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(54) Title: MYOMEGALIN VARIANT 8 AND USES THEREOF

(57) Abstract: The present invention provides a novel myomegalin isoform - myomegalin variant 8 (MMG8). The myomegalin variant 8 regulates microtubule organization at the Golgi apparatus, protein modification, secretion and trafficking, and cell proliferation. The present invention also provides nucleic acid molecules encoding the myomegalin isoforms, and vectors and host cells containing the nucleic acid molecules. Also provided are fusion constructs comprising the myomegalin isoform and antibodies that bind specifically to the myomegalin isoforms of the present invention. The present invention further provides uses of the myomegalin isoform as a diagnostic biomarker and as a target for screening for therapeutics for diseases such as cancer, diabetes, and lysosomal storage diseases.



MYOMEGALIN VARIANT 8 AND USES THEREOF

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CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application Serial No. 61/344,927, filed November 19, 2010, which is hereby incorporated by reference in its entirety, including all figures, tables and drawings.

10

BACKGROUND OF THE INVENTION

The Golgi apparatus is a membranous organelle that plays a pivotal role in protein post-translational modification, sorting, and transport. In interphase animal cells, the Golgi apparatus manifests a crescent moon shaped, ribbon-like morphology in the perinuclear region, normally situated closely with centrosomes.

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Microtubule cytoskeleton plays an essential role in the organization, positioning, and function of the Golgi apparatus (Rios and Bornens, *Curr. Opin. Cell Biol.* 15:60-66 (2003); Sutterlin and Colanzi, *J. Cell Biol.* 188:621-628 (2010); Lippincott-Schwartz, *Curr. Opin. Cell Biol.* 10:52-59 (1998)). Microtubule depolymerization causes severe Golgi defects, such as endoplasmic reticulum (ER)-to-Golgi transport blockage and Golgi fragmentation (Cole *et al.*, *Mol. Biol. Cell.* 7:631-650 (1996); Miller *et al.*, *Nat. Cell Biol.* 11:1069-1080 (2009)). The Golgi apparatus undergoes disassembly and reassembly during mitosis. During reassembly, microtubules derived from the Golgi apparatus and centrosomes facilitate the Golgi apparatus to form into a continuous ribbon structure with the central cellular positioning from Golgi ministacks (Miller *et al.*, *Nat. Cell Biol.* 11:1069-1080 (2009)).

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The Golgi apparatus serves as a major microtubule-organizing center (Chabin-Brion *et al.*, *Mol. Biol. Cell.* 12:2047-2060 (2001); Efimov *et al.*, *Dev. Cell.* 12:917-930 (2007); Miller *et al.*, *Nat. Cell Biol.* 11:1069-1080 (2009); Rivero *et al.*, *EMBO J.* 28:1016-1028 (2009)). In humans, almost half of cellular microtubules originate from the Golgi apparatus as observed in retinal pigment epithelial cells RPE1 (Efimov *et al.*, *Dev. Cell.* 12:917-930 (2007)). Golgi-associated microtubules are also required for Golgi ribbon assembly, directional trafficking, and cell motility (Miller *et al.*, *Nat. Cell Biol.* 11:1069-1080 (2009); Rivero *et al.*, *EMBO J.* 28:1016-1028 (2009)). Microtubule nucleation at the Golgi apparatus

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does not require centrosomes; rather, it depends on γ -tubulin (Efimov *et al.*, *Dev. Cell.* 12:917-930 (2007)), the principal microtubule nucleator that exists as γ -tubulin complexes (γ TuCs). γ TuCs associate with two *cis*-Golgi proteins, GMAP-210 and AKAP450 (AKAP450 is also known as AKAP350, CG-NAP, and hyperion) (Rios *et al.*, *Cell* 118:323-335 (2004);
5 Rivero *et al.*, *EMBO J.* 28:1016-1028 (2009); Takahashi *et al.*, *Mol. Biol. Cell* 13:3235-3245 (2002)). It is reported that silencing AKAP450 expression blocks the Golgi-associated microtubule nucleation (Rivero *et al.*, *EMBO J.* 28:1016-1028 (2009)).

Golgi-nucleated microtubules are required for post-Golgi secretion and directional cell migration. In the secretory pathway, protein transport from the ER to the Golgi apparatus
10 is initiated by cargo packaging into COPII-coated vesicles at ER exit sites, followed by the formation of vesicular-tubular clusters or the ER-Golgi intermediate compartment that move along microtubules towards the Golgi apparatus. Defects of protein secretion can cause a number of diseases, including neuronal disorders and diabetes. In addition, as microtubules are involved in cell migration, suppression of microtubule nucleation may be used to inhibit
15 tumor metastasis.

EB1 is the prototypic member of EB proteins, which are localized to microtubules and track growing microtubule plus-ends (Akhmanova and Steinmetz, *Nat. Rev. Mol. Cell Biol.* 9:309-322 (2008); Vaughan, *J. Cell Biol.* 171:197-200 (2005)). Among the EB proteins, EB1 and EB3 display similar tip-tracking properties, whereas EB2 appears to be distinct from the
20 other two, exhibiting a considerably weaker tip-tracking activity (Komarova *et al.*, *J. Cell Biol.* 184:691-706 (2009)).

EB1 is found on all growing microtubule tips, where it acts as a key component of plus-end protein complexes through its interaction with various plus-end tracking proteins (+TIPs). Many +TIPs contain the SxIP motif surrounded by basic and serine-rich sequences
25 for interaction with the EBH domain of EB1 (Honnappa *et al.*, *Cell* 138:366-376 (2009)). This SxIP-EB1 interaction relieves EB1 autoinhibition, and is required for EB1 in its action for promoting microtubule polymerization (Honnappa *et al.*, *EMBO J.* 24:261-269 (2005); Honnappa *et al.*, *Cell* 138:366-376 (2009); Slep *et al.*, *J. Cell Biol.* 168:587-598 (2005)). +TIPs in association with EB1 at the microtubule plus-ends have diverse functions, including
30 regulation of microtubule dynamics and microtubule attachment to subcellular targets (Akhmanova and Steinmetz, *Nat. Rev. Mol. Cell Biol.* 9:309-322 (2008)).

In a yeast two-hybrid screen, myomegalin (MMG) isoform 1 (Genbank accession: NP_055459) was cloned to interact with cyclic nucleotide phosphodiesterase 4D, and was identified as a phosphodiesterase 4D-interacting protein (Verde et al., *J. Biol. Chem.* 276:11189-11198 (2001)). The MMG1 nucleotide sequence encodes a ~230 kDa protein that is highly expressed in heart and skeletal muscles. In gene databases, MMG1 is the only known human homolog of CDK5RAP2 (CDK5 regulatory subunit-associated protein 2), a human microcephaly-related protein involved in microtubule organization in centrosomes as well as microtubule regulation at the growing tips (Fong et al., *Mol. Biol. Cell.* 19:115-125 (2008); Fong et al., *Mol. Biol. Cell.* 20:3660-3670 (2009)). MMG isoform 1 displays centrosome and Golgi-localizing patterns similar to CDK5RAP2 (Verde et al., *J. Biol. Chem.* 276:11189-11198 (2001); Wang et al., *J. Biol. Chem.* 285:22658-22665 (2010)).

Despite the apparent importance of the Golgi apparatus in various cellular activities such as protein modification and trafficking, microtubule organization, and cell migration, the precise mechanisms of how the Golgi apparatus exerts these functions have not been fully elucidated. Specifically, it remains unclear how γ TuCs are targeted to the Golgi apparatus. In addition, it remains unclear whether EB1 and other EB members are present at the Golgi apparatus to exert their functions. As will be clear from the disclosures that follow, these and other benefits are provided by the present invention.

SUMMARY OF THE INVENTION

The present invention provides novel myomegalin (MMG) isoforms, nucleic acid molecules encoding the myomegalin isoforms, fusion constructs comprising the myomegalin isoforms, and antibodies that bind specifically to the myomegalin isoforms. The myomegalin isoforms regulate microtubule organization and nucleation at the Golgi apparatus; protein modification, secretion, and transport; and cell proliferation.

In one embodiment, the present invention provides a myomegalin isoform, myomegalin variant 8 (MMG8), comprising SEQ ID NO:2. The present invention also provides MMG8 variants. In one embodiment, the MMG8 variant comprises amino acids 1-389 of SEQ ID NO:2. In some embodiments, the MMG8 variant comprises a polypeptide comprising amino acids 1-389 of SEQ ID NO:2, wherein the carboxy-terminus comprises amino acids 1098-1116 of SEQ ID NO:2 or a contiguous fragment of amino acids 1098-1116 of SEQ ID NO:2.

In one embodiment, the present invention provides a nucleic acid molecule encoding MMG8 comprising SEQ ID NO:2, or a fragment thereof, or any complement thereof. In an embodiment, the nucleic acid molecule comprises SEQ ID NO:1. Also provided are vectors, expression constructs, and host cells comprising the nucleic acid molecules of the invention.

5 In one embodiment, the present invention provides fusion proteins comprising MMG8 or an MMG8 variant, or a fragment thereof, a second protein, and optionally, a linker sequence that links the MMG8, MMG8 variant or fragment thereof to the second protein.

In one embodiment, the present invention provides antibodies that bind specifically to MMG8 comprising SEQ ID NO:2. In a specific embodiment, the antibody of the present
10 invention binds specifically to an epitope comprising 926-1116 of SEQ ID NO:2, or an epitope comprising a fragment of 926-1116 of SEQ ID NO:2. In a preferred embodiment, the anti-MMG8 antibody is a humanized antibody.

The present invention also provides uses of the myomegalin isoform as a diagnostic biomarker and as a target for screening for therapeutic agents for lysosomal storage diseases,
15 diabetes, and cancer. Also provided are methods for treating diseases associated with myomegalin by modulating the expression or activity of the myomegalin isoform of the invention.

BRIEF DESCRIPTION OF THE FIGURES

20 **Figure 1** shows that MMG8, a novel MMG isoform that localizes to *cis*-Golgi networks, is required for maintaining Golgi integrity. (A) An RT-PCR reaction was performed using oligonucleotide primers targeting a region encoding 474-762 of human MMG isoform 1. This targeted region is present in all currently-identified large sequences of MMG variants. The total RNA was extracted from HeLa cells. In the schematic
25 representation, the region amplified in the RT-PCR and those targeted by the antibodies are labeled. The hatched region corresponds to amino acids 474-762 of MMG isoform 1 and 637-925 of MMG8. M, DNA Marker. (B) HeLa extracts resolved by SDS-PAGE (6% and 15% gels) were probed with the anti-MMG8 antibody 443M or the preimmune serum (PS). (C) Immunoprecipitations (IPs) were performed with 443M and nonspecific IgG. The
30 immunoprecipitated proteins were resolved by SDS-PAGE for anti-MMG8 immunoblotting or Coomassie Blue staining. The protein of ~150 kDa was excised for sequencing by mass spectrometry. (D) MMG expression was examined in cell cultures by immunoblotting using

443M. (E) A schematic representation of the MMG variants. Identical sequences are labelled in same pattern. P1-13 denote peptides sequenced by mass spectrometry. (F) Immunoblotting of HeLa cells transfected with control or MMG8-targeting siRNAs. The transfection of si-MMG8-1 or si-MMG8-2 suppressed the expression of MMG8 by ~85%. (G) HeLa cells transfected with siRNAs were analyzed by immunofluorescence microscopy. Scale bars, 5 μ m.

Figure 2 shows that MMG8 interacts with AKAP450. (A) Anti-MMG8 and anti-AKAP450 immunoprecipitations (IPs) were performed with HeLa extracts. The immunoprecipitated proteins and inputs were analyzed by immunoblotting and quantified. The histogram shows the amount of the precipitated proteins relative to that of the respective inputs. Data shown are means $\pm$ S.D. from three independent experiments. (B-C) HEK293T cells expressing various MMG8 constructs containing a FLAG tag were subjected to anti-FLAG immunoprecipitation. The immunoprecipitates and inputs were immunoblotted (IB) for the MMG8 fragments (anti-FLAG) and AKAP450 (B) or the RII subunit of protein kinase A (C). (D) MMG8 fragments were transiently expressed in HeLa cells. The cells were stained for endogenous MMG8 and the Golgi markers as labeled. The arrow indicates a transfected cell. Boxed areas are enlarged. Scale bars, 5 μ m.

Figure 3 shows that MMG8 and AKAP450 are mutually dependent for stability. (A) hTERT-RPE1 cells were transfected with siRNAs as labeled. The cell extracts were examined by immunoblotting. After quantification, the amounts of MMG8 and AKAP450 in the siMMG8 or siAKAP450-transfected extracts were expressed as percentages of the respective amounts in the control extracts. Data from three independent experiments are shown. (B) Cells transfected with siRNAs were treated with MG132 at 72 h post-transfection. After treatment, the cells were analyzed on immunoblots for MMG8, AKAP450, and α -tubulin. The amounts of MMG8 and AKAP450 were plotted with quantification data of three independent experiments.

Figure 4 shows that MMG8 associates with γ -tubulin complexes at the Golgi apparatus. (A-B) anti-MMG8 (A) and anti-GCP3 (B) immunoprecipitations were performed with HeLa extracts. The immunoprecipitates and inputs were examined on immunoblots. (C) Cells transfected with siRNAs were subjected to immunostaining of γ -tubulin and mannosidase II (Man II). Scale bars, 5 μ m.

Figure 5 shows that MMG8 is required for microtubule nucleation at the Golgi apparatus. (A) hTERT-RPE1 cells were transfected with siRNAs. After nocodazole depolymerization of microtubules, microtubule regrowth was initiated by nocodazole washout. The cells were then stained for MMG8, TGN46, and microtubules (anti- α -tubulin). (B) Immunofluorescence micrographs of cells transfected with siRNAs. Boxed areas are enlarged. Scale bars, 5 μ m.

Figure 6 shows that MMG8 binds to EB1 and EB3, and mediates EB1 recruitment to the Golgi apparatus. (A) Co-immunoprecipitation of EB1 with MMG8. After anti-MMG8 immunoprecipitation, the immunoprecipitates and inputs were examined on immunoblots. (B-C) The full-length MMG8 (B) and its fragment 1-389 (C) were ectopically expressed with a FLAG tag. After anti-FLAG immunoprecipitation, the immunoprecipitates and inputs were probed on immunoblots. WT, MMG8 wild-type; 311/2A, MMG8(L311A/P312A). (D) HEK293T was double transfected with GFP-EB3 and a FLAG-tagged construct of MMG8 or its L311A/P312A mutant (311/2A). After anti-FLAG immunoprecipitation, the precipitated proteins and inputs were analyzed for EB3 (anti-GFP) and MMG8 (anti-FLAG). Vec, FLAG vector; WT, wild-type MMG8; 311/2A, MMG8(L311A/P312A) (E) HeLa cells transfected with siRNAs were triple stained for MMG8, EB1, and TGN46. Scale bars, 5 μ m.

Figure 7 shows that EB1 mediates the interaction between MMG8 and microtubules. Microtubules were polymerized with taxol in HEK293T extracts expressing GFP or GFP-EB1(185-268). Taxol was absent in control (Ctrl). After microtubule sedimentation, the supernatants and pellets were probed for MMG8, α -tubulin and the GFP proteins. GFP-EB1C, GFP-EB1(185-268).

Figure 8 shows that MMG8 is required for cell proliferation. HeLa cells were transfected with control siRNA or MMG8-targeting siRNA. Cell numbers were counted at various time points.

Figure 9 shows that suppression of MMG8 expression disrupts glycosylation of LAMP-1 and CD44. HeLa cells transfected with control or MMG8 siRNA were analyzed for LAMP-1 and CD44 on immunoblots.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is a nucleic acid sequence encoding the human myomegalin isoform of the present invention.

SEQ ID NO:2 is an amino acid sequence of the human myomegalin isoform of the present invention.

SEQ ID NO:3 is an amino acid sequence of a human myomegalin isoform (GenBank Accession No. NP_055459.4).

5 SEQ ID NO:4 is an amino acid sequence of a human myomegalin isoform (GenBank Accession No. NP_001002812.1).

SEQ ID NO:5 is an amino acid sequence of a human myomegalin isoform (GenBank Accession No. NP_071754.3).

10 SEQ ID NO:6 is an amino acid sequence of a human myomegalin isoform (GenBank Accession No. NP_001002810.1).

SEQ ID NO:7 is an amino acid sequence of a human myomegalin isoform (GenBank Accession No. NP_001002811.1).

SEQ ID NO:8 is an amino acid sequence of a human myomegalin isoform (GenBank Accession No. NP_001182189.1).

15 SEQ ID NO:9 is an amino acid sequence of a human myomegalin isoform (GenBank Accession No. NP_001182190.1).

SEQ ID NO:10 is an amino acid sequence of a human myomegalin isoform (GenBank Accession No. NP_001185761.1).

20 SEQ ID NO:11 is an amino acid sequence of a human myomegalin isoform (GenBank Accession No. NP_001185763.1).

SEQ ID NO:12 is an amino acid sequence of a *Pongo abelii* myomegalin isoform (GenBank Accession No. NP_001126198).

SEQ ID NO:13 is an amino acid sequence of a mouse myomegalin isoform (GenBank Accession No. AAI41173).

25 SEQ ID NO:14 is an amino acid sequence of a *Gallus gallus* myomegalin isoform (GenBank Accession No. XP_423459).

SEQ ID NO:15 is an amino acid sequence of an *Xenopus tropicalis* myomegalin isoform (GenBank Accession No. NP_001072886).

30 SEQ ID NO:16 is an amino acid sequence of a *Danio rerio* myomegalin isoform (GenBank Accession No. CAI11891).

SEQ ID NO:17 is an amino acid sequence of a *Danio rerio* myomegalin isoform (GenBank Accession No. NP_956195).

SEQ ID NO:18 is an amino acid sequence of a KIAA0477 protein (GenBank Accession No. KIAA0477).

SEQ ID NO:19 is a linker sequence useful according to the present invention.

SEQ ID NO:20 is a linker sequence useful according to the present invention.

5 SEQ ID NO:21 is a linker sequence useful according to the present invention.

SEQ ID NO:22 is a linker sequence useful according to the present invention.

SEQ ID NO:23 is a linker sequence useful according to the present invention.

SEQ ID NO:24 is a linker sequence useful according to the present invention.

SEQ ID NO:25 is an siRNA useful to the present invention.

10 SEQ ID NO:26 is an siRNA useful to the present invention.

SEQ ID NO:27 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

SEQ ID NO:28 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

15 SEQ ID NO:29 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

SEQ ID NO:30 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

20 SEQ ID NO:31 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

SEQ ID NO:32 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

SEQ ID NO:33 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

25 SEQ ID NO:34 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

SEQ ID NO:35 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

30 SEQ ID NO:36 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

SEQ ID NO:37 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

SEQ ID NO:38 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

SEQ ID NO:39 is an amino acid sequence of a fragment of the human myomegalin isoform of the present invention.

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DETAILED DESCRIPTION

The present invention provides novel myomegalin isoforms (MMG), nucleic acid molecules encoding the myomegalin isoforms, fusion constructs comprising the myomegalin isoforms, and antibodies that bind specifically to the myomegalin isoforms. In a specific
10 embodiment, the present invention provides a novel MMG isoform, myomegalin variant 8 (MMG8), comprising SEQ ID NO:2. The myomegalin isoforms of the present invention regulate microtubule organization and nucleation at the Golgi apparatus; protein modification, secretion, and transport; and cell proliferation.

The present invention also provides uses of the myomegalin isoform as a diagnostic
15 biomarker and as a target for screening for therapeutic agents for lysosomal storage diseases, diabetes, and cancer. Also provided are methods for treating diseases associated with myomegalin by modulating the expression or activity of the myomegalin isoform of the invention.

Specifically, the present invention provides methods for isolating protein complexes
20 containing MMG8 and uses of MMG8 for regulating protein transport and glycosylation, and inhibiting cell proliferation. MMG8 is a splicing variant of MMG gene (1q12, human genome), and is the major MMG isoform expressed in all cell lines examined by the present inventors and also in C2C12 myotubes. MMG8 is predominantly present in the *cis*-Golgi networks, and is required for Golgi organization and microtubule nucleation at the Golgi
25 apparatus. MMG8 forms a complex with AKAP450 at the *cis*-side of the Golgi apparatus to exert its functions. For example, MMG8 binds to γ TuCs and is required for γ TuCs localization to the Golgi apparatus for microtubule nucleation. MMG8 also interacts with EB1/EB3. The interaction recruits EB1 to the Golgi apparatus and mediates the association of microtubules with MMG8 at the Golgi apparatus.

30 MMG8 also plays an important role in ER-to-Golgi transport of the protein secretory pathway. The suppression of MMG8 expression induces the accumulation of LAMP-1 - a diagnostic marker of lysosomal storage diseases, and reduces glycosylated mature CD44 - a

therapeutic target of metastatic tumors. In addition, the suppression of MMG8 expression inhibits proliferation of cancer cell lines. Microtubules have been used as a target for screening for anti-cancer agents. MMG8 can be used as a diagnostic marker and target of screening for therapeutic agents.

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MMG8 Isoform and Variants

In one aspect, the present invention provides a novel human myomegalin (MMG), herein referred to as myomegalin variant 8 (MMG8), consisting of SEQ ID NO:2. The present invention also provides MMG8 “variants” comprising SEQ ID NO:2 or fragments thereof.

10

It is discovered that the naturally-occurring human MMG8 isoform is expressed in a variety of cell types, including proliferating epithelial, fibroblast, and neuroblastoma cells. In contrast, another human myomegalin isoform, MMG1 (SEQ ID NO: 3), is expressed in heart and skeletal muscles, but is not expressed or is only minimally expressed in proliferating epithelial, fibroblast, and cancer cells, when compared to the expression levels of the MMG8 isoform of SEQ ID NO:2.

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In some embodiments, the present invention provides MMG8 variants. In preferred embodiments, the MMG8 variants bind to one or more of AKAP450, EB1, EB3, and subunits of the γ -tubulin complexes such as GCP2 and GCP3. The MMG8 variant of the subject invention is preferably of mammalian origin, more preferably, of human origin.

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In an embodiment, the MMG8 variant comprises amino acids 1-389 of SEQ ID NO:2. In some embodiments, the MMG8 variant of the present invention comprises a polypeptide comprising amino acids 1-389 of SEQ ID NO:2, wherein the carboxy-terminus comprises amino acids 1098-1116 of SEQ ID NO:2 or a fragment comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 contiguous amino acids of 1098-1116 of SEQ ID NO:2. For example, in certain embodiments, the carboxy-terminus of the MMG8 variant comprises a contiguous fragment starting at any of the amino acids 1098 to 1115 of SEQ ID NO:2, extends through, and terminates at, any of the amino acids 1099 to 1116 of SEQ ID NO:2. For example, in a specific embodiment, the carboxy-terminus of the MMG8 variant comprises amino acids 1103-1116 of SEQ ID NO:2, or a fragment comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 contiguous amino acids of 1103-1116 of SEQ ID NO:2.

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In some embodiments, the MMG8 variant comprises a polypeptide having a sequence that is at least about 90%, 93%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, or 99.5% identical to SEQ ID NO:2. In some embodiments, the MMG8 variant comprises a sequence that is at least 90%, 93%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, or 99.5% identical to amino acids 1-389 of SEQ ID NO:2. In an embodiment, the MMG8 variant comprises the SxIP motif (Ser-x-Ile-Pro).

In some embodiments, the MMG8 variants of the present invention do not encompass any of SEQ ID NOs: 3-18. In some other embodiments, the MMG8 variants of the present invention do not encompass a polypeptide comprising a fragment of more than 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100 contiguous amino acids of any of SEQ ID NOs: 3-18.

A protein or polypeptide fragment, as used herein, unless explicitly specified otherwise, refers to a sequence comprising at least 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 350, 389, 400, 500, 600, 700, 800, 900, or 1000 contiguous amino acids of a given protein or polypeptide sequence.

Unless otherwise specified, as used herein, percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990). BLAST searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul *et al.* (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. See NCBI/NIH website.

In some embodiments, the present invention provides MMG8 variants, wherein one or more amino acids of SEQ ID NO:2, or of a fragment thereof, are modified. Modification of amino acid sequences includes, but is not limited to, addition, deletion, and conservative substitution of amino acids of a given protein or polypeptide sequence. Based on the common knowledge of MMG structure, some amino acids of SEQ ID NO:2 may be substituted, deleted, or added, without detracting from the biological activities of the protein.

In some embodiments, the modification of amino acids is performed at amino acids 390-1116 of SEQ ID NO:2, amino acids 390-1097 of SEQ ID NO:2, or amino acids 390-

1102 of SEQ ID NO:2. In an embodiment, the SxIP motif of SEQ ID NO:2 (amino acids 309, 311, and 312) is not modified.

In some embodiments, no more than 50 (*e.g.*, no more than: 50, 45, 40, 35, 30, 25, 20, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) amino acids of SEQ ID NO:2 are modified.

5 In some embodiments, no more than 50 (*e.g.*, no more than: 50, 45, 40, 35, 30, 25, 20, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) amino acids of 1-389 of SEQ ID NO:2 are modified.

In some embodiments, no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids of 1098-1116 of SEQ ID NO:2 are modified.

10 Methods of conservative modifications are known in the art. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine and threonine; lysine, histidine and arginine; and phenylalanine and tyrosine.

Conservative modifications can produce molecules having functional and chemical characteristics similar to those of the molecule from which such modifications are made. In addition, substantial modifications in the functional and/or chemical characteristics of the molecules may be accomplished by selecting substitutions in the amino acid sequence that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the size of the molecule. Therefore, it is possible to modify the amino acid sequences of the subject protein to obtain protein homologues with similar, or even more desirable properties. These modified proteins are within the scope of the present invention.

25 In certain embodiments, conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ -amino butyric acid, ϵ -amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, 30 sarcosine, homocitrulline, cysteic acid, τ -butylglycine, τ -butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acid analogues in

general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form.

Modifications of amino acid residues can also be achieved by direct mutation, phage display, or shuffling within the nucleic acids encoding the protein molecules, which are well known to those skilled in the art. In random mutagenesis, positions are chosen randomly or amino acid changes are made using simplistic rules. In addition, the amino acid derivative may contain substituted or non-substituted, linear, branched, or cyclic alkyl moieties, and may include one or more heteroatoms.

In an embodiment, the present invention provides isolated MMG8 and MMG8 variants. MMG8 and MMG8 variants can be isolated from epithelial cells, fibroblast cells, cancer or tumor cells, or cells undergoing division or proliferation.

The isolated MMG8 and MMG8 variants are substantially free of other cellular components, such as other biological molecules, proteins or peptides, nucleic acids, lipids and carbohydrates, which may normally be associated with MMG8 and MMG8 variants. The term “substantially free of,” as used herein, encompasses preparations having less than about 20%, 10%, and preferably less than 5% (by dry weight) contaminating factors (such as biological molecules, proteins or peptides, nucleic acids, lipids, and carbohydrates and other cellular components).

The present invention also provides recombinant forms of MMG8 and MMG8 variants. In one embodiment, the MMG8 is encoded by a polynucleotide comprising SEQ ID NO:1. Proteins or peptides that are recombinantly-produced may be subject to post-translational modification such as glycosylation. The nature and extent of such modifications in large part will be determined by the post-translational modification capacity of the particular host cell and the modification signals that are present in the amino acid sequence of the peptide or protein in question. For instance, glycosylation patterns may vary between different types of host cell. Alternatively, unglycosylated proteins may be generated by expression in a system which is defective for glycosylation.

Fusion Constructs

In another aspect, the present invention provides fusion proteins comprising MMG8 or an MMG8 variant or a fragment thereof, a second protein, and optionally, a linker sequence that links the MMG8, MMG8 variant, or fragment thereof to the second protein.

In an embodiment, the N-terminal of the MMG8 protein or polypeptide is fused with a second protein, optionally, through a linker sequence. In another embodiment, the C-terminal of the MMG8 protein or polypeptide is fused with a second protein, optionally, through a linker sequence.

In a specific embodiment, the fusion protein comprises an MMG8 protein, wherein the MMG8 protein comprises SEQ ID NO:2. In an embodiment, the fusion protein comprises an MMG8 fragment, wherein the fragment comprises amino acids 1-389 of SEQ ID NO:2. In an embodiment, the fusion protein comprises an MMG8 variant, wherein in the MMG8 variant, no more than 50 (*e.g.*, no more than: 50, 45, 40, 35, 30, 25, 20, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) amino acids of 1-389 of SEQ ID NO:2 are modified. In one embodiment, the fusion protein comprises MMG8 or an MMG8 variant, wherein the SxIP domain of MMG8 or MMG8 variant is not modified.

In an embodiment, the fusion protein comprises an MMG8 protein, the carboxy-terminus of which comprises a sequence comprising amino acids 1098-1116 of SEQ ID NO:2, or a fragment comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 contiguous amino acids of 1098-1116 of SEQ ID NO:2. In another embodiment, the fusion protein comprises an MMG8 protein, the carboxy-terminus of which comprises a sequence comprising amino acids 1103-1116 of SEQ ID NO:2, or a fragment comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 contiguous amino acids of 1103-1116 of SEQ ID NO:2.

In some embodiments, the MMG8 protein or polypeptide is fused with a second protein that is an Fc domain. The term "Fc domain" encompasses the full length and fragments of native human and animal Fc and Fc variant molecules and sequences, including for example IgG, IgM, IgD, IgE, IgA and subtypes such as for example IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. As with Fc variants and native Fc's, the term "Fc domain" includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

The term "Fc variant" refers to a molecule or sequence that is modified from a native Fc, but still comprises a binding site for the salvage receptor. Fc domains include molecules having two or more polypeptide chains associated covalently, noncovalently, or by both

covalent and non-covalent interactions. IgG molecules typically form dimers; IgM, pentamers; IgD, dimers; and IgA, monomers, dimers, trimers, or tetramers. Multimers may be formed by exploiting the sequence and resulting activity of the native Ig source of the Fc or by derivatizing (as defined below) such a native Fc.

5 The Fc domain within the scope of the invention can be of antibodies of any isotype, including IgG, IgA, IgE, IgD, and IgM. IgG isotype antibodies can be further subdivided into IgG1, IgG2, IgG3, and IgG4 subtypes. IgA antibodies can be further subdivided into IgA1 and IgA2 subtypes. In a specific embodiment, the Fc domain is IgG1.

10 In another embodiment, the fusion protein of the present invention comprises a linker sequence that links the MMG8 protein or polypeptide to a second protein such as an Fc domain. Linker would typically be a peptide chain. The length of the peptide may be, for example, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50 or more amino acid residues, but typically is between 5 and 25 residues. Depending upon the length and side chain composition, a linker may have, but need not have, greater than average
15 flexibility. Flexibility can be calculated using algorithms known in the art. Examples of useful linkers include, but are not limited to, 5GlyCys2ProCys (SEQ ID NO:19), 4Gly3Ser (SEQ ID NO:20), Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn (SEQ ID NO:21), Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn (SEQ ID NO:22), Gly Asp Leu Ile Tyr Arg Asn Gln Lys (SEQ ID NO:23), and
20 9GlyProSerCysValProLeuMetArgCysGlyGlyCysCysAsn (SEQ ID NO:24).

Nucleic Acid Molecules

Another aspect of the invention pertains to nucleic acid molecules that encode the MMG8 or MMG8 variants, fragments, fusion proteins, and antibodies of the present
25 invention. The nucleic acid molecules of the present invention encompass DNA molecules (*e.g.* genomic DNA and cDNA) and RNA molecules (*e.g.* mRNA including pre-mRNA and mature mRNA). In addition, the subject nucleic acid molecules may be single-stranded or double-stranded.

30 In an embodiment, the nucleic acid molecule of the present invention encodes MMG8 having an amino acid sequence comprising SEQ ID NO:2. In an embodiment, the nucleic acid molecule of the present invention encodes an MMG8 variant comprising amino acids 1-389 of SEQ ID NO:2. In an embodiment, the nucleic acid molecule encodes an MMG8

variant, the carboxy-terminus of which comprises amino acids 1098-1116 of SEQ ID NO:2, or a fragment comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 contiguous amino acids of 1098-1116 of SEQ ID NO:2. In an embodiment, the nucleic acid molecule encodes an MMG8 variant, the carboxy-terminus of which comprises amino acids
5 1103-1116 of SEQ ID NO:2, or a fragment comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 contiguous amino acids of 1103-1116 of SEQ ID NO:2.

In an embodiment, the nucleic acid molecule of the present invention comprises SEQ ID NO:1 or a fragment thereof, wherein the nucleic acid molecule encodes MMG8 or an MMG8 variant. In an embodiment, the nucleic acid molecule of the present invention
10 comprises nucleic acids 1-1167 of SEQ ID NO:1, or a fragment thereof, or any complement thereof. In some embodiments, the nucleic acid molecule comprises a polynucleotide, wherein the 3' end of the polynucleotide sequence comprises 3292-3348 of SEQ ID NO:1, or a contiguous fragment of 3292-3348 of SEQ ID NO:1, or any complement thereof. In some
15 embodiments, the nucleic acid molecule comprises a polynucleotide, wherein the 3' end of the polynucleotide sequence comprises 3309-3348 of SEQ ID NO:1, or a contiguous fragment of 3309-3348 of SEQ ID NO:1, or any complement thereof.

A polynucleotide fragment, as used herein, unless explicitly specified otherwise, refers to a sequence comprising at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000,
20 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, or 3400 contiguous nucleic acids of a given polynucleotide sequence.

In some embodiments, the nucleic acid molecule of the present invention comprises a polynucleotide having a sequence that is at least about 90%, 93%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, or 99.5% identical to SEQ ID NO:1, or a fragment thereof, or any
25 complement thereof. In some embodiments, the nucleic acid molecule of the present invention comprises a polynucleotide having a sequence that is at least about 90%, 93%, 95%, 96%, 97%, 97.5%, 98%, 98.5%, 99%, or 99.5% identical to nucleic acids 1-1167 of SEQ ID NO:1, or a fragment thereof, or any complement thereof. In an embodiment, the nucleic acid molecule encoding MMG8 or an MMG8 variant comprises the SxIP motif (Ser-x-Ile-Pro).

30 In an embodiment, the nucleic acid sequence of the present invention is identical to that of the mature mRNA that can be translated into MMG8 or an MMG8 variant of the present invention. In another embodiment, the nucleic acid molecule of the present invention

is a cDNA molecule, of which one strand is complementary to a mature mRNA that can be translated into MMG8 or an MMG8 variant of the present invention. The DNA and RNA sequences of the subject invention can be readily determined by those skilled in the art based on degeneracy of the genetic code.

5 In addition, one or more nucleotides of the subject invention may be substituted, deleted or inserted. Natural nucleotides of DNA can be substituted with nucleotides having a base moiety including, but not limited to, inosine, 5-fluorouracil, 5-bromouracil, hypoxanthine, 1-methylguanine, 5-methylcytosine, and tritylated bases. The sugar moiety of the nucleotide in a sequence can also be modified, and such modification includes, but is not
10 limited to, arabinose, xylulose, and hexose. In addition, the adenine, cytosine, guanine, thymine, and uracil bases of the nucleotides can be modified with acetyl, methyl, and/or thio groups. Sequences containing nucleotide substitutions, deletions, and/or insertions can be prepared and tested using standard techniques known in the art.

Further, the present invention provides nucleic acids, oligonucleotides, antisense
15 oligonucleotides, and synthetic oligonucleotides that hybridize to the nucleic acid encoding MMG8 or an MMG8 variant, useful as agents to detect the presence or level of MMG8 nucleic acid molecules in the biological samples. The present invention contemplates the use of nucleic acid sequences corresponding to the coding sequence of MMG8 or MMG8 variants of the invention, and to the complementary sequence thereof.

20 In an embodiment, such a nucleic acid molecule can be used as an oligonucleotide or polynucleotide probe. The preferred oligonucleotides for detecting the presence or level of the MMG8 nucleic acid molecule in biological samples are those that are complementary to at least part of the cDNA sequence encoding the MMG8 or an MMG8 variant of the present invention. These complementary sequences are also known in the art as "antisense"
25 sequences. These oligonucleotides may be oligoribonucleotides or oligodeoxyribonucleotides. In addition, oligonucleotides may be natural oligomers composed of the biologically significant nucleotides, *i.e.*, A (adenine), dA (deoxyadenine), G (guanine), dG (deoxyguanine), C (cytosine), dC (deoxycytosine), T (thymine) and U (uracil), or modified oligonucleotide species, substituting, for example, a methyl group or a sulfur
30 atom for a phosphate oxygen in the inter-nucleotide phosphodiester linkage.

The nucleic acid molecules of the present invention may be isolated from cells of interest (*e.g.* tissue cells of an organism or tissue-derived cell lines) or artificially created (*e.g.*

recombinant DNA and chemically-synthesized polynucleotide molecules). For example, the oligonucleotides can be prepared by using any of the commercially available, automated nucleic acid synthesizers. Alternatively, the oligonucleotides may be created by standard recombinant DNA techniques, for example, inducing transcription of the noncoding strand.

- 5 The DNA sequence encoding MMG8 or MMG8 variants may be inverted in a recombinant DNA system, *e.g.*, inserted in reverse orientation downstream of a suitable promoter, such that the noncoding strand now is transcribed.

Although any length oligonucleotide may be utilized to hybridize to a nucleic acid encoding MMG8 or an MMG8 variant, oligonucleotides typically within the range of 8-200,
10 15-100, or 15-50 nucleotides are preferred.

“Hybridization” refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between a particular purine and a particular pyrimidine in double-stranded nucleic acid molecules (DNA-DNA, DNA-RNA, or RNA-RNA). The major specific pairings are guanine with cytosine and adenine with
15 thymine or uracil. Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for duplex formation. Severity of conditions can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like.

Preferably, hybridization is conducted under high stringency conditions by techniques
20 well known in the art, as described, for example, in Keller, G.H. & M.M. Manak, *DNA Probes*, and the companion volume *DNA Probes: Background, Applications, Procedures* (various editions, including 2nd Edition, Nature Publishing Group, 1993). Hybridization is also described extensively in the Molecular Cloning manuals published by Cold Spring Harbor Laboratory Press, including Sambrook & Russell, *Molecular Cloning: A Laboratory*
25 *Manual* (2001).

An example of high stringency conditions for hybridization is at least about 6X SSC and 1% SDS at 65 °C, with a first wash for 10 minutes at about 42 °C with about 20% (v/v) formamide in 0.1X SSC, and with a subsequent wash with 0.2X SSC and 0.1% SDS at 65 °C. A non-limiting example of hybridization conditions are conditions selected to be about 1, 2, 3,
30 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 °C lower than the thermal melting point (T_m) for the specific sequence in the particular solution. T_m is the temperature (dependent upon ionic strength and pH) at which 50% of the target sequence

hybridizes to a perfectly matched probe. T_m typically increases with $[Na^+]$ concentration because the sodium cations electrostatically shield the anionic phosphate groups of the nucleotides and minimize their repulsion. The washes employed may be for about 5, 10, 15, 20, 25, 30, or more minutes each, and may be of increasing stringency if desired.

Calculations for estimating T_m are well-known in the art. For example, the melting temperature may be described by the following formula (Beltz, G.A., K.A. Jacobs, T.H. Eickbush, P.T. Cherbas, and F.C. Kafatos, *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285, 1983).

$T_m = 81.5^\circ\text{C} + 16.6 \text{ Log}[Na^+] + 0.41(\%G+C) - 0.61(\%\text{formamide}) - 600/\text{length of duplex in base pairs}.$

A more accurate estimation of T_m may be obtained using nearest-neighbor models. Breslauer, *et al.*, *Proc. Natl. Acad. Sci. USA*, 83:3746-3750 (1986); SantaLucia, *Proc. Natl. Acad. Sci. USA*, 95: 1460-1465 (1998); Allawi & SantaLucia, *Biochemistry* 36:10581-94 (1997); Sugimoto *et al.*, *Nucleic Acids Res.*, 24:4501-4505 (1996). T_m may also be routinely measured by differential scanning calorimetry (Duguid *et al.*, *Biophys J*, 71:3350-60, 1996) in a chosen solution, or by other methods known in the art, such as UV-monitored melting. As the stringency of the hybridization conditions is increased, higher degrees of homology are obtained.

The oligonucleotide selected for hybridizing to the MMG8 nucleic acid molecule, whether synthesized chemically or by recombinant DNA technology, can be isolated and purified using standard techniques and then preferably labeled (*e.g.*, with ^{35}S or ^{32}P) using standard labeling protocols.

The present invention also contemplates the use of oligonucleotide pairs in polymerase chain reactions (PCR) to detect the expression of MMG8 or MMG8 variants in biological samples. The oligonucleotide pairs include a forward MMG8 primer and a reverse MMG8 primer.

The presence of MMG8 or MMG8 variants in a sample from a patient may be determined by nucleic acid hybridization, such as but not limited to Northern blot analysis, dot blotting, Southern blot analysis, fluorescence *in situ* hybridization (FISH), and PCR. Chromatography, preferably HPLC, and other known assays may also be used to determine messenger RNA levels of MMG8 or MMG8 variants in a sample.

In one embodiment, the present invention contemplates the use of nucleic acids as agents for detecting MMG8 or an MMG8 variant in biological samples of patients, wherein the nucleic acids are labeled. The nucleic agents may be labeled with a radioactive label, a fluorescent label, an enzyme, a chemiluminescent tag, a colorimetric tag, or other labels or tags that are discussed above or that are known in the art.

In certain embodiment, the methods of detecting MMG8 nucleic acid in biological samples include Northern blot analysis, dot blotting, Southern blot analysis, FISH, and PCR.

In one embodiment, the present invention contemplates the use of Northern blot analysis to detect the presence of MMG8 mRNA in a sample of bodily fluid. The first step of the analysis involves separating a sample containing MMG8 nucleic acid by gel electrophoresis. The dispersed nucleic acids are then transferred to a nitrocellulose filter or another filter. Subsequently, the labeled oligonucleotide is exposed to the filter under suitable hybridizing conditions, *e.g.*, 50% formamide, 5 x SSPE, 2 x Denhardt's solution, 0.1% SDS at 42° C., as described in *Molecular Cloning: A Laboratory Manual*, Maniatis *et al.* (1982, CSH Laboratory). Other useful procedures known in the art include solution hybridization, dot and slot RNA hybridization, and probe based microarrays. Measuring the radioactivity of hybridized fragments, using standard procedures known in the art quantitates the amount of MMG8 nucleic acid present in the biological fluid of a subject.

Dot blotting involves applying samples containing the nucleic acid of interest to a membrane. The nucleic acid can be denatured before or after application to the membrane. The membrane is incubated with a labeled probe. Dot blot procedures are well known to the skilled artisan and are described more fully in U.S. Patent Nos. 4,582,789 and 4,617,261, the disclosures of which are incorporated herein by reference.

Polymerase chain reaction (PCR) is a process for amplifying one or more specific nucleic acid sequences present in a nucleic acid sample using primers and agents for polymerization and then detecting the amplified sequence. A specific example of PCR that is routinely performed by the skilled artisan to detect desired sequences is reverse transcript PCR (RT-PCR; Saiki *et al.*, *Science*, 1985, 230:1350; Scharf *et al.*, *Science*, 1986, 233:1076). RT-PCR involves isolating total RNA from biological fluid, denaturing the RNA in the presence of primers that recognize the desired nucleic acid sequence, using the primers to generate a cDNA copy of the RNA by reverse transcription, amplifying the cDNA by PCR

using specific primers, and detecting the amplified cDNA by electrophoresis or other methods known to the skilled artisan.

Vectors, Expression Constructs and Host Cells

5 In another aspect, the present invention provides vectors, expression constructs, and host cells comprising a nucleic acid molecule of the present invention. In an embodiment, the vector, expression construct, or host cell of the present invention comprises a nucleic acid molecule comprising SEQ ID NO:1, or a fragment thereof, or any complement thereof.

10 In an embodiment, the vector, expression construct, or host cell of the present invention comprises a nucleic acid molecule, wherein the nucleic acid molecule comprises nucleic acids 1-1167 of SEQ ID NO:1, or a complement thereof. In some embodiments, the vector, expression construct, or host cell of the present invention comprises a nucleic acid molecule, wherein the nucleic acid molecule comprises a polynucleotide comprising nucleic acids 1-1167 of SEQ ID NO:1, wherein the 3' end of the polynucleotide sequence comprises
15 3292-3348 of SEQ ID NO:1, or a contiguous fragment of nucleic acids 3292-3348 of SEQ ID NO:1, or any complement thereof.

In some embodiments, the vector, expression construct, or host cell of the present invention comprises a nucleic acid molecule, wherein the nucleic acid molecule comprises a polynucleotide comprising nucleic acids 1-1167 of SEQ ID NO:1, wherein the 3' end of the
20 polynucleotide sequence comprises 3292-3348 of SEQ ID NO:1, or a contiguous fragment having at least 75%, 80%, 85%, 90%, 95%, or 99% identity to nucleic acids 3292-3348 of SEQ ID NO:1, or any complement thereof.

In some embodiments, the vector, expression construct, or host cell of the present invention comprises a polynucleotide having a sequence that is at least about 90%, 93%, 95%,
25 96%, 97%, 97.5%, 98%, 98.5%, 99%, or 99.5% identical to SEQ ID NO:1, or a fragment thereof, or any complement thereof.

As used herein, the term "expression construct" refers to a combination of nucleic acid sequences that provides for transcription of an operably linked nucleic acid sequence. As used herein, the term "operably linked" refers to a juxtaposition of the components
30 described, wherein the components are in a relationship that permits them to function in their intended manner. In general, operably linked components are in contiguous relation.

Expression constructs of the invention will also generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Thus, a person of ordinary skill in the art can select regulatory elements for use in, for example, bacterial host cells, yeast host cells, mammalian host cells, and human host cells.

5 Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements.

An expression construct of the invention can comprise a promoter sequence operably linked to a polynucleotide sequence encoding a peptide of the invention. Promoters can be incorporated into a polynucleotide using standard techniques known in the art. Multiple
10 copies of promoters or multiple promoters can be used in an expression construct of the invention. In a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

15 Expression constructs of the invention may optionally contain a transcription termination sequence, a translation termination sequence, signal peptide sequence, and/or enhancer elements. Transcription termination regions can typically be obtained from the 3' untranslated region of a eukaryotic or viral gene sequence. Transcription termination sequences can be positioned downstream of a coding sequence to provide for efficient
20 termination. Signal peptides are a group of short amino terminal sequences that encode information responsible for the relocation of an operably linked peptide to a wide range of post-translational cellular destinations, ranging from a specific organelle compartment to sites of protein action and the extracellular environment.

Chemical enhancers are cis-acting elements that increase gene transcription and can
25 also be included in the expression construct. Chemical enhancer elements are known in the art, and include, but are not limited to, the CaMV 35S enhancer element, cytomegalovirus (CMV) early promoter enhancer element, and the SV40 enhancer element. DNA sequences which direct polyadenylation of the mRNA encoded by the structural gene can also be included in the expression construct.

30 Unique restriction enzyme sites can be included at the 5' and 3' ends of the expression construct to allow for insertion into a polynucleotide vector. As used herein, the term "vector" refers to any genetic element, including for example, plasmids, cosmids,

chromosomes, phage, virus, and the like, which is capable of replication when associated with proper control elements and which can transfer polynucleotide sequences between cells. Vectors contain a nucleotide sequence that permits the vector to replicate in a selected host cell. A number of vectors are available for expression and/or cloning, and include, but are not limited to, pBR322, pUC series, M13 series, and pBLUESCRIPT vectors (Stratagene, La Jolla, CA).

Host cells of the present invention include, for example, bacteria host cells, yeast host cells, plant host cells, insect host cells, mammalian host cells, and preferably human host cells. Host cells that can be used in the present invention include, but are not limited to, CHO cells, HEK-293 cells, 293T cells, COS cells, COS-7 cells, and NIH 3T3 cells.

MMG8 Antibodies and Aptamers

Another aspect of the present invention provides antibodies and aptamers that bind specifically to MMG8 or MMG8 variants of the present invention. In an embodiment, antibodies and aptamers of the subject invention bind specifically to the MMG8 isoform comprising SEQ ID NO:2. In certain embodiments, the antibodies and aptamers of the present invention bind specifically to an epitope at the carboxy-terminus of MMG8 of SEQ ID NO:2.

In certain embodiments, the antibodies and aptamers of the present invention bind specifically to an epitope comprising 926-1116 of SEQ ID NO:2, or an epitope comprising a fragment comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 contiguous amino acids of 1098-1116 of SEQ ID NO:2.

In certain embodiments, the antibodies and aptamers of the present invention bind specifically to an epitope comprising 926-1116 of SEQ ID NO:2, or an epitope comprising a fragment of 926-1116 of SEQ ID NO:2, wherein the fragment has at least 75%, 80%, 90%, or 95% identity to 926-1116 of SEQ ID NO:2.

In certain embodiments, the antibodies and aptamers of the present invention bind specifically to an epitope comprising 926-1116 of SEQ ID NO:2, or an epitope comprising a fragment of 1098-1116 of SEQ ID NO:2, wherein the fragment has at least 75%, 80%, 90%, or 95% identity to 1098-1116 of SEQ ID NO:2.

In certain embodiments, the antibodies and aptamers of the present invention bind specifically to an epitope comprising 926-1116 of SEQ ID NO:2, or an epitope comprising a

fragment of 1103-1116 of SEQ ID NO:2, wherein the fragment has at least 75%, 80%, 90%, or 95% identity to 1103-1116 of SEQ ID NO:2.

In a preferred embodiment, the anti-MMG8 antibody of the present invention is a humanized antibody.

5 In some embodiments, the antibodies and aptamers of the present invention do not bind to an epitope comprising amino acids 1-389 of SEQ ID NO:2, or an epitope comprising any fragment thereof. In some embodiments, the antibodies of the present invention do not bind to any one or more SEQ ID NOs: 3-18.

10 “Specific binding” or “specificity” refers to the ability of a protein to detectably bind an epitope presented on a protein or polypeptide molecule of interest, while having relatively little detectable reactivity with other proteins or structures. Specificity can be relatively determined by binding or competitive binding assays, using, *e.g.*, Biacore instruments. Specificity can be exhibited by, *e.g.*, an about 10:1, about 20:1, about 50:1, about 100:1, 10.000:1 or greater ratio of affinity/avidity in binding to the specific target molecule versus
15 nonspecific binding to other irrelevant molecules.

“Selectivity” refers to the preferential binding of a protein to a particular region, target, or peptide as opposed to one or more other biological molecules, structures, cells, tissues, etc. For example, selectivity can be determined by competitive ELISA or Biacore assays. The difference in affinity/avidity that marks selectivity can be any detectable preference (*e.g.*, a
20 ratio of more than 1:1.1, or more than about 1:5, if detectable).

Antibodies of the subject invention can be in any of a variety of forms, including intact immunoglobulin molecules, fragments of immunoglobulin molecules such as Fv, Fab and similar fragments; multimers of immunoglobulin molecules (*e.g.*, diabodies, triabodies, and bi-specific and tri-specific antibodies, as are known in the art; *see, e.g.*, Hudson and Kortt,
25 J. Immunol. Methods 231:177-189, 1999); fusion constructs containing an antibody or antibody fragment; and human or humanized immunoglobulin molecules or fragments thereof.

Antibodies within the scope of the invention can be of any isotype, including IgG, IgA, IgE, IgD, and IgM. IgG isotype antibodies can be further subdivided into IgG1, IgG2, IgG3, and IgG4 subtypes. IgA antibodies can be further subdivided into IgA1 and IgA2
30 subtypes.

Antibodies of the subject invention include polyclonal and monoclonal antibodies. The term "monoclonal antibody," as used herein, refers to an antibody or antibody fragment obtained from a substantially homogeneous population of antibodies or antibody fragments (*i.e.* the individual antibodies within the population are identical except for possible naturally occurring mutations that may be present in a small subset of the antibody molecules).

Anti-MMG8 antibodies of the present invention can be prepared using isolated native MMG8 or recombinant MMG8. Preferably, antibodies used in the methods of the invention are reactive against MMG8 if they bind with a K_a of greater than or equal to 10^7 M. In a sandwich immunoassay of the invention, mouse polyclonal antibodies and rabbit polyclonal antibodies are utilized.

For use in detection of MMG8 or an MMG8 variant from biological sample, the purified antibodies can be covalently attached, either directly or via linker, to a compound which serves as a reporter group to permit detection of the presence of MMG8 or an MMG8 variant. A variety of different types of substances can serve as the reporter group including, but not limited to, enzymes, dyes, radioactive metal and non-metal isotopes, fluorogenic compounds, fluorescent compounds, *etc.* Methods for preparation of antibody conjugates of the antibodies (or fragments thereof) of the invention are described in U.S. Patent Nos. 4,671,958; 4,741,900 and 4,867,973.

Assays for Detecting MMG8 Isoforms

Another aspect of the present invention provides assays for detecting the presence or level of MMG8 or an MMG8 variant in a sample. MMG8 proteins or MMG8 transcripts can be used to determine the level of MMG8 or an MMG8 variant in a sample. In an embodiment, the sample is a biological sample obtained from a subject.

In one embodiment, the method for detecting the presence or level of MMG8 or an MMG8 variant comprises:

- (a) contacting a sample with an agent that binds to MMG8 or an MMG8 variant; and
- (b) detecting the binding of the agent to the MMG8 or an MMG8 variant.

Agents capable of binding to MMG8 or an MMG8 variant in the samples encompass those that interact or bind with the MMG8 polypeptide or the nucleic acid molecule encoding MMG8. Examples of such agents (also referred to herein as binding agents) include, but are not limited to, MMG8 antibodies or fragments thereof that bind specifically to MMG8,

MMG8 binding partners, aptamers, and nucleic acid molecules that hybridize to the nucleic acid molecules encoding MMG8 polypeptides. Preferably, the binding agent is labeled with a detectable substance (*e.g.*, a detectable moiety). The binding agent may itself function as a label.

5 In a specific embodiment, the agent that detects MMG8 or an MMG8 variant is an antibody that binds specifically to MMG8 or the MMG8 variant. In a specific embodiment, the anti-MMG8 antibody binds specifically to an epitope at the carboxy-terminus of MMG8 of SEQ ID NO:2.

10 In certain embodiments, the antibody or aptamer of the present invention binds specifically to an epitope comprising amino acids 926-1116 of SEQ ID NO:2, or an epitope comprising a fragment of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 contiguous amino acids of 1098-1116 of SEQ ID NO:2.

15 In certain embodiments, the antibody or aptamer of the present invention binds specifically to an epitope comprising amino acids 926-1116 of SEQ ID NO:2, or an epitope comprising a fragment having at least 75%, 80%, 85%, 90%, or 95% identity to 926-1116 of SEQ ID NO:2.

20 In one embodiment, the agent (such as an anti-MMG8 antibody) that binds to the MMG8 or an MMG8 variant is labeled with a detectable substance. In a specific embodiment, the agent is fluorescently labeled. In one embodiment, the level of MMG8 or an MMG8 variant is determined by measuring the fluorescence level of the binding complex.

In a preferred embodiment, the invention provides a method for detecting the presence or level of MMG8 or an MMG8 variant, comprising:

25 (a) incubating a sample with a first antibody specific for MMG8 or an MMG8 variant wherein the first antibody is directly or indirectly labeled with a detectable substance, and a second antibody specific for MMG8 or the MMG8 variant wherein the second antibody is immobilized;

(b) separating the first antibody from the second antibody to provide a first antibody phase and a second antibody phase; and

30 (c) detecting the detectable substance in the first or second antibody phase thereby quantitating MMG8 or the MMG8 variant in the sample.

In an embodiment, MMG8 or MMG8 variants can be detected by incubating a sample with a first antibody or a first aptamer and a second antibody or a second aptamer. In an

embodiment, the first antibody or the first aptamer binds specifically to an epitope comprising amino acids 926-1116 of SEQ ID NO:2. In an embodiment, the first antibody or the first aptamer binds to an epitope comprising a fragment having at least 75%, 80%, 85%, 90%, or 95% identity to 926-1116 of SEQ ID NO:2. In an embodiment, the second antibody or the second aptamer that binds specifically to an epitope comprising a fragment comprising 50, 45, 40, 35, 30, 25, 20, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, or 5 amino acids of SEQ ID NO:2. In an embodiment, the second antibody or the second aptamer that binds specifically to an epitope having at least 75%, 80%, 85%, 90%, or 95% identity to a fragment comprising 50, 45, 40, 35, 30, 25, 20, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, or 5 amino acids of SEQ ID NO:2.

In another embodiment, the present invention provides a method for detecting the presence of a nucleic acid molecule encoding MMG8 or an MMG8 variant in a sample, comprising:

(a) contacting the sample with an agent that selectively binds to the nucleic acid molecule; and

(b) detecting whether the agent binds to the nucleic acid molecule in the sample.

In a specific embodiment, the binding agent is a second nucleic acid molecule comprising at least 8 nucleic acids of SEQ ID NO:1, or a complement thereof. In a specific embodiment, the binding agent is a second nucleic acid molecule comprising 3294-3348 of SEQ ID NO:1, or a fragment of 3294-3351 of SEQ ID NO:1, or any complement thereof.

The terms “detecting” or “detect” include assaying or otherwise establishing the presence or absence of the target MMG8 or MMG8 variant (nucleic acids encoding MMG8 or an MMG8 variant), subunits thereof, or combinations of agent bound targets, and the like. The term encompasses quantitative, semi-quantitative, and qualitative detection methodologies.

Methods for detecting MMG8 and other biomarkers (*e.g.* protein or peptide and nucleic acids) of the subject invention are well known in the art, including but not limited to, Western blots, Northern blots, Southern blots, ELISA, PCR, immunoprecipitation, immunofluorescence, radioimmunoassay, flow cytometry, immunocytochemistry, nucleic acid hybridization techniques, nucleic acid reverse transcription methods, and nucleic acid amplification methods.

Preferably, in the various embodiments of the invention, the detection method provides an output (*i.e.*, readout or signal) with information concerning the presence, absence,

or amount of MMG8 in a sample from a subject. For example, the output may be qualitative (*e.g.*, “positive” or “negative”), or quantitative (*e.g.*, a concentration such as nanograms per milliliter).

The terms “sample”, “biological sample”, and the like refer to a type of material known to or suspected of expressing or containing MMG8 or an MMG8 variant. The sample can be derived from any biological source, such as tissues or extracts, including cells (*e.g.*, tumor cells) and physiological fluids, such as, for example, whole blood, plasma, serum, peritoneal fluid, ascites, and the like. The sample can be obtained from animals, preferably mammals, most preferably humans. The sample can be pretreated by any method and/or can be prepared in any convenient medium that does not interfere with the assay. The sample can be treated prior to use, such as preparing plasma from blood, diluting viscous fluids, applying one or more protease inhibitors to samples. Sample treatment can involve filtration, distillation, extraction, concentration, inactivation of interfering components, the addition of reagents, and the like.

Drug Screening Assays

Another aspect of the present invention pertains to use of the MMG8 or an MMG8 variant for screening for therapeutic agents that can be used for treating diseases including, but not limited to, diseases involving defects in microtubule organization and/or nucleation; diseases associated with defects in protein modification, secretion, and trafficking; and diseases involving abnormal cell proliferation. In a specific embodiment, the MMG8 or MMG8 variants of the present invention can be used to screen for anti-cancer therapeutics. The therapeutic agent can be a drug, chemical, compound, protein or peptide, or a nucleic acid molecule (*e.g.* DNA, RNA such as siRNA).

In one embodiment, the subject methods can be used for screening for therapeutic agents that reduce levels of MMG8 or MMG8 transcripts (such as primary, intermediate, and mature mRNA transcripts of MMG8) in cells. In addition, the subject methods can be used for screening for therapeutic agents that inhibit or reduce MMG8 expression levels.

In one embodiment, the present invention provides a method for screening for therapeutics that inhibits MMG8 or an MMG8 variant, wherein the method comprises:

- a) providing a test sample containing cells expressing MMG8 or the MMG8 variant;
- b) contacting a candidate agent with the test sample;

- c) determining a level of MMG8 or the MMG8 variant in the test sample; and
- d) selecting the candidate agent if said agent reduces the level of MMG8 or the MMG8 variant in the test sample.

5 The level of MMG8 or an MMG8 variant can be determined by measuring the levels of MMG8 proteins and/or MMG8 transcripts.

In one embodiment, the methods can be used for screening for therapeutic agents that modulate the activity of MMG8 or MMG8 variants of the invention. In certain embodiments, the present invention provides methods for screening for therapeutic agents that modulate, enhance, or inhibit the association between MMG8 or MMG8 variants with proteins selected
10 from AKAP450, EB1, EB3 and γ -tubulin complexes such as GCP2 and GCP3. In certain embodiments, the present invention can be used for screening for therapeutic agents that modulate cellular activities including, but not limited to, microtubule organization; microtubule nucleation; post-translational protein modification, secretion and transport; and cell division, proliferation and migration.

15 In an embodiment, the present invention provides a method for selecting an agent that modulates the activity of MMG8 or an MMG8 variant, comprising:

- a) providing a first test sample containing cells expressing MMG8 or the MMG8 variant;
- b) contacting the first test sample with a protein that interacts with MMG8 or the
20 MMG8 variant;
- c) determining a first level of the binding of MMG8 or the MMG8 variant to said protein that interacts with MMG8 or the MMG8 variant in the first test sample;
- d) providing a second test sample containing cells expressing MMG8 or the MMG8 variant;
- 25 e) contacting the second sample with a candidate agent;
- f) after step e), contacting the second test sample with said protein that interacts with MMG8 or the MMG8 variant;
- g) determining a second level of the binding of MMG8 or the MMG8 variant to said protein that interacts with MMG8 or the MMG8 variant in the second test sample; and
- 30 h) selecting the candidate agent as a modulator of MMG8 or the MMG8 variant, if said second level is different from said first level.

In an embodiment, the protein that interacts with MMG8 or MMG8 variants is selected from AKAP450, EB1, EB3, GCP2, or GCP3. In an embodiment, the candidate agent is selected as an MMG8 modulator, if the difference between the first level and the second level is more than 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%. In an embodiment, if said second level is lower than said first level, the selected agent is an MMG8 inhibitor. In an embodiment, if said second level is higher than said first level, the selected agent is an MMG8 inhibitor.

Agents that modulate the level and/or activity of MMG8 or MMG8 variants of the invention can be used for treatment of diseases including, but not limited to, diseases involving defects in microtubule organization and/or nucleation; diseases associated with defects in protein modification, secretion, and trafficking; and diseases involving abnormal cell proliferation. In a specific embodiment, agents that modulate MMG8 level or activity can be used to treat diseases including, but not limited to, neuronal diseases, diabetes, cystic fibrosis, and lysosomal storage diseases (such as diseases involving LAMP-1).

In another specific embodiment, agents that modulate MMG8 level or activity can be used to treat or retard cancer cell division, proliferation, and/or migration. The MMG8-modulators can be used to treat cancer including, but not limited to, brain carcinoma, skin cancer, stomach cancer, and breast cancer.

In a further embodiment, the present invention provides a method for screening for anti-cancer agents, wherein the method comprises:

- a) providing a test sample containing tumor cells expressing MMG8 or an MMG8 variant;
- b) contacting a candidate agent with the test sample;
- c) determining a level of tumor cell population in a test sample;
- d) comparing said level in the test sample with that of a control sample; and
- e) selecting the candidate agent if said level in the test sample is lower than that of the control sample.

In an embodiment, the candidate agent is selected as an anti-cancer agent, if the level of tumor cell population in the test sample is more than 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% lower than that of the control sample.

Treatment of Diseases Associated with MMG8

In another aspect, the present invention provides prevention or treatment of diseases associated with defects in MMG8. In one embodiment, the present invention can be used to treat diseases including, but not limited to, neuronal diseases, diabetes, cystic fibrosis, lysosomal storage diseases, and cancer.

In one embodiment, the present invention provides a method for treating a disease associated with abnormally increased level of MMG8 or an MMG8 variant, comprising: inhibiting or reducing the expression of MMG8 or the MMG8 variant in a subject.

In a specific embodiment, the present invention provides a method for treating a disease associated with abnormally increased level of MMG8, comprising administering, to a subject in need of such treatment, an effective amount of an si-RNA that inhibits the expression of MMG8 or MMG8 variants in the subject. In another specific embodiment, the level or activity of MMG8 or MMG8 variants can be reduced or inhibited by administering, to a subject in need of such treatment, an effective amount of an anti-MMG8 antibody that binds specifically to MMG8 or an MMG8 variant. Diseases associated with abnormally increased level of MMG8 include, for example, cancer such as brain carcinoma, skin cancer, stomach cancer, and breast cancer. Specifically, the present invention can be used to inhibit tumor metastasis.

In another embodiment, the present invention provides a method for treating a disease associated with abnormally reduced level of MMG8, comprising: increasing the expression of MMG8 or an MMG8 variant.

In a specific embodiment, the present invention provides a method for treating a disease associated with abnormally reduced level of MMG8, comprising administering, to a subject in need of such treatment, an effective amount of an MMG8 protein or a fragment thereof, a nucleic acid molecule encoding the MMG8 protein or a fragment thereof, or a vector or host cell comprising said nucleic acid molecule. Diseases associated with abnormally decreased MMG8 level include lysosomal storage diseases.

In another aspect, the present invention provides a method for modulating a cellular activity, comprising modulating the level or activity of MMG8 in a subject. Cellular activities that can be modulated in accordance with the present invention include, but are not limited to, microtubule growth, organization, and/or nucleation at the Golgi apparatus; the

stability of AKAP450; the localization of γ TuCs to the Golgi apparatus; and post-translation modification (such as glycosylation), transport, and secretion of proteins.

In one embodiment, the present invention provides a method of inhibiting ER-to-Golgi transport of LAMP-1, by inhibiting the expression or activity of myomegalin, wherein the method comprises administering, to cells in which said inhibition is desired, an effective amount of an siRNA that inhibits the expression of the myomegalin isoform of the present invention, or an antibody or aptamer that binds specifically to the myomegalin isoform of present invention. In a specific embodiment, the method can be used to treat LAMP-1-related lysosomal storage diseases.

In another embodiment, the present invention provides a method of reducing the level of glycosylated CD44 having a molecular weight of about 90 kDa, comprising administering to cells in which said reduction is desired, an effective amount of an siRNA that inhibits the expression of the myomegalin isoform of the present invention, or an antibody or aptamer that binds specifically to the myomegalin isoform of the present invention.

In another embodiment, the present invention provides a method of increasing the level of glycosylated CD44 having a molecular weight of about 90 kDa, comprising administering to cells in which said increase is desired, an effective amount of the myomegalin isoform of the present invention, or a nucleic acid molecule encoding the myomegalin isoform of the present invention.

The term “subject,” as used herein, describes an organism, including mammals such as primates. Mammalian species that can benefit from the subject methods include, but are not limited to, apes, chimpanzees, orangutans, humans, monkeys; and domesticated and/or laboratory animals such as dogs, cats, horses, cattle, pigs, sheep, goats, chickens, mice, rats, guinea pigs, and hamsters. Typically, the subject is a human.

The term “treatment” or any grammatical variation thereof (*e.g.*, treat, treating, and treatment *etc.*), as used herein, includes but is not limited to, ameliorating or alleviating a symptom of a disease or condition, reducing, suppressing, inhibiting, lessening, or affecting the progression, severity, and/or scope of a condition.

The term “prevention” or any grammatical variation thereof (*e.g.*, prevent, preventing, and prevention *etc.*), as used herein, includes but is not limited to, delaying the onset of symptoms, preventing relapse to a disease, increasing latency between symptomatic episodes,

or a combination thereof. Prevention, as used herein, does not require the complete absence of symptoms.

“A subject in need of such treatment”, as used herein, includes a subject who is specifically at risk of, has symptoms of, or is diagnosed with, a diseases associated with MMG8 or an MMG isoform. In a specific embodiment, the present invention comprises diagnosing whether a subject has a disease associated with MMG8, wherein the therapeutic agents and compositions of the present invention are administered to the subject who is diagnosed with such disease.

The term “effective amount,” as used herein, refers to an amount that is capable of preventing, ameliorating, or treating a disease. In certain embodiments, the effective amount enables at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, or 100% modulation of the level and/or activity of MMG8.

Therapeutic Compositions and Formulations

The subject invention further provides therapeutic compositions that contain a therapeutically effective amount of the therapeutic agents and compositions and a pharmaceutically acceptable carrier or adjuvant.

The terms “pharmaceutically acceptable,” “physiologically tolerable,” and grammatical variations thereof, as used herein, include compositions, carriers, diluents, and reagents, are used interchangeably, and represent that the materials are capable of administration to or upon a subject such as mammal.

The term “carrier” refers to an adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum oil such as mineral oil, vegetable oil such as peanut oil, soybean oil, and sesame oil, animal oil, or oil of synthetic origin.

Suitable carriers also include ethanol, dimethyl sulfoxide, glycerol, silica, alumina, starch, sorbitol, inositol, xylitol, D-xylose, mannitol, powdered cellulose, microcrystalline cellulose, talc, colloidal silicon dioxide, calcium carbonate, calcium phosphate, calcium aluminium silicate, aluminium hydroxide, sodium starch phosphate, lecithin, and equivalent carriers and diluents. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions.

Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The therapeutic composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary, depending on the type of the condition and the subject to be treated. In general, a therapeutic composition contains from about 5% to about 95% active ingredient (w/w). More specifically, a therapeutic composition contains from about 20% (w/w) to about 80% or about 30% to about 70% active ingredient (w/w).

The therapeutic agents and compositions of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, Remington's Pharmaceutical Science by E. W. Martin describes formulations which can be used in connection with the subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of the bioactive compound(s) is combined with a suitable carrier in order to facilitate effective administration of the composition.

The therapeutic or pharmaceutical compositions of the subject invention can also be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectables either as liquid solutions or suspensions; however, solid forms suitable for solution, or suspensions, in liquid prior to use also can be prepared. The preparation also can be emulsified, such as oil-in-water emulsion.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients, *e.g.*, compound, carrier suitable for administration.

5 Routes of Administration

The therapeutic agents and compositions of the subject invention can be administered to the subject being treated by standard routes, including oral, inhalation, or parenteral administration including intravenous, subcutaneous, topical, transdermal, intradermal, transmucosal, intraperitoneal, intramuscular, intracapsular, intraorbital, intracardiac, 10 transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, and intrasternal injection, infusion, and electroporation, as well as co-administration as a component of any medical device or object to be inserted (temporarily or permanently) into a subject.

The amount of the therapeutic agent or pharmaceutical composition of the subject 15 invention which is effective in the treatment of a disease will depend on the nature of the disease, condition or disorder and can be determined by standard clinical techniques. In general, the dosage ranges from about 0.01 to about 2000 mg, about 0.01 to about 1000 mg, about 0.01 to about 500 mg, about 0.01 to about 300 mg, about 0.01 to about 200 mg, or about 0.01 to about 100 mg. Such a unit dose may be administered once to several times (*e.g.* 20 two, three and four times) every two weeks, every week, twice a week, or every day, according to the judgment of the practitioner and each patient's circumstances.

Illustratively, dosage levels of the administered active ingredients can be: intravenous, 0.01 to about 20 mg/kg; intraperitoneal, 0.01 to about 100 mg/kg; subcutaneous, 0.01 to about 100 mg/kg; intramuscular, 0.01 to about 100 mg/kg; orally 0.01 to about 200 mg/kg 25 and preferably about 1 to 100 mg/kg; intranasal instillation, 0.01 to about 20 mg/kg; and aerosol, 0.01 to about 20 mg/kg of animal (body) weight.

Once improvement of the patient's condition has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, may be reduced as a function of the symptoms to a level at which the improved 30 condition is retained. When the symptoms have been alleviated to the desired level, treatment should cease. Patients may however require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, condition or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

5 Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Diagnosis of Diseases Associated with MMG8

10 Another aspect of the present invention pertains to methods for diagnosis of a disease associated with an abnormal level of MMG8 or an MMG8 variant of the invention. In an embodiment, the method comprises:

- a) obtaining a biological sample from a subject;
- b) measuring a level of MMG8 or an MMG8 variant in the biological sample; and
- c) characterizing the subject's level of MMG8 or the MMG8 variant.

15 In a specific embodiment, the MMG8 protein biomarker comprises SEQ ID NO:2.

Detection of MMG8-associated diseases can be based on characterizing the level of MMG8 or an MMG8 variant in a sample with a predetermined level of the biomarker in a normal population sample and correlating such levels with factors such as the incidence, severity, and/or frequency of developing the specific disease in a population. In addition, the
20 predetermined value can be a single value, multiple values, a single range, or multiple ranges. Thus, diagnosis may be based on determining in which of the predetermined reference values or ranges the subject's level falls.

The MMG8 level can be determined based on the levels of MMG8 proteins or MMG8 transcripts. In a specifically exemplified embodiment, the MMG8 biomarker can be used for
25 diagnosis of lysosomal storage diseases.

Devices

The methods of the invention can be carried out on a solid support. The solid supports used may be those which are conventional for the purpose of assaying an analyte in
30 a biological sample, and are typically constructed of materials such as cellulose, polysaccharide such as Sephadex, and the like, and may be partially surrounded by a housing for protection and/or handling of the solid support. The solid support can be rigid, semi-rigid,

flexible, elastic (having shape-memory), *etc.*, depending upon the desired application. MMG8 can be detected in a sample *in vivo* or *in vitro* (*ex vivo*). When, according to an embodiment of the invention, the amount of MMG8 in a sample is to be determined without removing the sample from the body (*i.e.*, *in vivo*), the support should be one which is
5 harmless to the subject and may be in any form convenient for insertion into an appropriate part of the body. For example, the support may be a probe made of polytetrafluoroethylene, polystyrene or other rigid non-harmful plastic material and having a size and shape to enable it to be introduced into a subject. The selection of an appropriate inert support is within the competence of those skilled in the art, as are its dimensions for the intended purpose.

10 A contacting step in the assay (method) of the invention can involve contacting, combining, or mixing the biological sample and the solid support, such as a reaction vessel, microvessel, tube, microtube, well, multi-well plate, or other solid support. In an embodiment of the invention, the solid support to be contacted with the biological sample has an absorbent pad or membrane for lateral flow of the liquid medium to be assayed, such as
15 those available from Millipore Corp. (Bedford, MA), including but not limited to Hi-Flow Plus™ membranes and membrane cards, and SureWick™ pad materials.

Immunochromatographic assays, also known as lateral flow test strips or simply strip tests, for detecting various analytes of interest, have been known for some time, and may be used for detection of MMG8. The benefits of lateral flow tests include a user-friendly format,
20 rapid results, long-term stability over a wide range of climates, and relatively low cost to manufacture. These features make lateral flow tests ideal for applications involving home testing, rapid point of care testing, and testing in the field for various analytes. The principle behind the test is straightforward. Essentially, any ligand that can be bound to a visually detectable solid support, such as dyed microspheres, can be tested for, qualitatively, and in
25 many cases even semi-quantitatively. For example, a one-step lateral flow immunostrip for the detection of free and total prostate specific antigen in serum is described in Fernandez-Sanchez *et al.* (*J. Immuno. Methods*, 2005, 307(1-2):1-12, which is incorporated herein by reference) and may be adapted for detection of MMG8 in a biological sample.

30 Samples and/or MMG8-specific binding agents may be arrayed on the solid support, or multiple supports can be utilized, for multiplex detection or analysis. "Arraying" refers to the act of organizing or arranging members of a library (*e.g.*, an array of different samples or an array of devices that target the same target molecules or different target molecules), or

other collection, into a logical or physical array. Thus, an “array” refers to a physical or logical arrangement of, *e.g.*, biological samples. A physical array can be any “spatial format” or physically gridded format” in which physical manifestations of corresponding library members are arranged in an ordered manner, lending itself to combinatorial screening. For example, samples corresponding to individual or pooled members of a sample library can be arranged in a series of numbered rows and columns, *e.g.*, on a multi-well plate. Similarly, binding agents can be plated or otherwise deposited in microtitered, *e.g.*, 96-well, 384-well, or-1536 well, plates (or trays). Optionally, MMG8-specific binding agents may be immobilized on the solid support.

Detection of MMG8 and other biomarkers, and other assays that are to be carried out on samples, can be carried out simultaneously or sequentially with the detection of other target molecules, and may be carried out in an automated fashion, in a high-throughput format.

The MMG8-specific binding agents can be deposited but “free” (non-immobilized) in the conjugate zone, and be immobilized in the capture zone of a solid support. The MMG8-specific binding agents may be immobilized by non-specific adsorption onto the support or by covalent bonding to the support, for example. Techniques for immobilizing binding agents on supports are known in the art and are described for example in U.S. Patent Nos. 4,399,217, 4,381,291, 4,357,311, 4,343,312 and 4,260,678, which are incorporated herein by reference.

Kits

In another aspect, the present invention provides kits comprising the required elements for detecting the level or activity of MMG8 or an MMG8 variant, screening for therapeutic agents, and/or diagnosing or monitoring a disease associated with MMG8 or an MMG8 variant. Preferably, the kits comprise a container for collecting biological fluid from a patient and an agent for detecting the presence of MMG8 or its encoding nucleic acid in the fluid. The components of the kits can be packaged either in aqueous medium or in lyophilized form.

In a preferred embodiment, the present invention provides a protein chip comprising the MMG8 or MMG8 variants of the present invention. In a specific embodiment, the protein chip comprises the MMG8 isoform comprising SEQ ID NO:2.

The methods of the invention can be carried out using a diagnostic kit for qualitatively or quantitatively detecting MMG8 in a sample. By way of example, the kit can contain binding agents (*e.g.*, antibodies) specific for MMG8, antibodies against the antibodies labeled with an enzyme; and a substrate for the enzyme. The kit can also contain a solid support such as microtiter multi-well plates, standards, assay diluent, wash buffer, adhesive plate covers, and/or instructions for carrying out a method of the invention using the kit. In one embodiment, the kit includes one or more protease inhibitors (*e.g.*, a protease inhibitor cocktail) to be applied to the biological sample to be assayed.

The agent(s) can be packaged with a container for collecting the biological fluid from a patient. When the antibodies or binding partner are used in the kits in the form of conjugates in which a label is attached, such as a radioactive metal ion or a moiety, the components of such conjugates can be supplied either in fully conjugated form, in the form of intermediates or as separate moieties to be conjugated by the user of the kit.

Kits containing one or more agents that detect MMG8 nucleic acid molecules, such as but not limited to, the full length MMG8 nucleic acid, MMG8 oligonucleotides, and pairs of MMG8 primers can also be prepared. The agent(s) can be packaged with a container for collecting biological samples from a patient. The nucleic acid can be in the labeled form or to be labeled form.

Other components of the kit may include, but are not limited to, means for collecting biological samples, means for labeling the detecting agent (binding agent), membranes for immobilizing the MMG8 protein or MMG8 nucleic acid in the biological sample, means for applying the biological sample to a membrane, means for binding the agent to MMG8 in the biological sample of a subject, a second antibody, a means for isolating total RNA from a biological fluid of a subject, means for performing gel electrophoresis, means for generating cDNA from isolated total RNA, means for performing hybridization assays, and means for performing PCR, *etc.*

In these exemplary embodiments, the antibodies can be labeled with pairs of FRET dyes, bioluminescence resonance energy transfer (BRET) protein, fluorescent dye-quencher dye combinations, beta gal complementation assays protein fragments. The antibodies may participate in FRET, BRET, fluorescence quenching or beta-gal complementation to generate fluorescence, colorimetric or enhanced chemiluminescence (ECL) signals, for example.

These methods are routinely employed in the detection of antigen-specific antibody responses, and are well described in general immunology text books such as Immunology by Ivan Roitt, Jonathan Brostoff and David Male (London: Mosby, c1998. 5th ed. and Immunobiology: Immune System in Health and Disease/Charles A. Janeway and Paul Travers. Oxford: Blackwell Sci. Pub., 1994), the contents of which are herein incorporated by reference.

MATERIALS AND METHODS

Cloning of MMG8:

Based on peptide sequences revealed by mass spectrometry, the coding sequence of MMG8 was constructed by replacing a carboxy-terminal sequence of KIAA0477 with the corresponding sequence of MMG8 amplified by RT-PCR. Mutations were introduced into MMG8 by site-directed mutagenesis.

Generation of MMG8 antibodies:

Two MMG8 fragments, 637-925 and 926-1116, were cloned and bacterially expressed in fusion with a His₆-tag. The proteins were purified using Ni²⁺-nitrilotriacetic acid resins (Qiagen) in the presence of 6 M urea, and were dialyzed against phosphate-buffered saline. Thereafter, rabbits were immunized with the 637-925 or 926-1116 fragment. Antisera generated against 637-925 and 926-1116 were designated as 443M and 532C, respectively. Antibodies were purified from the sera using respective antigens immobilized onto nitrocellulose membranes.

Suppression of MMG8 expression:

Two siRNA duplexes were synthesized to target MMG8 (si-MMG8-1, AACCUCAGUGGCUGAAAGAA (SEQ ID NO: 25); si-MMG8-2, AAGCAGAGAGACAGCUCUAUA) (SEQ ID NO: 26). The siRNAs were delivered into the cells with Lipofectamine 2000 (Invitrogen). MMG8 expression was reduced by 80-90% at 70-80 hours post-transfection.

Isolation of MMG8 and its interacting proteins:

To immunoprecipitate MMG8, cell extracts were prepared in RIPA buffer (25 mM Tris-HCl, pH7.4, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS) supplemented with the Protease Inhibitor Cocktail (Roche) and clarified. After pre-cleared with Protein A-Agarose (Invitrogen), the extracts were incubated with MMG8 antibodies bound to Protein A beads for 4 h at 4°C with agitation. The beads were extensively washed before the immunoprecipitates were eluted for SDS-PAGE.

To prepare for mass spectrometry analysis, protein bands visualized by Coomassie Blue staining were excised and then subjected to in-gel tryptic digestion. Recovered peptides were introduced through a nanoelectrospray ion source into a quadrupole/time-of-flight mass spectrometer. Protein identity was revealed by searching a nonredundant sequence database with tandem mass spectra.

Anti-FLAG immunoprecipitation was performed using anti-FLAG-coupled beads (M2, Sigma) in cell extracts prepared with a lysis buffer (20 mM Tris-HCl, pH7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM dithiothreitol, and the Protease Inhibitor Cocktail). Immunoprecipitated proteins were detected by immunoblotting.

Immunofluorescence microscopy:

Cell cultures were maintained in media containing 10% fetal bovine serum at 37°C in 5% CO₂. HeLa, HEK293T, MCF-7, IMR-5 (human neuroblastoma cell line) and C2C12 (mouse myoblast) were grown in DMEM; human retinal pigment epithelial cells hTERT-RPE1 (RPE1) was in DMEM:Ham's F12 (1:1) (Invitrogen); human fetal lung fibroblasts MRC-5 were grown in MEM (Invitrogen). C2C12 was differentiated into myotubes in the medium of DMEM supplemented with 20% horse serum.

To perform immunostaining, cells were fixed either with cold methanol for 5 min at -20°C or with 4% paraformaldehyde/phosphate-buffered saline for 15 min at room temperature. After staining with primary and subsequently secondary antibodies, cell images were acquired on an epifluorescence microscope (Eclipse TE2000, Nikon).

To visualize γ -tubulin and EB1 on the Golgi apparatus, cells were extracted on ice for 30 min in a saponin extraction buffer (0.1 M K-PIPES, pH6.9, 2 M glycerol, 5 mM MgCl₂, 2 mM EGTA, and 0.1% saponin) before methanol fixation. To enhance γ -tubulin signals, the cells were stained sequentially with two secondary antibodies, AlexaFluor dye-labeled goat

secondary antibody (Invitrogen), followed by donkey anti-goat secondary antibody labeled with same dye.

Microtubule regrowth assays:

5 Cellular microtubules were completely depolymerized by placing cells on ice for 1 h or treating them with 10 μ g/ml nocodazole for 2 h. To initiate microtubule regrowth, cells treated under cold conditions were transferred to a 37°C water bath. Nocodazole-treated cells were washed several times with ice-cold phosphate-buffered saline and then incubated in medium prewarmed to 37°C. Before fixation, cells were extracted briefly with a
10 cytoskeleton-stabilizing buffer (50 mM imidazole, pH6.8, 50 mM KCl, 0.5 mM MgCl₂, 0.1 mM EGTA and 0.1 mM EDTA, 4% PEG4000, and 0.1% saponin).

Microtubule binding assay:

To test microtubule association, HEK293T extracts were prepared in PEM buffer (100
15 mm PIPES, pH6.9, 1 mM EGTA, 1 mM MgCl₂, and 1% Triton X-100) on ice and clarified by centrifugation at 100,000 g for 30 min. Microtubules were then polymerized in the extracts with 20 μ M taxol and 0.5 mM GTP at 37°C for 20 min. After polymerization, microtubules were overlaid on a cushion of 20% sucrose in the buffer for sedimentation by centrifugation (30,000 g; 30 min) at 25°C. The supernatants and pellets were resolved by SDS-PAGE for
20 immunoblotting.

Cell proliferation assay:

HeLa cells growing on tissue culture dishes were transfected with control or MMG8-targeting siRNA. Cells were then trypsinized at the indicated time points to count cell
25 numbers with a hemocytometer. Data presented are from three independent assays.

EXAMPLES

Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent
30 mixture proportions are by volume unless otherwise noted.

EXAMPLE 1 - IDENTIFICATION OF MMG8

To detect MMG in proliferating cell cultures, RT-PCR was performed with oligonucleotide primers targeting the region encoding 474-762 of human MMG isoform 1. This targeted region is present in all currently-identified large MMG variants based on searches of gene databases. An oligonucleotide band was specifically amplified at the
5 expected size from total RNA extracted from HeLa cells (Fig. 1A). The sequence of the RT-PCR product was verified. This fragment corresponds to the region encoding amino acids 474-762 of MMG isoform 1 and 637-925 of MMG8.

The RT-PCR product was also cloned into an expression vector and expressed in bacteria. The expressed protein was purified for immunizing rabbits. The resulting antibody,
10 designated as 443M, specifically detected a human MMG protein - a single band of ~150 kDa on the immunoblots of HeLa extracts (Fig. 1B).

The MMG protein was immunoprecipitated by 443M from HeLa extracts prepared in the RIPA buffer (Fig. 1C), and excised for mass spectrometric analysis. In total, 13 peptide sequences were revealed by tandem mass spectrometry. Most of the peptide sequences
15 matched the MMG variant GenBank Accession No. NP_001002811 (SEQ ID NO:7), and the last 4 peptides encoded by the cDNA sequence hCG1755149 (Fig. 1E and TABLE 1). In particular, Peptide 13 began with the last 9 residues encoded by the overlapping region of these two cDNA sequences followed by 7 residues present only in hCG1755149. The sequence of immunoprecipitated protein MMG8 is largely similar to NP_001002811 (SEQ
20 ID NO:7), with the exception of the carboxyl tail region.

Table 1. Peptides identified from MMG8 by mass spectrometry

Peptide No.	Sequence
P1	IEALSIER (SEQ ID NO: 27)
P2	IQQTEATNK (SEQ ID NO: 28)
P3	QDGTIQNLK (SEQ ID NO: 29)
P4	ALQQLQEELQNK (SEQ ID NO:30)
P5	EQLLQEFRELLQYR (SEQ ID NO: 31)
P6	EQESIIQQLQTS�HDR (SEQ ID NO: 32)
P7	NSELQALR (SEQ ID NO: 33)
P8	QTDQGSMQIPSR (SEQ ID NO: 34)
P9	STLGDLDTVAGLEK (SEQ ID NO: 35)
P10	LTQEVLLLR (SEQ ID NO: 36)
P11	LNEALQAER (SEQ ID NO: 37)
P12	TLQVELEGAQVLR (SEQ ID NO: 38)
P13	LETLAAIGGGELESVR (SEQ ID NO: 39)

The novel MMG isoform, designated as MMG8, was cloned based on protein
 5 sequencing and RT-PCR data. The carboxy-terminal region of MMG8 is different from all
 other MMG variants. The homolog of MMG8 was detected in *Pongo abelii* (Genbank
 accession: NP_001126198) (SEQ ID NO: 12) and mouse (Genbank accession: AAI41173)
 (SEQ ID NO: 13), with overall sequence homologies of 98% and 92%, respectively. MMG8
 also exhibits significant homology to *Gallus gallus* XP_423459 (SEQ ID NO: 14), *Xenopus*
 10 *tropicalis* NP_001072886 (SEQ ID NO: 15), and *Danio rerio* CAI11891 (SEQ ID NO: 16)
 and NP_956195 (SEQ ID NO: 17). MMG8 homologues have been observed in chicken,
Xenopus, and *zebrafish*, in addition to mammals, suggesting their conservation in vertebrates.
 In the cell cultures examined, MMG8 is the major isoform expressed from the human MMG

gene, which is multispliced. To date, the other known splice product of human MMG is MMG isoform 1 (Verde *et al.*, *J. Biol. Chem.* 276:11189-11198 (2001)).

A second antibody, 532C, was generated against the carboxy-terminus of MMG8. The 523C and 443M antibodies yielded identical results in immunoprecipitation and immunoblots; unless otherwise specified, the results shown herein were obtained with 532C.

A single protein band of MMG8 was detected on an immunoblot of proliferating epithelial, fibroblast, and neuroblastoma cells (Fig. 1D). In C2C12 myotubes, two smaller protein bands were found beside the ~150 kDa species (Fig. 1D), suggesting the existence of other isoforms or proteolytic products. However, MMG isoform 1, a ~230 kDa species, was not detectable in these cell cultures (Fig. 1D), although it was readily recognized by the antibody in rat heart tissue.

To explore the function of MMG8, RNAi-mediated suppression of MMG8 expression was performed. Two siRNA oligonucleotides were designed to target MMG8. Transfection of either siRNA effectively depleted the protein by ~90% (Fig. 1F). Cells expressing MMG8 showed prominent Golgi localization; whereas cells transfected with MMG8-targeting siRNA exhibited a weak background when labeled with anti-MMG8 antibodies and showed no Golgi localization pattern (Fig. 1G), corroborating the specificity of anti-MMG8 staining. In addition, in cells transfected with MMG8-siRNA, the Golgi ribbons broke into patches that overlapped largely with the nuclei (Fig. 1G). The results showed that MMG8 is required for the Golgi structural organization.

EXAMPLE 2 - ASSOCIATION OF MMG8 WITH AKAP450

To identify proteins that bind to MMG8, MMG8 immunoprecipitation was performed and the immunoprecipitates were analyzed by mass spectrometry. The results show that MMG8 interacts with the regulatory subunit of protein kinase A (PKA) and EB1.

To investigate the interaction between MMG8 and PKA, co-immunoprecipitation of MMG8 and AKAP450 was performed. AKAP450 is a *cis*-Golgi protein that binds to the regulatory subunit of protein kinase A (Keryer *et al.*, *Exp. Cell Res.* 204:230-240 (1993); Schmidt *et al.*, *J. Biol. Chem.* 274:3055-3066 (1999); Takahashi *et al.*, *J. Biol. Chem.* 274:17267-17274 (1999); Witczak *et al.*, *EMBO J.* 18:1858-1868 (1999); Rivero *et al.*, *EMBO J.* 28:1016-1028 (2009)). The reciprocal experiments revealed that MMG8 and AKAP450 co-precipitated each other specifically (Fig. 2A). The immunodepletion of MMG8

almost completely depleted AKAP450 from the HeLa lysates (Fig. 2A). In the reciprocal experiment, anti-AKAP450 immunoprecipitation proportionally co-precipitated MMG8 (Fig. 2A). The quantification of the co-immunoprecipitates revealed that MMG8 and AKAP450 formed a stoichiometric complex in lysates.

5 To identify the MMG8 domain that interacts with AKAP450, various MMG8 fragments were ectopically expressed for AKAP450 co-immunoprecipitation. The results showed that the head region consisting of amino acids 1-389 was essential for binding with AKAP450; whereas the MMG8 fragment comprising amino acids 390-1116 did not bind to AKAP450 (Fig. 2B). In addition, truncation of 1-389 significantly impaired the AKAP450
10 binding activity (Fig. 2B). Also, only the fragment that comprises 1-389 of MMG8 binds to protein kinase A (Fig. 2C). The results showed that MMG8 indirectly associates with protein kinase A through AKAP450.

When expressed at low levels, the MMG8 fragment comprising amino acids 1-389 displayed specific Golgi localization (Fig. 2D). When highly expressed, this MMG8 fragment
15 dislodged endogenous MMG8 from the Golgi apparatus (Fig. 2D). In contrast, the expression of 389-1116 did not display any specific pattern (Fig. 2D). The results show that amino acid residues 1-389 serve as the AKAP450-binding and Golgi-targeting domain of MMG8.

In experiments on RNAi-mediated MMG8 depletion, AKAP450 was probed on immunoblots. The effective suppression of MMG8 expression remarkably reduced the
20 protein level of AKAP450; the reduction of AKAP450 correlated with that of MMG8 (Fig. 3A). Similarly, the amount of MMG8 decreased significantly (by ~65%) when the expression of AKAP450 was suppressed by ~80% using siRNA (Fig. 3A). The results showed that the protein levels of MMG8 and AKAP450 are interrelated.

To investigate whether MMG8 and AKAP450 depend on each other for stability, a
25 proteasome inhibitor, MG132, was applied to cells either depleted of MMG8 or AKAP450. The protein level of AKAP450 in MMG8-depleted cells increased along the time course of MG132 treatment and eventually approached the level in control cells (Fig. 3B). The amount of MMG8 in AKAP450-depleted cells also increased significantly by the MG132 treatment (Fig. 3B). In control-transfected cells, the levels of MMG8 and AKAP450 were not
30 significantly altered by MG132 (Fig. 3B). The results reveal that in the absence of either MMG8 or AKAP450, the remaining protein becomes unstable and is degraded by the proteasome.

EXAMPLE 3 – EFFECTS OF MMG8 ON MICROTUBULE NUCLEATION AT THE GOLGI APPARATUS

This Example assesses whether MMG8 interacts with the γ TuCs, which associates with AKAP450 – a *cis*-Golgi protein indispensable for microtubule nucleation at the Golgi apparatus (Rivero *et al.*, *EMBO J.* 28:1016-1028 (2009); Takahashi *et al.*, *Mol. Biol. Cell* 13:3235-3245 (2002)).

Anti-MMG8 immunoprecipitation co-precipitated γ -tubulin and GCP2 (gamma-tubulin complex protein 2), which are core components of the γ TuCs examined (Fig. 4A). In a reciprocal experiment, MMG8 specifically co-precipitated with GCP3 (gamma-tubulin complex protein 3), another core component of γ TuCs (Fig. 4B). These results demonstrate that MMG8 associates with γ TuCs.

To examine whether the Golgi localization of γ TuCs requires MMG8, MMG8 expression was silenced and immunostaining of γ -tubulin was performed. RNAi-mediated depletion of MMG8 eliminated the Golgi attachment of γ -tubulin, without apparently affecting its centrosomal staining (Fig. 4C). The results show that MMG8 is required for the recruitment of γ TuCs to the Golgi apparatus, but is not required for the recruitment of γ TuCs to centrosomes.

This Example also examined the role of MMG8 in microtubule nucleation after nocodazole-induced depolymerization. Prior to the assays, RPE1 cells were transfected with either a control or MMG8-targeting siRNA. In nocodazole-treated control cells, short microtubules appeared at the fragmented Golgi apparatus as well as a centrosomal aster of microtubules 2 min after nocodazole washout (Fig. 5A). In comparison, the MMG8-depleted cells did not exhibit microtubule growth from the Golgi apparatus; the centrosomal regrowth was not discernibly affected by MMG8 depletion (Fig. 5A). The results showed that MMG8 depletion inhibits microtubule nucleation at the Golgi apparatus, but does not affect centrosomal nucleation.

In RPE1 cells, microtubules are focused on the Golgi apparatus and its colocalized centrosomes to form a radial pattern, and the Golgi region harbors a high density of microtubules (Fig. 5B). The suppression of MMG8 expression significantly reduced microtubule density at the Golgi apparatus (Fig. 5B). Also, in MMG8-depleted cells, the

microtubules were unfocused on the Golgi region (Fig. 5B). The data further support that MMG8 is required for microtubule growth and organization at the Golgi apparatus.

EXAMPLE 4 - ASSOCIATION OF MMG8 WITH EB1/EB3

5 The mass spectrometry results showed that MMG8 interacts with EB1. Anti-MMG8 immunoprecipitation was carried out to detect whether MMG8 co-precipitates with endogenous EB1. Immunoblotting of the immunoprecipitates revealed the specific association of EB1 with MMG8 (Fig. 6A).

MMG8 comprises a putative SxIP motif that is a microtubule plus-end-tracking signal associated with EB1/EB3. Within the motif, the Ile/Leu-Pro dipeptide is critical for EB1/EB3 binding (Honnappa *et al.*, *EMBO J.* 24:261-269 (2005)). A mutant MMG8 was constructed, wherein the dipeptide residue Leu311-Pro312 was substituted with two alanines. An MMG8 fragment (i.e., 1-389) that encompasses the SxIP motif (Ser-x-Ile-Pro) and its L311A/P312A mutant were constructed.

15 Fragment 1-389 displayed a similar EB1-binding activity as the full-length protein (Fig. 6, B-C). Unlike the wild-type MMG8, L311A/P312A mutants completely lost the binding activity (Fig. 6, B-C). The data confirmed that the SxIP motif of MMG8 is required for binding to EB1.

The Example also examines the interaction between MMG8 and EB3. EB3 was detected in the immunoprecipitates of wild-type MMG8, but not in the immunoprecipitates of the L311A/P312A mutant (Fig. 6D). The results show that the SxIP motif of MMG8 is required for EB3 binding.

EB1 resides in the cytoplasm and associates with microtubules. To visualize whether EB1 localizes at the Golgi apparatus, cells were extracted under a condition to preserve Golgi networks and to remove cytosolic and microtubule-associated proteins. Following extraction, cells were subjected to immunostaining. EB1 displayed Golgi patterns that merged well with the fluorescent signals of MMG8 (Fig. 6E). The RNAi-mediated MMG8 depletion eliminated the Golgi staining of EB1 (Fig. 6E), but did not discernibly affect attachment of EB1 to the growing microtubule tips. The results show that EB1 targets the Golgi apparatus in a manner dependent on its association with MMG8.

30 To test whether MMG8 associates with microtubules and whether EB1/EB3 is involved in such association, a microtubule binding assay was performed. Briefly,

microtubules were pelleted from cell extracts after taxol-induced polymerization. To disrupt the interaction between MMG8 and EB1/EB3, the carboxy-terminal fragment EB1(185-268), which harbors the EBH domain that binds to the SxIP motif (Askham *et al.*, *Mol. Biol. Cell* 13:3627-3645 (2002)), was over-expressed. As a control, the ectopic tag (GFP) was expressed.

The results showed that, in the absence of microtubules, MMG8 did not sediment (Fig. 7). In control cells, MMG8 was readily detected in the microtubule pellet (Fig. 7). The over-expression of the EB1 fragment 185-268 dramatically reduced the co-sedimentation of MMG8 with microtubules (Fig. 7). The results show that MMG8 associates with microtubules by forming a complex with EB1/EB3.

EXAMPLE 5 - MMG8 IS REQUIRED FOR CELL PROLIFERATION

This Example shows that MMG8 is required for cell proliferation. HeLa cells transfected with control siRNA exhibited normal cell growth (Fig. 8). The disruption of MMG8 expression blocked cell proliferation (Fig. 8). In addition, the expression disruption did not cause significant cell death. Similar results were obtained using the breast cancer line MCF-7. The results show that MMG8 inhibition suppresses cancer cell proliferation and MMG8 can serve as a target for screening of candidate anti-cancer therapeutics.

EXAMPLE 6 - KNOCKDOWN OF MMG8 CAUSES DEFECTIVE PROTEIN GLYCOSYLATION

Various membrane-bound or secreted proteins are transported from the ER to the Golgi apparatus, where they undergo post-translational modifications such as glycosylation. This Example investigates the effects of MMG8 on post-translational modification such as glycosylation.

To illustrate, the glycosylation of LAMP-1 and CD44 were examined. LAMP-1 is a lysosomal membrane protein that undergoes two-stage glycosylation: at the ER during synthesis (the first stage) and at the Golgi apparatus (the second stage) (Carlsson *et al.*, *J. Biol. Chem.* 263:18911-18919 (1988)). MMG8-knockdown cells contain both the LAMP-1 precursor and mature LAMP-1 (Fig. 9A), indicating that MMG8-knockdown impaired protein degradation in the lysosome (Meikle *et al.*, *Clin. Chem.* 43:1325-1335 (1997)) that might be resulted from the affected or slowed transport of proteases to the lysosome (Zhu *et*

al., *Mol. Biol. Cell.* 10:537-549 (1999)). Also, the amount of mature LAMP-1 was reduced in MMG8-knockdown cells by ~15% when compared to controls (Fig. 9A), indicating that the ER-to-Golgi transport of LAMP-1 is impaired.

CD44 is a plasma membrane protein that has various species of proteolysis and glycosylation isoforms ranging from ~42 kDa to ~90 kDa (Lokeshwar and Bourguignon, *J. Biol. Chem.* 266:17983-17989 (1991)). In MMG8-depleted cells, fully glycosylated CD44 running at ~90 kDa was significantly reduced (Fig. 9B).

LAMP-1 is a diagnostic marker of lysosomal storage diseases and CD44 has been used as a target for cancer therapy. Therefore, MMG8 can be used for the diagnosis and treatment of lysosomal storage diseases and cancer.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification. It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

CLAIMS

What is claimed is:

1. An isolated or recombinantly-produced myomegalin isoform comprising an amino acid
5 sequence having at least 95% identity to amino acids 1-389 of SEQ ID NO:2, wherein the
myomegalin isoform comprises a SxIP motif and wherein the carboxy-terminus of the
myomegalin isoform comprises:
 - i) a sequence identical to amino acids 1098-1116 of SEQ ID NO:2, or
 - ii) a sequence that is a variant of amino acids 1098-1116 of SEQ ID NO:2, wherein, in said
10 variant, no more than 5 amino acids of amino acids 1098-1116 of SEQ ID NO:2 are added,
deleted, or substituted, or
 - iii) a fragment of said sequence of i) or ii), wherein the fragment has at least 75% identity to
said sequence of i) or ii).
- 15 2. The isolated or recombinantly-produced myomegalin isoform of claim 1, comprising
amino acids 1-389 of SEQ ID NO:2, wherein the carboxy-terminus of the myomegalin
isoform comprises amino acids 1098-1116 of SEQ ID NO:2, or a contiguous fragment having
at least 75% identity to amino acids 1098-1116 of SEQ ID NO:2.
- 20 3. The isolated or recombinantly-produced myomegalin isoform of claim 2, comprising an
amino acid sequence having at least 90% identity to SEQ ID NO:2.
4. The isolated or recombinantly-produced myomegalin isoform of claim 1 comprising SEQ
ID NO:2.
- 25 5. An isolated or recombinant nucleic acid molecule comprising a polynucleotide encoding
the myomegalin isoform of claim 1, or a complement of said polynucleotide.
6. The isolated or recombinant nucleic acid molecule of claim 5, comprising a
30 polynucleotide having at least 95% identity to nucleic acids 1-1167 of SEQ ID NO:1,
wherein the 3' end of the polynucleotide has at least 95% identity to nucleic acids 3292-3348
of SEQ ID NO:1, or a complement of said polynucleotide.

7. The isolated or recombinant nucleic acid molecule of claim 6, comprising SEQ ID NO:1.
8. A vector comprising the nucleic acid molecule of claim 5.
9. A host cell comprising the nucleic acid molecule of claim 5.
10. A fusion protein comprising the myomegalin isoform of claim 1, a second protein, and, optionally, a linker sequence that links the myomegalin isoform with the second protein.
11. An antibody or aptamer that binds specifically to i) an epitope comprising an amino acid sequence identical to amino acids 926-1116 of SEQ ID NO:2; ii) an epitope comprising a variant of amino acids 926-1116 of SEQ ID NO:2 wherein, in said variant, no more than 20 amino acids of amino acids 926-1116 of SEQ ID NO:2 are added, deleted, or substituted; or
15 iii) an epitope comprising a contiguous fragment of amino acids 1098-1116 of SEQ ID NO:2, wherein said fragment has at least 75% identity to amino acids 1098-1116 of SEQ ID NO:2.
12. The antibody of claim 11, wherein the antibody binds specifically to an epitope comprising amino acids 926-1116 of SEQ ID NO:2, or an epitope comprising a fragment of
20 amino acids 926-1116 of SEQ ID NO:2, wherein said fragment has at least 75% identity to amino acids 1098-1116 of SEQ ID NO:2.
13. A protein chip comprising the myomegalin isoform of claim 1.
- 25 14. A pharmaceutical composition comprising the myomegalin isoform of claim 1 and a pharmaceutically acceptable carrier.
15. A method for detecting the presence or level of myomegalin, wherein the method comprises:
30 (a) contacting a sample with an agent that binds to the myomegalin isoform of claim 1;
and
 (b) detecting the binding of the agent to the myomegalin isoform;

wherein the agent is selected from:

1) a nucleic acid molecule comprising a polynucleotide encoding the myomegalin isoform of claim 1 or a complement of said polynucleotide or said fragment, or

2) an antibody or aptamer that binds specifically to i) an epitope comprising an amino acid sequence identical to amino acids 926-1116 of SEQ ID NO:2; ii) an epitope comprising a variant of amino acids 926-1116 of SEQ ID NO:2 wherein, in said variant, no more than 20 amino acids of amino acids 926-1116 of SEQ ID NO:2 are added, deleted, or substituted; or iii) an epitope comprising a contiguous fragment of amino acids 1098-1116 of SEQ ID NO:2, wherein said fragment has at least 75% identity to amino acids 1098-1116 of SEQ ID NO:2.

16. A method for screening for agents that inhibit the expression of myomegalin isoform, wherein the method comprises:

a) providing a test sample containing cells expressing the myomegalin isoform of claim 1;

b) contacting a candidate agent with the test sample;

c) determining a level of the myomegalin isoform in the test sample; and

d) selecting the candidate agent if said agent reduces the level of myomegalin isoform in the test sample.

17. A method for selecting an agent that modulates myomegalin activity, comprising:

a) providing a first test sample containing cells expressing the myomegalin isoform of claim 1;

b) contacting the first test sample with a protein that interacts with the myomegalin isoform;

c) determining a first level of the binding of the myomegalin isoform to said protein that interacts with the myomegalin isoform in the first test sample;

d) providing a second test sample containing cells expressing the myomegalin isoform;

e) contacting the second sample with a candidate agent;

f) after step e), contacting the second test sample with said protein that interacts with the myomegalin isoform;

g) determining a second level of the binding of the myomegalin isoform to said protein that interacts with the myomegalin isoform in the second test sample; and

h) selecting the candidate agent as a modulator of the myomegalin isoform, if said second level is different from said first level.

18. The method of claim 17, wherein the protein that interacts with the myomegalin isoform is selected from AKAP450, EB1, EB3, GCP2, or GCP3.

19. A method for diagnosis of a disease associated with myomegalin comprising:

a) obtaining a biological sample from a subject;

b) measuring a level of the myomegalin isoform of claim 1 in the biological sample;

and

c) characterizing the subject's level of the myomegalin isoform.

20. The method of claim 19, wherein the disease is selected from a neuronal disease, diabetes, cystic fibrosis, a lysosomal storage disease, or cancer.

21. A method of inhibiting ER-to-Golgi transport of LAMP-1, by inhibiting the expression or activity of myomegalin, wherein the method comprises administering to cells in which said inhibition is desired an effective amount of an siRNA that inhibits the expression of the myomegalin isoform of claim 1, or an antibody or aptamer that binds specifically to the myomegalin isoform of claim 1, and optionally, a pharmaceutically acceptable carrier.

22. The method of claim 21, used to treat a lysosomal storage disease.

23. A method of increasing the level of glycosylated CD44 having a molecular weight of about 90 kDa, comprising administering to cells in which said increase is desired an effective amount of the myomegalin isoform of claim 1, or a nucleic acid molecule encoding the myomegalin isoform of claim 1, and optionally, a pharmaceutically acceptable carrier.

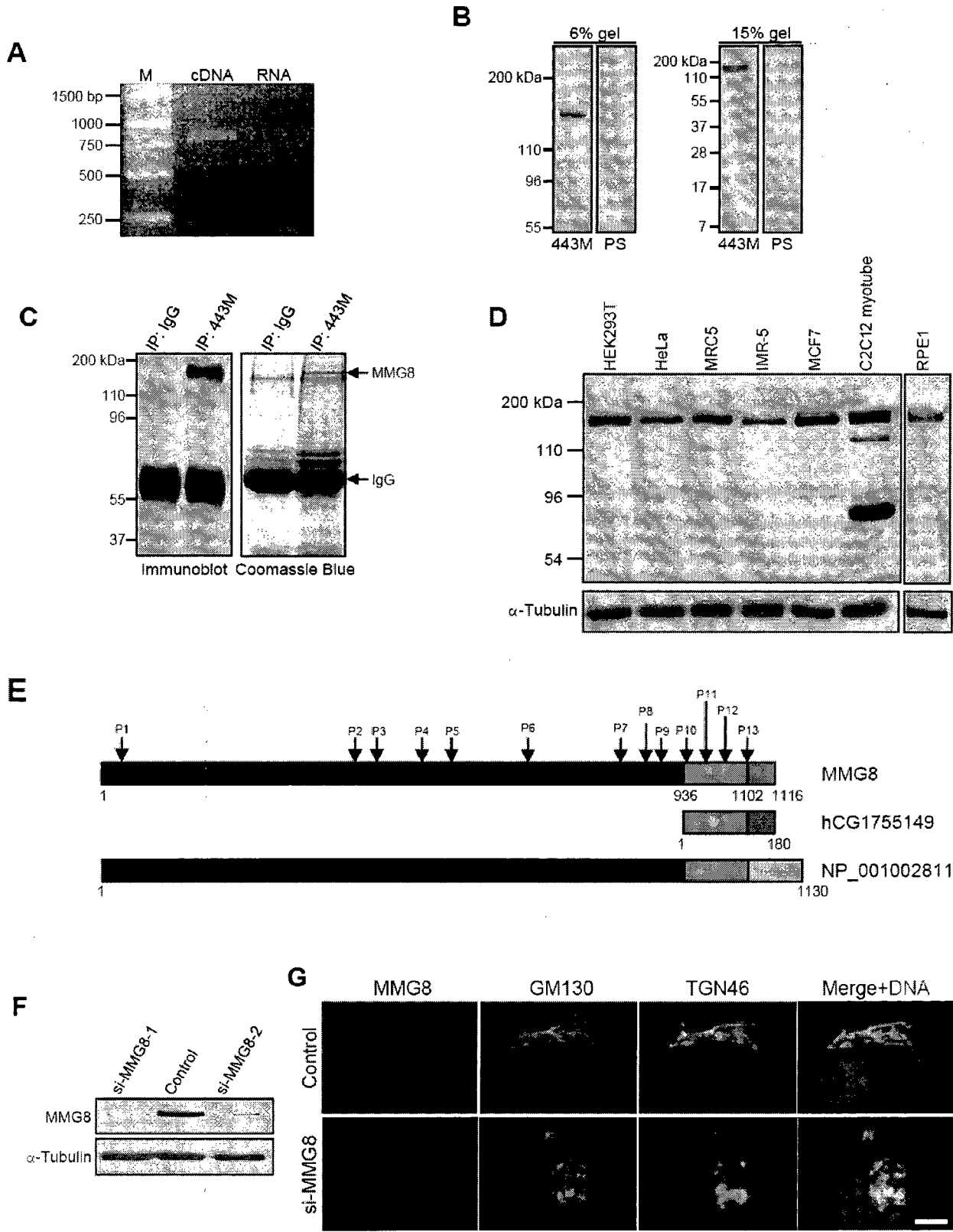


FIG. 1

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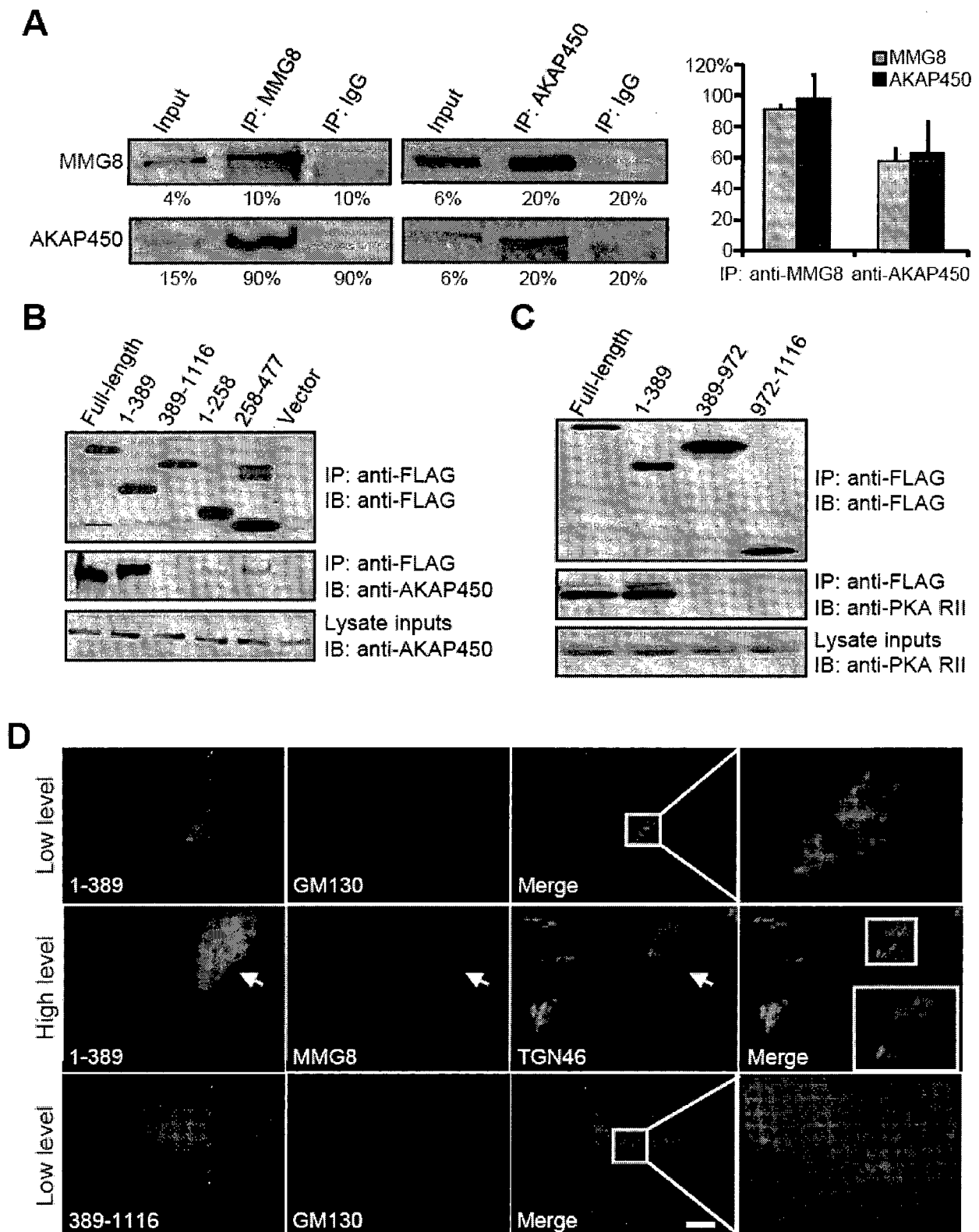


FIG. 2

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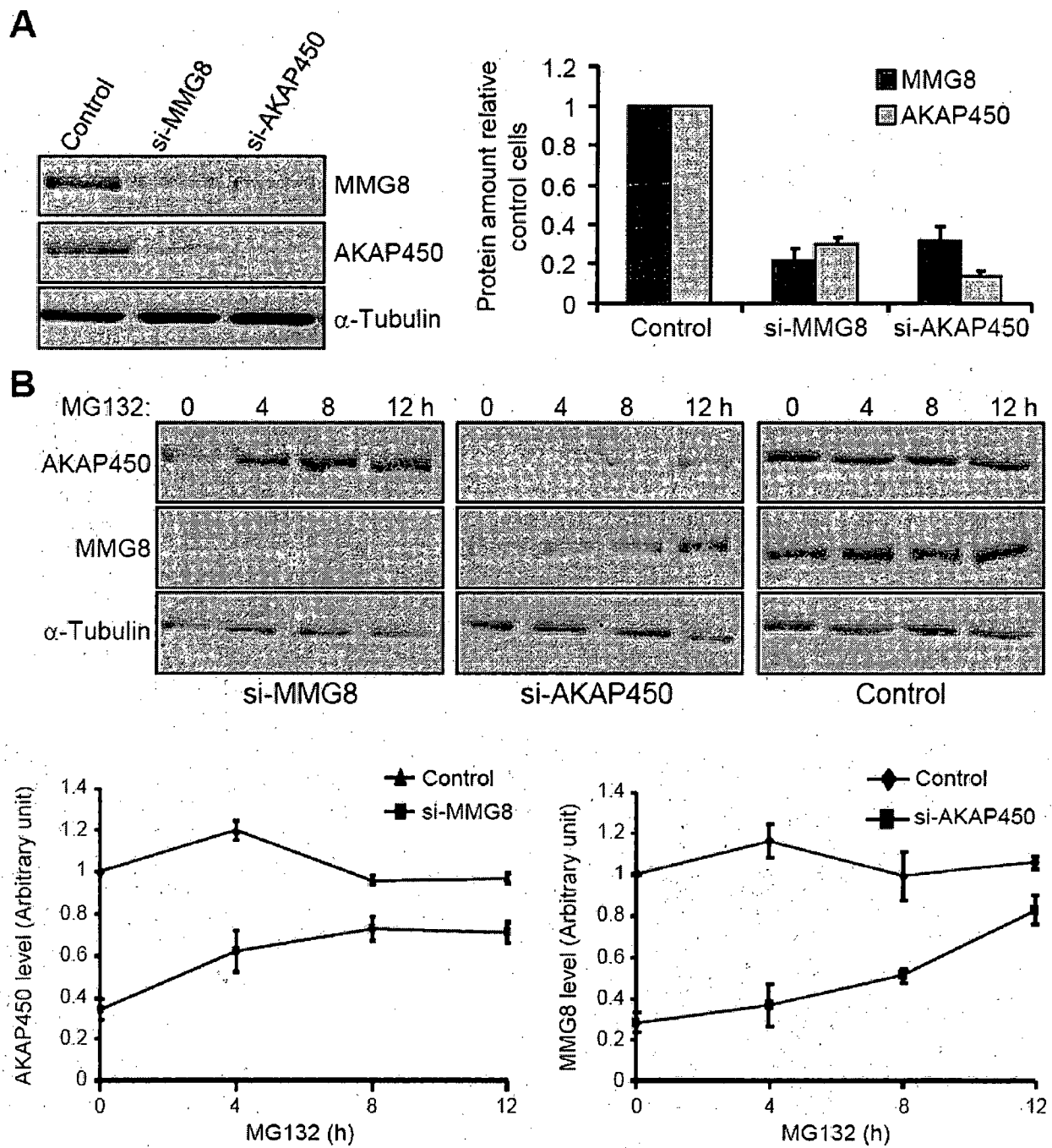


FIG. 3

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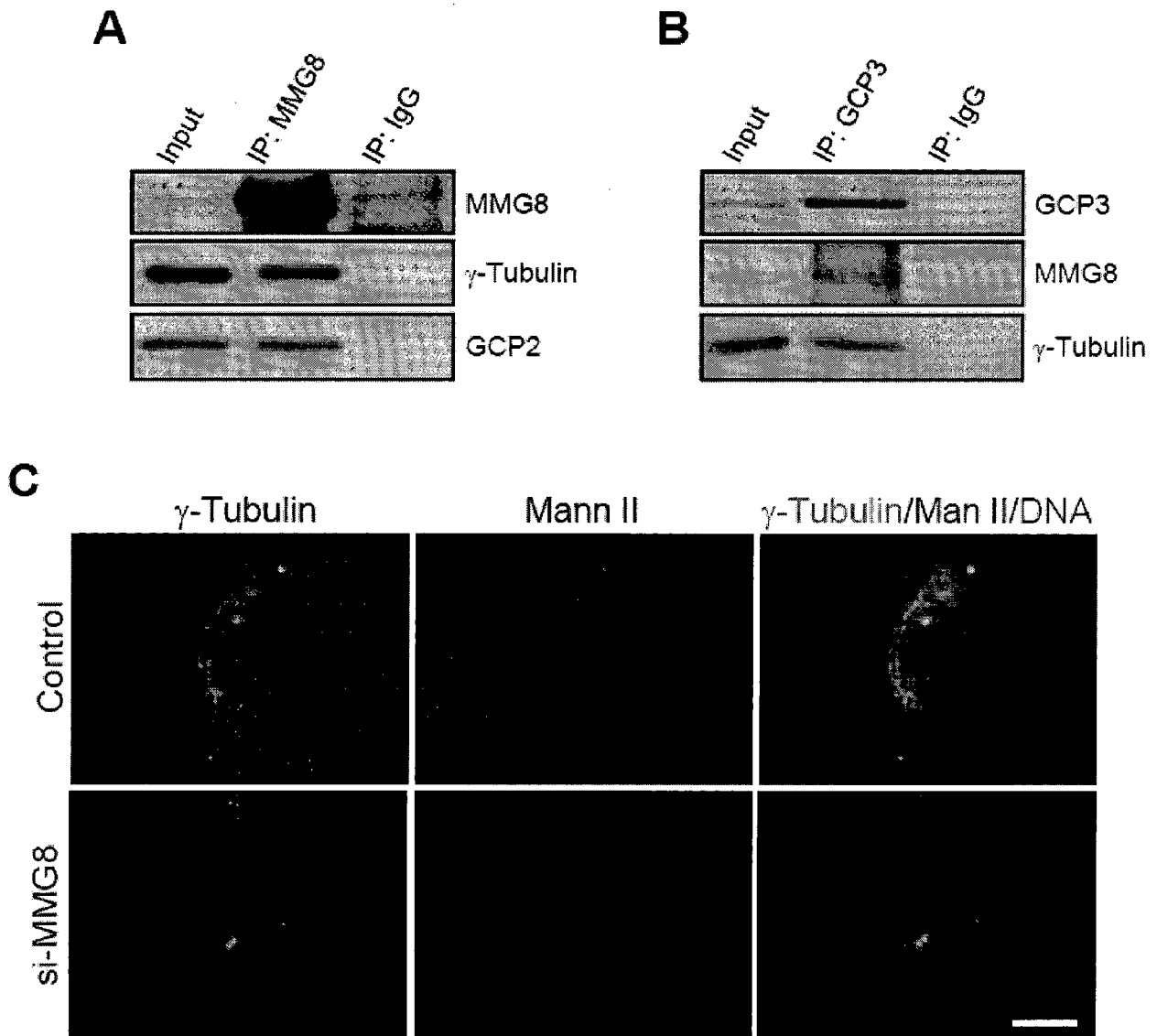


FIG. 4

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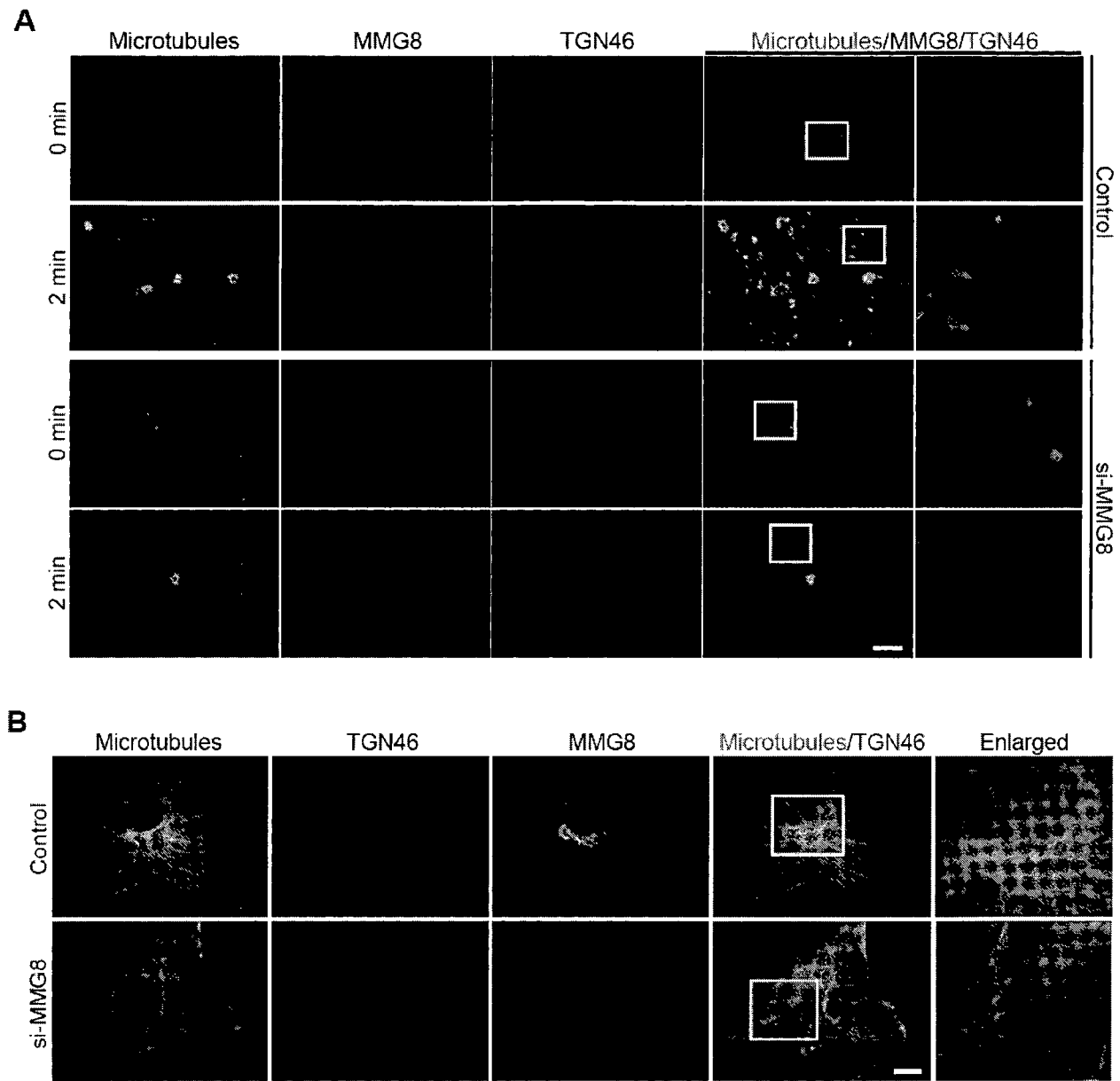


FIG. 5

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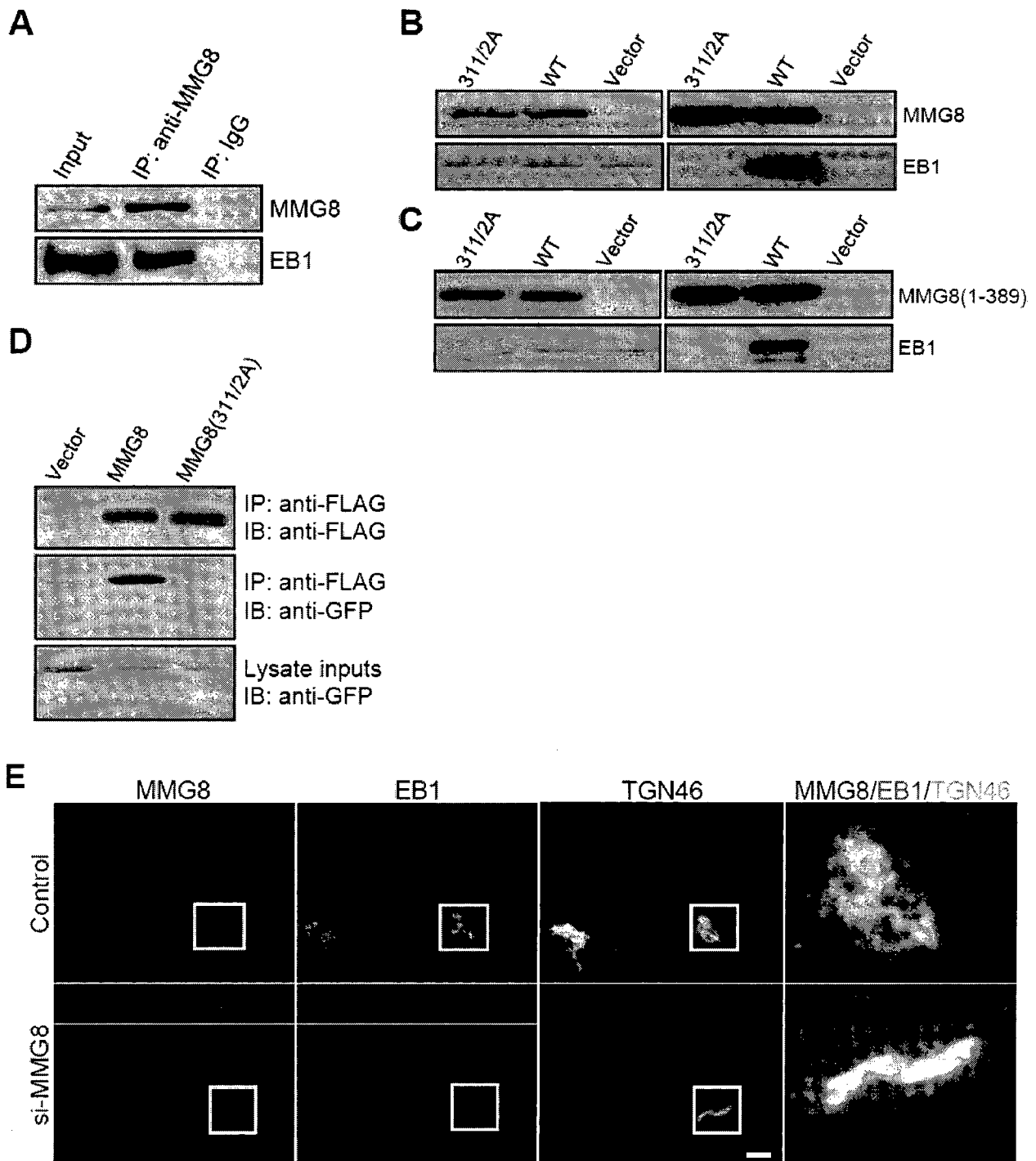


FIG. 6

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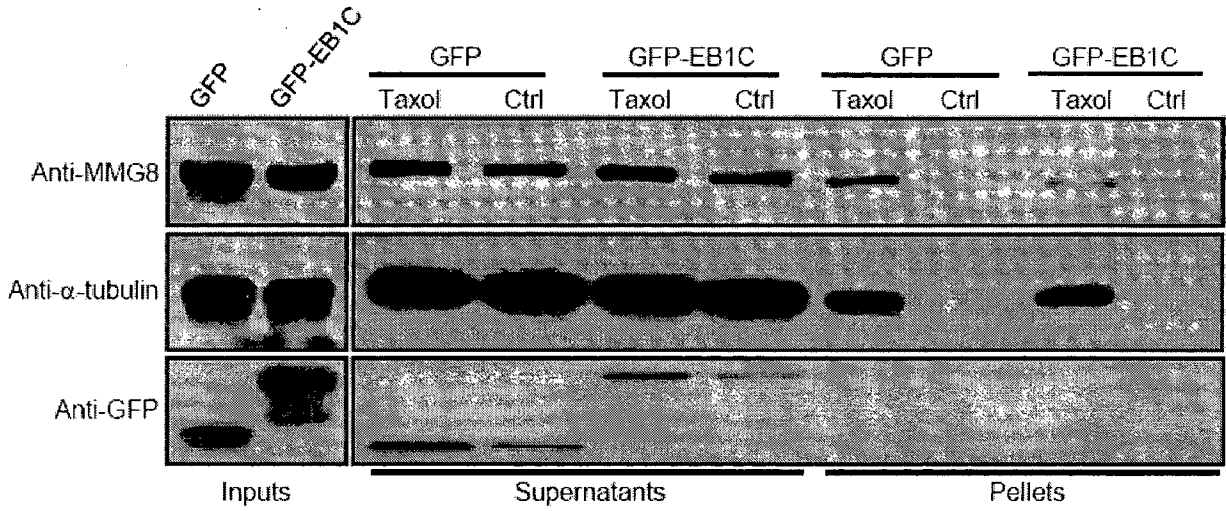


FIG. 7

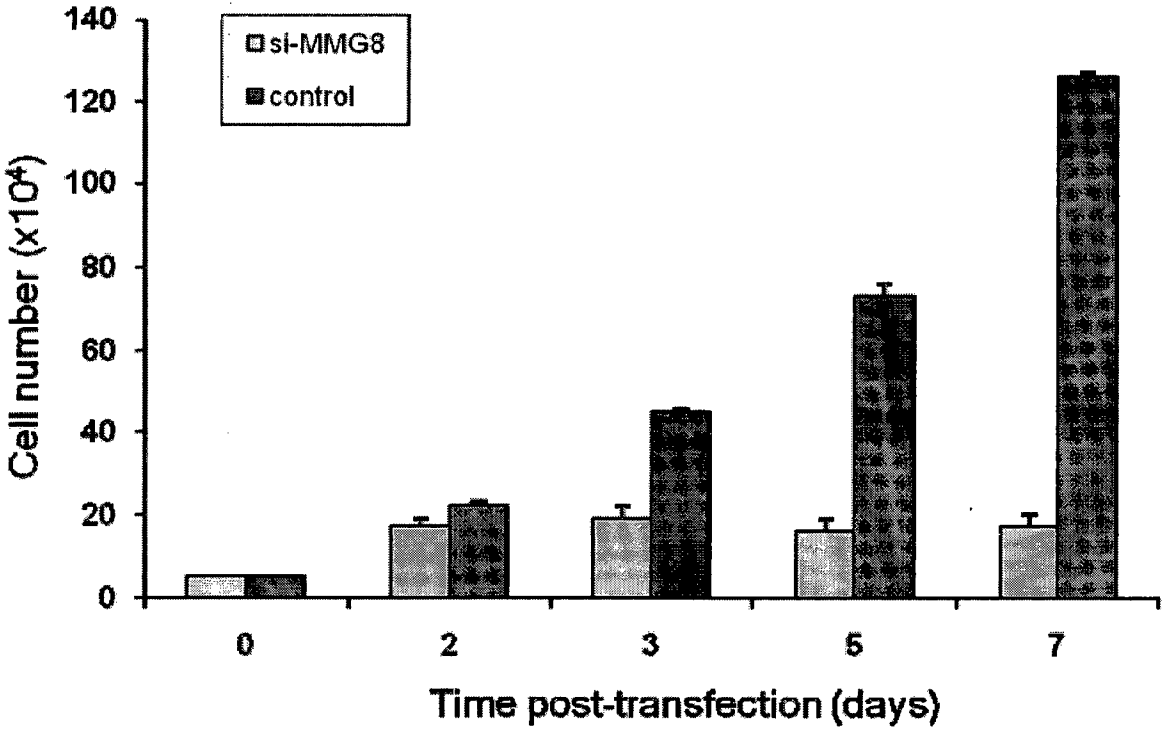


FIG. 8

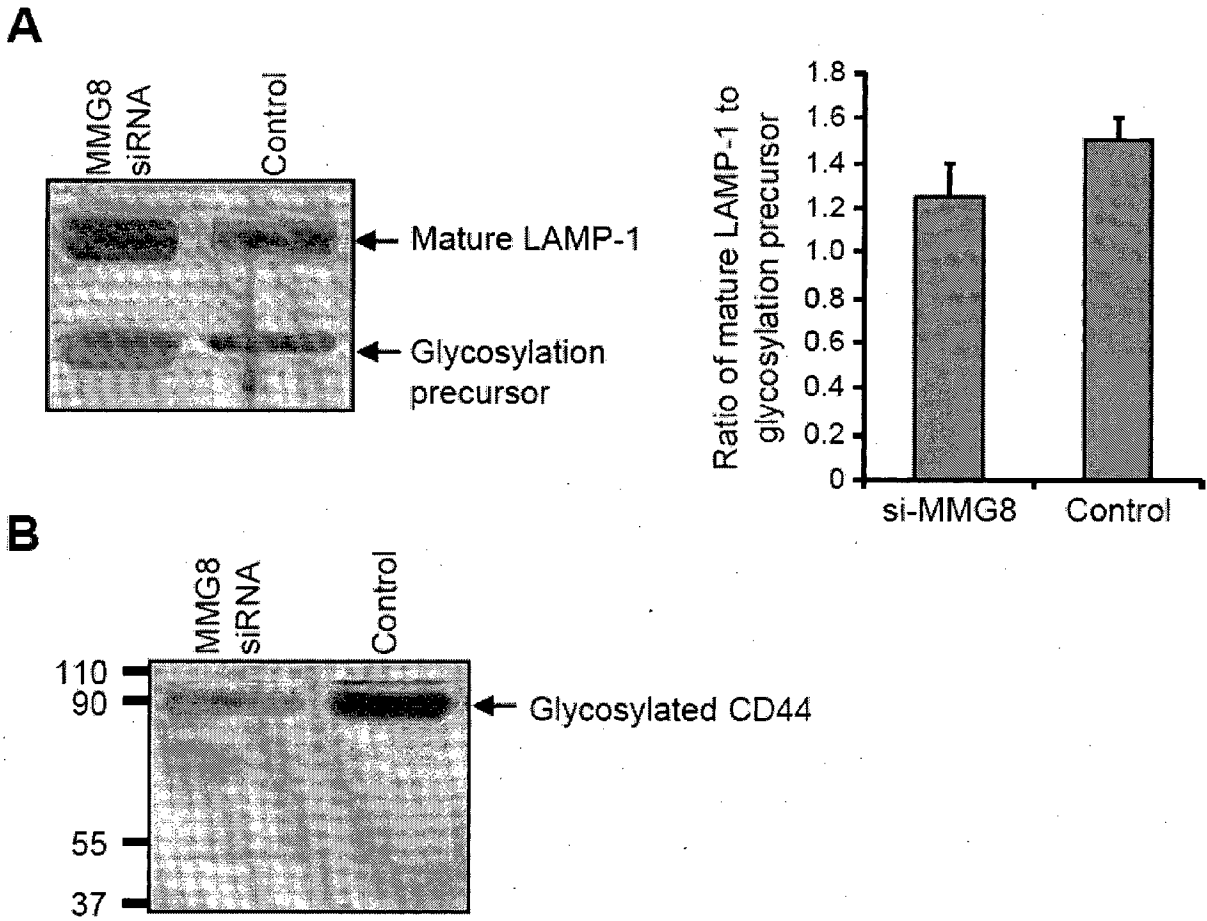


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2011/001907

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: C07K, C12N, A61K, A61P

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

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

CNPAT, WPI, EPODOC, GenBank:

Myomegalin, search of SEQ ID NO: 2 for amino acids 1-1116, 1-389, 1098-1116, 926-1116

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database GenBank Accession No. CAH92060. Bloecker, H. "hypothetical protein [Pongo abelii]". 01 May 2008 (01.05.2008).	1-23
X	Database GenBank Accession No. CR859904. Bloecker, H. "Pongo abelii mRNA; cDNA DKFZp469C1614 (from clone DKFZp469C1614)". 01 May 2008 (01.05.2008).	5-9
A	Srinivas Honnappa et al: "An EB1-Binding Motif Acts as a Microtubule Tip Localization Signal", Cell, 24 July 2009 (24.07.2009), Vol. 138, pages 366-376.	1-23

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search
14 February 2012 (14.02.2012)

Date of mailing of the international search report
08 Mar. 2012 (08.03.2012)

Name and mailing address of the ISA/CN
The State Intellectual Property Office, the P.R.China
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2011/001907

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

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

1. ☒ Claims Nos.: 22

because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 22 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the use of an siRNA, an antibody or an aptamer inhibiting myomegalin isoform of claim 1 for manufacturing a medicament for the treatment of lysosomal storage disease.

2. ☐ Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2011/001907

CLASSIFICATION OF SUBJECT MATTER

C07K 17/47 (2006.01) i

C12N 15/12 (2006.01) i

A61K 38/17 (2006.01) i

A61P 25/00 (2006.01) i

A61P 3/10 (2006.01) i

A61P 1/18 (2006.01) i

A61P 35/00 (2006.01) i