COMPOSITIONS AND METHODS FOR REDUCING NAV1.7 EXPRESSION TO TREAT NEUROPATHIC PAIN

CROSS-REFERENCING

[0001] This application claims the benefit of U.S. provisional application serial nos. 63/574,843 filed on April 4, 2024, and 63/670,071 filed on July 11, 2024, which applications are incorporated by reference in their entireties.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED AS A SEQUENCE LISTING XML FILE

[0002] A Sequence Listing is provided herewith as a Sequence Listing XML, "ENCO-010WO_SEQLIST" created on April 3, 2025 and having a size of 534,026 bytes. The contents of the Sequence Listing XML are incorporated by reference herein in their entirety.

BACKGROUND

[0003] NAV1.7 is a sodium channel which is encoded by SCN9A and is predominantly expressed in the peripheral somatic and visceral sensory neurons, olfactory sensory neurons and sympathetic ganglion neurons. NAV1.7 acts to set the threshold for action potential generation in primary sensory neurons.

[0004] NAV channel blockers are used to treat many neurological and cardiovascular disorders. However, these drugs generally lack isoform selectivity due to high sequence conservation across NAV channels, thereby limiting their therapeutic utility. About 30-50% of neuropathic pain patients have intractable pain.

[0005] Therefore, there is a need in the art for a therapeutic that can target specific NAV channel subtypes to treat neuropathic pain.

SUMMARY

[0006] The present disclosure relates to compositions, methods and processes for the design, preparation, manufacture, use and/or formulation of modulatory polynucleotides, e.g., polynucleotides encoding small interfering RNA (siRNA) molecules which target the transcript encoding the alpha (α) subunit of voltage-gated sodium channel (Na_v) 1.7 to treat chronic

neuropathic pain. In some embodiments, nucleic acid sequences encoding the siRNA molecules are inserted into recombinant adeno-associated virus (AAV) vectors. Methods for using the siRNA molecules to decrease the expression of mutated or wild-type $Na_v1.7$ in a subject with chronic pain (e.g., chronic neuropathic pain) are also disclosed.

[0007] The present disclosure further relates to nociceptor-selective regulatory elements and methods of using same to selectively express an operably linked transgene in nociceptors. In certain aspects, a nociceptor-selective regulatory element of the present disclosure in operable linkage with a therapeutic transgene are provided as expression cassettes in a gene therapy vector that finds use in treating a subject having a neurological disease or disorder, e.g., to treat a subject with chronic/neuropathic pain. In some embodiments, the therapeutic transgene is an siRNA molecule of the present disclosure that targets a transcript encoding Nav1.7.

[0008] These and other aspects will be described in greater detail below.

INCORPORATION BY REFERENCE

[0009] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Certain novel features of the invention may be set forth with particularity in the appended claims. A better understanding of some features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of some aspects of the invention are describes, and the accompanying drawings of which:

[0011] **Figure 1** is a graph showing knock down of exogenously-provided SCN9A transcript in HEK293 cells by miRNA candidates described herein as compared to a scrambled control (SCRM). The SEQ ID NO on the x-axis represents the RNA sequence of the targeting region of the miRNA candidate (the guide strand RNA). SEQ ID NO:120 represents a positive control sequence and SEQ ID NO:121 is a negative control sequence that does not bind to any target site in the SCN9A transcript (scrambled, or "SCRM").

[0012] **Figure 2** is a graph showing knock down of endogenous SCN9A transcript in iPSC glutamatergic neurons by miRNA candidates described herein as compared to the scrambled

sequence (SCRM). The SEQ ID NO on the x-axis represents the RNA sequence of the targeting region of the miRNA candidate (the guide strand RNA). SEQ ID NO:120 represents a positive control sequence and SEQ ID NO:121 is a negative control sequence that does not bind to any target site in the SCN9A transcript (scrambled, or "SCRM").

[0013] **Figure 3** is a graph showing knock down of endogenous SCN9A transcript in iPSC sensory neurons by miRNA candidates described herein as compared to the scrambled sequence (SCRM). The SEQ ID NO on the x-axis represents the RNA sequence of the targeting region of the miRNA candidate (the guide strand RNA). SEQ ID NO:120 represents a positive control sequence and SEQ ID NO:121 is a negative control sequence that does not bind to any target site in the SCN9A transcript (scrambled, or "SCRM").

[0014] **Figure 4** is a graph showing minimal off-target knock down effects in iPSC glutamatergic neurons by miRNA candidates described herein as compared to the scrambled sequence. The SEQ ID NO on the x-axis represents the RNA sequence of the targeting region of the miRNA candidate (the guide strand RNA). SEQ ID NO:121 is a negative control sequence that does not bind to any target site in the SCN9A transcript (scrambled, or "SCRM").

[0015] **Figure 5** is a graph showing minimal off-target knock down effects in iPSC sensory neurons by miRNA candidates described herein as compared to the scrambled sequence. The SEQ ID NO on the x-axis represents the RNA sequence of the targeting region of the miRNA candidate (the guide strand RNA). SEQ ID NO:121 is a negative control sequence that does not bind to any target site in the SCN9A transcript (scrambled, or "SCRM").

[0016] **Figure 6** provides a graph showing relative SCN9A expression in iPSC sensory neurons treated with AAVDJ vectors expressing the indicated scaffold/guide pri-miRNAs (scaffold and guide SEQ ID NOs are indicated at the bottom of the graph). Data represents biological replicates which are averages of 1-3 technical replicates. Error bars represent SEM.

[0017] **Figure 7** provides a table showing the level of knockdown of endogenous SCN9A transcript (the target gene) and transcript levels of closely related endogenous genes (listed at the left of the table) in iPSC sensory neurons treated with AAVDJ vectors expressing the indicated scaffold/guide pri-miRNAs relative to the Scramble control (scaffold/guides are indicated at the bottom of the graph). Higher numbers indicate higher knockdown (e.g., a value of 0.77 indicates 77% knockdown). A (-) indicates a knockdown value that is <0.01.

[0018] **Figures 8A, 8B, and 8C** provide off-target gene expression analyses (volcano plots) of select pri-miRNAs of the present disclosure. The target SCN9a transcript is indicated with an "x" while dots in the graphs represent off-target genes. Figure 8A shows volcano plots for miR-E-68 (SEQ ID NO:369; top panel) and miR-190a-68 (SEQ ID NO:378; bottom panel). Figure 8B shows volcano plots for miR-E-61 (SEQ ID NO:368; top panel) and miR-190a-61 (SEQ ID NO:380; bottom panel). Figure 8C shows volcano plots for miR-100-69 (SEQ ID NO:370; top panel) and miR-130a-69 (SEQ ID NO:373; bottom panel). Gene expression level change is on the x-axis and significance is on the y-axis. Dotted lines indicate 2-fold expression level change (both 2-fold lower and 20fold higher).

[0019] **Figure 9A** provides a graph showing relative Scn9a transcript levels (compared to PBS/Vehicle treated animals) *in vivo* in WT mice treated with miR-E-61 and miR-E-68 at different doses (3E9 vg/animal, 1E10 vg/animal, 3E10 vg/animal, and 1E11 vg/animal) and mice treated with miR-1-69 at 1E10 vg/animal (N=5 for each treatment; N=3 for PBS treatment). Statistical significance was assessed using one-way ANOVA with Dunnett's post-hoc test (**p<0.01; ****p < 0.0001).

[0020] **Figure 9B** provides images of DRG tissue from a PBS treated animal and an animal treated with 1E11 vg/animal of miR-E-68 that show: endogenous Nav1.7 expression using IHC (left), miRNA expression (SEQ ID NO:68) using ISH (middle), and H&E staining (right) to assess cellular integrity. Low and high magnification images are shown for each treatment (indicated on the right).

[0021] **Figure 9C** provides guide/passenger (G/P) ratios (top panel) and 5' processing precision (bottom panel) for each pri-miRNA tested at 1E10 vg/animal.

[0022] **Figure 10** provides graphs comparing SCN9A transcript expression levels from ssAAV vs. scAAV vectors expressing the indicated pri-miRNAs. SCN9A knockdown was equivalent at all doses for ssAAV and scAAV vectors expressing the miR-E-61 and miR-E-68 pri-miRNAs (left and middle panels). For miR-100-69, the scAAV vector showed a potency improvement at lower dose (1E2 vg/cell), with scAAV showing ~50% knockdown and ssAAV showed ~30% knockdown at this dose (right panel) (**** = $P \leq 0.0001$).

[0023] **Figure 11A** provides a graph of results of Von Frey tests for mechanical allodynia after SNI surgery was performed on adult male and female (2-months old) WT SD rats (n=10 vehicle; n=16 treatment) treated with the indicated pri-miRNA (in AAV9 vector). Vehicle and Vehicle +

gabapentin controls are also shown. The x-axis is days pre- and post-SNI surgery. The y-axis is mechanical threshold in grams (g) assessed using Von Frey testing as described herein.

[0024] **Figure 11B** shows results of the hot plate tests of the same animals in Figure 11A. The x-axis is days pre- and post-SNI surgery. The y-axis is withdrawal latency in seconds (s) from hot plate contact as described herein.

[0025] **Figure 12A** provides a graph showing viral copy number (VCN) per diploid genome from DRG tissues collected from the animals shown in Figure 11A. Each data point in represents an individual animal (n=8 per group). Statistical significance was assessed using one-way ANOVA with Dunnett's post-hoc test ((*p<0.05; **p<0.01; ****p < 0.0001).

[0026] **Figure 12B** provides a graph showing SCN9A mRNA levels in DRG tissues collected from the animals shown in Figure 11A. Each data point in represents an individual animal (n=8 per group). Statistical significance was assessed using one-way ANOVA with Dunnett's post-hoc test ((*p<0.05; **p<0.01; ****p < 0.0001).

[0027] **Figure 13** provides a graph showing SCN9A knockdown in iPSC sensory cells from expression vectors that express miR-E-68 pri-miRNA under the control of one or combinations of the following regulatory elements: Syn1, EF1α, candidate nociceptor-selective regulatory elements derived from the endogenous mouse (SEQ ID NO:451, 452) or human (SEQ ID NOs: 453, 454, 455, 461, 462, 463, 464, 465, and 467) SCN10A gene. Several of the sequences from the mouse and human SCN10A gene were able to knockdown SCN9A, with SEQ ID NO:466 showing the highest activity, indicating that this combination of human SCN10A elements is able to drive expression of an operably linked transgene in sensory neurons.

[0028] **Figure 14** provides graphs showing relative SCN9A expression in iPSC sensory neurons (iSensory; top panel) and glutamatergic neurons (iGluta; bottom panel) transduced with AAVDJ vectors expressing miR-E-68 or miR-100-69 under the control of the Syn1 promoter (SEQ ID NO:449) or SEQ ID NO:466. The results indicate that SEQ ID NO:466 has specificity for sensory neurons and is thus a nociceptor-selective regulatory element.

[0029] **Figure 15** provides a graph showing miRNA abundance in DRG, cortex (CTX), and olfactory bulb tissues for miR-E-68 under the control of Syn1 or SEQ ID NO:466 (upper panel) and a graph showing endogenous Scn9a transcript knockdown in the DRG tissue from the same treatment as in the upper panel. Statistical significance was assessed using one-way ANOVA with Dunnett's post-hoc test (*p<0.05; **p<0.01; ****p < 0.0001). SEQ ID NO:466 drives

strong selective expression of miR-E-68 in DRG tissue, which results in hig SCN9A transcript knockdown.

[0030] **Figure 16** provides a histogram showing SEQ ID NO:68 miR distribution expressed from either Syn1 or SEQ ID NO:466 on a per cell basis, showing the distribution of the number of ISH copies across DRG neuron area. Histograms are normalized for comparison purposes. The distribution of SEQ ID NO:68 expressed from SEQ ID NO:466 is biased towards smaller cell sizes suggestive of cell specificity for small-sized nociceptive neurons.

[0031] **Figure 17** provides graphs tabulating IHC co-staining data that localized SEQ ID NO:68 (miRNA) expression levels in DRG using peripherin, a marker for small sized unmyelinated nociceptive neuron (left panel), and NF200, a marker for large sized myelinated non-nociceptive neurons (right panel). This data shows that SEQ ID NO:68 (the expressed miRNA) is higher in small fiber neurons (peripherin positive) when operably linked to SEQ ID NO:466 as compared to when operably linked to Syn1 (left panel). Conversely, the number of copies of SEQ ID NO:68 (the miRNA) is lower in large fiber neurons (NF200 positive) when operably linked to SEQ ID NO:466 as compared to when operably linked to Syn1 (right panel). (*$p<0.05$; **$p<0.01$) This data further confirms the nociceptor-selectivity of SEQ ID NO:466.

[0032] **Figure 18** provides a graph showing relative EGFP expression in cardiomyocytes *in vitro*. EGFP expression was compared between constructs in which the EGFP gene was operably linked to either a nociceptor-selective regulatory element of the present disclosure (SEQ ID NO:466) or the CBA promoter (SEQ ID NO:479). Normalized EGFP expression was calculated by dividing EGFP transcript copies by GAPDH transcript copies for each sample. Relative EGFP was then calculated using CBA-treated samples at the lowest MOI of 1E3 set to a value of 1.0. EGFP expression was virtually undetectable from AAVDJ vectors in which its expression was controlled by SEQ ID NO:466, while expression from the CBA promoter showed the expected dose dependent activity.

DEFINITIONS

[0033] As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the

detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising".

[0034] The term "AAV" is an abbreviation for adeno-associated virus and may be used to refer to the virus itself or a derivative thereof. The term covers all serotypes, subtypes, and both naturally occurring and recombinant forms, except where required otherwise. The abbreviation "rAAV" refers to recombinant adeno-associated virus. The term "AAV" includes all serotypes of AAV, including AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hul4), AAV10, AAV11, AAV 12, AAV13, AAVrh8, AAVrhlO, AAV-DJ, and AAV-DJ8, and hybrids thereof (i.e., chimeric AAV vectors), as well as avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. The genomic sequences of various serotypes of AAV, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. A "rAAV vector" as used herein refers to an AAV vector comprising a polynucleotide sequence not of AAV origin (i.e., a polynucleotide heterologous to AAV), typically a sequence of interest for the genetic transformation of a cell. In general, the heterologous polynucleotide is flanked by at least one, and generally by two, AAV inverted terminal repeat sequences (ITRs). An rAAV vector may either be single-stranded (ssAAV) or self-complementary (scAAV). See, e.g., Raj et al., Expert Rev Hematol. 2011 Oct; 4(5): 539–549.An "AAV virus" or "AAV viral particle" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated polynucleotide rAAV vector. If the particle comprises a heterologous polynucleotide (i.e., a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "rAAV viral particle" or simply an "rAAV particle". AAVs may comprise genome components and capsids from multiple serotypes (e.g., pseudotyped vectors). For example, an AAV may comprise the genome of serotype 2 (e.g., ITRs) packaged in the capsid from serotype 5 or serotype 9. Pseudotyped vectors may demonstrate improved transduction efficiency as well as altered tropism. In some cases, an AAV serotype that can cross the blood brain barrier or infect cells of the CNS is preferred. In certain embodiments, variant AAV capsids that have improved CNS tropism and/or that cross the blood-brain barrier are employed, including, but not limited to: bCap1 (SEQ ID NO:2 from WO2023060264); AAV-B1 (SEQ ID NO: 5 from WO2016054557); AAV-S (AAV9 with insertion of SEQ ID NO:1 from WO2020198737);

AAV-TT (SEQ ID NO:2 from WO2015121501); and VCAP-101 or VCAP-102 (SEQ ID NOS: 981 and 982, respectively, from WO2023081648). In some aspects, the recombinant AAV vector is AAV1, AAV8, AAV9, AAVDJ, or chimeric AAV comprising features of two or more of these serotypes. In various embodiments, the AAV vector is an AAV9 vector or an scAAV9 vector. In certain embodiments, the AAV vector is an AAV9 vector or an scAAV9 vector and comprises a heterologous nucleic acid flanked by ITRs from a AAV serotype other than AAV9. In certain embodiments, the AAV vector is an AAV9 vector or an scAAV9 vector and comprises a heterologous nucleic acid flanked by AAV serotype 2 ITRs (i.e., ITR2).

[0035] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within one or more than one standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 15%, up to 10%, up to 5%, or up to 1%) of a given value.

[0036] In any of the embodiments described herein, "comprising" may be replaced with "consisting essentially of" or "consisting of." For example, an embodiment in which a particular element is included using the open-ended term “comprising” encompasses embodiments in which the element is included using the more restrictive terms “consisting essentially of” or “consisting of”.

[0037] The terms "determining", "measuring", "evaluating", "assessing", "assaying", "analyzing", and their grammatical equivalents can be used interchangeably herein to refer to any form of measurement and include determining if an element is present or not (for example, detection). These terms can include both quantitative and/or qualitative determinations. Assessing may be relative or absolute.

[0038] The term "expression" refers to the process by which a nucleic acid sequence or a polynucleotide is transcribed from a DNA template (such as into mRNA or other RNA transcript) and/or the process by which a transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts and encoded polypeptides may be collectively referred to as "gene product." If the polynucleotide includes introns or splice sites, e.g., is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[0039] An "expression cassette" refers to a nucleic molecule comprising one or more regulatory elements operably linked to a coding sequence (e.g., a gene or genes) for expression.

[0040] A "transgene" refers to a portion of a nucleic acid cassette that is designed to be expressed in a cell. In some embodiments, a transgene encodes functional RNA, e.g., an antisense RNA. In some embodiments, a transgene of the present disclosure encodes a therapeutic cargo, e.g., a therapeutic RNA.

[0041] The term "effective amount" or "therapeutically effective amount" refers to that amount of a composition described herein that is sufficient to affect the intended application, including but not limited to disease treatment, as defined below. The therapeutically effective amount may vary depending upon the intended treatment application (in a cell or *in vivo*), or the subject and disease condition being treated, e.g., the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term also applies to a dose that will induce a particular response in a target cell. The specific dose will vary depending on the particular composition chosen, the dosing regimen to be followed, whether it is administered in combination with other compounds, timing of administration, the tissue to which it is administered, and the physical delivery system in which it is carried.

[0042] A "fragment" of a nucleotide or peptide sequence is meant to refer to a sequence that is less than that believed to be the "full-length" sequence.

[0043] A "functional fragment" of a DNA, RNA, or protein sequence refers to a biologically active fragment of the sequence that is shorter than the full-length or reference DNA, RNA, or protein sequence, but which retains at least one biological activity (either functional or structural) that is substantially similar to a biological activity of the full-length or reference DNA, RNA, or protein sequence.

[0044] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0045] The term "human derived" as used herein refers to sequences that are found in a human genome (or a human genome build), or sequences homologous thereto. A homologous sequence may be a sequence which has a region with at least 80% sequence identity (e.g., as measured by BLAST) as compared to a region of the human genome. For example, a sequence that has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to a human sequence is deemed human derived. In some cases, a regulatory element contains a human derived sequence and a non-human derived sequence such that overall the regulatory element has low sequence identity to the human genome, while a part of the regulatory element has 100% sequence identity (or local sequence identity) to a sequence in the human genome.

[0046] The term "*in vitro*" refers to an event that takes places outside of a subject's body. For example, an *in vitro* assay encompasses any assay run outside of a subject. *In vitro* assays encompass cell-based assays in which cells alive or dead are employed. *In vitro* assays also encompass a cell-free assay in which no intact cells are employed.

[0047] The term "*in vivo*" refers to an event that takes place in a subject's body.

[0048] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally, at a chromosomal location that is different from its natural chromosomal location, or contains only coding sequences.

[0049] As used herein, "operably linked", "operable linkage", "operatively linked", or grammatical equivalents thereof refer to juxtaposition of genetic elements, e.g., a promoter, an enhancer, a polyadenylation sequence, etc., wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a regulatory element, which can comprise promoter and/or enhancer sequences, is operatively linked to a coding region if the regulatory element helps initiate transcription of the coding sequence. There may be intervening residues between the regulatory element and coding region so long as this functional relationship is maintained.

[0050] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation or composition, other than an active ingredient, which is nontoxic to a subject. A

pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0051] The terms "pharmaceutical formulation" or "pharmaceutical composition" refer to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0052] The term "regulatory element" refers to a nucleic acid sequence or genetic element which is capable of influencing (e.g., increasing, decreasing, or modulating) expression of an operably linked sequence, such as a gene, a coding sequence, or an RNA (e.g., an mRNA). Regulatory elements include, but are not limited to, promoter, enhancer, repressor, silencer, insulator sequences, an intron, UTR, an inverted terminal repeat (ITR) sequence, a long terminal repeat sequence (LTR), a stability element, a miRNA binding site, a posttranslational response element, or a polyA sequence, or a combination thereof. Regulatory elements can function at the DNA and/or the RNA level, e.g., by modulating gene expression at the transcriptional phase, post-transcriptional phase, or at the translational phase of gene expression; by modulating the level of translation (e.g., stability elements that stabilize mRNA for translation), RNA cleavage, RNA splicing, and/or transcriptional termination; by recruiting transcriptional factors to a coding region that increase gene expression; by increasing the rate at which RNA transcripts are produced, increasing or decreasing the stability of RNA produced, and/or increasing the rate of protein synthesis from RNA transcripts; and/or by preventing RNA degradation and/or increasing its stability to facilitate protein synthesis. In an exemplary embodiment, a regulatory element refers to an enhancer, repressor, promoter, or a combination thereof, particularly an enhancer plus promoter combination or a repressor plus promoter combination. In exemplary embodiments, the regulatory element is derived from a human sequence.

[0053] In general, "sequence identity" or "sequence homology", which can be used interchangeably, refer to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity", also referred to as "percent homology". The percent identity to a reference sequence (e.g., nucleic acid or amino acid sequence) may be calculated as the number of exact matches between two optimally aligned sequences divided by the length of the reference sequence and

multiplied by 100. Conservative substitutions are not considered as matches when determining the number of matches for sequence identity. It will be appreciated that where the length of a first sequence (A) is not equal to the length of a second sequence (B), the percent identity of A:B sequence will be different than the percent identity of B:A sequence. Sequence alignments, such as for the purpose of assessing percent identity, may be performed by any suitable alignment algorithm or program, including but not limited to the Needleman-Wunsch algorithm, the BLAST algorithm, the Smith-Waterman algorithm (see, e.g., the EMBOSS Water aligner), and Clustal Omega alignment program (F. Sievers et al., Mol Sys Biol. 7: 539 (2011)). Optimal alignment may be assessed using any suitable parameters of a chosen algorithm, including default parameters. The BLAST program is based on the alignment method of Karlin and Altschul, Proc. Natl. Acad. Sci. USA 87:2264-2268 (1990) and as discussed in Altschul, et al., J. Mol. Biol. 215:403-410 (1990); Karlin and Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5877 (1993); and Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997).

[0054] The terms "subject" and "individual" are used interchangeably herein to refer to a vertebrate, preferably a mammal, more preferably a human. The methods described herein can be useful in human therapeutics, veterinary applications, and/or preclinical studies in animal models of a disease or condition.

[0055] As used herein, the terms "treat", "treatment", "therapy" and the like refer to obtaining a desired pharmacologic and/or physiologic effect, including, but not limited to, alleviating, delaying or slowing progression, reducing effects or symptoms, preventing onset, preventing reoccurrence, inhibiting, ameliorating onset of a diseases or disorder, obtaining a beneficial or desired result with respect to a disease, disorder, or medical condition, such as a therapeutic benefit and/or a prophylactic benefit. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease or at risk of acquiring the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease. A therapeutic benefit includes eradication or amelioration of the underlying disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the subject, notwithstanding that the subject may still be afflicted with the

underlying disorder. In some cases, for prophylactic benefit, the compositions are administered to a subject at risk of developing a particular disease, or to a subject reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease may not have been made. The methods of the present disclosure may be used with any mammal. In some cases, the treatment can result in a decrease or cessation of symptoms. A prophylactic effect includes delaying or eliminating the appearance of a disease or condition, delaying or eliminating the onset of symptoms of a disease or condition, slowing, halting, or reversing the progression of a disease or condition, or any combination thereof.

[0056] A "variant" of a nucleotide sequence refers to a sequence having a genetic alteration or a mutation as compared to the most common wild-type DNA sequence (e.g., cDNA or a sequence referenced by its GenBank accession number) or a specified reference sequence (sometimes referred to herein as a "parent" sequence). A variant can be shorter or longer than the reference sequence and/or have one or more mutations relative to the reference sequence. In some cases, a variant may have a nucleotide sequence that is at least 80% identical, at least 90% identical or at least 95% identical to a reference sequence.

[0057] A "variant" of a polypeptide or protein sequence refers to a sequence having an amino acid difference as compared to a parent polypeptide or protein sequence, e.g., a wild-type polypeptide or protein sequence or a specified reference polypeptide or protein sequence. A variant can be shorter or longer than the parent sequence (i.e., include inserted or deleted amino acids) and/or have one or more substitutions relative to the parent sequence (i.e., a change of one or more amino acids in the parent sequence to a different amino acid). In some cases, a variant may have a polypeptide sequence that is at least 80% identical, at least 90% identical, or at least 95% identical to its parent sequence.

[0058] A "vector" as used herein refers to a nucleic acid molecule that can be used to mediate delivery of another nucleic acid molecule to which it is linked into a cell where it can be replicated or expressed. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors." Other examples of vectors include plasmids and viral vectors.

[0059] As used herein a "target cell" is generally a cell in which expression of RNA or protein product of the nucleic acid cassette is desired. A non-target cell is a cell in which expression of the RNA or protein product of the nucleic acid is not desired. As used herein "detargeting" generally refers to decreasing the expression in a non-target cell.

[0060] As used herein the term "SCN9A gene" refers to the sodium voltage-gated channel alpha subunit 9 gene which, in humans, is identified as Gene ID: 6335 in NCBI's Genbank database. This gene may be referred to as ETHA, FEB3B, GEFSP7, HSAN2D, NE-NA, NENA, Nav1.7, PN1, SFNP in other disclosures. The SCN9A gene encodes the Nav1.7 protein (see, e.g., Genbank accession no. NP_001352465.1), which is a voltage-gated sodium channel.

[0061] As used herein, the term "inhibitory RNA" refers to an RNA that decreases the expression of a target gene (which, in turn, results in the decrease of the protein encoded by the gene). Examples of inhibitory RNAs include miRNAs, shRNA, antisense oligonucleotides (ASOs), etc.

[0062] Unless otherwise indicated, all terms used herein have the same meaning as they would to one skilled in the art and the practice of the present invention will employ, conventional techniques of molecular biology, microbiology, and recombinant DNA technology, which are within the knowledge of those of skill of the art.

DETAILED DESCRIPTION

[0063] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, and as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0064] The upper and lower limits of ranges may independently be included in the ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0065] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and

materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0066] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" includes a plurality of such proteins and reference to "the nucleic acid" includes reference to one or more nucleic acids and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0067] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0068] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

[0069] As summarized above, the present disclosure relates to compositions, methods and processes for the design, preparation, manufacture, use and/or formulation of modulatory polynucleotides, e.g., polynucleotides encoding small interfering RNA (siRNA) molecules which target the transcript encoding the alpha (α) subunit of voltage-gated sodium channel (Na_v) 1.7 to

treat chronic neuropathic pain. In some embodiments, nucleic acid sequences encoding the siRNA molecules are inserted into recombinant adeno-associated virus (AAV) vectors. Methods for using the siRNA molecules to decrease the expression of mutated or wild-type $Na_v1.7$ in a subject with chronic pain (e.g., chronic neuropathic pain) are also disclosed.

[0070] Provided herein is a polynucleotide comprising a targeting region that binds to a target site in an endogenous mRNA encoding $Na_v1.7$, wherein the target site is from 10 to 30 nucleotides in length (e.g., 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 , 28, 29 or 30 nucleotides in length) and comprises a sequence having at least 90% sequence identity (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to any one of SEQ ID NOs: 1 to 59. In these embodiments, the endogenous mRNA is a transcript of an endogenous SCN9A gene. In some embodiments, the endogenous mRNA encoding $Na_v1.7$ may comprise the sequence having at least 95% sequence identity to SEQ ID NO: 305. In some embodiments, the target site may comprise the sequence of any one of SEQ ID NOs: 1 to 59.

[0071] In any embodiment, the targeting region may be at least 90% complementary (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary) to the target site. In any embodiment, the targeting region may be complementary to the target site with the optional exception of 1, 2, 3 or 4 mismatches.

[0072] In any embodiment, the targeting region may comprise a sequence having at least 90% sequence identity (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to any one of SEQ ID NOs: 61 to 119.

[0073] In any embodiment, the polynucleotide may comprises: at least one modified internucleoside linkage; at least one modified nucleoside; at least two different nucleoside residues selected from DNA, RNA, and arabino nucleic acid; or any combination thereof. In some embodiments, the oligonucleotide comprises at least 1 modified nucleoside, such as at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15 or at least 16 modified nucleosides. In an embodiment the oligonucleotide comprises from 1 to 10 modified nucleosides, such as from 2 to

9 modified nucleosides, such as from 3 to 8 modified nucleosides, such as from 4 to 7 modified nucleosides, such as 6 or 7 modified nucleosides.

[0074] In some embodiments, the oligonucleotide comprises at least one modified internucleoside linkage. In some embodiments, the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate or boranophosphate internucleoside linkages.

[0075] In some embodiments, the oligonucleotide is an RNA and does not comprise a modified nucleoside. In some cases, an RNA may be chemically synthesized, or may be expressed in a cell from a DNA template. In some embodiments, an RNA as described herein may be a miRNA, a siRNA, a shRNA, or an RNA oligonucleotide.

[0076] In any embodiment, the polynucleotide may selected from the group consisting of: a pri-miRNA, a pre-miRNA, a mature miRNA, an siRNA, an shRNA, and an antisense oligonucleotide. Such RNAs bind to a target sequence in another molecule such as an RNA transcript refers to an RNA molecule that can have an inhibitory effect on expression of the other molecule. Such inhibitory RNAs include but are not limited to antisense RNAs and inhibitory RNA (e.g., siRNAs and miRNAs). Such molecules are reviewed in a number of publications, including Hastings et al (RNA 2023 29: 393-395). Such RNA molecules can be made synthetically. Alternatively, RNA can be encoded by a transgene. If a transgene "encodes" such an RNA, then the transgene may contain all of the necessary sequence elements to effect expression of the RNA, or a precursor of the same that will be processed by the cell's endogenous machinery to produce the RNA.

[0077] Antisense oligonucleotides (ASOs) are small (~18–30 nucleotides) single-stranded nucleic acids of diverse chemistries, which can be employed to modulate gene expression via various mechanisms. ASOs can be subdivided into two major categories: RNase H competent and steric block. RnaseH competent ASOs comprise DNA. For RnaseH competent ASOs, the endogenous RNase H enzyme recognizes RNA–DNA heteroduplex substrates that are formed when DNA-based oligonucleotides bind to their cognate transcripts and catalyzes the degradation of the RNA. Cleavage at the site of ASO binding results in destruction of the target RNA, thereby silencing target gene expression.

[0078] Steric block oligonucleotides are typically RNA. These ASOs are designed to bind to target transcripts with high affinity but do not induce target transcript degradation as they lack

RNase H competence. Steric block oligonucleotides can mask specific sequences within a target transcript and thereby interfere with transcript RNA–RNA and/or RNA–protein interactions. The most widely used application of steric block ASOs is in the modulation of alternative splicing in order to selectively exclude or retain a specific exon. In these cases, the oligonucleotide 'masks' a splicing signal such that it becomes invisible to the spliceosome, leading to alterations in splicing.

[0079] ASOs are typically made synthetically, in which case they may contain any number of chemical modifications, including nucleobase modifications, terminal modifications and ribose sugar modifications (e.g., 2′-O-methoxyethyl or 2′-O-methyl bases) and can be directly administered, e.g., using a lipid-based carrier such as lipid nanoparticles.

[0080] Silencing RNAs, on the other hand, target the RNA transcript to which they bind for translational repression, destabilization or degradation, typically via the RISC complex. miRNAs (microRNAs) and siRNAs (small interfering RNAs) are types of silencing RNAs. Silencing RNAs are typically produced by transcribing a longer hairpin molecule (referred to as a 'pri-miRNA' or 'pre-miRNA' in the case of miRNAs or a 'shRNA' (short hairpin RNA) in the case of siRNAs) from an expression cassette in a cell, which is then processed by the cell's endogenous machinery. Pre- and pri-miRNAs are encoded by the human genome. miRNAs are initially transcribed as longer primary transcripts (or termed pri-miRNAs), containing a 60–120 nt RNA hairpin in which one of the two strands includes the miRNA. siRNAs can be designed using the sequence of a transcript. shRNAs have a 19–29 base pair stem, a small loop and 3′-terminal overhang, typically a UU overhang. In both cases, the hairpin is subsequently processed by Dicer to produce a duplex of 21 to 23 nucleotides and a 3' overhang. miRNAs have the same general structure as siRNAs, except that there may be mismatches in the duplex. Although either strand of the duplex may potentially act as a functional silencing RNA, only one strand is usually incorporated into the RNA-induced silencing complex (RISC) where the miRNA/siRNA and its target interact. Such molecules can also be produced by a 'mature' miRNA vector system that makes use of convergent promoters (e.g., the U6 and H1 promoters). A description of several strategies for expressing miRNAs and shRNAs can be found Fan et al (Cancer Gene Therapy 27: 424–437) and Herrera-Carrillo et al (Hum Gene Ther Methods 2017 28: 177–190).

[0081] Because strategies that rely on silencing RNA (particularly miRNAs) should be non-immunogenic and expression of the silencing RNA can be restricted to a particular tissue or cell-type by the use a tissue-specific or cell type-specific promoter, therapies that are based on administering silencing RNAs have the potential to have less side effects in certain cases. Further, multiple silencing RNAs can, in theory, be encoded on a single vector, allowing a single transcript to be targeted by multiple different silencing RNAs. Finally, silencing RNA-based strategies should result in long term effects, because, in theory, the vector should persist in the cells and should not diffuse away or degraded, which would be the case for certain other types of therapies.

Mismatches

[0082] miRNAs are known to regulate gene expression by binding to a target sequence. miRNAs contain a seed sequence, which is a conserved heptametrical sequence which is situated at positions 2-7 from the 5´-end of the miRNAs. The seed sequence should be perfectly complementary to the target sequence. The remainder of the miRNA sequence (i.e., the sequence that is 3' to the seed sequence) can be less than perfectly complementary to the target sequence. As such, in many cases, an RNA that 'binds', 'recognizes' or 'targets' a longer sequence may, in some embodiments, comprise 6, 7, 8, 9 or 10 contiguous nucleotides that perfectly base pair with the target sequence at the 5' end and a 3' and that contains mismatches. In any embodiment, an RNA of the invention may contain 0, 1, 2, 3 or 4 mismatches relative to the sequence to which it binds, particularly towards the 3' end.

Transgenes

[0083] In some embodiments, this disclosure provides a transgene which encodes an RNA. The transgene may comprise a sequence encoding the RNA as described herein and sequences that enable expression and processing of the RNA. In some cases, the transgene comprises a miRNA scaffold sequence. In some cases, the transgene comprises a sequence encoding a pri-miRNA. In some cases, the transgene comprises a sequence encoding a pre-miRNA. In some cases, the transgene comprises a sequence encoding an shRNA.

Scaffolds

[0084] In any embodiment, the RNA may be contained within a primary miRNA (pri-miRNA). In other words, the transgene may encode a primary miRNA (pri-miRNA) that comprises the RNA. In these embodiments, the term "pri-miRNA" is intended to describe any non-naturally

occurring RNA molecule that is efficiently processed by endogenous endonucleases to release the therapeutic RNA (e.g., by Drosha and/or Dicer and others) in a similar way to endogenous pri-miRNAs. Pri-miRNAs generally comprise a hairpin structure. In these embodiments, a pri-miRNA (which is a single molecule) may comprise the RNA sequence (which may be referred to as a "guide"), the complement of the RNA (with the optional exception of 1, 2, 3 or 4 nucleotide substitutions which produce "bulges") (which sequence may be referred to as a "passenger"), and a scaffold, where the scaffold comprises an optional 5' flanking sequence, a 5' stem, a loop, a 3' stem, and an optional 3' flanking sequence. In any embodiment, the scaffold may be derived from the scaffold of miR-E, miR-100, miR-130a, miR-132, miR-190/190a, or miR-451, the components parts of which are set forth in Table 2. In this context, the term "derived from" refers to a scaffold that has a nucleotide sequence that is at least 80% identical, at least 90% identical, or at least 95% identical to the scaffold. In these embodiments, the sequence of a scaffold may be the same as the scaffold of miR-E, miR-100, miR-130a, miR-132, miR-190, miR-190a, or miR-451, with the exception of up to 15 (e.g., up to 10, up to 8, or up to 5) nucleotide substitutions.

[0085] In some embodiments, a pri-miRNA may comprise an RNA of the present disclosure (e.g., any of SEQ ID NOS: 61 to 119, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions), the complement of the RNA (with the optional exception of 1, 2, 3, 4 or 5 nucleotide substitutions, which produce bulges) and the scaffold (which may comprises a 5' flanking sequence, a 5' stem, a loop, a 3' stem, and a 3' flanking sequence). When present in the context of a pri-miRNA, the RNA sequence is sometimes referred to herein as the "guide", "guide RNA", "guide strand", "guide sequence", or equivalent and the antisense of the RNA is sometimes referred to herein as the "passenger", "passenger RNA", "passenger strand", "passenger sequence", or equivalent. The guide RNA can be on either side of the loop. As such, in some embodiments, a pri-miRNA may comprise an optional 5' flanking sequence, a 5' stem, the guide RNA, a loop, the passenger RNA, a 3' stem, and an optional 3' flanking sequence. Alternatively, a pri-miRNA may comprise an optional 5' flanking sequence, a 5' stem, the passenger RNA, a loop, the guide RNA, a 3' stem, and an optional 3' flanking sequence. miRNA scaffolds are described in a variety of publications, including Xie et al. (Mol. Ther. 2020 28: 422–430), Bofill-De Ros et al. (Methods 2016 103: 157–166), Curtin et al. (Adv. Healthc. Mater. 2018 7) and Rao et al. (Adv. Drug Deliv. Rev. 2009 61: 746-59), Galka-Marciniak et al.

(Biochimica et Biophysica Acta 2016 1859: 639-649), and Fellman et al. (Cell 2013 5:1704-1713).

[0086] Table 1 provides examples of nucleotide substitutions in the complement of the targeting region (guide sequence) that may be included in different scaffolds.

TABLE 1

Scaffold	Nucleotide substitutions
miR-E	Bulge mismatch at base of RNA/antisense stem is formed by transversion of 1st base of antisense
miR-130a	Mismatch base 2, add GU wobble bases 18 and 19 (only if C at this position in the antisense, convert to a T), bulge mismatch transversion base 11, bulge mismatch transition base 22
miR-190a	Bulge mismatch transition base 21, bulge mismatch transversion base 22, 2bp deletion bases 4 and 5
miR-132	Mismatch transition base 2, mismatch transversion base 12 and 14

[0087] Mismatch refers to the following substitution rule: G -> C, C -> G, A -> T, T -> A. Bulge mismatch transition refers to the rule: T -> C, C - > A, A -> C, G-> A. Bulge mismatch transversion refers to the rule: G -> T, C -> A, A-> C, T -> G. Add GU wobble refers to the rule: If base is C, then convert to T.

[0088] Table 2 provides the annotated parts (regions) of several exemplary pri-miRNA scaffold sequences listed in 5' to 3' order in descending rows. The placement of a guide RNA sequence of the present disclosure and corresponding passenger RNA sequence of the present disclosure is indicated. The guide sequences of the present disclosure which target Sites 1 to 59 in SEQ ID NO:305 (SCN9A RNA transcript) are provided in Table 6 (both RNA and DNA). Examples of corresponding passenger sequences for the guide sequences in Table 6 are provided in Table 7 (both RNA and DNA). These guide/passenger pairs were used in the miR-E scaffold. Examples of passenger sequences for select guide sequences in different scaffolds are provided in Table 4. It is noted that the difference between scaffold miR-190 and miR-190a is in the loop sequence: miR-190 uses loop sequence of SEQ ID NO: 329 and miR-190a uses a loop sequence SEQ ID NO:330.

TABLE 2

Scaffold Name	Region	RNA sequence (5' to 3')	RNA SEQ ID NO	DNA SEQ ID NO
miR-E	5' Flank	UUGAAUGAGGCUUCAGUACUUU ACAGAAUCGUUGCCUGCACAUCU UGGAAACACUUGCUGGGAUUAC UUCGACUUCUUAACCCAACAGAA GGCUCGAGAAGGUAUAUUGCUG UU	307	337
	5' Stem	GACAGUGAGCG	308	338
	Passenger			
	Loop	UAGUGAAGCCACAGAUGUA	309	339
	Guide			
	3' Stem	UGCCUACUGCC	310	340
	3' Flank	UCGGACUUCAAGGGGCUAGAAU UCGAGCAAUUAUCUUGUUUACU AAAACUGAAUACCUUGCUAUCUC UUUGAUACAUUAUUACAAAGCU GAAUUAAAAUGGUAUAAAUUAA AUCAC	311	341
miR-100	5' Flank	CCCAAAAGAGAGAAGAUAUUGA GG	312	342
	5' Stem	CCUGUUGCCACA	313	343
	Guide			
	Loop	GUAUUAGUCCG	314	344
	Passenger			
	3' Stem	UGUGUCUGUUAGG	315	345
	3' Flank	CAAUCUCACGGACCUGGGGCUUU GCUUAUAUGCC	316	346
130a	5' Flank	GCAGGGCCGGCAUGCCUC	317	347
	5' Stem	UGCUGCUGGCCA	318	348
	Passenger			
	Loop	CUGUCUGCACCUGUCACUAG	319	349
	Guide			
	3' Stem	GUGGCCGUGUAGUG	320	350
	3' Flank	CUACCCAGCGCUGGCUGCCUCCU CAGCAUUG	321	351
132	5' Flank	GCCGUCCGCGCGCC	322	352

	5' Stem	CCGCCCCCGCGUCUCCAGGG	323	353
	Passenger			
	Loop	CUGUGGGAACUGGAGG	324	354
	Guide			
	3' Stem	CCCCGCAGCACGCCCACGCGC	325	355
	3' Flank	CGCGCCACGCCGCGCCCCGAGCC	326	356
miR-190	5' Flank	GAGCUCAGUCAAACCUGGAUGCCUUUUC	327	357
	5' Stem	UGCAGGCCUCUGUG	328	358
	Guide			
	Loop	UGUUAUUUAAUCCAC	329	359
	Passenger			
	3' Stem	CUACAGUGUCUUGCC	331	361
	3' Flank	CUGUCUCCGGGGGUUCCUAAUAAAG	332	362
miR-190a	5' Flank	GAGCUCAGUCAAACCUGGAUGCCUUUUC	327	357
	5' Stem	UGCAGGCCUCUGUG	328	359
	Guide			
	Loop	UGUUAUUUAAUCCA	330	360
	Passenger			
	3' Stem	CUACAGUGUCUUGCC	331	361
	3' Flank	CUGUCUCCGGGGGUUCCUAAUAAAG	332	362
miR-451	5' Flank	CUCUGCUCAGCCUGUCACAACCUACUGACUGCCAGGGCAC	333	363
	5' Stem	UUGGGAAUGGCAAGG	334	364
	Guide			
	Loop	*Loop is the four 3' nucleotides of the guide sequence*	NA	NA
	Passenger			
	3' Stem	UCUUGCUAUACCCAG	335	365
	3' Flank	AAAACGUGCCAGGAAGAGAACUCA	336	366

[0089] In some embodiments, the transgene may encode a pri-miRNA comprising:

(i) an optional 5' flanking sequence of SEQ ID NO: 307,

a 5' stem of SEQ ID NO: 308,
the complement of the RNA (with the optional exception of 1, 2, 3 or 4 nucleotide substitutions or bulges),
a loop of SEQ ID NO: 309,
an RNA of the present disclosure, e.g., any of SEQ ID NOS: 61 to 119, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions,
a 3' stem of SEQ ID NO: 310, and
an optional 3' flanking sequence of SEQ ID NO: 311;
(ii) an optional 5' flanking sequence of SEQ ID NO: 312,
a 5' stem of SEQ ID NO: 313,
an RNA of the present disclosure, e.g., any of SEQ ID NOS: 61 to 119, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions,
a loop of SEQ ID NO: 314,
the complement of the RNA (with the optional exception of 1, 2, 3 or 4 nucleotide substitutions or bulges),
a 3' stem of SEQ ID NO: 315, and
an optional 3' flanking sequence of SEQ ID NO: 316;
(iii) an optional 5' flanking sequence of SEQ ID NO: 317,
a 5' stem of SEQ ID NO: 318,
the complement of the RNA (with the optional exception of 1, 2, 3 or 4 nucleotide substitutions or bulges),
a loop of SEQ ID NO: 319,
an RNA of the present disclosure, e.g., any of SEQ ID NOS: 61 to 119, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions;
a 3' stem of SEQ ID NO: 320, and
an optional 3' flanking sequence of SEQ ID NO: 321;
(iv) an optional 5' flanking sequence of SEQ ID NO: 322,
a 5' stem of SEQ ID NO: 323,
the complement of the RNA (with the optional exception of 1, 2, 3 or 4 nucleotide substitutions or bulges),
a loop of SEQ ID NO: 324,

an RNA of the present disclosure, e.g., any of SEQ ID NOS: 61 to 119, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions,
a 3' stem of SEQ ID NO: 3235, and
an optional 3' flanking sequence of SEQ ID NO: 326;
(v) an optional 5' flanking sequence of SEQ ID NO: 327,
a 5' stem of SEQ ID NO: 328,
an RNA of the present disclosure, e.g., any of SEQ ID NOS: 61 to 119, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions;
a loop of SEQ ID NO: 329,
the complement of the RNA (with the optional exception of 1, 2, 3 or 4 nucleotide substitutions or bulges),
a 3' stem of SEQ ID NO: 331, and
an optional 3' flanking sequence of SEQ ID NO: 332; or
(vi) an optional 5' flanking sequence of SEQ ID NO: 327,
a 5' stem of SEQ ID NO: 328,
an RNA of the present disclosure, e.g., any of SEQ ID NOS: 61 to 119, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions,
a loop of SEQ ID NO: 330,
the complement of the RNA (with the optional exception of 1, 2, 3 or 4 nucleotide substitutions or bulges), and
a 3' stem of SEQ ID NO: 331, and
an optional 3' flanking sequence of SEQ ID NO: 332;
(vii) an optional 5' flanking sequence of SEQ ID NO: 333,
a 5' stem of sequence SEQ ID NO: 334,
an RNA of e.g., any of SEQ ID NOS: 61 to 119, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions (loop is 4 nucleotides of the RNA sequence),
the complement of the RNA (with the optional exception of 1, 2, 3 or 4 nucleotide substitutions or bulges,
a 3' stem of SEQ ID NO: 335, and
an optional 3' flanking sequence SEQ ID NO: 336.

[0090] In some embodiments, the transgene may encode a pri-miRNA comprising:

(i) an optional 5' flanking sequence of SEQ ID NO: 307;
a 5' stem of SEQ ID NO: 308,
an antisense (passenger) of SEQ ID NO: 183,
a loop of SEQ ID NO: 309,
an RNA (guide) RNA of 61,
a 3' stem of SEQ ID NO: 330, and
an optional 3' flanking sequence of SEQ ID NO: 331;
(ii) an optional 5' flanking sequence of SEQ ID NO: 307;
a 5' stem of SEQ ID NO: 308,
an antisense (passenger) of SEQ ID NO: 190,
a loop of SEQ ID NO: 309,
an RNA (guide) RNA of 68,
a 3' stem of SEQ ID NO: 330, and
an optional 3' flanking sequence of SEQ ID NO: 331;
(iii) an optional 5' flanking sequence of SEQ ID NO: 312,
a 5' stem of SEQ ID NO: 313,
an RNA (guide) of SEQ ID NO: 69,
a loop of SEQ ID NO: 262,
an antisense (passenger) of SEQ ID NO:403,
a 3' stem of SEQ ID NO: 263, and
an optional 3' flanking sequence of SEQ ID NO: 264.

[0091] In some embodiments, the transgene may encode a pri-miRNA comprising a sequence that is at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to any one of SEQ ID NOs: 310-333, shown in Table 3. Table 3 also provides SEQ ID NOs for the DNA sequence encoding these pri-miRNAs and the SEQ ID NOs for the guide and passenger RNA sequences.

TABLE 3

pri-mRNA Name	RNA Sequence	RNA SEQ ID NO	DNA SEQ ID NO	Guide RNA SEQ ID NO	Passenger RNA SEQ ID NO
miR-E-69	UUGAAUGAGGCUUCAGUAC UUUACAGAAUCGUUGCCUG	367	385	191	252

	CACAUCUUGGAAACACUUG CUGGGAUUACUUCGACUUC UUAACCCAACAGAAGGCUC GAGAAGGUAUAUUGCUGUU GACAGUGAGCGCUGUGCCU UAUUGUUUACAUGAUAGUG AAGCCACAGAUGUAUCAUG UAAACAAUAAGGCACAUUG CCUACUGCCUCGGACUUCA AGGGGCUAGAAUUCGAGCA AUUAUCUUGUUUACUAAAA CUGAAUACCUUGCUAUCUC UUUGAUACAUUAUUACAAA GCUGAAUUAAAAUGGUAUA AAUUAAAUCAC				
miR-E-61	UUGAAUGAGGCUUCAGUAC UUUACAGAAUCGUUGCCUG CACAUCUUGGAAACACUUG CUGGGAUUACUUCGACUUC UUAACCCAACAGAAGGCUC GAGAAGGUAUAUUGCUGUU GACAGUGAGCGGCAGAGUG CAGCACAGUUGAUAUAGUG AAGCCACAGAUGUAUAUCA ACUGUGCUGCACUCUGAUG CCUACUGCCUCGGACUUCA AGGGGCUAGAAUUCGAGCA AUUAUCUUGUUUACUAAAA CUGAAUACCUUGCUAUCUC UUUGAUACAUUAUUACAAA GCUGAAUUAAAAUGGUAUA AAUUAAAUCAC	368	386	183	244
miR-E-68	UUGAAUGAGGCUUCAGUAC UUUACAGAAUCGUUGCCUG CACAUCUUGGAAACACUUG CUGGGAUUACUUCGACUUC UUAACCCAACAGAAGGCUC GAGAAGGUAUAUUGCUGUU GACAGUGAGCGGUGCCCUC AUUGAACAACGCAUUAGUG AAGCCACAGAUGUAAUGCG UUGUUCAAUGAGGGCAAUG CCUACUGCCUCGGACUUCA AGGGGCUAGAAUUCGAGCA AUUAUCUUGUUUACUAAAA CUGAAUACCUUGCUAUCUC	369	387	190	251

	UUUGAUACAUUAUUACAAA GCUGAAUUAAAAUGGUAUA AAUUAAAUCAC				
miR-100-69	CCCAAAAGAGAGAAGAUAU UGAGGCCUGUUGCCACAUC AUGUAAACAAUAAGGCACA UGUAUUAGUCCGAUGUGCU UUAGUGUUUAUAUGUUGUG UCUGUUAGGCAAUCUCACG GACCUGGGGCUUUGCUUAU AUGCC	370	388	403	418
miR-100-61	CCCAAAAGAGAGAAGAUAU UGAGGCCUGUUGCCACAUA UCAACUGUGCUGCACUCUG AGUAUUAGUCCGUCAGAGC GCAUCACAGUCGAUUUGUG UCUGUUAGGCAAUCUCACG GACCUGGGGCUUUGCUUAU AUGCC	371	389	404	419
miR-100-68	CCCAAAAGAGAGAAGAUAU UGAGGCCUGUUGCCACAAU GCGUUGUUCAAUGAGGGCA AGUAUUAGUCCGUUGCCCC CAUGGAACAAUGCAAUGUG UCUGUUAGGCAAUCUCACG GACCUGGGGCUUUGCUUAU AUGCC	372	390	405	420
miR-130a-69	GCAGGGCCGGCAUGCCUCU GCUGCUGGCCAAAGUGCCU UAGUGUUUAUAUGGCUGUC UGCACCUGUCACUAGUCAU GUAAACAAUAAGGCACAUU GGCCGUGUAGUGCUACCCA GCGCUGGCUGCCUCCUCAG CAUUG	373	391	406	421
miR-130a-61	GCAGGGCCGGCAUGCCUCU GCUGCUGGCCAUGAGAGUG CAUCACAGUUGAUGCUGUC UGCACCUGUCACUAGUAUC AACUGUGCUGCACUCUGAU GGCCGUGUAGUGCUACCCA GCGCUGGCUGCCUCCUCAG CAUUG	374	392	407	422
miR-130a-68	GCAGGGCCGGCAUGCCUCU GCUGCUGGCCAUAGCCCUC AUGGAACAAUGCACCUGUC	375	393	408	423

	UGCACCUGUCACUAGAUGC GUUGUUCAAUGAGGGCAAU GGCCGUGUAGUGCUACCCA GCGCUGGCUGCCUCCUCAG CAUUG				
miR-132-69	GCCGUCCGCGCGCCCCGCC CCCGCGUCUCCAGGGACGU GCCUUAUGGGUUACAUGAC UGUGGGAACUGGAGGUCAU GUAAACAAUAAGGCACAUC CCCGCAGCACGCCCACGCG CCGCGCCACGCCGCGCCCC GAGCC	376	394	409	424
miR-132-61	GCCGUCCGCGCGCCCCGCC CCCGCGUCUCCAGGGUUAG AGUGCAGAAAAGUUGAUAC UGUGGGAACUGGAGGUAUC AACUGUGCUGCACUCUGAC CCCGCAGCACGCCCACGCG CCGCGCCACGCCGCGCCCC GAGCC	377	395	410	425
miR-132-68	GCCGUCCGCGCGCCCCGCC CCCGCGUCUCCAGGGUCGC CCUCAUUUACCAACGCAUC UGUGGGAACUGGAGGAUGC GUUGUUCAAUGAGGGCAAC CCCGCAGCACGCCCACGCG CCGCGCCACGCCGCGCCCC GAGCC	378	396	411	426
miR-190a-69	GAGCUCAGUCAAACCUGGA UGCCUUUUCUGCAGGCCUC UGUGUCAUGUAAACAAUAA GGCACAUUGUUAUUUAAUC CAAUGCCUUAUUGUUUACA UACCUACAGUGUCUUGCCC UGUCUCCGGGGGUUCCUAA UAAAG	379	397	412	427
miR-190a-61	GAGCUCAGUCAAACCUGGA UGCCUUUUCUGCAGGCCUC UGUGUAUCAACUGUGCUGC ACUCUGAUGUUAUUUAAUC CAUCAGUGCAGCACAGUUG ACCCUACAGUGUCUUGCCC UGUCUCCGGGGGUUCCUAA UAAAG	380	398	413	428

miR-190a-68	GAGCUCAGUCAAACCUGGA UGCCUUUUCUGCAGGCCUC UGUGAUGCGUUGUUCAAUG AGGGCAAUGUUAUUUAAUC CAUUGCUCAUUGAACAACG CGGCUACAGUGUCUUGCCC UGUCUCCGGGGGUUCCUAA UAAAG	381	399	414	429
miR-451-69	CUCUGCUCAGCCUGUCACA ACCUACUGACUGCCAGGGC ACUUGGGAAUGGCAAGGUC AUGUAAACAAUAAGGCACA UGCCUUAUUGUUUACAUGC UCUUGCUAUACCCAGAAAA CGUGCCAGGAAGAGAACUC A	382	400	415	430
miR-451-61	CUCUGCUCAGCCUGUCACA ACCUACUGACUGCCAGGGC ACUUGGGAAUGGCAAGGUA UCAACUGUGCUGCACUCUG AAGUGCAGCACAGUUGAUC UCUUGCUAUACCCAGAAAA CGUGCCAGGAAGAGAACUC A	383	401	416	431
miR-451-68	CUCUGCUCAGCCUGUCACA ACCUACUGACUGCCAGGGC ACUUGGGAAUGGCAAGGAU GCGUUGUUCAAUGAGGGCA ACCUCAUUGAACAACGCAG UCUUGCUAUACCCAGAAAA CGUGCCAGGAAGAGAACUC A	384	402	417	432

[0092] Passenger RNA and DNA sequences are provided in Table 4.

TABLE 4

Passenger Strand RNA Sequence (RNA encoded in vector)	SEQ ID NO	Passenger Strand DNA Sequence (present in expression vector)	SEQ ID NO
AUGUGCUUUAGUGUUUAUAUGU	403	ATGTGCTTTAGTGTTTATATGT	418
UCAGAGCGCAUCACAGUCGAUU	404	TCAGAGCGCATCACAGTCGATT	419

UUGCCCCCAUGGAACAAUGCAA	405	TTGCCCCCATGGAACAATGCAA	420
UCAUGUAAACAAUAAGGCACAU	406	TCATGTAAACAATAAGGCACAT	421
UAUCAACUGUGCUGCACUCUGA	407	TATCAACTGTGCTGCACTCTGA	422
AUGCGUUGUUCAAUGAGGGCAA	408	ATGCGTTGTTCAATGAGGGCAA	423
UCAUGUAAACAAUAAGGCACAU	409	TCATGTAAACAATAAGGCACAT	424
UAUCAACUGUGCUGCACUCUGA	410	TATCAACTGTGCTGCACTCTGA	425
AUGCGUUGUUCAAUGAGGGCAA	411	ATGCGTTGTTCAATGAGGGCAA	426
AUGCCUUAUUGUUUACAUAC	412	ATGCCTTATTGTTTACATAC	427
UCAGUGCAGCACAGUUGACC	413	TCAGTGCAGCACAGTTGACC	428
UUGCUCAUUGAACAACGCGG	414	TTGCTCATTGAACAACGCGG	429
GCCUUAUUGUUUACAUGC	415	GCCTTATTGTTTACATGC	430
AGUGCAGCACAGUUGAUC	416	AGTGCAGCACAGTTGATC	431

Expression cassettes

[0093] In some cases, a transgene may be contained within a nucleic acid expression cassette. An expression cassette may contain one or more additional regulatory elements (e.g., a promoter, a repressor, an insulator, a terminator, miRNA binding site, and/or an enhancer, etc.). In some instances, the regulatory element induces or represses expression of a transgene in a particular cell type, or a particular class of cell types, e.g., a central nervous system (CNS) cell or tissue selective promoter or enhancer (e.g., a Syn1 promoter [e.g., SEQ ID NO:449] or a nociceptor cell-selective promoter described herein [e.g., a combination of SEQ ID NOs:464, 465, and 455, e.g., SEQ ID NO:466]). For instance, a cell type selective regulatory element can induce gene expression in a particular cell type relative to one or more other cell types. Alternatively, or in addition, a cell type selective regulatory element can induce gene expression in a particular class of cells relative to one or more other classes of cells. In one embodiment, a cell type selective regulatory element of the invention enhances gene expression in a particular cell type, or a particular class of cells. In another embodiment, a cell type selective regulatory element suppresses gene expression in a particular cell type, or a particular class of cells. Cell type selective modulation of gene expression (e.g., enhancing or suppressing gene expression) does not require that gene expression is affected only in the target cell type or class of cells. Rather,

cell type selective modulation of gene expression (e.g., enhancing or suppressing gene expression) requires only that gene expression increase, or decrease, in the target cell type relative to one or more other cell types, or classes of cells.

[0094] In some embodiments, the regulatory element is constitutive, meaning that it does not demonstrate significantly different regulatory function in different cell types (for example, a human U6 promoter). As such, in certain embodiments, promoter that finds use in expression cassettes of the present disclosure have a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO:448 (a U6 promoter) or that contains a functional fragment of SEQ ID NO:448. Other constitutive promoter elements may be used, e.g., an EF1α promoter (e.g., SEQ ID NO:450), a CMV promoter, a CBA promoter (e.g., SEQ ID NO:479), and the like.

[0095] In certain embodiments, a promoter may be human derived or comprises a sequence that is human derived. In some cases, the promoter may be mouse derived or comprises a sequence that is mouse derived. In some cases, the promoter is non-naturally occurring or comprises a non-naturally occurring sequence. In some instances, the sequence of a promoter may be 100% human derived. In other instances, at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% of the promoter sequence is human derived. For example, a promoter can have 50% of its sequence derived from human, and the remaining 50% be non-human derived (e.g., mouse derived or fully synthetic).

[0096] In certain embodiments, the nucleic acid constructs described herein comprise another regulatory element in an addition to a promoter, such as, for example, sequences associated with transcription initiation or termination, enhancer sequences, and efficient RNA processing signals. Exemplary regulatory elements include, for example, an intron, an enhancer, UTR, stability element, WPRE sequence, a Kozak consensus sequence, or a combination thereof. Regulatory elements can function to modulate gene expression at the transcriptional phase or post-transcriptional phase of gene expression. At the RNA level, regulation can occur at the level of miRNA processing from pri-miRNA and pre-miRNA. In various embodiments, regulatory elements can recruit transcription factors that increase gene expression selectivity in a cell type of interest, increase the rate at which RNA transcripts are produced, and/or increase the rate of miRNA synthesis from RNA transcripts.

[0097] In certain embodiments, expression constructs include microRNA-based de-targeting elements that reduce expression of the transgene in a particular cell or tissue type. Examples

include de-targeting elements include those that reduce expression of an operably linked transcript in the liver (see, e.g., PCT/US2023/065801, published as WO2023/201354) as well as those that reduce expression of an operably linked transcript in dorsal root ganglion (DRG) cells of the CNS (see, e.g., PCT/US2019/067872, published as WO2020/132455; PCT/US2023/074878).

[0098] The cassette may be linear, circular and, in some embodiments, the nucleic acid cassette may be a vector such as a plasmid or viral vector, e.g., an adeno-associated virus (AAV) vector or lentiviral vector. The nucleic acid cassette may comprise sequences allowing for replication or packaging of the nucleic acid cassette. For example, a nucleic acid cassette may comprise viral vector sequences that allow for replication and/or packaging with capsid proteins. In certain embodiments, the viral vector may be an AAV vector selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hul4), AAV10, AAV11, AAV 12, AAV13, AAVrh8, AAVrhlO, AAV-DJ, and AAV-DJ8, and hybrids thereof. In some embodiments, the nucleic acid cassette may comprise an AAV ITR sequence. The AAV ITR sequence may be selected from an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hul4), AAV10, AAV11, AAV 12, AAV13, AAVrh8, AAVrhlO, AAV-DJ, or AAV-DJ8 ITR sequence, or may comprise a hybrid thereof. In some cases, the AAV ITR is an AAV2 ITR.

[0099] In any embodiment, the nucleic acid cassette may be non-naturally occurring, meaning that, for example, the miRNA sequence may be heterologous to the miRNA scaffold sequence. In any embodiment, the nucleic acid cassette may comprise a promoter and/or enhancer. In some embodiments, this nucleic acid cassette may be composed of a promoter, a coding sequence and a terminator, where the promoter, coding sequence and terminator are in operable linkage. In these embodiments, the promoter may be heterologous to the miRNA sequence, meaning that the promoter does not drive the expression of that miRNA sequence in a wild type cell. In any embodiment, the nucleic acid cassette may additionally comprise an enhancer.

Nociceptor-Selective Regulatory Element

[00100] Nociceptors are sensory neurons that responds to damaging or potentially damaging stimuli by sending "possible threat" signals to the spinal cord and the brain via action potentials driven by voltage-gated sodium channels, including Nav1.7. The brain creates the sensation of pain to direct attention to the body part, so the threat can be mitigated (a process called

nociception). Because of their involvement in pain signal propagation via Nav1.7 (encoded by the SCN9A gene), nociceptors are considered important target cells in aspects of the present disclosure.

[00101] In addition to Nav1.7, nociceptors express the Nav1.8 voltage-gated sodium channels encoded by the SCN10A gene, which also is involved in pain signal propagation. Nav1.8 is primarily expressed in small and medium diameter nociceptive neurons of the dorsal root ganglia (DRG) and cranial sensory ganglia (Akopian et al., Nature 1996, 379:257–262; and Djouhri et al., J. Physiol. 2003, 550:739–752). Regulatory element regions in the mouse SCN10A gene that provide nociceptive-selective gene expression have been investigated (Puhl et al., J Neurochem 2008, 106(3): 1209-1224).

[00102] The present disclosure provides nociceptor-selective regulatory element regions (promoter and/or enhancer regions) from the human SCN10A gene. In some cases, nociceptor-selective regulatory elements are associated with transgene expression levels in nociceptors that is higher than expression levels in non-nociceptor cell-types, including non-nociceptor neuronal cell types. In some cases, nociceptor-selective regulatory elements are associated with reduced gene expression in non-nociceptor cell types. In certain embodiments, nociceptor-selective regulatory elements result in expression levels of an operably-linked transgene that is about 1.5 times, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 7.5 times, 8 times, 9 times, or 10 times higher in nociceptors than in one or more non-target cell type, e.g., non-nociceptor neuronal cells in the CNS (e.g., DRG). In certain embodiments, the regulatory elements result in selective expression in nociceptors cells that is about 1.5 times, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 7.5 times, 8 times, 9 times, or 10 times higher than expected for natural distribution of nociceptor cells in the CNS.

[00103] In one aspect, nociceptor-selective regulatory elements provide herein can be operably linked to any heterologous transgene in an expression cassette to modulate gene expression in a cell, such as targeting expression of the transgene to nociceptors over one or more non-nociceptor cell type, e.g., nociceptors present in the CNS (e.g., DRG). In some cases, targeting expression of the transgene in a nociceptor includes increased gene expression in the nociceptor. Nociceptor-selective regulatory elements provided herein operably linked to a transgene can be part of an expression cassette, which can be a linear or a circular construct, a plasmid, a vector, a viral vector, e.g., a vector of an adeno-associated virus (AAV). Such expression cassettes can be

adapted for gene therapy or delivery into a subject (e.g., a human, a patient, or a mammal), e.g., for treatment of a disease or condition, e.g., neuropathic pain.

[00104] In some cases, selective expression of a transgene in nociceptors is used to treat a disease or condition associated with a genetic defect in an endogenous gene (gene therapy), wherein the genetic defect can be a mutation in the gene or dysregulation of the gene. Such genetic defect can result in a reduced or increased level of the gene product and/or a gene product with impaired function and/or activity or increased function and/or activity (e.g., hyperactivity). In some cases, an expression cassette comprises an RNAi agent (e.g., an engineered pri-miRNA specific for a target gene of interest as described herein), a gene, a subunit, a variant, or a functional fragment thereof, wherein gene expression from the expression cassette is used to treat the disease or condition associated with the genetic defect, impaired/increased function and/or activity, and/or dysregulation of the endogenous gene. In some cases, the disease or condition is neuropathic pain.

[00105] Selective expression generally refers to expression in a high fraction of cells of the cell type of interest (target cell type, e.g., nociceptors) as compared to other cells (or non-target cell type). Selective expression can also be viewed as preferential expression in a target cell or target cell type over one or more non-target cells or cell-types. In some cases, selective expression of one or more regulatory elements of this disclosure is compared to a known constitutive promoter or a non-selective regulatory element that is known to drive expression in any cell or cell type without selectivity (e.g., EF1α, U6, SV40, CMV, UBC, PGK, and CBA). In some cases, selective expression of one or more regulatory elements of this disclosure is compared to a constitutive promoter, a non-selective regulatory element, or an expression cassette without the regulatory elements (e.g., nociceptor-selective regulatory elements). In some cases, selective expression of one or more regulatory elements of this disclosure is compared to a regulatory element having selectivity for a different cell type or a broader range of cell types, e.g., a promoter that drives transgene expression in multiple different neuronal cell types, e.g., a Syn1 promoter.

[00106] Non-target cell types can include a different subset, subtype, or type of cells as compared to the target cell or target cell type, or all non-target cell types. In some cases, one or more regulatory elements operably linked to a gene result in selective expression in a target cell type over at least one type of non-target cells, or at least two, at least three, at least four, at least five,

or more than five types of non-target cells. In some cases, non-target cell types refer to all other cell types not including the target cell type. In some cases, non-target cell types are all other cell types within a relevant tissue or organ not including the target cell type, e.g., all non-target cell types in the CNS (e.g., all non-nociceptors in the DRG), etc. In some cases, a non-target cell or non-target cell type encompasses a subset or subtype of cells that is not the target cell. For example, non-nociceptor cell-types can include glutametargic neuronal cells. In some cases, cell types are distinguished by having a different cell marker, morphology, phenotype, genotype, function, and/or any other means for classifying cell types.

[00107] In some cases, nociceptor-selective regulatory elements of the present disclosure operably linked to a transgene result in selective expression of the transgene in nociceptors, wherein the percentage of nociceptors expressing the transgene is at a percentage higher than gene expression in nociceptors wherein the transgene is operably linked to an EF1α, a Syn1, a U6, and/or a CBA promoter. In some cases, nociceptor-selective regulatory elements of the present disclosure operably linked to a transgene result in selective expression of the transgene in nociceptors in the DRG, wherein the percentage of nociceptors expressing the transgene is at a percentage higher than gene expression in nociceptors wherein the transgene is operably linked to an EF1α, a Syn1, a U6, and/or a CBA promoter. In some cases, nociceptor-selective regulatory elements of the present disclosure operably linked to a transgene result in selective expression of the transgene in small-sized nociceptors of the DRG as compared to large-size myelinated non-nociceptor neurons, where the small-sized nociceptive neurons express peripherin and the large-size myelinated non-nociceptor neurons express neurofilament protein NF200.

[00108] In some cases, a nociceptor-selective regulatory element of the present disclosure comprises: (a) a combination of: (i) SEQ ID NOs:464, a functional fragment thereof, or sequences having at least 80%, at least 90%, at least 95%, or at least 99% sequence identity thereto; (ii) SEQ ID NO:465, a functional fragment thereof, or sequences having at least 80%, at least 90%, at least 95%, or at least 99% sequence identity thereto; (iii) and SEQ ID NO:455, a functional fragment thereof, or sequences having at least 80%, at least 90%, at least 95%, or at least 99% sequence identity thereto; or (b) SEQ ID NO:466, a functional fragment thereof, or sequences having at least 80%, at least 90%, at least 95%, or at least 99% sequence identity thereto. In some cases, nociceptor-selective regulatory elements comprise the regulatory element

of (a)(i) above, where the three sequences are operably linked to each other with or without nucleic acid linker sequences between them, e.g., a polynucleotide linker comprising 1-50 nucleotides. In some cases, the linker sequence is a human derived sequence. In some cases, the linker sequence is a result of restriction enzyme site, ligation, PCR, and/or cloning.

[00109] In certain aspects, the present disclosure provides a nucleic acid expression cassette comprising a nociceptor-selective regulatory element as described above operably linked to a transgene that results in selective expression of the transgene in nociceptor cells over one or more non-nociceptor cells.

[00110] In certain aspects, the present disclosure provides a method for selectively expressing a transgene in a nociceptor, comprising operably linking nucleic acid comprising a nociceptor-selective regulatory element as described above to the transgene to form an expression cassette and contacting the expression cassette to a cell population comprising nociceptor and non-nociceptor cells. In certain embodiments, the cells are in a subject, e.g., a subject being treated for a disease of disorder. In certain embodiments the nociceptor cells are in the central nervous system (CNS), e.g., the DRG. In certain embodiments, the transgene encodes an siRNA agent (e.g., a pri-miRNA of the present disclosure) that targets endogenous SCN9A transcript to reduce expression of Nav1.7. The present disclosure provides a method of treating a neurological condition or disorder in a subject in need thereof, the method comprising administering to the subject an expression cassette (e.g., in the form of an AAV vector) comprising: a nociceptor-selective regulatory element as described herein operably linked to a therapeutic transgene that results in selective expression of the therapeutic transgene in nociceptors in the subject. In certain embodiments, the neurological condition or disorder is neuropathic pain. In certain embodiments, the therapeutic transgene is an RNAi agent (e.g., a pri-miRNA) that targets an endogenous SCN9A transcript to reduce Nav1.8 expression.

Expression Cassette Examples

[00111] Examples of expression cassettes include those comprising a sequence that is at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to any one of SEQ ID NOs: 433, 436, 438, 441, 443, and 446, shown below:

[00112] SEQ ID NO:433 is a U6-miR-E-61 expression cassette (includes U6 promoter and pol-III terminator sequence) and has the following DNA sequence:

[00113] GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGT
TAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATA
CGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTA
AAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATA
TATCTTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATACTAGTCGA
CTAGGGATAACAGGGTAATTGTTTGAATGAGGCTTCAGTACTTTACAGAATCGTTGC
CTGCACATCTTGGAAACACTTGCTGGGATTACTTCGACTTCTTAACCCAACAGAAGG
CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGCAGAGTGCAGCACAGTTGATAT
AGTGAAGCCACAGATGTATATCAACTGTGCTGCACTCTGATGCCTACTGCCTCGGAC
TTCAAGGGGCTAGAATTCGAGCAATTATCTTGTTTACTAAAACTGAATACCTTGCTA
TCTCTTTGATACATTATTACAAAGCTGAATTAAAATGGTATAAATTAAATCACTTTTT
T

[00114] SEQ ID NO:436 is a nociceptor-selective miR-E-61 expression cassette (includes SEQ ID NO:466 and poly-A signal sequence) and has the following DNA sequence:

[00115] AGCAGGGCCTGGAAGAAAAAGTCAGAATTCCCCAGGCAGACAAACAGGGAG
TGGGGGGCAGCAGGTGAGCAAAAGGCCACCTTGAGGTGCTTGGGGATTGTCTTTCA
CACAGCTGTCCCATGCGCTCTGCCCAACCCAGGCTCTGTGCATGAGATGCTAATGAG
ATTTCTCTAAGAACAGGATGTTGTCCAAGAGCAAGGCTCATCTAATCTTTACCAGCA
TCCTAAGAAGTCCTGATTCTTATGTGACTCATTTAGCCAGCTTTTCCCCACAAGTGGT
ATCACAGGGAAATGACACGCTCCTTTTGGCACCAACTAATTTAAAGCACATTTGAGC
CATTTGATAATATATATTTTTAAATACTTGGCACTATTCCTTACTACCTTAAGCTGGT
ATATCAACCCTCGCTAACCTCTTCTCAGTAACTCAGGGCATTCACCTCTGCCCAACG
TGGGAAGGCAGGGAACCCTGTGAGGCCTCAGGAACATGCAGGGCTGCTGGCCAGGT
TACTAAGTGGTCCAGAAAAGCTGTGCTTTGAGTCTGTAGCTCTCCCATAGCCTTCTTC
CCTGCAGTCGTCACTTGTGTTCCAGCAGCTGCTGTTATCAGCTGCCATATTTTACATG
CCTTTGTCTAGGTAACAGCAGAGAGCATGAACTCTGTGACAGCTGTCTGCCACTCAG
AAGCATTTTCAGTTACTTTGGGGCTCTTAGGGAGCTCCAGGTGGCCAAGTTCCCTAC
AATTTTGACCTCTGGACAAATAACAATGGTCATGGCTAAAATCTATTGAGCCAAGCA
CTTCGTGCAAAGTGTTTTACAAGCGCTAACATATTTAGTCATCAAAACAGCTGAATG
ATGCAGATCTAGCCTTTTCAATTTTGAAGCTGGGAAAAATGAAGATTAGAGAGGTTA
AACAACTTGCCTAAGGTCACACAAGAGTGAGACTCCACAAGCTGTGGGACCTAAGA

TCAGTTCCCAAACCTCTCTGAGCCGCCATTTATTCATCTATGAAACTGAATGGTAAT
GCCTACTTGGTAGGATTATTTTGAAGCTTAAATGAGATGCTGCTTGTCAAATGTTTA
GGTCCTGCCTGAGACAAAGTAAGTGCCCAGGAAATGACAGCCAAGAAAAAAGGAA
ACGAAAGACAACGCACAAAGAAAGTCAAATATCTTTCAGAGCCAGCAAATAAGAGT
TGGAGGTGCTCACAGATGAAGAAGTTTTCCCTTGTGATTCTCCCCCACTTTCTTTTGT
CCACATCATTTTCTCTAGGCAAAAGTAGCCTGGGGCTAGGGAGAGGCTCTCTATAGG
TGAGTAGTGAAGTGACAGCCTCACAGAGACTGAGCTGGAAGATTAGAGGTTTAGGA
TTGTCTTATACAAGGTAAAAATAAATGTGGTTTCACTTACAAATCTTGAAGGAAACA
AGCTATAGGGAAAGAGGCATCTATAAAAGTTTAGCATCTTTAATAACACTGCCTTCA
ATTCAAGAATTTAATTTGACACCTAGCCACTGAGTGCTGACTTTGTGACAGGCACTG
GGCTCAACAGAGATAAGGAAGTCATAATTCATCCCCAAAGTTATCAAGCCCTTCAGC
TTGAAGAATGTCAGCTACTGGCTGTGTGCCCTAGGCAAGGCTGACCACAGCAGGGT
AAGATAAGAGGGAGGGCTGGGACGGGATTTGGGGGCTGTGGGTGAACAGAAGCCG
CTTGGTTCCCACTGGGTGAGGGCTGTCACATTCTGCACGTGGATGTCATGCTTCTTCC
CAAATGTTCCAAGGACTCCCCTGGCTAGTTGTCCCTACTCAATGTTTTCAGTACACA
ACTTCTGGGTGTTGGTTTTTTGTTTTTGTTTTCTTGTCCATGACCCCGGCTAGGCAGG
AGGGGCCTGACAGCGACAAATGGGGACCCGCCCCACCATTTGGGTCCATTAGCAAA
GCTATCAGGTTTACTAATATGAGAGCAACTTCAACCCCTAATTGAATTCATCTGGAG
ACATTATAGGGCTGGAGCTGCCAGGAGGGCAGCAGGCCCCTGCAGCATTACTCCAT
GATTAAATATTCAAGCCCAGTGAATGCTGAGAGTCGTTATGGGTGTAATTACGGTGT
CTCGGGCCATTGCCAGCACTCCATCAGGGGCCCCGAAGGTTTACAGTCTCACACAGC
TAAGCCTCTGGGGCTCCAGGGAGAAGAGCAGTGTTCCAGGCCTCAGCTGGCGAGGC
ACCAAACATAATGGAACCCTGACCCAGAGAAGCTGTTTTTTTGCTTGACTTGAGAGG
TTTTGAGTAAACAGAGTTGTAATGTAATAAAGCACCAAGCCAGTTTCCTCCTCTATT
AACTGGCATTGAGAGACAAGATGAATCAAGCAGCTGCAGGGGAGCTGGCTTGGCCT
GGGACCCCTTGCAGCACCTCCTCCACCTACCCCTCCCTTTTGGTCCTATGGCCCTGTG
GACAGGTTATTTTCTCCTGCTCTTTGTTTTGTTTAATCTCATCGGAGGTTTCAGATCTT
TTTTTTTTTTTTGTAATTTCATTTCAAACTATTATTTTATAAGACCTGGCCTATTACTG
AGTATGCAGGCAGAATATGAAAATTACTCCAAAACTTTTTTAAATGAAATTTTCAAG
ATGCAAAAAGTGAAACTTTAAAATTTCAGTGGAAGAAGGGGAACAAAAACATTTTA
ATAAATGAGAGTGTTTATTCCAGAATGGGAATATAGAGACAAGGAAGGTACCATGT

GAATGGGTGCACCTCGCTCTCTGGGGTCAATGATAGGAAACAGCCTGTCCCACAGTC
AAGGCAGCCTTGCCCAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGTTATCTAA
GTGCAGCCTCTTTGCTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTAAAATCAC
ATTTGCTATTTTACCATCTAATCACACATAAGCCTCTCCCCACACTCCCCCCGCCCTG
TTTCCATCCAAGGAGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAACTCGTGAC
GGGAAATGGGAATGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGAATGATAG
CAAGGCTCCCATCAGACAAGCTGAGCCACTGTCACTGAGGAGGACAAACGAGTGCA
AGTCTTTGCAGAGCTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCACACACTAG
CCTCTTGGCTAGAGAACAGACATCAGATGGAGTTTCTTCTGGCTATGCCTGAATGTT
AAGCTGAACGTATGTTCCAGGAGCTCGTGGTCTCCAGTAGAGGCAATCTGGGATAG
AAGAGAAGATATTTCTTACGTAGAAGACAAGCAAGATTGAGCAGGTGCTCGCTTCG
GCAGCACATATACTAGTCGACTAGGGATAACAGGGTAATTGTTTGAATGAGGCTTCA
GTACTTTACAGAATCGTTGCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCGA
CTTCTTAACCCAACAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGCA
GAGTGCAGCACAGTTGATATAGTGAAGCCACAGATGTATATCAACTGTGCTGCACTC
TGATGCCTACTGCCTCGGACTTCAAGGGGCTAGAATTCGAGCAATTATCTTGTTTAC
TAAAACTGAATACCTTGCTATCTCTTTGATACATTATTACAAAGCTGAATTAAAATG
GTATAAATTAAATCACAATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGTTTT
TTGTGTG

[00116] SEQ ID NO:438 is a U6-miR-E-68 expression cassette (includes U6 promoter and pol-III terminator sequence) and has the following DNA sequence:

[00117] GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGT
TAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATA
CGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTA
AAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATA
TATCTTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATACTAGTCGA
CTAGGGATAACAGGGTAATTGTTTGAATGAGGCTTCAGTACTTTACAGAATCGTTGC
CTGCACATCTTGGAAACACTTGCTGGGATTACTTCGACTTCTTAACCCAACAGAAGG
CTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGTGCCCTCATTGAACAACGCATTA
GTGAAGCCACAGATGTAATGCGTTGTTCAATGAGGGCAATGCCTACTGCCTCGGACT

TCAAGGGGCTAGAATTCGAGCAATTATCTTGTTTACTAAAACTGAATACCTTGCTAT
CTCTTTGATACATTATTACAAAGCTGAATTAAAATGGTATAAATTAAATCACTTTTTT

[00118] SEQ ID NO:441 is a nociceptor-selective miR-E-68 expression cassette (includes SEQ ID NO:466 and poly-A signal sequence) and has the following DNA sequence:

[00119] AGCAGGGCCTGGAAGAAAAAGTCAGAATTCCCCAGGCAGACAAACAGGGAG
TGGGGGGCAGCAGGTGAGCAAAAGGCCACCTTGAGGTGCTTGGGGATTGTCTTTCA
CACAGCTGTCCCATGCGCTCTGCCCAACCCAGGCTCTGTGCATGAGATGCTAATGAG
ATTTCTCTAAGAACAGGATGTTGTCCAAGAGCAAGGCTCATCTAATCTTTACCAGCA
TCCTAAGAAGTCCTGATTCTTATGTGACTCATTTAGCCAGCTTTTCCCCACAAGTGGT
ATCACAGGGAAATGACACGCTCCTTTTGGCACCAACTAATTTAAAGCACATTTGAGC
CATTTGATAATATATATTTTTAAATACTTGGCACTATTCCTTACTACCTTAAGCTGGT
ATATCAACCCTCGCTAACCTCTTCTCAGTAACTCAGGGCATTCACCTCTGCCCAACG
TGGGAAGGCAGGGAACCCTGTGAGGCCTCAGGAACATGCAGGGCTGCTGGCCAGGT
TACTAAGTGGTCCAGAAAAGCTGTGCTTTGAGTCTGTAGCTCTCCCATAGCCTTCTTC
CCTGCAGTCGTCACTTGTGTTCCAGCAGCTGCTGTTATCAGCTGCCATATTTTACATG
CCTTTGTCTAGGTAACAGCAGAGAGCATGAACTCTGTGACAGCTGTCTGCCACTCAG
AAGCATTTTCAGTTACTTTGGGGCTCTTAGGGAGCTCCAGGTGGCCAAGTTCCCTAC
AATTTTGACCTCTGGACAAATAACAATGGTCATGGCTAAAATCTATTGAGCCAAGCA
CTTCGTGCAAAGTGTTTTACAAGCGCTAACATATTTAGTCATCAAAACAGCTGAATG
ATGCAGATCTAGCCTTTTCAATTTTGAAGCTGGGAAAAATGAAGATTAGAGAGGTTA
AACAACTTGCCTAAGGTCACACAAGAGTGAGACTCCACAAGCTGTGGGACCTAAGA
TCAGTTCCCAAACCTCTCTGAGCCGCCATTTATTCATCTATGAAACTGAATGGTAAT
GCCTACTTGGTAGGATTATTTTGAAGCTTAAATGAGATGCTGCTTGTCAAATGTTTA
GGTCCTGCCTGAGACAAAGTAAGTGCCCAGGAAATGACAGCCAAGAAAAAAGGAA
ACGAAAGACAACGCACAAAGAAAGTCAAATATCTTTCAGAGCCAGCAAATAAGAGT
TGGAGGTGCTCACAGATGAAGAAGTTTTCCCTTGTGATTCTCCCCCACTTTCTTTTGT
CCACATCATTTTCTCTAGGCAAAAGTAGCCTGGGGCTAGGGAGAGGCTCTCTATAGG
TGAGTAGTGAAGTGACAGCCTCACAGAGACTGAGCTGGAAGATTAGAGGTTTAGGA
TTGTCTTATACAAGGTAAAAATAAATGTGGTTTCACTTACAAATCTTGAAGGAAACA
AGCTATAGGGAAAGAGGCATCTATAAAAGTTTAGCATCTTTAATAACACTGCCTTCA
ATTCAAGAATTTAATTTGACACCTAGCCACTGAGTGCTGACTTTGTGACAGGCACTG

GGCTCAACAGAGATAAGGAAGTCATAATTCATCCCCAAAGTTATCAAGCCCTTCAGC
TTGAAGAATGTCAGCTACTGGCTGTGTGCCCTAGGCAAGGCTGACCACAGCAGGGT
AAGATAAGAGGGAGGGCTGGGACGGGATTTGGGGGCTGTGGGTGAACAGAAGCCG
CTTGGTTCCCACTGGGTGAGGGCTGTCACATTCTGCACGTGGATGTCATGCTTCTTCC
CAAATGTTCCAAGGACTCCCCTGGCTAGTTGTCCCTACTCAATGTTTTCAGTACACA
ACTTCTGGGTGTTGGTTTTTTGTTTTTGTTTTCTTGTCCATGACCCCGGCTAGGCAGG
AGGGGCCTGACAGCGACAAATGGGGACCCGCCCCACCATTTGGGTCCATTAGCAAA
GCTATCAGGTTTACTAATATGAGAGCAACTTCAACCCCTAATTGAATTCATCTGGAG
ACATTATAGGGCTGGAGCTGCCAGGAGGGCAGCAGGCCCCTGCAGCATTACTCCAT
GATTAAATATTCAAGCCCAGTGAATGCTGAGAGTCGTTATGGGTGTAATTACGGTGT
CTCGGGCCATTGCCAGCACTCCATCAGGGGCCCCGAAGGTTTACAGTCTCACACAGC
TAAGCCTCTGGGGCTCCAGGGAGAAGAGCAGTGTTCCAGGCCTCAGCTGGCGAGGC
ACCAAACATAATGGAACCCTGACCCAGAGAAGCTGTTTTTTTGCTTGACTTGAGAGG
TTTTGAGTAAACAGAGTTGTAATGTAATAAAGCACCAAGCCAGTTTCCTCCTCTATT
AACTGGCATTGAGAGACAAGATGAATCAAGCAGCTGCAGGGGAGCTGGCTTGGCCT
GGGACCCCTTGCAGCACCTCCTCCACCTACCCCTCCCTTTTGGTCCTATGGCCCTGTG
GACAGGTTATTTTCTCCTGCTCTTTGTTTTGTTTAATCTCATCGGAGGTTTCAGATCTT
TTTTTTTTTTTTGTAATTTCATTTCAAACTATTATTTTATAAGACCTGGCCTATTACTG
AGTATGCAGGCAGAATATGAAAATTACTCCAAAACTTTTTTAAATGAAATTTTCAAG
ATGCAAAAAGTGAAACTTTAAAATTTCAGTGGAAGAAGGGGAACAAAAACATTTTA
ATAAATGAGAGTGTTTATTCCAGAATGGGAATATAGAGACAAGGAAGGTACCATGT
GAATGGGTGCACCTCGCTCTCTGGGGTCAATGATAGGAAACAGCCTGTCCCACAGTC
AAGGCAGCCTTGCCCAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGTTATCTAA
GTGCAGCCTCTTTGCTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTAAAATCAC
ATTTGCTATTTTACCATCTAATCACACATAAGCCTCTCCCCACACTCCCCCCGCCCTG
TTTCCATCCAAGGAGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAACTCGTGAC
GGGAAATGGGAATGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGAATGATAG
CAAGGCTCCCATCAGACAAGCTGAGCCACTGTCACTGAGGAGGACAAACGAGTGCA
AGTCTTTGCAGAGCTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCACACACTAG
CCTCTTGGCTAGAGAACAGACATCAGATGGAGTTTCTTCTGGCTATGCCTGAATGTT
AAGCTGAACGTATGTTCCAGGAGCTCGTGGTCTCCAGTAGAGGCAATCTGGGATAG

AAGAGAAGATATTTCTTACGTAGAAGACAAGCAAGATTGAGCAGTGACGGATCCAC
CGGTCGACTTCGAATAGGGATAACAGGGTAATTGTTTGAATGAGGCTTCAGTACTTT
ACAGAATCGTTGCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCGACTTCTTA
ACCCAACAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGTGCCCTCATT
GAACAACGCATTAGTGAAGCCACAGATGTAATGCGTTGTTCAATGAGGGCAATGCC
TACTGCCTCGGACTTCAAGGGGCTAGAATTCGAGCAATTATCTTGTTTACTAAAACT
GAATACCTTGCTATCTCTTTGATACATTATTACAAAGCTGAATTAAAATGGTATAAA
TTAAATCACATAATTAATGGTACCAGCTAGCGCTATAGTCAATAAAAGATCTTTATT
TTCATTAGATCTGTGTGTTGGTTTTTTGTGTG

[00120] SEQ ID NO:443 is a U6-miR-100-69 expression cassette (includes U6 promoter and pol-III terminator sequence) and has the following DNA sequence:

[00121] GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGT
TAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATA
CGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTA
AAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATA
TATCTTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATAGCTAGCAC
TAGTCGCCCAAAAGAGAGAAGATATTGAGGCCTGTTGCCACATCATGTAAACAATA
AGGCACATGTATTAGTCCGATGTGCTTTAGTGTTTATATGTTGTGTCTGTTAGGCAAT
CTCACGGACCTGGGGCTTTGCTTATATGCCTTTTTT

[00122] SEQ ID NO:446 is a nociceptor-selective miR-100-69 expression cassette (includes SEQ ID NO:466 and poly-A signal sequence) and has the following DNA sequence:

[00123] AGCAGGGCCTGGAAGAAAAAGTCAGAATTCCCCAGGCAGACAAACAGGGAG
TGGGGGGCAGCAGGTGAGCAAAAGGCCACCTTGAGGTGCTTGGGGATTGTCTTTCA
CACAGCTGTCCCATGCGCTCTGCCCAACCCAGGCTCTGTGCATGAGATGCTAATGAG
ATTTCTCTAAGAACAGGATGTTGTCCAAGAGCAAGGCTCATCTAATCTTTACCAGCA
TCCTAAGAAGTCCTGATTCTTATGTGACTCATTTAGCCAGCTTTTCCCCACAAGTGGT
ATCACAGGGAAATGACACGCTCCTTTTGGCACCAACTAATTTAAAGCACATTTGAGC
CATTTGATAATATATATTTTTAAATACTTGGCACTATTCCTTACTACCTTAAGCTGGT
ATATCAACCCTCGCTAACCTCTTCTCAGTAACTCAGGGCATTCACCTCTGCCCAACG
TGGGAAGGCAGGGAACCCTGTGAGGCCTCAGGAACATGCAGGGCTGCTGGCCAGGT
TACTAAGTGGTCCAGAAAAGCTGTGCTTTGAGTCTGTAGCTCTCCCATAGCCTTCTTC

CCTGCAGTCGTCACTTGTGTTCCAGCAGCTGCTGTTATCAGCTGCCATATTTTACATG
CCTTTGTCTAGGTAACAGCAGAGAGCATGAACTCTGTGACAGCTGTCTGCCACTCAG
AAGCATTTTCAGTTACTTTGGGGCTCTTAGGGAGCTCCAGGTGGCCAAGTTCCCTAC
AATTTTGACCTCTGGACAAATAACAATGGTCATGGCTAAAATCTATTGAGCCAAGCA
CTTCGTGCAAAGTGTTTTACAAGCGCTAACATATTTAGTCATCAAAACAGCTGAATG
ATGCAGATCTAGCCTTTTCAATTTTGAAGCTGGGAAAAATGAAGATTAGAGAGGTTA
AACAACTTGCCTAAGGTCACACAAGAGTGAGACTCCACAAGCTGTGGGACCTAAGA
TCAGTTCCCAAACCTCTCTGAGCCGCCATTTATTCATCTATGAAACTGAATGGTAAT
GCCTACTTGGTAGGATTATTTTGAAGCTTAAATGAGATGCTGCTTGTCAAATGTTTA
GGTCCTGCCTGAGACAAAGTAAGTGCCCAGGAAATGACAGCCAAGAAAAAAGGAA
ACGAAAGACAACGCACAAAGAAAGTCAAATATCTTTCAGAGCCAGCAAATAAGAGT
TGGAGGTGCTCACAGATGAAGAAGTTTTCCCTTGTGATTCTCCCCCACTTTCTTTTGT
CCACATCATTTTCTCTAGGCAAAAGTAGCCTGGGGCTAGGGAGAGGCTCTCTATAGG
TGAGTAGTGAAGTGACAGCCTCACAGAGACTGAGCTGGAAGATTAGAGGTTTAGGA
TTGTCTTATACAAGGTAAAAATAAATGTGGTTTCACTTACAAATCTTGAAGGAAACA
AGCTATAGGGAAAGAGGCATCTATAAAAGTTTAGCATCTTTAATAACACTGCCTTCA
ATTCAAGAATTTAATTTGACACCTAGCCACTGAGTGCTGACTTTGTGACAGGCACTG
GGCTCAACAGAGATAAGGAAGTCATAATTCATCCCCAAAGTTATCAAGCCCTTCAGC
TTGAAGAATGTCAGCTACTGGCTGTGTGCCCTAGGCAAGGCTGACCACAGCAGGGT
AAGATAAGAGGGAGGGCTGGGACGGGATTTGGGGGCTGTGGGTGAACAGAAGCCG
CTTGGTTCCCACTGGGTGAGGGCTGTCACATTCTGCACGTGGATGTCATGCTTCTTCC
CAAATGTTCCAAGGACTCCCCTGGCTAGTTGTCCCTACTCAATGTTTTCAGTACACA
ACTTCTGGGTGTTGGTTTTTTGTTTTTGTTTTCTTGTCCATGACCCCGGCTAGGCAGG
AGGGGCCTGACAGCGACAAATGGGGACCCGCCCCACCATTTGGGTCCATTAGCAAA
GCTATCAGGTTTACTAATATGAGAGCAACTTCAACCCCTAATTGAATTCATCTGGAG
ACATTATAGGGCTGGAGCTGCCAGGAGGGCAGCAGGCCCCTGCAGCATTACTCCAT
GATTAAATATTCAAGCCCAGTGAATGCTGAGAGTCGTTATGGGTGTAATTACGGTGT
CTCGGGCCATTGCCAGCACTCCATCAGGGGCCCCGAAGGTTTACAGTCTCACACAGC
TAAGCCTCTGGGGCTCCAGGGAGAAGAGCAGTGTTCCAGGCCTCAGCTGGCGAGGC
ACCAAACATAATGGAACCCTGACCCAGAGAAGCTGTTTTTTTGCTTGACTTGAGAGG
TTTTGAGTAAACAGAGTTGTAATGTAATAAAGCACCAAGCCAGTTTCCTCCTCTATT

AACTGGCATTGAGAGACAAGATGAATCAAGCAGCTGCAGGGGAGCTGGCTTGGCCT
GGGACCCCTTGCAGCACCTCCTCCACCTACCCCTCCCTTTTGGTCCTATGGCCCTGTG
GACAGGTTATTTTCTCCTGCTCTTTGTTTTGTTTAATCTCATCGGAGGTTTCAGATCTT
TTTTTTTTTTTTTGTAATTTCATTTCAAACTATTATTTTATAAGACCTGGCCTATTACTG
AGTATGCAGGCAGAATATGAAAATTACTCCAAAACTTTTTTAAATGAAATTTTCAAG
ATGCAAAAAGTGAAACTTTAAAATTTCAGTGGAAGAAGGGGAACAAAAACATTTTA
ATAAATGAGAGTGTTTATTCCAGAATGGGAATATAGAGACAAGGAAGGTACCATGT
GAATGGGTGCACCTCGCTCTCTGGGGTCAATGATAGGAAACAGCCTGTCCCACAGTC
AAGGCAGCCTTGCCCAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGTTATCTAA
GTGCAGCCTCTTTGCTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTAAAATCAC
ATTTGCTATTTTACCATCTAATCACACATAAGCCTCTCCCCACACTCCCCCCGCCCTG
TTTCCATCCAAGGAGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAACTCGTGAC
GGGAAATGGGAATGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGAATGATAG
CAAGGCTCCCATCAGACAAGCTGAGCCACTGTCACTGAGGAGGACAAACGAGTGCA
AGTCTTTGCAGAGCTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCACACACTAG
CCTCTTGGCTAGAGAACAGACATCAGATGGAGTTTCTTCTGGCTATGCCTGAATGTT
AAGCTGAACGTATGTTCCAGGAGCTCGTGGTCTCCAGTAGAGGCAATCTGGGATAG
AAGAGAAGATATTTCTTACGTAGAAGACAAGCAAGATTGAGCAGTGACGGATCCAC
CGGTCGACTTCGAATAGGGATAACAGGGTAATTGTCCCAAAAGAGAGAAGATATTG
AGGCCTGTTGCCACATCATGTAAACAATAAGGCACATGTATTAGTCCGATGTGCTTT
AGTGTTTATATGTTGTGTCTGTTAGGCAATCTCACGGACCTGGGGCTTTGCTTATATG
CCATAATTAATGGTACCAGCTAGCGCTATAGTCAATAAAAGATCTTTATTTTCATTA
GATCTGTGTGTTGGTTTTTTGTGTG

Vectors

[00124] Expression vectors may be used to deliver the nucleic acid molecule to a target cell via transfection or transduction. A vector may be an integrating or non-integrating vector, referring to the ability of the vector to integrate the expression cassette or transgene into the genome of the host cell. Examples of expression vectors include, but are not limited to, (a) non-viral vectors such as nucleic acid vectors including linear oligonucleotides and circular plasmids; artificial chromosomes such as human artificial chromosomes (HACs), yeast artificial chromosomes

(YACs), and bacterial artificial chromosomes (BACs or PACs)); episomal vectors; transposons (e.g., PiggyBac); and (b) viral vectors such as retroviral vectors, lentiviral vectors, adenoviral vectors, and adeno-associated viral vectors.

[00125] Expression vectors may be linear oligonucleotides or circular plasmids and can be delivered to a cell via various transfection methods, including physical and chemical methods. Physical methods generally refer to methods of delivery employing a physical force to counteract the cell membrane barrier in facilitating intracellular delivery of genetic material. Examples of physical methods include the use of a needle, ballistic DNA, electroporation, sonoporation, photoporation, magnetofection, and hydroporation. Chemical methods generally refer to methods in which chemical carriers deliver a nucleic acid molecule to a cell and may include inorganic particles, lipid-based vectors, polymer-based vectors and peptide-based vectors.

[00126] In some embodiments, an expression vector is administered to a target cell using an inorganic particle. Inorganic particles may refer to nanoparticles, such as nanoparticles that are engineered for various sizes, shapes, and/or porosity to escape from the reticuloendothelial system or to protect an entrapped molecule from degradation. Inorganic nanoparticles can be prepared from metals (e.g., iron, gold, and silver), inorganic salts, or ceramics (e.g., phosphate or carbonate salts of calcium, magnesium, or silicon). The surface of these nanoparticles can be coated to facilitate DNA binding or targeted gene delivery. Magnetic nanoparticles (e.g., supermagnetic iron oxide), fullerenes (e.g., soluble carbon molecules), carbon nanotubes (e.g., cylindrical fullerenes), quantum dots and supramolecular systems may also be used.

[00127] In some embodiments, an expression vector is administered to a target cell using a cationic lipid (e.g., cationic liposome). Various types of lipids have been investigated for gene delivery, such as, for example, a lipid nano emulsion (e.g., which is a dispersion of one immiscible liquid in another stabilized by emulsifying agent) or a solid lipid nanoparticle.

[00128] In some embodiments, an expression vector is administered to a target cell using a peptide-based delivery vehicle. Peptide based delivery vehicles can have advantages of protecting the genetic material to be delivered, targeting specific cell receptors, disrupting endosomal membranes and delivering genetic material into a nucleus. In some embodiments, an expression vector is administered to a target cell using a polymer-based delivery vehicle. Polymer based delivery vehicles may comprise natural proteins, peptides and/or polysaccharides or synthetic polymers. In one embodiment, a polymer-based delivery vehicle comprises

polyethylenimine (PEI). PEI can condense DNA into positively charged particles which bind to anionic cell surface residues and are brought into the cell via endocytosis. In other embodiments, a polymer based delivery vehicle may comprise poly-L-lysine (PLL), poly (DL-lactic acid) (PLA), poly (DL-lactide-co-glycoside) (PLGA), polyornithine, polyarginine, histones, protamines, dendrimers, chitosans, synthetic amino derivatives of dextran, and/or cationic acrylic polymers. In certain embodiments, polymer-based delivery vehicles may comprise a mixture of polymers, such as, for example PEG and PLL.

[00129] In certain embodiments, an expression vector may be a viral vector suitable for gene therapy. Preferred characteristics of viral gene therapy vectors or gene delivery vectors may include the ability to be reproducibly and stably propagated and purified to high titres; to mediate targeted delivery (e.g., to deliver the transgene specifically to the tissue or organ of interest without widespread vector dissemination elsewhere); and to mediate gene delivery and transgene expression without inducing harmful side effects.

[00130] Several types of viruses, for example the non-pathogenic parvovirus referred to as adeno-associated virus, have been engineered for the purposes of gene therapy by harnessing the viral infection pathway but avoiding the subsequent expression of viral genes that can lead to replication and toxicity. Such viral vectors can be obtained by deleting all, or some, of the coding regions from the viral genome, but leaving intact those sequences (e.g., terminal repeat sequences) that may be necessary for functions such as packaging the vector genome into the virus capsid or the integration of vector nucleic acid (e.g., DNA) into the host chromatin.

[00131] In various embodiments, suitable viral vectors include retroviruses (e.g., A-type, B-type, C-type, and D-type viruses), adenovirus, parvovirus (e.g. adeno-associated viruses or AAV), coronavirus, negative strand RNA viruses such as orthomyxovirus (e.g., influenza virus), rhabdovirus (e.g., rabies and vesicular stomatitis virus), paramyxovirus (e. g. measles and Sendai), positive strand RNA viruses such as picornavirus and alphavirus, and double-stranded DNA viruses including adenovirus, herpesvirus (e.g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e.g., vaccinia, fowlpox and canarypox). Examples of retroviruses include avian leukosis-sarcoma virus, human T-lymphotrophic virus type 1 (HTLV-1), bovine leukemia virus (BLV), lentivirus, and spumavirus. Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Viral vectors may be classified into two groups according to their ability to

integrate into the host genome – integrating and non-integrating. Oncoretroviruses and lentiviruses can integrate into host cellular chromatin while adenoviruses, adeno-associated viruses, and herpes viruses predominantly persist in the cell nucleus as extrachromosomal episomes.

[00132] In certain embodiments, a suitable viral vector is a retroviral vector. Retroviruses refer to viruses of the family Retroviridae. Examples of retroviruses include oncoretroviruses, such as murine leukemia virus (MLV), and lentiviruses, such as human immunodeficiency virus 1 (HIV-1). Retroviral genomes are single-stranded (ss) RNAs and comprise various genes that may be provided in cis or trans. For example, retroviral genome may contain cis-acting sequences such as two long terminal repeats (LTR), with elements for gene expression, reverse transcription and integration into the host chromosomes. Other components include the packaging signal (psi or ψ), for the specific RNA packaging into newly formed virions and the polypurine tract (PPT), the site of the initiation of the positive strand DNA synthesis during reverse transcription. In addition, the retroviral genome may comprise gag, pol and env genes. The gag gene encodes the structural proteins, the pol gene encodes the enzymes that accompany the ssRNA and carry out reverse transcription of the viral RNA to DNA, and the env gene encodes the viral envelope. Generally, the gag, pol and env are provided in trans for viral replication and packaging.

[00133] In certain embodiments, a retroviral vector provided herein may be a lentiviral vector. At least five serogroups or serotypes of lentiviruses are recognized. Viruses of the different serotypes may differentially infect certain cell types and/or hosts. Lentiviruses, for example, include primate retroviruses and non-primate retroviruses. Primate retroviruses include HIV and simian immunodeficiency virus (SIV). Non-primate retroviruses include feline immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV), caprine arthritis-encephalitis virus (CAEV), equine infectious anemia virus (EIAV) and visnavirus. Lentiviruses or lentivectors may be capable of transducing quiescent cells. As with oncoretrovirus vectors, the design of lentivectors may be based on the separation of cis- and trans-acting sequences.

[00134] In exemplary embodiments, a viral vector provided herein is an adeno-associated virus (AAV). AAV is a small, replication-defective, non-enveloped animal virus that infects humans and some other primate species. AAV is not known to cause human disease and induces a mild immune response. AAV vectors can also infect both dividing and quiescent cells without integrating into the host cell genome.

[00135] The AAV genome consists of a linear single stranded DNA which is ~4.7kb in length. The genome consists of two open reading frames (ORF) flanked by an inverted terminal repeat (ITR) sequence that is about 145bp in length. The ITR consists of a nucleotide sequence at the 5' end (5' ITR) and a nucleotide sequence located at the 3' end (3' ITR) that contain palindromic sequences. The ITRs function in cis by folding over to form T-shaped hairpin structures by complementary base pairing that function as primers during initiation of DNA replication for second strand synthesis. The two open reading frames encode for rep and cap genes that are involved in replication and packaging of the virion. In an exemplary embodiment, an AAV vector provided herein does not contain the rep or cap genes. Such genes may be provided in trans for producing virions as described further below.

[00136] In certain embodiments, an AAV vector may include a stuffer nucleic acid. In some embodiments, the stuffer nucleic acid may encode a green fluorescent protein or antibiotic resistance gene such as kanamycin or ampicillin. In certain embodiments, the stuffer nucleic acid may be located outside of the ITR sequences (e.g., as compared to the polynucleotide encoding a therapeutic protein, and regulatory sequences, which are located between the 5' and 3' ITR sequences).

[00137] Various serotypes of AAV exist, including AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hul4), AAV10, AAV11, AAV 12, AAV13, AAVrh8, AAVrhlO, AAV-DJ, and AAV-DJ8. These serotypes differ in their tropism, or the types of cells they infect. AAVs may comprise the genome and capsids from multiple serotypes (e.g., pseudotypes). For example, an AAV may comprise the genome of serotype 2 (e.g., 5' ITR containing SEQ ID NOs:470 and 3'ITR containing 471, provided elsewhere herein) packaged in the capsid from serotype 5 or serotype 9. Pseudotypes may improve transduction efficiency as well as alter tropism.

[00138] In some embodiments, an AAV vector or an AAV viral particle, or virion, may be used to deliver a construct comprising a cell selective regulatory element operably linked to a polynucleotide encoding functional therapeutic protein into a cell, cell type, or tissue, and may done either *in vivo*, *ex vivo*, or *in vitro*. In exemplary embodiments, such an AAV vector is replication-deficient. In some embodiments, an AAV virus is engineered or genetically modified so that it can replicate and generate virions only in the presence of helper factors.

[00139] In certain embodiments, a viral vector can be selected to produce a virion having high infectivity without selectivity for a particular cell type. In some cases, an AAV serotype that can cross the blood brain barrier or infect cells of the CNS is preferred. In certain embodiments, an rAAV particle of the present disclosure comprises an AAV capsid that has an enhanced tropism for a tissue or a cell, e.g., a CNS tissue or cell, where in some embodiments the AAV capsid is modified from a parent capsid, e.g., an AAV capsid with a variant polypeptide sequence and/or having a chemical modification (e.g., a covalently-modified AAV capsid). Examples of AAV capsids that have improved CNS tropism or that can cross the blood brain barrier include, but are not limited to, those disclosed in the following PCT publications, each of which is incorporated by reference herein in its entirety: WO2023060264 entitled "Capsid variants and methods of using the same" (Dyno Therapeutics, Inc.; see, e.g., SEQ ID NO: 2 disclosed therein, referred to herein as "bCap1"); WO2016054557 entitled "Novel high efficiency library-identified AAV vectors" (University of Massachusetts; see, e.g., SEQ ID NOs: 5 disclosed therein, referred to herein as "AAV-B1"); WO2020198737 entitled "Engineered adeno-associated (AAV) vectors for transgene expression" (Harvard College General Hospital Corp.; see, e.g., AAV9 parent with insertion of SEQ ID NO:1 disclosed therein, referred to herein as "AAV-S"); WO2015121501 entitled "Adeno-associated virus vector" (Kings College London; see, e.g., SEQ ID NO:2 disclosed therein, referred to herein as "AAV-TT"); WO2023081648 entitled "AAV capsid variants and uses thereof" (Voyager Therapeutics, Inc.; see, e.g., SEQ ID NOs: 981 and 982 disclosed therein, referred to herein as "VCAP-101" and "VCAP-102", respectively); WO2021041498 entitled "Adeno-Associated Viral Vectors for Crossing the Human Blood Brain Barrier"; WO2023168333 entitled "Compositions and Methods for Crossing Blood Brain Barrier"; WO2022235702 entitled "Recombinant AAVs for Delivery to Central Nervous System and Brain Vasculature"; WO2024017387 entitled "Novel AAV Capsids for Targeting Nervous System and Uses Thereof"; WO2024191877 entitled "Human Central Nervous System (CNS) Targeting AAV Variants"; WO2022221193 entitled "Recombinant AAV for treatment of neural disease"; WO2024030976 entitled "Compositions and methods for crossing the blood brain barrier"; WO2024218192 entitled "Novel Neurotropic Adeno-Associated Virus Capsids with Detargeting of Peripheral Organs"; WO2020072683 entitled "Redirection of Tropism of AAV Capsids"; WO19222441 entitled "AAV Serotypes for Brain Specific Payload Delivery"; WO2016081811 entitled "AAV vectors targeted to the central nervous system"; WO2021089856

entitled "Modified adeno-associated virus vectors and delivery thereof into the central nervous system"; and WO2022096681 entitled "Lactam-modified adeno-associated virus vectors".

[00140] In exemplary embodiments, the application provides expression vectors that have been designed for delivery by an AAV. The AAV can be any serotype, for examples, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hul4), AAV10, AAV11, AAV 12, AAV13, AAVrh8, AAVrhlO, AAV-DJ, and AAV-DJ8, or a chimeric, hybrid, or variant AAV. The AAV can also be a self-complementary AAV (scAAV), where a "self-complementary" AAV is one in which the coding region has been designed to form an intra-molecular double-stranded DNA template. Upon infection of such vectors, rather than waiting for cell mediated synthesis of the second strand, the two complementary halves of the scAAV will associate to form one double stranded DNA (dsDNA) unit that is ready for immediate replication and transcription. The design of scAAV vectors is described in a variety of publications, including McCarty et al Gene Therapy 2001 8: 1248–54. In some embodiments, an scAAV vector comprises a modified 3' ITR that contains SEQ ID NO:472 or a functional fragment thereof.

[00141] In certain embodiments, an expression vector designed for delivery by an AAV comprises a 5' ITR and a 3' ITR. In certain embodiments, an expression vector designed for delivery by an AAV comprises a 5' ITR, a promoter, a construct as described above and a 3' ITR. In certain embodiments, an expression vector designed for delivery by an AAV comprises a 5' ITR, an enhancer, a promoter, a construct as described above and a 3' ITR.

[00142] Examples of AAV vector sequences (full ITR to ITR genomes) include those comprising a sequence that is at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to any one of SEQ ID NOs:434, 439, and 444 (ssAAV genomes); SEQ ID NOs:435, 440, and 445 (scAAV genomes); and SEQ ID NOs:437, 442, and 447 (ssAAV nociceptor-selective), shown below:

[00143] SEQ ID NO:434 is an ITR-ITR ssAAV genome that includes a stuffer region and the U6-miR-E-61 expression cassette of SEQ ID NO:433 and has the following DNA sequence:

[00144] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGG
CGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCC
AACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATCTGTTCTATAAAT
TTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACATTACAGGAATGT

TTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGAACAGCCATCTGC
ATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACATGGATGTCACATTT
CTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGAGGAGATGTTCCTT
TTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATGGAGCAGTAGATAA
TAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAAATTTTGCTAGAGAC
TCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTGTCATATAATACAA
GGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTTAAAAGATGGCAA
GGTTATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTTGTTTGGTTTTTAG
GTTTTTCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTATTTGAATGAACA
AAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAATAAATTCATG
AAACATTGAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAATTGAGAAG
TTCATTATGCATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAAGAACTTA
TTGTAGTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTTGGAGA
CACAGAGTATTCCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAATATTA
TGCTTACTGCCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTCGA
GGCTTTTCAGTGCTTGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGATT
TATTCGTTGACATTTCATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTT
TTGGGAGTGTATATTTCTCATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAAC
TGTTTCTACTTTCATTTGTGACTTATGGCATTTGAATGTGTACATTCAATTGTGTTGG
CACATACTCTAGTCAAATGTGTGTGCCTGTATCTGTACATCCCTGTCAGGTATGTATA
CATATATCCAATTGTACATATTAGCCTTATAATATAATCCATCAAAATGTACACACC
TGTGTCAAATATGTTGTTTGTTTACTGGGATTTTATTTCCGTTAACTACTGCCAAAAT
TAAATAAACAGTTATTCTAAAAAGCACTAGATAACTCCCTATTGGTGTTTGAAGCCC
CAATGTGCTTACCGTTGCCTCATATATCAATGCAGGCTTTCCCCAGCTACCAAAGCT
GCCATTTGATTCAGATACCCAATCTGCCTCAGATGTGCTTGCGGTTGGGGAGTTACA
TTTGCGGCTATTTATTGGATGCTCAAAGAGATTAGTAAACACACACAGAGCTAGAGG
AATTTTATACATTATCCTGATAATAACTAAAGTATTGTTTAGTTGGATGCAAATGAA
GGAAATATGTCAAATGACTTACATATCTGACTACTAAAAAAATTATTGTATTATTAC
AGAGTATTACTGTAGTGTATTATTGTAGTGTATTATTATAGAGATAATAACTTAGAT
ACAATGTTAAGGAAAGTAAAATATTTCATTTTCCTCCAATAACAATAAAGGGAATGT
TCTTCTGATTGCAATTAACCTCAGATATTTTTGGATATGATCTATTTTTTAAAAATGA

TGATCCATGGGATAAAATATGAAAGAGACGAGGGAAAATAATTGGACGTGGAATAT
CCCCAGTTTTTATGTTATACAGGTGATGTAGTTTTGGACAAAAAATGATGGAAGAGA
GAGGCATGTAGAAAGTGTAAAGAAGTTCAGGGGAAGTTTTAAATTTAGCTCTCTTAA
TCTTGGGTCACTCATCAGAAAAAGGAAAGCACAATGTTAGAAATTCAGAGATCATA
TCACTGGTTGACTTCCGATCATATCACTGGTTGACTTCCTTAGTACTACAGGTTTAAA
ACTTAAGAGGCTTTTGTCTCAAAACAACATCAACAAAAAGCCAGGTGTGGTAGTGT
GCATCTGTAGTCTCAGCTACTGAGGAGACCAATGCAGGAAAATTGCTTAAGCCCAG
CAATTTGAGGCTGTAGTCAGTCAAGGTCTCACTACTGCACTGCAGACTGCGTTACAG
AACACTTTCTTAAAAAAAAGAAAGGAAAGAAAAGAAAAAGGGAAGGGAAAAGGGA
AAGGAGAAAGGGAAGGAGAAAGAGGTATTCTGTGTAGTTATATTAGTGGTCTAACA
AACAGACATCTACGAGATAAAATGAAACAAAACACACTGACAATTAAATAGAGAA
ATCAGACGAAAGTTGAGTCATGTAATCCAAAGCAAAATAGTATTTCAAGAATGAGG
GTGTGAATCAGTTTTACAGATACACACGACTGTTTGAGTAATACGCAATAGACAAGT
GATATATGGGATTTAGTGACAAGTATAAGATATGATTCCAAAAACAGGGTAAATGC
CTGAAGAAAGGAGAGTTAAGAAAAATAAAATTAAACCTTGTCACAGGCAACTCTTT
CCATGAGTCTTCCTATCATAGAAAATAAATAAATGAGTTGACAGTGGCATATCTAGA
ACTAAGACAATCCGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATA
CAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGT
ACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAAT
TATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTT
GGCTTTATATATCTTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATAT
ACTAGTCGACTAGGGATAACAGGGTAATTGTTTGAATGAGGCTTCAGTACTTTACAG
AATCGTTGCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCGACTTCTTAACCCA
ACAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGCAGAGTGCAGCACA
GTTGATATAGTGAAGCCACAGATGTATATCAACTGTGCTGCACTCTGATGCCTACTG
CCTCGGACTTCAAGGGGCTAGAATTCGAGCAATTATCTTGTTTACTAAAACTGAATA
CCTTGCTATCTCTTTGATACATTATTACAAAGCTGAATTAAAATGGTATAAATTAAAT
CACTTTTTTCAATTGGAAGACTAATGCGTTTATCTAGACACGTGCGGACCGAGCGGC
CGCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCA
CTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCA
GTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

[00145] SEQ ID NO:439 is an TR-ITR ssAAV genome that includes a stuffer region and the U6-miR-E-68 expression cassette of SEQ ID NO:438 and has the following DNA sequence:

[00146] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGG
CGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCC
AACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATCTGTTCTATAAAT
TTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACATTACAGGAATGT
TTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGAACAGCCATCTGC
ATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACATGGATGTCACATTT
CTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGAGGAGATGTTCCTT
TTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATGGAGCAGTAGATAA
TAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAAATTTTGCTAGAGAC
TCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTGTCATATAATACAA
GGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTTAAAAGATGGCAA
GGTTATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTTGTTTGGTTTTTAG
GTTTTTCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTATTTGAATGAACA
AAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAATAAATTCATG
AAACATTGAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAATTGAGAAG
TTCATTATGCATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAAGAACTTA
TTGTAGTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTTGGAGA
CACAGAGTATTCCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAATATTA
TGCTTACTGCCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTCGA
GGCTTTTCAGTGCTTGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGATT
TATTCGTTGACATTTCATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTT
TTGGGAGTGTATATTTCTCATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAAC
TGTTTCTACTTTCATTTGTGACTTATGGCATTTGAATGTGTACATTCAATTGTGTTGG
CACATACTCTAGTCAAATGTGTGTGCCTGTATCTGTACATCCCTGTCAGGTATGTATA
CATATATCCAATTGTACATATTAGCCTTATAATATAATCCATCAAAATGTACACACC
TGTGTCAAATATGTTGTTTGTTTACTGGGATTTTATTTCCGTTAACTACTGCCAAAAT
TAAATAAACAGTTATTCTAAAAAGCACTAGATAACTCCCTATTGGTGTTTGAAGCCC
CAATGTGCTTACCGTTGCCTCATATATCAATGCAGGCTTTCCCCAGCTACCAAAGCT
GCCATTTGATTCAGATACCCAATCTGCCTCAGATGTGCTTGCGGTTGGGGAGTTACA

TTTGCGGCTATTTATTGGATGCTCAAAGAGATTAGTAAACACACACAGAGCTAGAGG
AATTTTATACATTATCCTGATAATAACTAAAGTATTGTTTAGTTGGATGCAAATGAA
GGAAATATGTCAAATGACTTACATATCTGACTACTAAAAAAATTATTGTATTATTAC
AGAGTATTACTGTAGTGTATTATTGTAGTGTATTATTATAGAGATAATAACTTAGAT
ACAATGTTAAGGAAAGTAAAATATTTCATTTTCCTCCAATAACAATAAAGGGAATGT
TCTTCTGATTGCAATTAACCTCAGATATTTTTGGATATGATCTATTTTTTAAAAATGA
TGATCCATGGGATAAAATATGAAAGAGACGAGGGAAAATAATTGGACGTGGAATAT
CCCCAGTTTTTATGTTATACAGGTGATGTAGTTTTGGACAAAAAATGATGGAAGAGA
GAGGCATGTAGAAAGTGTAAAGAAGTTCAGGGGAAGTTTTAAATTTAGCTCTCTTAA
TCTTGGGTCACTCATCAGAAAAAGGAAAGCACAATGTTAGAAATTCAGAGATCATA
TCACTGGTTGACTTCCGATCATATCACTGGTTGACTTCCTTAGTACTACAGGTTTAAA
ACTTAAGAGGCTTTTGTCTCAAAACAACATCAACAAAAAGCCAGGTGTGGTAGTGT
GCATCTGTAGTCTCAGCTACTGAGGAGACCAATGCAGGAAAATTGCTTAAGCCCAG
CAATTTGAGGCTGTAGTCAGTCAAGGTCTCACTACTGCACTGCAGACTGCGTTACAG
AACACTTTCTTAAAAAAAAGAAAGGAAAGAAAAGAAAAAGGGAAGGGAAAAGGGA
AAGGAGAAAGGGAAGGAGAAAGAGGTATTCTGTGTAGTTATATTAGTGGTCTAACA
AACAGACATCTACGAGATAAAATGAAACAAAACACACTGACAATTAAATAGAGAA
ATCAGACGAAAGTTGAGTCATGTAATCCAAAGCAAAATAGTATTTCAAGAATGAGG
GTGTGAATCAGTTTTACAGATACACACGACTGTTTGAGTAATACGCAATAGACAAGT
GATATATGGGATTTAGTGACAAGTATAAGATATGATTCCAAAAACAGGGTAAATGC
CTGAAGAAAGGAGAGTTAAGAAAAATAAAATTAAACCTTGTCACAGGCAACTCTTT
CCATGAGTCTTCCTATCATAGAAAATAAATAAATGAGTTGACAGTGGCATATCTAGA
ACTAAGACAATCCGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATA
CAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGT
ACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAAT
TATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTT
GGCTTTATATATCTTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATAT
ACTAGTCGACTAGGGATAACAGGGTAATTGTTTGAATGAGGCTTCAGTACTTTACAG
AATCGTTGCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCGACTTCTTAACCCA
ACAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGTGCCCTCATTGAAC
AACGCATTAGTGAAGCCACAGATGTAATGCGTTGTTCAATGAGGGCAATGCCTACTG

CCTCGGACTTCAAGGGGCTAGAATTCGAGCAATTATCTTGTTTACTAAAACTGAATA
CCTTGCTATCTCTTTGATACATTATTACAAAGCTGAATTAAAATGGTATAAATTAAAT
CACTTTTTTCAATTGGAAGACTAATGCGTTTATCTAGACACGTGCGGACCGAGCGGC
CGCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCA
CTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCA
GTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

[00147] SEQ ID NO:444 is an ITR-ITR ssAAV genome that includes a stuffer region and the U6-miR-100-69 expression cassette of SEQ ID NO:443 and has the following DNA sequence:

[00148] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCC
CGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG
AGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATC
TGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACA
TTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGA
ACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACAT
GGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGA
GGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATG
GAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAA
ATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTG
TCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTT
AAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTT
GTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTA
TTTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAA
AATAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCA
TAAATTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGCACAGAATAAGGAG
ACAAAGAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTC
TTGTTTTGGAGACACAGAGTATTCCTTTGAATGAAGACTTAGGACAGATATTTTTAC
ATCACAATATTATGCTTACTGCCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAA
GAAAAATTTCGAGGCTTTTCAGTGCTTGAGTTCCTAAATAAATTTCACATACTTTAG
AAAGCATTTGATTTATTCGTTGACATTTCATTAATTAGCTGTAACATATTTATACTAA
GATGTGCCTTCTTTTGGGAGTGTATATTTCTCATTATAGTCCCATTGTGTACATTCTT
ATAGGCATGTAACTGTTTCTACTTTCATTTGTGACTTATGGCATTTGAATGTGTACAT

TCAATTGTGTTGGCACATACTCTAGTCAAATGTGTGTGCCTGTATCTGTACATCCCTG
TCAGGTATGTATACATATATCCAATTGTACATATTAGCCTTATAATATAATCCATCAA
AATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGATTTTATTTCCGTTAAC
TACTGCCAAAATTAAATAAACAGTTATTCTAAAAAGCACTAGATAACTCCCTATTGG
TGTTTGAAGCCCCAATGTGCTTACCGTTGCCTCATATATCAATGCAGGCTTTCCCCAG
CTACCAAAGCTGCCATTTGATTCAGATACCCAATCTGCCTCAGATGTGCTTGCGGTT
GGGGAGTTACATTTGCGGCTATTTATTGGATGCTCAAAGAGATTAGTAAACACACAC
AGAGCTAGAGGAATTTTATACATTATCCTGATAATAACTAAAGTATTGTTTAGTTGG
ATGCAAATGAAGGAAATATGTCAAATGACTTACATATCTGACTACTAAAAAAATTAT
TGTATTATTACAGAGTATTACTGTAGTGTATTATTGTAGTGTATTATTATAGAGATAA
TAACTTAGATACAATGTTAAGGAAAGTAAAATATTTCATTTTCCTCCAATAACAATA
AAGGGAATGTTCTTCTGATTGCAATTAACCTCAGATATTTTTGGATATGATCTATTTT
TTAAAAATGATGATCCATGGGATAAAATATGAAAGAGACGAGGGAAAATAATTGGA
CGTGGAATATCCCCAGTTTTTATGTTATACAGGTGATGTAGTTTTGGACAAAAAATG
ATGGAAGAGAGAGGCATGTAGAAAGTGTAAAGAAGTTCAGGGGAAGTTTTAAATTT
AGCTCTCTTAATCTTGGGTCACTCATCAGAAAAAGGAAAGCACAATGTTAGAAATTC
AGAGATCATATCACTGGTTGACTTCCGATCATATCACTGGTTGACTTCCTTAGTACTA
CAGGTTTAAAACTTAAGAGGCTTTTGTCTCAAAACAACATCATGGTACATGTTAGTT
TTCTCCTTTTTTTCTTTTCCCCAATATAATATATTTACTTAGTCCATTAATTTTTCAAT
GGTCTGTTCATTGTTTTCTTTTTTATGTGATCTTCTCATTCCCTCAATACTAGCTGTAC
ATTTTGAAAATATAAATGTTTGGTATATAATAAATATTATATGGGGGAAGGGAAAA
GGGAAAGGAGAAAGGGAAGGAGAAAGAGGTATTCTGTGTAGTTATATTAGTGGTCT
AACAAACAGACATCTACGAGATAAAATGAAACAAAACACACTGACAATTAAATAGA
GAAATCAGACGAAAGTTGAGTCATGTAATCCAAAGCAAAATAGTATTTCAAGAATG
AGGGTGTGAATCAGTTTTACAGATACACACGACTGTTTGAGTAATACGCAATAGACA
AGTGATATATGGGATTTAGTGACAAGTATAAGATATGATTCCAAAAACAGGGTAAA
TGCCTGAAGAAAGGAGAGTTAAGAAAAATAAAATTAAACCTTGTCACAGGCAACTC
TTTCCATGAGTCTTCCTATCATAGAAAATAAATAAATGAGTTGACAGTGGCATATCT
AGAACTAAGACAATCCGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACG
ATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATT
AGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAA

AATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTT
CTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACA
TATAGCTAGCACTAGTCGCCCAAAAGAGAGAAGATATTGAGGCCTGTTGCCACATC
ATGTAAACAATAAGGCACATGTATTAGTCCGATGTGCTTTAGTGTTTATATGTTGTGT
CTGTTAGGCAATCTCACGGACCTGGGGCTTTGCTTATATGCCTTTTTTATATATATTA
ATTAATATATAGGTACCCACGTGCGGACCGAGCGGCCGCAGGAACCCCTAGTGATG
GAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAG
GTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAG
CTGCCTGCAGG

[00149] SEQ ID NO:435 is an ITR-ITR scAAV genome that includes a stuffer region and the U6-miR-E-61 expression cassette of SEQ ID NO:433 and has the following DNA sequence:

[00150] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCC
CGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG
AGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATC
TGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACA
TTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGA
ACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACAT
GGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGA
GGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATG
GAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAA
ATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTG
TCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTT
AAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTT
GTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTA
TTTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAA
AATAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCA
TAAATTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGCACAGAATAAGGAG
ACAAAGAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTC
TTGTTTTGGAGACACAGAGTATTCCTTTGAATGAAGACTTAGGACAGATATTTTTAC
ATCACAATATTATGCTTACTGCCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAA
GAAAAATTTCGAGGCTTTTCAGTGCTTGAGTTCCTAAATAAATTTCACATACTTTAG

AAAGCATTTGATTTATTCGTTGACATTTCATTAATTAGCTGTAACATATTTATACTAA
GATGTGCCTTCTTTTGGGAGTGTATATTTCTCATTATAGTCCCATTGTGTACATTCTT
ATAGGCATGTAACTGTTTCTACTTTCATTTGTGACTTATGGCATTTGAATGTGTACAT
TCAATTGTGTTGGCACATACTCTAGTCAAATGTGTGTGCCTGTATCTGTACATCCCTG
TCAGGTATGTATACATATATCCAATTGTACATATTAGCCTTATAATATAATCCATCAA
AATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGATTTTATTTCCGTTAAC
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGA
GAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTG
ACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAAAAT
GGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATC
TTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATGCTAGCACTAGTC
GACTAGGGATAACAGGGTAATTGTTTGAATGAGGCTTCAGTACTTTACAGAATCGTT
GCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCGACTTCTTAACCCAACAGAA
GGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGCAGAGTGCAGCACAGTTGAT
ATAGTGAAGCCACAGATGTATATCAACTGTGCTGCACTCTGATGCCTACTGCCTCGG
ACTTCAAGGGGCTAGAATTCGAGCAATTATCTTGTTTACTAAAACTGAATACCTTGC
TATCTCTTTGATACATTATTACAAAGCTGAATTAAAATGGTATAAATTAAATCACTTT
TTTCAATTGGAAGACTAATGCGTTTATCTAGACACGTGCGGACCGAGCGGCCGCCCA
CTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGAC
GCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA

[00151] SEQ ID NO:440 is an ITR-ITR scAAV genome that includes a stuffer region and the U6-miR-E-68 expression cassette of SEQ ID NO:438 and has the following DNA sequence:

[00152] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCC
CGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG
AGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATC
TGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACA
TTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGA
ACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACAT
GGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGA
GGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATG
GAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAA

ATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTG
TCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTT
AAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTT
GTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTA
TTTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAA
AATAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCA
TAAATTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGCACAGAATAAGGAG
ACAAAGAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTC
TTGTTTTGGAGACACAGAGTATTCCTTTGAATGAAGACTTAGGACAGATATTTTTAC
ATCACAATATTATGCTTACTGCCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAA
GAAAAATTTCGAGGCTTTTCAGTGCTTGAGTTCCTAAATAAATTTCACATACTTTAG
AAAGCATTTGATTTATTCGTTGACATTTCATTAATTAGCTGTAACATATTTATACTAA
GATGTGCCTTCTTTTGGGAGTGTATATTTCTCATTATAGTCCCATTGTGTACATTCTT
ATAGGCATGTAACTGTTTCTACTTTCATTTGTGACTTATGGCATTTGAATGTGTACAT
TCAATTGTGTTGGCACATACTCTAGTCAAATGTGTGTGCCTGTATCTGTACATCCCTG
TCAGGTATGTATACATATATCCAATTGTACATATTAGCCTTATAATATAATCCATCAA
AATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGATTTTATTTCCGTTAAC
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGA
GAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTG
ACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAAAAT
GGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATC
TTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATGCTAGCACTAGTC
GACTAGGGATAACAGGGTAATTGTTTGAATGAGGCTTCAGTACTTTACAGAATCGTT
GCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCGACTTCTTAACCCAACAGAA
GGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGTGCCCTCATTGAACAACGCAT
TAGTGAAGCCACAGATGTAATGCGTTGTTCAATGAGGGCAATGCCTACTGCCTCGGA
CTTCAAGGGGCTAGAATTCGAGCAATTATCTTGTTTACTAAAACTGAATACCTTGCT
ATCTCTTTGATACATTATTACAAAGCTGAATTAAAATGGTATAAATTAAATCACTTTT
TTCAATTGGAAGACTAATGCGTTTATCTAGACACGTGCGGACCGAGCGGCCGCCCAC
TCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACG
CCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA

[00153] SEQ ID NO:445 is an ITR-ITR scAAV genome that includes a stuffer region and the U6-miR-100-69 expression cassette of SEQ ID NO:443 and has the following DNA sequence:

[00154] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCC
CGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG
AGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATC
TGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACA
TTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGA
ACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACAT
GGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGA
GGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATG
GAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAA
ATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTG
TCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTT
AAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTT
GTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTA
TTTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAA
AATAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCA
TAAATTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGCACAGAATAAGGAG
ACAAAGAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTC
TTGTTTTGGAGACACAGAGTATTCCTTTGAATGAAGACTTAGGACAGATATTTTTAC
ATCACAATATTATGCTTACTGCCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAA
GAAAAATTTCGAGGCTTTTCAGTGCTTGAGTTCCTAAATAAATTTCACATACTTTAG
AAAGCATTTGATTTATTCGTTGACATTTCATTAATTAGCTGTAACATATTTATACTAA
GATGTGCCTTCTTTTGGGAGTGTATATTTCTCATTATAGTCCCATTGTGTACATTCTT
ATAGGCATGTAACTGTTTCTACTTTCATTTGTGACTTATGGCATTTGAATGTGTACAT
TCAATTGTGTTGGCACATACTCTAGTCAAATGTGTGTGCCTGTATCTGTACATCCCTG
TCAGGTATGTATACATATATCCAATTGTACATATTAGCCTTATAATATAATCCATCAA
AATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGATTTTATTTCCGTTAAC
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGA
GAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTG
ACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAAAAT

GGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATC
TTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATGCTAGCACTAGTC
GCCCAAAAGAGAGAAGATATTGAGGCCTGTTGCCACATCATGTAAACAATAAGGCA
CATGTATTAGTCCGATGTGCTTTAGTGTTTATATGTTGTGTCTGTTAGGCAATCTCAC
GGACCTGGGGCTTTGCTTATATGCCTTTTTTATATATATTAATTAATATATAGGTACC
CACGTGCGGACCGAGCGGCCGCCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAG
GCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAG
CGAGCGAGCGCGCAGAGAGGGA

[00155] SEQ ID NO:437 is an ITR-ITR ssAAV genome that includes a nociceptor-selective miR-E-61 expression cassette of SEQ ID NO:436 and has the following DNA sequence:

[00156] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCC
CGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG
AGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTAGCAGGGCC
TGGAAGAAAAAGTCAGAATTCCCCAGGCAGACAAACAGGGAGTGGGGGGCAGCAG
GTGAGCAAAAGGCCACCTTGAGGTGCTTGGGGATTGTCTTTCACACAGCTGTCCCAT
GCGCTCTGCCCAACCCAGGCTCTGTGCATGAGATGCTAATGAGATTTCTCTAAGAAC
AGGATGTTGTCCAAGAGCAAGGCTCATCTAATCTTTACCAGCATCCTAAGAAGTCCT
GATTCTTATGTGACTCATTTAGCCAGCTTTTCCCCACAAGTGGTATCACAGGGAAAT
GACACGCTCCTTTTGGCACCAACTAATTTAAAGCACATTTGAGCCATTTGATAATAT
ATATTTTTAAATACTTGGCACTATTCCTTACTACCTTAAGCTGGTATATCAACCCTCG
CTAACCTCTTCTCAGTAACTCAGGGCATTCACCTCTGCCCAACGTGGGAAGGCAGGG
AACCCTGTGAGGCCTCAGGAACATGCAGGGCTGCTGGCCAGGTTACTAAGTGGTCC
AGAAAAGCTGTGCTTTGAGTCTGTAGCTCTCCCATAGCCTTCTTCCCTGCAGTCGTCA
CTTGTGTTCCAGCAGCTGCTGTTATCAGCTGCCATATTTTACATGCCTTTGTCTAGGT
AACAGCAGAGAGCATGAACTCTGTGACAGCTGTCTGCCACTCAGAAGCATTTTCAGT
TACTTTGGGGCTCTTAGGGAGCTCCAGGTGGCCAAGTTCCCTACAATTTTGACCTCT
GGACAAATAACAATGGTCATGGCTAAAATCTATTGAGCCAAGCACTTCGTGCAAAG
TGTTTTACAAGCGCTAACATATTTAGTCATCAAAACAGCTGAATGATGCAGATCTAG
CCTTTTCAATTTTGAAGCTGGGAAAAATGAAGATTAGAGAGGTTAAACAACTTGCCT
AAGGTCACACAAGAGTGAGACTCCACAAGCTGTGGGACCTAAGATCAGTTCCCAAA
CCTCTCTGAGCCGCCATTTATTCATCTATGAAACTGAATGGTAATGCCTACTTGGTAG

GATTATTTTGAAGCTTAAATGAGATGCTGCTTGTCAAATGTTTAGGTCCTGCCTGAG
ACAAAGTAAGTGCCCAGGAAATGACAGCCAAGAAAAAAGGAAACGAAAGACAACG
CACAAAGAAAGTCAAATATCTTTCAGAGCCAGCAAATAAGAGTTGGAGGTGCTCAC
AGATGAAGAAGTTTTCCCTTGTGATTCTCCCCCACTTTCTTTTGTCCACATCATTTTCT
CTAGGCAAAAGTAGCCTGGGGCTAGGGAGAGGCTCTCTATAGGTGAGTAGTGAAGT
GACAGCCTCACAGAGACTGAGCTGGAAGATTAGAGGTTTAGGATTGTCTTATACAA
GGTAAAAATAAATGTGGTTTCACTTACAAATCTTGAAGGAAACAAGCTATAGGGAA
AGAGGCATCTATAAAAGTTTAGCATCTTTAATAACACTGCCTTCAATTCAAGAATTT
AATTTGACACCTAGCCACTGAGTGCTGACTTTGTGACAGGCACTGGGCTCAACAGAG
ATAAGGAAGTCATAATTCATCCCCAAAGTTATCAAGCCCTTCAGCTTGAAGAATGTC
AGCTACTGGCTGTGTGCCCTAGGCAAGGCTGACCACAGCAGGGTAAGATAAGAGGG
AGGGCTGGGACGGGATTTGGGGGCTGTGGGTGAACAGAAGCCGCTTGGTTCCCACT
GGGTGAGGGCTGTCACATTCTGCACGTGGATGTCATGCTTCTTCCCAAATGTTCCAA
GGACTCCCCTGGCTAGTTGTCCCTACTCAATGTTTTCAGTACACAACTTCTGGGTGTT
GGTTTTTTGTTTTTGTTTTCTTGTCCATGACCCCGGCTAGGCAGGAGGGGCCTGACAG
CGACAAATGGGGACCCGCCCCACCATTTGGGTCCATTAGCAAAGCTATCAGGTTTAC
TAATATGAGAGCAACTTCAACCCCTAATTGAATTCATCTGGAGACATTATAGGGCTG
GAGCTGCCAGGAGGGCAGCAGGCCCCTGCAGCATTACTCCATGATTAAATATTCAA
GCCCAGTGAATGCTGAGAGTCGTTATGGGTGTAATTACGGTGTCTCGGGCCATTGCC
AGCACTCCATCAGGGGCCCCGAAGGTTTACAGTCTCACACAGCTAAGCCTCTGGGG
CTCCAGGGAGAAGAGCAGTGTTCCAGGCCTCAGCTGGCGAGGCACCAAACATAATG
GAACCCTGACCCAGAGAAGCTGTTTTTTTGCTTGACTTGAGAGGTTTTGAGTAAACA
GAGTTGTAATGTAATAAAGCACCAAGCCAGTTTCCTCCTCTATTAACTGGCATTGAG
AGACAAGATGAATCAAGCAGCTGCAGGGGAGCTGGCTTGGCCTGGGACCCCTTGCA
GCACCTCCTCCACCTACCCCTCCCTTTTGGTCCTATGGCCCTGTGGACAGGTTATTTT
CTCCTGCTCTTTGTTTTGTTTAATCTCATCGGAGGTTTCAGATCTTTTTTTTTTTTTGT
AATTTCATTTCAAACTATTATTTTATAAGACCTGGCCTATTACTGAGTATGCAGGCAG
AATATGAAAATTACTCCAAAACTTTTTTAAATGAAATTTTCAAGATGCAAAAAGTGA
AACTTTAAAATTTCAGTGGAAGAAGGGGAACAAAAACATTTTAATAAATGAGAGTG
TTTATTCCAGAATGGGAATATAGAGACAAGGAAGGTACCATGTGAATGGGTGCACC
TCGCTCTCTGGGGTCAATGATAGGAAACAGCCTGTCCCACAGTCAAGGCAGCCTTGC

CCAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGTTATCTAAGTGCAGCCTCTTT
GCTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTAAAATCACATTTGCTATTTTA
CCATCTAATCACACATAAGCCTCTCCCCACACTCCCCCCGCCCTGTTTCCATCCAAG
GAGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAACTCGTGACGGGAAATGGGA
ATGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGAATGATAGCAAGGCTCCCAT
CAGACAAGCTGAGCCACTGTCACTGAGGAGGACAAACGAGTGCAAGTCTTTGCAGA
GCTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCACACACTAGCCTCTTGGCTAG
AGAACAGACATCAGATGGAGTTTCTTCTGGCTATGCCTGAATGTTAAGCTGAACGTA
TGTTCCAGGAGCTCGTGGTCTCCAGTAGAGGCAATCTGGGATAGAAGAGAAGATAT
TTCTTACGTAGAAGACAAGCAAGATTGAGCAGGTGCTCGCTTCGGCAGCACATATAC
TAGTCGACTAGGGATAACAGGGTAATTGTTTGAATGAGGCTTCAGTACTTTACAGAA
TCGTTGCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCGACTTCTTAACCCAAC
AGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGCAGAGTGCAGCACAGT
TGATATAGTGAAGCCACAGATGTATATCAACTGTGCTGCACTCTGATGCCTACTGCC
TCGGACTTCAAGGGGCTAGAATTCGAGCAATTATCTTGTTTACTAAAACTGAATACC
TTGCTATCTCTTTGATACATTATTACAAAGCTGAATTAAAATGGTATAAATTAAATC
ACAATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGTTTTTTGTGTGCAATTGG
AAGACTAATGCGTTTATCTAGACACGTGCGGACCGAGCGGCCGCAGGAACCCCTAG
TGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGAC
CAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCG
CGCAGCTGCCTGCAGG

[00157] SEQ ID NO:442 is an ITR-ITR ssAAV genome that includes a nociceptor-selective miR-E-68 expression cassette of SEQ ID NO:441 and has the following DNA sequence:

[00158] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCC
CGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG
AGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGAGCAGGGCCT
GGAAGAAAAAGTCAGAATTCCCCAGGCAGACAAACAGGGAGTGGGGGGCAGCAGG
TGAGCAAAAGGCCACCTTGAGGTGCTTGGGGATTGTCTTTCACACAGCTGTCCCATG
CGCTCTGCCCAACCCAGGCTCTGTGCATGAGATGCTAATGAGATTTCTCTAAGAACA
GGATGTTGTCCAAGAGCAAGGCTCATCTAATCTTTACCAGCATCCTAAGAAGTCCTG
ATTCTTATGTGACTCATTTAGCCAGCTTTTCCCCACAAGTGGTATCACAGGGAAATG

ACACGCTCCTTTTGGCACCAACTAATTTAAAGCACATTTGAGCCATTTGATAATATA
TATTTTTAAATACTTGGCACTATTCCTTACTACCTTAAGCTGGTATATCAACCCTCGC
TAACCTCTTCTCAGTAACTCAGGGCATTCACCTCTGCCCAACGTGGGAAGGCAGGGA
ACCCTGTGAGGCCTCAGGAACATGCAGGGCTGCTGGCCAGGTTACTAAGTGGTCCA
GAAAAGCTGTGCTTTGAGTCTGTAGCTCTCCCATAGCCTTCTTCCCTGCAGTCGTCAC
TTGTGTTCCAGCAGCTGCTGTTATCAGCTGCCATATTTTACATGCCTTTGTCTAGGTA
ACAGCAGAGAGCATGAACTCTGTGACAGCTGTCTGCCACTCAGAAGCATTTTCAGTT
ACTTTGGGGCTCTTAGGGAGCTCCAGGTGGCCAAGTTCCCTACAATTTTGACCTCTG
GACAAATAACAATGGTCATGGCTAAAATCTATTGAGCCAAGCACTTCGTGCAAAGT
GTTTTACAAGCGCTAACATATTTAGTCATCAAAACAGCTGAATGATGCAGATCTAGC
CTTTTCAATTTTGAAGCTGGGAAAAATGAAGATTAGAGAGGTTAAACAACTTGCCTA
AGGTCACACAAGAGTGAGACTCCACAAGCTGTGGGACCTAAGATCAGTTCCCAAAC
CTCTCTGAGCCGCCATTTATTCATCTATGAAACTGAATGGTAATGCCTACTTGGTAG
GATTATTTTGAAGCTTAAATGAGATGCTGCTTGTCAAATGTTTAGGTCCTGCCTGAG
ACAAAGTAAGTGCCCAGGAAATGACAGCCAAGAAAAAAGGAAACGAAAGACAACG
CACAAAGAAAGTCAAATATCTTTCAGAGCCAGCAAATAAGAGTTGGAGGTGCTCAC
AGATGAAGAAGTTTTCCCTTGTGATTCTCCCCCACTTTCTTTTGTCCACATCATTTTCT
CTAGGCAAAAGTAGCCTGGGGCTAGGGAGAGGCTCTCTATAGGTGAGTAGTGAAGT
GACAGCCTCACAGAGACTGAGCTGGAAGATTAGAGGTTTAGGATTGTCTTATACAA
GGTAAAAATAAATGTGGTTTCACTTACAAATCTTGAAGGAAACAAGCTATAGGGAA
AGAGGCATCTATAAAAGTTTAGCATCTTTAATAACACTGCCTTCAATTCAAGAATTT
AATTTGACACCTAGCCACTGAGTGCTGACTTTGTGACAGGCACTGGGCTCAACAGAG
ATAAGGAAGTCATAATTCATCCCCAAAGTTATCAAGCCCTTCAGCTTGAAGAATGTC
AGCTACTGGCTGTGTGCCCTAGGCAAGGCTGACCACAGCAGGGTAAGATAAGAGGG
AGGGCTGGGACGGGATTTGGGGGCTGTGGGTGAACAGAAGCCGCTTGGTTCCCACT
GGGTGAGGGCTGTCACATTCTGCACGTGGATGTCATGCTTCTTCCCAAATGTTCCAA
GGACTCCCCTGGCTAGTTGTCCCTACTCAATGTTTTCAGTACACAACTTCTGGGTGTT
GGTTTTTTGTTTTTGTTTTCTTGTCCATGACCCCGGCTAGGCAGGAGGGGCCTGACAG
CGACAAATGGGGACCCGCCCCACCATTTGGGTCCATTAGCAAAGCTATCAGGTTTAC
TAATATGAGAGCAACTTCAACCCCTAATTGAATTCATCTGGAGACATTATAGGGCTG
GAGCTGCCAGGAGGGCAGCAGGCCCCTGCAGCATTACTCCATGATTAAATATTCAA

GCCCAGTGAATGCTGAGAGTCGTTATGGGTGTAATTACGGTGTCTCGGGCCATTGCC
AGCACTCCATCAGGGGCCCCGAAGGTTTACAGTCTCACACAGCTAAGCCTCTGGGG
CTCCAGGGAGAAGAGCAGTGTTCCAGGCCTCAGCTGGCGAGGCACCAAACATAATG
GAACCCTGACCCAGAGAAGCTGTTTTTTTGCTTGACTTGAGAGGTTTTGAGTAAACA
GAGTTGTAATGTAATAAAGCACCAAGCCAGTTTCCTCCTCTATTAACTGGCATTGAG
AGACAAGATGAATCAAGCAGCTGCAGGGGAGCTGGCTTGGCCTGGGACCCCTTGCA
GCACCTCCTCCACCTACCCCTCCCTTTTGGTCCTATGGCCCTGTGGACAGGTTATTTT
CTCCTGCTCTTTGTTTTGTTTAATCTCATCGGAGGTTTCAGATCTTTTTTTTTTTTTGT
AATTTCATTTCAAACTATTATTTTATAAGACCTGGCCTATTACTGAGTATGCAGGCAG
AATATGAAAATTACTCCAAAACTTTTTTAAATGAAATTTTCAAGATGCAAAAAGTGA
AACTTTAAAATTTCAGTGGAAGAAGGGGAACAAAAACATTTTAATAAATGAGAGTG
TTTATTCCAGAATGGGAATATAGAGACAAGGAAGGTACCATGTGAATGGGTGCACC
TCGCTCTCTGGGGTCAATGATAGGAAACAGCCTGTCCCACAGTCAAGGCAGCCTTGC
CCAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGTTATCTAAGTGCAGCCTCTTT
GCTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTAAAATCACATTTGCTATTTTA
CCATCTAATCACACATAAGCCTCTCCCCACACTCCCCCCGCCCTGTTTCCATCCAAG
GAGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAACTCGTGACGGGAAATGGGA
ATGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGAATGATAGCAAGGCTCCCAT
CAGACAAGCTGAGCCACTGTCACTGAGGAGGACAAACGAGTGCAAGTCTTTGCAGA
GCTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCACACACTAGCCTCTTGGCTAG
AGAACAGACATCAGATGGAGTTTCTTCTGGCTATGCCTGAATGTTAAGCTGAACGTA
TGTTCCAGGAGCTCGTGGTCTCCAGTAGAGGCAATCTGGGATAGAAGAGAAGATAT
TTCTTACGTAGAAGACAAGCAAGATTGAGCAGTGACGGATCCACCGGTCGACTTCG
AATAGGGATAACAGGGTAATTGTTTGAATGAGGCTTCAGTACTTTACAGAATCGTTG
CCTGCACATCTTGGAAACACTTGCTGGGATTACTTCGACTTCTTAACCCAACAGAAG
GCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGTGCCCTCATTGAACAACGCATT
AGTGAAGCCACAGATGTAATGCGTTGTTCAATGAGGGCAATGCCTACTGCCTCGGAC
TTCAAGGGGCTAGAATTCGAGCAATTATCTTGTTTACTAAAACTGAATACCTTGCTA
TCTCTTTGATACATTATTACAAAGCTGAATTAAAATGGTATAAATTAAATCACATAA
TTAATGGTACCAGCTAGCGCTATAGTCAATAAAAGATCTTTATTTTCATTAGATCTGT
GTGTTGGTTTTTTGTGTGTACCCACGTGCGGACCGAGCCGCAGGAACCCCTAGTGAT

GGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAA
GGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCA
GCTGCCTGCAGG

[00159] SEQ ID NO:447 is an ITR-ITR ssAAV genome that includes a nociceptor-selective miR-100-69 expression cassette of SEQ ID NO:446 and has the following DNA sequence:

[00160] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCC
CGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG
AGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGAGCAGGGCCT
GGAAGAAAAAGTCAGAATTCCCCAGGCAGACAAACAGGGAGTGGGGGGCAGCAGG
TGAGCAAAAGGCCACCTTGAGGTGCTTGGGGATTGTCTTTCACACAGCTGTCCCATG
CGCTCTGCCCAACCCAGGCTCTGTGCATGAGATGCTAATGAGATTTCTCTAAGAACA
GGATGTTGTCCAAGAGCAAGGCTCATCTAATCTTTACCAGCATCCTAAGAAGTCCTG
ATTCTTATGTGACTCATTTAGCCAGCTTTTCCCCACAAGTGGTATCACAGGGAAATG
ACACGCTCCTTTTGGCACCAACTAATTTAAAGCACATTTGAGCCATTTGATAATATA
TATTTTTAAATACTTGGCACTATTCCTTACTACCTTAAGCTGGTATATCAACCCTCGC
TAACCTCTTCTCAGTAACTCAGGGCATTCACCTCTGCCCAACGTGGGAAGGCAGGGA
ACCCTGTGAGGCCTCAGGAACATGCAGGGCTGCTGGCCAGGTTACTAAGTGGTCCA
GAAAAGCTGTGCTTTGAGTCTGTAGCTCTCCCATAGCCTTCTTCCCTGCAGTCGTCAC
TTGTGTTCCAGCAGCTGCTGTTATCAGCTGCCATATTTTACATGCCTTTGTCTAGGTA
ACAGCAGAGAGCATGAACTCTGTGACAGCTGTCTGCCACTCAGAAGCATTTTCAGTT
ACTTTGGGGCTCTTAGGGAGCTCCAGGTGGCCAAGTTCCCTACAATTTTGACCTCTG
GACAAATAACAATGGTCATGGCTAAAATCTATTGAGCCAAGCACTTCGTGCAAAGT
GTTTTACAAGCGCTAACATATTTAGTCATCAAAACAGCTGAATGATGCAGATCTAGC
CTTTTCAATTTTGAAGCTGGGAAAAATGAAGATTAGAGAGGTTAAACAACTTGCCTA
AGGTCACACAAGAGTGAGACTCCACAAGCTGTGGGACCTAAGATCAGTTCCCAAAC
CTCTCTGAGCCGCCATTTATTCATCTATGAAACTGAATGGTAATGCCTACTTGGTAG
GATTATTTTGAAGCTTAAATGAGATGCTGCTTGTCAAATGTTTAGGTCCTGCCTGAG
ACAAAGTAAGTGCCCAGGAAATGACAGCCAAGAAAAAAGGAAACGAAAGACAACG
CACAAAGAAAGTCAAATATCTTTCAGAGCCAGCAAATAAGAGTTGGAGGTGCTCAC
AGATGAAGAAGTTTTCCCTTGTGATTCTCCCCCACTTTCTTTTGTCCACATCATTTTCT
CTAGGCAAAAGTAGCCTGGGGCTAGGGAGAGGCTCTCTATAGGTGAGTAGTGAAGT

GACAGCCTCACAGAGACTGAGCTGGAAGATTAGAGGTTTAGGATTGTCTTATACAA
GGTAAAAATAAATGTGGTTTCACTTACAAATCTTGAAGGAAACAAGCTATAGGGAA
AGAGGCATCTATAAAAGTTTAGCATCTTTAATAACACTGCCTTCAATTCAAGAATTT
AATTTGACACCTAGCCACTGAGTGCTGACTTTGTGACAGGCACTGGGCTCAACAGAG
ATAAGGAAGTCATAATTCATCCCCAAAGTTATCAAGCCCTTCAGCTTGAAGAATGTC
AGCTACTGGCTGTGTGCCCTAGGCAAGGCTGACCACAGCAGGGTAAGATAAGAGGG
AGGGCTGGGACGGGATTTGGGGGCTGTGGGTGAACAGAAGCCGCTTGGTTCCCACT
GGGTGAGGGCTGTCACATTCTGCACGTGGATGTCATGCTTCTTCCCAAATGTTCCAA
GGACTCCCCTGGCTAGTTGTCCCTACTCAATGTTTTCAGTACACAACTTCTGGGTGTT
GGTTTTTTGTTTTTGTTTTCTTGTCCATGACCCCGGCTAGGCAGGAGGGGCCTGACAG
CGACAAATGGGGACCCGCCCCACCATTTGGGTCCATTAGCAAAGCTATCAGGTTTAC
TAATATGAGAGCAACTTCAACCCCTAATTGAATTCATCTGGAGACATTATAGGGCTG
GAGCTGCCAGGAGGGCAGCAGGCCCCTGCAGCATTACTCCATGATTAAATATTCAA
GCCCAGTGAATGCTGAGAGTCGTTATGGGTGTAATTACGGTGTCTCGGGCCATTGCC
AGCACTCCATCAGGGGCCCCGAAGGTTTACAGTCTCACACAGCTAAGCCTCTGGGG
CTCCAGGGAGAAGAGCAGTGTTCCAGGCCTCAGCTGGCGAGGCACCAAACATAATG
GAACCCTGACCCAGAGAAGCTGTTTTTTTGCTTGACTTGAGAGGTTTTGAGTAAACA
GAGTTGTAATGTAATAAAGCACCAAGCCAGTTTCCTCCTCTATTAACTGGCATTGAG
AGACAAGATGAATCAAGCAGCTGCAGGGGAGCTGGCTTGGCCTGGGACCCCTTGCA
GCACCTCCTCCACCTACCCCTCCCTTTTGGTCCTATGGCCCTGTGGACAGGTTATTTT
CTCCTGCTCTTTGTTTTGTTTAATCTCATCGGAGGTTTCAGATCTTTTTTTTTTTTTGT
AATTTCATTTCAAACTATTATTTTATAAGACCTGGCCTATTACTGAGTATGCAGGCAG
AATATGAAAATTACTCCAAAACTTTTTTAAATGAAATTTTCAAGATGCAAAAAGTGA
AACTTTAAAATTTCAGTGGAAGAAGGGGAACAAAAACATTTTAATAAATGAGAGTG
TTTATTCCAGAATGGGAATATAGAGACAAGGAAGGTACCATGTGAATGGGTGCACC
TCGCTCTCTGGGGTCAATGATAGGAAACAGCCTGTCCCACAGTCAAGGCAGCCTTGC
CCAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGTTATCTAAGTGCAGCCTCTTT
GCTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTAAAATCACATTTGCTATTTTA
CCATCTAATCACACATAAGCCTCTCCCCACACTCCCCCCGCCCTGTTTCCATCCAAG
GAGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAACTCGTGACGGGAAATGGGA
ATGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGAATGATAGCAAGGCTCCCAT

CAGACAAGCTGAGCCACTGTCACTGAGGAGGACAAACGAGTGCAAGTCTTTGCAGA
GCTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCACACACTAGCCTCTTGGCTAG
AGAACAGACATCAGATGGAGTTTCTTCTGGCTATGCCTGAATGTTAAGCTGAACGTA
TGTTCCAGGAGCTCGTGGTCTCCAGTAGAGGCAATCTGGGATAGAAGAGAAGATAT
TTCTTACGTAGAAGACAAGCAAGATTGAGCAGTGACGGATCCACCGGTCGACTTCG
AATAGGGATAACAGGGTAATTGTCCCAAAAGAGAGAAGATATTGAGGCCTGTTGCC
ACATCATGTAAACAATAAGGCACATGTATTAGTCCGATGTGCTTTAGTGTTTATATG
TTGTGTCTGTTAGGCAATCTCACGGACCTGGGGCTTTGCTTATATGCCATAATTAATG
GTACCAGCTAGCGCTATAGTCAATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTG
GTTTTTTGTGTGTACCCACGTGCGGACCGAGCCGCAGGAACCCCTAGTGATGGAGTT
GGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGC
CCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCC
TGCAGG

Method for reducing expression of Nav1.7

[00161] In some embodiments, the present disclosure provides methods of reducing expression of NAV1.7 in a cell wherein NAV1.7 is expressed, e.g., from an endogenous SCN9A gene. The methods may comprise contacting the cell with an oligonucleotide of this disclosure, an RNA of this disclosure, or a nucleic acid cassette or vector of this disclosure. The method of reducing expression of NAV1.7 may result in reduced expression compared to a comparable cell not treated with the oligonucleotide, RNA, nucleic acid cassette or vector. In some cases, the expression of NAV1.7 may be reduced by about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, or about 98% as compared to expression of NAV1.7 in a comparable cell not treated with the oligonucleotide, RNA, nucleic acid cassette or vector. In some cases, the expression of NAV1.7 may be reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 98% as compared to expression of NAV1.7 in a comparable cell not treated with the oligonucleotide, RNA, nucleic acid cassette or vector. In some cases, the expression of NAV1.7 may be reduced by about 5% to about 95%, about 10% to about 90%, about 10% to about 80%, about 10% to about 50%, about 15% to about

50%, or about 15% to about 50%, as compared to expression of NAV1.7 in a comparable cell not treated with the oligonucleotide, RNA, nucleic acid cassette or vector. The reduction in expression of NAV1.7 may be assessed by standard molecular techniques, including quantitative polymerase chain reaction.

Pharmaceutical compositions

[00162] Also disclosed are pharmaceutical compositions comprising any of the aforementioned viruses, vectors, expression cassettes, oligonucleotides and/or oligonucleotide conjugates and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS) and pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts. In some embodiments, the diluent is artificial cerebrospinal fluid (aCSF).

[00163] The disclosed viruses, vectors, expression cassettes, or oligonucleotides may be mixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

[00164] Those skilled in the art are aware of a variety of formulation strategies useful for storage and/or administration of viruses, vectors, expression cassettes, and nucleic acid therapeutics such as oligonucleotide therapeutics.

Methods of treatment

[00165] Also disclosed are methods for treating or preventing neurological pain, comprising administering a therapeutically or prophylactically effective amount of a pharmaceutical composition comprising an oligonucleotide, RNA, nucleic acid cassette, or vector, disclosed herein to a subject suffering from or susceptible to the pain.

[00166] Also disclosed is use of the disclosed oligonucleotides for the manufacture of a medicament for the treatment of a disorder as referred to herein, or for a method of the treatment of as a disorder as referred to herein.

[00167] The disclosed pharmaceutical compositions may be administered by topical (such as, to the skin, inhalation, ophthalmic or otic) or enteral (such as, orally or through the gastrointestinal tract) or parenteral (such as, intravenous, subcutaneous, intra-muscular, intracerebral, intracerebroventricular or intrathecal) administration. In some embodiments, the disclosed

pharmaceutical compositions are administered by a parenteral route including intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion, intrathecal or intracranial, e.g., intracerebral or intraventricular, administration. In some embodiments, the oligonucleotide is administered by intracerebral or intracerebroventricular injection. In another embodiment the active oligonucleotide or oligonucleotide conjugate is administered intrathecally. In some embodiments, the pharmaceutical composition is administered by intracisternae magna injection.

Methods of production

[00168] In certain embodiments, a recombinant AAV virion provided herein may be prepared by encapsidating an AAV genome derived from a particular AAV serotype in a viral particle formed by natural Cap proteins corresponding to an AAV of the same particular serotype. In other embodiments, an AAV viral particle provided herein comprises a viral vector comprising ITR(s) of a given AAV serotype packaged into proteins from a different serotype. See e.g., Bunning H et al. J Gene Med 2008; 10: 717-733. For example, a viral vector having ITRs from a given AAV serotype may be package into: a) a viral particle constituted of capsid proteins derived from a same or different AAV serotype (e.g. AAV2 ITRs and AAV9 capsid proteins; AAV2 ITRs and AAV8 capsid proteins; etc.); b) a mosaic viral particle constituted of a mixture of capsid proteins from different AAV serotypes or mutants (e.g. AAV2 ITRs with AAV1 and AAV9 capsid proteins); c) a chimeric viral particle constituted of capsid proteins that have been truncated by domain swapping between different AAV serotypes or variants (e.g. AAV2 ITRs with AAV8 capsid proteins with AAV9 domains); or d) a targeted viral particle engineered to display selective binding domains, enabling stringent interaction with target cell specific receptors (e.g. AAV5 ITRs with AAV9 capsid proteins genetically truncated by insertion of a peptide ligand; or AAV9 capsid proteins non-genetically modified by coupling of a peptide ligand to the capsid surface).

[00169] The skilled person will appreciate that an AAV virion provided herein may comprise capsid proteins of any AAV serotype. In one embodiment, the viral particle comprises capsid proteins from an AAV serotype selected from the group consisting of an AAV1, an AAV2, an AAV5, an AAV8, and an AAV9, which are more suitable for delivery to the CNS (M. Hocquemiller et al., Hum Gene Ther 27(7): 478-496 (2016)). In a particular embodiment, the viral particle comprises a nucleic acid construct of the invention wherein the 5'ITR and 3'ITR

sequences of the nucleic acid construct are of an AAV2 serotype and the capsid proteins are of an AAV9 serotype.

[00170] Numerous methods are known in the art for production of rAAV virions, including transfection, stable cell line production, and infectious hybrid virus production systems which include adenovirus-AAV hybrids, herpesvirus-AAV hybrids (Conway, J E et al., (1997) J. Virology 71(11):8780-8789) and baculovirus-AAV hybrids. rAAV production cultures for the production of rAAV virus particles all require; 1) suitable host cells, including, for example, human-derived cell lines such as HeLa, A549, or 293 cells, or insect-derived cell lines such as SF-9, in the case of baculovirus production systems; 2) suitable helper virus function, provided by wild-type or mutant adenovirus (such as temperature sensitive adenovirus), herpes virus, baculovirus, or a plasmid construct providing helper functions; 3) AAV rep and cap genes and gene products; 4) a transgene flanked by AAV ITR sequences; and 5) suitable media and media components to support rAAV production.

[00171] In various embodiments, the host cells described herein comprise the following three components: (1) a rep gene and a cap gene, (2) genes providing helper functions, and (3) a transgene flanked by ITRs. The AAV rep gene, AAV cap gene, and genes providing helper functions can be introduced into the cell by incorporating said genes into a vector such as, for example, a plasmid, and introducing said vector into the host cell. The rep, cap and helper function genes can be incorporated into the same plasmid or into different plasmids. In a preferred embodiment, the AAV rep and cap genes are incorporated into one plasmid and the genes providing helper functions are incorporated into another plasmid. The various plasmids for creation of a host cell for virion production (e.g., comprising AAV rep and cap genes, helper functions, or a transgene) can be introduced into the cell by using any suitable method well known in the art. Examples of transfection methods include, but are not limited to, co-precipitation with calcium phosphate, DEAE-dextran, polybrene, electroporation, microinjection, liposome-mediated fusion, lipofection, retrovirus infection and biolistic transfection. In certain embodiments, the plasmids providing the rep and cap genes, the helper functions and the transgene can be introduced into the cell simultaneously. In another embodiment, the plasmids providing the rep and cap genes and the helper functions can be introduced in the cell before or after the introduction of plasmid comprising the transgene. In an exemplary embodiment, the cells are transfected simultaneously with three plasmids (e.g., a triple transfection method): (1) a

plasmid comprising the transgene, (2) a plasmid comprising the AAV rep and cap genes, and (3) a plasmid comprising the genes providing the helper functions. Exemplary host cells may be 293, A549 or HeLa cells.

[00172] In other embodiments, one or more of (1) the AAV rep and cap genes, (2) genes providing helper functions, and (3) the transgene (e.g., a PV selective regulatory element operably linked to a polynucleotide encoding a therapeutic protein disclosed herein), may be carried by the packaging cell, either episomally and/or integrated into the genome of the packaging cell. In one embodiment, host cells may be packaging cells in which the AAV rep and cap genes and helper functions are stably maintained in the host cell and the host cell is transiently transfected with a plasmid containing a transgene. In another embodiment, host cells are packaging cells in which the AAV rep and cap genes are stably maintained in the host cell and the host cell is transiently transfected with a plasmid containing a transgene and a plasmid containing the helper functions. In another embodiment, host cells may be packaging cells in which the helper functions are stably maintained in the host cell and the host cell is transiently transfected with a plasmid containing a transgene and a plasmid containing rep and cap genes. In another embodiment, host cells may be producer cell lines that are stably transfected with rep and cap genes, helper functions and the transgene sequence. Exemplary packaging and producer cells may be derived from 293, A549 or HeLa cells.

[00173] In another embodiment, the producer cell line is an insect cell line (typically Sf9 cells) that is infected with baculovirus expression vectors that provide Rep and Cap proteins. This system does not require adenovirus helper genes (Ayuso E, et al., Curr. Gene Ther. 2010, 10:423-436).

[00174] The term "cap protein", as used herein, refers to a polypeptide having at least one functional activity of a native AAV Cap protein (e.g. VP1, VP2, VP3). Examples of functional activities of cap proteins include the ability to induce formation of a capsid, facilitate accumulation of single-stranded DNA, facilitate AAV DNA packaging into capsids (i.e. encapsidation), bind to cellular receptors, and facilitate entry of the virion into host cells. In principle, any Cap protein can be used in the context of the present invention.

[00175] Cap proteins have been reported to have effects on host tropism, cell, tissue, or organ specificity, receptor usage, infection efficiency, and immunogenicity of AAV viruses. Accordingly, an AAV cap for use in an rAAV may be selected taking into consideration, for

example, the subject's species (e.g. human or non-human), the subject's immunological state, the subject's suitability for long or short-term treatment, or a particular therapeutic application (e.g. treatment of a particular disease or disorder, or delivery to particular cells, tissues, or organs). In certain embodiments, the cap protein is derived from the AAV of the group consisting of AAV1, AAV2, AAV5, AAV8, and AAV9 serotypes. In an exemplary embodiment, the cap protein is derived from AAV9.

[00176] In some embodiments, an AAV Cap for use in the method of the invention can be generated by mutagenesis (i.e. by insertions, deletions, or substitutions) of one of the aforementioned AAV caps or its encoding nucleic acid. In some embodiments, the AAV cap is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% or more similar to one or more of the aforementioned AAV caps.

[00177] In some embodiments, the AAV cap is chimeric, comprising domains from two, three, four, or more of the aforementioned AAV caps. In some embodiments, the AAV cap is a mosaic of VP1, VP2, and VP3 monomers originating from two or three different AAV or a recombinant AAV. In some embodiments, a rAAV composition comprises more than one of the aforementioned caps.

[00178] In some embodiments, an AAV cap for use in a rAAV virion is engineered to contain a heterologous sequence or other modification. For example, a peptide or protein sequence that confers selective targeting or immune evasion may be engineered into a cap protein. Alternatively or in addition, the cap may be chemically modified so that the surface of the rAAV is polyethylene glycolated (i.e., pegylated), which may facilitate immune evasion. The cap protein may also be mutagenized (e.g., to remove its natural receptor binding, or to mask an immunogenic epitope).

[00179] The term “rep protein”, as used herein, refers to a polypeptide having at least one functional activity of a native AAV rep protein (e.g. rep 40, 52, 68, 78). Examples of functional activities of a rep protein include any activity associated with the physiological function of the protein, including facilitating replication of DNA through recognition, binding and nicking of the AAV origin of DNA replication as well as DNA helicase activity. Additional functions include modulation of transcription from AAV (or other heterologous) promoters and site-specific integration of AAV DNA into a host chromosome. In a particular embodiment, AAV rep genes may be from the serotypes AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9,

AAV10 or AAVrh10; more preferably from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV5, AAV8, and AAV9.

[00180] In some embodiments, an AAV rep protein for use in the method of the invention can be generated by mutagenesis (i.e. by insertions, deletions, or substitutions) of one of the aforementioned AAV reps or its encoding nucleic acid. In some embodiments, the AAV rep is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% or more similar to one or more of the aforementioned AAV reps.

[00181] The expressions "helper functions" or "helper genes", as used herein, refer to viral proteins upon which AAV is dependent for replication. The helper functions include those proteins required for AAV replication including, without limitation, those proteins involved in activation of AAV gene transcription, stage specific AAV mRNA splicing, AAV DNA replication, synthesis of cap expression products, and AAV capsid assembly. Viral-based accessory functions can be derived from any of the known helper viruses such as adenovirus, herpesvirus (other than herpes simplex virus type-1), and vaccinia virus. Helper functions include, without limitation, adenovirus E1, E2a, VA, and E4 or herpesvirus UL5, ULB, UL52, and UL29, and herpesvirus polymerase. In a preferred embodiment, the proteins upon which AAV is dependent for replication are derived from adenovirus.

[00182] In some embodiments, a viral protein upon which AAV is dependent for replication for use in the method of the invention can be generated by mutagenesis (i.e. by insertions, deletions, or substitutions) of one of the aforementioned viral proteins or its encoding nucleic acid. In some embodiments, the viral protein is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% or more similar to one or more of the aforementioned viral proteins.

[00183] Methods for assaying the functions of cap proteins, rep proteins and viral proteins upon which AAV is dependent for replication are well known in the art.

[00184] Host cells for expressing a transgene of interest may be grown under conditions adequate for assembly of the AAV virions. In certain embodiments, host cells are grown for a suitable period of time in order to promote the assembly of the AAV virions and the release of virions into the media. Generally, cells mfay be grown for about 24 hours, about 36 hours, about 48 hours, about 72 hours, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, or up to about 10 days. After about 10 days (or sooner, depending on the culture conditions and the particular host cell used), the level of production generally decreases

significantly. Generally, time of culture is measured from the point of viral production. For example, in the case of AAV, viral production generally begins upon supplying helper virus function in an appropriate host cell as described herein. Generally, cells are harvested about 48 to about 100, preferably about 48 to about 96, preferably about 72 to about 96, preferably about 68 to about 72 hours after helper virus infection (or after viral production begins).

[00185] rAAV production cultures can be grown under a variety of conditions (over a wide temperature range, for varying lengths of time, and the like) suitable to the particular host cell being utilized. rAAV production cultures include attachment-dependent cultures which can be cultured in suitable attachment-dependent vessels such as, for example, roller bottles, hollow fiber filters, microcarriers, and packed-bed or fluidized-bed bioreactors. rAAV vector production cultures may also include suspension-adapted host cells such as HeLa, 293, and SF-9 cells which can be cultured in a variety of ways including, for example, spinner flasks, stirred tank bioreactors, and disposable systems such as the Wave bag system.

[00186] Suitable media known in the art may be used for the production of rAAV virions. These media include, without limitation, media produced by Hyclone Laboratories and JRH including Modified Eagle Medium (MEM), Dulbecco's Modified Eagle Medium (DMEM), each of which is incorporated herein by reference in its entirety. In certain embodiments, rAAV production culture media may be supplemented with serum or serum-derived recombinant proteins at a level of 0.5%-20% (v/v or w/v). Alternatively, rAAV vectors may be produced in serum-free conditions which may also be referred to as media with no animal-derived products.

[00187] After culturing the host cells to allow AAV virion production, the resulting virions may be then be harvested and purified. In certain embodiments, the AAV virions can be obtained from (1) the host cells of the production culture by lysis of the host cells, and/or (2) the culture medium of said cells after a period of time post-transfection, preferably 72 hours. The rAAV virions may be harvested from the spent media from the production culture, provided the cells are cultured under conditions that cause release of rAAV virions into the media from intact cells (see e.g., U.S. Pat. No. 6,566,118). Suitable methods of lysing cells are also known in the art and include for example multiple freeze/thaw cycles, sonication, microfluidization, and treatment with chemicals, such as detergents and/or proteases.

[00188] After harvesting, the rAAV virions may be purified. The term "purified" as used herein includes a preparation of rAAV virions devoid of at least some of the other components that may

also be present where the rAAV virions naturally occur or are initially prepared from. Thus, for example, purified rAAV virions may be prepared using an isolation technique to enrich it from a source mixture, such as a culture lysate or production culture supernatant. Enrichment can be measured in a variety of ways, such as, for example, by the proportion of DNase-resistant particles (DRPs) or genome copies (gc) present in a solution, or by infectivity, or it can be measured in relation to a second, potentially interfering substance present in the source mixture, such as contaminants, including production culture contaminants or in-process contaminants, including helper virus, media components, and the like.

[00189] In certain embodiments, the rAAV production culture harvest may be clarified to remove host cell debris. In some embodiments, the production culture harvest may be clarified using a variety of standard techniques, such as, centrifugation or filtration through a filter of 0.2 μm or greater pore size (e.g., a cellulose acetate filter or a series of depth filters).

[00190] In certain embodiments, the rAAV production culture harvest is further treated with Benzonase™ to digest any high molecular weight DNA present in the production culture. In some embodiments, the Benzonase™ digestion is performed under standard conditions, for example, a final concentration of 1-2.5 units/ml of Benzonase™ at a temperature ranging from ambient to 37°C for a period of 30 minutes to several hours.

[00191] In certain embodiments, the rAAV virions may be isolated or purified using one or more of the following purification steps: equilibrium centrifugation; flow-through anionic exchange filtration; tangential flow filtration (TFF) for concentrating the rAAV particles; rAAV capture by apatite chromatography; heat inactivation of helper virus; rAAV capture by hydrophobic interaction chromatography; buffer exchange by size exclusion chromatography (SEC); nanofiltration; and rAAV capture by anionic exchange chromatography, cationic exchange chromatography, or affinity chromatography. These steps may be used alone, in various combinations, or in different orders. Methods to purify rAAV particles are found, for example, in Xiao et al., (1998) Journal of Virology 72:2224-2232; U.S. Pat. Nos. 6,989,264 and 8,137,948; and WO 2010/148143.

[00192] In certain embodiments, purified AAV virions can be dialyzed against PBS, filtered and stored at –80°C. Titers of viral genomes can be determined by quantitative PCR using linearized plasmid DNA as standard curve (see e.g., Lock M, et al., Hum. Gene Ther. 2010; 21:1273-1285).

SEQUENCES

[00193] The nucleotide sequence of the human voltage-gated channel alpha subunit 9 (SCN9A), transcript variant 2, DNA encoding full mRNA (as defined by NCBI Genbank Accession no. NM_001365536.1) is set forth in the sequence listing as SEQ ID NO: 306:

agtctgcttgcaggcggtcgccagcgctccagcggcggctgtcggctttccaattccgccagct
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cttaaatttgaattcaatctaccgttatttcaaactcacaaatataactgcattatgaatactt
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caggtctcgctctgtcacccaggctagagtgcaatggcatgatcatagctctctgcagcctcaa
ctctgggctcaagcaaccctcctgcctcagcctcccaagtagctaagactacaggtacatgcca
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gagccaccatgccccaaacaggtttgaacacaaatctttcggatgaaaattagagaacctaatt
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ctgattgttgaacatcacaacccaaaatacttagcatgattttatagagttttgatagctttat
taaaaagagtgaaaataaaatgcatatgtaaataaagcagttctaaatagctatttcagagaaa
tgttaatagaagtgctgaaagaagggccaactaaattaggatggccagggaattggcctgggtt
taggacctatgtatgaaggccaccaatttttaaaaatatctgtggtttattatgttattatct
tcttgaggaaaacaatcaagaattgcttcatgaaaataaataaatagccatgaatatcataaag
ctgtttacataggattctttacaaatttcatagatctatgaatgctcaaaatgtttgagtttgc
cataaattatattgtagttatattgtagttatacttgagactgacacattgtaatataatctaa
gaataaaagttatacaaaataaaa (SEQ ID NO: 306)

[00194] The nucleotide sequence of mRNA encoded by human voltage-gated channel alpha subunit 9 (SCN9A), transcript variant 2, mRNA (as defined by NCBI Genbank Accession no. NM_001365536.1) as shown above is set forth in the sequence listing as SEQ ID NO: 305:

AGUCUGCUUGCAGGCGGUCGCCAGCGCUCCAGCGGCGGCUGUCGGCUUUCCAAUUCCGCCAGCU
CGGCUGAGGCUGGGCUAGCCUGGGUGCCAGUGGCUGCUAGCGGCAGGCGUCCCCUGAGCAACAG
GAGCCCAGAGAAAAAGAAGCAGCCCUGAGAGAGCGCCGGGGAAGGAGAGGCCCGCGCCCUCUCC
UGGAGCCAGAUUCUGCAGGUGCACUGGGUGGGGAUGAUCGGCGGGCUAGGUUGCAAGCCUCUUA
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GACCUCAGAGCUUUGUCCAUUUCACAAAACAGUCUCUUGCCCUCAUUGAACAACGCAUUGCUGA
AAGAAAAUCAAAGGAACCCAAAGAAGAAAAGAAAGAUGAUGAUGAAGAAGCCCCAAAGCCAAGC
AGUGACUUGGAAGCUGGCAAACAGCUGCCCUUCAUCUAUGGGGACAUUCCUCCCGGCAUGGUGU
CAGAGCCCCUGGAGGACUUGGACCCCUACUAUGCAGACAAAAAGACUUUCAUAGUAUUGAACAA
AGGGAAAACAAUCUUCCGUUUCAAUGCCACACCUGCUUUAUAUAUGCUUUCUCCUUUCAGUCCU
CUAAGAAGAAUAUCUAUUAAGAUUUUAGUACACUCCUUAUUCAGCAUGCUCAUCAUGUGCACUA
UUCUGACAAACUGCAUAUUUAUGACCAUGAAUAACCCACCGGACUGGACCAAAAAUGUCGAGUA
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GGAGAAUUCACUUUUCUUCGUGACCCGUGGAACUGGCUGGAUUUUGUCGUCAUUGUUUUUGCGU
AUUUAACAGAAUUUGUAAACCUAGGCAAUGUUUCAGCUCUUCGAACUUUCAGAGUAUUGAGAGC
UUUGAAAACUAUUUCUGUAAUCCCAGGCCUGAAGACAAUUGUAGGGGCUUUGAUCCAGUCAGUG
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UACAGCUGUUCAUGGGAAACCUGAAGCAUAAAUGUUUUCGAAAUUCACUUGAAAAUAAUGAAAC
AUUAGAAAGCAUAAUGAAUACCCUAGAGAGUGAAGAAGACUUUAGAAAAUAUUUUUAUUACUUG
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AUCUAAUAAACUUGAUCCUGGCUGUGGUUGCCAUGGCAUAUGAAGAACAGAACCAGGCAAACAU
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GAAGAAGCUGAGGCAAUUGCAGCGGCAGCGGCUGAAUAUACAAGUAUUAGGAGAAGCAGAAUUA
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AAACAGAAGAAAGAAAAAGAAUCAAAAGAAGCUCUCCAGUGGAGAGGAAAAGGGAGAUGCUGAG
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GAUGGUCAAUCAUUUAUUCACAAUCCCAGCCUCACAGUGACAGUGCCAAUUGCACCUGGGGAAU
CCGAUUUGGAAAAUAUGAAUGCUGAGGAACUUAGCAGUGAUUCGGAUAGUGAAUACAGCAAAGU
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CAGGAAAACCUGCUACAAGAUUGUUGAACACAGUUGGUUUGAAAGCUUCAUUGUCCUCAUGAUC
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CCCUUCGGACACUGAGAGCUUUAAGACCUCUAAGAGCCUUAUCUAGAUUUGAAGGAAUGAGGGU
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UUUUGGAUAUACUUCAAUGUAUACAAUGAGGAAGAUGUCACCUUCUCCUUAAAAUUCUAUGAUG
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CUUAAAUUUGAAUUCAAUCUACCGUUAUUUCAAACUCACAAAUAUAACUGCAUUAUGAAUACUU
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CUGAAGAACUUAACUUAGAAUAAAAUGGAAGCAAACAGGUUUUCUAAAAACUUUUUUAAGUGAC
CAGGUCUCGCUCUGUCACCCAGGCUAGAGUGCAAUGGCAUGAUCAUAGCUCUCUGCAGCCUCAA
CUCUGGGCUCAAGCAACCCUCCUGCCUCAGCCUCCCAAGUAGCUAAGACUACAGGUACAUGCCA
CCAUGCCUGGCUAAUAUUUAAAUUUUUGUAGAUAAGGGGUCUUGCUAUGUUGCCCAGGCUAGUC
UCAAACUCCUGGCUUCAAGUGUUCCUACUGUCAUGACCUGCCAACAUGCUGGGGUUACAGGCAU
GAGCCACCAUGCCCCAAACAGGUUUGAACACAAAUCUUUCGGAUGAAAAUUAGAGAACCUAAUU
UUAGCUUUUUGAUAGUUACCUAGUUUGCAAAAGAUUUGGGUGACUUGUGAGCUGUUUUUAAAUG
CUGAUUGUUGAACAUCACAACCCAAAAUACUUAGCAUGAUUUUAUAGAGUUUUGAUAGCUUUAU
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UGUUAAUAGAAGUGCUGAAAGAAGGGCCAACUAAAUUAGGAUGGCCAGGGAAUUGGCCUGGGUU
UAGGACCUAUGUAUGAAGGCCACCAAUUUUUUAAAAAUAUCUGUGGUUUAUUAUGUUAUUAUCU
UCUUGAGGAAAACAAUCAAGAAUUGCUUCAUGAAAAUAAAUAAAUAGCCAUGAAUAUCAUAAAG
CUGUUUACAUAGGAUUCUUUACAAAUUUCAUAGAUCUAUGAAUGCUCAAAAUGUUUGAGUUUGC
CAUAAAUUAUAUUGUAGUUAUAUUGUAGUUAUACUUGAGACUGACACAUUGUAAUAUAAUCUAA
GAAUAAAAGUUAUACAAAAUAAAA (SEQ ID NO: 305)

[00195] The tables below provide other sequences that are referenced in this disclosure.

[00196] Table 5 below shows the target site sequences in the SCN9A transcript provided herein (SEQ ID NO: 305) that were tested in the present disclosure.

TABLE 5

Target Site No.	Target Site Sequence in SCN9A RNA Transcript (SEQ ID NO:305)	SEQ ID NO
Site 1	UCAGAGUGCAGCACAGUUGAUA	1

Site 2	UGCCCUCAUUGAACAACGCAUU	2
Site 3	AGCAGUGGAUUCUGUUAAUGUA	3
Site 4	UAGCAGUGAUUCGGAUAGUGAA	4
Site 5	CAGAGUGCAGCACAGUUGAUAA	5
Site 6	AGCCCUCAUGCUCCCCAAUGGA	6
Site 7	CAAUCUUACAGCAAUUGAAGAA	7
Site 8	UUGCCCUCAUUGAACAACGCAU	8
Site 9	AUGUGCCUUAUUGUUUACAUGA	9
Site 10	UUCUUCACUUUGAACUUGUUCA	10
Site 11	CCAUUAAGAUUAUCCUGGAGUA	11
Site 12	AUGGUAACCAUGAUGGUAGAAA	12
Site 13	GCAAACAACCUCCAGAUUGCAG	13
Site 14	CCCUCAUGCUCCCCAAUGGACA	14
Site 15	UUGCUGGCAAGUUCUAUGAGUG	15
Site 16	CUCUAAGAAGAAUAUCUAUUAA	16
Site 17	ACAGAAAGAAUUAGAAUUUCAA	17
Site 18	GCCAGACAAAGAGAAAUAUGAA	18
Site 19	CUAAGAAGAAUAUCUAUUAAGA	19
Site 20	AGCCAGACAAAGAGAAAUAUGA	20
Site 21	UGCUGGCAAGUUCUAUGAGUGU	21
Site 22	GACAAUCUUACAGCAAUUGAAG	22
Site 23	ACAAUCUUACAGCAAUUGAAGA	23
Site 24	CUUGCCCUCAUUGAACAACGCA	24
Site 25	UCGUUCACAGAUGGAAGAAAGG	25
Site 26	AACAGAAAGAAUUAGAAUUUCA	26
Site 27	UGUGCCUUAUUGUUUACAUGAU	27
Site 28	UCAGCCCUCAUGCUCCCCAAUG	28
Site 29	CUUCGUUCACAGAUGGAAGAAA	29
Site 30	GUUCACAGAUGGAAGAAAGGUU	30
Site 31	AGUGCAGCACAGUUGAUAACCC	31
Site 32	AGAAGAAUAUCUAUUAAGAUUU	32
Site 33	AAAGUGUAUCUAUUUUAUUGUA	33
Site 34	GUGCAGCACAGUUGAUAACCCU	34
Site 35	GCAAGUUCUAUGAGUGUAUUAA	35
Site 36	AACAUUGAAGAAGCUAAACAGA	36
Site 37	CCUCUAAGAAGAAUAUCUAUUA	37
Site 38	ACAGUCUCUUGCCCUCAUUGAA	38
Site 39	AGUCCUCUAAGAAGAAUAUCUA	39
Site 40	GUGCCUUAUUGUUUACAUGAUG	40

Site 41	UUGUGGUUUCAGCACAGAUUCA	41
Site 42	UAGAAUUUCAACAGAUGUUAGA	42
Site 43	CACAAUCCCAGCCUCACAGUGA	43
Site 44	GCUGGCAAGUUCUAUGAGUGUA	44
Site 45	GUUCAGACAAUCUUACAGCAAU	45
Site 46	AGACAAUCUUACAGCAAUUGAA	46
Site 47	CACAGUUGGUUUGAAAGCUUCA	47
Site 48	CAGACAAUCUUACAGCAAUUGA	48
Site 49	GAGCGACGCAGCAGUAACAUCA	49
Site 50	AUGGAUUCUCUUCGUUCACAGA	50
Site 51	AAAGACCAUUAAGAUUAUCCUG	51
Site 52	UUCAGACAAUCUUACAGCAAUU	52
Site 53	UUGCCACUGAAGAAAGUACUGA	53
Site 54	CUCAGAUCUUGGCCCCAUUAAA	54
Site 55	UAAUGAGAACUCAAGUCCAGAA	55
Site 56	AGCUCUUCGAACUUUCAGAGUA	56
Site 57	GGACCCCUACUAUGCAGACAAA	57
Site 58	UGGACCCCUACUAUGCAGACAA	58
Site 59	GCAAAGGUCACAAUUUCCUCAA	59
Site 60 Positive Control	UUUGUAGAUCUUGCAAUUACCA	60

[00197] Table 6 below provides targeting region polynucleotide sequences that were designed to bind to the corresponding target sites in the SCN9A transcript provided in Table 5. These targeting regions find use in reducing cellular expression of the SCN9A gene (which encodes Nav1.7). For example, the RNA sequences can be used as targeting regions in RNAi agents (e.g., miRNAs, antisense oligonucleotides, etc.). The RNA sequences in Table 6 represent examples of mature miRNA sequences and thus can be used as guide strand RNA sequences in engineered pri-miRNAs. Corresponding DNA sequences that that can be employed in expression cassettes or vectors, e.g., encoding pri-miRNAs or other antisense RNA agents, are also provided. The scramble (SCRM) sequence does not have a cognate binding site in the SCN9A transcript and has been used in the Examples as a negative control.

TABLE 6

Target Site No.	Targeting Region RNA Sequence	SEQ ID NO	Targeting Region DNA Sequence	SEQ ID NO
Site 1	UAUCAACUGUGCUGCACUCUGA	61	TATCAACTGTGCTGCACTCTGA	122
Site 2	AAUGCGUUGUUCAAUGAGGGCA	62	AATGCGTTGTTCAATGAGGGCA	123
Site 3	UACAUUAACAGAAUCCACUGCU	63	TACATTAACAGAATCCACTGCT	124
Site 4	UUCACUAUCCGAAUCACUGCUA	64	TTCACTATCCGAATCACTGCTA	125
Site 5	UUAUCAACUGUGCUGCACUCUG	65	TTATCAACTGTGCTGCACTCTG	126
Site 6	UCCAUUGGGGAGCAUGAGGGCU	66	TCCATTGGGGAGCATGAGGGCT	127
Site 7	UUCUUCAAUUGCUGUAAGAUUG	67	TTCTTCAATTGCTGTAAGATTG	128
Site 8	AUGCGUUGUUCAAUGAGGGCAA	68	ATGCGTTGTTCAATGAGGGCAA	129
Site 9	UCAUGUAAACAAUAAGGCACAU	69	TCATGTAAACAATAAGGCACAT	130
Site 10	UGAACAAGUUCAAAGUGAAGAA	70	TGAACAAGTTCAAAGTGAAGAA	131
Site 11	UACUCCAGGAUAAUCUUAAUGG	71	TACTCCAGGATAATCTTAATGG	132
Site 12	UUUCUACCAUCAUGGUUACCAU	72	TTTCTACCATCATGGTTACCAT	133
Site 13	CUGCAAUCUGGAGGUUGUUUGC	73	CTGCAATCTGGAGGTTGTTTGC	134
Site 14	UGUCCAUUGGGGAGCAUGAGGG	74	TGTCCATTGGGGAGCATGAGGG	135
Site 15	CACUCAUAGAACUUGCCAGCAA	75	CACTCATAGAACTTGCCAGCAA	136
Site 16	UUAAUAGAUAUUCUUCUUAGAG	76	TTAATAGATATTCTTCTTAGAG	137
Site 17	UUGAAAUUCUAAUUCUUUCUGU	77	TTGAAATTCTAATTCTTTCTGT	138
Site 18	UUCAUAUUUCUCUUUGUCUGGC	78	TTCATATTTCTCTTTGTCTGGC	139
Site 19	UCUUAAUAGAUAUUCUUCUUAG	79	TCTTAATAGATATTCTTCTTAG	140
Site 20	UCAUAUUUCUCUUUGUCUGGCU	80	TCATATTTCTCTTTGTCTGGCT	141
Site 21	ACACUCAUAGAACUUGCCAGCA	81	ACACTCATAGAACTTGCCAGCA	142
Site 22	CUUCAAUUGCUGUAAGAUUGUC	82	CTTCAATTGCTGTAAGATTGTC	143
Site 23	UCUUCAAUUGCUGUAAGAUUGU	83	TCTTCAATTGCTGTAAGATTGT	144
Site 24	UGCGUUGUUCAAUGAGGGCAAG	84	TGCGTTGTTCAATGAGGGCAAG	145
Site 25	CCUUUCUUCCAUCUGUGAACGA	85	CCTTTCTTCCATCTGTGAACGA	146
Site 26	UGAAAUUCUAAUUCUUUCUGUU	86	TGAAATTCTAATTCTTTCTGTT	147
Site 27	AUCAUGUAAACAAUAAGGCACA	87	ATCATGTAAACAATAAGGCACA	148
Site 28	CAUUGGGGAGCAUGAGGGCUGA	88	CATTGGGGAGCATGAGGGCTGA	149
Site 29	UUUCUUCCAUCUGUGAACGAAG	89	TTTCTTCCATCTGTGAACGAAG	150
Site 30	AACCUUUCUUCCAUCUGUGAAC	90	AACCTTTCTTCCATCTGTGAAC	151
Site 31	GGGUUAUCAACUGUGCUGCACU	91	GGGTTATCAACTGTGCTGCACT	152
Site 32	AAAUCUUAAUAGAUAUUCUUCU	92	AAATCTTAATAGATATTCTTCT	153
Site 33	UACAAUAAAAUAGAUACACUUU	93	TACAATAAAATAGATACACTTT	154
Site 34	AGGGUUAUCAACUGUGCUGCAC	94	AGGGTTATCAACTGTGCTGCAC	155
Site 35	UUAAUACACUCAUAGAACUUGC	95	TTAATACACTCATAGAACTTGC	156
Site 36	UCUGUUUAGCUUCUUCAAUGUU	96	TCTGTTTAGCTTCTTCAATGTT	157

Site 37	UAAUAGAUAUUCUUCUUAGAGG	97	TAATAGATATTCTTCTTAGAGG	158
Site 38	UUCAAUGAGGGCAAGAGACUGU	98	TTCAATGAGGGCAAGAGACTGT	159
Site 39	UAGAUAUUCUUCUUAGAGGACU	99	TAGATATTCTTCTTAGAGGACT	160
Site 40	CAUCAUGUAAACAAUAAGGCAC	100	CATCATGTAAACAATAAGGCAC	161
Site 41	UGAAUCUGUGCUGAAACCACAA	101	TGAATCTGTGCTGAAACCACAA	162
Site 42	UCUAACAUCUGUUGAAAUUCUA	102	TCTAACATCTGTTGAAATTCTA	163
Site 43	UCACUGUGAGGCUGGGAUUGUG	103	TCACTGTGAGGCTGGGATTGTG	164
Site 44	UACACUCAUAGAACUUGCCAGC	104	TACACTCATAGAACTTGCCAGC	165
Site 45	AUUGCUGUAAGAUUGUCUGAAC	105	ATTGCTGTAAGATTGTCTGAAC	166
Site 46	UUCAAUUGCUGUAAGAUUGUCU	106	TTCAATTGCTGTAAGATTGTCT	167
Site 47	UGAAGCUUUCAAACCAACUGUG	107	TGAAGCTTTCAAACCAACTGTG	168
Site 48	UCAAUUGCUGUAAGAUUGUCUG	108	TCAATTGCTGTAAGATTGTCTG	169
Site 49	UGAUGUUACUGCUGCGUCGCUC	109	TGATGTTACTGCTGCGTCGCTC	170
Site 50	UCUGUGAACGAAGAGAAUCCAU	110	TCTGTGAACGAAGAGAATCCAT	171
Site 51	CAGGAUAAUCUUAAUGGUCUUU	111	CAGGATAATCTTAATGGTCTTT	172
Site 52	AAUUGCUGUAAGAUUGUCUGAA	112	AATTGCTGTAAGATTGTCTGAA	173
Site 53	UCAGUACUUUCUUCAGUGGCAA	113	TCAGTACTTTCTTCAGTGGCAA	174
Site 54	UUUAAUGGGGCCAAGAUCUGAG	114	TTTAATGGGGCCAAGATCTGAG	175
Site 55	UUCUGGACUUGAGUUCUCAUUA	115	TTCTGGACTTGAGTTCTCATTA	176
Site 56	UACUCUGAAAGUUCGAAGAGCU	116	TACTCTGAAAGTTCGAAGAGCT	177
Site 57	UUUGUCUGCAUAGUAGGGGUCC	117	TTTGTCTGCATAGTAGGGGTCC	178
Site 58	UUGUCUGCAUAGUAGGGGUCCA	118	TTGTCTGCATAGTAGGGGTCCA	179
Site 59	UUGAGGAAAUUGUGACCUUUGC	119	TTGAGGAAATTGTGACCTTTGC	180
Site 60 Positive Control	UGGUAAUUGCAAGAUCUACAAA	120	TGGTAATTGCAAGATCTACAAA	181
Scramble (SCRM) Negative Control	UUACUGUAUGCCGGUCGUUGCU	121	TACTGTATGCCGGTCGTTGCTG	182

[00198] Table 7 below provides examples of passenger strand RNA sequences for use in engineered pri-miRNAs of the present disclosure as well as the corresponding DNA sequences that can be employed in expression cassettes or vectors encoding the pri-miRNAs.

TABLE 7

Target Site No.	**Passenger Strand RNA Sequence**	**SEQ ID NO**	**Passenger Strand DNA Sequence (in vector)**	**SEQ ID NO**

Site 1	GCAGAGUGCAGCACAGUUGAUA	183	GCAGAGTGCAGCACAGTTGATA	244
Site 2	GGCCCUCAUUGAACAACGCAUU	184	GGCCCTCATTGAACAACGCATT	245
Site 3	CGCAGUGGAUUCUGUUAAUGUA	185	CGCAGTGGATTCTGTTAATGTA	246
Site 4	GAGCAGUGAUUCGGAUAGUGAA	186	GAGCAGTGATTCGGATAGTGAA	247
Site 5	AAGAGUGCAGCACAGUUGAUAA	187	AAGAGTGCAGCACAGTTGATAA	248
Site 6	CGCCCUCAUGCUCCCCAAUGGA	188	CGCCCTCATGCTCCCCAATGGA	249
Site 7	AAAUCUUACAGCAAUUGAAGAA	189	AAATCTTACAGCAATTGAAGAA	250
Site 8	GUGCCCUCAUUGAACAACGCAU	190	GTGCCCTCATTGAACAACGCAT	251
Site 9	CUGUGCCUUAUUGUUUACAUGA	191	CTGTGCCTTATTGTTTACATGA	252
Site 10	GUCUUCACUUUGAACUUGUUCA	192	GTCTTCACTTTGAACTTGTTCA	253
Site 11	ACAUUAAGAUUAUCCUGGAGUA	193	ACATTAAGATTATCCTGGAGTA	254
Site 12	CUGGUAACCAUGAUGGUAGAAA	194	CTGGTAACCATGATGGTAGAAA	255
Site 13	UCAAACAACCUCCAGAUUGCAG	195	TCAAACAACCTCCAGATTGCAG	256
Site 14	ACCUCAUGCUCCCCAAUGGACA	196	ACCTCATGCTCCCCAATGGACA	257
Site 15	GUGCUGGCAAGUUCUAUGAGUG	197	GTGCTGGCAAGTTCTATGAGTG	258
Site 16	AUCUAAGAAGAAUAUCUAUUAA	198	ATCTAAGAAGAATATCTATTAA	259
Site 17	CCAGAAAGAAUUAGAAUUUCAA	199	CCAGAAAGAATTAGAATTTCAA	260
Site 18	UCCAGACAAAGAGAAAUAUGAA	200	TCCAGACAAAGAGAAATATGAA	261
Site 19	AUAAGAAGAAUAUCUAUUAAGA	201	ATAAGAAGAATATCTATTAAGA	262
Site 20	CGCCAGACAAAGAGAAAUAUGA	202	CGCCAGACAAAGAGAAATATGA	263
Site 21	GGCUGGCAAGUUCUAUGAGUGU	203	GGCTGGCAAGTTCTATGAGTGT	264
Site 22	UACAAUCUUACAGCAAUUGAAG	204	TACAATCTTACAGCAATTGAAG	265
Site 23	CCAAUCUUACAGCAAUUGAAGA	205	CCAATCTTACAGCAATTGAAGA	266
Site 24	AUUGCCCUCAUUGAACAACGCA	206	ATTGCCCTCATTGAACAACGCA	267
Site 25	GCGUUCACAGAUGGAAGAAAGG	207	GCGTTCACAGATGGAAGAAAGG	268
Site 26	CACAGAAAGAAUUAGAAUUUCA	208	CACAGAAAGAATTAGAATTTCA	269
Site 27	GGUGCCUUAUUGUUUACAUGAU	209	GGTGCCTTATTGTTTACATGAT	270
Site 28	GCAGCCCUCAUGCUCCCCAAUG	210	GCAGCCCTCATGCTCCCCAATG	271
Site 29	AUUCGUUCACAGAUGGAAGAAA	211	ATTCGTTCACAGATGGAAGAAA	272
Site 30	UUUCACAGAUGGAAGAAAGGUU	212	TTTCACAGATGGAAGAAAGGTT	273
Site 31	CGUGCAGCACAGUUGAUAACCC	213	CGTGCAGCACAGTTGATAACCC	274
Site 32	CGAAGAAUAUCUAUUAAGAUUU	214	CGAAGAATATCTATTAAGATTT	275
Site 33	CAAGUGUAUCUAUUUUAUUGUA	215	CAAGTGTATCTATTTTATTGTA	276
Site 34	UUGCAGCACAGUUGAUAACCCU	216	TTGCAGCACAGTTGATAACCCT	277
Site 35	UCAAGUUCUAUGAGUGUAUUAA	217	TCAAGTTCTATGAGTGTATTAA	278
Site 36	CACAUUGAAGAAGCUAAACAGA	218	CACATTGAAGAAGCTAAACAGA	279
Site 37	ACUCUAAGAAGAAUAUCUAUUA	219	ACTCTAAGAAGAATATCTATTA	280
Site 38	CCAGUCUCUUGCCCUCAUUGAA	220	CCAGTCTCTTGCCCTCATTGAA	281
Site 39	CGUCCUCUAAGAAGAAUAUCUA	221	CGTCCTCTAAGAAGAATATCTA	282

Site 40	UUGCCUUAUUGUUUACAUGAUG	222	TTGCCTTATTGTTTACATGATG	283
Site 41	GUGUGGUUUCAGCACAGAUUCA	223	GTGTGGTTTCAGCACAGATTCA	284
Site 42	GAGAAUUUCAACAGAUGUUAGA	224	GAGAATTTCAACAGATGTTAGA	285
Site 43	AACAAUCCCAGCCUCACAGUGA	225	AACAATCCCAGCCTCACAGTGA	286
Site 44	UCUGGCAAGUUCUAUGAGUGUA	226	TCTGGCAAGTTCTATGAGTGTA	287
Site 45	UUUCAGACAAUCUUACAGCAAU	227	TTTCAGACAATCTTACAGCAAT	288
Site 46	CGACAAUCUUACAGCAAUUGAA	228	CGACAATCTTACAGCAATTGAA	289
Site 47	AACAGUUGGUUUGAAAGCUUCA	229	AACAGTTGGTTTGAAAGCTTCA	290
Site 48	AAGACAAUCUUACAGCAAUUGA	230	AAGACAATCTTACAGCAATTGA	291
Site 49	UAGCGACGCAGCAGUAACAUCA	231	TAGCGACGCAGCAGTAACATCA	292
Site 50	CUGGAUUCUCUUCGUUCACAGA	232	CTGGATTCTCTTCGTTCACAGA	293
Site 51	CAAGACCAUUAAGAUUAUCCUG	233	CAAGACCATTAAGATTATCCTG	294
Site 52	GUCAGACAAUCUUACAGCAAUU	234	GTCAGACAATCTTACAGCAATT	295
Site 53	GUGCCACUGAAGAAAGUACUGA	235	GTGCCACTGAAGAAAGTACTGA	296
Site 54	AUCAGAUCUUGGCCCCAUUAAA	236	ATCAGATCTTGGCCCCATTAAA	297
Site 55	GAAUGAGAACUCAAGUCCAGAA	237	GAATGAGAACTCAAGTCCAGAA	298
Site 56	CGCUCUUCGAACUUUCAGAGUA	238	CGCTCTTCGAACTTTCAGAGTA	299
Site 57	UGACCCCUACUAUGCAGACAAA	239	TGACCCCTACTATGCAGACAAA	300
Site 58	GGGACCCCUACUAUGCAGACAA	240	GGGACCCCTACTATGCAGACAA	301
Site 59	UCAAAGGUCACAAUUUCCUCAA	241	TCAAAGGTCACAATTTCCTCAA	302
Site 60 Positive Control	GUUGUAGAUCUUGCAAUUACCA	242	GTTGTAGATCTTGCAATTACCA	303
Scramble (SCRM) Negative Control	AAGCAACGACCGGCAUACAGUA	243	AAGCAACGACCGGCATACAGTA	304

[00199] Additional sequences are provided below:

[00200] SEQ ID NO:448 is a U6 promoter and has the following DNA sequence:

[00201] GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTT AGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATAC GTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAA AATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATAT ATCTTGTGGAAAGGACGAAACACC

[00202] SEQ ID NO:449 is a Syn1 promoter and has the following DNA sequence:

[00203] TAGTATCTGCAGAGGGCCCTGCGTATGAGTGCAAGTGGGTTTTAGGACCAGGA
TGAGGCGGGGTGGGGGTGCCTACCTGACGACCGACCCCGACCCACTGGACAAGCAC
CCAACCCCCATTCCCCAAATTGCGCATCCCCTATCAGAGAGGGGGAGGGGAAACAG
GATGCGGCGAGGCGCGTGCGCACTGCCAGCTTCAGCACCGCGGACAGTGCCTTCGC
CCCCGCCTGGCGGCGCGCGCCACCGCCGCCTCAGCACTGAAGGCGCGCTGACGTCA
CTCGCCGGTCCCCCGCAAACTCCCCTTCCCGGCCACCTTGGTCGCGTCCGCGCCGCC
GCCGGCCCAGCCGGACCGCACCACGCGAGGCGCGAGATAGGGGGGCACGGGCGCG
ACCATCTGCGCTGCGGCGCCGGCGACTCAGCGCTGCCTCAGTCTGCGGTGGGCAGC
GGAGGAGTCGTGTCGTGCCTGAGAGCGCAGCTGTGCTCCTGGGCACCGCGCAGTCC
GCCCCCGCGGCTCCTGGCCAGACCACCCCTAGGACCCCCTGCCCCAAGTCGCAGCC

[00204] SEQ ID NO:450 is an EF1α promoter and has the following DNA sequence:

[00205] GAGTAATTCATACAAAAGGACTCGCCCCTGCCTTGGGGAATCCCAGGGACCGT
CGTTAAACTCCCACTAACGTAGAACCCAGAGATCGCTGCGTTCCCGCCCCCTCACCC
GCCCGCTCTCGTCATCACTGAGGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCG
TCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCG
GCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTC
GTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTA
GTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCC
GTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAA
TTACTTCCACGCCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGA
AGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGA
GTTGAGGCCTGGCTTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGC
GCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTG
CGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTG
GTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACAT
GTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCT
CAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCC
TGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCT
TCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGG
CGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCAT
GTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTT

GGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACA
CTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGG
AATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCA
AAGTTTTTTTCTTCCATTTCAGGTGTCGTGA

[00206] SEQ ID NO:451 is a candidate promoter region from mouse SCN10A gene (which expresses Nav1.8) and maps to nucleotides 119548095 to 119551493 of chromosome 9 of the C57BL/6 genome (GRCm39) and has the following sequence:

[00207] GGCCAGAGAAGCTCCTCTGAAAACAGAAGTCAAGAGGGTGGAGTGTGGTGCA
AGGACCATGCAGCTAATCCTGCGGAGCCCCTAGGATGAGAGCGCCAGAGAGGAGTC
ACATGACCACAGGGAGACCAGTAGAAACCTGTTAAGATTCCGGGTGTCTCAGGACT
GCCTCTGGATGCACACTTCTTCCTTCCTTGGGAAGTTACTTTTCTGTCACTGTGATGA
AATACCTTAACCAAGGTGACTCAAAGAAGAGAGGGTTTATCTGGGCTCACGGGTCC
AGAGGTAGAGGAACACATGGAGATCGTGGTGGGGAACCAGTGTAGCAGGAAAGCA
TGGTGGCTGGGGCTGAGGCTGAGAGCTTATATCTTTGTCTGTATACAGAAAGCAGAG
AGAGCCAACTGGGAATGACTTGTGGCTTTTGGAACCTGAAACCTGTCTTCGGTGACA
TGCTCCCTCCAGCGAAGGCAATGCCTCCTCAAACTCCCCAAAGGGCACCACAAACT
AGGAACCAAGCACTCAGATGCCCGAGACTATGAGCGACATCTCCTTCAGATCACCA
CACTTGGGTACACCCATTCTCCTGTCTCATCCAGTTTGCTCTTCTGGAAGGGTGGTGA
GAGGGATGACAGCTAGTGACAAGTTGGAGAGACTTTAGAATAATTGCCATCACACA
AAGCCTACCCTATCAGTTAGTGGCTGGCACGCTATCCCAACAGCTTGAGTCTGAACT
TGCCAGAAATGGCCTCCGTCTCACCTCTCCCAGGCTCCCCAGCACCCACAGGTGCCC
TCCCCCAAGACCTGACATCATCGGAGCACTGAAGAGATGCCTCCTCTGCCCCTTTCT
CCCCTGGTCTGATTGCTACCAGGCAGCTGATCCACATGCCCTGCTCCAAGTTTGACC
CCAGTCAGCAGGCTTCTCTGAAGAAGAGGGTCTGTTAGCATGACACACAGCATTTCC
CATGCAACAGAACCTTGGAACCTAGGACAGAACTCAAGATATCAACGTGACACACA
CATGTACATGTACCCTTACACACCTGAACGTGCATATACACACGTACACTTGTACAC
ACACTAAAATAACTGTGGTGCCAGGAGTGATCAGGGTACTGAGGTGACAGTCATTC
ACAAGGCTTGCCTTTCTCCACGTGACACACTTTGACCTTACCACCTGTTTCCACCTTG
CTCTGGCATTTTAAAAACATGACATTTTTAGTAAATTCTTTGAATTTTTTTGAGACAG
GGTCTTACACTACAGCTCAAGCTGATCTTGGGTTTGCAGCAATCCTCTTCCCTCGACC
ACCCCCCCAACTAGGATGTGAGCTGCCATGCCCAGTTTGACTCTTTCCCAGATGTTT

GTTTTTATTCTGTGTGTATGAGTGTTTTACTGTATGTATGTCTGTATATGCACCATGT
AGAGCCCAAAAGCAGTTGCTGAATGCTCCAGAGCTGGAGTTGTGGGTGGCTGTGAG
CTGCCACGTGGGTGCCAAGAATAGAACACAGGTCCTCTGCAAGAGCATCGAATGCT
CTTAACCACTGAGCTATCCCTCCAGGTATTAAAACATAAAATAAGCTTGACTTTTTA
CTCTGACATAATTTAAAATTCACAAAAAGTTTTAAAAAACGTAACAGCTTCCGTATA
CTCCCAACCCCATTTCCCCAGTTAGGATATTCTTTAACCATAGTACATTGTCAAATGA
GAAAACTAACATTCATACAACACGACTGATTTTGGTGAAAACCCTATTTGGAGTACA
CTACCTTTGACTGTGATTTCTTTTTACGCTATGGCCCAGGCTAGCCTGCAACTATTTA
TCATTATTTATCCCAGGGTGACTTGAACTTAGGGCAATTCTTCTACCTCAGCTCTGCC
CCCCACTCCCACCCCAACTCCAGTCCTGGGGTTACAGGAGCAAGCCATCAAGTTCTA
TAATATTTAATACACAAGGACACTGGTTAAACTCAGAAGGACCTAAATTAGCATAA
GACTGTGGGGACCAGAGAAGTGAGAAGTGAGGACAGGGGAGGGAGGGTAGGAGAG
GGAGGGCAGGGGAGGGAAGATGGGGAGGAATGATGGGGAAGGAGAATGAGGAGG
AAGGGGAGGGAGGGAGGAGGAGGAAGGGCAGTGGAGGGAGAATGGGAAGGGAGG
GAGTTGAGAGAAAGCAGGATCGGGAGCCATAGAATGTCTGTAGGAAACCATCAAAG
GCATTTAATTTAATAAAGCAACCAGGATTGTACATAATTCTACTGTGTACATACAAA
CATTCAAGTTTTGGGAGCAAGAATTTTAGCTTTCCTTCCCCTGCCCCTTTATGATTCA
GTCTCTGCTAGAAAAAGCGGAGCCTTGCAGGGTGCCGTGGTGCATGCCTTTAATTCC
AGCGTTTGGGAGGCAGAGGCAGGTGGATTTCTGTGAGTTCCCGGTCAACCAAATCTC
CGTAGTATGGTCCTTCGTGGAATACCAGCCAACCAAACAAGCAAACAAACAAACAA
AAATCCCAAACAAACCCCACCCCCACCCAAATAGAGGGGATTATTGACTCAAAGAA
GCCAATAATTTTGAGTTGGTTTGGGACATTTGAGTAAATGAAGCTGTAATGGGCAAG
CATGGGCCCTCGCCAGTTTCCTGCAGTATAGCATGGCTTCCTAAGGCTGGGTGGGTT
GCACTGTTACAGAGGGCTCAGCTCAGACAGGGGGTTCCCTGTGCAACCTCCCTTCTT
ATGGTCCCACAACCCCACAAATAGGGCACTTTCCCTACCCAGCTCCCTTCTCAGCTC
TCACTGGGGTCGGAGAACATTTTTGTTTCAGCATTTCATCTGAAGCCACGGTTTCAC
ATCATCAAGTCTGCAAAAAACCGTTCACAAACCACACCAAAACTTCTCGGTAAAGA
ACTCCTAAGGCCAAAGAGGGAGACTGGGTAGATTGTTTTTAATTTGTTTCTTTTTGTC
AAAGGGGGACAAAACACGCTTTGGTGAGTGCGAGTGTTTATTCTGGGACACAAAAC
CAGAGTCTGGAAGGGAGCATTCAACGGGTGCTGCTCTGCCACGCAGGGGCAGCGGT
GGGACTCAGCCCATCCTGCTAAGGACGGGCAGCCTGAGCCAGGCTTGGGAGTCTGT

CATGGCTGCCAGACGAATCATTATCTAATTGCAGCCTTTTCTCTTCCTTAGGTTTCAG
CAGGTCCCGAGAGAGCATTTAAAATCACATTTACTACTTTACCATCTAATCACACAT
AAGCCTCTCCCTATACCCTCCACCCTCCTTCCATTCAGAGTGTACTTTCTGGAGCACC
ATCCAGCAAGCAGGGTGGAACTCGTGACGGGAAATGGGAATGGCACCCACGAAGG
CGTGATTCCTTGTAGATCCTTGAGTGACGGACGGGTGAGGTTTCCGTCAGGCAAGCC
C

[00208] SEQ ID NO:452 (in SEQ ID NO:451 above) is a region from the mouse SCN10A gene (which expresses Nav1.8) and maps to nucleotides 119549695 to 119549794 of chromosome 9 of the C57BL/6 genome (GRCm39) and has the following sequence:

[00209] AGAAAACTAACATTCATACAACACGACTGATTTTGGTGAAAACCCTATTTGGA
GTACACTACCTTTGACTGTGATTTCTTTTTACGCTATGGCCCAGGCT

[00210] SEQ ID NO:453 is a candidate promoter region from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38816207 to 38819604 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00211] TTTGTGTTTGACTTCTTTTACTTAACATACTATTTTTTAAATTTTATTTATTTATT
TATTTATTTTATTATGCTTTAAGTTCTAGGGTACATGTGCACAATGTGCAGGTTTGCT
ACATATGTATACATGTGCCATACTTAACATACTATTTTTAAGCTTTGTCCAGGTTGTA
GCACTTATTAGTACTTTCTCTTTTTTTTTTTTTTTTTTTTTTTTTAGACAAAGTCTTGCT
CTGTCACCCAGGTTGGAGTGCAATGGCCCAACCTTGGCTCACTGCAACCTCCACCTC
CCGGGTTCAAGCAATTCTCTTGCCTCAGCCTCCTGAGTAGCTGGGACTACAGGGGCA
CGGCACCATGCCCGGCTAATTTTTTGTATTTTAGTAGAGAGCAGGTTTCACCATGTTG
CCTAGGCTGATCTCGAACTCCTGACCTCAGGCAATCTGCCCGCCTTGGCCTCCCAAA
TTGCTAGGATTACAGGCATGAGCCACGGCCCCCGGCCAGTAGTTTATTTCTTTTTATG
GCTGAACAATATTCTAGCAATGTATTTTTGAATGAGTATCCAGTGCAGAGCTGAGAG
AGTTTCTTCCAGTGAGACATCTAAAGTTCCCTGGAATGGCTCTTTTCACTCTGTCAGA
TGCACCCCCCCGACTCTAACCAGCATCCATAAGTGGTCCAAGAATAAGGAGGGGAG
TTGTGGGTGTTGAAATGAAGTTCCAGTAGGTGACTTTCCCCATGTGCCTTCCCTCTCT
GTGCCCTAAGCTTCCACTCTGCCTCAGATGGTGAAGGCATTTGGCACAGACCTTGCA
CACCAAAGAGCAAAAGTATTTCCATCCAAAGGGAACCACCATAAAAACAGCCATAT
TGCAGTGGCTCACACTTGTAATTCCAACACTTTGGGAGACAGGTGAGACGACTGCTT
GAAGCCAGGAGTTCAAGTCCAGCCTGGGCAACAAATCGAGACCTCCATCTCTATCA

AAAAAAAAAAAAACTTAAGAAATTAGCTGGGCATGGTGGTCCACGCCTATAGTCCC
AGCTGCTTAGGAGACTGAGGAGGGGTATCACTTAAGCCCAAGGATTCGAGACTTCA
GTGAGCTATGATAATGCCACTGCCCTCCAGCCCAGGAGACAGAGCAAAACCCCAAC
AACAACAACAACAAAAACACCAACAACAATATTGGCAGCTTCTCCTGAACCATCAA
TCTGCAAGTATATTCAGATGAACCCTGTGGTAATTAGTTGCAGGAGGGAAAAGATC
ACATCATACCAATAGTGTGGTAAGACTTCTGAACATCCACAAGGAAAGAGATGGTT
AATGATTTTTATGACATTTCTAAGTTCAAACCCAACTACAAAACCAGAAGGCAGCTA
AGAGGTCTCCTTGAACAATGTGTTTATTTCACAGGTGGAGAAAATGAGATCCAGAG
AAATTAAGACACAAAACTTACCAGTGGCAGCACTGACCCTCCATGACAAACTCCAC
AGCTATTTCCCCTAACAATGCCCTTCTGAGAACTCTGAAGCCCTAGTTCTAGGATGG
GAGGAAGAGAGATAGCAAGTGGAAAGGGTGGTTCCAAAAACCAGAGAGAAGCCCT
TTTCTAGCTGTGCAGATTCATTTGAGGGAAGATGACAGGGCTTAATGTATGTCCAGG
GGCACTCAGGGGATACCCTCTGAAGGAAGGATTCCCTTCTCCCAGTCAACACTCCCA
GCAATTACTCCTTCTTACTCGCCAGACCAAGGGGGGCAGAAATCCTGACAGAGCCCT
CACCATGCTCTTTAAATTTTCTGAAATTGCCAAGTTTAATATGGAGAGAAGTTTAAA
AAAAAGGGAGAAGAAAAAAAAAATTAGAATTACCCATAATTCTACTACACACATAC
AAACACTCAAACTTTGAGTGTCTTTCCTTCCACACAGTATTCTCTGCATGTACGCAGA
ATTTTGTTTTCGTAAAGTTAGAATCAACAAAGTGTCACAATTCAGTTTTCATGTAACA
GCCTAGTGTTAATGCCTTTGCATGAGCATCACAATTTAAAAACACTTTGTTACAGAA
AATTTCAAGAATATACCAAAGTAGAAAAAAATATATAATAAATTCTTACCTACTCCA
ATTCTACAATTATCTACTCAGAGCCAATAAATCATTTTGCAGCAAATTCCAGACCTC
AAGTCATTCCACCCATAAATATTTCAGAACCATCATCTTTCGAGGCAGTTCAGTTTTC
ATTGTGTGGATAGACCGACAGCAGAAGCACCTTAAAAAGCTTGCCGGGGTTGGGGA
AGCATCCTCCTCCTCCTCCTCTCTACCTTCCATGGAGCTCTCTCCCATACCCTCT
GACCTCTACCCTTTCTGCTGAGCTGTGCCTTGGAGCAGGGATGTGGCTGTCCTTGGCT
TGTCCCCTCACAAATTGCAGTCCCTTCAGGAAAAGGCCAAGGCTTCACAAGAATGG
AACCCTGACCCAGAGAAGCTGTTTTTTTGCTTGACTTGAGAGGTTTTGAGTAAACAG
AGTTGTAATGTAATAAAGCACCAAGCCAGTTTCCTCCTCTATTAACTGGCATTGAGA
GACAAGATGAATCAAGCAGCTGCAGGGGAGCTGGCTTGGCCTGGGACCCCTTGCAG
CACCTCCTCCACCTACCCCTCCCTTTTGGTCCTATGGCCCTGTGGACAGGTTATTTTC
TCCTGCTCTTTGTTTTGTTTAATCTCATCGGAGGTTTCAGATCTTTTTTTTTTTTTGTA

ATTTCATTTCAAACTATTATTTTATAAGACCTGGCCTATTACTGAGTATGCAGGCAGA
ATATGAAAATTACTCCAAAACTTTTTTAAATGAAATTTTCAAGATGCAAAAAGTGAA
ACTTTAAAATTTCAGTGGAAGAAGGGGAACAAAAACATTTTAATAAATGAGAGTGT
TTATTCCAGAATGGGAATATAGAGACAAGGAAGGTACCATGTGAATGGGTGCACCT
CGCTCTCTGGGGTCAATGATAGGAAACAGCCTGTCCCACAGTCAAGGCAGCCTTGCC
CAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGTTATCTAAGTGCAGCCTCTTTG
CTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTAAAATCACATTTGCTATTTTAC
CATCTAATCACACATAAGCCTCTCCCCACACTCCCCCCGCCCTGTTTCCATCCAAGG
AGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAACTCGTGACGGGAAATGGGAA
TGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGAATGATAGCAAGGCTCCCATC
AGACAAGCTGAGCCACTGTCACTGAGGAGGACAAACGAGTGCAAGTCTTTGCAGAG
CTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCA

[00212] SEQ ID NO:454 is a candidate promoter region from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38816042 to 38816601 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00213] GTCAAGGCAGCCTTGCCCAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGT
TATCTAAGTGCAGCCTCTTTGCTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTA
AAATCACATTTGCTATTTTACCATCTAATCACACATAAGCCTCTCCCCACACTCCCCC
CGCCCTGTTTCCATCCAAGGAGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAAC
TCGTGACGGGAAATGGGAATGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGA
ATGATAGCAAGGCTCCCATCAGACAAGCTGAGCCACTGTCACTGAGGAGGACAAAC
GAGTGCAAGTCTTTGCAGAGCTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCAC
ACACTAGCCTCTTGGCTAGAGAACAGACATCAGATGGAGTTTCTTCTGGCTATGCCT
GAATGTTAAGCTGAACGTATGTTCCAGGAGCTCGTGGTCTCCAGTAGAGGCAATCTG
GGATAGAAGAGAAGATATTTCTTACGTAGAAGACAAGCAAGATTGAGCAG

[00214] SEQ ID NO:455 is a candidate promoter region from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38816042 to 38817161 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00215] AATGGAACCCTGACCCAGAGAAGCTGTTTTTTTGCTTGACTTGAGAGGTTTTG
AGTAAACAGAGTTGTAATGTAATAAAGCACCAAGCCAGTTTCCTCCTCTATTAACTG
GCATTGAGAGACAAGATGAATCAAGCAGCTGCAGGGGAGCTGGCTTGGCCTGGGAC

CCCTTGCAGCACCTCCTCCACCTACCCCTCCCTTTTGGTCCTATGGCCCTGTGGACAG
GTTATTTTCTCCTGCTCTTTGTTTTGTTTAATCTCATCGGAGGTTTCAGATCTTTTTTTT
TTTTTTGTAATTTCATTTCAAACTATTATTTTATAAGACCTGGCCTATTACTGAGTAT
GCAGGCAGAATATGAAAATTACTCCAAAACTTTTTTAAATGAAATTTTCAAGATGCA
AAAAGTGAAACTTTAAAATTTCAGTGGAAGAAGGGGAACAAAAACATTTTAATAAA
TGAGAGTGTTTATTCCAGAATGGGAATATAGAGACAAGGAAGGTACCATGTGAATG
GGTGCACCTCGCTCTCTGGGGTCAATGATAGGAAACAGCCTGTCCCACAGTCAAGG
CAGCCTTGCCCAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGTTATCTAAGTGC
AGCCTCTTTGCTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTAAAATCACATTT
GCTATTTTACCATCTAATCACACATAAGCCTCTCCCCACACTCCCCCCGCCCTGTTTC
CATCCAAGGAGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAACTCGTGACGGG
AAATGGGAATGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGAATGATAGCAA
GGCTCCCATCAGACAAGCTGAGCCACTGTCACTGAGGAGGACAAACGAGTGCAAGT
CTTTGCAGAGCTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCACACACTAGCCT
CTTGGCTAGAGAACAGACATCAGATGGAGTTTCTTCTGGCTATGCCTGAATGTTAAG
CTGAACGTATGTTCCAGGAGCTCGTGGTCTCCAGTAGAGGCAATCTGGGATAGAAG
AGAAGATATTTCTTACGTAGAAGACAAGCAAGATTGAGCAG

[00216] SEQ ID NO:456 is an intronic region (and candidate enhancer) from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38815666 to 38816041 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00217] CAAAGGTTTGGCTGTGCGACTTTGTTAGTCTGTGGCTAACAGTGTAAAGATAT
GTGCTTGGGTATTTTGAGGGGGATTACAGATTTTATTCATAAACCAAAAACAAAGCA
TGTTTTGTGGGATGTTTTAATATATGACCTTCCTCAAACACCCATAGTTCTTGGAAAA
ATACTATTAATCTATATGCATAGAAGACTCCCACTGCATCTCTTCCTCTGAAGGATA
AGGGTGAAAATATAGCTAAATCCAAAAGCTATCAATTTCTACATGCTACATCAACGT
CAAGTTTATACTCTTCAAAGAGTTGTGTTGATATAAAAAGATGTTTTATTAGCAGAT
GTGATTTCAGACATTTAACAAAAACTGTGATAACAGA

[00218] SEQ ID NO:457 is an intronic region (and candidate enhancer) from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38814527 to 38815096 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00219] TAGCACACTTAGTGTAAATTTGATTCTTGATCATGATTTTGATCTTTGAGATTA CCTGATTAGATTTGTGCCAGAAAAGAGAAAACACTTGCTATGATTTTAAAACGTAGT TTGAATCAGCTTTACTGGACTTGCAAAATGAAGCAGGGAGAAGCAACCACGTGTAT ATATTTTAAGTAACAGAAGCCAAAGGTTGGGCTTTCTAGCCACATGTTGTGGAAGGA AGTTTGACACAGATAAATGAATTGCTTTTAAACTGGTGGAAATTTTTGGTTTGAAAT GGCATAAAGTTGGTTTGAAATGTTAGGAAGGTTTTAGAGTGGCCTTAAAAAGTAATT TCCTAGTTCCCTGTCCACTTAGTCAAATAATGGTAGTGATAATAGTAATAATAAATG AGCATATAAATTGAAACATTAGCATGTTTTTGCCCCAAGCATATGAAAGAGCACATA CCTACAAGGATAATTTTTGAGATAACAATCATCAGAAGGAAATGAAAAAGAAACCT GAGAATGGTCTGTAATGGTAAATGGTAGTAAAGGGTCTTTCACAAATAAATATTGCC AAGAA

[00220] SEQ ID NO:458 is an intronic region (and candidate enhancer) from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38809959 to 38810668 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00221] AGTCTAGTGGGGCAGACAGATATTAAACAAATACACAAATAAATATAATTTT AAACTGTGATAAGTGCCAGGAAAGAAATAAACTAGATTCTTTGGGAGATCTTGGGG CCACCTAATTTAGACAGACAAGACCAAGAAAGGCCTCCCTGATGAAGTGACATTTA AGCTTTAGCCTAAAAGGAGATACAATATTTTTGTCCAAATTTGCCATCTAGAAGCAG AATTTGAGGACCAAATCAGAATGTGACTTGTGAACAAAAGACAGATTCCTTCAATGT CAGAACTGCACAGCAACACTGAATTTCCTGCGTATCCTTTGCTTCCCTGGAATAAAC TGAAGGGCTGCGTTATACTGGTTGGCATTTACATAATGTTCACAGTGCTCTTGCTTAG AACCTTGCCCAAAACCCATTTCTACCTGTCAATTTTTATTATAATTTGCGTATTTTTTC TTTAGAAACTTAGAGAAAAAAGGGGGATTATTGTTCATGATAAACACTTTGGCTCTG AAATAATTAATGTTTAAGAAGATTAACAACCATACAGTTGCTTTATGAGCAGGGGCT CATGCTTTTGGTAGGTGTTCAACCAAGTTCCCAGTGGGGAACTTCAGTCTGGGCATG TATTTGTGTATTTTTCAGTGTCCCAAAGCCCAGAGTGCAATGTGTATGTGGCATTCAT ATTGTGACAAACACAGTCATAAAAACACT

[00222] SEQ ID NO:459 is an intronic region (and candidate enhancer) from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38806479 to 38806941 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00223] CCAGACCAATCCCCTCATTGTACAAATGAGAAAACTTTTATTTGGGGCAGCTG AACCATATTCAAAAGTATTTACAAACTAAATAAAGTGGAACATTGTAAATTCTTTAT ATTTAATTTAATTTAATTGCAAATGATCTCACATGTTTGTTTTGTTTATACACTGAGA GCTAGTGGTGTGGAATTGAAATAGGCTAAGTGGCTATTGGCTATTAAATCACATATG TAATGGAAATATTAAGGTCTATATTATGCATTTGTCTATAATTTACCTTACATAATGC TTCCATATACATTATTTGATTTTCCTACAGTGTCAGATAAGTAGGGCCGAGGTAATC ATCTCCAGTTTACAAATTACAGAAATGAGGGTCAAAGAATTCAAAAGACCCAGATA GCATCAGAACTCATGCTTCCTGTCTTCGAGTCCAGTGGCTATTTCAACAAAACCACA CTATCTCTCT

[00224] SEQ ID NO:460 is an intronic region (and candidate enhancer) from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38794043 to 38794240 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00225] AGGAGGGTTGAATAAACAGCCTCCCCTGAATGCCTTGCAGGGGAATGGGTTC CTGGGAGGAGCCAAGTGTGAGTTCAGGGAGGGGCCAGATATGAGGGTGGGAGAAG GGCTGTTCTGACAATCAAGATGGGACAGATGAGAGGGACAGGGCCAGTGGGCAAGC TGTCACCTCTCTGTGGTTATGTCCACTCTTATAAG

[00226] SEQ ID NO:461 is a combination of SEQ ID NOs:456, 457, 458. 459, and 460 and has the following sequence:

[00227] CAAAGGTTTGGCTGTGCGACTTTGTTAGTCTGTGGCTAACAGTGTAAAGATAT GTGCTTGGGTATTTTGAGGGGGATTACAGATTTTATTCATAAACCAAAAACAAAGCA TGTTTTGTGGGATGTTTTAATATATGACCTTCCTCAAACACCCATAGTTCTTGGAAAA ATACTATTAATCTATATGCATAGAAGACTCCCACTGCATCTCTTCCTCTGAAGGATA AGGGTGAAAATATAGCTAAATCCAAAAGCTATCAATTTCTACATGCTACATCAACGT CAAGTTTATACTCTTCAAAGAGTTGTGTTGATATAAAAAGATGTTTTATTAGCAGAT GTGATTTCAGACATTTAACAAAAACTGTGATAACAGATAGCACACTTAGTGTAAATT TGATTCTTGATCATGATTTTGATCTTTGAGATTACCTGATTAGATTTGTGCCAGAAAA GAGAAAACACTTGCTATGATTTTAAAACGTAGTTTGAATCAGCTTTACTGGACTTGC AAAATGAAGCAGGGAGAAGCAACCACGTGTATATATTTTAAGTAACAGAAGCCAAA GGTTGGGCTTTCTAGCCACATGTTGTGGAAGGAAGTTTGACACAGATAAATGAATTG CTTTTAAACTGGTGGAAATTTTTGGTTTGAAATGGCATAAAGTTGGTTTGAAATGTT AGGAAGGTTTTAGAGTGGCCTTAAAAAGTAATTTCCTAGTTCCCTGTCCACTTAGTC

AAATAATGGTAGTGATAATAGTAATAATAAATGAGCATATAAATTGAAACATTAGC
ATGTTTTTGCCCCAAGCATATGAAAGAGCACATACCTACAAGGATAATTTTTGAGAT
AACAATCATCAGAAGGAAATGAAAAAGAAACCTGAGAATGGTCTGTAATGGTAAAT
GGTAGTAAAGGGTCTTTCACAAATAAATATTGCCAAGAAAGTCTAGTGGGGCAGAC
AGATATTAAACAAATACACAAATAAATATAATTTTAAACTGTGATAAGTGCCAGGA
AAGAAATAAACTAGATTCTTTGGGAGATCTTGGGGCCACCTAATTTAGACAGACAA
GACCAAGAAAGGCCTCCCTGATGAAGTGACATTTAAGCTTTAGCCTAAAAGGAGAT
ACAATATTTTTGTCCAAATTTGCCATCTAGAAGCAGAATTTGAGGACCAAATCAGAA
TGTGACTTGTGAACAAAAGACAGATTCCTTCAATGTCAGAACTGCACAGCAACACTG
AATTTCCTGCGTATCCTTTGCTTCCCTGGAATAAACTGAAGGGCTGCGTTATACTGGT
TGGCATTTACATAATGTTCACAGTGCTCTTGCTTAGAACCTTGCCCAAAACCCATTTC
TACCTGTCAATTTTTATTATAATTTGCGTATTTTTTCTTTAGAAACTTAGAGAAAAAA
GGGGGATTATTGTTCATGATAAACACTTTGGCTCTGAAATAATTAATGTTTAAGAAG
ATTAACAACCATACAGTTGCTTTATGAGCAGGGGCTCATGCTTTTGGTAGGTGTTCA
ACCAAGTTCCCAGTGGGGAACTTCAGTCTGGGCATGTATTTGTGTATTTTTCAGTGTC
CCAAAGCCCAGAGTGCAATGTGTATGTGGCATTCATATTGTGACAAACACAGTCATA
AAAACACTCCAGACCAATCCCCTCATTGTACAAATGAGAAAACTTTTATTTGGGGCA
GCTGAACCATATTCAAAAGTATTTACAAACTAAATAAAGTGGAACATTGTAAATTCT
TTATATTTAATTTAATTTAATTGCAAATGATCTCACATGTTTGTTTTGTTTATACACTG
AGAGCTAGTGGTGTGGAATTGAAATAGGCTAAGTGGCTATTGGCTATTAAATCACAT
ATGTAATGGAAATATTAAGGTCTATATTATGCATTTGTCTATAATTTACCTTACATAA
TGCTTCCATATACATTATTTGATTTTCCTACAGTGTCAGATAAGTAGGGCCGAGGTA
ATCATCTCCAGTTTACAAATTACAGAAATGAGGGTCAAAGAATTCAAAAGACCCAG
ATAGCATCAGAACTCATGCTTCCTGTCTTCGAGTCCAGTGGCTATTTCAACAAAACC
ACACTATCTCTCTAGGAGGGTTGAATAAACAGCCTCCCCTGAATGCCTTGCAGGGGA
ATGGGTTCCTGGGAGGAGCCAAGTGTGAGTTCAGGGAGGGGCCAGATATGAGGGTG
GGAGAAGGGCTGTTCTGACAATCAAGATGGGACAGATGAGAGGGACAGGGCCAGT
GGGCAAGCTGTCACCTCTCTGTGGTTATGTCCACTCTTATAAG

[00228] SEQ ID NO:462 is a candidate enhancer region from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38738506 to 38739123 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00229] AGTCGTTTTTAAGATCCTCAGGGGTGGGTGAGGAAAAAGAATGGTTCCATGAT GACCCGAAAGTCTCCCATCTGCCAAGCAAGCGAATAAGCCACAGACCTGGATCTCA AATCCAGGTTGTCTGACTCCAGAGCCCCAGCTCCTTCCAGCCTCAAGGAAGTCATTC TCTGATTCCCTTGTGCCCGAAATCACAGCCCCAATGTCTCAAGCACCTTCAACCTGC CTATTAGTCTCACTCACATGCAATGATGGCTCTTCTCTAAACTCAGCTCCAGGACGC CCTCTACAGGAGAAGTGCCACAGGCCACCCACTTTTATCAGAGCCACAGGTTTGTCA GCCAAACCTGTCAGATTCCCAGGAGTGTCGGGTCACTAGGTCTCCAGGCCATCTGGA GGAAGCTTGGCATCTCACTGACTCTTGGGAATAGGAAAGAGGCCTGAAGAAATGTC ACGGCTTGTTAGCGCAGGCCGCCTGCTCTCAGGAATCTCTCAGTATTAACTGGGAGC CCTGCATCTAGACTTGAAGAGAGGTGACAAAACAAAGGGGAGTATCCAACAATCTT CCCAAGGTCCTGACAAGTGTTGGCATCCAGAGGTTGAAGGGGGTGCATGGTTCAG

[00230] SEQ ID NO:463 is a candidate enhancer region from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38805250 to 38806780 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00231] ACTGAGAGCTAGTGGTGTGGAATTGAAATAGGCTAAGTGGCTATTGGCTATTA AATCACATATGTAATGGAAATATTAAGGTCTATATTATGCATTTGTCTATAATTTACC TTACATAATGCTTCCATATACATTATTTGATTTTCCTACAGTGTCAGATAAGTAGGGC CGAGGTAATCATCTCCAGTTTACAAATTACAGAAATGAGGGTCAAAGAATTCAAAA GACCCAGATAGCATCAGAACTCATGCTTCCTGTCTTCGAGTCCAGTGGCTATTTCAA CAAAACCACACTATCTCTCTGGTTTTTTAAAATTATTATAACTCAAGGTTTCTGTAGC TGACTCAATCCACCATCCTGAGTTTTTTGGTTCACAACACTTTCAGATCATGTGATAA GGAACTTCTTTTCTTCCTTTGACACAAAAATTTATTCCTTCTTTCTGATCCCATTCTCA ATGGGTTGGAAGAGATGATACGGAGGTAAAGACTCAGGAGAAGTTAAGATTTATCC TCCTCATCTTCGTTTGGATCTCCATTCTGGTGGGCAGAGCTGTTTTAAGTGCCCTATT AGGCACTTTCTTCTCCAAACTCCAGCTTTCTTTCTTGAATTACGCTCTTCTTCCCTTTT AGCACCAATATAAATGCACCTATTCTCAGCATTTCAGCCTGACACTCTCATTCAGCA CTACAAACAGATCAATCATTCACCAAGCCCCACAGAAAGCCAGTCCCATTCAGCTCT CACCCATTCCTTCCTCTCTTGTTCAGTACCACGGTCAGCTCAAATCCAGACTAGGCG CTCACTAGCTATTTCAGAACCATGGATAGCGCACGCACTTTCGTCCGCCCTGTAAAT TACTTTGGTCCTGCTCCAACAACAGATTACCTAGCTATTATCTCTGAGATCATTCATA GGTGATTCAGTTAAGCAAGGATTTCCCTAACCCCATTCTTTGTTATCAGAGGCAACT

CAGGATTATTCAACTTCAAAGGTAGCTAAGTGCTATTTTTCCAGTGTCACAGATTGC
TGAGACTAGGTCTCGAAGGACTGGGCAGGATCATGTAGAGTGAGGTCTGGTCTCAC
TTTCGTCACCACAAACAGCTTATCACACTTTCTTAGTGGCTTATCTATTTCAAATGCT
ATATCTTTCCTAGACAGCTTACTCATATGTCCGGTGTCCTGTTGCTCCCATCAGATGT
CTAAAGATTAAAATTTTTGAGGCAATGGACAGCTTAGCTTGACACATATCAGTATCA
CTGGCTGTATGAAATGCTTACATCTTGGAGACAATCTTCTTAAAGTCTTGACCAGTCT
GGTCACATCTGCAAAACCAGGGACATTTAATCTCATGCTCACGTTGCCATGGAGAGC
CCAGCAATATCTTCAGTGCTAAAGCTAGCTTGGTTATTTGTTCCATGTATTAGAGAA
CCTAATCACACTCTCAGCACCATGCATAACACAGTTTCACTCAGACATAACAGTAGT
TCAGTTAGTATTTCTCAGCACCAGCTCAGTCACACTTTCACCC

[00232] SEQ ID NO:464 is a candidate enhancer region from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38725531 to 38726460 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00233] AGCAGGGCCTGGAAGAAAAAGTCAGAATTCCCCAGGCAGACAAACAGGGAG
TGGGGGGCAGCAGGTGAGCAAAAGGCCACCTTGAGGTGCTTGGGGATTGTCTTTCA
CACAGCTGTCCCATGCGCTCTGCCCAACCCAGGCTCTGTGCATGAGATGCTAATGAG
ATTTCTCTAAGAACAGGATGTTGTCCAAGAGCAAGGCTCATCTAATCTTTACCAGCA
TCCTAAGAAGTCCTGATTCTTATGTGACTCATTTAGCCAGCTTTTCCCCACAAGTGGT
ATCACAGGGAAATGACACGCTCCTTTTGGCACCAACTAATTTAAAGCACATTTGAGC
CATTTGATAATATATATTTTTAAATACTTGGCACTATTCCTTACTACCTTAAGCTGGT
ATATCAACCCTCGCTAACCTCTTCTCAGTAACTCAGGGCATTCACCTCTGCCCAACG
TGGGAAGGCAGGGAACCCTGTGAGGCCTCAGGAACATGCAGGGCTGCTGGCCAGGT
TACTAAGTGGTCCAGAAAAGCTGTGCTTTGAGTCTGTAGCTCTCCCATAGCCTTCTTC
CCTGCAGTCGTCACTTGTGTTCCAGCAGCTGCTGTTATCAGCTGCCATATTTTACATG
CCTTTGTCTAGGTAACAGCAGAGAGCATGAACTCTGTGACAGCTGTCTGCCACTCAG
AAGCATTTTCAGTTACTTTGGGGCTCTTAGGGAGCTCCAGGTGGCCAAGTTCCCTAC
AATTTTGACCTCTGGACAAATAACAATGGTCATGGCTAAAATCTATTGAGCCAAGCA
CTTCGTGCAAAGTGTTTTACAAGCGCTAACATATTTAGTCATCAAAACAGCTGAATG
ATGCAGATCTAGCCTTTTCAATTTTGAAGCTGGGAAAAATGAAGATTAGAGAGGTTA
AACAACTTGCCTAAGGTCACAC

[00234] SEQ ID NO:465 is a candidate enhancer region from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38723641 to 38724930 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00235] AAGAGTGAGACTCCACAAGCTGTGGGACCTAAGATCAGTTCCCAAACCTCTCT
GAGCCGCCATTTATTCATCTATGAAACTGAATGGTAATGCCTACTTGGTAGGATTAT
TTTGAAGCTTAAATGAGATGCTGCTTGTCAAATGTTTAGGTCCTGCCTGAGACAAAG
TAAGTGCCCAGGAAATGACAGCCAAGAAAAAAGGAAACGAAAGACAACGCACAAA
GAAAGTCAAATATCTTTCAGAGCCAGCAAATAAGAGTTGGAGGTGCTCACAGATGA
AGAAGTTTTCCCTTGTGATTCTCCCCCACTTTCTTTTGTCCACATCATTTTCTCTAGGC
AAAAGTAGCCTGGGGCTAGGGAGAGGCTCTCTATAGGTGAGTAGTGAAGTGACAGC
CTCACAGAGACTGAGCTGGAAGATTAGAGGTTTAGGATTGTCTTATACAAGGTAAA
AATAAATGTGGTTTCACTTACAAATCTTGAAGGAAACAAGCTATAGGGAAAGAGGC
ATCTATAAAAGTTTAGCATCTTTAATAACACTGCCTTCAATTCAAGAATTTAATTTGA
CACCTAGCCACTGAGTGCTGACTTTGTGACAGGCACTGGGCTCAACAGAGATAAGG
AAGTCATAATTCATCCCCAAAGTTATCAAGCCCTTCAGCTTGAAGAATGTCAGCTAC
TGGCTGTGTGCCCTAGGCAAGGCTGACCACAGCAGGGTAAGATAAGAGGGAGGGCT
GGGACGGGATTTGGGGGCTGTGGGTGAACAGAAGCCGCTTGGTTCCCACTGGGTGA
GGGCTGTCACATTCTGCACGTGGATGTCATGCTTCTTCCCAAATGTTCCAAGGACTC
CCCTGGCTAGTTGTCCCTACTCAATGTTTTCAGTACACAACTTCTGGGTGTTGGTTTT
TTGTTTTTGTTTTCTTGTCCATGACCCCGGCTAGGCAGGAGGGGCCTGACAGCGACA
AATGGGGACCCGCCCCACCATTTGGGTCCATTAGCAAAGCTATCAGGTTTACTAATA
TGAGAGCAACTTCAACCCCTAATTGAATTCATCTGGAGACATTATAGGGCTGGAGCT
GCCAGGAGGGCAGCAGGCCCCTGCAGCATTACTCCATGATTAAATATTCAAGCCCA
GTGAATGCTGAGAGTCGTTATGGGTGTAATTACGGTGTCTCGGGCCATTGCCAGCAC
TCCATCAGGGGCCCCGAAGGTTTACAGTCTCACACAGCTAAGCCTCTGGGGCTCCAG
GGAGAAGAGCAGTGTTCCAGGCCTCAGCTGGCGAGGCACCAAACAT

[00236] SEQ ID NO:466 is a combination of SEQ ID NOs:464, 465, and 455 and has the following sequence:

[00237] AGCAGGGCCTGGAAGAAAAAGTCAGAATTCCCCAGGCAGACAAACAGGGAG
TGGGGGGCAGCAGGTGAGCAAAAGGCCACCTTGAGGTGCTTGGGGATTGTCTTTCA
CACAGCTGTCCCATGCGCTCTGCCCAACCCAGGCTCTGTGCATGAGATGCTAATGAG

ATTTCTCTAAGAACAGGATGTTGTCCAAGAGCAAGGCTCATCTAATCTTTACCAGCA
TCCTAAGAAGTCCTGATTCTTATGTGACTCATTTAGCCAGCTTTTCCCCACAAGTGGT
ATCACAGGGAAATGACACGCTCCTTTTGGCACCAACTAATTTAAAGCACATTTGAGC
CATTTGATAATATATATTTTTAAATACTTGGCACTATTCCTTACTACCTTAAGCTGGT
ATATCAACCCTCGCTAACCTCTTCTCAGTAACTCAGGGCATTCACCTCTGCCCAACG
TGGGAAGGCAGGGAACCCTGTGAGGCCTCAGGAACATGCAGGGCTGCTGGCCAGGT
TACTAAGTGGTCCAGAAAAGCTGTGCTTTGAGTCTGTAGCTCTCCCATAGCCTTCTTC
CCTGCAGTCGTCACTTGTGTTCCAGCAGCTGCTGTTATCAGCTGCCATATTTTACATG
CCTTTGTCTAGGTAACAGCAGAGAGCATGAACTCTGTGACAGCTGTCTGCCACTCAG
AAGCATTTTCAGTTACTTTGGGGCTCTTAGGGAGCTCCAGGTGGCCAAGTTCCCTAC
AATTTTGACCTCTGGACAAATAACAATGGTCATGGCTAAAATCTATTGAGCCAAGCA
CTTCGTGCAAAGTGTTTTACAAGCGCTAACATATTTAGTCATCAAAACAGCTGAATG
ATGCAGATCTAGCCTTTTCAATTTTGAAGCTGGGAAAAATGAAGATTAGAGAGGTTA
AACAACTTGCCTAAGGTCACACAAGAGTGAGACTCCACAAGCTGTGGGACCTAAGA
TCAGTTCCCAAACCTCTCTGAGCCGCCATTTATTCATCTATGAAACTGAATGGTAAT
GCCTACTTGGTAGGATTATTTTGAAGCTTAAATGAGATGCTGCTTGTCAAATGTTTA
GGTCCTGCCTGAGACAAAGTAAGTGCCCAGGAAATGACAGCCAAGAAAAAAGGAA
ACGAAAGACAACGCACAAAGAAAGTCAAATATCTTTCAGAGCCAGCAAATAAGAGT
TGGAGGTGCTCACAGATGAAGAAGTTTTCCCTTGTGATTCTCCCCCACTTTCTTTTGT
CCACATCATTTTCTCTAGGCAAAAGTAGCCTGGGGCTAGGGAGAGGCTCTCTATAGG
TGAGTAGTGAAGTGACAGCCTCACAGAGACTGAGCTGGAAGATTAGAGGTTTAGGA
TTGTCTTATACAAGGTAAAAATAAATGTGGTTTCACTTACAAATCTTGAAGGAAACA
AGCTATAGGGAAAGAGGCATCTATAAAAGTTTAGCATCTTTAATAACACTGCCTTCA
ATTCAAGAATTTAATTTGACACCTAGCCACTGAGTGCTGACTTTGTGACAGGCACTG
GGCTCAACAGAGATAAGGAAGTCATAATTCATCCCCAAAGTTATCAAGCCCTTCAGC
TTGAAGAATGTCAGCTACTGGCTGTGTGCCCTAGGCAAGGCTGACCACAGCAGGGT
AAGATAAGAGGGAGGGCTGGGACGGGATTTGGGGGCTGTGGGTGAACAGAAGCCG
CTTGGTTCCCACTGGGTGAGGGCTGTCACATTCTGCACGTGGATGTCATGCTTCTTCC
CAAATGTTCCAAGGACTCCCCTGGCTAGTTGTCCCTACTCAATGTTTTCAGTACACA
ACTTCTGGGTGTTGGTTTTTTGTTTTTGTTTTCTTGTCCATGACCCCGGCTAGGCAGG
AGGGGCCTGACAGCGACAAATGGGGACCCGCCCCACCATTTGGGTCCATTAGCAAA

GCTATCAGGTTTACTAATATGAGAGCAACTTCAACCCCTAATTGAATTCATCTGGAG
ACATTATAGGGCTGGAGCTGCCAGGAGGGCAGCAGGCCCCTGCAGCATTACTCCAT
GATTAAATATTCAAGCCCAGTGAATGCTGAGAGTCGTTATGGGTGTAATTACGGTGT
CTCGGGCCATTGCCAGCACTCCATCAGGGGCCCCGAAGGTTTACAGTCTCACACAGC
TAAGCCTCTGGGGCTCCAGGGAGAAGAGCAGTGTTCCAGGCCTCAGCTGGCGAGGC
ACCAAACATAATGGAACCCTGACCCAGAGAAGCTGTTTTTTTGCTTGACTTGAGAGG
TTTTGAGTAAACAGAGTTGTAATGTAATAAAGCACCAAGCCAGTTTCCTCCTCTATT
AACTGGCATTGAGAGACAAGATGAATCAAGCAGCTGCAGGGGAGCTGGCTTGGCCT
GGGACCCCTTGCAGCACCTCCTCCACCTACCCCTCCCTTTTGGTCCTATGGCCCTGTG
GACAGGTTATTTTCTCCTGCTCTTTGTTTTGTTTAATCTCATCGGAGGTTTCAGATCTT
TTTTTTTTTTTTGTAATTTCATTTCAAACTATTATTTTATAAGACCTGGCCTATTACTG
AGTATGCAGGCAGAATATGAAAATTACTCCAAAACTTTTTTAAATGAAATTTTCAAG
ATGCAAAAAGTGAAACTTTAAAATTTCAGTGGAAGAAGGGGAACAAAAACATTTTA
ATAAATGAGAGTGTTTATTCCAGAATGGGAATATAGAGACAAGGAAGGTACCATGT
GAATGGGTGCACCTCGCTCTCTGGGGTCAATGATAGGAAACAGCCTGTCCCACAGTC
AAGGCAGCCTTGCCCAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGTTATCTAA
GTGCAGCCTCTTTGCTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTAAAATCAC
ATTTGCTATTTTACCATCTAATCACACATAAGCCTCTCCCCACACTCCCCCCGCCCTG
TTTCCATCCAAGGAGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAACTCGTGAC
GGGAAATGGGAATGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGAATGATAG
CAAGGCTCCCATCAGACAAGCTGAGCCACTGTCACTGAGGAGGACAAACGAGTGCA
AGTCTTTGCAGAGCTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCACACACTAG
CCTCTTGGCTAGAGAACAGACATCAGATGGAGTTTCTTCTGGCTATGCCTGAATGTT
AAGCTGAACGTATGTTCCAGGAGCTCGTGGTCTCCAGTAGAGGCAATCTGGGATAG
AAGAGAAGATATTTCTTACGTAGAAGACAAGCAAGATTGAGCAG

[00238] SEQ ID NO:467 is a candidate enhancer region from the human SCN10A gene (which expresses Nav1.8) and maps to nucleotides 38828592 to 38830159 of chromosome 3 of the human genome build GRCh38.p14 and has the following sequence:

[00239] GACCTCCCAATTTCATTCCTTGAAGAGAGTTCTTCCATGCCTTTGTCAGGAAGA
CATTTAGATGCTGCCAAAGAAACAACCCAGAAGGTGAGGCTCTATCCTAGGACTCA
GCCTCTGCAAGCCTGTAGGCGGACATTCTACCAGGAGTAAGTTCTAAATGGATTTCT

CCAGGACACCCAACAGTGTGCAGAGAACTGGCTGCCTCAGCATCTTGCTTGATCTTT
CTCTTGAAAAGTGTGTCTCTAACGCAATTTTCTCAGCATTAAAATATGAAAGATAAA
GCCACTTATCTCCCAGGGCTTCAGGGAGAACTCAGTGAGGGCTTGCAGGTCAAGGG
GCCAGGTGTGCTGCCAGCCGTCGAGTCCATGACTGTCCCTCCCTCATGGGGTTCACC
TGCTGGTCCCGTCCTCATTCAGATCCCTTCTCTACACCGCTATGCTGAGGGGTCCAGC
TGCCAGCCCCATGCGCTGCTTTGCAGAGGACTGCTCATGTGGGACGGGAGTATCCTT
CCTGGTGCTGGGTGTGGAGTTGTGGGAGGAGGAGGAGTCCCATGGGTAATTACAGC
AAACCTCCACTTATCCAAATAAATGAGTCAGGACTCTGCAAGGCAGGGAGGGCAGC
AGCTCCCTCCGAGGTCTGCCTGTATGAGGCCTGTCTGTGCTCAGCCCAGGACTTGCC
TCAGAGAAAGAACTGGAGGAAGTGAGCCTTTCTTCCCCTCTCTCTTTTGAGAGCTGA
CTGGTCTGCCCTCAATTCCTGGTAGCTGCCCCTCTGCCACCAGCCTGAATCAGGAAG
GTGGGGAGGGGAGAATGTGGCTTAGGGGCCAGAGTCAGGCAGGAAAGGGGTCCTG
ACAACAGACTCCTTCTCACTCTCCTTCTCCTCTCACTTCAGAGCTGTGGCCCACAGGA
CACAGGCATACTTGCTTTTTACCAGATGACTGGCTCCCAGCTGATGGGGTAAGTGTT
CATGGATAAGTTCAGGGGAAGGCCTGGAACTCATTTAGAAAATTTTATCAGAGGAA
CCTGGGAGCCACCATTGCCCCTCCCTACCACTGAGCCACCCACTGATCTTTGGGTGA
ACACTGTAAATCCCAGCCCCCCACATGCCATACATTGAGCCCCTCCTCTGCACCCCC
AGCCCAGCCATCCTCATTCTGGGAATTAATAAGATAATCCCGTAAGGCTCTTTATGA
ATGACAAGAACCAGTGTCAGCTGGTATCATTCATAGCATTAGTGTGATTCTCCCTGG
CTCTCATCATCCTTTGTGGCCTCACCCTTCCTCCATAGCCCAGAACAGCTTACTGCAG
AAGGCCATTGCTGATGAAGGAACCCAGCGCCACATCCAGATGCCCTGCCCGCTCTTC
CTGTGCTGCGGCATGCCCAGGTCAGGATGCACAGCTCCTGTGTCTCAGAGAAATGCA
CTAAGTGCCTTTCCTAATGGTTCATGGACTCTCCCCAGAGCATAGTAGGTTTAGTGCT
GCTGACGTAGTCTCTGGATCTTCTCCCTCCACTCTGTCGTCTGCCAGCATTCCCTTTG
TGGACACCCATTACCATTCCAAGCTTGGTGGGAG

[00240] SEQ ID NO:468 is a poly-A signal sequence and has the following DNA sequence:

[00241] AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGTTTTTTGTGTG

[00242] SEQ ID NO:469 is a Pol-III terminator and has the following DNA sequence:

[00243] TTTTTT.

[00244] SEQ ID NO:470 is a 5' AAV2 ITR and has the following DNA sequence:

[00245] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA GGGAGTGGCCAACTCCATCACTAGGGGTTCCT

[00246] SEQ ID NO:471 is a 3' AAV2 ITR (complement to SEQ ID NO:427) and has the following DNA sequence:

[00247] AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTC ACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTC AGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

[00248] SEQ ID NO:472 is an AAV ITR present in scAAV constructs (replaces 3' ITR in ssAAV) and has the following DNA sequence:

[00249] CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC GCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA GGGA

[00250] SEQ ID NO:473 is an example of a stuffer sequence and has the following DNA sequence:

[00251] ACAAGAATCTGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTC TTGGTCCCCCACATTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCAT GTCCTTTATTGGAACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGAT CCCATGTTACATGGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATA CCTAGAGCCAGAGGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAA TGTTGATAAATGGAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAA TCCCTGAAATAAATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTT GATTTGAGTACTGTCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGC CAAGATAATATTTAAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTT GTTTGTTTTTTTTTGTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTC ATTACTTTATCTTATTTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTG AAATAGTTTATGAAAATAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGT TAGCCTTCTCATCATAAATTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGC ACAGAATAAGGAGACAAAGAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAG AAATCTATTAACTCTTGTTTTGGAGACACAGAGTATTCCTTTGAATGAAGACTTAGG ACAGATATTTTTACATCACAATATTATGCTTACTGCCTGTCATTCCTCTTTGATTATT

ACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCTTGAGTTCCTAAATAAAT
TTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTCATTAATTAGCTGTAA
CATATTTATACTAAGATGTGCCTTCTTTTGGGAGTGTATATTTCTCATTATAGTCCCA
TTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGTGACTTATGGCATT
TGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGTGTGCCTGTAT
CTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGCCTTATAA
TATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGATTT
TATTTCCGTTAAC

[00252] SEQ ID NO:474 is an example of a stuffer sequence and has the following DNA sequence:

[00253] ACAAGAATCTGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTC
TTGGTCCCCCACATTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCAT
GTCCTTTATTGGAACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGAT
CCCATGTTACATGGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATA
CCTAGAGCCAGAGGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAA
TGTTGATAAATGGAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAA
TCCCTGAAATAAATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTT
GATTTGAGTACTGTCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGC
CAAGATAATATTTAAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTT
GTTTGTTTTTTTTTGTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTC
ATTACTTTATCTTATTTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTG
AAATAGTTTATGAAAATAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGT
TAGCCTTCTCATCATAAATTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGC
ACAGAATAAGGAGACAAAGAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAG
AAATCTATTAACTCTTGTTTTGGAGACACAGAGTATTCCTTTGAATGAAGACTTAGG
ACAGATATTTTTACATCACAATATTATGCTTACTGCCTGTCATTCCTCTTTGATTATT
ACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCTTGAGTTCCTAAATAAAT
TTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTCATTAATTAGCTGTAA
CATATTTATACTAAGATGTGCCTTCTTTTGGGAGTGTATATTTCTCATTATAGTCCCA
TTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGTGACTTATGGCATT
TGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGTGTGCCTGTAT

CTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGCCTTATAA
TATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGATTT
TATTTCCGTTAACTACTGCCAAAATTAAATAAACAGTTATTCTAAAAAGCACTAGAT
AACTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCGTTGCCTCATATATCAATGC
AGGCTTTCCCCAGCTACCAAAGCTGCCATTTGATTCAGATACCCAATCTGCCTCAGA
TGTGCTTGCGGTTGGGGAGTTACATTTGCGGCTATTTATTGGATGCTCAAAGAGATT
AGTAAACACACACAGAGCTAGAGGAATTTTATACATTATCCTGATAATAACTAAAGT
ATTGTTTAGTTGGATGCAAATGAAGGAAATATGTCAAATGACTTACATATCTGACTA
CTAAAAAAATTATTGTATTATTACAGAGTATTACTGTAGTGTATTATTGTAGTGTATT
ATTATAGAGATAATAACTTAGATACAATGTTAAGGAAAGTAAAATATTTCATTTTCC
TCCAATAACAATAAAGGGAATGTTCTTCTGATTGCAATTAACCTCAGATATTTTTGG
ATATGATCTATTTTTTAAAAATGATGATC

[00254] SEQ ID NO:475 is an example of a stuffer sequence and has the following DNA sequence:

[00255] GATAAAATATGAAAGAGACGAGGGAAAATAATTGGACGTGGAATATCCCCAG
TTTTTATGTTATACAGGTGATGTAGTTTTGGACAAAAAATGATGGAAGAGAGAGGCA
TGTAGAAAGTGTAAAGAAGTTCAGGGGAAGTTTTAAATTTAGCTCTCTTAATCTTGG
GTCACTCATCAGAAAAAGGAAAGCACAATGTTAGAAATTCAGAGATCATATCACTG
GTTGACTTCCGATCATATCACTGGTTGACTTCCTTAGTACTACAGGTTTAAAACTTAA
GAGGCTTTTGTCTCAAAACAACATCATGGTACATGTTAGTTTTCTCCTTTTTTTCTTTT
CCCCAATATAATATATTTACTTAGTCCATTAATTTTTCAATGGTCTGTTCATTGTTTTC
TTTTTTATGTGATCTTCTCATTCCCTCAATACTAGCTGTACATTTTGAAAATATAAAT
GTTTGGTATATAATAAATATTATATGGGGGAAGGGAAAAGGGAAAGGAGAAAGGG
AAGGAGAAAGAGGTATTCTGTGTAGTTATATTAGTGGTCTAACAAACAGACATCTAC
GAGATAAAATGAAACAAAACACACTGACAATTAAATAGAGAAATCAGACGAAAGT
TGAGTCATGTAATCCAAAGCAAAATAGTATTTCAAGAATGAGGGTGTGAATCAGTTT
TACAGATACACACGACTGTTTGAGTAATACGCAATAGACAAGTGATATATGGGATTT
AGTGACAAGTATAAGATATGATTCCAAAAACAGGGTAAATGCCTGAAGAAAGGAGA
GTTAAGAAAAATAAAATTAAACCTTGTCACAGGCAACTCTTTCCATGAGTCTTCCTA
TCATAGAAAATAAATAAATGAGTTGACAGTGGCATATCTAGAACTAAGACA

[00256] SEQ ID NO:476 is an example of a stuffer sequence and has the following DNA sequence:

[00257] ACAAGAATCTGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTC
TTGGTCCCCCACATTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCAT
GTCCTTTATTGGAACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGAT
CCCATGTTACATGGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATA
CCTAGAGCCAGAGGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAA
TGTTGATAAATGGAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAA
TCCCTGAAATAAATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTT
GATTTGAGTACTGTCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGC
CAAGATAATATTTAAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTT
GTTTGTTTTTTTTTGTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTC
ATTACTTTATCTTATTTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTG
AAATAGTTTATGAAAATAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGT
TAGCCTTCTCATCATAAATTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGC
ACAGAATAAGGAGACAAAGAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAG
AAATCTATTAACTCTTGTTTTGGAGACACAGAGTATTCCTTTGAATGAAGACTTAGG
ACAGATATTTTTACATCACAATATTATGCTTACTGCCTGTCATTCCTCTTTGATTATT
ACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCTTGAGTTCCTAAATAAAT
TTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTCATTAATTAGCTGTAA
CATATTTATACTAAGATGTGCCTTCTTTTGGGAGTGTATATTTCTCATTATAGTCCCA
TTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGTGACTTATGGCATT
TGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGTGTGCCTGTAT
CTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGCCTTATAA
TATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGATTT
TATTTCCGTTAACTACTGCCAAAATTAAATAAACAGTTATTCTAAAAAGCACTAGAT
AACTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCGTTGCCTCATATATCAATGC
AGGCTTTCCCCAGCTACCAAAGCTGCCATTTGATTCAGATACCCAATCTGCCTCAGA
TGTGCTTGCGGTTGGGGAGTTACATTTGCGGCTATTTATTGGATGCTCAAAGAGATT
AGTAAACACACACAGAGCTAGAGGAATTTTATACATTATCCTGATAATAACTAAAGT
ATTGTTTAGTTGGATGCAAATGAAGGAAATATGTCAAATGACTTACATATCTGACTA

CTAAAAAAATTATTGTATTATTACAGAGTATTACTGTAGTGTATTATTGTAGTGTATT
ATTATAGAGATAATAACTTAGATACAATGTTAAGGAAAGTAAAATATTTCATTTTCC
TCCAATAACAATAAAGGGAATGTTCTTCTGATTGCAATTAACCTCAGATATTTTTGG
ATATGATCTATTTTTTAAAAATGATGATCCATGGGATAAAATATGAAAGAGACGAG
GGAAAATAATTGGACGTGGAATATCCCCAGTTTTTATGTTATACAGGTGATGTAGTT
TTGGACAAAAAATGATGGAAGAGAGAGGCATGTAGAAAGTGTAAAGAAGTTCAGG
GGAAGTTTTAAATTTAGCTCTCTTAATCTTGGGTCACTCATCAGAAAAAGGAAAGCA
CAATGTTAGAAATTCAGAGATCATATCACTGGTTGACTTCCGATCATATCACTGGTT
GACTTCCTTAGTACTACAGGTTTAAAACTTAAGAGGCTTTTGTCTCAAAACAACATC
ATGGTACATGTTAGTTTTCTCCTTTTTTTCTTTTCCCCAATATAATATATTTACTTAGT
CCATTAATTTTTCAATGGTCTGTTCATTGTTTTCTTTTTTATGTGATCTTCTCATTCCCT
CAATACTAGCTGTACATTTTGAAAATATAAATGTTTGGTATATAATAAATATTATAT
GGGGGAAGGGAAAAGGGAAAGGAGAAAGGGAAGGAGAAAGAGGTATTCTGTGTAG
TTATATTAGTGGTCTAACAAACAGACATCTACGAGATAAAATGAAACAAAACACAC
TGACAATTAAATAGAGAAATCAGACGAAAGTTGAGTCATGTAATCCAAAGCAAAAT
AGTATTTCAAGAATGAGGGTGTGAATCAGTTTTACAGATACACACGACTGTTTGAGT
AATACGCAATAGACAAGTGATATATGGGATTTAGTGACAAGTATAAGATATGATTC
CAAAAACAGGGTAAATGCCTGAAGAAAGGAGAGTTAAGAAAAATAAAATTAAACC
TTGTCACAGGCAACTCTTTCCATGAGTCTTCCTATCATAGAAAATAAATAAATGAGT
TGACAGTGGCATATCTAGAACTAAGACA

[00258] SEQ ID NO:477 is an example of a stuffer sequence and has the following DNA sequence:

[00259] ACAAGAATCTGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTC
TTGGTCCCCCACATTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCAT
GTCCTTTATTGGAACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGAT
CCCATGTTACATGGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATA
CCTAGAGCCAGAGGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAA
TGTTGATAAATGGAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAA
TCCCTGAAATAAATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTT
GATTTGAGTACTGTCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGC
CAAGATAATATTTAAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTT

GTTTGTTTTTTTTTGTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTC
ATTACTTTATCTTATTTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTG
AAATAGTTTATGAAAATAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGT
TAGCCTTCTCATCATAAATTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGC
ACAGAATAAGGAGACAAAGAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAG
AAATCTATTAACTCTTGTTTTGGAGACACAGAGTATTCCTTTGAATGAAGACTTAGG
ACAGATATTTTTACATCACAATATTATGCTTACTGCCTGTCATTCCTCTTTGATTATT
ACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCTTGAGTTCCTAAATAAAT
TTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTCATTAATTAGCTGTAA
CATATTTATACTAAGATGTGCCTTCTTTTGGGAGTGTATATTTCTCATTATAGTCCCA
TTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGTGACTTATGGCATT
TGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGTGTGCCTGTAT
CTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGCCTTATAA
TATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGATTT
TATTTCCGTTAACTACTGCCAAAATTAAATAAACAGTTATTCTAAAAAGCACTAGAT
AACTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCGTTGCCTCATATATCAATGC
AGGCTTTCCCCAGCTACCAAAGCTGCCATTTGATTCAGATACCCAATCTGCCTCAGA
TGTGCTTGCGGTTGGGGAGTTACATTTGCGGCTATTTATTGGATGCTCAAAGAGATT
AGTAAACACACACAGAGCTAGAGGAATTTTATACATTATCCTGATAATAACTAAAGT
ATTGTTTAGTTGGATGCAAATGAAGGAAATATGTCAAATGACTTACATATCTGACTA
CTAAAAAAATTATTGTATTATTACAGAGTATTACTGTAGTGTATTATTGTAGTGTATT
ATTATAGAGATAATAACTTAGATACAATGTTAAGGAAAGTAAAATATTTCATTTTCC
TCCAATAACAATAAAGGGAATGTTCTTCTGATTGCAATTAACCTCAGATATTTTTGG
ATATGATCTATTTTTTAAAAATGATGATCCATGGGATAAAATATGAAAGAGACGAG
GGAAAATAATTGGACGTGGAATATCCCCAGTTTTTATGTTATACAGGTGATGTAGTT
TTGGACAAAAAATGATGGAAGAGAGAGGCATGTAGAAAGTGTAAAGAAGTTCAGG
GGAAGTTTTAAATTTAGCTCTCTTAATCTTGGGTCACTCATCAGAAAAAGGAAAGCA
CAATGTTAGAAATTCAGAGATCATATCACTGGTTGACTTCCGATCATATCACTGGTT
GACTTCCTTAGTACTACAGGTTTAAAACTTAAGAGGCTTTTGTCTCAAAACAACATC
AACAAAAAGCCAGGTGTGGTAGTGTGCATCTGTAGTCTCAGCTACTGAGGAGACCA
ATGCAGGAAAATTGCTTAAGCCCAGCAATTTGAGGCTGTAGTCAGTCAAGGTCTCAC

TACTGCACTGCAGACTGCGTTACAGAACACTTTCTTAAAAAAAAGAAAGGAAAGAA
AAGAAAAAGGGAAGGGAAAAGGGAAAGGAGAAAGGGAAGGAGAAAGAGGTATTC
TGTGTAGTTATATTAGTGGTCTAACAAACAGACATCTACGAGATAAAATGAAACAA
AACACACTGACAATTAAATAGAGAAATCAGACGAAAGTTGAGTCATGTAATCCAAA
GCAAAATAGTATTTCAAGAATGAGGGTGTGAATCAGTTTTACAGATACACACGACT
GTTTGAGTAATACGCAATAGACAAGTGATATATGGGATTTAGTGACAAGTATAAGA
TATGATTCCAAAAACAGGGTAAATGCCTGAAGAAAGGAGAGTTAAGAAAAATAAA
ATTAAACCTTGTCACAGGCAACTCTTTCCATGAGTCTTCCTATCATAGAAAATAAAT
AAATGAGTTGACAGTGGCATATCTAGAACTAAGACA

[00260] SEQ ID NO:478 is an example of a stuffer sequence and has the following DNA sequence:

[00261] ACAAGAATCTGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTATTTCACAG
TAGCTCCCCCACATTACAGGATTGTATAGGCAGAAACTCTCCATAGCTGCCATTCAA
GTCCTTTATTGGAACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGAT
CCCATGTTACATGGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATA
CCTAGAGCCAGAGGAGATGTTCCTTTTGAGAAACAGTGTCAAAAGTGTAGAAGAAA
TGTTGATAAATGGAGCAGTAGATAATACATCTGCAATCCCTTAATATTTATTTATAA
TCCCTGAAATAAATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTT
GATTTGAGTACTGTCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGC
CAAGATAATATTTAAAAGATGGCAAGGTTATCACAGAAAGAAAATGTAGAAATTTT
TGTTTGTTATATAATGTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCT
TCTTAAGTTTATCTTATTTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATG
TGAAATAGTTTATGAAAATAAATTCATGAAACATTGAAATAATTTATCTTCTCAGAA
GATAGCCTTCTCATCATAAATTGAGAAGTTCATTATGCATTTTGAGTATAACTTCAAT
GCACAGAATAAGGAGACAAAGAACTTATTGTAGTTTTGGGAAATCTGGATAATGGT
AGAAATCTATTAACTCTTGTTTTGGAGACACAGAGTATTCCTTAGAATCAAGACTTA
GGACAGATATTTTTACATCACAATATTATGCTTACTGCCTGTCATTCCTCTTTGATTA
TTACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCTTGAGTTCCTAAATAA
ATTTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTGATTAATTAGCTGT
AACATATTTATACTAAGATGTGCCTTCTTTTGGGACAGTTTATAACTCTTTTTACTCC
CATTGTGTACAAACTTATAGGCATGTAACTGTTTCTACTTTCTTTTGTGACTTATGGC

ATTTGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGTGTGCCTG
TATCTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGCCTTA
TAATATAATCCTTCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGA
TTTTATTTCCGTTAACTACTGCCAAAATTAAATAAACAGTTATTCTAAAAAGCACTA
GATAACTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCGTTGCCTCATATATCAA
TGCAGGCTTTCCCCAGCTACCAAAGCTGCCATTTGATTCACATACCCAATCTGCCTC
AGATGTGCTTGCGGTTGGGGAGTTACATTTGCGGCTATTTATTGGATGCTCAAAGAG
ATTAGTAAACACACACAGAGCTAGAGGAATTTTATAGATTATCCTGATAATAACTAA
AGTATTGTTTAGTTGGATGCTTATGTTGGAAATATGTCATATGACTTACATATCTGAC
TACTATATAAATTATTGTATTATTACAGAGTATTACTGTAGTGTATTATTGTAGTGTA
TTATTATAGAGATAATAACTTAGATACAATGTTAAGGAAAGTAAAATATTTCTTTTT
CCTCCAATAACAATAAAGGGAAAGTAGTTCTCAATGCAATTAACCTCACATATTTTT
GGATATGATCTATATTTTAAAAATGATGATCCATGGGATAAATTATCAAAGAGACGA
GGGAAAATAATTGGACGTGGAATATCCCCAGTATATATGTTATACAGGTGATGTAGT
TTTGGACAAAAATTGAAGGAAGAGAGAGGCAAGTAGTAAGAGAAAAGAAGTTCAG
GGGAAGTTTTAAATTTAGCTCTCTTAATCTTGGGTCACTGATCAGAAAAAGGAAAGC
ACAATGTTAGAAATTCAGAGATCATATCACTGGATGACTTCCGATCATATCACTGGT
TGACTTCCTTAGTACTACAGGTTTAAAACTTAAGAGGCTTTTGTCTCAAAACAACAT
CAGGGAAGGGAAAAGGGAAAGGAGAAAGGGAAGGAGAAAGAGGTATTCTGTGTAG
TTATATTAGTGGTCTAACAAACAGACATCTACGAGATATAATCAAACAAAACACACT
GACAATTAAATAGAGAAATGAGACGAAAGTTGAGTCATGTAATCCAAAGCAAAATA
GTATTTCAAGAATGAGGGTGTGAATCACTTAAACAGATACACACCTGTGATTGAGTT
TAACGCAATAGACAAGTGATATATGGGATTTAGTGACAAGTATAAGATATGATTCC
AAAAACAGGGTAAATGCCTGAAGAAAGGAGAGTTAAGAAAAATAAAATTAAACCT
TGTCACAGGCAACTCTTTCCATGAGTCTTCCTATCATAGAAAATAAATAAATGAGTT
GACAGTGGCATATCTAGAACTAAGACA

[00262] SEQ ID NO:479 is a CBA promoter and has the following DNA sequence:

[00263] CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAG
TGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCT

GGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACG
TATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCC
CCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGT
GCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGC
GAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCG
CGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAG
CGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGC
GCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGC
GGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTC
GTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGC
GGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGT
GCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTG
CGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGG
GGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCA
GGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGT
TGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGC
TCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCC
GCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCG
GCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGG
GCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCCGC
CGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAAT
GGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCC
TCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTT
CGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTT
CTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTGTTGTGCTGTCTCATCATTTTG
GCAAAGAATT

[00264] SEQ ID NO:480 is an expression cassette comprising a nociceptor-selective promoter of the present disclosure (SEQ ID NO:466) operably linked to a EGFP-KASH reporter gene and a poly-A signal sequence (SEQ ID NO:468) and has the following DNA sequence:

[00265] AGCAGGGCCTGGAAGAAAAAGTCAGAATTCCCCAGGCAGACAAACAGGGAG
TGGGGGGCAGCAGGTGAGCAAAAGGCCACCTTGAGGTGCTTGGGGATTGTCTTTCA

CACAGCTGTCCCATGCGCTCTGCCCAACCCAGGCTCTGTGCATGAGATGCTAATGAG
ATTTCTCTAAGAACAGGATGTTGTCCAAGAGCAAGGCTCATCTAATCTTTACCAGCA
TCCTAAGAAGTCCTGATTCTTATGTGACTCATTTAGCCAGCTTTTCCCCACAAGTGGT
ATCACAGGGAAATGACACGCTCCTTTTGGCACCAACTAATTTAAAGCACATTTGAGC
CATTTGATAATATATATTTTTAAATACTTGGCACTATTCCTTACTACCTTAAGCTGGT
ATATCAACCCTCGCTAACCTCTTCTCAGTAACTCAGGGCATTCACCTCTGCCCAACG
TGGGAAGGCAGGGAACCCTGTGAGGCCTCAGGAACATGCAGGGCTGCTGGCCAGGT
TACTAAGTGGTCCAGAAAAGCTGTGCTTTGAGTCTGTAGCTCTCCCATAGCCTTCTTC
CCTGCAGTCGTCACTTGTGTTCCAGCAGCTGCTGTTATCAGCTGCCATATTTTACATG
CCTTTGTCTAGGTAACAGCAGAGAGCATGAACTCTGTGACAGCTGTCTGCCACTCAG
AAGCATTTTCAGTTACTTTGGGGCTCTTAGGGAGCTCCAGGTGGCCAAGTTCCCTAC
AATTTTGACCTCTGGACAAATAACAATGGTCATGGCTAAAATCTATTGAGCCAAGCA
CTTCGTGCAAAGTGTTTTACAAGCGCTAACATATTTAGTCATCAAAACAGCTGAATG
ATGCAGATCTAGCCTTTTCAATTTTGAAGCTGGGAAAAATGAAGATTAGAGAGGTTA
AACAACTTGCCTAAGGTCACACAAGAGTGAGACTCCACAAGCTGTGGGACCTAAGA
TCAGTTCCCAAACCTCTCTGAGCCGCCATTTATTCATCTATGAAACTGAATGGTAAT
GCCTACTTGGTAGGATTATTTTGAAGCTTAAATGAGATGCTGCTTGTCAAATGTTTA
GGTCCTGCCTGAGACAAAGTAAGTGCCCAGGAAATGACAGCCAAGAAAAAAGGAA
ACGAAAGACAACGCACAAAGAAAGTCAAATATCTTTCAGAGCCAGCAAATAAGAGT
TGGAGGTGCTCACAGATGAAGAAGTTTTCCCTTGTGATTCTCCCCCACTTTCTTTTGT
CCACATCATTTTCTCTAGGCAAAAGTAGCCTGGGGCTAGGGAGAGGCTCTCTATAGG
TGAGTAGTGAAGTGACAGCCTCACAGAGACTGAGCTGGAAGATTAGAGGTTTAGGA
TTGTCTTATACAAGGTAAAAATAAATGTGGTTTCACTTACAAATCTTGAAGGAAACA
AGCTATAGGGAAAGAGGCATCTATAAAAGTTTAGCATCTTTAATAACACTGCCTTCA
ATTCAAGAATTTAATTTGACACCTAGCCACTGAGTGCTGACTTTGTGACAGGCACTG
GGCTCAACAGAGATAAGGAAGTCATAATTCATCCCCAAAGTTATCAAGCCCTTCAGC
TTGAAGAATGTCAGCTACTGGCTGTGTGCCCTAGGCAAGGCTGACCACAGCAGGGT
AAGATAAGAGGGAGGGCTGGGACGGGATTTGGGGGCTGTGGGTGAACAGAAGCCG
CTTGGTTCCCACTGGGTGAGGGCTGTCACATTCTGCACGTGGATGTCATGCTTCTTCC
CAAATGTTCCAAGGACTCCCCTGGCTAGTTGTCCCTACTCAATGTTTTCAGTACACA
ACTTCTGGGTGTTGGTTTTTTGTTTTTGTTTTCTTGTCCATGACCCCGGCTAGGCAGG

AGGGGCCTGACAGCGACAAATGGGGACCCGCCCCACCATTTGGGTCCATTAGCAAA
GCTATCAGGTTTACTAATATGAGAGCAACTTCAACCCCTAATTGAATTCATCTGGAG
ACATTATAGGGCTGGAGCTGCCAGGAGGGCAGCAGGCCCCTGCAGCATTACTCCAT
GATTAAATATTCAAGCCCAGTGAATGCTGAGAGTCGTTATGGGTGTAATTACGGTGT
CTCGGGCCATTGCCAGCACTCCATCAGGGGCCCCGAAGGTTTACAGTCTCACACAGC
TAAGCCTCTGGGGCTCCAGGGAGAAGAGCAGTGTTCCAGGCCTCAGCTGGCGAGGC
ACCAAACATAATGGAACCCTGACCCAGAGAAGCTGTTTTTTTGCTTGACTTGAGAGG
TTTTGAGTAAACAGAGTTGTAATGTAATAAAGCACCAAGCCAGTTTCCTCCTCTATT
AACTGGCATTGAGAGACAAGATGAATCAAGCAGCTGCAGGGGAGCTGGCTTGGCCT
GGGACCCCTTGCAGCACCTCCTCCACCTACCCCTCCCTTTTGGTCCTATGGCCCTGTG
GACAGGTTATTTTCTCCTGCTCTTTGTTTTGTTTAATCTCATCGGAGGTTTCAGATCTT
TTTTTTTTTTTTGTAATTTCATTTCAAACTATTATTTTATAAGACCTGGCCTATTACTG
AGTATGCAGGCAGAATATGAAAATTACTCCAAAACTTTTTTAAATGAAATTTTCAAG
ATGCAAAAAGTGAAACTTTAAAATTTCAGTGGAAGAAGGGGAACAAAAACATTTTA
ATAAATGAGAGTGTTTATTCCAGAATGGGAATATAGAGACAAGGAAGGTACCATGT
GAATGGGTGCACCTCGCTCTCTGGGGTCAATGATAGGAAACAGCCTGTCCCACAGTC
AAGGCAGCCTTGCCCAGGCTATGAGTCTATTGTGGATGCTGGAGCATTGTTATCTAA
GTGCAGCCTCTTTGCTTCCTCAGGTTTCAGCATTTCCCATGAGATCATTTAAAATCAC
ATTTGCTATTTTACCATCTAATCACACATAAGCCTCTCCCCACACTCCCCCCGCCCTG
TTTCCATCCAAGGAGTGCACTTTCTGGAGCACCAGCAACCAGGGTGGAACTCGTGAC
GGGAAATGGGAATGGCACCCAAGAAAGCATGATTTCTGTAGTTTCGTGAATGATAG
CAAGGCTCCCATCAGACAAGCTGAGCCACTGTCACTGAGGAGGACAAACGAGTGCA
AGTCTTTGCAGAGCTTGGCATCTCAGACTTGCCTCTCATTTCTTGCTTCACACACTAG
CCTCTTGGCTAGAGAACAGACATCAGATGGAGTTTCTTCTGGCTATGCCTGAATGTT
AAGCTGAACGTATGTTCCAGGAGCTCGTGGTCTCCAGTAGAGGCAATCTGGGATAG
AAGAGAAGATATTTCTTACGTAGAAGACAAGCAAGATTGAGCAGTGACGCGCCACC
ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCT
GGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGAT
GCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTG
CCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTAC
CCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTC

CAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGT
GAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA
AGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAA
CGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCC
GCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACC
CCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCC
GCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGT
GACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTCCGGACTCAGAT
CTCGAGAGGAGGAGGAGGAGACAGACAGCAGGATGCCCCACCTCGACAGCCCCGG
CAGCTCCCAGCCGAGACGCTCCTTCCTCTCAAGGGTGATCAGGGCAGCTCTACCGTT
GCAGCTGCTTCTGCTGCTGCTGCTGCTCCTGGCCTGCCTGCTACCTGCCTCTGAAGAT
GACTACAGCTGCACCCAGGCCAACAACTTTGCCCGATCCTTCTACCCCATGCTGCGG
TACACCAACGGGCCACCTCCCACCTAGACTAGCGCTATAGTCAATAAAAGATCTTTA
TTTTCATTAGATCTGTGTGTTGGTTTTTTGTGTG

EMBODIMENTS

[00266] Exemplary embodiments are listed below.

[00267] Embodiment 1. A polynucleotide comprising a targeting region that binds to a target site in an endogenous mRNA encoding Nav1.7, wherein the target site is from 10 to 30 nucleotides in length and comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 1 to 59.

[00268] Embodiment 2. The polynucleotide of embodiment 1, wherein the endogenous mRNA encoding Nav1.7 is transcribed from an endogenous SCN9A gene.

[00269] Embodiment 3. The polynucleotide of embodiment 1, wherein the endogenous mRNA encoding Nav1.7 comprises a sequence having at least 95% sequence identity to SEQ ID NO: 305.

[00270] Embodiment 4. The polynucleotide of any one of embodiments 1 to 3, wherein the target site comprises a sequence of any one of SEQ ID NOs: 1 to 59.

[00271] Embodiment 5. The polynucleotide of any one of embodiments 1 to 4, wherein the targeting region is at least 90% complementary to the target site.

[00272] Embodiment 6. The polynucleotide of any one of embodiments 1 to 4, wherein the targeting region is at least 95% complementary to the target site.

[00273] Embodiment 7. The polynucleotide of any one of embodiments 1 to 4, wherein the targeting region is complementary to the target site, with the optional exception of 1, 2, 3 or 4 mismatches.

[00274] Embodiment 8. The polynucleotide of any one of embodiments 1 to 4, wherein the targeting region comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 61 to 119 .

[00275] Embodiment 9. The polynucleotide of any one of embodiments 1 to 4, wherein the targeting region comprises a sequence having at least 95% sequence identity to any one of SEQ ID NOs: 61 to 119.

[00276] Embodiment 10. The polynucleotide of any one of embodiments 1 to 4, wherein the targeting region comprises any one of SEQ ID NOs: 61 to 119.

[00277] Embodiment 11. The polynucleotide of any one of embodiments 1 to 10, wherein the polynucleotide is selected from the group consisting of: a pri-miRNA, a pre-miRNA, a mature miRNA, an siRNA, an shRNA, and an antisense oligonucleotide.

[00278] Embodiment 12. The polynucleotide of embodiment 11, wherein the polynucleotide comprises: at least one modified internucleoside linkage; at least one modified nucleoside; at least two different nucleoside residues selected from DNA, RNA, and arabino nucleic acid; or any combination thereof.

[00279] Embodiment 13. An expression cassette comprising a promoter operably linked to a transgene encoding an RNA, wherein the RNA comprises the polynucleotide of any one of embodiments 1 to 11.

[00280] Embodiment 14. The expression cassette of embodiment 13, further comprising an enhancer operably linked to the promoter.

[00281] Embodiment 15. The expression cassette of embodiment 13 or 14, wherein the RNA is a pri-miRNA.

[00282] Embodiment 16. The expression cassette of embodiment 15, wherein the pri-miRNA comprises a miRNA scaffold .

[00283] Embodiment 17. A vector comprising the expression cassette of any one of embodiments 13 to 16.

[00284] Embodiment 18. The vector of embodiment 17, wherein the vector is a plasmid.

[00285] Embodiment 19. The vector of embodiment 18, wherein the vector is a viral vector.

[00286] Embodiment 20. The vector of embodiment 19, wherein the viral vector is an adeno-associated virus (AAV) vector.

[00287] Embodiment 21. The vector of embodiment 20, wherein the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV-DJ, variants thereof, or hybrids thereof.

[00288] Embodiment 22. The vector of embodiment 20 or 21, wherein the AAV is an scAAV.

[00289] Embodiment 23. The vector of embodiment 19, wherein the viral vector is a lentiviral vector.

[00290] Embodiment 24. A pharmaceutical composition comprising: (i) the polynucleotide of any one of embodiments 1 to 12, the expression cassette of any one of embodiments 13 to 16, or the vector of any one of embodiments 17 to 23, and (ii) a pharmaceutically acceptable carrier.

[00291] Embodiment 25. A method of reducing expression of a gene encoding Nav1.7 in a cell, comprising contacting the cell with an effective amount of the polynucleotide of any one of embodiments 1 to 12, the expression cassette of any one of embodiments 13 to 16, the vector of any one of embodiments 17 to 23, or the pharmaceutical composition of embodiment 24.

[00292] Embodiment 26. The method of embodiment 25, wherein expression of the mRNA encoding Nav1.7 and/or the Nav1.7 protein encoded by the mRNA in the contacted cell is reduced compared to a comparable cell not contacted with the expression cassette of any one of embodiments 12 to 15, the vector of any one of embodiments 16 to 22, or the pharmaceutical composition of embodiment 24.

[00293] Embodiment 27. The method of embodiment 26, wherein the expression of the mRNA encoding Nav1.7 and/or the Nav1.7 protein encoded by the mRNA is reduced at least a 5% in the contacted cell compared to the comparable non-contacted cell.

[00294] Embodiment 28. The method of embodiment 26, wherein the expression of the mRNA encoding Nav1.7 and/or the Nav1.7 protein encoded by the mRNA is reduced at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% in the contacted cell compared to the comparable non-contacted cell.

[00295] Embodiment 29. The method of any one of embodiments 25 to 28, wherein the expression of the mRNA encoding Nav1.7 is measured by quantitative polymerase chain reaction.

[00296] Embodiment 30. The method of any one of embodiments 25 to 29, wherein the cell is a cultured cell.

[00297] Embodiment 31. The method of any one of embodiments 30, wherein the cultured cell is a primary neuron, an iPSC derived neural cell, a neuronal cell line, an engineered cell line, or a neural stem cell.

[00298] Embodiment 32. The method of any one of embodiments 25 to 29, wherein the cell is in vivo.

[00299] Embodiment 33. The method of embodiment 32, wherein the cell is a neuron.

[00300] Embodiment 34. The method of embodiment 33, wherein the cell is a neuron in the central nervous system (CNS), or a cell in contact with cerebral spinal fluid.

[00301] Embodiment 35. The method of any one of embodiments 32 to 34, wherein the contacting comprises delivering the polynucleotide of any one of embodiments 1 to 12, the expression cassette of any one of embodiments 13 to 16, the vector of any one of embodiments 17 to 23, or the pharmaceutical composition of embodiment 24 to the CNS or cerebral spinal fluid (CSF).

[00302] Embodiment 36. The method of embodiment 35, wherein the delivering comprises intraparenchymal injection, intrathecal injection, intra-cisterna magna injection, or intracerebroventricular injection.

[00303] Embodiment 37. A method for treating or preventing neuropathic pain in a subject comprising administering a therapeutically or prophylactically effective amount of the pharmaceutical composition of embodiment 24 to a subject.

[00304] Embodiment 38. The method of embodiment 37, wherein the subject is a mammal.

[00305] Embodiment 39. The method of embodiment 38, wherein the mammal is a human.

[00306] Embodiment 40. The method of any one of embodiments 37 to 39, wherein the method comprises administering the pharmaceutical composition to the subject via intraparenchymal injection, intrathecal injection, intra-cisterna magna injection, intravenous injection, or intracerebroventricular injection.

[00307] Embodiment 41. A use of the polynucleotide of any one of embodiments 1 to 12, the expression cassette of any one of embodiments 13 to 16, the vector of any one of embodiments 17 to 23, or the pharmaceutical composition of embodiment 24, in the manufacture of a medicament for the treatment of neuropathic pain.

[00308] Embodiment 42. A cell comprising the polynucleotide of any one of embodiments 1 to 12, the expression cassette of any one of embodiments 13 to 16, or the vector of any one of embodiments 17 to 23.

[00309] Embodiment 43. The cell of embodiment 42, wherein the cell comprises the viral vector of any one of embodiments 19 to 23.

[00310] Embodiment 44. A method of producing a composition comprising viral particles, comprising: (i) culturing the cell of embodiment 43 under conditions to produce viral particles, and (ii) isolating the viral particles from the cells in the culture, thereby producing a composition comprising viral particles.

[00311] Embodiment 45. The method of embodiment 44, wherein the viral particles are AAV particles.

[00312] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

EXAMPLES

EXAMPLE 1

RNA OLIGONUCLEOTIDE SCREENING IN HEK293 CELLS

[00313] Expression vectors encoding engineered pri-miRNAs specific for Sites 1-60 under the control of the human U6 promoter were generated using the targeting regions and passenger strands provided in Tables 6 and 7 (the scramble construct was also generated). The miR-E scaffold was used to construct these pri-miRNA expression vectors (see Fellmann, et al., 2013; Cell Reports vol. 5, pp. 1704–1713). These constructs also included sequences that allowed for packaging in an AAV capsid to produce AAV vectors (as done in Examples 2 to 5).

[00314] A reporter system in HEK293T cells was used to evaluate the knockdown of exogenous SCN9A expression using a co-transfection method. Two biological replicates with three technical transfection replicates were performed. HEK293T cells were plated at 75,000 cells per well in tissue culture plates. A mixture of 300ng pri-miRNA expression vector plasmid DNA, 150ng SCN9A-GFP reporter plasmid DNA, and 25ng mCherry plasmid DNA (a transfection control vector) was transfected into the cells in each well with Fugene transfection reagent at a ratio of 3:1 (Fugene:DNA).

[00315] 24h post transfection, the media was changed. At 48h post transfection, cells were harvested in RLT+BME lysis buffer and processed with RNAeasy Plus extraction kit (Qiagen). 120ng total RNA was used for cDNA generation using VILO Superscript IV kit (ThermoFisher Scientific). qPCR was set up with template (RT+) and control (RT-) conditions for each sample using TaqMan probe-based detection of amplification. Data were analyzed with delta delta Ct method and all samples were first normalized to GAPDH control and then relative to scramble control miRNA (SCRM) treated sample.

[00316] The results of this experiment are shown in Figure 1, which shows that expression vectors that encode a microRNA with a guide strand from Table 6 (i.e., SEQ ID NOs: 61-120, with 120 being a positive control) reduce the amount of SCN9A transcript in the cell by approximately 50% to greater than 90% as compared to the scrambled sequence (SCRM).

EXAMPLE 2

RNA OLIGONUCLEOTIDE SCREENING IN IPSC GLUTAMATERGIC NEURONS

[00317] For this example, miR-E-based expression vectors were delivered to iPSC cells using an AAV virus approach. AAV particles were produced for each miR-E vector tested and a scrambled control sequence using the AAV serotype AAVDJ.

[00318] iPSC glutamatergic neurons were purchased from Fujifilm Cellular Dynamics (iCell GlutaNeurons, 01279 (Catalog #: R10) and thawed according to the manufacturer's directions. A 96 well plate was precoated with 0.01% Poly-L-Ornithine (PLO) and 0.28 mg/mL Matrigel (Corning Life Sciences). Cells were plated at 25,000 cells/well in the precoated 96 well plate. 50% of the culture media was replaced at after 24 hours, and AAVs were added to the respective wells, diluted according to the multiplicity of infection (MOI) calculations. Scrambled miRNA treated neurons and unmanipulated neurons were taken along as controls. Neurons were

maintained according to manufacturer's protocol, with 50% media replaced every 48h, until they were harvested at DIV14 (13-day transduction).

[00319] The iPSC derived glutamatergic neurons were infected with AAV at a MOI of 1E6. Biological replicates were included for each construct. Cells were lysed using ThermoFisher Cells-to-Ct kit and lysate used for cDNA generation using Cells-to-Ct reagents according to manufacturer's protocols. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample and Taqman probes were used for target detection. Normalized SCN9A expression was calculated by dividing SCN9A transcript copies by GAPDH transcript copies. Relative SCN9A expression was then calculated by dividing miRNA treated samples expression by scramble treated samples expression.

[00320] The results of this experiment are shown in Figure 2, which shows significant knock down of endogenous SCN9A expression by each of the miRNA candidates as compared to the scrambled sequence.

EXAMPLE 3

RNA OLIGONUCLEOTIDE SCREENING IN iPSC SENSORY NEURONS

[00321] iPSC Sensory neurons were purchased from Fujifilm Cellular Dynamics and thawed according to the manufacturer's directions. A 96 well plate was precoated with Geltrex. Cells were plated at 20,000/well in the precoated 96 well plate. 100% of the culture media was replaced at after 24 hours, and AAVs were added to the respective wells, diluted according to the multiplicity of infection (MOI) calculations. Neurons were maintained according to manufacturer's protocol, with 50% media replaced every 3-4 days, until they were harvested at DIV14 (13-day transduction).

[00322] The iPSC derived Sensory neurons were infected with AAV at a MOI of 1E4. Biological replicates were included for each construct. Cells were lysed using ThermoFisher Cells-to-Ct kit and lysate used for cDNA generation using Cells-to-Ct reagents according to manufacturer's protocols. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample and Taqman probes were used for target detection. Normalized SCN9A expression was calculated by dividing SCN9A transcript copies by GAPDH transcript copies. Relative SCN9A expression was then calculated by dividing miRNA treated samples expression by scramble treated samples expression.

[00323] The results of this experiment are shown in Figure 3, which shows significant knock down of SCN9A expression by miRNA candidates (from about 50% to about 75%) as compared to the scrambled sequence.

EXAMPLE 4

NAV CHANNELS OFF-TARGET EVALUATION IN IPSC GLUTAMATERGIC NEURONS

[00324] To determine if the miRNA knockdown of SCN9A also affects the expression of related sodium channel family members, an experiment was conducted in iPSC derived glutamatergic neurons. A 96 well plate was precoated with 0.01% Poly-L-Ornithine (PLO) and 0.28 mg/mL Matrigel (Corning Life Sciences). Cells were plated at 25,000 cells/well in the precoated 96 well plate. 50% of the culture media was replaced at after 24 hours, and AAVs were added to the respective wells, diluted according to the multiplicity of infection (MOI) calculations. Scrambled miRNA treated neurons and unmanipulated neurons were taken along as controls. Neurons were maintained according to manufacturer's protocol, with 50% media replaced every 48h, until they were harvested at DIV14 (13-day transduction).

[00325] The iPSC derived glutamatergic neurons were infected with AAV at a MOI of 10^6. Biological replicates were included for each construct. Cells were lysed using ThermoFisher Cells-to-Ct kit and lysate used for cDNA generation using Cells-to-Ct reagents according to manufacturer's protocols. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample and Taqman probes were used for target detection. Normalized SCN1A, SCN2A, SCN3A and SCN8A expression was calculated by dividing transcript copies by GAPDH transcript copies and then relative the normalized scramble control treated samples.

[00326] The results of this experiment are shown in Figure 4. While some miRNA candidates showed some off-target knockdown of SCN1A transcript (e.g., those with guide strands of SEQ ID NO: 66 or any one of 70-73), only minimal reductions in transcripts of other sodium channels was observed as compared to the scrambled sequence.

EXAMPLE 5

NAv CHANNELS OFF-TARGET EVALUATION IN iPSC SENSORY NEURONS

[00327] To determine if the miRNA knockdown of SCN9A also affects the expression of related sodium channel family members, an experiment was conducted in iPSC derived sensory neurons. A 96 well plate was precoated with Geltrex. Cells were plated at 20,000/well in the precoated 96 well plate. 100% of the culture media was replaced at after 24 hours, and AAVs were added to the respective wells, diluted according to the multiplicity of infection (MOI) calculations. Neurons were maintained according to manufacturer's protocol, with 50% media replaced every 3-4 days, until they were harvested at DIV14 (13-day transduction).

[00328] The iPSC derived sensory neurons were infected with AAV at a MOI of 10^4. Biological replicates were included for each construct. Cells were lysed using ThermoFisher Cells-to-Ct kit and lysate used for cDNA generation using Cells-to-Ct reagents according to manufacturer's protocols. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample and Taqman probes were used for target detection. Normalized SCN2A, SCN3A, SCN8A and SCN10A expression was calculated by dividing transcript copies by GAPDH transcript copies and then relative the normalized scramble control treated samples.

[00329] The results of this experiment are shown in Figure 5, which demonstrates that the the miRNA candidates tested had minimal to no off-target knockdown of the transcripts of other sodium channels as compared to the scrambled sequence.

[00330] TaqMan Assays used in Examples are shown in Table 8.

TABLE 8

Gene	TaqMan Assay
SCN1A	Hs003764696_m1
SCN2A	Hs01109871_m1
SCN3A	Hs00366902_m1
SCN4A	Hs01109480_m1
SCN5A	Hs0016593_m1
SCN7A	Hs00161546_m1
SCN8A	Hs00274075_m1
SCN9A	Hs01076699_m1
SCN10A	Hs01045137_m1

SCN11A	Hs00204222_m1
GAPDH	Hs99999905_m1

EXAMPLE 6

TESTING ALTERNATIVE SCAFFFOLDS

[00331] Three different miRNA guide sequences that target the SCN9A transcript were selected (SEQ ID NO:61, SEQ ID NO:68, and SEQ ID NO:69) and cloned into AAVDJ vectors in alternate scaffolds (miR-E, miR-190a, miR-100, miR-132, miR-451, and miR-130a) and tested in iPSC sensory neurons as described in Example 3. In brief, iPSC Sensory Neurons were plated in 96 well plate and treated at 1E4 AAVDJ on DIV6, lysed on DIV21. Lysate was used for cDNA generation using Cells-to-Ct (ThermoFisher) reagents according to manufacturer's protocols. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample and Taqman probes were used for target detection. Normalized SCN9A expression was calculated by dividing SCN9A transcript copies by GAPDH transcript copies. Relative SCN9A expression was then calculated by dividing miRNA treated samples expression by scramble treated samples expression.

[00332] Figure 6 provides a graph showing relative SCN9A expression in the treated cells (scaffold and SEQ ID NO indicated at the bottom of the graph). Data represents biological replicates which are averages of 1-3 technical replicates. Error bars represent SEM.

[00333] As shown in Figure 6, SEQ ID NO:69 miRNA shows significantly improved performance in scaffolds miR-190a, miR-100, miR-132, and miR-130a scaffolds as compared to the same RNA in the miR-E scaffold. SEQ ID NO:69 in the miR-451 scaffold had significantly reduced potency as compared to all other scaffolds used. Unlike for SEQ ID NO:69, miRNAs of SEQ ID NO:61 and 68 had only minor variations in potency in the scaffolds tested.

EXAMPLE 7

NAV CHANNELS OFF-TARGET EVALUATION IN IPSC SENSORY NEURONS FOR ALTERNATIVE SCAFFFOLDS

[00334] Select miRNA scaffold-guide constructs from Example 6 were assayed to determine if the miRNA knockdown of SCN9A also affects the expression of related sodium channel family members in iPSC derived sensory neurons as performed in Example 5. Genes assessed include:

SCN9A, SCN10A, SCN8A, SCN7A, SCN3A, and SCN2A. Assays for these genes were performed using the Taq Man assays listed in Table 8.

[00335] Figure 7 provides a table showing the average SCN9A transcript knockdown (the higher the number, the higher the knockdown) relative to the Scramble control across 2 biological replicates which are averages of 2 technical replicates for the indicated genes (listed at the left of the table) and scaffold-guide constructs (listed at the bottom of the table; (-) represents a value of < 0.01). The miR-130a-61 showed the highest off target activity for SCN10A, knocking down it expression by 32% (0.32). The remaining scaffold-guide combinations had very low off-target activity for these closely related genes.

EXAMPLE 8

SPECIFICITY OF SELECT CONSTRUCTS

[00336] Six scaffold-guide pri-miRNA constructs were selected for off-target gene expression: miR-E-68, miR-190a-68, miR-E-61, miR-190a-61, miR-100-69, and miR-130a-69. iPSC derived sensory neurons (Fujifilm Cellular Dynamics) were plated, cultured, and contacted with AAVDJ vectors expressing the indicated pri-miRNAs as previously described in Example 3. Cells contacted with AAVDJ vectors expressing miR-E, miR-190a, miR-100, or miR130a scaffolds with scrambled guide sequences served as controls. Neurons were maintained according to manufacturer's protocol, with 50% media replaced every 3-5 days, until they were harvested at DIV12. Total RNA was extracted using MagMax mirVana Total RNA Isolation Kit. Total RNA transcriptomic libraries were prepared with Illumina's TruSeq Stranded Total RNA Library (Illumina, 20020597) and sequenced using Illumina's NextSeq2000 platform. Differential expression analysis was performed using DEseq2 tool.

[00337] The volcano plots in Figure 8A demonstrate that miR-E-68 (SEQ ID NO:369) has higher specificity for the SCN9A gene (top panel) than miR-190a-68 (SEQ ID NO:378; bottom panel). Specifically, miR-E-68 shows no significant impact on the expression of non-target (i.e., non-SCN9A) genes, with the majority of the changes below two-fold (vertical dotted lines in each plot) while miR-190a-68 shows increased background gene expression changes. The SCN9A transcript is indicated with an "x" in the volcano plots and shows significantly reduced expression for both constructs.

[00338] The volcano plots in Figure 8B demonstrate that miR-E-61 (SEQ ID NO:368) has higher specificity for the SCN9A gene (top panel) than miR-190a-61 (SEQ ID NO:380; bottom panel). Specifically, miR-E-61 shows no significant impact on the expression of non-target (i.e., non-SCN9A) genes, with the majority of the changes below two-fold (vertical dotted lines in each plot) while miR-190a-68 shows increased background gene expression changes. The SCN9A transcript is indicated with an "x" in the volcano plots and shows significantly reduced expression for both constructs.

[00339] The volcano plots in Figure 8C show that miR-100-69 (SEQ ID NO:370; top panel) and miR-130a-69 (SEQ ID NO:373; bottom panel) have similar specificity for the SCN9A gene, showing a low level of off-target activity, the majority of which is below 2-fold (between the dotted lines). However, miR-100-69 has a stronger downregulatory effect on SCN9A transcript expression, indicated with an "x" in the volcano plots.

EXAMPLE 9

IN VIVO MOUSE ASSESSMENT

[00340] Three different pri-miRNAs were tested *in vivo* in mice: miR-E-61 (SEQ ID NO:368), miR-E-68 (SEQ ID NO:369), and miR-100-69 (SEQ ID NO:370). The full ssAAVDJ genomes used were SEQ ID NO:434, SEQ ID NO:439, and SEQ ID NO:444, respectively. WT B6 mice were dosed at P1 via IT injection with the indicated AAVDJ vectors as follows: miR-E-61 and miR-E-68 were dosed at 3E9, 1E10, 3E10, or 1E11 vg/animal; miR-100-69 was dosed at 1E10 vg/animal (5 animals per treatment; 3 animals were PBS treated). Lumbar DRG tissues were removed 4 weeks post injection and analyzed as described below.

[00341] A portion of the DRG tissue was used for total RNA extraction. Endogenous *Scn9a* mRNA levels were quantified using ddPCR, normalized to a housekeeping gene, and compared to vehicle controls. Figure 9A provides a graph showing that all three pri-miRNAs significantly reduce *Scn9a* transcript levels in vivo, with miR-E-61 and miR-E-68 showing dose-dependent activity. (n=5 per treatment; n=3 PBS). Statistical significance was assessed using one-way ANOVA with Dunnett's post-hoc test (**p<0.01; ****p < 0.0001).

[00342] DRG tissue from a PBS treated animal and an animal treated with 1E11 vg/animal of miR-E-68 were assessed for endogenous Nav1.7 expression using IHC, miRNA expression (SEQ ID NO:68) using ISH, and H&E staining to assess cellular integrity. Figure 9B shows the results,

with two panels for each treatment/assay. The higher panel of the two is at lower magnification and the bottom is at a higher magnification. The box in each upper panel includes a box showing the region magnified in the lower panel. As shown in Figure 9B, Nav1.7 protein is robustly expressed in DRGs after PBS treatment but reduced after miR-E-68 treatment (left panels). miRNA ISH staining clearly shows abundant miRNA expression in DRG neurons but not in the PBS treated mice (middle panels). H&E staining shows no signs of neuronal degeneration or toxicity in either treatment group (right panels). ISH used ACD methods and a custom probe to the miRNA candidate. ISH, IHC and H&E staining were performed on adjacent serial sections of mouse spinal cord. All histology was whole slide imaged at 20x magnification.

[00343] A portion of the DRG tissue from mice treated with 1E10 vg/animal was used to assess miRNA characteristics. Small RNA was isolated from total RNA extracted from the DRG tissue using the NEB Small RNA library prep kit. The small RNA was sequenced and read counts for the miRNAs were determined based on the artificial miRNA sequences, considering both the guide and passenger strands. These counts were used to compute the guide-to-passenger ratio and assess guide strand processing accuracy. As shown in Figure 9C, the miRNA candidates showed favorable guide/passenger (G/P) ratios (top panel) and high fidelity 5' processing precision, with each having at least 96% accuracy (bottom panel).

EXAMPLE 10

scAAV ASSAYS IN iPSC SENSORY NEURONS

[00344] Self-complementary AAVDJ (scAAV) versions of the single-stranded AAVDJ (ssAAV) pri-miRNA expression vectors used in Example 9 were tested in iPSC-derived sensory neurons as described in Example 5. The SEQ ID NOs for the complete genomes for each ssAAV and scAAV vectors are as follows: ssAAV miR-E-61 (SEQ ID NO:434), ssAAV miR-E-68 (SEQ ID NO:439), ssAAV miR-100-69 (SEQ ID NO:444), scAAV miR-E-61 (SEQ ID NO:435), ssAAV miR-E-68 (SEQ ID NO:440), and ssAAV miR-100-69 (SEQ ID NO:445). iPSC Sensory Neurons were plated in 96 well plate and treated at 1E2, 1E3, 1E4 AAVDJ on DIV6, lysed on DIV13. Lysate was used for cDNA generation using Cells-to-Ct (ThermoFisher) reagents according to manufacturer's protocols. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample and Taqman probes were used for target detection. Normalized SCN9A expression was calculated by dividing SCN9A transcript copies by GAPDH transcript

copies. Relative SCN9A expression was then calculated by dividing miRNA treated samples expression by scramble treated samples expression.

[00345] The iPSC derived GABAergic neurons were infected with AAVDJ vectors containing one of the ssAAV and scAAV genomes noted above at MOI of 1E2, 1E3, and 1E4. Biological replicates were included for each construct. Cells were lysed using ThermoFisher Cells-to-Ct kit and lysate used for cDNA generation using Cells-to-Ct reagents according to manufacturer's protocols. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample and Taqman probes were used for target detection. Normalized endogenous SCN9A transcript expression was calculated by dividing SCN9A transcript copies by GAPDH transcript copies. Relative SCN9A transcript expression was then calculated by dividing miRNA treated sample expression by scramble treated samples expression.

[00346] Figure 10 compares SCN9A transcript expression levels for the ssAAV and scAAV vectors. SCN9A knockdown was equivalent at all doses for ssAAV and scAAV vectors expressing the miR-E-61 and miR-E-68 pri-miRNAs (left and middle panels). For miR-100-69, the scAAV vector showed a potency improvement at lower dose (1E2 vg/cell), with scAAV showing ~50% knockdown and ssAAV showed ~30% knockdown at this dose (right panel) (**** = $P \leq 0.0001$).

EXAMPLE 11

IN VIVO RAT FUNCTIONAL ASSAYS

[00347] In preliminary *in vitro* studies, miR-E-68 and miR-100-69 pri-miRNAs were found to be effective at knocking down endogenous rat *Scn9a* (data not shown). ssAAV9 vectors expressing these pri-miRNAs under control of a U6 promoter were tested for their ability to reduce pain signal propagation in a spared nerve injury (SNI) model in WT Sprague-Dawley (SD) rats using Von Frey and hot plate tests (described below). The expression cassette for the miR-E-68 pri-miRNA in the AAV9 vector is SEQ ID NO:438 (full genome is SEQ ID NO:439) and the expression cassette for the miR-100-69 pri-miRNA in the AAV9 vector is SEQ ID NO:443 (full genome is SEQ ID NO:444). AAV9 vector treatments were via intrathecal (IT) injections as follows: (i) 1E12 vg/animal of AAV9 expressing miR-E-68, (ii) 1E11 vg/animal of AAV9

expressing miR-100-69, and (iii) 1E12 vg/animal of AAV9 expressing miR-100-69. Vehicle and Vehicle + Gabapentin control animals were also tested.

[00348] Spared Nerve Injury (SNI) Induced Model

[00349] Spared Nerve Injury (SNI) procedure consists of ligating and dissecting the tibial and common peroneal nerves, thus sparing the sural nerve, hence the name of the model. The common sciatic nerve is furcated into three distinct nerves: tibial, common peroneal and sural nerve. Of these, sural nerve is separately furcated from the two other nerves, and its bifurcation site is located slightly proximal to the bifurcation of tibial and common peroneal nerves. The tibial and common peroneal nerves were first tightly ligated by suture, at immediately distal to the sciatic bifurcation. Thereafter, the nerves were cut at sites close to the ligatures, thereby removing a length of approximately 2-4 mm of the nerves. This is necessary to avoid formation of any re-connection to the nerves. This procedure leads to complete denervation of areas of tibial and peroneal innervation in the plantar paw, while neuropathy is developed upon the sural territories.

[00350] Mechanical Allodynia Evaluation (Von Frey Testing)

[00351] Allodynia response to tactile stimulation was assessed using the manual Von Frey apparatus (Touch Test®). The Von Frey test operate on the principle that when the tip of a fiber of a given length and diameter is pressed against the skin at right angles, the force of application increases as long as the researcher continues to advance the probe until the fiber bends. After the fiber bends, the probe continues to advance, causing the fiber to bend more, but without additional force being applied. Rodents exhibit a paw withdrawal reflex when the paw is unexpectedly touched. The animal indicates sensation by pulling back its paw. The minimal force needed to elevate the withdrawal reflex is considered/designated as the value of reference. Decreases in force needed to induce withdrawal are indicative of allodynia, as the force applied is a non-painful stimulus under normal conditions. Animals were habituated to Von Frey on day -2 by placing the animals in the testing cabins without using VF filament. For testing, animals were placed in an enclosure and positioned on a metal mesh surface and allowed to move freely. The test began after the cessation of exploratory behavior and was performed on the hind left paw (lateral portion, operated side) on study days -1 (baseline), 10 (inclusion), 25, 32, 39, 46, 53, and 60. The forces tested (in grams) were: 2.00, 4.00, 6.00, 8.00, 10.00, 15.00, and 26.00.

[00352] Hot Plate Test

[00353] This test monitors the animal's natural response to heat. The temperature of the plate beneath the animal's paws was set to 50-52°C. Animals were placed on the plate and the latency time to withdrawal, shaking, or licking of the hind left paw was recorded. The cut off for the test was 30 seconds. The Hot plate test was performed on study days -1 (baseline), 10 (inclusion), 25, 32, 39, 46, 53, and 60.

[00354] Results

[00355] Figure 11A shows results of Von Frey tests after SNI surgery was performed on adult male and female (2-months old) WT SD rats as described above (n=10 vehicle; n=16 treatment). Neuropathic pain was evaluated 10 days post SNI surgery prior to AAV9 treatment to set a baseline. On day 11, the rats were treated with the indicated AAV9 vector (at indicated dose) via IT injection. Neuropathic pain was then evaluated weekly from D25 to D60 post SNI surgery using mechanical sensitivity that was assessed using the von Frey test (described above). As shown in the top panel, all three pri-miRNAs showed rescue of mechanical allodynia in male rats, with miR-100-69 at 1E12 vg/animal showing the strongest effect. Rescue was as effective as gabapentin treatment (IP injection, 100 mg/kg, 1 hour pre-assessment). In contrast, only miR-E-68 (1E12 dose) showed rescue in female rats (bottom panel).

[00356] Figure 11B shows results of the hot plate tests. While the baseline phenotype only emerged D39 post dosing, we were able to observe rescue in both males and females across all test articles and doses, showing equivalent rescue as treatment with Gabapentin.

[00357] It is noted that the sex differences in pain sensitivity across different phenotypes observed in the experiments above, especially mechanical allodynia, aligns with literature findings (see, e.g., Pharmacol Res. 2018 Nov 1;139:1–16 and Nat Neurosci. 2015 Jun 29;18(8):1081–1083). The ability of miR-E-68 to rescue mechanical allodynia in female rats is noteworthy.

[00358] Molecular analyses were performed on DRG tissues (Lumbar L3-L6; Thoracic T10-T12; Cervical C6-C8) collected from animals after D67 posted SNI surgery. Figure 12A shows viral copy number (VCN) per diploid genome as determined by ddPCR comparing viral genome DNA counts to an endogenous housekeeping gene. Figure 12B shows *Scn9a* mRNA levels in the harvested DRG tissues, which were quantified using ddPCR, normalized to a housekeeping gene, and compared to vehicle controls. Each data point in Figures 12A and 12B represents an individual animal (n=8 per group). Statistical significance was assessed using one-way ANOVA with Dunnett's post-hoc test ((*$p<0.05$; **$p<0.01$; ****$p < 0.0001$).

[00359] The strongest *Scn9a* knockdown for all constructs was in the Lumbar DGR tissue of each rat, with the 1E12 doses showing the strongest knockdown. All pri-miRNAs tested showed significant knockdown of SCN9A. The *Scn9a* knockdown observed correlates well with the VCN data.

EXAMPLE 12

IN VITRO NOCICEPTOR-SELECTIVE REGULATORY ELEMENT SCREEN

[00360] Regions from the mouse and human SCN10A gene (encoding Nav1.8) were selected to assess their nociceptive-selective expression activity (as promoter and/or enhancer elements). These regions are set forth in SEQ ID NOs:451-467, with their corresponding genomic locations shown in Table 9 below (the SEQ ID NOs are in 5' to 3' orientation with respect to the direction of transcription of the SCN10A gene in each genome).

TABLE 9

SEQ ID NO	Description	Genomic and location (nucleotides)
451	Mouse promoter region	Mus musculus strain C57BL/6J chromosome 9, GRCm39: 119548095 to 119551493
452	Possible response element in SEQ ID NO: 451	Mus musculus strain C57BL/6J chromosome 9, GRCm39: 119549695 to 119549794
453	Human promoter region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38816207 to 38819604
454	Short Core Promoter (inside SEQ ID NO:453)	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38816042 to 38816601
455	Long Core Promoter (inside SEQ ID NO:453)	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38816042 to 38817161
456	Possible intronic enhancer region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38815666 to 38816041
457	Possible intronic enhancer region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38814527 to 38815096
458	Possible intronic enhancer region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38809959 to 38810668
459	Possible intronic enhancer region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38806479 to 38806941
460	Possible intronic enhancer region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38794043 to 38794240
461	Combination of SEQ ID NOs: 456 to 460	See SEQ ID NOs: 456 to 460
462	Possible enhancer region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38738506 to 38739123

463	Possible enhancer region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38805250 to 38806780
464	Possible enhancer region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38725531 to 38726460
465	Possible enhancer region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38723641 to 38724930
466	Combination of SEQ ID NOs:464, 465, and 455	See SEQ ID NOs:464, 465, and 455
467	Possible enhancer region	Homo sapiens chromosome 3, GRCh38.p14 Primary Assembly: 38828592 to 38830159

[00361] iPSC Sensory Neurons were plated in 96 well plate and treated at DIV6 with 1E4 MOI AAVDJ vectors expressing miR-E-68 under the transcriptional control of one the following sequences/sequence combinations: Syn1 (SEQ ID NO:449), EF1α (SEQ ID NO:450), SEQ ID NO: 451, SEQ ID NO:453, SEQ ID NO:452 + SEQ ID NO:453, SEQ ID NO:454, SEQ ID NO:455, SEQ ID NO:455 + SEQ ID NO:461, SEQ ID NO:462 + SEQ ID NO:463 + SEQ ID NO:455, SEQ ID NO:466, and SEQ ID NO:467 + 462 + 455. Cells were lysed on DIV21 using ThermoFisher Cells-to-Ct kit and used for cDNA generation using Cells-to-Ct reagents according to manufacturer's protocols. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample and Taqman probes were used for target detection (SCN9A transcript). Normalized endogenous SCN9A transcript expression was calculated by dividing SCN9A transcript copies by GAPDH transcript copies. Relative SCN9A transcript expression was then calculated by dividing treated sample expression by unmanipulated samples.

[00362] As shown in Figure 13, Syn1 and EF1a regulatory elements operably linked to an miR-E-68 encoding transgene showed significant SCN9A knockdown, indicating their ability to drive expression of the miR-E-68 RNA in sensory neurons. Several of the sequences from the mouse and human SCN10A gene were able to knockdown SCN9A, with SEQ ID NO:466 showing the highest activity, indicating that this combination of human SCN10A elements is able to drive expression of an operably linked transgene in sensory neurons.

[00363] iPSC sensory neurons (iSensory) and glutamatergic neurons (iGluta) were transduced on DIV6 with AAVDJ vectors expressing miR-E-68 or miR-100-69 under the control of the Syn1 promoter (SEQ ID NO:449) or SEQ ID NO:466 for 2 weeks (MOI were between 1E2 to 1E5). Following incubation, the cells were lysed and RNA was extracted using ThermoFisher cell-to-ct kit. The expression levels of SCN9A mRNA were quantified using ddPCR and normalized to a

house keeping gene. A non-targeting scramble sequence miRNA was used as a control for each treatment group. All conditions were further normalized to scramble. As shown in Figure 14, SEQ ID NO:466 performed equivalently to the Syn1 promoter in sensory neurons (top panel), knocking down SCN9A transcript levels by ~50%-60% (each point is a biological replicate; error bars are SEM). In glutamatergic neurons, however, SEQ ID NO:466 showed no SCN9A knockdown while Syn 1 was able to show ~50% knockdown activity (bottom panel). These results indicate that SEQ ID NO:466 has specificity for sensory neurons and is thus a nociceptor-selective regulatory element.

EXAMPLE 13

IN VIVO ACTIVITY OF NOCICEPTOR-SELECTIVE REGULATORY ELEMENT

[00364] Wild-type B6 mice were treated at P1 via IT injection with AAV9 vectors expressing miR-E-68 under the control of either Syn1 (SEQ ID NO:449) or SEQ ID NO:466 (nociceptor-selective regulatory element identified in Example 13). Tissue was harvested (DRG, cortex, and olfactory bulb) four weeks later for multiple molecular analyses ase detailed below.

[00365] A subset of tissue was used for RNA extraction and miRNA and SCN9A transcript levels quantification (using ddPCR, normalized to a housekeeping gene, and compared to vehicle controls). Each data point represents an individual animal (n=5 per group). Figure 15, top panel, shows miRNA abundance in DRG, cortex (CTX), and olfactory bulb tissues for miR-E-68 under the control of Syn1 or SEQ ID NO:466. While Syn1 shows equivalent expression levels in all three assayed CNS tissues, SEQ ID NO:466 shows strong selective expression of miR-E-68 in DRG tissue, which includes significant numbers of nociceptors, while exhibiting very low levels of expression in cortex and olfactory bulb tissues. Figure 15, lower panel, shows that both Syn1 and SEQ ID NO:466 expression constructs were able to knockdown endogenous *Scn9a* mRNA in DRG tissue. Statistical significance was assessed using one-way ANOVA with Dunnett's post-hoc test (*$p<0.05$; **$p<0.01$; ****$p < 0.0001$).

[00366] Harvested DRG tissue was also used for assessing miRNA distribution expression using ISH signal for SEQ ID NO:68, the processed miR derived from miRNA miR-E-68 pri-miRNA expressed from the AAV vectors. Figure 16 shows the SEQ ID NO:68 distribution was quantified on a per cell basis and the distribution is shown of the number of ISH copies across DRG neuron area. Histograms are normalized for comparison purposes. The distribution of SEQ

ID NO:68 expressed from SEQ ID NO:466 is biased towards smaller cell sizes suggestive of cell specificity for small-sized nociceptive neurons.

[00367] IHC co-staining was performed to localize SEQ ID NO:68 expression pattern in DRG using peripherin, a marker for small sized unmyelinated nociceptive neuron, and NF200, a marker for large sized myelinated non-nociceptive neurons. Figure 17 shows that the number of copies of SEQ ID NO:68 (the miRNA) is higher in small fiber neurons (peripherin positive) when operably linked to SEQ ID NO:466 as compared to when operably linked to Syn1 (left panel). Conversely, the number of copies of SEQ ID NO:68 (the miRNA) is lower in large fiber neurons (NF200 positive) when operably linked to SEQ ID NO:466 as compared to when operably linked to Syn1 (right panel). (*$p<0.05$; **$p<0.01$) This data further confirms the nociceptor-selectivity of SEQ ID NO:466.

EXAMPLE 14

NOCICEPTOR-SELECTIVE REGULATORY ELEMENT TEST IN NON-NEURONAL TISSUE

[00368] Nociceptor-selective regulatory element (SEQ ID NO:466) was tested for its activity in a non-neuronal cell line (cardiomyocytes; Fujifilm Cellular Dynamics, Cat# 01434) as compared to a CBA promoter. A 96 well plate was precoated with 0.1% gelatin and cells were plated at 50,000 cells/well. 100% of the culture media was replaced at after 24 hours. On DIV4, AAVDJ vectors expressing EGFP-KASH operably linked to CBA promoter (SEQ ID NO:479) or SEQ ID NO:466 were added to the respective wells at MOIs of: 1E3, 1E4, or 1E5. Cells were maintained according to manufacturer's protocol, with 100% media replaced every 2-3 days, until they were harvested at DIV11 (7-day transduction). Cells were lysed using ThermoFisher Cells-to-Ct kit and lysate used for cDNA generation using Cells-to-Ct reagents according to manufacturer's protocols. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample and Taqman probes were used for target detection (GFP Probe Assay ID: APH6FJC; GAPDH Probe Assay ID: Hs99999905_m1). Normalized EGFP expression was calculated by dividing EGFP transcript copies by GAPDH transcript copies for each sample. Relative EGFP expression was then calculated using CBA-treated samples at the lowest MOI of 1E3 set to a value of 1.0. As shown in Figure 18, GFP expression was virtually undetectable from AAVDJ vectors in which its expression was controlled by SEQ ID NO:466, while expression from the CBA promoter showed the expected dose dependent activity. This result

further establishes the selectivity of SEQ ID NO:466, i.e., it does not promote expression of an operably linked transgene in non-nociceptor cells, including non-neuronal cells (in this case cardiomyocytes).

CLAIMS

What is claimed is:

1. A polynucleotide comprising a targeting region that binds to a target site in an endogenous mRNA encoding $Na_v1.7$, wherein the target site is from 10 to 30 nucleotides in length and comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 1 to 59.

2. The polynucleotide of claim 1, wherein the endogenous mRNA encoding $Na_v1.7$ is transcribed from an endogenous SCN9A gene.

3. The polynucleotide of claim 1, wherein the endogenous mRNA encoding $Na_v1.7$ comprises a sequence having at least 95% sequence identity to SEQ ID NO: 305.

4. The polynucleotide of any one of claims 1 to 3, wherein the target site comprises a sequence of any one of SEQ ID NOs: 1 to 59.

5. The polynucleotide of any one of claims 1 to 4, wherein the targeting region is at least 90% complementary to the target site.

6. The polynucleotide of any one of claims 1 to 4, wherein the targeting region is at least 95% complementary to the target site.

7. The polynucleotide of any one of claims 1 to 4, wherein the targeting region is complementary to the target site, with the optional exception of 1, 2, 3 or 4 mismatches.

8. The polynucleotide of any one of claims 1 to 4, wherein the targeting region comprises a sequence having at least 90% sequence identity to any one of SEQ ID NOs: 61 to 119.

9. The polynucleotide of any one of claims 1 to 4, wherein the targeting region comprises a sequence having at least 95% sequence identity to any one of SEQ ID NOs: 61 to 119.

10. The polynucleotide of any one of claims 1 to 4, wherein the targeting region comprises any one of SEQ ID NOs: 61 to 119.

11. The polynucleotide of any one of claims 1 to 10, wherein the polynucleotide is selected from the group consisting of: a pri-miRNA, a pre-miRNA, a mature miRNA, an siRNA, an shRNA, and an antisense oligonucleotide.

12. The polynucleotide of claim 11, wherein the polynucleotide comprises: at least one modified internucleoside linkage; at least one modified nucleoside; at least two different nucleoside residues selected from DNA, RNA, and arabino nucleic acid; or any combination thereof.

13. The polynucleotide of claim 11, wherein the polynucleotide is a pri-miRNA, wherein the pri-miRNA comprises a miRNA scaffold, a guide RNA sequence selected from any one of SEQ ID NOs: 61 to 119, and a passenger RNA sequence.

14. The polynucleotide of claim 11, wherein the miRNA scaffold is selected from the group consisting of: miR-E, miR-100, miR-130a, miR-132, miR-190a, miR-190, and miR-451.

15. The polynucleotide of claim 14, wherein the miRNA scaffold is an miR-E scaffold, wherein the pri-miRNA comprises: an optional 5' flanking sequence of SEQ ID NO:307, a 5' stem of SEQ ID NO:308, the passenger RNA sequence; a loop of SEQ ID NO:309, the guide RNA sequence, a 3' stem of SEQ ID NO:310, and an optional 3' flanking sequence of SEQ ID NO:311.

16. The polynucleotide of claim 15, wherein the guide RNA sequence is SEQ ID NO: 68, 61, or 69.

17. The polynucleotide of claim 16, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 369, 368, and 367.

18. The polynucleotide of claim 14, wherein the miRNA scaffold is an miR-100 scaffold, wherein the pri-miRNA comprises: an optional 5' flanking sequence of SEQ ID NO:312, a 5' stem of SEQ ID NO:313, the guide RNA sequence; a loop of SEQ ID NO:314, the passenger RNA sequence, a 3' stem of SEQ ID NO:315, and an optional 3' flanking sequence of SEQ ID NO:316.

19. The polynucleotide of claim 18, wherein the guide RNA sequence is SEQ ID NO: 69, 61, or 68.

20. The polynucleotide of claim 19, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 370, 371, and 372.

21. The polynucleotide of claim 14, wherein the miRNA scaffold is an miR-130a scaffold, wherein the pri-miRNA comprises: an optional 5' flanking sequence of SEQ ID NO:317, a 5' stem of SEQ ID NO:318, the passenger RNA sequence; a loop of SEQ ID NO:319, the guide RNA sequence, a 3' stem of SEQ ID NO:320, and an optional 3' flanking sequence of SEQ ID NO:321.

22. The polynucleotide of claim 21, wherein the guide RNA sequence is SEQ ID NO: 69, 61, or 68.

23. The polynucleotide of claim 22, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 373, 374, and 375.

24. The polynucleotide of claim 14, wherein the miRNA scaffold is an miR-132 scaffold, wherein the pri-miRNA comprises: an optional 5' flanking sequence of SEQ ID NO:322, a 5' stem of SEQ ID NO:323, the passenger RNA sequence; a loop of SEQ ID NO:324, the guide RNA sequence, a 3' stem of SEQ ID NO:325, and an optional 3' flanking sequence of SEQ ID NO:326.

25. The polynucleotide of claim 24, wherein the guide RNA sequence is SEQ ID NO: 69, 61, or 68.

26. The polynucleotide of claim 25, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 376, 377, and 378.

27. The polynucleotide of claim 14, wherein the miRNA scaffold is an miR-190 scaffold, wherein the pri-miRNA comprises: an optional 5' flanking sequence of SEQ ID NO:327, a 5' stem of SEQ ID NO:328, the guide RNA sequence; a loop of SEQ ID NO:329, the guide RNA sequence, a 3' stem of SEQ ID NO:331, and an optional 3' flanking sequence of SEQ ID NO:332.

28. The polynucleotide of claim 27, wherein the guide RNA sequence is SEQ ID NO: 69, 61, or 68.

29. The polynucleotide of claim 14, wherein the miRNA scaffold is an miR-190a scaffold, wherein the pri-miRNA comprises: an optional 5' flanking sequence of SEQ ID NO:327, a 5' stem of SEQ ID NO:328, the guide RNA sequence; a loop of SEQ ID NO:330, the passenger RNA sequence, a 3' stem of SEQ ID NO:331, and an optional 3' flanking sequence of SEQ ID NO:332.

30. The polynucleotide of claim 29, wherein the guide RNA sequence is SEQ ID NO: 69, 61, or 68.

31. The polynucleotide of claim 30, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 379, 380, and 381.

32. The polynucleotide of claim 14, wherein the miRNA scaffold is an miR-451 scaffold, wherein the pri-miRNA comprises: an optional 5' flanking sequence of SEQ ID NO:333, a 5' stem of SEQ ID NO:334, the guide RNA sequence; the passenger RNA sequence, a 3' stem of SEQ ID NO:335, and an optional 3' flanking sequence of SEQ ID NO:336.

33. The polynucleotide of claim 32, wherein the guide RNA sequence is SEQ ID NO: 69, 61, or 68.

34. The polynucleotide of claim 33, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 382, 383, and 384.

35. An expression cassette comprising a regulatory element operably linked to a transgene encoding an RNA, wherein the RNA comprises the polynucleotide of any one of claims 1 to 11.

36. The expression cassette of claim 35, wherein the regulatory element is a constitutive promoter.

37. The expression cassette of claim 36, wherein the is a constitutive promoter is a U6 promoter or an EF1a promoter .

38. The expression cassette of claim 35, wherein the regulatory element is a nociceptor-selective promoter comprising: (i) a sequence of SEQ ID NO:466, (ii) a functional fragment thereof, or (iii) a sequence with at least 80% sequence identity to (i) or (ii).

39. The expression cassette of claim 36, wherein the promoter is a CNS selective promoter.

40. The expression cassette of claim 39, wherein the CNS selective promoter is a Syn1 promoter.

41. The expression cassette of claim 35, wherein the expression cassette comprises SEQ ID NO: 433, 436, 438, 441, 443, or 446.

42. A vector comprising the expression cassette of any one of claims 35 to 41.

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

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

45. The vector of claim 44, wherein the viral vector is an adeno-associated virus (AAV) vector.

46. The vector of claim 45, wherein the vector further comprises AAV inverted terminal repeat (ITR) sequences flanking the expression cassette.

47. The vector of claim 46, wherein each of the AAV ITR sequences are selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV-DJ, variants thereof, or hybrids thereof.

48. The vector of claim 45 or 46, wherein the AAV vector is an scAAV vector.

49. The vector of any one of claims 45 to 48, wherein the AAV vector comprises a stuffer sequence, wherein the stuffer sequence is positioned between the AAV ITR sequences.

50. The vector of claim 49, wherein the stuffer sequence comprises any one or any combination of SEQ ID NOs:473 to 478

51. The vector of any one of claims 45 to 50, wherein the vector comprises any one of SEQ ID NOs: 434, 435, 437, 439, 440, 442, 444, 445, and 447.

52. The vector of any one of claims 45 to 51, wherein the vector further comprises an AAV capsid.

53. The vector of claim 52, wherein the capsid is selected from: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV-DJ, variants thereof, or hybrids thereof.

54. The vector of claim 52, wherein the AAV capsid has enhanced tropism for CNS cells and/or crosses the blood-brain barrier.

55. The vector of claim 54, wherein the AAV capsid that has enhanced tropism for CNS cells and/or crosses the blood-brain barrier is selected from: bCap1, AAV-B1, AAV-S, AAV-TT, VCAP-101, and VCAP-102.

56. The vector of claim 44, wherein the viral vector is a lentiviral vector.

57. A pharmaceutical composition comprising: (i) the polynucleotide of any one of claims 1 to 34, the expression cassette of any one of claims 35 to 41, or the vector of any one of claims 42 to 56, and (ii) a pharmaceutically acceptable carrier.

58. A method of reducing expression of a gene encoding $Na_v1.7$ in a cell, comprising contacting the cell with an effective amount of the polynucleotide of any one of claims 1 to 34, the expression cassette of any one of claims 35 to 41, the vector of any one of claims 42 to 56, or the pharmaceutical composition of claim 57.

59. The method of claim 58, wherein expression of the mRNA encoding $Na_v1.7$ and/or the Nav1.7 protein encoded by the mRNA in the contacted cell is reduced compared to a comparable cell not contacted with the polynucleotide of any one of claims 1 to 34, the expression cassette of any one of claims 35 to 41, the vector of any one of claims 42 to 56, or the pharmaceutical composition of claim 57.

60. The method of claim 59, wherein the expression of the mRNA encoding $Na_v1.7$ and/or the Nav1.7 protein encoded by the mRNA is reduced at least a 5% in the contacted cell compared to the comparable non-contacted cell.

61. The method of claim 59, wherein the expression of the mRNA encoding $Na_v1.7$ and/or the Nav1.7 protein encoded by the mRNA is reduced at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% in the contacted cell compared to the comparable non-contacted cell.

62. The method of any one of claims 58 to 61, wherein the expression of the mRNA encoding $Na_v1.7$ is measured by quantitative polymerase chain reaction.

63. The method of any one of claims 58 to 62, wherein the cell is a cultured cell.

64. The method of any one of claims 63, wherein the cultured cell is a primary neuron, an iPSC derived neural cell, a neuronal cell line, an engineered cell line, or a neural stem cell.

65. The method of any one of claims 58 to 62, wherein the cell is in vivo.

66. The method of claim 65, wherein the cell is a neuron.

67. The method of claim 66, wherein the cell is a neuron in the central nervous system (CNS), or a cell in contact with cerebral spinal fluid.

68. The method of any one of claims 65 to 67, wherein the contacting comprises delivering the polynucleotide of any one of claims 1 to 34, the expression cassette of any one of claims 35 to 41, the vector of any one of claims 42 to 56, or the pharmaceutical composition of claim 24 to the CNS or cerebral spinal fluid (CSF).

69. The method of claim 68, wherein the delivering comprises intraparenchymal injection, intrathecal injection, intra-cisterna magna injection, or intracerebroventricular injection.

70. A method for treating or preventing neuropathic pain in a subject comprising administering a therapeutically or prophylactically effective amount of the pharmaceutical composition of claim 57 to a subject.

71. The method of claim 70, wherein the subject is a mammal.

72. The method of claim 71, wherein the mammal is a human.

73. The method of any one of claims 70 to 72, wherein the method comprises administering the pharmaceutical composition to the subject via intraparenchymal injection, intrathecal injection, intra-cisterna magna injection, intravenous injection, or intracerebroventricular injection.

74. A use of the polynucleotide of any one of claims 1 to 34, the expression cassette of any one of claims 35 to 41, the vector of any one of claims 42 to 56, or the pharmaceutical composition of claim 57, in the manufacture of a medicament for the treatment of neuropathic pain.

75. A cell comprising the polynucleotide of any one of claims 1 to 34, the expression cassette of any one of claims 35 to 41, or the vector of any one of claims 42 to 56.

76. The cell of claim 75, wherein the cell comprises the viral vector of any one of claims 44 to 56.

77. A method of producing a composition comprising viral particles, comprising: (i) culturing the cell of claim 76 under conditions to produce viral particles, and (ii) isolating the viral particles from the cells in the culture, thereby producing a composition comprising viral particles.

78. The method of claim 77, wherein the viral particles are AAV particles.

79. A nucleic acid expression cassette comprising a regulatory element operably linked to a transgene that results in selective expression of the transgene in nociceptor cells over one or more non-nociceptor cells, wherein the regulatory element comprises (i) a sequence of SEQ ID NO:466, (ii) a functional fragment thereof, or (iii) a sequence with at least 80% sequence identity to (i) or (ii).

80. The nucleic acid expression cassette of claim 79, wherein the nociceptor cells are in a region of the central nervous system (CNS).

81. The nucleic acid expression cassette of claim 79 or 80, wherein the regulatory elements result in selective expression in nociceptors cells that is about 1.5 times, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 7.5 times, 8 times, 9 times, or 10 times higher than expected for natural distribution of nociceptor cells in the CNS.

82. The nucleic acid expression cassette of claim 80, wherein the region of the CNS is a dorsal root ganglion (DRG).

83. The nucleic acid expression cassette of claim 80, wherein the nociceptor cells are small sized unmyelinated nociceptive neurons that co-express peripherin.

84. The nucleic acid expression cassette of any one of claims 79-83, wherein the transgene encodes an RNAi agent with a targeting region specific for a transcript from a gene of interest.

85. The nucleic acid expression cassette of claim 84, wherein the RNAi agent is a pri-miRNA.

86. The nucleic acid expression cassette of claim 85, wherein the gene of interest is SCN9A.

87. The nucleic acid expression cassette of any one of claims 84 to 86, wherein the targeting region is selected SEQ ID NOs: 61-119.

88. A vector comprising the expression cassette of any one of claims 80-87.

89. The vector of claim 88, wherein the vector is a plasmid.

90. The vector of claim 88, wherein the vector is a viral vector.

91. The vector of claim 90, wherein the viral vector is an adeno-associated virus (AAV) vector.

92. A method of increasing selective expression of a transgene in nociceptor cells, comprising generating a nucleic acid expression cassette of any one of claims 80-87 and contacting a cell with the nucleic acid expression cassette.

93. A method of treating a neurological condition or disorder in a subject in need thereof, the method comprising contacting a cell with a nucleic acid cassette of any one of claims 80-87 or the vector of any one of claim 88-91.