

COMPOSITIONS AND METHODS FOR MODULATING Nav1.8**TECHNICAL FIELD**

[001] This disclosure is related to oligonucleotides and their use in inhibiting/modulating the expression of the Nav1.8 gene (*SCNA10A* gene/transcript). The disclosure is also related to a conjugate including the oligonucleotide and anti-TfR antibodies or antigen-binding fragments thereof and their use in treating or reducing pain and/or diseases associated with Nav1.8 channel.

SEQUENCE LISTING

[002] The present application is being filed along with a Sequence Listing in ST.26 XML format. The Sequence Listing is provided as a file titled "30152_WO" created November 20, 2024 and is 1132 kilobytes in size. The Sequence Listing information in the ST.26 XML format is incorporated herein by reference in its entirety.

BACKGROUND

[003] Treatment for chronic pain is a significant unmet medical problem; over one-quarter of United States citizens suffer from chronic pain (Nahin RL, "Estimates of Pain Prevalence and Severity in Adults: United States 2012," *J. Pain*. 16(8):769-80 (2015)). Moreover, pain is one of the most common complaints seen in outpatient clinics. There are multiple categories and types of pain, including neuropathic, nociceptive, musculoskeletal, inflammatory, psychogenic, and mechanical.

[004] There are three kinds of chronic pain: neuropathic, inflammatory, and mixed pain which includes both neuropathic and inflammatory. Neuropathic pain can be classified as peripheral and central neuropathic pain. Peripheral neuropathic pain is caused by injury or infection of peripheral sensory nerves, whereas central neuropathic pain is caused by damage to the CNS and/or the spinal cord. Both peripheral and central neuropathic pain can occur without obvious initial nerve damage. Pain due to diabetic peripheral neuropathy (DPN) is a classic example of peripheral neuropathic pain. A similar definition is given by the International Association for the Study of Pain (IASP, Seattle, Wash., USA): peripheral neuropathic pain is pain initiated or caused by a primary lesion or dysfunction in the peripheral nervous system. Central neuropathic pain is pain initiated or caused by a primary lesion or dysfunction in the central nervous system. Inflammatory pain refers to increased sensitivity due to the inflammatory response associated with tissue damage. Inflammatory pain results from the increased excitability of peripheral nociceptive sensory fibers produced by the action of

inflammatory mediators. This excitatory effect, in turn, is a result of the altered activity of ion channels within affected sensory fibers. Conditions that exhibit features of both nociceptive and neuropathic pain, such as chronic low back pain (CLBP), are categorized as mixed pain. [005] Sodium channels are central to the generation of action potentials in all excitable cells such as neurons and myocytes and play a role in disease states such as pain (Waxman et al. "Sodium Channels and Pain" *Proc. Natl. Acad. Sci. USA* 96(14): 7635-9 (1999) and Waxman et al. (2000) "Voltage-gated Sodium Channels and the Molecular Pathogenesis of Pain: A Review," *J. Rehabil. Res. Dev.* 37(5): 517-28 (2000)). Voltage-gated sodium channels play a significant role in regulating neuronal excitability in normal and pathological pain states. However, the function of sodium channels (such as Nav1.7 and Nav1.8) in the pathophysiology of chronic pain is not fully understood. Nonselective antagonists of Nav channels can attenuate pain signals and are useful for treating a variety of pain conditions; however, such pain-relieving drugs (such as, analgesics) cause a range of adverse effects and are often not effective in completely relieving pain.

[006] Common analgesics like opioids and non-steroidal anti-inflammatory drugs (NSAIDs) do not sufficiently improve pathological pain, such as, peripheral, and central neuropathic pain due to lack of adequate efficacy and/or dose limiting side effects. Side effects also limit the utility of nonselective Nav antagonists, including dizziness, somnolence, nausea, and vomiting (Tremont-Lukats et al., "Anticonvulsants for Neuropathic Pain Syndromes: Mechanisms of Action and Place in Therapy," *Drugs* 60:1029-1052 (2000)) that limit the utility of Nav antagonists for the treatment of pain. These side effects are thought to result at least in part from the block of multiple Nav subtypes.

[007] There remains a need for an agent that can selectively and efficiently modulate the activity of Nav1.8 channels for use in treating or reducing pain. An agent that inhibits the Nav1.8 channel selectively could provide a beneficial therapeutic benefit over non-selective Nav antagonists and reduce adverse effects. Also, there is a need for a pain-relieving agent that has both high biological activity and *in vivo* stability for treating or reducing pain.

SUMMARY OF THE INVENTION

[008] The present disclosure provides oligonucleotides, conjugates comprising the oligonucleotides and an anti-transferrin receptor (anti-TfR) antibody or antigen binding fragment thereof, pharmaceutical compositions, and methods for inhibiting the expression of the Nav1.8 gene in a cell or mammal. The invention also provides pharmaceutical compositions and methods for treating chronic pain and other diseases caused by the expression of Nav1.8 gene.

[009] Nav1.8 is a voltage-gated sodium channel expressed almost exclusively in the nociceptor population of primary afferent dorsal root ganglion neurons in humans. It is responsible for the terminal phase of the action potential upstroke in primary afferents. Several Nav1.8 gain of function mutations have been identified in human populations that lead to hyperexcitable primary afferents and a variety of chronic pain phenotypes (Xiao et al., “Increased Resurgent Sodium Currents in Nav1.8 Contribute to Nociceptive Sensory Neuron Hyperexcitability Associated with Peripheral Neuropathies,” *J. Neuroscience* 39, no. 8: 1539-1550 (2019)). In addition, selective small molecules targeting Nav1.8 have shown efficacy in both acute and chronic pain indications (Qin, H., et al., “Discovery of Selective Nav1. 8 Inhibitors Based on 5-chloro-2-(4, 4-difluoroazepan-1-yl)-6-methyl Nicotinamide Scaffold for the Treatment of Pain,” *European Journal of Medicinal Chemistry*, 254, p.115371 (2023); Jones, et al., “Selective Inhibition of Nav1. 8 with VX-548 for Acute Pain,” *New England Journal of Medicine* 389, no. 5: 393-405 (2023)), and multiple small molecule programs are currently in various stages of clinical testing. However, targeting peripheral sodium channels with small molecules has been historically challenging (Waxman, Stephen G., “Targeting a Peripheral Sodium Channel to Treat Pain,” *New England Journal of Medicine* 389: 466-469 (2023)). This may be due to several factors including molecular selectivity as well as the variable nature of the blood-nerve-barrier and inconsistent target engagement throughout the multiple compartments of primary afferent neurons. The approach of delivering therapeutic oligonucleotides directly to primary afferent neurons may offer the opportunity for both exquisite molecular selectivity as well as offering consistent suppression of Nav1.8 protein expression throughout the multiple compartments of primary afferent neurons (skin terminals, nerve axons, neuronal somas, and proximal dorsal horn synapses).

[0010] In some embodiments, the conjugates, described herein, have superior benefit in that they can deliver an oligonucleotide to dorsal root ganglion (DRG) neurons. In some aspects, the present disclosure provides conjugates that have superior benefit in that they can deliver an oligonucleotide to dorsal root ganglion (DRG) neurons with reduced immunogenicity risk as compared to other conjugates having anti-TfR antibodies. In some embodiments, the conjugates, as described herein, cause reduction of pain, and can be used in treatment of chronic pain in human subjects.

[0011] In some embodiments, the TfR antibody of the present disclosure is an anti-human/cynomolgus macaque reactive transferrin receptor bivalent antibody attached to a siRNA targeting Nav1.8 (*SCN10A* gene/transcript) via a linker to form a conjugate antibody-oligo nucleotide (AOC) that has been engineered for uptake into peripheral tissues with the

transferrin receptor. In some embodiments, when administered, the AOC binds to the apical domain of the transferrin receptor expressed on peripheral tissues including dorsal root ganglia, whereby it undergoes receptor-mediated internalization and enters lysosomal degradative compartments. The siRNA targeting Nav1.8 then is presumably released to the cytoplasm to elicit RNAi targeting mechanism.

[0012] One aspect of the present disclosure is related to oligonucleotides for inhibiting the expression of a human Nav1.8 gene (*SCN10A*) in a cell comprising a single stranded oligonucleotide or double stranded oligonucleotide that comprises a region of complementarity to Nav1.8 mRNA.

[0013] Another aspect of the disclosure is related to an oligonucleotide for inhibiting the expression of a human Nav1.8 gene (*SCN10A*) in a cell, the oligonucleotide comprising: a sense strand and an antisense strand, wherein the sense strand and/or the antisense strand form a duplex region and the antisense strand comprises a region of complementarity to Nav1.8 mRNA (i.e., *SCN10A* mRNA), wherein said region of complementarity is less than 30 nucleotides in length.

[0014] Another aspect of the disclosure is related to a cell comprising one of the oligonucleotides of the disclosure. The cell is preferably a mammalian cell, such as a human cell.

[0015] In some embodiments, the oligonucleotide of the present disclosure is a single stranded (ss) oligonucleotide that includes an antisense sense strand that includes a region of complementarity to Nav1.8 mRNA (i.e., *SCN10A* mRNA), wherein said region of complementarity is less than 30 nucleotides in length. In some embodiments, the antisense strand is 15 to 30 nucleotides in length, 18 to 25 nucleotides in length, 19 nucleotides in length, or 21 nucleotides in length. In some embodiments, the antisense strand comprises a region of complementarity to a target sequence of any one of SEQ ID NOs: 1 to 141. In some embodiments, the region of complementarity of the ss oligonucleotide is at least 15 contiguous nucleotides in length, at least 16 contiguous nucleotides in length, at least 17 contiguous nucleotides in length, at least 18 contiguous nucleotides in length, at least 19 contiguous nucleotides in length, or at least 20 contiguous nucleotides in length. In some embodiments, the antisense strand comprises a 3' sequence of 1 or more nucleotides in length. In some embodiments, the antisense strand includes at least 1 modified nucleotide. In some embodiments, all nucleotides of the antisense strand are modified. In some embodiments, the antisense strand has modified nucleotide, and the modified nucleotide comprises a 2'-modification on the sugar. In some embodiments, the 2'-modification is 2'-fluoro, 2'-O-

methyl, or 2'-O-methoxyethyl. In some embodiments, the modification is 2'-fluoro, vinyl phosphonate uridine (VpUq), or 2'-O-methyl. In some embodiments, the 5' end of the antisense strand comprises at least one vinyl phosphonate uridine (VpUq). In some embodiments, one or more nucleotides at positions 1-21 of the antisense strand are modified with 2'-fluoro. In some embodiments, one or more nucleotides at positions 1-21 of the antisense strand are modified with 2'-O-methyl. In some embodiments, the antisense strand has at least one modified internucleotide linkage. In some embodiments, at least one modified internucleotide linkage is a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, at least two terminal nucleotides on 5' end or 3' end of the antisense strand have a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, at least three terminal nucleotides on 5' end or 3' end of the antisense strand have phosphorothioate linkages or a phosphorodithioate linkages. In some embodiments, a linker moiety is attached to one or more termini of the antisense strand. In some embodiments, the linker is succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC). In some embodiments, at least one nucleotide of the oligonucleotide is conjugated to cholesterol, lipid, polypeptide, or an antibody or an antigen binding fragment thereof. In some embodiments, the antisense strand has the sequence of i) any one of the odd numbers of SEQ ID NOs: 143 to 423 or ii) any one of SEQ ID Nos: 424 to 564. In some embodiments, (i) the antisense strand has a sequence as set forth in Table 2 or (ii) the antisense strand has a sequence as set forth in Table 3.

[0016] In another aspect, the disclosure provides a pharmaceutical composition for inhibiting the expression of the Nav1.8 gene in an organism, comprising one or more of the oligonucleotides of the invention and a pharmaceutically acceptable carrier.

[0017] In another aspect, the disclosure provides a cell comprising one of the oligonucleotides of the invention. The cell is preferably a mammalian cell, such as a human cell.

[0018] In another aspect, the disclosure provides a cell comprising a vector for inhibiting the expression of the Nav1.8 gene in a cell. The vector comprises a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the oligonucleotides of the invention.

[0019] Another aspect of the disclosure is related to a conjugate comprising the oligonucleotide of the present disclosure and an anti-transferrin receptor antibody or antigen-binding fragment thereof conjugated to the sense strand of the Nav1.8 targeting nucleotide or the antisense strand of the Nav1.8 targeting nucleotide. One embodiment of the disclosure is

related to a pharmaceutical composition comprising the conjugate of the present disclosure and a pharmaceutically acceptable carrier.

[0020] Another aspect of the present disclosure is related to a method of preventing, treating, or reducing pain in a patient in need thereof, the method comprising administering to the patient an effective amount of the oligonucleotide, the conjugate, or the pharmaceutical composition of the present disclosure.

[0021] In another aspect, the invention provides a method for inhibiting the expression of the Nav1.8 gene in a cell, comprising the following steps: a) introducing into the cell the oligonucleotide or a conjugate of the present disclosure; and b) maintaining the cell produced in step a) for a time sufficient to obtain degradation of the mRNA transcript of the Nav1.8 gene, thereby inhibiting expression of the Nav1.8 gene in the cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figures 1A and 1B show modification patterns that may be used in the oligonucleotides (e.g., Nav1.8 targeting siRNAs) of the present disclosure. In the Figures, VpUq is vinyl phosphonate uridine, 2'-OMe is 2'-OMethyl modification, 2'F is 2'-Fluoro, and PS is phosphorothioate. The notations shown at the bottom of Figure 1B are applicable to both Figures 1A and 1B.

[0023] Figure 2 shows the comparison between modified siRNA 410 without VpUq (i.e., siRNA No. 285 in Table 4) and modified siRNA 410 with VpUq (i.e., siRNA No. 305 in Table 4).

[0024] Figure 3 shows the comparison between modified siRNA 535 without VpUq (i.e., siRNA No. 291 in Table 4) and modified siRNA 535 with VpUq (i.e., siRNA No. 311 in Table 4).

[0025] Figure 4 shows the potency of various Nav1.8 siRNA sequences in primary human dorsal root ganglia neurons (DRGs). The most potent siRNAs showed equal to or greater than 70% Nav1.8 mRNA reduction in the DRG neurons. The figure identifies the siRNA by the start position of target region on human SCN10A transcript.

[0026] Figure 5 shows the concentration-response of some Nav1.8 siRNAs. The figure identifies the siRNA by the start position of target region on human SCN10A transcript.

[0027] Figures 6A-C show RT-qPCR analysis of SCN10A expression and ELISA analysis of SCN10A expression for two siRNAs.

[0028] Figure 7 is a representative structure of one embodiment of a Nav1.8 antibody-oligonucleotide conjugate, also referred to herein as "AOC" or "conjugate". The antibody is shown as a "Y" shape in the figure connected to a siRNA molecule via an SMCC Linker. In

some embodiments, two siRNA molecules may be attached to the antibody molecule (e.g., using eCys or nCys to connect each siRNA molecule to each heavy chain of the antibody using a linker).

[0029] Figure 8 shows a representative analytical strong anion exchange column chromatography (SAX) trace of Nav1.8 AOC reaction mixture using SAX method 1.

[0030] Figure 9 shows a representative Fast Protein Liquid Chromatography (FPLC) trace of Nav1.8 AOC reaction mixture using FPLC method 1.

[0031] Figure 10 shows a representative Size Exclusion Chromatography (SEC) trace using SEC Method 1.

[0032] Figure 11 shows a representative Strong Anion Exchange (SAX) trace using SAX Method 1.

[0033] Figure 12 shows SDS-PAGE analysis of AOCs. MWM indicates the molecular weight marker, and the molecular weight (MW) of each band of the marker is annotated. TBP1-410 is shown in the left gel, and TBP2-410 is shown in the right gel.

[0034] Figure 13 shows that the TBP1-si410 AOC reduces Nav1.8 mRNA expression in human DRGs *in vitro*. The TBP1-si410 AOC includes TBP1 antibody and modified 410 siRNA linked via an SMCC linker.

[0035] Figure 14 shows the reversal of cold-induced nocifensive response following administration of OX26-410 and OX26-535 AOCs in humanized Nav1.8 rats following IV administration of AOCs (6 mg/kg single-dose IV, by oligo weight, 13-day exposure). Rats treated with OX26-410 AOC (grey bars) displayed a statistically significant reduction of cold-induced nocifensive behavior vs PBS controls (white bars) at both temperatures tested, 2 °C and -1 °C, ($p < 0.05$, one-way ANOVA, Dunnet's post-hoc). Rats treated with OX26-535 AOC (black bars) showed a decreased response to cold, but this effect did not reach statistical significance.

[0036] Figure 15 shows the reduction of Nav1.8 protein in humanized Nav1.8 rat DRGs following administration of OX26-410 and OX26-535 AOCs. Administration of Nav1.8-targeting AOCs, OX26-410 and OX26-535 (6 mg/kg, IV, 13 days exposure), resulted in a significant decrease in Nav1.8 protein measured in the dorsal root ganglia (DRG) and correlated with a reduction in cold-induced nocifensive behavior in humanized Nav1.8 rats. AOC OX26-535 treated rats (black square) showed a significant reduction in DRG Nav1.8 protein vs. PBS controls (white circle) but did not cause a significant decrease in cold behavior. Humanized Nav1.8 rats treated with AOC OX26-410 (grey triangle) showed a

statistically significant decrease in both Nav1.8 DRG protein levels as well as cold-induced nocifensive behavior ($p < 0.05$, one-way ANOVA, Dunnet's post-hoc).

[0037] Figure 16 shows reduction in Nav1.8 protein in rat glabrous skin following administration of OX26-410 and OX26-535 AOCs. Administration of Nav1.8-targeting AOCs, OX26-410 and OX26-535 (6 mg/kg, IV, 13 days exposure), resulted in a significant decrease in Nav1.8 protein measured in glabrous paw skin and correlated with a reduction in cold-induced nocifensive behavior in humanized Nav1.8 rats. AOC OX26-535 treated rats (black square) showed a significant reduction in glabrous paw skin Nav1.8 protein vs. PBS controls (white circle) but did not cause a significant decrease in cold behavior. Humanized Nav1.8 rats treated with AOC OX26-410 (grey triangle) showed a statistically significant decrease in both Nav1.8 glabrous paw skin protein levels as well as cold-induced nocifensive behavior (one-way ANOVA, Dunnet's post-hoc, $p < 0.05$).

[0038] Figure 17 shows reductions in Nav1.8 protein in glabrous paw skin in cynomolgus monkeys following a single dose of anti-Tfr-410 nCys mAb (dark grey bar; TBP1-siRNA410 AOC), anti-Tfr-410 eCys mAb (light grey bar; TBP2-siRNA410 AOC), anti-Tfr-410 Fab (shaded grey bar; TBP3-siRNA410 AOC), 2 doses of anti-Tfr-410 nCys mAb, or PBS (white bar). Single-dose groups received either 1.0 or 6.0 mg/kg (by oligo weight) of AOCs. The two-dose group of anti-Tfr-410 nCys mAb was administered AOC twice on day 1 and day 8 at 3.0 mg/kg (by oligo weight) and this resulted in a significant reduction of glabrous paw skin Nav1.8 protein levels ($p < 0.05$, one-way ANOVA). All exposures were 29 days.

[0039] Figure 18 shows the effect of TBP2-410 eCys AOC on Nav1.8 protein knockdown in NHP glabrous paw skin in cynomolgus monkeys. The Nav1.8 protein is reduced in glabrous paw skin following treatment with the TBP2-410 eCys AOC. Groups received either PBS (control), 0.3, 3.0, or 30.0 mg/kg (by oligo weight) of the TBP2-410 eCys AOC ($N=4$ /group, except for 30.0 mg/kg group which was $N=2$). The AOC was administered in four weekly IV doses. Administration of 3.0 and 30.0 mg/kg (by oligo weight) of AOC resulted in a significant reduction of glabrous paw skin Nav1.8 protein levels (52.3% and 42.2% of the PBS control group respectively, $*p < 0.05$, one-way ANOVA). All exposures were 27 days following final dose (49-day total study duration).

DETAILED DESCRIPTION

[0040] One aspect of the present disclosure is related to oligonucleotides, conjugates including such oligonucleotides, pharmaceutical compositions, and methods for inhibiting the expression of the Nav1.8 gene in a cell or mammal using the oligonucleotides, the conjugates,

or the compositions. The invention also provides compositions and methods for treating pathological conditions and diseases in a mammal caused by Nav1.8 gene using the oligonucleotides, conjugates, or compositions thereof. In some embodiments, the oligonucleotide directs the sequence-specific degradation of Nav1.8 mRNA (i.e., *SCN10A* mRNA) through a process known as RNA interference (RNAi).

[0041] The use of these oligonucleotides and the conjugates enables targeted degradation of Nav1.8 mRNA that is implicated in pain response in mammals. The present disclosure has demonstrated that these oligonucleotides or the conjugates can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of the Nav1.8 gene. The present disclosure has also demonstrated that the oligonucleotides and the conjugates (such as with anti-TfR antibodies) can be effectively delivered to neurons (such as dorsal root ganglion neurons) resulting in significant inhibition of expression of the Nav1.8 gene and in reduction/treatment of pain. Thus, the methods and compositions of the invention comprising these oligonucleotides and conjugates are useful for reducing or treating pain.

[0042] The following detailed description discloses how to make and use the oligonucleotides, the conjugates, and related compositions to inhibit the expression of a target Nav1.8 gene, as well as compositions and methods for treating diseases and disorders caused by the expression of Nav1.8, such as pain.

[0043] The conjugates of the present disclosure include an oligonucleotide of the present disclosure having an antisense strand comprising a region of complementarity to at least part of an RNA transcript of the Nav1.8 gene and an anti-TfR antibody (anti-transferrin receptor antibody) or an antigen binding fragment thereof.

[0044] The pharmaceutical compositions of the disclosure comprise an oligonucleotide having an antisense strand comprising a region of complementarity to at least part of an RNA transcript of the Nav1.8 gene, together with a pharmaceutically acceptable carrier. Another embodiment of the pharmaceutical compositions comprises the conjugate of the present disclosure together with a pharmaceutically acceptable carrier. Accordingly, certain aspects of the disclosure provide pharmaceutical compositions comprising the oligonucleotides or the conjugates of the present disclosure together with a pharmaceutically acceptable carrier, methods of using such pharmaceutical compositions to inhibit expression of the Nav1.8 gene, and methods of using the pharmaceutical compositions to treat diseases caused by expression of the Nav1.8 gene (e.g., treat pain or reduce pain).

[0045] One aspect of the present disclosure is related to an oligonucleotide (e.g., siRNA) for inhibiting the expression of a human Nav1.8 gene in a cell. This oligonucleotide includes: a

sense strand and/or an antisense strand, wherein the sense strand and/or the antisense strand form a duplex region and the antisense strand comprises a region of complementarity to Nav1.8 mRNA, wherein said region of complementarity is less than 30 nucleotides in length.

[0046] In some embodiments, the oligonucleotide of the present disclosure is such that the region of complementarity of the antisense strand to the Nav1.8 mRNA target sequence is at least 15 contiguous nucleotides in length, at least 18 contiguous nucleotides in length, at least 19 contiguous nucleotides in length, at least 20 contiguous nucleotides in length, or at least 21 contiguous nucleotides in length. In some embodiments, the region of complementarity of the antisense strand to the Nav1.8 mRNA target sequence is at least 16, 17, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

[0047] In some embodiments, the sense strand of the oligonucleotide is 15 to 30 nucleotides in length, 18 to 25 nucleotides in length, or 19 nucleotides in length. In some embodiments, the sense strand is 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In some embodiments, the sense strand is 19 nucleotides in length. In some embodiments, the sense strand is 21 nucleotides in length.

[0048] In some embodiments, the antisense strand of the oligonucleotide is 15 to 30 nucleotides in length, 18 to 25 nucleotides in length, 19 nucleotides in length, or 21 nucleotides in length. In some embodiments, the antisense strand is 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length. In some embodiments, the sense strand is 19 nucleotides in length and the antisense strand is 21 nucleotides in length. In some embodiments, the sense strand is 19 nucleotides in length and the antisense strand is 19 nucleotides in length.

[0049] In some embodiments, the oligonucleotide includes an antisense strand comprising a region of complementarity to a target sequence of any one of SEQ ID NOs: 1 to 141. Table 1 shows the target sequences present in Nav1.8 mRNA.

Table 1: Target Sequences (18-mers) for SCNA10A mRNA Transcript.

SEQ ID NO.	Target Sequence (5' to 3')	Start position of target region on human SCN10A transcript
1	UUACGGUCACUAUUUUGG	407
2	UGAUCUUGGCUGUAGUCA	1172
3	UAAUUGUGUGUGCAUGAC	426
4	CGGUCACUAUUUUGGUUA	410
5	AACCUGAUCAGAAGAACG	349
6	AAAUGAGUUCACGUACCU	537

7	CAACCCGGAUUUUAACUA	990
8	CUGACAACCCGGAUUUUA	986
9	AAUGAGUUCACGUACCUG	538
10	ACAACCCGGAUUUUAACU	989
11	AGAACGGCCAUCAAAGUG	361
12	CUUCUGACAACCCGGAUU	983
13	UGUCUAAAUGAGUUCACG	532
14	AGAAUACUUAGAUGAACC	1794
15	UACCUUCGAAGCCAUGCU	2073
16	GUGCAUGACCCGAACUGA	435
17	UGUGCAUGACCCGAACUG	434
18	CUUACAACCAGCGCAGGA	1442
19	CCUACCUUCGAAGCCAUG	2071
20	GCGUCAUUACCCUGGCAU	584
21	AAUUGUGUGUGCAUGACC	427
22	GGAUUUUAGCGUCAUUAC	576
23	CAGCAGAAUACUUAGAUG	1790
24	GCAUGAGCCCUACCUUCG	2063
25	UGACAACCCGGAUUUUA	987
26	CUAAAUGAGUUCACGUAC	535
27	UGUCAGCAGAAUACUUAG	1787
28	AACCAACUACUCAUCUCA	858
29	CGCUUUACUCCGGAGUCA	43
30	ACAACCAACUACUCAUCU	856
31	CUUGUACCUUUGUCGAUU	3958
32	CACGUACCUGAGAGAUCC	546
33	CCCAAACCCAAUCGAAA	5331
34	GGGUGAAGCUCAAGACAA	1952
35	GUUUUUUUUUACGGUCA	398
36	CAGUUUUUUUUUUACGGU	396
37	ACCAACUACUCAUCUCAC	859
38	ACCUGAUCAGAAGAACGG	350
39	CUUUUUUGUGCUCGUAUU	1125
40	GAAUUCCTCAUUGGAUCC	4
41	GGCAUAUGUUGGCACAGC	597
42	GCCUUAGUGAUAGAGUCA	5780
43	UCAUUACCCUGGCAUAUG	587
44	AAUGAGUGUUGUCAGUAU	1830
45	AGGACAUCUAGCUCAAUA	5803
46	UGAUCCUUACAACCAGCG	1437
47	UUAUUUUUUACGGUCACU	400
48	AAGAACGGCCAUCAAAGU	360
49	GACAACCCGGAUUUUAAC	988
50	UCUAGCUCAAUACAAAUA	5809

51	GAUCUCCGUGGGAUCUCA	619
52	CCGGUUUAGUGCCACUCG	306
53	CUCAUCCUUUUCUUGACG	2599
54	ACCCAAUCGAAAUAUACU	5337
55	UUUAUCUUUGUGUUCGAG	3583
56	ACAGAAGGAUGCAUUCGC	3346
57	GUCACUAGAGGCCUUAGU	5770
58	UCUUUUUUGUGCUCGUAA	1124
59	UUGUCGAUUGUGAAUAAAC	3967
60	AGGGCAAUGAGUGUUGUC	1825
61	UUUUUGUGCUCGUAAUCU	1127
62	UUCGAUGCAGGACAAAAG	1759
63	CAACCGAAAAAAUAUCUC	2445
64	ACGGUCACUAUUUUGGUU	409
65	GGAUGCAUUCGCCACUGU	3352
66	GGGCAAUGAGUGUUGUCA	1826
67	UGAUAGAGUCAACAUGAG	5787
68	AUCCUUACAACCAGCGCA	1439
69	UGUACCUUUGUCGAUUGU	3960
70	UUGUACCUUUGUCGAUUG	3959
71	ACUCGAGGAGUCUGAACA	1869
72	CAGUAUCAUAACCUCCGU	1842
73	AAGGGCAAUGAGUGUUGU	1824
74	CAAUGAGUGUUGUCAGUA	1829
75	UGCAUUCGCCACUGUCCC	3355
76	AUCUUUUUUGUGCUCGUA	1123
77	CAGUGACUUCAUCGCUAA	2952
78	GUUUUAUGGCAACUAAUCU	5484
79	CUUGUGCAUCGUGGUGAA	2016
80	CUUUGGGCUUGUGACGGA	1974
81	CUUGGGCUUCCUCUCAC	1028
82	CAUCUAGCUCAAUACAAA	5807
83	GUUGUCAGUAUCAUAACC	1837
84	CUCCGGAGUCACUGGUGG	50
85	AGGUCUUUGGCCAUCGUA	2753
86	GUGUGCAUGACCCGAACU	433
87	CACGGAUCCAGGUCUUUG	2744
88	ACUUAGAUGAACCUUCC	1799
89	AACAUGCAACCCAAGUGG	4147
90	UAGAGGCCUUAGUGAUAG	5775
91	AUCGAAAUAUACUGAUCC	5342
92	UUUCAGCCACUGUCAUUC	5558
93	AUAACCUCCGUCCUUGAG	1849
94	UUUAUGGCAACUAAUCUU	5485

95	CUAUGAACCAAUAGCAAC	5514
96	CCAACUACUCAUCUCACA	860
97	GUUCAUCGCCCUGCUAUU	2652
98	AUCCUUUUCUUGACGGUG	2602
99	CUAUCGGAGCUAUGUGCU	5583
100	CCAAGAAUGUCCUAGGAG	5423
101	UUCUACAGCACACACCGG	253
102	AGUUUAUUUAUUACGGUC	397
103	AUCAUCCUAUGAACCAAU	5508
104	CAGGUCUUUGGCCAUCGU	2752
105	GCACACACCGGACAUUUA	260
106	CAUCGUGACCAGACAAGC	4413
107	GCAAUGGGUUACCUUGCA	4060
108	UCCUUUUCUUGACGGUGA	2603
109	UCGAAAUAUACUGAUCCA	5343
110	UCGAUGCAGGACAAAAGA	1760
111	CAAAAGGCCUAUCGGAGC	5575
112	GGCGAAUGUGUCAUGAAG	4561
113	GCUAUGUGCUGCACCGCU	5591
114	UCCCAGCAGAACUGAUCG	209
115	CACACCGGACAUUUAUGG	263
116	GUGUCACUAGAGGCCUUA	5768
117	UGUCGAUUGUGAAUAACA	3968
118	AACUUCUGACAACCCGGA	981
119	CUAUGUGCUGCACCGCUC	5592
120	AAGUCUGACUGCAAGAUU	3985
121	CCUUUGUCGAUUGUGAAU	3964
122	GUUUAGUGCCACUCGGGC	309
123	UCCUUACAACCAGCGCAG	1440
124	AACCCAAUCGAAAUAUAC	5336
125	UGUGUGCAUGACCCGAAC	432
126	CAUCGCCCUGCUAUUGAA	2655
127	CCUUACAACCAGCGCAGG	1441
128	AUCUCCGCGCCCCAUGAA	2458
129	CUUUUCUUGACGGUGAUG	2605
130	UCACUAGAGGCCUUAGUG	5771
131	GCGAAUGUGUCAUGAAGA	4562
132	UCUGACAACCCGGAUUUU	985
133	AGUAUCAUAACCUCCGUC	1843
134	GCAUAUGUUGGCACAGCA	598
135	CCCUUACUGUGUGGCAAU	919
136	CUACAGCACACACCGGAC	255
137	CGAAAAAAUAUCUCCGCG	2449
138	CUUGGUGGGGCUGCAACU	783

139	CUGCAAGAUUCAAACUC	3993
140	CGUCGCUUUACUCCGGAG	40
141	UCAUCCUAUGAACCAUA	5509

[0050] In some embodiments, the oligonucleotide includes an antisense strand comprising a region of complementarity to a Nav1.8 mRNA target sequence wherein the region of complementarity of the antisense strand to the target sequence (e.g., as shown in Table 1) is at least 15 contiguous nucleotides in length, at least 16 contiguous nucleotides in length, at least 17 contiguous nucleotides in length, at least 18 contiguous nucleotides in length, at least 19 contiguous nucleotides in length, or at least 20 contiguous nucleotides in length.

[0051] In some embodiments, the oligonucleotide is such that the sense strand has the sequence of anyone of the even numbers of SEQ ID NOs: 142 to 422. In some embodiments, the antisense strand of the oligonucleotide has the sequence of any one of the odd numbers of SEQ ID NOs: 143 to 423. In some embodiments, the antisense strand of the oligonucleotide has the sequence of anyone of SEQ ID Nos: 424 to 564.

[0052] Tables 2 and 3 below show some exemplary oligonucleotides of the present disclosure.

Table 2: Nucleic Acid Sequences of 19-mer Nav1.8 siRNAs

dsRNA No. (or siRNA No.) (refers to duplexed SS and AS strand of siRNA)	Sense Strand (5' to 3')	SEQ ID NO.	Antisense Strand (5' to 3') (without 3' UU overhang)	SEQ ID NO.	Start position of target region on human SCN10A transcript
1	UUACGGUCACU AUUUUGGA	142	UCCAAAAUAGU GACCGUAA	143	407
2	UGAUCUUGGCU GUAGUCAA	144	UUGACUACAGC CAAGAUCA	145	1172
3	UAAUUGUGUGU GCAUGACA	146	UGUCAUGCACA CACAAUUA	147	426
4	CGGUCACUAUU UUGGUUAA	148	UUAACCAAAAU AGUGACCG	149	410
5	AACCUGAUCAG AAGAACGA	150	UCGUUCUUCUG AUCAGGUU	151	349
6	AAAUGAGUUCA CGUACCUA	152	UAGGUACGUGA ACUCAUUU	153	537
7	CAACCCGGAUU UUAACUAA	154	UUAGUUAAAAU CCGGGUUG	155	990
8	CUGACAACCCG GAUUUUAA	156	UUAAAAUCCGG GUUGUCAG	157	986

9	AAUGAGUUCAC GUACCUGA	158	UCAGGUACGUG AACUCAUU	159	538
10	ACAACCCGGAU UUUAACUA	160	UAGUAAAAAUC CGGGUUGU	161	989
11	AGAACGGCCAU CAAAGUGA	162	UCACUUUGAUG GCCGUUCU	163	361
12	CUUCUGACAAC CCGGAUUA	164	UAAUCCGGGUU GUCAGAAG	165	983
13	UGUCUAAAUGA GUUCACGA	166	UCGUGAACUCA UUUAGACA	167	532
14	AGAAUACUUAG AUGAACCA	168	UGGUUCAUCUA AGUAUUCU	169	1794
15	UACCUUCGAAG CCAUGCUA	170	UAGCAUGGCUU CGAAGGUA	171	2073
16	GUGCAUGACCC GAACUGAA	172	UUCAGUUCGGG UCAUGCAC	173	435
17	UGUGCAUGACC CGAACUGA	174	UCAGUUCGGGU CAUGCACA	175	434
18	CUUACAACCAG CGCAGGAA	176	UUCUGCGCUG GUUGUAAG	177	1442
19	CCUACCUUCGA AGCCAUGA	178	UCAUGGCUUCG AAGGUAGG	179	2071
20	GCGUCAUUACC CUGGCAUA	180	UAUGCCAGGGU AAUGACGC	181	584
21	AAUUGUGUGUG CAUGACCA	182	UGGUCAUGCAC ACACAAUU	183	427
22	GGAUUUUAGCG UCAUUACA	184	UGUAAUGACGC UAAAAUCC	185	576
23	CAGCAGAAUAC UUAGAUGA	186	UCAUCUAAGUA UUCUGCUG	187	1790
24	GCAUGAGCCCU ACCUUCGA	188	UCGAAGGUAGG GCUCAUGC	189	2063
25	UGACAACCCGG AUUUUAAA	190	UUUAAAAUCCG GGUUGUCA	191	987
26	CUAAAUGAGUU CACGUACA	192	UGUACGUGAAC UCAUUUAG	193	535
27	UGUCAGCAGAA UACUUAGA	194	UCUAAGUAUUC UGCUGACA	195	1787
28	AACCAACUACU CAUCUCAA	196	UUGAGAUGAGU AGUUGGUU	197	858
29	CGCUUUACUCC GGAGUCAA	198	UUGACUCCGGA GUAAAGCG	199	43
30	ACAACCAACUA CUCAUCUA	200	UAGAUGAGUAG UUGGUUGU	201	856
31	CUUGUACCUUU GUCGAUUA	202	UAAUCGACAAA GGUACAAG	203	3958
32	CACGUACCUGA GAGAUCCA	204	UGGAUCUCUCA GGUACGUG	205	546

33	CCCCAAACCCAA UCGAAAA	206	UUUUCGAUUGG GUUUUGGG	207	5331
34	GGGUGAAGCUC AAGACAAA	208	UUUGUCUUGAG CUUCACCC	209	1952
35	GUUUUUUAUU ACGGUCAA	210	UUGACCGUAAU AAAUAAAC	211	398
36	CAGUUUAUUUA UUACGGUA	212	UACCGUAAUAA AUAACUG	213	396
37	ACCAACUACUC AUCUCACA	214	UGUGAGAUGAG UAGUUGGU	215	859
38	ACCUGAUCAGA AGAACGGA	216	UCCGUUCUUCU GAUCAGGU	217	350
39	CUUUUUUGUGC UCGUAAUA	218	UAUUACGAGCA CAAAAAAG	219	1125
40	GAAUCCCCAU UGGAUCCA	220	UGGAUCCAAUG GGGAUUC	221	4
41	GGCAUAUGUUG GCACAGCA	222	UGCUGUGCCAA CAUAUGCC	223	597
42	GCCUUAGUGAU AGAGUCAA	224	UUGACUCUAUC ACUAAGGC	225	5780
43	UCAUUACCCUG GCAUAUGA	226	UCAUAUGCCAG GGUAAUGA	227	587
44	AAUGAGUGUUG UCAGUAUA	228	UAUACUGACAA CACUCAUU	229	1830
45	AGGACAUCUAG CUCAAUAA	230	UUAUUGAGCUA GAUGUCCU	231	5803
46	UGAUCCUACA ACCAGCGA	232	UCGCUGGUUGU AAGGAUCA	233	1437
47	UUAUUUAUUAC GGUCACUA	234	UAGUGACCGUA AUAAAUAA	235	400
48	AAGAACGGCCA UCAAAGUA	236	UACUUUGAUGG CCGUUCUU	237	360
49	GACAACCCGGA UUUUAACA	238	UGUAAAAAUCC GGGUUGUC	239	988
50	UCUAGCUCAAU ACAAAAUA	240	UAUUUUGUAUU GAGCUAGA	241	5809
51	GAUCUCCGUGG GAUCUCAA	242	UUGAGAUCCCA CGGAGAUC	243	619
52	CCGGUUUAGUG CCACUCGA	244	UCGAGUGGCAC UAAACCGG	245	306
53	CUCAUCCUUUU CUUGACGA	246	UCGUCAAGAAA AGGAUGAG	247	2599
54	ACCCAAUCGAA AUAUACUA	248	UAGUAUAUUUC GAUUGGGU	249	5337
55	UUUAUCUUUGU GUUCGAGA	250	UCUCGAACACA AAGAUAAA	251	3583
56	ACAGAAGGAUG CAUUCGCA	252	UGCGAAUGCAU CCUUCUGU	253	3346

57	GUCACUAGAGG CCUUAGUA	254	UACUAAGGCCU CUAGUGAC	255	5770
58	UCUUUUUUGUG CUCGUAAA	256	UUUACGAGCAC AAAAAAGA	257	1124
59	UUGUCGAUUGU GAAUAAACA	258	UGUUAUUCACA AUCGACAA	259	3967
60	AGGGCAAUGAG UGUUGUCA	260	UGACAACACUC AUUGCCCU	261	1825
61	UUUUUGUGCUC GUAAUCUA	262	UAGAUUACGAG CACAAAAA	263	1127
62	UUCGAUGCAGG ACAAAAGA	264	UCUUUUGUCCU GCAUCGAA	265	1759
63	CAACCGAAAAA AUAUCUCA	266	UGAGAUUUUUU UUCGGUUG	267	2445
64	ACGGUCACUAU UUUGGUUA	268	UAACCAAAAUA GUGACCGU	269	409
65	GGAUGCAUUCG CCACUGUA	270	UACAGUGGCCA AUGCAUCC	271	3352
66	GGGCAAUGAGU GUUGUCAA	272	UUGACAACACU CAUUGCCC	273	1826
67	UGAUAGAGUCA ACAUGAGA	274	UCUCAUGUUGA CUCUAUCA	275	5787
68	AUCCUUACAAC CAGCGCAA	276	UUGCGCUGGUU GUAAGGAU	277	1439
69	UGUACCUUUGU CGAUUGUA	278	UACAAUCGACA AAGGUACA	279	3960
70	UUGUACCUUUG UCGAUUGA	280	UCAAUCGACAA AGGUACAA	281	3959
71	ACUCGAGGAGU CUGAACAA	282	UUGUUCAGACU CCUCGAGU	283	1869
72	CAGUAUCAUAA CCUCCGUA	284	UACGGAGGUUA UGAUACUG	285	1842
73	AAGGGCAAUGA GUGUUGUA	286	UACAACACUCA UUGCCCUU	287	1824
74	CAAUGAGUGUU GUCAGUAA	288	UUACUGACAAC ACUCAUUG	289	1829
75	UGCAUUCGCCA CUGUCCCA	290	UGGGACAGUGG CGAAUGCA	291	3355
76	AUCUUUUUUGU GCUCGUAA	292	UUACGAGCACA AAAAAGAU	293	1123
77	CAGUGACUUCA UCGCUAAA	294	UUUAGCGAUGA AGUCACUG	295	2952
78	GUUUAUGGCAA CUAAUCUA	296	UAGAUUAGUUG CCAUA AAC	297	5484
79	CUUGUGCAUCG UGGUGAAA	298	UUUCACCACGA UGCACAAG	299	2016
80	CUUUGGGCUUG UGACGGAA	300	UUCCGUCACAA GCCCAAAG	301	1974

81	CUUGGGCUUUC CUCUCACA	302	UGUGAGAGGAA AGCCCAAG	303	1028
82	CAUCUAGCUCA AUACAAAA	304	UUUUGUAUUGA GCUAGAUG	305	5807
83	GUUGUCAGUAU CAUAACCA	306	UGGUUAUGAUA CUGACAAC	307	1837
84	CUCCGGAGUCA CUGGUGGA	308	UCCACCAGUGAC UCCGGAG	309	50
85	AGGUCUUUGGC CAUCGUAA	310	UUACGAUGGCC AAAGACCU	311	2753
86	GUGUGCAUGAC CCGAACUA	312	UAGUUCGGGUC AUGCACAC	313	433
87	CACGGAUCCAG GUCUUUGA	314	UCAAAGACCUG GAUCCGUG	315	2744
88	ACUUAGAUGAA CCUUUCCA	316	UGGAAAGGUUC AUCUAAGU	317	1799
89	AACAUGCAACC CAAGUGGA	318	UCCACUUGGGU UGCAUGUU	319	4147
90	UAGAGGCCUUA GUGAUAGA	320	UCUAUCACUAA GGCCUCUA	321	5775
91	AUCGAAAUUA CUGAUCCA	322	UGGAUCAGUAU AUUUCGAU	323	5342
92	UUUCAGCCACU GUCAUUCA	324	UGAAUGACAGU GGCUGAAA	325	5558
93	AUAACCUCCGU CCUUGAGA	326	UCUCAAGGACG GAGGUUAU	327	1849
94	UUUAUGGCAAC UAAUCUUA	328	UAAGAUUAGUU GCCAUAAA	329	5485
95	CUAUGAACCAA UAGCAACA	330	UGUUGCUAUUG GUUCAUAG	331	5514
96	CCAACUACUCA UCUCACAA	332	UUGUGAGAUGA GUAGUUGG	333	860
97	GUUCAUCGCCC UGCUAUUA	334	UAAUAGCAGGG CGAUGAAC	335	2652
98	AUCCUUUUCUU GACGGUGA	336	UCACCGUCAAG AAAAGGAU	337	2602
99	CUAUCGGAGCU AUGUGCUA	338	UAGCACAUAGC UCCGAUAG	339	5583
100	CCAAGAAUGUC CUAGGAGA	340	UCUCCUAGGAC AUUCUUGG	341	5423
101	UUCUACAGCAC ACACCGGA	342	UCCGGUGUGUG CUGUAGAA	343	253
102	AGUUUAUUUAU UACGGUCA	344	UGACCGUAAUA AAUAAACU	345	397
103	AUCAUCCUAUG AACC AAUA	346	UAUUGGUUCAU AGGAUGAU	347	5508
104	CAGGUCUUUGG CCAUCGUA	348	UACGAUGGCCA AAGACCUG	349	2752

105	GCACACACCGG ACAUUUAA	350	UUAAAUGUCCG GUGUGUGC	351	260
106	CAUCGUGACCA GACAAGCA	352	UGCUUGUCUGG UCACGAUG	353	4413
107	GCAAUGGGUUA CCUUGCAA	354	UUGCAAGGUAA CCCAUUGC	355	4060
108	UCCUUUUCUUG ACGGUGAA	356	UUCACCGUCA GAAAAGGA	357	2603
109	UCGAAAUAUAC UGAUCCAA	358	UUGGAUCAGUA UAUUUCGA	359	5343
110	UCGAUGCAGGA CAAAAGAA	360	UUCUUUUGUCC UGCAUCGA	361	1760
111	CAAAAGGCCUA UCGGAGCA	362	UGCUCCGAUAG GCCUUUUG	363	5575
112	GGCGAAUGUGU CAUGAAGA	364	UCUUCAUGACA CAUUCGCC	365	4561
113	GCUAUGUGCUG CACCGCUA	366	UAGCGGUGCAG CACAUAGC	367	5591
114	UCCCAGCAGAA CUGAUCGA	368	UCGAUCAGUUC UGCUGGGA	369	209
115	CACACCGGACA UUUAUGGA	370	UCCAUAAAUGU CCGGUGUG	371	263
116	GUGUCACUAGA GGCCUUA	372	UUAAGGCCUCU AGUGACAC	373	5768
117	UGUCGAUUGUG AAUAACAA	374	UUGUUAUUCAC AAUCGACA	375	3968
118	AACUUCUGACA ACCCGGAA	376	UUCCGGGUUGU CAGAAGUU	377	981
119	CUAUGUGCUGC ACCGCUCA	378	UGAGCGGUGCA GCACAUAG	379	5592
120	AAGUCUGACUG CAAGAUUA	380	UAAUCUUGCAG UCAGACUU	381	3985
121	CCUUUGUCGAU UGUGAAUA	382	UAUUCACAAUC GACAAAGG	383	3964
122	GUUUAGUGCCA CUCGGGCA	384	UGCCCGAGUGG CACUAAAC	385	309
123	UCCUUACAACC AGCGCAGA	386	UCUGCGCUGGU UGUAAGGA	387	1440
124	AACCCAAUCGA AAUAUACA	388	UGUAUAUUUCG AUUGGGUU	389	5336
125	UGUGUGCAUGA CCCGAACA	390	UGUUCGGGUCA UGCACACA	391	432
126	CAUCGCCCUGCU AUUGAAA	392	UUUCAUAGCA GGGCGAUG	393	2655
127	CCUUACAACCA GCGCAGGA	394	UCCUGCGCUGG UUGUAAGG	395	1441
128	AUCUCCGCGCCC CAUGAAA	396	UUUCAUGGGGC GCGGAGAU	397	2458

129	CUUUUCUUGAC GGUGAUGA	398	UCAUCACCGUCA AGAAAAG	399	2605
130	UCACUAGAGGC CUUAGUGA	400	UCACUAAGGCC UCUAGUGA	401	5771
131	GCGAAUGUGUC AUGAAGAA	402	UUCUUCAUGAC ACAUUCGC	403	4562
132	UCUGACAACCC GGAUUUUA	404	UAAAAUCCGGG UUGUCAGA	405	985
133	AGUAUCAUAAC CUCCGUCA	406	UGACGGAGGUU AUGAUACU	407	1843
134	GCAUAUGUUGG CACAGCAA	408	UUGCUGUGCCA ACAUAUGC	409	598
135	CCCUUACUGUG UGGCAUA	410	UAUUGCCACAC AGUAAGGG	411	919
136	CUACAGCACAC ACCGGACA	412	UGUCCGGUGUG UGCUGUAG	413	255
137	CGAAAAAAUAU CUCCGCGA	414	UCGCGGAGAU UUUUUUCG	415	2449
138	CUUGGUGGGGC UGCAACUA	416	UAGUUGCAGCC CCACCAAG	417	783
139	CUGCAAGAUUC AAAACUCA	418	UGAGUUUUGAA UCUUGCAG	419	3993
140	CGUCGCUUUAC UCCGGAGA	420	UCUCCGGAGUA AAGCGACG	421	40
141	UCAUCCUAUGA ACCAAUAA	422	UUAUUGGUUCA UAGGAUGA	423	5509

Table 3: Nucleic Acid Sequences of 19/21-mer Nav1.8 siRNAs

siRNA No.	Sense Strand (5' to 3')	SEQ ID NO.	Antisense Strand (5' to 3') (with 3' UU overhang)	SEQ ID NO.	Start position of target region on human SCN10A transcript
142	UUACGGUCACU AUUUUGGA	142	UCCAAAAUAGUG ACCGUAAUU	424	407
143	UGAUCUUGGCU GUAGUCAA	144	UUGACUACAGCC AAGAUCAUU	425	1172
144	UAAUUGUGUGU GCAUGACA	146	UGUCAUGCACAC ACAAUUAUU	426	426
145	CGGUCACUAUU UUGGUUAA	148	UUAACCAAAAUA GUGACCGUU	427	410
146	AACCUGAUCAG AAGAACGA	150	UCGUUCUUCUGA UCAGGUUUU	428	349
147	AAAUGAGUUCA CGUACCUA	152	UAGGUACGUGAA CUCAUUUUU	429	537
148	CAACCCGGAUU UUAACUAA	154	UUAGUAAAAUC CGGGUUGUU	430	990
149	CUGACAACCCG GAUUUUAA	156	UUAAAAUCCGGG UUGUCAGUU	431	986
150	AAUGAGUUCAC GUACCUGA	158	UCAGGUACGUGA ACUCAUUUU	432	538
151	ACAACCCGGAU UUUAACUA	160	UAGUUAAAAUCC GGGUUGUUU	433	989
152	AGAACGGCCAU CAAAGUGA	162	UCACUUUGAUGG CCGUUCUUU	434	361
153	CUUCUGACAAC CCGGAUUA	164	UAAUCCGGGUUG UCAGAAGUU	435	983
154	UGUCUAAAUGA GUUCACGA	166	UCGUGAACUCAU UUAGACAUU	436	532
155	AGAAUACUUAG AUGAACCA	168	UGGUUCAUCUAA GUAUUCUUU	437	1794
156	UACCUUCGAAG CCAUGCUA	170	UAGCAUGGCUUC GAAGGUAUU	438	2073
157	GUGCAUGACCC GAACUGAA	172	UUCAGUUCGGGU CAUGCACUU	439	435
158	UGUGCAUGACC CGAACUGA	174	UCAGUUCGGGUC AUGCACAUU	440	434
159	CUUACAACCAG CGCAGGAA	176	UCCUGCGCUGG UUGUAAGUU	441	1442
160	CCUACCUUCGA AGCCAUGA	178	UCAUGGCUUCGA AGGUAGGUU	442	2071
161	GCGUCAUUACC CUGGCAUA	180	UAUGCCAGGGUA AUGACGCUU	443	584

162	AAUUGUGUGUG CAUGACCA	182	UGGUCAUGCACA CACAAUUUU	444	427
163	GGAUUUUAGCG UCAUUACA	184	UGUAAUGACGCU AAAAUCCUU	445	576
164	CAGCAGAAUAC UUAGAUGA	186	UCAUCUAAGUAU UCUGCUGUU	446	1790
165	GCAUGAGCCCU ACCUUCGA	188	UCGAAGGUAGGG CUCAUGCUU	447	2063
166	UGACAACCCGG AUUUUAAA	190	UUUAAAAUCCGG GUUGUCAUU	448	987
167	CUAAAUGAGUU CACGUACA	192	UGUACGUGAACU CAUUUAGUU	449	535
168	UGUCAGCAGAA UACUUAGA	194	UCUAAGUAUUCU GCUGACAUU	450	1787
169	AACCAACUACU CAUCUCAA	196	UUGAGAUGAGUA GUUGGUUUU	451	858
170	CGCUUACUCC GGAGUCAA	198	UUGACUCCGGAG UAAAGCGUU	452	43
171	ACAACCAACUA CUCAUCUA	200	UAGAUGAGUAGU UGGUUGUUU	453	856
172	CUUGUACCUUU GUCGAUUA	202	UAAUCGACAAAG GUACAAGUU	454	3958
173	CACGUACCUGA GAGAUCCA	204	UGGAUCUCUCAG GUACGUGUU	455	546
174	CCCCAAACCCA AUCGAAAA	206	UUUUCGAUUGGG UUUUGGGUU	456	5331
175	GGGUGAAGCUC AAGACAAA	208	UUUGUCUUGAGC UUCACCCUU	457	1952
176	GUUUAAUUUAU ACGGUCAA	210	UUGACCGUAAUA AAUAAACUU	458	398
177	CAGUUUAAUUA UUACGGUA	212	UACCGUAAUAAA UAAACUGUU	459	396
178	ACCAACUACUC AUCUCACA	214	UGUGAGAUGAGU AGUUGGUUU	460	859
179	ACCUGAUCAGA AGAACGGA	216	UCCGUUCUUCUG AUCAGGUUU	461	350
180	CUUUUUUGUGC UCGUAAUA	218	UAUUACGAGCAC AAAAAAGUU	462	1125
181	GAAUUCCCCAU UGGAUCCA	220	UGGAUCCAAUGG GGAAUUCUU	463	4
182	GGCAUAUGUUG GCACAGCA	222	UGCUGUGCCAAC AUAUGCCUU	464	597
183	GCCUUAGUGAU AGAGUCAA	224	UUGACUCUAUCA CUAAGGCUU	465	5780
184	UCAUUACCCUG GCAUAUGA	226	UCAUAUGCCAGG GUAAUGAUU	466	587
185	AAUGAGUGUUG UCAGUAUA	228	UAUACUGACAAC ACUCAUUUU	467	1830

186	AGGACAUCUAG CUCAAUAA	230	UUAUUGAGCUAG AUGUCCUUU	468	5803
187	UGAUCCUUACA ACCAGCGA	232	UCGCUGGUUGUA AGGAUCAUU	469	1437
188	UUAUUUAUUAC GGUCACUA	234	UAGUGACCGUAA UAAAUAAUU	470	400
189	AAGAACGGCCA UCAAGUA	236	UACUUUGAUGGC CGUUCUUUU	471	360
190	GACAACCCGGA UUUUAACA	238	UGUUAAAAUCCG GGUUGUCUU	472	988
191	UCUAGCUCAAU ACAAAUA	240	UAUUUUGUAUUG AGCUAGA UU	473	5809
192	GAUCUCCGUGG GAUCUCAA	242	UUGAGAUGCCAC GGAGAUCUU	474	619
193	CCGGUUUAGUG CCACUCGA	244	UCGAGUGGCACU AAACCGGUU	475	306
194	CUCAUCCUUUU CUUGACGA	246	UCGUCAAGAAAA GGAUGAGUU	476	2599
195	ACCCAAUCGAA AUAUACUA	248	UAGUAUAUUUCG AUUGGGUUU	477	5337
196	UUUAUCUUUGU GUUCGAGA	250	UCUCGAACACAA AGAUAAA UU	478	3583
197	ACAGAAGGAUG CAUUCGCA	252	UGCGAAUGCAUC CUUCUGUUU	479	3346
198	GUCACUAGAGG CCUUAGUA	254	UACUAAGGCCUC UAGUGACUU	480	5770
199	UCUUUUUUGUG CUCGUAAA	256	UUUACGAGCACA AAAAAGAUU	481	1124
200	UUGUCGAUUGU GAAUAACA	258	UGUUAUUCACAA UCGACAAUU	482	3967
201	AGGGCAAUGAG UGUUGUCA	260	UGACAACACUCA UUGCCCUUU	483	1825
202	UUUUUGUGCUC GUAAUCUA	262	UAGAUUACGAGC ACAAAAAUU	484	1127
203	UUCGAUGCAGG ACAAAAGA	264	UCUUUUGUCCUG CAUCGAAUU	485	1759
204	CAACCGAAAAA AUAUCUA	266	UGAGAUUUUUUU UCGGUUGUU	486	2445
205	ACGGUCACUAU UUUGGUUA	268	UAACCAAAAUAG UGACCGUUU	487	409
206	GGAUGCAUUCG CCACUGUA	270	UACAGUGGCGAA UGCAUCCUU	488	3352
207	GGGCAAUGAGU GUUGUCAA	272	UUGACAACACUC AUUGCCCUU	489	1826
208	UGAUAGAGUCA ACAUGAGA	274	UCUCAUGUUGAC UCUAUCAUU	490	5787
209	AUCCUUACAAC CAGCGCAA	276	UUGCGCUGGUUG UAAGGAUUU	491	1439

210	UGUACCUUUGU CGAUUGUA	278	UACAAUCGACAA AGGUACAUI	492	3960
211	UUGUACCUUUG UCGAUUGA	280	UCAAUCGACAAA GGUACAAUI	493	3959
212	ACUCGAGGAGU CUGAACAA	282	UUGUUCAGACUC CUCGAGUII	494	1869
213	CAGUAUCAUAA CCUCCGUA	284	UACGGAGGUUAI GAUACUGUI	495	1842
214	AAGGGCAAUGA GUGUUGUA	286	UACAACACUCAU UGCCCUUII	496	1824
215	CAAUGAGUGUI GUCAGUAA	288	UUACUGACAACA CUCAUUGUI	497	1829
216	UGCAUUCGCCA CUGUCCCA	290	UGGGACAGUGGC GAAUGCAUI	498	3355
217	AUCUUUUUUGU GCUCGUAA	292	UUACGAGCACAA AAAAGAUUI	499	1123
218	CAGUGACUUCA UCGCUAAA	294	UUUAGCGAUGAA GUCACUGUI	500	2952
219	GUUUUAGGCAA CUAAUCUA	296	UAGAUUAGUUGC CAUAAACUI	501	5484
220	CUUGUGCAUCG UGGUGAAA	298	UUUCACCACGAU GCACAAGUI	502	2016
221	CUUUGGGCUUG UGACGGAA	300	UCCGUCACAAG CCCAAAGUI	503	1974
222	CUUGGGCUUUC CUCUCACA	302	UGUGAGAGGAAA GCCCAAGUI	504	1028
223	CAUCUAGCUCA AUACAAAA	304	UUUUGUAUUGAG CUAGAUGUI	505	5807
224	GUUGUCAGUAU CAUAACCA	306	UGGUUAUGAUAC UGACAACUI	506	1837
225	CUCCGGAGUCA CUGGUGGA	308	UCCACCAGUGAC UCCGGAGUI	507	50
226	AGGUCUUUGGC CAUCGUAA	310	UUACGAUGGCCA AAGACCUUI	508	2753
227	GUGUGCAUGAC CCGAACUA	312	UAGUUCGGGUCA UGCACACUI	509	433
228	CACGGAUCCAG GUCUUUGA	314	UCAAAGACCUGG AUCCGUGUI	510	2744
229	ACUUAGAUGAA CCUUUCCA	316	UGGAAAGGUUCA UCUAAGUII	511	1799
230	AACAUGCAACC CAAGUGGA	318	UCCACUUGGGUI GCAUGUII	512	4147
231	UAGAGGCCUUA GUGAUAGA	320	UCUAUCACUAAG GCCUCUAUI	513	5775
232	AUCGAAAUAA CUGAUCCA	322	UGGAUCAGUAUA UUUCGAUII	514	5342
233	UUUCAGCCACU GUCAUUCA	324	UGAAUGACAGUG GCUGAAUI	515	5558

234	AUAACCUCCGU CCUUGAGA	326	UCUCAAGGACGG AGGUUAAUUU	516	1849
235	UUUAUGGCAAC UAAUCUUA	328	UAAGAUUAGUUG CCAUAAAUU	517	5485
236	CUAUGAACCAA UAGCAACA	330	UGUUGCUAUUGG UUCAUAGUU	518	5514
237	CCAACUACUCA UCUCACAA	332	UUGUGAGAUGAG UAGUUGGUU	519	860
238	GUUCAUCGCCC UGCUAUUA	334	UAAUAGCAGGGC GAUGAACUU	520	2652
239	AUCCUUUUCUU GACGGUGA	336	UCACCGUCAAGA AAAGGAUUU	521	2602
240	CUAUCGGAGCU AUGUGCUA	338	UAGCACAUAGCU CCGAUAGUU	522	5583
241	CCAAGAAUGUC CUAGGAGA	340	UCUCCUAGGACA UUCUUGGUU	523	5423
242	UUCUACAGCAC ACACCGGA	342	UCCGGUGUGUGC UGUAGAAUU	524	253
243	AGUUUAUUUAU UACGGUCA	344	UGACCGUAAUAA AUAAACUUU	525	397
244	AUCAUCCUAUG AACCAAUA	346	UAUUGGUUCAUA GGAUGAUUU	526	5508
245	CAGGUCUUUGG CCAUCGUA	348	UACGAUGGCCAA AGACCUGUU	527	2752
246	GCACACACCGG ACAUUUAA	350	UUAAAUGUCCGG UGUGUGCUU	528	260
247	CAUCGUGACCA GACAAGCA	352	UGCUGUGUCUGGU CACGAUGUU	529	4413
248	GCAAUGGGUUA CCUUGCAA	354	UUGCAAGGUAAC CCAUUGCUU	530	4060
249	UCCUUUUCUUG ACGGUGAA	356	UUCACCGUCAAG AAAAGGAUU	531	2603
250	UCGAAAUAUAC UGAUCCAA	358	UUGGAUCAGUAU AUUUCGAUU	532	5343
251	UCGAUGCAGGA CAAAAGAA	360	UUCUUUUGUCCU GCAUCGAUU	533	1760
252	CAAAAGGCCUA UCGGAGCA	362	UGCUCCGAUAGG CCUUUUGUU	534	5575
253	GGCGAAUGUGU CAUGAAGA	364	UCUUCAUGACAC AUUCGCCUU	535	4561
254	GCUAUGUGCUG CACCGCUA	366	UAGCGGUGCAGC ACAUAGCUU	536	5591
255	UCCCAGCAGAA CUGAUCGA	368	UCGAUCAGUUCU GCUGGGAUU	537	209
256	CACACCGGACA UUUAUGGA	370	UCCAUAAAUGUC CGGUGUGUU	538	263
257	GUGUCACUAGA GGCCUUA	372	UUAAGGCCUCUA GUGACACUU	539	5768

258	UGUCGAUUGUG AAUAACAA	374	UUGUUAUUCACA AUCGACAUI	540	3968
259	AACUUCUGACA ACCCGGAA	376	UCCCGGUUGUC AGAAGUUUU	541	981
260	CUAUGUGCUGC ACCGCUCA	378	UGAGCGGUGCAG CACAUAGUU	542	5592
261	AAGUCUGACUG CAAGAUUA	380	UAAUCUUGCAGU CAGACUUUU	543	3985
262	CCUUUGUCGAU UGUGAAUA	382	UAUUCACAAUCG ACAAAGGUU	544	3964
263	GUUUAGUGCCA CUCGGGCA	384	UGCCCGAGUGGC ACUAAACUU	545	309
264	UCCUUAACAACC AGCGCAGA	386	UCUGCGCUGGUU GUAAGGAUU	546	1440
265	AACCCAAUCGA AAUAUACA	388	UGUAUAUUUCGA UUGGGUUUU	547	5336
266	UGUGUGCAUGA CCCGAACA	390	UGUUCGGGUCAU GCACACAUI	548	432
267	CAUCGCCUGC UAUUGAAA	392	UUUCAAUAGCAG GGCGAUGUU	549	2655
268	CCUUACAACCA GCGCAGGA	394	UCCUGCGCUGGU UGUAAGGUU	550	1441
269	AUCUCCGCGCC CCAUGAAA	396	UUUCAUGGGGCG CGGAGAUUU	551	2458
270	CUUUUCUUGAC GGUGAUGA	398	UCAUCACCGUCA AGAAAAGUU	552	2605
271	UCACUAGAGGC CUUAGUGA	400	UCACUAAGGCCU CUAGUGAUU	553	5771
272	GCGAAUGUGUC AUGAAGAA	402	UUCUUAUGACA CAUUCGCUU	554	4562
273	UCUGACAACCC GGAUUUUA	404	UAAAAUCCGGGU UGUCAGAUU	555	985
274	AGUAUCAUAAC CUCCGUCA	406	UGACGGAGGUUA UGAUACUUU	556	1843
275	GCAUAUGUUGG CACAGCAA	408	UUGCUGUGCCAA CAUAUGCUU	557	598
276	CCCUUACUGUG UGGCAAUA	410	UAUUGCCACACA GUAAGGGUU	558	919
277	CUACAGCACAC ACCGGACA	412	UGUCCGGUGUGU GCUGUAGUU	559	255
278	CGAAAAAAUAU CUCCGCGA	414	UCGCGGAGAUAU UUUUUCGUU	560	2449
279	CUUGGUGGGGC UGCAACUA	416	UAGUUGCAGCCC CACCAAGUU	561	783
280	CUGCAAGAUUC AAAACUCA	418	UGAGUUUUGAAU CUUGCAGUU	562	3993
281	CGUCGCUUUAC UCCGGAGA	420	UCUCCGGAGUAA AGCGACGUU	563	40

282	UCAUCCUAUGA ACCAAUAA	422	UUAUUGGUUCAU AGGAUGAUU	564	5509
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[0053] In some embodiments, the oligonucleotide of the present disclosure is such that the sense strand and the antisense strands comprise nucleotide sequences selected from the group consisting of:

- (a) SEQ ID NOs: 565 and 566, respectively;
- (b) SEQ ID NOs: 567 and 568, respectively;
- (c) SEQ ID NOs: 569 and 570, respectively;
- (d) SEQ ID NOs: 571 and 572, respectively;
- (e) SEQ ID NOs: 573 and 574, respectively;
- (f) SEQ ID NOs: 575 and 576, respectively;
- (g) SEQ ID NOs: 577 and 578 respectively;
- (h) SEQ ID NOs: 579 and 580, respectively;
- (i) SEQ ID NOs: 581 and 582, respectively;
- (j) SEQ ID NOs: 583 and 584, respectively;
- (k) SEQ ID NOs: 585 and 586, respectively;
- (l) SEQ ID NOs: 587 and 588, respectively;
- (m) SEQ ID NOs: 589 and 590 respectively;
- (n) SEQ ID NOs: 591 and 592, respectively;
- (o) SEQ ID NOs: 593 and 594, respectively;
- (p) SEQ ID NOs: 595 and 596, respectively;
- (q) SEQ ID NOs: 597 and 598, respectively;
- (r) SEQ ID NOs: 599 and 600, respectively;
- (s) SEQ ID NOs: 601 and 602 respectively; and
- (t) SEQ ID NOs: 603 and 604 respectively.

[0054] In some embodiments, the oligonucleotide of the present disclosure is such that the sense strand and the antisense strands comprise nucleotide sequences selected from the group consisting of:

- (a) SEQ ID NOs: 565 and 605, respectively;
- (b) SEQ ID NOs: 567 and 606, respectively;
- (c) SEQ ID NOs: 569 and 607, respectively;
- (d) SEQ ID NOs: 571 and 608, respectively;
- (e) SEQ ID NOs: 573 and 609, respectively;

- (f) SEQ ID NOs: 575 and 610, respectively;
- (g) SEQ ID NOs: 577 and 611 respectively;
- (h) SEQ ID NOs: 579 and 612, respectively;
- (i) SEQ ID NOs: 581 and 613, respectively;
- (j) SEQ ID NOs: 583 and 614, respectively;
- (k) SEQ ID NOs: 585 and 615, respectively;
- (l) SEQ ID NOs: 587 and 616, respectively;
- (m) SEQ ID NOs: 589 and 617 respectively;
- (n) SEQ ID NOs: 591 and 618, respectively;
- (o) SEQ ID NOs: 593 and 619, respectively;
- (p) SEQ ID NOs: 595 and 620, respectively;
- (q) SEQ ID NOs: 597 and 621, respectively;
- (r) SEQ ID NOs: 599 and 622, respectively;
- (s) SEQ ID NOs: 601 and 623 respectively; and
- (t) SEQ ID NOs: 603 and 624 respectively.

[0055] In some embodiments, the oligonucleotide of the present disclosure is such that i) the sense strand has a sequence as set forth in Table 2 and antisense strand has a sequence as set forth in Table 2; ii) the sense strand has a sequence set forth in Table 3 and the antisense strand has a sequence set forth in Table 3; iii) the oligonucleotide has a sequence set forth by any one of siRNA No. 1 to 141; iv) the oligonucleotide has a sequence set forth by any one of siRNA No. 142 to 282; or v) the oligonucleotide has a sequence set forth by any one of siRNA No. 283 to 322.

RNA Modifications

[0056] A variety of oligonucleotide types and/or structures are useful for targeting SCN10A mRNA including, but not limited to, oligonucleotides, antisense oligonucleotides (ASOs), miRNAs, *etc.* Any of the oligonucleotide types described herein or elsewhere are contemplated for use as a framework to incorporate a targeting sequence herein for the purposes of inhibiting SCN10A activity. In some embodiments, the oligonucleotide herein inhibits SCN10A activity by engaging with RNAi pathways upstream or downstream of Dicer involvement. For example, oligonucleotides have been developed with each strand having sizes of about 19-25 nucleotides with at least one 3' overhang of 1 to 5 nucleotides (*see, e.g.*, US Patent No. 8,372,968). Longer oligonucleotides also have been developed that are processed by Dicer to generate active RNAi products (*see, e.g.*, US Patent No. 8,883,996). Further work produced extended RNAi oligonucleotides where at least one end of at least one

strand is extended beyond a duplex targeting region, including structures where one of the strands includes a thermodynamically stabilizing tetraloop (*see, e.g.*, US Patent Nos. 8,513,207 and 8,927,705, as well as Intl. Patent Application Publication No. WO 2010/033225). Such structures include single strand extensions (on one or both sides of the molecule) as well as double strand extensions.

[0057] In some embodiments, the oligonucleotides herein engage with the RNAi pathway downstream of the involvement of Dicer (*e.g.*, Dicer cleavage). In some embodiments, the oligonucleotide has an overhang (*e.g.*, of 1, 2, or 3 nucleotides in length) in the 3' end of the sense strand. In some embodiments, the oligonucleotide (*e.g.*, siRNA) includes a 21-nucleotide antisense strand that is antisense to a target mRNA (*e.g.*, SCN10A mRNA) and a complementary sense strand, in which both strands anneal to form a 19-bp duplex and 2 nucleotide overhangs at either or both 3' ends. Longer oligonucleotide designs also are contemplated, including oligonucleotides having an antisense strand of 23 nucleotides and a sense strand of 21 nucleotides, where there is a blunt end on the right side of the molecule (3' end of sense strand/5' end of antisense strand) and a two nucleotide 3' antisense strand overhang on the left side of the molecule (5' end of the sense strand/3' end of the antisense strand). In such molecules, there is a 21 bp duplex region. *See, e.g.*, US Patent Nos. 9,012,138; 9,012,621 and 9,193,753.

[0058] In some embodiments, the oligonucleotide of the present disclosure has a modification pattern as shown in FIG. 1A, optionally without the amine or cholesterol handle. In some embodiments, the oligonucleotide has a modification pattern as shown in FIG. 1B, optionally without the amine or cholesterol handle.

[0059] In some embodiments, the oligonucleotide of the present disclosure has an antisense strand that includes a 3' overhang sequence of 1 or more nucleotides in length. In some embodiments, the 3' overhang sequence is 2 nucleotides in length. In some embodiments, the 3' overhang sequence is UU. In some embodiments, the 3' overhang sequence is AA, GG, or CC.

[0060] In some embodiments, the oligonucleotide of the present disclosure includes at least 1 modified nucleotide. In some embodiments, all nucleotides of the oligonucleotide are modified.

[0061] A modified sugar (also referred to herein as a sugar analog) includes a modified deoxyribose or ribose moiety in which, for example, one or more modifications occur at the 2', 3', 4', and/or 5' carbon position of the sugar. A modified sugar also includes non-natural, alternative, carbon structures such as those present in locked nucleic acids ("LNA"; *see, e.g.*,

Koshkin et al., “LNA (Locked Nucleic Acids): Synthesis of the Adenine, Cytosine, Guanine, 5-methylcytosine, Thymine and Uracil Bicyclonucleoside Monomers, Oligomerisation, and Unprecedented Nucleic Acid Recognition,” *Tetrahedron* 54, no. 14 (1998): 3607-3630), unlocked nucleic acids (“UNA”; *see, e.g.*, Snead et al., “5' Unlocked Nucleic Acid Modification Improves siRNA Targeting,” *Molecular Therapy-Nucleic Acids* 2 (2013)) and bridged nucleic acids (“BNA”; *see, e.g.*, Imanishi, Takeshi, and Satoshi Obika, “BNAs: Novel Nucleic Acid Analogs with a Bridged Sugar Moiety,” *Chemical Communications* 16 (2002): 1653-1659).

[0062] In some embodiments, the nucleotide modification in the sugar is a 2'-modification, for example, 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-F (2'-Fluoro), 2'-aminoethyl (EA), 2'-OMe (2'-OMethyl), 2'-MOE (2'-methoxyethyl), 2'-O-[2-(methylamino)-2-oxoethyl] (2'-O-NMA), or 2'-FANA (2'-Fluoro-arabinonucleic acid). In certain embodiments, the modification is 2'-F, 2'-OMe, or 2'-MOE. In other embodiments, the modification in the sugar is a modification of the sugar ring, which includes modification of one or more carbons of the sugar ring. For example, the modification in the sugar is a 2'-oxygen of the sugar linked to a 1'-carbon or 4'-carbon of the sugar, or a 2'-oxygen linked to the 1'-carbon or 4'-carbon *via* an ethylene or methylene bridge. In other embodiments, the modification is an acyclic sugar that lacks a 2'-carbon to 3'-carbon bond. In other embodiments, the modification is a thiol group such as, for example, in the 4' position of the sugar.

[0063] The oligonucleotides herein include at least 1 modified nucleotide (*e.g.*, at least 1, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, or more). In some embodiments, the sense strand comprises at least 1 modified nucleotide (*e.g.*, at least 1, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, or more). In some embodiments, the antisense strand comprises at least 1 modified nucleotide (*e.g.*, at least 1, at least 5, at least 10, at least 15, at least 20, or more).

[0064] In certain embodiments, all nucleotides of the sense strand except the tetraL (also referred to herein as tetraloop) are modified. Likewise, all nucleotides of the antisense strand are modified. In some embodiments, all the nucleotides of the oligonucleotide (*i.e.*, paired nucleotides of the sense strand and the antisense strand) are modified. As above, and in some embodiments, the modified nucleotide is a 2'-modification (*e.g.*, a 2'-F, 2'-OMe, 2'-MOE, and/or 2'-FANA). In certain embodiments, the modified nucleotide is a 2'-modification such as, for example, a 2'-F or a 2'-OMe.

[0065] In some embodiments, the modified nucleotide comprises a 2'-modification. In some embodiments, the 2'-modification can be, for example, 2'-aminoethyl, 2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl, or 2'-deoxy-2'-fluoro- β -d-arabinonucleic acid. In some embodiments, the oligonucleotide is modified and includes 2'-fluoro, vinyl phosphonate uridine (VpUq), or 2'-O-methyl modifications.

[0066] In some embodiments, the oligonucleotide of the present disclosure includes at least one vinyl phosphonate uridine (VpUq) at the 5' end of the antisense strand. In some embodiments, the oligonucleotide of the present disclosure includes at least two or more vinyl phosphonate uridine (VpUq) at the 5' end of the antisense strand. In some embodiments, the oligonucleotide is modified and contains a vinyl phosphonate (Vp).

[0067] In some embodiments, the oligonucleotide comprises a sense strand with about 50-90% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%) of the nucleotides of the sense strand comprising a 2'-OMe modification. In some embodiments, the oligonucleotide comprises a sense strand with about 50-60% of the nucleotides of the sense strand comprising a 2'-OMe modification. In some embodiments, the oligonucleotide comprises a sense strand with about 60%-70% of the nucleotides of the sense strand comprising a 2'-OMe modification. In some embodiments, the oligonucleotide comprises a sense strand with about 70%-80% of the nucleotides of the sense strand comprising a 2'-OMe modification. In some embodiments, the oligonucleotide comprises a sense strand with about 80%-90% of the nucleotides of the sense strand comprising a 2'-OMe modification. In some embodiments, the oligonucleotide comprises a sense strand with about 90%-100% of the nucleotides of the sense strand comprising a 2'-OMe modification. In some embodiments, about 78% of the nucleotides of the sense strand comprise a 2'-OMe modification. In some embodiments, about 79% of the nucleotides of the sense strand comprise a 2'-OMe modification. In some embodiments, about 80% of the nucleotides of the sense strand comprise a 2'-OMe modification. In some embodiments, about 81% of the nucleotides of the sense strand comprise a 2'-OMe modification. In some embodiments, about 84% of the nucleotides of the sense strand comprise a 2'-OMe modification.

[0068] In some embodiments, the oligonucleotide comprises an anti-sense strand with about 50-90% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%) of the nucleotides of the anti-sense strand comprising a 2'-OMe modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 50-60% of the nucleotides of the anti-sense strand comprising a 2'-OMe modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 60%-70% of the nucleotides of the

anti-sense strand comprising a 2'-OMe modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 70%-80% of the nucleotides of the anti-sense strand comprising a 2'-OMe modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 80%-90% of the nucleotides of the anti-sense strand comprising a 2'-OMe modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 90%-100% of the nucleotides of the anti-sense strand comprising a 2'-OMe modification. In some embodiments, about 78% of the nucleotides of the anti-sense strand comprise a 2'-OMe modification. In some embodiments, about 79% of the nucleotides of the anti-sense strand comprise a 2'-OMe modification. In some embodiments, about 80% of the nucleotides of the anti-sense strand comprise a 2'-OMe modification. In some embodiments, about 81% of the nucleotides of the anti-sense strand comprise a 2'-OMe modification.

[0069] In some embodiments, the oligonucleotide has about 15% to about 25% (*e.g.*, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, or 25%) of its nucleotides comprising a 2'-OMe modification. In some embodiments, the oligonucleotide has about 35-45% (*e.g.*, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45%) of its nucleotides comprising a 2'-OMe modification. In some embodiments, the oligonucleotide has about 45-85% (*e.g.*, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85%) of its nucleotides comprising a 2'-OMe modification. In some embodiments, about 70% of the nucleotides in the oligonucleotide comprise a 2'-OMe modification. In some embodiments, about 75% of the nucleotides in the oligonucleotide comprise a 2'-OMe modification. In some embodiments, about 80% of the nucleotides in the oligonucleotide comprise a 2'-OMe modification. In some embodiments, about 85% of the nucleotides in the oligonucleotide comprise a 2'-OMe modification. In some embodiments, about 90% of the nucleotides in the oligonucleotide comprise a 2'-OMe modification. In some embodiments, about 95% of the nucleotides in the oligonucleotide comprise a 2'-OMe modification.

[0070] In some embodiments, the oligonucleotide comprises a sense strand with about 10-20% (*e.g.*, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20%) of the nucleotides of the sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises a sense strand with about 21-30% (*e.g.*, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%) of the nucleotides of the sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises a sense strand with about 38-43% (*e.g.*, 38%, 39%, 40%, 41%, 42%, or 43%) of the nucleotides of the sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises a sense strand with about

38-43% (*e.g.*, 38%, 39%, 40%, 41%, 42%, or 43%) of the nucleotides of the sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises a sense strand with about 15% of the nucleotides of the sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises a sense strand with about 16% of the nucleotides of the sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises a sense strand with about 17% of the nucleotides of the sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises a sense strand with about 18% of the nucleotides of the sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises a sense strand with about 19% of the nucleotides of the sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises a sense strand with about 20% of the nucleotides of the sense strand comprising a 2'-F modification.

[0071] In some embodiments, the oligonucleotide comprises an anti-sense strand with about 10-20% (*e.g.*, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20%) of the nucleotides of the anti-sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 21-30% (*e.g.*, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%) of the nucleotides of the anti-sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 38-43% (*e.g.*, 38%, 39%, 40%, 41%, 42%, or 43%) of the nucleotides of the anti-sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 15% of the nucleotides of the anti-sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 16% of the nucleotides of the anti-sense strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 17% of the nucleotides comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 18% of the nucleotides of the strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 19% of the nucleotides of the strand comprising a 2'-F modification. In some embodiments, the oligonucleotide comprises an anti-sense strand with about 20% of the nucleotides of the strand comprising a 2'-F modification.

[0072] In some embodiments, about 14% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about 15% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about 16% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about

17% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about 18% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about 19% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about 20% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about 21% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about 22% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about 23% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about 24% of the nucleotides in the oligonucleotide comprise a 2'-F modification. In some embodiments, about 25% of the nucleotides in the oligonucleotide comprise a 2'-F modification.

[0073] Moreover, the oligonucleotides herein can have different modification patterns. In some embodiments, the modified oligonucleotide comprises a sense strand sequence having a modification pattern as set forth in Table 4 (as well as Figures 1A or 1B) and an antisense strand having a modification pattern as set forth in Table 4.

[0074] In some embodiments, the oligonucleotide of the present disclosure includes 2'-fluoro (2'-F) modifications at one or more nucleotides at positions 7, 8, or 9 of the sense strand. In some embodiments, the oligonucleotide is such that one or more nucleotides at positions 2, 6, 14, or 16 of the antisense strand are modified with 2'-fluoro. In some embodiments, the oligonucleotide is such that one or more nucleotides at positions 1, 2, 3, 4, 5, 6, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 of the sense strand are modified with 2'-O-methyl (2'-OMe). In some embodiments, the oligonucleotide is such that one or more nucleotides at positions 1, 3, 4, 5, 7 to 13, 15, 17, 18, 19, 20, or 21 of the antisense strand are modified with 2'-O-methyl.

[0075] In some embodiments, the oligonucleotide of the present disclosure includes at least one modified internucleotide linkage. The internucleotide linkage can be a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, at least two terminal nucleotides on 5' end or 3' end of the antisense strand or the sense strand have a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, at least three terminal nucleotides on 5' end or 3' end of the antisense strand or the sense strand have phosphorothioate linkages or a phosphorodithioate linkages.

[0076] In some embodiments, the oligonucleotide is such that the sense strand has 19 nucleotides and the internucleotide linkage of nucleotides between positions 1 and 2, 2 and 3, 17 and 18, or 18 and 19 of the sense strand are modified with the phosphorothioate linkage.

[0077] In some embodiments, the oligonucleotide is such that the antisense strand has 21 nucleotides and the internucleotide linkage of one or more nucleotides between positions 1 and 2, 2 and 3, 19 and 20, or 20 and 21 of the antisense strand are modified with the phosphorothioate linkage.

[0078] In some embodiments, the oligonucleotide of the present disclosure is selected from Table 4. Table 4 shows some embodiments of the modified siRNA sequences of the present disclosure.

Table 4: Nav1.8 siRNAs with Modifications

siRNA No.	Strand	Sequence (5' to 3')	SEQ ID NO.	Start position of target region on human SCN10A transcript
283	S	m(U)[ps].m(U)[ps].m(A)p.m(C)p.m(G)p.m(G)p.[fl2r](U)p.[fl2r](C)p.[fl2r](A)p.m(C)p.m(U)p.m(A)p.m(U)p.m(U)p.m(U)p.m(U)p.m(G)[ps].m(G)[ps].m(A)	565	407
	AS	m(U)[ps].[fl2r](C)[ps].m(C)p.m(A)p.m(A)p.[fl2r](A)p.m(A)p.m(U)p.m(A)p.m(G)p.m(U)p.m(G)p.m(A)p.[fl2r](C)p.m(C)p.[fl2r](G)p.m(U)p.m(A)p.m(A)[ps].m(U)[ps].m(U)	566	
284	S	m(A)[ps].m(C)[ps].m(G)p.m(G)p.m(U)p.m(C)p.[fl2r](A)p.[fl2r](C)p.[fl2r](U)p.m(A)p.m(U)p.m(U)p.m(U)p.m(U)p.m(G)p.m(G)p.m(U)[ps].m(U)[ps].m(A)	567	409
	AS	m(U)[ps].[fl2r](A)[ps].m(A)p.m(C)p.m(C)p.[fl2r](A)p.m(A)p.m(A)p.m(A)p.m(U)p.m(A)p.m(G)p.m(U)p.[fl2r](G)p.m(A)p.[fl2r](C)p.m(C)p.m(G)p.m(U)[ps].m(U)[ps].m(U)	568	
285	S	m(C)[ps].m(G)[ps].m(G)p.m(U)p.m(C)p.m(A)p.[fl2r](C)p.[fl2r](U)p.[fl2r](A)p.m(U)p.m(U)p.m(U)p.m(U)p.m(G)p.m(G)p.m(U)p.m(U)[ps].m(A)[ps].m(A)	569	410
	AS	m(U)[ps].[fl2r](U)[ps].m(A)p.m(A)p.m(C)p.[fl2r](C)p.m(A)p.m(A)p.m(A)p.m(A)p.m(U)p.m(A)p.m(G)p.[fl2r](U)p.m(G)p.[fl2r](A)p.m(C)p.m(C)p.m(G)[ps].m(U)[ps].m(U)	570	
286	S	m(U)[ps].m(A)[ps].m(A)p.m(U)p.m(U)p.m(G)p.[fl2r](U)p.[fl2r](G)p.[fl2r](U)p.m(G)p.m(U)p.m(G)p.m(C)p.m(A)p.m(U)p.m(G)p.m(A)[ps].m(C)[ps].m(A)	571	426
	AS	m(U)[ps].[fl2r](G)[ps].m(U)p.m(C)p.m(A)p.[fl2r](U)p.m(G)p.m(C)p.m(A)p.m(C)p.m(A)p.m(C)	572	

		C)p.m(A)p.[fl2r](C)p.m(A)p.[fl2r](A)p.m(U)p. m(U)p.m(A)[ps].m(U)[ps].m(U)		
287	S	m(A)[ps].m(A)[ps].m(U)p.m(U)p.m(G)p.m(U) p.[fl2r](G)p.[fl2r](U)p.[fl2r](G)p.m(U)p.m(G)p. m(C)p.m(A)p.m(U)p.m(G)p.m(A)p.m(C)[ps].m (C)[ps].m(A)	573	427
	AS	m(U)[ps].[fl2r](G)[ps].m(G)p.m(U)p.m(C)p.[fl 2r](A)p.m(U)p.m(G)p.m(C)p.m(A)p.m(C)p.m(A)p.m(C)p.[fl2r](A)p.m(C)p.[fl2r](A)p.m(A)p. m(U)p.m(U)[ps].m(U)[ps].m(U)	574	
288	S	m(U)[ps].m(G)[ps].m(U)p.m(G)p.m(C)p.m(A)p .[fl2r](U)p.[fl2r](G)p.[fl2r](A)p.m(C)p.m(C)p. m(C)p.m(G)p.m(A)p.m(A)p.m(C)p.m(U)[ps].m (G)[ps].m(A)	575	434
	AS	m(U)[ps].[fl2r](C)[ps].m(A)p.m(G)p.m(U)p.[fl 2r](U)p.m(C)p.m(G)p.m(G)p.m(G)p.m(U)p.m(C)p.m(A)p.[fl2r](U)p.m(G)p.[fl2r](C)p.m(A)p. m(C)p.m(A)[ps].m(U)[ps].m(U)	576	
289	S	m(G)[ps].m(U)[ps].m(G)p.m(C)p.m(A)p.m(U)p .[fl2r](G)p.[fl2r](A)p.[fl2r](C)p.m(C)p.m(C)p. m(G)p.m(A)p.m(A)p.m(C)p.m(U)p.m(G)[ps].m (A)[ps].m(A)	577	435
	AS	m(U)[ps].[fl2r](U)[ps].m(C)p.m(A)p.m(G)p.[fl 2r](U)p.m(U)p.m(C)p.m(G)p.m(G)p.m(G)p.m(U)p.m(C)p.[fl2r](A)p.m(U)p.[fl2r](G)p.m(C)p. m(A)p.m(C)[ps].m(U)[ps].m(U)	578	
290	S	m(U)[ps].m(G)[ps].m(U)p.m(C)p.m(U)p.m(A)p .[fl2r](A)p.[fl2r](A)p.[fl2r](U)p.m(G)p.m(A)p. m(G)p.m(U)p.m(U)p.m(C)p.m(A)p.m(C)[ps].m (G)[ps].m(A)	579	532
	AS	m(U)[ps].[fl2r](C)[ps].m(G)p.m(U)p.m(G)p.[fl 2r](A)p.m(A)p.m(C)p.m(U)p.m(C)p.m(A)p.m(U)p.m(U)p.[fl2r](U)p.m(A)p.[fl2r](G)p.m(A)p. m(C)p.m(A)[ps].m(U)[ps].m(U)	580	
291	S	m(C)[ps].m(U)[ps].m(A)p.m(A)p.m(A)p.m(U)p .[fl2r](G)p.[fl2r](A)p.[fl2r](G)p.m(U)p.m(U)p. m(C)p.m(A)p.m(C)p.m(G)p.m(U)p.m(A)[ps].m (C)[ps].m(A)	581	535
	AS	m(U)[ps].[fl2r](G)[ps].m(U)p.m(A)p.m(C)p.[fl 2r](G)p.m(U)p.m(G)p.m(A)p.m(A)p.m(C)p.m(U)p.m(C)p.[fl2r](A)p.m(U)p.[fl2r](U)p.m(U)p. m(A)p.m(G)[ps].m(U)[ps].m(U)	582	
292	S	m(A)[ps].m(A)[ps].m(A)p.m(U)p.m(G)p.m(A) p.[fl2r](G)p.[fl2r](U)p.[fl2r](U)p.m(C)p.m(A)p. m(C)p.m(G)p.m(U)p.m(A)p.m(C)p.m(C)[ps].m (U)[ps].m(A)	583	537
	AS	m(U)[ps].[fl2r](A)[ps].m(G)p.m(G)p.m(U)p.[fl 2r](A)p.m(C)p.m(G)p.m(U)p.m(G)p.m(A)p.m(A)p.m(C)p.[fl2r](U)p.m(C)p.[fl2r](A)p.m(U)p. m(U)p.m(U)[ps].m(U)[ps].m(U)	584	

293	S	m(G)[ps].m(G)[ps].m(A)p.m(U)p.m(U)p.m(U)p. p.[fl2r](U)p.[fl2r](A)p.[fl2r](G)p.m(C)p.m(G)p. m(U)p.m(C)p.m(A)p.m(U)p.m(U)p.m(A)[ps].m (C)[ps].m(A)	585	576
	AS	m(U)[ps].[fl2r](G)[ps].m(U)p.m(A)p.m(A)p.[fl 2r](U)p.m(G)p.m(A)p.m(C)p.m(G)p.m(C)p.m(U)p.m(A)p.[fl2r](A)p.m(A)p.[fl2r](A)p.m(U)p. m(C)p.m(C)[ps].m(U)[ps].m(U)	586	
294	S	m(A)[ps].m(C)[ps].m(A)p.m(A)p.m(C)p.m(C)p .[fl2r](A)p.[fl2r](A)p.[fl2r](C)p.m(U)p.m(A)p. m(C)p.m(U)p.m(C)p.m(A)p.m(U)p.m(C)[ps].m (U)[ps].m(A)	587	856
	AS	m(U)[ps].[fl2r](A)[ps].m(G)p.m(A)p.m(U)p.[fl 2r](G)p.m(A)p.m(G)p.m(U)p.m(A)p.m(G)p.m(U)p.m(U)p.[fl2r](G)p.m(G)p.[fl2r](U)p.m(U)p. m(G)p.m(U)[ps].m(U)[ps].m(U)	588	
295	S	m(A)[ps].m(A)[ps].m(C)p.m(U)p.m(U)p.m(C)p .[fl2r](U)p.[fl2r](G)p.[fl2r](A)p.m(C)p.m(A)p. m(A)p.m(C)p.m(C)p.m(C)p.m(G)p.m(G)[ps].m (A)[ps].m(A)	589	981
	AS	m(U)[ps].[fl2r](U)[ps].m(C)p.m(C)p.m(G)p.[fl 2r](G)p.m(G)p.m(U)p.m(U)p.m(G)p.m(U)p.m(C)p.m(A)p.[fl2r](G)p.m(A)p.[fl2r](A)p.m(G)p. m(U)p.m(U)[ps].m(U)[ps].m(U)	590	
296	S	m(C)[ps].m(U)[ps].m(U)p.m(C)p.m(U)p.m(G)p .[fl2r](A)p.[fl2r](C)p.[fl2r](A)p.m(A)p.m(C)p. m(C)p.m(C)p.m(G)p.m(G)p.m(A)p.m(U)[ps].m (U)[ps].m(A)	591	983
	AS	m(U)[ps].[fl2r](A)[ps].m(A)p.m(U)p.m(C)p.[fl 2r](C)p.m(G)p.m(G)p.m(G)p.m(U)p.m(U)p.m(G)p.m(U)p.[fl2r](C)p.m(A)p.[fl2r](G)p.m(A)p. m(A)p.m(G)[ps].m(U)[ps].m(U)	592	
297	S	m(C)[ps].m(U)[ps].m(G)p.m(A)p.m(C)p.m(A)p .[fl2r](A)p.[fl2r](C)p.[fl2r](C)p.m(C)p.m(G)p. m(G)p.m(A)p.m(U)p.m(U)p.m(U)p.m(U)[ps].m (A)[ps].m(A)	593	986
	AS	m(U)[ps].[fl2r](U)[ps].m(A)p.m(A)p.m(A)p.[fl 2r](A)p.m(U)p.m(C)p.m(C)p.m(G)p.m(G)p.m(G)p.m(U)p.[fl2r](U)p.m(G)p.[fl2r](U)p.m(C)p. m(A)p.m(G)[ps].m(U)[ps].m(U)	594	
298	S	m(U)[ps].m(G)[ps].m(A)p.m(C)p.m(A)p.m(A)p .[fl2r](C)p.[fl2r](C)p.[fl2r](C)p.m(G)p.m(G)p. m(A)p.m(U)p.m(U)p.m(U)p.m(U)p.m(A)[ps].m (A)[ps].m(A)	595	987
	AS	m(U)[ps].[fl2r](U)[ps].m(U)p.m(A)p.m(A)p.[fl 2r](A)p.m(A)p.m(U)p.m(C)p.m(C)p.m(G)p.m(G)p.m(G)p.[fl2r](U)p.m(U)p.[fl2r](G)p.m(U)p. m(C)p.m(A)[ps].m(U)[ps].m(U)	596	
299	S	m(A)[ps].m(C)[ps].m(A)p.m(A)p.m(C)p.m(C)p .[fl2r](C)p.[fl2r](G)p.[fl2r](G)p.m(A)p.m(U)p.	597	989

		m(U)p.m(U)p.m(U)p.m(A)p.m(A)p.m(C)[ps].m(U)[ps].m(A)		598
	AS	m(U)[ps].[fl2r](A)[ps].m(G)p.m(U)p.m(U)p.[fl2r](A)p.m(A)p.m(A)p.m(A)p.m(U)p.m(C)p.m(C)p.m(G)p.[fl2r](G)p.m(G)p.[fl2r](U)p.m(U)p.m(G)p.m(U)[ps].m(U)[ps].m(U)		
300	S	m(C)[ps].m(A)[ps].m(A)p.m(C)p.m(C)p.m(C)p.[fl2r](G)p.[fl2r](G)p.[fl2r](A)p.m(U)p.m(U)p.m(U)p.m(U)p.m(A)p.m(A)p.m(C)p.m(U)[ps].m(A)[ps].m(A)	599	990
	AS	m(U)[ps].[fl2r](U)[ps].m(A)p.m(G)p.m(U)p.[fl2r](U)p.m(A)p.m(A)p.m(A)p.m(A)p.m(U)p.m(C)p.m(C)p.[fl2r](G)p.m(G)p.[fl2r](G)p.m(U)p.m(U)p.m(G)[ps].m(U)[ps].m(U)	600	
301	S	m(U)[ps].m(G)[ps].m(A)p.m(U)p.m(C)p.m(U)p.[fl2r](U)p.[fl2r](G)p.[fl2r](G)p.m(C)p.m(U)p.m(G)p.m(U)p.m(A)p.m(G)p.m(U)p.m(C)[ps].m(A)[ps].m(A)	601	1172
	AS	m(U)[ps].[fl2r](U)[ps].m(G)p.m(A)p.m(C)p.[fl2r](U)p.m(A)p.m(C)p.m(A)p.m(G)p.m(C)p.m(C)p.m(A)p.[fl2r](A)p.m(G)p.[fl2r](A)p.m(U)p.m(C)p.m(A)[ps].m(U)[ps].m(U)	602	
302	S	m(U)[ps].m(A)[ps].m(C)p.m(C)p.m(U)p.m(U)p.[fl2r](C)p.[fl2r](G)p.[fl2r](A)p.m(A)p.m(G)p.m(C)p.m(C)p.m(A)p.m(U)p.m(G)p.m(C)[ps].m(U)[ps].m(A)	603	2073
	AS	m(U)[ps].[fl2r](A)[ps].m(G)p.m(C)p.m(A)p.[fl2r](U)p.m(G)p.m(G)p.m(C)p.m(U)p.m(U)p.m(C)p.m(G)p.[fl2r](A)p.m(A)p.[fl2r](G)p.m(G)p.m(U)p.m(A)[ps].m(U)[ps].m(U)	604	
303	S	m(U)[ps].m(U)[ps].m(A)p.m(C)p.m(G)p.m(G)p.[fl2r](U)p.[fl2r](C)p.[fl2r](A)p.m(C)p.m(U)p.m(A)p.m(U)p.m(U)p.m(U)p.m(G)[ps].m(G)[ps].m(A)	565	407
	AS	[5vp_una2moe](U)[ps].[fl2r](C)[ps].m(C)p.m(A)p.m(A)p.[fl2r](A)p.m(A)p.m(U)p.m(A)p.m(G)p.m(U)p.m(G)p.m(A)p.[fl2r](C)p.m(C)p.[fl2r](G)p.m(U)p.m(A)p.m(A)[ps].m(U)[ps].m(U)	605	
304	S	m(A)[ps].m(C)[ps].m(G)p.m(G)p.m(U)p.m(C)p.[fl2r](A)p.[fl2r](C)p.[fl2r](U)p.m(A)p.m(U)p.m(U)p.m(U)p.m(G)p.m(G)p.m(U)[ps].m(U)[ps].m(A)	567	409
	AS	[5vp_una2moe](U)[ps].[fl2r](A)[ps].m(A)p.m(C)p.m(C)p.[fl2r](A)p.m(A)p.m(A)p.m(A)p.m(U)p.m(A)p.m(G)p.m(U)p.[fl2r](G)p.m(A)p.[fl2r](C)p.m(C)p.m(G)p.m(U)[ps].m(U)[ps].m(U)	606	
305	S	m(C)[ps].m(G)[ps].m(G)p.m(U)p.m(C)p.m(A)p.[fl2r](C)p.[fl2r](U)p.[fl2r](A)p.m(U)p.m(U)p.m(U)p.m(U)p.m(G)p.m(G)p.m(U)p.m(U)[ps].m(A)[ps].m(A)	569	410

	AS	[5vp_una2moe](U)[ps].[fl2r](U)[ps].m(A)p.m(A)p.m(C)p.[fl2r](C)p.m(A)p.m(A)p.m(A)p.m(A)p.m(U)p.m(A)p.m(G)p.[fl2r](U)p.m(G)p.[fl2r](A)p.m(C)p.m(C)p.m(G)[ps].m(U)[ps].m(U)	607	
306	S	m(U)[ps].m(A)[ps].m(A)p.m(U)p.m(U)p.m(G)p.[fl2r](U)p.[fl2r](G)p.[fl2r](U)p.m(G)p.m(U)p.m(G)p.m(C)p.m(A)p.m(U)p.m(G)p.m(A)[ps].m(C)[ps].m(A)	571	426
	AS	[5vp_una2moe](U)[ps].[fl2r](G)[ps].m(U)p.m(C)p.m(A)p.[fl2r](U)p.m(G)p.m(C)p.m(A)p.m(C)p.m(A)p.m(C)p.m(A)p.[fl2r](C)p.m(A)p.[fl2r](A)p.m(U)p.m(U)p.m(A)[ps].m(U)[ps].m(U)	608	
307	S	m(A)[ps].m(A)[ps].m(U)p.m(U)p.m(G)p.m(U)p.[fl2r](G)p.[fl2r](U)p.[fl2r](G)p.m(U)p.m(G)p.m(C)p.m(A)p.m(U)p.m(G)p.m(A)p.m(C)[ps].m(C)[ps].m(A)	573	427
	AS	[5vp_una2moe](U)[ps].[fl2r](G)[ps].m(G)p.m(U)p.m(C)p.[fl2r](A)p.m(U)p.m(G)p.m(C)p.m(A)p.m(C)p.m(A)p.m(C)p.[fl2r](A)p.m(C)p.[fl2r](A)p.m(A)p.m(U)p.m(U)[ps].m(U)[ps].m(U)	609	
308	S	m(U)[ps].m(G)[ps].m(U)p.m(G)p.m(C)p.m(A)p.[fl2r](U)p.[fl2r](G)p.[fl2r](A)p.m(C)p.m(C)p.m(C)p.m(G)p.m(A)p.m(A)p.m(C)p.m(U)[ps].m(G)[ps].m(A)	575	434
	AS	[5vp_una2moe](U)[ps].[fl2r](C)[ps].m(A)p.m(G)p.m(U)p.[fl2r](U)p.m(C)p.m(G)p.m(G)p.m(G)p.m(U)p.m(C)p.m(A)p.[fl2r](U)p.m(G)p.[fl2r](C)p.m(A)p.m(C)p.m(A)[ps].m(U)[ps].m(U)	610	
309	S	m(G)[ps].m(U)[ps].m(G)p.m(C)p.m(A)p.m(U)p.[fl2r](G)p.[fl2r](A)p.[fl2r](C)p.m(C)p.m(C)p.m(G)p.m(A)p.m(A)p.m(C)p.m(U)p.m(G)[ps].m(A)[ps].m(A)	577	435
	AS	[5vp_una2moe](U)[ps].[fl2r](U)[ps].m(C)p.m(A)p.m(G)p.[fl2r](U)p.m(U)p.m(C)p.m(G)p.m(G)p.m(G)p.m(U)p.m(C)p.[fl2r](A)p.m(U)p.[fl2r](G)p.m(C)p.m(A)p.m(C)[ps].m(U)[ps].m(U)	611	
310	S	m(U)[ps].m(G)[ps].m(U)p.m(C)p.m(U)p.m(A)p.[fl2r](A)p.[fl2r](A)p.[fl2r](U)p.m(G)p.m(A)p.m(G)p.m(U)p.m(U)p.m(C)p.m(A)p.m(C)[ps].m(G)[ps].m(A)	579	532
	AS	[5vp_una2moe](U)[ps].[fl2r](C)[ps].m(G)p.m(U)p.m(G)p.[fl2r](A)p.m(A)p.m(C)p.m(U)p.m(C)p.m(A)p.m(U)p.m(U)p.[fl2r](U)p.m(A)p.[fl2r](G)p.m(A)p.m(C)p.m(A)[ps].m(U)[ps].m(U)	612	
311	S	m(C)[ps].m(U)[ps].m(A)p.m(A)p.m(A)p.m(U)p.[fl2r](G)p.[fl2r](A)p.[fl2r](G)p.m(U)p.m(U)p.m(C)p.m(A)p.m(C)p.m(G)p.m(U)p.m(A)[ps].m(C)[ps].m(A)	581	535
	AS	[5vp_una2moe](U)[ps].[fl2r](G)[ps].m(U)p.m(A)p.m(C)p.[fl2r](G)p.m(U)p.m(G)p.m(A)p.m(C)	613	

318	S	m(U)[ps].m(G)[ps].m(A)p.m(C)p.m(A)p.m(A)p. [fl2r](C)p.[fl2r](C)p.[fl2r](C)p.m(G)p.m(G)p. m(A)p.m(U)p.m(U)p.m(U)p.m(U)p.m(A)[ps].m (A)[ps].m(A)	595	987
	AS	[5vp_una2moe](U)[ps].[fl2r](U)[ps].m(U)p.m(A)p.m(A)p.[fl2r](A)p.m(A)p.m(U)p.m(C)p.m(C)p.m(G)p.m(G)p.m(G)p.[fl2r](U)p.m(U)p.[fl2 r](G)p.m(U)p.m(C)p.m(A)[ps].m(U)[ps].m(U)	620	
319	S	m(A)[ps].m(C)[ps].m(A)p.m(A)p.m(C)p.m(C)p. [fl2r](C)p.[fl2r](G)p.[fl2r](G)p.m(A)p.m(U)p. m(U)p.m(U)p.m(U)p.m(A)p.m(A)p.m(C)[ps].m (U)[ps].m(A)	597	989
	AS	[5vp_una2moe](U)[ps].[fl2r](A)[ps].m(G)p.m(U)p.m(U)p.[fl2r](A)p.m(A)p.m(A)p.m(A)p.m(U)p.m(C)p.m(C)p.m(G)p.[fl2r](G)p.m(G)p.[fl2 r](U)p.m(U)p.m(G)p.m(U)[ps].m(U)[ps].m(U)	621	
320	S	m(C)[ps].m(A)[ps].m(A)p.m(C)p.m(C)p.m(C)p. [fl2r](G)p.[fl2r](G)p.[fl2r](A)p.m(U)p.m(U)p. m(U)p.m(U)p.m(A)p.m(A)p.m(C)p.m(U)[ps].m (A)[ps].m(A)	599	990
	AS	[5vp_una2moe](U)[ps].[fl2r](U)[ps].m(A)p.m(G)p.m(U)p.[fl2r](U)p.m(A)p.m(A)p.m(A)p.m(A)p.m(U)p.m(C)p.m(C)p.[fl2r](G)p.m(G)p.[fl2 r](G)p.m(U)p.m(U)p.m(G)[ps].m(U)[ps].m(U)	622	
321	S	m(U)[ps].m(G)[ps].m(A)p.m(U)p.m(C)p.m(U)p. [fl2r](U)p.[fl2r](G)p.[fl2r](G)p.m(C)p.m(U)p. m(G)p.m(U)p.m(A)p.m(G)p.m(U)p.m(C)[ps].m (A)[ps].m(A)	601	1172
	AS	[5vp_una2moe](U)[ps].[fl2r](U)[ps].m(G)p.m(A)p.m(C)p.[fl2r](U)p.m(A)p.m(C)p.m(A)p.m(G)p.m(C)p.m(C)p.m(A)p.[fl2r](A)p.m(G)p.[fl2 r](A)p.m(U)p.m(C)p.m(A)[ps].m(U)[ps].m(U)	623	
322	S	m(U)[ps].m(A)[ps].m(C)p.m(C)p.m(U)p.m(U)p. [fl2r](C)p.[fl2r](G)p.[fl2r](A)p.m(A)p.m(G)p. m(C)p.m(C)p.m(A)p.m(U)p.m(G)p.m(C)[ps].m (U)[ps].m(A)	603	2073
	AS	[5vp_una2moe](U)[ps].[fl2r](A)[ps].m(G)p.m(C)p.m(A)p.[fl2r](U)p.m(G)p.m(G)p.m(C)p.m(U)p.m(U)p.m(C)p.m(G)p.[fl2r](A)p.m(A)p.[fl2 r](G)p.m(G)p.m(U)p.m(A)[ps].m(U)[ps].m(U)	624	

Legend: (N) = 2'-OH (ribose; ex: A, G, C, or U); m(N) = 2'-O-Methylribose; p=Phosphate; [fl2r] (N) = 2'-Fluororibose; [ps] = Phosphorothioate backbone modification; 5vp_una2moe = Vinylphosphonate-2'-O-MethoxyEthylRibose-UNA-U; "S" means the sense strand; "AS" means the antisense strand. On the 5'-end of the sense strand, there can be either a free C6-NH₂ or cholesterol-conjugated to the amino handle.

[0079] The sense strand and antisense strand of oligonucleotides of the present disclosure can be synthesized using any nucleic acid polymerization methods known in the art, for example,

solid-phase synthesis by employing phosphoramidite chemistry methodology (e.g., Current Protocols in Nucleic Acid Chemistry, Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA), H-phosphonate, phosphotriester chemistry, or enzymatic synthesis. Automated commercial synthesizers can be used, for example, MerMade™ 12 from LGC Biosearch Technologies, or other synthesizers from BioAutomation or Applied Biosystems. Phosphorothioate linkages can be introduced using a sulfurizing reagent such as phenylacetyl disulfide or DDTT (((dimethylaminomethylidene) amino)-3H-1,2,4-dithiazoine-3-thione). It is well known to use similar techniques and commercially available modified amidites and controlled-pore glass (CPG) products to synthesize modified oligonucleotides or conjugated oligonucleotides.

[0080] Purification methods can be used to exclude the unwanted impurities from the final oligonucleotide product. Commonly used purification techniques for single stranded oligonucleotides include reverse-phase ion pair high performance liquid chromatography (RP-IP-HPLC), capillary gel electrophoresis (CGE), anion exchange HPLC (AX-HPLC), and size exclusion chromatography (SEC). After purification, oligonucleotides can be analyzed by mass spectrometry and quantified by spectrophotometry at a wavelength of 260 nm. The sense strand and antisense strand can then be annealed to form a dsRNA.

[0081] In addition to the above modifications, the oligonucleotides herein also comprise one or more modified nucleobases. In some embodiments, modified nucleobases (also referred to herein as base analogs) are linked at the 1' position of a nucleotide sugar moiety. In some embodiments, the modified nucleobase is a nitrogenous base. In other embodiments, the modified nucleobase does not contain nitrogen atom. *See, e.g.*, US Patent Application Publication No. 2008/0274462. In certain other embodiments, the modified nucleotide is a universal base. However, in certain embodiments, the modified nucleotide does not contain a nucleobase (abasic).

[0082] With regard to universal bases, they comprise a heterocyclic moiety located at the 1' position of a nucleotide sugar moiety in a modified nucleotide, or the equivalent position in a nucleotide sugar moiety substitution, that, when present in a duplex, is positioned opposite more than one type of base without substantially altering structure of the duplex. Moreover, and compared to a reference single strand (ss) nucleic acid (*e.g.*, oligonucleotide) that is fully complementary to a target nucleic acid, a ss nucleic acid having a universal base forms a duplex with the target nucleic acid that has a lower melting temperature (T_m) than a duplex formed with the complementary nucleic acid. However, when compared to a reference ss nucleic acid in which the universal base has been replaced with a base to generate a single

mismatch, the ss nucleic acid having the universal base forms a duplex with the target nucleic acid that has a higher T_m than a duplex formed with the nucleic acid having the mismatched base.

[0083] The oligonucleotides herein, or a pharmaceutically acceptable salt thereof (e.g., trifluoroacetate salts, acetate salts, or hydrochloride salts), are incorporated into a formulation or pharmaceutical composition. Various formulations have been developed to facilitate oligonucleotide use. For example, oligonucleotides can be delivered to an individual or a cellular environment using a formulation that minimizes degradation, facilitates delivery and/or uptake, or provides another beneficial property to the oligonucleotides in the formulation. In some embodiments, the oligonucleotides are formulated in buffer solutions such as phosphate buffered saline solutions, liposomes, micellar structures, and capsids.

[0084] To improve *in vivo* compatibility and effectiveness, the oligonucleotides may be reacted with any of a number of inorganic and organic acids/bases to form pharmaceutically acceptable acid/base addition salts. Pharmaceutically acceptable salts and common methodologies for preparing them are well known in the art.

Antibodies (TfR Binding Proteins) and Antibody-Oligonucleotide Conjugates

[0085] One aspect of the present disclosure is based, at least in part, on the development of transferrin receptor binding proteins (also referred to herein as anti-TfR antibodies or antigen-binding fragments thereof) that bind transferrin receptor, have low immunogenicity risk, and may be used for delivery of a molecular payload (such as a therapeutic, single stranded RNA (ssRNA), ASO, dsRNA, siRNA, or a drug) to different tissues in a subject's body, such as, muscle, pancreas, brain, central nervous system (CNS), peripheral nervous system (PNS), or to different cell types, such as, cancerous cells, or dorsal root ganglion (DRG) neurons.

[0086] In another aspect, provided herein are conjugates comprising human TfR binding proteins described herein and an oligonucleotide. In some embodiments, the oligonucleotide is a single stranded RNA (ssRNA). In some embodiments, the oligonucleotide is a double stranded RNA (dsRNA). In some embodiments, the oligonucleotide comprises a sense strand and/or an antisense strand, wherein the antisense strand is complementary to at least a portion of the SCN10A mRNA. In some embodiments, the oligonucleotide comprises a sense strand and/or an antisense strand, wherein the antisense strand is complementary to a portion of SNC10A mRNA.

[0087] In some aspects, the present disclosure provides data demonstrating that certain conjugates including the transferrin binding proteins (TBPs; also described herein as anti-TfR antibodies) described herein have superior benefit in that they have reduced immunogenicity

risk as compared to other conjugates having anti-TfR antibodies. In some aspects, the present disclosure provides conjugates with TBPs that have superior benefit in that they can deliver a molecular payload to dorsal root ganglion (DRG) neurons. In some aspects, the present disclosure provides conjugates with TBPs that have superior benefit in that they can deliver a molecular payload to dorsal root ganglion (DRG) neurons with reduced immunogenicity risk as compared to other anti-TfR antibodies.

[0088] In some embodiments, transferrin receptor binding proteins (TBPs) described herein bind to human transferrin receptor with high specificity, affinity, and with reduced immunogenicity risk. In some embodiments, the TBPs provide for a range of affinities to TfR.

[0089] In some embodiments, the TBPs described herein may be used for targeting tissues and/or cells that express TfR. In some embodiments, the TBPs described herein may be used to deliver a molecular payload to a target cell or tissue (e.g., a cell or tissue that expresses transferrin receptor).

[0090] In some aspects the present disclosure is related to complexes (or conjugates) including at least one TBP of the present disclosure conjugated (e.g., covalently) to at least one molecular payload (e.g., a therapeutic agent, siRNA). In some embodiments, the TBPs of the present disclosure may be used to deliver the conjugate (which includes the TBP and a molecular payload) to a cell or a tissue that expresses TfR1 (e.g., DRG, or the brain) for treating or reducing pain; and/or treating a disease related to *SCN10A*.

[0091] One aspect of the present disclosure is related to a conjugate comprising the oligonucleotide of the present disclosure and an anti-transferrin receptor antibody (anti-TfR antibody) or fragment thereof conjugated to the sense strand of the oligonucleotide or the antisense strand of the oligonucleotide. In some embodiments, the anti-transferrin receptor antibody (anti-TfR antibody) or fragment thereof is conjugated to at least one sense strand of the oligonucleotide. In some embodiments, the anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to at least two sense strands of at least two oligonucleotides. In some embodiments, the anti-transferrin receptor antibody (anti-TfR antibody) or fragment thereof is conjugated to at least one antisense strand of the oligonucleotide. In some embodiments, the anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to at least two antisense strands of at least two oligonucleotides.

[0092] In some embodiments, provided herein are conjugates of Formula (I): $P-(L-R)_n$, wherein R is an oligonucleotide of the present disclosure comprising a sense strand and an antisense strand; wherein P is the anti-TfR antibody or antigen binding fragment thereof as

described in the present disclosure; and wherein L is a linker, which can be optionally absent; wherein “n” can be 1, 2 or more. In some embodiments, n is 1. In some embodiments, n is 2. [0093] In some embodiments, the R is selected from Table 2, 3, or 4. In some embodiments, R is selected from siRNA No. 1 to 322 disclosed in Table 2, 3, or 4. In some embodiments, R has an antisense strand that has 90% sequence similarity to any one of antisense strands disclosed in Table 2, 3, or 4. In some embodiments, R has an antisense strand that has 95% sequence similarity to any one of antisense strands disclosed in Table 2, 3, or 4. In some embodiments, R has a sense strand that has 90% sequence similarity to any one of sense strands disclosed in Table 2, 3, or 4. In some embodiments, R has a sense strand that has 95% sequence similarity to any one of sense strands disclosed in Table 2, 3, or 4. In some embodiments, the P is selected from Table 8.

[0094] In some embodiments, the oligonucleotide of the conjugate mediates RNA interference against the human Nav1.8 mRNA. In certain embodiments, the oligonucleotide of the conjugate mediates RNA interference against the human Nav1.8 mRNA preferentially in DRG cells in a human subject. In some embodiments, the conjugate of the present disclosure, which includes the oligonucleotide, mediates RNA interference against the human Nav1.8 mRNA and modulates pain in a subject. In some embodiments, the conjugate of the present disclosure, which includes the oligonucleotide, mediates RNA interference against the human Nav1.8 mRNA and treats pain in a subject. In some embodiments, the conjugate of the present disclosure, which includes the oligonucleotide, mediates RNA interference against the human Nav1.8 mRNA and reduces pain in a subject.

[0095] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein the HCVR comprises heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, and the LCVR comprises light chain complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the following sequences: HCDR1 comprises SEQ ID NO: 629, HCDR2 comprises SEQ ID NO: 630, HCDR3 comprises SEQ ID NO: 631, LCDR1 comprises SEQ ID NO: 632, LCDR2 comprises SEQ ID NO: 633, and LCDR3 comprises SEQ ID NO: 634. Some examples of HCDRs of the TBPs of the present disclosure are provided in Table 5.

Table 5: Examples of Transferrin Receptor Binding Protein (TBP) Heavy Chain Complementarity-determining Regions (HCDRs)

TBP #	HCDR1 (Kabat)	HCDR2 (Kabat)	HCDR3 (Kabat)
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1, 2, and 3	SEQ ID NO: 629 SYSMN	SEQ ID NO: 630 SISSSSSYIYYADSVKG	SEQ ID NO: 631 RHGYSNSDAFDT
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[0096] Some examples of LCDRs of the TBPs of the present disclosure are provided in Table 6.

Table 6: Examples of Transferrin Receptor Binding Protein (TBP) Light Chain Complementarity-determining Regions (LCDRs)

TBP #	LCDR1 (Kabat)	LCDR2 (Kabat)	LCDR3 (Kabat)
1, 2, and 3	SEQ ID NO: 632 RASQGISHYLV	SEQ ID NO: 633 AASSLQS	SEQ ID NO: 634 LQHNSYPWT

[0097] Some examples of HCVRs and LCVRs of the TBPs of the present disclosure are provided in Table 7.

Table 7: Transferrin Receptor Binding Protein (TBP) Heavy Chain Variable Region (HCVR) and Light Chain Variable Region (LCVR)

TBP #	HCVR	LCVR
1, 2, and 3	SEQ ID NO: 627	SEQ ID NO: 628

[0098] Some examples of HCs and LCs of the TBPs of the present disclosure are provided in Table 8. TBP2 is a heteromab and accordingly has two Heavy chains (namely, HCA and HCB). Such heteromab antibodies are described, *e.g.*, in Example 1 of US Patent Application Publication No. 2021/0054103, which is hereby incorporated by reference in its entirety. Heterodimeric antibodies such as heteromab, orthomab, or duobody have been described in WO2014150973, WO2016118742, WO2018118616, WO2011131746, which are hereby incorporated by reference in their entireties. The nucleic acid sequences for the TBPs are provided in Table 9 below.

Table 8: Transferrin Receptor Binding Protein Heavy Chain (HC) and Light Chain (LC)

TBP #	Type/Format	Conjugation Type	HCA (First Heavy Chain)	HCB (Second Heavy Chain)	LC (Light Chain)
1	211 Com29 B09 hIgG1-EN mAb	nCys DAR1	SEQ ID NO: 625		SEQ ID NO: 626
2	211 Com29 B09 hIgG1-AAS heteromab 124C	eCys DAR1 (Heteromab)	SEQ ID NO: 635 (124S)	SEQ ID NO: 636 (124C)	SEQ ID NO: 626
3	211 Com29 B09 hIgG1 124C Fab	eCys DAR1	SEQ ID NO: 637		SEQ ID NO: 638

[0099] In Table 8 and this disclosure, the term “EN” refers to effector null mutations. In some embodiment, the term “EN” refers to mutations L234A/L235E/G237A/A330S/P331S in the Fc region of the antibody or derivatives thereof.

[00100] In Table 8 and this disclosure, the term “AAS” refers to L234A/L235A/D265S mutations. This is an example of effector null mutations in the Fc region. For more information see, *e.g.*, Pejchal et al., “Profiling the Biophysical Developability Properties of Common IgG1 Fc Effector Silencing Variants,” *Antibodies*, 12, 54 (2023), which is hereby incorporated by reference in its entirety.

[00101] In Table 8 and this disclosure, the term “211” refers to an antibody or derivatives thereof that bind to only the apical domain of the TfR.

[00102] In Table 8 and this disclosure, “Com29 B09” (*e.g.*, TBP1) refers to antibody or derivative thereof engineered for reduced affinity and reduced immunogenicity risk. In Table 8 and this disclosure, “TBP2” has, among other things, an asymmetric/single eCys site. “TBP3” is a monovalent Fab fragment of TBP2 that has, among other things, an eCys site engineered in. In some embodiments, the conjugates (or AOCs) of the present disclosure include an antibody or antigen binding fragment thereof that includes at least one heavy chain constant region comprising cysteine at residue 124 (according to the EU Index numbering). In some embodiments, the conjugates (or AOCs) of the present disclosure include an antibody or antigen binding fragment thereof that includes two heavy chain constant regions comprising cysteine at residue 124 (according to the EU Index numbering).

Table 9: Nucleic Acid Sequences for Transferrin Receptor Binding Protein (TBP) Heavy Chain (HC) and Light Chain (LC)

TBP #	Heavy Chain (HC) DNA	Heavy Chain B (HCB) DNA	Light Chain (LC) DNA
1	SEQ ID NO: 639	Not Applicable	SEQ ID NO: 643
2	SEQ ID NO: 640 (HCA)	SEQ ID NO: 642	SEQ ID NO: 644
3	SEQ ID NO: 641	Not Applicable	SEQ ID NO: 645

[00103] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure include a HCVR and a LCVR, wherein the HCVR comprises a sequence having at least 95% sequence identity to SEQ ID NO: 627 and LCVR comprises a sequence having at least 95% sequence identity to SEQ ID NO: 628.

[00104] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure include a HCVR and a LCVR, and wherein the HCVR comprises a

sequence having at least 90% sequence identity to SEQ ID NO: 627 and LCVR comprises a sequence having at least 90% sequence identity to SEQ ID NO: 628.

[00105] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure is a human IgG1 antibody with effector null (EN) mutations that comprises a HC and a LC, wherein the HC comprises the sequence of SEQ ID NO: 625 and the LC comprises the sequence of SEQ ID NO: 626.

[00106] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure is a human IgG1 antibody with effector null (EN) mutations that comprises a HC and a LC, wherein the HC comprises a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 625 and the LC comprises a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 626.

[00107] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure is a human IgG1 antibody with effector null (EN) mutations that comprises a HC and a LC, wherein the HC comprises a sequence having at least 95% sequence identity to the sequence of SEQ ID NO: 625 and the LC comprises a sequence having at least 95% sequence identity to the sequence of SEQ ID NO: 626.

[00108] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure is a human IgG1 antibody heteromab with AAS effector null mutations and the TBP comprises two variants of HC (HCA and HCB) and a LC, wherein the HCA comprises the sequence of SEQ ID NO: 635 which includes 124S, HCB comprises the sequence of SEQ ID NO: 636 which includes 124C, and the LC comprises the sequence of SEQ ID NO: 626.

[00109] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure is a human IgG1 antibody heteromab with AAS effector null mutations and the TBP comprises two variants of HC (HCA and HCB) and a LC, wherein the HCA comprises a sequence having at least 95% sequence identity to the sequence of SEQ ID NO: 635 which includes 124S, HCB comprises a sequence having at least 95% sequence identity to the sequence of SEQ ID NO: 636 which includes 124C, and the LC comprises a sequence having at least 95% sequence identity to the sequence of SEQ ID NO: 626.

[00110] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure is a human IgG1 antibody heteromab with AAS effector null mutations and the TBP comprises two variants of HC (HCA and HCB) and a LC, wherein the HCA comprises a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 635 which includes 124S, HCB comprises a sequence having at least 90% sequence identity

to the sequence of SEQ ID NO: 636 which includes 124C, and the LC comprises a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 626.

[00111] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure is a human IgG1 Fab with a cysteine at position 124 of the HC and the Fab comprises a HC and a LC, wherein the HC comprises the sequence of SEQ ID NO: 637 and the LC comprises the sequence of SEQ ID NO: 638.

[00112] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure is a human IgG1 Fab with a cysteine at position 124 of the HC and the Fab comprises a HC and a LC, wherein the HC comprises a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 637 and the LC comprises a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 638.

[00113] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure is a human IgG1 Fab with a cysteine at position 124 of the HC and the Fab comprises a HC and a LC, wherein the HC comprises a sequence having at least 95% sequence identity to the sequence of SEQ ID NO: 637 and the LC comprises a sequence having at least 95% sequence identity to the sequence of SEQ ID NO: 638.

[00114] In some embodiments, the conjugate of the present disclosure is selected from the following Table 10. In some embodiments, the conjugate has one SMCC linker connecting the antibody to the oligonucleotide.

Table 10: Some embodiments of the Conjugates of the present disclosure.

“P” (anti-TfR Antibody)	“R” oligonucleotide*
TBP 1 Conjugate	
TBP1 (HC SEQ ID NO: 625 and LC 626). In some embodiments the “P” protein has at least 90% or 95% sequence identity to HC. In some embodiments, the “P” has at least 90% or 95% sequence identity to LC.	SEQ ID NOs: 565 and 605
	SEQ ID NOs: 567 and 606
	SEQ ID NOs: 569 and 607
	SEQ ID NOs: 571 and 608
	SEQ ID NOs: 573 and 609
	SEQ ID NOs: 575 and 610
	SEQ ID NOs: 577 and 611
	SEQ ID NOs: 579 and 612
	SEQ ID NOs: 581 and 613
	SEQ ID NOs: 583 and 614
	SEQ ID NOs: 585 and 615
	SEQ ID NOs: 587 and 616
	SEQ ID NOs: 589 and 617
	SEQ ID NOs: 591 and 618

	SEQ ID NOs: 593 and 619
	SEQ ID NOs: 595 and 620
	SEQ ID NOs: 597 and 621
	SEQ ID NOs: 599 and 622
	SEQ ID NOs: 601 and 623
	SEQ ID NOs: 603 and 624
TBP 2 Conjugate	
TBP2 (HCA SEQ ID NO: 635; HCB SEQ ID NO: 636; and LC 626)	SEQ ID NOs: 565 and 605
	SEQ ID NOs: 567 and 606
	SEQ ID NOs: 569 and 607
	SEQ ID NOs: 571 and 608
	SEQ ID NOs: 573 and 609
In some embodiments the "P" protein has at least 90% or 95% sequence identity to HCA.	SEQ ID NOs: 575 and 610
	SEQ ID NOs: 577 and 611
	SEQ ID NOs: 579 and 612
	SEQ ID NOs: 581 and 613
	SEQ ID NOs: 583 and 614
	SEQ ID NOs: 585 and 615
In some embodiments the "P" protein has at least 90% or 95% sequence identity to HCB.	SEQ ID NOs: 587 and 616
	SEQ ID NOs: 589 and 617
	SEQ ID NOs: 591 and 618
	SEQ ID NOs: 593 and 619
	SEQ ID NOs: 595 and 620
In some embodiments, the "P" has at least 90% or 95% sequence identity to LC.	SEQ ID NOs: 597 and 621
	SEQ ID NOs: 599 and 622
	SEQ ID NOs: 601 and 623
	SEQ ID NOs: 603 and 624
TBP 3 Conjugate	
TBP1 (HC SEQ ID NO: 637 and LC 638)	SEQ ID NOs: 565 and 605
	SEQ ID NOs: 567 and 606
	SEQ ID NOs: 569 and 607
	SEQ ID NOs: 571 and 608
In some embodiments the "P" protein has at least 90% or 95% sequence identity to HC.	SEQ ID NOs: 573 and 609
	SEQ ID NOs: 575 and 610
	SEQ ID NOs: 577 and 611
	SEQ ID NOs: 579 and 612
	SEQ ID NOs: 581 and 613
In some embodiments, the "P" has at least 90% or 95% sequence identity to LC.	SEQ ID NOs: 583 and 614
	SEQ ID NOs: 585 and 615
	SEQ ID NOs: 587 and 616
	SEQ ID NOs: 589 and 617
	SEQ ID NOs: 591 and 618
	SEQ ID NOs: 593 and 619
	SEQ ID NOs: 595 and 620

	SEQ ID NOs: 597 and 621
	SEQ ID NOs: 599 and 622
	SEQ ID NOs: 601 and 623
	SEQ ID NOs: 603 and 624

** In some embodiments the “R” oligonucleotide has at least 90% or 95% sequence identity to the listed sequences.*

[00115] The TfR binding proteins described herein can be recombinantly produced in a host cell, for example, using an expression vector. For example, an expression vector may include a sequence that encodes one or more signal peptides that facilitate secretion of the polypeptide(s) from a host cell. Expression vectors containing a polynucleotide of interest (e.g., a polynucleotide encoding a heavy chain or light chain of the TfR binding proteins) may be transferred into a host cell by well-known methods. Additionally, expression vectors may contain one or more selection markers, e.g., tetracycline, neomycin, and dihydrofolate reductase, to aide in detection of host cells transformed with the desired polynucleotide sequences.

[00116] A host cell (e.g., a mammalian cell) includes cells stably or transiently transfected, transformed, transduced, or infected with one or more expression vectors expressing all or a portion of the TfR binding proteins described herein. According to some embodiments, a host cell may be stably or transiently transfected, transformed, transduced, or infected with an expression vector expressing HC polypeptides and an expression vector expressing LC polypeptides of the TfR binding proteins described herein. In some embodiments, a host cell may be stably or transiently transfected, transformed, transduced, or infected with an expression vector expressing HC and LC polypeptides of the TfR binding proteins described herein. The TfR binding proteins may be produced in mammalian cells such as CHO, NS0, HEK293 or COS cells according to techniques well known in the art.

[00117] In some embodiments, the cell growth medium, into which the TfR binding proteins have been secreted, may be purified by conventional techniques, such as mixed-mode methods of ion-exchange and hydrophobic interaction chromatography. For example, the cell growth medium may be applied to and eluted from a Protein A or G column using conventional methods; mixed-mode methods of ion-exchange and hydrophobic interaction chromatography may also be used. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. Various methods of protein purification may be employed, and such methods are known in the art and described, for example, in Deutscher,

Methods in Enzymology 182: 83-89 (1990) and Scopes, Protein Purification: Principles and Practice, 3rd Edition, Springer, NY (1994).

[00118] Embodiments of the present disclosure also include antibody fragments or antigen-binding fragments that, as used herein, comprise at least a portion of an antibody retaining the ability to specifically interact with an antigen or an epitope of the antigen, such as, Fab, Fab', F(ab')₂, Fv fragments, scFv antibody fragments, scFv-Fc, diabody, scFab, scFv-CH3, Fv, scFa, disulfide-linked Fvs (sdFv), and a Fd fragment.

[00119] In some embodiments, the TfR binding protein is scFv. In some embodiments, the TfR binding protein is Fab. In some embodiments, the TfR binding protein is a Fab and a VHH linked to the Fab, wherein the VHH binds human serum albumin (HSA). In some embodiments, the TfR binding protein further comprises a heavy chain constant region comprising cysteine at residue 124 (according to the EU Index numbering). EU numbering system is used in the present disclosure for numbering the residues of antibodies or fragments thereof.

[00120] In some embodiments, the antibody or antigen binding fragment thereof is conjugated to the oligonucleotide using "nCys" method. "nCys" refers to a conjugation method where one or more native cysteine residues are used for conjugation of the antibody or antigen-binding fragment thereof to a molecular payload (*e.g.*, ASO or siRNA). In some embodiments, the human TfR binding proteins described herein comprise one or more native cysteine residues, which can be used for conjugation. For example, in some embodiments, the human TfR binding protein described herein comprises a native cysteine at position 214 of the light chain and/or a native cysteine at positions 220, 226, and/or 229 of the heavy chain, which can be used for conjugation (all residues according to the EU Index numbering).

[00121] In some embodiments, the antibody or antigen binding fragment thereof is conjugated to the oligonucleotide using "eCys" method. This is a method where one or more engineered cysteine residues are artificially incorporated into the antibody or antigen binding fragment thereof and are used for conjugation of the antibody or antigen-binding fragment thereof to a molecular payload (*e.g.*, siRNA). The approach of including engineered cysteines as a means for conjugation has been described in WO2018232088, which is hereby incorporated by reference in its entirety. In some embodiments, the human TfR binding proteins described herein comprise a heavy chain comprising one or more cysteines at the following residues: 124, 157, 162, 262, 373, 375, 397, 415 (all residues according to the EU Index numbering). In some embodiments, the human TfR binding proteins described herein comprise a light chain (*e.g.*, a kappa light chain) comprising one or more cysteines at the

following residues: 156, 171, 191, 193, 202, 208 (all residues according to the EU Index numbering). In some embodiments, the human TfR binding proteins described herein comprise a heavy chain constant region comprising cysteine at residue 124 (according to the EU Index numbering). In some embodiments, the human TfR binding proteins described herein comprise a light chain constant region comprising cysteine at residue 156 (according to the EU Index numbering). In some embodiments, the human TfR binding proteins described herein comprise an immunoglobulin Fc region comprising cysteine at residue 378 (according to the EU Index numbering).

[00122] In some embodiments, the antibody or the antigen-binding fragment thereof is conjugated to one payload (*e.g.*, oligonucleotide) and has a drug to antibody ratio (DAR) of 1. In some embodiments, the antibody or the antigen-binding fragment thereof is conjugated to two payloads and has a drug to antibody ratio (DAR) of 2. In some embodiments, two variants of heavy chain (HCA and HCB) are such that one variant includes an engineered cysteine (eCys) for site-specific conjugation to, *e.g.*, a payload. Such conjugation to one variant of the heavy or the light chain, *e.g.*, may be done to yield a highly homogenous DAR1 conjugate or product. In some embodiments, HCA has the eCys site for site-specific conjugation. In some embodiments, HCB has the eCys site for site-specific conjugation.

[00123] In some embodiments, the antibody or antigen-binding fragment thereof of the present disclosure further comprises a half-life extender, *e.g.*, an immunoglobulin Fc region or a VHH that binds human serum albumin (see, *e.g.*, WO2022169766, which is hereby incorporated by reference in its entirety). In some embodiments, the antibody or antigen-binding fragment thereof of the present disclosure comprises an immunoglobulin Fc region, *e.g.*, a modified human IgG4 Fc region or a modified human IgG1 Fc region. In some embodiments, the antibody or antigen-binding fragment thereof of the present disclosure comprises a modified human IgG4 Fc region comprising proline at residue 228, and alanine at residues 234 and 235 (all residues are numbered according to the EU Index numbering, also called hIgG4PAA Fc region). In some embodiments, the antibody or antigen-binding fragment thereof of the present disclosure comprises a modified human IgG1 Fc region comprising alanine at residues 234, 235, and 329, serine at position 265, aspartic acid at position 436 (all residues are numbered according to the EU Index numbering, all called hIgG1 effector null or hIgG1EN Fc region).

[00124] In some embodiments, the antibody or antigen-binding fragment thereof of the present disclosure comprises a VHH that binds HSA. In some embodiments, the VHH also binds mouse, rat, and/or cynomolgus monkey albumin.

Linkers

[00125] In some embodiments, the oligonucleotide of the present disclosure includes a linker moiety attached to one or more termini of the sense strand or the antisense strand. Such linker may be used to attach an antibody to the oligonucleotide. In some embodiments, the linker is SMCC and is optionally attached to the 5' end of the sense strand. In some embodiments, at least one nucleotide of the oligonucleotide is conjugated to cholesterol, lipid, polypeptide, or an antibody or a fragment thereof. In some embodiments, a linker is used to conjugate the cholesterol, lipid, polypeptide, or an antibody or a fragment thereof to the oligonucleotide.

[00126] In some embodiments, the anti-TfR antibody or antigen binding fragment thereof of the present disclosure is linked to the sense strand or the antisense strand of the oligonucleotide through a linker. In some embodiments, this is an SMCC linker. In some embodiments, the linker is SMCC linker attached to i) the 5' end of the sense strand, ii) the 5' end of the antisense strand, iii) the 3' end of the sense strand, or iv) the 3' end of the antisense strand.

[00127] In some embodiments, a linker described herein is a cleavable linker or a non-cleavable linker. In some instances, the linker is a cleavable linker. In other instances, the linker is a non-cleavable linker.

[00128] In some cases, the linker is a non-polymeric linker. A non-polymeric linker refers to a linker that does not contain a repeating unit of monomers generated by a polymerization process. Exemplary non-polymeric linkers include, but are not limited to, C₁-C₆ alkyl group (e.g., a C₅, C₄, C₃, C₂, or C₁ alkyl group), homobifunctional cross linkers, heterobifunctional cross linkers, peptide linkers, traceless linkers, self-immolative linkers, maleimide-based linkers, or combinations thereof. In some cases, the non-polymeric linker comprises a C₁-C₆ alkyl group (e.g., a C₅, C₄, C₃, C₂, or C₁ alkyl group), a homobifunctional cross linker, a heterobifunctional cross linker, a peptide linker, a traceless linker, a self-immolative linker, a maleimide-based linker, or a combination thereof.

[00129] In some embodiments, the linker comprises a maleimide group. In some instances, the maleimide group is also referred to as a maleimide spacer. In some instances, the maleimide group further encompasses a caproic acid, forming maleimidocaproyl (mc). In some cases, the linker comprises maleimidocaproyl (mc). In some cases, the linker is maleimidocaproyl (mc). In other instances, the maleimide group comprises a maleimidomethyl group, such as succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) or sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) described above.

[00130] In some embodiments, the maleimide group is a self-stabilizing maleimide. In some instances, the self-stabilizing maleimide utilizes diaminopropionic acid (DPR) to incorporate a basic amino group adjacent to the maleimide to provide intramolecular catalysis of tiosuccinimide ring hydrolysis, thereby eliminating maleimide from undergoing an elimination reaction through a retro-Michael reaction. In some instances, the self-stabilizing maleimide is a maleimide group described in Lyon, et al., "Self-hydrolyzing maleimides improve the stability and pharmacological properties of antibody-drug conjugates." *Nat. Biotechnol.* 32(10):1059-1062 (2014). In some instances, the linker comprises a self-stabilizing maleimide. In some instances, the linker is a self-stabilizing maleimide.

[00131] In some embodiments, the linker comprises a peptide moiety. In some instances, the peptide moiety comprises at least 2, 3, 4, 5, or 6 more amino acid residues. In some instances, the peptide moiety comprises at most 2, 3, 4, 5, 6, 7, or 8 amino acid residues. In some instances, the peptide moiety comprises about 2, about 3, about 4, about 5, or about 6 amino acid residues. In some instances, the peptide moiety is a cleavable peptide moiety (e.g., either enzymatically or chemically). In some instances, the peptide moiety is a non-cleavable peptide moiety.

[00132] In some embodiments, the linker comprises a benzoic acid group, or its derivatives thereof. In some instances, the benzoic acid group or its derivatives thereof comprise paraaminobenzoic acid (PABA). In some instances, the benzoic acid group or its derivatives thereof comprise gamma-aminobutyric acid (GABA).

[00133] In some embodiments, the linker comprises one or more of a maleimide group, a peptide moiety, and/or a benzoic acid group, in any combination. In some embodiments, the linker comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In some instances, the maleimide group is maleimidocaproyl (mc). In some instances, the peptide group is val-cit. In some instances, the benzoic acid group is PABA. In some instances, the linker comprises a mc-val-cit group. In some cases, the linker comprises a val-cit-PABA group. In additional cases, the linker comprises a mc-val-cit-PABA group.

[00134] In some embodiments, the linker is a self-immolative linker or a self-elimination linker. In some cases, the linker is a self-immolative linker. In other cases, the linker is a self-elimination linker (e.g., a cyclization self-elimination linker). In some instances, the linker comprises a linker described in U.S. Pat. No. 9,089,614 or PCT Publication No. WO2015038426.

[00135] In some instances, the linker comprises a homobifunctional linker. Exemplary homobifunctional linkers include, but are not limited to, Lomant's reagent dithiobis

(succinimidylpropionate) DSP, 3'3'-dithiobis(sulfosuccinimidyl propionate) (DTSSP), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl)suberate (BS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo DST), ethylene glycobis(succinimidylsuccinate) (EGS), disuccinimidyl glutarate (DSG), N,N'-disuccinimidyl carbonate (DSC), dimethyl adipimidate (DMA), dimethyl pimelimidate (DMP), dimethyl suberimidate (DMS), dimethyl-3,3'-dithiobispropionimidate (DTBP), 1,4-di-3'-(2'-pyridyldithio)propionamido)butane (DPDPB), bismaleimido-hexane (BMH), aryl halide-containing compound (DFDNB), such as e.g. 1,5-difluoro-2,4-dinitrobenzene or 1,3-difluoro-4,6-dinitrobenzene, 4,4'-difluoro-3,3'-dinitrophenylsulfone (DFDNPS), bis-[β-(4-azidosalicylamido)ethyl]disulfide (BASED), formaldehyde, glutaraldehyde, 1,4-butanediol diglycidyl ether, adipic acid dihydrazide, carbonyldiimidazole, o-toluidine, 3,3'-dimethylbenzidine, benzidine, α,α'-p-diaminodiphenyl, diiodo-p-xylene sulfonic acid, N,N'-ethylene-bis(iodoacetamide), or N,N'-hexamethylene-bis(iodoacetamide).

[00136] In some embodiments, the linker is a dendritic type linker. In some instances, the dendritic type linker comprises a branching, multifunctional linker moiety. In some instances, the dendritic type linker is used to increase the molar ratio of siRNA to the antibody. In some instances, the dendritic type linker comprises polyamidoamine (PAMAM) dendrimers.

[00137] In some instances, the linker is a linker described in U.S. Pat. Nos. 6,884,869; 7,498,298; 8,288,352; 8,609,105; or 8,697,688; U.S. Patent Publication Nos. 2014/0127239; 2013/028919; 2014/286970; 2013/0309256; 2015/037360; or 2014/0294851; or PCT Publication Nos. WO2015057699; WO2014080251; WO2014197854; WO2014145090; or WO2014177042.

Expression Vectors and Cells

[00138] In some embodiments, the invention provides vectors for inhibiting the expression of the Nav1.8 gene in a cell, comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the oligonucleotides of the invention.

[00139] In another embodiment, the invention provides a cell comprising a vector for inhibiting the expression of the Nav1.8 gene in a cell. The vector comprises a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the oligonucleotides of the invention.

[00140] In another aspect of the invention, Nav1.8 specific oligonucleotide molecules that modulate Nav1.8 gene expression activity are expressed from transcription units inserted into DNA or RNA vectors known in the art. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be incorporated and inherited as a

transgene integrated into the host genome. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, et al., *PNAS* (1995) 92:1292).

[00141] The individual strands of an oligonucleotide can be transcribed by promoters on two separate expression vectors and co-transfected into a target cell. Alternatively, each individual strand of the oligonucleotide can be transcribed by promoters both of which are located on the same expression plasmid. The recombinant oligonucleotide expression vectors are preferably DNA plasmids or viral vectors. oligonucleotide expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus (for a review, see Muzyczka, et al., *Curr. Topics Micro. Immunol.* (1992) 158:97-129)); adenovirus (see, for example, Berkner, et al., *BioTechniques* (1998) 6:616), Rosenfeld et al. (1991, *Science* 252:431-434), and Rosenfeld et al. (1992), *Cell* 68:143-155)); or alphavirus as well as others known in the art. The oligonucleotide molecules can also be inserted into vectors and used as gene therapy vectors for human patients.

[00142] A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the oligonucleotide transgene. In addition, expression of the transgene can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, e.g., circulating glucose levels, or hormones (Docherty et al., 1994, *FASEB J.* 8:20-24).

Pharmaceutical Compositions

[00143] In one embodiment, the disclosure provides pharmaceutical compositions comprising an oligonucleotide, as described herein, and a pharmaceutically acceptable carrier. In another embodiment, the disclosure provides pharmaceutical compositions comprising a conjugate, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical composition comprising the oligonucleotide, or the conjugate is useful for treating a disease or disorder associated with the expression or activity of the Nav1.8 gene, such as chronic pain, including, neuropathic, inflammatory, or mixed pain.

[00144] In another embodiment, the disclosure provides pharmaceutical compositions comprising at least two oligonucleotides, designed to target different regions of the Nav1.8 gene, and a pharmaceutically acceptable carrier. In yet another embodiment, the disclosure provides pharmaceutical compositions comprising at least two conjugates, designed to target different regions of the Nav1.8 gene, and a pharmaceutically acceptable carrier. In this embodiment, one oligonucleotide can have a nucleotide sequence which is substantially complementary to at least one part of the Nav1.8 gene and an additional oligonucleotide which

has a nucleotide sequence that is substantially complementary to different part of the Nav1.8 gene. The multiple oligonucleotides or conjugates may be combined in the same pharmaceutical composition or formulated separately. If formulated individually, the compositions containing the separate oligonucleotide or conjugates may comprise the same or different carriers and may be administered using the same or different routes of administration. Moreover, the pharmaceutical compositions comprising the individual oligonucleotides or conjugates may be administered substantially simultaneously, sequentially, or at preset intervals throughout the day or treatment period.

[00145] The pharmaceutical compositions of the disclosure are administered in dosages sufficient to inhibit expression of the Nav1.8 gene. In general, a suitable dose of oligonucleotide will be in the range of 0.01 to 10.0 milligrams per kilogram body weight of the recipient per day. A skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the individual oligonucleotides encompassed by the disclosure can be made using conventional methodologies or based on *in vivo* testing using an appropriate animal model.

[00146] The pharmaceutical compositions encompassed by the disclosure may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intravenous, intramuscular, intraperitoneal, epidural, intrathecal, intracerebroventricular, intraparenchymal (within the peripheral or central nervous system), subcutaneous, transdermal, intranasal, airway (aerosol), rectal, vaginal, and topical (including buccal and sublingual) administration.

[00147] For intrathecal, intracerebroventricular, intramuscular, intraparenchymal (within the peripheral or central nervous system), subcutaneous and intravenous use, the pharmaceutical compositions of the disclosure will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. In a preferred embodiment, the carrier consists exclusively of an aqueous buffer. In this context, "exclusively" means no auxiliary agents or encapsulating substances are present which might affect or mediate uptake of oligonucleotide in the cells that express the Nav1.8 gene. Such substances include, for example, micellar structures, such as liposomes or capsids, as described below.

[00148] The pharmaceutical compositions useful according to the disclosure also include encapsulated formulations to protect the oligonucleotide against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811; PCT publication WO 91/06309; and European patent publication EP-A-43075, which are incorporated by reference herein. Lipid nanoparticles (LNPs) may also be used for delivering the oligonucleotides or conjugates of the present disclosure.

[00149] In addition to their administration individually or as a plurality, as discussed above, the oligonucleotide or conjugates of the disclosure can be administered in combination with other known agents effective in treatment of pain. In any event, the administering physician can adjust the amount and timing of oligonucleotides or conjugates disclosed herein for administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

[00150] In some embodiments, the composition herein comprises a carrier, which can confer to a composition improved stability, improved absorption, improved solubility and/or therapeutic enhancement of the active ingredient. In some embodiments, the carrier is a buffering agent (*e.g.*, sodium citrate, sodium phosphate, a tris base, or sodium hydroxide) or a vehicle (*e.g.*, a buffered solution, petrolatum, dimethyl sulfoxide, or mineral oil). In some embodiments, the oligonucleotides herein are lyophilized for extending shelf-life and then made into a solution before use (*e.g.*, administration to an individual). Accordingly, the carrier in a pharmaceutical composition including one or more of the oligonucleotides is a lyoprotectant (*e.g.*, mannitol, lactose, polyethylene glycol, or polyvinylpyrrolidone) or a collapse temperature modifier (*e.g.*, dextran, Ficoll™, or gelatin).

[00151] Pharmaceutical compositions are formulated to be compatible with its intended route of administration. Routes of administration include, but are not limited to, parenteral (*e.g.*, intravenous, intramuscular, intraperitoneal, intradermal, and subcutaneous), oral (*e.g.*, inhalation), transdermal (*e.g.*, topical), transmucosal, and rectal administration.

Methods of Reducing *SCN10A* Activity in Cells, Tissue, Organs, and Organisms

[00152] The oligonucleotides, conjugates, or pharmaceutical compositions disclosed herein are used to reduce *SCN10A* mRNA, Nav1.8 protein and/or Nav1.8 activity in cells, tissues, organs, or individuals. The methods comprise the steps described herein, and these may be, but not necessarily, carried out in the sequence as described. Other sequences, however, also are conceivable. Moreover, individual, or multiple steps are carried out either in parallel and/or overlapping in time and/or individually or in multiply repeated steps. Furthermore, the methods comprise additional, unspecified steps.

[00153] The methods comprise contacting or delivering to a cell, population of cells, tissues, organs, or individuals an effective amount any of the oligonucleotides, conjugates, or pharmaceutical compositions disclosed herein for reducing *SCN10A* expression. In some embodiments, reduced *SCN10A* activity is determined by measuring a reduction in the amount or level of *SCN10A* mRNA, Nav1.8 protein, and/or Nav1.8 protein activity in a cell.

[00154] With regard to an appropriate cell type, the cell type is any cell that expresses *SCN10A* mRNA (e.g., DRG neurons). In some embodiments, the cell is a primary cell obtained from an individual. In some embodiments, the cell is a primary cell obtained from the nervous system (CNS or PNS) of an individual. In some embodiments, the primary cell has undergone a limited number of passages such that the cell substantially maintains its natural phenotypic properties. In some embodiments, the cell is an *ex vivo*, *in vivo*, or *in vitro* cell (*i.e.*, such that one or more of the oligonucleotides or conjugates described herein can be delivered to the cell in culture or to an organism in which the cell resides).

[00155] In some embodiments, the oligonucleotides herein are delivered to a cell or population of cells using a nucleic acid delivery method known in the art including, but not limited to, injecting a solution containing the oligonucleotides, bombarding by particles covered by the oligonucleotides, exposing the cell or population of cells to a solution containing the oligonucleotides, or electroporating cell membranes in the presence of the oligonucleotides. Other methods known in the art for delivering oligonucleotides to cells are used such as, for example, lipid-mediated carrier transport, chemical-mediated transport, and cationic liposome transfection such as calcium phosphate, and others. In some embodiments, the oligonucleotides are conjugated to anti-TfR antibodies or antigen-binding fragments thereof for transport into a cell, a population of cells, a tissue, or a subject's cells.

[00156] Reduced *SCN10A* activity is determined by an assay or technique that evaluates one or more molecules, properties or characteristics of a cell or population of cells associated with *SCN10A* gene expression (e.g., using a *SCN10A* expression biomarker) or by an assay or technique that evaluates molecules that are directly indicative of *SCN10A* activity in a cell or

population of cells (*e.g.*, *SCN10A* mRNA, Nav1.8 protein and/or Nav1.8 activity). In some embodiments, the extent to which the oligonucleotides reduce *SCN10A* activity are evaluated by comparing *SCN10A* activity in a cell or population of cells contacted with the oligonucleotides or conjugates to a control cell or population of cells (*e.g.*, a cell or population of cells not contacted with the oligonucleotides or contacted with a control oligonucleotide). In some embodiments, a control amount or level of *SCN10A* activity in a control cell or population of cells is predetermined, such that the control amount or level need not be measured in every instance the assay or technique is performed. The predetermined level or value takes a variety of forms including, but not limited to, a single cut-off value, such as a median or mean.

[00157] Contacting or delivering the oligonucleotides, conjugates, or pharmaceutical compositions disclosed herein to a cell or a population of cells result in reduced *SCN10A* activity. In some embodiments, reduced *SCN10A* activity is relative to a control amount or level of *SCN10A* activity in the cell or the population of cells not contacted with, *e.g.*, the oligonucleotides or contacted with a control oligonucleotide. In some embodiments, reduced *SCN10A* activity is about 1% or lower, about 5% or lower, about 10% or lower, about 15% or lower, about 20% or lower, about 25% or lower, about 30% or lower, about 35% or lower, about 40% or lower, about 45% or lower, about 50% or lower, about 55% or lower, about 60% or lower, about 70% or lower, about 80% or lower, or about 90% or lower relative to a control amount or level of *SCN10A* activity. In some embodiments, the control amount or level of *SCN10A* activity is an amount or level of *SCN10A* mRNA, Nav1.8 protein and/or Nav1.8 activity in the cell or the population of cells that has not been contacted with the oligonucleotides, conjugates, or pharmaceutical compositions disclosed herein. In some embodiments, the effect of delivery of the oligonucleotides, conjugates, or pharmaceutical compositions disclosed herein to the cell or the population of cells according to a method herein is assessed after any finite period or amount of time (*e.g.*, minutes, hours, days, weeks, and/or months). For example, *SCN10A* activity is determined in the cell or the population of cells at least about 4 hours, about 8 hours, about 12 hours, about 18 hours, or about 24 hours. Alternatively, *SCN10A* activity is determined in the cell or the population of cells at least about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 21 days, about 28 days, about 35 days, about 42 days, about 49 days, about 56 days, about 63 days, about 70 days, about 77 days, or about 84 days or more after contacting or delivering the oligonucleotides, conjugates, or pharmaceutical compositions

disclosed herein to the cell or population of cells. In other embodiments, *SCN10A* activity is determined in the cell or the population of cells at least about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months or more after contacting or delivering the oligonucleotides, conjugates, or pharmaceutical compositions disclosed herein to the cell or the population of cells.

Methods of Preventing, Reducing, or Treating:

[00158] One aspect of the present disclosure is related to a method of preventing, treating, or reducing pain in a patient in need thereof, the method comprising administering to the patient an effective amount of the oligonucleotide of the present disclosure; an effective amount of the conjugate of the present disclosure; or the pharmaceutical composition of the present disclosure. Another aspect of the present disclosure is related to use of the oligonucleotide of the present disclosure; the conjugate of the present disclosure; or the pharmaceutical composition of the present disclosure in the manufacture of a medicament for preventing, treating, or reducing pain. In yet another aspect, the oligonucleotide of the present disclosure; the conjugate of the present disclosure; or the pharmaceutical composition of the present disclosure is for use in preventing, treating, or reducing pain in a patient in need thereof. In some embodiments, the methods of treating an individual having, suspected of having, or at risk of developing a disease, disorder, or condition associated with *SCN10A* activity comprise administering at least one or more of the oligonucleotides or conjugates described herein to the individual.

[00159] In some embodiments, the individual is suffering from chronic pain, including, i) inflammatory pain, ii) neuropathic pain, or iii) mixed pain. In one embodiment, the oligonucleotide, the conjugate, or the pharmaceutical composition of the present disclosure is administered intravenously according to at least one of the methods described herein.

[00160] Additionally, methods of treating or attenuating an onset or progression of a disease, disorder, or condition associated with *SCN10A* activity in an individual comprise using one or more of oligonucleotides, pharmaceutical compositions, or conjugates described herein. Furthermore, methods of achieving one or more therapeutic benefits in an individual having a disease, disorder, or condition associated with *SCN10A* activity comprise providing one or more of the oligonucleotides, pharmaceutical compositions, or conjugates disclosed herein. In some embodiments, the individual can be treated by administering a therapeutically effective amount of any one or more of the oligonucleotides, pharmaceutical compositions, or conjugates herein. In some embodiments, the treatment comprises reducing *SCN10A*

activity. In some embodiments, the individual is treated therapeutically. In some embodiments, the individual is treated prophylactically.

[00161] In some embodiments, the one or more oligonucleotides, one or more conjugates, or a pharmaceutical composition including the same, is administered to the individual having a disease, disorder, or condition associated with *SCN10A* activity such that *SCN10A* activity is reduced in the individual, thereby treating the individual. In some embodiments, an amount or level of *SCN10A* mRNA is reduced in the individual. In other embodiments, an amount or level of *SCN10A* (or Nav1.8) protein is reduced in the individual. In still other embodiments, an amount or level of *SCN10A* activity is reduced in the individual.

[00162] In some embodiments, *SCN10A* activity is reduced in the individual by at least about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to *SCN10A* activity prior to administering the one or more oligonucleotides, conjugates, or pharmaceutical composition thereof. In other embodiments, *SCN10A* activity is reduced in the individual by at least about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to *SCN10A* activity in an individual (e.g., a reference or control individual) not receiving the one or more oligonucleotides, conjugates, or pharmaceutical composition or receiving a control oligonucleotide, conjugates, pharmaceutical composition or treatment.

[00163] In certain embodiments, an amount or level of *SCN10A* mRNA is reduced in the individual by at least about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of *SCN10A* mRNA prior to administering the one or more oligonucleotides, conjugates, or pharmaceutical composition thereof. In some embodiments, the amount or level of *SCN10A* mRNA is reduced in the individual by at least about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of *SCN10A* mRNA in an individual (e.g., a reference or control individual) not administered the one or more oligonucleotides, conjugates, or pharmaceutical composition or administered a control oligonucleotide, pharmaceutical composition, or treatment.

[00164] In certain embodiments, an amount or level of SCN10A (or Nav1.8) protein is reduced in the individual by at least about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of SCN10A protein prior to administering the one or more oligonucleotides, conjugates, or pharmaceutical composition thereof. In other embodiments, an amount or level of SCN10A protein is reduced in the individual by at least about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of SCN10A protein in an individual (*e.g.*, a reference or control individual) not administered the one or more oligonucleotides, conjugates, or pharmaceutical composition or administered a control oligonucleotide, pharmaceutical composition or treatment.

[00165] In certain embodiments, an amount or level of Nav1.8 activity is reduced in the individual by at least about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of Nav1.8 activity prior to administering the one or more oligonucleotides, conjugates, or pharmaceutical composition thereof. In some embodiments, the amount or level of Nav1.8 activity is reduced in the individual by at least about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of Nav1.8 activity in an individual (*e.g.*, a reference or control individual) not administered the one or more oligonucleotides or pharmaceutical composition or administered a control oligonucleotide, pharmaceutical composition or treatment.

[00166] Here, *SCN10A* activity, the amount or level of SCN10A mRNA, Nav1.8 protein, Nav1.8 activity, or any combination thereof, is reduced in a cell (*e.g.*, a DRG neuron), a population or a group of cells (*e.g.*, a nerve), a tissue, a sample, an organ, blood, or a fraction thereof, or any other biological material obtained or isolated from the individual. In some embodiments, *SCN10A* activity, the amount or level of SCN10A mRNA, Nav1.8 protein, Nav1.8 activity, or any combination thereof, is reduced in more than one type of cell, more than one groups of cells, more than one type of tissue, more than one type of sample, more than one organ, more than one fraction of blood obtained or isolated from the individual.

[00167] Examples of a disease, disorder, or condition associated with *SCN10A* activity include, but are not limited to, chronic pain, including neuropathic pain, inflammatory pain, mixed pain. Examples of diseases that may be related to activity of *SCN10A* activity include inflammatory CNS diseases such as multiple sclerosis, myelitis or syphilis, ischemia, hemorrhages, or arteriovenous malformations (*e.g.*, post-stroke neuropathic pain) located in the thalamus, spinothalamic pathway or thalamocortical projections, and syringomyelia (Koltzenburg, *Pain* 2002—An Updated Review: Refresher Course Syllabus; IASP Press, Seattle, 2002). The diseases or disorders related to *SCN10A* activity also include “pain and related disorders,” the term “related disorders” refers to disorders that either cause or are associated with pain or have been shown to have similar mechanisms to pain. These disorders include addiction, seizure, stroke, ischemia, a neurodegenerative disorder, anxiety, depression, headache, asthma, rheumatic disease, osteoarthritis, retinopathy, inflammatory eye disorders, pruritis, ulcer, gastric lesions, uncontrollable urination, an inflammatory or unstable bladder disorder, inflammatory bowel disease, irritable bowel syndrome (IBS), irritable bowel disease (IBD), gastroesophageal reflux disease (GERD), functional dyspepsia, functional chest pain of presumed oesophageal origin, functional dysphagia, non-cardiac chest pain, symptomatic gastroesophageal disease, gastritis, aerophagia, functional constipation, functional diarrhea, burbulence, chronic functional abdominal pain, recurrent abdominal pain (RAP), functional abdominal bloating, functional biliary pain, functional incontinence, functional anorectal pain, chronic pelvic pain, pelvic floor dyssynergia, unspecified functional anorectal disorder, interstitial cystitis, dysmenorrhea, and dyspareunia.

[00168] Because of their high specificity, the oligonucleotides or conjugates described herein specifically target mRNAs of target genes of cells, tissues, or organs. In preventing disease, the target gene is the one that is required for initiation or maintenance of the disease or that has been identified as being associated with a higher risk of contracting the disease. In treating disease, one or more of the oligonucleotides or conjugates described herein are brought into contact with the cells, tissue or organ exhibiting or responsible for mediating the disease. For example, an oligonucleotide substantially complimentary to all or part of a wild-type (*i.e.*, native) or mutated gene associated with a disease, disorder, or condition associated with *SCN10A* (or Nav1.8) activity is brought into contact with or introduced into a cell or tissue type of interest such as a DRG neuron.

[00169] In some embodiments, the target gene is from any mammal, such as a human. Any gene may be silenced according to the methods herein. Moreover, the methods herein typically involve administering to an individual a therapeutically effective amount of one or

more oligonucleotides herein, that is, an amount capable of producing a desirable therapeutic result. The therapeutically acceptable amount is an amount that therapeutically treats a disease or disorder or condition. The appropriate dosage for any one individual will depend on certain factors, including the individual's size, body surface area, age, the composition to be administered, the active ingredient(s) in the composition, time and route of administration, general health, and other therapeutic agents being administered concurrently.

[00170] In the methods, the individual is administered any one of the oligonucleotides, conjugates, or compositions herein either enterally (*e.g.*, orally, by gastric feeding tube, by duodenal feeding tube, *via* gastrostomy, or rectally), parenterally (*e.g.*, subcutaneous injection, intravenous injection or infusion, intra-arterial injection or infusion, intraosseous infusion, intramuscular injection, intracerebral injection, intracerebroventricular injection, or intrathecal), topically (*e.g.*, epicutaneous, inhalational, *via* eye drops, or through a mucous membrane), or by direct injection into a target organ (*e.g.*, the liver of an individual). Typically, the oligonucleotides or compositions are administered intravenously or subcutaneously.

[00171] As a non-limiting set of examples, the oligonucleotides or compositions herein typically are administered quarterly (once every three months), bi-monthly (once every two months), monthly or weekly. For example, the oligonucleotides or compositions are administered every week or at intervals of two, or three weeks. In certain embodiments, the oligonucleotides, or compositions are administered daily. In some embodiments, an individual is administered one or more loading doses of the oligonucleotides or compositions followed by one or more maintenance doses of the oligonucleotides or compositions.

[00172] In some embodiments, the individual is a human, a NHP, or other mammal. In other embodiments, the individual is a domesticated animal such as a dog or a cat; livestock such as a horse, cattle, pig, sheep, goat, or chicken; and animals such as a mouse, rat, guinea pig or hamster.

[00173] The oligonucleotides, conjugates, or compositions disclosed herein can be used, or adapted for use, to treat an individual (*e.g.*, a human having a disease, disorder, or condition associated with Nav1.8 activity) that would benefit from reducing *SCN10A* activity. In some embodiments, the oligonucleotides are provided for use, or adapted for use, to treat an individual having a disease, disorder, or condition associated with *SCN10A* activity. Also, the oligonucleotides are provided for use, or adaptable for use, in the manufacture of a medicament or pharmaceutical composition for treating a disease, disorder, or condition associated with *SCN10A* activity. In other embodiments, the oligonucleotides or conjugates

are provided for use, or adaptable for use, in targeting SCN10A mRNA and reducing Nav1.8 activity (*e.g.*, *via* the RNAi pathway). In other embodiments, the oligonucleotides or conjugates are provided for use, or adaptable for use, in targeting SCN10A mRNA and reducing an amount or level of SCN10A mRNA, Nav1.8 protein, and/or Nav1.8 activity.

[00174] The oligonucleotides, the conjugates, or the pharmaceutical compositions disclosed herein can be incorporated into a kit comprising one or more of the oligonucleotides, the conjugates, or the pharmaceutical compositions, and instructions for use. In some embodiments, the kit comprises one or more of the oligonucleotides, the conjugates, or the pharmaceutical compositions, and a package insert containing instructions for use of the kit and/or any component thereof. In other embodiments, the kit comprises a suitable container, one or more of the oligonucleotides, the conjugates, or the pharmaceutical compositions, one or more controls, and various buffers, reagents, enzymes, and other standard ingredients as are known in the art.

[00175] In some embodiments, the container can be at least one vial, well, test tube, flask, bottle, syringe, or other container means, into which the one or more the oligonucleotides, the conjugates, or the pharmaceutical compositions are placed, and in some embodiments, suitably aliquoted. In other embodiments, where an additional component is provided, the kit contains additional containers into which this component is placed. The kit also comprises a means for containing the one or more the oligonucleotides, the conjugates, or the pharmaceutical compositions and any other reagent in close confinement for commercial sale. Such containers include injection or blow-molded plastic containers into which the desired vials are retained. Containers and/or kits comprise labeling with instructions for use and/or warnings.

[00176] In some embodiments, the kit comprises one or more the oligonucleotides, the conjugates herein, and a pharmaceutically acceptable carrier, or a pharmaceutical composition comprising one or more of the oligonucleotides or the conjugates and instructions for treating or delaying progression of a disease, disorder, or condition associated with *SCN10A* activity in an individual in need thereof.

Abbreviations and Definitions

[00177] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of skill in the art to which the disclosure pertains. Moreover, reference to an element by the indefinite article “a” or “an” does not exclude the possibility that more than one element is present, unless the context clearly requires that there

be one and only one element. The indefinite article “a” or “an” thus usually means “at least one.” Furthermore, use of “including,” as well as other forms, such as “include,” “includes” and “included” is not limiting.

[00178] Certain definitions used herein are defined as follows:

[00179] As used herein, “about” means within a statistically meaningful range of a value or values such as, for example, a stated concentration, length, molecular weight, pH, sequence similarity, time frame, temperature, volume, *etc.* Such a value or range can be within an order of magnitude typically within 20%, more typically within 10%, and even more typically within 5% of a given value or range. The allowable variation encompassed by “about” will depend upon the particular system or subject under study, and can be readily appreciated by one of skill in the art.

[00180] As used herein, “administer,” “administering,” “administration” and the like refers to providing a substance (*e.g.*, an oligonucleotide, a conjugate, an AOC, or a composition herein) to an individual in a manner that is pharmacologically useful (*e.g.*, to treat a disease, disorder, or condition in the individual or patient).

[00181] As used herein, “target sequence” refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of the Nav1.8 gene (*SCN10A*), including mRNA that is a product of RNA processing of a primary transcription product.

[00182] As used herein, “antisense strand” means an oligonucleotide herein that is complementary to a region of a target sequence. Likewise, and as used herein, “sense strand” means an oligonucleotide herein that is complementary to a region of an antisense strand.

[00183] As used herein, “attenuate,” “attenuating,” “attenuation” and the like refers to reducing or effectively halting. As a non-limiting example, one or more of the treatments herein may reduce or effectively halt the onset or progression of pain, as well as related diseases, disorders, and conditions in an individual such as, for example, chronic pain. This attenuation may be exemplified by, for example, a decrease in one or more aspects (*e.g.*, symptoms, tissue characteristics, and cellular, inflammatory, or immunological activity, *etc.*), as well as related diseases, disorders, and conditions in an individual.

[00184] As used herein, and unless otherwise indicated, the term “complementary,” when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the

skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions may include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50 °C or 70 °C for 12-16 hours followed by washing. Other conditions, such as physiologically relevant conditions as may be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides. This includes base-pairing of the oligonucleotide or polynucleotide comprising the first nucleotide sequence to the oligonucleotide or polynucleotide comprising the second nucleotide sequence over the entire length of the first and second nucleotide sequence. Such sequences can be referred to as “fully complementary” with respect to each other herein. However, where a first sequence is referred to as “substantially complementary” with respect to a second sequence herein, the two sequences can be fully complementary, or they may form one or more, but preferably not more than 4, 3 or 2 mismatched base pairs upon hybridization, while retaining the ability to hybridize under the conditions most relevant to their ultimate application. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, may yet be referred to as “fully complementary” for the purposes of the invention.

[00185] “Complementary” sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled.

[00186] The terms “complementary”, “fully complementary” and “substantially complementary” herein may be used with respect to the base matching between the antisense of a ssRNA and target sequence, between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a dsRNA and a target sequence, as will be understood from the context of their use.

[00187] As used herein, a polynucleotide which is “substantially complementary to at least part of a messenger RNA (mRNA) refers to a polynucleotide which is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, encoding Nav1.8). For example, a polynucleotide is complementary to at least a part of a Nav1.8 mRNA if the

sequence is substantially complementary to a non-interrupted portion of an mRNA encoding Nav1.8.

[00188] As used herein, “contact,” “contacting” and the like means directly or indirectly introducing or delivering an oligonucleotide such as an oligonucleotide or an AOC into, for example, a cell by facilitating or effecting uptake or absorption into the cell.

[00189] “Introducing into a cell”, when referring to a ssRNA or dsRNA, means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of ssRNA or dsRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells *in vitro*; a ssRNA or a dsRNA may also be “introduced into a cell”, wherein the cell is part of a living organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for *in vivo* delivery, ssRNA or dsRNA can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection.

[00190] As used herein, “deoxyribonucleotide” means a nucleotide having a hydrogen in place of a hydroxyl at the 2' position of its pentose sugar when compared with a ribonucleotide. A modified deoxyribonucleotide has one or more modifications or substitutions of atoms other than at the 2' position, including modifications or substitutions in or of the nucleobase, sugar, or phosphate group.

[00191] The term “double-stranded RNA” or “dsRNA”, as used herein, refers to a ribonucleic acid molecule, or complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary, as defined above, nucleic acid strands. The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5' end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a “hairpin loop”. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5' end of the respective other strand forming the duplex structure, the connecting structure is referred to as a “linker”. The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA. In addition to the duplex structure, a dsRNA may comprise one or more nucleotide overhangs.

[00192] As used herein, a “nucleotide overhang” refers to the unpaired nucleotide or nucleotides that protrude from the duplex structure of a dsRNA when a 3'-end of one strand of the dsRNA extends beyond the 5'-end of the other strand, or vice versa. “Blunt” or “blunt end” means that there are no unpaired nucleotides at that end of the dsRNA, i.e., no nucleotide overhang. A “blunt ended” dsRNA is a dsRNA that is double stranded over its entire length, i.e., no nucleotide overhang at either end of the molecule.

[00193] As used herein, “duplex,” in reference to nucleic acids (*e.g.*, oligonucleotides), means a structure formed through complementary base pairing of two antiparallel sequences of nucleotides.

[00194] The term “antibody,” as used herein, refers to a molecule that binds an antigen. Embodiments of an antibody include a monoclonal antibody, polyclonal antibody, human antibody, humanized antibody, chimeric antibody, heterodimeric antibody, bispecific or multispecific antibody, or conjugated antibody. The antibodies can be of any class (*e.g.*, IgG, IgE, IgM, IgD, IgA), and any subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4). In some embodiments, the antibody of the present disclosure is an IgG1 antibody.

[00195] An immunoglobulin G (IgG) type antibody is comprised of four polypeptide chains: two heavy chains (HC) and two light chains (LC) that are cross-linked via inter-chain disulfide bonds. The amino-terminal portion of each of the four polypeptide chains includes a variable region of about 100-125 or more amino acids primarily responsible for antigen recognition. The carboxyl-terminal portion of each of the four polypeptide chains contains a constant region primarily responsible for effector function. Each heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (VL) and a light chain constant region. The IgG isotype may be further divided into subclasses (*e.g.*, IgG1, IgG2, IgG3, and IgG4).

[00196] The VH (also referred to herein as HCVR) and VL (also referred to herein as LCVR) regions can be further subdivided into regions of hyper-variability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). The CDRs are exposed on the surface of the protein and are important regions of the antibody for antigen binding specificity. Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Herein, the three CDRs of the heavy chain are referred to as “HCDR1, HCDR2, and HCDR3” and the three CDRs of the light chain are referred to as “LCDR1, LCDR2 and LCDR3”. The CDRs contain most of the residues that form specific interactions with the antigen. Assignment of amino acid

residues to the CDRs may be done according to the well-known schemes, including those described in Kabat (Kabat et al., “Sequences of Proteins of Immunological Interest,” *National Institutes of Health*, Bethesda, MD. (1991), which is hereby incorporated by reference in its entirety), Chothia (Chothia et al., “Canonical Structures for the Hypervariable Regions of Immunoglobulins,” *J. Mol. Biol.*, 196, 901-917 (1987); Al-Lazikani et al., “Standard Conformations for the Canonical Structures of Immunoglobulins,” *J. Mol. Biol.*, 273, 927-948 (1997), which are hereby incorporated by reference in their entirety), North (North et al., “A New Clustering of Antibody CDR Loop Conformations,” *J. Mol. Biol.*, 406, 228-256 (2011)), or IMGT® (the International ImMunoGeneTics database available on at imgt.org; see Lefranc et al., *Nucleic Acids Res.* 27:209-212 (1999), which are hereby incorporated by reference in their entireties).

[00197] The term “antigen-binding fragments”, as used herein, refers to a portion of an antibody that binds an antigen or an epitope of the antigen. For example, “TfR binding protein” refers to a portion of an antibody or antibody fragment that binds TfR or an epitope of TfR.

[00198] As referred to herein, the term “epitope” refers to the amino acid residues, of an antigen, that are bound by an antibody. An epitope can be a linear epitope, a conformational epitope, or a hybrid epitope. The term “epitope” may be used in reference to a structural epitope. A structural epitope, according to some embodiments, may be used to describe the region of an antigen which is covered by an antibody or antigen binding protein. In some embodiments, a structural epitope may describe the amino acid residues of the antigen that are within a specified proximity (*e.g.*, within a specified number of angstroms) of an amino acid residue of the antibody or antigen binding protein. The term “epitope” may also be used in reference to a functional epitope. A functional epitope, according to some embodiments, may be used to describe amino acid residues of the antigen that interact with amino acid residues of the antibody or antigen binding protein in a manner contributing to the binding energy between the antigen and the antibody or antigen binding protein.

[00199] An epitope can be determined according to different experimental techniques, also called “epitope mapping techniques.” It is understood that the determination of an epitope may vary based on the different epitope mapping techniques used and may also vary with the different experimental conditions used, *e.g.*, due to the conformational changes or cleavages of the antigen induced by specific experimental conditions. Epitope mapping techniques are known in the art (*e.g.*, Rockberg and Nilvebrant, *Epitope Mapping Protocols: Methods in Molecular Biology*, Humana Press, 3rd ed. 2018), including but not limited to, X-ray

crystallography, nuclear magnetic resonance (NMR) spectroscopy, site-directed mutagenesis, species swap mutagenesis, alanine-scanning mutagenesis, hydrogen-deuterium exchange (HDX) and cross-blocking assays.

[00200] The term “Fc region” as used herein refers to a polypeptide comprising the CH2 and CH3 domains of a constant region of an immunoglobulin, *e.g.*, IgG1, IgG2, IgG3, or IgG4. Optionally, the Fc region may include a portion of the hinge region or the entire hinge region of an immunoglobulin, *e.g.*, IgG1, IgG2, IgG3, or IgG4. In some embodiments, the Fc region is a human IgG Fc region, *e.g.*, a human IgG1 Fc region, human IgG2 Fc region, human IgG3 Fc region or human IgG4 Fc region. In some embodiments, the Fc region is a modified IgG Fc region with reduced or eliminated effector functions compared to the corresponding wild type IgG Fc region. The numbering of the residues in the Fc region is based on the EU index as described in Kabat (Kabat et al, *Sequences of Proteins of Immunological Interest*, 5th edition, Bethesda, MD: U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, 1991). The boundaries of the Fc region of an immunoglobulin heavy chain might vary, and the human IgG heavy chain Fc region is usually defined as the stretch from the N-terminus of the CH2 domain (*e.g.*, the amino acid residue at position 231 according to the EU index numbering) to the C-terminus of the CH3 domain (or the C-terminus of the immunoglobulin).

[00201] The term “knockdown” or “expression knockdown” refers to reduced mRNA or protein expression of a gene after treatment of a reagent.

[00202] The terms “bind” and “binds” as used herein are intended to mean, unless indicated otherwise, the ability of a protein or molecule to form a chemical bond or attractive interaction with another protein or molecule, which results in proximity of the two proteins or molecules as determined by common methods known in the art.

[00203] The term “% sequence identity” or “percentage sequence identity” with respect to a reference nucleic acid sequence is defined as the percentage of nucleotides, nucleosides, or nucleobases in a candidate sequence that are identical with the nucleotides, nucleosides, or nucleobases in the reference nucleic acid sequence, after optimally aligning the sequences and introducing gaps or overhangs, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software programs, for example, those described in Current Protocols in Molecular Biology (Ausubel *et al.*, eds., 1987, Supp. 30, section 7.7.18, Table 7.7.1), and including BLAST, BLAST-2, ALIGN, Megalign (DNASTAR), Clustal W2.0 or Clustal

X2.0 software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Percentage of “sequence identity” can be determined by comparing two optimally aligned sequences over a comparison window, where the fragment of the nucleic acid sequence in the comparison window may comprise additions or deletions (*e.g.*, gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage can be calculated by determining the number of positions at which the identical nucleotide, nucleoside, or nucleobase occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity. The output is the percent identity of the subject sequence with respect to the query sequence.

[00204] As used herein, “TfR” refers to a transferrin receptor protein or polypeptide, *e.g.*, a human transferrin receptor protein or polypeptide. The amino acid sequence of the human transferrin receptor protein (hTfR) can be found at NCBI Reference Sequence: NP_001121620.1, which is hereby incorporated by reference in its entirety.

[00205] The term “pharmaceutically acceptable carrier” refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid, or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

[00206] As used herein, “individual” means any mammal, including cats, dogs, mice, rats, and primates, especially humans. Moreover, “subject” or “patient” may be used interchangeably with “individual.”

[00207] As used herein, “labile linker” means a linker that can be cleaved (*e.g.*, by acidic pH). Likewise, “fairly stable linker” means a linker that cannot be cleaved.

[00208] As used herein, “modified internucleotide linkage” means an internucleotide linkage having one or more chemical modifications when compared with a reference internucleotide linkage having a phosphodiester bond. A modified nucleotide can be a non-naturally occurring linkage. Typically, a modified internucleotide linkage confers one or more desirable properties to a nucleic acid in which the modified internucleotide linkage is present. For example, a modified nucleotide may improve thermal stability, resistance to degradation, nuclease resistance, solubility, bioavailability, bioactivity, reduced immunogenicity, *etc.*

[00209] As used herein, “modified nucleotide” refers to a nucleotide having one or more chemical modifications when compared with a corresponding reference nucleotide selected from: adenine ribonucleotide, guanine ribonucleotide, cytosine ribonucleotide, uracil ribonucleotide, adenine deoxyribonucleotide, guanine deoxyribonucleotide, cytosine deoxyribonucleotide and thymidine deoxyribonucleotide. A modified nucleotide can be a non-naturally occurring nucleotide. A modified nucleotide can have, for example, one or more chemical modifications in its sugar, nucleobase and/or phosphate group. Additionally, or alternatively, a modified nucleotide can have one or more chemical moieties conjugated to a corresponding reference nucleotide. Typically, a modified nucleotide confers one or more desirable properties to a nucleic acid in which the modified nucleotide is present. For example, a modified nucleotide may improve thermal stability, resistance to degradation, nuclease resistance, solubility, bioavailability, bioactivity, reduced immunogenicity, *etc.*

[00210] As used herein, “nucleoside” means a nucleobase-sugar combination, where the nucleobase portion is normally a heterocyclic base. The two most common classes of such heterocyclic bases are purines and pyrimidines. The sugar is normally a pentose sugar such as a ribose or a deoxyribose (*e.g.*, 2'-deoxyribose).

[00211] As used herein, “nucleotide” means an organic molecule having a nucleoside (a nucleobase such as, for example, adenine, cytosine, guanine, thymine, or uracil; and a pentose sugar such as, *e.g.*, ribose or 2'-deoxyribose) and a phosphate group, which can serve as a monomeric unit of nucleic acid polymers such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

[00212] As used herein, “oligonucleotide” means a short nucleic acid molecule (*e.g.*, less than about 100 nucleotides in length). An oligonucleotide may be single-stranded (ss) or double-stranded (ds). An oligonucleotide may or may not have duplex regions. As a set of non-limiting examples, an oligonucleotide may be, but is not limited to, a small interfering RNA (siRNA), microRNA (miRNA), short hairpin RNA (shRNA), Dicer substrate interfering RNA (DsiRNA), antisense oligonucleotide (ASO), short siRNA or ss siRNA. In some instances,

the oligonucleotide is a phosphorodiamidate morpholino oligomers (PMO), which are short single-stranded oligonucleotide analogs that are built upon a backbone of morpholine rings connected by phosphorodiamidate linkages. An oligonucleotide is a single stranded (ss) oligonucleotide (e.g., ASO) or a double stranded (ds) oligonucleotide (e.g., siRNA).

[00213] As used herein, “phosphate analog” means a chemical moiety that mimics the electrostatic and/or steric properties of a phosphate group. In some embodiments, a phosphate analog is positioned at the 5' terminal nucleotide of an oligonucleotide in place of a 5'-phosphate, which is often susceptible to enzymatic removal. A 5' phosphate analog can include a phosphatase-resistant linkage. Examples of phosphate analogs include, but are not limited to, 5' phosphonates, such as 5' methylenephosphonate (5'-MP) and 5'-(E)-vinylphosphonate (5'-VP). An oligonucleotide can have a phosphate analog at a 4'-carbon position of the sugar (referred to as a “4'-phosphate analog”) at a 5'-terminal nucleotide. An example of a 4'-phosphate analog is oxymethylphosphonate, in which the oxygen atom of the oxymethyl group is bound to the sugar moiety (e.g., at its 4'-carbon) or analog thereof. *See, e.g.*, Intl. Patent Application Publication No. WO 2018/045317. Other modifications have been developed for the 5' end of oligonucleotides (*see, e.g.*, Intl. Patent Application No. WO 2011/133871; US Patent No. 8,927,513; and Prakash *et al.* (2015) *Nucleic Acids Res.* 43:2993-3011).

[00214] As used herein, “reduced expression” or “reduced activity,” and with respect to a gene (e.g., *SCN10A*), means a decrease in the amount or level of RNA transcript (e.g., *SCN10A* mRNA) or protein (e.g., Nav1.8 protein) encoded by the gene and/or a decrease in the amount or level of activity of the gene or protein in a cell, a population of cells, a sample or a subject, when compared to an appropriate reference (e.g., a reference cell, population of cells, sample or individual).

[00215] The terms “silence” and “inhibit the expression of”, in as far as they refer to the Nav1.8 gene, herein refer to the at least partial suppression of the expression of the Nav1.8 gene, as manifested by a reduction of the amount of mRNA transcribed from the Nav1.8 gene which may be isolated from a first cell or group of cells in which the Nav1.8 gene is transcribed and which has or have been treated such that the expression of the Nav1.8 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells).

[00216] Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to Nav1.8 gene transcription, e.g., the amount of protein encoded by the Nav1.8 gene which is secreted by a cell, or the number of cells displaying a

certain phenotype, *e.g.*, apoptosis. In principle, Nav1.8 gene silencing may be determined in any cell expressing the target, either constitutively or by genomic engineering, and by any appropriate assay. However, when a reference is needed in order to determine whether a given siRNA inhibits the expression of the Nav1.8 gene by a certain degree and therefore is encompassed by the instant invention.

[00217] As used herein, “region of complementarity” means a sequence of nucleotides of a nucleic acid (*e.g.*, a double stranded oligonucleotide) that is sufficiently complementary to an antiparallel sequence of nucleotides to permit hybridization between the two sequences of nucleotides under appropriate hybridization conditions (*e.g.*, in a phosphate buffer, in a cell, *etc.*). An oligonucleotide herein includes a targeting sequence having a region of complementary to a mRNA target sequence.

[00218] As used herein, “ribonucleotide” means a nucleotide having a ribose as its pentose sugar, which contains a hydroxyl group at its 2' position. A modified ribonucleotide is a ribonucleotide having one or more modifications or substitutions of atoms other than at the 2' position, including modifications or substitutions in or of the nucleobase, sugar, or phosphate group.

[00219] As used herein, siRNA includes a double stranded oligonucleotide having a sense strand and antisense strand, in which the antisense strand or part of the antisense strand is used by the Argonaute 2 (Ago2) endonuclease of the RNA induced silencing complex (RISC) in the cleavage of a target mRNA.

[00220] The term a “single stranded oligonucleotide” as used herein includes an antisense strand (or part of that antisense strand) that in combination with the ribonuclease H (RNase H) endonuclease mediates the cleavage of a target mRNA.

[00221] As used herein, “strand” refers to a single, contiguous sequence of nucleotides linked together through internucleotide linkages (*e.g.*, phosphodiester linkages or phosphorothioate linkages). A strand can have two free ends (*e.g.*, a 5' end and a 3' end).

[00222] As used herein, “synthetic” refers to a nucleic acid or other molecule that is artificially synthesized (*e.g.*, using a machine such as, for example, a solid-state nucleic acid synthesizer) or that is otherwise not derived from a natural source (*e.g.*, a cell or organism) that normally produces the nucleic acid or other molecule.

[00223] As used herein, “treat” or “treating” means an act of providing care to an individual in need thereof, for example, by administering a therapeutic agent (*e.g.*, an oligonucleotide herein) to the individual for purposes of improving the health and/or well-being of the individual with respect to an existing a disease, disorder, or condition, or to prevent or

decrease the likelihood of the occurrence of a disease, disorder, or condition. Treating also can involve reducing the frequency or severity of at least one sign, symptom or contributing factor of a disease, disorder, or condition experienced by the individual. The term “treat” or “treating” also includes relief from or alleviation of the perception of pain, including the relief from or alleviation of the intensity and/or duration of a pain (*e.g.*, burning sensation, tingling, electric-shock-like feelings, etc.) experienced by a subject in response to a given stimulus (*e.g.*, pressure, tissue injury, cold temperature, etc.). Relief from or alleviation of the perception of pain can be any detectable decrease in the intensity or duration of pain. Treatment can occur in a subject (*e.g.*, a human or companion animal) suffering from a pain condition or having one or more symptoms of a pain-related disorder that can be treated according to the present disclosure, or in an animal model of pain.

[00224] By “Nav1.8” as used herein is meant, any Nav1.8 protein, peptide, or polypeptide associated with the development or maintenance of an ion channel. The terms “Nav1.8” also refer to nucleic acid sequences encoding any Nav1.8 protein, peptide, or polypeptide. The gene encoding for Nav1.8 is referred to as *SCN10A*.

[00225] As used herein, a “pharmaceutical composition” comprises a pharmacologically effective amount of an oligonucleotide or conjugate and a pharmaceutically acceptable carrier. As used herein, “pharmacologically effective amount,” “therapeutically effective amount” or simply “effective amount” refers to that amount of an RNA effective to produce the intended pharmacological, therapeutic, or preventive result. For example, if a given clinical treatment is considered effective when there is at least a 25% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to affect at least a 25% reduction in that parameter.

[00226] As used herein, the phrases “therapeutically effective amount” and “prophylactically effective amount” refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of pain or an overt symptom of pain. The specific amount that is therapeutically effective can be readily determined by ordinary medical practitioner and may vary depending on factors known in the art, such as, *e.g.*, the type of pain, the patient's history and age, the stage of pain, and the administration of other anti-pain agents.

[00227] As used herein, a “transformed cell” is a cell into which a vector has been introduced from which a dsRNA molecule may be expressed.

[00228] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described

below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

[00229] The following non-limiting examples are offered for purposes of illustration, not limitation.

Example 1: Bioinformatics Based Testing/selection of siRNAs.

Design and in-silico Selection of siRNAs for Targeting of Human SCN10A Messenger RNA

An *in-silico* triage was conducted to select siRNA sequences targeting *SCN10A* (sodium voltage-gated channel alpha subunit 10, Gene ID:6336, Alias:*Nav1.8*) for *in vitro* testing. All possible 5856 19-mer siRNA sequences for the *SCN10A* human messenger RNA (mRNA) were created *in-silico* using the *SCN10A* reference sequence NM_006514.3 (SEQ ID NO: 648), which is hereby incorporated by reference in its entirety. For bioinformatics analysis, all transcript RNA sequences were obtained from NCBI RefSeq DB release 95 (July 2019) and Ensembl DB release 97 (July 2019). Table 11 shows the reference sequences used for *in-silico* screening and selection. The selection criteria used to select the initial round of siRNAs were:

- a. Perfect match (19-mer) with sequence of human *SCN10A* and cynomolgus/rhesus macaques *SCN10A* was required, and cross-reactivity with mouse or rat were analyzed but not required.
- b. No sequences with perfect matches or one mismatch against non-*SCN10A* genes within positions 2-18 (5'-3') of the antisense strand were included.
- c. Three or more mismatches in antisense strand were required for a set of other sodium channel sequences, including *SCN4A* (Nav1.4, Gene ID 6329); *SCN5A* (Nav1.5, Gene ID 6331); *SCN9A* (Nav1.7, Gene ID 6335), and *SCN11A* (Nav1.9, Gene ID 11280).
- d. Sequences with identical seed region of known miRNAs, based on miRBase R22 (human, rhesus, mouse, rat, March 2018), were excluded.
- e. siRNA sequences targeting regions with known SNPs of >1% minor allele frequency (MAF) based on human SNP database (NCBI dbSNP Build 151, Oct 2017) were excluded.

Table 11: Transcript Sequences Used for *in-silico* Nav1.8 siRNA Design and Triage.

Species	Transcript (Accession/ID)
cyno	XM_005546684.2 Mfa
cyno	XM_015445464.1 Mfa
cyno	XM_015445465.1 Mfa
cyno	ENSMFAT00000023288.1
human	NM_001293306.2
human	NM_001293307.2
human	NM_006514.3
human	XM_005265371.3
human	XM_011533993.2
human	XM_011533994.2
human	ENST00000643924.1
human	ENST00000449082.3
human	ENST00000643108.1
human	ENST00000644826.1
human	ENST00000655275.1
mouse	NM_001205321.1
mouse	NM_009134.3
mouse	XM_017313223.1
mouse	ENSMUST00000084787.5
mouse	ENSMUST00000213392.1
mouse	ENSMUST00000214408.1
mouse	XM_017313221.1
mouse	XM_017313222.1
mouse	ENSMUST00000216583.1
mouse	ENSMUST00000213401.1
rat	NM_017247.1
rat	XM_017595503.1
rat	XM_017595504.1
rat	XM_017595505.1
rat	ENSRNOT00000046864.4
rhesus	XM_015131314.2
rhesus	XM_015131316.2
rhesus	XM_015131317.2
rhesus	ENSMUT00000022236.3

[00230] With the exclusion criteria for selecting Nav1.8 siRNA, the *in-silico* screen identified a total of 141 siRNA sequences (see Table 2 above), approximately 2.4% of the total possible 19-mer sequences. Each selected siRNA matched the desired selection criteria of human and cyno/rhesus species predicted cross-reactivity (Table 12) with low predicted off-target effect and miRNA activity.

Table 12: Cross-reactivity of the 19-mer siRNAs from the *in-silico* Screen (“X” indicates the siRNA species showed *in-silico* predicted cross-reactivity).

siRNA NO.	NHP (predicted)	Mouse (predicted)	Rat (predicted)	Start position of target region on human SCN10A transcript
1	X			407
2	X			1172
3	X			426
4	X			410
5	X			349
6	X			537
7	X	X	X	990
8	X	X	X	986
9	X			538
10	X	X	X	989
11	X			361
12	X	X		983
13	X			532
14	X			1794
15	X			2073
16	X	X	X	435
17	X	X	X	434
18	X	X	X	1442
19	X			2071
20	X			584
21	X			427
22	X			576
23	X			1790
24	X			2063
25	X	X	X	987
26	X			535
27	X			1787
28	X			858
29	X			43
30	X			856
31	X			3958
32	X			546
33	X			5331
34	X			1952
35	X			398
36	X			396
37	X			859
38	X			350
39	X			1125
40	X			4
41	X			597

42	X			5780
43	X			587
44	X			1830
45	X			5803
46	X			1437
47	X			400
48	X			360
49	X	X	X	988
50	X			5809
51	X			619
52	X			306
53	X			2599
54	X			5337
55	X			3583
56	X			3346
57	X			5770
58	X			1124
59	X			3967
60	X	X		1825
61	X			1127
62	X			1759
63	X			2445
64	X			409
65	X			3352
66	X	X		1826
67	X			5787
68	X			1439
69	X			3960
70	X			3959
71	X			1869
72	X			1842
73	X	X		1824
74	X			1829
75	X			3355
76	X			1123
77	X			2952
78	X	X		5484
79	X			2016
80	X			1974
81	X			1028
82	X			5807
83	X			1837
84	X			50
85	X			2753

86	X	X	X	433
87	X			2744
88	X			1799
89	X			4147
90	X			5775
91	X			5342
92	X			5558
93	X			1849
94	X	X		5485
95	X	X		5514
96	X			860
97	X			2652
98	X			2602
99	X			5583
100	X			5423
101	X	X	X	253
102	X			397
103	X			5508
104	X			2752
105	X			260
106	X			4413
107	X			4060
108	X			2603
109	X			5343
110	X			1760
111	X			5575
112	X			4561
113	X			5591
114	X			209
115	X			263
116	X			5768
117	X			3968
118	X	X		981
119	X			5592
120	X			3985
121	X			3964
122	X			309
123	X	X	X	1440
124	X			5336
125	X			432
126	X			2655
127	X	X	X	1441
128	X			2458
129	X			2605

130	X			5771
131	X			4562
132	X	X	X	985
133	X			1843
134	X			598
135	X			919
136	X	X	X	255
137	X			2449
138	X			783
139	X			3993
140	X			40
141	X	X	X	5509

[00231] Of the 5856 possible 19-mer siRNAs sequences targeting the human Nav1.8 transcript (reference sequence NM_006514.3), 141 sequences matched the selected criteria such as low potential off-target toxicity to other NAV channels and genes as well as perfect 19-mer matching to known cynomolgus and rhesus SCN10A mRNA transcripts. These 141 siRNAs were then selected to be synthesized for *in vitro* screening.

Example 2: Methods of Making of siRNA Molecules and Description of RNA

Modifications

[00232] The Nav1.8 21-mer duplexes with 19 bases of complementarity and 3' dinucleotide overhangs on the guide strand targeting the human *SCN10A* mRNA (also called Nav1.8 mRNA) were synthesized (see Table 3). RNA modifications were used to optimize the potency of the siRNA duplex and reduce immunogenicity (as shown in Figure 1A or 1B; with or without the amine or cholesterol handle at the 5'-end of the passenger strand).

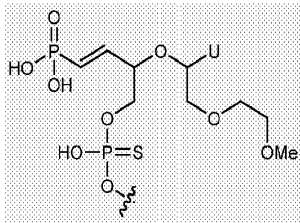
[00233] All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded 21-mer/19-mer siRNAs (Malecova et al., "Targeted Tissue Delivery of RNA Therapeutics using Antibody-oligonucleotide Conjugates (AOCs)," *Nucleic Acids Res.* Volume 51, Issue 12, Pages 5901–5910 (2023)).

[00234] A non-target control siRNA sequence (siNTC), a published non-targeting control sequence, having a 21-mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs on the guide strand sequence was synthesized (Burke et al., "siRNA-Mediated Knockdown of P450 Oxidoreductase in Rats: A Tool to Reduce Metabolism by CYPs and Increase Exposure of High Clearance Compounds," *Pharm. Res.* 31, 3445-3460 (2014)). The sequence for the siNTC is shown in Table 13.

Table 13: Non-target Control siRNA Sequence.

siRNA ID	Sense Strand (5' to 3')	Antisense Strand (5' to 3')
siNTC	CUAGCUGGACACGUCGAUA (SEQ ID NO: 646)	UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 647)

[00235] In some embodiments, the siRNA included vinylphosphonate-modified nucleotide (VpUq) in the antisense strand as illustrated in Figure 1B and shown in Table 4 (5vp_una2moe). VpUq is a vinylphosphonate-2'-O-methoxyethylribose unlocked nucleic acid with a uridine base. It was synthesized according to procedures found in U.S. Patent No. US 11,110,180, which is hereby incorporated by reference in its entirety. The chemical structure of the VpUq is shown below:



Example 3: Effect of VpUq Guide Strand Modification in Nav1.8 siRNAs.

[00236] The siRNAs with modification pattern with VpUq are described in Table 4 and illustrated in Figure 1B. To evaluate the activity and potency of the VpUq modification in the siRNAs, Nav1.8 siRNA sequences with and without the VpUq modification were tested in human primary neurons. Modified siRNA 410 without VpUq (i.e., siRNA No. 285 in Table 4) and modified siRNA 410 with VpUq (i.e., siRNA No. 305 in Table 4) and modified siRNA 535 without VpUq (i.e., siRNA No. 291 in Table 4) and modified siRNA 535 with VpUq (i.e., siRNA No. 311 in Table 4) were tested. All tested siRNAs were enclosed in lipid nanoparticles as described below.

[00237] Human dorsal root ganglia neurons (DRGs) were used as a source of primary cells expressing Nav1.8 mRNA. Fresh primary human DRGs were purchased from AnaBios (San Diego, CA) and maintained in serum-free NbActive4 neural maintenance medium (Axol, Easter Brush, United Kingdom) with 25 ng/mL of recombinant human nerve growth factor (NGF, Axol). Primary DRGs were treated with siRNA encapsulated in Lipid Nano Particles (LNP, Precision Nanosystems, Vancouver, Canada) following the manufacturer's

recommendation. LNP-siRNAs (with VpUq) modifications were introduced to growth medium at 150 – 0.008 nM for 3 days as-is, with no transfection reagent.

[00238] Treated human DRGs were kept at 37 °C in 5% CO₂ for 3 days, followed by a wash in phosphate-buffer saline (PBS, ThermoFisher™), and stored in 300 µL of TRIzol™ (ThermoFisher™) at -80 °C until RNA extraction. The total mRNA was isolated using the ZYMO 96-well RNA kit (Zymo Research, Irvine, CA), and 100-250 ng of purified mRNA was used to generate the cDNA with the iScript cDNA synthesis kit (BioRad, Hercules, CA). RNA expression levels were determined by quantitative reverse transcription polymerase chain reaction (RT-qPCR) using commercially available TaqMan probes (Life Technologies; *SCN10A*: Hs01045150_m1, *STMN2*: Hs00975900_m1, and *SNAP25*: Hs00938957_m1). The relative reduction in Nav1.8 transcripts was determined by normalizing the expression of siRNA treated DRGs to the mock-transfected group using the DDCT method using the following formula: [%hNav1.8 mRNA = 100*2^{-DCT (siRNA treated cells)-DCT (mock-transfected cells)}], with DCT determined by the difference in Ct values between *SCN10A* and the housekeeping genes *STMN2* and *SNAP25* {avg DCT=[((2*Ct(SCN10A))-Ct(STMN2)-Ct(SNAP25))/2]} (Green MR, Sambrook J. “Quantification of RNA by Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR),” *Cold Spring Harb Protoc.* 2018 Oct, 2018(10): pdb.prot095042; and Livak KJ, Schmittgen TD, “Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method,” *Methods*, 25(4), 402–8 (2001)).

[00239] The IC₅₀s were calculated by fitting the siRNA concentration-response data into a 3-parameter non-linear regression fitting model [log(concentration of siRNA) vs. %hNav1.8 mRNA] using the GraphPad Prism software (GraphPad Software, LLC).

[00240] The dose response curves are shown in Figures 2 and 3. The addition of the modified nucleotide VpUq to the siRNA had minimal effects in the maximum levels of mRNA reduction. However, the presence of VpUq at the 5'-end of the guide strand affected the siRNA IC₅₀ value in a sequence specific manner. For the siRNA No. 305 (start position of 410), minor differences in the IC₅₀ values were calculated whereas for siRNA No. 311 (start position of 535), the addition of VpUq in lowered the siRNA IC₅₀ value greater than 3-fold from 23.4 nM to 6.9 nM as shown in Table 14.

Table 14: VpUq Modification Pattern Effects

siRNA No.	VpUq modification	IC ₅₀ (pM)	Start position of target region on human SCN10A transcript
285	not present	3.787	410

305	present	3.931	410
291	not present	23.41	535
311	present	6.929	535

[00241] In primary human DRGs, incorporation of the VpUq modified nucleotide had a beneficial effect in a sequence-specific manner. The data showed that the IC₅₀ value of the Nav1.8 siRNA sequence siRNA No. 311 was improved by greater than ~3-fold. However, minor differences were observed Nav1.8 siRNA sequence siRNA No. 305. Small changes in the maximum mRNA reduction were observed with the incorporation of the modified nucleotide VpUq to the siRNA.

Example 4: Nav1.8 siRNAs Activity in HEK293 Cell *in vitro*.

[00242] HEK293 cells expressing the human Nav1.8 transcript (HEK293-hNav1.8) were used to determine the *in vitro* activity of the siRNA sequences. The cells were grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Billings, MT) supplemented with 10% of heat-inactivated fetal bovine serum (FBS; Corning, Corning, NY), 1% Pen/Strep (Gibco), 0.1 mg/mL of Zeocin™ (Gibco), and 0.5 µg/mL of Puromycin (ThermoFisher, Waltham, MA). For transfections, HEK293-hNav1.8 cells were seeded at 15,000 cells/well in 100 µL in 96-well tissue culture plates and transfected within 24 hours post-seeding. The siRNAs were gently mixed with Lipofectamine™ RNAiMAX transfection reagent (ThermoFisher) added to the wells to a final concentration of 10 nM or 1 nM according to manufacturer's recommendation.

[00243] Treated cells were kept at 37 °C in 5% CO₂ for 72 hours, followed by a wash in phosphate-buffer saline (PBS, ThermoFisher), and stored in 300 µL of TRIzol™ (ThermoFisher) at -80 °C until RNA extraction. The total mRNA was isolated using the ZYMO 96-well RNA kit (Zymo Research, Irvine, CA), and 100-250 ng of purified mRNA was used to generate the cDNA with the iScript cDNA synthesis kit (BioRad, Hercules, CA).

[00244] RNA expression levels were determined by quantitative reverse transcription polymerase chain reaction (RT-qPCR) using commercially available TaqMan probes (Life Technologies; *SCN10A*: Hs01045150_m1 and *PPIB*: Hs00168719_m1). The relative reduction in Nav1.8 transcripts was determined by normalizing the expression of siRNA treated HEK-hNav1.8 cells to the mock-transfected group using the DDCT method and the following formula: $[\%hNav1.8 \text{ mRNA} = 100 * 2^{-\text{DCt}(\text{siRNA treated cells}) - \text{DCt}(\text{mock-transfected cells})}]$, with DCt determined by the difference in Ct values between SCN10A and the housekeeping gene PPIB $[\text{DCt} = \text{Ct}(\text{SCN10A}) - \text{Ct}(\text{PPIB})]$ (Green MR, Sambrook J. "Quantification of RNA by

Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR),” *Cold Spring Harb. Protoc.*, 2018(10): pdb.prot095042; Livak KJ, Schmittgen TD, “Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2- $\Delta\Delta$ CT Method,” *Methods*, 25(4), 402–8 (2001)).

[00245] Table 15 summarizes the screening results, showing the Nav1.8 targeting siRNA sequences ranked based on the *in vitro* activity at the 10 nM concentration of siRNA. The %hNav1.8 mRNA represents the relative expression of human *Nav1.8* mRNA remaining after siRNA treatment compared to mock-transfected control cells. Accompanying the siRNA number in the table is the starting siRNA sequence position based on the *SCN10A* reference transcript sequence (NM_006514.3).

[00246] A non-target human siRNA sequence (siNTC, Table 13) was used as a transfection control to account for unspecific mRNA reduction. The control results are also shown in Table 15. Significant mRNA reduction cut-off was determined using the combined average of the siNTC group (10 nM and 1 nM siRNA concentrations), subtracted by 3 standard deviations (std.dev) (Average siNTC= 95.6%; std.dev=14.8%).

Table 15: *In vitro* Activity at Two siRNA Concentrations in HEK-293 hNav1.8 Cells. The siRNAs are modified as shown in Figure 1A but without the amino/cholesterol handle.

siRNA No.	%hNav1.8 mRNA at 10nM	%hNav1.8 mRNA at 1nM	Start position of target region on human SCN10A transcript
142	17.84	32.98	407
143	19.55	32.77	1172
144	22.59	48.88	426
145	25.71	30.56	410
146	27.11	65.56	349
147	27.32	31.37	537
148	30.89	37.77	990
149	31.82	37.30	986
150	32.46	51.47	538
151	33.14	46.34	989
152	33.18	46.49	361
153	33.58	38.86	983
154	34.14	45.02	532
155	34.28	51.22	1794
156	34.63	57.53	2073
157	35.08	45.33	435
158	35.45	43.51	434

159	36.02	55.63	1442
160	36.09	55.46	2071
161	36.48	58.97	584
162	36.55	58.42	427
163	36.76	56.63	576
164	36.99	40.63	1790
165	37.04	40.15	2063
166	37.64	43.22	987
167	38.29	51.17	535
168	39.65	37.18	1787
169	39.81	41.64	858
170	41.35	56.28	43
171	41.94	38.18	856
172	42.34	55.14	3958
173	42.72	66.55	546
174	42.74	53.86	5331
175	43.14	54.38	1952
176	43.22	71.53	398
177	43.39	71.96	396
178	44.20	49.82	859
179	44.20	52.20	350
180	44.67	57.52	1125
181	45.74	76.21	4
182	46.05	66.08	597
183	46.29	45.37	5780
184	46.40	46.51	587
185	47.40	43.23	1830
186	48.41	66.25	5803
187	48.42	63.52	1437
188	48.57	69.65	400
189	48.91	76.47	360
190	49.51	56.68	988
191	49.78	49.95	5809
192	51.04	81.72	619
193	51.24	72.83	306
194	51.73	64.94	2599
195	53.33	55.92	5337
196	54.79	75.38	3583
197	54.99	61.97	3346
198	55.20	68.33	5770
199	55.39	56.55	1124
200	55.62	67.05	3967
201	55.64	57.15	1825
202	56.04	53.50	1127

203	56.16	65.78	1759
204	56.48	68.04	2445
205	56.76	57.52	409
206	56.85	67.37	3352
207	57.02	55.99	1826
208	57.34	72.13	5787
209	57.48	63.17	1439
210	57.99	69.56	3960
211	58.31	72.54	3959
212	58.46	73.96	1869
213	58.58	69.56	1842
214	58.62	66.49	1824
215	58.68	58.20	1829
216	58.87	69.06	3355
217	59.51	65.56	1123
218	59.64	75.02	2952
219	60.10	62.27	5484
220	60.42	69.49	2016
221	60.57	73.50	1974
222	60.76	60.02	1028
223	62.60	65.58	5807
224	62.71	57.12	1837
225	63.15	77.17	50
226	63.97	66.33	2753
227	64.20	73.24	433
228	64.75	57.20	2744
229	64.93	71.93	1799
230	65.36	67.42	4147
231	65.76	84.60	5775
232	66.11	59.35	5342
233	66.22	72.64	5558
234	66.30	86.94	1849
235	66.54	62.96	5485
236	66.88	65.58	5514
237	67.14	37.56	860
238	67.34	70.56	2652
239	67.95	53.73	2602
240	69.16	82.02	5583
241	69.66	84.60	5423
242	69.76	75.41	253
243	70.94	81.22	397
244	70.96	78.95	5508
245	71.16	66.87	2752
246	74.64	74.06	260

247	75.13	54.74	4413
248	76.07	63.95	4060
249	76.39	63.98	2603
250	77.23	84.47	5343
251	78.37	90.37	1760
252	78.69	96.77	5575
253	79.20	63.59	4561
254	79.60	75.48	5591
255	79.95	81.01	209
256	80.80	84.54	263
257	82.25	79.49	5768
258	82.38	63.61	3968
259	82.79	86.62	981
260	82.92	102.21	5592
261	83.74	74.75	3985
262	85.50	68.20	3964
263	85.51	74.33	309
264	87.06	109.87	1440
265	87.57	65.61	5336
266	87.89	83.12	432
267	89.31	74.03	2655
268	89.52	82.54	1441
269	90.37	91.60	2458
270	91.65	70.58	2605
271	92.03	103.69	5771
272	94.04	67.76	4562
273	97.08	99.70	985
274	98.19	87.94	1843
275	99.42	78.43	598
276	99.94	106.80	919
277	101.80	92.03	255
278	107.04	61.69	2449
279	110.97	85.12	783
280	112.95	77.68	3993
281	113.99	85.41	40
282	241.69	259.71	5509
siNTC*	107.21	84.35	not applicable

*: indicates the non-target control siRNA (siNTC) sequence.

[00247] The selected 141 siRNA sequences from the *in-silico* screen were synthesized and were further evaluated *in vitro* in HEK293 cells expressing the human Nav1.8. The HEK293-hNav1.8 cells were transfected with Nav1.8 siRNA sequences using lipofectamine. Of the 141 siRNA sequences tested in HEK293-hNav1.8 cells, 52 siRNA sequences induced greater than 50% reduction in Nav1.8 mRNA levels (%hNav1.8 mRNA <51.2%) at 10 nM or 1 nM

(see Table 15). The siRNA sequence siRNA No. 142 (start position of target region on human SCN10A transcript at 407) had the best activity in decreasing Nav1.8 mRNA levels with more than 85% decrease in Nav1.8 mRNA levels at 10 nM (%hNav1.8 mRNA = 17.8%).

[00248] In the HEK293-hNav1.8 cells line, it was shown that Nav1.8 mRNA levels can be down regulated by Nav1.8 siRNA sequences. Although 141 19-mer siRNA sequences were identified the *in-silico* screen, 52 of the tested Nav1.8 siRNA sequences showed greater than 50% inhibitory activity at 10 nM or 1 nM (%hNav1.8 mRNA <51.2%).

Example 5: Nav1.8 siRNAs Concentration-dependent Activity in HEK293 Expressing Human Nav1.8.

[00249] HEK293 cells expressing the human Nav1.8 transcript (HEK293-hNav1.8) were used to determine the *in vitro* hNav1.8 mRNA reduction and the half-maximal inhibitory concentration (IC₅₀) of the selected lead siRNA sequences. The cells were grown in DMEM (Gibco, Billings, MT) supplemented with 10% of heat-inactivated fetal bovine serum (FBS; Corning, Corning, NY), 1% Pen/Strep (Gibco), 0.1 mg/mL of Zeocin™ (Gibco), and 0.5 µg/mL of Puromycin (ThermoFisher, Waltham, MA). For transfections, HEK293-hNav1.8 cells were seeded at 15,000 cells/well in 100 µL in 96-well tissue culture plates and transfected within 24 hours post-seeding. The siRNAs were gently mixed with Lipofectamine™ RNAiMAX transfection reagent (ThermoFisher) added to the wells to a final concentration ranging from 67-0.27 nM according to manufacturer's recommendation.

[00250] Treated cells were kept at 37 °C in 5% CO₂ for 72 hours, washed in phosphate buffer saline (PBS, ThermoFisher), and stored in 300 µL of TRIzol™ (ThermoFisher) at -80 °C until RNA extraction. The total mRNA was isolated using the ZYMO 96-well RNA kit (Zymo Research, Irvine, CA), and 100-250 ng of purified mRNA was used to generate the cDNA with the iScript cDNA synthesis kit (BioRad, Hercules, CA).

[00251] RNA expression levels were determined by RT-qPCR using commercially available TaqMan probes (Life Technologies; *SCN10A*: Hs01045150_m1 and *PPIB*: Hs00168719_m1). The relative reduction in Nav1.8 transcripts was determined by normalizing the expression of siRNA treated HEK-hNav1.8 cells to the mock-transfected group using the DDCT method with the following formula: [%hNav1.8 mRNA = 100*2^{-DCt (siRNA treated cells)-DCt (mock-transfected cells)}], with DCt determined by the difference in Ct values between SCN10A and the housekeeping gene PPIB [DCt=Ct(SCN10A)-Ct(PPIB)] (Green MR, Sambrook J." Quantification of RNA by Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)," *Cold Spring Harb Protoc.* 2018 Oct, 2018(10): pdb.prot095042;

and Livak KJ, Schmittgen TD, "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2- $\Delta\Delta$ CT Method," *Methods*, 25(4), 402-8 (2001)).

[00252] Table 16 summarizes the screening results, showing the Nav1.8 targeting siRNA sequences ordered based on the siRNA gene starting position of the *SCN10A* reference transcript sequence (NM_006514.3). The IC50 and percentage of maximum hNav1.8 knockdown (%maxKD) target mRNA were calculated by fitting the siRNA concentration-response data into a 3-parameter non-linear regression fitting model [log(concentration of siRNA) vs. %hNav1.8 mRNA] using the GraphPad Prism software (GraphPad Software, LLC). The maximum knockdown (maxKD) was calculated by the following formula: %maxKD=100-hNav1.8 eMax, with eMax representing the predicted lowest value of the fitted concentration-response curve (Bottom-value of the 3-parameter non-linear regression curve). The *in-silico* predictions of siRNAs with expected activity against mouse and or rat Nav1.8 mRNA based upon an analysis of the *in-silico* predictions are also included with the *in vitro* results.

Table 16: Nav1.8 siRNAs Summary of the Concentration-dependent Response Assays in HEK293-hNav1.8 Cells. The siRNAs are modified as shown in Figure 1A but without the amino/cholesterol handle.

siRN A No.	%hNav1.8 mRNA eMax	%maxKD (100- hNav1.8 mRNA eMax)	IC50 (nM)	R ²	<i>in- silico</i> predict ed rodent active	Start position of target region on human SCN10A transcript
170	36.23	63.77	0.56	0.92	X	43
146	41.18	58.82	1.36	0.93		349
152	33.96	66.04	0.28	0.97		361
142	24.85	75.15	0.13	0.98		407
145	20.27	79.73	0.2	0.98		410
144	31.68	68.32	0.37	0.92		426
162	38.46	61.54	0.22	0.92		427
158	29.01	70.99	1.01	0.96	X	434
157	38.71	61.29	0.66	0.81	X	435
154	30.76	69.24	0.18	0.97		532
167	32.96	67.04	0.28	0.98		535
147	28.17	71.83	0.26	0.96		537
150	34.4	65.6	0.35	0.97		538
163	34.67	65.33	0.25	0.97		576
161	41.09	58.91	0.66	0.97		584
171	36.4	63.6	0.26	0.97		856
169	42.56	57.44	1.07	0.94		858

237	37.78	62.22	0.19	0.97		860
153	29.41	70.59	0.73	0.98	X	983
149	28.78	71.22	0.37	0.97	X	986
166	41.74	58.26	0.71	0.94	X	987
151	40.52	59.48	0.46	0.94	X	989
148	38.88	61.12	0.23	0.92	X	990
143	28.8	71.2	0.7	0.85		1172
159	54.55	45.45	0.63	0.81		1442
168	43.09	56.91	0.47	0.73		1787
164	43.01	56.99	0.68	0.75		1790
155	52.93	47.07	1.49	0.72		1794
165	36.8	63.2	0.33	0.82		2063
160	35.01	64.99	1.24	0.8		2071
156	37.01	62.99	0.57	0.93		2073
siNT C	93.54	6.46	ND	<0.1		

Legend: ND means not determined; X indicates expected activity against mouse and/or rat Nav1.8 mRNA based on the *in-silico* analysis.

[00253] Based on the activities of the siRNA sequences from the *in vitro* screening in HEK cells as shown in Table 15, the 31 siRNA sequences with the highest inhibitory activity (lowest %hNav1.8 mRNA) at 10 nM or 1 nM were further assayed in concentration-dependent response assays in the HEK293-hNav1.8 cell line (top 30 rows from Table 15 plus siRNA No. 237 sequence (start position of 860)) to determine the maximum Nav1.8 transcript reduction and the siRNA half-maximal inhibitory concentration.

[00254] As shown in Table 16, most siRNA sequences induced greater than 50% decrease in Nav1.8 mRNA levels. The Nav1.8 siRNA No. 145 sequence (start position of 410) had the highest maxKD of 80%, and the Nav1.8 siRNA No. 142 sequence (start position of 407) had the lowest IC₅₀ with 0.13. Overall, the 31 Nav1.8 siRNA sequences had a maxKD average of 63.8% (std.dev=7.3%) and an average IC₅₀ of 0.54 nM (std.dev=0.37 nM). As expected, the siNTC negative control had a negligible effect, with a poor non-linear regression R-square ($R^2 < 0.1$). A summary of the 3-parameter non-linear regression is also shown in Table 16.

[00255] The selected siRNA sequences showed *in vitro* activities and potencies in the HEK293-hNav1.8 cell line, with 7 siRNA sequences reducing Nav1.8 transcript expression levels by more than 70%. Regarding the IC₅₀s, 26 of the 31 Nav1.8 siRNAs had activities in the subnanomolar range (<1 nM), with the remaining siRNA sequences in the low single-digit nanomolar range (<2 nM).

Example 6: Evaluation of Off Target Effects of Nav1.8 siRNAs.

[00256] Bulk RNA-seq analysis was performed on human skeletal myotubes (Institute of Myology, France), grown, and differentiated using respective medias from Promocell (Heidelberg, Germany). After differentiation, cells were treated with 50 nM LNP-siRNA negative control (siNTC) or Nav1.8 siRNAs. The tested siRNAs are: siRNA No. 283 of Table 4 (start position of 407), siRNA No. 285 of Table 4 (start position of 410), siRNA No. 291 of Table 4 (start position of 535) and siRNA No. 301 of Table 4 (start position of 1172)) for 24 hours (n=4, per LNP-siRNA). The modification pattern of the siRNA sequences is described in Table 4.

[00257] Total RNA was isolated using TRIzol™ (ThermoFisher™) followed by chloroform extraction and isopropanol precipitation for the QIAGEN RNeasy® mini kit following the manufacturer's recommendations (QIAGEN, Hilden Germany). Isolated RNA was DNaseI (QIAGEN) treated on-column and quantified via Nanodrop (ThermoFisher) after resuspension, using the 260/280nm ratio. cDNA libraries were prepared with Poly(A)+ selection. Sequencing reads were normalized to Transcripts Per Kilobase Million (TPM) for downstream analyses.

[00258] The siRNA sequences siRNA No. 283, siRNA No. 285, siRNA No. 291 and siRNA No. 301 were selected for off-target analysis in human skeletal myotube cells. Bulk RNA-seq analysis was performed and differentially expressed (DE) genes were identified using the DESeq2 package for R and a P-Adjusted value < 0.05 (Love MI, et al., "Moderated Estimation of Fold Change and Dispersion for RNA-seq Data with DESeq2," *Genome Biol.* 15(12):550 (2014)). The siNTC treated cells were used as the baseline comparison condition. The number of DE genes with a log2 fold-change (FC) greater than 2 are reported in Table 17.

[00259] Lipid nanoparticle delivery of the 4 Nav1.8 siRNA sequences *in vitro* to human skeletal myotubes showed a negligible to small downregulation of SCN10A non-related genes. The siRNA No. 291 (start position of 535) showed the highest undesired off-target profile (total of 14 DE genes, with a log 2 FC over siNTC greater than 2) while the siRNA No. 285 (start position of 410) sequence showed the lowest off target effects (no DE genes with a log 2 FC over siNTC greater than 2).

Table 17: Off-target Analysis of Human Skeletal Myotubes Treated with Nav1.8 siRNAs.

Cell type	Nav1.8 siRNA No.	Number of upregulated genes	Number of downregulated genes	Start position of target region on human SCN10A transcript
	283	0	1	407
	285	0	0	410

Human skeletal myotubes	291	0	14	535
	301	0	8	1172

[00260] Overall, all lead Nav1.8 siRNAs showed little to negligible off-target profile, with sequence siRNA No. 285 (start position of 410) showing the best overall performance *in vitro*, with a negligible off-target profile after 24 hours LNP-siRNA treatment.

Example 7: Activity of Lead Nav1.8-siRNAs in DRG Neurons.

[00261] Human neurons expressing functional Nav1.8 mRNA were used to confirm the activity of lead Nav1.8 siRNAs. The top 14 Nav1.8 siRNA sequences with the highest %maxKD and/or the lowest IC50s were selected for further evaluation. An additional 5 siRNA sequences were selected based on the *in-silico* predicted species cross-reactivity to mouse Nav1.8 mRNA. An extra siRNA sequence, siRNA No. 205 sequence of Table 3 (start position of 409), was selected based on its high sequence similarity to the siRNA No. 142 of Table 3 (start position of 407) and siRNA No. 145 of Table 3 (start position of 410). Thus, a total of 20 siRNA sequences were prioritized for synthesis, using the modification pattern as illustrated in Figure 1A, with a 5'-end cholesterol handle in the passenger strand to allow for efficient transfection in primary human neuron cells without the need of a lipofectamine reagent.

[00262] Human dorsal root ganglia neurons (DRGs) were used as a source of primary cells expressing functional Nav1.8 mRNA and protein. Fresh primary human DRGs were purchased from AnaBios (San Diego, CA) and maintained in serum-free NbActive4 neural maintenance medium (Axol, Easter Brush, United Kingdom) with 25 ng/mL of recombinant human NGF (Axol). Cholesterol-conjugated siRNAs (Chol-siRNA) were introduced to growth medium at 1 μ M twice (day 0 and day 3, for a total of 5 days) as-is, with no transfection reagent.

[00263] Treated human DRGs were kept at 37 °C in 5% CO₂ for 5 days, followed by a wash in phosphate-buffer saline (PBS, ThermoFisher), and stored in 300 μ L of TRIzol™ (ThermoFisher) at -80 °C until RNA extraction. The total mRNA was isolated using the ZYMO 96-well RNA kit (Zymo Research, Irvine, CA), and 100-250 ng of purified mRNA was used to generate the cDNA with the iScript cDNA synthesis kit (BioRad, Hercules, CA).

[00264] RNA expression levels were determined by quantitative reverse transcription polymerase chain reaction (RT-qPCR) using commercially available TaqMan probes (Life Technologies; *SCN10A*: Hs01045150_m1, *STMN2*: Hs00975900_m1, and *SNAP25*:

Hs00938957_m1). The relative reduction in Nav1.8 transcripts was determined by normalizing the expression of siRNA treated DRGs to the mock-transfected group using the DDCT method using the following formula: $[\%hNav1.8 \text{ mRNA} = 100 * 2^{-\text{DCt}(\text{siRNA treated cells}) - \text{DCt}(\text{mock-transfected cells})}]$, with DCt determined by the difference in Ct values between *SCN10A* and the housekeeping genes *STMN2* and *SNAP25* $\{\text{avg DCt} = [(2 * \text{Ct}(\text{SCN10A})) - \text{Ct}(\text{STMN2}) - \text{Ct}(\text{SNAP25})] / 2\}$ (Green MR, Sambrook J. “Quantification of RNA by Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR),” *Cold Spring Harb Protoc.* 2018 Oct, 2018(10): pdb.prot095042; and Livak KJ, Schmittgen TD, “Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2- $\Delta\Delta\text{CT}$ Method,” *Methods*, 25(4), 402–8 (2001)).

[00265] Figure 4 summarizes the results in human DRGs, showing the Nav1.8 targeting cholesterol modified siRNA sequences ranked based on the *in vitro* activity at the 1 μM concentration of siRNA. The %hNav1.8 mRNA represents the relative expression of human Nav1.8 mRNA remaining after siRNA treatment compared to mock-transfected control cells. The number below each bar on the bar graph indicates the starting siRNA sequence position based on the *SCN10A* reference transcript sequence (GenBank: NM_006514.3). The non-target cholesterol modified siRNA sequence (siNTC) was used as a transfection control to account for unspecific mRNA reduction. A commercially available Accell cholesterol modified Nav1.8 siRNA pool was used as positive control (Horizon Discovery Ltd, Waterbeach, UK). An ordinary one-way ANOVA with Dunnett’s multiple comparison test was used to determine the adjusted *P*-values, using the software tool GraphPad Prism (GraphPad Software, LLC).

[00266] From the *in vitro* assay with the HEK293-hNav1.8 cells, 14 Nav1.8 siRNA sequences with the highest %maxKD and or lowest IC50 values and 6 additional siRNA sequences from in the *in-silico* screen were further selected for synthesis and modified with cholesterol. These modified cholesterol siRNA sequences were further assayed in *in vitro* using human neurons expressing functional Nav1.8 mRNA transfected with cholesterol modified Nav1.8 siRNA sequences. As shown in Figure 4, the selected Nav1.8 cholesterol modified siRNA sequences decreased Nav1.8 mRNA levels in human DRGs at the siRNA concentration of 1 μM . Out of the 20 siRNA sequences tested in primary human DRGs, 10 siRNA sequences had at least 50% reduction in the levels of *Nav1.8* transcripts in human primary DRGs when compared to the siNTC sequence (*P*-adjusted value <0.05). The siRNA No. 285 sequence (start position of 410) had the best activity in decreasing Nav1.8 mRNA levels in in human primary DRGs.

[00267] Out of the 18 siRNA sequences identified in the preliminary screen in the HEK293-hNav1.8 cell line (Table 14), 10 showed significant Nav1.8 transcript reduction in primary human DRG cell cultures with cholesterol-modified siRNAs. Sequences siRNA No. 285 (start position of 410), siRNA No. 291 (start position of 535) and siRNA No. 301 (start position of 1172) showed consistent high inhibitory activity *in vitro*, with an overall reduction of Nav1.8 mRNA equal or greater than 70%.

Example 8: Concentration-response of Selected Lead Nav1.8 siRNAs in Human DRGs Show IC50 in the Subnanomolar Range.

[00268] To further characterize the activity and potency of the top 3 Nav1.8 siRNAs in human DRGs, a concentration-response assay was performed *in vitro*. The 3 siRNA sequences that showed the highest activity in primary neurons were tested. The tested siRNAs are: siRNA No. 285 of Table 4 (start position of 410), siRNA No. 291 of Table 4 (start position of 535), siRNA No. 301 of Table 4 (start position of 1172), and siRNA No. 283 of Table 4 (start position of 407). The siRNAs were encapsulated in Lipid Nano Particles.

[00269] Human dorsal root ganglia neurons (DRGs) were used as a source of primary cells expressing Nav1.8 mRNA. Fresh primary human DRGs obtained were purchased from AnaBios (San Diego, CA) and maintained in serum-free NbActive4 neural maintenance medium (Axol, Easter Brush, United Kingdom) with 25 ng/mL of recombinant human NGF (Axol). Primary DRGs were treated with siRNA encapsulated in Lipid Nano Particles (LNP, Precision Nanosystems, Vancouver, Canada) following manufacturer's recommendation. LNP-siRNAs were introduced to growth medium at 150 – 0.008 nM for 3 days as-is, with no transfection reagent. The modification patterns of the siRNA sequences are described in Table 4.

[00270] Treated human DRGs were kept at 37 °C in 5% CO₂ for 3 days, followed by a wash in phosphate-buffer saline (PBS, ThermoFisher), and stored in 300 µL of TRIzol™ (ThermoFisher) at -80 °C until RNA extraction. The total mRNA was isolated using the ZYMO 96-well RNA kit (Zymo Research, Irvine, CA), and 100-250 ng of purified mRNA was used to generate the cDNA with the iScript cDNA synthesis kit (BioRad, Hercules, CA).

[00271] RNA expression levels were determined by quantitative reverse transcription polymerase chain reaction (RT-qPCR) using commercially available TaqMan probes (Life Technologies; *SCN10A*: Hs01045150_m1, *STMN2*: Hs00975900_m1, and *SNAP25*: Hs00938957_m1). The relative reduction in Nav1.8 transcripts was determined by normalizing the expression of siRNA treated DRGs to the mock-transfected group using the DDCT method using the following formula: [%hNav1.8 mRNA = 100*2^{-DDCT (siRNA treated cells)-}

DCt (mock-transfected cells)], with DCt determined by the difference in Ct values between *SCN10A* and the housekeeping genes *STMN2* and *SNAP25* {avg DCt=[((2*Ct(SCN10A))-Ct(STMN2)-Ct(SNAP25))/2]} (Green MR, Sambrook J. “Quantification of RNA by Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR),” *Cold Spring Harb Protoc.* 2018 Oct, 2018(10): pdb.prot095042; and Livak KJ, Schmittgen TD, “Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method,” *Methods*, 25(4), 402–8 (2001)).

[00272] The IC50 and percentage of maximum hNav1.8 knockdown (%maxKD) target mRNA were calculated by fitting the siRNA concentration-response data into a 3-parameter non-linear regression fitting model [log(concentration of siRNA) vs. %hNav1.8 mRNA] using the GraphPad Prism software (GraphPad Software, LLC). The maximum knockdown (maxKD) was calculated by the following formula: %maxKD=100-hNav1.8 eMax, with eMax representing the predicted lowest value of the fitted concentration-response curve (Bottom-value of the 3-parameter non-linear regression curve).

[00273] As shown in Figure 5, the concentration-response curves of the lead top 3 siRNA sequences and siRNA No. 283 showed similar maximum Nav1.8 mRNA reduction in human DRGs, with greater than 75% target transcript reduction for all tested sequences in the LNP format. Nav1.8 siRNA IC50s were in the subnanomolar (<1 nM) range to 3 out of the 4 sequences tested (siRNA No.’s 285, 291 and 283), with siRNA No. 301 in the single digit nanomolar range (<2nM) (see Table 18).

Table 18: Summary Results of the Concentration-response Assay in Primary Human DRGs.

siRNA No.	% hNav1.8 mRNA eMax	%maxKD (100-hNav1.8 mRNA eMax)	IC50 (pM)	Start position of target region on human SCN10A transcript
285	7.15	92.85	71	410
291	10.03	89.97	48	535
283	10.81	89.19	990	407
301	23.33	76.67	1747	1172

[00274] All tested Nav1.8 siRNA sequences significantly reduced Nav1.8 mRNA in human primary DRGs by more than 70%. The siRNA No. 285 showed the highest activity, with a transcript reduction greater than 90% and an IC50 of 71 pM.

Example 9: Downregulation of SCN10A Transcripts Using siRNA-LNP Leads to Reduction in Nav1.8 Protein Expression in Human Primary Neurons.

[00275] Test siRNA sequences (i.e., siRNA No. 283 of Table 4 (start position of 407) and siRNA No. 285 of Table 4 (start position of 410)) were tested for their capability to reduce the expression of Nav1.8 protein in human primary neurons. The modification patterns of the siRNA sequences are described in Table 4.

[00276] Human dorsal root ganglia neurons (DRGs) were used as a source of primary cells expressing Nav1.8 mRNA. Fresh primary human DRGs were purchased from AnaBios (San Diego, CA) and maintained in serum-free Nucleoside Active4 neural maintenance medium (Axol, Easter Brush, United Kingdom) with 25 ng/mL of recombinant human NGF (Axol). Primary DRGs were treated with the test siRNA encapsulated in Lipid Nano Particles (LNP, Precision Nanosystems, Vancouver, Canada) following the manufacturer's recommendation. LNP-siRNAs were introduced to growth medium at 0.5 µg/mL twice for 6 days, with no transfection reagent.

[00277] Treated human DRGs were kept at 37 °C in 5% CO₂ for 6 days, followed by a wash in phosphate-buffer saline (PBS, ThermoFisher), and stored in 300 µL of TRIzol (ThermoFisher) at -80 °C until RNA extraction. The total mRNA was isolated using the Zymo 96-well RNA kit (Zymo Research, Irvine, CA), and 100-250 ng of purified mRNA was used to generate the cDNA with the iScript cDNA synthesis kit (BioRad, Hercules, CA).

[00278] RNA expression levels were determined by quantitative reverse transcription polymerase chain reaction (RT-qPCR) using commercially available TaqMan probes (Life Technologies; *SCN10A*: Hs01045150_m1, *STMN2*: Hs00975900_m1, and *SNAP25*: Hs00938957_m1). The relative reduction in Nav1.8 transcripts was determined by normalizing the expression of siRNA treated DRGs to the mock-transfected group using the DDCT method using the following formula: $[\% \text{hNav1.8 mRNA} = 100 * 2^{-\text{DCt}(\text{siRNA treated cells}) - \text{DCt}(\text{mock-transfected cells})}]$, with DCt determined by the difference in Ct values between *SCN10A* and the housekeeping genes *STMN2* and *SNAP25* $\{\text{avg DCt} = ((2 * \text{Ct}(\text{SCN10A})) - \text{Ct}(\text{STMN2}) - \text{Ct}(\text{SNAP25})) / 2\}$ (Green MR, Sambrook J. "Quantification of RNA by Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)," *Cold Spring Harb Protoc.* 2018 Oct, 2018(10): pdb.prot095042; and Livak KJ, Schmittgen TD, "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method," *Methods*, 25(4), 402–8 (2001)).

[00279] ELISA was used to measure the Nav1.8 protein concentration in cultured human DRG neuron protein lysate. Growth media was aspirated off and the cultured human DRG neurons were washed once in Dulbecco's phosphate buffered saline (DPBS; Gibco, Cat #14190-136). Whole cell lysates were prepared by suspending cultured human DRG neurons

in 100 μ L/well of the 1X lysis Buffer (CST, Cat #9803) along with Halt (Protease and Phosphatase Inhibitor (TFS, Cat #78440) diluted to 2X and PMSF (Sigma, #78830-25G, (17.4 mg in 1 mL isopropanol, diluted to 1X). Lysis was incubated on ice for five minutes, then centrifuged at 4 °C at 800 x g for 20 minutes. Supernatant was collected and frozen at -80 °C for analysis the next day. Protein concentration was measured with the Pierce™ BCA Protein Assay Kit (Cat #23227) by following the “Microplate Procedure” described in the manufacturer's manual. Samples were further diluted 1:3 in cell lysis buffer before plating. The Meso Scale Discovery (MSD) S-Plex platform was used to detect Nav1.8 protein levels in the cultured human DRG neuron protein lysate. The custom developed assay utilized S-PLEX Development Pack B, SECTOR (25 Plate) (Cat # K15601S-4) kit. Capture and detection antibodies were tagged by MSD. Capture antibody (NeuroMab, Cat #75-166) was diluted to 0.5 μ g/mL and a detection antibody was diluted to 0.2 μ g/mL. Calibrator was a recombinant C-terminal fragment of human Nav1.8. Sample lysates were diluted 4-fold in assay buffer. Assay buffer base was Diluent 39 (MSD, Cat# R5ABB2) with 100 μ L each of the components included in the MSD® Inhibitor Pack (Cat #R70AA-1). Washing steps utilized MSD Tris Wash Buffer (MSD, Cat #R61TX-1) diluted to 1X in double-distilled water (ddH₂O). Plates were read using an MSD plate reader MESO QuickPlex SQ 120MM with MSD Methodical Mind software. Data was analyzed using Discovery Workbench v4. Data was normalized by dividing the Nav1.8 protein concentration by the total protein concentration for each lysate sample. Statistical significance was determined by One-way ANOVA.

[00280] *Results:* The siRNA sequences siRNA No. 283 and siRNA No. 285 having a minimal off-target profile were further assayed *in vitro* in human DRGs transfected as the Lipid Nano Particle (LNP) siRNA sequences. Nav1.8 mRNA levels were determined by qPCR (Figure 6A) and Nav1.8 protein levels were determined by ELISA (Figure 6B). Negligible transcript regulation was observed with the LNP-siNTC negative control sequence, with an approximately 30% reduction in Nav1.8 protein expression when the assay is normalized by the total protein amount. No difference between the untreated and siNTC group was observed when Nav1.8 protein expression was corrected by CGRP protein expression (human DRG specific protein). The Nav1.8 LNP-siRNAs downregulated SCN10A transcripts by more than 90%. More importantly, reduction in Nav1.8 mRNA levels was correlated by more than 80% inhibition in protein expression levels, independent of the normalization method (total protein loading or human DRGs normalization).

[00281] Nav1.8 mRNA and protein expression are highly correlated in human primary DRGs *in vitro*. Lead siRNA sequences siRNA No. 283 and siRNA No. 285 showed great mRNA and protein reduction *in vitro* following treatment with LNP-Nav1.8 siRNAs. Nav1.8 protein reduction in the Nav1.8 siRNA treated groups, was independent of the protein normalization method. Confirming the linked association between Nav1.8 mRNA regulation and protein translation in human DRGs *in vitro*.

Example 10: Expression and Purification of Transferrin Receptor Antibody (TfR).

[00282] The exemplified anti-TfR antibodies and antibody fragments of the present disclosure were expressed and purified as follows. Antibodies TBP1, TBP2, and TBP3 (as shown in Table 8 above) are expressed in an appropriate host cell, such CHO cells, either transiently or stably transfected with an expression system for secreting the TBP1 antibody or TBP3 antibody using an optimal predetermined HC:LC vector ratio or a single vector system encoding both HC and LC.

[00283] For TBP2 antibody, an optimal predetermined HCA:HCB:LC vector ratio or a single vector system encoding HCA, HCB, and LC is used. The expression plasmids contain cDNA versions of the LC and HC genes for antibody TBP1, TBP2, or TBP3; and are expressed from a commonly used and suitable construct for this purpose, such as one based on human cytomegalovirus major immediate early promoters.

[00284] Medium, into which the antibody was secreted, may be purified by conventional techniques, such as mixed-mode methods of ion-exchange and hydrophobic interaction chromatography. For example, the medium containing TBP1 antibody or TBP2 antibody may be applied to and eluted from a Protein A column (Cytiva) using conventional methods. Medium containing TBP3 antibody may be applied to and eluted from a CaptureSelect™ CH1-XL column (Thermo Scientific™) using conventional methods.

[00285] Soluble aggregate, multimers, and fragments may be effectively removed by cation exchange chromatography using a POROS™ HS 50 column (Thermo Scientific™) using conventional methods. The product may be immediately frozen, for example at -70 °C, refrigerated, or may be lyophilized. Various methods of protein purification may be employed, and such methods are known in the art and described, for example, in Deutscher, Methods in Enzymology 182: 83-89 (1990) and Scopes, Protein Purification: Principles and Practice, 3rd Edition, Springer, NY (1994). Antibodies TBP1, TBP2 or TBP3 may be immediately frozen at -70 °C or stored at 2-8 °C for several months, or may be lyophilized, or preserved in 4 °C for immediate use. Amino acid sequences for the antibodies of the present

disclosure are shown in Tables 5-8. The nucleic acid sequences for the antibodies are provided in Table 9.

Example 11: Method of Making Native Cysteine (nCys) Conjugated Antibody Oligonucleotide Conjugates (AOCs)

[00286] Nav1.8-AOC (Figure 7) is an antibody-siRNA conjugate (also referred to herein as antibody oligonucleotide conjugate (AOC)) formed by the conjugation of a human IgG1 antibody specific to the human transferrin receptor 1 (anti-TFR1 antibody) to a double stranded siRNA oligonucleotide (Nav1.8 siRNA) targeting Nav1.8 mRNA. Any of the antibodies provided in Tables 5 to 8 may be used in the AOCs and conjugated to any one of the siRNAs provided in Tables 2 to 4. The conjugation may be done via a linker, e.g., a SMCC linker.

[00287] A succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) maleimide linker is located on 5' end of the passenger strand and it is conjugated to the antibody through one of the cysteines in the antibody amino acid sequence. The conjugate binds human transferrin receptor on the cell surface, internalizes into the cell and delivers the siRNA oligonucleotide to the intracellular compartment. Upon uptake by the cells, the siRNA loads into the RNA-induced silencing complex (RISC) and hydrolyses the intracellular Nav1.8 mRNA.

Step 1: Antibody interchain disulfide reduction with TCEP

[00288] Antibody (anti-human transferrin receptor 1, TBP1) was formulated in phosphate buffered saline (PBS) pH 7.4 and adjusted to 2 mM ethylenediamine tetra acetic acid (EDTA). To this solution, 2 equivalents (EQ) of tris(2-carboxyethyl)phosphine (TCEP) in water was added and rotated for 4 hours at room temperature (RT). To the resultant reaction mixture was added a solution of 4-(N-maleimidomethyl)cyclohexane-1-carboxylic acid (MCC)-siRNA (0.9 EQ) in pH 6 10 mM sodium acetate at RT and rotated for 1 hour. Analysis of the reaction mixture by analytical strong anion exchange (SAX) column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA (Figure 8). The reaction mixture was treated with 10 EQ of N-ethylmaleimide (in dimethyl sulfoxide (DMSO) at 10 mg/mL) to cap any remaining free cysteine residues.

Step 2: Purification

[00289] The crude reaction mixture was purified by ÄKTA explorer fast purification liquid chromatography (FPLC) using anion exchange chromatography (Figure 9). Fractions containing DAR1 siRNA conjugates were separated, concentrated, and formulated with pH 7.4 PBS.

FPLC Method 1 Details

Column: HiScale™ 50/20 column, TSKgel® SuperQ-5PW resin

Solvent A: 20 mM phosphate buffer, pH 7.2

Solvent B: 20 mM phosphate buffer, 1.5M NaCl, pH 7.2

Gradients used (%A/%B): 90:10; 81:19; 68:32

Volumes used: 0.5, 0.2, 6

Step 3: Analysis of the Purified Conjugate

[00290] The isolated conjugates were characterized by size exclusion (SEC) and SAX chromatography. The purity of the conjugate was assessed by analytical high pressure liquid chromatography (HPLC) with isolated DAR1 conjugates being greater than 90% pure (Figures 10 and 11).

SAX Method 1 Details

Column: Thermo Scientific, PropPac™ SAX-10, Bio LC, 4x250 mM

Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol

Solvent B: Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl

Flow Rate: 0.9 mL/minute

Gradients (%A/%B): 84:16; 60:40; 46.5:53.5; 40:60; 84:16; 84:16

Time associated to gradients: 0, 2, 4, 4.5, 5, 6.5

SEC Method 1 Details

Column: Waters™ Bioresolve SEC mAb, 2.5 µm, 4.6x150 mm

Solvent A: 55 mM KH₂PO₄, 62 mM Na₂HPO₄·7H₂O, 100 mM Na₂SO₄, 0.05% w/v NaN₃, pH 6.7

Flow Rate: 0.4 mL/minute

Isocratic (%A): 100

Time associated to isocratic: 0, 5.5

Example 12: Method of Generating of Engineered Cysteine (eCys) Conjugated AOCs.

[00291] Site-specific native or engineered cysteine amino acid residues in the antibody molecule were used to conjugate dsRNA (siRNA). Cysteines can be engineered into the primary amino acid sequence of the TfR binding proteins (TBPs) disclosed herein. The approach of introducing cysteines as a means for conjugation has been described in WO 2018/232088, which is both incorporated by reference in its entirety and incorporated specifically in relation to conjugation via cysteine residues. For engineered cysteine conjugation, the TfR binding proteins were first reduced with 10 molar equivalents reducing agent tris(2-carboxyethyl)phosphine (TCEP) at room temperature for two hours, followed by

buffer exchange by, for example, tangential flow filtration (TFF) to remove reduction reagent. For TBP2, this is followed by re-oxidation of the TfR binding protein to reform the interchain disulfides with 20 molar equivalent dehydroascorbic acid (DHAA) incubation at room temperature for two hours. Buffer exchange was performed again to remove oxidizing agent. For TBP3, the reoxidation step was omitted as scaffold does not contain interchain disulfide bonds.

[00292] Conjugation of dsRNA onto TfR binding proteins were done using the SMCC-functionalized dsRNA. The prepared TBP above was incubated with 0.9-1.0 molar equivalents of the SMCC-dsRNA for conjugation for 1-2 hours at room temperature. Conjugation was monitored using analytical anion exchange chromatography (AEX). A ProPac™ SAX-10 HPLC Column, 10 µm particle, 4 mm diameter, 50 mm length (Thermo Scientific™) was utilized with the following method. Flow rate of 1 mL/min, Buffer A: 20 mM TRIS pH 7.0, Buffer B: 20 mM TRIS pH 7.0 + 1 M NaCl, at 30 °C. The mobile phase gradient is shown in Table 19. Drug antibody/protein ratio (DAR) was calculated based on peak area % from the analytical anion exchange (aAEX) chromatogram.

Table 19 : HPLC Gradient used to Determine dsRNA Conjugation to TfR Binding Protein

Time [min]	A [%]	B [%]
0.00	85.0	15.0
8.00	0.0	100.0
9.00	0.0	100.0
9.10	85.0	15.0
10.00	85.0	15.0

Example 13: *In vitro* Stability of Antibody Oligonucleotide Conjugates (AOCs).

[00293] Stability of the native cysteine conjugated bivalent mAb (TBP1), the engineered cysteine conjugated bivalent mAb (TBP2), and engineered cysteine conjugated monovalent Fab (TBP3) DAR1 AOCs were assessed *in vitro*. The siRNA portion of the AOC was modified as shown in Figure 1B with the amine handle.

[00294] Samples were prepared at 1 mg/mL protein concentration in phosphate buffered saline (PBS) pH 7.2, then held at 4 °C and 40 °C for 2 weeks. Samples were analyzed by analytical size exclusion chromatography (SEC), AEX (as described in Example 12), and reduced and non-reduced CE-SDS. SEC was performed using an Acquity UPLC Protein BEH SEC 200Å 1.7µm particle size, 4.6mm diameter, 150mm length column (Waters™). Mobile

phase was 50 mM sodium phosphate, 0.3 M NaCl, 0.005% sodium azide, pH 6.8, and elution was isocratic at 0.3 mL/min for an 8-minute run time. Reduced CE-SDS was performed using a Maurice (BioTechne) following the manufacturer's protocol. Non-reduced CE-SDS was performed with a Labchip® GXII (PerkinElmer) following the manufacturer's protocol.

[00295] Results are shown in Table 20. Across all assays, the native cysteine conjugated TBP1-410 AOC showed decreased stability relative to the engineered cysteine conjugated TBP2-410 AOC. The monovalent Fab engineered cysteine AOC (TBP3-410 AOC) shows comparable stability relative to the bivalent mAb engineered cysteine AOC.

[00296] AOCs were analyzed by non-reduced and reduced SDS-PAGE to assess covalent assembly (Figure 12). NuPAGE™ 4-12% Bis-Tris gels, LDS sample buffer, and MES SDS running buffer were used (ThermoFisher Scientific). Mark12™ molecular weight marker (MWM) was used as a standard. Non-reduced samples were analyzed with and without alkylation by iodoacetamide (IAM) to confirm that the apparent fragmentation under denatured conditions was not a result of artifact reduction during SDS-PAGE sample preparation.

[00297] Multiple fragments are observed for the TBP1-410 AOC with and without IAM alkylation corresponding to light chain, heavy chain, half-antibody, heavy chain dimer, heavy chain dimer + light chain, and intact antibody are observed for the TBP1-410 while the TBP2-410 AOC is observed as majority intact antibody with only minor fragment species observed. In the reduced analysis, samples are comparable showing roughly equal intensity bands for heavy chain and heavy chain + RNA with little to no observed light chain + RNA. The fragmentation observed in the TBP1-410 AOC but not in the TBP2-410 AOC is an expected result from the conjugation approaches and may be causing the observed stability differences. Together, these results demonstrate that the engineered cysteine conjugation approach provides a more homogenous, more stable AOC compared to the native cysteine conjugation approach.

Table 20: *In vitro* Stability Results Showing the Change in Main Peak at 40 °C Relative to 4 °C Following 2-week Incubation.

AOC	SEC Δ%Main	AEX Δ%Main	CE-SDS Reduced Δ%Main	CE-SDS Non- Reduced Δ%Main
TBP1-410	-33.0	-37	-8.6	N/A ^a
TBP2-410	-1.7	-7	0	-0.4
TBP3-410	-2.7	-0.9	0 ^b	0 ^b

^a Non-reduced CE-SDS analysis could not be performed for the TBP1-410 due to significant peak heterogeneity.

^b No % change in peak area was observed, however main peak was shifted due to maleimide ring hydrolysis.

Example 14: *In vitro* Evaluation of the TfR1-Nav1.8 siRNA AOC in Human DRGs.

[00298] To confirm that the siRNA sequence can be delivered to human DRGs by an anti-transferrin antibody (anti-TfR1) of the present disclosure, an anti-TfR1 antibody conjugated to the siRNA 305 of Table 4 (TBP1-si410 AOC) was synthesized and evaluated in an *in vitro* assay using primary human DRGs. The siRNA sequence used in the AOC was siRNA 305 as shown in Table 4 and the siRNA was conjugated to the antibody via SMCC linker as shown in Figure 7. The antibody used in the AOC is TBP1 as shown in Table 8.

[00299] Human dorsal root ganglia neurons (DRGs) were used as a source of primary cells expressing Nav1.8 mRNA. Fresh primary human DRGs were purchased from AnaBios (San Diego, CA) and maintained in serum-free NbActive4 neural maintenance medium (Axol, Easter Brush, United Kingdom) with 25 ng/mL of recombinant human NGF (Axol). Primary DRGs were treated with AOCs at 100nM, twice, for 5 days.

[00300] Treated human DRGs were kept at 37 °C in 5% CO₂ for 5 days, followed by a wash in phosphate-buffer saline (PBS, ThermoFisher), and stored in 300 µL of TRIzol (ThermoFisher) at -80 °C until RNA extraction. The total mRNA was isolated using the ZYMO 96-well RNA kit (Zymo Research, Irvine, CA), and 100-250 ng of purified mRNA was used to generate the cDNA with the iScript cDNA synthesis kit (BioRad, Hercules, CA).

[00301] RNA expression levels were determined by quantitative reverse transcription polymerase chain reaction (RT-qPCR) using commercially available TaqMan probes (Life Technologies; *SCN10A*: Hs01045150_m1, *STMN2*: Hs00975900_m1, and *SNAP25*: Hs00938957_m1). The relative reduction in Nav1.8 transcripts was determined by normalizing the expression of TBP1-si410 AOC treated DRGs to the siNTC group using the DDCT method and the following formula: $[\%hNav1.8 \text{ mRNA} = 100 * 2^{-\text{DCt}(\text{si410 treated cells}) - \text{DCt}(\text{siNTC-treated cells})}]$, with the DCt determined by the difference in Ct values between *SCN10A* and the housekeeping genes *STMN2* and *SNAP25* $\{\text{avg DCt} = ((2 * \text{Ct}(\text{SCN10A})) - \text{Ct}(\text{STMN2}) - \text{Ct}(\text{SNAP25}))/2\}$ (Green MR, Sambrook J., “Quantification of RNA by Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR),” *Cold Spring Harb Protoc.* 2018 Oct, 2018(10): pdb.prot095042; and Livak KJ, Schmittgen TD, “Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method,” *Methods*, 25(4), 402–8 (2001)). A parametric unpaired t-test was performed using the software tool GraphPad Prism (GraphPad Software, LLC).

[00302] As illustrated in Figure 13, the tested AOC significantly reduced Nav1.8 mRNA levels (by approximately 30%) when compared to the negative control treated cells (TfR1-siNTC AOC) in primary human DRGs. In primary human DRGs, it was demonstrated that an AOC can deliver a Nav1.8 specific siRNA. AOC treatment results in receptor mediated uptake of the Nav1.8 siRNA, as human DRGs express TfR1 on their surface. The AOC uptake resulted in siRNA mediated knockdown of the Nav1.8 mRNA.

Example 15: *In vivo* Activity of OX26-410 and OX26-535 Tool AOCs in Humanized Nav1.8 Rats.

[00303] Humanized Nav 1.8 rats, in which the rat SCN10A gene was knocked out and replaced with the human SCN10A gene, were developed to allow testing of oligonucleotide therapeutics that only had predicted activity on human SCN10A transcript.

[00304] Test siRNAs-410 and siRNA-535 were conjugated to a commercial rat transferrin antibody (OX26 clone, BioXcell) using native cysteine conjugation and purified to a drug-antibody ratio of 1.0. Both the test siRNAs had the modifications as shown in Figure 1B without the amine or cholesterol handle.

Table 21: Description of the AOCs

Test AOC Name	Description
OX26-siRNA410	OX26 antibody linked to 410 siRNA via SMCC linker using nCys sites.
OX26-siRNA535	OX26 antibody linked to 535 siRNA via SMCC linker using nCys sites.

[00305] Female rats, approximately 4 weeks old, received an intravenous (IV) administration of either vehicle (PBS), OX26-siRNA410 (6 mg/kg oligo) or OX26-siRNA535 (6 mg/kg oligo). Thirteen days after administration, rats received 3 test sessions in the cold plate system. Rats first received a 5-minute session at room temperature (23 °C) followed by 10 minutes in the home cage. Half of the rats then received a 4-minute test session at 2 °C and the other half received a test session at -1 °C. After approximately 2 hours, rats received a test session at the other temperature. No effects of test order were observed. Nocifensive behavior was defined as the time spent licking either rear paw or time spent guarding the rear paws (paw lifted into the body). This time was summed into a Nocifensive Behavior score reported on the y-axis of the Figures 14, 15 and 16. Statistical analysis was performed in JMP version 15 via ANOVA of each temperature session, and in the case of the knockdown study, ANOVAs with p-values less than 0.05 were followed with a Dunnet's post-hoc analysis with the PBS control as the comparator. An alpha level of 0.05 was used to determine statistical significance in these

studies. Following the collection of behavioral data, rats were humanely euthanized and dorsal root ganglia and glabrous skin samples were collected and assayed for Nav1.8 protein levels.

[00306] Nav1.8 expression in glabrous skin is due completely to Nav1.8 in sensory nerve terminals, so assay of both DRG soma and skin allows confirmation of Nav1.8 protein knockdown throughout the entire dorsal root ganglia neuron. To assay Nav1.8 protein concentration, tissue lysates were prepared by suspending tissue in 200 µL/sample of the 1X lysis Buffer (CST, Cat #9803) along with Halt protease and phosphatase inhibitor (TFS, Cat #78440) diluted to 2X and phenylmethanesulfonyl fluoride (PMSF; Sigma, #78830-25G, (17.4 mg in 1 mL isopropanol, diluted to 1X) and two (2) 5 mm Tissuelyser beads (QIAGEN). Tissue tube was run in the Tissuelyser (QIAGEN) for 3 minutes at 30 1/s, then centrifuged at 4 °C at 5000 x g for 20 minutes. Supernatant was collected and frozen at -80 °C for analysis the next day. Protein concentration was measured with the Pierce™ BCA Protein Assay Kit (Cat #23227) by following the “Microplate Procedure” described in the manufacturer's manual. Samples were further diluted 1:3 in cell lysis buffer before plating.

[00307] The Meso Scale Discovery (MSD) S-plex platform was used to detect Nav1.8 protein levels. The custom developed assay utilized S-PLEX Development Pack B, SECTOR (25 Plate) (Cat # K15601S-4) kit. Capture and detection antibodies were tagged by MSD. Capture antibody (NeuroMab, Cat #75-166) was diluted to 0.5 µg/mL and the detection antibody (Eli Lilly) was diluted to 0.2 µg/mL. Calibrator was a recombinant C-terminal fragment of human Nav1.8 generated in-house. Sample lysates were diluted 4-fold in assay buffer. Assay buffer base was Diluent 39 (MSD, Cat #R5ABB2) with 100 µL each of the components included in the MSD® Inhibitor Pack (Cat #R70AA-1). Washing steps utilized MSD Tris Wash Buffer (MSD, Cat #R61TX-1) diluted to 1X in double-distilled water (ddH₂O). Plates were read using an MSD plate reader MESO QuickPlex SQ 120 with MSD Methodical Mind software. Data was analyzed using Discovery Workbench v4. Data was normalized by dividing the Nav1.8 protein concentration by the total protein concentration for each lysate sample. Statistical significance was determined by One-way ANOVA, p<0.05.

[00308] Effects on cold pressor have been previously reported with a selective Nav1.8 small molecule in early clinical testing (Hijma, et al., “A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Crossover Study to Evaluate the Pharmacodynamic Effects of VX-150, a Highly Selective Nav1.8 Inhibitor, in Healthy Male Adults,” *Pain Medicine*, 22 (8), pages 1814-26, (2021)). Similar effects in rodent cold behavior were observed following IV administration of AOCs targeting Nav1.8. Dosing with AOCs OX26-siRNA410 and OX26-

siRNA535 resulted in a decrease in cold-induced nocifensive behavior, however only animals receiving OX26-siRNA410 showed a statistically significant effect ($p < 0.05$, one-way ANOVA, Dunnet's post-hoc). This finding was consistent at both temperatures assayed (2 °C and -1 °C; Figure 14). Administration of OX26-siRNA410 and OX26-siRNA535 AOCs also resulted in a significant decrease in Nav1.8 protein in both DRG soma and glabrous paw (the latter finding confirming Nav1.8 protein knockdown in sensory nerve terminals) for both treatment groups ($p < 0.05$, one-way ANOVA).

[00309] Further, these data suggest a good correlation between the degree of Nav1.8 protein knockdown and the reversal of cold-induced behavior. These AOCs employed the same OX26 delivery antibody and only differed in the siRNA conjugated. The AOC containing siRNA410 displays superior *in vivo* efficacy as reflected by both a greater reversal of nocifensive cold behavior and a larger degree of Nav1.8 protein knockdown versus the AOC containing the siRNA535 and PBS controls.

Example 16: *In vivo* Activity of Tfr-410siRNA AOCs in Non-human Primates

[00310] The objective of this study was to determine the pharmacodynamic effects of anti-Tfr antibody-siRNA410 AOC (TBP1 anti-Tfr antibody conjugated to 410 siRNA via SMCC linker) when given by intravenous bolus injection in cynomolgus monkeys. Groups included PBS control, TBP1-410 nCys mAb, TBP2-410 eCys mAb, and TBP3-410 eCys Fab AOCs, N=4/group. The siRNA molecules were modified as shown in Figure 1B but without the amine or cholesterol handle.

Table 22: Description of Test AOCs

Test AOC Name	Description
TBP1-410 nCys mAb	Anti-Tfr antibody (TBP1) linked to modified 410 siRNA via SMCC linker using nCys linking site.
TBP2-410 eCys mAb	Anti-Tfr antibody (TBP2) linked to a modified 410 siRNA via SMCC linker using eCys linking site.
TBP3-410 eCys Fab	Fab Anti-Tfr antibody (TBP3) linked to modified 410 siRNA via SMCC linker using eCys linking site.

[00311] Female cynomolgus monkeys (age range, 2.4-4.0 years, and weight range 2.4-4.0 kgs) were dosed with a single IV dose of PBS control, or 1.0 or 6.0 mg/kg of the three test AOCs (by oligo weight). In addition, a single group was dosed twice with TBP1-siRNA410 nCys mAb at 3.0 mg/kg, by oligo weight, on day 1 and day 8. Humane euthanasia occurred on day 29, and glabrous paw skin was collected to assay for Nav1.8 protein levels. Nav1.8

protein in glabrous paw skin is indicative of protein expression in sensory nerve terminals. NHP skin lysates were prepared by suspending tissue in 150 μ L of the 1X lysis Buffer (CST, Cat #9803) along with Halt (Protease and Phosphatase Inhibitor (TFS, Cat #78440) diluted to 2X and PMSF (Sigma, #78830-25G, (17.4 mg in 1 mL isopropanol, diluted to 1x) and two (2) 5mm TissueLyser Beads (QIAGEN). Tissue tube was run in the TissueLyser (QIAGEN) for 3 minutes at 30 1/s, then centrifuged at 4 °C at 5000 x g for 20 minutes. Supernatant was collected and frozen at -80 °C for analysis the next day. Protein concentration was measured with the Pierce™ BCA Protein Assay Kit (Cat #23227) by following the “Microplate Procedure” described in the manufacturer's manual. Samples were further diluted 1:3 in cell lysis buffer before plating. The Meso Scale Discovery (MSD) S-Plex platform was used to detect Nav1.8 protein levels in the cultured human DRG neuron protein lysate. The custom developed assay utilized S-PLEX Development Pack B, SECTOR (25 Plate) (Cat #K15601S-4) kit. Capture and detection antibodies were tagged by MSD. Capture antibody (NeuroMab, Cat #75-166) was diluted to 0.5 μ g/mL and the detection antibody was diluted to 0.2 μ g/mL. Calibrator was a recombinant C-terminal fragment of human Nav1.8. Sample lysates were diluted 4-fold in assay buffer. Assay buffer base was Diluent 39 (MSD, Cat #R5ABB2) with 100 μ L each of the components included in the MSD® Inhibitor Pack (Cat #R70AA-1). Washing steps utilized MSD Tris Wash Buffer (MSD, Cat #R61TX-1) diluted to 1X in ddH₂O. Plates were read using an MSD plate reader MESO QuickPlex SQ 120 with MSD Methodical Mind software. Data was analyzed using Discovery Workbench v4. Data was normalized by dividing the Nav1.8 protein concentration by the total protein concentration for each lysate sample. Statistical significance was determined by one-way ANOVA.

[00312] All groups administered a single dose of test AOC showed a consistent, but modest decrease in Nav1.8 protein levels when compared to PBS controls (Figure 17), but none of these reductions reached statistical significance. However, the treatment group that received two doses of 3.0 mg/kg (by oligo weight) of TBP1-siRNA410 nCys mAb showed a significant reduction of Nav1.8 protein (47.3% of the PBS control group, $p < 0.05$, one-way ANOVA). While the effects of cold-induced behavior were not tested in these animals, the previous testing in humanized Nav1.8 rats suggests that this level of Nav1.8 protein knockdown in skin would be expected to have a significant effect and suggests that this is a physiologically relevant reduction in Nav1.8 protein.

Example 17: Dose-response Study of TBP2-410 eCys in Non-human Primates.

[00313] The objective of this study was to determine the pharmacodynamic effects of TBP2-410 eCys mAb DAR1 (referred to hereafter as TBP2-410 eCys) when given by intravenous bolus injection in cynomolgus monkeys.

Table 23: Description of AOC

AOC Name	Description
TBP2-410 eCys mAb DAR1	Anti-TfR antibody (TBP2) as shown in Table 8 linked to a modified 410 siRNA via SMCC linker using eCys linking site having DAR of 1. The modifications of the siRNA are described in Table 4 for siRNA No. 305 (which has a start position of target region on human SCN10A transcript at 410)

[00314] Female cynomolgus monkeys were dosed with four weekly IV doses of PBS control or 3 dose groups of TBP2-410 eCys at 0.3, 3.0, and 30.0 mg/kg (by oligo weight), N=4/group for the PBS control and two low dose groups, and N=2/group for high dose group. Humane euthanasia occurred on day 49 (27 days following the final dose), and glabrous paw skin was collected to assay for Nav1.8 protein levels. Nav1.8 protein in glabrous paw skin is indicative of protein expression in sensory nerve terminals.

[00315] NHP skin lysates were prepared by suspending tissue in 150 µl of the 1X lysis Buffer (CST, Cat#9803) along with Halt (Protease and Phosphatase Inhibitor (TFS, Cat#78440) diluted to 2X and PMSF (Sigma, #78830-25G, (17.4 mg in 1 mL isopropanol, diluted to 1X) and two (2) 5 mm TissueLyser Beads (QIAGEN). Tissue tube was run in the TissueLyser (QIAGEN) for 3 minutes at 30 1/s, then centrifuged at 4 °C at 5000 x g for 20 minutes. Supernatant was collected and frozen at -80 °C for analysis the next day. Protein concentration was measured with the Pierce™ BCA Protein Assay Kit (Catalog#23227) by following the “Microplate Procedure” described in the manufacturer's manual.

[00316] Samples were further diluted 1:3 in cell lysis buffer before plating. The Meso Scale Discovery (MSD) S-Plex platform was used to detect Nav1.8 protein levels in the cultured human DRG neuron protein lysate. The custom developed assay utilized S-PLEX Development Pack B, SECTOR (25 Plate) (Cat # K15601S-4) kit. Capture and detection antibodies were tagged by MSD. Capture antibody (NeuroMab, Cat#75-166) was diluted to 0.5 µg/mL and the detection antibody (6B2, produced onsite at Lilly Research Laboratories) was diluted to 0.2 µg/mL. Calibrator was a recombinant C-terminal fragment of human

Nav1.8 generated by Lilly at Lilly Research Laboratories. Sample lysates were diluted 4-fold in assay buffer. Assay buffer base was Diluent 39 (MSD, Cat# R5ABB2) with 100 μ L each of the components included in the MSD® Inhibitor Pack (Cat#R70AA-1). Washing steps utilized MSD Tris Wash Buffer (MSD, Cat#R61TX-1) diluted to 1X in ddH₂O. Plates were read using an MSD plate reader MESO QuickPlex SQ 120 with MSD Methodical Mind software. Data was analyzed using Discovery Workbench v4. Data was normalized by dividing the Nav1.8 protein concentration by the total protein concentration for each lysate sample. Statistical significance was determined by one-way ANOVA.

[00317] As shown in Figure 18, administration of the middle and high doses of TBP2-410 eCys (3.0 and 30.0 mg/kg by oligo weight) resulted in significant reduction in Nav1.8 protein in glabrous paw skin (52.3% and 42.2% of the PBS control group respectively, * $p < 0.05$, one-way ANOVA). While the effects of cold-induced behavior were not tested in these animals, the previous testing in humanized Nav1.8 rats suggests that this level of Nav1.8 protein knockdown in skin would be expected to have a significant effect and suggests that this is a physiologically relevant reduction in Nav1.8 protein.

SEQUENCES

[00318] The following nucleic and/or amino acid sequences of the antibodies of the present disclosure are provided below for reference.

TBP1; 211 Com29 B09 hIgG1-EN mAb:

Heavy Chain (HC) (SEQ ID NO: 625)

EVQLVESGGGLVKPGGSLRLSCVASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSS
YIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARRHGYSNSDAFDTW
GQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD
KTHHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
GK

Light Chain (LC) (SEQ ID NO: 626)

DIQMTQSPSAMSASVGDRVITTCRASQGISHYLVWFQQKPGKVPKRLIYAASSLQSG
VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQHNSYPWTFGQGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Heavy Chain Variable Region (HCVR) (SEQ ID NO: 627)

EVQLVESGGGLVKPGGSLRLSCVASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSS
YIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARRHGYSNSDAFDTW
GQGTLLTVSS

Light Chain Variable Region (LCVR) (SEQ ID NO: 628)

DIQMTQSPSAMSASVGDRVTITCRASQGISHYLVWFQQKPGKVPKRLIYAASSLQSG
VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

HCDR1 (SEQ ID NO: 629)

SYSMN

HCDR2 (SEQ ID NO: 630)

SISSSSSYIYYADSVKG

HCDR3 (SEQ ID NO: 631)

RHGYSNSDAFDT

LCDR1 (SEQ ID NO: 632)

RASQGISHYLV

LCDR2 (SEQ ID NO: 633)

AASSLQS

LCDR3 (SEQ ID NO: 634)

LQHNSYPWT

TBP2; 211 Com29 B09 hIgG1-AAS Heteromab 124C Antibody (for eCys DAR1 conjugation):**Heavy Chain A (HCA) (SEQ ID NO: 635)**

EVQLVESGGGLVKPGGSLRLSCVASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSS
YIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARRHGYSNSDAFDTW
GQGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD
KTHHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVTVSVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPRRPRVYTLPPSREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSDGSFFLYSVLTVDKSRWQQGNVVFSCVMHEALHNHYTQKSLSLSP
GK

Heavy Chain B (HCB) (SEQ ID NO: 636)

EVQLVESGGGLVKPGGSLRLSCVASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSS
YIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARRHGYSNSDAFDTW
GQGTLTVTVSSASTKGPCVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
DKTHHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVTVSVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTDNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLMSDGSFFLASKLTVDKSRWQQGNVVFSCVMHEALHNHYTQKSLSL
SPGK

Light Chain (LC) (SEQ ID NO: 626)

DIQMTQSPSAMSASVGDRVTITCRASQGISHYLVWFQQKPGKVPKRLIYAASSLQSG
VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQHNSYPWTFGQGTKVEIKRTVAAPSV

FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

HCVR (SEQ ID NO: 627)

EVQLVESGGGLVKPGGSLRLSCVASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSS
YIYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARRHGYSNSDAFDTW
GQGTLVTVSS

LCVR (SEQ ID NO: 628)

DIQMTQSPSAMSASVGDRVITTCRASQGISHYLVWFQQKPGKVPKRLIYAASSLQSG
VPSRFGSGSGTEFTLTISLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

HCDR1 (SEQ ID NO: 629)

SYSMN

HCDR2 (SEQ ID NO: 630)

SISSSSYIYYADSVKG

HCDR3 (SEQ ID NO: 631)

RHGYSNSDAFDT

LCDR1 (SEQ ID NO: 632)

RASQGISHYLV

LCDR2 (SEQ ID NO: 633)

AASSLQS

LCDR3 (SEQ ID NO: 634)

LQHNSYPWT

TBP3; 211 Com29 B09 hIgG1 124C Fab:

Heavy Chain (HC) (SEQ ID NO: 637)

EVQLVESGGGLVKPGGSLRLSCVASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSS
YIYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARRHGYSNSDAFDTW
GQGTLVTVSSASTKGPCVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV

Light Chain (LC) (SEQ ID NO: 638)

DIQMTQSPSAMSASVGDRVITTCRASQGISHYLVWFQQKPGKVPKRLIYAASSLQSG
VPSRFGSGSGTEFTLTISLQPEDFATYYCLQHNSYPWTFGQGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE

HCVR (SEQ ID NO: 627)

EVQLVESGGGLVKPGGSLRLSCVASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSS
YIYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARRHGYSNSDAFDTW
GQGTLVTVSS

LCVR (SEQ ID NO: 628)

DIQMTQSPSAMSASVGDRVTTTCRASQGISHYLVWFQQKPGKVPKRLIYAASSLQSG
VPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPWTFGQGTKVEIK

HCDR1 (SEQ ID NO: 629)
SYSMN

HCDR2 (SEQ ID NO: 630)
SISSSSSYIYYADSVKG

HCDR3 (SEQ ID NO: 631)
RHGYSNSDAFDT

LCDR1 (SEQ ID NO: 632)
RASQGISHYLV

LCDR2 (SEQ ID NO: 633)
AASSLQS

LCDR3 (SEQ ID NO: 634)
LQHNSYPWT

TBP1; Heavy Chain (SEQ ID NO: 639)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCCT
GAGACTCTCTGTGTAGCCTCTGGATTACCTTCAGTAGCTATAGCATGAACTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCATCCATTAGTTCCAG
TAGTAGTTACATATATTATGCAGACTCAGTGAAGGGCCGATTACCATCTCCAG
AGACAACGCCAAGAAGTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCTGTGTATTACTGTGCGAGAAGGCATGGCTACAGTAACTCCGATGCTT
TTGATACTTGGGGCCAAGGGACATTGGTCACCGTCTCTTCAGCCTCCACCAAGG
GCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAG
CGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGT
GGAACTCAGGCGCACTGACCAGCGGCGTGACACCTTCCCGGCTGTCTTACAGT
CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG
GCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTG
GACAAGAGAGTTGAGCCCAAATCTTGTGACAAAATCACAATGCCCACCGTGC
CCAGCACCTGAAGCCGAGGGGGCACCGTCAGTCTTCCTCTTCCCCCAAAACCC
AAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGAC
GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTATGTGGACGGCGTGGA
GGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACC
GTGTGGTCAGCGTCTCACCCTGCTGCACCAAGACTGGCTGAATGGCAAGGAGT
ACAAGTGCAAGGTCTCCAACAAGCCCTCCCATCCTCCATCGAGAAAACCATCT
CCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCC
CGGGAGGAGATGACCAAGAACCAAGTCAGCCTGACCTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA
ACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTATTC
CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT
CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT
CTCCGGGCAAA

TBP2; Heavy Chain A (SEQ ID NO: 640)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCCTGGTCAAGCCTGGGGGGTCCCT
GAGACTCTCCTGTGTAGCCTCTGGATTACCTTCAGTAGCTATAGCATGAACTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCATCCATTAGTTCCAG
TAGTAGTTACATATATTATGCAGACTCAGTGAAGGGCCGATTACCATCTCCAG
AGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCTGTGTATTACTGTGCGAGAAGGCATGGCTACAGTAACTCCGATGCTT
TTGATACTTGGGGCCAAGGGACATTGGTCACCGTCTCTTCAGCCTCCACCAAGG
GCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGACAG
CGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTG
GGAACCTCAGGCGCGCTGACCAGCGGCGTGACACACCTTCCCGGCTGTCTTACAGT
CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG
GCACCCAGACCTACATCTGCAACGTGAATCACAAAGCCCAGCAACACCAAGGTG
GACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACACACATGCCACCCGTGC
CCAGCACCTGAAGCCGCCGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCC
AAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGTCC
GTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTATGTGGACGGCGTGGA
GGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACC
GTGTGGTCAGCGTCTCACCCTCCTGCACCAAGACTGGCTGAATGGCAAGGAGT
ACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCT
CCAAAGCCAAAGGGCAGCCCCGAAGACCACGGGTGTACACCCCTGCCCCATCC
CGGGAGGAGATGACCAAGAACCAAGTCAGCCTGGTCTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA
ACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTATTC
CGTGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTC
TCCGGGCAAA

TBP3; Heavy Chain (SEQ ID NO: 641)

GAAGTGCAGCTGGTTCGAGTCAGGAGGCGGCCCTCGTGAAACCGGGTGGTAGCCT
GAGGCTGTCTGCGTGGCATCAGGGTTACCTTCTCGTCCTACTCCATGAACTGG
GTCAGACAGGCCCCCGAAAGGGACTTGAATGGGTGTCCAGCATCAGCAGCTC
CTCCTCGTACATCTACTACGCCGATTCCGTGAAGGGCCGGTTCACCATTAGCCG
CGACAATGCCAAGAACTCGCTGTATTTGCAAATGAACTCTCTGCGCGCCGAGGA
CACTGCTGTGTACTACTGTGCGCGGAGACACGGGTACTCCAACCTCCGATGCCTT
TGACACCTGGGGCCAGGGAACCTCTCGTGACCGTGTCTCGCCAGCACCAAGGG
CCCATGCGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGACAGC
GGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTG
GAACTCAGGCGCACTGACCAGCGGCGTGACACCTTCCCGGCTGTCTTACAGTC
CTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGG
CACCCAGACCTACATCTGCAACGTGAATCACAAAGCCCAGCAACACCAAGGTGG
ACAAGAGAGTT

TBP2; Heavy Chain B (SEQ ID NO: 642)

GAAGTGCAGCTGGTTCGAGTCAGGAGGCGGCCCTCGTGAAACCGGGTGGTAGCCT
GAGGCTGTCTGCGTGGCATCAGGGTTACCTTCTCGTCCTACTCCATGAACTGG
GTCAGACAGGCCCCCGAAAGGGACTTGAATGGGTGTCCAGCATCAGCAGCTC
CTCCTCGTACATCTACTACGCCGATTCCGTGAAGGGCCGGTTCACCATTAGCCG
CGACAATGCCAAGAACTCGCTGTATTTGCAAATGAACTCTCTGCGCGCCGAGGA
CACTGCTGTGTACTACTGTGCGCGGAGACACGGGTACTCCAACCTCCGATGCCTT
TGACACCTGGGGCCAGGGAACCTCTCGTGACCGTGTCTCGCCAGCACCAAGGG

CCCATGCGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGC
GGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTG
GAACTCAGGCGCGCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTC
CTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGG
CACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGG
ACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCCACCGTGCC
CAGCACCTGAAGCCGCCGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCA
AGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGTCCG
TGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTATGTGGACGGCGTGGAG
GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCG
TGTGGTCAGCGTCCTCACCGTCTGCACCAAGACTGGCTGAATGGCAAGGAGTA
CAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTC
CAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCC
GGGAGGAGATGACCGACAACCAAGTCAGCCTGACCTGCCTGGTCAAAGGCTTC
TATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA
CTACAAGACCACGCTCCCGTGTCTGATGTCCGACGGCTCCTTCTTCTCGCTCC
AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTC
TCCGGGCAAA

TBP1; Light Chain (SEQ ID NO: 643)

GACATCCAGATGACCCAGTCTCCATCTGCCATGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGTCTGGGCGAGTCAGGGCATTAGCCACTATTTAGTGTGGTTTC
AGCAGAAACCAGGGAAAGTCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTAC
AAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAATTCACCT
TCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTACAGC
ATAATAGTTACCCTTGGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAACGA
ACCGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT
CTGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA
AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGT
GTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGAC
GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCC
ATCAGGGCCTGAGCTCGCCCGTCAAAAGAGCTTCAACAGGGGAGAGTGC

TBP2; Light Chain (SEQ ID NO: 644)

GACATCCAGATGACCCAGTCTCCATCTGCCATGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGTCTGGGCGAGTCAGGGCATTAGCCACTATTTAGTGTGGTTTC
AGCAGAAACCAGGGAAAGTCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTAC
AAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAATTCACCT
TCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTACAGC
ATAATAGTTACCCTTGGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAACGA
ACCGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT
CTGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA
AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGT
GTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGAC
GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCC
ATCAGGGCCTGAGCTCGCCCGTCAAAAGAGCTTCAACAGGGGAGAGTGC

TBP3; Light Chain (SEQ ID NO: 645)

GACATCCAGATGACCCAGTCTCCATCTGCCATGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGTCTGGGCGAGTCAGGGCATTAGCCACTATTTAGTGTGGTTTC
AGCAGAAACCAGGGAAAGTCCCTAAGCGCCTGATCTATGCTGCATCCAGTTTAC
AAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAATTCACCTC
TCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTACAGC
ATAATAGTTACCCCTTGGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAACGA
ACCGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT
CTGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA
AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGT
GTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGAC
GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCC
ATCAGGGCCTGAGCTCGCCCGTCAAAAGAGCTTCAACAGGGGAGAG

siNTC (SEQ ID NO: 646)
CUAGCUGGACACGUCGAUA

siNTC (SEQ ID NO: 647)
UAUCGACGUGUCCAGCUAGUU

**NM_006514.3 Homo sapiens Sodium Voltage-gated Channel Alpha Subunit 10
(SCN10A), Transcript Variant 1, mRNA (SEQ ID NO: 648)**

ATGGAATTCCCCATTGGATCCCTCGAAACTAACAACCTCCGTCGCTTTACTCCGG
AGTCACTGGTGGAGATAGAGAAGCAAATTGCTGCCAAGCAGGGAACAAAGAAA
GCCAGAGAGAAGCATAGGGAGCAGAAGGACCAAGAAGAGAAGCCTCGGCCCC
AGCTGGACTTGAAAGCCTGCAACCAGCTGCCCAAGTTCTATGGTGAGCTCCAG
CAGAACTGATCGGGGAGCCCCTGGAGGATCTAGATCCGTTCTACAGCACACACC
GGACATTTATGGTGCTGAACAAAGGGAGGACCATTTCCCGGTTTATGTCCACTC
GGGCCCTGTGGCTATTCACTCCTTTCAACCTGATCAGAAGAACGGCCATCAAAG
TGTCTGTCCACTCGTGGTTCAGTTTATTATTACGGTCACTATTTTGGTTAATTGT
GTGTGCATGACCCGAACCTGACCTTCCAGAGAAAATTGAATATGTCTTCACTGTC
ATTTACACCTTTGAAGCCTTGATAAAGATACTGGCAAGAGGATTTTGTCTAAAT
GAGTTCACGTACCTGAGAGATCCTTGGAACTGGCTGGATTTTAGCGTCATTACC
CTGGCATATGTTGGCACAGCAATAGATCTCCGTGGGATCTCAGGCCTGCGGACA
TTCAGAGTTCTTAGAGCATTAAAAACAGTTTCTGTGATCCCAGGCCTGAAGGTC
ATTGTGGGGGCCCTGATTCAGTCACTGAGAAACTGGCTGATGTGACCATCCTC
ACCATCTTCTGCCTAAGTGTTTTTGCCTTGGTGGGGCTGCAACTCTTCAAGGGCA
ACCTCAAAAATAAATGTGTCAAGAATGACATGGCTGTCAATGAGACAACCAAC
TACTCATCTCACAGAAAACCAGATATCTACATAAATAAGCGAGGCACTTCTGAC
CCCTTACTGTGTGGCAATGGATCTGACTCAGGCCACTGCCCTGATGGTTATATCT
GCCTTAAAACTTCTGACAACCCGGATTTTAACTACACCAGCTTTGATTCTTTTGC
TTGGGCTTTTCTCTCACTGTTCCGCCTCATGACACAGGATTCTTGGGAACGCCTC
TACCAGCAGACCCTGAGGACTTCTGGGAAAATCTATATGATCTTTTTTGTGCTCG
TAATCTTCTGGGATCTTTCTACCTGGTCAACTTGATCTTGGCTGTAGTCACCAT
GGCGTATGAGGAGCAGAACCAGGCAACCACTGATGAAATTGAAGCAAAGGAGA
AGAAGTTCAGGAGGCCCTCGAGATGCTCCGGAAGGAGCAGGAGGTGCTAGCA
GCACTAGGGATTGACACAACCTCTCTCCACTCCCACAATGGATCACCTTTAACC
TCCAAAAATGCCAGTGAGAGAAGGCATAGAATAAAGCCAAGAGTGTGAGAGGG
CTCCACAGAAGACAACAAATCACCCCGCTCTGATCCTTACAACCAGCGCAGGAT

GTCTTTTCTAGGCCCTCGCCTCTGGAAAACGCCGGGCTAGTCATGGCAGTGTGTTTC
CATTTCCGGTCCCCCTGGCCGAGATATCTCACTCCCTGAGGGAGTCAACAGATGAT
GGAGTCTTTCTGGAGACCACGAAAGCCATCGGGGCTCTCTGCTGCTGGGTGGG
GGTGCTGGCCAGCAAGGCCCCCTCCCTAGAAGCCCTCTTCCTCAACCCAGCAAC
CCTGACTCCAGGCATGGAGAAGATGAACACCAACCGCCGCCCACTAGTGAGCTT
GCCCCTGGAGCTGTCGATGTCTCGGCATTTCGATGCAGGACAAAAGAAGACTTTC
TTGTCAGCAGAATACTTAGATGAACCTTTCCGGGCCCAAAGGGCAATGAGTGTT
GTCAGTATCATAACCTCCGTCCTTGAGGAACTCGAGGAGTCTGAACAGAAGTGC
CCACCCTGCTTGACCAGCTTGTCTCAGAAGTATCTGATCTGGGATTGCTGCCCCA
TGTGGGTGAAGCTCAAGACAATTCTCTTTGGGCTTGTGACGGATCCCTTTGCAG
AGCTCACCATCACCTTGTGCATCGTGGTGAACACCATCTTCATGGCCATGGAGC
ACCATGGCATGAGCCCTACCTTCGAAGCCATGCTCCAGATAGGCAACATCGTCT
TTACCATATTTTTTACTGCTGAAATGGTCTTCAAAATCATTGCCTTCGACCCATA
CTATTATTTCCAGAAGAAGTGGAATATCTTTGACTGCATCATCGTCACTGTGAGT
CTGCTAGAGCTGGGCGTGGCCAAGAAGGGAAGCCTGTCTGTGCTGCGGAGCTTC
CGCTTGCTGCGCGTATTCAAGCTGGCCAAATCCTGGCCCACCTTAAACACACTC
ATCAAGATCATCGGAACTCAGTGGGGGCACTGGGGAACTCACCATCATCCTG
GCCATCATTGTCTTTGTCTTTGCTCTGGTTGGCAAGCAGCTCCTAGGGGAAAAC
ACCGTAACAACCGAAAAAATATCTCCGCGCCCCATGAAGACTGGCCCCGCTGGC
ACATGCACGACTTCTTCCACTCTTTCCTCATTGTCTTCCGTATCCTCTGTGGAGA
GTGGATTGAGAACATGTGGGCCTGCATGGAAGTTGGCCAAAAATCCATATGCCT
CATCCTTTTCTTGACGGTGATGGTGCTAGGGAACCTGGTGGTGCTTAACCTGTTC
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CAGCCCAAGGCAGAGCCTGAGCTGGTGGTGAACCTCCCACTCTCCAGCTCCAAG
GCTGAGAACACATTGCTGCCAACACTGCCAGGGGGAGCTCTGGAGGGCTCCA
AGCTCCCAGAGGGCCCCAGGGATGAGCACAGTGACTTCATCGCTAATCCGACTGT
GTGGGTCTCTGTGCCCATTGCTGAGGGTGAATCTGATCTTGATGACTTGGAGGA
TGATGGTGGGGAAGATGCTCAGAGCTTCCAGCAGGAAGTGATCCCCAAAGGAC
AGCAGGAGCAGCTGCAGCAAGTCGAGAGGTGTGGGGACCACCTGACACCCAGG
AGCCCAGGCACTGGAACATCTTCTGAGGACCTGGCTCCATCCCTGGGTGAGACG
TGGAAGATGAGTCTGTTCCCTCAGGTCCCTGCTGAGGGAGTGGACGACACAAGC
TCCTCTGAGGGCAGCACGGTGGACTGCCTAGATCCTGAGGAAATCCTGAGGAA
GATCCCTGAGCTGGCAGATGACCTGGAAGAACCAGATGACTGCTTCACAGAAG
GATGCATTGCCCCTGTCCCTGCTGCAAACTGGATACCAACAGAGTCCATGGG
ATGTGGGCTGGCAGGTGCGCAAGACTTGCTACCGTATCGTGGAGCACAGCTGGT
TTGAGAGCTTCATCATCTTCATGATCCTGCTCAGCAGTGGATCTCTGGCCTTTGA
AGACTATTACCTGGACCAGAAGCCACGGTGAAAGCTTTGCTGGAGTACACTGA
CAGGGTCTTCACCTTTATCTTTGTGTTGAGATGCTGCTTAAGTGGGTGGCCTAT
GGCTTCAAAAAGTACTTCACCAATGCCTGGTGCTGGCTGGACTTCCTCATTGTG
AATATCTCACTGATAAGTCTCACAGCGAAGATTCTGGAATATTCTGAAGTGGCT
CCCATCAAAGCCCTTCGAACCCCTTCGCGCTCTGCGGCCACTGCGGGCTCTTTCTC
GATTTGAAGGCATGCGGGTGGTGGTGGATGCCCTGGTGGGCGCCATCCCATCCA
TCATGAATGTCTCCTCGTCTGCCTCATCTTCTGGCTCATCTTCAGCATCATGGG
TGTGAACCTCTTCGCAGGGAAGTTTTGGAGGTGCATCAACTATACCGATGGAGA
GTTTTCCCTTGTACCTTTGTGCGATTGTGAATAACAAGTCTGACTGCAAGATTCAA
AACTCCACTGGCAGCTTCTTCTGGGTCAATGTGAAAGTCAACTTTGATAATGTTG
CAATGGGTACCTTGCACCTTCTGCAGGTGGCAACCTTTAAAGGCTGGATGGACA
TTATGTATGCAGCTGTTGATTCCCGGGAGGTCAACATGCAACCAAGTGGGAGG

ACAACGTGTACATGTATTTGTACTTTGTTCATCTTCATCATTTTTGGAGGCTTCTTC
ACACTGAATCTCTTTGTTGGGGTCATAATTGACAACTTCAATCAACAGAAAAA
AAGTTAGGGGGCCAGGACATCTTCATGACAGAGGAGCAGAAGAAATACTACAA
TGCCATGAAGAAGTTGGGCTCCAAGAAGCCCCAGAAGCCCATCCCACGGCCCCCT
GAACAAGTTCCAGGGTTTTGTCTTTGACATCGTGACCAGACAAGCTTTTGACAT
CACCATCATGGTCCTCATCTGCCTCAACATGATCACCATGATGGTGGAGACTGA
TGACCAAAGTGAAGAAAAGACGAAAATTCTGGGCAAAAATCAACCAGTTCTTTG
TGGCCGTCTTCACAGGCGAATGTGTTCATGAAGATGTTTCGCTTTGAGGCAGTACT
ACTTCACAAATGGCTGGAATGTGTTTGACTTCATTGTGGTGGTTCTCTCCATTGC
GAGCCTGATTTTTTCTGCAATTCTTAAGTCACTTCAAAGTTACTTCTCCCCAACG
CTCTTCAGAGTCATCCGCCTGGCCCGAATTGGCCGCATCCTCAGACTGATCCGA
GCGGCCAAGGGGATCCGCACACTGCTCTTTGCCCTCATGATGTCCCTGCCTGCC
CTCTTCAACATCGGGCTGTTGCTATTCCTTGTTCATGTTTCATCTACTCTATCTTCGG
TATGTCCAGCTTTCCCATGTGAGGTGGGAGGCTGGCATCGACGACATGTTCAA
CTCCAGACCTTCGCCAACAGCATGCTGTGCCTCTTCCAGATTACCACGTCCGCC
GGCTGGGATGGCCTCCTCAGCCCCATCCTCAACACAGGGCCCCCTACTGTGAC
CCCAATCTGCCAACAGCAATGGCACCAGAGGGGACTGTGGGAGCCCAGCCGT
AGGCATCATCTTCTTCACCACCTACATCATCTCCTTCCTCATCGTGGTCAAC
ATGTACATTGCAGTGATTCTGGAGAACTTCAATGTGGCCACGGAGGAGAGCACT
GAGCCCCCTGAGTGAGGACGACTTTGACATGTTCTATGAGACCTGGGAGAAGTTT
GACCCAGAGGCCACTCAGTTTATTACCTTTTCTGCTCTCTCGGACTTTGCAGACA
CTCTCTCTGGTCCCCTGAGAATCCCAAACCCAATCGAAATATACTGATCCAGA
TGGACCTGCCTTTGGTCCCTGGAGATAAGATCCACTGCTTGGACATCCTTTTTGC
TTTCACCAAGAATGTCCTAGGAGAATCCGGGGAGTTGGATTCTCTGAAGGCAAA
TATGGAGGAGAAGTTTATGGCAACTAATCTTTCAAATCATCCTATGAACCAAT
AGCAACCACTCTCCGATGGAAGCAAGAAGACATTTAGCCACTGTCATTCAAAA
GGCCTATCGGAGCTATGTGCTGCACCGCTCCATGGCACTCTCTAACACCCCATG
TGTGCCCAGAGCTGAGGAGGAGGCTGCATCACTCCCAGATGAAGGTTTTGTTGC
ATTCACAGCAAATGAAAATTGTGTACTCCCAGACAAATCTGAAACTGCTTCTGC
CACATCATTCCCACCGTCCTATGAGAGTGTCACTAGAGGCCTTAGTGATAGAGT
CAACATGAGGACATCTAGCTCAATACAAAATGAAGATGAAGCCACCAGTATGG
AGCTGATTGCCCCCTGGGCCCTAGTGA

What is claimed is:

1. An oligonucleotide for inhibiting the expression of a human Nav1.8 gene (*SCN10A*) in a cell comprising a single stranded oligonucleotide or double stranded oligonucleotide that comprises a region of complementarity to Nav1.8 mRNA.
2. The oligonucleotide of claim 1, wherein the double stranded oligonucleotide comprises a sense strand and an antisense strand, wherein the sense strand and the antisense strand form a duplex region and the antisense strand comprises a region of complementarity to Nav1.8 mRNA, wherein said region of complementarity is less than 30 nucleotides in length.
3. The oligonucleotide of claim 2, wherein the region of complementarity of the antisense strand to the Nav1.8 mRNA target sequence is at least 15 contiguous nucleotides in length, at least 18 contiguous nucleotides in length, at least 19 contiguous nucleotides in length, at least 20 contiguous nucleotides in length, or at least 21 contiguous nucleotides in length.
4. The oligonucleotide of claim 2, wherein the sense strand is 15 to 30 nucleotides in length, 18 to 25 nucleotides in length, or 19 nucleotides in length.
5. The oligonucleotide of claim 2, wherein the antisense strand is 15 to 30 nucleotides in length, 18 to 25 nucleotides in length, 19 nucleotides in length, or 21 nucleotides in length.
6. The oligonucleotide of claim 2, wherein the sense strand is 19 nucleotides in length and the antisense strand is 21 nucleotides in length.
7. The oligonucleotide of claim 2, wherein the antisense strand comprises a region of complementarity to a target sequence of any one of SEQ ID NOs: 1 to 141.
8. The oligonucleotide of claim 2, wherein the region of complementarity is at least 15 contiguous nucleotides in length, at least 16 contiguous nucleotides in length, at least 17 contiguous nucleotides in length, at least 18 contiguous nucleotides in length, at least 19 contiguous nucleotides in length, or at least 20 contiguous nucleotides in length.

9. The oligonucleotide of any one of claims 2 to 8, wherein the antisense strand comprises a 3' overhang sequence of 1 or more nucleotides in length.
10. The oligonucleotide of claim 9, wherein the 3' overhang sequence is 2 nucleotides in length, and optionally wherein the 3' overhang sequence is UU.
11. The oligonucleotide of any one of claims 2 to 10 comprising at least 1 modified nucleotide.
12. The oligonucleotide of claim 11, wherein all nucleotides of the oligonucleotide are modified.
13. The oligonucleotide of claim 11 or 12, wherein the modified nucleotide comprises a 2'-modification on the sugar.
14. The oligonucleotide of claim 13, wherein the 2'-modification is 2'-fluoro, 2'-O-methyl, or 2'-O-methoxyethyl.
15. The oligonucleotide of claim 12, wherein the modification is 2'-fluoro, vinyl phosphonate uridine (VpUq), or 2'-O-methyl.
16. The oligonucleotide of any one of claims 11 to 15, wherein the 5' end of the antisense strand comprises at least one vinyl phosphonate uridine (VpUq).
17. The oligonucleotide of any one of claims 11 to 16, wherein one or more nucleotides at positions 7, 8, or 9 of the sense strand are modified with 2'-fluoro.
18. The oligonucleotide of any one of claims 11 to 17, wherein one or more nucleotides at positions 2, 6, 14, or 16 of the antisense strand are modified with 2'-fluoro.
19. The oligonucleotide of any one of claims 11 to 18, wherein one or more nucleotides at positions 1, 2, 3, 4, 5, 6, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 of the sense strand are modified with 2'-O-methyl.

20. The oligonucleotide of any one of claims 11 to 19, wherein one or more nucleotides at positions 1, 3, 4, 5, 7 to 13, 15, 17, 18, 19, 20, or 21 of the antisense strand are modified with 2'-O-methyl.
21. The oligonucleotide of any one of the preceding claims comprising at least one modified internucleotide linkage.
22. The oligonucleotide of claim 21, wherein at least one modified internucleotide linkage is a phosphorothioate linkage or a phosphorodithioate linkage.
23. The oligonucleotide of claim 22, wherein at least two terminal nucleotides on 5' end or 3' end of the antisense strand or the sense strand have a phosphorothioate linkage or a phosphorodithioate linkage.
24. The oligonucleotide of claim 22, wherein at least three terminal nucleotides on 5' end or 3' end of the antisense strand or the sense strand have phosphorothioate linkages or a phosphorodithioate linkages.
25. The oligonucleotide of any one of claims 21-24, wherein the sense strand has 19 nucleotides and the internucleotide linkage of nucleotides between positions 1 and 2, 2 and 3, 17 and 18, or 18 and 19 of the sense strand are modified with the phosphorothioate linkage.
26. The oligonucleotide of any one of claims 21-25, wherein the antisense strand has 21 nucleotides and the internucleotide linkage of one or more nucleotides between positions 1 and 2, 2 and 3, 19 and 20, or 20 and 21 of the antisense strand are modified with the phosphorothioate linkage.
27. The oligonucleotide of any one of claims 2-26, wherein a linker moiety is attached to one or more termini of the sense strand or the antisense strand.
28. The oligonucleotide of claim 26, wherein a linker is SMCC, optionally attached to the 5' end of the sense strand.

29. The oligonucleotide of any one of claims 2 to 28, wherein the oligonucleotide has a modification pattern as shown in FIG. 1A, optionally without the amine or cholesterol handle.
30. The oligonucleotide of any one of claims 2 to 28, wherein the oligonucleotide has a modification pattern as shown in FIG. 1B, optionally without the amine or cholesterol handle.
31. The oligonucleotide of any one of the preceding claims, wherein at least one nucleotide of the oligonucleotide is conjugated to cholesterol, lipid, polypeptide, or an antibody or an antigen binding fragment thereof.
32. The oligonucleotide of any one of claims 2-30, wherein the sense strand has the sequence of anyone of the even numbers of SEQ ID NOs: 1 to 422.
33. The oligonucleotide of any one of claims 2-30, wherein the antisense strand has the sequence of i) anyone of the odd numbers of SEQ ID NOs: 143 to 423 or ii) anyone of SEQ ID Nos: 424 to 564.
34. The oligonucleotide of any one of claims 2-30, wherein the sense strand and the antisense strands comprise nucleotide sequences selected from the group consisting of:
- (a) SEQ ID NOs: 565 and 566, respectively;
 - (b) SEQ ID NOs: 567 and 568, respectively;
 - (c) SEQ ID NOs: 569 and 570, respectively;
 - (d) SEQ ID NOs: 571 and 572, respectively;
 - (e) SEQ ID NOs: 573 and 574, respectively;
 - (f) SEQ ID NOs: 575 and 576, respectively;
 - (g) SEQ ID NOs: 577 and 578 respectively;
 - (h) SEQ ID NOs: 579 and 580, respectively;
 - (i) SEQ ID NOs: 581 and 582, respectively;
 - (j) SEQ ID NOs: 583 and 584, respectively;
 - (k) SEQ ID NOs: 585 and 586, respectively;
 - (l) SEQ ID NOs: 587 and 588, respectively;
 - (m) SEQ ID NOs: 589 and 590 respectively;
 - (n) SEQ ID NOs: 591 and 592, respectively;
 - (o) SEQ ID NOs: 593 and 594, respectively;

- (p) SEQ ID NOs: 595 and 596, respectively;
- (q) SEQ ID NOs: 597 and 598, respectively;
- (r) SEQ ID NOs: 599 and 600, respectively;
- (s) SEQ ID NOs: 601 and 602 respectively; and
- (t) SEQ ID NOs: 603 and 604 respectively.

35. The oligonucleotide of any one of claims 2-30, wherein the sense strand and the antisense strands comprise nucleotide sequences selected from the group consisting of:

- (a) SEQ ID NOs: 565 and 605, respectively;
- (b) SEQ ID NOs: 567 and 606, respectively;
- (c) SEQ ID NOs: 569 and 607, respectively;
- (d) SEQ ID NOs: 571 and 608, respectively;
- (e) SEQ ID NOs: 573 and 609, respectively;
- (f) SEQ ID NOs: 575 and 610, respectively;
- (g) SEQ ID NOs: 577 and 611 respectively;
- (h) SEQ ID NOs: 579 and 612, respectively;
- (i) SEQ ID NOs: 581 and 613, respectively;
- (j) SEQ ID NOs: 583 and 614, respectively;
- (k) SEQ ID NOs: 585 and 615, respectively;
- (l) SEQ ID NOs: 587 and 616, respectively;
- (m) SEQ ID NOs: 589 and 617 respectively;
- (n) SEQ ID NOs: 591 and 618, respectively;
- (o) SEQ ID NOs: 593 and 619, respectively;
- (p) SEQ ID NOs: 595 and 620, respectively;
- (q) SEQ ID NOs: 597 and 621, respectively;
- (r) SEQ ID NOs: 599 and 622, respectively;
- (s) SEQ ID NOs: 601 and 623 respectively; and
- (t) SEQ ID NOs: 603 and 624 respectively.

36. The oligonucleotide of any one of claims 2-30, wherein
- i) the sense strand has a sequence as set forth in Table 2 and antisense strand has a sequence as set forth in Table 2;
 - ii) the sense strand has a sequence set forth in Table 3 and the antisense strand has a sequence set forth in Table 3;

- iii) the oligonucleotide has a sequence set forth by any one of siRNA No. 1 to 141;
- iv) the oligonucleotide has a sequence set forth by any one of siRNA No. 142 to 282; or
- v) the oligonucleotide has a sequence set forth by any one of siRNA No. 283 to 322.

37. The oligonucleotide of claim 1, wherein the single stranded oligonucleotide comprises an antisense sense strand that includes a region of complementarity to Nav1.8 mRNA, wherein said region of complementarity is less than 30 nucleotides in length.

38. The oligonucleotide of claim 37, wherein the antisense strand is 15 to 30 nucleotides in length, 18 to 25 nucleotides in length, 19 nucleotides in length, or 21 nucleotides in length.

39. The oligonucleotide of claim 37, wherein the antisense strand comprises a region of complementarity to a target sequence of any one of SEQ ID NOs: 1 to 141.

40. The oligonucleotide of claim 37, wherein the region of complementarity is at least 15 contiguous nucleotides in length, at least 16 contiguous nucleotides in length, at least 17 contiguous nucleotides in length, at least 18 contiguous nucleotides in length, at least 19 contiguous nucleotides in length, or at least 20 contiguous nucleotides in length.

41. The oligonucleotide of any one of claims 37 to 40, wherein the antisense strand comprises a 3' sequence of 1 or more nucleotides in length.

42. The oligonucleotide of any one of claims 37 to 41 comprising at least 1 modified nucleotide.

43. The oligonucleotide of claim 42, wherein all nucleotides of the oligonucleotide are modified.

44. The oligonucleotide of claim 42 or 43, wherein the modified nucleotide comprises a 2'-modification on the sugar.

45. The oligonucleotide of claim 44, wherein the 2'-modification is 2'-fluoro, 2'-O-methyl, or 2'-O-methoxyethyl.
46. The oligonucleotide of claim 45, wherein the modification is 2'-fluoro, vinyl phosphonate uridine (VpUq), or 2'-O-methyl.
47. The oligonucleotide of any one of claims 41 to 46, wherein the 5' end of the antisense strand comprises at least one vinyl phosphonate uridine (VpUq).
48. The oligonucleotide of any one of claims 42 to 47, wherein one or more nucleotides at positions 1-21 of the antisense strand are modified with 2'-fluoro.
49. The oligonucleotide of any one of claims 42 to 48, wherein one or more nucleotides at positions 1-21 of the antisense strand are modified with 2'-O-methyl.
50. The oligonucleotide of any one of the preceding claims comprising at least one modified internucleotide linkage.
51. The oligonucleotide of claim 50, wherein at least one modified internucleotide linkage is a phosphorothioate linkage or a phosphorodithioate linkage.
52. The oligonucleotide of claim 51, wherein at least two terminal nucleotides on 5' end or 3' end of the antisense strand have a phosphorothioate linkage or a phosphorodithioate linkage.
53. The oligonucleotide of claim 51, wherein at least three terminal nucleotides on 5' end or 3' end of the antisense strand have phosphorothioate linkages or a phosphorodithioate linkages.
54. The oligonucleotide of any one of claims 2-53, wherein a linker moiety is attached to one or more termini of the antisense strand.

55. The oligonucleotide of claim 54, wherein a linker is SMCC.
56. The oligonucleotide of any one of the preceding claims, wherein at least one nucleotide of the oligonucleotide is conjugated to cholesterol, lipid, polypeptide, or an antibody or an antigen binding fragment thereof.
57. The oligonucleotide of any one of claims 37-56, wherein the antisense strand has the sequence of i) anyone of the odd numbers of SEQ ID NOs: 143 to 423 or ii) anyone of SEQ ID Nos: 424 to 564.
58. The oligonucleotide of any one of claims 37-56, wherein (i) the antisense strand has a sequence as set forth in Table 2 or (ii) the antisense strand has a sequence as set forth in Table 3.
59. A cell comprising the oligonucleotide of any one of claims 1 to 58.
60. A vector comprising a nucleotide sequence that encodes the oligonucleotide of any one of claims 1 to 58.
61. The vector of claim 60, wherein the nucleotide sequence that encodes the oligonucleotide is operably linked to a regulatory sequence.
62. A pharmaceutical composition comprising the oligonucleotide of any one of claims 1 to 58 and a pharmaceutically acceptable carrier.
63. A conjugate comprising the oligonucleotide of any one of claims 1-58 and an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to the sense strand of the oligonucleotide or the antisense strand of the oligonucleotide.
64. The conjugate of claim 63, wherein the antibody or antigen binding fragment thereof is linked to the sense strand or the antisense strand through a linker.
65. The conjugate of claim 64, wherein the linker is an SMCC linker.

66. The conjugate of claim 65, wherein the linker is SMCC linker attached to: i) the 5' end of the sense strand, ii) the 5' end of the antisense strand, iii) the 3' end of the sense strand, or iv) the 3' end of the antisense strand.

67. The conjugate of any one of claims 63 to 66, wherein the oligonucleotide mediates RNA interference against the human Nav1.8 mRNA preferentially in DRG cells in a human subject.

68. The conjugate of claim 67, wherein the mediation of RNA interference against the human Nav1.8 mRNA modulates pain in a subject.

69. The conjugate of claim 67, wherein the medication of RNA interference against the human Nav1.8 mRNA reduces pain in a subject.

70. The conjugate of any one of claims 63 to 67, wherein the antibody or antigen binding fragment thereof comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein the HCVR comprises heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, and the LCVR comprises light chain complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the following sequences: HCDR1 comprises SEQ ID NO: 629, HCDR2 comprises SEQ ID NO: 630, HCDR3 comprises SEQ ID NO: 631, LCDR1 comprises SEQ ID NO: 632, LCDR2 comprises SEQ ID NO: 633, and LCDR3 comprises SEQ ID NO: 634.

71. The conjugate of any one of claims 63 to 67, wherein the antibody or antigen binding fragment thereof comprises a HCVR of SEQ ID NO: 627 and a LCVR of SEQ ID NO: 628.

72. The conjugate of any one of claims 63 to 67, wherein the antibody comprises a heavy chain (HC) and a light chain (LC) of following sequences:

- (a) HC of SEQ ID NO: 625 and LC of SEQ ID NO: 626;
- (b) HCA (first heavy chain) of SEQ ID NO: 635, HCB (second heavy chain) of SEQ ID NO: 636, and a LC of SEQ ID NO: 626; or
- (c) HC of SEQ ID NO: 637 and LC of SEQ ID NO: 638.

73. The conjugate of any one of claims 63 to 72, wherein the antibody fragment is a Fab, scFv, Fv, or scFab.
74. The conjugate of any one of claims 63 to 73, wherein the antibody or antigen binding fragment thereof comprises at least one heavy chain constant region comprising cysteine at residue 124 (according to the EU Index numbering).
75. The conjugate of any one of claims 63 to 73, wherein the antibody or antigen binding fragment thereof comprises two heavy chain constant regions comprising cysteine at residue 124 (according to the EU Index numbering).
76. The conjugate of any one of claims 63 to 73, wherein the antibody or antigen binding fragment thereof further comprises a half-life extender.
77. The conjugate of claim 76, wherein the half-life extender is a VHH that binds human serum albumin (HSA).
78. A pharmaceutical composition comprising the conjugate of any one of claims 63 to 77 and a pharmaceutically acceptable carrier.
79. A method of preventing, treating, or reducing pain in a patient in need thereof, the method comprising administering to the patient an effective amount of the oligonucleotide of any one of claims 1 to 58; an effective amount of the conjugate of any one of claims 63-77; the pharmaceutical composition of claim 62; or the pharmaceutical composition of claim 78.
80. The method of claim 79, wherein the pain is chronic pain.
81. The method of claim 79, wherein the pain is: i) inflammatory pain, ii) neuropathic pain, or iii) mixed pain.
82. The method of any one of claims 79 to 81, wherein the oligonucleotide, the conjugate, or the pharmaceutical composition is administered intravenously.

83. The method of any one of claims 79 to 81, wherein the oligonucleotide, the conjugate, or the pharmaceutical composition is administered subcutaneously.
84. Use of the oligonucleotide of any one of claims 1 to 58; the conjugate of any one of claims 63-77; the pharmaceutical composition of claim 62; or the pharmaceutical composition of claim 78 in the manufacture of a medicament for preventing, treating, or reducing pain.
85. The use of claim 84, wherein the pain is chronic pain.
86. The use of claim 84, wherein the pain is: i) inflammatory pain, ii) neuropathic pain, or iii) mixed pain.
87. The use of any one of claims 84 to 86, wherein the oligonucleotide, the conjugate, or the pharmaceutical composition is administered intravenously.
88. The oligonucleotide of any one of claims 1 to 58; the conjugate of any one of claims 63-77; the pharmaceutical composition of claim 62; or the pharmaceutical composition of claim 78 for use in preventing, treating, or reducing pain in a patient in need thereof.
89. The oligonucleotide, the conjugate, or the pharmaceutical composition of claim 88, wherein the pain is chronic pain.
90. The oligonucleotide, the conjugate, or the pharmaceutical composition of claim 89, wherein the pain is: i) inflammatory pain, ii) neuropathic pain, or iii) mixed pain.
91. The oligonucleotide, the conjugate, or the pharmaceutical composition of any one of claims 88 to 90, wherein the conjugate is administered intravenously.