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(54) Title: P16 MEDIATED REGULATION OF NMDA RECEPTORS

(57) Abstract: Discovered is a novel protein and variants thereof whose activity at the NMDA receptor causes an increased efflux of calcium ions through the channel of said receptor. This activity is downregulated by the NR3A subunit of NMDA. Also discovered are the nucleic acid sequences encoding said novel protein and variants thereof. The discovery is useful for the diagnosing of NMDA receptor dysregulation and the treatment of NMDA receptor dysregulation related disorders. In addition, the discovery is useful for the further discovery of modulators affecting the activity of the novel protein and variants thereof at the NMDA receptor.

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p16 MEDIATED REGULATION OF NMDA RECEPTORS

Statement Regarding Federally Sponsored Research

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Related Application

Benefit of priority under 35 U.S.C. 119(e) is claimed herein to U.S. Provisional Application No.: 60/494,017, filed August 8, 2003. The disclosure of the above referenced application is incorporated by reference in its entirety herein.

Field of the Invention

This invention relates to the discovery of a novel protein, termed p16 and variants thereof, and the discovery that when expressed, p16 causes an increased efflux of cations through the NMDA receptor. The invention also relates to the discovery of novel nucleotide sequences that encode p16. The discovery of the current invention is useful for methods for diagnosing, treating and screening to identify agents useful for treating NMDA receptor dysregulation related diseases and pathological conditions and to compositions having an improved therapeutic profile identified using such screening methods.

Background of the Invention

Ionotropic glutamate receptors activate ligand-gated cation channels that mediate the predominant component of excitatory neurotransmission in the central nervous system (CNS). These receptors have been classified based on their preference for the glutamate-like agonists (RS)-2-amino-3- (3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), kainate (KA), and N-methyl-D-aspartate (NMDA). All three glutamate receptor subtypes are heteromultimeric complexes, and many of the subunits that comprise them have been

identified and characterized. To date, six NMDA receptor subunits (NR1, NR2A-2D and NR3A) have been reported.

The NMDA receptor (NMDAR) has unique properties distinguishing it from the other glutamate receptor subtypes. First, the activation of NMDAR requires the presence of dual agonists, glutamate (or NMDA) and glycine. The ligand-gated ion channel of the NMDA receptor is, thus, under the control of at least two distinct allosteric sites. In addition, the NMDA receptor controls the flow of both divalent (Ca.sup.2+) and monovalent (Na.sup.+, K.sup.+) ions into the postsynaptic neural cell through a receptor associated channel. (Foster et al., "Taking apart NMDA receptors", Nature, 329:395-396, 1987; Mayer et al., "Excitatory amino acid receptors, second messengers and regulation of intracellular Ca.sup.2+ in mammalian neurons," Trends in Pharmacol. Sci., 11:254-260, 1990). The activation of these receptors is regulated by Mg.sup.2+ in a voltage-dependent manner (i.e., the NMDAR is blocked at resting membrane potential and activated when depolarized). Most importantly; however, the NMDAR is extremely permeable to Ca.sup.2+, a key regulator of cell function.

NMDARs are believed to play a pivotal role in the transmission of excitatory signals from primary sensory neurons to the brain through the spinal cord (A. H. Dickenson (1990) Trends Pharmacol. Sci., 11. 307-309). NMDA receptors mediate Ca.sup.2+ influx into neurons, and its receptor-gated channel activity is blocked by Mg.sup.2+ in a voltage-dependent manner. These unique properties allow NMDARs to play a critical role in development of the nervous system, synaptic plasticity, memory, and other physiological processes in the CNS.

However, excessive stimulation of NMDARs has also been implicated in many pathological conditions including stroke, ischemia, head and spinal trauma, headache, epilepsy, neuropathic pain syndromes including diabetic neuropathy, glaucoma, depression and anxiety, drug addiction/withdrawal/tolerance, and in chronic neurodegenerative states, such as Alzheimer's disease, Huntington's disease, HIV-associated dementia, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis (ALS).

The molecular cloning and functional analysis of expressed NMDAR subunits, coupled with the examination of their temporal and spatial expression patterns in vivo, has led to significant advances in our understanding of NMDAR function at the molecular

level. However, the identification of these subunits alone has failed to explain the observed diversity in NMDAR function, particularly in motor neurons. Thus there is a need to further understand the role of NMDAR subunits in regulating these diverse functions.

Due to its broad-spectrum of neurological involvement, yet non-universal distribution, investigators are interested in the identification and development of drugs acting at the NMDA receptor. Drugs acting on the NMDA receptor are, therefore, expected to have enormous therapeutic potential. For instance, U.S. Pat. No. 4,904,681, issued to Cordi et al. (Cordi I), describes the use of D-Cycloserine, which was known to modulate the NMDA receptor, to improve/enhance memory and to treat cognitive deficits linked to a neurological disorder. D-Cycloserine is described as a glycine agonist which binds to the strychnine-insensitive glycine receptor.

U.S. Pat. No. 5,061,721, issued to Cordi et al. (Cordi II), describes the use of a combination of D-cycloserine and D-alanine to treat Alzheimer's disease, age-associated memory impairment, learning deficits, and psychotic disorders, as well as to improve memory or learning in healthy individuals.

U.S. Pat. No. 5,086,072, issued to Trullas et al., describes the use of 1-aminocyclopropanecarboxylic acid (ACPC), which was known to modulate the NMDA receptor as a partial agonist of the strychnine-insensitive glycine binding site, to treat mood disorders including major depression, bipolar disorder, dysthymia and seasonal effective disorder. It is also therein described that ACPC mimics the actions of clinically effective antidepressants in animal models. In addition, a copending U.S. patent application is cited that describes that ACPC and its derivatives may be used to treat neuropharmacological disorders resulting from excessive activation of the NMDA receptor.

None of the foregoing offers, however, a satisfactory mechanism for modulating NMDA receptor function. Development of drugs targeting the NMDA receptor, although desirous, has been hindered because the molecular pathway surrounding the NMDA receptor has not yet been completely elucidated. As mentioned above, the NMDAR consists of several protein chains (subunits) embedded in the postsynaptic membrane. Subunits NR1A and NR2A-D from a large extracellular region which probably contains most of the allosteric binding sites, several transmembrane regions looped and folded to

form a pore or channel which is permeable to Ca.sup.2+, and a carboxyl terminal region. It is believed that the channel is in constant motion, alternating between a cation passing (open) and a cation blocking (closed) state. The opening and closing of the channel is regulated by the binding of various ligands to domains of the protein residing on the extracellular surface and separate from the channel. As such, these ligands are all known as allosteric ligands. The binding of two co-agonist ligands--glycine and glutamate--is thought to effect a conformational change in the overall structure of the protein which is ultimately reflected in the channel opening, partially opening, partially closing, or closing. The binding of other allosteric ligands modulates the conformational change caused or effected by glutamate and glycine. The recently characterized subunit NR3A has been found to act in a novel manner, as compared to subunits NR1A-2D. NR3A downmodulates the NMDAR and this downmodulation has been correlated with a decreased unitary current and Ca.sup.2+ permeability of the channel. This unique regulatory behavior associated with the NR3A subunit is believed to have therapeutic importance. For example, studies in mice have shown that the NR3A subunit may protect the young nervous system from excitotoxic damage during development. Thus, it is desirable to further understand the NR3A molecular pathway, thereby allowing for the discovery of therapeutic compounds that modulate this same pathway.

Summary of the Invention

NR3A represents a dominant-interfering subunit of the conventional NMDA receptors (Das et al., Nature 393:377). NR3A expression is developmentally regulated, with its peak expression occurring during the first two weeks after birth. NR3A expression persists into adulthood at low levels in restricted areas of the brain. Neurons in NR3A knockout mice manifest increased NMDA-induced currents. Therefore, these mice allow us to identify signal transduction pathways downstream to NMDAR hyperactivation. To this end, Inventors identified genes whose expression is altered in NR3A-deficient brains using gene microarrays. Briefly, mRNAs were extracted from WT and NR3A-KO brains at postnatal day 15, and genes that displayed different levels of expression between the two samples were identified. Differential expression of these candidate genes was confirmed using real-time PCR and in situ hybridization. One gene identified in this manner encodes an ORF of 150 amino acids, representing a protein with a predicted MW of 16 kD. This gene was tentatively designated p16. Interestingly, p16

expression was up-regulated in NR3A-KO brains. As expected, up-regulation of p16 occurred in brain areas where NR3A expression is usually observed. These areas included the hippocampus; layer V of the cerebral cortex; and the amygdala. Exogenous p16 was then overexpressed in cultured cortical and hippocampal neurons. In the transfected neurons, Inventors observed that p16 protein was localized at synapses, and resulted in an increase in NMDA- but not AMPA- or GABA-induced currents. Intrestingly, p16 is a member of a large gene family. Based on the analysis of the mouse genome sequence, the estimated number of the gene family is 40-60. This gene family was named Takusan; however, in this current document the term "p16" will be used regardless of the actual molecular weight of the gene products. At least 32 different variants of p16 are expressed in the mouse brain. In addition, it is herein demonstrated that p16 can dimerize itself in cells, and, furthermore, select variants of p16 bind to PSD-95, a protein known to associate with NMDAR subunit 2 (NR2), while other variants do not. Therefore, there is a functional diversity among p16 variants.

Thus, the invention provides nucleotide sequences and amino acid sequences encoding and forming p16 and the variants thereof (hereinafter "p16"). The invention also provides methods for diagnosing and treating abnormalities in the p16:NMDAR molecular pathway. In addition, the invention provides a method of screening for modulators of said pathway which will increase or decrease signaling through an NMDA receptor. In a still further embodiment the invention provides a method of modulating NMDA receptor dysregulation associated with p16 using agents including, but not limited to, peptides, nucleic acids, small molecules and antibodies.

In one embodiment, the invention provides a method of modulating a cellular response to glycine or glutamate by introducing a nucleic acid molecule encoding a p16 polypeptide or functional fragment into a cell, and expressing the p16 functional fragment encoded by the nucleic acid molecule in the cell. In another embodiment, the invention provides a method of modulating a cellular response to glycine or glutamate by introducing an antisense nucleic acid molecule, a ribozyme molecule or a small interfering RNA (siRNA) molecule into the cell, wherein the molecule hybridizes to a p16 nucleic acid molecule and prevents translation of the encoded p16 polypeptide.

Brief Description of the Drawings

Figures 1a, 1b and 1c. Expression of p16 enhances NMDA currents in cultured hippocampal neurons. Representative NMDA, AMPA, and GABA currents from a control neuron (EGFP) and a neuron containing p16 (p16-EGFP) are shown in figures 1a and 1b. NMDA, AMPA and GABA current densities in p16-EGFP neurons is shown in figure 1c.

Figures 2a, 2b and 2c show that expression of p16 enhances recombinant NR1/NR2A currents in HEK293 cells.

Figure 3 shows that p16 expression is upregulated in NR3A knockout mice as compared to wild type using a p16 probe for in-situ hybridization.

Figures 4a and 4b. From the 90 cDNA clones amplified by RT-PCR of the C57BL/6 WT mouse brain (male, 6 week old); 34 variants of p16-related proteins were identified. Figure 4a shows these 34 amino-acid sequences, (SEQ ID Nos.: 4 through 37). In figure 4a, amino acid sequences are aligned against each other for comparison and, thus, are shown in 3 parts; line 1 of 3, line 2 of 3 and line 3 of 3. The nucleotide sequences for all clones are shown in Figure 4b, (SEQ ID Nos.: 38 through 71). In figure 4b, the nucleotide sequences are again aligned for comparison, and, thus, must again be shown in parts. Due to the size of the nucleotide sequences there are 18 parts.

Figure 5 is a schematic representation of many of the p16 variants identified in the current invention.

Figure 6 is an immunoblot showing that p16 protein can dimerize in cells.

Figure 7 is an immunoblot showing that select p16 variants can associate with PSD-95

Figure 8 is an illustration of a model for p16 function in a putative positive-feedback loop regulating NMDA receptor activity.

Detailed Description of the Invention

Definitions:

As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. For example, "a compound" refers to one or more of such compounds, while "the enzyme" includes a particular enzyme as well as other family members and equivalents thereof as known to those skilled in the art.

As used herein, the terms "polypeptide" and "polypeptides" refer to a genus of polypeptide or peptide fragments that encompass the amino acid sequences identified

herein, as well as smaller fragments. Alternatively, a polypeptide may be defined in terms of its antigenic relatedness to any peptide encoded by the nucleic acid sequences of the invention. Thus, in one embodiment, a polypeptide within the scope of the invention is defined as an amino acid sequence comprising a linear or 3-dimensional epitope shared with any peptide encoded by the nucleic acid sequences of the invention. Alternatively, a polypeptide within the scope of the invention is recognized by an antibody that specifically recognizes any peptide encoded by the nucleic acid sequences of the invention.

As used herein, the term "isolated," in reference to polypeptides or proteins, means that the polypeptide or protein is substantially removed from polypeptides, proteins, nucleic acids, or other macromolecules with which it, or its analogues, occurs in nature. Although the term "isolated" is not intended to require a specific degree of purity, typically, the protein will be at least about 75% pure, more typically at least about 90% pure, preferably at least about 95% pure, and more preferably at least about 99% pure.

Generally, the nomenclature used hereafter and the laboratory procedures in cell culture, molecular genetics, and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. Standard techniques are used for recombinant nucleic acid methods, polynucleotide synthesis, cell culture, and transgene incorporation (e.g., electroporation, microinjection, lipofection). Generally enzymatic reactions, oligonucleotide synthesis, and purification steps are performed according to the manufacturer's specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references which are provided throughout this document, as well as: Maniatis et al., Molecular Cloning: A Laboratory Manual (1989), 2nd Ed., Cold Spring Harbor, N.Y.; and Berger and Kimmel, Methods in Enzymology, Volume 152, Guide to Molecular Cloning Techniques (1987), Academic Press, Inc., San Diego, Calif., which are incorporated herein by reference. Oligonucleotides can be synthesized on an Applied Bio Systems oligonucleotide synthesizer according to specifications provided by the manufacturer. The procedures are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

As used herein, the term "agonist" refers to an agent which produces activation of p16 and provides for a substantial increase in NMDAR activation.

As used herein, the term "antagonist" refers to an agent which opposes the agonist activity of a known agonist of p16.

The term "candidate compound" refers to any molecule that potentially acts as a ligand, agonist or antagonist in the screening methods disclosed herein. A candidate compound can be a naturally occurring macromolecule, such as a polypeptide, amino acid, nucleic acid, carbohydrate, lipid, or any combination thereof. A candidate compound also can be a partially or completely synthetic derivative, analog or mimetic of such a macromolecule, or a small organic molecule prepared by combinatorial chemistry methods. If desired in a particular assay format, a candidate compound can be detectably labeled or attached to a solid support.

Methods for preparing large libraries of compounds, including simple or complex organic molecules, metal-containing compounds, carbohydrates, peptides, proteins, peptidomimetics, glycoproteins, lipoproteins, nucleic acids, antibodies, and the like, are well known in the art and are described, for example, in Huse, U.S. Patent No. 5,264,563; Francis et al., Curr. Opin. Chem. Biol. 2:422-428 (1998); Tietze et al., Curr. Biol., 2:363-371 (1998); Sofia, Mol. Divers. 3:75-94 (1998); Eichler et al., Med. Res. Rev. 15:481-496 (1995); and the like. Libraries containing large numbers of natural and synthetic compounds also can be obtained from commercial sources.

The number of different candidate compounds to test in the methods of the invention will depend on the application of the method. For example, one or a small number of candidate compounds can be advantageous in manual screening procedures, or when it is desired to compare efficacy among several predicted ligands, agonists or antagonists. However, it is generally understood that the larger the number of candidate compounds, the greater the likelihood of identifying a compound having the desired activity in a screening assay. Additionally, large numbers of compounds can be processed in high-throughput automated screening assays. Therefore, "one or more candidate compounds" can be, for example, 2 or more, such as 5, 10, 15, 20, 50 or 100 or more different compounds, such as greater than about 103, 105 or 107 different compounds, which can be assayed simultaneously or sequentially

The term "detectable label" refers to any moiety that can be selectively detected in a screening assay. Examples include without limitation, radiolabels, (e.g., .sup.3H, .sup.14C, .sup.35S, .sup.125I, .sup.131I), affinity tags (e.g. biotin / avidin or streptavidin, binding sites for antibodies, metal binding domains, epitope tags, FLASH binding domains - See US Patents 6,451,569; 6,054,271; 6,008,378 and 5,932,474 - glutathione or maltose binding domains) fluorescent or luminescent moieties (e.g. fluorescein and derivatives, GFP, rhodamine and derivatives, lanthanides etc.), and enzymatic moieties (e.g. horseradish peroxidase, .beta.-galactosidase, .beta.-lactamase, luciferase, alkaline phosphatase). Such detectable labels can be formed *in situ*, for example, through use of an unlabeled primary antibody which can be detected by a secondary antibody having an attached detectable label.

The methods of detecting a p16 nucleic acid molecule or peptide in a sample can be either qualitative or quantitative, and can detect the presence, abundance, integrity or structure of the nucleic acid molecule, as desired for a particular application. Suitable hybridization-based assay methods include, for example, in situ hybridization, Northern blots, RNase protection assays, Western blots and Southern blots, which can be used to determine the copy number and integrity of DNA. A hybridization probe can be labeled with any suitable detectable moiety such as those listed directly above. These methods are well known to those of ordinary skill in the art.

The term "DNA binding domain" or "DBD" refers to protein domain capable of binding to a specific DNA sequence, and comprising at least one zinc finger sequence.

The term "functional fragment" refers to a portion of a full-length p16 polypeptide that retains at least one biological activity characteristic of the full-length polypeptide. A functional fragment can contain, for example, at least about 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 110, 125, 150, 200 or more amino acids of a polypeptide. The remaining amino acid sequence is identical to, or exhibits substantial identity to, the corresponding positions in the naturally-occurring sequence.

As used herein, the term "functionally expressed" refers to a coding sequence which is transcribed, translated, post-translationally modified (if relevant), and positioned in a cell such that the protein provides the desired function. With reference to a reporter cassette, functional expression generally means production of a sufficient amount of the

encoded cell surface reporter protein to provide a statistically significant detectable signal to report transcriptional effects of a reporter polynucleotide.

As used herein, the term "LBD" or "ligand-binding domain" refers to the protein domain of a receptor, such as a NMDA receptor or other suitable receptor as discussed herein, which binds a physiological ligand and thereupon undergoes a conformational change and/or altered intermolecular interaction with an associated protein so as to confer a detectable activity.

As used herein, the term "ligand" refers to any biological or chemical compound that binds the recited polypeptide, fragment or receptor with high affinity. High affinity binding refers to binding with a Kd of less than about 10.sup.-3 M, such as less than 10.sup.-5 M, and often less than 10.sup.-7 M. p16 antibodies are examples of ligands of p16. As used herein, antibodies are defined to be "specifically binding" to a polypeptide if they bind polypeptides of the current invention with a K.sub.a of greater than or equal to about 10.sup.7 times M.sup.-1.

A "p16 ligand" can further be an agonist or antagonist of p16, as described below, or can be a compound having little or no effect on p16 biological activity. For example, a ligand without agonistic or antagonistic activity can be used to specifically target a diagnostic or therapeutic moiety to cells and tissues that express an excitatory glycine receptor. Thus, an identified ligand can be labeled with a detectable moiety, such as a radiolabel, fluorochrome, ferromagnetic substance, or luminescent substance, and used to detect normal or abnormal expression of an excitatory glycine receptor in an isolated sample or in in vivo diagnostic imaging procedures. Likewise, an identified ligand can be labeled with a therapeutic moiety, such as a cytotoxic or cytostatic agent or radioisotope, and administered in an effective amount to arrest proliferation or kill a cell or tissue that aberrantly expresses an excitatory glycine receptor for use in therapeutic applications described further below.

Binding assays, including high-throughput automated binding assays, are well known in the art and can be used in the invention methods. The assay format can employ a cell, cell membrane, artificial membrane system, or purified polypeptide, fragment or receptor, either in solution or attached to a solid phase. If desired, the binding assay can be performed in the presence of a known ligand of p16.

Suitable assays that can be used for detecting ligand binding include, for example, scintillation proximity assays (SPA) (Alouani, Methods Mol. Biol. 138:135-41 (2000)), UV or chemical cross-linking (Fancy, Curr. Opin. Chem. Biol. 4:28-33 (2000)), competition binding assays (Yamamura et al., Methods in Neurotransmitter Receptor Analysis, Raven Press, New York, 1990), biomolecular interaction analysis (BIA) (Weinberger et al., Pharmacogenomics 1:395-416 (2000)), mass spectrometry (MS) (McLafferty et al., Science 284:1289-1290 (1999) and Degterev, et al., Nature Cell Biology 3:173-182 (2001)), nuclear magnetic resonance (NMR) (Shuker et al., Science 274:1531-1534 (1996), Hajduk et al., J. Med. Chem. 42:2315-2317 (1999), and Chen and Shapiro, Anal. Chem. 71:669A-675A (1999)), fluorescence polarization assays (FPA) (Degterev et al., supra, 2001); surface plasmon resonance (SPR)(Liparoto et al., J. Mol Recognit. 12:316-321 (1999)); protein chip proteomic array analysis (e.g. ProteinChip™ System from Ciphergen Biosystems, which can be used in tandem with mass spectrometry analysis for sequence or structure determination), and in silico screening, whereby a library of compounds are screened using a computer based platform for an efficient method for filtering large virtual compound libraries.

An exemplary assay that has been used successfully to identify ligands of an NMDA receptor is phage display (see Li et al., Nature Biotech. 14:986-991 (1996)). A similar phage display approach can be applied to determine p16 ligands and excitatory glycine receptor ligands.

Exemplary high-throughput receptor binding assays are described, for example, in Mellentin-Micelotti et al., Anal. Biochem. 272:P182-190 (1999); Zuck et al., Proc. Natl. Acad. Sci. USA 96:11122-11127 (1999); and Zhang et al., Anal. Biochem. 268:134-142 (1999). Other suitable methods are well known in the art.

As used herein, "linked" means in polynucleotide linkage (i.e., phosphodiester linkage) or polypeptide linkage, depending upon the context of usage. "Unlinked" means not linked to another polynucleotide or polypeptide sequence; hence, two sequences are unlinked if each sequence has a free 5' terminus and a free 3' terminus.

As used herein, the term "modulator" refers to a wide range of candidate compounds, including, but not limited to natural, synthetic or semi-synthetic organic molecules, proteins, oligonucleotides and antisense, that directly or indirectly influence the activity of the p16 and or NR3A pathway. Furthermore, the precursor of a modulator (i.e.,

a compound that can be converted into a modulator) is also considered to be a modulator. Similarly, a compound which converts a precursor into a modulator is also considered to be a modulator.

"Naturally fluorescent protein" refers to proteins capable of forming a highly fluorescent, intrinsic chromophore either through the cyclization and oxidation of internal amino acids within the protein or via the enzymatic addition of a fluorescent co-factor. Typically such chromophores can be spectrally resolved from weakly fluorescent amino acids such as tryptophan and tyrosine. Endogenously fluorescent proteins have been isolated and cloned from a number of marine species including the sea pansies *Renilla reniformis, R. kollikeri and R. mullerei* and from the sea pens *Ptilosarcus, Stylatula and Acanthoptilum*, as well as from the Pacific Northwest jellyfish, *Aequorea victoria*; Szent-Gyorgyi *et al.* (SPIE conference 1999), D.C. Prasher *et al.*, Gene, 111:229-233 (1992) and red and yellow fluorescent proteins from coral. A variety of mutants of the GFP from *Aequorea victoria* have been created that have distinct spectral properties, improved brightness and enhanced expression and folding in mammalian cells compared to the native GFP, (*Green Fluorescent Proteins*, Chapter 2, pages 19 to 47, edited Sullivan and Kay, Academic Press, U.S. patent NOs: 5,625,048 to Tsien *et al.*, issued April 29, 1997; 5,777,079 to Tsien *et al.*, issued July 7, 1998; and U.S. Patent No. 5,804,387 to Cormack *et al.*, issued September 8, 1998). In many cases these functional engineered fluorescent proteins have superior spectral properties to wild–type proteins and are preferred for use as reporter genes in the present invention. Preferred naturally fluorescent proteins include without limitation, EGFP, YFP, Renilla GFP and DS red.

The terms "nucleotide sequence" "nucleic acid" or "nucleic acid molecule," as used herein, refer to a deoxyribonucleotide or ribonucleotide polymer in either single-or-double-stranded form, and unless otherwise limited, would fully encompass known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides.

Accordingly, a designated sequence identifier, unless specified otherwise, is intended to refer to the single-stranded molecule having the recited sequence, the single-stranded complement of the recited sequence, or a double stranded (or partially double-stranded) molecule in which one strand has the recited sequence. A nucleic acid molecule can optionally include one or more non-native nucleotides, having, for example,

modifications to the base, the sugar, or the phosphate portion, or having a modified phosphodiester linkage. Such modifications can be advantageous in increasing the stability of the nucleic acid molecule. Furthermore, a nucleic acid molecule can include, for example, a detectable moiety, such as a radiolabel, a fluorochrome, a ferromagnetic substance, a luminescent tag or a detectable binding agent such as biotin. Such modifications can be advantageous in applications where detection of a hybridizing nucleic acid molecule is desired.

Some of the nucleic acid molecules of the present invention are derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequence by standard biochemical methods. Examples of such methods, including methods for PCR, RT-PCR, SSCP analysis and coupled PCR transcription and translation analysis protocols that may be used herein, are disclosed in Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989), Ausubel, F.A., et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., New York (1987), and Innis, M., et al. (Eds.) PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, California (1990). Reference to a nucleic acid molecule also includes its complement as determined by the Standard Watson-Crick base-pairing rules, with Uracil (U) in RNA replacing Thymine (T) in DNA, unless the complement is specifically excluded.

As used herein, the nucleic acid molecules of the invention include DNA in both single-stranded and double-stranded form, as well as the DNA or RNA complement thereof (e.g., sense or antisense). DNA includes, for example, DNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and various combinations thereof. Genomic DNA, including translated, non-translated and control regions, may be isolated by conventional techniques, e.g., using any one of the cDNAs of the invention, or suitable fragments thereof, as a probe to identify a piece of genomic DNA which can then be cloned using methods commonly known in the art.

Polypeptides encoded by the nucleic acids of the invention are fully encompassed by the invention. As used herein, reference to a nucleic acid "encoding" a protein or a polypeptide encompasses not only cDNAs and other intronless nucleic acids, but also DNAs, such as genomic DNA, with introns, on the assumption that the introns included

have appropriate splice donor and acceptor sites that will ensure that the introns are spliced out of the corresponding transcript when the transcript is processed in a eukaryotic cell. Due to the degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, multiple DNA sequences can code for the same polypeptide. Such variant DNA sequences can result from genetic drift or artificial manipulation, such as occurring during PCR amplification or as the product of deliberate mutagenesis of a native sequence. Deliberate mutagenesis of a native sequence can be carried out using numerous techniques well known in the art. For example, oligonucleotide-directed site-specific mutagenesis procedures can be employed, particularly where it is desired to mutate a gene such that predetermined restriction nucleotides or codons are altered by substitution, deletion, or insertion. Exemplary methods of making such alteration are disclosed by Walder et al. (Gene 42:133, 1986); Bauer et al. (Gene 37:73, 1985); Craik (BioTechniques, January 12-19, 1985); Smith et al. (Genetic Engineering: Principles and Methods, Plenum Press, 1981); Kunkel (Proc. Natl. Acad. Sci. USA 82:488, 1985); Kunkel et al. (Methods in Enzymol. 154:367, 1987). The present invention thus fully encompasses any nucleic acid capable of encoding a protein of the current invention.

As used herein, the term "variant" refers to a polypeptide substantially homologous to a native polypeptide, but which has an amino acid sequence different from that encoded by any of the nucleic acid sequences of the invention because of one or more deletions, insertions or substitutions. Variants may be naturally occurring or artificially constructed. Variants can comprise conservatively substituted sequences, meaning that a given amino acid residue is replaced by a residue having similar physiochemical characteristics. See Zubay, Biochemistry, Addison-Wesley Pub. Co., (1983).

It is a well-established principle of protein and peptide chemistry that certain amino acids substitutions, entitled "conservative" amino acid substitutions, can frequently be made in a protein or a peptide without altering either the confirmation or the function of the protein or peptide. Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa.

The above-mentioned substitutions are not the only amino acid substitutions that can be considered "conservative." Other substitutions can also be considered conservative,

depending on the environment of the particular amino acid. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can be alanine and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pKs of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

The effects of such substitutions can be calculated using substitution score matrices such as PAM120, PAM-200, and PAM-250 as discussed in Altschul, (J. Mol. Biol. 219:55565 (1991)). Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Naturally-occurring and artificially constructed peptide variants are also encompassed by the present invention. Examples of such variants are proteins that result from alternate mRNA splicing events or from proteolytic cleavage of the polypeptides described herein. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the polypeptides encoded by the sequences of the invention.

As used herein, the term “splice variant” refers to a polypeptide generated from one of several RNA transcripts resulting from splicing of a primary transcript. Naturally-occurring and artificially constructed peptide splice variants are also encompassed by the present invention.

As used herein, the terms “hybridization” and “in situ hybridization” refer to conditions and washes under which nucleotide sequences that are significantly identical or homologous to each other remain bound to each other. Appropriate hybridization conditions can be selected by those skilled in the art with minimal experimentation as exemplified in Ausubel, F. A., et al., eds., Current Protocols in Molecular Biology Vol. 2, John Wiley and Sons, Inc., New York (1995). Additionally, stringency conditions are described in Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989). Variations on the conditions for low,

moderate, and high stringency are well known in the art and may be used with the current invention.

As used herein, the term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence using the coding sequence as a template. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. However, since enhancers generally function when separated from the promoter by several kilobases and intronic sequences may be of variable lengths, some polynucleotide elements may be operably linked but not contiguous. A structural gene (e.g., a HSV tk gene) which is operably linked to a polynucleotide sequence corresponding to a transcriptional regulatory sequence of an endogenous gene is generally expressed in substantially the same temporal and specific pattern as is the naturally-occurring gene. Methods for operatively linking a nucleic acid to a desired promoter are well known in the art and include, for example, cloning the nucleic acid into a vector containing the desired promoter, or appending the promoter to a nucleic acid sequence using PCR.

A vector of the invention can include a variety of elements useful for cloning and/or expression of the encoded nucleic acid molecule in the desired host cell, such as promoter and/or enhancer sequences, which can provide for constitutive, inducible or cell-specific RNA transcription; transcription termination and RNA processing signals, including polyadenylation signals, which provide for stability of a transcribed mRNA sequence; an origin of replication, which allows for proper episomal replication; selectable marker genes, such as a neomycin or hygromycin resistance gene, useful for selecting stable or transient transfectants in mammalian cells, or an ampicillin resistance gene, useful for selecting transformants in prokaryotic cells; and versatile multiple cloning sites for inserting nucleic acid molecules of interest.

Cloning vectors of the invention include, for example, viral vectors such as a bacteriophage, a baculovirus or a retrovirus; cosmids or plasmids; and, particularly for cloning large nucleic acid molecules, bacterial artificial chromosome vectors (BACs) and

yeast artificial chromosome vectors (YACs). Such vectors are commercially available, and their uses are well known in the art.

Thus, an invention nucleic acid molecule operatively linked to a promoter can be used to express p16 transcripts and polypeptides in a desired host cell, or in an in vitro system, such as an extract or lysate that supports transcription and translation.

For use in the gene therapy applications described further below, a nucleic acid molecule of the invention can be incorporated into suitable gene therapy vector, such as a viral vector or plasmid. Viral based vectors are advantageous in being able to introduce relatively high levels of a heterologous nucleic acid into a variety of cells, including nondividing cells.

Suitable viral vectors for gene therapy applications are well known in the art, and include, for example, Herpes simplex virus vectors (U.S. Patent No. 5,501,979), Vaccinia virus vectors (U.S. Patent No. 5,506,138), Cytomegalovirus vectors (U.S. Patent No. 5,561,063), Modified Moloney murine leukemia virus vectors (U.S. Patent No. 5,693,508), adenovirus vectors (U.S. Patent Nos. 5,700,470 and 5,731,172), adeno-associated virus vectors (U.S. Patent No. 5,604,090), constitutive and regulatable retrovirus vectors (U.S. Patent Nos. 4,405,712; 4,650,764 and 5,739,018, 5,646,013, 5,624,820, 5,693,508 and 5,674,703), papilloma virus vectors (U.S. Patent Nos. 5,674,703 and 5,719,054), lentiviral vectors (Kafri et al., Mol. Ther. 1:516-521 (2000), and the like. For targeting neural cells in the treatment of neuronal diseases, adenoviral vectors, Herpes simplex virus vectors and lentiviral vectors are particularly useful.

For gene therapy applications, the nucleic acid molecule can be administered to a subject by various routes. For example, local administration at the site of a pathology can be advantageous because there is no dilution effect and, therefore, the likelihood that a majority of the targeted cells will be contacted with the nucleic acid molecule is increased. This is particularly true in the eye, where either intravitreal or intraretinal administration is possible. In addition, administration can be systemic, such as via intravenous or subcutaneous injection into the subject. For example, following injection, viral vectors will circulate until they recognize host cells with the appropriate target specificity for infection.

Receptor-mediated DNA delivery approaches also can be used to deliver a nucleic acid molecule into cells in a tissue-specific manner using a tissue-specific ligand or an

antibody that is non-covalently complexed with the nucleic acid molecule via a bridging molecule. Direct injection of a naked nucleic acid molecule or a nucleic acid molecule encapsulated, for example, in cationic liposomes also can be used for stable gene transfer into non-dividing or dividing cells. In addition, a nucleic acid molecule can be transferred into a variety of tissues using the particle bombardment method.

Contemplated promoters and expression vectors provide for expression in bacterial cells, yeast cells, insect cells, amphibian cells, plant cells, mammalian cells (including human, non-human primate and rodent cells) and other vertebrate cells. A variety of promoters and expression vectors suitable for such purposes are commercially available, and can be further modified, if desired, to include appropriate regulatory elements to provide for the desired level of expression or replication in the host cell.

A "reporter gene" includes any gene that directly or indirectly produces a specific reporter gene product, detectable label, enzymatic moiety, or cellular phenotype, such as drug resistance that can be used to monitor transcription of that gene. Preferred reporter genes include proteins with an enzymatic activity that provides enzymatic amplification of gene expression such as .beta.-lactamase, luciferase, .beta.-galactosidase, catalytic antibodies and alkaline phosphatase. Other reporter genes include proteins such as naturally fluorescent proteins or homologs thereof, cell surface proteins or the native or modified forms of an endogenous gene to which a specific assay exists or can be developed in the future. Preferred reporter genes for use in the present invention provide for multiplexed analysis.

As used herein, the term "sample" is intended to mean any biological fluid, cell, tissue, organ or portion thereof that contains or potentially contains a p16 nucleic acid molecule or polypeptide. For example, a sample can be a histologic section of a specimen obtained by biopsy, or cells that are placed in or adapted to tissue culture. A sample further can be a subcellular fraction or extract, or a crude or substantially pure nucleic acid or protein preparation. A sample can be prepared by methods known in the art suitable for the particular format of the detection method employed.

As used herein, the phrase "system" refers to an intact organism or a cell-based system containing the various components required for analyzing the p16, NR3A and or p16/NR3A cellular pathway in response to the test compounds described herein.

The term "serial analysis" means that a test compound is analyzed and ranked based on a single activity. For example, compounds selected based solely on binding affinity, efficacy, ability to promote co-activator recruitment, ability to cause co-repressor dissociation or any other single factor, without reference to any other assay result or characteristic, are considered for the purposes here to be subject to "serial analysis." A compound may be subject to multiple rounds of serial analysis, each round being based on data created from a single activity. For purposes here this analysis strategy is not considered to be equivalent to parallel analysis so long as each analysis or ranking step is completed independently of each other.

The phrases "substantially identical," "substantial identity," "substantially similar" or "substantial similarity" mean that a relevant sequence is at least 70%, 75%, 80%, 85%, 90%, 92%, 95% 96%, 97%, 98%, or 99% identical to a given sequence. By way of example, such sequences may be allelic variants, sequences derived from various species, sequences derived from various loci within the same species, or they may be derived from the given sequence by truncation, deletion, amino acid substitution or addition. Percent identity between two sequences is determined by standard alignment algorithms such as ClustalX, GAP or BESTFIT when the two sequences are in best alignment according to the alignment algorithm. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains.

"Treating" or "treatment" as used herein covers the treatment of a disease-state associated with activity as disclosed herein, and includes:

a) preventing a disease-state associated with p16 activity from occurring;

b) inhibiting a disease-state associated with p16 activity, *i.e.*, arresting its development; or

c) relieving a disease-state associated with p16 activity, *i.e.*, causing regression of the condition.

The term "transcription activation domain" is used herein refers to a protein, or protein domain with the capacity to enhance transcription of a structural sequence in-trans. The ability to enhance transcription may affect the inducible transcription of a gene, or may effect the basal level transcription of a gene, or both. For example, a reporter

polynucleotide may comprise a minimal-promoter driving transcription of a sequence encoding a reporter gene. Such a reporter polypeptide may be transferred to a cell line for use in the creation of a modified host cell. Cloned sequences that silence expression of the reporter gene in cells cultured in the presence of an agonist also may be included (e.g., to reduce basal transcription and ensure detectable inducibility). Numerous other specific examples of transcription regulatory elements, such as specific minimal promoters and response elements are known to those of skill in the art and may be selected for use in the methods and polynucleotide constructs of the invention on the basis of the practitioner's desired application. Literature sources and published patent documents, as well as GenBank and other sequence information data sources can be consulted by those of skill in the art in selecting suitable transcription regulatory elements and other structural and functional sequences for use in the invention. Where necessary, a transcription regulatory element may be constructed by synthesis (and ligation, if necessary) of oligonucleotides made on the basis of available sequence information (e.g., GenBank sequences for a UAS, response element, minimal promoter etc).

Unless specified otherwise, the lefthand end of single-stranded polynucleotide sequences is the 5' end; the lefthand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences"; sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

As used herein, the term "transcriptional regulatory sequence" refers to a polynucleotide sequence or a polynucleotide segment which, when placed in operable linkage to a transcribable polynucleotide sequence, can produce transcriptional modulation of the operably linked transcribable polynucleotide sequence. A positive transcriptional regulatory element is a DNA sequence which activates transcription alone or in combination with one or more other DNA sequences. Typically, transcriptional regulatory sequences comprise a promoter, or minimal promoter and frequently a response element, and may include other positive and/or negative response elements as are known in the art or as can be readily identified by conventional transcription activity analysis (e.g., with

"promoter trap" vectors, transcription rate assays, and the like). Often, transcriptional regulatory sequences include a promoter and a transcription factor recognition site and / or response elements. The term often refers to a DNA sequence comprising a functional promoter and any associated transcription elements (e.g., enhancer, CCAAT box, TATA box, SP1 site, etc.) that are essential for transcription of a polynucleotide sequence that is operably linked to the transcription regulatory region. Enhancers and promoters include, but are not limited to, herpes simplex thymidine kinase promoter, cytomegalovirus (CMV) promoter / enhancer, SV40 promoters, pga promoter, regulatable promoters and systems (e.g., metallothionein promoter, the ecdysone promoter, the Tet on / Tet-off system, the PIP on / PIP off system, etc) adenovirus late promoter, vacinia virus 7.5 K promoter, and the like, as well as any permutations and variations thereof.

Since the list of technical and scientific terms cannot be all encompassing, any undefined terms shall be construed to have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. Reference to a "restriction enzyme" or a "high fidelity enzyme" may include mixtures of such enzymes and any other enzymes fitting the stated criteria, or reference to the method includes reference to one or more methods for obtaining cDNA sequences which will be known to those skilled in the art or will become known to them upon reading this specification.

Discovery – Cloning and Characterization of a Novel Peptide

The present invention relates to the cloning and characterization of a novel peptide, termed p16. The present invention also relates to the identification of amino acid sequences and nucleotide sequences comprising p16 and variants thereof (hereinafter "p16"). The present invention also relates to the determination that p16 is involved in the activation of NMDA receptors. The invention provides molecules and methods for screening candidate compounds to discover modulators of p16, NMDAR, NR3A and other proteins involved in the regulation of p16 mediates cation efflux in NMDAR. The invention provides molecules and methods that can be used to prevent or ameliorate conditions in which inappropriate NMDA receptor activation, or inappropriate responses to glycine or glutamate, are involved. The invention also provides molecules and methods used to diagnose conditions related to the dysregulation of p16 mediated cation efflux in NMDAR.

NR3A represents a dominant-interfering subunit of the conventional NMDA receptors (Das et al., Nature 393:377). NR3A expression is developmentally regulated, with its peak expression occurring during the first two weeks after birth. NR3A expression persists into adulthood at low levels in restricted areas of the brain. Neurons in NR3A knockout mice manifest increased NMDA-induced currents. Therefore, these mice allow Inventors to identify signal transduction pathways downstream to NMDAR hyperactivation. To this end, Inventors searched for genes having altered expression in NR3A-deficient brains. Inventors' selected to search using gene microarrays. Gene chips were obtained from the Ontario Cancer Center for microarray analysis between the NR3A knockout cells and wildtype cells (WT). mRNAs were extracted from WT and NR3A-KO brains at postnatal day 15, and genes that displayed different levels of expression between the two samples were identified. Inventors confirmed differential expression of these candidate genes using real-time PCR and in situ hybridization. One gene that was identified in this manner encodes an ORF of 150 amino acids, representing a protein with a predicted MW of 16 kD. The gene has the following sequence (SEQ ID No.: 1):

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1 ggcttggatc ccagagccca gcctgggagg aaccggggct cctggtgtac
catcatcatc
61 cccaacactc ctgttcagaa gatgggtgag gaaagtggaa agtctaacca
gtcagccgat
121 gaccagtggg aaaaatgagc tacaagatca cctgatcttc atcagtgaga
aagctttgca
181 caagagggtc tgctagaaat acttcaaccc aaaattccaa aatgaccaag
aagagatcaa
241 aaataaatga actagaagaa ctgaaattgg atatgaggaa gatcagcaat
gacatggagg
301 aaatgtgtgg aatcctgaac ctttacatgt atgaggattt gaactacagg
atgaacactg
361 aattcaacat cattaaatca caacatgaga agacaatgtt ggatatgaat
aaaatgatcc
421 agtccataat tggttccatg cagtactcca aggaactgat agaagataac
tattcctaca
481 gcattaagga ggaccacctc ctccgtgagt gcactcaact caacgaaaac
gtaaggatat
541 tactgaatga gaacagaagg ctgctggtgg agcaggctgg ccataagtgt
cctgtgggga
601 agaaaagagg ttctgtgagg aggccagcaa gaacatctgt gtcccaagtg
ccaaggaaca
661 gcagtgtgat atagtccagc agaaagcaga acatggcaca gaccacgaca
tgatctccct
721 caaagagaag tgctggagga agagcactga gtgtgcacag gaaatacacc
actgttgcct
781 ctcatcccta ataaccatgg ctgtaatggg ctgtatgctc ctcttttatt
ttgtttcttt
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841 ggtatgaaca ggccttaatt tcatctagcc tctggcccag gaagagtgca
catttaaagg
901 gactcagaga aatgctgaga cacatcaaga gctgctgggc atccaggaag
attctgagag
961 tttatattta tcttttcctg atgggtcatc atcaataatt acatggagat
cagtcaacaa
1021 aattgtaaaa ccttggatcc aagtctacaa catgtgttct gctttgactt
gggaggccat
1081 atccttcaga cccacactcc aaaaggagag tgttgcttaa atttctcctg
caaagtttgt
1141 tacctccagg aactactttt ctactaagtt gccaaggaca gccacaggct
gtaagtctgt
1201 gctacaaaat gagcagacta agaattttgc tttgcacaat ttttgtggtt
tgattttggt
1261 ttgagttttg attagtttag ttatttgttt tttcttgttt tcattcaaag
ttttgttatt
1321 tattggttat ttattgttct tttaattaat ttgatatttt gataaggtta
tacacagtac
1381 atattgactg tcagctttca gttacaattg agtacattgc atttttttctt
atgactaaca
1441 cagtgatctc caactcttca ctctaagagc cttgttattt cagttgtgat
catgaaatcc
1501 cacagatatc agacccagat ggatctctgc actcttcatg ggacttgggc
tccatagttt
1561 cttctgagcc ggacttaact acaaagtcct tcatacattc agtatggaga
gtttttctaa
1621 ctgtctgtat aggaacttaa tgatggaaaa cttacccatg ctgcatcgtt
gctgtcaaat
1681 atttagctac tgtgaaaatc ctgtggatta tggtgttgaa cgcattaatg
gcaaatacat
1741 cagtatttct gtaatagctc tcattaaatc aaagcatagt ctaagggaat
aaaaagctgt
1801 cagaaaacac agcagtgtat gcttctgcgt tccttcaaat atacaatcac
tggtaattgc
1861 aagtggtttc tgtgggggtc cttcaatgtt cattttatta ctttatgatt
cacctgtgtc
1921 tgccaaaaaa catcactcaa aaacaatgaa gattgtaatt aggtatcatc
ctataaaatc
1981 ctaacaaatg cc
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The ORF within SEQ ID No.: 1 has the following sequence (SEQ ID No.: 2):

ATGACCAAGAAGAGATCAAAAATAAATGAACTAGAAGAACTGAAATT
GGATATGAGGAAGATCAGCAATGACATGGAGGAAATGTGTGGAATCCT
GAACCTTTACATGTATGAGGATTTGAACTACAGGATGAACACTGAATTC
AACATCATTAAATCACAACATGAGAAGACAATGTTGGATATGAATAAA
ATGATCCAGTCCATAATTGGTTCCATGCAGTACTCCAAGGAACTGATAG
AAGATAACTATTCCTACAGCATTAAGGAGGACCACCTCCTCCGTGAGT
GCACTCAACTCAACGAAAACGTAAGGATATTACTGAATGAGAACAGAA
GGCTGCTGGTGGAGCAGGCTGGCCATAAGTGTCCTGTGGGGAAGAAAA

GAGGTTCTGTGAGGAGGCCAGCAAGAACATCTGTGTCCCAAGTGCCAA
GGAACAGCAGTGTGATATAG

The amino acid sequence of the p16 protein corresponding to SEQ ID No.: 2 is as follows (SEQ ID No.: 3):

MTKKRSKINELEELKLDMRKISNDMEEMCGILNLYMYEDLNYRMNTEFNI
IKSQHEKTMLDMNKMIQSIIGSMQYSKELIEDNYSYSIKEDHLLRECTQLNE
NVRILLNENRRLLVEQAGHKCPVGKKRGSVRRPARTSVSQVPRNSSVI

This gene, ORF and protein were tentatively designated p16; however, as is discussed below, because the discovered variants of this protein do not necessarily share the 16 kD molecular weight with SEQ ID No.: 3, Inventors have selected the more suitable name for the genes, ORFs and proteins of the current discovery: "Takusan". Nonetheless, for this disclosure the term p16 will be used herein to refer to the discovered genes, ORFs and proteins regardless of whether the molecular weight of a protein species is actually 16 kD.

The Inventors of the current application have discovered that the overexpression of p16 in cells having NMDA receptors (NMDAR) causes hyper-excitation of these cells and an increased efflux of cations through the associated ligand gated cation channel. The overexpression of endogenous p16 was further found to localize to the same areas of the brain where expression of the NMDAR subunit NR3A normally occurs, and p16 expression is up-regulated in NR3A-KO brains. Thus, the up-regulation of p16 occurred in brain areas where NR3A expression is usually observed. These areas included the hippocampus, layer V of the cerebral cortex, and the amygdala. The fact that p16 mRNA is up-regulated in NR3A-KO brains is consistent with the notion that p16 plays a role in the positive-feedback loop that allows sustained activation of NMDARs. Thus, Inventors have discovered a novel molecular pathway allowing for the diagnosis and treatment of NMDAR dysregulation and further providing a method of screening for agents that modulate NMDAR excitation.

Inventors have overexpressed exogenous p16 in cultured cortical and hippocampal neurons. In the transfected neurons, it is observed that p16 protein localizes at synapses, and results in an increase in NMDA- but not AMPA- or GABA-induced currents.

Low density primary hippocampal cultures were prepared from newborn rats, and maintained in cell culture for 1-3 weeks. Hippocampi were enzymatically (papain, Worthington Biochemical Corporation (Lakewood, NJ) Catalogue #3126) and mechanically dissociated into a single cell suspension, and plated onto glass coverslips coated with collagen/poly-D-lysine. Cells were then transfected with pSFV1-EGFP (control) or pSFV1/p16-EGFP (fusion protein between p16 and EGFP). Transfected cells were identified by fluorescence under microscopy. The vector pSFV1 is available from Invitrogen, Corp. (Carlsbad, CA) as catalogue no. 18488-019. The procedure to create pSFV1/p16-EGFP or pSFV1/EGFP is as follows: (1) the cDNA fragment corresponding to the coding region of p16 was subcloned into pEGFP-C3 (BD Biosciences Clontech, La Jolla, CA, catalogue no.: 6082-1) at Xho I/BamH I cloning sites. The resulting construct encodes the EGFP coding region fused at the N-terminal of p16 in frame; (2) the pEGFP-C3/EGFP-p16 was then digested with Nhe I and BamH I, which released a fragment encoding EGFP-p16 fusion protein. The cohesive ends of the fragment were blunted by the Klenow fragment of E.coli DNA polymerase I and then cloned into the Sma I site of the pSFV1 vector. The resulting plasmid is named pSFV1/p16-EGFP. pSFV1/EGFP was constructed by the same method without EGFP fused to p16.

For HEK293 cells, recombinant NR1/NR2A subunits were co-transfected with pSFV1/EGFP, or pSFV1/p16-EGFP. Whole cell recordings were made 18-25 hours after the transfection. NR1 and NR2A subunits were inserted into pCDNA1.1/Amp from Invitrogen (Carlsbad, CA). Catalogue number is V46020.

Whole cell recording of NMDA, AMPA, and GABA currents were made from cultured hippocampal neurons (DIV 8-10), 19 to 27 hours after being transfected with pSFV1/EGFP, or pSFV1/p16-EGFP. The patch pipettes (4-6 M.ohm.) were filled with an internal solution consisting of (in mM): 140 potassium gluconate, 17.5 KCl, 9 NaCl, 1 MgCl.sub.2, 10 Hepes, and 0.2 EGTA, at pH 7.4. The standard external solution contained 150 mM NaCl, 3 mM KCl, 10 mM Hepes, 5 mM glucose, 2 mM CaCl.sub.2, and 1 μM TTX. To isolate NMDA currents, 10 .micro.M CNQX (chemical name: 6-cyano-7-nitroquinoxaline-2,3-dione; which is available from numerous vendors, including A.G. Scientific, Inc., San Diego, CA 92121 as catalogue number C1053), 10 .micro.M glycine, and 10 .micro.M biccuculine were added to the solution. To isolate AMPA currents, 50 .micro.M APV and 10 .micro.M biccuculine were added to the solution. To

isolate GABA currents, 10 .micro.M CNQX and 50 .micro.M APV were added to the solution. NMDA (100 .micro.M), AMPA (10 .micro.M), or GABA (100 .micro.M) were applied every 15 seconds at a holding potential of –75 mV.

Solution exchange was made with computer controlled gravity-fed flow tubes, which is essentially comprised of a computer controlled, valve controller (Warner Instrument Co, Hamden CT, VC-6) controlling 3-way valves (The Lee Co, Essex, CT, LFAA1203618H). The flow tube is from Polymicro Technologies, Phoenix, AZ, 2000625). Data acquisition and analysis were made with PClamp 8 (Axon Instruments, Union City, CA). Currents were normalized to cell capacitance. Results are expressed as mean ± SEM in figures 1a-c. All experiments were performed at room temperature.

Expression of the p16 protein enhances NMDA currents in cultured hippocampal neurons. Representative NMDA, AMPA, and GABA currents from a control neuron (EGFP) and a neuron containing p16 (p16-EGFP) are shown in figures 1a and 1b. The straight, horizontal line in both figure 1a and figure 1b indicates the duration of agonist applications. Traces are averages of 4-5 responses. In figures 1a and 1b, the tracing that remains the uppermost tracing during the duration of agonist application represents AMPA, the middle tracing during the duration of agonist application represents NMDA, and the lowermost tracing during the duration of agonist application represent GABA. Figure 1c shows that NMDA current density, measured in pA/pF, in p16-EGFP neurons (n=10) was significantly larger than that of control neurons having EGFP only (n=10, $p<0.05$). In contrast, AMPA and GABA currents were not altered by p16 transfection.

Figure 2. Expression of p16 enhances recombinant NR1/NR2A currents in HEK293 cells. Representative NMDA currents from a HEK293 cell containing EGFP (figure 2a) or p16-EGFP (figure 2b) are shown. NMDA current density in cells containing p16-EGFP (n = 7) was significantly larger than that of cells containing on EGFP (n=8, $p<0.01$) (figure 2c).

Electrophysiological methods for detecting monovalent cation currents through an NMDA receptor are well known in the art. Exemplary methods for recording whole-cell and single-channel currents in Xenopus oocytes, brain slices, mammalian cells and cell-free membrane patches are described in Das et al., Nature 393:377-381 (1998); Sakmann and Neher, in Single-Channel Recording, 2nd ed., Ch. 15, pp. 341-355, (1995), edited by Bert Sakmann and Erwin Neher, Plenum Press, New York; Penner, in Single-Channel

Recording, 2nd ed., Ch. 1, pp. 3-28; Hamill et al., Pflugers Arch. 391:85-100 (1981); Ilers et al., in Single-Channel Recording, 2nd ed., Ch. 9, pp. 213-229, (1995), edited by Bert Sakmann and Erwin Neher, Plenum Press, New York.

Ionic currents can also be detected using suitable detectably labeled ion indicators. Ion indicators and methods for their use are known in the art. For example, monovalent cation currents through the NMDA receptor can be detected using Na.sup.+ or K.sup.+ ion indicators, which can be fluorescently labeled or radiolabeled (see, for example, Moore et al., Proc. Natl. Acad. Sci. USA 90:8058-8062 (1993); Paucek et al., J. Biol. Chem. 267:26062-26069 (1992); Xu et al., J. Biol. Chem. 270: 19606-19612 (1995)). Exemplary ion indicators include: SBFI sodium indicator, Sodium Green sodium indicator; CoroNa Red sodium indicator; PBFI potassium indicator; 6-Methoxy-N-(3-sulfopropyl)quinolinium (SPQ) chloride indicator; N-(Ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE) chloride indicator; 6-Methoxy-N-ethylquinolinium iodide (MEQ) chloride indicator; Lucigenin chloride indicator, which are available from Molecular Probes, Inc.

Subsequent to NMDA receptor activation and membrane depolarization, an influx of Ca.sup.2+ ions occurs if voltage-dependent Ca.sup.2+ channels are present in the cell being studied. If the cell of interest does not endogenously express voltage-dependent Ca.sup.2+ channels, the cell can be recombinantly engineered to express such channels, using voltage-dependent Ca.sup.2+ channel subunit gene sequences and molecular biology methods known in the art. Accordingly, ionic currents through the NMDA receptor can also be detected, indirectly, using detectably labeled Ca.sup.2+ ion indicators, which can be fluorescently labeled or radiolabeled. Exemplary Ca.sup.2+ ion indicators include FLUO-3 AM, FLUO-4 AM, FURA-2, INDO-1, FURA RED, CALCIUM GREEN, CALCIUM ORANGE, CALCIUM CRIMSON, BTC, and OREGON GREEN BAPTA (see, for example, Grynkiewitz et al., J. Biol. Chem. 260:3440-3450 (1985); Sullivan et al., in Calcium Signal Protocol, Methods in Molecular Biology 114: 125-133, Edited by David G. Lambert, Human Press, Totowa, New Jersey (1999); Miyawaki et al., Proc. Natl. Acad. Sci. USA 96:2135-2140 (1999); and Coward et al., Analyt. Biochem. 270:242-248 (1999)).

Figure 3 shows that p16 expression is upregulated in NR3A knockout mice. In this example, p16 was used as a hybridization probe to perform an in-situ hybridization. An

anti sense RNA probe was used for this in-situ hybridization. The probe sequence (SEQ ID No. 84) was produced from pCRII-TOPO included in TOPO-TA cloning kits (Invitrogen, Carlsbad, CA, Catalogue # KNM4500-40z). The difference in RNA levels between NR3A KO and WT was visually compared on the pictures taken from the brain tissue sections performed with in situ hybridization. The experiments for both NR3A KO and WT were performed under same conditions and at the same time. The pictures were taken under same exposusre conditions. Inventors have identified p16 as a gene whose expression is higher in NR3A knockout mice than WT mice using DNA microarray. The data shown here in figure 3 confirms that p16 expression is upregulated in the amygdala, cerebral cortex and hippocampus of NR3A KO mice compared to the NR3A wild type. These three areas (amygdala, cerebral cortex and hippocampus) are where NR3A expression normally occurs for WT mice.

Inventors' discovery of this novel genes, ORF and protein in the NMDAR molecular pathway presents a variety of uses, including, but not limited to: diagnosing the cause of disorders associated with NMDAR function; treating disorders associated with NMDAR function; and screening for novel agents that modulate the function of p16.

Screening of Complete Mouse Genome for p16 Loci

Inventors then examined the completed mouse genome sequence to identify sequences related to p16. Using the coding region (SEQ ID No.: 2) of the discovered p16 DNA sequence (SEQ ID No.: 1) as a query for BLASTN against the mouse genome found at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), Inventors discovered 40-60 different loci encoding putative proteins that are highly homologous to p16. Furthermore, a search of Genbank reveals multiple sequences highly related to p16. Some of these sequences are putative transcription units based on the genome sequence. Others are cDNAs isolated by the FANTOM Consortium and the RIKEN Genome Exploration Research. These cDNAs were isolated from embryonic whole body and various organs such as testis, ovary, uterus, mammary tumors and spinal cord. None of the cDNAs had been assigned functions prior to Inventors' current work.

To determine if any of these p16-related sequences are expressed in the mouse brain, RT-PCR was performed using whole brain of a wild-type C57BL6/J mouse (male, 6 week old). Briefly, total RNA was isolated from the mouse brain using RNA STAT-60 for the RNA extraction (TEL-TEST, INC., Friendswood, TX; Catalogue #CS-110) and

using RNeasy midi kit for RNA purification (Qiagen, Hilden, Germany; Catalogue #75144). cDNAs were synthesized using Superscript II RNase H.sup.- Reverse Transcriptase (Invitrogen Life Technologies, Carlsbad, CA, catalogue no.: 18064-022), and PCR was performed using PfuUltra High Fidelity DNA Polymerase (Stratagene, Inc., La Jolla, CA, catalogue no.: 600384). In order to amplify p16 and its related proteins, primer sequences were designed against the untranslated regions of the genomic and cDNA sequences that were deposited in Genbank. Four different 5' primers and one common 3' primer were used to set up four different PCR reactions. These four PCR reactions were further amplified using nested primers, again having four different 5' primers and one common 3' primer. Thus, reaction 1 used 5' primer CATCCCCAACACTCCTGTTC (SEQ ID No.: 72) and 3' primer GAGGAGCATACAGCCCATTAC (SEQ ID No.: 73), followed by 5' nested primer CTAGCTAGCAAGATGGGTGAGGAAAGTGG (SEQ ID No.: 74) and 3' nested primer CCGCTCGAGTGCACACTCAGTGCTCTTCC (SEQ ID No.: 75). Reaction 2 used 5' primer CAGCTGGAAGATAGCTTTTCTG (SEQ ID No.: 76) and 3' primer SEQ ID No.: 73, followed by 5' nested primer CTAGCTAGCTCCCTCCATCTTCTTCTTGG (SEQ ID No.: 77) and 3' nested primer SEQ ID No.: 75. Reaction 3 used 5' primer CCCCTCAAAAGCACATGAC (SEQ ID No.: 78) and 3' primer SEQ ID No.: 73, followed by 5' nested primer CTAGCTAGCGAAGGAGAGGTTGCCAAAGG (SEQ ID No.: 79) and 3' nested primer SEQ ID No.: 75. Reaction 4 used 5' primer ACTCGTCTCGCCACATGAAC (SEQ ID No.: 80) and 3' primer SEQ ID No.: 73, followed by 5' nested primer CTAGCTAGCTTCACAGAGATGTGAGATGGAG (SEQ ID No.: 81) and 3' nested primer SEQ ID No. 75.

In order to minimize the occurrence of mutations during the PCR, a DNA polymerase with proof-reading ability (PfuUltra – Stratagene, Inc.) was used and the number of PCR cycles was reduced. In addition, several lines of evidence suggest that most of these variations are authentic and were not introduced by PCR. First, variations occur at certain positions of the PCR products. Second, variations are reproducible from one PCR reaction to another. Third, most of these variations are present in genomic and Riken cDNA sequences that have been deposited to Genbank.

PCR products were cloned into pcDNA3.1/myc-His (Invitrogen, Corp., Carlsbad, CA, catalogue number V855-20) and the DNA sequences of the cloned products were

determined for both strands using a capillary ABI 3730 sequencer. DNA sequences were determined for 90 cDNA clones, 34 of which encoded different nucleotide sequences in their coding regions. All 34 deduced amino acid sequences are substantially similar to the amino acid sequence of SEQ ID No.: 3. These amino-acid sequences are presented in figure 4a, and are listed in the Sequence Listing below as Sequence ID Nos.: 4-37. In this figure 4a, the clone identification numbers are listed at the left, the sizes (number of amino acids) of the proteins are at the right. Note that the prototypical p16 starts at the position 56 in this alignment. In other words, there are multiple variants that contain as many as 55 amino acids at the N-termini. As mentioned above, there are 40-60 multiple loci encoding p16 and its related proteins in the mouse genome. Therefore, the multiplicity of these loci is likely a major contributor of the variations among p16 and its related proteins. Another likely source of the variations is alternative splicing, although it appears this occurs frequently via the usage of different acceptors (intronic GT sequences) from a single exon. As a result of these variations, the encoded p16 proteins differ in the following fashions: (1) at the N-terminal, the presence or absence of termination codons at the 5'-UTR creates variations in the starting ATG position; (2) multiple single amino-acid changes are present in the middle of the sequences, although many are conservative and may not alter protein functions; and (3) c-terminals vary in multiple forms, for example, many forms contain -SVI at the C-terminus, which is a motif that is likely to bind to a class I PDZ domain, while other forms contain C-terminal sequences (such as –SVK) that are unlikely to bind to a PDZ domain.

PDZ domains are regions of sequence homology found in diverse signaling proteins (Cho, K.O. et al. (1992) Neuron 9:929-942; Woods, D.F. and Bryant, P.J. (1993), Mech Dev 44:889, Kim, E. et al. (1995) Nature 378:85-88). The name "PDZ" derives from the first three proteins in which these domains were identified: PSD-95, a protein involved in signaling at the post-synaptic density; DLG, the Drosophila Discs Large protein; and ZO-1, the zonula occludens 1 protein. PDZ domains are also sometimes called DH domains or GLGF repeats.

These hypotheses were tested, and it has been determined that, indeed, some p16 variants bind to a PDZ-containing protein, while other variants do not (see discussion below).

Figure 4b shows the nucleotide sequences for the clones shown in figure 4a. Again, the clone identification is on the left; however, the number of nucleic acid residues is along the top. In figure 4b, the nucleotide sequences are again aligned for comparison. The nucleotide sequences are also listed in the Sequence Listing below as Sequence ID Nos.: 38-71.

Figure 5 is a schematic representation of many of the p16 variants identified in the current invention. The various forms of p16 are shown in modular form, having one or more of six modules. The module sizes are, from n-terminus to c-terminus, 55 aa, 17 aa, 72 aa, 42 aa, 19 aa and 22 aa. The prototypical p16 protein in the C57BL/6 mouse strain is PNN1131 (SEQ ID No.: 21), which has four modules. PNN1155 (SEQ ID No.: 9) is the longest variant having all six modules. Other p16 variants are also shown having different modules, which will vary in size, shape and, thus, interactions. These additional representative p16 variants are PNN1154 (SEQ ID No.: 4), PNN1159 (SEQ ID No.: 10); PNN1179 (SEQ ID No.: 33); PNN1143 (SEQ ID No.: 19); PNN1176 (SEQ ID No.: 13); PNN1101 (SEQ ID No.: 35); PNN1128 (SEQ ID No.: 36); and PNN1103 (SEQ ID No.: 34).

P16 is predicted to contain a coiled-coil domain, which is often used for self dimerization or oligomerization of proteins. To test if p16 dimerizes, oligomerizes or otherwise associates with a second p16 molecule, the following experiments were performed (see Figure 6). Prototypical p16 (SEQ ID No.: 21) was tagged with either myc or EGFP. The tagged p16 proteins were transfected separately into COS-7 cells or co-transfected into COS-7 cells. The p16-myc and p16-EGFP proteins were expressed within their respective cells, and following expression, the cells were lysed. Protein lysates were precipitated and the extracts were subjected to western immunoblot using anti-EGFP or anti-myc in a two-stage antibody detection reaction. In figure 6, lane 1 represents COS-7 transfected with p16-EGFP, lane 2 represents COS-7 transfected with p16-myc and lane 3 represents COS-7 transfected with both p16-EGFP and p16-myc. Also in figure 6, the top and middle panels represent an immunoblot of the cell lysates using anti-EGFP and anti-myc, respectively. In the bottom panel, the immunoprecipitates with anti-EGFP were blotted on the membrane and probed with anti-myc.

The top and middle panels of figure 6 show that p16-EGFP and p16-myc are expressed in COS-7 cells. Lane 3 of the bottom panel of Figure 6 shows that p16-myc is

co-immunoprecipitated with p16-EGFP. Both p16-EGFP and p16-myc stayed in the same complex during the procedure of immunoprecipitation. Before they were subjected to SDS-PAGE for the immunoblot, they were treated with SDS and mercaptoethanol for denaturation. By probing with anti-myc antibody on this blot, the monomerized p16-myc was visualized. The fact that p16-myc is present in the fraction precipitated by anti-GFP suggests p16-myc and p16-EGFP dimerizes, oligomerizes or otherwise associate in cells.

As discussed above, some p16 variants contain C-terminal sequences that are predicted to bind a class I PDZ domain, while other variants do not. PDZ domains are contained in proteins such as PSD-95, which is known to bind and regulate NMDAR-receptor subunit 2 (NR2). In Figure 7, the ability of p16 variants to bind PSD-95 was tested in co-immunoprecipitation experiments.

Six p16 variants were subjected to co-immunoprecipitation experiments with PSD-95. Briefly, six variants (p16-1 to p16-6) were cloned by RT-PCR as described above from NR3A KO mice whose genetic background is 129SV/J. Both DNA (SEQ ID Nos.: 85-90) and deduced-amino-acid sequences (SEQ ID Nos.: 91-96) of these clones are provided in the Sequence Listing, below. The sequences of p16 are slightly divergent from strain to strain, which is not surprising considering the unusual size of this gene family. COS-7 cells were then transfected with PDS-95 alone (lane 1 in figure 7), p16-EGFP variants alone (lanes 2-7 in figure 7), or the combination of PSD-95 and one of the p16-EGFP variants (Lanes 8-13 in figure 7). Based on antigenicity plots, it was determined to raise rabbit antisera against the following two peptides: N-terminal (2-20) TKKRSKINELEELKLDMRK (SEQ ID No.: 82) and C-terminal (123-141) CPVGKKRGSLRRPARTSVS (SEQ ID No.: 83). Antibodies against these peptides were predicted to recognize p16 and its structurally related proteins. The antibodies were produced by ABGENT (San Diego, CA). Briefly, the two peptides were synthesized and conjugated to keyhole limpet hematocyanin (KLH). Conjugated peptides were used to immunize two rabbits per peptide. Each rabbit was immunized 8 times with 100-200 mg antigen in the span of 10 weeks. Antisera against C-terminal and N-terminal peptides were named anti-p16N and p16C, respectively. These sera are useful for binding antibody against p16, which in turn is useful for a variety of purposes, including but not limited to immunoblotting and immunohistochemistry. For the immunoblot experiments presented in Figure 7, a mixture of anti-p16N and p16C sera was used and generally termed "anti-

p16". In the top panel of figure 7 the lysates were blotted with anti-p16 antibody in a two stage detection reaction to verify the expression of p16 in the transfected COS cells. In the lower panel of figure 7 the lysates were immunoprecipitated with anti PSD-95 antibody and then detected using a two stage antibody detection reaction, wherein the first stage was anti p16 and the second stage had a detectable label. Consistent with the predictions made by Scansite 2.0 program (available from Massachusetts Institute of Technology via their website at http://scansite.mit.edu/), the p16 variants that contain the C-terminal sequences such as –SVI (p16-2 (SEQ ID No.: 92)) and –VVL (p16-5 (SEQ ID No.: 95)) associate with PSD-95, while other p16 variants did not.

From these data, and without being held to any theory, Inventors have proposed the molecular mechanism presented in Figure 8. Briefly, p16 is upregulated in NR3A KO mice. The mechanism by which NR3A KO leads to the upregulation of p16 is possibly mediated by the increased Ca.sup.2+ permeability through the NMDA receptor. P16, in turn, dimerizes and binds to PSD-95 which is known to associate with NR2. These interactions underlie the mechanisms by which p16 upregulates NMDAR activity. The observation that p16 comes in many variant forms, some of which do not bind PSD-95 adds another layer of regulation diversity in the activity of this molecule.

Inventors have screened the human genome for a p16 homologue and have discovered that there is not a human homologue of p16. This is remarkable given the extensive expansion of p16 gene family in the rodents. It is possible that mouse and human sequences diverged quickly so that they no longer are homologous. It is also possible that p16 is unique to rodents (rats carry p16 orthologues) and mammals below human. Regardless, since NMDAR and its associated molecules such as PSD-95 are conserved between mouse and human, mouse p16 is still an effective reagent for regulating human NMDAR activity. In fact, the lack of endogenous p16 in human may account for increased efficiency of p16 in NMDAR regulation when applied to the human system, for example, through gene therapy techniques.

Endogenous p16

In the methods of the current invention, p16 can be endogenously and/or exogenously expressed in cells. Using NR3A knockout studies, endogenous expression of p16 was shown to occur in the hippocampus, in layer V of the cerebral cortex and in the

amygdala. Endogenous expression of p16 can be regulated using modulators (e.g., compounds that either directly or indirectly increase or reduce the expression of p16).

Exogenous p16

Exogenous expression of p16 is accomplished using techniques well known in the art. The invention provides an isolated nucleic acid molecule that encodes a functional fragment of a p16 polypeptide. For example, using knowledge of the rat or mouse p16-encoding nucleic acid sequences and polypeptides disclosed herein, those skilled in the art can readily clone p16-encoding nucleic acids from other mammalian or vertebrate species using conventional cDNA or expression library screening methods, or using the polymerase chain reaction (PCR). Additionally, using knowledge of the rat or mouse p16-encoding nucleic acid sequences and polypeptides disclosed herein, those skilled in the art can readily determine cDNA and coding sequences from other species from an analysis of ESTs and genomic sequences present in available databases.

Interference with p16 Expression

In addition to the effects a sequence mutation may have on the expression and/or function of p16, one may use a variety of other techniques well known in the art for disrupting p16 activity on the NMDAR, including, but not limited to siRNA, anti-sense RNA and ribozymes.

a. siRNA

Small interfering RNAs (siRNAs), which are short duplex RNAs with overhanging 3' ends, directed against p16 can also be effective in preventing or reducing p16 expression. Methods of preparing and using siRNAs are known in the art and described, for example, in Elbashir et al., Nature 411:494-498 (2001).

b. anti-sense

Antisense nucleotide sequences that are complementary to a nucleic acid molecule encoding a p16 polypeptide can be used to prevent or reduce p16 expression. Therefore, the method can be practiced with an antisense nucleic acid molecule complementary to at least a portion of the nucleotide sequence of p16. For example, the antisense nucleic acid molecule can be complementary to a region within the N-terminus of p16 such as within nucleotides 1-1000, 1-500, 1-100 or 1-18, and can optionally include sequences 5' to the start codon. Methods of preparing antisense nucleic acids molecules and using them

therapeutically are known in the art and described, for example, in Galderisi et al., J. Cell Physiol. 181:251-257 (1999).

c. **ribozyme**

Likewise, ribozymes that bind to and cleave p16 can also be effective in preventing or reducing p16 expression. Methods of preparing ribozymes and DNA encoding ribozymes, including hairpin and hammerhead ribozymes, and using them therapeutically are known in the art and described, for example, in Lewin et al., Trends Mol. Med. 7:221-228 (2001).

Screening Methods and Examples

Applicant's discovery of a novel pathway leading to NMDAR function is useful in a variety of methods for diagnosing and treating disorders and conditions relating to said pathway, and in screening for compounds that modulate said pathway.

The following non-limiting examples are useful in describing Applicant's discovery, and are in no way meant to limit the current invention. Those of ordinary skill in the art will readily adopt the underlying principles of applicant's discovery to design a variety of screening assays without departing from the spirit of the current invention.

Example One.

A first example shows a method wherein p16 modulators are discovered. Assay methods for identifying compounds (candidate compounds) that modulate p16 activity involve comparison to a control (modulators of p16 alter its biological activity, and can include, but are not limited to those that directly bind to the p16 protein, those that affect p16 gene expression and/or translation, and those that have an indirect effect on p16). For example, identical cells, both expressing p16, are plated in two separate tissue culture wells and one well is exposed to the candidate compound, while the control well is not exposed to the candidate compound. In this situation, the response of the test cell to a candidate compound is compared to the response (or lack of response) of the control cell to the same compound under substantially the same reaction conditions.

The effect of the candidate compound on the cell lines can be measure using a variety of techniques well known in the art. In the preferred embodiment, NMDAR channel current is measured to indicate the effect of a control compound. Techniques for measuring channel current, including but not limited to that described herein above are well known in the art.

Candidate compounds shown to have an effect on the channel current of NMDAR (e.g., modulators) are useful in treating the conditions associated with NMDAR dysregulation.

Example Two.

In a further assay, candidate compounds are screened for their ability to bind p16 (e.g., agonists, antagonists, ligands, etc). Assay methods for identifying compounds (candidate compounds) that bind to p16 involve comparison to a control. For example, identical cells, both expressing p16, are plated in two separate tissue culture wells and one well is exposed to the candidate compound, while the control well is not exposed to the candidate compound. In this situation, the response of the test cell to a candidate compound is compared to the response (or lack of response) of the control cell to the same compound under substantially the same reaction conditions.

The effect of the candidate compound on the cell lines can be measure using a variety of techniques well known in the art. In the current embodiment, ligand binding is measured to indicate the effect of a control compound. Techniques for measuring ligand binding, including but not limited to those described herein above are well known in the art.

Candidate compounds shown to bind p16 are useful in treating the conditions associated with NMDAR dysregulation.

Example Three

In a further example, treatments for the prevention and/or amelioration of conditions associated with inappropriate NMDAR activation, or inappropriate responses to glycine or glutamate are discussed.

Using the methods disclosed herein, it is possible to characterize and treat conditions associated with inappropriate NMDAR activation, or inappropriate responses to glycine or glutamate. For example, it is possible to isolate and sequence p16 from a sample belonging to one suffering from such conditions. It is further possible to screen for NMDA receptor subunits, including NR3A and knock-outs thereof. Nucleotide and/or protein sequence mutation are compared to the library of mutations and associated effects, described above. Alternatively, quantitative studies can be performed to uncover up or down regulation of p16 expression. Such studies are readily performed by those of skill in

the art using numerous well known techniques, including but not limited to RT-PCR, Northern Blot or Western Blot. The information is then used to determine a treatment.

Depending on the results from the sequencing studies, treatment options may include: gene therapy to introduce a functional wild-type p16; or the use of p16 antagonists or agonists, (which may be small molecules, nucleic acids, such as siRNA, anti-sense RNA or the like, proteins or other discovered modulators).

Those of ordinary skill in the art will uncover a number of treatments using the above disclosed invention. Such treatments are all within the spirit of the current invention.

P16 protein and conditions of NMDAR:

P16 is a cytoplasmic protein that causes excitation in cells expressing NMDAR. Upregulation of p16 expression, as is observed with the NR3A knockouts is knocked out, or when NMDAR otherwise loses its biological activity, causes NMDAR bearing cells to become hyperexcited, leading to a variety of conditions. Conditions in which inappropriate NMDAR activation, or inappropriate responses to glycine or glutamate, are implicated include, for example, acute neurologic condition, such as cerebral ischemia; stroke; hypoxia; anoxia; poisoning by carbon monoxide, manganese, cyanide or domoic acid; hypoglycemia; mechanical trauma to the nervous system such as trauma to the head or spinal cord; or epileptic seizure. Other conditions include, for example, chronic neurodegenerative disease, such as Huntington's disease; a disorder of photoreceptor degeneration such as retinitis pigmentosa; acquired immunodeficiency syndrome (AIDS) dementia complex (HIV-associated dementia); a neuropathic pain syndrome such as causalgia or a painful peripheral neuropathy; olivopontocerebellar atrophy; Parkinsonism; amyotrophic lateral sclerosis; a mitochondrial abnormality or other biochemical disorder such as MELAS syndrome, MERRF, Leber's disease, Wernicke's encephalopathy, Rett syndrome, homocysteinuria, hyperhomocysteinemia, hyperprolinemia, nonketotic hyperglycinemia, hydroxybutyric aminoaciduria, sulfite oxidase deficiency, combined systems disease, lead encephalopathy, Alzheimer's disease, hepatic encephalopathy, Tourette's syndrome, drug addiction/tolerance/dependency, glaucoma, depression, anxiety, multiple sclerosis and other demyelinating disorders. Other conditions are known in the art and reviewed, for example, in Lipton et al., New Engl. J. Med. 330:613-622 (1994) and Cull-Candy et al., Curr. Opin. Neurobiol. 11:327-335 (2001). Thus,

Applicant's current invention is useful in diagnosing and treating disorders and screening for modulating compounds relating to p16.

Pharmaceutical Compositions.

Methods of using the compounds and pharmaceutical compositions of the invention are also provided herein. The methods involve both *in vitro* and *in vivo* uses of the compounds and pharmaceutical compositions for altering preferred nuclear receptor activity, in a cell type specific fashion.

In certain embodiments, the claimed methods involve the discovery and use of modulating compounds including agonists, antagonists, ligands and nucleic acid molecules.

Once identified as a modulator using a method of the current invention, an agent can be put in a pharmaceutically acceptable formulation, such as those described in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990), incorporated by reference herein, to generate a pharmaceutical composition useful for specific treatment of diseases and pathological conditions.

Agents identified by the methods taught herein can be administered to a patient either by themselves or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of agent or agents such as these is administered. A therapeutically effective dose refers to that amount of the agent resulting in amelioration of symptoms or a prolongation of survival in a patient.

The agents also can be prepared as pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include, but are not limited to acid addition salts such as those containing hydrochloride, sulfate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*- toluenesulfonate, cyclohexylsulfamate and quinate. Such salts can be derived using acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid.

Pharmaceutically acceptable salts can be prepared by standard techniques. For example, the free base form of the agent is first dissolved in a suitable solvent such as an aqueous or aqueous-alcohol solution, containing the appropriate acid. The salt is then isolated by evaporating

the solution. In another example, the salt is prepared by reacting the free base and acid in an organic solvent.

Carriers or excipients can be used to facilitate administration of the agent, for example, to increase the solubility of the agent. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents.

For applications that require the compounds and compositions to cross the blood-brain barrier, or to cross the cell membrane, formulations that increase the lipophilicity of the compound are particularly desirable. For example, the compounds of the invention can be incorporated into liposomes (Gregoriadis, Liposome Technology, Vols. I to III, 2nd ed. (CRC Press, Boca Raton FL (1993)). Liposomes, which consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer. Additionally, the therapeutic compound can be conjugated to a peptide that facilitates cell entry, such as penetratin (also known as Antennapedia peptide), other homeodomain sequences, or the HIV protein Tat.

Toxicity and therapeutic efficacy of such agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Agents which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

For any agent identified by the methods taught herein, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test agent which achieves a half-maximal disruption of the protein complex, or a half-maximal inhibition of the cellular level and/or activity of a complex component). Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See *e.g.* Fingl *et al.*, in The Pharmacological Basis of Therapeutics, Ch. 1 p. 1 (1975)). It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may also be used in veterinary medicine.

Depending on the specific conditions being treated, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just to name a few.

For injection, the agents may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate the agents herein disclosed into dosages suitable for systemic administration is contemplated. With proper choice of carrier and suitable manufacturing practice, these agents, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The agents can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the agents of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be encapsulated into liposomes, and then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the context of the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active agents into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions contemplated by the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active agents in water-soluble form. Additionally, suspensions of the active agents may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the agents to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active agents with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or

sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active agent doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active agents may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

Some methods of delivery that may be used include:

a. encapsulation in liposomes,

b. transduction by retroviral vectors,

c. localization to nuclear compartment utilizing nuclear targeting site found on most nuclear proteins,

d. transfection of cells ex vivo with subsequent reimplantation or administration of the transfected cells,

e. a DNA transporter system.

Sequence Listing

Sequence

<212> Type : DNA

<211> Length : 1992

SequenceName : Gene Seq ID No.: 1

<213> OrganismName : mouse

<400> SEQ ID No.: 1:

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cccaacactc ctgttcagaa gatgggtgag gaaagtggaa agtctaacca gtcagccgat 120

gaccagtggg aaaaatgagc tacaagatca cctgatcttc atcagtgaga aagctttgca 180

caagagggtc tgctagaaat acttcaaccc aaaattccaa aatgaccaag aagagatcaa 240

aaataaatga actagaagaa ctgaaattgg atatgaggaa gatcagcaat gacatggagg 300

aaatgtgtgg aatcctgaac ctttacatgt atgaggattt gaactacagg atgaacactg 360

aattcaacat cattaaatca caacatgaga agacaatgtt ggatatgaat aaaatgatcc 420

agtccataat tggttccatg cagtactcca aggaactgat agaagataac tattcctaca 480

gcattaagga ggaccacctc ctccgtgagt gcactcaact caacgaaaac gtaaggatat 540

tactgaatga gaacagaagg ctgctggtgg agcaggctgg ccataagtgt cctgtgggga 600

agaaaagagg ttctgtgagg aggccagcaa gaacatctgt gtcccaagtg ccaaggaaca 660

gcagtgtgat atagtccagc agaaagcaga acatggcaca gaccacgaca tgatctccct 720

caaagagaag tgctggagga agagcactga gtgtgcacag gaaatacacc actgttgcct 780

ctcatcccta ataaccatgg ctgtaatggg ctgtatgctc ctcttttatt ttgtttcttt 840

ggtatgaaca ggccttaatt tcatctagcc tctggcccag gaagagtgca catttaaagg 900

gactcagaga aatgctgaga cacatcaaga gctgctgggc atccaggaag attctgagag 960

tttatattta tcttttcctg atgggtcatc atcaataatt acatggagat cagtcaacaa 1020

aattgtaaaa ccttggatcc aagtctacaa catgtgttct gctttgactt gggaggccat 1080

atccttcaga cccacactcc aaaaggagag tgttgcttaa atttctcctg caaagtttgt 1140

tacctccagg aactactttt ctactaagtt gccaaggaca gccacaggct gtaagtctgt 1200

gctacaaaat gagcagacta agaattttgc tttgcacaat ttttgtggtt tgattttggt 1260

ttgagttttg attagtttag ttatttgttt tttcttgttt tcattcaaag ttttgttatt 1320

tattggttat ttattgttct tttaattaat ttgatatttt gataaggtta tacacagtac 1380

atattgactg tcagctttca gttacaattg agtacattgc atttttttctt atgactaaca 1440

cagtgatctc caactcttca ctctaagagc cttgttattt cagttgtgat catgaaatcc 1500

cacagatatc agacccagat ggatctctgc actcttcatg ggacttgggc tccatagttt 1560
cttctgagcc ggacttaact acaaagtcct tcatacattc agtatggaga gtttttctaa 1620
ctgtctgtat aggaacttaa tgatggaaaa cttacccatg ctgcatcgtt gctgtcaaat 1680
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cagtatttct gtaatagctc tcattaaatc aaagcatagt ctaagggaat aaaaagctgt 1800
cagaaaacac agcagtgtat gcttctgcgt tccttcaaat atacaatcac tggtaattgc 1860
aagtggtttc tgtgggggtc cttcaatgtt cattttatta ctttatgatt cacctgtgtc 1920
tgccaaaaaa catcactcaa aaacaatgaa gattgtaatt aggtatcatc ctataaaatc 1980
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Sequence
<212> Type : DNA
<211> Length : 453
SequenceName : ORF Seq ID No.: 2
<213> OrganismName : Mouse
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aactacagga tgaacactga attcaacatc attaaatcac aacatgagaa gacaatgttg 180
gatatgaata aaatgatcca gtccataatt ggttccatgc agtactccaa ggaactgata 240
gaagataact attcctacag cattaaggag gaccacctcc tccgtgagtg cactcaactc 300
aacgaaaacg taaggatatt actgaatgag aacagaaggc tgctggtgga gcaggctggc 360
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tcccaagtgc caaggaacag cagtgtgata tag 453

Sequence
<212> Type : PRT
<211> Length : 150
SequenceName : p16 amino acid Seq ID No. 3
<213> OrganismName : mouse
<400> SEQ ID No.: 3:

MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL NENVRILLNE NRRLLVEQAG 120

HKCPVGKKRG SVRRPARTSV SQVPRNSSVI 150

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<212> Type : PRT

<211> Length : 205

SequenceName : PNN1154 aa SEQ ID No 4

<213> OrganismName : Mouse

<400> SEQ ID No.: 4:

MFSWLLRLFQ KENGDEGETR PTEKEEGILS HEKGRRKSFW RRHRSARNTS TQNSKMTKKR 60

SKINELEELK LDMRKISNDM EEMCGILNLY MYEDLNYRMN TEFNIIKSQH EKTMLDMNKM 120

IQSIIGSMQY SKELIEDNYS YSIKEDHLLR ECTQLNENVR ILLNENRRLL VEQAGHKCPV 180

GKKRGSLRKP ARTSVSQVPR NSSVK 205

Sequence

<212> Type : PRT

<211> Length : 205

SequenceName : PNN1152 aa SEQ ID No 5

<213> OrganismName : Mouse

<400> SEQ ID No.: 5:

MFSWLLRLFQ KENGDEGETR PTEKEEGILS HEKGRRKSFW RRHRSARNTS TQNSKMTKKR 60

SKINELEELK LDMRKISNDM EEMCGILNLY MYEDLNYRMN TEFNIIKSQH EKTMLDMNKM 120

IQSIIGSMQY SKELIEDNYS YSIKEDHLLR ECTQLNENVR ILLNENRRLL VEQAGYKCPV 180

GKKRGSLRRP ARTSVSQVPR NSSVK 205

Sequence

<212> Type : PRT

<211> Length : 205

SequenceName : PNN1157 aa SEQ ID No 6

<213> OrganismName : Mouse

<400> SEQ ID No.: 6:

MFSWLLRLFQ KENGDEGETR PTEKEEGILS HEKGRRKSFW RRHRSARNTS

TQNSKMTKKR 60

SKINELEELK LDMRKISNDM EEMCGILNLY MYEDLNYRMN TEFNIIKSQH

EKTMLDMNKM 120

IQSIIGSMQY SKELIEDNYS YSIKEDHLLR ECTQLNENVR ILLNENRRLL VEQAGHKCPV

180

GKKRGSLRRP ARTSVSQVPR NSSVI 205

Sequence

<212> Type : PRT

<211> Length : 205

SequenceName : PNN1170 aa SEQ ID No. 7

<213> OrganismName : Mouse

<400> SEQ ID No.: 7:

MFSWLLRLFQ KENGDEGETR PTEKEEGILS HEKGRRKSFW RRHRSARNTS

TQNSKMTKKR 60

SKINELEELK LDMRKISNDM EEMCGILNLY MYEDLNYRMN TEFNIIKSQH

EKTMLDMNKM 120

IQSIIGSMQY SKELIEDNYS YSIKEDHLLR ECTQLNENVR ILLNENRRLL VEQAGHKCPV

180

GKKRGSLRRP GSTSVSQVPR NSSVI 205

Sequence

<212> Type : PRT

<211> Length : 205

SequenceName : PNN1153 aa SEQ ID No. 8

<213> OrganismName : Mouse

<400> SEQ ID No.: 8:

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SKINELEELK LDMRKISNDM EEMCGILNLY MYEDLNYRMN TEFNIIKSQH EKTMLDMNKM 120

IQSIIGSMQY SKELIEDNYS YSIKEDHLLR ECTQLNENVR ILLNENRRLL VEQAGHKCPV 180

GKKRGSLRRP ARTSVSQVPR NSSVK 205

Sequence

<212> Type : PRT

<211> Length : 227

SequenceName : PNN1155 aa SEQ ID No 9

<213> OrganismName : Mouse

<400> SEQ ID No.: 9:

MFSWLLRLFQ KENGDEGETR PTEKEEGILS HEKGRRKSFW RRHRSARNTS TQNSKMTKKR 60

SKINELEELK LDMRKISNDM EEMCGILNLY MYEDLNYRMN TEFNIIKSQH EKTMLDMNKM 120

IQSIIGSMQY SMELIEDNYS YSIKEDHLLR ECTQLNENVR ILLNENRRLM VEQAGHKCPV 180

GKKRGSLRRP ARTSVSQVPR NSSPAESRTW HRPGNDLPQR EVLEEEH 227

Sequence

<212> Type : PRT

<211> Length : 171

SequenceName : PNN1159 aa SEQ ID No. 10

<213> OrganismName : Mouse

<400> SEQ ID No.: 10:

MFSWLLRLFQ KETGDEGETR PTEKEEGILS HEKGRRKSFW RRHRSARNTS
TQNSKMTKKR 60
SKINELEELK LDMRKISNDM EEMCGILNLY MYEDLNYRMN TEFNIIKSQH
EKTMLDMNKM 120
IQSIIGSMQY SKELIEDNYS YRALAGIIGL VGVASQWNLA GNHQFFFVDQ H 171

Sequence
<212> Type : PRT
<211> Length : 171
SequenceName : PNN1166 aa SEQ ID No. 11
<213> OrganismName : Mouse
<400> SEQ ID No.: 11:
MFSWLLRLFQ KENGDEGETR PTEKEEGILS HEKGRRKSFW RRHRSARNTS
TQNSKMTKKR 60
SKINELEELK LDMRKISNDM EEMCGILNLY MYEDLNYRMN TEFNIIKSQH
EKTMLDMNKM 120
IQSIIGSMQY SKELIEDNYS YRALAGIIGL VGVASQWNLA GNHQFFFVDQ H 171

Sequence
<212> Type : PRT
<211> Length : 156
SequenceName : PNN1137 aa SEQ ID No. 12
<213> OrganismName : Mouse
<400> SEQ ID No.: 12:
MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRRPARTSV SQVPRNSSPA ESRTWH 156

Sequence
<212> Type : PRT

<211> Length : 172

SequenceName : PNN1176 aa SEQ ID No. 13

<213> OrganismName : Mouse

<400> SEQ ID No.: 13:

MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNVYMYEDL NYRMNTEFNI IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE NRRLLVEQAG 120

HKCPVGKKRG SLRRPARTSV SQVPRNSSPA ESRTWHRPGH DLPQREVLEE EH 172

Sequence

<212> Type : PRT

<211> Length : 172

SequenceName : PNN1139 aa SEQ ID No. 14

<213> OrganismName : Mouse

<400> SEQ ID No.: 14:

MTKKRSKINE LEELKLDMRK ISNDIEEMCG ILNLYMYEDL NYRMNTEFNI IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE NRRLLVEQAG 120

HKCPVGKKRG SLRRPARTSV SQVPRNSSPA ESRTWHRPGH DLPQREVLEE EH 172

Sequence

<212> Type : PRT

<211> Length : 172

SequenceName : PNN1105 aa SEQ ID No 15

<213> OrganismName : Mouse

<400> SEQ ID No.: 15:

MTKKRSKINE LEELKLDMRK ISNDMEEMGG ILNLYMYEDL NYRMNTEFNI IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRDCTQL HENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRRPARTSV SQVPRNSSPA ESRTWHRPGH DLPQREVLEE EH
172

Sequence
<212> Type : PRT
<211> Length : 172
SequenceName : PNN1136 aa SEQ ID No. 16
<213> OrganismName : Mouse
<400> SEQ ID No.: 16:
MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL NENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SVRRPARTSV SQVPRNSSPA ESRTWHRPGH DLPQREVLEE EH
172

Sequence
<212> Type : PRT
<211> Length : 172
SequenceName : PNN1113 aa SEQ ID No. 17
<213> OrganismName : Mouse
<400> SEQ ID No.: 17:
MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRMPDRTSV SQVPRNSSPA ESRTWHRPGH DLPQREVLEE EH
172

Sequence

<212> Type : PRT

<211> Length : 172

SequenceName : PNN1168 aa SEQ ID No. 18

<213> OrganismName : Mouse

<400> SEQ ID No.: 18:

MTKKRSKRNE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE NRRLLVEQAG 120

HKCPVGKKRG SLRRPARTSV SQVPRNSSPA ESRTWHRPGH DLPQREVLEE EH 172

Sequence

<212> Type : PRT

<211> Length : 150

SequenceName : PNN1143 aa SEQ ID No. 19

<213> OrganismName : Mouse

<400> SEQ ID No.: 19:

MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL NEKVRILLNE NRRLLVEQAG 120

HKCPVGKKRG SLRKPARTSV SQVPRNSSVK 150

Sequence

<212> Type : PRT

<211> Length : 193

SequenceName : PNN1174 aa SEQ ID No. 20

<213> OrganismName : Mouse

<400> SEQ ID No.: 20:

MAMKERPDQQ RRKRESFLMK KEEGNHSGEG TGLLEILQPK IPKMTKKRSK INELEELKLD 60

MRKISNDMEE MCGILNLYMY EDLNYRMNTE FNIIKSQHEK TMLDMNKMIQ
SIIGSMQYSK 120
ELIEDNHSYS IKEDHLLREC TQLNENVRIL LNENRRLLVE QAGYKCPVGK
KRGSLRRPAR 180
TSVSQVPRNS SVK 193

Sequence
<212> Type : PRT
<211> Length : 150
SequenceName : PNN1131 aa SEQ ID No 21
<213> OrganismName : Mouse
<400> SEQ ID No.: 21:
MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL NENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRRPARTSV SQVPRNSSVI 150
Sequence
<212> Type : PRT
<211> Length : 150
SequenceName : PNN1149 aa SEQ ID No 22
<213> OrganismName : Mouse
<400> SEQ ID No.: 22:
MTKKRSKRNE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL NENVRILLNE
NRRLLVEQSG 120
HKCPVGKKRG SLRRPARTSV SQVPRNSSVI 150

Sequence
<212> Type : PRT
<211> Length : 148

SequenceName : PNN1132 aa SEQ ID No. 23

<213> OrganismName : Mouse

<400> SEQ ID No.: 23:

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IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL NENVRILLNE
NRRLLVEQAG 120

HKCPVGKKRG SLRRPARTSV SQVPRNSS 148

Sequence

<212> Type : PRT

<211> Length : 150

SequenceName : PNN1102 aa SEQ ID No. 24

<213> OrganismName : Mouse

<400> SEQ ID No.: 24:

MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE
NRRLLVEQAG 120

HKCPVGKKRG SLRRPARTSV PQVPRSSSVI 150

Sequence

<212> Type : PRT

<211> Length : 150

SequenceName : PNN1108 aa SEQ ID No. 25

<213> OrganismName : Mouse

<400> SEQ ID No.: 25:

MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60

DMNKMIQSII VSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE
NRRLLVDQAG 120

HKCPVGKKRG SLRRPARTSV SQVPRNTSVK 150

Sequence
<212> Type : PRT
<211> Length : 150
SequenceName : PNN1196 aa SEQ ID No. 26
<213> OrganismName : Mouse
<400> SEQ ID No.: 26:
MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRRPARTSV SQVPRNTSVK 150

Sequence
<212> Type : PRT
<211> Length : 150
SequenceName : PNN1118 aa SEQ ID No 27
<213> OrganismName : Mouse
<400> SEQ ID No.: 27:
MTKKRSKRNE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRRPARTSV SQVPRNTSVI 150

Sequence
<212> Type : PRT
<211> Length : 150
SequenceName : PNN1150 aa SEQ ID No. 28
<213> OrganismName : Mouse
<400> SEQ ID No.: 28:
MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRRPARTSV SQVPRNTSVI 150

Sequence
<212> Type : PRT
<211> Length : 150
SequenceName : PNN1183 aa SEQ ID No. 29
<213> OrganismName : Mouse
<400> SEQ ID No.: 29:
MTKKRSKRNE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRRPARTSV SQVPRNSSVK 150

Sequence
<212> Type : PRT
<211> Length : 148
SequenceName : PNN1130 aa SEQ ID No. 30
<213> OrganismName : Mouse
<400> SEQ ID No.: 30:
MTKKRSKRNE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL NENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRRPQEHLC PKCQGTAV 148

Sequence
<212> Type : PRT
<211> Length : 131
SequenceName : PNN1165 aa SEQ ID No. 31

<213> OrganismName : Mouse

<400> SEQ ID No.: 31:

MTKKRSKRNE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE
NRRLLVEQAG 120

HKCPVGKKKV L 131

Sequence

<212> Type : PRT

<211> Length : 131

SequenceName : PNN1104 aa SEQ ID No. 32

<213> OrganismName : Mouse

<400> SEQ ID No.: 32:

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IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE
NEGCWWSRLA 120

TSVLWGRKEV L 131

Sequence

<212> Type : PRT

<211> Length : 111

SequenceName : PNN1179 aa SEQ ID No. 33

<213> OrganismName : Mouse

<400> SEQ ID No.: 33:

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IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSPAE SRTWHRPGHD LPQREVLEEE H 111

Sequence

<212> Type : PRT

<211> Length : 146

SequenceName : PNN1103 aa SEQ ID No 34

<213> OrganismName : Mouse

<400> SEQ ID No.: 34:

MRKISNDMEE MGGILELYIY EDLNYRMNTE FNIIKSQHEK TMLDMNEMIQ SIIVSMQYSK 60

ELIEDNYSYS IKEDHLLREC TQLSEKVRIL LNENRKLLVE QAGTQLSHGE EKRFCEEASK 120

NICASSAKEQ QCVNSSRNRN MAQTTT 146

Sequence

<212> Type : PRT

<211> Length : 71

SequenceName : PNN1101 aa SEQ ID No. 35

<213> OrganismName : Mouse

<400> SEQ ID No.: 35:

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ELIEDNYSYS V 71

Sequence

<212> Type : PRT

<211> Length : 114

SequenceName : PNN1128 aa SEQ IN No. 36

<213> OrganismName : Mouse

<400> SEQ ID No.: 36:

MRKISNDMEE MCGILDLYIY EDLNYRMNTE FNIIKSQHEK TILDMNKMIQ SIIGSMQYSK 60

ELIEDNYSYS IKEDHLLREC TQLHENVRIL LNENRRLLVE QAGHKCPVGR KVVL 114

Sequence

<212> Type : PRT

<211> Length : 72

SequenceName : PNN1125 aa SEQ ID No. 37

<213> OrganismName : Mouse

<400> SEQ ID No.: 37:

MRKISNDMEE MCGILDLYMY EDLNYRMNTE FNIIKSQHEK TMLDMNKMIQ SIIGSMQYSK 60

ELIEDNYSYS VK 72

Sequence

<212> Type : DNA

<211> Length : 939

SequenceName : PNN1154 nt SEQ ID No. 38

<213> OrganismName : Mouse

<400> SEQ ID No.: 38:

ggatcccacg ggtccatgag aggctccagg agaggcaccc cagaaaagga ccaagctggg 60

cagaactagt taacaggtag gtcatggcag ttgctagtga catcatcagt aatgccttcc 120

ttggagaatc tcttgtatct aggatagaac tagaatcctc tgcgttgtca cctgtgttgt 180

ggtgacagca actgcctgtg gttcatccct tctgtttgct ctgggttcac cagcaggaat 240

gttttcctgg ctgctcaggc tatttcagaa agagaatggc gatgaaggag agaccagacc 300

aacagagaag gaagagggaa tcctttctca tgaaaaagga agaaggaaat cattctggag 360

aaggcacagg tctgctagaa atacttcaac ccaaaattcc aaaatgacta agaagagatc 420

aaaaataaat gaactagaag aactgaaatt ggatatgagg aagatcagca atgacatgga 480

ggaaatgtgt ggaatcctga acctttacat gtatgaggat ttgaactaca ggatgaacac 540

tgaattcaac atcattaaat cacaacatga gaagacaatg ttggatatga ataaaatgat 600

ccagtccata attggttcca tgcagtactc caaggaactg atagaagata actattccta 660

cagcattaag gaggaccacc tcctccgtga gtgcactcaa ctcaacgaaa acgtaaggat 720

attactgaat gagaacagaa ggctgctggt ggagcaggct ggccataagt gtcctgtggg 780

gaagaaaaga ggttctctga ggaagccagc aagaacatct gtgtcccaag tgccaaggaa 840

cagcagtgtg aaatagtcca gcagaaagca gaacacggca cagaccacga catgatctcc 900

ctcaaagaga agtgctggag gaagagcact gagtgtgca 939

Sequence

<212> Type : DNA

<211> Length : 939

SequenceName : PNN1152 nt SEQ ID No. 39

<213> OrganismName : Mouse

<400> SEQ ID No.: 39:

ggatcccacg ggtccatgag aggctccagg agaggcaccc cagaaaagga ccaagctggg 60

cagaactagt taacaggtag gtcatggcag ttgctagtga catcatcagt aatgccttcc 120

ttggagaatc tcttgtatct aggataaaac tagaatcctc tgcgttgtca cctgtgttgt 180

ggtgacagca actgcctgtg gttcatccct tctgtttgct ctgggttcac cagcaggaat 240

gttttcctgg ctgctcaggc tatttcagaa agagaatggc gatgaaggag agaccagacc 300

aacagagaag gaagagggaa tcctttctca tgaaaaagga agaaggaaat cattctggag 360

aaggcacagg tctgctagaa atacttcaac ccaaaattcc aaaatgacta agaagagatc 420

aaaaataaat gaactagaag aactgaaatt ggatatgagg aagatcagca atgacatgga 480

ggaaatgtgt ggaatcctga acctttacat gtatgaggat ttgaactaca ggatgaacac 540

tgaattcaac atcattaaat cacaacatga gaagacaatg ttggatatga ataaaatgat 600

ccagtccata attggttcca tgcagtactc caaggaactg atagaagata actattccta 660

cagcattaag gaggaccacc tcctccgtga gtgcactcaa ctcaacgaaa acgtaaggat 720

attactgaat gagaacagaa ggctgctggt ggagcaggct ggctataagt gtcctgtggg 780

gaagaaaaga ggttctctga ggaggccagc aagaacatct gtgtcccaag tgccaaggaa 840

cagcagtgtg aaatagtcca gctgaaagca gaacatggca cagaccagga catgatctcc 900

ctcaaagaga agtgctggag gaagagcact gagtgtgca 939

Sequence

<212> Type : DNA

<211> Length : 939

SequenceName : PNN1157 nt SEQ ID No. 40

<213> OrganismName : Mouse

<400> SEQ ID No.: 40:

ggatcccacg ggtccatgag aggctccagg agaggcaccc cagaaaagga ccaagctggg 60

cagaactagt taacaggtag gtcatggcag ttgctagtga catcatcagt aatgccttcc 120

ttggagaatc tcttgtatct aggatagaac tagaatcttc tgtgttgtca cctgtgttgt 180

ggtgacagca actgcctgtg gttcatccct tctgtttgct ctgggttcac cagcaggaat 240
gttttcctgg ctgctcaggc tatttcagaa agagaatggc gatgaaggag agaccagacc 300
aacagagaag gaagagggaa tcctttctca tgaaaaagga agaaggaaat cattctggag 360
aaggcacagg tctgctagaa atacttcaac ccaaaattcc aaaatgacca agaagagatc 420
aaaaataaat gaactagaag aactgaaatt ggatatgagg aagatcagca atgacatgga 480
ggaaatgtgt ggaatcctga acctttacat gtatgaggat ttgaactaca ggatgaacac 540
tgaattcaac atcattaaat cacaacatga gaagacaatg ttggatatga ataaaatgat 600
ccagtccata attggttcca tgcagtactc caaggaactg atagaagata actattccta 660
cagcattaag gaggaccacc tcctccgtga gtgcactcaa ctcaacgaaa acgtaaggat 720
attactgaat gagaacagaa ggctgctggt ggagcaggct ggccataagt gtcctgtggg 780
gaagaaaaga ggttctctga ggaggccagc aagaacatct gtgtcccaag tgccaaggaa 840
cagcagtgtg atatagtcca gcagaaagca gaacatggca cagaccacga catgatctcc 900
ctcaaagaga agtgctggag gaagagcact gagtgtgca 939

Sequence
<212> Type : DNA
<211> Length : 939
SequenceName : PNN1170 nt SEQ ID No. 41
<213> OrganismName : Mouse
<400> SEQ ID No.: 41:
ggatcccacg ggtccatgag aggctccagg agaggcaccc cagaaaagga ccaagctggg 60
cagaactagt taacaggtag gtcatggcag ttgctagtga catcatcagt aatgccttcc 120
ttggagaatc tcttgtatct aggatagaac tagaatcctc tgtgttgtca cctgtgttgt 180
ggtgacagca actgcctgtg gttcatccct tctgtttgct ctgggttcac cagcaggaat 240
gttttcctgg ctgctcaggc tatttcagaa agagaatggc gatgaaggag agaccagacc 300
aacagagaag gaagagggaa tcctttctca tgaaaaagga agaaggaaat cattctggag 360
aaggcacagg tctgctagaa atacttcaac ccaaaattcc aaaatgacta agaagagatc 420
aaaaataaat gaactagaag aactgaaatt ggatatgagg aagatcagca atgacatgga 480
ggaaatgtgt ggaatcctga acctttacat gtatgaggat ttgaactaca ggatgaacac 540
tgaattcaac atcattaaat cacaacatga gaagacaatg ttggatatga ataaaatgat 600
ccagtccata attggttcca tgcagtattc caaggaactg atagaagata actattccta 660
cagcattaag gaggaccacc tcctccgtga gtgcactcaa ctcaacgaaa acgtaaggat 720

attactgaat gagaacagaa ggctgctggt ggagcaggct ggccataagt gtcctgtggg 780
gaagaaaaga ggttctctga ggaggccagg aagtacatct gtgtcccaag tgccaaggaa 840
cagcagtgtg atatagtcca gcagaaagca gaacatggca cagaccacgg catgatctcc 900
ctcaaagaga agtgctggag gaagagcact gagtgtgca 939

Sequence
<212> Type : DNA
<211> Length : 939
SequenceName : PNN1153 nt SEQ ID No. 42
<213> OrganismName : Mouse
<400> SEQ ID No.: 42:
ggatcacacg ggtccatgag aagctccagg agaggcaccc cagaaaggga ccaagctggg 60
cagaactagt taacaggtag gtcatggcag ttgctagtga catcatcagt aatgccttcc 120
ttggagaatc tcttgtatct aggatagaac tagaatcctc tgcgttgtca cctgtgttgt 180
ggtgacagca actgcctgtg gttcatccct tctgtttgct ctgggttcac cagcaggaat 240
gttttcctgg ctgctcaggc tatttcagaa agagactggc gatgaaggag agaccagacc 300
aacagagaag gaagagggaa tcctttctca tgaaaaagga agaaggaaat cattctggag 360
aaggcacagg tctgctagaa atacttcaac ccaaaattcc aaaatgacca agaagagatc 420
aaaaataaat gaactagaag aactgaaatt ggatatgagg aagatcagca atgacatgga 480
ggaaatgtgt ggaatcctga acctttacat gtatgaggat ttgaactaca ggatgaacac 540
tgaattcaac atcattaaat cacaacatga gaagacaatg ttggatatga ataaaatgat 600
ccagtccata attggttcca tgcagtactc caaggaactg atagaagata actattccta 660
cagcattaag gaggaccacc tcctccgtga gtgcactcaa ctcaacgaaa acgtaaggat 720
attactgaat gagaacagaa ggctgctggt ggagcaggct ggccataagt gtcctgtggg 780
gaagaaaaga ggttctctga ggaggccagc aagaacatct gtgtcccaag tgccaaggaa 840
cagcagtgtg aaatagtcca acagaaagca gaacatggca cagaccacga catgatctcc 900
ctcaaagaga agtgctggag gaagagcact gagtgtgca 939

Sequence
<212> Type : DNA
<211> Length : 769
SequenceName : PNN1155 nt SEQ ID No. 43

<213> OrganismName : Mouse

<400> SEQ ID No.: 43:

ggatcccacg ggtccatgag aggctccagg agaggcaccc cagaaaagga ccaagctgga 60

cagaactagt taacaggaat gttttcctgg ctgctcaggc tatttcagaa agagaatggc 120

gatgaaggag agaccagacc aacagagaag gaagagggaa tcctttctca tgaaaaagga 180

agaaggaaat cattctggag aaggcacagg tctgctagaa atacttcaac ccaaaattcc 240

aaaatgacta agaagagatc aaaaataaat gaactagaag aactgaaatt ggatatgagg 300

aagatcagca atgacatgga ggaaatgtgt ggaatcctga acctttacat gtatgaggat 360

ttgaactaca ggatgaacac tgaattcaac atcattaaat cacaacatga gaagacaatg 420

ttggatatga ataaaatgat ccagtccata attggttcca tgcagtactc catggaactg 480

atagaagata actattccta cagcattaag gaggaccacc tcctccgtga gtgcactcaa 540

ctcaacgaaa acgtaaggat attactgaat gagaacagaa ggctgatggt ggagcaggct 600

ggccataagt gtcctgtggg gaagaaaaga ggttctctga ggaggccagc aagaacatct 660

gtgtcccaag tgccaaggaa cagcagtcca gcagaaagca gaacatggca cagaccagga 720

aatgatctcc ctcaaagaga agtgctggag gaagagcact gagtgtgca 769

Sequence

<212> Type : DNA

<211> Length : 988

SequenceName : PNN1159 nt SEQ ID No. 44

<213> OrganismName : Mouse

<400> SEQ ID No.: 44:

ggatcacacg ggtccatgag aagctccagg agaggcaccc cagaaaagga ccaagctggg 60

cagaactagt taacaggtag gtcatggcag ttgctagtga catcatcagt aatgccttcc 120

ttggagaatc tcttgtatct aggatagaac tagaatcctc tgcgttgtca cctgtgttgt 180

ggtgacagca actgcctgtg gttcatccct tctgtttgct ctgggttcac cagcaggaat 240

gttttcctgg ctgctcaggc tatttcagaa agagactggc gatgaaggag agaccagacc 300

aacagagaag gaagagggaa tcctttctca tgaaaaagga agaaggaaat cattctggag 360

aaggcacagg tctgctagaa atacttcaac ccaaaattcc aaaatgacca agaagagatc 420

aaaaataaat gaactagaag aactgaaatt ggatatgagg aagatcagca atgacatgga 480

ggaaatgtgt ggaatcctga acctttacat gtatgaggat ttgaactaca ggatgaacac 540

tgaattcaac atcattaaat cacaacatga gaagacaatg ttggatatga ataaaatgat 600

ccagtccata attggttcca tgcagtactc caaggaactg atagaagata actattccta 660
cagggccctt gcagggatca taggacttgt aggagtggca agccaatgga atctggctgg 720
gaatcaccaa tttttctttg tggatcagca ttaaggagga ccacctcctc cgtgagtgca 780
ctcaactcaa cgaaaacgta aggatattac tgaatgagaa cagaaggctg ctggtggagc 840
aggctggcca taagtgtcct gtggggaaga aaagaggttc tctgaggagg ccagcaagaa 900
catctgtgtc ccaagtgcca aggaacagca gtgtgaaata gtccaacaga aagcagaaca 960
tggcacagac cacgacatga tctccctc 988

Sequence
<212> Type : DNA
<211> Length : 867
SequenceName : PNN1166 nt SEQ ID No. 45
<213> OrganismName : Mouse
<400> SEQ ID No.: 45:
ggatcccacg ggtccatgag aggctccagg agaggcaccc cagaaaagga ccaagctggg 60
cagaactagt taacagcagg aatgttttcc tggctgctca ggctatttca gaaagagaat 120
ggcgatgaag gagagaccag accaacagag aaggaagagg gaatcctttc tcatgaaaaa 180
ggaagaagga aatcattctg gagaaggcac aggtctgcta gaaatacttc aacccaaaat 240
tccaaaatga ctaagaagag atcaaaaata aatgaactag aagaactgaa attggatatg 300
aggaagatca gcaatgacat ggaggaaatg tgtggaatcc tgaaccttta catgtatgag 360
gatttgaact acaggatgaa cactgaattc aacatcatta aatcacaaca tgagaagaca 420
atgttggata tgaataaaat gatccagtcc ataattggtt ccatgcagta ttccaaggaa 480
ctgatagaag ataactattc ctacagggcc cttgcaggga tcataggact tgtacgagtg 540
gcaagccaat ggaatctggc tgggaatcac caatttttct ttgtggatca gcattaagga 600
ggaccacctc ctccgtgagt gcactcaact caacgaaaac gtaaggatat tactgaatga 660
gaacagaagg ctgctggtgg agcaggctgg ccataagtgt cctgtgggga agaaaagagg 720
ttctctgagg aggccaggaa gtacatctgt gtcccaagtg ccaaggaaca gcagtgtgat 780
atagtccagc agaaagcaga acatggcaca gaccacggca tgatctccct caaagagaag 840
tgctggagga agagcactga gtgtgca 867

Sequence
<212> Type : DNA

<211> Length : 631

SequenceName : PNN1137 nt SEQ ID No. 46

<213> OrganismName : Mouse

<400> SEQ ID No.: 46:

gactcagaat ggtctcccct gcccctgggc catcaagtat gcaaattctt ttgtgtttac 60

ctatctgcag ggtctgctag aaatacttca acccaaaatt ccaaaatgac taagaagaga 120

tcaaaaataa atgaactaga agaactgaaa ttggatatga ggaagatcag caatgacatg 180

gaggaaatgt gtggaatcct gaacctttac atgtatgagg atttgaacta caggatgaac 240

actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat gaataaaatg 300

atccagtcaa taattggttc catgcagtac tctaaggaac tgatagaaga taactattcc 360

tacagcatta aggaggacca cctcctccgt gagtgcactc aactccacga aaacgtaagg 420

atattactga atgagaacag aaggctgctg gtggagcagg ctggccacaa gtgtcctgtg 480

gggaagaaaa gaggttctct gaggaggcca gcaagaacat ctgtgtccca agtgccaagg 540

aacagcagtc cagcagaaag cagaacatgg cactgaccac gacatgatct ccctcaaaga 600

gaagtgctgg aggaagagca ctgagtgtgc a 631

Sequence

<212> Type : DNA

<211> Length : 756

SequenceName : PNN1176 nt SEQ ID No. 47

<213> OrganismName : Mouse

<400> SEQ ID No.: 47:

caggagcagt ggctaaatgt tgaactcttc aaaacttggc gtatgggctg ctggtgtacc 60

accatcatcc ccaacactcc tgttcagaag atgggtgagg aaagtggaaa gtctaaccag 120

tcagccgatg accagtggga aaaatgagct acaagatcac ctgatcttca tcagtgagaa 180

agctttgcac aagagggtct gctagaaata cttcaaccca aaattccaaa atgactaaga 240

agagatcaaa aataaatgaa ctagaagaac tgaaattgga tatgaggaag atcagcaatg 300

acatggagga aatgtgtgga atcctgaacg tttacatgta tgaggatttg aactacagga 360

tgaacactga attcaacatc attaaatcac aacatgagaa gacaatgttg gatatgaata 420

aaatgatcca gtccataatt ggttccatgc agtactccaa ggaactgata gaagataact 480

attcctacag cattaaggag gaccacctcc tccgtgagtg cactcaactc cacgaaaacg 540

taaggatatt actgaatgag aacagaaggc tgctggtgga gcaggctggc cacaagtgtc 600

ctgtggggaa gaaaagaggt tctctgagga ggccagcaag aacatctgtg tcccaagtgc 660
caaggaacag cagtccagca gaaagcagaa catggcacag accaggacat gatctccctc 720
aaagagaagt gctggaggaa gagcactgag tgtgca 756

Sequence
<212> Type : DNA
<211> Length : 631
SequenceName : PNN1139 nt SEQ ID No. 48
<213> OrganismName : Mouse
<400> SEQ ID No.: 48:
gactcagaat ggtctcccct gcccctgggc catcaagtat gcaaattctt ttgtgtttac 60
ctatctgcag ggtctgctag aaatacttca acccaaaatt ccaaaatgac taagaagaga 120
tcaaaaataa atgaactaga agaactgaaa ttggatatga ggaagatcag caatgacatt 180
gaggaaatgt gtggaatcct aaacctttac atgtatgagg atttgaacta caggatgaac 240
actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat gaataaaatg 300
atccagtcaa taattggttc catgcagtac tctaaggaac tgatagaaga taactattcc 360
tacagcatta aggaggacca cctcctccgt gagtgcactc aactccacga aaacgtaagg 420
atattactga atgagaacag aaggctgctg gtggagcagg ctggccacaa gtgtcctgtg 480
gggaagaaaa gaggttctct gaggaggcca gcaagaacat ctgtgtccca agtgccaagg 540
aacagcagtc cagcagaaag cagaacatgg cacagaccag gacatgatct ccctcaaaga 600
gaagtgctgg aggaagagca ctgagtgtgc a 631

Sequence
<212> Type : DNA
<211> Length : 648
SequenceName : PNN1105 nt SEQ ID No. 49
<213> OrganismName : Mouse
<400> SEQ ID No.: 49:
aagcctaacc actcagccga tgacaagtgg gaaaaatgcg ctacaagatc acctgatatt 60
catcagtgag aaagctttgc acaagagggt ctgctagaaa tacttcaacc caaaattcca 120
aaatgactaa gaagagatca aaaataaatg aactagaaga actgaaattg gatatgagga 180
agatcagcaa tgacatggag gaaatgggtg gaatcctgaa cctttacatg tatgaggatt 240

tgaactacag gatgaacact gaattcaaca tcattaaatc acaacatgag aagacaatgt 300
tggatatgaa taaaatgatc cagtcaataa ttggttccat gcagtactct aaggaactga 360
tagaagataa ctattcctac agcattaagg aggaccacct cctccgtgac tgcactcaac 420
tccacgaaaa cgtaaggata ttactgaatg agaacagaag gctgctggtg gagcaggctg 480
gccacaagtg tcctgtgggg aagaaaagag gttctctgag gaggccagca agaacatctg 540
tgtcccaagt gccaaggaac agcagtccag cagaaagcag aacatggcac agaccaggac 600
atgatctccc tcaaagagaa gtgctggagg aagagcactg agtgtgca 648

Sequence
<212> Type : DNA
<211> Length : 631
SequenceName : PNN1136 nt SEQ ID No. 50
<213> OrganismName : Mouse
<400> SEQ ID No.: 50:
gactcagaat ggtctcccct gcccctgggc catcaagtat gcaaattctt ttgtgtttac 60
ctatctgcag ggtctgctag aaatacttca acccaaaatt ccaaaatgac taagaagaga 120
tcaaaaataa atgaactaga agaactgaaa ttggatatga ggaagatcag caatgacatg 180
gaggaaatgt gtggaatcct gaacctttac atgtatgagg atttgaacta caggatgaac 240
actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat gaataaaatg 300
atccagtcca taattggttc catgcagtac tccaaggaac tgatagaaga taactattcc 360
tacagcatta aggaggacca cctcctccgt gagtgcactc aactcaacga aaacgtaagg 420
atattactga atgagaacag aaggctgctg gtggagcagg ctggccataa gtgtcctgtg 480
gggaagaaaa gaggttctgt gaggaggcca gcaagaacat ctgtgtccca agtgccaagg 540
aacagcagtc cagcagaaag cagaacatgg cacagaccag gacatgatct ccctcaaaga 600
gaagtgctgg aggaagagca ctgagtgtgc a 631

Sequence
<212> Type : DNA
<211> Length : 648
SequenceName : PNN1113 nt SEQ ID No. 51
<213> OrganismName : Mouse
<400> SEQ ID No.: 51:

aagtctaacc agtcagccga tgaccagtgg gaacaatgag ctacaagatc acctgatctt 60
catcagtgag aaagctttgc acaagagggt ctgctagaaa tacttcaacc caaaattcca 120
aaatgactaa gaagagatca aaaataaatg aactagaaga actgaaattg gatatgagga 180
agatcagcaa tgacatggag gaaatgtgtg gaatcctgaa cctttacatg tatgaggatt 240
tgaactacag gatgaacact gaattcaaca tcattaaatc acaacatgag aagacaatgt 300
tggatatgaa taaaatgatc cagtccataa ttggttccat gcagtactcc aaggaactga 360
tagaagataa ctattcctac agcattaagg aggaccacct cctccgtgag tgcactcaac 420
tccacgaaaa cgtaaggata ttactgaatg agaacagaag gctgctggtg gagcaggctg 480
gccacaagtg tcctgtgggg aagaaaagag gttctctgag gatgccagat agaacatctg 540
tgtcccaagt gccaaggaac agcagtccag cagaaagcag aacatggcac agaccaggac 600
atgatctccc tcaaagagaa gtgctggagg aagagcactg agtgtgca 648

Sequence
<212> Type : DNA
<211> Length : 637
SequenceName : PNN1168 nt SEQ ID No. 52
<213> OrganismName : Mouse
<400> SEQ ID No.: 52:
ggatcacacg ggtccatgag aggctccagg agaggcaccc cagaaaagga ccaagctggg 60
cagaactagt taacagggtc tgctagaaat acttcaaccc aaaattccaa aatgactaag 120
aagagatcaa aaagaaatga actagaagaa ctgaaattgg atatgaggaa gatcagcaat 180
gacatggagg aaatgtgtgg aatcctgaac ctttacatgt atgaggattt gaactacagg 240
atgaacactg aattcaacat cattaaatca caacatgaga agacaatgtt ggatatgaat 300
aaaatgatcc agtccataat tggttccatg cagtattcca aggaactgat agaagataac 360
tattcctaca gcattaagga ggaccacctc ctccgtgagt gcactcaact ccacgaaaat 420
gtaaggatat tactgaatga gaacagaagg ctgctggtgg agcaggctgg ccacaagtgt 480
cctgtgggga agaaaagagg ttctctgagg aggccagcaa gaacatctgt gtcccaagtc 540
ccaaggaaca gcagtccagc agaaagcaga acatggcaca gaccaggaca tgatctccct 600
caaagagaag tgctggagga agagcactga gtgtgca 637

Sequence
<212> Type : DNA

<211> Length : 641

SequenceName : PNN1143 nt SEQ ID No. 53

<213> OrganismName : Mouse

<400> SEQ ID No.: 53:

gactcagaat ggtctcccct gcccctgggc cttcaagtat gcaaattctt ttgtgtttac 60

ctatctgcag ggtctgctag aaatacttca acccaaaatt ccaaaatgac taagaagaga 120

tcaaaaataa atgaactaga agaactgaaa ttggatatga ggaagatcag caatgacatg 180

gaggaaatgt gtggaatcct gaacctttac atgtatgagg atttgaacta caggatgaac 240

actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat gaataaaatg 300

atccagtcca taattggttc catgcagtac tccaaggaac tgatagaaga taactattcc 360

tacagcatta aggaggacca cctcctccgt gagtgcactc aactcaacga aaaagtaagg 420

atattactga atgagaacag aaggctgctg gtggagcagg ctggccataa gtgtcctgtg 480

gggaagaaaa gaggttctct gaggaagcca gcaagaacat ctgtgtccca agtgccaagg 540

aacagcagtg tgaaatagtc cagcagaaag cagaacatgg cactgaccac gacatgatct 600

ccctcaaaga gaagtgctgg aggaagagca ctgagtgtgc a 641

Sequence

<212> Type : DNA

<211> Length : 940

SequenceName : PNN1174 nt SEQ ID No. 54

<213> OrganismName : Mouse

<400> SEQ ID No.: 54:

ggatcccacg ggtccatgag aggctccagg agaggcaccc cagaaaagga ccaagctggg 60

cagaactagt taacaggtag gtcatggcag ttgctagtga catcatcagt aatgccttcc 120

ttggagaatc tcttgtatct aggataaaac tagaatcctc tgcgttgtca cctgtgttgt 180

ggtgacagca actgcctgtg gttcatccct tctgtttgct ctgggttcac cagcaggaat 240

gttttcctgg ctgctcaggc tatttcagaa agagaatggc gatgaaggag agaccagacc 300

aacagagaag gaagagggaa tcctttctca tgaaaaagga agaaggaaat cattctggag 360

aaggcacagg tctgctagaa atacttcaac ccaaaattcc aaaaatgact aagaagagat 420

caaaaataaa tgaactagaa gaactgaaat tggatatgag gaagatcagc aatgacatgg 480

aggaaatgtg tggaatcctg aacctttaca tgtatgagga tttgaactac aggatgaaca 540

ctgaattcaa catcattaaa tcacaacatg agaagacaat gttggatatg aataaaatga 600

tccagtccat aattggttcc atgcagtact ccaaggaact gatagaagat aaccattcct 660
acagcattaa ggaggaccac ctcctccgtg agtgcactca actcaacgaa aacgtaagga 720
tattactgaa tgagaacaga aggctgctgg tggagcaggc tggctataag tgtcctgtgg 780
ggaagaaaag aggttctctg aggaggccag caagaacatc tgtgtcccaa gtgccaagga 840
acagcagtgt gaaatagtcc agctgaaagc agaacatggc acagaccagg acatgatctc 900
cctcaaagag aagtgctgga ggaagagcac tgagtgtgca 940

Sequence
<212> Type : DNA
<211> Length : 641
SequenceName : PNN1131 nt SEQ ID No. 55
<213> OrganismName : Mouse
<400> SEQ ID No.: 55:
gactcagaat ggtctcccct gcccctgggc catcaagtat gcaaattctt ttgtgtttac 60
ctatctgcag ggtctgctag aaatacttca acccaaaatt ccaaaatgac caagaagaga 120
tcaaaaataa atgaactaga agaactgaaa ttggatatga ggaagatcag caatgacatg 180
gaggaaatgt gtggaatcct gaacctttac atgtatgagg atttgaacta caggatgaac 240
actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat gaataaaatg 300
atccagtcca taattggttc catgcagtac tccaaggaac tgatagaaga taactattcc 360
tacagcatta aggaggacca cctcctccgt gagtgcactc aactcaacga aaacgtaagg 420
atattactga atgagaacag aaggctgctg gtggagcagg ctggccataa gtgtcctgtg 480
gggaagaaaa gaggttctct gaggaggcca gcaagaacat ctgtgtccca agtgccaagg 540
aacagcagtg tgatatagtc cagcagaaag cagaacatgg cacagaccac gacatgatct 600
ccctcaaaga gaagtgctgg aggaagagca ctgagtgtgc a 641

Sequence
<212> Type : DNA
<211> Length : 641
SequenceName : PNN1149 nt SEQ ID No. 56
<213> OrganismName : Mouse
<400> SEQ ID No.: 56:
gactcagaat ggtctcccct gcccctgggc catcaagtat gcaaattctt ttgtgtttac 60

ctatctgcag ggtctgctag aaatacttca acccaaaatt ccaaaatgac taagaagaga 120
tcaaaaagaa atgaactaga agaactgaaa ttggatatga ggaagatcag caatgacatg 180
gaggaaatgt gtggaatcct gaacctttac atgtatgagg atttgaacta caggatgaac 240
actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat gaataaaatg 300
atccagtcca taattggttc catgcagtac tccaaggaac tgatagaaga taactattcc 360
tacagcatta aggaggacca cctcctccgt gagtgcactc aactcaacga aaacgtaagg 420
atattactga atgagaacag aaggctgctg gtggagcagt ctggccataa gtgtcctgtg 480
gggaagaaaa gaggttctct gaggaggcca gcaagaacat ctgtgtccca agtgccaagg 540
aacagcagtg tgatatagtc cagcagaaag cagaacatgg cacagaccac gacatgatct 600
ccctcaaaga gaagtgctgg aggaagagca ctgagtgtgc a 641

Sequence
<212> Type : DNA
<211> Length : 646
SequenceName : PNN1132 nt SEQ ID No. 57
<213> OrganismName : Mouse
<400> SEQ ID No.: 57:
gactcagaat ggtctcccct gcccctgggc catcaagtat gcaaattctt ttgtgtttac 60
ctatctgcag ggtctgctag aaatacttca accaaaaatt ccaaaatgac taagcagaga 120
tcaaaaataa atgaactaga agaactgaaa ttggatatga ggaagatcag caatgacatg 180
gaggaaatgt gtggaatcct gaacctttac atgtatgagg atttgaacta caggatgaac 240
actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat gaataaaatg 300
atccagtcca taattggttc catgcagtac tccaaggaac tgatagaaga taactattcc 360
tacagcatta aggaggacca cctcctccgt gagtgcactc aactcaacga aaacgtaagg 420
atattactga atgagaacag aaggctgctg gtggagcagg ctggccataa gtgtcctgtg 480
gggaagaaaa gaggttctct gaggaggcca gcaagaacat ctgtgtccca agtgccaagg 540
aacagcagct aagtgtgaaa tagtccagct gaaagcagaa catggcacag accaggacat 600
gatctccctc aaagagaagt gctggaggaa gagcactgag tgtgca 646

Sequence
<212> Type : DNA
<211> Length : 659

SequenceName : PNN1102 nt SEQ ID No. 58

<213> OrganismName : Mouse

<400> SEQ ID No.: 58:

aaagtctaat cagtcagccg atgaccagtg ggaaaaatga gctacaagat cacctgatct 60

tcatcagtga gaaagctttg cacaagaggg tctgctagaa atacttcaac ccaaaattcc 120

aaaatgacta agaagagatc aaaaataaat gaactagaag aactgaaatt ggatatgagg 180

aagatcagca atgacatgga ggaaatgtgt ggaatcctga acctttacat gtatgaggat 240

ttgaactaca ggatgaacac tgaattcaac atcattaaat cacaacatga gaagacaatg 300

ttggatatga ataaaatgat ccagtccata attggttcca tgcagtactc caaggaactg 360

atagaagata actattccta cagcattaag gaggaccacc tcctccgtga gtgcactcaa 420

ctccacgaaa acgtaaggat attactgaat gagaacagaa ggctgctggt ggagcaggct 480

ggccacaagt gtcctgtggg gaagaaaaga ggttctctga ggaggccagc aagaacatct 540

gtgcctcaag tgccaaggag cagcagtgtg atatagtcca gcagaaagca gaacatggca 600

cagaccacga catgatctcc ctcaaagaga agtgctggag gaagagcact gagtgtgca 659

Sequence

<212> Type : DNA

<211> Length : 658

SequenceName : PNN1108 nt SEQ ID No. 59

<213> OrganismName : Mouse

<400> SEQ ID No.: 59:

aagtctaatc agtcagccga tgaccagtgg gaaaaatgag ctacaagatc acctgatctt 60

catcagtgag aaagctttgc acaagagggt ctgctagaaa tacttcaacc caaaattcca 120

aaatgactaa gaagagatca aaaataaatg aactagaaga actgaaattg gatatgagga 180

agatcagcaa tgacatggag gaaatgtgtg gaatcctgaa cctttacatg tatgaggatt 240

tgaactacag gatgaacact gaattcaaca tcattaaatc acaacatgag aagacaatgt 300

tggatatgaa taaaatgatc cagtccataa ttgtttccat gcagtactcc aaggaactga 360

tagaagataa ctattcctac agcattaagg aggaccacct cctccgtgag tgcactcaac 420

tccacgaaaa tgtaaggata ttactgaatg agaacagaag gctgctggtg gatcaggctg 480

gccacaagtg tcctgtgggg aagaaaagag gttctctgag gaggccagca agaacatctg 540

tgtcccaagt gccaaggaac accagtgtga aatagtccag cagaaagcag aacatggcac 600

agaccaggac atgatctccc tcaaagagaa gtgctggagg aagagcactg agtgtgca 658

Sequence
<212> Type : DNA
<211> Length : 766
SequenceName : PNN1196 nt SEQ ID No. 60
<213> OrganismName : Mouse
<400> SEQ ID No.: 60:
caggagcagt ggctaaatgt tgaactcttc aaaacttggc gtatgggctg ctggtgtacc 60
accatcatcc ccaacactcc tgttcagaag atgggtgagg aaagtggaaa gtctacccag 120
tcagccgatg accagtggga aaaatgagct acaagatcac ctgatcttca tcagtgagaa 180
agctttgcac aagagggtct gctagaaata cttcaaccca aaattccaaa atgactaaga 240
agagatcaaa aataaatgaa ctagaagaac tgaaattgga tatgaggaag atcagcaatg 300
acatggagga aatgtgtgga atcctgaacc tttacatgta tgaggatttg aactacagga 360
tgaacactga attcaacatc attaaatcac aacatgagaa gacaatgttg gatatgaata 420
aaatgatcca gtccataatt ggttccatgc agtactccaa ggaactgata gaagataact 480
attcctacag cattaaggag gaccacctcc tccgtgagtg cactcaactc cacgaaaacg 540
taaggatatt actgaatgag aacagaaggc tgctggtgga gcaggctggc cacaagtgtc 600
ctgtggggaa gaaaagaggt tctctgagga ggccagcaag aacatctgtg tcccaagtgc 660
caaggaacac cagtgtgaaa tagtccagca gaaagcagaa catggcacag accaggacat 720
gatctccctc aaagagaagt gctggaggaa gagcactgag tgtgca 766

Sequence
<212> Type : DNA
<211> Length : 658
SequenceName : PNN1118 nt SEQ ID No. 61
<213> OrganismName : Mouse
<400> SEQ ID No.: 61:
aagtctaatc agtcagccga tgaccagtgg gaaaaatgag ctacaagatc acctgatctt 60
catcagtgag aaagctttgc acaagagggt ctgctagaaa tacttcaacc caaaattcca 120
aaatgactaa gaagagatcc aaaagaaatg aactagaaga actgaaattg gatatgagga 180
agatcagcaa tgacatggag gaaatgtgtg gaatcctgaa cctttacatg tatgaggatt 240
tgaactacag gatgaacact gaattcaaca tcattaaatc acaacatgag aagacaatgt 300

tggatatgaa taaaatgatc cagtccataa ttggttccat gcagtactcc aaggaactga 360
tagaagataa ctattcctac agcattaagg aggaccacct cctccgtgag tgcactcaac 420
tccacgaaaa cgtaaggata ttactgaatg agaacagaag gctgctggtg gagcaggctg 480
gccacaagtg tcctgtgggg aagaaaagag gttctctgag gaggccagca agaacatctg 540
tgtcccaagt gccaaggaac accagtgtga tatagtccag cagaaagcag aacatggcac 600
agaccacgac atgatctccc tcaaagagaa gtgctggagg aagagcactg agtgtgca 658

Sequence
<212> Type : DNA
<211> Length : 641
SequenceName : PNN1150 nt SEQ ID No. 62
<213> OrganismName : Mouse
<400> SEQ ID No.: 62:
gactcagaat ggtctcccct gcccctgggc catcaagtat gcaaattctt ttgtgtttac 60
ctatctgcag ggtctgctag aaatacttca acccaaaatt ccaaaatgac taagaagaga 120
tcaaaaataa atgaactaga agaactgaaa ttggatatga ggaagatcag caatgacatg 180
gaggaaatgt gtggaatcct gaacctttac atgtatgagg atttgaacta caggatgaac 240
actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat gaataaaatg 300
atccagtcca taattggttc catgcagtac tccaaggaac tgatagaaga taactattcc 360
tacagcatta aggaggacca cctcctccgt gagtgcactc aactccacga aaacgtaagg 420
atattactga atgagaacag aaggctgctg gtggagcagg ctggccacaa gtgtcctgtg 480
gggaagaaaa gaggttctct gaggaggcca gcaagaacat ctgtgtccca agtgccaagg 540
aacaccagtg tgatatagtc cagcagaaag cagaacatgg cacagaccac gacatgatct 600
ccctcaaaga gaagtgctgg aggaagagca ctgagtgtgc a 641

Sequence
<212> Type : DNA
<211> Length : 766
SequenceName : PNN1183 nt SEQ ID No. 63
<213> OrganismName : Mouse
<400> SEQ ID No.: 63:
caggagcagt ggctaaatgt tgaactcttc aaaacttggc gtatgggctg ctggtgtacc 60

accatcatcc ccaacactcc tgttcagaag atgggtgagg aaagtggaaa gtctaatcag 120
tcagccgatg accagtggga aaaatgagct acaagatcac ctgatcttca tcagtgagaa 180
agctttgcac aagagggtct gctagaaata cttcaaccca aaattccaaa atgactaaga 240
agagatcaaa aagaaatgaa ctagaagaac tgaaattgga tatgaggaag atcagcaatg 300
acatggagga aatgtgtgga atcctgaacc tttacatgta tgaggatttg aactacagga 360
tgaacactga attcaacatc attaaatcac aacatgagaa gacaatgttg gatatgaata 420
aaatgatcca gtccataatt ggttccatgc agtattccaa ggaactgata gaagataact 480
attcctacag cattaaggag gaccacctcc tccgtgagtg cactcaactc cacgaaaatg 540
taaggatatt actgaatgag aacagaaggc tgctggtgga gcaggctggc cacaagtgtc 600
ctgtggggaa gaaaagaggt tctctgagga ggccagcaag aacatctgtg tcccaagtcc 660
caaggaacag cagtgtgaaa tagtccagca gaaagcagaa catggcacag accaggacat 720
gatctccctc aaagagaagt gctggaggaa gagcactgag tgtgca 766

Sequence
<212> Type : DNA
<211> Length : 640
SequenceName : PNN1130 nt SEQ ID No. 64
<213> OrganismName : Mouse
<400> SEQ ID No.: 64:
gactcagaat ggtctcccct gcccctgggc cttcaagtat gcaaattctt ttgtgtttac 60
ctatctgcag ggtctgctag aaatacttca acccaaaatt ccaaaatgac caagaagaga 120
tcaaaaagaa atgaactaga agaactgaaa ttggatatga ggaagatcag caatgacatg 180
gaggaaatgt gtggaatcct gaacctttac atgtatgagg atttgaacta caggatgaac 240
actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat gaataaaatg 300
atccagtcca taattggttc catgcagtac tccaaggaac tgatagaaga taactattcc 360
tacagcatta aggaggacca cctcctccgt gagtgcactc aactcaacga aaacgtaagg 420
atattactga atgagaacag aaggctgctg gtggagcagg ctggccataa gtgtcctgtg 480
gggaagaaaa gaggttctct gaggaggcca caagaacatc tgtgtcccaa gtgccaagga 540
acagcagtgt gatatagtcc agcagaaagc agaacatggc acagaccacg acatgatctc 600
cctcaaagag aagtgctgga ggaagagcac tgagtgtgca 640

Sequence

<212> Type : DNA

<211> Length : 640

SequenceName : PNN1165 nt SEQ ID No. 65

<213> OrganismName : Mouse

<400> SEQ ID No.: 65:

gactcagaat ggtctcccct gcccctgggc catcaagtat gcaaattctt ttgtgtttac 60

ctatctgcag ggtctgctag aaatacttca acccaaaatt ccaaaatgac taagaagaga 120

tccaaaagaa atgaactaga agaactgaaa ttggatatga ggaagatcag caatgacatg 180

gaggaaatgt gtggaatcct gaacctttac atgtatgagg atttgaacta caggatgaac 240

actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat gaataaaatg 300

atccagtcca taattggttc catgcagtac tccaaggaac tgatagaaga taactattcc 360

tacagcatta aggaggacca cctcctccgt gagtgcactc aactccacga aaacgtaagg 420

atattactga atgagaacag aaggctgctg gtggagcagg ctggccacaa gtgtcctgtg 480

gggaagaaaa aggttctctg aggaggccag caagaacatc tgtgtcccaa gtgccaagga 540

acaccagtgt gatatagtct agcagaaagc agaacatggc acagaccacg acatgatctc 600

ccacaaagag aagtgctgga ggaagagcac tgagtgtgca 640

Sequence

<212> Type : DNA

<211> Length : 647

SequenceName : PNN1104 nt SEQ ID No. 66

<213> OrganismName : Mouse

<400> SEQ ID No.: 66:

aagtctaccc agtcagccga tgaccagtgg gaaaaatgag ctacaagatc acctgatctt 60

catcagtgag aaagctttgc acaagagggt ctgctagaaa tacttcaacc caaaattcca 120

aaatgactaa gaagagatca aaaataaatg aactagaaga actgaaattg gatatgagga 180

agatcagcaa tgacatggag gaaatgtgtg gaatcctgaa cctttacatg tatgaggatt 240

tgaactacag gatgaacact gaattcaaca tcattaaatc acaacatgag aagacaatgt 300

tggatatgaa taaaatgatc cagtccataa ttggttccat gcagtactcc aaggaactga 360

tagaagataa ctattcctac agcattaagg aggaccacct cctccgtgag tgcactcaac 420

tccacgaaaa cgtaaggata ttactgaatg agaacgaagg ctgctggtgg agcaggctgg 480

ccacaagtgt cctgtgggga agaaaagagg ttctctgagg aggccagcaa gaacatctgt 540

gtcccaagtg ccaaggaaca gcagtccagc agaaagcaga acatggcaca gaccaggaca 600
tgatctccct caaagagaag tgctggagga agagcactga gtgtgca 647

Sequence
<212> Type : DNA
<211> Length : 573
SequenceName : PNN1179 nt SEQ ID No. 67
<213> OrganismName : Mouse
<400> SEQ ID No.: 67:
caggagcagt ggctaaatgt tgaactcttc aaaacttggc gtatgggctg ctggtgtacc 60
accatcatcc ccaacactcc tgttcagaag atgggtgagg aaagtggaaa gtctaatcag 120
tcagccgatg accagtggga aaaatgagct acaagatcac ctgatcttca tcagtgagaa 180
agctttgcac aagagggtct gctagaaata cttcaaccca aaattccaaa atgactaaga 240
agagatcaaa aagaaatgaa ctagaagaac tgaaattgga tatgaggaag atcagcaatg 300
acatggagga aatgtgtgga atcctgaacc tttacatgta tgaggatttg aactacagga 360
tgaacactga attcaacatc attaaatcac aacatgagaa gacaatgttg gatatgaata 420
aaatgatcca gtccataatt ggttccatgc agtattccaa ggaactgata gaagataact 480
attcctacag tccagcagaa agcagaacat ggcacagacc aggacatgat ctccctcaaa 540
gagaagtgct ggaggaagag cactgagtgt gca 573

Sequence
<212> Type : DNA
<211> Length : 658
SequenceName : PNN1103 nt SEQ ID No. 68
<213> OrganismName : Mouse
<400> SEQ ID No.: 68:
aagtctaatc agtcagccga tgaccagtgg gaaaaatgag ctacaagatc acctgatctt 60
catcagtgag aaagctttgc acaagagggt ctgctagaaa tacttcaacc caaaattcca 120
aaatcactaa gcagagatca aaaataaatg aactagaaga actgaaattg gatatgagga 180
agatcagcaa tgacatggag gaaatgggtg gaatcctgga actttacata tatgaggatt 240
tgaactacag gatgaacact gaattcaaca tcattaaatc acaacatgag aagacaatgt 300
tggatatgaa tgaaatgatc cagtccataa ttgtttccat gcagtactcc aaggaactga 360

tagaagataa ctattcctac agcattaagg aggaccacct cctccgtgag tgcactcaac 420
tcagcgaaaa agtaaggata ttactgaatg agaacagaaa gctgctagtg gagcaggctg 480
gaacgcaatt gtctcatggg gaagaaaaga ggttctgtga ggaggccagc aagaacatct 540
gtgcctcaag tgccaaggag cagcagtgtg taaactccag tagaaaccgg aacatggcac 600
agaccacgac atgatctccc tcaaagagaa gtgctggagg aggagcactg agtgtgca 658

Sequence
<212> Type : DNA
<211> Length : 474
SequenceName : PNN1101 nt SEQ ID No. 69
<213> OrganismName : Mouse
<400> SEQ ID No.: 69:
aagtctaatc agtcagccga tgaccagtgg gaaaaatgag ctacaagatc acctgatctt 60
catcagtgag aaagctttgc acaagagggt ctgctagaaa tacttcaacc caaaattcca 120
aaatcactaa gcagagatca aaaataaatg aactagaaga actgaaattg gatatgagga 180
agatcagcaa tgacatggag gaaatgggtg gaatcctgga actttacata tatgaggatt 240
tgaactacag gatgaacact gaattcaaca tcattaaatc acaacatgag aagacaatgt 300
tggatatgaa tgaaatgatc cagtccataa ttgtttccat gcagtactcc aaggaactga 360
tagaagataa ctattcctac agtgtgtaaa ctccagtaga aaccggaaca tggcacagac 420
cacgacatga tctccctcaa agagaagtgc tggaggaaga gcactgagtg tgca 474

Sequence
<212> Type : DNA
<211> Length : 640
SequenceName : PNN1128 nt SEQ ID No. 70
<213> OrganismName : Mouse
<400> SEQ ID No.: 70:
gactcagaat ggtctcccct gcgcctgggc catcaagtat gcaaattctt ttgtgtttat 60
ctatctgcag ggtctgctag aaatacttca acccagaatt ccaaaatcac taagaagaga 120
tcaaaaataa atgaactaga agaattgaaa ttggatatga ggaagatcag caatgacatg 180
gaggaaatgt gtggaatcct ggacctttac atatatgagg atttgaacta caggatgaac 240
actgaattca acatcattaa atcacaacat gagaagacaa tattggatat gaataaaatg 300

atccagtcca taattggttc catgcagtac tccaaggaac tgatagaaga taactattcc 360
tacagcatta aggaggacca cctcctccgt gagtgcactc aactccacga aaacgtaagg 420
atattactga atgagaacag aaggctgctg gtggagcagg ctggccacaa gtgtcctgtg 480
ggaagaaaag tggttctctg aggaggccag caagaacatc tgtgtcccaa gtgccaagga 540
acagcagtgt gaaatagtcc agcagaaagc agaacatggc acagaccagg acatgatctc 600
cctcaaagag aagtgctgga ggaagagcac tgagtgtgca 640

Sequence
<212> Type : DNA
<211> Length : 475
SequenceName : PNN1125 nt SEQ ID No. 71
<213> OrganismName : Mouse
<400> SEQ ID No.: 71:
aagtctaatc agtcagccga tgaccagtgg gaaaaatgag ctacaagatc acctgatctt 60
catcagtgag aaagctttgc acaagaggat ctgctagaaa tacttcaacc caaaagtcca 120
aaatcactaa gcagaaatca aatataaatg aactagaaga attgaatttg gatatgagga 180
agatcagtaa tgacatggag gaaatgtgtg gaatcctgga cctttacatg tatgaggatt 240
tgaactacag gatgaacact gaattcaaca tcattaaatc acaacatgag aagacaatgt 300
tggatatgaa taaaatgatc cagtccataa ttggttccat gcagtactcc aaggaactga 360
tagaagataa ctattcctac agtgtgaaat aggcaggcag aaagcagaac atggcacaga 420
ccaggacatg atctccctca aagagaagtg ctggaggaag agcactgagt gtgca 475

Sequence
<212> Type : DNA
<211> Length : 20
SequenceName : Primer1 5prime SEQ ID No. 72
<213> OrganismName : Primer
<400> SEQ ID No.: 72:
catccccaac actcctgttc 20

Sequence
<212> Type : DNA

<211> Length : 21
SequenceName : Primer2 3prime SEQ ID No. 73
<213> OrganismName : Primer
<400> SEQ ID No.: 73:
gaggagcata cagcccatta c 21

Sequence
<212> Type : DNA
<211> Length : 29
SequenceName : Primer3 5prime SEQ ID No. 74
<213> OrganismName : Primer
<400> SEQ ID No.: 74:
ctagctagca agatgggtga ggaaagtgg 29

Sequence
<212> Type : DNA
<211> Length : 29
SequenceName : Primer4 3prime SEQ ID No. 75
<213> OrganismName : Primer
<400> SEQ ID No.: 75:
ccgctcgagt gcacactcag tgctcttcc 29

Sequence
<212> Type : DNA
<211> Length : 22
SequenceName : Primer5 5prime SEQ ID No. 76
<213> OrganismName : Primer
<400> SEQ ID No.: 76:
cagctggaag atagcttttc tg 22

Sequence
<212> Type : DNA

<211> Length : 29
SequenceName : Primer6 5prime SEQ ID No. 77
<213> OrganismName : Primer
<400> SEQ ID No.: 77:
ctagctagct ccctccatct tcttcttgg 29

Sequence
<212> Type : DNA
<211> Length : 19
SequenceName : Primer7 5prime SEQ ID No. 78
<213> OrganismName : Primer
<400> SEQ ID No.: 78:
cccctcaaaa gcacatgac 19

Sequence
<212> Type : DNA
<211> Length : 29
SequenceName : Primer8 5prime SEQ ID No. 79
<213> OrganismName : Primer
<400> SEQ ID No.: 79 :
ctagctagcg aaggagaggt tgccaaagg 29

Sequence
<212> Type : DNA
<211> Length : 20
SequenceName : Primer9 5prime SEQ ID No. 80
<213> OrganismName : Primer
<400> SEQ ID No.: 80 :
actcgtctcg ccacatgaac 20

Sequence
<212> Type : DNA

<211> Length : 31
SequenceName : Primer10 5prime SEQ ID No. 81
<213> OrganismName : Primer
<400> SEQ ID No.: 81:
ctagctagct tcacagagat gtgagatgga g 31

Sequence
<212> Type : PRT
<211> Length : 19
SequenceName : Anti-p16N aa SEQ ID No. 82
<213> OrganismName : Mouse
<400> SEQ ID No.: 82:
TKKRSKINEL EELKLDMRK 19

Sequence
<212> Type : PRT
<211> Length : 19
SequenceName : Anti-p16C aa SEQ ID No. 83
<213> OrganismName : Mouse
<400> SEQ ID No.: 83:
CPVGKKRGSL RRPARTSVS 19

Sequence
<212> Type : DNA
<211> Length : 644
SequenceName : Anti-sense RNA Probe SEQ ID No. 84
<213> OrganismName : Probe
<400> SEQ ID No.: 84:
gcatttctct gagtcccttt aaatgtgcac tcttcctggg ccagaggcta gatgaaatta 60
aggcctgttc ataccaaaga aacaaaataa aggaggagca tacagcccat tacagccatg 120
gttattaggg atgagaggca acagtggtgt atttcctgtg cacactcagt gctcttcctc 180
cagcacttct ttttgaggga gatcatgtcg tggtctgtgc cgtgttctgc tttctgctgg 240

actatttcac actgctgttc tttggcactt gggacacaga tgttcttgct ggcctcctca 300
gagaacctct tttcttcccc acaggacact tgtggccagc ctgctccacc agcagccttc 360
tgttctcatt cagaaatatc cttacgtttt cgttgagttg agtgcactca cggaggaggt 420
ggtcctcctt aatgctgtag gaatagttat cttctatcag ttccttggag tactgcatgg 480
aaccaattat ggactgggat catttttatt catatccaac attgncttct catgttgtga 540
tttaatgatg ttgaattcag tgttcatcct gtagttcaaa tcctcataca tgtaaaggtt 600
caggattcca cacatttcct ccatgtcatt gctgatcttc ctca 644

Sequence
<212> Type : DNA
<211> Length : 653
SequenceName : P16-1 nt SEQ ID No. 85
<213> OrganismName : Mouse
<400> SEQ ID No.: 85:
tcttcttctt gggactcaga atggtctccc ctgcccctgg tccatcaagt atgcaaattc 60
ttttgtgttt acctatctgc agggtctgct agaaatactt caacccaaaa ttccaaaatg 120
actaagaaga gatcaaaaat aaatgaacta gaagaactga aattggatat gaggaagatc 180
agcaatgaca tggaggaaat gtgtggaatc ctgaaccttt acatgtatga ggatttgaac 240
tacaggatga acactgaatt caacatcatt aaatcacaac atgagaagac aatgttggat 300
atgaataaaa tgatccagtc cataattggt tccatgcagt actccaagga actgatagaa 360
gataactatt cctacagcat taaggaggac cacctcctcc gtgagtgcac tcaactcaac 420
gaaaacgtaa ggatattact gaatgagaac agaaggctgc tggtggagca ggctggctat 480
aagtgtcctg tggggaagaa aagaggttct ctgaggaggc cagcaagaac atctgtgtcc 540
caagtgccaa ggaacagcag tgtgaaatag tccagctgaa agcagaacat ggcacagacc 600
aggacatgat ctccctcaaa gagaagtgct ggaggaagag cactgagtgt gca 653

Sequence
<212> Type : DNA
<211> Length : 652
SequenceName : P16-2 nt SEQ ID No. 86
<213> OrganismName : Mouse
<400> SEQ ID No.: 86:

cttcttcttg ggactcagaa tggtctcccc tgcccctggg ccatcaagta tgcaaattct 60
tttgtgttta cctatctgca gggtctgcta gaaatacttc aacccaaaat tccaaaatga 120
ccaagaagag atcaaaaata aatgaactag aagaactgaa attggatatg aggaagatca 180
gcaatgacat ggaggaaatg tgtggaatcc tgaaccttta catgtatgag gatttgaact 240
acaggatgaa cactgaattc aacatcatta aatcacaaca tgagaagaca atgttggata 300
tgaataaaat gatccagtca ataattggtt ccatgcagta ctctaaggaa ctgatagaag 360
ataactattc ctacagcatt aaggaggacc acctcctccg tgagtgcact caactcaacg 420
aaaacgtaag gatattactg aatgagaaca gaaggctgct ggtggagcag gctggccata 480
agtgtcctgt ggggaagaaa agaggttctc tgaggaggcc agcaagaaca tctgtgtccc 540
aagtgccaag gaacagcagt gtgatatagt ccagcagaaa gcagaacatg gcacagacca 600
cgacatgatc tccctcaaag agaagtgctg gaggaagagc actgagtgtg ca 652

Sequence
<212> Type : DNA
<211> Length : 784
SequenceName : P16-3 nt SEQ ID No. 87
<213> OrganismName : Mouse
<400> SEQ ID No.: 87:
atgtgagatg gagcaggagc agtggctaaa tgttgaactc ttcaaaactt ggcttatggg 60
ctgctggtgt atcaccatca tccccaacac tcctgttcag aagatgggtg aggaaagtgg 120
aaagcctaac cactcagccg atgaccagtg ggaaaaatga gctacaagat cacctgatct 180
tcatcagtga gaaagctttg cacaagaggg tctgctagaa atacttcaac ccaaaattcc 240
aaaatgacca agaagagatc aaaaataaat gaactagaag aactgaaatt ggatatgagg 300
aagatcagca atgacatgga ggaaatgtgt ggaatcctga acctttacat gtatgaggat 360
ttgaactaca ggatgaacac tgaattcaac atcattaaat cacaacatga gaagacaatg 420
ttggatatga ataaaatgat ccagtccata attggttcca tgcagtactc caaggaactg 480
atagaagata actattccta cagcattaag gaggaccacc tcctccgtga gtgcactcaa 540
ctcaacgaaa acgtaaggat attactgaat gagaacagaa ggctgctggt ggagcaggct 600
ggccataagt gtcctgtggg gaagaaaaga ggttctctga ggaggccagc aagaacatct 660
gtgtcccaag tgccaaggaa cagcagctaa gtgtgaaata gtccagctga aagcagaaca 720
tggcacagac caggacatga tctccctcaa agagaagtgc tggaggaaga gcactgagtg 780
tgca 784

Sequence

<212> Type : DNA

<211> Length : 643

SequenceName : P16-4 nt SEQ ID No. 88

<213> OrganismName : Mouse

<400> SEQ ID No.: 88:

tcttcttctt gggactcaga atggtctccc ctgcccctgg gccatcaagt atgcaaattc 60

ttttgtgttt acctatctgc agggtctgct agaaatactt caacccaaaa ttccaaaatg 120

actaagaaga gatcaaaaat aaatgaacta gaagaactga aattggatat gaggaagatc 180

agcaatgaca tggaggaaat gtgtggaatc ctgaaccttt acatgtatga ggatttgaac 240

tacaggatga acactgaatt caacatcatt aaatcacaac atgagaagac aatgttggat 300

atgaataaaa tgatccagtc aataattggt tccatgcagt actctaagga actgatagaa 360

gataactatt cctacagcat taaggaggac cacctcctcc gtgagtgcac tcaactccac 420

gaaaacgtaa ggatattact gaatgagaac agaaggctgc tggtggagca ggctggccac 480

aagtgtcctg tggggaagaa aagaggttct ctgaggaggc cagcaagaac atctgtgtcc 540

caagtcccaa ggaacagcag tccagcagaa agcagaacat ggcacagacc aggacatgat 600

ctccctcaaa gagaagtgct ggaggaagag cactgagtgt gca 643

Sequence

<212> Type : DNA

<211> Length : 650

SequenceName : P16-5 nt SEQ ID No. 89

<213> OrganismName : Mouse

<400> SEQ ID No.: 89:

aaggggatcc cacgggtcca tgagaggctc caggagaggc accccagaaa aggaccaagc 60

tgggcagaac tagttaacag ggtctgctag aaatacttca acccaaaatt ccaaaatgac 120

taagaagaga tcaaaaataa atgaactaga agaactgaaa ttggatatga ggaagatcag 180

caatgacatg gaggaaatgg gtggaatcct gaacctttac atgtatgagg atttgaacta 240

caggatgaac actgaattca acatcattaa atcacaacat gagaagacaa tgttggatat 300

gaataaaatg atccagtcaa taattggttc catgcagtac tctaaggaac tgatagaaga 360

taactattcc tacagcatta aggaggacca cctcctccgt gagtgcactc aactccacga 420

aaacgtaagg atattactga atgagaacag aaggctgctg gtggagcagg ctggccacaa 480
gtgtcctgtg ggaagaaaag tggttctctg aggaggccag caagaacatc tgtgtcccaa 540
gtgccaagga acagcagtgt gaaatagtcc agcagaaagc agaatatggc acagaccacg 600
acatgatctc cctcaaagag aggtgctgga ggaagagcac tgagtgtgca 650

Sequence
<212> Type : DNA
<211> Length : 657
SequenceName : P16-6 nt SEQ ID No. 90
<213> OrganismName : Mouse
<400> SEQ ID No.: 90:
aagtctaatc agtcagccga tgaccagtgg gaaaaatgag ctacaagatc acctgatctt 60
catcagtgag aaagctttgc acaagagggt ctgctagaaa tacttcaacc caaaattcca 120
aaatgactaa gaagagatca aaaataaatg aactagaaga actgaaattg gatatgagga 180
agatcagcaa tgacatggag gaaatgtgtg gaatcctgaa cctttacatg tatgaggatt 240
tgaactacag gatgaacact gaattcaaca tcattaaatc acaacatgag aagacaatgt 300
tggatatgaa taaaatgatc cagtccataa ttgtttccat gcagtactcc aaggaactga 360
tagaagataa ctattcctac agcattaagg aggaccacct cctccgtgag tgcactcaac 420
tcaacgaaaa cgtaaggata ttactgaatg agaacagaag gctgctggtg gagcaggctg 480
gccataagtg tcctgtgggg aagaaaagag gttctctgag gaggccacaa gaacatctgt 540
gtcccaagtg ccaaggaaca gcagtgtgat atagtccagc agaaagcaga acatggcaca 600
gaccacgaca tgatctccct caaagagaag tgctggagga agagcactga gtgtgca 657

Sequence
<212> Type : PRT
<211> Length : 150
SequenceName : P16-1 aa SEQ ID No. 91
<213> OrganismName : Mouse
<400> SEQ ID No.: 91:
MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL NENVRILLNE
NRRLLVEQAG 120
YKCPVGKKRG SLRRPARTSV SQVPRNSSVK 150

Sequence
<212> Type : PRT
<211> Length : 150
SequenceName : P16-2 aa SEQ ID No. 92
<213> OrganismName : Mouse
<400> SEQ ID No.: 92:
MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL NENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRRPARTSV SQVPRNSSVI 150

Sequence
<212> Type : PRT
<211> Length : 148
SequenceName : P16-3 aa SEQ ID No. 93
<213> OrganismName : Mouse
<400> SEQ ID No.: 93:
MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI
IKSQHEKTML 60
DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL NENVRILLNE
NRRLLVEQAG 120
HKCPVGKKRG SLRRPARTSV SQVPRNSS 148

Sequence
<212> Type : PRT
<211> Length : 172
SequenceName : P16-4 aa SEQ ID No. 94

<213> OrganismName : Mouse

<400> SEQ ID No.: 94:

MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE NRRLLVEQAG 120

HKCPVGKKRG SLRRPARTSV SQVPRNSSPA ESRTWHRPGH DLPQREVLEE EH 172

Sequence

<212> Type : PRT

<211> Length : 131

SequenceName : P16-5 aa SEQ ID No. 95

<213> OrganismName : Mouse

<400> SEQ ID No.: 95:

MTKKRSKINE LEELKLDMRK ISNDMEEMGG ILNLYMYEDL NYRMNTEFNI IKSQHEKTML 60

DMNKMIQSII GSMQYSKELI EDNYSYSIKE DHLLRECTQL HENVRILLNE NRRLLVEQAG 120

HKCPVGRKVV L 131

Sequence

<212> Type : PRT

<211> Length : 148

SequenceName : P16-6 aa SEQ ID No. 96

<213> OrganismName : Mouse

<400> SEQ ID No.: 96:

MTKKRSKINE LEELKLDMRK ISNDMEEMCG ILNLYMYEDL NYRMNTEFNI IKSQHEKTML 60

DMNKMIQSII VSMQYSKELI EDNYSYSIKE DHLLRECTQL NENVRILLNE NRRLLVEQAG 120

HKCPVGKKRG SLRRPQEHLC PKCQGTAV 148

What is claimed is:

1. A method for screening an agent that modulate p16 activity, comprising:
   (a) providing a format having an NMDA receptor that is detectably capable of cation efflux;
   (b) providing at least one amino acid sequence comprising an active domain of p16;
   (c) introducing a candidate compound; and
   (d) selecting those agents that modulate cation efflux activity of the NMDA receptor.
2. The method of claim 1 wherein the NMDA receptor comprises all subunits.
3. The method of claim 1 wherein the NR3A subunit is knocked out.
4. The method of claim 1 wherein the amino acid sequence comprising an active domain of p16 is an amino acid sequence substantially similar to an amino acid sequence selected from the group consisting of SEQ ID No.: 3, SEQ ID No.: 4, SEQ ID No.: 5, SEQ ID No.: 6, SEQ ID No.: 7, SEQ ID No.: 8, SEQ ID No.: 9, SEQ ID No.: 10, SEQ ID No.: 11, SEQ ID No.: 12, SEQ ID No.: 13, SEQ ID No.: 14, SEQ ID No.: 15, SEQ ID No.: 16, SEQ ID No.: 17, SEQ ID No.: 18, SEQ ID No.: 19, SEQ ID No.: 20, SEQ ID No.: 21, SEQ ID No.: 22, SEQ ID No.: 23, SEQ ID No.: 24, SEQ ID No.: 25, SEQ ID No.: 26, SEQ ID No.: 27, SEQ ID No.: 28, SEQ ID No.: 29, SEQ ID No.: 30, SEQ ID No.: 31, SEQ ID No.: 32, SEQ ID No.: 33, SEQ ID No.: 34, SEQ ID No.: 35, SEQ ID No.: 36, SEQ ID No.: 37, SEQ ID No.: 91, SEQ ID No.: 92, SEQ ID No.: 93, SEQ ID No.: 94, SEQ ID No.: 95 and SEQ ID No.: 96.
5. The method of claim 4 wherein said amino acid sequence comprising an active domain of p16 is a whole peptide.
6. The method of claim 4 wherein amino acid sequence comprising an active domain of p16 is a peptide fragment.
7. The method of claim 6 wherein the peptide fragment is an amino acid sequence that binds to a class I PDZ domain.
8. The method of claim 7 wherein the peptide fragment is an SVK sequence.
9. The method of claim 6 wherein the peptide fragment further comprises a coiled-coil domain.
10. The method of claim 1 wherein the active domain of p16 is a coiled-coil domain.

11. The method of claim 1 wherein the active domain of p16 is an amino acid sequence that binds to a class I PDZ domain.

12. The method of claim 11 wherein the active domain of p16 is an SVK sequence.

13. The method of claim 1 wherein a first amino acid sequence comprising an active domain of p16 is associated with a second amino acid sequence comprising an active domain of p16.

14. The method of claim 1, wherein the amino acid sequence comprising an active domain of p16 associates with a peptide having an amino acid sequence substantially similar to PSD-95.

15. The method of claim 1 wherein said agent is selected from the group consisting of a peptide, polypeptide, peptidomimetic, an antibody or antibody fragment, siRNA, anti-sense RNA, gene therapy products and a nucleotide sequence.

16. The method of claim 15 wherein said agent increases cation efflux activity of the NMDA receptor.

17. The method of claim 15 wherein said agent decreases cation efflux activity of the NMDA receptor.

18. The method of claim 1, wherein the format that provides the NMDA receptor that is detectably capable of cation efflux is a format selected from the group consisting of mammalian cell, insect cell, plant cell, prokaryotic cell, eukaryotic cell, yeast cell, lipid bilayer, lipid membrane, cell-free membrane patch and liposome.

19. The method of claim 18, wherein the format that provides the NMDA receptor that is detectably capable of cation efflux is a mammalian cell.

20. The method of claim 19, wherein the format that provides the NMDA receptor that is detectably capable of cation efflux is a mouse cell.

21. The method of claim 19, wherein the format that provides the NMDA receptor that is detectably capable of cation efflux is a human cell.

22. The method of claim 19, wherein the format that provides the NMDA receptor that is detectably capable of cation efflux is a neuronal cell selected from the group consisting of amygdala cells, hippocampus cells and cerebral cortex cells.

23. A modulator of p16 activity identified using the method of claim 1.

24. An isolated nucleic acid comprising the nucleic acid sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No.: 2, SEQ ID No.: 38, SEQ ID No.: 39, SEQ ID

No.: 40, SEQ ID No.: 41, SEQ ID No.: 42, SEQ ID No.: 43, SEQ ID No.: 44, SEQ ID No.: 45, SEQ ID No.: 46, SEQ ID No.: 47, SEQ ID No.: 48, SEQ ID No.: 49, SEQ ID No.: 50, SEQ ID No.: 51, SEQ ID No.: 52, SEQ ID No.: 53, SEQ ID No.: 54, SEQ ID No.: 55, SEQ ID No.: 56, SEQ ID No.: 57, SEQ ID No.: 58, SEQ ID No.: 59, SEQ ID No.: 60, SEQ ID No.: 61, SEQ ID No.: 62, SEQ ID No.: 63, SEQ ID No.: 64, SEQ ID No.: 65, SEQ ID No.: 66, SEQ ID No.: 67, SEQ ID No.: 68, SEQ ID No.: 69, SEQ ID No.: 70, SEQ ID No.: 71, SEQ ID No.: 85, SEQ ID No.: 86, SEQ ID No.: 87, SEQ ID No.: 88, SEQ ID No.: 89 and SEQ ID No.: 90.

25. The isolated nucleic acid of claim 24 wherein the nucleic acid is DNA.

26. An isolated nucleic acid that encodes an amino acid sequence substantially similar to the amino acid sequence of p16.

27. The isolated nucleic acid sequence of claim 26, wherein the amino acid sequence is selected from the group consisting of SEQ ID No.: 3, SEQ ID No.: 4, SEQ ID No.: 5, SEQ ID No.: 6, SEQ ID No.: 7, SEQ ID No.: 8, SEQ ID No.: 9, SEQ ID No.: 10, SEQ ID No.: 11, SEQ ID No.: 12, SEQ ID No.: 13, SEQ ID No.: 14, SEQ ID No.: 15, SEQ ID No.: 16, SEQ ID No.: 17, SEQ ID No.: 18, SEQ ID No.: 19, SEQ ID No.: 20, SEQ ID No.: 21, SEQ ID No.: 22, SEQ ID No.: 23, SEQ ID No.: 24, SEQ ID No.: 25, SEQ ID No.: 26, SEQ ID No.: 27, SEQ ID No.: 28, SEQ ID No.: 29, SEQ ID No.: 30, SEQ ID No.: 31, SEQ ID No.: 32, SEQ ID No.: 33, SEQ ID No.: 34, SEQ ID No.: 35, SEQ ID No.: 36, SEQ ID No.: 37, SEQ ID No.: 91, SEQ ID No.: 92, SEQ ID No.: 93, SEQ ID No.: 94, SEQ ID No.: 95 and SEQ ID No.: 96.

28. The isolated nucleic acid sequence of claim 26, wherein the nucleic acid is DNA.

29. An isolated nucleic acid sequence that encodes an amino acid sequence substantially similar to the amino acid sequence of p16 with 0-20 conservative amino acid substitutions.

30. The isolated amino acid sequence of claim 29 wherein the nucleic acid encodes an amino acid sequence with 0 to 10 conservative amino acid substitutions.

31. The isolated nucleic acid of claim 30 wherein the nucleic acid encodes an amino acid sequence with 0 to 5 conservative amino acid substitutions.

32. The isolated nucleic acid of claim 31 wherein the nucleic acid is DNA.

33. An isolated nucleic acid that hybridizes to a nucleic acid selected from the group consisting of SEQ ID No. 1, SEQ ID No.: 2, SEQ ID No.: 38, SEQ ID No.: 39, SEQ ID No.: 40, SEQ ID No.: 41, SEQ ID No.: 42, SEQ ID No.: 43, SEQ ID No.: 44, SEQ ID

No.: 45, SEQ ID No.: 46, SEQ ID No.: 47, SEQ ID No.: 48, SEQ ID No.: 49, SEQ ID No.: 50, SEQ ID No.: 51, SEQ ID No.: 52, SEQ ID No.: 53, SEQ ID No.: 54, SEQ ID No.: 55, SEQ ID No.: 56, SEQ ID No.: 57, SEQ ID No.: 58, SEQ ID No.: 59, SEQ ID No.: 60, SEQ ID No.: 61, SEQ ID No.: 62, SEQ ID No.: 63, SEQ ID No.: 64, SEQ ID No.: 65, SEQ ID No.: 66, SEQ ID No.: 67, SEQ ID No.: 68, SEQ ID No.: 69, SEQ ID No.: 70, SEQ ID No.: 71, SEQ ID No.: 85, SEQ ID No.: 86, SEQ ID No.: 87, SEQ ID No.: 88, SEQ ID No.: 89 and SEQ ID No.: 90.

34. The isolated nucleic acid of claim 33 wherein there is no more than about a 5% hybridization mismatch.

35. The isolated nucleic acid of claim 34 wherein there is no more than about a 2% hybridization mismatch.

36. The isolated nucleic acid of claim 35 wherein there is no more than about a 1% hybridization mismatch.

37. The isolated nucleic acid of claim 33 wherein the nucleic acid is DNA.

38. The isolated nucleic acid of claim 33 wherein the nucleic acid is RNA.

39. An isolated nucleic acid that encodes a first protein having an amino acid sequence substantially similar to p16, that: associates with a second protein having an amino acid sequence similar to p16; associates with a protein having an amino acid sequence substantially similar to PSD-95; and has NMDA receptor modulation activity.

40. The isolated nucleic acid of claim 39 wherein the nucleic acid is DNA.

41. The isolated nucleic acid of claim 39 wherein the nucleic acid is RNA.

42. The isolated nucleic acid of claim 39 wherein the first protein having an amino acid sequence similar to p16 forms a homodimer with the second protein having an amino acid sequence similar to p16.

43. An isolated polypeptide having an amino acid sequence substantially similar to p16, wherein the isolated polypeptide has activity modulating cation efflux through the NMDA receptor.

44. The isolated polypeptide of claim 43 wherein the amino acid sequence substantially similar to p16 is an amino acid sequences selected from the group consisting of SEQ ID No.: 3, SEQ ID No.: 4, SEQ ID No.: 5, SEQ ID No.: 6, SEQ ID No.: 7, SEQ ID No.: 8, SEQ ID No.: 9, SEQ ID No.: 10, SEQ ID No.: 11, SEQ ID No.: 12, SEQ ID No.: 13, SEQ ID No.: 14, SEQ ID No.: 15, SEQ ID No.: 16, SEQ ID No.: 17, SEQ ID No.: 18, SEQ ID

No.: 19, SEQ ID No.: 20, SEQ ID No.: 21, SEQ ID No.: 22, SEQ ID No.: 23, SEQ ID No.: 24, SEQ ID No.: 25, SEQ ID No.: 26, SEQ ID No.: 27, SEQ ID No.: 28, SEQ ID No.: 29, SEQ ID No.: 30, SEQ ID No.: 31, SEQ ID No.: 32, SEQ ID No.: 33, SEQ ID No.: 34, SEQ ID No.: 35, SEQ ID No.: 36, SEQ ID No.: 37, SEQ ID No.: 91, SEQ ID No.: 92, SEQ ID No.: 93, SEQ ID No.: 94, SEQ ID No.: 95 and SEQ ID No.: 96.

45. The isolated polypeptide of claim 43 wherein the amino acid sequence is coded by a nucleotide having a sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No.: 2, SEQ ID No.: 38, SEQ ID No.: 39, SEQ ID No.: 40, SEQ ID No.: 41, SEQ ID No.: 42, SEQ ID No.: 43, SEQ ID No.: 44, SEQ ID No.: 45, SEQ ID No.: 46, SEQ ID No.: 47, SEQ ID No.: 48, SEQ ID No.: 49, SEQ ID No.: 50, SEQ ID No.: 51, SEQ ID No.: 52, SEQ ID No.: 53, SEQ ID No.: 54, SEQ ID No.: 55, SEQ ID No.: 56, SEQ ID No.: 57, SEQ ID No.: 58, SEQ ID No.: 59, SEQ ID No.: 60, SEQ ID No.: 61, SEQ ID No.: 62, SEQ ID No.: 63, SEQ ID No.: 64, SEQ ID No.: 65, SEQ ID No.: 66, SEQ ID No.: 67, SEQ ID No.: 68, SEQ ID No.: 69, SEQ ID No.: 70, SEQ ID No.: 71, SEQ ID No.: 85, SEQ ID No.: 86, SEQ ID No.: 87, SEQ ID No.: 88, SEQ ID No.: 89 and SEQ ID No.: 90.

46. The isolated polypeptide of claim 43 wherein the isolated polypeptide forms a homodimer.

47. The isolated polypeptide of claim 43 wherein the isolated polypeptide forms an association with PSD-95.

48. The isolated polypeptide of claim 43 wherein the isolated polypeptide binds to a class I PDZ domain.

49. The isolated polypeptide of claim 48 wherein the isolated polypeptide further comprises an SVK sequence in the c-terminus.

50. The isolated polypeptide of claim 43 wherein the isolated polypeptide further comprises a coiled-coil domain.

51. The isolated polypeptide of claim 43 wherein a first isolated polypeptide is associated with a second isolated polypeptide.

52. The isolated polypeptide of claim 43 wherein the isolated polypeptide causes an increased efflux of cations through the NMDA receptor.

53. The isolated polypeptide of claim 52 wherein the cation efflux activity caused by the isolated polypeptide is negatively regulated by NR3A subunit of the NMDA receptor.

54. A method for diagnosing a dysfunctional NMDA receptor within a cell, comprising the steps of:

(a) determining cation flux through an NMDA receptor;

(b) determining the level of p16;

(c) identifying the condition associated with the dysfunction of the NMDA receptor in a cell; and

(d) correlating said in vivo characteristic to a known characteristic leading to a dysregulation of NMDAR channel current, such that the presence of at least one characteristic indicates an individual's susceptibility to a condition stemming from a dysregulation of NMDAR channel current.

55. The method of claim 54, wherein the cation is calcium.

56. The method of claim 55, wherein the cation flux through the NMDA receptor is increased.

57. The method of claim 56, wherein the level of p16 is increased.

58. The method of claim 56, wherein the level of a protein having an amino acid sequence substantially similar to the amino acid sequence selected from the group consisting of SEQ ID No.: 3, SEQ ID No.: 4, SEQ ID No.: 5, SEQ ID No.: 6, SEQ ID No.: 7, SEQ ID No.: 8, SEQ ID No.: 9, SEQ ID No.: 10, SEQ ID No.: 11, SEQ ID No.: 12, SEQ ID No.: 13, SEQ ID No.: 14, SEQ ID No.: 15, SEQ ID No.: 16, SEQ ID No.: 17, SEQ ID No.: 18, SEQ ID No.: 19, SEQ ID No.: 20, SEQ ID No.: 21, SEQ ID No.: 22, SEQ ID No.: 23, SEQ ID No.: 24, SEQ ID No.: 25, SEQ ID No.: 26, SEQ ID No.: 27, SEQ ID No.: 28, SEQ ID No.: 29, SEQ ID No.: 30, SEQ ID No.: 31, SEQ ID No.: 32, SEQ ID No.: 33, SEQ ID No.: 34, SEQ ID No.: 35, SEQ ID No.: 36, SEQ ID No.: 37, SEQ ID No.: 91, SEQ ID No.: 92, SEQ ID No.: 93, SEQ ID No.: 94, SEQ ID No.: 95 and SEQ ID No.: 96 is increased.

59. The method of claim 56, wherein the level of a protein having an amino acid sequence substantially similar to the amino acid sequence encoded by the nucleotide sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No.: 2, SEQ ID No.: 38, SEQ ID No.: 39, SEQ ID No.: 40, SEQ ID No.: 41, SEQ ID No.: 42, SEQ ID No.: 43, SEQ ID No.: 44, SEQ ID No.: 45, SEQ ID No.: 46, SEQ ID No.: 47, SEQ ID No.: 48, SEQ ID No.: 49, SEQ ID No.: 50, SEQ ID No.: 51, SEQ ID No.: 52, SEQ ID No.: 53, SEQ ID No.: 54, SEQ ID No.: 55, SEQ ID No.: 56, SEQ ID No.: 57, SEQ ID No.: 58, SEQ ID No.: 59, SEQ ID No.: 60, SEQ ID No.: 61, SEQ ID No.: 62, SEQ ID No.: 63,

SEQ ID No.: 64, SEQ ID No.: 65, SEQ ID No.: 66, SEQ ID No.: 67, SEQ ID No.: 68, SEQ ID No.: 69, SEQ ID No.: 70, SEQ ID No.: 71, SEQ ID No.: 85, SEQ ID No.: 86, SEQ ID No.: 87, SEQ ID No.: 88, SEQ ID No.: 89 and SEQ ID No.: 90 is increased.

60. The method of claim 55, wherein the cation flux through the NMDA receptor is decreased.

61. The method of claim 60, wherein the level of p16 is decreased.

62. The method of claim 60, wherein the level of a protein having an amino acid sequence substantially similar to the amino acid sequence selected from the group consisting of SEQ ID No.: 3, SEQ ID No.: 4, SEQ ID No.: 5, SEQ ID No.: 6, SEQ ID No.: 7, SEQ ID No.: 8, SEQ ID No.: 9, SEQ ID No.: 10, SEQ ID No.: 11, SEQ ID No.: 12, SEQ ID No.: 13, SEQ ID No.: 14, SEQ ID No.: 15, SEQ ID No.: 16, SEQ ID No.: 17, SEQ ID No.: 18, SEQ ID No.: 19, SEQ ID No.: 20, SEQ ID No.: 21, SEQ ID No.: 22, SEQ ID No.: 23, SEQ ID No.: 24, SEQ ID No.: 25, SEQ ID No.: 26, SEQ ID No.: 27, SEQ ID No.: 28, SEQ ID No.: 29, SEQ ID No.: 30, SEQ ID No.: 31, SEQ ID No.: 32, SEQ ID No.: 33, SEQ ID No.: 34, SEQ ID No.: 35, SEQ ID No.: 36, SEQ ID No.: 37, SEQ ID No.: 91, SEQ ID No.: 92, SEQ ID No.: 93, SEQ ID No.: 94, SEQ ID No.: 95 and SEQ ID No.: 96 is decreased.

63. The method of claim 60, wherein the level of a protein having an amino acid sequence substantially similar to the amino acid sequence encoded by the nucleotide sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No.: 2, SEQ ID No.: 38, SEQ ID No.: 39, SEQ ID No.: 40, SEQ ID No.: 41, SEQ ID No.: 42, SEQ ID No.: 43, SEQ ID No.: 44, SEQ ID No.: 45, SEQ ID No.: 46, SEQ ID No.: 47, SEQ ID No.: 48, SEQ ID No.: 49, SEQ ID No.: 50, SEQ ID No.: 51, SEQ ID No.: 52, SEQ ID No.: 53, SEQ ID No.: 54, SEQ ID No.: 55, SEQ ID No.: 56, SEQ ID No.: 57, SEQ ID No.: 58, SEQ ID No.: 59, SEQ ID No.: 60, SEQ ID No.: 61, SEQ ID No.: 62, SEQ ID No.: 63, SEQ ID No.: 64, SEQ ID No.: 65, SEQ ID No.: 66, SEQ ID No.: 67, SEQ ID No.: 68, SEQ ID No.: 69, SEQ ID No.: 70, SEQ ID No.: 71, SEQ ID No.: 85, SEQ ID No.: 86, SEQ ID No.: 87, SEQ ID No.: 88, SEQ ID No.: 89 and SEQ ID No.: 90 is decreased.

64. The method of claim 54 wherein said cell is a mammalian cell.

65. The method of claim 64, wherein the mammalian cell is a human cell.

66. The method of claim 54 wherein the condition associated with the dysfunction of the NMDA receptor in a cell is a dysfunctional NR3A subunit.

67. The method of claim 54 wherein the dysfunctional NR3A subunit is an NR3A knock-out.

68. A method for treating conditions relating to dysregulation of NMDA receptors comprising administering to a patient diagnosed with such condition an agent that is capable of modulating the consequences of dysfunctional p16 activity, wherein said agent is administered in a quantity sufficient to modulate p16 activity to treat such condition.

69. The treatment method of claim 68, wherein the agent that modulates the consequences of dysfunctional p16 activity is an agent selected from the group consisting of a peptide, polypeptide, peptidomimetic, an antibody or antibody fragment, siRNA, anti-sense RNA, gene therapy products and a nucleotide sequence.

70. The treatment method of claim 69, wherein the agent is an exogenous nucleotide sequence administered in a quantity sufficient to modulate p16 activity to treat the condition.

71. The treatment method of claim 70, wherein the nucleotide sequence is administered to a patient using an administration system selected from the group consisting of a nucleic acid vector system, microinjection, a gene gun and a liposome.

72. The treatment method of claim 70, wherein the nucleotide sequence is an RNA molecule that has a sequence that is antisense to a portion of the native p16 RNA transcript.

73. The treatment method of claim 72, wherein the antisense RNA molecule is specific and sensitive for the native p16 RNA transcript.

74. The treatment method of claim 68 wherein the patient is a mammal.

75. The treatment method of claim 68 wherein the patient is a human.

76. The treatment method of claim 68 wherein the patient is a human and the agent that modulates the consequences of dysfunctional p16 activity is a nucleotide sequence.

77. The treatment method of claim 76 wherein the nucleotide sequence is an RNA molecule that has a sequence that is antisense to a portion of the p16 peptide.

78. The treatment method of claim 77, wherein the antisense RNA molecule is specific and sensitive for the p16 RNA transcript.

79. The treatment method of claim 76 wherein the nucleotide sequence codes for an amino acid sequence substantially similar to the NR3A subunit of NMDA receptor with the provisio that the exogenous NR3A subunit downregulates p16 activity towards cation efflux through the NMDA receptor.

80. The treatment method of claim 76 wherein the nucleotide sequence codes for an amino acid sequence substantially similar to p16.

81. The treatment method of claim 80 wherein the nucleotide sequence is selected from the group consisting of SEQ ID No. 1, SEQ ID No.: 2, SEQ ID No.: 38, SEQ ID No.: 39, SEQ ID No.: 40, SEQ ID No.: 41, SEQ ID No.: 42, SEQ ID No.: 43, SEQ ID No.: 44, SEQ ID No.: 45, SEQ ID No.: 46, SEQ ID No.: 47, SEQ ID No.: 48, SEQ ID No.: 49, SEQ ID No.: 50, SEQ ID No.: 51, SEQ ID No.: 52, SEQ ID No.: 53, SEQ ID No.: 54, SEQ ID No.: 55, SEQ ID No.: 56, SEQ ID No.: 57, SEQ ID No.: 58, SEQ ID No.: 59, SEQ ID No.: 60, SEQ ID No.: 61, SEQ ID No.: 62, SEQ ID No.: 63, SEQ ID No.: 64, SEQ ID No.: 65, SEQ ID No.: 66, SEQ ID No.: 67, SEQ ID No.: 68, SEQ ID No.: 69, SEQ ID No.: 70, SEQ ID No.: 71, SEQ ID No.: 85, SEQ ID No.: 86, SEQ ID No.: 87, SEQ ID No.: 88, SEQ ID No.: 89 and SEQ ID No.: 90.

82 The treatment method of claim 80 wherein the amino acid sequence substantially similar to p16 is an amino acid sequences selected from the group of consisting of SEQ ID No.: 3, SEQ ID No.: 4, SEQ ID No.: 5, SEQ ID No.: 6, SEQ ID No.: 7, SEQ ID No.: 8, SEQ ID No.: 9, SEQ ID No.: 10, SEQ ID No.: 11, SEQ ID No.: 12, SEQ ID No.: 13, SEQ ID No.: 14, SEQ ID No.: 15, SEQ ID No.: 16, SEQ ID No.: 17, SEQ ID No.: 18, SEQ ID No.: 19, SEQ ID No.: 20, SEQ ID No.: 21, SEQ ID No.: 22, SEQ ID No.: 23, SEQ ID No.: 24, SEQ ID No.: 25, SEQ ID No.: 26, SEQ ID No.: 27, SEQ ID No.: 28, SEQ ID No.: 29, SEQ ID No.: 30, SEQ ID No.: 31, SEQ ID No.: 32, SEQ ID No.: 33, SEQ ID No.: 34, SEQ ID No.: 35, SEQ ID No.: 36, SEQ ID No.: 37, SEQ ID No.: 91, SEQ ID No.: 92, SEQ ID No.: 93, SEQ ID No.: 94, SEQ ID No.: 95 and SEQ ID No.: 96.

Figure 1

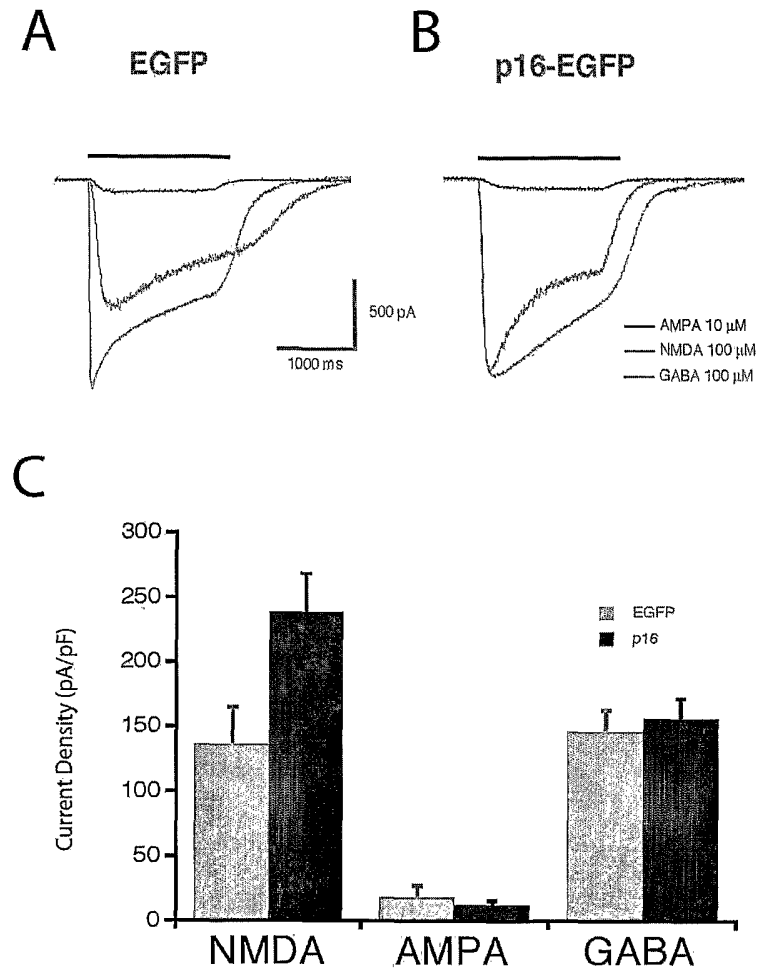

Figure 1

Figure 2

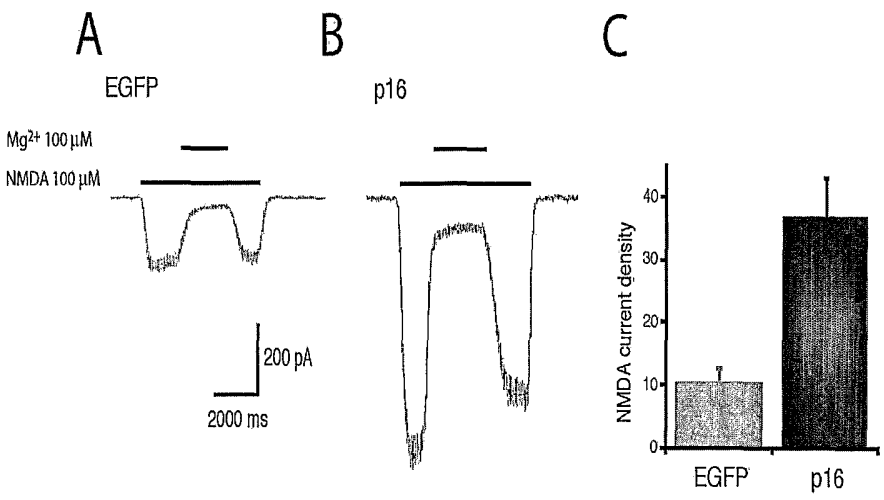

Figure 2

Figure 3

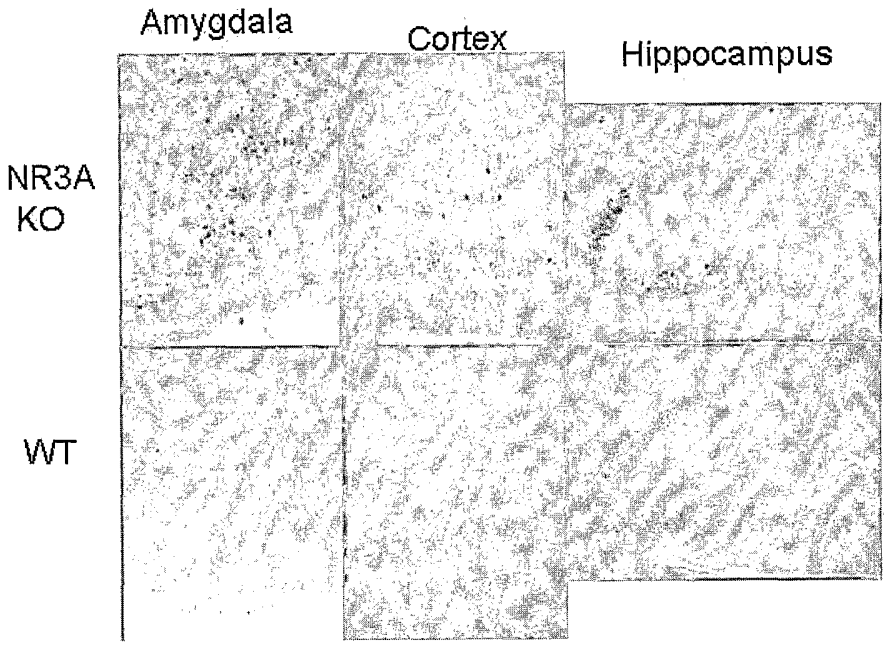

Figure 3

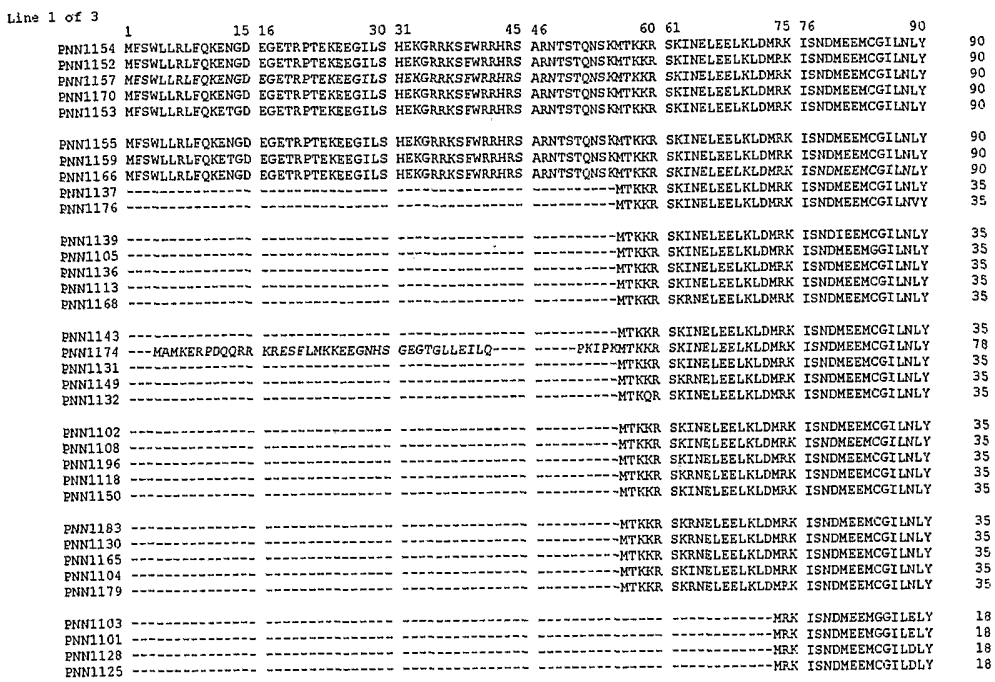

Figure 4a part 1 of 3

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Lines 2 of 3
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Figure 4a part 2 of 3

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Lines 3 of 3
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Figure 4a part 3 of 3

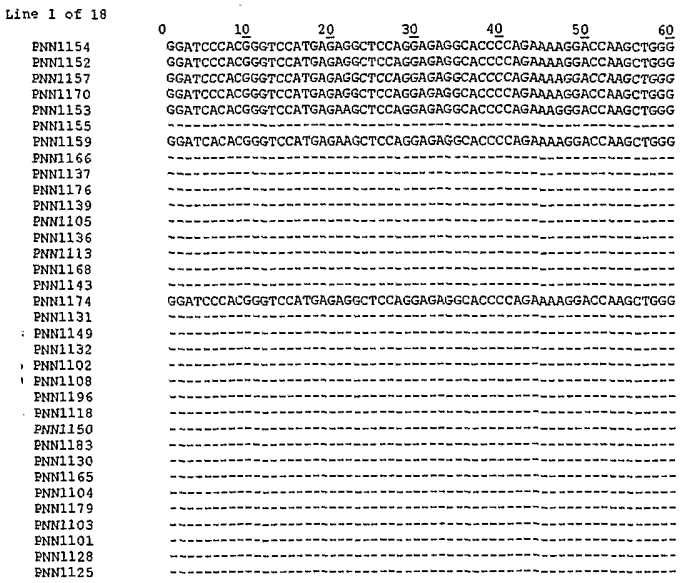
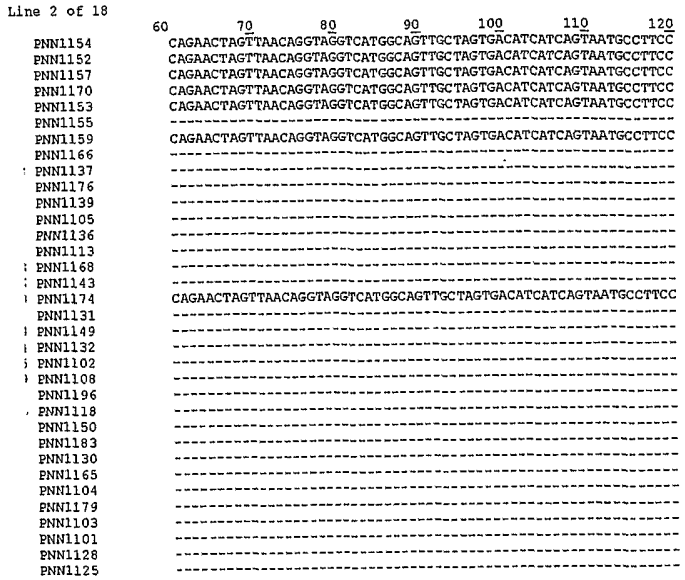


Figure 4b part 1 of 18



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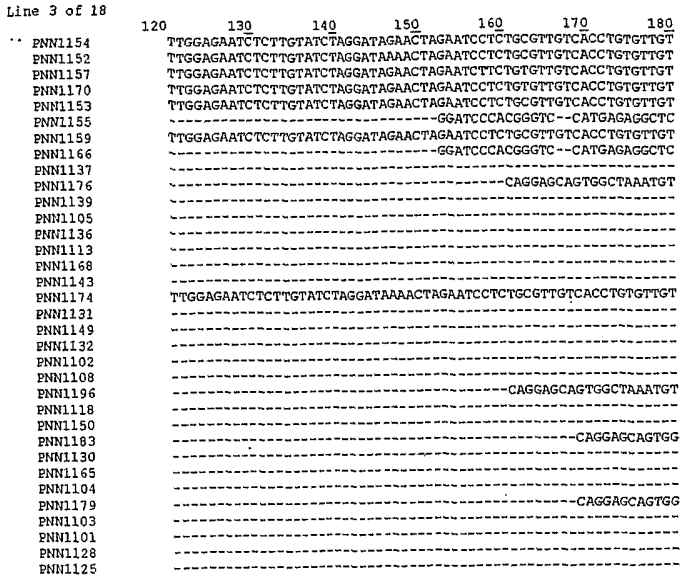

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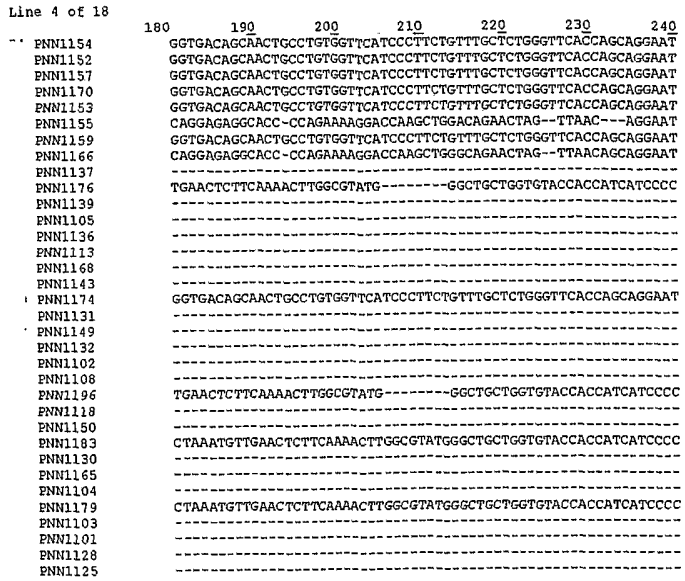

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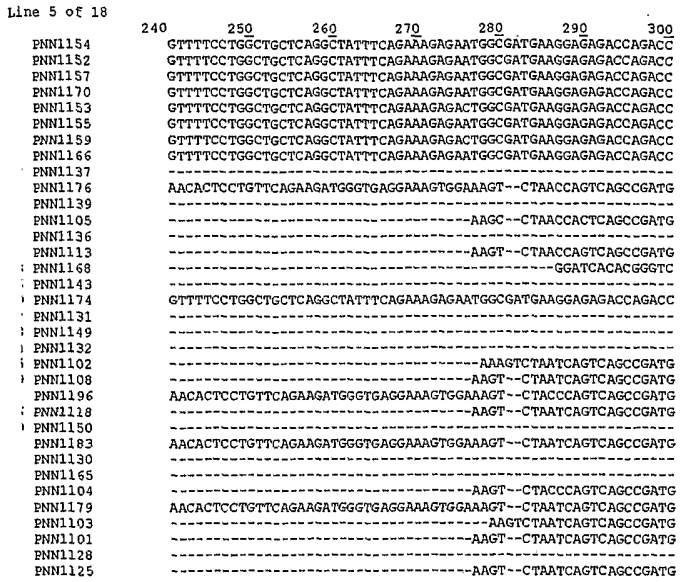

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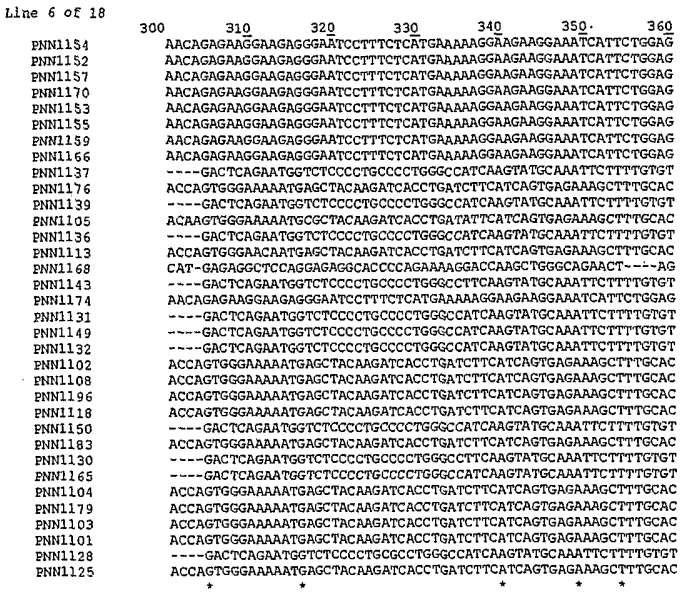

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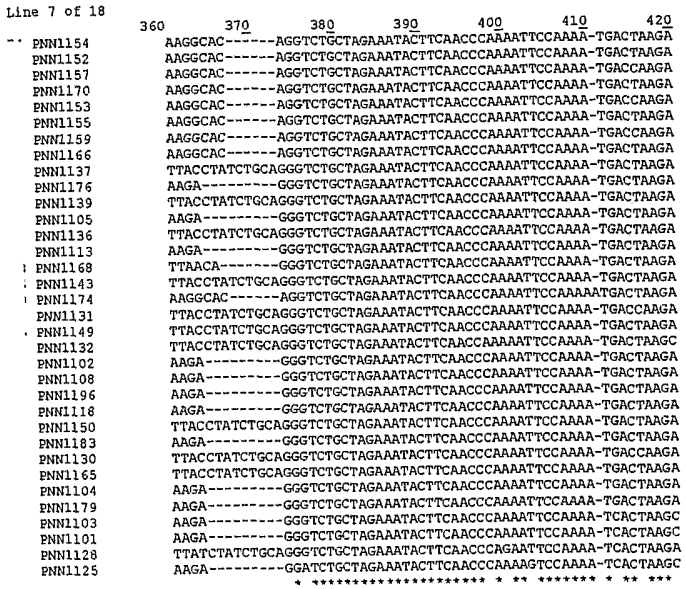
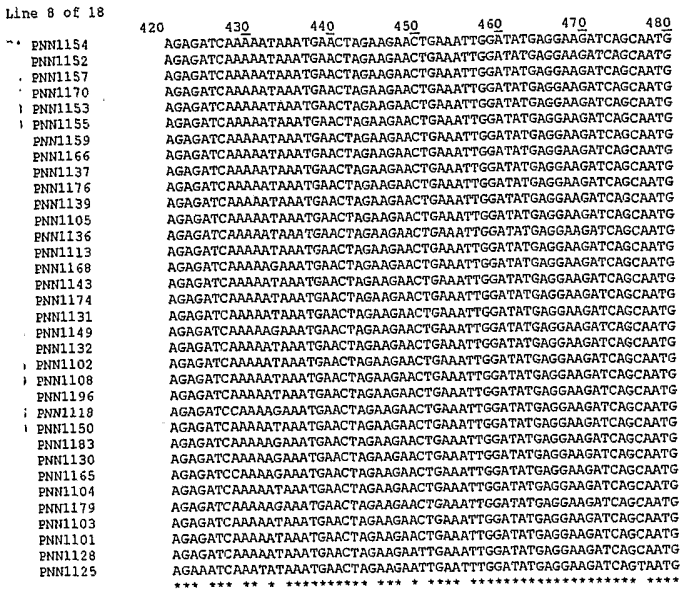


Figure 4b part 7 of 18



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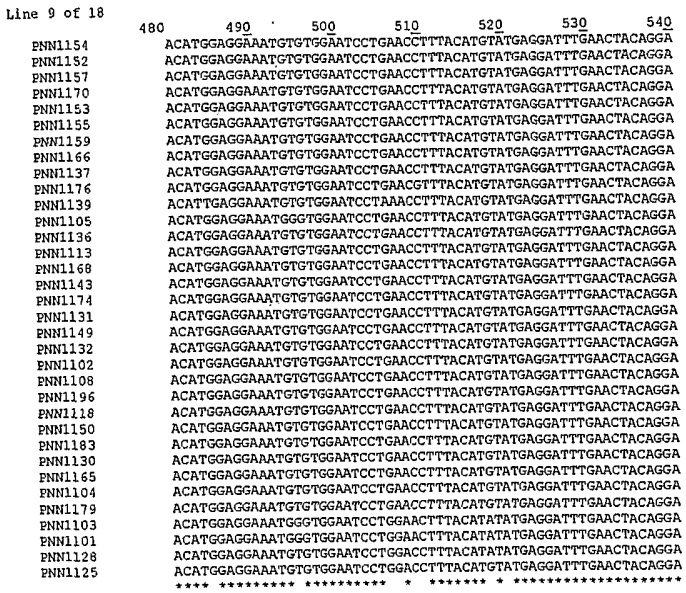

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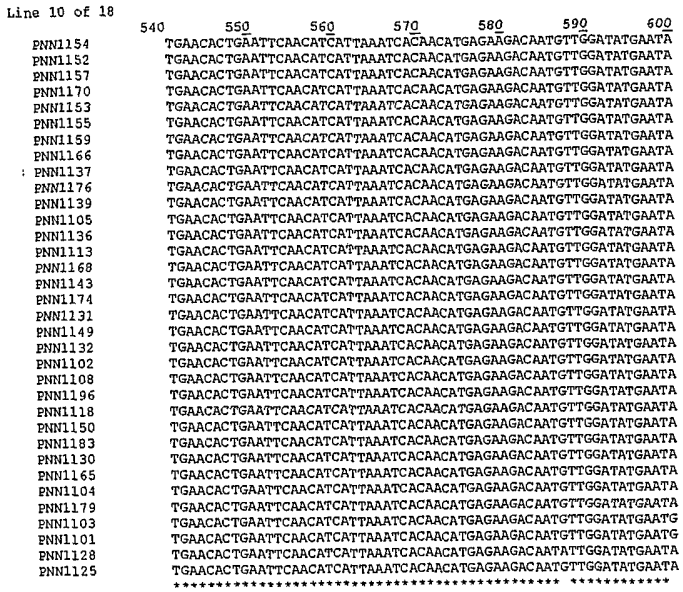
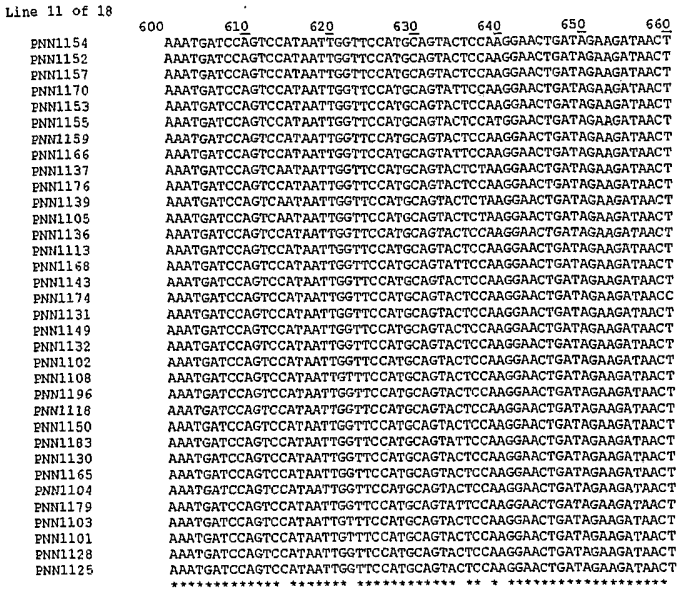


Figure 4b part 10 of 18



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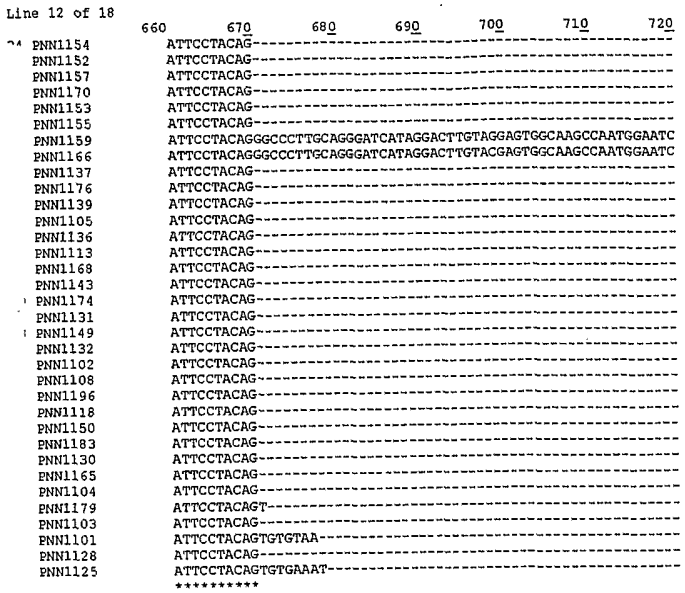

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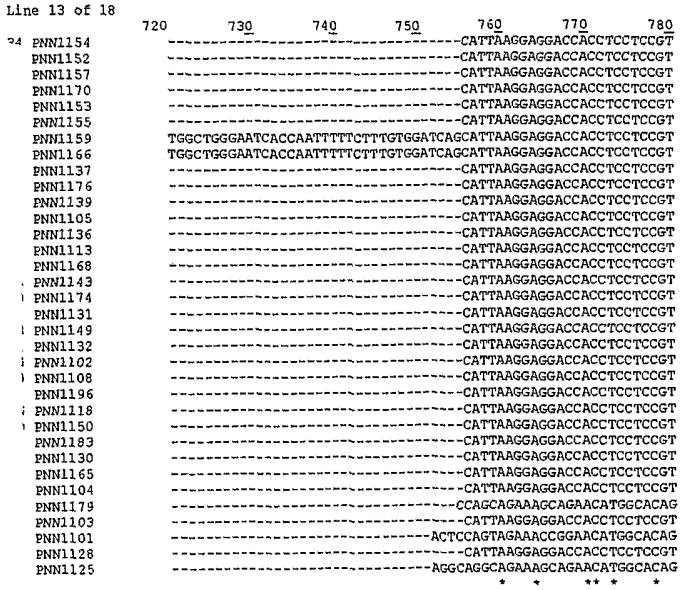

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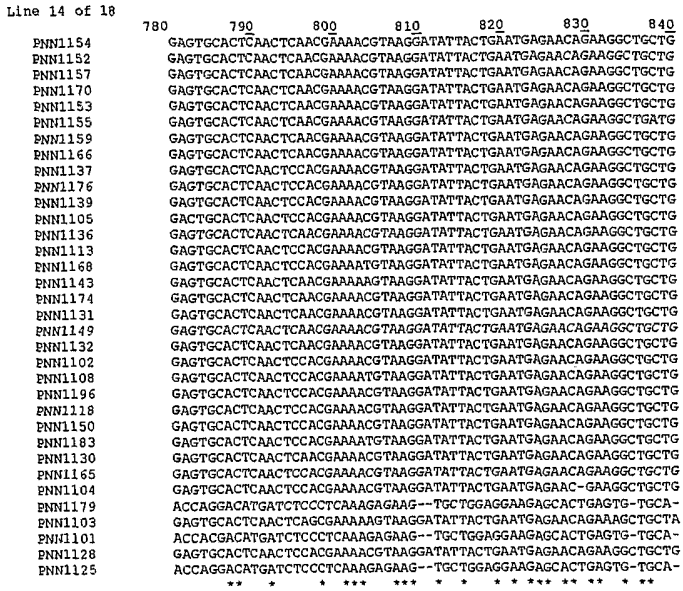

Figure 4b part 14 of 18

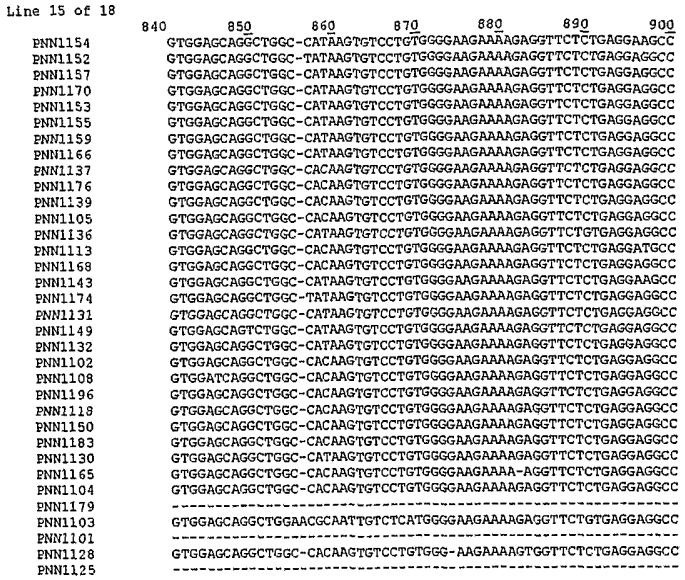

Figure 4b part 15 of 18

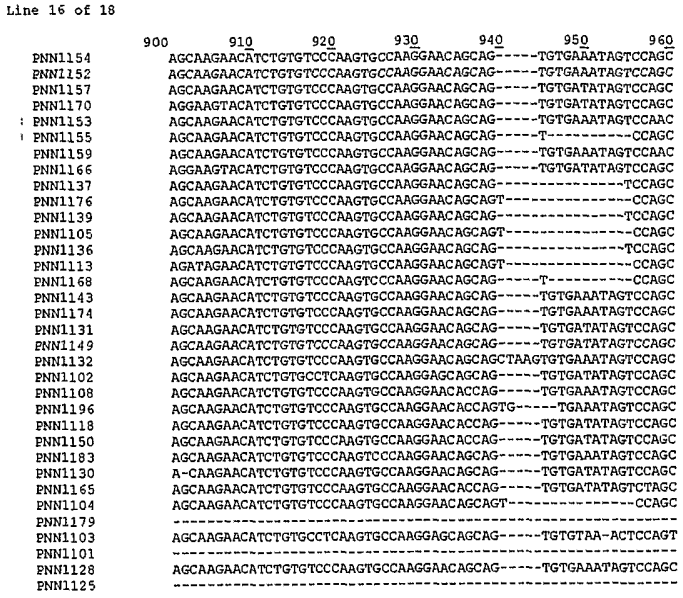

Figure 4b part 16 of 18

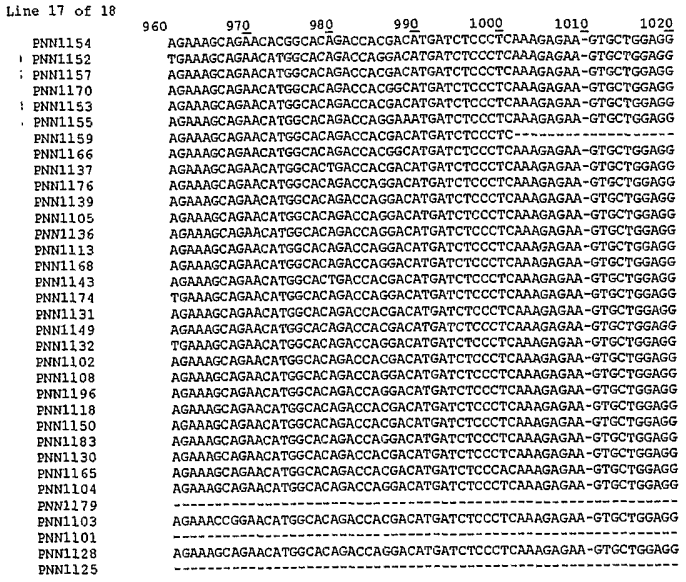

Figure 4b part 17 of 18

```
Line 18 of 18
                1020      1030    1038
   PNN1154          AAGAGCACTGAGTGTGCA
   PNN1152          AAGAGCACTGAGTGTGCA
   PNN1157          AAGAGCACTGAGTGTGCA
   PNN1170          AAGAGCACTGAGTGTGCA
   PNN1153          AAGAGCACTGAGTGTGCA
   PNN1155          AAGAGCACTGAGTGTGCA
   PNN1159          ------------------
   PNN1166          AAGAGCACTGAGTGTGCA
   PNN1137          AAGAGCACTGAGTGTGCA
   PNN1176          AAGAGCACTGAGTGTGCA
   PNN1139          AAGAGCACTGAGTGTGCA
   PNN1105          AAGAGCACTGAGTGTGCA
   PNN1136          AAGAGCACTGAGTGTGCA
   PNN1113          AAGAGCACTGAGTGTGCA
   PNN1168          AAGAGCACTGAGTGTGCA
   PNN1143          AAGAGCACTGAGTGTGCA
   PNN1174          AAGAGCACTGAGTGTGCA
   PNN1131          AAGAGCACTGAGTGTGCA
   PNN1149          AAGAGCACTGAGTGTGCA
   PNN1132          AAGAGCACTGAGTGTGCA
   PNN1102          AAGAGCACTGAGTGTGCA
   PNN1108          AAGAGCACTGAGTGTGCA
   PNN1196          AAGAGCACTGAGTGTGCA
   PNN1118          AAGAGCACTGAGTGTGCA
   PNN1150          AAGAGCACTGAGTGTGCA
   PNN1183          AAGAGCACTGAGTGTGCA
   PNN1130          AAGAGCACTGAGTGTGCA
   PNN1165          AAGAGCACTGAGTGTGCA
   PNN1104          AAGAGCACTGAGTGTGCA
   PNN1179          ------------------
   PNN1103          AGGAGCACTGAGTGTGCA
   PNN1101          ------------------
   PNN1128          AAGAGCACTGAGTGTGCA
   PNN1125          ------------------
```

Figure 4b part 18 of 18

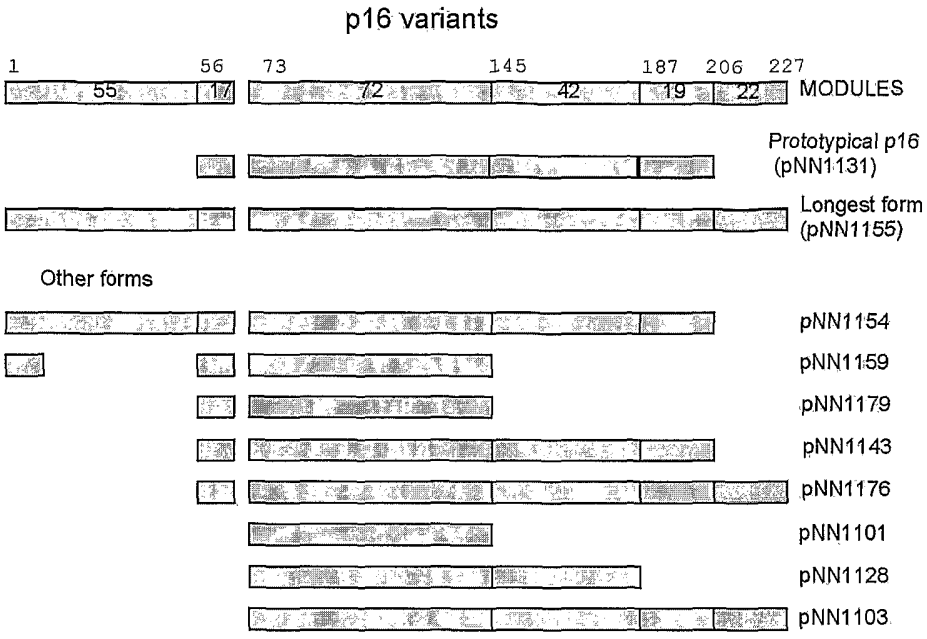
p16 variants
1
56
73
145
187
206
227
55
17
72
42
19
22
MODULES
Prototypical p16
(pNN1131)
Longest form
(pNN1155)
Other forms
pNN1154
pNN1159
pNN1179
pNN1143
pNN1176
pNN1101
pNN1128
pNN1103

Figure 5

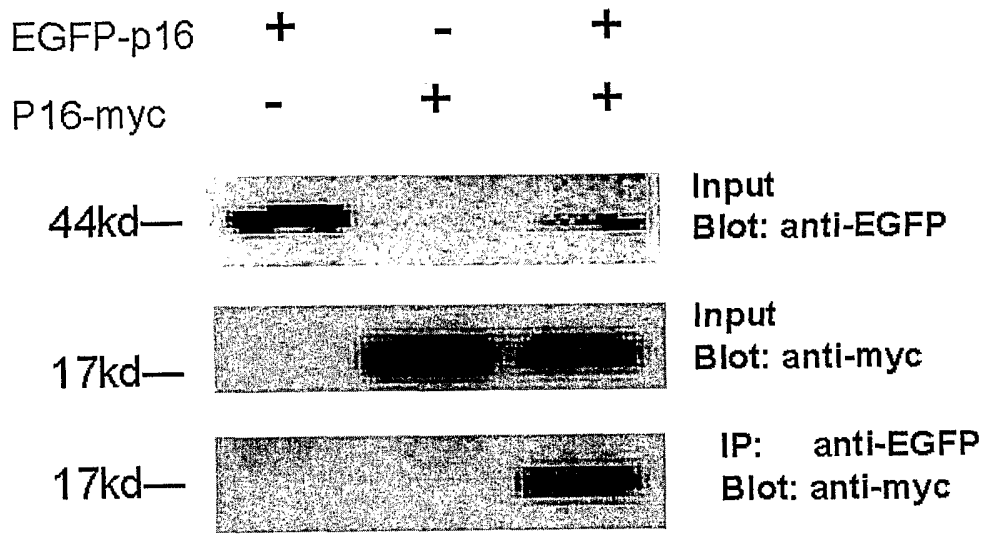
EGFP-p16 + - +
P16-myc - + +
44kd—
Input
Blot: anti-EGFP
17kd—
Input
Blot: anti-myc
17kd—
IP: anti-EGFP
Blot: anti-myc

Figure 6

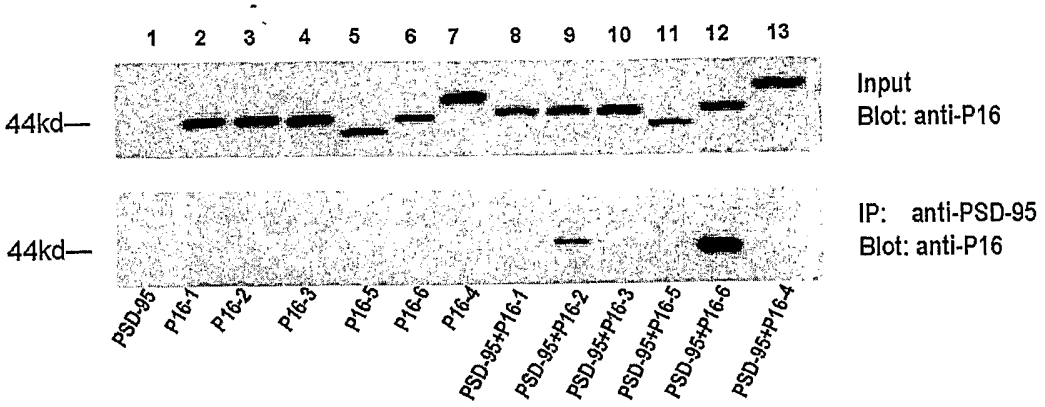
1 2 3 4 5 6 7 8 9 10 11 12 13
44kd—
Input
Blot: anti-P16
44kd—
IP: anti-PSD-95
Blot: anti-P16
PSD-95
P16-1
P16-2
P16-3
P16-5
P16-6
P16-4
PSD-95+P16-1
PSD-95+P16-2
PSD-95+P16-3
PSD-95+P16-5
PSD-95+P16-6
PSD-95+P16-4

Figure 7

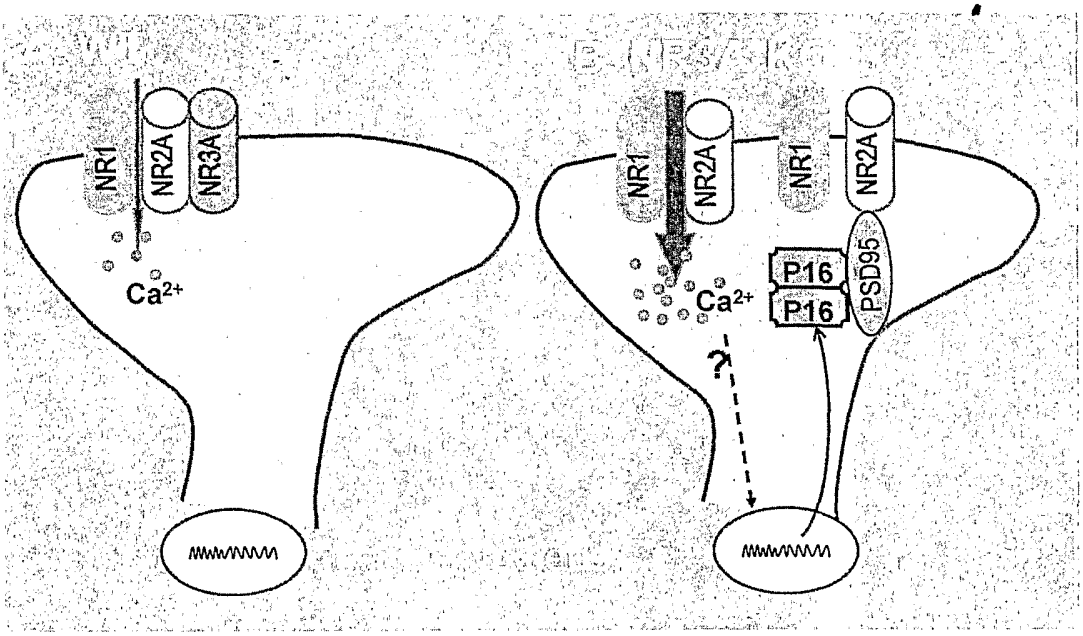

Figure 8