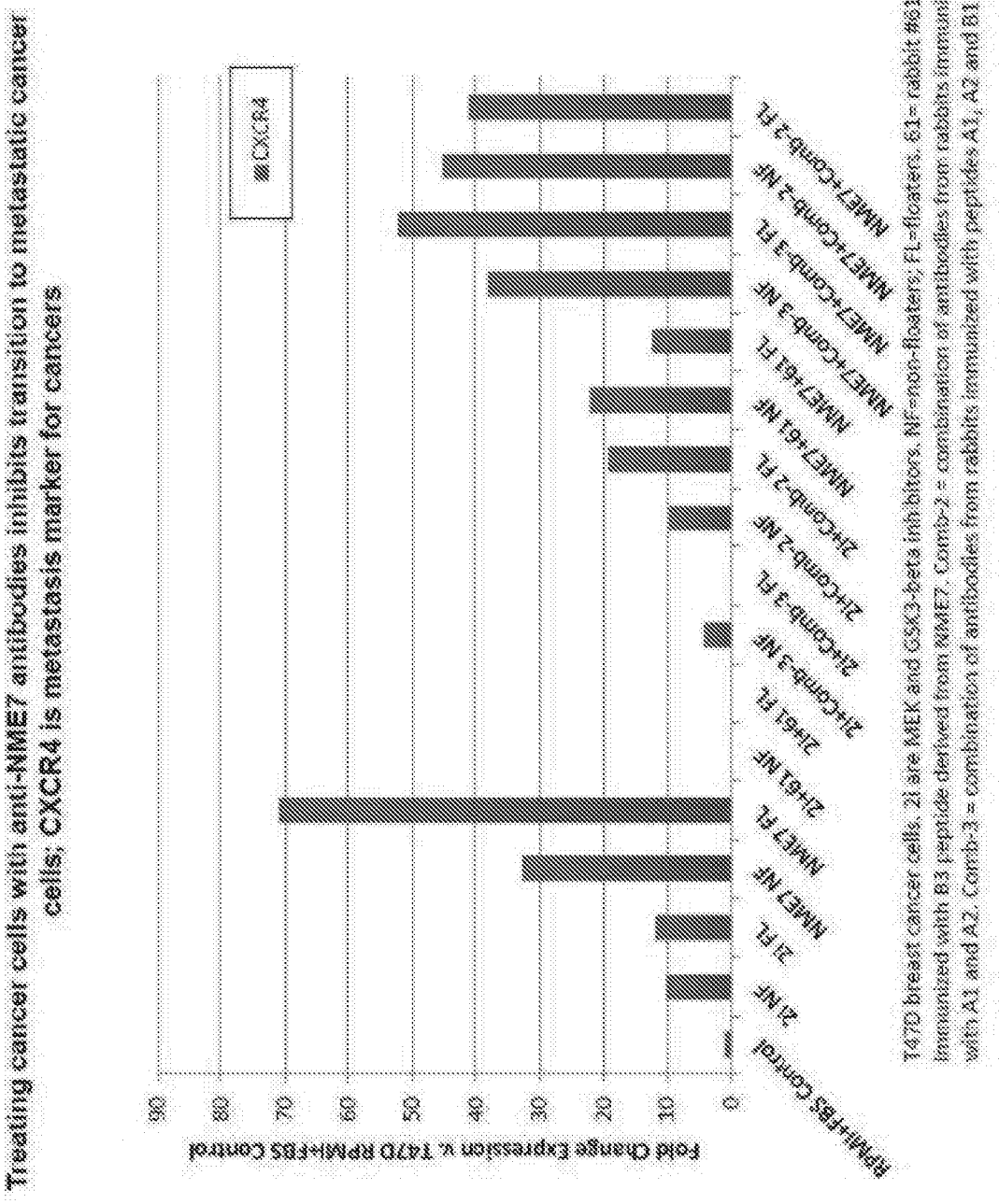

Treating cancer cells with anti-NME7 antibodies inhibits transition to metastatic cancer cells; CXCR4 is metastasis marker for cancers
Fold Change Expression v T47D RPMI+FBS Control
CXCR4
0
10
20
30
40
50
60
70
80
90
RPMI+FBS Control
2I NF
3I FL
NME7 NF
NME7 FL
Zt-61 NF
Zt-61 FL
2t+Comb-3 NF
2t+Comb-3 FL
2t+Comb-2 NF
2t+Comb-2 FL
NME7+61 NF
NME7+61 FL
NME7+Comb-3 NF
NME7+Comb-3 FL
NME7+Comb-2 NF
NME7+Comb-2 FL
T47D breast cancer cells. 2I are MEK and GSK3-beta inhibitors. NF=non-floaters; FL=floaters. 61= rabbit #61 immunized with B3 peptide derived from NME7. Comb-2 = combination of antibodies from rabbits immunized with A1 and A2. Comb-3 = combination of antibodies from rabbits immunized with peptides A1, A2 and B1.

Figure 15A

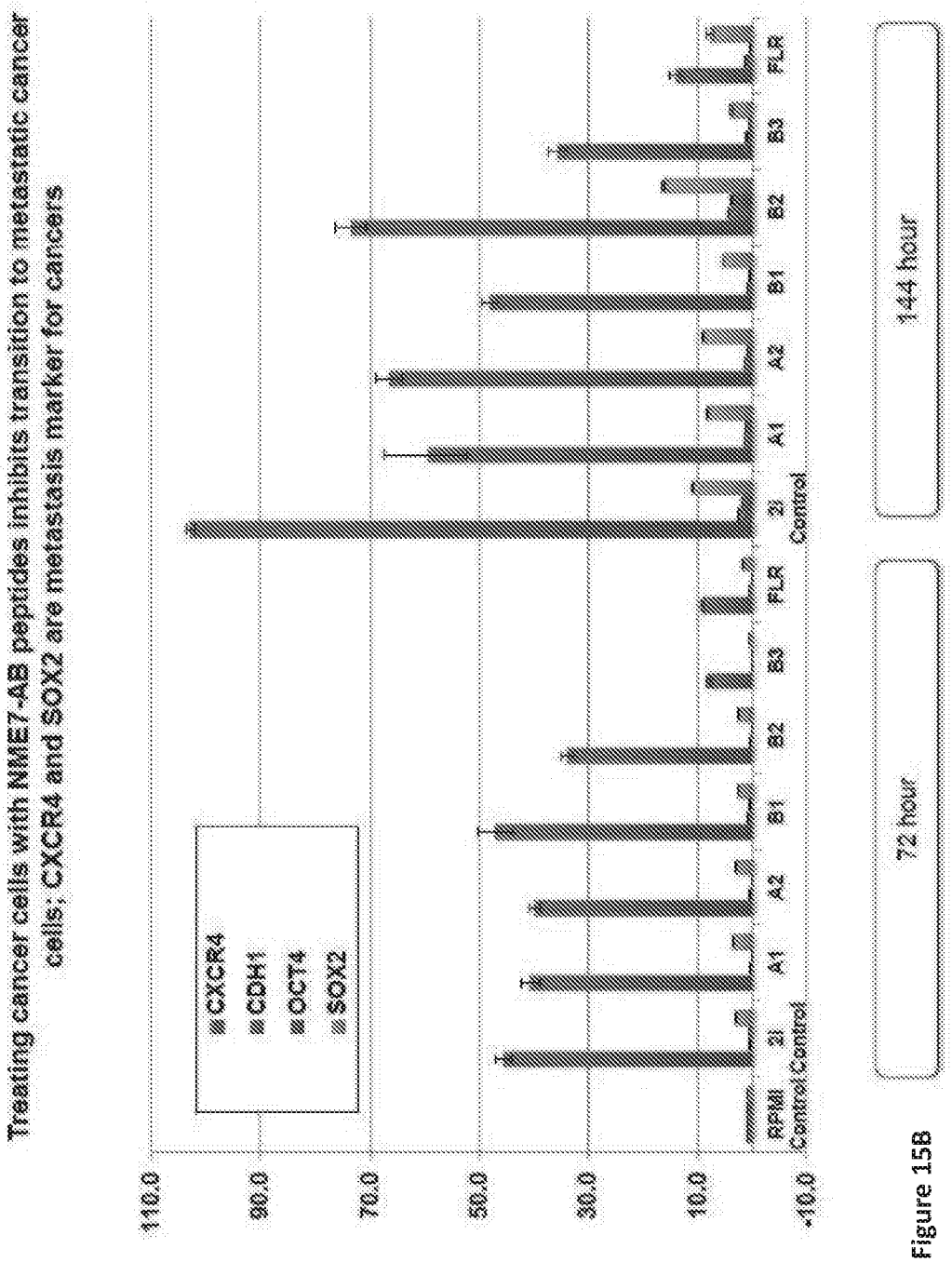

Treating cancer cells with NME7-AB peptides inhibits transition to metastatic cancer cells; CXCR4 and SOX2 are metastasis marker for cancers
CXCR4
CDH1
OCT4
SOX2
110.0
90.0
70.0
50.0
30.0
10.0
-10.0
RPMI Control
21 Control
A1
A2
B1
B2
B3
FLR
21 Control
A1
A2
B1
B2
B3
FLR
72 hour
144 hour

Figure 15B

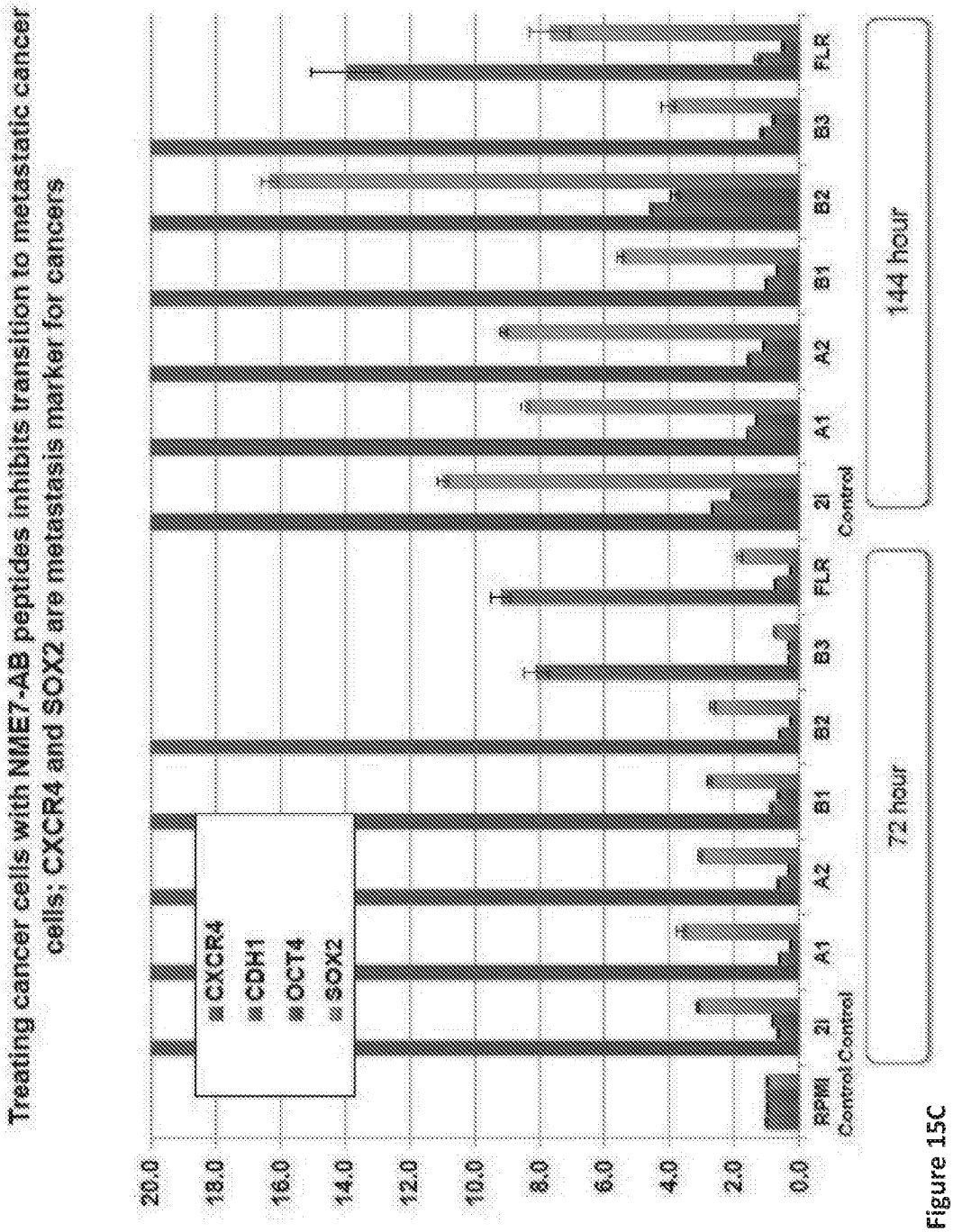
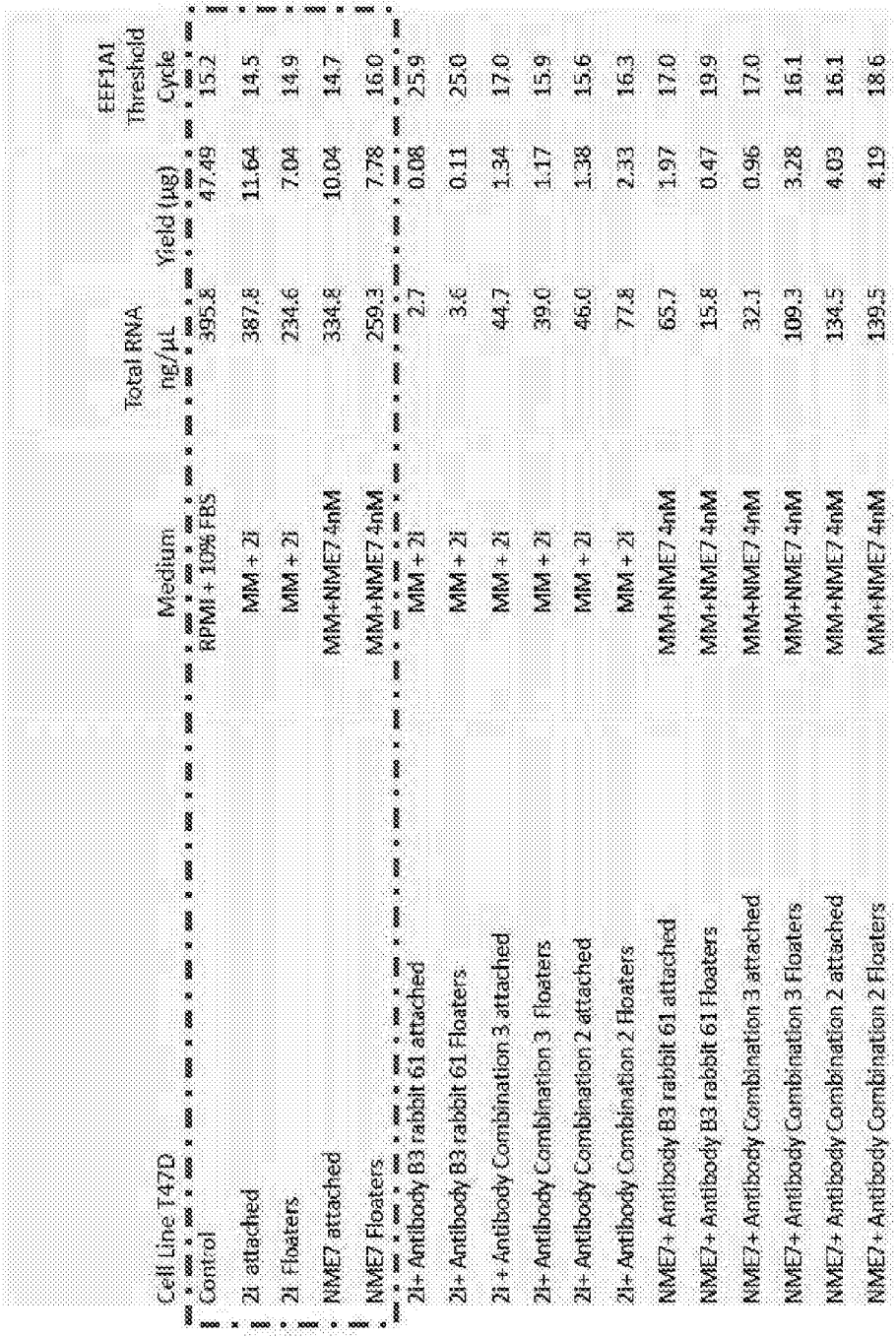

Treating cancer cells with NME7-AB peptides inhibits transition to metastatic cancer cells; CXCR4 and SOX2 are metastasis marker for cancers
CXCR4
CDH1
OCT4
SOX2
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
RPMI
2i
A1
A2
B1
B2
B3
FLR
Control Control
72 hour
144 hour

Figure 15C



	Cell Line T47D	Medium	Total RNA ng/µL	Yield (µg)	EEF1A1 Threshold Cycle
CONTROLS	Control	RPMI + 10% FBS	395.8	47.49	15.2
	2i attached	MM + 2i	387.8	11.64	14.5
	2i Floaters	MM + 2i	234.6	7.04	14.9
	NME7 attached	MM+NME7 4nM	334.8	10.04	14.7
	NME7 Floaters	MM+NME7 4nM	259.3	7.78	16.0
	2i+ Antibody B3 rabbit 61 attached	MM + 2i	2.7	0.08	25.9
	2i+ Antibody B3 rabbit 61 Floaters	MM + 2i	3.6	0.11	25.0
	2i + Antibody Combination 3 attached	MM + 2i	44.7	1.34	17.0
	2i+ Antibody Combination 3 Floaters	MM + 2i	39.0	1.17	15.9
	2i+ Antibody Combination 2 attached	MM + 2i	46.0	1.38	15.6
	2i+ Antibody Combination 2 Floaters	MM + 2i	77.8	2.33	16.3
	NME7+ Antibody B3 rabbit 61 attached	MM+NME7 4nM	65.7	1.97	17.0
	NME7+ Antibody B3 rabbit 61 Floaters	MM+NME7 4nM	15.8	0.47	19.9
	NME7+ Antibody Combination 3 attached	MM+NME7 4nM	32.1	0.96	17.0
	NME7+ Antibody Combination 3 Floaters	MM+NME7 4nM	109.3	3.28	16.1
	NME7+ Antibody Combination 2 attached	MM+NME7 4nM	134.5	4.03	16.1
	NME7+ Antibody Combination 2 Floaters	MM+NME7 4nM	139.5	4.19	18.6

Figure 16

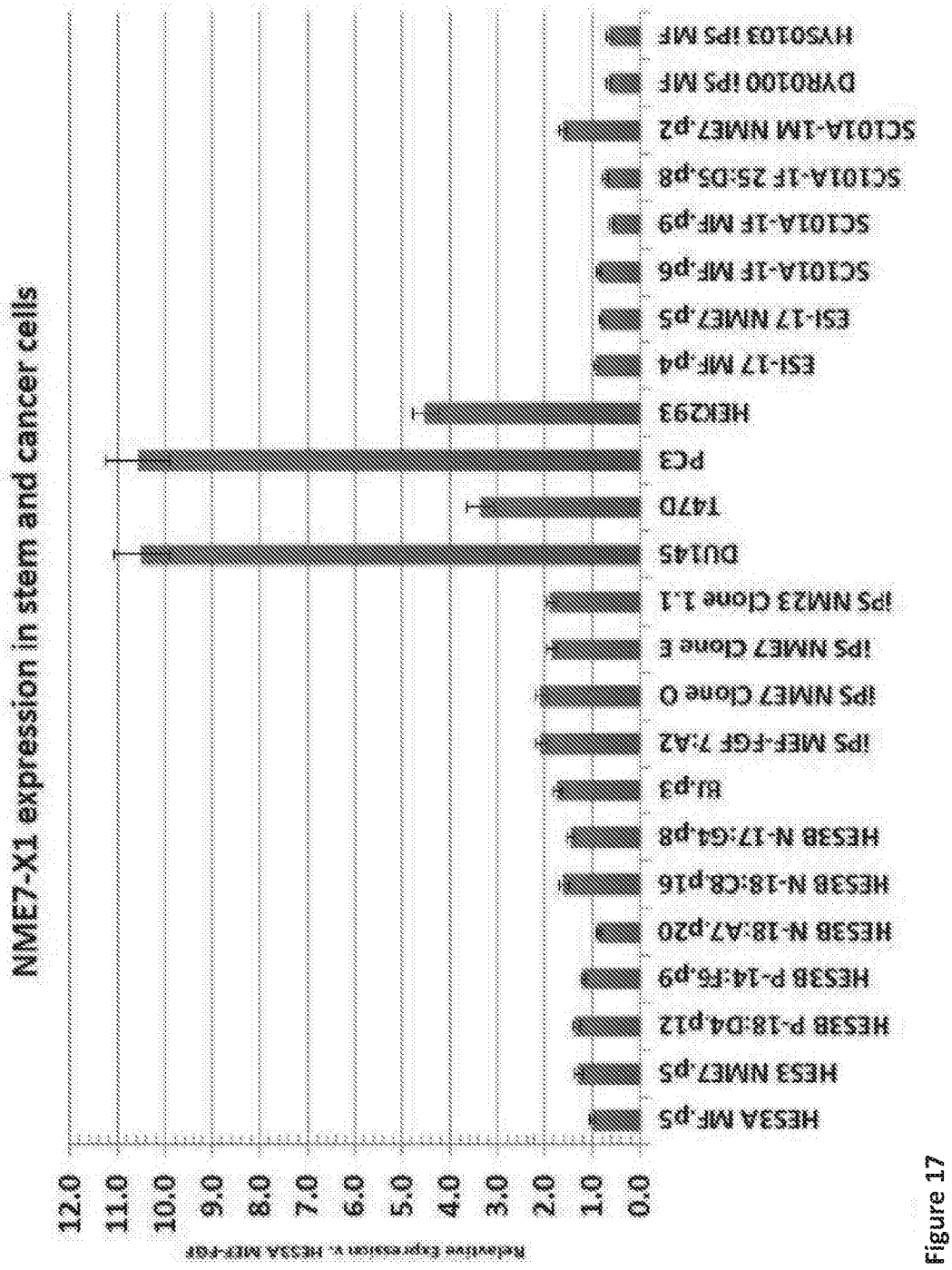

NME7-X1 expression in stem and cancer cells
Relative Expression v. HES3A MF.p5
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
HES3A MF.p5
HES3 NME7.p5
HES3B P-18:D4.p12
HES3B P-14:F5.p9
HES3B N-18:A7.p20
HES3B N-18:C8.p16
HES3B N-17:G4.p8
BJ.p3
iPS MEF-FGF 7:A2
iPS NME7 Clone O
iPS NME7 Clone E
iPS NM23 Clone 1.1
DU145
T47D
PC3
HEK293
ESI-17 MF.p4
ESI-17 NME7.p5
SC10IA-1F MF.p6
SC10IA-1F MF.p9
SC10IA-1F 25:D5.p8
SC10IA-1M NME7.p2
DYR0100 iPS MF
HYS0103 iPS MF

Figure 17

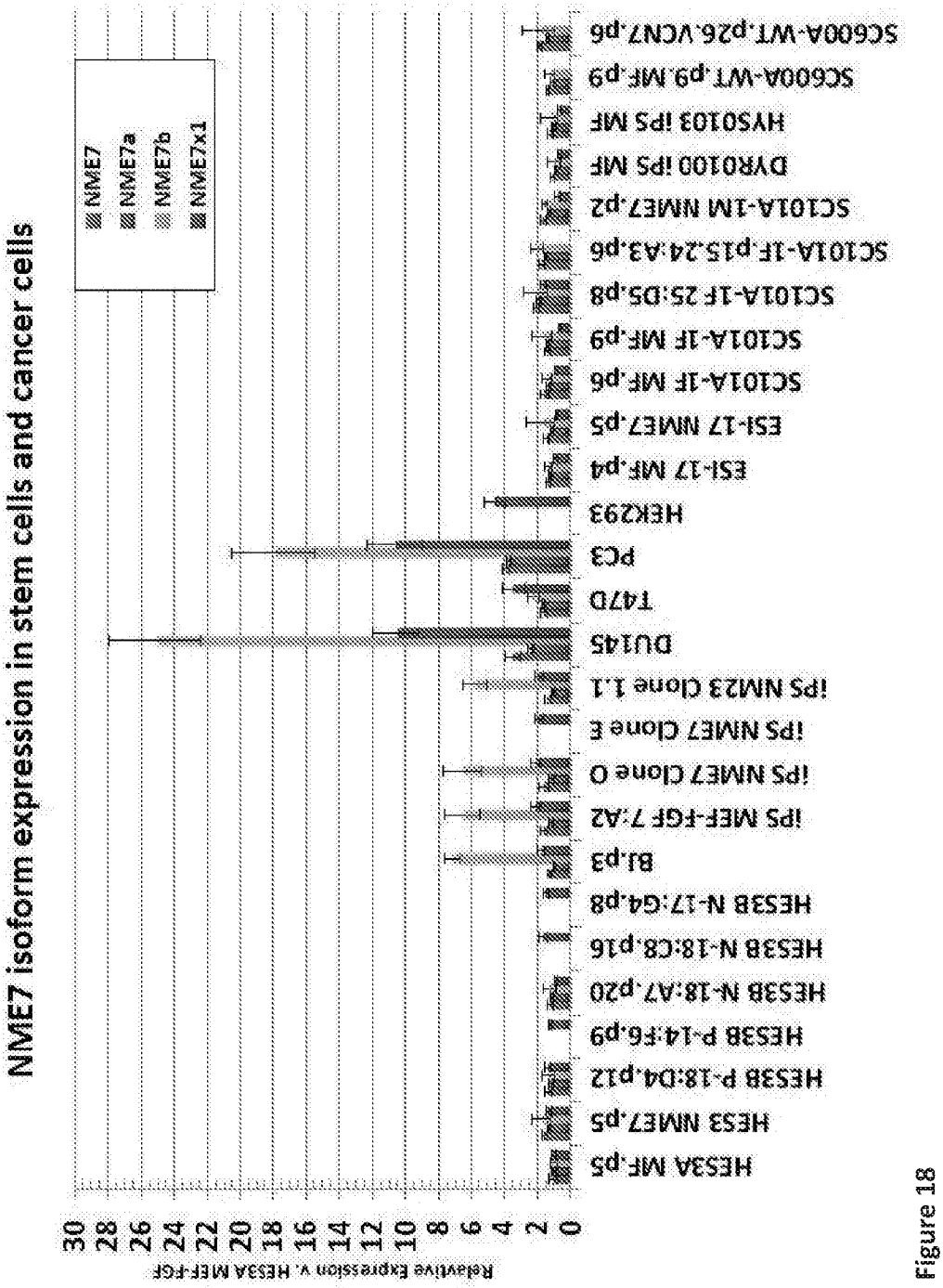
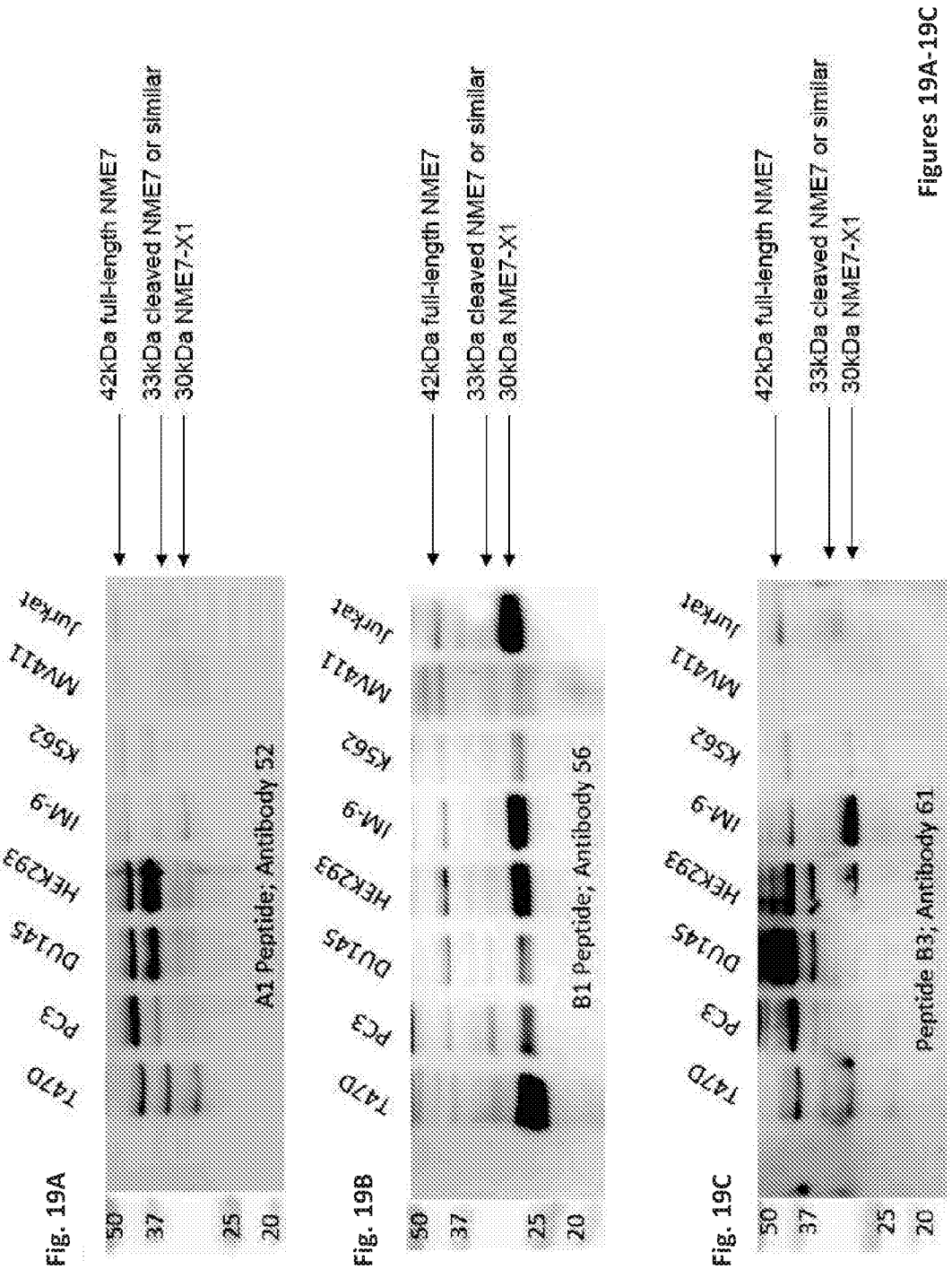

NME7 isoform expression in stem cells and cancer cells
NME7
NME7a
NME7b
NME7x1
Relative Expression v. HES3A MEF-FGF
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
HES3A MF.p5
HES3 NME7.p5
HES3B P-18:D4.p12
HES3B P-14:F6.p9
HES3B N-18:A7.p20
HES3B N-18:C8.p16
HES3B N-17:G4.p8
BJ.p3
iPS MEF-FGF 7:A2
iPS NME7 Clone O
iPS NME7 Clone E
iPS NM23 Clone 1.1
DU145
T47D
PC3
HEK293
ESI-17 MF.p4
ESI-17 NME7.p5
SC101A-1F MF.p6
SC101A-1F MF.p9
SC101A-1F 25:D5.p8
SC101A-1F.p15.24:A3.p6
SC101A-1M NME7.p2
DYR0100 iPS MF
HYS0103 iPS MF
SC600A-WT.p9.MF.p9
SC600A-WT.p26.VCN7.p6

Figure 18



Figures 19A-19C

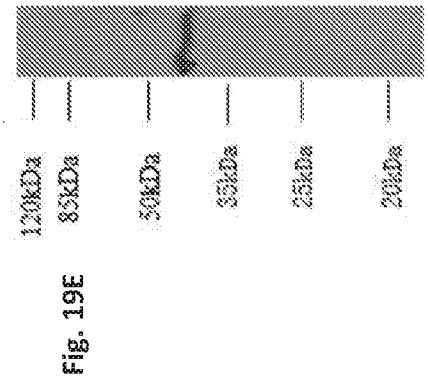


Fig. 19E

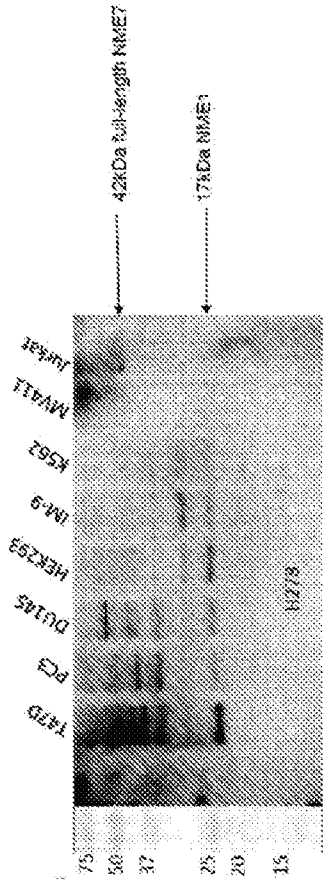


Fig. 19D

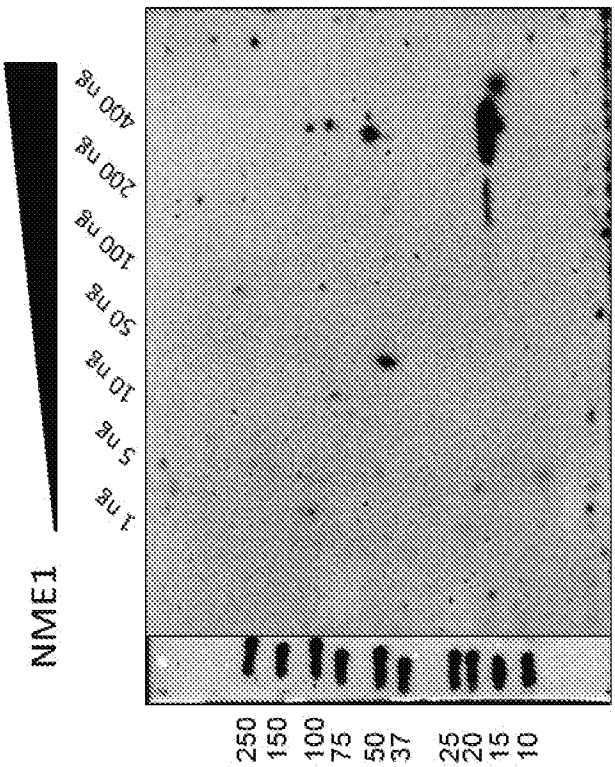
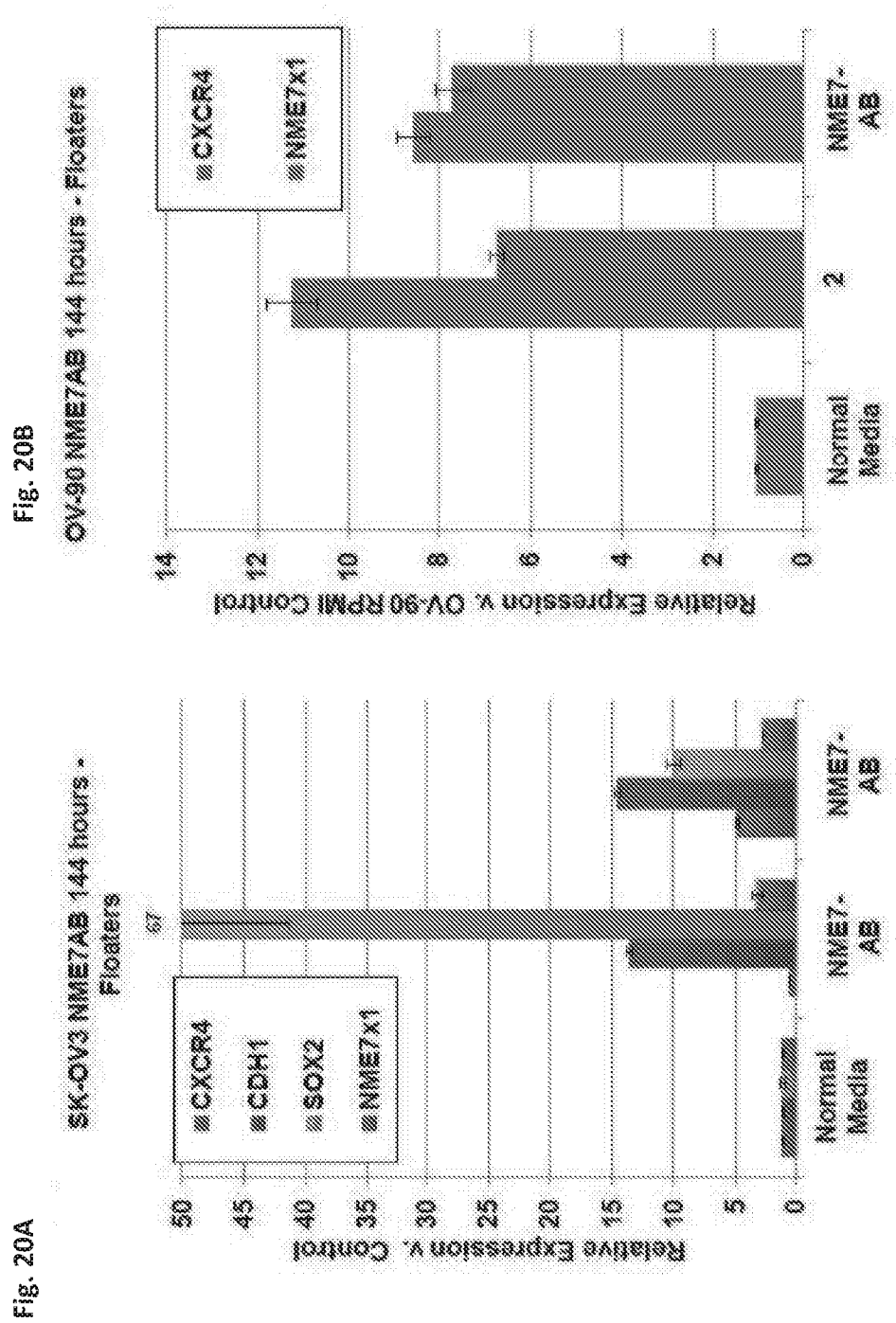


Fig. 19F

Figures 19D-19F



Figures 20A-20B

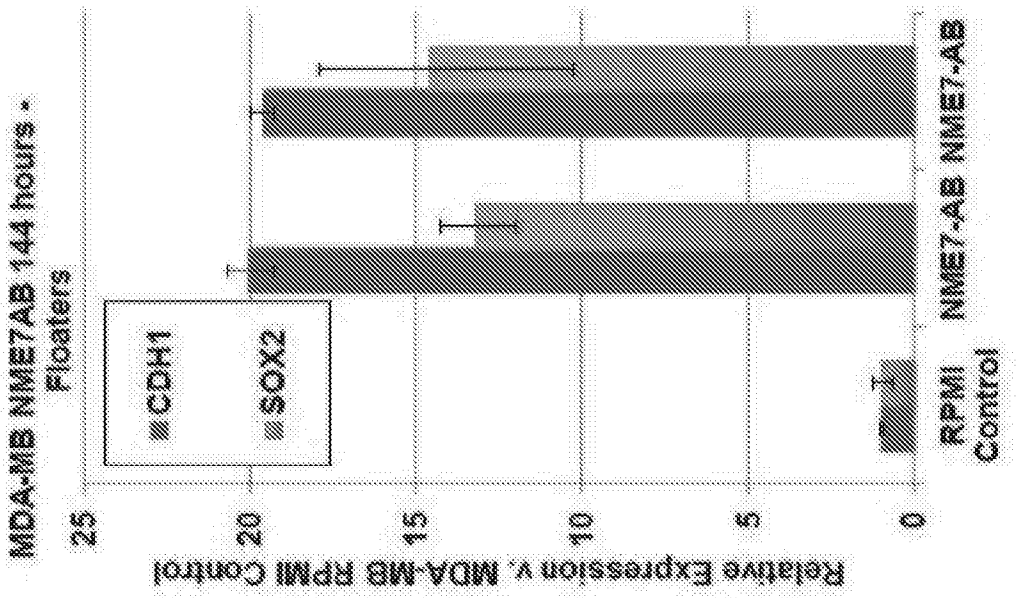

MDA-MB NME7AB 144 hours -
Floaters
CDH1
SOX2
Relative Expression v. MDA-MB RPMI Control
25
20
15
10
5
0
RPMI
Control
NME7-AB
NME7-AB

Figure 20C

Various cancer cell lines express both full-length MUC1 and MUC1*

Fig. 21A

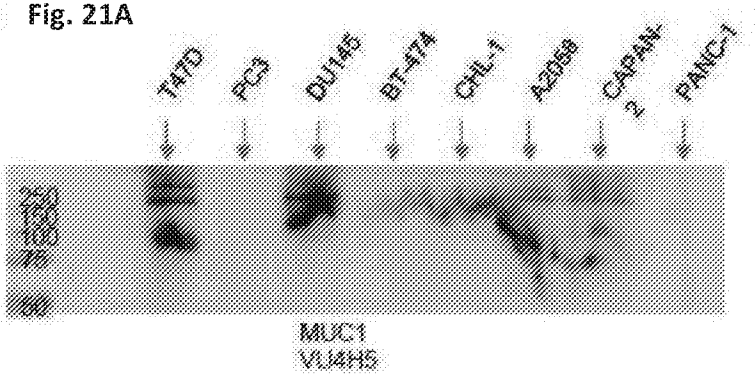


Fig. 21B

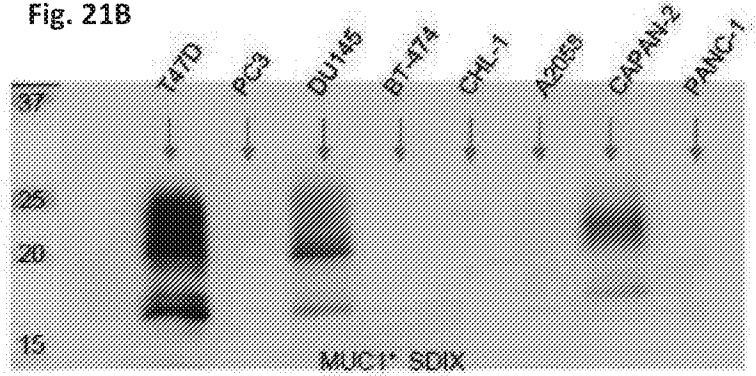
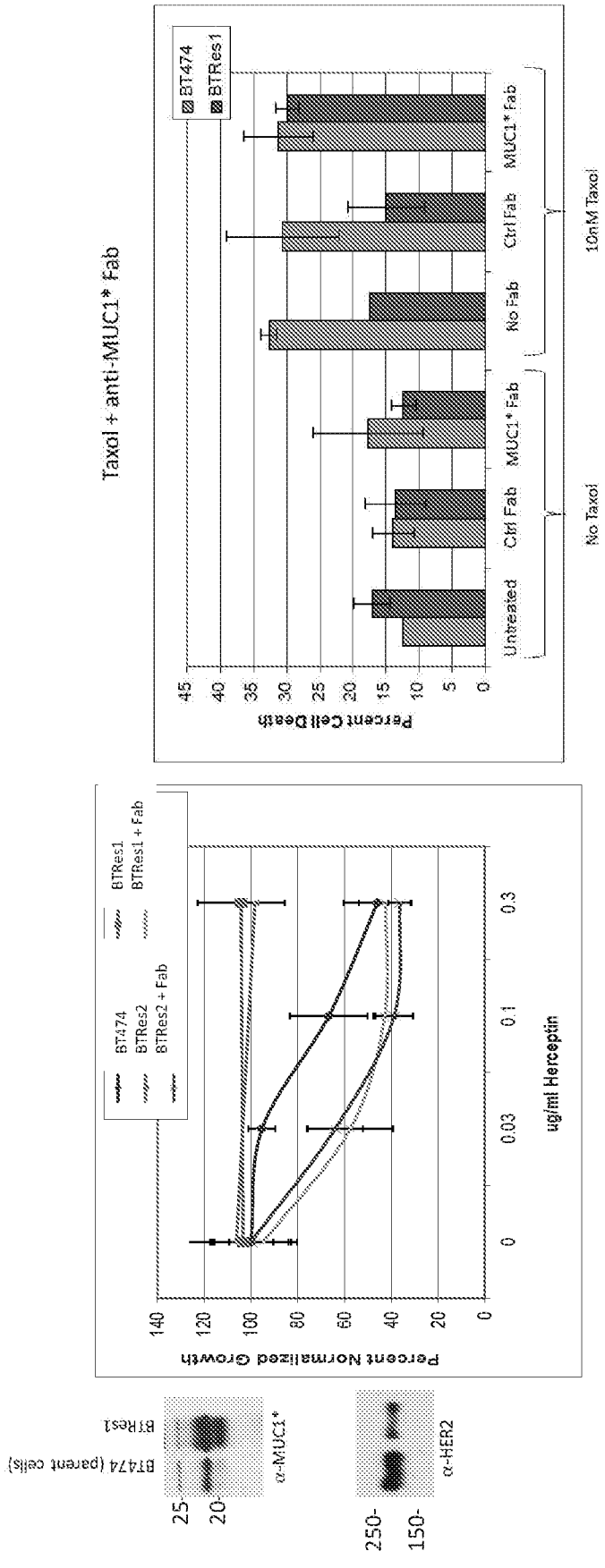


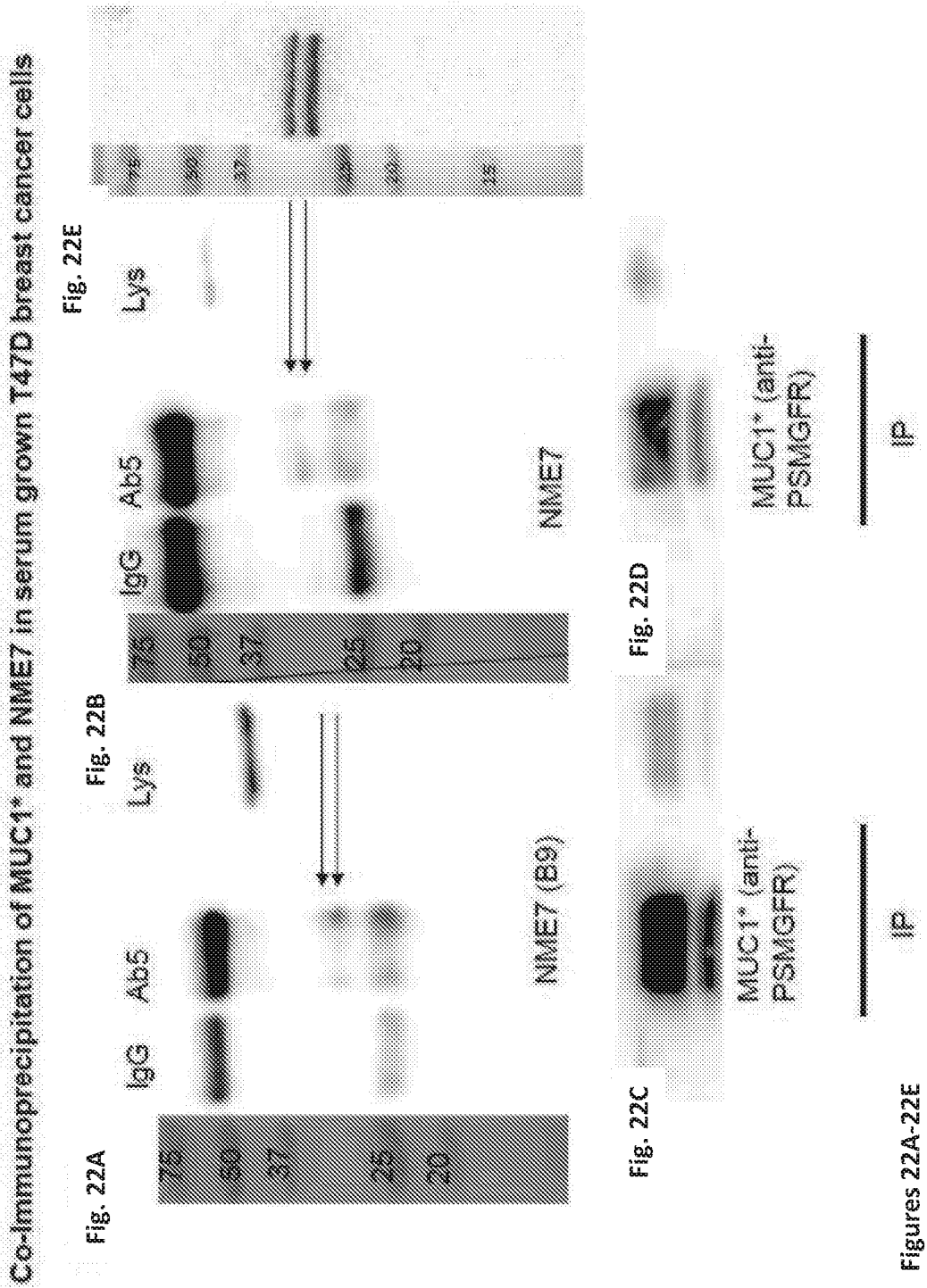
Fig. 21C

T47D – MUC1 positive breast cancer
PC3 – MUC1 negative prostate cancer
DU145 – MUC1 positive prostate cancer
BT-474 – HER2 positive breast cancer
CHL-1 – melanoma
A2058 – melanoma
CAPAN-2 – MUC1 positive pancreatic cancer
PANC-1 – MUC1 negative pancreatic cancer

Figures 21A-21C



Figures 21D-21F



Figures 22A-22E

Co-Immunoprecipitation of MUC1* and NME7 from human embryonic stem cells & iPS cells

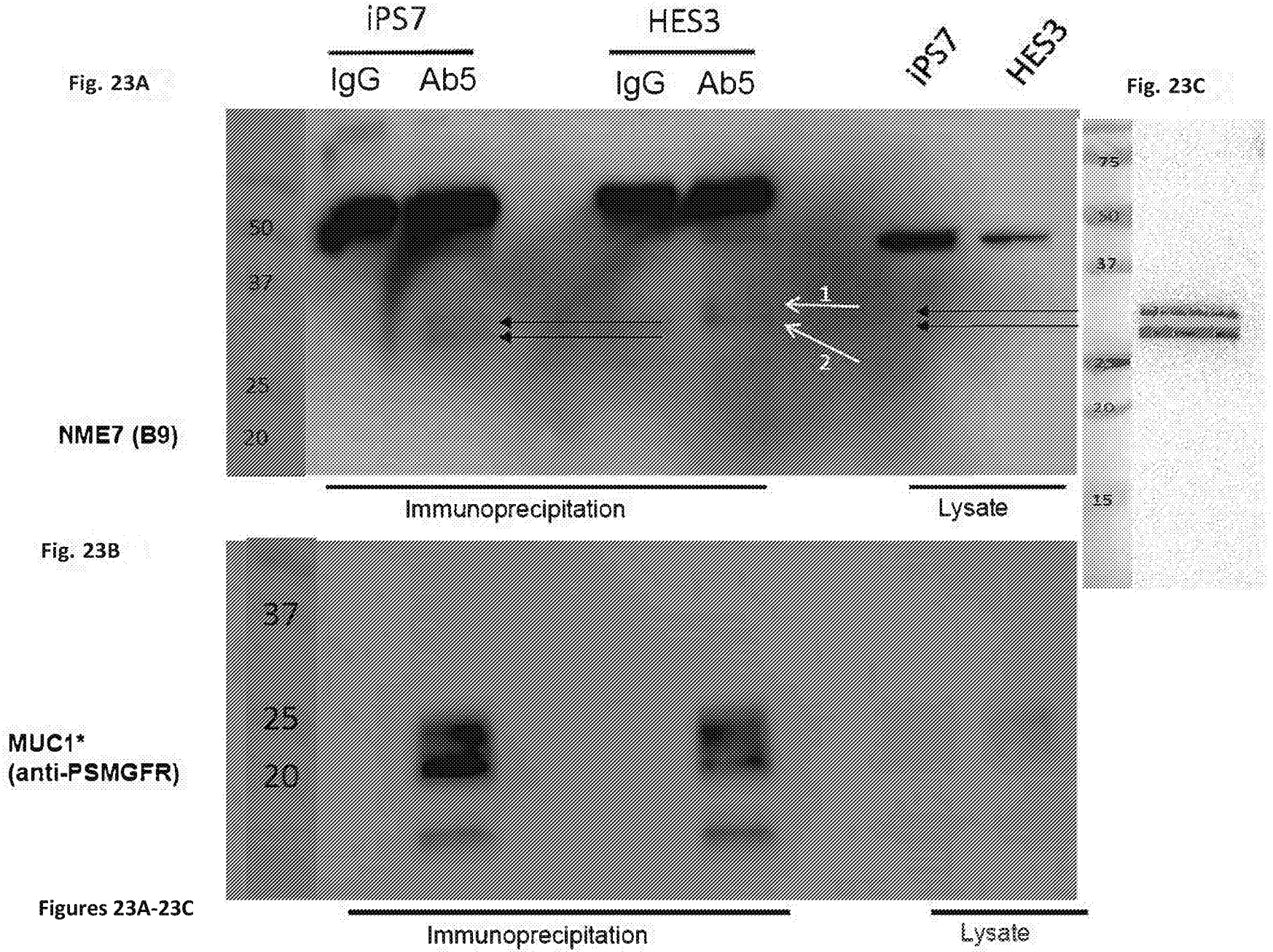


Fig. 23A

Fig. 23B

Fig. 23C

Figures 23A-23C

ELISA: Binding of anti-NME7 B3 antibodies to NME7-AB

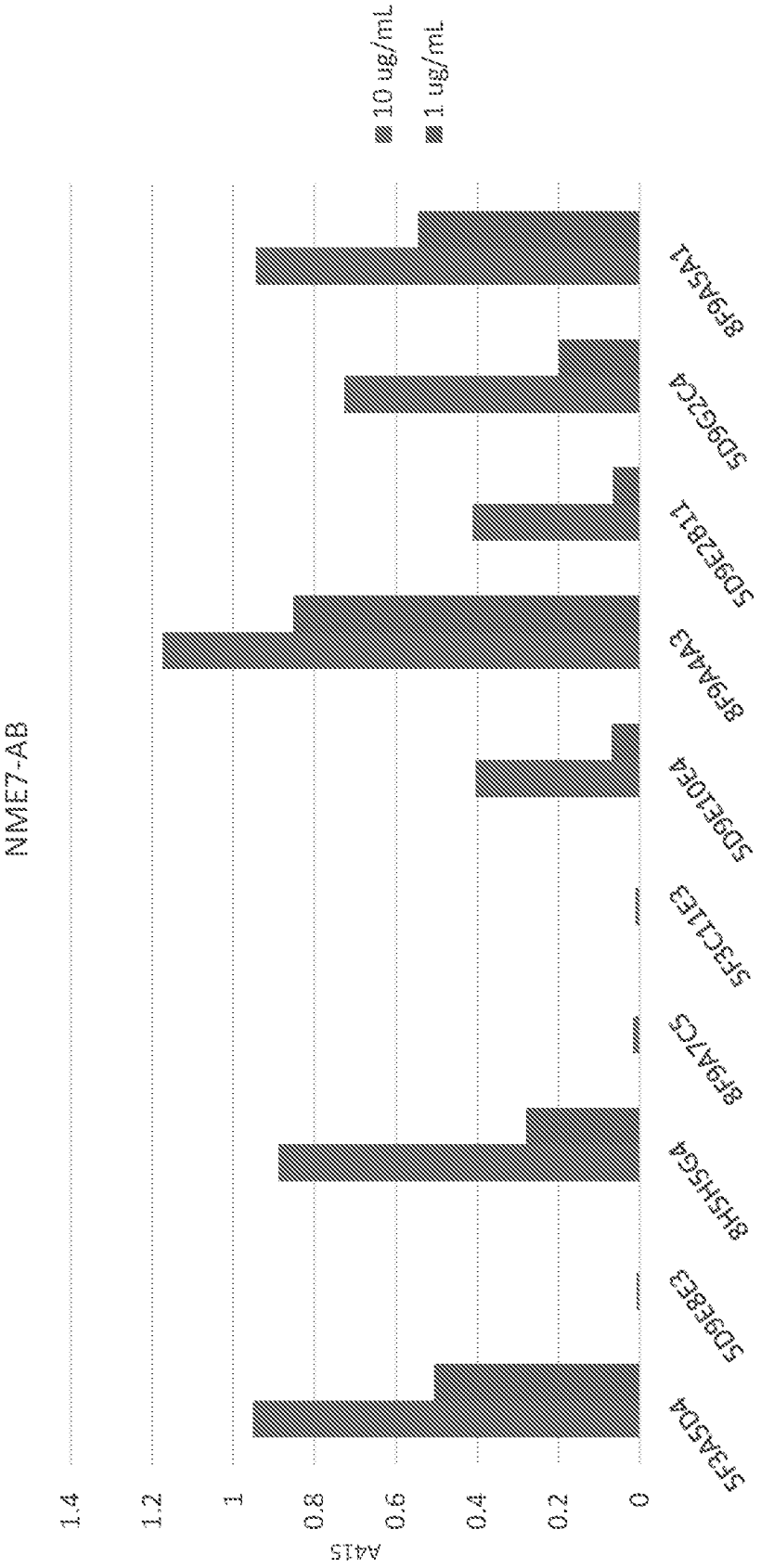


Figure 24

ELISA: Binding of anti-NME7 B3 antibodies to NME7-AB

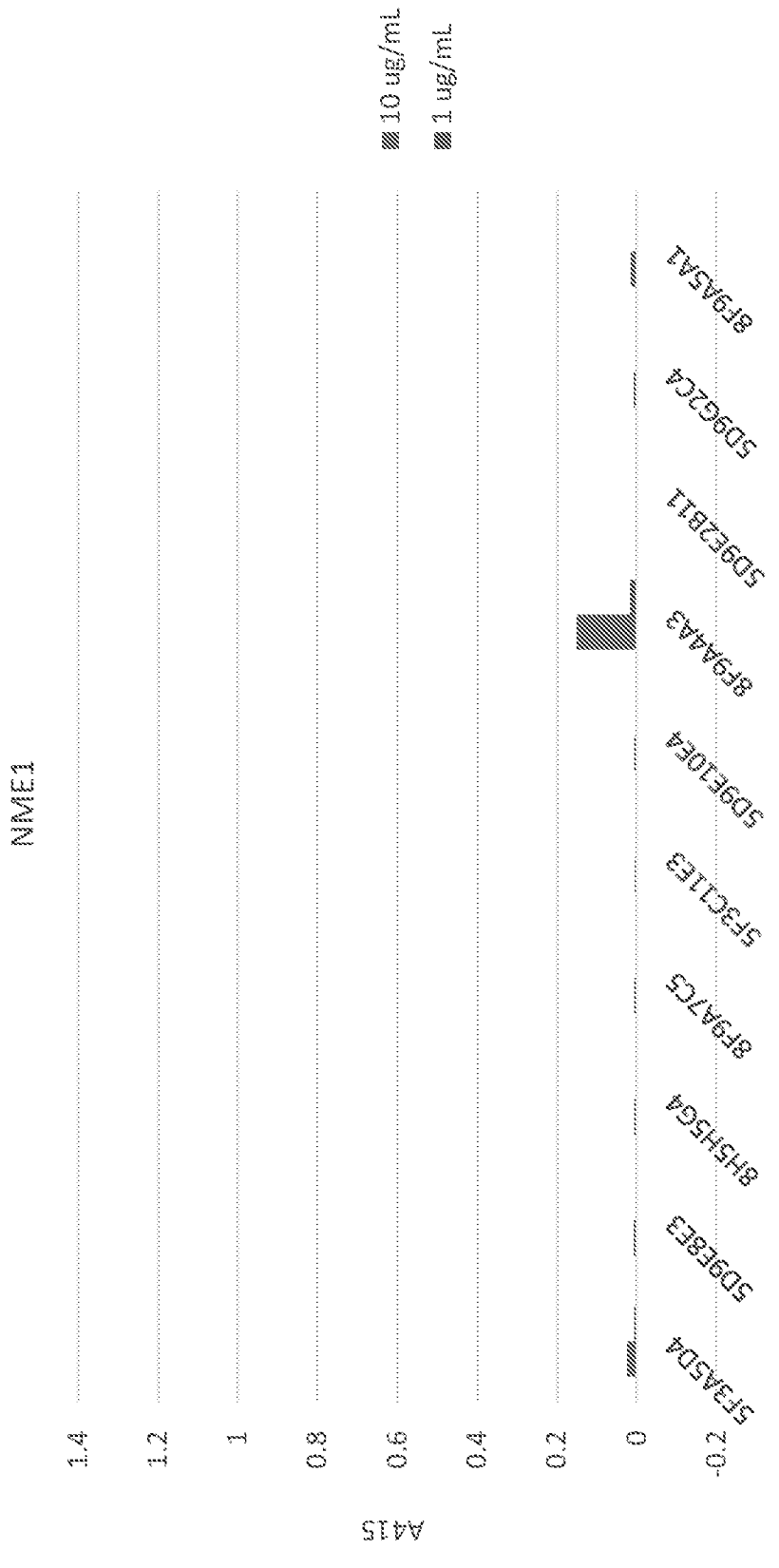


Figure 25

Displacement assay: Anti-NME7 B3 antibodies disrupt binding of NME7-AB to the MUC1* extra cellular domain peptide. The NME7-AB/anti-NME7 antibody complex was made before adding to plate coated with MUC1* extra cellular domain peptide.

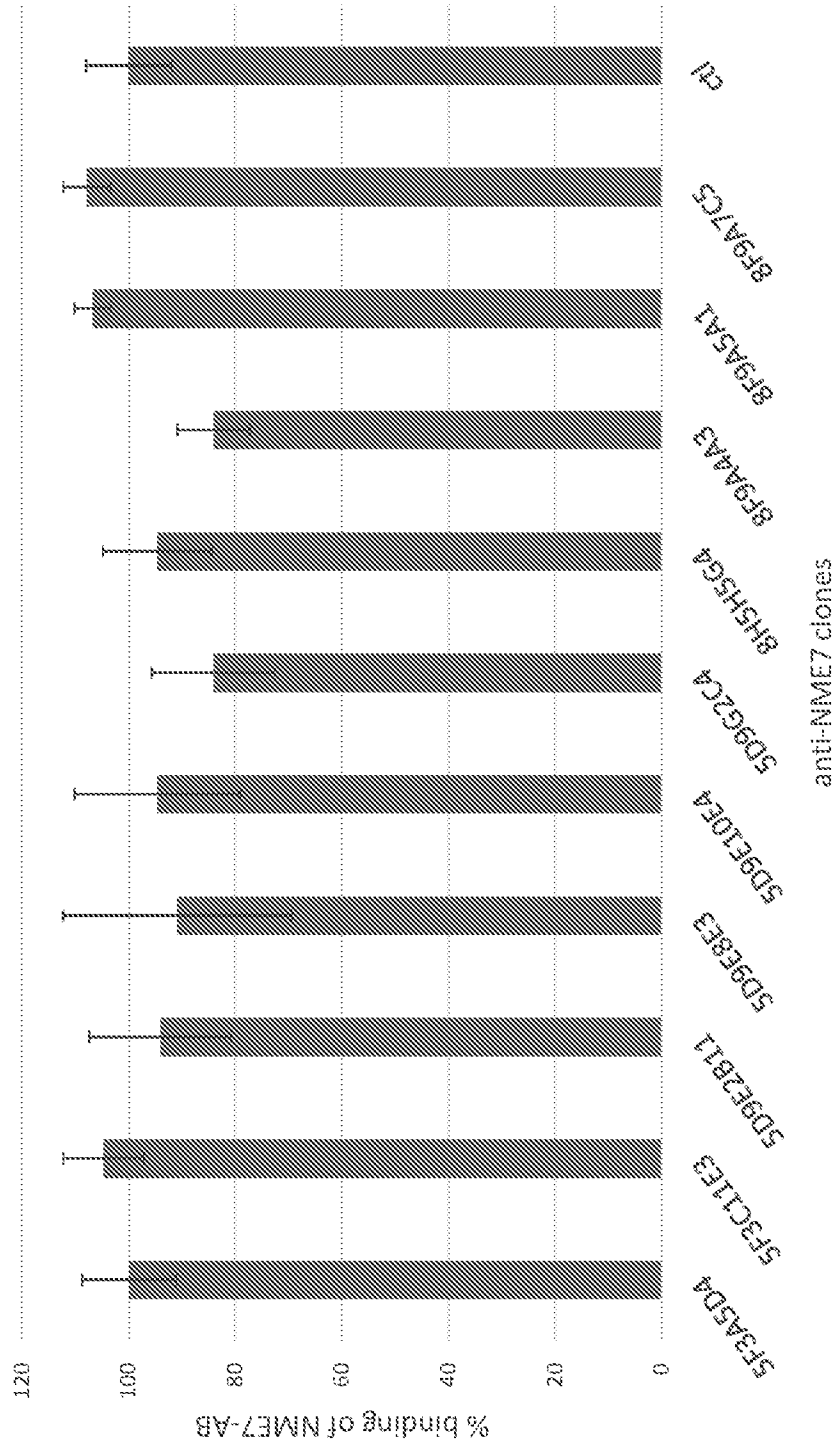


Figure 26

Displacement assay: Anti-NME7 B3 antibodies disrupt binding of NME7-AB to the MUC1* extra cellular domain peptide.
The NME7-AB was first bound to MUC1* extra cellular domain peptide on the plate, then disrupted by the addition of anti-NME7 antibodies.

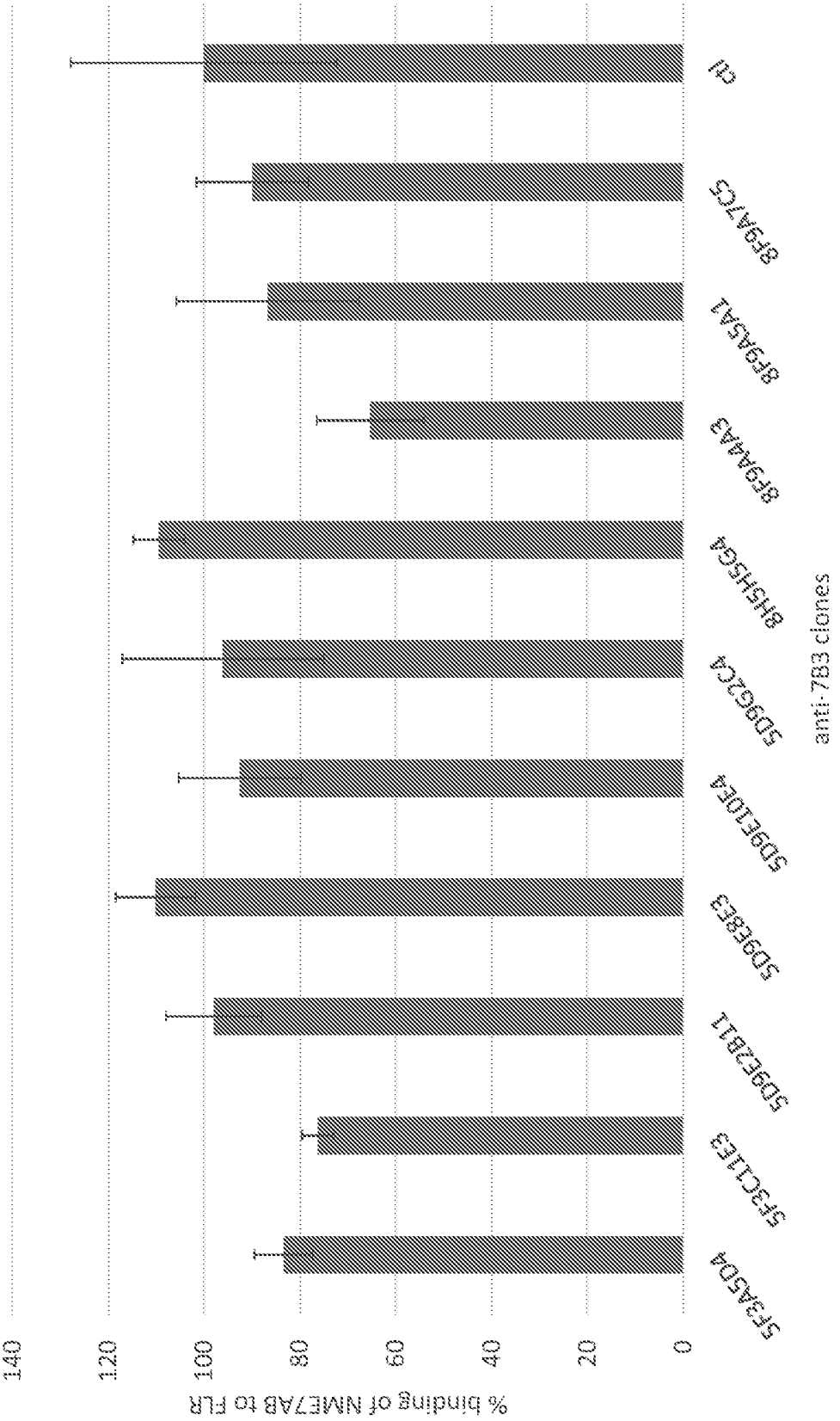


Figure 27

Displacement assay: Anti-NME7 B3 antibodies disrupt binding of NME7-AB to the MUC1* N-10 extra cellular domain peptide. The NME7-AB was first bound to MUC1* N-10 peptide on the plate, then disrupted by the addition of anti-NME7 antibodies.

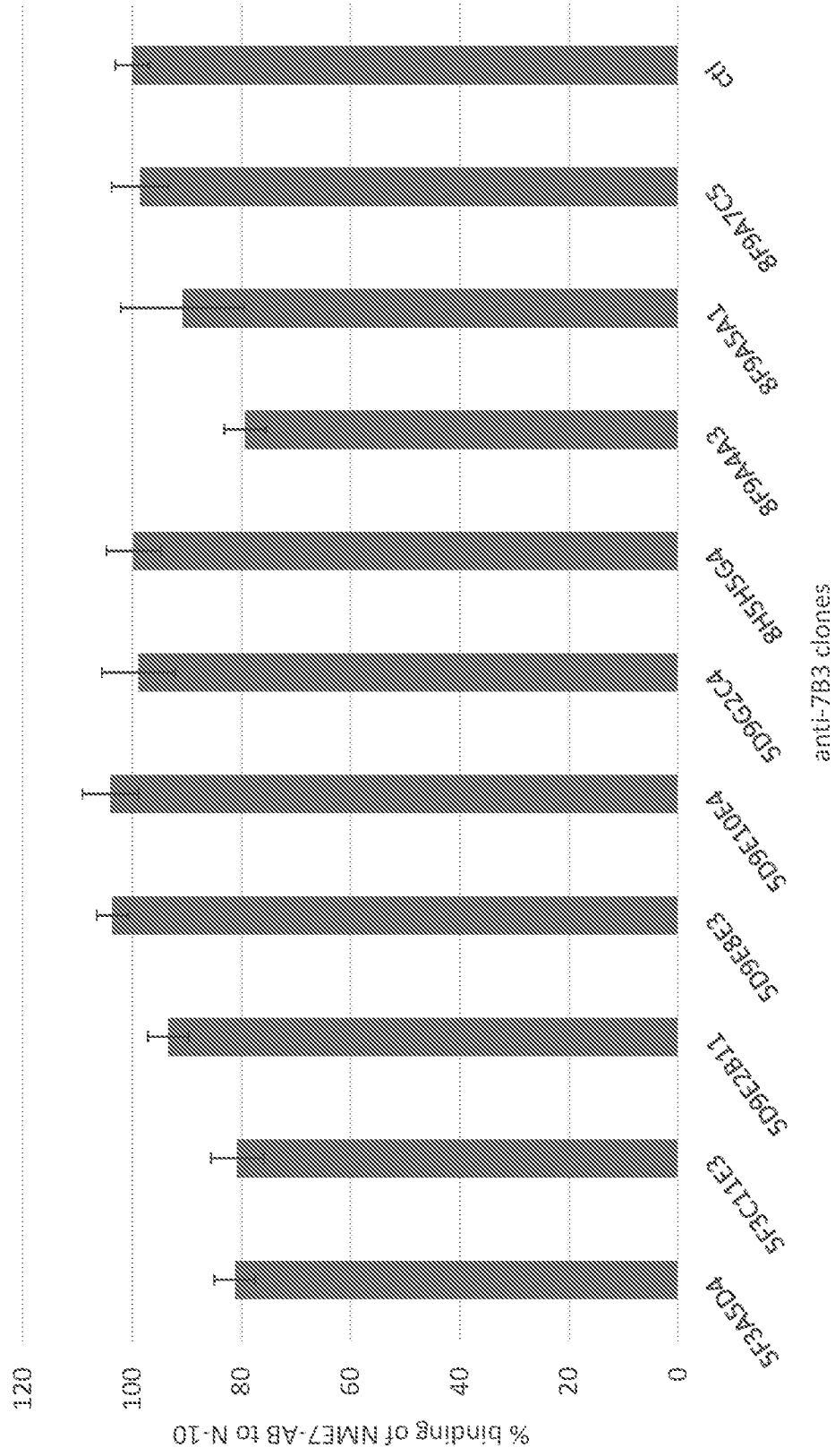


Figure 28

RNA yield, which is an indicator of cell number, from T47D breast cancer cells grown in NME7-AB media in the presence or absence of anti-NME7 B3 antibodies

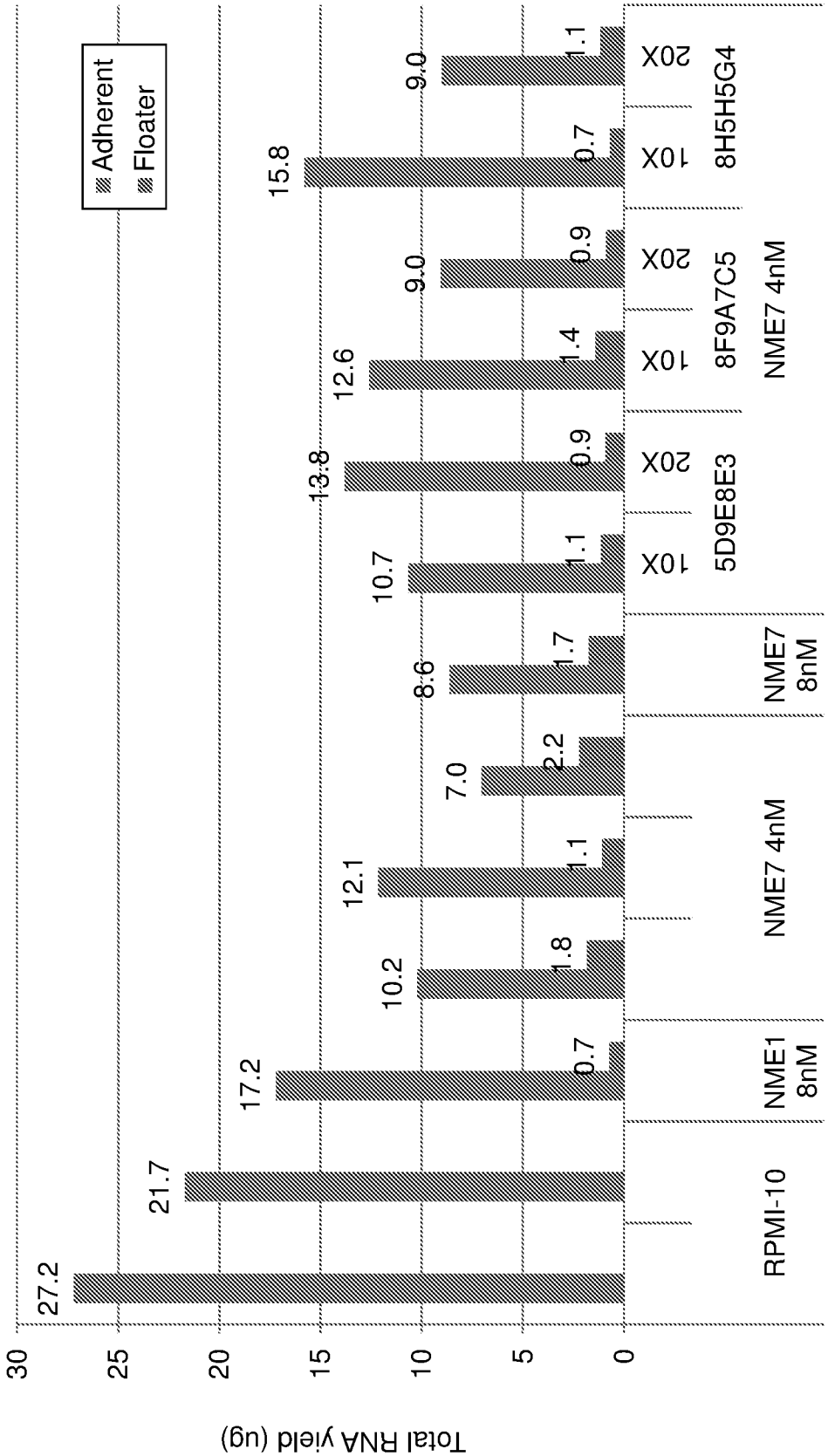


Figure 29

RT-PCR analysis of breast cancer metastasis marker CXCR4 in T47D breast cancer cells grown in NME7-AB media In the presence or absence of anti-NME7 B3 antibodies

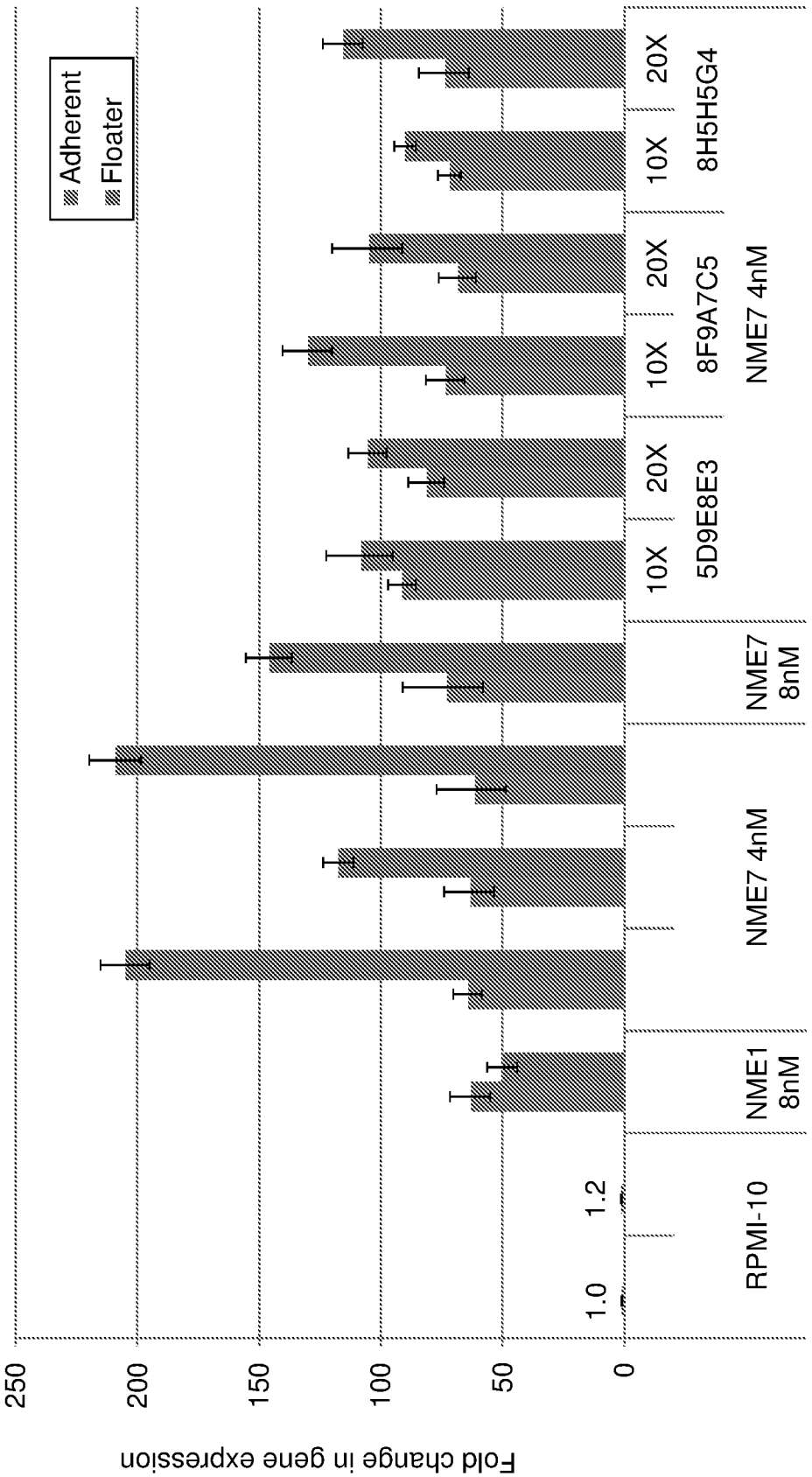


Figure 30

RT-PCR analysis of stem cell and metastasis marker SOX2 in T47D breast cancer cells grown in NME7-AB media In the presence or absence of anti-NME7 B3 antibodies

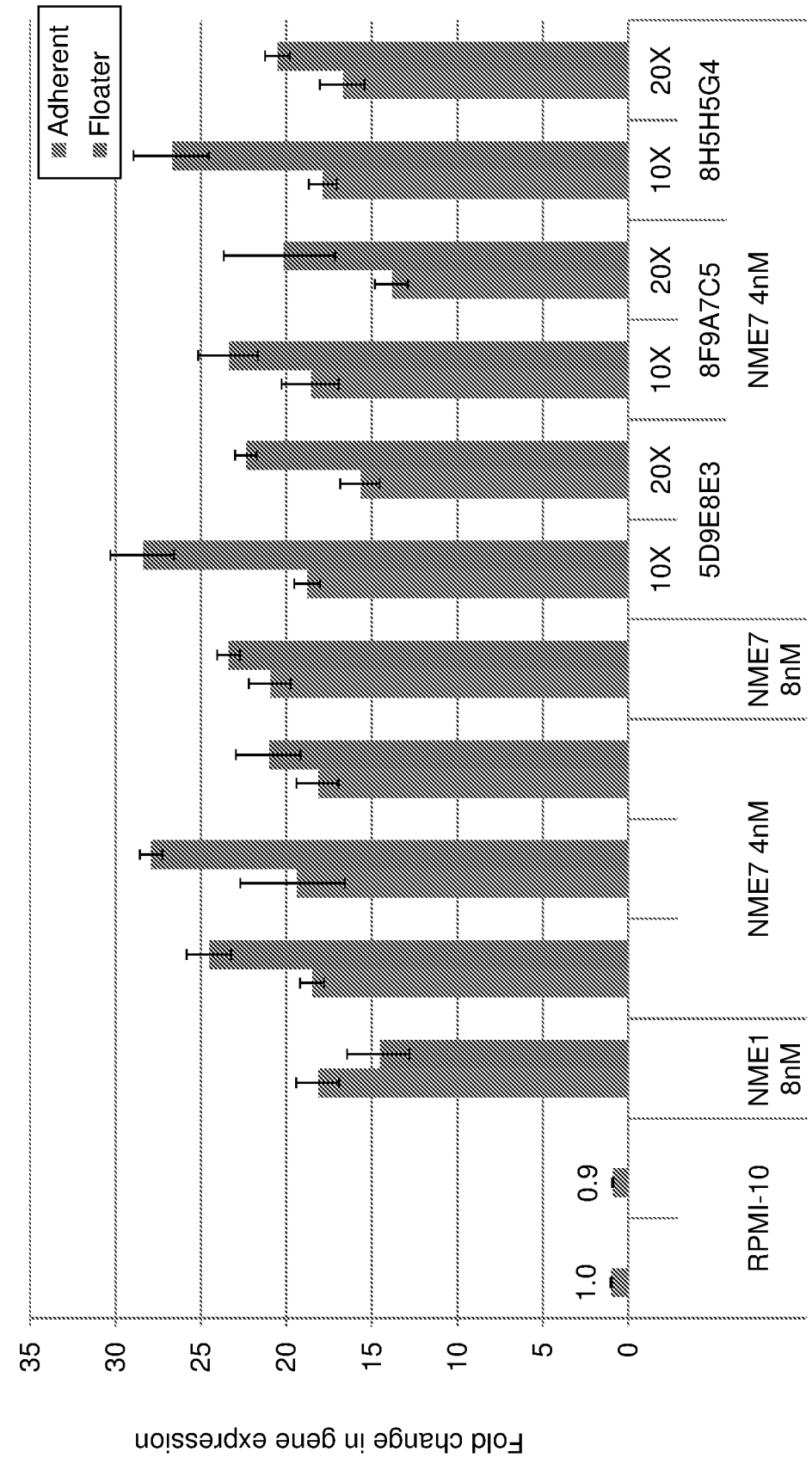


Figure 31

RT-PCR analysis of the NME7-AB cognate receptor MUC1* in T47D breast cancer cells grown in NME7-AB media In the presence or absence of anti-NME7 B3 antibodies

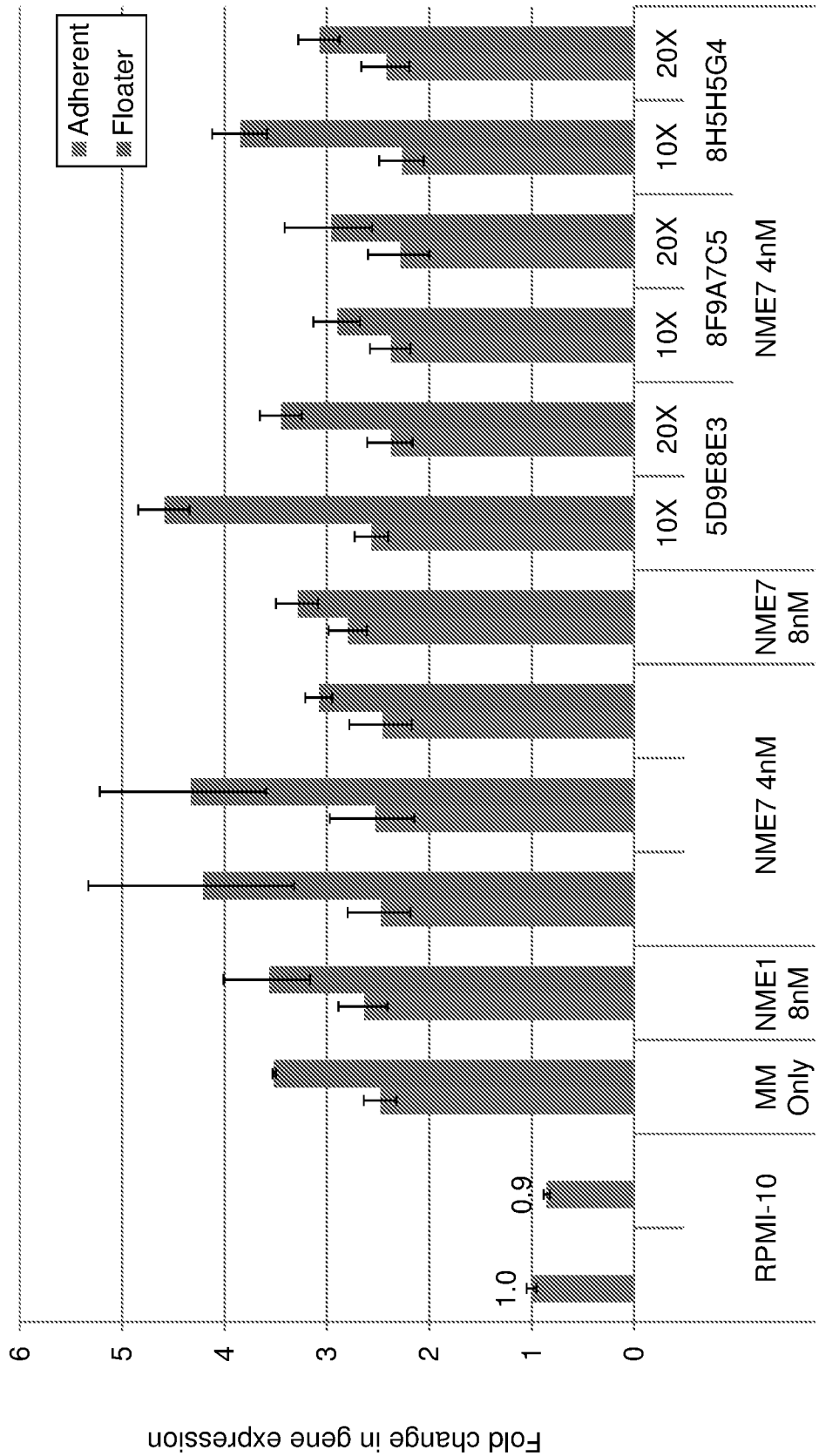


Figure 32

Onco-embryonic growth factor NME7 transforms cancer cells into metastatic cancer stem cells

Day 6

Fig. 33A

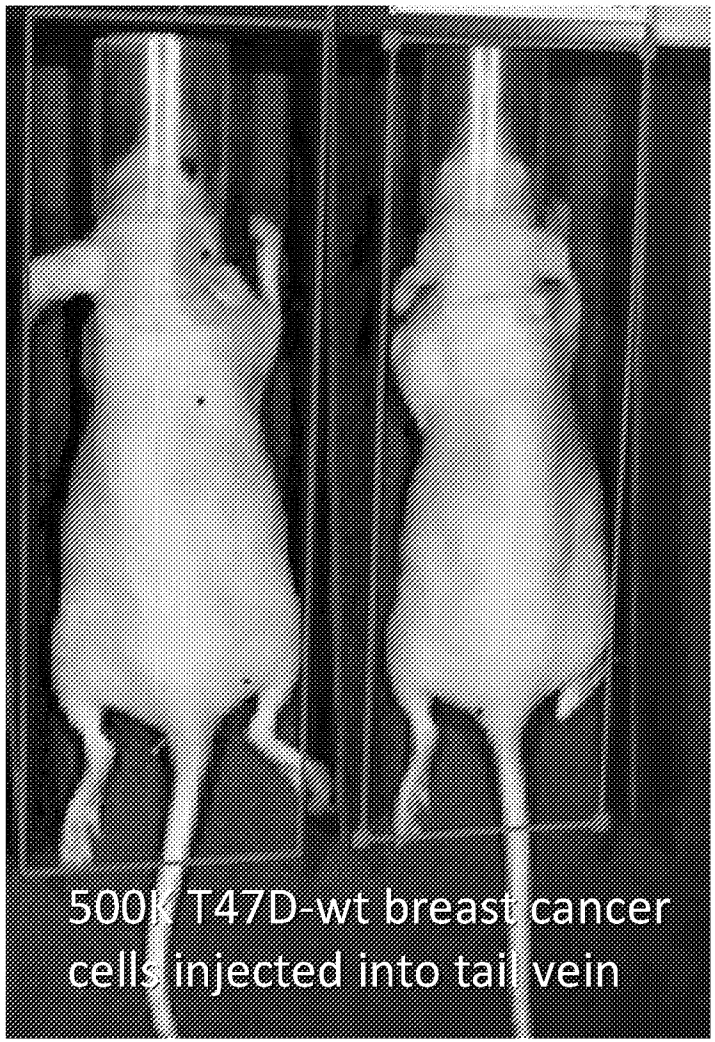
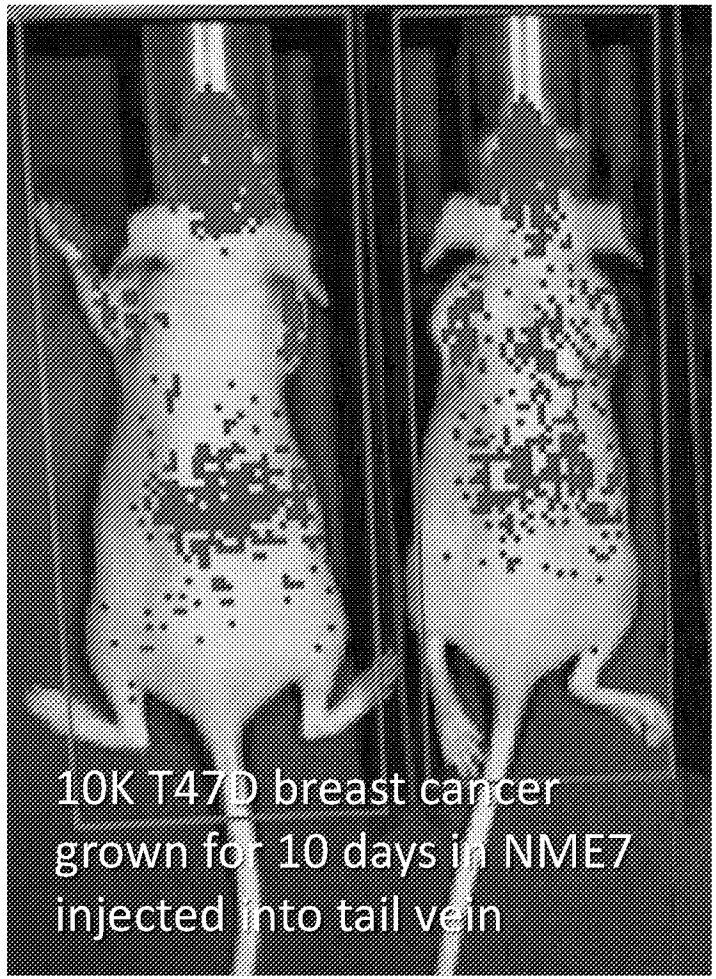


Fig. 33B



Figures 33A-33B

Day 10 T47D-CSC (cancer stem cells) @ 10K vs wt @ 500K +/- anti-NME7 treatment; Day 7 was 1st antibody injection but was done just after injection of NME7

Fig. 34A

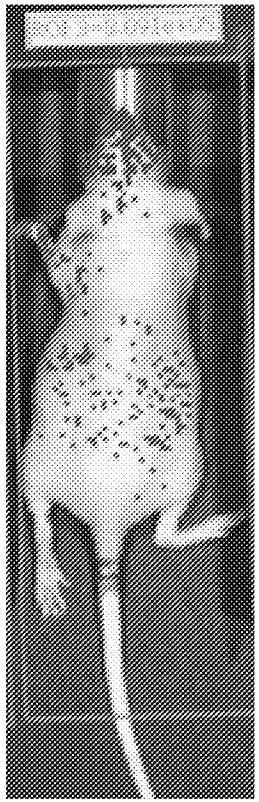
T47D-wt
500K i.v.
Day 10
No Antibody

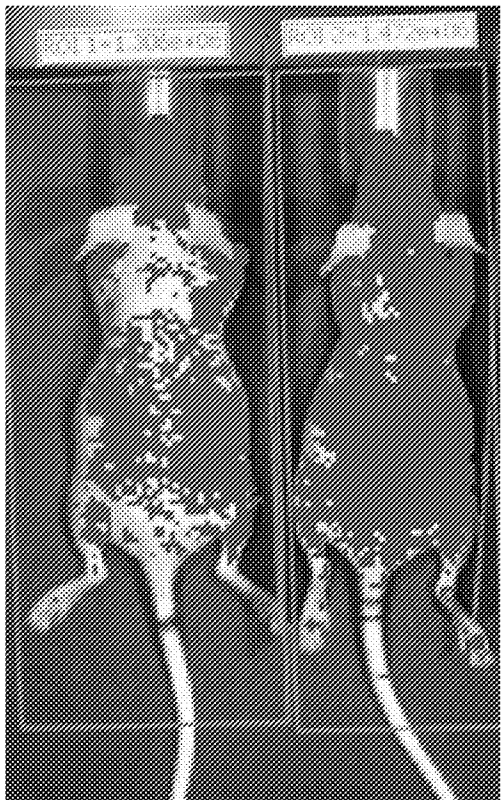
Fig. 34B

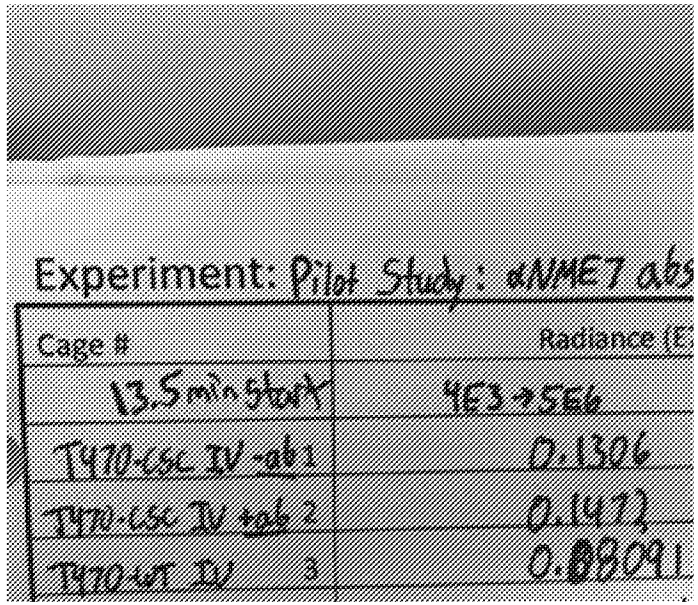
Fig. 34C

T47D-CSC
10K i.v.
Day 10
+ Antibody

Fig. 34D



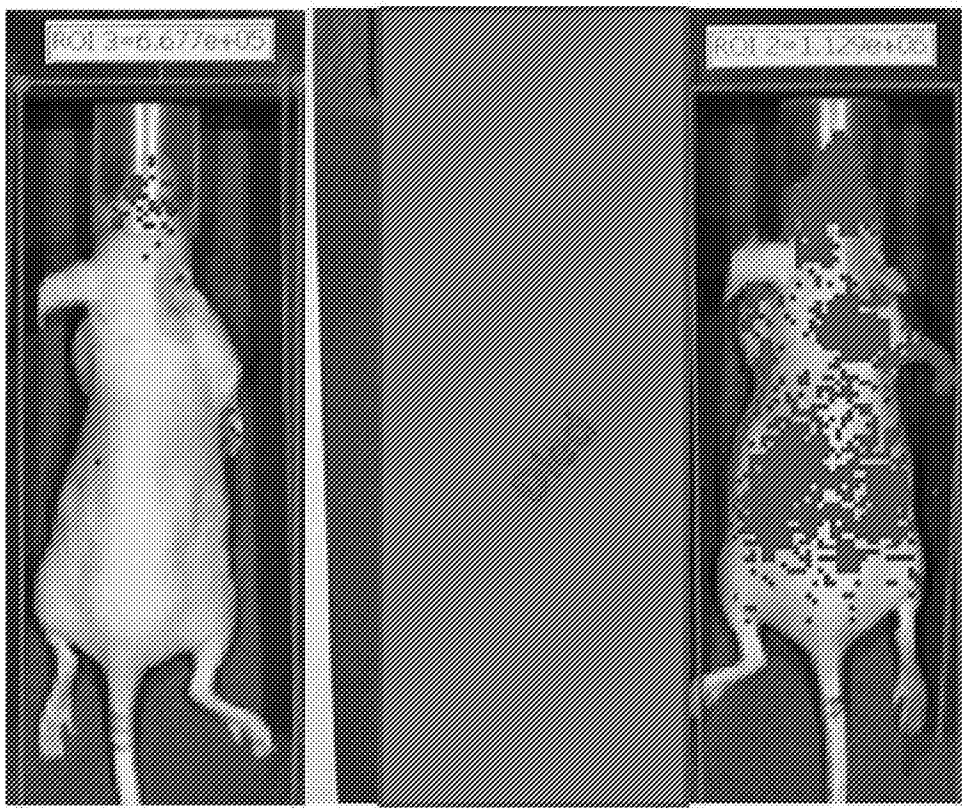




Figures 34A-34D

Day 12 T47D-CSC @ 10K vs wt @ 500K; CSC mouse was + anti-NME7 treatment;

Fig. 35A	Fig. 35B	Fig. 35C
T47D-wt 500K i.v. Day 12 No Antibody	T47D-CSC 10K i.v. Day 12 No Antibody	T47D-CSC 10K i.v. Day 12 + Antibody



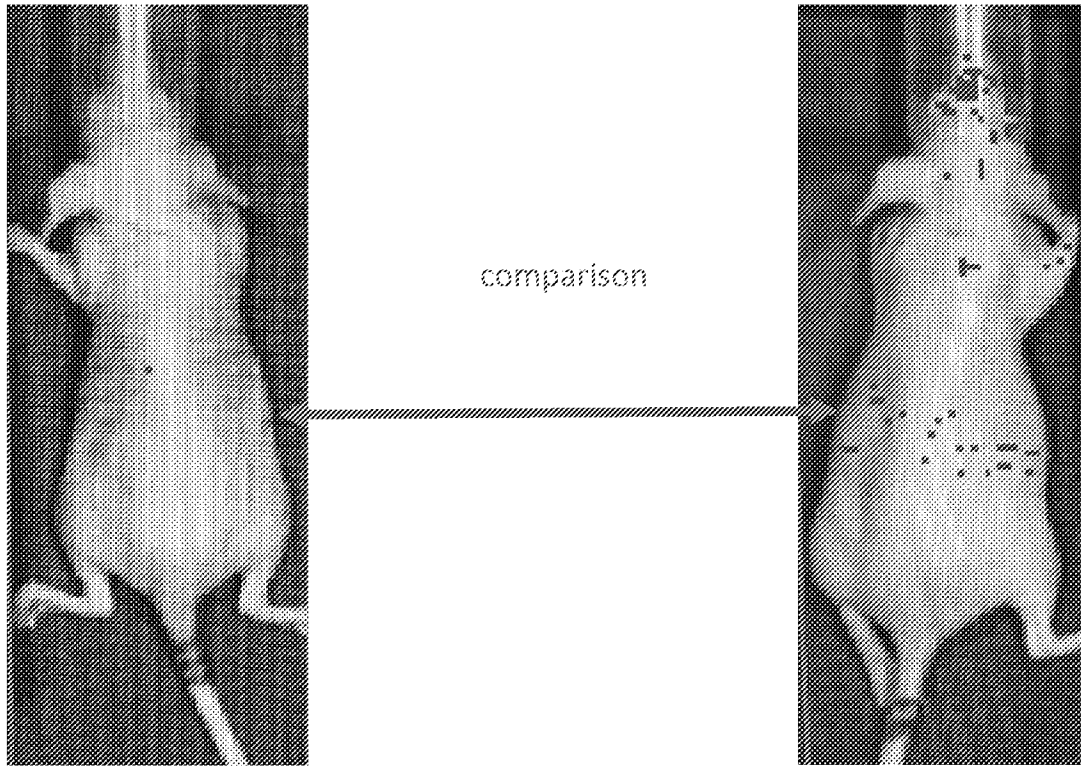
Figures 35A-35C

Day 14 T47D-CSC @ 10K vs wt @ 500K; CSC mouse had 3 anti-NME7 injections, although 1st injection was at same time as NME7 injection

Fig. 36A

Fig. 36B

T47D-wt
500K i.v.
Day 14
No Antibody



Figures 36A-36B

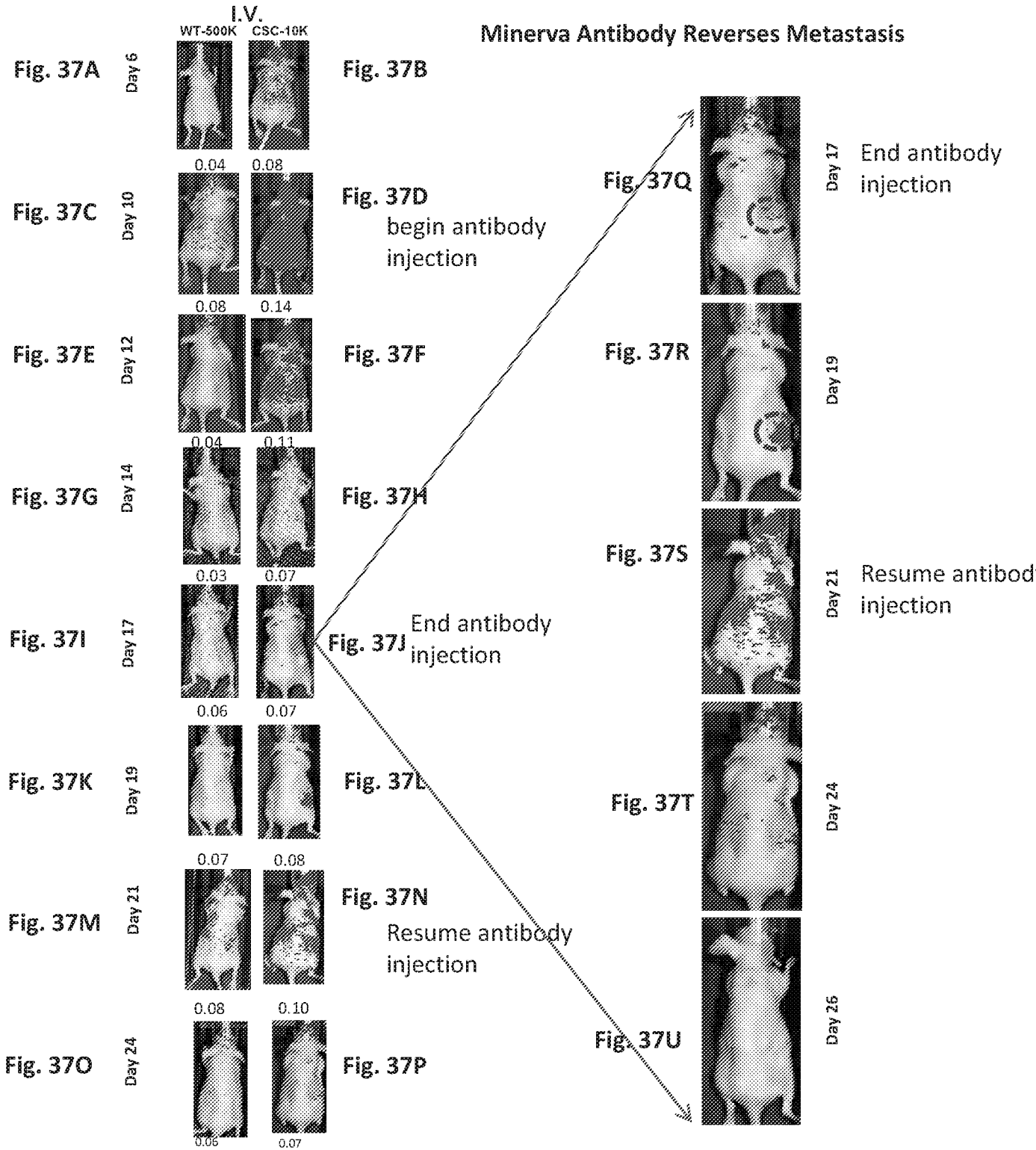
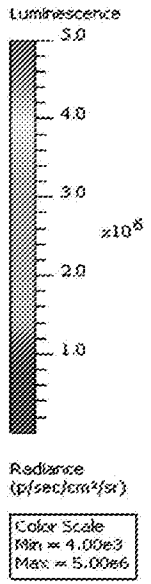


Fig. 37V



Figures 37A-37V

This was intended to be a pilot experiment to determine which cancer cells would metastasize and by what routes of administration. 24 female nu/nu mice implanted with 9-day release estrogen pellets. As of Day 20, no other cells induced metastases. These other cells included AsPC-1 pancreatic cells, reported to metastasize 28-30 days post i.v. injection of 500K cells.

* Indicates treated with anti-NME7 antibody

Day 0 – engraftment w 32nM NME7
Day 2, 4, 6 NME7 injections ip 32nM
Day 7, 10, 12, 14 iv, ip or sc anti-NME7 mAb injections

Figures 38A-38C

WO 2020/163325 PCT/US2020/016570

Lung

Normal

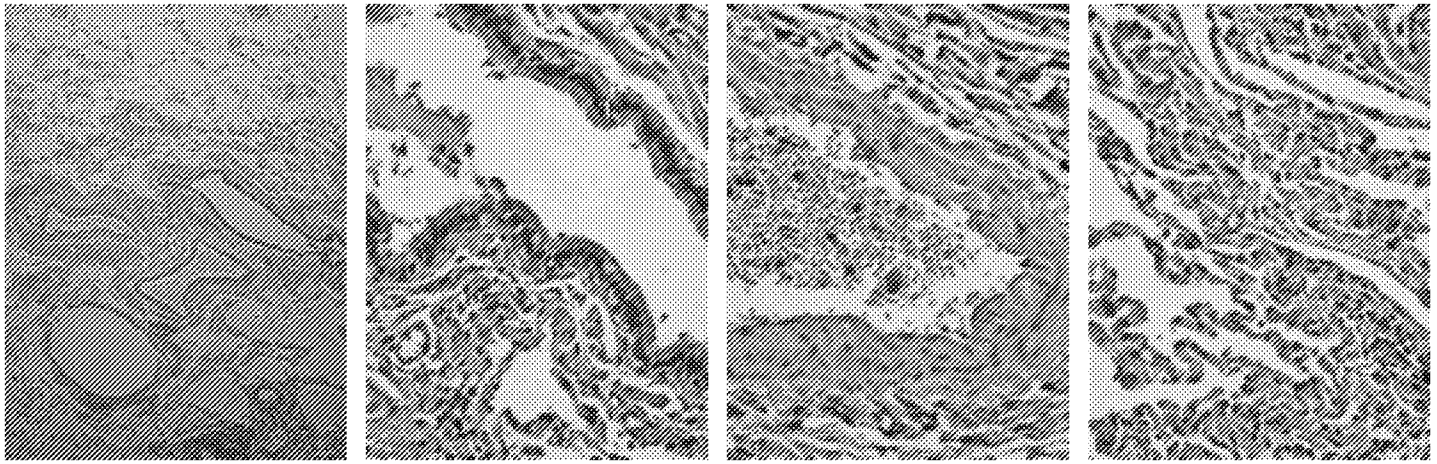
61

10 ug/ml

61 = Minerva's anti-NME7 antibody

Respiratory Epithelium | Bronchiole | Alveolae

Fig. 39A

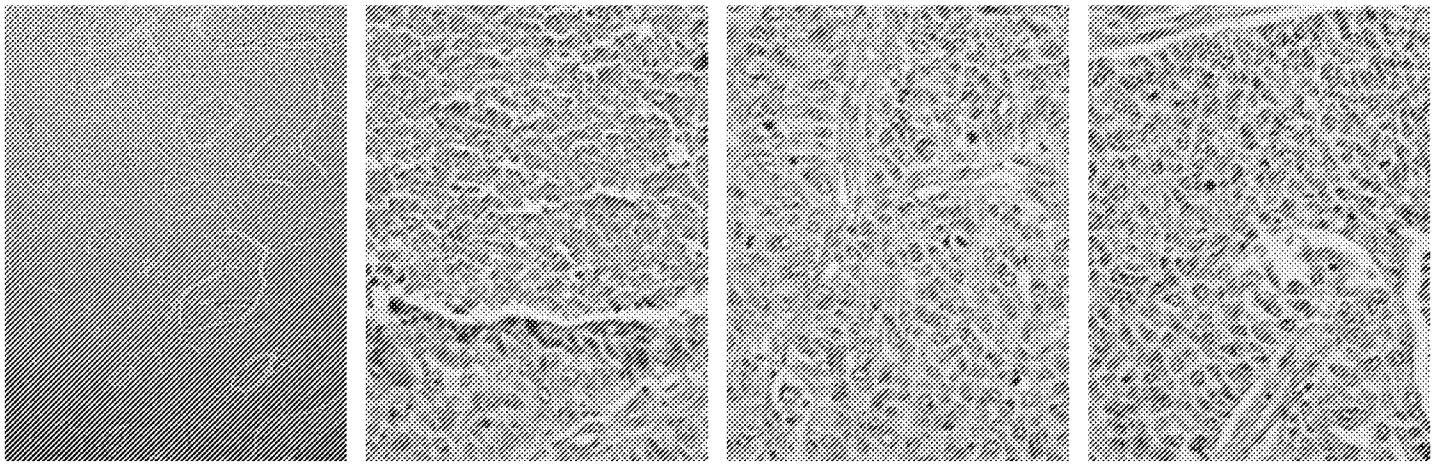


Tumor Grade 2

61

10 ug/ml

Fig. 39B

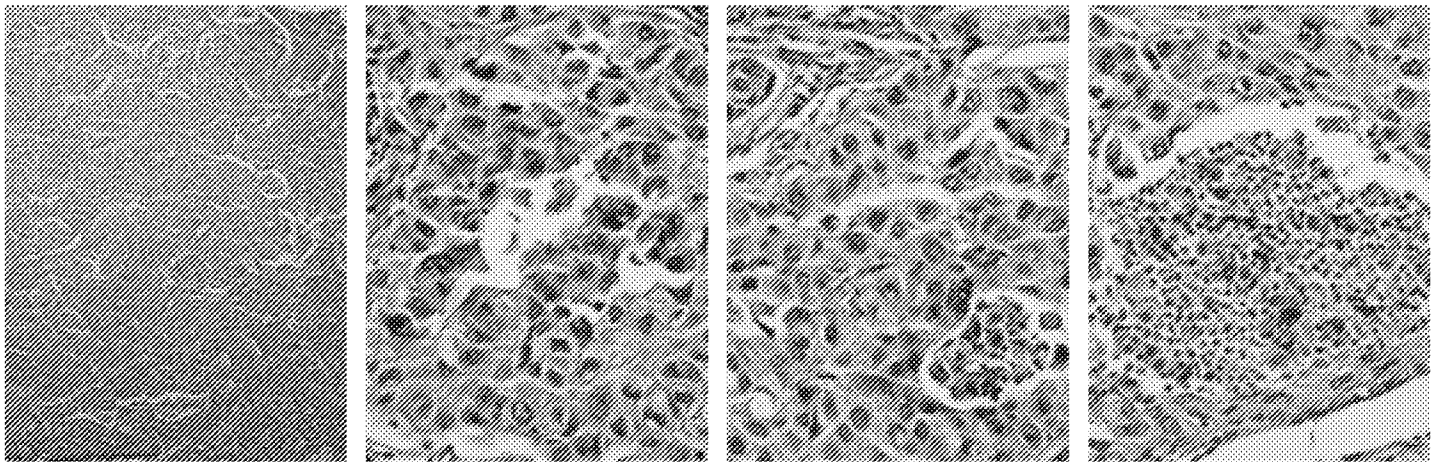


Tumor Grade 3
(T2N0M0) Clinical IB

61

10 ug/ml

Fig. 39C



Figures 39A-39C

Small Intestine

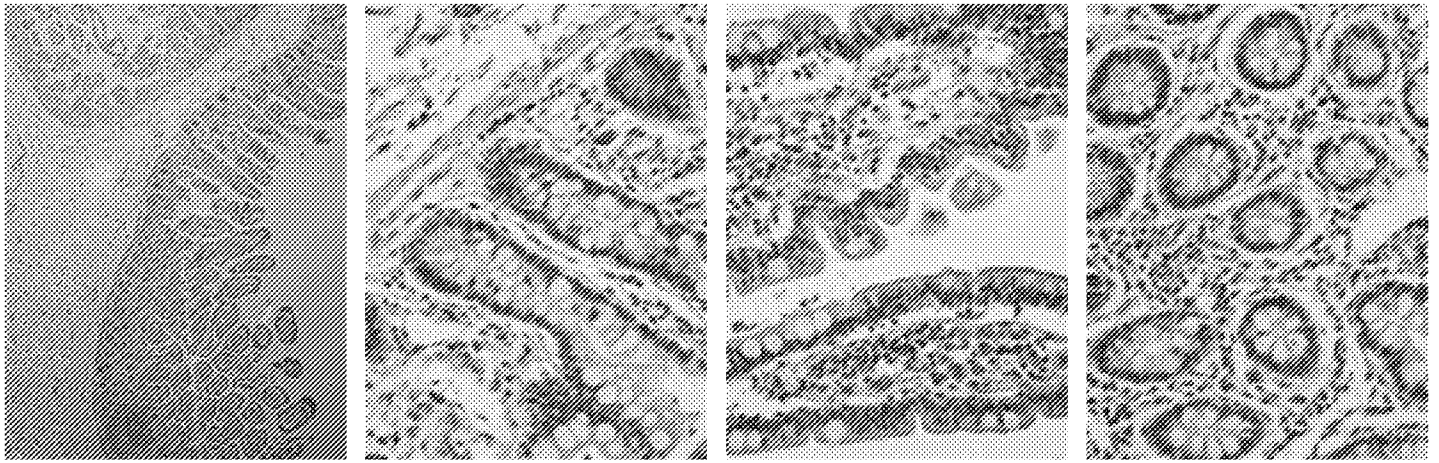
Normal

Fig. 40A

61

10 ug/ml

61 = Minerva's anti-NME7 antibody

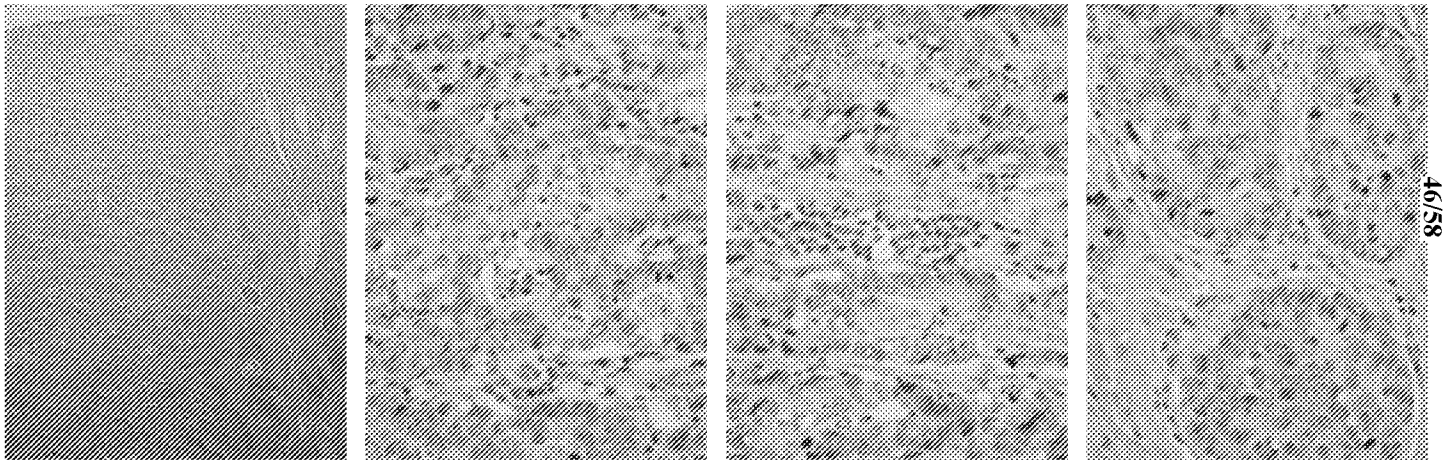


Tumor Grade 2

61

Fig. 40B

10 ug/ml



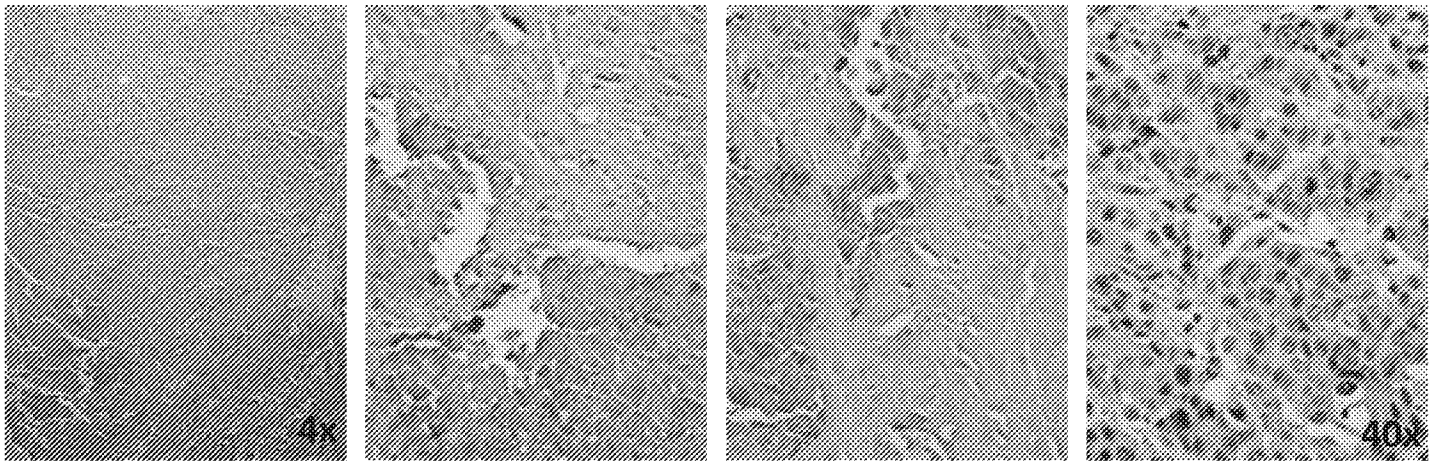
Tumor Grade 3

(T3N0M0) Clinical II

61

Fig. 40C

10 ug/ml



Figures 40A-40C

Colon

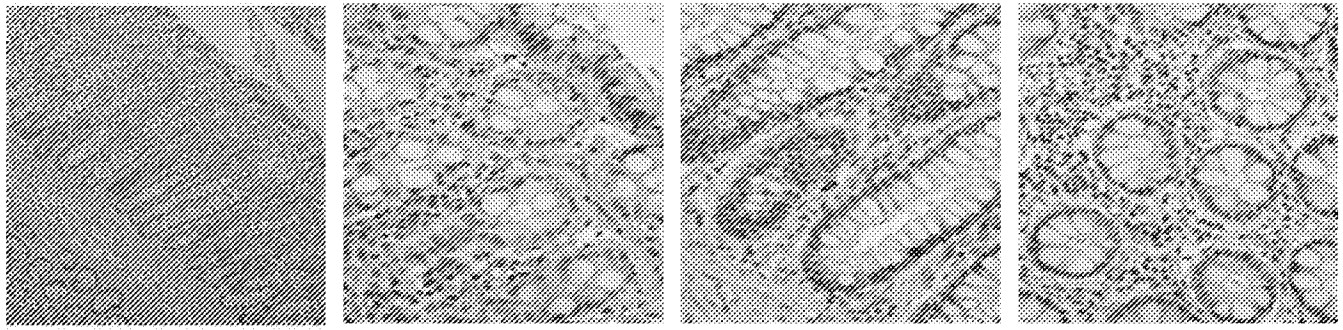
Normal

61

10 ug/ml

61 = Minerva's anti-NME7 antibody

Fig. 41A

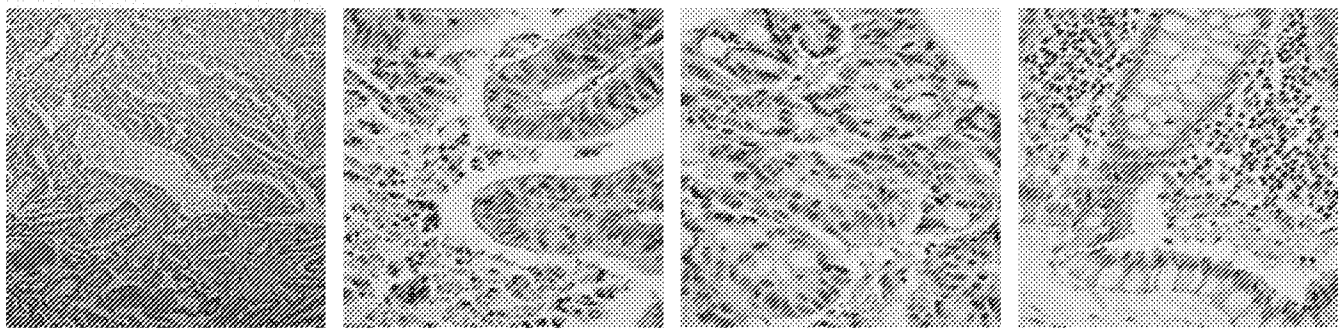


Tumor Grade 1
(T4N1M1, Clinical IV)

61

10 ug/ml

Fig. 41B

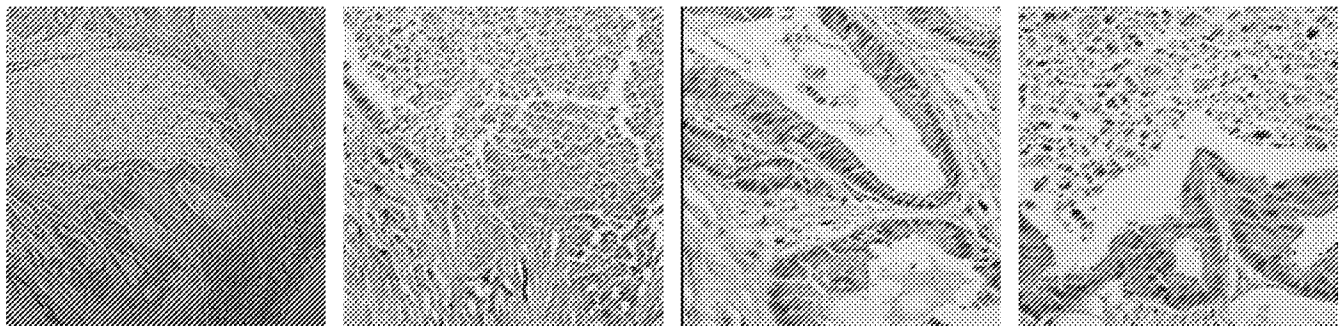


Tumor Grade 2
(T4N0M0, Clinical IB)

61

10 ug/ml

Fig. 41C

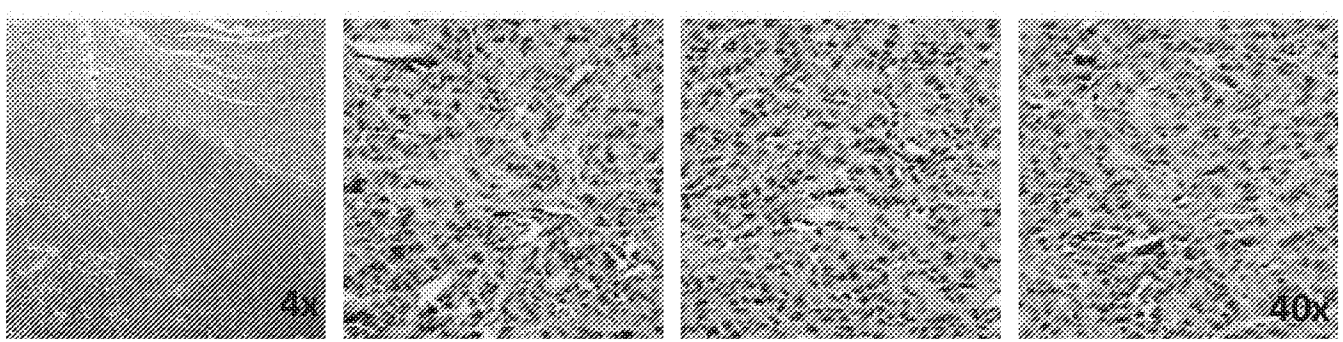


Tumor Grade 3
(T3N1M1, Clinical IV)

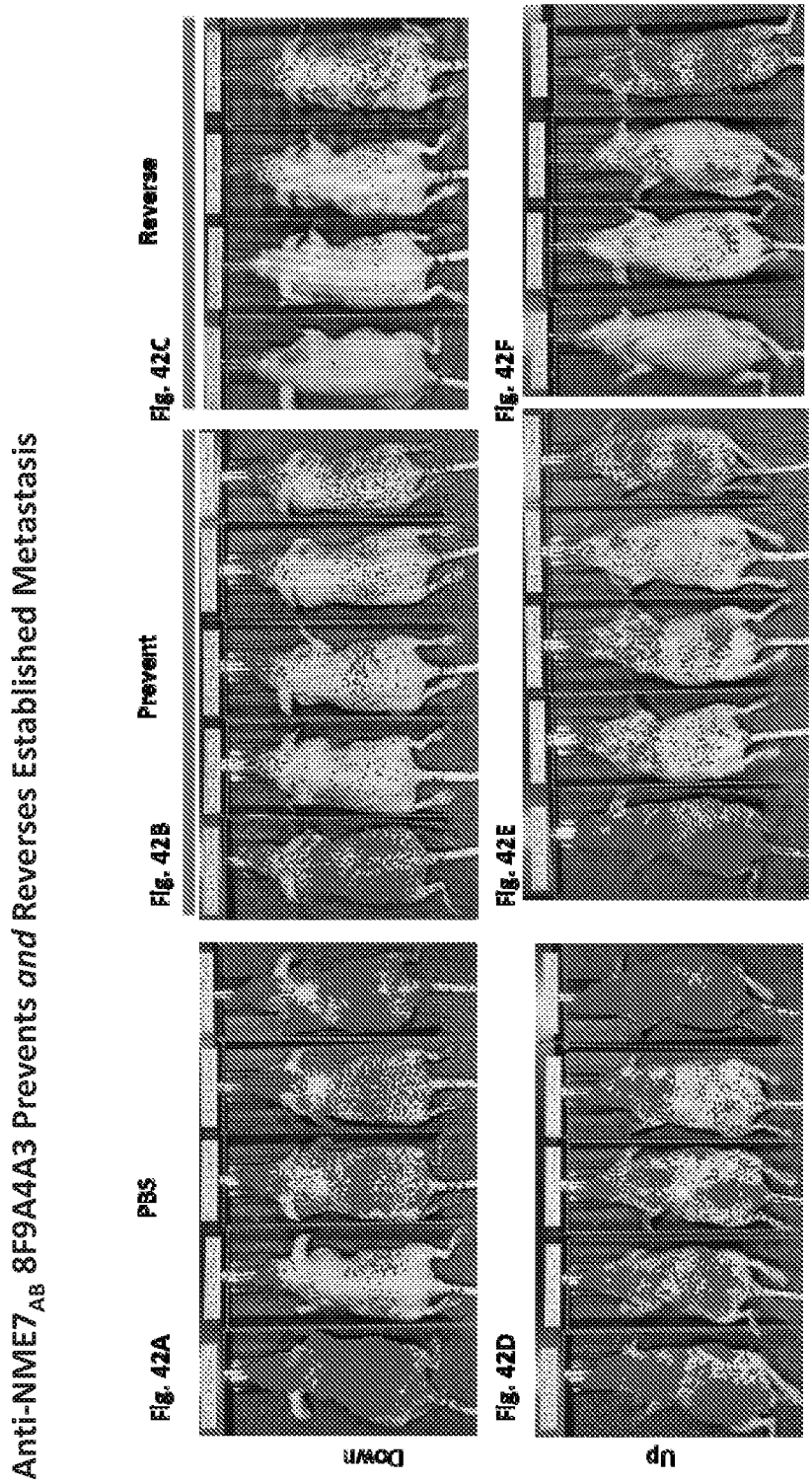
61

10 ug/ml

Fig. 41D

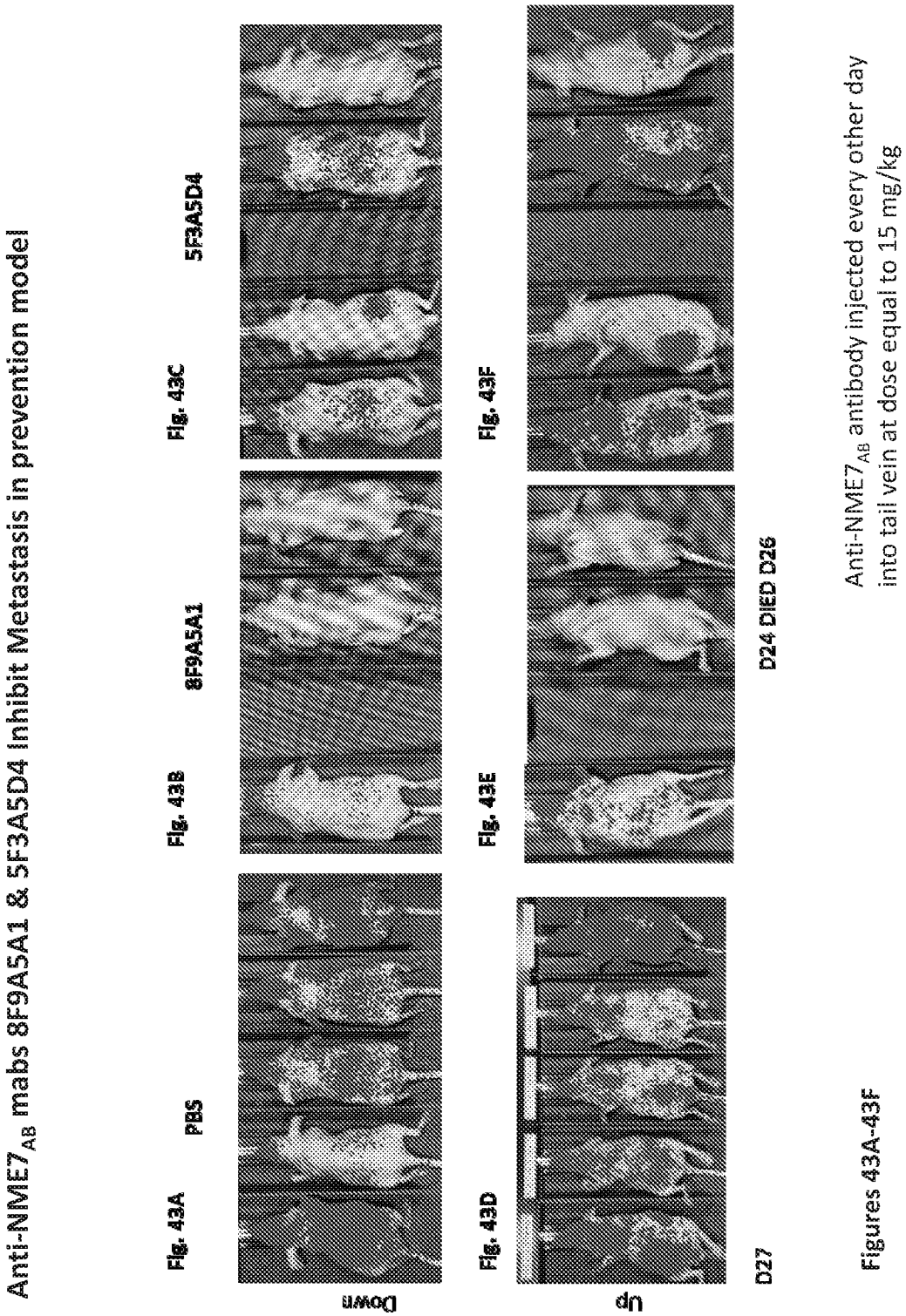


Figures 41A-41D

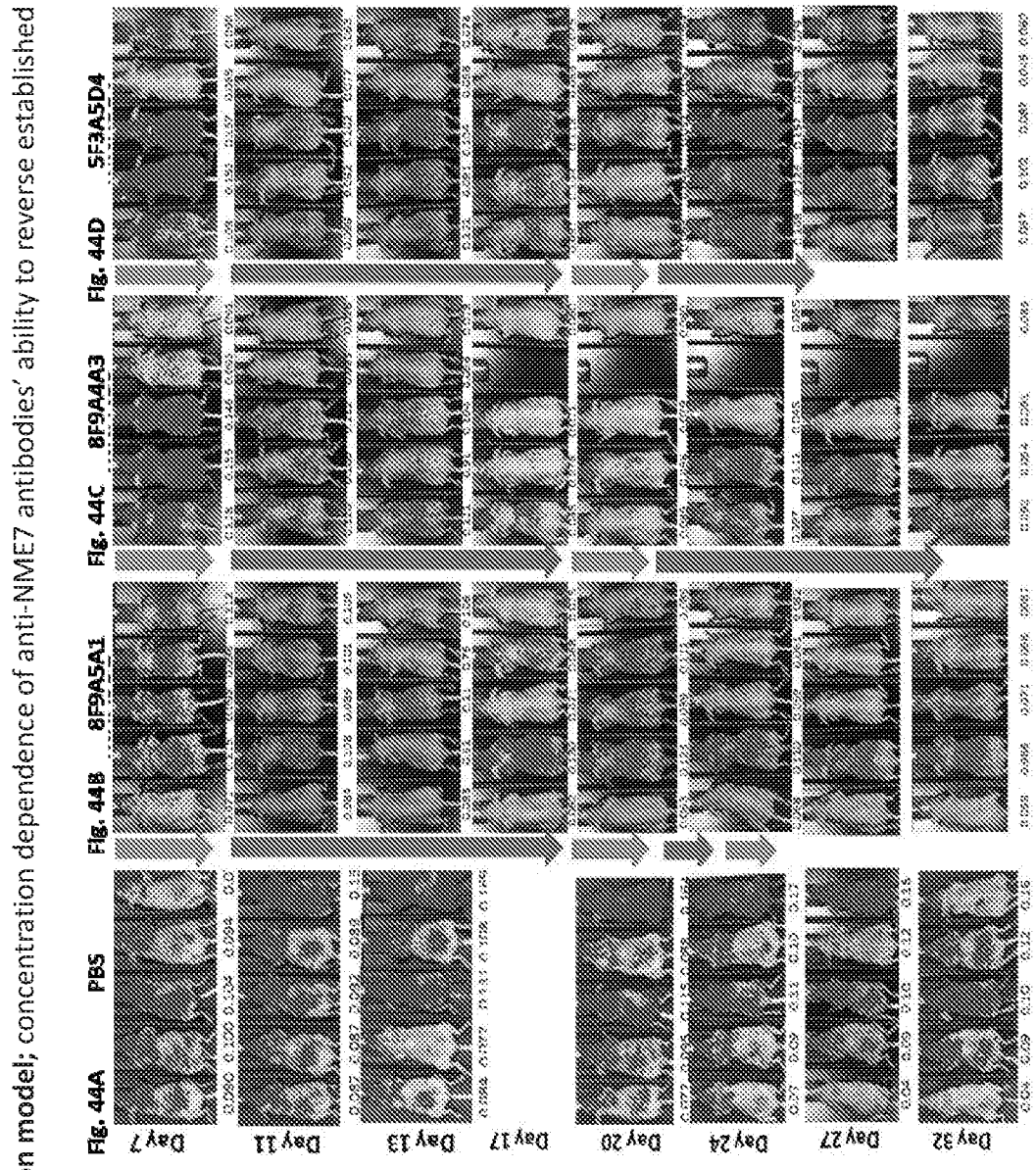


Anti-NME7$_{AB}$ antibody 4A3 injected every other day into tail vein at dose equal to 15 mg/kg

Figures 42A-42F



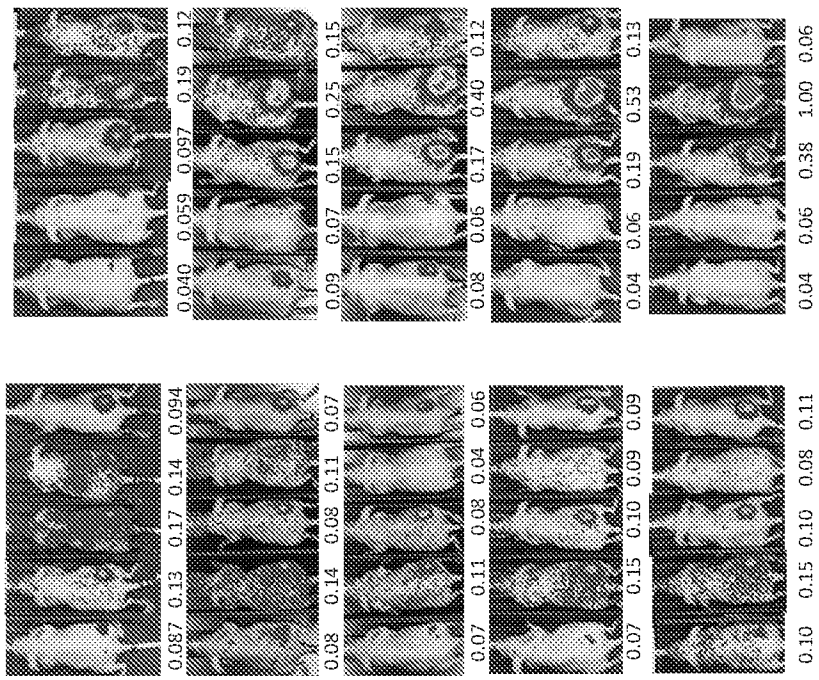
Figures 43A-43F



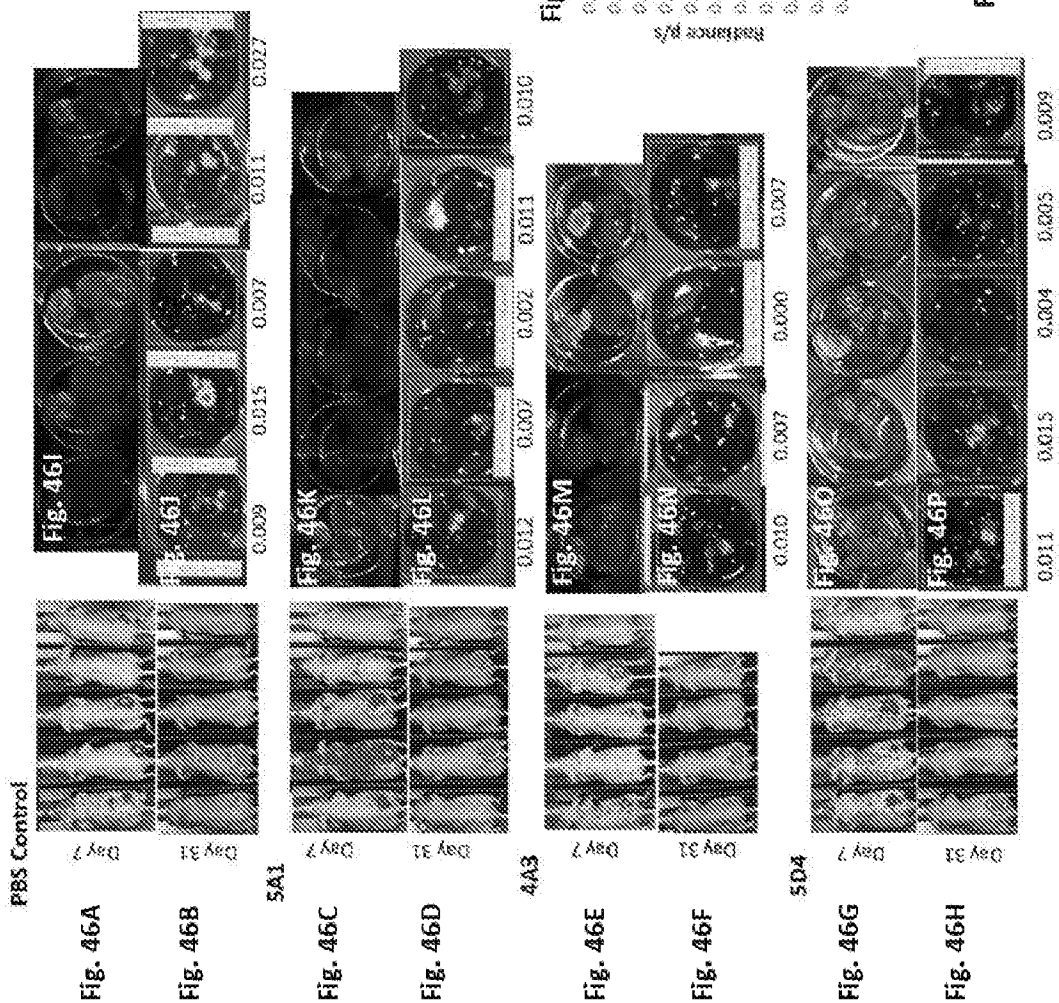
Metastasis reversion model; concentration dependence of anti-NME7 antibodies' ability to reverse established metastases

Figures 44A-44D

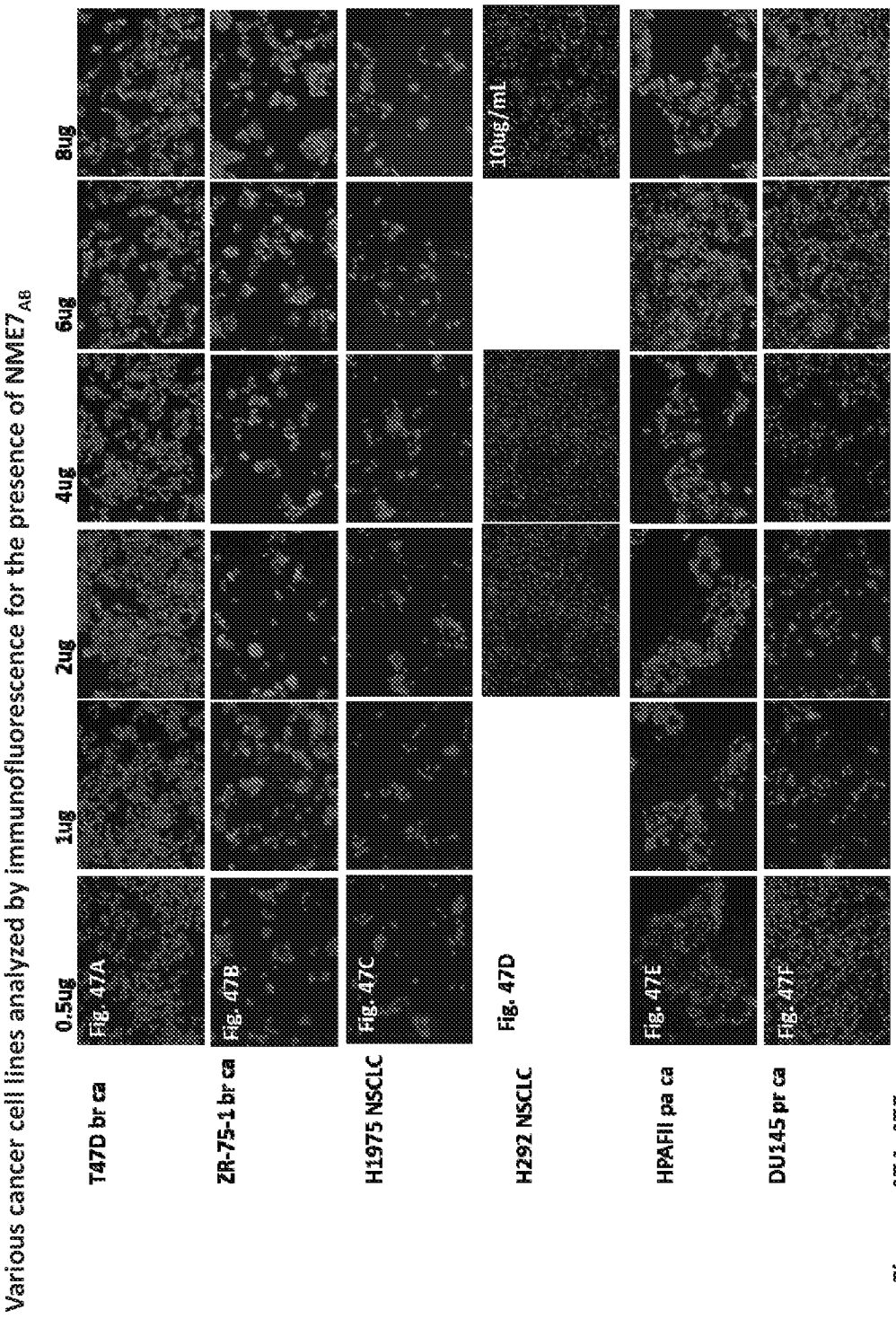
Sub-cu implantation of primary tumor comprised of T47D CSCs; conclusion: antibody cocktail inhibited metastases but did not inhibit growth of the primary



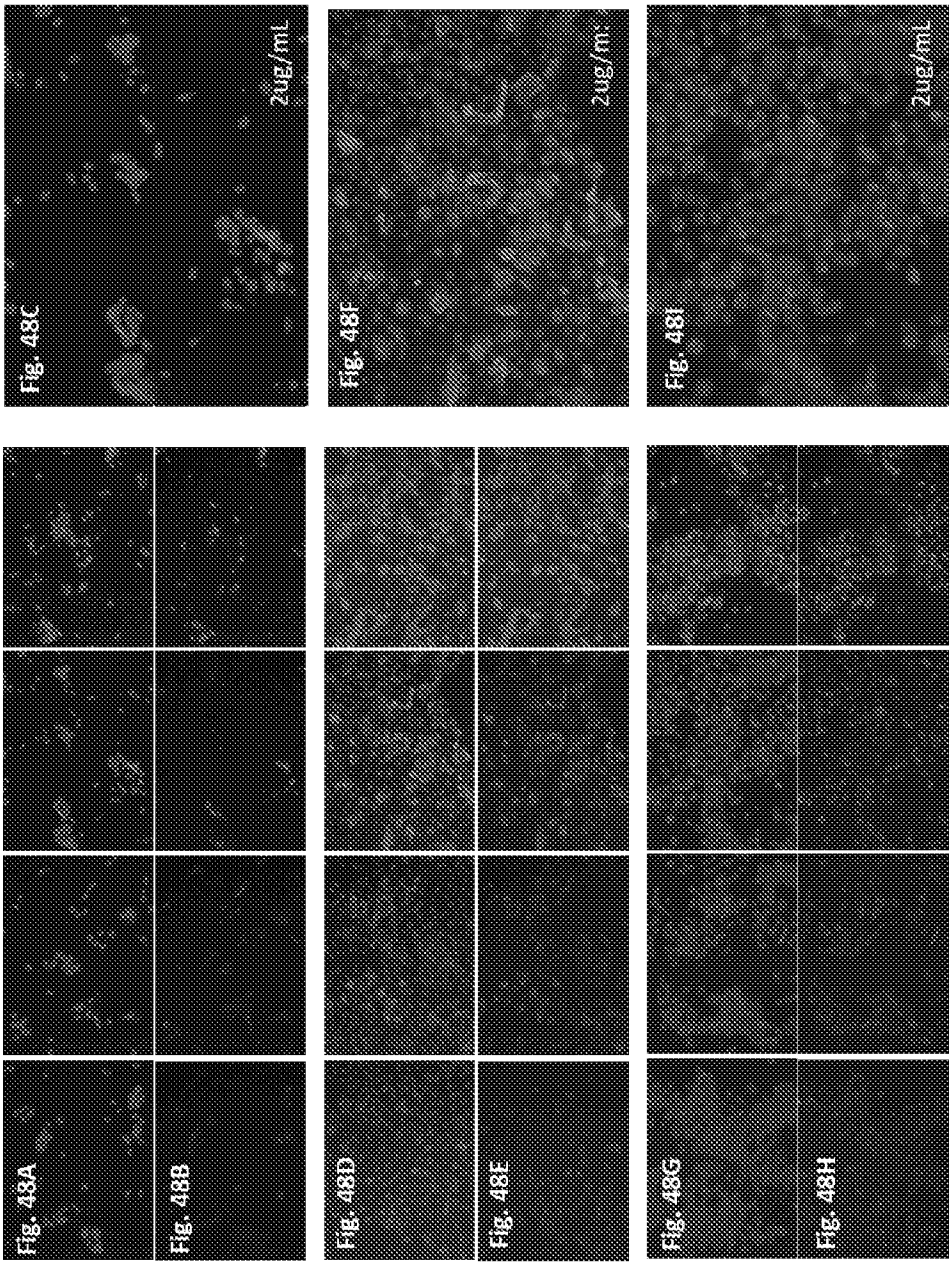
Figures 45A-45B



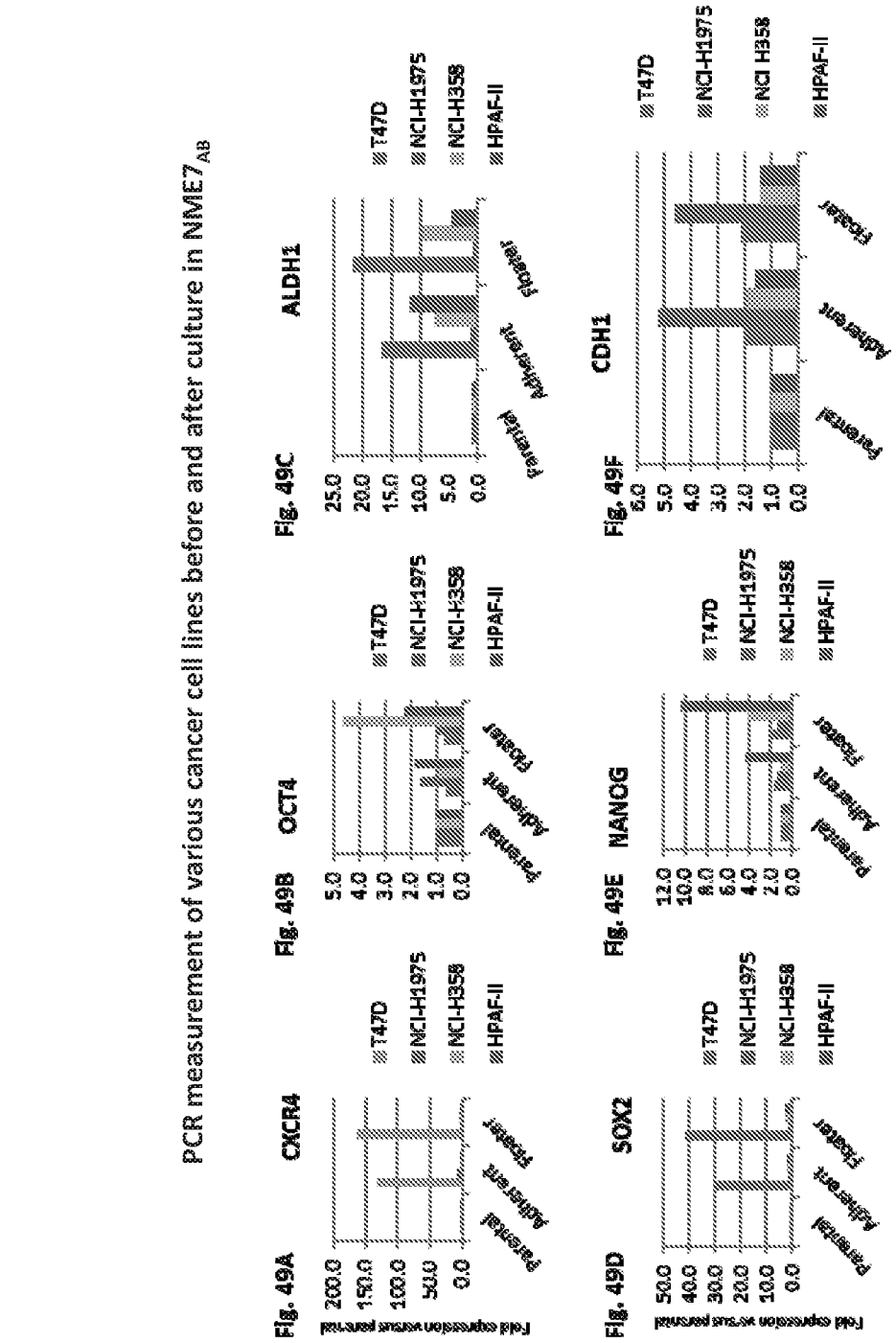
Figures 46A-46Q



Figures 47A-47F



Figures 48A-48I



Figures 49A-49F

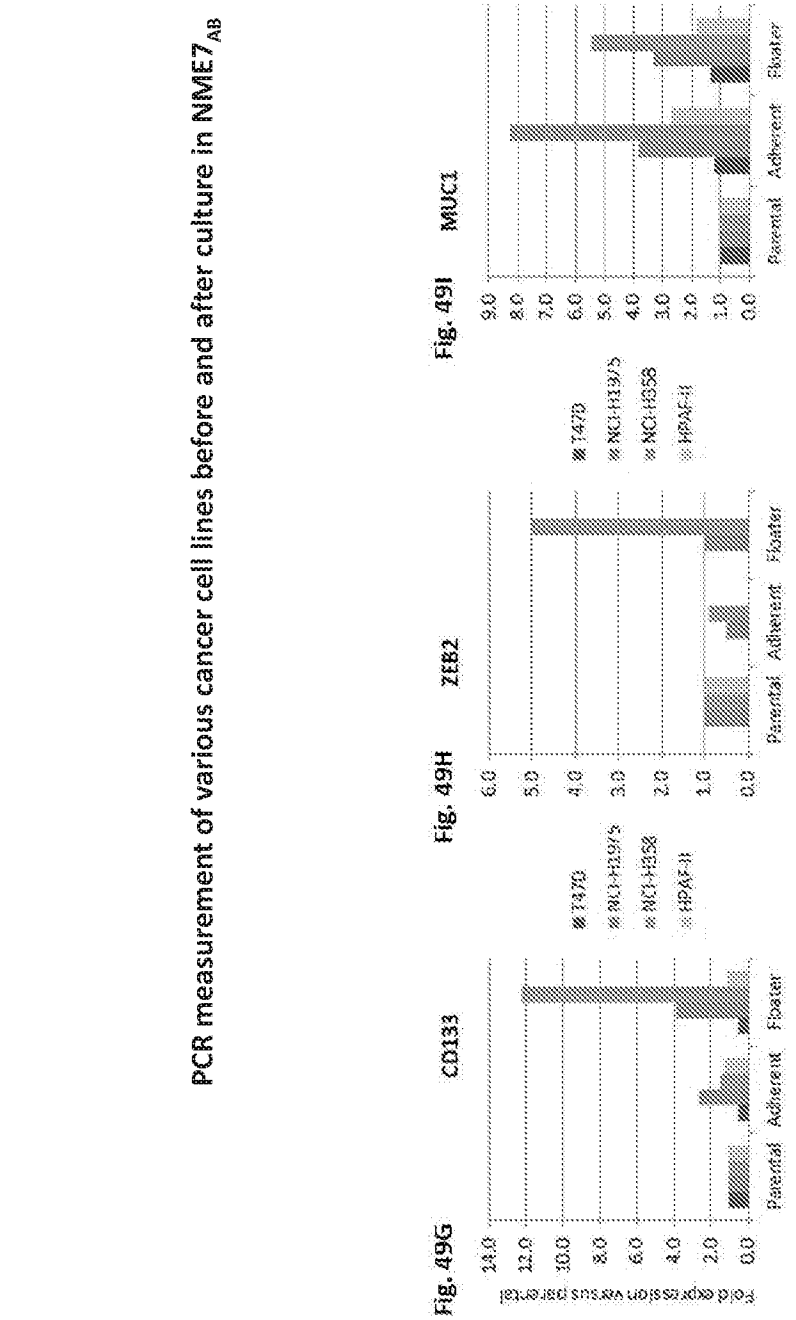
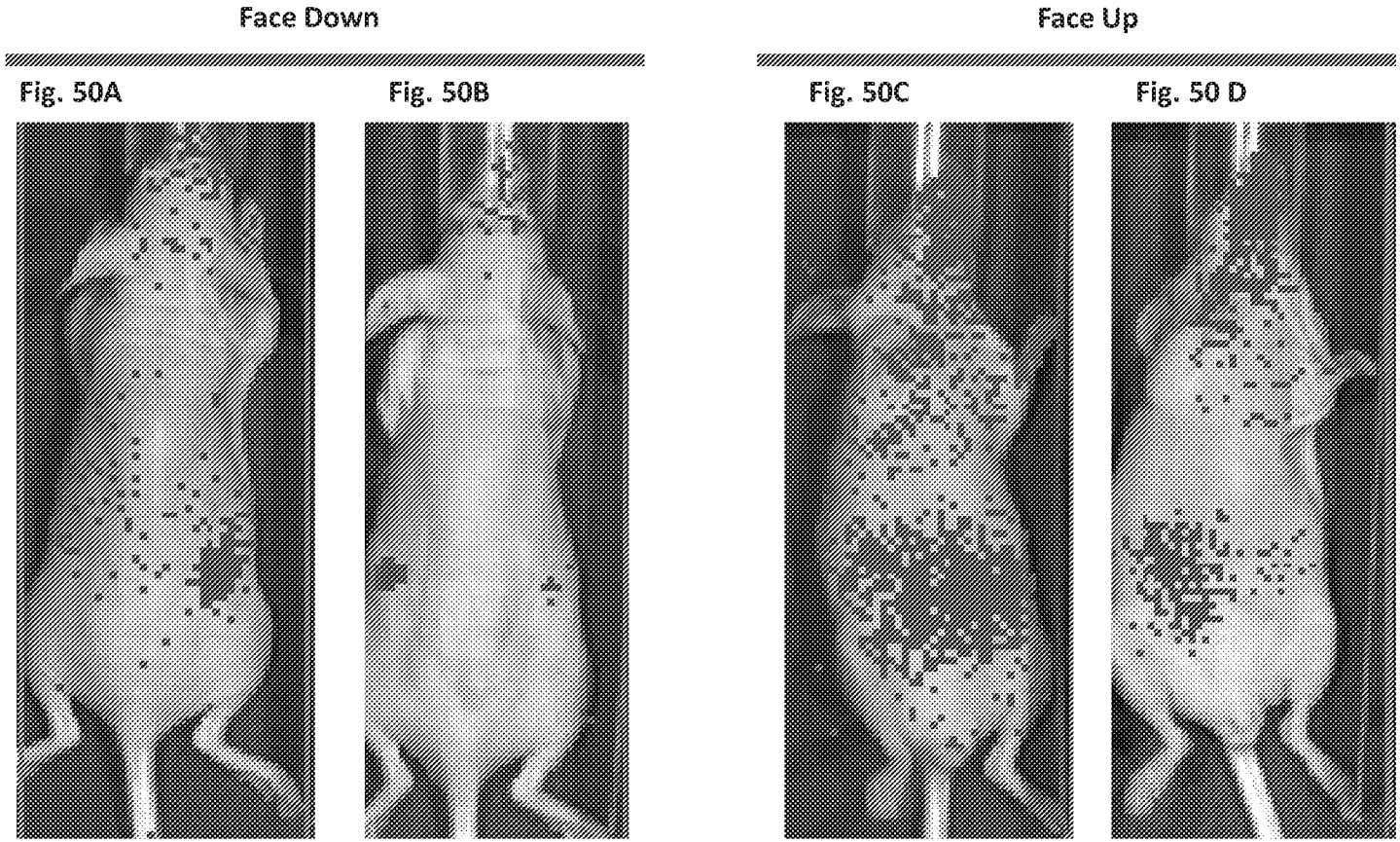


Figure 49G-49I

Day 6 IVIS photographs of NSG mouse injected into tail vein with NCI-H358 lung cancer cells that were cultured for 10 days in $NME7_{AB}$



Figures 50

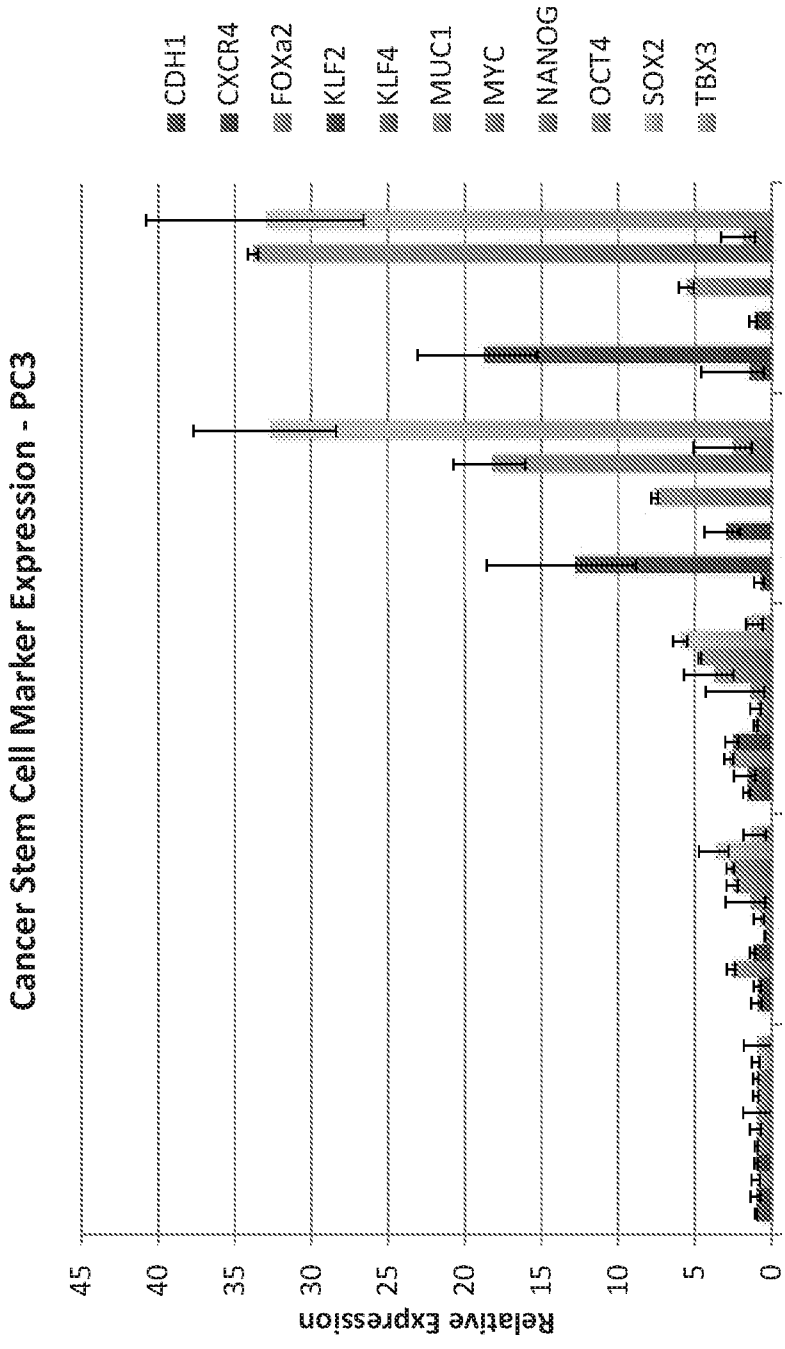

Cancer Stem Cell Marker Expression - PC3
Relative expression
0
5
10
15
20
25
30
35
40
45
CDH1
CXCR4
FOXa2
KLF2
KLF4
MUC1
MYC
NANOG
OCT4
SOX2
TBX3
PC3 Control
PC3 NM23 P2
PC3 NME7 P2
PC3 NM23 P3
PC3 NME7 P3

Figure 51