

OPA1 ANTISENSE OLIGOMERS FOR TREATMENT OF CONDITIONS AND DISEASES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/023,013, filed May 11, 2020, and U.S. Provisional Application No. 63/112,458, filed November 11, 2020, each of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Alternative splicing events in genes can lead to non-productive mRNA transcripts which in turn can lead to aberrant or reduced protein expression, and therapeutic agents which can target the alternative splicing events in genes can modulate the expression level of functional proteins in patients and/or inhibit aberrant protein expression. Such therapeutic agents can be used to treat a condition or disease caused by the protein deficiency.

[0003] Autosomal dominant optic atrophy (ADOA) is one of the most commonly diagnosed optic neuropathies. This optic nerve disease is associated with structural and functional mitochondrial deficits that lead to degeneration of the retinal ganglion cells and progressive, irreversible loss of vision. A majority of ADOA patients carry mutations in OPA1 and most mutations lead to haploinsufficiency (Lenaers G. et al. Orphanet J Rare Dis 2012). OPA1 encodes a mitochondrial GTPase with a critical role in mitochondrial fusion, ATP synthesis and apoptosis. Currently, there is no approved disease-modifying treatment for ADOA patients and there is a need for such treatments.

SUMMARY

[0004] Described herein, in some aspects, is a method of modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an OPA1 gene and that comprises a non-sense mediated RNA decay-inducing exon (NMD exon), the method comprising contacting an agent or a vector encoding the agent to the cell, whereby the agent modulates splicing of the NMD exon from the pre-mRNA, thereby modulating a level of processed mRNA that is processed from the pre-mRNA, and modulating the expression of the OPA1 protein in the cell, wherein the agent comprises an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299.

[0005] In some embodiments, the agent: (a) binds to a targeted portion of the pre-mRNA; (b) modulates binding of a factor involved in splicing of the NMD exon; or (c) a combination of (a)

and (b). In some embodiments, the agent interferes with binding of the factor involved in splicing of the NMD exon to a region of the targeted portion. In some embodiments, the targeted portion of the pre-mRNA is proximal to the NMD exon. In some embodiments, the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NMD exon. In some embodiments, the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NMD exon. In some embodiments, the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NMD exon. In some embodiments, the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NMD exon. In some embodiments, the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509. In some embodiments, the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509. In some embodiments, the targeted portion of the pre-mRNA is at most

about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616. In some embodiments, the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616. In some embodiments, the targeted portion of the pre-mRNA is located in an intronic region between two canonical exonic regions of the pre-mRNA, and wherein the intronic region contains the NMD exon. In some embodiments, the targeted portion of the pre-mRNA at least partially overlaps with the NMD exon. In some embodiments, the targeted portion of the pre-mRNA at least partially overlaps with an intron upstream or downstream of the NMD exon. In some embodiments, the targeted portion of the pre-mRNA comprises 5' NMD exon-intron junction or 3' NMD exon-intron junction. In some embodiments, the targeted portion of the pre-mRNA is within the NMD exon. In some embodiments, the targeted portion of the pre-mRNA comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[0006] In some embodiments, the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279. In some embodiments, the NMD exon comprises a sequence of SEQ ID NO: 279. In some embodiments, the targeted portion of the pre-mRNA is within the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616. In some embodiments, the targeted portion of the pre-mRNA is upstream or downstream of the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616. In some embodiments, the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193628509 to 193628616. In some embodiments, the OPA1 protein expressed from the processed mRNA is a full-length OPA1 protein or a wild-type OPA1 protein. In some embodiments, the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein. In some embodiments, the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein. In some embodiments, the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein. In some embodiments, the OPA1 protein expressed from the processed mRNA is an OPA1 protein

that lacks an amino acid sequence encoded by a nucleic acid sequence with at least 80% sequence identity to SEQ ID NO: 277.

[0007] In some embodiments, the method promotes exclusion of the NMD exon from the pre-mRNA. In some embodiments, the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent. In some embodiments, the method results in an increase in the level of the processed mRNA in the cell. In some embodiments, the level of the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent. In some embodiments, the method results in an increase in the expression of the OPA1 protein in the cell. In some embodiments, a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[0008] In some embodiments, the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292. In some embodiments, the agent further comprises a gene editing molecule. In some embodiments, the gene editing molecule comprises CRISPR-Cas9.

[0009] Described herein, in some aspects, is a method of modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an OPA1 gene, wherein the pre-mRNA comprises a coding exon, the method comprising contacting an agent or a vector encoding the agent to the cell, whereby the agent promotes exclusion of the coding exon from the pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon in the cell. In some embodiments, the agent: (a) binds to a targeted portion of the pre-mRNA; (b) modulates binding of a factor involved in splicing of the coding exon; or (c) a combination of (a) and (b). In some embodiments, the agent interferes with binding of the factor involved in splicing of the coding exon to a region of the targeted portion. In some embodiments, the targeted portion of the pre-mRNA is proximal to the coding exon. In some embodiments, the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the coding exon. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 100 to 50, from 90 to 50, from 80 to 50, from 70 to 50, from 60 to 50, from 60 to 40, from 60 to 30, from 60 to 20, from 60 to 10, from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon. In some embodiments, the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the coding exon. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, from 1 to 19, from 10 to 60, from 20 to 60, from 30 to 60, from 40 to 60, from 50 to 60, from 50 to 70, from 50 to 80, from 50 to 90, or from 50 to 100 nucleotides downstream of 3' end of the coding exon. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, or from 1 to 19 nucleotides downstream of 3' end of the coding exon. In some embodiments, the targeted portion of the pre-mRNA at least partially overlaps with the coding exon. In some embodiments, the targeted portion of the pre-mRNA at least partially overlaps with an intron immediately upstream or immediately downstream of the coding exon. In some embodiments, the targeted portion of the pre-mRNA comprises 5' coding exon-intron junction or 3' coding exon-intron junction. In some embodiments, the targeted

portion is within the coding exon of the pre-mRNA. In some embodiments, the coding exon is an alternatively spliced exon.

[0010] In some embodiments, the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277. In some embodiments, the coding exon comprises SEQ ID NO: 277. In some embodiments, the targeted portion of the pre-mRNA is immediately upstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092. In some embodiments, the targeted portion of the pre-mRNA is immediately downstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, or from 1 to 19 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202. In some embodiments, the targeted portion of the pre-mRNA is within the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some embodiments, the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193626092 to 193626202. In some embodiments, the targeted portion comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the coding exon. In some embodiments, the targeted portion of the pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 277.

[0011] In some embodiments, the exclusion of the coding exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent. In some embodiments, the method results in an increase in expression of the OPA1 protein in the cell. In some embodiments, a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to

about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent. In some embodiments, a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by at least about 1.5-fold compared to in the absence of the agent.

[0012] In some embodiments, the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein. In some embodiments, the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein. In some embodiments, the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein. In some embodiments, the agent promotes exclusion of a non-sense mediated RNA decay-inducing exon (NMD exon) from the pre-mRNA. In some embodiments, the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279. In some embodiments, the NMD exon comprises a sequence of SEQ ID NO: 279. In some embodiments, the OPA1 protein expressed from the processed mRNA comprises fewer proteolytic cleavage sites than an OPA1 protein encoded by a corresponding mRNA containing the coding exon.

[0013] In some embodiments, the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242, 250, 280-283, 288, and 290-292. In some embodiments, the agent comprises a gene editing molecule. In some embodiments, the gene editing molecule comprises CRISPR-Cas9.

[0014] Described herein, in some aspects, is a method of modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an OPA1 gene, wherein the pre-mRNA comprises a coding exon, the method comprising contacting an agent or a vector encoding the agent to the cell, wherein the agent comprises an antisense oligomer that binds to: (a) a targeted portion of the pre-mRNA within an intronic region immediately upstream of a 5' end of the coding exon of the pre-mRNA; or (b) a targeted portion of the pre-mRNA within an intronic region immediately downstream of a 3' end of the coding exon of the pre-mRNA; whereby the agent increases a level of a processed mRNA that is processed from the pre-mRNA and that contains the coding exon in the cell.

[0015] In some embodiments, the coding exon is an alternatively spliced exon. In some embodiments, the method promotes inclusion of the coding exon in the processed mRNA during

splicing of the pre-mRNA in the cell. In some embodiments, the target portion of the pre-mRNA is within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of a 5' end of the coding exon. In some embodiments, the target portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of a 3' end of the coding exon. In some embodiments, the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277. In some embodiments, the coding exon comprises SEQ ID NO: 277. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202. In some embodiments, the inclusion of the coding exon in the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent. In some embodiments, the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267.

[0016] Described herein, in some aspects, is a method of modulating expression of a target protein in a cell having a pre-mRNA transcribed from a gene that encodes the target protein, wherein the pre-mRNA comprises a coding exon and a non-sense mediated RNA decay-inducing exon (NMD exon), the method comprising contacting an agent or a vector encoding the agent to the cell, wherein the agent promotes exclusion of both the coding exon and the NMD exon from the pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks both the NMD exon and the coding exon in the cell.

[0017] In some embodiments, the agent: (a) binds to a targeted portion of the pre-mRNA; (b) modulates binding of a factor involved in splicing of the coding exon, the NMD exon, or both; or (c) a combination of (a) and (b). In some embodiments, the agent interferes with binding of the

factor involved in splicing of the coding exon, the NMD exon, or both, to a region of the targeted portion. In some embodiments, the NMD exon is within an intronic region adjacent to the coding exon. In some embodiments, the NMD exon is within an intronic region immediately upstream of the coding exon. In some embodiments, the NMD exon is within an intronic region immediately downstream of the coding exon. In some embodiments, the targeted portion of the pre-mRNA is proximal to the coding exon. In some embodiments, the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the coding exon. In some embodiments, the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the coding exon. In some embodiments, the targeted portion of the pre-mRNA is located within the coding exon. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the coding exon to 100 nucleotides downstream of the coding exon. In some embodiments, the coding exon is an alternatively spliced exon. In some embodiments, the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277.

[0018] In some embodiments, the coding exon comprises SEQ ID NO: 277. In some embodiments, the targeted portion of the pre-mRNA is immediately upstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some embodiments, the targeted portion of the pre-mRNA is immediately downstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of GRCh38/ hg38: chr3 193626092. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092. to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202. In some embodiments, the targeted portion of the pre-mRNA is within the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some embodiments, the targeted portion of the pre-mRNA comprises an exon-intron junction of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some embodiments, the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the coding exon. In some embodiments, the targeted portion of the pre-mRNA is proximal to the NMD exon. In some embodiments, the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the NMD exon. In some embodiments, the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the NMD exon. In some embodiments, the targeted portion of the pre-mRNA is

located within the NMD exon. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the NMD exon to 100 nucleotides downstream of the NMD exon. In some embodiments, the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279. In some embodiments, the NMD exon comprises SEQ ID NO: 279. In some embodiments, the targeted portion of the pre-mRNA is immediately upstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some embodiments, the targeted portion of the pre-mRNA is immediately downstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some embodiments, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616. In some embodiments, the targeted portion of the pre-mRNA is within the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some embodiments, the targeted portion of the pre-mRNA comprises an exon-intron junction of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some embodiments, the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[0019] In some embodiments, the exclusion of the coding exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent. In some embodiments, the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at

least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent. In some embodiments, the agent results in an increase in the level of the processed mRNA in the cell. In some embodiments, the level of the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent. In some embodiments, the method results in an increase in expression of the target protein in the cell. In some embodiments, a level of the target protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent. In some embodiments, the target protein is an OPA1 protein. In some embodiments, a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by at least about 1.5-fold compared to in the absence of the agent. In some embodiments, the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.

[0020] In some embodiments, the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein. In some embodiments, the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.

[0021] In some embodiments, the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 236, 242, 250, 280-283, 288, and 290-292. In some embodiments, the agent comprises

a gene editing molecule. In some embodiments, the gene editing molecule comprises CRISPR-Cas9.

[0022] In some embodiments, the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage. In some embodiments, the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, or a 2'-O-methoxyethyl moiety. In some embodiments, the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises at least one modified sugar moiety. In some embodiments, each sugar moiety is a modified sugar moiety. In some embodiments, the agent is an antisense oligomer (ASO) and wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases. In some embodiments, the vector comprises a viral vector encoding the agent. In some embodiments, the viral vector comprises an adenoviral vector, adeno-associated viral (AAV) vector, lentiviral vector, Herpes Simplex Virus (HSV) viral vector, or retroviral vector.

[0023] In some embodiments, the method further comprises assessing mRNA level or expression level of the OPA1 protein. In some embodiments, the agent is a therapeutic agent.

[0024] Described herein, in some aspects, is a pharmaceutical composition comprising the therapeutic agent as disclosed herein or a vector encoding the therapeutic agent as disclosed herein, and a pharmaceutically acceptable excipient.

[0025] Described herein, in some aspects, is a pharmaceutical composition, comprising a therapeutic agent or a vector encoding a therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent comprises an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299. In some embodiments, the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292. In some

embodiments, the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242, 250, 280-283, 288, and 290-292. In some embodiments, the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267. In some embodiments, the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 236, 242, 250, 280-283, 288, and 290-292.

[0026] Described herein, in some aspects, is a composition, comprising an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299, wherein the antisense oligomer comprises a backbone modification, a sugar moiety modification, or a combination thereof. In some embodiments, the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292. In some embodiments, the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242, 250, 280-283, 288, and 290-292. In some embodiments, the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267. In some embodiments, the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 236, 242, 250, 280-283, 288, and 290-292.

[0027] Described herein, in some aspects, is a pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent promotes exclusion of a coding exon from a pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon in a cell, wherein the pre-mRNA is transcribed from an OPA1 gene and that comprises the coding exon.

[0028] Described herein, in some aspects, is a pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent comprises an antisense oligomer that binds to a pre-mRNA that is transcribed from an OPA1 gene in a cell, wherein the antisense oligomer binds to: (a) a targeted portion of the pre-mRNA within an intronic region immediately upstream of a 5' end of the coding exon of the pre-mRNA; or (b) a targeted portion of the pre-mRNA within an intronic region immediately downstream of a 3' end of the coding exon of the pre-mRNA; whereby the therapeutic agent increases a level of a processed mRNA that is processed from the pre-mRNA and that contains the coding exon in the cell.

[0029] Described herein, in some aspects, is a pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent promotes exclusion of both a coding exon and a non-sense mediated RNA decay-inducing exon (NMD exon) from a pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon and the NMD exon in a cell, wherein the pre-mRNA is transcribed from an OPA1 gene in the cell and comprises the coding exon and the NMD exon.

[0030] In some embodiments, the pharmaceutical composition is formulated for intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection. In some embodiments, the pharmaceutical composition is formulated for intravitreal injection. In some embodiments, the pharmaceutical composition further comprises a second therapeutic agent. In some embodiments, the second therapeutic agent comprises a small molecule. In some embodiments, the second therapeutic agent comprises an antisense oligomer. In some embodiments, the second therapeutic agent corrects intron retention. In some embodiments, the antisense oligomer is selected from the group consisting of Compound ID NOs: 1-303.

[0031] Described herein, in some aspects, is a method of treating or reducing the likelihood of developing a disease or condition in a subject in need thereof by modulating expression of an OPA1 protein in a cell of the subject, comprising contacting to cells of the subject the therapeutic agent as disclosed herein. In some embodiments, the disease or condition is associated with a loss-of-function mutation in an OPA1 gene. In some embodiments, the disease or condition is associated with haploinsufficiency of the OPA1 gene, and wherein the subject has a first allele encoding a functional OPA1 protein, and a second allele from which the OPA1 protein is not produced or produced at a reduced level, or a second allele encoding a nonfunctional OPA1 protein or a partially functional OPA1 protein. In some embodiments, the disease or condition comprises an eye disease or condition. In some embodiments, the disease or condition comprises ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome;

Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis. In some embodiments, the disease or condition comprises Optic atrophy type 1. In some embodiments, the disease or condition comprises autosomal dominant optic atrophy (ADOA). In some embodiments, the disease or condition is associated with an autosomal recessive mutation of OPA1 gene, wherein the subject has a first allele encoding from which: (i) OPA1 protein is not produced or produced at a reduced level compared to a wild-type allele; or (ii) the OPA1 protein produced is nonfunctional or partially functional compared to a wild-type allele, and a second allele from which: (iii) the OPA1 protein is produced at a reduced level compared to a wild-type allele and the OPA1 protein produced is at least partially functional compared to a wild-type allele; or (iv) the OPA1 protein produced is partially functional compared to a wild-type allele.

[0032] In some embodiments, the subject is a human. In some embodiments, the subject is a non-human animal. In some embodiments, the subject is a fetus, an embryo, or a child. In some embodiments, the cells that the methods and compositions described herein are applicable to are ex vivo. In some embodiments, the therapeutic agent is administered by intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection. In some embodiments, the therapeutic agent is administered by intravitreal injection. In some embodiments, the method disclosed herein treats the disease or condition.

INCORPORATION BY REFERENCE

[0033] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be

obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0035] FIGs. 1A-1C depict a schematic representation of a target mRNA that contains a non-sense mediated mRNA decay-inducing exon (NMD exon mRNA) and therapeutic agent-mediated exclusion of the nonsense-mediated mRNA decay-inducing exon to increase expression of the full-length target protein or functional RNA. **FIG. 1A** shows a cell divided into nuclear and cytoplasmic compartments. In the nucleus, a pre-mRNA transcript of a target gene undergoes splicing to generate mRNA, and this mRNA is exported to the cytoplasm and translated into target protein. For this target gene, some fraction of the mRNA contains a nonsense-mediated mRNA decay-inducing exon (NMD exon mRNA) that is degraded in the cytoplasm, thus leading to no target protein production. **FIG. 1B** shows an example of the same cell divided into nuclear and cytoplasmic compartments. Treatment with a therapeutic agent, such as an antisense oligomer (ASO), promotes the exclusion of the nonsense-mediated mRNA decay-inducing exon and results in an increase in mRNA, which is in turn translated into higher levels of target protein. **FIG. 1C** shows an example schematic of a Novel NMD exon inclusion event (Exon X) identified in the *OPAI* gene which leads to the introduction of a premature termination codon (PTC) resulting in a non-productive mRNA transcript degraded by non-sense mediated decay (NMD).

[0036] FIG. 2 depicts identification of an exemplary nonsense-mediated mRNA decay (NMD)-inducing exon in the *OPAI* gene. The identification of the NMD-inducing exon in the *OPAI* gene using RNA sequencing is shown, visualized in the UCSC genome browser. The upper panel shows a graphic representation of the *OPAI* gene to scale. Peaks corresponding to RNA sequencing reads were identified in intron GRCh38/hg38: chr3 193626204 to 193631611, shown in the middle panel. Bioinformatic analysis identified an exon-like sequence (bottom panel, sequence highlighted in uppercase; GRCh38/hg38: chr3 193628509 to 193628616) flanked by 3' and 5' splice sites. Inclusion of this exon leads to the introduction of a premature termination codon rendering the transcript a target of NMD.

[0037] FIG. 3 depicts identification of an exemplary nonsense-mediated mRNA decay (NMD)-inducing exon in the *OPAI* gene. The identification of the NMD-inducing exon in the *OPAI* gene using RNA sequencing is shown, visualized in the UCSC genome browser. The upper panel shows a graphic representation of the *OPAI* gene to scale. Peaks corresponding to RNA sequencing reads were identified in intron GRCh38/hg38: chr3 193593374 to 193614710, shown in the middle panel. Bioinformatic analysis identified an exon-like sequence (bottom panel, sequence highlighted in uppercase; GRCh38/hg38: chr3 193603500 to 193603557) flanked by 3'

and 5' splice sites. Inclusion of this exon leads to the introduction of a premature termination codon rendering the transcript a target of NMD.

[0038] FIG. 4 depicts confirmation of NMD-inducing exon via puromycin or cycloheximide treatment in various cell lines, as well as the confirmation of NMD-inducing exon in brain and retina samples. RT-PCR analysis using total RNA from water-treated, DMSO-treated, puromycin-treated, or cycloheximide-treated cells confirmed the presence of a band corresponding to the NMD-inducing exon 7x (GRCh38/hg38: chr3 193628509 to 193628616) of *OPAI* gene

[0039] FIG. 5 depicts an exemplary ASO walk around *OPAI* exon 7x (GRCh38/hg38: chr3 193628509 193628616) region. A graphic representation of an ASO walk performed for around *OPAI* exon 7x (GRCh38/hg38: chr3 193628509 193628616) region targeting sequences upstream of the 3' splice site, across the 3' splice site, exon 7x, across the 5' splice site, and downstream of the 5' splice site is shown. ASOs were designed to cover these regions by shifting 5 nucleotides at a time or 3 nucleotides across the splice site regions.

[0040] FIG. 6 depicts an *OPAI* exon 7x (GRCh38/hg38: chr3 193628509 193628616) region ASO walk evaluated by Taqman RT-qPCR. Graphs of fold-change of the *OPAI* productive mRNA product relative to Sham are plotted.

[0041] FIG. 7 depicts an *OPAI* exon 7x (GRCh38/hg38: chr3 193628509 193628616) region ASO walk evaluated by Taqman RT-qPCR. Graphs of fold-change of the *OPAI* productive mRNA product relative to Sham are plotted.

[0042] FIG. 8 illustrates expression of *OPAI* transcripts containing the NMD exon in HEK293 cells treated with increasing amounts of cycloheximide.

[0043] FIG. 9A illustrates RT-PCR data from the posterior segment of the eye of *Chlorocebus sabaues* (green monkey) at postnatal data P93 (3 months) and postnatal day P942 (2.6 years). Fig. 9A confirms expression of *OPAI* transcripts containing the NMD exon in these cells.

[0044] FIG. 9B illustrates quantification of the NMD exon abundance from **FIG. 9A**.

[0045] FIG. 10A illustrates RT-PCR of the productive and non-productive *OPAI* mRNA after treatment of HEK293 cells with various ASOs and cycloheximide.

[0046] FIG. 10B illustrates quantification of the data in **FIG. 10A**.

[0047] FIG. 11 illustrates expression of productive *OPAI* mRNA by quantitative PCR in HEK293 cells treated with various ASOs and not treated with cycloheximide.

[0048] FIG. 12A illustrates RT-PCR for non-productive *OPAI* mRNAs in HEK293 cells after treatment with ASO-14 and cycloheximide.

[0049] FIG. 12B illustrates quantification of productive *OPAI* mRNAs in HEK293 cells after treatment with ASO-14 in the absence of cycloheximide.

[0050] **FIG. 12C** illustrates protein expression of OPA1 in HEK293 cells after treatment with ASO-14 in the absence of cycloheximide.

[0051] **FIG. 13A** illustrates mRNA and protein levels of *OPA1* gene in *OPA1* haploinsufficient (*OPA1*^{+/-}) HEK293 cells.

[0052] **FIG. 13B** illustrates OPA1 protein expression in the *OPA1* haploinsufficient (*OPA1*^{+/-}) HEK293 cells after treatment with ASO-14.

[0053] **FIG. 13C** illustrates quantification of OPA1 protein expression in the *OPA1* haploinsufficient (*OPA1*^{+/-}) HEK293 cells after treatment with ASO-14.

[0054] **FIG. 14A** illustrates study design for the *in vivo* rabbit experiment of Example 14.

[0055] **FIG. 14B** illustrates levels of productive and non-productive *OPA1* mRNA and protein.

[0056] **FIG. 14C** illustrates quantification of the data from **FIG. 14B**.

[0057] **FIG. 15** illustrates exemplary OPA1 ASOs of this disclosure. The right two columns in the chart illustrate the chemical modifications of the exemplary ASOs. Each nucleotide of all the ASOs has 2'-O-methoxyethyl (2'MOE) modification ("MOE") unless otherwise noted, for instance, letters of larger font size (*e.g.*, **G**) are locked nucleic acids ("LNA"), underlined letters (*e.g.*, C) are 5' methyl-cytosines that have 2'-MOE moiety ("5MeC-MOE"), and some ASOs are noted as phosphorodiamidate morpholino oligomers ("PMO").

[0058] **FIG. 16A** illustrates RT-PCR results for *OPA1* mRNAs using probes spanning exon 7 and exon 8 in HEK293 cells after treatment with ASO-14 and cycloheximide.

[0059] **FIG. 16B** illustrates quantification of *OPA1* mRNAs in HEK293 cells after treatment with ASO-14 in the absence of cycloheximide based on qPCR using probes spanning exons 6 and 8, probes spanning exons 7 and 8, or probes spanning exons 23 and 24.

[0060] **FIG. 16C** illustrates sequencing data on the relative amount of various *OPA1* mRNA transcripts in HEK293 cells transfected with ASO-14.

[0061] **FIG. 17A** illustrates RT-PCR results for *OPA1* mRNAs using probes spanning exon 6 and exon 8 in HEK293 cells after treatment with various exemplary OPA1 ASOs.

[0062] **FIG. 17B** illustrates relative ratio of *OPA1* mRNA transcripts having exons 6, 7, and 8 in tandem ("6-7-8") over the total amount of "6-7-8" transcripts and transcripts having exons 6 and 8 in tandem ("6-8"), in HEK293 cells after treatment with various exemplary OPA1 ASOs.

[0063] **FIGs. 17C and 17D** illustrate quantification of *OPA1* mRNAs using probes spanning exons 6 and 8, and probes spanning exons 7 and 8, respectively, in HEK293 cells after treatment with various exemplary OPA1 ASOs.

[0064] **FIG. 18A** illustrates RT-PCR results for *OPA1* mRNAs using probes spanning exon 6 and exon 8 ("Exon 6-8 PCR"), or probes spanning exon 7x and exon 8 ("Exon 7x-8 PCR"), in

HEK293 cells after treatment with various exemplary OPA1 ASOs and treatment with cycloheximide.

[0065] **FIG. 18B** illustrates expression level of OPA1 protein in HEK293 cells after treatment with various exemplary OPA1 ASOs.

[0066] **FIG. 18C** illustrates dose response in *OPA1* mRNAs using probes spanning exon 6 and exon 8 in HEK293 cells after treatment with various exemplary OPA1 ASOs.

[0067] **FIGs. 18D and 18E** illustrate quantification of the dose response in *OPA1* mRNAs using probes spanning exons 6 and 8, probes spanning exons 7 and 8, probes spanning exons 23 and 24, respectively, in HEK293 cells after treatment with various exemplary OPA1 ASOs. **FIG. 18D** summarizes the Ct values for the qPCR reactions, and **FIG. 18E** summarizes the relative amounts.

[0068] **FIG. 18F** illustrates dose response in expression level of OPA1 protein in HEK293 cells after treatment with various exemplary OPA1 ASOs.

[0069] **FIGs. 19A-19D** illustrate RT-PCR results for *OPA1* mRNAs using probes spanning exon 6 and exon 8 ("Exon 6-8"), or probes spanning exon 7x and exon 8 ("Exon 7-8"), in HEK293 cells after treatment with various exemplary OPA1 ASO 18-mers and treatment with or without cycloheximide.

[0070] **FIGs. 20A-20B** illustrate RT-PCR results for *OPA1* mRNAs using probes spanning exon 6 and exon 8 ("Exon 6-8"), or probes spanning exon 7x and exon 8 ("Exon 7x-8"), in HEK293 cells after treatment with various exemplary OPA1 ASO 18-mers and treatment with or without cycloheximide.

[0071] **FIGs. 21A-21D** illustrate RT-PCR results for *OPA1* mRNAs using probes spanning exon 6 and exon 8 ("Exon 6-8"), or probes spanning exon 7x and exon 8 ("Exon 7-8"), in HEK293 cells after treatment with various exemplary OPA1 ASO 16-mers and treatment with or without cycloheximide.

[0072] **FIGs. 22A-22C** illustrate RT-PCR results for *OPA1* mRNAs using probes spanning exon 6 and exon 8 ("Exon 6-8"), or probes spanning exon 7x and exon 8 ("Exon 7x-8"), in HEK293 cells after treatment with various exemplary OPA1 ASO 15-mers and treatment with or without cycloheximide.

[0073] **FIGs. 23A-23B** illustrate dose response in *OPA1* mRNAs having Exon 6 and Exon 8 ("6-8"), having Exon 7 and Exon 8 ("7-8"), or having Exon 7x and Exon 8 ("7x-8") in HEK293 cells after treatment with different concentrations of various exemplary OPA1 ASOs.

[0074] **FIG. 24A** is a histogram that demonstrates ATP level was reduced in mock-treated OPA1+/- HEK293 cells as compared to OPA1+/+ HEK293 cells, and ASO-14 treatment of OPA1+/- HEK293 cells increased the ATP level in the cells.

[0075] FIGS. 24B-24C demonstrate the OPA1 protein was increased by ASO-14 in OPA1+/+ HEK293 cells. FIG. 24B shows the immunoblot gel images of OPA1 and β -actin proteins, and FIG. 24C is a histogram that summarizes quantification of the immunoblot results.

[0076] FIGS. 25A-25B show histograms that demonstrate mRNA (FIG. 25A) and protein expression (FIG. 25B) of *OPA1* gene were reduced in fibroblast cells from diagnosed patients that have haploinsufficient mutation in *OPA1* gene as compared to wildtype (WT) fibroblast cells. FIG. 25C shows a representative immunoblot image of OPA protein expression level in diseased fibroblast cells.

[0077] FIGS. 26A, 26B, and 26D show histograms that demonstrate exemplary antisense oligomer, ASO-14, decreased *OPA1* NMD exon inclusion (FIG. 26A), increased *OPA1* total mRNA level (FIG. 26B), and protein level (FIG. 26D) in wildtype (WT) fibroblast cells and fibroblast cells from diagnosed patients that have haploinsufficient mutation in *OPA1* gene. FIG. 26C shows representative immunoblot images of OPA1 protein and loading control β -Tubulin under all types of conditions.

[0078] FIGS. 27A-27E demonstrate that patient fibroblast cells (cell lines F35 and F36) show deficiencies in mitochondrial bioenergetics. FIG. 27A shows representative time courses of the oxygen consumption rate of WT cells, F35 cells, and F36 cells at baseline level and when challenged sequentially with oligomycin, FCCP, rotenone and antimycin A. FIGS. 27B-27E show histograms demonstrating that patient fibroblast cells, F35 and F36 cells had reduced basal oxygen consumption rate (FIG. 27B), ATP linked respiration (FIG. 27C), maximal respiration (FIG. 27D), and spare respiratory capacity (FIG. 27E), as compared to WT fibroblast cells.

[0079] FIGS. 28A-28D show histograms demonstrating that treatment of ASO-14 at 20 nM, 40 nM, and 60 nM increased basal oxygen consumption rate (FIG. 28A), ATP linked respiration (FIG. 28B), maximal respiration (FIG. 28C), and spare respiratory capacity (FIG. 28D) of F35 patient cells in a dose-dependent manner.

[0080] FIGS. 29A-29D show histograms demonstrating that treatment of ASO-14 at 20 nM, 40 nM, and 60 nM increased basal oxygen consumption rate (FIG. 29A), ATP linked respiration (FIG. 29B), maximal respiration (FIG. 29C), and spare respiratory capacity (FIG. 29D) of F36 patient cells in a dose-dependent manner.

DETAILED DESCRIPTION

[0081] Alternative splicing events in the *OPA1* gene can lead to non-productive mRNA transcripts which in turn can lead to aberrant protein expression, and therapeutic agents which can target the alternative splicing events in the *OPA1* gene can modulate the expression level of

functional proteins in DS patients and/or inhibit aberrant protein expression. Such therapeutic agents can be used to treat a condition caused by OPA1 protein deficiency.

[0082] One of the alternative splicing events that can lead to non-productive mRNA transcripts is the inclusion of an extra exon in the mRNA transcript that can induce non-sense mediated mRNA decay. The present disclosure provides compositions and methods for modulating alternative splicing of *OPA1* to increase the production of protein-coding mature mRNA, and thus, translated functional OPA1 protein. These compositions and methods include antisense oligomers (ASOs) that can cause exon skipping, e.g., pseudoexon skipping, and promote constitutive splicing of *OPA1* pre-mRNA. In various embodiments, functional OPA1 protein can be increased using the methods of the disclosure to treat a condition caused by OPA1 protein deficiency.

mRNA Splicing

[0083] Intervening sequences in RNA sequences or introns are removed by a large and highly dynamic RNA-protein complex termed the spliceosome, which orchestrates complex interactions between primary transcripts, small nuclear RNAs (snRNAs) and a large number of proteins. Spliceosomes assemble ad hoc on each intron in an ordered manner, starting with recognition of the 5' splice site (5'ss) by U1 snRNA or the 3'splice site (3'ss) by the U2 pathway, which involves binding of the U2 auxiliary factor (U2AF) to the 3'ss region to facilitate U2 binding to the branch point sequence (BPS). U2AF is a stable heterodimer composed of a U2AF2-encoded 65-kD subunit (U2AF65), which binds the polypyrimidine tract (PPT), and a U2AF1-encoded 35-kD subunit (U2AF35), which interacts with highly conserved AG dinucleotides at 3'ss and stabilizes U2AF65 binding. In addition to the BPS/PPT unit and 3'ss/5'ss, accurate splicing requires auxiliary sequences or structures that activate or repress splice site recognition, known as intronic or exonic splicing enhancers or silencers. These elements allow genuine splice sites to be recognized among a vast excess of cryptic or pseudo-sites in the genome of higher eukaryotes, which have the same sequences but outnumber authentic sites by an order of magnitude. Although they often have a regulatory function, the exact mechanisms of their activation or repression are poorly understood.

[0084] The decision of whether to splice or not to splice can be typically modeled as a stochastic rather than deterministic process, such that even the most defined splicing signals can sometimes splice incorrectly. However, under normal conditions, pre-mRNA splicing proceeds at surprisingly high fidelity. This is attributed in part to the activity of adjacent cis-acting auxiliary exonic and intronic splicing regulatory elements (ESRs or ISRs). Typically, these functional elements are classified as either exonic or intronic splicing enhancers (ESEs or ISEs) or silencers (ESSs or ISSs) based on their ability to stimulate or inhibit splicing, respectively. Although there

is now evidence that some auxiliary cis-acting elements may act by influencing the kinetics of spliceosome assembly, such as the arrangement of the complex between U1 snRNP and the 5' splice site, it seems very likely that many elements function in concert with trans-acting RNA-binding proteins (RBPs). For example, the serine- and arginine-rich family of RBPs (SR proteins) is a conserved family of proteins that have a key role in defining exons. SR proteins promote exon recognition by recruiting components of the pre-spliceosome to adjacent splice sites or by antagonizing the effects of ESSs in the vicinity. The repressive effects of ESSs can be mediated by members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family and can alter recruitment of core splicing factors to adjacent splice sites. In addition to their roles in splicing regulation, silencer elements are suggested to have a role in repression of pseudo-exons, sets of decoy intronic splice sites with the typical spacing of an exon but without a functional open reading frame. ESEs and ESSs, in cooperation with their cognate trans-acting RBPs, represent important components in a set of splicing controls that specify how, where and when mRNAs are assembled from their precursors.

[0085] Alternative splicing is a regulated process during gene expression that can result in multiple isoforms of mature mRNA transcripts that are processed from a single primary mRNA transcript that is transcribed from a single gene, and the resultant multiple proteins that are translated from at least some of the multiple mature mRNA isoforms. In this process, particular exons of a gene may be included within or excluded from the final, processed mRNA produced from that gene. Consequently, the proteins translated from alternatively spliced mRNAs will contain differences in their amino acid sequence and, in some cases, in their biological functions.

[0086] As described herein, an “alternatively spliced exon” can refer to an exon of a gene that can be either included or excluded naturally from a mature mRNA transcript, thus resulting in different protein products that are translated from the different mature mRNA transcripts. The inclusion or skipping of an alternatively spliced exon can take place naturally in a cell, either randomly, or in a regulated manner, *e.g.*, subject to regulation by external physiological or pathological stimuli, or intracellular signaling. In some cases, the production of alternatively spliced mRNAs, *e.g.*, the splicing of the alternatively spliced exon, is regulated by a system of trans-acting proteins that bind to cis-acting sites on the primary transcript itself. In some cases, an alternatively spliced exon is a coding exon, *e.g.*, an exon that, when included in the mature mRNA transcript, is translated into an amino acid sequence as part of the protein product translated from the mature mRNA transcript. In some cases, the inclusion of an alternatively spliced exon in the mature mRNA transcript would maintain the canonical open reading frame as compared to a mature mRNA transcript without the alternatively spliced exon, *e.g.*, the number of nucleotides in the alternatively spliced exon is divisible by 3.

[0087] The sequences marking the exon-intron boundaries are degenerate signals of varying strengths that can occur at high frequency within human genes. In multi-exon genes, different pairs of splice sites can be linked together in many different combinations, creating a diverse array of transcripts from a single gene. This is commonly referred to as alternative pre-mRNA splicing. Although most mRNA isoforms produced by alternative splicing can be exported from the nucleus and translated into functional polypeptides, different mRNA isoforms from a single gene can vary greatly in their translation efficiency. Those mRNA isoforms with premature termination codons (PTCs) at least 50 bp upstream of an exon junction complex are likely to be targeted for degradation by the nonsense-mediated mRNA decay (NMD) pathway. Mutations in traditional (BPS/PPT/3'ss/5'ss) and auxiliary splicing motifs can cause aberrant splicing, such as exon skipping or cryptic (or pseudo-) exon inclusion or splice-site activation, and contribute significantly to human morbidity and mortality. Both aberrant and alternative splicing patterns can be influenced by natural DNA variants in exons and introns.

[0088] Given that exon-intron boundaries can occur at any of the three positions of a codon, it is clear that only a subset of alternative splicing events can maintain the canonical open reading frame. For example, only exons that are evenly divisible by 3 can be skipped or included in the mRNA without any alteration of reading frame. Splicing events that do not have compatible phases will induce a frame-shift. Unless reversed by downstream events, frame-shifts can certainly lead to one or more PTCs, probably resulting in subsequent degradation by NMD. NMD is a translation-coupled mechanism that eliminates mRNAs containing PTCs. NMD can function as a surveillance pathway that exists in all eukaryotes. NMD can reduce errors in gene expression by eliminating mRNA transcripts that contain premature stop codons. Translation of these aberrant mRNAs could, in some cases, lead to deleterious gain-of-function or dominant-negative activity of the resulting proteins. NMD targets not only transcripts with PTCs but also a broad array of mRNA isoforms expressed from many endogenous genes, suggesting that NMD is a master regulator that drives both fine and coarse adjustments in steady-state RNA levels in the cell.

[0089] A NMD-inducing exon ("NIE" or "NMD exon") is an exon or a pseudo-exon that is a region within an intron and can activate the NMD pathway if included in a mature RNA transcript. In constitutive splicing events, the intron containing an NMD exon is usually spliced out, but the intron or a portion thereof (e.g. NMD exon) may be retained during alternative or aberrant splicing events. Mature mRNA transcripts containing such an NMD exon may be non-productive due to frame shifts which induce the NMD pathway. Inclusion of a NMD exon in mature RNA transcripts may downregulate gene expression. mRNA transcripts containing an

NMD exon may be referred to as “NIE-containing mRNA” or “NMD exon mRNA” in the current disclosure.

[0090] Cryptic (or pseudo- splice sites) have the same splicing recognition sequences as genuine splice sites but are not used in splicing reactions. They outnumber genuine splice sites in the human genome by an order of a magnitude and are normally repressed by thus far poorly understood molecular mechanisms. Cryptic 5' splice sites have the consensus NNN/GUNNNN or NNN/GCNNNN where N is any nucleotide and / is the exon-intron boundary. Cryptic 3' splice sites have the consensus NAG/N. Their activation is positively influenced by surrounding nucleotides that make them more similar to the optimal consensus of authentic splice sites, namely MAG/GURAGU and YAG/G, respectively, where M is C or A, R is G or A, and Y is C or U.

[0091] Splice sites and their regulatory sequences can be readily identified by a skilled person using suitable algorithms publicly available, listed for example in Kralovicova, J. and Vorechovsky, I. (2007) Global control of aberrant splice site activation by auxiliary splicing sequences: evidence for a gradient in exon and intron definition. *Nucleic Acids Res.*, 35, 6399-6413 (www.ncbi.nlm.nih.gov/pmc/articles/PMC2095810/pdf/gkm680.pdf).

[0092] The cryptic splice sites or splicing regulatory sequences may compete for RNA-binding proteins, such as U2AF, with a splice site of the NMD exon. In some embodiments, an agent may bind to a cryptic splice site or splicing regulatory sequence to prevent binding of RNA-binding proteins and thereby favor binding of RNA-binding proteins to the NMD exon splice sites.

[0093] In some embodiments, the cryptic splice site may not comprise the 5' or 3' splice site of the NMD exon. In some embodiments, the cryptic splice site may be at least 10 nucleotides, at least 20 nucleotides, at least 50 nucleotides, at least 100 nucleotides or at least 200 nucleotides upstream of the NMD exon 5' splice site. In some embodiments, the cryptic splice site may be at least 10 nucleotides, at least 20 nucleotides, at least 50 nucleotides, at least 100 nucleotides, at least 200 nucleotides downstream of the NMD exon 3' splice site.

Target Transcripts

[0094] In some embodiments, the methods and compositions of the present disclosure exploit the presence of NMD exon in the pre-mRNA transcribed from the *OPAI* gene. Splicing of the identified *OPAI* NMD exon pre-mRNA species to produce functional mature *OPAI* mRNA may be induced using an agent such as an ASO that stimulates exon skipping of an NMD exon. Induction of exon skipping may result in inhibition of an NMD pathway. The resulting mature *OPAI* mRNA can be translated normally without activating NMD pathway, thereby increasing the amount of OPA1 protein in the patient's cells and alleviating symptoms of a condition or

disease associated with OPA1 deficiency, such as an eye disease or condition, Optic atrophy type 1, autosomal dominant optic atrophy (ADOA), ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

[0095] In some embodiments, the methods and compositions of the present disclosure exploit the alternative splicing of the pre-mRNA transcribed from the *OPA1* gene. In some cases, splicing of a coding exon, *e.g.*, an alternatively spliced exon, *e.g.*, *OPA1* exon 7 (or an exon encoded by genomic region spanning from GRCh38/ hg38: chr3 193626092 to 193626202), can modulate the level of OPA1 protein expressed from the *OPA1* gene. As described herein, the term “*OPA1* exon 7” or grammatically equivalents thereof, is used interchangeably with the term “exon (GRCh38/ hg38: chr3 193626092 to 193626202)” or “an exon encoded by genomic region spanning from GRCh38/ hg38: chr3 193626092 to 193626202.” Without wishing to be bound by a certain theory, the presence or absence of an amino acid sequence encoded by exon 7 or exon (GRCh38/ hg38: chr3 193626092 to 193626202) can modulate the stability of the OPA1 protein. For instance, in some cases, the OPA1 protein encoded by a mature mRNA transcript that lacks exon 7 can have fewer proteolytic cleavage sites as compared to an OPA1 protein encoded by a corresponding mature mRNA transcript that has contains exon 7. In some cases, the OPA1 protein an OPA1 protein encoded by a corresponding mature mRNA transcript that has contains encoded by a mature mRNA transcript that lacks exon 7 is a functional protein. The OPA1 protein encoded by a mature mRNA transcript that lacks exon 7 can be at least partially functional as compared to an OPA1 protein encoded by a corresponding mature mRNA transcript that has contains exon 7. In some cases, the OPA1 protein encoded by a mature

mRNA transcript that lacks exon 7 is at least partially functional as compared to a full-length wild-type OPA1 protein. In some cases, increase of OPA1 protein encoded by a mature mRNA transcript that lacks exon 7 in a cell can result in more functional OPA1 protein in the cell, due to the higher stability of the OPA1 protein lacking exon 7 and its at least partial functional equivalence.

[0096] In other embodiments, a coding exon of *OPA1* pre-mRNA other than exon 7 is targeted by an agent disclosed herein, which promotes exclusion of the coding exon other than exon 7. In these other embodiments, the agent that promotes exclusion of the coding exon other than exon 7 increases expression of OPA1 protein encoded by a mature mRNA transcript that lacks the excluded exon.

[0097] Alternative splicing of the *OPA1* pre-mRNA species, *e.g.*, skipping of a coding exon, *e.g.*, an alternatively spliced exon, *e.g.*, exon 7, to produce functional mature OPA1 protein may be induced using an agent such as an ASO that stimulates the exon skipping. Induction of exon skipping may result in modulation of levels of different alternatively spliced mRNA transcripts. The resulting mature *OPA1* mRNA can be translated into different OPA1 proteins, thereby modulating the amount of OPA1 protein in the patient's cells and alleviating symptoms of a condition or disease associated with OPA1 deficiency, such as an eye disease or condition, Optic atrophy type 1, autosomal dominant optic atrophy (ADOA), ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; charcot-Marie-tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

[0098] In some embodiments, the diseases or conditions that can be treated or ameliorated using the method or composition disclosed herein are not directly associated with the target protein (gene) that the therapeutic agent targets. In some embodiments, a therapeutic agent provided herein can target a protein (gene) that is not directly associated with a disease or condition, but the modulation of expression of the target protein (gene) can treat or ameliorate the disease or condition.

[0099] In various embodiments, the present disclosure provides a therapeutic agent which can target *OPAI* mRNA transcripts to modulate splicing or protein expression level. The therapeutic agent can be a small molecule, polynucleotide, or polypeptide. In some embodiments, the therapeutic agent is an ASO. Various regions or sequences on the *OPAI* pre-mRNA can be targeted by a therapeutic agent, such as an ASO. In some embodiments, the ASO targets an *OPAI* pre-mRNA transcript containing an NMD exon. In some embodiments, the ASO targets a sequence within an NMD exon of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence upstream (or 5') from the 5' end of an NMD exon (3'ss) of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence downstream (or 3') from the 3' end of an NMD exon (5'ss) of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking on the 5' end of the NMD exon of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking the 3' end of the NMD exon of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising an NMD exon-intron boundary of an *OPAI* pre-mRNA transcript. An NMD exon-intron boundary can refer to the junction of an intron sequence and an NMD exon region. The intron sequence can flank the 5' end of the NMD exon, or the 3' end of the NMD exon. In some embodiments, the ASO targets a sequence within an exon of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence within an intron of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising both a portion of an intron and a portion of an exon of an *OPAI* pre-mRNA transcript.

[0100] In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides upstream (or 5') from the 5' end of the NMD exon. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, or about 250 to about 300 nucleotides upstream (or 5') from the 5' end of the NMD exon region. In some embodiments, the ASO may target a sequence more than 300 nucleotides upstream from the 5' end of the NMD exon. In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides downstream (or 3') from the 3' end of the

NMD exon. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, or about 250 to about 300 nucleotides downstream from the 3' end of the NMD exon. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from the 3' end of the NMD exon.

[0101] In some embodiments, the *OPAI* NMD exon-containing pre-mRNA transcript is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO. 1. In some embodiments, the *OPAI* NMD exon pre-mRNA transcript comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs: 2-5.

[0102] In some embodiments, the *OPAI* NMD exon-containing pre-mRNA transcript (or NMD exon mRNA) comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 2-5. In some embodiments, *OPAI* NMD exon-containing pre-mRNA transcript (or NMD exon mRNA) is encoded by a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 2-5. In some embodiments, the targeted portion of the NMD exon mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5.

[0103] In some embodiments, the ASO targets exon 6x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 6, exon 7x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 7, or exon 28x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 28. In some embodiments, the ASO targets exon (GRCh38/ hg38: chr3 193628509 193628616) of *OPAI* pre-mRNA; or exon (GRCh38/ hg38: chr3 193603500 193603557) of *OPAI*. In some embodiments, the ASO targets an NMD exon of *OPAI* pre-mRNA other than NMD exon (GRCh38/hg38: chr3 193628509 193628616).

[0104] In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from the 5' end of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*. In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50

nucleotides upstream (or 5') from GRCh38/ hg38: chr3 193628509 of *OPAI*; or GRCh38/ hg38: chr3 193603500 of *OPAI*.

[0105] In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from the 5' end of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*. In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from GRCh38/ hg38: chr3 193628509 of *OPAI*; or GRCh38/ hg38: chr3 193603500 of *OPAI*.

[0106] In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from the 3' end of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*. In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from GRCh38/ hg38: chr3 193628616 of *OPAI*; or GRCh38/ hg38: chr3 193603557 of *OPAI*.

[0107] In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from the 3' end of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*. In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from GRCh38/ hg38: chr3 193628616 of *OPAI*; or GRCh38/ hg38: chr3 193603557 of *OPAI*.

[0108] In some embodiments, the ASO has a sequence complementary to the targeted portion of the NMD exon mRNA according to any one of SEQ ID NOs: 2-5, or 279.

[0109] In some embodiments, the ASO targets a sequence upstream from the 5' end of an NMD exon. For example, ASOs targeting a sequence upstream from the 5' end of an NMD exon (exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*) comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3. For example, ASOs targeting a sequence upstream from the 5' end of an NMD exon (e.g., exon (GRCh38/ hg38: chr3 193628509 to 193628616) of *OPAI*; or exon (GRCh38/ hg38: chr3 193603500 193603557) of *OPAI*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 4 or 5.

[0110] In some embodiments, the ASOs target a sequence containing an exon-intron boundary (or junction). For example, ASOs targeting a sequence containing an exon-intron boundary can comprise a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5. In some embodiments, the ASOs target a sequence downstream from the 3' end of an NMD exon. For example, ASOs targeting a sequence downstream from the 3' end of an NMD exon (e.g., exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 2 or 3, or at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3. For example, ASOs targeting a sequence downstream from the 3' end of an NMD exon (e.g., exon (GRCh38/ hg38: chr3 193628509 to 193628616) of *OPAI*; or exon (GRCh38/ hg38: chr3 193603500 to 193603557) of *OPAI*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 4 or 5, or at least 8 contiguous nucleic acids of SEQ ID NO: 4 or 5. In some embodiments, ASOs target a sequence within an NMD exon.

[0111] In some embodiments, the ASO targets exon 6x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 6, exon 7x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 7, or exon 28x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 28. In some embodiments, the ASO targets a sequence downstream (or 3') from the 5' end of exon 6x, exon 7x, or exon 28x of an *OPAI* pre-mRNA. In some embodiments, the ASO targets a sequence upstream (or 5') from the 3' end of exon 6x, exon 7x, or exon 28x of an *OPAI* pre-mRNA.

[0112] In some embodiments, the targeted portion of the *OPAI* NMD exon-containing pre-mRNA is in intron 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50. In some embodiments, hybridization of an ASO to the targeted portion of the NMD exon

pre-mRNA results in exon skipping of at least one of NMD exon within intron 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, and subsequently increases OPA1 protein production. In some embodiments, the targeted portion of the *OPA1* NMD exon-containing pre-mRNA is in intron 6 of *OPA1*, or intron 28 of *OPA1*. In some embodiments, the targeted portion of the *OPA1* NMD exon-containing pre-mRNA is intron (GRCh38/ hg38: chr3 193626203 to 193631611) of *OPA1*; or intron (GRCh38/ hg38: chr3 193593374 to 193614710) of *OPA1*.

[0113] In some embodiments, the methods and compositions of the present disclosure are used to increase the expression of OPA1 by inducing exon skipping of a pseudo-exon of an *OPA1* NMD exon-containing pre-mRNA. In some embodiments, the pseudo-exon is a sequence within any of introns 1-50. In some embodiments, the pseudo-exon is a sequence within any of introns 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50. In some embodiments, the pseudo-exon can be an *OPA1* intron or a portion thereof. In some embodiments, the pseudo-exon is within intron 6 of *OPA1*, or intron 28 of *OPA1*. In some embodiments, the pseudo-exon is within intron (GRCh38/ hg38: chr3 193626203 to 193631611) of *OPA1*; or intron (GRCh38/ hg38: chr3 193593374 to 193614710) of *OPA1*.

[0114] In some embodiments, the ASO targets an *OPA1* pre-mRNA transcript to induce exon skipping of a coding exon, *e.g.*, an alternatively spliced exon. In some embodiments, the ASO targets a sequence within a coding exon, *e.g.*, an alternatively spliced exon, of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence upstream (or 5') from the 5' end of a coding exon (3'ss) of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence downstream (or 3') from the 3' end of a coding exon (5'ss) of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking on the 5' end of the coding exon of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking the 3' end of the coding exon of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising an exon-intron boundary of an *OPA1* pre-mRNA transcript. An exon-intron boundary can refer to the junction of an intron sequence and an exon sequence. The intron sequence can flank the 5' end of the coding exon, or the 3' end of the coding exon. In some embodiments, the ASO targets a sequence within an exon of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence within an intron of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising both a portion of an intron and a portion of an exon of an *OPA1* pre-mRNA transcript.

[0115] In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides upstream (or 5') from the 5' end of the coding exon, *e.g.*, alternatively spliced exon. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, or about 250 to about 300 nucleotides upstream (or 5') from the 5' end of the coding exon region. In some embodiments, the ASO may target a sequence more than 300 nucleotides upstream from the 5' end of the coding exon. In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides downstream (or 3') from the 3' end of the coding exon. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, or about 250 to about 300 nucleotides downstream from the 3' end of the coding exon. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from the 3' end of the coding exon.

[0116] In some embodiments, the *OPAI* pre-mRNA transcript is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO. 1. In some embodiments, the *OPAI* pre-mRNA transcript comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs: 2-5.

[0117] In some embodiments, the *OPAI* pre-mRNA transcript (or NMD exon mRNA) comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 2-5. In some embodiments, *OPAI* pre-mRNA transcript (or NMD exon mRNA) is encoded by a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 2-5. In some embodiments, the targeted portion of the *OPAI* pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5.

[0118] In some embodiments, the ASO targets exon 7 of an *OPAI* pre-mRNA, *i.e.*, the ASO targets exon (GRCh38/ hg38: chr3 193626092 to 193626202) of *OPAI* pre-mRNA.

[0119] In some embodiments, the ASO targets a coding exon of an *OPAI* pre-mRNA other than exon 7, *i.e.*, the ASO targets an exon of *OPAI* pre-mRNA other than exon defined by (GRCh38/ hg38: chr3 193626092 to 193626202).

[0120] In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100

nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from the 5' end of exon 7 of *OPAI*. In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from GRCh38/ hg38: chr3 193626092 of *OPAI*.

[0121] In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from the 5' end of exon 7 of *OPAI*. In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from GRCh38/ hg38: 193626092 of *OPAI*.

[0122] In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from the 3' end of exon 7 of *OPAI*. In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from GRCh38/ hg38: chr3 193626202 of *OPAI*.

[0123] In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from the 3' end of exon 7 of *OPAI*. In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80

nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from GRCh38/ hg38: chr3 193626202 of *OPA1*.

[0124] In some embodiments, the ASO has a sequence complementary to the targeted portion of the NMD exon mRNA according to any one of SEQ ID NOs: 2-5, or 277.

[0125] In some embodiments, the ASO targets a sequence upstream from the 5' end of a coding exon, *e.g.*, an alternatively spliced exon. For example, ASOs targeting a sequence upstream from the 5' end of a coding exon (*e.g.*, exon 7 of *OPA1*) comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3. For example, ASOs targeting a sequence upstream from the 5' end of a coding exon (*e.g.*, exon (GRCh38/ hg38: 193626092 to 193626202) of *OPA1*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 4 or 5.

[0126] In some embodiments, the ASOs target a sequence containing an exon-intron boundary (or junction). For example, ASOs targeting a sequence containing an exon-intron boundary can comprise a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5. In some embodiments, the ASOs target a sequence downstream from the 3' end of a coding exon, *e.g.*, an alternatively spliced exon. For example, ASOs targeting a sequence downstream from the 3' end of a coding exon (*e.g.*, exon 7 of *OPA1*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 2 or 3, or at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3. For example, ASOs targeting a sequence downstream from the 3' end of a coding exon (*e.g.*, exon 7 of *OPA1*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 4 or 5, or at least 8 contiguous nucleic acids of SEQ ID NO: 4 or 5. In some embodiments, ASOs target a sequence within a coding exon, *e.g.*, an alternatively spliced exon.

Protein Expression

[0127] In some embodiments, the methods described herein are used to increase the production of a functional OPA1 protein or RNA. As used herein, the term "functional" refers to the amount of activity or function of an OPA1 protein or RNA that is necessary to eliminate any one or more symptoms of a treated condition or disease, *e.g.*, Optic atrophy type 1. In some embodiments, the methods are used to increase the production of a partially functional OPA1 protein or RNA. As used herein, the term "partially functional" refers to any amount of activity or function of the OPA1 protein or RNA that is less than the amount of activity or function that is necessary to eliminate or prevent any one or more symptoms of a disease or condition. In some embodiments, a partially functional protein or RNA will have at least 10%, at least 20%, at least 30%, at least

40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% less activity relative to the fully functional protein or RNA.

[0128] In some embodiments, the method is a method of increasing the expression of the OPA1 protein by cells of a subject having an *OPA1* pre-mRNA, wherein the subject has a disease or condition, *e.g.*, Optic atrophy type 1, caused by a deficient amount of activity of OPA1 protein, and wherein the deficient amount of the OPA1 protein is caused by haploinsufficiency of the OPA1 protein. In such an embodiment, the subject has a first allele encoding a functional OPA1 protein, and a second allele from which the OPA1 protein is not produced. In another such embodiment, the subject has a first allele encoding a functional OPA1 protein, and a second allele encoding a nonfunctional OPA1 protein. In another such embodiment, the subject has a first allele encoding a functional OPA1 protein, and a second allele encoding a partially functional OPA1 protein. In any of these embodiments, the antisense oligomer binds to a targeted portion of the *OPA1* pre-mRNA transcribed from the second allele, thereby inducing exon skipping of the pseudo-exon from the pre-mRNA, and causing an increase in the level of mature mRNA encoding functional OPA1 protein, and an increase in the expression of the OPA1 protein in the cells of the subject.

[0129] In some embodiments, the method is a method of increasing the expression of the OPA1 protein by cells of a subject having an *OPA1* pre-mRNA, wherein the subject has a disease or condition caused by a deficient amount of activity of OPA1 protein, and wherein the deficient amount of the OPA1 protein is caused by autosomal recessive inheritance.

[0130] In some embodiments, the method is a method of increasing the expression of the OPA1 protein by cells of a subject having an *OPA1* pre-mRNA, wherein the subject has a disease or condition, *e.g.*, Optic atrophy type 1, caused by a deficient amount of activity of OPA1 protein, and wherein the deficient amount of the OPA1 protein is caused by autosomal dominant inheritance.

[0131] In related embodiments, the method is a method of using an ASO to increase the expression of a protein or functional RNA. In some embodiments, an ASO may be used to increase the expression of OPA1 protein in cells of a subject having an *OPA1* pre-mRNA, wherein the subject has a deficiency, *e.g.*, Optic atrophy type 1; in the amount or function of an OPA1 protein.

[0132] In some embodiments, the pre-mRNA transcript that encodes the protein that is causative of the disease or condition is targeted by the agent, *e.g.*, the oligonucleotides, described herein. In some cases, it is the NMD exon-containing pre-mRNA transcript targeted by the agent, *e.g.*, the oligonucleotides, described herein. In some cases, the agent, *e.g.*, the oligonucleotides, described herein, are designed to target a coding exon of the pre-mRNA. In some cases, the

agent, *e.g.*, the oligonucleotides, described herein can induce skipping of the NMD exon, a coding exon, or both. In some embodiments, a NMD exon-containing pre-mRNA transcript that encodes a protein that is not causative of the disease is targeted by the ASOs. For example, a disease that is the result of a mutation or deficiency of a first protein in a particular pathway may be ameliorated by targeting a pre-mRNA that encodes a second protein, thereby increasing production of the second protein. In some embodiments, the function of the second protein is able to compensate for the mutation or deficiency of the first protein (which is causative of the disease or condition).

[0133] In some embodiments, the subject has:

(a) a first mutant allele from which

- (i) the OPA1 protein is produced at a reduced level compared to production from a wild-type allele,
- (ii) the OPA1 protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
- (iii) the OPA1 protein or functional RNA is not produced; and

(b) a second mutant allele from which

- (i) the OPA1 protein is produced at a reduced level compared to production from a wild-type allele,
- (ii) the OPA1 protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
- (iii) the OPA1 protein is not produced, and

wherein the NMD exon-containing pre-mRNA is transcribed from the first allele and/or the second allele. In these embodiments, the ASO binds to a targeted portion of the NMD exon-containing pre-mRNA transcribed from the first allele or the second allele, thereby inducing exon skipping of the pseudo-exon from the NMD exon-containing pre-mRNA, and causing an increase in the level of mRNA encoding OPA1 protein and an increase in the expression of the target protein or functional RNA in the cells of the subject. In these embodiments, the target protein or functional RNA having an increase in expression level resulting from the exon skipping of the pseudo-exon from the NMD exon-containing pre-mRNA may be either in a form having reduced function compared to the equivalent wild-type protein (partially-functional), or having full function compared to the equivalent wild-type protein (fully-functional).

[0134] In some embodiments, the subject has:

(a) a first mutant allele from which

- (i) the OPA1 protein is produced at a reduced level compared to production from a wild-type allele,

- (ii) the OPA1 protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
 - (iii) the OPA1 protein or functional RNA is not produced; and
- (b) a second mutant allele from which
- (i) the OPA1 protein is produced at a reduced level compared to production from a wild-type allele,
 - (ii) the OPA1 protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
 - (iii) the OPA1 protein is not produced, and

wherein the *OPA1* pre-mRNA is transcribed from the first allele and/or the second allele. In these embodiments, the ASO binds to a targeted portion of the *OPA1* pre-mRNA transcribed from the first allele or the second allele, thereby inducing exon skipping of a coding exon from the *OPA1* pre-mRNA, and causing an increase in the expression of the target OPA1 protein in the cells of the subject. In these embodiments, the target OPA1 protein having an increase in expression level resulting from the exon skipping of the coding exon from the *OPA1* pre-mRNA may be either in a form having reduced function compared to the equivalent full-length wild-type protein (partially-functional), or having full function compared to the equivalent full-length wild-type protein (fully-functional).

[0135] In some embodiments, the level of mRNA encoding OPA1 protein is increased 1.1 to 10-fold, when compared to the amount of mRNA encoding OPA1 protein that is produced in a control cell, *e.g.*, one that is not treated with the antisense oligomer or one that is treated with an antisense oligomer that does not bind to the targeted portion of the *OPA1* pre-mRNA.

[0136] In some embodiments, a subject treated using the methods of the present disclosure expresses a partially functional OPA1 protein from one allele, wherein the partially functional OPA1 protein may be caused by a frameshift mutation, a nonsense mutation, a missense mutation, or a partial gene deletion. In some embodiments, a subject treated using the methods of the disclosure expresses a nonfunctional OPA1 protein from one allele, wherein the nonfunctional OPA1 protein may be caused by a frameshift mutation, a nonsense mutation, a missense mutation, a partial gene deletion, in one allele. In some embodiments, a subject treated using the methods of the disclosure has an OPA1 whole gene deletion, in one allele.

Exon Inclusion

[0137] As used herein, a “NMD exon-containing pre-mRNA” is a pre-mRNA transcript that contains at least one pseudo-exon. Alternative or aberrant splicing can result in inclusion of the at least one pseudo-exon in the mature mRNA transcripts. The terms “mature mRNA,” and “fully-spliced mRNA,” are used interchangeably herein to describe a fully processed mRNA.

Inclusion of the at least one pseudo-exon can be non-productive mRNA and lead to NMD of the mature mRNA. NMD exon-containing mature mRNA may sometimes lead to aberrant protein expression.

[0138] In some embodiments, the included pseudo-exon is the most abundant pseudo-exon in a population of NMD exon-containing pre-mRNAs transcribed from the gene encoding the target protein in a cell. In some embodiments, the included pseudo-exon is the most abundant pseudo-exon in a population of NMD exon-containing pre-mRNAs transcribed from the gene encoding the target protein in a cell, wherein the population of NMD exon-containing pre-mRNAs comprises two or more included pseudo-exons. In some embodiments, an antisense oligomer targeted to the most abundant pseudo-exon in the population of NMD exon-containing pre-mRNAs encoding the target protein induces exon skipping of one or two or more pseudo-exons in the population, including the pseudo-exon to which the antisense oligomer is targeted or binds. In some embodiments, the targeted region is in a pseudo-exon that is the most abundant pseudo-exon in a NMD exon-containing pre-mRNA encoding the OPA1 protein.

[0139] The degree of exon inclusion can be expressed as percent exon inclusion, e.g., the percentage of transcripts in which a given pseudo-exon is included. In brief, percent exon inclusion can be calculated as the percentage of the amount of RNA transcripts with the exon inclusion, over the sum of the average of the amount of RNA transcripts with exon inclusion plus the average of the amount of RNA transcripts with exon exclusion.

[0140] In some embodiments, an included pseudo-exon is an exon that is identified as an included pseudo-exon based on a determination of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%, inclusion. In embodiments, a included pseudo-exon is an exon that is identified as a included pseudo-exon based on a determination of about 5% to about 100%, about 5% to about 95%, about 5% to about 90%, about 5% to about 85%, about 5% to about 80%, about 5% to about 75%, about 5% to about 70%, about 5% to about 65%, about 5% to about 60%, about 5% to about 55%, about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20%, about 5% to about 15%, about 10% to about 100%, about 10% to about 95%, about 10% to about 90%, about 10% to about 85%, about 10% to about 80%, about 10% to about 75%, about 10% to about 70%, about 10% to about 65%, about 10% to about 60%, about 10% to about 55%, about 10% to about 50%, about 10% to about 45%, about 10% to about 40%, about 10% to about 35%, about 10% to about 30%, about 10% to about 25%, about 10% to about 20%, about 15% to about 100%, about 15% to about 95%, about 15% to about 90%, about 15% to about 85%, about 15% to about 80%, about 15% to about 75%, about

15% to about 70%, about 15% to about 65%, about 15% to about 60%, about 15% to about 55%, about 15% to about 50%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 15% to about 30%, about 15% to about 25%, about 20% to about 100%, about 20% to about 95%, about 20% to about 90%, about 20% to about 85%, about 20% to about 80%, about 20% to about 75%, about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 25% to about 100%, about 25% to about 95%, about 25% to about 90%, about 25% to about 85%, about 25% to about 80%, about 25% to about 75%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, or about 25% to about 35%, inclusion. ENCODE data (described by, *e.g.*, Tilgner, *et al.*, 2012, “Deep sequencing of subcellular RNA fractions shows splicing to be predominantly co-transcriptional in the human genome but inefficient for lncRNAs,” *Genome Research* 22(9):1616-25) can be used to aid in identifying exon inclusion.

[0141] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of an *OPAI* pre-mRNA transcript results in an increase in the amount of OPA1 protein produced by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In some embodiments, the total amount of OPA1 protein produced by the cell to which the antisense oligomer is contacted is increased about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 10%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300%, compared to the amount of target protein produced by a control compound. In some embodiments, the total amount of OPA1 protein produced by the cell to which the antisense oligomer is contacted is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-

fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the amount of target protein produced by a control compound. A control compound can be, for example, an oligonucleotide that is not complementary to a targeted portion of the pre-mRNA.

[0142] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of an *OPA1* pre-mRNA transcript results in an increase in the amount of mRNA encoding OPA1, including the mature mRNA encoding the target protein. In some embodiments, the amount of mRNA encoding OPA1 protein, or the mature mRNA encoding the OPA1 protein, is increased by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In some embodiments, the total amount of the mRNA encoding OPA1 protein, or the mature mRNA encoding OPA1 protein produced in the cell to which the antisense oligomer is contacted is increased about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 10%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300%, compared to the amount of mature RNA produced in an untreated cell, *e.g.*, an untreated cell or a cell treated with a control compound. In some embodiments, the total amount of the mRNA encoding OPA1 protein, or the mature mRNA encoding OPA1 protein produced in the cell to which the antisense oligomer is contacted is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold compared to the amount of mature RNA produced in an untreated cell, *e.g.*, an untreated cell or a cell treated with a control compound. A control compound can be, for

example, an oligonucleotide that is not complementary to a targeted portion of the OPA1 NMD exon-containing pre-mRNA.

[0143] The NMD exon can be in any length. In some embodiments, the NMD exon comprises a full sequence of an intron, in which case, it can be referred to as intron retention. In some embodiments, the NMD exon can be a portion of the intron. In some embodiments, the NMD exon can be a 5' end portion of an intron including a 5'ss sequence. In some embodiments, the NMD exon can be a 3' end portion of an intron including a 3'ss sequence. In some embodiments, the NMD exon can be a portion within an intron without inclusion of a 5'ss sequence. In some embodiments, the NMD exon can be a portion within an intron without inclusion of a 3'ss sequence. In some embodiments, the NMD exon can be a portion within an intron without inclusion of either a 5'ss or a 3'ss sequence. In some embodiments, the NMD exon can be from 5 nucleotides to 10 nucleotides in length, from 10 nucleotides to 15 nucleotides in length, from 15 nucleotides to 20 nucleotides in length, from 20 nucleotides to 25 nucleotides in length, from 25 nucleotides to 30 nucleotides in length, from 30 nucleotides to 35 nucleotides in length, from 35 nucleotides to 40 nucleotides in length, from 40 nucleotides to 45 nucleotides in length, from 45 nucleotides to 50 nucleotides in length, from 50 nucleotides to 55 nucleotides in length, from 55 nucleotides to 60 nucleotides in length, from 60 nucleotides to 65 nucleotides in length, from 65 nucleotides to 70 nucleotides in length, from 70 nucleotides to 75 nucleotides in length, from 75 nucleotides to 80 nucleotides in length, from 80 nucleotides to 85 nucleotides in length, from 85 nucleotides to 90 nucleotides in length, from 90 nucleotides to 95 nucleotides in length, or from 95 nucleotides to 100 nucleotides in length. In some embodiments, the NMD exon can be at least 10 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides in length, at least 90 nucleotides, or at least 100 nucleotides in length. In some embodiments, the NMD exon can be from 100 to 200 nucleotides in length, from 200 to 300 nucleotides in length, from 300 to 400 nucleotides in length, from 400 to 500 nucleotides in length, from 500 to 600 nucleotides in length, from 600 to 700 nucleotides in length, from 700 to 800 nucleotides in length, from 800 to 900 nucleotides in length, from 900 to 1,000 nucleotides in length. In some embodiments, the NMD exon may be longer than 1,000 nucleotides in length.

[0144] Inclusion of a pseudo-exon can lead to a frameshift and the introduction of a premature termination codon (PIC) in the mature mRNA transcript rendering the transcript a target of NMD. Mature mRNA transcript containing NMD exon can be non-productive mRNA transcript which does not lead to protein expression. The PIC can be present in any position downstream of an NMD exon. In some embodiments, the PIC can be present in any exon downstream of an NMD exon. In some embodiments, the PIC can be present within the NMD exon. For example,

inclusion of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*, in an mRNA transcript encoded by the *OPAI* gene can induce a PIC in the mRNA transcript. For example, inclusion of exon (GRCh38/ hg38: chr3 193628509 193628616) of *OPAI*; or exon (GRCh38/ hg38: chr3 193603500 193603557) of *OPAI* in an mRNA transcript encoded by the *OPAI*.

[0145] In some aspects, provided herein is a method of modulating expression of an OPA1 protein by promoting inclusion of a coding exon. The method can comprise contacting an agent to a cell having an *OPAI* pre-mRNA, wherein the agent comprises an oligonucleotide that binds to: (a) a targeted portion of the pre-mRNA within an intronic region immediately upstream of a 5' end of the coding exon of the pre-mRNA; or (b) a targeted portion of the pre-mRNA within an intronic region immediately downstream of a 3' end of the coding exon of the pre-mRNA; whereby the agent increases a level of a processed mRNA that is processed from the pre-mRNA and that contains the coding exon in the cell. In some cases, the coding exon to be included is an alternatively spliced exon. In some cases, the method promotes inclusion of the coding exon in the processed mRNA during splicing of the pre-mRNA in the cell.

[0146] In some of these embodiments for inclusion of coding exon, the target portion of the pre-mRNA is within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of a 5' end of the coding exon. In some cases, the target portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of a 3' end of the coding exon. In some cases, the coding exon is exon 7 of *OPAI*. In some cases, the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277. In some cases, the coding exon comprises SEQ ID NO: 277. The targeted portion of the pre-mRNA can be within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202.

[0147] In some cases, the inclusion of the coding exon in the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about

2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

Exclusion of Both NMD Exon and Coding Exon

[0148] In some embodiments, provided herein is a method of modulating expression of a target protein by targeting a pre-mRNA and modulating exclusion of both a coding exon and a non-sense mediated RNA decay-inducing exon (NMD exon) from the pre-mRNA. In some cases, the method comprises contacting an agent to the cell, and the agent promotes exclusion of both the coding exon and the NMD exon from the pre-mRNA, thereby increasing level of a processed mRNA that is processed from the pre-mRNA and lacks both the coding exon and the NMD exon. In some cases, the agent binds to a targeted portion of the pre-mRNA, or modulates binding of a factor involved in splicing of the coding exon, the NMD exon, or both. In some cases, the agent interferes with binding of the factor involved in splicing of the coding exon, the NMD exon, or both, to a region of the targeted portion. In some cases, the NMD exon is within an intronic region adjacent to the coding exon. In some cases, the NMD exon is within an intronic region immediately upstream of the coding exon. In some cases, the NMD exon is within an intronic region immediately downstream of the coding exon. In some cases, the coding exon is an alternatively spliced exon.

[0149] In some cases, the targeted portion of the pre-mRNA is proximal to the coding exon. The targeted portion of the pre-mRNA can be located in an intronic region immediately upstream of the coding exon. The targeted portion of the pre-mRNA can be located in an intronic region immediately downstream of the coding exon. In some cases, the targeted portion of the pre-mRNA can be located within the coding exon. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the coding exon to 100 nucleotides downstream of the coding exon. In some cases, the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the coding exon.

[0150] In some cases, the targeted portion of the pre-mRNA is proximal to the NMD exon. In some cases, the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the NMD exon. In some cases, the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the NMD exon. In some cases, the targeted portion of the pre-mRNA is located within the NMD exon. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the NMD exon to 100 nucleotides downstream of the NMD exon.

[0151] In some embodiments, the method described herein is applicable to modulation of expression of OPA1 protein by modulating exclusion of both exon 7 and an NMD exon (e.g., exon 7x) of *OPA1* pre-mRNA that contains both exon 7 and exon 7x. In some cases, the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277. In some cases, the coding exon comprises SEQ ID NO: 277. In some cases, the targeted portion of the pre-mRNA is immediately upstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some cases, the targeted portion of the pre-mRNA is immediately downstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of GRCh38/ hg38: chr3 193626092. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202. In some cases, the targeted portion of the pre-mRNA is within the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some cases, the targeted portion of the pre-mRNA comprises an exon-intron junction of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some cases, the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279. In some cases, the NMD exon comprises SEQ ID NO: 279. In some cases, the targeted portion of the pre-mRNA is immediately upstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some cases, the targeted portion of the pre-mRNA is immediately downstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

[0152] In some cases, the targeted portion of the pre-mRNA is within the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some cases, the targeted portion of the pre-mRNA comprises an exon-intron junction of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some cases, the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[0153] In some cases, the exclusion of the coding exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold,

about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of contacting with the agent. In some cases, the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of contacting with the agent. In some cases, the method results in an increase in the level of the processed mRNA in the cell. The level of the processed mRNA in the cell contacted with the agent can be increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of contacting with the agent.

[0154] In some cases, the method results in an increase in expression of the OPA1 protein in the cell. A level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent can be increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold,

at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of contacting with the agent.

[0155] In some cases, a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by at least about 1.5-fold compared to in the absence of contacting with the agent.

[0156] In some cases, the OPA1 protein expressed from the processed mRNA that lacks exon 7 and exon 7x is a functional OPA1 protein. The OPA1 protein expressed from the processed mRNA that lacks exon 7 and exon 7x can be at least partially functional as compared to a wild-type OPA1 protein. The OPA1 protein expressed from the processed mRNA that lacks exon 7 and exon 7x can be at least partially functional as compared to a full-length wild-type OPA1 protein.

Therapeutic Agents

[0157] In various embodiments of the present disclosure, compositions and methods comprising a therapeutic agent are provided to modulate protein expression level of OPA1. In some embodiments, provided herein are compositions and methods to modulate alternative splicing of *OPA1* pre-mRNA. In some embodiments, provided herein are compositions and methods to induce exon skipping in the splicing of *OPA1* pre-mRNA, e.g., to induce skipping of a pseudo-exon during splicing of *OPA1* pre-mRNA. In other embodiments, therapeutic agents may be used to induce the inclusion of an exon in order to decrease the protein expression level.

[0158] A therapeutic agent disclosed herein can be a NIE repressor agent. A therapeutic agent may comprise a polynucleic acid polymer.

[0159] According to one aspect of the present disclosure, provided herein is a method of treatment or prevention of a condition or disease associated with a functional OPA1 protein deficiency, comprising administering a NIE repressor agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region of the pre-mRNA transcript to decrease inclusion of the NMD exon in the mature transcript. For example, provided herein is a method of treatment or prevention of a condition associated with a functional OPA1 protein deficiency, comprising administering a NIE repressor agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region of an intron containing an NMD exon (e.g., exon 6x of *OPA1*, exon 7x of *OPA1*, or exon 28x of *OPA1*) of the pre-mRNA transcript or to a NMD exon-activating regulatory sequence in the same intron. For example, provided herein is a method of treatment or prevention of a condition associated with a functional OPA1 protein deficiency, comprising administering a NIE repressor agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region of an intron containing an NMD exon (e.g., exon (GRCh38/ hg38: chr3 193628509 193628616) of *OPA1*; or

exon (GRCh38/ hg38: chr3 193603500 193603557) of *OPA1*) of the pre-mRNA transcript or to a NMD exon-activating regulatory sequence in the same intron. In some embodiments, the method comprises administering a NIE repressor agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region of an intron containing an NMD exon (e.g., exon of *OPA1* other than exon 7x defined by (GRCh38/ hg38: chr3 193628509 193628616) or exon defined by (GRCh38/ hg38: chr3 193603500 193603557)) of the pre-mRNA transcript or to a NMD exon-activating regulatory sequence in the same intron. In some embodiments, the therapeutic agent promotes exclusion of an NMD exon of *OPA1* pre-mRNA other than exon 7x defined by (GRCh38/ hg38: chr3 193628509 193628616) or exon defined by (GRCh38/ hg38: chr3 193603500 193603557). In some embodiments, the composition disclosed herein includes an agent that promotes exclusion of an NMD exon of *OPA1* pre-mRNA other than exon 7x defined by (GRCh38/ hg38: chr3 193628509 193628616) or exon defined by (GRCh38/ hg38: chr3 193603500 193603557).

[0160] Where reference is made to reducing NMD exon inclusion in the mature mRNA, the reduction may be complete, e.g., 100%, or may be partial. The reduction may be clinically significant. The reduction/correction may be relative to the level of NMD exon inclusion in the subject without treatment, or relative to the amount of NMD exon inclusion in a population of similar subjects. The reduction/correction may be at least 10% less NMD exon inclusion relative to the average subject, or the subject prior to treatment. The reduction may be at least 20% less NMD exon inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 40% less NMD exon inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 50% less NMD exon inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 60% less NMD exon inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 80% less NMD exon inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 90% less NMD exon inclusion relative to an average subject, or the subject prior to treatment.

[0161] According to one aspect of the present disclosure, provided herein is a method of treatment or prevention of a condition or disease associated with a functional OPA1 protein deficiency, comprising administering an agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region of the pre-mRNA transcript to decrease inclusion of a coding exon (e.g., exon 7) in the mature transcript. For example, provided herein is a method of treatment or prevention of a condition associated with a functional OPA1 protein deficiency, comprising administering an agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region containing a coding exon (e.g., exon 7 of *OPA1*) of the pre-

mRNA transcript. For example, provided herein is a method of treatment or prevention of a condition associated with a functional OPA1 protein deficiency, comprising administering an agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region containing a coding exon (e.g., exon (GRCh38/ hg38: chr3 193626092 to 193626202) of *OPA1*) of the pre-mRNA transcript. In some embodiments, the method comprises administering an agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region containing a coding exon (e.g., exon of *OPA1* other than exon 7 defined by (GRCh38/ hg38: chr3 193626092 to 193626202)) of the pre-mRNA transcript. In some embodiments, the therapeutic agent promotes exclusion of a coding exon of *OPA1* pre-mRNA other than exon 7 defined by (GRCh38/ hg38: chr3 193626092 to 193626202). In some embodiments, the composition disclosed herein includes an agent that promotes exclusion of a coding exon of *OPA1* pre-mRNA other than exon 7 defined by (GRCh38/ hg38: chr3 193626092 to 193626202).

[0162] Where reference is made to increasing active OPA1 protein levels, the increase may be clinically significant. The increase may be relative to the level of active OPA1 protein in the subject without treatment, or relative to the amount of active OPA1 protein in a population of similar subjects. The increase may be at least 10% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 20% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 40% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 50% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 80% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 100% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 200% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 500% more active OPA1 protein relative to the average subject, or the subject prior to treatment.

[0163] In embodiments wherein the NIE repressor agent comprises a polynucleic acid polymer, the polynucleic acid polymer may be about 50 nucleotides in length. The polynucleic acid polymer may be about 45 nucleotides in length. The polynucleic acid polymer may be about 40 nucleotides in length. The polynucleic acid polymer may be about 35 nucleotides in length. The polynucleic acid polymer may be about 30 nucleotides in length. The polynucleic acid polymer may be about 24 nucleotides in length. The polynucleic acid polymer may be about 25 nucleotides in length. The polynucleic acid polymer may be about 20 nucleotides in length. The polynucleic acid polymer may be about 19 nucleotides in length. The polynucleic acid polymer

may be about 18 nucleotides in length. The polynucleic acid polymer may be about 17 nucleotides in length. The polynucleic acid polymer may be about 16 nucleotides in length. The polynucleic acid polymer may be about 15 nucleotides in length. The polynucleic acid polymer may be about 14 nucleotides in length. The polynucleic acid polymer may be about 13 nucleotides in length. The polynucleic acid polymer may be about 12 nucleotides in length. The polynucleic acid polymer may be about 11 nucleotides in length. The polynucleic acid polymer may be about 10 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 50 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 45 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 40 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 35 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 30 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 25 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 20 nucleotides in length. The polynucleic acid polymer may be between about 15 and about 25 nucleotides in length. The polynucleic acid polymer may be between about 15 and about 30 nucleotides in length. The polynucleic acid polymer may be between about 12 and about 30 nucleotides in length.

[0164] The sequence of the polynucleic acid polymer may be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% complementary to a target sequence of an mRNA transcript, e.g., a partially processed mRNA transcript. The sequence of the polynucleic acid polymer may be 100% complementary to a target sequence of a pre-mRNA transcript.

[0165] The sequence of the polynucleic acid polymer may have 4 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have 3 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have 2 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have 1 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have no mismatches to a target sequence of the pre-mRNA transcript.

[0166] The polynucleic acid polymer may specifically hybridize to a target sequence of the pre-mRNA transcript. For example, the polynucleic acid polymer may have 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 100% sequence complementarity to a target sequence of the pre-mRNA transcript. The hybridization may be under high stringent hybridization conditions.

[0167] The polynucleic acid polymer comprising a sequence with at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 2-5. The polynucleic acid polymer may comprise a sequence with 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 2-5.

[0168] Where reference is made to a polynucleic acid polymer sequence, the skilled person will understand that one or more substitutions may be tolerated, optionally two substitutions may be tolerated in the sequence, such that it maintains the ability to hybridize to the target sequence; or where the substitution is in a target sequence, the ability to be recognized as the target sequence. References to sequence identity may be determined by BLAST sequence alignment using standard/default parameters. For example, the sequence may have 99% identity and still function according to the present disclosure. In other embodiments, the sequence may have 98% identity and still function according to the present disclosure. In another embodiment, the sequence may have 95% identity and still function according to the present disclosure. In another embodiment, the sequence may have 90% identity and still function according to the present disclosure.

Antisense Oligomers

[0169] Provided herein is a composition comprising an antisense oligomer that induces exon skipping by binding to a targeted portion of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA. As used herein, the terms “ASO” and “antisense oligomer” are used interchangeably and refer to an oligomer such as a polynucleotide, comprising nucleobases that hybridizes to a target nucleic acid (*e.g.*, an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA) sequence by Watson-Crick base pairing or wobble base pairing (G-U). The ASO may have exact sequence complementary to the target sequence or near complementarity (*e.g.*, sufficient complementarity to bind the target sequence and enhancing splicing at a splice site). ASOs are designed so that they bind (hybridize) to a target nucleic acid (*e.g.*, a targeted portion of a pre-mRNA transcript) and remain hybridized under physiological conditions. Typically, if they hybridize to a site other than the intended (targeted) nucleic acid sequence, they hybridize to a limited number of sequences that are not a target nucleic acid (to a few sites other than a target nucleic acid). Design of an ASO can take into consideration the occurrence of the nucleic acid sequence of the targeted portion of the pre-mRNA transcript or a sufficiently similar nucleic acid sequence in other locations in the genome or cellular pre-mRNA or transcriptome, such that the likelihood the ASO will bind other sites and cause “off-target” effects is limited. Any antisense oligomers known in the art (for example, in PCT Application No. PCT/US2014/054151, published as WO 2015/035091, titled “Reducing Nonsense-Mediated

mRNA Decay,” incorporated by reference herein), can be used to practice the methods described herein.

[0170] In some embodiments, ASOs “specifically hybridize” to or are “specific” to a target nucleic acid or a targeted portion of an *OPAI* pre-mRNA, *e.g.*, a NMD exon-containing pre-mRNA. Typically such hybridization occurs with a T_m substantially greater than 37 °C, preferably at least 50 °C, and typically between 60 °C to approximately 90 °C. Such hybridization preferably corresponds to stringent hybridization conditions. At a given ionic strength and pH, the T_m is the temperature at which 50% of a target sequence hybridizes to a complementary oligonucleotide.

[0171] Oligomers, such as oligonucleotides, are “complementary” to one another when hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides. A double-stranded polynucleotide can be “complementary” to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second. Complementarity (the degree to which one polynucleotide is complementary with another) is quantifiable in terms of the proportion (*e.g.*, the percentage) of bases in opposing strands that are expected to form hydrogen bonds with each other, according to generally accepted base-pairing rules. The sequence of an antisense oligomer (ASO) need not be 100% complementary to that of its target nucleic acid to hybridize. In certain embodiments, ASOs can comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an ASO in which 18 of 20 nucleobases of the oligomeric compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining non-complementary nucleobases may be clustered together or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. Percent complementarity of an ASO with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul, *et al.*, J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656).

[0172] An ASO need not hybridize to all nucleobases in a target sequence and the nucleobases to which it does hybridize may be contiguous or noncontiguous. ASOs may hybridize over one or more segments of a pre-mRNA transcript, such that intervening or adjacent segments are not involved in the hybridization event (*e.g.*, a loop structure or hairpin structure may be formed). In certain embodiments, an ASO hybridizes to noncontiguous nucleobases in a target pre-mRNA

transcript. For example, an ASO can hybridize to nucleobases in a pre-mRNA transcript that are separated by one or more nucleobase(s) to which the ASO does not hybridize.

[0173] The ASOs described herein comprise nucleobases that are complementary to nucleobases present in a target portion of an *OPAI* pre-mRNA, *e.g.*, a NMD exon-containing pre-mRNA. The term ASO embodies oligonucleotides and any other oligomeric molecule that comprises nucleobases capable of hybridizing to a complementary nucleobase on a target mRNA but does not comprise a sugar moiety, such as a peptide nucleic acid (PNA). The ASOs may comprise naturally-occurring nucleotides, nucleotide analogs, modified nucleotides, or any combination of two or three of the preceding. The term “naturally occurring nucleotides” includes deoxyribonucleotides and ribonucleotides. The term “modified nucleotides” includes nucleotides with modified or substituted sugar groups and/or having a modified backbone. In some embodiments, all of the nucleotides of the ASO are modified nucleotides. Chemical modifications of ASOs or components of ASOs that are compatible with the methods and compositions described herein will be evident to one of skill in the art and can be found, for example, in U.S. Patent No. 8,258,109 B2, U.S. Patent No. 5,656,612, U.S. Patent Publication No. 2012/0190728, and Dias and Stein, *Mol. Cancer Ther.* 2002, 347-355, herein incorporated by reference in their entirety.

[0174] One or more nucleobases of an ASO may be any naturally occurring, unmodified nucleobase such as adenine, guanine, cytosine, thymine and uracil, or any synthetic or modified nucleobase that is sufficiently similar to an unmodified nucleobase such that it is capable of hydrogen bonding with a nucleobase present on a target pre-mRNA. Examples of modified nucleobases include, without limitation, hypoxanthine, xanthine, 7-methylguanine, 5, 6-dihydrouracil, 5-methylcytosine, and 5-hydroxymethoylcytosine.

[0175] The ASOs described herein also comprise a backbone structure that connects the components of an oligomer. The term “backbone structure” and “oligomer linkages” may be used interchangeably and refer to the connection between monomers of the ASO. In naturally occurring oligonucleotides, the backbone comprises a 3’-5’ phosphodiester linkage connecting sugar moieties of the oligomer. The backbone structure or oligomer linkages of the ASOs described herein may include (but are not limited to) phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoramidate, and the like. See, *e.g.*, LaPlanche, *et al.*, *Nucleic Acids Res.* 14:9081 (1986); Stec, *et al.*, *J. Am. Chem. Soc.* 106:6077 (1984), Stein, *et al.*, *Nucleic Acids Res.* 16:3209 (1988), Zon, *et al.*, *Anti-Cancer Drug Design* 6:539 (1991); Zon, *et al.*, *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec, *et al.*, U.S. Pat. No. 5,151,510; Uhlmann and Peyman, *Chemical Reviews*

90:543 (1990). In some embodiments, the backbone structure of the ASO does not contain phosphorous but rather contains peptide bonds, for example in a peptide nucleic acid (PNA), or linking groups including carbamate, amides, and linear and cyclic hydrocarbon groups. In some embodiments, the backbone modification is a phosphorothioate linkage. In some embodiments, the backbone modification is a phosphoramidate linkage.

[0176] In some embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is random. In some embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is controlled and is not random. For example, U.S. Pat. App. Pub. No. 2014/0194610, “Methods for the Synthesis of Functionalized Nucleic Acids,” incorporated herein by reference, describes methods for independently selecting the handedness of chirality at each phosphorous atom in a nucleic acid oligomer. In some embodiments, an ASO used in the methods of the disclosure, including, but not limited to, any of the ASOs set forth herein in Tables 5 and 6, comprises an ASO having phosphorus internucleotide linkages that are not random. In some embodiments, a composition used in the methods of the disclosure comprises a pure diastereomeric ASO. In some embodiments, a composition used in the methods of the disclosure comprises an ASO that has diastereomeric purity of at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, about 90% to about 100%, about 91% to about 100%, about 92% to about 100%, about 93% to about 100%, about 94% to about 100%, about 95% to about 100%, about 96% to about 100%, about 97% to about 100%, about 98% to about 100%, or about 99% to about 100%.

[0177] In some embodiments, the ASO has a nonrandom mixture of Rp and Sp configurations at its phosphorus internucleotide linkages. For example, it has been suggested that a mix of Rp and Sp is required in antisense oligonucleotides to achieve a balance between good activity and nuclease stability (Wan, *et al.*, 2014, “Synthesis, biophysical properties and biological activity of second generation antisense oligonucleotides containing chiral phosphorothioate linkages,” *Nucleic Acids Research* 42(22): 13456-13468, incorporated herein by reference). In some embodiments, an ASO used in the methods of the disclosure, including, but not limited to, any of the ASOs set forth herein in SEQ ID NOs: 2-5, comprises about 5-100% Rp, at least about 5% Rp, at least about 10% Rp, at least about 15% Rp, at least about 20% Rp, at least about 25% Rp, at least about 30% Rp, at least about 35% Rp, at least about 40% Rp, at least about 45% Rp, at least about 50% Rp, at least about 55% Rp, at least about 60% Rp, at least about 65% Rp, at least about 70% Rp, at least about 75% Rp, at least about 80% Rp, at least about 85% Rp, at least about 90% Rp, or at least about 95% Rp, with the remainder Sp, or about 100% Rp. In some

embodiments, an ASO used in the methods of the disclosure, including, but not limited to, any of the ASOs set forth herein comprise a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5, comprises about 10% to about 100% Rp, about 15% to about 100% Rp, about 20% to about 100% Rp, about 25% to about 100% Rp, about 30% to about 100% Rp, about 35% to about 100% Rp, about 40% to about 100% Rp, about 45% to about 100% Rp, about 50% to about 100% Rp, about 55% to about 100% Rp, about 60% to about 100% Rp, about 65% to about 100% Rp, about 70% to about 100% Rp, about 75% to about 100% Rp, about 80% to about 100% Rp, about 85% to about 100% Rp, about 90% to about 100% Rp, or about 95% to about 100% Rp, about 20% to about 80% Rp, about 25% to about 75% Rp, about 30% to about 70% Rp, about 40% to about 60% Rp, or about 45% to about 55% Rp, with the remainder Sp.

[0178] In some embodiments, an ASO used in the methods of the disclosure, including, but not limited to, any of the ASOs set forth herein comprise a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5, comprises about 5-100% Sp, at least about 5% Sp, at least about 10% Sp, at least about 15% Sp, at least about 20% Sp, at least about 25% Sp, at least about 30% Sp, at least about 35% Sp, at least about 40% Sp, at least about 45% Sp, at least about 50% Sp, at least about 55% Sp, at least about 60% Sp, at least about 65% Sp, at least about 70% Sp, at least about 75% Sp, at least about 80% Sp, at least about 85% Sp, at least about 90% Sp, or at least about 95% Sp, with the remainder Rp, or about 100% Sp. In embodiments, an ASO used in the methods of the disclosure, including, but not limited to, any of the ASOs set forth herein comprise a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5, comprises about 10% to about 100% Sp, about 15% to about 100% Sp, about 20% to about 100% Sp, about 25% to about 100% Sp, about 30% to about 100% Sp, about 35% to about 100% Sp, about 40% to about 100% Sp, about 45% to about 100% Sp, about 50% to about 100% Sp, about 55% to about 100% Sp, about 60% to about 100% Sp, about 65% to about 100% Sp, about 70% to about 100% Sp, about 75% to about 100% Sp, about 80% to about 100% Sp, about 85% to about 100% Sp, about 90% to about 100% Sp, or about 95% to about 100% Sp, about 20% to about 80% Sp, about 25% to about 75% Sp, about 30% to about 70% Sp, about 40% to about 60% Sp, or about 45% to about 55% Sp, with the remainder Rp.

[0179] Any of the ASOs described herein may contain a sugar moiety that comprises ribose or deoxyribose, as present in naturally occurring nucleotides, or a modified sugar moiety or sugar analog, including a morpholine ring. Non-limiting examples of modified sugar moieties include

2' substitutions such as 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'MOE), 2'-O-aminoethyl, 2'F; N3'->P5' phosphoramidate, 2'dimethylaminooxyethoxy, 2'dimethylaminoethoxyethoxy, 2'-guanidinidum, 2'-O-guanidinium ethyl, carbamate modified sugars, and bicyclic modified sugars. In some embodiments, the sugar moiety modification is selected from 2'-O-Me, 2'F, and 2'MOE. In some embodiments, the sugar moiety modification is an extra bridge bond, such as in a locked nucleic acid (LNA). In some embodiments the sugar analog contains a morpholine ring, such as phosphorodiamidate morpholino (PMO). In some embodiments, the sugar moiety comprises a ribofuransyl or 2'deoxyribofuransyl modification. In some embodiments, the sugar moiety comprises 2'4'-constrained 2'-O-methoxyethyl (cMOE) modifications. In some embodiments, the sugar moiety comprises cEt 2', 4' constrained 2'-O ethyl BNA modifications. In some embodiments, the sugar moiety comprises tricycloDNA (tcDNA) modifications. In some embodiments, the sugar moiety comprises ethylene nucleic acid (ENA) modifications. In some embodiments, the sugar moiety comprises MCE modifications. Modifications are known in the art and described in the literature, *e.g.*, by Jarver, *et al.*, 2014, "A Chemical View of Oligonucleotides for Exon Skipping and Related Drug Applications," *Nucleic Acid Therapeutics* 24(1): 37-47, incorporated by reference for this purpose herein.

[0180] In some embodiments, each monomer of the ASO is modified in the same way, for example each linkage of the backbone of the ASO comprises a phosphorothioate linkage or each ribose sugar moiety comprises a 2'-O-methyl modification. Such modifications that are present on each of the monomer components of an ASO are referred to as "uniform modifications." In some examples, a combination of different modifications may be desired, for example, an ASO may comprise a combination of phosphorodiamidate linkages and sugar moieties comprising morpholine rings (morpholinos). Combinations of different modifications to an ASO are referred to as "mixed modifications" or "mixed chemistries."

[0181] In some embodiments, the ASO comprises one or more backbone modifications. In some embodiments, the ASO comprises one or more sugar moiety modification. In some embodiments, the ASO comprises one or more backbone modifications and one or more sugar moiety modifications. In some embodiments, the ASO comprises a 2'MOE modification and a phosphorothioate backbone. In some embodiments, the ASO comprises a phosphorodiamidate morpholino (PMO). In some embodiments, the ASO comprises a peptide nucleic acid (PNA). Any of the ASOs or any component of an ASO (*e.g.*, a nucleobase, sugar moiety, backbone) described herein may be modified in order to achieve desired properties or activities of the ASO or reduce undesired properties or activities of the ASO. For example, an ASO or one or more components of any ASO may be modified to enhance binding affinity to a target sequence on a pre-mRNA transcript; reduce binding to any non-target sequence; reduce degradation by cellular

nucleases (*i.e.*, RNase H); improve uptake of the ASO into a cell and/or into the nucleus of a cell; alter the pharmacokinetics or pharmacodynamics of the ASO; and/or modulate the half-life of the ASO.

[0182] In some embodiments, the ASOs are comprised of 2'-O-(2-methoxyethyl) (MOE) phosphorothioate-modified nucleotides. ASOs comprised of such nucleotides are especially well-suited to the methods disclosed herein; oligomers having such modifications have been shown to have significantly enhanced resistance to nuclease degradation and increased bioavailability, making them suitable, for example, for oral delivery in some embodiments described herein. See *e.g.*, Geary, *et al.*, J Pharmacol Exp Ther. 2001; 296(3):890-7; Geary, *et al.*, J Pharmacol Exp Ther. 2001; 296(3):898-904.

[0183] Methods of synthesizing ASOs will be known to one of skill in the art. Alternatively or in addition, ASOs may be obtained from a commercial source.

[0184] Unless specified otherwise, the left-hand end of single-stranded nucleic acid (*e.g.*, pre-mRNA transcript, oligonucleotide, ASO, etc.) sequences is the 5' end and the left-hand direction of single or double-stranded nucleic acid sequences is referred to as the 5' direction. Similarly, the right-hand end or direction of a nucleic acid sequence (single or double stranded) is the 3' end or direction. Generally, a region or sequence that is 5' to a reference point in a nucleic acid is referred to as "upstream," and a region or sequence that is 3' to a reference point in a nucleic acid is referred to as "downstream." Generally, the 5' direction or end of an mRNA is where the initiation or start codon is located, while the 3' end or direction is where the termination codon is located. In some aspects, nucleotides that are upstream of a reference point in a nucleic acid may be designated by a negative number, while nucleotides that are downstream of a reference point may be designated by a positive number. For example, a reference point (*e.g.*, an exon-exon junction in mRNA) may be designated as the "zero" site, and a nucleotide that is directly adjacent and upstream of the reference point is designated "minus one," *e.g.*, "-1," while a nucleotide that is directly adjacent and downstream of the reference point is designated "plus one," *e.g.*, "+1."

[0185] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is downstream (in the 3' direction) of the 5' splice site (or 3' end of the NMD exon) of the included exon in an *OPAI* pre-mRNA (*e.g.*, the direction designated by positive numbers relative to the 5' splice site). In some embodiments, the ASOs are complementary to a targeted portion of the *OPAI* pre-mRNA, *e.g.*, the *OPAI* NMD exon-containing pre-mRNA that is within the region about +1 to about +500 relative to the 5' splice site (or 3' end) of the included exon. In some embodiments, the ASOs may be complementary to a targeted portion of an *OPAI* pre-mRNA, *e.g.*, an *OPAI*

NMD exon-containing pre-mRNA, that is within the region between nucleotides +6 and +40,000 relative to the 5' splice site (or 3' end) of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about +1 to about +40,000, about +1 to about +30,000, about +1 to about +20,000, about +1 to about +15,000, about +1 to about +10,000, about +1 to about +5,000, about +1 to about +4,000, about +1 to about +3,000, about +1 to about +2,000, about +1 to about +1,000, about +1 to about +500, about +1 to about +490, about +1 to about +480, about +1 to about +470, about +1 to about +460, about +1 to about +450, about +1 to about +440, about +1 to about +430, about +1 to about +420, about +1 to about +410, about +1 to about +400, about +1 to about +390, about +1 to about +380, about +1 to about +370, about +1 to about +360, about +1 to about +350, about +1 to about +340, about +1 to about +330, about +1 to about +320, about +1 to about +310, about +1 to about +300, about +1 to about +290, about +1 to about +280, about +1 to about +270, about +1 to about +260, about +1 to about +250, about +1 to about +240, about +1 to about +230, about +1 to about +220, about +1 to about +210, about +1 to about +200, about +1 to about +190, about +1 to about +180, about +1 to about +170, about +1 to about +160, about +1 to about +150, about +1 to about +140, about +1 to about +130, about +1 to about +120, about +1 to about +110, about +1 to about +100, about +1 to about +90, about +1 to about +80, about +1 to about +70, about +1 to about +60, about +1 to about +50, about +1 to about +40, about +1 to about +30, or about +1 to about +20 relative to 5' splice site (or 3' end) of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region from about +1 to about +100, from about +100 to about +200, from about +200 to about +300, from about +300 to about +400, or from about +400 to about +500 relative to 5' splice site (or 3' end) of the included exon.

[0186] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is upstream (in the 5' direction) of the 5' splice site (or 3' end) of the included exon in an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA (*e.g.*, the direction designated by negative numbers relative to the 5' splice site). In some embodiments, the ASOs are complementary to a targeted portion of the *OPAI* pre-mRNA, *e.g.*, the *OPAI* NMD exon-containing pre-mRNA, that is within the region about -4 to about -270 relative to the 5' splice site (or 3' end) of the included exon. In some embodiments, the ASOs may be complementary to a targeted portion of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is within the region between nucleotides -1 and -40,000 relative to the 5' splice site (or 3' end) of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about -1 to about -40,000, about -1 to about -30,000, about -1 to about -20,000, about -1 to about -15,000,

about -1 to about -10,000, about -1 to about -5,000, about -1 to about -4,000, about -1 to about -3,000, about -1 to about -2,000, about -1 to about -1,000, about -1 to about -500, about -1 to about -490, about -1 to about -480, about -1 to about -470, about -1 to about -460, about -1 to about -450, about -1 to about -440, about -1 to about -430, about -1 to about -420, about -1 to about -410, about -1 to about -400, about -1 to about -390, about -1 to about -380, about -1 to about -370, about -1 to about -360, about -1 to about -350, about -1 to about -340, about -1 to about -330, about -1 to about -320, about -1 to about -310, about -1 to about -300, about -1 to about -290, about -1 to about -280, about -1 to about -270, about -1 to about -260, about -1 to about -250, about -1 to about -240, about -1 to about -230, about -1 to about -220, about -1 to about -210, about -1 to about -200, about -1 to about -190, about -1 to about -180, about -1 to about -170, about -1 to about -160, about -1 to about -150, about -1 to about -140, about -1 to about -130, about -1 to about -120, about -1 to about -110, about -1 to about -100, about -1 to about -90, about -1 to about -80, about -1 to about -70, about -1 to about -60, about -1 to about -50, about -1 to about -40, about -1 to about -30, or about -1 to about -20 relative to 5' splice site (or 3' end) of the included exon.

[0187] In some embodiments, the ASOs are complementary to a targeted region of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is upstream (in the 5' direction) of the 3' splice site (or 5' end) of the included exon in an *OPAI* pre-mRNA (*e.g.*, in the direction designated by negative numbers). In some embodiments, the ASOs are complementary to a targeted portion of the *OPAI* pre-mRNA, *e.g.*, the *OPAI* NMD exon-containing pre-mRNA, that is within the region about -1 to about -500 relative to the 3' splice site (or 5' end) of the included exon. In some embodiments, the ASOs are complementary to a targeted portion of the *OPAI* pre-mRNA that is within the region -1 to -40,000 relative to the 3' splice site of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about -1 to about -40,000, about -1 to about -30,000, -1 to about -20,000, about -1 to about -15,000, about -1 to about -10,000, about -1 to about -5,000, about -1 to about -4,000, about -1 to about -3,000, about -1 to about -2,000, about -1 to about -1,000, about -1 to about -500, about -1 to about -490, about -1 to about -480, about -1 to about -470, about -1 to about -460, about -1 to about -450, about -1 to about -440, about -1 to about -430, about -1 to about -420, about -1 to about -410, about -1 to about -400, about -1 to about -390, about -1 to about -380, about -1 to about -370, about -1 to about -360, about -1 to about -350, about -1 to about -340, about -1 to about -330, about -1 to about -320, about -1 to about -310, about -1 to about -300, about -1 to about -290, about -1 to about -280, about -1 to about -270, about -1 to about -260, about -1 to about -250, about -1 to about -240, about -1 to about -230, about -1 to about -220, about -1 to about -210, about -1 to about -200, about -1 to about -190, about -1 to about -

180, about -1 to about -170, about -1 to about -160, about -1 to about -150, about -1 to about -140, about -1 to about -130, about -1 to about -120, about -1 to about -110, about -1 to about -100, about -1 to about -90, about -1 to about -80, about -1 to about -70, about -1 to about -60, about -1 to about -50, about -1 to about -40, about -1 to about -30, or about -1 to about -20 relative to 3' splice site of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region from about -1 to about -100, from about -100 to about -200, from about -200 to about -300, from about -300 to about -400, or from about -400 to about -500 relative to 3' splice site of the included exon.

[0188] In some embodiments, the ASOs are complementary to a targeted region of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is downstream (in the 3' direction) of the 3' splice site (5' end) of the included exon in an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA (*e.g.*, in the direction designated by positive numbers). In some embodiments, the ASOs are complementary to a targeted portion of the *OPAI* pre-mRNA that is within the region of about +1 to about +40,000 relative to the 3' splice site of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about +1 to about +40,000, about +1 to about +30,000, about +1 to about +20,000, about +1 to about +15,000, about +1 to about +10,000, about +1 to about +5,000, about +1 to about +4,000, about +1 to about +3,000, about +1 to about +2,000, about +1 to about +1,000, about +1 to about +500, about +1 to about +490, about +1 to about +480, about +1 to about +470, about +1 to about +460, about +1 to about +450, about +1 to about +440, about +1 to about +430, about +1 to about +420, about +1 to about +410, about +1 to about +400, about +1 to about +390, about +1 to about +380, about +1 to about +370, about +1 to about +360, about +1 to about +350, about +1 to about +340, about +1 to about +330, about +1 to about +320, about +1 to about +310, about +1 to about +300, about +1 to about +290, about +1 to about +280, about +1 to about +270, about +1 to about +260, about +1 to about +250, about +1 to about +240, about +1 to about +230, about +1 to about +220, about +1 to about +210, about +1 to about +200, about +1 to about +190, about +1 to about +180, about +1 to about +170, about +1 to about +160, about +1 to about +150, about +1 to about +140, about +1 to about +130, about +1 to about +120, about +1 to about +110, about +1 to about +100, about +1 to about +90, about +1 to about +80, about +1 to about +70, about +1 to about +60, about +1 to about +50, about +1 to about +40, about +1 to about +30, or about +1 to about +20, or about +1 to about +10 relative to 3' splice site of the included exon.

[0189] In some embodiments, the targeted portion of the *OPAI* pre-mRNA, *e.g.*, the *OPAI* NMD exon-containing pre-mRNA, is within the region +100 relative to the 5' splice site (3' end) of the included exon to -100 relative to the 3' splice site (5' end) of the included exon. In some

embodiments, the targeted portion of the *OPAI* NMD exon-containing pre-mRNA is within the NMD exon. In some embodiments, the target portion of the *OPAI* NMD exon-containing pre-mRNA comprises a pseudo-exon and intron boundary.

[0190] The ASOs may be of any length suitable for specific binding and effective enhancement of splicing. In some embodiments, the ASOs consist of 8 to 50 nucleobases. For example, the ASO may be 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, or 50 nucleobases in length. In some embodiments, the ASOs consist of more than 50 nucleobases. In some embodiments, the ASO is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, 12 to 15 nucleobases, 13 to 50 nucleobases, 13 to 40 nucleobases, 13 to 35 nucleobases, 13 to 30 nucleobases, 13 to 25 nucleobases, 13 to 20 nucleobases, 14 to 50 nucleobases, 14 to 40 nucleobases, 14 to 35 nucleobases, 14 to 30 nucleobases, 14 to 25 nucleobases, 14 to 20 nucleobases, 15 to 50 nucleobases, 15 to 40 nucleobases, 15 to 35 nucleobases, 15 to 30 nucleobases, 15 to 25 nucleobases, 15 to 20 nucleobases, 20 to 50 nucleobases, 20 to 40 nucleobases, 20 to 35 nucleobases, 20 to 30 nucleobases, 20 to 25 nucleobases, 25 to 50 nucleobases, 25 to 40 nucleobases, 25 to 35 nucleobases, or 25 to 30 nucleobases in length. In some embodiments, the ASOs are 18 nucleotides in length. In some embodiments, the ASOs are 15 nucleotides in length. In some embodiments, the ASOs are 25 nucleotides in length.

[0191] In some embodiments, two or more ASOs with different chemistries but complementary to the same targeted portion of the pre-mRNA, *e.g.*, NMD exon-containing pre-mRNA, are used. In some embodiments, two or more ASOs that are complementary to different targeted portions of the pre-mRNA, *e.g.*, the NMD exon-containing pre-mRNA, are used.

[0192] In some embodiments, the antisense oligonucleotides of the disclosure are chemically linked to one or more moieties or conjugates, *e.g.*, a targeting moiety or other conjugate that enhances the activity or cellular uptake of the oligonucleotide. Such moieties include, but are not limited to, a lipid moiety, *e.g.*, as a cholesterol moiety, a cholesteryl moiety, an aliphatic chain, *e.g.*, dodecandiol or undecyl residues, a polyamine or a polyethylene glycol chain, or adamantane acetic acid. Oligonucleotides comprising lipophilic moieties and preparation

methods have been described in the published literature. In embodiments, the antisense oligonucleotide is conjugated with a moiety including, but not limited to, an abasic nucleotide, a polyether, a polyamine, a polyamide, a peptides, a carbohydrate, *e.g.*, N-acetylgalactosamine (GalNAc), N-Ac-Glucosamine (GluNAc), or mannose (*e.g.*, mannose-6-phosphate), a lipid, or a polyhydrocarbon compound. Conjugates can be linked to one or more of any nucleotides comprising the antisense oligonucleotide at any of several positions on the sugar, base or phosphate group, as understood in the art and described in the literature, *e.g.*, using a linker. Linkers can include a bivalent or trivalent branched linker. In embodiments, the conjugate is attached to the 3' end of the antisense oligonucleotide. Methods of preparing oligonucleotide conjugates are described, *e.g.*, in U.S. Pat. No. 8,450,467, "Carbohydrate conjugates as delivery agents for oligonucleotides," incorporated by reference herein.

[0193] In some embodiments, the nucleic acid to be targeted by an ASO is an *OPAI* pre-mRNA, *e.g.*, NMD exon-containing pre-mRNA expressed in a cell, such as a eukaryotic cell. In some embodiments, the term "cell" may refer to a population of cells. In some embodiments, the cell is in a subject. In some embodiments, the cell is isolated from a subject. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is a condition or disease-relevant cell or a cell line. In some embodiments, the cell is *in vitro* (*e.g.*, in cell culture).

Pharmaceutical Compositions

[0194] Pharmaceutical compositions or formulations comprising the agent, *e.g.*, antisense oligonucleotide, of the described compositions and for use in any of the described methods can be prepared according to conventional techniques well known in the pharmaceutical industry and described in the published literature. In embodiments, a pharmaceutical composition or formulation for treating a subject comprises an effective amount of any antisense oligomer as described herein, or a pharmaceutically acceptable salt, solvate, hydrate or ester thereof. The pharmaceutical formulation comprising an antisense oligomer may further comprise a pharmaceutically acceptable excipient, diluent or carrier.

[0195] Pharmaceutically acceptable salts are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, etc., and are commensurate with a reasonable benefit/risk ratio. (See, *e.g.*, S. M. Berge, et al., J. Pharmaceutical Sciences, 66: 1-19 (1977), incorporated herein by reference for this purpose. The salts can be prepared in situ during the final isolation and purification of the compounds, or separately by reacting the free base form with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric

acid, citric acid, succinic acid or malonic acid or by using other documented methodologies such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

[0196] In some embodiments, the compositions are formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. In embodiments, the compositions are formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers. In embodiments, a pharmaceutical formulation or composition of the present disclosure includes, but is not limited to, a solution, emulsion, microemulsion, foam or liposome-containing formulation (*e.g.*, cationic or noncationic liposomes).

[0197] The pharmaceutical composition or formulation described herein may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients as appropriate and well known to those of skill in the art or described in the published literature. In embodiments, liposomes also include sterically stabilized liposomes, *e.g.*, liposomes comprising one or more specialized lipids. These specialized lipids result in liposomes with enhanced circulation lifetimes. In embodiments, a sterically stabilized liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. In some embodiments, a surfactant is included in the pharmaceutical formulation or compositions. The use of surfactants in drug products, formulations and emulsions is well known in the art. In embodiments, the present disclosure employs a penetration enhancer to effect the efficient delivery of the antisense oligonucleotide, *e.g.*, to aid diffusion across cell membranes and /or enhance the permeability of a lipophilic drug. In some

embodiments, the penetration enhancers are a surfactant, fatty acid, bile salt, chelating agent, or non-chelating nonsurfactant.

[0198] In some embodiments, the pharmaceutical formulation comprises multiple antisense oligonucleotides. In embodiments, the antisense oligonucleotide is administered in combination with another drug or therapeutic agent.

Combination Therapies

[0199] In some embodiments, the ASOs disclosed in the present disclosure can be used in combination with one or more additional therapeutic agents. In some embodiments, the one or more additional therapeutic agents can comprise a small molecule. For example, the one or more additional therapeutic agents can comprise a small molecule described in WO2016128343A1, WO2017053982A1, WO2016196386A1, WO201428459A1, WO201524876A2, WO2013119916A2, and WO2014209841A2, which are incorporated by reference herein in their entirety. In some embodiments, the one or more additional therapeutic agents comprise an ASO that can be used to correct intron retention.

Treatment of Subjects

[0200] Any of the compositions provided herein may be administered to an individual.

“Individual” may be used interchangeably with “subject” or “patient.” An individual may be a mammal, for example a human or animal such as a non-human primate, a rodent, a rabbit, a rat, a mouse, a horse, a donkey, a goat, a cat, a dog, a cow, a pig, or a sheep. In embodiments, the individual is a human. In embodiments, the individual is a fetus, an embryo, or a child. In other embodiments, the individual may be another eukaryotic organism, such as a plant. In some embodiments, the compositions provided herein are administered to a cell *ex vivo*.

[0201] In some embodiments, the compositions provided herein are administered to an individual as a method of treating a disease or disorder. In some embodiments, the individual has a genetic disease, such as any of the diseases described herein. In some embodiments, the individual is at risk of having a disease, such as any of the diseases described herein. In some embodiments, the individual is at increased risk of having a disease or disorder caused by insufficient amount of a protein or insufficient activity of a protein. If an individual is “at an increased risk” of having a disease or disorder caused insufficient amount of a protein or insufficient activity of a protein, the method involves preventative or prophylactic treatment. For example, an individual may be at an increased risk of having such a disease or disorder because of family history of the disease. Typically, individuals at an increased risk of having such a disease or disorder benefit from prophylactic treatment (*e.g.*, by preventing or delaying the onset or progression of the disease or disorder). In embodiments, a fetus is treated in utero, *e.g.*, by administering the ASO composition to the fetus directly or indirectly (*e.g.*, via the mother).

[0202] In some cases, the subject pharmaceutical composition and method are applicable for treatment of a condition or disease associated with OPA1 deficiency. In some cases, the subject pharmaceutical composition and method are applicable for treatment of an eye disease or condition. In some cases, the subject pharmaceutical composition and method are applicable for treatment of Optic atrophy type 1, autosomal dominant optic atrophy (ADOA), ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

[0203] Autosomal dominant optic atrophy (ADOA) is the most common inherited optic nerve disorder and is characterized by retinal ganglion cell loss. In some cases, 65-90% of ADOA cases are caused by mutations in one allele of the *OPA1* gene. *OPA1* gene encodes an OPA1 protein that is a mitochondrial GTPase, which can have a critical maintenance role in mitochondria structure and function. Most *OPA1* mutations can lead to a haploinsufficiency, resulting in about a 50% decrease of normal OPA1 protein levels. Approximately 1 out of 30,000 people are affected globally with a higher incidence of ~1 out of 10,000 in Denmark due to a founder effect. ADOA can present within the first decade of life. 80% of ADOA patients are symptomatic before 10 years of age. The disease can cause progressive and irreversible vision loss and up to 46% of patients are registered as legally blind.

[0204] In some cases, a therapeutic agent comprises an oligonucleotide. In some cases, a therapeutic agent comprises a vector, *e.g.*, a viral vector, expressing a oligonucleotide that binds to the targeted region of a pre-mRNA the encodes the target peptide sequence. The methods provided herein can be adapted to contacting a vector that encodes an agent, *e.g.*, an

oligonucleotide, to a cell, so that the agent binds to a pre-mRNA in the cell and modulates the processing of the pre-mRNA. In some cases, the viral vector comprises an adenoviral vector, adeno-associated viral (AAV) vector, lentiviral vector, Herpes Simplex Virus (HSV) viral vector, retroviral vector, or any applicable viral vector. In some cases, a therapeutic agent comprises a gene editing tool that is configured to modify a gene encoding the target peptide sequence such that a gene region that encodes the inefficient translation region is deleted. In some cases, a gene editing tool comprises vector, *e.g.*, viral vector, for gene editing based on CRISPR-Cas9, TALEN, Zinc Finger, or other applicable technologies.

[0205] Suitable routes for administration of ASOs of the present disclosure may vary depending on cell type to which delivery of the ASOs is desired. Multiple tissues and organs are affected by ADOA, with the eye being the most significantly affected tissue. The ASOs of the present disclosure may be administered to patients parenterally, for example, by intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[0206] In embodiments, the antisense oligonucleotide is administered with one or more agents capable of promoting penetration of the subject antisense oligonucleotide across the blood-brain barrier by any method known in the art. For example, delivery of agents by administration of an adenovirus vector to motor neurons in muscle tissue is described in U.S. Pat. No. 6,632,427, “Adenoviral-vector-mediated gene transfer into medullary motor neurons,” incorporated herein by reference. Delivery of vectors directly to the brain, *e.g.*, the striatum, the thalamus, the hippocampus, or the substantia nigra, is described, *e.g.*, in U.S. Pat. No. 6,756,523, “Adenovirus vectors for the transfer of foreign genes into cells of the central nervous system particularly in brain,” incorporated herein by reference.

[0207] In some embodiments, the antisense oligonucleotides are linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. In embodiments, the antisense oligonucleotide is coupled to a substance, known in the art to promote penetration or transport across the blood-brain barrier, *e.g.*, an antibody to the transferrin receptor. In embodiments, the antisense oligonucleotide is linked with a viral vector, *e.g.*, to render the antisense compound more effective or increase transport across the blood-brain barrier. In embodiments, osmotic blood brain barrier disruption is assisted by infusion of sugars, *e.g.*, meso erythritol, xylitol, D(+) galactose, D(+) lactose, D(+) xylose, dulcitol, myo-inositol, L(-) fructose, D(-) mannitol, D(+) glucose, D(+) arabinose, D(-) arabinose, cellobiose, D(+) maltose, D(+) raffinose, L(+) rhamnose, D(+) melibiose, D(-) ribose, adonitol, D(+) arabitol, L(-) arabitol, D(+) fucose, L(-) fucose, D(-) lyxose, L(+) lyxose, and L(-) lyxose, or amino acids, *e.g.*, glutamine, lysine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine,

leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and taurine. Methods and materials for enhancing blood brain barrier penetration are described, *e.g.*, in U.S. Pat. No. 9,193,969, "Compositions and methods for selective delivery of oligonucleotide molecules to specific neuron types," U.S. Pat. No. 4,866,042, "Method for the delivery of genetic material across the blood brain barrier," U.S. Pat. No. 6,294,520, "Material for passage through the blood-brain barrier," and U.S. Pat. No. 6,936,589, "Parenteral delivery systems," each incorporated herein by reference.

[0208] In some embodiments, subjects treated using the methods and compositions are evaluated for improvement in condition using any methods known and described in the art.

Methods of Identifying Additional ASOs that Induce Exon Skipping

[0209] Also within the scope of the present disclosure are methods for identifying or determining ASOs that induce exon skipping of an *OPAI* NMD exon-containing pre-mRNA. For example, a method can comprise identifying or determining ASOs that induce pseudo-exon skipping of an *OPAI* NMD exon-containing pre-mRNA. ASOs that specifically hybridize to different nucleotides within the target region of the pre-mRNA may be screened to identify or determine ASOs that improve the rate and/or extent of splicing of the target intron. In some embodiments, the ASO may block or interfere with the binding site(s) of a splicing repressor(s)/silencer. Any method known in the art may be used to identify (determine) an ASO that when hybridized to the target region of the exon results in the desired effect (*e.g.*, pseudo-exon skipping, protein or functional RNA production). These methods also can be used for identifying ASOs that induce exon skipping of the included exon by binding to a targeted region in an intron flanking the included exon, or in a non-included exon. An example of a method that may be used is provided below.

[0210] A round of screening, referred to as an ASO "walk" may be performed using ASOs that have been designed to hybridize to a target region of a pre-mRNA. For example, the ASOs used in the ASO walk can be tiled every 5 nucleotides from approximately 100 nucleotides upstream of the 3' splice site of the included exon (*e.g.*, a portion of sequence of the exon located upstream of the target/included exon) to approximately 100 nucleotides downstream of the 3' splice site of the target/included exon and/or from approximately 100 nucleotides upstream of the 5' splice site of the included exon to approximately 100 nucleotides downstream of the 5' splice site of the target/included exon (*e.g.*, a portion of sequence of the exon located downstream of the target/included exon). For example, a first ASO of 15 nucleotides in length may be designed to specifically hybridize to nucleotides +6 to +20 relative to the 3' splice site of the target/included exon. A second ASO may be designed to specifically hybridize to nucleotides +11 to +25 relative to the 3' splice site of the target/included exon. ASOs are designed as such spanning the

target region of the pre-mRNA. In embodiments, the ASOs can be tiled more closely, *e.g.*, every 1, 2, 3, or 4 nucleotides. Further, the ASOs can be tiled from 100 nucleotides downstream of the 5' splice site, to 100 nucleotides upstream of the 3' splice site. In some embodiments, the ASOs can be tiled from about 1,160 nucleotides upstream of the 3' splice site, to about 500 nucleotides downstream of the 5' splice site. In some embodiments, the ASOs can be tiled from about 500 nucleotides upstream of the 3' splice site, to about 1,920 nucleotides downstream of the 3' splice site.

[0211] One or more ASOs, or a control ASO (an ASO with a scrambled sequence, sequence that is not expected to hybridize to the target region) are delivered, for example by transfection, into a disease-relevant cell line that expresses the target pre-mRNA (*e.g.*, a NMD exon-containing pre-mRNA described herein). The exon skipping effects of each of the ASOs may be assessed by any method known in the art, for example by reverse transcriptase (RT)-PCR using primers that span the splice junction, as described in **Example 4**. A reduction or absence of a longer RT-PCR product produced using the primers spanning the region containing the included exon (*e.g.* including the flanking exons of the NMD exon) in ASO-treated cells as compared to in control ASO-treated cells indicates that splicing of the target NMD exon has been enhanced. In some embodiments, the exon skipping efficiency (or the splicing efficiency to splice the intron containing the NMD exon), the ratio of spliced to unspliced pre-mRNA, the rate of splicing, or the extent of splicing may be improved using the ASOs described herein. The amount of protein or functional RNA that is encoded by the target pre-mRNA can also be assessed to determine whether each ASO achieved the desired effect (*e.g.*, enhanced functional protein production). Any method known in the art for assessing and/or quantifying protein production, such as Western blotting, flow cytometry, immunofluorescence microscopy, and ELISA, can be used.

[0212] A second round of screening, referred to as an ASO “micro-walk” may be performed using ASOs that have been designed to hybridize to a target region of a pre-mRNA. The ASOs used in the ASO micro-walk are tiled every 1 nucleotide to further refine the nucleotide acid sequence of the pre-mRNA that when hybridized with an ASO results in exon skipping (or enhanced splicing of NMD exon).

[0213] Regions defined by ASOs that promote splicing of the target intron are explored in greater detail by means of an ASO “micro-walk”, involving ASOs spaced in 1-nt steps, as well as longer ASOs, typically 18-25 nt.

[0214] As described for the ASO walk above, the ASO micro-walk is performed by delivering one or more ASOs, or a control ASO (an ASO with a scrambled sequence, sequence that is not expected to hybridize to the target region), for example by transfection, into a disease-relevant cell line that expresses the target pre-mRNA. The splicing-inducing effects of each of the ASOs

may be assessed by any method known in the art, for example by reverse transcriptase (RT)-PCR using primers that span the NMD exon, as described herein (see, *e.g.*, **Example 4**). A reduction or absence of a longer RT-PCR product produced using the primers spanning the NMD exon in ASO-treated cells as compared to in control ASO-treated cells indicates that exon skipping (or splicing of the target intron containing an NMD exon) has been enhanced. In some embodiments, the exon skipping efficiency (or the splicing efficiency to splice the intron containing the NMD exon), the ratio of spliced to unspliced pre-mRNA, the rate of splicing, or the extent of splicing may be improved using the ASOs described herein. The amount of protein or functional RNA that is encoded by the target pre-mRNA can also be assessed to determine whether each ASO achieved the desired effect (*e.g.*, enhanced functional protein production). Any method known in the art for assessing and/or quantifying protein production, such as Western blotting, flow cytometry, immunofluorescence microscopy, and ELISA, can be used.

[0215] ASOs that when hybridized to a region of a pre-mRNA result in exon skipping (or enhanced splicing of the intron containing a NMD exon) and increased protein production may be tested *in vivo* using animal models, for example transgenic mouse models in which the full-length human gene has been knocked-in or in humanized mouse models of disease. Suitable routes for administration of ASOs may vary depending on the disease and/or the cell types to which delivery of the ASOs is desired. ASOs may be administered, for example, by intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection. Following administration, the cells, tissues, and/or organs of the model animals may be assessed to determine the effect of the ASO treatment by for example evaluating splicing (*e.g.*, efficiency, rate, extent) and protein production by methods known in the art and described herein. The animal models may also be any phenotypic or behavioral indication of the disease or disease severity.

[0216] Also within the scope of the present disclosure is a method to identify or validate an NMD-inducing exon in the presence of an NMD inhibitor, for example, cycloheximide. An exemplary method is provided in **Example 2**.

SPECIFIC EMBODIMENTS (A)

[0217] Embodiment A1. A method of treating Optic atrophy type 1 in a subject in need thereof, by increasing the expression of a target protein or functional RNA by a cell of the subject, wherein the cell has an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA), and wherein the NMD exon mRNA encodes the target protein or functional RNA, the method comprising contacting the cell of the subject with a therapeutic agent that binds to a targeted portion of the NMD exon mRNA encoding the target protein or

functional RNA, whereby the non-sense mediated RNA decay-inducing exon is excluded from the NMD exon mRNA encoding the target protein or functional RNA, thereby increasing the level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cell of the subject.

[0218] Embodiment A2. The method of embodiment A1, wherein the target protein is OPA1.

[0219] Embodiment A3. A method of increasing expression of OPA1 protein by a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes OPA1 protein, the method comprising contacting the cell with an agent that binds to a targeted portion of the NMD exon mRNA encoding OPA1 protein, whereby the non-sense mediated RNA decay-inducing exon is excluded from the NMD exon mRNA encoding OPA1 protein, thereby increasing the level of mRNA encoding OPA1 protein, and increasing the expression of OPA1 protein in the cell.

[0220] Embodiment A4. The method of any one of embodiments A1 to A3, wherein the non-sense mediated RNA decay-inducing exon is spliced out from the NMD exon mRNA encoding the target protein or functional RNA.

[0221] Embodiment A5. The method of any one of embodiments A1 to A4, wherein the target protein does not comprise an amino acid sequence encoded by the non-sense mediated RNA decay-inducing exon.

[0222] Embodiment A6. The method of any one of embodiments A1 to A5, wherein the target protein is a full-length target protein.

[0223] Embodiment A7. The method of any one of embodiments A1 to A6, wherein the agent is an antisense oligomer (ASO) complementary to the targeted portion of the NMD exon mRNA.

[0224] Embodiment A8. The method of any one of embodiments A1 to A7, wherein the mRNA is pre-mRNA.

[0225] Embodiment A9. The method of any one of embodiments A1 to A8, wherein the contacting comprises contacting the therapeutic agent to the mRNA, wherein the mRNA is in a nucleus of the cell.

[0226] Embodiment A10. The method of any one of embodiments A1 to A9, wherein the target protein or the functional RNA corrects a deficiency in the target protein or functional RNA in the subject.

[0227] Embodiment A11. The method of any one of embodiments A1 to A10, wherein the cells are in or from a subject with a condition caused by a deficient amount or activity of an OPA1 protein.

[0228] Embodiment A12. The method of any one of embodiments A1 to A11, wherein the deficient amount of the target protein is caused by haploinsufficiency of the target protein, wherein the subject has a first allele encoding a functional target protein, and a second allele from which the target protein is not produced, or a second allele encoding a nonfunctional target protein, and wherein the antisense oligomer binds to a targeted portion of a NMD exon mRNA transcribed from the first allele.

[0229] Embodiment A13. The method of any one of embodiments A1 to A11, wherein the subject has a condition caused by a disorder resulting from a deficiency in the amount or function of the target protein, wherein the subject has

- (a) a first mutant allele from which
 - (i) the target protein is produced at a reduced level compared to production from a wild-type allele,
 - (ii) the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
 - (iii) the target protein is not produced, and
- (b) a second mutant allele from which
 - (i) the target protein is produced at a reduced level compared to production from a wild-type allele,
 - (ii) the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
 - (iii) the target protein is not produced, and

wherein when the subject has a first mutant allele (a)(iii), the second mutant allele is (b)(i) or (b)(ii) and wherein when the subject has a second mutant allele (b)(iii), the first mutant allele is (a)(i) or (a)(ii), and wherein the NMD exon mRNA is transcribed from either the first mutant allele that is (a)(i) or (a)(ii), and/or the second allele that is (b)(i) or (b)(ii).

[0230] Embodiment A14. The method of embodiment A13, wherein the target protein is produced in a form having reduced function compared to the equivalent wild-type protein.

[0231] Embodiment A15. The method of embodiment A13, wherein the target protein is produced in a form that is fully-functional compared to the equivalent wild-type protein.

[0232] Embodiment A16. The method of any one of embodiments A1 to A15, wherein the targeted portion of the NMD exon mRNA is within the non-sense mediated RNA decay-inducing exon.

[0233] Embodiment A17. The method of any one of embodiments A1 to A15, wherein the targeted portion of the NMD exon mRNA is either upstream or downstream of the non-sense mediated RNA decay-inducing exon.

[0234] Embodiment A18. The method of any one of embodiments A1 to A17, wherein the NMD exon mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 2 or 3.

[0235] Embodiment A19. The method of any one of embodiments A1 to A17, wherein the NMD exon mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 1.

[0236] Embodiment A20. The method of any one of embodiments A1 to A17, wherein the targeted portion of the NMD exon mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[0237] Embodiment A21. The method of any one of embodiments A1 to A20, wherein the agent is an antisense oligomer (ASO) and wherein the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[0238] Embodiment A22. The method of any one of embodiments A1 to A15, wherein the targeted portion of the NMD exon mRNA is within the non-sense mediated RNA decay-inducing exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[0239] Embodiment A23. The method of any one of embodiments A1 to A15, wherein the targeted portion of the NMD exon mRNA is upstream or downstream of the non-sense mediated RNA decay-inducing exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[0240] Embodiment A24. The method of any one of embodiments A1 to A15, wherein the targeted portion of the NMD exon mRNA comprises an exon-intron junction exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[0241] Embodiment A25. The method of any one of embodiments A1 to A24, wherein the target protein produced is full-length protein, or wild-type protein.

[0242] Embodiment A26. The method of any one of embodiments A1 to A25, wherein the total amount of the mRNA encoding the target protein or functional RNA produced in the cell contacted with the antisense oligomer is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least

about 5-fold, or at least about 10-fold, compared to the total amount of the mRNA encoding the target protein or functional RNA produced in a control cell.

[0243] Embodiment A27. The method of any one of embodiments A1 to A25, wherein the total amount of the mRNA encoding the target protein or functional RNA produced in the cell contacted with the antisense oligomer is increased about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 10%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300%, compared to the total amount of the mRNA encoding the target protein or functional RNA produced in a control cell.

[0244] Embodiment A28. The method of one any of embodiments A1 to A25, wherein the total amount of target protein produced by the cell contacted with the antisense oligomer is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the total amount of target protein produced by a control cell.

[0245] Embodiment A29. The method of one any of embodiments A1 to A25, wherein the total amount of target protein produced by the cell contacted with the antisense oligomer is increased about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 10%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least

about 250%, or at least about 300%, compared to the total amount of target protein produced by a control cell.

[0246] Embodiment A30. The method of any one of embodiments A1 to 29, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[0247] Embodiment A31. The method of any one of embodiments A1 to A30, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[0248] Embodiment A32. The method of any one of embodiments A1 to A31, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises at least one modified sugar moiety.

[0249] Embodiment A33. The method of embodiment A32, wherein each sugar moiety is a modified sugar moiety.

[0250] Embodiment A34. The method of any one of embodiments A1 to A33, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[0251] Embodiment A35. The method of any one of embodiments A1 to A34, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, complementary to the targeted portion of the NMD exon mRNA encoding the protein.

[0252] Embodiment A36. The method of any one of embodiments A1 to A35, wherein the method further comprises assessing *OPAI* mRNA or protein expression.

[0253] Embodiment A37. The method of any one of embodiments A1 to A36, wherein Optic atrophy type 1 is treated and wherein the antisense oligomer binds to a targeted portion of an *OPAI* NMD exon mRNA, wherein the targeted portion is within SEQ ID NO: 2 or 3.

- [0254] Embodiment A38. The method of any one of embodiments A1 to A37, wherein the subject is a human.
- [0255] Embodiment A39. The method of any one of embodiments A1 to A38, wherein the subject is a non-human animal.
- [0256] Embodiment A40. The method of any one of embodiments A1 to A39, wherein the subject is a fetus, an embryo, or a child.
- [0257] Embodiment A41. The method of any one of embodiments A1 to A40, wherein the cells are ex vivo.
- [0258] Embodiment A42. The method of any one of embodiments A1 to A41, wherein the therapeutic agent is administered by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection of the subject.
- [0259] Embodiment A43. The method of any of embodiments A1 to A42, wherein the method further comprises administering a second therapeutic agent to the subject.
- [0260] Embodiment A44. The method of embodiment A43, wherein the second therapeutic agent is a small molecule.
- [0261] Embodiment A45. The method of embodiment A43, wherein the second therapeutic agent is an ASO.
- [0262] Embodiment A46. The method of any one of embodiments A43 to A45, wherein the second therapeutic agent corrects intron retention.
- [0263] Embodiment A47. An antisense oligomer as used in a method of any of embodiments A1 to A46.
- [0264] Embodiment A48. An antisense oligomer comprising a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.
- [0265] Embodiment A49. A pharmaceutical composition comprising the antisense oligomer of embodiment A47 or A48 and an excipient.
- [0266] Embodiment A50. A method of treating a subject in need thereof, comprising administering the pharmaceutical composition of embodiment A49 to the subject, wherein the administering is by intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.
- [0267] Embodiment A51. A composition comprising a therapeutic agent for use in a method of increasing expression of a target protein or a functional RNA by cells to treat Optic atrophy type 1 in a subject in need thereof, associated with a deficient protein or deficient functional

RNA, wherein the deficient protein or deficient functional RNA is deficient in amount or activity in the subject, wherein the target protein is:

- (a) the deficient protein; or
 - (b) a compensating protein which functionally augments or replaces the deficient protein or in the subject;
- and wherein the functional RNA is:
- (c) the deficient RNA; or
 - (d) a compensating functional RNA which functionally augments or replaces the deficient functional RNA in the subject;

[0268] wherein the therapeutic agent enhances exclusion of the non-sense mediated RNA decay-inducing exon from the NMD exon mRNA encoding the target protein or functional RNA, thereby increasing production or activity of the target protein or the functional RNA in the subject.

[0269] Embodiment A52. A composition comprising a therapeutic agent for use in a method of treating a condition associated with OPA1 protein in a subject in need thereof, the method comprising the step of increasing expression of OPA1 protein by cells of the subject, wherein the cells have an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes OPA1 protein, the method comprising contacting the cells with the therapeutic agent, whereby the non-sense mediated RNA decay-inducing exon is excluded from the NMD exon mRNA that encodes OPA1 protein, thereby increasing the level of mRNA encoding OPA1 protein, and increasing the expression of OPA1 protein in the cells of the subject.

[0270] Embodiment A53. The composition of embodiment A52, wherein the condition is a disease or disorder.

[0271] Embodiment A54. The composition of embodiment A53, wherein the disease or disorder is Optic atrophy type 1.

[0272] Embodiment A55. The composition of any one of embodiments A52 to 54, wherein the OPA1 protein and NMD exon mRNA are encoded by the *OPA1* gene.

[0273] Embodiment A56. The composition of any one of embodiments A51 to A55, wherein the non-sense mediated RNA decay-inducing exon is spliced out from the NMD exon mRNA encoding the OPA1 protein.

[0274] Embodiment A57. The composition of any one of embodiments A51 to A56, wherein the OPA1 protein does not comprise an amino acid sequence encoded by the non-sense mediated RNA decay-inducing exon.

[0275] Embodiment A58. The composition of any one of embodiments A51 to A57, wherein the OPA1 protein is a full-length OPA1 protein.

[0276] Embodiment A59. The composition of any one of embodiments A51 to A58, wherein the therapeutic agent is an antisense oligomer (ASO) complementary to the targeted portion of the NMD exon mRNA.

[0277] Embodiment A60. The composition of any of embodiments A51 to A59, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer targets a portion of the NMD exon mRNA that is within the non-sense mediated RNA decay-inducing exon.

[0278] Embodiment A61. The composition of any of embodiments A51 to A59, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer targets a portion of the NMD exon mRNA that is upstream or downstream of the non-sense mediated RNA decay-inducing exon.

[0279] Embodiment A62. The composition of any one of embodiments A51 to A61, wherein the target protein is OPA1.

[0280] Embodiment A63. The composition of embodiment A62, wherein the NMD exon mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 2 or 3.

[0281] Embodiment A64. The composition of embodiment A62, wherein the NMD exon mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 1.

[0282] Embodiment A65. The composition of embodiment A62, wherein the targeted portion of the NMD exon mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[0283] Embodiment A66. The composition of any one of embodiments A62 to A65, wherein the targeted portion of the NMD exon mRNA is within the non-sense mediated RNA decay-inducing exon 6x of *OPA1*, exon 7x of *OPA1*, or exon 28x of *OPA1*.

[0284] Embodiment A67. The composition of any one of embodiments A62 to A65, wherein the targeted portion of the NMD exon mRNA is upstream or downstream of the non-sense mediated RNA decay-inducing exon 6x of *OPA1*, exon 7x of *OPA1*, or exon 28x of *OPA1*.

[0285] Embodiment A68. The composition of any one of embodiments A62 to A65, wherein the targeted portion of the NMD exon mRNA comprises an exon-intron junction of exon 6x of *OPA1*, exon 7x of *OPA1*, or exon 28x of *OPA1*.

[0286] Embodiment A69. The composition of any one of embodiments A62 to A68, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[0287] Embodiment A70. The composition of any one of embodiments A51 to A69, wherein the mRNA encoding the target protein or functional RNA is a full-length mature mRNA, or a wild-type mature mRNA.

[0288] Embodiment A71. The composition of any one of embodiments A51 to A70, wherein the target protein produced is full-length protein, or wild-type protein.

[0289] Embodiment A72. The composition of any one of embodiments A51 to A71, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[0290] Embodiment A73. The composition of any of embodiments A51 to A72, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein said antisense oligomer is an antisense oligonucleotide.

[0291] Embodiment A74. The composition of any of embodiments A51 to A73, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[0292] Embodiment A75. The composition of any of embodiments A51 to A74, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises at least one modified sugar moiety.

[0293] Embodiment A76. The composition of embodiment A75, wherein each sugar moiety is a modified sugar moiety.

[0294] Embodiment A77. The composition of any of embodiments A51 to A76, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40

nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[0295] Embodiment A78. A pharmaceutical composition comprising the therapeutic agent of any of the compositions of embodiments A51 to A77, and an excipient.

[0296] Embodiment A79. A method of treating a subject in need thereof, comprising administering the pharmaceutical composition of embodiment A78 to the subject, wherein the administering is by intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[0297] Embodiment A80. The method of any of embodiments A51 to A79, wherein the method further comprises administering a second therapeutic agent to the subject.

[0298] Embodiment A81. The method of embodiment A80, wherein the second therapeutic agent is a small molecule.

[0299] Embodiment A82. The method of embodiment A80, wherein the second therapeutic agent is an ASO.

[0300] Embodiment A83. The method of any one of embodiments A80 to A82, wherein the second therapeutic agent corrects intron retention.

[0301] Embodiment A84. A pharmaceutical composition comprising: an antisense oligomer that hybridizes to a target sequence of an *OPAI* mRNA transcript, wherein the *OPAI* mRNA transcript comprises a non-sense mediated RNA decay-inducing exon, wherein the antisense oligomer induces exclusion of the non-sense mediated RNA decay-inducing exon from the *OPAI* mRNA transcript; and a pharmaceutical acceptable excipient.

[0302] Embodiment A85. The pharmaceutical composition of embodiment A84, wherein the *OPAI* mRNA transcript is an *OPAI* NMD exon mRNA transcript.

[0303] Embodiment A86. The pharmaceutical composition of embodiment A84 or A85, wherein the targeted portion of the NMD exon mRNA is within the non-sense mediated RNA decay-inducing exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[0304] Embodiment A87. The pharmaceutical composition of embodiment A84 or A85, wherein the targeted portion of the NMD exon mRNA is upstream or downstream of the non-sense mediated RNA decay-inducing exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[0305] Embodiment A88. The pharmaceutical composition of embodiment A84 or A85, wherein the targeted portion of the NMD exon mRNA comprises an exon-intron junction exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[0306] Embodiment A89. The pharmaceutical composition of any one of embodiments A84 to A88, wherein the *OPAI* NMD exon mRNA transcript is encoded by a genetic sequence with

at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 1.

[0307] Embodiment A90. The pharmaceutical composition of embodiment A84 or A88, wherein the *OPAI* NMD exon mRNA transcript comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 2 or 3.

[0308] Embodiment A91. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[0309] Embodiment A92. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer is an antisense oligonucleotide.

[0310] Embodiment A93. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[0311] Embodiment A94. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises at least one modified sugar moiety.

[0312] Embodiment A95. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[0313] Embodiment A96. The pharmaceutical composition of embodiment A84 or A85, wherein the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or is 100% complementary to a targeted portion of the *OPAI* NMD exon mRNA transcript.

[0314] Embodiment A97. The pharmaceutical composition of embodiment A84 or A85, wherein the targeted portion of the *OPAI* NMD exon mRNA transcript is within SEQ ID NO: 2 or 3.

[0315] Embodiment A98. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises a nucleotide sequence that is at least about 80%, 85%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[0316] Embodiment A99. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises a nucleotide sequence that is identical a region comprising at least 8 contiguous nucleic acids SEQ ID NO: 2 or 3.

[0317] Embodiment A100. The pharmaceutical composition of any one of the embodiments A84 to A99, wherein the pharmaceutical composition is formulated for intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[0318] Embodiment A101. The method of any of embodiments A84 to A100, wherein the method further comprises administering a second therapeutic agent to the subject.

[0319] Embodiment A102. The method of embodiment A101, wherein the second therapeutic agent is a small molecule.

[0320] Embodiment A103. The method of embodiment A101, wherein the second therapeutic agent is an ASO.

[0321] Embodiment A104. The method of any one of embodiments A101 to A103, wherein the second therapeutic agent corrects intron retention.

[0322] Embodiment A105. A method of inducing processing of a deficient *OPAI* mRNA transcript to facilitate removal of a non-sense mediated RNA decay-inducing exon to produce a fully processed *OPAI* mRNA transcript that encodes a functional form of an OPA1 protein, the method comprising:

- (a) contacting an antisense oligomer to a target cell of a subject;
- (b) hybridizing the antisense oligomer to the deficient *OPAI* mRNA transcript, wherein the deficient *OPAI* mRNA transcript is capable of encoding the functional form of an OPA1 protein and comprises at least one non-sense mediated RNA decay-inducing exon;
- (c) removing the at least one non-sense mediated RNA decay-inducing exon from the deficient *OPAI* mRNA transcript to produce the fully processed *OPAI* mRNA transcript that encodes the functional form of OPA1 protein; and
- (d) translating the functional form of OPA1 protein from the fully processed *OPAI* mRNA transcript.

[0323] Embodiment A106. A method of treating a subject having a condition caused by a deficient amount or activity of OPA1 protein comprising administering to the subject an antisense oligomer comprising a nucleotide sequence with at least about 80%, 85%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[0324] Embodiment A107. A method of treating Optic atrophy type 1 in a subject in need thereof, by increasing the expression of a target protein or functional RNA by a cell of the subject, wherein the cell has an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA), and wherein the NMD exon mRNA encodes the target protein or functional RNA, the method comprising contacting the cell of the subject with a therapeutic agent that modulates splicing of the NMD exon mRNA encoding the target protein or functional RNA, whereby the non-sense mediated RNA decay-inducing exon is excluded from the NMD exon mRNA encoding the target protein or functional RNA, thereby increasing the level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cell of the subject.

[0325] Embodiment A108. A method of increasing expression of OPA1 protein by a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes OPA1 protein, the method comprising contacting the cell with an agent that modulates splicing of the NMD exon mRNA encoding OPA1 protein, whereby the non-sense mediated RNA decay-inducing exon is excluded from the NMD exon mRNA encoding OPA1 protein, thereby increasing the level of mRNA encoding OPA1 protein, and increasing the expression of OPA1 protein in the cell.

[0326] Embodiment A109. The method of embodiment A107 or A108, wherein the agent

- (a) binds to a targeted portion of the NMD exon mRNA encoding the target protein or functional RNA;
- (b) binds to one or more components of a spliceosome; or
- (c) a combination of (a) and (b).

[0327] Embodiment B1. A method of modulating expression of a target protein, by a cell having an mRNA that comprises a non-sense mediated RNA decay-inducing exon (NMD exon) and encodes the target protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the mRNA, thereby modulating level of processed mRNA encoding the target protein, and modulating the expression of the target protein in the cell, wherein the target protein is selected from the group consisting of: OPA1 proteins.

[0328] Embodiment B2. A method of treating a disease or condition in a subject in need thereof by modulating expression of a target protein in a cell of the subject, comprising: contacting the cell of the subject with a therapeutic agent that modulates splicing of a non-sense mediated mRNA decay-inducing exon (NMD exon) from an mRNA in the cell, wherein the

mRNA comprises the NMD exon and encodes the target protein, thereby modulating level of processed mRNA encoding the target protein, and modulating expression of the target protein in the cell of the subject, wherein the target protein is selected from the group consisting of: OPA1 proteins.

[0329] Embodiment B3. The method of embodiment B1 or B2, wherein the therapeutic agent

- (a) binds to a targeted portion of the mRNA encoding the target protein;
- (b) modulates binding of a factor involved in splicing of the NMD exon; or
- (c) a combination of (a) and (b).

[0330] Embodiment B4. The method of embodiment B3, wherein the therapeutic agent interferes with binding of the factor involved in splicing of the NMD exon to a region of the targeted portion.

[0331] Embodiment B5. The method of embodiment B3 or B4, wherein the targeted portion is proximal to the NMD exon.

[0332] Embodiment B6. The method of any one of embodiments B3 to B5, wherein the targeted portion is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NMD exon.

[0333] Embodiment B7. The method of any one of embodiments B3 to B6, wherein the targeted portion is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NMD exon.

[0334] Embodiment B8. The method of any one of embodiments B3 to B5, wherein the targeted portion is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NMD exon.

[0335] Embodiment B9. The method of any one of embodiments B3 to B5 or B8, wherein the targeted portion is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NMD exon.

[0336] Embodiment B10. The method of any one of embodiments B3 to B5, wherein the targeted portion is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site selected from the group consisting of: GRCh38/ hg38: chr3 193628509; and GRCh38/ hg38: chr3 193603500.

[0337] Embodiment B11. The method of any one of embodiments B3 to B5 or B10, wherein the targeted portion is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site selected from the group consisting of: GRCh38/ hg38: chr3 193628509; and GRCh38/ hg38: chr3 193603500.

[0338] Embodiment B12. The method of any one of embodiments B3 to B5, wherein the targeted portion is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site selected from the group consisting of: GRCh38/ hg38: chr3 193628616; and GRCh38/ hg38: chr3 193603557.

[0339] Embodiment B13. The method of any one of embodiments B3 to B5 or B12, wherein the targeted portion is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site selected

from the group consisting of: GRCh38/ hg38: chr3 193628616; and GRCh38/ hg38: chr3 193603557.

[0340] Embodiment B14. The method of any one of embodiments B3 to B13, wherein the targeted portion is located in an intronic region between two canonical exonic regions of the mRNA encoding the target protein, and wherein the intronic region contains the NMD exon.

[0341] Embodiment B15. The method of any one of embodiments B3 to B14, wherein the targeted portion at least partially overlaps with the NMD exon.

[0342] Embodiment B16. The method of any one of embodiments B3 to B15, wherein the targeted portion at least partially overlaps with an intron upstream or downstream of the NMD exon.

[0343] Embodiment B17. The method of any one of embodiments B3 to B16, wherein the targeted portion comprises 5' NMD exon–intron junction or 3' NMD exon–intron junction.

[0344] Embodiment B18. The method of any one of embodiments B3 to B16, wherein the targeted portion is within the NMD exon.

[0345] Embodiment B19. The method of any one of embodiments B1 to B18, wherein the targeted portion comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[0346] Embodiment B20. The method of any one of embodiments B1 to B19, wherein the mRNA encoding the target protein comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 4 or 5.

[0347] Embodiment B21. The method of any one of embodiments B1 to B20, wherein the mRNA encoding the target protein is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 1.

[0348] Embodiment B22. The method of any one of embodiments B3 to B21, wherein the targeted portion of the mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 4 or 5.

[0349] Embodiment B23. The method of any one of embodiments B1 to B22, wherein the agent is an antisense oligomer (ASO) and wherein the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to at least 8 contiguous nucleic acids of SEQ ID Ns: 4 or 5.

[0350] Embodiment B24. The method of any one of embodiments B3 to B23, wherein the targeted portion of the mRNA is within the non-sense mediated RNA decay-inducing exon selected from the group consisting of: GRCh38/ hg38: chr3 193628509 193628616; and GRCh38/ hg38: chr3 193603500 193603557;.

[0351] Embodiment B25. The method of any one of embodiments B3 to B23, wherein the targeted portion of the mRNA is upstream or downstream of the non-sense mediated RNA decay-inducing exon selected from the group consisting of: GRCh38/ hg38: chr3 193628509 193628616; and GRCh38/ hg38: chr3 193603500 193603557.

[0352] Embodiment B26. The method of any one of embodiments B3 to B23, wherein the targeted portion of the mRNA comprises an exon-intron junction of exon selected from the group consisting of: GRCh38/ hg38: chr3 193628509 193628616; and GRCh38/ hg38: chr3 193603500 193603557.

[0353] Embodiment B27. The method of any one of embodiments B1 to B26, wherein the target protein produced is a full-length protein or a wild-type protein.

[0354] Embodiment B28. The method of any one of embodiments B1 to B27, wherein the therapeutic agent promotes exclusion of the NMD exon from the pre-mRNA encoding the target protein.

[0355] Embodiment B29. The method of embodiment B28, wherein exclusion of the NMD exon from the pre-mRNA encoding the target protein in the cell contacted with the therapeutic agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to exclusion of the NMD exon from the pre-mRNA encoding the target protein in a control cell.

[0356] Embodiment B30. The method of embodiment B28 or B29, wherein the therapeutic agent increases the level of the processed mRNA encoding the target protein in the cell.

[0357] Embodiment B31. The method of any one of embodiments B28 to B30, wherein the level of the processed mRNA encoding the target protein produced in the cell contacted with the therapeutic agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about

9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to a level of the processed mRNA encoding the target protein in a control cell.

[0358] Embodiment B32. The method of any one of embodiments B28 to B31, wherein the therapeutic agent increases the expression of the target protein in the cell.

[0359] Embodiment B33. The method of any one of embodiments B28 to B32, wherein a level of the target protein produced in the cell contacted with the therapeutic agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to a level of the target protein produced in a control cell.

[0360] Embodiment B34. The method of any one of embodiments B2 to B33, wherein the disease or condition is induced by a loss-of-function mutation in the target protein.

[0361] Embodiment B35. The method of embodiment B34, wherein the disease or condition is associated with haploinsufficiency of a gene encoding the target protein, and wherein the subject has a first allele encoding a functional target protein, and a second allele from which the target protein is not produced or produced at a reduced level, or a second allele encoding a nonfunctional target protein or a partially functional target protein.

[0362] Embodiment B36. The method of any one of embodiments B2 to B35, wherein the disease or condition is selected from the group consisting of: Optic atrophy type 1.

[0363] Embodiment B37. The method of any one of embodiments B34 to B36, wherein the therapeutic agent promotes exclusion of the NMD exon from the pre-mRNA encoding the target protein and increases the expression of the target protein in the cell.

[0364] Embodiment B38. The method of any one of embodiments B1 to B27, wherein the therapeutic agent inhibits exclusion of the NMD exon from the pre-mRNA encoding the target protein.

[0365] Embodiment B39. The method of embodiment B38, wherein exclusion of the NMD exon from the pre-mRNA encoding the target protein in the cell contacted with the therapeutic agent is decreased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-

fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to exclusion of the NMD exon from the pre-mRNA encoding the target protein in a control cell.

[0366] Embodiment B40. The method of embodiment B38 or B39, wherein the therapeutic agent decreases the level of the processed mRNA encoding the target protein in the cell.

[0367] Embodiment B41. The method of any one of embodiments B38 to B40, wherein the level of the processed mRNA encoding the target protein in the cell contacted with the therapeutic agent is decreased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to a level of the processed mRNA encoding the target protein in a control cell.

[0368] Embodiment B42. The method of any one of embodiments B38 to B41, wherein the therapeutic agent decreases the expression of the target protein in the cell.

[0369] Embodiment B43. The method of any one of embodiments B38 to B42, wherein a level of the target protein produced in the cell contacted with the therapeutic agent is decreased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at

least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to a level of the target protein produced in a control cell.

[0370] Embodiment B44. The method of any one of embodiments B2 to B27 or B38 to B43, wherein the disease or condition is induced by a gain-of-function mutation in the target protein

[0371] Embodiment B45. The method of embodiment B44, wherein the subject has an allele from which the target protein is produced at an increased level, or an allele encoding a mutant target protein that exhibits increased activity in the cell.

[0372] Embodiment B46. The method of embodiment B44 or B45, wherein the therapeutic agent inhibits exclusion of the NMD exon from the pre-mRNA encoding the target protein and decreases the expression of the target protein in the cell.

[0373] Embodiment B47. The method of any one of embodiments B1 to B46, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[0374] Embodiment B48. The method of any one of embodiments B1 to B47, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[0375] Embodiment B49. The method of any one of embodiments B1 to B48, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises at least one modified sugar moiety.

[0376] Embodiment B50. The method of embodiment B49, wherein each sugar moiety is a modified sugar moiety.

[0377] Embodiment B51. The method of any one of embodiments B1 to B50, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[0378] Embodiment B52. The method of any one of embodiments B3 to B51, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, complementary to the targeted portion of the mRNA.

[0379] Embodiment B53. The method of any one of embodiments B1 to B52, wherein the method further comprises assessing mRNA level or expression level of the target protein.

[0380] Embodiment B54. The method of any one of embodiments B1 to B53, wherein the subject is a human.

[0381] Embodiment B55. The method of any one of embodiments B1 to B53, wherein the subject is a non-human animal.

[0382] Embodiment B56. The method of any one of embodiments B2 to B54, wherein the subject is a fetus, an embryo, or a child.

[0383] Embodiment B57. The method of any one of embodiments B1 to B56, wherein the cells are ex vivo.

[0384] Embodiment B58. The method of any one of embodiments B2 to B56, wherein the therapeutic agent is administered by intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, intravitreal, or intravenous injection of the subject.

[0385] Embodiment B59. The method of any one of embodiments B2 to B56 or B58, wherein the method further comprises administering a second therapeutic agent to the subject.

[0386] Embodiment B60. The method of any one of embodiments B1 to B59, wherein the second therapeutic agent is a small molecule.

[0387] Embodiment B61. The method of any one of embodiments B1 to B59, wherein the second therapeutic agent is an antisense oligomer.

[0388] Embodiment B62. The method of any one of embodiments B1 to B61, wherein the second therapeutic agent corrects intron retention.

[0389] Embodiment B63. The method of any one of embodiments B2 to B62, wherein the disease or condition is Optic atrophy type 1.

FURTHER SPECIFIC EMBODIMENTS

[0390] Embodiment 1. A method of modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an OPA1 gene and that comprises a non-sense mediated RNA decay-inducing exon (NMD exon), the method comprising contacting an agent or a vector encoding the agent to the cell, whereby the agent modulates splicing of the NMD exon from the pre-mRNA, thereby modulating a level of processed mRNA that is processed from the pre-mRNA, and modulating the expression of the OPA1 protein in the cell, wherein the agent

comprises an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299.

[0391] Embodiment 2. The method of embodiment 1, wherein the agent:

- (a) binds to a targeted portion of the pre-mRNA;
- (b) modulates binding of a factor involved in splicing of the NMD exon; or
- (c) a combination of (a) and (b).

[0392] Embodiment 3. The method of embodiment 2, wherein the agent interferes with binding of the factor involved in splicing of the NMD exon to a region of the targeted portion

[0393] Embodiment 4. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is proximal to the NMD exon.

[0394] Embodiment 5. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NMD exon.

[0395] Embodiment 6. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NMD exon.

[0396] Embodiment 7. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NMD exon.

[0397] Embodiment 8. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NMD exon.

[0398] Embodiment 9. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/hg38: chr3 193628509.

[0399] Embodiment 10. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/hg38: chr3 193628509.

[0400] Embodiment 11. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/hg38: chr3 193628616.

[0401] Embodiment 12. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/hg38: chr3 193628616.

[0402] Embodiment 13. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is located in an intronic region between two canonical exonic regions of the pre-mRNA, and wherein the intronic region contains the NMD exon.

[0403] Embodiment 14. The method of embodiment 2, wherein the targeted portion of the pre-mRNA at least partially overlaps with the NMD exon.

[0404] Embodiment 15. The method of embodiment 2, wherein the targeted portion of the pre-mRNA at least partially overlaps with an intron upstream or downstream of the NMD exon.

[0405] Embodiment 16. The method of embodiment 2, wherein the targeted portion of the pre-mRNA comprises 5' NMD exon-intron junction or 3' NMD exon-intron junction.

[0406] Embodiment 17. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is within the NMD exon.

- [0407]** Embodiment 18. The method of embodiment 2, wherein the targeted portion of the pre-mRNA comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.
- [0408]** Embodiment 19. The method of any one of embodiments 1 to 18, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.
- [0409]** Embodiment 20. The method of any one of embodiments 1 to 18, wherein the NMD exon comprises a sequence of SEQ ID NO: 279.
- [0410]** Embodiment 21. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is within the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.
- [0411]** Embodiment 22. The method of embodiment 2, wherein the targeted portion of the pre-mRNA is upstream or downstream of the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.
- [0412]** Embodiment 23. The method of embodiment 2, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193628509 to 193628616.
- [0413]** Embodiment 24. The method of any one of embodiments 1 to 23, wherein the OPA1 protein expressed from the processed mRNA is a full-length OPA1 protein or a wild-type OPA1 protein.
- [0414]** Embodiment 25. The method of any one of embodiments 1 to 23, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.
- [0415]** Embodiment 26. The method of any one of embodiments 1 to 23, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.
- [0416]** Embodiment 27. The method of any one of embodiments 1 to 23, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.
- [0417]** Embodiment 28. The method of any one of embodiments 1 to 23, or 25 to 27, wherein the OPA1 protein expressed from the processed mRNA is an OPA1 protein that lacks an amino acid sequence encoded by a nucleic acid sequence with at least 80% sequence identity to SEQ ID NO: 277.
- [0418]** Embodiment 29. The method of any one of embodiments 1 to 28, wherein the method promotes exclusion of the NMD exon from the pre-mRNA.

[0419] Embodiment 30. The method of embodiment 29, wherein the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[0420] Embodiment 31. The method of any one of embodiments 1 to 30, wherein the method results in an increase in the level of the processed mRNA in the cell.

[0421] Embodiment 32. The method of embodiment 31, wherein the level of the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[0422] Embodiment 33. The method of any one of embodiments 1 to 32, wherein the method results in an increase in the expression of the OPA1 protein in the cell.

[0423] Embodiment 34. The method of embodiment 33, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least

about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[0424] Embodiment 35. The method of any one of embodiments 1 to 34, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292.

[0425] Embodiment 36. The method of any one of embodiments 1 to 34, wherein the agent further comprises a gene editing molecule.

[0426] Embodiment 37. The method of embodiment 36, wherein the gene editing molecule comprises CRISPR-Cas9.

[0427] Embodiment 38. A method of modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an OPA1 gene, wherein the pre-mRNA comprises a coding exon, the method comprising contacting an agent or a vector encoding the agent to the cell, whereby the agent promotes exclusion of the coding exon from the pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon in the cell.

[0428] Embodiment 39. The method of embodiment 38, wherein the agent:

- (a) binds to a targeted portion of the pre-mRNA;
- (b) modulates binding of a factor involved in splicing of the coding exon; or
- (c) a combination of (a) and (b).

[0429] Embodiment 40. The method of embodiment 39, wherein the agent interferes with binding of the factor involved in splicing of the coding exon to a region of the targeted portion.

[0430] Embodiment 41. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is proximal to the coding exon.

[0431] Embodiment 42. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the coding exon.

[0432] Embodiment 43. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 to 50, from 90 to 50, from 80 to 50, from 70 to 50, from 60 to 50, from 60 to 40, from 60 to 30, from 60 to 20, from 60 to 10, from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon.

[0433] Embodiment 44. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon.

[0434] Embodiment 45. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the coding exon.

[0435] Embodiment 46. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, from 1 to 19, from 10 to 60, from 20 to 60, from 30 to 60, from 40 to 60, from 50 to 60, from 50 to 70, from 50 to 80, from 50 to 90, or from 50 to 100 nucleotides downstream of 3' end of the coding exon.

[0436] Embodiment 47. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, or from 1 to 19 nucleotides downstream of 3' end of the coding exon.

[0437] Embodiment 48. The method of embodiment 39, wherein the targeted portion of the pre-mRNA at least partially overlaps with the coding exon.

[0438] Embodiment 49. The method of embodiment 39, wherein the targeted portion of the pre-mRNA at least partially overlaps with an intron immediately upstream or immediately downstream of the coding exon.

[0439] Embodiment 50. The method of embodiment 39, wherein the targeted portion of the pre-mRNA comprises 5' coding exon-intron junction or 3' coding exon-intron junction.

[0440] Embodiment 51. The method of embodiment 39, wherein the targeted portion is within the coding exon of the pre-mRNA.

[0441] Embodiment 52. The method of any one of embodiments 39 to 51, wherein the coding exon is an alternatively spliced exon.

[0442] Embodiment 53. The method of any one of embodiments 39 to 52, wherein the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277.

[0443] Embodiment 54. The method of any one of embodiments 39 to 52, wherein the coding exon comprises SEQ ID NO: 277.

[0444] Embodiment 55. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is immediately upstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[0445] Embodiment 56. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092.

[0446] Embodiment 57. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is immediately downstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[0447] Embodiment 58. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, or from 1 to 19 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202.

[0448] Embodiment 59. The method of embodiment 39, wherein the targeted portion of the pre-mRNA is within the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[0449] Embodiment 60. The method of embodiment 39, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193626092 to 193626202.

[0450] Embodiment 61. The method of embodiment 39, wherein the targeted portion comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the coding exon.

[0451] Embodiment 62. The method of embodiment 39, wherein the targeted portion of the pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 277.

[0452] Embodiment 63. The method of any one of embodiments 38 to 62, wherein the exclusion of the coding exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[0453] Embodiment 64. The method of any one of embodiments 38 to 63, wherein the method results in an increase in expression of the OPA1 protein in the cell.

[0454] Embodiment 65. The method of embodiment 64, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

- [0455]** Embodiment 66. The method of embodiment 64, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by at least about 1.5-fold compared to in the absence of the agent.
- [0456]** Embodiment 67. The method of any one of embodiments 64 to 66, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.
- [0457]** Embodiment 68. The method of any one of embodiments 64 to 66, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.
- [0458]** Embodiment 69. The method of any one of embodiments 64 to 66, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.
- [0459]** Embodiment 70. The method of any one of embodiments 64 to 69, wherein the OPA1 protein expressed from the processed mRNA comprises fewer proteolytic cleavage sites than an OPA1 protein encoded by a corresponding mRNA containing the coding exon.
- [0460]** Embodiment 71. The method of any one of embodiments 38 to 70, wherein the agent promotes exclusion of a non-sense mediated RNA decay-inducing exon (NMD exon) from the pre-mRNA.
- [0461]** Embodiment 72. The method of embodiment 71, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.
- [0462]** Embodiment 73. The method of embodiment 71, wherein the NMD exon comprises a sequence of SEQ ID NO: 279.
- [0463]** Embodiment 74. The method of any one of embodiments 64 to 73, wherein the OPA1 protein expressed from the processed mRNA comprises fewer proteolytic cleavage sites than an OPA1 protein encoded by a corresponding mRNA containing the coding exon.
- [0464]** Embodiment 75. The method of any one of embodiments 38 to 74, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242, 250, 280-283, 288, and 290-292.
- [0465]** Embodiment 76. The method of any one of embodiments 38 to 74, wherein the agent comprises a gene editing molecule.
- [0466]** Embodiment 77. The method of embodiment 76, wherein the gene editing molecule comprises CRISPR-Cas9.
- [0467]** Embodiment 78. A method of modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an OPA1 gene, wherein the pre-mRNA comprises a

coding exon, the method comprising contacting an agent or a vector encoding the agent to the cell,

wherein the agent comprises an antisense oligomer that binds to:

(a) a targeted portion of the pre-mRNA within an intronic region immediately upstream of a 5' end of the coding exon of the pre-mRNA; or

(b) a targeted portion of the pre-mRNA within an intronic region immediately downstream of a 3' end of the coding exon of the pre-mRNA;

[0468] whereby the agent increases a level of a processed mRNA that is processed from the pre-mRNA and that contains the coding exon in the cell.

[0469] Embodiment 79. The method of embodiments 78, wherein the coding exon is an alternatively spliced exon.

[0470] Embodiment 80. The method of embodiments 78 or 79, wherein the method promotes inclusion of the coding exon in the processed mRNA during splicing of the pre-mRNA in the cell.

[0471] Embodiment 81. The method of any one of embodiments 78 to 80, wherein the target portion of the pre-mRNA is within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of a 5' end of the coding exon.

[0472] Embodiment 82. The method of any one of embodiments 78 to 80, wherein the target portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of a 3' end of the coding exon.

[0473] Embodiment 83. The method of any one of embodiments 78 to 80, wherein the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277.

[0474] Embodiment 84. The method of any one of embodiments 78 to 80, wherein the coding exon comprises SEQ ID NO: 277.

[0475] Embodiment 85. The method of any one of embodiments 78 to 80, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092.

[0476] Embodiment 86. The method of any one of embodiments 78 to 80, wherein the targeted portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202.

[0477] Embodiment 87. The method of any one of embodiments 78 to 86, wherein the inclusion of the coding exon in the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[0478] Embodiment 88. The method of any one of embodiments 78 to 87, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267.

[0479] Embodiment 89. A method of modulating expression of a target protein in a cell having a pre-mRNA transcribed from a gene that encodes the target protein, wherein the pre-mRNA comprises a coding exon and a non-sense mediated RNA decay-inducing exon (NMD exon), the method comprising contacting an agent or a vector encoding the agent to the cell, **[0480]** wherein the agent promotes exclusion of both the coding exon and the NMD exon from the pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks both the NMD exon and the coding exon in the cell.

[0481] Embodiment 90. The method of embodiment 89, wherein the agent:

- (a) binds to a targeted portion of the pre-mRNA;
- (b) modulates binding of a factor involved in splicing of the coding exon, the NMD exon, or both; or
- (c) a combination of (a) and (b).

[0482] Embodiment 91. The method of embodiment 90, wherein the agent interferes with binding of the factor involved in splicing of the coding exon, the NMD exon, or both, to a region of the targeted portion.

[0483] Embodiment 92. The method of any one of embodiments 89 to 91, wherein the NMD exon is within an intronic region adjacent to the coding exon.

[0484] Embodiment 93. The method of embodiment 92, wherein the NMD exon is within an intronic region immediately upstream of the coding exon.

[0485] Embodiment 94. The method of embodiment 92, wherein the NMD exon is within an intronic region immediately downstream of the coding exon.

- [0486] Embodiment 95. The method of any one of embodiments 90 to 94, wherein the targeted portion of the pre-mRNA is proximal to the coding exon.
- [0487] Embodiment 96. The method of any one of embodiments 90 to 94, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the coding exon.
- [0488] Embodiment 97. The method of any one of embodiments 90 to 94, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the coding exon.
- [0489] Embodiment 98. The method of any one of embodiments 90 to 94, wherein the targeted portion of the pre-mRNA is located within the coding exon.
- [0490] Embodiment 99. The method of any one of embodiments 90 to 94, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon.
- [0491] Embodiment 100. The method of any one of embodiments 90 to 94, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the coding exon to 100 nucleotides downstream of the coding exon.
- [0492] Embodiment 101. The method of any one of embodiments 89 to 100, wherein the coding exon is an alternatively spliced exon.
- [0493] Embodiment 102. The method of any one of embodiments 89 to 101, wherein the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277.
- [0494] Embodiment 103. The method of any one of embodiments 89 to 101, wherein the coding exon comprises SEQ ID NO: 277.
- [0495] Embodiment 104. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is immediately upstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.
- [0496] Embodiment 105. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is immediately downstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.
- [0497] Embodiment 106. The method of any one of embodiments 90 to 94, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of GRCh38/ hg38: chr3 193626092.
- [0498] Embodiment 107. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/

hg38: chr3 193626092. to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202. .

[0499] Embodiment 108. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is within the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[0500] Embodiment 109. The method of embodiment 90, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[0501] Embodiment 110. The method of embodiment 90, wherein the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the coding exon.

[0502] Embodiment 111. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is proximal to the NMD exon.

[0503] Embodiment 112. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the NMD exon.

[0504] Embodiment 113. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the NMD exon.

[0505] Embodiment 114. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is located within the NMD exon.

[0506] Embodiment 115. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the NMD exon to 100 nucleotides downstream of the NMD exon.

[0507] Embodiment 116. The method of any one of embodiments 89 to 115, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.

[0508] Embodiment 117. The method of embodiment 89, wherein the NMD exon comprises SEQ ID NO: 279.

[0509] Embodiment 118. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is immediately upstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.

[0510] Embodiment 119. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is immediately downstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.

[0511] Embodiment 120. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/

hg38: chr3 193628509 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

[0512] Embodiment 121. The method of embodiment 90, wherein the targeted portion of the pre-mRNA is within the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.

[0513] Embodiment 122. The method of embodiment 90, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.

[0514] Embodiment 123. The method of embodiment 90, wherein the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[0515] Embodiment 124. The method of any one of embodiments 89 to 123, wherein the exclusion of the coding exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[0516] Embodiment 125. The method of any one of embodiments 89 to 124, wherein the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[0517] Embodiment 126. The method of any one of embodiments 89 to 125, wherein the agent results in an increase in the level of the processed mRNA in the cell.

[0518] Embodiment 127. The method of embodiment 126, wherein the level of the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[0519] Embodiment 128. The method of any one of embodiments 89 to 127, wherein the method results in an increase in expression of the target protein in the cell.

[0520] Embodiment 129. The method of embodiment 128, wherein a level of the target protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[0521] Embodiment 130. The method of any one of embodiments 89 to 128, wherein the target protein is an OPA1 protein.

[0522] Embodiment 131. The method of embodiment 130, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by at least about 1.5-fold compared to in the absence of the agent.

[0523] Embodiment 132. The method of embodiment 130, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.

[0524] Embodiment 133. The method of embodiment 130, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.

[0525] Embodiment 134. The method of embodiment 130, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.

[0526] Embodiment 135. The method of any one of embodiments 89 to 127, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 236, 242, 250, 280-283, 288, and 290-292.

[0527] Embodiment 136. The method of any one of embodiments 78 to 135, wherein the agent comprises a gene editing molecule.

[0528] Embodiment 137. The method of embodiment 136, wherein the gene editing molecule comprises CRISPR-Cas9.

[0529] Embodiment 138. The method of any one of embodiments 1 to 75 or 78 to 135, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[0530] Embodiment 139. The method of any one of embodiments 1 to 75 or 78 to 138, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, or a 2'-O-methoxyethyl moiety.

[0531] Embodiment 140. The method of any one of embodiments 1 to 75 or 78 to 139, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises at least one modified sugar moiety.

[0532] Embodiment 141. The method of embodiment 140, wherein each sugar moiety is a modified sugar moiety.

[0533] Embodiment 142. The method of any one of embodiments 1 to 75 or 78 to 141, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[0534] Embodiment 143. The method of any one of embodiments 1 to 142, wherein the vector comprises a viral vector encoding the agent.

[0535] Embodiment 144. The method of embodiment 143, wherein the viral vector comprises an adenoviral vector, adeno-associated viral (AAV) vector, lentiviral vector, Herpes Simplex Virus (HSV) viral vector, or retroviral vector.

[0536] Embodiment 145. The method of any one of embodiments 1 to 144, wherein the method further comprises assessing mRNA level or expression level of the OPA1 protein.

[0537] Embodiment 146. The method of any one of embodiments 1 to 145, wherein the agent is a therapeutic agent.

[0538] Embodiment 147. A pharmaceutical composition comprising the therapeutic agent of embodiment 146 or a vector encoding the therapeutic agent of embodiment 146, and a pharmaceutically acceptable excipient.

[0539] Embodiment 148. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding a therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent comprises an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299.

[0540] Embodiment 149. The pharmaceutical composition of embodiment 148, wherein the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242 and 250.

[0541] Embodiment 150. The pharmaceutical composition of embodiment 148, wherein the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267.

[0542] Embodiment 151. The pharmaceutical composition of embodiment 148, wherein the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, and 280-299.

[0543] Embodiment 152. A composition, comprising an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299, wherein the antisense oligomer comprises a backbone modification, a sugar moiety modification, or a combination thereof.

[0544] Embodiment 153. The composition of embodiment 152, wherein the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242 and 250.

[0545] Embodiment 154. The composition of embodiment 152, wherein the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267.

[0546] Embodiment 155. The composition of embodiment 152, wherein the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, and 280-299.

[0547] Embodiment 156. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent promotes exclusion of a coding exon from a pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon in a cell, wherein the pre-mRNA is transcribed from an OPA1 gene and that comprises the coding exon.

[0548] Embodiment 157. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent comprises an antisense oligomer that binds to a pre-mRNA that is transcribed from an OPA1 gene in a cell, wherein the antisense oligomer binds to:

(a) a targeted portion of the pre-mRNA within an intronic region immediately upstream of a 5' end of the coding exon of the pre-mRNA; or

(b) a targeted portion of the pre-mRNA within an intronic region immediately downstream of a 3' end of the coding exon of the pre-mRNA;

whereby the therapeutic agent increases a level of a processed mRNA that is processed from the pre-mRNA and that contains the coding exon in the cell.

[0549] Embodiment 158. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent promotes exclusion of both a coding exon and a non-sense mediated RNA decay-inducing exon (NMD exon) from a pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon and the NMD exon in a cell, wherein the pre-mRNA is transcribed from an OPA1 gene in the cell and comprises the coding exon and the NMD exon.

[0550] Embodiment 159. The pharmaceutical composition of any one of embodiments 147 to 158, wherein the pharmaceutical composition is formulated for intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.

[0551] Embodiment 160. The pharmaceutical composition of any one of embodiments 147 to 158, wherein the pharmaceutical composition is formulated for intravitreal injection.

[0552] Embodiment 161. The pharmaceutical composition of any one of embodiments 147 to 160, wherein the pharmaceutical composition further comprises a second therapeutic agent.

[0553] Embodiment 162. The pharmaceutical composition of embodiment 161, wherein the second therapeutic agent comprises a small molecule.

[0554] Embodiment 163. The pharmaceutical composition of embodiment 161, wherein the second therapeutic agent comprises an antisense oligomer.

[0555] Embodiment 164. The pharmaceutical composition of embodiment 161, wherein the second therapeutic agent corrects intron retention.

[0556] Embodiment 165. The pharmaceutical composition or composition of any one of embodiments 147 to 160, wherein the antisense oligomer is selected from the group consisting of Compound ID NOs: 1-303.

[0557] Embodiment 166. A method of treating or reducing the likelihood of developing a disease or condition in a subject in need thereof by modulating expression of an OPA1 protein in a cell of the subject, comprising contacting to cells of the subject the therapeutic agent of any one of embodiments 147 to 165.

[0558] Embodiment 167. The method of embodiment 166, wherein the disease or condition is associated with a loss-of-function mutation in an OPA1 gene.

[0559] Embodiment 168. The method of embodiment 166 or 167, wherein the disease or condition is associated with haploinsufficiency of the OPA1 gene, and wherein the subject has a first allele encoding a functional OPA1 protein, and a second allele from which the OPA1 protein is not produced or produced at a reduced level, or a second allele encoding a nonfunctional OPA1 protein or a partially functional OPA1 protein.

[0560] Embodiment 169. The method of any one of embodiments 166 to 168, wherein the disease or condition comprises an eye disease or condition.

[0561] Embodiment 170. The method of any one of embodiments 166 to 168, wherein the disease or condition comprises ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease;

Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

[0562] Embodiment 171. The method of any one of embodiments 166 to 168, wherein the disease or condition comprises Optic atrophy type 1.

[0563] Embodiment 172. The method of any one of embodiments 166 to 168, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA).

[0564] Embodiment 173. The method of embodiment 166 or 167, wherein the disease or condition is associated with an autosomal recessive mutation of OPA1 gene, wherein the subject has a first allele encoding from which:

(i) OPA1 protein is not produced or produced at a reduced level compared to a wild-type allele; or

(ii) the OPA1 protein produced is nonfunctional or partially functional compared to a wild-type allele, and

a second allele from which:

(iii) the OPA1 protein is produced at a reduced level compared to a wild-type allele and the OPA1 protein produced is at least partially functional compared to a wild-type allele;

or

(iv) the OPA1 protein produced is partially functional compared to a wild-type allele.

[0565] Embodiment 174. The method of any one of embodiments 166 to 173, wherein the subject is a human.

[0566] Embodiment 175. The method of any one of embodiments 166 to 173, wherein the subject is a non-human animal.

[0567] Embodiment 176. The method of any one of embodiments 166 to 173, wherein the subject is a fetus, an embryo, or a child.

[0568] Embodiment 177. The method of any one of embodiments 166 to 173, wherein the cells are ex vivo.

[0569] Embodiment 178. The method of any one of embodiments 166 to 173, wherein the therapeutic agent is administered by intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.

[0570] Embodiment 179. The method of any one of embodiments 166 to 173, wherein the therapeutic agent is administered by intravitreal injection.

[0571] Embodiment 180. The method of any one of embodiments 166 to 179, wherein the method treats the disease or condition.

EXAMPLES

[0572] The present disclosure will be more specifically illustrated by the following Examples. However, it should be understood that the present disclosure is not limited by these examples in any manner.

Example 1: Identification of NMD-inducing Exon Inclusion Events in Transcripts by RNAseq using Next Generation Sequencing.

[0573] Whole transcriptome shotgun sequencing is carried out using next generation sequencing to reveal a snapshot of transcripts produced by the genes described herein to identify NMD exon inclusion events. For this purpose, polyA⁺ RNA from nuclear and cytoplasmic fractions of human cells is isolated and cDNA libraries are constructed using Illumina's TruSeq Stranded mRNA library Prep Kit. The libraries are pair-end sequenced resulting in 100-nucleotide reads that are mapped to the human genome (Feb. 2009, GRCh37/hg19 assembly). **FIGs. 2 and 3** depict identification of different exemplary nonsense-mediated mRNA decay (NMD)-inducing exons in various genes.

[0574] Exemplary genes and intron sequences are summarized in **Table 1** and **Table 2** (SEQ ID NOs indicate the corresponding nucleotide sequences represented by the Gene ID Nos). The sequence for each intron is summarized in **Table 3** and **Table 4**. **Table 5** lists sequences of OPA1 antisense oligomers of this disclosure.

Table 1. List of exemplary target gene sequences.

Gene Symbol	Gene ID No.	SEQ ID No.	Disease	OMIM	Genetics	Introns
<i>OPA1</i>	4976	1	Optic atrophy type 1; Autosomal dominant optic atrophy (ADOA)	165500	Haploinsufficient	ENST00000361908.7:6 ENST00000361908.7:28

Table 2. List of exemplary target gene sequences.

Gene Symbol	Gene ID No.	SEQ ID No.	Disease	OMIM	Genetics	Introns
<i>OPA1</i>	4976	1	Optic atrophy type 1; Autosomal dominant optic atrophy (ADOA)	165500	Haploinsufficient	GRCh38/ hg38: chr3 193626203 193631611 GRCh38/ hg38: chr3 193593374 193614710

Table 3. Sequences of exemplary target introns in pre-mRNA transcripts.

Gene	SEQ ID NO.	Intron
<i>OPA1</i>	2	<p><u>Intron 6:</u></p> <p>gtgatggatggttaagggggctaccgatacattcacactaatcagccattctgccaagatcatgtcacctcaatctgt tcatggactccaatacaagaataatttgacaaagtgaataataaaagatgcatcatataaatatgaactttctgg agtgggtagtataggtaagccaaagaacaattcaagcagaggaaatttggtttgaaaattaggtgtctgtag ggtcctgtatttatacttagaacaataataggaatttctgtttatgtgtccagtatttagtcaccctaagttgttaggca tcttacctactactgtctcccaagttttatttctaaaagaaaagcattgctgtatgaccagtttactactaagaata acatttatttattgttttagctaaagtatatggcaggggaacattcatattctgtagaagaaaatttttgacttttgggca aaagcatgtagtcttatacactttgacaaactcattgcgtacattttcacattaatcaaagtcagcacaaataaatttca ccttgaccacggagggttgaaactggaaattgatataattctggttgctaaagaacaagttctaataaaagcttaa gtgtataccaatatgtgctgttggtgcaatcagcaggtccgtaaaaatagattttatgttaggtaatccacaacg gagatcccaaagttcatgtttggaagagacttttgggtcaaagtgaatcagtgtaataatttaaaatactctgaga tcttgaatcagctaattatgttacatcttattagctcagaaaagtttgaagtatatacaatgctagtcaggaaaaag attcagtcagttaattctgtacattctactatttaaatcaaccaatattatagattatgatttagtcagtaattctgctggct aaccttatctcatttgggtggttagtactcagagtactaccatagtttcatttatgtttcagcatcacttctggttttc tcaattccatggctgtggaatcaattcatatgtatatttagcttcggtgagcaaaacatagctagaaaaagaaaagaa gtgagtttctactctggtaaaataaagtcgatgtgtaagccaaggaggacttctttgaatgtactttaacaacccctg ttctgtatactgtgaatatacatfttaaatagcctaataaattggatgcttaggctgagccacatactttagtttgtatg gaaagaaggagaggagcaagtatgttcttatgttacttagaataagaatgtagctgtagtacacattgttcttaa gttttttcgtaagacaactgaaatgagtcaccataggcctgctatttaacattctaagatatgacttaagggttaatgatga gcttttgaaatcgacaattcaagagatatccaatgaatactgattcattttctacattgctgaaagctaattgtcattttaa gcctactttagtagcctttatttgggcttagagatgtatttctcttctgatatttattgggttatctgttaaccctttatatct cccttcccgaattgtaaatagagactggcaagacttttaccctgagtagagcaccacaaataggtgttctgtccac</p>

Gene	SEQ ID NO.	Intron
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	3	<p><u>Intron 28:</u> gtgagtagttctactgcccctctaccttactacctttccaccttccatttccattgtttgtgatccatttaatctcaactt acagaaaagttacaaggaactgggctgagcacgggtggctcacgctgtaatccagcacttgggaggccaagatg gggtgaaaacaaggtcagaagatcaagaccatcctggctaacacagtgaaccccgctctactaaaaatacaaaa aacttagccagggtgtggtggtgggtgcctatagctccagctacttgggaggctgaggcaggagaatgggtgaacc cgggaggcgagcgtggtgagccaagatcctgcccactgcactccagcctgagcgacaggcgagattctgtct caaaaaaaaaaaaaaaaaaagttacaaggaatttttcttctgtaagtatttgagagtaagtgtgctgaccttaagtc ctatcacttccaagtaggttcattgtagttctagaacagattttctcatagcaaccgaacattgataaattacaatatct aattctcagaccccttcaagttcacccgtgtccagattatccctccatataacaagatgtccagggtcaatacctg accagcttctttttgaagaatgggtgttagaatggagacctagaaattatataatgctgttattggaatatcactgttc cctggtttctcagtgaaagagctaggaactaaggtgtgtgaatgtttgtgtgtgaggtgaatatacacacactgac atctgtattcctaatacatgtgtatatttatttataaaaactgtgagttgatgtgatacttccattttaatccagcattaca agggtttgtctagttctcccttcgataattgtcactgtcttctgatagaaaacgggcttctagatccttaatatatttc atattttggtagtctcctatcacgaaccaacttgaatgaagatatgtcctttccattgcagaatgttcttttcccgag ctcgactcaacactacacaccaggccaccacatggcgccgacccagcattgacacttctttacctgtctgggct ctgacatccgtgccagggtgtcttctgcatgagtgagcttcttactgagctctgctgacgcttgtgccagggtgctct ccatctcatcttcccaccgctagcctctgcccagcccagacagattccttctcactgaagccagaccatgctt tgtggagataccctcttaccctgctgtcttcgcccagcctgcaccaggccaccctcctgcacagatactctcctcag tactggaccaggctaccaacagccccatgtgaaccattgttaaccagggtcaggcattaacacctgcagtaggctac catggcttccccttcccaccccctagcttggccctactaataatcactttgtcactgtttgggtgataattgggtttct tgtagggtcctagctttaagataggattgcatactaaaatttacttagatctttgagaactcaaggaaatcagtgaacatt attgttataataaaaaataaacctgtagttggtacctctgtttgagcctgcttttacaagtttactgacttcagctt cgtgtaacaagtatcttttcttcaacgtgtacttaatttctgtcttattagttttctgatatctaaaaggaaaaaagc agatacgttaataaattagaagaaggttctgcaaatftaaaagtgcttctaagctgagttgtaggattacagtacaatc cataggggtatcctgaagaagccaggcagggtcttctgtgttacacctgtgctgctgcagcatgctcacccttgc catcagcgcttgcggccccattctctccctctagtaataatctaagttctgcattgttctcttcttcttcttcttctta aatattcttcttgcagacatatctcattttaaacttttattttctgtcacttttgggttttctatgcccacttggcaatgta gttaagttgtgctaacgtagaagatttagtgcataatctgaattgccattactactagctgtgtcatcttcggcaggga atctcccagagccttagcttcttatttgaataatgactattatagtggtatttctcaggattgttagaattactccgcaaa</p>

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Table 4. Exemplary target gene intron sequences

<p>SEQ ID NO: 4</p> <p>Gene: <i>OPA1</i></p> <p>Intron: GRCh38/ hg38:chr3 193626203 to 193631611</p> <p>Intron Sequence:</p> <p> gtgatggatggtttaaggggctaccgatacttcacactaatcagccatttctgccaagatcatgtcacctcaatctgttcatggactcca aatacaagaaattaattgacaaagtgaaaataaaaagatgcatcatataaataatgtaacttttctggagtgggtagtataggtaaagcca aaagaaacaaattcaagcagaggaattttggttctgaaaattaggtgtctgtagggtccctgatttatactagaacaaaattaggaattt ctgtttatgtgtccagttattgagtcaccctaagttttaggcattcttacctactgtctcccaagttttatttctaaaatgaaaagcat tgctgtagatgaccagtttactaaagaataacattattttttagctaaagtatatggacaggaacattcatattctgtagaaga aaattattttagcttttgggcaaaagcatgtagtcttatacactttgacaaactcattgcgtacattttcacattaatcaaagtcagcacaat aaatttcaccttgaccacggagggttgaacactggaatttgatataattctggttgctaaagaacaagtctaataaaaagcttaagtgt ataccaatatgtggctgttgggtcaatcagcaggtccgtaaaaatatgatttaattggttaggtaatcccacaacggagatccaaagttc atgtttgaagagacttttgggtcaaaagtgaatcagtgtaattgaaattatactctgagatctgaaatcagtaattatgttacatct tattagctcagaaaagtttgaagttatatacaaatgctagtcaggaaaaagattcagtcagtaattctgtacattctactatttaaatcaa ccaatattatagattatgatttagtcagtaattctgctggctaaccttatctcatttgggtggtggttagtacttcagagtactaccatagtctc </p>

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SEQ ID NO: 5

Gene: *OPAI*

Intron: GRCh38/ hg38:chr3 193593374 to 193614710

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SEQ ID NO: 276**Gene:** *OPA1*

Intron: GRCh38/ hg38:chr3 193618937 to 193626091

Intron Sequence:

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130

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SEQ ID NO: 277

Gene: *OPA1*

Exon: GRCh38/ hg38:chr3 193626092 to 193626202

Exon Sequence:

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SEQ ID NO: 278

Gene: *OPA1*

Intron: GRCh38/ hg38:chr3 193626203 to 193631611

Intron Sequence:

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SEQ ID NO: 279

Gene: *OPAI*

NMD Exon: GRCh38/ hg38:chr3 193628509 to 193628616

NMD Exon Sequence:

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CTTTAGCTTGTTTCTGCGGAGGATTCCGCTCTTTCTCCATCAGTTTCATAGCCCTG
GAATTGTAGAAAAGCTCTGGTTCAAGACCATTGATATCCATTCTGTCAGG
```

Example 2: Confirmation of NMD Exon via Cycloheximide Treatment.

[0575] RT-PCR analysis using cytoplasmic RNA from DMSO-treated or puromycin or cycloheximide-treated human cells and primers in exons was used to confirm the presence of a band corresponding to an NMD-inducing exon. The identity of the product was confirmed by sequencing. Densitometry analysis of the bands was performed to calculate percent NMD exon inclusion of total transcript. Treatment of cells with cycloheximide or puromycin to inhibit NMD can lead to an increase of the product corresponding to the NMD-inducing exon in the cytoplasmic fraction. **FIG. 4** depicts confirmation of exemplary NMD exons in *OPAI* gene transcripts using cycloheximide or puromycin treatment, respectively.

Example 3: NMD Exon Region ASO Walk.

[0576] An ASO walk was performed for NMD exon region targeting sequences immediately upstream of the 3' splice site, across the 3' splice site, the NMD exon, across the 5' splice site, and downstream of the 5' splice site using 2'-MOE ASOs, PS backbone. ASOs were designed to cover these regions by shifting 5 nucleotides at a time. **FIG. 5** depicts an ASO walk for an exemplary *OPAI* NMD exon region.

Example 4: NMD exon Region ASO Walk Evaluated by RT-PCR.

[0577] ASO walk sequences were evaluated by RT-PCR. HEK293 cells were transfected using Lipofectamine RNAiMax with control ASO treated (Ctrl), or with a 2'-MOE ASO targeting the *OPAI* NMD exon regions as described herein. Products corresponding to *OPAI* mRNA were quantified and normalized to RPL32 internal control, and fold-change relative to control was plotted. **FIG. 6** depicts evaluation via TaqMan qPCR of various exemplary ASO walk along

exemplary NMD exon regions. The measurement of the amount of *OPAI* mRNA was carried out with HEK293 cells 24 hours after treatment with 80nM of an exemplary ASO in the absence of cycloheximide, by Taqman qPCR using probes spanning exon 7 and exon 8.

Example 5: NMD exon Region ASO Microwalk Evaluated by RT-qPCR.

[0578] ASO microwalk sequences (across exon 7x) were evaluated by RT-PCR. HEK293 cells were transfected using Lipofectamine RNAiMax with control ASO treated (Ctrl), or with a 2'-MOE ASO targeting the *OPAI* NMD exon regions as described herein. Products corresponding to NMD exon inclusion and full-length were quantified and percent NMD exon inclusion was plotted. **FIG. 7** depicts evaluation of various exemplary ASO walk along exemplary NMD exon regions. The measurement of the amount of *OPAI* mRNA was carried out with HEK293 cells 24 hours after transfection with 80nM of an exemplary ASO in the absence of cycloheximide, by Taqman qPCR using probes spanning exon 7 and exon 8 (top panel of **FIG. 7**). qPCR amplification results were normalized to RPL32, and plotted as fold change relative to control. The measurement of exon 7x inclusion was carried out by quantifying exon 7x inclusion based on RT-PCR using probes spanning exon 7 and exon 8 (bottom panel of **FIG. 7**).

Example 6: Dose-dependent Effect of Selected ASO in CXH-treated Cells.

[0579] PAGE can be used to show SYBR-safe-stained RT-PCR products of mock-treated (Sham, RNAiMAX alone), or treated with 2'-MOE ASOs targeting NMD exons at 30 nM, 80 nM, and 200 nM concentrations in mouse or human cells by RNAiMAX transfection. Products corresponding to NMD exon inclusion and full-length are quantified and percent NMD exon inclusion can be plotted. The full-length products can also be normalized to HPRT internal control and fold-change relative to Sham can be plotted.

Example 7: Intravitreal (IVT) Injection of Selected ASOs.

[0580] PAGEs of SYBR-safe-stained RT-PCR products of mice from PBS-injected (1 μ L) (-) or ASOs or Cep290 (negative control ASO; Gerard et al, *Mol. Ther. Nuc. Ac.*, 2015) 2'-MOE ASO-injected (1 μ L) (+) at 10 mM concentration. Products corresponding to NMD exon inclusion and full-length (are quantified and percent NMD exon inclusion can be plotted Full-length products can be normalized to GAPDH internal control and fold-change of ASO-injected relative to PBS-injected can plotted.

Example 8: Intracerebroventricular (ICV) Injection of Selected ASOs.

[0581] PAGEs of SYBR-safe-stained RT-PCR products of mice from uninjected (-, no ASO control), or 300 μ g of Cep290 (negative control ASO; Gerard et al, *Mol. Ther. Nuc. Ac.*, 2015), 2'-MOE ASO-injected brains. Products corresponding to NMD exon inclusion and full-length can be quantified and percent NMD exon inclusion can be plotted. Taqman PCR can be performed using two different probes spanning NMD exon junctions and the products can be

normalized to GAPDH internal control and fold-change of ASO-injected relative to Cep290-injected brains can be plotted.

Example 9: *OPA1* Non-productive Splicing Event Identification and Validation.

[0582] A novel nonsense mediated decay (NMD) exon inclusion event (Exon X) was identified in the *OPA1* gene which leads to the introduction of a premature termination codon (PTC) resulting in a non-productive mRNA transcript degraded by NMD, as diagramed in **FIG. 1D**. As NMD is a translation-dependent process, the protein synthesis inhibitor cycloheximide (CHX) was used to evaluate the true abundance of the event. **FIG. 8** shows an increase in *OPA1* transcripts containing the NMD exon in HEK293 cells with increasing CHX dose. Other ocular cell lines also validated for the presence of the NMD exon (ARPE-19, Y79).

Example 10: *OPA1* NMD Event is Conserved in Primate Eyes.

[0583] **FIG. 9A** shows reverse transcription PCR data from the posterior segment of the eye of *Chlorocebus sabaeus* (green monkey) at postnatal data P93 (3 months) and postnatal day P942 (2.6 years) for the right eye (OD) and left eye (OS). **FIG. 9B** shows quantification of the NMD exon abundance at 3 months and 2.6 years of age (N=1/age). Data represents average of right eye and left eye values for each animal. The abundance of the event may be higher *in vivo*, given that NMD is presumed active in the tissue.

Example 11: *OPA1* Antisense Oligonucleotides Reduce Non-Productive Splicing and Increase Productive *OPA1* mRNA Levels In Vitro.

[0584] Exemplary antisense oligomers (ASOs) were transfected at 80 nM dose into HEK293 cells using Lipofectamine RNAiMax as a transfection agent. To assess the effect on the NMD exon, cells were treated with CHX (50 µg/ml, 3 hrs.) 21 hours after transfection. RNA was isolated for RT-PCR using probes spanning exon 7 and exon 8, as shown in **FIG. 10A**, and quantified in **FIG. 10B**. To assess levels of productive *OPA1* mRNA expression, non-cycloheximide treated cells were used for Taqman qPCR using probes spanning exon 23 and exon 24, and mRNA expression of *OPA1* was normalized to RPL32, as shown in **FIG. 11**. Arrows highlight ASOs that reduce non-productive splicing and increase *OPA1* mRNA expression by at least 20%. Among these, ASO-14 produces the most increase in *OPA1* mRNA (30%).

Example 12: ASO-14 Decreases Non-Productive *OPA1* mRNA and Increases *OPA1* Expression in a Dose-Dependent Manner In Vitro.

[0585] HEK293 cells were transfected with different doses of ASO-14 or non-targeting (NT) ASO. RNA was isolated 24 hours after transfection and analyzed for impact on non-productive *OPA1* mRNA (**FIG. 12A**) and *OPA1* mRNA expression (**FIG. 12B**) similarly to in Example 11. For protein analysis, cells were lysed with RIPA buffer 48 hours after transfection and western

blots were probed with antibodies targeting OPA1 and β -actin, as shown in **FIG. 12C**. Multiple bands correspond to different isoforms of OPA1. Data represent the average of three independent experiments (* $P < 0.05$ by one-way ANOVA compared to “NO ASO” group). The Non-targeting ASO targets an unrelated gene.

Example 13: ASO-14 Increases OPA1 Expression in an *OPA1* Haploinsufficient (*OPA1*^{+/-}) Cell Line.

[0586] *OPA1* haploinsufficient (*OPA1*^{+/-}) HEK293 cells were generated using CRISPR-Cas9 gene editing. Similar to ADOA patient cells, *OPA1*^{+/-} HEK293 cells show approximately 50% mRNA and protein levels of that observed in *OPA1*^{+/+} cells (**FIG. 13A**). The *OPA1*^{+/-} HEK293 cells were transfected with different doses of ASO-14 as indicated in **FIG. 13B**, and total protein was isolated 72 hours after transfection. Western blots were probed with antibodies targeting OPA1 and β -tubulin, a representative blot is shown in **FIG. 13B** and quantification of two independent experiments is shown in **FIG. 13C** (* $P < 0.05$ by one-way ANOVA compared to “No ASO” group). ASO-14 increases OPA1 protein levels in *OPA1*^{+/-} HEK293 cells by 50%, which translates to 75% of wild-type levels.

Example 14: Exemplary OPA1 ASOs Decrease Non-Productive Splicing and Increase OPA1 Expression in Wild-Type Rabbit Retinae Following Intravitreal Injection.

[0587] Female New Zealand White (NZW) adult rabbits were injected with either vehicle, non-targeting (NT), or test, antisense oligonucleotides. Animals were euthanized after 15 days to obtain retinal tissue. **FIG. 14A** outlines the study design, (*Final concentration in the vitreous calculated assuming vitreal volume in the rabbit as 1.5mL). **FIG. 14B** shows levels of productive and non-productive *OPA1* mRNA and protein, and **FIG. 14C** shows quantification of this data (* $P < 0.05$ by one-way ANOVA compared to Vehicle group). OD: oculus dextrus (right eye), OS: oculus sinister (left eye).

[0588] It was also found that the antisense oligonucleotides were well-tolerated in wild-type rabbit for up to 28 days after intravitreal injection.

Example 15: ASO-14 Modulates Inclusion of Both Exon 7 and Exon 7x in *OPA1* mRNA Transcript.

[0589] HEK293 cells were transfected with different doses of ASO-14 or no ASO, in the presence or absence of cycloheximide. RNA was isolated 24 hours after transfection and analyzed for impact on *OPA1* mRNA splicing and *OPA1* mRNA expression similarly to in Example 11. **FIG. 16A** shows gel image of PCR products from RT-PCR reaction using probes spanning exon 7 and 8. As shown in the figure, the dose of ASO-14 increased from 1 nM, 5 nM, to 20 nM, the amount of transcripts having exon 7x between exons 7 and 8 (“7+7x+8”) gradually decreased, as compared to relatively stable amount of transcripts lacking exon 7x between exons

7 and 8 (“7+8”). **FIG. 16B** shows plots summarizing the relative amount of various *OPA1* mRNA transcripts quantified by qPCR reactions using different pairs of probes: “Ex6-8,” probes spanning exons 6 and 8; “Ex7-8,” probes spanning exons 7 and 8; and “Ex23-24,” probes spanning exons 23 and 24. Results were normalized to RPL32 as an internal control. **FIG. 16C** shows a chart summarizing the quantification of various *OPA1* mRNA transcripts based on sequencing of the RNA extracts from the treated HEK293 cells in the absence of cycloheximide. As suggested by the figures, ASO-14 appeared to induce reduction in *OPA1* exon 7x inclusion, increase in *OPA1* Ex6-8 transcripts (transcripts having exon 6 and exon 8 in tandem, thus lacking exon 7 and exon 7x), modest decrease or no change in *OPA1* Ex7-8 transcripts (transcripts having exon 7 and exon 8 in tandem, thus lacking exon 7x).

Example 16: Exemplary OPA1 Antisense Oligomers Modulate Inclusion of Exon 7, Exon 7x, or Both in OPA1 mRNA Transcript.

[0590] HEK293 cells were transfected with different exemplary OPA1 modified 2'MOE-PS (2' methoxyethyl and phosphorothioate) ASOs. Each well of HEK 293 cells (about 100,000 cells/well) were treated with an exemplary ASO at 80 nM final concentration in the presence of 0.9 μ L of Lipofectamine® RNAiMax in the absence of cycloheximide. The cells were harvested 24 hours after transfection and RNA was isolated and analyzed for impact on *OPA1* mRNA splicing and *OPA1* mRNA expression similarly to in Example 11. **FIG. 17A** shows gel image of PCR products from RT-PCR reaction using probes spanning exon 6 and 8, and **FIG. 17B** is a plot summarizing the relative ratio of the amount of transcripts having exons 6, 7, and 8 in tandem (“6-7-8”) over the total amount of “6-7-8” transcripts and transcripts having exons 6 and 8 in tandem (“6-8”). As shown in the figures, certain ASOs, such as ASO-19, ASO-20, ASO-21, ASO-22, induced increase in the relative amount of “6-7-8” transcripts, suggesting an increase in the inclusion of exon 7 in mature *OPA1* mRNA transcripts. Some ASOs, such as ASO-23, ASO-24, ASO-25, ASO-26, ASO-28, ASO-29, ASO-30, ASO-31, ASO-32, ASO-33, ASO-34, ASO-35, ASO-36, ASO-37, and ASO-38, in contrast, induced reduction in the relative amount of “6-7-8” transcripts, suggesting a reduction in the inclusion of exon 7 in mature *OPA1* mRNA transcript. **FIGs. 17C** and **17D** show the Ct values for the qPCR reaction (upper plots) for, and quantification of the relative amount (bottom plots) of, *OPA1* transcripts having exons 6 and 8 (“Ex6-8”) and *OPA1* transcripts having exons 7 and 8 (“Ex7-8”), respectively. Cells treated with ASO-29, ASO20, ASO-21, and ASO-22 showed reduced amount of “Ex6-8” transcripts and increased amount of “Ex7-8” transcripts, consistent with the suggestion that these ASOs promote the inclusion of exon 7 in *OPA1* mature mRNA transcripts. Cells treated with ASO-23, ASO-24, ASO-25, ASO-26, ASO-28, ASO-29, ASO-30, ASO-31, ASO-32, ASO-33, ASO-34, ASO-35, ASO-36, ASO-37, and ASO-38 showed increase in the amount of “Ex6-8” transcripts and

decrease in the amount of “Ex7-8” transcripts, consistent with the suggestion that these ASOs promote the exclusion of exon 7 from *OPA1* mature mRNA transcripts.

Example 17: Exemplary *OPA1* Antisense Oligomers Modulate Inclusion of Exon 7, Exon 7x, or Both in *OPA1* mRNA Transcript And Modulate Expression Level of *OPA1* Protein.

[0591] HEK293 cells were transfected with different exemplary *OPA1* modified 2'MOE-PS (2' methoxyethyl and phosphorothioate) ASOs. Each well of HEK 293 cells (about 50,000 cells/well) were treated with an exemplary ASO at 80 nM final concentration in the presence of 0.9 μ L of Lipofectamine® RNAiMax. Here, the cells were harvested 72 hours after transfection to test ASO's effect on *OPA1* mRNA and protein expression. The cells were treated with cycloheximide (50 μ g/mL) for 3 hours prior to harvest for mRNA analysis. **FIG. 18A** shows gel image of PCR products from RT-PCR reaction using probes spanning exon 6 and 8. As shown in the figure, ASO-14 induced reduction in the amount of transcripts having exons 6, 7, 7x, and 8 in tandem (“6-7-7x-8”). ASO-32, ASO-38, and ASO-39 induced significant reduction in the amount of “6-7-8” transcripts, and modest reduction in the amount of “6-7-7x-8” transcripts, whereas ASO-40 induced increase in the amount of “6-7-8” transcripts. These data suggest that ASO-14 promotes exclusion of exon 7x from *OPA1* mRNA transcript, ASO-32, ASO-38, and ASO-39 promote exclusion of exon 7 from *OPA1* mRNA transcript, and they also promote exclusion of exon 7x from *OPA1* mRNA transcript. In contrast, the data suggest that ASO-40 promotes inclusion of exon 7 in *OPA1* mRNA transcript.

[0592] **FIG. 18B** shows image of Western blot using antibody against *OPA1* protein and antibody against β -tubulin protein in the cells after treatment with different ASOs or no ASO (control), as well as Ponceau staining image of the same blot. **FIG. 18B** also shows plots summarizing the amount of *OPA1* protein under different treatment conditions as normalized relative to the amount of β -tubulin or Ponceau staining intensity. The data suggest that ASO-14, ASO-32, ASO-38, and ASO-39 all may induce increase in *OPA1* protein expression, whereas ASO-40 may not significantly change the expression level of *OPA1* protein.

[0593] Dose response of ASO-32 and ASO-38 were also tested along with ASO-14. ASO treatment, cell harvest, and RNA isolation and analysis were conducted similarly to the experiment above in this example. Each well of HEK293 cells (about 50,000 cells/well) were treated with either 20 nM or 80 nM of ASO-14, ASO-32, ASO-38, or no ASO. **FIG. 18C** shows gel image of products from RT-PCR reaction using probes spanning exon 6 and 8. **FIG. 18D** shows quantification of qPCR Ct values for reactions under different experimental conditions using probes spanning exons 6 and 8 (“Ex6-8”), probes spanning exons 7 and 8 (“Ex7-8”), and probes spanning exons 23 and 24 (“Ex23-24”), and **FIG. 18E** shows quantification of relative amount of the corresponding transcripts. The data show consistent observation that ASO-32 and

ASO-38 promote exclusion of exon 7 from mature *OPA1* mRNA transcripts. **FIG. 18F** shows the data on the *OPA1* expression level after treatment of ASO-14, ASO-32, or ASO-38.

Consistently, ASO-32 and ASO-38 increased *OPA1* protein level.

Example 18: ASO Microwalk Evaluated by RT-qPCR.

[0594] In one experiment, microwalk was conducted to test ASOs that have sequences listed in **Table 7**. Briefly, about 30,000 HEK293 cells per well were treated gymnotically with 20 μ M one of the 20 exemplary ASOs (free uptake) listed in **Table 7** for 72 hours. After the treatment, the cells were harvested for analysis. RT-PCR reactions were conducted for products corresponding to Exon 7 or Exon 7x inclusion and full-length.

[0595] **FIGs. 19A-20B** demonstrate data from experiments with some of the 18-mers (named ASO-41 to ASO-48) listed in **Table 7**. **FIGs. 19A-19B** show the Ct values for the qPCR reaction (upper plots) for, and quantification of the relative amount (lower plots; normalized to Ct value of RPL32 qPCR product) of, *OPA1* transcripts having exons 6 and 8 (“Ex6-8”) and *OPA1* transcripts having exons 7 and 8 (“Ex7-8”), respectively. **FIG. 19C** shows the Ct values for the qPCR reaction (upper plots) for, and quantification of the relative amount (lower plots; normalized to Ct value of RPL32 qPCR product) of, *OPA1* transcripts having exons 23 and 24 (“Ex23-24”), and **FIG. 19D** shows the Ct values for RPL32 transcripts as a loading control. These data demonstrate that cells treated with ASO-41 to ASO-47 all showed increased amount of “Ex6-8” transcripts and decreased amount of “Ex7-8” transcripts, suggesting these ASOs promote exclusion of Exon 7 from *OPA1* transcripts. No cycloheximide was applied to the cells that were subject to these analyses for Exon 7 inclusion. **FIG. 20A** shows the Ct values for the qPCR reaction (upper plots) for, and quantification of the relative amount (lower plots; normalized to Ct value of RPL32 qPCR product) of, *OPA1* transcripts having exons 7x and 8 (“Ex7x-8”), and **FIG. 20B** shows the Ct values for RPL32 transcripts as a loading control. These data demonstrate that cells treated with ASO-41 to ASO-44 all showed decreased amount of “Ex7x-8” transcripts, suggesting these ASOs promote exclusion of Exon 7x from *OPA1* transcripts. Cycloheximide was applied to the cells for these analyses for Exon 7x inclusion.

[0596] **FIGs. 21A-22C** demonstrate data from experiments with some of the 16-mers (named ASO-49 to ASO-60) listed in **Table 7**. **FIGs. 21A-21B** show the Ct values for the qPCR reaction (upper plots) for, and quantification of the relative amount (lower plots; normalized to Ct value of RPL32 qPCR product) of, *OPA1* transcripts having exons 6 and 8 (“Ex6-8”) and *OPA1* transcripts having exons 7 and 8 (“Ex7-8”), respectively. **FIG. 21C** shows the Ct values for the qPCR reaction (upper plots) for, and quantification of the relative amount (lower plots; normalized to Ct value of RPL32 qPCR product) of, *OPA1* transcripts having exons 23 and 24 (“Ex23-24”), and **FIG. 21D** shows the Ct values for RPL32 transcripts. These data demonstrate

that cells treated with ASO-49 to ASO-60 all showed increased amount of “Ex6-8” transcripts and decreased amount of “Ex7-8” transcripts, suggesting these ASOs promote exclusion of Exon 7 from *OPAI* transcripts. No cycloheximide was applied to the cells that were subject to these analyses for Exon 7 inclusion. **FIG. 22A** shows the Ct values for the qPCR reaction (upper plots) for, and quantification of the relative amount (lower plots; normalized to Ct value of RPL32 qPCR product) of, *OPAI* transcripts having exons 7x and 8 (“Ex7x-8”), and **FIG. 22C** shows the Ct values for RPL32 transcripts as a loading control. These data demonstrate that cells treated with ASO-49 to ASO-56 all showed decreased amount of “Ex7x-8” transcripts, suggesting these ASOs promote exclusion of Exon 7x from *OPAI* transcripts. Cycloheximide was applied to the cells for these analyses for Exon 7x inclusion.

[0597] Another experiment was conducted to assess transfection dose response relationship with select ASOs among the ASOs tested above in the microwalk analyses. Briefly, 100,000 HEK293 cells per well were transfected with 1, 3, 10, or 30 nM of an exemplary ASO with 0.45 μ L lipofectamine for 24 hours. Cells were later harvested for qPCR analysis as above. **FIGs. 23A-23B** show plots depicting the dose response curves of relative amounts of different *OPAI* transcripts versus the transfection concentration of exemplary ASOs, ASO-14, 38, 41, 42, 43, 44, 49, 51, 52, and 53. The plots show that as a general trend, in cells treated with ASOs like ASO-38, 41, 42, 43, 44, 49, 51, 52, or 53, the amount of *OPAI* transcripts having Exon 6 and 8 (“6-8”) increased, while the amounts of *OPAI* transcripts having Exon 7 and 8 (“7-8”) and *OPAI* transcripts having Exon 7x and 8 (“7x-8”) decreased, as concentration of the exemplary ASO increased. In contrast, in cells treated with ASO-14, while “7x-8” decreased and “6-8” transcripts increased, “7-8” transcripts did not significantly change. These data suggest that ASO-38, 41, 42, 43, 44, 49, 51, 52, and 53 may all promote exclusion of both Exon 7 and Exon 7x, , while ASO-14 may promote exclusion of Exon 7x

Table 5. Exemplary OPA1 ASO sequences

SEQ ID NO.:	Sequence (5'-3')	Coordinates GRCh38/hg38: chr3	
		Oligo Start	Oligo End
6	AGGCCATTCTGAAATTCT	193628406	193628423
7	CATTGAGGCCATTCTGAA	193628411	193628428
8	TAAGGCATTGAGGCCATT	193628416	193628433
9	CCTATTAAGGCATTGAGG	193628421	193628438
10	TTCTTCCTATTAAGGCAT	193628426	193628443
11	AGTATTTCTTCCTATTAA	193628431	193628448

12	TTTCAAGTATTTCTTCCT	193628436	193628453
13	AAAAATTTCAAGTATTTTC	193628441	193628458
14	AATTTAAAAATTTCAAGT	193628446	193628463
15	GCCCTAATTTAAAAATTT	193628451	193628468
16	ACCAAGCCCTAATTTAAA	193628456	193628473
17	ACAAAACCAAGCCCTAAT	193628461	193628478
18	TCCTCACAAAACCAAGCC	193628466	193628483
19	CTAGTCCTCACAAAACC	193628471	193628488
20	CTTTACTAGCTCCTCACA	193628476	193628493
21	AAAACCTTTACTAGCTCC	193628481	193628498
22	AGAGAAAAACCTTTACTA	193628486	193628503
23	CTGAAAGAGAAAAACCTT	193628491	193628508
24	AAGCTGAAAGAGAAAAAC	193628494	193628511
25	CTAAAGCTGAAAGAGAAA	193628497	193628514
26	AAGCTAAAGCTGAAAGAG	193628500	193628517
27	AACAAGCTAAAGCTGAAA	193628503	193628520
28	AGAAACAAGCTAAAGCTG	193628506	193628523
29	CGCAGAAACAAGCTAAAG	193628509	193628526
30	TCCTCCGCAGAAACAAGC	193628514	193628531
31	CGGAATCCTCCGCAGAAA	193628519	193628536
32	AAGAGCGGAATCCTCCGC	193628524	193628541
33	GGAGAAAGAGCGGAATCC	193628529	193628546
34	CTGATGGAGAAAGAGCGG	193628534	193628551
35	TGAAACTGATGGAGAAAG	193628539	193628556
36	GGCTATGAAACTGATGGA	193628544	193628561
37	TCCAGGGCTATGAACTG	193628549	193628566
38	ACAATTCAGGGCTATGA	193628554	193628571
39	TTTCTACAATTCAGGGC	193628559	193628576
40	GAGCTTTTCTACAATTCC	193628564	193628581
41	AACCAGAGCTTTTCTACA	193628569	193628586
42	CTTGAAACCAGAGCTTTT	193628574	193628591
43	ATGGTCTTGAAACCAGAG	193628579	193628596
44	TATCAATGGTCTTGAAAC	193628584	193628601
45	ATGGATATCAATGGTCTT	193628589	193628606
46	CAGAAATGGATATCAATG	193628594	193628611
47	CCTGACAGAAATGGATAT	193628599	193628616
48	CACCCTGACAGAAATGGA	193628602	193628619

49	ACTCACCTGACAGAAAT	193628605	193628622
50	AAAACCTCACCTGACAGA	193628608	193628625
51	TTTAAAACTCACCTGAC	193628611	193628628
52	AAATTTAAAACTCACCT	193628614	193628631
53	AATAAATTTAAAACTCAC	193628617	193628634
54	CATGAAATAAATTTAAAA	193628622	193628639
55	TGCATCATGAAATAAATT	193628627	193628644
56	TTGTTTGCATCATGAAAT	193628632	193628649
57	ATATATTGTTTGCATCAT	193628637	193628654
58	GTTCAATATATTGTTTGC	193628642	193628659
59	CTGTTGTTCAATATATTG	193628647	193628664
60	ATGTCCTGTTGTTCAATA	193628652	193628669
61	AGTTCATGTCCTGTTGTT	193628657	193628674
62	GAACAAGTTCATGTCCTG	193628662	193628679
63	AACAAGAACAAGTTCATG	193628667	193628684
64	CTTACAACAAGAACAAGT	193628672	193628689
65	AGCCACTTACAACAAGAA	193628677	193628694
66	AATTCAGCCACTTACAAC	193628682	193628699
67	GATAAAATTCAGCCACTT	193628687	193628704
68	TTACTGATAAAATTCAGC	193628692	193628709
69	GTGCTTTACTGATAAAAT	193628697	193628714
70	TTGATGTGCTTTACTGAT	193628702	193628719
71	TGGAGAAAGAGCGGAATC	193628530	193628547
72	ATGGAGAAAGAGCGGAAT	193628531	193628548
73	GATGGAGAAAGAGCGGAA	193628532	193628549
74	TGATGGAGAAAGAGCGGA	193628533	193628550
75	ACTGATGGAGAAAGAGCG	193628535	193628552
76	AACTGATGGAGAAAGAGC	193628536	193628553
77	AACTGATGGAGAAAGAG	193628537	193628554
78	GAAACTGATGGAGAAAGA	193628538	193628555
79	ATGAAACTGATGGAGAAA	193628540	193628557
80	TATGAAACTGATGGAGAA	193628541	193628558
81	CTATGAAACTGATGGAGA	193628542	193628559
82	GCTATGAAACTGATGGAG	193628543	193628560
83	GGGCTATGAAACTGATGG	193628545	193628562
84	AGGGCTATGAAACTGATG	193628546	193628563
85	CAGGGCTATGAAACTGAT	193628547	193628564

86	CCAGGGCTATGAAACTGA	193628548	193628565
87	CTGATGGAGAAAGAGCGGAATC	193628530	193628551
88	CTGATGGAGAAAGAGCGGAA	193628532	193628551
89	AACTGATGGAGAAAGAGCGGAA	193628532	193628553
90	AACTGATGGAGAAAGAGCGG	193628534	193628553
91	GAAACTGATGGAGAAAGAGCGG	193628534	193628555
92	GGCTATGAAACTGATGGAGAAA	193628540	193628561
93	GGCTATGAAACTGATGGAGA	193628542	193628561
94	AGGGCTATGAAACTGATGGAGA	193628542	193628563
95	AGGGCTATGAAACTGATGGA	193628544	193628563
96	CCAGGGCTATGAAACTGATGGA	193628544	193628565
97	TTCTTACCCATTTAATTA	193655041	193655059
98	TGCTTCTTACCCATTTAA	193655044	193655062
99	TAATGCTTCTTACCCATT	193655047	193655065
100	AGATAATGCTTCTTACCC	193655050	193655068
101	CAGATAATGCTTCTTACC	193655051	193655069
102	CCCTTCAGATAATGCTTC	193655056	193655074
103	CTACTCCCTTCAGATAAT	193655061	193655079
104	AGCTCCTACTCCCTTCAG	193655066	193655084
105	TTACAGCTCCTACTCCC	193655071	193655089
106	TAAAATTCACAGCTCCTA	193655076	193655094
107	AAATCTAAAATTCACAGC	193655081	193655099
108	GAATAAAATCTAAAATTC	193655086	193655104
109	GATGGGAATAAAATCTAA	193655091	193655109
110	GCTGTGATGGGAATAAAA	193655096	193655114
111	TAGAGGCTGTGATGGGAA	193655101	193655119
112	AAAGATAGAGGCTGTGAT	193655106	193655124
113	AAAAGAAAGATAGAGGCT	193655111	193655129
114	GACCTAAAAGAAAGATAG	193655116	193655134
115	ATAAAGACCTAAAAGAAA	193655121	193655139
116	GAGATATAAAGACCTAAA	193655126	193655144
117	GGCTGTGATGGGAATAAA	193655097	193655115
118	AGGCTGTGATGGGAATAA	193655098	193655116
119	GAGGCTGTGATGGGAATA	193655099	193655117
120	AGAGGCTGTGATGGGAAT	193655100	193655118
121	ATAGAGGCTGTGATGGGA	193655102	193655120
122	GATAGAGGCTGTGATGGG	193655103	193655121

123	AGATAGAGGCTGTGATGG	193655104	193655122
124	AAGATAGAGGCTGTGATG	193655105	193655123
125	TAGAGGCTGTGATGGGAATAAA	193655097	193655119
126	ATAGAGGCTGTGATGGGAATAA	193655098	193655120
127	GATAGAGGCTGTGATGGGAATA	193655099	193655121
128	AGATAGAGGCTGTGATGGGAAT	193655100	193655122
129	AAGATAGAGGCTGTGATGGGAA	193655101	193655123
130	GAGGCTGTGATGGGAATAAA	193655097	193655117
131	AGAGGCTGTGATGGGAATAA	193655098	193655118
132	TAGAGGCTGTGATGGGAATA	193655099	193655119
133	ATAGAGGCTGTGATGGGAAT	193655100	193655120
134	GATAGAGGCTGTGATGGGAA	193655101	193655121
135	AGATAGAGGCTGTGATGGGA	193655102	193655122
136	AAGATAGAGGCTGTGATGGG	193655103	193655123
137	CTGTGATGGGAATAAA	193655097	193655113
138	GCTGTGATGGGAATAA	193655098	193655114
139	GGCTGTGATGGGAATA	193655099	193655115
140	AGGCTGTGATGGGAAT	193655100	193655116
141	GAGGCTGTGATGGGAA	193655101	193655117
142	AGAGGCTGTGATGGGA	193655102	193655118
143	TAGAGGCTGTGATGGG	193655103	193655119
144	ATAGAGGCTGTGATGG	193655104	193655120
145	GATAGAGGCTGTGATG	193655105	193655121
146	AGATAGAGGCTGTGAT	193655106	193655122
147	AAGATAGAGGCTGTGA	193655107	193655123
148	AGGCTGTGATGTGAATAA	193655099	193655116
149	AGAGGCTGTGATGTGAAT	193655101	193655118
150	TAGAGGCTGTGATGTGAA	193655102	193655119
151	GATAGAGGCTGTGATTGG	193655104	193655121
152	GGCTGTGATGTGAATA	193655100	193655115
153	GAGGCTGTGATGTGAA	193655102	193655117
154	TAGAGGCTGTGATTGG	193655104	193655119
155	ATGAAACTGATGGAGA	193628542	193628557
156	CTATGAAACTGATGGA	193628544	193628559
157	GGCTATGAAACTGATG	193628546	193628561
158	GAAACTGATGGAGA	193628542	193628555
159	ATGAAACTGATGGA	193628544	193628557

160	CTATGAAACTGATG	193628546	193628559
161	GGCTATGAACTGA	193628548	193628561
162	TAGAGGCTGTGATGGGAATAAAAT	193655096	193655119
163	ATAGAGGCTGTGATGGGAATAAAA	193655097	193655120
164	ATAGAGGCTGTGATGGGAATAAAAT	193655096	193655120
165	AAAGATAGAGGCTGTGATGGGAATA	193655100	193655124
166	GGCTATGAACTGATGGAGAA	193628541	193628561
167	GGCTATGAACTGATGGAGAAAGA	193628538	193628561
168	AGGGCTATGAACTGATGGAGAAAG	193628539	193628563
169	CATTTAATTAAATTATAT	193655033	193655051
170	CCATTTAATTAAATTATA	193655034	193655052
171	CCCATTTAATTAAATTAT	193655035	193655053
172	ACCCATTTAATTAAATTA	193655036	193655054
173	TACCCATTTAATTAAATT	193655037	193655055
174	TTACCCATTTAATTAAAT	193655038	193655056
175	CTTACCCATTTAATTAAA	193655039	193655057
176	TCTTACCCATTTAATTAA	193655040	193655058
177	GATAGAGGCTGTGATGG	193655104	193655122
178	GGCTGTGAACTGATGGA	89481662	89481679
179	GGCTGTGAACTGATGGAGA	89481660	89481679
180	GCTATGAACTGATGG	193628545	193628560
181	TATGAACTGATGG	193628545	193628558
182	GCTATGAACTGAT	193628547	193628560
183	GCTGTGAACTGATGGAGAA	89481659	89481678
184	GGGCTGTGAACTGATGGAG	89481661	89481680
185	TGTGAACTGATGGAGAA	89481659	89481676
186	CTGTGAACTGATGGAGA	89481660	89481677
187	GCTGTGAACTGATGGAG	89481661	89481678
188	GGGCTGTGAACTGATGG	89481663	89481680
189	TGAACTGATGGAGAA	89481659	89481674
190	GTGAACTGATGGAGA	89481660	89481675
191	TGTGAACTGATGGAG	89481661	89481676
192	CTGTGAACTGATGGA	89481662	89481677
193	GCTGTGAACTGATGG	89481663	89481678
194	GGCTGTGAACTGATG	89481664	89481679
195	GGGCTGTGAACTGAT	89481665	89481680
196	CGGTCCAGGAATGAC	193593285	193593303

197	CCGGTCCAGGAATGA	193593286	193593304
198	CCCGGTCCAGGAATG	193593287	193593305
199	TCCCGGTCCAGGAAT	193593288	193593306
200	CTCCCGGTCCAGGAA	193593289	193593307
201	GCTCCCGGTCCAGGA	193593290	193593308
202	GGCTCCCGGTCCAGG	193593291	193593309
203	CGGGAGCCCCGTGT	193593318	193593336
204	GCGGGAGCCCCGTG	193593319	193593337
205	CGCGGGAGCCCCGT	193593320	193593338
206	ACGCGGGAGCCCCG	193593321	193593339
207	CACGCGGGAGCCCC	193593322	193593340
208	CCACGCGGGAGCCCC	193593323	193593341
209	GCCACGCGGGAGCCC	193593324	193593342
210	GGCCACGCGGGAGCC	193593325	193593343
211	CGGCCACGCGGGAGC	193593326	193593344
212	ACGGCCACGCGGGAG	193593327	193593345
213	GACGGCCACGCGGGA	193593328	193593346
214	AGACGGCCACGCGGG	193593329	193593347
215	GCTAGGGAGGGATGGTTA	193625988	193626006
216	TGTAAGCTAGGGAGGGAT	193625993	193626011
217	ACAGATGTAAGCTAGGGA	193625998	193626016
218	AAGGAACAGATGTAAGCT	193626003	193626021
219	CAACAAAGGAACAGATGT	193626008	193626026
220	GGGTGCAACAAAGGAACA	193626013	193626031
221	ACCAAGGGTGCAACAAAG	193626018	193626036
222	GTTAAACCAAGGGTGCAA	193626023	193626041
223	ATAATGTTAAACCAAGGG	193626028	193626046
224	GGAGAATAATGTTAAACC	193626033	193626051
225	GGGGAGGAGAATAATGTT	193626038	193626056
226	AAATTGGGGAGGAGAATA	193626043	193626061
227	AGAGGAAATTGGGGAGGA	193626048	193626066
228	GGAGAAGAGGAAATTGGG	193626053	193626071
229	AATGAGGAGAAGAGGAAA	193626058	193626076
230	TTCACAATGAGGAGAAGA	193626063	193626081
231	ACGAGTTCACAATGAGGA	193626068	193626086
232	CTGCCACGAGTTCACAAT	193626073	193626091
233	AGACCCTGCCACGAGTTC	193626078	193626096

234	CAAGCAGACCCTGCCACG	193626083	193626101
235	CTCACCAAGCAGACCCTG	193626088	193626106
236	GAGCTACCAAGCAGACC	193626091	193626109
237	AGAATGAGCTACCAAGC	193626096	193626114
238	GTAAGAGAATGAGCTCAC	193626101	193626119
239	TTGTTGTAAGAGAATGAG	193626106	193626124
240	ATTTGTTGTTGTAAGAGA	193626111	193626129
241	CTTGAATTTGTTGTTGTA	193626116	193626134
242	ATGCTCTTGAATTTGTTG	193626121	193626139
243	TCTTCATGCTCTTGAATT	193626126	193626144
244	CTTCCTCTTCATGCTCTT	193626131	193626149
245	GCGCGCTTCCTCTTCATG	193626136	193626154
246	GCTCTGCGCGCTTCCTCT	193626141	193626159
247	GGCCAGCGGCTCTGCGCG	193626149	193626167
248	ATATTGGCCAGCGGCTCT	193626154	193626172
249	GTGCTATATTGGCCAGCG	193626159	193626177
250	AGCTCGTGCTATATTGGC	193626164	193626182
251	GGCATAGCTCGTGCTATA	193626169	193626187
252	TGTTGGGCATAGCTCGTG	193626174	193626192
253	GCTTCTGTTGGGCATAGC	193626179	193626197
254	CTTGCGCTTCTGTTGGGC	193626184	193626202
255	CACCTTGCGCTTCTGTTG	193626187	193626205
256	TCCATCACCTTGCGCTTC	193626192	193626210
257	AACCATCCATCACCTTGC	193626197	193626215
258	CCTTAAACCATCCATCAC	193626202	193626220
259	AGCCCCCTTAAACCATCC	193626207	193626225
260	TCGGTAGCCCCCTTAAAC	193626212	193626230
261	ATGTATCGGTAGCCCCCT	193626217	193626235
262	TGTGAATGTATCGGTAGC	193626222	193626240
263	ATTAGTGTGAATGTATCG	193626227	193626245
264	GGCTGATTAGTGTGAATG	193626232	193626250
265	GAAATGGCTGATTAGTGT	193626237	193626255
266	TGGCAGAAATGGCTGATT	193626242	193626260
267	GATCTTGGCAGAAATGGC	193626247	193626265
268	GACATGATCTTGGCAGAA	193626252	193626270
269	GAGGTGACATGATCTTGG	193626257	193626275
270	AGATTGAGGTGACATGAT	193626262	193626280

271	TGAACAGATTGAGGTGAC	193626267	193626285
272	GTCCATGAACAGATTGAG	193626272	193626290
273	TTGGAGTCCATGAACAGA	193626277	193626295
274	TGTATTTGGAGTCCATGA	193626282	193626300
275	TTTCTTGTATTTGGAGTC	193626287	193626305

Table 6. Exemplary OPA1 ASO sequences

SEQ ID NO	Region	Sequence (5'-3')	Coordinates: GRCh38/hg38: chr3	
			Oligo Start	Oligo End
215	OPA1-IVS6-86	GCTAGGGAGGGATGGTTA	193625988	193626006
216	OPA1-IVS6-81	TGTAAGCTAGGGAGGGAT	193625993	193626011
217	OPA1-IVS6-76	ACAGATGTAAGCTAGGGA	193625998	193626016
218	OPA1-IVS6-71	AAGGAACAGATGTAAGCT	193626003	193626021
227	OPA1-IVS6-26	AGAGGAAATTGGGGAGGA	193626048	193626066
228	OPA1-IVS6-21	GGAGAAGAGGAAATTGGG	193626053	193626071
229	OPA1-IVS6-16	AATGAGGAGAAGAGGAAA	193626058	193626076
230	OPA1-IVS6-11	TTCACAATGAGGAGAAGA	193626063	193626081
231	OPA1-IVS6-6	ACGAGTTCACAATGAGGA	193626068	193626086
232	OPA1-IVS6-1	CTGCCACGAGTTCACAAT	193626073	193626091
233	OPA1-IVS6-EX7+5	AGACCCTGCCACGAGTTC	193626078	193626096
234	OPA1-IVS6-EX7+10	CAAGCAGACCCTGCCACG	193626083	193626101
235	OPA1-IVS6-EX7+15	CTCACCAAGCAGACCCTG	193626088	193626106
236	OPA1-EX7+1	GAGCTCACCAAGCAGACC	193626091	193626109
237	OPA1-EX7+6	AGAATGAGCTCACCAAGC	193626096	193626114
238	OPA1-EX7+11	GTAAGAGAATGAGCTCAC	193626101	193626119
239	OPA1-EX7+16	TTGTTGTAAGAGAATGAG	193626106	193626124
240	OPA1-EX7+21	ATTGTTGTTGTAAGAGA	193626111	193626129
241	OPA1-EX7+26	CTTGAATTTGTTGTTGTA	193626116	193626134
242	OPA1-EX7+31	ATGCTCTTGAATTTGTTG	193626121	193626139
250	OPA1-EX7-21	AGCTCGTGCTATATTGGC	193626164	193626182
267	OPA1-IVS7+46	GATCTTGGCAGAAATGGC	193626247	193626265

Table 7. Exemplary OPA1 ASO sequences

SEQ ID NO	Region	Sequence (5'-3')	Coordinates: GRCh38/hg38: chr3	
			Oligo Start	Oligo End

280	OPA1-EX7+27	TCTTGAATTTGTTGTTGT	193626117	193626135
281	OPA1-EX7+28	CTCTTGAATTTGTTGTTG	193626118	193626136
282	OPA1-EX7+29	GCTCTTGAATTTGTTGTT	193626119	193626137
283	OPA1-EX7+30	TGCTCTTGAATTTGTTGT	193626120	193626138
284	OPA1-EX7+32	CATGCTCTTGAATTTGTT	193626122	193626140
285	OPA1-EX7+33	TCATGCTCTTGAATTTGT	193626123	193626141
286	OPA1-EX7+34	TTCATGCTCTTGAATTTG	193626124	193626142
287	OPA1-EX7+35	CTTCATGCTCTTGAATTT	193626125	193626143
288	OPA1-EX7+26	TGAATTTGTTGTTGTA	193626116	193626132
289	OPA1-EX7+27	TTGAATTTGTTGTTGT	193626117	193626133
290	OPA1-EX7+28	CTTGAATTTGTTGTTG	193626118	193626134
291	OPA1-EX7+29	TCTTGAATTTGTTGTT	193626119	193626135
292	OPA1-EX7+30	CTCTTGAATTTGTTGT	193626120	193626136
293	OPA1-EX7+31	GCTCTTGAATTTGTTG	193626121	193626137
294	OPA1-EX7+32	TGCTCTTGAATTTGTT	193626122	193626138
295	OPA1-EX7+33	ATGCTCTTGAATTTGT	193626123	193626139
296	OPA1-EX7+34	CATGCTCTTGAATTTG	193626124	193626140
297	OPA1-EX7+35	TCATGCTCTTGAATTT	193626125	193626141
298	OPA1-EX7+36	TTCATGCTCTTGAATT	193626126	193626142
299	OPA1-EX7+37	CTTCATGCTCTTGAAT	193626127	193626143

Example 19: ASO-14 Mediates ATP Upregulation in *OPA1* Haploinsufficient HEK293 Cell Line.

[0598] The ATP levels generated through mitochondrial oxidative phosphorylation and glycolytic pathway were measured in HEK293 cell lysates using a commercially available kit (Cat# ab83355, Abcam; USA) according to the manufacturer's instructions. Briefly, about 3×10^5 OPA1+/+ (wildtype) and OPA1+/- HEK293 cells were plated in a T-25 flask and treated with 10 μ M ASO-14. For the ATP test, 96-hrs after treatment, cells were harvested, and two aliquots of cell suspension were prepared. One aliquot was processed for deproteinizing using commercially available kit (Cat# ab204708, Abcam; USA) to remove residual protein for executing ATP fluorescence assay to measure total ATP level. The second aliquot was used for BCA assay (Cat# 23225, Thermo Fisher; USA) to measure total protein level. ATP level was then calculated by normalizing the measured total ATP level to the measured total protein level.

[0599] FIG. 24A summarizes the ATP level measured under each condition. In the mock group, untreated OPA1+/- HEK293 cells were found to have 0.79 ± 0.02 ATP level as compared to untreated OPA1+/+ HEK293 cells. There was about 20% ATP deficit in OPA1+/- HEK293

cells. In comparison, OPA1^{+/-} HEK293 cells treated with ASO-14 had ATP levels 0.88 ± 0.01 , significantly higher than the mock-treated OPA1^{+/-} HEK293 cells, suggesting that treatment of ASO-14 reduced the deficit by about 50%. Data were collected from three independent experiments. (Statistics: Ordinary one-way ANOVA; *** $P < 0.0001$; ** $P < 0.0080$).

[0600] FIGS. 24B-24C demonstrate the OPA1 protein under each condition. 96 hours after treatment with ASO-14 or no treatment (mock), cells were lysed with RIPA buffer and immunoblot blot was probed with antibodies targeting OPA1 and β -actin. The data show that treatment of ASO-14 upregulated about 18% OPA1 protein in OPA1^{+/-} cells. **FIG. 24B** shows the immunoblot gel images. Multiple bands on the immunoblot image represent various isoforms of OPA1. **FIG. 24C** summarizes quantification of the immunoblot results. Untreated (mock) OPA1^{+/-} HEK293 cells were found to have $46 \pm 0.5\%$ OPA1 protein level as compared to untreated (mock) OPA1^{+/+} HEK293 cells. OPA1^{+/+} cells treated with ASO-14 had OPA1 levels 123.2 ± 1.3 of untreated OPA1^{+/+} cells. OPA1^{+/-} cells treated with ASO-14 had OPA1 levels $54.54 \pm 0.6\%$ of untreated OPA1^{+/+} cells. Statistics performed with corresponding mock. *** $P < 0.0001$, by Ordinary one-Way ANOVA and ### $P < 0.0001$, by Welch's t test. Data represent average of three technical replicates.

Example 20: Exemplary Antisense Oligomers Restore OPA1 Expression in Cells with OPA1 Mutations from Diagnosed Patients.

[0601] This example examines *OPA1* mRNA and protein levels in cells with mutations in *OPA1* gene from patients diagnosed with Autosomal dominant optic atrophy (ADOA), as well as effects of exemplary antisense oligomer ASO-14 on OPA1 mRNA and protein levels, and mitochondrial bioenergetics in the patient cells.

[0602] FIGS. 25A-25C summarize mRNA and protein expression of *OPA1* gene in fibroblast cells from diagnosed patients that have haploinsufficient mutation in *OPA1* gene: F34 (*OPA1* canonical splice mutation at c.1608+1delGTGAGG); F35 (*OPA1* frameshift mutation at c.2873_2876del); F36 (*OPA1* frameshift mutation at c.635_636delAA). mRNA expression level of *OPA1* gene in patient cells is about 50% to 60% of the mRNA level in wildtype (WT) cells (**FIG. 25A**); OPA1 protein level in patient cells is about 30% to about 40% of the protein level in WT cells (**FIG. 25B**). Histograms in **FIGS. 25A-25B** show mean $\pm$ SEM of 3 independent experiments; one-way ANOVA compared to WT group (**** $P < 0.0001$). **FIG. 25C** shows a representative immunoblot image of OPA protein expression level in diseased fibroblast cells.

[0603] FIGS. 26A-26D demonstrate the effects of exemplary antisense oligomer, ASO-14, on *OPA1* NMD exon inclusion, mRNA level, and protein level in wildtype (WT) fibroblast cells and fibroblast cells from diagnosed patients that have haploinsufficient mutation in *OPA1* gene.

The fibroblast cells were transfected with ASO-14 (40nM), and RNA was isolated 24 hrs after transfection and analyzed. For non-productive *OPAI* mRNA measurement, cells were treated with cycloheximide (50µg/mL) for 3 hrs. prior to RNA isolation. Immunoblot was performed 72 hrs. post transfection with antibodies targeting OPA1 and β -tubulin. As shown in **FIG. 26A**, ASO-14 significantly decreased inclusion of NMD exon (exon 7x), measured by level of non-productive *OPAI* mRNA, in WT cells and all diseased cells to lower than 20% level of the normalized level in WT cells. There was a trend of increase in total *OPAI* mRNA level in all types of cells by the treatment of ASO-14 (**FIG. 26B**). Histograms in **FIGS. 26A-26B** show mean $\pm$ SEM of 2-3 independent experiments; one-way ANOVA vs. Mock for respective cell line (* $P < .05$; *** $P < .001$; **** $P < .0001$). Correspondingly, OPA1 protein level was significantly increased by the treatment of ASO-14 in all types of cells (**FIGS. 26C-26D**). **FIG. 26C** shows representative immunoblot images of OPA1 protein and loading control β -Tubulin under all types of conditions; **FIG. 26D** shows the statistical summary of the OPA1 protein levels, the histograms show mean $\pm$ SEM of 3 independent experiments; unpaired t-test vs. Mock for respective cell line (* $P < .05$, ** $P < .01$, *** $P < .001$).

[0604] FIGS. 27A-27E demonstrate that patient fibroblast cells (cell lines F35 and F36) show deficiencies in mitochondrial bioenergetics. **FIG. 27A** shows representative time courses of the oxygen consumption rate of WT cells, F35 cells, and F36 cells at baseline level and when challenged sequentially with oligomycin, FCCP, rotenone and antimycin A. Patient fibroblast cells, F35 and F36 cells, were found to have reduced basal oxygen consumption rate (**FIG. 27B**), ATP linked respiration (**FIG. 27C**), maximal respiration (**FIG. 27D**), and spare respiratory capacity (**FIG. 27E**), as compared to WT fibroblast cells. Units in **FIGS. 27B-27E** are pmol/min/cells, data normalized to wild-type (WT). Histograms in **FIGS. 27B-27E** show mean $\pm$ SEM of >18 individual measurements from 2 independent experiments; one-way ANOVA vs. WT (** $P < .01$; **** $P < .0001$).

[0605] FIGS. 28A-28D demonstrate that ASO-14 increased mitochondrial energetics in F35 patient cell line. As shown in the figures, treatment with 40nM or 60 nM ASO-14 increased basal oxygen consumption rate (**FIG. 28A**), ATP linked respiration (**FIG. 28B**), maximal respiration (**FIG. 28C**), and spare respiratory capacity (**FIG. 28D**) of F35 patient cells in a dose-dependent manner. Treatment with 20 nM ASO-14 also significantly increased spare respiratory capacity (**FIG. 28D**). In contrast, non-targeting ASO (NT ASO, targeting an unrelated gene) did not significantly alter the parameters at any of the tested concentrations. Units in the figures are pmol/min/cells; the Oxygen Consumption Rates (OCR) are normalized to total cell count and plotted to Mock (No ASO). The histograms show mean $\pm$ SEM of >20 individual measurements

from at least 3 independent experiments; one-way ANOVA vs. Mock (*P<.05; ***P<.001; ****P<.0001).

[0606] FIGS. 29A-29D demonstrate that ASO-14 increased mitochondrial energetics in F36 patient cell line. As shown in the figures, ASO-14 also increased basal oxygen consumption rate (FIG. 29A), ATP linked respiration (FIG. 29B), maximal respiration (FIG. 29C), and spare respiratory capacity (FIG. 29D) of F36 patient cells in a dose-dependent manner from 20 nM, 40 nM, to 60 nM. In contrast, non-targeting ASO did not significantly alter the parameters at 40 nM. Units in the figures are pmol/min/cells; the Oxygen Consumption Rates (OCR) are normalized to total cell count and plotted to Mock (No ASO). The histograms show mean $\pm$ SEM of >20 individual measurements from 2-5 independent experiments; one-way ANOVA vs. Mock (*P<.05; ** P<.01; *** P<.001 **** P<.0001).

[0607] The experiments in F35 and F36 cells suggest that the dose-dependent improvement in mitochondrial bioenergetics by ASO-14 is mutation-independent.

[0608] The foregoing preclinical data support the TANGO disease modifying approach in ADOA. As demonstrated by the data, the exemplary antisense oligomer, ASO-14, reduced non-productive exon inclusion, increased total *OPA1* mRNA and protein expression in all three patient fibroblast cell lines; increased ASO-14 dose increased mitochondrial respiration in two fibroblast cell lines. The data further suggest that the ASO mediated increase in OPA1 protein expression is disease modifying in ADOA in a mutation-independent manner.

[0609] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the present disclosure may be employed in practicing the present disclosure. It is intended that the following claims define the scope of the present disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

What is claimed is:

1. A method of modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an OPA1 gene and that comprises a non-sense mediated RNA decay-inducing exon (NMD exon), the method comprising contacting an agent or a vector encoding the agent to the cell, whereby the agent modulates splicing of the NMD exon from the pre-mRNA, thereby modulating a level of processed mRNA that is processed from the pre-mRNA, and modulating the expression of the OPA1 protein in the cell, wherein the agent comprises an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299.
2. The method of claim 1, wherein the agent:
 - (a) binds to a targeted portion of the pre-mRNA;
 - (b) modulates binding of a factor involved in splicing of the NMD exon; or
 - (c) a combination of (a) and (b).
3. The method of claim 2, wherein the agent interferes with binding of the factor involved in splicing of the NMD exon to a region of the targeted portion.
4. The method of claim 2, wherein the targeted portion of the pre-mRNA is proximal to the NMD exon.
5. The method of claim 2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NMD exon.
6. The method of claim 2, wherein the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NMD exon.
7. The method of claim 2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300

- nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NMD exon.
8. The method of claim 2, wherein the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NMD exon.
 9. The method of claim 2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509.
 10. The method of claim 2, wherein the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509.
 11. The method of claim 2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.
 12. The method of claim 2, wherein the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

13. The method of claim 2, wherein the targeted portion of the pre-mRNA is located in an intronic region between two canonical exonic regions of the pre-mRNA, and wherein the intronic region contains the NMD exon.
14. The method of claim 2, wherein the targeted portion of the pre-mRNA at least partially overlaps with the NMD exon.
15. The method of claim 2, wherein the targeted portion of the pre-mRNA at least partially overlaps with an intron upstream or downstream of the NMD exon.
16. The method of claim 2, wherein the targeted portion of the pre-mRNA comprises 5' NMD exon-intron junction or 3' NMD exon-intron junction.
17. The method of claim 2, wherein the targeted portion of the pre-mRNA is within the NMD exon.
18. The method of claim 2, wherein the targeted portion of the pre-mRNA comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.
19. The method of any one of claims 1 to 18, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.
20. The method of any one of claims 1 to 18, wherein the NMD exon comprises a sequence of SEQ ID NO: 279.
21. The method of claim 2, wherein the targeted portion of the pre-mRNA is within the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.
22. The method of claim 2, wherein the targeted portion of the pre-mRNA is upstream or downstream of the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.
23. The method of claim 2, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193628509 to 193628616.
24. The method of any one of claims 1 to 23, wherein the OPA1 protein expressed from the processed mRNA is a full-length OPA1 protein or a wild-type OPA1 protein.
25. The method of any one of claims 1 to 23, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.
26. The method of any one of claims 1 to 23, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.
27. The method of any one of claims 1 to 23, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.

28. The method of any one of claims 1 to 23, or 25 to 27, wherein the OPA1 protein expressed from the processed mRNA is an OPA1 protein that lacks an amino acid sequence encoded by a nucleic acid sequence with at least 80% sequence identity to SEQ ID NO: 277.
29. The method of any one of claims 1 to 28, wherein the method promotes exclusion of the NMD exon from the pre-mRNA.
30. The method of claim 29, wherein the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.
31. The method of any one of claims 1 to 30, wherein the method results in an increase in the level of the processed mRNA in the cell.
32. The method of claim 31, wherein the level of the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.
33. The method of any one of claims 1 to 32, wherein the method results in an increase in the expression of the OPA1 protein in the cell.
34. The method of claim 33, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold,

- about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.
35. The method of any one of claims 1 to 34, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292.
36. The method of any one of claims 1 to 34, wherein the agent further comprises a gene editing molecule.
37. The method of claim 36, wherein the gene editing molecule comprises CRISPR-Cas9.
38. A method of modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an OPA1 gene, wherein the pre-mRNA comprises a coding exon, the method comprising contacting an agent or a vector encoding the agent to the cell, whereby the agent promotes exclusion of the coding exon from the pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon in the cell.
39. The method of claim 38, wherein the agent:
- (a) binds to a targeted portion of the pre-mRNA;
 - (b) modulates binding of a factor involved in splicing of the coding exon; or
 - (c) a combination of (a) and (b).
40. The method of claim 39, wherein the agent interferes with binding of the factor involved in splicing of the coding exon to a region of the targeted portion.
41. The method of claim 39, wherein the targeted portion of the pre-mRNA is proximal to the coding exon.
42. The method of claim 39, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the coding exon.
43. The method of claim 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 to 50, from 90 to 50, from 80 to 50, from 70 to 50, from 60 to 50, from 60 to 40, from 60 to 30, from 60 to 20, from 60 to 10, from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon.

44. The method of claim 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon.
45. The method of claim 39, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the coding exon.
46. The method of claim 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, from 1 to 19, from 10 to 60, from 20 to 60, from 30 to 60, from 40 to 60, from 50 to 60, from 50 to 70, from 50 to 80, from 50 to 90, or from 50 to 100 nucleotides downstream of 3' end of the coding exon.
47. The method of claim 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, or from 1 to 19 nucleotides downstream of 3' end of the coding exon.
48. The method of claim 39, wherein the targeted portion of the pre-mRNA at least partially overlaps with the coding exon.
49. The method of claim 39, wherein the targeted portion of the pre-mRNA at least partially overlaps with an intron immediately upstream or immediately downstream of the coding exon.
50. The method of claim 39, wherein the targeted portion of the pre-mRNA comprises 5' coding exon-intron junction or 3' coding exon-intron junction.
51. The method of claim 39, wherein the targeted portion is within the coding exon of the pre-mRNA.
52. The method of any one of claims 39 to 51, wherein the coding exon is an alternatively spliced exon.
53. The method of any one of claims 39 to 52, wherein the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277.
54. The method of any one of claims 39 to 52, wherein the coding exon comprises SEQ ID NO: 277.
55. The method of claim 39, wherein the targeted portion of the pre-mRNA is immediately upstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.
56. The method of claim 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092.
57. The method of claim 39, wherein the targeted portion of the pre-mRNA is immediately downstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

58. The method of claim 39, wherein the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, or from 1 to 19 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202.
59. The method of claim 39, wherein the targeted portion of the pre-mRNA is within the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.
60. The method of claim 39, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193626092 to 193626202.
61. The method of claim 39, wherein the targeted portion comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the coding exon.
62. The method of claim 39, wherein the targeted portion of the pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 277.
63. The method of any one of claims 38 to 62, wherein the exclusion of the coding exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.
64. The method of any one of claims 38 to 63, wherein the method results in an increase in expression of the OPA1 protein in the cell.
65. The method of claim 64, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-

- fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.
66. The method of claim 64, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by at least about 1.5-fold compared to in the absence of the agent.
67. The method of any one of claims 64 to 66, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.
68. The method of any one of claims 64 to 66, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.
69. The method of any one of claims 64 to 66, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.
70. The method of any one of claims 64 to 69, wherein the OPA1 protein expressed from the processed mRNA comprises fewer proteolytic cleavage sites than an OPA1 protein encoded by a corresponding mRNA containing the coding exon.
71. The method of any one of claims 38 to 70, wherein the agent promotes exclusion of a non-sense mediated RNA decay-inducing exon (NMD exon) from the pre-mRNA.
72. The method of claim 71, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.
73. The method of claim 71, wherein the NMD exon comprises a sequence of SEQ ID NO: 279.
74. The method of any one of claims 64 to 73, wherein the OPA1 protein expressed from the processed mRNA comprises fewer proteolytic cleavage sites than an OPA1 protein encoded by a corresponding mRNA containing the coding exon.
75. The method of any one of claims 38 to 74, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242, 250, 280-283, 288, and 290-292.
76. The method of any one of claims 38 to 74, wherein the agent comprises a gene editing molecule.
77. The method of claim 76, wherein the gene editing molecule comprises CRISPR-Cas9.
78. A method of modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an OPA1 gene, wherein the pre-mRNA comprises a coding exon, the method comprising contacting an agent or a vector encoding the agent to the cell, wherein the agent comprises an antisense oligomer that binds to:

- (a) a targeted portion of the pre-mRNA within an intronic region immediately upstream of a 5' end of the coding exon of the pre-mRNA; or
- (b) a targeted portion of the pre-mRNA within an intronic region immediately downstream of a 3' end of the coding exon of the pre-mRNA;
- whereby the agent increases a level of a processed mRNA that is processed from the pre-mRNA and that contains the coding exon in the cell.
79. The method of claims 78, wherein the coding exon is an alternatively spliced exon.
80. The method of claims 78 or 79, wherein the method promotes inclusion of the coding exon in the processed mRNA during splicing of the pre-mRNA in the cell.
81. The method of any one of claims 78 to 80, wherein the target portion of the pre-mRNA is within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of a 5' end of the coding exon.
82. The method of any one of claims 78 to 80, wherein the target portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of a 3' end of the coding exon.
83. The method of any one of claims 78 to 80, wherein the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277.
84. The method of any one of claims 78 to 80, wherein the coding exon comprises SEQ ID NO: 277.
85. The method of any one of claims 78 to 80, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092.
86. The method of any one of claims 78 to 80, wherein the targeted portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202.
87. The method of any one of claims 78 to 86, wherein the inclusion of the coding exon in the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-

- fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.
88. The method of any one of claims 78 to 87, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267.
89. A method of modulating expression of a target protein in a cell having a pre-mRNA transcribed from a gene that encodes the target protein, wherein the pre-mRNA comprises a coding exon and a non-sense mediated RNA decay-inducing exon (NMD exon), the method comprising contacting an agent or a vector encoding the agent to the cell, wherein the agent promotes exclusion of both the coding exon and the NMD exon from the pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks both the NMD exon and the coding exon in the cell.
90. The method of claim 89, wherein the agent:
- (a) binds to a targeted portion of the pre-mRNA;
 - (b) modulates binding of a factor involved in splicing of the coding exon, the NMD exon, or both; or
 - (c) a combination of (a) and (b).
91. The method of claim 90, wherein the agent interferes with binding of the factor involved in splicing of the coding exon, the NMD exon, or both, to a region of the targeted portion.
92. The method of any one of claims 89 to 91, wherein the NMD exon is within an intronic region adjacent to the coding exon.
93. The method of claim 92, wherein the NMD exon is within an intronic region immediately upstream of the coding exon.
94. The method of claim 92, wherein the NMD exon is within an intronic region immediately downstream of the coding exon.
95. The method of any one of claims 90 to 94, wherein the targeted portion of the pre-mRNA is proximal to the coding exon.
96. The method of any one of claims 90 to 94, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the coding exon.
97. The method of any one of claims 90 to 94, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the coding exon.
98. The method of any one of claims 90 to 94, wherein the targeted portion of the pre-mRNA is located within the coding exon.
99. The method of any one of claims 90 to 94, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon.

100. The method of any one of claims 90 to 94, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the coding exon to 100 nucleotides downstream of the coding exon.
101. The method of any one of claims 89 to 100, wherein the coding exon is an alternatively spliced exon.
102. The method of any one of claims 89 to 101, wherein the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277.
103. The method of any one of claims 89 to 101, wherein the coding exon comprises SEQ ID NO: 277.
104. The method of claim 90, wherein the targeted portion of the pre-mRNA is immediately upstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.
105. The method of claim 90, wherein the targeted portion of the pre-mRNA is immediately downstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.
106. The method of any one of claims 90 to 94, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of GRCh38/ hg38: chr3 193626092.
107. The method of claim 90, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092. to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202. .
108. The method of claim 90, wherein the targeted portion of the pre-mRNA is within the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.
109. The method of claim 90, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.
110. The method of claim 90, wherein the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the coding exon.
111. The method of claim 90, wherein the targeted portion of the pre-mRNA is proximal to the NMD exon.
112. The method of claim 90, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the NMD exon.
113. The method of claim 90, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the NMD exon.
114. The method of claim 90, wherein the targeted portion of the pre-mRNA is located within the NMD exon.

115. The method of claim 90, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the NMD exon to 100 nucleotides downstream of the NMD exon.
116. The method of any one of claims 89 to 115, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.
117. The method of claim 89, wherein the NMD exon comprises SEQ ID NO: 279.
118. The method of claim 90, wherein the targeted portion of the pre-mRNA is immediately upstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.
119. The method of claim 90, wherein the targeted portion of the pre-mRNA is immediately downstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.
120. The method of claim 90, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.
121. The method of claim 90, wherein the targeted portion of the pre-mRNA is within the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.
122. The method of claim 90, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.
123. The method of claim 90, wherein the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.
124. The method of any one of claims 89 to 123, wherein the exclusion of the coding exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.
125. The method of any one of claims 89 to 124, wherein the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-

- fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.
126. The method of any one of claims 89 to 125, wherein the agent results in an increase in the level of the processed mRNA in the cell.
127. The method of claim 126, wherein the level of the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.
128. The method of any one of claims 89 to 127, wherein the method results in an increase in expression of the target protein in the cell.
129. The method of claim 128, wherein a level of the target protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.
130. The method of any one of claims 89 to 128, wherein the target protein is an OPA1 protein.

131. The method of claim 130, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by at least about 1.5-fold compared to in the absence of the agent.
132. The method of claim 130, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.
133. The method of claim 130, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.
134. The method of claim 130, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.
135. The method of any one of claims 89 to 127, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 236, 242, 250, 280-283, 288, and 290-292.
136. The method of any one of claims 78 to 135, wherein the agent comprises a gene editing molecule.
137. The method of claim 136, wherein the gene editing molecule comprises CRISPR-Cas9.
138. The method of any one of claims 1 to 75 or 78 to 135, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.
139. The method of any one of claims 1 to 75 or 78 to 138, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, or a 2'-O-methoxyethyl moiety.
140. The method of any one of claims 1 to 75 or 78 to 139, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises at least one modified sugar moiety.
141. The method of claim 140, wherein each sugar moiety is a modified sugar moiety.
142. The method of any one of claims 1 to 75 or 78 to 141, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40

- nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.
143. The method of any one of claims 1 to 142, wherein the vector comprises a viral vector encoding the agent.
144. The method of claim 143, wherein the viral vector comprises an adenoviral vector, adeno-associated viral (AAV) vector, lentiviral vector, Herpes Simplex Virus (HSV) viral vector, or retroviral vector.
145. The method of any one of claims 1 to 144, wherein the method further comprises assessing mRNA level or expression level of the OPA1 protein.
146. The method of any one of claims 1 to 145, wherein the agent is a therapeutic agent.
147. A pharmaceutical composition comprising the therapeutic agent of claim 146 or a vector encoding the therapeutic agent of claim 146, and a pharmaceutically acceptable excipient.
148. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding a therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent comprises an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299.
149. The pharmaceutical composition of claim 148, wherein the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242 and 250.
150. The pharmaceutical composition of claim 148, wherein the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267.
151. The pharmaceutical composition of claim 148, wherein the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, and 280-299.
152. A composition, comprising an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299, wherein the antisense oligomer comprises a backbone modification, a sugar moiety modification, or a combination thereof.
153. The composition of claim 152, wherein the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242 and 250.
154. The composition of claim 152, wherein the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267.

155. The composition of claim 152, wherein the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, and 280-299.
156. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent promotes exclusion of a coding exon from a pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon in a cell, wherein the pre-mRNA is transcribed from an OPA1 gene and that comprises the coding exon.
157. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent comprises an antisense oligomer that binds to a pre-mRNA that is transcribed from an OPA1 gene in a cell, wherein the antisense oligomer binds to:
- (a) a targeted portion of the pre-mRNA within an intronic region immediately upstream of a 5' end of the coding exon of the pre-mRNA; or
 - (b) a targeted portion of the pre-mRNA within an intronic region immediately downstream of a 3' end of the coding exon of the pre-mRNA;
- whereby the therapeutic agent increases a level of a processed mRNA that is processed from the pre-mRNA and that contains the coding exon in the cell.
158. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent promotes exclusion of both a coding exon and a non-sense mediated RNA decay-inducing exon (NMD exon) from a pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon and the NMD exon in a cell, wherein the pre-mRNA is transcribed from an OPA1 gene in the cell and comprises the coding exon and the NMD exon.
159. The pharmaceutical composition of any one of claims 147 to 158, wherein the pharmaceutical composition is formulated for intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.
160. The pharmaceutical composition of any one of claims 147 to 158, wherein the pharmaceutical composition is formulated for intravitreal injection.
161. The pharmaceutical composition of any one of claims 147 to 160, wherein the pharmaceutical composition further comprises a second therapeutic agent.

162. The pharmaceutical composition of claim 161, wherein the second therapeutic agent comprises a small molecule.
163. The pharmaceutical composition of claim 161, wherein the second therapeutic agent comprises an antisense oligomer.
164. The pharmaceutical composition of claim 161, wherein the second therapeutic agent corrects intron retention.
165. The pharmaceutical composition or composition of any one of claims 147 to 160, wherein the antisense oligomer is selected from the group consisting of Compound ID NOs: 1-303.
166. A method of treating or reducing the likelihood of developing a disease or condition in a subject in need thereof by modulating expression of an OPA1 protein in a cell of the subject, comprising contacting to cells of the subject the therapeutic agent of any one of claims 147 to 165.
167. The method of claim 166, wherein the disease or condition is associated with a loss-of-function mutation in an OPA1 gene.
168. The method of claim 166 or 167, wherein the disease or condition is associated with haploinsufficiency of the OPA1 gene, and wherein the subject has a first allele encoding a functional OPA1 protein, and a second allele from which the OPA1 protein is not produced or produced at a reduced level, or a second allele encoding a nonfunctional OPA1 protein or a partially functional OPA1 protein.
169. The method of any one of claims 166 to 168, wherein the disease or condition comprises an eye disease or condition.
170. The method of any one of claims 166 to 168, wherein the disease or condition comprises ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS

- (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.
171. The method of any one of claims 166 to 168, wherein the disease or condition comprises Optic atrophy type 1.
172. The method of any one of claims 166 to 168, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA).
173. The method of claim 166 or 167, wherein the disease or condition is associated with an autosomal recessive mutation of OPA1 gene, wherein the subject has a first allele encoding from which:
- (i) OPA1 protein is not produced or produced at a reduced level compared to a wild-type allele; or
 - (ii) the OPA1 protein produced is nonfunctional or partially functional compared to a wild-type allele, and
a second allele from which:
 - (iii) the OPA1 protein is produced at a reduced level compared to a wild-type allele and the OPA1 protein produced is at least partially functional compared to a wild-type allele; or
 - (iv) the OPA1 protein produced is partially functional compared to a wild-type allele.
174. The method of any one of claims 166 to 173, wherein the subject is a human.
175. The method of any one of claims 166 to 173, wherein the subject is a non-human animal.
176. The method of any one of claims 166 to 173, wherein the subject is a fetus, an embryo, or a child.
177. The method of any one of claims 166 to 173, wherein the cells are ex vivo.
178. The method of any one of claims 166 to 173, wherein the therapeutic agent is administered by intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.
179. The method of any one of claims 166 to 173, wherein the therapeutic agent is administered by intravitreal injection.
180. The method of any one of claims 166 to 179, wherein the method treats the disease or condition.