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(54) Title: COMPOSITIONS AND METHODS FOR OPA1 GENE THERAPY

(57) **Abstract:** Described herein are pharmaceutical compositions and methods for treating a subject diagnosed as having, e.g., a neuro-ocular disorder or OPA1 -deficiency disorder, such as Autosomal Dominant Optic Atrophy (ADOA) caused by mutations in the Optic Atrophy 1 (OPA1) gene. The pharmaceutical compositions and methods of the disclosure include polynucleotides, viral vectors (e.g., adeno-associated viral vectors), and non-viral vectors, that contain a codon-optimized transgene encoding OPA1 protein.



COMPOSITIONS AND METHODS FOR OPA1 GENE THERAPY

Sequence Listing

The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on April 16, 2024, is named "51037-084WO2_Sequence_Listing_4_16_24" and is 68,178 bytes in size.

Field of the Invention

The invention relates to the field of gene therapy of neuro-ocular disorders, such as optic neuropathies, and OPA1-deficiency disorders, such as Autosomal Dominant Optic Atrophy (ADOA), that are caused by mutations in the Optic Atrophy 1 (*OPA1*) gene.

Background of the Invention

Autosomal Dominant Optic Atrophy (ADOA) is one of the most common forms of hereditary optic neuropathy. Its symptoms include impaired vision, impaired color vision (color blindness), impaired central vision, central scotoma, reduced visual acuity, vision loss, and blindness, among others. Problems such as retinal damage, retinal degeneration, retinal ganglion cell loss, and optic nerve damage often arise out of this neuropathy. ADOA can cause blindness, vision loss, or vision impairment even starting in childhood, and has been diagnosed in patients as young as a one year of age.

The Optic Atrophy 1 (*OPA1*) gene offers the ability to treat patients suffering from ADOA. Sarzi et al., *Scientific Reports* 8:2468 (2018) discloses that OPA1 has therapeutic potential in the treatment of retinal ganglion cell degeneration, one of the primary phenotypes of ADOA. Maloney et al., *Frontiers in Neuroscience* 14:571479 (2020) discloses the potential therapeutic utility of OPA1 in ameliorating mitochondrial dysfunction, demonstrating that OPA1 has the ability to protect spatial visual function in a murine model of retinal ganglion cell degeneration caused by mitochondrial dysfunction. US 11,459,584 discloses methods for treating hereditary optic neuropathy, such as ADOA, using human type II mitochondrial dynein-like GTPase enzymes. WO 2019/084050 discloses methods for regulating expression of OPA1, namely, by way of modulating mRNA splicing. WO 2022/067398 discloses antisense oligomers for modulating mRNA translation of endogenous OPA1. WO 2022/074396 discloses compositions for upregulating OPA1 gene expression, namely, nucleic acid molecules that contain (1) a sequence reverse complementary to an OPA1 mRNA molecule, and (2) a downstream regulatory sequence.

Despite these disclosures, there remains a need in the art for improved approaches for the treatment of human patients diagnosed with or displaying one or more symptoms of ADOA.

Summary of the Invention

In a first aspect, the disclosure features a polynucleotide encoding optic atrophy 1 (OPA1), wherein the polynucleotide has a nucleic acid sequence that is at least 85% identical (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9%

identical, or more) to the nucleic acid sequence of any one of SEQ ID NOs: 3-5. In some embodiments, the polynucleotide has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 3. In some
5 embodiments, the polynucleotide has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide has a nucleic acid sequence that is at least 96% identical to the nucleic acid sequence of SEQ ID NO: 3. In some
10 embodiments, the polynucleotide has a nucleic acid sequence that is at least 97% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide has a nucleic acid
sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 3. In some
embodiments, the polynucleotide has a nucleic acid sequence that is at least 99% identical to the
nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide has the nucleic
acid sequence of SEQ ID NO: 3.

In some embodiments, the polynucleotide has a nucleic acid sequence that is at least 85%
15 identical to the nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide
has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO:
4. In some embodiments, the polynucleotide has a nucleic acid sequence that is at least 95% identical
to the nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide has a
nucleic acid sequence that is at least 96% identical to the nucleic acid sequence of SEQ ID NO: 4. In
20 some embodiments, the polynucleotide has a nucleic acid sequence that is at least 97% identical to
the nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide has a nucleic
acid sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 4. In some
embodiments, the polynucleotide has a nucleic acid sequence that is at least 99% identical to the
nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide has the nucleic
25 acid sequence of SEQ ID NO: 4.

In some embodiments, the polynucleotide has a nucleic acid sequence that is at least 85%
identical to the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide
has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO:
5. In some embodiments, the polynucleotide has a nucleic acid sequence that is at least 95% identical
30 to the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide has a
nucleic acid sequence that is at least 96% identical to the nucleic acid sequence of SEQ ID NO: 5. In
some embodiments, the polynucleotide has a nucleic acid sequence that is at least 97% identical to
the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide has a nucleic
acid sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 5. In some
35 embodiments, the polynucleotide has a nucleic acid sequence that is at least 99% identical to the
nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide has the nucleic
acid sequence of SEQ ID NO: 5.

In another aspect, the disclosure features a vector including the polynucleotide of the
foregoing aspect. The vector may be, for example, a viral vector or a non-viral vector. In some
40 embodiments, the vector is a viral vector. In some embodiments, the viral vector is selected from the

group consisting of an adeno-associated virus (AAV), an adenovirus, a lentivirus, a retrovirus, a poxvirus, a baculovirus, a herpes simplex virus, a vaccinia virus, and a synthetic virus. In some embodiments, the viral vector is an AAV. In some embodiments, the AAV includes capsid proteins from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, AAVrh74, and AAV2.7m8. In some embodiments, the AAV is a pseudotyped AAV. In some embodiments, the pseudotyped AAV is AAV2/8 or AAV2/9, optionally wherein the pseudotyped AAV is AAV2/8. In some embodiments, the AAV includes a recombinant capsid protein.

In some embodiments, the polynucleotide is operably linked to a promoter, optionally wherein the promoter is positioned 5' to the polynucleotide. In some embodiments, the promoter is a cytomegalovirus (CMV) promoter, cone-rod homeobox (CRX) promoter, retinal homeobox gene 1 (rx1) promoter, retinal pigment epithelium 65 (RPE65) promoter, CMV early enhancer/chicken beta actin (CAG, CBh, CBA) promoter, gamma-synuclein gene (SNCG) promoter, synapsin I (SYNI) promoter, rhodopsin kinase (GRK1) promoter, or a phosphoglycerate kinase (PGK) promoter.

In some embodiments, the promoter is a CMV promoter. In some embodiments, the CMV promoter has a nucleic acid sequence that is at least 85% identical (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identical, or more) to the nucleic acid sequence of SEQ ID NO: 20. In some embodiments, the CMV promoter has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 20. In some embodiments, CMV promoter has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 20, optionally wherein the CMV promoter has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of SEQ ID NO: 20. In some embodiments, the CMV promoter has the nucleic acid sequence of SEQ ID NO: 20.

In some embodiments, the polynucleotide is operably linked to a Woodchuck Hepatitis Virus (WHV) Posttranscriptional Regulatory Element (WPRE). In some embodiments, the WPRE has a nucleic acid sequence that is at least 85% identical (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identical, or more) to the nucleic acid sequence of SEQ ID NO: 10 or 11. In some embodiments, the WPRE has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 10 or 11. In some embodiments, the WPRE has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 10 or 11, optionally wherein the WPRE has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of SEQ ID NO: 10 or 11. In some embodiments, the WPRE has the nucleic acid sequence of SEQ ID NO: 10 or 11.

In some embodiments, the polynucleotide further includes a polyadenylation (PolyA) signal sequence. In some embodiments, the PolyA signal sequence includes the simian virus 40 (SV40) late PolyA signal sequence, the SV40 early PolyA signal sequence or human growth hormone PolyA signal sequence (hGH PolyA signal sequence), or a shortened hGH PolyA signal sequence. In some embodiments, the PolyA signal sequence includes the SV40 late PolyA signal sequence.

In some embodiments, the polynucleotide is comprised in a viral vector. The viral vector may be, for example, an AAV. The AAV may further include two inverted terminal repeats (ITRs), wherein

the two ITRs include a first ITR (ITR1) and a second ITR (ITR2), wherein ITR1 is positioned 5' to the polynucleotide and ITR2 is positioned 3' to the polynucleotide, thereby forming a cassette including the structure ITR1-OPA1-ITR2. In some embodiments, the two ITRs are AAV serotype 2 ITRs.

In another aspect, the disclosure features a plasmid encoding the viral vector comprising the polynucleotide of the foregoing aspect.

In another aspect, the disclosure features a pharmaceutical composition including the polynucleotide of any one of the foregoing aspects or the vector of any one of the foregoing aspects, and a pharmaceutically acceptable carrier, diluent, or excipient.

In another aspect, the disclosure features a nucleic acid molecule including: a CMV promoter (CMV), a codon-optimized transgene encoding an OPA1 protein (e.g., an OPA1 protein having an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 21) (OPA1), a WPRE, and a SV40 PolyA signal sequence (SV40 PolyA), wherein the components are operably linked to each other in the 5'-to-3' direction as CMV-OPA1-WPRE-SV40 PolyA.

In some embodiments, the CMV promoter has a nucleic acid sequence that is at least 85% identical (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identical, or more) to the nucleic acid sequence of SEQ ID NO: 20. In some embodiments, the CMV promoter has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 20. In some embodiments, the CMV promoter has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 20, optionally wherein the CMV promoter has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of SEQ ID NO: 20. In some embodiments, the CMV promoter has the nucleic acid sequence of SEQ ID NO: 20.

In some embodiments, the transgene encodes a protein having an amino acid sequence that is at least 85% identical (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identical, or more) to the amino acid sequence of SEQ ID NO: 21. In some embodiments, the transgene encodes a protein having an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 21. In some embodiments, the transgene encodes a protein having an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 21, optionally wherein the transgene encodes a protein having an amino acid sequence that is at least 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 21. In some embodiments, the transgene encodes a protein having the amino acid sequence of SEQ ID NO: 21.

In some embodiments, the transgene has a nucleic acid sequence that is at least 85% identical (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identical, or more) to the nucleic acid sequence of any one of SEQ ID Nos: 2-5. In some embodiments, the transgene has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of any one of SEQ ID Nos: 2-5. In some embodiments, the transgene has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of any one of SEQ ID Nos: 2-5, optionally wherein the transgene has a nucleic acid sequence that is at least 96%, 97%,

98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID Nos: 2-5. In some embodiments, the transgene has the nucleic acid sequence of any one of SEQ ID Nos: 2-5.

In some embodiments, the WPRE has a nucleic acid sequence that is at least 85% identical (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identical, or more) to the nucleic acid sequence of SEQ ID NO: 10 or 11. In some embodiments, the WPRE has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 10 or 11. In some embodiments, the WPRE has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 10 or 11, optionally wherein the WPRE has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of SEQ ID NO: 10 or 11. In some embodiments, the WPRE has the nucleic acid sequence of SEQ ID NO: 10 or 11.

In some embodiments, the SV40 PolyA signal sequence includes the SV40 late PolyA signal sequence or the SV40 early PolyA signal sequence. In some embodiments, the PolyA signal sequence includes the SV40 late PolyA signal sequence.

In another aspect, the disclosure features a vector including the nucleic acid molecule of the foregoing aspect. The vector may be, for example, a viral vector or a non-viral vector. In some embodiments, the vector is a viral vector. In some embodiments, the viral vector is selected from the group consisting of an AAV, an adenovirus, a lentivirus, a retrovirus, a poxvirus, a baculovirus, a herpes simplex virus, a vaccinia virus, and a synthetic virus. In some embodiments, the viral vector is an AAV. In some embodiments, the AAV includes capsid proteins from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, AAVrh74, and AAV2.7m8. In some embodiments, the AAV is a pseudotyped AAV. In some embodiments, the pseudotyped AAV is AAV2/8 or AAV2/9, optionally wherein the pseudotyped AAV is AAV2/8. In some embodiments, the AAV includes a recombinant capsid protein.

In some embodiments, the vector has a nucleic acid sequence that is at least 85% identical (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identical, or more) to the nucleic acid sequence of any one of SEQ ID NOs: 12-14. In some embodiments, the vector has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of any one of SEQ ID NOs: 12-14. In some embodiments, the vector has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of any one of SEQ ID NOs: 12-14, optionally wherein the vector has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 12-14. In some embodiments, the vector has the nucleic acid sequence of any one of SEQ ID NOs: 12-14.

In another aspect, the disclosure features a plasmid encoding the viral vector of the foregoing aspect. In some embodiments, the plasmid includes a promoter operably linked to a selectable marker gene. In some embodiments, the selectable marker gene is an antibiotic resistance gene.

In another aspect, the disclosure features a pharmaceutical composition including the nucleic acid molecule of any one of the foregoing aspects or the vector of any one of the foregoing aspects, and a pharmaceutically acceptable carrier, diluent, or excipient.

In another aspect, the disclosure features a method of treating a human patient diagnosed as having a neuro-ocular disorder or OPA1-deficiency disorder, the method including administering to the patient a therapeutically effective amount of the polynucleotide, the vector, the nucleic acid molecule, or the pharmaceutical composition of any one of the foregoing aspects.

5 In another aspect, the disclosure features a method of improving central and color vision in a human patient diagnosed as having a neuro-ocular disorder or OPA1-deficiency disorder, the method including administering to the patient a therapeutically effective amount of the polynucleotide, the vector, the nucleic acid molecule, or the pharmaceutical composition of any one of the foregoing aspects.

10 In another aspect, the disclosure features a method of improving retinal function in a human patient diagnosed as having a neuro-ocular disorder or OPA1-deficiency disorder, the method including administering to the patient a therapeutically effective amount of the polynucleotide, the vector, the nucleic acid molecule, or the pharmaceutical composition of any one of the foregoing aspects.

15 In another aspect, the disclosure features a method of improving visual acuity in a human patient diagnosed as having a neuro-ocular disorder or OPA1-deficiency disorder, the method including administering to the patient a therapeutically effective amount of the polynucleotide, the vector, the nucleic acid molecule, or the pharmaceutical composition of any one of the foregoing aspects.

20 In another aspect, the disclosure features a method of increasing the expression of OPA1 in a human patient diagnosed as having a neuro-ocular disorder or OPA1-deficiency disorder, the method including administering to the patient a therapeutically effective amount of the polynucleotide, the vector, the nucleic acid molecule, or the pharmaceutical composition of any one of the foregoing aspects.

25 In some embodiments of any one of the foregoing method aspects, the patient has impaired vision. In some embodiments, the patient has reduced visual acuity. In some embodiments, the patient has blindness. In some embodiments, the patient has retinal damage. In some embodiments, the patient has retinal degeneration. In some embodiments, the neuro-ocular disorder is an optic neuropathy. In some embodiments, the neuro-ocular disorder is a hereditary optic neuropathy. In
30 some embodiments, the OPA1-deficiency disorder is Autosomal Dominant Optic Atrophy (ADOA).

In another aspect, the disclosure features a method of ameliorating a symptom associated with a deficiency in OPA1 expression or activity in a human patient, the method including administering to the patient a therapeutically effective amount of the polynucleotide, the vector, the nucleic acid molecule, or the pharmaceutical composition of any one of the foregoing aspects. In
35 some embodiments, the symptom is selected from peripheral neuropathy, deafness, cerebellar ataxia, spastic paraparesis, and myopathy.

In another aspect, the disclosure features a kit including the polynucleotide, the vector, the nucleic acid molecule, or the pharmaceutical composition of any one of the foregoing aspects, and a package insert, wherein the package insert instructs a user of the kit to administer the polynucleotide,
40 nucleic acid, vector, or pharmaceutical composition to a human patient diagnosed as having a neuro-

ocular disorder, an OPA1-deficiency disorder, or a symptom associated with a deficiency in OPA1 expression or activity. In some embodiments, the symptom is selected from peripheral neuropathy, deafness, cerebellar ataxia, spastic paraparesis, and myopathy.

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Brief Description of the Drawings

FIG. 1 is a bar graph measuring expression levels of the OPA1 protein using western blot to quantify the expression ratio of OPA1 protein as expressed by each construct in Example 1 to GAPDH protein. Data were obtained by western blotting performed to visualize OPA1 protein expression levels in HEK293 cells after transfection with the plasmids. The measurement was made as described in Example 4, below. FIG. 1 abbreviations: OPA1: Optic Atrophy 1; pOPA1-A to I: plasmid OPA1-A to I; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

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Definitions

As used herein, the term “about” refers to a value that is within 10% above or below the value being described. For example, the phrase “about 100 nucleic acid residues” refers to a value of from 90 to 110 nucleic acid residues.

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As used herein, the terms “adeno-associated virus”, “AAV”, and the like refer to AAV vectors having one or more inverted terminal repeats (ITRs) and one or more capsid proteins of any AAV serotype. Exemplary AAV serotypes include, without limitation, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, snake AAV, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, and any other AAV now known or later discovered. See, e.g., Fields et al. *Virology*, 4th ed. Lippincott-Raven Publishers, Philadelphia, 1996. Additional AAV serotypes and clades have been identified recently. See, e.g., Gao et al. *J. Virol.* 78:6381 (2004); Moris et al. *Virol.* 33:375 (2004). The genomic sequences of various serotypes of AAV, as well as the sequences of the native ITRs, Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC—002077, NC—001401, NC—001729, NC—001863, NC—001829, NC—001862, NC—000883, NC—001701, NC—001510, NC—006152, NC—006261, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226, AY028223, AY631966, AX753250, EU285562, NC—001358, NC—001540, AF513851, AF513852 and AY530579; the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences. See also, e.g., Bantel-Schaal et al. *J. Virol.* 73:939 (1999); Chiorini et al. *J. Virol.* 71:6823 (1997); Chiorini et al. *J. Virol.* 73:1309 (1999); Gao et al. *Proc. Nat. Acad. Sci. USA* 99:11854 (2002); Moris et al. *Virol.* 33:375 (2004); Muramatsu et al. *Virol.* 221:208 (1996); Ruffing et al. *J. Gen. Virol.* 75:3385 (1994); Rutledge et al. *J. Virol.* 72:309 (1998); Schmidt et al. *J. Virol.* 82:8911 (2008); Shade et al. *J. Virol.* 58:921 (1986); Srivastava et al. *J. Virol.* 45:555 (1983); Xiao et al. *J. Virol.* 73:3994 (1999); WO 00/28061, WO 99/61601, WO 98/11244; and US 6,156,303; the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences.

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A “capsid protein” as used herein refers to any of the AAV capsid proteins that are components of AAV viral particles, including AAV8 and AAV9.

The term “codon” as used herein refers to any group of three consecutive nucleotide bases in a given messenger RNA molecule, or coding strand of DNA, that specifies a particular amino acid or a starting or stopping signal for translation. The term codon also refers to base triplets in a DNA strand.

As used herein, the term “codon optimization” refers a process of modifying a nucleic acid sequence in accordance with the principle that the frequency of occurrence of synonymous codons (e.g., codons that code for the same amino acid) in coding DNA is biased in different species. Such codon degeneracy allows an identical polypeptide to be encoded by a variety of nucleotide sequences. Sequences modified in this way are referred to herein as “codon-optimized” sequences. This process may be performed on any of the sequences described in this specification to enhance expression or stability. Codon optimization may be performed by any manner known in the art, such as, for example, that described in, e.g., U.S. Patent Nos. 7,561,972, 7,561,973, and 7,888,112, each of which is incorporated herein by reference in its entirety. The sequence surrounding the translational start site can be converted to a consensus Kozak sequence according to known methods. See, e.g., Kozak et al, *Nucleic Acids Res.* 15 (20): 8125-8148, incorporated herein by reference in its entirety. Multiple stop codons can be incorporated. Codon-optimized sequences are synthetic sequences, and preferably encode the identical polypeptide (or a biologically active fragment of a full-length polypeptide which has substantially the same activity as the full-length polypeptide) encoded by the non-codon-optimized parent polynucleotide.

Throughout the specification and claims, the word “comprise,” or variations such as “comprises” or “comprising,” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

An “inverted terminal repeat” or “ITR” is a palindromic nucleic acid, e.g., of from about 120 nucleotides to about 250 nucleotides in length, that is capable of forming a hairpin. The terms “inverted terminal repeat” and “ITR” include the site of the viral genome replication that can be recognized and bound by a parvoviral protein (e.g., Rep78/68). An ITR may be from any adeno-associated virus (AAV), for example, from AAV serotype 2, among others described herein. An ITR includes a replication protein binding element (RBE) and a terminal resolution sequence (TRS). The term “ITR” does not require a wild-type parvoviral ITR (e.g., a wild-type nucleic acid sequence may be altered by insertion, deletion, truncation, or missense mutations), as long as the ITR functions to mediate virus packaging, replication, integration, and/or provirus rescue, and the like. The term “5’ ITR” is intended to mean the parvoviral ITR located at the 5’ boundary of the nucleic acid molecule; similarly, the term “3’ ITR” is intended to mean the parvoviral ITR located at the 3’ boundary of the nucleic acid molecule.

As used herein, the “length” of a nucleic acid refers to the linear size of the nucleic acid as assessed by measuring the quantity of nucleotides from the 5’ to the 3’ end of the nucleic acid. Exemplary molecular biology techniques that may be used to determine the length of a nucleic acid of interest are known in the art.

As used herein, the term “operably linked” refers to a first molecule (e.g., a first nucleic acid) joined to a second molecule (e.g., a second nucleic acid), wherein the molecules are so arranged that the first molecule affects the function of the second molecule. The two molecules may or may not be part of a single contiguous molecule and may or may not be adjacent to one another. For example, a promoter is “operably linked” to a transcribable polynucleotide molecule if the promoter modulates transcription of the transcribable polynucleotide molecule of interest in target cells. Additionally, two portions of a transcription regulatory element are “operably linked” to one another if they are joined such that the transcription-activating functionality of one portion is not adversely affected by the presence of the other portion. Two transcription regulatory elements may be “operably linked” to one another by way of a linker nucleic acid (e.g., an intervening non-coding nucleic acid) or may be “operably linked” to one another with no intervening nucleotides present.

“Percent (%) sequence identity” with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values may be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a given nucleic acid or amino acid sequence, B) is calculated as follows:

$$100 \text{ multiplied by (the fraction } X/Y)$$

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program’s alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

As used herein, the term “pharmaceutical composition” refers to a mixture containing a therapeutic agent, such as a polynucleotide, a nucleic acid molecule or vector comprising the polynucleotide or the nucleic acid molecule described herein, optionally in combination with one or more pharmaceutically acceptable excipients, diluents, and/or carriers, to be administered to a subject, such as a mammal, e.g., a human, in order to prevent, treat or control a particular disease or condition affecting or that may affect the subject.

As used herein, the term “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms, which are suitable for contact with the tissues of a subject, such

as a mammal (e.g., a human) without excessive toxicity, irritation, allergic response, and other problem complications commensurate with a reasonable benefit/risk ratio.

As used herein, the terms “wild-type” and “non-mutant” form of a gene refer to a nucleic acid that encodes a protein associated with normal or non-pathogenic activity (e.g., a protein lacking a mutation that results in higher risk of developing, onset, or progression of a neurodegenerative disease).

As used herein, the term “mutation” refers to any change in the gene sequence, which may be passed onto subsequent generations (hereditary mutation) or not (somatic mutation) or any change in the amino acid sequence, for example, the substitution, insertion, or deletion of one or more amino acids. Gene mutations include the substitution, insertion, or deletion of a single base in a polynucleotide or the substitution, insertion, deletion, or rearrangement of multiple bases or larger sections of genes or chromosomes, including repeat expansions.

As used herein, “expression construct” refers to any type of genetic construct containing a nucleic acid (e.g., transgene) in which part or all the nucleic acid encoding sequence is capable of being transcribed. In some embodiments, expression includes transcription of the nucleic acid, for example, to generate a biologically active polypeptide product.

As used herein, the term “promoter” refers to a recognition site on DNA that is bound by an RNA polymerase. The polymerase drives transcription of the transgene. Exemplary promoters suitable for use with the compositions and methods described herein include ubiquitous promoters (e.g., a cytomegalovirus (CMV) promoter), among others described herein.

As used herein, the term “therapeutic protein” refers to (i) a protein whose deficiency or lack of activity is associated with a disorder (e.g., Optic Atrophy 1 (OPA1)), as well as (ii) a protein that is not necessarily deficient in a patient, but whose supplementation would nonetheless have a beneficial effect on the patient.

As used herein, the term “transgene” refers to an exogenous nucleic acid that has been transferred by genetic engineering means into another cell and is capable of being transcribed, and optionally translated.

As used herein, the terms “nucleic acid” and “polynucleotide” refer to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally-occurring nucleotides. As used herein, a polynucleotide encoding OPA1 may comprise DNA sequence encoding OPA1 and/or RNA equivalent thereof. As used herein, the term “RNA equivalent” refers to an RNA polynucleotide (i.e., a polynucleotide comprised of ribonucleotides or variants thereof) that encodes the same polypeptide sequence as is encoded by the corresponding DNA polynucleotide counterpart. RNA polynucleotides of the disclosure may include naturally occurring nucleosides and internucleoside linkages. Additionally or alternatively, RNA polynucleotides of the disclosure may include one or more chemically modified nucleosides or internucleoside linkages. For example, RNA polynucleotides of the disclosure may include a chemically modified nucleobase, such as one or more chemically modified forms of adenine, uracil, cytosine, and guanine.

As used herein, the term “endogenous” describes a molecule (e.g., a polypeptide, nucleic acid, or cofactor) that is found naturally in a particular organism (e.g., a human) or in a particular location within an organism (e.g., an organ, a tissue, or a cell, such as a human cell).

As used herein, the term “gene expression” refers to the process by which a nucleic acid is transcribed from a nucleic acid molecule, and often, translated into a peptide or protein. The process can include transcription, post-transcriptional control, post-transcriptional modification, translation, post-translational control, post translational modification, or any combination thereof. Reference to a measurement of “gene expression” may refer to measurement of the product of transcription (e.g., RNA or mRNA), the product of translation (e.g., peptides or proteins).

As used herein, the terms “subject” and “patient” refer to an organism that receives treatment for a particular disease or condition as described herein (such as a genetic disease described herein). The subject may be, for example, a patient suffering from a genetic disease, for example, an OPA1-deficiency disorder such as Autosomal Dominant Optic Atrophy (ADOA). As used herein, the term “OPA1-deficiency disorder” means a disorder or condition which is caused by mutations in the *OPA1* gene and shows at least one symptom selected from optic neuropathy, peripheral neuropathy, deafness, cerebellar ataxia, spastic paraparesis, and myopathy.

As used herein, the term “transcription regulatory element” refers to a nucleic acid that controls, at least in part, the transcription of a gene of interest. Transcription regulatory elements may include promoters, enhancers, and other nucleic acids (e.g., PolyA signal sequences) that control or help to control gene transcription. Examples of transcription regulatory elements are described, for example, in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego, CA, 1990).

As used herein, the terms “treat” and “treatment” refer to therapeutic treatment, in which the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of a genetic disease described herein.

As used herein, the term “vector” refers to a vehicle for the delivery of a gene of interest into a cell (e.g., a mammalian cell, such as a human cell), tissue, organ, or organism, such as a patient undergoing treatment for a disease or condition described herein, for purposes of expressing an encoded transgene. A vector can be non-viral or viral vector. Exemplary non-viral vectors are plasmids, closed-ended DNA (ceDNA), chromosomes, artificial chromosomes, naked nucleic acid (DNA or RNA) and lipid nanoparticles, such as lipid nanoparticles comprising a PEG-modified lipid, a non-cationic lipid, a sterol, an ionizable cationic lipid, or any combination thereof. Exemplary viral vectors are an adeno-associated virus (AAV), an adenovirus, a lentivirus, a retrovirus, a poxvirus, a baculovirus, a herpes simplex virus, a vaccinia virus, and a synthetic virus.

As used herein, “administration” refers to providing or giving a subject a therapeutic agent (e.g., a pharmaceutical composition containing a vector containing a polynucleotide that encodes OPA1), by any effective route. Exemplary routes of administration are described herein below.

As used herein, the terms “effective amount”, “therapeutically effective amount”, and a “sufficient amount” of a pharmaceutical composition described herein refer to a quantity sufficient to, when administered to the subject in need thereof, including a mammal, for example a human, effect

beneficial or desired results, including clinical results, and, as such, an “effective amount” or synonym thereto depends upon the context in which it is being applied. The amount of a given pharmaceutical composition described herein that will correspond to such an amount will vary depending upon various factors, such as the given agent, the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject (e.g., age, sex, weight) or host being treated, and the like, but can nevertheless be routinely determined by one skilled in the art. Also, as used herein, a “therapeutically effective amount” of a pharmaceutical composition of the present disclosure is an amount which results in a beneficial or desired result in a subject as compared to a control. Note that when a combination of active ingredients is administered, the effective amount of the combination may or may not include amounts of each ingredient that would have been effective if administered individually. As defined herein, a therapeutically effective amount of a pharmaceutical composition of the present disclosure may be readily determined by one of ordinary skill by routine methods known in the art. Dosage regime may be adjusted to provide the optimum therapeutic response.

As used herein, the terms “cytomegalovirus promoter” and “CMV promoter” refer to the cytomegalovirus immediate early enhancer/promoter. In some embodiments, the CMV promoter comprises the nucleic acid set forth in SEQ ID NO: 20, as well as nucleic acids having at least 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identity, or more) to the nucleic acid sequence of SEQ ID NO: 20 and that promote the expression of a transgene in target cells when the transgene is operably linked to the promoter. In some embodiments, the CMV promoter has the nucleic acid sequence of SEQ ID NO: 20.

As used herein, the terms “Optic Atrophy 1,” “OPA1,” and “OPA1 protein” refer to OPA1 mitochondrial dynamin like GTPase (e.g., NCBI Reference Sequence: NP_056375.2 and SEQ ID NO: 21). The term “OPA1” or “OPA1 gene” refers to a polynucleotide encoding the OPA1 protein. In some embodiments, the *OPA1* gene includes a wild-type *OPA1* gene (e.g., NCBI Reference Sequence: NC_000003.12:193593208-193697811 and SEQ ID NO: 2) or a variant thereof. Exemplary variants of wild-type *OPA1* are polynucleotides that have at least 85% nucleic acid sequence identity (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% nucleic acid sequence identity, or more) to the nucleic acid sequence of a wild-type *OPA1* gene (e.g., SEQ ID NO: 2) and/or that retain the therapeutic function of a wild-type OPA1 protein when translated in target cells. In some embodiments, an *OPA1* gene comprises a codon-optimized polynucleotide encoding an OPA1 protein, such as a codon-optimized polynucleotide having at least 85% nucleic acid sequence identity (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% nucleic acid sequence identity, or more) to the nucleic acid sequence of any one of SEQ ID NOs: 3-5. In some embodiments, the codon-optimized polynucleotide encoding an OPA1 protein has the nucleic acid sequence of any one of SEQ ID NOs: 3-5.

As used herein, the term “5' UTR” refers to the 5' untranslated region (also referred to as leader sequence, transcript leader, or leader RNA) in mRNA. It is placed right before the initiation codon and plays an important role in the regulation of translation. Exemplary 5' UTRs may include an internal ribosomal entry site (IRES), central polypurine tract (cPPT), RNA localization signal, small

RNA binding site, and RNA/RNA polymerase recognition site. Examples of 5' UTRs that may be used in conjunction with the pharmaceutical compositions and methods of the disclosure are those having the nucleic acid sequence of SEQ ID NO: 6 or 8.

As used herein, the term "3' UTR" refers to the 3' untranslated region in mRNA and is placed right after the translation termination codon. The 3' UTR may contain regulatory regions that affect gene expression in a post-transcriptional manner. Exemplary 3' UTRs may include a PolyA signal sequence and a WPRE. Examples of 3' UTRs that may be used in conjunction with the pharmaceutical compositions and methods of the disclosure are those having the nucleic acid sequence of any one of SEQ ID NOs: 7, 9, 10, and 11.

As used herein, the terms "woodchuck hepatitis virus posttranscriptional regulatory element" and "WPRE" refer to a DNA sequence, which when transcribed, creates a tertiary structure enhancing expression. The WPRE acts at the transcriptional level, by promoting nuclear export of transcripts and/or by increasing the efficiency of polyadenylation of the nascent transcript, thus increasing the total amount of mRNA in the cells. The addition of the WPRE may result in a substantial improvement in the level of transgene expression, both in vitro and in vivo. For example, "WPRE" elements that may be used herein include those having the nucleic acid sequence of SEQ ID NO: 10 or 11, as well as nucleic acids having at least 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identity, or more) to the nucleic acid sequences of SEQ ID NO: 10 or 11 and that promote the expression of a transgene in target cells. In some embodiments, the WPRE has the nucleic acid sequence of SEQ ID NO: 10 or 11.

As used herein, the term "ocular" refers to the eye and may designate a composition as being derived from, or related to, the eye.

As used herein, the terms "neuro-ocular disorder" and "neuro-visual disorder" refer to a disorder in which the neurons in the eye are affected, for example, a disorder caused by injury or damage to the retinal ganglion cells in the eye. Examples of neuro-ocular disorders include an optic neuropathy (for example, a hereditary optic neuropathy, such as ADOA) caused by mutations in the *OPA1* gene. In some instances, a neuro-ocular disorder may be associated with genetic defects (e.g., a mutation in the *OPA1* gene), protein misfolding, defects in protein degradation, programmed cell death, membrane damage, or other processes. Exemplary, non-limiting neuro-ocular disorders include optic neuropathy, ADOA, retinal degeneration, blindness, blindness-related disorders, retinopathy, optic neuropathies, optic neuritis, retinitis pigmentosa, chiasm disorders, giant cell (temporal) arteritis, eye movement disorders, double vision, nystagmus, oscillopsia, disorders of the pupils (for example, anisocoria), and temporary vision problems related to auras, among others.

As used herein, the term "central vision" refers to a subject's ability to see what is directly in front of the subject. One generally uses central vision to focus on the details of the things in the visual field. Central vision primarily relies upon the activity of cone cells.

As used herein, the term "color vision" refers to the ability of a subject to perceive color (i.e., the ability to perceive and distinguish between different wavelengths of light). Color vision primarily relies upon the activity of cone photoreceptors in the retina.

As used herein, the term “visual acuity” is a measure of the spatial resolution achieved by the eye (i.e., a measure of one’s sharpness and clarity of vision).

As used herein, the term “retinal degeneration” refers to the progressive loss of cells in the retina, for example, rod-cone degeneration leads to loss of rods and cones. Retinal degeneration can cause blindness.

As used herein, the term “optic neuropathy” refers to damage caused to the optic nerve, which carries visual information from the eye to the brain. Optic neuropathy can be hereditary in nature, for example, ADOA is a common hereditary optic neuropathy which can be caused by mutations in the *OPA1* gene. Optic neuropathy can arise due to a variety of reasons such as infections (e.g., toxoplasmosis, herpes simplex), inflammatory diseases, neurological disorders, certain medication, dietary deficiencies, and toxins (e.g., methanol, alcohol, tobacco).

As used herein, the term “retinal damage” refers to any damage to the retina that can be caused by, for example, old age, light, trauma, and retinal disorders, among others. Retinal damage is a common problem of the eye and can cause vision loss or blindness.

As used herein, the term “retinal function” refers to the ability of the retina to convert the light that enters the eye into electrical signals that can be sent to the brain using the optic nerve so that a visual image can be formed.

Detailed Description

Described herein are pharmaceutical compositions and methods for the treatment of optic neuropathy (for example, Autosomal Dominant Optic Atrophy (ADOA)) and other diseases (e.g., systemic diseases) caused by mutations in the Optic Atrophy 1 (*OPA1*) gene in a subject (such as a mammalian subject, for example, a human). Using the pharmaceutical compositions and methods described herein, one can treat ADOA in a subject by administering a vector comprising a codon-optimized transgene encoding the *OPA1* protein. The sections that follow describe the compositions and methods useful for the treatment of ADOA in further detail.

The pharmaceutical compositions described herein may include nucleic acid molecules that include a transgene encoding *OPA1*, and methods for the delivery of this transgene into target cells to express the resulting protein *OPA1*. In some embodiments, the target cells are eukaryotic cells, such as mammalian cells. In some embodiments, the mammalian cells are human cells, non-human primate cells or mouse cells. In some embodiments, the human cells are neurons, retinal cells (e.g., retinal ganglion cells, retinal pigment epithelium, or photoreceptor cells), cells in the inner ear, cells in muscle tissues. Without being limited by mechanism, the pharmaceutical compositions described herein may ameliorate the negative effects of ADOA by increasing the expression of *OPA1* in human patients diagnosed with or displaying one or more symptoms of ADOA. For example, the pharmaceutical compositions and methods described herein may be used to treat neuro-ocular disorders, such as ADOA, or symptoms of such disorders, such as blindness or reduced visual acuity.

The nucleic acids described herein may be carried in a vector, such as a viral vector. For example, described herein are AAV vectors, such as pseudotyped AAV vectors (e.g., an AAV2/8 vector) containing a transgene encoding *OPA1* operably linked to a promoter, e.g., a CMV promoter.

The present invention is based, at least in part, on the discovery that a codon-optimized polynucleotide encoding the OPA1 protein leads to a highly efficient and robust expression of OPA1.

Using the pharmaceutical compositions and methods described herein, the expression of OPA1 can be increased in patients diagnosed with or displaying one or more symptoms of ADOA, such as blindness or reduced visual acuity. This change in gene expression and subsequent shift in protein expression can mitigate symptoms of ADOA and may ameliorate the disease.

Autosomal Dominant Optic Atrophy

Autosomal Dominant Optic Atrophy (ADOA) is a form of optic neuropathy which can be hereditary in nature, for example, because of a mutation in the *OPA1* gene. It is one of the most common forms of hereditary optic neuropathy. The symptoms of this disease include impaired vision, impaired color vision (color blindness), impaired central vision, central scotoma, reduced visual acuity, vision loss, and/or blindness and phenotypes such as retinal damage, retinal degeneration, retinal ganglion cell loss, optic nerve damage, among others. ADOA can cause blindness, vision loss or vision impairment, starting in childhood, thus, resulting in childhood blindness. ADOA causes bilateral optic neuropathy, but other neurological phenotypes include peripheral neuropathy, deafness, cerebellar ataxia, spastic paraparesis, and myopathy. Some of these phenotypes are extra-ocular in nature. DOA generally affects both eyes in a roughly symmetrical manner and causes a slowly progressive pattern of vision loss starting in childhood. Vision examinations in ADOA patients reveal scotomas (areas of impaired visual acuity) in the central visual fields with sparing of peripheral vision. Visual acuity loss tends to vary from mild to severe. Symptoms of ADOA are typically observable between four to six years of age but ADOA has been diagnosed even in patients as young as a one-year-old. In some cases, DOA is not readily observable until early adulthood. The severity of ADOA by adolescence reflects the overall level of visual function that the patient can expect to have for the rest of the adult life.

The *OPA1* gene is found on chromosome 3, region q28-qter. Most ADOA cases are due to mutations in the *OPA1* gene. OPA1 is a mitochondrial dynamin-like GTPase protein and is ubiquitously expressed. It plays an important role in mitochondrial fusion dynamics. It is required for forming protein complexes that provide structural support to the cristae junctions in mitochondria and for keeping cytochrome C within the lumen of the cristae. OPA1 also functionally interacts with the oxidative phosphorylation complexes. Since OPA1 is required for normal mitochondrial fusion, mutations in this protein cause inherited neuropathies because the nervous system has a high metabolic rate and therefore, the proper functioning of the mitochondria is important for its optimal activity. OPA1 is located on the inner mitochondrial membrane and protects cells from apoptosis by binding cytochrome C. Mutations in OPA1 cause mitochondrial respiratory dysfunction and lead to the loss of retinal ganglion cells, the axons of which form the optic nerve, which is in turn responsible for carrying visual information from the eye to the brain. The pharmaceutical compositions and methods described herein target this dysfunction by providing codon-optimized transgenes encoding the OPA1 protein, as well as nucleic acid molecules, expression cassettes, and vectors containing the same.

Exemplary Nucleic Acids*Transgene*

Nucleic acid molecules described herein include codon-optimized polynucleotides encoding OPA1. The nucleic acid molecules described herein may further contain a promoter, as operably linking a codon-optimized version of the *OPA1* gene to a promoter (e.g., CMV promoter) advantageously and robustly increases the OPA1 protein in target cells. The nucleic acid molecule may be carried in a vector to optimize delivery into target cells. In some embodiments, the polynucleotide encoding OPA1 comprises a wild-type *OPA1* (e.g., SEQ ID NO: 2) or a variant thereof, such as a polynucleotide having at least 85% sequence identity (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identity, or more) to the nucleic acid sequence of the wild-type *OPA1* gene and retaining the therapeutic function of the wild-type OPA1 protein when translated in target cells. In some embodiments, the *OPA1* gene has the nucleic acid sequence of SEQ ID NO: 2. SEQ ID NO: 2 is shown below:

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15  ATGTGGCGACTACGTCGGGCGCTGTGGCCTGTGAGGTCTGCCAGTCTTTAGTGAAACACAGCT
    CTGGAATAAAAGGAAGTTTACCACTACAAAACTACATCTGGTTTCACGAAGCATTTATCATTAC
    ATCATCCTACCTTAAAGCTTCAACGACCCCAATTAAGGACATCCTTTCAGCAGTTCTCTTCTCTGA
    CAAACCTTCCTTTACGTAAACTGAAATTCTCTCCAATTAATATGGCTACCAGCCTCGCAGGAATT
    TTTGGCCAGCAAGATTAGCTACGAGACTCTTAAACTTCGCTATCTCATACTAGGATCGGCTGTT
20  GGGGGTGGCTACACAGCCAAAAAGACTTTTGATCAGTGGAAGATATGATACCGGACCTTAGTG
    AATATAAATGGATTGTGCCTGACATTGTGTGGGAAATTGATGAGTATATCGATTTTGAGAAAATTA
    GAAAAGCCCTTCCTAGTTTCAAGACCTTGTAAGTTAGCACCAGACTTTGACAAGATTGTTGAA
    AGCCTTAGCTTATTGAAGGACTTTTTTACCTCAGGTTCTCCGGAAGAAACGGCGTTTAGAGCAAC
    AGATCGTGATCTGAAAGTGACAAGCATTTTAGAAAGGTGTCAGACAAAGAGAAAATTGACCAAC
25  TTCAGGAAGAACTTCTGCACACTCAGTTGAAGTATCAGAGAATCTTGGAACGATTAGAAAAGGAG
    AACAAAGAATTGAGAAAATTAGTATTGCAGAAAGATGACAAAGGCATTTCATCATAGAAAGCTTAAG
    AAATCTTTGATTGACATGTATTCTGAAGTTCTTGATGTTCTCTCTGATTATGATGCCAGTTATAATA
    CGCAAGATCATCTGCCACGGGTTGTTGTGGTTGGAGATCAGAGTGCTGGAAGACTAGTGTGTT
    GGAAATGATTGCCAAGCTCGAATATTCCCAAGAGGATCTGGGGAGATGATGACACGTTCTCCA
30  GTTAAGGTGACTCTGAGTGAAGGTCCTCACCATGTGGCCCTATTTAAAGATAGTTCTCGGGAGTT
    TGATCTTACCAAAGAAGAAGATCTTGAGCATTAAAGACATGAAATAGAACTTCGAATGAGGAAAA
    ATGTGAAAGAAGGCTGTACCGTTAGCCCTGAGACCATATCCTTAAATGTAAAAGGCCCTGGACTA
    CAGAGGATGGTGCTTGTTGACTTACCAGGTGTGATTAATACTGTGACATCAGGCATGGCTCCTGA
    CACAAAGGAACTATTTTCAGTATCAGCAAAGCTTACATGCAGAATCCTAATGCCATCATACTGTG
35  TATTCAAGATGGATCTGTGGATGCTGAACGCAGTATTGTTACAGACTTGGTCAGTCAAATGGACC
    CTCATGGAAGGAGAACCATATTGTTTTGACCAAAGTAGACCTGGCAGAGAAAAATGTAGCCAGT
    CCAAGCAGGATTCAGCAGATAATTGAAGGAAAGCTCTTCCCAATGAAAGCTTTAGGTTATTTTGC
    TGTGTAAACAGGAAAAGGGAACAGCTCTGAAAGCATTGAAGCTATAAGAGAATATGAAGAAGAGT
    TTTTTCAGAATTCAAAGCTCCTAAAGACAAGCATGCTAAAGGCACACCAAGTGAAGTACAAGAAATT
40  TAAGCCTTGAGTATCAGACTGCTTTTGAAAATGGTACGAGAGTCTGTTGAACAACAGGCTGAT

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AGTTTCAAAGCAACACGTTTTAACCTTGAACTGAATGGAAGAATAACTATCCTCGCCTGCGGGA
 ACTTGACCGGAATGAACTATTTGAAAAAGCTAAAAATGAAATCCTTGATGAAGTTATCAGTCTGAG
 CCAGGTTACACCAAAACATTGGGAGGAAATCCTTCAACAATCTTTGTGGGAAAGAGTATCAACTC
 ATGTGATTGAAAACATCTACCTTCCAGCTGCGCAGACCATGAATTCAGGAACTTTTAACACCACA
 5 GTGGATATCAAGCTTAAACAGTGGACTGATAAACAACTTCCTAATAAAGCAGTAGAGGTTGCTTG
 GGAGACCCTACAAGAAGAATTTTCCCGCTTTATGACAGAACCGAAAGGGAAAGAGCATGATGAC
 ATATTTGATAAACTTAAAGAGGCTGTTAAGGAAGAAAGTATTAACGACACAAGTGGAATGACTTT
 GCGGAGGACAGCTTGAGGGTTATTCAACACAATGCTTTGGAAGACCGATCCATATCTGATAAACA
 GCAATGGGATGCAGCTATTTATTTTATGGAAGAGGCTCTGCAGGCTCGTCTCAAGGATACTGAAA
 10 ATGCAATTGAAAACATGGTGGGTCCAGACTGAAAAAGAGGTGGTTATACTGGAAGAATCGGAC
 CCAAGAACAGTGTGTTCACAATGAAACCAAGAATGAATTGGAGAAGATGTTGAAATGTAATGAGG
 AGCACCAGCTTATCTTGCAAGTGATGAAATAACCACAGTCCGGAAGAACCTTGAATCCCGAGG
 AGTAGAAGTAGATCCAAGCTTGATTAAGGATACTTGGCATCAAGTTTATAGAAGACATTTTTTAAA
 AACAGCTCTAAACCATTGTAACCTTTGTCGAAGAGGTTTTTATTACTACCAAAGGCATTTTGTAGA
 15 TTCTGAGTTGGAATGCAATGATGTGGTCTTGTTTTGGCGTATACAGCGCATGCTTGCTATCACCG
 CAAATACTTTAAGGCAACAACCTTACAAATACTGAAGTTAGGCGATTAGAGAAAAATGTTAAAGAGG
 TATTGGAAGATTTTGCTGAAGATGGTGAGAAGAAGATTAAATTGCTTACTGGTAAACGCGTTCAA
 CTGGCGGAAGACCTCAAGAAAGTTAGAGAAATTCAAGAAAACTTGATGCTTTCATTGAAGCTCT
 TCATCAGGAGAAATAA

In some embodiments, the *OPA1* gene comprises codon-optimized polynucleotides encoding the OPA1 protein. Exemplary codon-optimized polynucleotides encoding OPA1 are shown in Table 1, below.

Table 1. Exemplary polynucleotides encoding OPA1

SEQ ID NO.	Name	Nucleic Acid Sequence
3	OPA1-B	ATGTGGCGACTTAGAAGGGCTGCCGTGGCCTGTGAAGTGTGCCAGT CTCTGGTCAAGCACAGCAGCGGCATCAAGGGATCTCTGCCCCTGCA GAAGCTGCACCTGGTTTCCAGATCCATCTACCACAGCCACCATCCTA CACTGAAGCTGCAGAGGCCCCAGCTGAGAACCAGCTTCCAGCAGTT TAGCAGCCTGACCAACCTGCCTCTGAGAAAGCTGAAGTTCAGCCCC ATTAAGTACGGCTACCAGCCTCGGCGGAACCTTCTGGCCTGCTAGAC TGGCTACCAGGCTGCTGAAGCTGAGATACCTGATCCTGGGCTCTGC CGTCGGCGGAGGCTATACCGCCAAGAAAACCTTCGACCAGTGGAAG GACATGATCCCCGACCTGAGCGAGTACAAGTGATCGTGCCCGACA TCGTGTGGGAGATCGACGAGTACATCGACTTCGAGAAGATCCGGAA GGCCCTGCCTAGCAGCGAGGATCTGGTTAAGCTGGCCCCCTGATTT GACAAGATCGTGGAAGCCTGAGCCTGCTGAAGGACTTCTTCACCA GCGGCAGCCCAGAGGAAACCGCCTTTAGAGCCACAGACAGAGGCA

SEQ ID NO.	Name	Nucleic Acid Sequence
		GCGAGAGCGACAAGCACTTCAGAAAGGTGTCCGACAAAGAGAAGAT CGACCAGCTGCAAGAGGAACTGCTGCACACCCAGCTGAAGTACCAG AGAATCCTGGAACGGCTGGAAAAAGAGAACAAAGAACTGCGCAAGC TGGTGCTGCAGAAGGACGACAAGGGCATCCACCACCGGAAGCTGA AGAAAAGCCTGATCGACATGTACAGCGAGGTGCTGGACGTGCTGAG CGACTACGACGCCAGCTACAACACACAGGACCATCTGCCTAGAGTG GTGGTCGTGGGCGATCAGTCTGCCGGCAAAACAAGCGTGCTGGAAA TGATTGCCCAGGCCAGGATCTTCCCTAGAGGCTCCGGCGAGATGAT GACCAGATCTCCCGTGAAAGTGACCCTGTCCGAGGGACCTCATCAC GTGGCCCTGTTCAAGGACAGCTCCAGAGAGTTGACCTGACCAAAG AAGAGGACCTGGCCGCTCTGAGACACGAGATCGAGCTGCGGATGC GGAAGAACGTGAAAGAGGGCTGCACAGTCAGCCCCGAGACAATCA GCCTGAATGTGAAAGGCCAGGCCTGCAGAGAATGGTGCTGGTTGA TCTGCCCGGCGTGATCAACACAGTGACCTCTGGAATGGCCCCAGAC ACCAAAGAGACAATCTTCAGCATCAGCAAGGCCTACATGCAGAACC CCAACGCCATCATCCTGTGTATTGAGGACGGCAGCGTTGACGCCGA GAGATCCATCGTGACCGATCTGGTGTCCCAGATGGACCCTCATGGC AGACGGACCATCTTCGTGCTGACCAAGGTGGACCTGGCTGAGAAGA ATGTGGCTAGCCCCAGCAGAATCCAGCAGATCATCGAGGGCAAGCT GTTCCCCATGAAGGCTCTGGGCTACTTCGCCGTGGTTACCGGCAAG GGCAATAGCTCCGAGAGCATCGAGGCCATCAGAGAGTACGAGGAA GAGTTCTTCCAGAACAGCAAGCTGCTGAAAACCTCCATGCTGAAGG CCCACCAAGTGACCACCAGAAATCTGAGCCTGGCCGTGTCCGACTG CTTCTGGAAAATGGTCCGAGAGAGCGTGGAACAGCAGGCCGACAG CTTTAAGGCCACCAGATTCAACCTGGAACCGAGTGGAAGAACAAC ACCCAGACTGAGAGAGCTGGACCGGAACGAGCTGTTGAGAAGG CCAAGAACGAGATCCTGGACGAAGTGATCTCCCTGAGCCAAGTGAC ACCCAAGCACTGGGAAGAGATCCTGCAGCAGAGCCTGTGGGAGCG CGTTAGCACACACGTGATCGAGAACATCTACCTGCCTGCCGCTCAG ACCATGAACAGCGGCACCTTCAACACCACCGTGACATCAAGCTGA AGCAGTGGACCGACAAGCAGCTGCCCAACAAGGCTGTGGAAGTGG CCTGGGAGACACTGCAAGAAGAGTTCAGCCGTTTCATGACCGAGCC TAAGGGCAAAGAGCACGACGACATCTTCGACAAGCTGAAAGAAGCC GTGAAAGAAGAGAGCATCAAGCGGCACAAGTGGAACGACTTCGCCG AGGACAGCCTGAGAGTGATCCAGCACACGCCCTGGAAGATCGGA GCATCAGCGATAAGCAGCAGTGGGACGCCGCCATCTACTTCATGGA AGAAGCCCTGCAGGCCCGCCTGAAGGATACCGAGAATGCCATCGA GAATATGGTCGGACCCGACTGGAAGAAGAGATGGCTGTACTGGAAA

SEQ ID NO.	Name	Nucleic Acid Sequence
		AACAGGACCCAAGAGCAGTGCGTGCACAACGAGACAAAGAATGAGC TGGAAAAGATGCTCAAGTGCAACGAGGAACACCCCGCCTACCTGGC CTCTGATGAGATCACAACCGTGCGCAAGAACCTGGAATCCAGAGGC GTTGAGGTGGACCCAGCCTGATCAAGGATACCTGGCACCAGGTGT ACCGGCGGCACTTCCTGAAAACAGCCCTGAACCACTGCAACCTGTG CAGACGGGGCTTCTACTACTACCAGAGACACTTCGTGGACAGCGAG CTGGAATGCAACGACGTGGTGCTGTTTTGGCGCATCCAGCGGATGC TGGCCATCACCGCCAATACTCTCAGACAGCAGCTGACAAACACCGA AGTGCGGCGGCTCGAGAAAAACGTCAAAGAGGTGCTCGAGGATTTT GCCGAGGACGGCGAGAAGAAGATCAAAGTCTGACCGGCAAGCGG GTGCAGCTGGCCGAAGATCTGAAGAAAGTGCGCGAGATCCAAGAGA AGCTGGACGCCTTTATCGAGGCCCTGCACCAAGAGAAATGA

4	OPA1-C	ATGTGGCGGCTGCGCAGGGCAGCTGTGGCCTGTGAGGTGTGCCAG AGCCTGGTGAAGCACAGCTCTGGAATCAAGGGCAGCCTGCCTCTGC AGAAGCTGCACCTGGTGTCCAGAAGCATCTACCACTCTCACCACCC CACCTGAAGCTGCAGAGACCTCAGCTGAGAACCTCTTTCCAGCAG TTCTCTTCTCTGACCAACCTGCCTCTGAGGAAGCTGAAGTTCTCTCC CATCAAGTATGGATACCAGCCTAGAAGAACTTCTGGCCTGCCAGAC TGGCCACCAGACTGCTGAAGCTGAGATACCTGATCCTGGGCTCTGC TGTGGGAGGAGGCTACACAGCCAAGAAGACCTTTGACCAGTGGAAG GACATGATTCTGACCTGAGTGAATACAAGTGGATTGTGCCAGACAT TGTGTGGGAAATTGATGAATACATTGACTTTGAAAAGATCAGAAAGG CACTGCCTAGCTCAGAAGACCTGGTGAAGCTGGCTCCAGACTTTGA CAAGATTGTGGAATCTCTGTCTCTGCTGAAGGACTTCTTCACCTCTG GATCTCCAGAAGAGACAGCCTTCAGAGCCACAGACAGAGGCAGTGA AAGTGACAAGCACTTCAGGAAGGTGTCTGACAAGGAGAAGATTGAC CAGCTGCAGGAAGAGCTGCTGCACACACAGCTGAAGTACCAGAGAA TCCTGGAGAGACTGGAGAAGGAAAACAAGGAAGTGAAGGAGCTGGT TCTGCAGAAGGATGACAAAGGAATTCATCACAGGAAGCTGAAGAAG TCTCTGATTGACATGTACAGTGAAGTGCTGGATGTGCTGTCTGACTA TGATGCCTCTTACAACACTCAGGACCACCTGCCCAGAGTGGTGGTG GTTGGAGACCAGAGTGCAGGAAAGACCTCTGTGCTGGAAATGATTG CTCAGGCAAGAATCTTCCCAGAGGCTCTGGAGAAATGATGACAAG ATCTCCTGTGAAGGTGACTCTGTCTGAAGGACCTCACCATGTGGCTC TGTTCAAGGACAGCAGCAGAGAATTTGACCTGACCAAGGAAGAAGA CCTGGCTGCTCTGAGACATGAAATTGAACTGAGAATGAGGAAGAAT GTGAAGGAAGGCTGCACTGTGTCTCCAGAAACCATCTCTCTGAATGT GAAGGGACCTGGACTGCAGAGGATGGTGCTGGTGGATCTTCCTGGA GTCATCAACACTGTGACCTCTGGAATGGCTCCAGACACCAAGGAAA CCATCTTCTCCATCAGCAAAGCCTACATGCAGAACCCAAATGCCATC ATTCTGTGTATTCAAGATGGCTCTGTGGATGCAGAGAGAAGCATTGT GACAGACCTGGTGTCTCAGATGGACCCGCATGGAAGAAGAACCATC TTTGTGCTGACCAAAGTGGACCTGGCAGAGAAGAATGTGGCATCTC CTAGCAGAATCCAGCAGATCATTGAAGGAAAGCTGTTCCCATGAAG GCTCTGGGCTACTTTGCTGTGGTGACAGGAAAAGGAAACAGCTCAG AAAGCATTGAAGCCATCAGAGAATATGAAGAAGAATTCTTCCAGAAC AGCAAGCTGCTGAAGACCAGCATGCTGAAGGCTCACCAGGTGACCA CCAGGAACCTGTCTCTGGCTGTGTCTGACTGCTTCTGGAAGATGGT GAGAGAATCTGTGGAACAGCAAGCAGACAGCTTCAAGGCCACAAGA TTCAACCTGGAAACAGAATGGAAGAACAACCTACCCAGACTGAGAG AACTGGACAGAAATGAGCTGTTTGAAAAGGCAAAGAATGAAATCCTG
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SEQ ID NO.	Name	Nucleic Acid Sequence
		GATGAAGTGATCTCTCTGTCTCAGGTGACTCCCAAGCACTGGGAAG AAATCCTGCAGCAGAGCCTGTGGGAAAGAGTGTCCACTCATGTCATT GAAAACATCTACCTGCCTGCAGCTCAGACCATGAACTCTGGAACCTT CAACACCACTGTGGACATCAAGCTGAAGCAGTGGACAGACAAGCAG CTGCCCCAACAAGGCTGTGGAAGTGGCCTGGGAAACACTGCAGGAA GAGTTCTCTAGATTCATGACAGAACCCAAAGGAAAGGAACATGATGA CATCTTTGACAAGCTGAAGGAAGCTGTGAAGGAAGAAAGCATCAAG AGACACAAGTGGAATGACTTTGCAGAAGACTCTCTGAGAGTCATCCA GCACAATGCTCTGGAAGACAGAAGCATCAGTGACAAGCAGCAGTGG GATGCTGCCATCTACTTCATGGAAGAAGCTCTGCAGGCCAGACTGA AGGACACAGAAAATGCCATTGAAAACATGGTGGGACCAGACTGGAA GAAGAGATGGCTGTACTGGAAGAACAGAACACAGGAGCAGTGTGTG CACAATGAAACCAAGAATGAGCTGGAGAAGATGCTGAAGTGCAATG AAGAGCACCTGCCTACCTGGCCTCAGATGAAATCACCACAGTGAG GAAGAACCTGGAGAGCAGAGGAGTGAAGTGGACCCCTCTCTGATC AAGGACACCTGGCACCAGGTGTACAGGAGACACTTCCTGAAGACAG CCCTGAACCACTGCAACCTGTGCCGCAGAGGCTTCTACTACTACCA GAGACACTTTGTGGACTCTGAGCTGGAGTGCAATGATGTGGTGCTC TTCTGGAGAATCCAGAGGATGCTGGCCATCACAGCCAACACCCTGA GGCAGCAGCTGACCAACACAGAAGTGAGGAGACTGGAGAAGAATGT GAAGGAAGTGCTGGAAGACTTTGCAGAAGATGGAGAGAAGAAGATC AAGCTGCTGACAGGGAAGAGAGTGCAGCTGGCAGAAGACCTGAAG AAGGTGAGAGAAATCCAGGAGAAGCTGGATGCCTTCATTGAAGCTC TGCACCAGGAGAAGTAA
5	OPA1-D	ATGTGGAGGCTGAGGAGAGCTGCTGTGGCATGCGAGGTGTGTCAG AGCCTGGTGAAGCACTCCTCCGGCATCAAGGGGAGCCTGCCTCTCC AGAAGCTGCACCTGGTGTCCCGGTCTATCTATCATTCTCACCACCCA ACACTGAAATTGCAGCGGCCCCAGCTGAGGACCTCTTTCCAGCAGT TTAGCAGTCTGACCAATCTGCCCCCTGAGAAAGCTGAAATTCTCTCCA ATTAAATATGGATACCAGCCTCGCAGAACTTCTGGCCTGCCAGGCT GGCCACAAGGCTGCTGAACTGAGATACTTAATCCTGGGATCTGCC GTGGGGGGCGGATACACAGCCAAAAAACCTTCGACCAGTGGAAG GACATGATCCCCGATCTGTGAGAGTACAAATGGATTGTGCCCCGACAT CGTGTGGGAGATCGACGAGTACATCGACTTCGAGAAGATCCGGAAG GCCCTGCCTTCTTCAGAGGATCTGGTCAAGCTGGCGCCAGACTTCG ATAAGATCGTGGAAAGCCTGAGTTTGCTGAAGGATTTTTTACATCT GGCTCCCCTGAAGAAACCGCTTCCGCGCCACCGACCGGGGTAGC GAGTCCGACAAACACTTCCGGAAGGTCAGCGACAAGGAGAAGATTG

SEQ ID NO.	Name	Nucleic Acid Sequence
		ACCAGCTGCAGGAAGAGCTGCTGCACACACAGCTGAAGTACCAAAG AATCCTCGAAAGGCTGAAAAAGGAGAATAAGGAACTGAGAAAAGCTC GTGCTGCAGAAGGACGATAAGGGCATCCACCATAGAAAGCTGAAGA AATCTCTGATCGACATGTACTCTGAGGTGCTGGACGTCCTGTCCGAT TACGACGCCTCCTACAATACCCAGGATCACCTGCCCAGGGTGGTGG TTGTGGGGGACCAGTCCGCCGAAAGACAAGCGTGCTGGAGATGA TCGCCCAGGCAAGGATCTTCCCCCGGGGAGCGGCGAAATGATGA CTAGATCTCCAGTGAAGGTGACCCTGAGCGAGGGGCCTCATCACGT GGCACTGTTCAAGGACAGCTCTAGAGAGTTTGATCTTACAAAGGAG GAAGACCTGGCTGCACTGAGGCACGAGATTGAACTGAGAATGAGGA AGAACGTGAAGGAGGGATGCACCGTGTCCCCAGAGACCATCTCTCT CAATGTGAAGGGACCCGGCCTGCAGCGCATGGTGCTGGTTGACCT GCCAGGCGTGATCAACACAGTGACCAGTGGCATGGCCCCCGACAC CAAGGAAACAATTTTTAGTATTTCTAAGGCCTACATGCAGAACCCAAA CGCCATCATCCTGTGCATCCAGGATGGCAGCGTGATGCTGAACGC TCTATTGTGACAGACCTGGTGTCTCAGATGGACCCTCACGGACGCC GGACCATCTTCGTGCTGACCAAAGTGGACCTGGCCGAGAAAAACGT GGCCAGCCCTAGCAGAATCCAGCAGATCATCGAGGGCAAGCTGTTT CCAATGAAGGCCCTCGGTTACTTTGCCGTGGTGACCGGCAAGGGGA ACTCATCAGAGAGTATCGAGGCCATCCGCGAATATGAGGAGGAGTT CTTTCAGAACTCAAACTGCTGAAAACCAAGTATGCTGAAGGCCACC AGGTGACAACCAGGAACCTTCCCTCGCCGTGTCAGACTGCTTCTG GAAGATGGTTTCGGGAAAGTGTGGAGCAGCAGGCCGACTCTTTCAG GCTACCCGCTTCAATCTGGAGACGGAGTGGAAGAATAACTATCCAA GACTCAGGGAGCTGGACCGCAACGAGCTGTTTGAGAAAGCTAAGAA TGAGATCCTGGATGAGGTGATTTCCCTGAGCCAGGTGACCCCCAAG CACTGGGAGGAAATCCTGCAGCAGTCTCTTTGGGAACGCGTGAGCA CTCACGTCAATTGAGAACATTTACCTGCCCCGCCGCCAGACCATGAA CAGCGGAACATTCAACACCACCGTGGATATTAAGCTCAAACAGTGGA CCGACAAGCAGCTGCCCAACAAGGCCGTGAGGTGGCCTGGGAGA CCCTGCAGGAAGAGTTCTCTAGGTTTCATGACCGAGCCCAAAGGAAA GGAGCACGACGATATTTTTGACAAGCTGAAGGAGGCAGTGAAGGAA GAGAGCATTAAACGGCATAAGTGAACGACTTCGCCGAGGACAGCC TGCGCGTGATCCAGCACAAACGCCCTGGAGGACCGAAGCATTTCTGA TAAGCAGCAGTGGGACGCTGCTATTTACTTCATGGAAGAGGCCCTG CAAGCCCGGCTGAAAGACACCGAAAACGCCATCGAGAACATGGTGG GCCCAGACTGGAAAAAAAGGTGGCTGTACTGGAAAAATAGGACACA GGAGCAGTGTGTGCACAACGAGACCAAAAATGAGCTGGAGAAGATG

SEQ ID NO.	Name	Nucleic Acid Sequence
		CTGAAATGTAATGAGGAACACCCAGCCTACCTGGCCAGCGACGAGA TTACCACCGTGAGGAAGAACCTGGAGAGCCGCGGCGTGAGGTGG ACCCTTCACTGATCAAGGACACCTGGCACCAGGTGTACAGACGGCA CTTCTCAAGACAGCACTGAACCATTGCAATCTGTGTAGGAGAGGCT TCTACTATTACCAGAGACACTTTGTGGACTCCGAGCTGGAGTGCAAC GACGTGGTGCTGTTTTGGAGAATCCAGCGCATGCTGGCCATCACCG CCAACACCCTGCGGCAGCAGCTGACAAATACAGAGGTGAGAAGGCT GGAGAAGAATGTGAAGGAGGTGCTCGAGGACTTTGCCGAGGACGG AGAGAAGAAGATCAAGCTGCTTACAGGCAAGAGAGTGCAGCTGGCC GAAGACCTGAAGAAAGTGCGCGAGATCCAGGAGAACTGGACGCCT TCATCGAGGCCCTGCACCAGGAAAAGTGA

In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of any one of SEQ ID NOs: 3-5 (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleic acid sequence of any one of SEQ ID NOs: 3-5).

In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 96% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 97% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the polynucleotide encoding OPA1 has the nucleic acid sequence of SEQ ID NO: 3.

In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 96% identical to the nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 97% identical to the nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 4.

4. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the polynucleotide encoding OPA1 has the nucleic acid sequence of SEQ ID NO: 4.

In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 96% identical to the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 97% identical to the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide encoding OPA1 has a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the polynucleotide encoding OPA1 has the nucleic acid sequence of SEQ ID NO: 5.

The OPA1 protein may be wild-type OPA1 protein (e.g., NCBI Reference Sequence: NP_056375.2 and SEQ ID NO: 21) or a variant of the wild-type OPA1 protein, e.g., a variant protein having at least 85% amino acid sequence identity (e.g., at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% amino acid sequence identity, or more) to the amino acid sequence of a wild-type OPA1 protein (e.g., SEQ ID NO: 21), provided that the OPA1 variant retains the therapeutic function of wild-type OPA1 protein when translated in target cells. In some embodiments, the OPA1 protein has the amino acid sequence of SEQ ID NO: 21. SEQ ID NO: 21 is shown below:

MWRLRRAAVACEVCQSLVKHSSGIKGSPLQLKLHLVSRSIYHSHHPTLKLQRPQLRTSFQQFSSLTN
LPLRKLKFSPIKYGYQPRNFWPARLATRLLKLRYLILGSAVGGGYTAKKTFDQWKDMIPDLSEYKWI
VPDIVWEIDEYIDFEKIRKALPSSSEDLVKLAPDFDKIVESLSLLKDFFTSGSPEETAFRATDRGSESDKH
FRKVSDKEKIDQLQEELLHTQLKYQRILERLEKENKELRKLVLQKDDKGIHHRKLKSLIDMYSEVLDV
LSDYDASYNTQDHLPRVVVVG DQSAGKTSVLEMAQARIFPRGSGEMMTRSPVKVTLSEGP HHVALF
KDSSREFDLTKEEDLAALRHEIELMRKNVKEGCTVSPETISLVKGPGLQRMVLVDLPGVINTVTSG
MAPDTKETIFSISKAYMQNPNAILCIQDGSVDAERSIVTDLV SQMDPHGRRTIFVLTKVDLA EKNVASP
SRIQQIIEGKLFPMKALGYFAVVTGKGNSSIESIAIREYEEFFQNSKLLKTSMLKAHQVTTTRNLSLAVS
DCFWMVRESVEQQADSFKATRFNLET EWKNNYPRLRELD RNELFEKAKNEILDEVISLSQVTPKHW
EEILQQSLWERVSTHVIENIYLPAAQTMNSGTFNTTVDIKLKQWTDKQLPNKAVEVAWETLQEEFSRF
MTEPKGKEHDDIFDKLKEAVKEESIKRHKW NDFAE DSLRVIQHNALED RSISDKQQWDAAIYME EAL
QARLKDTENAIENMVGPDWKKRWLYWKNRTQE QCVHNETKNELEKMLKCNEEHPAYLASDEITT VR
KNLESRGVEVDPSLIKDTWHQVYRRHFLKTALNHCNLCRRGFYYYQRHFVDSELECNDVWLFWRIQ
RMLAITANTLRQQLTNTTEVRRLEKNVKEVLEDFAEDGEKKIKLLTGKRVQLAEDLKKVREIQEKLDAFI
EALHQEK

Transcription Regulatory Elements

Polynucleotides encoding an OPA1 protein disclosed herein may include a regulatory element capable of modulating (i.e., increasing or attenuating) gene expression. For example, the polynucleotide or nucleic acid molecule of the disclosure may further include a transcription regulatory element operably linked to the polynucleotide encoding OPA1 so as to regulate gene transcription. In some embodiments, the polynucleotide or nucleic acid molecule of the disclosure can comprise promoters, enhancers, and other nucleic acids (e.g., PolyA signal sequences and WPRE), and so on.

Promoters that may be used in conjunction with the polynucleotides of the disclosure include, without limitation, a cytomegalovirus (CMV) promoter, cone-rod homeobox (CRX) promoter, retinal homeobox gene 1 (rx1) promoter, retinal pigment epithelium 65 (RPE65) promoter, CMV early enhancer/chicken beta actin (CAG, CBh) promoter, gamma-synuclein gene (SNCG) promoter, human rhodopsin kinase (GRK1) promoter, or a phosphoglycerate kinase (PGK) promoter, a synapsin (Syn) promoter, synapsin I (SYNI) promoter, a chicken β -actin promoter with cytomegalovirus enhancer elements (CB7), a tetracycline-controlled transactivator protein (tTA) promoter, a reverse tetracycline-controlled transactivator protein (rTA) promoter, a U1 promoter, a U6 promoter, a U7 promoter, a prion promoter, an H1 promoter, a CMV–chicken β -actin (CBA) promoter, a glial fibrillary acidic protein (GFAP) promoter, a calcium/calmodulin-dependent protein kinase III promoter, a tubulin alpha I promoter, a microtubulin-associated protein IB (MAP IB) promoter, a neuron-specific enolase promoter, a platelet-derived growth factor beta chain promoter, a neurofilament light chain promoter, a neuron-specific VGF gene promoter, a neuronal nuclei (NeuN) promoter, a adenomatous polyposis coli (APC) promoter, an ionized calcium-binding adapter molecule 1 (Iba-1) promoter, or a homeobox protein 9 (HB9) promoter, elongation factor 1-alpha (EF1 α) promoter, CD68 molecule (CD68) promoter (see Dahl et al., *Mol. Ther.* 23:835 (2015), incorporated herein by reference as it pertains to the use of PGK and CD68 promoters to express *GRN*), C-X3-C motif chemokine receptor 1 (CX3CR1) promoter, integrin subunit alpha M (ITGAM) promoter, allograft inflammatory factor 1 (AIF1) promoter, purinergic receptor P2Y12 (P2Y12) promoter, transmembrane protein 119 (TMEM119) promoter, and colony stimulating factor 1 receptor (CSF1R) promoter.

In some embodiments, the CMV promoter has the nucleic acid sequence set forth in SEQ ID NO: 20, or a nucleic acid sequence having at least 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identity, or more) to the nucleic acid sequence of SEQ ID NO: 20 and that promotes the expression of a transgene in target cells when the transgene is operably linked to the promoter. In some embodiments, the CMV promoter has the nucleic acid sequence of SEQ ID NO: 20. SEQ ID NO: 20 is shown below:

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TTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACT
TACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATGACGTCAATAATGACG
TATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTA
AACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATG
ACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCA
GTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGC
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GTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTT
 GTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCTGTAACAACCTCCGCCCCATTGACGCAAA
 TGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCT

Other DNA sequence elements that may be included in the polynucleotide or nucleic acid molecule of the disclosure are enhancer sequences. Enhancers represent another class of regulatory elements that induce a conformational change in the polynucleotide containing the gene of interest such that the DNA adopts a three-dimensional orientation that is favorable for binding of transcription factors and RNA polymerase at the transcription initiation site. Many enhancer sequences are now known from mammalian genes or derived from the genetic material of a virus capable of infecting a eukaryotic cell. Exemplary enhancers from mammalian genes are those from the genes that encode mammalian globin, elastase, albumin, α -fetoprotein, and insulin. Exemplary enhancers derived from the genetic material of a virus are the SV40 enhancer on the late side of the replication origin (bp 100-270), the CMV early promoter enhancer, the cytomegalovirus immediate early enhancer, the cytomegalovirus early enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. Additional enhancer sequences that induce activation of eukaryotic gene transcription are disclosed in Yaniv et al., *Nature* 297:17 (1982).

In some embodiments, the polynucleotide or nucleic acid molecule of the disclosure may include a WPRE. In some embodiments, the WPRE has the nucleic acid of SEQ ID NO: 10 or 11, or a nucleic acid having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% sequence identity, or more) to the nucleic acid sequence of SEQ ID NO: 10 or 11 and that promotes the expression of a transgene in target cells when the transgene is operably linked to the WPRE. In some embodiments, the WPRE has the nucleic acid sequence of SEQ ID NO: 10 or 11.

SEQ ID NOs: 10 (mutant WPRE (mtWPRE)) and 11 (short WPRE (WPRES)) are shown in Table 2, below:

Table 2. Exemplary WPRE sequences

SEQ ID NO.	Name	Nucleic Acid Sequence
10	mtWPRE	ATAATCAACCTCTGGATTACAAATTTGTGAAAGATTGACTGGTATTCTTAACT ATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATG CTATTGCTTCCCGTATGGCTTTTCAATTTCTCCTCCTTGATAAATCCTGGTTGC TGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTG CACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGT CAGCTCCTTTCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAAC TCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCA CTGACAATTCCGTGGTGTGTCGGGGAAATCATCGTCCTTTCTTGGCTGCTC GCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTT

		CGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGC GGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTG GGCCGCCTCCCCGCATC
11	WPRES	AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACAT GTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCT ATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGATAAATCCTGGTTAGTT CTTGCCACGGCGGAACATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGG GCTCGGCTGTTGGGCACTGACAATTCCGTGGTGT

In some embodiments, the polynucleotide or nucleic acid molecule of the disclosure may further include a PolyA signal sequence. Exemplary PolyA signal sequence include those known in the art and described in the working examples, below. In some embodiments, the PolyA signal sequence includes the simian virus 40 (SV40) late PolyA signal sequence, the SV40 early PolyA signal sequence, human growth hormone PolyA signal sequence (hGH PolyA signal sequence), or shortened hGH PolyA signal sequence. In some embodiments, the PolyA signal sequence includes the SV40 late PolyA signal sequence.

Untranslated regions (UTRs) other than WPRE and PolyA signal sequence also occur on either side of a coding sequence in an mRNA. In some embodiments, the polynucleotide or nucleic acid molecule of the disclosure may comprise 5' UTR and/or 3' UTR. Exemplary 5' UTRs include the nucleic acids set forth in any one of SEQ ID NO: 6 or 8, as well as nucleic acids having at least 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identity, or more) to the nucleic acid sequence of any one of SEQ ID NO: 6 or 8 and that regulate the translation in target cells. In some embodiments, the 5' UTR has the nucleic acid sequence of any one of SEQ ID NO: 6 or 8. Exemplary 3' UTRs include the nucleic acids set forth in any one of SEQ ID NO: 7 or 9, as well as nucleic acids having at least 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identity, or more) to the nucleic acid sequence of any one of SEQ ID NO: 7 or 9 and that regulate the translation in target cells. In some embodiments, the 3' UTR has the nucleic acid sequence of any one of SEQ ID NO: 7 or 9.

SEQ ID NOs: 6-9 are shown in Table 3, below:

Table 3. Exemplary other UTR sequences

SEQ ID NO:	UTR	Nucleic Acid Sequence
6	5' UTR (Alpha-1-globin)	TCTTCTGGTCCCCACAGACTCAGAGAGAACCC
7	3' UTR (Alpha-1-globin)	GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCTTGGGCCTCCC CCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCGTGGTCTT TGAATAAAGTCTGAGTGGGCGGCA

8	5' UTR (virus derived sequence)	AGAGGGCCCTGAAGAGGGCCCTTTATATATGCCTGCGTCGGTA CTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGGTGGAA CTGACGAGTTCGGAACACCCGGCCGCGACCCTGGGAGACGTCC CAGGGACTTCGGG
9	3' UTR (virus derived sequence)	GCCTGCGTCGGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGG ACCCGTGGTGGAACTGACGAGTTCGGAACACCCGGCCGCGACC CTGGGAGACGTCCCAGGGACTTCGGGATATATAAAGGGCCCTC TTCAGGGCCCTCT

Vectors

The polynucleotide or nucleic acid molecule of the disclosure may be carried in a vector. A vector can be non-viral or viral vector. Examples of non-viral vectors are plasmids, closed-ended DNA (ceDNA), chromosomes, artificial chromosomes, naked nucleic acid (DNA or RNA) and lipid nanoparticles. Examples of viral vectors are a retrovirus (e.g., Retroviridae family viral vector), adenovirus (e.g., Ad5, Ad26, Ad34, Ad35, and Ad48), parvovirus (e.g., adeno-associated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e.g., influenza virus), rhabdovirus (e.g., rabies and vesicular stomatitis virus), paramyxovirus (e.g., measles and Sendai), positive strand RNA viruses, such as picornavirus and alphavirus, and double stranded DNA viruses including adenovirus, herpesvirus (e.g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e.g., vaccinia, modified vaccinia Ankara (MVA), fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, human papilloma virus, human foamy virus, and hepatitis virus, for example. Examples of retroviruses are avian leukosis-sarcoma, avian C-type viruses, mammalian C-type, B-type viruses, D-type viruses, oncoretroviruses, HTLV-BLV group, lentivirus, alpharetrovirus, gammaretrovirus, spumavirus (Coffin, J. M., Retroviridae: The viruses and their replication, Virology, Third Edition (Lippincott-Raven, Philadelphia, (1996)). Other examples are murine leukemia viruses, murine sarcoma viruses, mouse mammary tumor virus, bovine leukemia virus, feline leukemia virus, feline sarcoma virus, avian leukemia virus, human T-cell leukemia virus, baboon endogenous virus, Gibbon ape leukemia virus, Mason Pfizer monkey virus, simian immunodeficiency virus, simian sarcoma virus, Rous sarcoma virus and lentiviruses. Other examples of vectors are described, for example, in McVey et al., (US 5,801,030), the teachings of which are incorporated herein by reference. In some embodiments, the viral vector is selected from the group consisting of an adeno-associated virus (AAV), an adenovirus, a lentivirus, a retrovirus, a poxvirus, a baculovirus, a herpes simplex virus, a vaccinia virus, and a synthetic virus. In some embodiments, the viral vector is an AAV.

Exemplary AAV Vectors for Transgene Delivery

AAV vectors that may be used in conjunction with the pharmaceutical compositions and methods of the disclosure include those having one or more of the naturally occurring AAV genes deleted in whole or in part but retain functional flanking ITR sequences. The AAV ITRs may be of any serotype (e.g., derived from serotype 2) suitable for a particular application. Methods for using AAV

vectors are described, for example, in Tal et al., J. Biomed. Sci. 7:279-291 (2000), and Monahan and Samulski, Gene Delivery 7:24-30 (2000), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

The polynucleotide or nucleic acid molecule of the disclosure can be incorporated into a AAV virion to facilitate introduction of the polynucleotide or nucleic acid molecule into a cell. The capsid proteins of AAV compose the exterior, non-nucleic acid portion of the virion and are encoded by the AAV cap gene. The cap gene encodes three viral coat proteins, VP1, VP2, and VP3, which are required for virion assembly. The construction of AAV virions has been described, for example, in US Patent Nos. 5,173,414; 5,139,941; 5,863,541; 5,869,305; 6,057,152; and 6,376,237; as well as in Rabinowitz et al., J. Virol. 76:791-801 (2002) and Bowles et al., J. Virol. 77:423-432 (2003), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

AAV virions useful in conjunction with the pharmaceutical compositions and methods described herein include those derived from a variety of AAV serotypes including AAV 1, 2, 3, 4, 5, 6, 7, 8 and 9. Construction and use of AAV vectors and AAV proteins of different serotypes are described, for example, in Chao et al., Mol. Ther. 2 :619-623 (2000); Davidson et al., Proc. Natl. Acad. Sci. USA 97 :3428-3432 (2000); Xiao et al., J. Virol. 72:2224-2232 (1998); Halbert et al., J. Virol. 74:1524-1532 (2000); Halbert et al., J. Virol. 75:6615-6624 (2001); and Auricchio et al., Hum. Molec. Genet. 10:3075-3081 (2001), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery. An additional example of an AAV virion that may be used in conjunction with the pharmaceutical compositions and methods of the disclosure is AAV2.7m8, which is disclosed, e.g., in WO 2012/145601 and US Patent No. 11,236,402, the disclosures of each of which are incorporated herein by reference.

Also useful in conjunction with the pharmaceutical compositions and methods described herein are pseudotyped AAV vectors. Pseudotyped vectors include AAV vectors of a given serotype (e.g., AAV2) pseudotyped with a capsid gene derived from a serotype other than the given serotype (e.g., AAV1, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9, among others). For example, a representative pseudotyped vector is an AAV2 vector encoding a therapeutic protein pseudotyped with a capsid gene derived from AAV serotype 8 or AAV serotype 9. Techniques involving the construction and use of pseudotyped AAV virions are known in the art and are described, for example, in Duan et al., J. Virol. 75:7662-7671 (2001); Halbert et al., J. Virol. 74:1524-1532 (2000); Zolotukhin et al., Methods, 28:158-167 (2002); and Auricchio et al., Hum. Molec. Genet., 10:3075-3081 (2001).

AAV virions that have mutations within the virion capsid may be used to infect particular cell types more effectively than non-mutated capsid virions. For example, suitable AAV mutants may have ligand insertion mutations for the facilitation of targeting AAV to specific cell types. The construction and characterization of AAV capsid mutants including insertion mutants, alanine screening mutants, and epitope tag mutants is described in Wu et al., J. Virol. 74:8635-45 (2000). Other AAV virions that can be used in methods of the disclosure include those capsid hybrids that are generated by

molecular breeding of viruses as well as by exon shuffling. See, e.g., Soong et al., Nat. Genet., 25:436-439 (2000) and Kolman and Stemmer, Nat. Biotechnol. 19:423-428 (2001).

Using the pharmaceutical compositions and methods of the disclosure, a patient diagnosed with or displaying one or more symptoms of optic neuropathy or OPA1-deficiency disorder, for example Autosomal Dominant Optic Atrophy (ADOA), such as a patient experiencing reduced visual acuity, may be administered a pseudotyped AAV2/8 vector including a polynucleotide encoding, from 5' to 3', a CMV promoter, a transgene encoding OPA1, a WPRE, and a simian virus 40 late PolyA signal sequence. In some embodiments, the CMV promoter has a nucleic acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the nucleic acid sequence of SEQ ID NO: 20 and that promotes the expression of the transgene in target cells. In some embodiments, the transgene encoding OPA1 has a nucleic acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the nucleic acid sequence of any one of SEQ ID NOs: 3-5. In some embodiments, the OPA1 protein has an amino acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 21 and retains the therapeutic function of wild-type OPA1 protein. In some embodiments, the WPRE has a nucleic acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the nucleic acid sequence of any one of SEQ ID NO: 10 or 11 and that promote the expression of a transgene in target cells. In some embodiments, the AAV vector comprises a nucleic acid sequence that is at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the nucleic acid sequence of any one of SEQ ID NOs: 12-14 to express the OPA1 protein in target cells.

Exemplary AAV vectors of the disclosure include those having the nucleic acid sequence of any one of SEQ ID NOs: 1 and 12-19. SEQ ID NOs: 1 and 12-19 are shown in Table 4, below.

Table 4. Exemplary sequences comprised in AAV vectors of the disclosure

SEQ ID NO.	Name	Nucleic Acid Sequence
1	Promoter-5' UTR-CDS(OPA1)-3' UTR-polyA signal (pOPA1-A)	TCGCGATGTACGGGCCAGATATACGCGTTTTAATAGTAATCAATTAC GGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAAC TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTG ACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACAT GACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCA TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT

SEQ ID NO.	Name	Nucleic Acid Sequence
		GGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTG ACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACCTCCGCCCCATTGACGCAAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGGATATCCTTAAGCACCGGTG CCACCATGTGGCGACTACGTCGGGCCGCTGTGGCCTGTGAGGTCT GCCAGTCTTTAGTGAAACACAGCTCTGGAATAAAAGGAAGTTACCA CTACAAAACCTACATCTGGTTTCACGAAGCATTATCATTACATCAT CCTACCTTAAAGCTTCAACGACCCCAATTAAGGACATCCTTTCAGCA GTTCTCTTCTCTGACAAACCTTCCTTTACGTAAACTGAAATTCTCTCC AATTAAATATGGCTACCAGCCTCGCAGGAATTTTTGGCCAGCAAGAT TAGCTACGAGACTCTTAAACCTTCGCTATCTCATACTAGGATCGGCT GTTGGGGGTGGCTACACAGCCAAAAGACTTTTGATCAGTGGAAG ATATGATACCGGACCTTAGTGAATATAAATGGATTGTGCCTGACATT GTGTGGGAAATTGATGAGTATATCGATTTTGAGAAAATTAGAAAAGC CCTTCCTAGTTCAGAAGACCTTGTAAGTTAGCACCAGACTTTGACA AGATTGTTGAAAGCCTTAGCTTATTGAAGGACTTTTTTACCTCAGGTT CTCCGGAAGAAACGGCGTTTAGAGCAACAGATCGTGGATCTGAAAG TGACAAGCATTTTAGAAAGGTGTCAGACAAAGAGAAAATTGACCAAC TTCAGGAAGAATTCTGCACACTCAGTTGAAGTATCAGAGAATCTTG GAACGATTAGAAAAGGAGAACAAAGAATTGAGAAAATTAGTATTGCA GAAAGATGACAAAGGCATTTCATCATAGAAAGCTTAAGAAATCTTTGA TTGACATGTATTCTGAAGTTCTTGATGTTCTCTCTGATTATGATGCCA GTTATAATACGCAAGATCATCTGCCACGGGTTGTTGTGGTTGGAGAT CAGAGTGCTGGAAAGACTAGTGTGTTGGAAATGATTGCCCAAGCTC GAATATTCCCAAGAGGATCTGGGGAGATGATGACACGTTCTCCAGTT AAGGTGACTCTGAGTGAAGGTCCTCACCATGTGGCCCTATTTAAAGA TAGTTCTCGGGAGTTTGATCTTACCAAAGAAGAAGATCTTGACAGCAT TAAGACATGAAATAGAACTTCGAATGAGGAAAAATGTGAAAGAAGGC TGTACCGTTAGCCCTGAGACCATATCCTTAAATGTAAAAGGCCCTGG ACTACAGAGGATGGTGCTTGTTGACTTACCAGGTGTGATTAATACTG TGACATCAGGCATGGCTCCTGACACAAAGGAACTATTTTCAGTATC AGCAAAGCTTACATGCAGAATCCTAATGCCATCATACTGTGTATTCA AGATGGATCTGTGGATGCTGAACGCAGTATTGTTACAGACTTGGTCA GTCAAATGGACCCTCATGGAAGGAGAACCATATTCGTTTTGACCAA

SEQ ID NO.	Name	Nucleic Acid Sequence
		GTAGACCTGGCAGAGAAAAATGTAGCCAGTCCAAGCAGGATTCAGC AGATAATTGAAGGAAAGCTCTTCCCAATGAAAGCTTTAGGTTATTTTG CTGTTGTAAACAGGAAAAAGGGAACAGCTCTGAAAGCATTGAAGCTATA AGAGAATATGAAGAAGAGTTTTTTTCAGAATTCAAAAGCTCCTAAAGAC AAGCATGCTAAAGGCACACCAAGTGACTACAAGAAATTTAAGCCTTG CAGTATCAGACTGCTTTTGGAAAAATGGTACGAGAGTCTGTTGAACAA CAGGCTGATAGTTTCAAAGCAACACGTTTTAACCTTGAAACTGAATG GAAGAATAACTATCCTCGCCTGCGGGAACCTTGACCGGAATGAACAT TTGAAAAAGCTAAAAATGAAATCCTTGATGAAGTTATCAGTCTGAGC CAGGTTACACCAAAACATTGGGAGGAAATCCTTCAACAATCTTTGTG GGAAAGAGTATCAACTCATGTGATTGAAAACATCTACCTTCCAGCTG CGCAGACCATGAATTCAGGAACTTTTAACACCACAGTGGATATCAAG CTTAAACAGTGGACTGATAAACAACCTTCCTAATAAAGCAGTAGAGGT TGCTTGGGAGACCCTACAAGAAGAATTTTCCCGCTTTATGACAGAAC CGAAAGGGAAAGAGCATGATGACATATTTGATAAACTTAAAGAGGCT GTTAAGGAAGAAAGTATTAAACGACACAAGTGGAAATGACTTTGCGGA GGACAGCTTGAGGGTTATTCAACACAATGCTTTGGAAGACCGATCCA TATCTGATAAACAGCAATGGGATGCAGCTATTTATTTTATGGAAGAG GCTCTGCAGGCTCGTCTCAAGGATACTGAAAATGCAATTGAAAACAT GGTGGGTCCAGACTGGAAAAAGAGGTGGTTATACTGGAAGAATCGG ACCCAAGAACAGTGTGTTCACAATGAAACCAAGAATGAATTGGAGAA GATGTTGAAATGTAATGAGGAGCACCAGCTTATCTTGCAAGTGATG AAATAACCACAGTCCGGAAGAACCTTGAATCCCGAGGAGTAGAAGT AGATCCAAGCTTGATTAAGGATACTTGGCATCAAGTTTATAGAAGAC ATTTTTTAAAAACAGCTCTAAACCATTGTAACCTTTGTGCAAGAGGTT TTTATTACTACCAAAGGCATTTTGTAGATTCTGAGTTGGAATGCAATG ATGTGGTCTTGTTTTGGCGTATACAGCGCATGCTTGCTATCACCGCA AATACTTTAAGGCAACAACCTTACAAATACTGAAGTTAGGCGATTAGA GAAAAATGTTAAAGAGGTATTGGAAGATTTTGTGTAAGATGGTGAGA AGAAGATTAAATTGCTTACTGGTAAACGCGTTCAACTGGCGGAAGAC CTCAAGAAAGTTAGAGAAATTCAAGAAAACTTGATGCTTTCATTGAA GCTCTTCATCAGGAGAAATAAGGATCCACTAGTCCAGTGTGGTGGA ATTCTGCAGATATCCAGCACAGTGGCGGCCGCTCGAGTCTAGAGGG CCCGTTTAAACCGCTGATCAGCCTCGAAAATAAAATACGAAATGAA ATAAAATACGAAATGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTC TAGGGGGTATCCCC

SEQ ID NO.	Name	Nucleic Acid Sequence
12	Promoter-5' UTR-CDS(OPA1)-3' UTR-polyA signal (pOPA1-B)	TCGCGATGTACGGGCCAGATATACGCGTTTTAATAGTAATCAATTAC GGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAAC TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTG ACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACAT GACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCA TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTG ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACTCCGCCCCATTGACGCAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGGATATCCTTAAGCACCGGTG CCACCATGTGGCGACTTAGAAGGGCTGCCGTGGCCTGTGAAGTGTG CCAGTCTCTGGTCAAGCACAGCAGCGGCATCAAGGGATCTCTGCCC CTGCAGAAGCTGCACCTGGTTTTCCAGATCCATCTACCACAGCCACC ATCCTACACTGAAGCTGCAGAGGCCCCAGCTGAGAACCAGCTTCCA GCAGTTTAGCAGCCTGACCAACCTGCCTCTGAGAAAGCTGAAGTTC AGCCCCATTAAGTACGGCTACCAGCCTCGGCGGAACTTCTGGCCTG CTAGACTGGCTACCAGGCTGCTGAAGCTGAGATACCTGATCCTGGG CTCTGCCGTCGGCGGAGGCTATACCGCCAAGAAAACCTTCGACCAG TGGAAGGACATGATCCCCGACCTGAGCGAGTACAAGTGGATCGTGC CCGACATCGTGTGGGAGATCGACGAGTACATCGACTTCGAGAAGAT CCGGAAGGCCCTGCCTAGCAGCGAGGATCTGGTTAAGCTGGCCCC TGATTTGACAAGATCGTGGAAGCCTGAGCCTGCTGAAGGACTTC TTCACCAGCGGCAGCCCAGAGGAAACCGCCTTTAGAGCCACAGACA GAGGCAGCGAGAGCGACAAGCACTTCAGAAAGGTGTCCGACAAAG AGAAGATCGACCAGCTGCAAGAGGAACTGCTGCACACCCAGCTGAA GTACCAGAGAATCCTGGAACGGCTGGAAAAAGAGAACAAAGAACTG CGCAAGCTGGTGTCTGCAGAAGGACGACAAGGGCATCCACCACCGG AAGCTGAAGAAAAGCCTGATCGACATGTACAGCGAGGTGCTGGACG TGCTGAGCGACTACGACGCCAGCTACAACACACAGGACCATCTGCC TAGAGTGGTGGTCTGTTGGCGATCAGTCTGCCGGCAAAACAAGCGT GCTGGAAATGATTGCCCAGGCCAGGATCTTCCCTAGAGGCTCCGGC

SEQ ID NO.	Name	Nucleic Acid Sequence
		GAGATGATGACCAGATCTCCCGTGAAAGTGACCCTGTCCGAGGGAC CTCATCACGTGGCCCTGTTCAAGGACAGCTCCAGAGAGTTGACCT GACCAAAGAAGAGGACCTGGCCGCTCTGAGACACGAGATCGAGCT GCGGATGCGGAAGAACGTGAAAGAGGGCTGCACAGTCAGCCCCGA GACAATCAGCCTGAATGTGAAAGGCCAGGCCTGCAGAGAATGGTG CTGGTTGATCTGCCCCGGCGTGATCAACACAGTGACCTCTGGAATGG CCCCAGACACCAAAGAGACAATCTTCAGCATCAGCAAGGCCTACAT GCAGAACCCCAACGCCATCATCCTGTGTATTGAGGACGGCAGCGTT GACGCCGAGAGATCCATCGTGACCGATCTGGTGTCCAGATGGACC CTCATGGCAGACGGACCATCTTCGTGCTGACCAAGGTGGACCTGGC TGAGAAGAATGTGGCTAGCCCCAGCAGAATCCAGCAGATCATCGAG GGCAAGCTGTTCCCATGAAGGCTCTGGGCTACTTCGCCGTGGTTA CCGGCAAGGGCAATAGCTCCGAGAGCATCGAGGCCATCAGAGAGT ACGAGGAAGAGTTCTTCAGAACAGCAAGCTGCTGAAAACCTCCAT GCTGAAGGCCACCAAGTGACCACCAGAAATCTGAGCCTGGCCGTG TCCGACTGCTTCTGGAAAATGGTCCGAGAGAGCGTGGAACAGCAGG CCGACAGCTTTAAGGCCACCAGATTCAACCTGGAAACCGAGTGAA GAACAACCTACCCAGACTGAGAGAGCTGGACCGGAACGAGCTGTTT GAGAAGGCCAAGAACGAGATCCTGGACGAAGTGATCTCCCTGAGCC AAGTGACACCCAAGCACTGGGAAGAGATCCTGCAGCAGAGCCTGTG GGAGCGCGTTAGCACACACGTGATCGAGAACATCTACCTGCCTGCC GCTCAGACCATGAACAGCGGCACCTTCAACACCACCGTGGACATCA AGCTGAAGCAGTGGACCGACAAGCAGCTGCCCAACAAGGCTGTGG AAGTGGCCTGGGAGACACTGCAAGAAGAGTTCAGCCGGTTCATGAC CGAGCCTAAGGGCAAAGAGCAGCAGCAGCATCTTCGACAAGCTGAAA GAAGCCGTGAAAGAAGAGAGCATCAAGCGGCACAAAGTGGAACGACT TCGCCGAGGACAGCCTGAGAGTGATCCAGCACAACGCCCTGGAAG ATCGGAGCATCAGCGATAAGCAGCAGTGGGACGCCGCCATCTACTT CATGGAAGAAGCCCTGCAGGCCCGCCTGAAGGATACCGAGAATGC CATCGAGAATATGGTCGGACCCGACTGGAAGAAGAGATGGCTGTAC TGAAAAACAGGACCCAAGAGCAGTGCCTGCACAACGAGACAAAGA ATGAGCTGGAAGAAGATGCTCAAGTGCAACGAGGAACACCCCGCCTA CCTGGCCTCTGATGAGATCACAACCGTGCGCAAGAACCTGGAATCC AGAGGCGTTGAGGTGGACCCAGCCTGATCAAGGATACCTGGCAC CAGGTGTACCGGCGGCACTTCTGAAAACAGCCCTGAACCACTGCA ACCTGTGCAGACGGGGCTTCTACTACTACCAGAGACACTTCGTGGA

SEQ ID NO.	Name	Nucleic Acid Sequence
		CAGCGAGCTGGAATGCAACGACGTGGTGCTGTTTTGGCGCATCCAG CGGATGCTGGCCATCACCGCCAATACTCTCAGACAGCAGCTGACAA ACACCGAAGTGCGGCGGCTCGAGAAAAACGTCAAAGAGGTGCTCG AGGATTTTCCGAGGACGGCGAGAAGAAGATCAAAGTCTGACCGG CAAGCGGGTGCAGCTGGCCGAAGATCTGAAGAAAGTGCGCGAGAT CCAAGAGAAGCTGGACGCCTTTATCGAGGCCCTGCACCAAGAGAAA TGAGGATCCACTAGTCCAGTGTGGTGAATTCTGCAGATATCCAGC ACAGTGGCGGCCGCTCGAGTCTAGAGGGCCCGTTTAAACCCGCTG ATCAGCCTCGAAAATAAAATACGAAATGAAATAAAATACGAAATGCTT CTGAGGCGGAAAGAACCAGCTGGGGCTCTAGGGGGTATCCCC
13	Promoter-5' UTR- CDS(OPA1)-3' UTR-polyA signal (pOPA1-C)	TCGCGATGTACGGGCCAGATATACGCGTTTTAATAGTAATCAATTAC GGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAAC TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTG ACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACAT GACCTTATGGGACTTTTCTACTTGGCAGTACATCTACGTATTAGTCA TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTG ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGGATATCCTTAAGCACCGGTG CCACCATGTGGCGGCTGCGCAGGGCAGCTGTGGCCTGTGAGGTGT GCCAGAGCCTGGTGAAGCACAGCTCTGGAATCAAGGGCAGCCTGC CTCTGCAGAAGCTGCACCTGGTGTCCAGAAGCATCTACCACTCTCA CCACCCACCCCTGAAGCTGCAGAGACCTCAGCTGAGAACCCTCTTTC CAGCAGTTCTCTTCTCTGACCAACCTGCCTCTGAGGAAGCTGAAGTT CTCTCCCATCAAGTATGGATACCAGCCTAGAAGAACTTCTGGCCTG CCAGACTGGCCACCAGACTGCTGAAGCTGAGATACCTGATCCTGGG CTCTGCTGTGGGAGGAGGCTACACAGCCAAGAAGACCTTTGACCAG TGGAAGGACATGATTCCTGACCTGAGTGAATACAAGTGGATTGTGC CAGACATTGTGTGGGAAATTGATGAATACATTGACTTTGAAAAGATC AGAAAGGCACTGCCTAGCTCAGAAGACCTGGTGAAGCTGGCTCCAG

SEQ ID NO.	Name	Nucleic Acid Sequence
		ACTTTGACAAGATTGTGGAATCTCTGTCTCTGCTGAAGGACTTCTTC ACCTCTGGATCTCCAGAAGAGACAGCCTTCAGAGCCACAGACAGAG GCAGTGAAAGTGACAAGCACTTCAGGAAGGTGTCTGACAAGGAGAA GATTGACCAGCTGCAGGAAGAGCTGCTGCACACACAGCTGAAGTAC CAGAGAATCCTGGAGAGACTGGAGAAGGAAAACAAGGAAGTGAAGGA AGCTGGTTCTGCAGAAGGATGACAAAGGAATTCATCACAGGAAGCT GAAGAAGTCTCTGATTGACATGTACAGTGAAGTGCTGGATGTGCTGT CTGACTATGATGCCTCTTACAACACTCAGGACCACCTGCCCAGAGT GGTGGTGGTTGGAGACCAGAGTGCAGGAAAGACCTCTGTGCTGGA AATGATTGCTCAGGCAAGAATCTTCCCCAGAGGCTCTGGAGAAATG ATGACAAGATCTCCTGTGAAGGTGACTCTGTCTGAAGGACCTCACCA TGTGGCTCTGTTCAAGGACAGCAGCAGAGAATTTGACCTGACCAAG GAAGAAGACCTGGCTGCTCTGAGACATGAAATTGAACTGAGAATGA GGAAGAATGTGAAGGAAGGCTGCACTGTGTCTCCAGAAACCATCTC TCTGAATGTGAAGGGACCTGGACTGCAGAGGATGGTGCTGGTGGAT CTTCTGGAGTCATCAACACTGTGACCTCTGGAATGGCTCCAGACA CCAAGGAAACCATCTTCTCCATCAGCAAAGCCTACATGCAGAACCCA AATGCCATCATTCTGTGTATTCAAGATGGCTCTGTGGATGCAGAGAG AAGCATTGTGACAGACCTGGTGTCTCAGATGGACCCGCATGGAAGA AGAACCATCTTTGTGCTGACCAAAGTGGACCTGGCAGAGAAGAATG TGGCATCTCCTAGCAGAATCCAGCAGATCATTGAAGGAAAGCTGTTC CCCATGAAGGCTCTGGGCTACTTTGCTGTGGTGACAGGAAAAGGAA ACAGCTCAGAAAGCATTGAAGCCATCAGAGAATATGAAGAAGAATTC TTCCAGAACAGCAAGCTGCTGAAGACCAGCATGCTGAAGGCTCACC AGGTGACCACCAGGAACCTGTCTCTGGCTGTGTCTGACTGCTTCTG GAAGATGGTGAGAGAATCTGTGGAACAGCAAGCAGACAGCTTCAAG GCCACAAGATTCAACCTGGAAACAGAATGGAAGAACAACCTACCCCA GACTGAGAGAACTGGACAGAAATGAGCTGTTTGAAAAGGCAAAGAA TGAAATCCTGGATGAAGTGATCTCTCTGTCTCAGGTGACTCCCAAGC ACTGGGAAGAAATCCTGCAGCAGAGCCTGTGGGAAAGAGTGTCCAC TCATGTCATTGAAAACATCTACCTGCCTGCAGCTCAGACCATGAACT CTGGAACCTTCAACACCACTGTGGACATCAAGCTGAAGCAGTGGAC AGACAAGCAGCTGCCCAACAAGGCTGTGGAAGTGGCCTGGGAAAC ACTGCAGGAAGAGTTCTCTAGATTCATGACAGAACCCAAAGGAAAG GAACATGATGACATCTTTGACAAGCTGAAGGAAGCTGTGAAGGAAG AAAGCATCAAGAGACACAAGTGGAATGACTTTGCAGAAGACTCTCTG

SEQ ID NO.	Name	Nucleic Acid Sequence
		AGAGTCATCCAGCACAATGCTCTGGAAGACAGAAGCATCAGTGACA AGCAGCAGTGGGATGCTGCCATCTACTTCATGGAAGAAGCTCTGCA GGCCAGACTGAAGGACACAGAAAATGCCATTGAAAACATGGTGGGA CCAGACTGGAAGAAGAGATGGCTGTACTGGAAGAACAGAACACAGG AGCAGTGTGTGCACAATGAAACCAAGAATGAGCTGGAGAAGATGCT GAAGTGCAATGAAGAGCACCCCTGCCTACCTGGCCTCAGATGAAATC ACCACAGTGAGGAAGAACCTGGAGAGCAGAGGAGTGGAAGTGAC CCCTCTCTGATCAAGGACACCTGGCACCAGGTGTACAGGAGACACT TCCTGAAGACAGCCCTGAACCACTGCAACCTGTGCCGCAGAGGCTT CTACTACTACCAGAGACACTTTGTGGACTCTGAGCTGGAGTGCAATG ATGTGGTGCTCTTCTGGAGAATCCAGAGGATGCTGGCCATCACAGC CAACACCCTGAGGCAGCAGCTGACCAACACAGAAGTGAGGAGACTG GAGAAGAATGTGAAGGAAGTGCTGGAAGACTTTGCAGAAGATGGAG AGAAGAAGATCAAGCTGCTGACAGGGAAGAGAGTGCAGCTGGCAG AAGACCTGAAGAAGGTGAGAGAAATCCAGGAGAAGCTGGATGCCTT CATTGAAGCTCTGCACCAGGAGAAGTAAGGATCCACTAGTCCAGTG TGGTGGAAATTCTGCAGATATCCAGCACAGTGGCGGCCGCTCGAGTC TAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGAAAATAAAATACG AAATGAAATAAAATACGAAATGCTTCTGAGGCGGAAAGAACCAGCTG GGGCTCTAGGGGGTATCCCC
14	Promoter-5' UTR- CDS(OPA1)-3' UTR-polyA signal (pOPA1-D)	TCGCGATGTACGGGCCAGATATACGCGTTTTAATAGTAATCAATTAC GGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAAC TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTG ACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACAT GACCTTATGGGACTTTTCTACTTGGCAGTACATCTACGTATTAGTCA TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTG ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACTCCGCCCCATTGACGCAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGGATATCCTTAAGCACCGGTG CCACCATGTGGAGGCTGAGGAGAGCTGCTGTGGCATGCGAGGTGT

SEQ ID NO.	Name	Nucleic Acid Sequence
		GTCAGAGCCTGGTGAAGCACTCCTCCGGCATCAAGGGGAGCCTGC CTCTCCAGAAGCTGCACCTGGTGTCCCGGTCTATCTATCATTCTCAC CACCCAACACTGAAATTGCAGCGGCCCCAGCTGAGGACCTCTTTCC AGCAGTTTAGCAGTCTGACCAATCTGCCCCTGAGAAAGCTGAAATTC TCTCCAATTAATATGGATACCAGCCTCGCAGAACTTCTGGCCTGC CAGGCTGGCCACAAGGCTGCTGAAACTGAGATACTTAATCCTGGGA TCTGCCGTGGGGGGCGGATACACAGCCAAAAAACCTTCGACCAGT GGAAGGACATGATCCCCGATCTGTCAGAGTACAAATGGATTGTGCC CGACATCGTGTGGGAGATCGACGAGTACATCGACTTCGAGAAGATC CGGAAGGCCCTGCCTTCTTCAGAGGATCTGGTCAAGCTGGCGCCAG ACTTCGATAAGATCGTGGAAGCCTGAGTTTGCTGAAGGATTTTTTC ACATCTGGCTCCCCTGAAGAAACCGCCTTCGCGCCACCGACCGG GGTAGCGAGTCCGACAAACACTTCCGGAAGGTCAGCGACAAGGAG AAGATTGACCAGCTGCAGGAAGAGCTGCTGCACACACAGCTGAAGT ACCAAAGAATCCTCGAAAGGCTGGAAAAGGAGAATAAGGAACTGAG AAAGCTCGTGCTGCAGAAGGACGATAAGGGCATCCACCATAGAAAG CTGAAGAAATCTCTGATCGACATGTACTCTGAGGTGCTGGACGTCCT GTCCGATTACGACGCCTCCTACAATACCCAGGATCACCTGCCCAGG GTGGTGGTTGTGGGGGACCAGTCCGCCGGAAGACAAGCGTGCTG GAGATGATCGCCCAGGCAAGGATCTTCCCCGGGGGAGCGGCGAA ATGATGACTAGATCTCCAGTGAAGGTGACCCTGAGCGAGGGGCCTC ATCACGTGGCACTGTTCAAGGACAGCTCTAGAGAGTTTGATCTTACA AAGGAGGAAGACCTGGCTGCACTGAGGCACGAGATTGAACTGAGAA TGAGGAAGAACGTGAAGGAGGGATGCACCGTGTCCCCAGAGACCA TCTCTCTCAATGTGAAGGGACCCGGCCTGCAGCGCATGGTGCTGGT TGACCTGCCAGGCGTGATCAACACAGTGACCAGTGGCATGGCCCCC GACACCAAGGAAACAATTTTTAGTATTTCTAAGGCCTACATGCAGAA CCCAAACGCCATCATCCTGTGCATCCAGGATGGCAGCGTGATGCT GAACGCTCTATTGTGACAGACCTGGTGTCTCAGATGGACCCTCACG GACGCCGGACCATCTTCGTGCTGACCAAAGTGGACCTGGCCGAGAA AAACGTGGCCAGCCCTAGCAGAATCCAGCAGATCATCGAGGGCAAG CTGTTCCCAATGAAGGCCCTCGGTTACTTTGCCGTGGTGACCGGCA AGGGGAACATCATCAGAGAGTATCGAGGCCATCCGCGAATATGAGGA GGAGTTCTTTCAGAACTCAAACTGCTGAAAACCAGTATGCTGAAGG CCCACCAGGTGACAACCAGGAACCTTTCCCTCGCCGTGTCAGACTG CTTCTGGAAGATGGTTCGGGAAAGTGTGGAGCAGCAGGCCGACTCT

SEQ ID NO.	Name	Nucleic Acid Sequence
		TTCAAGGCTACCCGCTTCAATCTGGAGACGGAGTGGAAGAATAACT ATCCAAGACTCAGGGAGCTGGACCGCAACGAGCTGTTTGAGAAAGC TAAGAATGAGATCCTGGATGAGGTGATTTCCCTGAGCCAGGTGACC CCCAAGCACTGGGAGGAAATCCTGCAGCAGTCTCTTTGGGAACGCG TGAGCACTCACGTCATTGAGAACATTTACCTGCCCGCCGCCAGAC CATGAACAGCGGAACATTCAACACCACCGTGGATATTAAGCTCAAAC AGTGGACCGACAAGCAGCTGCCCAACAAGGCCGTGAGGTGGCCT GGGAGACCCTGCAGGAAGAGTTCTCTAGGTTTCATGACCGAGCCCAA AGGAAAGGAGCACGACGATATTTTTGACAAGCTGAAGGAGGCAGTG AAGGAAGAGAGCATTAAACGGCATAAGTGGAACGACTTCGCCGAGG ACAGCCTGCGCGTGATCCAGCACAAACGCCCTGGAGGACCGAAGCA TTTCTGATAAGCAGCAGTGGGACGCTGCTATTTACTTCATGGAAGAG GCCCTGCAAGCCCGGCTGAAAGACACCGAAAACGCCATCGAGAACA TGGTGGGCCCAGACTGGAAAAAAGGTGGCTGTACTGGAAAAATAG GACACAGGAGCAGTGTGTGCACAACGAGACCAAAAATGAGCTGGAG AAGATGCTGAAATGTAATGAGGAACACCCAGCCTACCTGGCCAGCG ACGAGATTACCACCGTGAGGAAGAACCTGGAGAGCCGCGGCGTGG AGGTGGACCTTCACTGATCAAGGACACCTGGCACCAGGTGTACAG ACGGCACTTCTCAAGACAGCACTGAACCATTGCAATCTGTGTAGGA GAGGCTTCTACTATTACCAGAGACACTTTGTGGACTCCGAGCTGGA GTGCAACGACGTGGTGCTGTTTTGGAGAATCCAGCGCATGCTGGCC ATCACCGCCAACACCCTGCGGCAGCAGCTGACAAATACAGAGGTGA GAAGGCTGGAGAAGAATGTGAAGGAGGTGCTCGAGGACTTTGCCG AGGACGGAGAGAAGAAGATCAAGCTGCTTACAGGCAAGAGAGTGCA GCTGGCCGAAGACCTGAAGAAAGTGCGCGAGATCCAGGAGAACT GGACGCCTTCATCGAGGCCCTGCACCAGGAAAAGTGAGGATCCACT AGTCCAGTGTGGTGGAATTCTGCAGATATCCAGCACAGTGGCGGCC GCTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGAAA ATAAAATACGAAATGAAATAAAATACGAAATGCTTCTGAGGCGGAAA GAACCAGCTGGGGCTCTAGGGGGTATCCCC
15	Promoter-5' UTR- CDS(OPA1)-3' UTR-polyA signal (pOPA1-E)	TCGCGATGTACGGGCCAGATATACGCGTTTTAATAGTAATCAATTAC GGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAAC TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTG

SEQ ID NO.	Name	Nucleic Acid Sequence
		ACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACAT GACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCA TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTG ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGGATATCCTTAAGCACCGGTT CTTCTGGTCCCCACAGACTCAGAGAGAACCCGCCACCATGTGGCGA CTACGTGCGGCGCTGTGGCCTGTGAGGTCTGCCAGTCTTTAGTGA AACACAGCTCTGGAATAAAAGGAAGTTTACCACTACAAAACTACAT CTGGTTTCACGAAGCATTATCATTACATCATCCTACCTTAAAGCTT CAACGACCCCAATTAAGGACATCCTTTCAGCAGTTCTCTTCTCTGAC AAACCTTCCTTTACGTAAACTGAAATTCTCTCCAATTAAATATGGCTA CCAGCCTCGCAGGAATTTTTGGCCAGCAAGATTAGCTACGAGACTC TTAAAACTTCGCTATCTCATACTAGGATCGGCTGTTGGGGGTGGCTA CACAGCCAAAAAGACTTTTGATCAGTGGAAAGATATGATACCGGACC TTAGTGAATATAAATGGATTGTGCCTGACATTGTGTGGGAAATTGAT GAGTATATCGATTTTGAGAAAATTAGAAAAGCCCTTCCTAGTTCAGAA GACCTTGTAAGTTAGCACCAGACTTTGACAAGATTGTTGAAAGCCT TAGCTTATTGAAGGACTTTTTTACCTCAGGTTCTCCGGAAGAAACGG CGTTTAGAGCAACAGATCGTGATCTGAAAGTGACAAGCATTTTAGA AAGGTGTCAGACAAAGAGAAAATTGACCAACTTCAGGAAGAAGTTCT GCACACTCAGTTGAAGTATCAGAGAATCTTGGAACGATTAGAAAAGG AGAACAAAGAATTGAGAAAATTAGTATTGCAGAAAGATGACAAAGGC ATTCATCATAGAAAGCTTAAGAAATCTTTGATTGACATGTATTCTGAA GTTCTTGATGTTCTCTCTGATTATGATGCCAGTTATAATACGCAAGAT CATCTGCCACGGGTTGTTGTGGTTGGAGATCAGAGTGCTGGAAAGA CTAGTGTGTTGGAAATGATTGCCCAAGCTCGAATATCCCAAGAGGA TCTGGGGAGATGATGACACGTTCTCCAGTTAAGGTGACTCTGAGTG AAGGTCCTCACCATGTGGCCCTATTTAAAGATAGTTCTCGGGAGTTT GATCTTACCAAAGAAGAAGATCTTGACGATTAAAGACATGAAATAGA ACTTCGAATGAGGAAAAATGTGAAAGAAGGCTGTACCGTTAGCCCT GAGACCATATCCTTAAATGTAAAGGCCCTGGACTACAGAGGATGGT GCTTGTTGACTTACCAGGTGTGATTAATACTGTGACATCAGGCATGG

SEQ ID NO.	Name	Nucleic Acid Sequence
		CTCCTGACACAAAGGAACTATTTTCAGTATCAGCAAAGCTTACATG CAGAATCCTAATGCCATCATACTGTGTATTCAAGATGGATCTGTGGA TGCTGAACGCAGTATTGTTACAGACTTGGTCAGTCAAATGGACCCTC ATGGAAGGAGAACCATATTCGTTTTGACCAAAGTAGACCTGGCAGA GAAAAATGTAGCCAGTCCAAGCAGGATTCAGCAGATAATTGAAGGAA AGCTCTTCCCAATGAAAGCTTTAGGTTATTTTGCTGTTGTAACAGGAA AAGGGAACAGCTCTGAAAGCATTGAAGCTATAAGAGAATATGAAGAA GAGTTTTTTCAGAATTCAAAGCTCCTAAAGACAAGCATGCTAAAGGC ACACCAAGTGACTACAAGAAATTTAAGCCTTGACAGTATCAGACTGCT TTTGAAAATGGTACGAGAGTCTGTTGAACAACAGGCTGATAGTTTC AAAGCAACACGTTTTAACCTTGAACTGAATGGAAGAATAACTATCCT CGCCTGCGGGAACCTTGACCGGAATGAACTATTTGAAAAAGCTAAAA TGAAATCCTTGATGAAGTTATCAGTCTGAGCCAGGTTACACCAAAAC ATTGGGAGGAAATCCTTCAACAATCTTTGTGGGAAAGAGTATCAACT CATGTGATTGAAAACATCTACCTTCCAGCTGCGCAGACCATGAATTC AGGAACTTTTAACACCACAGTGGATATCAAGCTTAAACAGTGGACTG ATAAACAACTTCCTAATAAAGCAGTAGAGGTTGCTTGGGAGACCCTA CAAGAAGAATTTCCCGCTTTATGACAGAACCGAAAGGGAAAGAGCA TGATGACATATTTGATAAACTTAAAGAGGCTGTTAAGGAAGAAAGTAT TAAACGACACAAGTGGAATGACTTTGCGGAGGACAGCTTGAGGGTT ATTCAACACAATGCTTTGGAAGACCGATCCATATCTGATAAACAGCA ATGGGATGCAGCTATTTATTTTATGGAAGAGGCTCTGCAGGCTCGTC TCAAGGATACTGAAAATGCAATTGAAAACATGGTGGGTCCAGACTGG AAAAAGAGGTGGTTATACTGGAAGAATCGGACCCAAGAACAGTGTG TTCACAATGAAACCAAGAATGAATTGGAGAAGATGTTGAAATGTAAT GAGGAGCACCCAGCTTATCTTGCAAGTGATGAAATAACCACAGTCC GGAAGAACCTTGAATCCCGAGGAGTAGAAGTAGATCCAAGCTTGAT TAAGGATACTTGGCATCAAGTTTATAGAAGACATTTTTTAAAAACAGC TCTAAACCATTGTAACCTTTGTCTGAAGAGGTTTTTATTACTACCAAAG GCATTTTGTAGATTCTGAGTTGGAATGCAATGATGTGGTCTTGTTTTG GCGTATACAGCGCATGCTTGCTATCACCGCAAATACTTTAAGGCAAC AACTTACAAATACTGAAGTTAGGCGATTAGAGAAAAATGTTAAAGAG GTATTGGAAGATTTTGTCTGAAGATGGTGAGAAGAAGATTAAATTGCT TACTGGTAAACGCGTTCAACTGGCGGAAGACCTCAAGAAAGTTAGA GAAATTCAAGAAAACTTGATGCTTTTCATTGAAGCTCTTCATCAGGA GAAATAAGGATCCACTAGTCCAGTGTGGTGGAAATTCTGCAGATATCC

SEQ ID NO.	Name	Nucleic Acid Sequence
		AGCACAGTGGCGGCCGCTCGAGTCTAGAGGGCCCGTTTAAACCCG CTGATCAGCCTCGAAAATAAAATACGAAATGAAATAAAATACGAAAT GCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCTAGGGGGTATCCC C
16	Promoter-5' UTR- CDS(OPA1)-3' UTR-polyA signal (pOPA1-F)	TCGCGATGTACGGGCCAGATATACGCGTTTTAATAGTAATCAATTAC GGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAAC TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTG ACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACAT GACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCA TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTG ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGGATATCCTTAAGCACCGGTG CCACCATGTGGCGACTACGTGCGGCCGCTGTGGCCTGTGAGGTCT GCCAGTCTTTAGTGAAACACAGCTCTGGAATAAAAGGAAGTTTACCA CTACAAAACTACATCTGGTTTCACGAAGCATTATCATTACATCAT CCTACCTTAAAGCTTCAACGACCCCAATTAAGGACATCCTTTCAGCA GTTCTCTTCTCTGACAAACCTTCCTTTACGTAAACTGAAATTCTCTCC AATTAATATGGCTACCAGCCTCGCAGGAATTTTTGGCCAGCAAGAT TAGCTACGAGACTCTTAAACTTCGCTATCTCATACTAGGATCGGCT GTTGGGGGTGGCTACACAGCCAAAAAGACTTTTGATCAGTGGAAAG ATATGATACCGGACCTTAGTGAATATAAATGGATTGTGCCTGACATT GTGTGGGAAATTGATGAGTATATCGATTTTGAGAAAATTAGAAAAGC CCTTCCTAGTTCAGAAGACCTTGTAAGTTAGCACCCAGACTTTGACA AGATTGTTGAAAGCCTTAGCTTATTGAAGGACTTTTTTACCTCAGGTT CTCCGGAAGAAACGGCGTTTAGAGCAACAGATCGTGATCTGAAAG TGACAAGCATTTTAGAAAAGGTGTCAGACAAAGAGAAAATTGACCAAC TTCAGGAAGAACTTCTGCACACTCAGTTGAAGTATCAGAGAATCTTG GAACGATTAGAAAAGGAGAACAAAGAATTGAGAAAATTAGTATTGCA GAAAGATGACAAAGGCATTCATCATAGAAAGCTTAAGAAATCTTTGA

SEQ ID NO.	Name	Nucleic Acid Sequence
		TTGACATGTATTCTGAAGTTCTTGATGTTCTCTCTGATTATGATGCCA GTTATAATACGCAAGATCATCTGCCACGGGTTGTTGTGGTTGGAGAT CAGAGTGCTGGAAAGACTAGTGTGTTGGAAATGATTGCCCAAGCTC GAATATTCCCAAGAGGATCTGGGGAGATGATGACACGTTCTCCAGTT AAGGTGACTCTGAGTGAAGGTCCTCACCATGTGGCCCTATTTAAAGA TAGTTCTCGGGAGTTTGATCTTACCAAAGAAGAAGATCTTGCAGCAT TAAGACATGAAATAGAACTTCGAATGAGGAAAAATGTGAAAGAAGGC TGTACCGTTAGCCCTGAGACCATATCCTTAAATGTAAAAGGCCCTGG ACTACAGAGGATGGTGCTTGTGACTTACCAGGTGTGATTAATACTG TGACATCAGGCATGGCTCCTGACACAAAGGAACTATTTTCAGTATC AGCAAAGCTTACATGCAGAATCCTAATGCCATCATACTGTGTATTCA AGATGGATCTGTGGATGCTGAACGCAGTATTGTTACAGACTTGGTCA GTCAAATGGACCCTCATGGAAGGAGAACCATATTCGTTTTGACCAA GTAGACCTGGCAGAGAAAAATGTAGCCAGTCCAAGCAGGATTCAGC AGATAATTGAAGGAAAGCTCTTCCAATGAAAGCTTTAGGTTATTTTG CTGTTGTAACAGGAAAAGGGAACAGCTCTGAAAGCATTGAAGCTATA AGAGAATATGAAGAAGAGTTTTTTCAGAATTCAAAGCTCCTAAAGAC AAGCATGCTAAAGGCACACCAAGTGACTACAAGAAATTTAAGCCTTG CAGTATCAGACTGCTTTTGAAAAATGGTACGAGAGTCTGTTGAACAA CAGGCTGATAGTTTCAAAGCAACACGTTTTAACCTTGAACTGAATG GAAGAATAACTATCCTCGCCTGCGGGAACCTTGACCGGAATGAACAT TTGAAAAAGCTAAAAATGAAATCCTTGATGAAGTTATCAGTCTGAGC CAGGTTACACCAAACATTGGGAGGAAATCCTTCAACAATCTTTGTG GGAAAGAGTATCAACTCATGTGATTGAAAACATCTACCTTCCAGCTG CGCAGACCATGAATTCAGGAACTTTTAACACCACAGTGGATATCAAG CTTAAACAGTGGACTGATAAACAACCTTCCTAATAAAGCAGTAGAGGT TGCTTGGGAGACCCTACAAGAAGAATTTTCCCGCTTTATGACAGAAC CGAAAGGGAAAGAGCATGATGACATATTTGATAAACTTAAAGAGGCT GTTAAGGAAGAAAGTATTAAACGACACAAGTGGAATGACTTTGCGGA GGACAGCTTGAGGGTTATTCAACACAATGCTTTGGAAGACCGATCCA TATCTGATAAACAGCAATGGGATGCAGCTATTTATTTTATGGAAGAG GCTCTGCAGGCTCGTCTCAAGGATACTGAAAATGCAATTGAAAACAT GGTGGGTCCAGACTGGAAAAAGAGGTGGTTATACTGGAAGAATCGG ACCCAAGAACAGTGTGTTCACAATGAAACCAAGAATGAATTGGAGAA GATGTTGAAATGTAATGAGGAGCACCAGCTTATCTTGCAAGTGATG AAATAACCACAGTCCGGAAGAACCTTGAATCCCGAGGAGTAGAAGT

SEQ ID NO.	Name	Nucleic Acid Sequence
		AGATCCAAGCTTGATTAAGGATACTTGGCATCAAGTTTATAGAAGAC ATTTTTTAAAAACAGCTCTAAACCATTGTAACCTTTGTCTGAAGAGGTT TTTATTACTACCAAAGGCATTTTGTAGATTCTGAGTTGGAATGCAATG ATGTGGTCTTGTTTTGGCGTATACAGCGCATGCTTGCTATCACCGCA AATACTTTAAGGCAACAACCTTACAAATACTGAAGTTAGGCGATTAGA GAAAAATGTTAAAGAGGTATTGGAAGATTTTGCTGAAGATGGTGAGA AGAAGATTAAATTGCTTACTGGTAAACGCGTTCAACTGGCGGAAGAC CTCAAGAAAGTTAGAGAAATTCAAGAAAACTTGATGCTTTCATTGAA GCTCTTCATCAGGAGAAATAAGGATCCGCTGGAGCCTCGGTGGCCA TGCTTCTTGCCCTTGCGCCTCCCCCAGCCCCTCCTCCCCTTCCT GCACCCGTACCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCA TCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGAAAATAAAATA CGAAATGAAATAAAATACGAAATGCTTCTGAGGCGGAAAGAACCAGC TGGGGCTCTAGGGGGTATCCCC
17	Promoter-5' UTR- CDS(OPA1)-3' UTR-polyA signal (pOPA1-G)	TCGCGATGTACGGGCCAGATATACGCGTTTTAATAGTAATCAATTAC GGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAAC TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTG ACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACAT GACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCA TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTG ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACCTCCGCCCCATTGACGCAAAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGGATATCCTTAAGCACCGGTA GAGGGCCCTGAAGAGGGCCCTTTATATATGCCTGCGTCCGTACTAG TTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGGTGGAAGTACG AGTTCGGAACACCCGGCCGCGACCCTGGGAGACGTCCCAGGGACT TCGGGGCCACCATGTGGCGACTACGTGGGGCCGCTGTGGCCTGTG AGGTCTGCCAGTCTTTAGTGAAACACAGCTCTGGAATAAAAGGAAGT TTACCACTACAAAACTACATCTGGTTTCACGAAGCATTTATCATTCA CATCATCCTACCTTAAAGCTTCAACGACCCCAATTAAGGACATCCTTT

SEQ ID NO.	Name	Nucleic Acid Sequence
		CAGCAGTTCTCTTCTCTGACAAACCTTCCTTTACGTAAACTGAAATTC TCTCCAATTAAATATGGCTACCAGCCTCGCAGGAATTTTTGGCCAGC AAGATTAGCTACGAGACTCTTAAAACTTCGCTATCTCATACTAGGATC GGCTGTTGGGGTGGCTACACAGCCAAAAAGACTTTTGATCAGTGG AAAGATATGATACCGGACCTTAGTGAATATAAATGGATTGTGCCTGA CATTGTGTGGGAAATTGATGAGTATATCGATTTTGAGAAAATTAGAAA AGCCCTTCCTAGTTTCTAGAACCTTGTAAGTTAGCACCAGACTTTG ACAAGATTGTTGAAAGCCTTAGCTTATTGAAGGACTTTTTTACCTCAG GTTCTCCGGAAGAAACGGCGTTTAGAGCAACAGATCGTGGATCTGA AAGTGACAAGCATTTTAGAAAGGTGTCAGACAAAGAGAAAATTGACC AACTTCAGGAAGAACTTCTGCACACTCAGTTGAAGTATCAGAGAATC TTGGAACGATTAGAAAAGGAGAACAAAGAATTGAGAAAATTAGTATT GCAGAAAGATGACAAAGGCATTCATCATAGAAAGCTTAAGAAATCTT TGATTGACATGTATTCTGAAGTTCTTGATGTTCTCTCTGATTATGATG CCAGTTATAATACGCAAGATCATCTGCCACGGGTGTTGTGGTTGGA GATCAGAGTGCTGGAAGACTAGTGTGTTGGAATGATTGCCCAAG CTCGAATATTCCCAAGAGGATCTGGGGAGATGATGACACGTTCTCC AGTTAAGGTGACTCTGAGTGAAGGTCTCACCATGTGGCCCTATTTA AAGATAGTTCTCGGGAGTTTGATCTTACCAAAGAAGAAGATCTTGCA GCATTAAGACATGAAATAGAACTTCGAATGAGGAAAAATGTGAAAGA AGGCTGTACCGTTAGCCCTGAGACCATATCCTTAAATGTAAAGGCC CTGGACTACAGAGGATGGTGCTTGTTGACTTACCAGGTGTGATTAAT ACTGTGACATCAGGCATGGCTCCTGACACAAAGGAACTATTTTCAG TATCAGCAAAGCTTACATGCAGAATCCTAATGCCATCATACTGTGTAT TCAAGATGGATCTGTGGATGCTGAACGCAGTATTGTTACAGACTTGG TCAGTCAAATGGACCCTCATGGAAGGAGAACCATATTCGTTTTGACC AAAGTAGACCTGGCAGAGAAAAATGTAGCCAGTCCAAGCAGGATTC AGCAGATAATTGAAGGAAAGCTCTTCCCAATGAAAGCTTTAGGTTAT TTTGCTGTTGTAACAGGAAAAGGGAACAGCTCTGAAAGCATTGAAGC TATAAGAGAATATGAAGAAGAGTTTTTTCAGAATCAAAGCTCCTAAA GACAAGCATGCTAAAGGCACACCAAGTGACTACAAGAAATTTAAGCC TTGCAGTATCAGACTGCTTTTGGAAAATGGTACGAGAGTCTGTTGAA CAACAGGCTGATAGTTTCAAAGCAACACGTTTTAACCTTGAACTGA ATGGAAGAATAACTATCCTCGCCTGCGGGAACCTTGACCGGAATGAA CTATTTGAAAAAGCTAAAAATGAAATCCTTGATGAAGTTATCAGTCTG AGCCAGGTTACACCAAAACATTGGGAGGAAATCCTTCAACAATCTTT

SEQ ID NO.	Name	Nucleic Acid Sequence
		GTGGGAAAGAGTATCAACTCATGTGATTGAAAACATCTACCTTCCAG CTGCGCAGACCATGAATTCAGGAACTTTTAACACCACAGTGATATC AAGCTTAAACAGTGGACTGATAAACAACTTCCTAATAAAGCAGTAGA GGTGCTTGGGAGACCCTACAAGAAGAATTTTCCCGCTTTATGACAG AACCGAAAGGGAAAGAGCATGATGACATATTTGATAAACTTAAAGAG GCTGTTAAGGAAGAAAGTATTAACGACACAAGTGGAATGACTTTGC GGAGGACAGCTTGAGGGTTATTCAACACAATGCTTTGGAAGACCGA TCCATATCTGATAAACAGCAATGGGATGCAGCTATTTATTTATGGAA GAGGCTCTGCAGGCTCGTCTCAAGGATACTGAAAATGCAATTGAAAA CATGGTGGGTCCAGACTGGAAAAAGAGGTGGTTATACTGGAAGAAT CGGACCCAAGAACAGTGTGTTCAACAATGAAACCAAGAATGAATTGGA GAAGATGTTGAAATGTAATGAGGAGCACCCAGCTTATCTTGCAAGTG ATGAAATAACCACAGTCCGGAAGAACCTTGAATCCCGAGGAGTAGA AGTAGATCCAAGCTTGATTAAGGATACTTGGCATCAAGTTTATAGAA GACATTTTTTAAAAACAGCTCTAAACCATTGTAACCTTTGTGGAAGAG GTTTTTATTACTACCAAAGGCATTTTGTAGATTCTGAGTTGGAATGCA ATGATGTGGTCTTGTTTTGGCGTATACAGCGCATGCTTGCTATCACC GCAAATACTTTAAGGCAACAACCTTACAAATACTGAAGTTAGGCGATT AGAGAAAAATGTAAAGAGGTATTGGAAGATTTTGCTGAAGATGGTG AGAAGAAGATTAAATTGCTTACTGGTAAACGCGTTCAACTGGCGGAA GACCTCAAGAAAGTTAGAGAAATTCAAGAAAAACTTGATGCTTTTCATT GAAGCTCTTCATCAGGAGAAATAAGGATCCGCCTGCGTCGGTACTA GTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGGTGGAAGTAC GAGTTCGGAACACCCGGCCGCGACCCTGGGAGACGTCCCAGGGAC TTCGGGATATATAAAGGGCCCTCTTCAGGGCCCTCTTCTAGAGGGC CCGTTTAAACCCGCTGATCAGCCTCGAAAATAAAATACGAAATGAAA TAAATACGAAATGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCT AGGGGGTATCCCC
18	Promoter-5' UTR- CDS(OPA1)-3' UTR-polyA signal (pOPA1-H)	TCGCGATGTACGGGCCAGATATACGCGTTTTAATAGTAATCAATTAC GGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAAC TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTG ACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACAT GACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCA

SEQ ID NO.	Name	Nucleic Acid Sequence
		TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTG ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGGATATCCTTAAGCACCGGTG CCACCATGTGGCGACTACGTCGGGCCGCTGTGGCCTGTGAGGTCT GCCAGTCTTTAGTGAAACACAGCTCTGGAATAAAAGGAAGTTTACCA CTACAAAACACTACATCTGGTTTCACGAAGCATTATCATTACATCAT CCTACCTTAAAGCTTCAACGACCCCAATTAAGGACATCCTTTCAGCA GTTCTCTTCTCTGACAAACCTTCCTTTACGTAAACTGAAATTCTCTCC AATTAAATATGGCTACCAGCCTCGCAGGAATTTTTGGCCAGCAAGAT TAGCTACGAGACTCTTAAACCTTCGCTATCTCATACTAGGATCGGCT GTTGGGGGTGGCTACACAGCCAAAAAGACTTTTGATCAGTGGAAAG ATATGATACCGGACCTTAGTGAATATAAATGGATTGTGCCTGACATT GTGTGGGAAATTGATGAGTATATCGATTTTGAGAAAATTAGAAAAGC CCTTCCTAGTTTCAAGACCTTGTAAGTTAGCACCCAGACTTTTGACA AGATTGTTGAAAGCCTTAGCTTATTGAAGGACTTTTTTACCTCAGGTT CTCCGGAAGAAACGGCGTTTAGAGCAACAGATCGTGGATCTGAAAG TGACAAGCATTTTAGAAAGGTGTCAGACAAAGAGAAAATTGACCAAC TTCAGGAAGAACTTCTGCACACTCAGTTGAAGTATCAGAGAATCTTG GAACGATTAGAAAAGGAGAACAAAGAATTGAGAAAATTAGTATTGCA GAAAGATGACAAAGGCATTCATCATAGAAAGCTTAAGAAATCTTTGA TTGACATGTATTCTGAAGTTCTTGATGTTCTCTCTGATTATGATGCCA GTTATAATACGCAAGATCATCTGCCACGGGTTGTTGTGGTTGGAGAT CAGAGTGCTGGAAAGACTAGTGTGTTGGAATGATTGCCCAAGCTC GAATATTCCCAAGAGGATCTGGGGAGATGATGACACGTTCTCCAGTT AAGGTGACTCTGAGTGAAGGTCCTCACCATGTGGCCCTATTTAAAGA TAGTTCTCGGGAGTTTGATCTTACCAAAGAAGAAGATCTTGACGCAT TAAGACATGAAATAGAACTTCGAATGAGGAAAAATGTGAAAGAAGGC TGTACCGTTAGCCCTGAGACCATATCCTTAAATGTAAAAGGCCCTGG ACTACAGAGGATGGTGCTTGTTGACTTACCAGGTGTGATTAATACTG TGACATCAGGCATGGCTCCTGACACAAAGGAACTATTTTCAGTATC AGCAAAGCTTACATGCAGAATCCTAATGCCATCATACTGTGTATTCA AGATGGATCTGTGGATGCTGAACGCAGTATTGTTACAGACTTGGTCA

SEQ ID NO.	Name	Nucleic Acid Sequence
		GTCAAATGGACCCTCATGGAAGGAGAACCATATTCGTTTTGACCAAA GTAGACCTGGCAGAGAAAAATGTAGCCAGTCCAAGCAGGATTCAGC AGATAATTGAAGGAAAGCTCTTCCCAATGAAAGCTTTAGGTTATTTTG CTGTTGTAACAGGAAAAAGGGAACAGCTCTGAAAGCATTGAAGCTATA AGAGAATATGAAGAAGAGTTTTTTTCAGAATTCAAAGCTCCTAAAGAC AAGCATGCTAAAGGCACACCAAGTGACTACAAGAAATTTAAGCCTTG CAGTATCAGACTGCTTTTGGAAAAATGGTACGAGAGTCTGTTGAACAA CAGGCTGATAGTTTCAAAGCAACACGTTTTAACCTTGAAACTGAATG GAAGAATAACTATCCTCGCCTGCGGGAACCTTGACCGGAATGAACAT TTGAAAAAGCTAAAAATGAAATCCTTGATGAAGTTATCAGTCTGAGC CAGGTTACACCAAAACATTGGGAGGAAATCCTTCAACAATCTTTGTG GGAAAGAGTATCAACTCATGTGATTGAAAACATCTACCTTCCAGCTG CGCAGACCATGAATTCAGGAACTTTTAACACCACAGTGGATATCAAG CTTAAACAGTGGACTGATAAACAACCTCCTAATAAAGCAGTAGAGGT TGCTTGGGAGACCCTACAAGAAGAATTTTCCCGCTTTATGACAGAAC CGAAAGGGAAAGAGCATGATGACATATTTGATAAACTTAAAGAGGCT GTTAAGGAAGAAAGTATTAACGACACAAGTGGAATGACTTTGCGGA GGACAGCTTGAGGGTTATTCAACACAATGCTTTGGAAGACCGATCCA TATCTGATAAACAGCAATGGGATGCAGCTATTTATTTTATGGAAGAG GCTCTGCAGGCTCGTCTCAAGGATACTGAAAATGCAATTGAAAACAT GGTGGGTCCAGACTGGAAAAAGAGGTGGTTATACTGGAAGAATCGG ACCCAAGAACAGTGTGTTACAATGAAACCAAGAATGAATTGGAGAA GATGTTGAAATGTAATGAGGAGCACCAGCTTATCTTGCAAGTGATG AAATAACCACAGTCCGGAAGAACCTTGAATCCCGAGGAGTAGAAGT AGATCCAAGCTTGATTAAGGATACTTGGCATCAAGTTTATAGAAGAC ATTTTTTAAAAACAGCTCTAAACCATTGTAACCTTTGTCGAAGAGGTT TTTATTACTACCAAAGGCATTTTGTAGATTCTGAGTTGGAATGCAATG ATGTGGTCTTGTTTTGGCGTATACAGCGCATGCTTGCTATCACCGCA AATACTTTAAGGCAACAACCTTACAAATACTGAAGTTAGGCGATTAGA GAAAAATGTTAAAGAGGTATTGGAAGATTTTGCTGAAGATGGTGAGA AGAAGATTAAATTGCTTACTGGTAAACGCGTTCAACTGGCGGAAGAC CTCAAGAAAGTTAGAGAAATTCAAGAAAAACTTGATGCTTTCATTGAA GCTCTTCATCAGGAGAAATAAGGATCCATAATCAACCTCTGGATTAC AAAATTTGTGAAAGATTGACTGGTATTCTTAACCTATGTTGCTCCTTTT ACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCT TCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTG

SEQ ID NO.	Name	Nucleic Acid Sequence
		TCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGG TGTGCACTGTGTTTGCTGACGCAACCCCCACTGTTGGGGCATTGC CACCACCTGTCAGCTCCTTTCCGGGACTTTGCTTTCCCCCTCCCTA TTGCCACGGCGGAACATCATCGCCGCCTGCCTTGCCCGCTGCTGGA CAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTGTCGG GGAAATCATCGTCCTTTCTTGGCTGCTCGCCTGTGTTGCCACCTG GATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAAT CCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCT CTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTT GGGCCGCCTCCCCGCATCTCTAGAGGGCCCGTTTAAACCCGCTGAT CAGCCTCGAAAATAAAATACGAAATGAAATAAAATACGAAATGCTTCT GAGGCGGAAAGAACCAGCTGGGGCTCTAGGGGGTATCCCC
19	Promoter-5' UTR- CDS(OPA1)-3' UTR-polyA signal (pOPA1-l)	TCGCGATGTACGGGCCAGATATACGCGTTTTAATAGTAATCAATTAC GGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAAC TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTG ACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACAT GACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCA TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTG ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCA AAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGGATATCCTTAAGCACCGGTG CCACCATGTGGCGACTACGTGCGGCCGCTGTGGCCTGTGAGGTCT GCCAGTCTTTAGTGAAACACAGCTCTGGAATAAAAGGAAGTTTACCA CTACAAAACTACATCTGTTTTACGAAGCATTATCATTACATCAT CCTACCTTAAAGCTTCAACGACCCCAATTAAGGACATCCTTTCAGCA GTTCTCTTCTCTGACAAACCTTCCTTTACGTAAACTGAAATTCTCTCC AATTAATATGGCTACCAGCCTCGCAGGAATTTTTGGCCAGCAAGAT TAGCTACGAGACTCTTAAAACCTTCGCTATCTCATACTAGGATCGGCT GTTGGGGGTGGCTACACAGCCAAAAAGACTTTTGATCAGTGGAAG ATATGATACCGGACCTTAGTGAATATAAATGGATTGTGCCTGACATT

SEQ ID NO.	Name	Nucleic Acid Sequence
		GTGTGGGAAATTGATGAGTATATCGATTTTGAGAAAATTAGAAAAGC CCTTCCTAGTTCAGAAGACCTTGTAAGTTAGCACCCAGACTTTTGACA AGATTGTTGAAAGCCTTAGCTTATTGAAGGACTTTTTTACCTCAGGTT CTCCGGAAGAAACGGCGTTTAGAGCAACAGATCGTGGATCTGAAAG TGACAAGCATTTTAGAAAGGTGTCAGACAAAGAGAAAATTGACCAAC TTCAGGAAGAACTTCTGCACACTCAGTTGAAGTATCAGAGAATCTTG GAACGATTAGAAAAGGAGAACAAAGAATTGAGAAAATTAGTATTGCA GAAAGATGACAAAGGCATTTCATCATAGAAAGCTTAAGAAATCTTTGA TTGACATGTATTCTGAAGTTCTTGATGTTCTCTCTGATTATGATGCCA GTTATAATACGCAAGATCATCTGCCACGGGTTGTTGTGGTTGGAGAT CAGAGTGCTGGAAAGACTAGTGTGTTGGAAATGATTGCCCAAGCTC GAATATTCCCAAGAGGATCTGGGGAGATGATGACACGTTCTCCAGTT AAGGTGACTCTGAGTGAAGGTCCTCACCATGTGGCCCTATTTAAAGA TAGTTCTCGGGAGTTTGATCTTACCAAAGAAGAAGATCTTGACGCAT TAAGACATGAAATAGAACTTCGAATGAGGAAAAATGTGAAAGAAGGC TGTACCGTTAGCCCTGAGACCATATCCTTAAATGTAAAAGGCCCTGG ACTACAGAGGATGGTGCTTGTTGACTTACCAGGTGTGATTAATACTG TGACATCAGGCATGGCTCCTGACACAAAGGAACTATTTTCAGTATC AGCAAAGCTTACATGCAGAATCCTAATGCCATCATACTGTGTATTCA AGATGGATCTGTGGATGCTGAACGCAGTATTGTTACAGACTTGGTCA GTCAAATGGACCCTCATGGAAGGAGAACCATATTCGTTTTGACCAAA GTAGACCTGGCAGAGAAAAATGTAGCCAGTCCAAGCAGGATTCAGC AGATAATTGAAGGAAAGCTCTTCCCAATGAAAGCTTTAGGTTATTTTG CTGTTGTAACAGGAAAAGGGAACAGCTCTGAAAGCATTGAAGCTATA AGAGAATATGAAGAAGAGTTTTTTTCAGAATTCAAAGCTCCTAAAGAC AAGCATGCTAAAGGCACACCAAGTGACTACAAGAAATTTAAGCCTTG CAGTATCAGACTGCTTTTGGAAAATGGTACGAGAGTCTGTTGAACAA CAGGCTGATAGTTTCAAAGCAACACGTTTTAACCTTGAACTGAATG GAAGAATAACTATCCTCGCCTGCGGGAACCTTGACCGGAATGAACAT TTGAAAAAGCTAAAAATGAAATCCTTGATGAAGTTATCAGTCTGAGC CAGGTTACACCAAAACATTGGGAGGAAATCCTTCAACAATCTTTGTG GGAAAGAGTATCAACTCATGTGATTGAAAACATCTACCTTCCAGCTG CGCAGACCATGAATTCAGGAACTTTTAACACCACAGTGGATATCAAG CTTAAACAGTGGACTGATAACAACCTTCCTAATAAAGCAGTAGAGGT TGCTTGGGAGACCCTACAAGAAGAATTTTCCCGCTTTATGACAGAAC CGAAAGGGAAAGAGCATGATGACATATTTGATAAACTTAAAGAGGCT

SEQ ID NO.	Name	Nucleic Acid Sequence
		GTTAAGGAAGAAAGTATTAAACGACACAAGTGGGAATGACTTTGCGGA GGACAGCTTGAGGGTTATTCAACACAATGCTTTGGAAGACCGATCCA TATCTGATAAACAGCAATGGGATGCAGCTATTTATTTTATGGAAGAG GCTCTGCAGGCTCGTCTCAAGGATACTGAAAATGCAATTGAAAACAT GGTGGGTCCAGACTGGAAAAAGAGGTGGTTATACTGGAAGAATCGG ACCCAAGAACAGTGTGTTTACAATGAAACCAAGAATGAATTGGAGAA GATGTTGAAATGTAATGAGGAGCACCCAGCTTATCTTGCAAGTGATG AAATAACCACAGTCCGGAAGAACCCTTGAATCCCGAGGAGTAGAAGT AGATCCAAGCTTGATTAAGGATACTTGGCATCAAGTTTATAGAAGAC ATTTTTTAAAAACAGCTCTAAACCATTGTAACCTTTGTCTGAAGAGGTT TTTATTACTACCAAAGGCATTTTGTAGATTCTGAGTTGGAATGCAATG ATGTGGTCTTGTGTTTGGCGTATACAGCGCATGCTTGCTATCACCGCA AATACTTTAAGGCAACAACCTTACAAATACTGAAGTTAGGCGATTAGA GAAAAATGTTAAAGAGGTATTGGAAGATTTTGCTGAAGATGGTGAGA AGAAGATTAAATTGCTTACTGGTAAACGCGTTCAACTGGCGGAAGAC CTCAAGAAAGTTAGAGAAATTCAAGAAAAACTTGATGCTTTCATTGAA GCTCTTCATCAGGAGAAATAAGGATCCAATCAACCTCTGGATTACAA AATTTGTGAAAGATTGACTGGTATTCTTAACCTATGTTGCTCCTTTTAC GCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTC CCGTATGGCTTTCATTTTCTCCTCCTTGATAAATCCTGGTTAGTTCT TGCCACGGCGGAACCTCATCGCCGCTGCCTTGCCCGCTGCTGGAC AGGGGCTCGGCTGTTGGGCACTGACAATCCGTGGTGTCTAGAGG GCCCGTTTAAACCCGCTGATCAGCCTCGAAAATAAAATACGAAATGA AATAAAATACGAAATGCTTCTGAGGCGGAAAGAACCAGCTGGGGCT CTAGGGGGTATCCCC

In some embodiments, AAV vector of the disclosure comprises the nucleic acid set forth in any one of SEQ ID NOs: 12-14, as well as nucleic acids having at least 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identity, or more) to the nucleic acid sequence of any one of SEQ ID NOs: 12-14 and that achieve expression of the *OPA1* gene in target cells.

The vector including the nucleic acid molecule may be delivered to desired tissues, such as the eye (e.g., to the retina), of a patient diagnosed with or displaying one or more symptoms of optic neuropathy and *OPA1*-deficiency disorder, for example ADOA. Upon administration of the viral vector including the *OPA1* gene to the patient, the patient may exhibit an increase in the expression of *OPA1*, an increase in visual acuity, improvement of retinal function, improvement of central vision, improvement of color vision and/or overall improvement in other symptoms within sometime after

treatment. Depending on the outcome of the evaluation, the patient may receive additional treatments.

Methods of Treatment

5 The pharmaceutical compositions and methods of the present disclosure may be used to treat a variety of subjects having a disease or condition associated with a mutation in one or more *OPA1* alleles. For example, subjects that may be treated as described herein include those subjects having an optic neuropathy, such as a hereditary optic neuropathy (e.g., ADOA). ADOA is hereditary in nature and can be caused, for example, because of a mutation in the *OPA1* gene. Additional
10 examples of subjects that may be treated using the pharmaceutical compositions and methods of the disclosure are those having diseases or conditions caused by *OPA1* protein deficiency. Significantly, *OPA1* deficiency is associated not only with optic neuropathy, but also with systemic pathologies, including peripheral neuropathy, deafness, cerebellar ataxia, spastic paraparesis, and myopathy. Accordingly, the pharmaceutical compositions and methods disclosed herein can be used to
15 ameliorate these systemic conditions.

 The pharmaceutical compositions and methods described herein may also be used as a preventative treatment for patients having a deleterious mutation in an *OPA1* gene, patients with reduced *OPA1* activity, and patients exhibiting symptoms of ADOA. Exemplary symptoms of ADOA include impaired vision, reduced visual acuity, vision loss, color blindness, central vision loss,
20 blindness, retinal damage, retinal degeneration, retinal ganglion cell loss, and optic nerve damage, among others. In some embodiments, a subject may be administered a vector comprising the polynucleotide or nucleic acid molecule of the disclosure (e.g., an AAV vector of the disclosure) to deliver the *OPA1* protein that supplements the expression of the *OPA1* protein that is already expressed in the subject, but whose supplementation gives rise to a therapeutic effect.

25 Exemplary transgenes that can be delivered using the pharmaceutical compositions and methods of the disclosure include a codon-optimized polynucleotide encoding *OPA1* operably linked to a promoter.

 Vectors, such as AAV vectors and others described herein, containing an *OPA1* gene operably linked to a promoter described herein may be administered to a patient (e.g., a human
30 patient) by a variety of routes of administration. Administration of an effective dose of the pharmaceutical composition comprising the vectors may be by exemplary routes of administration standard in the art, including, but not limited to, systemic (e.g., by intravenous administration), local (e.g., into the eye), and direct injection (e.g., subretinal). The route of administration may vary, for example, with the onset and severity of disease, and may include, e.g., subretinal, intraocular,
35 intravitreal, intravenous, intrathecal, intracerebroventricular, intraparenchymal, intracisternal, intradermal, transdermal, parenteral, intramuscular, intranasal, subcutaneous, percutaneous, intratracheal, intraperitoneal, intraarterial, intravascular, inhalation, perfusion, lavage, and administration.

 Treatment regimens may vary, and often depend on disease severity and the age, weight,
40 and sex of the patient. Treatment may include administration of vectors (e.g., viral vectors) or other

agents described herein as useful for the introduction of a transgene into a target cell in various unit doses. Each unit dose will ordinarily contain a predetermined quantity of the pharmaceutical composition. The AAV vector comprising the transgene encoding OPA1 described herein can be administered in an amount sufficient to improve one or more pathological features of ADOA.

- 5 Administration of the pharmaceutical compositions described herein may improve visual acuity in the subject, reduce the amount of vision loss in the subject, improve central vision of the subject, improve color vision of the subject, increase the expression of OPA1 in the subject, and/or improve retinal function in the subject.

10 **Pharmaceutical Compositions**

The polynucleotide, the nucleic acid molecule, or the vector comprising the polynucleotide or the nucleic acid molecule (e.g., the AAV vector) described herein can be formulated into a pharmaceutical composition for administration to a patient, such as a human patient having a disease or condition associated with a mutation in one or more *OPA1* alleles.

- 15 For example, the pharmaceutical compositions of the disclosure may be administered to a subject having an optic neuropathy, such as a subject diagnosed with or displaying one or more symptoms of a hereditary optic neuropathy (e.g., ADOA). The pharmaceutical compositions of the disclosure may also be administered to a subject having a systemic disease or condition caused by OPA1 protein deficiency, such as peripheral neuropathy, deafness, cerebellar ataxia, spastic
20 paraparesis, and myopathy.

- The pharmaceutical compositions of the disclosure may be provided in a biologically compatible form suitable for administration *in vivo*. A pharmaceutical composition containing a polynucleotide encoding OPA1 or a nucleic acid molecule including a transgene encoding OPA1, for example, the AAV vector comprising the polynucleotide or the nucleic acid molecule, typically
25 includes a pharmaceutically acceptable diluent or carrier. As the diluent or carrier, a sterile saline solution (typically a pharmaceutical grade saline), sterile water (typically a pharmaceutical grade water), or phosphate-buffered saline (PBS) (typically a pharmaceutical grade PBS) can be used. Pharmaceutical compositions of the disclosure can be formulated using, for example, physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences 16th edition,
30 Osol, A. Ed. (1980); incorporated herein by reference), and in a desired form, e.g., in the form of lyophilized formulations or aqueous solutions.

- Treatment regimens may vary, and often depend on disease severity and the age, weight, and sex of the patient. The disclosure provides the polynucleotide, the nucleic acid molecule, or the vector comprising the polynucleotide or the nucleic acid molecule (e.g., the AAV vector) for use in the
35 treatment or prevention of an optic neuropathy or OPA1-deficiency disorder, such as ADOA. Furthermore, the disclosure provides use of the polynucleotide, the nucleic acid molecule, or the vector comprising the polynucleotide or the nucleic acid molecule (e.g., the AAV vector) in the manufacture of a pharmaceutical composition for the treatment or prevention of an optic neuropathy or OPA1-deficiency disorder, such as ADOA.

Kits

The polynucleotide, nucleic acid molecule, vector, or pharmaceutical composition that achieves the expression of OPA1 protein in a subject described herein can be provided in a kit for use in treating OPA1-deficiency disorder, such as ADOA. The kit can include a package insert that instructs a user of the kit, such as a physician, to perform the methods described herein. The kit may optionally include a syringe or other device for administering the composition.

Examples

The following examples are put forth so as to provide those of ordinary skill in the art with a description of how the compositions and methods described herein may be used and evaluated and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention.

Example 1. Construction of plasmids for in vitro assay

Materials and Methods

The plasmid tested in this Example has a CMV promoter sequence, a nucleotide sequence encoding OPA1, and a PolyA signal sequence. The sequence from CMV promoter to PolyA signal was inserted between the NruI site and F1 ori start site of pcDNA3.1(+) (ThermoFisher Scientific V79020) from the 5' end to the 3' end, as shown in SEQ ID NO: 1. This plasmid is referred to as pOPA1-A. pOPA1-A includes the native codon nucleotide sequence of OPA1 (SEQ ID NO: 2).

Based on pOPA1-A, two codon-optimized sequences encoding OPA1 (SEQ ID NO: 3 and SEQ ID NO: 4) were inserted between the AgeI site and BamHI site. The plasmid containing the sequence of SEQ ID NO: 3 is referred to as pOPA1-B and the plasmid containing the sequence of SEQ ID NO: 4 is referred to as pOPA1-C. Each codon-optimized sequence is inserted to replace SEQ ID NO: 2 in pOPA1-A.

Based on pOPA1-A, a fragment was prepared by PCR from a plasmid containing a codon-optimized sequence encoding OPA1 (SEQ ID NO: 5) and inserted between the AgeI site and BamHI site. The plasmid containing the sequence of SEQ ID NO: 5 is referred to as pOPA1-D. The codon-optimized sequence was inserted to replace SEQ ID NO: 2 in pOPA1-A.

Based on pOPA1-A, human alpha-1-globin derived 5' UTR (SEQ ID NO: 6) was inserted between the SacI site and PflMI site. The plasmid containing the sequence of SEQ ID NO: 6 is referred to as pOPA1-E.

Based on pOPA1-A, a fragment was prepared by PCR from a plasmid containing human alpha-1-globin derived 3' UTR (SEQ ID NO: 7) and inserted between the BamHI site and XbaI site. The plasmid containing the sequence of SEQ ID NO: 7 is referred to as pOPA1-F.

Based on pOPA1-A, a virus derived sequence (SEQ ID NO: 8) was inserted between the SacI site and PflMI site. After this step, linked sequence of a virus derived sequence (SEQ ID NO: 9) was inserted between the BamHI site and XbaI site. The plasmid containing the sequence of SEQ ID NO: 8 and 9 is referred to as pOPA1-G.

Based on pOPA1-A, a fragment was prepared by PCR from a plasmid containing Woodchuck hepatitis virus (WHV) derived mutant WHV Posttranscriptional Regulatory Element (WPRE) sequence (SEQ ID NO: 10) and inserted between the BamHI site and XbaI site. The plasmid containing the sequence of SEQ ID NO: 10 is referred to as pOPA1-H.

Based on pOPA1-A, a fragment was prepared by PCR from a plasmid containing Woodchuck hepatitis virus derived short WPRE sequence (SEQ ID NO: 11) and inserted between the BamHI site and XbaI site. The plasmid containing the sequence of SEQ ID NO: 11 is referred to as pOPA1-I.

pOPA1-A to I were transformed using stbl3 (ThermoFisher Scientific C7373-03), and each colony was collected and incubated at 37°C for 1 day. Plasmid purification was performed for pOPA1-A using Nucleo Bond Xtra Maxi Plus EF (TaKaRa bio, 740426.50). pOPA1-B to I were purified using NucleoBond Xtra Midi Plus EF (TaKaRa bio, 740422.50).

The sequences contained by each plasmid are shown in Table 5 below. For the plasmid pOPA1-B to I, the full-length sequences from the promoter to the PolyA signal are SEQ ID NOs: 12-19, respectively.

Table 5. OPA1-Encoding Construct Sequences

Construct	5' UTR	OPA1-Coding Sequence	3' UTR	Promoter-5' UTR-CDS-3' UTR-PolyA Signal
pOPA1-A	--	SEQ ID NO: 2	--	SEQ ID NO: 1
pOPA1-B	--	SEQ ID NO: 3	--	SEQ ID NO: 12
pOPA1-C	--	SEQ ID NO: 4	--	SEQ ID NO: 13
pOPA1-D	--	SEQ ID NO: 5	--	SEQ ID NO: 14
pOPA1-E	SEQ ID NO: 6	SEQ ID NO: 2	--	SEQ ID NO: 15
pOPA1-F	--	SEQ ID NO: 2	SEQ ID NO: 7	SEQ ID NO: 16
pOPA1-G	SEQ ID NO: 8	SEQ ID NO: 2	SEQ ID NO: 9	SEQ ID NO: 17
pOPA1-H	--	SEQ ID NO: 2	SEQ ID NO: 10	SEQ ID NO: 18
pOPA1-I	--	SEQ ID NO: 2	SEQ ID NO: 11	SEQ ID NO: 19

Example 2. Homology analysis

Homology analysis was performed on the OPA1 encoding nucleotide sequences of the plasmids obtained in Example 1 using SnapGene (Ver5.3.2 GSL Biotech). Specifically, homology of SEQ ID NOs: 2, 3, 4, and 5 was confirmed. The percentages of homology are shown in Table 6 below.

Table 6. Sequence Homology of OPA1-Encoding Constructs

Homology (%)			
	pOPA1-A	pOPA1-B	pOPA1-C
pOPA1-B	74.39		
pOPA1-C	78.51	83.53	
pOPA1-D	75.32	84.12	83.11

Example 3. Preparation of in vitro samples

HEK293 cells (ATCC CRL-1573) were seeded into a collagen I-coated 24 well plate (IWAKI 4820-010) at a density of 100,000 cells per well and cultured with Dulbecco Modified Eagle Medium (Sigma D6429-500ML) containing 10% fetal bovine serum (FBS, Hyclone SH30084.03) and 1% Penicillin-Streptomycin (ThermoFisher Scientific 15070063) at 37°C with 5% CO₂ for 1 day.

Cells were transfected with 500 ng of each plasmid by using the ViaFect™ Transfection Reagent (Promega E4981). The cells were incubated for 2 days after transfection. Cells were lysed with 100 uL of RIPA buffer (ThermoFisher Scientific 89901) containing 1% Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (ThermoFisher scientific 78441) and 0.1% Benzonase® Nuclease, Purity > 99% (Millipore, 70664-10KUN). Cell lysates were centrifuged at 12,000 g for 10 mins at 4°C and the supernatants were collected in new 1.5 mL tubes.

The amount of total protein was calculated using the BCA protein assay kit (ThermoFisher Scientific 23227) on 96 well assay plates (IWAKI 3881-096). Absorbance was measured at 562 nm by Infinite M200PRO (TECAN). The concentrations of protein lysates were normalized with RIPA buffer (ThermoFisher Scientific 89901) containing 1% Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (ThermoFisher scientific 78441) and 0.1% Benzonase® Nuclease, Purity > 99% (Millipore, 70664-10KUN). Protein lysates were mixed with NuPAGE™ LDS Sample Buffer (ThermoFisher scientific NP0007) and NuPAGE™ Sample Reducing Agent (ThermoFisher scientific NP0009). The samples were heated at 95°C for 10 mins.

Example 4. Measurement of OPA1 protein using SDS-PAGE and Western Blotting*Materials and Methods*

Total protein was separated by SDS-PAGE on NuPAGE™ 4 to 12%, Bis-Tris, 1.0–1.5 mm, Mini Protein Gels (ThermoFisher scientific NP0329BOX) and then transferred onto PVDF membranes using Trans-Blot Turbo Mini 0.2 µm PVDF Transfer Packs (Bio-Rad 1704156). Non-specific protein binding was blocked using Blocking One (NACALAI TESQUE, INC. 03953-95). The proteins on the membranes were immunoblotted against anti-OPA1 (D6U6N) Rabbit mAb (Cell Signaling 80471S) and anti-GAPDH (14C10) Rabbit mAb (Cell Signaling 2118S). Immunoreactive bands were visualized using Anti-rabbit IgG, HRP-linked Antibody (Cell Signaling 7074S) and the ECL detection system (GE Healthcare RPN2236). Chemiluminescence signals were captured using a ChemiDoc Touch (Bio-Rad) and the protein expression levels were quantified using Image Lab (Bio-Rad).

Results

The expression level of OPA1 protein was normalized by GAPDH protein. The relative OPA1 protein expression level from each construct compared to the not treated sample was calculated (Fig. 1). The OPA1 expression levels of pOPA1-B, pOPA1-C, and pOPA1-D were higher than that of pOPA1-A.

Other Embodiments

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the invention that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are within the claims.

Claims

1. A polynucleotide encoding optic atrophy 1 (OPA1), wherein the polynucleotide has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of any one of SEQ ID NOs: 3-5.
2. The polynucleotide of claim 1, wherein the polynucleotide has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 3.
3. The polynucleotide of claim 2, wherein the polynucleotide has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 3.
4. The polynucleotide of claim 3, wherein the polynucleotide has a nucleic acid sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 3.
5. The polynucleotide of claim 4, wherein the polynucleotide has a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of SEQ ID NO: 3.
6. The polynucleotide of claim 5, wherein the polynucleotide has the nucleic acid sequence of SEQ ID NO: 3.
7. The polynucleotide of claim 1, wherein the polynucleotide has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 4.
8. The polynucleotide of claim 7, wherein the polynucleotide has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 4.
9. The polynucleotide of claim 8, wherein the polynucleotide has a nucleic acid sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 4.
10. The polynucleotide of claim 9, wherein the polynucleotide has a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of SEQ ID NO: 4.
11. The polynucleotide of claim 10, wherein the polynucleotide has the nucleic acid sequence of SEQ ID NO: 4.
12. The polynucleotide of claim 1, wherein the polynucleotide has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of SEQ ID NO: 5.
13. The polynucleotide of claim 12, wherein the polynucleotide has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NO: 5.
14. The polynucleotide of claim 13, wherein the polynucleotide has a nucleic acid sequence that is at least 98% identical to the nucleic acid sequence of SEQ ID NO: 5.

15. The polynucleotide of claim 14, wherein the polynucleotide has a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence of SEQ ID NO: 5.
16. The polynucleotide of claim 15, wherein the polynucleotide has the nucleic acid sequence of SEQ ID NO: 5.
17. A vector comprising the polynucleotide of any one of claims 1-16, optionally wherein the vector is a non-viral vector, or a viral vector.
18. The vector of claim 17, wherein the vector is a viral vector.
19. The vector of claim 18, wherein the viral vector is selected from the group consisting of an adeno-associated virus (AAV), an adenovirus, a lentivirus, a retrovirus, a poxvirus, a baculovirus, a herpes simplex virus, a vaccinia virus, and a synthetic virus.
20. The vector of claim 19, wherein the viral vector is an AAV.
21. The vector of claim 20, wherein the AAV comprises capsid proteins from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, AAVrh74, and AAV2.7m8.
22. The vector of any one of claims 17-21, wherein the polynucleotide is operably linked to a promoter.
23. The vector of claim 22, wherein the promoter is a cytomegalovirus (CMV) promoter, cone-rod homeobox (CRX) promoter, retinal homeobox gene 1 (rx1) promoter, retinal pigment epithelium 65 (RPE65) promoter, CMV early enhancer/chicken beta actin (CAG, CBh, CBA) promoter, gamma-synuclein gene (SNCG) promoter, synapsin I (SYNI) promoter, rhodopsin kinase (GRK1) promoter, or a phosphoglycerate kinase (PGK) promoter.
24. The vector of claim 23, wherein the promoter is a CMV promoter.
25. The vector of claim 24, wherein the CMV promoter has the nucleic acid sequence of SEQ ID NO: 20.
26. The vector of any one of claims 17-25, wherein the polynucleotide is operably linked to a Woodchuck Hepatitis Virus (WHV) Posttranscriptional Regulatory Element (WPRE).
27. The vector of claim 26, wherein the WPRE has the nucleic acid sequence of any one of SEQ ID Nos: 10 or 11.
28. The vector of any one of claims 17-27, wherein the polynucleotide further comprises a polyadenylation (PolyA) signal sequence.

29. The vector of claim 28, wherein the PolyA signal sequence comprises the simian virus 40 (SV40) late PolyA signal sequence, the SV40 early PolyA signal sequence, or human growth hormone PolyA signal sequence (hGH PolyA signal sequence), or shortened hGH PolyA signal sequence.

30. The vector of claim 29, wherein the PolyA signal sequence comprises the SV40 late PolyA signal sequence.

31. A plasmid encoding the viral vector of any one of claims 17-30.

32. A pharmaceutical composition comprising the polynucleotide of any one of claims 1-16, or the vector of any one of claims 17-30, and a pharmaceutically acceptable carrier, diluent, or excipient.

33. A nucleic acid molecule comprising:
(i) a CMV promoter (CMV);
(ii) a codon-optimized transgene encoding an OPA1 protein having the amino acid sequence of SEQ ID NO: 21 (OPA1);
(iii) a WPRE; and
(iv) a SV40 PolyA signal sequence (SV40 pA);
wherein the components are operably linked to each other in the 5'-to-3' direction as:
CMV-OPA1-WPRE-SV40 pA.

34. The nucleic acid molecule of claim 33, wherein the CMV promoter has the nucleic acid sequence of SEQ ID NO: 20.

35. The nucleic acid molecule of claim 33 or 34, wherein the transgene has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of any one of SEQ ID NOs: 2-5.

36. The nucleic acid molecule of claim 35, wherein the transgene has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of any one of SEQ ID NOs: 2-5.

37. The nucleic acid molecule of claim 36, wherein the transgene has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of any one of SEQ ID NOs: 2-5, optionally wherein the transgene has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 2-5.

38. The nucleic acid molecule of claim 37, wherein the transgene has the nucleic acid sequence of any one of SEQ ID NOs: 2-5.

39. The nucleic acid molecule of any one of claims 33-38, wherein the WPRE has the nucleic acid sequence of any one of SEQ ID NOs: 10 or 11.

40. The nucleic acid molecule of any one of claims 33-39, wherein the SV40 PolyA signal sequence comprises the SV40 late PolyA signal sequence or the SV40 early PolyA signal sequence.

41. The nucleic acid molecule of claim 40, wherein the PolyA signal sequence comprises the SV40 late PolyA signal sequence.

42. A vector comprising the nucleic acid molecule of any one of claims 33-41, optionally wherein the vector is a non-viral vector or a viral vector.

43. The vector of claim 42, wherein the vector is a viral vector.

44. The vector of claim 43, wherein the viral vector is selected from the group consisting of an AAV, an adenovirus, a lentivirus, a retrovirus, a poxvirus, a baculovirus, a herpes simplex virus, a vaccinia virus, and a synthetic virus.

45. The vector of claim 44, wherein the viral vector is an AAV.

46. The vector of claim 45, wherein the AAV comprises capsid proteins from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, AAVrh74, and AAV2.7m8.

47. A plasmid encoding the viral vector of any one of claims 43-46.

48. A pharmaceutical composition comprising the nucleic acid molecule of any one of claims 33-41 or the vector of any one of claims 42-46, and a pharmaceutically acceptable carrier, diluent, or excipient.

49. A method of treating a human patient diagnosed as having a neuro-ocular disorder or OPA1-deficiency disorder, the method comprising administering to the patient a therapeutically effective amount of the polynucleotide of any one of claims 1-16, the vector of any one of claims 17-30 and 42-46, the nucleic acid molecule of any one of claims 33-41, or the pharmaceutical composition of claim 32 or 48.

50. A method of improving central and color vision in a human patient diagnosed as having a neuro-ocular disorder or OPA1-deficiency disorder, the method comprising administering to the patient a therapeutically effective amount of the polynucleotide of any one of claims 1-16, the vector of any one of claims 17-30 and 42-46, the nucleic acid molecule of any one of claims 33-41, or the pharmaceutical composition of claim 32 or 48.

51. A method of improving retinal function in a human patient diagnosed as having a neuro-ocular disorder or OPA1-deficiency disorder, the method comprising administering to the patient a therapeutically effective amount of the polynucleotide of any one of claims 1-16, the vector

of any one of claims 17-30 and 42-46, the nucleic acid molecule of any one of claims 33-41, or the pharmaceutical composition of claim 32 or 48.

52. A method of improving visual acuity in a human patient diagnosed as having a neuro-ocular disorder or OPA1-deficiency disorder, the method comprising administering to the patient a therapeutically effective amount of the polynucleotide of any one of claims 1-16, the vector of any one of claims 17-30 and 42-46, the nucleic acid molecule of any one of claims 33-41, or the pharmaceutical composition of claim 32 or 48.

53. A method of increasing the expression of OPA1 in a human patient diagnosed as having a neuro-ocular disorder or OPA1-deficiency disorder, the method comprising administering to the patient a therapeutically effective amount of the polynucleotide of any one of claims 1-16, the vector of any one of claims 17-30 and 42-46, the nucleic acid molecule of any one of claims 33-41, or the pharmaceutical composition of claim 32 or 48.

54. The method of any one of claims 49-53, wherein the neuro-ocular disorder is an optic neuropathy.

55. The method of any one of claims 49-53, wherein the neuro-ocular disorder is a hereditary optic neuropathy.

56. The method of any one of claims 49-53, wherein the OPA1-deficiency disorder is Autosomal Dominant Optic Atrophy (ADOA).

57. A method of ameliorating a symptom associated with a deficiency in OPA1 expression or activity in a human patient, the method comprising administering to the patient a therapeutically effective amount of the polynucleotide of any one of claims 1-16, the vector of any one of claims 17-30 and 42-46, the nucleic acid molecule of any one of claims 33-41, or the pharmaceutical composition of claim 32 or 48.

58. The method of claim 57, wherein the symptom is selected from peripheral neuropathy, deafness, cerebellar ataxia, spastic paraparesis, and myopathy.

59. A kit comprising the polynucleotide of any one of claims 1-16, the vector of any one of claims 17-30 and 42-46, the nucleic acid molecule of any one of claims 33-41, or the pharmaceutical composition of claim 32 or 48, and a package insert, wherein the package insert instructs a user of the kit to administer the polynucleotide, nucleic acid, vector, or pharmaceutical composition to a human patient diagnosed as having a neuro-ocular disorder, an OPA1-deficiency disorder, or a symptom associated with a deficiency in OPA1 expression or activity.

60. The kit of claim 59, wherein the symptom is selected from peripheral neuropathy, deafness, cerebellar ataxia, spastic paraparesis, and myopathy.

FIG. 1

