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(54) Title: VARIANT NEURONAL NICOTINIC ALPHA-7 RECEPTOR AND METHODS OF USE

(57) Abstract: The present invention relates to a variant of the nicotinic acetylcholine receptor (nAChR) $\alpha 7$ subunit having a substitution within its second transmembrane (TM2) domain. Specifically, the sixth amino acid position within the TM2 domain has the point mutation T $\rightarrow$ S, such that threonine-244 becomes serine-244. Advantageously, the $\alpha 7$ variant of the present invention retains the essential drug sensitivities of the wild-type $\alpha 7$ receptor, but does not exhibit the response-limiting form of fast desensitization. Therefore, the $\alpha 7$ variant is a "gain of function" mutant that is particularly useful for testing new pharmacological agents. The present invention includes the T6'S variant TM2 domain, T6'S variant $\alpha 7$ subunit, and T6'S variant nACh receptor polypeptides, polynucleotides encoding these polypeptides, recombinant hosts expressing these polynucleotides, and assays utilizing the T6'S variant TM2 domain, T6'S variant $\alpha 7$ subunit, and/or T6'S variant nACh receptor.



WO 2005/074641 A2

DESCRIPTION

VARIANT NEURONAL NICOTINIC ALPHA-7 RECEPTOR

5

AND METHODS OF USE

Cross-Reference to Related Application

This application is a continuation-in-part of U.S. Application Serial No. 10/769,085, filed January 30, 2004, which is hereby incorporated by reference herein in its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences, and drawings.

The subject invention was made with government support under a research project supported by National Institutes of Health Grant No. GM57481-01A2. The federal government may have certain rights in this invention.

Background of Invention

Nicotinic acetylcholine receptors (nAChRs) have long been recognized as the primary postsynaptic effector in vertebrate neuromuscular transmission, as well as having a clearly established role in ganglionic neurotransmission. In recent years, nicotinic receptors have also been identified as being important functional molecules in central neurons (reviewed in (McGehee, D.S. and Role, L.W., *Annu. Rev. Physiol.*, 57:521-546, 1995; Dani, J.A., *Biol. Psychiatry*, 49:166-174, 2001)). There are multiple types of nAChRs in the brain associated with synaptic function, signal processing or cell survival. Based on differences in function, pharmacology, and tissue distribution, it is possible to categorize the larger family of nAChRs broadly into three major subfamilies. These are the muscle-type receptors, the neuronal beta subunit-containing receptors that bind agonist with relatively high affinity in the desensitized state, and the homomeric alpha subunit receptors, typified by $\alpha 7$.

Concomitant with the evolution of these diverse forms of the nAChR and the functional radiation of these receptor subtypes into various tissues where each subtype performs highly specialized functions, a large amount of sequence divergence has

emerged. There is less than 40% amino acid sequence identity between the alpha subunit of muscle type receptors ($\alpha 1$) and the $\alpha 7$ -type receptor found in the brain. It has generally been assumed that the functional differences that exist among these receptor subtypes are emergent properties of the collective sequence differences. Nonetheless, it is well appreciated that specific residues, conserved across multiple subtypes, can be key features common to all those subtypes. Several such foci of important conserved sequence are in the pore-forming second transmembrane domain, and in this domain the sequence identity between $\alpha 1$ and $\alpha 7$ is increased to 60%.

Both muscle-type and homomeric $\alpha 7$ nAChRs are widely expressed in mammals and bind the snake toxin, α -Btx. Despite these similarities, there are numerous important functional and pharmacological differences between the muscle and $\alpha 7$ receptors. For example, $\alpha 7$ receptors have high divalent ion permeability, show inward rectifying current-voltage relationships, and have unique agonist concentration-dependent macroscopic response kinetics (Decker, E.R. and Dani, J.A. 1990, *J. Neurosci.*, 10(10):3413-3420; Sands *et al.*, 1993, *Biophys. J.*, 65:2614-2621; Seguela *et al.*, 1993, *J. Neurosci.*, 13(2):596-604; Vernino *et al.*, 1994, *J. Neurosci.*, 14:5514-5524).

Previous work has indicated that amino acid residues in the TM2 domain of the beta subunit of heteromeric nAChRs are important regulators of nAChR pharmacology. Specifically, substitution of the TM2 6' (according to the numbering scheme proposed by Miller *et al.* (Miller, C. 1989, *Neuron*, 2:1195-1205)) phenylalanine of the muscle $\beta 1$ subunit for the neuronal $\beta 4$ subunit serine residue increased sensitivity to nicotine. This study also showed that substitution of $\beta 1$ subunit amino acid sequence at the TM2 6' and 10' positions of the neuronal $\beta 4$ subunit reduced inhibition by the ganglionic blocker mecamylamine (Webster *et al.*, 1999, *Br. J. Pharmacol.*, 127:1337-1348).

The therapeutic targeting of nicotinic receptors in the brain requires the identification of drugs which may be selective for their ability to activate or inhibit a limited range of these receptor subtypes. An important nAChR in the brain is the $\alpha 7$ subtype, which has high permeability to the calcium ion. This receptor subtype has been implicated to have an important role in improving memory-related behaviors and slowing neuronal degeneration, as may occur in aging or dementia. The activation of $\alpha 7$ receptors has also been suggested as a therapeutic approach for treating schizophrenia, Alzheimer's disease, inflammation, and other pathologies. (Salamone, F. *et al.*, *MJM*,

2000, 5:90-97; Cooper, E.C. and Jan, L.Y. *Proc. Natl. Acad. Sci. USA*, 1999, 96:4759-4766; Sharples, C. and Wonnacott, S. *Neuronal Nicotinic Receptors*, October 2001, 19:1-12; Mihailescu, S. and Drucker-Colin, R. *Arch. Med. Res.*, 2000, 31:131-144; Papke, R.L. *et al.*, *Euro. J. Pharm.*, 2000, 393:179-195; Newhouse, P.A. and Kelton, M. *Pharm. Acta Helv.*, 2000, 74:91-101; Newhouse, P.A. *et al.*, *Clin. Pharm.*, 1997, 11:206-228; Lloyd, G.K. and Williams, M. *J. Pharm. Exp. Therapies*, 2000, 292:461-467; Hollady, M.W. *et al.*, *J. Med. Chem.*, 1997, 40:4169-4194; Benowitz, N.L. *Annu. Rev. Pharm. Toxicol.*, 1996, 36:597-613; Freedman, R. *et al.*, *Harvard Rev. Psych.*, 1994, 2:179-192; and Freedman, R. *et al.*, *J. Chem. Neuroanatomy*, 2000, 20:299-306).

10 The development of drugs for targeting the $\alpha 7$ receptor therapeutically has been impeded by the fact that the wild-type receptor exhibits a unique concentration-dependent fast desensitization that makes it difficult to study with large-scale drug screens. It would be advantageous to have available a variant of the wild-type $\alpha 7$ nAChR that does not exhibit the response-limiting form of fast desensitization. Variant $\alpha 7$ nAChRs have been
15 reported previously (Galzi JL *et al.*, *Nature*, 359:500-505, 1992; Bertrand D. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 90(15):6971-6975, 1993; Revah F. *et al.*, *Nature* 353:846-849, 1991; Hogg R.C. *et al.*, *J. Biol. Chem.*, 278(29):26908-26912, July 2003, Epub May 13, 2003; Tonini R. *et al.*, *Neuropharmacology*, 44(6):765-771, May 2003; Eddins D. *et al.*, *Am. J. Cell Physiol.*, 283(5):C1454-C1460, 2003; Lyford L.K. *et al.*, *Biochem Biophys. Acta*. 10;1559(1):69-78, 2002; Orr-Urtreger A *et al.*, *J. Neurochem.*, 74(5):2154-2166,
20 2000; Palma E, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 96(23):13421-13426, 1999; Maggi L. *et al.*, *Mol. Psychiatry*, 3(4):350-355, 1998; Bertrand S. *et al.*, *Neuroreport*, 8(16):3591-3596, 1997; Palma E. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 94(4):1539-1543, 1997; Palma E. *et al.*, *Proc. Natl. Acad. Sci. U S A.*, 93(20):11231-11235, 1996; Bertrand D. *et al.*,
25 *Proc. Natl. Acad. Sci. U.S.A.*, 89(4):1261-1265, 1992; U.S. Patent No. 6,323,000 (Briggs *et al.*), filed December 20, 1996; and U.S. Patent No. 6,683,157 (Briggs *et al.*), filed September 18, 2001.

“Gain of function” mutants of the $\alpha 7$ receptor have previously been described; however, these mutants respond very differently to experimental drugs from wild-type
30 receptors. The ideal “gain of function” mutant would retain the essential drug sensitivities of the wild-type native $\alpha 7$ receptors and, therefore, provide a much sought after model for testing new drugs.

Brief Summary of the Invention

The present invention relates to a variant of the nicotinic acetylcholine receptor (nAChR) $\alpha 7$ subunit having a substitution at the 6' position of the second transmembrane domain (referred to as M2 or TM2). Specifically, a threonine has been substituted with serine. For example, in the case of the rat (*Rattus norvegicus*) $\alpha 7$ subunit, threonine-244 has been substituted with serine. In accordance with the numbering scheme proposed by Miller (*Neuron*, 1989, 2:1195-1205), the variant is referred to herein as the T6'S mutant or T6'S variant (because the point mutation T $\rightarrow$ S is in the sixth amino acid position of the TM2 domain).

Thus, the nucleotide sequence encoding the T6'S variant differs from the coding sequence of the wild-type rat $\alpha 7$ subunit in containing a codon (sequence of three nucleotides) encoding serine, instead of threonine, at nucleotides 799 to 781 of the coding sequence (nucleotides 733-735 excluding signal sequence).

The T6'S variant of the subject invention exhibits pharmacological and electrophysiological characteristics that are very similar to the wild-type $\alpha 7$ subunit. However, unexpectedly, the T6'S variant exhibits much slower desensitization in comparison to the wild-type $\alpha 7$ subunit and is, therefore, a "gain of function" mutant. In fact, the T6'S variant of the present invention substantially eliminates the fast desensitization rate observed in the wild-type $\alpha 7$ subunit. This distinguishing feature, combined with the variant's similarities to the wild-type $\alpha 7$ subunit, makes the T6'S variant particularly useful for drug screening purposes.

Accordingly, one aspect of the present invention is an isolated polynucleotide comprising a nucleotide sequence that encodes a variant of the nAChR TM2 domain of the $\alpha 7$ subunit, wherein the variant of the nAChR TM2 domain comprises the amino acid sequence of SEQ ID NO:2, or a functional fragment or homologue thereof, wherein the functional fragment or homologue contains the T $\rightarrow$ S substitution at the T6'S position. In one embodiment, the polynucleotide comprises the nucleic acid sequence of SEQ ID NO:18. In other embodiments, the polynucleotide comprises the nucleic acid sequence of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, each of which are degenerate variants of SEQ ID NO:18.

The present invention further includes an isolated polynucleotide comprising a nucleotide sequence that encodes a variant of the wild-type nAChR $\alpha 7$ subunit. In one embodiment, the polynucleotide comprises the nucleic acid sequence of SEQ ID NO:4 or the contiguous nucleotides 67 to 1509 of SEQ ID NO:4. In other embodiments, the polynucleotide is a degenerate variant of SEQ ID NO:4. For example, the degenerate variant can comprise the nucleic acid sequence of SEQ ID NO:26 or the contiguous nucleotides 67 to 1509 of SEQ ID NO:26; or SEQ ID NO:27 or the contiguous nucleotides 67 to 1509 of SEQ ID NO:27; or SEQ ID NO:28 or the contiguous nucleotides 67 to 1509 of SEQ ID NO:28; or SEQ ID NO:29 or nucleotides 67 to 1509 of SEQ ID NO:29; or SEQ ID NO:30 or the contiguous nucleotides 67 to 1509 of SEQ ID NO:30.

In another aspect, the present invention includes an isolated polypeptide that is a variant of the wild-type TM2 domain of the $\alpha 7$ subunit (SEQ ID NO:1), wherein the variant domain comprises the amino acid sequence of SEQ ID NO:2.

The present invention further includes isolated polypeptides that are variants of the wild-type nAChR $\alpha 7$ subunits of rat (amino acids 23 to 502 of SEQ ID NO:5), human (amino acids 23 to 502 of SEQ ID NO: 7), mouse (amino acids 23 to 502 of SEQ ID NO: 9) monkey (amino acids 23 to 502 of SEQ ID NO: 11), cow (amino acids 23 to 502 of SEQ ID NO: 13), chicken (amino acids 23 to 502 of SEQ ID NO: 13), zebra fish (amino acids 23 to 502 of SEQ ID NO: 15) wherein the variants of the wild-type nAChR $\alpha 7$ subunit comprise the contiguous amino acids 23 to 502 of SEQ ID NO:20, or a functional fragment or homologue thereof, wherein the functional fragment or homologue contains a TM2 domain with the T $\rightarrow$ S substitution at the T6'S position (*e.g.*, functional fragments or homologues comprising the amino acid sequence of SEQ ID NO:2).

In other aspects, the present invention concerns an isolated nAChR (also referred to herein as the T6'S variant nAChR) comprising the T6'S variant TM2 domain (SEQ ID NO:2) or a T6'S variant $\alpha 7$ subunit (such as the contiguous amino acids 23 to 502 of SEQ ID NO:20), and an isolated polynucleotide encoding the T6'S variant nAChR or $\alpha 7$ subunit. The isolated nAChR may comprise other wild-type nAChR subunits and/or nAChR variant subunits, in addition to the T6'S variant $\alpha 7$ subunit of the present invention.

In another aspect, the present invention concerns a recombinant vector comprising a polynucleotide of the invention, such as a vector comprising a nucleotide sequence encoding the T6'S variant, or encoding a variant $\alpha 7$ nAChR subunit having the T6'S variant TM2 domain, or encoding a variant nAChR comprising a variant $\alpha 7$ subunit having the T6'S variant TM2 domain. In another aspect, the present invention concerns genetically modified host cells that have been transformed or transfected with vectors comprising a polynucleotide of the invention, including genetically modified progeny of such cells. In another aspect, the present invention concerns methods of producing recombinant polypeptides for the treatment of neurological conditions (*e.g.*, neurodegenerative processes), enzymatic function, affective disorders, and immunofunction, using such genetically modified cells. In another aspect, the present invention concerns methods of treating pathologic conditions, such as neurological disorders, by administering to a subject a polynucleotide encoding a T6'S variant of the present invention, or a genetically modified cell of the invention that comprises and expresses the polynucleotide *in vivo*, or a polypeptide of the invention comprising the T6'S variant TM2 domain.

In another aspect, the present invention concerns agents (*e.g.*, antisense polynucleotides, compounds), such as antagonists, which are useful in treating conditions such as neurological conditions (*e.g.*, neurodegenerative processes), enzymatic function, affective disorders, and immunofunction. Methods of treating patients using these antisense polynucleotides and compounds are also provided.

In another aspect, the present invention pertains to methods and reagents for detecting the T6'S variant in a sample *in vitro*, or *in vivo*.

In another aspect, the present invention is directed to a method of expressing a polynucleotide of the subject invention in a cell. In one embodiment, a polynucleotide encoding the T6'S variant is expressed in the cell. In another embodiment, a polynucleotide encoding a variant $\alpha 7$ nAChR subunit having the T6'S variant TM2 domain is expressed in the cell.

In another embodiment, a polynucleotide encoding an nAChR comprising the T6'S variant subunit is expressed in the cell.

In another aspect, the present invention is directed to a method of identifying agents that modulate the activity of the T6'S variant and receptors containing the variant

subunit, and to a method of identifying cytoprotective or other therapeutic agents, using recombinant cells of the present invention.

Brief Description of Drawings

5 **Figure 1** shows a rendering of the nicotinic acetylcholine receptor (nAChR) accompanied by a hydrophobicity plot of the $\alpha 7$ nAChR subunit.

Figure 2 shows the amino acid sequences of the wild-type $\alpha 7$ TM2 domain, wild-type $\beta 2$ TM2 domain, wild-type $\beta 4$ TM2 domain, wild-type $\beta 1$ TM2 domain, and two $\alpha 7$ TM2 mutants having amino acid substitutions at the sixth and tenth amino acid positions
10 of the TM2 domain. The numbering of specific residues of the second membrane spanning region is in accordance with that proposed by Miller *et al.* (*Neuron*, 2:1195-1205, 1989).

Figure 3 shows acetylcholine (ACh) evoked responses from oocytes expressing wild-type $\alpha 7$ (300 μ M ACh), rat $\alpha 7$ TM2 T6'F (3 μ M ACh), rat $\alpha 7$ TM2 T6'S (100 μ M ACh). The inset represents the same current traces normalized to one another for the
15 purpose of comparison.

Figures 4A-4C show ACh evoked normalized responses from oocytes expressing wild-type $\alpha 7$, rat $\alpha 7$ TM2 T6'S, and rat $\alpha 7$ TM2 T6'F, respectively, at the ACh concentrations indicated.

20 **Figures 5A-5C** show electrophysiological characterization of wild-type and mutant $\alpha 7$. Figures 5A and 5B demonstrate that the T6'F mutant receptor shows a nearly total loss of permeability to barium. Figure 5C demonstrates that the T6'S mutant exhibits a confounding biphasic current that occurs near the chloride reversal potential.

Figures 6A-6D show electrophysiological traces demonstrating that substitution
25 of the muscle $\beta 1$ subunit phenylalanine at the TM2 6' position confers the predicted sensitivity to succinylcholine, whereas the T6'S mutant is similar to wild-type $\alpha 7$.

Figure 7 shows the concentration-response relationships for wild-type $\alpha 7$ and TM2 6' mutants showing selectivity of succinylcholine (SuCh) for the T6'F mutant.

Figures 8A and 8B show ACh evoked responses from GH4C1 cells expressing
30 the T6'S mutant and the wild-type $\alpha 7$ receptor, respectively.

Brief Description of Sequences

SEQ ID NO:1 is the amino acid sequence of the TM2 domain of the wild-type $\alpha 7$ subunit (the threonine in the sixth amino acid position of TM2 is underlined and bold for reference), which has 100% homology among *Homo sapiens* (human), *Mucaca mulatto* (rhesus monkey), *Rattus norvegicus* (rat), *Mus musculus* (mouse), *Bos taurus* (domestic cow), *Gallus gallus* (chicken), *Danio rerio* (zebrafish):

ISLGI**T**VLLSLTVFMLLVAE

SEQ ID NO:2 is the amino acid sequence of the T6'S variant of the second transmembrane (TM2) domain (the serine in the sixth amino acid position of TM2 is underlined and bold for reference):

ISLGI**S**VLLSLTVFMLLVAE

SEQ ID NO:3 is the nucleotide coding sequence of the TM2 domain of the wild-type rat $\alpha 7$ subunit (the codon encoding the threonine at the sixth amino acid of TM2 is underlined and bold for reference):

atctctcttgggaata**act**gtcttactttctctgactgtcttcatgctgcttggctgag

SEQ ID NO:4 is the nucleotide coding sequence of the wild-type rat $\alpha 7$ subunit, including nucleotides 1-66 (underlined), which encode a signal peptide that is not present in the mature $\alpha 7$ receptor subunit (the TM2 domain at nucleotides 784-843 is underlined and the codon for the sixth amino acid (6') of TM2 (threonine) is bold for reference):

atgtgcggcgggcggggagggcatctggctggctctggccgcgccgctgctgcacgtgtccctgcaaggcgagttccagagga
ggctgtacaaggagctggtcaagaactacaacccgctggagaggccggtggccaacgactcgcagccgctaccgtgtacttc
tcctgagctcctgcagatcatggatgtggatgagaagaaccaagtttaaccaccaacatttggctacaaatgtcttggacagat
cactatttgcagtgaacatgtctgagtaccccgagtgagaagaatgttcgtttccagatggccagatttggaaaccagacatttc
ctctataacagtgtgatgagcgcttggatgccacgttccacaccaatgtttggatgaatgcactctgggcattgccagtatctccctcc
aggcatattcaagagctcctgctacattgacgttcgctggttccctttgatgtgcagcagtgcaaaactgaagtgtggctcctggtcc
tatggagggtggtcactggacctgcaaatgcaagggcagatatcagcagctatatcccaacggagaatgggatctcatggga
atccctggcaaaagggaatgagaagttctatgagtgtcgaagagccataccagatgtcacctacacagtaaccatgcgccgt
aggacactctactatggcctcaatctgctcacccttgtgtactcatttcagccctggctctgctggtatttctgctgctgcagactct
ggagagaaaaatctctcttgggaata**act**gtcttactttctctgactgtcttcatgctgcttggctgagatcatgccagcaacatctga
ttctgtgcccttgatagcacaatacttcgccagcaccatgatcatcgtggccctctctgtagtggtgacagtgattgtgctgagatat
caccacatgaccctgatggtggcaaaatgcctaagtggaccagaatcattctcctgaactggtgtgcatggtttctgcgcataaa
gaggcccgagaggacaaggtgcggccagctgtcagcacaagcctcggcgctgcagcctggccagtggtggagctgagtgcc
aggtgctggggccaccaccagcaatggcaacctgctctacattggcttccgaggcctggagggcagtgactgtgccccaaactcc
agactctggggctgtagtggtggccgttggcctgctcccaacacatgatgagcacctcatgcacgggtgcacacccctctgatggg

gaccccgacctggccaagatcctggaggaggtccgctacatcgccaaccgcttcgctgccaggacgagagtgaggtgatct
gcagtgaatggaagtttcagcctgcgtggtggaccgcttgccatggccttttcggtctttaccatcatctgtaccatcgccat
cctcatgtcagctccaaactttgtggaggctgtgtccaaagactttgcttaa

- 5 (NCBI Accession No. NM_012832; Khirg S. *et al.*, *J. Physiol.* (Lond.) 540(Pt. 2):425-434, 2002; Oshikawa J. *et al.*, *Am. J. Physiol.*, Cell Physiol., 285(3):C567-C574, 2003; which are incorporated herein by reference in their entireties)

SEQ ID NO:5 is the amino acid sequence of the wild-type rat $\alpha 7$ subunit, including
10 amino acids 1-22 (underlined), which represent a signal peptide that is not present in the mature receptor subunit (the TM2 domain at amino acids 262-281 is underlined and the 6' threonine of TM2 is bold for reference):

MCGGRGGIWLALAAALLHVSLQGEFQRRLYKELVKNYNPLERPVANDSQPLTV
YFSLSLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNMSEYPGVKNVRFDPGQ
15 IWKPDILLYNSADERFDATFHTNVLVNASGHCQYLPPGIFKSSCYIDVRWFPPDV
QQCKLKFGWSYGGWSLDLQMQEADISSYIPNGEWDLMGIPGKRNEKFYECCKE
PYPDVITYTVMRRRTLYYGLNLLIPCVLISALALLVFLLPADSGEKISL**GITV**LLSL
TVFMLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVVTVIVLRYHHHDPDGGKM
PKWTRIILLNWCAWFLRMKRPGEDKVRPACQHKPRRCSLASVELSAGAGPPTS
20 GNLLYIGFRGLEGMHCAPTDSGVVCGRLACSPTHDEHLMHGAHPSDGDPLAK
ILEEVRYIANRFRQCDESEVICSEWKFAACVVDRLCLMAFSVFTIICTIGILMSAPN
FVEAVSKDFA

The $\alpha 7$ subunit protein has approximately 90% homology among rat, chick, and
25 humans.

SEQ ID NO:6 is the nucleotide coding sequence of the wild-type human $\alpha 7$ subunit,
including nucleotides 1-66 (underlined), which encode a signal peptide that is not present
in the mature $\alpha 7$ receptor subunit (the TM2 domain at nucleotides 784-843 is underlined
30 and the codon for the 6' amino acid of TM2 (threonine) is bold for reference):

atgcgctgctcgccgggaggcgtctggctggcgctggccgctgcctcctgcacgtgtccctgcaaggcgagttccagaggaa
gctttacaaggagctggtcaagaactacaatcccttgagagggccgctggccaatgactcgcaaccactaccgtctacttcc
ctgagcctcctgcagatcatggacgtggatgagaagaaccaagtttaaccaccaacatttggtgcaaatgtcttgacagatca
ctatttacagtggaatgtgcagaataccaggggtgaagactgttcgttccagatggccagatttggaaccagacattctctc

tataacagtgtgatgagcgctttgacgccacattccacactaacgtgttggtgaattcttctgggcattgccagtacctgcctccag
 gcatattcaagagttcctgctacatcgatgtacgtggttccctttgatgtgcagcactgcaaactgaagtttgggtcctggtcttac
 ggaggctggctccttgatctgcagatgcaggaggcagatatcagtggttatatccccaatggagaatgggacctagtgggaatc
 cccggcaagaggagtgaaggttctatgagtgtgcaaagagccctaccccgatgtcaccttcacagtgacctatgcgccgag
 5 gacgtctactatggcctcaacctgtgatccctgtgtgctcatctccgccctcgccctgctggtgttctgcttctgcagattcc
 ggggagaagatttccctggggata**acag**tttactctcttaccgtcttcatgctgctcgtggctgagatcatgcccgcaacatcc
 gattcgggtaccattgatagcccagttctgccagcaccatgatcatcgtgggcctctcggtggtggtgacgggtatcgtgctgca
 gtaccaccaccacgaccccgacgggggcaagatgcccaagtggaccagagtcaccttctgaactggtgctgctggttctgc
 gaatgaagaggcccgaggagacaaggtgccccggcctgccagcacaagcagcggcgctgcagcctggccagtgtgga
 10 gatgagcgccgtggcgccgcccggcagcaacgggaacctgctgtacatcggttccggcgccctggacggcgctgacgtg
 gtcccgacccccactctgggtagtgtgtggccgatggcctgctccccacgcacgatgagacctctgcacggcgggc
 aacccccgagggggacccggacttgccaagatcctggaggaggtccgctacattgccaaccgttccgctgccaggacga
 aagcgaggcggtctgcagcgagtgaagttcgccgctgtgtggtggaccgctgtgctcatggccttctcggttcttaccatc
 atctgcaccatcggtcctgatgtcggtcccaacttcgtggaggccgtgtccaaagactttgcgtaa

(NCBI Accession No. NM000746; Chini B. *et al.*, *Genomics* 19(2):379-381, 1994;
 Burhaus L. *et al.*, *Parkinsonism Relat. Disord.*, 9(5):243-246, 2003; which are
 incorporated by reference herein in their entireties)

20 SEQ ID NO:7 is the amino acid sequence of the wild-type human $\alpha 7$ subunit, including
 amino acids 1-22 (underlined), which represent a signal peptide that is not present in the
 mature receptor subunit (the TM2 domain at amino acids 262-281 is underlined and the
 codon for the 6' amino acid of TM2 (threonine) is bold for reference for reference):

MRCSPGGVWLALAASLLHVSLQGEFQRKLYKELVKKNYNPLERPVANDSQPLTV
 25 YFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDGQI
WKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPDVQ
HCKLKFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGIPGKRSERFYECCKEP
YPDVTFTVTMRRRTLYYGLNLLIPCVLISALLVFLLPADSGEKISLGITVLLSLT
VFMLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVTVIVLQYHHHDPDGKMP
 30 KWTRVILLNWCAWFLRMKRPGEDKVRPACHKQRRCSLASVEMSAVAPPPASN
GNLLYIGFRGLDGVHCVPTPDSGVVCGRMACSPTHDEHLLHGGQPPEGDPDLAK
ILEEVRYIANRFRCDESEAVCSEWKFAACVDRLCLMAFSVFTICTIGILMSAP
NFVEAVSKDFA

35 SEQ ID NO:8 is the nucleotide coding sequence of the wild-type mouse $\alpha 7$ subunit,
 including nucleotides 1-66 (underlined), which encode a signal peptide that is not present
 in the mature $\alpha 7$ receptor subunit (the TM2 domain at nucleotides 784-843 is underlined
 and the codon for the 6' amino acid of TM2 (threonine) is bold for reference):

atgtgcggccggcggggaggcatctggctggctctggccgcgcgctgctgcacgtgtccctgcaaggcgagttccagagga
 40 ggctgtacaaggagctggtcaagaactacaacccgctggagaggccggtggccaacgactgcagccgctcaccgtgtacttc
tccctgagcctcctgcagatcatggatgtggatgagaagaaccaagtttaaccaccaacatttggttcaaatgtcttgacagat

cactatttgcagtggaaacatgtctgagtaccccgagtgaaaaatgttcgtttccagatggccagatttgaaaccagacattctc
 ctctataacagtgcagatgaacgctttgatgccacattccacaccaacgtcttggtgaatgcattctggcattgccagtatctccctc
 caggcatattcaagagctcctgctacatcgatgtacgctggtccctttgatgtgcagcagtgcaaactgaagttgggtcctggtc
 ctatggagggtggccttgacatgcagatgcaagaggcagatatcagcagctatatcccaatggagaatgggatctcatggg
 5 aatccctggcaaaaggaatgagaagttctatgaatgtgcaaagagccataccagatgtcacctacacagtaaccatgcgccct
 aggacactctactatggcctcaacctgctcattcctgtgtgctcattcagccctggctttgctggtattcttgcctgcagactct
 ggagagaaaactctcttgaataactgtcttactttctgactgtcttcatgctgcttggctgagatcatgccagcaacatctga
 ttccgtgcccttgatagcacagtacttcgccagcaccatgatcatcgtgggcctctcagtggctcgtgacagtgattgtgctgcgata
 tcaccaccatgacctgatggtggcaaaatgcctaagtgaccaggaatcattcttgaattggtgtgcatggttctgcgcatgaa
 10 gaggccgggagaggacaaggtgcggccggctgtcagcacaagcctcggcgctgcagcctggccagtggtgagctgagcg
 caggtgcggggccacctccagcaatggcaacctgctctacattggcttccgaggcctggagggcatgactgtgccccaaact
 ccagactctggggctgctgtgtggtcgtttggcctgctcccaacacatgatgagcacctcatgcatggtacacacctctgatgg
 ggaccccgacctggccaagatcctggaggaggtccgctacatgcccaaccgctccgctgccaggatgagagtgaggtgatct
 gcagtgagtggaaagtttgcggcctgtgtggtggaccgctgtgcctcatggccttttcggttttaccatcatctgcaccatcggcat
 15 cctcatgtcagcccaaaactttgtggaggctgtgtccaaagactttgcttaa

(NCBI Accession No. NM_007390; Orr-Urtreger A. *et al.*, *Genomics* 26(2):399-402, 1995; Saragoza P.A. *et al.*, *Brain Res. Mol. Brain Res.* 117(1):15-26, 2003; which are incorporated by reference herein in their entireties)

SEQ ID NO:9 is the amino acid sequence of the wild-type mouse $\alpha 7$ subunit, including amino acids 1-22 (underlined), which represent a signal peptide that is not present in the mature receptor subunit (the TM2 domain at amino acids 262-281 is also underlined for reference):

MCGRRRGGIWLALAAALLHVS**LQGEFQRRLYKELVKNYNPLERP**VANDSQPLTV
 YFSLSLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNMSEYPGVKNVRFPDQG
 IWKPDILLYNSADERFDATFHTNVLVNASGHCQYLPPGIFKSSCYIDVRWFPFDV
 QQCKLKFGSWSYGGWSLDLQMQEADISSYIPNGEWDLMGIPGKRNEKFYECCKE
 30 PYPDVITYVTMRRRTLYYGLNLLIPCVLISALALLVFLPADSGEKISL**GITVLLSL**
TVFMLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVVTVIVLRYYHHHDPDGGKM
 PKWTRIILLNWCAWFLRMKRPGEDKVRPACQHKPRRCSLASVELSAGAGPPSSN
 GNLLYIGFRGLEGMHCAPTDSGVVCGRLACSPHDEHLMHGTHPSDGDPLAK
 ILEEVRYIANRFRQCDESEVICSEWKFAACVVDRLCLMAFSVFTI**ICTIGILMSAPN**
 35 FVEAVSKDFA

SEQ ID NO:10 is the nucleotide coding sequence of the wild-type rhesus monkey $\alpha 7$ subunit, including nucleotides 1-66 (underlined), which encode a signal peptide that is not present in the mature $\alpha 7$ receptor subunit (the TM2 domain at nucleotides 784-843 is underlined and the codon for the 6' amino acid of TM2 (threonine) is bold for reference):

atgcgctgctcgaggaggcgtctggctggctctggccgcgtcgctcctgcatgtgtccctgcaaggcgagttccagaggaa
gctttacaaggagctggtaagaactacaaccccttgagaggcccgaggccaatgactcgcaaccgctcaccgtctacttctcc
ctgagcctcctgcagatcatggacgtggatgagaagaaccaagtttaaccaccaacatttggctgcaaatgtcttggacagatca
ctatttacagtgaatgtgtcagaatatccaggggtgaagactgttcgtttccagatggccagatttgaaaccagacattctctc
5 tataacagtgcggatgagcgctttgacgccacattccacaccaacgtgttggaattcttctgggcattgccagtacctgcctcca
ggcatattcaagagttcctgtacatcgatgtgcgtggtttccctttgatgtgcagcattgcaaaactgaagtttggatcctggtcttat
ggaggctggccttggatctgcagatgcaggaggcagatatcagtggctatatccccagtgagaaatgggacctagtgggaatt
cccggaaggaggagtgaagttctatgagtgtgcaagagccctacccgatgtcaccttcacagtgacctgcgccgcag
gacctctactacggcctcaacctgctgatccctgtgtgctcatcttgccttgcctgtggtgttctgttctgcagattccg
10 gggagaagatttccctggggata**acag**tcttactctctcactgtcttcatgctgctcgtggctgagatcatgccgcaacatctg
attcagtaccattgatagcccagttctgccagcaccatgatcatctggggcctctccgtgggtgacgggtgatcgtgctgcag
taccaccaccagaccccgacgggggcaagatgccaaagtggaccagagtcaccttctgaactggtgcgcgtggttctgcg
catgaaggaggccgggagaggataaggtgcgccggcctgccagcacaagcagcgccgtgcagcctggccagcgtggaga
tgagcgccgtggcgccgccggccggcagcaacgggaacctgctgtacatcggttccgcggcctggacggcatgcattgcgc
15 cccgaccccgactccggggtggtgtgcggccgatggcctgctccccacgcacgacgagcacctcctgcacggtgggca
gcccccgagggggacccggacctggccaagatcctggaggaggtccgctacatcgccaaccgcttctgctgccaggacga
aagcgaggcggtctgagtgaagttcgccgctgctggtggaccgctgtgctcatggccttctcgttcttaccatc
atctgcacatcggtcctgatgtcggtcccaacttctggaggccgtgtccaaagactttgcgtaa

20 (NCBI Accession No. AF486623)

SEQ ID NO:11 is the amino acid sequence of the wild-type rhesus monkey $\alpha 7$ subunit,
 including amino acids 1-22 (underlined), which represent a signal peptide that is not
 present in the mature receptor subunit (the TM2 domain at amino acids 262-281 is also
 underlined for reference):

MRC SQGGVWLALAAASLLHVS LQGEFQRKLYKELVKNYNPLERP VANDS QPLTV
YFSL SLLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPD GQI
WKP DILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPD VQ
 30 HCKLKFGSWSYGGWSLDLQM QEADISGYIPSGEWDLVGIPGKRSEKFYECCKEP
YPDVTFTVTMRRRTLYYGLNLLIPC VLISALALLVFLLPADS GEKISL GITVLLSLT
VFMLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVVTVIVLQYHHHDPDGGKMP
KWTRVILLNWCAWFLRMKRPGEDKVRPACQHKQRRC SLASVEMSAVAPPPASN
GNLLYIGFRGLDGMHCAPT PDSGVVCGRMACSP THDEHLLHGGQPPEGDPDLAK
 35 ILEEVRYIANRFR CQDESEAVCSEWKFAACVVDRLCLMAFSVFTI CTIGILMSAP
NFVEAVSKDFA

SEQ ID NO:12 is the nucleotide coding sequence of the wild-type domestic cow $\alpha 7$
 subunit, -including nucleotides 1-57 (underlined), which encode a signal peptide that is
 not present in the mature $\alpha 7$ receptor subunit (the TM2 domain at nucleotides 775-834 is
 underlined and the codon for the 6' amino acid of TM2 (threonine) is bold for reference):

atgcgcggctcgttgccctggcgtggcagcgtcgatcttgacgtgtccctgcaaggcgagttccagaggaagctctacaaa
 gacctggtgaagaactacaaccccttggagaggccggtggccaacgactcgtgccgctcacagtctacttccctgagcctc
 ctgcagatcatggacgtggatgagaagaaccaggtgttgaccaccaacatttggctacaaatgacttggacagatcactatttaca
 gtggaatgcatccgagtatccaggcgtgaagacggttcgttccagacggccagatctggaagccagacattctctctacaac
 5 agtgcgtgatgaacggtttagctacgttccacaccaatgtgttagtgaattcttctgggactgccagtacctccctccaggcatct
 ttaagagctcctgctacatcgatgtgcgctggttccattttagtgtgcagcagtgcaaaactgaagtttgggtcctggtcttatggagg
 atggctccttgatctgcaaatgcaggaggcagacatcagcggctatatcccaaatggagaatgggacctgtgggggtcttggg
 caagcggagtgagaagtctacgagtgtgcaaaagaacctaccagacgtgaccttcacggtcagcatccgccgaggactc
 tctactacggcctcaacctgctcatccctgtgtgctcatctctgccctagccctcctcgtcttcttgcctcctgacactctggagag
 10 aaaatctccctgggaatc**acagttt**tactctccctcaccgtcttcatgctgctgtagctgagatcatgctgcgacctctgactcag
 tgccttaatagcccagttctgccagcaccatgatcatcgtgggcctctctgtggtgtcacgggtgatcgtgctacagtaccacc
 accacgacctgatggtggcaagatgcccaggtggaccagagtcgtcctcctgaactggtgtgcctggttctctcgcatgaaga
 ggccggggaggagacaaggtgcggccagcctgccagcacaacgagcggcgctgcagcctggccagcgtggagatgagcgc
 agtggcggggccgccggccaccaatggcaacctgctgtacatcggttctgcggcctggacacatgcactgcgcaccacc
 15 cctgactcaggggtcgtgtgcggccgctggcctgctcccaacccatgacgagcacctgctgcacgccggccagccctctga
 gggggaccggacctagccaagatcctggaggaggtgcgctacattgccaccgcttccgctgccaggatgagagtggggcc
 gtgtgcagtgagtgaagtcgcggcctgtgtggtggaccgctgtgtctcatggccttctccgtcttcaccatcctctgcaccatt
 ggcacctgatgtcggcgcccaacttcgtgaagctgtgtccaaagacttcgcttga

- 20 (NCBI Accession No. NM_174515; Garcia-Guzman, M. *et al.*, *Eur. J. Neurosci.*
 7(4):647-655, 1995; Maneu V. *et al.*, *Ann. N.Y. Acad. Sci.* 971:165-167, 2002; which are
 incorporated by reference herein in their entirety)

SEQ ID NO:13 is the amino acid sequence of the wild-type cow $\alpha 7$ subunit, including
 25 amino acids 1-19 (underlined), which represent a signal peptide that is not present in the
 mature receptor subunit (the TM2 domain at amino acids 259-278 is also underlined for
 reference):

MRGSLCLALAASILHVSLOGEFQRKLYKDLVKNYNPLERPVANDSLPLTVYFSL
 LLQIMDVDEKNQVLTTNIWLQMTWTDHYLQWNASEYPGVKTVRFPDGGQIWKPD
 30 ILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQQCKL
 KFGSWSYGGWSLDLQMQEADISGYIPNGEWDLVGVLGKRSEKFYECCKEPYPD
 VTFTVSIRRRRTLYYGLNLLIPCVLISALALLVFLLPADSGEKISLGI**TVLLSLTVFML**
 LVAEIMPATSDSVPLIAQYFASTMIIVGLSVVVTVIVLQYHHHDPDGGKMPKWTR
 VVLLNWCWFLRMKRPGEDKVRPACQHNERRCSLASVEMSAVAGPPATNGNL
 35 LYIGFRGLDTMHCAPTPDGSGVVCGRVACSPTHDEHLLHAGQPSEGDPDLAKILEE
 VRYIAHRFRCQDESEAVCSEWKFAACVVDRLCLMAFSVFTILCTIGILMSAPNFV
 EAVSKDFA

SEQ ID NO:14 is the nucleotide coding sequence of the wild-type chicken $\alpha 7$ subunit,
 40 including nucleotides 1-66 (underlined), which encode a signal peptide that is not present

in the mature $\alpha 7$ receptor subunit (the TM2 domain at nucleotides 784-843 is underlined and the codon for the 6' amino acid of TM2 (threonine) is bold for reference):

5 atgggcctccggcgctgatgctgtggctgctggcgccggcggggctcgtgcgcgagtccttgcgaaggagaggttccaaagga
agctgtacaaggagctgctgaagaactacaaccctctggaacgaccagtgcaaatgactcccagccgctcactgtctatttact
 10 ctcagcctcatgcagatcatggatgtggatgaaaagaatcaagtattacaacaaacatctggctacaaatgtactggacagatca
ttacttacagtggaatgtgtctgaataccctggagtgagaacgtccgtttcctgatggactgatttggagccagatattcttct
ataacagtgtgatgaaagatttgatgctacatttcacactaatgttttagtcaattcttcgggacactgccaatatctgccaccaggc
atatttaaaagctcatgctacatagacgtgcgttggttccatttgatgttcagaagtgcattctgaagtttggactcttgacatatgga
 15 ggctggccttagacttacaaatgcaagaagcagatatatccggctatatttcaaaggagagtggtgatttagtaggaattcctggg
aagagaactgagagctttatgagtgtgtaagaaccataccagatatcacattcacagtaacctgagacgcagaactctcta
ctacgggctcaaccttctattccctgtgtactgatatcagcacttgccttattagctttctgcttcagcagactcaggagaaaagat
ctcactaggtataacagttttattgtctctcaccgtcttcatgttactcgtggctgaaattatgccagcaacatctgattctgtgccctta
attgctcagtttttgcagcaccatgattattgttggcctctctgttgtgtcactgttatcgttctacaataccatcatcacgatccaga
 20 tgggggaaaaatgcctaaatggacaagagtcaccttctgaattggtgtgcttggttctgaggatgaagagaccaggggaagat
aaagtgcgtcccgcctgtcaacataaacagcgcggatgcagcctgtcaagcatggagatgaacactgtgagtgtcagcaatgc
agtaatgggaacatgctgtatattgggttcgagggctggatgggttactgcacaccaccactgattcaggggtgatctgtgg
gaggatgacctgttcaccaacagaggaagaaaatcttgcacagtggccaccctctgaaggcgaccagatttggctaagat
cctggaagaggtcagatacattgcaaacaggttcagagaccaggatgaagaagaagccatttgaacgaatggaagttgcag
cctctgtagtagatcggtctgctgatggcatttccgtcttcaccatcattgtacaattggcatcttaatgtcagcaccaaaacttgt
 25 agaggctgtctctaaagatttgcctaa

(NCBI Accession No. X52295; Schoepfer R. *et al.*, *Neuron* 5(1):35-48, 1990; which is incorporated herein by reference in its entirety)

25 SEQ ID NO:15 is the amino acid sequence of the wild-type chicken $\alpha 7$ subunit, including
 amino acids 1-22 (underlined), which represent a signal peptide that is not present in the
 mature receptor subunit (the TM2 domain at amino acids 262-281 is also underlined for
 reference):

30 MGLRALMLWLLAAAGLVRESLQGEFQRKLYKELLKNYNPLERPVAN
DSQPLTV
YFTLSLMQIMDVDEKNQVLTTNIWLQMYWTDHYLQWNVSEYPGVKNVRFPDG
LIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDV
QKCNLKFSGSWTYGGWSLDLQMQEADISGYISNGEWDLVGIPGKRTEFYECCKE
PYPDITFTVTMRRRTLYYGLNLLPCVLISALALLVFLLPADSGEKISLGITVLLSLT
 35 VFMLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVTVIVLQYHHHDPDGGKMP
KWTRVILLNWCAWFLRMKRPGEDKVRPACQHKQRRC
SLSSMEMNTVSGQQCS
NGNMLYIGFRGLDG
VHCTPTTDSGVICGRMTCSPTEENLLHSGHPSEGD
PD LAK
ILEEVRYIANRFRDQDEEEAICNEWKFAASVVDRLCLMAFSVFTI
ICTIGILMSAPN
FVEAVSKDFA

40 SEQ ID NO:16 is the nucleotide coding sequence of the wild-type zebrafish $\alpha 7$ subunit,
 including nucleotides 1-66 (underlined), which encode a signal peptide that is not present

in the mature $\alpha 7$ receptor subunit (the TM2 domain at nucleotides 784-843 is underlined and the codon for the 6' amino acid of TM2 (threonine) is bold for reference):

atgggaatttgggaatatactctttatttcaccaccacatgctgcctatggagtgtgtcacttcagggggagcatcagaggagactc
 tatagagacctgatgaaagattataaccactggagagacctgtgtcaatgacaccactcactcacggtgtatttcagcatgag
 5 cctcatgcagattatggatgtggatgaaaagaatcaagttcttacgaccaacatttggctacagctgtactggtatgactactatcttc
 aatggaacgcctcagaatacctggagtaaccaatgtaaggtttctgacagtcagatctggaagcctgacatcttactttataaca
 gtgctgatgaaaggtttgatgccacattcatactaatgtgctggtgaactcttctggtgcctgccaatacttaccgccagggatcttc
 aaaagcacctgctacatcgacgttcgctggtcccccttgatcttcagaggtgtgatctgaaatttggctcctggacgtatggaggat
 ggtctctggacctgcagatgattgatgccgacatcacaggatacattgctaagagagtgggacctgtggaagtccaggttag
 10 acggaatgagagggttctacgactgctgcaaggagccgtaccagatgtgacttttacggtggtgatgcggagacgaacactgtat
 tacggtcttaacttacttattccctgtgtgctcatcttactctggccctgctggtgttctgctgcctgctgactcaggagaaaagatc
 tcgctcgggatc**accg**ctcctgctcgcctgacagtttcatgcttttggtagcagagataatgccggcaacatctgactctgtgccttt
 aatagctcagtattttgcaccaccatggttattgttgactatctgtaatagccacagctctgggtttacaataccactaccatgatcc
 tgaaggaggaaaaatgccaaaatggacacgtgtggtgctgtgaactggtgtgcctggttctgcggatgaagagacctggcga
 15 agacaaagtgcgccagctgtcacaacaagcagccgcgcagcagcctctccagcgtggatctaaacatcagccctggagtcg
 cacagtcaccaatggaacctgctgtacatcggttccgcgggatggacactattcactatgctacttcacccgattctgggggtg
 atctgcagccggcttggtgcgaccggagaggaggtgtgctgctccccggagcccaggcattctcagtcagtagtagcggccc
 tggagaaccgaactgtccaagatcttgatgaggtgcggtacatttccaagcgcttcgggaccaagacgaggaagacactgt
 gtgtaatgagtgaagttgcagcgtctgtgattgaccgtctctgtctcatggcttttccctgttcaccatcctctgcaccattggtatc
 20 ttaatgtctgccctaactttgttgaggccatataaaagatttttccactga

(NCBI Accession No. NM_201219; Zirger J.M. *et al.*, *Gen Expr. Patterns* 3(6):747-754, 2003)

25 SEQ ID NO:17 is the amino acid sequence of the wild-type zebra fish $\alpha 7$ subunit, including amino acids 1-22 (underlined), which represent a signal peptide that is not present in the mature receptor subunit (the TM2 domain at amino acids 262-281 is also underlined for reference):

MGIWEYTLYFTTTCCLWSVSLQGEHQRRLYRDLMKDYNPLERPVFNDTHSLTVY
 30 FMSMLMQIMDVDEKNQVLTTNIWLQLYWYDYLLQWNASEYPGVTNVRFPDSQI
 WKPDILLYNSADERFDATFHTNVLVNSSGACQYLPPGIFKSTCYIDVRWFPFDLQ
 RCDLKFGSWTYGGWSLDLQMIADITGYIANGEWDLVEVPGRNRERFYDCCKE
 PYPDVTFTVVMRRRTLYYGLNLLIPCVLISLTLALLVFLLPADSGEKISL**LGITVLLSL**
 TVFMLLVAEIMPATSDSVPLIAQYFATTMVIVGLSVIATVWVLQYHYHDPEGGK
 35 MPKWTRVVLLNWCAWFLRMKRPGEDKVRPACHNKQPRSSLSSVDLNISPGVAQ
 STNGNLLYIGFRGMDTIHYATSPDSGVICSRLVATGEEDVLLPGAQASSVSSSGPG
 ETELSKILDEVRYISKRFRDQDEEDTVCNEWKFAASVIDRLCLMAFSLFTILCTIGI
 LMSAPNFVEAISKDFFT

SEQ ID NO:18 is a nucleotide coding sequence of the T6'S variant of the rat (and mouse) second transmembrane (TM2) domain from (the codon encoding serine, in place of threonine, at the sixth amino acid position of TM2 is underlined):

atctctcttgggaataatctgtcttactttctctgactgtcttcatgctgcttgggctgag

5

SEQ ID NO:19 is a nucleotide coding sequence of the T6'S variant $\alpha 7$ subunit, including nucleotides 1-66 (underlined), which encode a signal peptide that is not present in the mature receptor subunit (the TM2 domain at nucleotides 784-843 is underlined and the codon for the 6' amino acid of TM2 (threonine) is bold for reference):

10 atgtgcggcgggcggggaggcatctggctggctctggccgcgcgctgctgcacgtgtccctgcaaggcgagttccagagga
ggctgtacaaggagctggtaagaactacaacccgctggagaggccggtggccaacgactcgcagccgctcaccgtgtacttc
tccctgagtcctctgcagatcatggatgtggatgagaagaaccaagttaaccaccaacatttggtacaaatgtcttgacagat
cactatttgcaaggacatgtctgagtaccccgagtggaagaatgttcgtttccagatggccagatttgaaaccagacattctc
ctctataacagtgtgatgagcgcttggatgccacgttccacaccaatgtttggatgaatgcacatctgggcattgccagtatctccctcc
15 aggcatattcaagagctcctgctacattgacgttcgctgggtccctttgatgtgcagcagtgcaaaactgaagttgggtcctggctc
tatggagggtggctcactggacctgcaaatgcaagaggcagatatcagcagctatatccccaacggagaatgggatctcatggga
atccctggcaaaaggaatgagaagttctatgagtgtgcaaaagaccataccagatgtcacctacacagtaaccatgcgccgt
aggacactctactatggcctcaatctgctcatccctgtgtactcatttcagccctggctctgctgggtattctgctgcctgcagactct
ggagagaaaaatctctcttgaataatctgtcttactttctctgactgtcttcacgtctgtgctggctgagatcatgccagcaacatctgat
20 tctgtgcccttgatagcacaatacttcgccagcaccatgatcatcgtgggcctctctgtagtggtgacagtattgtgctgagatc
accaccatgacctgatgggtggcaaatgcctaagtggaccagaatcattctcctgaactggtgtgcatggttctgcgcgatgaag
aggcccgagaggacaaggtgcggccagctgtgcagcacaagcctcggcgctgcagcctggccagtggtggagctgagtgc
ggtgctggggccaccaccagcaatggcaacctgctctacattggcttcaggcctggaggcgatgcactgtccccaactcca
gactctggggctgtagtggtggcgtttggcctgctcccaacacatgatgagcacctcatgcacgggtgcacaccctctgatgggg
25 accccgacctggccaagatcctggaggaggtccgctacatgcccaaccgctccgctgccaggacgagagtgaggtgatctgc
agtgaatggaagttgcagcctgcgtggtggaccgctgtgcctcatggccttttcggtctttaccatcatctgtaccatcggcatcc
tcatgtcagctccaaactttgtggaggctgtgtccaaagactttgcttaa

SEQ ID NO:20 is an amino acid sequence of the T6'S variant $\alpha 7$ subunit, including amino acids 1-22 (underlined), which represent a signal peptide that is not present in the mature receptor subunit (the TM2 domain at amino acids 262-281 is also underlined for reference):

30 MCGGRGGIWLALAAALLHVSLQGEFQRRLYKELVKNYNPLERPVAN
DSQPLTVYFSLSLQIMDVDEKNQVLTTNIWLQMSWTDHYLQWNMSEYPGVKNVRFPD
GQIWKPDILLYNSADERFDATFHTNVLVNASGHCQYLPPGIFKSSCYIDVRWFP
FDVQQCKLKFGSWSYGGWSLDLQMQEADISSYIPNGEWDLMGIPGKRNEKFYE
CKEPYPDVTYTVTMRRTLYYGLNLLPCVLISALALLVFLLPADSGEKISL
GISVLLSLTVFMLLVAEIMPATSDSVPLIAQYFASTMIIVGLSVVTVIVLR
YHHHDPDGGKMPKWTRIILLNWCAWFLRMKRPGEDKVRPACQHKPRRCSLAS
VELSAGAGPPTSNGNLLYIGFRGLEGMHCAPTPDSGVVCGRLACSP
40 THDEHLMHGAHPSDGDPDLAK

ILEEVRYIANRFRQCDESEVICSEWKFAACVVDRLCLMAFSVFTIICTIGILMSAPN
FVEAVSKDFA

5 SEQ ID NO:21 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:18) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcc-)encoding serine at the sixth amino acid position of the TM2 domain:

atctctcttggaaatatccgtcttactttctctgactgtcttcacgtctgcttggtgctgag

10 SEQ ID NO:22 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:18) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tca-)encoding serine at the sixth amino acid position of the TM2 domain:

atctctcttggaaatatcaggtcttactttctctgactgtcttcacgtctgcttggtgctgag

15

SEQ ID NO:23 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:18) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcg-)encoding serine at the sixth amino acid position of the TM2 domain:

20 atctctcttggaaatatcgggtcttactttctctgactgtcttcacgtctgcttggtgctgag

25 SEQ ID NO:24 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:18) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-agt-)encoding serine at the sixth amino acid position of the TM2 domain:

atctctcttggaaataagtgtcttactttctctgactgtcttcacgtctgcttggtgctgag

30 SEQ ID NO:25 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:18) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-agc-)encoding serine at the sixth amino acid position of the TM2 domain:

atctctcttggaaataagcgtcttactttctctgactgtcttcacgtctgcttggtgctgag

SEQ ID NO:26 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:19) encoding a T6'S mutant $\alpha 7$ subunit (SEQ ID NO:20), containing a degenerate codon (-tcc-) encoding serine at the sixth amino acid position of the TM2 domain (the TM2 domain at nucleotides 784-843 is underlined and the codon for the 6' amino acid of

5 TM2 (threonine) is bold for reference):

atgtcgccggcgccggggaggcatctggctggctctggccgcggcgctgctgcacgtgtccctgcaaggcgagttccagagga
ggctgtacaaggagctgggtcaagaactacaacccgctggagaggccgggtggccaacgactgcagccgctcaccgtgtacttc
tccctgagctcctgcagatcatggatgtggatgagaagaaccaagttttaaccaccaacatttggctacaaatgtcttggacagat
cactatttgcagtggaaacatgtctgagtacccggagtgaaagtgttcgtttccagatggccagatttgaaaccagacattctc
10 ctctataacagtgtgatgagcgctttagtccacgttccacaccaatgtttggatgaatgcattctgggcattgccagtatctccctcc
aggcatattcaagagctcctgctacattgacgttcgctggttccctttttagtgtgcagcagtgcaaacgaagtttgggtcctggctcc
tatggagggtggtcactggacctgcaaatgcaagaggcagatatcagcagctatatcccaacggagaatgggatctcatggga
atccctggcaaaaggatgagaagttctatgagtgtgcaaaagagccataccagatgtcacctacacagtaaacatgcgccgt
aggacacttactatggcctcaatctgctcacccttgtgtactcatttcagccctggctctgctgggtattcttgcctgcagactct
15 ggagagaaaaatctcttggaaat**atccgtcttactttctctgactgtcttcattgctgcttggctgagatcatgccagcaacatctga**
tctgtgcccttgatagcacaatacttcgccagcaccatgatcatcgtgggcctctctgtagtggtgacagtattgtgctgagatac
accaccatgacctgatgtggcaaaatgcctaagtggaccagaatcattctcctgaactgggtgtgcatggtttctgcgcatgaag
aggcccgagaggacaagggtgcggccagcttgcagcacaagcctcggcgctgcagcctggccagtggtggagctgagtgc
20 ggtgctgggccaccaccagcaatggcaacctgctctacattggcttccgaggcctggagggcatgcactgtgccccaaactcca
gactctggggctgtagtggccgtttggcctgctcccaacacatgatgagcacctcatgcacggtgcacacccctctgatgggg
accccgacctggcaagatcctggaggaggtccgctacatgcgaaccgcttccgctgccaggacgagagtgggtgatctgc
agtgaatggaagtttgcagcctgcgtggtggaccgcttgcctcatggccttttgggtctttaccatcatctgtaccatggcatcc
tcatgtcagctccaaacttgtggaggtgtgtccaaagacttgcctaa

25 SEQ ID NO:27 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:19) encoding a T6'S mutant $\alpha 7$ subunit (SEQ ID NO:20), containing a degenerate codon (-tca-) encoding serine at the sixth amino acid position of the TM2 domain (the TM2 domain at nucleotides 784-843 is underlined and the codon for the 6' amino acid of

TM2 (threonine) is bold for reference):

30 atgtcgccggcgccggggaggcatctggctggctctggccgcggcgctgctgcacgtgtccctgcaaggcgagttccagagga
ggctgtacaaggagctgggtcaagaactacaacccgctggagaggccgggtggccaacgactgcagccgctcaccgtgtacttc
tccctgagctcctgcagatcatggatgtggatgagaagaaccaagttttaaccaccaacatttggctacaaatgtcttggacagat
cactatttgcagtggaaacatgtctgagtacccggagtgaaagtgttcgtttccagatggccagatttgaaaccagacattctc
ctctataacagtgtgatgagcgctttagtccacgttccacaccaatgtttggatgaatgcattctgggcattgccagtatctccctcc
35 aggcatattcaagagctcctgctacattgacgttcgctggttccctttttagtgtgcagcagtgcaaacgaagtttgggtcctggctcc
tatggagggtggtcactggacctgcaaatgcaagaggcagatatcagcagctatatcccaacggagaatgggatctcatggga
atccctggcaaaaggatgagaagttctatgagtgtgcaaaagagccataccagatgtcacctacacagtaaacatgcgccgt
aggacacttactatggcctcaatctgctcacccttgtgtactcatttcagccctggctctgctgggtattcttgcctgcagactct
ggagagaaaaatctcttggaaat**atcagcttactttctctgactgtcttcattgctgcttggctgagatcatgccagcaacatctga**
40 ttctgtgcccttgatagcacaatacttcgccagcaccatgatcatcgtgggcctctctgtagtggtgacagtattgtgctgagatat
caccaccatgacctgatgtggcaaaatgcctaagtggaccagaatcattctcctgaactgggtgtgcatggtttctgcgcatgaa
gaggcccgagaggacaagggtgcggccagcttgcagcacaagcctcggcgctgcagcctggccagtggtggagctgagtgc
aggtgctgggccaccaccagcaatggcaacctgctctacattggcttccgaggcctggagggcatgcactgtgccccaaactcc

agactctggggctgatatgtggccgtttggcctgctccccaacacatgatgagcacctcatgcacgggtgcacaccctctgatggg
gaccccgacctggccaagatcctggaggaggtccgctacatcgccaaccgcttccgctgccaggacgagagtgaggtgatct
gcagtgaatggaagtttgagcctgcgtggaggaccgcttgcctcatggccttttcggtctttaccatcatctgtaccatcggcat
cctcatgtcagctccaaactttgtggaggctgtgtccaaagactttgcttaa

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SEQ ID NO:28 is an example of a degenerate variant of the nucleotide sequence (SEQ ID
NO:19) encoding a T6'S mutant $\alpha 7$ subunit (SEQ ID NO:20), containing a degenerate
codon (-tcg-) encoding serine at the sixth amino acid position of the TM2 domain (the
TM2 domain at nucleotides 784-843 is underlined and the codon for the 6' amino acid of

10 TM2 (threonine) is bold for reference):

atgtgcggcgggcggggaggcatctggctggctctggccgcggcgctgctgcacgtgtccctgcaaggcgagttccagagga
ggctgtacaaggagctggtaagaactacaacccgctggagaggccggtggccaacgactcgcagccgctcaccgtgtacttc
tcctgagtcctctgcagatcatggatgtggatgagaagaaccaagtttaaccaccaacatttggctacaaatgtcttgacagat
cactatttgcaaggatgtctgagtagtaccggagtgagaatgttcgtttccagatggccagatttggaaccagacattctc
15 ctctataacagtgtgatgagcgctttagtccacgttccacaccaatgtttgggtgaatgcacatctgggcattgccagtatctccctcc
aggcatattcaagagctcctgtacattgacgttcgctgggtccctttttagtgtgcagcagtgcaaacgaagtgttggtcctggctc
tatggagggtggctcactggacctgcaaatgcaagaggcagatatcagcagctatatcccaacggagaatgggatctcatggga
atccctggcaaaaggaatgagaagttctatgagtgtgcaaaagaccataccagatgtcacctacacagtaaccatgcgccgt
aggacactctactatggcctcaatctgctcatcccttgtgtactcatttcagccctggctctgctggtattcttgcctgcagactct
20 ggagagaaaaatctctcttgaatat**tcg**gtcttacttctctgactgtcttcatgtgcttggctgagatcatgccagcaacatctga
ttctgtgcccttgatagcaatacttcgccagcaccatgatcatcgtggcctctctgtagtggtgacagtgattgtgctgagatat
caccaccatgacctgatggggcaaaatgcctaagtggaccagaatcattctcctgaactgggtgtgcatggttctgcgcagtaa
gagggccggagaggacaaggtgcggccagcttgtcagcacaagcctcggcgctgcagcctggccagtgaggagctgagtg
aggtgctggggccaccaccagcaatggcaacctgctctacattggcttccaggcctggagggtgcatgactgtgccccaaactcc
25 agactctggggctgatatgtggccgtttggcctgctccccaacacatgatgagcacctcatgcacgggtgcacaccctctgatggg
gaccccgacctggccaagatcctggaggaggtccgctacatcgccaaccgcttccgctgccaggacgagagtgaggtgatct
gcagtgaatggaagtttgagcctgcgtggaggaccgcttgcctcatggccttttcggtctttaccatcatctgtaccatcggcat
cctcatgtcagctccaaactttgtggaggctgtgtccaaagactttgcttaa

30 SEQ ID NO:29 is an example of a degenerate variant of the nucleotide sequence (SEQ ID
NO:19) encoding a T6'S mutant $\alpha 7$ subunit (SEQ ID NO:20), containing a degenerate
codon (-agt-) encoding serine at the sixth amino acid position of the TM2 domain (the
TM2 domain at nucleotides 784-843 is underlined and the codon for the 6' amino acid of
TM2 (threonine) is bold for reference):

35 atgtgcggcgggcggggaggcatctggctggctctggccgcggcgctgctgcacgtgtccctgcaaggcgagttccagagga
ggctgtacaaggagctggtaagaactacaacccgctggagaggccggtggccaacgactcgcagccgctcaccgtgtacttc
tcctgagtcctctgcagatcatggatgtggatgagaagaaccaagtttaaccaccaacatttggctacaaatgtcttgacagat
cactatttgcaaggatgtctgagtagtaccggagtgagaatgttcgtttccagatggccagatttggaaccagacattctc
ctctataacagtgtgatgagcgctttagtccacgttccacaccaatgtttgggtgaatgcacatctgggcattgccagtatctccctcc
40 aggcatattcaagagctcctgtacattgacgttcgctgggtccctttttagtgtgcagcagtgcaaacgaagtgttggtcctggctc
tatggagggtggctcactggacctgcaaatgcaagaggcagatatcagcagctatatcccaacggagaatgggatctcatggga
atccctggcaaaaggaatgagaagttctatgagtgtgcaaaagaccataccagatgtcacctacacagtaaccatgcgccgt

aggacactctactatggcctcaatctgctcatcccttgtgtactcattcagccctggctctgctggtattctgctgcctgcagactct
ggagagaaaaatctctcttgggaata**agt**gtcttactttctctgactgtcttcatgctgcttgtggctgagatcatgccagcaacatctga
ttctgtgcccttgatagcacaatacttcgccagcaccatgatcatcgtgggcctctctgtagtggtagacagtattgtgctgagatat
caccaccatgacctgatggtggcaaaatgcctaaagtggaccagaatcattctctgaactgggtgtgcatggtttctgcgcatgaa
5 gaggcccgaggagaggacaaggtgcccagcttgtagcacaagcctcggcgctgcagcctggccagtggtggagctgagtgc
aggtgctggggccaccaccagcaatggcaacctgctctacattggcttccgaggcctggagggcatgcactgtccccaaactcc
agactctggggctgatatgtggccgtttggcctgctcccaacacatgatgagcacctcatgcacgggtgcacacccctctgatggg
gaccccgacctggccaagatcctggaggagtccgctacatcgccaaccgcttccgctgccaggacgagagtgagtgatct
gcagtgaatggaagtttcgacgctgcgtgggtggaccgcttgctctcatggcctttcgggtctttaccatcatctgtaccatcggcat
10 cctcatgtcagctccaaactttgtggaggctgtgtccaaagactttgcttaa

SEQ ID NO:30 is an example of a degenerate variant of the nucleotide sequence (SEQ ID NO:19) encoding a T6'S mutant $\alpha 7$ subunit (SEQ ID NO:20), containing a degenerate codon (-agc-) encoding serine at the sixth amino acid position of the TM2 domain (the TM2 domain at nucleotides 784-843 is underlined and the codon for the 6' amino acid of TM2 (threonine) is bold for reference):

20 atgtgcggcgggcggggaggcatctggcttggctctggccgcggcgctgctgcacgtgtccctgcaaggcgagttccagaggga
ggctgtacaaggagctgtgaagaactacaaccgcgtggagaggccggtggccaacgactcgcagccgctaccggtgtacttc
tcctgagtgctcctgcagatcatggtatgtggatgagaagaaccaagtttaaccaccaacatttggctacaaatgtcttgacagat
25 cactatttgcaaggatgtctgagtaccccgagtgagaagaatgttctgtttccagatggccagatttggaaaccagacatttct
ctctataacagtgtgatgagcgcttgatgccacgttccacaccaatgttttggtgaatgcatctgggcatfgccagtatctccctcc
aggcatattcaagagctcctgctacattgacgttcgctgggttccctttgatgtgcagcagtgcaaaactgaagtttgggtcctgggtcc
tatggagggtggctactggacctgcaaatgcaagaggcagatatcagcagctatatcccaacggagaatgggatctcatggga
atccctggcaaaaggaatgagaagtctatgagtgtgcaagagccataccagatgtcacctacacagtaaccatgcgccgt
30 aggacactctactatggcctcaatctgctcatccctgtgtactcattcagccctggctctgctggtattcttctgcctgcagactct
ggagagaaaaatctctcttggaaata**agc**gtcttactttctctgactgtcttcatgctgcttgtggctgagatcatgccagcaacatctga
ttctgtgcccttgatagcacaatacttcgccagcaccatgatcatcgtgggcctctctgtagtgggtgacagtgttctgtgctgagatat
caccacatgaccctgatgggtggcaaatgcctaagtggaccagaatcatttctcctgaactgggtgctgcatggtttctgcgcatgaa
gaggcccgagaggacaagggtgcggccagcttgtagcacaagcctcggcgctgcagcctggccagtgaggagctgagtgc
35 aggtgctggggccaccaccagcaatggcaacctgctctacattggcttccgaggcctggagggcatgcactgtgccccaaactcc
agactctggggctgatatgtggccgfttggcctgctcccaacacatgatgagcacctcatgcacgggtgcacacccctctgatggg
gaccccgacctggccaagatcctggaggaggtccgctacatcgccaaccgcttccgctgccaggacgagagtgaggtgatct
gcagtgaatggaagtgtgcagcctgcgtgggtggaccgctgtgctctcatggcctttcgggtctttaccatcatctgtaccatcggc
cctcatgtcagctccaaactttgtggaggctgtgtccaaagactttgcttaa

35 SEQ ID NO:31 is a nucleotide coding sequence of the T6'S variant of the human second transmembrane (TM2) domain of (the codon encoding serine, in place of threonine, at the sixth amino acid position of TM2 is underlined):

atttcctggggatatctgtcttactctcttaccgtcttcatgctgctcgtggctgag

40

SEQ ID NO:32 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:31) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcc-) encoding serine at the sixth amino acid position of the TM2 domain:

5 atttcctggggatatccgtcttactctcttaccgtcttcatgctgctcgtggctgag

SEQ ID NO:33 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:31) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tca-) encoding serine at the sixth amino acid position of the TM2 domain:

10 atttcctggggatatcagtccttactctcttaccgtcttcatgctgctcgtggctgag

SEQ ID NO:34 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:31) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcg-) encoding serine at the sixth amino acid position of the TM2 domain:

15 atttcctggggatatcggcttactctcttaccgtcttcatgctgctcgtggctgag

SEQ ID NO:35 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:31) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-agt-) encoding serine at the sixth amino acid position of the TM2 domain:

20 atttcctggggataagtgcttactctcttaccgtcttcatgctgctcgtggctgag

SEQ ID NO:36 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:31) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-agc-) encoding serine at the sixth amino acid position of the TM2 domain:

25 atttcctggggataagcgttactctcttaccgtcttcatgctgctcgtggctgag

SEQ ID NO:37 is a nucleotide coding sequence of the T6'S variant of the rhesus monkey second transmembrane (TM2) domain of (the codon encoding serine, in place of threonine, at the sixth amino acid position of TM2 is underlined):

atttcctgggggatatctgtcttactctctctcactgtcttcacgtgctcgtggctgag

5

SEQ ID NO:38 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:37) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcc-) encoding serine at the sixth amino acid position of the TM2 domain:

10 atttcctgggggatatccgtcttactctctctcactgtcttcacgtgctcgtggctgag

SEQ ID NO:39 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:37) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tca-) encoding serine at the sixth amino acid position of the TM2 domain:

15

atttcctgggggatatcagtcttactctctctcactgtcttcacgtgctcgtggctgag

SEQ ID NO:40 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:37) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcg-) encoding serine at the sixth amino acid position of the TM2 domain:

20

atttcctgggggatatcggtcttactctctctcactgtcttcacgtgctcgtggctgag

SEQ ID NO:41 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:37) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-agt-) encoding serine at the sixth amino acid position of the TM2 domain:

25

atttcctgggggataagtgcttactctctctcactgtcttcacgtgctcgtggctgag

30

SEQ ID NO:42 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:37) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID

NO:2), containing a degenerate codon (-agc-) encoding serine at the sixth amino acid position of the TM2 domain:

atctccctggggataagcgtcttactctctcactgtcttcacgtctgctgtagctgag

- 5 SEQ ID NO:43 is a nucleotide coding sequence of the T6'S variant of the cow second transmembrane (TM2) domain of (the codon encoding serine, in place of threonine, at the sixth amino acid position of TM2 is underlined):

atctccctgggaatctctgtttactctccctcaccgtcttcacgtctgctgtagctgag

- 10 SEQ ID NO:44 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:43) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcc-) encoding serine at the sixth amino acid position of the TM2 domain:

atctccctgggaatctccggtttactctccctcaccgtcttcacgtctgctgtagctgag

15

SEQ ID NO:45 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:43) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tca-) encoding serine at the sixth amino acid position of the TM2 domain:

- 20 atctccctgggaatctcaggtttactctccctcaccgtcttcacgtctgctgtagctgag

SEQ ID NO:46 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:43) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcg-) encoding serine at the sixth amino acid position of the TM2 domain:

25

atctccctgggaatctcgggtttactctccctcaccgtcttcacgtctgctgtagctgag

SEQ ID NO:47 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:43) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-agt-) encoding serine at the sixth amino acid position of the TM2 domain:

30

atctccctgggaatcagtgtttactctccctcaccgtcttcacgtctgctgtagctgag

SEQ ID NO:48 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:43) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-agc-) encoding serine at the sixth amino acid position of the TM2 domain:

atctccctgggaatcagcgtttactctccctcaccgtcttcattgctgctggtagctgag

SEQ ID NO:49 is a nucleotide coding sequence of the T6'S variant of the chicken second transmembrane (TM2) domain of (the codon encoding serine, in place of threonine, at the sixth amino acid position of TM2 is underlined):

atctcactaggtatatctgtttattgtctctcaccgtcttcattgttactcgtggctgaa

SEQ ID NO:50 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:49) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcc-) encoding serine at the sixth amino acid position of the TM2 domain:

atctcactaggtatatccgtttattgtctctcaccgtcttcattgttactcgtggctgaa

SEQ ID NO:51 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:49) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tca-) encoding serine at the sixth amino acid position of the TM2 domain:

atctcactaggtatatcagttttattgtctctcaccgtcttcattgttactcgtggctgaa

SEQ ID NO:52 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:49) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcg-) encoding serine at the sixth amino acid position of the TM2 domain:

atctcactaggtatatcggttttattgtctctcaccgtcttcattgttactcgtggctgaa

SEQ ID NO:53 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:49) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID

NO:2), containing a degenerate codon (-agt-) encoding serine at the sixth amino acid position of the TM2 domain:

atctcactaggtataagtgtttattgtctctcaccgtcttcattactcgtggctgaa

- 5 SEQ ID NO:54 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:49) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-agc-) encoding serine at the sixth amino acid position of the TM2 domain:

atctcactaggtataagcgtttattgtctctcaccgtcttcattactcgtggctgaa

10

SEQ ID NO:55 is a nucleotide coding sequence of the T6'S variant of the zebra-fish second transmembrane (TM2) domain of (the codon encoding serine, in place of threonine, at the sixth amino acid position of TM2 is underlined):

atctcgctcgggatctctctctcgtctcgctgacagttttcatgcttttggtagcagag

15

SEQ ID NO:56 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:55) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcc-) encoding serine at the sixth amino acid position of the TM2 domain:

- 20 atctcgctcgggatcttccgtctcgtctcgctgacagttttcatgcttttggtagcagag

SEQ ID NO:57 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:55) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tca-) encoding serine at the sixth amino acid position of the TM2 domain:

25

atctcgctcgggatctcagtctcgtctcgctgacagttttcatgcttttggtagcagag

SEQ ID NO:58 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:55) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-tcg-) encoding serine at the sixth amino acid position of the TM2 domain:

30

atctcgctcgggatctcgctctcgtctcgctgacagttttcatgcttttggtagcagag

SEQ ID NO:59 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:55) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-agt-) encoding serine at the sixth amino acid position of the TM2 domain:

atctcgctcgggatcagtgctctcgtcgtgacagttttcatgcttttggtagcagag

SEQ ID NO:60 is an example of a degenerate variant of a nucleotide sequence (SEQ ID NO:55) encoding the T6'S mutant second transmembrane (TM2) domain (SEQ ID NO:2), containing a degenerate codon (-agc-) encoding serine at the sixth amino acid position of the TM2 domain:

atctcgctcgggatcagcgtcctcgtcgtgacagttttcatgcttttggtagcagag

Detailed Disclosure of the Invention

The T6'S variant of the $\alpha 7$ nicotinic receptor subunit contains a T $\rightarrow$ S substitution at the 6' position of the second transmembrane (TM2) domain (corresponding to residue 244 of the wild-type rat $\alpha 7$ subunit). The biophysical and pharmacological properties of the T6'S variant, including its slow rate of desensitization, make it particularly useful for drug screening.

The term "AChR", as used herein, refers to a receptor for the neurotransmitter acetylcholine ("Ach"). AChRs are broadly subclassified as nicotinic or muscarinic. These types differ in their pharmacology, structures, and signal transduction mechanisms.

The term "nAChR", as used herein, refers to a nicotinic acetylcholine receptor. Although nAChRs of various subunit structures are best known in muscle cells, neurons, and chromaffin cells, they are not necessarily excluded from other cells types (*e.g.*, glial cells, mast cells, blood cells, fibroblasts, *etc.*).

The term "nAChR subunit", as used herein, refers to a proteinaceous molecule that can combine with other such molecules in the formation of a nAChR. For example, the muscle nAChR is believed to be a pentamer comprised of four types of transmembrane subunit: two $\alpha 1$ subunits, one $\beta 1$ subunit, one δ subunit and one γ or ϵ subunit depending upon the nAChR form. Neuronal nAChR analogously are also thought to be pentameric and comprised of related but different subunits. At present, eight

neuronal α subunits ($\alpha 2$ - $\alpha 9$) and three neuronal β subunits ($\beta 2$ - $\beta 4$) have been isolated. Some neuronal nAChRs appear to require at least one α subunit and at least one β subunit for a functional complex (e.g., exhibiting ion channel response to ACh or other agonists). Some subunits, however, may assemble to form "homooligomeric" nAChR, as in the case
5 of $\alpha 7$ nAChR in *Xenopus* oocytes and in transfected mammalian cells. Although the combination of nAChR subunits with subunits related to other types of receptor (e.g., other classes of ligand-gated ion channel) has not been demonstrated, such combinations are possible and contemplated within the scope of the present invention.

The term "wild-type" (WT), as used herein, refers to the typical, most common or
10 conventional form as it occurs in nature. The human wild-type $\alpha 7$ nAChR was described by Doucette-Stamm *et al.* (*Drug Dev. Res.* 30: 252-256, 1993; and U.S. Patent No. 5,837,489, which are incorporated herein by reference in their entireties). The rat and mouse wild-type $\alpha 7$ nAChRs were described by Khiroug, S.S., *et al.* (*J. Physiol. (Lond.)* 540 (Pt 2): 425-434, 2002) (NCBI Accession No. L31629); and Orr-Urtreger, A. *et al.*
15 (*Genomics* 26 (2): 399-402, 1995) (NCBI Accession No. L37663), respectively. An abbreviation of the form " $\alpha 7XnnnO$ " intends an $\alpha 7$ subunit in which the amino acid X, located at position nnn, relative to the wild-type sequence, has been replaced by amino acid O. Thus, for example, the $\alpha 7T144S$ subunit indicates the threonine residue located at position 144 in the wild-type receptor has been replaced by a serine residue.

20 The term "nicotinic cholinergic agonist", as used herein, refers to an agent that binds to and activates a nicotinic acetylcholine receptor. By "activates" is intended the elicitation of one or more pharmacological, physiological, or electrophysiological responses. Such a response includes, but is not limited to, cell membrane depolarization and increased permeability to Ca_2^+ other cations.

25 The term "nicotinic cholinergic antagonist", as used herein, refers to a substance that binds to a nicotinic acetylcholine receptor and prevents agonists from activating the receptor. Pure antagonists do not activate the receptor, but some substances may have mixed agonist and antagonist properties. Nicotinic cholinergic channel blockers block the ability of agonists to elicit current flow through the nicotinic acetylcholine receptor
30 channel, but do so by blocking the channel rather than by preventing agonists from binding to and activating the receptor.

The term “nicotinic cholinergic modulator”, as used herein, refers to an agent that influences the activity of the nicotinic acetylcholine receptor through interaction at one or more sites other than the classic agonist binding site. The modulator may itself increase or decrease receptor activity, or may influence agonist activity (for example, potentiating responses) without itself eliciting an overt change in channel current. A single agent can have different properties at different nicotinic acetylcholine receptor subtypes, for example, being an agonist at one receptor and antagonist at another, or an antagonist at one and a channel blocker at another.

The term “nAChR regulator”, as used herein, refers to an agent that may act as an agonist, antagonist, channel blocker or modulator.

The term “polynucleotide”, as used herein, refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, the term includes double-stranded and single-stranded DNA, as well as double-stranded and single-stranded RNA. It also includes modifications, such as by methylation and/or by capping, and unmodified forms of the polynucleotide.

The term “variant”, as used herein, refers to an oligonucleotide sequence that differs from the related wild-type sequence in one or more nucleotides. Such a variant oligonucleotide is expressed as a protein variant which, as used herein, indicates a polypeptide sequence that differs from the wild-type polypeptide in the substitution, insertion or deletion of one or more amino acids. The variant polypeptide differs in primary structure (amino acid sequence), but may or may not differ significantly in secondary or tertiary structure or in function relative to the wild-type.

The term “mutant”, as used herein, generally refers to an organism or a cell displaying a new genetic character or phenotype as the result of change in its gene or chromosome. In some instances, however, the term “mutant” may be used in reference to a variant protein or oligonucleotide and “mutation” may refer to the change underlying the variant.

The terms “polypeptide” and “protein” are used interchangeably herein and indicate a molecular chain of amino acids of any length linked through peptide bonds. Thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. The terms include post-translational modifications of the polypeptide, for

example, glycosylations, acetylations, phosphorylations and the like. In addition, protein fragments, analogs, mutated or variant proteins, fusion proteins and the like are included within the meaning of polypeptide.

The polypeptides of the present invention are, optionally, N-acetylated and/or C-terminal amidated. The polypeptides of the present invention may be synthetically produced using methods well known in the art utilizing D or L isomeric amino acid precursors. Therapeutic peptides of the present invention preferably contain one, a few, or all of their amino acid residues in the D-isomer conformation. The polypeptides of the present invention may also be produced by recombinant technology, as small polypeptides, as small synthetic fusion proteins comprising one or more polypeptides of the present invention fused to a targeting polypeptide. The term "fusion protein" as used herein refers to protein constructs that are the result of combining multiple protein domains or linker regions for the purpose of gaining function of the combined functions of the domains or linker regions. This is most often accomplished by molecular cloning of the nucleotide sequences to result in the creation of a new polynucleotide sequence that codes for the desired protein. Alternatively, creation of a fusion protein may be accomplished by chemically joining two proteins together.

Unless otherwise specified, the terms "T6'S variant" or "T6'S mutant" are used herein interchangeably to refer a TM2 domain having a serine residue in the sixth amino acid position (6'S), an $\alpha 7$ subunit polypeptide having a TM2 domain with a serine in the 6'S position, and/or an nACh receptor having a TM2 domain with a serine in the 6'S position, functional fragments or variants of any of the foregoing polypeptides; or polynucleotides encoding any of the foregoing polypeptides, including functional fragments or variants thereof. Preferably, the TM2 domain is at least 20 amino acids in length. Preferably, the TM2 domain comprises the amino acid sequence of ISLGISVLLSLTVFMLLVAE (SEQ ID NO:2). The terms "T6'S variant" or "T6'S mutant" are used herein interchangeably to refer to any polynucleotide encoding an amino acid sequence comprising a TM2 domain having a serine residue in the sixth amino acid position (6'S). Preferably, the polynucleotide encodes the amino acid sequence of ISLGISVLLSLTVFMLLVAE (SEQ ID NO:2), such as the nucleic acid sequences of SEQ ID NO: 18, SEQ ID NO:19, 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: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
5 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, and SEQ ID NO:60, or degenerate variants (*i.e.*, structurally conservative mutants) of these nucleic acid sequences.

The nucleotide sequences encoding the T6'S variant, or encoding the T6'S variant
10 nAChR used in the subject invention include "homologous" or "modified" nucleotide sequences. Homologous nucleic acid sequences will be understood to include any nucleotide sequence obtainable by mutagenesis according to techniques well known to persons skilled in the art, and exhibiting modifications in relation to the normal sequences. For example, mutations in the regulatory and/or promoter sequences for the
15 expression of a polypeptide that result in a modification of the level of expression of a polypeptide according to the invention provide for a "modified nucleotide sequence". Likewise, substitutions, deletions, or additions of nucleic acid to the polynucleotides of the invention provide for "homologous" or "modified" nucleotide sequences. In various embodiments, "homologous" or "modified" nucleic acid sequences have substantially the
20 same biological function *in vitro* and/or *in vivo* as the T6'S variant of the present invention. The function of homologues of the invention can be assessed by a number of methods known for assessing nAChR receptor function known in the art, including those disclosed herein. For example, function of homologues can be determined in the *Xenopus* oocyte by a variety of electrophysiological techniques including intracellular voltage
25 recording, two-electrode voltage clamp, patch clamp methods, and the like. A "homologous" or "modified" nucleotide sequence will also be understood to include a splice variant of the polynucleotides of the instant invention or any nucleotide sequence encoding a "modified polypeptide" as defined below. A "homologue" or "modified" nucleotide or amino acid sequence will also be understood to include mammalian
30 homologues of the nucleotide sequences and amino acid disclosed herein.

Homologues of the present invention include those sequences having "functionally conservative mutations" and/or "structurally conservative mutations." The

phrase “functionally conservative mutation”, as used herein, intends a change in a polynucleotide encoding a derivative polypeptide in which the activity is not substantially altered compared to that of the polypeptide from which the derivative is made. Such derivatives may have, for example, amino acid insertions, deletions, or substitutions in the relevant molecule that do not substantially affect its properties (see Table 1). Functionally conservative mutants of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:20, amino acids 23 to 502 of SEQ ID NO:20, and other T6’S polypeptides disclosed herein, are encompassed by the present invention.

The phrase “structurally conservative mutant”, as used herein, refers to a polynucleotide containing changes in the nucleic acid sequence but encoding a polypeptide having the same amino acid sequence as the polypeptide encoded by the polynucleotide from which the degenerate variant is derived. This can occur because a specific amino acid may be encoded by more than one codon (sequence of three nucleotides). Structurally conservative mutants of the nucleic acid sequences encoding SEQ ID NO: 2, SEQ ID NO:20, amino acids 23 to 502 of SEQ ID NO:20, and other T6’S polypeptides disclosed herein are encompassed by the present invention. For example, degenerate variants of the T6’S TM2 domain (SEQ ID NO:2) having alternative codons that encode serine at the sixth amino acid position are SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25. Degenerate codons encoding serine at the sixth amino acid position include –tct-, –tcc-, –tca-, –tcg-, –agt-, and –agc-. Examples of degenerate variants of the T6’S $\alpha 7$ receptor having alternative codons that encode serine at the sixth amino acid position of the TM2 domain include, but are not limited to, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30. Specific embodiments of these degenerate variants of the T6’S $\alpha 7$ receptor are nucleotides 67 to 1509 of SEQ ID NO:26, nucleotides 67 to 1509 of SEQ ID NO:27, nucleotides 67 to 1509 of SEQ ID NO:28, nucleotides 67 to 1509 of SEQ ID NO:29, nucleotides 67 to 1509 of SEQ ID NO:30, and the nucleotide sequences of SEQ ID NOs. 31 to 60. Of course, the polynucleotides of the present invention may include further degenerate codons as well (*i.e.*, in addition to those codons encoding serine in the sixth amino acid position of the TM2 domain).

Accordingly, the polynucleotides of the present include degenerate variants which, because of the degeneracy of the genetic code, have different nucleic acid

sequences (*i.e.*, one or more differing codons) from those polynucleotides exemplified herein, but which nonetheless encode the same amino acid sequences. A listing of valid codons for any given amino acid can be readily determined by those having knowledge of, or access to, the genetic code. Alternatively, so-called "back-translation" tools can be
5 utilized to reverse-translate any given amino acid into its respective codons. One such tool is the "Utilities/Codon Calculator" feature of DNATOOLS for WINDOWS 95/98/NT/2000, which displays all possible degenerate codons for each amino acid input.

A homologous nucleotide sequence, for the purposes of the present invention, encompasses a nucleotide sequence having a percentage identity with the bases of the
10 nucleotide sequences of between at least (or at least about) 20.00% to 99.99% (inclusive). The aforementioned range of percent identity is to be taken as including, and providing written description and support for, any fractional percentage, in intervals of 0.01%, between 20.00% and 99.99%. These percentages are purely statistical and differences between two nucleic acid sequences can be distributed randomly and over the entire
15 sequence length.

In various embodiments, homologous sequences exhibiting a percentage identity with the bases of the nucleotide sequences of the present invention can have 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70,
20 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity with the polynucleotide sequences of the instant invention. Homologous nucleotide and amino acid sequences include mammalian homologues of the rat T6'S variant and mammalian homologues of the rat T6'S variant nAChR. In a specific embodiment, the mammalian homologue is a nucleotide sequence
25 encoding the human T6'S variant or the human T6'S variant nAChR. In another embodiment, the mammalian homologue is the amino acid sequence of the human T6'S variant or the human T6'S variant nAChR.

The T6'S variant homologues of the present invention include polypeptides containing, as a primary amino acid sequence, all or part of an exemplified T6'S variant
30 polypeptide sequence. The T6'S variant homologues thus include T6'S variant polypeptides having conservative substitutions, *i.e.*, altered sequences in which functionally equivalent amino acid residues are substituted for residues within the

sequence resulting in a peptide which is biologically active. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. In one aspect of the present invention, conservative substitutions for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs (see Table 1). Conservative substitutions also include substitutions by amino acids having chemically modified side chains that do not eliminate the biological function of the resulting T6'S variant homologue.

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Table 1.	
Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

15

Both protein and nucleic acid sequence homologies may be evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman *Proc. Natl. Acad. Sci. USA*, 1988, 85(8):2444-2448; Altschul *et al. J. Mol. Biol.*, 1990, 215(3):403-410; Thompson *et al. Nucleic Acids Res.*, 1994, 22(2):4673-4680; Higgins *et al. Methods Enzymol.*, 1996, 266:383-402; Altschul *et al. J. Mol. Biol.*, 1990, 215(3):403-410; Altschul *et al. Nature Genetics*, 1993, 3:266-272).

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Identity and similarity of related nucleic acid molecules and polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; York (1988); Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; York (1993); Computer Analysis of

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Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Jersey (1994); Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Press (1987); Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; York (1991); and Carillo *et al.*,
5 SIAM J. Applied Math., 48:1073 (1988).

The methods, vectors, and compositions of the present invention can utilize functional fragments of nucleic acid sequences encoding the T6'S variant or T6'S variant nAChR disclosed herein, so long as the functional fragments include those nucleotides encoding the second transmembrane (TM2) domain of the $\alpha 7$ subunit and have the T→S
10 substitution in the sixth amino acid position of the TM2 domain.

Representative fragments of the nucleotide sequences according to the invention will be understood to mean any polynucleotide fragment having at least 30 consecutive nucleotides, preferably at least 35 consecutive nucleotides, and still more preferably at least 40 or at least 50 consecutive nucleotides of the sequence from which it is derived.
15 The upper limit for such fragments is one nucleotide less than the total number of nucleotides found in the full-length sequence (or, in certain embodiments, of the full length open reading frame (ORF) identified herein).

In other embodiments, depending upon the relevant full-length sequence from which they are derived, fragments can comprise consecutive nucleotides of 31, 32, 33, 34,
20 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, and up to one nucleotide less than the full-length T6'S variant
25 TM2 domain, up to one nucleotide less than the full-length T6'S variant subunit, or up to one nucleotide less than the full-length T6'S variant nAChR.

It is also well known in the art that restriction enzymes can be used to obtain functional fragments of the nucleic acid sequences. For example, *Bal31* exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to
30 as "erase-a-base" procedures). See, for example, Maniatis *et al. Molecular Cloning: A Laboratory Manual*, 1982, Cold Spring Harbor Laboratory, New York; Wei *et al. J. Biol. Chem.*, 1983, 258:13006-13512.

The methods, vectors, and compositions of the present invention can utilize amino acid sequences that are functional fragments of the full-length T6'S variant TM2 domain, the T6'S variant subunit, or T6'S variant nAChR.

Representative fragments of the polypeptides according to the invention will be understood to mean any polypeptide fragment having at least 9 or 10 consecutive amino acids, preferably at least 15 amino acids, and still more preferably at least 20 or at least 30 consecutive amino acids of the polypeptide sequence from which it is derived. The upper limit for such fragments is one amino acid less than the total number of amino acids found in the full-length sequence.

In other embodiments, depending upon the relevant full-length sequence from which they are derived, fragments of the polypeptides can comprise consecutive amino acids of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, and up to one amino acid less than the full-length T6'S variant TM2 domain, up to one amino acid less than the full-length T6'S variant subunit, or up to one amino acid less than the full-length T6'S variant nAChR. Fragments of polypeptides can be any portion of the full-length amino acid sequence (including human or non-human mammalian homologues) that exhibit functional activity, *e.g.*, a C-terminally or N-terminally truncated version, or an intervening portion of the full-length sequence.

The terms "recombinant host cells", "host cells", "cells", "cell lines", "cell cultures", and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA, immaterial of the method by which the DNA is introduced into the cell or the subsequent disposition of the cell. The terms include the progeny of the original cell that has been transfected. Cells in primary culture as well as cells such as oocytes also can be used as recipients.

The term "genetic modification" as used herein refers to the stable or transient alteration of the genotype of a cell of the subject invention by intentional introduction of exogenous nucleic acids by any means known in the art (including for example, direct

transmission of a polynucleotide sequence from a cell or virus particle, transmission of infective virus particles, and transmission by any known polynucleotide-bearing substance) resulting in a permanent or temporary alteration of genotype. The nucleic acids may be synthetic, or naturally derived, and may contain genes, portions of genes, or other useful polynucleotides in addition to those encoding the T6'S variant TM2 domain, the T6'S variant $\alpha 7$ subunit, or T6'S variant nAChR. A translation initiation codon can be inserted as necessary, making methionine the first amino acid in the sequence.

The term "vector" is used to refer to any molecule (*e.g.*, nucleic acid, plasmid, or virus) usable to transfer coding sequence information (*e.g.*, nucleic acid sequence encoding the T6'S variant TM2 domain, the T6'S variant $\alpha 7$ subunit, or T6'S variant nACh receptor), such as to a host cell. A vector typically includes a replicon in which another polynucleotide segment is attached, such as to bring about the replication and/or expression of the attached segment. The term includes expression vectors, cloning vectors, and the like. Thus, the term includes gene expression vectors capable of delivery/transfer of exogenous nucleic acid sequences into a host cell. The term "expression vector" refers to a vector that is suitable for use in a host cell (*e.g.*, a patient's cell, tissue culture cell, cells of a cell line, *etc.*) and contains nucleic acid sequences which direct and/or control the expression of exogenous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present. Nucleic acid sequences can be modified according to methods known in the art to provide optimal codon usage for expression in a particular expression system. The vector may include elements to control targeting, expression and transcription of the nucleic acid sequence in a cell selective manner as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene may be replaced by the 5'UTR and/or 3'UTR of the expression vehicle. The vector can include a promoter for controlling transcription of the exogenous material and can be either a constitutive or inducible promoter to allow selective transcription. The expression vector can also include a selection gene.

A "coding sequence" is a polynucleotide sequence that is transcribed into mRNA and/or translated into a polypeptide. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to, mRNA, cDNA, and

recombinant polynucleotide sequences. Variants or analogs may be prepared by the deletion of a portion of the coding sequence, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are well known to those skilled
5 in the art (See, *e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, 1989; DNA Cloning, Vols. I and II, D.N. Glover ed., 1985). Optionally, the polynucleotides of the present invention, and composition and methods of the invention that utilize such polynucleotides, can include non-coding sequences.

The term “operably-linked” is used herein to refer to an arrangement of flanking
10 control sequences wherein the flanking sequences so described are configured or assembled so as to perform their usual function. Thus, a flanking control sequence operably-linked to a coding sequence may be capable of effecting the replication, transcription and/or translation of the coding sequence under conditions compatible with the control sequences. For example, a coding sequence is operably-linked to a promoter
15 when the promoter is capable of directing transcription of that coding sequence. A flanking sequence need not be contiguous with the coding sequence, so long as it functions correctly. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence, and the promoter sequence can still be considered “operably-linked” to the coding sequence.
20 Each nucleotide sequence coding for a polypeptide will typically have its own operably-linked promoter sequence.

The terms “transfection” and “transformation” are used interchangeably herein to refer to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, the molecular form of the polynucleotide that is inserted, or
25 the nature of the cell (*e.g.*, prokaryotic or eukaryotic). The insertion of a polynucleotide per se and the insertion of a plasmid or vector comprised of the exogenous polynucleotide are included. The exogenous polynucleotide may be directly transcribed and translated by the cell, maintained as a nonintegrated vector, for example, a plasmid, or alternatively, may be stably integrated into the host genome.

30 The term “isolated”, as used herein, when referring to a polynucleotide or a polypeptide, means that the indicated molecule is present in the substantial absence of other similar biological macromolecules. The term “isolated” as used herein means that

at least 75 wt. %, more preferably at least 85 wt. %, more preferably still at least 95 wt. %, and most preferably at least 98 wt. % of a composition is the isolated polynucleotide or polypeptide. An "isolated polynucleotide" that encodes a particular polypeptide refers to a polynucleotide that is substantially free of other nucleic acid molecules that do not
5 encode the subject polypeptide; however, the molecule may include functionally and/or structurally conservative mutations as defined herein.

The term "test sample", as used herein, refers to biological samples and non-biological samples (*e.g.*, synthetic fluids). Biological samples which can be evaluated by the methods of the present invention described herein include body fluids such as whole
10 blood, tissues and cell preparations.

The terms "cell" and "cells" are used interchangeably herein to refer to a single cell or plurality of cells (*i.e.*, at least one cell).

The following single-letter amino acid abbreviations are used throughout the text: Alanine: A; Arginine: R; Asparagine: N; Aspartic acid: D; Cysteine: C; Glutamine: Q;
15 Glutamic acid: E; Glycine: G; Histidine: H; Isoleucine: I; Leucine: L; Lysine: K; Methionine: M; Phenylalanine: F; Proline: P; Serine: S; Threonine: T; Tryptophan: W; Tyrosine: Y; and Valine: V.

The T6'S variant TM2 domain, the T6'S variant $\alpha 7$ subunit, the T6'S variant nACh receptor; polynucleotides encoding the T6'S variant TM2 domain, the T6'S variant
20 $\alpha 7$ subunit, and the T6'S variant nACh receptor; and methods of making the T6'S variant TM2 domain, the T6'S variant $\alpha 7$ subunit, and the T6'S variant nACh receptor, are provided herein, and encompassed by the subject invention. The invention includes not only the T6'S variant but also methods for screening compounds using the T6'S variant and cells expressing the T6'S variant TM2 domain. Further, polynucleotides and
25 antibodies which can be used in methods for detection of the variant subunit, as well as the reagents useful in these methods, are provided. Compounds and polynucleotides useful in regulating the variant and its expression also are provided as disclosed herein below.

Accordingly, one aspect of the present invention is an isolated polynucleotide that
30 encodes a variant of the nAChR TM2 domain of the $\alpha 7$ subunit, wherein the variant of the nAChR TM2 domain comprises the amino acid sequence of SEQ ID NO:2, or a functional fragment or homologue thereof, wherein the functional fragment or homologue

contains the T→S substitution at the T6'S position. In one embodiment, the polynucleotide comprises the nucleic acid sequence of SEQ ID NO:18. In other embodiments, the polynucleotide comprises the nucleic acid sequence of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, which are each a
5 degenerate variant of SEQ ID NO:18.

In other embodiments, the polynucleotides of the present invention comprise the nucleic acid coding sequences of the TM2 domains of human, mouse, rat, rhesus monkey, domestic cow, chicken, or zebrafish, or mammalian or non-mammalian homologs of these polynucleotides, wherein the codon encoding the sixth amino acid residue of the
10 TM2 domain has been altered to encode serine. Preferably, the altered TM2 domain comprises SEQ ID NO:2. In other embodiments, the polynucleotides of the present invention comprise the coding sequence of the nAChR α 7 subunit of human, mouse, rat, rhesus monkey, domestic cow, chicken, or zebrafish, or mammalian or non-mammalian homologs of these polynucleotides, wherein the codon encoding the sixth acid residue of
15 the TM2 domain has been altered to encode serine. Preferably, the altered TM2 domain comprises SEQ ID NO:2.

The present invention further includes an isolated polynucleotide that encodes a variant of the wild-type nAChR α 7 subunit, such as the wild-type nAChR α 7 subunit of rat, human, mouse, monkey, cow, chicken, and zebrafish (the coding sequences of SEQ
20 ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, and SEQ ID NO:16).

In another aspect, the present invention includes an isolated polypeptide that is a variant of the wild-type TM2 domain of the α 7 subunit, wherein the domain comprises the amino acid sequence of SEQ ID NO:2.

25 In other embodiments, the polypeptides of the present invention comprise the amino acid sequences of the mature α 7 subunit of human, mouse, rat, rhesus monkey, domestic cow, chicken, or zebrafish (*i.e.*, comprising the amino acid sequences of SEQ ID NO: 5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:17, with or without signal peptides), or mammalian or non-mammalian homologs of these polypeptides that have a TM2 domain, wherein the sixth
30 amino acid residue of the TM2 domain in the amino acid sequence has been altered to

encode serine. Preferably, the altered TM2 domain comprises SEQ ID NO:2. In a specific embodiment, the sixth amino acid is altered from a threonine to a serine (T→S).

A polynucleotide encoding the T6'S variant TM2 domain, the T6'S variant $\alpha 7$ subunit, or the T6'S variant nACh receptor can be derived from genomic or cDNA as a starting material, prepared by synthesis, or by a combination of techniques. The polynucleotide can then be used to express the T6'S variant TM2 domain, the T6'S variant $\alpha 7$ subunit, or the T6'S variant nACh receptor, or as a template for the preparation of RNA using methods well known in the art (see, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, 1989).

One method for obtaining the desired polynucleotide involves isolating cDNA encoding the wild-type $\alpha 7$ nAChR subunit as described by Doucette-Stamm *et al.* *Drug Dev. Res.*, 1993, 30: 252-256. The wild-type cDNA thus obtained can then be modified by site-directed mutagenesis using, for example QUICKCHANGE kits (STRATAGENE, LaJolla, CA) and amplified using the polymerase chain reaction ("PCR") and primer sequences designed to obtain the DNA encoding the T6'S variant.

PCR employs short oligonucleotide primers (generally 10-20 nucleotides in length) that match opposite ends of a desired sequence within the wild-type DNA molecule. The sequence between the primers need not be known. The initial template can be either RNA or DNA. If RNA is used, it is first reverse-transcribed to cDNA. The cDNA is then denatured, using well known techniques such as heat, and appropriate oligonucleotide primers are added in molar excess.

The primer can be made specific by keeping primer length and base composition within relatively narrow limits, and by keeping the mutant base corresponding to T6'S centrally located (Zoler *et al.* (1983) *Meth. Enzymol.* 100:468). Primer extension is effected using DNA polymerase in the presence of deoxynucleotide triphosphates or nucleotide analogs. The resulting product includes the respective primers at their 5'-termini, covalently linked to the newly synthesized complements of the original strands. The replicated molecule is again denatured, hybridized with primers, and so on, until the product is sufficiently amplified. Such PCR methods are described in the art, *e.g.*, U.S. Patent Nos. 4,965,188; 4,800,159; 4,683,202; 4,683,195; incorporated herein by reference in their entireties. The product of the PCR is cloned and the clones containing the

mutated DNA, derived by segregation of the primer extended strand, selected. Selection can be accomplished using the mutant primer as a hybridization probe.

By way of example, two complimentary oligonucleotides can be synthesized which contain the desired mutation flanked by 10-15 bases of unmodified nucleotide sequence. Using a thermal cycler, *Pfu* DNA polymerase extends the sequence around the whole vector, generating a plasmid with staggered nicks. Each cycle builds only off the parent strands, and therefore there is no amplification of misincorporations. After approximately 12-16 cycles, the product can then be treated with the restriction endonuclease *Dpn* I, which digests the methylated parent DNA into numerous small pieces. Optionally, the product can then be transformed into cells, such as *E. coli*, which repair the nicks. After linearization and purification of cloned cDNAs, RNA transcripts can be prepared *in vitro* using the appropriate mMessage mMachine kit from AMBION Inc. (Austin, TX).

Alternatively, the wild-type DNA may be obtained from an appropriate DNA library. DNA libraries may be probed using the procedure described by Grunstein *et al.* (*Proc. Natl. Acad. Sci. USA* 73:3961, 1975).

Synthetic oligonucleotides may be prepared using an automated oligonucleotide synthesizer such as that described by Warner (*DNA* 3:401, 1984). If desired, the synthetic strands may be labeled with ^{32}P by treatment with polynucleotide kinase in the presence of ^{32}P -ATP, using standard conditions for the reaction. DNA sequences, including those isolated from genomic or cDNA libraries, may be modified by known methods which include site-directed mutagenesis as described by Zoller (*Nucleic Acids Res.* 10:6487, 1982). Briefly, the DNA to be modified is packaged into phage as a single stranded sequence, and converted to a double stranded DNA with DNA polymerase using, as a primer, a synthetic oligonucleotide complementary to the portion of the DNA to be modified, and having the desired modification included in its own sequence. Culture of the transformed bacteria, which contain replications of each strand of the phage, are plated in agar to obtain plaques. Theoretically, 50% of the new plaques contain phage having the mutated sequence, and the remaining 50% have the original sequence. Replicates of the plaques are hybridized to labeled synthetic probe at temperatures and conditions suitable for hybridization with the correct strand, but not with the unmodified sequence. The sequences which have been identified by hybridization are recovered and

cloned. Alternatively, it may be necessary to identify clones by sequence analysis if there is difficulty in distinguishing the variant from wild-type by hybridization. In any case, the DNA can be sequence-confirmed.

Once produced, the DNA may then be incorporated into a cloning vector or an expression vector for replication in a suitable host cell. Vector construction employs methods known in the art. Generally, site-specific DNA cleavage is performed by treating with suitable restriction enzymes under conditions which generally are specified by the manufacturer of the commercially available enzymes. After incubation with the restriction enzyme, protein is removed by extraction and the DNA recovered by precipitation. The cleaved fragments may be separated using, for example, polyacrylamide or agarose gel electrophoresis methods, according to methods known by those of skill in the art.

Sticky end cleavage fragments may be blunt ended using *E. coli* DNA polymerase 1 (Klenow) in the presence of the appropriate deoxynucleotide triphosphates (dNTPs) present in the mixture. Treatment with S1 nuclease also may be used, resulting in the hydrolysis of any single stranded DNA portions.

Ligations are performed using standard buffer and temperature conditions using T4 DNA ligase and ATP. Alternatively, restriction enzyme digestion of unwanted fragments can be used to prevent ligation.

Standard vector constructions can include specific antibiotic resistance elements. Ligation mixtures are transformed into a suitable host, and successful transformants selected by antibiotic resistance or other markers. Plasmids from the transformants can then be prepared according to methods known to those in the art usually following a chloramphenicol amplification as reported by Clewell *et al.* (*J. Bacteriol.* 110:667, 1972) may be added. The DNA is isolated and analyzed, usually by restriction enzyme analysis and/or sequencing. Sequencing may be by the well-known dideoxy method of Sanger *et al.* (*Proc. Natl. Acad. Sci. USA* 74:5463, 1977) as further described by Messing *et al.* (*Nucleic Acid Res.* 2:309, 1981), or by the method reported by Maxam *et al.* (*Meth. Enzymol.* 65:499, 1980). Problems with band compression, which are sometimes observed in GC rich regions, can be overcome by use of, for example, T-deazoguanosine or inosine, according to the method reported by Barr *et al.* (*Biotechniques* 4:428, 1986).

Host cells can be genetically modified with the vectors of the present invention, which may be a cloning vector or an expression vector. The vector may be in the form of a plasmid, a viral particle, a phage, *etc.* The genetically modified host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants/transfectants or amplifying the subunit-encoding polynucleotide. The culture conditions, such as temperature, pH and the like, generally are similar to those previously used with the host cell selected for expression, and will be apparent to those of skill in the art.

Both prokaryotic and eukaryotic host cells may be used for expression of desired coding sequences when appropriate control sequences (*e.g.*, promoter sequences) that are compatible with the designated host are used. For example, among prokaryotic hosts, *Escherichia coli* is frequently used. Also, for example, expression control sequences for prokaryotes include but are not limited to promoters, optionally containing operator portions, and ribosome binding sites. Transfer vectors compatible with prokaryotic hosts can be derived from, for example, the plasmid pBR322 that contains operons conferring ampicillin and tetracycline resistance, and the various pUC vectors, that also contain sequences conferring antibiotic resistance markers. These markers may be used to obtain successful transformants by selection. Commonly used prokaryotic control sequences include but are not limited to the lactose operon system (Chang *et al. Nature* 198:1056, 1977), the tryptophan operon system (reported by Goeddel *et al. (Nucleic Acid Res.* 8:4057, 1980) and the lambda-derived P1 promoter and N gene ribosome binding site (Shimatake *et al. Nature* 292:128, 1981), the hybrid Tac promoter (De Boer *et al. Proc. Natl. Acad. Sci. USA* 292:128, 1983) derived from sequences of the trp and lac UV5 promoters. The foregoing systems are particularly compatible with *E. coli*; however, other prokaryotic hosts such as strains of *Bacillus* or *Pseudomonas* may be used if desired.

Eukaryotic hosts include yeast and mammalian cells in culture systems. *Pichia pastoris*, *Saccharomyces cerevisiae* and *S. carlsbergensis* are commonly used yeast hosts. Yeast-compatible vectors carry markers that permit selection of successful transformants by conferring protrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Yeast compatible vectors may employ the 2- μ origin of replication (Broach *et al. Meth. Enzymol.* 101:307, 1983), the combination of CEN3 and ARS1 or other

means for assuring replication, such as sequences that will result in incorporation of an appropriate fragment into the host cell genome. Control sequences for yeast vectors are known in the art and include but are not limited to promoters for the synthesis of glycolytic enzymes, including the promoter for 3-phosphoglycerate kinase. (See, for
5 example, Hess *et al.* *J. Adv. Enzyme Reg.* 7:149, 1968; Holland *et al.* *Biochemistry* 17:4900, 1978; and Hitzeman *J. Biol. Chem.* 255:2073, 1980). For example, some useful control systems are those that comprise the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter or alcohol dehydrogenase (ADH) regulatable promoter, terminators also derived from GAPDH, and, if secretion is desired, leader sequences from yeast alpha
10 factor. In addition, the transcriptional regulatory region and the transcriptional initiation region which are operably linked may be such that they are not naturally associated in the wild-type organism.

Thus, the host cells of the present invention may be used in a method of the invention (*e.g.*, methods for identifying agents (substances such as genetic material,
15 protein, lipids, carbohydrates, small molecules, and other compositions) that modulate nAChR activity, methods for screening for agents useful in treating disorders, and methods for identifying cytotoxic or cytoprotective agents). Preferably, the polynucleotide of the invention is stably expressed in the host cell such that a functional receptor is formed, wherein the functional receptor elicits electrophysiological currents *in*
20 *vitro* or *in vivo* when stimulated by a wild-type $\alpha 7$ agonist. Host cells useful for expression of the polynucleotides of the present invention may be primary cells or cells of cell lines. The host cells may be tumor cells or non-tumor cells. Mammalian cell lines available as hosts for expression are known in the art and are available from depositories such as the American Type Culture Collection. These include but are not limited to HeLa
25 cells, human embryonic kidney (HEK) cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and others. Preferably, the host cell is one capable of expressing the wild-type $\alpha 7$ receptor. For example, the host cell may be the GH4C1 (rat anterior pituitary tumor; GH₄C₁) cell line or SHEP1 (human epithelial; SH-EP1) cell line, or variants of these cell lines, that may be genetically modified to express the
30 polynucleotides of the present invention (Tashjian A.H., Jr., *Endocrinology*, 1968, 82:342-352; Tashjian, A.H., Jr., *Methods Enzymol.*, 58:527-535; Bancroft, F.C., in *Functionally Differentiated Cell Lines* (Sato, G., ed.), pp. 47-59, Alan R. Liss, Inc., New

York; Westendorf, J.M., *J. Biol. Chem.*, 1983, 258(12):7527-7535; Peng J-H. *et al.*, *Soc. Neurosci. Abstr.*, 1999, 25:1723; Eaton J.B. *et al.*, *Soc. Neurosci. Abstr.*, 2000, 26:371; Lukas R.J. *et al.*, Some methods for studies of nicotinic acetylcholine receptor pharmacology, in *Nicotinic Receptors and the Nervous System* (Levin ED ed.) pp. 3-27, 5 CRC Press, Boca Raton; Gentry C.L. *et al.*, *JPET*, 2003, 304:206-216, Peng J.H. *et al.*, *J. Pharmacol. Exp. Ther.*, "High affinity epibatidine binding of functional, human {alpha}7-nicotinic acetylcholine receptors stably and heterologously expressed de novo in human SH-EP1 cells, 2004 Dec. 8, Epub ahead of print; Pacheco M.A. *et al.*, *Neurochem. Res.*, 2001, 26(6):683-693; Grinevich V.P. *et al.*, *J. Pharmacol. Exp. Ther.*, 2004, Feb; 10 312(2):619-626, Epub 2004, Sept. 08; Ramsdell J.S. *et al.*, *Mol. Cell. Endocrinol.*, 1985, 43(2-3):173-180; Zao L. *et al.*, *JPET*, 2003, 305:1132-1141; Swileh W. *et al.*, *Brain Res. Mol. Brain Res.*, 2000, 75(2):293-302; Eaton J.B. *et al.*, *JPET*, 2003, 64:1283-1294, each of which are incorporated herein in their entirety).

Suitable promoters for mammalian cells also are known in the art and include 15 viral promoters such as that from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus (ADV), bovine papilloma virus (BPV) and cytomegalovirus (CMV). Mammalian cells also may require terminator sequences and poly A addition sequences; enhancer sequences which increase expression also may be included, and sequences which cause amplification of the gene also may be desirable. These sequences are known 20 in the art. Vectors suitable for replication in mammalian cells may include viral replicons, or sequences which ensure integration of the appropriate sequences encoding the T6'S variant TM2 domain, the T6'S variant $\alpha 7$ subunit, and/or the T6'S variant nACh receptor into the host genome. An example of such a mammalian expression system is described in Gopalakrishnan *et al.* *Eur. J. Pharmacol.-Mol. Pharmacol.* 290: 237-246, 25 1995).

Other eukaryotic systems available as hosts for expression are also known, as are methods for introducing polynucleotides into such systems, such as amphibian cells using methods described in Briggs *et al.* (*Neuropharmacol.* 34:583-590, 1995), insect cells using methods described in Summers and Smith, Texas Agricultural Experiment Station 30 Bulletin No. 1555 (1987), and the like.

The baculovirus expression system can be used to generate high levels of recombinant proteins in insect host cells. The baculovirus expression system allows for

high level of protein expression, while post-translationally processing the protein in a manner similar to mammalian cells. These expression systems use viral promoters that are activated following baculovirus infection to drive expression of cloned genes in the insect cells (O'Reilly *et al.* (1992) *Baculovirus Expression Vectors: A Laboratory*
5 *Manual*, IRL/Oxford University Press).

Transfection may be by any known method for introducing polynucleotides into a host cell, including packaging the polynucleotide in a virus and transducing a host cell with the virus, by direct uptake of the polynucleotide by the host cell, and the like, which methods are known to those skilled in the art. The transfection procedures selected
10 depend upon the host to be transfected.

The expression of the polynucleotide encoding the T6'S variant TM2 domain, the T6'S variant $\alpha 7$ subunit, and/or the T6'S variant nACh receptor may be detected by use of a radioligand selective for the receptor. For example, for the nicotinic cholinergic receptor, such a ligand may be [^{125}I] α -bungarotoxin. However, any radioligand binding
15 technique known in the art may be used to detect the receptor subunit (see, *e.g.*, Winzor *et al.* (1995) *Quantitative Characterization of Ligand Binding*, Wiley-Liss, Inc., N.Y.). Alternatively, expression can be detected by utilizing antibodies or functional measurements which are well known to those skilled in the art.

The T6'S variant TM2 domain polypeptide, the T6'S variant $\alpha 7$ subunit
20 polypeptide, or the T6'S variant nACh receptor polypeptide can be recovered and purified from genetically modified host cell cultures expressing the same by known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography or lectin chromatography. Protein
25 refolding steps can be used, as necessary, in completing configuration of the protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The T6'S variant TM2 domain polypeptide, the T6'S variant $\alpha 7$ subunit polypeptide, or the T6'S variant nACh receptor polypeptide, or functional fragments or
30 homologues thereof, of the present invention, also may be synthesized by conventional techniques known in the art, for example, by chemical synthesis such as solid phase peptide synthesis. In general, these methods employ either solid or solution phase

synthesis methods (See, *e.g.*, J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, 2nd Ed., Pierce Chemical Co., Rockford, Ill. (1984) and G. Barany and R. B. Merrifield, The Peptides: Analysis, Synthesis, Biology, editors E. Gross and J. Meienhofer, Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. Bodansky, Principles of Peptide Synthesis, Springer-Verlag, Berlin (1984) and E. Gross and J. Meienhofer, Eds., The Peptides: Analysis, Synthesis, Biology, *supra*, Vol. 1, for classical solution synthesis).

In one preferred system, either the DNA or the RNA derived therefrom, both of which encode the desired T6'S variant TM2 domain polypeptide, T6'S variant $\alpha 7$ subunit polypeptide, and/or T6'S variant nACh receptor polypeptide, may be expressed by direct injection into a cell, such as a *Xenopus laevis* oocyte. Using this method, the functionality of the polypeptide encoded by the DNA or the mRNA can be evaluated as follows (see Dascal *CRC Crit. Rev. Biochem.* 22:317-387, 1987). A T6'S variant-encoding polynucleotide is injected into an oocyte for translation into a functional receptor subunit. The function of the expressed T6'S variant TM2 domain polypeptide, T6'S variant $\alpha 7$ subunit polypeptide, and/or T6'S variant nAChR polypeptide can be assessed in the oocyte by a variety of electrophysiological techniques including intracellular voltage recording, two-electrode voltage clamp, patch clamp methods, and the like. The cation-conducting channel intrinsic to the nAChR opens in response to ACh or other nicotinic cholinergic agonists, permitting the flow of transmembrane current. This current can be monitored directly by voltage clamp techniques or indirectly by intracellular voltage recording, wherein changes in membrane potential due to the induced current are measured. Alternatives can include measurement of ion flux or fluorescent probes sensitive to transmembrane potential or changes in ion activity.

Receptors expressed in a genetically modified host cell may be used to identify compounds that modulate nAChR activity. In this regard, the specificity of the binding of a compound showing affinity for the receptor is demonstrated by measuring the affinity of the compound for cells expressing the receptor or membranes from these cells. This may be done by measuring specific binding of labeled (*e.g.*, radioactive) compound to the cells, cell membranes or isolated receptor, or by measuring the ability of the compound to displace the specific binding of a standard labeled ligand. Expression of variant receptors and screening for compounds that bind to, or inhibit the binding of labeled ligand to these

cells or membranes provides a method for rapid selection of compounds with high affinity for the receptor. These compounds may be agonists, antagonists or modulators of the receptor.

Expressed receptors also may be used to screen for agents, such as chemical compounds, that modulate nicotinic acetylcholine receptor activity. One method for identifying agents that modulate nAChR activity comprises providing a cell that expresses a polynucleotide encoding a T6'S variant (*e.g.*, T6'S variant TM2 domain polypeptide, a T6'S variant $\alpha 7$ subunit polypeptide, and/or a T6'S variant nACh receptor polypeptide of the present invention), contacting a test agent with the cell and measuring the effect of the test compound on the variant receptor activity. The cell may be a bacterial cell, a mammalian cell, a yeast cell, an amphibian cell or any other cell expressing the receptor. Preferably, the cell is a mammalian cell or an amphibian cell. Thus, for example, a test agent is evaluated for its ability to elicit an appropriate response, *e.g.*, the stimulation of transmembrane current flow, for its ability to inhibit the response to a cholinergic agonist, or for its ability to modulate the response to an agonist or antagonist. The contacting step in the methods of the invention can involve combining or mixing the test agent and the cell, for example.

In one embodiment, the present invention provides a method for identifying agents that modulate nicotinic acetylcholine receptor (nAChR) activity, comprising: (a) providing a host cell that expresses a polynucleotide encoding a variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the variant $\alpha 7$ nAChR polypeptide has a serine residue in the sixth amino acid position of the TM2 domain; (b) contacting a test agent with the host cell; and (c) determining: (i) the effect of the test agent on the variant $\alpha 7$ nAChR polypeptide or the host cell expressing the polynucleotide, or (ii) the binding of the test agent to the host cell or the variant $\alpha 7$ nAChR polypeptide, or (iii) both (c) (i) and (c) (ii).

In addition, expressed receptors may be used to screen agents, such as chemical compounds, that exhibit either a cytoprotective or cytotoxic effect. Abnormal activation of membrane channels is a potential cause of neurological disorders, such as neurodegenerative diseases (See, for example, Salamone, F. *et al.*, *MJM*, 2000, 5:90-97; Cooper, E.C. and Jan, L.Y. *Proc. Natl. Acad. Sci. USA*, 1999, 96:4759-4766; Sharples, C. and Wonnacott, S. *Neuronal Nicotinic Receptors*, October 2001, 19:1-12; Mihailescu, S.

and Drucker-Colin, R. *Arch. Med. Res.*, 2000, 31:131-144; Papke, R.L. *et al.*, *Euro. J. Pharm.*, 2000, 393:179-195; Newhouse, P.A. and Kelton, M. *Pharm. Acta Helv.*, 2000, 74:91-101; Newhouse, P.A. *et al.*, *Clin. Pharm.*, 1997, 11:206-228; Lloyd, G.K. and Williams, M. *J. Pharm. Exp. Therapies*, 2000, 292:461-467; Hollady, M.W. *et al.*, *J. Med. Chem.*, 1997, 40:4169-4194; Benowitz, N.L. *Annu. Rev. Pharm. Toxicol.*, 1996, 36:597-613; Freedman, R. *et al.*, *Harvard Rev. Psych.*, 1994, 2:179-192; and Freedman, R. *et al.*, *J. Chem. Neuroanatomy*, 2000, 20:299-306). Examples of such neurological disorders include, but are not limited to, presenile dementia (early onset Alzheimer's disease); senile dementia (dementia of the Alzheimer's type); movement disorders such as Parkinsonism including Parkinson's disease, Huntington's chorea, tardive dyskinesia, hyperkinesias, tremor, epilepsy, Tourette syndrome or other tic disorders; mania; attention deficit disorder; attention deficit hyperactivity disorder; sleep-wake disorder; chronic-fatigue syndrome; neuropathic pain; addiction (*e.g.*, nicotine/smoking addiction); anxiety; dyslexia; schizophrenia; and obsessive-compulsive disorder.

The T6'S variant of the present invention can be used to screen for agents useful in treating disorders caused by alterations in sensory gating, immunofunction and neuropathic pain (*e.g.*, pain associated with cancerous conditions, post herpetic neuralgia, diabetic neuropathy and osteoarthritis), as well as the foregoing list of neurological disorders.

Accordingly, nicotinic drugs are considered potential therapeutic agents in several neurological disorders, including the foregoing list of neurological disorders. Compounds that activate nAChRs, especially of the $\alpha 7$ subtype, have been found to have in vivo activity in models of cognition enhancement (U.S. Patent No. 5,741,802, issued April 21, 1998). Activation of the wild-type $\alpha 7$ nAChR appears to elicit cytoprotective properties (*e.g.*, reduced cell lysis, see Donnelly-Roberts *et al. Brain Res.*, 1996, 719:36-44). Although it is not yet conclusively established whether a full agonist or partial agonist is preferable, nor, if the latter, what type of partial agonist is best (*e.g.*, one that stabilizes the open and desensitized states or one that stabilizes the open and resting states of the receptor), the T6'S variant of the present invention can be used to evaluate these questions, and to select among ligands for specific types of partial agonists or specific types of antagonists.

Thus, $\alpha 7$ nAChR ligand pharmacology can be studied through the use of the T6'S variant of the present invention (*e.g.*, T6'S variant TM2 domain, T6'S variant $\alpha 7$ subunit, and/or T6'S variant nAChR). The ability of a ligand to stabilize the desensitized state of the receptor can be evaluated by comparing the ligand's potency and efficacy at the T6'S variant nAChR to its potency and efficacy at the wild-type $\alpha 7$ nAChR, for example. Alternatively, the ligand's interaction with the T6'S variant nAChR can be compared to other variants of the wild-type $\alpha 7$ nAChR. The interaction of agents with variants and the wild-type nAChR can be identified using several methods, including, but not limited to, electrophysiologic measurement of transmembrane current flow or electrical potential, measurement of the fluorescence of potential-sensitive or ion-sensitive dyes, or measurement of radioactive ion flux and a variety of $\alpha 7$ nAChR expression systems, for example, transfected mammalian cells in culture or injected amphibian cells. In preferred embodiments, the responses are measured with a high throughput electrophysiology system and/or rapid fluorescence assays such as the FlipR (fluorometric imaging plate reader) system.

In addition to screening test agents, the expressed T6'S variant (*e.g.*, T6'S variant TM2 domain, T6'S variant $\alpha 7$ subunit, and/or T6'S variant nACh receptor) may be used to investigate mechanisms of cytotoxicity and cytoprotection. The evidence that activation of $\alpha 7$ nAChR is cytoprotective comes from the finding that nAChR agonists elicit cytoprotection in cells expressing the wild-type $\alpha 7$ nAChR subunit and that this cytoprotection is inhibited by selective $\alpha 7$ antagonists (for example, see Donnelly-Roberts *et al. Brain Res.*, 719:36-44, 1996).

Cytoprotective or cytotoxic agents that interact with the T6'S variant (*e.g.*, T6'S variant TM2 domain, T6'S variant $\alpha 7$ subunit, and/or T6'S variant nACh receptor) of the present invention may be identified using several methods. One such method comprises providing a cell that expresses the T6'S variant TM2 domain, T6'S variant $\alpha 7$ subunit, and/or T6'S variant nACh receptor of the present invention, combining a test agent with the cell, and monitoring the cell for an indicator of cytotoxicity. If it is necessary to control spontaneous action of the variant nAChR subunit, it may be stably expressed in a recombinant mammalian cell line under the control of an inducible promoter, *e.g.*, the LacSwitch system which is inducible by isopropylthiogalactoside ("IPTG"). Expression of the T6'S variant TM2 domain, T6'S variant $\alpha 7$ subunit, and/or T6'S variant nACh

receptor would be maintained at a low level until induction by the addition of IPTG. Alternatively, with or without an inducible promoter, the transfected cells could be cultured in the presence of an $\alpha 7$ blocker, such as methyllycaconitine ("MLA") or mecamylamine, that would prevent or reduce cytotoxic action. Both blockers are reversible, permitting one to measure the effect of the test agent on $\alpha 7$ nAChR function after the blocker is removed (*e.g.*, washed out).

Cytoprotective agents can be identified by their ability to reduce cell death while cytotoxic agents can be identified by their ability to promote cell death. That these effects are mediated by the $\alpha 7$ subunit, T6'S variant or wild-type, can be identified by the ability of an $\alpha 7$ blocker to prevent the effect. Cell death, or cytotoxicity, can be monitored by a variety of techniques including but not limited to measurement of cell number or density in the culture, of cell growth rate (*e.g.*, incorporation of labeled nucleotide or amino acid), or of cell integrity for example by uptake of a dye (*e.g.*, trypan blue is excluded by healthy cells, or by inclusion of MTT by healthy cells), or by the release of a cytoplasmic constituent such as lactate dehydrogenase (LDH). Cytoprotective agents may also be screened for their ability to antagonize a variant nAChR to a greater extent than a wild-type nAChR, or for their ability to augment the decay rate of variant nAChR compared to the wild-type nAChR.

In one embodiment, the subject invention provides a method for identifying a cytoprotective agent, comprising: (a) providing a host cell that expresses a polynucleotide encoding a variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the variant $\alpha 7$ nAChR polypeptide has a serine residue in the sixth amino acid position of the TM2 domain (such as a T6'S variant $\alpha 7$ subunit comprising SEQ ID NO:2); contacting a test agent (such as a putative wild-type $\alpha 7$ receptor agonist) with the host cell; and (c) monitoring the host cell or cellular function for an indication of cytotoxicity. In a specific embodiment, whether the test agent overstimulates the variant $\alpha 7$ nAChR polypeptide is determined. Because the variant $\alpha 7$ nAChR polypeptide exhibits amplified pharmacologic responses compared to the wild-type $\alpha 7$ receptor, determination of overstimulation of the variant $\alpha 7$ nAChR polypeptide can indicate or connote that the test agent is sufficient to activate the wild-type $\alpha 7$ receptor at a cytoprotective level. In this way, a potential therapeutic agent may elicit a cytotoxic response in the variant $\alpha 7$

nAChR polypeptide of the present invention but elicit a cytoprotective response in the wild-type $\alpha 7$ receptor.

It has been determined that native and degenerate peptides derived from the $\alpha 7$ nicotinic receptor (such as the human $\alpha 7$ receptor) are useful as ligands for β amyloid peptides. The neurotoxic β amyloid₁₋₄₂ ($A\beta_{1-42}$) is abundantly present in the amyloid plaques of Alzheimer's disease (AD) brains and also modulates cholinergic functions which are critical in memory and cognitive neurophysiology (Auld D. *et al.*, *Trends Neurosci* 21:43-49, 1998). $A\beta_{1-42}$ interacts selectively with high affinity to the neuronal $\alpha 7$ nicotinic receptor. U.S. Patent No. 6,441,049 (Reitz *et al.*; issued August 27, 2002) describes methods of treating a neurodegenerative disorder in a subject by administering a compound that inhibits the interaction of $A\beta$ with the $\alpha 7$ receptor. U.S. Patent publication U.S. 2003/0092613 (Lee *et al.*; filed August 13, 2001) describes methods for diagnosing Alzheimer's disease involving the binding of $\alpha 7$ that allows the measurement of $A\beta$ peptides in biological samples, methods for designing a drug discovery assay for identifying $\alpha 7$ nAChR modulators based on the interaction of $\alpha 7$ with $A\beta$ (the disclosure of which is expressly incorporated herein by reference in its entirety). The subject invention encompasses these methods, wherein polypeptides comprising the T6'S domain of the present invention, and which interact with $A\beta$, are substituted for the $\alpha 7$ and variant peptides described in U.S. Patent publication U.S. 2003/0092613.

The present invention also includes isolated polynucleotides (*e.g.*, detection probes or primers) capable of selectively hybridizing to a nucleic acid sequence encoding a T6'S variant TM2 domain, T6'S variant $\alpha 7$ subunit, and/or T6'S variant nACh receptor. For example, the isolated polynucleotide can be capable of selectively hybridizing to a nucleic acid sequence comprising SEQ ID NO:18, 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, or a functional fragment or homologue of any of the foregoing. Such a detection probe will comprise a contiguous/consecutive span of at least 8, 9, 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 nucleotides. Labeled probes or primers are labeled with a radioactive compound or with another type of label as set forth above (*e.g.*, 1) radioactive labels, 2) enzyme labels, 3) chemiluminescent labels, 4) fluorescent labels, or 5) magnetic labels). Alternatively, non-labeled nucleotide sequences may be

used directly as probes or primers; however, the sequences are generally labeled with a radioactive element (^{32}P , ^{35}S , ^3H , ^{125}I) or with a molecule such as biotin, acetylaminofluorene, digoxigenin, 5-bromo-deoxyuridine, or fluorescein to provide probes that can be used in numerous applications. Preferably, the isolated polynucleotide specifically hybridizes with at least 8, 9, 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 nucleotides of the nucleotide sequence encoding the T6'S variant $\alpha 7$ subunit. More preferably, the isolated polynucleotide specifically hybridizes with at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, or 60 nucleotides of the T6'S variant TM2 domain (*e.g.*, of SEQ ID NOs: 18, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30). Optionally, the isolated polynucleotide can specifically hybridize with nucleotides within that portion of the target sequence which encodes the T6'S variant TM2 domain and outside that which encodes the TM2 domain. Optionally, hybridization outside the T6'S variant TM2 domain can include hybridization to non-coding nucleotides. Such polynucleotides can be produced by recombinant or synthetic techniques.

Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for duplex formation. Severity of conditions can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under low, intermediate, or high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak, 1987, *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

For example, hybridization of immobilized DNA on Southern blots with ^{32}P -labeled gene-specific probes can be performed by standard methods (Maniatis *et al.* [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York). In general, hybridization and subsequent washes can be carried out under intermediate to high stringency conditions that allow for detection of target sequences with homology to the exemplified polynucleotide sequence. For double-stranded DNA gene probes, hybridization can be carried out overnight at 20-25° C below the melting temperature (T_m) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula

(Beltz *et al.* [1983] *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

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

5 Washes are typically carried out as follows:

(1) twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash);

(2) once at $T_m - 20^\circ\text{C}$ for 15 minutes in 0.2X SSPE, 0.1% SDS (intermediate stringency wash).

10 For oligonucleotide probes, hybridization can be carried out overnight at 10-20°C below the melting temperature (T_m) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. T_m for oligonucleotide probes can be determined by the following formula:

$$T_m(^{\circ}\text{C}) = 2(\text{number T/A base pairs}) + 4(\text{number G/C base pairs}) \text{ (Suggs *et al.* [1981])}$$

15 *ICN-UCLA Symp. Dev. Biol. Using Purified Genes*, D.D. Brown [ed.], Academic Press, New York, 23:683-693).

Washes can be carried out as follows:

(1) twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash);

20 (2) once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (intermediate stringency wash).

In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment >70 or so bases in length, the following conditions can be used:

Low: 1 or 2X SSPE, room temperature

25 Low: 1 or 2X SSPE, 42°C

Intermediate: 0.2X or 1X SSPE, 65°C

High: 0.1X SSPE, 65°C.

By way of another non-limiting example, procedures using conditions of high stringency can also be performed as follows: Pre-hybridization of filters containing DNA
30 is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C, the preferred

hybridization temperature, in pre-hybridization mixture containing 100 µg/ml denatured salmon sperm DNA and $5-20 \times 10^6$ cpm of ^{32}P -labeled probe. Alternatively, the hybridization step can be performed at 65°C in the presence of SSC buffer, 1X SSC corresponding to 0.15M NaCl and 0.05 M Na citrate. Subsequently, filter washes can be
5 done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA, followed by a wash in 0.1X SSC at 50°C for 45 min. Alternatively, filter washes can be performed in a solution containing 2X SSC and 0.1% SDS, or 0.5X SSC and 0.1% SDS, or 0.1X SSC and 0.1% SDS at 68°C for 15 minute intervals. Following the wash steps, the hybridized probes are detectable by autoradiography. Other
10 conditions of high stringency which may be used are well known in the art and as cited in Sambrook *et al.*, 1989, Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y., pp. 9.47-9.57; and Ausubel *et al.*, 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. are incorporated herein in their entirety.

15 Another non-limiting example of procedures using conditions of intermediate stringency are as follows: Filters containing DNA are pre-hybridized, and then hybridized at a temperature of 60°C in the presence of a 5X SSC buffer and labeled probe. Subsequently, filters washes are performed in a solution containing 2X SSC at 50°C and the hybridized probes are detectable by autoradiography. Other conditions of
20 intermediate stringency which may be used are well known in the art and as cited in Sambrook *et al.*, 1989, Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Press, N.Y., pp. 9.47-9.57; and Ausubel *et al.*, 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. are incorporated herein in their entirety.

25 Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probe sequences of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable
30 hybrids with the target polynucleotide of interest. Mutations, insertions and deletions can be produced in a given polynucleotide sequence in many ways, and these methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

A “complementary” polynucleotide sequence, as used herein, generally refers to a sequence arising from the hydrogen bonding between a particular purine and a particular pyrimidine in double-stranded nucleic acid molecules (DNA-DNA, DNA-RNA, or RNA-RNA). The major specific pairings are guanine with cytosine and adenine with thymine or uracil. A “complementary” polynucleotide sequence may also be referred to as an “antisense” polynucleotide sequence or an “antisense sequence”.

The present invention also includes pharmaceutical compositions useful for treating conditions associated with neurodegenerative processes, enzymatic function, affective disorders or immuno function, comprising a pharmaceutically acceptable carrier and an agent that regulates the function of a T6’S variant TM2 domain comprising the amino acid sequence of SEQ ID NO:2, the function of the T6’S variant $\alpha 7$ subunit comprising the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:20, or amino acids 23 to 502 of SEQ ID NO:20, and/or the function of the T6’S variant nACh receptor comprising the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:20, or amino acids 23 to 502 of SEQ ID NO:20; or a functional fragment or homologue of any of the foregoing. Such homologues include, for example, the mature peptides of SEQ ID NOs. 7, 9, 11, 13, 15, and 17, wherein the threonine in the sixth amino acid position of the TM2 domain has been substituted with a serine residue.

The present invention also includes pharmaceutical compositions useful for treating conditions associated with neurodegenerative processes, enzymatic function, affective disorders or immuno function, comprising a pharmaceutically acceptable carrier and a polynucleotide encoding the T6’S variant TM2 domain comprising the amino acid sequence of SEQ ID NO:2, the T6’S variant $\alpha 7$ subunit comprising the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:20, or amino acids 23 to 502 of SEQ ID NO:20, and/or the T6’S variant nACh receptor comprising the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:20, or amino acids 23 to 502 of SEQ ID NO:20; or a functional fragment or homologue of any of the foregoing. The polynucleotide is preferably a component of a host cell or an expression vector, which further comprises a control sequence that directs expression of the polynucleotide, whereby the polynucleotide is expressed within the cell upon transplantation *in vivo*. In other embodiments, the polynucleotide T6’S variant TM2 domain can encode homologs of SEQ ID NO:20. Such homologues include, for example, the mature peptides of SEQ ID

NOs. 7, 9, 11, 13, 15, and 17, wherein the threonine in the sixth amino acid position of the TM2 domain has been substituted with a serine residue.

The present invention further includes methods for treating one or more of the aforementioned conditions by administering an agent that regulates the function of a T6'S variant of the present invention, or by administering a T6'S variant polypeptide of the present invention, or by administering a polynucleotide encoding a T6'S variant polypeptide of the present invention, to a patient in need thereof. Preferably, the agent, T6'S polypeptide, or T6'S polynucleotide administered is a pharmaceutical composition of the subject invention. The pharmaceutical compositions of the present invention can be administered to a patient by any route that results in the desired therapeutic effect. For example, the pharmaceutical compositions can be administered intravenously (I.V.), intramuscularly (I.M.), subcutaneously (S.C.), intradermally (I.D.), orally, intranasally, *etc.*

Thus, the present invention includes methods of gene therapy whereby polynucleotides encoding the T6'S variant of the present invention is delivered to a patient, and the T6'S variant-encoding polynucleotide is expressed *in vivo*. The term "gene therapy", as used herein, refers to the transfer of genetic material (*e.g.*, DNA or RNA) of interest into a host to treat or prevent a genetic or acquired disease or condition phenotype. The genetic material of interest encodes a product (*e.g.*, a protein, polypeptide, peptide, or functional RNA) whose production *in vivo* is desired. For example, the genetic material of interest can encode a hormone, receptor, enzyme, polypeptide or peptide of therapeutic value. For a review see, in general, the text "Gene Therapy" (*Advances in Pharmacology 40*, Academic Press, 1997).

Two basic approaches to gene therapy have evolved: (1) *ex vivo* and (2) *in vivo* gene therapy. The methods of the subject invention encompass either or both. In *ex vivo* gene therapy, cells are removed from a patient and, while being cultured, are treated *in vitro*. Generally, a functional replacement gene is introduced into the cell via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination, *etc.*) and an expression system as needed and then the modified cells are expanded in culture and returned to the host/patient. These genetically reimplanted cells have been shown to produce the transfected gene product *in situ*.

In *in vivo* gene therapy, target cells are not removed from the subject, rather the gene to be transferred is introduced into the cells of the recipient organism *in situ*, that is within the recipient. Alternatively, if the host gene is defective, the gene is repaired *in situ*. These genetically altered cells have been shown to produce the transfected gene product *in situ*.

The gene expression vector is capable of delivery/transfer of heterologous nucleic acid sequences into a host cell. The expression vector may include elements to control targeting, expression and transcription of the nucleic acid sequence in a cell selective manner as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene may be replaced by the 5'UTR and/or 3'UTR of the expression vehicle.

The expression vector can include a promoter for controlling transcription of the heterologous material and can be either a constitutive or inducible promoter to allow selective transcription. The expression vector can also include a selection gene.

Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang *et al.*, Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega *et al.*, Gene Targeting, CRC Press, Ann Arbor, Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and include, for example, stable or transient transfection, lipofection, electroporation, and infection with recombinant viral vectors.

Introduction of nucleic acids by infection offers several advantages over the other listed methods. Higher efficiency can be obtained due to their infectious nature. Moreover, viruses are very specialized and typically infect and propagate in specific cell types. Thus, their natural specificity can be used to target the vectors to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

A specific example of a DNA viral vector for introducing and expressing recombinant sequences is the adenovirus derived vector Adenop53TK. This vector expresses a herpes virus thymidine kinase (TK) gene for either positive or negative

selection and an expression cassette for desired recombinant sequences. This vector can be used to infect cells that have an adenovirus receptor which includes most cancers of epithelial origin as well as others. This vector as well as others that exhibit similar desired functions can be used to treat a mixed population of cells and can include, for example, an *in vitro* or *ex vivo* culture of cells, a tissue or a human subject.

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus. An example of such a negative selection marker is the TK gene described above that confers sensitivity to the antibiotic gancyclovir. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation will not occur. Features that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

In addition, recombinant viral vectors are useful for *in vivo* expression of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

The pharmaceutical compositions of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Sciences* (Martin EW [1995] Easton Pennsylvania, Mack Publishing Company, 19th ed.) describes formulations which can be used in connection with the subject invention. Formulations suitable for parenteral administration include, for example, aqueous sterile injection

solutions, which may contain antioxidants, buffers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powder, granules, tablets, *etc.* It should be understood that in addition to the ingredients particularly mentioned above, the formulations of the subject invention can include other agents conventional in the art having regard to the type of formulation in question.

The present invention also includes antibodies (*e.g.*, monoclonal or polyclonal) and antibody fragments that specifically bind to a T6'S variant $\alpha 7$ TM2 domain comprising the amino acid sequence of SEQ ID NO: 2. Antibodies that are immunospecific for the polypeptides as set forth herein are specifically contemplated. In various embodiments, antibodies that do not cross-react with other proteins (such as *A. marginale* MSP5) are also specifically contemplated. Preferably, the antibody or antibody fragment does not specifically bind to the wild-type $\alpha 7$ TM2 domain. The antibodies of the subject invention can be prepared using standard materials and methods known in the art (see, for example, *Monoclonal Antibodies: Principles and Practice*, 1983; *Monoclonal Hybridoma Antibodies: Techniques and Applications*, 1982; *Selected Methods in Cellular Immunology*, 1980; *Immunological Methods, Vol. II*, 1981; *Practical Immunology*, and Kohler *et al.* [1975] *Nature* 256:495). These antibodies can further comprise one or more additional components, such as a solid support, a carrier or pharmaceutically acceptable excipient, or a label.

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity, particularly neutralizing activity. "Antibody fragments" comprise a portion of a full-length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab',

F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multi-specific antibodies formed from antibody fragments.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.* [1975] *Nature* 256: 495, or may be made by recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.* [1991] *Nature* 352: 624-628 and Marks *et al.* [1991] *J. Mol. Biol.* 222: 581-597, for example.

The monoclonal antibodies described herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison *et al.* [1984] *Proc. Natl. Acad. Sci. USA* 81: 6851-6855). Also included are humanized antibodies, such as those taught in U.S. Patent Nos. 6,407,213 or 6,417,337 which are hereby incorporated by reference in their entirety.

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and

V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies* [1994] Vol. 113:269-315, Rosenberg and Moore eds. Springer-Verlag, New York.

An "isolated" antibody is one which has been identified and separated and/or
5 recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most
10 preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the
15 antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The terms "patient", "subject", and "individual" are used interchangeably herein to refer to human or non-human mammals. For example, the patient may be a human or non-human primate, rodent (such as rat or mouse), or other mammalian species.

20 The terms "comprising", "consisting of" and "consisting essentially of" are defined according to their standard meaning. The terms may be substituted for one another throughout the instant application in order to attach the specific meaning associated with each term. The phrases "isolated" or "biologically pure" refer to material that is substantially or essentially free from components which normally accompany the
25 material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment. "Link" or "join" refers to any method known in the art for functionally connecting peptides, including, without limitation, recombinant fusion, covalent bonding, disulfide bonding, ionic bonding, hydrogen bonding, and electrostatic
30 bonding.

As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise.

Thus, for example, a reference to "a receptor subunit" includes more than one such subunit, and the like.

The practice of the present invention can employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology, electrophysiology, and pharmacology, that are within the skill of the art. Such techniques are explained fully in the literature (see, *e.g.*, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989); DNA Cloning, Vols. I and II (D. N. Glover ed. 1985); Perbal, B., *A Practical Guide to Molecular Cloning* (1984); the series, *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Transcription and Translation* (Hames *et al.* eds. 1984); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller et al. eds. (1987) Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.); *Scopes, Protein Purification: Principles and Practice* (2nd ed., Springer-Verlag); and *PCR: A Practical Approach* (McPherson et al. eds. (1991) IRL Press)).

15

Materials and Methods

cDNA clones. These experiments used the rat neuronal nAChR and the mouse muscle cDNA clones, which were obtained from Dr. Jim Boulter (UCLA).

Site-directed mutagenesis. Site-directed mutagenesis was performed using QuickChange (TM) kits (Stratagene, LaJolla, CA). In brief, two complimentary oligonucleotides were synthesized which contained the desired mutation flanked by 10-15 bases of unmodified nucleotide sequence. Using a thermal cycler, *Pfu* DNA polymerase extends the sequence around the whole vector, generating a plasmid with staggered nicks. Each cycle builds only off the parent strands, and therefore there is no amplification of misincorporations. After 12-16 cycles, the product was treated with *Dpn* I, which digests the methylated parent DNA into numerous small pieces. The product was then transformed into *E. coli* cells, which repair the nicks.

Preparation of RNA. After linearization and purification of cloned cDNAs, RNA transcripts were prepared *in vitro* using the appropriate mMessage mMachine kit from Ambion Inc. (Austin, TX).

30

Expression in *Xenopus* oocytes. Mature (>9 cm) female *Xenopus laevis* African toads (Nasco, Ft. Atkinson, WI) were used as a source of oocytes. Prior to surgery, frogs

were anesthetized by placing the animal in a 2 g/L solution of MS222 (3-aminobenzoic acid ethyl ester). Oocytes were removed from an incision made in the abdomen.

In order to remove the follicular cell layer, harvested oocytes were treated with collagenase from Worthington Biochemical Corporation (Freehold, NJ) for 2 hours at room temperature in calcium-free Barth's solution (88 mM NaCl, 10 mM HEPES pH 7.6, 0.33 mM MgSO₄, 0.1 mg/ml gentamicin sulfate). Subsequently, stage 5 oocytes were isolated and injected with 50 nl each of a mixture of the appropriate subunit cRNAs following harvest. Recordings were made 3 to 21 days after injection depending on the cRNAs being tested.

Voltage-clamp recording of whole oocyte responses. Data were obtained by means of two-electrode voltage-clamp recording. Recordings were made at room temperature (21-24 deg. C) in Frog Ringer's solution (115 mM NaCl, 10 mM HEPES, 2.5 mM KCl, and 1.8 mM CaCl₂, pH 7.3) with 1 μ M atropine to inhibit muscarinic acetylcholine receptor responses. This extracellular solution was used for all experiments unless otherwise noted. Voltage electrodes were filled with 3M KCl, and current electrodes were filled with 250 mM CsCl, 250 mM CsF, and 100 mM EGTA (pH 7.3).

Bath solution and drug applications are applied through a linear perfusion system to oocytes placed in Lucite chamber with a total volume of 0.5 ml. Drug delivery involves pre-loading a 1.8 ml length of tubing at the terminus of the perfusion system, while a Mariotte flask filled with Ringer's solution is used to maintain constant perfusion. Applications of drug solutions are then synchronized with acquisition. Current responses are recorded using a PC interfaced to either a Warner OC-725C (Warner Instruments, Hamden, CT) or a GeneClamp 500 amplifier via a Digidata 1200 digitizer (Axon Instruments, Union City, CA). In addition, some oocyte recordings were made using a beta version of the OpusXpress 6000A (Axon Instruments, Union City, CA). OpusXpress is an integrated system that provides automated impalement and voltage clamp, which in our case permitted the study of four oocytes in parallel. Cells were automatically perfused with bath solution, and agonist solutions were delivered from a 96-well compound plate. In experiments using the OpusXpress system, the voltage and current electrodes were filled with 3 M KCl. In all experiments, bath flow rates were set at 6 ml/minute.

Experimental protocols and analysis of data from *Xenopus* oocytes. Current responses to drug application were studied under two-electrode voltage clamp at a holding potential of -50 mV unless otherwise noted (-60 mV for the OpusXpress system). Holding currents immediately prior to agonist application were subtracted from measurements of the peak response to agonist. All drug applications were separated by a wash period of 5 minutes unless otherwise noted. At the start of recording, all oocytes received two initial control applications of ACh. Subsequent drug applications were normalized to the second ACh application in order to control for the level of channel expression in each oocyte. Means and standard errors (SEM) were calculated from the normalized responses of at least four oocytes for each experimental concentration.

For concentration-response relations, data were plotted using Kaleidagraph 3.0.2 (Abelbeck Software; Reading, PA), and curves were generated using the Hill equation:

$$\text{Response} = \frac{I_{\max} [\text{agonist}]^n}{[\text{agonist}]^n + (EC_{50})^n}$$

where $I_{\max}$ denotes the maximal response for a particular agonist/subunit combination, and n represents the Hill coefficient. $I_{\max}$, n , and the EC_{50} were all unconstrained for the fitting procedures.

For experiments measuring barium permeability, oocytes were perfused with barium Ringers (low barium: 90.7 mM NaCl, 2.5 mM KCl, 10 mM HEPES pH 7.3, 1.8 mM BaCl₂, 48.6 mM sucrose; high barium: 90.7 mM NaCl, 2.5 mM KCl, 10 mM HEPES pH 7.3, 18 mM BaCl₂). Shifts in reversal potential were measured by changing the holding potential from -40 mV to +30 mV by 10 mV increments. Calculations of barium - sodium permeability ratios using the extended GHK equation were performed using the Clampfit analysis portion of the pClamp software suite (Axon Instruments, Union City, CA). Barium was used instead of calcium to minimize the contribution of endogenous calcium-activated chloride channels (Sands, S.B. *et al. Biophys. J.*, 1993, 65:2614-2621).

Calculations of peak amplitudes and net charge were made using pClamp either during acquisition or during subsequent Clampfit analysis. Note that measurement of net charge has been shown to be a more accurate indicator of fast $\alpha 7$ responses than measurement of peak response. An appropriate method using analysis of the area under the curve of agonist-evoked currents in oocytes has been previously published (Papke,

R.L. and J.K.P. Papke *Br. J. Pharmacol.*, 2002, 137:49-61). Baseline was defined for Clampfit statistics based on 20 s before drug application, the analysis region for peak and net charge analysis went from 5 s before the initiation of drug application and extended at least 135 s following. Area analysis data is provided for all receptor subtypes examined in this paper for comparison to wild-type $\alpha 7$.

Transfection and whole cell voltage clamp recording from GH4C1 cells. GH4C1 cells were cultured in F10 medium (Gibco, Carlsbad, CA) at 37 °C, 5% CO₂. Cells were transiently transfected using Fugene (Roche, Indianapolis, IN), according to the manufacturer instructions. One microgram of WT or T6'F mutant $\alpha 7$ cDNA (pTR-UF22, University of Florida, Gainesville, FL) was added to each 35-mm Petri dish, together with 0.5 or 1 μ g of the cDNA encoding the red fluorescent DsRed protein (BD Biosciences Clontech, Palo Alto, CA). Cells were used 48-72 hours after transfection. Typical transfection efficiency was 10-25% using this method.

Whole-cell currents were recorded using an Axopatch 200B amplifier (Axon Instruments, Union City, CA) at room temperature. Cells were bathed in a solution containing 140 mM NaCl, 2.8 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, 10 mM glucose, 10 mM HEPES/ NaOH (pH 7.3). Patch electrodes (tip resistances, 1-3 M Ω) were filled with 90 mM CsCl, 60 mM CsF, 10 mM HEPES/CsOH (pH 7.3). Cells were continuously superfused by a gravity-driven perfusion system connected to a PZ-150M piezoelectric switching device (EXFO Burleigh Products Group, Victor, NY), with independent tubes for each solution placed ca. 100 μ m from the cells. Currents were recorded at 2 KHz using pClamp 8 (Axon Instruments Union City, CA), at a membrane holding potential of -70 mV, unless otherwise indicated. Analysis was conducted using pClamp 9.

Example 1—The T6'S mutant receptor pharmacology closely resembles that of wild-type $\alpha 7$

Raw data traces from transiently transfected GH4C1 cells show the larger amplitude and slower macroscopic kinetics of the T6'S mutant compared to wild-type $\alpha 7$ (Figures 8A and 8B), and that this observation carries over from oocyte studies to a mammalian cell under whole-cell voltage clamp. Table 2 shows the results of oocyte experiments examining representative concentrations of a battery of compounds, indicating the ability of the T6'S mutant to effectively emulate wild-type $\alpha 7$. For the

drugs examined, the T6'S mutant is more like wild-type $\alpha 7$, particularly in regard to antagonists that have been converted to agonists in the L247T mutant.

Figures 8A and 8B and Table 2 show that the T6'S variant of the present invention yields much larger currents than wild-type $\alpha 7$ receptor, and provides a more faithful representation of the wild-type $\alpha 7$ pharmacology than does the commonly used L247T mutant.

Table 2

	L247T	Wild type	T6'S
Choline EC ₅₀	12 μ M	300 μ M	$\approx$ 360 μ M
ACh EC ₅₀	1 μ m	30 μ M	100 μ M
GTS-21 (DMXB)	full agonist	32% agonist 5 μ M	12% agonist 3 μ M
AR-R17779	?	78% agonist 10 μ M	90% agonist 30 μ M
Cytisine	?	73% agonist 13 μ M	80% agonist 43 μ M
Choline	?	100% agonist 300 μ M	95% agonist 2 mM
TC-2403	?	16 % agonist 240 μ M	18% agonist 400 μ M
4OH-GTS-21	?	46% agonist 1.4 μ M	20% agonist 3.3 μ M
Tropisetron	?	38% Agonist 0.3 μ M	30 % 0.9 μ M
Nor-Nicotine	?	50 % Agonist 6 μ M	50 % 64 μ M
Bicuculline	agonist	antagonist	antagonist
5-HT	agonist	antagonist	antagonist
Tubocurarine	agonist	antagonist	antagonist
Hexamethonium	agonist	antagonist	antagonist
Zinc	agonist	antagonist	antagonist
5HI (5-hydroxy-indole)	?	potentiates	does not potentiate

10 Example 2—Alpha7 TM2 6' mutants are functionally expressed in oocytes and impact absolute current amplitude

Raw data traces for each of the TM2 6' single point mutations and wild-type $\alpha 7$ are shown in Figure 3. A particularly obvious difference between the two 6' mutant

receptors and wild-type $\alpha 7$ is the consistently large peak current for the T6'S mutant and the relatively small peak for the T6'F response. Quantification of differences in absolute amplitude is complicated by variations in the degree of receptor expression from cell to cell (which is why each cell is used as it's own control), however variations in current
5 amplitude similar to those seen were regularly observed.

Example 3—The $\alpha 7$ T6'F mutation increases ACh potency while the T6'S mutant shows no significant change in ACh potency compared to wild type $\alpha 7$

Concentration-response functions for wild-type $\alpha 7$ and each of the two TM2 6' mutants are seen in Figures 4A-4C. Both net charge (area under the curve) and peak
10 CRCs are shown to illustrate the differences in apparent ACh potency as a function of the analysis method used. Comparison of these analyses shows that the wild-type $\alpha 7$ receptor shows the greatest difference in apparent potency, while the two 6' mutants show less difference between the two methods, probably due to their slower response kinetics (see
15 methods). In addition, a dramatic increase in ACh potency is seen for the net charge analysis if the T6'F mutant, whereas the T6'S mutant is not significantly different from wild-type.

Example 4—The $\alpha 7$ T6'F mutation abolishes divalent ion permeability and reduces inward current rectification
20

Current-voltage relationships in either high or low extracellular barium, show that the wild-type $\alpha 7$ receptor displays its characteristic inward rectification and permeability to divalent ions (Figures 5A and 5B). The extended GHK equation gives an estimated barium-to-sodium permeability ratio of approximately 4:1. By contrast, the T6'F mutant
25 receptor shows little current rectification, particularly under conditions of high extracellular barium and there was a complete lack of a shift in reversal potential with varying extracellular divalent ion concentrations. The GHK equation applied to these results gives a barium to sodium permeability ratio that is near zero. Measurements of barium ion permeability in the T6'S mutant were complicated by biphasic currents like
30 the example shown Figure 5C. These biphasic currents occurred near the reversal potential for chloride and possibly reflect an interaction between barium and the calcium gated chloride channels that are constitutively expressed by Xenopus oocytes. Given the

slow, large amplitude macroscopic responses of the $\alpha 7$ T6'S mutant, it is possible that low potency interactions between barium and these calcium gated chloride channels would be significantly enhanced.

5 Example 5—Succinylcholine is a selective agonist of muscle-type receptors, and is also an agonist of $\alpha 7$ T6'F mutant receptors, but not the T6'S mutant

 The comparative effect of 100 μ M succinylcholine on muscle or wild-type $\alpha 7$ receptors, shows that succinylcholine is relatively ineffective in activating wild-type $\alpha 7$ (Figures 6A and 6B). A dramatic increase in sensitivity to succinylcholine can be seen in
10 the T6'F mutant receptor, while the T6'S mutant shows no virtually no response at this concentration.

 The concentration-response functions for succinylcholine applied to wild-type $\alpha 7$, or the T6'F or T6'S mutant receptors show that both wild-type $\alpha 7$ and the T6'S mutant respond poorly through a full range of concentrations, while succinylcholine is a
15 relatively potent, full agonist of the T6'F mutant (Figure 7).

 All patents, patent applications, provisional applications, and publications referred to or cited herein, whether supra or infra, are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit
20 teachings of this specification.

 It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Claims

What is claimed is:

1. A method for identifying agents that modulate nicotinic acetylcholine receptor (nAChR) activity, comprising:

(a) providing a host cell that expresses a polynucleotide encoding a variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the variant $\alpha 7$ nAChR polypeptide has a serine residue in the sixth amino acid position of the TM2 domain;

(b) contacting a test agent with the host cell; and

(c) determining:

(i) the effect of the test agent on the variant $\alpha 7$ nAChR polypeptide or the host cell expressing the polynucleotide, or

(ii) the binding of the test agent to the host cell or the variant $\alpha 7$ nAChR polypeptide, or

(iii) both (c) (i) and (c) (ii).

2. The method of claim 1, wherein the TM2 domain comprises the amino acid sequence of SEQ ID NO:2.

3. The method of claim 1 or claim 2, wherein the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 18, SEQ ID NO:19, 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: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, and SEQ ID NO:60, or degenerate variants of any of the foregoing.

4. The method of any of claims 1-3, wherein the variant $\alpha 7$ nAChR polypeptide comprises a mammalian $\alpha 7$ nAChR polypeptide having a serine in the sixth amino acid position of the TM2 domain.

5. The method of any of claims 1-4, wherein the effect of the test agent on the $\alpha 7$ receptor activity of the variant $\alpha 7$ nAChR polypeptide is determined.

6. The method of any of claims 1-5, wherein the variant $\alpha 7$ nAChR polypeptide is a mammalian $\alpha 7$ nAChR polypeptide having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

7. The method of any of claims 1-5, wherein the variant $\alpha 7$ nAChR polypeptide is an $\alpha 7$ nAChR polypeptide of human, non-human primate, rodent, chicken, or zebrafish, having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

8. The method of any of claims 1-7, wherein the variant $\alpha 7$ nAChR polypeptide comprises the human $\alpha 7$ nAChR polypeptide having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

9. The method of any of claims 1-8, wherein said determining of step (c) (ii) is performed by measuring a signal generated by a detectable moiety.

10. The method of claim 9, wherein the detectable moiety is selected from the group consisting of a fluorescent label, a radiolabel, a chemiluminescent label, and an enzyme.

11. The method of any of claims 1-10, wherein said determining of step (c) (i) is performed by measuring a signal generated by a radiolabeled ion, a fluorescent probe, or an electrical current.

12. The method of any of claims 1-11, wherein the host cell is selected from the group consisting of a bacterial cell, a mammalian cell, a yeast cell, an amphibian cell, and a starfish cell.

- (ii) the binding of the test agent to the host cell or the variant $\alpha 7$ nAChR polypeptide, or
- (iii) both of the foregoing.

13. The method of any of claims 1-12, wherein the host cell is a mammalian cell, and wherein the polynucleotide is stably expressed in the mammalian cell such that a functional receptor is formed, wherein the functional receptor elicits electrophysiological currents *in vitro* or *in vivo* when stimulated by a wild-type $\alpha 7$ agonist.

14. The method of any of claims 1-13, wherein the serine residue is a substitution for a threonine residue (T→S).

15. A method for identifying a cytoprotective agent, comprising:

- (a) providing a host cell that expresses a polynucleotide encoding a variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the variant $\alpha 7$ nAChR polypeptide has a serine residue in the sixth amino acid position of the TM2 domain;
- (b) contacting a test agent with the host cell; and
- (c) monitoring the cell or cellular function for an indication of cytotoxicity.

16. The method of claim 15, wherein the TM2 domain comprises the amino acid sequence of SEQ ID NO:2.

17. The method of claim 15 or claim 16, wherein the polynucleotide comprises SEQ ID NO: 18, SEQ ID NO:19, 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: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, and SEQ ID NO:60, or degenerate variants of any of the foregoing.

18. The method of any of claims 15-17, wherein the host cell is selected from the group consisting of a bacterial cell, a mammalian cell, a yeast cell, an amphibian cell, and a starfish cell.

19. The method of any of claims 15-18, wherein the host cell is a mammalian cell, and wherein the polynucleotide is stably expressed in the mammalian cell such that a functional receptor is formed, wherein the functional receptor elicits electrophysiological currents *in vitro* or *in vivo* when stimulated by a wild-type $\alpha 7$ agonist.

20. The method of any of claims 15-19, wherein the serine residue is a substitution for a threonine residue (T $\rightarrow$ S).

21. The method of any of claims 15-20, wherein the variant $\alpha 7$ nAChR polypeptide comprises a mammalian $\alpha 7$ nAChR polypeptide having a serine in the sixth amino acid position of the TM2 domain.

22. The method of any of claims 15-21, wherein the variant $\alpha 7$ nAChR polypeptide is a mammalian $\alpha 7$ nAChR polypeptide having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

23. The method of any of claims 15-20, wherein the variant $\alpha 7$ nAChR polypeptide is an $\alpha 7$ nAChR polypeptide of human, non-human primate, rodent, chicken, or zebrafish, having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

24. The method of any of claims 15-23, wherein the variant $\alpha 7$ nAChR polypeptide comprises the human $\alpha 7$ nAChR polypeptide having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

25. The method of any of claims 15-24, wherein the host cell is maintained in the presence of a substance so as to minimize or block a cytotoxic effect on the host cell.

26. An isolated polynucleotide encoding a variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the variant $\alpha 7$ nAChR polypeptide has a serine residue in the sixth amino acid position of the TM2 domain.

27. The isolated polynucleotide of claim 26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2.

28. The isolated polynucleotide of claim 26, wherein the serine residue is a substitution for a threonine residue (T $\rightarrow$ S).

29. The isolated polynucleotide of any of claims 26-28, wherein the variant $\alpha 7$ nAChR polypeptide comprises a mammalian $\alpha 7$ nAChR polypeptide having a serine in the sixth amino acid position of the TM2 domain.

30. The isolated polynucleotide of claim 26, wherein the variant $\alpha 7$ nAChR polypeptide is a mammalian $\alpha 7$ nAChR polypeptide having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

31. The isolated polynucleotide of any of claims 26-28, wherein the variant $\alpha 7$ nAChR polypeptide is an $\alpha 7$ nAChR polypeptide of human, non-human primate, rodent, chicken, or zebrafish, having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

32. The isolated polynucleotide of any of claims 26-30, wherein the variant $\alpha 7$ nAChR polypeptide comprises the human $\alpha 7$ nAChR polypeptide having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

33. The isolated polynucleotide of any of claims 26-32, wherein the polynucleotide comprises SEQ ID NO: 18, SEQ ID NO:19, 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: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, and SEQ ID NO:60, or degenerate variants of any of the foregoing.

34. An isolated variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the variant $\alpha 7$ nAChR polypeptide has a serine residue in the sixth amino acid position of the TM2 domain.

35. The isolated polypeptide of claim 34, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2.

36. The isolated polypeptide of claim 34, wherein the serine residue is a substitution for a threonine residue (T $\rightarrow$ S).

37. The isolated polypeptide of any of claims 34-36, wherein the variant $\alpha 7$ nAChR polypeptide comprises a mammalian $\alpha 7$ nAChR polypeptide having a serine in the sixth amino acid position of the TM2 domain.

38. The isolated polypeptide of any of claims 34-37, wherein the variant $\alpha 7$ nAChR polypeptide is a mammalian $\alpha 7$ nAChR polypeptide having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

39. The isolated polypeptide of any of claims 34-36, wherein the variant $\alpha 7$ nAChR polypeptide is an $\alpha 7$ nAChR polypeptide of human, non-human primate, rodent, chicken, or zebrafish, having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

40. The isolated polypeptide of any of claims 34-39, wherein the variant $\alpha 7$ nAChR polypeptide comprises the human $\alpha 7$ nAChR polypeptide having a threonine to serine (T→S) substitution in the sixth amino acid position of the TM2 domain.

41. An expression vector comprising a polynucleotide encoding a variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the variant $\alpha 7$ nAChR polypeptide has a serine residue in the sixth amino acid position of the TM2 domain, and wherein said polynucleotide is operably linked to a control sequence that directs the expression of said polynucleotide whereby said polynucleotide is expressed in a host cell.

42. The expression vector of claim 41, wherein said polypeptide encoded by said polynucleotide comprises the amino acid sequence of SEQ ID NO:2.

43. The expression vector of claim 41, wherein the serine residue is a substitution for a threonine residue (T→S).

44. The expression vector of any of claims 41-43, wherein the variant $\alpha 7$ nAChR polypeptide comprises a mammalian $\alpha 7$ nAChR polypeptide having a serine in the sixth amino acid position of the TM2 domain.

45. The expression vector of any of claims 41-44, wherein the variant $\alpha 7$ nAChR polypeptide is a mammalian $\alpha 7$ nAChR polypeptide having a threonine to serine (T→S) substitution in the sixth amino acid position of the TM2 domain.

46. The expression vector of any of claims 41-43, wherein the variant $\alpha 7$ nAChR polypeptide is an $\alpha 7$ nAChR polypeptide of human, non-human primate, rodent, chicken, or zebrafish, having a threonine to serine (T→S) substitution in the sixth amino acid position of the TM2 domain.

47. The expression vector of any of claims 41-46, wherein the variant $\alpha 7$ nAChR polypeptide comprises the human $\alpha 7$ nAChR polypeptide having a threonine to serine (T→S) substitution in the sixth amino acid position of the TM2 domain.

48. The expression vector of any of claims 41-47, wherein the polynucleotide comprises SEQ ID NO: 18, SEQ ID NO:19, 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: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, and SEQ ID NO:60, or degenerate variants of any of the foregoing.

49. The expression vector of any of claims 41-48, wherein said control sequence comprises an inducible promoter.

50. An isolated host cell comprising a polynucleotide encoding a variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the variant $\alpha 7$ nAChR polypeptide has a serine residue in the sixth amino acid position of the TM2 domain, and wherein said polynucleotide is operably linked to a control sequence that directs the expression of said polynucleotide in said host cell.

51. The isolated host cell of claim 50, wherein said host cell further comprises a control sequence operably linked to said polynucleotide, wherein the control sequence directs expression of said polynucleotide in said host cell.

52. The isolated host cell of claim 50, wherein said polynucleotide encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2.

53. The isolated host cell of any of claims 50-52, wherein the serine residue is a substitution for a threonine residue (T→S).

54. The isolated host cell of any of claims 50-53, wherein the variant $\alpha 7$ nAChR polypeptide comprises a mammalian $\alpha 7$ nAChR polypeptide having a serine in the sixth amino acid position of the TM2 domain.

55. The isolated host cell of any of claims 50-54, wherein the variant $\alpha 7$ nAChR polypeptide is a mammalian $\alpha 7$ nAChR polypeptide having a threonine to serine (T→S) substitution in the sixth amino acid position of the TM2 domain.

56. The isolated host cell of claim 50, wherein the variant $\alpha 7$ nAChR polypeptide is an $\alpha 7$ nAChR polypeptide of human, non-human primate, rodent, chicken, or zebrafish, having a threonine to serine (T→S) substitution in the sixth amino acid position of the TM2 domain.

57. The isolated host cell of any of claims 50-55, wherein the variant $\alpha 7$ nAChR polypeptide comprises the human $\alpha 7$ nAChR polypeptide having a threonine to serine (T→S) substitution in the sixth amino acid position of the TM2 domain.

58. The isolated host cell of any of claims 50-57, wherein the polynucleotide comprises SEQ ID NO: 18, SEQ ID NO:19, 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: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, and SEQ ID NO:60, or degenerate variants of any of the foregoing.

59. The isolated host cell of any of claims 50-58, wherein said cell is selected from the group consisting of a bacterial cell, a mammalian cell, a yeast cell, and an amphibian cell.

60. The isolated host cell of any of claims 50-58, wherein said cell is a human cell.

61. The isolated host cell of any of claims 50-60, wherein said host cell is a mammalian cell, and wherein said polynucleotide is stably expressed in said mammalian cell such that a functional receptor is formed, wherein said functional receptor elicits electrophysiological currents *in vitro* or *in vivo* when stimulated by a wild-type $\alpha 7$ agonist.

62. A method for treating a neurological condition in a patient, said method comprising administering a polypeptide to the patient having a TM2 domain, wherein the polypeptide has a serine residue in the sixth amino acid position of the TM2 domain; or administering a polynucleotide encoding the polypeptide to the patient, wherein the polynucleotide is expressed within the patient.

63. The method of claim 62, wherein the TM2 domain comprises the amino acid of SEQ ID NO:2.

64. The method of claim 62 or claim 63, wherein a vector or host cell comprising the polynucleotide is administered to the patient.

65. The method of any of claims 62-64, wherein a viral vector or non-viral vector comprising the polynucleotide is administered to the patient.

66. The method of any of claims 62-65, wherein the serine residue is a substitution for a threonine residue (T $\rightarrow$ S).

67. The method of any of claims 62-66, wherein the variant $\alpha 7$ nAChR polypeptide comprises a mammalian $\alpha 7$ nAChR polypeptide having a serine in the sixth amino acid position of the TM2 domain.

68. The method of any of claims 62-67, wherein the variant $\alpha 7$ nAChR polypeptide is a mammalian $\alpha 7$ nAChR polypeptide having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

69. The method of any of claims 62-66, wherein the variant $\alpha 7$ nAChR polypeptide is an $\alpha 7$ nAChR polypeptide of human, non-human primate, rodent, chicken, or zebrafish, having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

70. The method of any of claims 62-69, wherein the variant $\alpha 7$ nAChR polypeptide comprises the human $\alpha 7$ nAChR polypeptide having a threonine to serine (T $\rightarrow$ S) substitution in the sixth amino acid position of the TM2 domain.

71. The method of any of claims 62-70, wherein the polynucleotide comprises SEQ ID NO: 18, SEQ ID NO:19, 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: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, and SEQ ID NO:60, or degenerate variants of any of the foregoing.

72. A composition comprising a variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the polypeptide has a serine residue in the sixth amino acid position of the TM2 domain; or a polynucleotide encoding said polypeptide; and a pharmaceutically acceptable carrier.

73. The composition of claim 72, wherein the TM2 domain comprises the amino acid of SEQ ID NO:2.

74. The composition of claim 72 or claim 73, wherein the serine residue is a substitution for a threonine residue (T $\rightarrow$ S).

75. The composition of any of claims 72-74, wherein the variant $\alpha 7$ nAChR polypeptide comprises a mammalian $\alpha 7$ nAChR polypeptide having a serine in the sixth amino acid position of the TM2 domain.

76. The composition of any of claims 72-75, wherein the variant $\alpha 7$ nAChR polypeptide is a mammalian $\alpha 7$ nAChR polypeptide having a threonine to serine (T→S) substitution in the sixth amino acid position of the TM2 domain.

77. The composition of any of claims 72-74, wherein the variant $\alpha 7$ nAChR polypeptide is an $\alpha 7$ nAChR polypeptide of human, non-human primate, rodent, chicken, or zebrafish, having a threonine to serine (T→S) substitution in the sixth amino acid position of the TM2 domain.

78. The composition of any of claims 72-77, wherein the variant $\alpha 7$ nAChR polypeptide comprises the human $\alpha 7$ nAChR polypeptide having a threonine to serine (T→S) substitution in the sixth amino acid position of the TM2 domain.

79. The composition of any of claims 72-78, wherein the polynucleotide comprises SEQ ID NO: 18, SEQ ID NO:19, 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: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, and SEQ ID NO:60, or degenerate variants of any of the foregoing.

80. A method for identifying $\alpha 7$ nAChR modulators, said method comprising: (a) providing a variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the polypeptide has a serine residue in the sixth amino acid position of the TM2 domain; (b) contacting the polypeptide with an A β peptide capable of interacting with the polypeptide; (c) exposing the

polypeptide and the A β peptide to a test agent; and (d) determining the effect of the test agent on the interaction of the polypeptide and the A β peptide.

81. The method of claim 80, wherein the TM2 domain comprises the amino acid sequence of SEQ ID NO:2.

82. The method of claim 80 or claim 81, wherein the A β peptide is comprises β -amyloid peptide₁₋₄₂, β -amyloid₁₋₄₀, or a functional fragment or variant thereof.

83. A method for identifying a cytoprotective agent, comprising:

(a) providing a host cell that expresses a polynucleotide encoding a variant $\alpha 7$ nAChR polypeptide having a TM2 domain, wherein the variant $\alpha 7$ nAChR polypeptide has a serine residue in the sixth amino acid position of the TM2 domain;

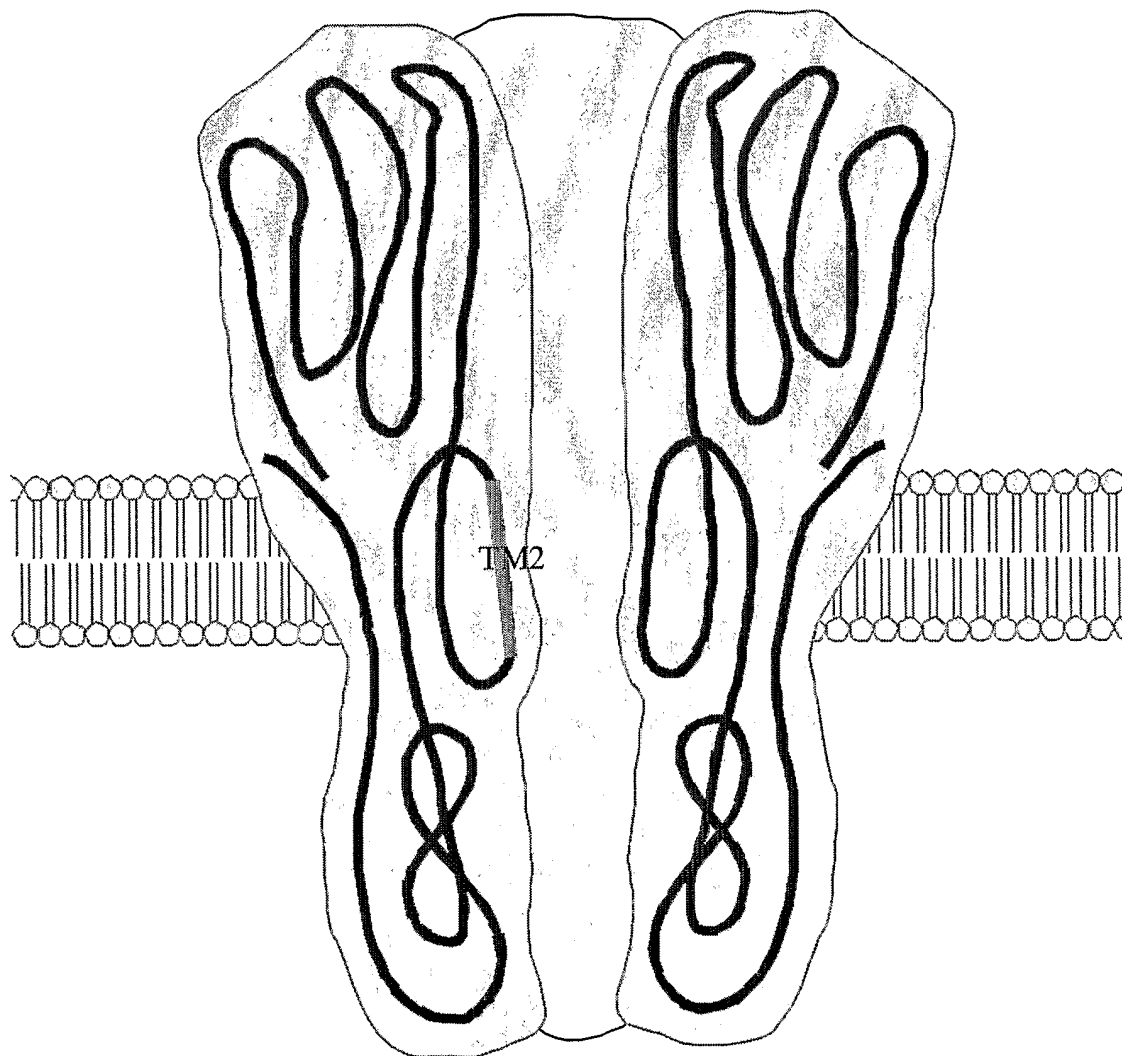
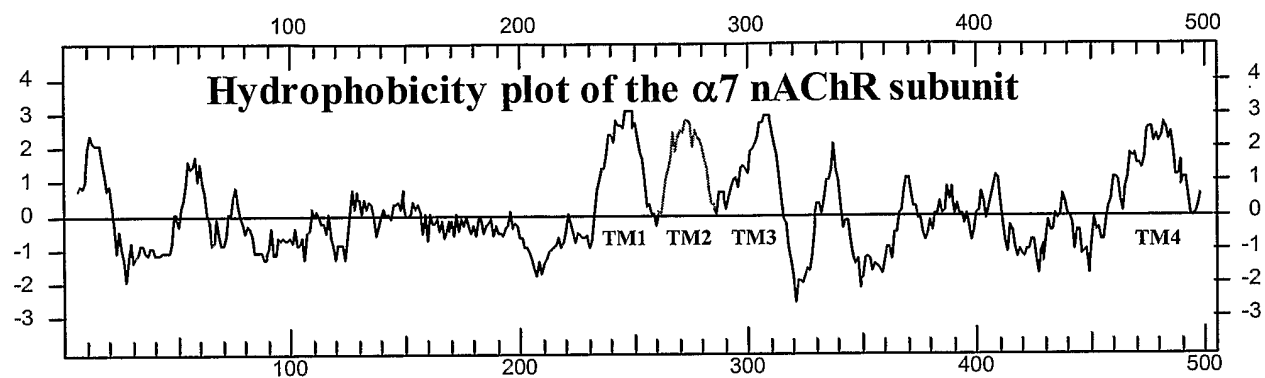
(b) contacting a test agent with the host cell; and

(c) monitoring the cell or cellular function for overstimulation of the variant $\alpha 7$ nAChR polypeptide, wherein overstimulation of the variant $\alpha 7$ nAChR polypeptide indicates that the test agent is sufficient to activate a wild-type $\alpha 7$ receptor at a cytoprotective level.

84. The method of claim 83, wherein the TM2 domain comprises the amino acid sequence of SEQ ID NO:2.

1/8

FIG. 1



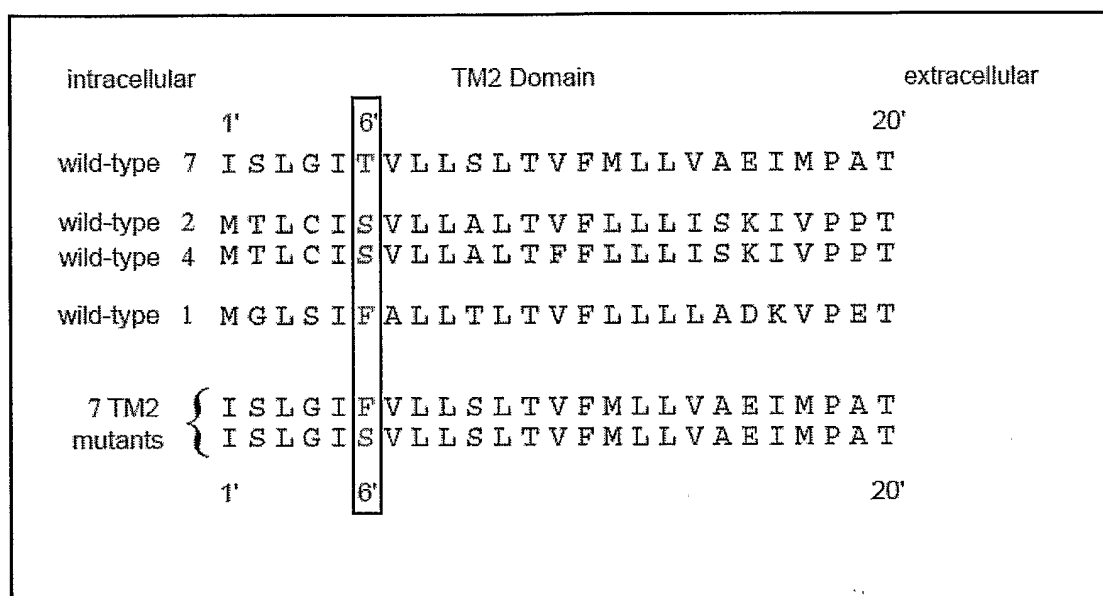


FIG. 2

3/8

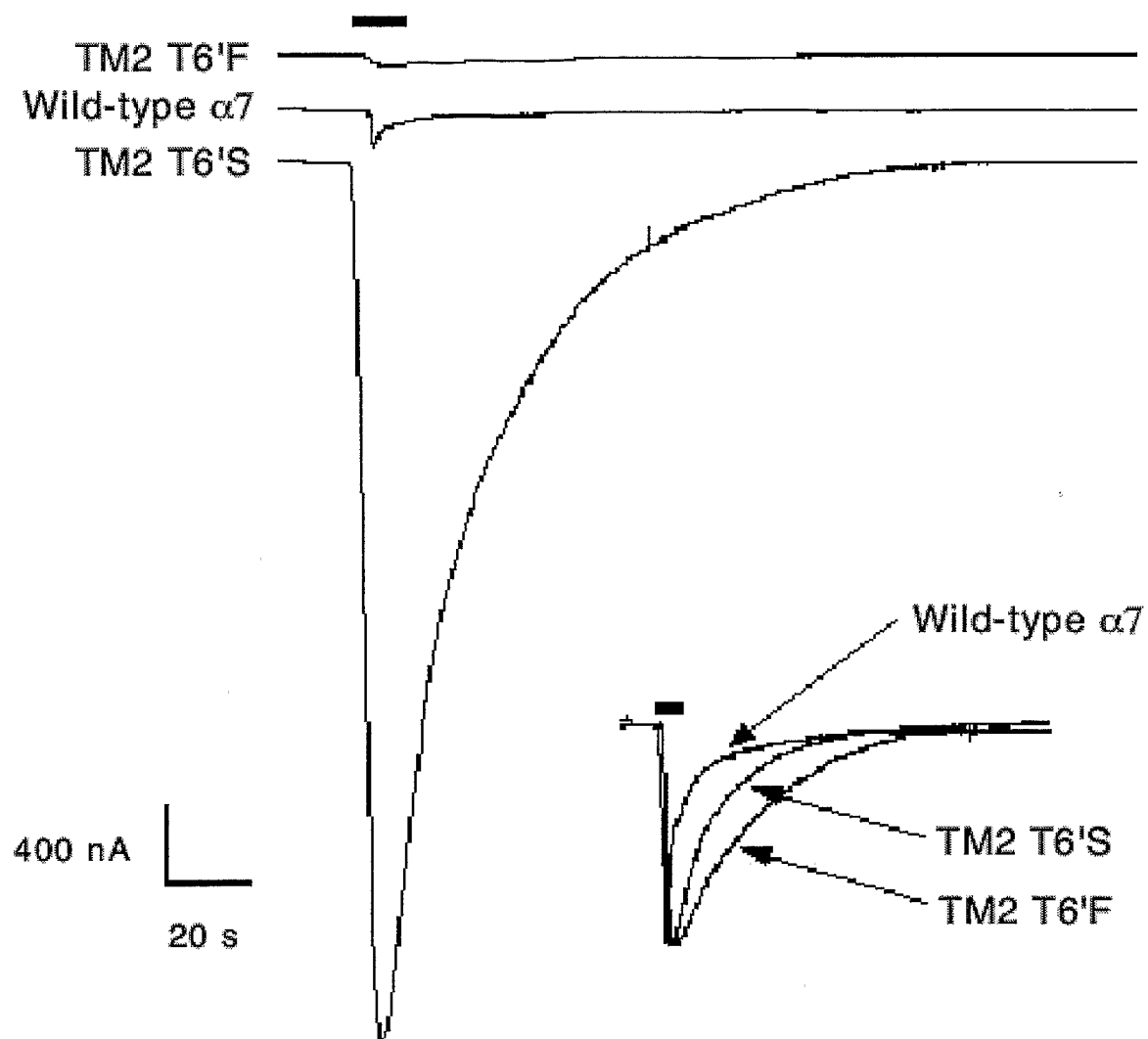
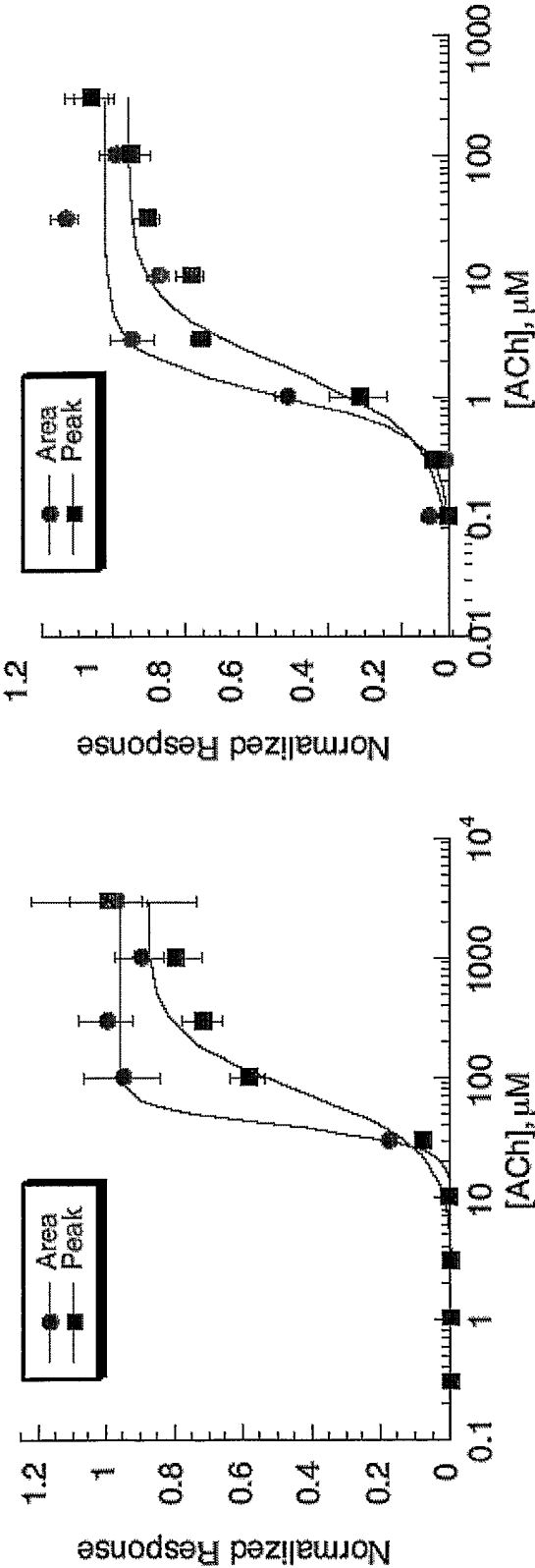
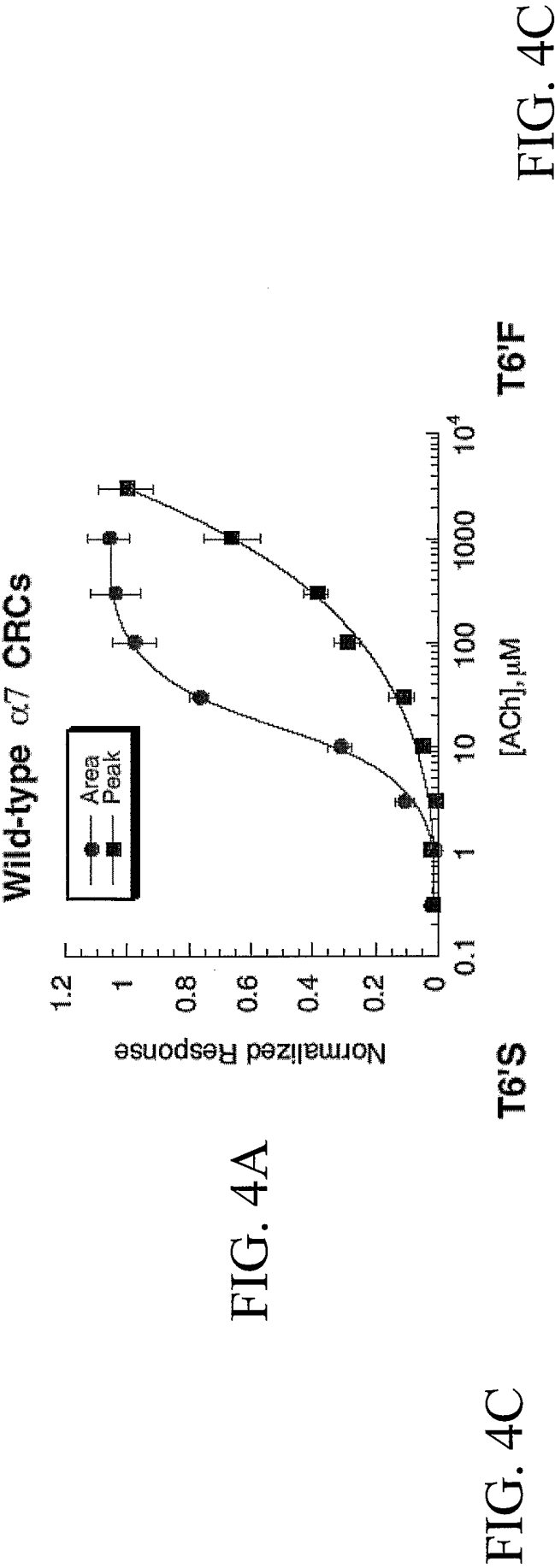


FIG. 3



5/8

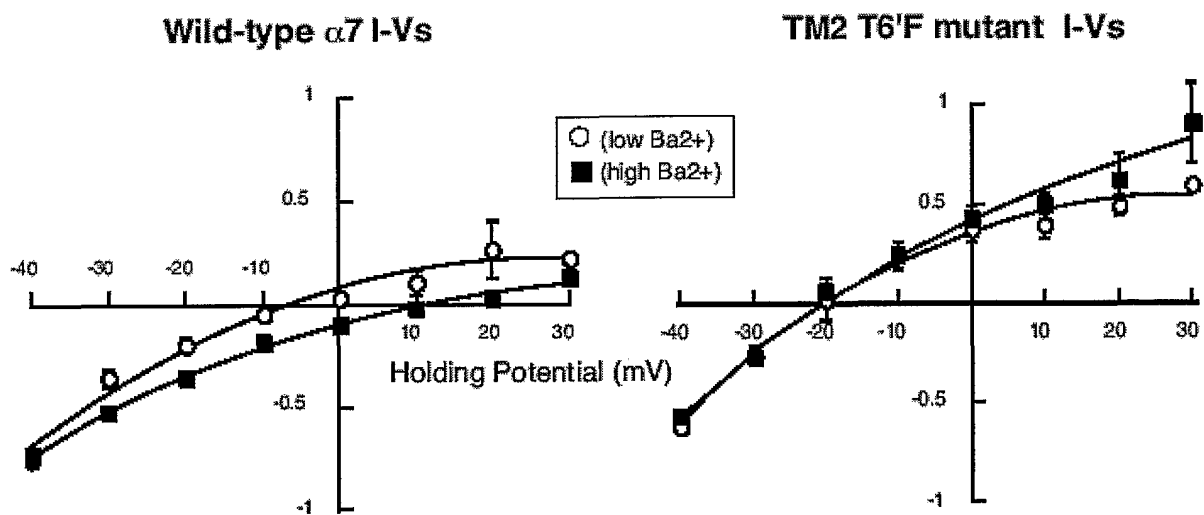


FIG. 5A

FIG. 5B

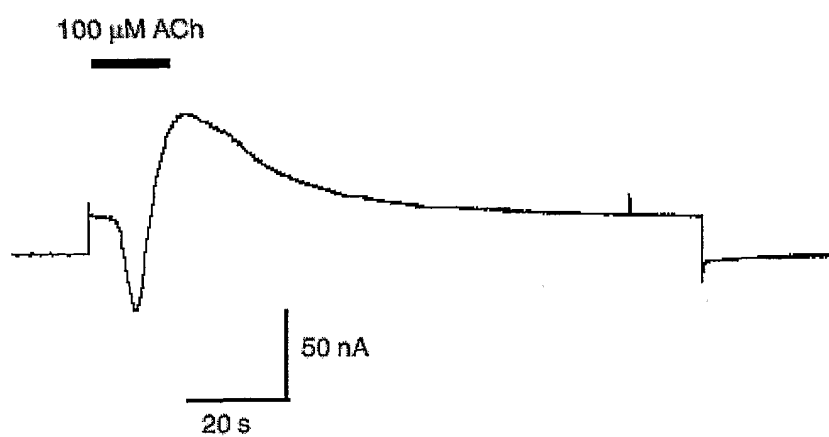
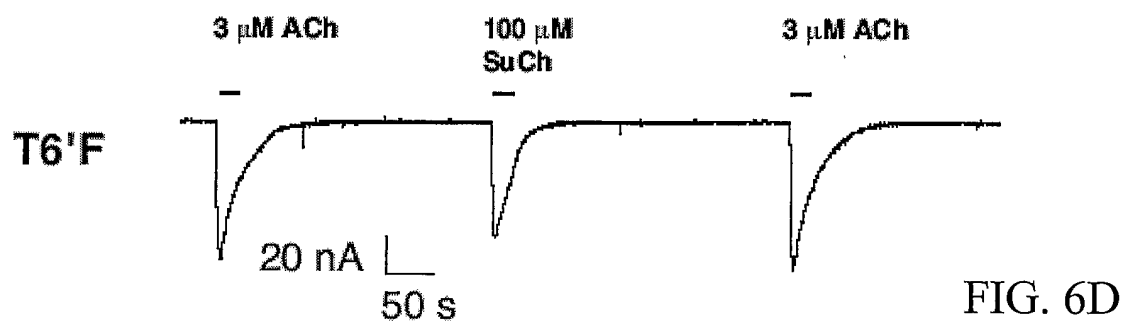
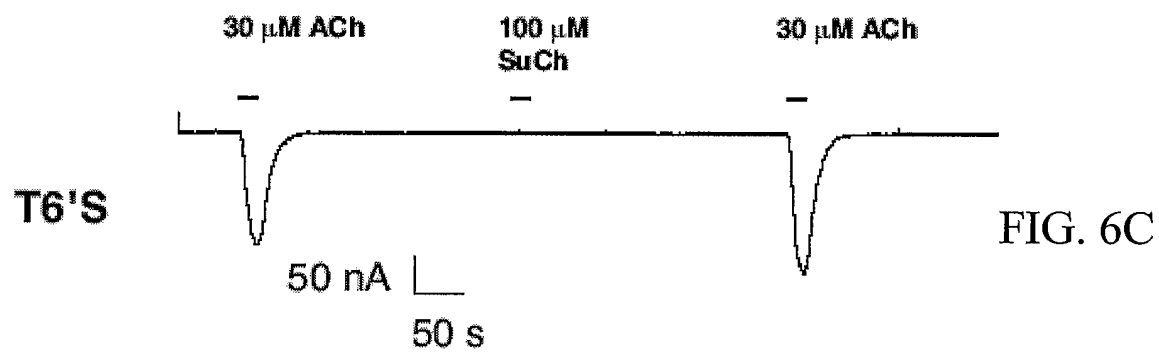
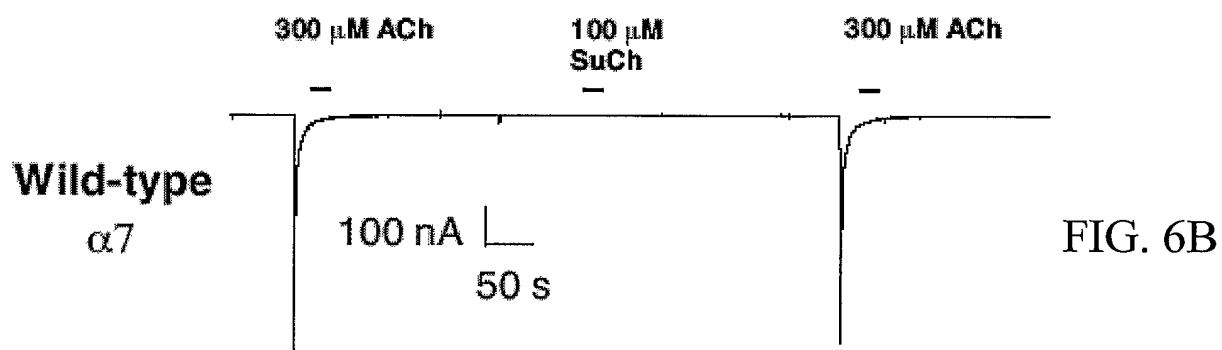
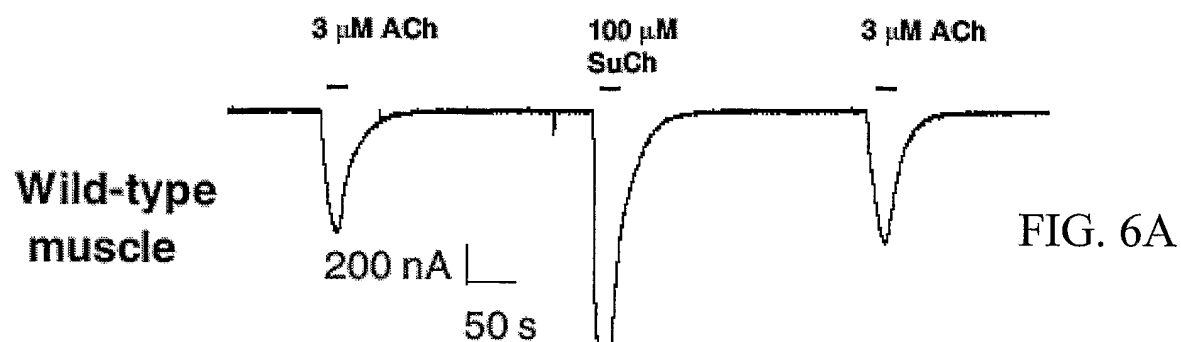


FIG. 5C

6/8



7/8

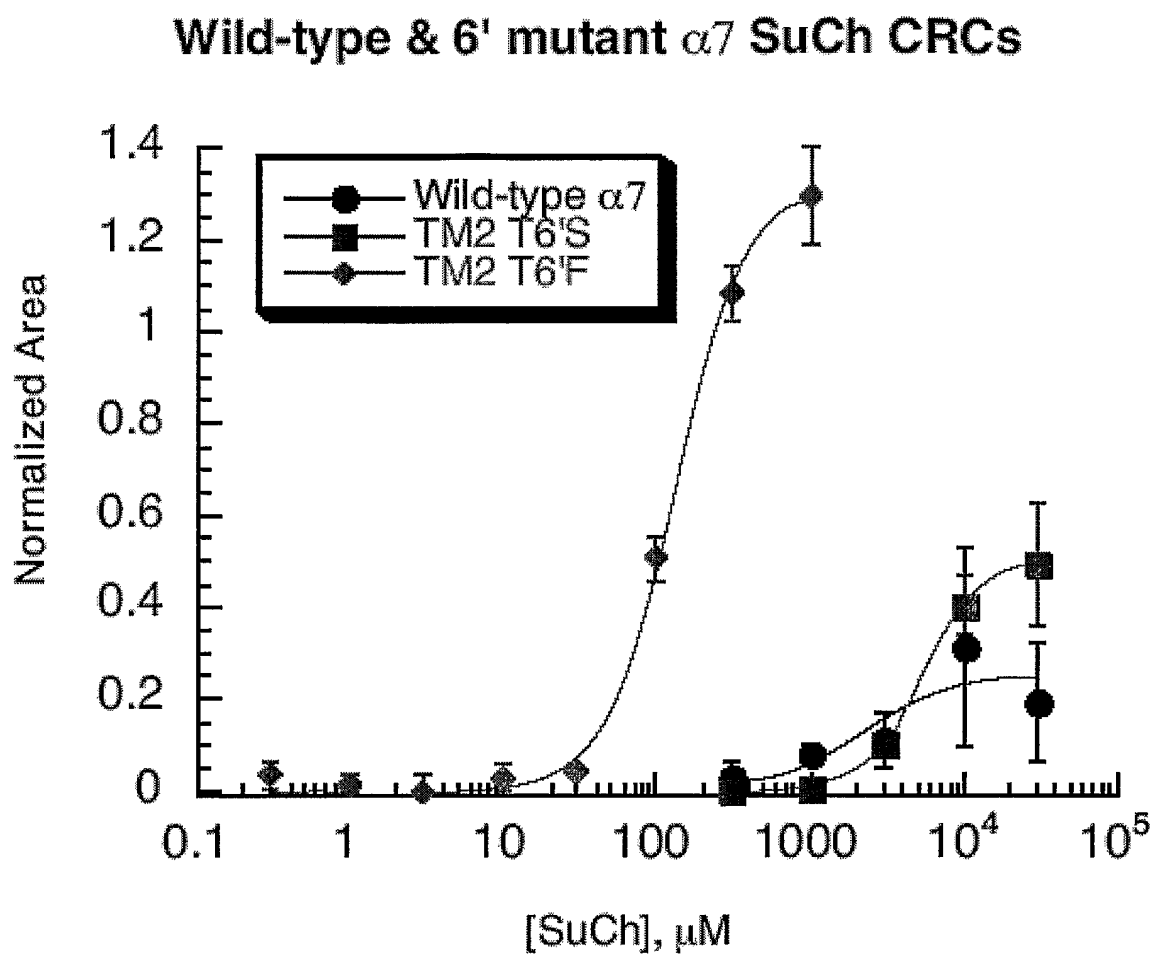
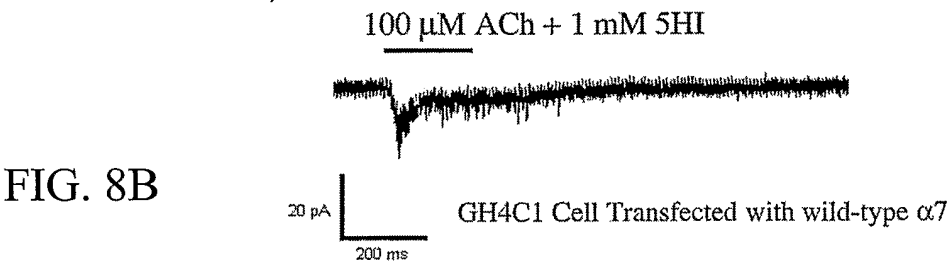
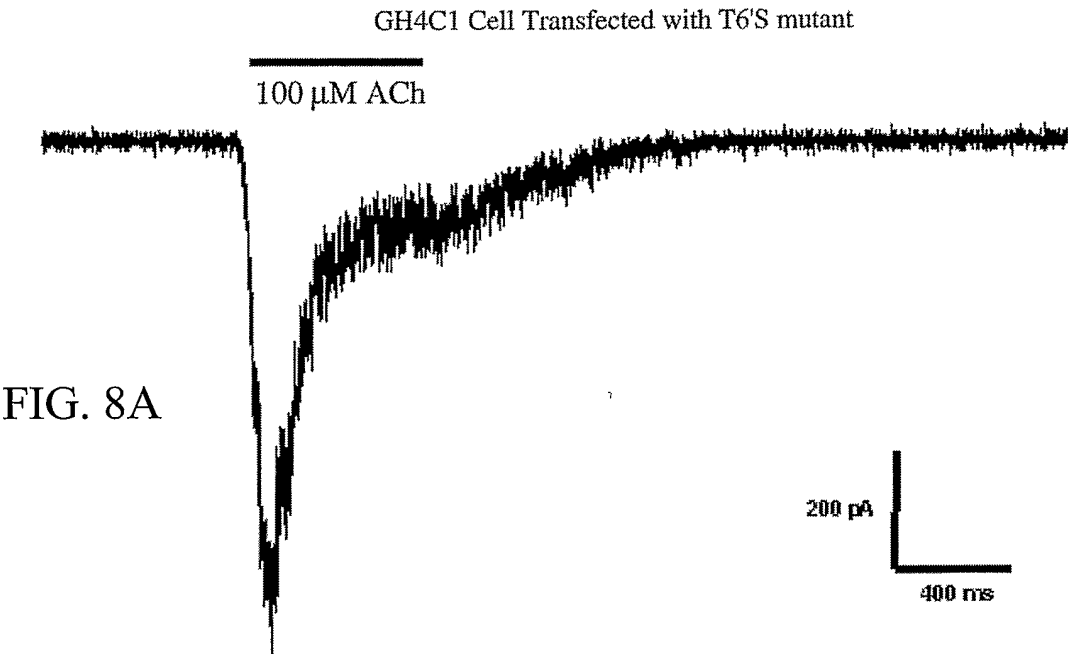


FIG. 7



SEQUENCE LISTING

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Papke, Roger
Placzek, Andon

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<130> UF-408C1

<150> 10/769,085

<151> 2004-01-30

<160> 73

<170> PatentIn version 3.2

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ctg cac gtg tcc ctg caa ggc gag ttc cag agg agg ctg tac aag gag 96
Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
20 25 30

ctg gtc aag aac tac aac ccg ctg gag agg ccg gtg gcc aac gac tcg 144
Leu Val Lys Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser
35 40 45

cag ccg ctc acc gtg tac ttc tcc ctg agt ctc ctg cag atc atg gat 192
Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
50 55 60

gtg gat gag aag aac caa gtt tta acc acc aac att tgg cta caa atg 240
Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
65 70 75 80

tct tgg aca gat cac tat ttg cag tgg aac atg tct gag tac ccc gga 288
Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
85 90 95

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Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile

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aat gtt ttg gtg aat gca tct ggg cat tgc cag tat ctc cct cca ggc Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly 130 135 140			432
ata ttc aag agc tcc tgc tac att gac gtt cgc tgg ttc cct ttt gat Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp 145 150 155 160			480
gtg cag cag tgc aaa ctg aag ttt ggg tcc tgg tcc tat gga ggg tgg Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp 165 170 175			528
tca ctg gac ctg caa atg caa gag gca gat atc agc agc tat atc ccc Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro 180 185 190			576
aac gga gaa tgg gat ctg atg gga atc cct ggc aaa agg aat gag aag Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys 195 200 205			624
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act gtc ttc atg ctg ctt gtg gct gag atc atg cca gca aca tct gat Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp 275 280 285			864
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aac tgg tgt gca tgg ttt ctg cgc atg aag agg ccc gga gag gac aag			1056

Asn	Trp	Cys	Ala	Trp	Phe	Leu	Arg	Met	Lys	Arg	Pro	Gly	Glu	Asp	Lys	
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Val	Arg	Pro	Ala	Cys	Gln	His	Lys	Pro	Arg	Arg	Cys	Ser	Leu	Ala	Ser	
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Val	Glu	Leu	Ser	Ala	Gly	Ala	Gly	Pro	Pro	Thr	Ser	Asn	Gly	Asn	Leu	
	370					375					380					
ctc	tac	att	ggc	ttc	cga	ggc	ctg	gag	ggc	atg	cac	tgt	gcc	cca	act	1200
Leu	Tyr	Ile	Gly	Phe	Arg	Gly	Leu	Glu	Gly	Met	His	Cys	Ala	Pro	Thr	
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cca	gac	tct	ggg	gtc	gta	tgt	ggc	cgt	ttg	gcc	tgc	tcc	cca	aca	cat	1248
Pro	Asp	Ser	Gly	Val	Val	Cys	Gly	Arg	Leu	Ala	Cys	Ser	Pro	Thr	His	
			405					410						415		
gat	gag	cac	ctc	atg	cac	ggg	gca	cac	ccc	tct	gat	ggg	gac	ccc	gac	1296
Asp	Glu	His	Leu	Met	His	Gly	Ala	His	Pro	Ser	Asp	Gly	Asp	Pro	Asp	
			420					425					430			
ctg	gcc	aag	atc	ctg	gag	gag	gtc	cgc	tac	atc	gcc	aac	cgc	ttc	cgc	1344
Leu	Ala	Lys	Ile	Leu	Glu	Glu	Val	Arg	Tyr	Ile	Ala	Asn	Arg	Phe	Arg	
		435					440					445				
tgc	cag	gac	gag	agt	gag	gtg	atc	tgc	agt	gaa	tgg	aag	ttt	gca	gcc	1392
Cys	Gln	Asp	Glu	Ser	Glu	Val	Ile	Cys	Ser	Glu	Trp	Lys	Phe	Ala	Ala	
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tgc	gtg	gtg	gac	cgc	ttg	tgc	ctc	atg	gcc	ttt	tcg	gtc	ttt	acc	atc	1440
Cys	Val	Val	Asp	Arg	Leu	Cys	Leu	Met	Ala	Phe	Ser	Val	Phe	Thr	Ile	
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atc	tgt	acc	atc	ggc	atc	ctc	atg	tca	gct	cca	aac	ttt	gtg	gag	gct	1488
Ile	Cys	Thr	Ile	Gly	Ile	Leu	Met	Ser	Ala	Pro	Asn	Phe	Val	Glu	Ala	
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 Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val
 210 215 220
 Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240
 Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
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 Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

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Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp
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Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
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Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
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Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
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Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
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Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
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Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
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Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
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Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
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Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys
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Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val
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Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
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Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
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Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
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Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu
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Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
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Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp
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Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
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 Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Lys Leu Tyr Lys Glu
 20 25 30

ctg gtc aag aac tac aat ccc ttg gag agg ccc gtg gcc aat gac tcg 144
 Leu Val Lys Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser

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gtg gat gag aag aac caa gtt tta acc acc aac att tgg ctg caa atg Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met 65 70 75 80			240
tct tgg aca gat cac tat tta cag tgg aat gtg tca gaa tat cca ggg Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Val Ser Glu Tyr Pro Gly 85 90 95			288
gtg aag act gtt cgt ttc cca gat ggc cag att tgg aaa cca gac att Val Lys Thr Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile 100 105 110			336
ctt ctc tat aac agt gct gat gag cgc ttt gac gcc aca ttc cac act Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr 115 120 125			384
aac gtg ttg gtg aat tct tct ggg cat tgc cag tac ctg cct cca ggc Asn Val Leu Val Asn Ser Ser Gly His Cys Gln Tyr Leu Pro Pro Gly 130 135 140			432
ata ttc aag agt tcc tgc tac atc gat gta cgc tgg ttt ccc ttt gat Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp 145 150 155 160			480
gtg cag cac tgc aaa ctg aag ttt ggg tcc tgg tct tac gga ggc tgg Val Gln His Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp 165 170 175			528
tcc ttg gat ctg cag atg cag gag gca gat atc agt ggc tat atc ccc Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Gly Tyr Ile Pro 180 185 190			576
aat gga gaa tgg gac cta gtg gga atc ccc ggc aag agg agt gaa agg Asn Gly Glu Trp Asp Leu Val Gly Ile Pro Gly Lys Arg Ser Glu Arg 195 200 205			624
ttc tat gag tgc tgc aaa gag ccc tac ccc gat gtc acc ttc aca gtg Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val 210 215 220			672
acc atg cgc cgc agg acg ctc tac tat ggc ctc aac ctg ctg atc ccc Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro 225 230 235 240			720
tgt gtg ctc atc tcc gcc ctc gcc ctg ctg gtg ttc ctg ctt cct gca Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala 245 250 255			768
gat tcc ggg gag aag att tcc ctg ggg ata aca gtc tta ctc tct ctt Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu 260 265 270			816
acc gtc ttc atg ctg ctc gtg gct gag atc atg ccc gca aca tcc gat			864

<210> 8

<211> 502

<212> PRT

<213> Homo sapiens

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Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Lys Leu Tyr Lys Glu
 20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
 50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
 65 70 75 80

Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Val Ser Glu Tyr Pro Gly
 85 90 95

Val Lys Thr Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
 100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

Asn Val Leu Val Asn Ser Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Val Gln His Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Gly Tyr Ile Pro
 180 185 190

Asn Gly Glu Trp Asp Leu Val Gly Ile Pro Gly Lys Arg Ser Glu Arg
 195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val
 210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
 290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Gln Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Val Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Gln Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Met Ser Ala Val Ala Pro Pro Pro Ala Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Asp Gly Val His Cys Val Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Met Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Leu His Gly Gly Gln Pro Pro Glu Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

14

Cys Gln Asp Glu Ser Glu Ala Val Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
 500

<210> 9
 <211> 502
 <212> PRT
 <213> Homo sapiens

<220>
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<220>
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 <222> (262)..(281)
 <223> TM2 domain

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 20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
 50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
 65 70 75 80

Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Val Ser Glu Tyr Pro Gly
 85 90 95

15

Val Lys Thr Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
 100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

Asn Val Leu Val Asn Ser Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Val Gln His Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Gly Tyr Ile Pro
 180 185 190

Asn Gly Glu Trp Asp Leu Val Gly Ile Pro Gly Lys Arg Ser Glu Arg
 195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val
 210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
 290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Gln Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Val Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Gln Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Met Ser Ala Val Ala Pro Pro Pro Ala Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Asp Gly Val His Cys Val Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Met Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Leu His Gly Gly Gln Pro Pro Glu Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Cys Gln Asp Glu Ser Glu Ala Val Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
 500

<210> 10
 <211> 1509
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(1509)

<220>
 <221> sig_peptide
 <222> (1)..(66)

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<220>
<221> misc_feature
<222> (784)..(843)
<223> TM2 domain

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Met Cys Gly Arg Arg Gly Gly Ile Trp Leu Ala Leu Ala Ala Ala Leu
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ctg cac gtg tcc ctg caa ggc gag ttc cag agg agg ctg tac aag gag      96
Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
          20          25          30

ctg gtc aag aac tac aac ccg ctg gag agg ccg gtg gcc aac gac tcg     144
Leu Val Lys Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser
          35          40          45

cag ccg ctc acc gtg tac ttc tcc ctg agc ctc ctg cag atc atg gat     192
Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
          50          55          60

gtg gat gag aag aac caa gtt tta acc acc aac att tgg ctt caa atg     240
Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
65          70          75          80

tct tgg aca gat cac tat ttg cag tgg aac atg tct gag tac ccc gga     288
Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
          85          90          95

gtg aaa aat gtt cgt ttt cca gat ggc cag att tgg aaa cca gac att     336
Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
          100          105          110

ctc ctc tat aac agt gca gat gaa cgc ttt gat gcc aca ttc cac acc     384
Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
          115          120          125

aac gtc ttg gtg aat gca tct ggg cat tgc cag tat ctc cct cca ggc     432
Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
          130          135          140

ata ttc aag agc tcc tgc tac atc gat gta cgc tgg ttc cct ttt gat     480
Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
145          150          155          160

gtg cag cag tgc aaa ctg aag ttt ggg tcc tgg tcc tat gga ggg tgg     528
Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
          165          170          175

tcc ttg gac ctg cag atg caa gag gca gat atc agc agc tat atc ccc     576
Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
          180          185          190

aat gga gaa tgg gat ctc atg gga atc cct ggc aaa agg aat gag aag     624
Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys
          195          200          205

ttc tat gaa tgc tgc aaa gag cca tac cca gat gtc acc tac aca gta     672

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Phe	Tyr	Glu	Cys	Cys	Lys	Glu	Pro	Tyr	Pro	Asp	Val	Thr	Tyr	Thr	Val	
210						215					220					
acc	atg	cgc	cgt	agg	aca	ctc	tac	tat	ggc	ctc	aac	ctg	ctc	att	cct	720
Thr	Met	Arg	Arg	Arg	Thr	Leu	Tyr	Tyr	Gly	Leu	Asn	Leu	Leu	Ile	Pro	
225					230					235					240	
tgt	gtg	ctc	att	tca	gcc	ctg	gct	ttg	ctg	gta	ttc	ttg	ctg	cct	gca	768
Cys	Val	Leu	Ile	Ser	Ala	Leu	Ala	Leu	Leu	Val	Phe	Leu	Leu	Pro	Ala	
				245					250					255		
gac	tct	gga	gag	aaa	atc	tct	ctt	gga	ata	act	gtc	tta	ctt	tct	ctg	816
Asp	Ser	Gly	Glu	Lys	Ile	Ser	Leu	Gly	Ile	Thr	Val	Leu	Leu	Ser	Leu	
			260					265					270			
act	gtc	ttc	atg	ctg	ctt	gtg	gct	gag	atc	atg	cca	gca	aca	tct	gat	864
Thr	Val	Phe	Met	Leu	Leu	Val	Ala	Glu	Ile	Met	Pro	Ala	Thr	Ser	Asp	
	275						280					285				
tcc	gtg	ccc	ttg	ata	gca	cag	tac	ttc	gcc	agc	acc	atg	atc	atc	gtg	912
Ser	Val	Pro	Leu	Ile	Ala	Gln	Tyr	Phe	Ala	Ser	Thr	Met	Ile	Ile	Val	
	290					295					300					
ggc	ctc	tca	gtg	gtc	gtg	aca	gtg	att	gtg	ctg	cga	tat	cac	cac	cat	960
Gly	Leu	Ser	Val	Val	Val	Thr	Val	Ile	Val	Leu	Arg	Tyr	His	His	His	
305					310					315					320	
gac	cct	gat	ggc	ggc	aaa	atg	cct	aag	tgg	acc	agg	atc	att	ctt	ctg	1008
Asp	Pro	Asp	Gly	Gly	Lys	Met	Pro	Lys	Trp	Thr	Arg	Ile	Ile	Leu	Leu	
			325					330					335			
aat	tgg	tgt	gca	tgg	ttt	ctg	cgc	atg	aag	agg	ccg	gga	gag	gac	aag	1056
Asn	Trp	Cys	Ala	Trp	Phe	Leu	Arg	Met	Lys	Arg	Pro	Gly	Glu	Asp	Lys	
			340					345					350			
gtg	cgg	ccg	gct	tgt	cag	cac	aag	cct	cgg	cgc	tgc	agc	ctg	gcc	agt	1104
Val	Arg	Pro	Ala	Cys	Gln	His	Lys	Pro	Arg	Arg	Cys	Ser	Leu	Ala	Ser	
	355						360					365				
gtg	gag	ctg	agc	gca	ggc	gcg	ggg	cca	ccc	tcc	agc	aat	ggc	aac	ctg	1152
Val	Glu	Leu	Ser	Ala	Gly	Ala	Gly	Pro	Pro	Ser	Ser	Asn	Gly	Asn	Leu	
	370					375					380					
ctc	tac	att	ggc	ttc	cga	ggc	ctg	gag	ggc	atg	cac	tgt	gcc	cca	act	1200
Leu	Tyr	Ile	Gly	Phe	Arg	Gly	Leu	Glu	Gly	Met	His	Cys	Ala	Pro	Thr	
385					390					395					400	
cca	gac	tct	ggg	gtc	gtg	tgt	ggc	cgt	ttg	gcc	tgc	tcc	cca	aca	cat	1248
Pro	Asp	Ser	Gly	Val	Val	Cys	Gly	Arg	Leu	Ala	Cys	Ser	Pro	Thr	His	
			405					410					415			
gat	gag	cac	ctc	atg	cat	ggc	aca	cac	ccc	tct	gat	ggg	gac	ccc	gac	1296
Asp	Glu	His	Leu	Met	His	Gly	Thr	His	Pro	Ser	Asp	Gly	Asp	Pro	Asp	
			420				425					430				
ctg	gcc	aag	atc	ctg	gag	gag	gtc	cgc	tac	atc	gcc	aac	cgc	ttc	cgc	1344
Leu	Ala	Lys	Ile	Leu	Glu	Glu	Val	Arg	Tyr	Ile	Ala	Asn	Arg	Phe	Arg	
		435					440					445				

19

tgc cag gat gag agt gag gtg atc tgc agt gag tgg aag ttt gcg gcc 1392
 Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460
 tgt gtg gtg gac cgc ttg tgc ctc atg gcc ttt tcg gtt ttt acc atc 1440
 Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480
 atc tgc acc atc ggc atc ctc atg tca gcc cca aac ttt gtg gag gct 1488
 Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495
 gtg tcc aaa gac ttt gct taa 1509
 Val Ser Lys Asp Phe Ala
 500

<210> 11
 <211> 502
 <212> PRT
 <213> Mus musculus

<400> 11

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Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
65 70 75 80

Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
85 90 95

Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
115 120 125

Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
130 135 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
 180 185 190

Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys
 195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val
 210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
 290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Ser Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Met His Gly Thr His Pro Ser Asp Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
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<210> 12
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 <212> PRT
 <213> Mus musculus

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<220>
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 <222> (262)..(281)
 <223> TM2 domain

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Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
 20 25 30

22

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

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
 50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
 65 70 75 80

Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
 85 90 95

Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
 100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
 180 185 190

Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys
 195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val
 210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
 290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Ser Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Met His Gly Thr His Pro Ser Asp Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
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Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
 500

<210> 13
 <211> 1509
 <212> DNA
 <213> Macaca mulatta

<220>
 <221> CDS
 <222> (1)..(1509)

<220>
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 <222> (1)..(66)

<220>
 <221> misc_feature
 <222> (784)..(843)
 <223> TM2 domain

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 ctg cat gtg tcc ctg caa ggc gag ttc cag agg aag ctt tac aag gag 96
 Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Lys Leu Tyr Lys Glu
 20 25 30
 ctg gtc aag aac tac aac ccc ttg gag agg ccc gtg gcc aat gac tcg 144
 Leu Val Lys Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser
 35 40 45
 caa ccg ctc acc gtc tac ttc tcc ctg agc ctc ctg cag atc atg gac 192
 Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
 50 55 60
 gtg gat gag aag aac caa gtt tta acc acc aac att tgg ctg caa atg 240
 Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
 65 70 75 80
 tct tgg aca gat cac tat tta cag tgg aat gtg tca gaa tat cca ggg 288
 Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Val Ser Glu Tyr Pro Gly
 85 90 95
 gtg aag act gtt cgt ttc cca gat ggc cag att tgg aaa cca gac att 336
 Val Lys Thr Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
 100 105 110
 ctt ctc tat aac agt gcg gat gag cgc ttt gac gcc aca ttc cac acc 384
 Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125
 aac gtg ttg gtg aat tct tct ggg cat tgc cag tac ctg cct cca ggc 432
 Asn Val Leu Val Asn Ser Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140
 ata ttc aag agt tcc tgc tac atc gat gtg cgc tgg ttt ccc ttt gat 480

25

Ile	Phe	Lys	Ser	Ser	Cys	Tyr	Ile	Asp	Val	Arg	Trp	Phe	Pro	Phe	Asp	
145					150					155					160	
gtg	cag	cat	tgc	aaa	ctg	aag	ttt	gga	tcc	tgg	tct	tat	gga	ggc	tgg	528
Val	Gln	His	Cys	Lys	Leu	Lys	Phe	Gly	Ser	Trp	Ser	Tyr	Gly	Gly	Trp	
				165					170					175		
tcc	ttg	gat	ctg	cag	atg	cag	gag	gca	gat	atc	agt	ggc	tat	atc	ccc	576
Ser	Leu	Asp	Leu	Gln	Met	Gln	Glu	Ala	Asp	Ile	Ser	Gly	Tyr	Ile	Pro	
			180					185					190			
agt	gga	gaa	tgg	gac	cta	gtg	gga	att	ccc	ggc	aag	agg	agt	gaa	aag	624
Ser	Gly	Glu	Trp	Asp	Leu	Val	Gly	Ile	Pro	Gly	Lys	Arg	Ser	Glu	Lys	
		195					200				205					
ttc	tat	gag	tgc	tgc	aaa	gag	ccc	tac	ccc	gat	gtc	acc	ttc	aca	gtg	672
Phe	Tyr	Glu	Cys	Cys	Lys	Glu	Pro	Tyr	Pro	Asp	Val	Thr	Phe	Thr	Val	
	210					215				220						
acc	atg	cgc	cgc	agg	acc	ctc	tac	tac	ggc	ctc	aac	ctg	ctg	atc	ccc	720
Thr	Met	Arg	Arg	Arg	Thr	Leu	Tyr	Tyr	Gly	Leu	Asn	Leu	Leu	Ile	Pro	
225					230					235					240	
tgt	gtg	ctc	atc	tct	gcc	ctt	gcc	ctg	ctg	gtg	ttc	ctg	ctt	cct	gca	768
Cys	Val	Leu	Ile	Ser	Ala	Leu	Ala	Leu	Leu	Val	Phe	Leu	Leu	Pro	Ala	
				245					250					255		
gat	tcc	ggg	gag	aag	att	tcc	ctg	ggg	ata	aca	gtc	tta	ctc	tct	ctc	816
Asp	Ser	Gly	Glu	Lys	Ile	Ser	Leu	Gly	Ile	Thr	Val	Leu	Leu	Ser	Leu	
		260						265				270				
act	gtc	ttc	atg	ctg	ctc	gtg	gct	gag	atc	atg	ccc	gca	aca	tct	gat	864
Thr	Val	Phe	Met	Leu	Leu	Val	Ala	Glu	Ile	Met	Pro	Ala	Thr	Ser	Asp	
		275					280					285				
tca	gta	cca	ttg	ata	gcc	cag	tac	ttc	gcc	agc	acc	atg	atc	atc	gtg	912
Ser	Val	Pro	Leu	Ile	Ala	Gln	Tyr	Phe	Ala	Ser	Thr	Met	Ile	Ile	Val	
	290					295					300					
ggc	ctc	tcc	gtg	gtg	gtg	acg	gtg	atc	gtg	ctg	cag	tac	cac	cac	cac	960
Gly	Leu	Ser	Val	Val	Val	Thr	Val	Ile	Val	Leu	Gln	Tyr	His	His	His	
305					310				315					320		
gac	ccc	gac	ggg	ggc	aag	atg	ccc	aag	tgg	acc	aga	gtc	atc	ctt	ctg	1008
Asp	Pro	Asp	Gly	Gly	Lys	Met	Pro	Lys	Trp	Thr	Arg	Val	Ile	Leu	Leu	
				325					330					335		
aac	tgg	tgc	gcg	tgg	ttc	ctg	cgc	atg	aag	agg	ccg	gga	gag	gat	aag	1056
Asn	Trp	Cys	Ala	Trp	Phe	Leu	Arg	Met	Lys	Arg	Pro	Gly	Glu	Asp	Lys	
			340					345				350				
gtg	cgc	ccg	gcc	tgc	cag	cac	aag	cag	cgc	cgc	tgc	agc	ctg	gcc	agc	1104
Val	Arg	Pro	Ala	Cys	Gln	His	Lys	Gln	Arg	Arg	Cys	Ser	Leu	Ala	Ser	
		355					360					365				
gtg	gag	atg	agc	gcc	gtg	gcg	ccg	ccg	ccc	gcc	agc	aac	ggg	aac	ctg	1152
Val	Glu	Met	Ser	Ala	Val	Ala	Pro	Pro	Pro	Ala	Ser	Asn	Gly	Asn	Leu	
	370					375				380						

26

ctg tac atc ggc ttc cgc ggc ctg gac ggc atg cat tgc gcc ccg acc 1200
 Leu Tyr Ile Gly Phe Arg Gly Leu Asp Gly Met His Cys Ala Pro Thr
 385 390 395 400

 ccc gac tcc ggg gtg gtg tgc ggc cgc atg gcc tgc tcc ccc acg cac 1248
 Pro Asp Ser Gly Val Val Cys Gly Arg Met Ala Cys Ser Pro Thr His
 405 410 415

 gac gag cac ctg ctg cac ggt ggg cag ccc ccc gag ggg gac ccg gac 1296
 Asp Glu His Leu Leu His Gly Gly Gln Pro Pro Glu Gly Asp Pro Asp
 420 425 430

 ctg gcc aag atc ctg gag gag gtc cgc tac atc gcc aac cgc ttt cgc 1344
 Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

 tgc cag gac gaa agc gag gcg gtc tgc agt gag tgg aag ttc gcc gcc 1392
 Cys Gln Asp Glu Ser Glu Ala Val Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

 tgc gtg gtg gac cgc ctg tgc ctg atg gcc ttc tcg gtc ttc acc atc 1440
 Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

 atc tgc acc atc ggc atc ctg atg tcg gct ccc aac ttc gtg gag gcc 1488
 Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

 gtg tcc aaa gac ttt gcg taa 1509
 Val Ser Lys Asp Phe Ala
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 <211> 502
 <212> PRT
 <213> Macaca mulatta

<400> 14

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Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Lys Leu Tyr Lys Glu
20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
65 70 75 80


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<221> TRANSMEM

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Leu	His	Val	Ser	Leu	Gln	Gly	Glu	Phe	Gln	Arg	Lys	Leu	Tyr	Lys	Glu
			20					25					30		

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

Gln	Pro	Leu	Thr	Val	Tyr	Phe	Ser	Leu	Ser	Leu	Leu	Gln	Ile	Met	Asp
	50					55					60				

Val	Asp	Glu	Lys	Asn	Gln	Val	Leu	Thr	Thr	Asn	Ile	Trp	Leu	Gln	Met
65					70					75					80

Ser	Trp	Thr	Asp	His	Tyr	Leu	Gln	Trp	Asn	Val	Ser	Glu	Tyr	Pro	Gly
				85					90					95	

Val	Lys	Thr	Val	Arg	Phe	Pro	Asp	Gly	Gln	Ile	Trp	Lys	Pro	Asp	Ile
			100					105					110		

Leu	Leu	Tyr	Asn	Ser	Ala	Asp	Glu	Arg	Phe	Asp	Ala	Thr	Phe	His	Thr
		115					120					125			

Asn	Val	Leu	Val	Asn	Ser	Ser	Gly	His	Cys	Gln	Tyr	Leu	Pro	Pro	Gly
	130					135					140				

Ile	Phe	Lys	Ser	Ser	Cys	Tyr	Ile	Asp	Val	Arg	Trp	Phe	Pro	Phe	Asp
145					150					155					160

Val	Gln	His	Cys	Lys	Leu	Lys	Phe	Gly	Ser	Trp	Ser	Tyr	Gly	Gly	Trp
				165					170					175	

Ser	Leu	Asp	Leu	Gln	Met	Gln	Glu	Ala	Asp	Ile	Ser	Gly	Tyr	Ile	Pro
			180					185					190		

Ser	Gly	Glu	Trp	Asp	Leu	Val	Gly	Ile	Pro	Gly	Lys	Arg	Ser	Glu	Lys
		195					200					205			

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val
 210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
 290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Gln Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Val Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Gln Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Met Ser Ala Val Ala Pro Pro Pro Ala Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Asp Gly Met His Cys Ala Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Met Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Leu His Gly Gly Gln Pro Pro Glu Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg

435 440 445
 Cys Gln Asp Glu Ser Glu Ala Val Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460
 Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480
 Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495
 Val Ser Lys Asp Phe Ala
 500

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 <213> Bos taurus

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<220>
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 <222> (775)..(834)
 <223> TM2 domain

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 tcc ctg caa ggc gag ttc cag agg aag ctc tac aaa gac ctg gtg aag 96
 Ser Leu Gln Gly Glu Phe Gln Arg Lys Leu Tyr Lys Asp Leu Val Lys
 20 25 30
 aac tac aac ccc ttg gag agg ccg gtg gcc aac gac tcg ctg ccg ctc 144
 Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser Leu Pro Leu
 35 40 45
 aca gtc tac ttc tcc ctg agc ctc ctg cag atc atg gac gtg gat gag 192
 Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp Val Asp Glu
 50 55 60
 aag aac cag gtg ttg acc acc aac att tgg cta caa atg act tgg aca 240
 Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met Thr Trp Thr
 65 70 75 80

gat cac tat tta cag tgg aat gca tcc gag tat cca ggc gtg aag acg	288
Asp His Tyr Leu Gln Trp Asn Ala Ser Glu Tyr Pro Gly Val Lys Thr	
85 90 95	
ggt cgt ttc cca gac ggc cag atc tgg aag cca gac att ctc ctc tac	336
Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile Leu Leu Tyr	
100 105 110	
aac agt gct gat gaa cgg ttt gat gct acg ttc cac acc aat gtg tta	384
Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr Asn Val Leu	
115 120 125	
gtg aat tct tct ggg cac tgc cag tac ctc cct cca ggc atc ttt aag	432
Val Asn Ser Ser Gly His Cys Gln Tyr Leu Pro Pro Gly Ile Phe Lys	
130 135 140	
agc tcc tgc tac atc gat gtg cgc tgg ttc cca ttt gat gtg cag cag	480
Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp Val Gln Gln	
145 150 155 160	
tgc aaa ctg aag ttt ggg tcc tgg tct tat gga gga tgg tcc ttg gat	528
Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp Ser Leu Asp	
165 170 175	
ctg caa atg cag gag gca gac atc agc ggc tat atc cca aat gga gaa	576
Leu Gln Met Gln Glu Ala Asp Ile Ser Gly Tyr Ile Pro Asn Gly Glu	
180 185 190	
tgg gac ctt gtg ggg gtc ttg ggc aag cgg agt gag aag ttc tac gag	624
Trp Asp Leu Val Gly Val Leu Gly Lys Arg Ser Glu Lys Phe Tyr Glu	
195 200 205	
tgc tgc aaa gaa ccc tac cca gac gtg acc ttc acg gtc agc atc cgc	672
Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val Ser Ile Arg	
210 215 220	
cgc agg act ctc tac tac ggc ctc aac ctg ctc atc ccc tgt gtg ctc	720
Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro Cys Val Leu	
225 230 235 240	
atc tct gcc cta gcc ctc ctc gtc ttc ttg ctc cct gca gac tct gga	768
Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala Asp Ser Gly	
245 250 255	
gag aaa atc tcc ctg gga atc aca gtt tta ctc tcc ctc acc gtc ttc	816
Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu Thr Val Phe	
260 265 270	
atg ctg ctg gta gct gag atc atg cct gcg acc tct gac tca gtg ccc	864
Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp Ser Val Pro	
275 280 285	
tta ata gcc cag tac ttc gcc agc acc atg atc atc gtg ggc ctc tct	912
Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val Gly Leu Ser	
290 295 300	
gtg gtt gtc acg gtg atc gtg cta cag tac cac cac cac gac cct gat	960

33

Val	Val	Val	Thr	Val	Ile	Val	Leu	Gln	Tyr	His	His	His	Asp	Pro	Asp	
305					310					315					320	
ggg	ggc	aag	atg	ccc	aag	tgg	acc	aga	gtc	gtc	ctc	ctg	aac	tgg	tgt	1008
Gly	Gly	Lys	Met	Pro	Lys	Trp	Thr	Arg	Val	Val	Leu	Leu	Asn	Trp	Cys	
				325					330					335		
gcc	tgg	ttc	ctt	cgc	atg	aag	agg	ccg	ggg	gag	gac	aag	gtg	cgg	cca	1056
Ala	Trp	Phe	Leu	Arg	Met	Lys	Arg	Pro	Gly	Glu	Asp	Lys	Val	Arg	Pro	
			340					345					350			
gcc	tgc	cag	cac	aac	gag	cgg	cgc	tgc	agc	ctg	gcc	agc	gtg	gag	atg	1104
Ala	Cys	Gln	His	Asn	Glu	Arg	Arg	Cys	Ser	Leu	Ala	Ser	Val	Glu	Met	
		355					360					365				
agc	gca	gtg	gcg	ggg	ccg	ccg	gcc	acc	aat	ggc	aac	ctg	ctg	tac	atc	1152
Ser	Ala	Val	Ala	Gly	Pro	Pro	Ala	Thr	Asn	Gly	Asn	Leu	Leu	Tyr	Ile	
	370					375					380					
ggc	ttt	cgc	ggc	ctg	gac	acc	atg	cac	tgc	gca	ccc	acc	cct	gac	tca	1200
Gly	Phe	Arg	Gly	Leu	Asp	Thr	Met	His	Cys	Ala	Pro	Thr	Pro	Asp	Ser	
385					390					395					400	
ggg	gtc	gtg	tgc	ggc	cgc	gtg	gcc	tgc	tcc	cca	acc	cat	gac	gag	cac	1248
Gly	Val	Val	Cys	Gly	Arg	Val	Ala	Cys	Ser	Pro	Thr	His	Asp	Glu	His	
			405					410						415		
ctg	ctg	cac	gcc	ggc	cag	ccc	tct	gag	ggg	gac	ccg	gac	cta	gcc	aag	1296
Leu	Leu	His	Ala	Gly	Gln	Pro	Ser	Glu	Gly	Asp	Pro	Asp	Leu	Ala	Lys	
			420					425					430			
atc	ctg	gag	gag	gtg	cgc	tac	att	gcc	cac	cgc	ttc	cgc	tgc	cag	gat	1344
Ile	Leu	Glu	Glu	Val	Arg	Tyr	Ile	Ala	His	Arg	Phe	Arg	Cys	Gln	Asp	
		435					440					445				
gag	agt	gag	gcc	gtg	tgc	agt	gag	tgg	aag	ttc	gcg	gcc	tgt	gtg	gtg	1392
Glu	Ser	Glu	Ala	Val	Cys	Ser	Glu	Trp	Lys	Phe	Ala	Ala	Cys	Val	Val	
	450					455					460					
gac	cgc	ctg	tgt	ctc	atg	gcc	ttc	tcc	gtc	ttc	acc	atc	ctc	tgc	acc	1440
Asp	Arg	Leu	Cys	Leu	Met	Ala	Phe	Ser	Val	Phe	Thr	Ile	Leu	Cys	Thr	
465					470				475					480		
att	ggc	atc	ctg	atg	tcg	gcg	ccc	aac	ttc	gtt	gaa	gct	gtg	tcc	aaa	1488
Ile	Gly	Ile	Leu	Met	Ser	Ala	Pro	Asn	Phe	Val	Glu	Ala	Val	Ser	Lys	
			485					490						495		
gac	ttc	gct	tga													1500
Asp	Phe	Ala														

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 <211> 499
 <212> PRT
 <213> Bos taurus

<400> 17

Met Arg Gly Ser Leu Cys Leu Ala Leu Ala Ala Ser Ile Leu His Val
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Ser Leu Gln Gly Glu Phe Gln Arg Lys Leu Tyr Lys Asp Leu Val Lys
 20 25 30

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

Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp Val Asp Glu
 50 55 60

Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met Thr Trp Thr
 65 70 75 80

Asp His Tyr Leu Gln Trp Asn Ala Ser Glu Tyr Pro Gly Val Lys Thr
 85 90 95

Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile Leu Leu Tyr
 100 105 110

Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr Asn Val Leu
 115 120 125

Val Asn Ser Ser Gly His Cys Gln Tyr Leu Pro Pro Gly Ile Phe Lys
 130 135 140

Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp Val Gln Gln
 145 150 155 160

Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp Ser Leu Asp
 165 170 175

Leu Gln Met Gln Glu Ala Asp Ile Ser Gly Tyr Ile Pro Asn Gly Glu
 180 185 190

Trp Asp Leu Val Gly Val Leu Gly Lys Arg Ser Glu Lys Phe Tyr Glu
 195 200 205

Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val Ser Ile Arg
 210 215 220

Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro Cys Val Leu

35

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

36

Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile Leu Cys Thr
 465 470 475 480

Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala Val Ser Lys
 485 490 495

Asp Phe Ala

<210> 18
 <211> 499
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<220>
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<400> 18

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Ser Leu Gln Gly Glu Phe Gln Arg Lys Leu Tyr Lys Asp Leu Val Lys
 20 25 30

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

Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp Val Asp Glu
 50 55 60

Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met Thr Trp Thr
 65 70 75 80

Asp His Tyr Leu Gln Trp Asn Ala Ser Glu Tyr Pro Gly Val Lys Thr
 85 90 95

Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile Leu Leu Tyr
 100 105 110

Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr Asn Val Leu
 115 120 125

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Val Asn Ser Ser Gly His Cys Gln Tyr Leu Pro Pro Gly Ile Phe Lys
  130                      135                      140

Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp Val Gln Gln
  145                      150                      155                      160

Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp Ser Leu Asp
          165                      170                      175

Leu Gln Met Gln Glu Ala Asp Ile Ser Gly Tyr Ile Pro Asn Gly Glu
          180                      185                      190

Trp Asp Leu Val Gly Val Leu Gly Lys Arg Ser Glu Lys Phe Tyr Glu
          195                      200                      205

Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val Ser Ile Arg
  210                      215                      220

Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro Cys Val Leu
  225                      230                      235                      240

Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala Asp Ser Gly
          245                      250                      255

Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu Thr Val Phe
          260                      265                      270

Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp Ser Val Pro
          275                      280                      285

Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val Gly Leu Ser
          290                      295                      300

Val Val Val Thr Val Ile Val Leu Gln Tyr His His His Asp Pro Asp
  305                      310                      315                      320

Gly Gly Lys Met Pro Lys Trp Thr Arg Val Val Leu Leu Asn Trp Cys
          325                      330                      335

Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys Val Arg Pro
          340                      345                      350

Ala Cys Gln His Asn Glu Arg Arg Cys Ser Leu Ala Ser Val Glu Met
          355                      360                      365

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38

Ser Ala Val Ala Gly Pro Pro Ala Thr Asn Gly Asn Leu Leu Tyr Ile
 370 375 380

Gly Phe Arg Gly Leu Asp Thr Met His Cys Ala Pro Thr Pro Asp Ser
 385 390 395 400

Gly Val Val Cys Gly Arg Val Ala Cys Ser Pro Thr His Asp Glu His
 405 410 415

Leu Leu His Ala Gly Gln Pro Ser Glu Gly Asp Pro Asp Leu Ala Lys
 420 425 430

Ile Leu Glu Glu Val Arg Tyr Ile Ala His Arg Phe Arg Cys Gln Asp
 435 440 445

Glu Ser Glu Ala Val Cys Ser Glu Trp Lys Phe Ala Ala Cys Val Val
 450 455 460

Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile Leu Cys Thr
 465 470 475 480

Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala Val Ser Lys
 485 490 495

Asp Phe Ala

<210> 19
 <211> 1509
 <212> DNA
 <213> Gallus gallus

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 <222> (1)..(1509)

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 <222> (1)..(66)

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 <222> (1)..(66)
 <223> TM2 domain

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48

Met	Gly	Leu	Arg	Ala	Leu	Met	Leu	Trp	Leu	Leu	Ala	Ala	Ala	Gly	Leu	
1				5					10					15		
gtg	cgc	gag	tcc	ctg	caa	gga	gag	ttc	caa	agg	aag	ctg	tac	aag	gag	96
Val	Arg	Glu	Ser	Leu	Gln	Gly	Glu	Phe	Gln	Arg	Lys	Leu	Tyr	Lys	Glu	
			20					25					30			
ctg	ctg	aag	aac	tac	aac	cct	ctg	gaa	cga	cca	gtt	gca	aat	gac	tcc	144
Leu	Leu	Lys	Asn	Tyr	Asn	Pro	Leu	Glu	Arg	Pro	Val	Ala	Asn	Asp	Ser	
		35					40					45				
cag	ccg	ctc	act	gtc	tat	ttc	act	ctc	agc	ctc	atg	cag	atc	atg	gat	192
Gln	Pro	Leu	Thr	Val	Tyr	Phe	Thr	Leu	Ser	Leu	Met	Gln	Ile	Met	Asp	
	50					55					60					
gtg	gat	gaa	aag	aat	caa	gta	tta	aca	aca	aac	atc	tgg	cta	caa	atg	240
Val	Asp	Glu	Lys	Asn	Gln	Val	Leu	Thr	Thr	Asn	Ile	Trp	Leu	Gln	Met	
65					70					75					80	
tac	tgg	aca	gat	cat	tac	tta	cag	tgg	aat	gtg	tct	gaa	tac	cct	gga	288
Tyr	Trp	Thr	Asp	His	Tyr	Leu	Gln	Trp	Asn	Val	Ser	Glu	Tyr	Pro	Gly	
				85					90					95		
gtg	aag	aac	gtc	cgt	ttt	cct	gat	gga	ctg	att	tgg	aag	cca	gat	att	336
Val	Lys	Asn	Val	Arg	Phe	Pro	Asp	Gly	Leu	Ile	Trp	Lys	Pro	Asp	Ile	
			100					105					110			
ctt	ctc	tat	aac	agt	gct	gat	gaa	aga	ttt	gat	gct	aca	ttt	cac	act	384
Leu	Leu	Tyr	Asn	Ser	Ala	Asp	Glu	Arg	Phe	Asp	Ala	Thr	Phe	His	Thr	
		115					120					125				
aat	gtt	tta	gtc	aat	tct	tcg	gga	cac	tgc	caa	tat	ctg	cca	cca	ggc	432
Asn	Val	Leu	Val	Asn	Ser	Ser	Gly	His	Cys	Gln	Tyr	Leu	Pro	Pro	Gly	
	130					135					140					
ata	ttt	aaa	agc	tca	tgc	tac	ata	gac	gtg	cgt	tgg	ttt	cca	ttt	gat	480
Ile	Phe	Lys	Ser	Ser	Cys	Tyr	Ile	Asp	Val	Arg	Trp	Phe	Pro	Phe	Asp	
145					150					155					160	
gtt	cag	aag	tgc	aat	ctg	aag	ttt	gga	tct	tgg	aca	tat	gga	ggc	tgg	528
Val	Gln	Lys	Cys	Asn	Leu	Lys	Phe	Gly	Ser	Trp	Thr	Tyr	Gly	Gly	Trp	
				165					170					175		
tcc	tta	gac	tta	caa	atg	caa	gaa	gca	gat	ata	tcc	ggc	tat	att	tca	576
Ser	Leu	Asp	Leu	Gln	Met	Gln	Glu	Ala	Asp	Ile	Ser	Gly	Tyr	Ile	Ser	
			180					185					190			
aat	gga	gag	tgg	gat	tta	gta	gga	att	cct	ggg	aag	aga	act	gag	agc	624
Asn	Gly	Glu	Trp	Asp	Leu	Val	Gly	Ile	Pro	Gly	Lys	Arg	Thr	Glu	Ser	
		195					200					205				
ttt	tat	gag	tgc	tgt	aaa	gaa	cca	tac	cca	gat	atc	aca	ttc	aca	gta	672
Phe	Tyr	Glu	Cys	Cys	Lys	Glu	Pro	Tyr	Pro	Asp	Ile	Thr	Phe	Thr	Val	
	210					215					220					
acc	atg	aga	cgc	aga	act	ctc	tac	tac	ggg	ctc	aac	ctt	ctt	att	ccc	720
Thr	Met	Arg	Arg	Arg	Thr	Leu	Tyr	Tyr	Gly	Leu	Asn	Leu	Leu	Ile	Pro	
225					230					235					240	

tgt gta ctg ata tca gca ctt gcc tta tta gtc ttt ctg ctt cca gca Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala 245 250 255	768
gac tca gga gaa aag atc tca cta ggt ata aca gtt tta ttg tct ctc Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu 260 265 270	816
acc gtc ttc atg tta ctc gtg gct gaa att atg cca gca aca tct gat Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp 275 280 285	864
tct gtg ccc tta att gct cag tat ttt gcc agc acc atg att att gtt Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val 290 295 300	912
ggc ctc tct gtt gtt gtc act gtt atc gtt cta caa tac cat cat cac Gly Leu Ser Val Val Val Thr Val Ile Val Leu Gln Tyr His His His 305 310 315 320	960
gat cca gat ggg gga aaa atg cct aaa tgg aca aga gtc atc ctt ctg Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Val Ile Leu Leu 325 330 335	1008
aat tgg tgt gct tgg ttt ctg agg atg aag aga cca ggg gaa gat aaa Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys 340 345 350	1056
gtg cgt ccc gcc tgt caa cat aaa cag cgc cga tgc agc ctg tca agc Val Arg Pro Ala Cys Gln His Lys Gln Arg Arg Cys Ser Leu Ser Ser 355 360 365	1104
atg gag atg aac act gtg agt ggt cag caa tgc agt aat ggg aac atg Met Glu Met Asn Thr Val Ser Gly Gln Gln Cys Ser Asn Gly Asn Met 370 375 380	1152
ctg tat att ggg ttt cga ggg ctg gat ggg gtt cac tgc aca ccc acc Leu Tyr Ile Gly Phe Arg Gly Leu Asp Gly Val His Cys Thr Pro Thr 385 390 395 400	1200
act gat tca ggg gtg atc tgt ggg agg atg acc tgt tca cca aca gag Thr Asp Ser Gly Val Ile Cys Gly Arg Met Thr Cys Ser Pro Thr Glu 405 410 415	1248
gaa gaa aat ctt ctg cac agt ggc cac ccc tct gaa ggc gac cca gat Glu Glu Asn Leu Leu His Ser Gly His Pro Ser Glu Gly Asp Pro Asp 420 425 430	1296
ttg gct aag atc ctg gaa gag gtc aga tac att gca aac agg ttc aga Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg 435 440 445	1344
gac cag gat gaa gaa gaa gcc att tgc aac gaa tgg aag ttt gca gcc Asp Gln Asp Glu Glu Glu Ala Ile Cys Asn Glu Trp Lys Phe Ala Ala 450 455 460	1392
tct gta gta gat cgg ctc tgc ttg atg gca ttt tcg gtc ttc acc atc Ser Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile 465 470 475 480	1440

att tgt aca att ggc atc tta atg tca gca cca aac ttt gta gag gct 1488
 Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

gtc tct aaa gat ttt gct taa 1509
 Val Ser Lys Asp Phe Ala
 500

<210> 20
 <211> 502
 <212> PRT
 <213> Gallus gallus

<400> 20

Met Gly Leu Arg Ala Leu Met Leu Trp Leu Leu Ala Ala Ala Gly Leu
 1 5 10 15

Val Arg Glu Ser Leu Gln Gly Glu Phe Gln Arg Lys Leu Tyr Lys Glu
 20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Thr Leu Ser Leu Met Gln Ile Met Asp
 50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
 65 70 75 80

Tyr Trp Thr Asp His Tyr Leu Gln Trp Asn Val Ser Glu Tyr Pro Gly
 85 90 95

Val Lys Asn Val Arg Phe Pro Asp Gly Leu Ile Trp Lys Pro Asp Ile
 100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

Asn Val Leu Val Asn Ser Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Val Gln Lys Cys Asn Leu Lys Phe Gly Ser Trp Thr Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Gly Tyr Ile Ser
180 185 190

Asn Gly Glu Trp Asp Leu Val Gly Ile Pro Gly Lys Arg Thr Glu Ser
195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Ile Thr Phe Thr Val
210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Gln Tyr His His His
305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Val Ile Leu Leu
325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
340 345 350

Val Arg Pro Ala Cys Gln His Lys Gln Arg Arg Cys Ser Leu Ser Ser
355 360 365

Met Glu Met Asn Thr Val Ser Gly Gln Gln Cys Ser Asn Gly Asn Met
370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Asp Gly Val His Cys Thr Pro Thr
385 390 395 400

Thr Asp Ser Gly Val Ile Cys Gly Arg Met Thr Cys Ser Pro Thr Glu

43

405

410

415

Glu Glu Asn Leu Leu His Ser Gly His Pro Ser Glu Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Asp Gln Asp Glu Glu Glu Ala Ile Cys Asn Glu Trp Lys Phe Ala Ala
 450 455 460

Ser Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
 500

<210> 21
 <211> 502
 <212> PRT
 <213> Gallus gallus

<220>
 <221> SIGNAL
 <222> (1)..(22)

<220>
 <221> TRANSMEM
 <222> (262)..(281)

<400> 21

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Val Arg Glu Ser Leu Gln Gly Glu Phe Gln Arg Lys Leu Tyr Lys Glu
 20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Thr Leu Ser Leu Met Gln Ile Met Asp
 50 55 60

44

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
65 70 75 80

Tyr Trp Thr Asp His Tyr Leu Gln Trp Asn Val Ser Glu Tyr Pro Gly
85 90 95

Val Lys Asn Val Arg Phe Pro Asp Gly Leu Ile Trp Lys Pro Asp Ile
100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
115 120 125

Asn Val Leu Val Asn Ser Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
130 135 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
145 150 155 160

Val Gln Lys Cys Asn Leu Lys Phe Gly Ser Trp Thr Tyr Gly Gly Trp
165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Gly Tyr Ile Ser
180 185 190

Asn Gly Glu Trp Asp Leu Val Gly Ile Pro Gly Lys Arg Thr Glu Ser
195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Ile Thr Phe Thr Val
210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
290 295 300

45

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Gln Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Val Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Gln Arg Arg Cys Ser Leu Ser Ser
 355 360 365

Met Glu Met Asn Thr Val Ser Gly Gln Gln Cys Ser Asn Gly Asn Met
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Asp Gly Val His Cys Thr Pro Thr
 385 390 395 400

Thr Asp Ser Gly Val Ile Cys Gly Arg Met Thr Cys Ser Pro Thr Glu
 405 410 415

Glu Glu Asn Leu Leu His Ser Gly His Pro Ser Glu Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Asp Gln Asp Glu Glu Glu Ala Ile Cys Asn Glu Trp Lys Phe Ala Ala
 450 455 460

Ser Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
 500

<210> 22
 <211> 1530
 <212> DNA
 <213> Danio rerio

<220>
 <221> CDS
 <222> (1)..(1530)

<220>
 <221> sig_peptide
 <222> (1)..(66)

<220>
 <221> misc_feature
 <222> (784)..(843)
 <223> TM2 domain

<400> 22
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 Met Gly Ile Trp Glu Tyr Thr Leu Tyr Phe Thr Thr Thr Cys Cys Leu
 1 5 10 15
 tgg agt gtg tca ctt cag ggg gag cat cag agg aga ctc tat aga gac 96
 Trp Ser Val Ser Leu Gln Gly Glu His Gln Arg Arg Leu Tyr Arg Asp
 20 25 30
 ctg atg aaa gat tat aac cca ctg gag aga cct gtg ttc aat gac acc 144
 Leu Met Lys Asp Tyr Asn Pro Leu Glu Arg Pro Val Phe Asn Asp Thr
 35 40 45
 cac tca ctc acg gtg tat ttc agc atg agc ctc atg cag att atg gat 192
 His Ser Leu Thr Val Tyr Phe Ser Met Ser Leu Met Gln Ile Met Asp
 50 55 60
 gtg gat gaa aag aat caa gtt ctt acg acc aac att tgg cta cag ctg 240
 Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Leu
 65 70 75 80
 tac tgg tat gac tac tat ctt caa tgg aac gcc tca gaa tat cct gga 288
 Tyr Trp Tyr Asp Tyr Tyr Leu Gln Trp Asn Ala Ser Glu Tyr Pro Gly
 85 90 95
 gta acc aat gta agg ttt cct gac agt cag atc tgg aag cct gac atc 336
 Val Thr Asn Val Arg Phe Pro Asp Ser Gln Ile Trp Lys Pro Asp Ile
 100 105 110
 tta ctt tat aac agt gct gat gaa agg ttt gat gcc aca ttt cat act 384
 Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125
 aat gtg ctg gtg aac tct tct ggt gcc tgc caa tac tta ccg cca ggg 432
 Asn Val Leu Val Asn Ser Ser Gly Ala Cys Gln Tyr Leu Pro Pro Gly
 130 135 140
 atc ttc aaa agc acc tgc tac atc gac gtt cgc tgg ttc ccc ttt gat 480
 Ile Phe Lys Ser Thr Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160
 ctt cag agg tgt gat ctg aaa ttt ggc tcc tgg acg tat gga gga tgg 528
 Leu Gln Arg Cys Asp Leu Lys Phe Gly Ser Trp Thr Tyr Gly Gly Trp
 165 170 175

47

tct ctg gac ctg cag atg att gat gcc gac atc aca gga tac att gct	576
Ser Leu Asp Leu Gln Met Ile Asp Ala Asp Ile Thr Gly Tyr Ile Ala	
180 185 190	
aat gga gag tgg gac ctt gtg gaa gtt cca ggt aga cgg aat gag agg	624
Asn Gly Glu Trp Asp Leu Val Glu Val Pro Gly Arg Arg Asn Glu Arg	
195 200 205	
ttc tac gac tgc tgc aag gag ccg tac cca gat gtg act ttt acg gtg	672
Phe Tyr Asp Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val	
210 215 220	
gtg atg cgg aga cga aca ctg tat tac ggt ctt aat cta ctt att ccc	720
Val Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro	
225 230 235 240	
tgt gtg ctc atc tct act ctg gcc ctg ctg gtg ttc ctg ctg cct gct	768
Cys Val Leu Ile Ser Thr Leu Ala Leu Leu Val Phe Leu Leu Pro Ala	
245 250 255	
gac tca gga gaa aag atc tcg ctc ggg atc acc gtc ctg ctc tcg ctg	816
Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu	
260 265 270	
aca gtt ttc atg ctt ttg gta gca gag ata atg ccg gca aca tct gac	864
Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp	
275 280 285	
tct gtg cct tta ata gct cag tat ttt gcc acc acc atg gtt att gtt	912
Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Thr Thr Met Val Ile Val	
290 295 300	
gga cta tct gta ata gcc aca gtc tgg gtt tta caa tac cac tac cat	960
Gly Leu Ser Val Ile Ala Thr Val Trp Val Leu Gln Tyr His Tyr His	
305 310 315 320	
gat cct gaa gga gga aaa atg cca aaa tgg aca cgt gtg gtg ctg ttg	1008
Asp Pro Glu Gly Gly Lys Met Pro Lys Trp Thr Arg Val Val Leu Leu	
325 330 335	
aac tgg tgt gcc tgg ttc ctg ccg atg aag aga cct ggc gaa gac aaa	1056
Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys	
340 345 350	
gtt cgc cca gct tgt cac aac aag cag ccg cgc agc agc ctc tcc agc	1104
Val Arg Pro Ala Cys His Asn Lys Gln Pro Arg Ser Ser Leu Ser Ser	
355 360 365	
gtg gat cta aac atc agc cct gga gtc gca cag tcc acc aat gga aac	1152
Val Asp Leu Asn Ile Ser Pro Gly Val Ala Gln Ser Thr Asn Gly Asn	
370 375 380	
ctg ctg tac atc ggc ttc cgc ggg atg gac act att cac tat gct act	1200
Leu Leu Tyr Ile Gly Phe Arg Gly Met Asp Thr Ile His Tyr Ala Thr	
385 390 395 400	
tca ccc gat tct ggg gtg atc tgc agc cgg ctt gtg gcg acc gga gag	1248
Ser Pro Asp Ser Gly Val Ile Cys Ser Arg Leu Val Ala Thr Gly Glu	
405 410 415	

gag gat gtg ctg ctc ccc gga gcc cag gca tct tca gtc agt agt agc 1296
 Glu Asp Val Leu Leu Pro Gly Ala Gln Ala Ser Ser Val Ser Ser Ser
 420 425 430

ggc cct gga gaa acc gaa ctg tcc aag atc ttg gat gag gtg cgg tac 1344
 Gly Pro Gly Glu Thr Glu Leu Ser Lys Ile Leu Asp Glu Val Arg Tyr
 435 440 445

att tcc aag cgc ttt cgg gac caa gac gag gaa gac act gtg tgt aat 1392
 Ile Ser Lys Arg Phe Arg Asp Gln Asp Glu Glu Asp Thr Val Cys Asn
 450 455 460

gag tgg aag ttt gca gcg tct gtg att gac cgt ctc tgt ctc atg gct 1440
 Glu Trp Lys Phe Ala Ala Ser Val Ile Asp Arg Leu Cys Leu Met Ala
 465 470 475 480

ttt tcc ctg ttc acc atc ctc tgc acc att ggt atc tta atg tct gcc 1488
 Phe Ser Leu Phe Thr Ile Leu Cys Thr Ile Gly Ile Leu Met Ser Ala
 485 490 495

cct aac ttt gtt gag gcc ata tca aaa gat ttt ttc acc tga 1530
 Pro Asn Phe Val Glu Ala Ile Ser Lys Asp Phe Phe Thr
 500 505

<210> 23
 <211> 509
 <212> PRT
 <213> Danio rerio

<400> 23

Met Gly Ile Trp Glu Tyr Thr Leu Tyr Phe Thr Thr Thr Cys Cys Leu
 1 5 10 15

Trp Ser Val Ser Leu Gln Gly Glu His Gln Arg Arg Leu Tyr Arg Asp
 20 25 30

Leu Met Lys Asp Tyr Asn Pro Leu Glu Arg Pro Val Phe Asn Asp Thr
 35 40 45

His Ser Leu Thr Val Tyr Phe Ser Met Ser Leu Met Gln Ile Met Asp
 50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Leu
 65 70 75 80

Tyr Trp Tyr Asp Tyr Tyr Leu Gln Trp Asn Ala Ser Glu Tyr Pro Gly
 85 90 95

Val Thr Asn Val Arg Phe Pro Asp Ser Gln Ile Trp Lys Pro Asp Ile
 100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

Asn Val Leu Val Asn Ser Ser Gly Ala Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

Ile Phe Lys Ser Thr Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Leu Gln Arg Cys Asp Leu Lys Phe Gly Ser Trp Thr Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Ile Asp Ala Asp Ile Thr Gly Tyr Ile Ala
 180 185 190

Asn Gly Glu Trp Asp Leu Val Glu Val Pro Gly Arg Arg Asn Glu Arg
 195 200 205

Phe Tyr Asp Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val
 210 215 220

Val Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Thr Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Thr Thr Met Val Ile Val
 290 295 300

Gly Leu Ser Val Ile Ala Thr Val Trp Val Leu Gln Tyr His Tyr His
 305 310 315 320

Asp Pro Glu Gly Gly Lys Met Pro Lys Trp Thr Arg Val Val Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys

Val	Asp	Leu	Asn	Ile	Ser	Pro	Gly	Val	Ala	Gln	Ser	Thr	Asn	Gly	Asn
	370					375					380				

Leu Leu Tyr Ile Gly Phe Arg Gly Met Asp Thr Ile His Tyr Ala Thr
385 390 395 400

Ser Pro Asp Ser Gly Val Ile Cys Ser Arg Leu Val Ala Thr Gly Glu
405 410 415

Glu Asp Val Leu Leu Pro Gly Ala Gln Ala Ser Ser Val Ser Ser Ser
420 425 430

Gly Pro Gly Glu Thr Glu Leu Ser Lys Ile Leu Asp Glu Val Arg Tyr
435 440 445

Ile Ser Lys Arg Phe Arg Asp Gln Asp Glu Glu Asp Thr Val Cys Asn
450 455 460

Glu Trp Lys Phe Ala Ala Ser Val Ile Asp Arg Leu Cys Leu Met Ala
465 470 475 480

Phe Ser Leu Phe Thr Ile Leu Cys Thr Ile Gly Ile Leu Met Ser Ala
485 490 495

Pro Asn Phe Val Glu Ala Ile Ser Lys Asp Phe Phe Thr
500 505

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<210> 24
<211> 509
<212> PRT
<213> Danio rerio
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<220>
<221> SIGNAL
<222> (1) .. (22)
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<220>
<221> TRANSMEM
<222> (262) .. (281)
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<400> 24

51

Met Gly Ile Trp Glu Tyr Thr Leu Tyr Phe Thr Thr Thr Cys Cys Leu
 1 5 10 15
 Trp Ser Val Ser Leu Gln Gly Glu His Gln Arg Arg Leu Tyr Arg Asp
 20 25 30
 Leu Met Lys Asp Tyr Asn Pro Leu Glu Arg Pro Val Phe Asn Asp Thr
 35 40 45
 His Ser Leu Thr Val Tyr Phe Ser Met Ser Leu Met Gln Ile Met Asp
 50 55 60
 Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Leu
 65 70 75 80
 Tyr Trp Tyr Asp Tyr Tyr Leu Gln Trp Asn Ala Ser Glu Tyr Pro Gly
 85 90 95
 Val Thr Asn Val Arg Phe Pro Asp Ser Gln Ile Trp Lys Pro Asp Ile
 100 105 110
 Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125
 Asn Val Leu Val Asn Ser Ser Gly Ala Cys Gln Tyr Leu Pro Pro Gly
 130 135 140
 Ile Phe Lys Ser Thr Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160
 Leu Gln Arg Cys Asp Leu Lys Phe Gly Ser Trp Thr Tyr Gly Gly Trp
 165 170 175
 Ser Leu Asp Leu Gln Met Ile Asp Ala Asp Ile Thr Gly Tyr Ile Ala
 180 185 190
 Asn Gly Glu Trp Asp Leu Val Glu Val Pro Gly Arg Arg Asn Glu Arg
 195 200 205
 Phe Tyr Asp Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val
 210 215 220
 Val Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Thr Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Thr Thr Met Val Ile Val
 290 295 300

Gly Leu Ser Val Ile Ala Thr Val Trp Val Leu Gln Tyr His Tyr His
 305 310 315 320

Asp Pro Glu Gly Gly Lys Met Pro Lys Trp Thr Arg Val Val Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys His Asn Lys Gln Pro Arg Ser Ser Leu Ser Ser
 355 360 365

Val Asp Leu Asn Ile Ser Pro Gly Val Ala Gln Ser Thr Asn Gly Asn
 370 375 380

Leu Leu Tyr Ile Gly Phe Arg Gly Met Asp Thr Ile His Tyr Ala Thr
 385 390 395 400

Ser Pro Asp Ser Gly Val Ile Cys Ser Arg Leu Val Ala Thr Gly Glu
 405 410 415

Glu Asp Val Leu Leu Pro Gly Ala Gln Ala Ser Ser Val Ser Ser Ser
 420 425 430

Gly Pro Gly Glu Thr Glu Leu Ser Lys Ile Leu Asp Glu Val Arg Tyr
 435 440 445

Ile Ser Lys Arg Phe Arg Asp Gln Asp Glu Glu Asp Thr Val Cys Asn
 450 455 460

Glu Trp Lys Phe Ala Ala Ser Val Ile Asp Arg Leu Cys Leu Met Ala

Pro Asn Phe Val Glu Ala Ile Ser Lys Asp Phe Phe Thr
500 505

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<211> 60
<212> DNA
<213> Artificial Sequence
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<220>
<223> degenerate variant encoding T6'S mutant TM2 domain
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<400> 25
atctctcttg gaatatctgt cttactttct ctgactgtct tcatgctgct tgtggctgag 60

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<210> 26
<211> 1509
<212> DNA
<213> Artificial Sequence
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<220>
<223> degenerate variant encoding T6'S mutant alpha7 subunit

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<220>
<221> CDS
<222> (1) .. (1509)
```

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<220>
<221>  sig_peptide
<222>  (1)..(66)
```

```
<220>
<221> misc_feature
<222> (784)..(843)
<223> TM2 domain
```

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<400> 26
atg tgc ggc ggg cgg gga ggc atc tgg ctg gct ctg gcc gcg gcg ctg
Met Cys Gly Gly Arg Gly Gly Ile Trp Leu Ala Leu Ala Ala Ala Leu
1          5          10          15          48
```

ctg cac gtg tcc ctg caa ggc gag ttc cag agg agg ctg tac aag gag 96
Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
20 25 30

ctg gtc aag aac tac aac ccg ctg gag agg ccg gtg gcc aac gac tcg 144
Leu Val Lys Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser
35 40 45

caq ccq ctc acc gtg tac ttc tcc ctg agt ctc ctg cag atc atg gat 192

Gln	Pro	Leu	Thr	Val	Tyr	Phe	Ser	Leu	Ser	Leu	Leu	Gln	Ile	Met	Asp	
50						55					60					
gtg	gat	gag	aag	aac	caa	gtt	tta	acc	acc	aac	att	tgg	cta	caa	atg	240
Val	Asp	Glu	Lys	Asn	Gln	Val	Leu	Thr	Thr	Asn	Ile	Trp	Leu	Gln	Met	
65					70					75					80	
tct	tgg	aca	gat	cac	tat	ttg	cag	tgg	aac	atg	tct	gag	tac	ccc	gga	288
Ser	Trp	Thr	Asp	His	Tyr	Leu	Gln	Trp	Asn	Met	Ser	Glu	Tyr	Pro	Gly	
				85					90					95		
gtg	aag	aat	gtt	cgt	ttt	cca	gat	ggc	cag	att	tgg	aaa	cca	gac	att	336
Val	Lys	Asn	Val	Arg	Phe	Pro	Asp	Gly	Gln	Ile	Trp	Lys	Pro	Asp	Ile	
			100					105					110			
ctc	ctc	tat	aac	agt	gct	gat	gag	cgc	ttt	gat	gcc	acg	ttc	cac	acc	384
Leu	Leu	Tyr	Asn	Ser	Ala	Asp	Glu	Arg	Phe	Asp	Ala	Thr	Phe	His	Thr	
		115					120					125				
aat	gtt	ttg	gtg	aat	gca	tct	ggg	cat	tgc	cag	tat	ctc	cct	cca	ggc	432
Asn	Val	Leu	Val	Asn	Ala	Ser	Gly	His	Cys	Gln	Tyr	Leu	Pro	Pro	Gly	
	130					135					140					
ata	ttc	aag	agc	tcc	tgc	tac	att	gac	gtt	cgc	tgg	ttc	cct	ttt	gat	480
Ile	Phe	Lys	Ser	Ser	Cys	Tyr	Ile	Asp	Val	Arg	Trp	Phe	Pro	Phe	Asp	
145					150					155					160	
gtg	cag	cag	tgc	aaa	ctg	aag	ttt	ggg	tcc	tgg	tcc	tat	gga	ggg	tgg	528
Val	Gln	Gln	Cys	Lys	Leu	Lys	Phe	Gly	Ser	Trp	Ser	Tyr	Gly	Gly	Trp	
				165					170					175		
tca	ctg	gac	ctg	caa	atg	caa	gag	gca	gat	atc	agc	agc	tat	atc	ccc	576
Ser	Leu	Asp	Leu	Gln	Met	Gln	Glu	Ala	Asp	Ile	Ser	Ser	Tyr	Ile	Pro	
			180					185					190			
aac	gga	gaa	tgg	gat	ctc	atg	gga	atc	cct	ggc	aaa	agg	aat	gag	aag	624
Asn	Gly	Glu	Trp	Asp	Leu	Met	Gly	Ile	Pro	Gly	Lys	Arg	Asn	Glu	Lys	
	195						200				205					
ttc	tat	gag	tgc	tgc	aaa	gag	cca	tac	cca	gat	gtc	acc	tac	aca	gta	672
Phe	Tyr	Glu	Cys	Cys	Lys	Glu	Pro	Tyr	Pro	Asp	Val	Thr	Tyr	Thr	Val	
	210					215					220					
acc	atg	cgc	cgt	agg	aca	ctc	tac	tat	ggc	ctc	aat	ctg	ctc	atc	cct	720
Thr	Met	Arg	Arg	Arg	Thr	Leu	Tyr	Tyr	Gly	Leu	Asn	Leu	Leu	Ile	Pro	
225					230					235					240	
tgt	gta	ctc	att	tca	gcc	ctg	gct	ctg	ctg	gta	ttc	ttg	ctg	cct	gca	768
Cys	Val	Leu	Ile	Ser	Ala	Leu	Ala	Leu	Leu	Val	Phe	Leu	Leu	Pro	Ala	
				245					250					255		
gac	tct	gga	gag	aaa	atc	tct	ctt	gga	ata	tct	gtc	tta	ctt	tct	ctg	816
Asp	Ser	Gly	Glu	Lys	Ile	Ser	Leu	Gly	Ile	Ser	Val	Leu	Leu	Ser	Leu	
			260					265				270				
act	gtc	ttc	atg	ctg	ctt	gtg	gct	gag	atc	atg	cca	gca	aca	tct	gat	864
Thr	Val	Phe	Met	Leu	Leu	Val	Ala	Glu	Ile	Met	Pro	Ala	Thr	Ser	Asp	
		275					280					285				

55

tct gtg ccc ttg ata gca caa tac ttc gcc agc acc atg atc atc gtg	912
Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val	
290 295 300	
ggc ctc tct gta gtg gtg aca gtg att gtg ctg aga tat cac cac cat	960
Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His	
305 310 315 320	
gac cct gat ggt ggc aaa atg cct aag tgg acc aga atc att ctc ctg	1008
Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu	
325 330 335	
aac tgg tgt gca tgg ttt ctg cgc atg aag agg ccc gga gag gac aag	1056
Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys	
340 345 350	
gtg cgg cca gct tgt cag cac aag cct cgg cgc tgc agc ctg gcc agt	1104
Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser	
355 360 365	
gtg gag ctg agt gca ggt gct ggg cca ccc acc agc aat ggc aac ctg	1152
Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu	
370 375 380	
ctc tac att ggc ttc cga ggc ctg gag ggc atg cac tgt gcc cca act	1200
Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr	
385 390 395 400	
cca gac tct ggg gtc gta tgt ggc cgt ttg gcc tgc tcc cca aca cat	1248
Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His	
405 410 415	
gat gag cac ctc atg cac ggt gca cac ccc tct gat ggg gac ccc gac	1296
Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp	
420 425 430	
ctg gcc aag atc ctg gag gag gtc cgc tac atc gcc aac cgc ttc cgc	1344
Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg	
435 440 445	
tgc cag gac gag agt gag gtg atc tgc agt gaa tgg aag ttt gca gcc	1392
Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala	
450 455 460	
tgc gtg gtg gac cgc ttg tgc ctc atg gcc ttt tcg gtc ttt acc atc	1440
Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile	
465 470 475 480	
atc tgt acc atc ggc atc ctc atg tca gct cca aac ttt gtg gag gct	1488
Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala	
485 490 495	
gtg tcc aaa gac ttt gct taa	1509
Val Ser Lys Asp Phe Ala	
500	

<210> 27

<211> 502

56

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 27

Met Cys Gly Gly Arg Gly Gly Ile Trp Leu Ala Leu Ala Ala Ala Leu
 1 5 10 15

Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
 20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
 50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
 65 70 75 80

Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
 85 90 95

Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
 100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
 180 185 190

Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys
 195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val
 210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Ser Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
 290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
 500

<210> 28
 <211> 502
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> T6'S mutant alpha7 subunit

<220>
 <221> SIGNAL
 <222> (1)..(22)

<220>
 <221> TRANSMEM
 <222> (262)..(281)

<400> 28

Met Cys Gly Gly Arg Gly Gly Ile Trp Leu Ala Leu Ala Ala Ala Leu
 1 5 10 15

Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
 20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
 50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
 65 70 75 80

60

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
 500

<210> 29
 <211> 60
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> degenerate variant encoding T6'S mutant TM2 domain

<400> 29
 atctctcttg gaatatccgt cttacttttct ctgactgtct tcatgctgct tgtggctgag

60

<210> 30
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 30
atctctcttg gaatatcagt cttactttct ctgactgtct tcatgctgct tgtggctgag 60

<210> 31
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 31
atctctcttg gaatatcggg cttactttct ctgactgtct tcatgctgct tgtggctgag 60

<210> 32
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 32
atctctcttg gaataagtgt cttactttct ctgactgtct tcatgctgct tgtggctgag 60

<210> 33
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 33
atctctcttg gaataagcgt cttactttct ctgactgtct tcatgctgct tgtggctgag 60

<210> 34
<211> 1509
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant alpha7 subunit

<220>
 <221> CDS
 <222> (1) .. (1509)

<220>
 <221> sig_peptide
 <222> (1) .. (66)

<220>
 <221> misc_feature
 <222> (784) .. (843)
 <223> TM2 domain

<400> 34
 atg tgc ggc ggg cgg gga ggc atc tgg ctg gct ctg gcc gcg gcg ctg 48
 Met Cys Gly Gly Arg Gly Gly Ile Trp Leu Ala Leu Ala Ala Leu
 1 5 10 15

ctg cac gtg tcc ctg caa ggc gag ttc cag agg agg ctg tac aag gag 96
 Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
 20 25 30

ctg gtc aag aac tac aac ccg ctg gag agg ccg gtg gcc aac gac tcg 144
 Leu Val Lys Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser
 35 40 45

cag ccg ctc acc gtg tac ttc tcc ctg agt ctc ctg cag atc atg gat 192
 Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
 50 55 60

gtg gat gag aag aac caa gtt tta acc acc aac att tgg cta caa atg 240
 Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
 65 70 75 80

tct tgg aca gat cac tat ttg cag tgg aac atg tct gag tac ccc gga 288
 Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
 85 90 95

gtg aag aat gtt cgt ttt cca gat ggc cag att tgg aaa cca gac att 336
 Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
 100 105 110

ctc ctc tat aac agt gct gat gag cgc ttt gat gcc acg ttc cac acc 384
 Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

aat gtt ttg gtg aat gca tct ggg cat tgc cag tat ctc cct cca ggc 432
 Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

ata ttc aag agc tcc tgc tac att gac gtt cgc tgg ttc cct ttt gat 480
 Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

gtg cag cag tgc aaa ctg aag ttt ggg tcc tgg tcc tat gga ggg tgg 528
 Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

63

tca ctg gac ctg caa atg caa gag gca gat atc agc agc tat atc ccc	576
Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro	
180 185 190	
aac gga gaa tgg gat ctc atg gga atc cct ggc aaa agg aat gag aag	624
Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys	
195 200 205	
ttc tat gag tgc tgc aaa gag cca tac cca gat gtc acc tac aca gta	672
Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val	
210 215 220	
acc atg cgc cgt agg aca ctc tac tat ggc ctc aat ctg ctc atc cct	720
Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro	
225 230 235 240	
tgt gta ctc att tca gcc ctg gct ctg ctg gta ttc ttg ctg cct gca	768
Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala	
245 250 255	
gac tct gga gag aaa atc tct ctt gga ata tcc gtc tta ctt tct ctg	816
Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Ser Val Leu Leu Ser Leu	
260 265 270	
act gtc ttc atg ctg ctt gtg gct gag atc atg cca gca aca tct gat	864
Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp	
275 280 285	
tct gtg ccc ttg ata gca caa tac ttc gcc agc acc atg atc atc gtg	912
Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val	
290 295 300	
ggc ctc tct gta gtg gtg aca gtg att gtg ctg aga tat cac cac cat	960
Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His	
305 310 315 320	
gac cct gat ggt ggc aaa atg cct aag tgg acc aga atc att ctc ctg	1008
Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu	
325 330 335	
aac tgg tgt gca tgg ttt ctg cgc atg aag agg ccc gga gag gac aag	1056
Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys	
340 345 350	
gtg cgg cca gct tgt cag cac aag cct cgg cgc tgc agc ctg gcc agt	1104
Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser	
355 360 365	
gtg gag ctg agt gca ggt gct ggg cca ccc acc agc aat ggc aac ctg	1152
Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu	
370 375 380	
ctc tac att ggc ttc cga ggc ctg gag ggc atg cac tgt gcc cca act	1200
Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr	
385 390 395 400	
cca gac tct ggg gtc gta tgt ggc cgt ttg gcc tgc tcc cca aca cat	1248
Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His	
405 410 415	

64

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gat gag cac ctc atg cac ggt gca cac ccc tct gat ggg gac ccc gac      1296
Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp
      420                      425                      430

ctg gcc aag atc ctg gag gag gtc cgc tac atc gcc aac cgc ttc cgc      1344
Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
      435                      440                      445

tgc cag gac gag agt gag gtg atc tgc agt gaa tgg aag ttt gca gcc      1392
Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
      450                      455                      460

tgc gtg gtg gac cgc ttg tgc ctc atg gcc ttt tcg gtc ttt acc atc      1440
Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
      465                      470                      475                      480

atc tgt acc atc ggc atc ctc atg tca gct cca aac ttt gtg gag gct      1488
Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
      485                      490                      495

gtg tcc aaa gac ttt gct taa      1509
Val Ser Lys Asp Phe Ala
      500

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<210> 35
<211> 502
<212> PRT
<213> Artificial Sequence

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<220>
<223> Synthetic Construct

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<400> 35

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Met Cys Gly Gly Arg Gly Gly Ile Trp Leu Ala Leu Ala Ala Ala Leu
1          5          10          15

```

```

Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
      20          25          30

```

```

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

```

```

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
      50          55          60

```

```

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
      65          70          75          80

```

```

Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
      85          90          95

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Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
 100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
 180 185 190

Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys
 195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val
 210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Ser Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
 290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
 500

<210> 36
 <211> 1509
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> degenerate variant encoding T6'S mutant alpha7 subunit

<220>
 <221> CDS
 <222> (1)..(1509)

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<220>
<221> sig_peptide
<222> (1)..(66)

<220>
<221> misc_feature
<222> (784)..(843)
<223> TM2 domain

<400> 36
atg tgc ggc ggg cgg gga ggc atc tgg ctg gct ctg gcc gcg gcg ctg      48
Met Cys Gly Gly Arg Gly Gly Ile Trp Leu Ala Leu Ala Ala Ala Leu
1          5          10          15

ctg cac gtg tcc ctg caa ggc gag ttc cag agg agg ctg tac aag gag      96
Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
          20          25          30

ctg gtc aag aac tac aac ccg ctg gag agg ccg gtg gcc aac gac tcg      144
Leu Val Lys Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser
          35          40          45

cag ccg ctc acc gtg tac ttc tcc ctg agt ctc ctg cag atc atg gat      192
Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
          50          55          60

gtg gat gag aag aac caa gtt tta acc acc aac att tgg cta caa atg      240
Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
65          70          75          80

tct tgg aca gat cac tat ttg cag tgg aac atg tct gag tac ccc gga      288
Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
          85          90          95

gtg aag aat gtt cgt ttt cca gat ggc cag att tgg aaa cca gac att      336
Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
          100          105          110

ctc ctc tat aac agt gct gat gag cgc ttt gat gcc acg ttc cac acc      384
Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
          115          120          125

aat gtt ttg gtg aat gca tct ggg cat tgc cag tat ctc cct cca ggc      432
Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
          130          135          140

ata ttc aag agc tcc tgc tac att gac gtt cgc tgg ttc cct ttt gat      480
Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
145          150          155          160

gtg cag cag tgc aaa ctg aag ttt ggg tcc tgg tcc tat gga ggg tgg      528
Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
          165          170          175

tca ctg gac ctg caa atg caa gag gca gat atc agc agc tat atc ccc      576
Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
          180          185          190

aac gga gaa tgg gat ctc atg gga atc cct ggc aaa agg aat gag aag      624

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68

Asn	Gly	Glu	Trp	Asp	Leu	Met	Gly	Ile	Pro	Gly	Lys	Arg	Asn	Glu	Lys		
		195					200					205					
ttc	tat	gag	tgc	tgc	aaa	gag	cca	tac	cca	gat	gtc	acc	tac	aca	gta	672	
Phe	Tyr	Glu	Cys	Cys	Lys	Glu	Pro	Tyr	Pro	Asp	Val	Thr	Tyr	Thr	Val		
	210					215					220						
acc	atg	cgc	cgt	agg	aca	ctc	tac	tat	ggc	ctc	aat	ctg	ctc	atc	cct	720	
Thr	Met	Arg	Arg	Arg	Thr	Leu	Tyr	Tyr	Gly	Leu	Asn	Leu	Leu	Ile	Pro		
225					230					235					240		
tgt	gta	ctc	att	tca	gcc	ctg	gct	ctg	ctg	gta	ttc	ttg	ctg	cct	gca	768	
Cys	Val	Leu	Ile	Ser	Ala	Leu	Ala	Leu	Leu	Val	Phe	Leu	Leu	Pro	Ala		
				245					250					255			
gac	tct	gga	gag	aaa	atc	tct	ctt	gga	ata	tca	gtc	tta	ctt	tct	ctg	816	
Asp	Ser	Gly	Glu	Lys	Ile	Ser	Leu	Gly	Ile	Ser	Val	Leu	Leu	Ser	Leu		
			260					265					270				
act	gtc	ttc	atg	ctg	ctt	gtg	gct	gag	atc	atg	cca	gca	aca	tct	gat	864	
Thr	Val	Phe	Met	Leu	Leu	Val	Ala	Glu	Ile	Met	Pro	Ala	Thr	Ser	Asp		
	275						280					285					
tct	gtg	ccc	ttg	ata	gca	caa	tac	ttc	gcc	agc	acc	atg	atc	atc	gtg	912	
Ser	Val	Pro	Leu	Ile	Ala	Gln	Tyr	Phe	Ala	Ser	Thr	Met	Ile	Ile	Val		
	290					295					300						
ggc	ctc	tct	gta	gtg	gtg	aca	gtg	att	gtg	ctg	aga	tat	cac	cac	cat	960	
Gly	Leu	Ser	Val	Val	Val	Thr	Val	Ile	Val	Leu	Arg	Tyr	His	His	His		
305					310					315					320		
gac	cct	gat	ggc	ggc	aaa	atg	cct	aag	tgg	acc	aga	atc	att	ctc	ctg	1008	
Asp	Pro	Asp	Gly	Gly	Lys	Met	Pro	Lys	Trp	Thr	Arg	Ile	Ile	Leu	Leu		
				325					330					335			
aac	tgg	tgt	gca	tgg	ttt	ctg	cgc	atg	aag	agg	ccc	gga	gag	gac	aag	1056	
Asn	Trp	Cys	Ala	Trp	Phe	Leu	Arg	Met	Lys	Arg	Pro	Gly	Glu	Asp	Lys		
			340					345					350				
gtg	cgg	cca	gct	tgt	cag	cac	aag	cct	cgg	cgc	tgc	agc	ctg	gcc	agt	1104	
Val	Arg	Pro	Ala	Cys	Gln	His	Lys	Pro	Arg	Arg	Cys	Ser	Leu	Ala	Ser		
		355					360					365					
gtg	gag	ctg	agt	gca	ggc	gct	ggg	cca	ccc	acc	agc	aat	ggc	aac	ctg	1152	
Val	Glu	Leu	Ser	Ala	Gly	Ala	Gly	Pro	Pro	Thr	Ser	Asn	Gly	Asn	Leu		
	370					375					380						
ctc	tac	att	ggc	ttc	cga	ggc	ctg	gag	ggc	atg	cac	tgt	gcc	cca	act	1200	
Leu	Tyr	Ile	Gly	Phe	Arg	Gly	Leu	Glu	Gly	Met	His	Cys	Ala	Pro	Thr		
385					390					395					400		
cca	gac	tct	ggg	gtc	gta	tgt	ggc	cgt	ttg	gcc	tgc	tcc	cca	aca	cat	1248	
Pro	Asp	Ser	Gly	Val	Val	Cys	Gly	Arg	Leu	Ala	Cys	Ser	Pro	Thr	His		
				405					410					415			
gat	gag	cac	ctc	atg	cac	ggc	gca	cac	ccc	tct	gat	ggg	gac	ccc	gac	1296	
Asp	Glu	His	Leu	Met	His	Gly	Ala	His	Pro	Ser	Asp	Gly	Asp	Pro	Asp		
			420					425					430				

69

ctg gcc aag atc ctg gag gag gtc cgc tac atc gcc aac cgc ttc cgc	1344
Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg	
435 440 445	
tgc cag gac gag agt gag gtg atc tgc agt gaa tgg aag ttt gca gcc	1392
Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala	
450 455 460	
tgc gtg gtg gac cgc ttg tgc ctc atg gcc ttt tcg gtc ttt acc atc	1440
Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile	
465 470 475 480	
atc tgt acc atc ggc atc ctc atg tca gct cca aac ttt gtg gag gct	1488
Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala	
485 490 495	
gtg tcc aaa gac ttt gct taa	1509
Val Ser Lys Asp Phe Ala	
500	

<210> 37
 <211> 502
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 37

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

70

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
 180 185 190

Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys
 195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val
 210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Ser Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
 290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

71

Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
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<210> 38
 <211> 1509
 <212> DNA
 <213> Artificial Sequence

<220>
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 <222> (1)..(1509)

<220>
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 <222> (1)..(66)

<220>

<221> misc_feature
 <222> (784)..(843)
 <223> TM2 domain

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 1 5 10 15

ctg cac gtg tcc ctg caa ggc gag ttc cag agg agg ctg tac aag gag 96
 Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
 20 25 30

ctg gtc aag aac tac aac ccg ctg gag agg ccg gtg gcc aac gac tcg 144
 Leu Val Lys Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser
 35 40 45

cag ccg ctc acc gtg tac ttc tcc ctg agt ctc ctg cag atc atg gat 192
 Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
 50 55 60

gtg gat gag aag aac caa gtt tta acc acc aac att tgg cta caa atg 240
 Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
 65 70 75 80

tct tgg aca gat cac tat ttg cag tgg aac atg tct gag tac ccc gga 288
 Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
 85 90 95

gtg aag aat gtt cgt ttt cca gat ggc cag att tgg aaa cca gac att 336
 Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
 100 105 110

ctc ctc tat aac agt gct gat gag cgc ttt gat gcc acg ttc cac acc 384
 Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

aat gtt ttg gtg aat gca tct ggg cat tgc cag tat ctc cct cca ggc 432
 Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

ata ttc aag agc tcc tgc tac att gac gtt cgc tgg ttc cct ttt gat 480
 Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

gtg cag cag tgc aaa ctg aag ttt ggg tcc tgg tcc tat gga ggg tgg 528
 Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

tca ctg gac ctg caa atg caa gag gca gat atc agc agc tat atc ccc 576
 Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
 180 185 190

aac gga gaa tgg gat ctc atg gga atc cct ggc aaa agg aat gag aag 624
 Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys
 195 200 205

ttc tat gag tgc tgc aaa gag cca tac cca gat gtc acc tac aca gta 672
 Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val

210	215	220	
acc atg cgc cgt agg aca ctc tac tat ggc ctc aat ctg ctc atc cct			720
Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro			
225	230	235	240
tgt gta ctc att tca gcc ctg gct ctg ctg gta ttc ttg ctg cct gca			768
Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala			
	245	250	255
gac tct gga gag aaa atc tct ctt gga ata tcg gtc tta ctt tct ctg			816
Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Ser Val Leu Leu Ser Leu			
	260	265	270
act gtc ttc atg ctg ctt gtg gct gag atc atg cca gca aca tct gat			864
Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp			
	275	280	285
tct gtg ccc ttg ata gca caa tac ttc gcc agc acc atg atc atc gtg			912
Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val			
	290	295	300
ggc ctc tct gta gtg gtg aca gtg att gtg ctg aga tat cac cac cat			960
Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His			
305	310	315	320
gac cct gat ggt ggc aaa atg cct aag tgg acc aga atc att ctc ctg			1008
Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu			
	325	330	335
aac tgg tgt gca tgg ttt ctg cgc atg aag agg ccc gga gag gac aag			1056
Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys			
	340	345	350
gtg cgg cca gct tgt cag cac aag cct cgg cgc tgc agc ctg gcc agt			1104
Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser			
	355	360	365
gtg gag ctg agt gca ggt gct ggg cca ccc acc agc aat ggc aac ctg			1152
Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu			
	370	375	380
ctc tac att ggc ttc cga ggc ctg gag ggc atg cac tgt gcc cca act			1200
Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr			
385	390	395	400
cca gac tct ggg gtc gta tgt ggc cgt ttg gcc tgc tcc cca aca cat			1248
Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His			
	405	410	415
gat gag cac ctc atg cac ggt gca cac ccc tct gat ggg gac ccc gac			1296
Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp			
	420	425	430
ctg gcc aag atc ctg gag gag gtc cgc tac atc gcc aac cgc ttc cgc			1344
Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg			
	435	440	445
tgc cag gac gag agt gag gtg atc tgc agt gaa tgg aag ttt gca gcc			1392

74

Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

tgc gtg gtg gac cgc ttg tgc ctc atg gcc ttt tcg gtc ttt acc atc 1440
 Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

atc tgt acc atc ggc atc ctc atg tca gct cca aac ttt gtg gag gct 1488
 Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

gtg tcc aaa gac ttt gct taa 1509
 Val Ser Lys Asp Phe Ala
 500

<210> 39
 <211> 502
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 39

Met Cys Gly Gly Arg Gly Gly Ile Trp Leu Ala Leu Ala Ala Ala Leu
 1 5 10 15

Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
 20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
 50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
 65 70 75 80

Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
 85 90 95

Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
 100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly

75

130		135		140
Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp				
145		150	155	160
Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp				
	165		170	175
Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro				
	180	185	190	
Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys				
	195	200	205	
Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val				
	210	215	220	
Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro				
225		230	235	240
Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala				
	245	250	255	
Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Ser Val Leu Leu Ser Leu				
	260	265	270	
Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp				
	275	280	285	
Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val				
	290	295	300	
Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His				
305		310	315	320
Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu				
	325	330	335	
Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys				
	340	345	350	
Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser				
	355	360	365	

76

Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
 500

<210> 40
 <211> 1509
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> degenerate variant encoding T6'S mutant alpha7 subunit

<220>
 <221> CDS
 <222> (1)..(1509)

<220>
 <221> sig_peptide
 <222> (1)..(66)

<220>
 <221> misc_feature
 <222> (784)..(843)
 <223> TM2 domain

<400> 40

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Met Cys Gly Gly Arg Gly Gly Ile Trp Leu Ala Leu Ala Ala Leu	
1 5 10 15	
ctg cac gtg tcc ctg caa ggc gag ttc cag agg agg ctg tac aag gag	96
Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu	
20 25 30	
ctg gtc aag aac tac aac ccg ctg gag agg ccg gtg gcc aac gac tcg	144
Leu Val Lys Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser	
35 40 45	
cag ccg ctc acc gtg tac ttc tcc ctg agt ctc ctg cag atc atg gat	192
Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp	
50 55 60	
gtg gat gag aag aac caa gtt tta acc acc aac att tgg cta caa atg	240
Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met	
65 70 75 80	
tct tgg aca gat cac tat ttg cag tgg aac atg tct gag tac ccc gga	288
Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly	
85 90 95	
gtg aag aat gtt cgt ttt cca gat ggc cag att tgg aaa cca gac att	336
Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile	
100 105 110	
ctc ctc tat aac agt gct gat gag cgc ttt gat gcc acg ttc cac acc	384
Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr	
115 120 125	
aat gtt ttg gtg aat gca tct ggg cat tgc cag tat ctc cct cca ggc	432
Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly	
130 135 140	
ata ttc aag agc tcc tgc tac att gac gtt cgc tgg ttc cct ttt gat	480
Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp	
145 150 155 160	
gtg cag cag tgc aaa ctg aag ttt ggg tcc tgg tcc tat gga ggg tgg	528
Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp	
165 170 175	
tca ctg gac ctg caa atg caa gag gca gat atc agc agc tat atc ccc	576
Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro	
180 185 190	
aac gga gaa tgg gat ctc atg gga atc cct ggc aaa agg aat gag aag	624
Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys	
195 200 205	
ttc tat gag tgc tgc aaa gag cca tac cca gat gtc acc tac aca gta	672
Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val	
210 215 220	
acc atg cgc cgt agg aca ctc tac tat ggc ctc aat ctg ctc atc cct	720

78

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro	
225 230 235 240	
tgt gta ctc att tca gcc ctg gct ctg ctg gta ttc ttg ctg cct gca	768
Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala	
245 250 255	
gac tct gga gag aaa atc tct ctt gga ata agt gtc tta ctt tct ctg	816
Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Ser Val Leu Leu Ser Leu	
260 265 270	
act gtc ttc atg ctg ctt gtg gct gag atc atg cca gca aca tct gat	864
Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp	
275 280 285	
tct gtg ccc ttg ata gca caa tac ttc gcc agc acc atg atc atc gtg	912
Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val	
290 295 300	
ggc ctc tct gta gtg gtg aca gtg att gtg ctg aga tat cac cac cat	960
Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His	
305 310 315 320	
gac cct gat ggt ggc aaa atg cct aag tgg acc aga atc att ctc ctg	1008
Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu	
325 330 335	
aac tgg tgt gca tgg ttt ctg cgc atg aag agg ccc gga gag gac aag	1056
Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys	
340 345 350	
gtg cgg cca gct tgt cag cac aag cct cgg cgc tgc agc ctg gcc agt	1104
Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser	
355 360 365	
gtg gag ctg agt gca ggt gct ggg cca ccc acc agc aat ggc aac ctg	1152
Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu	
370 375 380	
ctc tac att ggc ttc cga ggc ctg gag ggc atg cac tgt gcc cca act	1200
Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr	
385 390 395 400	
cca gac tct ggg gtc gta tgt ggc cgt ttg gcc tgc tcc cca aca cat	1248
Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His	
405 410 415	
gat gag cac ctc atg cac ggt gca cac ccc tct gat ggg gac ccc gac	1296
Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp	
420 425 430	
ctg gcc aag atc ctg gag gag gtc cgc tac atc gcc aac cgc ttc cgc	1344
Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg	
435 440 445	
tgc cag gac gag agt gag gtg atc tgc agt gaa tgg aag ttt gca gcc	1392
Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala	

79

450	455	460	
tgc gtg gtg gac cgc ttg tgc ctc atg gcc ttt tcg gtc ttt acc atc			1440
Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile			
465	470	475	480
atc tgt acc atc ggc atc ctc atg tca gct cca aac ttt gtg gag gct			1488
Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala			
	485	490	495
gtg tcc aaa gac ttt gct taa			1509
Val Ser Lys Asp Phe Ala			
	500		

<210> 41
 <211> 502
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 41

Met Cys Gly Gly Arg Gly Gly Ile Trp Leu Ala Leu Ala Ala Ala Leu
1 5 10 15

Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
65 70 75 80

Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
85 90 95

Val Lys Asn Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
115 120 125

Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
130 135 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
 180 185 190

Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys
 195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val
 210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Ser Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
 290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu

81

370 375 380
 Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr
 385 390 395 400
 Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His
 405 410 415
 Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp
 420 425 430
 Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445
 Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460
 Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480
 Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495
 Val Ser Lys Asp Phe Ala
 500

<210> 42
 <211> 1509
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> degenerate variant encoding T6'S mutant alpha7 subunit

<220>
 <221> CDS
 <222> (1) .. (1509)

<220>
 <221> sig_peptide
 <222> (1) .. (66)

<220>
 <221> misc_feature
 <222> (784) .. (843)
 <223> TM2 domain

<400> 42
 atg tgc ggc ggg cgg gga ggc atc tgg ctg gct ctg gcc gcg gcg ctg

48

82

Met	Cys	Gly	Gly	Arg	Gly	Gly	Ile	Trp	Leu	Ala	Leu	Ala	Ala	Ala	Leu	
1				5					10					15		
ctg	cac	gtg	tcc	ctg	caa	ggc	gag	ttc	cag	agg	agg	ctg	tac	aag	gag	96
Leu	His	Val	Ser	Leu	Gln	Gly	Glu	Phe	Gln	Arg	Arg	Leu	Tyr	Lys	Glu	
			20					25					30			
ctg	gtc	aag	aac	tac	aac	ccg	ctg	gag	agg	ccg	gtg	gcc	aac	gac	tcg	144
Leu	Val	Lys	Asn	Tyr	Asn	Pro	Leu	Glu	Arg	Pro	Val	Ala	Asn	Asp	Ser	
		35					40					45				
cag	ccg	ctc	acc	gtg	tac	ttc	tcc	ctg	agt	ctc	ctg	cag	atc	atg	gat	192
Gln	Pro	Leu	Thr	Val	Tyr	Phe	Ser	Leu	Ser	Leu	Leu	Gln	Ile	Met	Asp	
	50					55					60					
gtg	gat	gag	aag	aac	caa	ggt	tta	acc	acc	aac	att	tgg	cta	caa	atg	240
Val	Asp	Glu	Lys	Asn	Gln	Val	Leu	Thr	Thr	Asn	Ile	Trp	Leu	Gln	Met	
65				70						75				80		
tct	tgg	aca	gat	cac	tat	ttg	cag	tgg	aac	atg	tct	gag	tac	ccc	gga	288
Ser	Trp	Thr	Asp	His	Tyr	Leu	Gln	Trp	Asn	Met	Ser	Glu	Tyr	Pro	Gly	
				85					90					95		
gtg	aag	aat	ggt	cgt	ttt	cca	gat	ggc	cag	att	tgg	aaa	cca	gac	att	336
Val	Lys	Asn	Val	Arg	Phe	Pro	Asp	Gly	Gln	Ile	Trp	Lys	Pro	Asp	Ile	
			100					105					110			
ctc	ctc	tat	aac	agt	gct	gat	gag	cgc	ttt	gat	gcc	acg	ttc	cac	acc	384
Leu	Leu	Tyr	Asn	Ser	Ala	Asp	Glu	Arg	Phe	Asp	Ala	Thr	Phe	His	Thr	
		115					120					125				
aat	ggt	ttg	gtg	aat	gca	tct	ggg	cat	tgc	cag	tat	ctc	cct	cca	ggc	432
Asn	Val	Leu	Val	Asn	Ala	Ser	Gly	His	Cys	Gln	Tyr	Leu	Pro	Pro	Gly	
	130					135					140					
ata	ttc	aag	agc	tcc	tgc	tac	att	gac	ggt	cgc	tgg	ttc	cct	ttt	gat	480
Ile	Phe	Lys	Ser	Ser	Cys	Tyr	Ile	Asp	Val	Arg	Trp	Phe	Pro	Phe	Asp	
145				150						155					160	
gtg	cag	cag	tgc	aaa	ctg	aag	ttt	ggg	tcc	tgg	tcc	tat	gga	ggg	tgg	528
Val	Gln	Gln	Cys	Lys	Leu	Lys	Phe	Gly	Ser	Trp	Ser	Tyr	Gly	Gly	Trp	
			165					170					175			
tca	ctg	gac	ctg	caa	atg	caa	gag	gca	gat	atc	agc	agc	tat	atc	ccc	576
Ser	Leu	Asp	Leu	Gln	Met	Gln	Glu	Ala	Asp	Ile	Ser	Ser	Tyr	Ile	Pro	
			180					185					190			
aac	gga	gaa	tgg	gat	ctc	atg	gga	atc	cct	ggc	aaa	agg	aat	gag	aag	624
Asn	Gly	Glu	Trp	Asp	Leu	Met	Gly	Ile	Pro	Gly	Lys	Arg	Asn	Glu	Lys	
		195					200					205				
ttc	tat	gag	tgc	tgc	aaa	gag	cca	tac	cca	gat	gtc	acc	tac	aca	gta	672
Phe	Tyr	Glu	Cys	Cys	Lys	Glu	Pro	Tyr	Pro	Asp	Val	Thr	Tyr	Thr	Val	
	210					215					220					
acc	atg	cgc	cgt	agg	aca	ctc	tac	tat	ggc	ctc	aat	ctg	ctc	atc	cct	720
Thr	Met	Arg	Arg	Arg	Thr	Leu	Tyr	Tyr	Gly	Leu	Asn	Leu	Leu	Ile	Pro	
225					230					235					240	

tgt gta ctc att tca gcc ctg gct ctg ctg gta ttc ttg ctg cct gca	768
Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala	
245 250 255	
gac tct gga gag aaa atc tct ctt gga ata agc gtc tta ctt tct ctg	816
Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Ser Val Leu Leu Ser Leu	
260 265 270	
act gtc ttc atg ctg ctt gtg gct gag atc atg cca gca aca tct gat	864
Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp	
275 280 285	
tct gtg ccc ttg ata gca caa tac ttc gcc agc acc atg atc atc gtg	912
Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val	
290 295 300	
ggc ctc tct gta gtg gtg aca gtg att gtg ctg aga tat cac cac cat	960
Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His	
305 310 315 320	
gac cct gat ggt ggc aaa atg cct aag tgg acc aga atc att ctc ctg	1008
Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu	
325 330 335	
aac tgg tgt gca tgg ttt ctg cgc atg aag agg ccc gga gag gac aag	1056
Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys	
340 345 350	
gtg cgg cca gct tgt cag cac aag cct cgg cgc tgc agc ctg gcc agt	1104
Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser	
355 360 365	
gtg gag ctg agt gca ggt gct ggg cca ccc acc agc aat ggc aac ctg	1152
Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu	
370 375 380	
ctc tac att ggc ttc cga ggc ctg gag ggc atg cac tgt gcc cca act	1200
Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr	
385 390 395 400	
cca gac tct ggg gtc gta tgt ggc cgt ttg gcc tgc tcc cca aca cat	1248
Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His	
405 410 415	
gat gag cac ctc atg cac ggt gca cac ccc tct gat ggg gac ccc gac	1296
Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp	
420 425 430	
ctg gcc aag atc ctg gag gag gtc cgc tac atc gcc aac cgc ttc cgc	1344
Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg	
435 440 445	
tgc cag gac gag agt gag gtg atc tgc agt gaa tgg aag ttt gca gcc	1392
Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala	
450 455 460	
tgc gtg gtg gac cgc ttg tgc ctc atg gcc ttt tcg gtc ttt acc atc	1440
Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile	
465 470 475 480	

atc tgt acc atc ggc atc ctc atg tca gct cca aac ttt gtg gag gct 1488
 ile Cys Thr ile Gly ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

gtg tcc aaa gac ttt gct taa 1509
 Val Ser Lys Asp Phe Ala
 500

<210> 43
 <211> 502
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 43

Met Cys Gly Gly Arg Gly Gly ile Trp Leu Ala Leu Ala Ala Ala Leu
 1 5 10 15

Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Arg Leu Tyr Lys Glu
 20 25 30

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

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln ile Met Asp
 50 55 60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn ile Trp Leu Gln Met
 65 70 75 80

Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Met Ser Glu Tyr Pro Gly
 85 90 95

Val Lys Asn Val Arg Phe Pro Asp Gly Gln ile Trp Lys Pro Asp ile
 100 105 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
 115 120 125

Asn Val Leu Val Asn Ala Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
 130 135 140

Ile Phe Lys Ser Ser Cys Tyr ile Asp Val Arg Trp Phe Pro Phe Asp
 145 150 155 160

Val Gln Gln Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
 165 170 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Ser Tyr Ile Pro
 180 185 190

Asn Gly Glu Trp Asp Leu Met Gly Ile Pro Gly Lys Arg Asn Glu Lys
 195 200 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Tyr Thr Val
 210 215 220

Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
 225 230 235 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
 245 250 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Ser Val Leu Leu Ser Leu
 260 265 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
 275 280 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
 290 295 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Arg Tyr His His His
 305 310 315 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Ile Ile Leu Leu
 325 330 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
 340 345 350

Val Arg Pro Ala Cys Gln His Lys Pro Arg Arg Cys Ser Leu Ala Ser
 355 360 365

Val Glu Leu Ser Ala Gly Ala Gly Pro Pro Thr Ser Asn Gly Asn Leu
 370 375 380

Leu Tyr Ile Gly Phe Arg Gly Leu Glu Gly Met His Cys Ala Pro Thr
 385 390 395 400

Pro Asp Ser Gly Val Val Cys Gly Arg Leu Ala Cys Ser Pro Thr His
 405 410 415

Asp Glu His Leu Met His Gly Ala His Pro Ser Asp Gly Asp Pro Asp
 420 425 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
 435 440 445

Cys Gln Asp Glu Ser Glu Val Ile Cys Ser Glu Trp Lys Phe Ala Ala
 450 455 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
 465 470 475 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
 485 490 495

Val Ser Lys Asp Phe Ala
 500

<210> 44
 <211> 60
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> degenerate variant encoding T6'S mutant TM2 domain

<400> 44
 atttccctgg ggatatctgt cttactctct cttaccgtct tcatgctgct cgtggctgag 60

<210> 45
 <211> 60
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> degenerate variant encoding T6'S mutant TM2 domain

<400> 45
 atttccctgg ggatatccgt cttactctct cttaccgtct tcatgctgct cgtggctgag 60

<210> 46
 <211> 60
 <212> DNA
 <213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 46
atttccctgg ggatatcagt cttactctct cttaccgtct tcatgctgct cgtggctgag 60

<210> 47
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 47
atttccctgg ggatatcgggt cttactctct cttaccgtct tcatgctgct cgtggctgag 60

<210> 48
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 48
atttccctgg ggataagtgt cttactctct cttaccgtct tcatgctgct cgtggctgag 60

<210> 49
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 49
atttccctgg ggataagcgt cttactctct cttaccgtct tcatgctgct cgtggctgag 60

<210> 50
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 50
atttccctgg ggatatctgt cttactctct ctcactgtct tcatgctgct cgtggctgag 60

<210> 51
<211> 60

<212> DNA
<213> Artificial Sequence

<220>

<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 51

atttccctgg ggatatccgt cttactctct ctcactgtct tcatgctgct cgtggctgag 60

<210> 52

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 52

atttccctgg ggataccagt cttactctct ctcactgtct tcatgctgct cgtggctgag 60

<210> 53

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 53

atttccctgg ggataccggt cttactctct ctcactgtct tcatgctgct cgtggctgag 60

<210> 54

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 54

atttccctgg ggataagtgt cttactctct ctcactgtct tcatgctgct cgtggctgag 60

<210> 55

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 55

atttccctgg ggataagcgt cttactctct ctcactgtct tcatgctgct cgtggctgag 60

<210> 56

<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 56
atctccctgg gaatctctgt tttactctcc ctcaccgtct tcatgctgct ggtagctgag 60

<210> 57
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 57
atctccctgg gaatctccgt tttactctcc ctcaccgtct tcatgctgct ggtagctgag 60

<210> 58
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 58
atctccctgg gaatctcagt tttactctcc ctcaccgtct tcatgctgct ggtagctgag 60

<210> 59
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 59
atctccctgg gaatctcggg tttactctcc ctcaccgtct tcatgctgct ggtagctgag 60

<210> 60
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 60
atctccctgg gaatcagtggt tttactctcc ctcaccgtct tcatgctgct ggtagctgag 60

<210> 61
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 61
atctccctgg gaatcagcgt tttactctcc ctcaccgtct tcatgctgct ggtagctgag 60

<210> 62
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 62
atctcactag gtatatctgt tttattgtct ctcaccgtct tcatgttact cgtggctgaa 60

<210> 63
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 63
atctcactag gtatatccgt tttattgtct ctcaccgtct tcatgttact cgtggctgaa 60

<210> 64
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 64
atctcactag gtatatcagt tttattgtct ctcaccgtct tcatgttact cgtggctgaa 60

<210> 65
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 65
atctcactag gtatatcggt tttattgtct ctcaccgtct tcatgttact cgtggctgaa 60

<210> 66
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 66
atctcactag gtataagtgt tttattgtct ctcaccgtct tcatgttact cgtggctgaa 60

<210> 67
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 67
atctcactag gtataagcgt tttattgtct ctcaccgtct tcatgttact cgtggctgaa 60

<210> 68
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 68
atctcgctcg ggatctctgt cctgctctcg ctgacagttt tcatgctttt ggtagcagag 60

<210> 69
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 69
atctcgctcg ggatctccgt cctgctctcg ctgacagttt tcatgctttt ggtagcagag 60

<210> 70
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 70

atctcgctcg ggatctcagt cctgctctcg ctgacagttt tcatgctttt ggtagcagag 60

<210> 71
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 71
atctcgctcg ggatctcggg cctgctctcg ctgacagttt tcatgctttt ggtagcagag 60

<210> 72
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 72
atctcgctcg ggatcagtggt cctgctctcg ctgacagttt tcatgctttt ggtagcagag 60

<210> 73
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> degenerate variant encoding T6'S mutant TM2 domain

<400> 73
atctcgctcg ggatcagcgt cctgctctcg ctgacagttt tcatgctttt ggtagcagag 60