(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
03 September 2020 (03.09.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/176776 A1

(51) International Patent Classification:
C12N 15/113 (2010.01) *C12Q 1/68* (2018.01)
C12N 15/11 (2006.01)

(21) International Application Number:
PCT/US2020/020175

(22) International Filing Date:
27 February 2020 (27.02.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/811,511 27 February 2019 (27.02.2019) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

(54) Title: ANTISENSE OLIGOMERS FOR TREATMENT OF CONDITIONS AND DISEASES

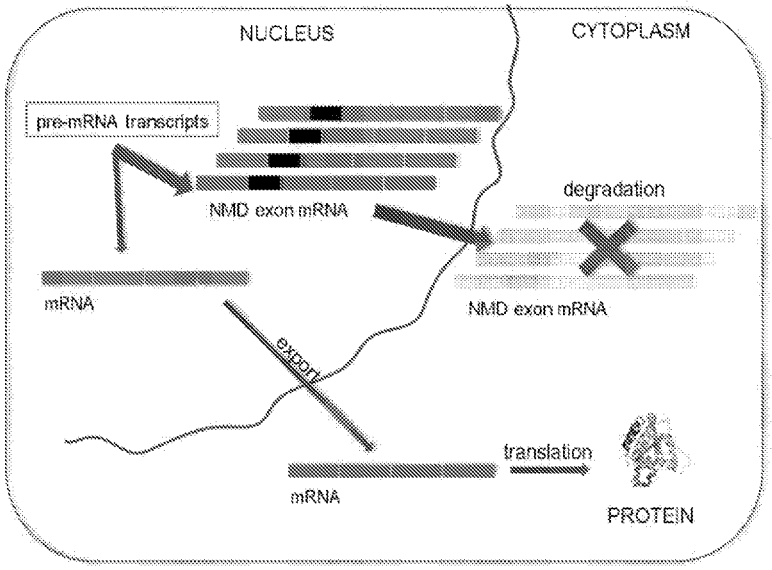

FIG. 1A

(57) Abstract: Alternative splicing events in *SCN1A* 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 *SCN1A* gene can modulate the expression level of functional proteins in Dravet Syndrome patients and/or inhibit aberrant protein expression. Such therapeutic agents can be used to treat a condition caused by SCN1A, SCN8A or SCN5A protein deficiency.

WO 2020/176776 A1

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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

ANTISENSE OLIGOMERS FOR TREATMENT OF CONDITIONS AND DISEASES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/811,511, filed on February 27, 2019 which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Nervous system disorders are often associated with channelopathy, characterized by the disturbed function of ion channels that mediate neuronal excitability, neuronal interactions, and brain functions at large. Mutations in the SCN1A gene, which is part of the SCN1A-SCN2A-SCN3A gene cluster that encodes alpha-pore forming subunits of the neuronal voltage gated sodium channel, are associated with development of disease number of diseases and conditions, such as Dravet Syndrome (DS) (Miller, et al., 1993-2015, GeneReviews, Eds. Pagon RA, et al. Seattle (WA): University of Washington, Seattle, Bookshelf ID: NBK1318, and Mulley, et al., 2005, Hum. Mutat. 25: 535-542).

SUMMARY

[0003] Disclosed herein, in certain embodiments, is a method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is: from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE. In some embodiments, the therapeutic agent interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted portion. In some embodiments, the targeted portion is at most 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 NIE. In some embodiments, the targeted portion is at least 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 NIE. In some embodiments, the targeted portion is at most 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 NIE. In some embodiments, the targeted portion is at least 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 NIE. In some embodiments, the therapeutic agent is an antisense oligomer (ASO). In some embodiments, the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731. In some embodiments, the therapeutic agent promotes exclusion of the NMD exon from the processed mRNA encoding SCN1A protein. In some embodiments, exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent 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 exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in a control cell. In some embodiments, the therapeutic agent increases level of the processed mRNA encoding SCN1A protein in the cell. In some embodiments, an amount of the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent 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 an total amount of the processed mRNA encoding SCN1A protein in a control cell.

[0004] Disclosed herein, in certain embodiments, is a method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A 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 that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is: from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE. In some embodiments, the therapeutic agent interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted portion. In some embodiments, the targeted portion is at most 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 NIE. In some embodiments, the targeted portion is at least 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 NIE. In some embodiments, the targeted portion is at most 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 NIE. In some embodiments, the targeted portion is at least 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 NIE. In some embodiments, the therapeutic agent is an antisense oligomer (ASO). In some embodiments, the ASO comprises a sequence that is at least about

80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731. In some embodiments, the therapeutic agent promotes exclusion of the NMD exon from the processed mRNA encoding SCN1A protein. In some embodiments, exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent 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 exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in a control cell. In some embodiments, the therapeutic agent increases level of the processed mRNA encoding SCN1A protein in the cell. In some embodiments, an amount of the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent 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 an total amount of the processed mRNA encoding SCN1A protein in a control cell. In some embodiments, the disease or condition is induced by a loss-of-function mutation in $Na_v1.1$. In some embodiments, the disease or condition is associated with haploinsufficiency of the *SCN1A* gene, and wherein the subject has a first allele encoding a functional SCN1A, and a second allele from which SCN1A is not produced or produced at a reduced level, or a second allele encoding a nonfunctional SCN1A or a partially functional SCN1A. In some embodiments, the disease or condition is encephalopathy. In some embodiments, the encephalopathy is epileptic encephalopathy. In some embodiments, the disease or condition is Dravet Syndrome (DS); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive

myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; autism; or malignant migrating partial seizures of infancy. In some embodiments, GEFS+ is epilepsy, generalized, with febrile seizures plus, type 2. In some embodiments, the Febrile seizure is Febrile seizures, familial, 3A. In some embodiments, SMEB is SMEB without generalized spike wave (SMEB-SW), SMEB without myoclonic seizures (SMEB-M), SMEB lacking more than one feature of SMEI (SMEB-O), or intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC).

[0005] Disclosed herein, in certain embodiments, is a method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

[0006] Disclosed herein, in certain embodiments, is a method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A 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 that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

[0007] Disclosed herein, in certain embodiments, is an antisense oligomer (ASO) comprising a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731.

[0008] Disclosed herein, in certain embodiments, is an antisense oligomer (ASO) consisting of a sequence selected from SEQ ID NOs: 12-731.

[0009] Disclosed herein, in certain embodiments, is a method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising: contacting the cell of the subject with an ASO comprising a sequence that is at least about 80%, 85%,

90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731; or an ASO consisting of a sequence selected from SEQ ID NOs: 12-731.

[0010] Disclosed herein, in certain embodiments, is a kit comprising an ASO comprising a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731; or an ASO consisting of a sequence selected from SEQ ID NOs: 12-731.

INCORPORATION BY REFERENCE

[0011] 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

[0012] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0013] FIG. 1 depicts a schematic representation of a target mRNA that contains a non-sense mediated RNA 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** is a schematic representation of therapeutic ASO-mediated exclusion of a nonsense-mediated mRNA decay-inducing exon, which decreases non-productive mRNA and increases productive mRNA and increases expression of the full-length target protein from the productive mRNA.

[0014] FIG. 2 depicts identification of an exemplary nonsense-mediated mRNA decay (NMD)-inducing exon in the *SCN1A* gene. The identification of the NMD-inducing exon in the *SCN1A* gene using comparative genomics is shown, visualized in the UCSC genome browser. The upper panel

shows a graphic representation of the *SCN1A* gene to scale. The conservation level across 100 vertebrate species is shown as peaks. The highest peaks correspond to exons (black boxes), while no peaks are observed for the majority of the introns (lines with arrow heads). Peaks of conservation were identified in intron 20 (NM_006920), shown in the middle panel. Inspection of the conserved sequences identified an exon-like sequence of 64 bp (bottom panel, sequence highlighted in grey) flanked by 3' and 5' splice sites (underlined sequence), which we refer to as exon 20x. Inclusion of this exon leads to a frameshift and the introduction of a premature termination codon in exon 21 rendering the transcript a target of NMD.

[0015] FIG. 3A depicts confirmation of NMD-inducing exon via cycloheximide treatment. RT-PCR analysis using cytoplasmic RNA from DMSO-treated (CHX-) or cycloheximide-treated (CHX+) Neuro 2A (mouse neural progenitor cells) and primers in exon 21 and a downstream exon confirmed the presence of a band corresponding to the NMD-inducing exon (21x). The identity of the product was confirmed by sequencing. Densitometry analysis of the bands was performed to calculate percent exon 21x inclusion of total *SCN1A* transcript. Treatment of Neuro 2A with cycloheximide (CHX+) to inhibit NMD led to a 2-fold increase of the product corresponding to the NMD-inducing exon 21x in the cytoplasmic fraction (cf. light grey bar, CHX-, to dark grey bar, CHX+).

[0016] FIG. 3B depicts confirmation of NMD-inducing exon via cycloheximide treatment. RT-PCR analysis using cytoplasmic RNA from DMSO-treated (CHX-) or cycloheximide-treated (CHX+) RenCell VM (human neural progenitor cells) and primers in exon 20 and exon 23 confirmed the presence of a band corresponding to the NMD-inducing exon (20x). The identity of the product was confirmed by sequencing. Densitometry analysis of the bands was performed to calculate percent exon 20x inclusion of total *SCN1A* transcript. Treatment of RenCell VM with cycloheximide (CHX+) to inhibit NMD led to a 2-fold increase of the product corresponding to the NMD-inducing exon 20x in the cytoplasmic fraction (cf. light grey bar, CHX-, to dark grey bar, CHX+).

[0017] FIG. 4. depicts an exemplary graphic representation of an ASO walk performed for SCN1A exon 20x region targeting two indicated regions (region 1 and region 2) upstream of the 3' splice site of exon 20x and two indicated regions (region 3 and region 4) downstream of the 5' splice site of exon 20x. ASOs were designed to cover these regions by shifting 5 nucleotides at a time.

[0018] FIG. 5A depicts SCN1A exon 20x region ASOs selected from an extended ASO walk evaluated by RT-PCR. A representative PAGE shows SYBR-safe-stained RT-PCR products of SCN1A mock-treated, control ASO treated (NT), *SCN1A* exon 20x region ASOs from an extended walk in RenCells via nucleofection at 1uM for 24 hrs. Mock = No ASO; control NT = non-targeting control; Posctrl = positive control.

[0019] FIG. 5B depicts a graph plotting the percent exon 20x inclusion from the data in FIG. 5A.

[0020] FIG. 5C depicts a graph of qPCR results of an extended ASO walk using the samples of FIG. 5A normalized to RPL32 internal control and the fold-change of SCN1A mRNA relative to mock is plotted.

DETAILED DESCRIPTION OF THE INVENTION

Splicing and Nonsense-mediated mRNA Decay

[0021] Intervening 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.

[0022] 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'ss, 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.

[0023] 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.

[0024] 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.

[0025] A NMD-inducing exon (NIE) 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 the constitutive splicing events, the intron containing an NIE is usually spliced out, but the intron or a portion thereof (e.g. NIE) can be retained during alternative or aberrant splicing events. Mature mRNA transcripts containing such an NIE can be non-productive due to frame shift which induce NMD pathway. Inclusion of a NIE in mature RNA transcripts can downregulate gene expression. mRNA transcripts containing an NIE can be referred as "NIE containing mRNA" or "NMD exon mRNA" in the current disclosure.

[0026] Cryptic (or pseudo- splice sites) have the same splicing recognition sequences as genuine splice sites but are not used in the 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.

[0027] 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,(http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2095810/pdf/gkm680.pdf)

[0028] The cryptic splice sites or splicing regulatory sequences may compete for RNA-binding proteins such as U2AF with a splice site of the NIE. In one embodiment, an agent may bind to the cryptic splice site or splicing regulatory sequences to prevent the binding of RNA-binding proteins and thereby favoring utilization of the NIE splice sites.

[0029] In one embodiment, the cryptic splice site may not comprise the 5' or 3' splice site of the NIE. The cryptic splice site may be at least 10 nucleotides upstream of the NIE 5' splice site. The cryptic splice site may be at least 20 nucleotides upstream of the NIE 5' splice site. The cryptic splice site may be at least 50 nucleotides upstream of the NIE 5' splice site. The cryptic splice site may be at least 100 nucleotides upstream of the NIE 5' splice site. The cryptic splice site may be at least 200 nucleotides upstream of the NIE 5' splice site.

[0030] The cryptic splice site may be at least 10 nucleotides downstream of the NIE 3' splice site. The cryptic splice site may be at least 20 nucleotides downstream of the NIE 3' splice site. The cryptic splice site may be at least 50 nucleotides downstream of the NIE 3' splice site. The cryptic splice site

may be at least 100 nucleotides downstream of the NIE 3' splice site. The cryptic splice site may be at least 200 nucleotides downstream of the NIE 3' splice site.

Target Transcripts

[0031] In some embodiments, the methods of the present disclosure exploit the presence of NIE in the pre-mRNA transcribed from the *SCN1A* gene. Splicing of the identified *SCN1A* NIE pre-mRNA species to produce functional mature *SCN1A* mRNA can be induced using a therapeutic agent such as an ASO that stimulates exon skipping of an NIE. Induction of exon skipping can result in inhibition of an NMD pathway. The resulting mature *SCN1A* mRNA can be translated normally without activating NMD pathway, thereby increasing the amount of SCN1A protein in the patient's cells and alleviating symptoms of a condition associated with SCN1A deficiency, such as Dravet Syndrome (DS); Epilepsy, generalized, with febrile seizures plus, type 2; Febrile seizures, familial, 3A; Autism; Epileptic encephalopathy, early infantile, 13; Sick sinus syndrome 1; Alzheimer's disease; or SUDEP.

[0032] In various embodiments, the present disclosure provides a therapeutic agent which can target *SCN1A* mRNA transcripts to modulate, e.g., enhance or inhibit, 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 *SCN1A* pre-mRNA can be targeted by a therapeutic agent, such as an ASO. In some embodiments, the ASO targets a *SCN1A* pre-mRNA transcript containing an NIE. In some embodiments, the ASO targets a sequence within an NIE of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence upstream (or 5') from the 5' end of an NIE (3'ss) of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence downstream (or 3') from the 3' end of an NIE (5'ss) of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking on the 5' end of the NIE of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking the 3' end of the NIE of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising an NIE-intron boundary of a *SCN1A* pre-mRNA transcript. An NIE-intron boundary can refer to the junction of an intron sequence and an NIE region. The intron sequence can flank the 5' end of the NIE, or the 3' end of the NIE. In some embodiments, the ASO targets a sequence within an exon of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence within an intron of a *SCN1A* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising both a portion of an intron and a portion of an exon.

[0033] In some embodiments, a therapeutic agent described herein modulates binding of a factor involved in splicing of the NMD exon mRNA.

[0034] In some embodiments, a therapeutic agent described herein interferes with binding of a factor involved in splicing of the NMD exon mRNA.

[0035] In some embodiments, a therapeutic agent described herein prevents binding of a factor involved in splicing of the NMD exon mRNA.

[0036] In some embodiments, a therapeutic agent targets a targeted portion located in an intronic region between two canonical exonic regions of the NMD exon mRNA encoding SCN1A, and wherein the intronic region contains the NMD exon.

[0037] In some embodiments, a therapeutic agent targets a targeted portion at least partially overlaps with the NMD exon.

[0038] In some embodiments, a therapeutic agent targets a targeted portion that is at least partially overlaps with an intron upstream of the NMD exon.

[0039] In some embodiments, a therapeutic agent targets a targeted portion within the NMD exon.

[0040] In some embodiments, a therapeutic agent targets a targeted portion comprising at least 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. In some embodiments, a therapeutic agent targets a targeted portion comprising at most 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. In some embodiments, a therapeutic agent targets a targeted portion comprising 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.

[0041] In some embodiments, a therapeutic agent targets a targeted portion proximal to the NMD exon.

[0042] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides downstream from the 5' end of the intron comprising the NIE. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, downstream from the 5' end of the intron comprising the NIE. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides downstream from the 5' end of the intron comprising the NIE.

[0043] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200 nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides downstream from the 5' end of the intron comprising the NIE.

[0044] In some embodiments, the ASO targets a sequence from about 1 to about 2000 nucleotides upstream (or 5') from the 5' end of the NIE. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, or about 1950 to about 2000 nucleotides upstream (or 5') from the 5' end of the NIE. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides upstream (or 5') from the 5' end of the NIE.

[0045] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200 nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, or at least about 2000 nucleotides upstream (or 5') from the 5' end of the NIE.

[0046] In some embodiments, the ASO targets a sequence from about 1 to about 500 nucleotides downstream from the 5' end of the NIE. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, or at least about 500 nucleotides downstream from the 5' end of the NIE region.

[0047] In some embodiments, the ASO targets a sequence from about 1 to about 500 nucleotides upstream from the 3' end of the NIE. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides,

at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, or at least about 500 nucleotides upstream from the 3' end of the NIE region.

[0048] In some embodiments, the ASO targets a sequence from about 1 to about 2000 nucleotides downstream (or 3') from the 3' end of the NIE. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, or about 1950 to about 2000 nucleotides downstream (or 3') from the 3' end of the NIE. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides downstream (or 3') from the 3' end of the NIE.

[0049] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200

nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, or at least about 2000 nucleotides downstream (or 3') from the 3' end of the NIE.

[0050] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides upstream from the 3' end of the intron comprising the NIE. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, upstream from the 3' end of the intron comprising the NIE. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides upstream from the 3' end of the intron comprising the NIE.

[0051] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200 nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600

nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides upstream from the 3' end of the intron comprising the NIE.

[0052] In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from the 5' end of the NIE. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, or about 1450 to about 1500 nucleotides upstream (or 5') from the 5' end of the NIE region. In some embodiments, the ASO may target a sequence more than 300 nucleotides upstream from the 5' end of the NIE. In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides downstream (or 3') from the 3' end of the NIE. 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, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, or about 1450 to about 1500 nucleotides downstream from the 3' end of the NIE. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from the 3' end of the NIE.

[0053] In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from the 5' end of the NIE. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300

nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, or at least about 1000 nucleotides upstream (or 5') from the 5' end of the NIE region. In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides downstream (or 3') from the 3' end of the NIE. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, or at least about 1000 nucleotides downstream from the 3' end of the NIE. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from the 3' end of the NIE.

[0054] In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from the 5' end of the NIE. In some embodiments, the ASO targets a sequence at most about 10 nucleotides, at most about 20 nucleotides, at most about 50 nucleotides, at most about 80 nucleotides, at most about 85 nucleotides, at most about 90 nucleotides, at most about 95 nucleotides, at most about 96 nucleotides, at most about 97 nucleotides, at most about 98 nucleotides, at most about 99 nucleotides, at most about 100 nucleotides, at most about 101 nucleotides, at most about 102 nucleotides, at most about 103 nucleotides, at most about 104 nucleotides, at most about 105 nucleotides, at most about 110 nucleotides, at most about 120 nucleotides, at most about 150 nucleotides, at most about 200 nucleotides, at most about 300 nucleotides, at most about 400 nucleotides, at most about 500 nucleotides, at most about 600 nucleotides, at most about 700 nucleotides, at most about 800 nucleotides, at most about 900 nucleotides, at most about 1000 nucleotides, at most about 1100 nucleotides, at most about 1200 nucleotides, at most about 1300 nucleotides, at most about 1400 nucleotides, or at most about 1500 nucleotides upstream (or 5') from the 5' end of the NIE region. In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides downstream (or 3') from the 3' end of the NIE. In some embodiments, the ASO targets a sequence at most about 10 nucleotides, at most about 20 nucleotides, at most about 50 nucleotides, at most about 80 nucleotides, at most about 85 nucleotides, at most about 90 nucleotides, at most about 95 nucleotides, at most about 96 nucleotides, at most about 97 nucleotides, at most about 98

nucleotides, at most about 99 nucleotides, at most about 100 nucleotides, at most about 101 nucleotides, at most about 102 nucleotides, at most about 103 nucleotides, at most about 104 nucleotides, at most about 105 nucleotides, at most about 110 nucleotides, at most about 120 nucleotides, at most about 150 nucleotides, at most about 200 nucleotides, at most about 300 nucleotides, at most about 400 nucleotides, at most about 500 nucleotides, at most about 600 nucleotides, at most about 700 nucleotides, at most about 800 nucleotides, at most about 900 nucleotides, or at most about 1000 nucleotides, at most about 1100 nucleotides, at most about 1200 nucleotides, at most about 1300 nucleotides, at most about 1400 nucleotides, or at most about 1500 nucleotides downstream from the 3' end of the NIE. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from the 3' end of the NIE.

[0055] In some embodiments, the NIE (exon 23) as described herein is located between GRCh38/hg38: chr2: 166007230 and chr2: 166007293. In some embodiments, the 5' end of the NIE is located at GRCh38/hg38: chr2: 166007230. In some embodiments, the 3' end of the NIE is located at GRCh38/hg38: chr2: 166007293.

[0056] In some embodiments, the ASO targets a sequence from about 1 to about 2000 nucleotides upstream (or 5') from genomic site GRCh38/hg38: chr2: 166007230. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, or about 1950 to about 2000 nucleotides upstream (or 5') from genomic site GRCh38/hg38: chr2: 166007230. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides upstream (or 5') from genomic site GRCh38/hg38: chr2: 166007230.

[0057] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200 nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, or at least about 2000 nucleotides upstream (or 5') from genomic site GRCh38/hg38: chr2: 166007230.

[0058] In some embodiments, the ASO targets a sequence from about 1 to about 500 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166007230. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, or at least about 500 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166007230.

[0059] In some embodiments, the ASO targets a sequence from about 1 to about 500 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166007293. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300

nucleotides, at least about 400 nucleotides, or at least about 500 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166007293.

[0060] In some embodiments, the ASO targets a sequence from about 1 to about 2000 nucleotides downstream (or 3') from genomic site GRCh38/hg38: chr2: 166007293. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, or about 1950 to about 2000 nucleotides downstream (or 3') from genomic site GRCh38/hg38: chr2: 166007293. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides downstream (or 3') from genomic site GRCh38/hg38: chr2: 166007293.

[0061] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200 nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, or at least about 2000 nucleotides downstream (or 3') from genomic site GRCh38/hg38: chr2: 166007293.

[0062] In some embodiments, the intron comprising the NIE is located between GRCh38/hg38: chr2: 166002754 and chr2: 166009718. In some embodiments, the 5' end of the intron comprising the NIE is located at GRCh38/hg38: chr2: 166002754. In some embodiments, the 3' end of the intron comprising the NIE is located at GRCh38/hg38: chr2: 166009718.

[0063] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166002754. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, downstream from genomic site GRCh38/hg38: chr2: 166002754. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166002754.

[0064] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200

nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166002754.

[0065] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166007229. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, upstream from genomic site GRCh38/hg38: chr2: 166007229. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166007229.

[0066] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200

nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166007229.

[0067] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166007294. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, downstream from genomic site GRCh38/hg38: chr2: 166007294. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166007294.

[0068] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200

nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides downstream from genomic site GRCh38/hg38: chr2: 166007294.

[0069] In some embodiments, the ASO targets a sequence from about 1 to about 5000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166009718. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, about 1450 to about 1500 nucleotides, about 1550 to about 1600 nucleotides, about 1650 to about 1700 nucleotides, about 1750 to about 1800 nucleotides, about 1850 to about 1900 nucleotides, about 1950 to about 2000 nucleotides, about 2000 to about 3000 nucleotides, about 3000 to about 4000 nucleotides, or about 4000 to about 5000 nucleotides, upstream from genomic site GRCh38/hg38: chr2: 166009718. In some embodiments, the ASO targets a sequence from 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, or about 950 to about 1000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166009718.

[0070] In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least about 1200

nucleotides, at least about 1400 nucleotides, at least about 1500 nucleotides, at least about 1600 nucleotides, at least about 1800 nucleotides, at least about 2000 nucleotides, at least about 3000 nucleotides, at least about 4000 nucleotides, or at least about 5000 nucleotides upstream from genomic site GRCh38/hg38: chr2: 166009718.

[0071] In some embodiments, the NIE as described herein is located between GRCh37/hg19: chr2:166,863,740 and GRCh37/hg19: chr2:166,863,803, as depicted in **FIG. 2**. In some embodiments, the 5' end of the NIE is located at GRCh37/hg19: chr2:166,863,803. In some embodiments, the 3' end of the NIE is located at GRCh37/hg19: chr2:166,863,740.

[0072] In some embodiments, In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. 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, about 250 to about 300, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, or about 1450 to about 1500 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO may target a sequence more than 300 nucleotides upstream from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides downstream (or 3') from GRCh37/hg19: chr2:166,863,740. 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, about 250 to about 300 nucleotides, about 350 to about 400 nucleotides, about 450 to about 500 nucleotides, about 550 to about 600 nucleotides, about 650 to about 700 nucleotides, about 750 to about 800 nucleotides, about 850 to about 900 nucleotides, about 950 to about 1000 nucleotides, about 1050 to about 1100 nucleotides, about 1150 to about 1200 nucleotides, about 1250 to about 1300 nucleotides, about 1350 to about 1400 nucleotides, or about 1450 to about 1500 nucleotides downstream from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from GRCh37/hg19: chr2:166,863,740.

[0073] In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20

nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, or at least about 1000 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides downstream (or 3') from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence at least about 1 nucleotide, at least about 10 nucleotides, at least about 20 nucleotides, at least about 50 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 96 nucleotides, at least about 97 nucleotides, at least about 98 nucleotides, at least about 99 nucleotides, at least about 100 nucleotides, at least about 101 nucleotides, at least about 102 nucleotides, at least about 103 nucleotides, at least about 104 nucleotides, at least about 105 nucleotides, at least about 110 nucleotides, at least about 120 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, at least about 300 nucleotides, at least about 400 nucleotides, at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, or at least about 1000 nucleotides downstream from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from GRCh37/hg19: chr2:166,863,740.

[0074] In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence at most about 10 nucleotides, at most about 20 nucleotides, at most about 50 nucleotides, at most about 80 nucleotides, at most about 85 nucleotides, at most about 90 nucleotides, at most about 95 nucleotides, at most about 96 nucleotides, at most about 97 nucleotides, at most about 98 nucleotides, at most about 99 nucleotides, at most about 100 nucleotides, at most about 101 nucleotides, at most about 102 nucleotides, at most about 103 nucleotides, at most about 104 nucleotides, at most about 105 nucleotides, at most about 110 nucleotides, at most about 120 nucleotides, at most about 150 nucleotides, at most about 200 nucleotides, at most about 300 nucleotides, at most about 400 nucleotides, at most about 500 nucleotides, at most about 600 nucleotides, at most about 700 nucleotides, at most about 800 nucleotides, at most about 900

nucleotides, at most about 1000 nucleotides, at most about 1100 nucleotides, at most about 1200 nucleotides, at most about 1300 nucleotides, at most about 1400 nucleotides, or at most about 1500 nucleotides upstream (or 5') from genomic site GRCh37/hg19: chr2:166,863,803. In some embodiments, the ASO targets a sequence from about 4 to about 300 nucleotides downstream (or 3') from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence at most about 10 nucleotides, at most about 20 nucleotides, at most about 50 nucleotides, at most about 80 nucleotides, at most about 85 nucleotides, at most about 90 nucleotides, at most about 95 nucleotides, at most about 96 nucleotides, at most about 97 nucleotides, at most about 98 nucleotides, at most about 99 nucleotides, at most about 100 nucleotides, at most about 101 nucleotides, at most about 102 nucleotides, at most about 103 nucleotides, at most about 104 nucleotides, at most about 105 nucleotides, at most about 110 nucleotides, at most about 120 nucleotides, at most about 150 nucleotides, at most about 200 nucleotides, at most about 300 nucleotides, at most about 400 nucleotides, at most about 500 nucleotides, at most about 600 nucleotides, at most about 700 nucleotides, at most about 800 nucleotides, at most about 900 nucleotides, or at most about 1000 nucleotides, at most about 1100 nucleotides, at most about 1200 nucleotides, at most about 1300 nucleotides, at most about 1400 nucleotides, or at most about 1500 nucleotides downstream from GRCh37/hg19: chr2:166,863,740. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from GRCh37/hg19: chr2:166,863,740.

[0075] As described herein in the Examples, the *SCN1A* gene (**SEQ ID NO. 1**) was analyzed for NIE and inclusion of a portion of intron 20 (see **SEQ ID NO. 6** which encodes the Intron 20 pre-mRNA) (this portion is referred as Exon 23 or Exon 20x throughout the present disclosure) was observed. In some embodiments, the ASOs disclosed herein target a NIE containing pre-mRNA (**SEQ ID NO. 2**) transcribed from a *SCN1A* genomic sequence. In some embodiments, the ASO targets a NIE containing pre-mRNA transcript from a *SCN1A* genomic sequence comprising a portion of intron 20. In some embodiments, the ASO targets a NIE containing pre-mRNA transcript from a *SCN1A* genomic sequence comprising exon 23 (or exon 20x) (**SEQ ID NO. 4**). In some embodiments, the ASO targets a NIE containing pre-mRNA transcript of **SEQ ID NO. 2 or 9**. In some embodiments, the ASO targets a NIE containing pre-mRNA transcript of **SEQ ID NO. 2 or 9** comprising an NIE. In some embodiments, the ASO targets a NIE containing pre-mRNA transcript of **SEQ ID NO. 2** comprising exon 23 (or exon 20x) (**SEQ ID NO.** 7). In some embodiments, the ASOs disclosed herein target a *SCN1A* pre-mRNA sequence (**SEQ ID NO. 2 or 9**). In some embodiments, the ASO targets a *SCN1A* pre-mRNA sequence comprising an NIE (**SEQ ID NO. 7 or 11**). In some embodiments, the ASO targets a *SCN1A* pre-mRNA sequence according to any one of **SEQ ID NOs: 6, 7, 10, or 11**. In some embodiments, the ASO has a sequence according to any one of **SEQ ID NOs: 12-731**. In some

embodiments, the ASO has a sequence according to any one of **SEQ ID NOs: 12-371**. In some embodiments, the ASO has a sequence according to any one of **SEQ ID NOs: 372-731.**

[0076] In some embodiments, the *SCN1A* NIE 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 or 8**. In some embodiments, the *SCN1A* NIE 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.: 2-7 and 9-11**.

[0077] In some embodiments, the *SCN1A* NIE 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, 6, 7, 9, 10, and 12**. In some embodiments, *SCN1A* NIE 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 **SEQ ID NOs: 1 and 8**. 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 **SEQ ID NOs: 2, 6, 7, 9, 10, and 12**.

[0078] In some embodiments, the ASO targets a sequence upstream from the 5' end of an NIE. For example, ASOs targeting a sequence upstream from the 5' end of an NIE (e.g. exon 23 (or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 12-191 or 372-551**. For another example, ASOs targeting a sequence upstream from the 5' end of an NIE (e.g. exon 23 (or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 12-191**. For an additional example, ASOs targeting a sequence upstream from the 5' end of an NIE (e.g. exon 23 (or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 372-551**.

[0079] In some embodiments, the ASO targets exon 23 (or exon 20x) in a *SCN1A* NIE containing pre-mRNA comprising exon 23. In some embodiments, the ASO targets an exon 23 sequence downstream (or 3') from the 5' end of the exon 23 of a SCN1A pre-mRNA. In some embodiments, the ASO targets an exon 23 sequence upstream (or 5') from the 3' end of the exon 20x of a *SCN1A* pre-mRNA.

[0080] In some embodiments, the ASO targets a sequence downstream from the 3' end of an NIE. For example, ASOs targeting a sequence downstream from the 3' end of an NIE (e.g. exon 23 (or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 192-371 or 552-731.** For another example, ASOs targeting a sequence downstream from the 3' end of an NIE (e.g. exon 23

(or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 192-371**. For an additional example, ASOs targeting a sequence downstream from the 3' end of an NIE (e.g. exon 23 (or exon 20x) in human *SCN1A*, or exon 21x in mouse *SCN1A*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of **SEQ ID NOs: 552-731**.

[0081] In some embodiments, the targeted portion of the *SCN1A* NIE 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, or 25 (intron numbering corresponding to the mRNA sequence at NM_006920). In some embodiments, hybridization of an ASO to the targeted portion of the NIE pre-mRNA results in exon skipping of at least one of NIE 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, or 25, and subsequently increases SCN1A protein production. In some embodiments, hybridization of an ASO to the targeted portion of the NIE pre-mRNA inhibits or blocks exon skipping of at least one of NIE 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, or 25, and subsequently decreases SCN1A protein production. In some embodiments, the targeted portion of the *SCN1A* NIE containing pre-mRNA is in intron 20. One of skill in the art can determine the corresponding intron number in any isoform based on an intron sequence provided herein or using the number provided in reference to the mRNA sequence at NM_006920, NM_001202435, NM_001165964, or NM_001165963. One of skill in the art also can determine the sequences of flanking exons in any *SCN1A* isoform for targeting using the methods of the invention, based on an intron sequence provided herein or using the intron number provided in reference to the mRNA sequence at NM_006920, NM_001202435, NM_001165964, or NM_001165963.

[0082] In some embodiments, the methods and compositions of the present disclosure are used to modulate, e.g., increase or decrease, the expression of SCN1A by inducing or inhibiting exon skipping of a pseudo-exon of an *SCN1A* NIE containing pre-mRNA. In some embodiments, the pseudo-exon is a sequence within any of introns 1-25. In some embodiments, the pseudo-exon is a sequence within any of introns 2, 4, 6, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, and 25. In some embodiments, the pseudo-exon is a sequence within any of introns 15, 18, and 19. In some embodiments, the pseudo-exon can be any *SCN1A* intron or a portion thereof. In some embodiments, the pseudo-exon is within intron 20. The *SCN1A* intron numbering used herein corresponds to the mRNA sequence at NM_006920. It is understood that the intron numbering may change in reference to a different *SCN1A* isoform sequence.

SCN1A Protein

[0083] The *SCN1A* gene can encode SCN1A (sodium channel, voltage-gated, type I, alpha subunit) protein, which can also be referred to as alpha-subunit of voltage-gated sodium channel $Na_v1.1$. Also

described above, SCN1A mutations in DS are spread across the entire protein. More than 100 novel mutations have been identified throughout the gene with the more debilitating arising de novo. These comprise of truncations (47%), missense (43%), deletions (3%), and splice site mutations (7%). The percentage of subjects carrying SCN1A mutations varies between 33 and 100%. The majority of mutations are novel changes (88%).

[0084] In some embodiments, the methods described herein are used to modulate, e.g., increase or decrease, the production of a functional SCN1A protein. As used herein, the term "functional" refers to the amount of activity or function of a SCN1A protein that is necessary to eliminate any one or more symptoms of a treated condition, e.g., Dravet syndrome; Epilepsy, generalized, with febrile seizures plus, type 2; Febrile seizures, familial, 3A; Autism; Epileptic encephalopathy, early infantile, 13; Sick sinus syndrome 1; Alzheimer's disease; or SUDEP. In some embodiments, the methods are used to increase the production of a partially functional SCN1A protein. As used herein, the term "partially functional" refers to any amount of activity or function of the SCN1A protein 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.

[0085] In some embodiments, the method is a method of increasing the expression of the SCN1A protein by cells of a subject having a NIE containing pre-mRNA encoding the SCN1A protein, wherein the subject has Dravet syndrome caused by a deficient amount of activity of SCN1A protein, and wherein the deficient amount of the SCN1A protein is caused by haploinsufficiency of the SCN1A protein. In such an embodiment, the subject has a first allele encoding a functional SCN1A protein, and a second allele from which the SCN1A protein is not produced. In another such embodiment, the subject has a first allele encoding a functional SCN1A protein, and a second allele encoding a nonfunctional SCN1A protein. In another such embodiment, the subject has a first allele encoding a functional SCN1A protein, and a second allele encoding a partially functional SCN1A protein. In any of these embodiments, the antisense oligomer binds to a targeted portion of the NIE containing 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 SCN1A protein, and an increase in the expression of the SCN1A protein in the cells of the subject.

[0086] 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 is used to increase the expression of SCN1A protein in cells of a subject having a NIE containing pre-mRNA encoding SCN1A protein,

wherein the subject has a deficiency, e.g., Dravet Syndrome (DS) (also known as SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; early infantile SCN1A encephalopathy; early infantile epileptic encephalopathy (EIEE); or autism, in the amount or function of a SCN1A protein. In some embodiments, an ASO is used to increase the expression of SCN1A protein in cells of a subject, wherein the subject has a deficiency, e.g., Epileptic encephalopathy, early infantile, 13; in the amount or function of a SCN8A protein. In some embodiments, an ASO is used to increase the expression of SCN1A protein in cells of a subject, wherein the subject has a deficiency, e.g., Sick sinus syndrome 1; in the amount or function of a SCN5A protein.

[0087] In some embodiments, the NIE containing pre-mRNA transcript that encodes the protein that is causative of the disease or condition is targeted by the ASOs described herein. In some embodiments, a NIE 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 NIE containing 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).

[0088] In some embodiments, the subject has:

(a) a first mutant allele from which

(i) the SCN1A protein is produced at a reduced level compared to production from a wild-type allele,

(ii) the SCN1A protein is produced in a form having reduced function compared to an equivalent wild-type protein, or

(iii) the SCN1A protein or functional RNA is not produced; and

(b) a second mutant allele from which

(i) the SCN1A protein is produced at a reduced level compared to production from a wild-type allele,

(ii) the SCN1A protein is produced in a form having reduced function compared to an equivalent wild-type protein, or

(iii) the SCN1A protein is not produced, and

wherein the NIE 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 NIE containing pre-mRNA transcribed from the first allele or the second allele, thereby inducing exon skipping of the pseudo-exon from the NIE containing pre-mRNA, and causing an increase in the level of mRNA encoding SCN1A 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 NIE containing pre-mRNA is 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).

[0089] In some embodiments, the level of mRNA encoding SCN1A protein is increased 1.1 to 10-fold, when compared to the amount of mRNA encoding SCN1A 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 *SCN1A* NIE containing pre-mRNA.

[0090] In some embodiments, a subject treated using the methods of the present disclosure expresses a partially functional SCN1A protein from one allele, wherein the partially functional SCN1A protein is 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 invention expresses a nonfunctional SCN1A protein from one allele, wherein the nonfunctional SCN1A protein is 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 invention has a *SCN1A* whole gene deletion, in one allele.

[0091] In some embodiments, the method is a method of decreasing the expression of the SCN1A protein by cells of a subject having a NIE containing pre-mRNA encoding the SCN1A protein, and wherein the subject has a gain-of-function mutation in $Na_v1.1$. In such an embodiment, the subject has an allele from which the SCN1A protein is produced in an elevated amount or an allele encoding a mutant SCN1A that induces increased activity of $Na_v1.1$ in the cell. In some embodiments, the increased activity of $Na_v1.1$ is characterized by a prolonged or near persistent sodium current mediated by the mutant $Na_v1.1$ channel, a slowing of fast inactivation, a positive shift in steady-state inactivation, higher channel availability during repetitive stimulation, increased non-inactivated depolarization-induced persistent sodium currents, delayed entry into inactivation, accelerated recovery from fast inactivation, and/or rescue of folding defects by incubation at lower temperature or co-expression of interacting proteins. In any of these embodiments, the antisense oligomer binds to a targeted portion of the NIE containing pre-mRNA transcribed from the second allele, thereby inhibiting or blocking exon skipping of the pseudo-exon from the pre-mRNA, and causing a decrease

in the level of mature mRNA encoding functional SCN1A protein, and a decrease in the expression of the SCN1A protein in the cells of the subject.

[0092] In related embodiments, the method is a method of using an ASO to decrease the expression of a protein or functional RNA. In some embodiments, an ASO is used to decrease the expression of SCN1A protein in cells of a subject having a NIE containing pre-mRNA encoding SCN1A protein. In some embodiments, the subject has a gain-of-function mutation in $Na_v1.1$, e.g., migraine. In some embodiments, an ASO is used to decrease the expression of SCN1A protein in cells of a subject, the subject has a gain-of-function mutation in $Na_v1.1$, e.g., migraine, familial hemiplegic, 3.

[0093] In some embodiments, the level of mRNA encoding SCN1A protein is decreased 1.1 to 10-fold, when compared to the amount of mRNA encoding SCN1A 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 *SCN1A* NIE containing pre-mRNA.

[0094] In some embodiments, a subject treated using the methods of the present disclosure expresses a mutant SCN1A protein from one allele, wherein the mutant SCN1A protein is caused by a frameshift mutation, a nonsense mutation, a missense mutation, or a partial gene deletion, and wherein the mutant SCN1A protein causes an elevated activity level of $Na_v1.1$. In some embodiments, a subject treated using the methods of the present disclosure expresses an elevated amount of SCN1A protein from one allele due to a frameshift mutation, a nonsense mutation, a missense mutation, or a partial gene deletion.

[0095] In embodiments of the present invention, a subject can have a mutation in *SCN1A*. Mutations in *SCN1A* can be spread throughout said gene. SCN1A protein can consist of four domains. Said SCN1A domains can have transmembrane segments. Mutations in said SCN1A protein may arise throughout said protein. Said SCN1A protein may consist of at least two isoforms. Mutations in SCN1A may comprise of R931C, R946C, M934I, R1648C, or R1648H. In some cases, mutations may be observed in a C-terminus of a SCN1A protein. Mutations in a SCN1A protein may also be found in loops between segments 5 and 6 of the first three domains of said SCN1A protein. In some cases, mutations may be observed in an N-terminus of a SCN1A protein. Exemplary mutations within SCN1A include, but are not limited to, R222X, R712X, I227S, R1892X, W952X, R1245X, R1407X, W1434R, c.4338+1G>A, S1516X, L1670fsX1678, or K1846fsX1856. Mutations that can be targeted with the present invention may also encode a pore of an ion channel.

[0096] In some embodiments, the methods and compositions described herein can be used to treat DS. In other embodiments, the methods and compositions described herein can be used to treat severe myclonic epilepsy of infancy (SMEI). In other embodiments, the methods and compositions described herein can be used to treat borderline Dravet syndrome; Epilepsy, generalized, with febrile seizures

plus, type 2; Febrile seizures, familial, 3A; Migraine, familial hemiplegic, 3; Autism; Epileptic encephalopathy, early infantile, 13; Sick sinus syndrome 1; Alzheimer's disease or SUDEP. The methods and compositions described herein can also be used to treat borderline SMEI. Additionally, the methods and compositions described herein can be used to treat generalized epilepsy with febrile seizures plus (GEFS+). GEFS+ may be associated with mutations in epilepsy-associated ion channel subunits such as SCN1B or GABRG2. The methods and compositions described herein can also be used to treat sodium channelopathies. Sodium channelopathies may be associated with mutations in SCN1A. Sodium channelopathies may also be associated with subunits of SCN1A, such as the beta subunit, SCN1B. In some cases, additional diseases associated with SCN1A mutations may also be treated with the present disclosure. Related SCN1A diseases associated with SCN1A mutations include, but are not limited to, atypical myotonia congenita, hyperkalemic periodic paralysis, and paramyotonia congenita.

[0097] In some embodiments, a subject having any SCN1A mutation known in the art and described in the literature referenced above (*e.g.*, by Hamdan, *et al.*, 2009, Mulley, *et al.*, 2005) can be treated using the methods and compositions described herein. In some embodiments, the mutation is within any *SCN1A* intron or exon.

Exon Inclusion

[0098] As used herein, a "NIE 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. NIE containing mature mRNA may sometimes lead to aberrant protein expression.

[0099] In some embodiments, the included pseudo-exon is the most abundant pseudo-exon in a population of NIE 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 NIE containing pre-mRNAs transcribed from the gene encoding the target protein in a cell, wherein the population of NIE 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 NIE 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 embodiments, the targeted region is in a pseudo-exon that is the most abundant pseudo-exon in a NIE containing pre-mRNA encoding the SCN1A protein.

[00100] 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.

[00101] 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.

[00102] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of a *SCN1A* pre-mRNA transcript results in an increase in the amount of SCN1A 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 SCN1A 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.

[00103] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of a *SCN1A* pre-mRNA transcript results in a decrease in the amount of SCN1A 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 SCN1A protein produced by the cell to which the antisense oligomer is contacted is decreased 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.

[00104] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of a *SCN1A* pre-mRNA transcript results in an increase in the amount of mRNA encoding SCN1A, including the mature mRNA encoding the target protein. In some embodiments, the amount of mRNA encoding SCN1A protein, or the mature mRNA encoding the SCN1A 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 SCN1A protein, or the mature mRNA encoding SCN1A 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 SCN1A NIE containing pre-mRNA.

[00105] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of a *SCN1A* pre-mRNA transcript results in a decrease in the amount of mRNA encoding SCN1A, including the mature mRNA encoding the target protein. In some embodiments, the amount of mRNA encoding SCN1A protein, or the mature mRNA encoding the SCN1A protein, is decreased 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 SCN1A protein, or the mature mRNA encoding SCN1A protein produced in the cell to which the antisense oligomer is contacted is decreased 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 SCN1A NIE containing pre-mRNA.

[00106] The NIE can be in any length. In some embodiments, the NIE comprises a full sequence of an intron, in which case, it can be referred to as intron retention. In some embodiments, the NIE can be a portion of the intron. In some embodiments, the NIE can be a 5' end portion of an intron including a 5'ss sequence. In some embodiments, the NIE can be a 3' end portion of an intron including a 3'ss sequence. In some embodiments, the NIE can be a portion within an intron without inclusion of a 5'ss sequence. In some embodiments, the NIE can be a portion within an intron without inclusion of a 3'ss sequence. In some embodiments, the NIE can be a portion within an intron without inclusion of either a 5'ss or a 3'ss sequence. In some embodiments, the NIE 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 NIE 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 nucleoids, 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 NIE 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 NIE may be longer than 1,000 nucleotides in length.

[00107] 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 NIE can be non-productive mRNA transcript which does not lead to protein expression. The PIC can be present in any position downstream of an NIE. In some embodiments, the PIC can be present in any exon downstream of an NIE. In some embodiments, the PIC can be present within the NIE. For example, inclusion of exon 20x in an mRNA transcript encoded by the *SCN1A* gene can induce a PIC in the mRNA transcript, e.g., a PIC in exon 21 of the mRNA transcript.

Therapeutic Agents

[00108] In various embodiments of the present disclosure, compositions and methods comprising a therapeutic agent are provided to modulate protein expression level of SCN1A. In some embodiments, provided herein are compositions and methods to modulate alternative splicing of *SCNA1* pre-mRNA. In some embodiments, provided herein are compositions and methods to induce exon skipping in the splicing of SCN1A pre-mRNA, e.g., to induce skipping of a pseudo-exon during splicing of SCN1A 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.

[00109] In some embodiment, a therapeutic agent disclosed herein is a small molecule, a polypeptide, or a polynucleic acid polymer. In some instances, the therapeutic agent is a small molecule. In some instances, the therapeutic agent is a polypeptide. In some instances, the therapeutic agent is a polynucleic acid polymer. In some cases, the therapeutic agent is a repressor agent. In additional cases, the therapeutic agent is an enhancer agent.

[00110] A therapeutic agent disclosed herein can be a NIE repressor agent. A therapeutic agent may comprise a polynucleic acid polymer.

[00111] According to one aspect of the present disclosure, provided herein is a method of treatment or prevention of a condition associated with a functional-SCN1A protein deficiency, comprising administering a NIE repressor agent to a subject to increase levels of functional SCN1A protein, wherein the agent binds to a region of the pre-mRNA transcript to decrease inclusion of the NIE in the mature transcript. For example, provided herein is a method of treatment or prevention of a condition associated with a functional-SCN1A protein deficiency, comprising administering a NIE repressor agent to a subject to increase levels of functional SCN1A protein, wherein the agent binds to a region of an intron containing an NIE (e.g., intron 20 in human *SCN1A* gene) of the pre-mRNA transcript or to a NIE-activating regulatory sequence in the same intron.

[00112] Where reference is made to reducing NIE 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 NIE inclusion in the subject without treatment, or relative to the amount of NIE inclusion in a population of similar subjects. The reduction/correction may be at least 10% less NIE inclusion relative to the average subject, or the subject prior to treatment. The reduction may be at least 20% less NIE inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 40% less NIE inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 50% less NIE inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 60% less NIE inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 80% less

NIE inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 90% less NIE inclusion relative to an average subject, or the subject prior to treatment.

[00113] Where reference is made to increasing active-SCN1A protein levels, the increase may be clinically significant. The increase may be relative to the level of active-SCN1A protein in the subject without treatment, or relative to the amount of active-SCN1A protein in a population of similar subjects. The increase may be at least 10% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 20% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 40% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 50% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 80% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 100% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 200% more active-SCN1A protein relative to the average subject, or the subject prior to treatment. The increase may be at least 500% more active-SCN1A protein relative to the average subject, or the subject prior to treatment.

[00114] 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.

[00115] 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.

[00116] 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.

[00117] 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.

[00118] The polynucleic acid polymer may have 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: 12-731**. The polynucleic acid polymer may have a sequence with 100% sequence identity to a sequence selected from the group consisting of **SEQ ID NOs: 12-731**. In some instances, the polynucleic acid polymer may have 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: 12-371**. In some cases, the polynucleic acid polymer may have a sequence with 100% sequence identity to a sequence selected from the group consisting of **SEQ ID NOs: 12-371**. In some instances, the polynucleic acid polymer may have 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: 372-731**. In some cases, the

polynucleic acid polymer may have a sequence with 100% sequence identity to a sequence selected from the group consisting of **SEQ ID NOs: 372-731**.

[00119] 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

[00120] Provided herein is a composition comprising an antisense oligomer that induces exon skipping by binding to a targeted portion of a *SCN1A* NIE 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.*, a *SCN1A* NIE 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.

[00121] In some embodiments, ASOs "specifically hybridize" to or are "specific" to a target nucleic acid or a targeted portion of a NIE 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.

[00122] 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).

[00123] 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.

[00124] The ASOs described herein comprise nucleobases that are complementary to nucleobases present in a targeted portion of a NIE 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.

[00125] 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.

[00126] 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 phosphothioate linkage. In some embodiments, the backbone modification is a phosphoramidate linkage.

[00127] In embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is random. In 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 embodiments, an ASO used in the methods of the invention, 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 embodiments, a composition used in the methods of the invention comprises a pure diastereomeric ASO. In embodiments, a composition used in the methods of the invention 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%.

[00128] In 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 embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in **SEQ ID NOs: 12-731**, 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 embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in **SEQ ID NOs: 12-731**, 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.

[00129] In embodiments, an ASO used in the methods of the invention, including, but not limited to, any of the ASOs set forth herein in **SEQ ID NOs: 12-731**, 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 invention, including, but not limited to, any of the ASOs set forth herein in **SEQ ID NOs: 12-731**, 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.

[00130] 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'-guanidinidium, 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-methyloxyethyl (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.

[00131] 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."

[00132] 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.

[00133] 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.

[00134] 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.

[00135] 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."

[00136] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of a *SCN1A* NIE containing pre-mRNA that is downstream (in the 3' direction) of the 5' splice site (or 3' end of the NIE) of the included exon in a *SCN1A* NIE containing 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 *SCN1A* NIE 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 a *SCN1A* NIE containing pre-mRNA that is within the region between nucleotides +6 and +496 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 +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.

[00137] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of a *SCN1A* NIE containing pre-mRNA that is upstream (in the 5' direction) of the 5' splice site (or 3' end) of the included exon in a *SCN1A* NIE 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 *SCN1A* NIE 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 a *SCN1A* NIE containing pre-mRNA that is within the region between nucleotides -1 and -264 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 -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 -50, from about -50 to about -100, from about -100 to about -150, from about -150 to about -200, or from about -200 to about -250 relative to 5' splice site (or 3' end) of the included exon.

[00138] In some embodiments, the ASOs are complementary to a targeted region of a *SCN1A* NIE containing pre-mRNA that is upstream (in the 5' direction) of the 3' splice site (or 5' end) of the included exon in a *SCN1A* NIE containing pre-mRNA (*e.g.*, in the direction designated by negative numbers). In some embodiments, the ASOs are complementary to a targeted portion of the *SCN1A* NIE 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 *SCN1A* NIE containing pre-mRNA that is within the region -1 to -496 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 -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, or about -1 to about -30 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.

[00139] In some embodiments, the ASOs are complementary to a targeted region of a *SCN1A NIE containing* pre-mRNA that is downstream (in the 3' direction) of the 3' splice site (5' end) of the included exon in a *SCN1A NIE 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 *SCN1A NIE containing* pre-mRNA that is within the region of about +1 to about +100 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 +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, about +1 to about +20, or about +1 to about +10 relative to 3' splice site of the included exon.

[00140] In some embodiments, the targeted portion of the *SCN1A* NIE 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 *SCN1A* NIE containing pre-mRNA is within the NIE. In some embodiments, the targeted portion of the *SCN1A* NIE containing pre-mRNA comprises a pseudo-exon and intron boundary.

[00141] 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.

[00142] In some embodiments, two or more ASOs with different chemistries but complementary to the same targeted portion of the NIE containing pre-mRNA are used. In some embodiments, two or more ASOs that are complementary to different targeted portions of the NIE containing pre-mRNA are used.

[00143] In embodiments, the antisense oligonucleotides of the invention 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.

[00144] In some embodiments, the nucleic acid to be targeted by an ASO is a *SCN1A* NIE 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

[00145] 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.

[00146] 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 function 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.

[00147] In 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 invention includes, but is not limited to, a solution, emulsion, microemulsion, foam or liposome-containing formulation (*e.g.*, cationic or noncationic liposomes).

[00148] 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 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 invention 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 embodiments, the penetration enhancers are a surfactant, fatty acid, bile salt, chelating agent, or non-chelating nonsurfactant.

[00149] In 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

[00150] 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. In some embodiments, the one or more other agents are selected from the ASOs listed in **Table 4**.

Treatment of Subjects

[00151] 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*.

[00152] 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).

[00153] Suitable routes for administration of ASOs of the present invention may vary depending on cell type to which delivery of the ASOs is desired. Multiple tissues and organs are affected by Dravet syndrome; Epilepsy, generalized, with febrile seizures plus, type 2; Febrile seizures, familial, 3A; Migraine, familial hemiplegic, 3; Autism; Epileptic encephalopathy, early infantile, 13; Sick sinus syndrome 1; Alzheimer's disease or SUDEP, with the brain being the most significantly affected tissue. The ASOs of the present invention may be administered to patients parenterally, for example, by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, intravitreal injection, or intravenous injection.

[00154] In some embodiments, the disease or condition is induced by a mutation in $Na_v1.1$ (a protein encoded by the *SCN1A* gene). In some instances, the mutation is a loss-of-function mutation in $Na_v1.1$. In some cases, the loss-of-function mutation in $Na_v1.1$ comprises one or more mutations that decreases

or impairs the function of $Na_v1.1$ (e.g., by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more) relative to the function of a wild-type $Na_v1.1$. In some cases, the loss-of-function mutation in $Na_v1.1$ comprises one or more mutations that result in a disease phenotype. Exemplary loss-of-function mutations include, but are not limited to, R859C, T875M, V1353L, I1656M, R1657C, A1685V, M1841T, and R1916G.

[00155] In other instances, the mutation is a gain-of-function mutation in $Na_v1.1$. In such cases, the gain-of-function mutation comprises one or more mutations that prolongs activation of $Na_v1.1$ (e.g., by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more) relative to the function of a wild-type $Na_v1.1$. In such cases, the gain-of-function mutation in $Na_v1.1$ comprises one or more mutations that result in a disease phenotype. Exemplary gain-of-function mutations include, but are not limited to, D188V, W1204R, R1648H, and D1866Y.

[00156] In some embodiments, the disease or condition is an encephalopathy. In some cases, the encephalopathy is induced by a loss-of-function mutation in $Na_v1.1$.

[00157] In some embodiments, the encephalopathy is epileptic encephalopathy. Exemplary epileptic encephalopathies include, but are not limited to, Dravet Syndrome (DS) (also known as severe myoclonic epilepsy of infancy or SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); early infantile SCN1A encephalopathy; early infantile epileptic encephalopathy (EIEE); or sick sinus syndrome 1. In some embodiments, the disease or condition is epileptic encephalopathy, optionally selected from Dravet Syndrome (DS) (also known as severe myoclonic epilepsy of infancy or SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); and sick sinus syndrome 1.

[00158] In some instances, GEFS+ is epilepsy, generalized, with febrile seizures plus, type 2.

[00159] In some instances, the Febrile seizure is Febrile seizures, familial, 3A.

[00160] In some instances, SMEB is SMEB without generalized spike wave (SMEB-SW), SMEB without myoclonic seizures (SMEB-M), SMEB lacking more than one feature of SMEI (SMEB-O), or intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC).

[00161] In some embodiments, the diseases or conditions induced by a loss-of-function mutation in $Na_v1.1$ include, but are not limited to, Dravet Syndrome (DS) (also known as SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; early infantile *SCN1A* encephalopathy; early infantile epileptic encephalopathy (EIEE); autism; or malignant migrating partial seizures of infancy.

[00162] In some embodiments, the disease or condition is induced by a gain-of-function mutation in $Na_v1.1$. Exemplary diseases or conditions associated with a gain-of-function mutation in $Na_v1.1$ include, but are not limited to, migraine. In some instances, the disease or condition induced by a gain-of-function mutation in $Na_v1.1$ is migraine.

[00163] In some instances, the migraine is migraine, familial hemiplegic, 3.

[00164] In some embodiments, the disease or condition is a $Na_v1.1$ genetic epilepsy. The $Na_v1.1$ genetic epilepsy can include a loss-of-function mutation in $Na_v1.1$ or a gain-of-function mutation in $Na_v1.1$. In some cases, the $Na_v1.1$ genetic epilepsy includes one or more hereditary mutations. In other cases, the $Na_v1.1$ genetic epilepsy includes one or more de novo mutations. In some cases, the $Na_v1.1$ genetic epilepsy includes Dravet Syndrome (DS) (also known as severe myoclonic epilepsy of infancy or SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; early infantile *SCN1A* encephalopathy; early infantile epileptic encephalopathy (EIEE); sudden unexpected death in epilepsy (SUDEP); or malignant migrating partial seizures of infancy. In some cases, the $Na_v1.1$ genetic epilepsy associated with a loss-of-function mutation in $Na_v1.1$ includes Dravet Syndrome (DS) (also known as severe myoclonic epilepsy of infancy or SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West

syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; early infantile *SCN1A* encephalopathy; early infantile epileptic encephalopathy (EIEE); sudden unexpected death in epilepsy (SUDEP); malignant migrating partial seizures of infancy.

[00165] In some embodiments, the disease or condition is associated with a haploinsufficiency of the *SCN1A* gene. Exemplary diseases or conditions associated with a haploinsufficiency of the *SCN1A* gene include, but are not limited to, Dravet Syndrome (DS) (also known as SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; early infantile *SCN1A* encephalopathy; early infantile epileptic encephalopathy (EIEE); or malignant migrating partial seizures of infancy. In some cases, the disease or condition is Dravet Syndrome (DS) (also known as SMEI); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; early infantile *SCN1A* encephalopathy; early infantile epileptic encephalopathy (EIEE); or malignant migrating partial seizures of infancy.

[00166] In some cases, the disease or condition is Dravet Syndrome (DS).

[00167] Dravet syndrome (DS), otherwise known as severe myoclonic epilepsy of infancy (SMEI), is an epileptic encephalopathy presenting in the first year of life. Dravet syndrome is an increasingly recognized epileptic encephalopathy in which the clinical diagnosis is supported by the finding of sodium channel gene mutations in approximately 70–80% of patients. Mutations of ion channel genes play a major role in the pathogenesis of a range of epilepsy syndromes, resulting in some epilepsies being regarded as channelopathies. Voltage-gated sodium channels (VGSCs) play an essential role in neuronal excitability; therefore, it is not surprising that many mutations associated with DS have been identified in the gene encoding a VGSC subunit. The disease is described by, e.g., Mulley, et al., 2005, and the disease description at OMIM #607208 (Online Mendelian Inheritance in Man, Johns Hopkins University, 1966-2015), both incorporated by reference herein.

[00168] Between 70% and 80% of patients carry sodium channel α1 subunit gene (*SCN1A*) abnormalities, and truncating mutations account for about 40%, and have a significant correlation with an earlier age of seizures onset. Sequencing mutations are found in about 70% of cases and comprise truncating (40%) and missense mutations (40%) with the remaining being splice-site changes. Most mutations are de novo, but familial mutations occur in 5–10% of cases and are usually missense in nature. The remaining *SCN1A* mutations comprise splice-site and missense mutations, most of which fall into the pore-forming region of the sodium channel. At present, over 500 mutations have been associated with DS and are randomly distributed along the gene (Mulley, et al., *Neurol.* 2006, 67, 1094-1095).

[00169] The *SCN1A* gene is located in the cluster of sodium channel genes on human chromosome 2q24 and encodes the α-pore forming subunits known as Nav1.1 of the neuronal voltage gated sodium channel. The *SCN1A* gene spans approximately 100 kb of genomic DNA and comprises 26 exons. The SCN1A protein consists of four domains, each with six-transmembrane segments. Two splice variants have been identified that result in a long and short isoform that differ in the presence or absence of 11 amino acids in the cytoplasmic loop between domains 1 and 2, in exon 11 (Miller, et al., 1993-2015, and Mulley, et al., 2005, 25, 535-542, incorporated herein by reference).

[00170] Alternative splicing events in *SCN1A* 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 *SCN1A* 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 SCN1A protein deficiency.

[00171] 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 *SCN1A* to increase the production of protein-coding mature mRNA, and thus, translated functional SCN1A protein. These compositions and methods include antisense oligomers (ASOs) that can cause exon skipping and promote constitutive splicing of *SCN1A* pre-mRNA. In various embodiments, functional SCN1A protein can be increased using the methods of the disclosure to treat a condition caused by SCN1A protein deficiency.

[00172] In some cases, the disease or condition is SMEB.

[00173] In some cases, the disease or condition is GEFS+.

[00174] In some cases, the disease or condition is a Febrile seizure (e.g., Febrile seizures, familial, 3A).

[00175] In some cases, the disease or condition is autism (also known as autism spectrum disorder or ASD).

[00176] In some cases, the disease or condition is migraine (e.g., migraine, familial hemiplegic, 3).

[00177] In some cases, the disease or condition is Alzheimer's disease.

[00178] In some embodiments, the disease or condition is *SCN2A* encephalopathy.

[00179] In some embodiments, the disease or condition is *SCN8A* encephalopathy.

[00180] In some embodiments, the disease or condition is *SCN5A* arrhythmia.

[00181] 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.

[00182] In 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.

[00183] In embodiments, an ASO of the invention is coupled to a dopamine reuptake inhibitor (DRI), a selective serotonin reuptake inhibitor (SSRI), a noradrenaline reuptake inhibitor (NRI), a norepinephrine-dopamine reuptake inhibitor (NDRI), and a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI), using methods described in, *e.g.*, U.S. Pat. No. 9,193,969, incorporated herein by reference.

[00184] In 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

[00185] Also within the scope of the present disclosure are methods for identifying or determining ASOs that induce exon skipping of a *SCN1A* NIE containing pre-mRNA. For example, a method can comprise identifying or determining ASOs that induce pseudo-exon skipping of a *SCN1A* NIE 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.

[00186] 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.

[00187] 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 NIE 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 NIE) in ASO-treated cells as compared to in control ASO-treated cells indicates that splicing of the target NIE has been enhanced. In some embodiments, the exon skipping efficiency (or the splicing efficiency to splice the intron containing the NIE), 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.

[00188] 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 NIE).

[00189] 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.

[00190] 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 NIE, as described herein (see, *e.g.*, **Example 4**). A reduction or absence of a longer RT-PCR product produced using the primers spanning the NIE in ASO-treated cells as compared to in control ASO-treated cells indicates that exon skipping (or splicing of the target intron containing an NIE) has

been enhanced. In some embodiments, the exon skipping efficiency (or the splicing efficiency to splice the intron containing the NIE), 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.

[00191] ASOs that when hybridized to a region of a pre-mRNA result in exon skipping (or enhanced splicing of the intron containing a NIE) 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 intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, intravitreal 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 (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.

[00192] As described herein in various examples, exon 20x in human *SCN1A* gene is equivalent to exon 21x in mouse *SCN1A* gene.

[00193] 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 **FIG. 3** and **Example 2**.

SPECIFIC EMBODIMENTS

[00194] Embodiment 1. A method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is: from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE.

[00195] Embodiment 2. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A 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 that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is: from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE.

[00196] Embodiment 3. The method of embodiment 1 or 2, wherein the therapeutic agent interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted portion.

[00197] Embodiment 4. The method of embodiment 1 or 2, wherein the targeted portion is at most 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 NIE.

[00198] Embodiment 5. The method of embodiment 1 or 2, wherein the targeted portion is at least 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 NIE.

[00199] Embodiment 6. The method of embodiment 1 or 2, wherein the targeted portion is at most 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 NIE.

[00200] Embodiment 7. The method of embodiment 1 or 2, wherein the targeted portion is at least 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 NIE.

[00201] Embodiment 8. The method of any one of the embodiments 1-7, wherein the therapeutic agent is an antisense oligomer (ASO).

[00202] Embodiment 9. The method of embodiment 8, wherein the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731.

[00203] Embodiment 10. The method of any one of the embodiments 1-9, wherein the therapeutic agent promotes exclusion of the NMD exon from the processed mRNA encoding SCN1A protein.

[00204] Embodiment 11. The method of embodiment 10, wherein exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent 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 exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in a control cell.

[00205] Embodiment 12. The method of embodiment 10, wherein the therapeutic agent increases level of the processed mRNA encoding SCN1A protein in the cell.

[00206] Embodiment 13. The method of embodiment 10, wherein an amount of the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent 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 an total amount of the processed mRNA encoding SCN1A protein in a control cell.

[00207] Embodiment 14. The method of embodiment 2, wherein the disease or condition is induced by a loss-of-function mutation in Nav1.1.

[00208] Embodiment 15. The method of embodiment 14, wherein the disease or condition is associated with haploinsufficiency of the SCN1A gene, and wherein the subject has a first allele

encoding a functional SCN1A, and a second allele from which SCN1A is not produced or produced at a reduced level, or a second allele encoding a nonfunctional SCN1A or a partially functional SCN1A.

[00209] Embodiment 16. The method of embodiment 14, wherein the disease or condition is encephalopathy.

[00210] Embodiment 17. The method of embodiment 16, wherein the encephalopathy is epileptic encephalopathy.

[00211] Embodiment 18. The method of embodiment 14, wherein the disease or condition is Dravet Syndrome (DS); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; autism; or malignant migrating partial seizures of infancy.

[00212] Embodiment 19. The method of embodiment 18, wherein GEFS+ is epilepsy, generalized, with febrile seizures plus, type 2.

[00213] Embodiment 20. The method of embodiment 18, wherein the Febrile seizure is Febrile seizures, familial, 3A.

[00214] Embodiment 21. The method of embodiment 18, wherein SMEB is SMEB without generalized spike wave (SMEB-SW), SMEB without myoclonic seizures (SMEB-M), SMEB lacking more than one feature of SMEI (SMEB-O), or intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC).

[00215] Embodiment 22. The method of embodiments 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 72 or 432.

[00216] Embodiment 23. The method of embodiments 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 73 or 433.

[00217] Embodiment 24. The method of embodiments 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 76 or 436.

[00218] Embodiment 25. The method of embodiments 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 181 or 541.

[00219] Embodiment 26. The method of embodiments 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 220 or 580.

[00220] Embodiment 27. A method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and

encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

[00221] Embodiment 28. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A 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 that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

[00222] Embodiment 29. An antisense oligomer (ASO) comprising a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731.

[00223] Embodiment 30. An antisense oligomer (ASO) consisting of a sequence selected from SEQ ID NOs: 12-731.

[00224] Embodiment 31. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising: contacting the cell of the subject with an ASO of embodiment 29 or embodiment 30.

[00225] Embodiment 32. A kit comprising an ASO of embodiment 29 or embodiment 30.

[00226] While preferred embodiments of the present invention 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 invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[00227] The present invention will be more specifically illustrated by the following Examples. However, it should be understood that the present invention is not limited by these examples in any manner.

Example 1: Identification of NMD-inducing Exon Inclusion Events in *SCN1A* Transcripts by RNAseq using Next Generation Sequencing

[00228] Whole transcriptome shotgun sequencing was carried out using next generation sequencing to reveal a snapshot of transcripts produced by the *SCN1A* gene to identify NIE inclusion events. For this purpose, polyA+ RNA from nuclear and cytoplasmic fractions of HCN (human cortical neurons) was isolated and cDNA libraries constructed using Illumina's TruSeq Stranded mRNA library Prep Kit. The libraries were pair-end sequenced resulting in 100-nucleotide reads that were mapped to the human genome (Feb. 2009, GRCh37/hg19 assembly). The sequencing results for *SCN1A* are shown in **Fig. 2**. Briefly, **Fig. 2** shows the mapped reads visualized using the UCSC genome browser (operated by the UCSC Genome Informatics Group (Center for Biomolecular Science & Engineering, University of California, Santa Cruz, 1156 High Street, Santa Cruz, CA 95064) and described by, *e.g.*, Rosenbloom, *et al.*, 2015, "The UCSC Genome Browser database: 2015 update," Nucleic Acids Research 43, Database Issue, doi: 10.1093/nar/gku1177) and the coverage and number of reads can be inferred by the peak signals. The height of the peaks indicates the level of expression given by the density of the reads in a particular region. The upper panel shows a graphic representation of the *SCN1A* gene to scale. The conservation level across 100 vertebrate species is shown as peaks. The highest peaks correspond to exons (black boxes), while no peaks are observed for the majority of the introns (lines with arrow heads). Peaks of conservation were identified in intron 20 (NM_006920), shown in the middle panel. Inspection of the conserved sequences identified an exon-like sequence of 64 bp (bottom panel, sequence highlighted in grey) flanked by 3' and 5' splice sites (underlined sequence). Inclusion of this exon leads to a frameshift and the introduction of a premature termination codon in exon 21 rendering the transcript a target of NMD.

[00229] Exemplary *SCN1A* gene, pre-mRNA, exon, and intron sequences are summarized in **Table 1**. The sequence for each exon or intron is summarized in **Table 2**.

Table 1. List of target SCN1A gene and pre-mRNA sequences.

Species	SEQ ID NO.	Sequence Type
Human	SEQ ID NO. 1	SCN1A gene (NC_000002.12)
	SEQ ID NO. 2	SCN1A pre-mRNA (encoding e.g., SCN1A mRNA NM_006920.5)
	SEQ ID NO. 3	Intron 22 gene (GRCh38/hg38 assembly) (coordinate: chr2 166002754 166007229)

	SEQ ID NO. 4	Exon 23 (Exon 20x) gene (GRCh38/hg38 assembly) (coordinate: chr2 166007230 166007293)
	SEQ ID NO. 5	Intron 23 gene (GRCh38/hg38 assembly) (coordinate: chr2 166007294 166009718)
	SEQ ID NO. 6	IVS 22+IVS 23 pre-mRNA (pre-mRNA sequence of introns 22 and 23)
	SEQ ID NO. 7	Exon 23 (Exon 20x) pre-mRNA
Mouse	SEQ ID NO. 8	SCN1A gene (NC_000068.7)
	SEQ ID NO. 9	SCN1A pre-mRNA (encoding e.g., SCN1A mRNA NM_001313997.1)
	SEQ ID NO. 10	Intron 21 pre-mRNA
	SEQ ID NO. 11	Exon 21x pre-mRNA

Table 2. Sequences of target exon or intron in SCN1A pre-mRNA transcripts.

SEQ ID NO.	Sequence Type	Sequence
3	Intron 22 gene	gtaagaaaaatgaaagaacctgaagtattgtatatagccaaaattaaactaaattaaatttagaaaaaggaaaatctatgcatgcaaaaggaatggcaaattcttgcaaaattgctactttattgttttatctgttgcatatttacttctaggtgatatgcaagagaaataggcctctcttgaaatgatataatatcatttatctgctgtgcttatttaaatgactttatttcctaatccatcttgggagtttccttacaaatctatatacaaaaaaaagctgatgcattattaaagtactatgtgtaatgatataatggtaatctaaagtaaattctatatcaggtacttattctttgtgatgatatactgtacttaacgagttttcctgaaaataatgtgaatcacacatgtgcctaagtatgagtgttaagaaaaaaatgaaaggagttgttaaaacttttgtctgtataatgccaaagtttgcattatttgaatatattcaagattagatggttagatattaagtgttgactgaatttataaaactagtaatactaacttaaagattacatacaaatccacatcatttttataacaataaagtaaaacacttataatgaacagaaaatataattttgactcattactataggtaatttatacattaaccttaacttgcatcttattggtcagagtcacacaaaatgttattttatccttttcaaagatgcaataatcattttccatcatgcataacagattagaaattttgccattattgacttattttccatgccttttttttacggcatgaagcattagtttatagatatataatataaaaaattagttctgctttttttttaaaaaaaaaatattatcaaaacaaaacactgaattgtggattccaatagaaaaacactgctctttcacctcctaaggtgtagttacttttatggaaactaagctgtattgtagacttccatttgcactttgtagattgtttatagccttatgttctcttctcaagtcttattataaatgtcactttgtaagaacgtaggacttgtcttcgatttccctaacatatatgaaaactttgtcctcattatcgacaactcagaacaataataatacaagtagtcctcttttatttctcacagagagcctcaaattttcaccaaaatgttaacagaaattatctctggggtgtataagaattaagtctgttttccaattaaatgtcactttgttttgtttcagactggcagtttcagttctggagaaaaaaaaatgtcatttgtgtacattctacttgaaaacatgttgcctgaatcaaaataatatatttttatatggcttgtgaaatctgaacaatgctaaacatttgaaaatattataaaccttttacatttgaccatttgaaagtttattaaattcattggtcaagtgctcagatatttccatacattacacttcatttctataaaaaaagctgatcttatcggtatactttaattttctcagaaataaccatatctataattattaatcaataatgccttttatattaaaagaggttagttttggaaacttggagttttagacataaaatccttataaatgctgatagtgatataactaatagtttaaatggtcagatttatgaatatggctctattcctcataatgacaacatacacacagcactaaaatgactaatctcttcaatacgtgtttggcattgtagagtcaaaataacgttataattgattctatttttatacttctagtgtttggatattttattttgtaaaaatataatcatgaatgatggtgaggttggatataagaatgatgattatgattgggaagtgagatttgaacatgctcagaaactctcatttaattctttgccctagcagcataaaatcacaatagctgcgtcaaagcgtaactcaggcactcatttttatttttgttgttctgttatttttttcaaagcatgtgcttttatgcaacattactgaataaagcatgttgtacagtgcttgataagaagttagaaagtaacaaataaattatcatcacgttgcactttgtgttttgcatgttttatgcacatttctggctgacagcttttaaacatttattgtatttcaaatttccagtccaaatttttcaacttgtaaaattaaactgagtgaattgatgtcgtgaatatctagggtaaaataaaatttgtgtttaaatttgtatttttaatttcctaacctaggaaatcttaaatacctttcttttttcaaaagaactcaagtcttaatggatagggaaacagacggagagcatcatgaacaaaaagtaacaccaaatgttctgtcatatcagatttctaactaataacaaactatatatttctattttgtatag

4	Exon 23 (Exon 20x) gene	GATAATCTTGCTCCAACTTGGATGGGGTGGAGCGCTGGTTCCT CCCCTGAGCCCTTTATTATGG
5	Intron 23 gene	gtactgtattaccccttttgctacctttaatccttgcactgtgacttatgtgtagtggggtgagggagggattg ggaagggtactattattgcaccacagtagggaaaatacattatttacatcctaatccccctcttttcaattgtctt aaatttcatttgaaaaaaaaaaaaaccttttatgaatttaccctctgtggattttaaccccaatggttgatatcttt attaagtttcattgaatatgatttagttatgtgtatatggagttatccatctttggggagattactggattggtga gggcgggggaccctggtgtagaatgattatgtgaaaaaacaatttaacttgttaagctcatgatactgtttg aggcatacagcccctgctgtttagtacattggtctgggtcctgaaaattaccagttagataccatcagttgat tattgatatgtatgagcagatactagggtgcaatatttcaggtttcataagactggtattgattgtgaccactc tcatttttattgtgtaagttcatatggggttattttcaaaatgttaacaaggcaaaaatatattaagaaatagtt gaataagcacatgtgaattgtgttgtaaacaaaaagttagaataaaaaaatccacttatttgaattatgcaga atagaatacatacctagaaataaaacaaaaacgtcttatcatgagtattaagataaaatttaaggcataaact cacttcttagaataagtaactcccaactaactttctaggattttaaaacataacacagtgaaaacatacataa acataactctacattttatttattcttaaagtttaagtgtattatacaagaagaagagtttatattcgagagaca gaaaaagtcagaatttttgtttggatcaccaatatatcatagcttacaaaaaaactgtcttaattaaaacccac aacataatttttttagatttttaagaaagattctattattcttctttatacttaaaaatggatgattcctactttgccc actttttatttttattcacatagattttctttatttctattagagaagcactagaattcatgaatagtgttgatttgaa gttcaaagtaattaattcagataaaaagacatttctgcatgtatgaaaatttctaatgtgaatttgcatatttaat tatcaatccttcatttagtgtagacttatttttaaaaatgcaggtaatgaaccagaaatagaattggttgtgcta gagtagagaaactttatttgatgattgttttgaaaaaaaagcttctgagaagaaacaacctctagtacagtat taattcattaagatagctcctttctcagacatttcctttcatgtagcctgaaagttcaatttgaaatttgttctttcc aatttattcagactaattctgcctactttcttccccccataagaaccaattactgcagctttattgagactgaaa aaagttaatacacctccttctttgctgaaccaaggaatggcttggaactcttgggaaaagacaatcttttcta tgatctttcattgtctaatttaatacatcataaatatgactatagctttgtataataaactccccaatactgtgcc agatgttttctaagataaagttattttatgttcacaaaaaaaataaaactttctctgggccaaatgtatgccaa ctttgcaaatcatatcctgaagtgcactgctgcagagtacatgcttgcgtcataaattccatagagttcgcttt aactctaaatcaatccccagtttcaaagtaaacctctcaaacatattacctaagcacaaacttctccctgtgc tcagttccttaattattctcatcccatattcagaaataacatttaaaaattatgctttgatcaataaatactaatct aaactttgcttcattaacccattcatttttgtcaaccattattttattcctatattcaaagctctctggtatgttctta tattcaagacactcaaggccctggaagattcacgaacatatgtttgcatcttaaatttttagaaaatcttacaa tctgtcaggattacactgaactctagtacagagtaatatgggtaccagataagtgggagcaactcttccac gtagactggaaacagcactaaatgctatttataggctactttctgaacttaacttgttttaacctcatttttctca tatgccaaatgagaacgcaatactgaattatctgtacagttctgttcagtactagaattctgattcttgaattca aaggggaaaacattcctctttattttggaggctaaactgggggacaaagttaggctccatgaaagaagtg ctatttgaactaaagcctttaagaggggagagtatttcagaagaggagctattagacaaggaatttcaatgt aaatggcatctcaatcacctggcaattatattagcacacggttattatattaattgaagtggcatgaagtata gatgaccagggaagttaaaactggaaatatagattgtggagtgatgtgaataccaaggtaagaaaaatat ttgttagttaccagagagccaataaataactttcaagtgggacttggggaagattaattcatcttacatagat taaatgaaggagaaggttaggagacagatgacagtgcaagtatgaaataacagagggcagttctaggt ggtgactgtgagaatggaaaagaggtggcaaagctgagaaacgtttcaaagaaaaaatgtgagacagg taatgtgaaaagaaaatcgagaaataggtatagataatcagtgttctgctcatactctaaattgggtgttgaa ggcaaaatacgtattttaattagtactctgtgtatacacactagaaacagcattgtaatctggatagtggaca aaatattcagaaaagaagggaaatagtaacttgatttcaatttccaaatctctaatctgaaagaaatctaattc tattcatccatttaaaataaattatataacgagaatttatgaagtccattgtattaatgcagacagtcagatga gataaggcaaagtgtcacgtgtcagcttggtagttgcatcggccacatcatttggttctgcctggataactc aaccaaattaatttttcatactcatcccctccacctttgtcattactggtattcttatttttctttggcccacttatca cactgttttatgttccccagaaggcctagagttctttacaggctttaaacagggatcagaagtataagaaatt ggctcatgtatttttttttcagacaggcagttaaaaaaaattgttctaaaaatacactggcatcaaatggcaa atagaagatgttttgacgactacttccattggatcagactgacaagaataatacaagcacataggtggaatt aaacttagctattaatgtccaagtttgaggcagctgccccttataagcattttagggtctgttttttagcttccct cttagccactcctgtgcagctccagtgggaggtatggaggaaaaagcaaggaagccatccctatgttgtt tccaaacatgaacactcaagatttttaactagtggtccagaagtaaagaggggggaaaacatccttctatag aaaaaaaaaaaagtagataataattgaacacagaacttcatgtgatcacatcagatttgagaactatgtatg gcatccctcttttttcttatttttcctaagaaatgatttctattatgtttcatttgaaataagttttttgaattaaactcag taaatgaaacaactgacatgactggagcttgaaataaacgatgtgatgatctaatgaaatacataatgcaa

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6	IVS 22+IVS 23 pre-mRNA	guaagaaaaaugaaagaaccugaaguauuguauauagccaaaauuaaacuaaauuaaaauuuag aaaaaggaaaaucuaugcaugcaaaaggaauggcaaaauucuugcaaaauugcuacuuuauuugu uuuaucuguugcauauuuacuucuaggugauaugcaagagaaauaggccucucuugaaauga uauaauaucauuuaucugcugugcuuauuuaaaugacuuuauuuccuaauccaucuugggag uuuccuuacaaaucuauauacaaaaaaaagcugaugcauuauuaaaguacuauguguaaugau auaaugguaaucuaaaguaaaauucuauaucagguacuuauucuuugugaugauauacuguac uuaacgaguuuuccugaaaauaaugugaaucacacaugugccuaaguaugaguguuaagaaa aaaaugaaaggaguuguuaaaacuuuugucuguauaaugccaaaguuugcauuauuugaaua uauucaagauuagaugguuagauauuaaguguugacugaauuuauaaaacuaguaauacuaa cuuaaagauuacauacaaauccacaucauuuuuauaacaauaaaguaaaacacuuauaaugaac agaaaauauaauuuugacucauuacuauagguaauuuauacauuaaccuuaacuugcaucuua uuggucagagucacacaaaauguuauuuuauccuuuucaaagaugcaauaaucauuuuccau caugcauaacagauuagaaauuuugccauuauugacuuauuuuccaugccuuuuuuuacggc augaagcauuaguuuauagauauauaauauaaaaaauuaguucugcuuuuuuuuaaaaaaaa auauuaucaaaacaaaacacugaauugugugauuccaauagaaaaacacugcucuuucaccuc cuaagguguaguuacuuuuauggaaacuaagcuguauuguagacuuccauuugcacuuugua gauuguuuauagccuuauguucucuucucaagucuuauuauaaaaugucacuuuguaagaacg uaggacuugucuucgauuucccuaacauauaugaaaacuuuguccucauuaucgacaacucag aacaauauaauacaaguaguccucuuuuauuucucacagagagccucaaauuuucaccaaaau guuaacagaaauuaucucugggguguauaagaauuaagucuguuuuccaauuaaaugucacu uuguuuuguuucagacuggcaguuucaguucuggagaaaaaaaaugucauuuguguacauuc uacuugaaaacauguugccugaaucaaaauaauauauuuuauauggcuugugaaaucugaac aaugcuaaacauuugaaaauauuauaaaccuuuuacauuugaccauuugaaaguuuauuaaau ucauuggucaagugcucagauauuuccauacauuacacuucauuucuauaaaaaagcugaucu uaucgguauacuuuuaauuuucucagaaauaaccauaucuauaaauuauuaaucaauaaugccu uuuauauuaaaagagguuaguuuuugaaacuuggaguuuuagacauaaaauccuuauaaaug cugauagugauauaacuaauaguuuaaauggucagauuuaugaauauggcucuauuccucau aaugacaacauacacacagcacuaaaaugacuaaucucuucaauacguguuuggcauuguaga gucaaaauaacguuauaauugauucuauuuuuuauacuucuaguguuuggauauuuuauuu uguaaaaauauaaucaugaaugauggugagguuggauauaagaaugaugauuaugauuggga agugagauuugaacaugcucagaaacucucauuuaauucuuugcccuagcagcauaaaaucac aauagcugcgucaaagcguaacucaggcacucauuuuauuuuuguuguucuguuauuuuuuc aaagcaugugcuuuuaugcaacauuacugaauaaagcauguuguacagugcuugauaagaag uuagaaaguaacaaauaaaauuaucaucacguugcacuuuguguuuugcauguuuuaugcaca uuucuggcugacagcuuuuaaacauuuauuguauuucaaauuuccaguccaaauuuuucaac uuguaaaauuaaacugagugaauugaugucgugaauaucuaggguaaaauaaaauuuguguu uaaauuuguauuuuuaauuuccuaaccuaggaaaucuuaaauaccuucuuuuucaaaagaac ucaagucuuaauggauagggaaacagacggagagcaucaugaacaaaaaguaacaccaaaugu ucugucauaucagauuucuaacuaauaacaaacuauauauuucuauuuuguauagguacugu auuaccccuuuugcuaccuuuaauccuugcacugugacuuauguguaguggggugagggag ggauugggaaggguacuauuauugcaccacaguagggaaaauacauuauuuacauccuaauc cccucuuuucaauugucuuaaauuucauuugaaaaaaaaaaaaaccuuuaugaauuuacccuc uguggauuuuaaccccaaugguugauaucuuuauuaaguuucauugaauaugauuuaguua uguguauauggaguuauccaucuuuggggagauuacuggauuggugagggcgggggacccu gguguagaaugauuauugugaaaaaacaauuuaacuuguuaagcucaugauacuguuugaggc auacagccccugcuguuuaguacauuggucuggguccugaaaauuaccaguuagauaccauc aguugauuauugauauguaugagcagauacuagggugcaauauuucagguuucauaagacug

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7	Exon 23 (Exon 20x) pre-mRNA	GAUAAUCUUGCUCCAACUUGGAUGGGGUGGAGCGCUGGUUC CUCCCCUGAGCCCUUUAUUAUGG
10	Intron 21 pre-mRNA	guaagaaaaaggaaaacucugcagcguuguauauugucaaagcuaggcugaguucaacuuaac uaacgaaaaacacgugcaugcaaaaggaauggcaacccuuugcaaacuugcuacuuuacccuu uucucuguugcauauuuacuucuuggugauaugcaagagaaaaucggccucuuugaaaauga uuuaauaucauuuaucugcuuugcuaauuaaaaugaccuuaguucauaaucgaucuugggag uuuccuuauaauuccuaauacaaaggggagggcagauacucucuuaaagaacuaaguuga gucauguaauaauuaccuagagauaauuuuguuucauaucguucuccucuaugacagcccau caguacuuaagggauccuauggaaaguaaugugaaucacaaaugugguaugaauacaaaggaaa aaaugaagaauuguuaaaugguuuugucuuuacaaugccaaaauucucauuauuugaauauau ccaagggcagauauuaaccauugacuggaguauaauaauacugccucaacuguaacuaaauua augacauugaauaaguaagacacuaauuuaauuacuauaaaauacauacacaucuuaugacaau acagcugauaaggaaaaagaacauguauuuuuauucauugccauacauggcucgucaaccuua acuuaaaccucgguucucaguuacacagaguuuuauguugcucuuuugagcaaagcauuauu ccccucuccauaauucaacacguaucagauuuuugcauuuauuggcucauuguuaugaugau uauuucaaagcauuauacaaucauuuauagaagaugugccgugugaaaauuauuuuuuuauu aagauccaaaauuuacgcucuuuaaaccaaucagaugaaauguauaaggcaaagagugcucau uguccugacacuuacaaaccaaggcuccaacaaacaggcuccucucugcaccacauagagggcu uucagccuguguccccccauaaaaaccuauuauaaaauuuuauuauacuauacuguaagaaacc uguccaauuuuuaauuucucuagcacauaugaaaacuucucuucaguagauccaaguaagcac aaaggagcuuugauucucacacaagaaaucacauuuguauuaaaaauguaucauaaaauuuucu cccaauuaugcaaaacuuaaaugcuuuuccaauuaaaagagcacuuucguuucagaauagcaa uuucaguugucaaggaaaacauuguuuuuauacauuuuauauaaaaauacaugagcuaaauu uaaauucacauuuuucaacuuuuuaugguuuuuuuauguuucuuuuucuuucgcauuuuuu aacaauccagccauuaccucuccucccuguccccccuccuauaguuucucaucucauuccuccu cccccuugccucugagaggaugcucccucccucacuaggccucccucuucccccagggcuucaa guuccucaaggauuauacacaucuucucccacugagaccaggccaggcaguccucugcuucug cccagccuguguauguuccugcuugguagcucagucucuggaagcucccuggggucugggu uaguugggacugcuggucuuccuauggaguuacccucccccuucaacuucuucaauccuuccc cuaauucaaccacagguauccagacuucaguccaaugguugaguguaaauauuggcaucugu cucugucagcuguuguuaggggcucagaggacagccaugcuaggcuccugccuacaagcagc acaccauaacaucaguaauagugucaggccuuuaaugaauaaugcuacguauauaaggguugu uagauuauacuucaacuuugaucuuuuagaauauuauuaaaauccagucguuuauuuuuuaua uguaauauugacuuuccauaacaaaugagucuauuuuccuuuuguguagaaauaacuuuauc aauuauuguuaauaaugcuuuugucaauuauuguugauaugcuucucuuuuuuaaaacugg agucaucaaaacaaaaauucuggguagauauuacgaagcugacuccuuggucagcuuggcaca uagugagaccacaaauaucucaaggucacggcaauuccucaccaccaguuuggcauugugaag ucaaaaccaaccuucuguugauucuggguuuuuguauuucuaguaugagacauuuucuacuuu guaagaguauauaacuguggauggcggcgaggguugggcaugaugaugauuguaagcgggaa gugagcuagaguaucuucagaaacucucacuuuauccucccuuggcagcauagaaccgcaaucg cuguguccgagugucaaccaggcagucauuuuguuuuuggguuuuuugucacucuuucaaag caugugcuucuacgcaacacuaccaaacacagcaugcugcauagugcuugagaaggaguuaga aaguaacaaacgaguuaucaucacguugcccuuuguguuuugcaugucuuaugcacacuuuu ggcugacagcuuuugaacauuuauuguauuucaaauuuccaguccaaauuuuuuuuucaacu ugugaaauugaacggaaugaaccgaugucgugaauaccuagggucaaauaaaacuuguauuu aaauucguaguuuuaauuucccaagcugggaaaaucguaaaaaccuuuuccaaagaacucaag

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11	Exon 21x pre-mRNA	GAUAAUCUUGCUCCAACUUGGAUGGGGUGGAGCGGUGG UUCCUCCCCUCAGCCCUUUAUUAUGG

Example 2: Confirmation of NIE via Cycloheximide Treatment

[00230] RT-PCR analysis using cytoplasmic RNA from DMSO-treated (CHX-) or cycloheximide-treated (CHX+) mouse Neuro 2A cells (**FIG. 3A**) and RenCell VM (human neuroprogenitor cells) (**FIG. 3B**) and primers in exon 20 and exon 23 confirmed the presence of a band corresponding to the NMD-inducing exon (20x). The identity of the product was confirmed by sequencing. Densitometry analysis of the bands was performed to calculate percent exon 20x inclusion of total SCN1A transcript. Treatment of RenCell VM with cycloheximide (CHX+) to inhibit NMD led to a 2-fold increase of the product corresponding to the NMD-inducing exon 20x in the cytoplasmic fraction (cf. light grey bar, CHX-, to dark grey bar, CHX+).

Example 3: SCN1A Exon 23 (Exon 20x) Region ASO Walk

[00231] ASOs were designed to cover a region from about 1000 to about 100 nucleotides upstream of the 5' end of the *SCN1A* exon 23 (exon 20x) gene by shifting 5 nucleotides at a time. ASOs were also designed to cover a second region from about 1000 to about 100 nucleotides downstream from the 3' end of the *SCN1A* exon 23 (exon 20x) gene by shifting 5 nucleotides at a time. A list of ASOs targeting *SCN1A* is summarized in **Table 3**. Sequences of ASOs are summarized in **Table 4**.

Table 3. List of ASOs targeting *SCN1A*

Gene	Pre-mRNA	ASOs	NIE

SEQ ID NO.	SEQ ID NO.	SEQ ID NO.	
SEQ ID NO. 1	SEQ ID NO. 2	SEQ ID NOs: 12-731	Exon 23 (Exon 20x)

Table 4. Sequences of ASOs targeting human *SCN1A*

SEQUENCE NAME	Chr2 Start	Chr2 End	SEQ ID NO:	ASO sequence	SEQ ID NO:	ASO sequence
SCN1A-IVS22-986	166864788	166864806	12	ATGAATTTAATAAACTTT	372	AUGAAUUUAAUAAACUUU
SCN1A-IVS22-981	166864783	166864801	13	GACCAATGAATTTAATAA	373	GACCAAUGAAUUUAAUAA
SCN1A-IVS22-976	166864778	166864796	14	CACTTGACCAATGAATTT	374	CACUUGACCAAUGAAUUU
SCN1A-IVS22-971	166864773	166864791	15	CTGAGCACTTGACCAATG	375	CUGAGCACUUGACCAAUG
SCN1A-IVS22-966	166864768	166864786	16	AATATCTGAGCACTTGAC	376	AAUAUCUGAGCACUUGAC
SCN1A-IVS22-961	166864763	166864781	17	ATGGAAATATCTGAGCAC	377	AUGGAAAUAUCUGAGCAC
SCN1A-IVS22-956	166864758	166864776	18	AATGTATGGAAATATCTG	378	AAUGUAUGGAAAUAUCUG
SCN1A-IVS22-951	166864753	166864771	19	AGTGTAATGTATGGAAAT	379	AGUGUAAUGUAUGGAAAU
SCN1A-IVS22-946	166864748	166864766	20	AATGAAGTGTAATGTATG	380	AAUGAAGUGUAAUGUAUG
SCN1A-IVS22-941	166864743	166864761	21	ATAGAAATGAAGTGTAAT	381	AUAGAAAUGAAGUGUAAU
SCN1A-IVS22-936	166864738	166864756	22	TTTTTATAGAAATGAAGT	382	UUUUUAUAGAAAUGAAGU
SCN1A-IVS22-931	166864733	166864751	23	CAGCTTTTTTATAGAAAT	383	CAGCUUUUUUAUAGAAAU
SCN1A-IVS22-926	166864728	166864746	24	AAGATCAGCTTTTTTATA	384	AAGAUCAGCUUUUUUAUA
SCN1A-IVS22-921	166864723	166864741	25	CCGATAAGATCAGCTTTT	385	CCGAUAAGAUCAGCUUUU
SCN1A-IVS22-916	166864718	166864736	26	GTATACCGATAAGATCAG	386	GUAUACCGAUAAGAUCAG
SCN1A-IVS22-911	166864713	166864731	27	TAAAAGTATACCGATAAG	387	UAAAAGUAUACCGAUAAG
SCN1A-IVS22-906	166864708	166864726	28	AAAATTAAAAGTATACCG	388	AAAAUUAAAAGUAUACCG
SCN1A-IVS22-901	166864703	166864721	29	CTGAGAAAATTAAAAGTA	389	CUGAGAAAAUUAAAAGUA
SCN1A-IVS22-896	166864698	166864716	30	TATTTCTGAGAAAATTAA	390	UAUUUCUGAGAAAAUUAA
SCN1A-IVS22-891	166864693	166864711	31	ATGGTTATTTCTGAGAAA	391	AUGGUUAUUUCUGAGAAA
SCN1A-IVS22-886	166864688	166864706	32	TAGATATGGTTATTTCTG	392	UAGAUAUGGUUAUUUCUG
SCN1A-IVS22-881	166864683	166864701	33	AATTATAGATATGGTTAT	393	AAUUAUAGAUAUGGUUAU

SCN1A-IVS22-876	166864678	166864696	34	TTAATAATTATAGATATG	394	UUAAUAAUUAUAGAUAUG
SCN1A-IVS22-871	166864673	166864691	35	ATTGATTAATAATTATAG	395	AUUGAUUAAUAAUUAUAG
SCN1A-IVS22-866	166864668	166864686	36	GCATTATTGATTAATAAT	396	GCAUUAUUGAUUAAUAAU
SCN1A-IVS22-861	166864663	166864681	37	AAAAGGCATTATTGATTA	397	AAAAGGCAUUAUUGAUUA
SCN1A-IVS22-856	166864658	166864676	38	AATATAAAAGGCATTATT	398	AAUAUAAAAGGCAUUAUU
SCN1A-IVS22-851	166864653	166864671	39	CTTTTAATATAAAAGGCA	399	CUUUUAAUAUAAAAGGCA
SCN1A-IVS22-846	166864648	166864666	40	AACCTCTTTTAATATAAA	400	AACCUCUUUUAAUAUAAA
SCN1A-IVS22-841	166864643	166864661	41	AAACTAACCTCTTTTAAT	401	AAACUAACCUCUUUUAAU
SCN1A-IVS22-836	166864638	166864656	42	TTCAAAAACTAACCTCTT	402	UUCAAAAACUAACCUCUU
SCN1A-IVS22-831	166864633	166864651	43	CAAGTTTCAAAAACTAAC	403	CAAGUUUCAAAAACUAAC
SCN1A-IVS22-826	166864628	166864646	44	AACTCCAAGTTTCAAAAA	404	AACUCCAAGUUUCAAAAA
SCN1A-IVS22-821	166864623	166864641	45	TCTAAAACTCCAAGTTTC	405	UCUAAAACUCCAAGUUUC
SCN1A-IVS22-816	166864618	166864636	46	TTATGTCTAAAACTCCAA	406	UUAUGUCUAAAACUCCAA
SCN1A-IVS22-811	166864613	166864631	47	GGATTTTATGTCTAAAAC	407	GGAUUUUAUGUCUAAAAC
SCN1A-IVS22-806	166864608	166864626	48	TATAAGGATTTTATGTCT	408	UAUAAGGAUUUUAUGUCU
SCN1A-IVS22-801	166864603	166864621	49	GCATTTATAAGGATTTTA	409	GCAUUUAUAAGGAUUUUA
SCN1A-IVS22-796	166864598	166864616	50	TATCAGCATTTATAAGGA	410	UAUCAGCAUUUAUAAGGA
SCN1A-IVS22-791	166864593	166864611	51	ATCACTATCAGCATTTAT	411	AUCACUAUCAGCAUUUAU
SCN1A-IVS22-786	166864588	166864606	52	GTTATATCACTATCAGCA	412	GUUAUAUCACUAUCAGCA
SCN1A-IVS22-781	166864583	166864601	53	TATTAGTTATATCACTAT	413	UAUUAGUUAUAUCACUAU
SCN1A-IVS22-776	166864578	166864596	54	TAAACTATTAGTTATATC	414	UAAACUAUUAGUUAUAUC
SCN1A-IVS22-771	166864573	166864591	55	CCATTTAAACTATTAGTT	415	CCAUUUAAACUAUUAGUU
SCN1A-IVS22-766	166864568	166864586	56	TCTGACCATTTAAACTAT	416	UCUGACCAUUUAAACUAU
SCN1A-IVS22-761	166864563	166864581	57	ATAAATCTGACCATTTAA	417	AUAAAUCUGACCAUUUAA
SCN1A-IVS22-756	166864558	166864576	58	TATTCATAAATCTGACCA	418	UAUUCAUAAAUCUGACCA
SCN1A-IVS22-751	166864553	166864571	59	AGCCATATTCATAAATCT	419	AGCCAUAUUCAUAAAUCU
SCN1A-IVS22-746	166864548	166864566	60	AATAGAGCCATATTCATA	420	AAUAGAGCCAUAUUCAUA

SCN1A-IVS22-741	166864543	166864561	61	TGAGGAATAGAGCCATAT	421	UGAGGAAUAGAGCCAUAU
SCN1A-IVS22-736	166864538	166864556	62	CATTATGAGGAATAGAGC	422	CAUUAUGAGGAAUAGAGC
SCN1A-IVS22-731	166864533	166864551	63	GTTGTCATTATGAGGAAT	423	GUUGUCAUUAUGAGGAAU
SCN1A-IVS22-726	166864528	166864546	64	TGTATGTTGTCATTATGA	424	UGUAUGUUGUCAUUAUGA
SCN1A-IVS22-721	166864523	166864541	65	CTGTGTGTATGTTGTCAT	425	CUGUGUGUAUGUUGUCAU
SCN1A-IVS22-716	166864518	166864536	66	TAGTGCTGTGTGTATGTT	426	UAGUGCUGUGUGUAUGUU
SCN1A-IVS22-711	166864513	166864531	67	CATTTTAGTGCTGTGTGT	427	CAUUUUAGUGCUGUGUGU
SCN1A-IVS22-706	166864508	166864526	68	TTAGTCATTTTAGTGCTG	428	UUAGUCAUUUUAGUGCUG
SCN1A-IVS22-701	166864503	166864521	69	AGAGATTAGTCATTTTAG	429	AGAGAUUAGUCAUUUUAG
SCN1A-IVS22-696	166864498	166864516	70	ATTGAAGAGATTAGTCAT	430	AUUGAAGAGAUUAGUCAU
SCN1A-IVS22-691	166864493	166864511	71	CACGTATTGAAGAGATTA	431	CACGUAUUGAAGAGAUUA
SCN1A-IVS22-686	166864488	166864506	72	CCAAACACGTATTGAAGA	432	CCAAACACGUAUUGAAGA
SCN1A-IVS22-681	166864483	166864501	73	CAATGCCAAACACGTATT	433	CAAUGCCAAACACGUAUU
SCN1A-IVS22-676	166864478	166864496	74	CTCTACAATGCCAAACAC	434	CUCUACAAUGCCAAACAC
SCN1A-IVS22-671	166864473	166864491	75	TTTGACTCTACAATGCCA	435	UUUGACUCUACAAUGCCA
SCN1A-IVS22-666	166864468	166864486	76	GTTATTTTGACTCTACAA	436	GUUAUUUUGACUCUACAA
SCN1A-IVS22-661	166864463	166864481	77	ATAACGTTATTTTGACTC	437	AUAACGUUAUUUUGACUC
SCN1A-IVS22-656	166864458	166864476	78	CAATTATAACGTTATTTT	438	CAAUUAUAACGUUAUUUU
SCN1A-IVS22-651	166864453	166864471	79	AGAATCAATTATAACGTT	439	AGAAUCAAUUAUAACGUU
SCN1A-IVS22-646	166864448	166864466	80	AAAATAGAATCAATTATA	440	AAAAUAGAAUCAAUUAUA
SCN1A-IVS22-641	166864443	166864461	81	TATAAAAAATAGAATCAA	441	UAUAAAAAAUAGAAUCAA
SCN1A-IVS22-636	166864438	166864456	82	AGAAGTATAAAAAATAGA	442	AGAAGUAUAAAAAAUAGA
SCN1A-IVS22-631	166864433	166864451	83	ACACTAGAAGTATAAAAA	443	ACACUAGAAGUAUAAAAA
SCN1A-IVS22-626	166864428	166864446	84	TCCAAACACTAGAAGTAT	444	UCCAAACACUAGAAGUAU
SCN1A-IVS22-621	166864423	166864441	85	AAATATCCAAACACTAGA	445	AAAUAUCCAAACACUAGA
SCN1A-IVS22-616	166864418	166864436	86	AAATAAAATATCCAAACA	446	AAAUAAAAUAUCCAAACA
SCN1A-IVS22-611	166864413	166864431	87	TTACAAAATAAAATATCC	447	UUACAAAAUAAAAUAUCC

SCN1A-IVS22-606	166864408	166864426	88	TATTTTTACAAAATAAAA	448	UAUUUUUACAAAAUAAAA
SCN1A-IVS22-601	166864403	166864421	89	GATTATATTTTTACAAAA	449	GAUUAUAUUUUUACAAAA
SCN1A-IVS22-596	166864398	166864416	90	TTCATGATTATATTTTTA	450	UUCAUGAUUAUAUUUUUA
SCN1A-IVS22-591	166864393	166864411	91	CATCATTCATGATTATAT	451	CAUCAUUCAUGAUUAUAU
SCN1A-IVS22-586	166864388	166864406	92	CTCACCATCATTCATGAT	452	CUCACCAUCAUUCAUGAU
SCN1A-IVS22-581	166864383	166864401	93	CCAACCTCACCATCATTC	453	CCAACCUCACCAUCAUUC
SCN1A-IVS22-576	166864378	166864396	94	TATATCCAACCTCACCAT	454	UAUAUCCAACCUCACCAU
SCN1A-IVS22-571	166864373	166864391	95	ATTCTTATATCCAACCTC	455	AUUCUUAUAUCCAACCUC
SCN1A-IVS22-566	166864368	166864386	96	TCATCATTCTTATATCCA	456	UCAUCAUUCUUAUAUCCA
SCN1A-IVS22-561	166864363	166864381	97	CATAATCATCATTCTTAT	457	CAUAAUCAUCAUUCUUAU
SCN1A-IVS22-556	166864358	166864376	98	CCAATCATAATCATCATT	458	CCAAUCAUAAUCAUCAUU
SCN1A-IVS22-551	166864353	166864371	99	ACTTCCCAATCATAATCA	459	ACUUCCCAAUCAUAAUCA
SCN1A-IVS22-546	166864348	166864366	100	ATCTCACTTCCCAATCAT	460	AUCUCACUUCCCAAUCAU
SCN1A-IVS22-541	166864343	166864361	101	TTCAAATCTCACTTCCCA	461	UUCAAAUCUCACUUCCCA
SCN1A-IVS22-536	166864338	166864356	102	GCATGTTCAAATCTCACT	462	GCAUGUUCAAAUCUCACU
SCN1A-IVS22-531	166864333	166864351	103	TCTGAGCATGTTCAAATC	463	UCUGAGCAUGUUCAAAUC
SCN1A-IVS22-526	166864328	166864346	104	GAGTTTCTGAGCATGTTC	464	GAGUUUCUGAGCAUGUUC
SCN1A-IVS22-521	166864323	166864341	105	AATGAGAGTTTCTGAGCA	465	AAUGAGAGUUUCUGAGCA
SCN1A-IVS22-516	166864318	166864336	106	AATTAAATGAGAGTTTCT	466	AAUUAAAUGAGAGUUUCU
SCN1A-IVS22-511	166864313	166864331	107	CAAAGAATTAAATGAGAG	467	CAAAGAAUUAAAUGAGAG
SCN1A-IVS22-506	166864308	166864326	108	TAGGGCAAAGAATTAAAT	468	UAGGGCAAAGAAUUAAAU
SCN1A-IVS22-501	166864303	166864321	109	GCTGCTAGGGCAAAGAAT	469	GCUGCUAGGGCAAAGAAU
SCN1A-IVS22-496	166864298	166864316	110	TTTATGCTGCTAGGGCAA	470	UUUAUGCUGCUAGGGCAA
SCN1A-IVS22-491	166864293	166864311	111	GTGATTTTATGCTGCTAG	471	GUGAUUUUAUGCUGCUAG
SCN1A-IVS22-486	166864288	166864306	112	CTATTGTGATTTTATGCT	472	CUAUUGUGAUUUUAUGCU
SCN1A-IVS22-481	166864283	166864301	113	CGCAGCTATTGTGATTTT	473	CGCAGCUAUUGUGAUUUU
SCN1A-IVS22-476	166864278	166864296	114	TTTGACGCAGCTATTGTG	474	UUUGACGCAGCUAUUGUG

SCN1A-IVS22-471	166864273	166864291	115	TACGCTTTGACGCAGCTA	475	UACGCUUUGACGCAGCUA
SCN1A-IVS22-466	166864268	166864286	116	TGAGTTACGCTTTGACGC	476	UGAGUUACGCUUUGACGC
SCN1A-IVS22-461	166864263	166864281	117	GTGCCTGAGTTACGCTTT	477	GUGCCUGAGUUACGCUUU
SCN1A-IVS22-456	166864258	166864276	118	AATGAGTGCCTGAGTTAC	478	AAUGAGUGCCUGAGUUAC
SCN1A-IVS22-451	166864253	166864271	119	AATAAAATGAGTGCCTGA	479	AAUAAAAUGAGUGCCUGA
SCN1A-IVS22-446	166864248	166864266	120	ACAAAAATAAAATGAGTG	480	ACAAAAAUAAAAUGAGUG
SCN1A-IVS22-441	166864243	166864261	121	GAACAACAAAAATAAAAT	481	GAACAACAAAAAUAAAAU
SCN1A-IVS22-436	166864238	166864256	122	TAACAGAACAACAAAAAT	482	UAACAGAACAACAAAAAU
SCN1A-IVS22-431	166864233	166864251	123	AAAAATAACAGAACAACA	483	AAAAAUAACAGAACAACA
SCN1A-IVS22-426	166864228	166864246	124	TTTGAAAAAATAACAGAA	484	UUUGAAAAAAUAACAGAA
SCN1A-IVS22-421	166864223	166864241	125	CATGCTTTGAAAAAATAA	485	CAUGCUUUGAAAAAAUAA
SCN1A-IVS22-416	166864218	166864236	126	AAGCACATGCTTTGAAAA	486	AAGCACAUGCUUUGAAAA
SCN1A-IVS22-411	166864213	166864231	127	CATAAAAGCACATGCTTT	487	CAUAAAAGCACAUGCUUU
SCN1A-IVS22-406	166864208	166864226	128	TGTTGCATAAAAGCACAT	488	UGUUGCAUAAAAGCACAU
SCN1A-IVS22-401	166864203	166864221	129	AGTAATGTTGCATAAAAG	489	AGUAAUGUUGCAUAAAAG
SCN1A-IVS22-396	166864198	166864216	130	TATTCAGTAATGTTGCAT	490	UAUUCAGUAAUGUUGCAU
SCN1A-IVS22-391	166864193	166864211	131	TGCTTTATTCAGTAATGT	491	UGCUUUAUUCAGUAAUGU
SCN1A-IVS22-386	166864188	166864206	132	CAACATGCTTTATTCAGT	492	CAACAUGCUUUAUUCAGU
SCN1A-IVS22-381	166864183	166864201	133	CTGTACAACATGCTTTAT	493	CUGUACAACAUGCUUUAU
SCN1A-IVS22-376	166864178	166864196	134	AAGCACTGTACAACATGC	494	AAGCACUGUACAACAUGC
SCN1A-IVS22-371	166864173	166864191	135	TTATCAAGCACTGTACAA	495	UUAUCAAGCACUGUACAA
SCN1A-IVS22-366	166864168	166864186	136	ACTTCTTATCAAGCACTG	496	ACUUCUUAUCAAGCACUG
SCN1A-IVS22-361	166864163	166864181	137	TTCTAACTTCTTATCAAG	497	UUCUAACUUCUUAUCAAG
SCN1A-IVS22-356	166864158	166864176	138	TTACTTTCTAACTTCTTA	498	UUACUUUCUAACUUCUUA
SCN1A-IVS22-351	166864153	166864171	139	ATTTGTTACTTTCTAACT	499	AUUUGUUACUUUCUAACU
SCN1A-IVS22-346	166864148	166864166	140	AATTTATTTGTTACTTTC	500	AAUUUAUUUGUUACUUUC
SCN1A-IVS22-341	166864143	166864161	141	ATGATAATTTATTTGTTA	501	AUGAUAAUUUAUUUGUUA

SCN1A-IVS22-336	166864138	166864156	142	ACGTGATGATAATTTATT	502	ACGUGAUGAUAAUUUAUU
SCN1A-IVS22-331	166864133	166864151	143	GTGCAACGTGATGATAAT	503	GUGCAACGUGAUGAUAAU
SCN1A-IVS22-326	166864128	166864146	144	ACAAAGTGCAACGTGATG	504	ACAAAGUGCAACGUGAUG
SCN1A-IVS22-321	166864123	166864141	145	AAAACACAAAGTGCAACG	505	AAAACACAAAGUGCAACG
SCN1A-IVS22-316	166864118	166864136	146	CATGCAAAACACAAAGTG	506	CAUGCAAAACACAAAGUG
SCN1A-IVS22-311	166864113	166864131	147	TAAAACATGCAAAACACA	507	UAAAACAUGCAAAACACA
SCN1A-IVS22-306	166864108	166864126	148	GTGCATAAAACATGCAAA	508	GUGCAUAAAACAUGCAAA
SCN1A-IVS22-301	166864103	166864121	149	GAAATGTGCATAAAACAT	509	GAAAUGUGCAUAAAACAU
SCN1A-IVS22-296	166864098	166864116	150	AGCCAGAAATGTGCATAA	510	AGCCAGAAAUGUGCAUAA
SCN1A-IVS22-291	166864093	166864111	151	CTGTCAGCCAGAAATGTG	511	CUGUCAGCCAGAAAUGUG
SCN1A-IVS22-286	166864088	166864106	152	AAAAGCTGTCAGCCAGAA	512	AAAAGCUGUCAGCCAGAA
SCN1A-IVS22-281	166864083	166864101	153	TGTTTAAAAGCTGTCAGC	513	UGUUUAAAAGCUGUCAGC
SCN1A-IVS22-276	166864078	166864096	154	ATAAATGTTTAAAAGCTG	514	AUAAAUGUUUAAAAGCUG
SCN1A-IVS22-271	166864073	166864091	155	ATACAATAAATGTTTAAA	515	AUACAAUAAAUGUUUAAA
SCN1A-IVS22-266	166864068	166864086	156	TTGAAATACAATAAATGT	516	UUGAAAUACAAUAAAUGU
SCN1A-IVS22-261	166864063	166864081	157	GAAATTTGAAATACAATA	517	GAAAUUUGAAAUACAAUA
SCN1A-IVS22-256	166864058	166864076	158	GACTGGAAATTTGAAATA	518	GACUGGAAAUUUGAAAUA
SCN1A-IVS22-251	166864053	166864071	159	ATTTGGACTGGAAATTTG	519	AUUUGGACUGGAAAUUUG
SCN1A-IVS22-246	166864048	166864066	160	GAAAAATTTGGACTGGAA	520	GAAAAAUUUGGACUGGAA
SCN1A-IVS22-241	166864043	166864061	161	AAGTTGAAAAATTTGGAC	521	AAGUUGAAAAAUUUGGAC
SCN1A-IVS22-236	166864038	166864056	162	TTTACAAGTTGAAAAATT	522	UUUACAAGUUGAAAAAUU
SCN1A-IVS22-231	166864033	166864051	163	TTAATTTTACAAGTTGAA	523	UUAAUUUUACAAGUUGAA
SCN1A-IVS22-226	166864028	166864046	164	TCAGTTTAATTTTACAAG	524	UCAGUUUAAUUUUACAAG
SCN1A-IVS22-221	166864023	166864041	165	TTCACTCAGTTTAATTTT	525	UUCACUCAGUUUAAUUUU
SCN1A-IVS22-216	166864018	166864036	166	ATCAATTCACTCAGTTTA	526	AUCAAUUCACUCAGUUUA
SCN1A-IVS22-211	166864013	166864031	167	ACGACATCAATTCACTCA	527	ACGACAUCAAUUCACUCA
SCN1A-IVS22-206	166864008	166864026	168	TATTCACGACATCAATTC	528	UAUUCACGACAUCAAUUC

SCN1A-IVS22-201	166864003	166864021	169	CTAGATATTCACGACATC	529	CUAGAUAUUCACGACAUC
SCN1A-IVS22-196	166863998	166864016	170	TTACCCTAGATATTCACG	530	UUACCCUAGAUAUUCACG
SCN1A-IVS22-191	166863993	166864011	171	TTATTTTACCCTAGATAT	531	UUAUUUUACCCUAGAUAU
SCN1A-IVS22-186	166863988	166864006	172	AAATTTTATTTTACCCTA	532	AAAUUUUAUUUUACCCUA
SCN1A-IVS22-181	166863983	166864001	173	AACACAAATTTTATTTTA	533	AACACAAAUUUUAUUUUA
SCN1A-IVS22-176	166863978	166863996	174	ATTTAAACACAAATTTTA	534	AUUUAAACACAAAUUUUA
SCN1A-IVS22-171	166863973	166863991	175	TACAAATTTAAACACAAA	535	UACAAAUUUAAACACAAA
SCN1A-IVS22-166	166863968	166863986	176	AAAAATACAAATTTAAAC	536	AAAAAUACAAAUUUAAAC
SCN1A-IVS22-161	166863963	166863981	177	AAATTAAAAATACAAATT	537	AAAUUAAAAAUACAAAUU
SCN1A-IVS22-156	166863958	166863976	178	TTAGGAAATTAAAAATAC	538	UUAGGAAAUUAAAAAUAC
SCN1A-IVS22-151	166863953	166863971	179	CTAGGTTAGGAAATTAAA	539	CUAGGUUAGGAAAUUAAA
SCN1A-IVS22-146	166863948	166863966	180	ATTTCCTAGGTTAGGAAA	540	AUUUCCUAGGUUAGGAAA
SCN1A-IVS22-141	166863943	166863961	181	TTAAGATTTCCTAGGTTA	541	UUAAGAUUUCCUAGGUUA
SCN1A-IVS22-136	166863938	166863956	182	GGTATTTAAGATTTCCTA	542	GGUAUUUAAGAUUUCCUA
SCN1A-IVS22-131	166863933	166863951	183	AAGAAGGTATTTAAGATT	543	AAGAAGGUAUUUAAGAUU
SCN1A-IVS22-126	166863928	166863946	184	TGAAAAAGAAGGTATTTA	544	UGAAAAAGAAGGUAUUUA
SCN1A-IVS22-121	166863923	166863941	185	TCTTTTGAAAAAGAAGGT	545	UCUUUUGAAAAAGAAGGU
SCN1A-IVS22-116	166863918	166863936	186	TGAGTTCTTTTGAAAAAG	546	UGAGUUCUUUUGAAAAAG
SCN1A-IVS22-111	166863913	166863931	187	AGACTTGAGTTCTTTTGA	547	AGACUUGAGUUCUUUUGA
SCN1A-IVS22-106	166863908	166863926	188	CATTAAGACTTGAGTTCT	548	CAUUAAGACUUGAGUUCU
SCN1A-IVS22-101	166863903	166863921	189	CTATCCATTAAGACTTGA	549	CUAUCCAUUAAGACUUGA
SCN1A-IVS22-096	166863898	166863916	190	TTTCCCTATCCATTAAGA	550	UUUCCCUAUCCAUUAAGA
SCN1A-IVS22-091	166863893	166863911	191	GTCTGTTTCCCTATCCAT	551	GUCUGUUUCCCUAUCCAU
SCN1A-IVS23+091	166863631	166863649	192	TTTCCCTACTGTGGTGCA	552	UUUCCCUACUGUGGUGCA
SCN1A-IVS23+096	166863626	166863644	193	TGTATTTTCCCTACTGTG	553	UGUAUUUUCCCUACUGUG
SCN1A-IVS23+101	166863621	166863639	194	AATAATGTATTTTCCCTA	554	AAUAAUGUAUUUUCCCUA
SCN1A-IVS23+106	166863616	166863634	195	ATGTAAATAATGTATTTT	555	AUGUAAAUAAUGUAUUUU

SCN1A-IVS23+111	166863611	166863629	196	TTAGGATGTAAATAATGT	556	UUAGGAUGUAAAUAAUGU
SCN1A-IVS23+116	166863606	166863624	197	AGGGATTAGGATGTAAAT	557	AGGGAUUAGGAUGUAAAU
SCN1A-IVS23+121	166863601	166863619	198	AAAGAGGGAATTAGGATG	558	AAAGAGGGAAUUAGGAUG
SCN1A-IVS23+126	166863596	166863614	199	ATTGAAAAGAAGGGATTA	559	AUUGAAAAGAAGGGAUUA
SCN1A-IVS23+131	166863591	166863609	200	AGACAATTGAAAAGAGGG	560	AGACAAUUGAAAAGAGGG
SCN1A-IVS23+136	166863586	166863604	201	ATTTAAGACAATTGAAAA	561	AUUUAAGACAAUUGAAAA
SCN1A-IVS23+141	166863581	166863599	202	ATGAAATTTAAGACAATT	562	AUGAAAUUUAAGACAAUU
SCN1A-IVS23+146	166863576	166863594	203	TTCAAATGAAATTTAAGA	563	UUCAAAUGAAAUUUAAGA
SCN1A-IVS23+151	166863571	166863589	204	TTTTTTTCAAATGAAATT	564	UUUUUUUCAAAUGAAAUU
SCN1A-IVS23+156	166863566	166863584	205	TTTTTTTTTTTTCAAATG	565	UUUUUUUUUUUUCAAAUG
SCN1A-IVS23+161	166863561	166863579	206	AAGGTTTTTTTTTTTTTC	566	AAGGUUUUUUUUUUUUUC
SCN1A-IVS23+166	166863556	166863574	207	TCATAAAGGTTTTTTTTT	567	UCAUAAAGGUUUUUUUUU
SCN1A-IVS23+171	166863551	166863569	208	TAAATTCATAAAGGTTTT	568	UAAAUUCAUAAAGGUUUU
SCN1A-IVS23+176	166863546	166863564	209	GAGGGTAAATTCATAAAG	569	GAGGGUAAAUUCAUAAAG
SCN1A-IVS23+181	166863541	166863559	210	CCACAGAGGGTAAATTCA	570	CCACAGAGGGUAAAUUCA
SCN1A-IVS23+186	166863536	166863554	211	AAAATCCACAGAGGGTAA	571	AAAAUCCACAGAGGGUAA
SCN1A-IVS23+191	166863531	166863549	212	GGGTTAAAATCCACAGAG	572	GGGUUAAAAUCCACAGAG
SCN1A-IVS23+196	166863526	166863544	213	CATTGGGATTAAAATCCA	573	CAUUGGGAUUAAAAUCCA
SCN1A-IVS23+201	166863521	166863539	214	TCAACCATTAGGGTTAAA	574	UCAACCAUUAGGGUUAAA
SCN1A-IVS23+206	166863516	166863534	215	AGATATCAACCATTGGGA	575	AGAUAUCAACCAUUGGGA
SCN1A-IVS23+211	166863511	166863529	216	AATAAAGATATCAACCAT	576	AAUAAAGAUAUCAACCAU
SCN1A-IVS23+216	166863506	166863524	217	AACTTAATAAAGATATCA	577	AACUUAAUAAAGAUAUCA
SCN1A-IVS23+221	166863501	166863519	218	AATGAAACTTAATAAAGA	578	AAUGAAACUUAAUAAAGA
SCN1A-IVS23+226	166863496	166863514	219	TATTCAATGAAACTTAAT	579	UAUUCAAUGAAACUUAAU
SCN1A-IVS23+231	166863491	166863509	220	AATCATATTCAATGAAAC	580	AAUCAUAUUCAAUGAAAC
SCN1A-IVS23+236	166863486	166863504	221	AACTAAATCATATTCAAT	581	AACUAAAUCAUAUUCAAU
SCN1A-IVS23+241	166863481	166863499	222	CACATAACTAAATCATAT	582	CACAUAACUAAAUCAUAU

SCN1A-IVS23+246	166863476	166863494	223	ATATACACATAACTAAAT	583	AUAUACACAUAACUAAAU
SCN1A-IVS23+251	166863471	166863489	224	ACTCCATATACACATAAC	584	ACUCCAUAUACACAUAAC
SCN1A-IVS23+256	166863466	166863484	225	GGATAACTCCATATACAC	585	GGAUAACUCCAUAUACAC
SCN1A-IVS23+261	166863461	166863479	226	AAGATGGATAACTCCATA	586	AAGAUGGAUAACUCCAUA
SCN1A-IVS23+266	166863456	166863474	227	CCCCAAAGATGGATAACT	587	CCCCAAAGAUGGAUAACU
SCN1A-IVS23+271	166863451	166863469	228	AATCTCCCCAAAGATGGA	588	AAUCUCCCCAAAGAUGGA
SCN1A-IVS23+276	166863446	166863464	229	CCAGTAATCTCCCCAAAG	589	CCAGUAAUCUCCCCAAAG
SCN1A-IVS23+281	166863441	166863459	230	CCAATCCAGTAATCTCCC	590	CCAAUCCAGUAAUCUCCC
SCN1A-IVS23+286	166863436	166863454	231	CCTCACCAATCCAGTAAT	591	CCUCACCAAUCCAGUAAU
SCN1A-IVS23+291	166863431	166863449	232	CCCGCCCTCACCAATCCA	592	CCCGCCCUCACCAAUCCA
SCN1A-IVS23+296	166863426	166863444	233	GGTCCCCCGCCCTCACCA	593	GGUCCCCCGCCCUCACCA
SCN1A-IVS23+301	166863421	166863439	234	ACCAGGGTCCCCCGCCCT	594	ACCAGGGUCCCCCGCCCU
SCN1A-IVS23+306	166863416	166863434	235	TCTACACCAGGGTCCCCC	595	UCUACACCAGGGUCCCCC
SCN1A-IVS23+311	166863411	166863429	236	ATCATTCTACACCAGGGT	596	AUCAUUCUACACCAGGGU
SCN1A-IVS23+316	166863406	166863424	237	ACATAATCATTCTACACC	597	ACAUAAUCAUUCUACACC
SCN1A-IVS23+321	166863401	166863419	238	TTTTCACATAATCATTCT	598	UUUUCACAUAAUCAUUCU
SCN1A-IVS23+326	166863396	166863414	239	TTGTTTTTTCACATAATC	599	UUGUUUUUUCACAUAAUC
SCN1A-IVS23+331	166863391	166863409	240	TTAAATTGTTTTTTCACA	600	UUAAAUUGUUUUUUCACA
SCN1A-IVS23+336	166863386	166863404	241	ACAAGTTAAATTGTTTTT	601	ACAAGUUAAAUUGUUUUU
SCN1A-IVS23+341	166863381	166863399	242	GCTTAACAAGTTAAATTG	602	GCUUAACAAGUUAAAUUG
SCN1A-IVS23+346	166863376	166863394	243	CATGAGCTTAACAAGTTA	603	CAUGAGCUUAACAAGUUA
SCN1A-IVS23+351	166863371	166863389	244	AGTATCATGAGCTTAACA	604	AGUAUCAUGAGCUUAACA
SCN1A-IVS23+356	166863366	166863384	245	CAAACAGTATCATGAGCT	605	CAAACAGUAUCAUGAGCU
SCN1A-IVS23+361	166863361	166863379	246	TGCCTCAAACAGTATCAT	606	UGCCUCAAACAGUAUCAU
SCN1A-IVS23+366	166863356	166863374	247	CTGTATGCCTCAAACAGT	607	CUGUAUGCCUCAAACAGU
SCN1A-IVS23+371	166863351	166863369	248	AGGGACTGTATGCCTCAA	608	AGGGACUGUAUGCCUCAA
SCN1A-IVS23+376	166863346	166863364	249	ACAGCAGGGACTGTATGC	609	ACAGCAGGGACUGUAUGC

SCN1A-IVS23+381	166863341	166863359	250	ACTAAACAGCAAGGGCTG	610	ACUAAACAGCAAGGGCUG
SCN1A-IVS23+386	166863336	166863354	251	AATGTACTAAACAGCAGG	611	AAUGUACUAAACAGCAGG
SCN1A-IVS23+391	166863331	166863349	252	AGACCAATGTACTAAACA	612	AGACCAAUGUACUAAACA
SCN1A-IVS23+396	166863326	166863344	253	GACCCAGACCAATGTACT	613	GACCCAGACCAAUGUACU
SCN1A-IVS23+401	166863321	166863339	254	TTCAGGACCCAGACCAAT	614	UUCAGGACCCAGACCAAU
SCN1A-IVS23+406	166863316	166863334	255	TAATTTTCAGGACCCAGA	615	UAAUUUUCAGGACCCAGA
SCN1A-IVS23+411	166863311	166863329	256	ACTGGTAATTTTCAGGAC	616	ACUGGUAAUUUUCAGGAC
SCN1A-IVS23+416	166863306	166863324	257	ATCTAACTGGTAATTTTC	617	AUCUAACUGGUAAUUUUC
SCN1A-IVS23+421	166863301	166863319	258	ATGGTATCTAACTGGTAA	618	AUGGUAUCUAACUGGUAA
SCN1A-IVS23+426	166863296	166863314	259	AACTGATGGTATCTAACT	619	AACUGAUGGUAUCUAACU
SCN1A-IVS23+431	166863291	166863309	260	TAATCAACTGATGGTATC	620	UAAUCAACUGAUGGUAUC
SCN1A-IVS23+436	166863286	166863304	261	ATCAATAATCAACTGATG	621	AUCAAUAAUCAACUGAUG
SCN1A-IVS23+441	166863281	166863299	262	TACATATCAATAATCAAC	622	UACAUAUCAAUAAUCAAC
SCN1A-IVS23+446	166863276	166863294	263	GCTCATACATATCAATAA	623	GCUCAUACAUAUCAAUAA
SCN1A-IVS23+451	166863271	166863289	264	TATCTGCTCATACATATC	624	UAUCUGCUCAUACAUAUC
SCN1A-IVS23+456	166863266	166863284	265	CCTAGTATCTGCTCATAC	625	CCUAGUAUCUGCUCAUAC
SCN1A-IVS23+461	166863261	166863279	266	TGCACCCTAGTATCTGCT	626	UGCACCCUAGUAUCUGCU
SCN1A-IVS23+466	166863256	166863274	267	AATATTGCACCCTAGTAT	627	AAUAUUGCACCCUAGUAU
SCN1A-IVS23+471	166863251	166863269	268	CCTGAAATATTGCACCCT	628	CCUGAAAUAUUGCACCCU
SCN1A-IVS23+476	166863246	166863264	269	TGAAACCTGAAATATTGC	629	UGAAACCUGAAAUAUUGC
SCN1A-IVS23+481	166863241	166863259	270	TCTTATGAAACCTGAAAT	630	UCUUAUGAAACCUGAAAU
SCN1A-IVS23+486	166863236	166863254	271	ACCAGTCTTATGAAACCT	631	ACCAGUCUUAUGAAACCU
SCN1A-IVS23+491	166863231	166863249	272	TCAATACCAGTCTTATGA	632	UCAAUACCAGUCUUAUGA
SCN1A-IVS23+496	166863226	166863244	273	CACAATCAATACCAGTCT	633	CACAAUCAAUACCAGUCU
SCN1A-IVS23+501	166863221	166863239	274	GTGGTCACAATCAATACC	634	GUGGUCACAAUCAAUACC
SCN1A-IVS23+506	166863216	166863234	275	TGAGAGTGGTCACAATCA	635	UGAGAGUGGUCACAAUCA
SCN1A-IVS23+511	166863211	166863229	276	AAAAATGAGAGTGGTCAC	636	AAAAAUGAGAGUGGUCAC

SCN1A-IVS23+516	166863206	166863224	277	CAATAAAAAATGAGAGTG	637	CAAUAAAAAAUGAGAGUG
SCN1A-IVS23+521	166863201	166863219	278	TTACACAATAAAAAATGA	638	UUACACAAUAAAAAAUGA
SCN1A-IVS23+526	166863196	166863214	279	TGAACTTACACAATAAAA	639	UGAACUUACACAAUAAAA
SCN1A-IVS23+531	166863191	166863209	280	CCATATGAACTTACACAA	640	CCAUAUGAACUUACACAA
SCN1A-IVS23+536	166863186	166863204	281	TAACCCCATATGAACTTA	641	UAACCCCAUAUGAACUUA
SCN1A-IVS23+541	166863181	166863199	282	GAAAATAACCCCATATGA	642	GAAAAUAACCCCAUAUGA
SCN1A-IVS23+546	166863176	166863194	283	ATTTTGAAAATAACCCCA	643	AUUUUGAAAAUAACCCCA
SCN1A-IVS23+551	166863171	166863189	284	TTAACATTTTGAAAATAA	644	UUAACAUUUUGAAAAUAA
SCN1A-IVS23+556	166863166	166863184	285	CCTTGTTAACATTTTGAA	645	CCUUGUUAACAUUUUGAA
SCN1A-IVS23+561	166863161	166863179	286	TTTTGCCTTGTTAACATT	646	UUUUGCCUUGUUAACAUU
SCN1A-IVS23+566	166863156	166863174	287	TATATTTTTGCCTTGTTA	647	UAUAUUUUUGCCUUGUUA
SCN1A-IVS23+571	166863151	166863169	288	CTTAATATATTTTTGCCT	648	CUUAAUAUAUUUUUGCCU
SCN1A-IVS23+576	166863146	166863164	289	TATTTCTTAATATATTTT	649	UAUUUCUUAAUAUAUUUU
SCN1A-IVS23+581	166863141	166863159	290	TCAACTATTTCTTAATAT	650	UCAACUAUUUCUUAAUAU
SCN1A-IVS23+586	166863136	166863154	291	CTTATTCAACTATTTCTT	651	CUUAUUCAACUAUUUCUU
SCN1A-IVS23+591	166863131	166863149	292	ATGTGCTTATTCAACTAT	652	AUGUGCUUAUUCAACUAU
SCN1A-IVS23+596	166863126	166863144	293	TTCACATGTGCTTATTCA	653	UUCACAUGUGCUUAUUCA
SCN1A-IVS23+601	166863121	166863139	294	CACAATTCACATGTGCTT	654	CACAAUUCACAUGUGCUU
SCN1A-IVS23+606	166863116	166863134	295	TACAACACAATTCACATG	655	UACAACACAAUUCACAUG
SCN1A-IVS23+611	166863111	166863129	296	TTGTTTACAACACAATTC	656	UUGUUUACAACACAAUUC
SCN1A-IVS23+616	166863106	166863124	297	ACTTTTTGTTTACAACAC	657	ACUUUUUGUUUACAACAC
SCN1A-IVS23+621	166863101	166863119	298	TTCTAACTTTTTGTTTAC	658	UUCUAACUUUUUGUUUAC
SCN1A-IVS23+626	166863096	166863114	299	TTTTATTCTAACTTTTTG	659	UUUUAUUCUAACUUUUUG
SCN1A-IVS23+631	166863091	166863109	300	GATTTTTTTATTCTAACT	660	GAUUUUUUUAUUCUAACU
SCN1A-IVS23+636	166863086	166863104	301	AAGTGGATTTTTTTATTC	661	AAGUGGAUUUUUUUAUUC
SCN1A-IVS23+641	166863081	166863099	302	CAAATAAGTGGATTTTTT	662	CAAAUAAGUGGAUUUUUU
SCN1A-IVS23+646	166863076	166863094	303	TAATTCAAATAAGTGGAT	663	UAAUUCAAAUAAGUGGAU

SCN1A-IVS23+651	166863071	166863089	304	CTGCATAATTCAAATAAG	664	CUGCAUAAUUCAAAUAAG
SCN1A-IVS23+656	166863066	166863084	305	CTATTCTGCATAATTCAA	665	CUAUUCUGCAUAAUUCAA
SCN1A-IVS23+661	166863061	166863079	306	GTATTCTATTCTGCATAA	666	GUAUUCUAUUCUGCAUAA
SCN1A-IVS23+666	166863056	166863074	307	GGTATGTATTCTATTCTG	667	GGUAUGUAUUCUAUUCUG
SCN1A-IVS23+671	166863051	166863069	308	TTCTAGGTATGTATTCTA	668	UUCUAGGUAUGUAUUCUA
SCN1A-IVS23+676	166863046	166863064	309	TTTATTTCTAGGTATGTA	669	UUUAUUUCUAGGUAUGUA
SCN1A-IVS23+681	166863041	166863059	310	TTTGTTTTATTTCTAGGT	670	UUUGUUUUAUUUCUAGGU
SCN1A-IVS23+686	166863036	166863054	311	ACGTTTTTGTTTTATTTC	671	ACGUUUUUGUUUUAUUUC
SCN1A-IVS23+691	166863031	166863049	312	ATAAGACGTTTTTGTTTT	672	AUAAGACGUUUUUGUUUU
SCN1A-IVS23+696	166863026	166863044	313	TCATGATAAGACGTTTTT	673	UCAUGAUAAGACGUUUUU
SCN1A-IVS23+701	166863021	166863039	314	AATACTCATGATAAGACG	674	AAUACUCAUGAUAAGACG
SCN1A-IVS23+706	166863016	166863034	315	ATCTTAATACTCATGATA	675	AUCUUAAUACUCAUGAUA
SCN1A-IVS23+711	166863011	166863029	316	ATTTTATCTTAATACTCA	676	AUUUUAUCUUAAUACUCA
SCN1A-IVS23+716	166863006	166863024	317	CTTAAATTTTATCTTAAT	677	CUUAAAUUUUAUCUUAAU
SCN1A-IVS23+721	166863001	166863019	318	TATGCCTTAAATTTTATC	678	UAUGCCUUAAAUUUUAUC
SCN1A-IVS23+726	166862996	166863014	319	GAGTTTATGCCTTAAATT	679	GAGUUUAUGCCUUAAAUU
SCN1A-IVS23+731	166862991	166863009	320	GAAGTGAGTTTATGCCTT	680	GAAGUGAGUUUAUGCCUU
SCN1A-IVS23+736	166862986	166863004	321	TCTAAGAAGTGAGTTTAT	681	UCUAAGAAGUGAGUUUAU
SCN1A-IVS23+741	166862981	166862999	322	CTTATTCTAAGAAGTGAG	682	CUUAUUCUAAGAAGUGAG
SCN1A-IVS23+746	166862976	166862994	323	AGTTACTTATTCTAAGAA	683	AGUUACUUAUUCUAAGAA
SCN1A-IVS23+751	166862971	166862989	324	TTGGGAGTTACTTATTCT	684	UUGGGAGUUACUUAUUCU
SCN1A-IVS23+756	166862966	166862984	325	GTTAGTTGGGAGTTACTT	685	GUUAGUUGGGAGUUACUU
SCN1A-IVS23+761	166862961	166862979	326	AGAAAGTTAGTTGGGAGT	686	AGAAAGUUAGUUGGGAGU
SCN1A-IVS23+766	166862956	166862974	327	ATCCTAGAAAGTTAGTTG	687	AUCCUAGAAAGUUAGUUG
SCN1A-IVS23+771	166862951	166862969	328	TTAAAATCCTAGAAAGTT	688	UUAAAAUCCUAGAAAGUU
SCN1A-IVS23+776	166862946	166862964	329	ATGTTTTAAAATCCTAGA	689	AUGUUUUAAAAUCCUAGA
SCN1A-IVS23+781	166862941	166862959	330	GTGTTATGTTTTAAAATC	690	GUGUUAUGUUUUAAAAUC

SCN1A-IVS23+786	166862936	166862954	331	TCACTGTGTTATGTTTTA	691	UCACUGUGUUAUGUUUUA
SCN1A-IVS23+791	166862931	166862949	332	TGTTTTCACTGTGTTATG	692	UGUUUUCACUGUGUUAUG
SCN1A-IVS23+796	166862926	166862944	333	ATGTATGTTTTCACTGTG	693	AUGUAUGUUUUCACUGUG
SCN1A-IVS23+801	166862921	166862939	334	TGTTTATGTATGTTTTCA	694	UGUUUAUGUAUGUUUUCA
SCN1A-IVS23+806	166862916	166862934	335	AGTTATGTTTATGTATGT	695	AGUUAUGUUUAUGUAUGU
SCN1A-IVS23+811	166862911	166862929	336	TGTAGAGTTATGTTTATG	696	UGUAGAGUUAUGUUUAUG
SCN1A-IVS23+816	166862906	166862924	337	TAAAATGTAGAGTTATGT	697	UAAAAUGUAGAGUUAUGU
SCN1A-IVS23+821	166862901	166862919	338	ATAAATAAAATGTAGAGT	698	AUAAAUAAAAUGUAGAGU
SCN1A-IVS23+826	166862896	166862914	339	TAAGAATAAATAAAATGT	699	UAAGAAUAAAUAAAAUGU
SCN1A-IVS23+831	166862891	166862909	340	AACTTTAAGAATAAATAA	700	AACUUUAAGAAUAAAUAA
SCN1A-IVS23+836	166862886	166862904	341	ACTTAAACTTTAAGAATA	701	ACUUAAACUUUAAGAAUA
SCN1A-IVS23+841	166862881	166862899	342	AATACACTTAAACTTTAA	702	AAUACACUUAAACUUUAA
SCN1A-IVS23+846	166862876	166862894	343	TGTATAATACACTTAAAC	703	UGUAUAAUACACUUAAAC
SCN1A-IVS23+851	166862871	166862889	344	CTTCTTGTATAATACACT	704	CUUCUUGUAUAAUACACU
SCN1A-IVS23+856	166862866	166862884	345	CTCTTCTTCTTGTATAAT	705	CUCUUCUUCUUGUAUAAU
SCN1A-IVS23+861	166862861	166862879	346	ATAAACTCTTCTTCTTGT	706	AUAAACUCUUCUUCUUGU
SCN1A-IVS23+866	166862856	166862874	347	CGAATATAAACTCTTCTT	707	CGAAUAUAAACUCUUCUU
SCN1A-IVS23+871	166862851	166862869	348	TCTCTCGAATATAAACTC	708	UCUCUCGAAUAUAAACUC
SCN1A-IVS23+876	166862846	166862864	349	TTCTGTCTCTCGAATATA	709	UUCUGUCUCUCGAAUAUA
SCN1A-IVS23+881	166862841	166862859	350	ACTTTTTCTGTCTCTCGA	710	ACUUUUUCUGUCUCUCGA
SCN1A-IVS23+886	166862836	166862854	351	TTCTGACTTTTTCTGTCT	711	UUCUGACUUUUUCUGUCU
SCN1A-IVS23+891	166862831	166862849	352	AAAAATTCTGACTTTTTC	712	AAAAAUUCUGACUUUUUC
SCN1A-IVS23+896	166862826	166862844	353	CAAACAAAAATTCTGACT	713	CAAACAAAAAUUCUGACU
SCN1A-IVS23+901	166862821	166862839	354	TGATCCAAACAAAAATTC	714	UGAUCCAAACAAAAAUUC
SCN1A-IVS23+906	166862816	166862834	355	ATTGGTGATCCAAACAAA	715	AUUGGUGAUCCAAACAAA
SCN1A-IVS23+911	166862811	166862829	356	GATATATTGGTGATCCAA	716	GAUAUAUUGGUGAUCCAA
SCN1A-IVS23+916	166862806	166862824	357	GCTATGATATATTGGTGA	717	GCUAUGAUAUAUUGGUGA

SCN1A-IVS23+921	166862801	166862819	358	TGTAAGCTATGATATATT	718	UGUAAGCUAUGAUAUAUU
SCN1A-IVS23+926	166862796	166862814	359	TTTTTTGTAAGCTATGAT	719	UUUUUUGUAAGCUAUGAU
SCN1A-IVS23+931	166862791	166862809	360	ACAGTTTTTTTGTAAGCT	720	ACAGUUUUUUUGUAAGCU
SCN1A-IVS23+936	166862786	166862804	361	TTAAGACAGTTTTTTTGT	721	UUAAGACAGUUUUUUUGU
SCN1A-IVS23+941	166862781	166862799	362	TTTAATTAAGACAGTTTT	722	UUUAAUUAAGACAGUUUU
SCN1A-IVS23+946	166862776	166862794	363	TGGGTTTTAATTAAGACA	723	UGGGUUUUAAUUAAGACA
SCN1A-IVS23+951	166862771	166862789	364	TGTTGTGGGTTTTAATTA	724	UGUUGUGGGUUUUAAUUA
SCN1A-IVS23+956	166862766	166862784	365	AATTATGTTGTGGGTTTT	725	AAUUAUGUUGUGGGUUUU
SCN1A-IVS23+961	166862761	166862779	366	AAAAAAATTATGTTGTGG	726	AAAAAAAUUAUGUUGUGG
SCN1A-IVS23+966	166862756	166862774	367	AATCTAAAAAAATTATGT	727	AAUCUAAAAAAAUUAUGU
SCN1A-IVS23+971	166862751	166862769	368	TTAAAAATCTAAAAAAAT	728	UUAAAAAUCUAAAAAAAU
SCN1A-IVS23+976	166862746	166862764	369	CTTTCTTAAAAATCTAAA	729	CUUUCUUAAAAAUCUAAA
SCN1A-IVS23+981	166862741	166862759	370	AGAATCTTTCTTAAAAAT	730	AGAAUCUUUCUUAAAAAU
SCN1A-IVS23+986	166862736	166862754	371	ATAATAGAATCTTTCTTA	731	AUAAUAGAAUCUUUCUUA

Example 4: SCN1A Exon 23 (Exon 20x) Region Extended ASO Walk Evaluated by RT-PCR

[00232] ASO walk sequences can be evaluated by for example RT-PCR. In FIG. 5A, a representative PAGE shows SYBR-safe-stained RT-PCR products of SCN1A mock-treated, control ASO treated, targeting the exon 20x region as described herein in the Example 3 and in the description of FIG. 3, at 1 μM concentration in RenCells by nucleofection. Two products, one comprising the exon 20x and one excluding exon 20x were quantified and the percent exon 20x inclusion is plotted in the bar graph (FIG. 5B). Taqman q PCR products were also normalized to RPL32 internal control and fold-change relative to mock is plotted in the bar graph (FIG. 5C).

[00233] While preferred embodiments of the present invention 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 invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention 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 SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is:

from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or

from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE.

2. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A 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 that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is:

from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 100 nucleotides upstream from the 5' end of the NIE; or

from about 100 nucleotides downstream of the 3' end of the NIE to about 1000 nucleotides downstream of the 3' end of the NIE.

3. The method of claim 1 or 2, wherein the therapeutic agent interferes with binding of a factor involved in splicing of the NMD exon from a region of the targeted portion.

4. The method of claim 1 or 2, wherein the targeted portion is at most 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 NIE.

5. The method of claim 1 or 2, wherein the targeted portion is at least 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 NIE.
6. The method of claim 1 or 2, wherein the targeted portion is at most 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 NIE.
7. The method of claim 1 or 2, wherein the targeted portion is at least 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 NIE.
8. The method of any one of the claims 1-7, wherein the therapeutic agent is an antisense oligomer (ASO).
9. The method of claim 8, wherein the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731.
10. The method of any one of the claims 1-9, wherein the therapeutic agent promotes exclusion of the NMD exon from the processed mRNA encoding SCN1A protein.
11. The method of claim 10, wherein exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent 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 exclusion of the NMD exon from the processed mRNA encoding SCN1A protein in a control cell.

12. The method of claim 10, wherein the therapeutic agent increases level of the processed mRNA encoding SCN1A protein in the cell.

13. The method of claim 10, wherein an amount of the processed mRNA encoding SCN1A protein in the cell contacted with the therapeutic agent 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 an total amount of the processed mRNA encoding SCN1A protein in a control cell.

14. The method of claim 2, wherein the disease or condition is induced by a loss-of-function mutation in $Na_v1.1$.

15. The method of claim 14, wherein the disease or condition is associated with haploinsufficiency of the *SCN1A* gene, and wherein the subject has a first allele encoding a functional SCN1A, and a second allele from which SCN1A is not produced or produced at a reduced level, or a second allele encoding a nonfunctional SCN1A or a partially functional SCN1A.

16. The method of claim 14, wherein the disease or condition is encephalopathy.

17. The method of claim 16, wherein the encephalopathy is epileptic encephalopathy.

18. The method of claim 14, wherein the disease or condition is Dravet Syndrome (DS); severe myoclonic epilepsy of infancy (SMEI)-borderland (SMEB); Febrile seizure (FS); epilepsy, generalized, with febrile seizures plus (GEFS+); epileptic encephalopathy, early infantile, 13; cryptogenic generalized epilepsy; cryptogenic focal epilepsy; myoclonic-astatic epilepsy; Lennox-Gastaut syndrome; West syndrome; idiopathic spasms; early myoclonic encephalopathy; progressive myoclonic epilepsy; alternating hemiplegia of childhood; unclassified epileptic encephalopathy; sudden unexpected death in epilepsy (SUDEP); sick sinus syndrome 1; autism; or malignant migrating partial seizures of infancy.

19. The method of claim 18, wherein GEFS+ is epilepsy, generalized, with febrile seizures plus, type 2.

20. The method of claim 18, wherein the Febrile seizure is Febrile seizures, familial, 3A.

21. The method of claim 18, wherein SMEB is SMEB without generalized spike wave (SMEB-SW), SMEB without myoclonic seizures (SMEB-M), SMEB lacking more than one feature of SMEI (SMEB-O), or intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC).

22. The method of claims 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 72 or 432.

23. The method of claims 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 73 or 433.

24. The method of claims 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 76 or 436.

25. The method of claims 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 181 or 541.

26. The method of claims 1 or 2, wherein the ASO consists of a sequence selected from SEQ ID NOs: 220 or 580.

27. A method of modulating expression of SCN1A protein in a cell having an mRNA that contains a non-sense mediated RNA decay-inducing exon (NMD exon mRNA) and encodes SCN1A protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the NMD exon mRNA encoding SCN1A protein, thereby modulating the level of processed mRNA encoding SCN1A protein, and modulating expression of SCN1A protein in the cell, wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

28. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A 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 that contains the NMD exon and encodes SCN1A, thereby modulating the level of processed mRNA encoding the SCN1A protein, and modulating expression of SCN1A protein in the cell of the subject; wherein the therapeutic agent binds to a targeted portion of the NMD exon mRNA encoding SCN1A, and wherein the targeted portion is from about 1000 nucleotides upstream from the 5' end of an NMD-inducing exon (NIE) to about 1000 nucleotides downstream of the 3' end of the NIE.

29. An antisense oligomer (ASO) comprising a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% identity to any one of SEQ ID NOs: 12-731.

30. An antisense oligomer (ASO) consisting of a sequence selected from SEQ ID NOs: 12-731.

31. A method of treating a disease or condition in a subject in need thereof by modulating expression of SCN1A protein in a cell of the subject, comprising:

contacting the cell of the subject with an ASO of claim 29 or claim 30.

32. A kit comprising an ASO of claim 29 or claim 30.

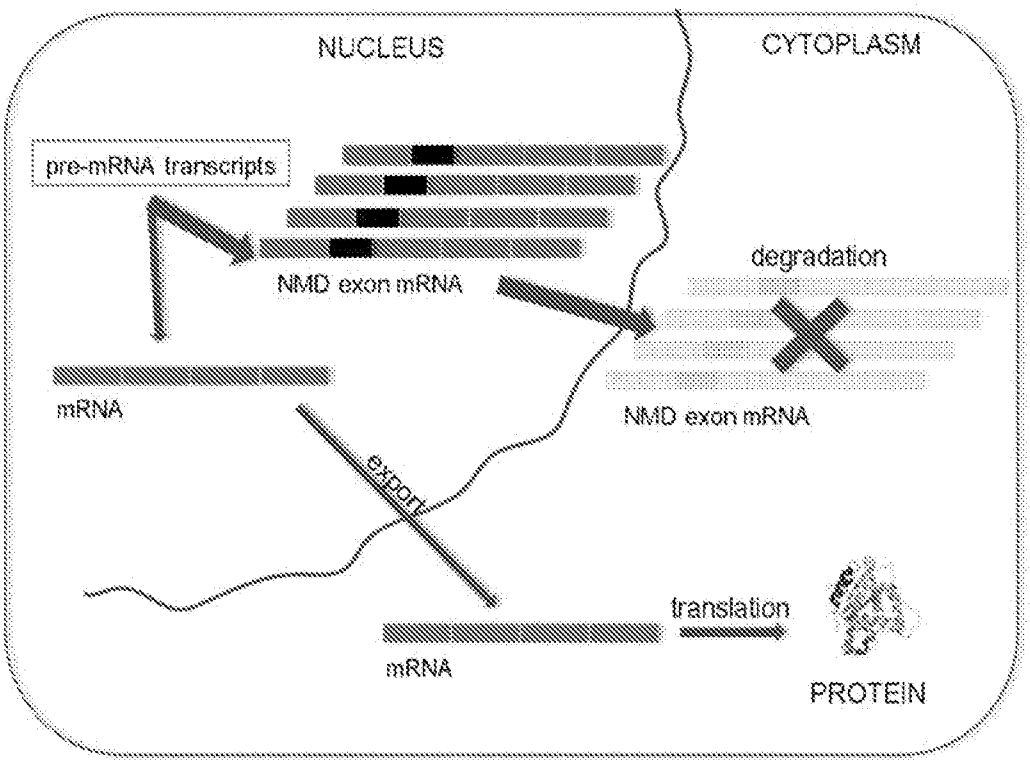

NUCLEUS
CYTOPLASM
pre-mRNA transcripts
NMD exon mRNA
degradation
mRNA
NMD exon mRNA
export
translation
mRNA
PROTEIN

FIG. 1A

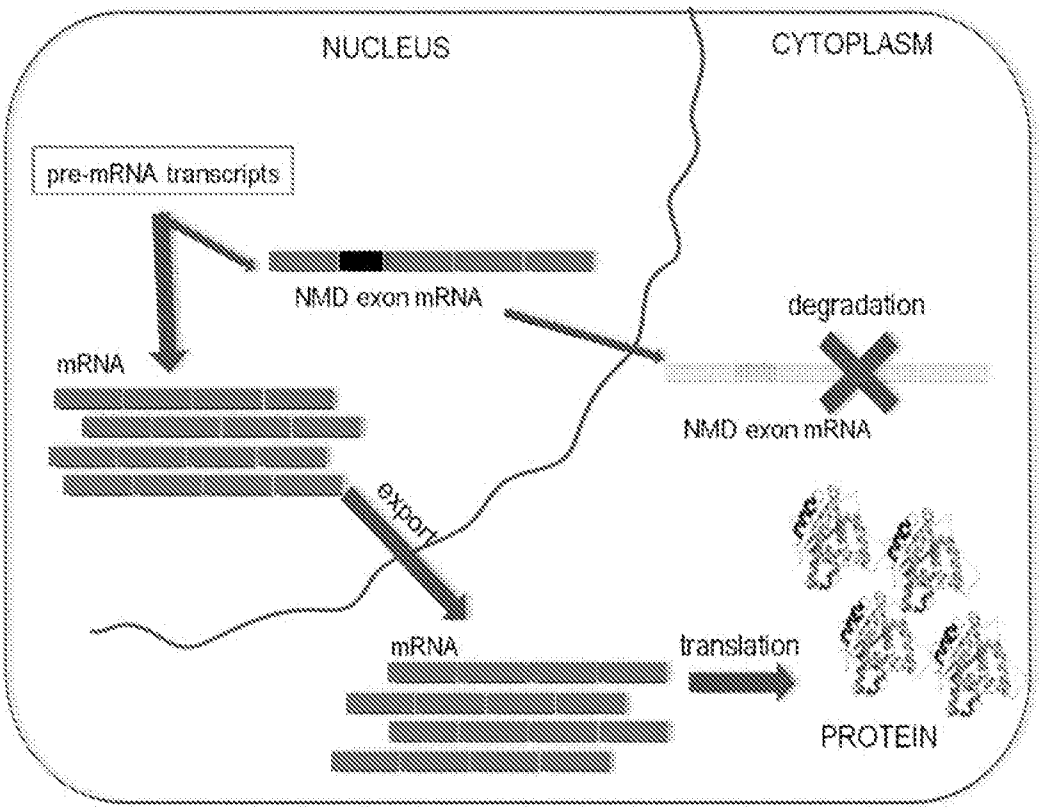

NUCLEUS
CYTOPLASM
pre-mRNA transcripts
NMD exon mRNA
degradation
mRNA
NMD exon mRNA
export
mRNA
translation
PROTEIN

FIG. 1B

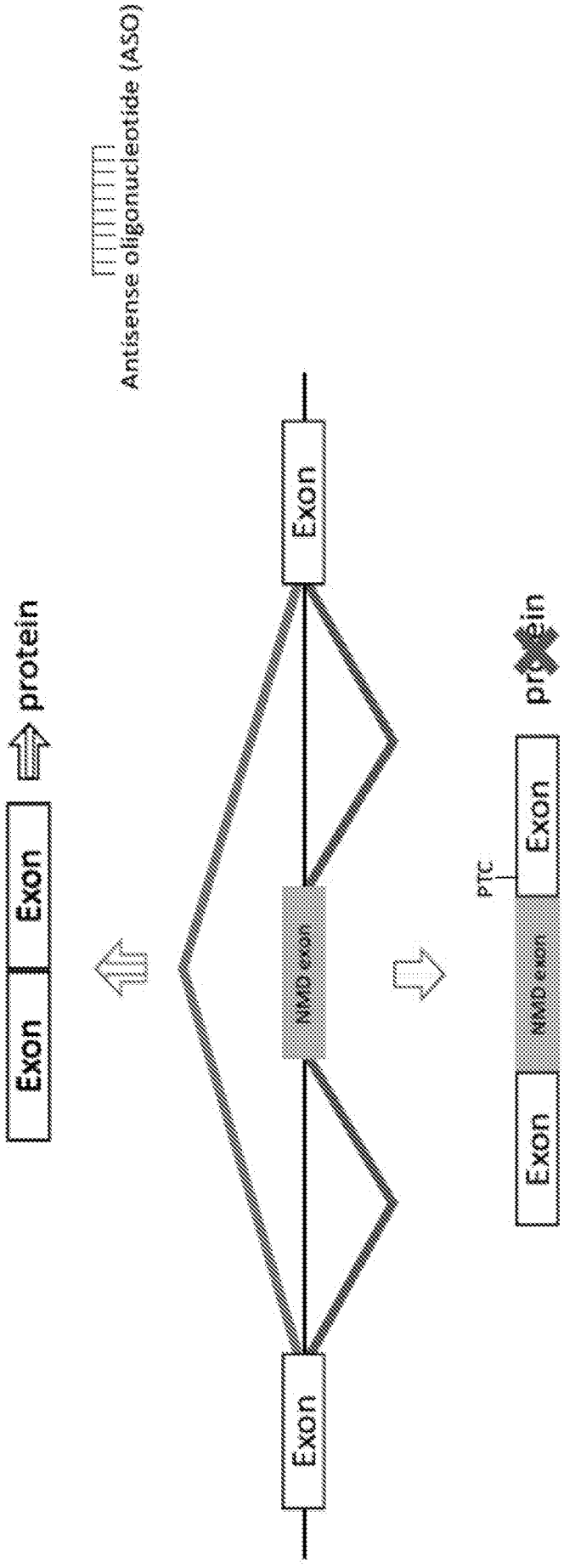

Antisense oligonucleotide (ASO)
Exon
Exon
protein
Exon
NMD exon
Exon
PTC
Exon
NMD exon
Exon
protein

FIG. 1C

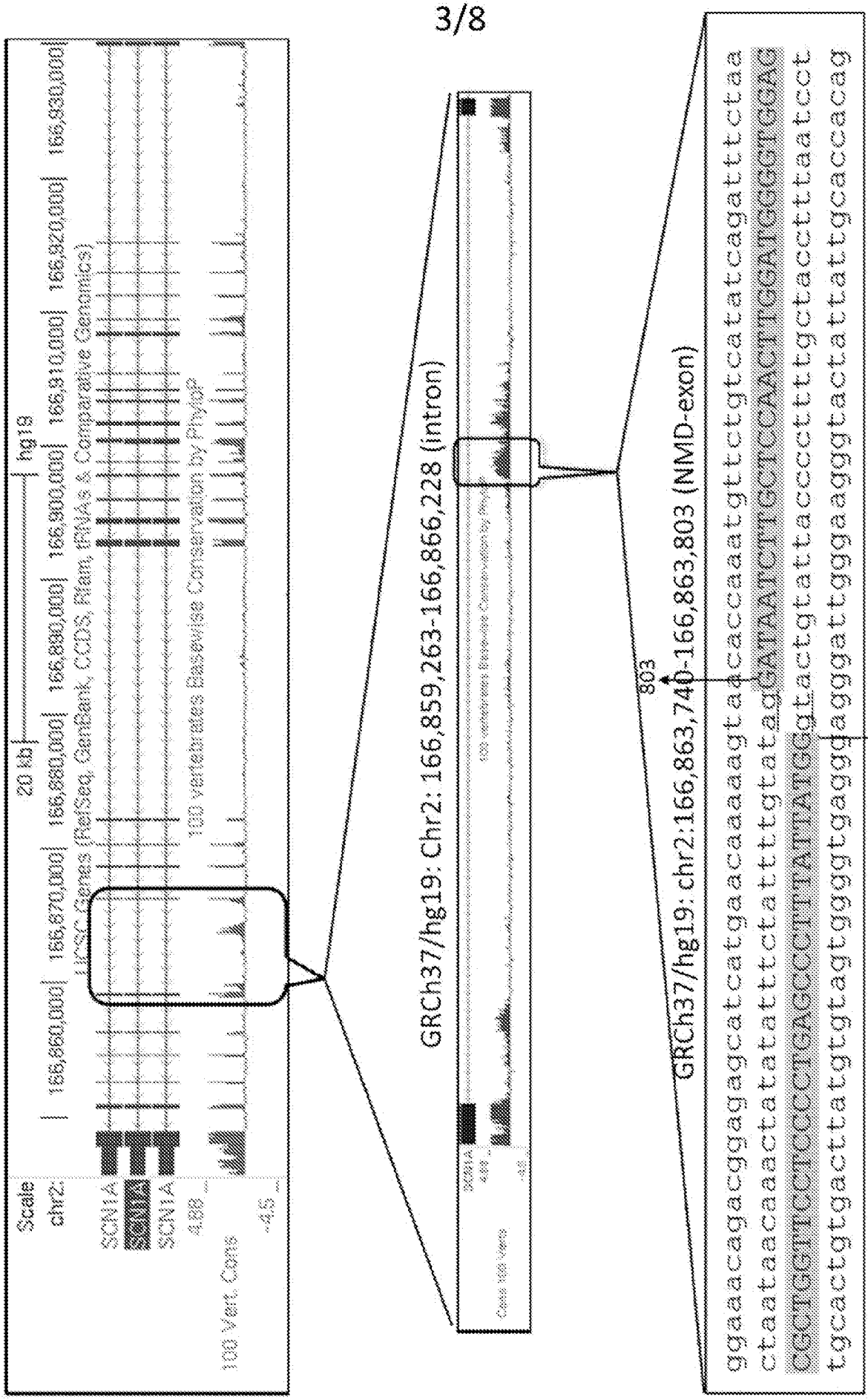

Scale
chr2:
166,860,000
166,870,000
166,880,000
166,890,000
166,900,000
166,910,000
166,920,000
166,930,000
hg19
20 kb
UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)
SCN1A
SCN1A
SCN1A
100 vertebrates Basewise Conservation by PhyloP
100 Vert. Cons
GRCh37/hg19: Chr2: 166,859,263-166,866,228 (intron)
GRCh37/hg19: chr2:166,863,740-166,863,803 (NMD-exon)
803
740
ggaaacagacggagagcatcatgaacaaaaaagtatcaccaaaatgttctgtcatatcagatttctaa
ctaataaacaaaactatatatttctatttgtatagGATAATCTTGCTCCAACTTGGATGGGTGGAG
CGCTGGTTCCTCCCCCTGAGCCCTTTATTATGGgtactgtattacccctttgctacctttaatcct
tgcactgtgacttatgtgtagtgggtgaggtaggggaagggtactattattgcaccacag

FIG. 2

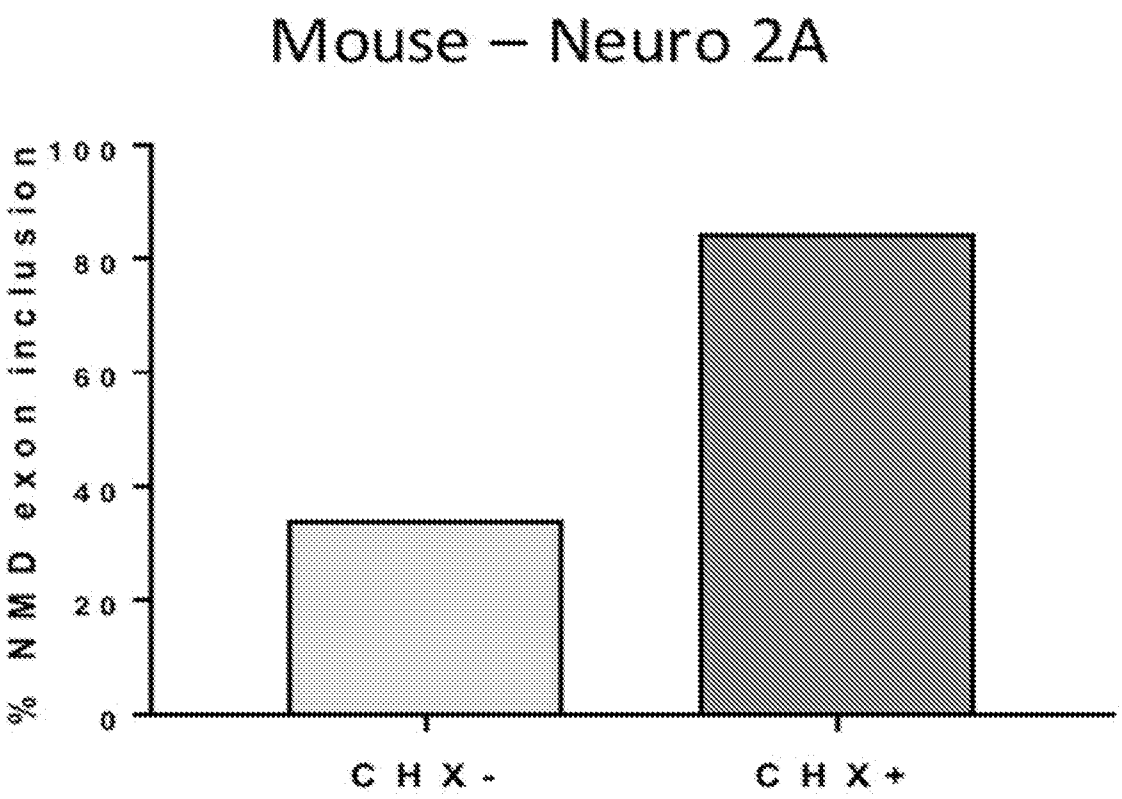

Mouse – Neuro 2A
% NMD exon inclusion
100
80
60
40
20
0
CHX-
CHX+

FIG. 3A

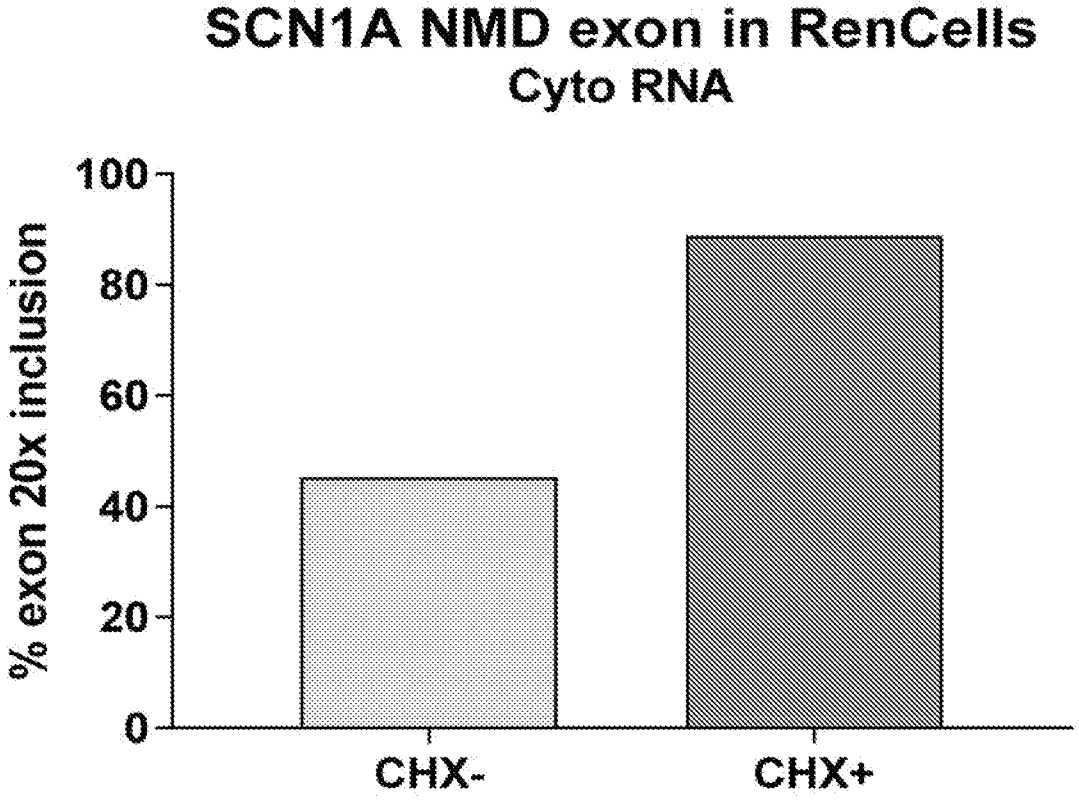
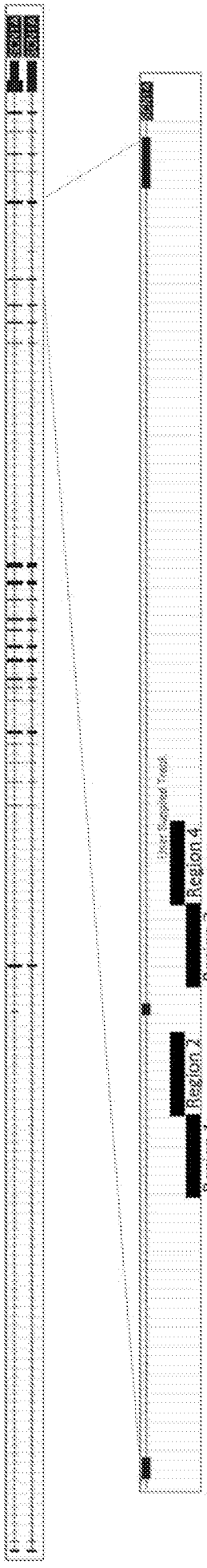

SCN1A NMD exon in RenCells
Cyto RNA
% exon 20x inclusion
100
80
60
40
20
0
CHX-
CHX+

FIG. 3B



	Coordinates	
Region 1	chr2:166007834-166008296	90 ASOs
Region 2	chr2:166007384-166007846	90 ASOs
Region 3	chr2:166006677-166007139	90 ASOs
Region 4	chr2:166006227-166006689	90 ASOs
NMD-exon	chr2:166007230-166007293	

FIG. 4

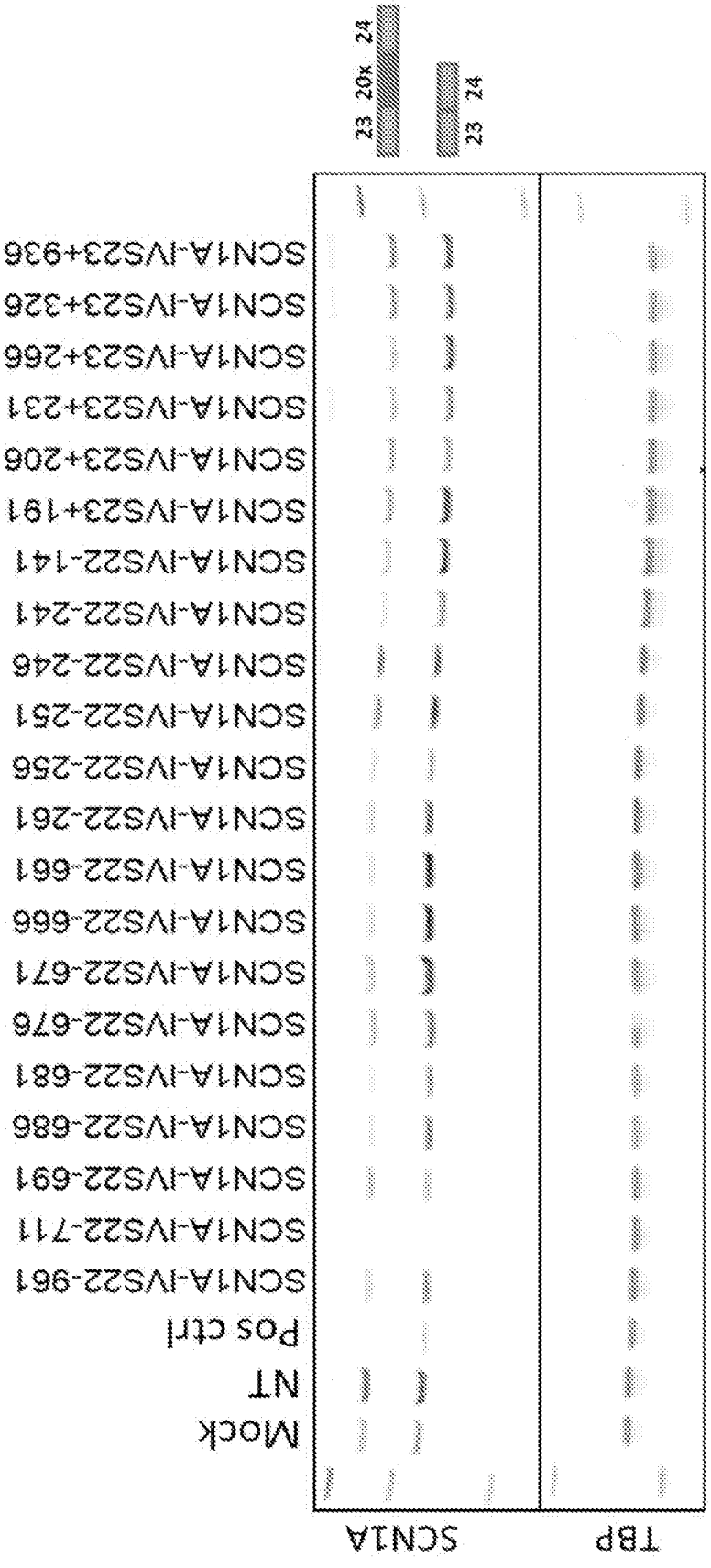

23 20x 24
23 24
SCN1A
TBP
Mock
NT
Pos ctrl
SCN1A-IVS22-961
SCN1A-IVS22-711
SCN1A-IVS22-691
SCN1A-IVS22-686
SCN1A-IVS22-681
SCN1A-IVS22-676
SCN1A-IVS22-671
SCN1A-IVS22-666
SCN1A-IVS22-661
SCN1A-IVS22-261
SCN1A-IVS22-256
SCN1A-IVS22-251
SCN1A-IVS22-246
SCN1A-IVS22-241
SCN1A-IVS22-141
SCN1A-IVS23+191
SCN1A-IVS23+206
SCN1A-IVS23+231
SCN1A-IVS23+266
SCN1A-IVS23+326
SCN1A-IVS23+936

FIG. 5A

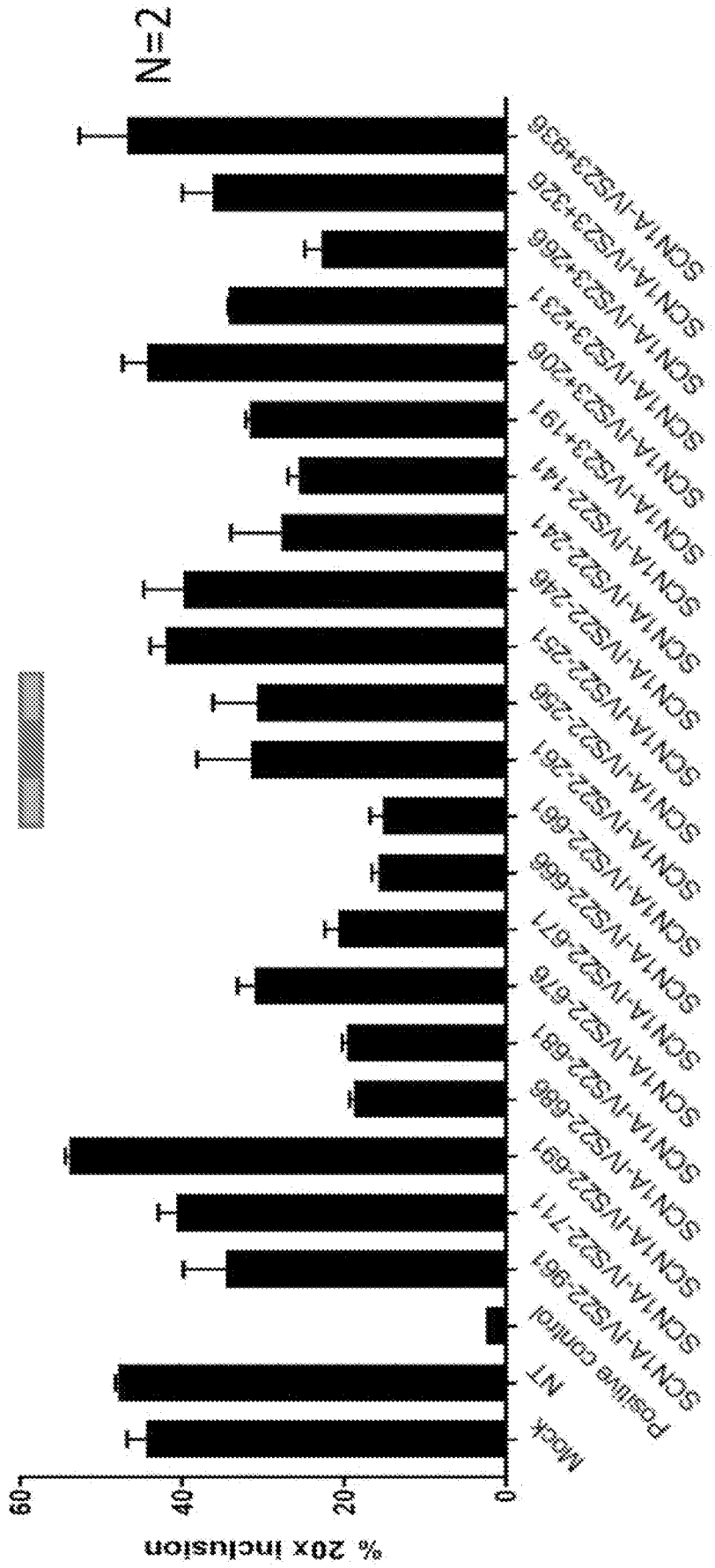

N=2
% 20x inclusion
0
20
40
60
Block
NT
Positive control
SON1A-IVS22-98i
SON1A-IVS22-77i
SON1A-IVS22-69i
SON1A-IVS22-68e
SON1A-IVS22-68i
SON1A-IVS22-67e
SON1A-IVS22-67i
SON1A-IVS22-66e
SON1A-IVS22-66i
SON1A-IVS22-61i
SON1A-IVS22-26i
SON1A-IVS22-25i
SON1A-IVS22-24e
SON1A-IVS22-24i
SON1A-IVS22-14i
SON1A-IVS23+19i
SON1A-IVS23+20e
SON1A-IVS23+23i
SON1A-IVS23+26e
SON1A-IVS23+32e
SON1A-IVS23+95e

FIG. 5B

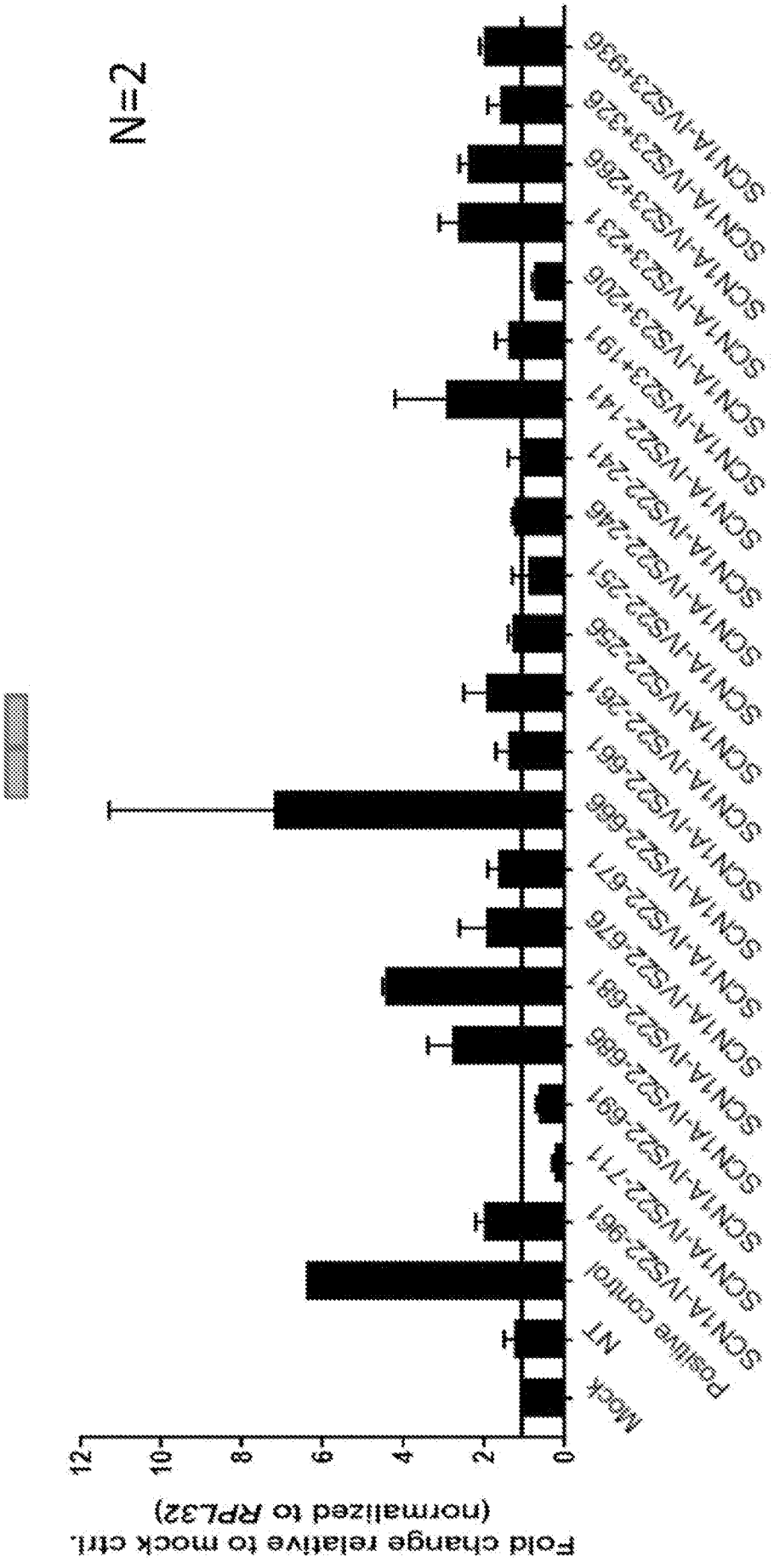

N=2
Fold change relative to mock ctrl.
(normalized to RPL32)
0
2
4
6
8
10
12
Mock
NT
Positive control
SON1A-IVS22-66I
SON1A-IVS22-71I
SON1A-IVS22-69I
SON1A-IVS22-66E
SON1A-IVS22-68I
SON1A-IVS22-67E
SON1A-IVS22-67I
SON1A-IVS22-68E
SON1A-IVS22-66I
SON1A-IVS22-25I
SON1A-IVS22-25E
SON1A-IVS22-24E
SON1A-IVS22-24I
SON1A-IVS22-14I
SON1A-IVS23+19I
SON1A-IVS23+20E
SON1A-IVS23+23I
SON1A-IVS23+26E
SON1A-IVS23+32E
SON1A-IVS23+93E

FIG. 5C