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(54) Title: COMPOSITIONS USEFUL FOR TREATMENT OF CHARCOT-MARIE-TOOTH DISEASE

(57) Abstract: Provided herein are rAAV and other vectors and compositions useful for treating a patient having CMT2 comprising:

(a) a recombinant nucleic acid sequence encoding an engineered human mitofusin 2 coding sequence operably linked to regulatory sequences which direct expression thereof in a human target cell. Also provided are rAAV and other vectors and compositions useful for treating a patient having CMT2 comprising: (b) a nucleic acid sequence encoding at least one miRNA specific for an endogenous human mitofusin 2 sequence in a human CMT2A subject, wherein the miRNA coding sequence is operably linked to regulatory sequences which direct expression thereof in the subject. Further provided are compositions containing both the engineered hMfn2 coding sequence and the at least one miRNA coding sequence, wherein the engineered human mitofusin 2 coding sequence has a sequence which differs from endogenous human mitofusin 2 in the CMT2A patient in the target site of the encoded miRNA.



COMPOSITIONS USEFUL FOR TREATMENT OF CHARCOT-MARIE-TOOTH DISEASE

BACKGROUND OF THE INVENTION

5 Charcot-Marie-Tooth (CMT) neuropathy is a heterogeneous group of inherited diseases found in peripheral nerves. CMT is a common disorder affecting both children and adults. Charcot-Marie Tooth disease (CMT) or hereditary motor and sensory neuropathy (HMSN) are the most commonly used names for inherited neuropathies that are not part of a syndrome (Klein, C. J., Duan, X., Shy, M. E., 2013. Inherited neuropathies: Clinical overview
10 and update. *Muscle Nerve*; Bassam, B., 2014. Charcot-Marie-Tooth Disease Variants—Classification, Clinical, and Genetic Features and Rational Diagnostic Evaluation. *J. Clin. Neuromusc. Dis.* 15, 117-128; Scherer, S. S., Shy, M. E., 2015. CMT Subtypes and Disease Burden in Patients Enrolled in the INC Natural History Study (6601) from 2009-2013. *J. Neurol. Neurosurg. Psychiat.* 86, 873-878).

15 The dominantly inherited axonal forms of CMT neuropathy (type 2 CMT disorders) have normal or slightly reduced velocities with reduced motor and sensory compound action potential amplitudes, and axonal loss is the chief finding in biopsied nerves. Dominant mutations in the Mitofusin2 (MFN2) gene, a protein required for mitochondrial fusion and transport along axons, cause CMT2A (Zuchner, S., et al., 2004. Mutations in the
20 mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat Genet* 36, 449-451). CMT2A is estimated to cause up to 7% of all CMT, and is the most common form of type 2 CMT (Freidman, V., et al., 2015. CMT Subtypes and Disease Burden in Patients Enrolled in the INC Natural History Study (6601) from 2009-2013. *J. Neurol. Neurosurg. Psychiat.* 86, 873-878). Different MFN2 mutations cause different degrees of
25 neuropathy.

Most MFN2 mutations cause a severe, early onset, axonal neuropathy, and are de novo mutations. Other MFN2 dominant mutations cause a milder axonal neuropathy with a later onset (Lawson, V. H., et al, 2005. Clinical and electrophysiologic features of CMT2A with mutations in the mitofusin 2 gene. *Neurology* 65, 197-204; Chung, K. W., et al., 2006. Early
30 onset severe and late-onset mild Charcot-Marie-Tooth disease with mitofusin 2 (MFN2) mutations. *Brain* 129, 2103-2118; Verhoeven, K., et al., 2006. MFN2 mutation distribution and genotype/phenotype correlation in Charcot-Marie-Tooth type 2. *Brain* 129, 2093-2102;

Feely, S. M. E., et al., 2011, MFN2 mutations cause severe phenotypes in most patients with CMT2A. *Neurology* 76, 1690-1696). More rarely, recessive MFN2 mutations may cause severe, early onset, axonal neuropathy. Mfn2 mutations selectively toxic to lower motor neurons and primary sensory neurons in dorsal root ganglia (DRG).

5 Some dominant mutations in MFN2 produce myelopathy or optic atrophy; these complicated forms of CMT2 are sometimes referred to HMSN-V and HMSN-VI, respectively, but are not only caused by MFN2 mutations (Scherer, S. S., et al., 2015. *Peripheral neuropathies in Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease*, 5th ed. Elsevier, Philadelphia, pp. 1051-1074).

10 Mammals have two mitofusin genes, Mfn1 and Mfn2, which have distinct but overlapping distributions, and both of which can promote mitochondrial fusion through trans-interactions (Chen, H., Chan, D. C., 2005. Emerging functions of mammalian mitochondrial fusion and fission. *Hum. Mol. Genet.* 14, R283-R289). Nearly all of the mutations in the MFN2 gene cause amino acid substitutions as single point mutations, including but not
15 restricted to the GTPase domain (Cartoni, R., Martinou, J. C., 2009. Role of mitofusin 2 mutations in the physiopathology of Charcot-Marie-Tooth disease type 2A. *Exp. Neurol.* 218, 268-273). Because loss-of-function mutations of MFN2 also cause a severe axonal neuropathy (Nicholson, G. A., et al, 2008. Severe early-onset axonal neuropathy with homozygous and compound heterozygous MFN2 mutations. *Neurology* 70, 1678-1681), and
20 result in reduced mitochondrial fusion (Chen, H., Chan, D. C., 2005. Emerging functions of mammalian mitochondrial fusion and fission. *Hum. Mol. Genet.* 14, R283-R289), it is possible that dominant MFN2 mutations have a dominant-negative effect on mitochondrial fusion (Detmer, S. A., Chan, D. C., 2007. Complementation between mouse Mfn1 and Mfn2 protects mitochondrial fusion defects caused by CMT2A disease mutations. *J. Cell Biol.* 176, 405-414;
25 Baloh, R. H., et al., 2007. Altered axonal mitochondrial transport in the pathogenesis of Charcot-Marie-Tooth disease from mitofusin 2 mutations. *J Neurosci* 27, 422-430; Misko, A., et al., 2010. Mitofusin 2 is necessary for transport of axonal mitochondria and interacts with the Miro/Milton complex. *J Neurosci* 30, 4232-4240; Misko, A. L., et al., 2012. Mitofusin2 mutations disrupt axonal mitochondrial positioning and promote axon degeneration. *J*
30 *Neurosci* 32, 4145-4155).

What are needed are treatments useful for reducing the symptoms, severity and/or progression of CMT2A and related disorders.

Summary of the Invention

5 Viral and non-viral vectors and compositions useful for treating patients having symptoms associated with defects in human mitofusin 2 expression and/or patients having CMT2A are provided herein.

 In certain embodiments, a recombinant adeno-associated virus (rAAV) comprising an AAV capsid and a vector genome is provided. The rAAV comprises: (a) an engineered
10 nucleic acid sequence encoding human mitofusin 2; (b) a spacer sequence located between (a) and (c); (c) a nucleic acid sequence encoding at least one miRNA sequence specific for endogenous human mitofusin 2 in a CMT2 patient located 3' to the sequence of (a) and (b); wherein the engineered nucleic acid sequence of (a) lacks the target site for the encoded at least one miRNA, thereby preventing the encoded miRNA from targeting the engineered
15 human mitofusin 2 coding sequence; and (c) regulatory sequences operably linked to (a) and (c). In certain embodiments, the AAV capsid is selected from AAV9, AAVhu68, AAV1 or AAVrh91. In certain embodiments, the spacer is 75 nucleotides to about 250 nucleotides in length. In one aspect, a vector is provided which comprises an engineered human mitofusin 2 coding sequence operably linked to regulatory sequences which direct expression thereof in a
20 human target cell. In certain embodiments, a vector is provided which comprises a nucleic acid sequence encoding at least one hairpin miRNA, wherein the encoded miRNA is specific for endogenous human mitofusin 2 in a human subject operably linked to regulatory sequences which direct expression thereof in the subject. In certain embodiments, a vector or other composition comprises both the engineered human mitofusin 2 coding sequence and the at least one miRNA coding sequence. In such an embodiment, the engineered mitofusin 2
25 coding sequence lacks the target site for the at least one miRNA, thereby preventing the miRNA from targeting the engineered human mitofusin 2 coding sequence.

 In certain embodiments, the vector is a replication-defective viral vector which comprises a vector genome comprising the human mitofusin 2 coding sequences, the coding
30 sequence for the at least one miRNA and the regulatory sequences. In certain embodiments, the viral vector is a recombinant adeno-associated virus (rAAV) particle having an AAV

capsid which has the packaged therein the vector genome. In certain embodiments, the AAV capsid is AAVhu68, AAV1 or AAVrh91.

In certain embodiments, a vector is provided which comprises a engineered mitofusin 2 coding sequence has the nucleic acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto, provided that the nucleic acid sequences targeted by the encoded miRNA are different from the endogenous human mitofusin 2 sequence.

In certain embodiments, a vector is provided which comprises a nucleic acid sequence comprising at least one miRNA coding sequence which comprises one or more of: (a) an miRNA coding sequence comprising SEQ ID NO 15 (miR1693, 64 nt); (b) an miRNA coding sequence comprising at least 60 consecutive nucleotides of SEQ ID NO: 15; (c) an miRNA coding sequence comprising at least 99% identity to SEQ ID NO: 15 which comprises a sequence with 100% identity to about nucleotide 6 to about nucleotide 26 of SEQ ID NO: 15 (or SEQ ID NO: 68); (d) an miRNA coding sequence comprising one or more of:

(i) TTGACGTCCAGAACCTGTTCT, SEQ ID NO: 27; (ii) AGAAGTGGGCACTTAGAGTTG, SEQ ID NO: 28; (iii) TTCAGAAGTGGGCACTTAGAG, SEQ ID NO: 29; (iv) TTGTCAATCCAGCTGTCCAGC, SEQ ID NO: 30; (v) CAAACTTGGTCTTCACTGCAG, SEQ ID NO: 31 ; (vi) AAACCTTGAGGACTACTGGAG, SEQ ID NO: 32; (vii) TAACCATGGAAACCATGAACT, SEQ ID NO: 33; (viii) ACAACAAGAATGCCCATGGAG, SEQ ID NO: 34; (ix) AAAGGTCCCAGACAGTTCCTG, SEQ ID NO: 35; (x) TGTTTCATGGCGGCAATTCCT, SEQ ID NO: 36; (xi) TGAGGTTGGCTATTGATTGAC, SEQ ID NO: 37; (xii) TTCTCACACAGTCAACACCTT, SEQ ID NO: 38; (xiii) TTTCTCGCAGTAAACCTGCT, SEQ ID NO: 39; (xiv) AGAAATGGAATCAATGTCTT, SEQ ID NO: 40; (xv) TGAACAGGACATCACCTGTGA, SEQ ID NO: 41; (xvi) AATACAAGCAGGTATGTGAAC, SEQ ID NO: 42; (xvii) TAAACCTGCTGCTCCCGAGCC, SEQ ID NO: 43; (xviii) TAGAGGAGGCCATAGAGCCCA, SEQ ID NO: 44; (xix) TCTACCCGCAGGAAGCAATTG, SEQ ID NO: 45; or (xx) CTCCTTAGCAGACACAAAGAA, SEQ ID NO: 46, or combinations of any of (i) through (xx). In certain embodiments, a single nucleic acid molecule comprises both the human

mitofusin 2 coding sequence and the miRNA coding sequence and the nucleic acid molecule further comprises a spacer of at least 75 nucleotides between the hMfn2 coding sequence and the coding sequence at least one miRNA. In certain embodiments, the vector is a non-viral vector.

5 In certain embodiments, the composition comprises a recombinant nucleic acid sequence encoding an engineered human mitofusin 2 coding sequence operably linked to regulatory sequences which direct expression thereof in a human target cell and a nucleic acid sequence encoding at least one miRNA specific for endogenous human mitofusin 2 in a CMT2A patient operably linked to regulatory sequences which direct expression thereof in the
10 subject, wherein the engineered mitofusin 2 coding sequence lacks a target site for the encoded at least one miRNA, thereby preventing the miRNA from targeting the engineered human mitofusin 2 coding sequence.

 In certain embodiments, a pharmaceutical composition comprising the vector, rAAV, or a composition, and a pharmaceutically acceptable aqueous suspending liquid, excipient,
15 and/or diluent.

 In certain embodiments, a method for treating a patient having Charcot-Marie-Tooth (CMT) neuropathy is provided comprising delivering an effective amount of the vector, a recombinant AAV, or a composition to a patient in need thereof. In certain embodiments, a method for reducing neuropathy in a patient having Charcot-Marie-Tooth (CMT) neuropathy
20 is provided which comprises delivering an effective amount of the vector, a recombinant AAV, or a composition to a patient in need thereof. In certain embodiments, a method for treating a patient is provided, wherein the method additionally comprising a combination with one or more co-therapies selected from: acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), tricyclic antidepressants or antiepileptic drugs, such as carbamazepine or
25 gabapentin.

 In certain embodiments, a combination regimen for treating a patient having CMT2A is provided which comprises co-administering (a) a recombinant nucleic acid sequence encoding an engineered human mitofusin 2 coding sequence operably linked to regulatory sequences which direct expression thereof in a human target cell, wherein the human
30 mitofusin 2 coding sequence has the sequence of SEQ ID NO: 11 or a sequence at least 95% identical thereto and which differs from endogenous human mitofusin 2 in the CMT2A patient

by having a mismatch in the miRNA target sequence of (b), (b) at least one miRNA specific for an endogenous human mitofusin 2 sequence in a human CMT2A subject, wherein the mRNA is operably linked to regulatory sequences which direct expression thereof in the subject.

5 In certain embodiments, a combination regimen for treating a patient having CMT2A is provided which comprises co-administering: (a) a recombinant nucleic acid sequence encoding an engineered human mitofusin 2 coding sequence operably linked to regulatory sequences which direct expression thereof in a human target cell, wherein the human mitofusin 2 coding sequence is engineered to differs from endogenous human mitofusin 2 in
10 the CMT2A patient by having a mismatch in the miRNA target sequence of (b), (b) at least one miRNA specific for an endogenous human mitofusin 2 sequence in a human CMT2A subject, wherein the miRNA is operably linked to regulatory sequences which direct expression thereof in the subject. In certain embodiments, a first vector comprises the nucleic acid (a) and a second, different vector, comprises at least one miRNA (b). In certain
15 embodiments, the first vector and/or the second vector are each a viral vector which may be same or different. In certain embodiments, the first and/or second vector are a non-viral vector.

These and other advantages will be apparent from the Detailed Description of the Invention which follows.

20

Brief Description of the Drawings

FIGs 1A to 1B illustrate mitofusin 2 (Mfn2) miRNA selection (knockdown of endogenous Mfn2 with various miRNA). FIG. 1A illustrates knockdown of endogenous Mfn2 RNA, as measured by qPCR, in mouse brain in B6 mice following intravenous delivery of
25 AAV-mediated delivery of miRNA. FIG. 1B illustrates knock down of endogenous mfn2 RNA, as measured by qPCR, in mouse spinal cord in B6 mice following intravenous delivery of AAV-mediated delivery of miRNA.

FIG 2A to FIG 2C illustrate Mfn2 RNA fold expression following delivery AAV vector comprising Mfn2 cDNA transgene (i.e., engineered nucleic acid sequence encoding
30 Mfn2) and miR1518, wherein AAV vector was administered in mice intravenously at a dose of 3×10^{11} GC. FIG. 2A illustrates mouse Mfn (mMfn2) RNA fold expression in spinal cord.

FIG. 2B illustrates rat Mfn engineered (rMfn2co) RNA fold expression in spinal cord. FIG. 2C illustrates miR1518 RNA fold expression in spinal cord.

FIG 3 illustrates plotted quantitation of western blot signal measuring percent expression of mitofurin-2 protein following miRNA treatment of B104 rat cells. Mitofurin-2
5 expression is plotted from calculated value of percent total over loading control of beta-actin.

FIG 4 illustrates plotted quantitation of fold-expression of rat Mfn2 (rMfn2) cDNA expression in spinal cord of treated mice following AAV vector delivery of engineered rMfn2 cDNA transgene with miR1518.

FIG 5 illustrates plotted quantitation of fold-expression of human Mfn2 (hMfn2)
10 cDNA expression in spinal cord of treated mice following AAV vector delivery of engineered hMfn2 cDNA transgene with miR1693.

FIG 6A and FIG 6B illustrate total amount of mature miRNA processed from the AAV vectors following intravenous delivery in mice. FIG 6A illustrates fold expression of miR1518 and miR1693, as measured by qPCR with miR1518 primers. FIG 6B illustrates fold
15 expression of miR1518 and miR1693, as measured by qPCR with miR1693 primers.

FIG 7 shows expression levels of Mfn2 (Mfn2 expressed from vector) in Mfn2-null MEF cell line following transfection with various vectors comprising CB7 promoter; expression levels shown as plotted quantitation of western blot signal measuring expression of mitofurin-2 (Mfn2) following transfections with CB7.CI.hMfn2.GA.WPRE.RBG;
20 CB7.CI.hMfn2.GA.LINK.miR1518.RBG; CB7.CI.hMfn2.GA.LINK.miR538.RBG.

FIG 8 shows expression levels of Mfn2 (Mfn2 expressed from vector) in Mfn2-null MEF cell line following transfection with various vectors comprising CAG promoter; expression levels shown as plotted quantitation of western blot signal measuring expression of mitofurin-2 (Mfn2) following transfections with CAG.CI.hMfn2.GA.WPRE.SV40 ;
25 CAG.CI.hMfn2.GA.LINK.miR1518.WPRE.SV40 ; CAG.CI.hMfn2.GA.LINK.miR538.WPRE.SV40. FIG.

FIG 9A and FIG 9B show expression levels of Mfn2 in HEK293 cell line following transfection with various vectors comprising either CB7 or CAG promoter. FIG 9A show endogenous Mfn2 knockdown in HEK293 cells as measured by qPCR and plotted as fold
30 expression, following transfection with various vectors comprising CB7 promoter, (CB7.CI.hMfn2.GA.WPRE.RBG; CB7.CI.hMfn2.GA.LINK.miR1518.RBG ;

CB7.CI.hMfn2.GA.LINK.miR538.RBG. FIG 9B show endogenous Mfn2 knockdown in HEK293 cells as measured by qPCR and plotted as fold expression, following transfection with various vectors comprising a CAG promoter CAG.CI.hMfn2.GA.WPRE.SV40; CAG.CI.hMfn2.GA.LINK.miR1518.WPRE.SV40;
 5 CAG.CI.hMfn2.GA.LINK.miR538.WPRE.SV40.

FIG 10 shows expression levels of Mfn2 (endogenous Mfn2 and Mfn2 expressed from vector) in HEK293 cell line following transfection with various vectors comprising CB7 promoter; expression levels shown as plotted quantitation of western blot signal measuring expression of mitofurin-2 (Mfn2) following transfections with
 10 CB7.CI.hMfn2.GA.WPRE.RBG ; CB7.CI.hMfn2.GA.LINK.miR1518.RBG ; CB7.CI.hMfn2.GA.LINK.miR538.RBG . Quantitation is plotted as percent expression; transfection efficiency was determined to be about 95%.

FIG 11 shows expression levels of Mfn2 (endogenous Mfn2 and Mfn2 expressed from vector) in HEK293 cell line following transfection with various vectors comprising CAG
 15 promoter; expression levels shown as plotted quantitation of western blot signal measuring expression of mitofurin-2 (Mfn2) following transfections with CAG.CI.hMfn2.GA.WPRE.SV40 (p6168); CAG.CI.hMfn2.GA.LINK.miR1518.WPRE.SV40 (p6169); CAG.CI.hMfn2.GA.LINK.miR538.WPRE.SV40 (p6170). Quantitation is plotted as percent expression; transfection efficiency was determined to be about 95%.

FIG 12A to FIG 12C show expression levels, as measured by qPCR, of mature miRNA (miR1518 or miR538) in Mfn2-null MEF cell line (ATCC; CRL-2933), following transfection with various vectors comprising either CB7 or CAG promoter. FIG 12A shows a comparison of expression levels, as measured by qPCR and plotted as fold expression, of mature miR1518 in Mfn2-null MEF cell line, following transfection with vectors comprising
 25 either CB7 or CAG promoter. FIG 12B shows a comparison of expression levels, as measured by qPCR and plotted as fold expression, of mature miR538 in Mfn2-null MEF cell line, following transfection with vectors comprising either CB7 or CAG promoter. FIG 12C shows a comparison of expression levels, as measured by qPCR and plotted as fold expression, of mature miR1518 and miR538 in Mfn2-null MEF cell line, following transfection with vectors
 30 comprising either CB7 or CAG promoter.

FIG 13A to FIG 13F show characterization of a mouse model. FIG 13A show schematic representation of the mice genotype. FIG 13B shows mice phenotype characterization, characterized by relative expression levels endogenous and FLAG-tagged MFN2 in brain as measured by western blotting. FIG 13C shows mice phenotype characterization, characterized by relative expression levels endogenous and FLAG-tagged MFN2 in spinal cord as measured by western blotting. FIG 13D shows measured weight in (g) of the mice in the CMT2A mouse model (nTg, MFN2^{WT}, and MFN2^{R49Q}). FIG 13E shows mice phenotype characterization, as measured by the latency to fall (sec). FIG 13F shows mice phenotype characterization, as measured by grip strength (g).

FIG 14A and FIG 14B show results of the pharmacological study in MFN2^{R94Q} mice (Study groups: G1 - wild type (WT) mice, PBS; G2 - MFN2^{R94Q} mice, PBS; G3 - MFN2^{R94Q} mice, CB7.MFN2; G4 - MFN2^{R94Q} mice, CB7.MFN2.miR1518; G5 - MFN2^{R94Q} mice, CB7.MFN2.miR538; G6 - MFN2^{R94Q} mice, CAG.MFN2.miR1518; G7 - MFN2^{R94Q} mice, CAG.MFN2.miR538). FIG 14A shows body weight results (plotted as (g)) as measured in mice groups G1 to G7. FIG 14B shows survival results (plotted as probability of survival over day 0 to 50) as measured in mice groups G1 to G7.

FIG 15 shows grip strength results (plotted as (kg)) of the pharmacological study in MFN2^{R94Q} mice.

Detailed Description of the Invention

Sequences, vectors and compositions are provided here for co-administering to a patient a nucleic acid sequence which expresses human mitofusin 2 (or hMfn2) protein and a nucleic acid sequence encoding at least one miRNA which specifically targets a site in the endogenous human mitofusin 2 gene of the patient which target site is not present on human mitofusin 2 engineered coding sequence. Suitably, the human mitofusin 2 coding sequence is engineered to remove the specific target site for the encoded miRNA. Novel engineered human mitofusin 2 coding sequences and novel miRNA sequences are provided herein. These may be used alone or in combination with each other and/or other therapeutics for the treatment of CMT2A.

As used herein the term “endogenous mitofusin 2” refers to the mitofusin 2 gene which encodes the mitofusin 2 protein in humans having CMT2A. Patients with CMT2A may

have a number of missense mutations or allelic variants. See, also, omim.org/allelicVariants/608507, describing various allelic variants. Autosomal dominant Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A) is caused by heterozygous mutation in the MFN2 gene (608507) on chromosome 1p36.2. Homozygous or compound
 5 heterozygous mutation in the MFN2 gene causes autosomal recessive CMT2A2B (617087), a more severe disorder with earlier onset. Another form of CMT2A mapping to chromosome 1p36.2, CMT2A1 (118210), is caused by mutation in the KIF1B gene (605995). See also hereditary motor and sensory neuropathy VI (HMSN6; 601152), an allelic disorder with overlapping features.

10 The native, functional, human Mfn2a gene, such as is found endogenously in patients without CMT2A, is reproduced in SEQ ID NO: 18 and the native, functional, human Mf2A protein is reproduced in SEQ ID NO: 19. Mitofusin 2 is made in many types of cells and tissues, including muscles, the spinal cord, and the nerves that connect the brain and spinal
 15 (peripheral nerves). This gene may alternatively be called: CMT2A2, CRPP1, KIAA0214, MARF, MFN2_Human or mitochondrial assembly regulatory factor. See, OMIM.ORG/entry/609260, accessed July 12, 2020. In certain embodiments, functional Mfn2 proteins having less than 100% identity to the amino acid sequence of SEQ ID NO: 19 may be delivered by the compositions provided herein (e.g., Mfn2 having 97% to 100% identity to
 20 SEQ ID NO: 19). In such embodiments, the enzymatic and binding function of native functional human Mfn2 are preferably retained. See, also, UniProtKB-09140 (e.g., binding sites at position 305 and 307 are conserved and/or nucleotide binding sites at nucleotides 106-111 and/or 258-261 are conserved).

In one embodiment, an engineered mitofusin 2 coding sequence is provided which has
 25 the nucleic acid sequence of SEQ ID NO: 11 or a sequence of about 90%, at least 95% identical, at least 97% identical, at least 98% identical, or 99% to 100% identical to SEQ ID NO: 11 and which expresses the human mitofusin 2 protein found in non-CMT2A patients. See, e.g., SEQ ID NO: 19. See, also SEQ ID NO: 2 and SEQ ID NO: 4.

In certain embodiments, an engineered mitofusin 2 coding sequence is provided which
 30 has the nucleic acid sequence of SEQ ID NO: 11 or a sequence at least 80% identical, provided that nt 216 to 236 of SEQ ID NO: 11 are conserved (e.g., 100% identical, or at least

99% identical), e.g., when the engineered coding sequence is co-administered with the miR538 coding sequence.

In certain embodiments, an engineered mitofusin 2 coding sequence is provided which has the nucleic acid sequence of SEQ ID NO: 11 or a sequence at least 80% identical, provided that nt 1371 to 1391 of SEQ ID NO: 11 are conserved (e.g., 100% identical, or at least 99% identical), e.g., when the engineered coding sequence is co-administered with the miR1518 coding sequence. Suitably, the sequence having identity to SEQ ID NO: 11 expresses the same protein. See, e.g., SEQ ID NO: 19; SEQ ID NO: 2 and SEQ ID NO: 4.

In one embodiment, an engineered mitofusin 2 coding sequence is provided which has the nucleic acid sequence of SEQ ID NO: 28 or a sequence at least 90%, at least 95% identical, at least 97% identical, at least 98% identical, at least 99% identical, and/or at least 99% to 100% identical to SEQ ID NO: 24.

A “5’ UTR” is upstream of the initiation codon for a gene product coding sequence. The 5’ UTR is generally shorter than the 3’ UTR. Generally, the 5’ UTR is about 3 nucleotides to about 200 nucleotides in length, but may optionally be longer.

A “3’ UTR” is downstream of the coding sequence for a gene product and is generally longer than the 5’ UTR. In certain embodiments, the 3’ UTR is about 200 nucleotides to about 800 nucleotides in length, but may optionally be longer or shorter.

As used herein, an “miRNA” refers to a microRNA which is a small non-coding RNA molecule which regulates mRNA and stops it from being translated to protein. Generally, hairpin-forming RNAs have a self-complementary “stem-loop” structure that includes a single nucleic acid encoding a stem portion having a duplex comprising a sense strand (e.g., passenger strand) connected to an antisense strand (e.g., guide strand) by a loop sequence. The passenger strand and the guide strand share complementarity. In some embodiments, the passenger strand and guide strand share 100% complementarity. In some embodiments, the passenger strand and guide strand share at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% complementarity. A passenger strand and a guide strand may lack complementarity due to a base-pair mismatch. In some embodiments, the passenger strand and guide strand of a hairpin-forming RNA have at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7 at least 8, at least 9, or at least 10 mismatches. Generally, the first 2-8 nucleotides of the stem (relative to the loop) are referred to as “seed”

residues and play an important role in target recognition and binding. The first residue of the stem (relative to the loop) is referred to as the “anchor” residue. In some embodiments, hairpin-forming RNA have a mismatch at the anchor residue. As used herein, the miRNA contains a “seed sequence” which is a region of nucleotides which specifically binds to mRNA (e.g., in the endogenous hMfn2) by complementary base pairing, leading to destruction or silencing of the mRNA. Such silencing may result in downregulation rather than complete extinguishing of the endogenous hMfn2. Unless otherwise specified, the term “miRNA” encompasses artificial microRNA (amiRNA), which are artificially designed.

A “self-complementary nucleic acid” refers to a nucleic acid capable of hybridizing with itself (i.e., folding back upon itself) to form a single-stranded duplex structure, due to the complementarity (e.g., base-pairing) of the nucleotides within the nucleic acid strand. Self-complementary nucleic acids can form a variety of secondary structures, such as hairpin loops, loops, bulges, junctions and internal bulges. Certain self-complementary nucleic acids (e.g., miRNA or AmiRNA) perform regulatory functions, such as gene silencing.

The encoded miRNA provided herein have been designed to specifically target the endogenous human mitofusin 2 gene in patients having CMT2A. In certain embodiments the miRNA coding sequence comprises an anti-sense sequence in the following table 1, SEQ ID NOs: 27-46, 68, and 89.

Table 1.

miR #	Target hMfn2 Sequence	SEQ ID NO:	miRNA Antisense sequence	SEQ ID NO:
538	AGAACAGGTTCTGGACG TCAA	47	TTGACGTCCAG AACCTGTTCT	27
874	CAACTCTAAGTGCCAC TTCT	48	AGAAGTGGGCA CTTAGAGTTG	28
877	CTCTAAGTGCCCACTTCT GAA	49	TTCAGAAGTGG GCACTTAGAG	29
946	GCTGGACAGCTGGATTG ACAA	50	TTGTCAATCCA GCTGTCCAGC	30

miR #	Target hMfn2 Sequence	SEQ ID NO:	miRNA Antisense sequence	SEQ ID NO:
1377	CTGCAGTGAAGACCAAG TTTG	51	CAAACCTTGGTC TTCAGTGCAG	31
1686	CTCCAGTAGTCCTCAAG GTTT	52	AAACCTTGAGG ACTACTGGAG	32
2115	AGTTCATGGTTTCCATG GTTA	53	TAACCATGGAA ACCATGAACT	33
2164	CTCCATGGGCATTCTTGT TGT	54	ACAACAAGAAT GCCCATGGAG	34
2390	CAGGAAGTGTCTGGGAC CTTT	55	AAAGGTCCCAG ACAGTTCCTG	35
2457	AGGAAATTGCCGCCATG AACA	56	TGTTTCATGGCG GCAATTTCTT	36
1693	AGTCCTCAAGGTTTATA AGAA	57	TTCTTATAAACC TTGAGGACT	68
	GTCAATCAATAGCCAAC CTCA	58	TGAGGTTGGCT ATTGATTGAC	37
	AAGGTGTTGACTGTGTG AGAA	59	TTCTCACACAGT CAACACCTT	38
	AGCAGGTTTACTGCGAG GAAA	60	TTTCCTCGCAGT AAACCTGCT	39
	AAGACATTGAGTTCCAT TTCT	61	AGAAATGGAAC TCAATGTCTT	40
	TCACAGGTGATGTCCTG TTCA	62	TGAACAGGACA TCACCTGTGA	41
	GTTTACATACCTGCTTGT ATT	63	AATACAAGCAG GTATGTGAAC	42
	GGCTCGGGAGCAGCAGG TTTA	64	TAAACCTGCTG CTCCCGAGCC	43

miR #	Target hMfn2 Sequence	SEQ ID NO:	miRNA Antisense sequence	SEQ ID NO:
	TGGGCTCTATGGCCTCC TCTA	65	TAGAGGAGGCC ATAGAGCCCA	44
	CAATTGCTTCCTGCGGG TAGA	66	TCTACCCGCAG GAAGCAATTG	45
	TTCTTTGTGTCTGCTAAG GAG	67	CTCCTTAGCAG ACACAAAGAA	46

In certain embodiments, the seed sequence is 100% identical to the antisense sequence describe in the table. In certain embodiments, the seed sequence is located on the mature miRNA (5' to 3') and is generally starts at position 2 to 7, 2 to 8, or about 6 nucleotides from the 5' end of the miRNA sense strand (from the 5' end of the sense (+) strand) of the miRNA, although it may be longer than in length. In certain embodiments, the length of the seed sequence is no less than about 30% of the length of the miRNA sequence, which may be at least 7 nucleotides to about 28 nucleotides in length, at least 8 nucleotides to about 28 nucleotides in length, 7 nucleotides to 28 nucleotides, 8 nucleotides to 18 nucleotides, 12 nucleotides to 28 nucleotides in length, about 20 to about 26 nucleotides, about 21 nucleotides, about 24 nucleotides, or about 26 nucleotides. In the examples provided herein, the miRNA is delivered in the form of a stem-loop miRNA precursor sequences, e.g., about 50 to about 80 nucleotides in length, or about 55 nucleotides to about 70 nucleotides, or 60 to 65 nucleotides in length. In certain embodiments, this miRNA precursor comprises about 5 nucleotides, about a 21-nucleotide seed sequence, about a 19 nucleotide stem loop and about a 19 nucleotide sense sequence, wherein the sense sequence corresponds to the anti-sense sequence with one or two nucleotides being mismatched. An example of a suitable miRNA coding sequence is the miR1693 sequence of SEQ ID NO: 15. In certain embodiments, the miRNA coding sequence comprises SEQ ID NO: 15 (miR1693, 64 nt); an miRNA coding sequence comprising at least 60 consecutive nucleotides of SEQ ID NO: 15; or an miRNA coding sequence comprising at least 99% identity to SEQ ID NO: 15 which comprises a sequence with 100% identity to about nucleotide 6 to about nucleotide 26 of SEQ ID NO: 15 (or SEQ ID NO: 68). In certain embodiments, another sequences of the table above may be substituted

- in positions 6 to 26 of SEQ ID NO: 15 (or SEQ ID NO: 68). In still another embodiment, positions 6 to 26 of SEQ ID NO: 15 are retained, and an alternative sequence is selected for the stem-loop sequence. In certain embodiments, the miRNA coding sequence comprises SEQ ID NO: 16 (miR1518, 59 nt), a sequence comprising at least 99% identity to SEQ ID NO: 16.
- 5 In certain embodiments, the miRNA coding sequence comprises SEQ ID NO: 89 (miR538, 59 nt), or a sequence comprising at least 99% identity to SEQ ID NO: 89. In certain embodiments, alternative stem-loop sequences are selected, wherein an antisense strand of stem is nt 6 to 26 of SEQ ID NO: 16, or nt 1 to 21 of SEQ ID NO: 89, and wherein loop sequence is nt 27 to 45 of SEQ ID NO: 41 or nt 22 to 40 of SEQ ID NO: 89.
- 10 In certain embodiments, the nucleic acid molecules (e.g., an expression cassette or vector genome) may contain more than one miRNA coding sequence. Such may comprise an miRNA coding sequence having the sequence of one, two or more of: (a) an miRNA coding sequence comprising SEQ ID NO: 15 (miR1693, 64 nt); (b) an miRNA coding sequence comprising at least 60 consecutive nucleotides of SEQ ID NO: 15; (c) an miRNA coding
- 15 sequence comprising at least 99% identity to SEQ ID NO: 15 which comprises a sequence with 100% identity to about nucleotide 6 to about nucleotide 26 of SEQ ID NO: 15 (or SEQ ID NO: 68); and/or (d) an miRNA coding sequence comprising one or more of:
- (i) TTGACGTCCAGAACCTGTTCT, SEQ ID NO: 27; (ii) AGAAGTGGGCACTTAGAGTTG, SEQ ID NO: 28; (iii) TTCAGAAGTGGGCACTTAGAG, SEQ ID NO: 29; (iv) TTGTCAATCCAGCTGTCCAGC, SEQ ID NO: 30; (v) CAAACTTGGTCTTCACTGCAG, SEQ ID NO: 31; (vi) AAACCTTGAGGACTACTGGAG, SEQ ID NO: 32; (vii) TAACCATGGAAACCATGAACT, SEQ ID NO: 33; (viii) ACAACAAGAATGCCCATGGAG, SEQ ID NO: 34; (ix) AAAGGTCCCAGACAGTTCCTG, SEQ ID NO: 35; (x) TGTTTCATGGCGGCAATTCCT, SEQ ID NO: 36; (xi) TGAGGTGGCTATTGATTGAC, SEQ ID NO: 37; (xii) TTCTCACACAGTCAACACCTT, SEQ ID NO: 38; (xiii) TTTCTCGCAGTAAACCTGCT, SEQ ID NO: 39; (xiv) AGAAATGGAATCAATGTCTT, SEQ ID NO: 40; (xv) TGAACAGGACATCACCTGTGA, SEQ ID NO: 41; (xvi) AATACAAGCAGGTATGTGAAC, SEQ ID NO: 42; (xvii) TAAACCTGCTGCTCCCGAGCC, SEQ ID NO: 43; (xviii)
- 20
- 25
- 30

TAGAGGAGGCCATAGAGCCCA, SEQ ID NO: 44; (xix)

TCTACCCGCAGGAAGCAATTG, SEQ ID NO: 45; or (xx)

CTCCTTAGCAGACACAAAGAA, SEQ ID NO: 46, or combinations of any of (i) through

(xx). In certain embodiments, the nucleic acid molecules (e.g., an expression cassette or vector
5 genome) may contain one, two or more miRNA coding sequence of SEQ ID NO: 16
(miR1518). In certain embodiments, the nucleic acid molecules (e.g., an expression cassette or
vector genome) may contain one, two or more miRNA coding sequence of SEQ ID NO: 89
(miR538).

As used herein, an “miRNA target sequence” is a sequence located on the DNA
10 positive strand (5' to 3') (e.g., of hMfn2) and is at least partially complementary to a miRNA
sequence, including the miRNA seed sequence. The miRNA target sequence is exogenous to
the untranslated region of the encoded transgene product and is designed to be specifically
targeted by miRNA in cells in which repression of transgene expression is desired. Without
wishing to be bound by theory, because hMfn2 is a ubiquitous protein and excess expression
15 may be associated with toxicity and/or other negative side effects, the miRNA preferentially
target the endogenous hMfn2 gene while avoiding targeting the engineered hMfn2 gene which
is delivered to the CMT2A patent. More particularly, the sequences encoding the hMfn2
which are delivered via a vector are designed to contain altered codon sequences at the target
site.

20 Typically, the miRNA target sequence is at least 7 nucleotides to about 28 nucleotides
in length, at least 8 nucleotides to about 28 nucleotides in length, 7 nucleotides to 28
nucleotides, 8 nucleotides to 18 nucleotides, 12 nucleotides to 28 nucleotides in length, about
20 to about 26 nucleotides, about 22 nucleotides, about 24 nucleotides, or about 26
nucleotides, and which contains at least one consecutive region (e.g., 7 or 8 nucleotides) which
25 is complementary to the miRNA seed sequence. In certain embodiments, the target sequence
comprises a sequence with exact complementarity (100%) or partial complementarity to the
miRNA seed sequence with some mismatches. In certain embodiments, the target sequence
comprises at least 7 to 8 nucleotides which are 100% complementary to the miRNA seed
sequence. In certain embodiments, the target sequence consists of a sequence which is 100%
30 complementary to the miRNA seed sequence. In certain embodiments, the target sequence
contains multiple copies (e.g., two or three copies) of the sequence which is 100%

complementary to the seed sequence. In certain embodiments, the region of 100% complementarity comprises at least 30% of the length of the target sequence. In certain embodiments, the remainder of the target sequence has at least about 80 % to about 99% complementarity to the miRNA. In certain embodiments, in an expression cassette containing
 5 a DNA positive strand, the miRNA target sequence is the reverse complement of the miRNA.

Thus, the sequences provided herein which are 95% to 99.9% identical to the Mfn2 coding sequences of SEQ ID NO: 11 and 24, are designed to avoid reverting to a native human sequence to which a selected miRNA in the construct is targeted.

In certain embodiments, the miRNA preferentially targets the endogenous hMfn2 gene
 10 while avoiding targeting the engineered hMfn2 gene, wherein the endogenous hMfn2 nucleic acid sequence is of SEQ ID NO: 18. In certain embodiments, the miRNA coding sequence comprises one or more of : (i) TTGACGTCCAGAACCTGTTCT, SEQ ID NO: 27, targeting nt 216-236 of SEQ ID NO: 18; (ii) AGAAGTGGGCACTTAGAGTTG, SEQ ID NO: 28, targeting nt 552-572 of SEQ ID No: 18 ; (iii) TTCAGAAGTGGGCACTTAGAG, SEQ ID
 15 NO: 29, targeting nt 555-575 of SEQ ID NO: 18; (iv) TTGTCAATCCAGCTGTCCAGC, SEQ ID NO: 30, targeting nt 624-644 of SEQ ID NO: 18; (v) CAAACTTGGTCTTCACTGCAG, SEQ ID NO: 31, targeting nt 1055-1075 of SEQ ID NO: 18; (vi) AAACCTTGAGGACTACTGGAG, SEQ ID NO: 32, targeting nt 1364-1384 of SEQ ID NO: 18; (vii) TAACCATGGAAACCATGAACT, SEQ ID NO: 33, targeting nt 1793-1813
 20 of SEQ ID NO: 18; (viii) ACAACAAGAATGCCCATGGAG, SEQ ID NO: 34, targeting nt 1842-1862 of SEQ ID NO: 18; (ix) AAAGGTCCCAGACAGTTCCTG, SEQ ID NO: 35, targeting nt 2068-2088 of SEQ ID NO: 181; (x) TGTTCATGGCGGCAATTCCT, SEQ ID NO: 36, targeting nt 2135-2155 of SEQ ID NO: 18; (xi) TGAGGTTGGCTATTGATTGAC, SEQ ID NO: 37, targeting 5'UTR; (xii) TTCTCACACAGTCAACACCTT, SEQ ID NO: 38,
 25 targeting 3'UTR; (xiii) TTTCTCGCAGTAAACCTGCT, SEQ ID NO: 39, targeting nt 1157-1177 of SEQ ID NO: 18; (xiv) AGAAATGGAATCAATGTCTT, SEQ ID NO: 40, targeting nt 1616-1636 of SEQ ID NO: 18; (xv) TGAACAGGACATCACCTGTGA, SEQ ID NO: 41, targeting 3'UTR; (xvi) AATACAAGCAGGTATGTGAAC, SEQ ID NO: 42, targeting 3'UTR; (xvii) TAAACCTGCTGCTCCCGAGCC, SEQ ID NO: 43, targeting nt 1146-1166 of
 30 SEQ ID NO: 18; (xviii) TAGAGGAGGCCATAGAGCCCA, SEQ ID NO: 44, targeting nt 1914-1934 of SEQ ID NO: 18; (xix) TCTACCCGCAGGAAGCAATTG, SEQ ID NO: 45,

targeting nt 390-410 of SEQ ID NO: 18; or (xx) CTCCTTAGCAGACACAAAGAA, SEQ ID NO: 46, targeting nt 904-924 of SEQ ID NO: 18.

In certain embodiments, the engineered hMfn2 nucleic acid sequence is of SEQ ID NO: 11 or 24. In certain embodiments the engineered hMfn2 nucleic acid sequence is of SEQ ID NO: 18 wherein 1, 2, 3, or 4 nucleotide mismatches are present in the regions of nucleotides: (i) nt 216-236 of SEQ ID NO: 18; (ii) nt 552-572 of SEQ ID NO: 18; (iii) nt 555-575 of SEQ ID NO: 18; (iv) nt 624-644 of SEQ ID NO: 18; (v) nt 1055-1075 of SEQ ID NO: 18; (vi) nt 1364-1384 of SEQ ID NO: 18; (vii) nt 1793-1813 of SEQ ID NO: 18; (viii) nt 1842-1862 of SEQ ID NO: 18; (ix) nt 2068-2088 of SEQ ID NO: 18; (x) nt 2135-2155 of SEQ ID NO: 18; (xi) nt 1157-1177 of SEQ ID NO: 18; (xii) nt 1616-1636 of SEQ ID NO: 18; (xiii) nt 1146-1166 of SEQ ID NO: 18; (xiv) nt 1914-1934 of SEQ ID NO: 18; (xv) nt 390-410 of SEQ ID NO: 18; or (xvi) nt 904-924 of SEQ ID NO: 18.

In certain embodiments, a single nucleic acid (e.g., an expression cassette or vector genome containing same) contains both the engineered hMfn2 coding sequence and at least one miRNA coding sequence, wherein the miRNA is specifically targeted to a region of the endogenous human Mfn2 sequence not present in the engineered hMfn2 sequence. In certain embodiments, the human mitofusin 2 coding sequence is upstream (5') of the at least one miRNA and these two elements are separated by a spacer or linker sequence. In certain embodiments, there is at least 75 nucleotides between the stop codon of the hMfn2 coding sequence and the start of the most 5' miRNA coding sequence. In certain embodiments, the spacer is about 75 nucleotides to about 300 nucleotides, or about 75 nucleotides to about 250 nucleotides, or about 75 nucleotides to about 200 nucleotides, or about 75 nucleotides to about 150 nucleotides, or about 75 nucleotides to about 100 nucleotides, or about 80 nucleotides to about 300 nucleotides, or about 80 nucleotides to about 250 nucleotides, or about 80 nucleotides to about 200 nucleotides, or about 80 nucleotides to about 150 nucleotides, or about 80 nucleotides to about 100 nucleotides,. Optionally, the engineered hMfn2 coding sequence and the at least one miRNA coding sequence are separated by about 75 nucleotides. Suitably, the spacer sequence is a non-coding sequence which lacks any restriction enzyme sites. Optionally, the spacer may include one or more intron sequences. In certain embodiments, one or more of the miRNA sequences may be located within the intron. In

certain embodiments, the linker sequence is SEQ ID NO: 17. In certain embodiments, the linker sequence is SEQ ID NO: 90.

In certain embodiments, the engineered hMfn2 coding sequence and the miRNA coding sequence(s) are delivered via different nucleic acid sequences, e.g., two or more
 5 different vectors, a combination comprising a vector and an LNP, etc. In certain embodiments, the two different vectors are AAV vectors. In certain embodiments, these vectors have different expression cassettes. In other embodiments, these vectors have the same capsid. In other embodiments, the vectors have different embodiments. In certain
 10 embodiments, the miRNA coding sequence(s) are delivered via an LNP or another non-viral delivery system. In certain embodiments, the engineered hMfn2 sequence is delivered via an LNP or another non-viral delivery system. In certain embodiments, combinations of two or more different delivery systems (e.g., viral and non-viral, two different non-viral) are used. In these and other embodiments, the two or more different vectors or other delivery systems may be administered substantially simultaneously, or one or more of these systems may be
 15 delivered before the other. In certain embodiments, the engineered hMfn2 sequence is SEQ ID NO: 11, or a sequence 90% to 100% identical thereto which encodes an mRNA which is not bound by the miR with which it is co-administered and which encodes hMfn2. In certain embodiments, the engineered hMfn2 sequence is SEQ ID NO: 24, or a sequence 90% to 100% identical thereto which encodes an mRNA which is not bound by the miR with which it is co-
 20 administered and which encodes hMfn2. In certain embodiments, the miR is miR538 having the sequence of SEQ ID NO: 89 which targets endogenous hMfn2 in the subject, but which does not target the engineered hMfn2 cDNA sequence or engineered encoded mRNA sequence.

As used herein, the terms “AAV.hMfn2” or “rAAV.hMfn2” are used to refer to a
 25 recombinant adeno-associated virus which has an AAV capsid having therewithin a vector genome comprising a human mitofusin 2 coding sequence (e.g., a cDNA) under the control of regulatory sequences. As used herein, the terms “AAV.hMfn2 miRXXX” or “rAAV.hMfn2.miRXXX” are used to refer to a recombinant adeno-associated virus which has an AAV capsid having therewithin a vector genome comprising an miR targeting an
 30 endogenous human mitofusin 2 coding sequence.

Specific capsid types may be specified, such as, e.g., AAV1.hMfn2 or rAAV1.hMfn2, which refers to a recombinant AAV having an AAV1 capsid; AAVhu68.hMfn2 or AAVhu68.Mfn2, which refers to recombinant AAV having an AAVhu68 capsid. AAVrh91.hMfn2 or AAVrh91.Mfn2, which refers to recombinant AAV having an AAVrh91 capsid.

A “recombinant AAV” or “rAAV” is a DNase-resistant viral particle containing two elements, an AAV capsid and a vector genome containing at least non-AAV coding sequences packaged within the AAV capsid. Unless otherwise specified, this term may be used interchangeably with the phrase “rAAV vector”. The rAAV is a “replication-defective virus” or “viral vector”, as it lacks any functional AAV rep gene or functional AAV cap gene and cannot generate progeny. In certain embodiments, the only AAV sequences are the AAV inverted terminal repeat sequences (ITRs), typically located at the extreme 5’ and 3’ ends of the vector genome in order to allow the gene and regulatory sequences located between the ITRs to be packaged within the AAV capsid. Generally, an AAV capsid is composed of 60 capsid (cap) protein subunits, VP1, VP2, and VP3, that are arranged in an icosahedral symmetry in a ratio of approximately 1:1:10 to 1:1:20, depending upon the selected AAV. Various AAVs may be selected as sources for capsids of AAV viral vectors as identified above. In one embodiment, the AAV capsid is an AAV9 capsid or an engineered variant thereof. In certain embodiments, the variant AAV9 capsid is an AAV9.PhP.eB capsid (nucleic acid sequence of SEQ ID NO: 84; amino acid sequence of SEQ ID NO: 85). In certain embodiments, the PhP.eB capsid is selected for use in mouse studies and is a suitable model for a clade F vector (e.g., AAVhu68) in humans. In certain embodiments, the capsid protein is designated by a number or a combination of numbers and letters following the term “AAV” in the name of the rAAV vector. Unless otherwise specified, the AAV capsid, ITRs, and other selected AAV components described herein, may be readily selected from among any AAV, including, without limitation, the AAVs identified as AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, AAVhu37, AAVrh32.33, AAV8bp, AAV7M8 and AAVAnc80, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV12, AAVrh8, AAVrh74, AAV-DJ8, AAV-DJ, AAVhu68, and AAV9 variants (e.g., US Provisional Application No. 63/119,863, filed December 1, 2020), without limitation. See, e.g., WO 2019/168961 and WO 2019/169004,

both for Novel AAV Vectors Having Reduced Capsid Deamidation and Uses Therefor; US Published Patent Application No. 2007-0036760-A1; US Published Patent Application No. 2009-0197338-A1; EP 1310571. See also, WO 2003/042397 (AAV7 and other simian AAV), US Patent 7790449 and US Patent 7282199 (AAV8), WO 2005/033321 and US 7,906,111 (AAV9), and WO 2006/110689, and WO 2003/042397 (rh.10), WO 2005/033321, WO 2018/160582 (AAVhu68), which are incorporated herein by reference. See, also WO 2019/168961 and WO 2019/169004, describing deamidation profiles for these and other AAV capsids. Other suitable AAVs may include, without limitation, AAVrh90 [PCT/US20/30273, filed April 28, 2020], AAVrh91 [PCT/US20/30266, filed April 28, 2020 and US Provisional Patent Applications No. 63/109,734, filed November 4, 2020 and US Provisional Patent Application No. 63/065,616, filed August 14, 2020] AAVrh92, AAVrh93, AAVrh91.93 [PCT/US20/30281, filed April 28, 2020], which are incorporated by reference herein. Other suitable AAV include AAV3B variants which are described in PCT/US20/56511, filed October 20, 2020, which claims the benefit of US Provisional Patent Application No. 62/924,112, filed October 21, 2019, and US Provisional Patent Application No. 63/025,753, filed May 15, 2020, describing AAV3B.AR2.01, AAV3B.AR2.02, AAV3B.AR2.03, AAV3B.AR2.04, AAV3B.AR2.05, AAV3B.AR2.06, AAV3B.AR2.07, AAV3B.AR2.08, AAV3B.AR2.10, AAV3B.AR2.11, AAV3B.AR2.12, AAV3B.AR2.13, AAV3B.AR2.14, AAV3B.AR2.15, AAV3B.AR2.16, or AAV3B.AR2.17, which are incorporated herein by reference. These documents also describe other AAV capsids which may be selected for generating rAAV and are incorporated by reference. Among the AAVs isolated or engineered from human or non-human primates (NHP) and well characterized, human AAV2 is the first AAV that was developed as a gene transfer vector; it has been widely used for efficient gene transfer experiments in different target tissues and animal models.

As used herein, a “vector genome” refers to the nucleic acid sequence packaged inside a parvovirus (e.g., rAAV) capsid which forms a viral particle. Such a nucleic acid sequence contains AAV inverted terminal repeat sequences (ITRs). In the examples herein, a vector genome contains, at a minimum, from 5' to 3', an AAV 5' ITR, coding sequence(s) (i.e., transgene(s)), and an AAV 3' ITR. ITRs from AAV2, a different source AAV than the capsid, or other than full-length ITRs may be selected. In certain embodiments, the ITRs are from the same AAV source as the AAV which provides the rep function during production or a

transcomplementing AAV. Further, other ITRs, e.g., self-complementary (scAAV) ITRs, may be used. Both single-stranded AAV and self-complementary (sc) AAV are encompassed with the rAAV. The transgene is a nucleic acid coding sequence, heterologous to the vector sequences, which encodes a polypeptide, protein, functional RNA molecule (e.g., miRNA, 5 miRNA inhibitor) or other gene product, of interest. The nucleic acid coding sequence is operatively linked to regulatory components in a manner which permits transgene transcription, translation, and/or expression in a cell of a target tissue. Suitable components of a vector genome are discussed in more detail herein.

In one example, a “vector genome” contains, at a minimum, from 5’ to 3’, a vector-specific sequence, a nucleic acid sequence comprising an engineered human Mfn2 coding 10 sequence and optionally an miRNA sequences targeting the endogenous Mfn2 operably linked to regulatory control sequences (which direct their expression in a target cell), where the vector-specific sequence may be a terminal repeat sequence which specifically packages the vector genome into a viral vector capsid or envelope protein. For example, AAV inverted 15 terminal repeats are utilized for packaging into AAV and certain other parvovirus capsids.

In certain embodiments, a composition is provided which comprises an aqueous liquid suitable for intrathecal injection and a stock of vector (e.g., rAAV having a AAV capsid which preferentially targets cells in the central nervous system and/or the dorsal root ganglia (e.g., CNS, including, e.g., nerve cells (such as, pyramidal, purkinje, granule, spindle, and 20 interneuron cells) and glia cells (such as astrocytes, oligodendrocytes, microglia, and ependymal cells), wherein the vector having an engineered hMfn2 coding sequence and/or an at least one miRNA specific endogenous hMfn2 for delivery to the central nervous system (CNS). In certain embodiments, the composition comprising one or more vectors as described herein is formulated for sub-occipital injection into the cisterna magna (intra-cisterna magna). 25 In certain embodiments, the composition is administered via a computed tomography- (CT-) rAAV injection. In certain embodiments, the composition is administered using Ommaya reservoir. In certain embodiments, the patient is administered a single dose of the composition.

As used herein, an “expression cassette” refers to a nucleic acid molecule which comprises a biologically useful nucleic acid sequence (e.g., a gene cDNA encoding a protein, 30 enzyme or other useful gene product, mRNA, etc.) and regulatory sequences operably linked thereto which direct or modulate transcription, translation, and/or expression of the nucleic

acid sequence and its gene product. As used herein, “operably linked” sequences include both regulatory sequences that are contiguous or non-contiguous with the nucleic acid sequence and regulatory sequences that act in trans or cis nucleic acid sequence. Such regulatory sequences typically include, e.g., one or more of a promoter, an enhancer, an intron, a Kozak sequence, a polyadenylation sequence, and a TATA signal. The expression cassette may contain regulatory sequences upstream (5’ to) of the gene sequence, e.g., one or more of a promoter, an enhancer, an intron, etc., and one or more of an enhancer, or regulatory sequences downstream (3’ to) a gene sequence, e.g., 3’ untranslated region (3’ UTR) comprising a polyadenylation site, among other elements. In certain embodiments, the regulatory sequences are operably linked to the nucleic acid sequence of a gene product, wherein the regulatory sequences are separated from nucleic acid sequence of a gene product by an intervening nucleic acid sequences, i.e., 5’-untranslated regions (5’UTR). In certain embodiments, the expression cassette comprises nucleic acid sequence of one or more of gene products. In some embodiments, the expression cassette can be a monocistronic or a bicistronic expression cassette. In other embodiments, the term “transgene” refers to one or more DNA sequences from an exogenous source which are inserted into a target cell.

Typically, such an expression cassette can be used for generating a viral vector and contains the coding sequence for the gene product described herein flanked by packaging signals of the viral genome and other expression control sequences such as those described herein. In certain embodiments, a vector genome may contain two or more expression cassettes.

In certain embodiment expression cassette comprises the hMfn2 coding sequences (and/or miRNA sequences targeting the endogenous Mfn2), promoter, and may include other regulatory sequences therefor, which cassette may be packaged into a vector (e.g., rAAV, lentivirus, retrovirus, etc.).

AAV

Recombinant parvoviruses are particularly well suited as vectors for treatment of CMT2A. As described herein, recombinant parvoviruses may contain an AAV capsid (or bocavirus capsid). In certain embodiments, the capsid targets cells within the dorsal root ganglion and/or cells within the lower motor neurons and/or primary sensory neurons. In

certain embodiments, compositions provided herein may have a single rAAV stock which comprises an rAAV comprising an engineered hMfn2 and an miRNA specifically targeting endogenous hMfn2 in order to downregulate the endogenous hMfn2 levels and to reduce any toxicity associated with overexpression of hMfn2. In other embodiments, an rAAV may be
5 comprise the hMfn2 and may be co-administered with a different vector comprising an miRNA which downregulates endogenous hMfn2. In other embodiments, an rAAV may be comprise the at least one miRNA which downregulates endogenous hMfn2 and a second vector (or other composition) delivers the hMfn2.

For example, vectors generated using AAV capsids from Clade F (e.g., AAVhu68 or
10 AAV9) can be used to produce vectors which target and express hMfn2 in the CNS. Alternatively, vectors generated using AAV capsids from Clade A (e.g., AAV1, AAVrh91) may be selected. In still other embodiments, other parvovirus or other AAV viruses may be suitable sources of AAV capsids.

An AAV1 capsid refers to a capsid having AAV vp1 proteins, AAV vp2 proteins and
15 AAV vp3 proteins. In particular embodiments, the AAV1 capsid comprises a pre-determined ratio of AAV vp1 proteins, AAV vp2 proteins and AAV vp3 proteins of about 1:1:10 assembled into a T1 icosahedron capsid of 60 total vp proteins. An AAV1 capsid is capable of packaging genomic sequences to form an AAV particle (e.g., a recombinant AAV where the genome is a vector genome). Typically, the capsid nucleic acid sequences encoding the
20 longest of the vp proteins, i.e., VP1, is expressed in trans during production of an rAAV having an AAV1 capsid are described in, e.g., US Patent 6,759,237, US Patent 7,105,345, US Patent 7,186,552, US Patent 8,637,255, and US Patent 9,567,607, which are incorporated herein by reference. See, also, WO 2018/168961, which is incorporated by reference. In certain embodiments, AAV1 is characterized by a capsid composition of a heterogeneous
25 population of VP isoforms which are deamidated as defined in WO 2018/160582, incorporated herein by reference in its entirety, based on the total amount of VP proteins in the capsid, as determined using mass spectrometry. In certain embodiments, the AAV capsid is modified at one or more of the following positions, in the ranges provided below, as determined using mass spectrometry. Suitable modifications include those described in the
30 paragraph above labelled modulation of deamidation, which is incorporated herein. In certain embodiments, one or more of the following positions, or the glycine following the N is

modified as described herein. In certain embodiments, an AAV1 mutant is constructed in which the glycine following the N at position 57, 383, 512 and/or 718 are preserved (i.e., remain unmodified). In certain embodiments, the NG at the four positions identified in the preceding sentence are preserved with the native sequence. In certain embodiments, an
5 artificial NG is introduced into a different position than one of the positions as defined and identified in WO 2018/160582, incorporated herein by reference.

As used herein, an AAVhu68 capsid refers to a capsid as defined in WO 2018/160582, incorporated herein by reference. As described herein, a rAAVhu68 has a rAAVhu68 capsid produced in a production system expressing capsids from an AAVhu68 nucleic acid. In
10 certain embodiments, the AAVhu68 nucleic acid sequence is SEQ ID NO: 81, encoding and for an amino acid sequence of SEQ ID NO 82. In certain embodiments, the AAVhu68 nucleic acid sequence is SEQ ID NO: 83, encoding for an amino acid sequence of SEQ ID NO: 82. The rAAVhu68 resulting from production using a single nucleic acid sequence vp1 produces the heterogenous populations of vp1 proteins, vp2 proteins and vp3 proteins. These
15 subpopulations include, at a minimum, deamidated asparagine (N or Asn) residues. For example, asparagines in asparagine - glycine pairs are highly deamidated. In certain embodiments, the vp2 and/or vp3 proteins may be expressed additionally or alternatively from different nucleic acid sequences than the vp1, e.g., to alter the ratio of the vp proteins in a selected expression system.

20 Genomic sequences which are packaged into an AAV capsid and delivered to a host cell are typically composed of, at a minimum, a transgene and its regulatory sequences, and AAV inverted terminal repeats (ITRs). Both single-stranded AAV and self-complementary (sc) AAV are encompassed with the rAAV. The transgene is a nucleic acid coding sequence, heterologous to the vector sequences, which encodes a polypeptide, protein, functional RNA
25 molecule (e.g., miRNA, miRNA inhibitor) or other gene product, of interest. The nucleic acid coding sequence is operatively linked to regulatory components in a manner which permits transgene transcription, translation, and/or expression in a cell of a target tissue.

The AAV sequences of the vector typically comprise the cis-acting 5' and 3' inverted terminal repeat sequences (See, e.g., B. J. Carter, in "Handbook of Parvoviruses", ed., P. Tijsser, CRC Press, pp. 155 168 (1990)). The ITR sequences are about 145 bp in length.
30 Preferably, substantially the entire sequences encoding the ITRs are used in the molecule,

although some degree of minor modification of these sequences is permissible. The ability to modify these ITR sequences is within the skill of the art. (See, e.g., texts such as Sambrook et al, "Molecular Cloning. A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory, New York (1989); and K. Fisher et al., J. Virol., 70:520-532 (1996)). An example of such a molecule employed in the present invention is a "cis-acting" plasmid containing the transgene, in which the selected transgene sequence and associated regulatory elements are flanked by the 5' and 3' AAV ITR sequences.

The ITRs are the genetic elements responsible for the replication and packaging of the genome during vector production and are the only viral cis elements required to generate rAAV. In one embodiment, the ITRs are from an AAV different than that supplying a capsid. In a preferred embodiment, the ITR sequences from AAV2, or the deleted version thereof (Δ ITR), which may be used for convenience and to accelerate regulatory approval. However, ITRs from other AAV sources may be selected. Where the source of the ITRs is from AAV2 and the AAV capsid is from another AAV source, the resulting vector may be termed pseudotyped. Typically, AAV vector genome comprises an AAV 5' ITR, the nucleic acid sequences encoding the gene product(s) and any regulatory sequences, and an AAV 3' ITR. However, other configurations of these elements may be suitable. In one embodiment, a self-complementary AAV is provided. A shortened version of the 5' ITR, termed Δ ITR, has been described in which the D-sequence and terminal resolution site (trs) are deleted. In certain embodiments, the vector genome includes a shortened AAV2 ITR of 130 base pairs, wherein the external "a" element is deleted. The shortened ITR is reverted back to the wild-type length of 145 base pairs during vector DNA amplification using the internal A element as a template. In other embodiments, the full-length AAV 5' and 3' ITRs are used.

In addition to the major elements identified above for the vector (e.g., an rAAV), the vector also includes conventional control elements necessary which are operably linked to the transgene in a manner which permits its transcription, translation and/or expression in a cell. As used herein, the term "expression" or "gene expression" refers to the process by which information from a gene is used in the synthesis of a functional gene product. The gene product may be a protein, a peptide, or a nucleic acid polymer (such as an RNA, a DNA or a PNA).

As used herein, the term "regulatory sequence", or "expression control sequence" refers to nucleic acid sequences, such as initiator sequences, enhancer sequences, and promoter sequences, which induce, repress, or otherwise control the transcription of protein encoding nucleic acid sequences to which they are operably linked. The regulatory control elements typically contain a promoter sequence as part of the expression control sequences, e.g., located between the selected 5' ITR sequence and the coding sequence. In particularly desirable embodiments, a tissues specific promoter for the central nervous system is selected. For example, the promoter may be a neural cell promoter, e.g., gfaABC(1)D promoter (Addgene #50473), or the human Syn promoter (the sequence is available from Addgene, Ref. #50465).

Other suitable promoters may include, e.g., constitutive promoters, regulatable promoters [see, e.g., WO 2011/126808 and WO 2013/04943], tissue specific promoters, or a promoter responsive to physiologic cues may be used may be utilized in the vectors described herein. The promoter(s) can be selected from different sources, e.g., human cytomegalovirus (CMV) immediate-early enhancer/promoter, the SV40 early enhancer/promoter, the JC polymovirus promoter, myelin basic protein (MBP) or glial fibrillary acidic protein (GFAP) promoters, herpes simplex virus (HSV-1) latency associated promoter (LAP), rouse sarcoma virus (RSV) long terminal repeat (LTR) promoter, neuron-specific promoter (NSE), platelet derived growth factor (PDGF) promoter, hSYN, melanin-concentrating hormone (MCH) promoter, CBA, matrix metalloprotein promoter (MPP), and the chicken beta-actin promoter. In addition to a promoter a vector may contain one or more other appropriate transcription initiation, termination, enhancer sequences, efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA for example WPRE; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. An example of a suitable enhancer is the CMV enhancer. Other suitable enhancers include those that are appropriate for desired target tissue indications. In one embodiment, the expression cassette comprises one or more expression enhancers. In one embodiment, the expression cassette contains two or more expression enhancers. These enhancers may be the same or may differ from one another. For example, an enhancer may include a CMV immediate early enhancer. This enhancer may be present in two copies which

are located adjacent to one another. Alternatively, the dual copies of the enhancer may be separated by one or more sequences. In still another embodiment, the expression cassette further contains an intron, e.g., the chicken beta-actin intron. Other suitable introns include those known in the art, e.g., such as are described in WO 2011/126808. Examples of suitable polyA sequences include, e.g., SV40, SV50, bovine growth hormone (bGH), human growth hormone, and synthetic polyAs. In certain embodiments, the polyA is SV40 polyA. In certain embodiments, the polyA is rabbit globin poly A (RBG). Optionally, one or more sequences may be selected to stabilize mRNA. An example of such a sequence is a modified WPRE sequence, which may be engineered upstream of the polyA sequence and downstream of the coding sequence [see, e.g., MA Zanta-Boussif, et al, Gene Therapy (2009) 16: 605-619].

In certain embodiments, the vector genome comprises a tissue specific promoter. In some embodiments, the tissue specific promoter is a human synapsin promoter. In certain embodiments, the human synapsin promoter comprises nucleic acid sequence of SEQ ID NO: 6. In certain embodiments, the vector genome comprises a constitutive promoter, wherein the promoter is a CB7 promoter or a CAG promoter. In certain embodiments, the CB7 promoter comprises nucleic acid sequence of SEQ ID NO: 86. In certain embodiments, the CAG promoter comprises nucleic acid sequence of SEQ ID NO: 87.

In one embodiment, the vector genome comprises: an AAV 5' ITR, a promoter, an optional enhancer, an optional intron, a coding sequence for human Mfn2 (hMfn2 or huMfn2) comprising same, a poly A, and an AAV 3' ITR. In certain embodiments, the vector genome comprises: a AAV 5' ITR, a promoter, an optional enhancer, an optional intron, a coding sequence for human Mfn2 comprising same, a poly A, and an AAV 3' ITR. In certain embodiments, the vector genome comprises: a AAV 5' ITR, a promoter, an optional enhancer, an optional intron, a huMfn2 coding sequence, a poly A, and an AAV 3' ITR. In certain embodiments, the vector genome comprises: an AAV2 5' ITR, an EF1a promoter, an optional enhancer, an optional promoter, huMfn2, an SV40 poly A, and an AAV2 3' ITR. In certain embodiments, the vector genome is AAV2 5' ITR, UbC promoter, optional enhancer, optional intron, huMfn2, an SV40 poly A, and an AAV2 3' ITR. In certain embodiments, the vector genome is AAV2 5' ITR, CB7 promoter, an intron, huMfn2, an SV40 poly A, and an AAV2 3' ITR. In certain embodiment, the vector genome is an AAV2 5' ITR, CB7 promoter, intron, huMfn2, a rabbit beta globin poly A, and an AAV2 3' ITR. In certain embodiment, the vector

genome is an AAV2 5' ITR, CB7 promoter, intron, an engineered huMfn2, a linker, a miR targeted to endogenous huMfn2 sequence, a rabbit beta globin poly A, and an AAV2 3' ITR. In certain embodiment, the vector genome is an AAV2 5' ITR, CB7 promoter, intron, an engineered huMfn2, a linker, a miR1518 sequence, a rabbit beta globin poly A, and an AAV2 3' ITR. In certain embodiment, the vector genome is an AAV2 5' ITR, CB7 promoter, intron, an engineered huMfn2, a linker, a miR538, a rabbit beta globin poly A, and an AAV2 3' ITR. See, e.g., SEQ ID NOs: 1, 3, 69, 71, 73, 75, 77, and 79. The huMfn2 coding sequences are selected from those defined in the present specification. See, e.g., SEQ ID NO: 11 or a sequence 95% to 99.9% identical thereto, or SEQ ID NO: 11 or a sequence 95% to 99.9% identical thereto, or a fragment thereof as defined herein. Other elements of the vector genome or variations on these sequences may be selected for the vector genomes for certain embodiments of this invention.

Vector Production

For use in producing an AAV viral vector (e.g., a recombinant (r) AAV), the expression cassettes can be carried on any suitable vector, e.g., a plasmid, which is delivered to a packaging host cell. The plasmids useful in this invention may be engineered such that they are suitable for replication and packaging *in vitro* in prokaryotic cells, insect cells, mammalian cells, among others. Suitable transfection techniques and packaging host cells are known and/or can be readily designed by one of skill in the art.

Table 2.

AAV Vector	SEQ ID NOs (vector genome)
Syn.PI.hMfn2eng.link.hMfn2.miR1693.WPRE.bGH	1
Syn.PI.rMfn2eng.link.rMfn2 miR1518 WPRE.BGH	3
CB7.CI.hMfn2.GA.RBG	79
CB7.CI.hMfn2.GA.LINK.miR1518.RBG	77
CB7.CI.hMfn2.GA.LINK.miR538.RBG	75
CAG.CI.hMfn2.GA.WPRE.SV40	73
CAG.CI.hMfn2.GA.LINK.miR1518.WPRE.SV40	71

CAG.CI.hMfn2.GA.LINK.miR538.WPRE.SV40	69
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In certain embodiments, the production plasmid comprises a vector genome for packaging into a capsid which comprises: (a) an engineered nucleic acid sequence encoding human mitofusin 2; (b) a spacer sequence located between (a) and (c); (c) at least one miRNA sequence specific for endogenous human mitofusin 2 in a CMT2 patient located 3' to the sequence of (a) and (b); wherein the engineered nucleic acid sequence of (a) lacks the target site for the at least one miRNA, thereby preventing the miRNA from targeting the engineered human mitofusin 2 coding sequence; (c) regulatory sequences operably linked to (a) and (c). In certain embodiments, the production plasmid comprises a vector genome comprising nucleic acid sequence of SEQ ID NO: 1, 3, 69, 71, 73, 75, 77, or 79.

Methods for generating and isolating AAVs suitable for use as vectors are known in the art. See generally, e.g., Grieger & Samulski, 2005, "Adeno-associated virus as a gene therapy vector: Vector development, production and clinical applications," *Adv. Biochem. Engin/Biotechnol.* 99: 119-145; Buning et al., 2008, "Recent developments in adeno-associated virus vector technology," *J. Gene Med.* 10:717-733; and the references cited below, each of which is incorporated herein by reference in its entirety. For packaging a transgene into virions, the ITRs are the only AAV components required in cis in the same construct as the nucleic acid molecule containing the expression cassettes. The cap and rep genes can be supplied in trans.

In one embodiment, the expression cassettes described herein are engineered into a genetic element (e.g., a shuttle plasmid) which transfers the immunoglobulin construct sequences carried thereon into a packaging host cell for production a viral vector. In one embodiment, the selected genetic element may be delivered to an AAV packaging cell by any suitable method, including transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion. Stable AAV packaging cells can also be made. Alternatively, the expression cassettes may be used to generate a viral vector other than AAV, or for production of mixtures of antibodies in vitro. The methods used to make such constructs are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic

techniques. See, e.g., *Molecular Cloning: A Laboratory Manual*, ed. Green and Sambrook, Cold Spring Harbor Press, Cold Spring Harbor, NY (2012).

The term “AAV intermediate” or “AAV vector intermediate” refers to an assembled rAAV capsid which lacks the desired genomic sequences packaged therein. These may also
5 be termed an “empty” capsid. Such a capsid may contain no detectable genomic sequences of an expression cassette, or only partially packaged genomic sequences which are insufficient to achieve expression of the gene product. These empty capsids are non-functional to transfer the gene of interest to a host cell.

The recombinant adeno-associated virus (AAV) described herein may be generated
10 using techniques which are known. See, e.g., WO 2003/042397; WO 2005/033321, WO 2006/110689; US 7588772 B2. Such a method involves culturing a host cell which contains a nucleic acid sequence encoding an AAV capsid protein; a functional rep gene; an expression cassette as described herein flanked by AAV inverted terminal repeats (ITRs) and a transgene; and sufficient helper functions to permit packaging of the expression cassette into the AAV
15 capsid protein. Also provided herein is the host cell which contains a nucleic acid sequence encoding an AAV capsid; a functional rep gene; a vector genome as described; and sufficient helper functions to permit packaging of the vector genome into the AAV capsid protein. In one embodiment, the host cell is a HEK 293 cell. These methods are described in more detail in WO2017160360 A2, which is incorporated by reference herein. Methods of generating the
20 capsid, coding sequences therefor, and methods for production of rAAV viral vectors have been described. See, e.g., Gao, et al, *Proc. Natl. Acad. Sci. U.S.A.* 100 (10), 6081-6086 (2003) and US 2013/0045186A1.

In one embodiment, a production cell culture useful for producing a recombinant AAV is provided. Such a cell culture contains a nucleic acid which expresses the AAV capsid
25 protein in the host cell; a nucleic acid molecule suitable for packaging into the AAV capsid, e.g., a vector genome which contains AAV ITRs and a non-AAV nucleic acid sequence encoding a gene product operably linked to sequences which direct expression of the product in a host cell; and sufficient AAV rep functions and adenovirus helper functions to permit packaging of the nucleic acid molecule into the recombinant AAV capsid. In one
30 embodiment, the cell culture is composed of mammalian cells (e.g., human embryonic kidney 293 cells, among others) or insect cells (e.g., baculovirus).

Typically, the rep functions are from the same AAV source as the AAV providing the ITRs flanking the vector genome. In the examples herein, the AAV2 ITRs are selected and the AAV2 rep is used. Optionally, other rep sequences or another rep source (and optionally another ITR source) may be selected. For example, the rep may be, but is not limited to, AAV1 rep protein, AAV2 rep protein; or rep 78, rep 68, rep 52, rep 40, rep68/78 and rep40/52; or a fragment thereof; or another source. Optionally, the rep and cap sequences are on the same genetic element in the cell culture. There may be a spacer between the rep sequence and cap gene. Any of these AAV or mutant AAV capsid sequences may be under the control of exogenous regulatory control sequences which direct expression thereof in a host cell.

In one embodiment, cells are manufactured in a suitable cell culture (e.g., HEK 293) cells. Methods for manufacturing the gene therapy vectors described herein include methods well known in the art such as generation of plasmid DNA used for production of the gene therapy vectors, generation of the vectors, and purification of the vectors. In some embodiments, the gene therapy vector is an AAV vector and the plasmids generated are an AAV cis-plasmid encoding the AAV genome and the gene of interest, an AAV trans-plasmid containing AAV rep and cap genes, and an adenovirus helper plasmid. The vector generation process can include method steps such as initiation of cell culture, passage of cells, seeding of cells, transfection of cells with the plasmid DNA, post-transfection medium exchange to serum free medium, and the harvest of vector-containing cells and culture media.

In certain embodiments, the manufacturing process for rAAV.hMfn2 involves transient transfection of HEK293 cells with plasmid DNA. A single batch or multiple batches are produced by PEI-mediated triple transfection of HEK293 cells in PALL iCELLis bioreactors. Harvested AAV material are purified sequentially by clarification, TFF, affinity chromatography, and anion exchange chromatography in disposable, closed bioprocessing systems where possible.

The harvested vector-containing cells and culture media are referred to herein as crude cell harvest. In yet another system, the gene therapy vectors are introduced into insect cells by infection with baculovirus-based vectors. For reviews on these production systems, see generally, e.g., Zhang et al., 2009, "Adenovirus-adenovirus hybrid for large-scale recombinant adeno-associated virus production," Human Gene Therapy 20:922-929, the

contents of each of which is incorporated herein by reference in its entirety. Methods of making and using these and other AAV production systems are also described in the following U.S. patents, the contents of each of which is incorporated herein by reference in its entirety: 5,139,941; 5,741,683; 6,057,152; 6,204,059; 6,268,213; 6,491,907; 6,660,514; 6,951,753; 5 7,094,604; 7,172,893; 7,201,898; 7,229,823; and 7,439,065, which are incorporated herein by reference.

The crude cell harvest may thereafter be subject to additional method steps such as concentration of the vector harvest, diafiltration of the vector harvest, microfluidization of the vector harvest, nuclease digestion of the vector harvest, filtration of microfluidized 10 intermediate, crude purification by chromatography, crude purification by ultracentrifugation, buffer exchange by tangential flow filtration, and/or formulation and filtration to prepare bulk vector.

A two-step affinity chromatography purification at high salt concentration followed anion exchange resin chromatography are used to purify the vector drug product and to 15 remove empty capsids. These methods are described in more detail in International Patent Application No. PCT/US2016/065970, filed December 9, 2016, which is incorporated by reference herein. Purification methods for AAV8, International Patent Application No. PCT/US2016/065976, filed December 9, 2016, and rh10, International Patent Application No. PCT/US16/66013, filed December 9, 2016, entitled "Scalable Purification Method for 20 AAVrh10", also filed December 11, 2015, and for AAV1, International Patent Application No. PCT/US2016/065974, filed December 9, 2016, for "Scalable Purification Method for AAV1", filed December 11, 2015, are all incorporated by reference herein.

To calculate empty and full particle content, VP3 band volumes for a selected sample (*e.g.*, in examples herein an iodixanol gradient-purified preparation where # of GC = # 25 of particles) are plotted against GC particles loaded. The resulting linear equation ($y = mx + c$) is used to calculate the number of particles in the band volumes of the test article peaks. The number of particles (pt) per 20 μ L loaded is then multiplied by 50 to give particles (pt) /mL. Pt/mL divided by GC/mL gives the ratio of particles to genome copies (pt/GC). Pt/mL–GC/mL gives empty pt/mL. Empty pt/mL divided by pt/mL and x 100 gives the percentage of 30 empty particles.

Generally, methods for assaying for empty capsids and AAV vector particles with packaged genomes have been known in the art. See, e.g., Grimm et al., *Gene Therapy* (1999) 6:1322-1330; Sommer et al., *Molec. Ther.* (2003) 7:122-128. To test for denatured capsid, the methods include subjecting the treated AAV stock to SDS-polyacrylamide gel electrophoresis, consisting of any gel capable of separating the three capsid proteins, for example, a gradient gel containing 3-8% Tris-acetate in the buffer, then running the gel until sample material is separated, and blotting the gel onto nylon or nitrocellulose membranes, preferably nylon. Anti-AAV capsid antibodies are then used as the primary antibodies that bind to denatured capsid proteins, preferably an anti-AAV capsid monoclonal antibody, most preferably the B1 anti-AAV-2 monoclonal antibody (Wobus et al., *J. Virol.* (2000) 74:9281-9293). A secondary antibody is then used, one that binds to the primary antibody and contains a means for detecting binding with the primary antibody, more preferably an anti-IgG antibody containing a detection molecule covalently bound to it, most preferably a sheep anti-mouse IgG antibody covalently linked to horseradish peroxidase. A method for detecting binding is used to semi-quantitatively determine binding between the primary and secondary antibodies, preferably a detection method capable of detecting radioactive isotope emissions, electromagnetic radiation, or colorimetric changes, most preferably a chemiluminescence detection kit. For example, for SDS-PAGE, samples from column fractions can be taken and heated in SDS-PAGE loading buffer containing reducing agent (e.g., DTT), and capsid proteins were resolved on pre-cast gradient polyacrylamide gels (e.g., Novex). Silver staining may be performed using SilverXpress (Invitrogen, CA) according to the manufacturer's instructions or other suitable staining method, i.e., SYPRO ruby or coomassie stains. In one embodiment, the concentration of AAV vector genomes (vg) in column fractions can be measured by quantitative real time PCR (Q-PCR). Samples are diluted and digested with DNase I (or another suitable nuclease) to remove exogenous DNA. After inactivation of the nuclease, the samples are further diluted and amplified using primers and a TaqMan™ fluorogenic probe specific for the DNA sequence between the primers. The number of cycles required to reach a defined level of fluorescence (threshold cycle, Ct) is measured for each sample on an Applied Biosystems Prism 7700 Sequence Detection System. Plasmid DNA containing identical sequences to that contained in the AAV vector is employed to generate a standard curve in the Q-PCR reaction. The cycle threshold (Ct) values obtained from the samples are used to

determine vector genome titer by normalizing it to the Ct value of the plasmid standard curve. End-point assays based on the digital PCR can also be used.

In one aspect, an optimized q-PCR method is used which utilizes a broad-spectrum serine protease, e.g., proteinase K (such as is commercially available from Qiagen). More particularly, the optimized qPCR genome titer assay is similar to a standard assay, except that after the DNase I digestion, samples are diluted with proteinase K buffer and treated with proteinase K followed by heat inactivation. Suitably samples are diluted with proteinase K buffer in an amount equal to the sample size. The proteinase K buffer may be concentrated to 2-fold or higher. Typically, proteinase K treatment is about 0.2 mg/mL, but may be varied from 0.1 mg/mL to about 1 mg/mL. The treatment step is generally conducted at about 55 °C for about 15 minutes, but may be performed at a lower temperature (e.g., about 37 °C to about 50 °C) over a longer time period (e.g., about 20 minutes to about 30 minutes), or a higher temperature (e.g., up to about 60 °C) for a shorter time period (e.g., about 5 to 10 minutes). Similarly, heat inactivation is generally at about 95 °C for about 15 minutes, but the temperature may be lowered (e.g., about 70 to about 90 °C) and the time extended (e.g., about 20 minutes to about 30 minutes). Samples are then diluted (e.g., 1000-fold) and subjected to TaqMan analysis as described in the standard assay.

Additionally, or alternatively, droplet digital PCR (ddPCR) may be used. For example, methods for determining single-stranded and self-complementary AAV vector genome titers by ddPCR have been described. See, e.g., M. Lock et al, *Hu Gene Therapy Methods*, *Hum Gene Ther Methods*. 2014 Apr;25(2):115-25. doi: 10.1089/hgtb.2013.131. Epub 2014 Feb 14.

In brief, the method for separating rAAV particles having packaged genomic sequences from genome-deficient AAV intermediates involves subjecting a suspension comprising recombinant AAV viral particles and AAV capsid intermediates to fast performance liquid chromatography, wherein the AAV viral particles and AAV intermediates are bound to a strong anion exchange resin equilibrated at a high pH, and subjected to a salt gradient while monitoring eluate for ultraviolet absorbance at about 260 and about 280. The pH may be adjusted depending upon the AAV selected. See, e.g., WO2017/160360 (AAV9), WO2017/100704 (AAVrh10), WO 2017/100676 (e.g., AAV8), and WO 2017/100674 (AAV1), which are incorporated by reference herein. In this method, the AAV full capsids are

collected from a fraction which is eluted when the ratio of A260/A280 reaches an inflection point. In one example, for the Affinity Chromatography step, the diafiltered product may be applied to a Capture Select™ Poros- AAV2/9 affinity resin (Life Technologies) that efficiently captures the AAV2 serotype. Under these ionic conditions, a significant percentage of residual cellular DNA and proteins flow through the column, while AAV particles are efficiently captured.

NON-AAV AND NON-VIRAL VECTORS

A “vector” as used herein is a biological or chemical moiety comprising a nucleic acid sequence which can be introduced into an appropriate target cell for replication or expression of said nucleic acid sequence. Examples of a vector includes but not limited to a recombinant virus, a plasmid, Lipoplexes, a Polymersome, Polyplexes, a dendrimer, a cell penetrating peptide (CPP) conjugate, a magnetic particle, or a nanoparticle. In one embodiment, a vector is a nucleic acid molecule into which an exogenous or heterologous or engineered hMfn2 coding sequence (and/or at least one miRNA) may be inserted, which can then be introduced into an appropriate target cell. Such vectors preferably have one or more origin of replication, and one or more site into which the recombinant DNA can be inserted. Vectors often have means by which cells with vectors can be selected from those without, e.g., they encode drug resistance genes. Common vectors include plasmids, viral genomes, and "artificial chromosomes". Conventional methods of generation, production, characterization or quantification of the vectors are available to one of skill in the art.

In one embodiment, the vector is a non-viral plasmid that comprises an expression cassette described thereof, e.g., “naked DNA”, “naked plasmid DNA”, RNA, mRNA, shRNA, RNAi, etc. Optionally the plasmid or other nucleic acid sequence is delivered via a suitable device, e.g., via electrospray, electroporation. In other embodiments, the nucleic acid molecule is coupled with various compositions and nano particles, including, e.g., micelles, liposomes, cationic lipid - nucleic acid compositions, poly-glycan compositions and other polymers, lipid and/or cholesterol-based - nucleic acid conjugates, and other constructs such as are described herein. See, e.g., WO2014/089486, US 2018/0353616A1, US2013/0037977A1, WO2015/074085A1, US9670152B2, and US 8,853,377B2, X. Su et al, Mol. Pharmaceutics,

2011, 8 (3), pp 774–787; web publication: March 21, 2011; WO2013/182683, WO 2010/053572 and WO 2012/170930, all of which are incorporated herein by reference.

In certain embodiment, a non-viral vector is used for delivery of an miRNA transcript targeting endogenous hMfn2 at a site not present in the co-administered engineered hMfn2
5 sequence. In some embodiments, the miRNA is delivered at an amount greater than about 0.5 mg/kg (e.g., greater than about 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg, 2.5 mg/kg, 3.0 mg/kg, 4.0 mg/kg, 5.0 mg/kg, 6.0 mg/kg, 7.0 mg/kg, 8.0 mg/kg, 9.0 mg/kg, or 10.0 mg/kg) body weight of miRNA per dose. In some embodiments, the miRNA is delivered at an amount ranging from about 0.1-100 mg/kg (e.g., about 0.1-90 mg/kg, 0.1-80 mg/kg, 0.1-70 mg/kg, 0.1-60
10 mg/kg, 0.1-50 mg/kg, 0.1-40 mg/kg, 0.1-30 mg/kg, 0.1-20 mg/kg, 0.1-10 mg/kg) body weight of miRNA per dose. In some embodiments, the miRNA is delivered at an amount of or greater than about 1 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, or 500 mg per dose.

15 In certain embodiments, miRNA transcripts are encapsulated in a lipid nanoparticle (LNP). As used herein, the phrase "lipid nanoparticle" refers to a transfer vehicle comprising one or more lipids (e.g., cationic lipids, non- cationic lipids, and PEG-modified lipids). Preferably, the lipid nanoparticles are formulated to deliver one or more miRNA to one or more target cells (e.g., dorsal root ganglion, lower motor neurons and/or upper motor neurons,
20 or the cell types identified above in the CNS). Examples of suitable lipids include, for example, the phosphatidyl compounds (e.g., phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebroside, and gangliosides). Also contemplated is the use of polymers as transfer vehicles, whether alone or in combination with other transfer vehicles. Suitable polymers may include, for example, polyacrylates,
25 polyalkyanoacrylates, polylactide, polylactide- polyglycolide copolymers, polycaprolactones, dextran, albumin, gelatin, alginate, collagen, chitosan, cyclodextrins, dendrimers and polyethylenimine. In one embodiment, the transfer vehicle is selected based upon its ability to facilitate the transfection of a miRNA to a target cell. Useful lipid nanoparticles for miRNA comprise a cationic lipid to encapsulate and/or enhance the delivery
30 of miRNA into the target cell that will act as a depot for protein production. As used herein, the phrase "cationic lipid" refers to any of a number of lipid species that carry a net positive

charge at a selected pH, such as physiological pH. The contemplated lipid nanoparticles may be prepared by including multi-component lipid mixtures of varying ratios employing one or more cationic lipids, non-cationic lipids and PEG- modified lipids. Several cationic lipids have been described in the literature, many of which are commercially available. See, e.g.,

5 WO2014/089486, US 2018/0353616A1, and US 8,853,377B2, which are incorporated by reference. In certain embodiments, LNP formulation is performed using routine procedures comprising cholesterol, ionizable lipid, helper lipid, PEG-lipid and polymer forming a lipid bilayer around encapsulated mRNA (Kowalski et al., 2019, Mol. Ther. 27(4):710-728). In some embodiments, LNP comprises a cationic lipids (i.e. N-[1-(2,3-dioleoyloxy)propyl]-

10 N,N,N-trimethylammonium chloride (DOTMA), or 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)) with helper lipid DOPE. In some embodiments, LNP comprises an ionizable lipid Dlin-MC3-DMA ionizable lipids, or diketopiperazine-based ionizable lipids (cKK-E12). In some embodiments, polymer comprises a polyethyleneimine (PEI), or a poly(β -amino)esters (PBAEs). See, e.g., WO2014/089486, US 2018/0353616A1,

15 US2013/0037977A1, WO2015/074085A1, US9670152B2, and US 8,853,377B2, which are incorporated by reference.

In certain embodiments, the vector described herein is a "replication-defective virus" or a "viral vector" which refers to a synthetic or artificial viral particle in which an expression cassette containing a nucleic acid sequence encoding an engineered hMfn2 and/or at least one

20 miRNA targeting endogenous hMfn2 at a site not present on the sequence of the engineered hMfn2. Replication-defective viruses cannot generate progeny virions but retain the ability to infect target cells. In one embodiment, the genome of the viral vector does not include genes encoding the enzymes required to replicate (the genome can be engineered to be "gutless" - containing only the nucleic acid sequence encoding E2 flanked by the signals required for

25 amplification and packaging of the artificial genome), but these genes may be supplied during production. Therefore, it is deemed safe for use in gene therapy since replication and infection by progeny virions cannot occur except in the presence of the viral enzyme required for replication.

As used herein, a recombinant viral vector may be any suitable replication-defective

30 viral vector, including, e.g., a recombinant adeno-associated virus (AAV), an adenovirus, a bocavirus, a hybrid AAV/bocavirus, a herpes simplex virus or a lentivirus.

As used herein, the term “host cell” may refer to the packaging cell line in which a vector (e.g., a recombinant AAV) is produced. A host cell may be a prokaryotic or eukaryotic cell (e.g., human, insect, or yeast) that contains exogenous or heterologous DNA that has been introduced into the cell by any means, e.g., electroporation, calcium phosphate precipitation, microinjection, transformation, viral infection, transfection, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion. Examples of host cells may include, but are not limited to an isolated cell, a cell culture, an *Escherichia coli* cell, a yeast cell, a human cell, a non-human cell, a mammalian cell, a non-mammalian cell, an insect cell, an HEK-293 cell, a liver cell, a kidney cell, a cell of the central nervous system, a neuron, a glial cell, or a stem cell.

As used herein, the term “target cell” refers to any target cell in which expression of the hMfn2 and/or miRNA is desired. In certain embodiments, the term “target cell” is intended to reference the cells of the subject being treated for CMT2A. Examples of target cells may include, but are not limited to, cells within the central nervous system.

15

Compositions

Provided herein are compositions containing at least one vector comprising hMfn2.miR (e.g., an rAAV.hMfn2.miR stock) and/or at least one vector comprising miR and/or at least one vector comprising stock, and an optional carrier, excipient and/or preservative.

As used herein, a “stock” of rAAV refers to a population of rAAV. Despite heterogeneity in their capsid proteins due to deamidation, rAAV in a stock are expected to share an identical vector genome. A stock can include rAAV having capsids with, for example, heterogeneous deamidation patterns characteristic of the selected AAV capsid proteins and a selected production system. The stock may be produced from a single production system or pooled from multiple runs of the production system. A variety of production systems, including but not limited to those described herein, may be selected.

In certain embodiments, a composition comprises at least virus stock which is a recombinant AAV (rAAV) suitable for use in treating CMT2A alone or in combination with other vector stock or composition. In certain embodiments, the composition is suitable for use in preparing a medicament for treating CMT2A. In certain embodiments, a composition

comprises a virus stock which is a recombinant AAV (rAAV) suitable for use in treating CMT2A, said rAAV comprising: (a) an adeno-associated virus capsid, and (b) a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a coding sequence for an engineered human mitofusin 2, a spacer sequence, a coding sequence for at least one miRNA specifically targeted to endogenous human mitofusin at a site not present in the engineered human mitofusin coding sequence, and regulatory sequences which direct expression of the encoded gene products. In certain embodiments, a composition comprises separate vector stock comprising rAAV comprising: (a) an adeno-associated virus capsid, and (b) a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a coding sequence for an engineered human mitofusin 2, and regulatory sequences which direct expression of the encoded gene product and/or a separate vector stock comprising (a) an adeno-associated virus capsid, and (b) a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a coding sequence for at least one miRNA specifically targeted to endogenous human mitofusin 2 at a site not present in the engineered human mitofusin 2 coding sequence, and regulatory sequences which direct expression of the encoded gene product. In certain embodiments, the vector genome comprises a promoter, an enhancer, an intron, a human Mfn2 coding sequence, and a polyadenylation signal. In certain embodiments, the intron consists of a chicken beta actin splice donor and a rabbit β splice acceptor element. In certain embodiments, the vector genome further comprises an AAV2 5' ITR and an AAV2 3' ITR which flank all elements of the vector genome.

The rAAV.hMfn2.miR (rAAV.hMfn2 or another vector) may be suspended in a physiologically compatible carrier to be administered to a human CMT2A patient. In certain embodiments, for administration to a human patient, the vector is suitably suspended in an aqueous solution containing saline, a surfactant, and a physiologically compatible salt or
5 mixture of salts. Suitably, the formulation is adjusted to a physiologically acceptable pH, e.g., in the range of pH 6 to 9, or pH 6.5 to 7.5, pH 7.0 to 7.7, or pH 7.2 to 7.8. As the pH of the cerebrospinal fluid is about 7.28 to about 7.32, or a pH of 7.2 to 7.4, for intrathecal delivery, a pH within this range may be desired; whereas for intravenous delivery, a pH of about 6.8 to about 7.2 may be desired. However, other pHs within the broadest ranges and these subranges
10 may be selected for other route of delivery.

In certain embodiments, the formulation may contain a buffered saline aqueous solution not comprising sodium bicarbonate. Such a formulation may contain a buffered saline aqueous solution comprising one or more of sodium phosphate, sodium chloride, potassium chloride, calcium chloride, magnesium chloride and mixtures thereof, in water, such as a Harvard's buffer. The aqueous solution may further contain Kolliphor® P188, a poloxamer which is commercially available from BASF which was formerly sold under the trade name Lutrol® F68. The aqueous solution may have a pH of 7.2 or a pH of 7.4.

In another embodiment, the formulation may contain a buffered saline aqueous solution comprising 1 mM Sodium Phosphate (Na_3PO_4), 150 mM sodium chloride (NaCl), 3mM potassium chloride (KCl), 1.4 mM calcium chloride (CaCl_2), 0.8 mM magnesium chloride (MgCl_2), and 0.001% Kolliphor® 188. See, e.g., harvardapparatus.com/harvard-apparatus-perfusion-fluid.html. In certain embodiments, Harvard's buffer is preferred.

In other embodiments, the formulation may contain one or more permeation enhancers. Examples of suitable permeation enhancers may include, e.g., mannitol, sodium glycocholate, sodium taurocholate, sodium deoxycholate, sodium salicylate, sodium caprylate, sodium caprate, sodium lauryl sulfate, polyoxyethylene-9-laurel ether, or EDTA.

In another embodiment, the composition includes a carrier, diluent, excipient and/or adjuvant. Suitable carriers may be readily selected by one of skill in the art in view of the indication for which the transfer virus is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (e.g., phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. The buffer/carrier should include a component that prevents the rAAV, from sticking to the infusion tubing but does not interfere with the rAAV binding activity in vivo.

Optionally, the compositions may contain, in addition to the vector (e.g., rAAV) and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable chemical stabilizers include gelatin and albumin.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents,

buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a host. Delivery vehicles such as liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, may be used for the introduction of the compositions of the present invention into suitable host cells. In particular, the rAAV vector delivered transgenes may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

In one embodiment, a composition includes a final formulation suitable for delivery to a subject, e.g., is an aqueous liquid suspension buffered to a physiologically compatible pH and salt concentration. Optionally, one or more surfactants are present in the formulation. In another embodiment, the composition may be transported as a concentrate which is diluted for administration to a subject. In other embodiments, the composition may be lyophilized and reconstituted at the time of administration.

A suitable surfactant, or combination of surfactants, may be selected from among non-ionic surfactants that are nontoxic. In one embodiment, a difunctional block copolymer surfactant terminating in primary hydroxyl groups is selected, e.g., such as Pluronic® F68 [BASF], also known as Poloxamer 188, which has a neutral pH, has an average molecular weight of 8400. Other surfactants and other Poloxamers may be selected, i.e., nonionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly(propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide)), SOLUTOL HS 15 (Macrogol-15 Hydroxystearate), LABRASOL (Polyoxy caprylic glyceride), polyoxy 10 oleyl ether, TWEEN (polyoxyethylene sorbitan fatty acid esters), ethanol and polyethylene glycol. In one embodiment, the formulation contains a poloxamer. These copolymers are commonly named with the letter "P" (for poloxamer) followed by three digits: the first two digits x 100 give the approximate molecular mass of the polyoxypropylene core, and the last digit x 10 gives the percentage polyoxyethylene content. In one embodiment Poloxamer 188 is selected. The surfactant may be present in an amount up to about 0.0005 % to about 0.001% of the suspension.

The vectors are administered in sufficient amounts to transfect the cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse effects, or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Optionally, routes other than intrathecal administration
 5 may be used, such as, e.g., direct delivery to a desired organ (*e.g.*, the liver (optionally via the hepatic artery), lung, heart, eye, kidney), oral, inhalation, intranasal, intratracheal, intraarterial, intraocular, intravenous, intramuscular, subcutaneous, intradermal, and other parental routes of administration. Routes of administration may be combined, if desired.

Dosages of the vector will depend primarily on factors such as the condition being
 10 treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective human dosage of viral vector is generally in the range of from about 25 to about 1000 microliters to about 100 mL of solution containing concentrations of from about 1×10^9 to 1×10^{16} genomes virus vector (to treat an average subject of 70 kg in body weight) including all integers or fractional amounts within the range, and preferably 1.0×10^{12} GC to 1.0×10^{14} GC for a human patient. In one embodiment, the compositions are
 15 formulated to contain at least 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , or 9×10^9 GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , 5×10^{10} , 6×10^{10} , 7×10^{10} , 8×10^{10} , or 9×10^{10} GC per dose including all integers or
 20 fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1×10^{11} , 2×10^{11} , 3×10^{11} , 4×10^{11} , 5×10^{11} , 6×10^{11} , 7×10^{11} , 8×10^{11} , or 9×10^{11} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1×10^{12} , 2×10^{12} , 3×10^{12} , 4×10^{12} , 5×10^{12} , 6×10^{12} , 7×10^{12} , 8×10^{12} , or 9×10^{12} GC per dose including all integers or fractional amounts
 25 within the range. In another embodiment, the compositions are formulated to contain at least 1×10^{13} , 2×10^{13} , 3×10^{13} , 4×10^{13} , 5×10^{13} , 6×10^{13} , 7×10^{13} , 8×10^{13} , or 9×10^{13} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1×10^{14} , 2×10^{14} , 3×10^{14} , 4×10^{14} , 5×10^{14} , 6×10^{14} , 7×10^{14} , 8×10^{14} , or 9×10^{14} GC per dose including all integers or fractional amounts within the
 30 range. In another embodiment, the compositions are formulated to contain at least 1×10^{15} , 2×10^{15} , 3×10^{15} , 4×10^{15} , 5×10^{15} , 6×10^{15} , 7×10^{15} , 8×10^{15} , or 9×10^{15} GC per dose including all

integers or fractional amounts within the range. In one embodiment, for human application the dose can range from 1×10^{10} to about 1×10^{12} GC per dose including all integers or fractional amounts within the range.

In certain embodiments, the dose is in the range of about 1×10^9 GC/g brain mass to about 1×10^{12} GC/g brain mass. In certain embodiments, the dose is in the range of about 1×10^{10} GC/g brain mass to about 3.33×10^{11} GC/g brain mass. In certain embodiments, the dose is in the range of about 3.33×10^{11} GC/g brain mass to about 1.1×10^{12} GC/g brain mass. In certain embodiments, the dose is in the range of about 1.1×10^{12} GC/g brain mass to about 3.33×10^{13} GC/g brain mass. In certain embodiments, the dose is lower than 3.33×10^{11} GC/g brain mass. In certain embodiments, the dose is lower than 1.1×10^{12} GC/g brain mass. In certain embodiments, the dose is lower than 3.33×10^{13} GC/g brain mass. In certain embodiments, the dose is about 1×10^{10} GC/g brain mass. In certain embodiments, the dose is about 2×10^{10} GC/g brain mass. In certain embodiments, the dose is about 2×10^{10} GC/g brain mass. In certain embodiments, the dose is about 3×10^{10} GC/g brain mass. In certain embodiments, the dose is about 4×10^{10} GC/g brain mass. In certain embodiments, the dose is about 5×10^{10} GC/g brain mass. In certain embodiments, the dose about 6×10^{10} GC/g brain mass. In certain embodiments, the dose is about 7×10^{10} GC/g brain mass. In certain embodiments, the dose about 8×10^{10} GC/g brain mass. In certain embodiments, the dose is about 9×10^{10} GC/g brain mass. In certain embodiments, the dose is about 1×10^{11} GC/g brain mass. In certain embodiments, the dose is about 2×10^{11} GC/g brain mass. In certain embodiments, the dose is about 3×10^{11} GC/g brain mass. In certain embodiments, the dose is about 4×10^{11} GC/g brain mass. In certain embodiments, the dose is administered to humans as a flat dose in the range of about 1.44×10^{13} to 4.33×10^{14} GC of the rAAV. In certain embodiments, the dose is administered to humans as a flat dose in the range of about 1.44×10^{13} to 2×10^{14} GC of the rAAV. In certain embodiments, the dose is administered to humans as a flat dose in the range of about 3×10^{13} to 1×10^{14} GC of the rAAV. In certain embodiments, the dose is administered to humans as a flat dose in the range of about 5×10^{13} to 1×10^{14} GC of the rAAV. In some embodiments, the compositions can be formulated in dosage units to contain an amount of AAV that is in the range of about 1×10^{13} to 8×10^{14} GC of the rAAV. In some embodiments, the compositions can be formulated in dosage units to contain an amount of rAAV that is in the range of about 1.44×10^{13} to 4.33×10^{14} GC of the

rAAV. In some embodiments, the compositions can be formulated in dosage units to contain an amount of rAAV that is in the range of about 3×10^{13} to 1×10^{14} GC of the rAAV. In some embodiments, the compositions can be formulated in dosage units to contain an amount of rAAV that is in the range of about 5×10^{13} to 1×10^{14} GC of the rAAV.

5 In certain embodiments, the vector is administered to a subject in a single dose. In certain embodiments, vector may be delivered via multiple injections (for example 2 doses) is desired.

 The dosage will be adjusted to balance the therapeutic benefit against any side effects and such dosages may vary depending upon the therapeutic application for which the
10 recombinant vector is employed. The levels of expression of the transgene can be monitored to determine the frequency of dosage resulting in viral vectors, preferably AAV vectors containing the minigene. Optionally, dosage regimens similar to those described for therapeutic purposes may be utilized for immunization using the compositions provided herein.

15 As used herein, the terms “intrathecal delivery” or “intrathecal administration” refer to a route of administration via an injection into the spinal canal, more specifically into the subarachnoid space so that it reaches the cerebrospinal fluid (CSF). Intrathecal delivery may include lumbar puncture, intraventricular (including intracerebroventricular (ICV)), suboccipital/intracisternal, and/or C1-2 puncture. For example, material may be introduced for
20 diffusion throughout the subarachnoid space by means of lumbar puncture. In another example, injection may be into the cisterna magna.

 As used herein, the terms “intracisternal delivery” or “intracisternal administration” refer to a route of administration directly into the cerebrospinal fluid of the cisterna magna cerebellomedullaris, more specifically via a suboccipital puncture or by direct injection into the
25 cisterna magna or via permanently positioned tube.

 Compositions comprising the miR target sequences described herein for repressing endogenous hMfn2 (e.g., in CMT2A patients) are generally targeted to one or more different cell types within the central nervous system, including, but not limited to, neurons (including, e.g., lower motor neurons and/or primary sensory neurons. These may include, e.g.,
30 pyramidal, purkinje, granule, spindle, and interneuron cells).

Uses

The vectors and compositions provided herein are useful for treating a patient having Charcot-Marie-Tooth (CMT) disorder, neuropathy, or various symptoms associated therewith. A combination regimen or co-therapy for treating a patient having CMT2A is provided. In certain embodiments, this regimen or co-therapy comprises co-administering (a) a recombinant nucleic acid sequence encoding an engineered human mitofusin 2 coding sequence operably linked to regulatory sequences which direct expression thereof in a human target cell, wherein the human mitofusin 2 coding sequence has the sequence of SEQ ID NO: 15 or a sequence at least 95% identical thereto and which differs from endogenous human mitofusin 2 in the CMT2A patient by having a mismatch in the miRNA target sequence of (b), and (b) a coding sequence for at least one miRNA specific for an endogenous human mitofusin 2 sequence in a human CMT2A subject, wherein the mRNA is operably linked to regulatory sequences which direct expression thereof in the subject.

In certain embodiments, this regimen or co-therapy comprises co-administering (a) a recombinant nucleic acid sequence encoding an engineered human mitofusin 2 coding sequence operably linked to regulatory sequences which direct expression thereof in a human target cell, wherein the engineered human mitofusin 2 coding sequence has the sequence of SEQ ID NO: 11 or a sequence at least 95% identical thereto and which differs from endogenous human mitofusin 2 in the CMT2A patient by having a mismatch in the miRNA target sequence of (b), and (b) a coding sequence for at least one miRNA specific for an endogenous human mitofusin 2 sequence in a human CMT2A subject, wherein the at least one miRNA coding sequence is operably linked to regulatory sequences which direct expression thereof in the subject, and wherein the at least one miRNA coding sequence comprises a sequence of one or more of an miRNA targeting sequence comprising SEQ ID NO: 89 (miR538, 59 nt) or an miRNA comprising one or more of antisense sequence of SEQ ID NO: 27.

In certain embodiments, this regimen or co-therapy for treating a patient having CMT2A comprises co-administering (a) a recombinant nucleic acid sequence encoding an engineered human mitofusin 2 coding sequence operably linked to regulatory sequences which direct expression thereof in a human target cell, wherein the human mitofusin 2 coding sequence is engineered to differs from endogenous human mitofusin 2 in the CMT2A patient

by having a mismatch in the miRNA target sequence of (b), and (b) a coding sequence for at least one miRNA specific for an endogenous human mitofusin 2 sequence in a human CMT2A subject, wherein the miRNA coding sequence is operably linked to regulatory sequences which direct expression thereof in the subject, and wherein the at least one miRNA coding

5 sequence has a sequence of one or more of: an miRNA coding sequence comprising SEQ ID NO: 15 (miR1693, 64 nt); an miRNA coding sequence comprising at least 60 consecutive nucleotides of SEQ ID NO: 15; an miRNA coding sequence comprising at least 99% identity to SEQ ID NO: 15 which comprises a sequence with 100% identity to about nucleotide 6 to about nucleotide 26 of SEQ ID NO: 15 (or SEQ ID NO: 68); or an miRNA coding sequence

10 comprising one or more of:

(i) TTGACGTCCAGAACCTGTTCT, SEQ ID NO: 27; (ii) AGAAGTGGGCACTTAGAGTTG, SEQ ID NO: 28; (iii) TTCAGAAGTGGGCACTTAGAG, SEQ ID NO: 29; (iv) TTGTCAATCCAGCTGTCCAGC, SEQ ID NO: 30; (v) CAAACTTGGTCTTCACTGCAG, SEQ ID NO: 31 ; (vi)

15 AAACCTTGAGGACTACTGGAG, SEQ ID NO: 32; (vii) TAACCATGGAAACCATGAACT, SEQ ID NO: 33; (viii) ACAACAAGAATGCCCATGGAG, SEQ ID NO: 34; (ix) AAAGGTCCCAGACAGTTCCTG, SEQ ID NO: 35; (x) TGTTTCATGGCGGCAATTCCT, SEQ ID NO: 36; (xi) TGAGGTTGGCTATTGATTGAC, SEQ ID NO: 37; (xii)

20 TTCTCACACAGTCAACACCTT, SEQ ID NO: 38; (xiii) TTTCTCGCAGTAAACCTGCT, SEQ ID NO: 39; (xiv) AGAAATGGAATCAATGTCTT, SEQ ID NO: 40; (xv) TGAACAGGACATCACCTGTGA, SEQ ID NO: 41; (xvi) AATACAAGCAGGTATGTGAAC, SEQ ID NO: 42; (xvii) TAAACCTGCTGCTCCCGAGCC, SEQ ID NO: 43; (xviii)

25 TAGAGGAGGCCATAGAGCCCA, SEQ ID NO: 44; (xix) TCTACCCGCAGGAAGCAATTG, SEQ ID NO: 45; or (xx) CTCCTTAGCAGACACAAAGAA, SEQ ID NO: 46, or combinations of any of (i) through (xx). In certain embodiments, a first vector comprises the nucleic acid (a) and a second, different vector, comprises at least one miRNA (b). In certain embodiments, the first vector

30 is a viral vector and/or the second vector is a viral vector and the first and the second viral vector may be from the same virus source or may be different. In certain embodiments, the

first vector is a non-viral vector, the second vector is a non-viral vector and the first and the second vectors may be same composition or may be different.

In certain embodiments, the vectors and compositions provided herein are useful for treating patients having Mfn2-induced lipomatosis. In certain embodiments, the vectors and compositions provided herein are useful for treating patients having multiple symmetric lipomatosis. Multiple symmetric lipomatosis is associated with rare genetic mutation in Mfn2 gene, and characterizable by large deposits and accumulate of fat tissue in upper bodies and gradually lose fat tissue in arms and legs (Rocha, N., et al., Human biallelic MFN2 mutations induce mitochondrial dysfunction, upper body adipose hyperplasia, and suppression of leptin expression, *eLife*, 2017, 6:1-27, April 19, 2017). In certain embodiments, the vectors and compositions provided herein are useful for treating patients having severe early-onset neuropathy due to Mfn2 deficiency. In certain embodiments, the vectors and compositions provided herein are useful for treating patients having Alzheimer's Disease (AD), Parkinson's Disease (PD), cardiomyopathies, and Mfn2-associated pathogenesis in various cancers. It has been evidenced that there is a suggested link between Mfn2 deregulation and AD and PD, e.g., link between single nucleotide polymorphism in the Mfn2 gene and AD risk (Filadi, R., et al., *Cell Death and Disease*, 2018, 9:330).

Optionally, the vectors and compositions provided herein may be used in combination with one or more co-therapies selected from: acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), tricyclic antidepressants or antiepileptic drugs, such as carbamazepine or gabapentin. In still other embodiments, the vectors may be delivered in a combination with an immunomodulatory regimen involving one or more steroids, e.g., prednisone.

As used herein, the term Computed Tomography (CT) refers to radiography in which a three-dimensional image of a body structure is constructed by computer from a series of plane cross-sectional images made along an axis.

The term "substantial homology" or "substantial similarity," when referring to a nucleic acid, or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95 to 99% of the aligned sequences. Preferably, the homology is over full-length sequence, or an open reading frame thereof, or

another suitable fragment which is at least 15 nucleotides in length. Examples of suitable fragments are described herein.

The terms “sequence identity” “percent sequence identity” or “percent identical” in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length of the genome, the full-length of a gene coding sequence, or a fragment of at least about 500 to 5000 nucleotides, is desired. However, identity among smaller fragments, e.g., of at least about nine nucleotides, usually at least about 20 to 24 nucleotides, at least about 28 to 32 nucleotides, at least about 36 or more nucleotides, may also be desired. Similarly, “percent sequence identity” may be readily determined for amino acid sequences, over the full-length of a protein, or a fragment thereof. Suitably, a fragment is at least about 8 amino acids in length and may be up to about 700 amino acids. Examples of suitable fragments are described herein.

By the term “highly conserved” is meant at least 80% identity, preferably at least 90% identity, and more preferably, over 97% identity. Identity is readily determined by one of skill in the art by resort to algorithms and computer programs known by those of skill in the art.

Unless otherwise specified by an upper range, it will be understood that a percentage of identity is a minimum level of identity and encompasses all higher levels of identity up to 100% identity to the reference sequence. Unless otherwise specified, it will be understood that a percentage of identity is a minimum level of identity and encompasses all higher levels of identity up to 100% identity to the reference sequence. For example, “95% identity” and “at least 95% identity” may be used interchangeably and include 95, 96, 97, 98, 99 up to 100% identity to the referenced sequence, and all fractions therebetween.

Unless otherwise specified, numerical values will be understood to be subject to conventional mathematic rounding rules.

Generally, when referring to “identity”, “homology”, or “similarity” between two different adeno-associated viruses, “identity”, “homology” or “similarity” is determined in reference to “aligned” sequences. “Aligned” sequences or “alignments” refer to multiple nucleic acid sequences or protein (amino acids) sequences, often containing corrections for missing or additional bases or amino acids as compared to a reference sequence. In the

examples, AAV alignments are performed using the published AAV9 sequences as a reference point. Alignments are performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs. Examples of such programs include, “Clustal Omega”, “Clustal W”, “CAP Sequence Assembly”, “MAP”, and “MEME”, which are
5 accessible through Web Servers on the internet. Other sources for such programs are known to those of skill in the art. Alternatively, Vector NTI utilities are also used. There are also a number of algorithms known in the art that can be used to measure nucleotide sequence identity, including those contained in the programs described above. As another example, polynucleotide sequences can be compared using Fasta™, a program in GCG Version 6.1.
10 Fasta™ provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta™ with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference. Multiple sequence alignment programs are also available
15 for amino acid sequences, *e.g.*, the “Clustal Omega”, “Clustal X”, “MAP”, “PIMA”, “MSA”, “BLOCKMAKER”, “MEME”, and “Match-Box” programs. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program which provides at least the level of identity or alignment as that provided by the referenced
20 algorithms and programs. See, *e.g.*, J. D. Thomson et al, Nucl. Acids. Res., “A comprehensive comparison of multiple sequence alignments”, 27(13):2682-2690 (1999).

It is to be noted that the term “a” or “an” refers to one or more. As such, the terms “a (or “an”), “one or more,” and “at least one” are used interchangeably herein.

The words “comprise”, “comprises”, and “comprising” are to be interpreted
25 inclusively rather than exclusively. The words “consist”, “consisting”, and its variants, are to be interpreted exclusively, rather than inclusively. While various embodiments in the specification are presented using “comprising” language, under other circumstances, a related embodiment is also intended to be interpreted and described using “consisting of” or “consisting essentially of” language.

As used herein, the term “about” means a variability of 10 % ($\pm 10\%$, e.g., ± 1 , ± 2 , ± 3 , ± 4 , ± 5 , ± 6 , ± 7 , ± 8 , ± 9 , ± 10 , or values therebetween) from the reference given, unless otherwise specified.

As used herein, “disease”, “disorder” and “condition” are used interchangeably, to
5 indicate an abnormal state in a subject.

As used herein, the term “CMT2A-related symptom(s)” or “symptom(s)” refers to symptom(s) found in CMT2A patients as well as in CMT2A animal models. Early symptoms of CMT may include one or more of clumsiness, slight difficulty in walking because of trouble picking up the feet, weak leg muscles, fatigue, absence of reflexes. Common symptoms of
10 CMT2A include, foot deformity (very high arched foot/feet), difficulty lifting foot at the ankle (foot drop), curled toes (known as hammer toes), loss of lower leg muscle, which leads to skinny calves, numbness or burning sensation in the feet or hands, “Slapping” when walking (feet hit the floor hard when walking), weakness of the hips, legs, or feet, leg and hand cramps, Loss of balance, tripping, and falling, difficulty grasping and holding objects and
15 opening jars and bottles, pain (both nerve pain and arthritic pain). Later symptoms of CMT2A may include, e.g., a similar symptoms in the arms and hands, curvature of the spine (scoliosis). Other reported/known symptoms of CMT2A may include, e.g., speech and swallowing difficulties, breathing difficulties, especially when lying flat, hearing loss, vision loss, vocal cord paralysis.

20 “Patient” or “subject” as used herein means a male or female human, and animal models (including, e.g., dogs, non-human primates, rodents, or other suitable models) used for clinical research. In one embodiment, the subject of these methods and compositions is a human diagnosed with CMT2A. In certain embodiments, the human subject of these methods and compositions is a prenatal, a newborn, an infant, a toddler, a preschool, a grade-schooler,
25 a teen, a young adult or an adult. In a further embodiment, the subject of these methods and compositions is a pediatric CTM2A patient.

As used herein, the term “a therapeutic level” means an Mfn2 activity at least about 5%, about 8%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%,
30 about 80%, about 85%, about 90%, about 95%, about 100%, more than 100%, about 2-fold, about 3-fold, or about 5-fold of a healthy control. Suitable assays for measuring the activity of

an hMfn2 are known in the art. In some embodiments, such therapeutic levels of the one or more subunit protein may result in alleviation of the CMT2A related symptom(s); reversal of certain CMT2A-related symptoms and/or prevention of progression of CMT2A-related certain symptoms; or any combination thereof. In certain embodiments, the human Mfn2 delivered
5 by the compositions and regimens provided herein has the amino acid sequence of a functional endogenous wild-type protein. In certain embodiments, the sequence is the amino acid sequence of SEQ ID NO: 19 or a functional protein which is at about 95 to 100% identity to functional, human Mfn2 protein.

The term “expression” is used herein in its broadest meaning and comprises the
10 production of RNA or of RNA and protein. With respect to RNA, the term “expression” or “translation” relates in particular to the production of peptides or proteins. Expression may be transient or may be stable.

Additionally optionally, an expression cassette (and a vector genome) may comprise one or more dorsal root ganglion (drg)- miRNA targeting sequences in the UTR, e.g., to
15 reduce drg toxicity and/or axonopathy. See, e.g., PCT/US2019/67872, filed December 20, 2019 and now published as WO 2020/132455, US Provisional Patent Application No. 63/023593, filed May 12, 2020, and US Provisional Patent Application No. 63/038488, filed June 12, 2020, all entitled “Compositions for Drg-Specific Reduction of Transgene Expression”, which are incorporated herein in their entireties. In some embodiments, an
20 expression cassette may be delivered via a genetic element (e.g., a plasmid) to a packaging host cell and packaged into the capsid of a viral vector (e.g., a viral particle).

As used herein, the term “operably linked” refers to both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest.

25 The term “heterologous” when used with reference to a protein or a nucleic acid indicates that the protein or the nucleic acid comprises two or more sequences or subsequences which are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid. For example, in one embodiment, the nucleic
30 acid has a promoter from one gene arranged to direct the expression of a coding sequence from a different gene. Thus, with reference to the coding sequence, the promoter is heterologous.

As described herein, regulatory elements comprise but not limited to: promoter; enhancer; transcription factor; transcription terminator; efficient RNA processing signals such as splicing and polyadenylation signals (polyA); sequences that stabilize cytoplasmic mRNA, for example Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE); sequences that enhance translation efficiency (i.e., Kozak consensus sequence).

The term “translation” in the context of the present invention relates to a process at the ribosome, wherein an mRNA strand controls the assembly of an amino acid sequence to generate a protein or a peptide.

Expression cassettes containing engineered hMfn2 coding sequences are provided herein, e.g., Syn.PI.hMfn2eng.link.hMfn2.miR1693.WPRE.bGH (nt 223 to 4455 of SEQ ID NO: 1); CB7.CI.hMfn2.GA.RBG (nt 259 to 4370 of SEQ ID NO: 79); CB7.CI.hMfn2.GA.LINK.miR1518.RBG (nt 259 to 4710 of SEQ ID NO: 77); CB7.CI.hMfn2.GA.LINK.miR538.RBG (nt 259 to 4626 of SEQ ID NO: 75); CAG.CI.hMfn2.GA.WPRE.SV40 (nt 192 to 4262 of SEQ ID NO: 73); CAG.CI.hMfn2.GA.LINK.miR1518.WPRE.SV40 (nt 192 to 4474 of SEQ ID NO: 71); and CAG.CI.hMfn2.GA.LINK.miR538.WPRE.SV40 (nt 192 to 4474 of SEQ ID NO: 69).

Expression cassettes containing engineered rMfn2 coding sequences are provided herein, e.g., Syn.PI.rMfn2eng.link.rMfn2 miR1518 WPRE.BGH (nt 223 to 4430 of SEQ ID NO: 3).

Unless defined otherwise in this specification, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art and by reference to published texts, which provide one skilled in the art with a general guide to many of the terms used in the present application.

The following examples are illustrative only and are not intended to limit the present invention.

EXAMPLES

The following examples are illustrative and are not intended to limit the present invention.

Charcot-Marie-Tooth neuropathy type 2A (CMT2A) is caused by mutations in the gene encoding mitofusin 2 (Mfn2), a protein required for mitochondrial fusion and transport along axons. In most cases of CMT2A is caused by autosomal dominant missense mutations that act in a dominant-negative manner. In rare cases, CMT2A is caused by recessive loss-of-
 5 function mutations. A Mfn2 mutations are selectively toxic to lower motor neurons and primary sensory neurons in dorsal root ganglia (DRG).

A gene therapy was developed to restore mitofusin-2 expression by overexpression of Mfn2 to overcome dominant negative activity of mutant Mfn2. An AAV gene therapy technology is used to transfer an Mfn2 expression cassette into neurons. In some embodiments
 10 an Mfn2 expression cassette contains a miRNA connected via a linker, wherein miRNA is targeted to knock down mutant (defective) Mfn2, to eliminate mutant Mfn2 and supply with normal mitofurin-2 protein. The injection of the therapeutic article is delivered via intra-cisterna magna (ICM), or intravenously. ICM delivery of AAV efficiently targets lower motor neurons and primary sensor neurons. A Mfn2 expression cassette, with engineered Mfn2 gene,
 15 highly optimized for expression in humans was used in combination with an AAV capsid with highly improved central nervous system (CNS) transduction.

EXAMPLE 1: CMT2A Vector Strategy

In an AAV transgene cassette strategy 1, we generated a Mfn expression cassette
 20 comprising a promoter, an intron, an engineered Mfn2 cDNA and polyA. In AAV transgene cassette strategy 2, we generated a Mfn2 expression cassette comprising a promoter, an intron, an engineered Mfn2 cDNA, which was engineered to optimize for expression and resistant to the miRNA, an miRNA targeting endogenous mutant Mfn2 and a polyA. Additionally, in an AAV transgene strategy 2, a construct was identified for efficient expression of a miRNA and
 25 cDNA in vivo, wherein the construct comprised a synapsin promoter, a cDNA (engineered Mfn2), a linker, a miRNA, wherein a miRNA located at the 3' end of cDNA, a WRPE enhancer, and polyA (data not shown).

To knock down endogenous Mfn2 gene, various miRNA sequences various miRNA sequences were examined: miR238, miR1202, miR1518, and miR2282, among other, data for
 30 which is not shown, were examined. For western blot analysis, rat B104 cells were transfected with in vitro Block-iT plasmids (ThermoFisher) containing a CMV promoter. emGFP

transgene, a cloning site for miRNA and TK polyA. miRNAs were designed using Block-iT online software. Cells were transfected and selected for expression with blasticidin. Surviving cells were mostly positive for GFP and overall transfection efficiency could be determined. B104 cells were harvested after 72 hours and prepared for western blot analysis. Western blot

5 was probed with antibodies against Mfn2 and b-actin as loading control. FIG 1A and FIG 1B illustrate knockdown of endogenous Mfn2 RNA, as measured by qPCR, in mouse brain (FIG 1A) and spinal cord (FIG 1B) in B6 mice following intravenous administration of AAV-mediated delivery of miRNA. Mice were necropsied at day 14 post administration, following which brain and spinal cord tissues were harvested and homogenized. RNA was extracted

10 from the samples and used for qPCR with TaqMan primers against Mfn2. Four treatment groups were examined (N=5 per group): PBS, AAV9.PHP.eB.CB7.miR_NT (non-targeted at a dose of 3×10^{11} GC), AAV9.PHP.eB.CB7.miR_1518 (at a dose of 3×10^{11} GC), and AAV9.PHP.eB.CB7.miR_1202 (at a dose of 3×10^{11} GC). Among the sequences examined, miR1518 illustrated best knockdown of endogenous Mfn2.

15 FIG 2A to FIG 2C illustrate Mfn2 RNA fold expression following delivery AAV vector comprising rat Mfn2 (rMfn2) cDNA transgene and mR1518, wherein AAV vector was administered in mice intravenously at a dose of 3×10^{11} GC. Mice were necropsied at day 14 post administration, following which brain and spinal cord tissues were harvested and homogenized. RNA was extracted from the samples and used for qPCR with TaqMan primers

20 against Mfn2. Two treatment groups were examined (N=5 per group): PBS and AAV9.PHP.eB.CB7.miR_1518+rMfn2.

EXAMPLE 2: Mouse study of AAV vector comprising expression cassette of an engineered Mfn2 transgene with miRNA

25 In initial study, wildtype mice were injected intravenously with 3×10^{11} GC of AAV vectors, comprising an engineered rat Mfn2 (rMfn2) cDNA nucleic acid sequence (SEQ ID NO: 12) and miRNA1518 (SEQ ID NO: 16). Four treatment groups were examined (N=5 in each group): PBS, AAV9-PHP.eB.CAG.rMfn2 opt.link.rMfn2miR1518.WPRE.SV40, AAV9-PHP.eB.CB7.Cl.rMfn2 opt.link.rMfn2miR1518.rBG, and AAV9-

30 PHP.eB.hSyn.PI.rMfn2opt.link.rMfn2miR1518.WPRE.bGH (wherein the vector genome is of SEQ ID NO: 3). Mice were sacrificed 14 days post injection. Brain and spinal cord were

harvested and homogenized. RNA was extracted from the samples and used for qPCR with TaqMan primers against rat Mfn2 engineered cDNA. FIG 4 illustrates plotted quantitation of fold-expression of rat Mfn2 (rMfn2) cDNA expression in spinal cord of treated mice following AAV vector delivery of engineered rMfn2 cDNA transgene with miR1518.

5 In a further examination, wildtype mice were injected intravenously with 3×10^{11} GC of AAV vectors, comprising an engineered human Mfn2 (hMfn2) cDNA nucleic acid sequence (SEQ ID NO: 11) and miRNA1693 (SEQ ID NO: 15). Four treatment groups were examined (N=5 in each group): PBS, AAV9-
 10 PHP.eB.CAG.hMfn2opt.link.hMfn2miR1693.WPRE.SV40, AAV9-
 PHP.eB.CB7.CI.hMfn2opt.link.hMfn2miR1693.rBG, AAV9-
 PHP.eB.hSyn.PI.hMfn2opt.link.hMfn2miR1693.WPRE.SV40 (wherein vector genome is of SEQ ID NO: 1). Mice were sacrificed 14 days post injection. Brain and spinal cord were harvested and homogenized. RNA was extracted from the samples and used for qPCR with TaqMan primers against human Mfn2 engineered cDNA. FIG 5 illustrates plotted quantitation
 15 of fold-expression of human Mfn2 (hMfn2) cDNA expression in spinal cord of treated mice following AAV vector delivery of engineered hMfn2 cDNA transgene with miR1693.

In both studied examining human and rat Mfn2 expression, among AAV vectors delivered, various promoters were examined, such as CB7, CAG and neuron specific human synapsin (hSyn). AAV vectors comprising hSyn promoters exhibited highest fold-expression
 20 of Mfn2 cDNA in spinal cord tissue.

Furthermore, total amount of mature miRNA processed from AAV vectors were examined using an established procedure disclosed in Chen et al., Real-time quantification of micr-RNAs by stem-loop RT-PCR, Nucleic Acid Research, 2005, 33 (20). Wildtype mice were injected intravenously with 3×10^{11} GC of AAV vectors or control group PBS. AAV
 25 genome vector of treatment group 1 comprised expression cassette for hMfn2 with miR1693 (SEQ ID NO: 1) which further comprising an engineered human Mfn2 (hMfn2) cDNA nucleic acid sequence (SEQ ID NO: 11) connected via linker (SEQ ID NO: 17) to miRNA1693 (SEQ ID NO: 15). AAV vector of treatment group 2 comprising an expression cassette for rMfn2 with miR1518 (SEQ ID NO: 3), further comprising an engineered rat Mfn2 (rMfn2) cDNA
 30 nucleic acid sequence (SEQ ID NO: 12) connected via linker (SEQ ID NO: 17) to miRNA1518 (SEQ ID NO: 16). Following treatment, mice were sacrificed at 14 days post

injection. Brain and spinal cord were harvested and homogenized. RNA was extracted from the samples and used for custom miRNA Assay. The custom miRNA assay consists of a custom stem-loop RT primer to create the cDNA and then custom small RNA:stem loop TaqMan primers to amplify the miRNA. This assay measures the total amount of mature
 5 miRNA processed from the vector. FIG 6A and FIG 6B illustrate total amount of mature miRNA processed from the AAV vectors following intravenous delivery in mice.

In summary, hSyn promoter (SEQ ID NO: 6) appears to be best promoter for maximum spinal cord expression. AAV vectors comprising “link” (for “linker”) is an important spacer between the cDNA and miRNA necessary for proper miRNA excision and
 10 processing for RNAi. A smaller linker was tried due to cloning ease but was not functional. qPCR analysis suggests adequate expression of rat Mfn2 and human Mfn2 when co-delivered with miRNA targeting endogenous mutant Mfn2.

EXAMPLE 3. Transfection efficiency of AAV vectors in HEK293 and Mfn2-Null Cells

15 In this study we generated and examined various AAV vectors comprising a transgene of human Mfn2 engineered sequence, as listed in Table 3 below.

Table 3.

AAV Vector	SEQ ID NOs (AAV vector)
CB7.CI.hMfn2.GA.RBG	79
CB7.CI.hMfn2.GA.LINK.miR1518.RBG	77
CB7.CI.hMfn2.GA.LINK.miR538.RBG	75
CAG.CI.hMfn2.GA.WPRE.SV40	73
CAG.CI.hMfn2.GA.LINK.miR1518.WPRE.SV40	71
CAG.CI.hMfn2.GA.LINK.miR538.WPRE.SV40	69

20 Mfn2 Null MEF is a mouse cell line lacking Mfn2 which was used to detect expression of Mfn2 cDNA in vectors. Additionally, HEK293 cells, which is a human cell line which express Mfn 2, was used in transfection with above-identified vectors. Following transfection cell lysates were analyzed for overall Mfn protein expression via western blot,

endogenous Mfn2 knockdown via qPCR, and presence of miRNA via qPCR. Western blot quantification was performed with Wes platform. The analysis of Mfn expression in Mfn2-null MEF cells quantified only Mfn2 protein produced by vector because cell line lacks Mfn2 expression. For qPCR miRNA detection assay, a stem-loop primer was used for reverse transcription (RT) of mature miRNA, a TaqMan probe set was used for amplification of mature miRNA to show expression and/or processing of miRNA. For qPCR for measuring endogenous human Mfn2 knockdown, a TaqMan primer/probe set was used, which distinguishes between HEK293 endogenous Mfn2 and vector Mfn2. For Wes-based Mfn2 protein quantitation and antibody to Mfn2 was used which cross-reacts with endogenous and vector produced Mfn2, therefore probing for overall Mfn2 overexpression.

FIG 7 shows expression levels of Mfn2 in Mfn2-null MEF cell line following transfection with various vectors comprising CB7 promoter. Furthermore, FIG 7 shows plotted quantitation of western blot signal measuring expression of mitofurin-2 (Mfn2) following transfections with CB7.CI.hMfn2.GA.WPRE.RBG (p6165); CB7.CI.hMfn2.GA.LINK.miR1518.RBG (p6166); CB7.CI.hMfn2.GA.LINK.miR538.RBG (p6167). Quantitation is plotted as percent expression; transfection efficiency was determined to be about 40% as measured by flow cytometry and approximated by visual observation. For a western blot probed for expression levels of Mfn2 (data not shown), b-actin was used as a loading control (Mfn2: 2 µg loaded; b-actin: 0.27 µg loaded; exposure used for quantification 4 seconds).

FIG 8 shows expression levels of Mfn2 in Mfn2-null MEF cell line following transfection with various vectors comprising CAG promoter. Furthermore, FIG 8 shows plotted quantitation of western blot signal measuring expression of mitofurin-2 (Mfn2) following transfections with CAG.CI.hMfn2.GA.WPRE.SV40 (p6168); CAG.CI.hMfn2.GA.LINK.miR1518.WPRE.SV40 (p6169); CAG.CI.hMfn2.GA.LINK.miR538.WPRE.SV40 (p6170). Quantitation is plotted as percent expression; transfection efficiency was determined to be about 40% as measured by flow cytometry and approximated by visual observation. For a western blot probed for expression levels of Mfn2, b-actin was used as a loading control (Mfn2: 2 µg loaded; b-actin: 0.27 µg loaded; exposure used for quantification 4 seconds).

FIG 9A and FIG 9B show expression levels of Mfn2 in HEK293 cell line following transfection with various vectors comprising either CB7 or CAG promoter. High transfection efficiency was observed. FIG 9A show endogenous Mfn2 knockdown in HEK293 cells as measured by qPCR and plotted as fold expression, following transfection with various vectors comprising CB7 promoter, (CB7.CI.hMfn2.GA.WPRE.RBG (p6165); CB7.CI.hMfn2.GA.LINK.miR1518.RBG (p6166); CB7.CI.hMfn2.GA.LINK.miR538.RBG(p6167)). FIG 9B show endogenous Mfn2 knockdown in HEK293 cells as measured by qPCR and plotted as fold expression, following transfection with various vectors comprising CAG promoter (CAG.CI.hMfn2.GA.WPRE.SV40 (p6168); CAG.CI.hMfn2.GA.LINK.miR1518.WPRE.SV40 (p6169); CAG.CI.hMfn2.GA.LINK.miR538.WPRE.SV40 (p6170)).

FIG 10 shows expression levels of Mfn2 (endogenous Mfn2 and Mfn2 expressed from vector) in HEK293 cell line following transfection with various vectors comprising CB7 promoter. Furthermore, FIG 10 shows plotted quantitation of western blot signal measuring expression of mitofurin-2 (Mfn2) following transfections with CB7.CI.hMfn2.GA.WPRE.RBG (p6165); CB7.CI.hMfn2.GA.LINK.miR1518.RBG (p6166); CB7.CI.hMfn2.GA.LINK.miR538.RBG (p6167). Quantitation is plotted as percent expression; transfection efficiency was determined to be about 95%. For a western blot probed for expression levels of Mfn2, b-actin was used as a loading control (Mfn2: 0.78 µg loaded; b-actin: 0.78 µg loaded; exposure used for quantification 4 seconds).

FIG 11 shows expression levels of Mfn2 (endogenous Mfn2 and Mfn2 expressed from vector) in HEK293 cell line following transfection with various vectors comprising CAG promoter. Furthermore, FIG 11 shows plotted quantitation of western blot signal measuring expression of mitofurin-2 (Mfn2) following transfections with CAG.CI.hMfn2.GA.WPRE.SV40 (p6168); CAG.CI.hMfn2.GA.LINK.miR1518.WPRE.SV40 (p6169); CAG.CI.hMfn2.GA.LINK.miR538.WPRE.SV40 (p6170). Quantitation is plotted as percent expression; transfection efficiency was determined to be about 95%. For a western blot probed for expression levels of Mfn2, b-actin was used as a loading control (Mfn2: 0.78 µg loaded; b-actin: 0.78 µg loaded; exposure used for quantification 4 seconds).

FIG 12A to FIG 12C show expression levels, as measured by qPCR, of mature miRNA (miR1518 or MiR538) in Mfn2-null MEF cell line, following transfection with

various vectors comprising either CB7 or CAG promoter. FIG 12A shows a comparison of expression levels, as measured by qPCR and plotted as fold expression, of mature miR1518 in Mfn2-null MEF cell line, following transfection with vectors comprising either CB7 or CAG promoter. FIG 12B shows a comparison of expression levels, as measured by qPCR and plotted as fold expression, of mature miR538 in Mfn2-null MEF cell line, following transfection with vectors comprising either CB7 or CAG promoter. FIG 12C shows a comparison of expression levels, as measured by qPCR and plotted as fold expression, of mature miR1518 and miR538 in Mfn2-null MEF cell line, following transfection with vectors comprising either CB7 or CAG promoter.

EXAMPLE 4. Gene therapy vectors for efficacy in MFN2^{R94Q} mice (C57BL/6J-Tg(Thy1-MFN2*), a model of Charcot-Marie-Tooth Disease Type 2A

Seven (7) hemizygous male MFN2^{R94Q} mice (C57BL/6J-Tg(Thy1-MFN2*)44Balo/J, JAX stock# 029745), two (2) male C57BL/6J mice (JAX stock# 000664), and eighteen (18) female C57BL/6J mice (JAX stock# 000664) were transferred to our *in vivo* research laboratory in Bar Harbor, ME. The mice were ear notched for identification, genotype(s) confirmed and housed in individually and positively ventilated polysulfonate cages with HEPA filtered air at a density of 3 mice per cage (two females with one male). The animal room was lighted entirely with artificial fluorescent lighting, with a controlled 12 h light/dark cycle (6 am to 6 pm light). The normal temperature and relative humidity ranges in the animal rooms were 22 ± 4°C and 50 ± 15%, respectively. The animal rooms were set to have 15 air exchanges per hour. Filtered tap water, acidified to a pH of 2.5 to 3.0, and normal rodent chow were provided ad libitum. The mice were used as breeders to raise the study cohort of 10 male C57BL/6J mice and 60 male hemizygous MFN2^{R94Q} mice in two rounds of breeding. At P0-P1, a total of seventy (70) mice were enrolled in the study.

Table 4.

Group	#Mice	Genotype	Compound	Age at Dosing	Dosing Route	Dosing Frequency	Tissue Collection
1	10	WT	Vehicle	P0-P1	ICV injection	Single Dose	Spinal cord;
2	10	HEMI	Vehicle				

3	10	HEMI	Vector 1				Tibial nerve
4	10	HEMI	Vector 2				
5	10	HEMI	Vector 3				
6	10	HEMI	Vector 4				
7	10	HEMI	Vector 5				
Total	70						

FIG13A to FIG 13F show characterization of mouse model. FIG 13A show schematic representation of the mice genotype. FIG 13B shows mice phenotype characterization, characterized by relative expression levels endogenous and FLAG-tagged MFN2 in brain as measured by western blotting. FIG 13C shows mice phenotype characterization, characterized by relative expression levels endogenous and FLAG-tagged MFN2 in spinal cord as measured by western blotting. FIG 13D shows measured weight in (g) of the mice in CMT2A mouse model (nTg, MFN2^{WT}, and MFN2^{R49Q}). FIG 13E shows mice phenotype characterization, as measured by the latency to fall (sec). FIG 13F shows mice phenotype characterization, as measured by grip strength (g).

In this study, mice are dosed at P0-P1 by neonatal ICV injection according to the table 4 above. Body weights of mice are measured weekly. At 15-17 weeks of age, the following tests are performed on each mouse: rotarod test, grip strength test, visual acuity test (optokinetic response), optional procedure. Additionally, Compound Muscle Action Potential (CMAP) testing is performed. At 17-19 weeks of age, mice are necropsied and the following tissues are collected: spinal cord, tibial nerve. The collected tissues are fixed in EM fixative and embedded in epoxy resin. One section is cut from each tissue. Sections are stained with Toluidine Blue. Stained slides are scanned. For each of the scanned sections, the following parameters are determined: axon size distributions, axon counts, axon areas. The axon area distributions are then plotted for each group.

In a pharmacological study, for the first cohort, 4-7 mice per group were used. The study was a blinded study. Briefly, male MFN2^{R94W} mice were used in the treatment groups to which candidate AAVhu68 vectors were administered. Newborn ICV injection was used as a route of administration, with bilaterally administered dose of 3 μ L of 7.5×10^{10} GC AAV vectors. Weight was measured weekly. Grip strength was measured at 6 weeks. FIG 14A and

FIG 14B show results of the pilot pharmacological study in MFN2^{R94Q} mice (Study groups: G1 - wild type (WT) mice, PBS; G2 - MFN2^{R94Q} mice, PBS; G3 - MFN2^{R94Q} mice, CB7.MFN2; G4 - MFN2^{R94Q} mice, CB7.MFN2.miR1518; G5 - MFN2^{R94Q} mice, CB7.MFN2.miR538; G6 - MFN2^{R94Q} mice, CAG.MFN2.miR1518; G7 - MFN2^{R94Q} mice, CAG.MFN2.miR538). FIG 14A shows body weight results (plotted as (g)) as measured in mice groups G1 to G7. FIG 14B shows survival results (plotted as probability of survival over day 0 to 50) as measured in mice groups G1 to G7. FIG 15 shows grip strength results (plotted as (kg)) of the pilot pharmacological study in MFN2^{R94Q} mice.

10 EXAMPLE 5. AAV vector co-administering hMfn2 and miR targeting endogenous hMfn2

In this study, AAVhu68.CB7.CI.hMfn2.miR538.RBG (also referenced as AAVhu68.CB7.CI.hMfn2.GA.LINK.miR538.RBG) vector is administered in Rhesus Macaque (*Macaca mulatta*) or *Cynomolgus Macaque* (non-human primates (NHP) via Intra-Cisterna Magna (ICM) injection, utilizing cerebrospinal fluid (CSF) to achieve widespread distribution from a single injection.

Test Article. The AAVhu68.CB7.CI.hMfn2.GA.LINK.miR538.RBG is used in this study. A certificate of analysis verifying quality and purity of the test article will be included in the final study report.

20 **Preparation.** Calculations for test article dilutions are performed and verified by trained GTP personnel prior to making dilutions. Test article dilutions are performed by designated personnel on the day of injection. Test article dilutions are verified by additional designated personnel. Diluted test article are kept on wet ice or at 2-8 °C for up to 8 hours until injection.

25 **Archival Samples.** Archival samples of test and control articles are retained by designated personnel and stored at $\leq -60^{\circ}\text{C}$.

Test Article Analysis. The designated personnel is responsible for assuring that the test article meets the release requirements. A certificate of analysis is provided by Vector Core for inclusion with study records. All results are recorded. Copies of the data are provided to
30 for inclusion in the study notebook.

Unused Test Article. Unused test article provided to NPRP personnel are stored on wet ice prior to being returned for archiving. Archival samples are stored at $\leq -60^{\circ}\text{C}$.

Test System. Rhesus Macaque (*Macaca mulatta*) or *Cynomolgus Macaque* is used in this study.

5 **Justification for Test System Selection.** This study involves intra-cisterna magna (ICM) delivery of a miRNA test article (vector) for CNS diseases. The dimensions of the CNS in the NHP act as a representative model of our clinical target population. This study provides critical data on the dose and route of administration-related pharmacokinetics and safety of the test article after ICM injection in rhesus macaques. In this study, 2 animals are
10 used. The NHPs are selected from male or female, 4-5 years of age, and about 3-10 kg.

Acclimation Period and Care. Quarantine and acclimation, animal husbandry and care are conducted in accordance with Standard Operating Procedures (SOP) procedures. Macaques are housed in stainless steel caging in CTRB. Husbandry and care are provided by DVR personnel.

15 Certified Primate Diet 5048 or a similar diet appropriate for nonhuman primates is supplied to study animals. Water is available from an automatic watering system and is accessible to all macaques *ad libitum*. Macaques are monitored by the veterinary staff for any conditions requiring possible intervention. The Study Director is consulted whenever possible to determine the appropriate course of action. However, in emergency situations, including
20 possible euthanasia, decisions are made by the veterinary staff as needed and the Study Director is advised as soon as possible. Enrichment in the form of food rewards, conspecific interaction, and manipulanda are provided. Animals are maintained on a 12-hour light/dark cycle controlled by Building Automation. The desired temperature range is 64-84°F (18-29°C). Temperature is maintained in this range to the maximum extent possible. The desired
25 humidity range is 30-70%. Humidity is maintained in this range to the maximum extent possible. Each macaque has been previously identified with a unique identification number that has been tattooed on its chest by the supplier. Both animals are included in a single treatment group.

Study design procedures. Two rhesus macaques are used in the study. Both
30 macaques are included in a single treatment group. Baseline blood samples, including CSF,

CBC, serum chemistry, and baseline biomarker assessment are collected from all macaques on day 0 prior to dosing. Vitals are also obtained from each macaque.

On day 0, macaques are sedated; weighed and vital signs are recorded prior to test article administration.

- 5 For test Article Administration (AAVhu68.CB7.CI.hMfn2.GA.LINK.miR538.RBG), macaques are anesthetized and appropriate analgesics are administered in accordance with SOP. Macaques are dosed with test article via sub-occipital puncture into the cisterna magna. For the Intra-Cisterna Magna (ICM) Puncture Procedure, anesthetized macaques are transferred from animal holding to the Procedure Room and placed on an x-ray table in the
- 10 lateral decubitus position with the head flexed forward for CSF collection and dosing into the cisterna magna. The site of injection is aseptically prepared. Using aseptic technique, a 21-27 gauge, 1-1.5 inch Quincke spinal needle (Becton Dickinson) is advanced into the sub-occipital space until the flow of CSF is observed. 1 mL of CSF is collected for baseline analysis, prior to dosing. The anatomical structures that is traversed include the skin, subcutaneous fat,
- 15 epidural space, dura and atlanto-occipital fascia. The needle is directed at the wider superior gap of the cisterna magna to avoid blood contamination and potential brainstem injury. Correct placement of needle puncture is verified via myelography, using a fluoroscope (OEC9800 C-Arm, GE). After CSF collection, a small bore T extension catheter is connected to the spinal needle to facilitate dosing of Iohexol (Trade Name: Omnipaque 180 mg/mL,
- 20 General Electric Healthcare) contrast media and test article. Up to 2 mL of Iohexol is administered via the catheter and spinal needle. After verifying needle placement via CSF return and visualization of the needle by fluoroscopy, a syringe containing the test article is connected to a flexible linker containing the test article and injected at a rate of approximately 2ml/minute. After administration, the needle is removed, and direct pressure is applied to the
- 25 puncture site. The macaques receive ICM administration of test article (AAVhu68.CB7.CI.hMfn2.GA.LINK.miR538.RBG) at a dose of 3×10^{13} GC (3.33×10^{11} GC/g brain). The total volume of injected per macaque is 1.0 mL. Doses and volumes are documented in the study records.

- For observations, on selected time points as listed on the Study Schedule (Table 5), the
- 30 macaque(s) are monitored for additional parameters beyond the daily observations, including but not limited to vital signs, clinical pathology, collection of serum and CSF.

For blood draws, animals are bled from a peripheral vein for a general safety panel which includes: neutralizing antibodies to AAV, hematology, clinical chemistry. Procedures for blood collection are performed in accordance with SOP. Additional blood samples are collected to measure changes in biomarkers (pharmacodynamic markers). The frequency of blood draws for complete blood counts, serum chemistry, biomarkers, neutralizing antibodies to AAV are as defined in the Study Schedule (Table 5) of the study protocol.

For antibody assays (neutralizing antibodies to AAV, serum), the blood sample (up to 2 mL) is collected via red top tubes (w/ or w/o serum separator), allowed to clot, and centrifuged. Study personnel isolates the serum. Serum is divided into two individually marked microcentrifuge tubes (labeled with Study number, Animal ID, Group number, time point, neutralizing Abs, and date) for every Antibody time point and stored at $\leq -60^{\circ}\text{C}$.

For cell counts and differentials, blood samples for complete blood counts with differentials and platelet count are collected in labeled lavender top tubes (Study number, Animal ID, Group number, time point, CBC and date of collection), up to 2 mL and stored at 4°C . Blood is sent to Antech Diagnostics, Inc. overnight (with ice packs for lavender top tubes) for blood cell counts including platelet counts and differentials. The following parameters are analyzed for red cell count, hemoglobin, hematocrit, platelet count, leukocyte count, leukocyte differential, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell morphology.

For clinical chemistries, blood samples of (up to 2 mL) are collected in labeled red top tubes, allowed to clot, and centrifuged. The serum is separated, put into labeled microcentrifuge tubes (Study number, Animal ID, Group number, time point, Chemistry and date of collection, and sent to Antech Diagnostics, Inc. overnight on ice packs for analysis. The following parameters are analyzed: alkaline phosphatase, bilirubin (total), creatinine, gamma-Glutamyl Transpeptidase, glucose, serum Alanine Aminotransferase, serum aspartate aminotransferase, albumin, albumin/globulin ratio (calculated), aldolase, blood urea nitrogen, calcium, chloride, creatinine kinase and isoforms, globulin (calculated as total protein minus albumin = globulin), lactate dehydrogenase, phosphorus, inorganic, potassium, sodium, total protein.

For biomarker analysis, blood sample (up to 2 mL) is collected via red top tubes (w/ or w/o serum separator), allowed to clot, and centrifuged. Study personnel isolates the serum.

Serum is then divided into 2 – individually marked microcentrifuge tubes (Study number, Animal ID, Group number, time point, serum biomarker, and date of collection). Samples are be stored at $\leq -60^{\circ}\text{C}$.

For PBMC/tissue lymphocyte isolation and ELISPOT, blood samples (6-10 mL) are collected into sodium heparin (green top tubes) and PBMCs are isolated according to SOP. Blood collection tubes are labeled with Study number, Animal ID, Group number, Species, Time point, PBMC, and Date of Collection. Lymphocytes are harvested from spleen, liver and bone marrow according to SOPs. ELISPOT for capsid and transgene T cell responses are performed according to SOP.

For CSF collection, up to 1 mL of CSF is collected for analysis and biomarker assessment. For all CSF collection time points including Study Day 0, macaques are anesthetized in accordance with SOP and transferred from animal holding to the Procedure Room where they are placed in the lateral decubitus position with the head flexed forward for CSF collection. The hair over the back of the head and cervical spine is clipped. The occipital protuberance at the back of the skull and the wings of the atlas (C1) is palpated. The site of injection is aseptically prepared. Using aseptic technique, a 21-27 gauge, 1-1.5 inch Quincke spinal needle (BD) or 21-27 gauge needle is advanced into the cisterna magna until the flow of CSF is observed. The anatomical structures that are traversed include the skin, subcutaneous fat, epidural space, dura and atlanto-occipital fascia. The needle is directed at the wider superior gap of the cisterna magna to avoid blood contamination and potential brainstem injury. Post CSF collection, the needle is removed and direct pressure applied to the puncture site. Samples are collected in sterile 1.5 mL Eppendorf tubes labeled with the Study number, Animal ID, group number, time point, CSF, and date of collection. Samples are placed on wet ice, and immediately aliquoted for CSF clin pathology (blood cell counts and differentials and total protein quantification) and CSF biomarkers. CSF is collected at frequencies outlined in the Study Schedule (Table 5) of the study protocol.

For CSF clin pathology, CSF (0.5 mL) is aliquoted into lavender top tubes (labeled with the Study number, Animal ID, group number, time point, CSF clin path, and date of with ice packs) for blood cell counts and differentials and total protein quantification.

For CSF biomarkers, all remaining CSF (unused for CSF clin path – up to 1 mL) collected in 2 sterile 1.5 mL Eppendorf tubes is centrifuged at 800g for 5 minutes. Study

personnel isolates the supernatant and aliquot into cryovials (labeled with the Study number, Animal ID, group number, time point, CSF biomarker, and date of collection). Samples are shipped on wet ice.

- 5 Additionally, on all days selected for monitoring, each macaque is sedated, weighed and have its respiratory and heart rates monitored, and body temperature taken via rectal thermometers prior to any blood samples being taken. The blood samples for PBMC isolation are transported at room temperature. Other samples are transported on wet ice.

The study is performed according to the schedule as described in Table 5 below.

10

Table 5.

Study Timepoint	Sample/Procedure	N
Study Day 0	Body Weight, Temperature, Respiratory Rate, Heart Rate	2
	Biomarker (baseline) (RC)	2
	CSF (baseline) (CV + LT)	2
	Neurological Monitoring	2
	Clin path (baseline) (LT, RC)	2
	Nerve conduction velocity testing	2
	Antibodies (baseline) (RC)	2
	Immunology (baseline) (PBMC) (GT)	2
	ICM vector 3×10^{13} GC	2
Study Day 7 (± 2 days)	Body Weight, Temperature, Respiratory Rate, Heart Rate	2
	Biomarker (RC)	2
	Clin path (LT, RC)	2
Study Day 14 (± 2 days)	Body Weight, Temperature, Respiratory Rate, Heart Rate	2
	Biomarker (RC)	2
	CSF (CV + LT)	2
	Clin path (LT, RC)	2

Study Timepoint	Sample/Procedure	N
Study Day 21 (± 2 days)	Body Weight, Temperature, Respiratory Rate, Heart Rate	2
	Biomarker (RC)	2
	Clin path (LT, RC)	2
Study Day 28 (± 2 days)	Body Weight, Temperature, Respiratory Rate, Heart Rate	2
	Biomarker (RC)	2
	Clin path (LT, RC)	2
	Neurological Monitoring	2
	Nerve conduction velocity testing	2
	Body Weight, Temperature, Respiratory Rate, Heart Rate	2
	Neurological monitoring	2
Terminal Day 35 (± 2 days)	Biomarker (RC)	2
	CSF (CV + LT)	2
	Clin path (LT, RC)	2
	Antibodies (RC)	2
	Immunology (PBMC) (GT)	2
	Nerve conduction velocity testing	2

Study Timepoint	Sample/Procedure	N
	Necropsy	2
	Tissues to be harvested	2

When macaques are necropsied, tissues described in Table 6 below are collected.

5 Table 6 Tissues to be Collected at Time of Necropsy for Histopathology and Biodistribution

Organ	Histopathology		Biodistribution		Lymphocyte isolation	
	Req.	Coll.	Req.	Coll.		
Chest tattoo	√					
Ascending aorta (proximal)	√		√			
Bone marrow, rib	√					
Bone marrow, femur			√		√	
Brain right hemisphere ^A	√					
Frontal Cortex ^A			√			
Parietal Cortex ^A			√			
Temporal Cortex ^A			√			
Occipital Cortex ^A			√			
Hippocampus ^A			√			
Cerebellum ^A			√			
Medulla ^A			√			
Cranial nerves IX, X, XI	√ R&L					
Dorsal root ganglia, cervical ^B	√		√			
Dorsal root ganglia, thoracic ^B	√		√			
Dorsal root ganglia, lumbar ^B	√		√			
Epididymis	√ right		√ left			

Organ	Histopathology		Biodistribution		Lymphocyte isolation	
	Req.	Coll.	Req.	Coll.		
Eye	√ right		√ left			
Gall bladder	√		√			
Heart ^C	√		√ left ventricle			
Kidney, right	√		√			
Kidney, left	√		√			
Liver, left lobe	√		√		√	
Liver, right lobe	√		√			
Lung, left	√		√			
Lung, right	√		√			
Lymph node, deep cervical			√			
Lymph node, mandibular	√		√			
Lymph node, mesenteric	√		√			
Muscle, quadriceps femoris	√ right		√ left			
Nerve, sciatic	√ R&L		√ left			
Nerve, tibial	√ R&L		√ left			
Nerve, peroneal	√ R&L		√ left			
Nerve, median Proximal	√ R&L		√ left			
Nerve, median Distal	√ R&L					
Nerve, radial	√ R&L		√ left			
Nerve, sural	√ R&L		√ left			
Nerve, ulnar	√ R&L		√ left			
Optic nerve (CN II)	√ right		√ left			
Ovary	√ right		√ left			
Pancreas	√		√			
Pituitary	√		√			
Skin with mammary	√					
Injection site	√					
Spinal Cord, cervical ^D	√		√			

Organ	Histopathology		Biodistribution		Lymphocyte isolation	
	Req.	Coll.	Req.	Coll.		
Spinal cord, thoracic ^D	√		√			
Spinal cord, lumbar ^D	√		√			
Spleen	√		√		√	
Testis	√ right		√ left			
Thymus	√		√			
Thyroid gland (with parathyroid)	√ right		√ left			
Trigeminal nerve ganglia	√ right		√ left ^E			
Gross lesions (if any) ^F	√		√			

√ = Tissue will be collected and processed for indicated assay, Req. = Required by Protocol, Coll. = Collected, N/A = Not Applicable

Abbreviations: L, left; R, right

- 5 A. The brain is collected whole and then divided in half along the midsagittal plane. The entire right half of the brain is placed in 10% neutral buffered formalin for histopathology and further trimmed and processed according to “Pardo et al., 2012, Technical Guide for Nervous System Sampling of the Cynomolgus Monkey for General Toxicity Studies, Toxicologic Pathology 40: 624-636”. Samples for biodistribution are collected from the left
- 10 hemisphere and stored at $\leq -60^{\circ}\text{C}$.

B. A minimum of 3 cervical, 3 thoracic and 3 lumbar dorsal root ganglia are sampled and fixed in three tissue cassettes for histopathology (cervical, thoracic, lumbar). In addition, the 3 contralateral cervical dorsal root ganglia, 3 contralateral thoracic dorsal root ganglia, and 3 contralateral 3 lumbar dorsal root ganglia are sampled and frozen $\leq -60^{\circ}\text{C}$ for biodistribution.

- 15 C. Sections of heart should include the right and left ventricles (with AV valves) and interventricular septum (with valves).

D. Each spinal cord is divided into cervical, thoracic, and lumbar segments labeled C, T, or L, respectively. Each spinal cord segment is divided into three sections. The 3 C, T, or L sections will each be numbered 1-3. From each animal, there is a total of 9 spinal cord sections

generated, numbered C1-3, T1-3, and L1-3. From each spinal cord segment, section 1 (C1, T1, L1) is used for histopathological analysis. From each spinal cord segment, section 2 (C2, T2, L2) are used for biodistribution analysis (RNA and protein analysis, 2 tubes). From each spinal cord segment, section 3 (C3, T3, L3) are formalin fixed and paraffin embedded for LCM.

E. Portion of trigeminal nerve is collected for possible occupational health exposure and stored in $\leq -60^{\circ}\text{C}$.

F. Gross observations made at necropsy for which histopathology is not appropriate (e.g. fluid, matted hair, missing anatomic parts) are collected.

Experimental evaluation: observations

For viability checks (In-Cage), the macaques are monitored by daily visual observation for general appearance, signs of toxicity which may include but are not limited to neurologic signs or lethargy, distress and changes in behavior. This is performed by personnel in accordance with SOPs. The Veterinarian and Study Director are notified for any unusual conditions. Treatment are conducted only after approval by the Veterinarian and Study Director, except in case of emergency imperiling the macaque or for humanely sacrificing the macaque if the Secondary Veterinarian and Study Director cannot be contacted in a timely manner.

Macaques found dead or euthanized due to their moribund state are evaluated in the same manner as terminal sacrificed macaques. A complete set of tissues will be collected and any gross lesions recorded.

Additionally, all macaques are visually examined each time they are anesthetized. At the time of necropsy, the macaques are examined for gross abnormalities. All changes are noted. Macaques are weighed at the beginning of the study, at necropsy, and at every time point in which they are sedated.

Food consumption is not be monitored. Water is administered ad lib and standard diet with enrichment foods are given to the macaques.

On the days selected for monitoring, Sensory Nerve Conduction Studies are performed.

For neurological exam, on the days selected for monitoring, a Nonhuman Primate Neurological Assessment is performed. The neurological assessment is divided into 5 sections: Mentation, Posture and Gait, Proprioception, Cranial Nerves, and Spinal Reflexes.

For postmortem analysis, gross postmortem examinations are performed on all
5 macaques, including macaques euthanized in a moribund condition or found dead. All abnormal observations are recorded.

Since this study requires an ongoing evaluation of animals, therefore time of necropsy is determined at a later time point. Any animals that die unexpectedly are necropsied as soon as practical. For all unscheduled deaths, clinical pathology, extensive gross pathology, and
10 histopathology are performed on a complete list of tissues, which are collected under the discretion of the study pathologist. Other analyses (immune response, etc.) are included as appropriate in an attempt to determine possible cause of death. Tissues are collected on all animals as outlined in table 6. Gross observations made at necropsy for which histopathology is not appropriate (e.g., fluid, matted hair, missing anatomic parts) are not collected.

15 The macaques surviving to the end of their study period are euthanized. The macaques are first be sedated and are then euthanized. Death is confirmed by absence of heartbeat and respiration. The macaque may be exsanguinated to help ensure death.

All tissues are preserved which are listed in Table 6. Tissues are fixed by placement in 10% neutral buffered formalin for paraffin embedding. Eyes used for histopathologic
20 examination are fixed in modified Davidson's solution prior to paraffin embedding.

For biodistribution analysis, a section of each tissue in table 6 is frozen down to -80°C as quickly as possible. At the discretion of the Study Director, DNA is extracted from tissues, and vector biodistribution is evaluated by quantitative PCR.

For histopathological analysis, a subset or all tissues in Table 6 are stained
25 with hematoxylin and eosin (H&E). Additional procedures and/or other stains may be employed at the discretion of the Study Pathologist to identify/clarify histologic features and are documented in the final report. All data documenting experimental details and study procedures and observations are recorded and maintained in the study binder. At the completion of the study, all reports and raw data are maintained in the archives. Preserved
30 specimens and tissues are archived and stored. Study Director determines the need for final disposition of these materials. All data documenting experimental details and study

procedures and observations are recorded and maintained by the GTP personnel in dedicated notebooks and study binders.

Assays which are performed on tissues collected at necropsy

5 Following conclusion of the study, macaques are necropsied with tissues harvested for full pathology (for list of tissues collected at necropsy see Table 6).

For histopathology, hematoxylin and eosin (H&E) stains are performed on all or a subset of tissues listed for collection for histopathology, at the discretion of the Study Director. Additional appropriate stains may be employed based on histopathological findings. For Mfn2
10 cDNA and miRNA expression, tissue sections preserved in formalin and paraffin embedded (FFPE) are processed and motor neurons are laser-capture microdissected. Motor neurons isolated from the spinal cord are RNA extracted and qPCR is performed to determine level of Mfn2 knockdown, levels of Mfn2 cDNA expression and levels of miRNA expression using specific primer sets. Knockdown of Mfn2 and expression of Mfn2 cDNA may also be
15 evaluated in tissue sample lysates by qPCR and Western blot. Immunohistochemistry for Mfn2 may be performed on brain and spinal cord tissues. Briefly, paraffin sections are incubated with an antibody raised against the Mfn2 protein. For biodistribution, a section of a section of each tissue in Table 6 is frozen down to $\leq -60^{\circ}\text{C}$ as quickly as possible. DNA may be extracted from tissues, and vector biodistribution is evaluated by quantitative PCR. For
20 tissue lymphocyte isolation and ELISPOT, lymphocytes may be harvested from spleen and bone marrow and ELISPOT for capsid and transgene T cell responses are performed.

(Sequence Listing Free Text)

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25 numeric identifier <223>.

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SEQ ID NO:	Free Text under <223>
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73	<223> Vector genome CAG.CI.hMfn2.GA.WPRE.SV40 <220> <221> repeat_region <222> (1)..(188) <223> ITR <220> <221> misc_feature <222> (192)..(1032) <223> CAG (CBA) <220> <221> enhancer <222> (194)..(1032) <223> CMV enhancer <220> <221> promoter <222> (439)..(720) <223> CB promoter <220> <221> Intron <222> (720)..(1127) <223> Hybrid intron in CAG <220> <221> Intron <222> (720)..(950) <223> b-actin intron <220>

SEQ ID NO:	Free Text under <223>
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SEQ ID NO:	Free Text under <223>
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SEQ ID NO:	Free Text under <223>
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80	<223> Synthetic Construct
81	<223> AAVhu68
84	<223> AAV-PHP.eB

SEQ ID NO:	Free Text under <223>
86	<223> CB7 Promoter with CMV enhancer
87	<223> CAG promoter
88	<223> rabbit beta-globin
89	<223> miR1538
90	<223> linker

All patents, patent publications, and other publications listed in this specification, as well as the sequence listing file “20-9141PCT_SeqListing_ST25” are incorporated herein by reference. US Provisional Patent Application No. 63/051,336, filed July 13, 2020, and US Provisional Patent Application No. 63/173,045 filed April 9, 2021 are incorporated herein by reference. While the invention has been described with reference to a particularly preferred embodiment, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the appended claims.

CLAIMS:

1. A recombinant adeno-associated virus (rAAV) comprising an AAV capsid and packaged therein a vector genome, wherein the vector genome comprises:
 - (a) an engineered nucleic acid sequence encoding human mitofusin 2 (hMfn2);
 - (b) a spacer sequence located between (a) and (c);
 - (c) at least one miRNA coding sequence which is specific for a target site in an endogenous human mitofusin 2 nucleic acid sequence in a CMT2 patient;
wherein the engineered nucleic acid sequence of (a) lacks the target site for the at least one miRNA of (c), thereby preventing the at least one miRNA from targeting the engineered hMfn2 coding sequence; and
 - (d) regulatory sequences operably linked to (a) and (c) which direct expression thereof in a cell.
2. The rAAV according to claim 1, wherein the AAV capsid is selected from AAVrh91, AAV9, AAV9.PHP.eB, AAVhu68, or AAV1 capsid .
3. The rAAV according to claim 1 or 2, wherein the engineered hMfn2 coding sequence has a nucleic acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto.
4. The rAAV according to claim 1 to 3, wherein the engineered hMfn2 coding sequence has the nucleic acid sequence of SEQ ID NO: 11 or a sequence at least about 80% identical thereto, wherein nt 216 to 236 and/or nt 1371 to 1391 of the engineered hMfn2 coding sequence are conserved.
5. The rAAV according to any one of claims 1 to 4, wherein the at least one miRNA comprises a sequence of one or more of an miRNA targeting sequence comprising SEQ ID NO: 89 (miR538, 59 nt) or a sequence at least 99% identical to SEQ ID NO: 89, or an miRNA comprising one or more of an antisense sequence of:
AGAACAGGTTCTGGACGTCAA, SEQ ID NO: 27, wherein the at least one miRNA does

not bind to the engineered hMfn2 coding sequence of (a) or its encoded messenger RNA (mRNA).

6. The rAAV according to any one of claims 1 to 4, wherein the at least one miRNA coding sequence comprises a sequence of one or more of an miRNA targeting sequence comprising SEQ ID NO: 15 (miR1693, 64 nt), an miRNA comprising one or more of an antisense sequence of: AAACCTTGAGGACTACTGGAG, SEQ ID NO: 32, or an miRNA targeting sequence comprising SEQ ID NO: 16 (miR1518, 59 nt) or a sequence at least 99% identity to SEQ ID NO: 16, wherein the at least one miRNA does not bind to the engineered hMfn2 coding sequence of (a) or its encoded mRNA.

7. The rAAV according to any one of claims 1 to 6, wherein the spacer is 75 nucleotides to about 250 nucleotides in length.

8. The rAAV according to any one of claims 1 to 7, wherein the at least one miRNA coding sequence is 3' to the engineered hMfn2 coding sequence.

9. The rAAV according to any one of claims 1 to 7, wherein the at least one miRNA coding sequence is located within an intron sequence.

10. The rAAV according to any one of claims 1 to 9, wherein the at least one miRNA coding sequence further comprising more than one miRNA coding sequence.

11. The rAAV according to any one of claims 1 to 10, wherein the vector genome further comprises a constitutive promoter, optionally a CB7 promoter or a CAG promoter.

12. The rAAV according to any one of claims 1 to 10, wherein the vector genome further comprises a tissue specific promoter which is a human synapsin (hSyn) promoter.

13. A vector comprising:

(a) an engineered nucleic acid sequence encoding human mitofusin 2 (hMfn2) operably linked to regulatory sequences which direct expression thereof in a human target cell; and/or

(b) a nucleic acid sequence encoding at least one hairpin forming miRNA which inhibits expression of endogenous human mitofusin 2 by specifically targeting a site on the mRNA of an endogenous human mitofusin 2 coding sequence which is not present in the engineered hMfn2 coding sequence, wherein the at least one hairpin miRNA coding sequence is operably linked to regulatory sequences which direct expression thereof in the human target cell, thereby preventing the at least one hairpin miRNA from reducing expression from the engineered hMfn2 coding sequence,

wherein the engineered hMfn2 coding sequence of (a) lacks a target site for the at least one hairpin miRNA encoded by (b).

14. The vector according to claim 13, wherein the vector is a replication-defective viral vector which comprises the engineered hMfn2 coding sequences, the at least one hairpin miRNA coding sequence is one or more of an miRNA coding sequence or one or more artificial miRNA (A miRNA) coding sequence, and the regulatory sequences.

15. The vector according to claim 14, wherein the viral vector is a recombinant adeno-associated virus (rAAV) particle having an AAV capsid which has a vector genome packaged therein which comprises the engineered hMfn2 coding sequences, at least one hairpin miRNA coding sequence and the regulatory sequences.

16. The vector according to claim 15, wherein the AAV capsid is selected from AAVrh91 capsid, AAV9, AAV9.PHB.eB, AAVhu68, or AAV1.

17. The vector to any one of claims 13 to 16, wherein the engineered hMfn2 coding sequence has the nucleic acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical to SEQ ID NO: 11.

18. The vector according to claim 13 to 17, wherein the engineered hMfn2 coding sequence has the nucleic acid sequence of SEQ ID NO: 11, or a sequence at least 80% identical to SEQ ID NO: 11, wherein nt 216 to 236 and/or nt 1371 to 1391 of the engineered hMfn2 coding sequence are conserved.

19. The vector according to any one of claims 13 to 18, wherein the at least one hairpin miRNA is miR538, the coding sequence of which comprises the nucleic acid sequence of sequence of SEQ ID NO: 89 or a sequence at least 99% identical to SEQ ID NO: 89, wherein the at least one hairpin miRNA does not bind to the engineered hMfn2 coding sequence of (a) or its encoded mRNA.

20. The vector according to any one of claims 13 to 18, wherein the at least one hairpin miRNA coding sequence comprises the sequence of one or more of:

- (a) SEQ ID NO 15 (miR1693, 64 nt);
- (b) at least 60 consecutive nucleotides of SEQ ID NO: 15;
- (c) at least 99% identity to SEQ ID NO: 15 which comprises a sequence with 100% identity to about nucleotide 6 to about nucleotide 26 of SEQ ID NO: 15 (or SEQ ID NO: 68);
- (d) a hairpin miRNA coding sequence which comprises a seed sequence of one or more of:

- (i) TTGACGTCCAGAACCTGTTCT, SEQ ID NO: 27;
- (ii) AGAAGTGGGCACTTAGAGTTG, SEQ ID NO: 28;
- (iii) TTCAGAAGTGGGCACTTAGAG, SEQ ID NO: 29;
- (iv) TTGTCAATCCAGCTGTCCAGC, SEQ ID NO: 30;
- (v) CAAACTTGGTCTTCACTGCAG, SEQ ID NO: 31;
- (vi) AAACCTTGAGGACTACTGGAG, SEQ ID NO: 32;
- (vii) TAACCATGGAAACCATGAACT, SEQ ID NO: 33;
- (viii) ACAACAAGAATGCCCATGGAG, SEQ ID NO: 34;
- (ix) AAAGGTCCCAGACAGTTCCTG, SEQ ID NO: 35;
- (x) TGTTCATGGCGGCAATTCCT, SEQ ID NO: 36;

- (xi) TGAGGTTGGCTATTGATTGAC, SEQ ID NO: 37;
- (xii) TTCTCACACAGTCAACACCTT, SEQ ID NO: 38;
- (xiii) TTTCTCGCAGTAAACCTGCT, SEQ ID NO: 39;
- (xiv) AGAAATGGAACTCAATGTCTT, SEQ ID NO: 40;
- (xv) TGAACAGGACATCACCTGTGA, SEQ ID NO: 41;
- (xvi) AATACAAGCAGGTATGTGAAC, SEQ ID NO: 42;
- (xvii) TAAACCTGCTGCTCCCGAGCC, SEQ ID NO: 43;
- (xviii) TAGAGGAGGCCATAGAGCCCA, SEQ ID NO: 44;
- (xix) TCTACCCGCAGGAAGCAATTG, SEQ ID NO: 45; or
- (xx) CTCCTTAGCAGACACAAAGAA, SEQ ID NO: 46, or

combinations of any of (i) through (xx)

wherein the hairpin miRNA does not bind to the engineered hMfn2 coding sequence or its encoded mRNA.

21. The vector according to any one of claims 13 to 20, comprising the engineered hMfn2 coding sequence and the at least one miRNA coding sequence with a single nucleic acid molecule and further comprising a spacer of at least 75 nucleotides between the engineered hMfn2 coding sequence and the at least one miRNA coding sequence.

22. The vector according to any one of claims 13 to 21, wherein the miRNA coding sequence is 3' to the engineered hMfn2 coding sequence.

23. The vector according to any one of claims 13 to 21, wherein the miRNA coding sequence is located within an intron sequence.

24. The vector according to any one of claims 13 to 23, further comprising more than one miRNA coding sequence.

25. The vector according to any one of claims 13 to 24, wherein the regulatory sequences comprise a constitutive promoter, optionally a CB7 promoter or CAG promoter.

26. The vector according to any one of claims 13 to 24, wherein the regulatory sequences comprise a neuronal specific promoter, optionally a human synapsin promoter.
27. The vector according to claim 13, wherein the vector is a non-viral vector.
28. The vector according to claim 27, wherein the at least one non-viral vector is a liposome.
29. The vector according to any one of claims 13, 14 or 17 to 26, wherein the vector is a replication-incompetent, recombinant viral vector selected from a recombinant parvovirus, a recombinant lentivirus, or a recombinant herpes simplex virus.
30. A vector comprising an engineered human mitofusin 2 (hMfn2) coding sequence having the nucleic acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical to SEQ ID NO: 11, operably linked to regulatory sequences which direct expression thereof in a human target cell.
31. The vector according to claim 30 which is a replication-defective viral vector selected from a recombinant adeno-associated virus, a recombinant lentivirus, or a recombinant herpes simplex virus.
32. The vector according to claim 31, wherein the viral vector is a recombinant adeno-associated virus (rAAV) particle having an AAV capsid which has a vector genome packaged therein which comprises the engineered hMfn2 coding sequences and the regulatory sequences.
33. The vector to claim 32, wherein the AAV capsid is selected from AAVrh91, AAV9, AAV9.PHP.eB, AAVhu68, or AAV1 capsid.

34. The vector according to claim 13 to 17, wherein the engineered hMfn2 coding sequence has the nucleic acid sequence of SEQ ID NO: 11 and further comprises a CB7 promoter.

35. The vector according to claim 13 to 17, wherein the engineered hMfn2 coding sequence has the nucleic acid sequence of SEQ ID NO: 11 and further comprises a human synapsin (hSyn) promoter.

36. The vector according to claim 13 to 17, wherein the engineered hMfn2 coding sequence has the nucleic acid sequence of SEQ ID NO: 11 and further comprises a CAG promoter.

37. A composition comprising a recombinant nucleic acid sequence comprising an engineered human mitofusin 2 (hMfn2) coding sequence operably linked to regulatory sequences which direct expression thereof in a human target cell and at least one miRNA specific for a target site in an endogenous human mitofusin 2 nucleic acid sequence in a Charcot-Marie-Tooth 2A (CMT2A) patient operably linked to regulatory sequences which direct expression thereof in the subject, wherein the engineered hMfn2 coding sequence lacks the target site for the at least one miRNA, thereby preventing the miRNA from targeting the engineered Mfn2 coding sequence.

38. A pharmaceutical composition comprising the rAAV according to any one of claims 1 to 12, the vector according to any one of claims 13 to 36, or the composition according to claim 37, and a pharmaceutically acceptable aqueous suspending liquid, excipient, and/or diluent.

39. A method for treating a patient having Charcot-Marie-Tooth 2A (CMT2A) neuropathy comprising delivering an effective amount of the recombinant AAV according to any one of claims 1 to 12, the vector according to any one of claims 13 to 36, or the composition according to claim 37 to a patient in need thereof.

40. A method for reducing neuropathy in a patient having Charcot-Marie-Tooth 2A (CMT2A) neuropathy comprising delivering an effective amount of the recombinant AAV according to any one of claim 1 to 12, the vector according to any one of claims 13 to 36, or the composition according to claim 37 to a patient in need thereof.

41. A recombinant AAV according to any one of claims 1 to 12, a vector according to any one of claims 13 to 36, or a composition according to claim 37 suitable for treatment of a patient having Charcot-Marie-Tooth 2A (CMT2A) disease or neuropathy.

42. A recombinant AAV according to claim 1 to 12, a vector according to any one of claims 13 to 36, or a composition according to claim 37 for use in preparing a medicament for treatment of a patient having Charcot-Marie-Tooth 2A (CMT2A) disease or neuropathy.

43. A method for treating a patient according to claim 39 or 40 or use of a vector according to claim 41 or 42 in combination with one or more co-therapies selected from: acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), tricyclic antidepressants or antiepileptic drugs, such as carbamazepine or gabapentin.

44. A combination regimen for treating a patient having CMT2A which comprises co-administering

(a) a recombinant nucleic acid sequence comprising an engineered human mitofusin 2 (hMfn2) coding sequence operably linked to regulatory sequences which direct expression thereof in a human target cell, wherein the engineered hMfn2 coding sequence has the sequence of SEQ ID NO: 11 or a sequence at least 95% identical thereto and which differs from an endogenous human mitofusin 2 coding sequence in the CMT2A patient by having a mismatch in an miRNA target site of (b),

(b) a nucleic acid comprising at least one coding sequence for an miRNA specific for the endogenous hMfn2 coding sequence in a human CMT2A subject, wherein the miRNA coding sequence is operably linked to regulatory sequences which direct expression thereof in the subject, and wherein the miRNA does not bind to the engineered hMfn2 coding sequence of (a) or its encoded mRNA.

45. The combination regimen according to claim 44, wherein the miRNA coding sequence comprises of SEQ ID NO: 89 or at least 99% identical to SEQ ID NO: 89.

46. A combination regimen for treating a patient having CMT2A which comprises co-administering

(a) a recombinant nucleic acid sequence comprising an engineered human mitofusin 2 (hMfn2) coding sequence operably linked to regulatory sequences which direct expression thereof in a human target cell, wherein the engineered hMfn2 coding sequence is engineered to differ from an endogenous human mitofusin 2 in the CMT2A patient by having a mismatch in the miRNA target site of sequence of (b)),

(b) at least one nucleic acid sequence comprising an miRNA specific for the endogenous human mitofusin 2 coding sequence in a human CMT2A subject, wherein the miRNA coding sequence is operably linked to regulatory sequences which direct expression thereof in the subject, and wherein the at least one miRNA has a sequence of one or more of: an miRNA coding sequence comprising SEQ ID NO 15 (miR1693, 64 nt); an miRNA coding sequence comprising at least 60 consecutive nucleotides of SEQ ID NO: 15; an miRNA coding sequence comprising at least 99% identity to SEQ ID NO: 15 which comprises a sequence with 100% identity to about nucleotide 6 to about nucleotide 26 of SEQ ID NO: 15 (or SEQ ID NO: 68); or an miRNA coding sequence comprising one or more of:

- (i) TTGACGTCCAGAACCTGTTCT, SEQ ID NO: 27;
- (ii) AGAAGTGGGCACTTAGAGTTG, SEQ ID NO: 28;
- (iii) TTCAGAAGTGGGCACTTAGAG, SEQ ID NO: 29;
- (iv) TTGTCAATCCAGCTGTCCAGC, SEQ ID NO: 30;
- (v) CAAACTTGGTCTTCACTGCAG, SEQ ID NO: 31;
- (vi) AAACCTTGAGGACTACTGGAG, SEQ ID NO: 32;
- (vii) TAACCATGGAAACCATGAACT, SEQ ID NO: 33;
- (viii) ACAACAAGAATGCCCATGGAG, SEQ ID NO: 34;
- (ix) AAAGGTCCCAGACAGTTCCTG, SEQ ID NO: 35;
- (x) TGTTTCATGGCGGCAATTCCT, SEQ ID NO: 36;
- (xi) TGAGGTTGGCTATTGATTGAC, SEQ ID NO: 37;
- (xii) TTCTCACACAGTCAACACCTT, SEQ ID NO: 38;

- (xiii) TTTCTCGCAGTAAACCTGCT, SEQ ID NO: 39;
- (xiv) AGAAATGGAACTCAATGTCTT, SEQ ID NO: 40;
- (xiv) TGAACAGGACATCACCTGTGA, SEQ ID NO: 41;
- (xvi) AATACAAGCAGGTATGTGAAC, SEQ ID NO: 42;
- (xvii) TAAACCTGCTGCTCCCGAGCC, SEQ ID NO: 43;
- (xviii) TAGAGGAGGCCATAGAGCCCA, SEQ ID NO: 44;
- (xix) TCTACCCGCAGGAAGCAATTG, SEQ ID NO: 45; or
- (xx) CTCCTTAGCAGACACAAAGAA, SEQ ID NO: 46, or

combinations of any of (i) through (xx).

47. The combination regimen according to any one of claims claim 44 to 46, wherein a first vector comprises the nucleic acid (a) and a second, different vector, comprises at least one miRNA (b).

48. The combination regimen according to claim 47, wherein the first vector is a viral vector and/or the second vector is a viral vector and the first and the second viral vector may be from the same virus source or may be different.

49. The combination regimen according to claims 47 or 48, wherein the first vector is a non-viral vector, the second vector is a non-viral vector and the first and the second vectors may be same composition or may be different.

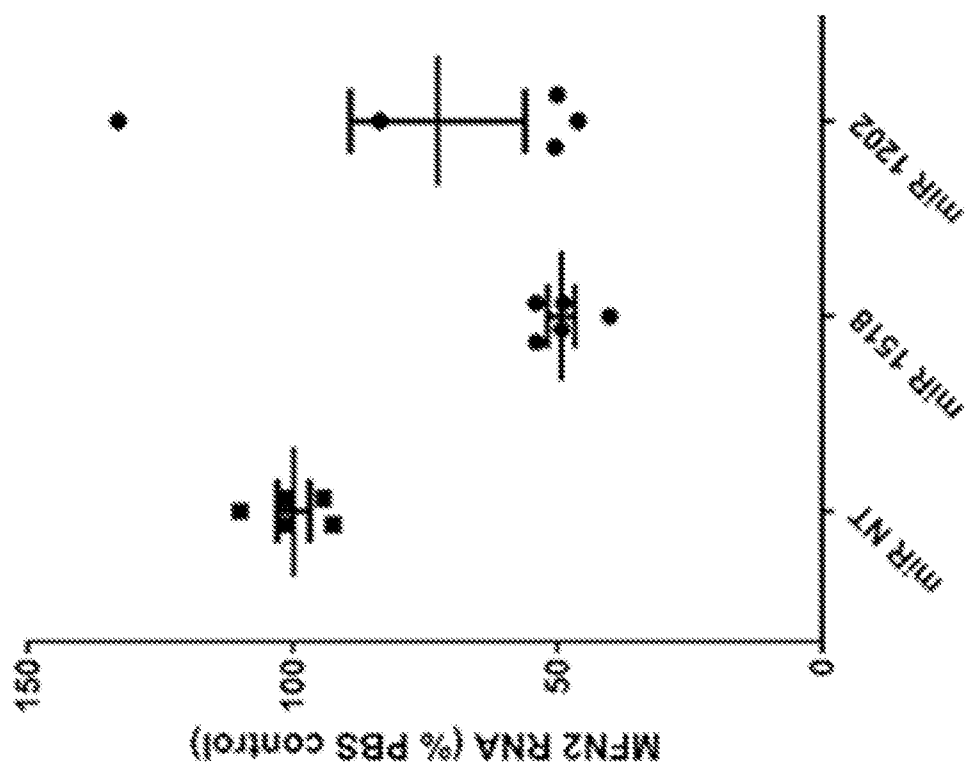
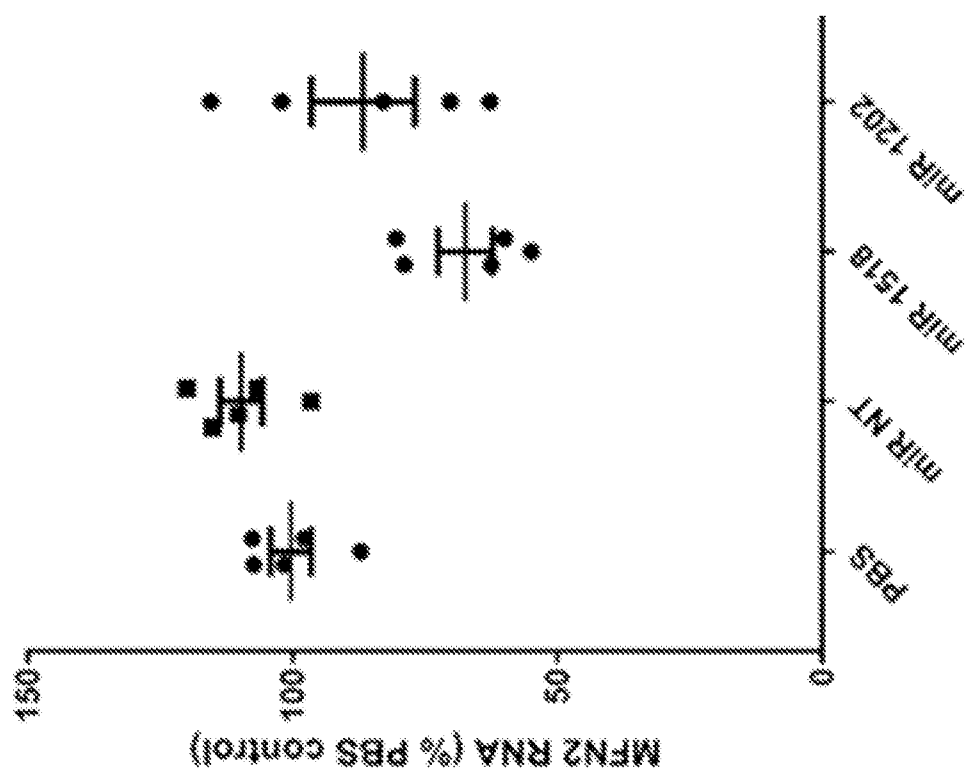


FIG 2A

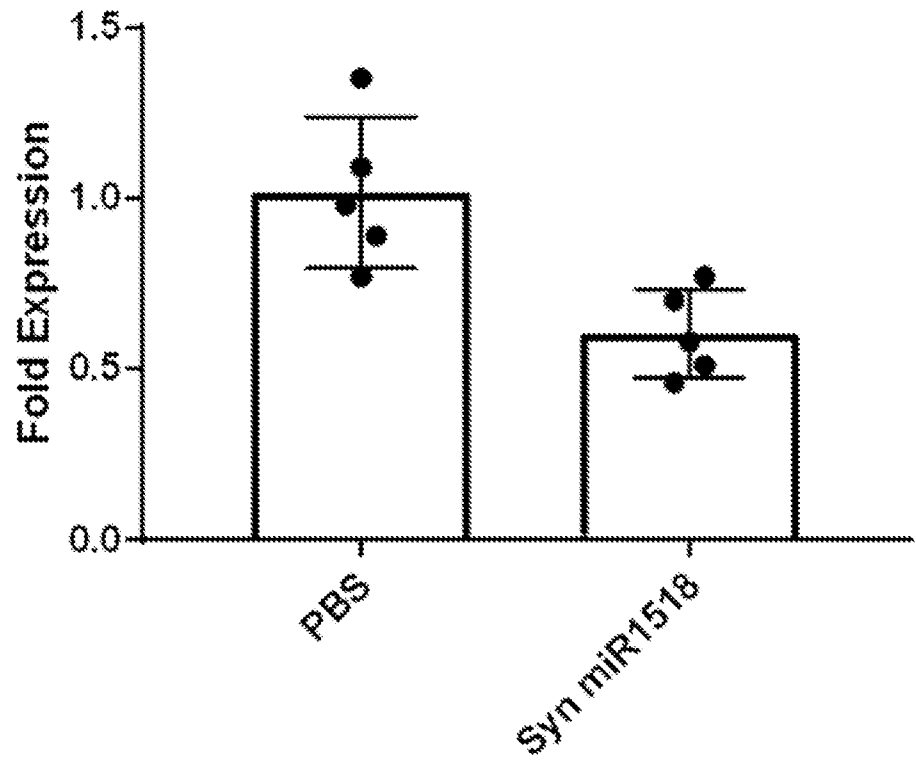


FIG 2B

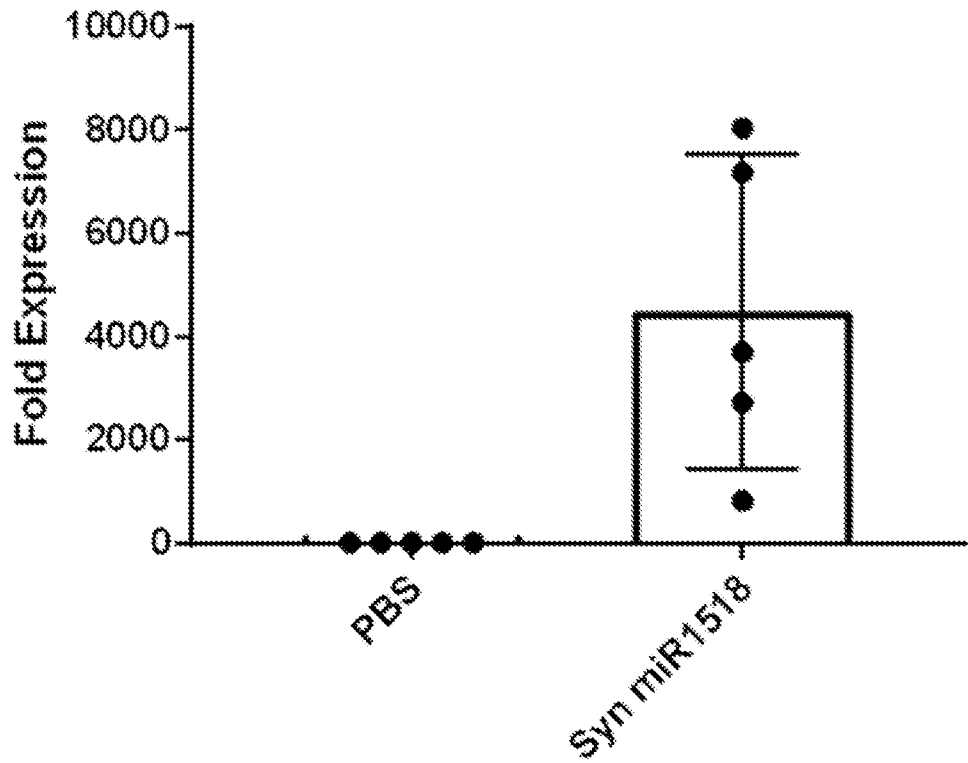


FIG 2C

Spinal Cord
miR1518

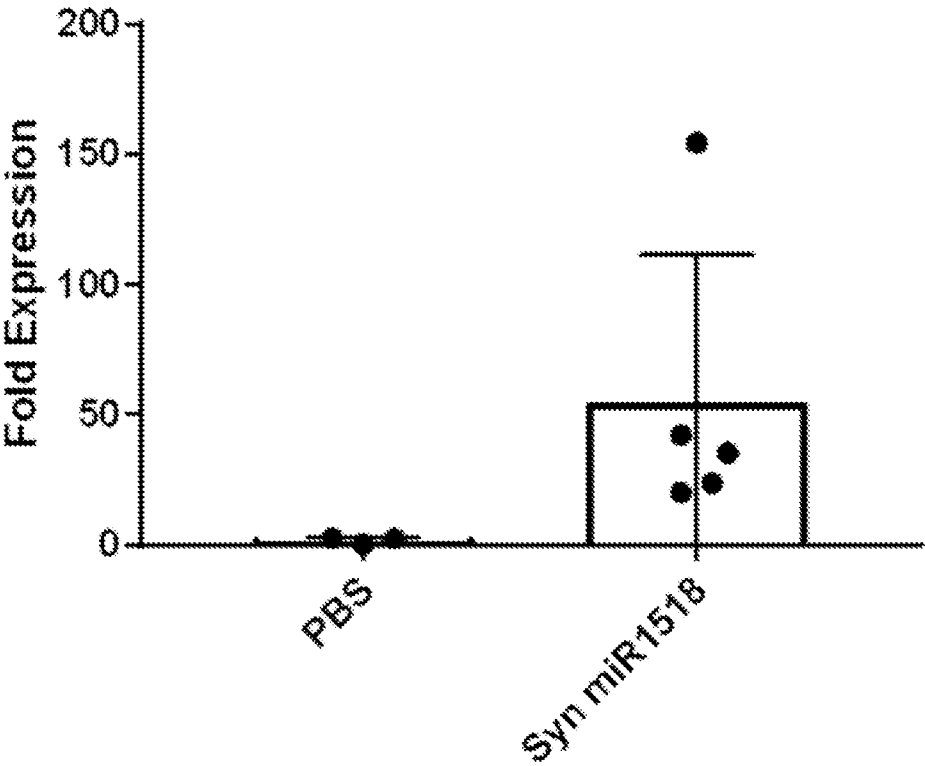


FIG 3

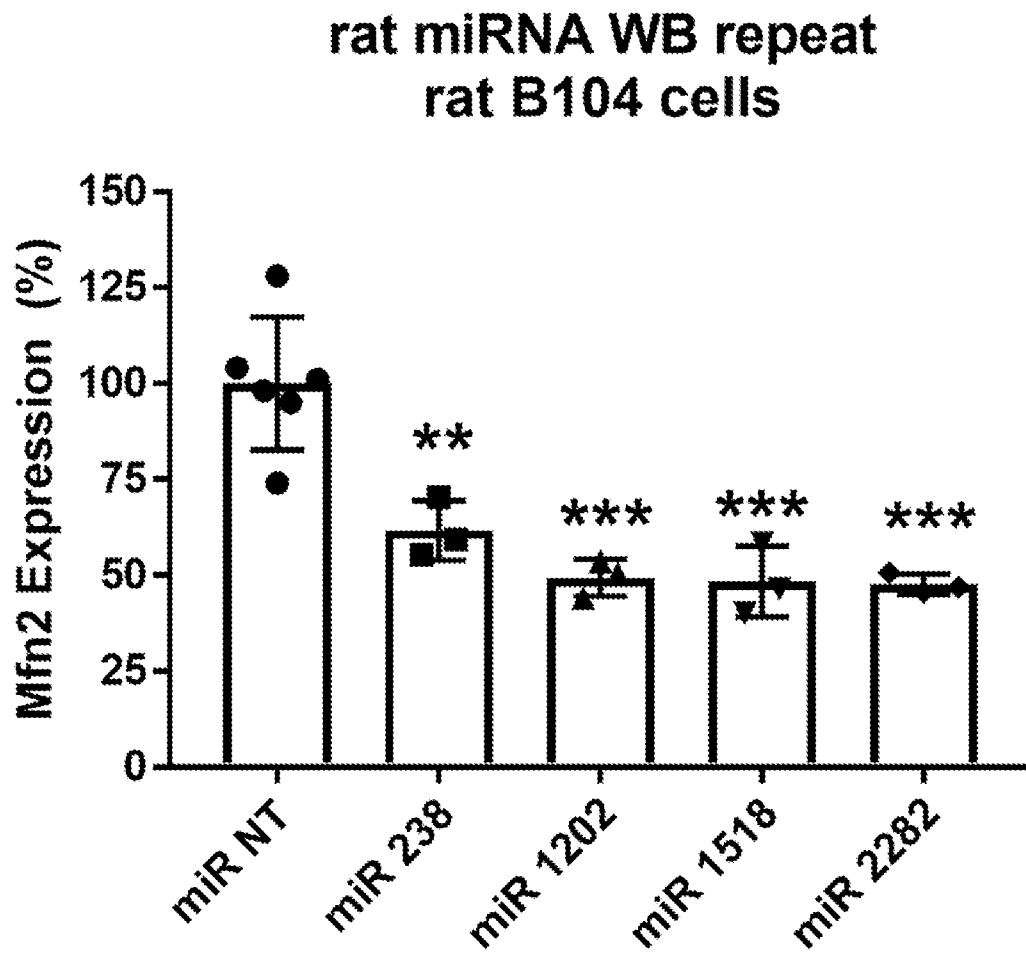


FIG 4

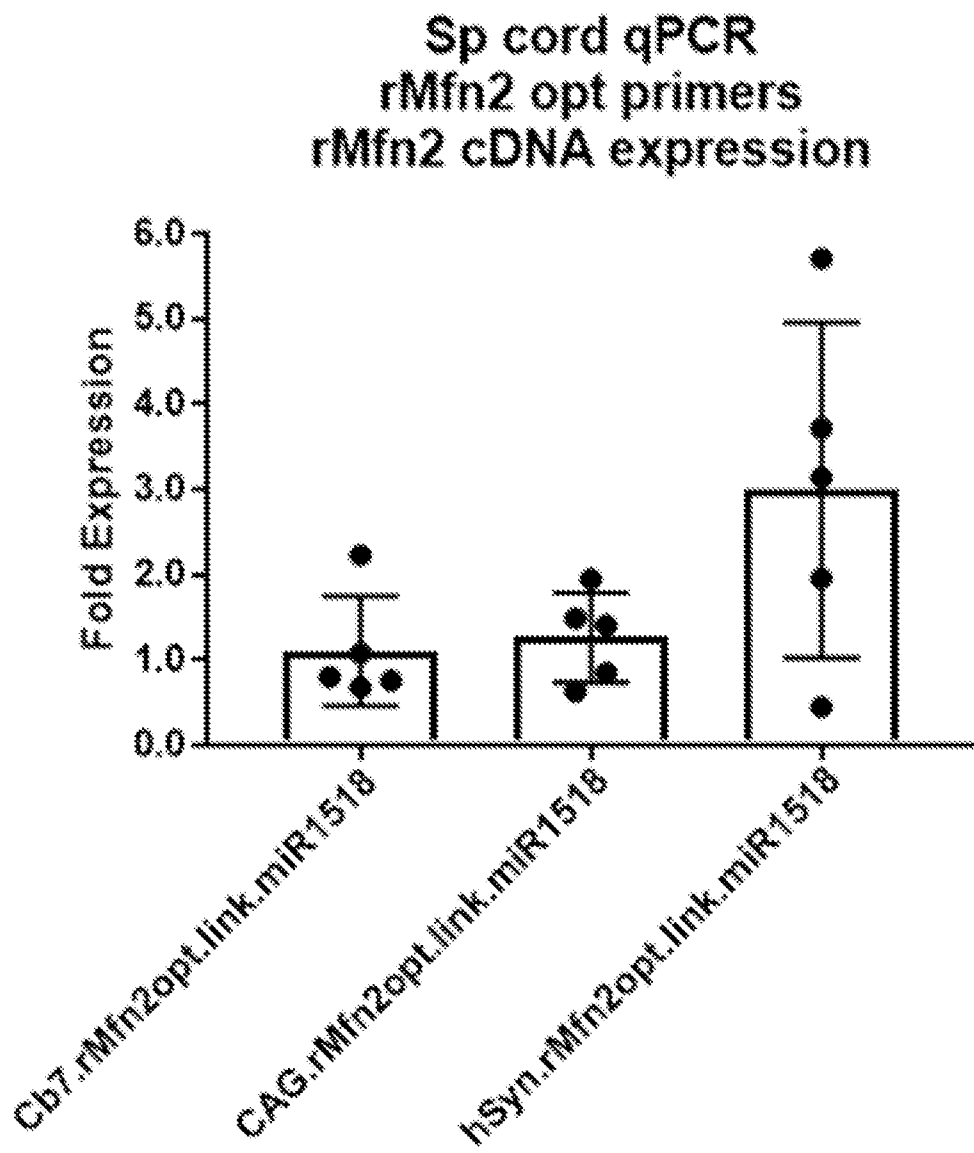


FIG 5

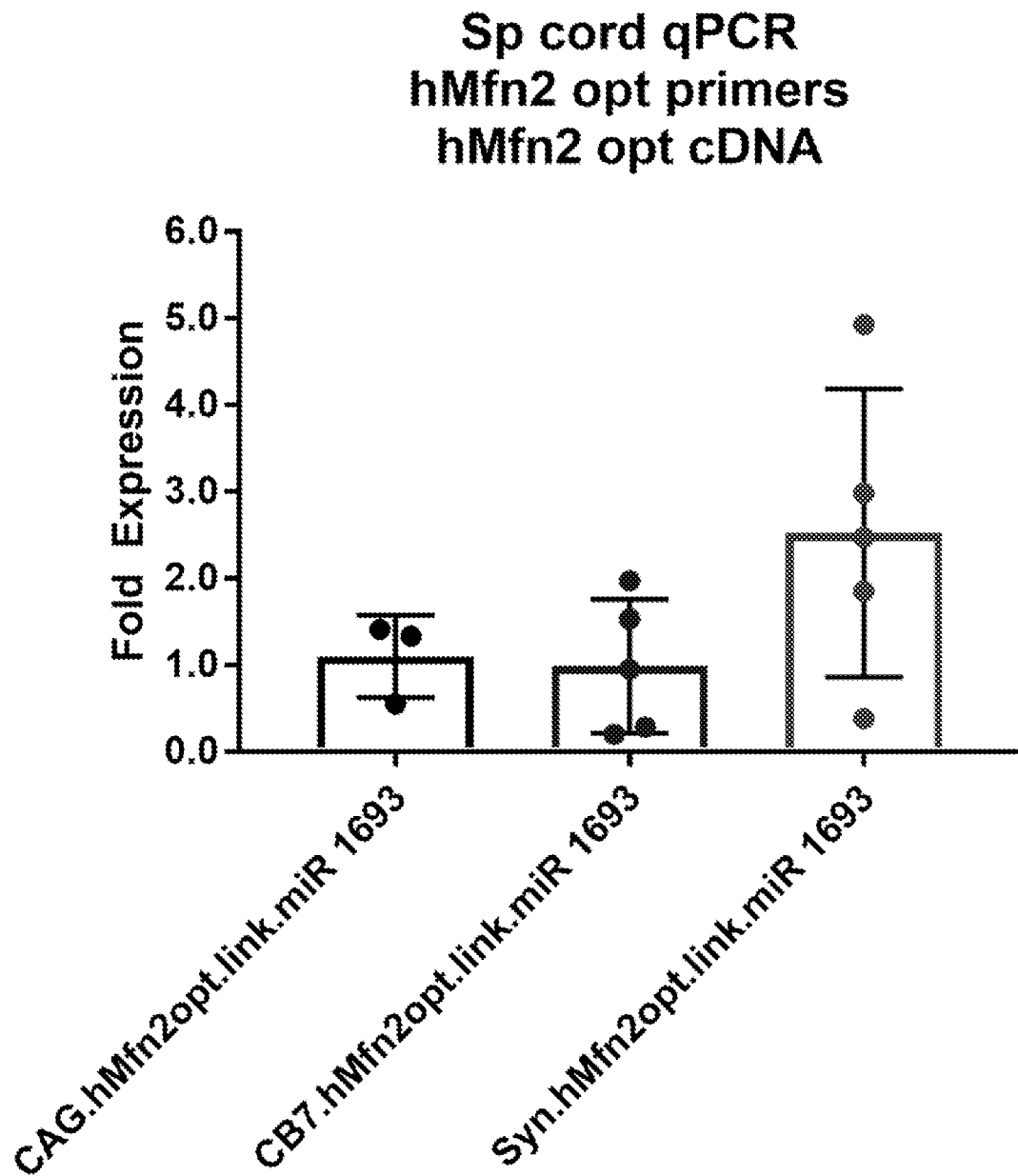


FIG 6A

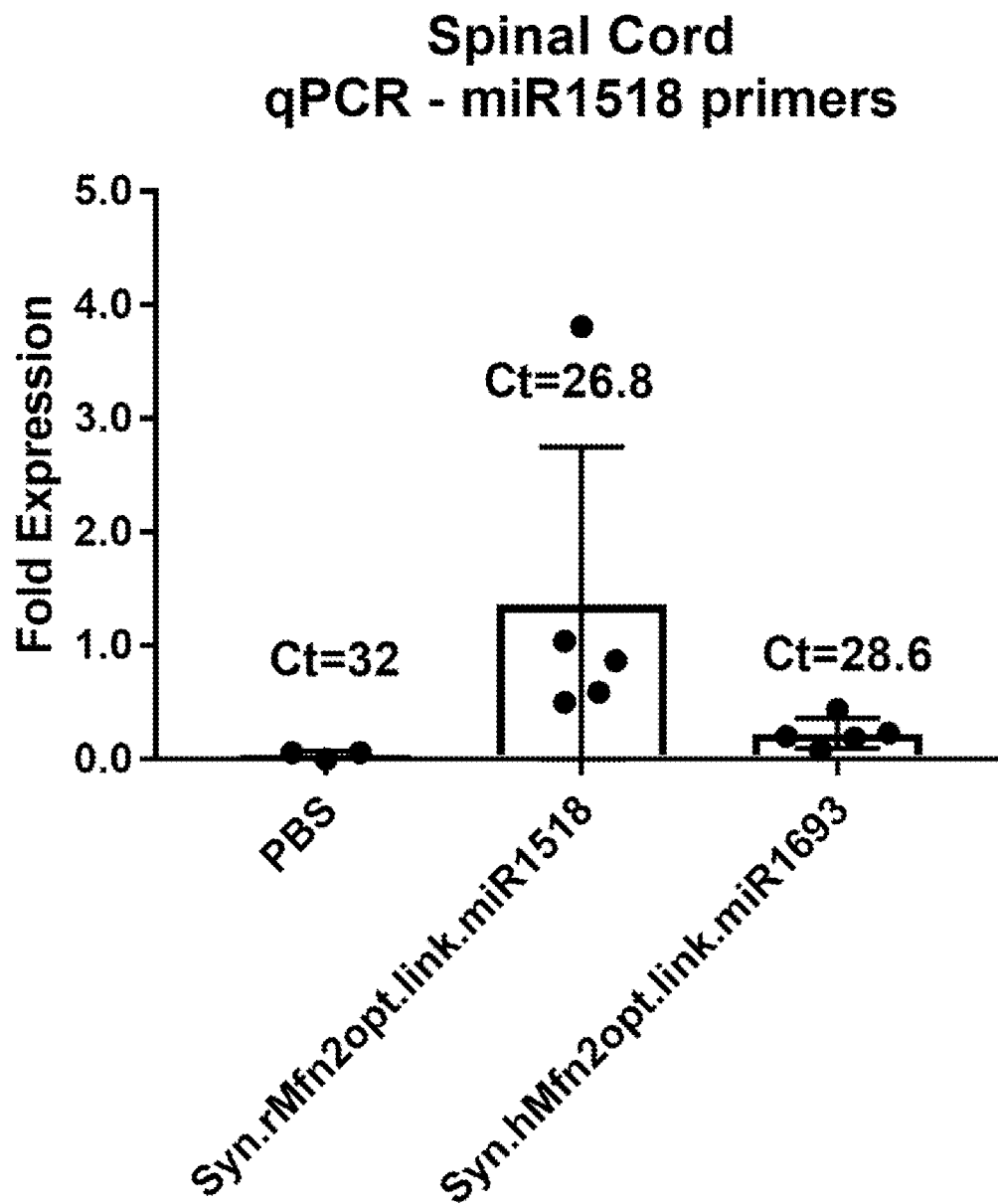


FIG 6B

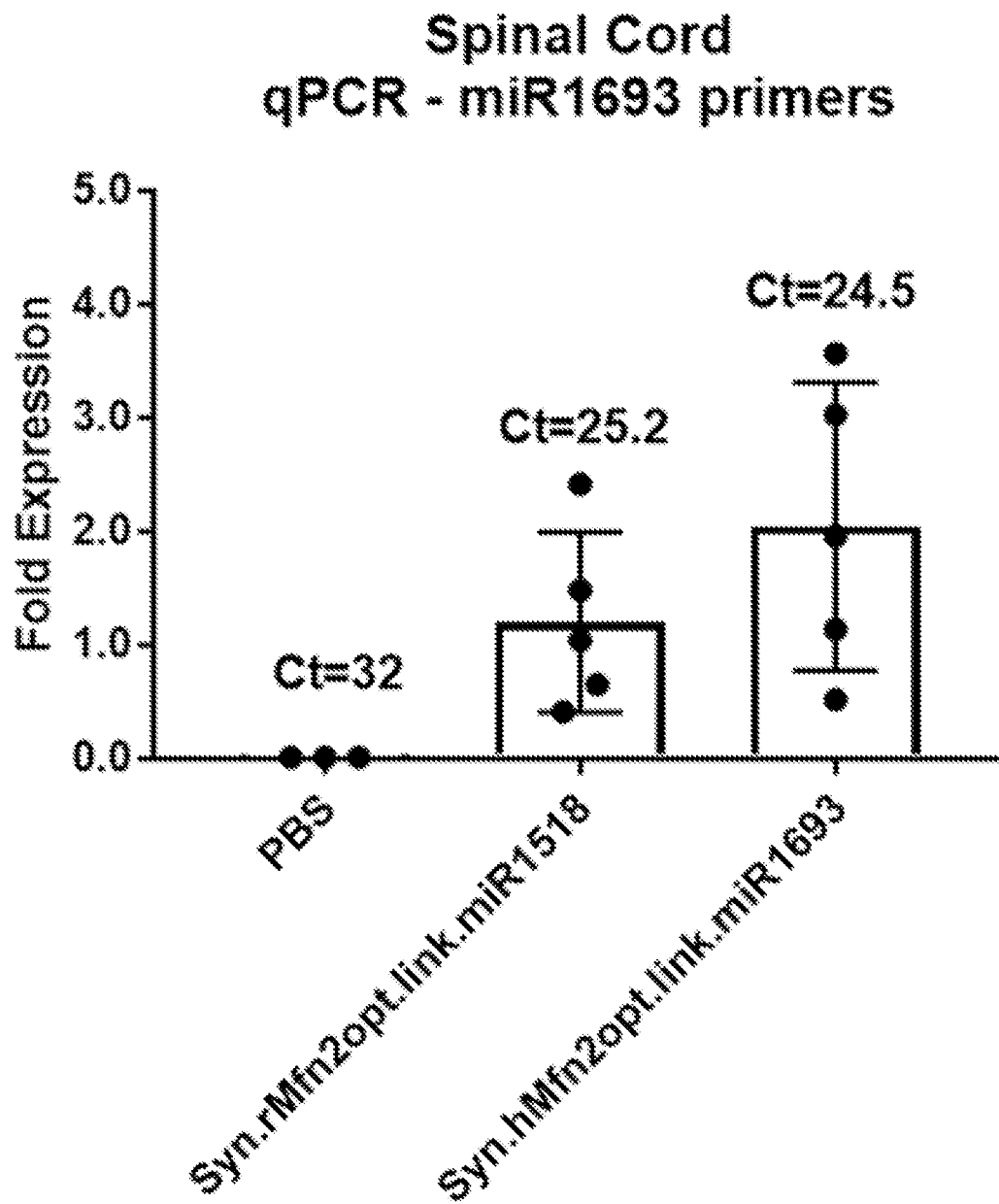


FIG 7

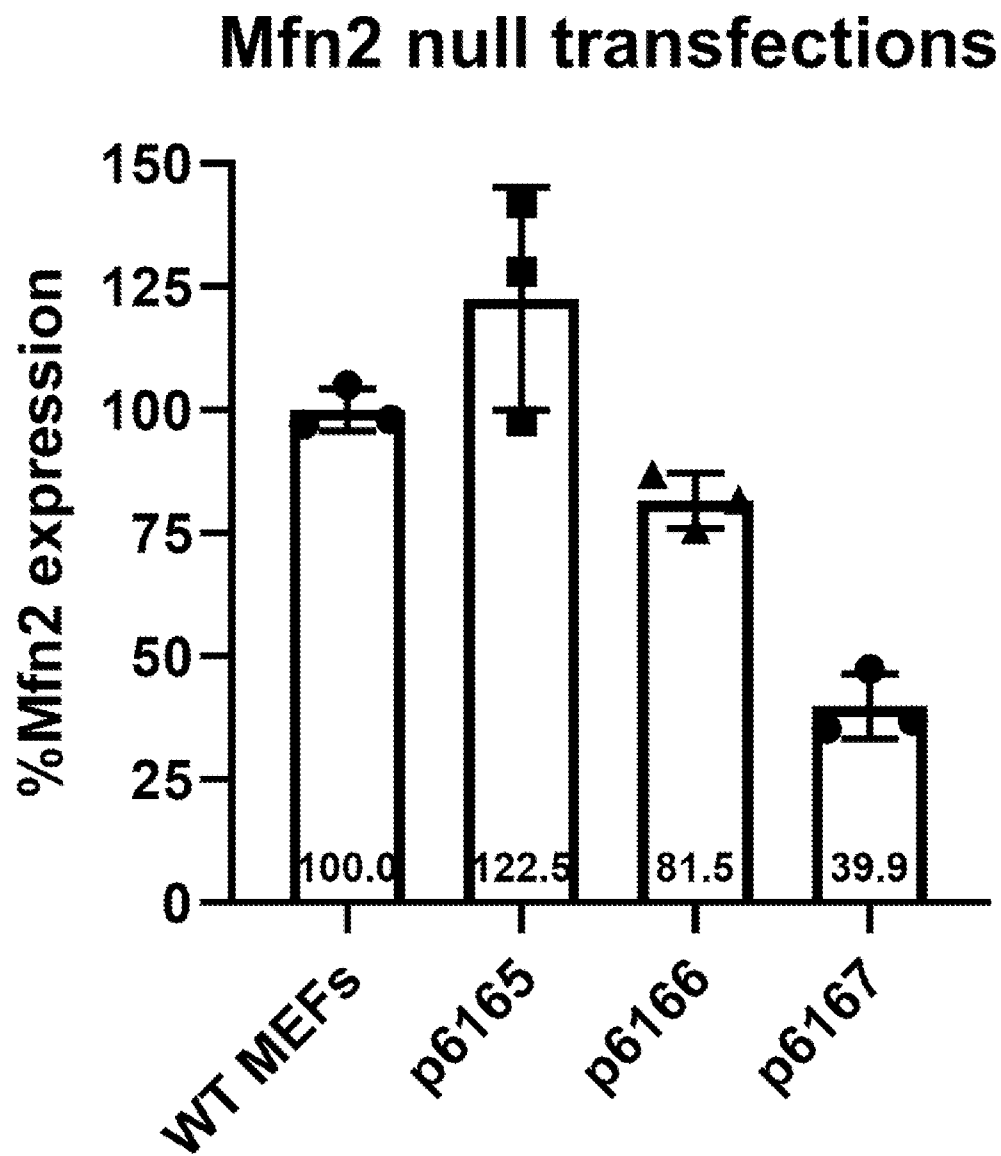


FIG 8

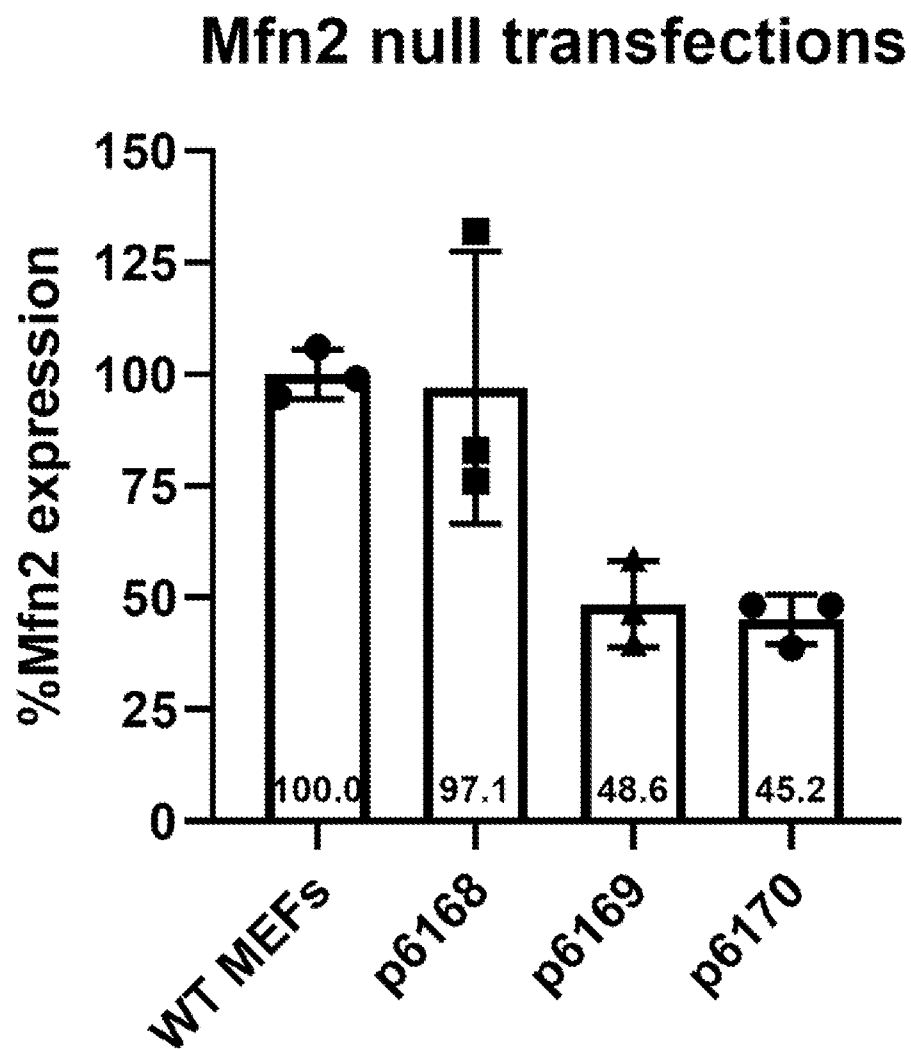


FIG 9A

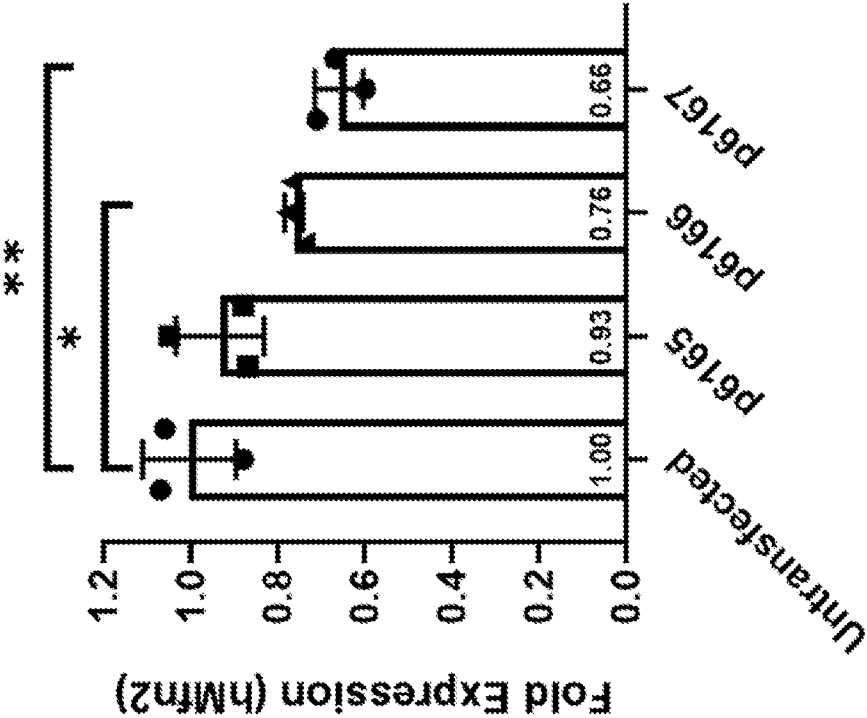


FIG 9B

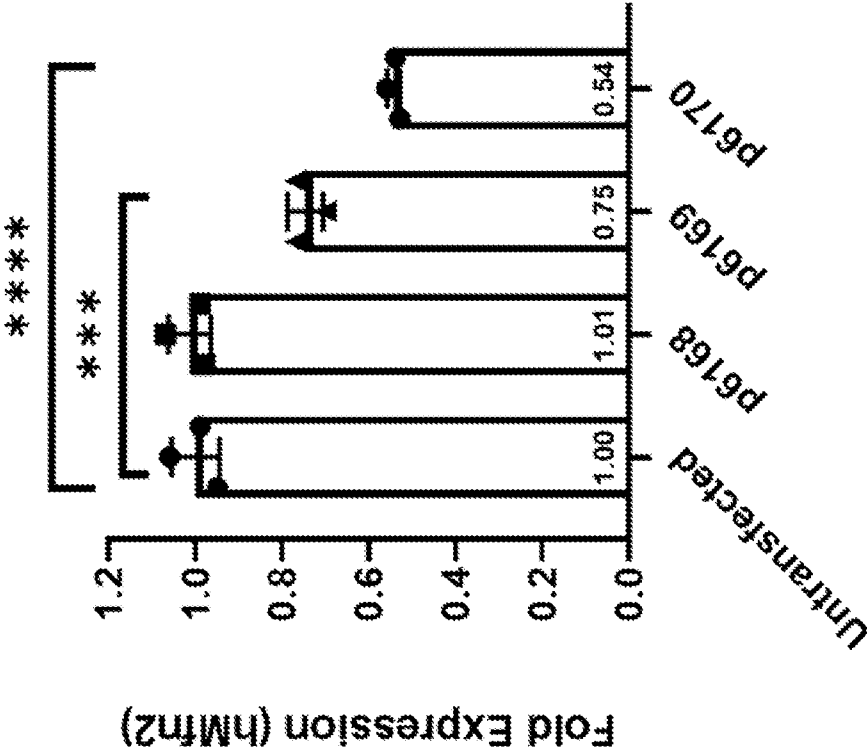


FIG 10

**HEK293 transfections
new Cb7 vectors
Wes protein quantitation**

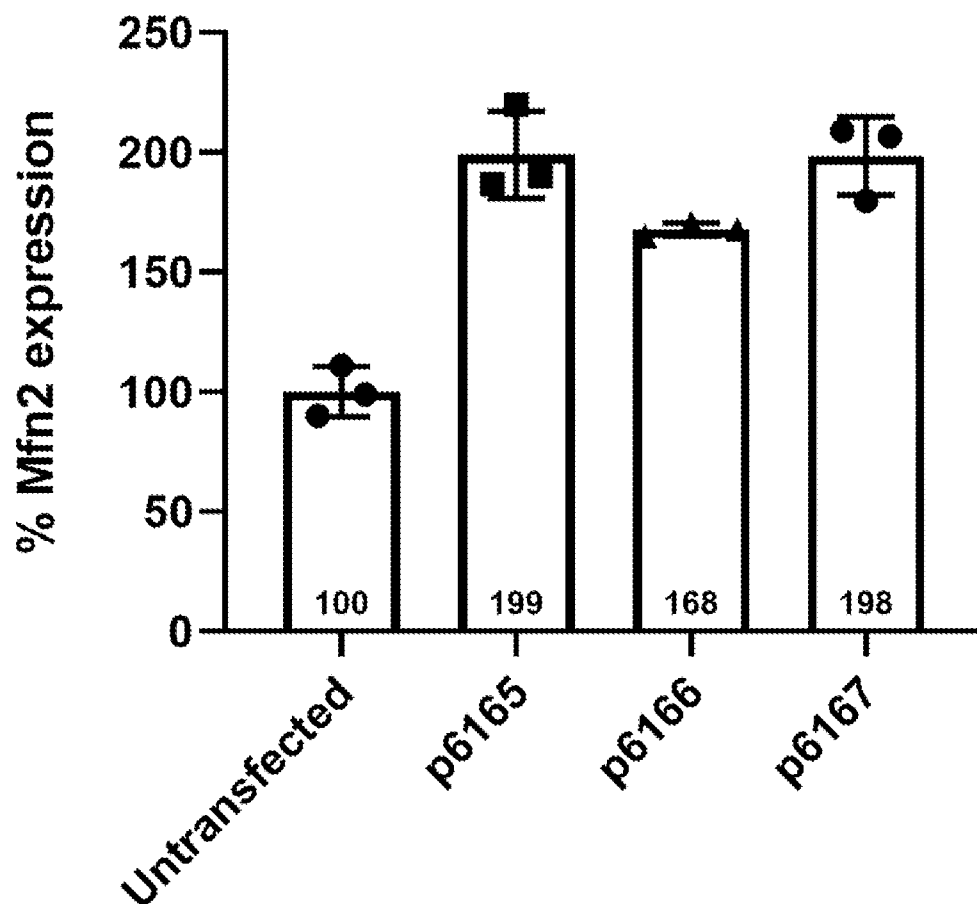


FIG 11

**HEK293 transfections
new CAG vectors
Wes protein quantitation**

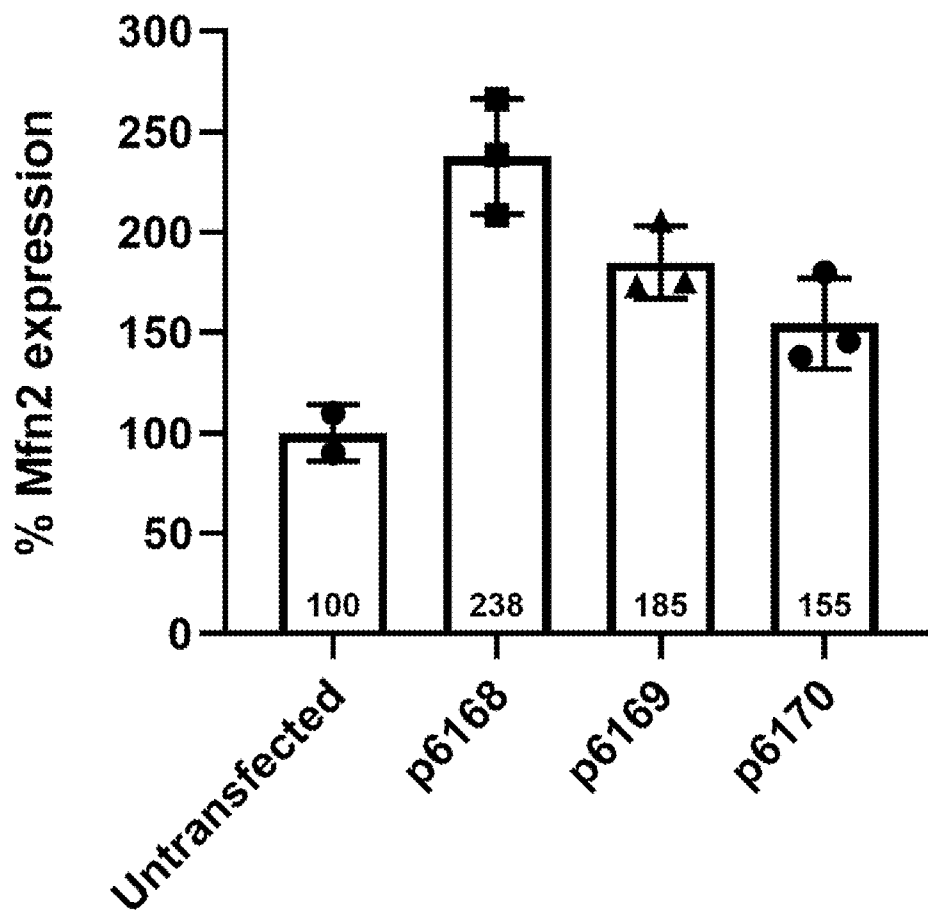


FIG 12A

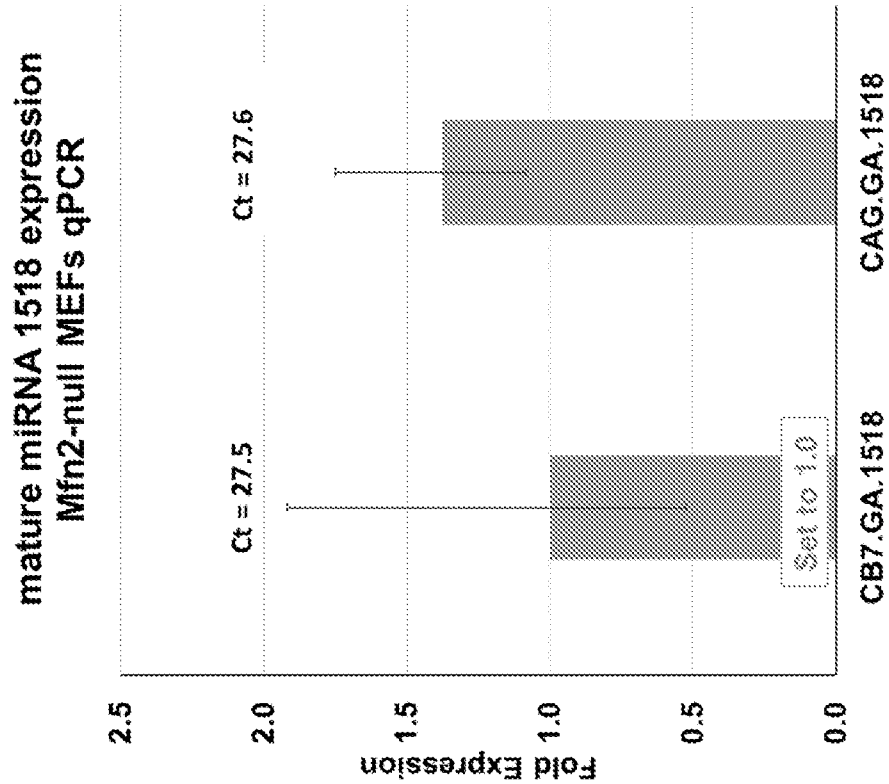


FIG 12B

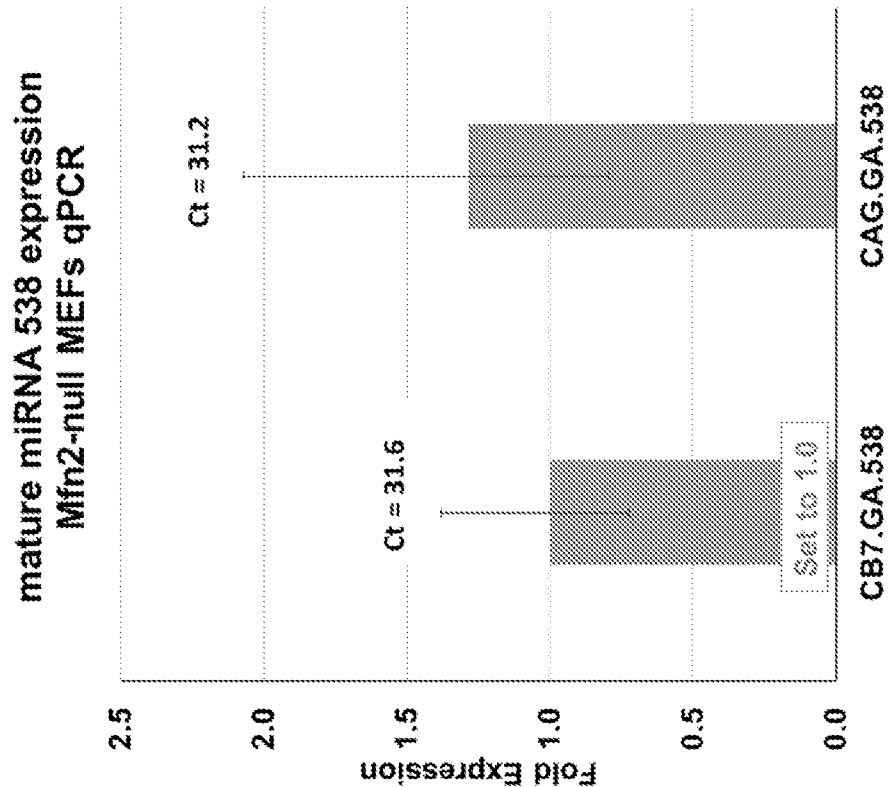


FIG 12C

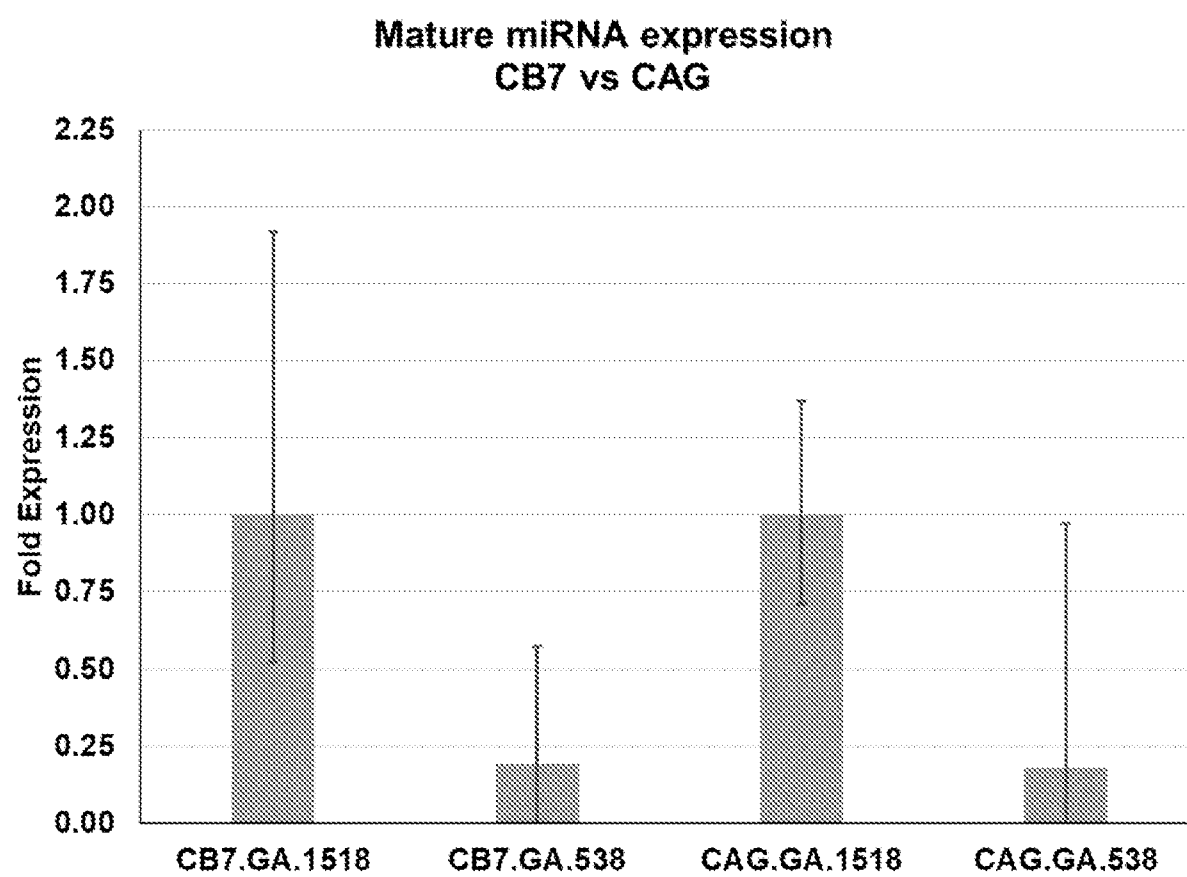


FIG 13A

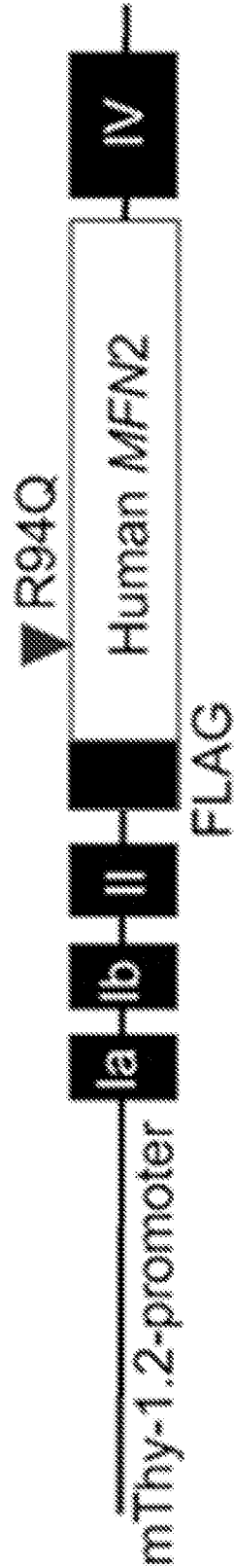


FIG 13B

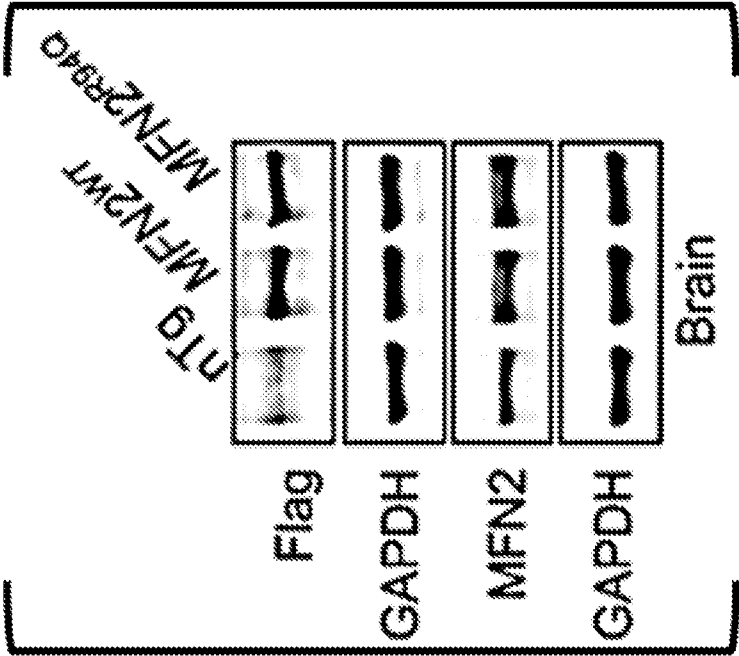


FIG 13C

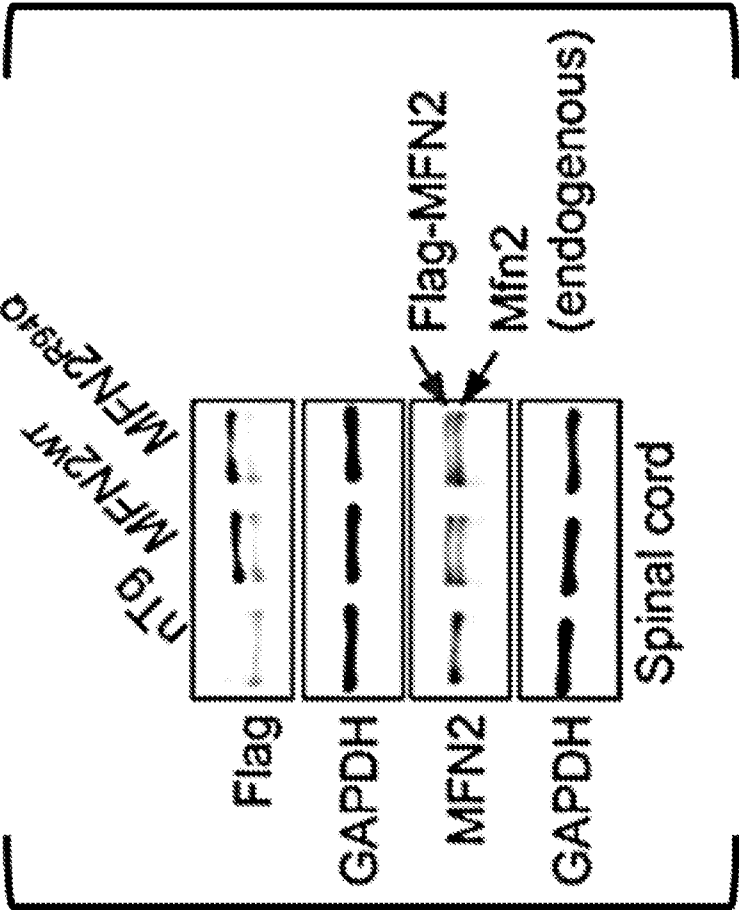


FIG 13D

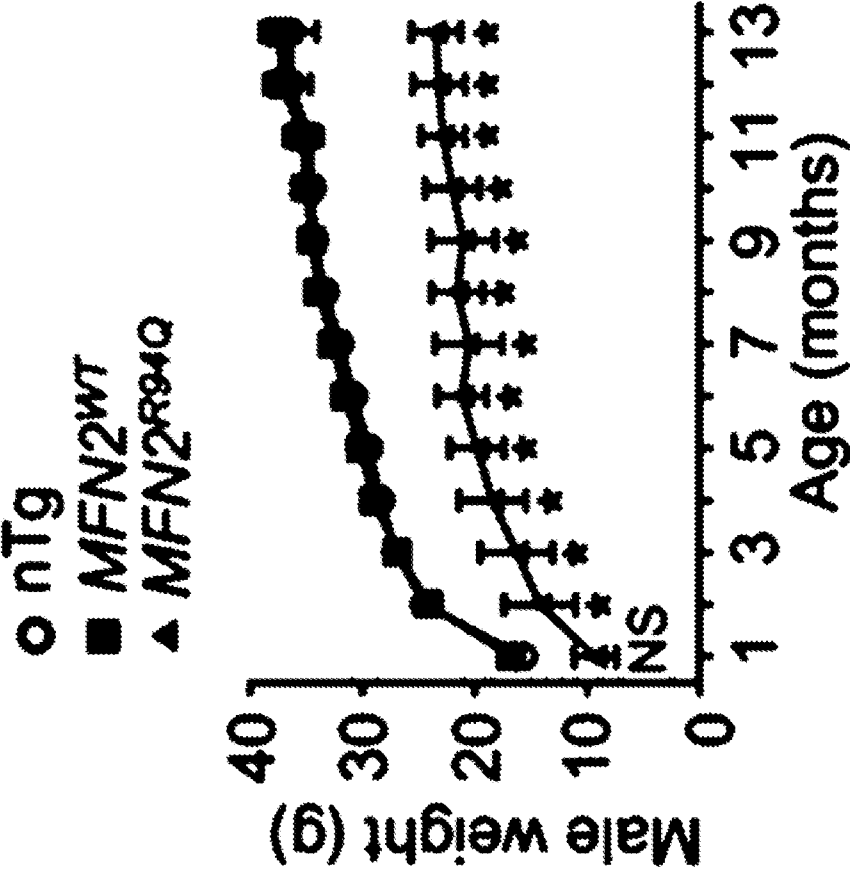


FIG 13E

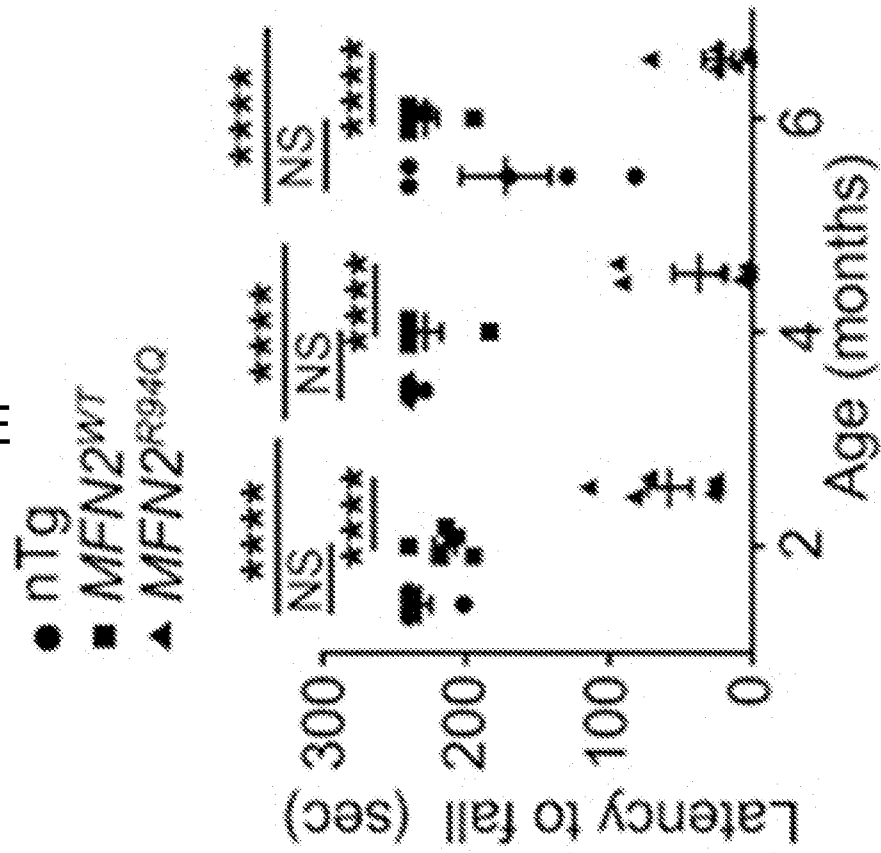


FIG 13F

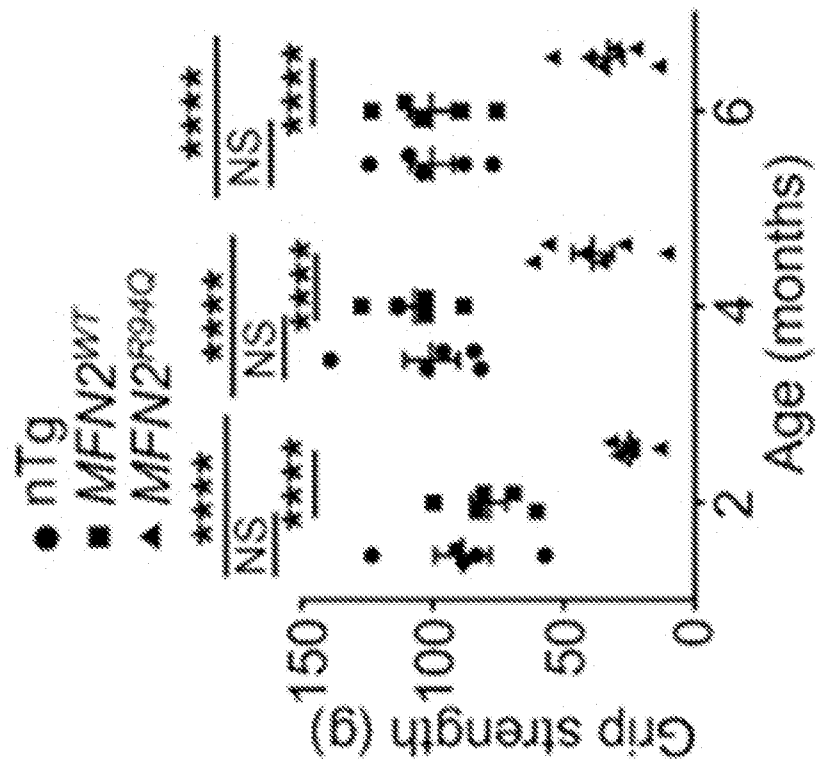


FIG 14A

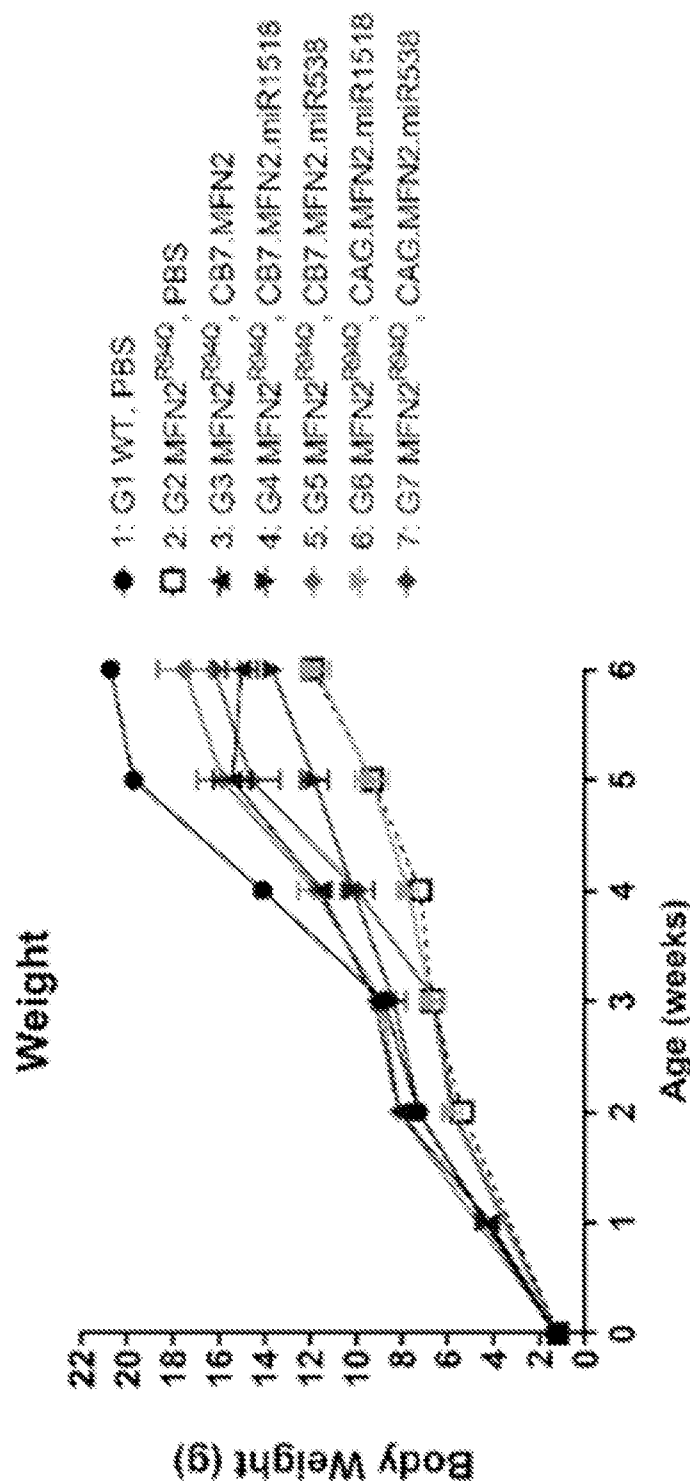
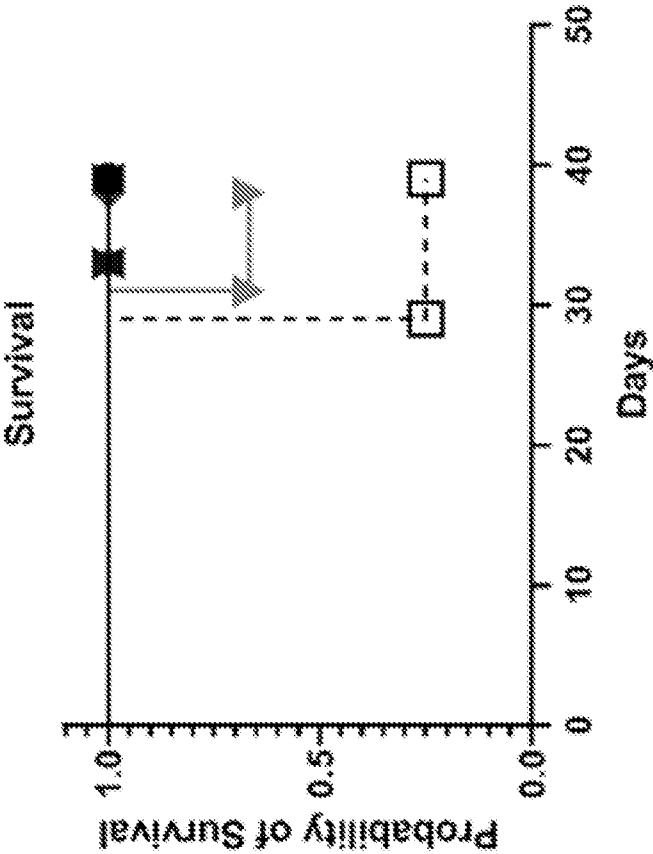
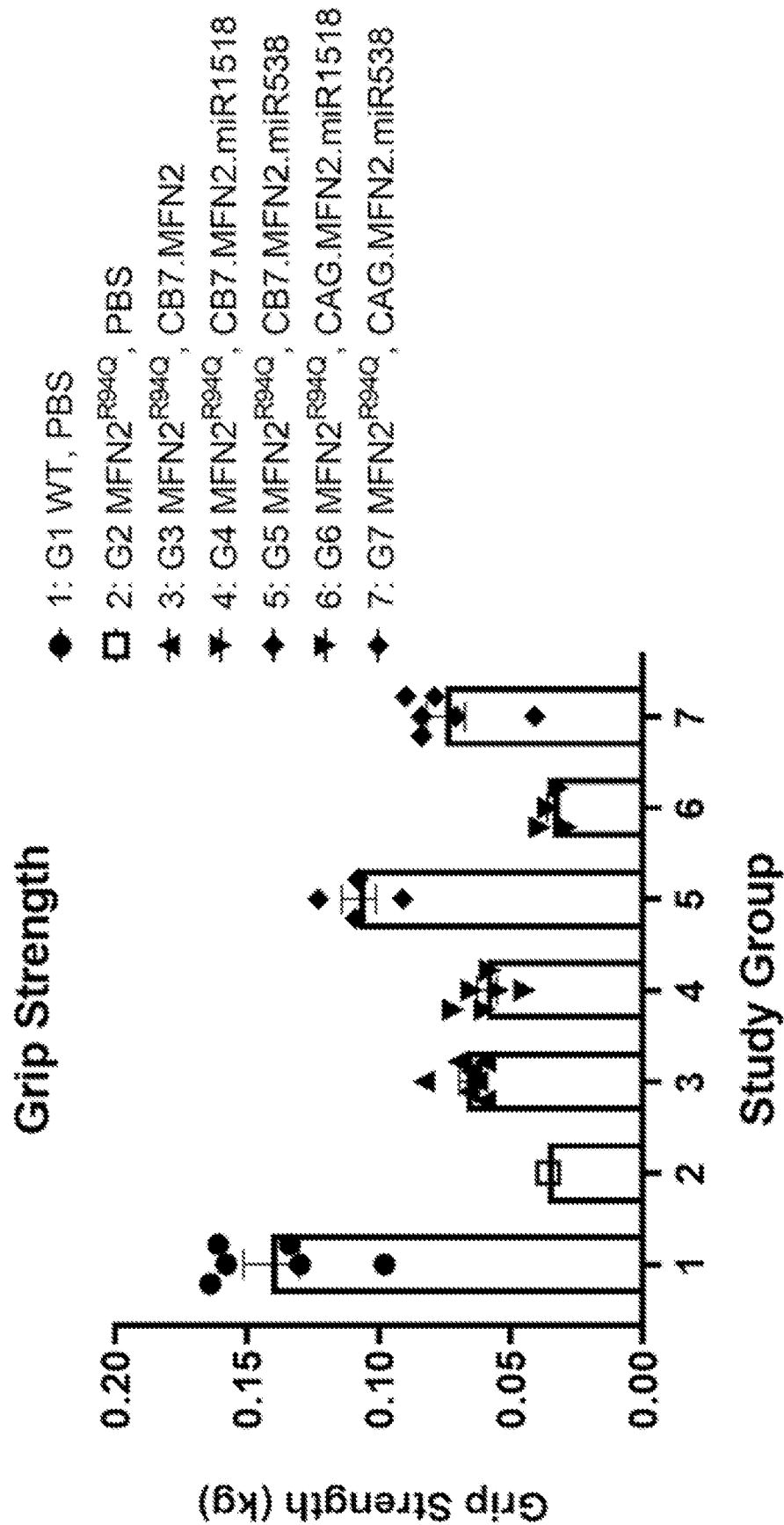


FIG 14B



- 1: G1 WT, PBS
- 2: G2 MFN2^{+/+}, PBS
- 3: G3 MFN2^{+/+}, CB7.MFN2
- 4: G4 MFN2^{+/+}, CB7.MFN2.mir1518
- 5: G5 MFN2^{+/+}, CB7.MFN2.mir538
- 6: G6 MFN2^{+/+}, CAG.MFN2.mir1518
- 7: G7 MFN2^{+/+}, CAG.MFN2.mir538

FIG 15



SEQUENCE LISTING

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<151> 2020-07-13

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<151> 2021-04-09

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tct cca ctg aag cac ttc gtg acc gcc aag aag aag atc aac ggc atc	988
Ser Pro Leu Lys His Phe Val Thr Ala Lys Lys Lys Ile Asn Gly Ile	
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ttc gag cag ctg ggc gcc tac atc caa gag agc gcc acc ttc ctg gaa	1036
Phe Glu Gln Leu Gly Ala Tyr Ile Gln Glu Ser Ala Thr Phe Leu Glu	
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Asp Thr Tyr Arg Asn Ala Glu Leu Asp Pro Val Thr Thr Glu Glu Gln	
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Val Leu Asp Val Lys Gly Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu	
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gtg ctg gcc aga cgg cat atg aag gtg gcc ttt ttc ggc cgg acc agc	1180
Val Leu Ala Arg Arg His Met Lys Val Ala Phe Phe Gly Arg Thr Ser	
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Thr Asp Gly His Glu Ala Phe Leu Leu Thr Glu Gly Ser Glu Glu Lys	
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gaa	cac	gcc	tcc	gag	aaa	ctg	cag	ctg	gtg	atc	agc	tac	acc	ggc	agc	2908	
Glu	His	Ala	Ser	Glu	Lys	Leu	Gln	Leu	Val	Ile	Ser	Tyr	Thr	Gly	Ser		
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aat	tgc	agc	cac	cag	gtg	cag	caa	gag	ctg	tcc	gga	aca	ttc	gcc	cac	2956	
Asn	Cys	Ser	His	Gln	Val	Gln	Gln	Glu	Leu	Ser	Gly	Thr	Phe	Ala	His		
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Tyr Ile Gln Glu Ser Ala Thr Phe Leu Glu Asp Thr Tyr Arg Asn Ala
50 55 60

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

Met Lys Val Ala Phe Phe Gly Arg Thr Ser Asn Gly Lys Ser Thr Val
100 105 110

Ile Asn Ala Met Leu Trp Asp Lys Val Leu Pro Ser Gly Ile Gly His
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Thr Thr Asn Cys Phe Leu Arg Val Glu Gly Thr Asp Gly His Glu Ala
130 135 140

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

Asn Gln Leu Ala His Ala Leu His Gln Asp Lys Gln Leu His Ala Gly
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Ser Leu Val Ser Val Met Trp Pro Asn Ser Lys Cys Pro Leu Leu Lys
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Asp Asp Leu Val Leu Met Asp Ser Pro Gly Ile Asp Val Thr Thr Glu
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Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu Asp Ala Asp Val Phe Val
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Leu Val Ala Asn Ser Glu Ser Thr Leu Met Gln Thr Glu Lys His Phe
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Phe His Lys Val Ser Glu Arg Leu Ser Arg Pro Asn Ile Phe Ile Leu
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Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly Phe Gln Val Arg Met Phe
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Leu Arg Ile Lys Gln Ile Thr Glu Glu Val Glu Arg Gln Val Ser Thr
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Gln Met Asp Phe His Pro Ser Pro Val Val Leu Lys Val Tyr Lys Asn
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645 650 655

Lys Glu Arg Ala Phe Lys Arg Gln Phe Val Glu His Ala Ser Glu Lys
660 665 670

Leu Gln Leu Val Ile Ser Tyr Thr Gly Ser Asn Cys Ser His Gln Val
675 680 685

Gln Gln Glu Leu Ser Gly Thr Phe Ala His Leu Cys Gln Gln Val Asp
690 695 700

Val Thr Arg Glu Asn Leu Glu Gln Glu Ile Ala Ala Met Asn Lys Lys
705 710 715 720

Ile Glu Val Leu Asp Ser Leu Gln Ser Lys Ala Lys Leu Leu Arg Asn
725 730 735

Lys Ala Gly Trp Leu Asp Ser Glu Leu Asn Met Phe Thr His Gln Tyr
740 745 750

Leu Gln Pro Ser Arg
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gcgtgctagc accggtgccca cc atg tct ctg ctg ttc agc cgg tgc aac agc	892
Met Ser Leu Leu Phe Ser Arg Cys Asn Ser	
1 5 10	
atc gtg acc gtg aag aaa gac aag agg cac atg gcc gaa gtg aac gcc	940
Ile Val Thr Val Lys Lys Asp Lys Arg His Met Ala Glu Val Asn Ala	
15 20 25	
tct cca ctg aag cac ttc gtg acc gcc aag aag aag atc aac ggc atc	988
Ser Pro Leu Lys His Phe Val Thr Ala Lys Lys Lys Ile Asn Gly Ile	
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ttc gag cag ctg ggc gcc tac atc caa gag tct gcc ggc ttc ctg gaa	1036
Phe Glu Gln Leu Gly Ala Tyr Ile Gln Glu Ser Ala Gly Phe Leu Glu	
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gat acc cac aga aac acc gag ctg gac ccc gtg acc aca gag gaa cag	1084
Asp Thr His Arg Asn Thr Glu Leu Asp Pro Val Thr Thr Glu Glu Gln	
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Val Leu Asp Val Lys Gly Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu	
75 80 85 90	
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Val Leu Ala Arg Arg His Met Lys Val Ala Phe Phe Gly Arg Thr Ser	
95 100 105	
aac ggc aag agc acc gtg atc aac gcc atg ctg tgg gac aaa gtg ctg	1228
Asn Gly Lys Ser Thr Val Ile Asn Ala Met Leu Trp Asp Lys Val Leu	
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cct tct ggc atc ggc cac acc acc aac tgc ttt ctg aga gtc ggc ggc	1276
Pro Ser Gly Ile Gly His Thr Thr Asn Cys Phe Leu Arg Val Gly Gly	
125 130 135	
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Thr Asp Gly His Glu Ala Phe Leu Leu Thr Glu Gly Ser Glu Glu Lys	
140 145 150	

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Lys Ser Val Lys Thr Val Asn Gln Leu Ala His Ala Leu His Gln Asp	
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Glu Gln Leu His Ala Gly Ser Leu Val Ser Val Met Trp Pro Asn Ser	
175 180 185	
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Lys Cys Pro Leu Leu Lys Asp Asp Leu Val Leu Met Asp Ser Pro Gly	
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Ile Asp Val Thr Thr Glu Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu	
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Asp Ala Asp Val Phe Val Leu Val Ala Asn Ser Glu Ser Thr Leu Met	
220 225 230	
cag acc gag aag cag ttc ttc cac aag gtg tcc gag agg ctg agc agg	1612
Gln Thr Glu Lys Gln Phe Phe His Lys Val Ser Glu Arg Leu Ser Arg	
235 240 245 250	
ccc aat atc ttt atc ctg aac aac aga tgg gac gcc agc gcc agc gag	1660
Pro Asn Ile Phe Ile Leu Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu	
255 260 265	
ccc gag tat atg gaa gaa gtg cgg agg cag cat atg gaa agg tgt acc	1708
Pro Glu Tyr Met Glu Glu Val Arg Arg Gln His Met Glu Arg Cys Thr	
270 275 280	
agc ttc ctg gtg gac gag ctg gga gtc gtg gat aga gca cag gcc ggc	1756
Ser Phe Leu Val Asp Glu Leu Gly Val Val Asp Arg Ala Gln Ala Gly	
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Asp Arg Ile Phe Phe Val Ser Ala Lys Glu Val Leu Ser Ala Arg Val	
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Gln Lys Ala Gln Gly Met Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly	
315 320 325 330	
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Phe Gln Val Arg Met Phe Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu	
335 340 345	
gaa tgc atc agc cag agc gcc gtc aag aca aag ttc gag cag cac aca	1948
Glu Cys Ile Ser Gln Ser Ala Val Lys Thr Lys Phe Glu Gln His Thr	
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gtg cgg gcc aag cag atc gct gag gct gtg cgg ctg atc atg gac agc	1996
Val Arg Ala Lys Gln Ile Ala Glu Ala Val Arg Leu Ile Met Asp Ser	

365	370	375	
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gaa aga cag gtg tcc acc gcc atg gcc gag gaa atc cgt aga ctg tct Glu Arg Gln Val Ser Thr Ala Met Ala Glu Glu Ile Arg Arg Leu Ser 430 435 440			2188
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cag aca atg cag cag gac atg atc gac ggc ctg aag cct ctg ctg cct Gln Thr Met Gln Gln Asp Met Ile Asp Gly Leu Lys Pro Leu Leu Pro 495 500 505			2380
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cct cct ctg cct caa ggc tct ctg acc caa gag gaa ctg atg gtg tcc Pro Pro Leu Pro Gln Gly Ser Leu Thr Gln Glu Glu Leu Met Val Ser 590 595 600	2668
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ctg gtc gtc ggc gga gtt gtg tgg aaa gct gtt ggc tgg cgc ctg atc Leu Val Val Gly Gly Val Val Trp Lys Ala Val Gly Trp Arg Leu Ile 620 625 630	2764
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acc tgg acc acc agg gcc aaa gag agg gcc ttc aag agg cag ttc gtg Thr Trp Thr Thr Arg Ala Lys Glu Arg Ala Phe Lys Arg Gln Phe Val 655 660 665	2860
gaa tac gcc tcc gag aaa ctg cag ctg att atc tcc tac acc ggc agc Glu Tyr Ala Ser Glu Lys Leu Gln Leu Ile Ile Ser Tyr Thr Gly Ser 670 675 680	2908
aac tgc agc cac cag gtg cag caa gag ctg tct gga aca ttc gcc cac Asn Cys Ser His Gln Val Gln Gln Glu Leu Ser Gly Thr Phe Ala His 685 690 695	2956
ctg tgc cag cag gtc gac atc acc aga gac aac ctg gaa caa gag atc Leu Cys Gln Gln Val Asp Ile Thr Arg Asp Asn Leu Glu Gln Glu Ile 700 705 710	3004
gcc gct atg aat aag aag gtg gaa gcc ctg gac tcc ctg cag agc aag Ala Ala Met Asn Lys Lys Val Glu Ala Leu Asp Ser Leu Gln Ser Lys 715 720 725 730	3052
gct aag ctg ctg aga aac aag gcc ggc tgg ctg gac tcc gag ctg aat Ala Lys Leu Leu Arg Asn Lys Ala Gly Trp Leu Asp Ser Glu Leu Asn 735 740 745	3100
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Asp Lys Arg His Met Ala Glu Val Asn Ala Ser Pro Leu Lys His Phe

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25

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Val Thr Ala Lys Lys Lys Ile Asn Gly Ile Phe Glu Gln Leu Gly Ala
 35 40 45

Tyr Ile Gln Glu Ser Ala Gly Phe Leu Glu Asp Thr His Arg Asn Thr
 50 55 60

Glu Leu Asp Pro Val Thr Thr Glu Glu Gln Val Leu Asp Val Lys Gly
 65 70 75 80

Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu Val Leu Ala Arg Arg His
 85 90 95

Met Lys Val Ala Phe Phe Gly Arg Thr Ser Asn Gly Lys Ser Thr Val
 100 105 110

Ile Asn Ala Met Leu Trp Asp Lys Val Leu Pro Ser Gly Ile Gly His
 115 120 125

Thr Thr Asn Cys Phe Leu Arg Val Gly Gly Thr Asp Gly His Glu Ala
 130 135 140

Phe Leu Leu Thr Glu Gly Ser Glu Glu Lys Lys Ser Val Lys Thr Val
 145 150 155 160

Asn Gln Leu Ala His Ala Leu His Gln Asp Glu Gln Leu His Ala Gly
 165 170 175

Ser Leu Val Ser Val Met Trp Pro Asn Ser Lys Cys Pro Leu Leu Lys
 180 185 190

Asp Asp Leu Val Leu Met Asp Ser Pro Gly Ile Asp Val Thr Thr Glu
 195 200 205

Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu Asp Ala Asp Val Phe Val
 210 215 220

Leu Val Ala Asn Ser Glu Ser Thr Leu Met Gln Thr Glu Lys Gln Phe
 225 230 235 240

Phe His Lys Val Ser Glu Arg Leu Ser Arg Pro Asn Ile Phe Ile Leu
245 250 255

Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu Pro Glu Tyr Met Glu Glu
260 265 270

Val Arg Arg Gln His Met Glu Arg Cys Thr Ser Phe Leu Val Asp Glu
275 280 285

Leu Gly Val Val Asp Arg Ala Gln Ala Gly Asp Arg Ile Phe Phe Val
290 295 300

Ser Ala Lys Glu Val Leu Ser Ala Arg Val Gln Lys Ala Gln Gly Met
305 310 315 320

Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly Phe Gln Val Arg Met Phe
325 330 335

Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu Glu Cys Ile Ser Gln Ser
340 345 350

Ala Val Lys Thr Lys Phe Glu Gln His Thr Val Arg Ala Lys Gln Ile
355 360 365

Ala Glu Ala Val Arg Leu Ile Met Asp Ser Leu His Ile Ala Ala Gln
370 375 380

Glu Gln Arg Val Tyr Cys Leu Glu Met Arg Glu Glu Arg Gln Asp Arg
385 390 395 400

Leu Arg Phe Ile Asp Lys Gln Leu Glu Leu Leu Ala Gln Asp Tyr Lys
405 410 415

Leu Arg Ile Lys Gln Met Thr Glu Glu Val Glu Arg Gln Val Ser Thr
420 425 430

Ala Met Ala Glu Glu Ile Arg Arg Leu Ser Val Leu Val Asp Glu Tyr
435 440 445

Gln Met Asp Phe His Pro Ser Pro Val Val Leu Lys Val Tyr Lys Asn

450

455

460

Glu Leu His Arg His Ile Glu Glu Gly Leu Gly Arg Asn Met Ser Asp
 465 470 475 480

Arg Cys Ser Thr Ala Ile Ala Ser Ser Leu Gln Thr Met Gln Gln Asp
 485 490 495

Met Ile Asp Gly Leu Lys Pro Leu Leu Pro Val Ser Val Arg Asn Gln
 500 505 510

Ile Asp Met Leu Val Pro Arg Gln Cys Phe Ser Leu Ser Tyr Asp Leu
 515 520 525

Asn Cys Asp Lys Leu Cys Ala Asp Phe Gln Glu Asp Ile Glu Phe His
 530 535 540

Phe Ser Leu Gly Trp Thr Met Leu Val Asn Arg Phe Leu Gly Pro Lys
 545 550 555 560

Asn Ser Arg Arg Ala Leu Leu Gly Tyr Asn Asp Gln Val Gln Arg Pro
 565 570 575

Leu Pro Leu Thr Pro Ala Asn Pro Ser Met Pro Pro Leu Pro Gln Gly
 580 585 590

Ser Leu Thr Gln Glu Glu Leu Met Val Ser Met Val Thr Gly Leu Ala
 595 600 605

Ser Leu Thr Ser Arg Thr Ser Met Gly Ile Leu Val Val Gly Gly Val
 610 615 620

Val Trp Lys Ala Val Gly Trp Arg Leu Ile Ala Leu Ser Phe Gly Leu
 625 630 635 640

Tyr Gly Leu Leu Tyr Val Tyr Glu Arg Leu Thr Trp Thr Thr Arg Ala
 645 650 655

Lys Glu Arg Ala Phe Lys Arg Gln Phe Val Glu Tyr Ala Ser Glu Lys
 660 665 670

Leu Gln Leu Ile Ile Ser Tyr Thr Gly Ser Asn Cys Ser His Gln Val
675 680 685

Gln Gln Glu Leu Ser Gly Thr Phe Ala His Leu Cys Gln Gln Val Asp
690 695 700

Ile Thr Arg Asp Asn Leu Glu Gln Glu Ile Ala Ala Met Asn Lys Lys
705 710 715 720

Val Glu Ala Leu Asp Ser Leu Gln Ser Lys Ala Lys Leu Leu Arg Asn
725 730 735

Lys Ala Gly Trp Leu Asp Ser Glu Leu Asn Met Phe Ile His Gln Tyr
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Leu Gln Pro Ser Arg
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<212> DNA
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<220>
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 <213> Artificial Sequence

<220>
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<220>

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Asn	Lys	Arg	His	Met	Ala	Glu	Val	Asn	Ala	Ser	Pro	Leu	Lys	His	Phe	
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gtc	act	gcc	aag	aag	aag	atc	aat	ggc	att	ttt	gag	cag	ctg	ggg	gcc	144
Val	Thr	Ala	Lys	Lys	Lys	Ile	Asn	Gly	Ile	Phe	Glu	Gln	Leu	Gly	Ala	
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Tyr	Ile	Gln	Glu	Ser	Ala	Thr	Phe	Leu	Glu	Asp	Thr	Tyr	Arg	Asn	Ala	
	50					55					60					

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Glu	Leu	Asp	Pro	Val	Thr	Thr	Glu	Glu	Gln	Val	Leu	Asp	Val	Lys	Gly	
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Tyr	Leu	Ser	Lys	Val	Arg	Gly	Ile	Ser	Glu	Val	Leu	Ala	Arg	Arg	His	
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atg	aaa	gtg	gct	ttt	ttt	ggc	cgg	acg	agc	aat	ggg	aag	agc	acc	gtg	336
Met	Lys	Val	Ala	Phe	Phe	Gly	Arg	Thr	Ser	Asn	Gly	Lys	Ser	Thr	Val	
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Ile	Asn	Ala	Met	Leu	Trp	Asp	Lys	Val	Leu	Pro	Ser	Gly	Ile	Gly	His	
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Phe 145	Leu	Leu	Thr	Glu	Gly 150	Ser	Glu	Glu	Lys	Arg 155	Ser	Ala	Lys	Thr	Val 160	
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agc Ser	cta Leu	gtg Val	agt Ser 180	gtg Val	atg Met	tgg Trp	ccc Pro	aac Asn 185	tct Ser	aag Lys	tgc Cys	cca Pro	ctt Leu 190	ctg Leu	aag Lys	576
gat Asp	gac Asp	ctc Leu 195	gtt Val	ttg Leu	atg Met	gac Asp	agc Ser 200	cct Pro	ggg Gly	att Ile	gat Asp	gtc Val 205	acc Thr	aca Thr	gag Glu	624
ctg Leu 210	gac Asp	agc Ser	tgg Trp	att Ile	gac Asp	aag Lys 215	ttt Phe	tgt Cys	ctg Leu	gat Asp	gct Ala 220	gat Asp	gtg Val	ttt Phe	gtg Val	672
ctg Leu 225	gtg Val	gcc Ala	aac Asn	tca Ser 230	gag Glu	tcc Ser	acc Thr	ctg Leu	atg Met	cag Gln 235	acg Thr	gaa Glu	aag Lys	cac His	ttc Phe 240	720
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gag cag cag gtt tac tgc gag gaa atg cgt gaa gag cgg caa gac cga Glu Gln Gln Val Tyr Cys Glu Glu Met Arg Glu Glu Arg Gln Asp Arg 385 390 395 400	1200
ctg aaa ttt att gac aaa cag ctg gag ctc ttg gct caa gac tat aag Leu Lys Phe Ile Asp Lys Gln Leu Glu Leu Ala Gln Asp Tyr Lys 405 410 415	1248
ctg cga att aag cag att acg gag gaa gtg gag agg cag gtg tcg act Leu Arg Ile Lys Gln Ile Thr Glu Glu Val Glu Arg Gln Val Ser Thr 420 425 430	1296
gca atg gcc gag gag atc agg cgc ctc tct gta ctg gtg gac gat tac Ala Met Ala Glu Glu Ile Arg Arg Leu Ser Val Leu Val Asp Asp Tyr 435 440 445	1344
cag atg gac ttc cac cct tct cca gta gtc ctc aag gtt tat aag aat Gln Met Asp Phe His Pro Ser Pro Val Val Leu Lys Val Tyr Lys Asn 450 455 460	1392
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cgc tgc tcc acg gcc atc acc aac tcc ctg cag acc atg cag cag gac Arg Cys Ser Thr Ala Ile Thr Asn Ser Leu Gln Thr Met Gln Gln Asp 485 490 495	1488
atg ata gat ggc ttg aaa ccc ctc ctt cct gtg tct gtg cgg agt cag Met Ile Asp Gly Leu Lys Pro Leu Leu Pro Val Ser Val Arg Ser Gln 500 505 510	1536
ata gac atg ctg gtc cca cgc cag tgc ttc tcc ctc aac tat gac cta Ile Asp Met Leu Val Pro Arg Gln Cys Phe Ser Leu Asn Tyr Asp Leu 515 520 525	1584
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Ser	Leu	Thr	Ser	Arg	Thr	Ser	Met	Gly	Ile	Leu	Val	Val	Gly	Gly	Val		
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Val	Trp	Lys	Ala	Val	Gly	Trp	Arg	Leu	Ile	Ala	Leu	Ser	Phe	Gly	Leu		
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Tyr	Gly	Leu	Leu	Tyr	Val	Tyr	Glu	Arg	Leu	Thr	Trp	Thr	Thr	Lys	Ala		
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Lys	Glu	Arg	Ala	Phe	Lys	Arg	Gln	Phe	Val	Glu	His	Ala	Ser	Glu	Lys		
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Gln	Gln	Glu	Leu	Ser	Gly	Thr	Phe	Ala	His	Leu	Cys	Gln	Gln	Val	Asp		
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Val	Thr	Arg	Glu	Asn	Leu	Glu	Gln	Glu	Ile	Ala	Ala	Met	Asn	Lys	Lys		
705				710						715					720		
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Ile	Glu	Val	Leu	Asp	Ser	Leu	Gln	Ser	Lys	Ala	Lys	Leu	Leu	Arg	Asn		
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Lys	Ala	Gly	Trp	Leu	Asp	Ser	Glu	Leu	Asn	Met	Phe	Thr	His	Gln	Tyr		
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Leu	Gln	Pro	Ser	Arg													
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<223> Synthetic Construct

<400> 19

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Val Thr Ala Lys Lys Lys Ile Asn Gly Ile Phe Glu Gln Leu Gly Ala
35 40 45

Tyr Ile Gln Glu Ser Ala Thr Phe Leu Glu Asp Thr Tyr Arg Asn Ala
50 55 60

Glu Leu Asp Pro Val Thr Thr Glu Glu Gln Val Leu Asp Val Lys Gly
65 70 75 80

Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu Val Leu Ala Arg Arg His
85 90 95

Met Lys Val Ala Phe Phe Gly Arg Thr Ser Asn Gly Lys Ser Thr Val
100 105 110

Ile Asn Ala Met Leu Trp Asp Lys Val Leu Pro Ser Gly Ile Gly His
115 120 125

Thr Thr Asn Cys Phe Leu Arg Val Glu Gly Thr Asp Gly His Glu Ala
130 135 140

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

Asn Gln Leu Ala His Ala Leu His Gln Asp Lys Gln Leu His Ala Gly
165 170 175

Ser Leu Val Ser Val Met Trp Pro Asn Ser Lys Cys Pro Leu Leu Lys
180 185 190

Asp Asp Leu Val Leu Met Asp Ser Pro Gly Ile Asp Val Thr Thr Glu
195 200 205

Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu Asp Ala Asp Val Phe Val
210 215 220

Leu Val Ala Asn Ser Glu Ser Thr Leu Met Gln Thr Glu Lys His Phe
225 230 235 240

Phe His Lys Val Ser Glu Arg Leu Ser Arg Pro Asn Ile Phe Ile Leu
245 250 255

Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu Pro Glu Tyr Met Glu Glu
260 265 270

Val Arg Arg Gln His Met Glu Arg Cys Thr Ser Phe Leu Val Asp Glu
275 280 285

Leu Gly Val Val Asp Arg Ser Gln Ala Gly Asp Arg Ile Phe Phe Val
290 295 300

Ser Ala Lys Glu Val Leu Asn Ala Arg Ile Gln Lys Ala Gln Gly Met
305 310 315 320

Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly Phe Gln Val Arg Met Phe
325 330 335

Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu Glu Cys Ile Ser Gln Ser
340 345 350

Ala Val Lys Thr Lys Phe Glu Gln His Thr Val Arg Ala Lys Gln Ile
355 360 365

Ala Glu Ala Val Arg Leu Ile Met Asp Ser Leu His Met Ala Ala Arg
370 375 380

Glu Gln Gln Val Tyr Cys Glu Glu Met Arg Glu Glu Arg Gln Asp Arg
385 390 395 400

Leu Lys Phe Ile Asp Lys Gln Leu Glu Leu Leu Ala Gln Asp Tyr Lys
405 410 415

Leu Arg Ile Lys Gln Ile Thr Glu Glu Val Glu Arg Gln Val Ser Thr
420 425 430

Ala Met Ala Glu Glu Ile Arg Arg Leu Ser Val Leu Val Asp Asp Tyr
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Gln Met Asp Phe His Pro Ser Pro Val Val Leu Lys Val Tyr Lys Asn
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Glu Leu His Arg His Ile Glu Glu Gly Leu Gly Arg Asn Met Ser Asp
465 470 475 480

Arg Cys Ser Thr Ala Ile Thr Asn Ser Leu Gln Thr Met Gln Gln Asp
485 490 495

Met Ile Asp Gly Leu Lys Pro Leu Leu Pro Val Ser Val Arg Ser Gln
500 505 510

Ile Asp Met Leu Val Pro Arg Gln Cys Phe Ser Leu Asn Tyr Asp Leu
515 520 525

Asn Cys Asp Lys Leu Cys Ala Asp Phe Gln Glu Asp Ile Glu Phe His
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Phe Ser Leu Gly Trp Thr Met Leu Val Asn Arg Phe Leu Gly Pro Lys
545 550 555 560

Asn Ser Arg Arg Ala Leu Met Gly Tyr Asn Asp Gln Val Gln Arg Pro
565 570 575

Ile Pro Leu Thr Pro Ala Asn Pro Ser Met Pro Pro Leu Pro Gln Gly
580 585 590

Ser Leu Thr Gln Glu Glu Phe Met Val Ser Met Val Thr Gly Leu Ala
595 600 605

Ser Leu Thr Ser Arg Thr Ser Met Gly Ile Leu Val Val Gly Gly Val
610 615 620

Val Trp Lys Ala Val Gly Trp Arg Leu Ile Ala Leu Ser Phe Gly Leu
625 630 635 640

Tyr Gly Leu Leu Tyr Val Tyr Glu Arg Leu Thr Trp Thr Thr Lys Ala
645 650 655

Lys Glu Arg Ala Phe Lys Arg Gln Phe Val Glu His Ala Ser Glu Lys
660 665 670

Leu Gln Leu Val Ile Ser Tyr Thr Gly Ser Asn Cys Ser His Gln Val
675 680 685

Gln Gln Glu Leu Ser Gly Thr Phe Ala His Leu Cys Gln Gln Val Asp
690 695 700

Val Thr Arg Glu Asn Leu Glu Gln Glu Ile Ala Ala Met Asn Lys Lys
705 710 715 720

Ile Glu Val Leu Asp Ser Leu Gln Ser Lys Ala Lys Leu Leu Arg Asn
725 730 735

Lys Ala Gly Trp Leu Asp Ser Glu Leu Asn Met Phe Thr His Gln Tyr
740 745 750

Leu Gln Pro Ser Arg
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Met Ser Leu Leu Phe Ser Arg Cys Asn Ser Ile Val Thr Val Lys Lys
1 5 10 15

gat aag cga cac atg gct gag gtg aat gct tcc cct ctc aag cac ttt 96
Asp Lys Arg His Met Ala Glu Val Asn Ala Ser Pro Leu Lys His Phe

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

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Phe His Lys Val Ser Glu Arg Leu Ser Arg Pro Asn Ile Phe Ile Leu	
245 250 255	
aac aac cgc tgg gat gcg tct gcc tcg gag cct gag tac atg gag gag	816
Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu Pro Glu Tyr Met Glu Glu	
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gtg cgg cgg cag cac atg gaa cgc tgc acc agc ttt ctg gtg gat gag	864
Val Arg Arg Gln His Met Glu Arg Cys Thr Ser Phe Leu Val Asp Glu	
275 280 285	
cta ggc gtg gtg gat cga gct cag gcc ggg gac cgg atc ttc ttt gtg	912
Leu Gly Val Val Asp Arg Ala Gln Ala Gly Asp Arg Ile Phe Phe Val	
290 295 300	
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Ser Ala Lys Glu Val Leu Ser Ala Arg Val Gln Lys Ala Gln Gly Met	
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Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly Phe Gln Val Arg Met Phe	
325 330 335	
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Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu Glu Cys Ile Ser Gln Ser	
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gca gta aag acc aaa ttt gag cag cac aca gtc cgg gcc aag cag att	1104
Ala Val Lys Thr Lys Phe Glu Gln His Thr Val Arg Ala Lys Gln Ile	
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gca gag gcc gtc cgt ctc atc atg gat tcc ctg cac att gcg gct cag	1152
Ala Glu Ala Val Arg Leu Ile Met Asp Ser Leu His Ile Ala Ala Gln	
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Glu Gln Arg Val Tyr Cys Leu Glu Met Arg Glu Glu Arg Gln Asp Arg	
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Leu Arg Phe Ile Asp Lys Gln Leu Glu Leu Leu Ala Gln Asp Tyr Lys	
405 410 415	
cta cgg att aag cag atg aca gag gaa gtg gaa agg cag gtg tcc aca	1296
Leu Arg Ile Lys Gln Met Thr Glu Glu Val Glu Arg Gln Val Ser Thr	
420 425 430	
gcc atg gct gaa gag atc agg cgc ctc tct gtg ctg gtt gac gag tac	1344
Ala Met Ala Glu Glu Ile Arg Arg Leu Ser Val Leu Val Asp Glu Tyr	
435 440 445	
cag atg gac ttt cac cca tcc cca gtt gtc ctc aag gtt tat aag aat	1392
Gln Met Asp Phe His Pro Ser Pro Val Val Leu Lys Val Tyr Lys Asn	

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cag	caa	gaa	ttg	tct	ggg	aca	ttt	gct	cat	ctg	tgc	cag	caa	gtt	gac	2112
Gln	Gln	Glu	Leu	Ser	Gly	Thr	Phe	Ala	His	Leu	Cys	Gln	Gln	Val	Asp	
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atc	acc	cgg	gat	aat	ctg	gag	cag	gaa	att	gct	gcc	atg	aac	aag	aaa	2160
Ile	Thr	Arg	Asp	Asn	Leu	Glu	Gln	Glu	Ile	Ala	Ala	Met	Asn	Lys	Lys	
		705				710				715					720	

gtt	gag	gct	ctg	gat	tca	ctt	cag	agc	aaa	gcc	aaa	ttg	ctc	agg	aat	2208
Val	Glu	Ala	Leu	Asp	Ser	Leu	Gln	Ser	Lys	Ala	Lys	Leu	Leu	Arg	Asn	
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aaa	gct	ggc	tgg	ttg	gac	agc	gaa	ctc	aac	atg	ttc	ata	cac	cag	tac	2256
Lys	Ala	Gly	Trp	Leu	Asp	Ser	Glu	Leu	Asn	Met	Phe	Ile	His	Gln	Tyr	
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Leu	Gln	Pro	Ser	Arg												
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Val	Thr	Ala	Lys	Lys	Lys	Ile	Asn	Gly	Ile	Phe	Glu	Gln	Leu	Gly	Ala
		35					40					45			

Tyr	Ile	Gln	Glu	Ser	Ala	Gly	Phe	Leu	Glu	Asp	Thr	His	Arg	Asn	Thr
	50					55					60				

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

Met Lys Val Ala Phe Phe Gly Arg Thr Ser Asn Gly Lys Ser Thr Val
100 105 110

Ile Asn Ala Met Leu Trp Asp Lys Val Leu Pro Ser Gly Ile Gly His
115 120 125

Thr Thr Asn Cys Phe Leu Arg Val Gly Gly Thr Asp Gly His Glu Ala
130 135 140

Phe Leu Leu Thr Glu Gly Ser Glu Glu Lys Lys Ser Val Lys Thr Val
145 150 155 160

Asn Gln Leu Ala His Ala Leu His Gln Asp Glu Gln Leu His Ala Gly
165 170 175

Ser Leu Val Ser Val Met Trp Pro Asn Ser Lys Cys Pro Leu Leu Lys
180 185 190

Asp Asp Leu Val Leu Met Asp Ser Pro Gly Ile Asp Val Thr Thr Glu
195 200 205

Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu Asp Ala Asp Val Phe Val
210 215 220

Leu Val Ala Asn Ser Glu Ser Thr Leu Met Gln Thr Glu Lys Gln Phe
225 230 235 240

Phe His Lys Val Ser Glu Arg Leu Ser Arg Pro Asn Ile Phe Ile Leu
245 250 255

Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu Pro Glu Tyr Met Glu Glu
260 265 270

Val Arg Arg Gln His Met Glu Arg Cys Thr Ser Phe Leu Val Asp Glu
275 280 285

Leu Gly Val Val Asp Arg Ala Gln Ala Gly Asp Arg Ile Phe Phe Val

290

295

300

Ser Ala Lys Glu Val Leu Ser Ala Arg Val Gln Lys Ala Gln Gly Met
 305 310 315 320

Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly Phe Gln Val Arg Met Phe
 325 330 335

Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu Glu Cys Ile Ser Gln Ser
 340 345 350

Ala Val Lys Thr Lys Phe Glu Gln His Thr Val Arg Ala Lys Gln Ile
 355 360 365

Ala Glu Ala Val Arg Leu Ile Met Asp Ser Leu His Ile Ala Ala Gln
 370 375 380

Glu Gln Arg Val Tyr Cys Leu Glu Met Arg Glu Glu Arg Gln Asp Arg
 385 390 395 400

Leu Arg Phe Ile Asp Lys Gln Leu Glu Leu Leu Ala Gln Asp Tyr Lys
 405 410 415

Leu Arg Ile Lys Gln Met Thr Glu Glu Val Glu Arg Gln Val Ser Thr
 420 425 430

Ala Met Ala Glu Glu Ile Arg Arg Leu Ser Val Leu Val Asp Glu Tyr
 435 440 445

Gln Met Asp Phe His Pro Ser Pro Val Val Leu Lys Val Tyr Lys Asn
 450 455 460

Glu Leu His Arg His Ile Glu Glu Gly Leu Gly Arg Asn Met Ser Asp
 465 470 475 480

Arg Cys Ser Thr Ala Ile Ala Ser Ser Leu Gln Thr Met Gln Gln Asp
 485 490 495

Met Ile Asp Gly Leu Lys Pro Leu Leu Pro Val Ser Val Arg Asn Gln
 500 505 510

Ile Asp Met Leu Val Pro Arg Gln Cys Phe Ser Leu Ser Tyr Asp Leu
515 520 525

Asn Cys Asp Lys Leu Cys Ala Asp Phe Gln Glu Asp Ile Glu Phe His
530 535 540

Phe Ser Leu Gly Trp Thr Met Leu Val Asn Arg Phe Leu Gly Pro Lys
545 550 555 560

Asn Ser Arg Arg Ala Leu Leu Gly Tyr Asn Asp Gln Val Gln Arg Pro
565 570 575

Leu Pro Leu Thr Pro Ala Asn Pro Ser Met Pro Pro Leu Pro Gln Gly
580 585 590

Ser Leu Thr Gln Glu Glu Leu Met Val Ser Met Val Thr Gly Leu Ala
595 600 605

Ser Leu Thr Ser Arg Thr Ser Met Gly Ile Leu Val Val Gly Gly Val
610 615 620

Val Trp Lys Ala Val Gly Trp Arg Leu Ile Ala Leu Ser Phe Gly Leu
625 630 635 640

Tyr Gly Leu Leu Tyr Val Tyr Glu Arg Leu Thr Trp Thr Thr Arg Ala
645 650 655

Lys Glu Arg Ala Phe Lys Arg Gln Phe Val Glu Tyr Ala Ser Glu Lys
660 665 670

Leu Gln Leu Ile Ile Ser Tyr Thr Gly Ser Asn Cys Ser His Gln Val
675 680 685

Gln Gln Glu Leu Ser Gly Thr Phe Ala His Leu Cys Gln Gln Val Asp
690 695 700

Ile Thr Arg Asp Asn Leu Glu Gln Glu Ile Ala Ala Met Asn Lys Lys
705 710 715 720

Val Glu Ala Leu Asp Ser Leu Gln Ser Lys Ala Lys Leu Leu Arg Asn

725

730

735

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 cattgtctga gtaggtgtca ttctattctg ggggggtgggg tggggcagga cagcaagggg 180
 gaggattggg aagacaatag caggcatgct gggga 215

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 tgcaataaac aagttaacaa caacaattgc attcatttta tgtttcaggt tcaggggggag 180
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atc	gtg	acc	gtg	aag	aaa	aac	aag	cgg	cac	atg	gcc	gaa	gtg	aac	gcc	96
Ile	Val	Thr	Val	Lys	Lys	Asn	Lys	Arg	His	Met	Ala	Glu	Val	Asn	Ala	
			20					25					30			

tct	cca	ctg	aag	cac	ttc	gtg	acc	gcc	aag	aag	aag	atc	aac	ggc	atc	144
Ser	Pro	Leu	Lys	His	Phe	Val	Thr	Ala	Lys	Lys	Lys	Ile	Asn	Gly	Ile	
		35					40					45				

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Phe	Glu	Gln	Leu	Gly	Ala	Tyr	Ile	Gln	Glu	Ser	Ala	Thr	Phe	Leu	Glu	
	50					55					60					

gat	acc	tac	aga	aac	gcc	gag	ctg	gac	ccc	gtg	acc	aca	gag	gaa	cag	240
Asp	Thr	Tyr	Arg	Asn	Ala	Glu	Leu	Asp	Pro	Val	Thr	Thr	Glu	Glu	Gln	
65					70				75						80	

gtg	ctg	gat	gtg	aag	ggc	tac	ctg	agc	aaa	gtg	cgg	ggc	atc	tct	gaa	288
Val	Leu	Asp	Val	Lys	Gly	Tyr	Leu	Ser	Lys	Val	Arg	Gly	Ile	Ser	Glu	
				85					90					95		

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Val	Leu	Ala	Arg	Arg	His	Met	Lys	Val	Ala	Phe	Phe	Gly	Arg	Thr	Ser	
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Asn	Gly	Lys	Ser	Thr	Val	Ile	Asn	Ala	Met	Leu	Trp	Asp	Lys	Val	Leu	
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ccc	tct	gga	atc	ggc	cac	acc	acc	aac	tgc	ttt	ctg	aga	gtg	gaa	ggc	432
Pro	Ser	Gly	Ile	Gly	His	Thr	Thr	Asn	Cys	Phe	Leu	Arg	Val	Glu	Gly	
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Thr	Asp	Gly	His	Glu	Ala	Phe	Leu	Leu	Thr	Glu	Gly	Ser	Glu	Glu	Lys	
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Arg	Ser	Ala	Lys	Thr	Val	Asn	Gln	Leu	Ala	His	Ala	Leu	His	Gln	Asp	
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Lys	Gln	Leu	His	Ala	Gly	Ser	Leu	Val	Ser	Val	Met	Trp	Pro	Asn	Ser	
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Leu	Ala	Gln	Asp	Tyr	Lys	Leu	Arg	Ile	Lys	Gln	Ile	Thr	Glu	Glu	Val		
			420					425					430				
gaa	aga	cag	gtg	tcc	acc	gct	atg	gcc	gag	gaa	atc	aga	cgg	ctg	agc		1344
Glu	Arg	Gln	Val	Ser	Thr	Ala	Met	Ala	Glu	Glu	Ile	Arg	Arg	Leu	Ser		
		435					440					445					
gtg	ctg	gtc	gac	gac	tac	cag	atg	gac	ttt	cac	ccc	tct	cca	gtg	gtg		1392
Val	Leu	Val	Asp	Asp	Tyr	Gln	Met	Asp	Phe	His	Pro	Ser	Pro	Val	Val		
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ctg	aag	gtc	tac	aag	aac	gag	ctg	cac	cgg	cac	atc	gag	gaa	ggc	ctg		1440
Leu	Lys	Val	Tyr	Lys	Asn	Glu	Leu	His	Arg	His	Ile	Glu	Glu	Gly	Leu		
465					470				475						480		
ggc	aga	aac	atg	agc	gac	aga	tgc	agc	acc	gcc	atc	acc	aat	agc	ctg		1488
Gly	Arg	Asn	Met	Ser	Asp	Arg	Cys	Ser	Thr	Ala	Ile	Thr	Asn	Ser	Leu		
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cag	acc	atg	cag	cag	gac	atg	atc	gac	ggc	ctg	aaa	cct	ctg	ctg	cct		1536
Gln	Thr	Met	Gln	Gln	Asp	Met	Ile	Asp	Gly	Leu	Lys	Pro	Leu	Leu	Pro		
			500					505				510					
gtg	tcc	gtc	aga	tcc	cag	atc	gat	atg	ctg	gtg	ccc	cgg	cag	tgc	ttt		1584
Val	Ser	Val	Arg	Ser	Gln	Ile	Asp	Met	Leu	Val	Pro	Arg	Gln	Cys	Phe		
	515					520					525						
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Ser	Leu	Asn	Tyr	Asp	Leu	Asn	Cys	Asp	Lys	Leu	Cys	Ala	Asp	Phe	Gln		
	530					535				540							
gag	gac	atc	gag	ttt	cac	ttc	agc	ctc	ggc	tgg	aca	atg	ctg	gtc	aac		1680
Glu	Asp	Ile	Glu	Phe	His	Phe	Ser	Leu	Gly	Trp	Thr	Met	Leu	Val	Asn		
545					550				555						560		
aga	ttt	ctg	ggc	ccc	aag	aac	agc	aga	cgg	gcc	ctg	atg	ggc	tac	aac		1728
Arg	Phe	Leu	Gly	Pro	Lys	Asn	Ser	Arg	Arg	Ala	Leu	Met	Gly	Tyr	Asn		
			565					570					575				
gat	cag	gtg	cag	agg	ccc	att	cct	ctg	aca	ccc	gcc	aat	cct	agc	atg		1776
Asp	Gln	Val	Gln	Arg	Pro	Ile	Pro	Leu	Thr	Pro	Ala	Asn	Pro	Ser	Met		
			580					585					590				
cct	cca	ctg	cct	cag	ggc	agc	ctg	aca	caa	gag	gaa	ttc	atg	gtg	tct		1824
Pro	Pro	Leu	Pro	Gln	Gly	Ser	Leu	Thr	Gln	Glu	Glu	Phe	Met	Val	Ser		
		595					600					605					
atg	gtc	acc	ggc	ctg	gcc	agc	ctg	acc	agc	aga	aca	tct	atg	ggc	atc		1872
Met	Val	Thr	Gly	Leu	Ala	Ser	Leu	Thr	Ser	Arg	Thr	Ser	Met	Gly	Ile		
	610					615					620						

ctg gtc gtc ggc ggc gtt gtg tgg aaa gct gtt ggc tgg cgg ctg atc	1920
Leu Val Val Gly Gly Val Val Trp Lys Ala Val Gly Trp Arg Leu Ile	
625 630 635 640	

gcc ctg agc ttt ggc ctg tat ggc ctg ctg tac gtg tac gag aga ctg	1968
Ala Leu Ser Phe Gly Leu Tyr Gly Leu Leu Tyr Val Tyr Glu Arg Leu	
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acc tgg acc acc aag gcc aaa gag cgg gcc ttc aag cgg cag ttt gtg	2016
Thr Trp Thr Thr Lys Ala Lys Glu Arg Ala Phe Lys Arg Gln Phe Val	
660 665 670	

gaa cac gcc tcc gag aaa ctg cag ctg gtg atc agc tac acc ggc agc	2064
Glu His Ala Ser Glu Lys Leu Gln Leu Val Ile Ser Tyr Thr Gly Ser	
675 680 685	

aat tgc agc cac cag gtg cag caa gag ctg tcc gga aca ttc gcc cac	2112
Asn Cys Ser His Gln Val Gln Gln Glu Leu Ser Gly Thr Phe Ala His	
690 695 700	

ctg tgt cag cag gtc gac gtg acc aga gag aac ctg gaa caa gag atc	2160
Leu Cys Gln Gln Val Asp Val Thr Arg Glu Asn Leu Glu Gln Glu Ile	
705 710 715 720	

gcc gcc atg aac aag aaa atc gag gtg ctc gac tcc ctg cag agc aag	2208
Ala Ala Met Asn Lys Lys Ile Glu Val Leu Asp Ser Leu Gln Ser Lys	
725 730 735	

gcc aag ctg ctg aga aac aag gcc ggc tgg ctg gac agc gag ctg aat	2256
Ala Lys Leu Leu Arg Asn Lys Ala Gly Trp Leu Asp Ser Glu Leu Asn	
740 745 750	

atg ttc acc cac cag tac ctgcagccta gccgctaata atgtacagcg	2304
Met Phe Thr His Gln Tyr	
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35 40 45

Phe Glu Gln Leu Gly Ala Tyr Ile Gln Glu Ser Ala Thr Phe Leu Glu
50 55 60

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

Val Leu Asp Val Lys Gly Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu
85 90 95

Val Leu Ala Arg Arg His Met Lys Val Ala Phe Phe Gly Arg Thr Ser
100 105 110

Asn Gly Lys Ser Thr Val Ile Asn Ala Met Leu Trp Asp Lys Val Leu
115 120 125

Pro Ser Gly Ile Gly His Thr Thr Asn Cys Phe Leu Arg Val Glu Gly
130 135 140

Thr Asp Gly His Glu Ala Phe Leu Leu Thr Glu Gly Ser Glu Glu Lys
145 150 155 160

Arg Ser Ala Lys Thr Val Asn Gln Leu Ala His Ala Leu His Gln Asp
165 170 175

Lys Gln Leu His Ala Gly Ser Leu Val Ser Val Met Trp Pro Asn Ser
180 185 190

Lys Cys Pro Leu Leu Lys Asp Asp Leu Val Leu Met Asp Ser Pro Gly
195 200 205

Ile Asp Val Thr Thr Glu Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu
210 215 220

Asp Ala Asp Val Phe Val Leu Val Ala Asn Ser Glu Ser Thr Leu Met
225 230 235 240

Gln Thr Glu Lys His Phe Phe His Lys Val Ser Glu Arg Leu Ser Arg
245 250 255

Pro Asn Asn Phe Ile Leu Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu
260 265 270

Pro Glu Tyr Met Glu Glu Val Arg Arg Gln His Met Glu Arg Cys Thr
275 280 285

Ser Phe Leu Val Asp Glu Leu Gly Val Val Asp Arg Ser Gln Ala Gly
290 295 300

Asp Arg Ile Phe Phe Val Ser Ala Lys Glu Val Leu Asn Ala Arg Ile
305 310 315 320

Gln Lys Ala Gln Gly Met Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly
325 330 335

Phe Gln Val Arg Met Phe Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu
340 345 350

Glu Cys Ile Ser Gln Ser Ala Val Lys Thr Lys Phe Glu Gln His Thr
355 360 365

Val Arg Ala Lys Gln Ile Ala Glu Ala Val Arg Leu Ile Met Asp Ser
370 375 380

Leu His Met Ala Ala Arg Glu Gln Gln Val Tyr Cys Glu Glu Met Arg
385 390 395 400

Glu Glu Arg Gln Asp Arg Leu Lys Phe Ile Asp Lys Gln Leu Glu Leu
405 410 415

Leu Ala Gln Asp Tyr Lys Leu Arg Ile Lys Gln Ile Thr Glu Glu Val
420 425 430

Glu Arg Gln Val Ser Thr Ala Met Ala Glu Glu Ile Arg Arg Leu Ser
435 440 445

Val Leu Val Asp Asp Tyr Gln Met Asp Phe His Pro Ser Pro Val Val
450 455 460

Leu Lys Val Tyr Lys Asn Glu Leu His Arg His Ile Glu Glu Gly Leu
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Gly Arg Asn Met Ser Asp Arg Cys Ser Thr Ala Ile Thr Asn Ser Leu
485 490 495

Gln Thr Met Gln Gln Asp Met Ile Asp Gly Leu Lys Pro Leu Leu Pro
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Val Ser Val Arg Ser Gln Ile Asp Met Leu Val Pro Arg Gln Cys Phe
515 520 525

Ser Leu Asn Tyr Asp Leu Asn Cys Asp Lys Leu Cys Ala Asp Phe Gln
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Glu Asp Ile Glu Phe His Phe Ser Leu Gly Trp Thr Met Leu Val Asn
545 550 555 560

Arg Phe Leu Gly Pro Lys Asn Ser Arg Arg Ala Leu Met Gly Tyr Asn
565 570 575

Asp Gln Val Gln Arg Pro Ile Pro Leu Thr Pro Ala Asn Pro Ser Met
580 585 590

Pro Pro Leu Pro Gln Gly Ser Leu Thr Gln Glu Glu Phe Met Val Ser
595 600 605

Met Val Thr Gly Leu Ala Ser Leu Thr Ser Arg Thr Ser Met Gly Ile
610 615 620

Leu Val Val Gly Gly Val Val Trp Lys Ala Val Gly Trp Arg Leu Ile
625 630 635 640

Ala Leu Ser Phe Gly Leu Tyr Gly Leu Leu Tyr Val Tyr Glu Arg Leu
645 650 655

Thr Trp Thr Thr Lys Ala Lys Glu Arg Ala Phe Lys Arg Gln Phe Val
660 665 670

Glu His Ala Ser Glu Lys Leu Gln Leu Val Ile Ser Tyr Thr Gly Ser
675 680 685

Asn Cys Ser His Gln Val Gln Gln Glu Leu Ser Gly Thr Phe Ala His
690 695 700

Leu Cys Gln Gln Val Asp Val Thr Arg Glu Asn Leu Glu Gln Glu Ile
705 710 715 720

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

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ccc tct gga atc ggc cac acc acc aac tgc ttt ctg aga gtg gaa ggc Pro Ser Gly Ile Gly His Thr Thr Asn Cys Phe Leu Arg Val Glu Gly	125	130	135	1577
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cgg agc gcc aag aca gtg aac cag ctg gcc cat gct ctg cac cag gat Arg Ser Ala Lys Thr Val Asn Gln Leu Ala His Ala Leu His Gln Asp	155	160	165	1673
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ccc gag tat atg gaa gaa gtg cgg cgg cag cat atg gaa cgg tgc aca Pro Glu Tyr Met Glu Glu Val Arg Arg Gln His Met Glu Arg Cys Thr	270	275	280	2009
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ctg aaa gtc tac aaa aac gag ctg cac cgg cac atc gag gaa ggc ctg Leu Lys Val Tyr Lys Asn Glu Leu His Arg His Ile Glu Glu Gly Leu 460 465 470	2585
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cag acc atg cag cag gac atg atc gac ggc ctg aaa cct ctg ctg cct Gln Thr Met Gln Gln Asp Met Ile Asp Gly Leu Lys Pro Leu Leu Pro 495 500 505	2681
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gag	gac	atc	gag	ttt	cac	ttc	agc	ctc	ggc	tgg	aca	atg	ctg	gtc	aac	2825				
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aga	ttt	ctg	ggc	ccc	aag	aac	agc	aga	cgg	gcc	ctg	atg	ggc	tac	aac	2873				
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Asp	Gln	Val	Gln	Arg	Pro	Ile	Pro	Leu	Thr	Pro	Ala	Asn	Pro	Ser	Met					
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cct	cca	ctg	cct	cag	ggc	agc	ctg	aca	caa	gag	gaa	ttc	atg	gtg	tct	2969				
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Leu	Val	Val	Gly	Gly	Val	Val	Trp	Lys	Ala	Val	Gly	Trp	Arg	Leu	Ile					
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Ala	Leu	Ser	Phe	Gly	Leu	Tyr	Gly	Leu	Leu	Tyr	Val	Tyr	Glu	Arg	Leu					
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gcc	gcc	atg	aat	aag	aaa	atc	gag	gtg	ctc	gac	tcc	ctg	cag	agc	aag	3353				
Ala	Ala	Met	Asn	Lys	Lys	Ile	Glu	Val	Leu	Asp	Ser	Leu	Gln	Ser	Lys					
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atg ttc acc cac cag tac ctg cag cct agc cgc taa taaagcggcc	3447
Met Phe Thr His Gln Tyr Leu Gln Pro Ser Arg	
750 755	
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Tyr Ile Gln Glu Ser Ala Thr Phe Leu Glu Asp Thr Tyr Arg Asn Ala
50 55 60

Glu Leu Asp Pro Val Thr Thr Glu Glu Gln Val Leu Asp Val Lys Gly
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Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu Val Leu Ala Arg Arg His
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Met Lys Val Ala Phe Phe Gly Arg Thr Ser Asn Gly Lys Ser Thr Val
100 105 110

Ile Asn Ala Met Leu Trp Asp Lys Val Leu Pro Ser Gly Ile Gly His
115 120 125

Thr Thr Asn Cys Phe Leu Arg Val Glu Gly Thr Asp Gly His Glu Ala
130 135 140

Phe Leu Leu Thr Glu Gly Ser Glu Glu Lys Arg Ser Ala Lys Thr Val
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Asn Gln Leu Ala His Ala Leu His Gln Asp Lys Gln Leu His Ala Gly
165 170 175

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

Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu Asp Ala Asp Val Phe Val
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Leu Val Ala Asn Ser Glu Ser Thr Leu Met Gln Thr Glu Lys His Phe
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Phe His Lys Val Ser Glu Arg Leu Ser Arg Pro Asn Ile Phe Ile Leu
245 250 255

Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu Pro Glu Tyr Met Glu Glu
260 265 270

Val Arg Arg Gln His Met Glu Arg Cys Thr Ser Phe Leu Val Asp Glu
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Leu Gly Val Val Asp Arg Ser Gln Ala Gly Asp Arg Ile Phe Phe Val
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Ser Ala Lys Glu Val Leu Asn Ala Arg Ile Gln Lys Ala Gln Gly Met
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Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly Phe Gln Val Arg Met Phe
325 330 335

Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu Glu Cys Ile Ser Gln Ser
340 345 350

Ala Val Lys Thr Lys Phe Glu Gln His Thr Val Arg Ala Lys Gln Ile
355 360 365

Ala Glu Ala Val Arg Leu Ile Met Asp Ser Leu His Met Ala Ala Arg
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Glu Gln Gln Val Tyr Cys Glu Glu Met Arg Glu Glu Arg Gln Asp Arg

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Gln	Met	Asp	Phe	His	Pro	Ser	Pro	Val	Val	Leu	Lys	Val	Tyr	Lys	Asn
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Val Trp Lys Ala Val Gly Trp Arg Leu Ile Ala Leu Ser Phe Gly Leu
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Tyr Gly Leu Leu Tyr Val Tyr Glu Arg Leu Thr Trp Thr Thr Lys Ala
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Lys Glu Arg Ala Phe Lys Arg Gln Phe Val Glu His Ala Ser Glu Lys
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Leu Gln Leu Val Ile Ser Tyr Thr Gly Ser Asn Cys Ser His Gln Val
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Gln Gln Glu Leu Ser Gly Thr Phe Ala His Leu Cys Gln Gln Val Asp
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Val Thr Arg Glu Asn Leu Glu Gln Glu Ile Ala Ala Met Asn Lys Lys
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Ile Glu Val Leu Asp Ser Leu Gln Ser Lys Ala Lys Leu Leu Arg Asn
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Val Leu Ala Arg Arg His Met Lys Val Ala Phe Phe Gly Arg Thr Ser	
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Asn Gly Lys Ser Thr Val Ile Asn Ala Met Leu Trp Asp Lys Val Leu	
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Gly	Arg	Asn	Met	Ser	Asp	Arg	Cys	Ser	Thr	Ala	Ile	Thr	Asn	Ser	Leu					
			475				480						485	490						
cag	acc	atg	cag	cag	gac	atg	atc	gac	ggc	ctg	aaa	cct	ctg	ctg	cct	2681				
Gln	Thr	Met	Gln	Gln	Asp	Met	Ile	Asp	Gly	Leu	Lys	Pro	Leu	Leu	Pro					
			495				500						505							
gtg	tcc	gtc	aga	tcc	cag	atc	gat	atg	ctg	gtg	ccc	cgg	cag	tgc	ttt	2729				
Val	Ser	Val	Arg	Ser	Gln	Ile	Asp	Met	Leu	Val	Pro	Arg	Gln	Cys	Phe					
			510				515						520							
agc	ctg	aac	tac	gac	ctg	aac	tgc	gac	aag	ctg	tgc	gcc	gat	ttc	caa	2777				
Ser	Leu	Asn	Tyr	Asp	Leu	Asn	Cys	Asp	Lys	Leu	Cys	Ala	Asp	Phe	Gln					
			525				530						535							
gag	gac	atc	gag	ttt	cac	ttc	agc	ctc	ggc	tgg	aca	atg	ctg	gtc	aac	2825				
Glu	Asp	Ile	Glu	Phe	His	Phe	Ser	Leu	Gly	Trp	Thr	Met	Leu	Val	Asn					
			540				545						550							

aga ttt ctg ggc ccc aag aac agc aga cgg gcc ctg atg ggc tac aac	2873
Arg Phe Leu Gly Pro Lys Asn Ser Arg Arg Ala Leu Met Gly Tyr Asn	
555 560 565 570	
gat cag gtg cag agg ccc att cct ctg aca ccc gcc aat cct agc atg	2921
Asp Gln Val Gln Arg Pro Ile Pro Leu Thr Pro Ala Asn Pro Ser Met	
575 580 585	
cct cca ctg cct cag ggc agc ctg aca caa gag gaa ttc atg gtg tct	2969
Pro Pro Leu Pro Gln Gly Ser Leu Thr Gln Glu Glu Phe Met Val Ser	
590 595 600	
atg gtc acc ggc ctg gcc agc ctg acc agc aga aca tct atg ggc atc	3017
Met Val Thr Gly Leu Ala Ser Leu Thr Ser Arg Thr Ser Met Gly Ile	
605 610 615	
ctg gtc gtc ggc ggc gtt gtg tgg aaa gct gtt ggc tgg cgg ctg atc	3065
Leu Val Val Gly Gly Val Val Trp Lys Ala Val Gly Trp Arg Leu Ile	
620 625 630	
gcc ctg agc ttt ggc ctg tat ggc ctg ctg tac gtg tac gag aga ctg	3113
Ala Leu Ser Phe Gly Leu Tyr Gly Leu Leu Tyr Val Tyr Glu Arg Leu	
635 640 645 650	
acc tgg acc acc aag gcc aaa gag cgg gcc ttc aag cgg cag ttt gtg	3161
Thr Trp Thr Thr Lys Ala Lys Glu Arg Ala Phe Lys Arg Gln Phe Val	
655 660 665	
gaa cac gcc tcc gag aaa ctg cag ctg gtg atc agc tac acc ggc agc	3209
Glu His Ala Ser Glu Lys Leu Gln Leu Val Ile Ser Tyr Thr Gly Ser	
670 675 680	
aat tgc agc cac cag gtg cag caa gag ctg tcc gga aca ttc gcc cac	3257
Asn Cys Ser His Gln Val Gln Gln Glu Leu Ser Gly Thr Phe Ala His	
685 690 695	
ctg tgt cag cag gtc gac gtg acc aga gag aac ctg gaa caa gag atc	3305
Leu Cys Gln Gln Val Asp Val Thr Arg Glu Asn Leu Glu Gln Glu Ile	
700 705 710	
gcc gcc atg aat aag aaa atc gag gtg ctc gac tcc ctg cag agc aag	3353
Ala Ala Met Asn Lys Lys Ile Glu Val Leu Asp Ser Leu Gln Ser Lys	
715 720 725 730	
gcc aag ctg ctg aga aac aag gcc ggc tgg ctg gac agc gag ctg aat	3401
Ala Lys Leu Leu Arg Asn Lys Ala Gly Trp Leu Asp Ser Glu Leu Asn	
735 740 745	
atg ttc acc cac cag tac ctg cag cct agc cgc taa taaagcggcc	3447
Met Phe Thr His Gln Tyr Leu Gln Pro Ser Arg	
750 755	
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aataaacaag ttaacaacaa caattgcatt cattttatgt ttcaggttca gggggagatg	4527
tgggagggtt tttaaagcaa gtaaaacctc taaaaatgtg gtaaaatcga taaggatctt	4587
cctagagcat ggctacgtag ataagtagca tggcgggtta atcattaact acaaggaacc	4647
cctagtgatg gagttggcca ctccctctct gcgcgctcgc tcgctcactg aggccgggcg	4707
accaaaggtc gcccgacgcc cgggctttgc ccgggcggcc tcagtgagcg agcgagcgcg	4767
cagc	4771

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 <213> Artificial Sequence

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 <223> Synthetic Construct

<400> 72

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

Tyr Ile Gln Glu Ser Ala Thr Phe Leu Glu Asp Thr Tyr Arg Asn Ala
50 55 60

Glu Leu Asp Pro Val Thr Thr Glu Glu Gln Val Leu Asp Val Lys Gly
65 70 75 80

Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu Val Leu Ala Arg Arg His
85 90 95

Met Lys Val Ala Phe Phe Gly Arg Thr Ser Asn Gly Lys Ser Thr Val
100 105 110

Ile Asn Ala Met Leu Trp Asp Lys Val Leu Pro Ser Gly Ile Gly His
115 120 125

Thr Thr Asn Cys Phe Leu Arg Val Glu Gly Thr Asp Gly His Glu Ala
130 135 140

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

Asn Gln Leu Ala His Ala Leu His Gln Asp Lys Gln Leu His Ala Gly
165 170 175

Ser Leu Val Ser Val Met Trp Pro Asn Ser Lys Cys Pro Leu Leu Lys
180 185 190

Asp Asp Leu Val Leu Met Asp Ser Pro Gly Ile Asp Val Thr Thr Glu
195 200 205

Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu Asp Ala Asp Val Phe Val

210

215

220

Leu Val Ala Asn Ser Glu Ser Thr Leu Met Gln Thr Glu Lys His Phe
 225 230 235 240

Phe His Lys Val Ser Glu Arg Leu Ser Arg Pro Asn Ile Phe Ile Leu
 245 250 255

Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu Pro Glu Tyr Met Glu Glu
 260 265 270

Val Arg Arg Gln His Met Glu Arg Cys Thr Ser Phe Leu Val Asp Glu
 275 280 285

Leu Gly Val Val Asp Arg Ser Gln Ala Gly Asp Arg Ile Phe Phe Val
 290 295 300

Ser Ala Lys Glu Val Leu Asn Ala Arg Ile Gln Lys Ala Gln Gly Met
 305 310 315 320

Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly Phe Gln Val Arg Met Phe
 325 330 335

Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu Glu Cys Ile Ser Gln Ser
 340 345 350

Ala Val Lys Thr Lys Phe Glu Gln His Thr Val Arg Ala Lys Gln Ile
 355 360 365

Ala Glu Ala Val Arg Leu Ile Met Asp Ser Leu His Met Ala Ala Arg
 370 375 380

Glu Gln Gln Val Tyr Cys Glu Glu Met Arg Glu Glu Arg Gln Asp Arg
 385 390 395 400

Leu Lys Phe Ile Asp Lys Gln Leu Glu Leu Leu Ala Gln Asp Tyr Lys
 405 410 415

Leu Arg Ile Lys Gln Ile Thr Glu Glu Val Glu Arg Gln Val Ser Thr
 420 425 430

Ala Met Ala Glu Glu Ile Arg Arg Leu Ser Val Leu Val Asp Asp Tyr
435 440 445

Gln Met Asp Phe His Pro Ser Pro Val Val Leu Lys Val Tyr Lys Asn
450 455 460

Glu Leu His Arg His Ile Glu Glu Gly Leu Gly Arg Asn Met Ser Asp
465 470 475 480

Arg Cys Ser Thr Ala Ile Thr Asn Ser Leu Gln Thr Met Gln Gln Asp
485 490 495

Met Ile Asp Gly Leu Lys Pro Leu Leu Pro Val Ser Val Arg Ser Gln
500 505 510

Ile Asp Met Leu Val Pro Arg Gln Cys Phe Ser Leu Asn Tyr Asp Leu
515 520 525

Asn Cys Asp Lys Leu Cys Ala Asp Phe Gln Glu Asp Ile Glu Phe His
530 535 540

Phe Ser Leu Gly Trp Thr Met Leu Val Asn Arg Phe Leu Gly Pro Lys
545 550 555 560

Asn Ser Arg Arg Ala Leu Met Gly Tyr Asn Asp Gln Val Gln Arg Pro
565 570 575

Ile Pro Leu Thr Pro Ala Asn Pro Ser Met Pro Pro Leu Pro Gln Gly
580 585 590

Ser Leu Thr Gln Glu Glu Phe Met Val Ser Met Val Thr Gly Leu Ala
595 600 605

Ser Leu Thr Ser Arg Thr Ser Met Gly Ile Leu Val Val Gly Gly Val
610 615 620

Val Trp Lys Ala Val Gly Trp Arg Leu Ile Ala Leu Ser Phe Gly Leu
625 630 635 640

Tyr Gly Leu Leu Tyr Val Tyr Glu Arg Leu Thr Trp Thr Thr Lys Ala

645

650

655

Lys Glu Arg Ala Phe Lys Arg Gln Phe Val Glu His Ala Ser Glu Lys
 660 665 670

Leu Gln Leu Val Ile Ser Tyr Thr Gly Ser Asn Cys Ser His Gln Val
 675 680 685

Gln Gln Glu Leu Ser Gly Thr Phe Ala His Leu Cys Gln Gln Val Asp
 690 695 700

Val Thr Arg Glu Asn Leu Glu Gln Glu Ile Ala Ala Met Asn Lys Lys
 705 710 715 720

Ile Glu Val Leu Asp Ser Leu Gln Ser Lys Ala Lys Leu Leu Arg Asn
 725 730 735

Lys Ala Gly Trp Leu Asp Ser Glu Leu Asn Met Phe Thr His Gln Tyr
 740 745 750

Leu Gln Pro Ser Arg
 755

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 <223> b-actin intron

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 <223> SV 40 PA temrinator

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 <223> ITR

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taggggttcc ttgtagttaa tgattaaccc gccatgctac ttatctacgt agccatgctc	180
taggaagagt accattgacg tcaataatga cgtatgttcc catagtaacg ccaataggga	240
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aagtgtatca tatgccaagt acgcccccta ttgacgtcaa tgacggtaaa tggcccgctt	360
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cgctgctag caccggtgcc acc atg agc ctg ctg ttc agc cgg tgc aac agc	1193
Met Ser Leu Leu Phe Ser Arg Cys Asn Ser	
1 5 10	
atc gtg acc gtg aag aaa aac aag cgg cac atg gcc gaa gtg aac gcc	1241
Ile Val Thr Val Lys Lys Asn Lys Arg His Met Ala Glu Val Asn Ala	
15 20 25	
tct cca ctg aag cac ttc gtg acc gcc aag aag aag atc aac ggc atc	1289
Ser Pro Leu Lys His Phe Val Thr Ala Lys Lys Lys Ile Asn Gly Ile	
30 35 40	
ttc gag cag ctg ggc gcc tac atc caa gag agc gcc acc ttc ctg gaa	1337
Phe Glu Gln Leu Gly Ala Tyr Ile Gln Glu Ser Ala Thr Phe Leu Glu	
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gat acc tac aga aac gcc gag ctg gac ccc gtg acc aca gag gaa cag	1385
Asp Thr Tyr Arg Asn Ala Glu Leu Asp Pro Val Thr Thr Glu Glu Gln	

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gtg ctg gat gtg aag ggc tac ctg agc aaa gtg cgg ggc atc tct gaa Val Leu Asp Val Lys Gly Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu 75 80 85 90			1433
gtg ctg gcc aga cgg cat atg aag gtg gcc ttt ttc ggc cgg acc agc Val Leu Ala Arg Arg His Met Lys Val Ala Phe Phe Gly Arg Thr Ser 95 100 105			1481
aac ggc aag agc acc gtg atc aat gcc atg ctg tgg gac aaa gtg ctg Asn Gly Lys Ser Thr Val Ile Asn Ala Met Leu Trp Asp Lys Val Leu 110 115 120			1529
ccc tct gga atc ggc cac acc acc aac tgc ttt ctg aga gtg gaa ggc Pro Ser Gly Ile Gly His Thr Thr Asn Cys Phe Leu Arg Val Glu Gly 125 130 135			1577
acc gac ggc cac gag gca ttt ctg ctg aca gag ggc tcc gag gaa aag Thr Asp Gly His Glu Ala Phe Leu Leu Thr Glu Gly Ser Glu Glu Lys 140 145 150			1625
cgg agc gcc aag aca gtg aac cag ctg gcc cat gct ctg cac cag gat Arg Ser Ala Lys Thr Val Asn Gln Leu Ala His Ala Leu His Gln Asp 155 160 165 170			1673
aag cag ctg cat gcc gga agc ctg gtg tcc gtg atg tgg ccc aat agc Lys Gln Leu His Ala Gly Ser Leu Val Ser Val Met Trp Pro Asn Ser 175 180 185			1721
aag tgc cct ctg ctg aag gac gac ctg gtg ctg atg gat agc ccc ggg Lys Cys Pro Leu Leu Lys Asp Asp Leu Val Leu Met Asp Ser Pro Gly 190 195 200			1769
atc gat gtg acc acc gaa ctg gac agc tgg atc gac aag ttc tgc ctg Ile Asp Val Thr Thr Glu Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu 205 210 215			1817
gac gcc gac gtg ttc gtg ctg gtg gcc aat agc gag agc acc ctg atg Asp Ala Asp Val Phe Val Leu Val Ala Asn Ser Glu Ser Thr Leu Met 220 225 230			1865
cag acc gag aag cac ttt ttc cac aag gtg tcc gag cgg ctg agc aga Gln Thr Glu Lys His Phe Phe His Lys Val Ser Glu Arg Leu Ser Arg 235 240 245 250			1913
ccc aat atc ttt atc ctg aac aac aga tgg gac gcc agc gcc agc gag Pro Asn Ile Phe Ile Leu Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu 255 260 265			1961
ccc gag tat atg gaa gaa gtg cgg cgg cag cat atg gaa cgg tgc aca Pro Glu Tyr Met Glu Glu Val Arg Arg Gln His Met Glu Arg Cys Thr 270 275 280			2009

tcc ttt ctg gtg gac gag ctg ggc gtc gtg gat aga tct cag gcc ggc	2057
Ser Phe Leu Val Asp Glu Leu Gly Val Val Asp Arg Ser Gln Ala Gly	
285 290 295	
gac aga atc ttc ttt gtg tcc gcc aaa gag gtg ctg aac gcc cgg att	2105
Asp Arg Ile Phe Phe Val Ser Ala Lys Glu Val Leu Asn Ala Arg Ile	
300 305 310	
cag aaa gcc cag gga atg cct gaa ggc gga ggt gca ctg gcc gag gga	2153
Gln Lys Ala Gln Gly Met Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly	
315 320 325 330	
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Phe Gln Val Arg Met Phe Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu	
335 340 345	
gag tgc atc agc cag agc gcc gtc aag acc aag ttc gag cag cat acc	2249
Glu Cys Ile Ser Gln Ser Ala Val Lys Thr Lys Phe Glu Gln His Thr	
350 355 360	
gtg cgg gcc aag cag att gcc gaa gcc gtc aga ctg atc atg gac agc	2297
Val Arg Ala Lys Gln Ile Ala Glu Ala Val Arg Leu Ile Met Asp Ser	
365 370 375	
ctg cat atg gcc gcc aga gag cag cag gtc tac tgc gag gaa atg cgg	2345
Leu His Met Ala Ala Arg Glu Gln Gln Val Tyr Cys Glu Glu Met Arg	
380 385 390	
gaa gag aga cag gac cgg ctg aag ttc atc gac aag cag ctg gaa ctg	2393
Glu Glu Arg Gln Asp Arg Leu Lys Phe Ile Asp Lys Gln Leu Glu Leu	
395 400 405 410	
ctg gcc cag gac tac aag ctg cgg atc aag cag atc acc gaa gag gtg	2441
Leu Ala Gln Asp Tyr Lys Leu Arg Ile Lys Gln Ile Thr Glu Glu Val	
415 420 425	
gaa aga cag gtg tcc acc gct atg gcc gag gaa atc aga cgg ctg agc	2489
Glu Arg Gln Val Ser Thr Ala Met Ala Glu Glu Ile Arg Arg Leu Ser	
430 435 440	
gtg ctg gtc gac gac tac cag atg gac ttt cac ccc tct cca gtg gtg	2537
Val Leu Val Asp Asp Tyr Gln Met Asp Phe His Pro Ser Pro Val Val	
445 450 455	
ctg aaa gtc tac aaa aac gag ctg cac cgg cac atc gag gaa ggc ctg	2585
Leu Lys Val Tyr Lys Asn Glu Leu His Arg His Ile Glu Glu Gly Leu	
460 465 470	
ggc aga aac atg agc gac aga tgc agc acc gcc atc acc aat agc ctg	2633
Gly Arg Asn Met Ser Asp Arg Cys Ser Thr Ala Ile Thr Asn Ser Leu	
475 480 485 490	
cag acc atg cag cag gac atg atc gac ggc ctg aaa cct ctg ctg cct	2681
Gln Thr Met Gln Gln Asp Met Ile Asp Gly Leu Lys Pro Leu Leu Pro	

495				500				505								
gtg	tcc	gtc	aga	tcc	cag	atc	gat	atg	ctg	gtg	ccc	cgg	cag	tgc	ttt	2729
Val	Ser	Val	Arg	Ser	Gln	Ile	Asp	Met	Leu	Val	Pro	Arg	Gln	Cys	Phe	
			510					515					520			
agc	ctg	aac	tac	gac	ctg	aac	tgc	gac	aag	ctg	tgc	gcc	gat	ttc	caa	2777
Ser	Leu	Asn	Tyr	Asp	Leu	Asn	Cys	Asp	Lys	Leu	Cys	Ala	Asp	Phe	Gln	
		525					530					535				
gag	gac	atc	gag	ttt	cac	ttc	agc	ctc	ggc	tgg	aca	atg	ctg	gtc	aac	2825
Glu	Asp	Ile	Glu	Phe	His	Phe	Ser	Leu	Gly	Trp	Thr	Met	Leu	Val	Asn	
	540					545					550					
aga	ttt	ctg	ggc	ccc	aag	aac	agc	aga	cgg	gcc	ctg	atg	ggc	tac	aac	2873
Arg	Phe	Leu	Gly	Pro	Lys	Asn	Ser	Arg	Arg	Ala	Leu	Met	Gly	Tyr	Asn	
555					560					565					570	
gat	cag	gtg	cag	agg	ccc	att	cct	ctg	aca	ccc	gcc	aat	cct	agc	atg	2921
Asp	Gln	Val	Gln	Arg	Pro	Ile	Pro	Leu	Thr	Pro	Ala	Asn	Pro	Ser	Met	
				575					580					585		
cct	cca	ctg	cct	cag	ggc	agc	ctg	aca	caa	gag	gaa	ttc	atg	gtg	tct	2969
Pro	Pro	Leu	Pro	Gln	Gly	Ser	Leu	Thr	Gln	Glu	Glu	Phe	Met	Val	Ser	
			590					595					600			
atg	gtc	acc	ggc	ctg	gcc	agc	ctg	acc	agc	aga	aca	tct	atg	ggc	atc	3017
Met	Val	Thr	Gly	Leu	Ala	Ser	Leu	Thr	Ser	Arg	Thr	Ser	Met	Gly	Ile	
		605					610					615				
ctg	gtc	gtc	ggc	ggc	gtt	gtg	tgg	aaa	gct	gtt	ggc	tgg	cgg	ctg	atc	3065
Leu	Val	Val	Gly	Gly	Val	Val	Trp	Lys	Ala	Val	Gly	Trp	Arg	Leu	Ile	
	620					625					630					
gcc	ctg	agc	ttt	ggc	ctg	tat	ggc	ctg	ctg	tac	gtg	tac	gag	aga	ctg	3113
Ala	Leu	Ser	Phe	Gly	Leu	Tyr	Gly	Leu	Leu	Tyr	Val	Tyr	Glu	Arg	Leu	
635					640					645					650	
acc	tgg	acc	acc	aag	gcc	aaa	gag	cgg	gcc	ttc	aag	cgg	cag	ttt	gtg	3161
Thr	Trp	Thr	Thr	Lys	Ala	Lys	Glu	Arg	Ala	Phe	Lys	Arg	Gln	Phe	Val	
				655					660					665		
gaa	cac	gcc	tcc	gag	aaa	ctg	cag	ctg	gtg	atc	agc	tac	acc	ggc	agc	3209
Glu	His	Ala	Ser	Glu	Lys	Leu	Gln	Leu	Val	Ile	Ser	Tyr	Thr	Gly	Ser	
			670					675					680			
aat	tgc	agc	cac	cag	gtg	cag	caa	gag	ctg	tcc	gga	aca	ttc	gcc	cac	3257
Asn	Cys	Ser	His	Gln	Val	Gln	Gln	Glu	Leu	Ser	Gly	Thr	Phe	Ala	His	
			685				690					695				
ctg	tgt	cag	cag	gtc	gac	gtg	acc	aga	gag	aac	ctg	gaa	caa	gag	atc	3305
Leu	Cys	Gln	Gln	Val	Asp	Val	Thr	Arg	Glu	Asn	Leu	Glu	Gln	Glu	Ile	
	700					705					710					

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<212>	PRT

<213> Artificial Sequence

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<223> Synthetic Construct

<400> 74

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

Tyr Ile Gln Glu Ser Ala Thr Phe Leu Glu Asp Thr Tyr Arg Asn Ala
50 55 60

Glu Leu Asp Pro Val Thr Thr Glu Glu Gln Val Leu Asp Val Lys Gly
65 70 75 80

Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu Val Leu Ala Arg Arg His
85 90 95

Met Lys Val Ala Phe Phe Gly Arg Thr Ser Asn Gly Lys Ser Thr Val
100 105 110

Ile Asn Ala Met Leu Trp Asp Lys Val Leu Pro Ser Gly Ile Gly His
115 120 125

Thr Thr Asn Cys Phe Leu Arg Val Glu Gly Thr Asp Gly His Glu Ala
130 135 140

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

Asn Gln Leu Ala His Ala Leu His Gln Asp Lys Gln Leu His Ala Gly
165 170 175

Ser Leu Val Ser Val Met Trp Pro Asn Ser Lys Cys Pro Leu Leu Lys
180 185 190

Asp Asp Leu Val Leu Met Asp Ser Pro Gly Ile Asp Val Thr Thr Glu
195 200 205

Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu Asp Ala Asp Val Phe Val
210 215 220

Leu Val Ala Asn Ser Glu Ser Thr Leu Met Gln Thr Glu Lys His Phe
225 230 235 240

Phe His Lys Val Ser Glu Arg Leu Ser Arg Pro Asn Ile Phe Ile Leu
245 250 255

Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu Pro Glu Tyr Met Glu Glu
260 265 270

Val Arg Arg Gln His Met Glu Arg Cys Thr Ser Phe Leu Val Asp Glu
275 280 285

Leu Gly Val Val Asp Arg Ser Gln Ala Gly Asp Arg Ile Phe Phe Val
290 295 300

Ser Ala Lys Glu Val Leu Asn Ala Arg Ile Gln Lys Ala Gln Gly Met
305 310 315 320

Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly Phe Gln Val Arg Met Phe
325 330 335

Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu Glu Cys Ile Ser Gln Ser
340 345 350

Ala Val Lys Thr Lys Phe Glu Gln His Thr Val Arg Ala Lys Gln Ile
355 360 365

Ala Glu Ala Val Arg Leu Ile Met Asp Ser Leu His Met Ala Ala Arg
370 375 380

Glu Gln Gln Val Tyr Cys Glu Glu Met Arg Glu Glu Arg Gln Asp Arg
385 390 395 400

Leu Lys Phe Ile Asp Lys Gln Leu Glu Leu Leu Ala Gln Asp Tyr Lys

405

410

415

Leu Arg Ile Lys Gln Ile Thr Glu Glu Val Glu Arg Gln Val Ser Thr
 420 425 430

Ala Met Ala Glu Glu Ile Arg Arg Leu Ser Val Leu Val Asp Asp Tyr
 435 440 445

Gln Met Asp Phe His Pro Ser Pro Val Val Leu Lys Val Tyr Lys Asn
 450 455 460

Glu Leu His Arg His Ile Glu Glu Gly Leu Gly Arg Asn Met Ser Asp
 465 470 475 480

Arg Cys Ser Thr Ala Ile Thr Asn Ser Leu Gln Thr Met Gln Gln Asp
 485 490 495

Met Ile Asp Gly Leu Lys Pro Leu Leu Pro Val Ser Val Arg Ser Gln
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Ile Asp Met Leu Val Pro Arg Gln Cys Phe Ser Leu Asn Tyr Asp Leu
 515 520 525

Asn Cys Asp Lys Leu Cys Ala Asp Phe Gln Glu Asp Ile Glu Phe His
 530 535 540

Phe Ser Leu Gly Trp Thr Met Leu Val Asn Arg Phe Leu Gly Pro Lys
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Asn Ser Arg Arg Ala Leu Met Gly Tyr Asn Asp Gln Val Gln Arg Pro
 565 570 575

Ile Pro Leu Thr Pro Ala Asn Pro Ser Met Pro Pro Leu Pro Gln Gly
 580 585 590

Ser Leu Thr Gln Glu Glu Phe Met Val Ser Met Val Thr Gly Leu Ala
 595 600 605

Ser Leu Thr Ser Arg Thr Ser Met Gly Ile Leu Val Val Gly Gly Val
 610 615 620

Val Trp Lys Ala Val Gly Trp Arg Leu Ile Ala Leu Ser Phe Gly Leu
625 630 635 640

Tyr Gly Leu Leu Tyr Val Tyr Glu Arg Leu Thr Trp Thr Thr Lys Ala
645 650 655

Lys Glu Arg Ala Phe Lys Arg Gln Phe Val Glu His Ala Ser Glu Lys
660 665 670

Leu Gln Leu Val Ile Ser Tyr Thr Gly Ser Asn Cys Ser His Gln Val
675 680 685

Gln Gln Glu Leu Ser Gly Thr Phe Ala His Leu Cys Gln Gln Val Asp
690 695 700

Val Thr Arg Glu Asn Leu Glu Gln Glu Ile Ala Ala Met Asn Lys Lys
705 710 715 720

Ile Glu Val Leu Asp Ser Leu Gln Ser Lys Ala Lys Leu Leu Arg Asn
725 730 735

Lys Ala Gly Trp Leu Asp Ser Glu Leu Asn Met Phe Thr His Gln Tyr
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Leu Gln Pro Ser Arg
755

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<211> 4821
<212> DNA
<213> Artificial Sequence

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<223> Vector genome CB7.CI.hMfn2.GA.link.miR538.rBG

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<222> (1)..(130)
<223> ITR

<220>
<221> enhancer
<222> (259)..(562)

<223> CMV enhancer

<220>

<221> promoter

<222> (561)..(842)

<223> CB7 promoter

<220>

<221> Intron

<222> (935)..(1907)

<223> chicken beta-actin intron

<220>

<221> misc_feature

<222> (1953)..(1958)

<223> Kozak

<220>

<221> CDS

<222> (1959)..(4232)

<223> human Mfn2 engineered

<220>

<221> misc_feature

<222> (2174)..(2194)

<223> miR538 mut

<220>

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<222> (3329)..(3349)

<223> miR1518

<220>

<221> misc_feature

<222> (4233)..(4314)

<223> linker p5271

<220>

<221> misc_feature

<222> (4315)..(4342)

<223> 5' flank

<220>

<221> misc_feature

<222> (4343)..(4363)

<223> miR538 AS

<220>

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<222> (4364)..(4382)

<223> loop

<220>

<221> misc_feature

<222> (4383)..(4401)
<223> miR538 Sense

<220>
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<222> (4402)..(4446)
<223> 3' flank

<220>
<221> polyA_signal
<222> (4500)..(4626)
<223> rabbit globin poly A

<220>
<221> repeat_region
<222> (4692)..(4821)
<223> ITR

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aggggttcct tgtagttaat gattaacccg ccatgctact tatctacgta gccatgctct 180
aggaagatct gatatcacta gttattaata gtaatcaatt acgggggtcat tagttcatag 240
cccatatatg gagttccgcg ttacataact tacggtaaata ggcccgccctg gctgaccgcc 300
caacgacccc cgcccattga cgtcaataat gacgtatgtt cccatagtaa cgccaatagg 360
gactttccat tgacgtcaat ggggtggagta ttacggtaa actgcccact tggcagtaca 420
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gccccggctc tgactgaccg cgttactccc acaggtgagc gggcgggacg gcccttctcc 960
tccgggctgt aattagcgct tggtttaatg acggcttggt tcttttctgt ggctgcgtga 1020
aagccttgag gggctccggg agggcccttt gtgcgggggg agcggctcgg ggggtgcgtg 1080

cgtgtgtgtg tgcgtgggga ggcgcgctg cggctccgcg ctgcccggcg gctgtgagcg	1140
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ccccctccc cgagttgctg agcacggccc ggcttcgggt gcggggctcc gtacggggcg	1380
tggcgcgggg ctgccgtgc cgggcggggg gtggcggcag gtgggggtgc cgggcggggc	1440
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cggctgtcga ggcgcggcga gccgcagcca ttgcctttta tggtaatcgt gcgagagggc	1560
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cccctctagc gggcgcgggg cgaagcggtg cggcgccggc aggaaggaaa tgggcgggga	1680
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gaccggcggc tctagagcct ctgctaacca tgttcatgcc ttcttctttt tcctacagct	1860
cctgggcaac gtgctggtta ttgtgctgtc tcatcatttt ggcaaagaat tcacgcgtgc	1920
taggatcccg tacgacgcgt gctagcaccg gtgccacc atg agc ctg ctg ttc agc	1976
	Met Ser Leu Leu Phe Ser
	1 5
cgg tgc aac agc atc gtg acc gtg aag aaa aac aag cgg cac atg gcc	2024
Arg Cys Asn Ser Ile Val Thr Val Lys Lys Asn Lys Arg His Met Ala	
	10 15 20
gaa gtg aac gcc tct cca ctg aag cac ttc gtg acc gcc aag aag aag	2072
Glu Val Asn Ala Ser Pro Leu Lys His Phe Val Thr Ala Lys Lys Lys	
	25 30 35
atc aac ggc atc ttc gag cag ctg ggc gcc tac atc caa gag agc gcc	2120
Ile Asn Gly Ile Phe Glu Gln Leu Gly Ala Tyr Ile Gln Glu Ser Ala	
	40 45 50
acc ttc ctg gaa gat acc tac aga aac gcc gag ctg gac ccc gtg acc	2168
Thr Phe Leu Glu Asp Thr Tyr Arg Asn Ala Glu Leu Asp Pro Val Thr	
	55 60 65 70
aca gag gaa cag gtg ctg gat gtg aag ggc tac ctg agc aaa gtg cgg	2216
Thr Glu Glu Gln Val Leu Asp Val Lys Gly Tyr Leu Ser Lys Val Arg	
	75 80 85
ggc atc tct gaa gtg ctg gcc aga cgg cat atg aag gtg gcc ttt ttc	2264

Gly	Ile	Ser	Glu	Val	Leu	Ala	Arg	Arg	His	Met	Lys	Val	Ala	Phe	Phe		
			90					95					100				
ggc	cgg	acc	agc	aac	ggc	aag	agc	acc	gtg	atc	aat	gcc	atg	ctg	tgg		2312
Gly	Arg	Thr	Ser	Asn	Gly	Lys	Ser	Thr	Val	Ile	Asn	Ala	Met	Leu	Trp		
		105					110					115					
gac	aaa	gtg	ctg	ccc	tct	gga	atc	ggc	cac	acc	acc	aac	tgc	ttt	ctg		2360
Asp	Lys	Val	Leu	Pro	Ser	Gly	Ile	Gly	His	Thr	Thr	Asn	Cys	Phe	Leu		
	120					125					130						
aga	gtg	gaa	ggc	acc	gac	ggc	cac	gag	gca	ttt	ctg	ctg	aca	gag	ggc		2408
Arg	Val	Glu	Gly	Thr	Asp	Gly	His	Glu	Ala	Phe	Leu	Leu	Thr	Glu	Gly		
	135				140					145					150		
tcc	gag	gaa	aag	cgg	agc	gcc	aag	aca	gtg	aac	cag	ctg	gcc	cat	gct		2456
Ser	Glu	Glu	Lys	Arg	Ser	Ala	Lys	Thr	Val	Asn	Gln	Leu	Ala	His	Ala		
				155					160					165			
ctg	cac	cag	gat	aag	cag	ctg	cat	gcc	gga	agc	ctg	gtg	tcc	gtg	atg		2504
Leu	His	Gln	Asp	Lys	Gln	Leu	His	Ala	Gly	Ser	Leu	Val	Ser	Val	Met		
			170					175					180				
tgg	ccc	aat	agc	aag	tgc	cct	ctg	ctg	aag	gac	gac	ctg	gtg	ctg	atg		2552
Trp	Pro	Asn	Ser	Lys	Cys	Pro	Leu	Leu	Lys	Asp	Asp	Leu	Val	Leu	Met		
		185				190						195					
gat	agc	ccc	ggg	atc	gat	gtg	acc	acc	gaa	ctg	gac	agc	tgg	atc	gac		2600
Asp	Ser	Pro	Gly	Ile	Asp	Val	Thr	Thr	Glu	Leu	Asp	Ser	Trp	Ile	Asp		
	200					205					210						
aag	ttc	tgc	ctg	gac	gcc	gac	gtg	ttc	gtg	ctg	gtg	gcc	aat	agc	gag		2648
Lys	Phe	Cys	Leu	Asp	Ala	Asp	Val	Phe	Val	Leu	Val	Ala	Asn	Ser	Glu		
	215				220					225					230		
agc	acc	ctg	atg	cag	acc	gag	aag	cac	ttt	ttc	cac	aag	gtg	tcc	gag		2696
Ser	Thr	Leu	Met	Gln	Thr	Glu	Lys	His	Phe	Phe	His	Lys	Val	Ser	Glu		
				235					240				245				
cgg	ctg	agc	aga	ccc	aat	atc	ttt	atc	ctg	aac	aac	aga	tgg	gac	gcc		2744
Arg	Leu	Ser	Arg	Pro	Asn	Ile	Phe	Ile	Leu	Asn	Asn	Arg	Trp	Asp	Ala		
			250					255					260				
agc	gcc	agc	gag	ccc	gag	tat	atg	gaa	gaa	gtg	cgg	cgg	cag	cat	atg		2792
Ser	Ala	Ser	Glu	Pro	Glu	Tyr	Met	Glu	Glu	Val	Arg	Arg	Gln	His	Met		
		265				270						275					
gaa	cgg	tgc	aca	tcc	ttt	ctg	gtg	gac	gag	ctg	ggc	gtc	gtg	gat	aga		2840
Glu	Arg	Cys	Thr	Ser	Phe	Leu	Val	Asp	Glu	Leu	Gly	Val	Val	Asp	Arg		
	280					285					290						
tct	cag	gcc	ggc	gac	aga	atc	ttc	ttt	gtg	tcc	gcc	aaa	gag	gtg	ctg		2888
Ser	Gln	Ala	Gly	Asp	Arg	Ile	Phe	Phe	Val	Ser	Ala	Lys	Glu	Val	Leu		
					300					305					310		

aac gcc cgg att cag aaa gcc cag gga atg cct gaa ggc gga ggt gca Asn Ala Arg Ile Gln Lys Ala Gln Gly Met Pro Glu Gly Gly Gly Ala 315 320 325	2936
ctg gcc gag gga ttt caa gtg cgg atg ttc gag ttc cag aac ttc gag Leu Ala Glu Gly Phe Gln Val Arg Met Phe Glu Phe Gln Asn Phe Glu 330 335 340	2984
cgg aga ttc gag gag tgc atc agc cag agc gcc gtc aag acc aag ttc Arg Arg Phe Glu Glu Cys Ile Ser Gln Ser Ala Val Lys Thr Lys Phe 345 350 355	3032
gag cag cat acc gtg cgg gcc aag cag att gcc gaa gcc gtc aga ctg Glu Gln His Thr Val Arg Ala Lys Gln Ile Ala Glu Ala Val Arg Leu 360 365 370	3080
atc atg gac agc ctg cat atg gcc gcc aga gag cag cag gtc tac tgc Ile Met Asp Ser Leu His Met Ala Ala Arg Glu Gln Gln Val Tyr Cys 375 380 385 390	3128
gag gaa atg cgg gaa gag aga cag gac cgg ctg aag ttc atc gac aag Glu Glu Met Arg Glu Glu Arg Gln Asp Arg Leu Lys Phe Ile Asp Lys 395 400 405	3176
cag ctg gaa ctg ctg gcc cag gac tac aag ctg cgg atc aag cag atc Gln Leu Glu Leu Leu Ala Gln Asp Tyr Lys Leu Arg Ile Lys Gln Ile 410 415 420	3224
acc gaa gag gtg gaa aga cag gtg tcc acc gct atg gcc gag gaa atc Thr Glu Glu Val Glu Arg Gln Val Ser Thr Ala Met Ala Glu Glu Ile 425 430 435	3272
aga cgg ctg agc gtg ctg gtc gac gac tac cag atg gac ttt cac ccc Arg Arg Leu Ser Val Leu Val Asp Asp Tyr Gln Met Asp Phe His Pro 440 445 450	3320
tct cca gtg gtg ctg aaa gtc tac aaa aac gag ctg cac cgg cac atc Ser Pro Val Val Leu Lys Val Tyr Lys Asn Glu Leu His Arg His Ile 455 460 465 470	3368
gag gaa ggc ctg ggc aga aac atg agc gac aga tgc agc acc gcc atc Glu Glu Gly Leu Gly Arg Asn Met Ser Asp Arg Cys Ser Thr Ala Ile 475 480 485	3416
acc aat agc ctg cag acc atg cag cag gac atg atc gac ggc ctg aaa Thr Asn Ser Leu Gln Thr Met Gln Gln Asp Met Ile Asp Gly Leu Lys 490 495 500	3464
cct ctg ctg cct gtg tcc gtc aga tcc cag atc gat atg ctg gtg ccc Pro Leu Leu Pro Val Ser Val Arg Ser Gln Ile Asp Met Leu Val Pro 505 510 515	3512
cgg cag tgc ttt agc ctg aac tac gac ctg aac tgc gac aag ctg tgc	3560

Arg	Gln	Cys	Phe	Ser	Leu	Asn	Tyr	Asp	Leu	Asn	Cys	Asp	Lys	Leu	Cys		
520						525					530						
gcc	gat	ttc	caa	gag	gac	atc	gag	ttt	cac	ttc	agc	ctc	ggc	tgg	aca	3608	
Ala	Asp	Phe	Gln	Glu	Asp	Ile	Glu	Phe	His	Phe	Ser	Leu	Gly	Trp	Thr		
535					540					545					550		
atg	ctg	gtc	aac	aga	ttt	ctg	ggc	ccc	aag	aac	agc	aga	cgg	gcc	ctg	3656	
Met	Leu	Val	Asn	Arg	Phe	Leu	Gly	Pro	Lys	Asn	Ser	Arg	Arg	Ala	Leu		
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atg	ggc	tac	aac	gat	cag	gtg	cag	agg	ccc	att	cct	ctg	aca	ccc	gcc	3704	
Met	Gly	Tyr	Asn	Asp	Gln	Val	Gln	Arg	Pro	Ile	Pro	Leu	Thr	Pro	Ala		
			570					575					580				
aat	cct	agc	atg	cct	cca	ctg	cct	cag	ggc	agc	ctg	aca	caa	gag	gaa	3752	
Asn	Pro	Ser	Met	Pro	Pro	Leu	Pro	Gln	Gly	Ser	Leu	Thr	Gln	Glu	Glu		
		585					590				595						
ttc	atg	gtg	tct	atg	gtc	acc	ggc	ctg	gcc	agc	ctg	acc	agc	aga	aca	3800	
Phe	Met	Val	Ser	Met	Val	Thr	Gly	Leu	Ala	Ser	Leu	Thr	Ser	Arg	Thr		
600					605						610						
tct	atg	ggc	atc	ctg	gtc	gtc	ggc	ggc	gtt	gtg	tgg	aaa	gct	gtt	ggc	3848	
Ser	Met	Gly	Ile	Leu	Val	Val	Gly	Gly	Val	Val	Trp	Lys	Ala	Val	Gly		
615					620					625					630		
tgg	cgg	ctg	atc	gcc	ctg	agc	ttt	ggc	ctg	tat	ggc	ctg	ctg	tac	gtg	3896	
Trp	Arg	Leu	Ile	Ala	Leu	Ser	Phe	Gly	Leu	Tyr	Gly	Leu	Leu	Tyr	Val		
				635				640						645			
tac	gag	aga	ctg	acc	tgg	acc	acc	aag	gcc	aaa	gag	cgg	gcc	ttc	aag	3944	
Tyr	Glu	Arg	Leu	Thr	Trp	Thr	Thr	Lys	Ala	Lys	Glu	Arg	Ala	Phe	Lys		
			650					655					660				
cgg	cag	ttt	gtg	gaa	cac	gcc	tcc	gag	aaa	ctg	cag	ctg	gtg	atc	agc	3992	
Arg	Gln	Phe	Val	Glu	His	Ala	Ser	Glu	Lys	Leu	Gln	Leu	Val	Ile	Ser		
		665					670					675					
tac	acc	ggc	agc	aat	tgc	agc	cac	cag	gtg	cag	caa	gag	ctg	tcc	gga	4040	
Tyr	Thr	Gly	Ser	Asn	Cys	Ser	His	Gln	Val	Gln	Gln	Glu	Leu	Ser	Gly		
		680				685					690						
aca	ttc	gcc	cac	ctg	tgt	cag	cag	gtc	gac	gtg	acc	aga	gag	aac	ctg	4088	
Thr	Phe	Ala	His	Leu	Cys	Gln	Gln	Val	Asp	Val	Thr	Arg	Glu	Asn	Leu		
695					700					705					710		
gaa	caa	gag	atc	gcc	gcc	atg	aat	aag	aaa	atc	gag	gtg	ctc	gac	tcc	4136	
Glu	Gln	Glu	Ile	Ala	Ala	Met	Asn	Lys	Lys	Ile	Glu	Val	Leu	Asp	Ser		
				715					720					725			
ctg	cag	agc	aag	gcc	aag	ctg	ctg	aga	aac	aag	gcc	ggc	tgg	ctg	gac	4184	
Leu	Gln	Ser	Lys	Ala	Lys	Leu	Leu	Arg	Asn	Lys	Ala	Gly	Trp	Leu	Asp		
			730					735					740				

agc gag ctg aat atg ttc acc cac cag tac ctg cag cct agc cgc taa	4232
Ser Glu Leu Asn Met Phe Thr His Gln Tyr Leu Gln Pro Ser Arg	
745 750 755	
taaagcggcc gcaagcttgc acccgtacct ttgggagcgc gcgccctcgt cgtgtcgtga	4292
cgtcacccgt tctgttggat ccctggaggc ttgctgaagg ctgtatgctg ttgacgtcca	4352
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acgtagataa gtagcatggc gggttaatca ttaactacaa ggaacccta gtgatggagt	4712
tggccactcc ctctctgcgc gctcgtcgc tctactgaggc cgggcgacca aaggctgccc	4772
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 <211> 757
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 76

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

Tyr Ile Gln Glu Ser Ala Thr Phe Leu Glu Asp Thr Tyr Arg Asn Ala
50 55 60

Glu Leu Asp Pro Val Thr Thr Glu Glu Gln Val Leu Asp Val Lys Gly
65 70 75 80

Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu Val Leu Ala Arg Arg His
85 90 95

Met Lys Val Ala Phe Phe Gly Arg Thr Ser Asn Gly Lys Ser Thr Val
100 105 110

Ile Asn Ala Met Leu Trp Asp Lys Val Leu Pro Ser Gly Ile Gly His
115 120 125

Thr Thr Asn Cys Phe Leu Arg Val Glu Gly Thr Asp Gly His Glu Ala
130 135 140

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

Asn Gln Leu Ala His Ala Leu His Gln Asp Lys Gln Leu His Ala Gly
165 170 175

Ser Leu Val Ser Val Met Trp Pro Asn Ser Lys Cys Pro Leu Leu Lys
180 185 190

Asp Asp Leu Val Leu Met Asp Ser Pro Gly Ile Asp Val Thr Thr Glu
195 200 205

Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu Asp Ala Asp Val Phe Val
210 215 220

Leu Val Ala Asn Ser Glu Ser Thr Leu Met Gln Thr Glu Lys His Phe
225 230 235 240

Phe His Lys Val Ser Glu Arg Leu Ser Arg Pro Asn Ile Phe Ile Leu
245 250 255

Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu Pro Glu Tyr Met Glu Glu
260 265 270

Val Arg Arg Gln His Met Glu Arg Cys Thr Ser Phe Leu Val Asp Glu
275 280 285

Leu Gly Val Val Asp Arg Ser Gln Ala Gly Asp Arg Ile Phe Phe Val
290 295 300

Ser Ala Lys Glu Val Leu Asn Ala Arg Ile Gln Lys Ala Gln Gly Met
305 310 315 320

Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly Phe Gln Val Arg Met Phe
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Gln Gln Glu Leu Ser Gly Thr Phe Ala His Leu Cys Gln Gln Val Asp
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Ile Glu Val Leu Asp Ser Leu Gln Ser Lys Ala Lys Leu Leu Arg Asn
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Asp Asp Leu Val Leu Met Asp Ser Pro Gly Ile Asp Val Thr Thr Glu
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Gln Gln Glu Leu Ser Gly Thr Phe Ala His Leu Cys Gln Gln Val Asp
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Val Thr Arg Glu Asn Leu Glu Gln Glu Ile Ala Ala Met Asn Lys Lys
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gatgggggcg gggggggggg gggggcgcg gccaggcggg gcggggcggg gcgaggggcg	720
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gccccggctc tgactgaccg cgttactccc acaggtagc gggcgggacg gcccttctcc	960
tccgggctgt aattagcgct tggtttaatg acggcttggt tcttttctgt ggctgcgtga	1020
aagccttgag gggctccggg agggcccttt gtgcgggggg agcggctcgg ggggtgcgtg	1080
cgtgtgtgtg tgcgtgggga gcgccgctg cggctccgcg ctgcccggcg gctgtgagcg	1140
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tagcaaggat ccaccggtcg ccacc atg agc ctg ctg ttc agc cgg tgc aac	1972
Met Ser Leu Leu Phe Ser Arg Cys Asn	
1 5	
agc atc gtg acc gtg aag aaa aac aag cgg cac atg gcc gaa gtg aac	2020
Ser Ile Val Thr Val Lys Lys Asn Lys Arg His Met Ala Glu Val Asn	
10 15 20 25	
gcc tct cca ctg aag cac ttc gtg acc gcc aag aag aag atc aac gga	2068
Ala Ser Pro Leu Lys His Phe Val Thr Ala Lys Lys Lys Ile Asn Gly	
30 35 40	
atc ttc gag cag ctg ggc gcc tac atc caa gag agc gcc acc ttc ctg	2116
Ile Phe Glu Gln Leu Gly Ala Tyr Ile Gln Glu Ser Ala Thr Phe Leu	
45 50 55	
gaa gat acc tac aga aac gcc gag ctg gac ccc gtg acc aca gag gaa	2164
Glu Asp Thr Tyr Arg Asn Ala Glu Leu Asp Pro Val Thr Thr Glu Glu	
60 65 70	
cag gtg ctg gat gtg aag ggc tac ctg agc aaa gtg cgg ggc atc tct	2212
Gln Val Leu Asp Val Lys Gly Tyr Leu Ser Lys Val Arg Gly Ile Ser	
75 80 85	
gaa gtg ctg gcc aga cgg cat atg aag gtg gcc ttt ttc ggc cgg acc	2260
Glu Val Leu Ala Arg Arg His Met Lys Val Ala Phe Phe Gly Arg Thr	
90 95 100 105	
agc aac ggc aag agc acc gtg atc aat gcc atg ctg tgg gac aaa gtg	2308
Ser Asn Gly Lys Ser Thr Val Ile Asn Ala Met Leu Trp Asp Lys Val	
110 115 120	
ctg ccc tct gga atc ggc cac acc acc aac tgc ttt ctg aga gtg gaa	2356
Leu Pro Ser Gly Ile Gly His Thr Thr Asn Cys Phe Leu Arg Val Glu	
125 130 135	
ggc acc gac ggc cac gag gca ttt ctg ctg aca gag ggc tcc gag gaa	2404
Gly Thr Asp Gly His Glu Ala Phe Leu Leu Thr Glu Gly Ser Glu Glu	
140 145 150	
aag cgg agc gcc aag aca gtg aac cag ctg gcc cat gct ctg cac cag	2452
Lys Arg Ser Ala Lys Thr Val Asn Gln Leu Ala His Ala Leu His Gln	

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gat aag cag ctg cat gcc gga agc ctg gtg tcc gtg atg tgg ccc aat	2500				
Asp Lys Gln Leu His Ala Gly Ser Leu Val Ser Val Met Trp Pro Asn					
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agc aag tgc cct ctg ctg aag gac gac ctg gtg ctg atg gat agc ccc	2548				
Ser Lys Cys Pro Leu Leu Lys Asp Asp Leu Val Leu Met Asp Ser Pro					
190 195 200					
ggg atc gat gtg acc acc gaa ctg gac agc tgg atc gac aag ttc tgc	2596				
Gly Ile Asp Val Thr Thr Glu Leu Asp Ser Trp Ile Asp Lys Phe Cys					
205 210 215					
ctg gac gcc gac gtg ttc gtg ctg gtg gcc aat agc gag agc acc ctg	2644				
Leu Asp Ala Asp Val Phe Val Leu Val Ala Asn Ser Glu Ser Thr Leu					
220 225 230					
atg cag acc gag aag cac ttt ttc cac aag gtg tcc gag cgg ctg agc	2692				
Met Gln Thr Glu Lys His Phe Phe His Lys Val Ser Glu Arg Leu Ser					
235 240 245					
aga ccc aat atc ttt atc ctg aac aac aga tgg gac gcc agc gcc agc	2740				
Arg Pro Asn Ile Phe Ile Leu Asn Asn Arg Trp Asp Ala Ser Ala Ser					
250 255 260 265					
gag ccc gag tat atg gaa gaa gtg cgg cgg cag cat atg gaa cgg tgc	2788				
Glu Pro Glu Tyr Met Glu Glu Val Arg Arg Gln His Met Glu Arg Cys					
270 275 280					
aca tcc ttt ctg gtg gac gag ctg ggc gtc gtg gat aga tct cag gcc	2836				
Thr Ser Phe Leu Val Asp Glu Leu Gly Val Val Asp Arg Ser Gln Ala					
285 290 295					
ggc gac aga atc ttc ttt gtg tcc gcc aaa gag gtg ctg aac gcc cgg	2884				
Gly Asp Arg Ile Phe Phe Val Ser Ala Lys Glu Val Leu Asn Ala Arg					
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att cag aaa gcc cag gga atg cct gaa ggc gga ggt gca ctg gcc gag	2932				
Ile Gln Lys Ala Gln Gly Met Pro Glu Gly Gly Gly Ala Leu Ala Glu					
315 320 325					
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Gly Phe Gln Val Arg Met Phe Glu Phe Gln Asn Phe Glu Arg Arg Phe					
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gag gag tgc atc agc cag agc gcc gtc aag acc aag ttc gag cag cat	3028				
Glu Glu Cys Ile Ser Gln Ser Ala Val Lys Thr Lys Phe Glu Gln His					
350 355 360					
acc gtg cgg gcc aag cag att gcc gaa gcc gtc aga ctg atc atg gac	3076				
Thr Val Arg Ala Lys Gln Ile Ala Glu Ala Val Arg Leu Ile Met Asp					
365 370 375					

agc	ctg	cat	atg	gcc	gcc	aga	gag	cag	cag	gtc	tac	tgc	gag	gaa	atg	3124
Ser	Leu	His	Met	Ala	Ala	Arg	Glu	Gln	Gln	Val	Tyr	Cys	Glu	Glu	Met	
		380					385					390				
cgg	gaa	gag	aga	cag	gac	cgg	ctg	aag	ttc	atc	gac	aag	cag	ctg	gaa	3172
Arg	Glu	Glu	Arg	Gln	Asp	Arg	Leu	Lys	Phe	Ile	Asp	Lys	Gln	Leu	Glu	
	395					400					405					
ctg	ctg	gcc	cag	gac	tac	aag	ctg	cgg	atc	aag	cag	atc	acc	gaa	gag	3220
Leu	Leu	Ala	Gln	Asp	Tyr	Lys	Leu	Arg	Ile	Lys	Gln	Ile	Thr	Glu	Glu	
410					415					420					425	
gtg	gaa	aga	cag	gtg	tcc	acc	gct	atg	gcc	gag	gaa	atc	aga	cgg	ctg	3268
Val	Glu	Arg	Gln	Val	Ser	Thr	Ala	Met	Ala	Glu	Glu	Ile	Arg	Arg	Leu	
				430					435					440		
agc	gtg	ctg	gtc	gac	gac	tac	cag	atg	gac	ttt	cac	ccc	tct	cca	gtg	3316
Ser	Val	Leu	Val	Asp	Asp	Tyr	Gln	Met	Asp	Phe	His	Pro	Ser	Pro	Val	
			445					450					455			
gtg	ctg	aaa	gtc	tac	aaa	aac	gag	ctg	cac	cgg	cac	atc	gag	gaa	ggc	3364
Val	Leu	Lys	Val	Tyr	Lys	Asn	Glu	Leu	His	Arg	His	Ile	Glu	Glu	Gly	
		460					465					470				
ctg	ggc	aga	aac	atg	agc	gac	aga	tgc	agc	acc	gcc	atc	acc	aat	agc	3412
Leu	Gly	Arg	Asn	Met	Ser	Asp	Arg	Cys	Ser	Thr	Ala	Ile	Thr	Asn	Ser	
	475					480					485					
ctg	cag	acc	atg	cag	cag	gac	atg	atc	gac	ggc	ctg	aaa	cct	ctg	ctg	3460
Leu	Gln	Thr	Met	Gln	Gln	Asp	Met	Ile	Asp	Gly	Leu	Lys	Pro	Leu	Leu	
490					495					500					505	
cct	gtg	tcc	gtc	aga	tcc	cag	atc	gat	atg	ctg	gtg	ccc	cgg	cag	tgc	3508
Pro	Val	Ser	Val	Arg	Ser	Gln	Ile	Asp	Met	Leu	Val	Pro	Arg	Gln	Cys	
				510				515						520		
ttt	agc	ctg	aac	tac	gac	ctg	aac	tgc	gac	aag	ctg	tgc	gcc	gat	ttc	3556
Phe	Ser	Leu	Asn	Tyr	Asp	Leu	Asn	Cys	Asp	Lys	Leu	Cys	Ala	Asp	Phe	
			525					530					535			
caa	gag	gac	atc	gag	ttt	cac	ttc	agc	ctc	ggc	tgg	aca	atg	ctg	gtc	3604
Gln	Glu	Asp	Ile	Glu	Phe	His	Phe	Ser	Leu	Gly	Trp	Thr	Met	Leu	Val	
		540					545					550				
aac	aga	ttt	ctg	ggc	ccc	aag	aac	agc	aga	cgg	gcc	ctg	atg	ggc	tac	3652
Asn	Arg	Phe	Leu	Gly	Pro	Lys	Asn	Ser	Arg	Arg	Ala	Leu	Met	Gly	Tyr	
	555					560					565					
aac	gat	cag	gtg	cag	agg	ccc	att	cct	ctg	aca	ccc	gcc	aat	cct	agc	3700
Asn	Asp	Gln	Val	Gln	Arg	Pro	Ile	Pro	Leu	Thr	Pro	Ala	Asn	Pro	Ser	
570					575					580					585	
atg	cct	cca	ctg	cct	cag	ggc	agc	ctg	aca	caa	gag	gaa	ttc	atg	gtg	3748
Met	Pro	Pro	Leu	Pro	Gln	Gly	Ser	Leu	Thr	Gln	Glu	Glu	Phe	Met	Val	

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tct atg gtc acc ggc ctg gcc agc	ctg acc agc aga aca tct atg ggc	3796	
Ser Met Val Thr Gly Leu Ala Ser	Leu Thr Ser Arg Thr Ser Met Gly		
605	610	615	
atc ctg gtc gtc ggc ggc gtt gtg tgg aaa gct gtt ggc tgg cgg ctg	3844		
Ile Leu Val Val Gly Gly Val Val Trp Lys Ala Val Gly Trp Arg Leu			
620	625	630	
atc gcc ctg agc ttt ggc ctg tat ggc ctg ctg tac gtg tac gag aga	3892		
Ile Ala Leu Ser Phe Gly Leu Tyr Gly Leu Leu Tyr Val Tyr Glu Arg			
635	640	645	
ctg acc tgg acc acc aag gcc aaa gag cgg gcc ttc aag cgg cag ttt	3940		
Leu Thr Trp Thr Thr Lys Ala Lys Glu Arg Ala Phe Lys Arg Gln Phe			
650	655	660	
gtg gaa cac gcc tcc gag aaa ctg cag ctg gtg atc agc tac acc ggc	3988		
Val Glu His Ala Ser Glu Lys Leu Gln Leu Val Ile Ser Tyr Thr Gly			
670	675	680	
agc aat tgc agc cac cag gtg cag caa gag ctg tcc gga aca ttc gcc	4036		
Ser Asn Cys Ser His Gln Val Gln Gln Glu Leu Ser Gly Thr Phe Ala			
685	690	695	
cac ctg tgt cag cag gtc gac gtg acc aga gag aac ctg gaa caa gag	4084		
His Leu Cys Gln Gln Val Asp Val Thr Arg Glu Asn Leu Glu Gln Glu			
700	705	710	
atc gcc gcc atg aat aag aaa atc gag gtg ctc gac tcc ctg cag agc	4132		
Ile Ala Ala Met Asn Lys Lys Ile Glu Val Leu Asp Ser Leu Gln Ser			
715	720	725	
aag gcc aag ctg ctg aga aac aag gcc ggc tgg ctg gac agc gag ctg	4180		
Lys Ala Lys Leu Leu Arg Asn Lys Ala Gly Trp Leu Asp Ser Glu Leu			
730	735	740	
aat atg ttc acc cac cag tac ctg cag cct agc cgc taa taaagcggcc	4229		
Asn Met Phe Thr His Gln Tyr Leu Gln Pro Ser Arg			
750	755		
gcctaaggct cgaggatctt tttccctctg ccaaaaatta tggggacatc atgaagcccc	4289		
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<211> 757
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 80

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

Tyr Ile Gln Glu Ser Ala Thr Phe Leu Glu Asp Thr Tyr Arg Asn Ala
50 55 60

Glu Leu Asp Pro Val Thr Thr Glu Glu Gln Val Leu Asp Val Lys Gly
65 70 75 80

Tyr Leu Ser Lys Val Arg Gly Ile Ser Glu Val Leu Ala Arg Arg His
85 90 95

Met Lys Val Ala Phe Phe Gly Arg Thr Ser Asn Gly Lys Ser Thr Val
100 105 110

Ile Asn Ala Met Leu Trp Asp Lys Val Leu Pro Ser Gly Ile Gly His
115 120 125

Thr Thr Asn Cys Phe Leu Arg Val Glu Gly Thr Asp Gly His Glu Ala
130 135 140

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

Asn Gln Leu Ala His Ala Leu His Gln Asp Lys Gln Leu His Ala Gly
165 170 175

Ser Leu Val Ser Val Met Trp Pro Asn Ser Lys Cys Pro Leu Leu Lys
180 185 190

Asp Asp Leu Val Leu Met Asp Ser Pro Gly Ile Asp Val Thr Thr Glu
195 200 205

Leu Asp Ser Trp Ile Asp Lys Phe Cys Leu Asp Ala Asp Val Phe Val
210 215 220

Leu Val Ala Asn Ser Glu Ser Thr Leu Met Gln Thr Glu Lys His Phe
225 230 235 240

Phe His Lys Val Ser Glu Arg Leu Ser Arg Pro Asn Ile Phe Ile Leu
245 250 255

Asn Asn Arg Trp Asp Ala Ser Ala Ser Glu Pro Glu Tyr Met Glu Glu
260 265 270

Val Arg Arg Gln His Met Glu Arg Cys Thr Ser Phe Leu Val Asp Glu
275 280 285

Leu Gly Val Val Asp Arg Ser Gln Ala Gly Asp Arg Ile Phe Phe Val
290 295 300

Ser Ala Lys Glu Val Leu Asn Ala Arg Ile Gln Lys Ala Gln Gly Met
305 310 315 320

Pro Glu Gly Gly Gly Ala Leu Ala Glu Gly Phe Gln Val Arg Met Phe
325 330 335

Glu Phe Gln Asn Phe Glu Arg Arg Phe Glu Glu Cys Ile Ser Gln Ser
340 345 350

Ala Val Lys Thr Lys Phe Glu Gln His Thr Val Arg Ala Lys Gln Ile
355 360 365

Ala Glu Ala Val Arg Leu Ile Met Asp Ser Leu His Met Ala Ala Arg
370 375 380

Glu Gln Gln Val Tyr Cys Glu Glu Met Arg Glu Glu Arg Gln Asp Arg

385		390		395		400									
Leu	Lys	Phe	Ile	Asp	Lys	Gln	Leu	Glu	Leu	Leu	Ala	Gln	Asp	Tyr	Lys
				405					410					415	
Leu	Arg	Ile	Lys	Gln	Ile	Thr	Glu	Glu	Val	Glu	Arg	Gln	Val	Ser	Thr
			420					425					430		
Ala	Met	Ala	Glu	Glu	Ile	Arg	Arg	Leu	Ser	Val	Leu	Val	Asp	Asp	Tyr
		435					440					445			
Gln	Met	Asp	Phe	His	Pro	Ser	Pro	Val	Val	Leu	Lys	Val	Tyr	Lys	Asn
	450					455					460				
Glu	Leu	His	Arg	His	Ile	Glu	Glu	Gly	Leu	Gly	Arg	Asn	Met	Ser	Asp
465					470					475					480
Arg	Cys	Ser	Thr	Ala	Ile	Thr	Asn	Ser	Leu	Gln	Thr	Met	Gln	Gln	Asp
				485					490					495	
Met	Ile	Asp	Gly	Leu	Lys	Pro	Leu	Leu	Pro	Val	Ser	Val	Arg	Ser	Gln
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Ile	Asp	Met	Leu	Val	Pro	Arg	Gln	Cys	Phe	Ser	Leu	Asn	Tyr	Asp	Leu
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Asn	Cys	Asp	Lys	Leu	Cys	Ala	Asp	Phe	Gln	Glu	Asp	Ile	Glu	Phe	His
	530					535					540				
Phe	Ser	Leu	Gly	Trp	Thr	Met	Leu	Val	Asn	Arg	Phe	Leu	Gly	Pro	Lys
545					550					555					560
Asn	Ser	Arg	Arg	Ala	Leu	Met	Gly	Tyr	Asn	Asp	Gln	Val	Gln	Arg	Pro
				565					570					575	
Ile	Pro	Leu	Thr	Pro	Ala	Asn	Pro	Ser	Met	Pro	Pro	Leu	Pro	Gln	Gly
			580					585					590		
Ser	Leu	Thr	Gln	Glu	Glu	Phe	Met	Val	Ser	Met	Val	Thr	Gly	Leu	Ala
		595					600					605			

Ser Leu Thr Ser Arg Thr Ser Met Gly Ile Leu Val Val Gly Gly Val
610 615 620

Val Trp Lys Ala Val Gly Trp Arg Leu Ile Ala Leu Ser Phe Gly Leu
625 630 635 640

Tyr Gly Leu Leu Tyr Val Tyr Glu Arg Leu Thr Trp Thr Thr Lys Ala
645 650 655

Lys Glu Arg Ala Phe Lys Arg Gln Phe Val Glu His Ala Ser Glu Lys
660 665 670

Leu Gln Leu Val Ile Ser Tyr Thr Gly Ser Asn Cys Ser His Gln Val
675 680 685

Gln Gln Glu Leu Ser Gly Thr Phe Ala His Leu Cys Gln Gln Val Asp
690 695 700

Val Thr Arg Glu Asn Leu Glu Gln Glu Ile Ala Ala Met Asn Lys Lys
705 710 715 720

Ile Glu Val Leu Asp Ser Leu Gln Ser Lys Ala Lys Leu Leu Arg Asn
725 730 735

Lys Ala Gly Trp Leu Asp Ser Glu Leu Asn Met Phe Thr His Gln Tyr
740 745 750

Leu Gln Pro Ser Arg
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<212> DNA
<213> adeno-associated virus hu68

<220>
<221> CDS
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Glu	Gly	Ile	Arg	Glu	Trp	Trp	Ala	Leu	Lys	Pro	Gly	Ala	Pro	Gln	Pro	
			20					25					30			
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aag	gca	aat	caa	caa	cat	caa	gac	aac	gct	cgg	ggt	ctt	gtg	ctt	ccg	
Lys	Ala	Asn	Gln	Gln	His	Gln	Asp	Asn	Ala	Arg	Gly	Leu	Val	Leu	Pro	
		35					40					45				
																192
ggt	tac	aaa	tac	ctt	gga	ccc	ggc	aac	gga	ctc	gac	aag	ggg	gag	ccg	
Gly	Tyr	Lys	Tyr	Leu	Gly	Pro	Gly	Asn	Gly	Leu	Asp	Lys	Gly	Glu	Pro	
	50					55					60					
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gtc	aac	gaa	gca	gac	gcg	gcg	gcc	ctc	gag	cac	gac	aag	gcc	tac	gac	
Val	Asn	Glu	Ala	Asp	Ala	Ala	Ala	Leu	Glu	His	Asp	Lys	Ala	Tyr	Asp	
65					70				75						80	
																288
cag	cag	ctc	aag	gcc	gga	gac	aac	ccg	tac	ctc	aag	tac	aac	cac	gcc	
Gln	Gln	Leu	Lys	Ala	Gly	Asp	Asn	Pro	Tyr	Leu	Lys	Tyr	Asn	His	Ala	
				85					90					95		
																336
gac	gcc	gag	ttc	cag	gag	cgg	ctc	aaa	gaa	gat	acg	tct	ttt	ggg	ggc	
Asp	Ala	Glu	Phe	Gln	Glu	Arg	Leu	Lys	Glu	Asp	Thr	Ser	Phe	Gly	Gly	
			100					105					110			
																384
aac	ctc	ggg	cga	gca	gtc	ttc	cag	gcc	aaa	aag	agg	ctt	ctt	gaa	cct	
Asn	Leu	Gly	Arg	Ala	Val	Phe	Gln	Ala	Lys	Lys	Arg	Leu	Leu	Glu	Pro	
		115					120					125				
																432
ctt	ggt	ctg	gtt	gag	gaa	gcg	gct	aag	acg	gct	cct	gga	aag	aag	agg	
Leu	Gly	Leu	Val	Glu	Glu	Ala	Ala	Lys	Thr	Ala	Pro	Gly	Lys	Lys	Arg	
	130					135					140					
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cct	gta	gag	cag	tct	cct	cag	gaa	ccg	gac	tcc	tcc	gtg	ggt	att	ggc	
Pro	Val	Glu	Gln	Ser	Pro	Gln	Glu	Pro	Asp	Ser	Ser	Val	Gly	Ile	Gly	
145					150				155						160	
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aaa	tcg	ggt	gca	cag	ccc	gct	aaa	aag	aga	ctc	aat	ttc	ggt	cag	act	
Lys	Ser	Gly	Ala	Gln	Pro	Ala	Lys	Lys	Arg	Leu	Asn	Phe	Gly	Gln	Thr	
				165					170					175		
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ggc	gac	aca	gag	tca	gtc	ccc	gac	cct	caa	cca	atc	gga	gaa	cct	ccc	
Gly	Asp	Thr	Glu	Ser	Val	Pro	Asp	Pro	Gln	Pro	Ile	Gly	Glu	Pro	Pro	
			180					185					190			
																624
gca	gcc	ccc	tca	ggt	gtg	gga	tct	ctt	aca	atg	gct	tca	ggt	ggt	ggc	
Ala	Ala	Pro	Ser	Gly	Val	Gly	Ser	Leu	Thr	Met	Ala	Ser	Gly	Gly	Gly	
		195				200						205				
																672
gca	cca	gtg	gca	gac	aat	aac	gaa	ggt	gcc	gat	gga	gtg	ggt	agt	tcc	
Ala	Pro	Val	Ala	Asp	Asn	Asn	Glu	Gly	Ala	Asp	Gly	Val	Gly	Ser	Ser	
	210					215					220					

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

Asp	Arg	Leu	Met	Asn	Pro	Leu	Ile	Asp	Gln	Tyr	Leu	Tyr	Tyr	Leu	Ser		
		435					440					445					
aag	act	att	aac	ggt	tct	gga	cag	aat	caa	caa	acg	cta	aaa	ttc	agt	1392	
Lys	Thr	Ile	Asn	Gly	Ser	Gly	Gln	Asn	Gln	Gln	Thr	Leu	Lys	Phe	Ser		
		450				455					460						
gtg	gcc	gga	ccc	agc	aac	atg	gct	gtc	cag	gga	aga	aac	tac	ata	cct	1440	
Val	Ala	Gly	Pro	Ser	Asn	Met	Ala	Val	Gln	Gly	Arg	Asn	Tyr	Ile	Pro		
465					470					475					480		
gga	ccc	agc	tac	cga	caa	caa	cgt	gtc	tca	acc	act	gtg	act	caa	aac	1488	
Gly	Pro	Ser	Tyr	Arg	Gln	Gln	Arg	Val	Ser	Thr	Thr	Val	Thr	Gln	Asn		
				485				490						495			
aac	aac	agc	gaa	ttt	gct	tgg	cct	gga	gct	tct	tct	tgg	gct	ctc	aat	1536	
Asn	Asn	Ser	Glu	Phe	Ala	Trp	Pro	Gly	Ala	Ser	Ser	Trp	Ala	Leu	Asn		
			500					505					510				
gga	cgt	aat	agc	ttg	atg	aat	cct	gga	cct	gct	atg	gcc	agc	cac	aaa	1584	
Gly	Arg	Asn	Ser	Leu	Met	Asn	Pro	Gly	Pro	Ala	Met	Ala	Ser	His	Lys		
		515					520					525					
gaa	gga	gag	gac	cgt	ttc	ttt	cct	ttg	tct	gga	tct	tta	att	ttt	ggc	1632	
Glu	Gly	Glu	Asp	Arg	Phe	Phe	Pro	Leu	Ser	Gly	Ser	Leu	Ile	Phe	Gly		
		530				535					540						
aaa	caa	gga	act	gga	aga	gac	aac	gtg	gat	gcg	gac	aaa	gtc	atg	ata	1680	
Lys	Gln	Gly	Thr	Gly	Arg	Asp	Asn	Val	Asp	Ala	Asp	Lys	Val	Met	Ile		
545					550					555					560		
acc	aac	gaa	gaa	gaa	att	aaa	act	acc	aac	cca	gta	gca	acg	gag	tcc	1728	
Thr	Asn	Glu	Glu	Glu	Ile	Lys	Thr	Thr	Asn	Pro	Val	Ala	Thr	Glu	Ser		
				565					570					575			
tat	gga	caa	gtg	gcc	aca	aac	cac	cag	agt	gcc	caa	gca	cag	gcg	cag	1776	
Tyr	Gly	Gln	Val	Ala	Thr	Asn	His	Gln	Ser	Ala	Gln	Ala	Gln	Ala	Gln		
			580					585				590					
acc	ggc	tgg	gtt	caa	aac	caa	gga	ata	ctt	ccg	ggg	atg	gtt	tgg	cag	1824	
Thr	Gly	Trp	Val	Gln	Asn	Gln	Gly	Ile	Leu	Pro	Gly	Met	Val	Trp	Gln		
		595				600					605						
gac	aga	gat	gtg	tac	ctg	caa	gga	ccc	att	tgg	gcc	aaa	att	cct	cac	1872	
Asp	Arg	Asp	Val	Tyr	Leu	Gln	Gly	Pro	Ile	Trp	Ala	Lys	Ile	Pro	His		
		610				615					620						
acg	gac	ggc	aac	ttt	cac	cct	tct	ccg	ctg	atg	gga	ggg	ttt	gga	atg	1920	
Thr	Asp	Gly	Asn	Phe	His	Pro	Ser	Pro	Leu	Met	Gly	Gly	Phe	Gly	Met		
625					630				635						640		
aag	cac	ccg	cct	cct	cag	atc	ctc	atc	aaa	aac	aca	cct	gta	cct	gcg	1968	
Lys	His	Pro	Pro	Pro	Gln	Ile	Leu	Ile	Lys	Asn	Thr	Pro	Val	Pro	Ala		
				645					650						655		

gat cct cca acg gct ttc aac aag gac aag ctg aac tct ttc atc acc	2016
Asp Pro Pro Thr Ala Phe Asn Lys Asp Lys Leu Asn Ser Phe Ile Thr	
660 665 670	

cag tat tct act ggc caa gtc agc gtg gag att gag tgg gag ctg cag	2064
Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln	
675 680 685	

aag gaa aac agc aag cgc tgg aac ccg gag atc cag tac act tcc aac	2112
Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn	
690 695 700	

tat tac aag tct aat aat gtt gaa ttt gct gtt aat act gaa ggt gtt	2160
Tyr Tyr Lys Ser Asn Asn Val Glu Phe Ala Val Asn Thr Glu Gly Val	
705 710 715 720	

tat tct gaa ccc cgc ccc att ggc acc aga tac ctg act cgt aat ctg	2208
Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu	
725 730 735	

taa	2211
-----	------

<210> 82
 <211> 736
 <212> PRT
 <213> adeno-associated virus hu68

<400> 82

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Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Ala Pro Gln Pro
20 25 30

Lys Ala Asn Gln Gln His Gln Asp Asn Ala Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Leu Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Val Gly Ile Gly
145 150 155 160

Lys Ser Gly Ala Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175

Gly Asp Thr Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro Pro
180 185 190

Ala Ala Pro Ser Gly Val Gly Ser Leu Thr Met Ala Ser Gly Gly Gly
195 200 205

Ala Pro Val Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser Ser
210 215 220

Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu Gly Asp Arg Val Ile
225 230 235 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
245 250 255

Tyr Lys Gln Ile Ser Asn Ser Thr Ser Gly Gly Ser Ser Asn Asp Asn
260 265 270

Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
275 280 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
290 295 300

Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile

305		310		315		320
Gln Val Lys Glu Val Thr Asp Asn Asn Gly Val Lys Thr Ile Ala Asn						
		325		330		335
Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Asp Tyr Gln Leu						
		340		345		350
Pro Tyr Val Leu Gly Ser Ala His Glu Gly Cys Leu Pro Pro Phe Pro						
		355		360		365
Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asp						
		370		375		380
Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe						
385		390		395		400
Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Glu						
		405		410		415
Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu						
		420		425		430
Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser						
		435		440		445
Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln Thr Leu Lys Phe Ser						
		450		455		460
Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly Arg Asn Tyr Ile Pro						
465		470		475		480
Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser Thr Thr Val Thr Gln Asn						
		485		490		495
Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser Ser Trp Ala Leu Asn						
		500		505		510
Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala Met Ala Ser His Lys						
		515		520		525

Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly Ser Leu Ile Phe Gly
530 535 540

Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala Asp Lys Val Met Ile
545 550 555 560

Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu Ser
565 570 575

Tyr Gly Gln Val Ala Thr Asn His Gln Ser Ala Gln Ala Gln Ala Gln
580 585 590

Thr Gly Trp Val Gln Asn Gln Gly Ile Leu Pro Gly Met Val Trp Gln
595 600 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
610 615 620

Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Met
625 630 635 640

Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
645 650 655

Asp Pro Pro Thr Ala Phe Asn Lys Asp Lys Leu Asn Ser Phe Ile Thr
660 665 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
675 680 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn
690 695 700

Tyr Tyr Lys Ser Asn Asn Val Glu Phe Ala Val Asn Thr Glu Gly Val
705 710 715 720

Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu
725 730 735

<211> 2211
<212> DNA
<213> adeno-associated virus hu68

<400> 83
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aacgctcggg gtcttgtgct tccgggttac aaataccttg gacccggcaa cggactcgac 180
aagggggagc cgggtcaacga agcagacgcg gcggccctcg agcacgacaa ggcctacgac 240
cagcagctca aggccggaga caacccttac ctcaagtaca accacgccga cgccgagttc 300
caggagcggc tcaaagaaga tacgtctttt gggggcaacc tcgggcgagc agtcttccag 360
gccaaaaaga ggcttcttga acctcttggg ctggttgagg aagcggctaa gacggctcct 420
ggaaagaaga ggcctgtaga gcagtctcct caggaaccgg actcctccgt gggatttggc 480
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tcagtccccg acctcaacc aatcggagaa cctcccgag cccctcagg tgtgggatct 600
cttacaatgg cttcaggtgg tggcgacca gtggcagaca ataacgaagg tgccgatgga 660
gtgggtagtt cctcgggaaa ttggcattgc gattcccaat ggctggggga cagagtcac 720
accaccagca cccgaacctg ggccctgccc acctacaaca atcacctcta caagcaaadc 780
tccaacagca catctggagg atcttcaa gacaacgcct acttcggcta cagcaccccc 840
tgggggtatt ttgacttcaa cagattccac tgccacttct caccacgtga ctggcaaaga 900
ctcatcaaca acaactgggg attccggcct aagcgactca acttcaagct cttcaacatt 960
caggtcaaag aggttacgga caacaatgga gtcaagacca tcgctaataa ccttaccagc 1020
acggtccagg tcttcacgga ctgagactat cagctcccgt acgtgctcgg gtcggctcac 1080
gagggctgcc tcccgccgtt cccagcggac gttttcatga ttcctcagta tggatactc 1140
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ccttcgcaga tgctgaggac tggcaacaac ttccagttca gctacgagtt cgagaacgtc 1260
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ctgaagttca gcgtggccgg gcctagcaac atggccgtcc agggcagaaa ctacatccct 1440
gggcccagct accggcagca gagagtctca accactgtga ctcagaacaa caacagttag 1500

ttcgctggc ctggcgccag ctcttgggcc ctcaacggcc gcaactcgct gatgaacca	1560
ggcccagcca tggccagtca caaggagggc gaggaccgtt tcttcccttt gtctggctct	1620
ctgatcttcg gcaagcaggg gaccggcaga gacaacgtgg acgcggacaa ggtcatgac	1680
acgaacgagg aggagatcaa gaccaccaac cctgtggcaa ccgagtccta cggccaggtg	1740
gcaaccaacc accagagcgc ccaggcacag gcgcagactg gctgggtcca gaaccagggg	1800
atcctgcctg gcatggtgtg gcaggaccgt gacgtgtacc tgcaggggccc tatctgggca	1860
aagatccctc acacggacgg caacttcac ctttctcctc tgatgggcgg cttcggcatg	1920
aagcaccgc ctcctcagat cctcatcaag aacactccgg tcccggcaga ccctccgacg	1980
gccttcaaca aggacaagct gaactcattc atcactcagt actccactgg ccaggtcagc	2040
gtggagatcg agtgggagct gcagaaggag aacagcaagc gttggaaccc agagatccag	2100
tacacttcca actactacaa gtctaacaac gtggagttcg ccgtcaacac tgagggtgtg	2160
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<210> 84
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 <212> DNA
 <213> adeno-associated virus PHP.eB

<220>
 <221> CDS
 <222> (1)..(2229)
 <223> AAV-PHP.eB

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aag gca aat caa caa cat caa gac aac gct aga ggt ctt gtg ctt ccg Lys Ala Asn Gln Gln His Gln Asp Asn Ala Arg Gly Leu Val Leu Pro 35 40 45	144
ggt tac aaa tac ctt gga ccc ggc aac gga ctc gac aag ggg gag ccg Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro 50 55 60	192

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

275	280	285	
ttc cac tgc cac ttc tca cca cgt gac tgg cag cga ctc atc aac aac Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn 290 295 300			912
aac tgg gga ttc cgg cct aag cga ctc aac ttc aag ctc ttt aac att Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile 305 310 315 320			960
cag gtc aaa gag gtt acg gac aac aat gga gtc aag acc atc gcc aat Gln Val Lys Glu Val Thr Asp Asn Asn Gly Val Lys Thr Ile Ala Asn 325 330 335			1008
aac ctt acc agc acg gtc cag gtc ttc acg gac tca gac tat cag ctc Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Asp Tyr Gln Leu 340 345 350			1056
ccg tac gtg ctc ggg tcg gct cac gag ggc tgc ctc ccg ccg ttc cca Pro Tyr Val Leu Gly Ser Ala His Glu Gly Cys Leu Pro Pro Phe Pro 355 360 365			1104
gcg gac gtt ttc atg att cct cag tac ggg tat ctg acg ctt aat gat Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asp 370 375 380			1152
gga agc cag gcc gtg ggt cgt tcg tcc ttt tac tgc ctg gaa tat ttc Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe 385 390 395 400			1200
ccg tcg caa atg cta aga acg ggt aac aac ttc cag ttc agc tac gag Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Glu 405 410 415			1248
ttt gag aac gta cct ttc cat agc agc tac gct cac agc caa agc ctg Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu 420 425 430			1296
gac cga cta atg aat cca ctc atc gac caa tac ttg tac tat ctc tct Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser 435 440 445			1344
aga act att aac ggt tct gga cag aat caa caa acg cta aaa ttc agt Arg Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln Thr Leu Lys Phe Ser 450 455 460			1392
gtg gcc gga ccc agc aac atg gct gtc cag gga aga aac tac ata cct Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly Arg Asn Tyr Ile Pro 465 470 475 480			1440
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Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser Ser Trp Ala Leu Asn	
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gga cgt aat agc ttg atg aat cct gga cct gct atg gcc tct cac aaa	1584
Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala Met Ala Ser His Lys	
515 520 525	
gaa gga gag gac cgt ttc ttt cct ttg tct gga tct tta att ttt ggc	1632
Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly Ser Leu Ile Phe Gly	
530 535 540	
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Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala Asp Lys Val Met Ile	
545 550 555 560	
acc aac gaa gaa gaa att aaa act act aac ccg gta gca acg gag tcc	1728
Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu Ser	
565 570 575	
tat gga caa gtg gcc aca aac cac cag agt gat ggg act ttg gcg gtg	1776
Tyr Gly Gln Val Ala Thr Asn His Gln Ser Asp Gly Thr Leu Ala Val	
580 585 590	
cct ttt aag gca cag gcg cag acc ggt tgg gtt caa aac caa gga ata	1824
Pro Phe Lys Ala Gln Ala Gln Thr Gly Trp Val Gln Asn Gln Gly Ile	
595 600 605	
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Leu Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro	
610 615 620	
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Ile Trp Ala Lys Ile Pro His Thr Asp Gly Asn Phe His Pro Ser Pro	
625 630 635 640	
ctg atg gga ggg ttt gga atg aag cac ccg cct cct cag atc ctc atc	1968
Leu Met Gly Gly Phe Gly Met Lys His Pro Pro Pro Gln Ile Leu Ile	
645 650 655	
aaa aac aca cct gta cct gcg gat cct cca acg gcc ttc aac aag gac	2016
Lys Asn Thr Pro Val Pro Ala Asp Pro Pro Thr Ala Phe Asn Lys Asp	
660 665 670	
aag ctg aac tct ttc atc acc cag tat tct act ggt caa gtc agc gtg	2064
Lys Leu Asn Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val	
675 680 685	
gag atc gag tgg gag ctg cag aag gaa aac agc aag cgc tgg aac ccg	2112
Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro	
690 695 700	
gag atc cag tac act tcc aac tat tac aag tct aat aat gtt gaa ttt	2160
Glu Ile Gln Tyr Thr Ser Asn Tyr Tyr Lys Ser Asn Asn Val Glu Phe	

705		710		715		720	
gct gtt aat act	gaa ggt gta tat	agt gaa ccc cgc	ccc att ggc acc	2208			
Ala Val Asn Thr	Glu Gly Val Tyr	Ser Glu Pro Arg	Pro Ile Gly Thr				
	725	730	735				

aga tac ctg act	cgt aat ctg	2229
Arg Tyr Leu Thr	Arg Asn Leu	
	740	

<210> 85
 <211> 743
 <212> PRT
 <213> adeno-associated virus PHP.eB

<400> 85

Met Ala Ala Asp	Gly Tyr Leu Pro	Asp Trp Leu Glu	Asp Asn Leu Ser
1	5	10	15

Glu Gly Ile Arg	Glu Trp Trp Ala	Leu Lys Pro Gly	Ala Pro Gln Pro
20	25	30	

Lys Ala Asn Gln	Gln His Gln Asp	Asn Ala Arg Gly	Leu Val Leu Pro
35	40	45	

Gly Tyr Lys Tyr	Leu Gly Pro Gly	Asn Gly Leu Asp	Lys Gly Glu Pro
50	55	60	

Val Asn Ala Ala	Asp Ala Ala Ala	Leu Glu His Asp	Lys Ala Tyr Asp
65	70	75	80

Gln Gln Leu Lys	Ala Gly Asp Asn	Pro Tyr Leu Lys	Tyr Asn His Ala
85	90	95	

Asp Ala Glu Phe	Gln Glu Arg Leu	Lys Glu Asp Thr	Ser Phe Gly Gly
100	105	110	

Asn Leu Gly Arg	Ala Val Phe Gln	Ala Lys Lys Arg	Leu Leu Glu Pro
115	120	125	

Leu Gly Leu Val	Glu Glu Ala Ala	Lys Thr Ala Pro	Gly Lys Lys Arg
130	135	140	

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ala Gly Ile Gly
145 150 155 160

Lys Ser Gly Ala Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175

Gly Asp Thr Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro Pro
180 185 190

Ala Ala Pro Ser Gly Val Gly Ser Leu Thr Met Ala Ser Gly Gly Gly
195 200 205

Ala Pro Val Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser Ser
210 215 220

Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu Gly Asp Arg Val Ile
225 230 235 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
245 250 255

Tyr Lys Gln Ile Ser Asn Ser Thr Ser Gly Gly Ser Ser Asn Asp Asn
260 265 270

Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
275 280 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
290 295 300

Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile
305 310 315 320

Gln Val Lys Glu Val Thr Asp Asn Asn Gly Val Lys Thr Ile Ala Asn
325 330 335

Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Asp Tyr Gln Leu
340 345 350

Pro Tyr Val Leu Gly Ser Ala His Glu Gly Cys Leu Pro Pro Phe Pro
355 360 365

Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asp
370 375 380

Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
385 390 395 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Glu
405 410 415

Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
420 425 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser
435 440 445

Arg Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln Thr Leu Lys Phe Ser
450 455 460

Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly Arg Asn Tyr Ile Pro
465 470 475 480

Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser Thr Thr Val Thr Gln Asn
485 490 495

Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser Ser Trp Ala Leu Asn
500 505 510

Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala Met Ala Ser His Lys
515 520 525

Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly Ser Leu Ile Phe Gly
530 535 540

Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala Asp Lys Val Met Ile
545 550 555 560

Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu Ser
565 570 575

Tyr Gly Gln Val Ala Thr Asn His Gln Ser Asp Gly Thr Leu Ala Val
580 585 590

Pro Phe Lys Ala Gln Ala Gln Thr Gly Trp Val Gln Asn Gln Gly Ile
595 600 605

Leu Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro
610 615 620

Ile Trp Ala Lys Ile Pro His Thr Asp Gly Asn Phe His Pro Ser Pro
625 630 635 640

Leu Met Gly Gly Phe Gly Met Lys His Pro Pro Pro Gln Ile Leu Ile
645 650 655

Lys Asn Thr Pro Val Pro Ala Asp Pro Pro Thr Ala Phe Asn Lys Asp
660 665 670

Lys Leu Asn Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val
675 680 685

Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro
690 695 700

Glu Ile Gln Tyr Thr Ser Asn Tyr Tyr Lys Ser Asn Asn Val Glu Phe
705 710 715 720

Ala Val Asn Thr Glu Gly Val Tyr Ser Glu Pro Arg Pro Ile Gly Thr
725 730 735

Arg Tyr Leu Thr Arg Asn Leu
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<210> 86

<211> 841

<212> DNA

<213> Artificial Sequence

<220>

<223> CB7 Promoter with CMV enhancer

<400> 86

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atgccaagta cgccccctat	tgacgtcaat gacggtaa	at ggcccgccctg gcattatgcc	180
cagtacatga ccttatggga	ctttcctact tggcagtaca	tctacgtatt agtcatcgct	240
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<210> 87
 <211> 841
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> CAG promoter

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cagtacatga ccttatggga	ctttcctact tggcagtaca	tctacgtatt agtcatcgct	240
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ccacccccaa ttttgtat	ttttat	gtgcagcgat gggggcgggg	360
gggggggggg ggcgcgcgcc	aggcggggcg gggcggggcg	aggggcgggg cggggcgagg	420
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a	841

<210> 88
 <211> 127
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> rabbit beta-globin

<400> 88	
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tcactcg	127

<210> 89
 <211> 59
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> miR1538

<400> 89	
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<210> 90
 <211> 82
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> linker

<400> 90	
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cgtcacccgt tctgttgat cc