METHODS AND COMPOSITIONS FOR REDUCING EXPRESSION OF TAU

CROSS-REFERENCING

[0001] This application claims the benefit of U.S. provisional application serial no. 63/574,847 filed on April 4, 2024, which application in incorporated by reference in its entirety.

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-011WO_SEQLIST" created on April 3, 2025, and having a size of 498,306 bytes. The contents of the Sequence Listing XML are incorporated by reference herein in their entirety.

BACKGROUND

[0003] A common pathological hallmark of neurodegenerative disease is the misfolding and deposition of specific proteins into insoluble proteinaceous deposits in the CNS accompanied by a progressive loss of neurons in the affected regions. Tauopathies are a group of neurodegenerative disorders that are broadly defined by the aggregation of hyperphosphorylated, filamentous Tau protein, the most common of which is Alzheimer's Disease (AD). While AD is characterized by the aggregation of both β-amyloid as plaques and hyperphosphorylated Tau as neurofibrillary tangles (NFTs), only NFT pathology closely correlates with cognitive decline. Tau fibrils stably propagate and spread Tau pathology transcellularly from different brain regions, depending on the disease (Braak et al. Acta Neuropathol 82, 239-259, doi:10.1007/bf00308809 (1991); Braak et al, Acta Neuropathol. 2011 121, 589-595; Irwin et al, Parkinsonism Related Disorders 2016 22: S29-33).

[0004] Though the MAPT gene encoding tau is not genetically linked to AD, mutations in MAPT cause other tauopathies such as Frontotemporal Dementia and Parkinsonism Linked to Chromosome 17 (FTDP-17) showing that disrupting Tau homeostasis is sufficient to cause neurodegeneration.

[0005] Currently, there are no approved therapies for tauopathies that significantly slow disease progression. Therefore, there remains a great need for therapeutics for the treatment of tauopathies.

SUMMARY

[0006] The present disclosure provides methods and compositions for the treatment of Tauopathies, including Alzheimer's Disease (AD) and Frontotemporal Dementia and Parkinsonism Linked to Chromosome 17 (FTDP-17), by inhibiting expression of the MAPT gene. Aspects of the disclosure provide engineered inhibitory RNA agents, e.g., siRNA, miRNA, and antisense oligonucleotides, that knock down endogenous mutated or wild-type MAPT transcripts in neuronal cells in a subject. Expression vectors encoding the engineered inhibitory RNA agents, e.g., in an AAV vector, are also provided.

[0007] These and other aspects will be described in greater detail below.

INCORPORATION BY REFERENCE

[0008] 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

[0009] 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:

[0010] **Figure 1** is a graph showing knock down of exogenously-provided MAPT 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: 99 represents a negative control sequence that does not bind to any target site in the MAPT transcript (scrambled, or "SCRM").

[0011] **Figure 2** is a graph showing knock down of endogenous MAPT transcript in iPSC GABAergic neurons by miRNA candidates described herein as compared to the 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: 98 represents a positive

control sequence and SEQ ID NO: 99 is a negative control sequence that does not bind to any target site in the MAPT transcript (scrambled, or "SCRM").

[0012] **Figure 3** is a graph showing knock down of endogenous MAPT transcript in iPSC glutamatergic neurons by miRNA candidates described herein as compared to the 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: 99 represents a negative control sequence that does not bind to any target site in the MAPT transcript (scrambled, or "SCRM").

[0013] **Figure 4A and 4B** are graphs showing relative endogenous MAPT mRNA expression (top panels) and relative endogenous Tau protein expression (bottom panels) in the hippocampus (Figure 4A) and Cortex (Figure 4B) of C57BL/6 mice at 4 weeks and 8 weeks post ICV treatment with AAV9 vectors that express pri-miRNAs with a miR-E scaffold and the indicate guide sequence (SEQ ID NOs: 50, 51, 53, or 55). Horizontal dotted lines in each graph indicate the levels of MAPT mRNA and Tau protein in PBS treated mice that were used to set baseline expression (i.e., 1.0).

[0014] **Figure 5** provides tables showing the percent knockdown (%KD) of endogenous MAPT mRNA expression (compared to untreated controls) in iPSC derived GABAergic cells treated with 1E4 (low level; top panel) or 1E6 (high level; bottom panel) of AAVDJ vectors that express pri-miRNAs with the indicated scaffold/guide combinations (scaffold names listed at the bottom of each table; guide SEQ ID NOs listed at the left of each panel). ND = not done.

[0015] **Figure 6** provides graphs showing relative endogenous MAPT mRNA expression (top panel) and relative endogenous Tau protein expression (bottom panel) in the hippocampus of C57BL/6 mice at 4 weeks and 8 weeks post ICV treatment with AAV9 vectors that express pri-miRNAs with the indicated scaffold/guide combinations, which are indicated at the bottom of each graph (scaffold names and guide sequence SEQ ID NOs are used). Horizontal dotted lines in each graph indicate the levels of MAPT mRNA and Tau protein in PBS treated mice that were used to set baseline expression (i.e., 1.0).

[0016] **Figure 7** provides graphs showing body weight in grams (top panel) and hind limb clasping score (bottom panel) in a subset of mice from Figure 6 treated with the indicated scaffold/guide combinations (listed at the bottom of each graph) as compared to PBS treated mice (vehicle).

[0017] **Figure 8** provides graphs showing differential expression analysis (volcano plots) in human iPSC-derived GABAergic neurons treated with AAVDJ vectors that express pri-miRNAs having the indicated scaffold/guide combinations: miR-130a-51 (SEQ ID NO:331), miR-100-50 (SEQ ID NO:316), and miR-100-55 (SEQ ID NO:317). AAVDJs with a scrambled guide sequence in each corresponding scaffold were used as controls. MAPT is indicated with an "x" in the volcano plots and shows significantly reduced expression.

[0018] **Figure 9** provides graphs showing viral copy number (VCN; top panel) and relative human MAPT mRNA expression (bottom panel) as compared to PBS controls in the cortex, hippocampus, and hindbrain of *htau* transgenic mice treated intravenously (IV) with PHP.eb AAV vectors that express the indicated scaffold/guide pri-miRNAs: miR-130a-51 (SEQ ID NO:331) and miR-100-50 (SEQ ID NO:316). The *htau* transgenic mice used in these experiments lack endogenous mouse MAPT and express all six isoforms of human MAPT (Andorfer et al, J Neurochem 86(3):582-90; Jackson Lab Stock #005491).

[0019] **Figure 10** provides graphs showing relative human Tau protein expression as compared to PBS controls (vehicle) in the cortex (left panel), hippocampus (center panel), and hindbrain (right panel) of *htau* transgenic mice treated intravenously (IV) with PHP.eB AAV vectors that express the indicated scaffold/guide pri-miRNAs: miR-130a-51 (SEQ ID NO:331) and miR-100-50 (SEQ ID NO:316). Data is from the same mice shown in Figure 9.

[0020] **Figure 11A** provides a table showing relative endogenous MAPT mRNA expression levels in the indicated brain tissues of cynomolgus monkeys (non-human primate, NHP) treated with AAV9 vectors that express the indicated scaffold/guide pri-miRNAs: miR-130a-51 (SEQ ID NO:331), miR-100-50 (SEQ ID NO:316), and miR-100-55 (SEQ ID NO:317). The AAV9 vectors were introduced via unilateral intracerebroventricular injection (ICV; 1E14vg/animal) and multiple cortical intraparenchymal injections (IP; total of 1.2E12vg/animal). Frontal cortex, amygdala, and thalamus data are from the left hemisphere only. The remaining data are the average of left and right hemispheres per region. Statistical comparisons by t-test: * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; **** = $P \leq 0.0001$.

[0021] **Figure 11B** provides a table showing relative endogenous Tau protein expression levels in the indicated brain tissues of cynomolgus monkeys (non-human primate, NHP) treated with AAV9 vectors that express the indicated scaffold/guide pri-miRNAs: miR-130a-51 (SEQ ID NO:331), miR-100-50 (SEQ ID NO:316), and miR-100-55 (SEQ ID NO:317). The AAV9

vectors were introduced via unilateral intracerebroventricular injection (ICV; 1E14vg/animal) and multiple cortical intraparenchymal injections (IP; total of 1.2E12vg/animal). Frontal cortex, amygdala, and thalamus data are from the left hemisphere only. The remaining data are the average of left and right hemispheres per region.

[0022] **Figure 12** provides graphs showing endogenous MAPT mRNA levels in iPSC derived GABAergic neurons infected with AAVDJ vectors expressing the indicated pri-miRNAs having either single stranded AAV (ssAAV) or self-complementary AAV (scAAV) genomes as compared to control AAVDJ vectors having scrambled guide sequences. Infections were performed at MOIs of 1E2, 1E3, and 1E4. SEQ ID NOs for the complete genomes for each ssAAV and scAAV are as follows: ssAAV miR-130a-51 is SEQ ID NO:419, scAAV miR-130a-51 is SEQ ID NO:424 (left panel); ssAAV miR-100-50 is SEQ ID NO:417, scAAV miR-100-50 is SEQ ID NO:422 (center panel), ssAAV miR-100-55 is SEQ ID NO:421, scAAV miR-100-55 is SEQ ID NO:423 (right panel). **** = $P \leq 0.0001$

[0023] **Figure 13** provides a graph showing relative endogenous MAPT expression in iPSC derived GABAergic neurons for AAVDJ vectors expressing (i) a pri-miRNA having an miR-E scaffold and a guide having SEQ ID NO: 50 guide sequence, and (ii) multiple different 3' variants of SEQ ID NO:50 (SEQ ID NOs: 398 to 406, listed at the bottom of the graph). Data shown is relative to an AAVDJ with an miR-E scaffold and a scramble guide (SEQ ID NO:99).

[0024] **Figures 14A and 14B** provide metrics of processed miRNAs expressed in hippocampus tissues from mice treated with AAVs expressing miR-130a-51 (SEQ ID NO:331), miR-100-50 (SEQ ID NO:316), or miR-100-55 (SEQ ID NO:317). Figure 14A shows the guide/passenger ratio of miRNA processed from the indicated AAV vectors in hippocampus tissue. Figure 14B, top panel, shows the 5' processing precision of the processed miRNA expressed from the indicated AAVs in hippocampus tissue. Figure 14B, bottom panel, shows the proportion of guide sequences (1.0 = 100%) having the indicated bp length (indicted at the bottom of the table) of miRNAs expressed from the indicated AAVs (a value of (-) indicates a value less than 0.01).

DEFINITIONS

[0025] 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".

[0026] 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).

[0027] 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.

[0028] 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".

[0029] 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.

[0030] 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.

[0031] 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.

[0032] 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.

[0033] 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.

[0034] 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.

[0035] 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.

[0036] 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.

[0037] 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.

[0038] 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.

[0039] The term "*in vivo*" refers to an event that takes place in a subject's body.

[0040] 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.

[0041] 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.

[0042] 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.

[0043] 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.

[0044] 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.

[0045] 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).

[0046] 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.

[0047] 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.

[0048] 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.

[0049] 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.

[0050] 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.

[0051] 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.

[0052] As used herein the term "MAPT gene" refers to the microtubule-associated protein tau gene, which, in humans, is identified as Gene ID: 4137 in NCBI's Genbank database. This gene may be referred to as TAU, MSTD, PPND, DDPAC, MAPTL, MTBT1, MTBT2, tau-40, FTDP-17, PPP1R103 or Tau-PHF6 in other disclosures. The MAPT gene encodes Tau protein. Mutations in the MAPT gene mutations have been associated with several neurodegenerative disorders such as Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy.

[0053] 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.

[0054] 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

[0055] 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.

[0056] 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.

[0057] 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.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] As summarized above, the present disclosure provides methods and compositions for the treatment of Tauopathies, including Alzheimer's Disease (AD) and Frontotemporal Dementia

and Parkinsonism Linked To Chromosome 17 (FTDP-17). The methods and compositions of the present disclosure comprise isolated nucleic acid molecules, recombinant adeno-associated virus (rAAV) vectors and rAAV viral vectors comprising polynucleotide sequences encoding for artificial micro RNAs (amiRNAs) directed to MAPT.

[0062] Provided herein is a polynucleotide comprising a targeting region that binds to a target site in an endogenous mRNA encoding Tau, 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 48. In these embodiments, the endogenous mRNA is a transcript of an endogenous MAPT gene. In some embodiments, the endogenous mRNA encoding Tau may comprise the sequence having at least 95% sequence identity to SEQ ID NO: 249. In some embodiments, the target site may comprise the sequence of any one of SEQ ID NOs: 1 to 48.

[0063] 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.

[0064] 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: 50-97.

[0065] In any embodiment, the polynucleotide may comprise: 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.

[0066] 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.

[0067] 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.

[0068] In any embodiment, the polynucleotide may be 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.

[0069] 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.

[0070] 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.

[0071] 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.

[0072] 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).

[0073] 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

[0074] 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

[0075] 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

[0076] 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.

[0077] In some embodiments, a pri-miRNA may comprise an RNA of the present disclosure (e.g., any of SEQ ID NOS: 50-97, 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).

[0078]

[0079] 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

[0080] 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.

[0081] 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 48 in SEQ ID NO:249 (MAPT 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: 272 and miR-190a uses a loop sequence SEQ ID NO:273.

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	250	280
	5' Stem	GACAGUGAGCG	251	281
	Passenger			
	Loop	UAGUGAAGCCACAGAUGUA	252	282
	Guide			
	3' Stem	UGCCUACUGCC	253	283
	3' Flank	UCGGACUUCAAGGGGCUAGAAU UCGAGCAAUUAUCUUGUUUACU AAAACUGAAUACCUUGCUAUCUC UUUGAUACAUUAUUACAAAGCU GAAUUAAAAUGGUAUAAAUUAA AUCAC	254	284
miR-100	5' Flank	CCCAAAAGAGAGAAGAUAUUGA GG	255	285
	5' Stem	CCUGUUGCCACA	256	286
	Guide			
	Loop	GUAUUAGUCCG	257	287
	Passenger			
	3' Stem	UGUGUCUGUUAGG	258	288
	3' Flank	CAAUCUCACGGACCUGGGGCUUU GCUUAUAUGCC	259	289
130a	5' Flank	GCAGGGCCGGCAUGCCUC	260	290
	5' Stem	UGCUGCUGGCCA	261	291
	Passenger			
	Loop	CUGUCUGCACCUGUCACUAG	262	292
	Guide			
	3' Stem	GUGGCCGUGUAGUG	263	293

	3' Flank	CUACCCAGCGCUGGCUGCCUCCU CAGCAUUG	264	294
132	5' Flank	GCCGUCCGCGCGCC	265	295
	5' Stem	CCGCCCCCGCGUCUCCAGGG	266	296
	Passenger			
	Loop	CUGUGGGAACUGGAGG	267	297
	Guide			
	3' Stem	CCCCGCAGCACGCCCACGCGC	268	298
	3' Flank	CGCGCCACGCCGCGCCCCGAGCC	269	299
miR-190	5' Flank	GAGCUCAGUCAAACCUGGAUGCC UUUUC	270	300
	5' Stem	UGCAGGCCUCUGUG	271	301
	Guide			
	Loop	UGUUAUUUAAUCCAC	272	302
	Passenger			
	3' Stem	CUACAGUGUCUUGCC	274	304
	3' Flank	CUGUCUCCGGGGGUUCCUAAUAA AG	275	305
miR-190a	5' Flank	GAGCUCAGUCAAACCUGGAUGCC UUUUC	270	300
	5' Stem	UGCAGGCCUCUGUG	271	301
	Guide			
	Loop	UGUUAUUUAAUCCA	273	303
	Passenger			
	3' Stem	CUACAGUGUCUUGCC	274	304
	3' Flank	CUGUCUCCGGGGGUUCCUAAUAA AG	275	305
miR-451	5' Flank	CUCUGCUCAGCCUGUCACAACCU ACUGACUGCCAGGGCAC	276	306
	5' Stem	UUGGGAAUGGCAAGG	277	307
	Guide			
	Loop	*Loop is the four 3' nucleotides of the guide sequence*	NA	NA
	Passenger			
	3' Stem	UCUUGCUAUACCCAG	278	308
	3' Flank	AAAACGUGCCAGGAAGAGAACUC A	279	309

[0082] In some embodiments, the transgene may encode a pri-miRNA comprising:

(i) an optional 5' flanking sequence of SEQ ID NO: 250,

a 5' stem of SEQ ID NO: 251,

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: 252,

an RNA of the present disclosure, e.g., any of SEQ ID NOS: 50-97, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions,

a 3' stem of SEQ ID NO: 253, and

an optional 3' flanking sequence of SEQ ID NO: 254;

(ii) an optional 5' flanking sequence of SEQ ID NO: 255,

a 5' stem of SEQ ID NO: 256,

an RNA of the present disclosure, e.g., any of SEQ ID NOS: 50-97, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions,

a loop of SEQ ID NO: 257,

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: 258, and

an optional 3' flanking sequence of SEQ ID NO: 259;

(iii) an optional 5' flanking sequence of SEQ ID NO: 260,

a 5' stem of SEQ ID NO: 261,

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: 262,

an RNA of the present disclosure, e.g., any of SEQ ID NOS: 50-97, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions;

a 3' stem of SEQ ID NO: 263, and

an optional 3' flanking sequence of SEQ ID NO: 264;

(iv) an optional 5' flanking sequence of SEQ ID NO: 265,

a 5' stem of SEQ ID NO: 266,

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: 267,
an RNA of the present disclosure, e.g., any of SEQ ID NOS: 50-97, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions,
a 3' stem of SEQ ID NO: 268, and
an optional 3' flanking sequence of SEQ ID NO: 269;

(v) an optional 5' flanking sequence of SEQ ID NO: 270,
a 5' stem of SEQ ID NO: 271,
an RNA of the present disclosure, e.g., any of SEQ ID NOS: 50-97, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions;
a loop of SEQ ID NO: 272,
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: 274, and
an optional 3' flanking sequence of SEQ ID NO: 275; or

(vi) an optional 5' flanking sequence of SEQ ID NO: 270,
a 5' stem of SEQ ID NO: 271,
an RNA of the present disclosure, e.g., any of SEQ ID NOS: 50-97, with the optional exception of 1, 2, 3 or 4 nucleotide substitutions,
a loop of SEQ ID NO: 273,
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: 274, and
an optional 3' flanking sequence of SEQ ID NO: 275;

(vii) an optional 5' flanking sequence of SEQ ID NO: 276,
a 5' stem of sequence SEQ ID NO: 277,
an RNA of e.g., any of SEQ ID NOS: 50-97, 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: 278, and

an optional 3' flanking sequence SEQ ID NO: 279.

[0083] In some embodiments, the transgene may encode a pri-miRNA comprising:

(i) an optional 5' flanking sequence of SEQ ID NO: 255;

a 5' stem of SEQ ID NO: 256,

an RNA (guide) of SEQ ID NO: 50,

a loop of SEQ ID NO: 257,

an antisense (passenger) RNA of 360,

a 3' stem of SEQ ID NO: 258, and

an optional 3' flanking sequence of SEQ ID NO: 259;

(ii) an optional 5' flanking sequence of SEQ ID NO: 255,

a 5' stem of SEQ ID NO: 256,

an RNA (guide) of SEQ ID NO: 55,

a loop of SEQ ID NO: 257,

an antisense RNA (passenger) of SEQ ID NO: 361,

a 3' stem of SEQ ID NO: 258, and

an optional 3' flanking sequence of SEQ ID NO: 259;

(iii) an optional 5' flanking sequence of SEQ ID NO: 260,

a 5' stem of SEQ ID NO: 261,

an antisense RNA (passenger) of SEQ ID NO: 375,

a loop of SEQ ID NO: 262,

an RNA (guide) of SEQ ID NO:51,

a 3' stem of SEQ ID NO: 263, and

an optional 3' flanking sequence of SEQ ID NO: 264.

[0084] 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-53	UUGAAUGAGGCUUCAGUAC UUUACAGAAUCGUUGCCUG CACAUCUUGGAAACACUUG CUGGGAUUACUUCGACUUC UUAACCCAACAGAAGGCUC GAGAAGGUAUAUUGCUGUU GACAGUGAGCGUCCAAGCA GGGUUUGUGAUCAGUAGUG AAGCCACAGAUGUACUGAU CACAAACCCUGCUUGGCUG CCUACUGCCUCGGACUUCA AGGGGCUAGAAUUCGAGCA AUUAUCUUGUUUACUAAAA CUGAAUACCUUGCUAUCUC UUUGAUACAUUAUUACAAA GCUGAAUUAAAAUGGUAUA AAUUAAAUCAC	310	334	53	153
miR-E-51	UUGAAUGAGGCUUCAGUAC UUUACAGAAUCGUUGCCUG CACAUCUUGGAAACACUUG CUGGGAUUACUUCGACUUC UUAACCCAACAGAAGGCUC GAGAAGGUAUAUUGCUGUU GACAGUGAGCGCCCAGAGU GACUAUGAUAGUGAUAGUG AAGCCACAGAUGUAUCACU AUCAUAGUCACUCUGGUUG CCUACUGCCUCGGACUUCA AGGGGCUAGAAUUCGAGCA AUUAUCUUGUUUACUAAAA CUGAAUACCUUGCUAUCUC UUUGAUACAUUAUUACAAA GCUGAAUUAAAAUGGUAUA AAUUAAAUCAC	311	335	51	151
miR-E-50	UUGAAUGAGGCUUCAGUAC UUUACAGAAUCGUUGCCUG CACAUCUUGGAAACACUUG CUGGGAUUACUUCGACUUC UUAACCCAACAGAAGGCUC GAGAAGGUAUAUUGCUGUU GACAGUGAGCGUAGGUGGC CAGGUGGAAGUAAAUAGUG	312	336	50	150

	AAGCCACAGAUGUAUUUAC UUCCACCUGGCCACCUCUG CCUACUGCCUCGGACUUCA AGGGGCUAGAAUUCGAGCA AUUAUCUUGUUUACUAAAA CUGAAUACCUUGCUAUCUC UUUGAUACAUUAUUACAAA GCUGAAUUAAAAUGGUAUA AAUUAAAUCAC				
miR-E-55	UUGAAUGAGGCUUCAGUAC UUUACAGAAUCGUUGCCUG CACAUCUUGGAAACACUUG CUGGGAUUACUUCGACUUC UUAACCCAACAGAAGGCUC GAGAAGGUAUAUUGCUGUU GACAGUGAGCGACAGGUGC AAAUAGUCUACAAAUAGUG AAGCCACAGAUGUAUUUGU AGACUAUUUGCACCUGGUG CCUACUGCCUCGGACUUCA AGGGGCUAGAAUUCGAGCA AUUAUCUUGUUUACUAAAA CUGAAUACCUUGCUAUCUC UUUGAUACAUUAUUACAAA GCUGAAUUAAAAUGGUAUA AAUUAAAUCAC	313	337	55	155
miR-100-53	CCCAAAAGAGAGAAGAUAU UGAGGCCUGUUGCCACACU GAUCACAAACCCUGCUUGG CGUAUUAGUCCGGCCAAGU AGGUUUUGUGGUCACUGUG UCUGUUAGGCAAUCUCACG GACCUGGGGCUUUGCUUAU AUGCC	314	338	53	358
miR-100-51	CCCAAAAGAGAGAAGAUAU UGAGGCCUGUUGCCACAUC ACUAUCAUAGUCACUCUGG UGUAUUAGUCCGACCAGAA UGAAUAUGAUGGUGUUGUG UCUGUUAGGCAAUCUCACG GACCUGGGGCUUUGCUUAU AUGCC	315	339	51	359
miR-100-50	CCCAAAAGAGAGAAGAUAU UGAGGCCUGUUGCCACAUU UACUUCCACCUGGCCACCU CGUAUUAGUCCGGAGGUGA	316	340	50	360

	CCAUGUGGAAAUAAUUGUG UCUGUUAGGCAAUCUCACG GACCUGGGGCUUUGCUUAU AUGCC				
miR-100-55	CCCAAAAGAGAGAAGAUAU UGAGGCCUGUUGCCACAUU UGUAGACUAUUUGCACCUG GGUAUUAGUCCGCCAGGUA CAACUAGUCUGCAAUUGUG UCUGUUAGGCAAUCUCACG GACCUGGGGCUUUGCUUAU AUGCC	317	341	55	361
miR-132-53	GCCGUCCGCGCGCCCCGCC CCCGCGUCUCCAGGGGUCA AGCAGGGGUGGUGAUCAGC UGUGGGAACUGGAGGCUGA UCACAAACCCUGCUUGGCC CCCGCAGCACGCCCACGCG CCGCGCCACGCCGCGCCCC GAGCC	318	342	53	362
miR-132-51	GCCGUCCGCGCGCCCCGCC CCCGCGUCUCCAGGGAUCA GAGUGACGAGGAUAGUGAC UGUGGGAACUGGAGGUCAC UAUCAUAGUCACUCUGGUC CCCGCAGCACGCCCACGCG CCGCGCCACGCCGCGCCCC GAGCC	319	343	51	363
miR-132-50	GCCGUCCGCGCGCCCCGCC CCCGCGUCUCCAGGGGGGG UGGCCAGUUUGAAGUAAAC UGUGGGAACUGGAGGUUUA CUUCCACCUGGCCACCUCC CCCGCAGCACGCCCACGCG CCGCGCCACGCCGCGCCCC GAGCC	320	344	50	364
miR-132-55	GCCGUCCGCGCGCCCCGCC CCCGCGUCUCCAGGGCUAG GUGCAAAGAUUCUACAAAC UGUGGGAACUGGAGGUUUG UAGACUAUUUGCACCUGGC CCCGCAGCACGCCCACGCG CCGCGCCACGCCGCGCCCC GAGCC	321	345	55	365
miR-190a-53	GAGCUCAGUCAAACCUGGA UGCCUUUUCUGCAGGCCUC	322	346	53	366

	UGUGCUGAUCACAAACCCU GCUUGGCUGUUAUUUAAUC CAGCCGCAGGGUUUGUGAU CGUCUACAGUGUCUUGCCC UGUCUCCGGGGGUUCCUAA UAAAG				
miR-190a-51	GAGCUCAGUCAAACCUGGA UGCCUUUUCUGCAGGCCUC UGUGUCACUAUCAUAGUCA CUCUGGUUGUUAUUUAAUC CAACCAGUGACUAUGAUAG UACCUACAGUGUCUUGCCC UGUCUCCGGGGGUUCCUAA UAAAG	323	347	51	367
miR-190a-50	GAGCUCAGUCAAACCUGGA UGCCUUUUCUGCAGGCCUC UGUGUUUACUUCCACCUGG CCACCUCUGUUAUUUAAUC CAGAGGGCCAGGUGGAAGU AGCCUACAGUGUCUUGCCC UGUCUCCGGGGGUUCCUAA UAAAG	324	348	50	368
miR-190a-55	GAGCUCAGUCAAACCUGGA UGCCUUUUCUGCAGGCCUC UGUGUUUGUAGACUAUUUG CACCUGGUGUUAUUUAAUC CACCAUGCAAAUAGUCUAC AGCCUACAGUGUCUUGCCC UGUCUCCGGGGGUUCCUAA UAAAG	325	349	55	369
miR-451-53	CUCUGCUCAGCCUGUCACA ACCUACUGACUGCCAGGGC ACUUGGGAAUGGCAAGGCU GAUCACAAACCCUGCUUGG CAGCAGGGUUUGUGAUCAU UCUUGCUAUACCCAGAAAA CGUGCCAGGAAGAGAACUC A	326	350	53	370
miR-451-51	CUCUGCUCAGCCUGUCACA ACCUACUGACUGCCAGGGC ACUUGGGAAUGGCAAGGUC ACUAUCAUAGUCACUCUGG UGAGUGACUAUGAUAGUGC UCUUGCUAUACCCAGAAAA CGUGCCAGGAAGAGAACUC A	327	351	51	371

miR-451-50	CUCUGCUCAGCCUGUCACA ACCUACUGACUGCCAGGGC ACUUGGGAAUGGCAAGGUU UACUUCCACCUGGCCACCU CUGGCCAGGUGGAAGUAAC UCUUGCUAUACCCAGAAAA CGUGCCAGGAAGAGAACUC A	328	352	50	372
miR-451-55	CUCUGCUCAGCCUGUCACA ACCUACUGACUGCCAGGGC ACUUGGGAAUGGCAAGGUU UGUAGACUAUUUGCACCUG GGUGCAAAUAGUCUACAAC UCUUGCUAUACCCAGAAAA CGUGCCAGGAAGAGAACUC A	329	353	55	373
miR-130a-53	GCAGGGCCGGCAUGCCUCU GCUGCUGGCCAGGCAAGCA GGUUUUGUGAUCAACUGUC UGCACCUGUCACUAGCUGA UCACAAACCCUGCUUGGCU GGCCGUGUAGUGCUACCCA GCGCUGGCUGCCUCCUCAG CAUUG	330	354	53	374
miR-130a-51	GCAGGGCCGGCAUGCCUCU GCUGCUGGCCAAGCAGAGU GAAUAUGAUAGUGGCUGUC UGCACCUGUCACUAGUCAC UAUCAUAGUCACUCUGGUU GGCCGUGUAGUGCUACCCA GCGCUGGCUGCCUCCUCAG CAUUG	331	355	51	375
miR-130a-50	GCAGGGCCGGCAUGCCUCU GCUGCUGGCCAGUGGUGGC CAUGUGGAAGUAAGCUGUC UGCACCUGUCACUAGUUUA CUUCCACCUGGCCACCUCU GGCCGUGUAGUGCUACCCA GCGCUGGCUGCCUCCUCAG CAUUG	332	356	50	376
miR-130a-55	GCAGGGCCGGCAUGCCUCU GCUGCUGGCCACGAGGUGC AACUAGUCUAUAAGCUGUC UGCACCUGUCACUAGUUUG UAGACUAUUUGCACCUGGU GGCCGUGUAGUGCUACCCA	333	357	55	377

	GCGCUGGCUGCCUCCUCAG CAUUG				

[0085] 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
GCCAAGUAGGUUUUGUGGUCAC	358	GCCAAGTAGGTTTTGTGGTCAC	378
ACCAGAAUGAAUAUGAUGGUGU	359	ACCAGAATGAATATGATGGTGT	379
GAGGUGACCAUGUGGAAAUAAU	360	GAGGTGACCATGTGGAAATAAT	380
CCAGGUACAACUAGUCUGCAAU	361	CCAGGTACAACTAGTCTGCAAT	381
GUCAAGCAGGGGUGGUGAUCAG	362	GTCAAGCAGGGGTGGTGATCAG	382
AUCAGAGUGACGAGGAUAGUGA	363	ATCAGAGTGACGAGGATAGTGA	383
GGGGUGGCCAGUUUGAAGUAAA	364	GGGGTGGCCAGTTTGAAGTAAA	384
CUAGGUGCAAAGAUUCUACAAA	365	CTAGGTGCAAAGATTCTACAAA	385
GCCGCAGGGUUUGUGAUCGU	366	GCCGCAGGGTTTGTGATCGT	386
ACCAGUGACUAUGAUAGUAC	367	ACCAGTGACTATGATAGTAC	387
GAGGGCCAGGUGGAAGUAGC	368	GAGGGCCAGGTGGAAGTAGC	388
CCAUGCAAAUAGUCUACAGC	369	CCATGCAAATAGTCTACAGC	389
AGCAGGGUUUGUGAUCAU	370	AGCAGGGTTTGTGATCAT	390
GAGUGACUAUGAUAGUGC	371	GAGTGACTATGATAGTGC	391
UGGCCAGGUGGAAGUAAC	372	TGGCCAGGTGGAAGTAAC	392
GUGCAAAUAGUCUACAAC	373	GTGCAAATAGTCTACAAC	393
GGCAAGCAGGUUUUGUGAUCAA	374	GGCAAGCAGGTTTTGTGATCAA	394
AGCAGAGUGAAUAUGAUAGUGG	375	AGCAGAGTGAATATGATAGTGG	395
GUGGUGGCCAUGUGGAAGUAAG	376	GTGGTGGCCATGTGGAAGTAAG	396
CGAGGUGCAACUAGUCUAUAAG	377	CGAGGTGCAACTAGTCTATAAG	397

Expression cassettes

[0086] 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. 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, e.g., a central nervous system (CNS) cell or tissue selective promoter or enhancer (e.g., a Syn1 promoter). 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.

[0087] 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:425 (a U6 promoter) or that contains a functional fragment of SEQ ID NO:425. Other constitutive promoter elements may be used, e.g., an EF1α promoter, a CMV promoter, a CBA promoter, and the like.

[0088] 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).

[0089] 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.

[0090] 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).

[0091] 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.

[0092] 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.

[0093] 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: 416, 418, and 420 shown below:

[0094] SEQ ID NO:416 is a U6-miR-100-50 expression cassette (includes U6 promoter and pol-III terminator sequence) and has the following DNA sequence:

[0095] GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAG
AGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGA
CGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAAAATGG
ACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTG
GAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATAGCTAGCACTAGTCGCCC
AAAAGAGAGAAGATATTGAGGCCTGTTGCCACATTTACTTCCACCTGGCCACCTCGTA
TTAGTCCGGAGGTGACCATGTGGAAATAATTGTGTCTGTTAGGCAATCTCACGGACCT
GGGGCTTTGCTTATATGCCTTTTTT

[0096] SEQ ID NO:418 is a U6-miR-130a-51 expression cassette (includes U6 promoter and pol-III terminator sequence) and has the following DNA sequence:

[0097] GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAG
AGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGA
CGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAAAATGG
ACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTG
GAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATAGCTAGCACTAGTCGGCA
GGGCCGGCATGCCTCTGCTGCTGGCCAAGCAGAGTGAATATGATAGTGGCTGTCTGC

ACCTGTCACTAGTCACTATCATAGTCACTCTGGTTGGCCGTGTAGTGCTACCCAGCGC
TGGCTGCCTCCTCAGCATTGTTTTTT

[0098] SEQ ID NO:420 is a U6-miR-100-55 expression cassette (includes U6 promoter and pol-III terminator sequence) and has the following DNA sequence:

[0099] GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAG
AGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGA
CGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAAAATGG
ACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTG
GAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATAGCTAGCACTAGTCGCCC
AAAAGAGAGAAGATATTGAGGCCTGTTGCCACATTTGTAGACTATTTGCACCTGGGTA
TTAGTCCGCCAGGTACAACTAGTCTGCAATTGTGTCTGTTAGGCAATCTCACGGACCT
GGGGCTTTGCTTATATGCCTTTTTT

Vectors

[00100] 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.

[00101] 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.

[00102] 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.

[00103] 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.

[00104] 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.

[00105] 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.

[00106] 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.

[00107] 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.

[00108] 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.

[00109] 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.

[00110] 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.

[00111] 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.

[00112] 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).

[00113] 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:427 and 3'ITR containing 428, provided elsewhere herein) packaged in the capsid from serotype 5 or serotype 9. Pseudotypes may improve transduction efficiency as well as alter tropism.

[00114] 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.

[00115] 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".

[00116] 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:429 or a functional fragment thereof.

[00117] 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.

[00118] 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:417, 419, and 421 (ssAAV genomes) and SEQ ID NOs:422, 423, and 424 (scAAV genomes), shown below:

[00119] SEQ ID NO:417 is an ITR-ITR ssAAV genome that includes a stuffer region and the U6-miR-100-50 expression cassette of SEQ ID NO:416 and has the following DNA sequence:

[00120] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATCTG
TTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACATTAC
AGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGAACAGC
CATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACATGGATGTCA
CATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGAGGAGATGTT
CCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATGGAGCAGTAGA
TAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAAATTTTGCTAGAGA
CTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTGTCATATAATACAAGG
ACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTTAAAAGATGGCAAGGTT
ATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTTGTTTGGTTTTTAGGTTTT
TCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTATTTGAATGAACAAAATTT
GAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAATAAATTCATGAAACATT

GAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAATTGAGAAGTTCATTATG
CATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAAGAACTTATTGTAGTTTT
GGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTTGGAGACACAGAGTATT
CCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAATATTATGCTTACTGCCTG
TCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCT
TGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTC
ATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTTTTGGGAGTGTATATTTCTC
ATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGTGA
CTTATGGCATTTGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGT
GTGCCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGC
CTTATAATATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGG
GATTTTATTTCCGTTAACTACTGCCAAAATTAAATAAACAGTTATTCTAAAAAGCACTA
GATAACTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCGTTGCCTCATATATCAAT
GCAGGCTTTCCCCAGCTACCAAAGCTGCCATTTGATTCAGATACCCAATCTGCCTCAG
ATGTGCTTGCGGTTGGGGAGTTACATTTGCGGCTATTTATTGGATGCTCAAAGAGATTA
GTAAACACACACAGAGCTAGAGGAATTTTATACATTATCCTGATAATAACTAAAGTATT
GTTTAGTTGGATGCAAATGAAGGAAATATGTCAAATGACTTACATATCTGACTACTAAA
AAAATTATTGTATTATTACAGAGTATTACTGTAGTGTATTATTGTAGTGTATTATTATAGA
GATAATAACTTAGATACAATGTTAAGGAAAGTAAAATATTTCATTTTCCTCCAATAACA
ATAAAGGGAATGTTCTTCTGATTGCAATTAACCTCAGATATTTTTGGATATGATCTATTT
TTTAAAAATGATGATCCATGGGATAAAATATGAAAGAGACGAGGGAAAATAATTGGAC
GTGGAATATCCCCAGTTTTTATGTTATACAGGTGATGTAGTTTTGGACAAAAAATGATG
GAAGAGAGAGGCATGTAGAAAGTGTAAAGAAGTTCAGGGGAAGTTTTAAATTTAGCT
CTCTTAATCTTGGGTCACTCATCAGAAAAAGGAAAGCACAATGTTAGAAATTCAGAG
ATCATATCACTGGTTGACTTCCGATCATATCACTGGTTGACTTCCTTAGTACTACAGGT
TTAAAACTTAAGAGGCTTTTGTCTCAAAACAACATCATGGTACATGTTAGTTTTCTCCT
TTTTTTCTTTTCCCCAATATAATATATTTACTTAGTCCATTAATTTTTCAATGGTCTGTTC
ATTGTTTTCTTTTTTATGTGATCTTCTCATTCCCTCAATACTAGCTGTACATTTTGAAAA
TATAAATGTTTGGTATATAATAAATATTATATGGGGGAAGGGAAAAGGGAAAGGAGAA
AGGGAAGGAGAAAGAGGTATTCTGTGTAGTTATATTAGTGGTCTAACAAACAGACATC
TACGAGATAAAATGAAACAAAACACACTGACAATTAAATAGAGAAATCAGACGAAAG

TTGAGTCATGTAATCCAAAGCAAAATAGTATTTCAAGAATGAGGGTGTGAATCAGTTT
TACAGATACACACGACTGTTTGAGTAATACGCAATAGACAAGTGATATATGGGATTTAG
TGACAAGTATAAGATATGATTCCAAAAACAGGGTAAATGCCTGAAGAAAGGAGAGTT
AAGAAAAATAAAATTAAACCTTGTCACAGGCAACTCTTTCCATGAGTCTTCCTATCAT
AGAAAATAAATAAATGAGTTGACAGTGGCATATCTAGAACTAAGACAATCCGAGGGC
CTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATT
AGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGT
AATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAAAATGGACTATCATATG
CTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACG
AAACACCGTGCTCGCTTCGGCAGCACATATAGCTAGCACTAGTCGCCCAAAAGAGAG
AAGATATTGAGGCCTGTTGCCACATTTACTTCCACCTGGCCACCTCGTATTAGTCCGG
AGGTGACCATGTGGAAATAATTGTGTCTGTTAGGCAATCTCACGGACCTGGGGCTTTG
CTTATATGCCTTTTTTATATATATTAATTAATATATAGGTACCCACGTGCGGACCGAGCGG
CCGCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTC
ACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTC
AGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG (SEQ ID NO:417)

[00121] SEQ ID NO:419 is an ITR-ITR ssAAV genome that includes a stuffer region and the U6-miR-130a-51 expression cassette of SEQ ID NO:418 and has the following DNA sequence:

[00122] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATCTG
TTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACATTAC
AGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGAACAGC
CATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACATGGATGTCA
CATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGAGGAGATGTT
CCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATGGAGCAGTAGA
TAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAAATTTTGCTAGAGA
CTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTGTCATATAATACAAGG
ACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTTAAAAGATGGCAAGGTT
ATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTTGTTTGGTTTTTAGGTTTT
TCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTATTTGAATGAACAAAATTT

GAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAATAAATTCATGAAACATT
GAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAATTGAGAAGTTCATTATG
CATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAAGAACTTATTGTAGTTTT
GGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTTGGAGACACAGAGTATT
CCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAATATTATGCTTACTGCCTG
TCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCT
TGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTC
ATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTTTTGGGAGTGTATATTTCTC
ATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGTGA
CTTATGGCATTTGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGT
GTGCCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGC
CTTATAATATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGG
GATTTTATTTCCGTTAACTACTGCCAAAATTAAATAAACAGTTATTCTAAAAAGCACTA
GATAACTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCGTTGCCTCATATATCAAT
GCAGGCTTTCCCCAGCTACCAAAGCTGCCATTTGATTCAGATACCCAATCTGCCTCAG
ATGTGCTTGCGGTTGGGGAGTTACATTTGCGGCTATTTATTGGATGCTCAAAGAGATTA
GTAAACACACACAGAGCTAGAGGAATTTTATACATTATCCTGATAATAACTAAAGTATT
GTTTAGTTGGATGCAAATGAAGGAAATATGTCAAATGACTTACATATCTGACTACTAAA
AAAATTATTGTATTATTACAGAGTATTACTGTAGTGTATTATTGTAGTGTATTATTATAGA
GATAATAACTTAGATACAATGTTAAGGAAAGTAAAATATTTCATTTTCCTCCAATAACA
ATAAAGGGAATGTTCTTCTGATTGCAATTAACCTCAGATATTTTTGGATATGATCTATTT
TTTAAAAATGATGATCCATGGGATAAAATATGAAAGAGACGAGGGAAAATAATTGGAC
GTGGAATATCCCCAGTTTTTATGTTATACAGGTGATGTAGTTTTGGACAAAAAATGATG
GAAGAGAGAGGCATGTAGAAAGTGTAAAGAAGTTCAGGGGAAGTTTTAAATTTAGCT
CTCTTAATCTTGGGTCACTCATCAGAAAAAGGAAAGCACAATGTTAGAAATTCAGAG
ATCATATCACTGGTTGACTTCCGATCATATCACTGGTTGACTTCCTTAGTACTACAGGT
TTAAAACTTAAGAGGCTTTTGTCTCAAAACAACATCATGGTACATGTTAGTTTTCTCCT
TTTTTTCTTTTCCCCAATATAATATATTTACTTAGTCCATTAATTTTTCAATGGTCTGTTC
ATTGTTTTCTTTTTTTATGTGATCTTCTCATTCCCTCAATACTAGCTGTACATTTTGAAAA
TATAAATGTTTGGTATATAATAAATATTATATGGGGGAAGGGAAAAGGGAAAGGAGAA
AGGGAAGGAGAAAGAGGTATTCTGTGTAGTTATATTAGTGGTCTAACAAACAGACATC

TACGAGATAAAATGAAACAAAACACACTGACAATTAAATAGAGAAATCAGACGAAAG
TTGAGTCATGTAATCCAAAGCAAAATAGTATTTCAAGAATGAGGGTGTGAATCAGTTT
TACAGATACACACGACTGTTTGAGTAATACGCAATAGACAAGTGATATATGGGATTTAG
TGACAAGTATAAGATATGATTCCAAAAACAGGGTAAATGCCTGAAGAAAGGAGAGTT
AAGAAAAATAAAATTAAACCTTGTCACAGGCAACTCTTTCCATGAGTCTTCCTATCAT
AGAAAATAAATAAATGAGTTGACAGTGGCATATCTAGAACTAAGACAATCCGAGGGC
CTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATT
AGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGT
AATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAAAATGGACTATCATATG
CTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACG
AAACACCGTGCTCGCTTCGGCAGCACATATAGCTAGCACTAGTCGGCAGGGCCGGCA
TGCCTCTGCTGCTGGCCAAGCAGAGTGAATATGATAGTGGCTGTCTGCACCTGTCACT
AGTCACTATCATAGTCACTCTGGTTGGCCGTGTAGTGCTACCCAGCGCTGGCTGCCTC
CTCAGCATTGTTTTTTATATATATTAATTAATATATAGGTACCCACGTGCGGACCGAGCG
GCCGCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCT
CACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCT
CAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG (SEQ ID NO:419)

[00123] SEQ ID NO:421 is an ITR-ITR ssAAV genome that includes a stuffer region and the U6-miR-100-55 expression cassette of SEQ ID NO:420 and has the following DNA sequence:

[00124] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATCTG
TTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACATTAC
AGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGAACAGC
CATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACATGGATGTCA
CATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGAGGAGATGTT
CCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATGGAGCAGTAGA
TAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAAATTTTGCTAGAGA
CTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTGTCATATAATACAAGG
ACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTTAAAAGATGGCAAGGTT
ATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTTTGTTTGGTTTTTAGGTTTT

TCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTATTTGAATGAACAAAATTT
GAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAATAAATTCATGAAACATT
GAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAATTGAGAAGTTCATTATG
CATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAAGAACTTATTGTAGTTTT
GGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTTGGAGACACAGAGTATT
CCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAATATTATGCTTACTGCCTG
TCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCT
TGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTC
ATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTTTTGGGAGTGTATATTTCTC
ATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGTGA
CTTATGGCATTTGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGT
GTGCCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGC
CTTATAATATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGG
GATTTTATTTCCGTTAACTACTGCCAAAATTAAATAAACAGTTATTCTAAAAAGCACTA
GATAACTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCGTTGCCTCATATATCAAT
GCAGGCTTTCCCCAGCTACCAAAGCTGCCATTTGATTCAGATACCCAATCTGCCTCAG
ATGTGCTTGCGGTTGGGGAGTTACATTTGCGGCTATTTATTGGATGCTCAAAGAGATTA
GTAAACACACACAGAGCTAGAGGAATTTTATACATTATCCTGATAATAACTAAAGTATT
GTTTAGTTGGATGCAAATGAAGGAAATATGTCAAATGACTTACATATCTGACTACTAAA
AAAATTATTGTATTATTACAGAGTATTACTGTAGTGTATTATTGTAGTGTATTATTATAGA
GATAATAACTTAGATACAATGTTAAGGAAAGTAAAATATTTCATTTTCCTCCAATAACA
ATAAAGGGAATGTTCTTCTGATTGCAATTAACCTCAGATATTTTTGGATATGATCTATTT
TTTAAAAATGATGATCCATGGGATAAAATATGAAAGAGACGAGGGAAAATAATTGGAC
GTGGAATATCCCCAGTTTTTATGTTATACAGGTGATGTAGTTTTGGACAAAAAATGATG
GAAGAGAGAGGCATGTAGAAAGTGTAAAGAAGTTCAGGGGAAGTTTTAAATTTAGCT
CTCTTAATCTTGGGTCACTCATCAGAAAAAGGAAAGCACAATGTTAGAAATTCAGAG
ATCATATCACTGGTTGACTTCCGATCATATCACTGGTTGACTTCCTTAGTACTACAGGT
TTAAAACTTAAGAGGCTTTTGTCTCAAAACAACATCATGGTACATGTTAGTTTTCTCCT
TTTTTTCTTTTCCCCAATATAATATATTTACTTAGTCCATTAATTTTTCAATGGTCTGTTC
ATTGTTTTCTTTTTTATGTGATCTTCTCATTCCCTCAATACTAGCTGTACATTTTGAAAA
TATAAATGTTTGGTATATAATAAATATTATATGGGGGAAGGGAAAAGGGAAAGGAGAA

AGGGAAGGAGAAAGAGGTATTCTGTGTAGTTATATTAGTGGTCTAACAAACAGACATC
TACGAGATAAAATGAAACAAAACACACTGACAATTAAATAGAGAAATCAGACGAAAG
TTGAGTCATGTAATCCAAAGCAAAATAGTATTTCAAGAATGAGGGTGTGAATCAGTTT
TACAGATACACACGACTGTTTGAGTAATACGCAATAGACAAGTGATATATGGGATTTAG
TGACAAGTATAAGATATGATTCCAAAAACAGGGTAAATGCCTGAAGAAAGGAGAGTT
AAGAAAAATAAAATTAAACCTTGTCACAGGCAACTCTTTCCATGAGTCTTCCTATCAT
AGAAAATAAATAAATGAGTTGACAGTGGCATATCTAGAACTAAGACAATCCGAGGGC
CTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATT
AGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGT
AATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAAAATGGACTATCATATG
CTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACG
AAACACCGTGCTCGCTTCGGCAGCACATATAGCTAGCACTAGTCGCCCAAAAGAGAG
AAGATATTGAGGCCTGTTGCCACATTTGTAGACTATTTGCACCTGGGTATTAGTCCGCC
AGGTACAACTAGTCTGCAATTGTGTCTGTTAGGCAATCTCACGGACCTGGGGCTTTGC
TTATATGCCTTTTTTATATATATTAATTAATATATAGGTACCCACGTGCGGACCGAGCGGC
CGCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCA
CTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCA
GTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG (SEQ ID NO:421)

[00125] SEQ ID NO:422 is an ITR-ITR scAAV genome that includes a stuffer region and the U6-miR-100-50 expression cassette of SEQ ID NO:416 and has the following DNA sequence:

[00126] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATCTG
TTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACATTAC
AGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGAACAGC
CATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACATGGATGTCA
CATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGAGGAGATGTT
CCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATGGAGCAGTAGA
TAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAAATTTTGCTAGAGA
CTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTGTCATATAATACAAGG
ACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTTAAAAGATGGCAAGGTT

ATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTTGTTTGGTTTTTAGGTTTT
TCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTATTTGAATGAACAAAATTT
GAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAATAAATTCATGAAACATT
GAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAATTGAGAAGTTCATTATG
CATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAAGAACTTATTGTAGTTTT
GGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTTGGAGACACAGAGTATT
CCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAATATTATGCTTACTGCCTG
TCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCT
TGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTC
ATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTTTTGGGAGTGTATATTTCTC
ATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGTGA
CTTATGGCATTTGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGT
GTGCCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGC
CTTATAATATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGG
GATTTTATTTCCGTTAACGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGAT
ACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGT
ACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTA
TGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCT
TTATATATCTTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATGCTAG
CACTAGTCGCCCAAAAGAGAGAAGATATTGAGGCCTGTTGCCACATTTACTTCCACCT
GGCCACCTCGTATTAGTCCGGAGGTGACCATGTGGAAATAATTGTGTCTGTTAGGCAA
TCTCACGGACCTGGGGCTTTGCTTATATGCCTTTTTTATATATATTAATTAATATATAGGT
ACCCACGTGCGGACCGAGCGGCCGCCCACTCCCTCTCTGCGCGCTCGCTCGCTCACT
GAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGT
GAGCGAGCGAGCGCGCAGAGAGGGA (SEQ ID NO:422)

[00127] SEQ ID NO:423 is an ITR-ITR scAAV genome that includes a stuffer region and the U6-miR-100-55 expression cassette of SEQ ID NO:420 and has the following DNA sequence:

[00128] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATCTG
TTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACATTAC

AGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGAACAGC
CATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACATGGATGTCA
CATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGAGGAGATGTT
CCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATGGAGCAGTAGA
TAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAAATTTTGCTAGAGA
CTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTGTCATATAATACAAGG
ACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTTAAAAGATGGCAAGGTT
ATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTTGTTTGGTTTTTAGGTTTT
TCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTATTTGAATGAACAAAATTT
GAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAATAAATTCATGAAACATT
GAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAATTGAGAAGTTCATTATG
CATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAAGAACTTATTGTAGTTTT
GGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTTGGAGACACAGAGTATT
CCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAATATTATGCTTACTGCCTG
TCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCT
TGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTC
ATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTTTTGGGAGTGTATATTTCTC
ATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGTGA
CTTATGGCATTTGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGT
GTGCCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGC
CTTATAATATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGG
GATTTTATTTCCGTTAACGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGAT
ACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGT
ACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTA
TGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCT
TTATATATCTTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATGCTAG
CACTAGTCGCCCAAAAGAGAGAAGATATTGAGGCCTGTTGCCACATTTGTAGACTATT
TGCACCTGGGTATTAGTCCGCCAGGTACAACTAGTCTGCAATTGTGTCTGTTAGGCAA
TCTCACGGACCTGGGGCTTTGCTTATATGCCTTTTTTATATATATTAATTAATATATAGGT
ACCCACGTGCGGACCGAGCGGCCGCCCACTCCCTCTCTGCGCGCTCGCTCGCTCACT

GAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGT
GAGCGAGCGAGCGCGCAGAGAGGGA (SEQ ID NO:423)

[00129] SEQ ID NO:424 is an ITR-ITR scAAV genome that includes a stuffer region and the U6-miR-130a-51 expression cassette of SEQ ID NO:418 and has the following DNA sequence:

[00130] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC
GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGTACAAGAATCTG
TTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCTTGGTCCCCCACATTAC
AGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGTCCTTTATTGGAACAGC
CATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCCATGTTACATGGATGTCA
CATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAGAGCCAGAGGAGATGTT
CCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAATGGAGCAGTAGA
TAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAAATTTTGCTAGAGA
CTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTACTGTCATATAATACAAGG
ACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTTAAAAGATGGCAAGGTT
ATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTTGTTTGGTTTTTAGGTTTT
TCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTATTTGAATGAACAAAATTT
GAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAATAAATTCATGAAACATT
GAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAATTGAGAAGTTCATTATG
CATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAAGAACTTATTGTAGTTTT
GGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTTGGAGACACAGAGTATT
CCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAATATTATGCTTACTGCCTG
TCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAGTGCT
TGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGATTTATTCGTTGACATTTC
ATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTTTTGGGAGTGTATATTTCTC
ATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGTGA
CTTATGGCATTTGAATGTGTACATTCAATTGTGTTGGCACATACTCTAGTCAAATGTGT
GTGCCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATCCAATTGTACATATTAGC
CTTATAATATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGG
GATTTTATTTCCGTTAACGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGAT
ACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGT

ACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTA
TGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCT
TTATATATCTTGTGGAAAGGACGAAACACCGTGCTCGCTTCGGCAGCACATATGCTAG
CACTAGTCGGCAGGGCCGGCATGCCTCTGCTGCTGGCCAAGCAGAGTGAATATGATA
GTGGCTGTCTGCACCTGTCACTAGTCACTATCATAGTCACTCTGGTTGGCCGTGTAGT
GCTACCCAGCGCTGGCTGCCTCCTCAGCATTGTTTTTTATATATATTAATTAATATATAGG
TACCCACGTGCGGACCGAGCGGCCGCCCACTCCCTCTCTGCGCGCTCGCTCGCTCAC
TGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAG
TGAGCGAGCGAGCGCGCAGAGAGGGA (SEQ ID NO:424)

Method for reducing expression of Tau

[00131] In some embodiments, the present disclosure provides methods of reducing expression of Tau in a cell wherein Tau is expressed, e.g., from an endogenous MAPT 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 Tau 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 Tau 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 Tau in a comparable cell not treated with the oligonucleotide, RNA, nucleic acid cassette or vector. In some cases, the expression of Tau 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 Tau in a comparable cell not treated with the oligonucleotide, RNA, nucleic acid cassette or vector. In some cases, the expression of Tau 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 Tau in a comparable cell not treated with the oligonucleotide, RNA, nucleic acid cassette or vector. The reduction in expression of Tau

may be assessed by standard molecular techniques, including quantitative polymerase chain reaction.

Pharmaceutical compositions

[00132] 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).

[00133] 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.

[00134] 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

[00135] Also disclosed are methodss for treating or preventing a tauopathy, including Alzheimer's Disease (AD) and Frontotemporal Dementia and Parkinsonism Linked To Chromosome 17 (FTDP-17), where the method comprises 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 tauopathy.

[00136] 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.

[00137] 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

[00138] 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).

[00139] 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.

[00140] 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.

[00141] 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.

[00142] 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.

[00143] 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).

[00144] 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.

[00145] 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.

[00146] 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.

[00147] 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.

[00148] 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).

[00149] 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.

[00150] 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.

[00151] 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.

[00152] 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.

[00153] 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.

[00154] 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).

[00155] 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.

[00156] 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.

[00157] 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.

[00158] 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.

[00159] 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).

[00160] 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.

[00161] 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.

[00162] 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

[00163] The nucleotide sequence of the human microtubule associated protein tau (MAPT) gene (as defined by 5092-138,872 of NCBI Reference Sequence: NG_007398.2RefSeqGene (LRG_660) of chromosome 17) is set forth in the sequence listing as SEQ ID NO: 248.

[00164] The nucleotide sequence of the RNA encoded by SEQ ID NO: 248 is set forth in the sequence listing as SEQ ID NO: 249.

[00165] The tables below provide other sequences that are referenced in this disclosure.

[00166] Table 5 provides the target site sequences in the MAPT transcript provided herein (SEQ ID NO: 249) that were tested in the present disclosure.

TABLE 5

Target Site No.	Target Site Sequence in MAPT RNA Transcript (SEQ ID NO:249)	SEQ ID NO
Site 1	GAGGUGGCCAGGUGGAAGUAAA	1
Site 2	ACCAGAGUGACUAUGAUAGUGA	2
Site 3	AAAGGACGCAUGUAUCUUGAAA	3
Site 4	GCCAAGCAGGGUUUGUGAUCAG	4
Site 5	UCAAUAGUUCCAUUUAAAUUGA	5
Site 6	CCAGGUGCAAAUAGUCUACAAA	6
Site 7	GCUGCUCCUCGCAGUUCGGUUA	7
Site 8	ACAAGCUGACCUUCCGCGAGAA	8
Site 9	CCGUCCUCGCCUCUGUCGACUA	9
Site 10	UCCUCGCCUCUGUCGACUAUCA	10
Site 11	CUCCCGUCCUCGCCUCUGUCGA	11
Site 12	AAGUCCCAUGAUUUCUUCGGUA	12
Site 13	UGAGACUGUAUCCUGUUUGCUA	13
Site 14	GCUGGAAGCCAUGCUGUCUGUU	14
Site 15	GGGCUAACCAGUUCUCUUUGUA	15
Site 16	AGCAGCUGAACAUAUACAUAGA	16
Site 17	GGCAGCAACAAAGGAUUUGAAA	17

Site 18	GCUUACAACUCCUGCAUCACAA	18
Site 19	UGGGUGGGCUAGAUAGGAUAUA	19
Site 20	GAAGGCCUGAAGCACAGGAUUA	20
Site 21	UCCCAUGAUUUCUUCGGUAAUU	21
Site 22	GUCCCAUGAUUUCUUCGGUAAU	22
Site 23	UGCUGGAGCAGCUGAACAUAUA	23
Site 24	CCCCAUGAGUUUGCCAUGUUGA	24
Site 25	GACCUUCCGCGAGAACGCCAAA	25
Site 26	CCCUGGCCAAGCAGGGUUUGUG	26
Site 27	UAACCAGUUCUCUUUGUAAGGA	27
Site 28	UGCCCUCUUUCAGGGGUCCUAA	28
Site 29	UCCAUACUGAGGGUGAAAUUAA	29
Site 30	ACAGCAGGGAUUGGGAUGAAUU	30
Site 31	CUGAUUUCUCUUCAGCUUUGAA	31
Site 32	CCCCCAGCUGCUCCUCGCAGUU	32
Site 33	UUCUGGUUUGGGUACAGUUAAA	33
Site 34	CUGUGAAUGUCUAUAUAGUGUA	34
Site 35	CUCCAUACUGAGGGUGAAAUUA	35
Site 36	GGCUACAGCAGCCCCGGCUCCC	36
Site 37	ACGCCACCAGGAUUCCAGCAAA	37
Site 38	CCGGCAGCAUCGACAUGGUAGA	38
Site39	UGGAAGAUCACGCUGGGACGUA	39
Site 40	GCAGCAUCGACAUGGUAGACUC	40
Site 41	GUGGUGUCUGGGGACACGUCUC	41
Site 42	ACCGGCAGCAUCGACAUGGUAG	42
Site 43	GUCAAGUCCAAGAUCGGCUCCA	43
Site 44	CACCGGCAGCAUCGACAUGGUA	44
Site 45	GGGUCCCUGGACAAUAUCACCC	45
Site 46	CCCAUGUUUAAAUGUACACACA	46
Site 47	CAGGAUGGCUGAGCCCCGCCAG	47
Site 48	AAGACCAAGAGGGUGACACGGA	48
Positive Control	GUAAAAGUGAAUUUGGAAAU	49

[00167] Table 6 provides targeting region polynucleotide sequences that were designed to bind to the corresponding target sites in the MAPT transcript provided in Table 5. These targeting regions find use in reducing cellular expression of the MAPT gene (which encodes Tau protein). 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 MAPT 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	UUUACUUCCACCUGGCCACCUC	50	TTTACTTCCACCTGGCCACCTC	100
Site 2	UCACUAUCAUAGUCACUCUGGU	51	TCACTATCATAGTCACTCTGGT	101
Site 3	UUUCAAGAUACAUGCGUCCUUU	52	TTTCAAGATACATGCGTCCTTT	102
Site 4	CUGAUCACAAACCCUGCUUGGC	53	CTGATCACAAACCCTGCTTGGC	103
Site 5	UCAAUUUAAAUGGAACUAUUGA	54	TCAATTTAAATGGAACTATTGA	104
Site 6	UUUGUAGACUAUUUGCACCUGG	55	TTTGTAGACTATTTGCACCTGG	105
Site 7	UAACCGAACUGCGAGGAGCAGC	56	TAACCGAACTGCGAGGAGCAGC	106
Site 8	UUCUCGCGGAAGGUCAGCUUGU	57	TTCTCGCGGAAGGTCAGCTTGT	107
Site 9	UAGUCGACAGAGGCGAGGACGG	58	TAGTCGACAGAGGCGAGGACGG	108
Site 10	UGAUAGUCGACAGAGGCGAGGA	59	TGATAGTCGACAGAGGCGAGGA	109
Site 11	UCGACAGAGGCGAGGACGGGAG	60	TCGACAGAGGCGAGGACGGGAG	110
Site 12	UACCGAAGAAAUCAUGGGACUU	61	TACCGAAGAAATCATGGGACTT	111
Site 13	UAGCAAACAGGAUACAGUCUCA	62	TAGCAAACAGGATACAGTCTCA	112
Site 14	AACAGACAGCAUGGCUUCCAGC	63	AACAGACAGCATGGCTTCCAGC	113
Site 15	UACAAAGAGAACUGGUUAGCCC	64	TACAAAGAGAACTGGTTAGCCC	114
Site 16	UCUAUGUAUAUGUUCAGCUGCU	65	TCTATGTATATGTTCAGCTGCT	115
Site 17	UUUCAAAUCCUUUGUUGCUGCC	66	TTTCAAATCCTTTGTTGCTGCC	116
Site 18	UUGUGAUGCAGGAGUUGUAAGC	67	TTGTGATGCAGGAGTTGTAAGC	117
Site 19	UAUAUCCUAUCUAGCCCACCCA	68	TATATCCTATCTAGCCCACCCA	118
Site 20	UAAUCCUGUGCUUCAGGCCUUC	69	TAATCCTGTGCTTCAGGCCTTC	119
Site 21	AAUUACCGAAGAAAUCAUGGGA	70	AATTACCGAAGAAATCATGGGA	120
Site 22	AUUACCGAAGAAAUCAUGGGAC	71	ATTACCGAAGAAATCATGGGAC	121
Site 23	UAUAUGUUCAGCUGCUCCAGCA	72	TATATGTTCAGCTGCTCCAGCA	122
Site 24	UCAACAUGGCAAACUCAUGGGG	73	TCAACATGGCAAACTCATGGGG	123
Site 25	UUUGGCGUUCUCGCGGAAGGUC	74	TTTGGCGTTCTCGCGGAAGGTC	124
Site 26	CACAAACCCUGCUUGGCCAGGG	75	CACAAACCCTGCTTGGCCAGGG	125
Site 27	UCCUUACAAAGAGAACUGGUUA	76	TCCTTACAAAGAGAACTGGTTA	126
Site 28	UUAGGACCCCUGAAAGAGGGCA	77	TTAGGACCCCTGAAAGAGGGCA	127
Site 29	UUAAUUUCACCCUCAGUAUGGA	78	TTAATTTCACCCTCAGTATGGA	128
Site 30	AAUUCAUCCCAAUCCCUGCUGU	79	AATTCATCCCAATCCCTGCTGT	129
Site 31	UUCAAAGCUGAAGAGAAAUCAG	80	TTCAAAGCTGAAGAGAAATCAG	130

Site 32	AACUGCGAGGAGCAGCUGGGGG	81	AACTGCGAGGAGCAGCTGGGGG	131
Site 33	UUUAACUGUACCCAAACCAGAA	82	TTTAACTGTACCCAAACCAGAA	132
Site 34	UACACUAUAUAGACAUUCACAG	83	TACACTATATAGACATTCACAG	133
Site 35	UAAUUUCACCCUCAGUAUGGAG	84	TAATTTCACCCTCAGTATGGAG	134
Site 36	GGGAGCCGGGGCUGCUGUAGCC	85	GGGAGCCGGGGCTGCTGTAGCC	135
Site 37	UUUGCUGGAAUCCUGGUGGCGU	86	TTTGCTGGAATCCTGGTGGCGT	136
Site 38	UCUACCAUGUCGAUGCUGCCGG	87	TCTACCATGTCGATGCTGCCGG	137
Site39	UACGUCCCAGCGUGAUCUUCCA	88	TACGTCCCAGCGTGATCTTCCA	138
Site 40	GAGUCUACCAUGUCGAUGCUGC	89	GAGTCTACCATGTCGATGCTGC	139
Site 41	GAGACGUGUCCCCAGACACCAC	90	GAGACGTGTCCCCAGACACCAC	140
Site 42	CUACCAUGUCGAUGCUGCCGGU	91	CTACCATGTCGATGCTGCCGGT	141
Site 43	UGGAGCCGAUCUUGGACUUGAC	92	TGGAGCCGATCTTGGACTTGAC	142
Site 44	UACCAUGUCGAUGCUGCCGGUG	93	TACCATGTCGATGCTGCCGGTG	143
Site 45	GGGUGAUAUUGUCCAGGGACCC	94	GGGTGATATTGTCCAGGGACCC	144
Site 46	UGUGUGUACAUUUAAACAUGGG	95	TGTGTGTACATTTAAACATGGG	145
Site 47	CUGGCGGGGCUCAGCCAUCCUG	96	CTGGCGGGGCTCAGCCATCCTG	146
Site 48	UCCGUGUCACCCUCUUGGUCUU	97	TCCGTGTCACCCTCTTGGTCTT	147
Positive Control	AUUUCCAAAUUCACUUUUAC	98	ATTTCCAAATTCACTTTAC	148
Negative cont. (SCRM)	UACUGUAUGCCGGUCGUUGCU	99	TACTGTATGCCGGTCGTTGCT	150

[00168] Table 7 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	UAGGUGGCCAGGUGGAAGUAAA	150	TAGGTGGCCAGGTGGAAGTAAA	199
Site 2	CCCAGAGUGACUAUGAUAGUGA	151	CCCAGAGTGACTATGATAGTGA	200
Site 3	CAAGGACGCAUGUAUCUUGAAA	152	CAAGGACGCATGTATCTTGAAA	201
Site 4	UCCAAGCAGGGUUUGUGAUCAG	153	TCCAAGCAGGGTTTGTGATCAG	202
Site 5	GCAAUAGUUCCAUUUAAAUUGA	154	GCAATAGTTCCATTTAAATTGA	203
Site 6	ACAGGUGCAAAUAGUCUACAAA	155	ACAGGTGCAAATAGTCTACAAA	204
Site 7	UCUGCUCCUCGCAGUUCGGUUA	156	TCTGCTCCTCGCAGTTCGGTTA	205
Site 8	CCAAGCUGACCUUCCGCGAGAA	157	CCAAGCTGACCTTCCGCGAGAA	206
Site 9	ACGUCCUCGCCUCUGUCGACUA	158	ACGTCCTCGCCTCTGTCGACTA	207
Site 10	GCCUCGCCUCUGUCGACUAUCA	159	GCCTCGCCTCTGTCGACTATCA	208

Site 11	AUCCCGUCCUCGCCUCUGUCGA	160	ATCCCGTCCTCGCCTCTGTCGA	209
Site 12	CAGUCCCAUGAUUUCUUCGGUA	161	CAGTCCCATGATTTCTTCGGTA	210
Site 13	GGAGACUGUAUCCUGUUUGCUA	162	GGAGACTGTATCCTGTTTGCTA	211
Site 14	UCUGGAAGCCAUGCUGUCUGUU	163	TCTGGAAGCCATGCTGTCTGTT	212
Site 15	UGGCUAACCAGUUCUCUUUGUA	164	TGGCTAACCAGTTCTCTTTGTA	213
Site 16	CGCAGCUGAACAUAUACAUAGA	165	CGCAGCTGAACATATACATAGA	214
Site 17	UGCAGCAACAAAGGAUUUGAAA	166	TGCAGCAACAAAGGATTTGAAA	215
Site 18	UCUUACAACUCCUGCAUCACAA	167	TCTTACAACTCCTGCATCACAA	216
Site 19	GGGGUGGGCUAGAUAGGAUAUA	168	GGGGTGGGCTAGATAGGATATA	217
Site 20	UAAGGCCUGAAGCACAGGAUUA	169	TAAGGCCTGAAGCACAGGATTA	218
Site 21	GCCCAUGAUUUCUUCGGUAAUU	170	GCCCATGATTTCTTCGGTAATT	219
Site 22	UUCCCAUGAUUUCUUCGGUAAU	171	TTCCCATGATTTCTTCGGTAAT	220
Site 23	GGCUGGAGCAGCUGAACAUAUA	172	GGCTGGAGCAGCTGAACATATA	221
Site 24	ACCCAUGAGUUUGCCAUGUUGA	173	ACCCATGAGTTTGCCATGTTGA	222
Site 25	UACCUUCCGCGAGAACGCCAAA	174	TACCTTCCGCGAGAACGCCAAA	223
Site 26	ACCUGGCCAAGCAGGGUUUGUG	175	ACCTGGCCAAGCAGGGTTTGTG	224
Site 27	GAACCAGUUCUCUUUGUAAGGA	176	GAACCAGTTCTCTTTGTAAGGA	225
Site 28	GGCCCUCUUUCAGGGGUCCUAA	177	GGCCCTCTTTCAGGGGTCCTAA	226
Site 29	GCCAUACUGAGGGUGAAAUUAA	178	GCCATACTGAGGGTGAAATTAA	227
Site 30	CCAGCAGGGAUUGGGAUGAAUU	179	CCAGCAGGGATTGGGATGAATT	228
Site 31	AUGAUUUCUCUUCAGCUUUGAA	180	ATGATTTCTCTTCAGCTTTGAA	229
Site 32	ACCCCAGCUGCUCCUCGCAGUU	181	ACCCCAGCTGCTCCTCGCAGTT	230
Site 33	GUCUGGUUUGGGUACAGUUAAA	182	GTCTGGTTTGGGTACAGTTAAA	231
Site 34	AUGUGAAUGUCUAUAUAGUGUA	183	ATGTGAATGTCTATATAGTGTA	232
Site 35	AUCCAUACUGAGGGUGAAAUUA	184	ATCCATACTGAGGGTGAAATTA	233
Site 36	UGCUACAGCAGCCCCGGCUCCC	185	TGCTACAGCAGCCCCGGCTCCC	234
Site 37	CCGCCACCAGGAUUCCAGCAAA	186	CCGCCACCAGGATTCCAGCAAA	235
Site 38	ACGGCAGCAUCGACAUGGUAGA	187	ACGGCAGCATCGACATGGTAGA	236
Site39	GGGAAGAUCACGCUGGGACGUA	188	GGGAAGATCACGCTGGGACGTA	237
Site 40	UCAGCAUCGACAUGGUAGACUC	189	TCAGCATCGACATGGTAGACTC	238
Site 41	UUGGUGUCUGGGGACACGUCUC	190	TTGGTGTCTGGGGACACGTCTC	239
Site 42	CCCGGCAGCAUCGACAUGGUAG	191	CCCGGCAGCATCGACATGGTAG	240
Site 43	UUCAAGUCCAAGAUCGGCUCCA	192	TTCAAGTCCAAGATCGGCTCCA	241
Site 44	AACCGGCAGCAUCGACAUGGUA	193	AACCGGCAGCATCGACATGGTA	242
Site 45	UGGUCCCUGGACAAUAUCACCC	194	TGGTCCCTGGACAATATCACCC	243
Site 46	ACCAUGUUUAAAUGUACACACA	195	ACCATGTTTAAATGTACACACA	244
Site 47	AAGGAUGGCUGAGCCCCGCCAG	196	AAGGATGGCTGAGCCCCGCCAG	245
Site 48	CAGACCAAGAGGGUGACACGGA	197	CAGACCAAGAGGGTGACACGGA	246
(SCRM) Negative Control	AAGCAACGACCGGCAUACAGUA	198	AAGCAACGACCGGCATACAGTA	247

[00169] Additional sequences are provided below:

[00170] SEQ ID NO:425 is U6 promoter and has the following DNA sequence:

[00171] GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAG AGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGA CGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTTAAAATTATGTTTTAAAATGG ACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTG GAAAGGACGAAACACC (SEQ ID NO:425)

[00172] SEQ ID NO:426 is a Pol-III terminator and has the following DNA sequence: TTTTTT (SEQ ID NO:426).

[00173] SEQ ID NO:427 is a 5' AAV2 ITR and has the following DNA sequence:

[00174] CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCC GGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA GGGAGTGGCCAACTCCATCACTAGGGGTTCCT (SEQ ID NO:427).

[00175] SEQ ID NO:428 is a 3' AAV2 ITR (complement to SEQ ID NO:427) and has the following DNA sequence:

[00176] AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCA CTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCA GTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG (SEQ ID NO:428)

[00177] SEQ ID NO:429 is an AAV ITR present in scAAV constructs (replaces 3' ITR in ssAAV) and has the following DNA sequence:

[00178] CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC GCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA GGGA (SEQ ID NO:429)

[00179] SEQ ID NO:430 is an example of a stuffer sequence and has the following DNA sequence:

[00180] ACAAGAATCTGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCT TGGTCCCCCACATTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGT CCTTTATTGGAACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCC ATGTTACATGGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAG AGCCAGAGGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTG ATAAATGGAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAA

ATAAATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTAC
TGTCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTT
AAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTT
GTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTAT
TTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAA
TAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAA
TTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAA
GAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTT
GGAGACACAGAGTATTCCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAAT
ATTATGCTTACTGCCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTC
GAGGCTTTTCAGTGCTTGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGAT
TTATTCGTTGACATTTCATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTTTT
GGGAGTGTATATTTCTCATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAACTGTT
TCTACTTTCATTTGTGACTTATGGCATTTGAATGTGTACATTCAATTGTGTTGGCACATA
CTCTAGTCAAATGTGTGTGCCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATC
CAATTGTACATATTAGCCTTATAATATAATCCATCAAAATGTACACACCTGTGTCAAATA
TGTTGTTTGTTTACTGGGATTTTATTTCCGTTAAC (SEQ ID NO:430)

[00181] SEQ ID NO:431 is an example of a stuffer sequence and has the following DNA sequence:

[00182] ACAAGAATCTGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCT
TGGTCCCCCACATTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGT
CCTTTATTGGAACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCC
ATGTTACATGGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAG
AGCCAGAGGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTG
ATAAATGGAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAA
ATAAATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTAC
TGTCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTT
AAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTT
GTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTAT
TTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAA
TAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAA

TTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAA
GAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTT
GGAGACACAGAGTATTCCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAAT
ATTATGCTTACTGCCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTC
GAGGCTTTTCAGTGCTTGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGAT
TTATTCGTTGACATTTCATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTTTT
GGGAGTGTATATTTCTCATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAACTGTT
TCTACTTTCATTTGTGACTTATGGCATTTGAATGTGTACATTCAATTGTGTTGGCACATA
CTCTAGTCAAATGTGTGTGCCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATC
CAATTGTACATATTAGCCTTATAATATAATCCATCAAAATGTACACACCTGTGTCAAATA
TGTTGTTTGTTTACTGGGATTTTATTTCCGTTAACTACTGCCAAAATTAAATAAACAGT
TATTCTAAAAAGCACTAGATAACTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCG
TTGCCTCATATATCAATGCAGGCTTTCCCCAGCTACCAAAGCTGCCATTTGATTCAGAT
ACCCAATCTGCCTCAGATGTGCTTGCGGTTGGGGAGTTACATTTGCGGCTATTTATTGG
ATGCTCAAAGAGATTAGTAAACACACACAGAGCTAGAGGAATTTTATACATTATCCTG
ATAATAACTAAAGTATTGTTTAGTTGGATGCAAATGAAGGAAATATGTCAAATGACTTA
CATATCTGACTACTAAAAAAATTATTGTATTATTACAGAGTATTACTGTAGTGTATTATTG
TAGTGTATTATTATAGAGATAATAACTTAGATACAATGTTAAGGAAAGTAAAATATTTCA
TTTTCCTCCAATAACAATAAAGGGAATGTTCTTCTGATTGCAATTAACCTCAGATATTT
TTGGATATGATCTATTTTTTTAAAAATGATGATC (SEQ ID NO:431)

[00183] SEQ ID NO:432 is an example of a stuffer sequence and has the following DNA sequence:

[00184] GATAAAATATGAAAGAGACGAGGGAAAATAATTGGACGTGGAATATCCCCAGTT
TTTATGTTATACAGGTGATGTAGTTTTGGACAAAAAATGATGGAAGAGAGAGGCATGT
AGAAAGTGTAAAGAAGTTCAGGGGAAGTTTTAAATTTAGCTCTCTTAATCTTGGGTCA
CTCATCAGAAAAAGGAAAGCACAATGTTAGAAATTCAGAGATCATATCACTGGTTGA
CTTCCGATCATATCACTGGTTGACTTCCTTAGTACTACAGGTTTAAAACTTAAGAGGCT
TTTGTCTCAAAACAACATCATGGTACATGTTAGTTTTCTCCTTTTTTTCTTTTCCCCAAT
ATAATATATTTACTTAGTCCATTAATTTTTCAATGGTCTGTTCATTGTTTTCTTTTTTATGT
GATCTTCTCATTCCCTCAATACTAGCTGTACATTTTGAAAATATAAATGTTTGGTATATA
ATAAATATTATATGGGGGAAGGGAAAAGGGAAAGGAGAAAGGGAAGGAGAAAGAGG

TATTCTGTGTAGTTATATTAGTGGTCTAACAAACAGACATCTACGAGATAAAATGAAAC
AAAACACACTGACAATTAAATAGAGAAATCAGACGAAAGTTGAGTCATGTAATCCAA
AGCAAAATAGTATTTCAAGAATGAGGGTGTGAATCAGTTTTACAGATACACACGACTG
TTTGAGTAATACGCAATAGACAAGTGATATATGGGATTTAGTGACAAGTATAAGATATG
ATTCCAAAAACAGGGTAAATGCCTGAAGAAAGGAGAGTTAAGAAAAATAAAATTAA
ACCTTGTCACAGGCAACTCTTTCCATGAGTCTTCCTATCATAGAAAATAAATAAATGA
GTTGACAGTGGCATATCTAGAACTAAGACA (SEQ ID NO:432)

[00185] SEQ ID NO:433 is an example of a stuffer sequence and has the following DNA sequence:

[00186] ACAAGAATCTGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCT
TGGTCCCCCACATTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGT
CCTTTATTGGAACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCC
ATGTTACATGGATGTCACATTTCTAGCATAAACAAGTGTTCTCATGACTGTATACCTAG
AGCCAGAGGAGATGTTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTG
ATAAATGGAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAA
ATAAATTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGAGTAC
TGTCATATAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTT
AAAAGATGGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTT
GTTTGGTTTTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTAT
TTGAATGAACAAAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAA
TAAATTCATGAAACATTGAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAA
TTGAGAAGTTCATTATGCATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAA
GAACTTATTGTAGTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTT
GGAGACACAGAGTATTCCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAAT
ATTATGCTTACTGCCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTC
GAGGCTTTTCAGTGCTTGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGAT
TTATTCGTTGACATTTCATTAATTAGCTGTAACATATTTATACTAAGATGTGCCTTCTTTT
GGGAGTGTATATTTCTCATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAACTGTT
TCTACTTTCATTTGTGACTTATGGCATTTGAATGTGTACATTCAATTGTGTTGGCACATA
CTCTAGTCAAATGTGTGTGCCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATC
CAATTGTACATATTAGCCTTATAATATAATCCATCAAAATGTACACACCTGTGTCAAATA

TGTTGTTTGTTTACTGGGATTTTATTTCCGTTAACTACTGCCAAAATTAAATAAACAGT
TATTCTAAAAAGCACTAGATAACTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCG
TTGCCTCATATATCAATGCAGGCTTTCCCCAGCTACCAAAGCTGCCATTTGATTCAGAT
ACCCAATCTGCCTCAGATGTGCTTGCGGTTGGGGAGTTACATTTGCGGCTATTTATTGG
ATGCTCAAAGAGATTAGTAAACACACACAGAGCTAGAGGAATTTTATACATTATCCTG
ATAATAACTAAAGTATTGTTTAGTTGGATGCAAATGAAGGAAATATGTCAAATGACTTA
CATATCTGACTACTAAAAAAATTATTGTATTATTACAGAGTATTACTGTAGTGTATTATTG
TAGTGTATTATTATAGAGATAATAACTTAGATACAATGTTAAGGAAAGTAAAATATTTCA
TTTTCCTCCAATAACAATAAAGGGAATGTTCTTCTGATTGCAATTAACCTCAGATATTT
TTGGATATGATCTATTTTTTTAAAAATGATGATCCATGGGATAAAATATGAAAGAGACGA
GGGAAAATAATTGGACGTGGAATATCCCCAGTTTTTATGTTATACAGGTGATGTAGTTT
TGGACAAAAAATGATGGAAGAGAGAGGCATGTAGAAAGTGTAAAGAAGTTCAGGGG
AAGTTTTAAATTTAGCTCTCTTAATCTTGGGTCACTCATCAGAAAAAGGAAAGCACAA
TGTTAGAAATTCAGAGATCATATCACTGGTTGACTTCCGATCATATCACTGGTTGACTT
CCTTAGTACTACAGGTTTAAAACTTAAGAGGCTTTTGTCTCAAAACAACATCATGGTA
CATGTTAGTTTTCTCCTTTTTTTCTTTTCCCCAATATAATATATTTACTTAGTCCATTAATT
TTTCAATGGTCTGTTCATTGTTTTCTTTTTTATGTGATCTTCTCATTCCCTCAATACTAGC
TGTACATTTTGAAAATATAAATGTTTGGTATATAATAAATATTATATGGGGGAAGGGAAA
AGGGAAAGGAGAAAGGGAAGGAGAAAGAGGTATTCTGTGTAGTTATATTAGTGGTCT
AACAAACAGACATCTACGAGATAAAATGAAACAAAACACACTGACAATTAAATAGAG
AAATCAGACGAAAGTTGAGTCATGTAATCCAAAGCAAAATAGTATTTCAAGAATGAG
GGTGTGAATCAGTTTTACAGATACACACGACTGTTTGAGTAATACGCAATAGACAAGT
GATATATGGGATTTAGTGACAAGTATAAGATATGATTCCAAAAACAGGGTAAATGCCTG
AAGAAAGGAGAGTTAAGAAAAATAAAATTAAACCTTGTCACAGGCAACTCTTTCCAT
GAGTCTTCCTATCATAGAAAATAAATAAATGAGTTGACAGTGGCATATCTAGAACTAA
GACA (SEQ ID NO:433)

[00187] SEQ ID NO:434 is an example of a stuffer sequence and has the following DNA sequence:

[00188] ACAAGAATCTGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTTTTTCAGTCT
TGGTCCCCCACATTACAGGAATGTTTAGGCAGAAACTCTCCAAAGCTGCCATTCATGT
CCTTTATTGGAACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCC

ATGTTACATGGATGTCACATTTCTAGCATAAACAAGTGTTCtcatgaCTGTATACCTAGAG
CCAGAGGagatgtTCCTTTTGAGAAACAGTGACAAAAGTGTAGAAGAAATGTTGATAAA
TGGAGCAGTAGATAATAGATCAGCAATCCCTTAATATTTATTTATAATCCCTGAAATAAA
TTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGagtactGTCATA
TAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTTAAAAGAT
GGCAAGGTTATGACAGAAAGAAAATGTAGTTTTTTTTGTTTGTTTTTTTTTGTTTGGTT
TTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTCATTACTTTATCTTATTTGAATGA
ACAAAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAATAAATtcatga
AACATTGAAAAAATTTATCTTCTCAGAAGTTAGCCTTCTCATCATAAATTGAGAAGTTC
ATTATGCATTTTCAGATTAACTTCAATGCACAGAATAAGGAGACAAAGAACTTATTGTA
GTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTTGGAGACACAGA
GTATTCCTTTGAATGAAGACTTAGGACAGATATTTTTACATCACAATATTATGCTTACTG
CCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCAG
TGCTTGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGATTTATTCGTTGAC
ATTTCATTAATTAGCTGTAACATATTTATACTAagatgtGCCTTCTTTTGGGAGTGTATATTT
CTCATTATAGTCCCATTGTGTACATTCTTATAGGCATGTAACTGTTTCTACTTTCATTTGT
GACTTATGGCATTTGAATGTGTACATTcaattgTGTTGGCACATACtctagtCAAATGTGTGTG
CCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATCcaattgTACATATTAGCCTTAT
AATATAATCCATCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGATTT
TATTTCCGTTAACTACTGCCAAAATTAAATAAACAGTTATTCTAAAAAGCACTAGATAA
CTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCGTTGCCTCATATATCAATGCAGG
CTTTCCCCAGCTACCAAAGCTGCCATTTGATTCAGATACCCAATCTGCCTCagatgtGCTT
GCGGTTGGGGAGTTACATTTGCGGCTATTTATTGGATGCTCAAAGAGATTAGTAAACA
CACACAGAGCTAGAGGAATTTTATACATTATCCTGATAATAACTAAAGTATTGTTTAGT
TGGATGCAAATGAAGGAAATATGTCAAATGACTTACATATCTGACTACTAAAAAAATT
ATTGTATTATTACAGAGTATTACTGTAGTGTATTATTGTAGTGTATTATTATAGAGATAAT
AACTTAGATACAATGTTAAGGAAAGTAAAATATTTCATTTTCCTCCAATAACAATAAAG
GGAATGTTCTTCTGATTGCAATTAACCTCAGATATTTTTGGATATGATCTATTTTTTAAA
AATGATGATCCATggGATAAAATATGAAAGAGACGAGGGAAAATAATTGGACGTGGAA
TATCCCCAGTTTTTATGTTATACAGGTGATGTAGTTTTGGACAAAAAATGATGGAAGAG
AGAGGCATGTAGAAAGTGTAAAGAAGTTCAGGGGAAGTTTTAAATTTAGCTCTCTTA

ATCTTGGGTCACTCATCAGAAAAAGGAAAGCACAATGTTAGAAATTCAGAGATCATAT
CACTGGTTGAcTTCCGATCATATCACTGGTTGAcTTCCTTAGTACTACAGGTTTAAAAC
TTAAGAGGCTTTTGTCTCAAAACAACATCAACAAAAAGCCAGGTGTGGTAGTGTGCA
TCTGTAGTCTCAGCTACTGAGGAGACCAATGCAGGAAAATTGCTTAAGCCCAGCAAT
TTGAGGCTGTAGTCAGTCAAGGTCTCACTACTGCACTGCAGACTGCGTTACAGAACA
CTTTCTTAAAAAAAAGAAAGGAAAGAAAAGAAAAAGGGAAGGGAAAAGGGAAAGG
AGAAAGGGAAGGAGAAAGAGGTATTCTGTGTAGTTATATTAGTGGTCTAACAAACAG
ACATCTACGAGATAAAATGAAACAAAACACACTGACAATTAAATAGAGAAATCAGAC
GAAAGTTGAGTCATGTAATCCAAAGCAAAATAGTATTTCAAGAATGAGGGTGTGAATC
AGTTTTACAGATACACACGACTGTTTGAGTAATACGCAATAGACAAGTGATATATGGG
ATTTAGTGACAAGTATAAGATATGATTCCAAAAACAGGGTAAATGCCTGAAGAAAGG
AGAGTTAAGAAAAATAAAATTAAACCTTGTCACAGGCAACTCTTTCCATGAGTCTTCC
TATCATAGAAAATAAATAAATGAGTTGACAGTGGCATATCTAGAACTAAGACA (SEQ
ID NO:434)

[00189] SEQ ID NO:435 is an example of a stuffer sequence and has the following DNA sequence:

[00190] ACAAGAATCTGTTCTATAAATTTCAGATAATCTTTTTGAAGAGTTATTTCACAGT
AGCTCCCCCACATTACAGGATTGTATAGGCAGAAACTCTCCATAGCTGCCATTCAAGT
CCTTTATTGGAACAGCCATCTGCATCTATACACCAGTACATCATTGTTACCCAGATCCC
ATGTTACATGGATGTCACATTTCTAGCATAAACAAGTGTTCtcatgaCTGTATACCTAGAG
CCAGAGGagatgtTCCTTTTGAGAAACAGTGTCAAAAGTGTAGAAGAAATGTTGATAAA
TGGAGCAGTAGATAATACATCTGCAATCCCTTAATATTTATTTATAATCCCTGAAATAAA
TTTTGCTAGAGACTCTTGTTAATATGAGATAAAATATTTAACTTGATTTGagtactGTCATA
TAATACAAGGACTATGTAGATGCTTTAGAAAACATTGGCCAAGATAATATTTAAAAGAT
GGCAAGGTTATCACAGAAAGAAAATGTAGAAATTTTTGTTTGTTATATAATGTTTGGTT
TTTAGGTTTTTCACATTGTAGTGCAGGGTGTGGCTTCTTAAGTTTATCTTATTTGAATG
AACAAAATTTGAGAACAGCTATGTCTAGGAATGTGAAATAGTTTATGAAAATAAATtcat
gaAACATTGAAATAATTTATCTTCTCAGAAGATAGCCTTCTCATCATAAATTGAGAAGTT
CATTATGCATTTTGAGTATAACTTCAATGCACAGAATAAGGAGACAAAGAACTTATTGT
AGTTTTGGGAAATCTGGATAATGGTAGAAATCTATTAACTCTTGTTTTGGAGACACAG
AGTATTCCTTAGAATCAAGACTTAGGACAGATATTTTTACATCACAATATTATGCTTACT

GCCTGTCATTCCTCTTTGATTATTACATTTTGGGGGAAGAAAAATTTCGAGGCTTTTCA
GTGCTTGAGTTCCTAAATAAATTTCACATACTTTAGAAAGCATTTGATTTATTCGTTGA
CATTTGATTAATTAGCTGTAACATATTTATACTAagatgtGCCTTCTTTTGGGACAGTTTATA
ACTCTTTTTTACTCCCATTGTGTACAAACTTATAGGCATGTAACTGTTTCTACTTTCTTTT
GTGACTTATGGCATTTGAATGTGTACATTcaattgTGTTGGCACATACtctagtCAAATGTGTG
TGCCTGTATCTGTACATCCCTGTCAGGTATGTATACATATATCcaattgTACATATTAGCCTT
ATAATATAATCCTTCAAAATGTACACACCTGTGTCAAATATGTTGTTTGTTTACTGGGAT
TTTATTTCCGTTAACTACTGCCAAAATTAAATAAACAGTTATTCTAAAAAGCACTAGAT
AACTCCCTATTGGTGTTTGAAGCCCCAATGTGCTTACCGTTGCCTCATATATCAATGCA
GGCTTTCCCCAGCTACCAAAGCTGCCATTTGATTCACATACCCAATCTGCCTCagatgtGC
TTGCGGTTGGGGAGTTACATTTGCGGCTATTTATTGGATGCTCAAAGAGATTAGTAAA
CACACACAGAGCTAGAGGAATTTTATAGATTATCCTGATAATAACTAAAGTATTGTTTA
GTTGGATGCTTATGTTGGAAATATGTCATATGACTTACATATCTGACTACTATATAAATTA
TTGTATTATTACAGAGTATTACTGTAGTGTATTATTGTAGTGTATTATTATAGAGATAATA
ACTTAGATACAATGTTAAGGAAAGTAAAATATTTCTTTTTCCTCCAATAACAATAAAGG
GAAAGTAGTTCTCAATGCAATTAACCTCACATATTTTTGGATATGATCTATATTTTAAAA
ATGATGATCCATggGATAAATTATCAAAGAGACGAGGGAAAATAATTGGACGTGGAAT
ATCCCCAGTATATATGTTATACAGGTGATGTAGTTTTGGACAAAAATTGAAGGAAGAG
AGAGGCAAGTAGTAAGAGAAAAGAAGTTCAGGGGAAGTTTTAAATTTAGCTCTCTTA
ATCTTGGGTCACTGATCAGAAAAAGGAAAGCACAATGTTAGAAATTCAGAGATCATAT
CACTGGATGAcTTCCGATCATATCACTGGTTGAcTTCCTTAGTACTACAGGTTTAAAAC
TTAAGAGGCTTTTGTCTCAAAACAACATCAGGGAAGGGAAAAGGGAAAGGAGAAAG
GGAAGGAGAAAGAGGTATTCTGTGTAGTTATATTAGTGGTCTAACAAACAGACATCTA
CGAGATATAATCAAACAAAACACACTGACAATTAAATAGAGAAATGAGACGAAAGTT
GAGTCATGTAATCCAAAGCAAAATAGTATTTCAAGAATGAGGGTGTGAATCACTTAAA
CAGATACACACCTGTGATTGAGTTTAACGCAATAGACAAGTGATATATGGGATTTAGT
GACAAGTATAAGATATGATTCCAAAAACAGGGTAAATGCCTGAAGAAAGGAGAGTTA
AGAAAAATAAAATTAAACCTTGTCACAGGCAACTCTTTCCATGAGTCTTCCTATCATA
GAAAATAAATAAATGAGTTGACAGTGGCATATCTAGAACTAAGACA (SEQ ID NO:435)

EMBODIMENTS

[00191] Exemplary embodiments are listed below.

[00192] Embodiment 1. A polynucleotide comprising a targeting region that binds to a target site in an endogenous mRNA encoding Tau, 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 48.

[00193] Embodiment 2. The polynucleotide of embodiment 1, wherein the endogenous mRNA encoding Tau is transcribed from an endogenous MAPT gene.

[00194] Embodiment 3. The polynucleotide of embodiment 1, wherein the endogenous mRNA encoding Tau comprises a sequence having at least 95% sequence identity to SEQ ID NO: 249.

[00195] 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 48.

[00196] 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.

[00197] 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.

[00198] 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.

[00199] 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: 50-97.

[00200] 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: 50-97.

[00201] Embodiment 10. The polynucleotide of any one of embodiments 1 to 4, wherein the targeting region comprises any one of SEQ ID NOs: 50-97.

[00202] 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.

[00203] 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.

[00204] 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.

[00205] Embodiment 14. The expression cassette of embodiment 13, further comprising an enhancer operably linked to the promoter.

[00206] Embodiment 15. The expression cassette of embodiment 13 or 14, wherein the RNA is a pri-miRNA.

[00207] Embodiment 16. The expression cassette of embodiment 15, wherein the pri-miRNA comprises a miRNA scaffold.

[00208] Embodiment 17. A vector comprising the expression cassette of any one of embodiments 13 to 16.

[00209] Embodiment 18. The vector of embodiment 17, wherein the vector is a plasmid.

[00210] Embodiment 19. The vector of embodiment 18, wherein the vector is a viral vector.

[00211] Embodiment 20. The vector of embodiment 19, wherein the viral vector is an adeno-associated virus (AAV) vector.

[00212] 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.

[00213] Embodiment 22. The vector of embodiment 20 or 21, wherein the AAV is an scAAV.

[00214] Embodiment 23. The vector of embodiment 19, wherein the viral vector is a lentiviral vector.

[00215] 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.

[00216] Embodiment 25. A method of reducing expression of a gene encoding Tau 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.

[00217] Embodiment 26. The method of embodiment 25, wherein expression of the mRNA encoding Tau and/or the Tau 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.

[00218] Embodiment 27. The method of embodiment 26, wherein the expression of the mRNA encoding Tau and/or the Tau protein encoded by the mRNA is reduced at least 5% in the contacted cell compared to the comparable non-contacted cell.

[00219] Embodiment 28. The method of embodiment 26, wherein the expression of the mRNA encoding Tau and/or the Tau 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.

[00220] Embodiment 29. The method of any one of embodiments 25 to 28, wherein the expression of the mRNA encoding Tau is measured by quantitative polymerase chain reaction.

[00221] Embodiment 30. The method of any one of embodiments 25 to 29, wherein the cell is a cultured cell.

[00222] 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.

[00223] Embodiment 32. The method of any one of embodiments 25 to 29, wherein the cell is in vivo.

[00224] Embodiment 33. The method of embodiment 32, wherein the cell is a neuron.

[00225] 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.

[00226] 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).

[00227] Embodiment 36. The method of embodiment 35, wherein the delivering comprises intraparenchymal injection, intrathecal injection, intra-cisterna magna injection, or intracerebroventricular injection.

[00228] Embodiment 37. A method for treating or preventing a tauopathy in a subject comprising administering a therapeutically or prophylactically effective amount of the pharmaceutical composition of embodiment 24 to a subject.

[00229] Embodiment 38. The method of embodiment 37, wherein the subject is a mammal.

[00230] Embodiment 39. The method of embodiment 38, wherein the mammal is a human.

[00231] Embodiment 40. The method of embodiment 37, wherein the subject has Alzheimer's Disease (AD) or Frontotemporal Dementia and Parkinsonism Linked To Chromosome 17 (FTDP-17).

[00232] Embodiment 41. The method of any one of embodiments 37 to 40, 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.

[00233] Embodiment 42. 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 a tauopathy.

[00234] Embodiment 43. 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.

[00235] Embodiment 44. The cell of embodiment 43, wherein the cell comprises the viral vector of any one of embodiments 19 to 23.

[00236] Embodiment 45. A method of producing a composition comprising viral particles, comprising: (i) culturing the cell of embodiment 44 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.

[00237] Embodiment 46. The method of embodiment 45, wherein the viral particles are AAV particles.

[00238] 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

[00239] Expression vectors encoding engineered pri-miRNAs specific for Sites 1-48 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).

[00240] A reporter system in HEK293T cells was used to evaluate the knockdown of exogenous MAPT expression using 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 MAPT-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).

[00241] 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 treated sample (represented by horizontal dotted line).

[00242] The results of this experiment are shown in Figure 1, which shows that expression vectors that encode a microRNA with guide strand selected from Table 6 (i.e., SEQ ID NOs: 50 and 85-

90, with 99 being a positive control) reduce the amount of MAPT transcript in the cell by approximately 20% to 90% as compared to the scrambled sequence (SCRM).

EXAMPLE 2

RNA OLIGONUCLEOTIDE SCREENING IN IPSC GABAERGIC NEURONS

[00243] 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.

[00244] iPSC GABAergic neurons were purchased from Fujifilm Cellular Dynamics and thawed according to the manufacturer's directions. A 96 well plate was precoated with Poly-L-Ornithine (PLO) and laminin and cells were plated at 60,000 cells/well. 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 3-5 days, until they were harvested at DIV14 (13-day transduction).

[00245] Positive control Roche-ASO-001933 was purchased from IDT with the same chemical modifications as described in Easton et al, 2022. GABAergic neurons were incubated with 5uM of ASO-001933 diluted in media beginning 24hrs post plating. Neurons were maintained according to manufacturer's protocol, and media containing 5uM ASO-001933 was replaced on DIV7 and DIV11 before harvested on DIV14 (13-day treatment).

[00246] The iPSC derived GABAergic 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 MAPT expression was calculated by dividing MAPT transcript copies by GAPDH transcript copies. Relative MAPT expression was then calculated by dividing miRNA treated samples expression by scramble treated samples expression.

[00247] The results of this experiment are shown in Figure 2, which shows significant knock down of endogenous MAPT expression by each of the miRNA candidates as compared to the scrambled sequence.

EXAMPLE 3

RNA OLIGONUCLEOTIDE SCREENING IN IPSC GLUTAMATERGIC NEURONS

[00248] A subset of the miRNA candidates were screened and validated for endogenous MAPT transcript knock down in iPSC glutamatergic neurons using an AAV delivery approach (as in Example 2). 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).

[00249] 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 MAPT expression was calculated by dividing MAPT transcript copies by GAPDH transcript copies. Relative MAPT expression was then calculated by dividing miRNA treated samples expression by scramble treated samples expression.

[00250] The results of this experiment are shown in Figure 3, which shows significant knock down of endogenous MAPT expression by the miRNA candidates as compared to the scrambled sequence.

[00251] TaqMan Assays from ThermoFisher Scientific: MAPT - Hs00902194_m1; GAPDH - Hs99999905_m1.

EXAMPLE 4

IN VIVO TESTING IN WTMICE

[00252] A subset of miR-E constructs were tested *in vivo* in WT C57BL6 Mice. At post-natal day 1 (P1), WT mice were dosed with AAV9 vectors expressing one of four miR-E-based pri-miRNAs

constructs via bilateral sICV (stereotaxic intracerebral ventricle) injection at 1E11 viral genomes (vg) per animal. The guide RNA sequences used in the four different miR-E constructs are: SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:53, and SEQ ID NO:55. Tissues were collected at 4 weeks and 8 weeks post injection to assess endogenous *mMapt* mRNA levels and mTau protein levels.

[00253] As shown in Figures 4A and 4B, all four miR-E candidates were effective at reducing *mMapt* mRNA levels (top panels) and Tau protein (bottom panels) at both 4-week and 8-week timepoints in the hippocampus (Figure 4A) and cortex (Figure 4B) of the treated mice (results are relative to PBS control treated animals). SEQ ID NOs:50 and 51 guide sequences had the strongest performance. *mMapt* mRNA knockdown (KD) was similar for all candidates at both timepoints (ns = no significant difference). For the SEQ ID NO:50 guide, mTau protein KD was significantly stronger at the 8-week timepoint in both cortex and hippocampus ($P \leq 0.05$, indicated with *; $P \leq 0.001$ indicated with ***). For SEQ ID NO:50, mTau protein KD was significantly stronger at the 8-week timepoint in the cortex ($P \leq 0.05$ indicated with *). It is noted that mi70 is not 100% conserved in mouse, so the level of KD was not expected to be as strong in this assay as it would be in human tissues/cells.

[00254] mMAPT mRNA levels from the WT C57BL/6 tissues were detected using TaqMan Assays from ThermoFisher Scientific: mMapt – Mm00521988_m1(Gene of interest) and mGusb - Mm00446953_m1(Housekeeping Control) using digital-droplet PCR quantification methods. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample. Normalized *mMapt* transcript expression was calculated by dividing *mMapt* transcript copies by *mGusb* transcript copies. Relative *mMapt* expression was then calculated by dividing AAV9-treated samples expression by PBS-treated samples expression. Total mouse Tau protein levels were detected using R-PLEX Mouse Tau (total) Assay (Meso Scale Discovery, K1528ER). Relative mTau expression was then calculated by dividing AAV9-treated animals by the PBS-treated animals.

EXAMPLE 5

ALTERNATIVE SCAFFOLD SCREEN IN IPSC GABAERGIC NEURONS

[00255] The MAPT transcript KD performance of guide sequences of SEQ ID NO:50, 51, 53, and 55 in the context of different scaffolds was tested in iPSC GABAergic neurons (as described in

Example 2). Scaffolds tested (in addition to miR-E) were derived from the following: miR-100, miR-132, miR-190a, miR-451, and miR-130a (listed at the bottom of each table). These constructs were linked to a ubiquitous U6 promoter and used to prepare AAV-DJ virons and used to transduce the iPSC GABAergic neurons at either low (1E4) or high (1E6) dose. MAPT mRNA KD activity for each treatment were determined as described in Example 2 (higher numbers in the tables indicate higher knockdown; thus, higher numbers indicate better performance).

[00256] As shown in Figure 5, the miR-451 scaffold had the lowest MAPT KD activity at both low (upper panel) and high (lower panel) doses as compared to the same targeting region in all other scaffolds. In addition, miR-E-53 and miR-190a-53 showed decreased performance over other guides in the miR-E and miR-190a scaffolds. This demonstrates that both the selection of the scaffold sequence and guide sequence can impact the KD activity of the disclosed MAPT targeting inhibitory RNAs of the present disclosure.

EXAMPLE 6

SCAFFOLD SCREEN IN WTMICE

[00257] The following scaffold/guide pri-miRNA sequences were tested *in vivo* in WT mice: miR-E-50, miR-E-51, miR-E-55, miR-100-50, miR-100-51, miR-100-55, miR-132-51, miR-132-55, miR-130a-50, miR-130a-51, miR-130a-55, and miR-190-50.

[00258] P1 WT C57BL/6 animals were dosed with the AAV9-vectorized miRNA candidates noted above via bilateral sICV at 6E10vg/mouse. Hippocampus tissues were collected at two timepoints, 4 weeks and 8 weeks, post injection to assess endogenous *mMapt* transcript (top panel) and mTau protein levels (lower panel). *mMapt* transcript levels from the WT C57BL/6 tissues were detected using TaqMan Assays from ThermoFisher Scientific: mMapt – Mm00521988_m1(Gene of interest) and mGusb -Mm00446953_m1(Housekeeping Control) using digital-droplet PCR quantification methods. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample. Normalized *mMapt* transcript expression was calculated by dividing *mMapt* transcript copies by *mGusb* transcript copies. Relative *mMapt* expression was then calculated by dividing AAV9-treated samples expression by PBS-treated samples expression. Total mouse Tau protein levels were detected using R-PLEX Mouse Tau (total) Assay (Meso Scale Discovery, K1528ER). Relative mTau expression was then calculated by dividing AAV9-treated animals by the PBS-treated animals.

[00259] As shown in Figure 6, each of the pri-miRNAs tested showed reduced expression of both endogenous *mMapt* transcript (top panel) and endogenous Tau protein (lower panel). Many of the pri-miRNAs tested demonstrated increased activity in Tau protein reduction at the 8-week timepoint as compared to the 4-week timepoint, including all miR-E and miR-100 constructs.

[00260] Figure 7, top panel, shows body weight of a subset of the treated mice (indicated at the bottom of the plot), demonstrating that there was no significant impact of the treatment.

[00261] Figure 7, bottom panel, shows the hind limb clasping score of a subset of the treated mice (indicated at the bottom of the plot). In this assay, the mouse is gently lifted by its tail, allowing it to hang freely, and the hindlimb posture is observed over approximately 30 seconds. The degree of hindlimb clasping is scored as follows: 0 = both hindlimbs are splayed outward, away from the abdomen; 1 = one or both hindlimbs are partially retracted towards the abdomen, but not touching it; 2 = both hindlimbs are partially retracted and touching the abdomen, but not each other; 3 = both hindlimbs are fully retracted and touching the abdomen. Healthy mice typically extend their hindlimbs outwards when suspended while mice with motor deficits or neurological dysfunction may exhibit hindlimb clasping, with the degree of clasping correlating with the severity of the impairment. As seen in this plot, several of the treated mice shows increased hind limb clasping indicating some level of motor/neurological deficits. miR-100-50, miR-130a-51, miR-E-50, miR-130a-50, miR-190-50, show no/low hindlimb clasping.

EXAMPLE 7

SPECIFICITY OF SELECT CONSTRUCTS

[00262] iPSC derived GABAergic neurons (Fujifilm Cellular Dynamics) were plated in a 6-well plate, pre-coated with 0.01% PLO and 10 µg/ml laminin, at $1x10^6$ cells/well. 100% of the culture media was replaced at DIV1 with media containing AAVDJ expression one of the following pri-miRNAs at an MOI of 1E3 vg/cell: miR-130a-51, miR-100-50, or miR-100-55. Cells contacted with AAVDJ vectors expressing 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.

[00263] The volcano plots in Figure 8 demonstrates that miR-100-50 (SEQ ID NO:316) and miR-100-55 (SEQ ID NO:317) are specific for the MAPT gene, having no significant impact on the expression of non-target (i.e., non-MAPT) genes. miR-130a-51 (SEQ ID NO:331) shows low level, low significant changes in off-target genes expression, with the majority of the changes below two-fold (vertical dotted lines in each plot). The MAPT transcript is indicated with an "x" in the volcano plots and shows significantly reduced expression.

EXAMPLE 8

KNOCKDOWN OF TAU IN VIVO IN TRANSGENIC MICE

[00264] Transgenic hTau mice (Andorfer et al, J Neurochem 86(3):582-90; Jackson Lab Stock #005491) were used to assess human MAPT and human Tau protein expression levels in adult animals via intravenous delivery of PHP.eB AAV vectors. Mice at 12 to 14 weeks of age were dosed with PHP.eB AAV vectors (Mathiesen et al., Methods & Clinical Development, 2020, Volume 19, p447-458) expressing either miR-100-50 or miR-130a-51 at 1E12 vg/animal via tail vein injection. Tissues were collected for vector copy number per diploid genome (VCN/dg), human MAPT transcript expression level, and total human Tau protein expression level.

[00265] Vector biodistribution was assessed by droplet-digital PCR detection using gene of interest specific TaqMan Assays from ThermoFisher Scientific (Assay ID: APDJ6UF) and mTfrc (4458367, Housekeeping Control). The top panel of Figure 9 shows strong expression of the AAV gene delivered via IV injection in the cortex, hippocampus, and hindbrain.

[00266] Human MAPT transcript levels in the cortex, hippocampus, and hindbrain tissues of test and control mice were detected using TaqMan Assays from ThermoFisher Scientific: MAPT - Hs00902194_m1 (Gene of interest) and mGusb -Mm00446953_m1 (Housekeeping Control) using digital-droplet PCR quantification methods. ddPCR was set up with template (RT+) and control (RT-) conditions for each sample. Normalized human MAPT transcript expression was calculated by dividing human MAPT transcript copies by *mGusb* transcript copies. Relative human MAPT transcript expression was then calculated by dividing PHP.eB-AAV-treated samples expression by PBS-treated sample expression. The bottom panel of Figure 9 shows

significant reduction of human MAPT transcript expression in all three tissues in mice treated with miR-100-50 or miR-130a-51.

[00267] Total human Tau protein levels in the cortex, hippocampus, and hindbrain tissues of test and control mice were detected using R-PLEX Human Tau (total) Assay (Meso Scale Discovery, K151AGTR). Relative hTau expression was then calculated by dividing PHP.eB-AAV-treated animals by the PBS-treated animals. Figure 10 shows significant reduction of human Tau protein expression in all three tissues in mice treated with miR-100-50 or miR-130a-51.

EXAMPLE 9

KNOCKDOWN OF TAU IN NHP

[00268] The following AAV9 vectors were tested for the ability to knockdown endogenous MAPT transcript and endogenous Tau protein expression in cynomolgus monkeys (non-human primate, NHP) administered via both ICV and IP infusion: (i) miR-130a-51 (pri-miRNA is SEQ ID NO:331; expression cassette is SEQ ID NO:418; full genome is SEQ ID NO:419), (ii) miR-100-50 (pri-miRNA is SEQ ID NO:316; expression cassette is SEQ ID NO:416; full genome is SEQ ID NO:417), and (iii) miR-100-55 (pri-miRNA is SEQ ID NO:317; expression cassette is SEQ ID NO:420; full genome is SEQ ID NO:421).

[00269] Animals were administered a single unilateral ICV infusion (total of 1E14 vg/animal) using stereotaxic techniques to target the lateral ventricle in the left hemisphere as well as six IP intracortical infusions in the right contralateral hemisphere (the six intracortical injections spanned 8 mm along the anterior-posterior axis of the cortex). A total of 1.2E12 vg/animal was delivered in the six IP injections.

[00270] Necropsies were performed 60±7 days post-injection. Following euthanasia, animals were transcardially perfused with chilled saline until clear of blood. Following perfusion, the brain was removed and cut into 4-6 mm coronal sections. Tissue punches (4 mm) were collected from coronal brain slabs and frozen in RNAlater for subsequent DNA and RNA isolation to use in subsequent analyses. Additional coronal slabs were placed in 4% paraformaldehyde (PFA) for 24 to 48 hours and then transferred to 70% ethanol (stored at 2 to 8 °C).

[00271] Extracted DNA was evaluated for biodistribution by a droplet digital PCR (ddPCR)-based assay which confirmed the presence of viral DNA in each animal (data not shown).

[00272] Extracted RNA was evaluated for processed miRNA abundance by two-tailed RT-qPCR-based method with additional custom Taqman probes. All three AAV constructs demonstrated expression of their respective miRNAs in brain tissue of treated animals (data not shown). The custom Thermo Fisher probes/primers used are shown in Table 8:

TABLE 8

miRNA SEQ ID NO	Probe / primer	SEQ ID NO	Sequence (5' – 3')
51	RT primer	436	GATAGTGACCTAGCTCTCCAGGTACAGTTGG TACCTGTCTCCACTTCCAGAG
	Forward	437	GATAGTGACCTAGCTCTCC
	Probe	438	CAGGTACAGTTGGTACCTGTCTCCACTTC
	Reverse	439	TCACTATCATAGTCACTCTGG
50	RT primer	440	GGAAGTAACTATGCTCTCCAGGTACAGTTGG TACCTGTCTCCACTTGTGGCC
	Forward	441	GGAAGTAACTATGCTCTCC
	Probe	442	CAAGTGGAGACAGGTACCAACTGTACCT
	Reverse	443	GACTTTACTTCCACCTGGC
55	RT primer	444	TCTACAAACTATGCTCTCCAGGTACAGTTGG TACCTGTCTCCACTTCAGGT
	Forward	445	TCTACAAACTATGCTCTCCA
	Probe	446	TACAGTTGGTACCTGTCTCCACTT
	Reverse	447	TTTGTAGACTATTTGCACCT

[00273] Extracted RNA from different regions of the brain was evaluated for transcript levels of endogenous MAPT and compared to vehicle treated controls. The assay tested for exons 15-16 of the MAPT transcript (Thermo Fisher assay ID: Hs00902194_m1). Figure 11A shows that miR-100-50 treated animals had measurable knockdown of MAPT transcript across all brain tissues, with several tissues having significantly reduced MAPT transcript levels (amygdala, cingulate cortex, and inferior parietal cortex). miR-130a-51 treated animals also had measurable knockdown of MAPT transcript across all tissues, with several tissues having significantly

reduced MAPT transcript levels (entorhinal cortex, hippocampus, inferior temporal cortex, inferior parietal cortex, and frontal cortex). miR-100-55 treated animals also had measurable knockdown of MAPT transcript across most brain tissues, with two tissues having significantly reduced MAPT transcript levels (amygdala and inferior parietal cortex). Frontal cortex, amygdala, and thalamus data are from the left hemisphere only. The remaining data are the average of left and right hemispheres per region. Statistical comparisons by t-test: * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; **** = $P \leq 0.0001$.

[00274] Expression levels of total endogenous Tau protein in multiple brain regions was quantified using hTau R-PLEX MSD (Meso Scale Diagnostics, LLC, Rockville, MD) and normalized to vehicle control. As shown in Figure 11B, endogenous Tau protein levels were reduced in numerous brain regions, including but not limited to the hippocampus, thalamus, and lateral occipital cortex.

[00275] These results demonstrate that AAV vector delivery of the MAPT-targeting pri-miRNAs of the present disclosure, for example the pri-miRNAs of SEQ ID NO:331, SEQ ID NO:316, and SEQ ID NO:317, can reduce both endogenous MAPT transcript and endogenous Tau protein levels.

EXAMPLE 10

SCAAV IN IPSC GABAERGIC NEURONS

[00276] Self-complementary AAV (scAAV) versions of the single-stranded AAV (ssAAV) pri-miRNA expression vectors used in Example 9 above (NHP studies) were tested in iPSC-derived GABAergic neurons. The SEQ ID NOs for the complete genomes for each ssAAV and scAAV vectors are as follows: ssAAV miR-130a-51 is SEQ ID NO:419, scAAV miR-130a-51 is SEQ ID NO:424; ssAAV miR-100-50 is SEQ ID NO:417, scAAV miR-100-50 is SEQ ID NO:422, ssAAV miR-100-55 is SEQ ID NO:421, scAAV miR-100-55 is SEQ ID NO:423. iPSC GABAergic neurons were purchased from Fujifilm Cellular Dynamics and thawed according to the manufacturer's directions. A 96 well plate was precoated with Poly-L-Ornithine (PLO) and laminin and cells were plated at 60,000 cells/well. 100% of the culture media was replaced at after 24 hours, and AAVs were added to the respective wells (DIV1), diluted according to the multiplicity of infection (MOI) calculations. Neurons were maintained according to

manufacturer's protocol, with 50% media replaced every 3-5 days, until they were harvested at DIV7 (6-day transduction).

[00277] The iPSC derived GABAergic neurons were infected with AAVDJ vectors containing one of the ssAAV and scAAV genomes noted above at a range of 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 MAPT transcript expression was calculated by dividing MAPT transcript copies by GAPDH transcript copies. Relative MAPT transcript expression was then calculated by dividing miRNA treated sample expression by scramble treated samples expression. Figure 12 compares MAPT transcript expression levels of ssAAV miR-130a-51 and scAAV miR-130a-51 (left panel), ssAAV miR-100-50 and scAAV miR-100-50 (center panel), and ssAAV miR-100-55 and scAAV miR-100-55 (right panel) (**** = $P \leq 0.0001$). As shown in this figure, the scAAV versions perform at least as well as (miR-100-50), and in some cases better than (miR-130a-51 and miR-100-55), the ssAAV versions.

EXAMPLE 11

3' GUIDE VARIANT TESTING IN IPSC GABAERGIC NEURONS

[00278] AAVDJ vectors were generated that included the miR-100 scaffold with either (i) the SEQ ID NO:50 guide, or (ii) a variant of SEQ ID NO:50 that includes a two nucleotide 3' mismatch to the target sequence (SEQ ID NOs:398-406; see Table 8).

TABLE 8

3' Mismatch Guide RNA Sequence for Target Site 1 (Variant of SEQ ID NO: 50)	RNA SEQ ID NO	DNA SEQ ID NO
UUUACUUCCACCUGGCCACCAA	398	407
UUUACUUCCACCUGGCCACCAU	399	408
UUUACUUCCACCUGGCCACCAG	400	409
UUUACUUCCACCUGGCCACCCA	401	410
UUUACUUCCACCUGGCCACCCU	402	411
UUUACUUCCACCUGGCCACCCG	403	412
UUUACUUCCACCUGGCCACCGA	404	413
UUUACUUCCACCUGGCCACCGU	405	414

UUUACUUCCACCUGGCCACCGG	406	415

[00279] The iPSC derived GABAergic neurons were infected with AAVDJ on DIV1 at MOI of 1E5. Biological replicates were included for each construct. Cells were lysed on DIV7 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 MAPT transcript expression was calculated by dividing MAPT transcript copies by GAPDH transcript copies. Relative MAPT expression was then calculated by dividing miRNA treated samples expression by scramble treated samples expression. As shown in Figure 13, all of the variants tested were able to significantly knockdown endogenous MAPT mRNA expression, with some having slightly improved performance over SEQ ID NO:50 (the non-variant parent guide sequence). The SEQ ID NO:402 variant shows the most increase in potency (~10% improvement). These results demonstrate that downregulation of mRNA expression can be achieved using guide sequences that are not 100% complimentary to the target sequence to which they were designed.

EXAMPLE 12

miRNA ASSESSMENT IN MOUSE HIPPOCAMPUS

[00280] Wild-type B6 mice were treated with AAV9 expressing miR-130a-51 (SEQ ID NO:331), miR-100-50 (SEQ ID NO:316), or miR-100-55 (SEQ ID NO:317) pri-miRNAs via ICV at P1 for 4 weeks. The hippocampal tissue was extracted and RNA libraries were prepared using NEB Small RNA library prep kit using Total RNA. The small libraries were sequenced and read counts were determined based on the artificial miRNA sequences, considering both the guide and passenger strands. These counts were used to compute the miRNA amount, guide-to-passenger ratio, and to assess guide strand processing accuracy.

[00281] Figure 14A shows that all three of the tested pri-miRNA constructs have optimal guide/passenger (G/P) ratios (a G/P ratio over 100 indicates strong preference for producing guide miRNA over passenger RNA).

[00282] Figure 14B, top panel, shows that all three of the tested pri-miRNA constructs exhibited favorable 5' processing precision, with each having at least 99% accuracy.

[00283] Figure 14B, bottom panel, shows that all three of the tested pri-miRNA constructs (listed at the left of the table) produced predominately 22bp guide miRNA species as the result of DROSHA/DICER miRNA processing pathways. Numbers in the cells indicate the proportion of guide sequences (1.0 = 100%) having the indicated bp length (indicted at the bottom of the table). A (-) in a cell indicates a value less than 0.01.

CLAIMS

What is claimed is:

1. A polynucleotide comprising a targeting region that binds to a target site in an endogenous mRNA encoding Tau, 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 48.

2. The polynucleotide of claim 1, wherein the endogenous mRNA encoding Tau is transcribed from an endogenous MAPT gene.

3. The polynucleotide of claim 1, wherein the endogenous mRNA encoding Tau comprises a sequence having at least 95% sequence identity to SEQ ID NO: 249.

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 48.

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: 50-97.

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: 50-97.

10. The polynucleotide of any one of claims 1 to 4, wherein the targeting region comprises any one of SEQ ID NOs: 50-97.

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: 50-97, and a passenger RNA sequence.

14. The polynucleotide of any of claims 11-13, wherein the miRNA scaffold is selected from the group consisting of: miR-100, miR-E, miR-130a, miR-132, miR-190a, miR-190, and miR-451.

15. 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:255, a 5' stem of SEQ ID NO:256, the guide RNA sequence; a loop of SEQ ID NO:257, the passenger RNA sequence, a 3' stem of SEQ ID NO:258, and an optional 3' flanking sequence of SEQ ID NO:259.

16. The polynucleotide of claim 15, wherein the guide RNA sequence is SEQ ID NO: 50, 51, 53, or 55.

17. The polynucleotide of claim 16, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 316, 315, 314, and 317.

18. 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:250, a 5' stem of SEQ ID NO:251, the passenger RNA sequence; a loop of SEQ ID NO:252, the guide

RNA sequence, a 3' stem of SEQ ID NO:253, and an optional 3' flanking sequence of SEQ ID NO:254.

19. The polynucleotide of claim 18, wherein the guide RNA sequence is SEQ ID NO: 50, 51, 53, or 55.

20. The polynucleotide of claim 19, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 312, 311, 310, and 313.

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:260, a 5' stem of SEQ ID NO:261, the passenger RNA sequence; a loop of SEQ ID NO:262, the guide RNA sequence, a 3' stem of SEQ ID NO:263, and an optional 3' flanking sequence of SEQ ID NO:264.

22. The polynucleotide of claim 21, wherein the guide RNA sequence is SEQ ID NO: 50, 51, 53, or 55.

23. The polynucleotide of claim 22, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 332, 331, 330, and 333.

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:265, a 5' stem of SEQ ID NO:266, the passenger RNA sequence; a loop of SEQ ID NO:267, the guide RNA sequence, a 3' stem of SEQ ID NO:268, and an optional 3' flanking sequence of SEQ ID NO:269.

25. The polynucleotide of claim 24, wherein the guide RNA sequence is SEQ ID NO: 50, 51, 53, or 55.

26. The polynucleotide of claim 25, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 320, 319, 318, and 321.

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:270, a 5'

stem of SEQ ID NO:271, the guide RNA sequence; a loop of SEQ ID NO:272, the passenger RNA sequence, a 3' stem of SEQ ID NO:274, and an optional 3' flanking sequence of SEQ ID NO:275.

28. The polynucleotide of claim 27, wherein the guide RNA sequence is SEQ ID NO: 50, 51, 53, or 55.

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:270, a 5' stem of SEQ ID NO:271, the guide RNA sequence; a loop of SEQ ID NO:273, the passenger RNA sequence, a 3' stem of SEQ ID NO:274, and an optional 3' flanking sequence of SEQ ID NO:275.

30. The polynucleotide of claim 29, wherein the guide RNA sequence is SEQ ID NO: 50, 51, 53, or 55.

31. The polynucleotide of claim 30, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 324, 323, 322, and 325.

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:276, a 5' stem of SEQ ID NO:277, the guide RNA sequence; the passenger RNA sequence, a 3' stem of SEQ ID NO:278, and an optional 3' flanking sequence of SEQ ID NO:279.

33. The polynucleotide of claim 32, wherein the guide RNA sequence is SEQ ID NO: 50, 51, 53, or 55.

34. The polynucleotide of claim 33, wherein the pri-miRNA comprises a sequence selected from the group consisting of: SEQ ID NO: 328, 327, 326, and 329.

35. An expression cassette comprising a promoter operably linked to a transgene encoding an RNA, wherein the RNA comprises the polynucleotide of any one of claims 1 to 11 or 13 to 34.

36. The expression cassette of claim 35, further comprising an enhancer operably linked to the promoter.

37. The expression cassette of claim 35 or 36, wherein the promoter is a constitutive promoter.

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

39. The expression cassette of claim 37, wherein the promoter is a central nervous system (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: 416, 418, or SEQ ID NO:420.

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 any one of claims 45 to 47, 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:430, 431, 432, 433, 434, and 435.

51. The vector of any one of claims 45 to 50, wherein the vector comprises any one of SEQ ID NOs: 417, 419, 421, 422, 423, and 424, or a sequence having at least 80%, 90%, 95%, 98%, or 99% sequence identity thereto.

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 Tau 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 25, wherein expression of the mRNA encoding Tau and/or the Tau 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 Tau and/or the Tau protein encoded by the mRNA is reduced at least 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 Tau and/or the Tau 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 Tau 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 57 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 a tauopathy 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 claim 72, wherein the subject has Alzheimer's Disease (AD) or Frontotemporal Dementia and Parkinsonism Linked To Chromosome 17 (FTDP-17).

74. The method of any one of claims 70 to 73, 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.

75. 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 a tauopathy.

76. 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.

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

78. A method of producing a composition comprising viral particles, comprising: (i) culturing the cell of claim 77 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.

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