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(54) Title: MRHOS AS MODIFIERS OF THE RHO PATHWAY AND METHODS OF USE

(57) Abstract: Human MRHO genes are identified as modulators of the RHO pathway, and thus are therapeutic targets for disorders associated with defective RHO function. Methods for identifying modulators of RHO, comprising screening for agents that modulate the activity of MRHO are provided.



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MRHOS AS MODIFIERS OF THE RHO PATHWAY AND METHODS OF USE

BACKGROUND OF THE INVENTION

[0001] The Rho family of small GTPases comprises a group of about 20 mammalian proteins, which include Rho, Rac, and CDC42. These ubiquitous and highly conserved proteins are key regulators of many cellular processes, including cell migration and shape changes, cell-cell and cell-matrix adhesion, cell-cycle progression and cytokinesis, and gene expression (see Burridge K and Wennerberg (2004) *Cell* 116(2):167-179). Like their close relative Ras, Rho GTPases act as molecular switches that cycle between a biochemically-active GTP-bound state and an inactive GDP-bound state. Regulation of cycling is mediated predominantly through 3 classes of proteins: guanine exchange factors (GEFs) which promote the active GTP-bound state by facilitating exchange of GDP for GTP; GTPase-activating proteins (GAPs) which promote the inactive state by stimulating the relatively weak intrinsic hydrolase activity of GTPases; and guanine-dissociation inhibitors (GDIs) which bind to and sequester GTPase in their GDP-bound form. Activation of many GEFs and their target GTPase occurs in response to a variety of upstream stimuli such as growth factor engagement and cell-cell or cell-matrix adhesion. As regulators, activated Rho proteins bind and usually activate one or more of a variety of downstream effector proteins (e.g., rho kinases, formins, p21-activated kinases) that elicit a cellular response.

[0002] Potential roles of Rho proteins in promoting cancer progression have been suggested based on the well-established functions of individual family members in promoting cell-cycle progression and cellular motility and tissue invasion, as well of the oncogenic properties associated with different GEFs acting on Rho proteins. Constitutively-activated forms of many GEFs result in hyperactivation of one or more Rho family members and are oncogenic for rodent cells. Further, overexpression of specific Rho proteins or Rho effectors has been observed for several human cancers, including breast, lung, and colorectal (see Croft DR et al (2004) *Can Res* 64:8994-9001, and references therein). In several cases, in vivo animal model studies have provided compelling evidence that overexpression of either a Rho protein or effector contributes significantly to tumor cell invasiveness and/or metastasis (See Sahai, E. (2005) *Cur Opin Genet & Dev.* (2005) 15:87-96).

- [0003]** Studies of model systems such as *C. elegans* and *Drosophila* have identified invertebrate orthologues of Rho, Rac, and CDC42 that show conservation in function as well as structure. *C. elegans* contains 3 Rac-encoding genes (*ced-10*, *mig-2*, *rac-2*) and single genes encoding CDC42 (*cdc-42*) or Rho (*rho-1*). The three nematode Rac genes have redundant roles in cell motility and migration (Lundquist EA et al., *Development* (2001) 128(22):4475-88), while *rho-1* is required for cytokinesis (Jantsch-Plunger V et al (2000) *J Cell Biol* 149(7):1291-404) and *cdc-42* has an essential role in cell polarity (Gotta M et al (2001) *Curr Biol* 11(7):482-488). *C. elegans* orthologues of several mammalian GEFs have also been characterized, including Dock180/ELMO (*ced-2/ced-12*) and Ect2 (*let-21*). These particular GEFs appear conserved in function as well as they exhibit loss-of-function mutant phenotypes that overlap those associated with Rho, Rac, or CDC42 mutants. For example, like their mammalian orthologues, *C. elegans* Rho (*rho-1*) and Ect2 (*let-21*) are required at the end of mitosis for cleavage furrow ingression. Reflecting this role, cytokinesis-defective phenotypes are observed in reduction-of-function *let-21* mutants or after RNA inhibition (RNAi) of either *let-21* or *rho-1* (R. Francis, unpublished observations; T. Schedl, personal communication).
- [0004]** KIF23 (kinesin family member 23) is a member of kinesin-like protein family. This family includes microtubule-dependent molecular motors that transport organelles within cells and move chromosomes during cell division. KIF23 has been shown to cross-bridge antiparallel microtubules and drive microtubule movement in vitro. Alternate splicing of this gene results in two transcript variants encoding two different isoforms.
- [0005]** PPIE (peptidylprolyl isomerase E; cyclophilin E) is a member of the peptidylprolyl cis-trans isomerase (PPIase) family. PPIases catalyze the cis-trans isomerization of proline imidic peptide bonds in oligopeptides and accelerate the folding of proteins. PPIE contains a highly conserved cyclophilin (CYP) domain as well as an RNA-binding domain, and possesses PPIase and protein folding activities and also exhibit RNA-binding activity.
- [0006]** FDPS (farnesyl diphosphate synthase) catalyzes the conversion of geranyl diphosphate and isopentenyl diphosphate to diphosphate and trans, trans-farnesyl diphosphate in the isoprene biosynthetic pathway.
- [0007]** Phosphatidylinositol (PI) 4-kinase (PIK4CA) catalyzes the first committed step in the biosynthesis of phosphatidylinositol 4,5-bisphosphate. The mammalian PI

4-kinases have been classified into two types, II and III, based on their molecular mass, and modulation by detergent and adenosine. Two transcript variants encoding different isoforms have been described for this gene.

[0008] Protein kinases with no lysine (lysine deficient protein kinase; PRKWNK) are cytoplasmic serine-threonine kinases that contain cysteine in place of lysine at a conserved location, yet have kinase activity. Mutations in two of PRKWNKs, the WNK1 and WNK4, cause type II pseudohypoaldosteronism, an autosomal dominant disorder featuring hypertension, hyperkalemia, and renal tubular acidosis.

[0009] Intercellular communication is often mediated by receptors on the surface of one cell that recognize and are activated by specific protein ligands released by other cells. Members of one class of cell surface receptors, receptor tyrosine kinases (RTKs), are characterized by having a cytoplasmic domain containing intrinsic tyrosine kinase activity. This kinase activity is regulated by the binding of a cognate ligand to the extracellular portion of the receptor. RTKs are expressed in cell type-specific fashions and play a role critical for the growth and differentiation of those cell types. ROR1 (Neurotrophic tyrosine kinase receptor related 1) is expressed in neural tissues and may be involved in transmembrane receptor protein tyrosine kinase signaling pathways (Oishi, I., et al (1999) *Genes Cells* 4:41-56; Masiakowski, P., and Carroll, R. D. (1992) *J Biol Chem* 267:26181-90; Reddy, U. R., et al (1996) *Oncogene* 13:1555-9). ROR2 (Receptor tyrosine kinase-like orphan receptor 2) is another neuronal-specific member of the RTK family. Mutations in ROR2 are associated with skeletal disorders, including dominant brachydactyly type B1 and recessive Robinow syndrome (Afzal, A. R., et al (2000) *Nat Genet* 25:419-22; Oldridge, M., et al (2000) *Nat Genet* 24:275-8).

[0010] MELK (maternal embryonic leucine zipper kinase) is a member of the evolutionarily conserved KIN1/PAR-1/MARK kinase family which is involved in cell polarity and microtubule dynamics.

[0011] The ability to manipulate the genomes of model organisms such as *C. elegans* provides a powerful means to analyze biochemical processes that, due to significant evolutionary conservation, have direct relevance to more complex vertebrate organisms. Due to a high level of gene and pathway conservation, the strong similarity of cellular processes, and the functional conservation of genes between these model organisms and mammals, identification of the involvement of novel genes in particular pathways and their functions in such model organisms can directly

contribute to the understanding of the correlative pathways and methods of modulating them in mammals (see, for example, Dulubova I, et al, J Neurochem 2001 Apr;77(1):229-38; Cai T, et al., Diabetologia 2001 Jan;44(1):81-8; Pasquinelli AE, et al., Nature. 2000 Nov 2;408(6808):37-8; Ivanov IP, et al., EMBO J 2000 Apr 17;19(8):1907-17; Vajo Z et al., Mamm Genome 1999 Oct;10(10):1000-4). For example, a genetic screen can be carried out in an invertebrate model organism having underexpression (e.g. knockout) or overexpression of a gene (referred to as a "genetic entry point") that yields a visible phenotype. Additional genes are mutated in a random or targeted manner. When a gene mutation changes the original phenotype caused by the mutation in the genetic entry point, the gene is identified as a "modifier" involved in the same or overlapping pathway as the genetic entry point. When the genetic entry point is an ortholog of a human gene implicated in a disease pathway, such as RHO, modifier genes can be identified that may be attractive candidate targets for novel therapeutics.

[0012] All references cited herein, including patents, patent applications, publications, and sequence information in referenced Genbank identifier numbers, are incorporated herein in their entireties.

SUMMARY OF THE INVENTION

[0013] We have discovered genes that modify the RHO pathway in *C. elegans*, and identified their human orthologs, hereinafter referred to as Modifiers of RHO (MRHO). The invention provides methods for utilizing these RHO modifier genes and polypeptides to identify MRHO-modulating agents that are candidate therapeutic agents that can be used in the treatment of disorders associated with defective or impaired RHO function and/or MRHO function. Preferred MRHO-modulating agents specifically bind to MRHO polypeptides and restore RHO function. Other preferred MRHO-modulating agents are nucleic acid modulators such as antisense oligomers and RNAi that repress MRHO gene expression or product activity by, for example, binding to and inhibiting the respective nucleic acid (i.e. DNA or mRNA).

[0014] MRHO modulating agents may be evaluated by any convenient *in vitro* or *in vivo* assay for molecular interaction with an MRHO polypeptide or nucleic acid. In one embodiment, candidate MRHO modulating agents are tested with an assay system comprising an MRHO polypeptide or nucleic acid. Agents that produce a change in the activity of the assay system relative to controls are identified as candidate RHO

modulating agents. The assay system may be cell-based or cell-free. MRHO-modulating agents include MRHO related proteins (e.g. dominant negative mutants, and biotherapeutics); MRHO -specific antibodies; MRHO -specific antisense oligomers and other nucleic acid modulators; and chemical agents that specifically bind to or interact with MRHO or compete with MRHO binding partner (e.g. by binding to an MRHO binding partner). In one specific embodiment, a small molecule modulator is identified using a binding assay. In specific embodiments, the screening assay system is selected from an apoptosis assay, a cell proliferation assay, and an angiogenesis assay.

[0015] In another embodiment, candidate RHO pathway modulating agents are further tested using a second assay system that detects changes in the RHO pathway, such as angiogenic, apoptotic, or cell proliferation changes produced by the originally identified candidate agent or an agent derived from the original agent. The second assay system may use cultured cells or non-human animals. In specific embodiments, the secondary assay system uses non-human animals, including animals predetermined to have a disease or disorder implicating the RHO pathway, such as an angiogenic, apoptotic, or cell proliferation disorder (e.g. cancer).

[0016] The invention further provides methods for modulating the MRHO function and/or the RHO pathway in a mammalian cell by contacting the mammalian cell with an agent that specifically binds an MRHO polypeptide or nucleic acid. The agent may be a small molecule modulator, a nucleic acid modulator, or an antibody and may be administered to a mammalian animal predetermined to have a pathology associated with the RHO pathway.

DETAILED DESCRIPTION OF THE INVENTION

[0017] A *C. elegans* genetic screen was designed which employed RNAi of specific genes to identify genetic modifiers of Rho pathway function. Methods for using RNAi to silence genes in *C. elegans* are known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); WO9932619). Genes causing altered phenotypes in the worms were identified as modifiers of the RHO pathway, followed by identification of their orthologs. Accordingly, vertebrate orthologs of these modifiers, and preferably the human orthologs, MRHO genes (i.e., nucleic acids and polypeptides) are attractive drug targets for the treatment of

pathologies associated with a defective RHO signaling pathway, such as cancer.

Table 1 (Example II) lists the modifiers and their orthologs.

[0018] In vitro and in vivo methods of assessing MRHO function are provided herein. Modulation of the MRHO or their respective binding partners is useful for understanding the association of the RHO pathway and its members in normal and disease conditions and for developing diagnostics and therapeutic modalities for RHO related pathologies. MRHO-modulating agents that act by inhibiting or enhancing MRHO expression, directly or indirectly, for example, by affecting an MRHO function such as enzymatic (e.g., catalytic) or binding activity, can be identified using methods provided herein. MRHO modulating agents are useful in diagnosis, therapy and pharmaceutical development.

Nucleic acids and polypeptides of the invention

[0019] Sequences related to MRHO nucleic acids and polypeptides that can be used in the invention are disclosed in Genbank (referenced by Genbank identifier (GI) or RefSeq number), shown in Table 1 and in the appended sequence listing.

[0020] The term "MRHO polypeptide" refers to a full-length MRHO protein or a functionally active fragment or derivative thereof. A "functionally active" MRHO fragment or derivative exhibits one or more functional activities associated with a full-length, wild-type MRHO protein, such as antigenic or immunogenic activity, enzymatic activity, ability to bind natural cellular substrates, etc. The functional activity of MRHO proteins, derivatives and fragments can be assayed by various methods known to one skilled in the art (Current Protocols in Protein Science (1998) Coligan *et al.*, eds., John Wiley & Sons, Inc., Somerset, New Jersey) and as further discussed below. In one embodiment, a functionally active MRHO polypeptide is an MRHO derivative capable of rescuing defective endogenous MRHO activity, such as in cell based or animal assays; the rescuing derivative may be from the same or a different species. For purposes herein, functionally active fragments also include those fragments that comprise one or more structural domains of an MRHO, such as a kinase domain or a binding domain. Protein domains can be identified using the PFAM program (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2). Methods for obtaining MRHO polypeptides are also further described below. In some embodiments, preferred fragments are functionally active, domain-containing fragments comprising at least 25 contiguous amino acids, preferably at least 50, more

preferably 75, and most preferably at least 100 contiguous amino acids of an MRHO. In further preferred embodiments, the fragment comprises the entire functionally active domain.

[0021] The term "MRHO nucleic acid" refers to a DNA or RNA molecule that encodes an MRHO polypeptide. Preferably, the MRHO polypeptide or nucleic acid or fragment thereof is from a human, but can also be an ortholog, or derivative thereof with at least 70% sequence identity, preferably at least 80%, more preferably 85%, still more preferably 90%, and most preferably at least 95% sequence identity with human MRHO. Methods of identifying orthologs are known in the art. Normally, orthologs in different species retain the same function, due to presence of one or more protein motifs and/or 3-dimensional structures. Orthologs are generally identified by sequence homology analysis, such as BLAST analysis, usually using protein bait sequences. Sequences are assigned as a potential ortholog if the best hit sequence from the forward BLAST result retrieves the original query sequence in the reverse BLAST (Huynen MA and Bork P, Proc Natl Acad Sci (1998) 95:5849-5856; Huynen MA *et al.*, Genome Research (2000) 10:1204-1210). Programs for multiple sequence alignment, such as CLUSTAL (Thompson JD *et al.*, 1994, Nucleic Acids Res 22:4673-4680) may be used to highlight conserved regions and/or residues of orthologous proteins and to generate phylogenetic trees. In a phylogenetic tree representing multiple homologous sequences from diverse species (e.g., retrieved through BLAST analysis), orthologous sequences from two species generally appear closest on the tree with respect to all other sequences from these two species. Structural threading or other analysis of protein folding (e.g., using software by ProCeryon, Biosciences, Salzburg, Austria) may also identify potential orthologs. In evolution, when a gene duplication event follows speciation, a single gene in one species, such as *C. elegans*, may correspond to multiple genes (paralogs) in another, such as human. As used herein, the term "orthologs" encompasses paralogs. As used herein, "percent (%) sequence identity" with respect to a subject sequence, or a specified portion of a subject sequence, is defined as the percentage of nucleotides or amino acids in the candidate derivative sequence identical with the nucleotides or amino acids in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul *et al.*, J. Mol. Biol. (1997) 215:403-410) with all the search parameters set to default values.

The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. A % identity value is determined by the number of matching identical nucleotides or amino acids divided by the sequence length for which the percent identity is being reported. "Percent (%) amino acid sequence similarity" is determined by doing the same calculation as for determining % amino acid sequence identity, but including conservative amino acid substitutions in addition to identical amino acids in the computation.

[0022] A conservative amino acid substitution is one in which an amino acid is substituted for another amino acid having similar properties such that the folding or activity of the protein is not significantly affected. Aromatic amino acids that can be substituted for each other are phenylalanine, tryptophan, and tyrosine; interchangeable hydrophobic amino acids are leucine, isoleucine, methionine, and valine; interchangeable polar amino acids are glutamine and asparagine; interchangeable basic amino acids are arginine, lysine and histidine; interchangeable acidic amino acids are aspartic acid and glutamic acid; and interchangeable small amino acids are alanine, serine, threonine, cysteine and glycine.

[0023] Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, *Advances in Applied Mathematics* 2:482-489; database: European Bioinformatics Institute; Smith and Waterman, 1981, *J. of Molec.Biol.*, 147:195-197; Nicholas et al., 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" (www.psc.edu) and references cited therein.; W.R. Pearson, 1991, *Genomics* 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff (Dayhoff: *Atlas of Protein Sequences and Structure*, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 *Nucl. Acids Res.* 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."

[0024] Derivative nucleic acid molecules of the subject nucleic acid molecules include sequences that hybridize to the nucleic acid sequence of an MRHO. The

stringency of hybridization can be controlled by temperature, ionic strength, pH, and the presence of denaturing agents such as formamide during hybridization and washing. Conditions routinely used are set out in readily available procedure texts (e.g., Current Protocol in Molecular Biology, Vol. 1, Chap. 2.10, John Wiley & Sons, Publishers (1994); Sambrook *et al.*, Molecular Cloning, Cold Spring Harbor (1989)).

- ~ In some embodiments, a nucleic acid molecule of the invention is capable of hybridizing to a nucleic acid molecule containing the nucleotide sequence of an MRHO under high stringency hybridization conditions that are: prehybridization of filters containing nucleic acid for 8 hours to overnight at 65° C in a solution comprising 6X single strength citrate (SSC) (1X SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5X Denhardt's solution, 0.05% sodium pyrophosphate and 100 µg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C in a solution containing 6X SSC, 1X Denhardt's solution, 100 µg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C for 1h in a solution containing 0.1X SSC and 0.1% SDS (sodium dodecyl sulfate).

[0025] In other embodiments, moderately stringent hybridization conditions are used that are: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA; hybridization for 18-20h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C in a solution containing 2X SSC and 0.1% SDS.

[0026] Alternatively, low stringency conditions can be used that are: incubation for 8 hours to overnight at 37° C in a solution comprising 20% formamide, 5 x SSC, 50 mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured sheared salmon sperm DNA; hybridization in the same buffer for 18 to 20 hours; and washing of filters in 1 x SSC at about 37° C for 1 hour.

Isolation, Production, Expression, and Mis-expression of MRHO Nucleic Acids and Polypeptides

[0027] MRHO nucleic acids and polypeptides are useful for identifying and testing agents that modulate MRHO function and for other applications related to the involvement of MRHO in the RHO pathway. MRHO nucleic acids and derivatives

and orthologs thereof may be obtained using any available method. For instance, techniques for isolating cDNA or genomic DNA sequences of interest by screening DNA libraries or by using polymerase chain reaction (PCR) are well known in the art. In general, the particular use for the protein will dictate the particulars of expression, production, and purification methods. For instance, production of proteins for use in screening for modulating agents may require methods that preserve specific biological activities of these proteins, whereas production of proteins for antibody generation may require structural integrity of particular epitopes. Expression of proteins to be purified for screening or antibody production may require the addition of specific tags (*e.g.*, generation of fusion proteins). Overexpression of an MRHO protein for assays used to assess MRHO function, such as involvement in cell cycle regulation or hypoxic response, may require expression in eukaryotic cell lines capable of these cellular activities. Techniques for the expression, production, and purification of proteins are well known in the art; any suitable means therefore may be used (*e.g.*, Higgins SJ and Hames BD (eds.) *Protein Expression: A Practical Approach*, Oxford University Press Inc., New York 1999; Stanbury PF et al., *Principles of Fermentation Technology*, 2nd edition, Elsevier Science, New York, 1995; Doonan S (ed.) *Protein Purification Protocols*, Humana Press, New Jersey, 1996; Coligan JE et al, *Current Protocols in Protein Science* (eds.), 1999, John Wiley & Sons, New York). In particular embodiments, recombinant MRHO is expressed in a cell line known to have defective RHO function. The recombinant cells are used in cell-based screening assay systems of the invention, as described further below.

[0028] The nucleotide sequence encoding an MRHO polypeptide can be inserted into any appropriate expression vector. The necessary transcriptional and translational signals, including promoter/enhancer element, can derive from the native MRHO gene and/or its flanking regions or can be heterologous. A variety of host-vector expression systems may be utilized, such as mammalian cell systems infected with virus (*e.g.* vaccinia virus, adenovirus, *etc.*); insect cell systems infected with virus (*e.g.* baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, plasmid, or cosmid DNA. An isolated host cell strain that modulates the expression of, modifies, and/or specifically processes the gene product may be used.

[0029] To detect expression of the MRHO gene product, the expression vector can comprise a promoter operably linked to an MRHO gene nucleic acid, one or more

origins of replication, and, one or more selectable markers (*e.g.* thymidine kinase activity, resistance to antibiotics, *etc.*). Alternatively, recombinant expression vectors can be identified by assaying for the expression of the MRHO gene product based on the physical or functional properties of the MRHO protein in *in vitro* assay systems (*e.g.* immunoassays).

[0030] The MRHO protein, fragment, or derivative may be optionally expressed as a fusion, or chimeric protein product (*i.e.* it is joined via a peptide bond to a heterologous protein sequence of a different protein), for example to facilitate purification or detection. A chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other using standard methods and expressing the chimeric product. A chimeric product may also be made by protein synthetic techniques, *e.g.* by use of a peptide synthesizer (Hunkapiller *et al.*, Nature (1984) 310:105-111).

[0031] Once a recombinant cell that expresses the MRHO gene sequence is identified, the gene product can be isolated and purified using standard methods (*e.g.* ion exchange, affinity, and gel exclusion chromatography; centrifugation; differential solubility; electrophoresis). Alternatively, native MRHO proteins can be purified from natural sources, by standard methods (*e.g.* immunoaffinity purification). Once a protein is obtained, it may be quantified and its activity measured by appropriate methods, such as immunoassay, bioassay, or other measurements of physical properties, such as crystallography.

[0032] The methods of this invention may also use cells that have been engineered for altered expression (mis-expression) of MRHO or other genes associated with the RHO pathway. As used herein, mis-expression encompasses ectopic expression, over-expression, under-expression, and non-expression (*e.g.* by gene knock-out or blocking expression that would otherwise normally occur).

Genetically modified animals

[0033] Animal models that have been genetically modified to alter MRHO expression may be used in *in vivo* assays to test for activity of a candidate RHO modulating agent, or to further assess the role of MRHO in a RHO pathway process such as apoptosis or cell proliferation. Preferably, the altered MRHO expression results in a detectable phenotype, such as decreased or increased levels of cell proliferation, angiogenesis, or apoptosis compared to control animals having normal MRHO

expression. The genetically modified animal may additionally have altered RHO expression (e.g. RHO knockout). Preferred genetically modified animals are mammals such as primates, rodents (preferably mice or rats), among others. Preferred non-mammalian species include zebrafish, *C. elegans*, and *Drosophila*. Preferred genetically modified animals are transgenic animals having a heterologous nucleic acid sequence present as an extrachromosomal element in a portion of its cells, i.e. mosaic animals (see, for example, techniques described by Jakobovits, 1994, Curr. Biol. 4:761-763.) or stably integrated into its germ line DNA (i.e., in the genomic sequence of most or all of its cells). Heterologous nucleic acid is introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal.

[0034] Methods of making transgenic animals are well-known in the art (for transgenic mice see Brinster et al., Proc. Nat. Acad. Sci. USA 82: 4438-4442 (1985), U.S. Pat. Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Pat. No. 4,873,191 by Wagner et al., and Hogan, B., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); for particle bombardment see U.S. Pat. No., 4,945,050, by Sandford *et al.*; for transgenic *Drosophila* see Rubin and Spradling, Science (1982) 218:348-53 and U.S. Pat. No. 4,670,388; for transgenic insects see Berghammer A.J. *et al.*, A Universal Marker for Transgenic Insects (1999) Nature 402:370-371; for transgenic Zebrafish see Lin S., Transgenic Zebrafish, Methods Mol Biol. (2000);136:375-3830); for microinjection procedures for fish, amphibian eggs and birds see Houdebine and Chourrout, Experientia (1991) 47:897-905; for transgenic rats see Hammer *et al.*, Cell (1990) 63:1099-1112; and for culturing of embryonic stem (ES) cells and the subsequent production of transgenic animals by the introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation and direct injection see, e.g., Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, E. J. Robertson, ed., IRL Press (1987)). Clones of the nonhuman transgenic animals can be produced according to available methods (see Wilmut, I. *et al.* (1997) Nature 385:810-813; and PCT International Publication Nos. WO 97/07668 and WO 97/07669).

[0035] In one embodiment, the transgenic animal is a "knock-out" animal having a heterozygous or homozygous alteration in the sequence of an endogenous MRHO gene that results in a decrease of MRHO function, preferably such that MRHO

expression is undetectable or insignificant. Knock-out animals are typically generated by homologous recombination with a vector comprising a transgene having at least a portion of the gene to be knocked out. Typically a deletion, addition or substitution has been introduced into the transgene to functionally disrupt it. The transgene can be a human gene (e.g., from a human genomic clone) but more preferably is an ortholog of the human gene derived from the transgenic host species. For example, a mouse MRHO gene is used to construct a homologous recombination vector suitable for altering an endogenous MRHO gene in the mouse genome. Detailed methodologies for homologous recombination in mice are available (see Capecchi, *Science* (1989) 244:1288-1292; Joyner *et al.*, *Nature* (1989) 338:153-156). Procedures for the production of non-rodent transgenic mammals and other animals are also available (Houdebine and Chourrout, *supra*; Pursel *et al.*, *Science* (1989) 244:1281-1288; Simms *et al.*, *Bio/Technology* (1988) 6:179-183). In a preferred embodiment, knock-out animals, such as mice harboring a knockout of a specific gene, may be used to produce antibodies against the human counterpart of the gene that has been knocked out (Claesson MH *et al.*, (1994) *Scan J Immunol* 40:257-264; Declerck PJ *et al.*, (1995) *J Biol Chem.* 270:8397-400).

[0036] In another embodiment, the transgenic animal is a "knock-in" animal having an alteration in its genome that results in altered expression (e.g., increased (including ectopic) or decreased expression) of the MRHO gene, e.g., by introduction of additional copies of MRHO, or by operatively inserting a regulatory sequence that provides for altered expression of an endogenous copy of the MRHO gene. Such regulatory sequences include inducible, tissue-specific, and constitutive promoters and enhancer elements. The knock-in can be homozygous or heterozygous.

[0037] Transgenic nonhuman animals can also be produced that contain selected systems allowing for regulated expression of the transgene. One example of such a system that may be produced is the cre/loxP recombinase system of bacteriophage P1 (Lakso *et al.*, *PNAS* (1992) 89:6232-6236; U.S. Pat. No. 4,959,317). If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase. Another example of a recombinase system is the FLP recombinase

system of *Saccharomyces cerevisiae* (O'Gorman et al. (1991) Science 251:1351-1355; U.S. Pat. No. 5,654,182). In a preferred embodiment, both Cre-LoxP and Flp-Frt are used in the same system to regulate expression of the transgene, and for sequential deletion of vector sequences in the same cell (Sun X et al (2000) Nat Genet 25:83-6).

[0038] The genetically modified animals can be used in genetic studies to further elucidate the RHO pathway, as animal models of disease and disorders implicating defective RHO function, and for *in vivo* testing of candidate therapeutic agents, such as those identified in screens described below. The candidate therapeutic agents are administered to a genetically modified animal having altered MRHO function and phenotypic changes are compared with appropriate control animals such as genetically modified animals that receive placebo treatment, and/or animals with unaltered MRHO expression that receive candidate therapeutic agent.

[0039] In addition to the above-described genetically modified animals having altered MRHO function, animal models having defective RHO function (and otherwise normal MRHO function), can be used in the methods of the present invention. For example, a RHO knockout mouse can be used to assess, *in vivo*, the activity of a candidate RHO modulating agent identified in one of the *in vitro* assays described below. Preferably, the candidate RHO modulating agent when administered to a model system with cells defective in RHO function, produces a detectable phenotypic change in the model system indicating that the RHO function is restored, i.e., the cells exhibit normal cell cycle progression.

Modulating Agents

[0040] The invention provides methods to identify agents that interact with and/or modulate the function of MRHO and/or the RHO pathway. Modulating agents identified by the methods are also part of the invention. Such agents are useful in a variety of diagnostic and therapeutic applications associated with the RHO pathway, as well as in further analysis of the MRHO protein and its contribution to the RHO pathway. Accordingly, the invention also provides methods for modulating the RHO pathway comprising the step of specifically modulating MRHO activity by administering an MRHO-interacting or -modulating agent.

[0041] As used herein, an "MRHO-modulating agent" is any agent that modulates MRHO function, for example, an agent that interacts with MRHO to inhibit or enhance MRHO activity or otherwise affect normal MRHO function. MRHO

function can be affected at any level, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In a preferred embodiment, the MRHO - modulating agent specifically modulates the function of the MRHO. The phrases "specific modulating agent", "specifically modulates", etc., are used herein to refer to modulating agents that directly bind to the MRHO polypeptide or nucleic acid, and preferably inhibit, enhance, or otherwise alter, the function of the MRHO. These phrases also encompass modulating agents that alter the interaction of the MRHO with a binding partner, substrate, or cofactor (e.g. by binding to a binding partner of an MRHO, or to a protein/binding partner complex, and altering MRHO function). In a further preferred embodiment, the MRHO- modulating agent is a modulator of the RHO pathway (e.g. it restores and/or upregulates RHO function) and thus is also a RHO-modulating agent.

[0042] Preferred MRHO-modulating agents include small molecule compounds; MRHO-interacting proteins, including antibodies and other biotherapeutics; and nucleic acid modulators such as antisense and RNA inhibitors. The modulating agents may be formulated in pharmaceutical compositions, for example, as compositions that may comprise other active ingredients, as in combination therapy, and/or suitable carriers or excipients. Techniques for formulation and administration of the compounds may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, 19th edition.

Small molecule modulators

[0043] Small molecules are often preferred to modulate function of proteins with enzymatic function, and/or containing protein interaction domains. Chemical agents, referred to in the art as "small molecule" compounds are typically organic, non-peptide molecules, having a molecular weight up to 10,000, preferably up to 5,000, more preferably up to 1,000, and most preferably up to 500 daltons. This class of modulators includes chemically synthesized molecules, for instance, compounds from combinatorial chemical libraries. Synthetic compounds may be rationally designed or identified based on known or inferred properties of the MRHO protein or may be identified by screening compound libraries. Alternative appropriate modulators of this class are natural products, particularly secondary metabolites from organisms such as plants or fungi, which can also be identified by screening compound libraries for MRHO-modulating activity. Methods for generating and obtaining compounds

are well known in the art (Schreiber SL, Science (2000) 151: 1964-1969; Radmann J and Gunther J, Science (2000) 151:1947-1948).

[0044] Small molecule modulators identified from screening assays, as described below, can be used as lead compounds from which candidate clinical compounds may be designed, optimized, and synthesized. Such clinical compounds may have utility in treating pathologies associated with the RHO pathway. The activity of candidate small molecule modulating agents may be improved several-fold through iterative secondary functional validation, as further described below, structure determination, and candidate modulator modification and testing. Additionally, candidate clinical compounds are generated with specific regard to clinical and pharmacological properties. For example, the reagents may be derivatized and re-screened using *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

Protein Modulators

[0045] Specific MRHO-interacting proteins are useful in a variety of diagnostic and therapeutic applications related to the RHO pathway and related disorders, as well as in validation assays for other MRHO-modulating agents. In a preferred embodiment, MRHO-interacting proteins affect normal MRHO function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In another embodiment, MRHO-interacting proteins are useful in detecting and providing information about the function of MRHO proteins, as is relevant to RHO related disorders, such as cancer (e.g., for diagnostic means).

[0046] An MRHO-interacting protein may be endogenous, i.e. one that naturally interacts genetically or biochemically with an MRHO, such as a member of the MRHO pathway that modulates MRHO expression, localization, and/or activity. MRHO-modulators include dominant negative forms of MRHO-interacting proteins and of MRHO proteins themselves. Yeast two-hybrid and variant screens offer preferred methods for identifying endogenous MRHO-interacting proteins (Finley, R. L. et al. (1996) in DNA Cloning-Expression Systems: A Practical Approach, eds. Glover D. & Hames B. D (Oxford University Press, Oxford, England), pp. 169-203; Fashema SF et al., Gene (2000) 250:1-14; Drees BL Curr Opin Chem Biol (1999) 3:64-70; Vidal M and Legrain P Nucleic Acids Res (1999) 27:919-29; and U.S. Pat. No. 5,928,868). Mass spectrometry is an alternative preferred method for the

elucidation of protein complexes (reviewed in, e.g., Pandley A and Mann M, *Nature* (2000) 405:837-846; Yates JR 3rd, *Trends Genet* (2000) 16:5-8).

- [0047] An MRHO-interacting protein may be an exogenous protein, such as an MRHO-specific antibody or a T-cell antigen receptor (see, e.g., Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory; Harlow and Lane (1999) *Using antibodies: a laboratory manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press). MRHO antibodies are further discussed below.
- [0048] In preferred embodiments, an MRHO-interacting protein specifically binds an MRHO protein. In alternative preferred embodiments, an MRHO-modulating agent binds an MRHO substrate, binding partner, or cofactor.

Antibodies

- [0049] In another embodiment, the protein modulator is an MRHO specific antibody agonist or antagonist. The antibodies have therapeutic and diagnostic utilities, and can be used in screening assays to identify MRHO modulators. The antibodies can also be used in dissecting the portions of the MRHO pathway responsible for various cellular responses and in the general processing and maturation of the MRHO.
- [0050] Antibodies that specifically bind MRHO polypeptides can be generated using known methods. Preferably the antibody is specific to a mammalian ortholog of MRHO polypeptide, and more preferably, to human MRHO. Antibodies may be polyclonal, monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab').sub.2 fragments, fragments produced by a FAb expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Epitopes of MRHO which are particularly antigenic can be selected, for example, by routine screening of MRHO polypeptides for antigenicity or by applying a theoretical method for selecting antigenic regions of a protein (Hopp and Wood (1981), *Proc. Natl. Acad. Sci. U.S.A.* 78:3824-28; Hopp and Wood, (1983) *Mol. Immunol.* 20:483-89; Sutcliffe et al., (1983) *Science* 219:660-66) to the amino acid sequence of an MRHO. Monoclonal antibodies with affinities of 10^8 M^{-1} preferably 10^9 M^{-1} to 10^{10} M^{-1} , or stronger can be made by standard procedures as described (Harlow and Lane, *supra*; Goding (1986) *Monoclonal Antibodies: Principles and Practice* (2d ed) Academic Press, New York; and U.S. Pat. Nos. 4,381,292; 4,451,570; and 4,618,577). Antibodies may be generated against crude

cell extracts of MRHO or substantially purified fragments thereof. If MRHO fragments are used, they preferably comprise at least 10, and more preferably, at least 20 contiguous amino acids of an MRHO protein. In a particular embodiment, MRHO-specific antigens and/or immunogens are coupled to carrier proteins that stimulate the immune response. For example, the subject polypeptides are covalently coupled to the keyhole limpet hemocyanin (KLH) carrier, and the conjugate is emulsified in Freund's complete adjuvant, which enhances the immune response. An appropriate immune system such as a laboratory rabbit or mouse is immunized according to conventional protocols.

[0051] The presence of MRHO-specific antibodies is assayed by an appropriate assay such as a solid phase enzyme-linked immunosorbant assay (ELISA) using immobilized corresponding MRHO polypeptides. Other assays, such as radioimmunoassays or fluorescent assays might also be used.

[0052] Chimeric antibodies specific to MRHO polypeptides can be made that contain different portions from different animal species. For instance, a human immunoglobulin constant region may be linked to a variable region of a murine mAb, such that the antibody derives its biological activity from the human antibody, and its binding specificity from the murine fragment. Chimeric antibodies are produced by splicing together genes that encode the appropriate regions from each species (Morrison et al., *Proc. Natl. Acad. Sci.* (1984) 81:6851-6855; Neuberger et al., *Nature* (1984) 312:604-608; Takeda et al., *Nature* (1985) 31:452-454). Humanized antibodies, which are a form of chimeric antibodies, can be generated by grafting complementary-determining regions (CDRs) (Carlos, T. M., J. M. Harlan. 1994. *Blood* 84:2068-2101) of mouse antibodies into a background of human framework regions and constant regions by recombinant DNA technology (Riechmann LM, et al., 1988 *Nature* 323: 323-327). Humanized antibodies contain ~10% murine sequences and ~90% human sequences, and thus further reduce or eliminate immunogenicity, while retaining the antibody specificities (Co MS, and Queen C. 1991 *Nature* 351: 501-501; Morrison SL. 1992 *Ann. Rev. Immun.* 10:239-265). Humanized antibodies and methods of their production are well-known in the art (U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,762, and 6,180,370).

[0053] MRHO-specific single chain antibodies which are recombinant, single chain polypeptides formed by linking the heavy and light chain fragments of the Fv regions via an amino acid bridge, can be produced by methods known in the art (U.S. Pat. No.

4,946,778; Bird, Science (1988) 242:423-426; Huston et al., Proc. Natl. Acad. Sci. USA (1988) 85:5879-5883; and Ward et al., Nature (1989) 334:544-546).

[0054] Other suitable techniques for antibody production involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors (Huse et al., Science (1989) 246:1275-1281). As used herein, T-cell antigen receptors are included within the scope of antibody modulators (Harlow and Lane, 1988, *supra*).

[0055] The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, antibodies will be labeled by joining, either covalently or non-covalently, a substance that provides for a detectable signal, or that is toxic to cells that express the targeted protein (Menard S, et al., Int J. Biol Markers (1989) 4:131-134). A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, fluorescent emitting lanthanide metals, chemiluminescent moieties, bioluminescent moieties, magnetic particles, and the like (U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241). Also, recombinant immunoglobulins may be produced (U.S. Pat. No. 4,816,567). Antibodies to cytoplasmic polypeptides may be delivered and reach their targets by conjugation with membrane-penetrating toxin proteins (U.S. Pat. No. 6,086,900).

[0056] When used therapeutically in a patient, the antibodies of the subject invention are typically administered parenterally, when possible at the target site, or intravenously. The therapeutically effective dose and dosage regimen is determined by clinical studies. Typically, the amount of antibody administered is in the range of about 0.1 mg/kg –to about 10 mg/kg of patient weight. For parenteral administration, the antibodies are formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion) in association with a pharmaceutically acceptable vehicle. Such vehicles are inherently nontoxic and non-therapeutic. Examples are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils, ethyl oleate, or liposome carriers may also be used. The vehicle may contain minor amounts of additives, such as buffers and preservatives, which enhance isotonicity and chemical stability or otherwise enhance therapeutic potential. The antibodies' concentrations in such vehicles are typically in

the range of about 1 mg/ml to about 10 mg/ml. Immunotherapeutic methods are further described in the literature (US Pat. No. 5,859,206; WO0073469).

Specific biotherapeutics

- [0057] In a preferred embodiment, an MRHO-interacting protein may have biotherapeutic applications. Biotherapeutic agents formulated in pharmaceutically acceptable carriers and dosages may be used to activate or inhibit signal transduction pathways. This modulation may be accomplished by binding a ligand, thus inhibiting the activity of the pathway; or by binding a receptor, either to inhibit activation of, or to activate, the receptor. Alternatively, the biotherapeutic may itself be a ligand capable of activating or inhibiting a receptor. Biotherapeutic agents and methods of producing them are described in detail in U.S. Pat. No. 6,146,628.
- [0058] When the MRHO is a ligand, it may be used as a biotherapeutic agent to activate or inhibit its natural receptor. Alternatively, antibodies against MRHO, as described in the previous section, may be used as biotherapeutic agents.
- [0059] When the MRHO is a receptor, its ligand(s), antibodies to the ligand(s) or the MRHO itself may be used as biotherapeutics to modulate the activity of MRHO in the RHO pathway.

Nucleic Acid Modulators

- [0060] Other preferred MRHO-modulating agents comprise nucleic acid molecules, such as antisense oligomers or double stranded RNA (dsRNA), which generally inhibit MRHO activity. Preferred nucleic acid modulators interfere with the function of the MRHO nucleic acid such as DNA replication, transcription, translocation of the MRHO RNA to the site of protein translation, translation of protein from the MRHO RNA, splicing of the MRHO RNA to yield one or more mRNA species, or catalytic activity which may be engaged in or facilitated by the MRHO RNA.
- [0061] In one embodiment, the antisense oligomer is an oligonucleotide that is sufficiently complementary to an MRHO mRNA to bind to and prevent translation, preferably by binding to the 5' untranslated region. MRHO-specific antisense oligonucleotides, preferably range from at least 6 to about 200 nucleotides. In some embodiments the oligonucleotide is preferably at least 10, 15, or 20 nucleotides in length. In other embodiments, the oligonucleotide is preferably less than 50, 40, or 30 nucleotides in length. The oligonucleotide can be DNA or RNA or a chimeric

mixture or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, agents that facilitate transport across the cell membrane, hybridization-triggered cleavage agents, and intercalating agents.

[0062] In another embodiment, the antisense oligomer is a phosphothioate morpholino oligomer (PMO). PMOs are assembled from four different morpholino subunits, each of which contain one of four genetic bases (A, C, G, or T) linked to a six-membered morpholine ring. Polymers of these subunits are joined by non-ionic phosphodiamidate intersubunit linkages. Details of how to make and use PMOs and other antisense oligomers are well known in the art (e.g. see WO99/18193; Probst JC, Antisense Oligodeoxynucleotide and Ribozyme Design, Methods. (2000) 22(3):271-281; Summerton J, and Weller D. 1997 Antisense Nucleic Acid Drug Dev. :7:187-95; US Pat. No. 5,235,033; and US Pat No. 5,378,841).

[0063] Alternative preferred MRHO nucleic acid modulators are double-stranded RNA species mediating RNA interference (RNAi). RNAi is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. Methods relating to the use of RNAi to silence genes in *C. elegans*, *Drosophila*, plants, and humans are known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); Sharp, P. A. RNA interference 2001. Genes Dev. 15, 485-490 (2001); Hammond, S. M., et al., Nature Rev. Genet. 2, 110-1119 (2001); Tuschl, T. Chem. Biochem. 2, 239-245 (2001); Hamilton, A. et al., Science 286, 950-952 (1999); Hammond, S. M., et al., Nature 404, 293-296 (2000); Zamore, P. D., et al., Cell 101, 25-33 (2000); Bernstein, E., et al., Nature 409, 363-366 (2001); Elbashir, S. M., et al., Genes Dev. 15, 188-200 (2001); WO0129058; WO9932619; Elbashir SM, et al., 2001 Nature 411:494-498; Novina CD and Sharp P. 2004 Nature 430:161-164; Soutschek J et al 2004 Nature 432:173-178; Hsieh AC et al. (2004) NAR 32(3):893-901).

[0064] Nucleic acid modulators are commonly used as research reagents, diagnostics, and therapeutics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used to elucidate the function of particular genes (see, for example, U.S. Pat. No. 6,165,790). Nucleic acid modulators are also used, for example, to distinguish between functions of various members of a

biological pathway. For example, antisense oligomers have been employed as therapeutic moieties in the treatment of disease states in animals and man and have been demonstrated in numerous clinical trials to be safe and effective (Milligan JF, *et al.*, Current Concepts in Antisense Drug Design, J Med Chem. (1993) 36:1923-1937; Tonkinson JL *et al.*, Antisense Oligodeoxynucleotides as Clinical Therapeutic Agents, Cancer Invest. (1996) 14:54-65). Accordingly, in one aspect of the invention, an MRHO-specific nucleic acid modulator is used in an assay to further elucidate the role of the MRHO in the RHO pathway, and/or its relationship to other members of the pathway. In another aspect of the invention, an MRHO-specific antisense oligomer is used as a therapeutic agent for treatment of RHO-related disease states.

Assay Systems

[0065] The invention provides assay systems and screening methods for identifying specific modulators of MRHO activity. As used herein, an "assay system" encompasses all the components required for performing and analyzing results of an assay that detects and/or measures a particular event. In general, primary assays are used to identify or confirm a modulator's specific biochemical or molecular effect with respect to the MRHO nucleic acid or protein. In general, secondary assays further assess the activity of an MRHO modulating agent identified by a primary assay and may confirm that the modulating agent affects MRHO in a manner relevant to the RHO pathway. In some cases, MRHO modulators will be directly tested in a secondary assay.

[0066] In a preferred embodiment, the screening method comprises contacting a suitable assay system comprising an MRHO polypeptide or nucleic acid with a candidate agent under conditions whereby, but for the presence of the agent, the system provides a reference activity (e.g. binding activity), which is based on the particular molecular event the screening method detects. A statistically significant difference between the agent-biased activity and the reference activity indicates that the candidate agent modulates MRHO activity, and hence the RHO pathway. The MRHO polypeptide or nucleic acid used in the assay may comprise any of the nucleic acids or polypeptides described above.

Primary Assays

[0067] The type of modulator tested generally determines the type of primary assay.

Primary assays for small molecule modulators

[0068] For small molecule modulators, screening assays are used to identify candidate modulators. Screening assays may be cell-based or may use a cell-free system that recreates or retains the relevant biochemical reaction of the target protein (reviewed in Sittampalam GS *et al.*, Curr Opin Chem Biol (1997) 1:384-91 and accompanying references). As used herein the term "cell-based" refers to assays using live cells, dead cells, or a particular cellular fraction, such as a membrane, endoplasmic reticulum, or mitochondrial fraction. The term "cell free" encompasses assays using substantially purified protein (either endogenous or recombinantly produced), partially purified or crude cellular extracts. Screening assays may detect a variety of molecular events, including protein-DNA interactions, protein-protein interactions (*e.g.*, receptor-ligand binding), transcriptional activity (*e.g.*, using a reporter gene), enzymatic activity (*e.g.*, via a property of the substrate), activity of second messengers, immunogenicity and changes in cellular morphology or other cellular characteristics. Appropriate screening assays may use a wide range of detection methods including fluorescent, radioactive, colorimetric, spectrophotometric, and amperometric methods, to provide a read-out for the particular molecular event detected.

[0069] Cell-based screening assays usually require systems for recombinant expression of MRHO and any auxiliary proteins demanded by the particular assay. Appropriate methods for generating recombinant proteins produce sufficient quantities of proteins that retain their relevant biological activities and are of sufficient purity to optimize activity and assure assay reproducibility. Yeast two-hybrid and variant screens, and mass spectrometry provide preferred methods for determining protein-protein interactions and elucidation of protein complexes. In certain applications, when MRHO-interacting proteins are used in screens to identify small molecule modulators, the binding specificity of the interacting protein to the MRHO protein may be assayed by various known methods such as substrate processing (*e.g.* ability of the candidate MRHO-specific binding agents to function as negative effectors in MRHO-expressing cells), binding equilibrium constants (usually at least about 10^7 M^{-1} , preferably at least about 10^8 M^{-1} , more preferably at least about 10^9 M^{-1}), and immunogenicity (*e.g.* ability to elicit MRHO specific antibody in a

heterologous host such as a mouse, rat, goat or rabbit). For enzymes and receptors, binding may be assayed by, respectively, substrate and ligand processing.

[0070] The screening assay may measure a candidate agent's ability to specifically bind to or modulate activity of an MRHO polypeptide, a fusion protein thereof, or to cells or membranes bearing the polypeptide or fusion protein. The MRHO polypeptide can be full length or a fragment thereof that retains functional MRHO activity. The MRHO polypeptide may be fused to another polypeptide, such as a peptide tag for detection or anchoring, or to another tag. The MRHO polypeptide is preferably human MRHO, or is an ortholog or derivative thereof as described above. In a preferred embodiment, the screening assay detects candidate agent-based modulation of MRHO interaction with a binding target, such as an endogenous or exogenous protein or other substrate that has MRHO -specific binding activity, and can be used to assess normal MRHO gene function.

[0071] Suitable assay formats that may be adapted to screen for MRHO modulators are known in the art. Preferred screening assays are high throughput or ultra high throughput and thus provide automated, cost-effective means of screening compound libraries for lead compounds (Fernandes PB, *Curr Opin Chem Biol* (1998) 2:597-603; Sundberg SA, *Curr Opin Biotechnol* 2000, 11:47-53). In one preferred embodiment, screening assays uses fluorescence technologies, including fluorescence polarization, time-resolved fluorescence, and fluorescence resonance energy transfer. These systems offer means to monitor protein-protein or DNA-protein interactions in which the intensity of the signal emitted from dye-labeled molecules depends upon their interactions with partner molecules (*e.g.*, Selvin PR, *Nat Struct Biol* (2000) 7:730-4; Fernandes PB, *supra*; Hertzberg RP and Pope AJ, *Curr Opin Chem Biol* (2000) 4:445-451).

[0072] A variety of suitable assay systems may be used to identify candidate MRHO and RHO pathway modulators (*e.g.* U.S. Pat. No. 6,165,992 and U.S. Pat. No. 6,720,162 (kinase assays); U.S. Pat. Nos. 5,550,019 and 6,133,437 (apoptosis assays); WO 01/25487 (Helicase assays), U.S. Pat. No. 6,114,132 and U.S. Pat. No. 6,720,162 (phosphatase and protease assays), U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434 (angiogenesis assays), among others). Specific preferred assays are described in more detail below.

[0073] Protein kinases, key signal transduction proteins that may be either membrane-associated or intracellular, catalyze the transfer of gamma phosphate from adenosine

triphosphate (ATP) to a serine, threonine or tyrosine residue in a protein substrate. Radioassays, which monitor the transfer from [γ - ^{32}P or ^{33}P]ATP, are frequently used to assay kinase activity. For instance, a scintillation assay for p56 (lck) kinase activity monitors the transfer of the gamma phosphate from [γ - ^{33}P] ATP to a biotinylated peptide substrate. The substrate is captured on a streptavidin coated bead that transmits the signal (Beveridge M *et al.*, J Biomol Screen (2000) 5:205-212). This assay uses the scintillation proximity assay (SPA), in which only radio-ligand bound to receptors tethered to the surface of an SPA bead are detected by the scintillant immobilized within it, allowing binding to be measured without separation of bound from free ligand. Other assays for protein kinase activity may use antibodies that specifically recognize phosphorylated substrates. For instance, the kinase receptor activation (KIRA) assay measures receptor tyrosine kinase activity by ligand stimulating the intact receptor in cultured cells, then capturing solubilized receptor with specific antibodies and quantifying phosphorylation via phosphotyrosine ELISA (Sadick MD, Dev Biol Stand (1999) 97:121-133). Another example of antibody based assays for protein kinase activity is TRF (time-resolved fluorometry). This method utilizes europium chelate-labeled anti-phosphotyrosine antibodies to detect phosphate transfer to a polymeric substrate coated onto microtiter plate wells. The amount of phosphorylation is then detected using time-resolved, dissociation-enhanced fluorescence (Braunwalder AF, et al., Anal Biochem 1996 Jul 1;238(2):159-64). Yet other assays for kinases involve uncoupled, pH sensitive assays that can be used for high-throughput screening of potential inhibitors or for determining substrate specificity. Since kinases catalyze the transfer of a gamma-phosphoryl group from ATP to an appropriate hydroxyl acceptor with the release of a proton, a pH sensitive assay is based on the detection of this proton using an appropriately matched buffer/indicator system (Chapman E and Wong CH (2002) Bioorg Med Chem. 10:551-5).

[0074] Protein phosphatases catalyze the removal of a gamma phosphate from a serine, threonine or tyrosine residue in a protein substrate. Since phosphatases act in opposition to kinases, appropriate assays measure the same parameters as kinase assays. In one example, the dephosphorylation of a fluorescently labeled peptide substrate allows trypsin cleavage of the substrate, which in turn renders the cleaved substrate significantly more fluorescent (Nishikata M *et al.*, Biochem J (1999) 343:35-391). In another example, fluorescence polarization (FP), a solution-based,

homogeneous technique requiring no immobilization or separation of reaction components, is used to develop high throughput screening (HTS) assays for protein phosphatases. This assay uses direct binding of the phosphatase with the target, and increasing concentrations of target- phosphatase increase the rate of dephosphorylation, leading to a change in polarization (Parker GJ *et al.*, (2000) J Biomol Screen 5:77-88).

- [0075] Proteases are enzymes that cleave protein substrates at specific sites. Exemplary assays detect the alterations in the spectral properties of an artificial substrate that occur upon protease-mediated cleavage. In one example, synthetic caspase substrates containing four amino acid proteolysis recognition sequences, separating two different fluorescent tags are employed; fluorescence resonance energy transfer detects the proximity of these fluorophores, which indicates whether the substrate is cleaved (Mahajan NP *et al.*, Chem Biol (1999) 6:401-409).
- [0076] Helicases are involved in unwinding double stranded DNA and RNA. In one embodiment, an assay for DNA helicase activity detects the displacement of a radio-labeled oligonucleotide from single stranded DNA upon initiation of unwinding (Sivaraja M *et al.*, Anal Biochem (1998) 265:22-27). An assay for RNA helicase activity uses the scintillation proximity (SPA) assay to detect the displacement of a radio-labeled oligonucleotide from single stranded RNA (Kyono K *et al.*, Anal Biochem (1998) 257:120-126).
- [0077] Peptidyl-prolyl isomerase (PPIase) proteins, which include cyclophilins, FK506 binding proteins and paravulins, catalyze the isomerization of *cis-trans* proline peptide bonds in oligopeptides and are thought to be essential for protein folding during protein synthesis in the cell. Spectrophotometric assays for PPIase activity can detect isomerization of labeled peptide substrates, either by direct measurement of isomer-specific absorbance, or by coupling isomerization to isomer-specific cleavage by chymotrypsin (Scholz C *et al.*, FEBS Lett (1997) 414:69-73; Janowski B *et al.*, Anal Biochem (1997) 252:299-307; Kullertz G *et al.*, Clin Chem (1998) 44:502-8). Alternative assays use the scintillation proximity or fluorescence polarization assay to screen for ligands of specific PPIases (Graziani F *et al.*, J Biomol Screen (1999) 4:3-7; Dubowchik GM *et al.*, Bioorg Med Chem Lett (2000) 10:559-562). Assays for 3,2-trans-enoyl-CoA isomerase activity have also been described (Binstock, J. F., and Schulz, H. (1981) Methods Enzymol. 71:403-411; Geisbrecht BV *et al* (1999) J Biol Chem. 274:21797-803). These assays use 3-cis-octenoyl-CoA as a substrate, and

reaction progress is monitored spectrophotometrically using a coupled assay for the isomerization of 3-cis-octenoyl-CoA to 2-trans-octenoyl-CoA.

- [0078] Ubiquitination is a process of attaching ubiquitin to a protein prior to the selective proteolysis of that protein in the cell. Assays based on fluorescence resonance energy transfer to screen for ubiquitination inhibitors are known in the art (Boisclair MD et al., J Biomol Screen 2000 5:319-328).
- [0079] Hydrolases catalyze the hydrolysis of a substrate such as esterases, lipases, peptidases, nucleotidases, and phosphatases, among others. Enzyme activity assays may be used to measure hydrolase activity. The activity of the enzyme is determined in presence of excess substrate, by spectrophotometrically measuring the rate of appearance of reaction products. High throughput arrays and assays for hydrolases are known to those skilled in the art (Park CB and Clark DS (2002) Biotech Bioeng 78:229-235).
- [0080] Kinesins are motor proteins. Assays for kinesins involve their ATPase activity, such as described in Blackburn et al (Blackburn CL, et al., (1999) J Org Chem 64:5565-5570). The ATPase assay is performed using the EnzCheck ATPase kit (Molecular Probes). The assays are performed using an Ultraspec spectrophotometer (Pharmacia), and the progress of the reaction are monitored by absorbance increase at 360 nm. Microtubules (1.7 mM final), kinesin (0.11 mM final), inhibitor (or DMSO blank at 5% final), and the EnzCheck components (purine nucleotide phosphorylase and MESG substrate) are premixed in the cuvette in a reaction buffer (40 mM PIPES pH 6.8, 5 mM paclitaxel, 1 mM EGTA, 5 mM MgCl₂). The reaction is initiated by addition of MgATP (1 mM final).
- [0081] High-throughput assays, such as scintillation proximity assays, for synthase enzymes involved in fatty acid synthesis are known in the art (He X et al (2000) Anal Biochem 2000 Jun 15;282(1):107-14).
- [0082] **Apoptosis assays.** Apoptosis or programmed cell death is a suicide program is activated within the cell, leading to fragmentation of DNA, shrinkage of the cytoplasm, membrane changes and cell death. Apoptosis is mediated by proteolytic enzymes of the caspase family. Many of the altering parameters of a cell are measurable during apoptosis. Assays for apoptosis may be performed by terminal deoxynucleotidyl transferase-mediated digoxigenin-11-dUTP nick end labeling (TUNEL) assay. The TUNEL assay is used to measure nuclear DNA fragmentation

characteristic of apoptosis (Lazebnik *et al.*, 1994, Nature 371, 346), by following the incorporation of fluorescein-dUTP (Yonehara *et al.*, 1989, J. Exp. Med. 169, 1747). Apoptosis may further be assayed by acridine orange staining of tissue culture cells (Lucas, R., et al., 1998, Blood 15:4730-41). Other cell-based apoptosis assays include the caspase-3/7 assay and the cell death nucleosome ELISA assay. The caspase 3/7 assay is based on the activation of the caspase cleavage activity as part of a cascade of events that occur during programmed cell death in many apoptotic pathways. In the caspase 3/7 assay (commercially available Apo-ONE™ Homogeneous Caspase-3/7 assay from Promega, cat# 67790), lysis buffer and caspase substrate are mixed and added to cells. The caspase substrate becomes fluorescent when cleaved by active caspase 3/7. The nucleosome ELISA assay is a general cell death assay known to those skilled in the art, and available commercially (Roche, Cat# 1774425). This assay is a quantitative sandwich-enzyme-immunoassay which uses monoclonal antibodies directed against DNA and histones respectively, thus specifically determining amount of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. Mono and oligonucleosomes are enriched in the cytoplasm during apoptosis due to the fact that DNA fragmentation occurs several hours before the plasma membrane breaks down, allowing for accumulation in the cytoplasm. Nucleosomes are not present in the cytoplasmic fraction of cells that are not undergoing apoptosis. The Phospho-histone H2B assay is another apoptosis assay, based on phosphorylation of histone H2B as a result of apoptosis. Fluorescent dyes that are associated with phosphohistone H2B may be used to measure the increase of phosphohistone H2B as a result of apoptosis. Apoptosis assays that simultaneously measure multiple parameters associated with apoptosis have also been developed. In such assays, various cellular parameters that can be associated with antibodies or fluorescent dyes, and that mark various stages of apoptosis are labeled, and the results are measured using instruments such as Cellomics™ ArrayScan® HCS System. The measurable parameters and their markers include anti-active caspase-3 antibody which marks intermediate stage apoptosis, anti-PARP-p85 antibody (cleaved PARP) which marks late stage apoptosis, Hoechst labels which label the nucleus and are used to measure nuclear swelling as a measure of early apoptosis and nuclear condensation as a measure of late apoptosis, TOTO-3 fluorescent dye which labels DNA of dead cells with high cell membrane permeability, and anti-alpha-tubulin or F-actin labels, which assess cytoskeletal changes in cells and correlate well with TOTO-3 label.

These assays may also be used for involvement of a gene in cell cycle and assessment of alterations in cell morphology.

[0083] An apoptosis assay system may comprise a cell that expresses an MRHO, and that optionally has defective RHO function (e.g. RHO is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the apoptosis assay system and changes in induction of apoptosis relative to controls where no test agent is added, identify candidate RHO modulating agents. In some embodiments of the invention, an apoptosis assay may be used as a secondary assay to test a candidate RHO modulating agents that is initially identified using a cell-free assay system. An apoptosis assay may also be used to test whether MRHO function plays a direct role in apoptosis. For example, an apoptosis assay may be performed on cells that over- or under-express MRHO relative to wild type cells. Differences in apoptotic response compared to wild type cells suggests that the MRHO plays a direct role in the apoptotic response. Apoptosis assays are described further in US Pat. No. 6,133,437.

[0084] **Cell proliferation and cell cycle assays.** Cell proliferation may be assayed via bromodeoxyuridine (BRDU) incorporation. This assay identifies a cell population undergoing DNA synthesis by incorporation of BRDU into newly-synthesized DNA. Newly-synthesized DNA may then be detected using an anti-BRDU antibody (Hoshino *et al.*, 1986, *Int. J. Cancer* 38, 369; Campana *et al.*, 1988, *J. Immunol. Meth.* 107, 79), or by other means.

[0085] Cell proliferation is also assayed via phospho-histone H3 staining, which identifies a cell population undergoing mitosis by phosphorylation of histone H3. Phosphorylation of histone H3 at serine 10 is detected using an antibody specific to the phosphorylated form of the serine 10 residue of histone H3. (Chadlee, D.N. 1995, *J. Biol. Chem* 270:20098-105). Cell Proliferation may also be examined using [³H]-thymidine incorporation (Chen, J., 1996, *Oncogene* 13:1395-403; Jeoung, J., 1995, *J. Biol. Chem.* 270:18367-73). This assay allows for quantitative characterization of S-phase DNA syntheses. In this assay, cells synthesizing DNA will incorporate [³H]-thymidine into newly synthesized DNA. Incorporation can then be measured by standard techniques such as by counting of radioisotope in a scintillation counter (e.g., Beckman LS 3800 Liquid Scintillation Counter). Another proliferation assay uses the dye Alamar Blue (available from Biosource International), which fluoresces when reduced in living cells and provides an indirect measurement of cell number (Voytik-

Harbin SL et al., 1998, *In Vitro Cell Dev Biol Anim* 34:239-46). Yet another proliferation assay, the MTS assay, is based on in vitro cytotoxicity assessment of industrial chemicals, and uses the soluble tetrazolium salt, MTS. MTS assays are commercially available, for example, the Promega CellTiter 96[®] AQueous Non-Radioactive Cell Proliferation Assay (Cat.# G5421).

[0086] Cell proliferation may also be assayed by colony formation in soft agar, or clonogenic survival assay (Sambrook et al., *Molecular Cloning*, Cold Spring Harbor (1989)). For example, cells transformed with MRHO are seeded in soft agar plates, and colonies are measured and counted after two weeks incubation.

[0087] Cell proliferation may also be assayed by measuring ATP levels as indicator of metabolically active cells. Such assays are commercially available, for example Cell Titer-Glo[™], which is a luminescent homogeneous assay available from Promega.

[0088] Involvement of a gene in the cell cycle may be assayed by flow cytometry (Gray JW et al. (1986) *Int J Radiat Biol Relat Stud Phys Chem Med* 49:237-55). Cells transfected with an MRHO may be stained with propidium iodide and evaluated in a flow cytometer (available from Becton Dickinson), which indicates accumulation of cells in different stages of the cell cycle.

[0089] Involvement of a gene in the cell cycle, cell movement, or cell morphology may further be assessed using the Cellomics[™] ArrayScan[®] HCS System, as described above. For cell morphology assessments, a further measurable parameter and marker is fluorescent phospho-Cofilin. Cofilin is a gene involved downstream in the RHO pathway that is phosphorylated by LIMK. Decreased LIMK levels lead to reduction of phospho-cofilin, and reduced fluorescent phospho-cofilin in the assay. Genes whose expression pattern is consistent with that of LIMK are members of the RHO pathway. For cell motility, cells are seeded in 96 well plates, then treated with modulator of interest, such as RNAi, then transferred to collagen plates containing fluorescent microspheres. Replated cells are later fixed and stained with rhodamine-Alexa546, and motility tracks are viewed and measured using the HCS system.

[0090] Accordingly, a cell proliferation, cell movement, cell morphology, or cell cycle assay system may comprise a cell that expresses an MRHO, and that optionally has defective RHO function (e.g. RHO is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the assay system and changes in cell proliferation or cell cycle relative to controls where no test agent is added, identify

candidate RHO modulating agents. In some embodiments of the invention, the cell proliferation or cell cycle assay may be used as a secondary assay to test a candidate RHO modulating agents that is initially identified using another assay system such as a cell-free assay system. A cell proliferation assay may also be used to test whether MRHO function plays a direct role in cell proliferation or cell cycle. For example, a cell proliferation or cell cycle assay may be performed on cells that over- or under-express MRHO relative to wild type cells. Differences in proliferation or cell cycle compared to wild type cells suggests that the MRHO plays a direct role in cell proliferation or cell cycle.

[0091] Angiogenesis. Angiogenesis may be assayed using various human endothelial cell systems, such as umbilical vein, coronary artery, or dermal cells. Suitable assays include Alamar Blue based assays (available from Biosource International) to measure proliferation; migration assays using fluorescent molecules, such as the use of Becton Dickinson Falcon HTS FluoroBlock cell culture inserts to measure migration of cells through membranes in presence or absence of angiogenesis enhancer or suppressors; and tubule formation assays based on the formation of tubular structures by endothelial cells on Matrigel® (Becton Dickinson). Accordingly, an angiogenesis assay system may comprise a cell that expresses an MRHO, and that optionally has defective RHO function (e.g. RHO is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the angiogenesis assay system and changes in angiogenesis relative to controls where no test agent is added, identify candidate RHO modulating agents. In some embodiments of the invention, the angiogenesis assay may be used as a secondary assay to test a candidate RHO modulating agents that is initially identified using another assay system. An angiogenesis assay may also be used to test whether MRHO function plays a direct role in cell proliferation. For example, an angiogenesis assay may be performed on cells that over- or under-express MRHO relative to wild type cells. Differences in angiogenesis compared to wild type cells suggests that the MRHO plays a direct role in angiogenesis. U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434, among others, describe various angiogenesis assays.

[0092] Hypoxic induction. The alpha subunit of the transcription factor, hypoxia inducible factor-1 (HIF-1), is upregulated in tumor cells following exposure to

hypoxia in vitro. Under hypoxic conditions, HIF-1 stimulates the expression of genes known to be important in tumour cell survival, such as those encoding glycolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells transfected with MRHO in hypoxic conditions (such as with 0.1% O₂, 5% CO₂, and balance N₂, generated in a Napco 7001 incubator (Precision Scientific)) and normoxic conditions, followed by assessment of gene activity or expression by Taqman®. For example, a hypoxic induction assay system may comprise a cell that expresses an MRHO, and that optionally has defective RHO function (e.g. RHO is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the hypoxic induction assay system and changes in hypoxic response relative to controls where no test agent is added, identify candidate RHO modulating agents. In some embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate RHO modulating agents that is initially identified using another assay system. A hypoxic induction assay may also be used to test whether MRHO function plays a direct role in the hypoxic response. For example, a hypoxic induction assay may be performed on cells that over- or under-express MRHO relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the MRHO plays a direct role in hypoxic induction.

[0093] Cell adhesion. Cell adhesion assays measure adhesion of cells to purified adhesion proteins, or adhesion of cells to each other, in presence or absence of candidate modulating agents. Cell-protein adhesion assays measure the ability of agents to modulate the adhesion of cells to purified proteins. For example, recombinant proteins are produced, diluted to 2.5g/mL in PBS, and used to coat the wells of a microtiter plate. The wells used for negative control are not coated. Coated wells are then washed, blocked with 1% BSA, and washed again. Compounds are diluted to 2× final test concentration and added to the blocked, coated wells. Cells are then added to the wells, and the unbound cells are washed off. Retained cells are labeled directly on the plate by adding a membrane-permeable fluorescent dye, such as calcein-AM, and the signal is quantified in a fluorescent microplate reader.

[0094] Cell-cell adhesion assays measure the ability of agents to modulate binding of cell adhesion proteins with their native ligands. These assays use cells that naturally or recombinantly express the adhesion protein of choice. In an exemplary assay, cells

expressing the cell adhesion protein are plated in wells of a multiwell plate. Cells expressing the ligand are labeled with a membrane-permeable fluorescent dye, such as BCECF, and allowed to adhere to the monolayers in the presence of candidate agents. Unbound cells are washed off, and bound cells are detected using a fluorescence plate reader.

[0095] High-throughput cell adhesion assays have also been described. In one such assay, small molecule ligands and peptides are bound to the surface of microscope slides using a microarray spotter, intact cells are then contacted with the slides, and unbound cells are washed off. In this assay, not only the binding specificity of the peptides and modulators against cell lines are determined, but also the functional cell signaling of attached cells using immunofluorescence techniques in situ on the microchip is measured (Falsey JR et al., *Bioconjug Chem.* 2001 May-Jun;12(3):346-53).

Primary assays for antibody modulators

[0096] For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the MRHO protein. Methods for testing antibody affinity and specificity are well known in the art (Harlow and Lane, 1988, 1999, *supra*). The enzyme-linked immunosorbent assay (ELISA) is a preferred method for detecting MRHO-specific antibodies; others include FACS assays, radioimmunoassays, and fluorescent assays.

[0097] In some cases, screening assays described for small molecule modulators may also be used to test antibody modulators.

Primary assays for nucleic acid modulators

[0098] For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance MRHO gene expression, preferably mRNA expression. In general, expression analysis comprises comparing MRHO expression in like populations of cells (*e.g.*, two pools of cells that endogenously or recombinantly express MRHO) in the presence and absence of the nucleic acid modulator. Methods for analyzing mRNA and protein expression are well known in the art. For instance, Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR (*e.g.*, using the TaqMan®, PE Applied Biosystems), or

microarray analysis may be used to confirm that MRHO mRNA expression is reduced in cells treated with the nucleic acid modulator (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, eds., John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm DH and Guiseppi-Elie, A Curr Opin Biotechnol 2001, 12:41-47). Protein expression may also be monitored. Proteins are most commonly detected with specific antibodies or antisera directed against either the MRHO protein or specific peptides. A variety of means including Western blotting, ELISA, or in situ detection, are available (Harlow E and Lane D, 1988 and 1999, *supra*).

[0099] In some cases, screening assays described for small molecule modulators, particularly in assay systems that involve MRHO mRNA expression, may also be used to test nucleic acid modulators.

Secondary Assays

[0100] Secondary assays may be used to further assess the activity of MRHO-modulating agent identified by any of the above methods to confirm that the modulating agent affects MRHO in a manner relevant to the RHO pathway. As used herein, MRHO-modulating agents encompass candidate clinical compounds or other agents derived from previously identified modulating agent. Secondary assays can also be used to test the activity of a modulating agent on a particular genetic or biochemical pathway or to test the specificity of the modulating agent's interaction with MRHO.

[0101] Secondary assays generally compare like populations of cells or animals (*e.g.*, two pools of cells or animals that endogenously or recombinantly express MRHO) in the presence and absence of the candidate modulator. In general, such assays test whether treatment of cells or animals with a candidate MRHO-modulating agent results in changes in the RHO pathway in comparison to untreated (or mock- or placebo-treated) cells or animals. Certain assays use "sensitized genetic backgrounds", which, as used herein, describe cells or animals engineered for altered expression of genes in the RHO or interacting pathways.

Cell-based assays

[0102] Cell based assays may detect endogenous RHO pathway activity or may rely on recombinant expression of RHO pathway components. Any of the aforementioned

assays may be used in this cell-based format. Candidate modulators are typically added to the cell media but may also be injected into cells or delivered by any other efficacious means.

Animal Assays

- [0103] A variety of non-human animal models of normal or defective RHO pathway may be used to test candidate MRHO modulators. Models for defective RHO pathway typically use genetically modified animals that have been engineered to mis-express (*e.g.*, over-express or lack expression in) genes involved in the RHO pathway. Assays generally require systemic delivery of the candidate modulators, such as by oral administration, injection, etc.
- [0104] In a preferred embodiment, RHO pathway activity is assessed by monitoring neovascularization and angiogenesis. Animal models with defective and normal RHO are used to test the candidate modulator's affect on MRHO in Matrigel® assays. Matrigel® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen IV, and heparin sulfate proteoglycan. It is provided as a sterile liquid at 4° C, but rapidly forms a solid gel at 37° C. Liquid Matrigel® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the MRHO. The mixture is then injected subcutaneously(SC) into female athymic nude mice (Taconic, Germantown, NY) to support an intense vascular response. Mice with Matrigel® pellets may be dosed via oral (PO), intraperitoneal (IP), or intravenous (IV) routes with the candidate modulator. Mice are euthanized 5 - 12 days post-injection, and the Matrigel® pellet is harvested for hemoglobin analysis (Sigma plasma hemoglobin kit). Hemoglobin content of the gel is found to correlate the degree of neovascularization in the gel.
- [0105] In another preferred embodiment, the effect of the candidate modulator on MRHO is assessed via tumorigenicity assays. Tumor xenograft assays are known in the art (see, *e.g.*, Ogawa K et al., 2000, *Oncogene* 19:6043-6052). Xenografts are typically implanted SC into female athymic mice, 6-7 week old, as single cell suspensions either from a pre-existing tumor or from *in vitro* culture. The tumors which express the MRHO endogenously are injected in the flank, 1×10^5 to 1×10^7 cells per mouse in a volume of 100 μ L using a 27gauge needle. Mice are then ear tagged and tumors are measured twice weekly. Candidate modulator treatment is initiated on the day the mean tumor weight reaches 100 mg. Candidate modulator is

delivered IV, SC, IP, or PO by bolus administration. Depending upon the pharmacokinetics of each unique candidate modulator, dosing can be performed multiple times per day. The tumor weight is assessed by measuring perpendicular diameters with a caliper and calculated by multiplying the measurements of diameters in two dimensions. At the end of the experiment, the excised tumors may be utilized for biomarker identification or further analyses. For immunohistochemistry staining, xenograft tumors are fixed in 4% paraformaldehyde, 0.1M phosphate, pH 7.2, for 6 hours at 4°C, immersed in 30% sucrose in PBS, and rapidly frozen in isopentane cooled with liquid nitrogen.

[0106] In another preferred embodiment, tumorigenicity is monitored using a hollow fiber assay, which is described in U.S. Pat No. US 5,698,413. Briefly, the method comprises implanting into a laboratory animal a biocompatible, semi-permeable encapsulation device containing target cells, treating the laboratory animal with a candidate modulating agent, and evaluating the target cells for reaction to the candidate modulator. Implanted cells are generally human cells from a pre-existing tumor or a tumor cell line. After an appropriate period of time, generally around six days, the implanted samples are harvested for evaluation of the candidate modulator. Tumorigenicity and modulator efficacy may be evaluated by assaying the quantity of viable cells present in the macrocapsule, which can be determined by tests known in the art, for example, MTT dye conversion assay, neutral red dye uptake, trypan blue staining, viable cell counts, the number of colonies formed in soft agar, the capacity of the cells to recover and replicate in vitro, etc.

[0107] In another preferred embodiment, a tumorigenicity assay use a transgenic animal, usually a mouse, carrying a dominant oncogene or tumor suppressor gene knockout under the control of tissue specific regulatory sequences; these assays are generally referred to as transgenic tumor assays. In a preferred application, tumor development in the transgenic model is well characterized or is controlled. In an exemplary model, the "RIP1-Tag2" transgene, comprising the SV40 large T-antigen oncogene under control of the insulin gene regulatory regions is expressed in pancreatic beta cells and results in islet cell carcinomas (Hanahan D, 1985, Nature 315:115-122; Parangi S et al, 1996, Proc Natl Acad Sci USA 93: 2002-2007; Bergers G et al, 1999, Science 284:808-812). An "angiogenic switch," occurs at approximately five weeks, as normally quiescent capillaries in a subset of hyperproliferative islets become angiogenic. The RIP1-TAG2 mice die by age 14

weeks. Candidate modulators may be administered at a variety of stages, including just prior to the angiogenic switch (e.g., for a model of tumor prevention), during the growth of small tumors (e.g., for a model of intervention), or during the growth of large and/or invasive tumors (e.g., for a model of regression). Tumorigenicity and modulator efficacy can be evaluating life-span extension and/or tumor characteristics, including number of tumors, tumor size, tumor morphology, vessel density, apoptotic index, etc.

Diagnostic and therapeutic uses

- [0108] Specific MRHO-modulating agents are useful in a variety of diagnostic and therapeutic applications where disease or disease prognosis is related to defects in the RHO pathway, such as angiogenic, apoptotic, or cell proliferation disorders. Accordingly, the invention also provides methods for modulating the RHO pathway in a cell, preferably a cell pre-determined to have defective or impaired RHO function (e.g. due to overexpression, underexpression, or misexpression of RHO, or due to gene mutations), comprising the step of administering an agent to the cell that specifically modulates MRHO activity. Preferably, the modulating agent produces a detectable phenotypic change in the cell indicating that the RHO function is restored. The phrase “function is restored”, and equivalents, as used herein, means that the desired phenotype is achieved, or is brought closer to normal compared to untreated cells. For example, with restored RHO function, cell proliferation and/or progression through cell cycle may normalize, or be brought closer to normal relative to untreated cells. The invention also provides methods for treating disorders or disease associated with impaired RHO function by administering a therapeutically effective amount of an MRHO -modulating agent that modulates the RHO pathway. The invention further provides methods for modulating MRHO function in a cell, preferably a cell pre-determined to have defective or impaired MRHO function, by administering an MRHO -modulating agent. Additionally, the invention provides a method for treating disorders or disease associated with impaired MRHO function by administering a therapeutically effective amount of an MRHO -modulating agent.
- [0109] The discovery that MRHO is implicated in RHO pathway provides for a variety of methods that can be employed for the diagnostic and prognostic evaluation of diseases and disorders involving defects in the RHO pathway and for the identification of subjects having a predisposition to such diseases and disorders.

[0110] Various expression analysis methods can be used to diagnose whether MRHO expression occurs in a particular sample, including Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR, and microarray analysis. (e.g., Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, eds., John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm and Guiseppi-Elie, Curr Opin Biotechnol 2001, 12:41-47). Tissues having a disease or disorder implicating defective RHO signaling that express an MRHO, are identified as amenable to treatment with an MRHO modulating agent. In a preferred application, the RHO defective tissue overexpresses an MRHO relative to normal tissue. For example, a Northern blot analysis of mRNA from tumor and normal cell lines, or from tumor and matching normal tissue samples from the same patient, using full or partial MRHO cDNA sequences as probes, can determine whether particular tumors express or overexpress MRHO. Alternatively, the TaqMan® is used for quantitative RT-PCR analysis of MRHO expression in cell lines, normal tissues and tumor samples (PE Applied Biosystems).

[0111] Various other diagnostic methods may be performed, for example, utilizing reagents such as the MRHO oligonucleotides, and antibodies directed against an MRHO, as described above for: (1) the detection of the presence of MRHO gene mutations, or the detection of either over- or under-expression of MRHO mRNA relative to the non-disorder state; (2) the detection of either an over- or an under-abundance of MRHO gene product relative to the non-disorder state; and (3) the detection of perturbations or abnormalities in the signal transduction pathway mediated by MRHO.

[0112] Kits for detecting expression of MRHO in various samples, comprising at least one antibody specific to MRHO, all reagents and/or devices suitable for the detection of antibodies, the immobilization of antibodies, and the like, and instructions for using such kits in diagnosis or therapy are also provided.

[0113] Thus, in a specific embodiment, the invention is drawn to a method for diagnosing a disease or disorder in a patient that is associated with alterations in MRHO expression, the method comprising: a) obtaining a biological sample from the patient; b) contacting the sample with a probe for MRHO expression; c) comparing results from step (b) with a control; and d) determining whether step (c) indicates a

likelihood of the disease or disorder. Preferably, the disease is cancer, most preferably a cancer as shown in TABLE 2. The probe may be either DNA or protein, including an antibody.

EXAMPLES

[0114] The following experimental section and examples are offered by way of illustration and not by way of limitation.

I. C. elegans RHO screen

[0115] A *C. elegans* genetic screen was designed which employed RNAi of specific genes to identify genetic modifiers of Rho pathway function. The Ect2-encoding gene, *let-21*, was chosen a genetic entrypoint for Rho pathway signaling since a weak reduction of mutant was available that shows phenotypic abnormalities similar to those of *rho-1*(RNAi) animals. Like *rho-1* (RNAi), the weak *let-21* allele, *oz93*, results in a sterile germline phenotype characterized by several pathway-diagnostic defects, including defective cytokinesis and nuclear/cytoplasmic partitioning, and a meiotic cell-cycle defect that prevents germ cells from proceeding past the pachytene-stage of meiotic prophase. These phenotypes are also shared by the stronger *let-21* mutant, *e1778*. However, the strong mutant also confers two somatic phenotypes not seen in the weak Ect2 mutant – a protruding vulva (Pvl) phenotype and an Uncoordinated (Unc) movement phenotype. As the basis for the genetic screen, we exploited the genetic principle that weak reduction-of-function mutants in a given biological pathway often show genetic synergy with perturbations elsewhere in the pathway. The weak *let-21* (*oz93*) mutant shows this behavior as demonstrated by combinations of it with certain other known Rho pathway components. For example, the combination of *let-21* (*oz93*) with *rho-1* (RNAi) results in Pvl and Unc phenotypes (similar to the strong *let-21* mutant) that are much more penetrant than observed with *let-21* (*oz93*) or *rho-1* (RNAi) alone. A similar genetic synergy is also observed with other genes whose mammalian counterparts function upstream or downstream of *rho-1*. These include genes for nematode orthologues of MGCRacGAP (*cyk-4* gene), Rho kinase (*let-502*); nonmuscle myosin heavy chain II (*nmy-2*), myosin light chain (MLC-2) and a formin protein (*cyk-1*). Based on these genetic interaction phenotypes, we postulated that a large scale enhancer screen using

the let-21 (oz93) mutant should identify additional novel genes whose normal function is to promote Rho/Ect2 pathway signaling

[0116] The let-21/Ect2 enhancer screen combined the use of a genetically-optimized let-21 (oz93) strain together with a library of double-stranded RNAs (dsRNA) made to approximately 3100 *C. elegans* genes. Genes represented in the RNA library were selected predominantly based on their containing enzymatic domains as determined by such informatic methods as PFAM searches and annotations in databases of *C. elegans* genomic information (e.g., WormBase, Worm Proteosome Database). The let-21 (oz93) strain is a genetically-balanced strain of the genotype let-21 (oz93) *sqt-1* (*sc13*)/ *mnC1*; *eri-1*(*mg366*). This strain segregates both let-21 *sqt-1* double homozygotes, which are recognizable by their Roller behavioral phenotype (conferred by the *sqt-1* mutation), and let-21 *sqt-1*/ + + heterozygotes, which are non-Roller. The *mnC1* chromosomal inversion serves to prevent recombination, while the *eri-1* mutation confers enhanced sensitivity to RNAi (Kennedy S et al (2004) *Nature* 427(6975):645-649).

[0117] In the genetic screen, a mixture of let-21 (oz93) homozygotes and heterozygotes were collected at L1 diapause, incubated 24 hr at 200 °C with a 3.5x volume of dsRNA corresponding to individual genes, and then plated onto nematode growth plates. Three to four days later, the plates were scored for the presence of worms exhibiting Pvl and/or Unc phenotypes; where appropriate the percentage of worms expressing each phenotype was determined separately for both let-21 homozygotes and heterozygotes. Genes were scored as showing genetic synergy with let-21 if let-21 (oz93) homozygotes expressed the Pvl and/or Unc phenotypes at a significantly higher frequency than did let-21 (oz93) heterozygotes. Statistical significance ($p < 0.05$) for genetic synergy was determined by a modified Test Stat function (where the Test stat = [(fraction mutant let-21 (oz93); geneX(RNAi) animals) – (fraction mutant let-21(oz93) + fraction mutant geneX(RNAi) animals)]/ square root(total invariance); p was determined as the Chi Square distribution of the Test Stat value squared divided by 2).

[0118] The screen identified genes that showed significant genetic synergy with let-21 (oz93) for either the Pvl phenotype alone or for both the Pvl and Unc phenotypes. A partial validation of the screen was provided by observation that 20 modifiers from the screen are homologous to mammalian genes whose products have been linked in published literature to signaling pathways involving Rho, Rac, or CDC42. As an

additional method of pathway validation, two genetic secondary assays were designed and used to test the modifiers. In one, a constitutively activated rho-1 transgene, containing a glycine-to-valine substitution at position 12 was constructed and expressed in worms under control the lin-31 promoter, which drives expression in the developing vulval cells. A strain containing this transgene integrated into the genome expressed a Multi-vulva phenotype that was approximately 80% penetrant (in a rrf-3 RNAi hypersensitivity background). A subset of 12 modifiers from the screen partially suppressed the activated rho-1 phenotype, indicating that at a genetic level these genes function downstream of (or in parallel with) rho-1. The second genetic validation assay utilized a temperature-sensitive mutant of myosin light phosphate encoding gene, mel-11. This mutant, mel-11(it26) displayed an embryonic lethal phenotype that was strongly suppressed by RNAi of the *C. elegans* orthologues of rho kinase (let-502), myosin heavy chain (nmy-1) or myosin light chain (mlc-4), but not by RNAi of Rho (rho-1) or Ect2 (let-21). Based on this differential sensitivity, suppression of mel-11(it26) appears to identify genes that act downstream of rho-1 in a Rock/nonmuscle myosin signaling pathway.

II. Analysis of Table 1

[0119] BLAST analysis (Altschul et al., *supra*) was employed to identify orthologs of *C. elegans* modifiers. The columns "MRHO symbol", and "MRHO name aliases " provide a symbol and the known name abbreviations for the Targets, where available, from Genbank. "MRHO RefSeq_NA or GI_NA", "MRHO GI_AA", "MRHO NAME", and "MRHO Description" provide the reference DNA sequences for the MRHOs as available from National Center for Biology Information (NCBI), MRHO protein Genbank identifier number (GI#), MRHO name, and MRHO description, all available from Genbank, respectively. The length of each amino acid is in the "MRHO Protein Length" column.

[0120] Names and Protein sequences of *C. elegans* modifiers of RHO from screen (Example I), are represented in the "Modifier Name" and "Modifier GI_AA" column by GI#, respectively.

Table 1

MRHO symbol	MRHO name aliases	MRHO RefSeq_N A or GI NA	MRHO GI_AA	MRHO NAME	MRHO Description	MRHO Protein Length	Modifier Name	Modifie GI_AA
AURKC	aurora-C AIE2 AIK3 serine/threonine kinase 13 (aurora/IPL1-like) AUR3 AIE1_m STK13 AurC aurora kinase C AURKC	NM_003160 NM_001015878 NM_001015879	4507273	aurora kinase C	ATP binding; nucleotide binding; protein serine/threonine kinase activity; transferase activity	275	air-2	175052
AURKB	Mutations: 1034:OK AURKB aurora kinase B AurB aurora-B aurora-1 Aik2 STK12 STK-1 AUR1 serine/threonine kinase 12 AIM-1 IPL1 ARK2 AIM1 AIK2 STK5	NM_004217	4759178	aurora kinase B	ATP binding; nucleotide binding; protein binding; protein serine/threonine kinase activity; transferase activity	344	air-2	175052
STK6	serine/threonine protein kinase 6 AUR2 STK6 AIK BTAK STK15 ARK1_m IAK1_m AYK1_m serine/threonine protein kinase 15,serine/threonine kinase 15 aurora-A serine/threonine protein kinase-6 serine/threonine kinase 6 IPL1-related kinase AurA ARK1 AURA MGC34538 aurora-related kinase 1 aurora/IPL1-like kinase serine/threonine kinase 15 breast-tumor-amplified kinase	NM_003600 NM_198433 NM_198435 NM_198434 NM_198437 NM_198436	38327562	serine/threonine kinase 6	ATP binding; nucleotide binding; protein binding; protein serine/threonine kinase activity; transferase activity	403	air-2	175052

DHX38	PRP16 pre-mRNA splicing factor ATP-dependent RNA helicase PRP16 PRP16 homolog of S.cerevisiae KIA A0224 DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 38 DEAH (Asp-Glu-Ala-His) box polypeptide 38 PRPF16 DDX38 DHX38	NM_014003	17999539	DEAH (Asp-Glu-Ala-His) box polypeptide 38	ATP binding; ATP-dependent RNA helicase activity; hydrolase activity; nucleic acid binding; nucleotide binding	1227	mog-1	175543
DYRK4	DYRK-4 Dyrk4B Dyrk4A DYRK4 dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4	NM_003845	28827774	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4	ATP binding; nucleotide binding; protein serine/threonine kinase activity; protein-tyrosine kinase activity; transferase activity	520	mbk-2 [Dr_dyrk2a	1754050
DYRK3	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 5 regulatory erythroid kinase protein kinase Dyrk3 DYRK5 RED dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3 DYRK3 REDK DYRK-3	NM_003582 NM_001004023	4503429	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	ATP binding; nucleotide binding; protein serine/threonine kinase activity; protein-tyrosine kinase activity; transferase activity	553	mbk-2 [Dr_dyrk2a	1754050
DYRK2	DYRK-2 DYRK2 dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	NM_006482 NM_003583	5922004	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	ATP binding; magnesium ion binding; manganese ion binding; nucleotide binding; protein serine/threonine kinase activity; protein-tyrosine kinase activity; transferase activity	601	mbk-2 [Dr_dyrk2a	1754050

LOC441261	similar to Farnesyl pyrophosphate synthetase (FPP synthetase) (FPS) (Farnesyl diphosphate synthetase) similar to Farnesyl pyrophosphate synthetase (FPP synthetase) (FPS) LOC441261	XM_496902 XM_499334	51466663	similar to Farnesyl pyrophosphate synthetase (FPP synthetase) (FPS) (Farnesyl diphosphate synthetase)	na	405	1M510	1750850
FDPS	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltransferase, geranyltransferase) FDPS farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase) FPS farnesyl diphosphate synthase	NM_002004	4503685	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltransferase, geranyltransferase)	geranyltransferase activity; transferase activity	419	1M510	1750850
ITGA3	integrin alpha 3 ITGA3 VL3A CD49C CD49c GAPB3 integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor) MSK18 VCA-2 VLA3a GAPB3 antigen identified by monoclonal antibody J143	NM_002204 NM_005501	4504747	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	calcium ion binding; protein binding; receptor activity	1051	ina-1	175539
ITGA7	integrin alpha 7 integrin alpha 7 chain ITGA7 integrin, alpha 7	NM_002206	4504753	integrin, alpha 7	calcium ion binding; protein binding; receptor activity	1137	ina-1	175539
ITGA6	Integrin alpha-6 integrin, alpha 6 CD49f ITGA6 integrin alpha chain, alpha 6	NM_000210	4557675	integrin, alpha 6	calcium ion binding; protein binding; receptor activity	1073	ina-1	175539
KIAA1463	MGC104005 KIAA1463 protein KIAA1463 FLJ34278	NM_173602 XM_498447	55749758	KIAA1463 protein	transcription factor binding; ligase activity	1576	1F517	175069

C21orf106	DIP2-like protein C21orf106 DIP2 disco-interacting protein 2 (Drosophila) homolog chromosome 21 open reading frame 106	NM_015151 NM_206889 NM_206890 NM_206891	45827692	chromosome 21 open reading frame 106	catalytic activity	1571	1F517	175069
KIAA0934	hypothetical protein LOC22982 OTTHUMP00000044556 KIAA0934 RP11-486H9.2	NM_014974	44888818	KIAA0934	catalytic activity; transcription factor binding	1556	1F517	175069
KIF20A	mitotic kinesin-like protein 2 MKLP2 KIF20A RAB6 KIFL RAB6 interacting, kinesin-like (rabkinesin6) kinesin family member 20A	NM_005733	5032013	kinesin family member 20A	ATP binding; microtubule motor activity; nucleotide binding; transporter activity	890		
KIF23	kinesin family member 23 kinesin-like 5 (mitotic kinesin-like protein 1) mitotic kinesin-like 1 MKLP-1 MKLP1 KNSL5 CHO1 KIF23	NM_138555 NM_004856	6754472	kinesin family member 23	ATP binding; microtubule motor activity; nucleotide binding	856	zen-4	251536
MPHOSP1	DKFZp434P0810 MPHOSP1 MPP1 KRMP1 MPP-1 DKFZp434B0435 mitotic kinesin-like protein M-phase phosphoprotein 1	NM_016195	46049114	M-phase phosphoprotein 1	na	1780	zen-4	251536
MELK	OTTHUMP00000046113 KIAA0175 maternal embryonic leucine zipper kinase MELK MPK38 HPK38 EG3 pEg3 kinase	NM_014791	7661974	maternal embryonic leucine zipper kinase	ATP binding; nucleotide binding; protein serine/threonine kinase activity; protein-tyrosine kinase activity; transferase activity	651	4B260	175428
MYH10	myosin heavy chain, nonmuscle type B nonmuscle myosin heavy chain IIB NMMHCB m myosin, heavy polypeptide 10, non-muscle nonmuscle myosin II heavy	NM_005964	41406064	myosin, heavy polypeptide 10, non-muscle	ATP binding; actin binding; calmodulin binding; motor activity; nucleotide binding	1976	nmy-2	251500

	chain-B cellular myosin heavy chain, type B type B nonmuscle myosin heavy chain-B near to the ATP binding region MYH10							
MYH14	DKFZp667A1311 NMHC-II-C MYH14 DFNA4 NHMCII FP17425 KIAA2034 myosin, heavy polypeptide 14 nonmuscle myosin heavy chain II-C FLJ13881 myosin in heavy chain 14	NM_024729	33563340	myosin, heavy polypeptide 14	na	1995	nmy-2	251500
MYH9	nonmuscle myosin heavy chain II-A NMHC-II-A MYH9 FTNS NMMHCA MHA OTTTHUMP00000028706 DFNA17 non-muscle myosin heavy polypeptide 9 myosin, heavy polypeptide 9, non-muscle myosin human non-muscle myosin heavy chain cellular myosin heavy chain, type A	NM_002473	12667788	myosin, heavy polypeptide 9, non-muscle	ATP binding; actin binding; calmodulin binding; motor activity; nucleotide binding	1960	nmy-2	251500
MYH11	smooth muscle myosin heavy chain 11, isoform SM2 DKFZp686D10126 MGC126726 MGC32963 MYH11 smooth muscle myosin heavy chain 11 SMHC SMMHC myosin, heavy polypeptide 11, smooth muscle	NM_002474 NM_022844	13124879	myosin, heavy polypeptide 11, smooth muscle	ATP binding; actin binding; calmodulin binding; motor activity; nucleotide binding	1972	nmy-2	251500
PPIE	rotamase E MGC3736 peptidyl-prolyl cis-trans isomerase E PPIase E cyclophilin 33 peptidylprolyl isomerase	NM_203456 NM_006112 NM_203457	45439316	peptidylprolyl isomerase E (cyclophilin E)	RNA binding; cyclosporin A binding; isomerase activity; nucleotide binding; peptidyl-prolyl	296	cyp-13	175394

	E cyclophilin E CYP- 33 peptidylprolyl isomerase E (cyclophilin E) PPIE				cis-trans isomerase activity; protein binding			
PPP2CA	PP2A-alpha RP- C replication protein C serine/threonine protein phosphatase 2A, catalytic subunit, alpha isoform protein phosphatase 2, catalytic subunit, alpha isoform PP2Ac pr otein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform PP2Aalph a PPP2CA PP2CA	NM_0027 15	450601 7	protein phosphat ase 2 (formerly 2A), catalytic subunit, alpha isoform	hydrolase activity; iron ion binding; manganese ion binding; metal ion binding; phosphoprotein phosphatase activity; protein heterodimerizati on activity	309	mts][4M6 23	171366 175403
PPP2CB	protein phosphatase type 2A catalytic subunit protein phosphatase 2, catalytic subunit, beta isoform PP2A- beta serine/threoni ne protein phosphatase 2A, catalytic subunit, beta isoform protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform PP2Abeta PPP2CB PP2CB	NM_0041 56 NM_00 1009552	475895 2	protein phosphat ase 2 (formerly 2A), catalytic subunit, beta isoform	hydrolase activity; iron ion binding; manganese ion binding; metal ion binding; phosphoprotein phosphatase activity; contributes_to protein phosphatase type 2A activity	309	mts][4M6 23	171366 175403
SRCAP	Snf2-related CBP activator protein KIAA030 9 SRCAP	NM_0066 62	573006 7	Snf2- related CBP activator protein	na	2971	pqn- 81][SRCA P	175567 573006
STK23	STK23 serine/thre onine kinase 23 MSSK1	NM_0143 70	765762 9	serine/thr eonine kinase 23	ATP binding; nucleotide binding; protein serine/threonine kinase activity; transferase activity	533	SRPK][C G11489][s pk-1	730307 857472 514401

SRPK1	SRPK1 SFRS protein kinase 1 SR protein kinase 1 SFRSK1	NM_003137	47419936	SFRS protein kinase 1	na	655	SRPK][C G11489][SRPK][C G11489][s pk-1][SRPK][CG11489][spk-1][SRPK2][SRPK1][SRPK1	730307: 857472: 303073: 574726: 144015: 030732: 747262: 440153: 884474: 199364: 19936
SRPK2	H_RG152G17.1a H_RG152G17.1b WUGSC:H_RG152G17.1a serine kinase SRPK2 SFRSK2 SFRS protein kinase 2 SRPK2	NM_182692 NM_182691	33188447	SFRS protein kinase 2	na	688	SRPK][C G11489][s pk-1][SRPK2	730307: 857472: 514401: 318844
TRRAP	FLJ10671 TRRAP transformation/transcription domain-associated protein PAF400 TR-AP H_DJ0808A01.1 WUGSC:H_DJ0808A01.1	NM_003496	4507691	transfor mation/tr anscripti on domain-associate d protein	molecular function unknown; phosphotransferase activity, alcohol group as acceptor; protein binding; transcription cofactor activity	3830	2L952	175324
USP7	ubiquitin specific peptidase 7 (herpes virus-associated) USP7 TEF1 HAUSP Herpes virus-associated ubiquitin-specific protease Ubiquitin-specific protease-7, herpes virus-associated ubiquitin specific protease 7 (herpes virus-associated)	NM_003470	4507857	ubiquitin specific peptidase 7 (herpes virus-associated)	cysteine-type endopeptidase activity; protein binding; ubiquitin thiolesterase activity	1102	5L597	175619

III. Kinase assay

[0121] A purified or partially purified MRHO is diluted in a suitable reaction buffer, e.g., 50 mM Hepes, pH 7.5, containing magnesium chloride or manganese chloride (1-20 mM) and a peptide or polypeptide substrate, such as myelin basic protein or casein (1-10 µg/ml). The final concentration of the kinase is 1-20 nM. The enzyme reaction is conducted in microtiter plates to facilitate optimization of reaction conditions by increasing assay throughput. A 96-well microtiter plate is employed using a final volume 30-100 µl. The reaction is initiated by the addition of ³³P-

gamma-ATP (0.5 μ Ci/ml) and incubated for 0.5 to 3 hours at room temperature. Negative controls are provided by the addition of EDTA, which chelates the divalent cation (Mg^{2+} or Mn^{2+}) required for enzymatic activity. Following the incubation, the enzyme reaction is quenched using EDTA. Samples of the reaction are transferred to a 96-well glass fiber filter plate (MultiScreen, Millipore). The filters are subsequently washed with phosphate-buffered saline, dilute phosphoric acid (0.5%) or other suitable medium to remove excess radiolabeled ATP. Scintillation cocktail is added to the filter plate and the incorporated radioactivity is quantitated by scintillation counting (Wallac/Perkin Elmer). Activity is defined by the amount of radioactivity detected following subtraction of the negative control reaction value (EDTA quench).

IV. Expression analysis

- [0122] All cell lines used in the following experiments are NCI (National Cancer Institute) lines, and are available from ATCC (American Type Culture Collection, Manassas, VA 20110-2209). Normal and tumor tissues were obtained from Impath, UC Davis, Clontech, Stratagene, Ardais, Genome Collaborative, and Ambion.
- [0123] TaqMan[®] analysis was used to assess expression levels of the disclosed genes in various samples.
- [0124] RNA was extracted from each tissue sample using Qiagen (Valencia, CA) RNeasy kits, following manufacturer's protocols, to a final concentration of 50ng/ μ l. Single stranded cDNA was then synthesized by reverse transcribing the RNA samples using random hexamers and 500ng of total RNA per reaction, following protocol 4304965 of Applied Biosystems (Foster City, CA).
- [0125] Primers for expression analysis using TaqMan[®] assay (Applied Biosystems, Foster City, CA) were prepared according to the TaqMan[®] protocols, and the following criteria: a) primer pairs were designed to span introns to eliminate genomic contamination, and b) each primer pair produced only one product. Expression analysis was performed using a 7900HT instrument.
- [0126] TaqMan[®] reactions were carried out following manufacturer's protocols, in 25 μ l total volume for 96-well plates and 10 μ l total volume for 384-well plates, using 300nM primer and 250 nM probe, and approximately 25ng of cDNA. The standard curve for result analysis was prepared using a universal pool of human cDNA samples, which is a mixture of cDNAs from a wide variety of tissues so that the

chance that a target will be present in appreciable amounts is good. The raw data were normalized using 18S rRNA (universally expressed in all tissues and cells).

[0127] For each expression analysis, tumor tissue samples were compared with matched normal tissues from the same patient. A gene was considered overexpressed in a tumor when the level of expression of the gene was 2 fold or higher in the tumor compared with its matched normal sample. In cases where normal tissue was not available, a universal pool of cDNA samples was used instead. In these cases, a gene was considered overexpressed in a tumor sample when the difference of expression levels between a tumor sample and the average of all normal samples from the same tissue type was greater than 2 times the standard deviation of all normal samples (i.e., $\text{Tumor} - \text{average}(\text{all normal samples}) > 2 \times \text{STDEV}(\text{all normal samples})$).

[0128] Results are shown in Table 2. Number of pairs of tumor samples and matched normal tissue from the same patient are shown for each tumor type. Percentage of the samples with at least two-fold overexpression for each tumor type is provided. A modulator identified by an assay described herein can be further validated for therapeutic effect by administration to a tumor in which the gene is overexpressed. A decrease in tumor growth confirms therapeutic utility of the modulator. Prior to treating a patient with the modulator, the likelihood that the patient will respond to treatment can be diagnosed by obtaining a tumor sample from the patient, and assaying for expression of the gene targeted by the modulator. The expression data for the gene(s) can also be used as a diagnostic marker for disease progression. The assay can be performed by expression analysis as described above, by antibody directed to the gene target, or by any other available detection method.

TABLE 2

	FD PS	KIF 23	ME LK	PPI E	US P7	AU RK B	AU RK C	C2 1or f10 6	DY RK 2_e 1	DY RK 2_e 2	DY RK 3	DY RK 4	KI AA 093 4	KI AA 146 3	MP HO SP H1	PP P2 CA	SR PK 1	SR PK 2	ST K2 3
Breast	26 %	71 %	67 %	3%	15 %	58 %	7%	15 %	19 %	28 %	11 %	28 %	9%	19 %	29 %	17 %	44 %	19 %	47 %
# of Pairs	34	34	36	34	34	29	29	34	36	36	36	36	34	32	31	36	36	36	35
Colon	15 %	45 %	49 %	10 %	18 %	39 %	6%	8%	5%	10 %	38 %	15 %	23 %	20 %	43 %	17 %	38 %	23 %	31 %
# of Pairs	40	40	41	40	40	33	32	40	39	39	39	39	40	36	40	38	40	40	39
Head And Neck	31 %	77 %	75 %	8%	8%	ND	ND	8%	31 %	38 %	31 %	23 %	23 %	38 %	46 %	0%	15 %	31 %	17 %

# of Pairs	13	13	12	13	13	ND	ND	13	13	13	13	13	13	13	13	13	13	13	12
Kidney	5%	10%	90%	10%	5%	85%	5%	14%	43%	48%	26%	13%	29%	43%	43%	9%	14%	10%	0%
# of Pairs	21	21	20	21	21	20	20	21	23	23	23	23	21	21	21	22	21	21	19
Liver	33%	89%	89%	11%	44%	ND	ND	44%	38%	50%	25%	12%	33%	22%	56%	0%	33%	33%	10%
# of Pairs	9	9	9	9	9	ND	ND	9	8	8	8	8	9	9	9	8	9	9	1
Lung	3%	70%	79%	8%	15%	84%	3%	2%	12%	25%	2%	15%	15%	28%	51%	0%	38%	12%	38%
# of Pairs	40	40	42	40	40	32	31	40	40	40	40	40	40	36	39	38	40	41	39
Lymph node	50%	75%	75%	0%	50%	ND	ND	0%	0%	0%	50%	0%	0%	0%	25%	33%	50%	0%	0%
# of Pairs	4	4	4	4	4	ND	ND	4	4	4	4	4	4	4	4	3	4	4	3
Ovary	47%	72%	68%	26%	5%	70%	12%	5%	26%	47%	5%	5%	5%	24%	47%	10%	58%	10%	37%
# of Pairs	19	18	19	19	19	17	17	19	19	19	19	19	19	17	19	20	19	21	19
Pancreas	58%	83%	75%	50%	58%	ND	ND	50%	62%	62%	69%	38%	75%	67%	67%	46%	50%	58%	55%
# of Pairs	12	12	12	12	12	ND	ND	12	13	13	13	13	12	12	12	13	12	12	11
Prostate	8%	17%	ND	0%	13%	14%	0%	17%	17%	12%	8%	12%	4%	13%	8%	13%	8%	13%	4%
# of Pairs	24	24	ND	24	24	14	14	24	24	24	24	24	24	23	24	24	24	24	24
Skin	29%	86%	50%	17%	0%	ND	ND	14%	57%	86%	71%	14%	43%	0%	29%	29%	14%	43%	0%
# of Pairs	7	7	24	7	7	ND	ND	7	7	7	7	7	7	7	7	7	7	7	7
Stomach	9%	100%	82%	9%	27%	ND	ND	0%	36%	0%	18%	18%	18%	27%	91%	36%	55%	9%	9%
# of Pairs	11	11	11	11	11	ND	ND	8	11	11	11	11	11	11	11	11	11	11	11
Testis	0%	38%	29%	0%	0%	ND	ND	7%	12%	25%	0%	0%	0%	0%	25%	0%	13%	0%	25%
# of Pairs	8	8	7	8	8	ND	ND	14	8	8	8	8	8	8	8	8	8	8	8
Thyroid Gland	0%	36%	58%	0%	0%	ND	ND	17%	0%	7%	0%	21%	7%	7%	0%	7%	21%	0%	56%
# of Pairs	14	14	12	14	14	ND	ND	23	14	14	14	14	14	14	14	14	14	14	9
Uterus	22%	43%	77%	0%	4%	61%	16%		9%	26%	4%	0%	4%	25%	26%	17%	22%	13%	30%
# of Pairs	23	23	22	23	23	18	19		23	23	23	23	23	20	23	24	23	23	23

V. MRHO functional assays

[0129] RNAi experiments were carried out to knock down expression of various MRHO sequences in various cell lines using small interfering RNAs (siRNA, Elbashir

et al, *supra*). The following cell lines were used in the experiments: A549 lung cancer cells, U87MG glioblastoma cells, MDA231 breast cancer cells, AGS gastric cancer cells, PC3 prostate cancer cells, SW480 colon cancer cells, and HELA cervical cancer cells.

[0130] Effect of MRHO RNAi on cell proliferation and growth. BrdU, Caspase 3, and Cell Titer-Glo™ assays, as described above, were employed to study the effects of decreased MRHO expression on cell proliferation.

[0131] Results: RNAi of MRHO PPIE decreased cell proliferation in A549, HELA, and U87MG cells in BRDU and Caspase 3 assays; RNAi of MRHO KIF23 decreased cell proliferation in A549, PC3, and SW480 cells; RNAi of MRHO MPHOSPH1 decreased proliferation in A549, PCe, SW480, and U87MG cells; RNAi of MRHO FDPS decreased cell proliferation in A549, SW480, PC3, and U87MG cells; RNAi of MRHO PIK4CA decreased proliferation in A549, SW480, and PC3 cells; RNAi of MRHO MELK decreased proliferation in A549, PC3, SW480, and U87MG cells; RNAi of MRHO SRPK1 decreased proliferation in A549, PC3, SW480, and U87MG cells; RNAi of MRHO SRPK2 decreased proliferation in A549 and PC3 cells; RNAi of MRHO DYRK2, MRHO DYRK3, and MRHO DYRK4 each decreased proliferation in A549, PC3, and U87MG cells; RNAi of MRHO USP7 decreased proliferation in A549, PC3, and U87MG cells; RNAi of MRHO PRKWNK2 and MRHO PRKWNK3 each decreased proliferation in A549, SW480, PC3, and U87MG cells; RNAi of MRHO PRKWNK4 decreased proliferation in A549 and U87MG cells.

[0132] Standard colony growth assays, as described above, were employed to study the effects of decreased MRHO expression on cell growth.

[0133] Results: RNAi of MRHO PPIE decreased proliferation in A549 cells; RNAi of MRHO KIF23 decreased proliferation in A549, PC3, and SW480 cells.

[0134] Effect of MRHO RNAi on apoptosis. The Phospho-histone H2B assay, as described above, was employed to study the effects of decreased MRHO expression on apoptosis.

[0135] Results indicate that RNAi of MRHO PPIE increased apoptosis in U87MG, HELA, and A549 cells; RNAi of MRHO PIK4CA increased apoptosis in SW480 cells; RNAi of MRHO MELK increased apoptosis in SW480 cells; RNAi of MRHO SRPK1 increased apoptosis in A549, PC3, SW480, and U87MG cells; RNAi of MRHO SRPK2 increased apoptosis in A549 and U87MG cells; RNAi of MRHO

PRKWINK2 increased apoptosis in SW480 and A549 cells; RNAi of MRHO

PRKWINK3 increased apoptosis in A549 and U87MG cells; RNAi of MRHO

PRKWINK4 increased apoptosis in A549 cells.

[0136] Multiple paramater apoptosis assay, as described above, was also used to study the effects of decreased MRHO expression on apoptosis, using Caspase 3 cleavage readout.

[0137] Results of this experiment indicated that RNAi of MRHO PPIE increased apoptosis in A549, PC3, HELA, and U87MG cells; RNAi of MRHO KIF23 increased apoptosis in A549 and PC3 cells; RNAi of MRHO MPHOSPH1 increased apoptosis in PC3, A549, SW480, and U87MG cells; RNAi of MRHO FDPS increased apoptosis in A549, PC3, MDA231, AGS, and SW480 cells; RNAi of MRHO PIK4CA increased apoptosis in SW480, A549, MDA231, and U87MG cells; RNAi of MRHO MELK increased apoptosis in PC3 and SW480 cells; RNAi of MRHO SRPK1 increased apoptosis in A549 cells; RNAi of MRHO SRPK2 increased apoptosis in PC3, A549, SW480, and U87MG cells; RNAi of MRHO DYRK4 increased apoptosis in A549, PC3, and SW480 cells; RNAi of MRHO PRKWINK1 increased apoptosis in A549, AGS, and MDA231T cells; RNAi of MRHO PRKWINK2 increased apoptosis in A549, and AGS cells; RNAi of MRHO PRKWINK3 increased apoptosis in A549, AGS, and MDA231T cells; RNAi of MRHO PRKWINK4 increased apoptosis in A549 and MDA231cells.

[0138] **Effect of MRHO RNAi on cell cycle, cell motility, and cell morphology.**

Since RHO and members of the ECT2/RHO pathway are involved in cell cycle and cell morphology, in vertebrates, mammals, C.elegans, and generally throughout evolution, and since the modifiers of the C.elegans screen were identified and validated in the RHO pathway, involvement of any MRHO sequence in cell cycle and cell morphology, consistent with results observed with RHO and ECT2, is compelling evidence of an MRHO involvement in RHO pathway.

[0139] In cell cycle experiments ECT2 and RHO sequences were knocked down with RNAi, resulting in elevated multinucleate phenotypes, indicative of increased levels of cytokinesis. These results were used as positive control in these experiments. Propidium iodide (PI) cell cycle assay, as described above, was employed to study the effects of decreased MRHO expression on cell cycle.

[0140] Results indicated that RNAi of MRHO PPIE increased cytokinesis in AGS, MDA231, and A549 cells, consistent with the control experiments; RNAi of MRHO

KIF23 also caused multinucleate phenotype (increased cytokinesis) in A549, AGS, PC3, SW480, and MB231 cells; RNAi of MRHO MPHOSPH1 caused multinucleate phenotype (increased cytokinesis) in A549 and AGS cells; RNAi of MRHO FDPS caused multinucleate phenotype (increased cytokinesis) and abnormal nuclear morphology in A549 and PC3 cells; RNAi of MRHO PIK4CA caused multinucleate phenotype (increased cytokinesis) in AGS cells, and multinucleate phenotype or irregular shape in A549 and MDA231 cells; RNAi of MRHO MELK caused multinucleate phenotype (increased cytokinesis) in A549, MDA231, and AGS cells, and further, produced micronuclear like structures in A549 and AGS cells; RNAi of MRHO PRKWNK1 and MRHO PRKWNK2 caused multinucleate phenotype (increased cytokinesis) in A549 and AGS cells, and further, caused stretched fibers in A549 cells; RNAi of MRHO PRKWNK3 and MRHO PRKWNK4 each caused multinucleate phenotype (increased cytokinesis) in A549 cells and multinuclear giant cells in AGS cells;

[0141] In further cell cycle experiments, Phospho-Cofilin assays as described above were employed to assess involvement of MRHOs in RHO pathway.

[0142] Results indicated that RNAi of MRHO PPIE produced decreased fluorescent Phospho-Cofilin in A549 and AGS cells, consistent with a decrease in LIMK levels; RNAi of MRHO KIF23 decreased phospho-cofilin levels in A549, AGS, and MDA231T cells; RNAi of MRHO MPHOSPH1 decreased phospho-cofilin levels in A549, AGS, and MDA231 cells; RNAi of MRHO FDPS decreased phospho-cofilin levels in A549, AGS, and MDA231T cells; RNAi of MRHO PIK4CA decreased phospho-cofilin levels in A549 and AGS cells; RNAi of MRHO MELK decreased phospho-cofilin levels in A549 and AGS cells; RNAi of MRHO USP7 decreased phospho-cofilin levels in A549 and AGS cells; RNAi of MRHO PRKWNK1, MRHO PRKWNK2, MRHO PRKWNK3, and MRHO PRKWNK4 each decreased phospho-cofilin levels in A549, MDA231, and AGS cells. Taken together, the results of these experiments are consistent with decrease in LIMK levels and indicate involvement of MRHO in the RHO pathway.

[0143] For cell morphology experiments, Cellomics™ assays were used as described above, using actin antibodies.

[0144] Results indicated that RNAi of MRHO PPIE caused irregular cell shape, increase in cell spreading, and variable patterns of actin staining in A549, PC3, and SW480 cells; RNAi of MRHO KIF23 caused irregular cell shapes and actin

reorganization in A549, AGS, and MDA231T cells; RNAi of MRHO PIK4CA caused highly irregular cell shapes and long cell processes and projections in A549 cells; RNAi of MRHO PRKWINK2 caused patchy, nonuniform actin staining in PC3 and A549 cells; RNAi of MRHO PRKWINK4 caused punctuate and patchy actin staining throughout the cell, and cell rounding.

[0145] Cell motility assays, as described above, were also employed to assess effects of RNAi of MRHO sequences on cell motility.

[0146] Results indicated that RNAi of MRHO KIF23 reduced motility in AGS cells; RNAi of MRHO MPHOSPH1 reduced motility in AGS and A549 cells; RNAi of MRHO FDPS reduced motility in AGS and A549 cells; RNAi of MRHO PIK4CA reduced motility in AGS and A549 cells; RNAi of MRHO MELK reduced motility in AGS and A549 cells; RNAi of MRHO USP7 decreased motility in AGS and A549 cells; RNAi of MRHO PRKWINK1, MRHO PRKWINK3, and MRHO PRKWINK3 each decreased motility in AGS and A549 cells; RNAi of MRHO PRKWINK2 decreased motility in AGS cells.

[0147] **Transcriptional reporter assays.** Effects of overexpressed MRHO on expression of various transcription factors was also studied. In this assay, rat intestinal epithelial cells (RIEs) or NIH3T3 cells were co-transfected with reporter constructs containing various transcription factors and luciferase along with MRHO. Luciferase intensity was then measured as the readout for transcriptional activation due to overexpression of the MRHO. Overexpressed MRHO PPIE caused increased expression of AP1 (Activator protein 1); Overexpressed MRHO KIF23 caused activation of AP1, NFkB, and SRE (Serum response element); Overexpressed MRHO MELK activated AP1, EGR (Early growth response), SRE, and STAT6 (signal transducer and activator of transcription 6); and overexpressed MRHO PRKWINK4 activated SRE, E2F, EGR, AP1, NFkB, and STAT6.

WHAT IS CLAIMED IS:

1. A method of identifying a candidate RHO pathway modulating agent, said method comprising the steps of:
 - (a) providing an assay system comprising an MRHO polypeptide or nucleic acid;
 - (b) contacting the assay system with a test agent under conditions whereby, but for the presence of the test agent, the system provides a reference activity; and
 - (c) detecting a test agent-biased activity of the assay system, wherein a difference between the test agent-biased activity and the reference activity identifies the test agent as a candidate RHO pathway modulating agent.
2. The method of Claim 1 wherein the assay system comprises cultured cells that express the MRHO polypeptide.
3. The method of Claim 2 wherein the cultured cells additionally have defective RHO function.
4. The method of Claim 1 wherein the assay system includes a screening assay comprising an MRHO polypeptide, and the candidate test agent is a small molecule modulator.
5. The method of Claim 4 wherein the assay is a binding assay.
6. The method of Claim 1 wherein the assay system is selected from the group consisting of an apoptosis assay system, a cell proliferation assay system, and an angiogenesis assay system.
7. The method of Claim 1 wherein the assay system includes a binding assay comprising an MRHO polypeptide and the candidate test agent is an antibody.

8. The method of Claim 1 wherein the assay system includes an expression assay comprising an MRHO nucleic acid and the candidate test agent is a nucleic acid modulator.
9. The method of claim 8 wherein the nucleic acid modulator is an antisense oligomer.
10. The method of Claim 8 wherein the nucleic acid modulator is a PMO.
11. The method of Claim 1 additionally comprising:
 - (d) administering the candidate RHO pathway modulating agent identified in (c) to a model system comprising cells defective in RHO function and, detecting a phenotypic change in the model system that indicates that the RHO function is restored.
12. The method of Claim 11 wherein the model system is a mouse model with defective RHO function.
13. A method for modulating a RHO pathway of a cell comprising contacting a cell defective in RHO function with a candidate modulator that specifically binds to an MRHO polypeptide, whereby RHO function is restored.
14. The method of claim 13 wherein the candidate modulator is administered to a vertebrate animal predetermined to have a disease or disorder resulting from a defect in RHO function.
15. The method of Claim 13 wherein the candidate modulator is selected from the group consisting of an antibody and a small molecule.
16. The method of Claim 1, comprising the additional steps of:
 - (d) providing a secondary assay system comprising cultured cells or a non-human animal expressing MRHO ,

(e) contacting the secondary assay system with the test agent of (b) or an agent derived therefrom under conditions whereby, but for the presence of the test agent or agent derived therefrom, the system provides a reference activity; and

(f) detecting an agent-biased activity of the second assay system,
wherein a difference between the agent-biased activity and the reference activity of the second assay system confirms the test agent or agent derived therefrom as a candidate RHO pathway modulating agent,

and wherein the second assay detects an agent-biased change in the RHO pathway.

17. The method of Claim 16 wherein the secondary assay system comprises cultured cells.

18. The method of Claim 16 wherein the secondary assay system comprises a non-human animal.

19. The method of Claim 18 wherein the non-human animal mis-expresses a RHO pathway gene.

20. A method of modulating RHO pathway in a mammalian cell comprising contacting the cell with an agent that specifically binds an MRHO polypeptide or nucleic acid.

21. The method of Claim 20 wherein the agent is administered to a mammalian animal predetermined to have a pathology associated with the RHO pathway.

22. The method of Claim 20 wherein the agent is a small molecule modulator, a nucleic acid modulator, or an antibody.

23. A method for diagnosing a disease in a patient comprising:
obtaining a biological sample from the patient;
contacting the sample with a probe for MRHO expression;
comparing results from step (b) with a control;
determining whether step (c) indicates a likelihood of disease.

24. The method of claim 23 wherein said disease is cancer.

25. The method according to claim 24, wherein said cancer is a cancer as shown in Table 2 as having >25% expression level.

Nucleic Acid and Polypeptide sequences

SEQ ID NO:1

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SEQ ID NO:2

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SEQ ID NO:3

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SEQ ID NO:4

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SEQ ID NO:5

>gi|28827773|ref|NM_003845.1| Homo sapiens dual-specificity tyrosine-
(Y)-phosphorylation regulated kinase 4 (DYRK4), mRNA
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SEQ ID NO:6

>gi|51702239|ref|NM_003582.2| Homo sapiens dual-specificity tyrosine-
(Y)-phosphorylation regulated kinase 3 (DYRK3), transcript variant 1,
mRNA

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SEQ ID NO:7

>gi|5922003|ref|NM_006482.1| Homo sapiens dual-specificity tyrosine-
(Y)-phosphorylation regulated kinase 2 (DYRK2), transcript variant 2,
mRNA

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SEQ ID NO:8

>gi|51466662|ref|XM_496902.1| PREDICTED: Homo sapiens similar to
Farnesyl pyrophosphate synthetase (FPP synthetase) (FPS) (Farnesyl
diphosphate synthetase) (LOC441261), mRNA
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SEQ ID NO:9

>gi|41281370|ref|NM_002004.2| Homo sapiens farnesyl diphosphate
synthase (farnesyl pyrophosphate synthetase,
dimethylallyltranstransferase, geranyltranstransferase) (FDPS), mRNA
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C
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SEQ ID NO:10

>gi|4504746|ref|NM_002204.1| Homo sapiens integrin, alpha 3 (antigen
CD49C, alpha 3 subunit of VLA-3 receptor) (ITGA3), transcript variant
a, mRNA
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SEQ ID NO:11

>gi|4504752|ref|NM_002206.1| Homo sapiens integrin, alpha 7 (ITGA7),
mRNA

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SEQ ID NO:12

>gi|4557674|ref|NM_000210.1| Homo sapiens integrin, alpha 6 (ITGA6),
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SEQ ID NO:13

>gi|55749757|ref|NM_173602.2| Homo sapiens KIAA1463 protein
(KIAA1463), mRNA

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SEQ ID NO:16

>gi|5032012|ref|NM_005733.1| Homo sapiens kinesin family member 20A
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SEQ ID NO:17

>gi|20143966|ref|NM_138555.1| Homo sapiens kinesin family member 23
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SEQ ID NO:18

>gi|46049113|ref|NM_016195.2| Homo sapiens M-phase phosphoprotein 1
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SEQ ID NO:19

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SEQ ID NO:20

>gi|41406063|ref|NM_005964.1| Homo sapiens myosin, heavy polypeptide
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SEQ ID NO:21

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SEQ ID NO:22

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SEQ ID NO:23

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SEQ ID NO:24

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SEQ ID NO:26

>gi|57634540|ref|NM_004156.2| Homo sapiens protein phosphatase 2
(formerly 2A), catalytic subunit, beta isoform (PPP2CB), transcript
variant 1, mRNA

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SEQ ID NO:27

>gi|5730066|ref|NM_006662.1| Homo sapiens Snf2-related CBP activator
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SEQ ID NO:28

>gi|63025195|ref|NM_014370.2| Homo sapiens serine/threonine kinase 23
(STK23), mRNA

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SEQ ID NO:29

>gi|47419935|ref|NM_003137.3| Homo sapiens SFRS protein kinase 1
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SEQ ID NO:32

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>gi|4507856|ref|NM_003470.1| Homo sapiens ubiquitin specific  
peptidase 7 (herpes virus-associated) (USP7), mRNA
```

[illegible]

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SEQ ID NO:33

>gi|4507273|ref|NP_003151.1| aurora kinase C [Homo sapiens]
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SEQ ID NO:34

>gi|4759178|ref|NP_004208.1| aurora kinase B [Homo sapiens]
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HFTIDDFEIGRPLGKGKFGNVYLAREKKSHFIVALKVLFKSQIEKEGVEHQLRREIEIQAHLHHPNILR
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SEQ ID NO:35

>gi|38327562|ref|NP_003591.2| serine/threonine protein kinase 6 [Homo sapiens]
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VQNQKQKQLOATSVPHPVSRPLNNTQKSKQPLPSAPENNPEEELASKQKNEESKKRQWALED FEIGRPL
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KGKFGNVYLAREKQSKFILALKVLFKAQLEKAGVEHQLRREVEIQSHLRHPNILRLYGYFHDATRVYLI
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H
APSSRRTTLCGTLDYLPPEMIEGRMHDEKVDLWSLGVLCYEFLVGKPPFEANTYQETYKRISRVEFTFP
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SEQ ID NO:36

>gi|17999539|ref|NP_054722.2| DEAH (Asp-Glu-Ala-His) box polypeptide 38 [Homo sapiens]
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SRSTWEEEDSGYGSSRRSQWESPSPTPSYRDSERSHRLSTRDRDRSVRGKYSDDTPLPTPSYKYNEWAD
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RRHLGSTPRLSRGRGRREEGEGISFDTEBERQQWEDDQRQADRDWYMMDEGYDEFHNPLAYSSSEDYVR
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A
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SEQ ID NO:37

>gi|28827774|ref|NP_003836.1| dual-specificity tyrosine-(Y)-
phosphorylation regulated kinase 4 [Homo sapiens]
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RGKKRYPDSKDLTMVLKTYDTSFLDFLRRLVWEPRLMTPDQALKHAWIHQSRNLKPQRPQTLRKS
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SEQ ID NO:38

>gi|4503429|ref|NP_003573.1| dual-specificity tyrosine-(Y)-
phosphorylation regulated kinase 3 [Homo sapiens]
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SEQ ID NO:39

>gi|5922004|ref|NP_006473.1| dual-specificity tyrosine-(Y)-
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SEQ ID NO:40

>gi|51466663|ref|XP_496902.1| PREDICTED: similar to Farnesyl pyrophosphate synthetase (FPP synthetase) (FPS) (Farnesyl diphosphate synthetase) [Homo sapiens]
MVPAPVTYNSLVVNQLLQNEPTGSGWVAKSNLTTCQSEGGALVYGPTQRAKPNGNQKSDIYAQEKQDF
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QHYSQIVRVLTEDEMGHPETGDATARLKEVLEYNAIGGKYHRGLMVLVAFRELVEPRKLDADSLQWAPT
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KKILLEIQEFFQIQDDYLDFFGDPSVTGRVGNDFQDNKCSWLVVQCLLQATPEQYQILKENYRQKEAEK
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SEQ ID NO:41

>gi|4503685|ref|NP_001995.1| farnesyl diphosphate synthase [Homo sapiens]
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SEQ ID NO:42

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LRDKLRPIIISMNYSPLRMPDRPRLGLRSLDAYPILNQAQALENHTEVQFQKECGPDNKCESNLQMRA
A
FVSEQQQLSRLQYSRDVRKLLLSINVTNTRTSERSGEDAHEALLTLVVPALLSSVRPPGACQANET
I
FCELGNPFKRNQRMELLIAFEVIGVTLHTRDLQVQLQLSTSSHQDNLWPMILTLLVDYTLQTSLSMVNH
R
LQSFFGGTVMGESGMKTVEDVGSPLKYEYFQVGPMEGLVGLGTLVLGLEWPYEVSNKGWLLYPTEITVH
G
NGSWPCRPPGDLINPLNLTLSDPGDRPSSPQRRRRQLDPGGGQGGPPVTLAAKKAKSETVLT CATGRA
H
CVWLECPIDAPVVTNVTVKARVWNSTFIEDYRDFDRVRVNGWATLFLRTSIPTINMENKTTWFSVDID
S

ELVEELPAEIELWLVLVAVGAGLLLLGLIILLWKCGLFFKRARTRALYEAKRQKAEMKSQPSETERLTD
D
Y

SEQ ID NO:43

>gi|4504753|ref|NP_002197.1| integrin alpha 7 precursor [Homo
sapiens]
MAGARSRDPWGASGICYLFGSLLVELLFSRAVAFNLDVMGALRKEGEPGSLFGFSVALHRQLQPRPQSW
L
LVGAPQALALPGQQANRTGGLFACPLSLEETDCYRVDIDQGADMQKESKENQWLGVSVRSQGPQGGKIVT
C
AHRYEARQQRVDQILETRDMIGRCFVLSQDLAIRDELDDGGEWKFCGRPQGHEQFGFCQQGTAAAFSPDS
H
YLLFGAPGTYNWKGLLFVTNIDSSDPDQLVYKTLDPADRLPGPAGDLALNSYLGFSIDSGKGLVRAEEL
S
FVAGAPRANHKGAVVILRKDSASRLVPEVMLSGERLTSGFGYSLAVADLNSDGWPDLLIVGAPYFFERQE
E
LGGAVVYVLNQQGHWAGISPLRLCGSPDSMFGISLAVLGDLNQDGFDPDIAVGAPFDGDGKVFYHGS
G
VVAKPSQVLEGEAVGIKSGFYSLSGSLDMDGNQYPDLLVGSGLADTAVLFRARPILHVSHEVSIAPRSID
L
EQPNCAGGHSVCVDLRVCFSYIAVPSSYSPTVALDYVLDADTDRLRGQVPRVTFLSRNLEEPKHQASG
T
VWLKHQHDRVCGDAMFQLQENVKDKLRAIVVTLSYSLQTPRLRRQAPGQGLPPVAPILNAHQPSQRAE
I
HFLKQCGCEDKICQSNLQLVHARFCTRVSDTEFQPLEMDVDGTTALFALSGQPVIQLELMVTNLPSPDPA
Q
PQADGDDAHEAQLLVMLPDSLHYSGVRALDPAEKPLCLSNENASHVECELGNPMKRGAAQVTFYLILSTS
G
ISIETTELEVELLLATISEQELHPVSARARVFIELPLSIAGMAIPQQLFFSGVVRRGERAMQSERDVGSK
V
KYEVTVSNOGQSLRTLGS AFLNIMWPHEIANGKWLLYPMQVELEGGQGPQKGLCSRPNNILHLDVDSR
D
RRRRELEPPEQQEPGERQEPSMSWWPVSSAEKKKNITLDCARGTANCVVFSCLPLYSFDRAAVLHVWGR
L
NSTFLEEYS AVKSLEIVRANITVKSSIKNMLRDASTVIPVMVYLDPMVVAEGVPWWVILLAVLAGL
L
VLALLVLLLWKMGFFKRAKHPEATVPQYHAVKIPREDRQQFKEEKTGTILRNNWGS PRREGPDAHPILA
A
DGHPELGPDPGHPGPGTA

SEQ ID NO:44

>gi|4557675|ref|NP_000201.1| integrin alpha chain, alpha 6 [Homo
sapiens]
MAAAGQLCLLYLSAGLLSRLGA AFNLDTREDNVIRKYGDPSLFGFSLAMHWQLQPEDKRLLLVGAPRG
E
ALPLQRANRTGGLYSCDITARGPCTRIEFDNDADPTSESKEDQWMGVTVQSQGPQGGKVVTCAHRYEKRO
H
VNTKQESRDIFGRCYVLSQNLRIEDDMDGGDWSFCDGRLRGHEKFGSCQQGVAATFTKDFHYIVFGAPG
T
YNWKGIVRVEQKNNTFFDMNIFEDGPYEVGGETEHDESLVPVPANSYLGFSLSGKGVSKDEITFVSG
A
PRANHSGAVVLLKRDMSAHLLEPHIFDGEGLASSFGYDVAVVDLNKDGWQDIVIGAPQYFDRDGEVGG
A
VYVYMNQQGRWNNVKPIRLNGTKDSMFGIAVKNIGDINQDGYPDIAVGAPYDDLKGVFIYHGSANGINT
K
PTQVLKGISPYFGYSIAGNMDLDRNSYPDAVAGSLSDSVTIFRSRPVINIQKTITVTNRIIDLKQKTAC
G
APSGICLQVKSCFEYTANPAGYNPSISIVGTLEAEKERRKSGLS SRVQFRNQGSEPKYTQELTLKRQKQ
K

VCMEETLWLQDNIRDCLRPIPIITASVEIQEPSSRRRVNSLPEVLPTLNSDEPKTAHIDVHFLKEGCGDD
N
VCNSNLKLEYKFCTREGNQDKFSYLPQKGVPELVLDQKDIALEITVTNSPSNPRNPTKGDGDAHEAK
L
IATFPDTLTYSAYRELRAFPEKQLSCVANQNGSQADCELGNPFRNSNVTFYLVLSTTEVTFDTPYLDI
N
LKLETTSNQDNLAPITAKAKVVIELLLSVSGVAKPSQVYFGGTVVGEQAMKSEDEVGSLIEYEFRVINL
G
KPLTNLGTATLNIQWPKEISNGKWLlyLVKVESKGLEKVTCEPQKEINSLNLTESHNSRKKREITEKQI
D
DNRKFSLFAERKYQTLNCSVNVNCVNIRCPLRGLDSKASLILRSRLWNSTFLEEYSKLNyLDILMRAFI
D
VTAAAEENIRLPNAGTQVRVTVPFSKTVAQYSGVPWWIILVAILAGILMLALLVFILWKC GFFKRNKKDH
Y
DATYHKAEIHAQPSDKERLTSDA

SEQ ID NO:45

>gi|55749758|ref|NP_775873.2| hypothetical protein LOC57609 [Homo sapiens]

MAERGLEPSPAAVAALPPEVRAQLAELELELSEGDITQKGYEKKRSKLLSPYSPQTQETDSAVQKELRN
Q
TPAPSAAQTSAPSKYHRTRSGGARDERYRSDIHEAVQAALAKHKEQKMALPMPTKRRSTFVQSPADAC
T
PDTSSASEDEGSLRRQAALSAALQQSLQNAESWINRSIQGSSTSSSASSTLSHGVEVKGTSGSLADVFA
N
TRIENFSAPPDVTTTTTSSSSSSSSIRPANIDLPPSGIVKGMHKGSNRSSLMDTADGVPVSSRVSTKIQQ
L
LNTLKRPKRPPLKEFFVDDSEEIVEVPQDPFNQPKPEGRQMTVPKGEPLGVICNWPPALESALQRWGTT
Q
AKCSCLTALDMTGKPVYTLTYGKLWSRSLKLAYTLLNKLGTKNEPVLKPGDRVALVYPNNDPVMFMVAF
Y
GCLLAEVIPVPIEVPLTRKDAGGQQIGFLLGSCGIALALTSEVCLKGLPKTQNGEIVQFKGWPRLKWVV
T
DSKYLKPPKDWQPHISPAGTEPAYIEYKTSKEGSVMGVTVSRLAMLSHCQALSQACNYSEGETIVNVL
D
FKKDAGLWHGMFANVMNMHTISVPYSVMKTCPLSWVQRVHAHKAKVALVKCRDLHWAMMAHRDQRDVS
L
SSLRMLIVTDGANPWSVSSCDAFLSLFQSHGLKPEAICPCATSAEAMTVAIRRPGVPGAPLPGRAILSM
N
GLSYGVIRVNTEDKNSALTVQDVGHVMPGMMCIKPDGPPQLCKTDEIGEICVSSRTGGMMYFGLAGV
T
KNTFEVIPVNSAGSPVGDVPFIRSGLLGFVGPGLVFFVVGKMDGLLMVSGRRHNADDIVATGLAVESIK
T
VYRGRIAVFSVSVFYDERIVVVAEQRPDASEEDSFQWMSRVLQAIDS IHQVG VYCLALVPANTLPKTPL
G
GIHISQTKQLFLEGLHPCNILMCPHTCVTNLPKPRQKQPGVGPASVMVGNLVAGKRIAQAAGRDLGQI
E
ENDLVRKHQFLAEILQWRAQATPDHVLFMILLNAKGTTVCTASCLQLHKRAERIASVLGDKGHLNAGDNV
V
LLYPPGIELIAAFYGCYAGCIPVTVRPPHAQNLATLPTVRMIVDVSKAACILTSQTLMRLLRSREAA
A
AVDVKTWPTIIDTDDLPRKRLPQLYKPPTPEMLAYLDFSVSTTGMLTGVMKSHSAVNALCRAIKLQCEL
Y
SSRQIAICLDPYCGLGFALWCLCSVYSGHQSVLIPMELENNLFLWLSTVNQYKIRDTFCSYSVMELCT
K
GLGNQVEVLKTRGINLSCVRTCVVVAERPRVALQQSFSKLFKDIGLSPRAVSTTFGSRVNVAICLQGT
S
GPDPTTVYVDLKSRLRHDRLVERGAPQSLLLSESGKILPGVKVIVNPETKGPVGDSHLGEIWNVNSPH
T
ASGYTYIDSETLQADHFNTRL SFGDAAQTLWARTGYLGFVRRTELTAATGERHDALYVVGALDETLEL
R

GLRYHPIDIETSVSRIHRSIAECAVFTWTNLLVVVVVELCGSEQEALDLVPLVTNVVLEEHYLVGVVVV
V
DPGVIPINSRGEKQRMHLRDSFLADQLDPIYVAYNM

SEQ ID NO:46

>gi|45827692|ref|NP_055966.2| DIP2-like protein isoform a [Homo
sapiens]
MADRGCPLAAPLPAEVRESLAELELELSEGDITQKGYEKKRAKLLARYIPLIQGIDPSLQAENRIPGP
S
QTAAAPKQKSRPTASRDERFRSDVHTEAVQAALAKYKERKMPMPKRRSVLVHSSVETYPPTSSA
S
EDEGSLRRPGRLTSTPLQSHSSVEPWLDREVIGSSSTSSSSASSTSSHGGRPTTAPSAAATPGAAATTAL
A
GLEANTHIDLHSAPPDVTGLVEHSYFERPQVASVRSVPRGCSGSMLETADGVFVNSRVSSKIQQLLNT
L
KRPKRPPLKEFFVDDFEELLEVOQDPNPQKPEGSETSVLRGEPLTAGVPRPPSLLATLQRWGTTPKPS
P
CLTALDTTGKAVYTLTYGKLWSRSLKLAYTLLNKLTSKNEPLLKPGDRVALVFPNSDPVMFMVAFYGCL
L
AELVPVPIEVPLTRKDAGSQQVGFLGSGCVFLALTTDACQKGLPKAQTGEVAAFKGWPPLSWLVIDGK
H
LAKPPKDWHPAQDTGTGTAYIEYKTSKEGSTVGVTVSHASLLAQCRALTQACGYSEAETLTNVLDfKR
D
AGLWHGVLTSVMNRMHVSVPYALMKANPLSWIQKVCIFYKARAALVKSRDMHWSLLAQRGQRDVSLSSL
R
MLIVADGANPWSISSCDAFLNVFQSRGLRPEVICPCASSPEALTVAIRRPPDLGGPPPRKAVLSMNGLS
Y
GVIRVDTEEKLSVLTVQDVGVMPGANVCVVKLEGTPYLCKTDEVGEICVSSSATGTAYYGLLGITKNV
F
EAVPVTGGAPIFDRPFTRTGLLGFIGPDNLVFIVGKLDGLMVTGVRRHNAADDVVATALAVEPMKFVYR
G
RIAVFSVTVLHDDRIVLVAEQRPDASEEDSFQWMSRVLQAIDSIHQVGVCYCLALVPANTLPKAPLGGIH
I
SETKQRFLEGTTLHPCNVLMCPHTCVTNLPKPRQKQPEVGPASMI VGNLVAGKRIAQASGRELAHLEDSD
Q
ARKFLFLADVLQWRAHTTPDHPLFLLLNAGKTVTSTATCVQLHKRAERVAAALMEKGRLSVGDHVALVY
P
PGVDLIAAFYGCCLYCGCVPTVRPPHPQNLGTTLPVKMIVEVSKSACVLTQAVTRLLRSKEAAAAMD
I
RTWPTILDTDIPKKKIASVFRPPSPDLAYLDFSVSTTGILAGVKMSHAATSALCRSIKLQCELYPSR
Q
IAICLDPYCGLGFALWCLCSVYSGHQSVLVPPLELESNVSLWLSAVSQYKARVTFCSYSVMEMCTKGLG
A
QTGVLRMKGVNLS CVRTCMVVAEERPRIALTQSFSKLFKDLGLPARAVSTTFGCRVNVAICLQGTAGPD
P
TTVYVDMRALRHDRVRLVERGSPHSLPLMESGKILPGVKVIAHTETKGPLGDSHLGEI WVSSPHNATG
Y
YTVYGEEALHADHFSARLSFGDTQTIWARTGYLGFLRRTELTDASGGRHDALYVVGSLDETLELRGMRY
H
PIDIETSVIRAHRSIAECAVFTWTNLLVVVVVELDGLEQDALDLVALVTNVVLEEHYLVGVVVIVDPGV
I
PINSRGEKQRMHLRDGFLADQLDPIYVAYNM

SEQ ID NO:47

>gi|44888818|ref|NP_055789.1| hypothetical protein LOC22982 [Homo
sapiens]
MADRSLGMLPLEVRARLAELELELSEGDITQKGYEKKRSKLIGAYLPQPPRVDQALPQERRAPVTPS
S
ASRYHRRRSSGSRDERYSRVHTEAVQAALAKHKERKMAVPMPKRRSLVVQTSMDAYTPPTSSGSED
E

GSVQGDSQGTPTSSQGSINMEHWISQAIHGSTTSTTSSSSSTQSGGSGAAHRLADVMAQTHIENHSAPPD
 V
 TTYTSEHSIQVERPQGSTGSRTPAPKYGNAELMETGDGVPVSSRVSAKIQQLVNTLKRPKRPPLREFFVD
 D
 FEELLEVOQPDNPQPKPEGAQMLAMRGEQLGVVTNWPPSLEAALQRWGTISPKAPCLTTMDTNGKPLYI
 L
 TYGKLWTRSMKVAYSILHKLGTKQEPMPVRPGDRVALVFPNNDPAAFMAAFYGCLLAEVVPVPIEVPLTR
 K
 DAGSQQIGFLLGSCGVTVALTSDACHKGLPKSPTGEIPQFKGWPKLLWFVTESKHLSPPRDWFPHIKD
 A
 NNDTAYIEYKTCCKDGSVLGVTVTRTALLTHCQALTQACGYTEAETIVNVLDFFKDVGLWHGILTSVMNM
 M
 HVISIPYSLMKVNPLSWIQKVCQYKAKVACVKSRDMHWALVAHRDQRDINLSSLRMLIVADGANPWSIS
 S
 CDAFLNVFQSKGLRQEVICPCASSPEALTVAIRRPTDDSNQPPGRGVLSMHGLTYGVIRVDSEEKLSVL
 T
 VQDVGLVMPGAIMCSVKPDGVPQLCRTDEIGELCVCAVATGTSYYGLSGMTKNTFEVFPMTSSGAPISE
 Y
 PFIRTGLLGFGVGPGLVVFVVGKMDGLMVVSGRRHNADDIVATALAVEPMKFVYRGRIAVFSVTVLHDER
 I
 VIVAEQRPDSTEEDSFQWMSRVLQAIDSIHQVGVYCLALVPANTLPKTPPLGGIHLSETKQLFLEGLSHP
 C
 NVLMCPHTCVTNLPKPRQKQPEIGPASVMVGNLVSGKRIAQASGRDLGQIEDNDQARKFLFLSEVLQWR
 A
 QTPPDHILYTLNCRGAIANSLTCVQLHKRAEKIAVMLMERGHLQDGDHVALVYPPGIDLIAAFYGCCLY
 A
 GCVPIITVRPPHPQNIATTLPTVKMIVEVSRSACLMTTQLICKLLRSREAAAADVVRTWPLILDTDLLPK
 K
 RPAQICKPCNPDTLAYLDFSVSTTGMLAGVKMSHAATSACFRSILKQCELYPSREVAICLDPYCGLGFV
 L
 WCLCSVYSGHQSSILIPPSELETNPALWLLAVSQYKVRDTFCSYSVMELCTKGLGSQTESLKARGLDLSR
 V
 RTCVVVAEERPRIALTQSFSLFKDLGLHPRAVSTSFSGCRVNLAIQLOGTSGPDPTTVYVDMRALRHDR
 V
 RLVERGSPHSLPLMESGKILPGVRIIIANPETKGPLGDSHLGEIWHVSAHNASGYFTIYGDESLOSDHF
 N
 SRLSFGDTQTIWARTGYLGFLRRTTELTDANGERHDALYVVGALDEAMELRGMRYHPIDIETSVIRAHKS
 V
 TECAVFTWTNLLVVVVVELDGSEQEALDLVPLVTNVVLEEYHLIVGVVVVVDIGVIPINSRGEKQRMHLR
 D
 GFLADQLDPIYVAYNM

SEQ ID NO:48

>gi|5032013|ref|NP_005724.1| kinesin family member 20A [Homo sapiens]
 MSQGILSPPAGLLSDDDVVVSMPFESTAADLGSVVRKNLLSDCSVVSTSLEDKQQVPSEDSMEKVKVYL
 R
 VRPLLPSELERQEDQGCVRIVENVETLVLPAPKDSFALKSNERGIGQATHRFTFSQIFGPEVGQASFFNL
 T
 VKEMVKDVLKGQNWLIYTYGVTNSGKTHTIQGTIKDGGILPRSLALIFNSLQGLHPTPDLKPLLSNEV
 I
 WLDSKQIRQEEMKKLSLLNGGLQEEELSTSLKRSVYIESRIGTSTSFDSGIAGLSSISQCTSSSQLDDET
 S
 HRWAQPDTPALPVPANIRFSIWISFFBIYNELLYDLLEPPSQQRKRQTLRLCEDQNGNPYVKDLNWIHV
 Q
 DAEAWKLLKVGRKNQSFATHLNQNSSRSHSIFSIRILHLQGECDIVPKISELSLCDLAGSERCKDQK
 S
 GERLKEAGNINTSLHTLGRCIAALRQNNQNRSKQNLVPFRDSKLTRVFQGGFTGRGRSCMIVNVNPCAS
 T
 YDETLHVAKFSATIASQLVHAPPMQLGFPSLHSFIKEHSLQVSPSLEKGAKADTGLDDDIENEADISMYG
 K

EELLQVVEAMKTLILLKERQEKLEMLHLRDEICNEMVEQMQRREQWCSEHLDTQKELLEEMYEKLNIL
K
ESLTSFYQEEIQERDEKIEELEALLQEARQQSVAHQQSGSELALRRSQRLAASASTQQLOEVKAKLQQC
K
AELNSTTEELHKYQKMLEPPPSAKPFTIDVDKKLEEGQKNIRLLRTELQKLGESLQSAERACCHSTGAG
K
LRQALTTCDDILIKQDQTLAELQNNMVLVKLDLRKKAACIAEQYHTVLKLQGGQVSAKKRLGTNQENQQP
N
QQPPGKKPFRLRNLLPRTPTCQSSSTDCSPYARILRSRRSPLLKSGPFGKKY

SEQ ID NO:49

>gi|6754472|ref|NP_004847.2| kinesin family member 23 isoform 2 [Homo
sapiens]
MKSARAKTPRKPTVKKGSQTNLKDPVGVYCRVRPLGFPDQECCEVINNTTVQLHTPEGYRLNRNGDYK
E
TQYSFKQVFGTHTTQKELFDVVANPLVNDLIHGKNGLLFTYGVGTSGKTHMTGSPGEGGLLPRCLDMI
F
NSIGSFQAKRYVFKSNDNRNSMDIQCEVDALLERQKREAMPNPKTSSSKRQVDPEFADMITVQEFCKAE
V
DEDSVYGVFVSYIEIYNNYIYDLLEEVFPDPIKPKPPQSKLLREDKNHNMYVAGCTEVEVKSTEEAFEV
F
WRGQKKRRIANTHLNRESSRSHSVFNIKLVQAPLDADGDNVLQEQEQTISQLSLVDLAGSERTNRTRA
E
GNRLREAGNINQSLMTLRTCMDVLRENQMYGTNKMVPYRDSKLTHLFKNYFDGEGKVRMIVCVNPKAED
Y
EENLQVMRFAEVTQEVEVARPVDKAICGLTPGRRYRNQPRGFPVNEPLVTDVVLQSFPLPSCEILDIN
D
EQTLPLRIEAEKRNHLRQMMIDEFNKQSNAFKALLQEFDNAVLSENHMQGLNEKEKISMISGQKLEIE
R
LEKKNKTLEYKIEILEKTTTIIYEEDKRNLLQEQLETONQKLQRQFSDKRRLEARLQGMVTETMTKWEKEC
E
RRVAAKQLEMQNKLWVKDEKLKQLKAIVTEPKTEKPERPSRERDREKVTQRSVSPSPVPLLFPQDQNA
P
IRLRHRRSRSAGDRWVDHKPASNMQTETVMQPHVPHAITVSVANEKALAKCEKYMLTHQELASDGEIET
K
LIKGDYKTRGGGQSVQFTDIETLKQESPNGSRKRRSSTVAPAPQPDGAESEWTDVETRCVAVEMRAGS
Q
LGPQYQHHAQPKRKKP

SEQ ID NO:50

>gi|46049114|ref|NP_057279.2| M-phase phosphoprotein 1 [Homo sapiens]
MESNFNQEGVPRPSYVFSADPIARPSEINFDGIKLDLSHEPSLVAPNTEANSFESKDYLOVCLRIRPFT
Q
SEKELESEGCVHILDSQTVVLKEPQCILGRLSEKSSGQMAQKFSFSKVFGPATTQKEFFQGCIMQPVKD
L
LKGQSRLIFTYGLTNSGKTYTFQGTEENIGILPRTLNVLFDSLQERLYTKMNLKPHRSREYLRLSSEQE
K
EEIASKSALLRQIKEVTVHNDSDDTLYGSLTNSLNISEFEESIKDYEQANLNMANSIKFSVWVSFFFIY
N
EYIYDLFVPVSSKFQKRKMLRLSQDVKGYSFIKDLQWIVQVSDSKEAYRLLKLGIKHQSVAF TKLNNASS
R
SHSIFTVKILQIEDSEMSRVIRVSELSLCDLAGSERTMKTQNEGERLRETGNINTSLTLGKCINVLKN
S
EKSKFQQHVFPFRESKLTHYFQSFNGKGKICMIVNISQCYLAYDET LNVLKFSIAIAQKVCVPDTLNSSQ
E
KLFGPVKSSQDVSLDSNSNSKIILNVKRATISWENSLEDLMEDEDLVEELENAEETQNVETKLLDEDLDK
T
LEENKAFISHEEKRLLDLIEDLKKKLINEKKEKLTLEFKIREEVTQEFTQYWAQREADFKETLLQERE
I
LEENAERRLAIFKDLVGKCDTREEAAKD ICATKVETEATACLELKFNQIKAELAKTKGELIKTKEELK
K

RENESDSLIELETSNKKIITQNQRIKELINIIDQKEDTINEFQNLKSHMENTFKCNDKADTSSLIINN
 K
 LICNETVEVPKDSKSKICSERKRVNENELQODEPPAKKGSIHVSSAITEDQKKSEEVPRPNIAEIEDIRV
 L
 QENNEGLRAPLLTIENELKNEKEEKAELNKQIVHFQOELSLSEKKNLTLSKEVQQIQSNYDIAIAELHV
 Q
 KSKNQEQEEKIMKLSNEIETATRSITNNVSQIKLMHTKIDELRTLDSVSQISNIDLLNLRDLSNGSEED
 N
 LPNTQLDLLGNDYLVSKQVKEYRIQEPNRENSFHSSIEAIWEECKEIVKASSKKSHQIEELEQQIEKLO
 A
 EVKGYKDENNRLKEKEHKNQDDLLKEKETLIQQLKEELOEKNVTLDVQIQHVVEGKRALSELTOGVTCY
 K
 AKIKELETILETQKVECSHSAKLEQDILEKESIILKLERNLKEFQEHLQDSVKNTKDLNVKELKLKEEI
 T
 QLTNNLQDMKHLLQLKEEEEETNRQETEKLKEELSASSARTQNLKADLQKKEEDYADLKEKLTDAKKQI
 K
 QVQKEVSVMRDEDKLLRIKINELEKKKNQCSQELDMKQRTIQQLKEQLNNQKVEEAIQQYERACKDLNV
 K
 EKIIEDMRMTLEEQEQTQVEQDQVLEAKLEEVEERLATELEKWKCKNDLETKNQSRNKEHENNTDVLG
 K
 LTNLQDELQESEQKYNADRKKWLEEKMMMLITQAKEAENIRNKEMKKYAEDRERFFKQQNEMEILTAQLT
 E
 KSDSLQKWREERDQLVAALEIQLKALISSNVQKDNEIEQLKRIISETSKIETQIMDIKPKRISSADPK
 L
 QTEPLSTSFEISRNKIEDGSGVVLDSCEVSTENDQSTRFPKPELEIQFTPLQPNKMAVKHHPGCTTPVTVK
 I
 PKARKRKSNEEEDLVKCNENKKNATPRTNLKFPISDDRNSSVKKEQKVAIKPSKKTYSLRSQASIIGV
 N
 LATKKKEGTLQKFGDFLQHSPILOSKAKKIIETMSSSKLSNVEASKENVSQPKRAKRKLYTSEISSPI
 D
 ISGQVILMDQMKESDHQIIKRLRLTKTAK

SEQ ID NO:51

>gi|7661974|ref|NP_055606.1| maternal embryonic leucine zipper kinase
 [Homo sapiens]
 MKDYDELLKYYELHETIGTGGFAKVKLACHILTGMVAIKIMDKNTLGSDLPRIKTEIEALKNLRHQHI
 C
 QLYHVLETANKIFMVLEYCPGGELFDYIISQDRLSSEETRVVFRQIVSAVAYVHSQGYAHRDLKPENLL
 F
 DEYHKLKLIDFGLCAKPKGNKDYHLQTCGSLAYAAPELIQGKSYLGSEADVWSMGILLYVLMCGFLPF
 D
 DDNVMALYKKIMRGKYDVPKWLSPSSILLQQLQVDPKKRISMKNLLNHPWIMQDYNYPVEWQSKNPF
 I
 HLDDDCVTELSVHHRNNRQTMEDLISLWQYDHLTATYLLLLAKKARGKPVRLRLSSFSCGQASATPFTD
 I
 KSNNWSLEDVTASDKNYVAGLIDYDWCEDDLSTGAATPRTSQFTKYWTESNGVESKSLTPALCRTPANK
 L
 KNKENVYTPKSAVKNEEYFMFPEPKTPVNKNQHKREILTTPNRYTTPSKARNQCLKETPIKIPVNSTGT
 D
 KLMTGVISPERRCRSVELDLNQAHEETPKRKGAKVFGSLERGLDKVITVLTRSKRKGSAARDGPRRLKL
 H
 YNVTTTRLVNPDLQLLNEIMSILPKKHVDFVQKGYTLKCQTQSDFGKVTMQFELEVCLQKQKPDVVGIRRO
 R
 LKGDWVYKRLVEDILSSCKV

SEQ ID NO:52

>gi|41406064|ref|NP_005955.1| myosin, heavy polypeptide 10, non-
 muscle [Homo sapiens]
 MAQRTGLEDPERYLFVDRAVIYNPATQADWTAKKLVWIPSERHGFEEAASKEERGDEVMVELAENGKKA
 M

SEQ ID NO:53
>gi|33563340|ref|NP_079005.2| myosin, heavy polypeptide 14 [Homo sapiens]
MAAVTMSVPGRKAPPRPGFVPEAAQPFLFTPRGPGSAGGGPGSGTSPQVEWVTARRLVVWPSELHGFEAAA
I.

RDEGEEAEVELAESGRRLRLPRDQIQRMNPPKFSKAEDMAELTCLNEASVLHNLRRERYVSGLIYTYSG
L
FCVVINPYKQLPIYTEAIVEMYRGKKRHEVPPHVYAVTEGAYRSMLQDREDQSILCTGESGAGKTENTK
K
VIQYLAHVASSPKGRKEPGVPGELERQLLQANPILEAFGNAKTVKNDNSSRFGKFIRINFVAGYIVGA
N
IETYLLEKSRAIRQAKDECSFHIFYQLLGGAGEQLKADLLLEPCSHYRFLTNGPSSSPGQERELFQETL
E
SLRVLGFSHEEIIISMLRMVSAVLQFGNIALKRERNTDQATMPDNTAAQKLCRLLGLGVTDfsralltpr
I
KVGRDYVQKAQTKEQADFALEALAKATYERLFRWLVLRLNRLDRSPRQGASFLGILDIAGFEIFQLNS
F
EQLCINYTNEKLQQLFNHTMFVLEQBEYQREGIPWTFldfGLDLQPCIDLIERPANPPGLLALLDEECW
F
PKATDKSFVEKVAQEQQGHPKFQRPRLRDQADFSVLHYAGKVDYKANEWLMKNMDPLNDNVAALLHQS
T
DRLTAEIWKDVEGIVGLEQVSSLGDGPPGGRPRRGMFRTVGQLYKESLSRLMATLSNTNPSFVRCIVPN
H
EKRAKLEPRLVLDQLRCNGVLEGIRICRQGFNRLFQEFQRQYEILTPNAIPKGFMDGKQACEKMIQ
A
LELDPNLYRVGQSKIFFRAGVLAQLEEERDLKVTDIIVSFQAAARGYLARRAFQKRQQQSALRVMQRN
C
AAYLKLRLHWQWRLFTKVKPLQVTRQDEVLQARAQELQKVQELQQQSAREVGELQGRVAQLEBEERARL
A
EQLRAEAEELCAEAEETRGRLAARKQELELVSELEARVGEEECsrQMqTEKKRLQOHIOELEAHLEAE
E
GARQKLQLEKVTTEAKMKKFEEDLLLLLEDQNSKLSKSGSCWKIWPSSHPRLRRRRRSRASISYGSNM
R
PQSQTWRDLRKEEKGRQELEKLKRRLDGESSELQEQMVEQQQRAEELRAQLGRKEEELQAALARAED
G
GARAQLLKSLEAQAALAEAEQEDLESERVARTKAQKQRRDLGEELEALRGELEDTLSTNAQQELRSKR
E
QEVTELKKTLEETRIHEAAVQELRQRHGQALGELAEQLEQARRGKGAWEKTRLALEAEVSELRAELSS
L
QTARQEGEQRRRRLLELQLOEVQGRAGDGERARAEAAEKLQRAQAELENVSGALNEAESKTIRLSKELSS
T
EAQLHDAQELLQEETRAKLALGSRVRAMEAAAGLREQLLEEEAAARERAGRELQTAQAQLSEWRRRQEE
E
AGALEAGEEARRRAAREAEALTQRLAEKTETVDRLERGRRRLQOELDDATMDLEQORQLVSTLEKKQRK
F
DQLLAEEKAAVLRAVEERERAEAEGREREARALSLTRALEEEQEAREELERQNRALRAELEALLSSKDD
V
GKSVHELERACRVAEQAANDLRAQVTELEDELTAEDAKLRLEVTVQALKTQHERDLQGRDEAGEERRR
Q
LAKQLRDAEVERDEERKQRTLAVAARKKLEGELEELKAQMASAGQGKEEAVKQLRKMQAQMKELWREVE
E
TRTSREEIFSQNRESEKRLKGLEAEVLRLEQELAASDRARRQAQQDRDEMADEVANGNLSKAAILEEKR
Q
LEGRLGQLEEELEEEQSNSELLNDRYRKLLLQVESLTTELSAERSFSAKAESGRQQLERQIQELRGRLG
E
EDAGARARHKMTIAALESKLAQAEQLEQETRERILSGKLVRRAEKRLKEVVLQVEEERRVADQLRDQL
E
KGNLRVKQLKRQLEEAEEEEASRAQAGRRRLQRELEDVTESAESMNREVTTLRNRLRRGPLTFTTRTVRQ
V
FRLEEGVASDEEAEEAQP GSGPSPEPEGSPPAHPQ

SEQ ID NO:54

>gi|12667788|ref|NP_002464.1| myosin, heavy polypeptide 9, non-muscle
[Homo sapiens]MAQQAADKYLVDKNFINNPLAQADWAAKKLVWVPSDKSGFEPASLKEEVGEEAIVELVENGKKVKVKNK
D

DIQKMNPFFSKVEDMAELTCLNEASVLHNLKERYYSGLIYTYSGLFCVVINPYKNLPIYSEEIVEMYK
G
KKRHEMPPHIYAITDTAYRSMMDREDQSILCTGESGAGKTENTKKVIQYLAYVASSHKSKKDQGELER
Q
LLQANPILEAFGNAKTVKNDNSSRFGKFIRINFVNGYIVGANIETYLLEKSRAIRQAKEERTFHIFY
L
LSGAGEHLKTDLLLEPYNKYRFLSNGHVTIPGQQDKDMFQETMEAMRIMGIPEEEEQMGLLRVISGVLQL
G
NIVFKKERNTDQASMPDNTAAQKVSHLLGINVTDFTRGILTPIKVGGRDYVQKAQTKEQADFAIEALAK
A
TYERMFRWLVLIRINKALDKTKRQGASFIGILDIAGFEIFDLNSFEQLCINYTNEKLQQLFNHTMFILEQ
E
EYQREGIEWNFIDFGLDLQPCIDLIEKPAGPPGILALLDEECWFPKATDKSFVEKVMQEQGTHPKFQKP
K
QLKDKADFCIIHYAGKVDYKADEWLMKNMDPLNDNIATLLHQSSDKFVSELWKDVDRIIGLDQVAGMSE
T
ALPGAFKTRKGMFRTVGQLYKEQLAKLMATLRNTNPNFVRCIIPNHEKKAGKLDPHLVLDQLRCNGVLE
G
IRICRQGFPNRVVFQEFQRQRYEILTSPNSIPKGFMDGKQACVLMIKALELDSNLYRIGQSKVFFRAGVLA
H
LEEEERDLKITDVIIGFQACCRGYLARKAFAKRQQQLTAMKVLQRNCAAYLKLNRWQWWRLFTKVKPLLQ
V
SRQBEEMMAKEEELVKVREKQLAAENRLTEMETLQSQLMAEKLQLQEQLQAETELCAEAEELRARLTAK
K
QELEEICHDLARVEEEEEERCQHLQAEKKMQQNIQELEEQLEEEEESARQKLQLEKVTTEAKLKKLEEE
Q
IILEDQONCKLAKEKKLLEDRIAFTTNLTBEEEEKSKSLAKLKNKHEAMITDLEERLRREEKQORQELEKT
R
RKLEGDSTDLSQIAELQAQIAELKMQLAKKEEELQAALARVEEEAAQKNMALKKIRELESQISELQED
L
ESERASRNKAQKQKRDLEGELEALKTELEDTLDDSTAAQQLRSKREQEVNILKKTLEEEAKTHEAQIQE
M
RQKHSQAVEELAEQLEQTKRVKANLEKAKQTLENERGELANEVKVLLQKGKGDSEHKRKKVEAQLQELQV
K
FNEGERVRTTELADKVTKLQVELDNVTGLLSQSDSKSSKLTKDFSALESQLODTQELLQEEENRQKLSLST
K
LKQVEDEKNSFREQLEEEEEAHNLEKQIATLHAQVADMKKKMEDSVGCLETAEEVKRKLQKDLEGLSQ
R
HEEKVAAYDKLEKTKTRLQQELDDLLVDLDHQRQSACNLEKKQKKFDQLLAEEKTISAKYAEERDRAEA
E
AREKETKALSLARALEEAMEQKAELERLNKQFRTEMEDLMSSKDDVGKSVHELEKSKRALEQQVEEMKT
Q
LEEELEDELQATEDAKLRLEVNLAQAMKAQFERDLQGRDEQSEBKKKQLVRQVREMEAELEDERKQORSMAV
A
ARKKLEMDLKDLEAHIDSANKNRDEAIKQLRKLQAQMKDCMRELDLTRASREEILAQAKENEKKLKSME
A
EMIQLQEELAAAERAKRQAQQRDELADEIANSSGKGALALEEKKRRLARIAQLEEELEEEQGNTELIN
D
RLKKANLQIDQINTDLNLSHAQKNENARQQLERQNKELKVKLQEMEGTVKSKYKASITALEAKIAQL
E
EQLDNETKERQAACKQVRRTEKKLKDVLQVDDERRNAEQYKDQADKASTRLKQLKRQLEEAEEEAQRA
N
ASRRKLQRELEDATETADAMNREVSSLKNKLRRGDLFPVPRRMARKGAGDGSDEEVDGKADGAEPKA
E

SEQ ID NO:55

>gi|13124879|ref|NP_002465.1| smooth muscle myosin heavy chain 11
isoform SM1 [Homo sapiens]

MAQKGQLSDDEKFLFVDKNFINSPVAQADWAAKRLVWVPSEKQGFEEAASIKKEKGDEVVVELVENGKKV
T

VGKDDIQKMNPPKFSKVEDMAELTCLNEASVLHNLRRERYFSGLIYTYSGLFCVVVNPYKHLPIYSEKIV
D
MYKGKKRHEMPPHIYAIADTAYRSMQLDREDQSILCTGESGAGKTENTKKVIQYLAVVASSHKGKKDTS
I
TGELEKQLLQANPILEAFGNAKTVKNDNSSRFGKFIRINFVDVTGYIVGANIETYLLEKSRAIRQARDER
T
FHIFYYMIAGAKEKMRSDLLLEGFNNTFLSNGFVPIPAAQDDEMFOETVEAMAIMGFSEEEQLSILKV
V
SSVLQGLNIVFKKERNTDQASMPDNTAAQKVCHLMGINVTDFTRSILTPRIKVG RDVVQKAQTKEQADF
A
VEALAKATYERLFRWILTRVNKALDKTHRQGASFLGILDIA GFEIFEVNSFEQLCINYTNEKLQQLFNH
T
MFILEQEEYQREGIEWNFIDFGLDLQPCIELIERPNNPPGVLALLDEECWFPKATDKSFVEKLCTEQGS
H
PKFQPKQLKDKTEFSIIHYAGKVDYNASAWLTKNMDPLNDNVTSLLNASSDKFVADLWKDVDRIVGLD
Q
MAKMTESLPSASKTKKGMFRTVGQLYKEQLGKLMTTLRNTTPNFVRCIIPNHEKRSGKLD A FLVLEQL
R
CNGVLEGIRICRQGFPNRIVFQEFRQRYEILANAIPKGFMDGKQACILMIKALELDPNLYRIGQSKIF
F
RTGVLAHLEEERDLKITDVIMAFQAMCRGYLARKAF AKRQQLTAMKVIQRNCAAYLKLRNWQWRLFT
K
VKPLLQVTRQEEEMQAKEDELQKTKERQQAENELKELEQKHSQLTEKNLLQEQLQAETELYAEAEEM
R
VRLAAKKQELEEILHEMEARLEEEEDRGQQLQAERKKMAQQMLDLEEQL EEEEEAARQKLQLEKVTA EAK
I
KKLEDEILVMDDQNNKLSKERKLL EERISDLTTNLAE EEEKAKNLT K LKNKHESMISELEVRLKKEEKS
R
QELEKLKRKLEGDASDFHEQIADLQAQIAELKMQLAKKEEELQAALARLDDEIAQKNNALKKIRELEGH
I
SDLQEDLDSEARAARKAEKQKRDLGEELEALKTELED TLDSTATQQELRAKREQEVTVLKKALDEETRS
H
EAQVQEMRQKHAQAVEELTEQLEQFKRAKANLDKNKQ TLEKENADLAGELRVLGQAKQEVEHKKKKLEA
Q
VQELQSKCSDGERARAELNDKVHKLQNEVESVTGMLNEAEGKA IKLAKDVASLSSQLQDTQELLQEETR
Q
KLNVS TKLRQLEEEERNSLQDQLDEEMEAKQNLERHISTLNIQLSDSKKKLQDFASTVEALEEGKKRFQK
E
IENLTQQYEEKAAAYDKLEKTKNRLQQELDDLVDLDNQRQLVSNLEKKQRKFDQLLAEEKNISSKYAD
E
RDRAEAEAREKETKALSLARALEEAL EAKEELERTNKMLKAEMEDLVSSKDDVGKNVHELEKSKRALET
Q
MEEMKTQLEEELEDELQATEDAKLRLEVNMQALKGQFERDLQARDEQNEEKRRQLQRQLHEYETELEDER
K
QRALAAA AKKKLEGDLKDLELQADSAIKGREEAIKQLRK LQAQMKDFQRELEDARASRDEIFATAKENE
K
KAKSLEADLMQLQEDLAAAERARKQADLEKEELAEELASSLSGRNALQDEKRRLEARIAQLEEELEEEEQ
G
NMEAMSDRVRKATQQAEQLSNELATERSTAQKNESARQQLERQNKELRSKLHEMEGAVKSKFKSTIAAL
E
AKIAQLEEQVEQEAREKQAATKSLKQKDKKLKEILLQVEDERKMAEQYKEQAEKGNARVKQLKRQLEEA
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EESQRINANRRKLQRELDEATESNEAMGREVNALSKSLRRGNETS FVPSRRSGGRRVIENADGSEETD
T
RDADFNGTKASE

SEQ ID NO:56

>gi|45439316|ref|NP_982281.1| peptidylprolyl isomerase E isoform 2
[Homo sapiens]MATTKRVLYVGGLAEEVDDKVLHAAFIPFGDITDIQIPLDYETEKH RGF AFVEFELAEDAAA AIDNMNE
S

ELFGRTIRVNLAKPMRIKEGSSRPVWSDDDLKKFSGKTEENKEEEGSEPPKAETQEGEPIAKKARSN
P
QVYMDIKIGNKPAGRIQMLLRSDVVPMTAENFRCLCTHEKGFGFKGSSFHRIIPQFMCQGGDFTNHNGT
G
GKSIYGKKFDDENFILKHTGPGLLSMANSGPNTNGSQFFLTCDKTDWLDGKHVVFGEVTEGLDVLRQIE
K
QEESAITSQPRSWKLT

SEQ ID NO:57

>gi|4506017|ref|NP_002706.1| protein phosphatase 2, catalytic
subunit, alpha isoform [Homo sapiens]
MDEKVFTKELDQWIEQLNECKQLSESVKSLCEKAKEILTKEENVQEVRCPVTVCGDVHGGQFHDLMELF
R
IGGKSPDTNYLFMGDYVDRGYYSVETVTLLVALKVRYRERITILRGNHESRQITQVYGFYDECLRKYGN
A
NVWKYFTDLFDYLPLTALVDGQIFCLHGGGLSPSIDTLDHIRALDRLQEVPHGPMCDLLWSDPDDRGGW
G
ISPRGAGYTFGQDISETFNHANGLTLVSRHQVMEGYNWCHDRNVVTIFSAPNYCYRCGNQAAIMELD
D
TLKYSFLQFDPAPRRGEPHVTRRTPDYFL

SEQ ID NO:58

>gi|4758952|ref|NP_004147.1| protein phosphatase 2, catalytic
subunit, beta isoform [Homo sapiens]
MDDKAFTKELDQWVEQLNECKQLNENQVRTLCEKAKEILTKEENVQEVRCPVTVCGDVHGGQFHDLMELF
R
IGGKSPDTNYLFMGDYVDRGYYSVETVTLLVALKVRYPERITILRGNHESRQITQVYGFYDECLRKYGN
A
NVWKYFTDLFDYLPLTALVDGQIFCLHGGGLSPSIDTLDHIRALDRLQEVPHGPMCDLLWSDPDDRGGW
G
ISPRGAGYTFGQDISETFNHANGLTLVSRHQVMEGYNWCHDRNVVTIFSAPNYCYRCGNQAAIMELD
D
TLKYSFLQFDPAPRRGEPHVTRRTPDYFL

SEQ ID NO:59

>gi|5730067|ref|NP_006653.1| Snf2-related CBP activator protein [Homo
sapiens]
MAKDVRQFWSNVEKVVFQKQSRLEEKRRKALDLHLDFIVGQTEKYSDDLSSQLNQPLTSSKAGSSPCL
G
SSSAASSPPPPASRLDDEDGDFQFQDEDEEEDDEETIEVEEQQEGNDAAEQRRRIELLRREGELPLEELL
R
SLPPQLLEGPPSSPSTPSSHSDTRDGPEEGAEPEPPQVLEIKPPPSAVTQRNKQPHWPDEDDEEFTAN
E
EEAEDEEDTIAAEQLEGEVDHAMELSELAREGELSMEELLQQYAGAYAPGSGSSEDEDEDEVDANSSD
C
EPEGPVEAEPEPPQEDSSSQSDSVEDRSEDEDEHSEEEETSGSSASEESESESESEDAQSQSQADEEEED
D
DFGVEYLLARDEEQSEADAGSGPPTPGPTTLGPKKEITDIAAAESLQPKGYTLATTQVKTPIPLLLRLG
Q
LREYQHIGLDWLVTMYEKKLNGILADEMGLGKTIQTISLLAHLACEKGNWGPPLIIVPTSVMLNWEMEL
K
RWCPSFKILTYGAQKERKLRQGWTKPNAFHVCIITSYKLVLDHQAFRRKNWRYLILDEAQNIKNFKS
Q
RWQSLNLFNSQRRLLLTGTPLQNSLMELWLSLHFLMPHFVQSHREFKEWFSNPLTGMIEGSQYNEGLV
K
RLHKVLRPFLLRRVKVDVEKQMPKKYEHVIRCRLSKRQRCLYDDFMAQTTHKETLATGHFMSVINILMQ
L
RKVCNHPNLFDRPVTSPPFITPGICFSTASLVLRAVDHPLQRIDMGRFDLIGLEGRVSRYEADTFLLPR
H
RLSRRVLLEVATAPDPPRPKPKVMKVNRLQVPVKQEGRTVVVVNNPRAPLGPVVRPPPGPELSAQPT
T

PGPVPQVLPASLMVSASPAGPPLIPASRPPGPVLLPPLQPNSSGSLPQVLPSPGLVLSGTSRPPTPTLSL
K
FTPPAPVRLSPAPPPGSSSLLKPLTVPPGYTFPPAAATTTSTTTATATTTAVPAPTAPQRLILSPDMQ
A
RLPSGEVVSIGQLASLAQRFPVANAGGSKPLTFQIQGNKLTLTGAQVRQLAVGQPRFLQMPPTMVNNTGV
V
KIVVRQAPRDGLTPVPPLAPAPRPPSSGLPAVLNPRPTLTTPGRLEPTPTLGTARAMPPTPTLVRPLLKLV
H
SPSPEVSASAPGAAPLTISSPLHVPSSLPGPASSPMPPIPNSSPLASPVSSSTVSVPLSSSLPISVPTTL
A
PASAPLTIPISAPLTVSASGPALLTSVTPPLAPVVPAAPGPPSLQPSGASPSASALTGLLATAPSLSSS
Q
TPGHPLLLAPTSSHVPGLNSTVAPACSPVLVPASALASFFPSAPNPAPAQASLLAPASSASQALATPLA
P
MAAPQTAILAPSPAPPLAPLPVLAPSPGAAPVLASSQTFVPVMAPSSSTPGTSLASASPVPAPTPVLAPS
S
TQTMLPAPVPSPLPSPASTQTLALAPALAPTLGGSSPSQTLSTLGTGNPQGPFPTQTLSTLPASSLVPTP
A
QTLSTLAPGPPPLGPTQTLSTLAPAPPLAPASPVGPAPAHTLTTLAPASSSASLLAPASVQTLTSLPAPVPTL
G
FAAAQTLALAPASTQSPASQASSLVVSASGAAPLVTMVSRLPVSKDEPDTLTLRSGPPSPSTATSFG
G
PRRRQPPPPRSFFYLDSELEKRRQRSERLERIFQLSEAHGALAPVYGTEVLDFCTLPQPVASPIGR
R
SPGPSHPTFWTYTEAAHRAVLFPQQRDLQSEIIERFIFVMPPVEAPPSLHACHPPPWLAPRQAQAEQ
Q
LASELWPRARPLHRIVCNMRTQFPDLRLIQYDCGKLQTLAVLLRQLKAEGHRVLIPTQMTRMLDVLEQF
L
TYHGHLYLRLDGSTRVEQRQALMERFNADKRIFCFILSTRSGGVGNLTGADTVFVYSDWNPTMDAQ
Q
DRCHRIGQTRDVHIYRLISERTVEENILKKANQKRMLGDMIEGGNFTTAYFKQQTIRELFDMPLEEPS
S
SSVPSAPEEEEEETVASKQTHILEQALCRAEDEEDIRAATQAKAEQVAELAEFNENDGFPAGEGEEAGR
G
AEDEEMSRAEQEIAALVEQLTPIERYAMKFLEASLEEVSRLEELKQAEQVEAARKDLDOAKEEVFRLPQ
E
EEEGPGAGDESSCGTGGGTHRRSKKAKAPERPGTRVSERLRGARAETQGANHTPVISAHQTRSTTTPP
C
SPARERVPRPAPRPRPTASAPAAIPALVPVPVSAPVPISAPNPITILPVHILPSPPPPSQIPPCSSPA
C
TPPPACTPPPAHTPPPAQTCLVTPSSPLLLGPPSVFISASVTNLPLGLRPEAELCAQALASPESLELAS
V
ASSETSSLSLVPPKDLLPVAVEILPVSEKNLSLTFAPSALTLEAGSIPNGQEQAEPDSAEGTTLTVLPE
G
EELPLCVSESNGLLEPPSAASDEPLQEPLADRTSEELTEAKTPTSSPEKPQELVTA EVAAPSTSSSAT
S
SPEGPSPARPPRRRTSADVEIRGQGTGRPGQPPGPKVLRLKLPGRLVTVVEEKELVQRRRQQRGAASTLV
P
GVSETSASPGSPSVRMSGPESPPIGGPCEAAPSSSLPTPPQPPFIARRHIELGVTGGGSPENGDGAL
L
AITPPAVKRRRGRPPKKNRSPADAGRGVDEAPSSTLKGKTNGADVPVGPETLIVADPVLEPQLIPGPQP
L
GPQPVHRPNLLSPVEKRRRGRPPKARDLPPIPGTISSAGDGNSESRTPPPHPSPLTLPPLLVCPAT
V
ANTVTTVTISTSPPKRKRGRPPKNPPSPRPSQLPVLDRDSTSVLESCGLGRRRQPPQGQGESEGGSSDED
G
SRPLTRLARLRLEAEGMRGRKSGGSMVVAVIQDDLDLADSGPGLLELTPPVVSLTPKLRSTRLRPGSLV
P
PLETEKLPRKRAGAPVGGSPGLAKRGRLQPPSPGLPEGSVEESEAEASGEEEEGDGTPRRRPGPRRLVG
T
TNQGDQRILRSSAPPSLAGPAVSHRGRKAKT

SEQ ID NO:60

>gi|7657629|ref|NP_055185.1| serine/threonine kinase 23 [Homo sapiens]
MSASTGGGGDSGGSGGSSSSSQASCGPESSGSELALATPVPQMLQGLLGSDDEEQEDPKDYCKGGYHPV
K
IGDVFNGRYHVVRKLGWGHFSTVWLCWDIQRKRFVALKVVKSAAGHYTETAVDEIKLLKCVRSDPSDPK
R
ETIVQLIDDFRISGVNGVHVCMVLEVLGHQLLKWIIKSNYQGLPVPCKSIVRQVLHGLDYLHTKCKII
H
TDIKPENILLCVGDAYIRRLAAEATEWQQAGAPPPSRIVSTAPQEVLTGKLSKNKRKKMRRKRKQQR
L
LEERLRDLQRLEAMEAATQAEDSGLRLDGGSGSTSSSGFSGSLFSPASCSILSGSSNQRETGGLLSPST
P
FGASNLLVNPLEPQNADKIKIKIADLGNACWVHKHFTEDIQTRQYRAVEVLIGAEGPPADIWSTACMA
F
ELATGDYLFEPHSGEDYSRDEDHIAHIVELLGDIPPAFALSGRYSREFFNRRGELRHIHNLKHGWLVEV
L
MEKYEWPLEQATQFSAFLLPMMEYIPEKRASAADCLQHPWLNP

SEQ ID NO:61

>gi|47419936|ref|NP_003128.3| SFRS protein kinase 1 [Homo sapiens]
MERKVLALQARKKRTAKKDKAQRKSETQHRGSAPHSEDLPEQEEIILGSDDEEQEDPNDYCKGGYHL
V
KIGDLFNGRYHVIRKLGWGHFSTVWLSWDIQQKKFVAMKVVKSAEHYTETALDEIRLLKSVRNSDPNDP
N
REMVVQLLDDFKISGVNGTHICMVFEVLGHLLKWIIKSNYQGLPLPCVKKIIQQVLQGLDYLHTKCRI
I
HTDIKPENILLSVNEQYIRRLAAEATEWQSRGAPPPSGSAVSTAPQPKPADKMSKNKKKKLKKKQKRQA
E
LLEKRMQEIEEMEKEGSGPGQKRPNKQEESESPVERPLKENPPNKMTOEKLEESSTIGDQDTLMERDTEG
G
AAEINCNGVIEVINYTQNSNNETLRHKEDLHNANDCDVQNLNQESSFLSSQNGDSSTSQETDCTPITS
E
VSDTMVCQSSSTVQSFSEQHISQLQESIRAEIPCEDEQEQEHNGLDNGKSTAGNFLVNPLEPKNAE
K
LKVKIADLGNACWVHKHFTEDIQTRQYRSLEVLIGSGYNTPADIWSTACMAFELATGDYLFEPHSGEEY
T
RDEDHIALIIELLGKVPRKLIVAGKYSKEFFTCKGDLKHITKLKPWGLFEVLVEKYEWSQEEAAGFTDF
L
LPMLELIPEKRATAAECLRHPWLNS

SEQ ID NO:62

>gi|33188447|ref|NP_872633.1| SFRS protein kinase 2 isoform b [Homo sapiens]
MSVNSEKSSSSSERPEPQQKAPLVPPPPPPPPPPPPPLPDPTPPEPEEEIILGSDDEEQEDPADYCKGGYH
P
VKIGDLFNGRYHVIRKLGWGHFSTVWLCWDMQGRFVAMKVVKSAQHYTETALDEIKLLKCVRESDPSP
P
NKDMVVQLIDDFKISGMNGIHVCMVFEVLGHLLKWIIKSNYQGLPVRCVKSIIIRQVLQGLDYLHCKCK
I
IHTDIKPENILMCVDDAYVRRMAAEATEWQKAGAPPPSGSAVSTAPQPKPIGKISKNNKKKKLKKKQKRQ
A
ELLEKRLQEIEELEREAERKII EENITSAAPSNDQDGEYCPEVKLKTGLEEAAEAETAKDNGEAEDEQE
E
KEDAEKENIEKDEDDVDQELANIDPTWIESPKTNNGHIENGPFSLQQLDDEDDDEEDCPNPEEYNLDEP
N
AESDYTYSSSYEQFNGELPNGRHKIPESQFPEFSTSLFSGSLEPVACGSVLSEGSPLTEQEESSPSHDR
S
RTVSASSTGDLPAKTRAADLLVNPLDPRNADKIRVKIADLGNACWVHKHFTEDIQTRQYRSIEVLIGA
G

YSTPADIWSTACMAFELATGDYLFEPHSGEDYSRDEDHIAHIIELLGSI PRHFALSGKYSREFFNRRGE
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RHITKLKPWSLFDVLVEKYGWPHEAQAQFTDFLIPMLEMVPEKRASAGECLRHPWLNS

SEQ ID NO:63

>gi|4507691|ref|NP_003487.1| transformation/transcription domain-
associated protein [Homo sapiens]

MAFVATQGATVVDQTTLMKKYLQFVAALTDVNTPDETKLKMMQEVSENFENVNTSSPOYSTFLEHIIPRF
L
TFLQDGEVQFLQEKPAQQLRKLVLLEIIRIPTNEHLRPHTKNVLSVMFRFLETENEENVLICLRRIIBL
H
KQFRPPITQEIHHFLDFVKQIYKELPKVVNRYFENPQVIPENTVPPPEMVGMITTIIVKVNPEREDSET
R
THSIIPRGSLSLKVLAELPIIVVLMYQLYKLNIHNVVAEFVPLIMNTIAIQVSAQARQHKLYNKELYAD
F
IAAQIKTSLFLAYIIRIYQELVTKYSSQMVKGMLQLLSNCPAETAHLRKELLIAAKHILTTTELNRNQFIP
C
MDKLFDESILIGSGYTARETLRPLAYSTLADLVHHVRQHPLPLSDLSLAVQLFAKNIDDESLPSSIQTMS
C
KLLNLVDCIRSKSEQESGNGRDVLMRMLEVFVLKFHTIARYQLSAIFKKCKPQSELGAVEAALPGVPT
A
PAAPGPAPSPAPVPAPPPPPPPPPATPVTAPVPPFEKQGEKDKEDKQTFQVTD CRSLVKTLVCGVKT
I
TWGITSCAPGEAQFIPNKQLQPKETQIYIKLVKYAMQALDIYQVQIAGNGQTYIRVANCQTVRMKEEK
E
VLEHFAGVFTMMNPLTFKEIFQTTVPYMERISKNYALQIVANSFLANPTTSALFATILVEYLLDRLPE
M
GSNVELSNLYLKLFLKLVFGSVSLFAAENEQMLKPHLHKIVNSSMELAQTAKPEPYNIFLLLRALFRSIGG
G
SHDLLYQEFPLPLPNLLQGLNMLQSGLHKQHMKDLFVELCLTVPVRLSSLLPYLPMLMDPLVSALNGSQ
T
LVSQGLRTLELCVDNLQPDFLYDHIQPVRAELMQALWRTL RNPADSISHVAYRVLGKFGGSRNRMKLKES
Q
KLHYVVTEVQGPSITVEFSDCKASLQPLMEKAIETALDCLKSANTEPYRRQAWEVKCFIVAMMSLED
N
KHALYQLLAHPNFTEKTIPNVIISHRYKAQDTPARKTTEQALTGAFMSAVIKDLRPSALPFVASLIRHY
T
MVAVAQQCGPFLPCYQVGSQPSTAMFHSEENGSKGMDPLVLIDAIACMAYEEKELCKIGEVALAVIF
D
VASIILGSKERACQLPLFSYIVERLCACCYEQAWYAKLGGVVSIFLMEERLPLTWVLQNQQTFLKALLF
V
MMDLTGEVSN GAVAMAKTTLEQLLMRCATPLKDEERAEIIVAAQEKSFHHVTHDLVREVTSPNSTVRKQ
A
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EHKVFTYELLNLCEAEDSALTCLPCYKSLPSLVPLRIAALNALAACNYLPQSRKIIAALFKALNSTNS
E
LQEAGEACMRKFLEGATIEVDQIHTHMRPLLMMLGDYRSLTLNVVNRLTSVTRLFPNSFNDKFCDQMMQ
H
LRKWMEVVVITHKGGQRSDGNEMKICSAIINLFHLIPAAPQTLVKPLLEVVMKTERAMLEAGSPFREP
L
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S
PSTSTMRLDLQFQAIIKIIISIIIVKNDDSWLASQHSLSVQLRRVWVSENFQERHRKENMAATNWKEPKLLA
Y
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L
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LKAHAMEARAIVRQAMAILTPAVPARMEDGHQMLTHWTRKIIIVEEGHTVPQLVHILHLIVQHFKVYYPV
R
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GLSVDSAQEVKRFRTATGAISAVFGRSQSLPGADSLAKPIDKQHTD TVVNFLIRVACQVNDNTNTAGS
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GEVLSRRVCVNLKLTALRPDMWPKSELKLQWFDKLLMTVEQPNQVNYGNICTGLEVL SFLLTVLQSPAIL
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L
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RVYERLLYVTCSONWEAMGNHFWIKQCIELLLAVCEKSTPIGTSCQGAMLP SITNVINLADSHDRAAFA
M
VTHVKQEPRERENSESKEEDVEIDIELAPGDQTSTPKTKELSEKDIGNQLHMLTNRHDKFLDTLREVKT
G
ALLSAFVQLCHISTTLAEKTWVQLFPRLWKILSDRQQHALAGEISPFLCSGSHQVQRDCQPSALNCFVE
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MSQCVPPIPIRPCVLKYLKGNLWFRSTLMLEHQAFKGLSLQIKPKQTTEFYEQESITPPQQEILDS
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LWEDHWIRCSKELNQWEALTEYGQSKGHINPYLVLECAWRVSNWTAMKEALVQVEVSCPKEMAWKVNMY
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L
GRNNSLHDMKTVVKTWRNRLPIVSDDL SHWSSIFMWRQH HYQAIVTAYENSSQHDPSSNNAMLGVHASA
S
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N
LK YFTKEMTAEFYALKGMFLAQINKSEEANKAFSAAVQMHDVLVKAWAMWGDYLENIFVKERQLHLGV
A
ITCYLHACRHNQNESKSRKYLAKVLWLLSFDDDKNTLADAVDKYCIGVPPIQLWLAWIPQLLTCLVGSE
L
LLNLISQVGRVYPQAVYFP IRTLYLT LKIEQRERYKSDPGPIRATAPMWRC SRIMHMQRELHPTLLSSL
E
GIVDQMVWFRENWHEEVLRLQQLQGLAKCYSVAFEKSGAVSDAKITPHTLNFVKKLVSTFGVGLENVSNV
S
TMFSSAASESLARRAQATAQDPVFQKLKGQFTTDFDFSVP GSMKLHNLISK LKKWIKILEAKTKQLPKF
F
LIEBKCRFLSNFSAQTA EVEIPGEFLMPKPTHYIYIKIARFMPRVEIVQKHNTAARRLYIRGHNGKIYPY
L
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KMFTIQLALIGFAEFVLHLNRLNPEMLQIAQDTGKLVAYFRFDINDATGDLDANRPVFPRLTPNISEF
L
TTIGVSGPLTASMIAVARCFAQPNFKVDGILKTVLRDEIIAWHKKQTQEDTSSPLSAGQPENMDSQQLV
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SEQ ID NO:64

>gi|4507857|ref|NP_003461.1| ubiquitin specific protease 7 (herpes
virus-associated) [Homo sapiens]

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H
TGYVGLKNQGATCYMNSLLQTLFFTNQLRKAVYMMPTGDDSSKSVPLALQRVFYELQHSDKPVGTKKL
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KSFGWETLDSFMQHDVQELCRVLLDNVENKMGTCVEGTIPKLFRGKMVSYIQCKEVDYRSDRREDYYD
I
QLSIKGGKNIFESFVDYVAVEQLDGDNKYDAGEHGLQEAEGVKFLTLPPVLHLQLMRMYDPQTDQNI
K
INDRFEFPEQLPLDEFLQKTDPKDPANYILHAVLVHSGDNHGGHYVVYLNPKGDGKWCKFDDDVVSRC
K
EEAIEHNYGGHDDLSVRHCTNAYMLVYIRESKLSEVLQAVTDHDIPOQLVERLQEEKRIEAQKRKERO
E
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GTRKPAMLDNEADGNKTMIELSDNENPWTIFLETVDPELAASGATLPKFDKDHDVMLFLKMYDPKTRSL
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K
DDPENDNSELPTAKEYFRDLYHRVDVIFCDKTIPNDPGFVVTLNRMNYFQVAKTVAQRLNTDPMLLQF
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L
YPDKHGCVRDLLBECKKAVELGEKASGKLRLLLEIVSYKIIIGVHQEDELLECLSPATSRTFRIEEIPLDQ
V
DIDKENEMLVTVAHFHKEVFGTFGIPFLLRIHQGEHFREVMKRIQSLLDIQEKEFEKFKFAIVMTGRHQ
Y
INEDEYEVNLKDFEPQPGNMSHPRPWLGLDHFNKAPKRSRYTYLEKAIKIHN

RDEGEEAEVELAESGRRLRLPRDQIQRMNPPKFSKAEDMAELTCLNEASVLHNLRERYYSGLIYTYSG
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VIQYLAHVASSPKGRKEPGVPGELERQLLQANPILEAFGNAKTVKNDNSSRFGKFIRINFDVAGYIVGA
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IETYLLEKSRAIROAKDECSFHIFYQLLGGAGEQLKADLLEPCSHYRFLTNGPSSSPGQERELFQETL
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I
KVGRDYVQKAQTKEQADFALEALAKATYERLFRWLVLRLNRLDRSPRQGASFLGILDIAGFEIFQLNS
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EQLCINYTNEKLQQLFNHTMFVLEQEYQREGIPWTFDGLDLQPCIDLIERPANPPGLLALLDEECW
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PKATDKSFVEKVAQEQQGHPKFQRPRLRDQADFSVLHYAGKVDYKANEWLMKNMDPLNDNVAALLHQS
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C
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LEGRLGQLEEELEEEQSNSELLNDRYRKLLQVESLTTELSAERSFSAKAESGRQQLERQIQELRGLG
E
EDAGARARHKMTIAALESKLAQAEQLEQETREIRILSGKLVRAEKRLKEVVLQVEEERRVADQLRDQL
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SEQ ID NO:54

>gi|12667788|ref|NP_002464.1| myosin, heavy polypeptide 9, non-muscle
[Homo sapiens]MAQQAADKYLYVDKNFINNPLAQADWAAKKLVWVPSDKSGFEPASLKEEVGEEAIVELVENGKKVKVKNK
D

DIQKMNPFPKFSKVEDMAELTCLNEASVLHNLKERYYSGLIYTYSGLFCVWINPYKNLPYSEEIVEMYK
G
KKRHEMPPHIYAITDTAYRSMMQDREDQSILCTGESGAGKTENTKKVIQYLAYVASSHKSKKDQGELER
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L
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G
NIVFKKERNTDQASMPDNTAAQKVSHLLGINVTDFTRGILTPRIKVG RDYVQKAQTKEQADFAIEALAK
A
TYERMFRWLVL RINKALDKTKRQGASFIGILDIA GFEIFDLNSFEQLCINYTNEKLQQLFNHTMFILEQ
E
EYQREGIEWNFIDFGLDLQPCIDLIEKPAGPPGILALLDEECWF PKATDKSFVEKVMQEQQGTHPKFQKP
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QLKDKADFCIIHYAGKVDYKADEWLMKNMDPLNDNIATLLHQSSDKFVSELWKDVDR IIGLDQVAGMSE
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SRQEEEMMAKEEELVKVREKQLAAENRLTEMETLQSQLMAEKLQLQEQLQAETELCAEAEELRARLTAK
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QELEEICHDL EARVEEEEEERCQHLQAEKKKMQQNIQELEEQL EEEESARQKLQLEKVTTEAKLKKLEEE
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IILEDQNC KLAKEKKLLEDRIAFTTNLT EEEESKSLAKLKNKHEAMITDLEERLRREEKQRQELEKT
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RKLEGDSTDLS DQIAELQAQIAELKMQLAKKEEELQAALARVEEEAAQKNMALKKIRELESQISELQED
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RQKHSQAVEELAEQLEQTKRVKANLEKAKQTL ENER GELANEV KVLQKGKGDSEHKRKKVEAQLQELQV
K
FNEGERV RTE LADKVT KLQVELDNVTGLLSQSDSKSSKLT KD FSALESQLODTQELLQ EENRQKLSLST
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HEEKVAAYDKLEKTKTRLQQELDDLVDLDHQRQSACNLEKKQKKFDQLLA BEKTISAKYAEERDRAEA
E
AREKETKALSLARALEEAMEQKAELERLNKQFRTEMEDLMSSKDDVGKSVHELEKSKRALEQQVEEMKT
Q
LEELEDELQATEDAKLRLEVN LQAMKAQFERDLQGRDEQSE EKKKQLVRQVREMEAELEDERKQRSMAV
A
ARKKLEMDLKDLEAHIDSANKNRDEAIKQLRK LQAQMKDCMRELD DTRASREEILAQAKENEKKLSME
A
EMIQLQEELAAAE RAKRQAQQERDELADEIANS SSGKGALALEEKRRLEARIAQLEEELEEEQGNTELIN
D
RLKKANLQIDQINTDLN LERSHAQKNENARQQLERQNKELKVKLQEMEGTVKSKYKASITALEAKIAQL
E
EQLDNETKERQAACKQVRRTEKKLKDVL LQVDDERRNAEQYKDQADKASTRLKQLKRQLEEAEEEAQRA
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ASRRKLQRELE DATETADAMNREVSSLKNKLRRGDLPFVVP RR MARKGAGDGSDEEVDGKADGA EAKPA
E

SEQ ID NO:55

>gi|13124879|ref|NP_002465.1| smooth muscle myosin heavy chain 11
isoform SM1 [Homo sapiens]

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T

VGKDDIQKMNPFPKFSKVEDMAELTCLNEASVLHNLRRERYFSGLIYTYSGLFCVVVNPPYKHLPIYSEKIV
D
MYKGKKRHEMPPHIYAIADTAYRSMQLDREDQSILCTGESGAGKTENTKKVIQYLAVVASSHKGKKDTS
I
TGELEKQLLQANPILEAFGNAKTVKNDNSSRFGKFIRINFDVTGYIVGANIETYLLEKSRAIRQARDER
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FHIFYMYIAGAKEKMRSDLLLEGFNNTFSLNGFVPIPAQQDDMFQETVEAMAIMGFSEEEQLSILKV
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SSVLQLGNIVFKKERNTDQASMPDNTAAQKVCHLMGINVTDFTRSILTPIKVG RDVVQKAQTKEQADF
A
VEALAKATYERLFRWILTRVNKALDKTHRQASFLGILDIA GFEIFEVNSFEQLCINYTNEKLQQLFNH
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PKFQKPKQLKDKTEFSIIHYAGKVDYNASAWLTKNMDPLNDNVTSLLNASSDKFVADLWKDVDRIVGLD
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I
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KLNVTSLRQLLEEERNSLQDQLDEEMEAKQNLERHISTLNIQLSDSKKKLQDFASTVEALEEGKKRFQK
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E
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NMEAMSDRVRKATQQAEQLSNELATERSTAQKNESARQQLERQNKELRSKLHEMEGAVKSKFKSTIAAL
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RDADFN GTKASE

SEQ ID NO:56

>gi|45439316|ref|NP_982281.1| peptidylprolyl isomerase E isoform 2
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S

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GKSIYGKKFDDENFILKHTGPGLLSMANS GPNTNGSQFFLTCDKTDWLDGKHVVFGDEVTEGLDVLRLQIE
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SEQ ID NO:57

>gi|4506017|ref|NP_002706.1| protein phosphatase 2, catalytic
subunit, alpha isoform [Homo sapiens]
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R
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A
NVWKYFTDLFDYLPLTALVDGQIFCLHGGLSPSIDTLDHIRALDRLQEVPHGPMCDLLWSDPDDRGGW
G
ISPRGAGYTFGQDISETFNHANGLTLVSRAHQLVMEGYNWCHDRNVVTIFSAPNYCYRCGNQAAIMELD
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SEQ ID NO:58

>gi|4758952|ref|NP_004147.1| protein phosphatase 2, catalytic
subunit, beta isoform [Homo sapiens]
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IGGKSPDTNYLFMGDYVDRGYYSVETVTLLVALKVRYPERITILRGNHESRQITQVYGFYDECLRKYGN
A
NVWKYFTDLFDYLPLTALVDGQIFCLHGGLSPSIDTLDHIRALDRLQEVPHGPMCDLLWSDPDDRGGW
G
ISPRGAGYTFGQDISETFNHANGLTLVSRAHQLVMEGYNWCHDRNVVTIFSAPNYCYRCGNQAAIMELD
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SEQ ID NO:59

>gi|5730067|ref|NP_006653.1| Snf2-related CBP activator protein [Homo
sapiens]
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SSSAASSPPPPASRLDDEDGDFQPQDEDEEEDDEETIEVEEQQEGNDAAEQRRREIELLRREGELPLEELL
R
SLPPQLLEGPPSSPSQTPSSHDS DTRDGP EEGAE EEPQVLEIKPPPSAVTQRNKQ PWH PDEDDEEFTAN
E
EEAEDEEDTIAAEEQLEGEVDHAMELSELAREGELSMEELLQYAGAYAPGSGSSEDEDEDEVDANSSD
C
EPEGPVEAE EEPQEDSSSQSDSVEDRSEDEEEDHSEEEETSGSSASEESESESESEDAQSQSQADEEEED
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Q
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DRCHRIGQTRDVHIYRLISERTVEENILKKANQKRM LGDMAIEGNGFTTAYFKQQTIRELFDMPLEPS
S
SSVPSAPEEEEEETVASKQTHILEQALCRAEDEEDIRAATQAKAEQVAELAEFNENDGFPAGEGEEAGRP
G
AEDEEMSRAEQEIAALVEQLTPIERYAMKFLEASLEEVSRLELKQAEQVEAARKOLDQAKEEVFRLPQ
E
EEEGPGAGDESSCGTGGGTHRRSKKAKAPERPGTRVSERLRGARAETQGANHTPVISAHQTRSTTPPR
C
SPARERVPRPAPRPRPTPASAPAAIPALVPVPVSAPVPISAPNPITILPVHILPSPPPPSQIPPCSSPA
C
TPPPACTPPPAHTPPPAQTCLVTPSSPLLLGPPSVPIASVTNLPLGLRPEAELCAQALASPESLELAS
V
ASSETSSLSLVPPKDLLPVAVEILPVSEKNLSLTPSAPSLTLEAGSIPNGQEQEAPDSAEGTTLTVLPE
G
EELPLCVSESNGLLEPPSAASDEPLQEPLLEADRTSEELTEAKTPTSSPEKQELVTAEVAAPSTSSSAT
S
SPEGPSPARPPRRRTSADVEIRGQGTGRPGQPPGPKVLRKLPGRLLVTVVEEKELVQRRRQQRGAASTLV
P
GVSETASPGSPSVRMSGPESPPIGGPCEAAPSSSLPTPPQPPFIARRHIELGVTGGGSPENGDGAL
L
AITPPAVKRRRGRPPKKNRSPADAGRGVDEAPSSTLKGKTNGADPVPGPETLIVADPVLEPQLIPGPQP
L
GPQPVHRPNPLLSPEKRRRGRPPKARDLPIPGTISSAGDGNSESRTPPPPHPSPLTPLPPLLVCPTAT
V
ANTVTTVTISTSPPKRKRGRPPKNPPSPRPSQLPVLDRDSTSVLESCGLGRRRQPPQGGESEGGSSDED
G
SRPLTRLARLRLEAEGMRGRKSGGSMVVAVIQDDLDLADSGPGGLELTPPVVSLTPKLRSTRLRPGSLV
P
PLETEKLPRKRAGAPVGGSPGLAKRGRLQPPSPLGPEGSVEESEAEASGEEEEGDGTPRRRPGPRRLVG
T
TNQGDQRILRSSAPPSLAGPAVSHRGRKAKT

SEQ ID NO:60

>gi|7657629|ref|NP_055185.1| serine/threonine kinase 23 [Homo sapiens]
MSASTGGGDSGGSGGSSSSSQASCGPSSGSELALATPVPQMLQGLLGSDDEEQEDPKDYCKGGYHPV
K
IGDVFNGRYHVVRKLGWGHFSTVWLCWDIQRKRFVAMKVVKSAGHYTETAVDEIKLLKCVRSDPSDPK
R
ETIVQLIDDFRISGVNGVHVCMVLEVLGHQLLKWIIKSNYQGLPVPCVKSIVRQVLHGLDYLHTKCKII
H
TDIKPENILLCVGDAYIRRLAAEATEWQQAGAPPPSRIVSTAPQEVLTGKLSKNKRKKMRRKRKQQR
L
LEERLRDLQRLEAMEAATQAEDSGLRLDGGSGSTSSSGFSGSLFSPASCSILSGSSNQRETGGLLSPST
P
FGASNLLVNPLEPQNADKIKIKIADLGNACWVHKHFTEDIQTRQYRAVEVLIGAEYGPADIWSTACMA
F
ELATGDYLFEPHSGEDYSRDEDHIAHIVELLGDIPPAFALSGRYSREFFNRRGELRHHNLKHWGLYEV
L
MEKYEWPLEQATQFSAFLLPMEYIPEKRASAADCLQHPWLN

SEQ ID NO:61

>gi|47419936|ref|NP_003128.3| SFRS protein kinase 1 [Homo sapiens]
MERKVLALQARKKRTAKKDKAQRKSETQHRGSAPHSESDLPEQEEIILGSDDEEQEDPNDYCKGGYHL
V
KIGDLFNGRYHVIRKLGWGHFSTVWLSWDIQQKKFVAMKVVKSAEHYTETALDEIRLLKSVRNSDPNDP
N
REMVVQLLDDFKISGVNGTHICMVFEVLGHLLKWIIKSNYQGLPLPCVKKIIQQVLQGLDYLHTKCR
I
HTDIKPENILLSVNEQYIRRLAAEATEWQSRGAPPPSGSAVSTAPQPKPADKMSKNKKKKLKKKQKRQA
E
LLEKRMQEIEEMEKESGPGQKRPNKQEESESPVERPLKENPPNKMTQEKLEESSTIGDQTLMERDTEG
G
AAEINCNGVIEVINYTQNSNNETLRHKEDLHNANDCDVQNLNQESSFLSSQNGDSSTSQETDSCPTITS
E
VSDTMVCQSSSTVGQSFSEQHISQLQESIRAEIPCEDEQEHEHNGPLDNKGKSTAGNFLVNPLEPKNAE
K
LKVKIADLGNACWVHKHFTEDIQTRQYRSLEVLIGSGYNTPADIWSTACMAFELATGDYLFEPHSGEEY
T
RDEDHIALIIELLGKVPRKLIVAGKYSKEFFTCKGDLKHITKLKPWGLFEVLVEKYEWSQEEAAGFTDF
L
LPMLELIPEKRATAAECLRHPWLNS

SEQ ID NO:62

>gi|33188447|ref|NP_872633.1| SFRS protein kinase 2 isoform b [Homo sapiens]
MSVNSEKSSSSSERPEPQOKAPLVPPPPPPPPPPPPPLPDPTPEPEEEIILGSDDEEQEDPADYCKGGYH
P
VKIGDLFNGRYHVIRKLGWGHFSTVWLCWDMQGRFVAMKVVKSAQHYTETALDEIKLLKCVRESDP
P
NKDMVVQLIDDFKISGMNGIHVCMVFEVLGHLLKWIIKSNYQGLPVRCVKSIIIRQVLQGLDYLH
SKCK
I
IHTDIKPENILMCVDDAYVRRMAAEATEWQKAGAPPPSGSAVSTAPQQKPIGKISKNNKKKKLKKKQKRQ
A
ELLEKRLQEIEELEREAERKIIENITSAAPSNDQDGEYCPEVKLKTGLEEAAEAETAKDNGEAEDQE
E
KEDAEKENIEKDEDDVDQELANIDPTWIESPKTNGHIENGPFSLQQLDDEDDDEEDCPNPPEEYNLDEP
N
AESDYTYSSSYEQFNGELPNGRHKIPESQFPEFSTSLFSGSLEPVACGSVLSEGSPLTEQEESSPSHDR
S
RTVSASSTGDLPAKATRAADLLVNPLDPRNADKIRVKIADLGNACWVHKHFTEDIQTRQYRSIEVLIG
A
G

YSTPADIWSTACMAFELATGDYLFEPHSGEDYSRDEDHIAHIIELLGSI PRHFALSGKYSREFFNRRGE
L
RHITKLKPWSLFDVLVEKYGWPHEAQAQFTDFLI PMLEMVPEKRASAGECLRHPWLNS

SEQ ID NO:63

>gi|4507691|ref|NP_003487.1| transformation/transcription domain-
associated protein [Homo sapiens]
MAFVATQGATVVDQTTLMKKYLQFVAALTDVNTPDETKLMMQEVSENFENVNTSSPQYSTFLEHIIPRF
L
TFLQDGEVQFLQEKPAQQLRKLVLLEIIHRIPTNEHLRPHTKNVLSVMFRFLETENEENVLICLRIIEL
H
KQFRPPITQEIHHFLDFVKQIYKELPKVVNRYFENPQVIPENTVPPPEMVGMITTIKVNPEREDSET
R
THSII PRGSLSLKVLAE LPIIVVLMYQLYKLNHNVAEFVPLIMNTIAIQVSAQARQHKLYNKELYAD
F
IAAQIKTSLFLAYIIRIYQELVTKYSQQMVKGMLQLLSNCPAETAHLRKELLIAAKHILTTELNRNQFIP
C
MDKLFDESILIGSGYTARETLRPLAYSTLADLVHHVRQHLP LSLDLSLAVQLFAKNIDDES LPS SIQTMS
C
KLLLNLDVCIRSKSEQESGNGRDVLMRMLEVFVLKFHTIARYQLSAIFKKCKPQSELGAVEAALPGVPT
A
PAAPGPAPSPAPVPAPPPPPPPPPATPVTPAPVPPFEKQGEKDKEDKQTFQVTD CRSLVKT LVC GVKT
I
TWGITSCKAPGEAQFIPNKQLQPKETQIYIKLVKYAMQALDIYQVQIAGNGQTYIRVANCQTVRMKEEK
E
VLEHFAGVFTMMNPLTFKEIFQTTVPYMERISKNYALQIVANSFLANPTTSALFATILVEYLLDRLPE
M
GSNVELSNLYLKLFLKLVFGSVSLFAAENEQMLKPHLHKIVNSSMELAQTAKEPYNFLLLRALFRSIGG
G
SHDLLYQEFPLLPNLLQGLNMLQSGLHKQHMKDLFVELCLTPVRLSSLLPYLPMLMDPLVSALNGSQ
T
LVSQGLRTLELCVDNLQPDFLYDHIQPVRAELMQALWRTL RNPADSISHVAYRVLGKF GGSNRKMLKES
Q
KLHYVVTEVQGPSITVEFSDCKASLQLPMEKAIETALDCLKSANTEPYRRQAWEVIKCFVAMMSLED
N
KHALYQLLAHPNFTEKTIPNVIISHRYKAQDTPARKTFEQALTGAFMSAVIKDLRPSALPFVASLIRHY
T
MVAVAQQCGPFLPCYQVGSQPSTAMFHSEENGSKGMDPLVLIDAI AICMAYEEKELCKIGEVALAVIF
D
VASIILGSKERACQLPLFSYIVERLCACCYEQAWYAKLGGVVS IKFLMERLPLTWVLQNNQOTFLKALLF
V
MMDLTGEVSN GAVAMAKTTLEQLLMRCATPLKDEERAE EIVAAQEKSFHHVTHDLVREVTSPNSTVRKQ
A
MHS LQVLAQVTGKSVTVIMEPHKEVLQDMVPPKKHLLRHQPANAQIGLMEGNTFCTTLQPR LFTMDLNV
V
EHKVFYTELLNLCEAEDSALTKLPCYKSLPSLVPLRIAALNALAACNYLPQSREKIIAALFKALNSTNS
E
LQEAGEACMRKFLEGATIEVDQIHTHMRPLMMMLGDYRSLTLNVVNRLTSVTRLFPNSFNDKFC DQMMQ
H
LRKWM EVVVITHKGGQRSDGNEMKICSAIINLFHLIPAAPQTLVKPLLEVVMKTERAMLIEAGSPFREP
L
IKFLTRHPSQTVELFMMEATLNDPQWSRMFMSFLKHKDARPLRDVLAANPNRFITLLLPGGAQTAVRPG
S
PSTSTMRLDLQFOAIKIISIIVKNDDSWLASQHSLSVQLRRVWVSENFQERHRKENMAATNWKEPKLLA
Y
CLLNYCKRNYGDIELLFQLLRAFTGRFLCNMTFLKEYMEE EIPKNYSIAQKRALFFRFVDFNDPNFGDE
L
KAKVLQHILNPAFLYSFEKGEQEQLLGPPNPEGDN PESITSVFITKVLDPEKQADMLDSLRIYLLQYAT
L
LVEHAPHHHIHDNNKNRNSKLRLMTFAWPCLLSKACVDPACKYSGHLLLAHIIAKFAIHKKIVLQVFHS
L

LKAHAMEARAIVRQAMAILTPAVPARMEDGHQMLTHWTRKIIVEEGHTVPQLVHILHLIVQHFKVYYFPV
R
HHLVQHMVSAMQRLGFTPSVTIEQRRRLAVDLSEVVIKWELORIKDQQPDSMDPNSSGEGVNSVSSSIK
R
GLSVDSAQEVKRFRATATGAISAVFGRSQSLPGADSLAKPIDKQHTDTVVNFLIRVACQVNDNTNTAGS
P
GEVLSRRCVNLLKTALRPDMWPKSELKLQWFDKLLMTVEQPNQVNYGNICTGLEVL SFLLTVLQSPAIL
S
SFKPLQRGIAACMTCGNTKVLRAVHSLLSRLMSIFPTEPSTSSVASKYEELECLYAAVGKVIYEGLTNY
E
KATNANPSQLFGTLMILKSACSNNPSYIDRLISVFMRSLOKMOVREHLNPQAASGSTEATSGTSELVMLS
L
ELVKTRLAVMSMEMRKNFIQAILTSLIEKSPDAKILRAVVKIVEEWVKNNSPMAANQTPTLREKSILLV
K
MMTYIEKRFPEDLELNAQFLDLVNYVYRDETLSGSELTALEPAFLSGLRCAQPLIRAKFFEVDNSMK
R
RVYERLLYVTCSONWEAMGNHFWIKQCIELLLAVCEKSTPIGTSCQGAMLP SITNVINLADSHDRAAFA
M
VTHVKQEPRERENSESKEEDVEIDIELAPGDQTSTPKTKELSEKDIGNQLHMLTNRHDKFLDTLREVKT
G
ALLSAFVQLCHISTTLAEKTVWQLFPRWLKILSDRQQHALAGEISPFLCSGSHQVQRDCQPSALNCFVE
A
MSQCVPPPIRCPVLKYLKGNLWFRSTLMLEHQAFKGLSLQIKPKQTTEFYEQESITPPQQEILDS
L
AELYSLLQEEDMWAGLWQKRCKYSETATAIAYEQHGFFEQAQESYEKAMDKAKKEHERSNASPAIFPEY
Q
LWEDHWIRCSKELNQEALTEYGQSKGHINPYLVLECAWRVSNWTAMKEALVQVEVSCPKEMAWKVNMY
R
GYLAICHPEEQQLSFIERLVEMASSLAIREWRRLPHVVSHVHTPLLQAAQQIIELQEAAQINAGLQPTN
L
GRNNSLHDMKTVVKTWRNRLPIVSDDL SHWSSIFMWRQHYYQAIVTAYENSSQHDPSSNNAMLGVHASA
S
AIIQYGKIARKQGLVNVALDILSRIHTIPTVPIVDCFQKIRQQVKCYLQLAGVMGKNECMQGLEVIEST
N
LKYFTKEMTAEFYALKGMFLAQINKSEEANKAFSAVQMHDLVKAWAMWGDYLENIFVKERQLHLGVS
A
ITCYLHACRHQNESKSRKYLAQVLWLLSFDDDKNTLADAVDKYCIGVPPIQWLAWIPQLLTCLVGSEGL
L
LLNLISQVGRVYPQAVYFPIRTLYLTTLKIEQRERYKSDPGPIRATAPMWRC SRIMHMQRELHPTLLSSL
E
GIVDQMVWFRENWHEEVLRLQLQQGLAKCYSVAFEKSGAVSDAKITPHTLNFVKKLVSTFGVGLENVSNV
S
TMFSSAASESLARRAQATAQDPVFQKLKGQFTTDFDFSVP GSMKLHNLISKLLKKWIKILEAKTKQLPKF
F
LIEEKCRLSNFSAQTAEVEIPGEFLMPKPTHYYIKIARFMPRVEIVQKHNTAARRLYIRGHNGKIYPY
L
VMNDACLTESRREERVQLLRLLNPCLEKRKETT KRHLFFTVPRVVAVSPQMRLVEDNPSSLSLVEIYK
Q
RCAKKGIEHDNPISRYYDRLATVQARGTQASHQVLRDILKEVQSNMVPR SMLKEWALHTFPNATDYWTF
R
KMFTIQLALIGFAEFVLHLNRLNPEMLQIAQDTGKLVAYFRFDINDATGDLDANRPVPFRITPNISEF
L
TTIGVSGPLTASMIAVARCFAPNFKVDGILKTVLRDEIIAWHKKQTQEDTSSPLSAAGQFENMDSQQLV
S
LVQKAVTAIMTRLHNLAQFEGGESKVNTLVAAANSLDNL CRMDPAWHPWL

SEQ ID NO: 64

>gi|4507857|ref|NP_003461.1| ubiquitin specific protease 7 (herpes
virus-associated) [Homo sapiens]
MNHQQQQQQKAGEQQLSEPEDMEMEAGDTDDPPRITQNPVINGNVALSDGHNTAEEDMEDDTSWRSEA
T

FQFTVERFSRLSESVLSPPCFVRNLPWKIMVMPRFYFDRPHQKSVGFFLQCNAESDSTSWSCHAQAVLK
I
INYRDDEKSFSRRISHLFFHKENDWGFSNFMWSEVTDPEKGFIDDDKVTFEVQADAPHGVAWDSKK
H
TGYVGLKNQGATCYMNSLLQTLFFTNQLRKAVYMMPTGDDSSKSVPLALQRVFYELQHSDKPVGTTKKL
T
KSFGWETLDSFMQHDVQELCRVLLDNVENKMKGTCEGTIPKLFGRGKMVSIIQCKEVDYRSDRREDYYD
I
QLSIKGGKNIFESFVDYVAVEQLDGDNKYDAGEHGLQEAEKGVKFLTLPPVLHLQLMRMYDPQTDQNI
K
INDRFEFPEQLPLDEFLQKTDPKDPANYILHAVLVHSGDNHGGHYVVYLNPKGDGKWKCFDDDVVSRC
K
EEAIEHNYGGHDDDLVRHCTNAYMLVYIRESKLSEVLQAVTDHDI PQQLVERLQEEKRIEAKRKERQ
E
AHLVMQVQIVAEDQFCGHQGNMYDEEKVKYTVFKVLKNSSLAEFVQSLSQTMGFPQDQIRLWPMQARS
N
GTKRPAMLDNEADGNKTMIELSDNENPWTIFLETVDPELAASGATLPKFDDKDHVMLFLKMYDPKTRSL
N
YCGHIYTPISCKIRDLLPVMCDRAGFIQDTSLLIYEEVKPNLTERIQDYDVSLDKALDELMDGDIIVFQ
K
DDPENDNSELPTAKEYFRDLYHRVDVIFCDKTIPNDPGFVVTLNRMNYFQVAKTVAQRLNTDPMLLQF
F
KSQGYRDGPGNPLRHNYEGTLRDLLQFFKPRQPKKLYYQQLKMKITDFENRRSFKCIWLNSQFREEEIT
L
YPDKHGCVRDLLEECKKAVELGEKASGKLRLLEIVSYKIIIGVHQEDELLECLSPATSRTFRIEIIPLDQ
V
DIDKENEMLVTVAHFHKEVFGTGIPFLLRIHQGEHFREVMKRIQSLLDIQEKEFEKFKFAIVMTGRHQ
Y
INEDEYEVNLKDFEPQPGNMSHPRPWLGLDHFNKAPKRSRYTYLEKAIKIH