

NME VARIANT SPECIES EXPRESSION AND SUPPRESSION

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention:

[0002] The present application relates to the field of manipulating the expression of NME family proteins and their associated factors to regulate stem-like growth and treat cancer.

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

[0004] NM23 exists as a family of proteins wherein the commonality among these proteins is the presence of a nucleoside diphosphate kinase (NDPK) domain that catalyzes the conversion of ATP to ADP. NM23 has previously been known as Tumor Metastasis Factor. With the recent identification of ten NM23 family members, they are now also known as NME proteins 1-10 (Mol Cell Biochem (2009) 329:51–62, “The mammalian Nm23/NDPK family: from metastasis control to cilia movement,” Mathieu Boissan , Sandrine Dabernat, Evelyne Peuchant, Uwe Schlattner, Ioan Lascu, and Marie-Lise Lacombe).

[0005] Scientists first isolated a differentiation inhibition factor from human leukemia cells and showed that the addition of this factor blocked chemically induced differentiation of certain types of leukemia and myeloid cells (Okabe-Kado, 1985, Cancer Research **45**, 4848-4852, “Characterization of a Differentiation-inhibitory Activity from Nondifferentiating Mouse Myeloid Leukemia Cells); this inhibitory factor was later identified as NME1 (NM23-H1) (Okabe-Kado, 1992, “Identity of a differentiation inhibiting factor for mouse myeloid leukemia cells with NM23/nucleoside diphosphate kinase”, Biochem Biophys Res Comm, 182 No.3 987-994). Leukemia cells are blood cells that are blocked from terminal differentiation. Interestingly, the ability to inhibit differentiation of leukemia cells was shown to be independent of its catalytic domain. Mutations in the NDPK domain that abrogated its enzymatic activity had no effect on the protein’s ability to block differentiation of some types of leukemia cells. However, the scientific literature of the following decades paints a picture of total confusion as to whether NM23 inhibits differentiation, accelerates differentiation or has no effect at all.

[0006] Many research articles provided evidence indicating that NM23 induces differentiation. Rosengard et al, 1989, Dearolf et al 1993, and Timmons et al 1993 reported that *in vivo* the Drosophila NM23 homologue, *awd*, is required for proper differentiation. Lakso et al 1992 reported that NM23 (mouse *in vivo*) increases with initiation of tissue differentiation, implying that it induces differentiation. Yamashiro et al 1994 reported that *in vitro* NM23 levels increase during differentiation of human erythroleukemia cells. Lombardi

et al 1995 concluded that NM23 (mouse *in vitro*) increases with initiation of cellular differentiation, again indicating that NM23 induces differentiation. Gervasi 1996, reported that overexpression of NM23 (rat *in vitro*) induced neuronal differentiation and down regulation of NM23 with anti-sense DNA inhibited differentiation. Amendola et al 1997 showed that transfection of NM23 in human neuroblastoma cells increased differentiation.

[0007] In direct contradiction to the many research articles that reported that NM23 induced differentiation, an equal number of papers published in the same time frame reported the opposite: that NM23 inhibited differentiation. Munoz-Dorado et al 1990 found that *ndk* (*Myxococcus* NM23 homologue, *in vivo*) was essential for growth but was down regulated during development, implying that its presence would inhibit differentiation. Okabe-Kado 1992 showed that *in vitro* a differentiation inhibitory factor (later identified by same group as NM23) inhibited differentiation of mouse leukemia. Yamashiro et al 1994 reported that NM23 levels decrease during differentiation of human megakaryoblasts, consistent with NM23 inhibiting differentiation. Okabe-Kado 1995 showed that recombinant NM23 inhibited erythroid differentiation of leukemia cell lines HEL, KU812, and K562 but not monocyte or granulocyte differentiation of progenitors HL60, U937, or HEL/S cells. Venturelli et al 1995 reported that NM23 overexpression inhibited G-CSF dependent granulocyte differentiation of human hematopoietic progenitors. Willems et al 1998 found that NM23 expression decreases as human CD34⁺ hematopoietic progenitors from the bone marrow cells differentiate. In 2002, Willems et al showed that NM23 had no effect on cell proliferation, did not induce or inhibit differentiation but skewed differentiation of CD34⁺ cells toward the erythroid lineage.

[0008] In a 2000 review article, Lombardi summarized these and other contradictory results pertaining to the role of NM23 in differentiation and concluded, “Although the role of the NM23 genes in the control of cell differentiation is widely under investigation, the functional connection between NM23 expression levels and such processes remains to be completely elucidated.” In other words, the functional connection between NM23 and differentiation was not understood.

[0009] In biological systems, proteins often make up complicated signaling cascades that direct the cell to behave in a particular way. For example, a common way that cells are directed to begin the process of dividing is that a protein (ligand) binds to the extra cellular domain of a transmembrane protein receptor wherein binding of the ligand to the extra cellular domain confers a change in the conformation of the receptor. The ligand-induced

conformational change can take place in the extra cellular domain, the intra cellular domain or both and results in a change in which proteins or molecules are able to bind to the receptor. This outside to inside signaling is a common mechanism that is used to signal cells to divide, initiate programmed cell death and many other processes.

[0010] One commonly used mechanism that regulates the activity of growth factor receptors is ligand-induced dimerization of the receptor's extra cellular domain which in turn brings the intracellular tails close together which makes a good docking site for modifying proteins such as kinases that initiate a signaling cascade that eventuates in a signal to the cell's nucleus that causes the cell to divide.

[0011] Ligand-induced dimerization of the extra cellular domain of growth factor receptors is often accomplished through the binding of ligand dimers; that is two ligands non-covalently bind to each other to form homo- or hetero-dimers which then bind to two receptors that are either the same (homo) or different (hetero).

[0012] An important example of ligand-induced receptor dimerization is NM23 dimers binding to and dimerizing the extra cellular domain of MUC1*, which is the truncated form of the MUC1 transmembrane protein that is tumor and stem cell specific. Whether or not the ligand is a monomer, dimer or a higher order multimer is a function of, among other things, its concentration. For many growth factor receptors, only the dimeric form of the ligand activates the growth factor receptor. Additionally, in primitive biological systems, there are feedback loops wherein the higher order multimers turn off the function that is promoted by the dimer. For example, the CI protein of Phage lambda turns on transcription of one set of genes when it is bound to DNA as a tetramer but turns off transcription of those genes when, as a function of increased concentration, the CI protein becomes an octamer. Therefore, it would be advantageous to discover if such multimerization-regulated feedback loops that regulate function exist in higher order organisms, such as humans, and to develop methods to manipulate function based on the knowledge of the function of each multimer.

SUMMARY OF THE INVENTION

[0013] In one aspect, the present invention is directed to a method for generating less mature cells from starting cells comprising inducing the starting cells to revert to a less mature state comprising increasing the amount of an NME family member whose multimerization state is the biologically active state or decreasing the relative amount of an NME family member whose multimerization state is the biologically inactive state.

[0014] In another aspect, the invention is directed to a method of inhibiting differentiation of embryonic stem cell, induced pluripotent stem cell or progenitor cell comprising increasing the amount of an NME family member whose multimerization state is the biologically active state or decreasing the relative amount of an NME family member whose multimerization state is the biologically inactive state.

[0015] In another aspect, the invention is directed to a method of maintaining or inducing pluripotency in cells comprising decreasing the relative amount of an NME family member whose multimerization state is the biologically inactive state.

[0016] In yet another aspect, the invention is directed to a method of inhibiting differentiation in embryonic stem cell, induced pluripotent stem cell, or progenitor cell, comprising decreasing the relative amount of an NME family member whose multimerization state is the biologically inactive state.

[0017] In any of the methods described above, the NME family member's multimerization state in the biologically inactive state may be a hexamer or higher order multimer. In one aspect, such NME family member may be NME1 (NM23-H1) or NME2 (NM23-H2).

[0018] In any of the methods described above, the NME family member's multimerization state in the biologically active state may be a dimer or a monomer that contains two units able to bind to the same target receptor. In this regard, such NME family member may be a mutant or variant of NME1 that favors dimerization or has two binding domains for its cognate ligand, or NME6 or NME7.

[0019] In any of the methods described above, the relative amount of the NME family member whose multimerization state is the biologically inactive state may be decreased by adding an NME family member whose multimerization state is the biologically active state. The NME family member whose multimerization state is the biologically active state may be increased by introducing a nucleic acid or small molecule that causes it to be expressed.

[0020] And further, in any of the methods described above, the relative amount of the NME family member whose multimerization state is the biologically inactive state may be decreased by introducing nucleic acids or small molecules that down-regulate its expression.

[0021] In another aspect, in any of the methods described above, the NME family member whose multimerization state is the biologically inactive state may be decreased and the NME family member whose multimerization state is the biologically active state may be increased by simultaneously introducing a first nucleic acid that down-regulates a first NME

that forms the inactive state and a second nucleic acid that up-regulates the NME that forms the active state.

[0022] In any of the methods described above, the nucleic acid that down-regulates may be an anti-sense DNA, an inhibitory RNA, siRNA or shRNA and the nucleic acid that up-regulates is an encoding DNA, RNA, mRNA, or plasmid. Such nucleic acid may be modified to facilitate entry into the cell. In this regard, the NME family member that may be down regulated may include NME1, NME2 or NME1 and NME2. In this regard, the NME family member that may be up-regulated may be a mutant or variant of NME1, NME2 that prefers dimerization, NME6 or NME7.

[0023] In another aspect, the present invention is directed to a nucleic acid that causes expression of NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand; and/or

[0024] a. NME7; and/or

[0025] b. a nucleic acid that causes expression of NME7; and/or

[0026] c. NME6; and/or

[0027] d. a nucleic acid that causes expression of NME6; and/or

[0028] e. a nucleic acid that down-regulates NME1 or NME1 and NME2.

[0029] In still another aspect, the invention is directed to a media for culturing stem or progenitor cells wherein the media contains:

[0030] a. NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand; and/or

[0031] b. a nucleic acid that causes expression of NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand; and/or

[0032] c. NME7; and/or

[0033] d. a nucleic acid that causes expression of NME7; and/or

[0034] e. NME6; and/or

[0035] f. a nucleic acid that causes expression of NME6; and/or

[0036] g. a nucleic acid that down-regulates NME1 or NME1 and NME2.

[0037] In yet another aspect, the invention is directed to a media for inducing pluripotency or for inducing cells to revert to a less mature state wherein the media contains:

[0038] a. NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand; and/or

- [0039] b. a nucleic acid that causes expression of NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand; and/or
- [0040] c. NME7; and/or
- [0041] d. a nucleic acid that causes expression of NME7; and/or
- [0042] e. NME6; and/or
- [0043] f. a nucleic acid that causes expression of NME6; and/or
- [0044] g. contains a nucleic acid that down-regulates NME1 or NME1 and NME2.
- [0045] The media may also contain nucleic acids that encode some or all of the pluripotency-inducing genes or small molecules including OCT4, SOX2, NANOG, KLF4, and c-Myc.
- [0046] In still another aspect, the invention is directed to a host cell that carries a synthetic nucleic acid that causes decreased expression of an NME family member whose multimerization state is the biologically inactive state. The NME family member may be NME1, NME2 or NME1 and NME2.
- [0047] In yet another aspect, the invention is directed to a host cell that carries a synthetic nucleic acid that causes increased expression of an NME family member whose multimerization state is the biologically active state. In this regard, the NME family member may include an NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand, NME6, or NME7.
- [0048] In another aspect, the invention is directed to a host cell that carries a synthetic nucleic acid that causes decreased expression of an NME family member whose multimerization state is the biologically inactive state and a synthetic nucleic acid that causes increased expression of an NME family member whose multimerization state is the biologically active state. In this regard, the NME family member whose expression will be decreased may be NME1 or NME1 and NME2 and the NME family member whose expression will be increased may be an NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand, and/or NME6, and/or NME7. The cell may also carry a nucleic acid that causes the expression of a gene that is either not expressed in the host cell or is mutated in the host cell. The host cell may also carry a nucleic acid to down-regulate an unwanted gene and up-regulates a desired or corrected gene. The host cell may be an embryonic stem cell, induced pluripotent stem cell, progenitor cell, or somatic cell.
- [0049] In another aspect, the invention is directed to a method of treating a patient wherein the cells described above are administered to the patient. The cells may be

administered by bone marrow transplant, transplant into a specific site, transfusion, injection, or topical treatment. The treatment may be for any disease or condition that would be alleviated by treatment with stem cells, progenitors, or by correction of a genetic abnormality or defect. Or, the cells may be differentiated prior to administration to patient and wherein NME1, NME6 or NME7 are withdrawn during differentiation process.

[0050] In yet another aspect, the invention is directed to a method of treating cancer in a patient comprising administering an agent to the patient such that the agent increases the amount of an NME family member whose multimerization state is the biologically inactive state or decreases the relative amount of an NME family member whose multimerization state is the biologically active state. In this regard, the NME family member whose multimerization state is the biologically active state may be NME7, and the NME family member whose multimerization state is the biologically inactive state may be NME1, NME2 or NME8.

BRIEF DESCRIPTION OF THE DRAWINGS

[0051] 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;

[0052] Figures 1A-F show photographs of Western blots detecting the presence of NME1 (A,D), NME6 (B,E) or NME7 (C,F) in human stem cells cultured in NM23-S120G dimers, cultured in bFGF over MEFs or human breast cancer cells or the presence of the NME isoforms in a MUC1* pull-down assay.

[0053] Figure 2 is a photograph of a Western blot of human embryonic stem cell lysates probed with an antibody specific for NME7.

[0054] Figure 3 shows photographs of human BGO1v embryonic stem cells that remain pluripotent and undifferentiated after several days in culture in NME1-S120G dimers present at a range of concentrations.

[0055] Figure 4 shows photographs of human BGO1v embryonic stem cells that have differentiated and are no longer pluripotent after several days in culture in NME1-wild type hexamers present at a range of concentrations.

[0056] Figure 5 shows photographs of human BGO1v embryonic stem cells that have differentiated and are no longer pluripotent after several days in culture in a mixture of 20%

NME1-S120G dimers and 80% NME1-wild type hexamers present at a range of concentrations.

[0057] Figure 6 is a graph showing quantitative PCR measurements of the expression levels of pluripotent, naïve and primed genes in human embryonic H9 stem cells cultured for up to 37 passages in NME1-S120G dimers, compared to the same source cells cultured in bFGF.

[0058] Figures 7A-B show photographs of human stem cells in which siRNAs specific for NME1, NME6 and NME7 have been used to suppress expression of the three NM23 isoforms.

[0059] Figures 8A-F show photographs of human stem cells in which siRNAs specific for NME1, NME6 and NME7 have been used to suppress expression of the three NM23 isoforms either separately or in combinations.

[0060] Figures 9A-D show photographs of human stem cells in which differentiation induced by suppression of NME7 is rescued if cells are treated with recombinant NME1 purified as stable dimers.

[0061] Figure 10A-B show photographs of human stem cells in which siRNAs specific for NME1, NME6 and NME7 have been used to suppress expression of all three NM23 isoforms and that inhibition of cell growth and differentiation induced by suppressing all three NMEs is rescued by treating cells with recombinant NME1 purified as stable dimers.

[0062] Figures 11A-D show magnified photographs of human stem cells in which expression of NME1 (A,C) or NME7 (B,D) has been suppressed using siRNAs, or suppressed but wherein the suppressed cells are treated with recombinant NME1 purified as stable dimers (C,D).

[0063] Figures 12A-D show magnified photographs of human stem cells in which expression of NME1 (A,C) or NME7 (B,D) has been suppressed using siRNAs, or suppressed but rescued by also treating cells with recombinant NME1 purified as stable dimers (C,D).

[0064] Figures 13A-D show magnified photographs of human stem cells in which expression of NME1, NME6 and NME7 are suppressed (A,C) or NME1 and NME7 are suppressed (B,D), wherein cells are rescued to some extent by culturing the cells in recombinant NME1 purified as stable dimers (C,D).

[0065] Figure 14 shows a graph of an RT-PCR experiment in which expression of pluripotency genes NANOG, OCT4 and KLF4 or differentiation marker FOXA2, plus

NME1, NME6 and NME7 genes are measured in human stem cells in which either NME1, NME6 or NME7 has been suppressed or “Knocked Out”; controls are a mock transfection and the same stem cells that have differentiated. A striking increase in differentiation marker FOXA2 is seen in NME knock out cells and in the differentiation control. A significant increase in the expression of NME1 is observed when NME6 or NME7 is suppressed.

[0066] Figure 15 shows a graph of an RT-PCR experiment in which expression of pluripotency genes NANOG, OCT4 and KLF4 or differentiation marker FOXA2, plus NME1, NME6 and NME7 genes are measured in human stem cells in which NME1 has been Knocked Out (KO) wherein one set of NME1 suppressed cells has been cultured in media supplemented with human recombinant NME1 dimer form; control is the same stem cells that have been allowed to differentiate. A striking increase in differentiation marker FOXA2 is seen in NME1 knock out cells and in the differentiation control.

[0067] Figure 16 shows a graph of an RT-PCR experiment in which expression of pluripotency genes NANOG, OCT4 and KLF4 or differentiation marker FOXA2, plus NME1, NME6 and NME7 genes are measured in human stem cells in which NME6 has been Knocked Out (KO) wherein one set of NME6 suppressed cells has been cultured in media supplemented with human recombinant NME1 dimer form; control is the same stem cells that have been allowed to differentiate.

[0068] Figure 17 shows a graph of an RT-PCR experiment in which expression of pluripotency genes NANOG, OCT4 and KLF4 or differentiation marker FOXA2, plus NME1, NME6 and NME7 genes are measured in human stem cells in which NME7 has been Knocked Out (KO) wherein one set of NME7 suppressed cells has been cultured in media supplemented with human recombinant NME1 dimer form; control is the same stem cells that have been allowed to differentiate. A striking increase in differentiation marker FOXA2 is seen in NME7 suppressed cells in addition to a significant increase in the expression of NME1. Both return to an almost normal state when the suppressed cells are cultured in a minimal media supplemented with NME1 in dimer form.

[0069] Figure 18 shows a graph of an RT-PCR experiment in which expression of pluripotency genes NANOG, OCT4 and KLF4 or differentiation marker FOXA2, plus NME1, NME6 and NME7 genes are measured in human stem cells in which NME1, NME6 and NME7 have been suppressed (KO) wherein one set of suppressed cells has been cultured in media supplemented with human recombinant NME1 dimer form; control is the same stem cells that have been allowed to differentiate. A striking increase in differentiation marker

FOXA2 is seen in the suppressed cells, wherein suppressed cells that are cultured in a minimal media supplemented with NME1 in dimer form return to a near normal gene profile.

[0070] Figure 19 shows a graph of an RT-PCR experiment in which expression of pluripotency genes NANOG, OCT4 and KLF4 or differentiation marker FOXA2, plus NME1, NME6 and NME7 genes are measured in human stem cells in which NME1 and NME7 have been suppressed (KO) wherein one set of suppressed cells has been cultured in media supplemented with human recombinant NME1 dimer form; control is the same stem cells that have been allowed to differentiate.

[0071] Figure 20 shows a graph of an ELISA assay in which a synthetic PSMGFR peptide of the MUC1* extra cellular domain is immobilized on the surface of a multi-well plate and three different recombinant human NME6 proteins are assayed for binding to the peptide, wherein one is the wild type human NME6 that has been denatured and refolded (NME6 WT RS), the second is a human NME6 wherein the region that contributes to dimerization is swapped to a sea sponge sequence (NME6 HuToS), and the third is a mutant NME6 S139G, which is in a comparable position to the NME1 mutant identified in cancers that prefers dimerization. All demonstrate binding to the peptide of the extra cellular domain of MUC1*.

[0072] Figures 21A-B show graphs of ELISA assays in which a synthetic PSMGFR peptide of the MUC1* extra cellular domain is immobilized on the surface of a multi-well plate and different recombinant human NME8 proteins are assayed for binding to the peptide, wherein one is a construct containing only NME8 domains A and B (NME8 1-2) and the other is a construct containing only NME8 domains B and C (NME8 2-3). The NME variants tested were either expressed in soluble form or were refolded (RS). In some cases, DTT was added to break up large oligomers. All demonstrate binding to the peptide of the extra cellular domain of MUC1*. NM23 S120G dimers are added for comparison to the binding of the NME8 constructs (A).

[0073] Figures 22A-D show magnified photographs of human stem cells at 96 hours post transfection of siRNA to suppress NME1 expression (A,B) and control cells wherein transfection reagents were added but with either no siRNA or a scrambled sequence siRNA (C,D). Comparison of NME1 suppressed cells to the control cells shows very little difference. Note that stem cells were plated over a layer of bivalent anti-MUC1* antibody which also functions as a MUC1* stimulating growth factor.

[0074] Figures 23A-D show magnified photographs of human stem cells at 96 hours post transfection of siRNA to suppress NME7 expression (A,B) and control cells wherein pluripotency gene Oct4 was suppressed (C,D). Comparison of NME7 suppressed cells to the OCT4 suppressed cells shows very little difference, wherein both have severely inhibited cell viability and remaining cells have taken on a fibroblast morphology.

[0075] Figure 24 shows magnified photographs of human stem cells at 96 hours post transfection of siRNA to suppress NME6 expression (A,B) and control cells wherein transfection reagents were added but with either no siRNA or a scrambled sequence siRNA (C,D). Comparison of NME6 suppressed cells to the control cells shows very little difference, although NME6 suppressed cells had areas of differentiating cells as can be seen as the brighter, thickening center of the cell cluster in Panel B. Note that stem cells were plated over a layer of bivalent anti-MUC1* antibody which also functions as a MUC1* stimulating growth factor.

[0076] Figure 25 shows a graph of an RT-PCR experiment in which expression of pluripotency genes NANOG, OCT4 and KLF4 or differentiation marker FOXA2, plus NME1, NME6 and NME7 genes are measured in human stem cells 96 hours post transfection of siRNA to suppress NME1, NME6 or NME7. A minimal media free of growth factors, serum or cytokines was changed every 48 hours, but note that stem cells were plated over a layer of bivalent anti-MUC1* antibody which also functions as a MUC1* stimulating growth factor. Suppression of NME7 shows a marked increase in the expression of differentiation marker FOXA2.

[0077] Figures 26A-D show magnified photographs of human HES-3 stem cells that have been cultured in a minimal media free of growth factors, serum or cytokines and supplemented with either NME7 monomers (A,C) or NME1 (NM23-H1) dimers (B,D).

[0078] Figures 27A-B show graphs of an ELISA sandwich assay that shows that NME7 monomers have two binding sites for the PSMGFR peptide of the MUC1* extra cellular domain. A synthetic PSMGFR peptide is immobilized on the surface of a multi-well plate and recombinant human NME7 (A and B domains and devoid of the M leader sequence) proteins are assayed for binding to the peptide (A), then bound by a histidine-tagged PSMGFR peptide, which is detected using an antibody to its histidine tag (B).

[0079] Figure 28 shows a graph of T47D breast cancer cell growth as a function of concentration of NME1 hexamers; experiment shows NME1 hexamers inhibit cancer cell growth.

[0080] Figures 29A-E show a graph of percent invasion of DU145 prostate cancer cells in response to treatment with NME1 hexamers, over a range of concentrations (A) and photographs of the cell migration assay wells (B-E) in which a cross is etched across a field of growing cancer cells and their ability to migrate across the gap is measured as a function of time. Photographs were taken at time zero (B,C) and at 16 hours (D,E), wherein NME1 hexamers at 12 μ M were added to the cells (C,E) and buffer alone was added to control cells (B,D). NME1 hexamers inhibited cancer cell migration.

[0081] Figures 30A-K show a graph of percent invasion of DU145 prostate cancer cells in response to treatment with NME1 dimers, over a range of concentrations (A) and photographs of the cell migration assay wells (B-K) in which a slash mark is etched across a field of growing cancer cells at time zero (B-F) and their ability to migrate across the gap is measured at 16 hours (G-K). NME1 dimers did not inhibit cancer cell migration. Note that NME1 dimers induce cancer cell growth at optimal concentrations (4-16 nM) wherein one nME1 dimer dimerizes two MUC1* receptors; at very high concentrations, each NME1 dimer binds to a single MUC1* receptor and inhibits cancer cell growth and migration.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0082] In the present application, “a” and “an” are used to refer to both single and a plurality of objects.

[0083] As used herein, “biologically active” NME multimer includes NME multimer that induces the starting cells to revert to a less mature state or inhibits differentiation of immature cells, or maintains the immature state of a cell.

[0084] As used herein, “biologically inactive” NME multimer includes NME multimer that does not induce the starting cells to revert to a less mature state or does not inhibit differentiation of immature cells, or does not maintain an immature state of the cell.

[0085] As used herein, “increasing MUC1* activity” refers to directly or indirectly increasing MUC1* signaling, and includes without limitation the dimerization of MUC1* receptor and also increased production of MUC1* by cleavage of the MUC1 receptor. MUC1* activity may be also increased by higher transcriptional expression of MUC1 receptor, which is further cleaved and dimerized. Therefore, in one aspect, MUC1* activity may be increased by a higher activity of the effector molecule that dimerizes MUC1*, or the higher activity of the cleavage molecule that cleaves MUC1 so that MUC1* is formed, or increased expression of the MUC1. Therefore, any chemical or biological species that is able

to increase the activity of the MUC1* dimerizing ligand, MUC1 cleavage enzyme to form MUC1*, or any transcriptional activator that enhances expression of MUC1, is encompassed as a species that “increases MUC1* activity”.

[0086] As used herein, “MUC1 Growth Factor Receptor” (MGFR) is a functional definition meaning that portion of the MUC1 receptor that interacts with an activating ligand, such as a growth factor or a modifying enzyme such as a cleavage enzyme. The MGFR region of MUC1 is that extracellular portion that is closest to the cell surface and is defined by most or all of the PSMGFR, as defined below. The MGFR is inclusive of both unmodified peptides and peptides that have undergone enzyme modifications, such as, for example, phosphorylation, glycosylation and so forth.

[0087] As used herein, "Primary Sequence of the MUC1 Growth Factor Receptor" (PSMGFR) refers to peptide sequence that defines most or all of the MGFR in some cases, and functional variants and fragments of the peptide sequence. The PSMGFR is defined as SEQ ID NO:6, and all functional variants and fragments thereof having any integer value of amino acid substitutions up to 20 (i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) and/or any integer value of amino acid additions or deletions up to 20 at its N-terminus and/or C-terminus. A "functional variant or fragment" in the above context refers to such variant or fragment having the ability to specifically bind to, or otherways specifically interact with, ligands that specifically bind to, or otherwise specifically interact with, the peptide of SEQ ID NO:6, while not binding strongly to identical regions of other peptide molecules identical to themselves, such that the peptide molecules would have the ability to aggregate (i.e. self-aggregate) with other identical peptide molecules. One example of a PSMGFR that is a functional variant of the PSMGFR peptide of SEQ NO:6 is SEQ ID NO:8, which differs from SEQ ID NO:6 by including an -SPY- sequence instead of the -SRY-.

[0088] As used herein, “MUC1*” refers to the MUC1 protein with the N-terminus truncated such that the extracellular domain is essentially comprised of the PSMGFR (SEQ ID NO:5).

[0089] As used herein “MUC1* associated factors” refers to agents that modify, activate, modulate the activity of, or modulate the expression of MUC1*. MUC1* associated factors include, without limitation, agents that affect dimerization of MUC1* receptor, increased production of MUC1*, induce cleavage of the MUC1 receptor, agents that increase MUC1* activity by higher transcriptional expression of MUC1 receptor, which is further cleaved and dimerized.

[0090] As used herein, "effective amount" is an amount sufficient to effect beneficial or desired clinical or biochemical results. An effective amount can be administered one or more times. For purposes of this invention, an effective amount of an inhibitor compound is an amount that is sufficient to induce or maintain pluripotency of a cell or activate MUC1*

[0091] As used herein, "fragments" or "functional derivatives" refers to biologically active amino acid sequence variants and fragments of the native ligands or receptors 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.

[0092] As used herein, "immature" cells refers to cells that can undergo at least one more step of differentiation and expresses markers of a particular cell type that is known to be able to undergo at least one more step of differentiation.

[0093] As used herein, "cell having less mature state than starting cell" refers to a cell that has de-differentiated so that it has an increased ability to differentiate into a different cell type than the starting cell or has an increased ability to differentiate into more cell types than the starting cell. A cell in a less mature state can be identified by measuring an increase in the expression of pluripotency markers, by a determination that the expression levels of pluripotency markers are closer to those of pluripotent stem cells or by measuring markers of a less mature state than the starting cells. For example, hematopoietic stem cells that can differentiate into any blood cell type are characterized by the expression of CD34 and the absence of CD38. As these cells differentiate, they go from CD34+/CD38- to CD34+/CD38- then to CD34-/CD38+. If one were to induce the CD34-/CD38+ cells to revert to a less mature state, the cells would regain expression of CD34. The technique of transdifferentiation involves reverting starting cells to a less mature state wherein the cells become unstable and can be directed to differentiate into a different cell type than the starting cell, even if the starting cell was at the same relative level of differentiation as the resultant cell (Ieda et al 2010; Efe et al 2011). For example, cardio fibroblasts have been reverted to a less mature state by brief ectopic expression of OCT4, SOX2, KLF4 and c-MYC, then from this unstable state, differentiated into cardiomyocytes.

[0094] As used herein, "ligand" refers to any molecule or agent, or compound that specifically binds covalently or transiently to a molecule such as a polypeptide. When used in certain context, ligand may include antibody. In other context, "ligand" may refer to a molecule sought to be bound by another molecule with high affinity, such as but not limited

to a natural or unnatural ligand for MUC1* or a cleaving enzyme binding to MUC1 or MUC1* or a dimerizing ligand for MUC1*.

[0095] As used herein, “Naïve stem cells” are those that resemble and share quantifiable characteristics with cells of the inner mass of a blastocyst. Naïve stem cells have quantifiable differences in expression of certain genes compared to primed stem cells, which resemble and share traits and characteristics of cells from the epiblast portion of a blastocyst. Notably, naïve stem cells of a female source have two active X chromosomes, referred to as XaXa, whereas the later primed stem cells of a female source have one of the X chromosomes inactivated (Nichols and Smith,).

[0096] As used herein, “NME” family proteins is a family of ten (10) proteins, some of which have been recently discovered, wherein they are categorized by their shared sequence homology to nucleoside diphosphate kinase (NDPK) domains, even though many of the NME family members are incapable of kinase activity. NME proteins were previously known as NM23-H1 and NM23-H2 then NM23-H3 through NM23-10 as they were being discovered. The different NME proteins function differently. Herein, NME1 and NME6 bind to and dimerize the MUC1* receptor (wherein its extra cellular domain is comprised essentially of the PSMGFR sequence) when they are in dimer form; NME7 has two (2) binding sites for MUC1* receptor extra cellular domain and also dimerizes the receptor. NME1 dimers, NME6 dimers and NME7 are the preferred NME family members for use as MUC1* ligands to induce or maintain cells in a less mature state than the starting cells. Other NME family members that are able to bind to and dimerize the MUC1* receptor are also contemplated for use as MUC1* ligands to induce or maintain cells in a less mature state than the starting cells.

[0097] As used herein, “pluripotency markers” are those genes and proteins whose expression is increased when cells revert to a less mature state than the starting cells. Pluripotency markers include OCT4, SOX2, NANOG, KLF4, KLF2, Tra 1-60, Tra 1-81, SSEA4, and REX-1 as well as others previously described and those currently being discovered. For example, fibroblast cells express no detectable or low levels of these pluripotency markers, but express a fibroblast differentiation marker called CD13. To determine if a cell is becoming less mature than the starting cells, one could measure a difference in the expression levels of the pluripotency markers between the starting cells and the resultant cells.

[0098] As used herein, “primed stem cells” are cells that resemble and share traits and characteristics of cells from the epiblast portion of a blastocyst.

[0099] As used herein, the term "specifically binds" refers to a non-random binding reaction between two molecules, for example between an antibody molecule immunoreacting with an antigen, or a non-antibody ligand reacting with another polypeptide, such as NM23 specifically binding with MUC1* or an antibody binding to MUC1* or a cleaving enzyme binding to MUC1 or MUC1*.

[00100] As used herein, “pluripotent” stem cell refers to stem cells that can differentiate to all three germ lines, endoderm, ectoderm and mesoderm, to differentiate into any cell type in the body, but cannot give rise to a complete organism. A totipotent stem cell is one that can differentiate or mature into a complete organism such as a human being. With reference to embryonic pluripotent stem cells, they are cells derived from the inner cell mass of a blastocyst. Typical markers of pluripotency are OCT4, KLF4, NANOG, Tra 1-60, Tra 1-81 and SSEA4.

[00101] As used herein, “multipotent” stem cells refer to stem cells that can differentiate into other cell types wherein the number of different cell types is limited.

[00102] As used herein, “semi-pluripotent” or “pre-iPS state” refers to a cell that has some or all of the morphological characteristics of a pluripotent stem cell, but its level of expression of the pluripotency markers or its ability to differentiate to all three germ lines is less than that of a pluripotent stem cell.

[00103] As used herein, “stem-like” morphology refers to a morphology that resembles that of a stem cell, a level of expression of one or more of the pluripotency genes, or an ability to differentiate into multiple cell types. Stem-like morphology is when the cells have a rounded shape, and are rather small compared to the size of their nucleus, which is often has a large nucleus to cytoplasm ratio, which is characteristic of pluripotent stem cells. By contrast, fibroblast morphology is when cells have a long, spindly shape and do not have a large nucleus to cytoplasm ratio. Additionally, pluripotent stem cells are non-adherent, whereas other cell types, such as fibroblasts, are adherent.

[00104] 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-associated virus (AAV)), DNA

encapsulated in liposomes, DNA complexed with polylysine, complexed with synthetic polycationic molecules, complexed with compounds such as polyethylene glycol (PEG) to immunologically "mask" the molecule and/or increase half-life, or conjugated to a non-viral protein. Preferably, the polynucleotide is DNA. As used herein, "DNA" includes not only bases A, T, C, and G, but also includes any of their analogs or modified forms of these bases, such as methylated nucleotides, internucleotide modifications such as uncharged linkages and thioates, use of sugar analogs, and modified and/or alternative backbone structures, such as polyamides.

[00105] As used herein, "multimer" refers to a plurality of monomers that are covalently linked together or non-covalently fused to each other.

[00106] As used herein, "higher order multimer" refers to a plurality of monomers that are covalently linked together or non-covalently fused to each other, which is greater than a dimer.

[00107] Sequence Listing Free Text

[00108] 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.

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MTPGTQSPFF LLLLLTLVLT V TGS GHASST PGGEKETSAT QRSSVPSSTE
KNAVSM TSSV LSSHSPGSGS STTQGQDVT L APATEPASGS AATWGQDVTS
VPVTRPALGS TTPPAHDVTS APDNKPAPGS TAPPAHGVTS APDTRPAPGS
TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS
TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
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TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
TAPPVHNVT ASGSASGSAS TLVHNGTSAR ATTTASKST PFSIPSHSD
TPTTLASHST KTDASSTHHS SVPLTSSNH STSPQLSTGV SFFFLSFHIS
NLQFNSSLED PSTDYYQELQ RDISEMFLQI YKQGGFLGLS NIKFRPGSVV
VQLTLAFREG TINVHDVETQ FNQYKTEAAS RYNLTISDVS VSDVPFPFSA

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QSGAGVPGWG IALLVLVCVL VALAIVYLIA LAVCQCRKN YGQLDIFPAR
 DTYHPMSEYP TYHTHGRYVP PSSTDRSPYE KVSAGNGGSS LSYTNPAVAA
 ASANL (SEQ ID NO: 1) describes full-length MUC1 Receptor (Mucin 1 precursor, Genbank
 Accession number: P15941).

MTPGTQSPFFLLLLLLTVLT (SEQ ID NO: 2)

MTPGTQSPFFLLLLLLTVLT VVTA (SEQ ID NO: 3)

MTPGTQSPFFLLLLLLTVLT VVTG (SEQ ID NO: 4)

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.

GTINVHDTVETQFNQYKTEAASRYNLTISDVSVDVPFFPFSQSGAGVPGWGIALLLVLVCVLV
 ALAIVYLIALAVCQCRKNYGQLDIFPARDTYHPMSEYPTYHTHGRYVPPSSTDRSPYEKVS
 AGNGGSSLSYTNPAVAAASANL (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.

GTINVHDTVETQFNQYKTEAASRYNLTISDVSVDVPFFPFSQSGA (SEQ ID NO:6) describes
 Native Primary Sequence of the MUC1 Growth Factor Receptor (nat-PSMGFR – an example
 of “PSMGFR”):

TINVHDTVETQFNQYKTEAASRYNLTISDVSVDVPFFPFSQSGA (SEQ ID NO:7) describes
 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).

GTINVHDTVETQFNQYKTEAASRYNLTISDVSVDVPFFPFSQSGA (SEQ ID NO:8) describes
 “SPY” functional variant of the native Primary Sequence of the MUC1 Growth Factor
 Receptor having enhanced stability (var-PSMGFR – An example of “PSMGFR”).

TINVHDTVETQFNQYKTEAASRYNLTISDVSVDVPFFPFSQSGA (SEQ ID NO: 9) describes
 “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).

tgtcagtgccgccgaaagaactacgggcagctggacatctttccagccccgggatacctacca
 tcctatgagcgagtagcccccacctaccacacccatggcgctatgtgccccctagcagtagccg
 atcgtagccccctatgagaagggtttctgcaggtaacgggtggcagcagcctctcttacacaaac
 ccagcagtggcagccgcttctgccaaacttg (SEQ ID NO:10) describes MUC1 cytoplasmic
 domain nucleotide sequence.

CQCRKNYGQLDIFPARDTYHPMSEYPTYHTHGRYVPPSSTDRSPYEKVSAGNGGSSLSYTN
 PAVAAASANL (SEQ ID NO:11) describes MUC1 cytoplasmic domain amino acid sequence.

[00109] Human NM23 H1

[00110] (DNA)

[00111] atggccaactgtgagcgtaccttcattgcgatcaaacagatgggggtccagcgggggtctgtgggagagattatcaag
 cgttttgagcagaaaggattccgccttggtgtgaaattcatgcaagctccgaagatcttctcaaggaacactacgttgacctgaagg

accgtccattctttgccggcctggtgaaatacatgcactcagggccggtagttgccatggtctgggaggggctgaatgtggtgaagac
ggggcgagtcagctcggggagaccaaacctgcagactccaagcctgggaccatccgtggagacttctgcatacaagttggcagga
acattatacatggcagtgattctgtggagagtgagagaaggagatcggttgggttcaccctgaggaactggtagattacacgagc
tgtgtcagaactggatctatgaatga (SEQ ID NO:12)

[00112] (amino acids)

[00113] MANCERTFIAIKPDGVQRGLVGEIHKRFEQKGFRLVGLKFMQASEDLLKEH
YVDLKDRPFFAGLVKYMHS GPVVAMVWEGLNVVKTGRVMLGETNPADSKPGTIRG
DFCIQVGRNIIHGSDSVESAEKEIGLWFHPEELVDYTSCAQNWIYE- (SEQ ID NO:13)

[00114] Mouse NM23 H1

[00115] (DNA)

[00116] atggccaacagtgagcgcaccttcattgccatcaagcctgatggggtccagcgggggctggtgggagatcatca
agcgggtcgagcagaaggggtccgccttgttggtctgaagttctgcaggcttcagaggaccttctcaaggagcactacactgacctg
aaggaccgcccccttcttactggcctggtgaaatacatgcactcaggaccagtggttgcctatggtctgggagggctgaatgtggtgaa
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ggaacatcattcatggcagcgattctgtaaagagcgcagagaaggagatcagcttgggttcagcctgaggagctggtggagtacaa
gagctgtgcgcagaactggatctatgagtga (SEQ ID NO:14)

[00117] (amino acids)

[00118] MANSERTFIAIKPDGVQRGLVGEIHKRFEQKGFRLVGLKFLQASEDLLKEH
YTDLKDRPFFTGLVKYMHS GPVVAMVWEGLNVVKTGRVMLGETNPADSKPGTIRG
DFCIQVGRNIIHGSDSVKSAEKEISLWFQPEELVEYKSCAQNWIYE- (SEQ ID NO:15)

[00119] Human NM23 H2

[00120] (DNA)

[00121] atggccaacctggagcgcaccttcacgccatcaagccggacggcgtgcagcgcggcctggtgggagatcatc
aagcgttcgagcagaagggtccgcctcgtggccatgaagttcctccgggcctctgaagaacctgaagcagcactacattgac
ctgaaagaccgaccattcttcctgggctggtgaagtacataactcagggccggttggccatggtctgggaggggctgaacgtg
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ggcaggaacatcattcatggcagtgattcagtaaaaagtgtgaaaaagaaatcacctatggtttaagcctgaagaactggttgacta
caagtcttgtgctcatgactgggtctatgaataa (SEQ ID NO:16)

[00122] (amino acids)

[00123] MANLERTFIAIKPDGVQRGLVGEIHKRFEQKGFRLVAMKFLRASEEHLKQH
YIDLKDRPFFPGLVKYMNS GPVVAMVWEGLNVVKTGRVMLGETNPADSKPGTIRG
DFCIQVGRNIIHGSDSVKSAEKEISLWFKPEELVDYKSCAHDWVYE- (SEQ ID NO:17)

[00124] Mouse NM23 H2

[00125] (DNA)

[00126] atggccaacctcgagcgtaccttcattgccatcaagccagatggcgtgcagcgcggcctgggtgggcgagatcatca
aacggttcgagcagaaggggtccgcctgggtggccatgaagttcctcgggcctctgaagaacacctgaagcagcattacatcgacct
gaaagaccgtcctttctccggggctgggtgaagtacatgaactcggggcccgtgggtggccatggctctgggaggggctcaatgtggt
gaaaacggggccgagtgatgctgggggagaccaatccagctgattcaaaaccaggcaccatccgtggggatttctgattcaagtgg
caggaacatcattcatggcagtgattcagtgagagtgctgagaaagagatccatctgtggttaagcccgaagaactgatcgactaca
agtcttgcccatgactgggtgtacgagtag (SEQ ID NO:18)

[00127] (amino acids)

[00128] MANLERTFIAIKPDGVQRGLVGEIHKRFEQKGFRLVAMKFLRASEEHLKQH
YIDLKDRPFFPGLVKYMNSGPVAMVWEGLNVVKTRVVMLGETNPADSKPGTIRG
DFCIQVGRNIIHGSDSVESAEKEIHLWFKPEELIDYKSCAHDWVYE- (SEQ ID NO:19)

[00129] Mouse NME6

[00130] (DNA)

[00131] Atgacctccatcttgcgaagtccccaagctcttcagctcacactagccctgatcaagcctgatgcagttgccaccca
ctgatcctggaggctgttcacagcagattctgagcaacaagttcctcattgtacgaacgagggaactgcagtggaagctggaggact
gccggaggtttaccgagagcatgaagggcggtttttctatcagcggctgggtggagttcatgacaagtgggccaatccgagcctatc
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ccctgacttcagtgaacagcgctggtatgaggaggaggaacccagctgcggtgtgtcctgtgcactacagtcagaggaaggtat
ccactgtgcagctgaaacaggaggccacaaacaacctaacaaaacctag (SEQ ID NO:20)

[00132] (amino acids)

[00133] MTSILRSPQALQLTLALIKPDVAHPLILEAVHQQILSNKFLIVRTRELQWK
LEDCCRFFYREHEGRFFYQRLVEFMTSGPIRAYILAHKDAIQLWRTLTMGPTRVFRARY
IAPDSIRGSLGLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEEEPQLRCGPVHY
SPEEGIHCAAETGGHKQPNKT- (SEQ ID NO:21)

[00134] Human NME6:

[00135] (DNA)

[00136] Atgaccagaatctggggagtgagatggcctcaatcttgcgaagccctcaggctctccagctcactctagccctgat
caagcctgacgcagtcgcccacccactgattctggaggctgttcacagcagattctaagcaacaagttcctgattgtacgaatgagag
aactactgtggagaaaggaagattgccagaggtttaccgagagcatgaagggcggtttttctatcagaggctgggtggagttcatggcc
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agccagcagagagattgcagccttcttcctgacttcagtgaacagcgctggtatgaggaggaagagccccagttgcgctgtggccct
gtgtgctatagccagagggaggtgtccactatgtagctggaacaggaggcctaggaccagcctga (SEQ ID NO:22)

[00137] (amino acids)

[00138] MTQNLGSEMASILRSPQALQLTLALIKPDAVAHPLILEAVHQILSNKFLIV
RMRELLWRKEDCQRFYREHEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTL
MGPTRVFRARHVAPDSIRGSFGLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEE
PQLRCGPVCYSPEGGVHYVAGTGGLGPA- (SEQ ID NO:23)

[00139] Human NME6 1:

[00140] (DNA)

[00141] Atgaccagaatctggggagtgagatggcctcaatcttgcgaagccctcaggctctccagctcactctagccctgat
caagcctgacgcagtcgcccactccactgattctggaggctgttcacgcagattctaagcaacaagttcctgattgtacgaatgagag
aactactgtggagaaaggaagattgccagaggtttaccgagagcatgaagggcggttttctatcagaggctggtggagttcatggcc
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gcacgccatgtggccccagattctatccgtgggagtttcggcctcactgacaccgcaacaccacccatggttcggactctgtggttc
agccagcagagagattgcagccttcttcctgacttcagtgaacagcgctggtatgaggaggaagagccccagttgcgctgtggccct
gtgtga (SEQ ID NO:24)

[00142] (amino acids)

[00143] MTQNLGSEMASILRSPQALQLTLALIKPDAVAHPLILEAVHQILSNKFLIV
RMRELLWRKEDCQRFYREHEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTL
MGPTRVFRARHVAPDSIRGSFGLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEE
PQLRCGPV- (SEQ ID NO:25)

[00144] Human NME6 2:

[00145] (DNA)

[00146] Atgctcactctagccctgatcaagcctgacgcagtcgcccactccactgattctggaggctgttcacgcagattctaa
gcaacaagttcctgattgtacgaatgagagaactactgtggagaaaggaagattgccagaggtttaccgagagcatgaagggcggttt
ttctatcagaggctggtggagttcatggccagcgggccaatccgagcctacatccttgcccacaaggatgccatccagctctggagga
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caccacccatggttcggactctgtggttcagccagcagagagattgcagccttcttcctgacttcagtgaacagcgctggtatgagg
aggaagagccccagttgcgctgtggccctgtgtga (SEQ ID NO:26)

[00147] (amino acids)

[00148] MLTLALIKPDAVAHPLILEAVHQILSNKFLIVRMRELLWRKEDCQRFYRE
HEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTLMGPTRVFRARHVAPDSIRGSF

GLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEEPQLRCGPV- (SEQ ID NO:27)

[00149] Human NME6 3:

[00150] (DNA)

[00151] Atgtcactctagccctgatcaagcctgacgcagtcgccatccactgattctggaggctgttcacagcagattctaa
gcaacaagttcctgattgtacgaatgagagaactactgtggagaaaggaagattgccagaggttttaccgagagcatgaaggcggttt
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caccacccatggttcggactctgtggttcagccagcagagagattgcagccttctccctgacttcagtgaacagcgctggatgagg
aggaagagccccagttgcgctgtgtgccctgtgtgctatagcccagaggaggtgtccactatgtagctggaacaggaggcctagga
ccagcctga (SEQ ID NO:28)

[00152] (amino acids)

[00153] MLTLALIKPDAVAHPLILEAVHQQILSNKFLIVRMRELLWRKEDCQRFYRE
HEGRFFYQRLVEFMASGPIRAYILAHKDAIQLWRTLMPTRVFRARHVAPDSIRGSF
GLTDTRNTTHGSDSVVSASREIAAFFPDFSEQRWYEEEEPQLRCGPVCYSPEGGVHY
VAGTGGLGPA- (SEQ ID NO:29)

[00154] Human NM23-H7-1

[00155] (DNA)

[00156] atgaatcatagtgaagattcgttttcattgcagagtggatgatccaaatgcttcacttctcgacgttatgagctttat
accagggggatggatctgttgaaatgcatgatgtaaagaatcgcacctttttaagcggaccaaataatgataacctgcacttgaag
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cggcatttacgccctggaactctcagagcaatcttggtaaaactaagatccagaatgctgttcactgtactgatctgccagaggatggc
ctattagaggttcaatacttctcaagatcttggataattag (SEQ ID NO:30)

[00157] (amino acids)

[00158] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR
TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAISKA
GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDD
AICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAAREMELFFPSS
GGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFMNMDRVNV
EEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLR
PGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDN- (SEQ ID NO:31)

[00159] Human NM23-H7-1 sequence optimized for *E. coli* expression

[00160] (DNA)

[00161] atgaatcactccgaacgcttgttttatcgccgaatggtagacccgaatgcttccctgctgcgccgctacgaactgct
gttttatccggcgatggtagcgtggaaatgcatgacgttaaaatcacctgaccttctgaaacgcacgaaatgataatctgcatctg
gaagacctgtttattggcaacaaagtaaatgtgttctctcgtcagctgggtgtagcattatggcgaccagtacaccgcgcgtcaactg
ggtagtcgcaaaagaaaaacgctggccctgattaaaccggatgcaatctccaaagctggcgaaattatcgaattatcaacaaagcgg
gtttcaccatcacgaaactgaaaatgatgatgctgagccgtaagaagccctggattttcatgctgaccaccagtctcgcctgttttcaa
tgaactgattcaattcatcaccacgggtccgattatcgcaatggaaattctcgctgtagacgctatctgcgaatggaaacgcctgctggg
cccgcaaacctcaggtgttgcgcgtaccgatccagtgaatccattcgcgctctgtttggcaccgatggatccgtaatgcagcacatg
gtccggactcattcgcacgcagctcgtgaaatggaactgttttcccgagctctggcggttgcggtccggcaaacaccgccaaattt
accaattgtacgtgctgtattgtcaaacgcacgcagtgatcagaaggcctgctgggtaaaattctgatggcaatccgtgatgctgcttt
gaaatctcgccatgcagatgttcaacatggaccgcgttaacgtcgaagaattctacgaagttacaaggcgtggttaccgaatatca
cgatatggttacggaaatgtactccggtccgtgcgtcgcgatgaaattcagcaaaacaatgccacaaaacgttctgtgaattctgtgg
tccggcagatccggaaatcgacgtcatctgcgtccgggtaccctgcgcgcaatttttgtaaaacgaaaatccagaacgctgtgcact
gtaccgatctgccggaagacggtctgctggaagtcaatacttttcaaaattctggataattag (SEQ ID NO:32)

[00162] (amino acids)

[00163] MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKR
TKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTARQLGSRKEKTLALIKPDAISKA
GEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFFNELIQFITTGPIIAMEILRDD
AICEWKRLLGPANSGVARTDASESIRALFGTDGIRNAAHGPDSFASAAAREMELFFPSS
GGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFMNMDRVNV
EEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQQNNATKTFREFCGPADPEIARHLR
PGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILDN- (SEQ ID NO:33)

[00164] Human NM23-H7-2

[00165] (DNA)

[00166] atgcatgatgtaagaatcatcgcaccttttaaagcggaccaaatatgataacctgcacttggagattttataggc
aacaaagtgaatgtctttctcgacaactggatattgactatggggatcaatatacagctcgccagctgggcagtaggaaagaaaa
acgctagccctaattaaccagatgcaatatcaaaggctggagaaataattgaaataataacaaagctggattactataaccaaactc
aaaatgatgatgtttcaaggaaagaagcattggattttcatgtagatcaccagtcaagacccttttcaatgagctgatccagttattaca
actggtcctattattgccatggagattttaagagatgatgctatatgtgaatggaaaagactgctgggacctgcaaaactctggagtggca
cgcacagatgcttctgaaagcattagagccctcttggacagatggcataagaaatgcagcgcattggccctgattctttgctctgcg
gccagagaaatggagttgttttcttcaagtggaggtgtgggccggcaaacactgctaaatttactaattgtacctgttcattgttaa
ccccatgctgtcagtgaaggactgttgggaaagatcctgatggctatccgagatgcaggtttgaaatctcagctatgcagatgttcaata
tggatcgggttaattgttgaggaaattctatgaagttataaaggagtagtgaccgaatatcatgacatggtgacagaaatgtattctggccct
tgtgtagcaatggagattcaacagaataatgctacaagacatttcgagaattttgtggacctgctgacctgaaattgcccgccatttac
gccctggaactctcagagcaatcttggtaaaactaagatccagaatgctgttactgtactgatctgccagaggatggcctattagagg
ttcaataacttctcaagatcttggataattga (SEQ ID NO:34)

[00167] (amino acids)

[00168] MHDVKNHRTFLKRTKYDNLHLEDLFIGNKVNVFSRQLVLIDYGDQYTAR
QLGSRKEKTLALIKPDAISKAGEIIEIINKAGFTTTKLKMMMLSRKEALDFHVDHQSRP
FFNELIQFITTGPIIAMEILRDDAICEWKRL LGPANSVARTDASESIRALFGTDGIRNA
AHGPDSFASAAREMELFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIR
DAGFEISAMQMFNMDRVNVEEFYEVYKGVVTEYHDMVTEMYSGPCVAMEIQNN
ATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLLEVQYFFKILD
N- (SEQ ID NO:35)

[00169] Mouse NM23-H7-1

[00170] (DNA)

[00171] atgagagcctgtcagcaggaagaagtccagtttggtttctccatatatggcacccaagaatcagagcgagagattc
gctttcattgcagagtggatgatccaaatgcttcattgctccgacgctatgagctgctgtttacccacagacggatctgtgaaatgca
tgatgtaaagaatcgtcgcaccttcttaaagcggaccaagtatgaggacctgcgcctggaagatctattataggcaacaaagtcaatgt
gttttctcgacagctggtgttgattgactatggggaccaatacacagcccgcagctgggcagcaggaaagagaaaacttttagccctg
atcaaaccagatgcagtgtaaaaggccggagaaatcattgagatgataaaciaaagtggattactataaccaaactccgaatgatgac
tctgacaaggaaagaagcagcggactttcatgtagaccatcactcaagaccttttataacgaactgatccagtttatcacaagtggcct
gttattgcatggagatcttaagagatgacgcgactgtgagtggaaggttgcctggacccgcaaactctgggctatcacggacaga
tgccccgggaagcatccgagccctcttgggacagatggcgtgagaaatgcagctcacggccctgatactttgcatctgctgccaga
gaaatggaattgttttcttcaagtggaggtgtgggccagcgaacactgctaaatttaccattgcacctgtgcatcattaagcctcat
gctatcagtgaaggaaatgttgggaaagattttaatgctattcgggatgcatgcttggaaatgtcagcgatacagatgttcaattggatcg
ggctaattgtgaagaattctatgaagctataaagggtgagtgtctgagtataatgatatggtgacagagctgtgctccggcccttgcgta

gcaatagagatccaacagagcaaccctacaaagacatttcgagaattctgcggacctgctgatcctgaaatgcccgccatttacgac
ctgagaccctcagggaatttttgtaaaactaaggttcaaaatgctgttcattgcacggatctgccggaggatgggctcctggaggtcc
agtatttctcaagatcttgataattag (SEQ ID NO:36)

[00172] (amino acids)

[00173] MRACQQGRSSSLVSPYMAPKNQSERFAFIAEWYDPNASLLRRYELLYPT
DGSVEMHDVKNRRTFLKRTKYEDLRLEDLFIGNKVNVFSRQLVLIDYGDQYTARQL
GSRKEKTLALIKPDVSKAGEHEMINKSGFTITKLMMTLTRKEAADFHVDHHSRPF
YNELIQFITSGPVIAMEILRDDAICEWKRLGPNANGLSRTDAPGSIRALFGTDGVRNA
AHGPDTFASAAREMELFFPSSGGCGPANTAKFTNCTCCIIKPHASEGMLGKILAIIRD
ACFGMSAIQMFNLDRANVEEFYEVYKGVVSEYNDMVTELCSGPCVAIEIQSNPTKT
FREFCGPADPEIARHLRPETLRAIFGKTKVQNAVHCTDLPEDGLLEVQYFFKILDN-
(SEQ ID NO:37)

[00174] Mouse NM23-H7-2

[00175] (DNA)

[00176] atgagagcctgtcagcaggaagaagtccagtttggtttctccatatatggcacccaagaatcagagcgagagattc
gcttcattgcagagtggatgatccaaatgcttcattgctccgacgctatgagctgctgtttacccacagacggatctgtgaaatgca
tgatgtaaagaatcgtcgacaccttctaaagcggaccaagtatgaggacctgcgcctggaagatctattataggcaacaaagtcaatgt
gtttctcgacagctggtgttgattgactatggggaccaatacacagcccgccagctgggcagcaggaaagagaaaaacttttagccctg
atcaaaccagatgcagtgtcaaaggccggagaaatcattgagatgataaaciaaagtggattactataacaaaactccgaatgatgac
tctgacaaggaaagaagcagcggacttcatgtagaccatcactcaagaccttttataacgaactgatccagttatcacaagtgggctt
gttattgcatggagatcttaagagatgacgcgactgtgagtggaagggttgctggacccgcaaactctgggctatcacggacaga
tgcccccggaagcatccgagccctcttgggacagatggcggtgagaaatgcagctcacggccctgatactttgcatctgctgccaga
gaaatggaattgttttcttcaagtggaggtgtgggacagcgaacactgctaaattaccaattgcacctgtgcatcattaagcctcat
gctatcagtgaagatttatttattcattatatgtaa (SEQ ID NO:38)

[00177] (amino acids)

[00178] MRACQQGRSSSLVSPYMAPKNQSERFAFIAEWYDPNASLLRRYELLYPT
DGSVEMHDVKNRRTFLKRTKYEDLRLEDLFIGNKVNVFSRQLVLIDYGDQYTARQL
GSRKEKTLALIKPDVSKAGEHEMINKSGFTITKLMMTLTRKEAADFHVDHHSRPF
YNELIQFITSGPVIAMEILRDDAICEWKRLGPNANGLSRTDAPGSIRALFGTDGVRNA
AHGPDTFASAAREMELFFPSSGGCGPANTAKFTNCTCCIIKPHASEDLFIHYM- (SEQ
ID NO:39)

[00179] Mouse NME8 variant1

[00180] (DNA)

[00181] atggcaagcaaaaagcgtgaagtccagctacagtcagtcgcaatagtcagaacttgaggatgagatgttgctgaac
aaaggcttaacagtgattgatgtttaccaagcctgggtggacctgcaaaagccgtgcaaagttattcagaaaactaaaaatgaactg
aacgaagatgagattctcacttcgtcgttgctgaagctgacaacattgtgactctccagccatttagagataaatgtgagcccgtgttct
ctttagtcttaatggtaaaatcattgcaaagattcagggcgcaaatgctccacttatcaatagaaaagtcattaccttgatagatgaagaga
ggaaaattgtagcaggtgaaatggatcgtcctcagtatgttgaaattccactagtagatgcaatcgatgaagaatatggggaagtacagt
atgaaagtgtcggaagttacaatatggcaattatcaaacctgatgctgtactcatgagaaaaatatagaagttagggaataatag
ccaaagaaggatttggtatagaatacaagaaaacctgattctccctgaagaggtagtgagggaattctacactcatatagcagaccag
cctgacttgaagagtttgcgtttctatgacaaatggcctcagctgtgtgctcattgtatctcaagaagactccgaggttattcaggaaga
aacctccccgcagactgatacagaagaagaacctggcggtttggaagagcctcacgtaggtttgcacctgtgatataaagaagaac
gggacagtttgaagagtatatggaccgacagcatatgtctgattactgcgatgtcaggacgatgcggtaaggtctctaagctcattg
acatattattccctgattttaaaactatgaaaagcacgaatgtacaaacgacgctagcattactgcacccagacatctgtgaggaagaga
aagatgacgtgtgaacgttattcacaatgaaggggtaccatactgatgcagaggcaaactgtattatcagaggaagaagcaagaaca
gtgtgcaagatccatgaaaacgaagagtattttgataatcttatagggcacatgaccagtaatactcttatgtccttgcctctacggagg
aaaatgggtgtggaatattgaaaacattaattgggccaaaaacgattgaggaagcttatgcatctcatccacagagtttatgtgtacagttt
gcttcaggaattttcctaccaaccagttctacgggagcagttcaaaagcagcagctgagaaggaaatagcgcatttcttccctcccca
gagcacacttgcatgatcaagcctcatgtgacacacaaagaaagaatggagatcctgaagaccattaaagaggcaggatttgagctg
acctgatgaaggaaatgcactgactccagagcatgcaaacaaaatttttcaaaataacaggaaaagattttataaaaatgtattgg
aagtcttatctttgggcatgtcgttagtcattgtttgaccaagtgggaatgctgttcagaatggaggcgaatgggtggccagtagacc
cagaagaagcaaaaactgctctccccagaatccctccgagccaaatatggactagacattttgagaaatgctgtccatggggcgtctaac
ttttctgaagcatcagaaatcattagtaatgtgttcacagagggtaatcctgagaactag (SEQ ID NO:40)

[00182] (amino acids)

[00183] MASKKREVQLQSVVNSQNLWDEMLLNKGLTVIDVYQAWCGPCKAVQSL
FRKLKNELNEDEILHFVVAEADNIVTLQPFDRDKCEPVFLFSLNGKIIAKIQGANAPLIN
RKVITLIDEERKIVAGEMDRPQYVEIPLVDAIDEEYGEVQYESAAEVYNMAIHKPDAV
LMRKNIEVREKIAKEGFVIEIQENLILPEEVVREFYTHIADQPDFEEFVVSMTNGLSCV
LIVSQEDSEVIQEETLPQTDTEEPGVLEEPHVRFAVPMIKKKRDSLQEYMDRQHMS
DYCDVEDDAVKVSKLIDILFPDFKTMKSTNVQTTLALLHPDICEEEKDDVLNVIHNE
GFTILMQRQIVLSEEEARTVCKIHENEEYFDNLIGHMTSNHSYVLALRRENGVEYWK
TLIGPKTIEEAYASHPQSLCVQFASGNFPTNQFYGSSSKAAAEKEIAHFFPPQSTLALIK
PHVTHKERMEILKTIKEAGFELTLMKEMHLTPEHANKIYFKITGKDFYKNVLEVL
GMSLVMVLTKWNAVAEWRRMVGPDPEEAKLLSPESLRAKYGLDILRNAVHGASN
FSEASEIISNVFTEGNPEN* (SEQ ID NO:41)

[00184] Mouse NME8 variant2

[00185] (DNA)

[00186] atggcaagcaaaaagcgtgaagtcagctacagtcagtcgcaatagtcagaactgtgggatgagatgttgctgaac
aaaggcttaacagtgattgatgtttaccaagcctgggtgtggacctgcaaaagccgtgcaaagttattcagaaaactaaaaatgaactg
aacgaagatgagattcttacttcgtgctgaagctgacaacattgtgactctccagccatttagagataaatgtgagcccgtgtttct
cttagtcttaatggtaaaatcattgcaaagattcagggtgcaaagtctccacttatcaatagaaaagtcattaccttgatagatgaagaga
ggaaaattgtagcaggtgaaatggatcgtcctcagtatgttgaaattccactagtagatgcaatcgatgaagaatatggggaagtacagt
atgaaagtgtcgcggaagttacaatatggcaattatcaaacctgatgctgtactcatgagaaaaatatagaagttagggaataatag
ccaaagaaggatttgtatagaatacaagaaaacctgattctccctgaagaggtagtgagggaattctacactcatatagcagaccag
cctgactttgaagagttgtcgtttctatgacaaatggcctcagctgtgtgctcattgtatctcaagaagactccgaggttattcaggaaga
aactctcccgagactgatacagaagaagaacctggcgttttgaagagcctcacgttaggttgcacctgtgatataaagaagaac
gggacagtttgaagagtatatggaccgacagcatatgtctgattactgcgatgtcgaggacgatcggttaaggctcttaagctcattg
acatatattccctgattttaaaactatgaaaagcacgaatgtacaaacgacgctagcattactgcacccagacatctgtgaggaagaga
aagatgacgtgttgacgttattcacaatgaaggggtcaccatactgatgcagaggcaaatcgattatcagaggaagaagcaagaaca
gtgtgcaagatccatgaaaacgaagagtattttgataatcttatagggcacatgaccagtaatcactcttatgtccttgctctacggaggg
aaaatggtgtggaatattggaacattaattgggccaaaaacgattgaggaagcttatgcatctcatccacagagtttatgtgtacagttt
gcttcaggaatttctaccaaccagttctacgggagcagttcaaaagcagcagctgagaaggaaatagcgcatttctccctcccca
gagcacacttgattgatcaagcctcatgtgacacacaaagaagaattcacagaagctcaaggaggtaatga (SEQ ID
NO:42)

[00187] (amino acids)

[00188] MASKKREVQLQSVVNSQNLWDEMLLNKGLTVIDVYQAWCGPCKAVQSL
FRKLKNELNEDEILHFVVAEADNIVTLQPFDRDKCEPVFLFSLNGKIIAKIQGANAPLIN
RKVITLIDEERKIVAGEMDRPQYVEIPLVDAIDEEYGEVQYESAAEVYNMAIIKPDVAV
LMRKNIEVREKIAKEGFVIEIQENLILPEEVVREFYTHIADQPDFEEFVVSMTNGLSCV
LIVSQEDSEVIQEETLPQTDTEEPGVLEEPHVRFAFVMIKKKRDSLQEYMDRQHMS
DYCDVEDDAVKVSKLIDILFPDFKTMKSTNVQTTLALLHPDICEEEKDDVLNVIHNE
GFTILMQRQIVLSEEEARTVCKIHENEEYFDNLIGHMTSNHSYVLALRRENGVEYWK
TLIGPKTIEEAYASHPQSLCVQFASGNFPTNQFYGSSSKAAAEKEIAHFFPPQSTLALIK
PHVTHKERIHRSSRR** (SEQ ID NO:43)

[00189] Human NME8

[00190] (DNA)

[00191] atggcaagcaaaaacgagaagtcagttacagacagtcacatcaataatcaaagcctgtgggatgagatgttgacagaa
caaaggcttaacagtgattgatgtttaccaagcctgggtgtggacctgcagagcaatgcaacctttattcagaaaattgaaaaatgaactg
aacgaagacgaaattctgcattttctgtcgcagaagctgacaacattgtgactttgcagccatttagagataaatgtgaacctgtttttctc

tttagtgtaaatggcaaaattatcgaaaagattcaggggtgcaaatgcaccgcttgtaataaaaaagttattaatttgatcgatgaggagag
aaaaattgcagcaggtgaaatggctcgacctcagtcctgaaattccattagtagactcagattcagaagttagtgaagaatcacatg
tgaaagtgttcaggaattatacagtattgctattatcaaaccggatgctgtgattagtaaaaaagttctagaattaaaagaaaaattacca
aagctggatttattatagaagcagagcataagacagtgctcactgaagaacaagttgtcaacttctatagtcgaatagcagaccagcgt
gacttcgaagagtttgtctcttttatgacaagtggcttaagctatattctagtgtatctcaaggaaagtaaacacaatcctccctctgaagaa
accgaaccacagactgacaccgaacctaacgaacgatctgaggatcaacctgaggtcgaagcccagggttacacctggaatgatgaa
gaacaaacaagacagtttacaagaatatctggaaagacaacatttagctcagctctgtgacattgaagaggatgcagctaattgttctaa
gttcatggatgctttctccccgattttaaaaaatgaaaagcatgaaattagaaaagacattggcattacttcgaccaaactctttcatga
aaggaaagatgatgttttgcgtattattaaagatgaagacttcaaaatactggagcaaaagacaagtagtattatcgaaaaagaagcaca
agcactgtgcaaggaatatgaaaatgaagactattttaataaactatagaaaacatgaccagtggctcatctctagccctgtttattgag
agacaatggcttgaatactggaacaattactgggaccaagaactgttgaagaagccattgaatattttcagagagtttatgtgcaca
gtttgcgatggacagtttcccggtcaaccagttgtatggcagcgattcattagaaccgctgaaagggaaatacagcatttcttctcttc
aaagcacttttagcttgattaaacctcatgcaacaagtgaacaaagagagcagatcctgaagatagttaaggaggttgatttgatctg
acacaggtgaagaaaatgttctaactcctgagcaaacggagaaaattatccaaaagtaacaggaaaagactttataaagattattg
gaaatgttatctgtgggtccatctatgtgcatgattctgaccaagtgggaatgctgttgagaatggagacgattgatgggcccacagac
ccagaagaagcaaaattactttccctgactccatccgagcccagtttgaataagtaaatgaaaaacattgtccatggagcatctaac
gcctatgaagcaaaagaggttgtaatagactctttgaggatcctgaggaaaactaa (SEQ ID NO:44)

[00192] (amino acids)

[00193] MASKKREVQLQTVINNQSLWDEMLQNKGLTVIDVYQAWCGPCRAMQPL
FRKLKNELNEDEILHFAVAEADNIVTLQPFDRDKCEPVFLFSVNGKIIIEKIQGANAPLVN
KKVINLIDEERKIAAGEMARPQYPEIPLVDSSEVSEESPCESVQELYSIAIHKPDAVISK
KVLEIKRKITKAGFIIIEAEHKTVLTEEQVNVNFYSRIADQRDFEEFVSFMTSGLSYILVV
SQGSKHNPPSEETEPQTDTEPNERSEDQPEVEAQVTPGMMKNKQDSLQEYLERQHL
AQLCDIEEDAANVAKFMDAFFPDFKKMKSMKLEKTLALLRPNLFHERKDDVLRRIKD
EDFKILEQRQVVLSEKEAQALCKEYENEDYFNKLIENMTSGPSLALVLLRDNGLQYW
KQLLGPRTVEEAIEYFPESLCAQFAMDSL PVNQLYGSDSLETAEREIQHFFPLQSTLGL
IKPHATSEQREQILKIVKEAGFDLTQVKKMFLTPEQTEKIYPKVTGKDFYKDLLEMLS
VGPSMVMILTKWNAVAEWRRMLMGPTDPEEAKLLSPDSIRAQFGISKLNIVHGASNA
YEAKEVVNRLFEDPEEN* (SEQ ID NO:45)

[00194] NME8 *E. coli* optimized

[00195] (DNA)

[00196] atgctggtcaataaaaaagtcataacctgatcgacgaagaacgcaaaatgccgctggtgaaatggcacgccgc
aatacccggaatcccgtggtgatagcgactctgaagttcagaagaatgccgctgcaatcagtcaggaactgtattcgatcgca

acctggaacgccagcatctggctcaactgtgtgatcgaagaagacgcggccaacgtggcgaaattcatggatgccttttcccgga
cttcaagaaaatgaaagcatgaaactggaaaaaacctggccctgctgcgtccgaacctgtccacgaacgtaaagatgacgttctg
cgcatcatcaaagatgaagacttcaaaatcctggaacagcgccaagttgtcctgtctgaaaaagaagcacaggctctgtgcaaagaat
acgaaaaagaagattactcaacaaactgatcgaaaacatgacctcaggtccgctcgtggcactggttctgctgcgtgataatggcctg
cagtattggaacaactgctgggtccgcgcacggtcgaagaagccattgaatacttccggaaaagcctgtgtgcacagtttgctatgga
ttctctgccgggtgaaccaactgtatggcagtgactccctggaaaccgcggaacgtgaaatccagcatttcttctcgagcaccaccacc
accaccactga (SEQ ID NO:48)

[00202] (amino acids)

[00203] MLVNKKVINLIDEERKIAAGEMARPQYPEIPLVDSSEVSEESPCESVQEL
YSIAIIKPDAVISKKVLEIKRKITKAGFIIAEHKTVLTEEQVVNFYSRIADQRDFEEFVS
FMTSGLSYILVVSQGSKHNPPSEETEPQTDTEPNERSEDQPEVEAQVTPGMMKNKQD
SLQEYLERQHILAQLCDIEEDAANVAKFMDAFFPDFKKMKSMKLEKTLALLRPNFLH
ERKDDVLRRIKDEDFKILEQRQVVLSEKEAQALCKEYENEDYFNKLIENMTSGPSLAL
VLLRDNGLQYWKQLLGPRTVEEAIEYFPESLCAQFAMDSL PVNQLYGSDSLETAERE
IQHFFLEHHHHHH* (SEQ ID NO:49)

[00204] NME8 2-3 E coli optimized

[00205] (DNA)

[00206] Atgtctggaaaaaacctggccctgctgcgtccgaacctgtccacgaacgtaaagatgacgttctgcgcacatcatcaa
agatgaagacttcaaaatcctggaacagcgccaagttgtcctgtctgaaaaagaagcacaggctctgtgcaaagaatacgaaaacga
agattacttcaacaaactgatcgaaaacatgacctcaggtccgctcgtggcactggttctgctgcgtgataatggcctgcagtattgga
acaactgctgggtccgcgcacggtcgaagaagccattgaatacttccggaaaagcctgtgtgcacagtttgctatggatttctgccgg
tgaaccaactgtatggcagtgactccctggaaaccgcggaacgtgaaatccagcatttcttccgctgcaaagtaccctgggtctgatta
aaccgcacgcgacgtccgaacagcgcgaaacaaattctgaaaatcgtaagaagccggcttcgatctgaccaggtgaagaaaatg
tttctgaccccggaacaaacggaaaaaatctatccgaaagtcacgggcaaagatttctacaaagacctgctggaaatgctgagtgttg
tccgtccatggtcatgattctgaccaaatggaatgcggttcgagaatggcgctgcctgatgggtccgacggatccggaagaagcaaaa
ctgctgagcccggaactctattcgcgctcagtttgcatcagcaaaactgaaaacattgttcattggtgcgccaatgcgtatgaagcgaaa
gaagttgtgaaccgcctgtttgaagaccggaagaaaatctcgagcaccaccaccaccaccactga (SEQ ID NO:50)

[00207] (amino acids)

[00208] MLEKTLALLRPNFLHERKDDVLRRIKDEDFKILEQRQVVLSEKEAQALCKE
YENEDYFNKLIENMTSGPSLALVLLRDNGLQYWKQLLGPRTVEEAIEYFPESLCAQF
AMDSL PVNQLYGSDSLETAEREIQHFFPLQSTLGLIKPHATSEQREQILKIVKEAGFDL
TQVKKMFLTPEQTEKIYPKVTGKDFYKDLLEMLSVGPSMVMILTKWNAVAEWRRLL

MGPTDPEEAKLLSPDSIRAQFGISKLNIVHGASNAYEAKVNVNRLFEDPEENLEHHH
HHH* (SEQ ID NO:51)

[00209] Structure and Function of NME family proteins

[00210] The inventor previously discovered that the growth factor receptor function of a MUC1 cleavage product, MUC1*, is activated by ligand induced dimerization of its extra cellular domain and that the ligand of MUC1* was NM23 (Mahanta et al, 2008). The inventor further demonstrated that it was the dimer form of NM23-H1 that binds to the PSMGFR portion of MUC1* (Figures 21 and 27) and inhibits differentiation and supports the growth of stem and progenitor cells (Hikita et al, 2008; Smagghe et al, 2013). Conversely, NM23-H1 as a hexamer does not promote or maintain pluripotency; rather it induces differentiation. Exemplary experiments are shown in Figures 3-5. Unlike the dimeric form, NM23-H1 hexamers do not bind to the PSMGFR portion of the MUC1 extra cellular domain. Thus, although NME1 hexamers have a biological function in that they induce differentiation, for brevity herein we refer to NME1 hexamers as a “biologically inactive” multimer because it is an NME multimer that does not induce the starting cells to revert to a less mature state or does not inhibit differentiation of immature cells, or does not maintain an immature state of the cell. Similarly, herein we refer to NME1 dimers as a “biologically active” NME multimer, being an NME multimer that induces the starting cells to revert to a less mature state or inhibits differentiation of immature cells, or maintains the immature state of a cell.

[00211] In addition, the inventor discovered that, like NME1 (NM23-H1) dimers, NME7 (NM23-H7) monomers (Lacombe et al, 2000) also bind to and dimerize the extra cellular domain of MUC1* and alone is sufficient to promote or maintain pluripotency in stem or stem-like cells (see Figure 26 and Figure 27). Further, the inventor discovered that NME1 dimers or NME7 monomers induce pluripotency or induce cells to revert to a less mature state.

[00212] We have now discovered that several NM23 family members have stem-related functions in that they promote or induce pluripotency while others or other multimerization states of the same NME protein promote or induce differentiation. NME1 (NM23-H1) promotes stem cell growth and inhibits differentiation when it is a dimer only. NME6 is roughly the same molecular weight as NME1 and in sea sponge (*Suberites domuncula*) it is reported to exist as a dimer (Perina et al, 2011, “Characterization of Nme6-like gene/protein from marine sponge *Suberites domuncula*” *Naunyn-Schmiedeberg’s Arch Pharmacol*, 384:451–460). We expressed NME6 and showed in an ELISA assay that NME6 binds to the

PSMGFR peptide derived from the MUC1* extra cellular domain (Figure 20). In this binding experiment, human NME6 wild type protein was tested along with a variant (S139G) in which a mutation similar to the S120G mutation that made NME1 prefer dimer formation (Chang et al, 1986) and another variant in which that same region that made NME1 prefer dimer formation was mutated (S139A, V142D, V143A) to match the sequence that occurs in sea sponge, as NME6 in sea sponge reportedly exists as a dimer.

[00213] Although NME7 (NM23-H7) is a monomeric protein, the inventor has discovered that it functions like a dimer. It contains two NDPK catalytic domains, is approximately twice the molecular weight of an NME1 or NME6 monomer and is expressed and secreted by human embryonic stem (ES) and induced pluripotent stem (iPS) cells, as well as cancer cells (see Figure 1, Figure 2 and Figure 27). NME8 is another NME family member that has some binding affinity for the MUC1 extra cellular domain. NME8 (Miranda-Vizuite et al, 2003) has 3 domains: A, B and C. When NME8 is expressed as only domains A and B, it is monomeric, while B with C forms oligomers. NME8 may bind to and re-cluster MUC1 to turn off stem-like growth.

[00214] An ELISA experiment was performed in which NME8, domains A and B (“1” and “2” in Figure 21) or domains B and C (“2” and “3” in Figure 21) were tested for their ability to bind to the PSMGFR peptide from MUC1* extra cellular domain and compared to the binding of NME1 dimers (NM23 S120G in Figure 21A). As can be seen in the figure, some binding is observed, but it is far less than the binding of NME1 when it is in dimer form. As can be seen in Figure 21B, the most binding is seen when NME8 is an oligomer, which may indicate that like NME1 hexamers, NME8 multimers or oligomers induce differentiation by binding to the extra cellular domain of MUC1 and re-clustering the receptors to occlude the binding site of the biologically active NME multimer.

[00215] NME family proteins are differentially expressed at different times of cell and tissue development. Whereas we detect NME6, NME7 and NME1 in embryonic stem cells, only NME1 and NME2 are routinely expressed in adult cells or adult stem cells. Because NME1 forms hexamers which induce rather than inhibit stem cell differentiation, it follows that NME7 is expressed earlier in embryogenesis and in naïve state stem cells because they cannot form the differentiation-inducing hexamers. In the earliest stages of embryogenesis, growth and inhibition of differentiation would be the default, with the regulatory function of the hexamer being important in later stages when one wants to initiate differentiation when a certain density of stem cells is reached. In support of our findings, Boyer et al (Boyer et al,

2005, “Core Transcriptional Regulatory Circuitry in Human Embryonic Stem Cells”, *Cell*, Vol. 122, 947–956) reported that pluripotency inducing proteins SOX2 and NANOG bind to the promoter of NME7 but not other NME family members, indicating that it is the first NME protein expressed and is consistent with the notion that it can induce or maintain pluripotency but cannot form the hexamers that turn it off. Boyer et al also report that pluripotency inducing proteins SOX2 and OCT4 bind to the promoter of MUC1, the target receptor of NME7 following cleavage to MUC1*. SOX2 and OCT4 also bind to the promoter of the MUC1 cleavage enzyme MMP-16. The fact that these pluripotency inducing proteins redundantly bind to the promoters of MUC1, its cleavage enzyme and its ligand, NME7, argues that this subset of proteins is critical to pluripotency and that NME7 is the first of the pluripotency proteins expressed in the developing embryo. NME7 is highly expressed by human stem cells if they are cultured in NM23 variants that prefer dimer formation.

[00216] Example 1 describes an experiment in which BGO1v human embryonic stem cells are grown in either NM23-S120G, which has been refolded and purified to exist primarily as a dimer, over a coating of anti-MUC1* monoclonal antibody MN-C3 or cultured in bFGF over mouse fibroblast feeder cells. Western blots of the resultant cells shows that NME7 is highly expressed in stem cells that have been cultured in NM23-S120G dimers (Figure 1, Part I. C – Lane 1) but only weakly expressed in stem cells cultured in bFGF (Lane 2). We have shown that stem cells cultured in NM23 dimers revert to the naïve state (Smagghe et al, 2013), which is a less mature state than the primed state of human stem cells cultured in bFGF as are all commercially available stem cell lines. The fact that more NME7 is expressed in naïve state stem cells than primed state stem cells is consistent with NME7 being preferentially expressed in very early stages of embryogenesis but not later in embryo or fetal development.

[00217] Analysis of adult tissues has shown that NME7 is either not present at all or barely detectable in adult tissues. Example 4 describes an experiment in which human embryonic stem cells were cultured in either NM23-S120G dimers over a MUC1* antibody surface or in bFGF over a surface of mouse fibroblast feeder cells, then analyzed by RT-PCR to measure expression levels of the naïve genes versus the primed genes. Figure 6 shows that stem cells cultured in NM23 dimers express higher levels of the naïve genes and lower levels of the differentiated, primed genes. These results are consistent with the findings shown in Figure 1C. Comparing Lane 1 to Lane 2 shows that NME7 is expressed to a greater degree in the desirable naïve stem cells, which are able to differentiate into any cell type in the human

body. Therefore, strategies that increase expression of NME7 in a cell, for example via introduction of nucleic acids capable of causing expression of NME7 or methods that add NME7 protein, or NME1 mutants or variants that prefer dimer formation are strategies that maintain pluripotency, maintain the naïve stem cell state in embryonic or induced pluripotent stem cells, and/or induce pluripotency in more mature cell types, including somatic cells, dermablasts and fibroblasts. These strategies may include ectopic expression of one or more of the pluripotency genes Oct4, Sox2, Nanog, Klf4 or c-myc in addition to NME7 or NME1 dimer forming or dimer mimicking variants. These experiments conclude that NME7 is an earlier form of NM23 that is expressed in a more naïve stem cell.

[00218] We have already shown that NM23 in dimer form induces stem cells to revert to a more pluripotent state often called the naïve state. Our experiments and those of others have shown that culturing stem cells in bFGF or culturing stem cells over a layer of mouse fibroblast feeder cells (MEFs) drives or maintains stem cells in the less pluripotent state called the “primed” state. Referring to Figure 1(C), these primed stem cells express much less NME7, consistent with the idea that NME7 is associated with a more naïve and thus truly pluripotent stem cell state. Since the naïve state human stem cells are predicted to be better able to differentiate into functional adult cells, the naïve stem cells are the desired cells for research as well as for therapeutic use. Thus, strategies that involve inducing expression of NME7 are desired to obtain cells for therapeutic uses. Conversely, strategies that decrease expression of NME7 in cancers would be anti-cancer therapies.

[00219] NME7 exists as a single protein but structurally is comprised of two monomers and so functions as a dimer. NME7 contains two NDPK domains, portions of which bind to the MUC1* growth factor receptor. Example 1 also describes a binding experiment called a pull-down assay. In this experiment, the MUC1* extra cellular domain peptide was attached to beads which were then incubated with lysates from BGO1v human embryonic stem cells. After wash steps and release from the beads, species captured by interaction with the MUC1* peptide were separated on an SDS-PAGE gel then probed with an anti-NME7 antibody. Figure 1(F) – Lane 1 shows that NME7 binds to the MUC1* extra cellular domain. Portions of the double NDPK domains in NME7 bind to MUC1* growth factor receptor and dimerize it which activates pathways that maintain pluripotency, induce pluripotency and inhibit differentiation of stem and progenitor cells.

[00220] NME6 exists as a dimer, and resists formation of higher order multimers. NME6 dimers bind to MUC1* growth factor receptor and dimerize it which activates pathways that

maintain pluripotency, induce pluripotency and inhibit differentiation of stem and progenitor cells. Like NME1 mutants and variants that prefer dimer formation, both NME6 and NME7 are capable of maintaining and inducing pluripotency and inhibiting differentiation of stem and progenitor cells, including iPS cells.

[00221] Like NME1 mutants and variants that prefer dimer formation, NME6 and NME7 can be added exogenously to stem cells (embryonic or induced pluripotent) or progenitor cells to induce growth, maintain them in an undifferentiated state or inhibit their differentiation. NME6 and NME7 can be added exogenously to stem or progenitor cells to induce pluripotency. In addition, nucleic acids encoding NME1 mutants and variants that prefer dimer formation, NME6 and/or NME7, or variants thereof, including single chain variants that behave as dimers, can be introduced into cells to induce the cells to revert to a less differentiated state or to maintain cells in a less mature state.

[00222] NME1 and NME2 wild type proteins form tetramers, hexamers and higher order multimers. We have discovered that only NME dimers bind to the PSMGFR portion of the MUC1* growth factor receptor to support stem and progenitor cell growth while inhibiting differentiation. We have also discovered that NME dimers or NME7 monomers cause cells to revert to a less mature state, for example to induce pluripotency in more mature cells including somatic cells, dermablasts, fibroblasts and the like. We also discovered that NME1 wild type proteins that exist primarily as hexamers and higher order multimers, do not bind to PSMGFR portion of the MUC1* growth factor receptor and induce differentiation of stem and progenitor cells [Smagghe et al, 2013]. NME1 forms multimers, including dimers, tetramers and hexamers. We have shown that the dimer form of NME1 maintains stem cells in an undifferentiated state and also induces pluripotency in more mature cells. However, as stem cell density increases, the amount of NME1 secreted from the stem cells causes an increase in the local concentration, favoring the formation of hexamers that do not support pluripotency and may in fact bind to a different cell surface receptor and actively trigger differentiation. Therefore, it is advantageous for the maintenance or induction of pluripotency to suppress the NME1 isoform to avoid accumulation of the deleterious hexamers. In contrast, NME7 appears to function as the NME1 dimers. Therefore, it is advantageous to increase expression of, or to add exogenously, NME7 for the maintenance or induction of pluripotency. Even more preferred for the maintenance or induction of pluripotency is to suppress NME1 and increase expression of NME7 or to suppress NME1 and increase relative concentration of NME7 or NME1 dimers. These experiments also show that a method for

purposely inducing differentiation is to add NME1 hexamers, or to suspend suppression of NME1. Therefore, it would be a great advantage to down-regulate expression of wild type NME1 in stem or progenitor cells *in vitro* or *in vivo* when it is desirable to have the stem or progenitor cells self-replicate without differentiating. Since NME2 also forms hexamers and higher order multimers, it is also desirable to down-regulate NME2 in addition to down regulation of NME1 while maintaining or inducing pluripotency and where inhibition of differentiation is desired. In another embodiment, NME1 or NME1 and NME2 are down regulated while NME1 mutants and variants that prefer dimer formation, such as NME1 S120G or P96S or similar mutations in NME6 or NME7 are added exogenously or nucleic acids encoding them are caused to be expressed in the cells.

[00223] In a preferred embodiment, NME family members that can form higher order multimers, greater than dimer, are suppressed. In a more preferred embodiment, expression of NME1 is suppressed in order to promote or maintain cells in a pluripotent state or to induce cells to revert to a less mature state. Suppression of a particular species can be carried out by a number of methods known to those skilled in the art. A target species can be suppressed at the protein level or nucleic acid level: DNA or RNA. The expressed protein can be suppressed by the use of antibodies or small molecules that inhibit or block the activity or binding site of the protein. Alternatively, expression of protein can be inhibited by using anti-sense nucleic acids, anti-sense DNA, anti-sense RNA, inhibitory RNAs, such as RNAi, shRNA, or siRNA. Small molecules may also be used to block or inhibit expression of the targeted protein.

[00224] There are several techniques known in the art for introducing nucleic acids into cells to cause expression or repression of a targeted gene. For example, cells can be transfected or transduced with vectors that include sequences that encode the desired or undesirable gene. Vectors can be viruses, DNA or RNA in nature. Other methods involve introducing to cells mRNA that encodes the gene of interest. Genes targeted for repression can be down-regulated using shRNA or siRNA, anti-sense nucleic acids and the like. Some methods for facilitating entry of nucleic acids encoding genes involve the use of detergents so that the encoding nucleic acid is encapsulated in a liposome. Still other methods involve modifying nucleic acids that encode the genes with moieties such as O-methylation or cholesterol, which facilitate entry of the nucleic acid into the cell (See US Patent Application No. US 2010/0173359, filed July 11, 2008, the contents of which are incorporated by reference herein for its disclosure of the moieties). These latter methods are less harsh than

detergents so can be used repeatedly to cause a cell to continuously express or repress a targeted gene. For example, Accell™ siRNA is specially modified for stability, target specificity, and uptake by cells without a transfection reagent (Dharmacon, Inc. - Lafayette, Colorado). The Accell™ system can be used to down-regulate genes such NME1 or NME1 and NME2 that inhibit pluripotency. Modifications of the Accell™ system could be used to modify nucleic acids bearing sequences of genes that are beneficial for stem or progenitor cell growth or induction of pluripotency. Many techniques for gene up-regulation or down-regulation are known to those skilled in the art and can be used to down-regulate NME family proteins that are capable of forming higher order multimers, such as hexamers that induce differentiation. For example, to promote pluripotency or reversion to a less mature state, NME1 and/or NME2 are suppressed with or without up-regulation of genes that maintain or induce pluripotency such as NME1 mutants and variants that prefer dimer formation, NME6 and/or NME7, or variants thereof, including single chain variants that behave as dimers.

[00225] Whether down-regulating NME1 or NME2 or up-regulating NME1 mutants and variants that prefer dimer formation, NME6 and/or NME7, or variants thereof, including single chain variants that behave as dimers, or adding exogenous protein forms of NME1 mutants and variants that prefer dimer formation, NME6 and/or NME7, or variants thereof, including single chain variants that behave as dimers, these proteins and nucleic acids can be prepared in time release formulations or encapsulated for delivery to cells over time. Using the methods described above, cell culture, especially stem or progenitor cell culture, can be greatly simplified with greatly reduced time and labor. If expression of the differentiation-inducing NME1 and/or NME2 are suppressed, then one merely needs to add sufficient buffer, with or without adding exogenous NME1 mutants and variants that prefer dimer formation, NME6 and/or NME7 to promote pluripotent stem or progenitor cell growth, inhibit differentiation or to induce pluripotency in more mature cells. In a preferred embodiment, NME1 and/or NME2 are suppressed, while NME7 is upregulated or added exogenously to promote, maintain or induce pluripotency or to induce cells to revert to a less mature state.

[00226] Suppression of NME7 greatly inhibits stem-like growth. siRNA suppression of NME7 in human stem cells resulted in great inhibition of growth and induction of differentiation in those cells that remained. Quantification of growth was observed and documented photographically. The amount of inhibition of cell growth as well as the change from stem-like morphology to fibroblast-like morphology was similar to that of cells in which the pluripotency gene Oct4 was suppressed. In addition, RT-PCR showed that

suppression of NME7 resulted in a marked increase in the expression of FoxA2, which is a marker of the differentiated state. In these experiments, no growth factor was added and cells were characterized every 24 hours until 96 hours after addition of transfection agents. Exemplary images can be seen in Figure 23 and Figure 25.

[00227] Conversely, suppression of NME1 had very little effect on stem-like growth or morphology. RT-PCR showed no significant increase in the expression of differentiation marker, FoxA2. The amount of cell growth and the persistence of stem-like morphology was consistent with that of the negative control experiments in which transfection reagents were added but a scrambled siRNA replaced the NME1-targeting siRNA. Exemplary images can be seen in Figure 22 and Figure 25. Suppression of NME6 had a modestly adverse effect on stem-like growth, with some areas of differentiation observed. Areas of differentiation are seen as thickened areas that appear very thick white or as darkened areas of thick cells piled on top of each other. Exemplary images can be seen in Figures 24 and Figure 25 .

[00228] These results show that NME1 can be suppressed to eliminate the negative effect of the hexamers that NME1 forms at increased concentration. In fact, when NME1 is suppressed, which is typically only reduced by 50-75% in siRNA knockdown experiments, this likely decreases the local concentration of NME1 and thus decreases the probability of hexamer formation. If NME1 is suppressed but NME7, or NME6 and NME7 are not knocked down, stem cell growth proceeds and is even enhanced such that no additional recombinant growth factor needs to be added. In fact, the temporary suppression of NME1 causes an increase in the concentration of NME7 which promotes pluripotency and does not form the hexamers that can turn off pluripotent growth and induce differentiation.

[00229] Therefore, in a preferred embodiment, NME1 but not NME7 is suppressed in pluripotent stem cells, which enables their growth and inhibits differentiation without the addition of exogenously added growth factors, including the addition of recombinant NMEs. NME7 or NME1 in dimer form may be added exogenously, however to increase cell number.

[00230] Knockdown of NME1 had very little effect even after 96 hours with no growth factor added into the media. It is well known that if stem cells are cultured in minimal media absent a stem cell growth factor such as bFGF or NME1 in dimer form, the stem cells rapidly differentiate by 48 hours. This did not occur when only NME1 was knocked down, which left NME6 and NME7, suspected of functioning as NME1 dimers would, available to promote pluripotency (See Figure 11, Figure 22 and Figure 25).

[00231] Expression of the NME family member, isoform or variant may be transient, stable or controlled as enabled via inducible expression systems so that expression of the NME family member or variant is transient and can be turned on or off in a controllable manner. *Sustained* expression of a biologically active NME family member or variant, especially NME6 or NME7 isoforms or variants, promotes self-renewal, maintains or induces pluripotency while inhibiting differentiation. Conversely, *transient* expression of the biologically active NME family members or variants, especially NME6 or NME7 isoforms or variants maintains or induces pluripotency while inhibiting differentiation for a limited time period, thereafter, cells would differentiate. Alternatively, expression of NME1 wild type can be activated in order to cause cells to enter differentiation as the biologically inactive hexamer form of NME1 is produced. Similarly, expression of NME6 and/or NME7 can be suppressed to induce differentiation. The expression or suppression of NME proteins can be carried out using several techniques known to those skilled in the art, including the use of expression plasmids, linear nucleic acids for expression or suppression, e.g. siRNA, that may be derivatized with moieties such as cholesterol to facilitate entrance into the cells or nucleus.

[00232] Cells to which an NME family member, isoform or variant of the invention is added exogenously or which are caused to express the NME family member or variant include but are not limited to somatic cells, dermablasts, fibroblasts, stem cells, pluripotent stem cells, bone marrow cells, peripheral mobilized blood cells, hematopoietic stem cells, progenitor cells, patient cells, cells bearing a genetic defect or alteration, or feeder cells meant to provide neighboring cells with the secreted NME family member or variant. In addition, the cell to which an NME family member, isoform or variant of the invention is added exogenously or which are caused to express the NME family member or variant can be a patient cell. The patient cell may bear a genetic defect or alteration for which it is desired to revert the cell to a less mature state, to correct or replace defective gene, and cause self-replication of the corrected gene bearing cell. The patient cells may be induced to become pluripotent, using NME family members or variants of the invention for treatments for the patient or another patient that requires treatment with stem or progenitor cells. NME family members or variants added exogenously or made to be expressed by patient cells for generating iPS cells, which can then be directed to differentiate into any desirable cell type. Such cells may then be administered to the patient or another patient for therapeutic purposes.

[00233] NME Family Proteins and Treatment of Cancers

[00234] NME7 is secreted by cancer cells as well as stem cells. Some cancer cells express mutant NME1 that preferentially forms dimers and resists the formation of hexamers, meaning that in these cases, the cancer cells exhibit stem-like growth due to the dimeric NME1 growth factor, which does not form hexamers, and as a result does not induce differentiation. In other cancers, the stem-like growth factor function of NME7 can overcome the differentiation-inducing effects of the hexamer. Thus, as a treatment for cancers, NME7 is suppressed. Alternatively, NME1 or NME2, which readily form hexamers are overexpressed as a treatment for cancers. In one aspect of the invention, NME7 is suppressed and NME1 and/or NME2 are overexpressed or exogenously introduced to the affected cells to inhibit stem-like growth. In general, any NME family member that resists differentiation-inducing multimerization states, such as the hexamer form, are suppressed to treat cancers. NME family members that induce differentiation, such as high concentrations of NME1 or NME2, are introduced or overexpressed to treat cancers or other maladies characterized by stem-like growth. The invention contemplates practicing these methods *in vitro*, *ex vivo* and *in vivo*. The invention also contemplates administering agents that suppress or induce expression of NME family members in a patient to either induce stem-like growth, for example at a site of injury, or to inhibit stem-like growth, for example as a treatment for cancers.

[00235] We have shown that NME1 in dimer form functions as a pluripotency factor and as a growth factor, stimulating the growth of both stem cells and cancer cells when added to a minimal serum-free media. Here we report that treating cancer cells with NME1 in hexamer form inhibits cancer cell growth and migration, while treatment with the NME1 dimer did not. Exemplary experiments are shown in Figures 28-30.

[00236] Different NME multimers may bind to MUC1 at different sites. Different cleavage enzymes may cleave MUC1 such that the binding site for a differentiation-inducing multimer is not present. For example, on cancer cells, or other cells exhibiting stem-like growth, the enzyme that cleaves MUC1 may be different from the cleavage enzyme in stem cells and thus may cleave MUC1 below the binding site for the multimer that turns off stem-like growth. In such cases, it would be advantageous to suppress the MUC1 cleavage enzyme that is not present in primed stem cells. To treat cancer, it may be advantageous to cause expression in cancer cells of the cleavage enzyme that is present in primed stem cells.

[00237] Thus, increasing the amount of an NME family member whose multimerization state is the biologically active state or decreasing the relative amount of an NME family

member whose multimerization state is the biologically inactive state is a method for generating less mature cells from starting cells or for maintaining cells in a stem-like state, which may be a pluripotent state. Similarly, increasing the amount of an NME family member whose multimerization state is the biologically active state or decreasing the relative amount of an NME family member whose multimerization state is the biologically inactive state is a method of inhibiting differentiation of embryonic stem cells, induced pluripotent stem cells or progenitor cells.

[00238] Conversely, for the treatment of cancers, agents that increase the amount of an NME family member whose multimerization state is the biologically inactive state or agents that decrease the relative amount of an NME family member whose multimerization state is the biologically active state are administered to a patient.

[00239] Nucleic acids that cause the expression of an NME family member whose multimerization state is the biologically active state, or nucleic acids that suppress expression of an NME family member whose multimerization state is the biologically inactive state, can be introduced to a host cell to promote induction to or maintain it in a less mature or stem-like state. The host cell may also carry a nucleic acid that causes the expression of a gene that is either not expressed in the host cell or is mutated in the host cell. In the case of a defective gene, a nucleic acid that down-regulates the unwanted gene and up-regulates a desired or corrected gene can also be introduced to a host cell. The source of host cells can be a donor or a patient and may be derived from the skin, tooth, bone marrow, blood, placenta, amniotic fluid, a blastocyst, fetus and the like and can be stem cells, iPS cells, progenitor cells or somatic cells. Host cells used with methods of the invention can be administered to a patient. Methods of administering the cells to a patient include by bone marrow transplant, transplant into a specific site, transfusion, injection, or topical treatment. These methods can be used to treat any condition or disease wherein the disease or condition would be alleviated by treatment with stem cells, progenitors, or by correction of a genetic abnormality or defect. Cells subjected to methods of the invention can also be differentiated to progenitors or somatic cells prior to administering to a patient. In cases where differentiation is desired, NME family members whose multimerization state is the biologically active state would be withdrawn to allow differentiation.

[00240] Because NME is highly conserved among all species, the methods described herein are not intended to be limited to human NME species nor limited to use with human cells.

[00241] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. The following examples are offered by way of illustration of the present invention, and not by way of limitation.

EXAMPLES

[00242] Example 1. To determine whether or not human stem cells express NME6 or NME7 in addition to NME1 (H1) and NME2 (H2), we performed Western blot analysis on lysates and supernatant from various human stem cell lines. Human embryonic stem cell line BGO1v cells were cultured either in a) NM23-S120G in dimer form only on a cell culture plate coated with anti-MUC1* monoclonal antibody MN-C3; or b) bFGF at 4 ng/mL on mouse feeder cells (MEFs). After 3 days in culture, the stem cells were harvested and lysed, then analyzed by Western blot using antibodies to probe for the presence of NME1, NME6 and NME7. For comparison, the same analysis was done in parallel on T47D MUC1*-positive breast cancer cells. As a control, recombinant NM23-H1 wild type (NM23-wt) protein was loaded onto the gel and also probed with antibodies that recognize the 3 different NMEs. Note that the gel is a denaturing gel so that the apparent molecular weight of the NM23-S120G dimer and the wild type hexamer will both appear to be the weight of a monomer. The antibodies used to probe the gel were: for NME1: NM23-H1 (C-20); NME6: NM23-H6 (L-17) and NME7: nm23-H7 (B9) (all purchased from Santa Cruz Biotechnology, Inc).

[00243] Figure 1 shows photos of 6 Western blot gels. Part I. A-C shows the Western blots wherein the cell lysate was separated by gel electrophoresis and then probed with antibodies for: A) NME1, B) NME6 and C) NME7. In each panel, Lane 1 corresponds to BGO1v stem cells cultured in NM23-S120G (in dimer form) on a cell culture plate coated with anti-MUC1* monoclonal antibody MN-C3; Lane 2 corresponds to BGO1v stem cells cultured in bFGF on MEFs; Lane 3 corresponds to T47D breast cancer cells; Lane 4 corresponds to purified recombinant NM23-H1 wild type (NM23-wt).

[00244] Figure 1(A) shows that NME1 is present in BGO1v human embryonic stem cells, whether cultured in NM23 in dimer form on an anti-MUC1* antibody surface (Lane 1) or cultured in bFGF on a surface of mouse feeder cells (MEFs) (Lane 2). NME1 is also present

in human breast cancer cells (Lane 3). And the positive control, Lane 4, shows that the antibody used does in fact recognize NME1 purified protein.

[00245] Figure 1(B) shows that NME6 is not present in any of the samples tested, using these antibodies.

[00246] Figure 1(C) shows that NME7 is strongly expressed in human stem cells if they are cultured in NM23 (dimers) on an anti-MUC1* surface (Lane 1) but only weakly expressed in stem cells cultured in bFGF on MEF feeder cells (Lane 2). NME7 is also strongly expressed in human breast cancer cells (Lane 3), but is not recognized by the C-20 antibody purportedly specific for the H1 isoform.

[00247] Figures 1(D-F) show photos of Western blots of pull-down assays to determine which NMEs bound to the MUC1* extra cellular domain peptide. Here, a histidine-tagged MUC1* extra cellular domain peptide (GTINVHDTVETQFNQYKTEAASRYNLTISDVSVDVPFPFSAQSGA-HHHHHH) was attached to NTA-Ni agarose beads and then incubated with the same cell lysates as in Part I of Figure 1. After 1 hour incubation at 4 degrees C, beads were centrifuged for 5 minutes at 15000 RPMs. Supernatant was discarded and beads were washed with PBS to remove species bound by non-specific binding. Imidazole was added to release the complex from the beads. After centrifugation, the supernatant was separated by gel electrophoresis and analyzed as in Figure 1, Part I with antibodies against NME1 (D), NME6 (E) and NME7 (F). Figure 1(D) shows that NME1 in stem cells, whether cultured in NM23 (dimers) (Lane 1) or in bFGF (Lane 2), binds to MUC1* extra cellular domain peptide, as the inventor has previously shown. Lane 3 shows that NME1 in breast cancer cell lysates also binds to MUC1* extra cellular domain peptide and Lane 4 shows that the C-20 NME1 specific antibody binds to the purified recombinant wild type NME1. Referring to Figure 1(E), this gel shows that NME6, which in Part I of Figure 1 was shown not to be in these cell lysates, was also not pulled down by the MUC1* peptide. Figure 1(F) importantly shows that NME7 binds to the MUC1* extra cellular domain peptide. NME7 from stem cells cultured in NM23 dimers and over a MUC1* antibody surface expressed greater amounts of NME7 than stem cells cultured in bFGF over MEFs. Consistent with Part I of Figure 1, NME7 was shown to bind to MUC1* peptide and was pulled down in the assay by that interaction (Lane 1). However, NME7 does not appear in Lane 2, which is likely due to the reduced expression in cells cultured in bFGF. Lane 3 shows that NME7 expressed in breast cancer cells binds to MUC1* and Lane 4 shows no protein because the NME7 antibody does not recognize the

NME1 isotype. NME7 likely binds to two MUC1* peptides to dimerize MUC1* receptors on cells, thus stimulating pluripotency, growth and inhibition of differentiation.

[00248] Example 2. Western blot analysis of human stem cell lines BGO1v and HES-3 cells shows that an NME antibody, purportedly specific for NME7 recognized NME7 and another species having an apparent molecular weight of ~22-25kDa (Figure 2).

[00249] Example 3. The inventor previously showed that NM23 in dimer form or in a form that dimerized MUC1* receptor on cells promoted pluripotent stem cell growth, inhibited differentiation and induced pluripotency in more mature cells including somatic cells. Binding experiments showed that NM23 in dimer form bound to the MUC1* receptor on cells and to the free MUC1* extra cellular domain peptide. However, experiments using the hexamer form of either the wild type protein or the hexamer form of the S120G mutant caused stem cells to differentiate. Cellular localization experiments showed that the hexamer could bind to cells and was translocated to the nucleus where it presumably bound to elements that induce differentiation. Therefore, it is clear that expression of NME1 (NM23-H1) that forms hexamers at very low concentration as well as other NME family members that form hexamers and higher order multimers are detrimental to the process of culturing stem cells *in vitro*, inducing pluripotency in more mature cells such as somatic cells and detrimental to the process of seeding a patient with a stem or progenitor cell wherein expansion to reconstitute a stem cell population or to overtake an endogenous stem cell population are desired.

[00250] To show the deleterious effect of NME1 wild type, we cultured human stem cells, BGO1vs, in either all NME1-S120G dimers (Figure 3), all NME-wt which are hexamers (Figure 4), or mixtures of the two wherein the NME1-S120G dimer was at 20% and the NME-wt was at 80% and the total NME concentration ranged from 8nM – 104nM (Figure 5). Figures 3-5 are photographs of the resultant stem cells on Day 4 post plating. Areas of darkened and thickened cells indicate that the stem cells have differentiated in contrast to the pluripotent stem cells which are a single layer of clear, small cells with large nucleus to cytoplasm ratio. The images clearly show that NM23 dimers (NME1-S120G dimers) fully support pluripotent stem cell growth (Figure 3) while the hexameric form makes cells differentiate (Figure 4). Figure 5 shows that the presence of a significant amount of NME1 wild type, which is hexameric, causes the stem cells to differentiate despite the presence of the dimer. Thus, the wild type NME1 endogenous to human stem cells and secreted by them

inhibits the growth and maintenance of stem and progenitor cells and inhibits the induction of pluripotency in more mature cells such as somatic cells.

[00251] Example 4. RT-PCR of naïve versus primed markers for human stem cells cultured in NM23 dimers compared to cells cultured in bFGF. Human embryonic H9 stem cells were plated onto plasticware coated with an anti-MUC1* monoclonal antibody MN-C3 then cultured in NM23-S120G, purified to dimer population only, for 37 passages. Samples of the growing cells were withdrawn at passages 8, 12, 14, 21, 26 and 37. For comparison, cells from the same starting stock were cultured in bFGF over a layer of mouse feeder cells (MEFs). Cells were analyzed using standard quantitative PCR techniques. Expression levels of OCT4 and NANOG (pluripotency genes), KLF4 and KLF2 (naïve genes) and LHX2, OTX2, XIST and FOXa2 (primed genes) were measured relative to GAPDH and normalized to their expression in cells cultured in bFGF over MEFs. As can be seen in Figure 6, stem cells cultured in NM23 dimers (NME1-S120G dimers) express high levels of the naïve genes and lower levels of the primed genes, indicating the cells are in the more primitive, naïve state than stem cells culture in bFGF. Together with the results of Example 1 shown in Figure 1, Part I. C), NME7 is characteristic of the naïve state, not the primed state, and is better able to support pluripotent stem cell growth. These data provide rationale for adding exogenous NME7 or nucleic acids that induce its expression for the maintenance of pluripotency in cells, inhibition of differentiation, or the induction of pluripotency in cells. Such introduction of NME7 can be accompanied by repression of the differentiation-inducing NME1.

[00252] Example 5. Suppression of NME family members.

[00253] To demonstrate the deleterious effect of some NMEs and the beneficial effects of other NMEs on the maintenance or induction of pluripotency, we performed knockdown experiments in which siRNA was used to knock down one or more of NME1, NME6 and NME7 in pluripotent stem cells. siRNA was added to established ES and iPS cells that are commercially available. Cells were cultured in serum-free minimal stem cell growth media and pluripotent stem cells were plated over a layer of an antibody to a stem cell surface receptor, MUC1*, to avoid introduction of random growth factors that are present in feeder cells and Matrigel.

[00254] NME1, NME6 and NME7 were all knocked down together to assess the effect of knocking down NM23s without recognizing that some promote differentiation and others inhibit it. As can be seen in Figure 7A, suppressing expression of NME1, NME6 and NME7 results in poor cell growth and induces differentiation as can be seen by thickened areas of

cell growth, in which cells have smaller nuclei and take on fibroblast-like morphology. Knocking down NM23, without distinguishing among which isoforms are deleterious and which are good for the maintenance of pluripotency, causes differentiation within 48 hours. In the control experiment, equal amounts of a scrambled siRNA were added but no induction of differentiation is observed (Figure 7B).

[00255] In single knockdown experiments, suppression of NME1 had little or no effect and there was no significant differentiation within the first 48 hours (Figure 8A). Suppression of NME6 alone, has a modest effect, but at 48 hours does not appear to be significantly different from the NME1 knockdown (Figure 8B). In stark contrast, down regulation of NME7 has a dramatic and negative effect on stem cell growth and induces differentiation (Figure 8C). In the triple and double knockdown experiments (Figures 8D-F), suppressing NME1 and NME7 together induced differentiation and suppressed cell growth (Figure 8F), but comparison to Figure 8A in which NME1 alone is suppressed implies that the effect is due to 2-times as much siRNA added rather than a synergistic effect between NME1 and NME7.

[00256] We next sought to determine if the addition of recombinant NME1 dimers could rescue differentiation induced by suppression of NME7. siRNA specific for either NME1 or NME7 (Figure 9A, B respectively) was added to pluripotent stem cells in culture as described above. In identical wells the siRNA was added but in addition, recombinant NME1 dimer was added. As can be seen in Figure 9D, addition of recombinant NME1 dimer prevented that differentiation that occurs when NME7 is suppressed. Addition of recombinant NME1 dimers increases the growth of pluripotent cells wherein NME1 is suppressed, but there is no significant differentiation in either case.

[00257] Inhibition of cell growth and differentiation induced by knock down of all three NM23 isoforms (Figure 10A) is rescued in part by the addition of recombinant NME1 purified as stable dimers (Figure 10B).

[00258] siRNAs specific for NME1, NME6 and NME7 were purchased from Santa Cruz Laboratories, Santa Cruz, CA. The procedure used was the StemgenTM siRNA transfection kit. The method uses lipids to form siRNA micelles, which will permeate the cell membrane and then act on the targeted RNA to downregulate it.

[00259] As negative controls, a scrambled sequence siRNA was used and mock transfection was done in which reagents were added but no siRNA was added. As a positive control, OCT4 siRNA was added. To assess the effects of multiple knockdowns in the same

cell, scrambled siRNA was added at 3-times the concentration of a single knockdown. OCT4 is a pluripotency gene so suppressing its expression should result in stem cell differentiation.

[00260] Example 6. The experiment of Example 5 was continued to 96 hours. Evaluation of cell morphology confirmed the 48 hour observations, but with additional caveats. After 96 hours, the NME variant knockdown experiment was extended to analyze the expression of pluripotency genes as well as to analyze the effect of suppressing one NME isoform on the other NME isoforms. Stem cells from each experimental condition were separately pelleted and analyzed by RT-PCR. Expression levels of pluripotency genes Nanog, Oct4, and Klf4, along with differentiation gene Foxa2 and NME isoforms NME1, NME6 and NME7 were measured. Expression levels were normalized to a control mock transfection of siRNA. siRNA typically suppresses the targeted gene by 50-75%. As a positive control, siRNA specific for Oct4 was used. Stem cells transfected with Oct4 siRNA differentiated as expected even if cultured in normal stem cell media and growth factor.

[00261] Results: Referring to Figure 14, it can be seen that in the differentiation control in which pluripotency gene Oct4 is knocked down, differentiation gene Foxa2 is elevated and expression of all three NME genes is suppressed. The same differentiation gene Foxa2 is also elevated when NME1, NME6 or NME7 is knocked down. However, it can also be seen that when one NME gene is knocked down, there appears to be an increase in one or both of the other NME genes. When NME1 is knocked down, NME6 is increased. When NME6 is knocked down NME 1 and NME7 are increased. When NME7 is knocked down NME1 is increased.

[00262] When the recombinant dimer form of NME1 is added into the media there is a rescue effect. Figure 15 shows that when NME1 is knocked down there is an increase in the expression of differentiation gene Foxa2, although the increase is minimal compared to its expression level in the differentiation control. This is consistent with the photos of Figure 11A and Figure 12A which show no visible signs of differentiation in stem cells in which NME1 has been knocked down. When the recombinant NME1 dimer is added into the media, Figure 15 shows that there is a significant decrease in the differentiation gene Foxa2 and an increase in the expression of NME1 and NME7. The photos of Figure 11B and Figure 12B are consistent with this result in that they show that there is an increase in the number of undifferentiated stem cells, but no significant differentiation of the cells whether the recombinant NME1 dimers are added in or not. This result indicates that NME1 can be down

regulated or knocked out in pluripotent stem cells without a significant adverse effect, because NME6 or NME7, which function as dimers are still present.

[00263] Referring now to Figure 16, siRNA suppression of NME6 results in only a modest increase in the differentiation marker Foxa2. However, when recombinant NME1 dimers are added into the media, there is a large increase in expression of the differentiation gene Foxa2, which is however accompanied by a large increase in expression of the pluripotency gene Klf4 and stimulation of expression of NME6 itself. Inspection of the corresponding cells showed that when NME6 was suppressed, there was a decrease in cell growth and a very modest increase in differentiation. The results suggest that NME6 is present in pluripotent stem cells, but its role may not be critical in that NME6 alone can be knocked down without a deleterious effect. However, adding in recombinant NME1 dimers may not improve growth or inhibition of differentiation.

[00264] Knock down of NME7 had the largest and most negative effect on cell growth and cells differentiated rapidly (see Figure 11B and Figure 12B). The addition of recombinant NME1 dimers had a modest rescue effect, wherein the cells appear to be less differentiated (Figure 11D and Figure 12D). Figure 17 shows that the NME7 knockdown has increased levels of differentiation gene Foxa2 which are reduced when NME1 dimers are added into the media. Addition of recombinant NME1 dimers also reduced the level of NME1 expression to compensate for the absence of NME7.

[00265] Figure 13 shows that all three NMEs cannot be knocked down without having a disastrous effect on cell growth and differentiation. The triple knockdown gave rise to very little cells that were alive after 96 hours and those cells were entirely differentiated (Figure 13A). Knock down of NME1 and NME7 had an identical disastrous effect (Figure 13B), implying that the role of NME6 is not as important as that of NME1 and NME7. The addition of recombinant NME1 in dimer form improved cell growth but only to a limited degree (Figure 13C, D). Figure 18 shows that the triple knockdown causes an increase in the differentiation gene Foxa2 and greatly reduced expression of all the NMEs. When recombinant NME1 dimers are added to the media, the level of Foxa2 is reduced but the levels of the pluripotency genes and the NME genes is comparable to the levels expressed in the differentiation control. Figure 19 shows that there is a very similar pattern of gene expression when NME1 and NME7 are knocked down together.

[00266] **Example 7.** In another NME knockdown experiment performed as described above, human embryonic stem cells (BGO1V) were plated over a surface of an anti-MUC1*

antibody (MN-C3). Specific NME proteins were knocked down using siRNA and images as well as RT-PCR quantified the results at 96 hours post transfection of the siRNA. No growth factor or serum was added to the cells after siRNA transfection. A minimal stem cell media was changed every 48 hours. Figures 22A,B show photographs of the stem cells 96 hours after NME1 was suppressed and in the absence of any growth factors or serum added. Note that the cell number and cell morphology appear stem-like and indistinguishable from the negative control experiment shown in Figure 22C,D in which transfection reagents were added but a nonsense siRNA was added. These results show that suppressing NME1 has no deleterious effect on stem-like growth. By contrast, Figure 23A,B shows that suppression of NME7 inhibited stem-like growth and inhibited stem cell growth in general. The one remaining cluster of cells is differentiating as can be seen by the thicker, whiter clustering of cells at the center of Figure 23B. By comparison to the positive control, suppressing pluripotency gene Oct4 (Figures 23C,D), it can be seen that suppression of NME7 is comparable to suppression of the key pluripotency gene Oct 4. Suppression of NME6, shown in Figures 24A,B inhibited stem-like growth to a modest extent as areas of differentiation can be seen in the view shown in Figure 24B. However, NME6 suppression looks similar to the negative control cells wherein scrambled siRNA was added (Figures 24C,D).

[00267] RT-PCR of the resultant NME suppression experiment described immediately above, shows that only suppression of NME7 resulted in a significant increase in the expression of Foxa2, which is a marker of differentiation.

[00268] Example 8. Recombinant human NME7 promotes and maintains pluripotent stem cell growth

[00269] Testing recombinant NME7 for ability to maintain pluripotency and inhibit differentiation. A soluble variant of NME7, NME7-AB, was generated and purified as previously described (PCT/US12/60684, filed October 17, 2012, the contents of which are incorporated by reference in their entirety). 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. Figure 26 shows that culturing human stem cells in NM23-H1 dimers or in NME7 monomers results in pluripotent stem cell growth. 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.

[00270] Example 9. ELISA assay showing NME7-AB simultaneously binds to two MUC1* extra cellular domain peptides

[00271] The PSMGFR peptide bearing a C-terminal Cysteine (PSMGFR-Cys) was covalently coupled to BSA using Inject 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 room temperature the plate was washed twice with PBS-T and NME7, diluted in PBS-T + 1% BSA, was added at different concentrations. After 1h at room temperature 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 room temperature 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 room temperature the plate was washed 3x with PBS-T and binding of NME7 was measured at 415nm using a ABTS solution (Pierce).

[00272] ELISA MUC1* dimerization: The protocol for NME7 binding was used and NME7 was used at 11.6ug/mL.

[00273] After 1h at room temperature the plate was washed 3x with PBS-T and HisTagged PSMGFR peptide (PSMGFR-His) or biotinylated PSMGFR peptide (PSMGFR-biotin), diluted in PBS-T + 1% BSA, was added at different concentration. After 1h at room temperature 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 room temperature 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).

[00274] Example 10. NME1 in hexamer form inhibits cancer cell growth and migration.

[00275] T47D breast cancer cells secrete both NME1 and NME7. T47D MUC1*-positive breast cancer cells were cultured in varying amounts of NME1 that had been purified as a stable population of essentially all hexamers in order to determine whether or not hexameric NME1 inhibited cancer cell growth and stem-like growth in the same manner stem cell growth is inhibited. T47D cells were grown in 10% FBS RPMI and collected with trypsin. Cells were spun down and counted with a hemacytometer. 6,000 cells per well were seeded into 96 well plates. 24 hours later the media was changed to 1% FBS RPMI plus the varying concentrations of hexamer. Media was again changed 48 hours later. Cell viability was measured at 96 hrs using the Vialight Plus (Lonza, Rockland ME) cell assay which measures the amount of ATP present in the cells. Data is graphed as the percent cell growth relative to the media only control. As another control, a small molecule (MN547) that we identified by virtue of its ability to inhibit cancer cell growth was included as a positive control. Figure 28 shows that NME1 in hexamer form inhibits the proliferation of breast cancer cells in a concentration dependent manner.

[00276] Example 11. NME1 in hexamer form inhibits cancer cell migration; NME1 dimers do not.

[00277] Cancer cell migration is often regarded as one of the methods by which cancer cells invade other tissues or implant at various locations to start occult metastases. One method of testing for inhibitors of cancer cell migration is a “scratch test.” In these assays, cancer cells are plated and allowed to proliferate to near confluency. A pipette tip or other instrument is used to scrape off a section of cells. Dislodged cells are washed away, leaving a background of growing cancer cells with an “slash” or “cross” shaped region in each well that is devoid of cells. NME1 purified as a pure population of either hexamers or dimers was added and allowed to grow for an additional 16 or 24 hours.

[00278] DU145 MUC1*-positive prostate cancer cells were grown in 10% FBS RPMI and collected with trypsin. Cells were spun down and counted with a hemacytometer. Approximately 1×10^6 cells per well were plated into 6 well plates in 10% FBS RPMI. The following day either a cross mark (NME1 hexamers) or a slash mark (NME1 dimers) was

made using a sterile 1000 uL pipette tip to remove cells in a defined region. Dislodged cells were gently washed away. The media was changed to 10% FBS plus the varying concentrations of hexamer or dimer. Photographs were taken at the time of the scratch ($t = 0$) and again 16 hours later using the cross of the lines as the center of the picture. For time zero and for time 16 hours, the area of the scratch was quantitated using ImageJ software. Data is expressed as the percent of cellular invasion into the scratch relative to its own zero hour photograph.

[00279] Figure 29A shows the graph of the Image J quantification of the percent invasion for cancer cells treated with NME1 hexamers at the concentrations shown. Figures 29B-E show photographs at time zero (B,C) and at 16 hours (D,E). By comparison to the control (D), it is clear that NME1 hexamer (E) inhibits cancer cell invasion.

[00280] Figure 30A shows the graph of the Image J quantification of the percent invasion for cancer cells treated with NME1 dimers at the concentrations shown. Figures 30B-K show photographs at time zero (B-F) and at 16 hours (G-K). By comparison to the control (G), it is clear that NME1 dimers (H,I) do not inhibit cancer cell invasion. Recall that NME1 dimers bind to and dimerize the extra cellular domain of the MUC1* growth factor receptor on cancer cells to stimulate growth. As we have shown with stem cells, concentrations of the NME1 dimers of ~4-16nM are sufficient such that one NME1 dimer binds to two (2) MUC1* receptors. At higher concentrations (J,K), there would be one dimer bound to each MUC1* receptor rather than dimerizing the two receptors. Thus at very high NME1 dimer concentration, there is an inhibition of invasion.

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- [00309] All of the references cited herein are incorporated by reference in their entirety.

* * * * *

[00310] 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. A method for generating less mature cells from starting cells comprising inducing the starting cells to revert to a less mature state comprising increasing the amount of an NME family member whose multimerization state is the biologically active state or decreasing the relative amount of an NME family member whose multimerization state is the biologically inactive state.
2. A method of inhibiting differentiation of embryonic stem cell, induced pluripotent stem cell or progenitor cell comprising increasing the amount of an NME family member whose multimerization state is the biologically active state or decreasing the relative amount of an NME family member whose multimerization state is the biologically inactive state.
3. A method of maintaining or inducing pluripotency in cells comprising decreasing the relative amount of an NME family member whose multimerization state is the biologically inactive state.
4. A method of inhibiting differentiation in embryonic stem cell, induced pluripotent stem cell, or progenitor cell, comprising decreasing the relative amount of an NME family member whose multimerization state is the biologically inactive state.
5. A method according to any of the preceding claims, wherein the NME family member's multimerization state in the biologically inactive state is a hexamer or higher order multimer.
6. A method according to any of the preceding claims, wherein the NME family member's multimerization state in the biologically active state is a dimer or a monomer that contains two units able to bind to the same target receptor.
7. The method according to claim 5, wherein the NME family member is NME1 (NM23-H1).
8. The method according to claim 5, wherein the NME family member is NME2 (NM23-H2).
9. The method according to claim 6, wherein the NME family member is a mutant or variant of NME1 that favors dimerization or has two binding domains for its cognate ligand.
10. The method according to claim 6, wherein the NME family member is NME7.
11. The method according to claim 6, wherein the NME family member is NME6.
12. The method according to any of the preceding claims, wherein the relative amount of the NME family member whose multimerization state is the biologically inactive state is

decreased by adding an NME family member whose multimerization state is the biologically active state.

13. The method according to claim 12, wherein the NME family member whose multimerization state is the biologically active state is increased by introducing a nucleic acid or small molecule that causes it to be expressed.

14. The method according to any of the preceding claims wherein the relative amount of the NME family member whose multimerization state is the biologically inactive state is decreased by introducing nucleic acids or small molecules that down-regulate its expression.

15. The method according to any of the preceding claims, wherein the NME family member whose multimerization state is the biologically inactive state is decreased and the NME family member whose multimerization state is the biologically active state is increased by simultaneously introducing a first nucleic acid that down-regulates a first NME that forms the inactive state and a second nucleic acid that up-regulates the NME that forms the active state.

16. The method according to claims 14 or claim 15, in which the nucleic acid that down-regulates is an anti-sense DNA, an inhibitory RNA, siRNA or shRNA and the nucleic acid that up-regulates is an encoding DNA, RNA, mRNA, or plasmid.

17. The method according to claim 16, in which the nucleic acid is modified to facilitate entry into the cell.

18. The method according to claim 14 to 17, wherein the NME family member that is down regulated is NME1, NME2 or NME1 and NME2.

19. The method according to claim 14 to 17, wherein the NME family member that is up-regulated is a mutant or variant of NME1, NME2 that prefers dimerization, NME6 or NME7.

20. A nucleic acid that causes expression of NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand; and/or

- a. NME7; and/or
- b. a nucleic acid that causes expression of NME7; and/or
- c. NME6; and/or
- d. a nucleic acid that causes expression of NME6; and/or
- e. A nucleic acid that down-regulates NME1 or NME1 and NME2.

21. A media for culture of stem or progenitor cells wherein the media contains:

- a. NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand; and/or

- b. a nucleic acid that causes expression of NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand; and/or
 - c. NME7; and/or
 - d. a nucleic acid that causes expression of NME7; and/or
 - e. NME6; and/or
 - f. a nucleic acid that causes expression of NME6; and/or
 - g. a nucleic acid that down-regulates NME1 or NME1 and NME2.
- 22. A media for inducing pluripotency or for inducing cells to revert to a less mature state wherein the media contains:
 - a. NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand; and/or
 - b. a nucleic acid that causes expression of NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand; and/or
 - c. NME7; and/or
 - d. a nucleic acid that causes expression of NME7; and/or
 - e. NME6; and/or
 - f. a nucleic acid that causes expression of NME6; and/or
 - g. contains a nucleic acid that down-regulates NME1 or NME1 and NME2.
- 23. A media as in claim 22 wherein the media also contains nucleic acids that encode some or all of the pluripotency-inducing genes or small molecules including OCT4, SOX2, NANOG, KLF4, and c-Myc.
- 24. A host cell that carries a synthetic nucleic acid that causes decreased expression of an NME family member whose multimerization state is the biologically inactive state.
- 25. A host cell as in claim 24, wherein the NME family member is NME1
- 26. A host cell as in claim 24, wherein the NME family member is NME1 and NME2.
- 27. A host cell that carries a synthetic nucleic acid that causes increased expression of an NME family member whose multimerization state is the biologically active state.
- 28. A host cell as in claim 27, wherein the NME family member is an NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand
- 29. A host cell as in claim 27, wherein the NME family member is NME7.
- 30. A host cell as in claim 27, wherein the NME family member is NME6.
- 31. A host cell that carries a synthetic nucleic acid that causes decreased expression of an NME family member whose multimerization state is the biologically inactive state and a

synthetic nucleic acid that causes increased expression of an NME family member whose multimerization state is the biologically active state.

32. A host cell as in claim 31, wherein the NME family member whose expression will be decreased is NME1 or NME1 and NME2 and the NME family member whose expression will be increased is an NME1 mutant or variant that prefers dimer formation or has two binding domains of the same ligand, and/or NME6, and/or NME7.

33. A host cell as in any of the claims 24 to 32 above, wherein the cell also carries a nucleic acid that causes the expression of a gene that is either not expressed in the host cell or is mutated in the host cell.

34. A host cell as in claim 33, that also carries a nucleic acid to down-regulate an unwanted gene and up-regulates a desired or corrected gene.

35. A host cell as in any of claims 24 to 34 above, wherein the host cell is embryonic stem cell, induced pluripotent stem cell, progenitor cell, or somatic cell.

36. A method of treating a patient wherein the cells of any of the claims 23 to 35 above are administered to the patient.

37. The method according to claim 36, wherein the cells are administered by bone marrow transplant, transplant into a specific site, transfusion, injection, or topical treatment.

38. The method according to claim 36, wherein the treatment is for any disease or condition that would be alleviated by treatment with stem cells, progenitors, or by correction of a genetic abnormality or defect.

39. The method according to claim 36, wherein the cells are differentiated prior to administration to patient and wherein NME1, NME6 or NME7 are withdrawn during differentiation process.

40. A method of treating cancer in a patient comprising administering an agent to the patient such that the agent increases the amount of an NME family member whose multimerization state is the biologically inactive state or decreases the relative amount of an NME family member whose multimerization state is the biologically active state.

41. The method according to claim 40, wherein the NME family member whose multimerization state is the biologically active state is NME7.

42. The method according to claim 40, wherein NME family member whose multimerization state is the biologically inactive state NME1, NME2 or NME8.

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Thr Glu Lys Asn Ala Val Ser Met Thr Ser Ser Val Leu Ser Ser His
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Pro Pro Ala His Asp Val Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro
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260 265 270

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Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala
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Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro
355 360 365

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
370 375 380

Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
385 390 395 400

Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His
405 410 415

Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala
420 425 430

Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro
435 440 445

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
450 455 460

Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
465 470 475 480

Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His
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Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala
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Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro
515 520 525

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
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Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
545 550 555 560

Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His
565 570 575

Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala
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Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro
595 600 605

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
610 615 620

Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
625 630 635 640

Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His
645 650 655

Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala
660 665 670

Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro
675 680 685

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
690 695 700

Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
705 710 715 720

Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His
725 730 735

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 740 745 750
 Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro
 755 760 765
 Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
 770 775 780
 Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
 785 790 795 800
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 Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro
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 Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
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 Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
 865 870 875 880
 Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His
 885 890 895
 Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala
 900 905 910
 Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro
 915 920 925
 Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Asn
 930 935 940
 Arg Pro Ala Leu Gly Ser Thr Ala Pro Pro Val His Asn Val Thr Ser
 945 950 955 960
 Ala Ser Gly Ser Ala Ser Gly Ser Ala Ser Thr Leu Val His Asn Gly
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 Thr Ser Ala Arg Ala Thr Thr Thr Pro Ala Ser Lys Ser Thr Pro Phe
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 Ser Ile Pro Ser His His Ser Asp Thr Pro Thr Thr Leu Ala Ser His
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Ser Asp Val Pro Phe Pro Phe Ser Ala Gln Ser Gly Ala Gly Val Pro
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Gly Trp Gly Ile Ala Leu Leu Val Leu Val Cys Val Leu Val Ala Leu
50 55 60

Ala Ile Val Tyr Leu Ile Ala Leu Ala Val Cys Gln Cys Arg Arg Lys
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Asn Tyr Gly Gln Leu Asp Ile Phe Pro Ala Arg Asp Thr Tyr His Pro
85 90 95

Met Ser Glu Tyr Pro Thr Tyr His Thr His Gly Arg Tyr Val Pro Pro
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Ser Ser Thr Asp Arg Ser Pro Tyr Glu Lys Val Ser Ala Gly Asn Gly
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Ser Asp Val Pro Phe Pro Phe Ser Ala Gln Ser Gly Ala
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 Gly Arg Tyr Val Pro Pro Ser Ser Thr Asp Arg Ser Pro Tyr Glu Lys
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Phe Arg Leu Val Gly Leu Lys Phe Met Gln Ala Ser Glu Asp Leu Leu
 35 40 45

Lys Glu His Tyr Val Asp Leu Lys Asp Arg Pro Phe Phe Ala Gly Leu
 50 55 60

Val Lys Tyr Met His Ser Gly Pro Val Val Ala Met Val Trp Glu Gly
 65 70 75 80

Leu Asn Val Val Lys Thr Gly Arg Val Met Leu Gly Glu Thr Asn Pro
 85 90 95

Ala Asp Ser Lys Pro Gly Thr Ile Arg Gly Asp Phe Cys Ile Gln Val
 100 105 110

Gly Arg Asn Ile Ile His Gly Ser Asp Ser Val Glu Ser Ala Glu Lys
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35 40 45

Lys Glu His Tyr Thr Asp Leu Lys Asp Arg Pro Phe Phe Thr Gly Leu
50 55 60

Val Lys Tyr Met His Ser Gly Pro Val Val Ala Met Val Trp Glu Gly
65 70 75 80

Leu Asn Val Val Lys Thr Gly Arg Val Met Leu Gly Glu Thr Asn Pro
85 90 95

Ala Asp Ser Lys Pro Gly Thr Ile Arg Gly Asp Phe Cys Ile Gln Val
100 105 110

Gly Arg Asn Ile Ile His Gly Ser Asp Ser Val Lys Ser Ala Glu Lys
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Cys Ala Gln Asn Trp Ile Tyr Glu
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Gln Arg Gly Leu Val Gly Glu Ile Ile Lys Arg Phe Glu Gln Lys Gly
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Phe Arg Leu Val Ala Met Lys Phe Leu Arg Ala Ser Glu Glu His Leu
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Lys Gln His Tyr Ile Asp Leu Lys Asp Arg Pro Phe Phe Pro Gly Leu
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Val Lys Tyr Met Asn Ser Gly Pro Val Val Ala Met Val Trp Glu Gly
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Leu Asn Val Val Lys Thr Gly Arg Val Met Leu Gly Glu Thr Asn Pro
85          90          95

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Ala Asp Ser Lys Pro Gly Thr Ile Arg Gly Asp Phe Cys Ile Gln Val
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Gly Arg Asn Ile Ile His Gly Ser Asp Ser Val Lys Ser Ala Glu Lys
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Glu Ile Ser Leu Trp Phe Lys Pro Glu Glu Leu Val Asp Tyr Lys Ser
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Cys Ala His Asp Trp Val Tyr Glu
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<400> 19

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Gln Arg Gly Leu Val Gly Glu Ile Ile Lys Arg Phe Glu Gln Lys Gly	20	25	30	
Phe Arg Leu Val Ala Met Lys Phe Leu Arg Ala Ser Glu Glu His Leu	35	40	45	
Lys Gln His Tyr Ile Asp Leu Lys Asp Arg Pro Phe Phe Pro Gly Leu	50	55	60	
Val Lys Tyr Met Asn Ser Gly Pro Val Val Ala Met Val Trp Glu Gly	65	70	75	80
Leu Asn Val Val Lys Thr Gly Arg Val Met Leu Gly Glu Thr Asn Pro	85	90	95	
Ala Asp Ser Lys Pro Gly Thr Ile Arg Gly Asp Phe Cys Ile Gln Val	100	105	110	
Gly Arg Asn Ile Ile His Gly Ser Asp Ser Val Glu Ser Ala Glu Lys	115	120	125	
Glu Ile His Leu Trp Phe Lys Pro Glu Glu Leu Ile Asp Tyr Lys Ser	130	135	140	

Cys Ala His Asp Trp Val Tyr Glu
145 150

<210> 20
<211> 570
<212> DNA
<213> Artificial Sequence

<220>
<223> Mouse NME6

<400> 20
atgacctcca tcttgcaag tccccaagct cttcagctca cactagccct gatcaagcct 60
gatgcagttg cccaccact gatcctggag gctgttcac agcagattct gagcaacaag 120
ttcctcattg tacgaacgag ggaactgcag tggaagctgg aggactgccg gaggttttac 180
cgagagcatg aagggcgttt tttctatcag cggctggtgg agttcatgac aagtgggcca 240
atccgagcct atatccttgc ccacaaagat gccatccaac tttggaggac actgatggga 300
cccaccagag tatttcgagc acgctatata gcccagatt caattcgtgg aagtttgggc 360
ctcactgaca cccgaaatac taccatggc tcagactccg tggtttccgc cagcagagag 420
attgcagcct ttttcctga cttcagtga cagcgctggt atgaggagga ggaacccag 480
ctgcggtgtg gtcctgtgca ctacagtcca gaggaaggta tccactgtgc agctgaaaca 540
ggaggccaca aacaacctaa caaacctag 570

<210> 21
<211> 189
<212> PRT
<213> Artificial Sequence

<220>
<223> Mouse NME6

<400> 21

Met Thr Ser Ile Leu Arg Ser Pro Gln Ala Leu Gln Leu Thr Leu Ala
1 5 10 15

Leu Ile Lys Pro Asp Ala Val Ala His Pro Leu Ile Leu Glu Ala Val
20 25 30

His Gln Gln Ile Leu Ser Asn Lys Phe Leu Ile Val Arg Thr Arg Glu
35 40 45

Leu Gln Trp Lys Leu Glu Asp Cys Arg Arg Phe Tyr Arg Glu His Glu
50 55 60

Gly Arg Phe Phe Tyr Gln Arg Leu Val Glu Phe Met Thr Ser Gly Pro
65 70 75 80

Ile Arg Ala Tyr Ile Leu Ala His Lys Asp Ala Ile Gln Leu Trp Arg
85 90 95

Thr Leu Met Gly Pro Thr Arg Val Phe Arg Ala Arg Tyr Ile Ala Pro
 100 105 110

Asp Ser Ile Arg Gly Ser Leu Gly Leu Thr Asp Thr Arg Asn Thr Thr
 115 120 125

His Gly Ser Asp Ser Val Val Ser Ala Ser Arg Glu Ile Ala Ala Phe
 130 135 140

Phe Pro Asp Phe Ser Glu Gln Arg Trp Tyr Glu Glu Glu Glu Pro Gln
 145 150 155 160

Leu Arg Cys Gly Pro Val His Tyr Ser Pro Glu Glu Gly Ile His Cys
 165 170 175

Ala Ala Glu Thr Gly Gly His Lys Gln Pro Asn Lys Thr
 180 185

<210> 22
 <211> 585
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Human NME6

<400> 22
 atgacccaga atctggggag tgagatggcc tcaatcttgc gaagccctca ggctctccag 60
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 catcagcaga ttctaagcaa caagttcctg attgtacgaa tgagagaact actgtggaga 180
 aaggaagatt gccagagggt ttaccgagag catgaagggc gttttttcta tcagaggctg 240
 gtggagttca tggccagcgg gccaatccga gcctacatcc ttgcccacaa ggatgccatc 300
 cagctctgga ggacgctcat gggacccacc agagtgttcc gagcacgcca tgtggcccca 360
 gattctatcc gtgggagttt cggcctcact gacacccgca acaccacca tggttcggac 420
 tctgtggttt cagccagcag agagattgca gccttcttcc ctgacttcag tgaacagcgc 480
 tggatatgagg aggaagagcc ccagttgcgc tgtggccctg tgtgctatag cccagaggga 540
 ggtgtccact atgtagctgg aacaggaggc ctaggaccag cctga 585

<210> 23
 <211> 194
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Human NME6

<400> 23

Met Thr Gln Asn Leu Gly Ser Glu Met Ala Ser Ile Leu Arg Ser Pro
 1 5 10 15

Gln Ala Leu Gln Leu Thr Leu Ala Leu Ile Lys Pro Asp Ala Val Ala
20 25 30

His Pro Leu Ile Leu Glu Ala Val His Gln Gln Ile Leu Ser Asn Lys
35 40 45

Phe Leu Ile Val Arg Met Arg Glu Leu Leu Trp Arg Lys Glu Asp Cys
50 55 60

Gln Arg Phe Tyr Arg Glu His Glu Gly Arg Phe Phe Tyr Gln Arg Leu
65 70 75 80

Val Glu Phe Met Ala Ser Gly Pro Ile Arg Ala Tyr Ile Leu Ala His
85 90 95

Lys Asp Ala Ile Gln Leu Trp Arg Thr Leu Met Gly Pro Thr Arg Val
100 105 110

Phe Arg Ala Arg His Val Ala Pro Asp Ser Ile Arg Gly Ser Phe Gly
115 120 125

Leu Thr Asp Thr Arg Asn Thr Thr His Gly Ser Asp Ser Val Val Ser
130 135 140

Ala Ser Arg Glu Ile Ala Ala Phe Phe Pro Asp Phe Ser Glu Gln Arg
145 150 155 160

Trp Tyr Glu Glu Glu Glu Pro Gln Leu Arg Cys Gly Pro Val Cys Tyr
165 170 175

Ser Pro Glu Gly Gly Val His Tyr Val Ala Gly Thr Gly Gly Leu Gly
180 185 190

Pro Ala

<210> 24
<211> 525
<212> DNA
<213> Artificial Sequence

<220>
<223> Human NME6 1

<400> 24
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ctcactctag ccctgatcaa gcctgacgca gtcgcccata cactgattct ggaggctgtt 120
catcagcaga ttctaagcaa caagttcctg attgtacgaa tgagagaact actgtggaga 180
aaggaagatt gccagagggt ttaccgagag catgaagggc gttttttcta tcagaggctg 240
gtggagttca tggccagcgg gccaatccga gcctacatcc ttgcccacaa ggatgccatc 300
cagctctgga ggacgctcat gggacccacc agagtgttcc gagcacgcca tgtggcccca 360

gattctatcc gtgggagttt cggcctcact gacacccgca acaccacca tggttcggac 420
 tctgtggttt cagccagcag agagattgca gccttcttcc ctgacttcag tgaacagcgc 480
 tggtatgagg aggaagagcc ccagttgcgc tgtggccctg tgtga 525

<210> 25
 <211> 174
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Human NME6 1

<400> 25

Met Thr Gln Asn Leu Gly Ser Glu Met Ala Ser Ile Leu Arg Ser Pro
 1 5 10 15

Gln Ala Leu Gln Leu Thr Leu Ala Leu Ile Lys Pro Asp Ala Val Ala
 20 25 30

His Pro Leu Ile Leu Glu Ala Val His Gln Gln Ile Leu Ser Asn Lys
 35 40 45

Phe Leu Ile Val Arg Met Arg Glu Leu Leu Trp Arg Lys Glu Asp Cys
 50 55 60

Gln Arg Phe Tyr Arg Glu His Glu Gly Arg Phe Phe Tyr Gln Arg Leu
 65 70 75 80

Val Glu Phe Met Ala Ser Gly Pro Ile Arg Ala Tyr Ile Leu Ala His
 85 90 95

Lys Asp Ala Ile Gln Leu Trp Arg Thr Leu Met Gly Pro Thr Arg Val
 100 105 110

Phe Arg Ala Arg His Val Ala Pro Asp Ser Ile Arg Gly Ser Phe Gly
 115 120 125

Leu Thr Asp Thr Arg Asn Thr Thr His Gly Ser Asp Ser Val Val Ser
 130 135 140

Ala Ser Arg Glu Ile Ala Ala Phe Phe Pro Asp Phe Ser Glu Gln Arg
 145 150 155 160

Trp Tyr Glu Glu Glu Glu Pro Gln Leu Arg Cys Gly Pro Val
 165 170

<210> 26
 <211> 468
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Human NME6 2

<400> 26

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atgctcactc tagccctgat caagcctgac gcagtcgccc atccactgat tctggaggct      60
gttcatacagc agattctaag caacaagttc ctgattgtac gaatgagaga actactgtgg      120
agaaaggaag attgccagag gttttaccga gagcatgaag ggcgtttttt ctatcagagg      180
ctggtggagt tcatggccag cgggccaatc cgagcctaca tccttgccca caaggatgcc      240
atccagctct ggaggacgct catgggaccc accagagtgt tccgagcacg ccatgtggcc      300
ccagattcta tccgtgggag tttcggcctc actgacaccc gcaacaccac ccatggttcg      360
gactctgtgg tttcagccag cagagagatt gcagccttct tccctgactt cagtgaacag      420
cgctggtatg aggaggaaga gccccagttg cgctgtggcc ctgtgtga      468

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<210> 27

<211> 155

<212> PRT

<213> Artificial Sequence

<220>

<223> Human NME6 2

<400> 27

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Met Leu Thr Leu Ala Leu Ile Lys Pro Asp Ala Val Ala His Pro Leu
1           5           10          15

```

```

Ile Leu Glu Ala Val His Gln Gln Ile Leu Ser Asn Lys Phe Leu Ile
20          25          30

```

```

Val Arg Met Arg Glu Leu Leu Trp Arg Lys Glu Asp Cys Gln Arg Phe
35          40          45

```

```

Tyr Arg Glu His Glu Gly Arg Phe Phe Tyr Gln Arg Leu Val Glu Phe
50          55          60

```

```

Met Ala Ser Gly Pro Ile Arg Ala Tyr Ile Leu Ala His Lys Asp Ala
65          70          75          80

```

```

Ile Gln Leu Trp Arg Thr Leu Met Gly Pro Thr Arg Val Phe Arg Ala
85          90          95

```

```

Arg His Val Ala Pro Asp Ser Ile Arg Gly Ser Phe Gly Leu Thr Asp
100         105         110

```

```

Thr Arg Asn Thr Thr His Gly Ser Asp Ser Val Val Ser Ala Ser Arg
115        120        125

```

```

Glu Ile Ala Ala Phe Phe Pro Asp Phe Ser Glu Gln Arg Trp Tyr Glu
130        135        140

```

```

Glu Glu Glu Pro Gln Leu Arg Cys Gly Pro Val
145        150        155

```

<210> 28
 <211> 528
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Human NME6 3

<400> 28
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 agaaaggaag attgccagag gttttaccga gagcatgaag ggcgtttttt ctatcagagg 180
 ctggttgaggt tcatggccag cgggccaatc cgagcctaca tccttgccca caaggatgcc 240
 atccagctct ggaggacgct catgggaccc accagagtgt tccgagcacg ccatgtggcc 300
 ccagattcta tccgtgggag tttcggcctc actgacaccc gcaacaccac ccatggttcg 360
 gactctgtgg tttcagccag cagagagatt gcagccttct tccctgactt cagtgaacag 420
 cgctggtatg aggaggaaga gccccagttg cgctgtggcc ctgtgtgcta tagcccagag 480
 ggaggtgtcc actatgtagc tggaacagga ggcctaggac cagcctga 528

<210> 29
 <211> 175
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Human NME6 3

<400> 29

Met Leu Thr Leu Ala Leu Ile Lys Pro Asp Ala Val Ala His Pro Leu
 1 5 10 15
 Ile Leu Glu Ala Val His Gln Gln Ile Leu Ser Asn Lys Phe Leu Ile
 20 25 30
 Val Arg Met Arg Glu Leu Leu Trp Arg Lys Glu Asp Cys Gln Arg Phe
 35 40 45
 Tyr Arg Glu His Glu Gly Arg Phe Phe Tyr Gln Arg Leu Val Glu Phe
 50 55 60
 Met Ala Ser Gly Pro Ile Arg Ala Tyr Ile Leu Ala His Lys Asp Ala
 65 70 75 80
 Ile Gln Leu Trp Arg Thr Leu Met Gly Pro Thr Arg Val Phe Arg Ala
 85 90 95
 Arg His Val Ala Pro Asp Ser Ile Arg Gly Ser Phe Gly Leu Thr Asp
 100 105 110

Thr Arg Asn Thr Thr His Gly Ser Asp Ser Val Val Ser Ala Ser Arg
115 120 125

Glu Ile Ala Ala Phe Phe Pro Asp Phe Ser Glu Gln Arg Trp Tyr Glu
130 135 140

Glu Glu Glu Pro Gln Leu Arg Cys Gly Pro Val Cys Tyr Ser Pro Glu
145 150 155 160

Gly Gly Val His Tyr Val Ala Gly Thr Gly Gly Leu Gly Pro Ala
165 170 175

<210> 30
<211> 1131
<212> DNA
<213> Artificial Sequence

<220>
<223> Human NM23-H7-1

<400> 30
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cttcgacgtt atgagctttt attttaccga ggggatggat ctgttgaaat gcatgatgta 120
aagaatcatc gcaccttttt aaagcggacc aaatatgata acctgcactt ggaagattta 180
tttataggca acaaagtga tgtcttttct cgacaactgg tattaattga ctatggggat 240
caatatacag ctgccagct gggcagtagg aaagaaaaaa cgctagccct aattaaacca 300
gatgcaatat caaaggctgg agaaataatt gaaataataa acaaagctgg atttactata 360
accaaactca aaatgatgat gctttcaagg aaagaagcat tggattttca ttagatcac 420
cagtcaagac cttttttcaa tgagctgac cagtttatta caactgggtcc tattattgcc 480
atggagattt taagagatga tgctatatgt gaatggaaaa gactgctggg acctgcaaac 540
tctggagtgg cacgcacaga tgcttctgaa agcattagag ccctctttgg aacagatggc 600
ataagaaatg cagcgcagtg ccctgattct ttgtcttctg cggccagaga aatggagtgg 660
ttttttcctt caagtggagg ttgtgggccg gcaaacactg ctaaatttac taattgtacc 720
tgttgcattg ttaaacccca tgctgtcagt gaaggactgt tgggaaagat cctgatggct 780
atccgagatg caggttttga aatctcagct atgcagatgt tcaatatgga tcgggttaat 840
gttgaggaat tctatgaagt ttataaagga gtagtgaccg aatatcatga catggtgaca 900
gaaatgtatt ctggcccttg tgtagcaatg gagattcaac agaataatgc taaaagaca 960
tttcgagaat ttgtggacc tgctgacct gaaattgccc ggcatttacg ccctggaact 1020
ctcagagcaa tctttggtaa aactaagatc cagaatgctg ttactgtac tgatctgcca 1080
gaggatggcc tattagaggt tcaatacttc ttcaagatct tggataatta g 1131

<210> 31
<211> 376
<212> PRT
<213> Artificial Sequence

<220>

<223> Human NM23-H7-1

<400> 31

Met Asn His Ser Glu Arg Phe Val Phe Ile Ala Glu Trp Tyr Asp Pro
 1 5 10 15

Asn Ala Ser Leu Leu Arg Arg Tyr Glu Leu Leu Phe Tyr Pro Gly Asp
 20 25 30

Gly Ser Val Glu Met His Asp Val Lys Asn His Arg Thr Phe Leu Lys
 35 40 45

Arg Thr Lys Tyr Asp Asn Leu His Leu Glu Asp Leu Phe Ile Gly Asn
 50 55 60

Lys Val Asn Val Phe Ser Arg Gln Leu Val Leu Ile Asp Tyr Gly Asp
 65 70 75 80

Gln Tyr Thr Ala Arg Gln Leu Gly Ser Arg Lys Glu Lys Thr Leu Ala
 85 90 95

Leu Ile Lys Pro Asp Ala Ile Ser Lys Ala Gly Glu Ile Ile Glu Ile
 100 105 110

Ile Asn Lys Ala Gly Phe Thr Ile Thr Lys Leu Lys Met Met Met Leu
 115 120 125

Ser Arg Lys Glu Ala Leu Asp Phe His Val Asp His Gln Ser Arg Pro
 130 135 140

Phe Phe Asn Glu Leu Ile Gln Phe Ile Thr Thr Gly Pro Ile Ile Ala
 145 150 155 160

Met Glu Ile Leu Arg Asp Asp Ala Ile Cys Glu Trp Lys Arg Leu Leu
 165 170 175

Gly Pro Ala Asn Ser Gly Val Ala Arg Thr Asp Ala Ser Glu Ser Ile
 180 185 190

Arg Ala Leu Phe Gly Thr Asp Gly Ile Arg Asn Ala Ala His Gly Pro
 195 200 205

Asp Ser Phe Ala Ser Ala Ala Arg Glu Met Glu Leu Phe Phe Pro Ser
 210 215 220

Ser Gly Gly Cys Gly Pro Ala Asn Thr Ala Lys Phe Thr Asn Cys Thr
 225 230 235 240

Cys Cys Ile Val Lys Pro His Ala Val Ser Glu Gly Leu Leu Gly Lys
 245 250 255

Ile Leu Met Ala Ile Arg Asp Ala Gly Phe Glu Ile Ser Ala Met Gln
260 265 270

Met Phe Asn Met Asp Arg Val Asn Val Glu Glu Phe Tyr Glu Val Tyr
275 280 285

Lys Gly Val Val Thr Glu Tyr His Asp Met Val Thr Glu Met Tyr Ser
290 295 300

Gly Pro Cys Val Ala Met Glu Ile Gln Gln Asn Asn Ala Thr Lys Thr
305 310 315 320

Phe Arg Glu Phe Cys Gly Pro Ala Asp Pro Glu Ile Ala Arg His Leu
325 330 335

Arg Pro Gly Thr Leu Arg Ala Ile Phe Gly Lys Thr Lys Ile Gln Asn
340 345 350

Ala Val His Cys Thr Asp Leu Pro Glu Asp Gly Leu Leu Glu Val Gln
355 360 365

Tyr Phe Phe Lys Ile Leu Asp Asn
370 375

<210> 32
<211> 1131
<212> DNA
<213> Artificial sequence

<220>
<223> Human NM23-H7-1 sequence optimized for E. coli expression

<400> 32
atgaatcact ccgaacgctt tgtttttatc gccgaatggt atgacccgaa tgcttcctg 60
ctgcgccgct acgaactgct gttttatccg ggcgatggta gcgtggaaat gcatgacgtt 120
aaaaatcacc gtacctttct gaaacgcacg aaatatgata atctgcatct ggaagacctg 180
tttattggca acaaagtcaa tgtgttctct cgtcagctgg tgctgatcga ttatggcgac 240
cagtacaccg cgcgtcaact gggtagtcgc aaagaaaaaa cgctggccct gattaaaccg 300
gatgcaatct ccaaagctgg cgaaattatc gaaattatca acaaagcggg tttcaccatc 360
acgaaactga aaatgatgat gctgagccgt aaagaagccc tggattttca tgtcgaccac 420
cagtctcgcc cgtttttcaa tgaactgatt caattcatca ccacgggtcc gattatcgca 480
atggaaattc tgcgtgatga cgctatctgc gaatggaaac gcctgctggg cccggcaaac 540
tcagggtgttg cgcgtaccga tgccagtga tccattcgcg ctctgttttg caccgatggt 600
atccgtaatg cagcacatgg tccggactca ttcgcatcgg cagctcgtga aatggaactg 660
tttttcccgga gctctggcgg ttgcggtccg gcaaacaccg ccaaatttac caattgtacg 720
tgctgtattg tcaaaccgca cgcagtgatc gaaggcctgc tgggtaaaat tctgatggca 780

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atccgtgatg ctggctttga aatctcggcc atgcagatgt tcaacatgga ccgcgttaac      840
gtcgaagaat tctacgaagt ttacaaaggc gtggttaccg aatatcacga tatgggttacg      900
gaaatgtact ccggtccgtg cgtcgcgatg gaaattcagc aaaacaatgc caccaaaacg      960
tttcgtgaat tctgtggtcc ggcagatccg gaaatcgcac gtcactctgcg tccgggtacc     1020
ctgcgcgcaa tttttggtaa aacgaaaatc cagaacgctg tgcactgtac cgatctgccg     1080
gaagacgggtc tgctggaagt tcaatacttt ttcaaaattc tggataatta g              1131

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<210> 33
 <211> 376
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Human NM23-H7-1 sequence optimized for E. coli expression
 <400> 33

Met Asn His Ser Glu Arg Phe Val Phe Ile Ala Glu Trp Tyr Asp Pro
1 5 10 15

Asn Ala Ser Leu Leu Arg Arg Tyr Glu Leu Leu Phe Tyr Pro Gly Asp
20 25 30

Gly Ser Val Glu Met His Asp Val Lys Asn His Arg Thr Phe Leu Lys
35 40 45

Arg Thr Lys Tyr Asp Asn Leu His Leu Glu Asp Leu Phe Ile Gly Asn
50 55 60

Lys Val Asn Val Phe Ser Arg Gln Leu Val Leu Ile Asp Tyr Gly Asp
65 70 75 80

Gln Tyr Thr Ala Arg Gln Leu Gly Ser Arg Lys Glu Lys Thr Leu Ala
85 90 95

Leu Ile Lys Pro Asp Ala Ile Ser Lys Ala Gly Glu Ile Ile Glu Ile
100 105 110

Ile Asn Lys Ala Gly Phe Thr Ile Thr Lys Leu Lys Met Met Met Leu
115 120 125

Ser Arg Lys Glu Ala Leu Asp Phe His Val Asp His Gln Ser Arg Pro
130 135 140

Phe Phe Asn Glu Leu Ile Gln Phe Ile Thr Thr Gly Pro Ile Ile Ala
145 150 155 160

Met Glu Ile Leu Arg Asp Asp Ala Ile Cys Glu Trp Lys Arg Leu Leu
165 170 175

Gly Pro Ala Asn Ser Gly Val Ala Arg Thr Asp Ala Ser Glu Ser Ile
180 185 190

Arg Ala Leu Phe Gly Thr Asp Gly Ile Arg Asn Ala Ala His Gly Pro
195 200 205

Asp Ser Phe Ala Ser Ala Ala Arg Glu Met Glu Leu Phe Phe Pro Ser
210 215 220

Ser Gly Gly Cys Gly Pro Ala Asn Thr Ala Lys Phe Thr Asn Cys Thr
225 230 235 240

Cys Cys Ile Val Lys Pro His Ala Val Ser Glu Gly Leu Leu Gly Lys
245 250 255

Ile Leu Met Ala Ile Arg Asp Ala Gly Phe Glu Ile Ser Ala Met Gln
260 265 270

Met Phe Asn Met Asp Arg Val Asn Val Glu Glu Phe Tyr Glu Val Tyr
275 280 285

Lys Gly Val Val Thr Glu Tyr His Asp Met Val Thr Glu Met Tyr Ser
290 295 300

Gly Pro Cys Val Ala Met Glu Ile Gln Gln Asn Asn Ala Thr Lys Thr
305 310 315 320

Phe Arg Glu Phe Cys Gly Pro Ala Asp Pro Glu Ile Ala Arg His Leu
325 330 335

Arg Pro Gly Thr Leu Arg Ala Ile Phe Gly Lys Thr Lys Ile Gln Asn
340 345 350

Ala Val His Cys Thr Asp Leu Pro Glu Asp Gly Leu Leu Glu Val Gln
355 360 365

Tyr Phe Phe Lys Ile Leu Asp Asn
370 375

<210> 34
<211> 1023
<212> DNA
<213> Artificial Sequence

<220>
<223> Human NM23-H7-2

<400> 34
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ttggaagatt ttttatagg caacaaagtg aatgtctttt ctcgacaact ggtattaatt 120
gactatgggg atcaatatac agctcgccag ctgggcagta ggaaagaaaa aacgctagcc 180
ctaattaaac cagatgcaat atcaaaggct ggagaaataa ttgaaataat aaacaaagct 240

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ggatttacta taaccaaact caaaatgatg atgctttcaa ggaaagaagc attggatttt      300
catgtagatc accagtcaag accctttttc aatgagctga tccagtttat tacaactggt      360
cctattattg ccatggagat ttttaagagat gatgctatat gtgaatggaa aagactgctg      420
ggacctgcaa actctggagt ggcacgcaca gatgcttctg aaagcattag agccctcttt      480
ggaacagatg gcataagaaa tgcagcgcac ggccctgatt cttttgcttc tgcggccaga      540
gaaatggagt tgttttttcc ttcaagtgga ggttggtgggc cggcaaacac tgctaaattt      600
actaattgta cctgttgcac tggttaaacc catgctgtca gtgaaggact gttgggaaag      660
atcctgatgg ctatccgaga tgcaggtttt gaaatctcag ctatgcagat gttcaatatg      720
gatcgggtta atgttgagga attctatgaa gtttataaag gagtagtgac cgaatatcat      780
gacatggtga cagaaatgta ttctggccct tgtgtagcaa tggagattca acagaataat      840
gctacaaaga catttcgaga attttgtgga cctgctgac ctgaaattgc ccggcattta      900
cgccctggaa ctctcagagc aatctttggt aaaactaaga tccagaatgc tgttcactgt      960
actgatctgc cagaggatgg cctattagag gttcaatact tcttcaagat cttggataat     1020
tga                                                                    1023

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<210> 35
<211> 340
<212> PRT
<213> Artificial Sequence

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<220>
<223> Human NM23-H7-2

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<400> 35

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Met His Asp Val Lys Asn His Arg Thr Phe Leu Lys Arg Thr Lys Tyr
1          5          10          15

```

```

Asp Asn Leu His Leu Glu Asp Leu Phe Ile Gly Asn Lys Val Asn Val
20          25          30

```

```

Phe Ser Arg Gln Leu Val Leu Ile Asp Tyr Gly Asp Gln Tyr Thr Ala
35          40          45

```

```

Arg Gln Leu Gly Ser Arg Lys Glu Lys Thr Leu Ala Leu Ile Lys Pro
50          55          60

```

```

Asp Ala Ile Ser Lys Ala Gly Glu Ile Ile Glu Ile Ile Asn Lys Ala
65          70          75          80

```

```

Gly Phe Thr Ile Thr Lys Leu Lys Met Met Met Leu Ser Arg Lys Glu
85          90          95

```

```

Ala Leu Asp Phe His Val Asp His Gln Ser Arg Pro Phe Phe Asn Glu
100         105         110

```

Leu Ile Gln Phe Ile Thr Thr Gly Pro Ile Ile Ala Met Glu Ile Leu
 115 120 125

Arg Asp Asp Ala Ile Cys Glu Trp Lys Arg Leu Leu Gly Pro Ala Asn
 130 135 140

Ser Gly Val Ala Arg Thr Asp Ala Ser Glu Ser Ile Arg Ala Leu Phe
 145 150 155 160

Gly Thr Asp Gly Ile Arg Asn Ala Ala His Gly Pro Asp Ser Phe Ala
 165 170 175

Ser Ala Ala Arg Glu Met Glu Leu Phe Phe Pro Ser Ser Gly Gly Cys
 180 185 190

Gly Pro Ala Asn Thr Ala Lys Phe Thr Asn Cys Thr Cys Cys Ile Val
 195 200 205

Lys Pro His Ala Val Ser Glu Gly Leu Leu Gly Lys Ile Leu Met Ala
 210 215 220

Ile Arg Asp Ala Gly Phe Glu Ile Ser Ala Met Gln Met Phe Asn Met
 225 230 235 240

Asp Arg Val Asn Val Glu Glu Phe Tyr Glu Val Tyr Lys Gly Val Val
 245 250 255

Thr Glu Tyr His Asp Met Val Thr Glu Met Tyr Ser Gly Pro Cys Val
 260 265 270

Ala Met Glu Ile Gln Gln Asn Asn Ala Thr Lys Thr Phe Arg Glu Phe
 275 280 285

Cys Gly Pro Ala Asp Pro Glu Ile Ala Arg His Leu Arg Pro Gly Thr
 290 295 300

Leu Arg Ala Ile Phe Gly Lys Thr Lys Ile Gln Asn Ala Val His Cys
 305 310 315 320

Thr Asp Leu Pro Glu Asp Gly Leu Leu Glu Val Gln Tyr Phe Phe Lys
 325 330 335

Ile Leu Asp Asn
 340

<210> 36
 <211> 1188
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Mouse NM23-H7-1

<400> 36
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 aatcagagcg agagattcgc ttctattgca gagtggtatg atccaaatgc ttcattgctc 120
 cgacgctatg agctgctggt ttacccccaca gacggatctg ttgaaatgca tgatgtaaag 180
 aatcgtcgca ccttcttaaa gcggaccaag tatgaggacc tgcgcctgga agatctattt 240
 ataggcaaca aagtcaatgt gttttctcga cagctggtgt tgattgacta tggggaccaa 300
 tacacagccc gccagctggg cagcaggaaa gagaaaactt tagccctgat caaaccagat 360
 gcagtgtcaa aggccggaga aatcattgag atgataaaca aaagtggatt tactataacc 420
 aaactccgaa tgatgactct gacaaggaaa gaagcagcgg actttcatgt agaccatcac 480
 tcaagacctt ttataacga actgatccag tttatcaca gtgggcctgt tattgccatg 540
 gagatcttaa gagatgacgc gatctgtgag tggaaaaggt tgcttgacc cgaaaactct 600
 gggctatcac ggacagatgc ccccggaagc atccgagccc tctttgggac agatggcgtg 660
 agaaatgcag ctacagggcc tgatactttt gcatctgctg ccagagaaat ggaattgttt 720
 tttccttcaa gtggaggctg tgggccagcg aacactgcta aatttaccaa ttgcacctgt 780
 tgcattatta agcctcatgc tatcagtga ggaatgttgg gaaagatttt aatagctatt 840
 cgggatgcat gctttggaat gtcagcgata cagatgttca atttgatcg ggctaattgt 900
 gaagaattct atgaagtcta taaagggtga gtgtctgagt ataattgat ggtgacagag 960
 ctgtgctccg gcccttgctg agcaatagag atccaacaga gcaaccctac aaagacattt 1020
 cgagaattct gcggacctgc tgatcctgaa atcgcccgcc atttacgacc tgagaccctc 1080
 agggcaattt ttggtaaaac taagggtcaa aatgctgttc attgcacgga tctgccggag 1140
 gatgggctcc tggagggtcca gtatttcttc aagatcttgg ataattag 1188

<210> 37
 <211> 395
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Mouse NM23-H7-1

<400> 37

Met Arg Ala Cys Gln Gln Gly Arg Ser Ser Ser Leu Val Ser Pro Tyr
 1 5 10 15

Met Ala Pro Lys Asn Gln Ser Glu Arg Phe Ala Phe Ile Ala Glu Trp
 20 25 30

Tyr Asp Pro Asn Ala Ser Leu Leu Arg Arg Tyr Glu Leu Leu Phe Tyr
 35 40 45

Pro Thr Asp Gly Ser Val Glu Met His Asp Val Lys Asn Arg Arg Thr
 50 55 60

Phe Leu Lys Arg Thr Lys Tyr Glu Asp Leu Arg Leu Glu Asp Leu Phe
 65 70 75 80
 Ile Gly Asn Lys Val Asn Val Phe Ser Arg Gln Leu Val Leu Ile Asp
 85 90 95
 Tyr Gly Asp Gln Tyr Thr Ala Arg Gln Leu Gly Ser Arg Lys Glu Lys
 100 105 110
 Thr Leu Ala Leu Ile Lys Pro Asp Ala Val Ser Lys Ala Gly Glu Ile
 115 120 125
 Ile Glu Met Ile Asn Lys Ser Gly Phe Thr Ile Thr Lys Leu Arg Met
 130 135 140
 Met Thr Leu Thr Arg Lys Glu Ala Ala Asp Phe His Val Asp His His
 145 150 155 160
 Ser Arg Pro Phe Tyr Asn Glu Leu Ile Gln Phe Ile Thr Ser Gly Pro
 165 170 175
 Val Ile Ala Met Glu Ile Leu Arg Asp Asp Ala Ile Cys Glu Trp Lys
 180 185 190
 Arg Leu Leu Gly Pro Ala Asn Ser Gly Leu Ser Arg Thr Asp Ala Pro
 195 200 205
 Gly Ser Ile Arg Ala Leu Phe Gly Thr Asp Gly Val Arg Asn Ala Ala
 210 215 220
 His Gly Pro Asp Thr Phe Ala Ser Ala Ala Arg Glu Met Glu Leu Phe
 225 230 235 240
 Phe Pro Ser Ser Gly Gly Cys Gly Pro Ala Asn Thr Ala Lys Phe Thr
 245 250 255
 Asn Cys Thr Cys Cys Ile Ile Lys Pro His Ala Ile Ser Glu Gly Met
 260 265 270
 Leu Gly Lys Ile Leu Ile Ala Ile Arg Asp Ala Cys Phe Gly Met Ser
 275 280 285
 Ala Ile Gln Met Phe Asn Leu Asp Arg Ala Asn Val Glu Glu Phe Tyr
 290 295 300
 Glu Val Tyr Lys Gly Val Val Ser Glu Tyr Asn Asp Met Val Thr Glu
 305 310 315 320
 Leu Cys Ser Gly Pro Cys Val Ala Ile Glu Ile Gln Gln Ser Asn Pro
 325 330 335

Thr Lys Thr Phe Arg Glu Phe Cys Gly Pro Ala Asp Pro Glu Ile Ala
340 345 350

Arg His Leu Arg Pro Glu Thr Leu Arg Ala Ile Phe Gly Lys Thr Lys
355 360 365

Val Gln Asn Ala Val His Cys Thr Asp Leu Pro Glu Asp Gly Leu Leu
370 375 380

Glu Val Gln Tyr Phe Phe Lys Ile Leu Asp Asn
385 390 395

<210> 38
<211> 834
<212> DNA
<213> Artificial Sequence

<220>
<223> Mouse NM23-H7-2

<400> 38
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aatcagagcg agagattcgc ttctattgca gagtggtatg atccaaatgc ttctattgctc 120
cgacgctatg agctgctggt ttaccccaca gacggatctg ttgaaatgca tgatgtaaag 180
aatcgtcgca ctttcttaaa gcggaccaag tatgaggacc tgcgcctgga agatctattt 240
ataggcaaca aagtcaatgt gttttctcga cagctgggtg tgattgacta tggggaccaa 300
tacacagccc gccagctggg cagcaggaaa gagaaaactt tagccctgat caaaccagat 360
gcagtgtcaa aggccggaga aatcattgag atgataaaca aaagtggatt tactataacc 420
aaactccgaa tgatgactct gacaaggaaa gaagcagcgg actttcatgt agaccatcac 480
tcaagacctt ttataacga actgatccag ttatcacaa gtgggcctgt tattgccatg 540
gagatcttaa gagatgacgc gatctgtgag tggaaaagggt tgcttggacc cgcaaactct 600
gggctatcac ggacagatgc ccccggaagc atccgagccc tctttgggac agatggcgtg 660
agaaatgcag ctcacggccc tgatactttt gcatctgctg ccagagaaat ggaattgttt 720
tttccttcaa gtggaggctg tgggccagcg aacactgcta aatttaccaa ttgcacctgt 780
tgcattatta agcctcatgc tatcagtga gatttattta ttctattat gtaa 834

<210> 39
<211> 277
<212> PRT
<213> Artificial Sequence

<220>
<223> Mouse NM23-H7-2

<400> 39

Met Arg Ala Cys Gln Gln Gly Arg Ser Ser Ser Leu Val Ser Pro Tyr
1 5 10 15

Met Ala Pro Lys Asn Gln Ser Glu Arg Phe Ala Phe Ile Ala Glu Trp
 20 25 30
 Tyr Asp Pro Asn Ala Ser Leu Leu Arg Arg Tyr Glu Leu Leu Phe Tyr
 35 40 45
 Pro Thr Asp Gly Ser Val Glu Met His Asp Val Lys Asn Arg Arg Thr
 50 55 60
 Phe Leu Lys Arg Thr Lys Tyr Glu Asp Leu Arg Leu Glu Asp Leu Phe
 65 70 75 80
 Ile Gly Asn Lys Val Asn Val Phe Ser Arg Gln Leu Val Leu Ile Asp
 85 90 95
 Tyr Gly Asp Gln Tyr Thr Ala Arg Gln Leu Gly Ser Arg Lys Glu Lys
 100 105 110
 Thr Leu Ala Leu Ile Lys Pro Asp Ala Val Ser Lys Ala Gly Glu Ile
 115 120 125
 Ile Glu Met Ile Asn Lys Ser Gly Phe Thr Ile Thr Lys Leu Arg Met
 130 135 140
 Met Thr Leu Thr Arg Lys Glu Ala Ala Asp Phe His Val Asp His His
 145 150 155 160
 Ser Arg Pro Phe Tyr Asn Glu Leu Ile Gln Phe Ile Thr Ser Gly Pro
 165 170 175
 Val Ile Ala Met Glu Ile Leu Arg Asp Asp Ala Ile Cys Glu Trp Lys
 180 185 190
 Arg Leu Leu Gly Pro Ala Asn Ser Gly Leu Ser Arg Thr Asp Ala Pro
 195 200 205
 Gly Ser Ile Arg Ala Leu Phe Gly Thr Asp Gly Val Arg Asn Ala Ala
 210 215 220
 His Gly Pro Asp Thr Phe Ala Ser Ala Ala Arg Glu Met Glu Leu Phe
 225 230 235 240
 Phe Pro Ser Ser Gly Gly Cys Gly Pro Ala Asn Thr Ala Lys Phe Thr
 245 250 255
 Asn Cys Thr Cys Cys Ile Ile Lys Pro His Ala Ile Ser Glu Asp Leu
 260 265 270
 Phe Ile His Tyr Met
 275

<210> 40
 <211> 1761
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Mouse NME8 variant1

<400> 40
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 ccttgcaaag ccgtgcaaag tttattcaga aaactaaaaa atgaactgaa cgaagatgag 180
 attcttcact tcgtcgttgc tgaagctgac aacattgtga ctctccagcc atttagagat 240
 aaatgtgagc ccgtgtttct ctttagtctt aatggtaaaa tcattgcaaa gattcagggt 300
 gcaaatgctc cacttatcaa tagaaaagtc attacctga tagatgaaga gaggaaaatt 360
 gtagcagggtg aaatggatcg tcctcagtat gttgaaattc cactagtaga tgcaatcgat 420
 gaagaatatg gggaagtaca gtatgaaagt gctgcggaag ttacaatat ggcaattatc 480
 aaacctgatg ctgtactcat gagaaaaaat atagaagtta gggaaaaaat agccaaagaa 540
 ggattttgta tagaaataca agaaaacctg attctccctg aagaggtagt gaggggaattc 600
 tacactcata tagcagacca gcctgacttt gaagagtttg tcgtttctat gacaaatggc 660
 ctgagctgtg tgctcattgt atctcaagaa gactccgagg ttattcagga agaaactctc 720
 ccgcgactg atacagaaga agaacctggc gttttggaag agcctcacgt taggtttgca 780
 cctgtgatga taaagaagaa acgggacagt ttgcaagagt atatggaccg acagcatatg 840
 tctgattact gcgatgtcga ggacgatgcg gttaaggctc ctaagctcat tgacatatta 900
 ttccctgatt ttaaaactat gaaaagcacg aatgtacaaa cgacgctagc attactgcat 960
 ccagacatct gtgaggaaga gaaagatgac gtgttgaaacg ttattcacia tgaagggttc 1020
 accatactga tgcagaggca aatcgtatta tcagagggaag aagcaagaac agtgtgcaag 1080
 atccatgaaa acgaagagta ttttgataat cttatagggc acatgaccag taatcactct 1140
 tatgtccttg ctctacggag ggaaaatggg gtggaatatt ggaaaacatt aattgggcca 1200
 aaaacgattg aggaagctta tgcattctcat ccacagagtt tatgtgtaca gtttgcttca 1260
 gggaattttc ctaccaacca gttctacggg agcagttcaa aagcagcagc tgagaaggaa 1320
 atagcgcatt tcttccctcc ccagagcaca cttgcattga tcaagcctca tgtgacacac 1380
 aaagaaagaa tggagatcct gaagaccatt aaagaggcag gatttgagct gaccctgatg 1440
 aaggaaatgc acctgactcc agagcatgca aacaaaattt atttcaaaat aacaggaaaa 1500
 gatTTTTata aaaatgtatt ggaagtctta tctttgggca tgtcgctagt catggTTTTg 1560
 accaagtgga atgctgttgc agaattggagg cgaatggttg gccagtaga cccagaagaa 1620
 gcaaaaactgc tctccccaga atccctccga gccaaatatg gactagacat tttgagaaat 1680
 gctgtccatg gggcgtctaa cttttctgaa gcatcagaaa tcattagtaa tgtgttcaca 1740
 gagggtaatc ctgagaacta g 1761

<210> 41
 <211> 586
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Mouse NME8 variant1

<400> 41

Met Ala Ser Lys Lys Arg Glu Val Gln Leu Gln Ser Val Val Asn Ser
 1 5 10 15

Gln Asn Leu Trp Asp Glu Met Leu Leu Asn Lys Gly Leu Thr Val Ile
 20 25 30

Asp Val Tyr Gln Ala Trp Cys Gly Pro Cys Lys Ala Val Gln Ser Leu
 35 40 45

Phe Arg Lys Leu Lys Asn Glu Leu Asn Glu Asp Glu Ile Leu His Phe
 50 55 60

Val Val Ala Glu Ala Asp Asn Ile Val Thr Leu Gln Pro Phe Arg Asp
 65 70 75 80

Lys Cys Glu Pro Val Phe Leu Phe Ser Leu Asn Gly Lys Ile Ile Ala
 85 90 95

Lys Ile Gln Gly Ala Asn Ala Pro Leu Ile Asn Arg Lys Val Ile Thr
 100 105 110

Leu Ile Asp Glu Glu Arg Lys Ile Val Ala Gly Glu Met Asp Arg Pro
 115 120 125

Gln Tyr Val Glu Ile Pro Leu Val Asp Ala Ile Asp Glu Glu Tyr Gly
 130 135 140

Glu Val Gln Tyr Glu Ser Ala Ala Glu Val Tyr Asn Met Ala Ile Ile
 145 150 155 160

Lys Pro Asp Ala Val Leu Met Arg Lys Asn Ile Glu Val Arg Glu Lys
 165 170 175

Ile Ala Lys Glu Gly Phe Val Ile Glu Ile Gln Glu Asn Leu Ile Leu
 180 185 190

Pro Glu Glu Val Val Arg Glu Phe Tyr Thr His Ile Ala Asp Gln Pro
 195 200 205

Asp Phe Glu Glu Phe Val Val Ser Met Thr Asn Gly Leu Ser Cys Val
 210 215 220

Leu Ile Val Ser Gln Glu Asp Ser Glu Val Ile Gln Glu Glu Thr Leu
 225 230 235 240
 Pro Gln Thr Asp Thr Glu Glu Glu Pro Gly Val Leu Glu Glu Pro His
 245 250 255
 Val Arg Phe Ala Pro Val Met Ile Lys Lys Lys Arg Asp Ser Leu Gln
 260 265 270
 Glu Tyr Met Asp Arg Gln His Met Ser Asp Tyr Cys Asp Val Glu Asp
 275 280 285
 Asp Ala Val Lys Val Ser Lys Leu Ile Asp Ile Leu Phe Pro Asp Phe
 290 295 300
 Lys Thr Met Lys Ser Thr Asn Val Gln Thr Thr Leu Ala Leu Leu His
 305 310 315 320
 Pro Asp Ile Cys Glu Glu Glu Lys Asp Asp Val Leu Asn Val Ile His
 325 330 335
 Asn Glu Gly Phe Thr Ile Leu Met Gln Arg Gln Ile Val Leu Ser Glu
 340 345 350
 Glu Glu Ala Arg Thr Val Cys Lys Ile His Glu Asn Glu Glu Tyr Phe
 355 360 365
 Asp Asn Leu Ile Gly His Met Thr Ser Asn His Ser Tyr Val Leu Ala
 370 375 380
 Leu Arg Arg Glu Asn Gly Val Glu Tyr Trp Lys Thr Leu Ile Gly Pro
 385 390 395 400
 Lys Thr Ile Glu Glu Ala Tyr Ala Ser His Pro Gln Ser Leu Cys Val
 405 410 415
 Gln Phe Ala Ser Gly Asn Phe Pro Thr Asn Gln Phe Tyr Gly Ser Ser
 420 425 430
 Ser Lys Ala Ala Ala Glu Lys Glu Ile Ala His Phe Phe Pro Pro Gln
 435 440 445
 Ser Thr Leu Ala Leu Ile Lys Pro His Val Thr His Lys Glu Arg Met
 450 455 460
 Glu Ile Leu Lys Thr Ile Lys Glu Ala Gly Phe Glu Leu Thr Leu Met
 465 470 475 480
 Lys Glu Met His Leu Thr Pro Glu His Ala Asn Lys Ile Tyr Phe Lys
 485 490 495

Ile Thr Gly Lys Asp Phe Tyr Lys Asn Val Leu Glu Val Leu Ser Leu
500 505 510

Gly Met Ser Leu Val Met Val Leu Thr Lys Trp Asn Ala Val Ala Glu
515 520 525

Trp Arg Arg Met Val Gly Pro Val Asp Pro Glu Glu Ala Lys Leu Leu
530 535 540

Ser Pro Glu Ser Leu Arg Ala Lys Tyr Gly Leu Asp Ile Leu Arg Asn
545 550 555 560

Ala Val His Gly Ala Ser Asn Phe Ser Glu Ala Ser Glu Ile Ile Ser
565 570 575

Asn Val Phe Thr Glu Gly Asn Pro Glu Asn
580 585

<210> 42
<211> 1416
<212> DNA
<213> Artificial Sequence

<220>
<223> Mouse NME8 variant2

<400> 42
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gatgagatgt tgctgaacaa aggcttaaca gtgattgatg ttaccaagc ctggtgtgga 120
ccttgcaaag ccgtgcaaag ttatttcaga aaactaaaa atgaactgaa cgaagatgag 180
attcttcact tcgtcgttgc tgaagctgac aacattgtga ctctccagcc atttagagat 240
aaatgtgagc ccgtgtttct ctttagtctt aatggtaaaa tcattgcaaa gattcagggt 300
gcaaatgctc cacttatcaa tagaaaagtc attaccttga tagatgaaga gaggaaaatt 360
gtagcagggtg aaatggatcg tcctcagtat gttgaaattc cactagtaga tgcaatcgat 420
gaagaatatg gggaagtaca gtatgaaagt gctgcggaag ttacaatat ggcaattatc 480
aaacctgatg ctgtactcat gagaaaaaat atagaagtta gggaaaaaat agccaaagaa 540
ggatttgtaa tagaaataca agaaaacctg attctccctg aagaggtagt gagggaaattc 600
tacactcata tagcagacca gcctgacttt gaagagtttg tcgtttctat gacaaatggc 660
ctcagctgtg tgctcattgt atctcaagaa gactccgagg ttattcagga agaaactctc 720
ccgcagactg atacagaaga agaacctggc gttttggaag agcctcacgt taggtttgca 780
cctgtgatga taaagaagaa acgggacagt ttgcaagagt atatggaccg acagcatatg 840
tctgattact gcgatgtcga ggacgatgcg gttaagggtct ctaagctcat tgacatatta 900
ttccctgatt ttaaaactat gaaaagcacg aatgtacaaa cgacgctagc attactgcat 960
ccagacatct gtgaggaaga gaaagatgac gtgttgaacg ttattcacia tgaagggttc 1020
accatactga tgcagaggca aatcgtatta tcagaggaag aagcaagaac agtgtgcaag 1080

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atccatgaaa acgaagagta ttttgataat cttatagggc acatgaccag taatcactct      1140
tatgtccttg ctctacggag ggaaaatggg gtggaatatt ggaaaacatt aattgggcca      1200
aaaacgattg aggaagctta tgcattctcat ccacagagtt tatgtgtaca gtttgcttca      1260
gggaattttc ctaccaacca gttctacggg agcagttcaa aagcagcagc tgagaaggaa      1320
atagcgcatt tcttccctcc ccagagcaca cttgcattga tcaagcctca tgtgacacac      1380
aaagaaagaa ttcacagaag ctcaaggagg taatga                                1416

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<210> 43
<211> 470
<212> PRT
<213> Artificial sequence

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<220>
<223> Mouse NME8 variant2

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<400> 43

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Met Ala Ser Lys Lys Arg Glu Val Gln Leu Gln Ser Val Val Asn Ser
1          5          10          15

```

```

Gln Asn Leu Trp Asp Glu Met Leu Leu Asn Lys Gly Leu Thr Val Ile
20          25          30

```

```

Asp Val Tyr Gln Ala Trp Cys Gly Pro Cys Lys Ala Val Gln Ser Leu
35          40          45

```

```

Phe Arg Lys Leu Lys Asn Glu Leu Asn Glu Asp Glu Ile Leu His Phe
50          55          60

```

```

Val Val Ala Glu Ala Asp Asn Ile Val Thr Leu Gln Pro Phe Arg Asp
65          70          75          80

```

```

Lys Cys Glu Pro Val Phe Leu Phe Ser Leu Asn Gly Lys Ile Ile Ala
85          90          95

```

```

Lys Ile Gln Gly Ala Asn Ala Pro Leu Ile Asn Arg Lys Val Ile Thr
100         105         110

```

```

Leu Ile Asp Glu Glu Arg Lys Ile Val Ala Gly Glu Met Asp Arg Pro
115         120         125

```

```

Gln Tyr Val Glu Ile Pro Leu Val Asp Ala Ile Asp Glu Glu Tyr Gly
130         135         140

```

```

Glu Val Gln Tyr Glu Ser Ala Ala Glu Val Tyr Asn Met Ala Ile Ile
145         150         155         160

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```

Lys Pro Asp Ala Val Leu Met Arg Lys Asn Ile Glu Val Arg Glu Lys
165         170         175

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Ile Ala Lys Glu Gly Phe Val Ile Glu Ile Gln Glu Asn Leu Ile Leu
 180 185 190

Pro Glu Glu Val Val Arg Glu Phe Tyr Thr His Ile Ala Asp Gln Pro
 195 200 205

Asp Phe Glu Glu Phe Val Val Ser Met Thr Asn Gly Leu Ser Cys Val
 210 215 220

Leu Ile Val Ser Gln Glu Asp Ser Glu Val Ile Gln Glu Glu Thr Leu
 225 230 235 240

Pro Gln Thr Asp Thr Glu Glu Glu Pro Gly Val Leu Glu Glu Pro His
 245 250 255

Val Arg Phe Ala Pro Val Met Ile Lys Lys Lys Arg Asp Ser Leu Gln
 260 265 270

Glu Tyr Met Asp Arg Gln His Met Ser Asp Tyr Cys Asp Val Glu Asp
 275 280 285

Asp Ala Val Lys Val Ser Lys Leu Ile Asp Ile Leu Phe Pro Asp Phe
 290 295 300

Lys Thr Met Lys Ser Thr Asn Val Gln Thr Thr Leu Ala Leu Leu His
 305 310 315 320

Pro Asp Ile Cys Glu Glu Glu Lys Asp Asp Val Leu Asn Val Ile His
 325 330 335

Asn Glu Gly Phe Thr Ile Leu Met Gln Arg Gln Ile Val Leu Ser Glu
 340 345 350

Glu Glu Ala Arg Thr Val Cys Lys Ile His Glu Asn Glu Glu Tyr Phe
 355 360 365

Asp Asn Leu Ile Gly His Met Thr Ser Asn His Ser Tyr Val Leu Ala
 370 375 380

Leu Arg Arg Glu Asn Gly Val Glu Tyr Trp Lys Thr Leu Ile Gly Pro
 385 390 395 400

Lys Thr Ile Glu Glu Ala Tyr Ala Ser His Pro Gln Ser Leu Cys Val
 405 410 415

Gln Phe Ala Ser Gly Asn Phe Pro Thr Asn Gln Phe Tyr Gly Ser Ser
 420 425 430

Ser Lys Ala Ala Ala Glu Lys Glu Ile Ala His Phe Phe Pro Pro Gln
 435 440 445

Ser Thr Leu Ala Leu Ile Lys Pro His Val Thr His Lys Glu Arg Ile
 450 455 460

His Arg Ser Ser Arg Arg
 465 470

<210> 44
 <211> 1767
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Human NME8

<400> 44
 atggcaagca aaaaacgaga agtccagtta cagacagtca tcaataatca aagcctgtgg 60
 gatgagatgt tgcagaacaa aggcttaaca gtgattgatg tttaccaagc ctggtgtgga 120
 ccttgacagag caatgcaacc tttattcaga aaattgaaaa atgaactgaa cgaagacgaa 180
 attctgcatt ttgctgtcgc agaagctgac aacattgtga ctttgacagcc atttagagat 240
 aaatgtgaac ctgtttttct ctttagtggt aatggcaaaa ttatcgaaaa gattcagggt 300
 gcaaatgcac cgcttgtaa taaaaaagtt attaatttga tcgatgagga gagaaaaatt 360
 gcagcagggtg aaatggctcg acctcagtat cctgaaattc cattagtaga ctcagattca 420
 gaagttagtg aagaatcacc atgtgaaagt gttcaggaat tatacagtat tgctattatc 480
 aaaccggatg ctgtgattag taaaaaagtt ctagaaatta aaagaaaaat taccaaagct 540
 ggatttatta tagaagcaga gcataagaca gtgctcactg aagaacaagt tgtcaacttc 600
 tatagtcgaa tagcagacca gcgtgacttc gaagagtttg tctcttttat gacaagtggc 660
 ttaagctata ttctagttgt atctcaagga agtaaacaca atcctccctc tgaagaaacc 720
 gaaccacaga ctgacaccga acctaacgaa cgatctgagg atcaacctga ggtcgaagcc 780
 caggttacac ctggaatgat gaagaacaaa caagacagtt tacaagaata tctggaaaga 840
 caacatttag ctcagctctg tgacattgaa gaggatgcag ctaatgttgc taagttcatg 900
 gatgctttct tccccgattt taaaaaatg aaaagcatga aattagaaaa gacattggca 960
 ttacttcgac caaatctctt tcatgaaagg aaagatgatg ttttgcgtat tattaaagat 1020
 gaagacttca aaatactgga gcaagacaaa gtagtattat cggaaaaaga agcacaagca 1080
 ctgtgcaagg aatatgaaaa tgaagactat tttaataaac ttatagaaaa catgaccagt 1140
 ggtccatctc tagcccttgt tttattgaga gacaatggct tgcaatactg gaaacaatta 1200
 ctgggaccaa gaactgttga agaagccatt gaatattttc cagagagttt atgtgcacag 1260
 tttgcgatgg acagtttgcc ggtcaaccag ttgtatggca gcgattcatt agaaaccgct 1320
 gaaagggaaa tacagcattt ctttcctctt caaagcactt taggcttgat taaacctcat 1380
 gcaacaagtg aacaaagaga gcagatcctg aagatagtta aggaggctgg atttgatctg 1440
 acacaggtga agaaaatggt cctaactcct gagcaaacgg agaaaattta tccaaaagta 1500
 acaggaaaag acttttataa agatttattg gaaatgttat ctgtgggtcc atctatggtc 1560

atgattctga ccaagtggaa tgctgttgca gaatggagac gattgatggg cccaacagac 1620
 ccagaagaag caaaattact ttcccctgac tccatccgag cccagtttgg aataagtaaa 1680
 ttgaaaaaca ttgtccatgg agcatctaac gcctatgaag caaaagaggt tgtaataga 1740
 ctctttgagg atcctgagga aaactaa 1767

<210> 45
 <211> 588
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Human NME8

<400> 45

Met Ala Ser Lys Lys Arg Glu Val Gln Leu Gln Thr Val Ile Asn Asn
 1 5 10 15

Gln Ser Leu Trp Asp Glu Met Leu Gln Asn Lys Gly Leu Thr Val Ile
 20 25 30

Asp Val Tyr Gln Ala Trp Cys Gly Pro Cys Arg Ala Met Gln Pro Leu
 35 40 45

Phe Arg Lys Leu Lys Asn Glu Leu Asn Glu Asp Glu Ile Leu His Phe
 50 55 60

Ala Val Ala Glu Ala Asp Asn Ile Val Thr Leu Gln Pro Phe Arg Asp
 65 70 75 80

Lys Cys Glu Pro Val Phe Leu Phe Ser Val Asn Gly Lys Ile Ile Glu
 85 90 95

Lys Ile Gln Gly Ala Asn Ala Pro Leu Val Asn Lys Lys Val Ile Asn
 100 105 110

Leu Ile Asp Glu Glu Arg Lys Ile Ala Ala Gly Glu Met Ala Arg Pro
 115 120 125

Gln Tyr Pro Glu Ile Pro Leu Val Asp Ser Asp Ser Glu Val Ser Glu
 130 135 140

Glu Ser Pro Cys Glu Ser Val Gln Glu Leu Tyr Ser Ile Ala Ile Ile
 145 150 155 160

Lys Pro Asp Ala Val Ile Ser Lys Lys Val Leu Glu Ile Lys Arg Lys
 165 170 175

Ile Thr Lys Ala Gly Phe Ile Ile Glu Ala Glu His Lys Thr Val Leu
 180 185 190

Thr Glu Glu Gln Val Val Asn Phe Tyr Ser Arg Ile Ala Asp Gln Arg
 195 200 205
 Asp Phe Glu Glu Phe Val Ser Phe Met Thr Ser Gly Leu Ser Tyr Ile
 210 215 220
 Leu Val Val Ser Gln Gly Ser Lys His Asn Pro Pro Ser Glu Glu Thr
 225 230 235 240
 Glu Pro Gln Thr Asp Thr Glu Pro Asn Glu Arg Ser Glu Asp Gln Pro
 245 250 255
 Glu Val Glu Ala Gln Val Thr Pro Gly Met Met Lys Asn Lys Gln Asp
 260 265 270
 Ser Leu Gln Glu Tyr Leu Glu Arg Gln His Leu Ala Gln Leu Cys Asp
 275 280 285
 Ile Glu Glu Asp Ala Ala Asn Val Ala Lys Phe Met Asp Ala Phe Phe
 290 295 300
 Pro Asp Phe Lys Lys Met Lys Ser Met Lys Leu Glu Lys Thr Leu Ala
 305 310 315 320
 Leu Leu Arg Pro Asn Leu Phe His Glu Arg Lys Asp Asp Val Leu Arg
 325 330 335
 Ile Ile Lys Asp Glu Asp Phe Lys Ile Leu Glu Gln Arg Gln Val Val
 340 345 350
 Leu Ser Glu Lys Glu Ala Gln Ala Leu Cys Lys Glu Tyr Glu Asn Glu
 355 360 365
 Asp Tyr Phe Asn Lys Leu Ile Glu Asn Met Thr Ser Gly Pro Ser Leu
 370 375 380
 Ala Leu Val Leu Leu Arg Asp Asn Gly Leu Gln Tyr Trp Lys Gln Leu
 385 390 395 400
 Leu Gly Pro Arg Thr Val Glu Glu Ala Ile Glu Tyr Phe Pro Glu Ser
 405 410 415
 Leu Cys Ala Gln Phe Ala Met Asp Ser Leu Pro Val Asn Gln Leu Tyr
 420 425 430
 Gly Ser Asp Ser Leu Glu Thr Ala Glu Arg Glu Ile Gln His Phe Phe
 435 440 445
 Pro Leu Gln Ser Thr Leu Gly Leu Ile Lys Pro His Ala Thr Ser Glu
 450 455 460

Gln Arg Glu Gln Ile Leu Lys Ile Val Lys Glu Ala Gly Phe Asp Leu
465 470 475 480

Thr Gln Val Lys Lys Met Phe Leu Thr Pro Glu Gln Thr Glu Lys Ile
485 490 495

Tyr Pro Lys Val Thr Gly Lys Asp Phe Tyr Lys Asp Leu Leu Glu Met
500 505 510

Leu Ser Val Gly Pro Ser Met Val Met Ile Leu Thr Lys Trp Asn Ala
515 520 525

Val Ala Glu Trp Arg Arg Leu Met Gly Pro Thr Asp Pro Glu Glu Ala
530 535 540

Lys Leu Leu Ser Pro Asp Ser Ile Arg Ala Gln Phe Gly Ile Ser Lys
545 550 555 560

Leu Lys Asn Ile Val His Gly Ala Ser Asn Ala Tyr Glu Ala Lys Glu
565 570 575

Val Val Asn Arg Leu Phe Glu Asp Pro Glu Glu Asn
580 585

<210> 46
<211> 1458
<212> DNA
<213> Artificial Sequence

<220>
<223> NME8 E. coli optimized

<400> 46
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gaaatggcac gcccgaata cccggaaatc ccgctgggtg atagcgactc tgaagtttca 120
gaagaatcgc cgtgcgaatc agtgcaggaa ctgtattcga tcgcaattat caaaccggat 180
gctgtcattt ccaaaaaagt gctggaaatc aaacgtaaaa tcaccaaagc gggtttcatt 240
atcgaagccg aacataaaac cgtgctgacg gaagaacagg tggttaattt ttattcacgt 300
atcgcggatc agcgcgactt tgaagaattt gtttcgttta tgaccagcgg cctgtcttac 360
attctggtcg tgagtcaggg ttccaaacac aatccgccga gcgaagaaac ggaaccgcag 420
accgatacgg aaccgaacga acgttctgaa gaccagccgg aagtggaagc acaagttacc 480
ccgggcatga tgaaaaataa acaggatagt ctgcaagaat acctggaacg ccagcatctg 540
gctcaactgt gtgatatcga agaagacgcg gccaacgtgg cgaaattcat ggatgccttt 600
ttcccggact tcaagaaaat gaaaagcatg aaactggaaa aaaccctggc cctgctgcgt 660
ccgaacctgt tccacgaacg taaagatgac gttctgcgca tcatcaaaga tgaagacttc 720
aaaatcctgg aacagcgcca agttgtcctg tctgaaaaag aagcacaggc tctgtgcaaa 780
gaatacgaaa acgaagatta cttcaacaaa ctgatcgaaa acatgacctc aggtccgtcg 840

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ctggcactgg ttctgctgcg tgataatggc ctgcagtatt ggaaacaact gctgggtccg      900
cgcacggtcg aagaagccat tgaatacttc ccggaaaagcc tgtgtgcaca gtttgctatg      960
gatttctctgc cgggtgaacca actgtatggc agtgactccc tggaaaccgc ggaacgtgaa    1020
atccagcatt tctttccgct gcaaagtacc ctgggtctga ttaaaccgca cgcgacgtcc    1080
gaacagcgcg aacaaattct gaaaatcgtc aaagaagccg gcttcgatct gacccagggtg    1140
aagaaaatgt ttctgacccc ggaacaaacg gaaaaaatct atccgaaagt cacgggcaaa    1200
gatttctaca aagacctgct ggaaatgctg agtgttggtc cgtccatggt catgattctg    1260
accaaatgga atgcggttgc agaatggcgt cgcctgatgg gtccgacgga tccggaagaa    1320
gcaaaactgc tgagcccgga ctctattcgc gctcagtttg gcatcagcaa actgaaaaac    1380
attgttcatg gtgcggtcaa tgcgtatgaa gcgaaagaag ttgtgaaccg cctgtttgaa    1440
gacccggaag aaaattaa                                     1458

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<210> 47
<211> 485
<212> PRT
<213> Artificial Sequence

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<220>
<223> NME8 E. coli optimized

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<400> 47

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Met Leu Val Asn Lys Lys Val Ile Asn Leu Ile Asp Glu Glu Arg Lys
1           5           10          15

```

```

Ile Ala Ala Gly Glu Met Ala Arg Pro Gln Tyr Pro Glu Ile Pro Leu
          20          25          30

```

```

Val Asp Ser Asp Ser Glu Val Ser Glu Glu Ser Pro Cys Glu Ser Val
          35          40          45

```

```

Gln Glu Leu Tyr Ser Ile Ala Ile Ile Lys Pro Asp Ala Val Ile Ser
          50          55          60

```

```

Lys Lys Val Leu Glu Ile Lys Arg Lys Ile Thr Lys Ala Gly Phe Ile
65          70          75          80

```

```

Ile Glu Ala Glu His Lys Thr Val Leu Thr Glu Glu Gln Val Val Asn
          85          90          95

```

```

Phe Tyr Ser Arg Ile Ala Asp Gln Arg Asp Phe Glu Glu Phe Val Ser
          100         105         110

```

```

Phe Met Thr Ser Gly Leu Ser Tyr Ile Leu Val Val Ser Gln Gly Ser
          115         120         125

```

```

Lys His Asn Pro Pro Ser Glu Glu Thr Glu Pro Gln Thr Asp Thr Glu
130          135          140

```

Pro Asn Glu Arg Ser Glu Asp Gln Pro Glu Val Glu Ala Gln Val Thr
145 150 155 160

Pro Gly Met Met Lys Asn Lys Gln Asp Ser Leu Gln Glu Tyr Leu Glu
165 170 175

Arg Gln His Leu Ala Gln Leu Cys Asp Ile Glu Glu Asp Ala Ala Asn
180 185 190

Val Ala Lys Phe Met Asp Ala Phe Phe Pro Asp Phe Lys Lys Met Lys
195 200 205

Ser Met Lys Leu Glu Lys Thr Leu Ala Leu Leu Arg Pro Asn Leu Phe
210 215 220

His Glu Arg Lys Asp Asp Val Leu Arg Ile Ile Lys Asp Glu Asp Phe
225 230 235 240

Lys Ile Leu Glu Gln Arg Gln Val Val Leu Ser Glu Lys Glu Ala Gln
245 250 255

Ala Leu Cys Lys Glu Tyr Glu Asn Glu Asp Tyr Phe Asn Lys Leu Ile
260 265 270

Glu Asn Met Thr Ser Gly Pro Ser Leu Ala Leu Val Leu Leu Arg Asp
275 280 285

Asn Gly Leu Gln Tyr Trp Lys Gln Leu Leu Gly Pro Arg Thr Val Glu
290 295 300

Glu Ala Ile Glu Tyr Phe Pro Glu Ser Leu Cys Ala Gln Phe Ala Met
305 310 315 320

Asp Ser Leu Pro Val Asn Gln Leu Tyr Gly Ser Asp Ser Leu Glu Thr
325 330 335

Ala Glu Arg Glu Ile Gln His Phe Phe Pro Leu Gln Ser Thr Leu Gly
340 345 350

Leu Ile Lys Pro His Ala Thr Ser Glu Gln Arg Glu Gln Ile Leu Lys
355 360 365

Ile Val Lys Glu Ala Gly Phe Asp Leu Thr Gln Val Lys Lys Met Phe
370 375 380

Leu Thr Pro Glu Gln Thr Glu Lys Ile Tyr Pro Lys Val Thr Gly Lys
385 390 395 400

Asp Phe Tyr Lys Asp Leu Leu Glu Met Leu Ser Val Gly Pro Ser Met
405 410 415

Val Met Ile Leu Thr Lys Trp Asn Ala Val Ala Glu Trp Arg Arg Leu
420 425 430

Met Gly Pro Thr Asp Pro Glu Glu Ala Lys Leu Leu Ser Pro Asp Ser
435 440 445

Ile Arg Ala Gln Phe Gly Ile Ser Lys Leu Lys Asn Ile Val His Gly
450 455 460

Ala Ser Asn Ala Tyr Glu Ala Lys Glu Val Val Asn Arg Leu Phe Glu
465 470 475 480

Asp Pro Glu Glu Asn
485

<210> 48
<211> 1062
<212> DNA
<213> Artificial Sequence

<220>
<223> NME8 1-2 E. coli optimized

<400> 48
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gaagaatcgc cgtgcgaatc agtgcaggaa ctgtattcga tcgcaattat caaaccggat 180
gctgtcattt ccaaaaaagt gctggaaatc aaacgtaaaa tcaccaaagc gggtttcatt 240
atcgaagccg aacataaaac cgtgctgacg gaagaacagg tggttaattt ttattcacgt 300
atcgcggtatc agcgcgactt tgaagaattt gtttcgttta tgaccagcgg cctgtcttac 360
attctggtcg tgagtcaggg ttccaaacac aatccgccga gcgaagaaac ggaaccgcag 420
accgatacgg aaccgaacga acgttctgaa gaccagccgg aagtgggaagc acaagttacc 480
ccgggcatga tgaaaaataa acaggatagt ctgcaagaat acctggaacg ccagcatctg 540
gctcaactgt gtgatatcga agaagacgcg gccaacgtgg cgaaattcat ggatgccttt 600
ttcccggact tcaagaaaat gaaaagcatg aaactggaaa aaaccctggc cctgctgcgt 660
ccgaacctgt tccacgaacg taaagatgac gttctgcgca tcatcaaaga tgaagacttc 720
aaaatcctgg aacagcgcca agttgtcctg tctgaaaaag aagcacaggc tctgtgcaaa 780
gaatacgaaa acgaagatta cttcaacaaa ctgatcgaaa acatgacctc aggtccgtcg 840
ctggcactgg ttctgtgctg tgataatggc ctgcagtatt ggaaacaact gctgggtccg 900
cgcacggctg aagaagccat tgaatacttc ccggaaaagcc tgtgtgcaca gtttgctatg 960
gattctctgc cggatgaacca actgtatggc agtgactccc tggaaaccgc ggaacgtgaa 1020
atccagcatt tctttctcga gcaccaccac caccaccact ga 1062

<210> 49
 <211> 353
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> NME8 1-2 E. coli optimized

<400> 49

Met Leu Val Asn Lys Lys Val Ile Asn Leu Ile Asp Glu Glu Arg Lys
 1 5 10 15

Ile Ala Ala Gly Glu Met Ala Arg Pro Gln Tyr Pro Glu Ile Pro Leu
 20 25 30

Val Asp Ser Asp Ser Glu Val Ser Glu Glu Ser Pro Cys Glu Ser Val
 35 40 45

Gln Glu Leu Tyr Ser Ile Ala Ile Ile Lys Pro Asp Ala Val Ile Ser
 50 55 60

Lys Lys Val Leu Glu Ile Lys Arg Lys Ile Thr Lys Ala Gly Phe Ile
 65 70 75 80

Ile Glu Ala Glu His Lys Thr Val Leu Thr Glu Glu Gln Val Val Asn
 85 90 95

Phe Tyr Ser Arg Ile Ala Asp Gln Arg Asp Phe Glu Glu Phe Val Ser
 100 105 110

Phe Met Thr Ser Gly Leu Ser Tyr Ile Leu Val Val Ser Gln Gly Ser
 115 120 125

Lys His Asn Pro Pro Ser Glu Glu Thr Glu Pro Gln Thr Asp Thr Glu
 130 135 140

Pro Asn Glu Arg Ser Glu Asp Gln Pro Glu Val Glu Ala Gln Val Thr
 145 150 155 160

Pro Gly Met Met Lys Asn Lys Gln Asp Ser Leu Gln Glu Tyr Leu Glu
 165 170 175

Arg Gln His Leu Ala Gln Leu Cys Asp Ile Glu Glu Asp Ala Ala Asn
 180 185 190

Val Ala Lys Phe Met Asp Ala Phe Phe Pro Asp Phe Lys Lys Met Lys
 195 200 205

Ser Met Lys Leu Glu Lys Thr Leu Ala Leu Leu Arg Pro Asn Leu Phe
 210 215 220

His Glu Arg Lys Asp Asp Val Leu Arg Ile Ile Lys Asp Glu Asp Phe
 225 230 235 240

Lys Ile Leu Glu Gln Arg Gln Val Val Leu Ser Glu Lys Glu Ala Gln
245 250 255

Ala Leu Cys Lys Glu Tyr Glu Asn Glu Asp Tyr Phe Asn Lys Leu Ile
260 265 270

Glu Asn Met Thr Ser Gly Pro Ser Leu Ala Leu Val Leu Leu Arg Asp
275 280 285

Asn Gly Leu Gln Tyr Trp Lys Gln Leu Leu Gly Pro Arg Thr Val Glu
290 295 300

Glu Ala Ile Glu Tyr Phe Pro Glu Ser Leu Cys Ala Gln Phe Ala Met
305 310 315 320

Asp Ser Leu Pro Val Asn Gln Leu Tyr Gly Ser Asp Ser Leu Glu Thr
325 330 335

Ala Glu Arg Glu Ile Gln His Phe Phe Leu Glu His His His His His
340 345 350

His

<210> 50
<211> 852
<212> DNA
<213> Artificial sequence

<220>
<223> NME8 2-3 E coli optimized

<400> 50
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tctgaaaaag aagcacaggc tctgtgcaaa gaatacgaaa acgaagatta cttcaacaaa 180
ctgatcgaaa acatgacctc aggtccgtcg ctggcactgg ttctgctgcg tgataatggc 240
ctgcagtatt ggaaacaact gctgggtccg cgcacggctg aagaagccat tgaatacttc 300
ccggaaagcc tgtgtgcaca gtttgctatg gatttctctgc cggatgaacca actgtatggc 360
agtgactccc tggaaaccgc ggaacgtgaa atccagcatt tctttccgct gcaaagtacc 420
ctgggtctga ttaaaccgca cgcgacgtcc gaacagcgcg aacaaattct gaaaatcgtc 480
aaagaagccg gcttcgatct gaccaggtg aagaaaatgt ttctgacccc ggaacaaacg 540
gaaaaaatct atccgaaagt cacgggcaaa gatttctaca aagacctgct ggaaatgctg 600
agtgttggtc cgtccatggc catgattctg accaaatgga atgcggttgc agaatggcgt 660
cgctgatgg gtccgacgga tccggaagaa gcaaaactgc tgagcccgga ctctattcgc 720
gctcagtttg gcatcagcaa actgaaaaac attgttcattg gtgcgtccaa tgcgtatgaa 780

gcgaaagaag ttgtgaaccg cctgtttgaa gacccggaag aaaatctcga gcaccaccac 840
caccaccact ga 852

<210> 51
<211> 283
<212> PRT
<213> Artificial Sequence

<220>
<223> NME8 2-3 E coli optimized

<400> 51

Met Leu Glu Lys Thr Leu Ala Leu Leu Arg Pro Asn Leu Phe His Glu
1 5 10 15

Arg Lys Asp Asp Val Leu Arg Ile Ile Lys Asp Glu Asp Phe Lys Ile
20 25 30

Leu Glu Gln Arg Gln Val Val Leu Ser Glu Lys Glu Ala Gln Ala Leu
35 40 45

Cys Lys Glu Tyr Glu Asn Glu Asp Tyr Phe Asn Lys Leu Ile Glu Asn
50 55 60

Met Thr Ser Gly Pro Ser Leu Ala Leu Val Leu Leu Arg Asp Asn Gly
65 70 75 80

Leu Gln Tyr Trp Lys Gln Leu Leu Gly Pro Arg Thr Val Glu Glu Ala
85 90 95

Ile Glu Tyr Phe Pro Glu Ser Leu Cys Ala Gln Phe Ala Met Asp Ser
100 105 110

Leu Pro Val Asn Gln Leu Tyr Gly Ser Asp Ser Leu Glu Thr Ala Glu
115 120 125

Arg Glu Ile Gln His Phe Phe Pro Leu Gln Ser Thr Leu Gly Leu Ile
130 135 140

Lys Pro His Ala Thr Ser Glu Gln Arg Glu Gln Ile Leu Lys Ile Val
145 150 155 160

Lys Glu Ala Gly Phe Asp Leu Thr Gln Val Lys Lys Met Phe Leu Thr
165 170 175

Pro Glu Gln Thr Glu Lys Ile Tyr Pro Lys Val Thr Gly Lys Asp Phe
180 185 190

Tyr Lys Asp Leu Leu Glu Met Leu Ser Val Gly Pro Ser Met Val Met
195 200 205

Ile Leu Thr Lys Trp Asn Ala Val Ala Glu Trp Arg Arg Leu Met Gly
 210 215 220

Pro Thr Asp Pro Glu Glu Ala Lys Leu Leu Ser Pro Asp Ser Ile Arg
 225 230 235 240

Ala Gln Phe Gly Ile Ser Lys Leu Lys Asn Ile Val His Gly Ala Ser
 245 250 255

Asn Ala Tyr Glu Ala Lys Glu Val Val Asn Arg Leu Phe Glu Asp Pro
 260 265 270

Glu Glu Asn Leu Glu His His His His His His
 275 280