

**INHIBITORY RNA TARGETING HUNTINGTIN EXPRESSION****CROSS REFERENCE TO RELATED APPLICATION**

**[0001]** The present application claims priority to U.S. Provisional Application No. 63/511,187, filed June 30, 2023, U.S. Provisional Application No. 63/591,868, filed October 20, 2023, U.S. Provisional Application No. 63/557,370, filed February 23, 2024, and U.S. Provisional Application No. 63/654,508 filed May 31, 2024, the disclosures of each of the provisional applications are incorporated by reference herein in their entirety.

**REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY**

**[0002]** The contents of the electronic sequence listing (065830.19WO.xml; size: 294,358 bytes; and date of creation: June 20, 2024) is incorporated herein by reference in its entirety.

**BACKGROUND OF THE INVENTION**

**[0003]** Huntington disease is a neurodegenerative disease caused by an abnormal CAG expansion in exon 1 of the huntingtin gene. CAG expansion produces a mutant huntingtin protein having an increased polyglutamine tract. Huntington disease symptoms include motor impairment, cognitive impairment and psychiatric disorders. A large number of different inhibitory RNA have been proposed to treat Huntington disease. (See *e.g.*, Prister et al., *Mol. Ther. Nucleic Acids*. (2017) Jun 16;7:324-334; Aguiar et al., *Transl. Neurodegener.* (2017) Nov 27;6:30; Miniarikova et al., *Mol. Ther. Nucleic Acids* (2016) Mar 22;5(3):e297; Paul et al., *Cells* (2020) Jul 15;9(7):1698; Wang et al., *Hum. Gene. Ther.* 2022 Jan;33(1-2):37-60; U.S. Patent No. 7,947,658; U.S. Patent No. 10,457,940; U.S. Patent No. 10,767,180; U.S. Patent No. 10,774,327; U.S. Patent No. 10,174,321; U.S. Patent Application Publication No. 2020/0155624; U.S. Patent Application Publication No. 2020/0377887; International Publication No. WO 2021/127455; and International Publication No. WO 2021/016505.)

**BRIEF SUMMARY OF THE INVENTION**

**[0004]** The present invention features RNA polynucleotide constructs comprising sequences targeting HTT mRNA, along with nucleotides sequences that can be used, for example, as scaffolds for the targeting sequences; and encoding nucleic acid. RNA polynucleotide constructs include inhibitory RNA polynucleotides comprising a sequence targeting Huntingtin mRNA, such as artificial primary microRNA (pri-miRNA), pre-microRNA (pre-miRNA), short hairpin RNA (shRNA), artificial microRNA (amiRNA) and optionally modified amiRNA. Constructs comprising sequences targeting HTT mRNA and/or encoding sequences can be used,

for example, in methods for inhibiting mutant HTT expression and/or treating Huntington disease.

**[0005]** A HTT mRNA “targeting” sequence is substantially complementary to a HTT mRNA target sequence. HTT mRNA target sequences described herein include those of any of SEQ ID Nos: 119-137. Preferred targeting sequences can serve as guide sequence for inhibitory RNA. Examples of targeting sequences described herein include sequences at least 80% identical to any of SEQ ID NOs: 1-19.

**[0006]** Reference to “inhibitory” RNA polynucleotide indicates the polynucleotide comprises a sequence targeting an RNA that can inhibit RNA activity. Inhibition of RNA activity results in a decrease in protein expression from the targeted mRNA. Inhibitory RNA includes, for example, amiRNA and amiRNA precursors such as pre-amiRNA, sh-RNA, and pri-amiRNA

**[0007]** Thus, a first aspect of the present invention describes an RNA polynucleotide comprising an RNA sequence targeting HTT mRNA. The RNA polynucleotide comprises a targeting RNA sequence at least 80% identical with the sequence of any of SEQ ID NOs: 1-19; provided that if the targeting sequence is at least 80% identical to SEQ ID NO: 18, then the RNA polynucleotide is either (a) a pri-amiRNA comprising the targeting sequence embedded in a scaffold selected from a S155e scaffold, a S26a scaffold, or a S33 scaffold, or (b) the RNA polynucleotide comprises the sequence of SEQ ID NOs: 117 or 118.

**[0008]** A second aspect of the present invention is directed to an optionally modified inhibitory RNA duplex comprising (a) a guide strand able to hybridize to the target sequence of any of SEQ ID NOs: 119-137; and (b) a substantially complementary passenger sequence; wherein one or more nucleotides of the guide strand and the passenger strand are optionally modified RNA.

**[0009]** A third aspect of the present invention is directed to a polynucleotide comprising a nucleic acid sequence encoding an RNA polynucleotide comprising a HTT mRNA targeting sequence.

**[0010]** A fourth aspect of the present invention is directed to an expression cassette comprising a nucleic acid sequence encoding an RNA polynucleotide comprising a HTT mRNA targeting sequence and one or more expression control elements operably coupled to the encoding nucleic acid sequence.

**[0011]** A fifth aspect of the present invention is directed to recombinant viral vector nucleic acid comprising (a) an expression cassette comprising a nucleic acid sequence encoding an RNA polynucleotide comprising a HTT mRNA targeting sequence and one or more expression control elements operably coupled to the encoding nucleic acid sequence and (b) 5' and/or 3' viral elements providing for viral packaging and/or replication.

**[0012]** A sixth aspect of the present invention is directed to a delivery vehicle comprising a viral or a non-viral vector and (a) an RNA polynucleotide comprising a sequence targeting HTT mRNA; (b) an optionally modified inhibitory RNA duplex targeting HTT mRNA; or (c) a polynucleotide, expression cassette, or recombinant viral nucleic acid comprising a sequence encoding for an RNA polynucleotide comprising a HTT mRNA targeting sequence.

**[0013]** A seventh aspect of the present invention is directed to a pharmaceutical compositions comprising (a) an RNA polynucleotide comprising a sequence targeting HTT mRNA, (b) an optionally modified inhibitory RNA duplex targeting HTT mRNA, (c) a polynucleotide, expression cassette, or recombinant viral nucleic acid comprising a sequence encoding for a HTT mRNA targeting sequence or (d) a delivery vehicle comprising (a), (b) or (c); and a pharmaceutically acceptable carrier.

**[0014]** An eighth aspect of the present invention is directed an RNA sequence comprising SEQ ID NO: 255, wherein  $N_{01}$  to  $N_{42}$  are ribonucleotides,  $N_{01}$  is complementary to  $N_{41}$ ,  $N_{02}$  is not complementary to  $N_{41}$ ,  $N_{03}$ - $N_{10}$  is complementary to  $N_{33}$ - $N_{40}$ ,  $N_{11}$  is not complementary to  $N_{32}$ , and  $N_{12}$ - $N_{21}$  is complementary to  $N_{22}$ - $N_{31}$ ; or the corresponding DNA.

**[0015]** DNA corresponding to ribonucleotides, provides deoxyribonucleotides comprising thymine (T), cytidine (C), adenosine (A), and guanosine (G) nitrogenous bases with complementary deoxyribonucleotides being A:T or C:G.

**[0016]** A ninth aspect of the present invention is directed to a method of reducing mutant HTT expression and/or treating Huntington disease is a subject comprising administration of (a) an RNA polynucleotide comprising a sequence targeting HTT mRNA, (b) an optionally modified inhibitory RNA duplex targeting HTT mRNA, (c) a polynucleotide, expression cassette, or recombinant viral nucleic acid comprising a sequence encoding for a HTT mRNA targeting sequence; (d) a pharmaceutical composition comprising (a), (b), or (c) and a pharmaceutically acceptable carrier; or (e) a delivery vehicle comprising (a), (b), (c), or (d).

**[0017]** A tenth aspect is directed to a DNA polynucleotide comprising in a 5' to 3' direction:

- (a) a 5' inverted terminal repeat (ITR) sequence comprising the sequence of SEQ ID NO: 262;
- (b) a CAG promoter;
- (c) a pre-miRNA encoding sequence comprising the sequence of SEQ ID NO: 261, wherein the CAG promoter is operably linked to the pre-miRNA encoding sequence and a polyadenylation signal; and
- (d) a 3' inverted terminal repeat (ITR) sequence comprising the sequence of SEQ ID NO: 263.

**[0018]** An eleventh aspect is directed to a recombinant adeno-associated viral (rAAV) vector comprising:

(a) an rAAV nucleic acid comprising a pre-miRNA encoding sequence comprising the sequence of SEQ ID NO: 261 operably linked to a promoter and a polyadenylation signal, and

(b) a rAAV capsid comprising: VP1 comprising the amino acid sequence of SEQ ID NO: 257, VP2 comprising the amino acid sequence of SEQ ID NO: 258, and VP3 comprising the amino acid sequence of SEQ ID NO: 259.

**[0019]** An twelfth aspect is directed to a method of treating Huntington disease in a subject comprising

(a) determining in the subject, the putamen and/or caudate volume in the right hemisphere and/or left hemisphere; and

(b) intraparenchymally administering to the subject a recombinant adeno-associated viral (rAAV) vector at a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> to the right hemisphere and/or a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> to the left hemisphere; wherein said dose is based on the volume determined in step (a).

**[0020]** Reference to “based on” with respect to the volume determined in step (a), provides a dose taking into account the measured volume for the putamen and/or caudate in the right and/or left hemisphere. For example,  $2.0 \times 10^7$  vg/mm<sup>3</sup> provides a ratio of (a)  $10^7$  vg to (b) mm<sup>3</sup> of the putamen and caudate volume in a hemisphere. The actual units for the ratio need not be vg/mm<sup>3</sup>, as long as the ratio converts to the referenced vg to mm<sup>3</sup>.

**[0021]** Additional aspects of the present invention include (a) an RNA polynucleotide comprising a sequence targeting HTT mRNA, (b) an optionally modified inhibitory RNA duplex targeting HTT mRNA, (c) a polynucleotide, expression cassette, or recombinant viral nucleic acid comprising a sequence encoding for a HTT mRNA targeting sequence or (d) a delivery vehicle comprising (a), (b) or (c) and a pharmaceutically acceptable carrier, for use in medicine, reducing HTT, or treating Huntington disease; and use of (a), (b), (c), or (d) in preparation of a medicament (*e.g.*, for use in medicine, reducing HTT or treating Huntington disease).

**[0022]** Other features and advantages of the present invention are apparent from additional descriptions provided herein, including different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. Such examples do not limit the claimed invention. Based on the present disclosure, the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.



**BRIEF DESCRIPTION OF THE DRAWINGS**

[0023] FIG. 1 illustrates SEQ ID NO: 256 in a pri-miRNA configuration. Predicted Dicer and Drosha cuts are shown.

[0024] FIGs. 2A, 2B, and 2C illustrate encoding constructs and results from a miRNA screen. FIG. 2A illustrates some of the regions present in the recombinant adeno-associated viral (rAAV) nucleic acid used in the experiments: 5' ITR, CAG promoter, pri-miRNA comprising a miR155 scaffold, a polyadenylation site, and a 3'-ITR. FIG. 2B illustrates endogenous HTT protein levels in HEK293 cells transfected with plasmids encoding for different pri-miRNAs comprising a miR155 scaffold. FIG. 2C illustrates total HTT mRNA levels from HEK293 cells transfected with different plasmids encoding for different pri-miRNAs comprising an S155 scaffold. Reference to \* indicates a P-value of <0.05, \*\* indicates a P-value <0.01, \*\*\* indicates a P value <0.001 and \*\*\*\* indicates a P-value <0.0001.

[0025] FIGs. 3A, 3B, 3C, 3D and 3E illustrate results of a miRNA screen in a YAC128 HD mouse model. FIG. 3A illustrates rAAV nucleic acid biodistribution from mouse striatum injected with different rAAV vectors comprising nucleic acid encoding for different pri-miRNAs. FIG. 3B illustrate miRNA abundance from mouse striatum injected with different rAAV vectors encoding for different pri-miRNAs. FIG. 3C illustrates mutant HTT protein level in striatum of rAAV vector injected mice, detected via WES immunoassay using poly-Q specific antibody MW1. FIG. 3D illustrates total human HTT mRNA analysis by RT-qPCR in mice injected with dilutant, or the ss.CAG.S155.miR18 vector at either 1e10 vg/mouse or 3.4e10 vg/mouse. FIG. 3E illustrates mutant HTT protein levels in striatum of mice injected with dilutant or the ss.CAG.S155.miR18 vector at either 1e10 vg/mouse or 3.4e10 vg/mouse.

[0026] FIGs. 4A, 4B, 4C and 4D illustrate miR18 embedded into different pri-miRNA scaffolds and predicted Drosha and Dices cleavage sites. FIG. 4A illustrates miR18 in a S155 scaffold. FIG. 4B illustrates miR18 embedded in a S26a scaffold. FIG. 4C illustrates miR18 embedded an S155e scaffold. FIG. 4D illustrates miR18 embedded in a S33 scaffold. The figures illustrate complementary bases and, in some cases, G:U wobbles.

[0027] FIGs. 5A, 5B and 5C illustrate screening results using miR18 embedded into different pri-miRNA scaffolds. FIG. 5A illustrates miR18 expression as measured by RT-qPCR from cells transfected with indicated constructs. FIG. 5B illustrates HTT mRNA levels as measured by RT-qPCR from cells transfected with indicated constructs. FIG. 5C illustrates HTT protein levels as measured by WES capillary electrophoresis using mab2166 and cells transfected with indicated constructs. Reference to \* indicates a P-value of <0.05, \*\* indicates a P-value <0.01, \*\*\* indicates a P value <0.001 and \*\*\*\* indicates a P-value <0.0001.

**[0028]** FIGs. 6A, 6B, and 6C illustrate small RNA sequencing results of HEK293 cells transfected with different miR18 expressing constructs. FIG. 6A illustrates small RNA sequencing reads that align to either guide (G) or passenger (P) strand of the miRNA duplex from HEK293 cells transfected with the indicated miRNA expressing constructs. FIG. 6B illustrates guide to passenger sequence ratio of different constructs. FIG. 6C illustrates read pileups along in cells transfected with the indicated constructs.

**[0029]** FIGs. 7A and 7B illustrate results from *in vitro* testing of different miRNA and scaffold combinations. FIG. 7A illustrates miRNA expression from HEK293 cells transfected with indicated constructs and analyzed at 48 hours post transfection, where the results are provided as copy number (CN) of miRNA per microgram of total RNA. FIG. 7B illustrates HTT protein levels as measured by WES capillary electrophoresis using mab2166 from cells transfected with indicated constructs at 72 hours post transfection, where the provided results are HTT protein/vinculin. Reference to \* indicates a P-value of <0.05, \*\*indicates a P-value <0.01, \*\*\*indicates a P value <0.001 and \*\*\*\* indicates a P-value <0.0001.

**[0030]** FIGs. 8A, 8B, 8C, and 8D illustrate *in vitro* testing results of different miRNA and scaffold combinations expressed using the EF1 $\alpha$  long promoter or CAG promoter. FIG. 8A illustrates miRNA expression from HEK293 cells transfected with the indicated constructs. FIG. 8B illustrates miRNA expression from HEK293 cells transfected with indicated constructs. FIG. 8C illustrates HTT mRNA levels as measured by RT-qPCR from cells transfected with indicated constructs. FIG. 8D illustrates HTT mRNA levels as measured by RT-qPCR from cells transfected with different constructs.

**[0031]** FIGs. 9A, 9B, 9C, 9D, and 9E illustrate results for guide and passenger processing and biodistribution for different miRNA sequences and pri-miRNA scaffold combinations in YAC128 mice. The different constructs were expressed using the EF-1 $\alpha$  promoter. FIG. 9A illustrates guide and passenger read counts from YAC128 mice injected with vectorized miRNA/scaffold constructs as determined by small RNA sequencing. FIG. 9B illustrates guide to passenger ratios as determined by small RNA sequencing. FIG. 9C illustrates the decrease in mutant HTT protein in the striatum. FIG. 9D illustrates quantification of total mutant HTT protein from YAC128 mice injected with each miRNA/scaffold construct or dilutant. FIG. 9E illustrates vector biodistribution and mutant HTT protein levels for constructs containing miR18. Reference to \* indicates a P-value of <0.05, \*\*indicates a P-value <0.01, \*\*\*indicates a P value <0.001 and \*\*\*\* indicates a P-value <0.0001.

**[0032]** FIG. 10 illustrates the effect of rAAV-miR21 administered at different doses to nonhuman primates (NHP) using different routes of administration, three month post injection. NHPs (N=6 per group) were injected via the following routes: IP to caudate and putamen, ICV

to the lateral ventricles, or IT to the lumbar intrathecal space with the indicated doses.

HTT=huntingtin; ICV=intracerebroventricular; IP=intraparenchymal; IT=intrathecal.

**[0033]** FIG. 11 illustrates the effect and durability of different doses of rAAV-miR21 on HTT lowering in non-human primates (NHP) after three and twelve months. HTT=huntingtin protein; vg=vector genome. The 25% dotted line and 50% dotted line are relative to 0 vg/brain, Control.

**[0034]** FIGs. 12A-12D illustrate an analysis of caudate and putamen tissue sample for vector copy number, in NHP administered different amounts of rAAV-miR21. FIG. 12A and 12C provide results in the caudate. FIG. 12B and FIG. 12D provide results in the putamen. rAAV-miR21 levels were reported as VGCN/ $\mu$ g of total genomic DNA and plotted as individual values (symbol) or group mean  $\pm$  SD (bar and whiskers). M=month; VGCN=vector genome copy number.

**[0035]** FIGs. 13A-13D illustrate an analysis of caudate and putamen tissue sample for HTT protein, in NHP administered different amounts of rAAV-miR21. FIG. 13A and 13C provide results in the caudate. FIG. 13B and FIG. 13D provide results in the putamen. HTT= huntingtin protein; M=month; vg=vector genome.

**[0036]** FIG. 14A and FIG. 14B illustrate an analysis of caudate and putamen tissue samples for vector copy number, in NHP administered different amounts of rAAV-miR21. FIG. 14A provides results in the caudate. FIG. 14B provides results in the putamen. rAAV-miR21 levels were reported as VGCN/ $\mu$ g of total genomic DNA and plotted as individual values (symbol) or group mean  $\pm$  SD (bar and whiskers). Reference to "x" indicates the absence of a  $1 \times 10^9$  twelve month cohort. M=month; VGCN=vector genome copy number; BLOQ=below limit of quantification.

**[0037]** FIGs. 15A and 15B illustrate HTT protein percent knockdown in the caudate (FIG. 15A) and putamen (FIG. 15B) at different rAAV-miR21 doses at three month and twelve months. HTT= huntingtin protein; SD=standard deviation; vg=vector genome; BLOQ=below limit of quantification. HTT protein levels were reported as ng/mg of total protein and plotted as individual values (symbol) or group mean  $\pm$  SD (bar and whiskers). Reference to "x" indicates the absence of a  $1 \times 10^9$  twelve month cohort.

**[0038]** FIGs. 16A-16E illustrate results from Study 005-017. Fig. 16A illustrates a quantitative analysis of mature miR21 in CSF of NHP administered different amounts of rAAV-miR21, miR21 levels were reported as RCN/ $\mu$ g of total RNA and plotted as individual values (symbol) or group mean  $\pm$  SD (bar and whiskers). M=month; RCN=RNA copy number. FIG. 16B illustrates a quantitative analysis of NF-L in CSF of NHP administered different amounts of rAAV-miR21, D=day; D0=pre-injection, D92=terminal for Cohort 1, D365=terminal for Cohort 2, NF-L= neurofilament light chain protein, and vg=vector genome. FIG. 16C illustrates NF-L

% change in CSF from baseline (pre-injection) up to terminal sample collection (3 or 12 months after injection) in NHP administered different amounts of rAAV-miR21, M=month, NF-L=neurofilament light chain protein, and Vg=vector genome. FIG. 16D illustrates longitudinal measurements of caudate structure volume from D0 (pre-injection) up to D365 (12 months post injection) in NHP administered different amounts of rAAV-miR21, each line represents 1 animal, D=day, M=month, L=left, R=right, and vg=vector genome. FIG. 16E illustrates longitudinal measurements of putamen structure volume from D0 (pre-injection) up to D365 (12 months post injection) in NHP administered different amounts of rAAV-miR21, each line represents 1 animal, D=day, M=month, L=left, R=right, and vg=vector genome. FIG. 16F illustrates longitudinal measurements of lateral ventricle volume from D0 (pre-injection) up to D365 (12 months post injection) in NHP administered different amounts of rAAV-miR21, each line represents 1 animal, D=day, M=month, L=left, R=right, and vg=vector genome.

**[0039]** FIG. 17 illustrates an estimation, based on NHP studies, of HTT lowering at 3 months after rAAV-miR21 administration in the putamen expressed as vector copy number per cubic millimeter (vg/mm<sup>3</sup>) in Putamen. CI=confidence interval; HTT=huntingtin protein; KD%=percent knockdown.

**[0040]** FIGs. 18A, 18B and 18C illustrate the impact of rAAV.EF1 $\alpha$ .S26.miR18 on improving motor impairment, assessed by average time on rotarod. FIG. 18A, depicts the time in seconds male mice remained on an accelerating rotarod for an average of 3 trials at each timepoint. FIG. 18B depicts the average time in seconds on the rotarod across all timepoints in males. FIG. 18C shows the average time on a rotarod in seconds across all timepoints for female mice. Reference to \*\*indicates a P-value <0.01, \*\*\*indicates a P value <0.001 and ns=not significant.

## DETAILED DESCRIPTION OF THE INVENTION

**[0041]** The present invention features RNA polynucleotide constructs comprising sequences targeting HTT mRNA and encoding DNA, along with nucleotides sequences that can be used, for example, as scaffolds for pri-miRNA and pre-miRNA. RNA polynucleotide constructs targeting HTT mRNA provided herein include inhibitory RNA polynucleotides, pre-miRNA, pre-miRNA, shRNA, and optionally modified inhibitory RNA.

**[0042]** In certain embodiments the inhibitory RNA polynucleotide is an inhibitory RNA duplex. Reference to an “inhibitory RNA duplex” indicates a double-stranded RNA comprising a guide strand targeting an RNA target region and a passenger strand. The passenger strand is sufficiently complementary to the guide strand to hybridize under physiological conditions. The guide strand in association with an RNA-induced silencing complex (RISC) can inhibit target

RNA activity. In the case of an mRNA target, for example, protein expression from the targeted mRNA can be inhibited.

**[0043]** Inhibitory RNA duplexes such as amiRNA can be produced, for example, through gene expression producing pri-amiRNA, pre-amiRNA, or shRNA followed by biogenesis; and chemical synthesis.

**[0044]** Pri-miRNA scaffolds can be embedded with different with guide and passenger sequences to produce pri-amiRNA. Guide strands comprise a targeting sequence and can be selected to target different RNA sequences or regions such as 5' UTR, 3' UTR, or mRNA. Different passenger sequences can be embedded into a pri-miRNA scaffold for a given guide sequence. The passenger strand can be optimized for a particular scaffold and to provide sufficient complementarity to the guide strand to hybridize under physiological conditions.

**[0045]** Constructs comprising a sequence targeting HTT mRNA can be used, for example, in inhibitory RNA or to produce inhibitory RNA, where the inhibitory RNA can inhibit mutant HTT expression and/or treat Huntington disease. Inhibiting mutant HTT expression can be carried out, for example, for therapeutic and research purposes. Research purposes include examining the impact of inhibiting mutant HTT expression in an animal model.

**[0046]** Reference to “subject” indicates a mammal, such as a human; a non-human primate such as an ape, gibbon, gorilla, chimpanzee, orangutan, or macaques; a domestic animal such as a dog and cat; a farm animal such as poultry, duck, horse, cow, goat, sheep and pig; and an experimental animal such as a mouse, rat, rabbit, or guinea pig. A preferred subject is a human.

**[0047]** Reference to a percent “identical”, “identity” and similar terminology are with respect to two sequences having maximal alignment in a particular area. The provided area is with respect to the indicated reference sequence. For example, sequence “identical” or “identity” to a targeting sequence can be calculated by determining the number of identical nucleotides in sequences aligned to provide the maximum identity and dividing by the total number of nucleotides in the targeting sequence and multiplying by 100. Differences between aligned sequence can include deletions, substitutions, and additions.

**[0048]** Reference to an indicated percent identity or identical to one or more reference sequences, and similar language throughout the specification providing for an indicated percent identity or identical to one or more reference sequences, provides the indicated percent or percent range independently to each of the referenced sequences. In determining percent identity or identical, RNA and the corresponding DNA are considered the same, unless otherwise indicated (*e.g.*, referring to the molecule as DNA or RNA). Corresponding RNA for DNA include uracil instead of thymine and replacement of the ribose backbone for the deoxyribose backbone.

**[0049]** The terms “nucleic acid” and “polynucleotide” are used interchangeably herein to refer to all forms of nucleic acid, oligonucleotides, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) unless otherwise indicated. In discussing nucleic acids, a sequence or structure of a particular polynucleotide can be described herein according to the convention of providing the sequence in the 5' to 3' direction.

**[0050]** In certain embodiments, nucleic acids include naturally occurring, synthetic, and intentionally modified or altered polynucleotides. Unless indicated otherwise by the context, nucleic acids can be single, double, or triplex, linear or circular, and can be of any length.

**[0051]** According to certain embodiments, the polynucleotide is a single-stranded (ssDNA) or a double-stranded DNA (dsDNA) molecule. According to certain embodiments, the dsDNA molecule is a minicircle, a nanoplasid, open linear duplex DNA or a closed-ended linear duplex DNA (CELiD/ceDNA/doggybone DNA). According to certain embodiments, the ssDNA molecule is a closed circular or an open linear DNA.

**[0052]** A “transgene” refers to a nucleic acid encoding an RNA sequence that, for example, is intended or has been introduced into a cell and operably linked to a promoter. Transgenes include, for example, nucleic acid encoding a heterologous polynucleotide sequence and/or an inhibitory RNA, and a heterologous promoter.

**[0053]** In certain embodiments encoding polynucleotide constructs are “CpG reduced” or “CpG depleted”. In certain embodiments, regions that are “CpG reduced” or “CpG depleted” are those outside of the pri-miRNA or pre-miRNA sequences. “CpG reduced” or “CpG depleted” refer to (i) a nucleotide sequence wherein one or more of the CpG dinucleotides (or motifs) are removed from a reference nucleic acid sequence; and/or (ii) the percentage of CpGs in a referred to polynucleotide is 0% to 10%. In different embodiments, the CpG percentage is 0-5%, 0%, about 0.5%, about 1.0%, about 2.0%, about 3.0%, about 4.0%, about 5.0%, about 6%, about 7%, about 8%, about 9% or about 10%.

**[0054]** In certain embodiments CpG motifs are reduced in the 5' and/or 3' untranslated regions (UTRs), stuffer sequences, promoter, enhancer, polyadenylation signal, 5' and/or 3' ITRs, and/or introns.

**[0055]** The singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

**[0056]** The conjunctive term “and/or” between multiple recited elements encompasses both individual and combined options. For instance, where two elements are conjoined by “and/or”, a first option refers to the applicability of the first option without the second, a second option refers to the applicability of the second option without the first, and a third option refers to the applicability of the first and second options together. Any one of the options is understood to fall

within the meaning, and therefore satisfy the requirement of the term “and/or”. Concurrent applicability of more than one of the options is also understood to fall within the meaning of the term “and/or.”

**[0057]** Unless clearly indicated otherwise by the context employed the terms “or” and “and” have the same meaning as “and/or”.

**[0058]** Reference to terms such as “including”, “for example”, “e.g.,” “such as” followed by different members or examples, are open-ended descriptions where the listed members or examples are illustrative and other member or examples can be provided or used.

**[0059]** The terms “polypeptide,” “protein” and “peptide” can be used interchangeably to refer to an amino acid sequence without regard to function. Polypeptides and peptides contain at least two amino acids, while proteins contain at least about 10 amino acid acids. The provided amino acids include naturally occurring amino acids and amino acids provided by cellular modification.

**[0060]** Reference to “comprise”, and variations such as “comprises” and “comprising”, used with respect to an element or group of elements is open-ended and does not exclude additional unrecited elements or method steps. Terms such as “including”, “containing” and “characterized by” are synonymous with comprising. In the different aspects and embodiments described herein reference to an open-ended term such as “comprising” can be replaced by “consisting” or “consisting essentially of”.

**[0061]** Reference to “consisting of” excludes any element, step, or ingredient not specified in the listed claim elements, where such element, step or ingredient is related to the claimed invention.

**[0062]** Reference to “consisting essentially of” limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claimed invention.

**[0063]** The term “about” refers to a value within 10% of the underlying parameter (*i.e.*, plus or minus 10%). For example, “about 1:10” includes 1.1:10.1 or 0.9:9.9, and “about 5 hours” includes 4.5 hours or 5.5 hours. The term “about” at the beginning of a string of values modifies each of the values by 10%. In certain the term “about” refers to a value within 10% of the underlying parameter.

**[0064]** All numerical values or numerical ranges include integers within such ranges and fractions of the values or the integers within ranges unless the context clearly indicates otherwise. Thus, to illustrate, reference to reduction of 95% or more includes 95%, 96%, 97%, 98%, 99%, 100%, as well as 95.1%, 95.2%, 95.3%, 95.4%, 95.5%, etc., 96.1%, 96.2%, 96.3%, 96.4%, 96.5% and so forth and reference to a numerical range, such as “1-4” includes 1, 2, 3, 4

as well as 1.1, 1.2, 1.3, 1.4 and so forth. As a further illustration, “1 to 4 weeks” includes 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 days.

**[0065]** Further, reference to a numerical range, such as “0.01 to 10” includes 0.011, 0.012, 0.013 etc., as well as 9.5, 9.6, 9.7, 9.8, 9.9 and so forth. For example, a dosage of about “0.01 mg/kg to about 10 mg/kg” body weight of a subject includes 0.011 mg/kg, 0.012 mg/kg, 0.013 mg/kg, 0.014 mg/kg, 0.015 mg/kg etc., as well as 9.5 mg/kg, 9.6 mg/kg, 9.7 mg/kg, 9.8 mg/kg, 9.9 mg/kg and so forth.

**[0066]** Reference to an integer with more (greater) or less than includes numbers greater or less than the reference number, respectively. Thus, for example, reference to more than 2 includes 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more; and administration “two or more” times includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more times.

**[0067]** Various references including articles and patent publications are cited or described in the background and throughout the specification. Each of these references is herein incorporated by reference in their entirety. None of the references are admitted to be prior art with respect to any inventions disclosed or claimed. In some cases, particular references are indicated to be incorporated by reference herein to highlight the incorporation.

**[0068]** The definitions provided herein, including those in the present section, and other sections of the application apply throughout the present application.

**[0069]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning commonly understood to one of ordinary skill in the art to which this invention pertains.

**[0070]** The description has been separated into various sections and paragraphs, and provides examples of various embodiments. These separations should not be considered as disconnecting the substance of a paragraph or section or embodiment from the substance of another paragraph or section or embodiment. The provided descriptions have broad application and encompasses all the combinations of the various sections, paragraphs and sentences that can be contemplated. The discussion of any embodiment is meant only to be exemplary and is not intended to suggest the scope of the disclosure, including the claims (unless otherwise provided in the claims), is limited to these examples.

**[0071]** The instant invention is generally disclosed herein using affirmative language to describe the numerous embodiments of the instant invention. The instant invention also specifically includes embodiments in which particular subject matter is excluded, in full or in part, such as substances or materials, method steps and conditions, protocols, or procedures. For example, in certain embodiments of the instant invention, materials and/or method steps are excluded. Thus, even though the instant invention is generally not expressed herein in terms of what the instant



invention does not include, embodiments that are not expressly excluded in the instant invention are nevertheless disclosed herein.

**[0072] I. RNA Polynucleotides Targeting HTT mRNA**

**[0073]** Different HTT mRNA target regions are provided by SEQ ID NOs: 119-137. RNA polynucleotides targeting a particular target region comprise a sequence substantially complementary to the target region. In certain embodiments, the construct comprising an HTT mRNA targeting sequence is an inhibitory RNA duplex, pri-miRNA, pre-miRNA, or shRNA. The HTT mRNA targeting sequence present in these constructs provides a guide strand sequence able to hybridize to HTT mRNA. Such constructs also comprise a passenger sequence substantially complementary to the guide sequence.

**[0074]** Reference to “substantially complementary” and similar terms indicates a region of at least 10 nucleotides that are at least 70% complementary, at least 80% complementary, at least 90% complementary, or 100% complementary. The degree of complementarity is determined based on sequences aligned for maximum complementarity. Differences between complementary sequences include addition, deletion, and/or non-complementary bases. Complementary RNA bases are A:U and C:G. Preferably, substantial complementary sequences can hybridize to each other under physiological conditions.

**[0075]** In further embodiments, substantially complementary sequences comprise a region of 10-25 nucleotides, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides, that when aligned for maximum complementarity are perfectly complementary, at least 70% complementary, at least 80% complementary, at least 90% complementary, or 100% complementary.

**[0076]** In further embodiments, substantially complementary sequences comprise a region of 10-25 nucleotides, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides, that when aligned for maximum complementarity are perfectly complementary or have 1, 2, or 3 nucleotide differences from being perfectly complementary.

**[0077]** In certain embodiments, directed to the RNA polynucleotide: (1) comprises a region at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% identical to the sequence of any of SEQ ID NO: 1-19; (2) comprises at least 15, at least 16, at least 17, at least 18, or at least 19 contiguous nucleotides of any of SEQ ID NOs: 1-19; differs from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18,

or SEQ ID NO: 19 by 0, 1, 2 or 3 nucleotides; and/or (3) consists of a sequence of any one SEQ ID NOs: 1-19 or differing from any one of SEQ ID NOs: 1-19 by 1, 2 or 3 nucleotides.

**[0078]** In certain embodiments, with respect to targeting and substantially complementary targeted sequence, nucleotide differences between the targeting and targeted sequence, if present, are preferably located outside the seed region (e.g., nucleotides 2-8).

**[0079]** In certain embodiments, the guide sequence comprises, consists or consists essentially of the targeting sequence and the RNA polynucleotide further comprises passenger sequence substantially complementary to the guide.

**[0080]** In certain embodiments, the guide sequence comprising the targeting sequence, and the passenger sequence comprising a substantially complementary sequences, are provided in a combination indicated in Table 1, wherein the guide strand comprises, consists essentially of, or consists the indicated sequences and the passenger sequences comprises, consists essentially of, or consists of the indicated sequences or a sequence differing from the indicated sequence by 1, 2, or 3 nucleotides.

Table 1

| Targeting  |                       | Passenger  |                        |
|------------|-----------------------|------------|------------------------|
| SEQ ID NO: |                       | SEQ ID NO: |                        |
| 1          | UCUUGGUAGCUGAAAGUUCUU | 20         | AAGAACUUAGCUACCAAGA    |
|            |                       | 21         | AAGAACUUUCUGCUACCACGA  |
|            |                       | 22         | GAACUUAGAACUACCAAGACA  |
| 2          | UAUUGUCAGACAAUGAUUCAC | 23         | GUGAAUCAGUCUGACAAUA    |
|            |                       | 24         | GUGAAUCAUUAUCUGACACUA  |
|            |                       | 25         | GAAUCAAAAGCCUGACAAUACA |
| 3          | UUCACACGGUCUUUCUUGGUA | 26         | UACCAAGAGACCGUGUGAA    |
|            |                       | 27         | UACCAAGAAACACCGUGUAAA  |
|            |                       | 28         | CCAAGAUUGGCCGUGUGAACAC |
| 4          | AUGAUUCACACGGUCUUUCUU | 29         | AAGAAAGAGUGUGAAUCAU    |
| 5          | UUCGAAGGCCUUCUACAGCUU | 30         | AAGCUGAUAGGCCUUCGAA    |
| 6          | UGAUUCACACGGUCUUUCUUG | 31         | CAAGAAAGCGUGUGAAUCA    |
|            |                       | 32         | CAAGAAAGAACGUGUGAAGCA  |
|            |                       | 33         | CAAGAAAGUGCAUGUGAAUCA  |
| 7          | ACAAUGAUUCACACGGUCUUU | 34         | AAAGACCGUGAAUCAUUGU    |
| 8          | UUCUUGGUAGCUGAAAGUUCU | 35         | AGAACUUUGCUACCAAGAA    |
| 9          | UGUCAGACAAUGAUUCACACG | 36         | CGUGUGAAAUUGUCUGACA    |
| 10         | UCACACGGUCUUUCUUGGUAG | 37         | CUACCAAGAGACCGUGUGA    |
| 11         | UGAAAGUUCUUUCUUGGUUCG | 38         | CGACCAAAAAGAACUUUCA    |
| 12         | UACGGUCUUUCUUGGUAGCUG | 39         | CAGCUACCGAAAGACCGUA    |
| 13         | UCAGACAAUGAUUCACACGGU | 40         | ACCGUGUGUCAUUGUCUGA    |
| 14         | UUGUCAGACAAUGAUUCACAC | 41         | GUGUGAAUUUGUCUGACAA    |

| Targeting   |                               | Passenger   |                       |
|-------------|-------------------------------|-------------|-----------------------|
| SEQ ID NO : |                               | SEQ ID NO : |                       |
| 15          | AUAUUGUCAGACAAUGAUUCA         | 42          | UGAAUCAUUCUGACAAUUAU  |
| 16          | UCGAAGGCCUUCAUCAGCUUU         | 43          | AAAGCUGAAAGGCCUUCGA   |
|             |                               | 44          | AAAGCUGAUGCAGGCCUUGGA |
|             |                               | 45          | AGCUGAACAGGGCCUUCGACA |
| 17          | UCGAAGGCCUUCAUCAGCU <u>AG</u> | 46          | GCUGAUGAAGGCCUUCG     |
| 19          | UCGAAGGCCUUCAUCAGCU <u>CC</u> | 47          | GGAGCUGAAAGGCCUUCGA   |
| 18          | UAGCGUUGAAGUACUGUCCCC         | 48          | GGGGACAGCUUCAACGCUA   |
|             |                               | 49          | GGGGACAGUAAUUCAACGCGA |
|             |                               | 50          | GGACAGAUCCUCAACGCUACA |

Nucleotides in bold underline are not complementary to target.

**[0081]** In certain embodiments, the encoding passenger strand and guide strand combination provided in Table 1, is a present in a pri-miRNA, pre-miRNA, shRNA or inhibitory RNA duplex.

**[0082]** Nucleotide differences between the guide and targeted sequence complement can be provided, for example, for embedding the guide into a particular scaffold, to enhance guide strand processing (e.g., incorporated into RISC for targeting and inhibition of targeted RNA) of a particular scaffold and/or decreasing complementarity of the guide strand to a host RNA.

**[0083]** Nucleotide differences between the guide and passenger strand complement, can be introduced into the passenger sequence for different purposes, such as, embedding into a particular scaffold, to enhance passenger strand processing (e.g., passenger strand degradation and not being incorporated in RISC for targeting and inhibition of RNA), and/or decreasing complementarity of the passenger strand to a host mRNA.

**[0084]** I.A. Inhibitory RNA Duplex

**[0085]** An inhibitory RNA duplex can inhibit mRNA activity in conjunction with the RNA-induced silencing complex (RISC). Reference to RISC includes associated proteins such the core argonaute (AGO) protein. Without being limited to any particular theory, the guide strand is loaded onto an RISC and hybridizes to the target mRNA, wherein hybridization is initiated at the seed region, located at n-2 to approximately n-8 of the guide strand 5' end.

**[0086]** In certain embodiments, the seed region is perfectly complementary to its mRNA target or different from perfect complementarity by 1 nucleotide.

**[0087]** Preferably, the passenger strand is discarded by the RISC and is degraded. In different embodiment the guide strand ratio to passenger strand ratio is about 100:1, about 1000:1 or about 3000:1.

**[0088]** Depending upon the degree of complementarity and the location of non-complementary regions, the RISC associated with the guide strand can cause mRNA cleavage or translation

repression. (See, *e.g.*, Lam et al., Mol. Ther. Nucleic Acids. (2015) 4(9):e252 and Kobayashi et al., ACS Omega. (2022) 7(2):2398-2410, each of which are hereby incorporated by reference herein in their entirety.)

**[0089]** In certain embodiments, the guide strand and the passenger strand are independently 18 to 25 nucleotides in length and each comprises a 3' overhang of 1 to 5 nucleotides. In further embodiments each overhang is 1, 2, 3, or 4 nucleotides. In further embodiments each guide strand and passenger strand is independently 20, 21, 22, or 23 nucleotides; and each overhang is 2 or 3 nucleotides. In further embodiments, each the guide and passenger strand are 21, 22, or 23 nucleotides and each overhang is 2 nucleotides.

**[0090]** I.B. amiRNA

**[0091]** Delivery of inhibitory RNA duplexes to a subject is facilitated using precursor constructs such as pri-amiRNA, pre-amiRNA and shRNA. These different constructs comprise a stem loop structure, a guide strand sequence and a passenger strand sequence; and make use of intracellular miRNA biogenesis mechanisms to form an inhibitory RNA duplex. Such constructs can be provided, for example, using transgenes encoding for inhibitory RNA.

**[0092]** Naturally occurring miRNA production and processing involves pri-miRNA transcription from an miRNA gene and cleavage of 3' and 5' regions mediated by drosha-DGCR8, forming pre-miRNA. The resulting pre-miRNA contains a stem of about 22 nucleotides with 2 nucleotide overhangs at the 3' ends and an apical stem loop. (See, *e.g.*, Jin *et al.*, (2020) Mol. Cell. 2020 May 7;78(3):423-433, hereby incorporated by reference herein in its entirety.)

**[0093]** Pri-amiRNA comprises a 5' flanking sequence, a guide/passenger sequence, an apical loop, a guide/passenger sequence and 3' flanking sequence. The guide strand sequence can be either 5' or 3' of the loop. When the guide strand sequence is 5' of the loop, the passenger strand sequence is 3' of the apical loop; and when the guide strand sequence is 3' of the loop the passenger strand sequence is 5' of the loop.

**[0094]** The 5' and 3' flanking regions make up part of a stem region and further comprise single-stranded regions. The length of the flanking regions can independently vary. In certain embodiments the 5' and 3' pri-miRNA flanking regions are each at least 20 nucleotides, at least 50 nucleotides, or at least 100 nucleotides.

**[0095]** A pri-miRNA scaffold refers to pri-miRNA regions excluding guide and passenger sequences. A scaffold can serve as a delivery vehicle for the biogenesis of different inhibitory RNA duplexes, where guide and passenger strand sequences are embedded into the scaffold.

**[0096]** Scaffolds able to incorporate guide and passenger strands sequences can be based on naturally occurring pri-miRNA, modification to a naturally occurring miRNA, or artificially

designed taking into account pri-miRNA features. (See, *e.g.*, Xie *et al.*, Mol. Ther. (2020) 28(2):422-430; Fowler *et al.*, Nucleic Acids Research (2016), 44(5):e48; Roden *et al.*, (2017) Genome Res. 27(3):374-384; Jin *et al.*, (2020) Mol. Cell. 7;78(3): 423-433; and Fang and Bartel Genes. Mol Cell. (2015) 60(1):131-145; each of which is hereby incorporated by reference herein in their entirety.

**[0097]** Design considerations of pri-miRNA scaffolds include the stem length, loop size and presence of particular motifs that may enhance biogenesis and/or inhibitory RNA. Pri-miRNA stems may contain different structures such as G-U or U-G wobbles, single base pair mismatches, bulges, and multiple base pair mismatches.

**[0098]** In certain embodiments the pri-miRNA scaffold comprises in the basal stem a UG motif in the 5' arm at a position -14 or -13 relative to the drosha 5' cleavage site; and/or a CNNC in the 3' arm between positions +14 and +18 relative to the drosha 3' cleavage site.

**[0099]** In certain embodiments, the pri-miRNA scaffold is a miR-1, miR-26, miR16-1, miR-30, miR-33, miR101, miR-64, miR-122, miR-125, miR-135, miR-155, enhanced miR-155 (eSIBR), or miR-451 scaffold. (U.S. Patent No. 10,457,940; Miniarikova *et al.*, Mol. Ther. Nucleic Acids (2016) Mar 22;5(3):e297; Fowler *et al.*, Nucleic Acids Research (2016), 44(5):e48; Xie *et al.*, Molecular Therapy (2020) 28:2 422-430; Fang and Bartel Molecular Cell (2015) 60, 131-145; Roden *et al.* (2017) Genome Res. 27, 374-384; and Calloni and Bonatto, Human Gene Therapy Methods (2015) 25(5):162-174).

**[00100]** In certain embodiments, the pri-miRNA scaffold is either S155, S155e, S26, or S33. The S155 scaffold corresponds to the scaffold present in miR155, while the S155e scaffold corresponds to an enhanced miR155 scaffold. (See Fowler *et al.*, Nucleic Acids Research (2016), 44(5):e48). The S33 scaffold corresponds to the scaffold present in miR33. (See Xie *et al.*, Molecular Therapy (2020) 28:2 422-430 2020.) The S26a scaffold is an artificial scaffold produced using the miR26a scaffold as a starting point. Examples of pri-miRNA scaffolds comprising embedded guide and passenger sequence are illustrated in FIG. 4A (S155), FIG. 4B (S26), FIG. 4C (S155e), and FIG. 4D (S33).

**[00101]** In certain embodiments, the pri-miRNA comprises a mismatched GHG motif in the 3' arm of the stem

**[00102]** In certain embodiments the pri-miRNA stem length is 33, 34, 35, 36, 37, 38, or 39 nucleotides.

**[00103]** In certain embodiments the pri-miRNA stem length is 34, 35, or 36 nucleotides.

**[00104]** In certain embodiments the pri-miRNA apical loop is 3 to 23 nucleotides. In a further embodiment the loop is 10 to 23 nucleotides. In certain embodiments the guide and passenger

strands do not extend into the loop. In certain embodiments, the guide and/or passenger extend into the loop.

**[00105]** In certain embodiments, the pri-miRNA comprises a 5' 7-methyl guanylate (m7G) cap.

**[00106]** In certain embodiments the pri-miRNA comprises, consists, or consists essentially of a sequence of any of SEQ ID NOs: 51-87; or a sequence differing from any of SEQ ID NOs: 51-87 by 0, 1, 2, 3, or 4 nucleotides. Nucleotide differences can be introduced in different locations taking into account the guidance provides herein, for example, different pri-miRNA scaffold features, guide complementarity to target, passenger strand complementarity to guide strand and adjustment for particular scaffolds.

**[00107]** Pre-miRNA produced by drosha cleavage of pri-miRNA is exported from the nucleus to the cytoplasm, where it is cleaved by dicer to produce an inhibitory RNA duplex. Reference to "pre-miRNA" indicates the RNA polynucleotide, such as those comprising guide and passenger strands described herein, can be cleaved by dicer to produce an inhibitory RNA duplex.

**[00108]** In certain embodiments, the dicer produced inhibitory RNA duplex comprises a guide and passenger strand each about 22 nucleotides in length, wherein each strand contains a nucleotide overhang of 2 bases at the each 3' end. (Fang and Bartel (2015) Molecular Cell, 60:131-145.)

**[00109]** In certain embodiments the pre-miRNA comprises, consists, or consists essentially of a sequence of any of SEQ ID NOs: 88-118; or a sequence differing from any of SEQ ID NOs: 88-118 by 0, 1, 2, 3, or 4 nucleotides. Nucleotide differences can be introduced in different locations such as the guide sequence, loop sequence, and/or passenger sequence.

**[00110]** In certain embodiments, pri-miRNA is introduced into a cell using a transgene comprising a sequence encoding for the pri-miRNA sequence. Biogenesis of the pri-miRNA leads to the production of inhibitory RNA duplex. Expression cassettes comprising the transgene can be introduced to a cell using viral or non-viral delivery vehicles.

**[00111]** In certain embodiments, shRNA is introduced into a cell using a transgene comprising a sequence encoding for the shRNA sequence. shRNA is similar in structure and function to pre-miRNA. shRNA comprises an RNA duplex comprising substantially complementary arms and a loop, where the shRNA can be exported from the nucleus to the cytoplasm and be cleaved by dicer to form an inhibitory RNA duplex. (Aguilar et al., Transl. Neurodegener. (2017) Nov 27;6:30.)

**[00112]** I.C. S126 Based Scaffold

[00113] The S126 (also referred to herein as S26a or S26) scaffold can be used to incorporate different guide and passenger strands. Certain embodiments are directed to RNA comprising the sequence of SEQ ID NO: 255:

N<sub>01</sub>N<sub>02</sub>N<sub>03</sub>N<sub>04</sub>N<sub>05</sub>N<sub>06</sub>N<sub>07</sub>N<sub>08</sub>N<sub>09</sub>N<sub>10</sub>N<sub>11</sub>N<sub>12</sub>N<sub>13</sub>N<sub>14</sub>N<sub>15</sub>N<sub>16</sub>N<sub>17</sub>N<sub>18</sub>N<sub>19</sub>N<sub>20</sub>N<sub>21</sub>UGUGCAGGUCCCA  
N<sub>22</sub>N<sub>23</sub>N<sub>24</sub>N<sub>25</sub>N<sub>26</sub>N<sub>27</sub>N<sub>28</sub>N<sub>29</sub>N<sub>30</sub>N<sub>31</sub>N<sub>32</sub>N<sub>33</sub>N<sub>34</sub>N<sub>35</sub>N<sub>36</sub>N<sub>37</sub>N<sub>38</sub>N<sub>39</sub>N<sub>40</sub>N<sub>41</sub>N<sub>42</sub>CG,

wherein N<sub>01</sub> to N<sub>42</sub> are ribonucleotides, N<sub>01</sub> is complementary to N<sub>42</sub>, N<sub>02</sub> is not complementary to N<sub>41</sub>, N<sub>03</sub>-N<sub>10</sub> is complementary to N<sub>33</sub>-N<sub>40</sub>, N<sub>11</sub> is not complementary to N<sub>32</sub>, and N<sub>12</sub>-N<sub>21</sub> is complementary to N<sub>22</sub>-N<sub>31</sub>. In certain embodiments, the guide strand after cellular processing (e.g., Dasha and Dicer cutting) is N<sub>01</sub>-N<sub>21</sub>U  $\pm$  1 nucleotide (in a further embodiment N<sub>01</sub>-N<sub>21</sub>U), wherein targeting sequences can be inserted into N<sub>01</sub>-N<sub>21</sub>. In certain embodiments the passenger strand after cellular processing is provided by N<sub>23</sub>-N<sub>42</sub>C  $\pm$  1 nucleotide, in further embodiments the passenger strand after processing is N<sub>23</sub>-N<sub>42</sub>C or N<sub>23</sub>-N<sub>42</sub>CG.

[00114] In certain embodiments, the RNA sequence comprising SEQ ID NO: 255 has the following structure:

|                      |                                                                                                                                 |                                                                                                                                                                   |                 |  |       |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|--|-------|
|                      | N <sub>02</sub>                                                                                                                 |                                                                                                                                                                   | N <sub>11</sub> |  | GUGCA |
| 5'-N <sub>01</sub>   | N <sub>03</sub> N <sub>04</sub> N <sub>05</sub> N <sub>06</sub> N <sub>07</sub> N <sub>08</sub> N <sub>09</sub> N <sub>10</sub> | N <sub>12</sub> N <sub>13</sub> N <sub>14</sub> N <sub>15</sub> N <sub>16</sub> N <sub>17</sub> N <sub>18</sub> N <sub>19</sub> N <sub>20</sub> N <sub>21</sub> U |                 |  | G     |
| 3'-GCN <sub>42</sub> | N <sub>40</sub> N <sub>39</sub> N <sub>38</sub> N <sub>37</sub> N <sub>36</sub> N <sub>35</sub> N <sub>34</sub> N <sub>33</sub> | N <sub>31</sub> N <sub>30</sub> N <sub>29</sub> N <sub>28</sub> N <sub>27</sub> N <sub>26</sub> N <sub>25</sub> N <sub>24</sub> N <sub>23</sub> N <sub>22</sub> A |                 |  | G     |
|                      | N <sub>41</sub>                                                                                                                 |                                                                                                                                                                   | N <sub>32</sub> |  | ACCCU |

[00115] In different embodiments, the RNA sequence comprising SEQ ID NO: 255 can be cut by Dicer; or can be by cut by Drosha and Dicer. FIG. 1 illustrates predicted Dasha and Dicer cut sites for an RNA sequence comprising SEQ ID NO: 255, and further comprises a 5' flanking region and 3' flanking region.

[00116] In certain embodiments directed to an RNA sequence comprising SEQ ID NO: 255, the RNA sequence further comprises a 5' flanking region and 3' flanking region, wherein the polynucleotide comprises the RNA sequence of SEQ ID NO: 256:

GUGGCCGN<sub>01</sub>N<sub>02</sub>N<sub>03</sub>N<sub>04</sub>N<sub>05</sub>N<sub>06</sub>N<sub>07</sub>N<sub>08</sub>N<sub>09</sub>N<sub>10</sub>N<sub>11</sub>N<sub>12</sub>N<sub>13</sub>N<sub>14</sub>N<sub>15</sub>N<sub>16</sub>N<sub>17</sub>N<sub>18</sub>N<sub>19</sub>N<sub>20</sub>N<sub>21</sub>UGUGCA  
GGUCCCAN<sub>22</sub>N<sub>23</sub>N<sub>24</sub>N<sub>25</sub>N<sub>26</sub>N<sub>27</sub>N<sub>28</sub>N<sub>29</sub>N<sub>30</sub>N<sub>31</sub>N<sub>32</sub>N<sub>33</sub>N<sub>34</sub>N<sub>35</sub>N<sub>36</sub>N<sub>37</sub>N<sub>38</sub>N<sub>39</sub>N<sub>40</sub>N<sub>41</sub>N<sub>42</sub>CGGGGA  
CGC,

wherein N<sub>01</sub> to N<sub>42</sub> are ribonucleotides, N<sub>01</sub> is complementary to N<sub>42</sub>, N<sub>02</sub> is not complementary to N<sub>41</sub>, N<sub>03</sub>-N<sub>10</sub> is complementary to N<sub>33</sub>-N<sub>40</sub>, N<sub>11</sub> is not complementary to N<sub>32</sub>, and N<sub>12</sub>-N<sub>21</sub> is complementary to N<sub>22</sub>-N<sub>31</sub>. The corresponding DNA can be used to encode the RNA sequence.

#### [00117] I.D. Modified Inhibitory RNA

[00118] An alternative to providing an inhibitory RNA duplex through transgene expression and biogenesis, is direct administration of the inhibitory RNA duplex. Direct administration can be facilitated using modified RNA. Modified inhibitory RNA duplex can be produced, for example, by modifying one or more one or nucleotides of an inhibitory RNA duplex. Modifications can

be made to inhibitory RNA duplex to, for example, improve pharmacokinetics, enhance activity, suppress innate immune activation, improve targeting and reduce off target toxicity.

**[00119]** Modification to inhibitory RNA can be made at different positions such as the 5'-end, 3'-end, sugar moiety, phosphate group, and nucleobase. A variety of different modifications can be made allowing, or facilitating, Watson-Crick hybridization to target and inhibitory RNA activity. References describing different modifications and modification patterns include Hu *et al.*, *Sig Transduct Target Ther.*, (2020) 5, 101; Varley and Desautels (2021) *RSC Adv.*, 11, 2415; Alterman *et al.*, *Nature Biotechnology* (2019) 37:884-894; International Patent Publication No. WO 2021/252649; and U.S. Patent Publication No. 2022/125823; each of which are hereby incorporated by reference herein in their entirety.

**[00120]** In certain embodiments, the inhibitory RNA comprises one or more modifications selected from: a 5' phosphate mimic (*e.g.*, 5-(E)-vinylphosphate, 5'-methylene phosphonate, 5'-(R)-methyl phosphate, 5'-(S)-methyl phosphate, 5'(R)-MeOCH<sub>3</sub> phosphate, 5'-(S)-methyl-F phosphate, 5 deoxy-5'-morpholino-2'O-methyl uridine, and phosphorothioate); internal phosphate modification (*e.g.*, Rp phosphorothioate, Sp phosphorothioate, phosphorodithioate, methoxyphosphonate, phenylethyl phosphate, 2'-5' phosphate linkage, and amide linkage); one or more nucleobase modification (*e.g.*, 5'-nitroindole, pseudouridine, 2'-thiouridine, N6'-methyladenosine, 5'-methylcytidine, 5'-fluoro-2'-deoxyuridine, N-ethylpiperidine 7'-EAA triazole modified adenine, N-ethylpiperidine 6'-triazole modified adenine, 6'-phenylpyrrolocytosine, or 2',4'-difluorotoluidyl ribonucleotide); sugar modification (*e.g.*, 2'-O-methyl, 2'-O-methoxyethyl, 2'-deoxy-2'-fluoro, 2'-O-(p-oxazol-2-yl)benzyl, (2'S)-2-deoxy-2'-C-methyl, (2'R)-2-deoxy-2'-C-methyl, 2'-arabino-fluoro, 2'O-benzyl, 2'O-methyl-4-pyridine, locked nucleic acid, (s)-cET-BNA, tricyclo-DNA, PMO, unlocked nucleic acid, glycol nucleic acid, altritol and nucleic acid); and conjugation group (*e.g.*, lipophilic group, hydrophilic group, cholesterol, GalNAc, docosahexaenoic, or docosahexaenoic with a phosphocholine head group).

**[00121]** In certain embodiments, at least 50%, at least 60%, at least 70%, at least 90% or 100% of the nucleotides are modified.

**[00122]** In certain embodiments the inhibitory RNA duplex is a modified divalent inhibitory RNA duplex. In further embodiments the modified divalent inhibitory RNA duplex is fully chemically stabilized and comprises one or more of 2'-OMe, 2'-F, phosphorothioate, phosphodiester, and 5'vinyl phosphonate. (See, *e.g.*, Alterman *et al.*, *Nature Biotechnology* (2019) 37:884-894 hereby incorporated by reference herein in its entirety.)

**[00123]** Modified RNA can be produced using different techniques such as step-wise synthesis of the RNA and/or modifying a produced RNA sequence. The produced RNA sequence itself can be made by, for example, step-wise synthesis or using encoding nucleic acid.



**[00124]** II. Nucleic Acid Encoding RNA Polynucleotides Comprising a Targeting Sequence

**[00125]** RNA polynucleotides comprising a targeting sequence can be produced from a polynucleotide using a nucleic acid sequence encoding for the RNA polynucleotide. With respect to an RNA polynucleotide, an encoding nucleic acid sequence provides the same sequence as the corresponding the RNA polynucleotide, wherein if the encoding nucleic acid is DNA, the DNA would have the nucleobase thymine instead of uracil and deoxyribose instead of ribose. The RNA polynucleotide is produced from a template strand, complementary to the encoding strand. The encoding strand can, for example, be provided with the template and/or used to produce the template strand.

**[00126]** A polynucleotide comprising a nucleic acid sequence encoding an RNA polynucleotide may comprise additional components such as those facilitating production of the RNA polynucleotide, facilitating delivery of the polynucleotide as a viral vector, and/or providing for additional activity. Additional activity can be provided, for example, by encoding for proteins and/or encoding for sequences providing one or more additional inhibitory RNAs.

**[00127]** Sequences providing for additional inhibitory RNAs can be the same or different and can be directed to the same or different target. Examples of different configurations include encoding for two or more pri-miRNA of the same sequence; encoding for two or more pri-miRNA having the same guide strand, but different scaffolds; and/or encoding for two or more two or more pri-miRNA with different guide strands. The different guide strands can be directed to the same target, for example, the same mRNA; or different targets, for example different mRNA. In certain embodiments, the polynucleotide encodes for 1, 2, 3, 4, or 5 pri-miRNA where each can be the same or different.

**[00128]** The same types of encoding configurations providing pri-miRNA can be used for a polynucleotide encoding for shRNA. In certain embodiments, the polynucleotide encodes for 1, 2, 3, 4, or 5 shRNA, where each can be the same or different.

**[00129]** Production of RNA polynucleotides comprising a targeting sequence is facilitated using expression cassettes. The expression cassette comprises a nucleic acid sequence encoding the RNA polynucleotide and one or more expression control elements operably linked to the nucleic acid sequence encoding the RNA polynucleotide. The expression cassette can comprise, for example, nucleic acid sequence encoding for different RNA polynucleotides (*e.g.*, different pri-miRNA or shRNA), which are the same or different, where the different polynucleotides can be operably linked to the same or different expression elements. For example, the same promoter can be coupled to a nucleic acid sequence providing for multiple pri-miRNA, or two or more multiple pri-miRNA are operably linked to different promoters.

**[00130]** In certain embodiments, the polynucleotide encoding for an RNA polynucleotide comprises a sequence (1) at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% identical to the sequence of any of SEQ ID NO: 138-156; (2) comprises at least 15, at least 16, at least 17, at least 18, or at least 19 contiguous nucleotides of any of SEQ ID NOs: 138-156; and/or (3) comprises a sequence that differs from SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, or SEQ ID NO: 156, by 0, 1, 2 or 3 nucleotides.

**[00131]** In certain embodiments, the targeting sequence is a guide strand sequence and the sequence encoding the RNA polynucleotide further comprises passenger sequence substantially complementary to the targeting sequence.

**[00132]** In certain embodiments, the guide sequence comprising the targeting sequence, and the passenger sequence comprising a substantially complementary sequences are provided in a combination indicated in Table 2, wherein the guide strand comprises, consists essentially of, or consists the indicated sequences and the passenger sequences comprises, consists essentially of, or consists of the indicated sequences or a sequence differing from the indicated sequence by 1, 2, or 3 nucleotides.

Table 2

| Targeting  |                       | Passenger  |                        |
|------------|-----------------------|------------|------------------------|
| SEQ ID NO: |                       | SEQ ID NO: |                        |
| 138        | TCTTGGTAGCTGAAAGTTCTT | 157        | AAGAACTTAGCTACCAAGA    |
|            |                       | 158        | AAGAACTTTCTGCTACCACGA  |
|            |                       | 159        | GAACCTAGAACTACCAAGACA  |
| 139        | TATTGTCAGACAATGATTCAC | 160        | GTGAATCAGTCTGACAATA    |
|            |                       | 161        | GTGAATCATTATCTGACACTA  |
|            |                       | 162        | GAATCAAAGCCTGACAATACA  |
| 140        | TTCACACGGTCTTTCTTGGA  | 163        | TACCAAGAGACCGTGTGAA    |
|            |                       | 164        | TACCAAGAAACACCGTGTA    |
|            |                       | 165        | CCAAGATTGGCCGTGTGAACAC |
| 141        | ATGATTACACGGTCTTTCTT  | 166        | AAGAAAGAGTGTGAATCAT    |
| 142        | TTCGAAGGCCTTCATCAGCTT | 167        | AAGCTGATAGGCCTTCGAA    |

| Targeting |                                      | Passenger |                       |
|-----------|--------------------------------------|-----------|-----------------------|
| 143       | TGATTACACGGTCTTTCTTG                 | 168       | CAAGAAAGCGTGTGAATCA   |
|           |                                      | 169       | CAAGAAAGAACGTGTGAAGCA |
|           |                                      | 170       | CAAGAAAGTGCATGTGAATCA |
| 144       | ACAATGATTACACGGTCTTT                 | 171       | AAAGACCGTGAATCATTGT   |
| 145       | TTCTTGGTAGCTGAAAGTTCT                | 172       | AGAACTTTGCTACCAAGAA   |
| 146       | TGTCAGACAATGATTCACACG                | 173       | CGTGTGAAATTGTCTGACA   |
| 147       | TCACACGGTCTTTCTTGGTAG                | 174       | CTACCAAGAGACCGTGTGA   |
| 148       | TGAAAGTTCTTTCTTGGTCG                 | 175       | CGACCAAAAAGAACTTTCA   |
| 149       | <u>T</u> ACGGTCTTTCTTGGTAGCTG        | 176       | CAGCTACCGAAAGACCGTA   |
| 150       | TCAGACAATGATTCACACGGT                | 177       | ACCGTGTGTCAATTGTCTGA  |
| 151       | TTGTCAGACAATGATTCACAC                | 178       | GTGTGAATTTGTCTGACAA   |
| 152       | ATATTGTCAGACAATGATTCA                | 179       | TGAATCATTCTGACAATAT   |
| 153       | TCGAAGGCCTTCATCAGCTTT                | 180       | AAAGCTGAAAGGCCTTCGA   |
|           |                                      | 181       | AAAGCTGATGCAGGCCTTGGA |
|           |                                      | 182       | AGCTGAACAGGGCCTTCGACA |
| 154       | TCGAAGGCCTTCATCAGCT <b><u>AG</u></b> | 183       | GCTGATGAAGGCCTTCG     |
| 155       | TCGAAGGCCTTCATCAGCT <b><u>CC</u></b> | 184       | GGAGCTGAAAGGCCTTCGA   |
| 156       | TAGCGTTGAAGTACTGTCCCC                | 185       | GGGGACAGCTTCAACGCTA   |
|           |                                      | 186       | GGGGACAGTAATTCAACGCGA |
|           |                                      | 187       | GGACAGATCCTCAACGCTACA |

**[00133]** Bolded underline indicates not complementary to target sequence.

**[00134]** In certain embodiments, the encoding passenger strand and guide strand combination provided in Table 2, encodes for a pri-miRNA, pre-miRNA, or shRNA.

**[00135]** In certain embodiments, the polynucleotide encoding for an RNA polynucleotide encodes comprising a sequence (1) at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% identical to the sequence of any of SEQ ID NO: 188-224 and 226-229; (2) comprises at least 15, at least 16, at least 17, at least 18, or at least 19 contiguous nucleotides of any of SEQ ID NOs: 188-230; and/or (3) comprises a sequence that differs from SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228 or SEQ ID NO: 229 by 0, 1, 2 or 3 nucleotides.

**[00136]** II.A. Expression Cassettes Elements

**[00137]** An expression cassette comprises a nucleic acid sequence encoding a polynucleotide operably linked to an expression control element. Expression cassettes described herein comprise a nucleotide sequence encoding for an RNA polynucleotide targeting HTT mRNA along with one or more regulatory sequences and may also comprises additional sequences. Example of additional sequences include non-coding sequences such as stuffer sequences, and encoding sequences such a sequence encoding for additional RNA polynucleotides and/or sequences encoding one or more polypeptides.

**[00138]** An “expression control element” influences expression of sequence to which it is operably linked. Protein expression control elements can affect, for example, transcription, translation, splicing, and message stability. Expression control elements impacting production of RNA polynucleotides can affect, for example, the level of transcription.

**[00139]** Expression control elements are typically located 5’ (“upstream”) or 3’ (“downstream”) of a transcribed nucleic acid. Expression control elements can also be located within the transcript (*e.g.*, in an intron). Expression control elements can be located adjacent to or at a distance away from the transcribed sequence. One or more expression control elements may be present. Examples of expression control elements include a promoter, enhancer, an intron, polyadenylation signal, a Kozak sequence, post-transcriptional regulator elements and a termination sequence.

**[00140]** A promoter is a DNA region where transcription is initiated. In general, transcribed nucleic acid are located 3’ of a promoter sequence. In certain embodiments, a promoter sequence is coupled to an enhancer. Enhancers are DNA regions that increase promoter transcription. Enhancers can be adjacent to a promoter or can be distal. Typically, enhancers are located upstream of a promoter, but can be located downstream or within a promoter sequence.

**[00141]** Expression control elements such a promoter and an enhancer can be chosen to preferentially drive expression in a particular cell or tissue type. Expression control elements are typically active in particular cells, tissues or organs because they are recognized by transcriptional activator proteins, or other regulators of transcription, that are unique to a specific cell, tissue or organ type. (*See, e.g.*, Green, M. and Sambrook, J. (2012) *Molecular Cloning: A Laboratory Manual*. 4th Edition, Vol. II, Cold Spring Harbor Laboratory Press, New York; and Ausubel *et al.*, (2010) *Current protocols in molecular biology*, John Wiley & Sons, New York.)

**[00142]** The incorporation of tissue specific regulatory elements in expression constructs provide for at least partial tissue tropism for the expression of encoded RNA polynucleotide. Reference to a promoter or enhancer specific for a particular cell type or tissue, indicates the promoter or enhancer provides higher levels of expression and/or secretion in the indicated cell or tissue type. Examples of the CNS specific promoters include: neuron specific promoters such

as the NSE (neuronal specific enolase), synapsin or NeuN, platelet-derived growth factor (PDGF), platelet-derived growth factor B-chain (PDGF- $\beta$ ), methyl-CpG binding protein 2 (MeCP2), Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII), metabotropic glutamate receptor 2 (mGluR2), neurofilament light (NFL) or heavy (NFH),  $\beta$ -globin minigene n $\beta$ 2, preproenkephalin (PPE), enkephalin (Enk), and excitatory amino acid transporter 2 (EAAT2) promoters; astrocyte specific promoters such as the glial fibrillar acidic protein (GFAP) and EAAT2 promoters; oligodendrocyte specific promoters such as the myelin basic protein (MBP)/myelin-associated glycoprotein and oligodendrocyte transcription factor 2 promoter; neurons/hypothalamus specific promoters such as the proopiomelanocortin (POMC) promoter; and neurons/spinal cord specific promoter such as superoxide dismutase 1 (SOD1). (See, *e.g.*, U.S. Patent Publication No. 2021/214749 and Adeno-Associated Virus Vectors (2019), Ed. Castle., 1<sup>st</sup> Edition, Springer New York, New York, NY.; both of which are hereby incorporated by reference herein in their entirety.)

**[00143]** Expression control elements also include ubiquitous or promiscuous promoters and promoters/enhancers capable of driving polynucleotide expression in many different cell types. Such elements include the cytomegalovirus (CMV) immediate early promoter/enhancer sequences, the Rous sarcoma virus (RSV) promoter/enhancer sequences, phosphoglycerate kinase (PKG) promoter, CAG (composite of the CMV enhancer, the chicken beta actin promoter (CBA) and the rabbit beta globin intron) (*see, e.g.*, Boshart *et al.*, (1985) Cell, 41:521-530), the SV40 promoter, the dihydrofolate reductase promoter, and the cytoplasmic b-actin promoter.

**[00144]** Additional promoters include the U6 promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), herpes simplex virus (HSV) promoter, SFFV promoter, rat insulin promoter, TBG promoter, the desmin promoter and similar muscle-specific promoters, synthetic promoters, hybrid promoters, and promoters with multi-tissue specificity.

**[00145]** In certain embodiments the promoter is a EF-1 $\alpha$  promoter (*see, e.g.*, Wang *et al.*, J. Cell Mol. Med. (2017) 21(11):3044-3054, hereby incorporated by reference herein in its entirety) and/or comprises, consists, or consists essentially of a sequence at least 95%, 97%, 99% or 100% identical to SEQ ID NO: 251.

**[00146]** Expression control elements also can impact expression in a manner that is regulatable by a signal or stimuli increasing or decreasing expression. A regulatable element increasing expression of transcribed nucleic acid in response to a signal or stimuli is also referred to as an “inducible element” (*i.e.*, is induced by a signal). Typically, the amount of increase or decrease conferred by such elements is proportional to the amount of signal or stimuli present. Particular examples include zinc-inducible sheep metallothioneine (MT) promoter; the steroid hormone-

inducible mouse mammary tumor virus (MMTV) promoter; the T7 polymerase promoter system (International Patent Publication No. WO1998/10088); the tetracycline-repressible system (Gossen, *et al.*, Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992)); the tetracycline-inducible system (Gossen *et al.*, Science 268: 1766-1769 (1995); see also Harvey *et al.*, Curr. Opin. Chem. Biol. 2:512-518 (1998)); the RU486-inducible system (Wang *et al.*, Nat. Biotech. 15:239-243 (1997) and Wang *et al.*, Gene Ther. 4:432-441 (1997); and the rapamycin-inducible system (Magari *et al.*, J. Clin. Invest. 100:2865-2872 (1997); and Rivera *et al.*, Nat. Medicine. 2:1028-1032 (1996)). Other examples of regulatable control elements include those regulated by a specific physiological state such as temperature, acute phase, or development.

**[00147]** In certain embodiments the expression cassette further comprises one or more introns. A variety of different introns can be used. Examples of introns that may be used include the rabbit  $\beta$ -globin intron with splice donor/splice acceptor, SV40 intron with splice donor/splice acceptor, human  $\beta$ -globin introns, intron 2 of the human hemoglobin beta gene, hFIX int1 (intron 1 of the human coagulation factor IX gene), CBA-rHHB (synthetic intron derived from the fusion of the intron 1 of the chicken beta actin gene and intron 2 of the rabbit hemoglobin beta), CBA (intron 1 of the chicken beta actin gene), hGH (intron 1 of the human growth hormone gene), hFIX synth (synthetic intron derived from different portions of the human coagulation factor IX gene and present in the pLIVE vector, Mirus Bio, Madison, WI); human hemoglobin subunit beta (HBB2) synthetic intron, and optimized HBB2; and chimeric introns such as introns made up of the 5'-splice donor of the first human  $\beta$ -globin intron and the branch and 3'-acceptor site from the intron that is between the leader and the body of the immunoglobulin gene heavy chain variable region. (Buck *et al.*, Int. J. Mol. Sci. (2020), 21, 4197; Ronzitti *et al.* Mol. Ther. Methods Clin Dev. (2016) Jul 20;3:16049; and the HBB-IGG intron provided by the pCMVNT<sup>TM</sup> vector.)

**[00148]** In certain embodiments, nucleic acid encoding for an RNA polynucleotide comprising a targeting sequence, a pri-miRNA or a shRNA is positioned within an intron.

**[00149]** In certain embodiments the expression cassette comprises a post-transcriptional regulatory element. Post-translational regulatory elements such as Woodchuck post-transcriptional regulatory element (WPRE) and Hepatitis B regulatory element can increase gene expression. (Buck *et al.*, Int. J. Mol. Sci. (2020), 21, 4197.)

**[00150]** Polyadenylation signal sequences provide for the formation of a polyA tail, which facilitates nuclear export, translation and/or mRNA stability, and may also be involved in transcription termination. Examples of polyadenylation signal sequences include SV40 late polyadenylation signal, bovine growth hormone polyA (bGHpA) signal sequence, synthetic

polyA, mouse  $\beta$ -globin pA, rabbit  $\beta$ -globin pA, and H4-based pA, (Buck *et al.*, Int. J. Mol. Sci. (2020), 21, 4197).

**[00151]** In certain embodiments, the expression cassette comprises a Kozak consensus sequence or a variation thereof. Kozak consensus sequences play a role in translation initiation. The Kozak consensus sequence and variations are provided in, for example, McClements *et al.*, (2021) Molecular vision, 27, 233–242.

**[00152]** In certain embodiments, the expression cassette comprises an upstream promoter and a downstream polyadenylation signal, operably linked to the nucleic acid sequence encoding the RNA polynucleotide.

**[00153]** In certain embodiments, the expression cassette comprises 5' to 3', operably linked to the nucleic acid sequence encoding the RNA polynucleotide, a promoter or promoter/enhancer, an intron, the nucleic acid sequence encoding the RNA polynucleotide, and a polyadenylation signal.

**[00154]** In certain embodiments the expression cassettes further comprises an miRNA target sequences, which in further embodiments is incorporated into the 3' UTR of the expression cassette. An miRNA target sequence is recognized by miRNA present in particular cells or tissues leading to degradation of mRNA transcripts. Based on the presence of certain miRNA in particular cells, incorporating a miRNA target sequences can be used to reduce expression in certain cell or tissue types. Multiple tandem repeats of miRNA target sequences can be used to increase degradation. (Geisle *et al.*, (2016) World Journal of Experimental Medicine 6(2): 37–54.)

**[00155]** In certain embodiments, the expression cassette encoding nucleotide sequence, not including the sequence encoding for a pri-miRNA or shRNA contains any of 0-5, 0-10, 0-15, 0-50, or 0-100 CpGs; 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 CpG's; and/or contains 0% to 5%, 0%, about 0.5%, about 1.0%, about 2.0%, about 3.0%, about 4.0%, or about 5.0% CpGs.

**[00156]** II.B. Recombinant Viral Vector Nucleic Acid

**[00157]** Polynucleotide recombinant viral nucleic acid contain 5' and/or 3' viral elements providing for viral packaging and replication, and may provide for additional activities such as promoter activity, genome integration, or episomal concatemerization. The 5' and 3' elements are generally located at or near the 5' and 3' terminal end of the recombinant viral nucleic acid and can be naturally occurring or modified versions of naturally occurring sequences. Examples of 5' and 3' elements include adenovirus ITRs, adeno-associated virus ITRs and packaging sequence; and retrovirus 5' and 3' long terminal repeats (LTRs) and packaging sequence. (Naso

*et al.*, (2017) BioDrugs, 31(4), 317--334; Bulcha *et al.*, (2021) Sig. Transduct. Target Ther. 6:53 (2021); and Liu and Seol (2020) BMB Reports; 53(11):565-575.)

**[00158]** The term “recombinant,” as a modifier of nucleic acid or a vector indicates a combination of elements that does not occur in nature. For example, a recombinant viral vector nucleic acid provides 5’ and/or 3’ viral elements along with an expression cassette containing one or more elements not naturally associated with the 5’ and/or 3’ elements. Similarly, a viral vector, such as a rAAV vector may contain a naturally occurring or modified capsid, encapsidating recombinant viral vector nucleic acid.

**[00159]** Polynucleotides, expression cassettes and viral vector nucleic acid are compatible with the particular viral vector. For example, rAAV can be produced comprising ssDNA or dsDNA, adenovirus vectors can be produced comprising dsDNA, and retrovirus vectors can be produced comprising ssRNA.

**[00160]** In certain embodiments, the viral vector nucleic acid, outside of the pri-miRNA or shRNA encoding region, contains any of 0-5, 0-10, 0-15, 0-50, 0-100, or 0 to 150 CpGs; 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 44, 46, 47, 48, 49 or 50 CpG’s; and/or 0% to 10%, 0%, about 0.5%, about 1.0%, about 2.0%, about 3.0%, about 4.0%, about 5.0% CpGs, about 6%, about 7%, about 8%, about 9% or about 10% CpGs.

**[00161]** II.C. miR21

**[00162]** Included in the Examples provided below is data illustrating the effect of an rAAV vector comprising an miR21 encoding nucleic acid (SEQ ID NO: 261) in a Huntington disease animal model and NHPs. miR21 employs a miR155 scaffold.

**[00163]** Certain embodiments are directed to a DNA polynucleotide comprising in a 5’ to 3’ direction:

(a) a 5’ inverted terminal repeat (ITR) sequence comprising the sequence of SEQ ID NO: 262;

(b) a CAG promoter;

(c) a pre-miRNA encoding sequence comprising the sequence of SEQ ID NO: 261, wherein the CAG promoter is operably linked to the pre-miRNA encoding sequence and a polyadenylation signal; and

(d) a 3’ inverted terminal repeat (ITR) sequence comprising the sequence of SEQ ID NO: 263.

**[00164]** In certain further embodiments the polyadenylation signal comprises the sequence of SEQ ID NOs: 252 or 264.



[00165] In further embodiment the CAG promoter comprising the sequence of SEQ ID NOs: 250 or 265.

[00166] In certain embodiments the DNA polynucleotide is a self-complementary rAAV and is up to about 2.5 kb.

[00167] In certain further embodiments, the DNA polynucleotide comprises the sequence of SEQ ID NO: 266.

[00168] In certain embodiments the polynucleotide is plasmid further comprising an origin of replication and a selectable marker.

[00169] In certain embodiment the polynucleotide is a recombinant adeno-associated viral (rAAV) nucleic acid comprising the 5' ITR at the 5' terminus and 3' ITR at the 3' terminus.

### III. Viral Vectors

[00170] In certain embodiments the gene delivery vehicle is a viral vector. Viral vectors comprises a protein capsid encapsidating recombinant viral nucleic acid and can deliver the nucleic acid to cells or tissues. Depending on the particular vector, the viral vector may further comprise a viral envelope. Examples of viral vectors that can be used include adenovirus vectors, rAAV, retrovirus vectors and herpes simplex vectors.

[00171] Different serotypes exist within different types of viruses. The different serotypes can provide for different activities, such as cell or tissue tropism and likelihood of generating a host immune response. The term "serotype" broadly refers to both serologically distinct viruses as well as viruses not serologically distinct that can be within a subgroup or a variant of a given serotype. Serologic distinctiveness can be determined based on the lack of cross-reactivity between antibodies to one capsid as compared to another capsid. Such cross-reactivity differences are usually due to differences in capsid protein sequences/antigenic determinants (e.g., due to VP1, VP2, and/or VP3 sequence differences of AAV serotypes).

[00172] As more naturally occurring virus isolates are discovered or capsid mutants generated, there may or may not be serological differences with any of the currently existing serotypes. Thus, in cases where the new virus has no serological difference, this new virus would be a subgroup or variant of the corresponding serotype.

#### [00173] III.A. Adenovirus Vectors

[00174] Adenoviruses are non-enveloped double-stranded DNA viruses. Recombinant adenovirus vectors comprise recombinant adenovirus nucleic acid lacking one or more protein involved in viral replication, and further comprise an adenoviral capsid. Recombinant adenovirus vectors can be produced containing different amounts of adenoviral DNA. The Ad genome is flanked by hairpin-like inverted terminal repeats (ITRs) varying in length from 30–371 bp at its termini. The ITR's serve as self-priming structures that promote primase-

independent DNA replication. The 5' and 3' inverted repeats need not be exact inverted repeats. A packaging signal located at the left arm of the genome is required for viral genome packaging. (Liu and Seol (2020) BMB Reports; 53(11):565-575; and Bulcha *et al.*, (2021) Sig. Transduct. Target Ther. 6:53.)

**[00175]** In certain embodiments, the recombinant adenovirus vector is a third-generation vector, also referred to as “gutless” or “helper-dependent”. Gutless vectors can be produced from recombinant adenoviral nucleic acid where all, or substantially all viral sequences, except for the ITRs and the packaging signal are not present. Gutless adenovirus vectors are high capacity vectors able to accommodate up to about 36 kb of DNA insert. Preferred recombinant adenovirus nucleic acid is about 27 kb to about 37 kb. Stuffer sequences can be added to recombinant adenovirus nucleic acid to increase nucleic acid size and capsid incorporation. Preferred stuffer sequences avoid coding sequences, repetitive sequences, recombination sequences, and immunogenic sequences. (Liu and Seol (2020) BMB Reports; 53(11):565-575; Bulcha *et al.*, (2021) Sig. Transduct. Target Ther. 6:53; and Sandig *et al.*, PNAS (2000) 97(3):1002-1007, each of which are hereby incorporated by reference herein in their entirety.)

**[00176]** In certain embodiments, recombinant adenovirus vectors can be produced based on rare human serotypes or chimpanzee serotypes. The use of chimpanzee and rare human serotypes may be helpful in reducing host immune response against recombinant adenovirus vectors due to preexisting immunity. (Guo *et al.*, (2018) Human vaccines & immunotherapeutics, 14(7):1679–1685 and Bulcha *et al.*, (2021) Sig. Transduct. Target Ther. 6:53.)

**[00177]** Adenovirus vectors can be produced by supplying viral proteins needed for vector production *in trans* using for example, appropriate helper viruses or plasmids and cell lines. (Liu and Seol (2020) BMB Reports; 53(11):565-575; and Bulcha *et al.*, (2021) Sig. Transduct. Target Ther. 6:53.)

**[00178]** III.B. AAV Vectors

**[00179]** A recombinant adeno-associated viral (also referred to herein as “rAAV”) vector is based on the adeno-associated virus. The adeno-associated virus is a single-strand DNA virus containing a 4.7-kb genome flanked by 145-nt ITRs on both ends of the genome. ITR activity is important for self-priming and packaging, and may also provide additional activity such as promoter activity. rAAV 5' and 3' ITR's can vary in size and the 5' and 3' inverted repeats need not be exact inverted repeats.

**[00180]** A rAAV vector contains AAV recombinant nucleic acid and a viral capsid. The rAAV recombinant nucleic acid lacks one or more AAV proteins involved in viral replication. In certain embodiments, the rAAV vector contains an AAV 5' and/or 3' ITR along with a DNA insert. In certain embodiments rAAV nucleic acid comprise a 5' ITR and/or 3' ITR

independently selected from 5' and 3' ITRs provided in AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.10, AAVrh.74 and AAV3B ITRs. In further embodiments 5' and 3' ITRs are present, and both ITRs are from the same serotype genome.

**[00181]** In further embodiments the 5' ITR comprises a sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 254 and the 3' ITR independently comprises a sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 253.

**[00182]** Recombinant adeno-associated viral vectors typically accept inserts of DNA having a size range generally about 4 kb to about 5.2 kb. If needed, stuffer sequence can be used to increase rAAV nucleic acid size and packaging efficiency. In different embodiments, the rAAV nucleic acid including stuffer is 4-5.2kb, 3.0-5.5 kb, 4.0-5.0 kb, 4.3-4.8 kb, about 4.2 kb, about 4.3 kb, about 4.4 kb about 4.5 kb, about 4.6 kb, or about 4.7 kb. Preferred stuffer sequences avoid coding sequences, repetitive sequences, recombination sequences, and immunogenic sequences.

**[00183]** In certain embodiments the rAAV is a self-complementary adeno-associated virus vector (scAAV) or short hairpin adeno-associated virus vector (shAAV). scAAV and shAAV provide for a double-stranded recombinant adeno-associated virus nucleic acid that can be incorporated into AAV capsid. scAAV and shAAV comprise inverted dimeric repeats providing intramolecular double-stranded DNA. scAAV can be produced by mutating an ITR terminal resolution site so that Rep fails to nick the terminal resolution site. shAAV can utilize a short hairpin to produce the dsAAV. scAAV and shAAV being double stranded DNA, provide an advantage in circumventing the DNA synthesis step required for single-stranded rAAV nucleic acid upon entry into a cell. A potential disadvantage of the scAAV and shAAV is the size of DNA inserts that can be incorporated is reduced by about half compared to single-stranded rAAV nucleic acid. (U.S. Patent No. 10,457,940; Xie *et al.*, Mol Ther. (2017) Jun 7;25(6):1363-1374; and McCarty Mol. Ther. (2008), 16(10):1648-1656; each of which are hereby incorporated by reference herein in their entirety.)

**[00184]** Naturally occurring AAV capsids contain viral proteins VP1, VP2 and VP3 in a ratio of about 1:1:10. AAV vectors can be produced where all three viral proteins are based upon a particular serotype or where one, two or all three viral protein are based on different serotypes.

**[00185]** Recombinant AAV capsid and nucleic acid can be based on the same serotype (or subgroup or variant), or can be based on different serotypes. In certain embodiments, a rAAV nucleic acid has the same serotype genome (*e.g.*, ITRs) as the encapsidating capsid protein.

**[00186]** In different embodiments, the rAAV capsid comprises a protein having a sequence at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, at least 99.1%, at least 99.2%, at least 99.3%, at least at least 99.4%, at least 99.5%, at least 99.9% or 100% identical to a VP1, VP2 or VP3 of any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.74, AAV3B, AAV-2i8, AAVrh.10, AAVrh.8, AAVHSC, AAV-B1, AAV-AS, or AAV1/rh.10; or VP1 of SEQ ID NO: 257 or SEQ ID NO: 260.

**[00187]** In certain embodiments, AAV capsids comprises VP1, VP2 and VP3 each independently having a sequence at least 80%, at least 90%, at least 95% or 100% identical to a VP1, VP2 or VP3 of any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.74, AAV3B, AAV-2i8, AAVrh.10, AAVrh.8, AAVHSC, AAV-B1, AAV-AS, AAV1/rh.10; or VP1 of SEQ ID NO: 257 or SEQ ID NO: 260.; as well as variants (*e.g.*, capsid variants, such as amino acid insertions, additions, substitutions and deletions) thereof. (See for example, U.S. Patent Nos. 9,909,142 and 9,840,719 disclosing RHM4-1, RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4 and RHM15-6; U.S. Patent Publication No. 2013/0059732 and U.S. Patent No. 9,169,299, disclosing LK01, LK02, and LK03; and U.S. Patent No. 11,110,153; the disclosures of which are herein incorporated in their entirety.)

**[00188]** In certain embodiments, the capsid comprises VP1 having the sequence of SEQ ID NO: 257; VP2 having the sequence of SEQ ID NO: 258; and VP3 having the sequence of SEQ ID NO: 259.

**[00189]** In certain embodiments, the rAAV vector comprises (1) rAAV nucleic acid comprising a sequence encoding for miR21 operably linked to a promoter and an polyadenylation signal and (2) a rAAV capsid comprising (a) VP1 comprising the amino acid sequence of SEQ ID NO: 257, (b) VP2 comprising the amino acid sequence of SEQ ID NO: 258, and (c) VP 3 comprising the amino acid sequence of SEQ ID NO: 259.

**[00190]** In certain embodiments, the AAV capsid can cross the blood brain barrier and provide for CNS expression. Examples of such AAV capsids and the design of AAV capsids able to provide for CNS expression are provided in Chen *et al.*, (2021) J. Control. Release 333, 129-138 (*e.g.*, AAV9, AAV-PHP-B, AAV-PHP.eB, AAVrh.10, AAVrh.8, AAVHSC, AAV-B1, AAV-AS, and AAV1/rh.10), U.S. Patent No. 9,585,971, and Goertsen *et al.*, (2022) Nat. Neurosci. 25, 106–115 (2022), each of which are incorporated by reference herein in its entirety.

**[00191]** The AAV genome contains two main genes: *rep* and *cap*. Transcription from the *rep* gene is initiated from two different promoters resulting in the production of nonstructural

proteins designated Rep78, Rep68, Rep52, and Rep40. The rep proteins function in genome replication and/or encapsidation. The *cap* gene encodes for structural proteins making up the capsid (VP1, VP2 and Vp3); a non-structural assembly-activating protein (APP), which performs functions related to capsid assembly; and the membrane-associated accessory protein, which may be associated with production phases of the replication cycle. (Maurer and Weitzman (2020) Hum. Gene Ther. 31(9-10):499-511, hereby incorporated by reference herein in its entirety.)

**[00192]** AAV requires helper virus functions to complete its replication cycle. Helper virus functions can be supplied by different viruses in permissive cell lines. Permissive cell lines are cell lines able to support viral replication. Examples of helper viruses for AAV include adenovirus, HSV-1, HPV-16, and HBov1 which can be used in conjunction with, for example, permissive primate cells; and baculovirus which can be used in conjunction with, for example, permissive insect cells such as sf9. (Maurer and Weitzman (2020) Hum. Gene Ther. (2020) 31(9-10):499-511 and Meier *et al.*, (2020) Viruses 19;12(6):662, both of which are herein incorporated by reference herein in their entirety.)

**[00193]** Recombinant AAV can be produced by supplying viral proteins needed for vector production *in trans* using for example, appropriate helper viruses or plasmids and cell lines. In certain embodiments, rAAV is produced using a rAAV vector genome plasmid. The plasmid comprises that portion of the rAAV nucleic acid ultimately packaged or encapsidated to form a viral (e.g., rAAV) vector. The “plasmid backbone,” contains elements important for propagation and recombinant virus production. Except for possible 3' ITR and/or 5' ITR cloning remnants the plasmid backbone is not itself packaged or encapsidated into virus particles.

**[00194]** The vector genome plasmid may contain regions such as an origin of replication and a selectable marker. Additional sites that may be present include cloning sites.

**[00195]** Recombinant AAV can be produced from different types of cell lines including HeLa, A549, BHK, Vero, and HEK293, or derivatives thereof. In certain embodiments, HEK293 cells are used (American Type Culture Collection Accession Number ATCC CRL1573). Other host cell lines appropriate for rAAV vector production are described in, for example, Robert *et al.*, Biotechnol. J. (2017) 12(3), 1600193; and International Application No. PCT/US2017/024951, the disclosures of which are herein incorporated in its entirety.

**[00196]** Recombinant AAV can be cultured under a variety of different conditions suitable for providing cell growth and gene expression. References describing rAAV manufacturing include Clément and Grieger (2016) Mol. Ther. Methods Clin. Dev. 16;3:16002; Robert *et al.*, (2017) Biotechnol. J. 12(3), 1600193; and Adeno-Associated Virus Vectors (2019), Ed. Castle., 1<sup>st</sup>

Edition, Springer New York, New York, NY.; each of which are hereby incorporated by reference herein in their entirety.)

**[00197]** In certain embodiments, AAV helper functions are introduced into the host cell by transfecting the host cell with an AAV helper construct either prior to, or concurrently with, the transfection of an AAV expression vector. A host cell having AAV helper functions can be referred to as a “helper cell” or “packaging helper cell.” AAV helper constructs are thus sometimes used to provide at least transient expression of AAV *rep* and/or *cap* genes to complement missing AAV functions necessary for productive AAV transduction. AAV helper constructs often lack AAV ITRs and can neither replicate nor package themselves. These constructs can be, for example, in the form of a plasmid, phage, transposon, cosmid, virus, or virion. A number of AAV helper constructs have been described, such as the commonly used plasmids pAAV/Ad and pIM29+45 which encode both *rep* and *cap* expression products. A number of other vectors are known which encode *rep* and/or *cap* expression products. Recombinant AAV can be produced, for example, as described in US Patent 9,408,904; and International Application Nos. PCT/US2017/025396 and PCT/US2016/064414, the disclosures of which are herein incorporated in their entirety.

**[00198]** In certain embodiments, a rAAV vector is produced by a rAAV production cell comprising rAAV helper virus activity. The genome of the rAAV production cell comprises rAAV nucleic acid, the *rep* gene and the *cap* gene.

**[00199]** In certain embodiments, a rAAV vector is produced by culturing a rAAV permissive cell comprising an AAV genome plasmid, where the rAAV permissive cell further comprises *rep* and *cap* genes provided either as part of the cell genome and/or by one or more separate plasmids; and helper virus activity either as part of the cell genome and/or provided by one or more separate plasmids. In further embodiments, (a) the rAAV permissive cell line is a packaging cell, wherein the genome of the packaging cell comprises the *cap* gene and the *rep* gene; (b) the *rep* gene, *cap* gene, and helper activity are provided from the same plasmid; or (c) the *rep* gene and *cap* gene are provided by a *rep/cap* plasmid and helper activity is provided by a helper plasmid.

**[00200]** In certain embodiments involving the use of HSV helper functions, the helper functions are provided by genes encoding for at least UL5, UL8, UL52, and ICP8.

**[00201]** In certain embodiments involving the use of adenovirus helper functions, the helper function are provided by genes encoding for at least E1A, E1B19K, E1B55K, E2A, E4orf6 and VA RNA. In certain embodiments E1, E2A and VR RNA functions are provided by a helper plasmid, where additional helper functions are provided by a host strain.

[00202] In certain embodiments, rAAV vector is obtained by producing rAAV using methods described herein and purifying the rAAV. Purification of rAAV can be performed using techniques such as gradient-based purification, column-based, and combined methods. (See, *e.g.*, Ayuso *et al.*, *Curr Gene Ther.* (2010) 10(6):423-36, hereby incorporated by reference herein in its entirety.)

[00203] III.C. Retrovirus Vectors

[00204] Retroviruses are enveloped, single-stranded RNA viruses comprising 5' and 3' LTRs, and a signal packaging sequence located just outside of the LTR. Different types of retrovirus vectors can contain different amounts of viral genome. In certain embodiments, the retrovirus vector is a lentiviral vector based on HIV, retaining all cis-acting sequences needed for viral RNA packaging, reverse transcription and proviral DNA integration, while removing all HIV protein-coding genes. Lentiviral vectors have a packaging capacity of up to about 9 kb. If needed, stuffer sequence can be used to increase rAAV nucleic acid size and packaging efficiency. Lentiviral vectors can be produced by supplying viral proteins needed for vector production *in trans* using appropriate plasmids and cell lines. (Bulcha *et al.*, (2021) *Sig. Transduct. Target Ther.* 6:53.)

IV. Non-Viral Vectors

[00205] In certain embodiments, the gene delivery vehicle is a non-viral vector. Preferred non-viral vectors are nanoparticles. A variety of different nanoparticles can be employed including lipid nanoparticles (LNP), polymeric nanoparticles, lipid polymer nanoparticles (LPNP), protein and peptide-based nanoparticles, DNA dendrimers and DNA-based nanocarriers, carbon nanotubes, microparticles, microcapsules, inorganic nanoparticles, peptide cage nanoparticles, and exosomes. (See, *e.g.*, Riley and Vermerris *Nanomaterials* (2017) 201, 7, 94; Thomas *et al.*, *Molecules* (2019), 24, 3744; Bochicchio *et al.*, (2021), 13, 198; Munagala *et al.*, *Cancer Letters* (2021), 505, 58; Fu *et al.*, (2020) *NanoImpact* 20, 100261; Neshat *et al.* (2020) *Current Opin. Biotechnol.* 66:1-10; Ouranidis *et al.*, (2022) *Biomedicines*, 10, 50; and Qin *et al.*, *Signal Transduct Target Ther.* (2022) May 21;7(1):166, each of which are hereby incorporated by reference herein in their entirety.)

[00206] If desired, a nanoparticle can target a cell type using, for example, targeting ligands recognizing a target cell receptor. Examples of targeting ligands include carbohydrates (*e.g.*, galactose, mannose, glucose, and galactomannan), endogenous ligands (*e.g.*, folic acid and transferrin), antibodies and protein/peptides (*e.g.*, RGD, epidermal growth factor, and low density lipoprotein) and peptides. (For example, Teo *et al.*, *Advanced Drug Delivery Reviews* (2016), 98, 41.)

[00207] Nanoparticles can be used to deliver inhibitory RNA or encoding polynucleotide constructs to a cell. In different embodiments, nanoparticles can deliver additional therapeutic compounds; and one or more additional compounds is provided in different nanoparticles. Reference to compound includes small molecules and large molecules (*e.g.*, therapeutic proteins and antibodies).

[00208] The production of different nanoparticles and incorporation of nucleic acid and other compounds is well known in the art. Examples of publications illustrating incorporation of nucleic acid in a particular nanoparticle such as an LPNP and a LNP include Teo *et al.*, *Advanced Drug Delivery Reviews* (2016) 98, 41; Bochicchio *et al.*, *Pharmaceutics* (2021) 13, 198; Mahzabin and Das, *IJPSR* (2021) 12(1), 65; and Teixeira *et al.*, (2017) *Prog. Lipid Res.* Oct;68:1-11 (each of which are hereby incorporated by reference herein in their entirety). Factors that may impact small molecule incorporation into a nanoparticle include hydrophobicity and the presence of an ionizable moiety. (See, *e.g.*, Nii and Ishii *International Journal of Pharmaceutics* (2005) 298, 198; and Chen *et al.*, *Journal of Controlled Release* (2018) 286, 46.)

#### IV.A. Lipid-Based Delivery Systems

[00209] Lipid-based delivery systems include the use of a lipid as a component. Examples of lipid-based delivery systems include liposomes, LNPs, micelles, and extracellular vesicles.

[00210] A “lipid nanoparticle” or “LNP” refers to a lipid-based vesicle useful for delivery of nucleic acid molecules and having dimensions on the nanoscale. In different embodiments the nanoparticle is from about 10 nm to about 1000 nm, about 50 nm to about 500 nm, or about 50 nm to about 200 nm.

[00211] DNA is negatively charged. Thus, it can be beneficial for the LNP to comprise a cationic lipid such as, for example, an amino lipid. Exemplary amino lipids are described in U.S. Patent Nos. 9,352,042, 9,220,683, 9,186,325, 9,139,554, 9,126,966, 9,018,187, 8,999,351, 8,722,082, 8,642,076, 8,569,256, 8,466,122, and 7,745,651 and U.S. Patent Publication Nos. 2016/0213785, 2016/0199485, 2015/0265708, 2014/0288146, 2013/0123338, 2013/0116307, 2013/0064894, 2012/0172411, and 2010/0117125, all of which are incorporated herein in their entirety. In certain embodiments, the LNP comprises amino lipids described in U.S. Patent No. 9,512,073, hereby incorporated herein in its entirety.

[00212] The terms “cationic lipid” and “amino lipid” are used interchangeably herein to include lipids and salts thereof having one, two, three, or more fatty acid or fatty alkyl chains and a pH-titratable amino group (*e.g.*, an alkylamino or dialkylamino group). The cationic lipid is typically protonated (*i.e.*, positively charged) at a pH below the pKa of the cationic lipid and is substantially neutral at a pH above the pKa. The cationic lipid can also be a titratable cationic



lipid. In certain embodiments, the cationic lipids comprise a protonatable tertiary amine (*e.g.*, pH-titratable) group; C18 alkyl chains, wherein each alkyl chain independently can have one or more double bonds or one or more triple bonds; and ether, ester, or ketal linkages between the head group and alkyl chains.

**[00213]** Cationic lipids include 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-di- $\gamma$ -linolenyloxy-N,N-dimethylaminopropane ( $\gamma$ -DLenDMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-K-C2-DMA, also known as DLin-C2K-DMA, XTC2, and C2K), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), dilinoleylmethyl-3-dimethylaminopropionate (DLin-M-C2-DMA, also known as MC2), (6Z,9Z,28Z,31 Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-M-C3-DMA, also known as MC3), salts thereof, and mixtures thereof. Other cationic lipids also include 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane (DSDMA), 1,2-dioleyloxy-N,N-dimethyl-3-aminopropane (DODMA), 2,2-dilinoleyl-4-(3-dimethylaminopropyl)-[1,3]-dioxolane (DLin-K-C3-DMA), 2,2-dilinoleyl-4-(3-dimethylaminobutyl)-[1,3]-dioxolane (DLin-K-C4-DMA), DLen-C2K-DMA,  $\gamma$ -DLen-C2K-DMA, and (DLin-MP-DMA) (also known as 1-B11).

**[00214]** Still further cationic lipids include 2,2-dilinoleyl-5-dimethylaminomethyl-[1,3]-dioxane (DLin-K6-DMA), 2,2-dilinoleyl-4-N-methylpepiazino-[1,3]-dioxolane (DLin-K-MPZ), 1,2-dilinoleylcarbamoxyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-dilinoleyloxy-3-(dimethylamino)acetoxyp propane (DLin-DAC), 1,2-dilinoleyloxy-3-morpholinopropane (DLin-MA), 1,2-dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), 3-(N,N-dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-dioleylamino)-1,2-propanedio (DOAP), 1,2-dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), 3-(N—(N',N'-dimethylaminoethane)-carbamoxy)cholesterol (DC-Chol), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), dioctadecylamidoglycyl spermine (DOGS), 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-octadecadienoxy)propane (CLinDMA), 2-[5'-(cholest-5-en-3-beta-oxy)-

3'-oxapentoxo)-3-dimethyl-1-(cis,cis-9',1'-octadecadienoxy)propane (CpLinDMA), N,N-dimethyl-3,4-dioleoyloxybenzylamine (DMOBA), 1,2-N,N'-dioleoylcarbamy-3-dimethylaminopropane (DOcarbDAP), 1,2-N,N'-dilinoleoylcarbamy-3-dimethylaminopropane (DLincarbDAP), dexamethasone-sperimine (DS) and disubstituted spermine (D2S) or mixtures thereof.

**[00215]** A number of commercial preparations of cationic lipids can be used, such as, LIPOFECTIN® (including DOTMA and DOPE, available from GIBCO/BRL), and LIPOFECTAMINE® (comprising DOSPA and DOPE, available from GIBCO/BRL).

**[00216]** Additional ionizable lipids that can be used include C12-200, 306Oi10, MC3, cKK-E12, bCKK-E12, Lipid 5, Lipid 9, ATX-002, ATX-003, and Merck-32. U.S. Patent Application Publication No. 2017/0367988, describes Merck-32.

**[00217]** In further embodiments, cationic lipid can be present in an amount from about 10% by molar ratio of the LNP to about 85% by molar ratio of the LNP, or from about 50% by molar ratio of the LNP to about 75% by molar ratio of the LNP.

**[00218]** LNP can comprise a neutral lipid. Neutral lipids can comprise a lipid species existing either in an uncharged or neutral zwitterionic form at physiological pH. Such lipids include diacylphosphatidylcholine, diacylphosphatidylethanolamine, ceramide, sphingomyelin, dihydrosphingomyelin, cephalin, and cerebroside. The selection of neutral lipids is generally guided by considerations including particle size and stability. In certain embodiments, the neutral lipid component can be a lipid having two acyl groups (*e.g.*, diacylphosphatidylcholine and diacylphosphatidylethanolamine).

**[00219]** Lipids having a variety of acyl chain groups of varying chain length and degree of saturation are available or can be isolated or synthesized. In certain embodiments, lipids containing saturated fatty acids with carbon chain lengths in the range of C14 to C22 can be used. In certain embodiments lipids with mono or di-unsaturated fatty acids with carbon chain lengths in the range of C14 to C22 are used. Additionally, lipids having mixtures of saturated and unsaturated fatty acid chains can be used. Exemplary neutral lipids include 1,2-dioleoyl-sn-glycero-3-phosphatidyl-ethanolamine (DOPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), or a phosphatidylcholine. The neutral lipids can also be composed of sphingomyelin, dihydrosphingomyelin, or phospholipids with other head groups, such as serine and inositol.

**[00220]** In further embodiments, providing for neutral lipids, the neutral lipid can be present in an amount from about 0.1% by weight of the LNP to about 99% by weight of the LNP, or from about 5% by weight of the LNP to about 15% by weight of the LNP, *e.g.*, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, 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%, or about 99%.

**[00221]** An LNP can contain additional components such as sterols and polyethylene glycol. Sterols can confer fluidity to the LNP. As used herein “sterol” refers to a naturally occurring sterol of plant (phytosterols) or animal (zoosterols) origin as well as non-naturally occurring synthetic sterols, all of which are characterized by the presence of a hydroxyl group at the 3-position of the steroid A-ring. Suitable sterols include those conventionally used in the field of liposome, lipid vesicle or lipid particle preparation, most commonly cholesterol. Phytosterols include campesterol, sitosterol, and stigmasterol. Sterols also include sterol-modified lipids, such as those described in U.S. Patent Application Publication No. 2011/0177156. In different embodiments providing for a sterol, the sterol is present in an amount from about 1% by weight of the LNP to about 80% by weight of the LNP or from about 10% by weight of the LNP to about 25% by weight of the LNP.

**[00222]** Polyethylene glycol (PEG) is a water-soluble polymer of ethylene PEG repeating units with terminal hydroxyl groups. PEGs are classified by their molecular weights, for example, PEG 2000 has an average molecular weight of about 2,000 daltons, and PEG 5000 has an average molecular weight of about 5,000 daltons. PEGs commercially available from Sigma Chemical Co. and other companies include monomethoxypolyethylene glycol (MePEG-OH), monomethoxypolyethylene glycol-succinate (MePEG-S), monomethoxypolyethylene glycol-succinimidyl succinate (MePEG-S-NHS), monomethoxypolyethylene glycol-amine (MePEG-NH<sub>2</sub>), monomethoxypolyethylene glycol-tresylate (MePEG-TRES), and monomethoxypolyethylene glycol-imidazolyl-carbonyl (MePEG-IM).

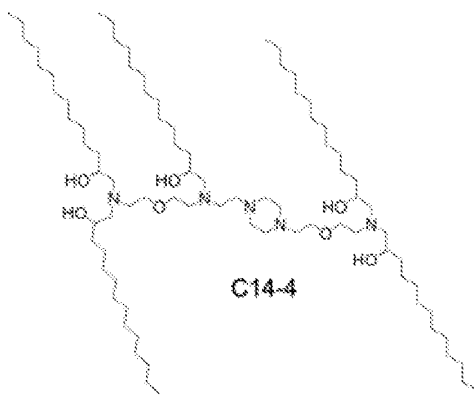
**[00223]** In certain embodiments concerning PEG, PEG has an average molecular weight of about 550 to about 10,000 daltons and is optionally substituted by alkyl, alkoxy, acyl or aryl. In further embodiments, the PEG is substituted with methyl at the terminal hydroxyl position. In further embodiments, the PEG has an average molecular weight from about 750 to about 5,000 daltons, or from about 1,000 to about 5,000 daltons, or from about 1,500 to about 3,000 daltons, or from about 2,000 daltons, or from about 750 daltons.

**[00224]** PEG-modified lipids include the PEG-dialkyloxypropyl conjugates (PEG-DAA) described in U.S. Patent Nos. 8,936,942 and 7,803,397. PEG-modified lipids (or lipid-polyoxyethylene conjugates) can have a variety of “anchoring” lipid portions to secure the PEG portion to the surface of the lipid vesicle. Examples of suitable PEG-modified lipids include PEG-modified phosphatidylethanolamine and phosphatidic acid, PEG-ceramide conjugates (*e.g.*, PEG-CerC14 or PEG-CerC20) which are described in U.S. Patent No. 5,820,873, PEG-modified

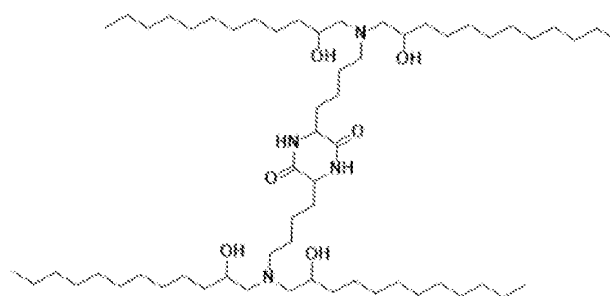
dialkylamines and PEG-modified 1,2-diacyloxypropan-3-amines. In certain embodiments, the PEG-modified lipid can be PEG-modified diacylglycerols and dialkylglycerols. In certain embodiments, the PEG can be in an amount from about 0.1% by weight of the LNP to about 50% by weight of the LNP, or from about 5% by weight of the LNP to about 15% by weight of the LNP.

**[00225]** In further embodiments concerning LNP size, prior to encapsulating nucleic acid, LNPs have a size range from about 10 nm to 500 nm, or from about 50 nm to about 200 nm, or from 75 nm to about 125 nm.

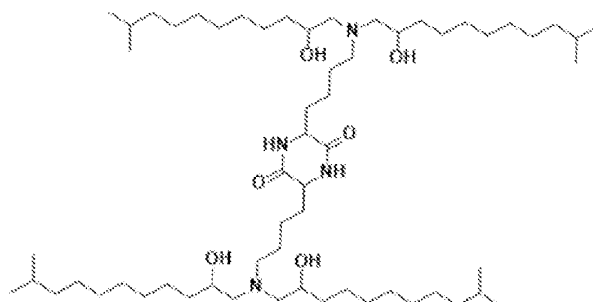
**[00226]** In certain embodiments concerning LNP, the LNP is described by Billingsley *et al.*, Nano Lett. 2020, 20, 1578 or Billingsley *et al.*, International Patent Publication No. WO 2021/077066 (both of which are hereby incorporated by reference herein in their entirety). Billingsley *et al.*, and WO2021/077066 describe LNPs containing lipid-anchored PEG, cholesterol, phospholipid and ionizable lipids. In certain embodiments, the LNP contains a C14-4 polyamine core and/or has a particle size of about 70 nm. C14-4 has the following structure.



**[00227]** In certain embodiments the LNP is made up of a cationic lipid or lipopeptide described by U.S. Patent No. 10,493,031, U.S. Patent No. 10,682,374 or WO2021/077066 (each of which is hereby incorporated by reference herein in its entirety). In certain embodiments, the LNP contains a cationic lipid, a cholesterol-based lipid, and/or one or more PEG-modified lipids. In certain embodiments the LNP contains cKK-E12 (Dong *et al.*, PNAS (2014) 111(11), 3955):

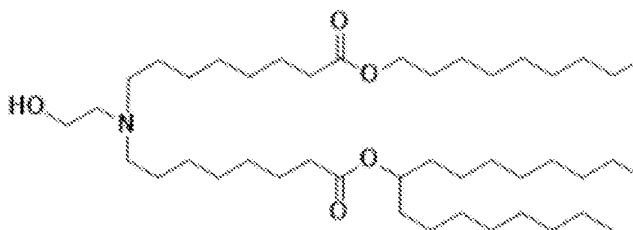


[00228] In certain embodiments the LNP comprises a modified form of cKK-E12 referred to herein as “bCKK-E12,” having the following structure:

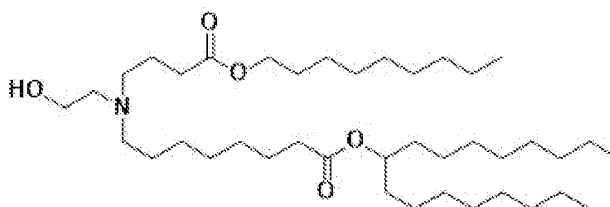


[00229] In certain embodiments the LNP comprises Lipid 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 as described by Sabnis *et al.*, Molecular Therapy 2018, 26:6, 1509-1519 (hereby incorporated by reference herein in its entirety). In certain embodiments the LNP comprises Lipid 5, 8, 9, 10, or 11 described in Sabnis *et al.*

[00230] Lipid 5 of Sabnis *et al.* has the structure:



[00231] Lipid 9 of Sabnis *et al.* has the structure:



[00232] Additional lipids that may be utilized include those described by Roces *et al.*, Pharmaceutics, 2020, 12,1095; Jayaraman *et al.*, Angew. Chem. Int. Ed., 2012, 51, 8529-8533; Maier *et al.*, www.moleculartherapy.org, 2013, Vol.21, No. 8, 1570-1578; Liu *et al.*, Adv. Mater. 2019, 31, 1902575, *e.g.*, BAMEA-O16B; Cheng *et al.*, Adv. Mater., 2018, 30, 1805308, *e.g.*, 5A2-SC8; Hajj and Ball, Small, 2019 15, 1805097, *e.g.*, 306Oi10; Du *et al.*, U.S. Patent Application Publication No. 20160376224; and Tanaka *et al.*, Adv. Funct. Mater., 2020, 30, 1910575; each of which are hereby incorporated by reference herein in their entirety.

[00233] In further embodiments, the nanoparticle is an LNP. In further embodiments the LPN in mol% comprises, consists essentially, or consists, of the following components: (1) one or more cationic lipids from about 20% to about 65%, one or more phospholipids from about 1% to about 50%, one or more PEG-conjugated lipids from about 0.1 % to about 10%, and cholesterol from 0% to about 70%; or (2) one or more cationic lipids from about 20% to about 50%, one or more phospholipids from about 5% to about 20%, one or more PEG-conjugated lipids from about 0.1 % to about 5%, and cholesterol from about 20% to about 60%. In further embodiments the phospholipid lipid is a neutral lipid; and the phospholipid lipid is DOPE or DSPC.

[00234] In further embodiments the LNP, in mole %, comprises, consists essentially, or consists of the following components: (1) cKK-E12 about 35%; C14-PEG2000, about 2.5%; cholesterol, about 46.5%; and DOPE, about 16%; (2) bCKK-E12 about 35%; C14-PEG2000, about 2.5%; cholesterol, about 46.5%; and DOPE, about 16%; (3) Lipid 9 about 50%; C14-PEG2000, about 1.5%; cholesterol, about 38.5%; and DSPC about 10%; (4) Lipid 5 about 50%; C14-PEG2000 about 1.5%; cholesterol about 38.5%; and DSPC about 10%; (5) ionizable lipid, about 50%; DSPC, about 10%; cholesterol, about 37.5%; and stabilizer (PEG-Lipid), about 2.5%; or (6) is GenVoy-ILM™ LNP (Precision NanoSystems).

#### IV.B. Polymer-Based Nanoparticles

[00235] Polymer-based delivery systems can be made from a variety of different natural and synthetic materials. DNA and other compounds can be entrapped into the polymeric matrix of polymeric nanoparticles or can be adsorbed or conjugated on the surface of the nanoparticles. Examples of commonly used polymers for nucleic acid delivery include poly(lactic-co-glycolic acid) (PLGA), poly lactic acid (PLA), poly(ethylene imine) (PEI) and PEI derivatives, chitosan, dendrimers, polyanhydride, polycaprolactone, polymethacrylates, poly-L-lysine, pullulan, dextran, and hyaluronic acid, poly-β-aminoesters. (Thomas *et al.*, (2019) Molecules 24, 3744.)

[00236] Polymeric-based nanoparticles can have different sizes, ranging from about 1 nm to about 1000 nm, from about 10 nm to about 500 nm, from about 50 nm to about 200 nm, from about 100 nm to about 150 nm, and from about 150 nm or less.

#### V.C. Lipid Polymer Nanoparticles

[00237] Lipid polymer nanoparticles are hybrid nanoparticles providing both a lipid component and a polymer component, and as such can be considered to be an LNP or LPNP. The LPNP configuration can provide an outer polymer and inner lipid or an outer lipid and inner polymer. The presence of two different types of material facilitates designing nanoparticles to provide for delayed release of a component. Different lipid and polymer components can be selected taking into account the material to be delivered. (For example, see Teo *et al.*, *Advanced Drug Delivery Reviews* (2016) 98, 41; Bochicchio *et al.*, *Pharmaceutics* (2021) 13, 198; Mahzabin and Das, *IJPSR* (2021) 12(1), 65; and Teixeira *et al.*, (2017) *Prog. Lipid Res.* Oct;68:1-11.)

#### IV.D. Protein and Peptide-Based Nanoparticles

[00238] Protein and peptide-based systems can employ a variety of different proteins and peptides. Examples of proteins that can be employed include gelatin and elastin. Peptide-based systems can employ, for example, CPPs.

[00239] CPPs are short peptides (6–30 amino acid residues) potentially capable of intracellular penetration to deliver therapeutic molecules. The majority of CPPs consists mainly of arginine and lysine residues, making them cationic and hydrophilic, but CPPs can also be amphiphilic, anionic, or hydrophobic. CPPs can be derived from natural biomolecules (*e.g.*, HIV-1 Tat protein), or obtained by synthetic methods (*e.g.*, poly-L-lysine, polyarginine) (Singh *et al.*, *Drug Deliv.* 2018;25(1):1996-2006). Examples of CPPs include cationic CPPs (highly positively charged) such as the Tat peptide, penetratin, protamine, poly-L-lysine, and polyarginine; amphipathic CPPs (chimeric or fused peptides, constructed from different sources, containing both positively and negatively charged amino acid sequences), such as transportan, VT5, battenecin-7 (Bac7), proline-rich peptide (PPR), SAP (VRLPPP)<sub>3</sub>, TP10, pep-1, and MPG; membranotropic CPPs (exhibits both hydrophobic and amphipathic nature simultaneously, and comprise both large aromatic residues and small residues) such as H625, SPIONs-PEG-CPP and NPs; and hydrophobic CPPs (contain only non-polar motifs or residues) such as SG3, PFVYLI, pep-7, and fibroblast growth factors.

[00240] Protein and peptide nanoparticles can be provided in different sizes, for example, ranging from about 1 nm to about 1000 nm, from about 10 nm to about 500 nm, from about 50 nm to about 200 nm, from about 100 nm to about 150 nm, or from about 150 nm or less.

#### IV.E. Peptide Cage Nanoparticles

[00241] Peptide cage-based delivery systems can be produced from proteinaceous material able to assemble into a cage-like structure forming a constrained internal environment. Peptide cages can comprise a proteinaceous shell that self-assembles to form a protein cage (*e.g.*, a structure with an interior cavity that is either naturally accessible to the solvent or can be made so by altering solvent concentration, pH, or equilibria ratios). The monomers of the protein cages can

be naturally occurring or variant forms, including amino acid substitutions, insertions, and deletions (*e.g.*, fragments).

[00242] Different types of protein “shells” can be assembled and loaded with different types of materials. Protein cages can be produced using viral coat protein(s) (*e.g.*, from the Cowpea Chlorotic Mottle Virus protein coat), as well non-viral proteins (*e.g.*, U.S. Patent Nos. 6,180,389 and 6,984,386, U.S. Patent Publication No. 20040028694, and U.S. Patent Publication No. 20090035389, each of which is incorporated by reference herein in their entirety).

[00243] Examples of protein cages derived from non-viral proteins include: eukaryotic or prokaryotic derived ferritins and apoferritins such as 12 and 24 subunit ferritins; and heat shock proteins (HSPs), such as the class of 24 subunit heat shock proteins that form an internal core space, the small HSP of *Methanococcus jannaschii*, the dodecameric Dsp HSP of *E. coli*; and the MrgA protein.

[00244] Protein cages can have different core sizes, such as ranging from about 1 nm to about 1000 nm, from about 10 nm to about 500 nm, from about 50 nm to about 200 nm, from about 100 nm to about 150 nm, or from about 150 nm or less.

#### IV.F. Exosomes

[00245] Exosomes are small biological membrane vesicles. Exosome have been utilized to deliver various cargoes including small molecules, peptides, proteins and nucleic acids. Exosomes generally range in size from about 30 nm to 100 nm and can be taken up by a cell and deliver its cargo (*e.g.*, expression cassette comprising nucleic acid encoding for RNA polynucleotide comprises an HTT targeting sequence). Cargoes can be associated with exosome surface structure or may be encapsulated within the exosome bilayer.

[00246] Various modifications can be made to exosomes facilitating cargo delivery and cell targeting. Modifications for facilitating cargo delivery include structures for associating with cargoes such as protein scaffolds and polymers. Modifications for cell targeting include targeting ligands and modifying surface charge. Publications describing production, modification, and use of exosomes for delivery of different cargoes include Munagala *et al.*, Cancer Letters (2021), 505, 58; Fu *et al.*, (2020) NanoImpact 20, 100261; and Dooley *et al.*, (2021) Molecular Therapy 29(5), 1729 (each of which is hereby incorporated by reference herein).

#### V. Pharmaceutical Compositions

[00247] Pharmaceutical compositions can be used to facilitate storage and/or delivery of an agent being administered to a subject. In certain embodiments, the pharmaceutically composition comprises: (a) an RNA polynucleotide comprising a sequence targeting HTT mRNA, (b) an optionally modified inhibitory RNA duplex targeting HTT mRNA, (c) a



polynucleotide, expression cassette, or recombinant viral nucleic acid comprising a sequence encoding a HTT mRNA targeting sequence, or (d) a delivery vehicle comprising (a), (b) or (c); and a pharmaceutically acceptable carrier.

[00248] Reference to “pharmaceutically acceptable” indicates the components do not cause substantial undesirable biological effects at the amount utilized. Pharmaceutically acceptable carriers can contain different components such as one or more pharmaceutically acceptable excipients such as a salt, sugar, buffer, solvent, preservative, protein and surfactant. A particular excipient can have more than one function. Examples of pharmaceutically acceptable excipients and carriers that can be used for viral vectors are provided in, for example, International Patent Publication No. WO2021/071835.

[00249] In certain embodiments the formulation comprises about 10 mM sodium phosphate, about 150 mM sodium chloride, and about 0.001% Kolliphor®; and a pH or about pH 7.3.

[00250] Pharmaceutical compositions can be formulated to be compatible with a particular route of administration or delivery. Compositions suitable for parenteral administration include aqueous and non-aqueous solutions, suspensions or emulsions, which preparations are typically sterile and can be isotonic with the blood of the intended recipient. Illustrative examples include water, buffered saline, Hanks’ solution, Ringer’s solution, dextrose, fructose, ethanol, animal vegetable and synthetic oils. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran.

[00251] In an embodiment, the pharmaceutical composition contains a formulation capable of injection into a subject. Examples of injectable formulation components include isotonic, sterile, saline solutions, salts (*e.g.*, monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and mixtures of such salts), buffered saline, sugars (*e.g.*, dextrose), and water for injection. Pharmaceutical compositions include dry, for example, freeze-dried compositions which upon addition of sterilized water or physiological saline, permit the constitution of solutions suitable for administration.

[00252] Additionally, suspensions can be prepared as appropriate oil injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension can also contain suitable stabilizers or agents which increase compound solubility facilitating the preparation of concentrated solutions.

[00253] An “effective amount” or “sufficient amount” refers to an amount providing an indicated or desired effect. The effective amount can be administered, in single or multiple doses, alone or in combination, with one or more other compositions (*e.g.*, additional therapeutic

or immunosuppressive agents), treatments, protocols, or therapeutic regimens; and provide for a long or short term response.

**[00254]** Pharmaceutical compositions comprising transgenes encoding an RNA polynucleotide targeting HTT mRNA can be delivered to a subject, so as to allow production of the encoded polynucleotide. Delivery can be *in vivo* or *ex vivo*. In certain embodiments, pharmaceutical compositions comprise sufficient genetic material to enable a recipient to produce a therapeutically effective amount in the subject.

**[00255]** A “therapeutically effective amount” refers to an amount that elicits the desired or indicated biological or medicinal response in a subject. A therapeutically effective amount can be determined based on observed symptoms and/or through the use of biomarkers associated with a particular disease or disorder. Selection of a particular effective dose can be optimized taking into account different factors, including the disease to be treated or prevented, the symptoms involved, the targeted disease or disorder, safety and effectiveness in animal models, the patient’s body mass, and the patient’s immune status. The optimal dose to be employed in the formulation will also depend on the route of administration, and the severity of the disease or disorder, and can be evaluated depending upon patient’s circumstances. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

**[00256]** In certain embodiments, a pharmaceutical composition comprising a rAAV vector comprises empty AAV capsids. In certain embodiments, in a pharmaceutical composition comprising rAAV vectors and empty AAV capsids, the ratio of the empty AAV capsids to the rAAV vector is within or between about 100:1-50:1, from about 50:1-25:1, from about 25:1-10:1, from about 10:1-1:1, from about 1:1-1:10, from about 1:10-1:25, from about 1:25-1:50, or from about 1:50-1:100. In certain embodiments, the ratio of the of the empty AAV capsids to the rAAV vector is about 2:1, 3:1, 4:1, 5:1, 6:1, 7: 1, 8:1, 9: 1, or 10:1.

**[00257]** Additional guidance and examples of pharmaceutical compositions and delivery systems are provided in, for example, Remington: The Science and Practice of Pharmacy (2020) 23th ed., University of the Science in Philadelphia, published by Elsevier; The Merck Index (2013) 15th ed., Whitehouse, NJ; Pharmaceutical Principles of Solid Dosage Forms (1993), Technomic Publishing Co., Inc., Lancaster, Pa.; and Ansel and Stoklosa, Pharmaceutical Calculations (2001) 11th ed., Lippincott Williams & Wilkins, Baltimore, MD.

#### VI. Administration and Treatment

**[00258]** RNA polynucleotides comprising an inhibitory sequence targeting HTT can be used in methods for reducing mutant HTT expression and/or treating Huntington disease in a subject. Such methods can, for example, comprise administration of (a) an RNA polynucleotide comprising a sequence targeting HTT mRNA, (b) an optionally modified inhibitory RNA duplex

targeting HTT mRNA, (c) a polynucleotide, expression cassette, or recombinant viral nucleic acid comprising a sequence encoding for a HTT mRNA targeting sequence or (d) a delivery vehicle comprising (a), (b) or (c) and a pharmaceutically acceptable carrier.

**[00259]** Huntington disease symptoms include motor impairment, cognitive impairment and psychiatric disorders. Diagnosis of Huntington disease can be confirmed, for example, by an analysis of the HTT protein (also referred to herein as huntingtin protein) measuring glutamine repeats or encoding nucleic acid measuring CAG repeats. In certain embodiments subjects are selected based on biomarker or genetic makers associated with Huntington's disease; and/or subjects are diagnosed with Huntington's disease.

**[00260]** Measuring glutamine or CAG repeats can also be used to identify patients at an increased likelihood of contracting Huntington disease and as a prognostic indicator. The CAG-repeat length in the HTT gene appears to be inversely correlated with age of onset and initial progression of severity. Partial penetrance and late disease onset have been associated with subjects having 36-39 CAG repeats. (Langbehn *et al.*, Am. J. Med. Genet. (2009) Part B 153B:397-408; and Langbehn Am. J. Med. Genet. (2022) 109:172-179, both of which are hereby incorporated by reference herein in its entirety.)

**[00261]** In certain embodiments the subject being treated has an HTT gene encoding for HTT comprising a CAG repeat segment of 36 or more. In further embodiments, the subject has a CAG repeat segment of at least 40, at least 45, at least 50, at least 55, at least 70, or at least 100.

**[00262]** In certain embodiments the subject being treated has an HTT protein comprising a glutamine repeat segment of 36 or more. In further embodiments the subject has a glutamine repeat of at least 40, at least 45, at least 50, at least 55, at least 70, or at least 100.

**[00263]** In certain embodiments the subject is diagnosed with an HTT gene comprising a CAG repeat segment of 36 or more prior to initial treatment. In further embodiments the subject is diagnosed has a CAG repeat segment of at least 40, at least 45, at least 50, at least 55, at least 70 or at least 100 prior to initial treatment.

**[00264]** In certain embodiments the subject is diagnosed with HTT protein comprising a glutamine repeat segment of 36 or more prior to initial treatment. In further embodiments the subject is diagnosed with glutamine repeats of at least 40, at least 45, at least 50, at least 55, at least 70 or at least 100 prior to initial treatment.

**[00265]** Administration can be by different routes, such as, subcutaneously, epidermally, intradermally, intrathecally, intraorbitally, intramucosally, intranasally, intraperitoneally, intravenously, intra-pleurally, intraarterially, intracavitary, orally, intrahepatically, via the portal vein, intramuscularly, intraparenchymal, intracisternal or intraventricular administration. In certain embodiments administration to a patient is via infusion in a pharmaceutical carrier.

**[00266]** In certain embodiment CNS delivery is achieved using techniques and/or agents that facilitate the crossing of the blood brain barrier; bypassing the blood brain barrier; or direct administration to the brain.

**[00267]** Techniques facilitating transport across the blood brain barrier, include disruption of the blood brain barrier, use of blood brain barrier carriers and the use of vectors able to cross the blood brain barrier. General techniques facilitating crossing the blood brain barrier can be utilized on viral and non-viral delivery vehicles. In addition, particular delivery vehicles such as certain AAV serotypes can facilitate crossing the blood brain barrier. (Chen et al., (2021) Journal of Controlled Release 333, 129-138; Bellettato and Scrapa, Italian Journal of Pediatrics (2018) 44(Suppl 2):131; Haumann et al., (2020) CNS Drugs 34, 1121-1131; Cammalleri et al., J. Clin. Neurophysiol. (2020) Mar;37(2):104-117); and Fischell and Fishman, Front. Neurosci., 23 September 2021, volume 15, Article 747726, doi: 10.3389/fnins.2021.747726; each of which are hereby incorporated by reference herein in their entirety.)

**[00268]** In certain embodiments, an AAV capsid facilitating CNS entry is used. Examples of such capsids are provided in Chen *et al.*, (2021) Journal of Controlled Release 333, 129-138 (e.g., AAV9, AAVrh.10, AAVrh.8, AAVHSC, AAV-B1, AAV-AS, and AAV1/rh.10), U.S. Patent No. 9,585,971, and U.S. Patent Publication No. US202/1214749, each of which are hereby incorporated by reference herein in their entirety.

**[00269]** In certain embodiments, CNS delivery involves the use of focused ultrasound and microbubbles. Focused ultrasound combined with microbubbles can temporarily disrupt the blood brain barrier facilitating entry of therapeutic agents, including transgene delivery vehicles. Focused ultrasound can deposit energy to a selected area of the human anatomy, and its use can be facilitated by MRI guidance. Different types of microbubbles can be used with focused ultrasound to temporarily disrupt the blood brain barrier. (Cammalleri *et al.*, J Clin Neurophysiol. (2020) Mar;37(2):104-117; Noroozian *et al.*, Methods Mol Biol. (2019) 1950, 177-197; U.S. Patent No. 10,322,178; and International Patent Publication No. WO 2019/113538; each of which are hereby incorporated by reference herein in its entirety.)

**[00270]** Microbubbles are typically injected intravenously. In certain embodiments, microbubbles are either Definity™ (Lantheus Medical Imaging, North Billerica, MA, USA) containing perflutren lipid microspheres (mean diameter of 1.1-3.3 µm with a maximum diameter of 20 µm); SonoVue™ (Bracco Imaging, Milan, Italy) a suspension of phospholipid microspheres containing sulfur hexafluoride gas (mean diameter of ~2.5 µm; more than 90% of the bubbles are smaller than 8 µm); and Optison™ (GE Healthcare, Princeton, NJ, USA) made up of a sterile non-pyrogenic suspension of microspheres of human serum albumin with perflutren (mean diameter of 3.0-4.5 µm with a maximum diameter of 32 µm). (See,

Cammalleri *et al.*, J Clin Neurophysiol. (2020) Mar;37(2):104-117, hereby incorporated by reference herein in its entirety.)

[00271] In certain embodiments microbubbles are produced and administered as described in U.S. Patent No. 10,322,178, hereby incorporated by reference herein in its entirety.

[00272] In certain embodiments rAAV vectors are selected, or engineered, to facilitate use with focused ultrasound delivery. (Kofed et al., Journal of Controlled Release 351 (2022) 667–680; Kofed et al., Molecular Therapy: Methods & Clinical Development Vol. 27 December 2022, 167-195; U.S. Patent Publication No. 2023/0047753; and International Patent Publication No. WO2023/0044161; each of which are incorporated by reference herein in their entirety.)

[00273] In certain embodiments, rAAV vectors comprise an acoustic targeting peptide described in U.S. Patent Publication No. 2023/0047753 and/or International Patent Publication No. WO2023/0044161.

[00274] In certain embodiments intranasal administration is used to achieve CNS vector delivery. Intranasal delivery bypasses the blood brain barrier and reduces systemic exposure. In further embodiments, intranasal delivery comprises the use of focused ultrasound and microbubbles. The use of focused ultrasound and microbubbles in combination with intranasal delivery has been indicated to enhance penetration of therapeutic agents already at the perivascular space beyond the blood brain barrier. (Ye et al., The Lancet (2022) Oct: Vol 84: 1-14, hereby incorporated by reference in its entirety.).

[00275] In certain embodiments, administration is direct to the CNS, for example, by intraparenchymal, intracisternal or intraventricular administration.

[00276] In certain embodiments CNS administration is carried out, for example, by direct administration to the brain using needles or catheter. (For example, International Publication No. WO 2021/108809, Cohen-Pferrer *et al.*, Pediatric Neurology 67 (2017) 23-35; and U.S. Patent No 10369329; each of which are hereby incorporated by reference herein in their entirety.)

[00277] Another example of a technique for CNS administration is convection enhanced delivery. Convection enhanced delivery comprises surgical exposure of the brain, placement of a catheter directly into the target area, followed by infusion of a therapeutic agent. (U.S. Patent Publication No. 2022/010001; and Debinski et al. (2009) Expert Rev Neurother. 9(10):1519-27; both of which are hereby incorporated by reference herein in their entirety.)

[00278] In different embodiments intraparenchymal administration is performed via IP infusions to the caudate and putamen employing either: a transfrontal trajectory via along axis of both structures; using a parietal trajectory (delivery to caudate) and/or using an occipital trajectory (delivery to putamen). In a further embodiment, vector delivery was at a steady rate of about 5 µl/min. Administration can be carried out, for example, by bilateral infusion using convection-

enhanced delivery. In further embodiments administration employs bilateral stereotactic infusions. In further embodiments administration is carried under MRI-guidance, wherein the rAAV is provided in a formulation comprising an imaging agent. Different devices are available for infusion including, for example, the ClearPoint® SmartFlow cannula via convection-enhanced delivery (CED) under MRI-guidance and ClearPoint® Neuro Navigation System.

**[00279]** In certain embodiments, after IP infusion, for example to the caudate and/or putamen, the presence of inhibitory nucleic acid encoded by a transgene is measured in cerebrospinal fluid (CSF). The presence of inhibitory nucleic acid in the CSF can be monitored at different time points, for example at baseline, and months 3, 6, 12, 18, and 24. In a further embodiment, a vector encoding miR21 is administered and presence of miR21 is measured in cerebrospinal fluid (CSF). Measuring inhibitory nucleic acid in CSF can be used, for example, as a durability marker.

**[00280]** CNS delivery devices, systems and techniques also include those described in, for example, U.S. Patent No. 8128600, U.S. Patent Publication No. 2020/0324089, U.S. Patent No. 11129643, U.S. Patent No. 11154377, U.S. Patent Publication No. 2021/0343397, U.S. Patent Publication No. 2021/0282866, U.S. Patent No. 9572928, U.S. Patent No. 8337458, U.S. Patent No. 10722265, and US Patent publication No. 2021/214749, each of which are incorporated by reference herein in their entirety.

**[00281]** In certain embodiments providing for CNS expression, the polynucleotide expressing the RNA polynucleotide comprises the PGK promoter, CBh promoter, and/or EF1- $\alpha$  promoter.

**[00282]** Optimal doses can vary depending upon different factors such as the particular therapeutic, and desired endpoint. The dose amount, number, frequency or duration can be proportionally increased or reduced, taking into account adverse side effects, complications or other risk factors of the treatment or therapy and the status of the subject.

**[00283]** A “unit dosage form” refers to a physically discrete unit containing a predetermined effective amount of active ingredient in combination with a pharmaceutically acceptable carrier. Unit dosage forms can be provided within, for example, ampules and vials, which can include a pharmaceutically acceptable carrier, or a composition in a freeze-dried or lyophilized state. In the case of a freeze-dried or lyophilized state, a sterile liquid carrier can be added prior to administration. Individual unit dosage forms can be included in multi-dose kits or containers.

**[00284]** An “effective amount” achieves the desired or indicated effect. For example, an effective amount for treatment decreases one or more adverse symptoms, reduces the likelihood of one or more symptoms associated with a disease or disorder, or reduces disease or disorder progression. Preferred effective amounts for treatment are effective to decrease multiple or all adverse symptoms.

**[00285]** In certain embodiments, pharmaceutical compositions comprising a viral or non-viral vector is administered to a subject at a dose suitable to decrease mutant HTT or mutant HTT expression. In different embodiments mutant HTT or mutant HTT expression is decreased by at least 50%, at least 60%, at least 70%, or at least 90%.

**[00286]** In certain embodiments, total HTT protein and/or total HTT mRNA expression is reduced by about 20% to about 90%, about 20% to about 65%, about 25% to about 60%, about 25% to about 50%, about 25% to about 45%, about 25% to 40%, about 25% to 35%, about 25% to 30%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, or about 30% to about 35%.

**[00287]** In different embodiments a suitable dosage is from about 0.01 mg/kg to about 10 mg/kg of vector per kg body weight of a subject, about 0.01 mg/kg to about 0.1 mg/kg of vector per kg body weight of a subject, about 0.1 mg/kg to about 1.0 mg/kg of vector per kg body weight of a subject, or about 1.0 mg/kg to about 10 mg/kg of vector per body weight of a subject.

**[00288]** In certain embodiments, rAAV vector doses range from at least  $1 \times 10^8$  vector genomes per kilogram (vg/kg) of the weight of the subject, or more, for example,  $1 \times 10^9$ ,  $1 \times 10^{10}$ ,  $1 \times 10^{11}$ ,  $1 \times 10^{12}$ ,  $1 \times 10^{13}$  or  $1 \times 10^{14}$ , or more, vector genomes per kilogram (vg/kg) of the weight of the subject, to achieve a therapeutic effect. In different embodiments the rAAV dose is about  $5 \times 10^{11}$  rAAV vg/kg or greater than about  $5 \times 10^{11}$  rAAV vg/kg; about  $1 \times 10^{12}$  rAAV vg/kg or greater than about  $1 \times 10^{12}$  rAAV vg/kg; about  $2 \times 10^{12}$  rAAV vg/kg or greater than about  $2 \times 10^{12}$  rAAV vg/kg; about  $3 \times 10^{12}$  rAAV vg/kg or greater than about  $3 \times 10^{12}$  rAAV vg/kg; about  $4 \times 10^{12}$  rAAV vg/kg or greater than about  $4 \times 10^{12}$  rAAV vg/kg; about  $5 \times 10^{12}$  rAAV vg/kg or greater than about  $5 \times 10^{12}$  rAAV vg/kg; about  $1 \times 10^{13}$  rAAV vg/kg or greater than about  $1 \times 10^{13}$  rAAV vg/kg; about  $2 \times 10^{13}$  rAAV vg/kg or greater than about  $2 \times 10^{13}$  rAAV vg/kg; about  $3 \times 10^{13}$  rAAV vg/kg or greater than about  $3 \times 10^{13}$  rAAV vg/kg; about  $4 \times 10^{13}$  rAAV vg/kg or greater than about  $4 \times 10^{13}$  rAAV vg/kg; about  $5 \times 10^{13}$  rAAV vg/kg or greater than about  $5 \times 10^{13}$  rAAV vg/kg; about  $6 \times 10^{13}$  rAAV vg/kg or greater than about  $6 \times 10^{13}$  rAAV vg/kg.

**[00289]** Examples of dose ranges of rAAV vg/kg include a dose range from about  $5 \times 10^{11}$  to about  $6 \times 10^{13}$  rAAV vg/kg; a dose range from about  $5 \times 10^{11}$  to about  $5.5 \times 10^{11}$  rAAV vg/kg; a dose range from about  $5.5 \times 10^{11}$  to about  $6 \times 10^{11}$  rAAV vg/kg; a dose range from about  $6 \times 10^{11}$  to about  $6.5 \times 10^{11}$  rAAV vg/kg; a dose range from about  $6.5 \times 10^{11}$  to about  $7 \times 10^{11}$  rAAV vg/kg; a dose range from about  $7 \times 10^{11}$  to about  $7.5 \times 10^{11}$  rAAV vg/kg; a dose range from about  $7.5 \times 10^{11}$  to about  $8 \times 10^{11}$  rAAV vg/kg; a dose range from about  $8 \times 10^{11}$  to about  $8.5 \times 10^{11}$  rAAV vg/kg; a dose range from about  $8.5 \times 10^{11}$  to about  $9 \times 10^{11}$  rAAV vg/kg; a dose range from about  $9 \times 10^{11}$  to about  $9.5 \times 10^{11}$  rAAV vg/kg; a dose range from about  $9.5 \times 10^{11}$  to about  $1 \times 10^{12}$  rAAV vg/kg; a dose range from about  $1 \times 10^{12}$  to about  $1.5 \times 10^{12}$  rAAV vg/kg; a dose range from about  $1.5 \times 10^{12}$

to about  $2 \times 10^{12}$  rAAV vg/kg; a dose range from about  $2 \times 10^{12}$  to about  $2.5 \times 10^{12}$  rAAV vg/kg; a dose range from about  $2.5 \times 10^{12}$  to about  $3 \times 10^{12}$  rAAV vg/kg; a dose range from about  $3 \times 10^{12}$  to about  $3.5 \times 10^{12}$  rAAV vg/kg; a dose range from about  $3.5 \times 10^{12}$  to about  $4 \times 10^{12}$  rAAV vg/kg; a dose range from about  $4 \times 10^{12}$  to about  $4.5 \times 10^{12}$  rAAV vg/kg; a dose range from about  $4.5 \times 10^{12}$  to about  $5 \times 10^{12}$  rAAV vg/kg; a dose range from about  $5 \times 10^{12}$  to about  $5.5 \times 10^{12}$  rAAV vg/kg; a dose range from about  $5.5 \times 10^{12}$  to about  $6 \times 10^{12}$  rAAV vg/kg; a dose range from about  $6 \times 10^{12}$  to about  $6.5 \times 10^{12}$  rAAV vg/kg; a dose range from about  $6.5 \times 10^{12}$  to about  $7 \times 10^{12}$  rAAV vg/kg; a dose range from about  $7 \times 10^{12}$  to about  $7.5 \times 10^{12}$  rAAV vg/kg; a dose range from about  $7.5 \times 10^{12}$  to about  $8 \times 10^{12}$  rAAV vg/kg; a dose range from about  $8 \times 10^{12}$  to about  $8.5 \times 10^{12}$  rAAV vg/kg; a dose range from about  $8.5 \times 10^{12}$  to about  $9 \times 10^{12}$  rAAV vg/kg; a dose range from about  $9 \times 10^{12}$  to about  $9.5 \times 10^{12}$  rAAV vg/kg; a dose range from about  $9.5 \times 10^{12}$  to about  $1 \times 10^{13}$  rAAV vg/kg; a dose range from about  $1 \times 10^{13}$  to about  $1.5 \times 10^{13}$  rAAV vg/kg; a dose range from about  $1.5 \times 10^{13}$  to about  $2 \times 10^{13}$  rAAV vg/kg; a dose range from about  $2 \times 10^{13}$  to about  $2.5 \times 10^{13}$  rAAV vg/kg; a dose range from about  $2.5 \times 10^{13}$  to about  $3 \times 10^{13}$  rAAV vg/kg; a dose range from about  $3 \times 10^{13}$  to about  $3.5 \times 10^{13}$  rAAV vg/kg; a dose range from about  $3.5 \times 10^{13}$  to about  $4 \times 10^{13}$  rAAV vg/kg; a dose range from about  $4 \times 10^{13}$  to about  $4.5 \times 10^{13}$  rAAV vg/kg; a dose range from about  $4.5 \times 10^{13}$  to about  $5 \times 10^{13}$  rAAV vg/kg; a dose range from about  $5 \times 10^{13}$  to about  $5.5 \times 10^{13}$  rAAV vg/kg; a dose range from about  $5.5 \times 10^{13}$  to about  $6 \times 10^{13}$  rAAV vg/kg; a dose range from about  $6 \times 10^{13}$  to about  $1 \times 10^{14}$  rAAV vg/kg.

**[00290]** In certain embodiments, rAAV vg/kg are administered at a dose of about  $5 \times 10^{11}$  vg/kg, about  $6 \times 10^{11}$  vg/kg, about  $7 \times 10^{11}$  vg/kg, about  $8 \times 10^{11}$  vg/kg, about  $9 \times 10^{11}$  vg/kg, about  $1 \times 10^{12}$  vg/kg, about  $2 \times 10^{12}$  vg/kg, about  $3 \times 10^{12}$  vg/kg, about  $4 \times 10^{12}$  vg/kg, about  $5 \times 10^{12}$  vg/kg, about  $6 \times 10^{12}$  vg/kg, about  $7 \times 10^{12}$  vg/kg, about  $8 \times 10^{12}$  vg/kg, about  $9 \times 10^{12}$  vg/kg, about  $1 \times 10^{13}$  vg/kg, about  $2 \times 10^{13}$  vg/kg, about  $3 \times 10^{13}$  vg/kg, about  $4 \times 10^{13}$  vg/kg, about  $5 \times 10^{13}$  vg/kg, or about  $6 \times 10^{13}$  vg/kg.

**[00291]** In certain embodiments doses and dose ranges for other viral vectors is as provided herein with respect to rAAV. For example, in certain embodiments the dose and dose range for recombinant adenovirus vectors, recombinant retrovirus vectors (*e.g.*, lentivirus), and recombinant herpes simplex virus vectors is the same as illustrated above with respect to rAAV.

**[00292]** In certain embodiments, the rAAV vector dose is about  $1.0 \times 10^{10}$  vg to about  $1.0 \times 10^{13}$  vg, about  $1.0 \times 10^{11}$  vg to about  $1.0 \times 10^{12}$  vg, about  $1.0 \times 10^{11}$  vg to about  $1.8 \times 10^{11}$  vg, about  $1.0 \times 10^{11}$  vg to about  $1.5 \times 10^{11}$  vg, about  $1.0 \times 10^{11}$  vg, about  $1.1 \times 10^{11}$  vg, about  $1.3 \times 10^{11}$  vg, about  $1.4 \times 10^{11}$  vg, about  $1.5 \times 10^{11}$  vg, about  $1.6 \times 10^{11}$  vg, about  $1.7 \times 10^{11}$  vg, about  $1.8 \times 10^{11}$  vg, about  $1.9 \times 10^{11}$  vg, about  $1.0 \times 10^{12}$  vg, about  $1.1 \times 10^{12}$  vg, about  $1.3 \times 10^{12}$  vg, about



$1.4 \times 10^{12}$  vg, about  $1.5 \times 10^{12}$  vg, about  $1.6 \times 10^{12}$  vg, about  $1.7 \times 10^{12}$  vg, about  $1.8 \times 10^{12}$  vg, about  $1.9 \times 10^{12}$  vg, or about  $1.0 \times 10^{13}$  vg.

**[00293]** Certain embodiments are directed to treating patient taking into account the putamen and/or caudate volume of the (i) the right hemisphere; (2) the left hemisphere; or (3) both hemispheres. Preferably, the putamen and the caudate volume are measured.

**[00294]** Putamen and caudate can significantly vary among Huntington disease patients. Typically, the putamen and caudate volumes do not significantly vary within a particular patient.

**[00295]** In certain embodiments a viral vector, such as rAAV is intraparenchymally administered to a subject to provide a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> to the right hemisphere or the left hemisphere; or a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to each of the right hemisphere and the left; wherein mm<sup>3</sup> is the putamen and caudate volume in a hemisphere.

**[00296]** In certain embodiments, a subject is treated for Huntington disease in a method comprising:

(a) determining in the subject, the putamen and/or caudate volume in the right hemisphere and/or left hemisphere; and

(b) intraparenchymally administering to the subject a recombinant adeno-associated viral (rAAV) vector at a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> to the right hemisphere and/or a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> to the left hemisphere; wherein said dose is based on the volume determined in step (a).

**[00297]** Reference to “and/or” provides for each possible combination of variables, which may be combined with other combinations. For example, reference to determining the putamen and/or caudate volume in the right hemisphere and/or left hemisphere includes: (1) determining the putamen volume in the right hemisphere; (2) determining the caudate volume in the right hemisphere; (3) determining the putamen volume in the left hemisphere; (4) determining the caudate volume in the left hemisphere; (5) determining the caudate and putamen volume in the right hemisphere; (6) determining the caudate and putamen volume in the left hemisphere; and (7) determining the putamen and caudate volume in the right and left hemisphere.

**[00298]** In certain embodiments, a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> is independently provided to both hemisphere. Reference to “independently” indicates the particular dose to the right and left hemisphere can vary within the indicated range.

**[00299]** In certain embodiments the dose is about  $2.0 \times 10^7$  vg/mm<sup>3</sup>, about  $2.5 \times 10^7$  vg/mm<sup>3</sup>, about  $3.0 \times 10^7$  vg/mm<sup>3</sup>, about  $3.5 \times 10^7$  vg/mm<sup>3</sup>, about  $4.0 \times 10^7$  vg/mm<sup>3</sup>, about  $4.5 \times 10^7$  vg/mm<sup>3</sup>, about  $5.0 \times 10^7$  vg/mm<sup>3</sup>, about  $5.5 \times 10^7$  vg/mm<sup>3</sup>, about  $6.0 \times 10^7$  vg/mm<sup>3</sup>, about  $6.5 \times$

$10^7$  vg/mm<sup>3</sup>, about  $7.0 \times 10^7$  vg/mm<sup>3</sup>, about  $7.5 \times 10^7$  vg/mm<sup>3</sup>, about  $8.0 \times 10^7$  vg/mm<sup>3</sup>, about  $8.5 \times 10^7$  vg/mm<sup>3</sup>, about  $9.0 \times 10^7$  vg/mm<sup>3</sup>, about  $9.5 \times 10^7$  vg/mm<sup>3</sup>, or about  $1.0 \times 10^8$  vg/mm<sup>3</sup>.

**[00300]** In certain embodiments, the putamen and caudate volume is obtained for a first hemisphere (right or left), and the dose for the second hemisphere is about the same as the dose for first hemisphere.

**[00301]** In certain embodiments administration to the right hemisphere comprises direct administration to the right hemisphere putamen and caudate; and administration to the left hemisphere comprises direct administration to the left hemisphere putamen and caudate.

**[00302]** In certain embodiments, the putamen and caudate volume of the right hemisphere is measured and the putamen and caudate volume of the left hemisphere is measured, and each hemisphere independently receives a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> based on the measured volume.

**[00303]** In certain embodiments a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the right hemisphere putamen and caudate in approximate proportion to the volume of the putamen and caudate of the right hemisphere and/or a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the left hemisphere putamen and caudate in approximate proportion to the volume of the putamen and caudate of the left hemisphere.

**[00304]** Reference to approximate proportion indicates within 10% of each value, to obtain 100%. For example, if the measured volume of the putamen to caudate is 60%:40%, approximate proportion of 10% provides a range of 70%:30% to 50%:50%. In further embodiments approximate proportion is within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%.

**[00305]** In certain embodiments, a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the right hemisphere putamen and caudate in a ratio of about 67% putamen to about 33% caudate; and/or a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the left hemisphere putamen and caudate in a ratio of about 67% putamen to about 33% caudate.

**[00306]** In certain embodiments, the same dose is administered to the right hemisphere and left hemisphere.

**[00307]** In certain embodiments administration to the caudate comprises a parietal trajectory and/or administration to the putamen comprises an occipital trajectory.

**[00308]** In certain embodiments each hemisphere is administered  $1.0 \times 10^{10}$  vg to  $1.0 \times 10^{13}$  vg of rAAV vector.

**[00309]** In certain embodiments, the method of treating Huntington disease in a subject comprises (a) determining the putamen and/or caudate volume in the right hemisphere and/or left hemisphere; and (b) intraparenchymally administering to the subject a recombinant adeno-associated viral (rAAV) vector, wherein a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$

vg/mm<sup>3</sup> is administered to the right hemisphere and/or the left hemisphere; wherein mm<sup>3</sup> is the putamen and caudate volume in a hemisphere.

**[00310]** The administered dose to each hemisphere can be the same or different. In certain embodiments, different amounts are provided to each hemisphere.

**[00311]** In certain embodiments, the polynucleotide constructs, viral vectors and non-viral vectors described herein are administered in combination with additional compounds or treatments for a particular disease or disorder; and/or in combination with a compound decreasing an immune response generated against the polynucleotide, delivery vehicle and/or produced protein. Additional compounds or treatments can be provided in different modalities such as administered separately; and administered or performed prior to, substantially contemporaneously with or following administration of the polynucleotide constructs, viral vectors and non-viral vectors described herein.

**[00312]** In certain embodiments, administration of polynucleotide constructs, viral vectors and non-viral vectors described herein is in combination with an immunosuppressive agent or regimen. Such agents and regimens can be utilized, as needed, to achieve immune tolerance or mitigate the immune response to the provided polynucleotides or delivery vehicles. Examples of immunosuppressive agents and regimens include methotrexate, rituximab, intravenous gamma globulin (IVIG), omalizumab, ImmTOR<sup>®</sup> (synthetic vaccine particle (SVP)-rapamycin (rapamycin encapsulated in a biodegradable nanoparticle)), ImmTOR-IL<sup>TM</sup> (ImmTOR with Treg-selective IL-2 agonist), B-cell depletion, immunoadsorption, and plasmapheresis.

**[00313]** In certain embodiments, the polynucleotide construct, viral vector or non-viral vector is administered in conjunction with one or more immunosuppressive agents, where one or more immunosuppressive agent is administered prior to, substantially at the same time as, or after, administering the polynucleotide construct, viral vector or non-viral vector. In certain embodiments, the one or more immunosuppressive agent is administered concomitantly with the polynucleotide construct, viral vector or non-viral vector. In certain embodiments, the one or more immunosuppressive agent is administered 1-12, 12-24 or 24-48 hours; or 2-4, 4-6, 6-8, 8-10, 10-14, 14-20, 20-25, 25-30, 30-50 days, or more than 50 days prior to polynucleotide construct, viral vector or non-viral vector administration. In certain embodiments, the one or more immunosuppressive agent is administered 1-12, 12-24 or 24-48 hours; or 2-4, 4-6, 6-8, 8-10, 10-14, 14-20, 20-25, 25-30, 30-50 days, or more than 50 days; following polynucleotide construct, viral vector or non-viral vector.

**[00314]** In certain embodiments, the immunosuppressive agent is an anti-inflammatory agent. In certain embodiments, the immunosuppressive agent is a steroid, e.g., a corticosteroid. In certain embodiments, the immunosuppressive agent is prednisone, prednisolone, calcineurin inhibitor

(*e.g.*, cyclosporine, tacrolimus), MMF (mycophenolic acid, *e.g.* CellCept®, Myfortic®), CD52 inhibitor (*e.g.*, alemtuzumab), CTLA4-Ig (*e.g.*, abatacept, belatacept), anti-CD3 mAb, anti-LFA-1 mAb (*e.g.*, efalizumab), anti-CD40 mAb (*e.g.*, ASKP1240), anti-CD22 mAb (*e.g.*, epratuzumab), anti-CD20 mAb (*e.g.*, rituximab, orelizumab, ofatumumab, veltuzumab), proteasome inhibitor (*e.g.*, bortezomib), TACI-Ig (*e.g.*, atacicept), anti-C5 mAb (*e.g.*, eculizumab), mycophenolate, azathioprine, sirolimus everolimus, TNFR-Ig, anti-TNF mAb, tofacitinib, anti-IL-2R (*e.g.*, basiliximab), anti-IL-17 mAb (*e.g.*, secukinumab), anti-IL-6 mAb (*e.g.*, anti-IL-6 antibody sirukumab, anti-IL-6 receptor antibody tocilizumab (Actemra®), IL-10 inhibitor, TGF-beta inhibitor, a B cell targeting antibody (*e.g.*, rituximab), a mammalian target of rapamycin (mTOR) inhibitor (*e.g.*, rapamycin), synthetic vaccine particle (SVP™)-rapamycin (rapamycin encapsulated in a biodegradable nanoparticle), intravenous gamma globulin (IVIG), omalizumab, methotrexate, a tyrosine kinase inhibitor (*e.g.*, ibrutinib), cyclophosphamide, fingolimod, an inhibitor of B-cell activating factor (BAFF) (*e.g.*, anti-BAFF mAb, *e.g.*, belimumab), an inhibitor of a proliferation-inducing ligand (APRIL), anti-IL-1b mAb (*e.g.*, canakinumab (Haris®)), a C3a inhibitor, a Tregitope (see, *e.g.*, U.S. Patent No. 10,213,496), or a combination and/or derivative thereof. In certain embodiments, the immunosuppressive agent is a granulocyte-macrophage colony-stimulating factor (GM-CSF) inhibitors, for example, gimsilumab, lenzilumab, namilumab, otilimab, or mavrilimumab.

**[00315]** Strategies to reduce (overcome), avoid or mitigate the effect of humoral immunity to viral vectors, such as rAAV vectors, include administering high vector doses; use of AAV empty capsids as decoys to adsorb anti-AAV antibodies; administration of immunosuppressive drugs to decrease, reduce, inhibit, prevent or eradicate the humoral immune response to rAAV; changing the rAAV capsid serotype or engineering the rAAV capsid to be less susceptible to neutralizing antibodies; use of plasma exchange cycles to adsorb anti-AAV immunoglobulins, thereby reducing anti-AAV antibody titer; and use of delivery techniques such as balloon catheters followed by saline flushing. Such strategies are described, for example, in Mingozzi *et al.*, (2013) Blood, 122:23-36. Similar techniques and strategies can be used for other types of viral vectors.

**[00316]** Empty capsids used as decoy probes, provided in the same or separate formulation as the rAAV vector, can be provided in different ratios to viral vectors. In certain embodiments the decoy probes is provided contemporaneously with the rAAV. Amounts of empty capsids administered can be calibrated based upon the amount (titer) of antibodies produced in a particular subject. In certain embodiments, the ratio of the empty AAV capsids to the rAAV vector is within or between about 100:1-50:1, from about 50:1 to 25:1, from about 25:1 to 10:1, from about 10:1 to 1:1, from about 1:1 to 1:10, from about 1:10 to 1:25, from about 1:25 to 1:50,

or from about 1:50 to 1:100. In particular aspects, the ratio of the of the administered empty AAV capsids to rAAV vector is about 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, or 10:1. Preferably, the serotype of the empty capsids is the same as the rAAV serotype.

**[00317]** Additional strategies to reduce humoral immunity to rAAV (which can be applied to other viral vectors) include methods to remove, deplete, capture, and/or inactivate AAV antibodies, commonly referred to as apheresis and more particularly, plasmapheresis where blood products are involved. Apheresis or plasmapheresis, is a process in which a human subject's plasma is circulated *ex vivo* (extracorporeal) through a device that modifies the plasma through addition, removal and/or replacement of components before its return to the patient. Plasmapheresis can be used to remove human immunoglobulins (*e.g.*, IgG, IgE, IgA, IgD) from a blood product (*e.g.*, plasma). This procedure can be employed to deplete, capture, inactivate, reduce or remove immunoglobulins (antibodies) that bind AAV thereby reducing the titer of AAV antibodies in the treated subject that can contribute to rAAV neutralization. An example is using a device composed of an AAV capsid affinity matrix column, and passing blood product (*e.g.*, plasma) through an AAV capsid affinity matrix resulting in binding of AAV antibodies of different isotypes. (See, *e.g.*, Bertin *et al.*, 2020, Sci. Rep. 10, 864, hereby incorporated by reference herein in its entirety.)

**[00318]** In certain embodiments the polynucleotide constructs, viral vectors and non-viral vectors can be used in combination with an agent that blocks, inhibits, or reduces the interaction of IgG with the neonatal Fc receptor (FcRn), such as an anti-FcRn antibody, to reduce IgG recycling and enhance IgG clearance *in vivo*; and/or an agent that decreases circulating antibodies that bind to a recombinant viral vector, or that binds to a nucleic acid or a polypeptide, protein or peptide encoded by a polynucleotide encapsidated by a recombinant viral vector, or that bind to the polynucleotide. In certain embodiments, antibody binding to a viral vector is reduced or inhibited by an agent that reduces interaction of IgG with FcRn, a protease or a glycosidase.

**[00319]** In certain embodiments, the polynucleotide constructs, viral vectors and non-viral vectors described herein can be used in combination with an endopeptidase (*e.g.*, IdeS from *Streptococcus pyogenes*) or a modified variant thereof, or an endoglycosidase (*e.g.*, *S. pyogenes* EndoS) or a modified variant thereof. Such treatment can, for example, be carried out to reduce or clear neutralizing antibodies against the gene delivery vehicle (*e.g.*, viral vector capsid) and enable treatment of patients previously viewed as not eligible for gene therapy or that develop antibodies resulting from gene therapy. Such strategies are described in, for example, Leborgne *et al.*, (2020) Nat. Med., 26:1096–1101.

**[00320]** VII. Kits

**[00321]** The present invention includes kits with packaging material and one or more components therein. A kit typically includes a label or packaging insert including a description of the components or instructions for use *in vitro*, *in vivo*, or *ex vivo*, of the components therein. A kit can contain a collection of such components, *e.g.*, polynucleotide construct, a viral or a non-viral vector, and optionally a second active, such as another compound, agent, drug or composition.

**[00322]** A kit refers to a physical structure housing one or more components. Packaging material can maintain the components sterily, and can be made of material commonly used for such purposes such as paper, corrugated fiber, glass, plastic, foil, ampules, vials, and tubes.

**[00323]** Labels or inserts can include identifying information of one or more components therein, dose amounts, clinical pharmacology of the active ingredient(s) including mechanism of action, pharmacokinetics and pharmacodynamics. Labels or inserts can include information identifying manufacturer information, lot numbers, manufacture location and date, and expiration dates. Labels or inserts can include information on a disease for which a kit component can be used. Labels or inserts can include instructions for the clinician or subject for using one or more of the kit components in a method, use, or treatment protocol or therapeutic regimen. Instructions can include dosage amounts, frequency or duration, and instructions for practicing any of the methods, uses, treatment protocols or prophylactic or therapeutic regimes described herein.

**[00324]** Labels or inserts can include information on one or more benefits a component can provide, such as a prophylactic or therapeutic benefit. Labels or inserts can include information on potential adverse side effects, complications or reactions, such as warnings to the subject or clinician regarding situations where it would not be appropriate to use a particular composition. Adverse side effects or complications could also occur when the subject has, will be or is currently taking one or more other medications that can be incompatible with the composition, or the subject has, will be or is currently undergoing another treatment protocol or therapeutic regimen which would be incompatible with the composition and, therefore, instructions could include information regarding such incompatibilities.

**[00325]** Labels or inserts include “printed matter,” *e.g.*, paper or cardboard, or separate or affixed to a component, a kit or packing material (*e.g.*, a box), or attached to an ampule, tube or vial containing a kit component. Labels or inserts can additionally include a computer readable medium, such as a bar-coded printed label, a disk, optical disk such as CD- or DVD-ROM/RAM, DVD, MP3, magnetic tape, or an electrical storage media such as RAM and ROM or hybrids of these such as magnetic/optical storage media, FLASH media or memory type cards.

VIII. Additional Aspects and Embodiments

[00326] Additional aspects, embodiments, and combinations thereof include the following:

1. An RNA polynucleotide comprising a targeting RNA sequence at least 80% identical with the sequence of any of SEQ ID NOs: 1-19; provided that if the targeting sequence is at least 80% identical to SEQ ID NO: 18, then the RNA polynucleotide is either (a) a pri-miRNA comprising the targeting sequence embedded in a scaffold selected from an S155e scaffold, a S-26a scaffold, and S-33 scaffold; or (b) the RNA polynucleotide comprises the sequence of SEQ ID NOs: 117 or 118. In different embodiments the sequence is at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% identical to the sequence of any of SEQ ID NO: 1-19.
2. The RNA polynucleotide of 1, wherein the targeting RNA sequence is at least 90% identical with any of SEQ ID NOs: 1-3, 5, 6, and 16-19.
3. The RNA polynucleotide of 2, wherein the targeting RNA sequence comprises the sequence of any of SEQ ID NOs: 1-3, 5, 6, 16-19.
4. The RNA polynucleotide of 2 or 3, wherein the RNA polynucleotide further comprises a second RNA sequence, wherein the second RNA sequence is substantially complementary to the targeting RNA sequence.
5. The RNA polynucleotide of 4, wherein the RNA polynucleotide is a pri-miRNA comprising a pri-miRNA scaffold, a guide sequence and a passenger sequence, wherein the guide sequence comprises the targeting sequence and the passenger sequence comprises the second RNA sequence.
6. The RNA polynucleotide of 5, wherein the scaffold is an S155e scaffold, S26a scaffold, a S33 scaffold or S155 scaffold.
7. The RNA polynucleotide of 5, wherein the RNA polynucleotide comprises a sequence at least 90% or at least 95% identical to any of SEQ ID NOs: 51-62, 64-68, and 78-87.
8. The RNA polynucleotide of 1, wherein the RNA polynucleotide comprises the sequence of any of SEQ ID NOs: 51-62, 64-68, and 78-87.
9. The RNA polynucleotide of 1, wherein the RNA polynucleotide consists of the sequence of any of SEQ ID NOs: 51-62, 64-68, and 78-87.
10. The RNA polynucleotide of 4, wherein the RNA polynucleotide is a pre-miRNA comprising a guide sequence and a passenger sequence, wherein the guide sequence comprises the targeting sequence and the passenger sequence comprises the second RNA sequence.
11. The RNA polynucleotide of 4, wherein the RNA polynucleotide is a shRNA comprising a guide sequence and a passenger sequence, wherein the guide sequence comprises the targeting

sequence and the passenger sequence comprises the second RNA sequence.

12. The RNA polynucleotide of 10, wherein the RNA polynucleotide comprises a sequence at least 90% or at least 95% identical to any of SEQ ID NOs: 88-96, 98-101, and 111-118.

13. The RNA polynucleotide of 1, wherein the RNA polynucleotide comprises the sequence of any of SEQ ID NOs: 88-96, 98-101, and 111-118.

14. The RNA polynucleotide of 4, wherein the RNA polynucleotide is an inhibitory RNA duplex comprising a guide sequence and a passenger sequence, wherein the guide sequence comprises the targeting sequence and the passenger sequence comprises the second RNA sequence.

15. The RNA polynucleotide of 14, wherein the inhibitory RNA duplex has a guide stand to passenger strand combination selected from group consisting of:

a) a guide strand comprising the sequence of SEQ ID NO: 1 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to any of SEQ ID NOs: 20, 21, and 22;

b) a guide strand comprising the sequence of SEQ ID NO: 2 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to any of SEQ ID NOs: 23, 24 and 25;

c) a guide strand comprising the sequence of SEQ ID NO: 3 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to any of SEQ ID NOs: 26, 27 and 28;

d) a guide strand comprising the sequence of SEQ ID NO: 4 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 29;

e) a guide strand comprising the sequence of SEQ ID NO: 5 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 30;

f) a guide strand comprising the sequence of SEQ ID NO: 6 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to any of SEQ ID NOs: 31, 32 and 33;

g) a guide strand comprising the sequence of SEQ ID NO: 7 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 34;

h) a guide strand comprising the sequence of SEQ ID NO: 8 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 35;



i) a guide strand comprising the sequence of SEQ ID NO: 9 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 36;

j) a guide strand comprising the sequence of SEQ ID NO: 10 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 37;

k) a guide strand comprising the sequence of SEQ ID NO: 11 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 38;

l) a guide strand comprising the sequence of SEQ ID NO: 12 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 39;

m) a guide strand comprising the sequence of SEQ ID NO: 13 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 40;

n) a guide strand comprising the sequence of SEQ ID NO: 14 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 41;

o) a guide strand comprising the sequence of SEQ ID NO: 15 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 42;

p) a guide strand comprising the sequence of SEQ ID NO: 16 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to any of SEQ ID NOs: 43, 44 and 45.

q) a guide strand comprising the sequence of SEQ ID NO: 17 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 46;

r) a guide strand comprising the sequence of SEQ ID NO: 19 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 47; and

s) a guide strand comprising the sequence of SEQ ID NO: 18 and a passenger strand comprising a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or 100% identical to any of SEQ ID NOs: 48, 49, and 50.

16. The RNA polynucleotide of 1, wherein the targeting sequence is a guide sequence at least 90%, at least 95%, at least 97%, or 100% identical to SEQ ID NO: 18 and the guide

sequence is embedded an S26a scaffold or a S33 scaffold.

17. The RNA polynucleotide of 1, wherein the RNA polynucleotide comprises the sequence of SEQ ID NOs: 117 or 118.

18. The RNA polynucleotide of 17, wherein the RNA polynucleotide comprises the sequence of SEQ ID NO: 86 or 87.

19. An optionally modified inhibitory RNA comprising (a) a guide strand able to hybridize to the target sequence of any of SEQ ID NOs: 119-137; and (b) a substantially complementary passenger sequence; wherein one or more nucleotides of the guide strand and the passenger strand are optionally modified RNA.

20. A polynucleotide comprising a nucleic acid sequence encoding the RNA polynucleotide of any of 1-19.

21. An expression cassette comprising a nucleic acid sequence encoding the RNA polynucleotide of any one of 1-19, and one or more expression control elements operably linked to the nucleic acid sequence encoding the RNA polynucleotide.

22. The expression cassette of 21, wherein the expression cassette comprises an upstream promoter and a downstream polyadenylation signal, operably linked to the nucleic acid sequence encoding the RNA polynucleotide.

23. The expression cassette of 22, wherein the expression cassette comprises 5' to 3', operably linked to the nucleic acid sequence encoding the RNA polynucleotide, a promoter or promoter/enhancer, the nucleic acid sequence encoding the RNA polynucleotide, and a polyadenylation signal.

24. The expression cassette of 22 or 23, wherein the promoter provides for high CNS expression.

25. The expression cassette of 22 or 23, wherein the promoter is an EF-1 $\alpha$  promoter.

26. The expression cassette of any one of 21-25, wherein the expression cassette is DNA.

27. The expression cassette of 26, wherein the expression cassette comprises a sequence at least 90%, at least 95%, at least 97% or 100% identical with any one of SEQ ID NOs: 188-199, 201-205, and 215-224.

28. The expression cassette of 26, comprising the sequence of SEQ ID NOs: 223 or 224.

29. A recombinant viral vector nucleic acid comprising the expression cassette of any one of 21-28, and 5' and/or 3' viral elements providing for viral packaging and/or replication.

30. The recombinant viral vector nucleic acid of 29, wherein the recombinant viral vector nucleic acid is a DNA and comprises an adeno-associated virus (AAV) inverted repeat (ITR) flanking the 5' terminus of the recombinant viral vector nucleic acid and an AAV ITR flanking the 3' terminus of the recombinant viral vector nucleic acid.

31. The recombinant viral vector nucleic acid of 30, wherein the 5' ITR and 3' ITR are selected from the 5' ITR and the 3' ITR of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.10, AAVrh.74 and AAV3B.
32. The recombinant viral vector nucleic acid of 30, wherein the recombinant viral vector nucleic acid is self-complementary.
33. The recombinant viral vector nucleic acid of 30 or 32, wherein the 5' ITR comprises a sequence at least 90%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 254, and the 3' ITR comprises a sequence at least 95%, at least 95%, at least 97% or 100% identical to SEQ ID NO: 253.
34. The recombinant viral vector nucleic acid of 30, comprising a sequence at least 90%, at least 95%, at least 97% or 100% identical to the sequence of any of SEQ ID NOs: 233-235 and 237-242.
35. A recombinant viral nucleotide comprising a sequence of any of SEQ ID NOs: 237-242
36. A delivery vehicle comprising a viral or a non-viral vector and the inhibitory RNA of 19, the polynucleotide of 20, the expression cassette of any one of 21-28 or the recombinant viral vector nucleic acid of any one of 29-35.
37. The delivery vehicle of 36, wherein the delivery vehicle is the viral vector.
38. The delivery vehicle of 37, wherein the viral vector is a recombinant AAV, a recombinant lentivirus vector, or a recombinant adenovirus vector.
39. The delivery vehicle of 38, wherein the viral vector is a recombinant AAV.
40. The delivery vehicle of 39, wherein the recombinant AAV vector comprises a capsid comprising a VP1, VP2 or VP3 having a sequence at least 90% identical to a VP1, VP2 or VP3 of any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.74, AAV3B, AAV-2i8, AAVrh.10, AAVrh.8, AAVHSC, AAV-B1, AAV-AS, or AAV1/rh.10; or VP1 of SEQ ID NO: 257 or SEQ ID NO: 260.
41. The delivery vehicle of 40, wherein the capsid is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.74, AAV3B, AAV-2i8, AAVrh.10, AAVrh.8, AAVHSC, AAV-B1, AAV-AS, or AAV1/rh.10 capsid; or the capsid comprises VP1 of SEQ ID NO: 257 or SEQ ID NO: 260.
42. The delivery vehicle of 36, wherein the delivery vehicle is a nanoparticle selected from the group consisting of a lipid nanoparticle (LNP), a polymeric nanoparticle, a lipid polymer nanoparticle (LPNP), a protein or peptide-based nanoparticle, a DNA dendrimer or DNA-based nanocarrier, a carbon nanotube, a microparticle, a microcapsule, an inorganic nanoparticle, a peptide cage nanoparticle, and an exosome.
43. The delivery of vehicle of 42, wherein the delivery vehicle is an LNP or LPNP.

44. A pharmaceutical composition comprising the inhibitory RNA of 19, the polynucleotide of 20, the expression cassette of any one of 21-28, the recombinant viral vector nucleic acid of any one of 29-35, or the delivery vehicle of any one of 36-43 and a pharmaceutically acceptable carrier.

45. A method of reducing huntingtin expression in a cell or subject comprising administering to the cell or subject the inhibitory RNA of 19, the polynucleotide of 20, the expression cassette of any one of 21-28, the recombinant viral vector nucleic acid of any one of 29-35, the delivery vehicle of any one of 36-43, or the pharmaceutical composition of 44.

46. A method of treating a subject for Huntington disease comprising administering to the subject the inhibitory RNA of 19, the polynucleotide of 20, the expression cassette of any one of 21-28, the recombinant viral vector nucleic acid of any one of 29-35, the delivery vehicle of any one of 36-43, or the pharmaceutical composition of 44.

47. The method of 45 or 46, wherein administering comprises direct intraparenchymal, intracisternal or intraventricular administration.

48. The method of 45 or 46, wherein initial administration is outside the CNS.

49. The method of any one of 45-48, wherein the subject is a human.

50. An AAV vector genome plasmid comprising the recombinant viral nucleic acid of any one of 30-35.

51. The AAV genome plasmid of 50, wherein the plasmid lacks *rep* and *cap* genes.

52. A method of producing a rAAV vector comprising the step of culturing a rAAV production cell line comprising a rAAV helper virus activity, wherein the genome of said production cell comprises the nucleic acid of any of 30-35, a *rep* gene and a *cap* gene, wherein said rAAV vector is produced.

53. A method of producing rAAV vector comprising the step of culturing a rAAV permissive cell comprising the rAAV genome plasmid of 51, wherein the rAAV permissive cell further comprises (a) *rep* and *cap* genes provided either as part of the cell genome and/or by one or more separate plasmids, and (b) helper virus activity provided by the cell genome and/or provided by one or more separate plasmids.

54. The method of 53, wherein the rAAV permissive cell is a packaging cell, wherein the genome of said packaging cell comprises a *cap* gene and a *rep* gene.

55. The method of 53, wherein either (a) said *rep* gene, said *cap* gene and said helper activity is provided in a single plasmid or (b) said *rep* gene and said *cap* gene is provided in a *rep/cap* plasmid and said helper activity is provided by a helper plasmid.

56. A method of obtaining a rAAV vector comprising the steps of (a) producing the rAAV vector using the method of any one of 52-55 and (b) purifying the rAAV vector.

57. A polynucleotide comprising the RNA sequence of SEQ ID NO: 255 wherein N<sub>01</sub> to N<sub>42</sub> are ribonucleotides, N<sub>01</sub> is complementary to N<sub>42</sub>, N<sub>02</sub> is not complementary to N<sub>41</sub>, N<sub>03</sub>-N<sub>10</sub> is complementary to N<sub>33</sub>-N<sub>40</sub>, N<sub>11</sub> is not complementary to N<sub>32</sub>, and N<sub>12</sub>-N<sub>21</sub> is complementary to N<sub>22</sub>-N<sub>31</sub>; or the corresponding DNA.

58. The polynucleotide of 57, further comprising a 5' flanking region and a 3' flanking region, wherein the polynucleotide comprises the RNA sequence of SEQ ID NO: 256; or the corresponding DNA sequence.

59. A DNA polynucleotide comprising in a 5' to 3' direction:

(a) a 5' inverted terminal repeat (ITR) sequence comprising the sequence of SEQ ID NO: 262;

(b) a CAG promoter;

(c) a pre-miRNA encoding sequence comprising the sequence of SEQ ID NO: 261, wherein the CAG promoter is operably linked to the pre-miRNA encoding sequence and a polyadenylation signal; and

(d) a 3' inverted terminal repeat (ITR) sequence comprising the sequence of SEQ ID NO: 263.

60) The polynucleotide of 59, wherein the polyadenylation signal comprises the sequence of SEQ ID NOs: 264 or 252.

61) The polynucleotide of 59 or 60, wherein the CAG promoter comprises the sequence of SEQ ID NO: 250.

62) The polynucleotide of 59 or 60, wherein the CAG promoter comprises the sequence of SEQ ID NO: 265.

63) The polynucleotide of any of 59-62, wherein the end of the 5' ITR to the end 3' ITR is up to about 2.5 kb.

64) The polynucleotide of 59, comprising the sequence of SEQ ID NO: 266.

65) The polynucleotide of any one of 59-64, wherein the polynucleotide is plasmid further comprising an origin of replication and a selectable marker.

66) The polynucleotide of any one of 59-64, wherein the polynucleotide is a recombinant adeno-associated viral (rAAV) nucleic acid comprising the 5' ITR at the 5' terminus and 3' ITR at the 3' terminus.

67) A recombinant adeno-associated viral (rAAV) vector comprising:

(a) the rAAV nucleic acid of 66, and

(b) a rAAV capsid comprising: VP1 comprising the amino acid sequence of SEQ ID NO: 257, VP2 comprising the amino acid sequence of SEQ ID NO: 258, and VP3 comprising the amino acid sequence of SEQ ID NO: 259.

- 68) A recombinant adeno-associated viral (rAAV) vector comprising:
- (a) an rAAV nucleic acid comprising a pre-miRNA encoding sequence comprising the sequence of SEQ ID NO: 261 operably linked to a promoter and a polyadenylation signal, and
  - (b) a rAAV capsid comprising: VP1 comprising the amino acid sequence of SEQ ID NO: 257, VP2 comprising the amino acid sequence of SEQ ID NO: 258, and VP3 comprising the amino acid sequence of SEQ ID NO: 259.
- 69) A pharmaceutical composition comprising about  $1.0 \times 10^{10}$  vg to about  $1.0 \times 10^{13}$  vg of the rAAV vector of 67 or 68, and a pharmaceutically acceptable carrier.
- 70) A pharmaceutical composition of 69, comprising about  $1.0 \times 10^{11}$  vg to about  $1.0 \times 10^{12}$  vg, and a pharmaceutically acceptable carrier.
- 71) The pharmaceutical composition of 69, comprising about  $1.0 \times 10^{11}$  vg, about  $1.1 \times 10^{11}$  vg, about  $1.2 \times 10^{11}$  vg, about  $1.3 \times 10^{11}$  vg, about  $1.4 \times 10^{11}$  vg, about  $1.5 \times 10^{11}$  vg, about  $1.6 \times 10^{11}$  vg, about  $1.7 \times 10^{11}$  vg, about  $1.8 \times 10^{11}$  vg, about  $1.9 \times 10^{11}$  vg, about  $1.0 \times 10^{12}$  vg, about  $1.1 \times 10^{12}$  vg, about  $1.2 \times 10^{12}$  vg, about  $1.3 \times 10^{12}$  vg, about  $1.4 \times 10^{12}$  vg, about  $1.5 \times 10^{12}$  vg, about  $1.6 \times 10^{12}$  vg, about  $1.7 \times 10^{12}$  vg, about  $1.8 \times 10^{12}$  vg, about  $1.9 \times 10^{12}$  vg, or about  $1.0 \times 10^{13}$  vg.
- 72) The pharmaceutical composition of any one of 44, or 69-71, further comprising a MRI imaging agent.
- 73) The pharmaceutical composition of 72, wherein the MRI imaging agent is gadoteridol.
- 74) A pharmaceutical composition comprising a sufficient amount of the rAAV vector of any one of 38-41, 67 or 68 to provide a 20% to 90% reduction in total huntingtin protein, and a pharmaceutically acceptable carrier.
- 75) The pharmaceutical composition of 74, wherein the pharmaceutical composition comprises a sufficient amount of the rAAV vector to provide a 20% to 65% reduction in total huntingtin protein.
- 76) The pharmaceutical composition of 75, wherein the pharmaceutical composition comprises a sufficient amount of the rAAV vector to provide a 25% to 40% reduction in total huntingtin protein, and a pharmaceutically acceptable carrier.
- 77) A pharmaceutical composition of comprising a sufficient amount of the rAAV vector of any one of 39-41, 67 or 68, to reduce total HTT protein and/or total HTT mRNA to about 25% to about 60%, about 25% to about 50%, about 25% to about 45%, about 25% to 40%, about 25% to 35%, about 25% to 30%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, or about 30% to about 35%; and a pharmaceutically acceptable carrier.

78) A method of treating Huntington disease in a subject comprising intraparenchymal administration to the subject the rAAV vector of claim 67 or 68, or the pharmaceutical composition of any one of 69-77.

79) A method of treating Huntington disease in a subject comprising

(a) determining in the subject, the putamen and/or caudate volume in the right hemisphere and/or left hemisphere; and

(b) intraparenchymally administering to the subject a recombinant adeno-associated viral (rAAV) vector at a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> to the right hemisphere and/or a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> to the left hemisphere; wherein said dose is based on the volume determined in step (a). In further embodiments volume determination is carried out for: (1) the putamen in the right hemisphere; (2) the caudate in the right hemisphere; (3) the putamen in the left hemisphere; (4) the caudate volume in the left hemisphere; (5) the caudate and putamen volume in the right hemisphere; (6) the caudate and putamen volume in the left hemisphere; or (7) the caudate and putamen volume in the right and left hemisphere.

80) The method of 79, wherein administration to the right hemisphere comprises direct administration to the right hemisphere putamen and caudate; and administration to the left hemisphere comprises direct administration to the left hemisphere putamen and caudate.

81) The method of 80, wherein the putamen and caudate volume of the right hemisphere is measured and the putamen and caudate volume of the left hemisphere is measured, and each hemisphere independently receives a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> based on the measured volume.

82) The method of any one of 79-81, wherein a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the right hemisphere putamen and caudate in approximate proportion to the volume of the putamen and caudate of the right hemisphere.

83) The method of any one of 79-82, wherein a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the right hemisphere putamen and caudate in a ratio of about 67% putamen to about 33% caudate.

84) The method of any one of 79-83, wherein for the left hemisphere a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the putamen and to the caudate of the left hemisphere in approximate proportion to the volume of the putamen and caudate of the left hemisphere.

85) The method of any one of 79-84, wherein a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the left hemisphere putamen and caudate in a ratio of about 67% putamen to about 33% caudate.

86) The method of any one of 79-85, wherein the same dose is administered to the right hemisphere and left hemisphere.

87) The method of any one of 79-86, wherein administration to the caudate comprises a parietal trajectory.

88) The method of any one of 79-87, wherein administration to the putamen comprises an occipital trajectory.

89) The method of any one of 79-88, wherein each hemisphere is administered  $1.0 \times 10^{10}$  vg to  $1.0 \times 10^{13}$  vg of rAAV vector.

90) The method of 89, wherein each hemisphere is administered  $1.0 \times 10^{11}$  vg to  $1.0 \times 10^{12}$  vg of rAAV vector.

91) The method of any one of 79-88, wherein each hemisphere is administered about  $1.0 \times 10^{11}$  vg, about  $1.1 \times 10^{11}$  vg, about  $1.2 \times 10^{11}$  vg, about  $1.3 \times 10^{11}$  vg, about  $1.4 \times 10^{11}$  vg, about  $1.5 \times 10^{11}$  vg, about  $1.6 \times 10^{11}$  vg, about  $1.7 \times 10^{11}$  vg, about  $1.8 \times 10^{11}$  vg, about  $1.9 \times 10^{11}$  vg, about  $1.0 \times 10^{12}$  vg, about  $1.1 \times 10^{12}$  vg, about  $1.2 \times 10^{12}$  vg, about  $1.3 \times 10^{12}$  vg, about  $1.4 \times 10^{12}$  vg, about  $1.5 \times 10^{12}$  vg, about  $1.6 \times 10^{12}$  vg, about  $1.7 \times 10^{12}$  vg, about  $1.8 \times 10^{12}$  vg, about  $1.9 \times 10^{12}$  vg, or about  $1.0 \times 10^{13}$  vg.

92) The method of any one of 79-91, wherein the rAAV vector is the rAAV vector of any one of claims 39-41, 67 and 68.

93) The method of any one of 79-92, wherein administration comprises convection enhanced delivery.

94) The method of any one of 79-93, comprising:

(a) determining the putamen and/or caudate volume in  $\text{mm}^3$  in the right hemisphere and/or the left hemisphere; and

(b) multiplying said volume obtained in step (a) by a desired dose in  $\text{vg}/\text{mm}^3$ , further obtaining said desired dose in vg per putamen and caudate; and

(c) manipulating said dose obtained in the step (b) using a given drug concentration in  $\text{vg}/\text{ml}$  to obtain a required drug dose volume; and

(d) intraparenchymally administering to the subject a dose of the rAAV vector, wherein said dose in ml is obtained according to the steps (b) and (c).

95) The method of claim 94, wherein the desired drug concentration is about  $6.2 \times 10^{11}$   $\text{vg}/\text{ml}$ .

96) A method of treating Huntington disease in a subject comprising:

(a) determining the putamen and/or caudate volume in  $\text{mm}^3$  the right hemisphere and/or the left hemisphere; and



(b) multiplying said volume obtained in step (a) by a desired dose in  $\text{vg}/\text{mm}^3$ , further obtaining said desired dose in  $\text{vg}$  per putamen and/or caudate; and

(c) manipulating said dose obtained in the step (b) using a given drug concentration in  $\text{vg}/\text{ml}$  to obtain a required drug dose volume; and

(d) intraparenchymally administering to the subject a dose of a recombinant adeno-associated viral (rAAV) vector, wherein said dose in  $\text{ml}$  is obtained according to said steps (b) and (c).

97) The method of claim 96, wherein the desired dose is about  $2.0 \times 10^7 \text{ vg}/\text{mm}^3$  to about  $2.0 \times 10^8 \text{ vg}/\text{mm}^3$ .

98) The method of claim 97, wherein the desired drug concentration is about  $6.2 \times 10^{11} \text{ vg}/\text{ml}$ .

99) The method of any one of 96-98, where step (a) measures the putamen and caudate in one or both hemispheres and step (b) obtains a dose for both the putamen and caudate.

100) The method of any one of 78-99, wherein the presence of inhibitory nucleic acid encoded for by the rAAV vector is measured in cerebrospinal fluid. In further embodiments, the presence of inhibitory nucleic acid encoded for by the rAAV vector is measured prior to or contemporaneously with vector administration, and at least one month after vector administration; or the presence of inhibitory nucleic acid encoded for by the rAAV vector is measured prior to or contemporaneously with vector administration, and at least 3, at least 6, at least 12, at least 18, and/or at least 24 months after vector administration.

101) The method of 100, wherein the inhibitory nucleic acid is encoded by rAAV nucleic acid comprising an miR155 scaffold and expressed inhibitory RNA is measured in cerebrospinal fluid by detecting the miR155 scaffold.

102) The method of 101, wherein the inhibitory nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 261.

103) The method of 101 or 102, wherein detection of expressed inhibitory nucleic acid comprises the steps of (a) RNA purification from cerebrospinal fluid; (b) reverse transcriptase; and (c) polymerase Chain Reaction (qPCR).

104) The method of 103, wherein step (a) comprises incubation of cerebrospinal fluid in lysis buffer and ethanol for about 5 minutes.

105) The method of any one of 102-104, wherein said step (b) comprises about  $20 \mu\text{L}$  RNA and about  $40 \mu\text{L}$  RT.

106) The method of any one of 102-105, wherein step (c) comprises cycling between about  $95^\circ\text{C}$  and about  $60^\circ\text{C}$ . In further embodiments, cycling at  $95^\circ\text{C}$  is for about 15 seconds and cycling at  $60^\circ\text{C}$  is for about one minute; and/or there are about 40 cycles. In additional

embodiments, prior to cycling, the qPCR mixture is placed at 50 °C for about two minutes, and then 95 °C for about 10 minutes.

107) The method of any one of 78-106, wherein the subject is a human.

### IX. Sequences

[00327] Table 3 provides different nucleic acid and amino acid sequences. In certain embodiments, a polynucleotide comprises a nucleic acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any of the nucleic acid sequences provided in Table 3.

Table 3

| SEQ ID NO: |                                                                                                                                                                                                                                                                                  |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Pri-miRNA  |                                                                                                                                                                                                                                                                                  |
| 51         | S155.miR1<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUCUUGGUAGCUGAAAGUUCUUGUUUUGGCCACUGACUGACCAAGAACUUAGCUACCAAGACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                 |
| 52         | S155e.miR1<br>CUGGAGGCUUGCUCUUGGGCUGUAUGCUGUCUUGGUAGCUGAAAGUUCUUGUUUUGGCCACUGACUGACAAGAACUUAGCUACCAAGACAGGACACAAGGCCCUUAUCAGCACUCACAUGGAACAAAUGGCC                                                                                                                               |
| 53         | S126.miR1<br>GAGGGAUGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCUGGGCGAAGGCCGUGGCCUCGUCUUGGUAGCUGAAAGUUCUUGUGCAGGUCCAAAAGAACUUUCUGCUACCACGACGGGGACGCGGGCCUGGACGCCGGCAUCCGGGCUCAGGACCCCCUCUCUGCCAGAGGC                                                                                    |
| 54         | S33.miR1<br>AGGGCUCUGCGUUGCUCAGGUAGUCCGUCGUCUCCUUGGGCCUGGGCCACUGACAGCCUGGUGCCUCUGGCCGGCCACACCUCUGGCGGGCAGCUGUGUCUUGGUAGCUGAAAGUUCUUGUUCUGGCAAUACUGAAGAACUUAGAACUACCAAGACACGGAGGCCUGCCUGACUGCCCACGGUGCCGUGGCCAAAGAGGAUCUAAGGGCACCGCUGAGGGCCUACCUAACCAUCGUGGGGAUAAGGACAGUGUCACCC   |
| 55         | S155.miR2<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUCUUAUGUCAGACAAUGAUUCACGUUUUGGCCACUGACUGACGUGAAUCAGUCUGACAAUACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                |
| 56         | S155e.miR2<br>CUGGAGGCUUGCUCUUGGGCUGUAUGCUGUAUUGUCAGACAAUGAUUCACGUUUUGGCCACUGACUGACGUGAAUCAGUCUGACAAUACAGGACACAAGGCCCUUAUCAGCACUCACAUGGAACAAAUGGCC                                                                                                                               |
| 57         | S126.miR2<br>GAGGGAUGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCUGGGCGAAGGCCGUGGCCUCGUUUUGUCAGACAAUGAUUCACUGUGCAGGUCCAAUGUGAAUCAUUAUCUGACACUACGGGGACGCGGGCCUGGACGCCGGCAUCCGGGCUCAGGACCCCCUCUCUGCCAGAGGC                                                                                  |
| 58         | S33.miR2<br>AGGGCUCUGCGUUGCUCAGGUAGUCCGUCGUCUCCUUGGGCCUGGGCCACUGACAGCCUGGUGCCUCUGGCCGGCCACACCUCUGGCGGGCAGCUGUGUAUUGUCAGACAAUGAUUCACUGUUCUGGCAAUACUGUGUGAAUCAAAGCCUGACAAUACACGGAGGCCUGCCUGACUGCCCACGGUGCCGUGGCCAAAGAGGAUCUAAGGGCACCGCUGAGGGCCUACCUAACCAUCGUGGGGAUAAGGACAGUGUCACCC |
| 59         | S155.miR3<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUCUUCACACGGUCUUCUUGGUAGUUUUGGCCACUGACUGACUACCAAGAGACCGUGUGAACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                 |
| 60         | S155e.miR3<br>CUGGAGGCUUGCUCUUGGGCUGUAUGCUGUUCACACGGUCUUCUUGGUAGUUUUGGCCACUGACUGACUACCAAGAGACCGUGUGAACAGGACACAAGGCCCUUAUCAGCACUCACAUGGAACAAAUGGCC                                                                                                                                |
| 61         | S126.miR3<br>GAGGGAUGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCUGGGCGAAGGCCGUGGCCUCGUUCAACGGUCUUCUUGGUUUGUGCAGGUCCAAUACCAAGAAACACCGUGUAAACGGGGACGCGGGCCUGGACGCCGGCAUCCGGGCUCAGGACCCCCUCUCUGCCAGAGGC                                                                                     |
| 62         | S33.miR3<br>AGGGCUCUGCGUUGCUCAGGUAGUCCGUCGUCUCCUUGGGCCUGGGCCACUGACAGCCUGGUGCCUCUGGCCGGCCACACCUCUGGCGGGCAGCUGUGUUCACACGGUCUUCUUGGUUUGUUCUGGCAAUAC                                                                                                                                 |

|    |                                                                                                                                                                                                                                                                                         |
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|    | CUGUACCAAGAUUGGCCGUGUGAACACGGAGGCCUGCCCUGACUGCCCACGGUGCCGUGGCCAAAGAGG<br>AUCUAAGGGCACCUGAGGGCCUACCUAACCAUCGUGGGGAUAAGGACAGUGUCACCC                                                                                                                                                      |
| 63 | S155.miR4<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUAUGAUUCACACGGUCUUUCUUGUUUUGGCCACUGACUGACAA<br>GAAAGAGUGUGAAUCAUCAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                    |
| 64 | S155.miR5<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUUUCGAAGGCCUUAUCAGCUUGUUUUGGCCACUGACUGACAA<br>GCUGAUAGGCCUUCGAACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                     |
| 65 | S155.miR6<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUUGAUUCACACGGUCUUUCUUGGUUUUGGCCACUGACUGACCA<br>AGAAAGCGUGUGAAUCAUCAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                   |
| 66 | S155e.miR6<br>CUGGAGGCUUGCUGUUUGGCCUGUAUGCUUGAUUCACACGGUCUUUCUUGGUUUUGGCCACUGACUGACC<br>AAGAAAGCGUGUGAAUCAUCAGGACACAAGGCCUUAUCAGCACUCACAUGGAACAAAUGGCC                                                                                                                                  |
| 67 | S126.miR6<br>GAGGGAAUGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCUGGGCGAAGGCCGUGGCCUCGUGAU<br>UCACACGGUCUUUCUUGUGUGCAGGUCCCAACAAGAAAGAACGUGUGAAGCACGGGGACGCGGGCCUGG<br>ACGCCGGCAUCCGGGCUAGGACCCCCUCUCUGCCAGAGGC                                                                                 |
| 68 | S33.miR6<br>AGGGCUCUGCGUUUGCUCAGGUAGUCCGUGCUCUUUGGGCCUGGGCCACUGACAGCCUGGUGCC<br>UCUGGCCGGCCACACCUCUGGCGGGCAGCUGUGUGAUUCACACGGUCUUUCUUGUUCUGGCAUAC<br>CUGCAAGAAAGUGCAUGUGAAUCACACGGAGGCCUGCCCUGACUGCCCACGGUGCCGUGGCCAAAGAGG<br>AUCUAAGGGCACCUGAGGGCCUACCUAACCAUCGUGGGGAUAAGGACAGUGUCACCC |
| 69 | S155.miR7<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUACAAUGAUUCACACGGUCUUUGUUUUGGCCACUGACUGACAA<br>AGACCGUGAAUCAUUGUCAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                    |
| 70 | S155.miR8<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUUUCUUGGUAGCUGAAAGUUCUGUUUUGGCCACUGACUGACAG<br>AACUUUGCUCACCAAGAACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                   |
| 71 | S155.miR9<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUUGUCAGACAAUGAUUCACACGGUUUUGGCCACUGACUGACCG<br>UGUGAAAUUGUCUGACACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                    |
| 72 | S155.miR10<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUUCACACGGUCUUUCUUGGUAGGUUUUGGCCACUGACUGACCU<br>ACCAAGAGACCGUGUGACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                   |
| 73 | S155.miR11<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUUGAAAGUUCUUUCUUGGUCGGUUUUGGCCACUGACUGACCG<br>ACCAAAAAGAACUUUCACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                    |
| 74 | S155.miR12<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUUACGGUCUUUCUUGGUAGCUGGUUUUGGCCACUGACUGACCA<br>GCUACCGAAAGACCGUACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                   |
| 75 | S155.miR13<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUUCAGACAAUGAUUCACACGGUGUUUUGGCCACUGACUGACAC<br>CGUGUGUCAUUGUCUGACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                   |
| 76 | S155.miR14<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUUUGUCAGACAAUGAUUCACACGUUUUGGCCACUGACUGACGU<br>GUGAAUUUGUCUGACAACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                   |
| 77 | S155.miR15<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUAUUUGUCAGACAAUGAUUCAGUUUUGGCCACUGACUGACUG<br>AAUCAUUCUGACAAUAUCAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                    |
| 78 | S155.miR16<br>CUGGAGGCUUGCUGAAGGCUGUAUGCUUCGAAGGCCUUAUCAGCUUGUUUUGGCCACUGACUGACAA<br>AGCUGAAAGGCCUUCGACAGGACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                     |
| 79 | S155e.miR16<br>CUGGAGGCUUGCUGUUUGGCCUGUAUGCUUGUCGAAGGCCUUAUCAGCUUGUUUUGGCCACUGACUGACA<br>AAGCUGAAAGGCCUUCGACAGGACACAAGGCCUUAUCAGCACUCACAUGGAACAAAUGGCC                                                                                                                                  |
| 80 | S126.miR16<br>GAGGGAAUGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCUGGGCGAAGGCCGUGGCCUCGUGCA<br>AGGCCUUCUACAGCUUUUGUGCAGGUCCAAAAAGCUGAUGCAGGCCUUGGACGGGGACGCGGGCCUGG<br>ACGCCGGCAUCCGGGCUAGGACCCCCUCUCUGCCAGAGGC                                                                                 |
| 81 | S33.miR16<br>AGGGCUCUGCGUUUGCUCAGGUAGUCCGUGCUCUUUGGGCCUGGGCCACUGACAGCCUGGUGCC<br>UCUGGCCGGCCACACCUCUGGCGGGCAGCUGUGUGCAAGGCCUUAUCAGCUUUUGUUCUGGCAUAC                                                                                                                                     |

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|            | CUGAAAGCUGAACAGGGCCUUCGACACGGAGGCCUGCCCUGACUGCCCACGGUGCCGUGGCCAAAGAGG<br>AUCUAAGGGCACCGCUGAGGGCCUACCUAACCAUCGUGGGGAUAAGGACAGUGUCACCC                                                                                                                                                              |
| 82         | S451.miR17<br>UGCCCUGGCAGUCAGUAGGUUGUGACAGGCUGAGCAGAGAGCUUCUUGGGCUUGGGAAUGGCAAGGUCG<br>AAGGCCUUCaucagcuagugcugaugaaggccuucgcucucuaauaccagagucucccuuccaguag<br>UCUGCUUCAGGGUCCUGAGUUCUCUCCUGGCACGUUU                                                                                               |
| 83         | S155.miR19<br>CUGGAGGCCUUGCUGAAGGCUGUAUGCUUCGAAGGCCUUCaucagcuccguuuuggccacugacugacgg<br>AGCUGAAAGGCCUUCGACAGGACACAAGGCCUGUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                             |
| 84         | S155.miR18<br>CUGGAGGCCUUGCUGAAGGCUGUAUGCUUAGCGUUGAAGUACUGUCCCCGUUUUGGCCACUGACUGACGG<br>GGACAGCUUCAACGCUACAGGACACAAGGCCUGUACUAGCACUCACAUGGAACAAAUGGCC                                                                                                                                             |
| 85         | S155e.miR18<br>CUGGAGGCCUUGCUUUGGGCUGUAUGCUGUAGCGUUGAAGUACUGUCCCCGUUUUGGCCACUGACUGACG<br>GGGACAGCUUCAACGCUACAGGACACAAGGCCCUUUAUCAGCACUCACAUGGAACAAAUGGCC                                                                                                                                          |
| 86         | S26.miR18<br>GAGGGAUUGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCUGGGCGAAGGCCGUGGCCUCGUAGC<br>GUUGAAGUACUGUCCCCUGUGCAGGUCCCAAGGGGACAGUAAUACAACGCGACGGGGACGCGGGCCUGG<br>ACGCCGGCAUCCGGGCUCAGGACCCCCUCUCUGCCAGAGGC                                                                                          |
| 87         | S33.miR18<br>AGGGCUCUGCGUUUGCUCCAGGUAGUCCGCGUCUCCCUUGGGCCUGGGCCACUGACAGCCCUGGUGCC<br>UCUGGCCGGCCACACCUCUGGGCGGCAGCUGUGUAGCGUUGAAGUACUGUCCCCUGUUCUGGCAAUAC<br>CUGAAGGACAGAUCCUCAACGCUACACGGAGGCCUGCCCUGACUGCCCACGGUGCCGUGGCCAAAGAGG<br>AUCUAAGGGCACCGCUGAGGGCCUACCUAACCAUCGUGGGGAUAAGGACAGUGUCACCC |
| Pre-amiRNA |                                                                                                                                                                                                                                                                                                   |
| 88         | S155/S155e.miR1<br>UCUUGGUAGCUGAAAGUUCUUGUUUUGGCCACUGACUGACAAGAACUUAGCUACCAAGACA                                                                                                                                                                                                                  |
| 89         | S26.miR1<br>UCUUGGUAGCUGAAAGUUCUUGUGCAGGUCCAAAAGAACUUUCUGCUACCACGACG                                                                                                                                                                                                                              |
| 90         | S33.miR1<br>UCUUGGUAGCUGAAAGUUCUUGUUCUGGCAAUACCUGAAGAACUUAGAACUACCAAGACA                                                                                                                                                                                                                          |
| 91         | S155/S155e.miR2<br>UAUUGUCAGACAAUGAUUCACGUUUUGGCCACUGACUGACGUGAAUCAGUCUGACAAUACA                                                                                                                                                                                                                  |
| 92         | S26.miR2<br>UAUUGUCAGACAAUGAUUCACUGUGCAGGUCCCAAGUGAAUCAUUAUCUGACACUACG                                                                                                                                                                                                                            |
| 93         | S33.miR2<br>UAUUGUCAGACAAUGAUUCACUGUUCUGGCAAUACCUGGUGAAUCAAAGCCUGACAAUACA                                                                                                                                                                                                                         |
| 94         | S155/S155e.miR3<br>UUCACACGGUCUUUCUUGGUAGUUUUGGCCACUGACUGACUACCAAGAGACCGUGUGAACA                                                                                                                                                                                                                  |
| 95         | S126.miR3<br>UUCACACGGUCUUUCUUGGUAGUGCAGGUCCAAUACCAAGAAACACCGUGUAAACG                                                                                                                                                                                                                             |
| 96         | S33.miR3<br>UUCACACGGUCUUUCUUGGUAGUUCUGGCAAUACCUGUACCAAGAUUGGCCGUGUGAACAC                                                                                                                                                                                                                         |
| 97         | miR4<br>AUGAUUCACACGGUCUUUCUUGUUUUGGCCACUGACUGACAAGAAAGAGUGUGAAUCAUCA                                                                                                                                                                                                                             |
| 98         | miR5<br>UUCGAAGGCCUUCaucagcuuguuuuggccacugacugacaagcugauaggccuucgaaca                                                                                                                                                                                                                             |
| 99         | S155/S155e.miR6<br>UGAUUCACACGGUCUUUCUUGGUUUUGGCCACUGACUGACCAAGAAAGCGUGUGAAUCACA                                                                                                                                                                                                                  |
| 100        | S26.miR6<br>UGAUUCACACGGUCUUUCUUGUGUGCAGGUCCCAACAAGAAAGAACGUGUGAAGCACG                                                                                                                                                                                                                            |
| 101        | S33.miR6<br>UGAUUCACACGGUCUUUCUUGUGUUCUGGCAAUACCUGCAAGAAAGUGCAUGUGAAUCA                                                                                                                                                                                                                           |
| 102        | S155.miR7<br>ACAAUGAUUCACACGGUCUUUGUUUUGGCCACUGACUGACAAGACCGUGAAUCAUUGUCA                                                                                                                                                                                                                         |
| 103        | S155.miR8<br>UUCUUGGUAGCUGAAAGUUCUGUUUUGGCCACUGACUGACAGAACUUUGCUACCAAGAACA                                                                                                                                                                                                                        |
| 104        | S155.miR9<br>UGUCAGACAAUGAUUCACACGGUUUUGGCCACUGACUGACCGUGUGAAAUUGUCUGACACA                                                                                                                                                                                                                        |
| 105        | S155.miR10<br>UCACACGGUCUUUCUUGGUAGGUUUUGGCCACUGACUGACCUACCAAGAGACCGUGUGACA                                                                                                                                                                                                                       |
| 106        | S155.miR11                                                                                                                                                                                                                                                                                        |

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|                             | UGAAAGUUCUUUCUUUGGUCGGUUUUUGGCCACUGACUGACCGACCAAAAAGAACUUUCACA                                                                                                                                                                                                                               |
| 107                         | S155.miR12<br>UACGGUCUUUCUUGGUAGCUGGUUUUGGCCACUGACUGACCGAGCUACCGAAAGACCGUACA                                                                                                                                                                                                                 |
| 108                         | S155.miR13<br>UCAGACAAUGAUUCACACGGUGUUUUUGGCCACUGACUGACACCGUGUGUCAUUGUCUGACA                                                                                                                                                                                                                 |
| 109                         | S155.miR14<br>UUGUCAGACAAUGAUUCACACGUUUUGGCCACUGACUGACGUGUGAAUUUGUCUGACAACA                                                                                                                                                                                                                  |
| 110                         | S155.miR15<br>AUAUUGUCAGACAAUGAUUCAGUUUUUGGCCACUGACUGACUGAAUCAUUCUGACAAUAUCA                                                                                                                                                                                                                 |
| 111                         | S155/S155e.miR16<br>UCGAAGGCCUUCAUCAGCUUUUGUUUUUGGCCACUGACUGACAAAGCUGAAAGGCCUUCGACA                                                                                                                                                                                                          |
| 112                         | S26.miR16<br>UCGAAGGCCUUCAUCAGCUUUUGUGCAGGUCCCAAAAAGCUGAUGCAGGCCUUGGACG                                                                                                                                                                                                                      |
| 113                         | S33.miR16<br>UCGAAGGCCUUCAUCAGCUUUUGUUCUGGCCAAUACCUGAAAGCUGAACAGGGCCUUCGACA                                                                                                                                                                                                                  |
| 114                         | S451.miR17<br>UCGAAGGCCUUCAUCAGCUAGUGCUGAUGAAGGCCUUCGCU                                                                                                                                                                                                                                      |
| 115                         | S155.miR19<br>UCGAAGGCCUUCAUCAGCUCCGUUUUGGCCACUGACUGACGGAGCUGAAAGGCCUUCGACA                                                                                                                                                                                                                  |
| 116                         | S155/155e.miR18<br>UAGCGUUGAAGUACUGUCCCCGUUUUGGCCACUGACUGACGGGGACAGCUUCAACGCUACA                                                                                                                                                                                                             |
| 117                         | S26.miR18<br>UAGCGUUGAAGUACUGUCCCCUGUGCAGGUCCCAAGGGGACAGUAAUUAACGCGACG                                                                                                                                                                                                                       |
| 118                         | S33.miR18<br>UAGCGUUGAAGUACUGUCCCCUGUUCUGGCCAAUACCUGGGGGACAGAUCCUCAACGCUACA                                                                                                                                                                                                                  |
| Target                      |                                                                                                                                                                                                                                                                                              |
| 119                         | AAGAACUUUCAGCUACCAAGA                                                                                                                                                                                                                                                                        |
| 120                         | GUGAAUCAUUGUCUGACAAUA                                                                                                                                                                                                                                                                        |
| 121                         | UACCAAGAAAGACCGUGUGAA                                                                                                                                                                                                                                                                        |
| 122                         | AAGAAAGACCGUGUGAAUCAU                                                                                                                                                                                                                                                                        |
| 123                         | AAGCUGAUGAAGGCCUUCGA                                                                                                                                                                                                                                                                         |
| 124                         | CAAGAAAGACCGUGUGAAUCA                                                                                                                                                                                                                                                                        |
| 125                         | AAAGACCGUGUGAAUCAUUGU                                                                                                                                                                                                                                                                        |
| 126                         | AGAACUUUCAGCUACCAAGAA                                                                                                                                                                                                                                                                        |
| 127                         | CGUGUGAAUCAUUGUCUGACA                                                                                                                                                                                                                                                                        |
| 128                         | CUACCAAGAAAGACCGUGUGA                                                                                                                                                                                                                                                                        |
| 129                         | CGACCAAGAAAGAACUUUCA                                                                                                                                                                                                                                                                         |
| 130                         | CAGCUACCAAGAAAGACCGU                                                                                                                                                                                                                                                                         |
| 131                         | ACCGUGUGAAUCAUUGUCUGA                                                                                                                                                                                                                                                                        |
| 132                         | GUGUGAAUCAUUGUCUGACAA                                                                                                                                                                                                                                                                        |
| 133                         | UGAAUCAUUGUCUGACAAUAU                                                                                                                                                                                                                                                                        |
| 134                         | AAAGCUGAUGAAGGCCUUCGA                                                                                                                                                                                                                                                                        |
| 135                         | GCUGAUGAAGGCCUUCG                                                                                                                                                                                                                                                                            |
| 136                         | AGCUGAUGAAGGCCUUCGA                                                                                                                                                                                                                                                                          |
| 137                         | GGGGACAGUACUUAACGCUA                                                                                                                                                                                                                                                                         |
| Pri-miRNA corresponding DNA |                                                                                                                                                                                                                                                                                              |
| 188                         | S155.miR1<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTCTTGGTAGCTGAAAGTTCTTGTTTTGGCCACTGACTGACCA<br>AGAACTTAGCTACCAAGACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                        |
| 189                         | S155e.miR1<br>CTGGAGGCTTGCTTTGGGCTGTATGCTGTCTTGGTAGCTGAAAGTTCTTGTTTTGGCCACTGACTGACA<br>AGAACTTAGCTACCAAGACAGGACACAAGGCCCTTTATCAGCACTCACATGGAACAAATGGCC                                                                                                                                       |
| 190                         | S126.miR1<br>GAGGGAATGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGTCTT<br>GGTAGCTGAAAGTTCTTTGTGCAGGTCCAAAAGAATTTCTGCTACCACGACGGGGACGCGGGCCTGG<br>ACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCAGAGGC                                                                                      |
| 191                         | S33.miR1<br>AGGGCTCTGCGTTTGCTCCAGGTAGTCCGCTGCTCCCTTGGGCCTGGGCCACTGACAGCCCTGGTGCC<br>TCTGGCCGGCCACACCTCCTGGCGGGCAGCTGTGTCTTGGTAGCTGAAAGTTCTTGTCTGGCAATAC<br>CTGAAGAACTTAGAACTACCAAGACACGGAGGCCTGCCCTGACTGCCACGGTGCCGTGGCCAAAGAGG<br>ATCTAAGGGCACCGCTGAGGGCTACCTAACCATCGTGGGAATAAGGACAGTGTACCC |
| 192                         | S155.miR2                                                                                                                                                                                                                                                                                    |

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|     | CTGGAGGCTTGCTGAAGGCTGTATGCTTATTGTCAGACAATGATTCACGTTTTGGCCACTGACTGACGTGAATCAGTCTGACAATACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                                    |
| 193 | S155e.miR2<br>CTGGAGGCTTGCTTTGGGCTGTATGCTGTATTGTCAGACAATGATTCACGTTTTGGCCACTGACTGACGTGAATCAGTCTGACAATACAGGACACAAGGCCCTTTATCAGCACTCACATGGAACAAATGGCC                                                                                                                                     |
| 194 | S126.miR2<br>GAGGGAATGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGTATTGTCAGACAATGATTCAGTGTGCAGGTCCCAAGTGAATCATTATCTGACACTACGGGGACGCGGGCCTGGACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCAGAGGC                                                                                      |
| 195 | S33.miR2<br>AGGGCTCTGCGTTTGCTCCAGGTAGTCCGCTGCTCCCTTGGGCCTGGGCCCACTGACAGCCCTGGTGCCCTCTGGCCGGCCACACCTCCTGGCGGGCAGCTGTGTATTGTCAGACAATGATTCAGTGTCTGGCAATACCTGGTGAATCAAAGCCTGACAATACAGGAGGCCTGCCCTGACTGCCCACGGTGCCGTGGCCAAAGAGGATCTAAGGGCACCGCTGAGGGCCTACCTAACCATCGTGGGAATAAGGACAGTGTCAACC  |
| 196 | S155.miR3<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTTCACACGGTCTTTCTTGGTAGTTTTGGCCACTGACTGACTACCAAGAGACCGTGTGAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                       |
| 197 | S155e.miR3<br>CTGGAGGCTTGCTTTGGGCTGTATGCTGTTACACGGTCTTTCTTGGTAGTTTTGGCCACTGACTGACTACCAAGAGACCGTGTGAACAGGACACAAGGCCCTTTATCAGCACTCACATGGAACAAATGGCC                                                                                                                                      |
| 198 | S126.miR3<br>GAGGGAATGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGTTCAACCGGTCTTTCTTGATGTGCAGGTCCCAATACCAAGAAACACCGTGTAACGGGGACGCGGGCCTGGACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCAGAGGC                                                                                         |
| 199 | S33.miR3<br>AGGGCTCTGCGTTTGCTCCAGGTAGTCCGCTGCTCCCTTGGGCCTGGGCCCACTGACAGCCCTGGTGCCCTCTGGCCGGCCACACCTCCTGGCGGGCAGCTGTGTTCACACGGTCTTTCTTGGTATGTTCTGGCAATACCTGTACCAAGATTGGCCGTGTGAACACGAGGCCTGCCCTGACTGCCCACGGTGCCGTGGCCAAAGAGGATCTAAGGGCACCGCTGAGGGCCTACCTAACCATCGTGGGAATAAGGACAGTGTCAACC |
| 200 | S155.miR4<br>CTGGAGGCTTGCTGAAGGCTGTATGCTATGATTACACGGTCTTTCTTGTTTTGGCCACTGACTGACAAAGAAAGTGTGAATCATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                         |
| 201 | S155.miR5<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTTCGAAGGCCTTCATCAGCTTGTTTTGGCCACTGACTGACAAAGCTGATAGGCCTTCGAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                      |
| 202 | S155.miR6<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTGATTACACGGTCTTTCTTGTTTTGGCCACTGACTGACCAAGAAAGCGTGTGAATCACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                         |
| 203 | S155e.miR6<br>CTGGAGGCTTGCTTTGGGCTGTATGCTGTGATTACACGGTCTTTCTTGTTTTGGCCACTGACTGACCAGAAAGCGTGTGAATCACAGGACACAAGGCCCTTTATCAGCACTCACATGGAACAAATGGCC                                                                                                                                        |
| 204 | S126.miR6<br>GAGGGAATGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGTGATTCACACGGTCTTTCTTGTTGTGCAGGTCCCAACAAGAAAGACGTGTGAAGCACGGGGACGCGGGCCTGGACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCAGAGGC                                                                                      |
| 205 | S33.miR6<br>AGGGCTCTGCGTTTGCTCCAGGTAGTCCGCTGCTCCCTTGGGCCTGGGCCCACTGACAGCCCTGGTGCCCTCTGGCCGGCCACACCTCCTGGCGGGCAGCTGTGTGATTACACGGTCTTTCTTGTTCTGGCAATACCTGCAAGAAAGTGCATGTGAATCACACGGAGGCCTGCCCTGACTGCCCACGGTGCCGTGGCCAAAGAGGATCTAAGGGCACCGCTGAGGGCCTACCTAACCATCGTGGGAATAAGGACAGTGTCAACC   |
| 206 | S155.miR7<br>CTGGAGGCTTGCTGAAGGCTGTATGCTACAATGATTCACACGGTCTTTGTTTTGGCCACTGACTGACAAAGACCGTGAATCATTGTGACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                     |
| 207 | S155.miR8<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTTCTTGGTAGCTGAAAGTTCTGTTTTGGCCACTGACTGACAGAACTTTGCTACCAAGAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                       |
| 208 | S155.miR9<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTGTCAGACAATGATTCACACGGTTTTGGCCACTGACTGACCGTGTGAATTTGTCTGACACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                       |
| 209 | S155.miR10<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTTCACACGGTCTTTCTTGGTAGGTTTTGGCCACTGACTGACCTACCAAGAGACCGTGTGACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                     |
| 210 | S155.miR11                                                                                                                                                                                                                                                                             |

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|     | CTGGAGGCTTGCTGAAGGCTGTATGCTTGAAAGTTCTTTCTTTGGTCGGTTTTGGCCACTGACTGACCG<br>ACCAAAAAGAACTTTCACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                                              |
| 211 | S155.miR12<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTACGGTCTTTCTTGGTAGCTGGTTTTGGCCACTGACTGACCA<br>GCTACCGAAAGACCGTACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                                |
| 212 | S155.miR13<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTCAGACAATGATTCACACGGTGGTTTTGGCCACTGACTGACAC<br>CGTGTGTCTTGTCTGACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                                |
| 213 | S155.miR14<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTGTGACACAATGATTCACACGGTTTTGGCCACTGACTGACGT<br>GTGAATTTGTCTGACAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                                |
| 214 | S155.miR15<br>CTGGAGGCTTGCTGAAGGCTGTATGCTATATTGTGACACAATGATTCAGTTTTGGCCACTGACTGACTG<br>AATCATTTCTGACAATATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                               |
| 215 | S155.miR16<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTCGAAGGCCTTCATCAGCTTGTGTTTTGGCCACTGACTGACAA<br>AGCTGAAAGGCCTTCGACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                               |
| 216 | S155e.miR16<br>CTGGAGGCTTGCTTTGGGCTGTATGCTGTGCAAGGCCTTCATCAGCTTGTGTTTTGGCCACTGACTGACA<br>AAGCTGAAAGGCCTTCGACAGGACACAAGGCCTTTATCAGCACTCACATGGAACAAATGGCC                                                                                                                                              |
| 217 | S126.miR16<br>GAGGGAATGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGTCGA<br>AGGCCTTCATCAGCTTTTGTGAGGTCCCAAAAGCTGATGCAGGCCTTGGACGGGGACGCGGGCCTGG<br>ACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCAGAGGC                                                                                             |
| 218 | S33.miR16<br>AGGGCTCTGCGTTTGCTCCAGGTAGTCCGCTGCTCCCTTGGGCCTGGGCCCCTGACAGCCCTGGTGCC<br>TCTGGCCGGCCACACCTCCTGGCGGGCAGCTGTGTGCAAGGCCTTCATCAGCTTTTGTCTGGCAATAC<br>CTGAAAGCTGAACAGGGCCTTCGACACGGAGGCCTGCCCTGACTGCCCACGGTGCCGTGGCCAAAGAGG<br>ATCTAAGGGCACCGCTGAGGGCCTACCTAACCATCGTGGGGAATAAGGACAGTGTCAACC   |
| 219 | S451.miR17<br>TGCCCTGGCAGTCAGTAGGTTGTGACAGGCTGAGCAGAGAGCTTCTTGGGCTTGGGAATGGCAAGGTGCG<br>AAGGCCTTCATCAGCTAGTGCTGATGAAGGCCTTCGCTCTTGCTATACCCAGAGTCTCCCTTCCAGTAG<br>TCTGCTTCAGGGTCCTGAGTTCTCTTCTGGCACGTTT                                                                                               |
| 220 | S155.miR19<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTCGAAGGCCTTCATCAGCTCCGTTTTGGCCACTGACTGACGG<br>AGCTGAAAGGCCTTCGACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                                |
| 221 | S155.miR18<br>CTGGAGGCTTGCTGAAGGCTGTATGCTTAGCGTTGAAGTACTGTCCCCGTTTTGGCCACTGACTGACGG<br>GGACAGCTTCAACGCTACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                                |
| 222 | S155e.miR18<br>CTGGAGGCTTGCTTTGGGCTGTATGCTGTAGCGTTGAAGTACTGTCCCCGTTTTGGCCACTGACTGACG<br>GGGACAGCTTCAACGCTACAGGACACAAGGCCTTTATCAGCACTCACATGGAACAAATGGCC                                                                                                                                               |
| 223 | S26.miR18<br>GAGGGAATGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGTAGC<br>GTTGAAGTACTGTCCCCGTGTGACAGTCCCAAGGGGACAGTAATTCAACGCGACGGGGACGCGGGCCTGG<br>ACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCAGAGGC                                                                                           |
| 224 | S33.miR18<br>AGGGCTCTGCGTTTGCTCCAGGTAGTCCGCTGCTCCCTTGGGCCTGGGCCCCTGACAGCCCTGGTGCC<br>TCTGGCCGGCCACACCTCCTGGCGGGCAGCTGTGTAGCGTTGAAGTACTGTCCCCGTGTTCTGGCAATAC<br>CTGAAGGACAGATCCTCAACGCTACACGGAGGCCTGCCCTGACTGCCCACGGTGCCGTGGCCAAAGAGG<br>ATCTAAGGGCACCGCTGAGGGCCTACCTAACCATCGTGGGGAATAAGGACAGTGTCAACC |
| 225 | S451.miR18<br>TGCCCTGGCAGTCAGTAGGTTGTGACAGGCTGAGCAGAGAGCTTCTTGGGCTTGGGAATGGCAAGGTAG<br>CGTTGAAGTACTGTCCCCGTGACAGTACTTCAACGCTCTCTTGCTATACCCAGAAGTCTCCCTTCCAGTA<br>GTCTGCTTCAGGGTCCTGAGTTCTCTTCTGGCACGTTT                                                                                              |
| 226 | S186.miR18<br>ATGAAAAATGTTTGGGAAGAATATGATGGGTGTTCTGTAAATGCTTGTAACCTTCTAGCGTTGAAGTA<br>CTGTCCCCCTTCTGGTTTTATTTTAAGGGGTGAGTAATTCAACGCTAGGAAGTTTGAAGTCAAATGTTT<br>AAATACAAATTTTTTTTCTCACTTGTATATTTTGGTTGAAGGAATTT                                                                                       |
| 227 | S122.miR18<br>CAATGGTGAATGTGGAGGTGAAGTTAACACCTTCGTGGCTACAGAGTTTCCTTAGCAGAGCTGTAGCG<br>TTGAAGTACTGTCCCCGTGTCTAACTATCGGGGACAGCACTTCAAACTACAGTACTGCTAGGCAAT<br>CCTTCCCTCGATAAATGTCTTGGCATCGTTTGCTTTGAGCAAGAAGG                                                                                          |

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| 228               | S10a.miR18<br>TTCTTGGCCAGAAATTTTCCAGTTCTCCTACTTTCCGCGCCTTCTTCGTTTGGGGTGTGGTTTGGAGAT<br>CTGTCTGTCTTCTGTATATAGCGTTGAAGTACTGTCCCTGTAAGGAATTTTGTGGTCAGGGGACAGGA<br>CTTCACGCAATATGTAGTTGACATAAACACTCCGCTCT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 229               | S16.miR18<br>TTGCTATCATAAGAGCTATGAATAAAAAAGAAATATATTTTCTTCAGAAAGATCGTCAGCAGTGCCTTAGC<br>GTTGAAGTACTGTCCCTTAAGATCTAAAATTATCTCGTGGACAATAATTCAACGCTAAAGTAAGGTTG<br>ACAAAACACATTAGTAACATTAATATACATTAAAACACAACCTGTAGAGTATG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 230               | S1et7.miR18<br>GGGAGCATGCAGTCACAATGGCTGGAAAACCTACCATTCTTAAGCAGGGAAATAGGTTAGCGTTGAAGT<br>ACTGTCCCTTAGAATTACATCAAGGGAGATAAGGGGACAGTACTCCAAGGCTTCTCGGAGGAATCATGA<br>TCGTTCTCACCATGTTGTTTAGTGCAAGACCCA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| 231               | miR22<br>CTGGAGGCTTGTCTGAAGGCTGTATGCTTTGAGGGACTCGAAGGCCTTCGTTTGGCCACTGACTGACGA<br>AGGCCTGAGTCCCTCAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 232               | miR23<br>CTGGAGGCTTGTCTGAAGGCTGTATGCTTTGGTCGGTGCAGCGGCTCCTGTTTGGCCACTGACTGACAG<br>GAGCCGGCACCGACCAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| rAAV nucleic acid |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 233               | rAAV.ss.CAG.S155.miR1<br>CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCT<br>TTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTT<br>CCTACGCGTCCGCGGATCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCCATATAT<br>GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATT<br>GACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGA<br>GTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGA<br>CGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTG<br>GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCT<br>CCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTATTTTAAATATTTTGTGCAGCGAT<br>GGGGGCGGGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG<br>GCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCG<br>GCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGGAGTCTGCGCGCTGCCTTCGCCCCGT<br>GCCCCGCTCCGCGCGCCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAG<br>CGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTAATGACGGCTTGTTCCTTTTCT<br>GTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTGTGCGGGGGGAGCGGCTCGGGGGGTG<br>GTGCGTGTGTGTGCGTGAGGAGCGCGCTGCGGCTCCGCGCTGCCGCGGCTGTGAGCGGCGGCGG<br>GCGCGGCGCGGGGCTTTGTGCGCTCCGCGAGTGTGCGCGAGGGGAGCGCGGCGGGGGCGGTGCCCGCG<br>GTGCGGGGGGGGCTGCGAGGGGAACAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGTGAGAGGGG<br>TGTGGGCGCGTCCGTGCGGCTGCAACCCCCCTGCACCCCCCTCCCGAGTTGCTGAGCACGGCCCGG<br>TTCGGGTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAAGT<br>GGGGGTGCCGGGCGGGGCGGGGCGCCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGGCCCCG<br>GAGCGCCGGCGGCTGTGAGGCGCGGCGAGCCGAGCATTGCTTTTATGGTAATCGTGCGAGAGGGC<br>GCAGGGACTTCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCGCGGCGACCCCCCTAG<br>CGGGCGCGGGGCGAAGCGGTGCGGCGCGGCGAGGAAGAAATGGGCGGGGAGGGCCTTCTGCGCTCGCC<br>GCGCCGCGTCCCTTCTCCTCTCCAGCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGG<br>ACGGGGCAGGGCGGGGTTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCA<br>TGCTTCTTCTTTTCTTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATATTTGGCAA<br>AGAATTAACCAAAGAATTATCGATACCTTAGGCTCGACGATCTAGCGTCGACCAAGTGGATCCTGGA<br>GGCTTGTGTAAGGCTGTATGCTGTCTTGGTAGCTGAAAGTTCTTGTTTTGGCCACTGACTGACAAGAAC<br>TTAGCTACCAAGACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCAGATCCGATC<br>TTTTTCCCTCTGCCAAAAATATGGGGACATCATGAAGCCCTTGAGCATCTGACTTCTGGCTAATAAA<br>GGAAATTTATTTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGATCGCGGCCGAGAT<br>CTATCTGAGGAACCCCTAGTGTGTGTTGGTTTTTGTGTAGGAACCCCTAGTGATGGAGTTGGCCACTC<br>CCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAGGTGCGCCGACGCCCGGGCTTTGCC<br>GGGCGGCTCAGTGAGCGAGCGAGCGCGAGCTGCCTGCAGGC |
| 234               | rAAV.ss.CAG.S155.miR2<br>CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCT<br>TTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTT<br>CCTACGCGTCCGCGGATCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCCATATAT<br>GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATT<br>GACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGA<br>GTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |



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|     | <p>CGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTG<br/>GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCT<br/>CCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTATTTTAAATTATTTTGTGCAGCGAT<br/>GGGGGCGGGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG<br/>GCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCCTTTATGGCGAGGCGGCG<br/>GCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTCTGCTGCGCGCTGCCCTTCGCCCCGT<br/>GCCCCGCTCCGCGCCCGCTCGCGCCCGCCCGCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAG<br/>CGGGCGGGACGGCCCTTCTCCTCCGGCTGTAATTAGCGCTTGGTTTAAATGACGGCTTGTTCCTTTCT<br/>GTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGC<br/>GTGCGTGTGTGTGCGTGCGGAGCGCCGCTGCGGCTCCGCGCTGCCGCGGGCTGTGAGCGCTGCGG<br/>GCGCGGCGCGGGGCTTTGTGCGCTCCGCACTGTGCGGAGGGGAGCGCGGCCGGGGCGGTGCCCGCG<br/>GTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGCGGGGGGTGAGCAGGGGG<br/>TGTGGGCGCGTGGTCGGGCTGCAACCCCCCTGCACCCCCCTCCCGAGTTGCTGAGCACGGCCCCGGC<br/>TTCGGGTGCGGGGCTCCGTACGGGGCGTGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGCGGCGAGGT<br/>GGGGGTGCCGGGCGGGGCGGGGCGCCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGCGGCCCCCCG<br/>GAGCGCCGGCGGCTGTGAGGGCGGGCGAGCCGCGAGCCATTGCCCTTTATGGTAATCGTGCGAGAGGGC<br/>GCAGGGACTTCCCTTTGCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCACCCCTCTAG<br/>CGGGCGCGGGGCGAAGCGGTGCGGCGCCGCGAGGAAGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCC<br/>GCGCCGCGCTCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCCTTCGGGGGG<br/>ACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCA<br/>TGCCTTCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTATTTGTGCTGTCTCATCATTTTGGCAA<br/>AGAATTAACCAAGAATTATCGATACCCTAGGCGTCGACGATCTAGCGTCGACCACTGGATCCTGGA<br/>GGCTTGTGAAGGCTGTATGCTGTATTGTGAGACAATGATTACGTTTTTGCCCACTGACTGACGTGAAT<br/>CAGTCTGACAATACAGGACACAAGGCTGTACTAGCACTCACATGGAACAAATGGCCAGATCCGATC<br/>TTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCCTTGAGCATCTGACTTCTGGCTAATAAA<br/>GGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGATCGCGGCCGAGAT<br/>CTATCTGAGGAACCCCTAGTGTGTGTTGGTTTTTGTGTAGGAACCCCTAGTGATGGAGTTGGCCACTC<br/>CCTCTCTGCGGCTCGCTCGCTCACTGAGGCCGGGCGACCAAGGTGCCCCGACGCCCGGGCTTTGCC<br/>GGCGGCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG</p>                                                                                                                                                                                                                                                                                                                                                                         |
| 235 | <p>rAAV.ss.CAG.S155.miR16</p> <p>CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCT<br/>TTGGTCGCCCCGGCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCCACTCCATCACTAGGGGT<br/>TCCTACGCGTCCGCGGATCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATA<br/>TGGAGTTCGCGGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCAT<br/>TGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGG<br/>AGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTG<br/>ACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTT<br/>GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTC<br/>TCCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTATTTTAAATTATTTTGTGCAGCGA<br/>TGGGGGCGGGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGA<br/>GGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCCTTTATGGCGAGGCGGC<br/>GGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGCGGAGTCTGCTGCGCGCTGCCCTTCGCCCCG<br/>TGCCCCGTCCGCGCGCGCTCGCGCGCGCCCGCCCGGCTCTGACTGACCGGCTTACTCCACAGGTGA<br/>GCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAAATGACGGCTTGTTCCTTTTC<br/>TGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTG<br/>CGTGCGTGTGTGTGCGTGCGGAGCGCGCGCTGCGGCTCCGCGCTGCCGCGGGCTGTGAGCGCTGCG<br/>GGCGGCGCGCGGGGCTTTGTGCGCTCCGCACTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCGCG<br/>GGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGCGGGGGGTGAGCAGGGG<br/>GTGTGGGCGCGTGGTCGGGCTGCAACCCCCCTGCACCCCCCTCCCGAGTTGCTGAGCACGGCCCCGG<br/>TTTCGGGTGCGGGGCTCCGTACGGGGCGTGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCCCG<br/>TGGGGGTGCCGGGCGGGGCGGGGCGCCCTCGGGCCGGGGAGGGCTCGGGGAGGGGCGGGGCGGGGCGA<br/>GGAGCGCCGGCGGCTGTGAGGCGCGGCGAGCCGAGCCATTGCCCTTTATGGTAATCGTGCGAGAGGG<br/>CGCAGGGACTTCCCTTTGCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCACCCCTCTA<br/>GCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGAAATGGGCGGGGAGGGCCTTCGTGCGTCGC<br/>CGCGCGCCGTCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCCTTCGGGGGG<br/>GACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCT<br/>ATGCCCTCTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCACTCTGTTGGCA<br/>AAGAATTAACCAAGAATTATCGATACCCTAGGCGTCGACGATCTAGCGTCGACCACTGGATCCTGG<br/>AGGCTTGTGAAGGCTGTATGCTGTGCAAGGCCCTTATCAGCTTTGTTTTGGCCACTGACTGACAAAGC<br/>TGAAAGGCTTCGACAGGACACAAGGCTGTTACTAGCACTCATGGAACAAATGGCCAGATCCGAT<br/>CTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCCTTGAGCATCTGACTTCTGGCTAATAA<br/>AGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGATCGCGGCCGAG<br/>TCTATCTGAGGAACCCCTAGTGTGTGTTGGTTTTTGTGTAGGAACCCCTAGTGATGGAGTTGGCCACT</p> |

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|     | CCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCACGCCCGGGCTTTGCC<br>CGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGGC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 236 | <p>rAAV.ss.CAG.S155.miR18</p> <p>CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCCGGGCGTCCGGCGACCT<br/>TTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTT<br/>CCTACGCGTCCGCGGATCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATAT<br/>GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCCCGCCATT<br/>GACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGA<br/>GTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGA<br/>CGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTG<br/>GCAGTACATCTACGTATTAGTCATCGCTATTACCATTGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCT<br/>CCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTAAATTATTTTGTGCAGCGAT<br/>GGGGGCGGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG<br/>GCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCG<br/>GCGGCGGCGGCCCTATAAAAAAGCAAGCGCGCGGCGGGGAGTTCGCTGCGCGCTGCCTTCGCCCCGT<br/>GCCCCGTCCGCGCGCCCTCGCGCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAG<br/>CGGGCGGGACGGCCCTTCCTCCGGGCTGTAATTAGCGCTTGGTTAATGACGCTTGTTCCTTTCT<br/>GTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGAGCGGCTCGGGGGTGC<br/>GTGCGTGTGTGTGTGCGTGGGAGCGCGCGTGC GGCTCCGCGCTGCCGCGGGCTGTGAGCGCTGCGG<br/>GCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGCGGTGCCCGCG<br/>GTGCGGGGGGGGCTGCGAGGGGAACAAGGCTGCGTGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGG<br/>TGTGGGCGCGTGGTTCGGGCTGCAACCCCCCTGCACCCCCCTCCCGAGTTGCTGAGCACGGCCCCGG<br/>TTCGGGTGCGGGGCTCCGTACGGGGCTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGTGGCGGCAGGT<br/>GGGGGTGCCGGGCGGGGCGGGGCGGCCCTCGGGCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCGG<br/>GAGCGCGCGGCGGTGTGAGGCGCGGCGAGCCGCGAGCCATTGCCCTTTATGGTAATCGTGCGAGAGGGC<br/>GCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCGCGCGCACCCCCCTAG<br/>CGGGCGCGGGGCGAAGCGGTGCGGCGCGCGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTGC<br/>CGCGCGCGTCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGG<br/>ACGGGGCAGGGCGGGGTTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCA<br/>TGCTTCTTCTTTTCTTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATATTTTGGCAA<br/>AGAATTAACCAAAGAATTATCGATACCCTAGGCGTCGACGATCTAGCGTCGACCAGTGGATCCTGGA<br/>GGCTTGCTGAAGGCTGTATGCTGTAGCGTTGAAGTACTGTCCCCGTTTGGCCACTGACTGACGGGGAC<br/>AGCTTCAACGCTACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCCAGATCCGATC<br/>TTTTTCCCTTGCCAAAAATTATGGGGACATCATGAAGCCCCCTTGAGCATCTGACTTCTGCTAATAAA<br/>GGAAATTTATTTTATTGCAATAGTGTGTTGGAATTTTGTGTCTCTCACTCGATCGCGGCGCAGAT<br/>CTATCTGAGGAACCCCTAGTGTGTGTTGGTTTTTGTGTAGGAACCCCTAGTGATGGAGTTGGCCACTC<br/>CCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCACGCCCGGGCTTTGCC<br/>GGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG</p> |
| 237 | <p>rAAV.EF1α.S155.miR2</p> <p>CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCCGGGCGTCCGGCGACCT<br/>TTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTT<br/>CCTACGCGTTACAGAAACCAAGAAAGCACCATAGCGTTTGCCTTCAACTGCTTCCCAACTTGTTTTCT<br/>CTTCCAATTTGATTGTGGTTTCTCTCCAGAAGGAACTCCACAGTACTTAGCGTTGGCCACATAGTAGG<br/>TTCTCAAATACTTGTTAATAAATAAGTTTGTTCGAGAAGCTGGGCAATGATATTTACAGCTGGAAGAA<br/>GAAACATAATGATCTAGTAATTAGCTCATTAATAAATAAACGTTCTTCTTCTCAGAGGAGCATTTC<br/>AAGGCCTGCCTTGATAGCCATCCAAAAAGGCCAAGCTCATCCAATCTGCCCTAGATTTATGCTAATAA<br/>GCAGTTACAATCGATAGGATGACAGAAAACGACAGCACTTATTTAATAATAATAGGCACTTATTTAATA<br/>AGGAGAAGCTGTGACTTCATAGCAAGTGTGGGGTTAGGAAACTGGGTGGATAAACTTGCTGATGCTGT<br/>AGATCTTAGCCTCTACATGAGATCATGTGGAATACTGAAAGCATTTTAGGTTCCCTATGTTTGAATC<br/>AAATAACTGTACACCTTTTAATTTAAAAAGTACCATGAGGCACACACACACTCCGAGGAACCTTTT<br/>GCGTAACAAAACCTAGAAATAGATCTAAAGCTAACTGTAGGACTGAGTCTATTCTAAACTGAAAGCCTG<br/>ACATCTGGAGTACCAGGGGGAGATGACGTGTTACGGGCTTCCATAAAAGCAGCTGGCTTTGAATGGAAG<br/>GAGCCAAGAGGCCAGCACAGGAGCGGATTCGTGCTTTCACGGCCATCGAGCCGAACCTCTCGCAAGTC<br/>CGTGAGCCGTTAAGGAGGCCCCAGTCCCGACCTTCGCCCCAAGCCCCCTCGGGTCCCCGGGCTGGT<br/>ACTCCTTGCCACACGGGAGGGGCGCGGAAGCCGGGCGGAGGAGGAGCCAACCCCGGGCTGGGCTGAGA<br/>CCCGCAGAGGAAGACGCTCTAGGGATTTGTCCCGACTAGCGAGATGGCAAGGCTGAGGACGGGAGGCT<br/>GATTGAGAGGCGAAGGTACACCCTAATCTCAATACAACCTTTGGAGCTAAGCCAGCAATGGTAGAGGGA<br/>AGATTTGCACGTCCCTTCCAGGCGGCTCCCGCTACCCACCCCCCAACCCCGGAGCTGAGGAGCTG<br/>AGAGTAATTCATACAAAAGGACTCGCCCTGCCTTGGGGAATCCCAGGGACCGTCGTTAACTCCCACT<br/>AACGTAGAACCAGAGATCGCTGCGTTCGCGCCCCCTCACCGCCCGCTCTCGTCATCACTGAGGTGGA<br/>GAAGAGCATGCGTGAGGCTCCGGTGCCGTGAGTGGGCGAGAGCGCACATCGCCACAGTCCCCGAGAAG<br/>TTGGGGGGAGGGGTCCGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAACTGGGAAAGTGAT</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

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|     | <p>GTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTG AACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCT GGCCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCCCTGGCTGCAGTACGTGAT TCTTGATCCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTTCGAGGCCCTTGCCTTAAGGAGCCCCCTC GCCTCGTGCTTGAGTTGAGGCCTGGCTTGGGCGCTGGGGCCGCCGCTGCGAATCTGGTGGCACCTTCG CGCCTGTCTCGCTGCTTTTCGATAAGTCTCTAGCCATTTAAAATTTTGTATGACCTGCTGCGACGCTTTT TTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCCGC GGGCGGCGACGGGGCCCCGTGCGTCCCAGCGCACATGTTTCGGCGAGCGGGGCCCTGCGAGCGCGGCCACC GAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGGCCTGTAT CGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAAGTTGCGTGAGCGGAAAGATGGCCGCTTCC CGGCCCTGCTGACGGGAGCTCAAATGGAGGACGCGCGCTCGGGAGAGCGGGCGGGTGAGTCACCAC ACAAGGAAAAGGGCCTTTCCGTCTCAGCCGTGCGTTTATGTGACTCCACGGAGTACGGGGCGCCGTC CAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTGCTCTTTAGGTTGGGGGGAGGGGTTTTATGC GATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTC CTTGGAATTTGCCCTTTTGTAGTTTGGATCTTGGTTTATTCTCAAGCCTCAGACAGTGGTTCAAAGTTT TTTTCTCCATTTAGGTGTCGTGAAAACAAACCCTAGGCGTCGACGATCTAGGATCTAGGATCTAGGATCT GGGAGGCTTGTCTGAAGGCTGTATGCTGTATTGTGAGACAATGATTCACGTTTTTGGCCACTGACTGACG TGAATCAGTCTGACAATACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCAGATC CGATCTTTTTTCCCTCTGCCAAAATATAGGGGACATCATGAAGCCCTTGAGCATCTGACTTCTGGCTA ATAAAGGAAATTTATTTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGATCGCGGCCG CAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGA CCAAAGGTGCGCCGACGCCGGGCTTTGCCCGGGCGGCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTG CAGG</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 238 | <p>rAAV.EF1<math>\alpha</math>.S.26.miR2<br/> CCTGCAGGACGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCCGGCGACCT TTGGTTCGCCCCGCCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTT CCTACGCGTTACAGAAACCAAGAAAGCACCATAGCGTTTGCCTTCAACTGCTTCCCAACTTGTTTTCCT CTTCCAATTTGATTGTGGTTTCTCTCCAGAAGGAACTCCACAGTACTTAGCGTTGGCCACATAGTAGG TTCTCAAATACTTGTAAATAAATAAGTTTGTTCGAGAAGCTGGGCAATGATATTCTACAGCTGGAAGAA GAAACATAATGATCTAGTAATTAGCTCATTAATAAATAAACGTTCTTCTTTCTCAGAGGAGCATTTCCC AAGGCCTGCCTTGATAGCCATCCAAAAGGCCAAGCTCATCCAATCTTGCCCTAGATTTATGCTAAAT CGAGTTACAATCGATAGGATGACAGAAAACGACAGCACTTATTTAAATATAATAGGCACTTATTTAAAT AGGAGAAGCTGTGACTTTCATAGCAAGTGTGGGGTTAGGAAACTGGGTGGATAAACTTGCTGATGCTGT AGATCTTAGCCTCTACATGAGATCATGTGGAATACTGAAAGCATTTTAGGTTCCCTATGTTTGCAATC AAATAACTGTACACCTTTTAATTTAAAAAGTACCATGAGGCACACACACACTCGCAGGAACCTTTTGG CCGTAACAAAACCTAGAAATAGATCTAAAGCTAACTGTAGGACTGAGTCTATTCTAAACTGAAAGCCTGG ACATCTGGAGTACCAGGGGAGATGACGTGTTACGGGCTTCCATAAAAGCAGCTGGCTTTGAATGGAAG GAGCCAAGAGGCCAGCACAGGAGCGGATTTCGTGCTTTTACGGCCATCGAGCCGAACCTCTCGCAAGTC CGTGAGCCGTTAAGGAGGCCCCAGTCCCGACCCTTCGCCCAAGCCCTCGGGGTCCCGGGGCTGGT ACTCCTTGCCACACGGGAGGGGCGCGGAAGCCGGGGCGGAGGAGGAGCCAACCCCGGGCTGGGCTGAGA CCCGCAGAGGAAGACGCTCTAGGGATTTGTCCCGACTAGCGAGATGGCAAGGCTGAGGACGGGAGGCT GATTGAGAGGCGAAGGTACACCCTAATCTCAATACAACTTTGGAGCTAAGCCAGCAATGGTAGAGGGA AGATTCTGACGCTCCCTTCCAGGCGGCCCTCCCGTCAACACCCCCCAACCCGCCCCGACCGGAGCTG AGAGTAATTCATACAAAAGGACTCGCCCTGCCTTGGGAATCCAGGGACCGTCGTTAACTCCCACT AACGTAGAACCCAGAGATCGCTGCGTTCCCGCCCCCTCACCCGCCCGCTCTCGTCATCACTCAGGTGGA GAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCGAGCGCACATCGCCACAGTCCCCGAGAAG TTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAACTGGGAAAGTGAT GTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTG AACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCT GGCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCCCTGGCTGCAGTACGTGAT TCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTTCGAGGCCCTTGCCTTAAGGAGGCCCTTC GCCTCGTGCTTGAGTTGAGGCCTGGCTTGGGCGCTGGGGCCGCCGCTGCGAATCTGGTGGCACCTTCG CGCCTGTCTCGCTGCTTTTCGATAAGTCTCTAGCCATTTAAAATTTTGTATGACCTGCTGCGACGCTTTT TTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCCGC GGGCGGCGACGGGGCCCCGTGCGTCCCAGCGCACATGTTTCGGCGAGGCGGGGCCCTGCGAGCGCGGCCACC GAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCTGCTCTGGTGCCTGGCCTCGCGCCCGGTGTAT CGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTGCGCACCAAGTTGCGTGAGCGGAAAGATGGCCGCTTCC CGGCCCTGCTGCAGGGAGCTCAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCAC ACAAGGAAAAGGGCCTTTCCGTCTCAGCCGTGCGTTTATGTGACTCCACGGAGTACGGGGCGCCGTC CAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTGCTCTTTAGGTTGGGGGGAGGGGTTTTATGC GATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTC CTTGGAATTTGCCCTTTTGTAGTTTGGATCTTGGTTTATTCTCAAGCCTCAGACAGTGGTTCAAAGTTT TTTTCTTCCATTTAGGTGTCGTGAAAACAAACCCTAGGCGAGGGAATGAAGCCAGGAGCCAAAGAGC</p> |

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|     | AGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGTATTGTCAGACAATGATTCACCTGTGCAGGTCCC<br>AAGTGAATCATTATCTGACACTACGGGGACGCGGGCCTGGACGCCGGCATCCGGGCTCAGGACCCCCCT<br>CTCTGCCAGAGGCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCCTTGAGCATCTGACT<br>TCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTGTGTCTCTCACTCGAT<br>CGCGGCCGACGAAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGG<br>CCGGGCGACCAAAGGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCA<br>GCTGCCTGCAGG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| 239 | rAAV.EF1 $\alpha$ .S33.miR2<br>CCTGCAGGCAGCTGCGCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCCGGGCGTCGGGCGACCT<br>TTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTT<br>CCTACGCGTTACAGAAACCAAGAAAGCACCATAGCGTTTGCCTTCAACTGCTTCCCAACTTGTTTTCT<br>CTTCCAATTTGATTGTGGTTTCTCTCCAGAAGGAACTCCACAGTACTTAGCGTTGGCCACATAGTAGG<br>TTCTCAAATACTTGTTAATAAATAAGTTTGTTCGAGAAGCTGGGCAATGATATTCTACAGCTGGAAGAA<br>GAAACATAATGATCTAGTAATTAGCTCATTAATAAATAAACGTTCTTCTTCTCAGAGGAGCATTTCCC<br>AAGGCCTGCCTTGATAGCCATCCAAAAAGGCCAAGCTCATCCAATCTTGCCCTAGATTTATGCTAAAAAT<br>GCAGTTACAATCGATAGGATGACAGAAAACGACAGCACTTATTTAAATATAATAGGCACTTATTTAAAT<br>AGGAGAAGCTGTGACTTCATAGCAAGTGTGGGGTTAGGAAACTGGGTGGATAAACTTGCTGATGCTGT<br>AGATCTTAGCCTCTACATGAGATCATGTGGAATACTGAAAGCATTTTAGGTTCCCTATGTTTGCAATC<br>AAATAACTGTACACCTTTTAATTTAAAAAGTACCATGAGGCACACACACACTCGCAGGAACTTTTTG<br>GCGTAACAAAACCTAGAATTAGATCTAAAGCTAACTGTAGGACTGAGTCTATTCTAAACTGAAAGCCTGG<br>ACATCTGGAGTACCAGGGGGAGATGACGTGTTACGGGCTTCCATAAAAGCAGCTGGCTTTGAATGGAAG<br>GAGCCAAGAGGCCAGCACAGGAGCGGATTCGTGCTTTACGGCCATCGAGCCGAACCTCTCGCAAGT<br>CGTGAGCCGTTAAGGAGGCCCCCAAGTCCCGACCTTCGCCCCAAGCCCCCTCGGGTCCCCGGGCTGGT<br>ACTCCTTGCCACACGGGAGGGGCGCGGAAGCCGGGGCGGAGGAGGAGCCAACCCCGGGCTGGGCTGAGA<br>CCCGCAGAGGAAGACGCTCTAGGGATTTGTCCCGACTAGCGAGATGGCAAGGCTGAGGACGGGAGGCT<br>GATTGAGAGGCGAAGGTACACCCTAATCTCAATACACCTTTGGAGCTAAGCCAGCAATGGTAGAGGGA<br>AGATTCTGACAGTCCCTTCCAGGCGGCCCTCCCCGTCAACACCCCCCAACCCGCCCCGACCGAGCTG<br>AGAGTAATTCATACAAAAGGACTCGCCCCCTGCCTTGGGAATCCAGGGACCGTCGTTAACTCCCACT<br>AACGTAGAACCCAGAGATCGCTGCGTTCCCGCCCCCTCACCCGCCCGCTCTCGTCACTCACTGAGGTGGA<br>GAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAG<br>TTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAACTGGGAAAAGTGAT<br>GTCGTGTACTGGCTCCGCCCTTTTCCCAGGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTG<br>AACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCT<br>GGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCTGGCTGCAGTAGCTGAT<br>TCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTTCGAGGCCCTTGCGCTTAAGGAGCCCCCTC<br>GCCTCGTGCTTGAGTTGAGGCCTGGCTTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCG<br>CGCCTGTCTCGCTGCTTTTCGATAAGTCTTAGCCATTTAAAATTTTGTATGACCTGCTGCGACGCTTTT<br>TTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCCGC<br>GGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCCGCGAGGCGGGGCTGCGAGCGCGGCCACC<br>GAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCTTGGCTGCGCGCCCGCTGTAT<br>CGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCC<br>CGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCAC<br>ACAAAGGAAAAGGGCCTTTCCGTCTCAGCCGTGCGTTCATGTGACTCCACGGAGTACCGGGCGCCGTC<br>CAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTGCTCTTAGGTTGGGGGGAGGGGTTTTATGC<br>GATGGAGTTTTCCCACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTTCTC<br>CTTGGAATTTGCCCTTTTGGATTGGATCTTGGTTCACTCTCAAGCCTCAGACAGTGGTTCAAAGTTT<br>TTTTCTTCCATTTAGGTGTCGTGAAAACAAACCTAGGAGGGCTCTGCGTTTGCTCCAGGTAGTCCGC<br>TGCTCCCTTGGGCTGGGCCACTGACAGCCCTGGTGCCTCTGGCCGGCTGCACACCTCCTGGCGGGCA<br>GCTGTGTATGTGAGACAATGATTCACTGTTCTGGCAATACCTGGTGAATCAAAGCCTGACAATACACG<br>GAGGCCTGCCCTGACTGCCACGGTGCCGTGGCCAAAGAGGATCTAAGGGCACCGCTGAGGGCTACCT<br>AACCATCGTGGGGAATAAGGACAGTGTACCCCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAA<br>GCCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTT<br>TTTGTGTCTCTCACTCGATCGCGGCCGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGC<br>GCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCTCA<br>GTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG |
| 240 | rAAV.EF1 $\alpha$ .S155.miR18<br>CCTGCAGGCAGCTGCGCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCCGGGCGTCGGGCGACCT<br>TTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTT<br>CCTACGCGTCCGCGGATCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATAT<br>GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCCCTGGCTGACCGCCCAACGACCCCCGCCCAT<br>GACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

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|                   | CGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTGCGCACCAAGTTGCGTGAGCGGAAAGATGGCCGCTTCC<br>CGGCCCTGCTGCAGGGAGCTCAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCAC<br>ACAAAGGAAAAGGGCCTTTCCGTCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTC<br>CAGGCACCTCGATTAGTTCTCAGACTTTTGGAGTACGTCGTCCTTAGGTTGGGGGGAGGGGTTTATGC<br>GATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTC<br>CTTGGAAATTTGCCCTTTTGGATTTGGATCTTGGTTTATTCTCAAGCCTCAGACAGTGGTTCAAAGTTT<br>TTTTCTTCCATTTAGGTGTCGTGAAAACAAACCCTAGGCGAGGGAATGAAGCCACAGGAGCCAAGAGC<br>AGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGTAGCGTTGAAGTACTGTCCCTGTGCAGGTCCC<br>AAGGGGACAGTAATCAACGCGACGGGGACGCGGGCCTGGACGCCGGCATCCGGGCTCAGGACCCCCCT<br>CTCTGCCAGAGGCTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCCTTGAGCATCTGACT<br>TCTGGCTAATAAAGGAAATTTATTTTCTTGAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGAT<br>CGCGGCCGACGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGG<br>CCGGGCGACCAAGGTGCGCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCA<br>GCTGCCTGCAGG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 242               | rAAV.EFlα.S33.miR18<br>CCTGCAGGACAGTGCAGCGCTCGCTCGCTCACTGAGGCCGCGCCGGGCAAAGCCCCGGGCGTCGGGCGACCT<br>TTGGTCGCCCCGCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCATCACTAGGGGTT<br>CCTACGCGTTACAGAAACCAAGAAAGCACCATAGCGTTTGCCTTCAACTGCTTCCCACTGTTTTCTCT<br>CTTCCAATTTGATTGTGGTTTCTCTCCAGAAGGAACCTCACAGTACTTAGCGTTGGCCACATAGTAGG<br>TTCTCAAATACTTGTTAATAAATAAGTTTGTTCGAGAAGCTGGGCAATGATATTTACAGCTGGAAGAA<br>GAAACATAATGATCTAGTAATTAGCTCATTAAAAATAAACGTTCTTCTTCTCAGAGGAGCATTTCCC<br>AAGGCCTGCCTTGATAGCCATCCAAAAGGCCAAGCTCATCCAATCTTGCCCTAGATTTATGCTAAAAAT<br>GCAGTTACAATCGATAGGATGACAGAAAACGACAGCACTTATTTAAATATAATAGGCACTTATTTAAAT<br>AGGAGAAGCTGTGACTTCATAGCAAGTGTGGGGTTAGGAAACTGGGTGGATAAACTTGCTGATGCTGT<br>AGATCTTAGCCTCTACATGAGATCATGTGGAATACTGAAAGCATTTTAGGTTCTTATGTTTGCAATC<br>AAATAACTGTACACCTTTTAATTTAAAAAGTACCATGAGGCACACACACACTCGCAGGAACCTTTTGT<br>GCGTAACAAAACCTAGAAATAGATCTAAAGCTAACTGTAGGACTGAGTCTATTCTAAACTGAAAGCCTGG<br>ACATCTGGAGTACCAGGGGGAGATGACGTGTTACGGGCTTCCATAAAAGCAGCTGGCTTTGAATGGAAG<br>GAGCCAAGAGGCCAGCACAGGAGCGGATTGTCGCTTTCACGGCCATCGAGCCGAACCTCTCGCAAGT<br>CGTGAGCCGTTAAGGAGGCCCCAGTCCCGACCCCTCGCCCCAAGCCCCCTCGGGGTCCCCGGGCTGGT<br>ACTCCTTGCCACACGGGAGGGGCGCGGAAGCCGGGGCGGAGGAGGAGCCAACCCCGGGCTGGGTGAGA<br>CCCGCAGAGGAAGACGCTCTAGGGATTTGTCCCGACTAGCGAGATGGCAAGGCTGAGGACGGGAGGCT<br>GATTGAGAGGCGAAGGTACACCCTAATCTCAATACAACTTTGGAGCTAAGCCAGCAATGGTAGAGGGA<br>AGATTCTGCACGTCCCTTCCAGGCGGCTCCCCGTCAACACCCCCCAACCCGCCCCGACCGGAGCTG<br>AGATAATTCATACAAAAGGACTCGCCCTGCCTTGGGAATCCAGGGACCGTGGCTTAAACTCCCCT<br>AACGTAGAACCCAGAGATCGCTGCGTTCCCGCCCCCTCACCCGCCCGCTCTCGTCATCACTAGGTTGGA<br>GAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCCAGTCCCCGAGAAG<br>TTGGGGGGAGGGGTGCGCAATTGAACCGGTGCTTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGAT<br>GTCGTGTACTGGCTCCGCCTTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTG<br>AACGTTCTTTTTTCGCAACGGGTTTGGCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCT<br>GGCCTCTTTACGGGTTATGGCCCTTGGCTGCTTGAATTACTTCCACGCCCTGGCTGAGTAACTGAT<br>TCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTGAGGCTTGCCTTAAGGAGCCCCCTTC<br>GCCTCGTGCTTGAGTTGAGGCTGGCTTGGGCGCTGGGGCCGCCGCTGCGAATCTGGTGGCACCTTCG<br>CGCCTGTCTGCTGCTTTGATAAGTCTTAGCCATTTAAATTTTTTGATGACCTGCTGCGACGCTTTT<br>TTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCCGC<br>GGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTGGCGAGGCGGGGCTGCGAGCGCGGCCACC<br>GAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCTGCTCTGGTGCTTGGCTGCGCGCCCGCTGTAT<br>CGCCCCGCTTGGGCGGCAAGGCTGGCCCGGTGCGCACCAAGTTGCGTGAGCGGAAAGATGGCCGCTTCC<br>CGGCCCTGCTGCAGGGAGCTCAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCAC<br>ACAAAGGAAAAGGGCCTTTCCGTCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTC<br>CAGGCACCTCGATTAGTTCTCAGACTTTTGGAGTACGTCGTCCTTAGGTTGGGGGGAGGGGTTTATGC<br>GATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTC<br>CTTGGAAATTTGCCCTTTTGGATTTGGATCTTGGTTTATTCTCAAGCCTCAGACAGTGGTTCAAAGTTT<br>TTTTCTTCCATTTAGGTGTCGTGAAAACAAACCCTAGGAGGGCTTGCCTTGTCTCCAGGTAGTCCGC<br>TGCTCCCTTGGGCTGGGCCACTGACAGCCCTGGTGCTCTGGCCGGCTGCACACCTCTGGCGGGCA<br>GCTGTGTAGCGTTGAAGTACTGTCCCTGTTCTGGCAATACTGGGGGACAGATCCTCAACGCTACACG<br>GAGGCCTGCCCTGACTGCCACGGTGCCGTGGCCAAAGAGGATCTAAGGGCACCGCTGAGGGCTACCT<br>AACCATCGTGGGAATAAGGACAGTGTACCCCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAA<br>GCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCTTGAATGTTGTTGGAATTT<br>TTTGTGTCTCTCACTCGATCGCGGCCGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGC<br>GCTCGCTCGCTCACTGAGGCCGGGCGACCAAGGTGCGCCGACGCCGGGCTTTGCCCGGGCGGCTCA<br>GTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG |
| Vector components |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

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| 250 | <p>CAG Promoter</p> <p>ATCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCCGCGTTAC<br/> ATAACTTACGGTAATGCCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGAC<br/> GTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAAC<br/> TGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAA<br/> ATGGCCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGT<br/> ATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCCTCCCATCTCCCCCCC<br/> CTCCCCACCCCCAATTTTGTATTTATTTATTTTAAATTATTTTGTGCAGCGATGGGGCGGGGGGGG<br/> GGGGGGGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGAGGCGGAGAGGTGCGGC<br/> GGCAGCCAATCAGAGCGGCGCGCTCCGAAAAGTTTCCTTTTATGGCGAGGCGGGCGGGCGGGCCCTA<br/> TAAAAGCGAAGCGCGGGCGGGGCGGAGTCTGCTGCGCGCTGCCCTCGCCCCGTGCCCGCTCCGCCG<br/> CGCCTCGCGCCGCCCCGCGCTCTGACTGACCGGTTACTCCACAGGTGAGCGGGCGGGACGGCCC<br/> TTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAAATGACGGCTTGTCTTTCTGTGGCTGCGTGAAAG<br/> CCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGAGCGGCTCGGGGGGTGCGTGCCTGTGTGTG<br/> CGTGGGGAGCGCGCTGCGGCTCCGCGCTGCCGCGGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCT<br/> TTGTGCGCTCCGCGAGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCGCGGTGCGGGGGGGCTG<br/> CGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCCGT<br/> CGGGCTGCAACCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTTCGGGGCT<br/> CCGTACGGGGCGTGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGG<br/> GGCGGGGCGCCTCGGGCGGGGAGGGCTCGGGGAGGGGCGCGGCGGCCCGGAGCGCGCGGCGGCTG<br/> TCGAGGCGCGCGAGCCGAGCCATTGCCTTTTATGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTT<br/> GTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCGCGCACCCCCCTCTAGCGGGCGCGGGGCGAA<br/> CGCGTGGCGCCGCGAGGAAGAAATGGCGGGGAGGGCCTTCGTGCGTCGCGCGCGCGCGCTCCCT<br/> TCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGACGGCTGCCCTCGGGGGGAGGGGCGAGGGCGGG<br/> GTTCCGGCTTCTGGCGTGTGACCGGGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTT<br/> CCTACAGCTCCTGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATT</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 251 | <p>EFlα-promoter</p> <p>TACAGAAACCAAGAAAGCACCATAGCGTTTGCCTTCAACTGCTTCCCAACTTGTTTTCTCTTCCAATT<br/> TGATTGTGGTTTCTCTCCAGAAGGAAGTCCACAGTACTTAGCGTTGGCCACATAGTAGGTTCTCAAAT<br/> ACTTGTTAATAAATAAGTTTGTTCGAGAAGCTGGGCAATGATATTCTACAGCTGGAAGAAGAAACATAA<br/> TGATCTAGTAATTAGCTCATTAAAAATAACGTTCTTCTTCTCAGAGGAGCATTTCCCAAGGCTGC<br/> CTTGATAGCCATCCAAAAAGGCCAAGCTCATCCAATCTTGCCCTAGATTTATGCTAAAATGCAGTTACA<br/> ATCGATAGGATGACAGAAAACGACAGCACTTATTTAAATATAATAGGCACTTATTTAAATAGGAGAAGC<br/> TGTGACTTCTATAGCAAGTTTGGGTTAGGAAACTGGGTGGATAAACTTGCTGCTGTAGATCTTAG<br/> CCTCTACATGAGATCATGTGGAATACTGAAAGCATTTTAGGTTCTTATGTTTGCAATCAAATAACTG<br/> TACACCTTTTAAATTTAAAAAGTACCATGAGGCACACACACACTCGCAGGAACCTTTTGGCGTAACAA<br/> AACTAGAATTAGATCTAAAGCTAACTGTAGGACTGAGTCTATTCTAAACTGAAAGCCTGGACATCTGGA<br/> GTACCAGGGGAGATGACGTGTTACGGGCTTCCATAAAAGCAGCTGGCTTTGAATGGAAGGAGCCAAGA<br/> GGCCAGCACAGGAGCGGATTTCGTGCTTTCACGGCCATCGAGCCGAACCTCTCGCAAGTCCGTGAGCCG<br/> TTAAGGAGGCCCCAGTCCCGACCTTCGCCCCAAGCCCTCGGGGTCCCGGGCTTGGTACTCCTTGC<br/> CACACGGGAGGGGCGCGAAGCCGGGGCGGAGGAGGCCAACCCGGGCTGGGCTGAGACCCGACAGAG<br/> GAAGACGCTCTAGGGATTTGTCCCGACTAGCGAGATGGCAAGGCTGAGGACGGGAGGCTGATTGAGAG<br/> GCGAAGGTACACCCTAATCTCAATACAACTTTGGAGCTAAGCCAGCAATGGTAGAGGGAAGATTCTGC<br/> ACGTCCCTTCCAGGCGGCCCTCCCGTCACCAACCCCCCAACCCGCCCCGACCGGAGCTGAGAGTAATT<br/> CATACAAAAGGACTCGCCCTGCCTTGGGGAATCCAGGGACCGTTCGTTAACTCCCACTAACGTAGAA<br/> CCCAGAGATCGCTGCGTTCCCGCCCCCTCACCGCCGCTCTCGTCATCACTGAGGTGGAGAAGAGCAT<br/> GCGTGAGGCTCCGGTGCCCGTCAGTGGGCGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGA<br/> GGGGTTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGGGGTAACTGGGAAAGTGATGTCGTGTAC<br/> TGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAAGTAGTCGCCGTGAACGTTCTT<br/> TTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGGCTGGCCTCTTT<br/> ACGGGTTATGGCCCTTGCCTGCCTTGAATTACTTCCACGCCCCCTGGCTGCAGTACGTGATCTTGATCC<br/> CGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTTCAGGCGCTTGCCTTAAGGAGCCCCCTTCGCTCGTGC<br/> TTGAGTTGAGGCTGGCTTGGGCGCTGGGGCGCGCGGTGCGAATCTGGTGGCACCTTCGCGCTGTCT<br/> CGCTGCTTTCGATAAGTCTCTAGCCATTTAAATTTTGTGACCTGCTGCGACGCTTTTTTCTGGCA<br/> AGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCGCGGGCGCGCA<br/> CGGGGCCCCGTGCGTCCCAGCGCACATGTTCCGCGAGGCGGGGCGCTGCGAGCGCGGCCACCGAGAATCGG<br/> ACGGGGGTAGTCTCAAGCTGGCCGGCTGCTCTGGTGCCTGGCTCGCGCGCCGTGATCGCCCCGCG<br/> CTGGGCGGCAAGGCTGGCCCGGTGCGCACCAAGTTGCTGAGCGGAAAGATGGCCGCTTCCCGCCCTGC<br/> TGCAGGGAGCTCAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCAACACACAAAGGAA<br/> AAGGGCCTTTCCGTCTCAGCCGTGCTTCATGTGACTCCACGGAGTACGGGGCGCGCTCCAGGCACCT<br/> CGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGAGGGGTTTTATGCGATGGAGTT<br/> TCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGAATT</p> |

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|                           | TGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCC<br>ATTTTCAGGTGTCGTGAAAAAC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 252                       | RBG polyA<br>TTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAAGCATCTGACTTCTGGCTAATAAA<br>GGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGATC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 253                       | 5' ITR<br>CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTGGGGCGACCT<br>TTGGTGCCTCGCCGCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTT<br>CCT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 254                       | 3' ITR<br>AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGGCGAC<br>CAAAGGTGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGC<br>AGG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 257                       | VP1<br>MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDNNGRLVLPGYKYLPGFNGLDKGEPVNAAD<br>AAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVESPVKT<br>APGKKRPVEPSQRSPPDSSTGIGKKGQQPAKKRLNFGQTGDSESVDPDQPIGEPPAAPSGVGPNTMAAG<br>GGAPMADNNEGADGVGSSSGNWHCDSTWLGDRIITSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYF<br>GYSTPWGYFDENRFHCHFSPRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTQNEGKTIANNLSTIQV<br>FTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFEF<br>SYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLP<br>PCYRQQRVSTTSLQNNNSNFAWTGATKYHLNGRDSLNVNPGVAMATHKDDEERFFPSSGVLMTFGKQGAGK<br>DNVDYSSVMLTSEEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGP<br>WAKIPHTDGNFHPSPMLMGFGFLKHPPQILIKNTVPADPPTTFNQAKLASFITQYSTGQVSVEIEWELQ<br>KENSKRWNPETQYTSNYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL |
| 258                       | VP2<br>MAPGKKRPVEPSQRSPPDSSTGIGKKGQQPAKKRLNFGQTGDSESVDPDQPIGEPPAAPSGVGPNTMAA<br>GGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRIITSTRTWALPTYNNHLYKQISNGTSGGSTNDNTY<br>FGYSTPWGYFDENRFHCHFSPRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTQNEGKTIANNLSTIQV<br>FTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFE<br>FSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLP<br>GPCYRQQRVSTTSLQNNNSNFAWTGATKYHLNGRDSLNVNPGVAMATHKDDEERFFPSSGVLMTFGKQGAG<br>KDNVDYSSVMLTSEEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGP<br>WAKIPHTDGNFHPSPMLMGFGFLKHPPQILIKNTVPADPPTTFNQAKLASFITQYSTGQVSVEIEWEL<br>QKENSKRWNPEIQYTSNYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL                                                                                                                                                |
| 259                       | VP3<br>MAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRIITSTRTWALPTYNNHLYKQISNGTSGGSTND<br>NTYFGYSTPWGYFDENRFHCHFSPRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTQNEGKTIANNLST<br>TIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGN<br>NFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTQSTGGTAGTQQLLFSQAGPNNMSAQAKN<br>WLPGPCYRQQRVSTTSLQNNNSNFAWTGATKYHLNGRDSLNVNPGVAMATHKDDEERFFPSSGVLMTFGKQ<br>GAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQ<br>GPIWAKIPHTDGNFHPSPMLMGFGFLKHPPQILIKNTVPADPPTTFNQAKLASFITQYSTGQVSVEIE<br>WELQKENSKRWNPEIQYTSNYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL                                                                                                                                                                                                                    |
| 260                       | VP1<br>MAADGYLPDWLEDNLSEGIREWALQPGAPKPKANQQHQDNARGLVLPGYKYLPGNGLDKGEPVNAAD<br>AAALEHDKAYDQQLKAGDNPYLYKNHADAEFQERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKT<br>APGKKRPVDQSPQEPDSSSGVGKSGKQPARKRLNFGQTGDSESVDPDQPLGEPPAAPTSLGSNTMASGG<br>GAPMADNNEGADGVGNSSGNWHCDSQWLGDRIITSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYS<br>TPWGYFDENRFHCHFSPRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTQNDGTTTTIANNLSTVQVFTD<br>SEYQLPYVLGSAHQGCLPPFPADVFMVQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNMFQFSYT<br>FEDVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQGTTSGTTNQSRLLFSQAGPQMSLSLQARNWLPGPC<br>YRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLNVNPGPAMASHKDDEEKFFPMHGNLIFGKEGTTASN<br>AELDNVMITDEEEIIRTNPVATEQYGTVANLQSSNTAPTTRTVNDQGALPGMVWQDRDVYLQGP<br>IPIHTDGHFHPSPMLMGFGFLKHPPQIMIKNTVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKE<br>NSKRWNPEIQYTSNYKSVNVDFVDTNGVYSEPRPIGTRYLTRPL      |
| miRNA21 vector components |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 261                       | miR21<br>CTGGAGGCTTGCTGAAGGCTGTATGCTGTAAGCATGGAGCTAGCAGGCTGTTTTGGCCACTGACTGACA<br>GCCTGCTCTCCATGCTTACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| 262                       | 5' ITR<br>CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCTCAGTGAGCGAGCGAGCGC<br>GCAGAGAGGGAGTG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |



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| 263 | 3' ITR<br>AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGAC<br>CAAAGGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAG<br>TGGCC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| 264 | RGB (2)<br>GATCTTTTTCCCTCTGCCAAAAATTATGGGACATCATGAAGCCCCCTTGAGCATCTGACTTCTGGCTAA<br>TAAAGGAAATTTATTTTCATTGCAATAGTGTGTGGAAATTTTTTGTGTCTCTCACTCG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 265 | CAG (2)<br>CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAAT<br>AATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACG<br>GTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGA<br>CGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTTGGCAGTACAT<br>CTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTC<br>CCCCCCTCCCCACCCCAATTTTGTATTTATTTATTTTAAATTATTTTGTGCAGCGATGGGGGCGGG<br>GGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGG<br>TGCGGCGGCGACCAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGCGGCGGCGCG<br>GCCCTATAAAAAGCGAAGCGCGCGGGGCGGGGAGTCTGCTGCGCGCTGCCTTCGCCCCGTGCCCGCTC<br>CGCCGCGCCTCGCGCGCGCCCGCCCGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGGA<br>CGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTAATGACGGCTTGTTCCTTTCTGTGGCTGCG<br>TGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGTGCCTGCGTGTG<br>TGTGTGCGTGGGGAGCGCGCGTGCCTGCGCGCTGCCGCGGCTGTGAGCGCTGCGGGCGCGCGCG<br>GGGGCTTTGTGCGCTCCGCGAGTGTGCGCGAGGGGAGCGCGCGCGGGGGCGGTGCCCGCGGTGCGGGG<br>GGCTGCGAGGGGAACAAAGGCTGCGTGCGGGTGTGTGCGTGGGGGGTGAAGAGGGGTGTGGGCGC<br>GTCGGTCGGGTGCAACCCCCCTGCACCCCCCTCCCGAGTTGCTGAGCACGGCCCGCTTCGGGTGC<br>GGGGCTCCGTACGGGGCTGGCGCGGGGCTCGCGTGCCTGCGGGCGGGGGTGGCGGAGGTGGGGGTGCC<br>GGGCGGGGCGGGGCGCCTCGGGCGGGGAGGGCTCGGGGAGGGGCGCGCGGCCCCCGAGCGCGCG<br>CGGCTGTGAGGCGCGGCGAGCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACT<br>TCCTTTGTCCCAATCTGTGCGGAGCCGAAATCTGGGAGGCGCGCGCGCACCCCCCTAGCGGGCGCGG<br>GGCGAAGCGGTGCGGCGCGCGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTGCCTGCGCGCGCG<br>TCCCCTTCTCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGACGGGCGAG<br>GGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCCTCTT<br>CTTTTTCTTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATATTTTGGCAAAGAATTCA                                                                                                                                                                                                                                                                                                                                                                       |
| 266 | ITR to ITR<br>CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCCGGGCGTCCTCAGTGAGCGAGCGAGCGC<br>GCAGAGAGGGAGTGTAGCCATGCTCTAGGAAGATCAATTCAATTACGCGTCGACATTGATTATTGACT<br>AGCTCTGGTCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATT<br>GACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGA<br>GTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGA<br>CGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTACGGGACTTTCCTACTTG<br>CGAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCT<br>CCCCCTTCCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTTAAATTATTTTGTGACGCGAT<br>GGGGGCGGGGGGGGGGGGGGGCGCGGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG<br>GCGGAGAGGTGCGGCGGCGACCAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGCG<br>GCGGCGGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTCTGCTGCGCGCTGCCTTCGCCCCGT<br>GCCCCGCTCCGCGCGCGCTCGCGCGCGCGCGCGCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAG<br>CGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTAATGACGGCTTGTTCCTTTCT<br>GTGGCTGCGTGAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGC<br>GTGCGTGTGTGTGCGTGGGGAGCGCGCGTGCCTGCGCGTCCGCGCTGCCGCGGCTGTGAGCGCTGCGG<br>GCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGCGGGGGCGGTGCCCGCG<br>GTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGTGTGTGCGTGGGGGGGTGAGCAGGGGG<br>TGTGGGCGCGTCCGTCCGGCTGCAACCCCCCTGCACCCCCCTCCCGAGTTGCTGAGCACGGCCCGGC<br>TTCGGGTGCGGGGCTCCGTACGGGGCTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGTGGCGGCAAGT<br>GGGGGTGCCGGGCGGGGCGGGGCGCCCTCGGGCGGGGAGGGCTCGGGGAGGGGCGCGGCGGCCCCG<br>GAGCGCGGGGCTGTGAGGCGCGGCGAGCGCGAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGC<br>GCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCGCGCACCCCCCTTAG<br>CGGGCGCGGGGCGAAGCGGTGCGGCGCGGCGAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTGC<br>GCGCGCGGTCCCCCTTCTCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGG<br>ACGGGCGAGGGCGGGGTTGCGCTTCTGGCGTGTGACGGCGGCTCTAGAGCCTGTGCTAACCATGTTCA<br>TGCTTCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATATTTTGGCAA<br>AGAATTCATCGATACCGTCGACGATCTAGCGTCGACCGTGGATCCTGGAGGCTTGTGAAGGCTGTAT<br>GCTGTAAGCATGGAGCTAGCAGGCTGTTTGGCCACTGACTGACAGCCTGCTCTCATGCTTACAGGAC<br>ACAAGGCCTGTACTAGCACTCACATGGAACAAATGGCCAGATCCGATCTTTTCCCTCTGCCAAAAA |

TTATGGGGACATCATGAAGCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCG  
AATAGTGTGTTGGAATTTTTGTGTCTCTCACTCGATCAGATCTGAGGAACCCCTAGTGATGGAGTTGG  
CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGACGCCCCGGCT  
TTGCCCCGGGCGGCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCCCCCCCCCCCCCCCCTG  
CATTGCGCAGATGCACCACCTGTCTCAGTGCAAAGCCCTGCCTAAGTAGGCTGGTCATAAGACCATGTGT  
CTGGCTGTAACCTCCAATTGATTGTCAGCATCAATAAACTTGGCCAACACTGTTATATACTGGTATTGA  
TAGTTACAACGAACATATTTGTTTAAAGCAATTGGAATTAAGAATTCACATGCAATGATATCAGGGTCC  
TTCTCCTCTGGTTAGTGTATTGGGGGAAATTGGACATCTCTCAGCTCAGTAGGCTAGTTAGGCCAGGA  
TGGATGACATCCACAGCCCTGGGCAGAGAGATTATGATGTAGCTAGTCTGACTCCTGACAAAGACTTG  
CTTCTGGAGCTTCTACTACTTTCTGGTGGATGGCTAAGAAATATGGTTGTGTTCTTTTAAAGTCTGAAG  
AGCATTATTTTTGCCAACCCCTGACCAAACATCCTTGCCAAGGAAAAGGCCTAAAATATATTTGCATTT  
AAAGATATTACAACTACTTGGTTTTGGAATGTTTGGCCTTTCAGGATCATAGCTATCAAATATTAGC  
TATTTGGGGTATGAGATGTCTGCTTGGTCAAGGACAAGTTCTTAAAGACATCATGTTGGGGAATAATGG  
GGAAAATGGGAAGGCTTATGCTCTGAGTAAGACATCTGAGTTATCATCTGTCAAACATTTTTGTAGTC  
ATAGTCTAATGGGAGCCTGTTTTCCCTCTTAAATATACATTACATCTGAATTTATGCTCTTCATTGAC  
AATGCCAGCCAGAACACAGCTCTTACCCTTTGGTTTTCTTCTAACCTTTAACTCCAATGTAACCAT  
TACCTGCCATTTTCAAGTAAACCATTTATCTCCCTACTTACCACCCAAAGTTGTACAATAAGAGTGT  
GCTCTCACTCATATACAAAGCAAATTCATTTGTTTGTGATGTACAGCTTGCTATGCCACAGATGTGGT  
TTGCCCTAGTCCTTTGCTCTAGGTCATTTGACTGGGAAACAGATGGGATGCTCATTGTTTTTAATGGT  
TAACTAGTCATTGAAATGCATTTTCATCAAATAATCTTAGAGGATAATTGTTTAAATGTCTGTCCAGACT  
AGCTTTGTAGAGCCAGGTGCCATTACACATGTCACCTTCTTATTTCTCTTAATTGAATTTTTATCATCT  
GAGATAGGAATAATAGAGGGCTTTTTCAAGTGAAGATATTACTATAGTCTAAAGACCTTAGTGTAACAT  
CCTGGCCCCCTAAGGAAAAACAAGTTCTGGTTCATACATATAATAACTTTGCATGTTATCTGCCACTGAG  
ATGTGTCTTAATCCAACAGAAAGGATGAATCTCTGTAGCTAGGTGTACAGGGCAAGAGCTGTACAGGG  
AACCTTTAAAGATAGCTTCAGGCCAAAGCTGAGGAAAGTGGATGGAGACTGGGAAAATGCTAAGACAT  
TTTAAAGATTTCTTTAGGTCAAAAATAGAATAAGAATAGACCATTTCCCTGGACATTTTCTGTAGGT  
TAATACTGTTAACTATTTGGTAAATGCATATGCTACAACCTTAATATGTCTGCTTTGTGAGTTTAGCATTG  
TCTCCTTGTCAATTCAGAAATGAAATGGCAAATACATTTAAATCAGAACTAAAAGGGGAACAGGGTAT  
AAAGGCTCAATTTAGTCACATCATTTCCCTTTCTCACCCACCCCTTTAAACCAGATGTTTGCCAATGC  
ATTAACAATGCAGATGTTTCTGAAAGAAAGTTTAGTAACTCAAGCAGACACCTTATTTCTTTTCAAG  
CAGAAAAGACTATGAGATGGTGGTTGTGGTTGTTCTGGGAGGGAGAGATATAAATGATACACATTATT  
TCAAATCATTTTCATGACCTCACTGCACACTTATAGTTATTGTACCTGTTGTCTTTTGTCTGTCAAGCCT  
AGCTAAGATCATTTGGAATGTTCAAGATCACTCATACATGCATGTGCACACATACACATGCACATATGT  
TCACTCCCTATTTTCATCCACATGAACCTAAGATTACATGATGTGTACAGATTCAAAGCACTTTTATCTTT  
TCCAAAGGCAAGAAGCTGAGCTACTTTCCAGAATAGTTGTGAAAGACCCTGTCTACTTCTGCATTGTT  
TCCTCCACACCACCTCCATCCAGTTCCTTATGAATGGTTACTGGTTTTCAAATATGAGATAAATTGA  
GTGTATAAAAGTCATTTTGTAGACAAAATGAAACAGGAAATGAAAGAAACCAGAACTCTCTCCTCATTTGT  
GGATGGGCCAGCTCCACCATGTCATGGTTAATCTGCAGGGAGGAAATACTAGATTTGATTGCAGATCAG  
ACTGCAGCAAACCTGCTGTGACTAAGGCATCAAGAGAAAGCAAGCAACAGCTGGGGCTTCAGTGGTGAA  
AACATTATATATCTAGCTTTGAAATATGAAATACCTTTAGCAGTGTACCTAGAAAAGAGTGTTCAA  
AATGCTGATGCTTCATAAGAACCTTTCTCTTCAGAGTTGTTTCTTTTATCTTTCAAATAGCCAGGGTG  
GGAAATAAAGTGATCACTTGGTGAAGAAATCTCACAAAGAAGACATAGAGAGTTCATTTTCATCTGGA  
GTAATGAACAGATTGAACAACTAGAAATGGTTAGTCTGTTAAAGAAAAGGTGTAGGTGAGCTGTTTGC  
AAGAGCCACAAGGGAAAGGGGAAGACAACCTTTTGTGGACTTAAGGGTGAAAGTTGCAAGCAGGCAAG  
ACCATTCTGACCTCCATTAAGAAAGCCCTTTCCAACCAACAACTGGGTTGGTTACTCAGGTTGGGC  
AGCATTGGGAGCAAATGTTGATTGAACAAATGTTTGTGAGAATGTTGACTTAAGAGCTGTTCTGTCA  
CTGGGGACAGCAGCAGCTAGATAGCCCCATTGAGGGAGAGGGCATTGTTACCTGGCCAGAGATCAGA  
GCAGGCTAAGGGACTGCTGGGATCCTGTCCAGCTTTGAGACCTACAGAGCCATGTTACCTAGCAGGT  
ATCCCTTCTGAGGTCACTCTCATTTCTTACCTTATTCAGGGCTTTACCTCAGCTTGCCAGGCTGGAG  
CCAAGGGCCAAGGCAGCCTCACCTTGTGGCTATGGTAGCTTCCCAGGAGCCCCCTATGTTTCAAGAAC  
AGCTCTGCCCTGCCCCATCCTGTTTGTACCTCTTAAAGCCAAAGGCACTGGTGGGCAAGGCCAGCTTCT  
AAAGTACACAAGGTTAGAGGTTCTGACAGGAAGGGCTTGAGGCCAATGGAAGGAGGTACTTCAGTT  
TCCCTCCAGATGCCAGTGATGGGCTCAGAGCTCCTTGAGAATTTGGGAAAGGAAGCAGGGTCTCTGAA  
GAAATACTTCAGGAGTAGAAAGAGGAAGCTAGAGGGTTAAATGCACTACACAGGAACAGAAATGAGTTT  
TTCTTAGAGTTAGTATATGTCTAGAGGTGTAGTAACTAAAACAAGTCTTGAATTGCATACAGCCACTT  
AGGGAAGAAATGAAAACCTTTGAATATTAGTGAAGAAAGGGAACTGCAACCCCTGTATTACTAGATAG  
CTTTTCATCAACAGCTCAAAACAGACAGATTTTTATAGGTTTACTGTGTGCACTTTAATAAGGGCAGT  
GGTTCAGAACTAGTCAGGTCTGAAAAGGATTTACCAAATGTTGAGTGTGCCCTCTAGTGTTCACACTT  
CCCAGCTTTCTTCTATAAAGGTGGATCAAGGCACCTTGCTTACAACCTGGAAGTGAATCCTCCAAGTGG  
AACTAGACATTGAGATGGAGAAAATATTATTGTCCACTGTAATTATGCAAGGAATATCCAGTTGAGAT  
AATGGACTTGCTCTTATCTAATAATACCCAGGCTCAATGGGTCACTGCTTTGTCCACTTTGCCAAAA  
TTCAAGCACAGCTAAGTTGATATTTTAGGACAAAGGCAGCTTACTATCCAGCCAGAGGGAGTAGAATA  
TGTTTAAAGAGAGGTGGAAGAATGAATGAGCCCTGCTATTCCTCACTGCCTGGATGGCTATAAGCACA  
GCCCTTATGGAGGCCTTAGGTCTTGCTTCATAATATTCAGTTTGAAGAGGGTTGAAAAGACCTCCTA

|  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | GAAAAATCAGTAGTTTTCTCTTTTGTAGTAACATGTAGCAAAAAAATTTTCATCATGTAGGTACAGGGA<br>ACACCCTAATAACTATTAATCTCAAGGAGTCAAGCCAGTGTGTTTCTAATGTATCTGCTGTATCCCCA<br>TGAAGCAAATTTTGCCATCAGAGAACTGACTCATGGGGAAAAAATCCAAGGACCTCAAATCACCAGAA<br>GAAGCCATTCCCTCAGATTTGCCTAAGCTTAAGCTTCCCTGTCTCTCATTGTGTGTTGCTTTCAATGCAG<br>TTACATAAATGGCTTTTTTGTATTATGCACCAAAAACTAATTTCATCTGCAAAGCTATAGGTCAAAGCA<br>ACCATAGTATGCACCTGCTAGCTGGCGCATTAAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGACC<br>GCTACACTTGCCAGCGCCTTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCTTCTCGCCACGTTTCGCC<br>GGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTC<br>GACCCCAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGC<br>CCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACTCT<br>ATCTCGGGCTATTCTTTGATTTAGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTGTTTTACAACG<br>TCGTGACTGGGAAAACCCCTGGCGTTACCCAACCTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTG<br>GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGCGA<br>TTTATTCAACAAAGCCCGCTCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGTTACAACCAATTAACCA<br>ATTCTGATTAGAAAACTCATCGAGCATCAAATGAACTGCAATTTATTTCATATCAGGATTATCAATAC<br>CATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAA<br>GATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCTCGTCAA<br>AAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGCTTAT<br>GCATTTCTTTCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCA<br>AACCGTTATTCAATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAGGACAATTAC<br>AAACAGGAATCGAATGCAACCGCGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAG<br>GATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAG<br>GAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCGGTCAGCCAGTTTAGTCTGACCATCT<br>CATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCGGCGCATCGGGCTTCC<br>CATACAATCGATAGATTGTCGCACCTGATTGCCCCGACATTATCGCGAGGCCATTTATACCCATATAAAT<br>CAGCATCCATGTTGGAATTTAATCGCGGCTTCGAGCAAGACGTTTCCCGTTGAATATGGCTCATAACAC<br>CCCTTGATATCTGTTTATGTAAGCAGACAGTTTTATTTGTTTCATGATGATATATTTTTATCTTGTCAA<br>TGTAACATCAGAGATTTTGAGACACAACGTGGCTTTGTTGAATAAATCGAACTTTTGCTGAGTTGAAGG<br>ATCAGATCAGCATCTTCCGACAACGACGACCGTTCCGTGGCAAAGCAAAGTTCAAATCACCACCT<br>GGTCCACCTACAACAAAGCTCTCATCAACCGTGGCTCCCTCACTTTCTGGCTGGATGATGGGGCGATT<br>AGGCCTGGTATGAGTCAGCAACACCTTCTTCACGAGGCAGACCTCTCGACGGAGTTCCACTGAGCGTCA<br>GACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTATCTGCTGCTTGCAA<br>ACAAAAAACCAACCGCTACCGAGCGGTGGTTGTTTGGCGGATCAAGAGCTACCAACTCTTTTCCGAAG<br>GTAACCTGGCTTCAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCAC<br>TTCAAGAACTCTGTAGCACCGCTACATACCTCGCTGCTAATCCTGTTACCACTGGCTGCTGCCAGT<br>GGCGATAAGTCGTGCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGC<br>TGAACGGGGGGTTCTGTCACACAGCCAGCTTGAGCGAACGACCTACACCGAACTGAGATACCTACAG<br>CGTGAGCTATGAGAAAGCGCCACGCTTCCGAAAGGAGAAAGGCGACAGGTATCCGGTAAGCGGCAGG<br>GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCGCTGGTATCTTTATAGTCCTGTGCGG<br>TTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAAC<br>GCCAGCAACGCGGCCTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACTTAATTAAGGCCTTA<br>ATTAGG |
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[00328] Table 4 provides a summary of the correspondence between guide RNA, passenger RNA, pri-amiRNA, pre-amiRNA, and target sequence: and corresponding/encoding DNA guide, passenger and pri-amiRNA.

Table 4

|      | Guide RNA | Passenger RNA        | Pri-ami RNA                                     | Pre-amiRNA           | Target sequence | Guide DNA | Passenger DNA            | DNA for pri-ami-RNA      |
|------|-----------|----------------------|-------------------------------------------------|----------------------|-----------------|-----------|--------------------------|--------------------------|
| miR1 | 1         | 20<br>20<br>21<br>22 | 51 (S155)<br>52 (S155e)<br>53 (S26)<br>54 (S33) | 88<br>88<br>89<br>90 | 119             | 138       | 157<br>157<br>158<br>159 | 188<br>189<br>190<br>191 |
| miR2 | 2         | 23<br>23<br>24<br>25 | 55 (S155)<br>56 (S155e)<br>57 (S26)<br>58 (S33) | 91<br>91<br>92<br>93 | 120             | 139       | 160<br>160<br>161<br>162 | 192<br>193<br>194<br>195 |
| miR3 | 3         | 26<br>26             | 59 (S155)<br>60 (S155E)                         | 94<br>94             | 121             | 140       | 163<br>163               | 196<br>197               |

|       | Guide RNA | Passenger RNA        | Pri-ami RNA                                     | Pre-amiRNA               | Target sequence | Guide DNA | Passenger DNA            | DNA for pri-ami-RNA                                                                                         |
|-------|-----------|----------------------|-------------------------------------------------|--------------------------|-----------------|-----------|--------------------------|-------------------------------------------------------------------------------------------------------------|
|       |           | 27<br>28             | 61 (S26)<br>62 (S33)                            | 95<br>96                 |                 |           | 164<br>165               | 198<br>199                                                                                                  |
| miR4  | 4         | 29                   | 63                                              | 97                       | 122             | 141       | 166                      | 200                                                                                                         |
| miR5  | 5         | 30                   | 64                                              | 98                       | 123             | 142       | 167                      | 201                                                                                                         |
| miR6  | 6         | 31<br>31<br>32<br>33 | 65 (S155)<br>66 (S155e)<br>67 (S26)<br>68 (S33) | 99<br>99<br>100<br>101   | 124             | 143       | 168<br>168<br>169<br>170 | 202<br>203<br>204<br>205                                                                                    |
| miR7  | 7         | 34                   | 69                                              | 102                      | 125             | 144       | 171                      | 206                                                                                                         |
| miR8  | 8         | 35                   | 70                                              | 103                      | 126             | 145       | 172                      | 207                                                                                                         |
| miR9  | 9         | 36                   | 71                                              | 104                      | 127             | 146       | 173                      | 208                                                                                                         |
| miR10 | 10        | 37                   | 72                                              | 105                      | 128             | 147       | 174                      | 209                                                                                                         |
| miR11 | 11        | 38                   | 73                                              | 106                      | 129             | 148       | 175                      | 210                                                                                                         |
| miR12 | 12        | 39                   | 74                                              | 107                      | 130             | 149       | 176                      | 211                                                                                                         |
| miR13 | 13        | 40                   | 75                                              | 108                      | 131             | 150       | 177                      | 212                                                                                                         |
| miR14 | 14        | 41                   | 76                                              | 109                      | 132             | 151       | 178                      | 213                                                                                                         |
| miR15 | 15        | 42                   | 77                                              | 110                      | 133             | 152       | 179                      | 214                                                                                                         |
| miR16 | 16        | 43<br>43<br>44<br>45 | 78 (S155)<br>79 (S155e)<br>80 (S26)<br>81 (S33) | 111<br>111<br>112<br>113 | 134             | 153       | 180<br>180<br>181<br>182 | 215<br>216<br>217<br>218                                                                                    |
| miR17 | 17        | 46                   | 82 (S451)                                       | 114                      | 135             | 154       | 183                      | 219                                                                                                         |
| miR19 | 19        | 47                   | 83 (S155)                                       | 115                      | 136             | 155       | 184                      | 220                                                                                                         |
| miR18 | 18        | 48<br>49<br>49<br>50 | 84 (S155)<br>85 (S155e)<br>86 (S26)<br>87 (S33) | 116<br>116<br>117<br>118 | 137             | 156       | 185<br>185<br>186<br>187 | 221<br>222<br>223<br>224<br>225 (S451)<br>226 (S186)<br>227 (S122)<br>228 (S10)<br>229 (S16)<br>230 (Slet7) |

[00329] The scaffold designations in Table 4 are provided for pri-amiRNA, and apply for the pri-miRNA corresponding/encoding DNA.

### EXAMPLES

[00330] Examples are provided below further illustrating different features of the present invention and methodology for practicing the invention. The provided examples do not limit the claimed invention.

#### [00331] Example 1: miRNA Screen for HTT Protein Knockdown

[00332] The ability of different miRNA guide sequences to inhibit HTT expressions was evaluated using plasmids comprising rAAV nucleic acid encoding different pri-amiRNA constructs. Pri-amiRNA constructs were designed by embedding different guide sequences and passenger sequences into an S155 scaffold. Passenger sequences were designed taking the

complement of the guide and removing 2 base pairs at positions 9 and 10 to create a central bulge. The S155 scaffold provides a 27 base 5' flanking region and 41 base 3' flanking region.

**[00333]** FIG. 2A illustrates the location of different components for a rAAV nucleic acid encoding pri-miRNA: 5'-ITR, CAG promoter, pri-miRNA based on the S155 scaffold, polyadenylation signal (pA) and 3' ITR.

**[00334]** Recombinant AAV nucleic acids encoding different pri-miRNAs were cloned into a plasmid to obtain a miRNA expressing plasmid. The rAAV included AAV2 ITRs, rabbit beta-globin (RBG) polyA, and a CAG promoter. Encoded pri-miRNA were inserted downstream of the promoter. Table 5 provides the (a) construct designation indicating the produced rAAV nucleic acid is single-stranded ("ss"), the promoter, the scaffold, and miRNA designation; and (b) the targeting sequence; and (c) the DNA encoding the pri-miRNA for the different constructs.

Table 5

| rAAV nucleic acid designation | Targeting SEQ ID NO: | DNA encoding Pri-miRNA SEQ ID NO: |
|-------------------------------|----------------------|-----------------------------------|
| ss.CAG.S155.miR1              | 1                    | 188                               |
| ss.CAG.S155.miR2              | 2                    | 192                               |
| ss.CAG.S155.miR3              | 3                    | 196                               |
| ss.CAG.S155.miR4              | 4                    | 200                               |
| ss.CAG.S155.miR5              | 5                    | 201                               |
| ss.CAG.S155.miR6              | 6                    | 202                               |
| ss.CAG.S155.miR7              | 7                    | 206                               |
| ss.CAG.S155.miR8              | 8                    | 207                               |
| ss.CAG.S155.miR9              | 9                    | 208                               |
| ss.CAG.S155.miR10             | 10                   | 209                               |
| ss.CAG.S155.miR11             | 11                   | 210                               |
| ss.CAG.S155.miR12             | 12                   | 211                               |
| ss.CAG.S155.miR13             | 13                   | 212                               |
| ss.CAG.S155.miR14             | 14                   | 213                               |
| ss.CAG.S155.miR15             | 15                   | 214                               |
| ss.CAG.S155.miR16             | 16                   | 215                               |
| ss.CAG.S155.miR17             | 17                   | 219                               |
| ss.CAG.S155.miR18             | 19                   | 221                               |
| ss.CAG.S155.miR19             | -                    | 231                               |

| rAAV nucleic acid designation | Targeting SEQ ID NO: | DNA encoding Pri-amiRNA SEQ ID NO: |
|-------------------------------|----------------------|------------------------------------|
| ss.CAG.S155.miR20             | -                    | 232                                |

**[00335]** Plasmids were transfected into HEK293 cells, and cell lysates were collected 72 hours post transfection. FIG. 2B illustrates HTT protein content in lysates quantified using the Bicinchoninic acid assay (BCA) method and normalized. Protein quantification was carried out using a custom WES capillary electrophoresis assay with an antibody (Millipore mab2166-clone 1HU-4C8) that recognizes total human HTT protein. Data was normalized to scrambled control. N = 3 experiments. One way ANOVA with comparisons to control (Dunnett's test).

**[00336]** Several constructs summarized in Table 5 were further evaluated by transfecting HEK293 cells with miRNA expressing plasmids and measuring HTT mRNA levels. FIG. 2C illustrates HTT total mRNA levels from HEK293 cells transfected with different miRNA expressing plasmids. RNA was extracted at 72 hours post transfection from cells transfected with 1 µg of plasmid and analyzed using TaqMan RT-qPCR primer/probes. HTT mRNA levels were plotted relative to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and normalized to scrambled control. N = 3-6 transfections. ss.CAG.S155.miR18 had the highest level of HTT mRNA reduction.

**[00337]** Example 2: Screening in YAC128 HD mouse model

**[00338]** Recombinant AAV encoding different miRNAs were produced using triple transfection and tested in a YAC128 HD mouse model S155.miR1, S155.miR2, S155.miR16, and S155.miR18 constructs were encapsidated into AAV particles and delivered at a dose of 1e10 vector genomes total per mouse bilaterally into the striatum using a stereotactic injection device. Mice were 6 weeks old at injection and incubated two-month post injection. The AAV particles comprised VP1 SEQ ID NO: 257, VP2 SEQ ID NO: 258, and VP 3 SEQ ID NO: 259. The different constructs are summarized in Table 6.

Table 6.

| nucleic acid designation | Targeting Sequence SEQ ID NO: | DNA encoding Pri-amiRNA SEQ ID NO: | rAAV SEQ ID NO: |
|--------------------------|-------------------------------|------------------------------------|-----------------|
| rAAV.ss.CAG.S155.miR1    | 1                             | 188                                | 233             |
| rAAV.ss.CAG.S155.miR2    | 2                             | 192                                | 234             |
| rAAV.ss.CAG.S155.miR16   | 16                            | 215                                | 235             |
| rAAV.ss.CAG.S155.miR18   | 18                            | 221                                | 236             |

**[00339]** Two months post injection, copy numbers of the injected vector were assessed by extracting DNA from the mouse striatal tissue and performing qPCR using primers targeting the vector DNA. Total DNA was extracted from freshly frozen mouse striatum tissue and measured using TaqMan qPCR primer/probes against the transgene polyA sequence. N = 6 mice per group. No significant differences between any of the miRNA vector injected groups were observed (Kruskal-Wallis non-parametric ANOVA + Dunnett's Test). The results are shown in FIG. 3A.

**[00340]** The miRNA abundance from mouse striatum injected with rAAV vector encoding different miRNA constructs is shown in FIG. 3B. Total RNA was extracted from freshly frozen mouse striatum tissue and miRNA levels were assessed using custom TaqMan MicroRNA Reverse Transcription Kit T-qPCR. N = 5-6 mice per group. rAAV.ss.CAG.S155.miR18 had the highest miRNA abundance when compared to other vectorized miRNA injected groups (Kruskal-Wallis non-parametric ANOVA + Dunnett's Test). miR16 had significantly lower miRNA levels than the other groups, whereas miR18, miR1 and miR2 had similar levels of miRNA in the striatum.

**[00341]** Mutant HTT protein was evaluated from striatal lysates using WES capillary electrophoresis with an antibody specific for the polyQ mutation (Millipore, MABN2427). All tested vectors were able to significantly reduce HTT mutant protein, with miR18 exhibiting the most reduction, ~40% of the dilutant control (FIG. 3C).

**[00342]** Total HTT mRNA levels were measured in mice injected with dilutant or r.AAV.ss.CAG.S155.miR18 vector at  $1 \times 10^{10}$  vg/mouse or  $3.4 \times 10^{10}$  vg/mouse. HTT mRNA levels were assessed RT-qPCR (FIG. 3D). N = 5-12 mice per group. HTT mRNA levels as assessed by RT-qPCR were reduced by about 30.1% at the low dose and about 61.4% at the high dose.

**[00343]** Mutant HTT protein was assessed in the striatum of mice injected with dilutant or ss.CAG.S155.miR18 vector at either  $1 \times 10^{10}$  vg/mouse or  $3.4 \times 10^{10}$  vg/mouse. Protein was detected via WES immunoassay using poly-Q specific antibody MW1 and normalized to dilutant control. N = 5-12 per group. Mutant HTT protein levels as assessed by WES capillary electrophoresis were reduced by 40.3% and 65.5% at the low and high doses test (FIG. 3E).

**[00344]** Example 3: In vitro Screening of miRNA Scaffolds

**[00345]** Guide and passenger sequences from miR18 were embedded into 10 different miRNA scaffolds: Scr6 (scramble control), S451 (miR451 scaffold), S122 (miR122 scaffold), S155e (eSIBR scaffold), S26, S33 (miR33 scaffold), S186 (miR186 scaffold), S10, S16 (miR-16 scaffold), S155 (miR155 scaffold), Slet7. The S451, S122, S33, S186, SR16 and S155 scaffolds were generated from endogenous human or mouse miRNA sequences, by replacing the endogenous guide/passenger sequences with artificial guide and passenger sequences. The

“S155e” backbone is a modified miR155 backbone described by Fang and Bartel Genes. Mol Cell. (2015) 60(1):131-1452015. The S26, S10 and Slet7 scaffolds are artificial scaffolds produced through modification of miR26, miR10, and Slet7. The artificial passenger sequences were manipulated to maintain endogenous miRNA secondary structure by generating mismatches to the guide.

**[00346]** Nucleic acid encoding for scaffolds with embedded guide and passenger sequences, along with flanking sequences on the 5' and 3' ends, were used to produce miRNA expressing plasmids designed according to Example 1 (CAG promoter) and transfected into HEK293 cells as described in Example 1. Constructs are summarized in Table 7.

Table 7

| rAAV nucleic acid designation | Targeting SEQ ID NO: | DNA encoding Pri-miRNA SEQ ID NO: |
|-------------------------------|----------------------|-----------------------------------|
| ss.CAG.S155.miR18             | 18                   | 221                               |
| ss.CAG.S155e.miR18            | 18                   | 222                               |
| ss.CAG.S451.miR18             | 18                   | 225                               |
| ss.CAG.S26.miR18              | 18                   | 223                               |
| ss.CAG.S33.miR18              | 18                   | 224                               |
| ss.CAG.S122.miR18             | 18                   | 227                               |
| ss.CAG.S186.miR18             | 18                   | 226                               |
| ss.CAG.SR10.miR18             | 18                   | 228                               |
| ss.CAG.S16.miR18              | 18                   | 229                               |
| ss.CAG.Slet7.miR18            | 18                   | 230                               |

**[00347]** FIG. 5A illustrates miRNA guide levels as measured by RT-qPCR, from cells transfected with indicated constructs. Data was normalized to miR18 expressed from the S155 scaffold. N = 3-6 transfections. S26a and S33 scaffolds showed significantly higher miRNA levels (~2.5 fold and 3.5 fold, respectively) than the S155 scaffold. The S155e scaffold also trended towards higher miRNA levels (~2 fold) than the original S155 scaffold.

**[00348]** FIG. 5B illustrates HTT mRNA levels as measured by RT-qPCR from cells transfected with the indicated constructs. Data was normalized to scrambled control. N = 3-6 transfections. S155e, S26a and S33 scaffolds exhibited the most potent knock down of HTT mRNA, with reductions of ~60%.

**[00349]** FIG. 5C illustrates protein levels as measured by WES capillary electrophoresis using mab2166 from cells transfected with the indicated constructs. Data was normalized to scrambled control. N = 3-6 transfections. 155e, S26a and S33 scaffolds trended toward having the greatest



knock down of HTT protein, with levels of ~40% compared to scrambled control. The results indicate the miRNA scaffold in which miRNA guide and passenger sequences are embedded can influence potency.

**[00350]** Example 4: Assessment of miR18 Processing

**[00351]** To further characterize the influence of the miRNA scaffold on miRNA processing, small RNA sequencing was carried out on HEK293 cells transfected with an miR18 guide embedded in the S155, S155e, S451, S26a or S33 scaffolds as described in Table 7. Sequencing was performed using Illumina TruSeq libraries with single end 150 base pair reads. Read counts were normalized to reads per million, with ~2 million total reads per sample. Scr6 group expresses a scrambled control whereas other groups express miR18. N = 3 samples/group.

**[00352]** FIG. 6A illustrates read counts for guide and passenger strands. miR18 guide levels were ~2-3 fold higher when expressed from the S26a or S33 scaffolds as compared to the S155 scaffold. The S26a scaffold showed the lowest amount of passenger strand expression.

**[00353]** FIG. 6B illustrates the guide to passenger sequence ratio of miRNA constructs. N = 3 samples/group. S26, S33 and S451 scaffolds all produced higher guide to passenger ratios than the S155 scaffold.

**[00354]** FIG. 6C illustrates read pileups along the miRNA transgene in cells transfected with the indicated constructs. Analysis of the read pileups showed that S26a and S33 scaffolds had the most precise processing of guide miRNA, with almost non-detectable levels of passenger strand. Since passenger strand activity could lead to dysregulation of off-target genes, the low amounts of passenger produced by these scaffolds indicates an improvement in potential safety and efficacy of these constructs.

**[00355]** Example 5: In Vitro Testing of Different miRNA and Scaffold Combinations

**[00356]** miR1, miR2, miR16 and miR18 guide and passenger sequences were embedded into the S155, S155e, S26a or S33 scaffolds. miRNA encoding nucleic acid were cloned into plasmid constructs described in Example 1. The constructs contained a CAG promoter, and are summarized in Table 8. Plasmids were transfected into HEK293 cells, miRNA expression and HTT protein levels were measured.

Table 8

| Designation | Targeting<br>SEQ ID NO: | DNA encoding Pri-<br>amiRNA<br>SEQ ID NO: |
|-------------|-------------------------|-------------------------------------------|
| miR1.S155   | 1                       | 188                                       |
| miR1.S155e  | 1                       | 189                                       |
| miR1.S26    | 1                       | 190                                       |

|             |    |     |
|-------------|----|-----|
| miR1.S33    | 1  | 191 |
| miR2.S155   | 2  | 192 |
| miR2.S155e  | 2  | 193 |
| miR2.S26    | 2  | 194 |
| miR2.S33    | 2  | 195 |
| miR16.S155  | 16 | 215 |
| miR16.S155e | 16 | 216 |
| miR16.S26   | 16 | 217 |
| miR16.S33   | 16 | 218 |
| miR18.S155  | 18 | 221 |
| miR18.S155e | 18 | 222 |
| miR18.S26   | 18 | 223 |
| miR18.S33   | 18 | 224 |

**[00357]** FIG. 7A and FIG. 7B, illustrate the impact of the different combinations of miRNA guide, passenger sequence, and scaffold on processing efficiency and potency of target knockdown. FIG. 7A illustrates miRNA expression from HEK293 cells transfected with indicated constructs and analyzed at 48 hours post transfection. Levels of miRNA were detected by RT-qPCR. N = 2-3 transfections per group. Indicated groups are significantly different from each other (1 Way ANOVA + Bonferroni test). miRNA expression levels varied depending upon the particular miRNA sequence and scaffold. For example, the S33 scaffold produced higher levels of miR1, miR2 and miR18 as compared to the S155 scaffold; and the S26a scaffold produced higher levels of miR1 and miR2 compared to the S155 scaffold.

**[00358]** FIG. 7B illustrates HTT protein levels as measured by WES capillary electrophoresis using mab2166 from cells transfected with indicated constructs at 72 hours post transfection. Data was plotted relative to loading control protein vinculin (a cytoskeletal protein) and then normalized to scrambled control. N = 6 transfections, 2 experiments. Indicated groups are significantly different from each other (1 way ANOVA + Dunnett's test). For example, S33 scaffold induced greater knockdown in the miR2 and miR16 groups, compared to the S155 scaffold. Knockdown of HTT protein levels depended upon the particular miRNA sequence and scaffold.

**[00359]** Example 6: EF-1 $\alpha$  Promoter

**[00360]** When generating AAV vectors for *in vivo* testing of the CAG promoter-miRNA constructs, heterogeneity of the AAV genome was observed both by cesium chloride gradient and capillary electrophoresis of the extracted AAV genomic DNA. New constructs were

designed incorporating ~2.5kb of the human EEF1A1 gene (abbreviated EF-1 $\alpha$ , SEQ ID NO: 251) promoter in place of the CAG promoter construct (Example 1 plasmid). The use of EF-1 $\alpha$  promoter increased the rAAV nucleic acid insert from 2.2kb to 3.1kb and lowered the GC content of the promoter. The EF-1 $\alpha$  constructs are summarized in Table 9.

Table 9

| Designation              | Targeting<br>SEQ ID NO: | DNA encoding Pri-<br>amiRNA<br>SEQ ID NO: |
|--------------------------|-------------------------|-------------------------------------------|
| EF1 $\alpha$ .miR1.S155  | 1                       | 188                                       |
| EF1 $\alpha$ .miR1.S26   | 1                       | 190                                       |
| EF1 $\alpha$ .miR1.S33   | 1                       | 191                                       |
| EF1 $\alpha$ .miR2.S155  | 2                       | 192                                       |
| EF1 $\alpha$ .miR2.S26   | 2                       | 194                                       |
| EF1 $\alpha$ .miR2.S33   | 2                       | 195                                       |
| EF1 $\alpha$ .miR3.S155  | 3                       | 196                                       |
| EF1 $\alpha$ .miR3.S26   | 3                       | 198                                       |
| EF1 $\alpha$ .miR3.S33   | 3                       | 199                                       |
| EF1 $\alpha$ .miR16.S155 | 16                      | 215                                       |
| EF1 $\alpha$ .miR16.S26  | 16                      | 217                                       |
| EF1 $\alpha$ .miR16.S33  | 16                      | 218                                       |
| CAG.miR18.S155           | 18                      | 221                                       |
| EF1 $\alpha$ .miR18.S155 | 18                      | 221                                       |
| EF1 $\alpha$ .miR18.S26  | 18                      | 223                                       |
| EF1 $\alpha$ .miR18.S33  | 18                      | 224                                       |

**[00361]** FIG. 8A illustrates miRNA expression from HEK293 cells transfected with CAG and EF-1 $\alpha$  promoter plasmids expressing miR18 from the S155 scaffold. miRNA was assessed by RT-qPCR. Reference to mm11 indicates a miRNA having a scrambled guide sequence, intended not to target a gene. Data were normalized to intracellular plasmid DNA content to account for differences in transfection efficiency. N = 6 transfections per group. The EF-1 $\alpha$  promoter construct produced similar levels of miR18 as the CAG promoter construct (1 Way ANOVA + Tukey post-hoc test).

**[00362]** FIG. 8B illustrates miRNA expression from HEK293 cells transfected with different miRNA guide sequences embedded in either the S155, 26a or 33 scaffolds and cloned into a plasmid with the 2.5kb EF-1 $\alpha$  promoter; miR18 in a S155 scaffold with the CAG promoter; and a mm11 in a CAG promoter. miRNA was assessed by RT-qPCR. N = 2-3 transfections per

group. Indicated groups are significantly different from each other (Welch non-parametric ANOVA + Dunnett's test). The EF-1 $\alpha$  construct produced similar levels of miR18 as the CAG promoter construct. miR18 embedded in the S26a or S33 scaffold showed ~2.7 fold higher miRNA abundance than the S155 scaffold. Similarly, miR1 showed ~4.6 fold increase in miRNA levels when embedded in either the S26a or S33 scaffold. As illustrated in FIG. 8B, miRNA expression levels were also impacted by choice of guide sequence.

**[00363]** FIG. 8C illustrates HTT mRNA levels as measured by RT-qPCR from cells transfected with indicated constructs. Data was normalized to mismatched control. N = 6 transfections, 2 experiments. All miRNA plasmid transfected groups show highly significant ( $p < 0.0001$ ) HTT downregulation (1 way ANOVA + Dunnett's test). miR18 embedded in the S33 scaffold showed the highest level of knockdown, with HTT mRNA levels reduced by ~65%. This is around the theoretical maximum knockdown, given that transfection efficiency was ~70% for these experiments. miR2 and miR3 also trended towards more potency when embedded in the S26a or S33 scaffolds but the high level of knockdown in all samples suggests that the *in vitro* system was saturated, thus making comparisons difficult. The miRNA levels of miR16 were not improved by incorporation into the S26a and S33 scaffolds (FIG. 8C), and small RNA next generation sequencing (NGS) confirmed that miR16 is not processed efficiently in any of the tested scaffolds (data not shown).

**[00364]** The miR16 constructs were modified to optimize the pyrimidine content. miR17 provides a SEQ ID NO: 17 guide in an S451 scaffold (pri-miRNA of SEQ ID NO: 82), where the resulting modification increased the loop pyrimidine content. miR19 provides a SEQ ID NO: 19 guide in the S155 scaffold, along with a corresponding modification to the passenger sequence providing GG, thereby increasing the GC content (pri-miRNA of SEQ ID NO: 83). FIG. 8D illustrates HTT mRNA levels as measured by RT-qPCR from cells transfected with the constructs. N = 2 transfections per group.

**[00365]** Example 7: miR18 and miR2 Construct Testing in YAC Mouse Model

**[00366]** Recombinant AAV constructs comprising an EF-1 $\alpha$  promoter operably linked to different DNA encoding pri-miRNAs (Table 10) were further tested *in vivo* using the YAC128 mouse model. Mice were bilaterally injected with rAAV viral particles (prepared by standard triple transfection method) into the striatum at a dose of 5e9 vg per hemisphere (1e10 vg/mouse) and incubated for 2 months post injection. Small RNA sequencing on striatum of injected mice were performed to characterize the miRNA processing. Sequencing was carried out using Illumina TruSeq libraries with single end 150 base pair reads and ~3-5 million reads per sample were obtained.

Table 10

| rAAV nucleic acid designation | Embedded Guide SEQ ID NO: | DNA encoding Pri-miRNA SEQ ID NO: | rAAV nucleic acid SEQ ID NO: |
|-------------------------------|---------------------------|-----------------------------------|------------------------------|
| rAAV.EF1 $\alpha$ .S155.miR2  | 2                         | 192                               | 237                          |
| rAAV.EF1 $\alpha$ .S26.miR2   | 2                         | 194                               | 238                          |
| rAAV.EF1 $\alpha$ .S33.miR2   | 2                         | 195                               | 239                          |
| rAAV.EF1 $\alpha$ .S155.miR18 | 18                        | 221                               | 240                          |
| rAAV.EF1 $\alpha$ .S26.miR18  | 18                        | 223                               | 241                          |
| rAAV.EF1 $\alpha$ .S33.miR18  | 18                        | 224                               | 242                          |

**[00367]** FIG. 9A illustrates guide and passenger read counts from YAC128 mice injected with vectorized miRNA/scaffold constructs as determined by small RNA sequencing using the Illumina TruSeq platform. RNA was extracted from the striatum, N = 3 mice per group were sequenced. Constructs containing the S26a or S33 scaffolds produced higher guide levels and lower passenger levels than those with the S155 scaffold. miR18 overall produced higher levels of miRNA than miR2. The S26a scaffold produced a modest decrease in passenger strand levels for miR18, but decreased passenger levels by 93 fold in the miR2 construct.

**[00368]** FIG. 9B illustrates guide to passenger ratios as determined by small RNA sequencing. RNA was extracted from the striatum, N = 3 mice per group were sequenced. Guide sequences matching at least 19 out of the expected 21 bp were considered in this analysis. The relative amount of guide to passenger, can be used as an indicator of miRNA processing efficiency (FIG. 9B). The S26a scaffold increased the guide to passenger ratio by 2.6 fold for miR18 and ~293 fold for miR2. The S33 scaffold increased the guide to passenger ratio by 3.3 fold for miR18 and 23.6 fold for miR2.

**[00369]** Table 11 provides guide and passenger read counts from YAC128 mice injected with vectorized miRNA/scaffold constructs as determined by small RNA sequencing using the Illumina TruSeq platform. RNA was extracted from the striatum, N = 3 mice per group were sequenced. miR18 in a S155 scaffold produced the largest heterogenous mix of guides with extra bases on the 3' end.

**[00370]** The results are provided in Table 11. For miR2, the expected guide from the algorithm (21 bases) was produced <1% of the time in the S155 scaffold construct, whereas the S26a and S33 scaffolds produced >94% of guides matching the expected sequence. The results provide sequenced DNA (labeled as guide in Table 11), produced from the RNA guide.

Table 11

| Expected Guide                                                               | Most Abundant Guides                                         | % of Guide Reads |
|------------------------------------------------------------------------------|--------------------------------------------------------------|------------------|
| EF1 $\alpha$ .S155.miR18<br><b>TAGCGTTGAAGTACTGTCCCC</b><br>(SEQ ID NO: 156) | TAGCGTTGAAGTACTGTCCCCG <u>T</u><br>(SEQ ID NO: 243)          | 52.70            |
|                                                                              | TAGCGTTGAAGTACTGTCCCCG <u>G</u><br>(SEQ ID NO: 244)          | 28.37            |
|                                                                              | TAGCGTTGAAGTACTGTCCCCG <u>A</u><br>(SEQ ID NO: 245)          | 9.65             |
|                                                                              | TAGCGTTGAAGTACTGTCCCCG <u>T</u> <u>A</u><br>(SEQ ID NO: 246) | 3.86             |
|                                                                              | <b>TAGCGTTGAAGTACTGTCCCC</b><br>(SEQ ID NO: 156)             | 0.90             |
| EF1 $\alpha$ .S26.miR18<br><b>TAGCGTTGAAGTACTGTCCCC</b><br>(SEQ ID NO: 156)  | <b>TAGCGTTGAAGTACTGTCCCC</b><br>(SEQ ID NO: 156)             | <b>94.67</b>     |
|                                                                              | TAGCGTTGAAGTACTGTCCCCG <u>G</u><br>(SEQ ID NO: 243)          | 1.12             |
|                                                                              | TAGCGTTGAAGTACTGTCCCC <u>T</u><br>(SEQ ID NO: 247)           | 1.11             |
| EF1 $\alpha$ .S33.miR18<br><b>TAGCGTTGAAGTACTGTCCCC</b><br>(SEQ ID NO: 156)  | <b>TAGCGTTGAAGTACTGTCCCC</b><br>(SEQ ID NO: 156)             | <b>94.71</b>     |
|                                                                              | TAGCGTTGAAGTACTGTCCCCG <u>G</u><br>(SEQ ID NO: 244)          | 1.08             |
|                                                                              | TAGCGTTGAAGTACTGTCCCC <u>T</u><br>(SEQ ID NO: 247)           | 1.06             |
| EF1 $\alpha$ .S155.miR2<br><b>TATTGTCAGACAATGATTCAC</b><br>(SEQ ID NO: 139)  | TATTGTCAGACAATGATTCACG <u>G</u><br>(SEQ ID NO: 248)          | 73.15            |
|                                                                              | TATTGTCAGACAATGATTCACG <u>T</u><br>(SEQ ID NO: 249)          | 23.15            |
|                                                                              | <b>TATTGTCAGACAATGATTCAC</b><br>(SEQ ID NO: 139)             | <b>0.42</b>      |
| EF1 $\alpha$ .S26.miR2<br><b>TATTGTCAGACAATGATTCAC</b><br>(SEQ ID NO: 139)   | <b>TATTGTCAGACAATGATTCAC</b><br>(SEQ ID NO: 139)             | <b>98.86</b>     |
| EF1 $\alpha$ .S33.miR2<br><b>TATTGTCAGACAATGATTCAC</b><br>(SEQ ID NO: 139)   | <b>TATTGTCAGACAATGATTCAC</b><br>(SEQ ID NO: 139)             | <b>98.92</b>     |

[00371] FIG. 9C illustrates quantification of total mutant HTT protein from YAC128 mice injected with each miRNA/Scaffold construct or dilutant. N = 2-6 mice per group. Due to variability in intra-striatal injections, only mice with vector biodistribution > 1e7 CN/ug DNA were included in this analysis. One Way ANOVA (p < 0.0001) followed by Tukey post hoc tests showed a significant decrease in mutant HTT protein compared to dilutant for all the tested

groups ( $p < 0.0001$ ). ss.EF1 $\alpha$ .S26.miR18 showed a significant difference when compared to ss.EF1 $\alpha$ .S33.miR18 ( $p < 0.05$ ), but not when compared to ss.EF1 $\alpha$ .S155.miR18. None of the miR2 containing constructs were significantly different from each other.

**[00372]** To further investigate the variability in vector biodistribution arising from the intra-striatal injections the correlation between vector biodistribution in the striatum and mutant HTT knockdown was examined. FIG. 9D illustrates the relationship between vector biodistribution from the right hemisphere of the brain and mutant HTT protein levels from the same hemisphere for constructs containing miR18. All mice ( $n=6-8$  per group) were included in this analysis. Linear regression analysis showed a highly linear correlation (EF1 $\alpha$ .S155.miR18;  $R^2 = 0.6455$ ; EF1 $\alpha$ .S26.miR18;  $R^2 = 0.6831$ ; EF1 $\alpha$ .S33.miR18;  $R^2 = 0.8683$ ). Slopes are significantly different from each other ( $p = 0.0411$ ).

**[00373]** FIG. 9E illustrates the relationship between vector biodistribution and mutant HTT protein levels for constructs containing miR18. All mice ( $n=6$  per group) were included in this analysis. Linear regression analysis showed a highly linear correlation (EF1 $\alpha$ .S155.miR2;  $R^2 = 0.8644$ ; EF1 $\alpha$ .26.miR2;  $R^2 = 0.9379$ ; EF1 $\alpha$ .S33.miR2;  $R^2 = 0.8944$ ). Slopes are not significantly different from each other ( $p = 0.0674$ ). EF1 $\alpha$ .S155.miR18 and EF1 $\alpha$ .26.miR18 had similar overall potency, but EF1 $\alpha$ .26.miR18 had a steeper slope, indicating it was more efficient at reducing HTT protein at lower biodistributions than the other constructs (FIG. 9E). The EF1 $\alpha$ .26.miR construct also trended toward increased potency when compared to the other miR2 constructs, indicating that the improved miRNA processing from the scaffold may also improve efficacy.

**[00374]** The results indicate that miRNA processing efficiency is impacted by the particular guide and scaffold selection. miRNA processing efficiency lowering unwanted passenger strand levels and raising the levels of active guide miRNA can impact the safety and efficacy profile of a therapeutic miRNA vector targeting HTT.

**[00375]** Example 8: HTT suppression in YAC128 HD mice

**[00376]** The ability of rAAV vectors comprising nucleic acid encoding miR21 was evaluated in YAC128 HD mice. The YAC128 HD strain encodes the human HTT gene with an expanded exon1. Administered rAAV vectors were either: (1) rAAV-miR21 comprising a capsid comprising VP1 of SEQ ID NO: 257, VP2 of SEQ ID NO: 258, and VP3 of SEQ ID NO: 259; and rAAV nucleic acid of SEQ ID NO: 266, comprising miR21 nucleic acid (SEQ ID NO: 261); or (2) an AAV9 capsid and rAAV nucleic acid which also comprises an miR21 encoding nucleic acid (AAV9). Recombinant AAV vectors were produced by triple-transfection.

**[00377]** YAC128 HD were administered at different doses of either rAAV-miR21 or AAV9. The effect of the rAAV vectors on HTT protein and HTT mRNA are shown in Table 12. The results

provided in Table 12 illustrate percent knockdown compared to untreated mice.

**[00378]** Table 12

| Analysis       | Dose<br>vg/brain   | Mean±SD Percent Knockdown Relative to Naïve Animals <sup>a</sup> |                |           |                  |                |         |
|----------------|--------------------|------------------------------------------------------------------|----------------|-----------|------------------|----------------|---------|
|                |                    | 1-month analysis                                                 |                |           | 6-month analysis |                |         |
|                |                    | AAV9                                                             | rAAV-<br>miR21 | P-value   | AAV9             | rAAV-<br>miR21 | P-value |
| HTT<br>mRNA    | $6 \times 10^8$    | 27.09±12.89                                                      | 0.84±12.65     | *0.001    | 17.70±7.90       | 3.97±9.99      | 0.083   |
|                | $6 \times 10^9$    | 20.58±9.83                                                       | 25.51±10.97    | 0.995     | 33.17±10.25      | 24.38±6.57     | 0.534   |
|                | $6 \times 10^{10}$ | 34.18±11.59                                                      | 39.22±6.97     | 0.992     | 34.94±10.13      | 21.46±14.79    | 0.110   |
| HTT<br>Protein | $6 \times 10^8$    | 22.76±7.92                                                       | 7.53±3.90      | *0.040    | 13.45±16.65      | 8.73±7.89      | 0.997   |
|                | $6 \times 10^9$    | 39.27±10.44                                                      | 10.66±11.58    | ***0.0001 | 27.07±17.00      | 21.06±7.55     | 0.982   |
|                | $6 \times 10^{10}$ | 65.88±6.52                                                       | 52.55±8.21     | 0.112     | 55.35±21.60      | 63.36±17.72    | 0.941   |

Statistical pairwise comparisons between vector AAV9 and rAAV-miR21 were performed using Tukeys HSD test and reported as follows: \* $p < 0.05$ , and \*\*\* $p < 0.001$

**[00379]** The mid ( $6 \times 10^9$ ) and low ( $6 \times 10^{10}$ ) doses of rAAV-miR21 and AAV9 were well tolerated, while the high dose ( $6 \times 10^{10}$ ) show adverse neurodegeneration. An additional study using a dose of  $1.9 \times 10^{10}$  vg/brain was well tolerated and decreased HTT mRNA and protein.

**[00380]** Doses of  $\geq 1.9 \times 10^{10}$  vg/brain resulted in a dose-dependent increase in vacuolation, microgliosis, and astrogliosis in the striatum with additional signs of neurodegeneration at  $6 \times 10^{10}$  vg/brain, which were considered adverse. Neurodegeneration observed in mice at higher doses may be a result of the higher vector load at increasing doses, which could have been exacerbated by tissue disruption due to surgical trauma. Histopathological findings at  $6.0 \times 10^{10}$  vg/brain, showed an impact on motor impairment, as observed by decreased rotarod activity, as well as increased mortality rate.

**[00381]** Example 9: HTT suppression in Non-Human Primates

**[00382]** rAAV-miR21 was further evaluated in a series of non-human primates (NHP) studies. The studies included an examination of different doses, routes of administrations, and surgical procedures. Both efficacy and adverse effects were examined. Adverse effects can potentially arise from different sources including the rAAV, vector dose, route of administration, surgical techniques and study conduct.

**[00383]** Study 005-001 compared efficiency of HTT protein reduction 3 and 6 months post injection at different doses administered intrathecal (IT), intraparenchymal (IP), and intracerebroventricular (ICV). IP administration was the most effective route, with potent HTT lowering and minimal spread of rAAV to nontarget brain or peripheral regions.



Histopathological evaluation revealed adverse changes including inflammation and tissue damage along the needle track and cannula terminus.

**[00384]** Study 005-004 tested the safety and efficacy of rAAV-miR21 at doses of  $3.17 \times 10^9$  to  $3.17 \times 10^{12}$  vg/brain for 3 and 12 months as a single IP injection with a transfrontal trajectory (caudate: 1 deposit, 0.050 mL; putamen: 2 deposits, 0.125 mL/deposit; at a 5  $\mu$ L/min speed) using the ClearPoint-like system to rhesus monkeys. Male and female rhesus monkeys were administered a single IP dose (qPCR titer) of rAAV-miR21 or a vehicle control. .

**[00385]** IP administration of rAAV-miR21 in Study 005-001 translated to higher levels of vector-derived transgene, and higher HTT mRNA and HTT protein reduction (> 50% and 65% reduction in the caudate and putamen, respectively) up to 6-months compared with single-route IT or ICV administration. FIG. 10 illustrates the effect of rAAV-miR21 administered at different doses and different routes of administration 3 months post injection.

**[00386]** In Study 005-004, rAAV-miR21 biodistribution analyses showed abundant vector levels were found consistently in regions close to the injection sites such as putamen, external globus pallidus, caudate, and internal globus pallidus of animals across all dosing groups. Due to the high variability of biodistribution readout within single groups and between 3- and 12-months post-surgery groups, additional biodistribution analysis was conducted. The additional results confirmed that rAAV vector DNA was detectable in a dose-dependent manner and that its concentrations were steady in the injected brain structures up to 12 months post-surgery. No statistically significant difference was identified between vector copy numbers at 3 and 12 months. Peripheral tissues contained lower vector levels.

**[00387]** Results of Study 005-004 are illustrated in FIGs. 12A, 12B, 12C, 12D, 13A, 13B, 13C, and 13D. Biodistribution in caudate and putamen following IP administration is illustrated in FIGs. 12A, 12B, 12C, and 12D. The additional analysis (FIGs. 12C and 12D) refers to caudate and putamen levels that were not included in the first round of analysis (FIGs. 12A and 12B). miR21 levels for a given brain region within the same dose group were as variable as the biodistribution values of the first round of analysis. Overall, mean miR21 levels were dose-dependent at each time point with variability in levels from Day 92 to Day 365  $\pm$  5 within animals receiving the same dose. Regions with the highest levels of miR21 corresponded to regions with the greatest reduction in HTT mRNA and greatest reduction of HTT protein. rAAV administration up to  $3.17 \times 10^{12}$  vg/brain was associated with dose-dependent reductions of HTT mRNA in the investigated brain tissues at both the Day 92 and Day 365  $\pm$  5 necropsy intervals.

**[00388]** Analysis of tissue samples from Study 005-004 for HTT protein (FIGs. 13A and 13B) found decreased levels of HTT protein localized primarily to the sites of administration in the caudate and putamen and surrounding structures. The decrease tended to be dose-dependent with

significant decreases noted in the putamen at all dose levels. At 90 days, other structures found to have decreased protein levels included the medial prefrontal cortex, motor cortex, caudate, and internal globus pallidus. At 12 months, decreases in HTT protein levels were found to follow a similar pattern. The greatest decrease in protein levels were noted in the putamen, external globus pallidus, caudate, internal globus pallidus, dorsal and medial prefrontal cortex, motor cortex and somatosensory cortex. Due to the high variability of HTT protein readout within single groups and between 3 and 12 months post-surgery groups, additional samples were collected and analyzed. These additional results, shown in FIG. 13C and FIG. 13D, confirmed that HTT protein is reduced in a dose-dependent manner and its concentrations were steady in the injected brain structures up to 12 months post-surgery. No statistically significant difference was identified between 3 and 12 months HTT protein concentration at each dose.

**[00389]** A dose dependent increase in the extent and severity of local tissue damage was observed in both Studies 005-001 and 005-004, which employed transfrontal trajectories/unoptimized surgical procedures. The highest dose cohort in Study 005-004 ( $3.17 \times 10^{12}$  vg/brain) developed severe neurological observations including limb misplacement/weakness, weakness in grasp, ataxia, proprioceptive deficits, dehydration, and progressive paresis/paralysis of limbs (Category 1). Neurological findings at lower doses included transient jerks and tremors and more severe, but still transient findings of convulsions (Category 2), from which the animal recovered. Category 2 effects were attributed to surgical and study conduct. Tissue damage was observed at doses  $\geq 1 \times 10^{11}$  vg/brain.

**[00390]** Adverse results are summarized in Table 13A: N/A=no animals were dosed; vg=vector genome. No Category 1 findings were noted in animals receiving a dose of  $1 \times 10^{12}$  vg/brain or less.

**[00391]** Table 13A

| vg/Brain              | 3-Month | 12-Month | Total events |
|-----------------------|---------|----------|--------------|
| Diluent               | 0/5     | 0/5      | 0/10         |
| Category 1            |         |          |              |
| $3.17 \times 10^{12}$ | 4/6     | N/A      | 4/6          |
| Category 2            |         |          |              |
| $3.17 \times 10^9$    | N/A     | 0/6      | 0/6          |
| $1 \times 10^{10}$    | 2/6     | 2/5      | 4/11         |
| $3.17 \times 10^{10}$ | 4/6     | 1/5      | 5/11         |
| $1 \times 10^{11}$    | 1/6     | 0/6      | 1/12         |
| $3.17 \times 10^{11}$ | 2/6     | 0/6      | 2/12         |
| $1 \times 10^{12}$    | 1/6     | 0/6      | 1/12         |
| $3.17 \times 10^{12}$ | 0/6     | N/A      | 0/6          |

**[00392]** Studies 005-010, 005-016, 005-017 employed improved surgical procedures significantly reducing histopathological findings. The improvements include real-time magnetic

resonance imaging (MRI) of infusions and refinement of infusion trajectories/parameters. The placement of the cannula along the parietal and occipital trajectories to caudate and putamen, respectively, instead of transfrontal trajectories (2 instead of 3 trajectories per hemisphere, respectively), achieved equivalent or higher coverage of the target structure (Table 13B), compared with a fewer number of injections and fewer safety findings. The trajectory modification enabled the infusate to be released in multiple smaller steps rather than in a single bolus. Additionally, the infusion speed was decreased to better preserve the integrity of brain parenchyma.

Table 13B

| Brain Target Structure | Study 005-010     |                      | Study 005-016     |                      |
|------------------------|-------------------|----------------------|-------------------|----------------------|
|                        | (n) Trajectory(s) | Structure Coverage % | (n) Trajectory(s) | Structure Coverage % |
| Caudate                | (1) Transfrontal  | 22.35±12.66          | (1) Parietal      | 41.25±13.96          |
| Putamen                | (2) Transfrontal  | 67.42±9.31           | (1) Occipital     | 62.85±13.73          |

**[00393]** Improvements in the study conduct included use of additional stereotactic equipment which reduced overall surgical time, thereby limiting the duration an animal was exposed to anesthesia. In the modified study protocol, animals recovered in a darkened and quiet room for the first night after surgery and were monitored more frequently.

**[00394]** Study 005-010 included injection along transfrontal trajectories and assessed the impact of surgery parameters, such as injection speed rate, on safety and efficacy of IP administration of rAAV-miR21 into the caudate/putamen. Two groups received rAAV-miR21 at  $1 \times 10^{11}$  or  $1 \times 10^{12}$  vg/brain with the infusion delivered at a ramping speed (1-3-5  $\mu\text{L}/\text{min}$ ) and a third group was injected with rAAV-miR21 at  $1 \times 10^{12}$  vg/brain delivered at a steady 5  $\mu\text{L}/\text{min}$  speed. Previous studies 005-001 and 005-004 employed 5  $\mu\text{L}/\text{min}$  injection speed rate. Reducing IP infusion rate led to increased safety for brain parenchyma. In Study 005-010,  $1 \times 10^{11}$  vg/brain was highest rAAV-miR21 dose not associated to test article-related findings.

**[00395]** Study 005-016 involved injection at a ramping speed (1-3-5  $\mu\text{L}/\text{min}$ ) along parietal and occipital trajectories to the caudate and putamen, respectively, instead of transfrontal trajectories. Cannula placement and diffusion of rAAV formulations admixed with gadoteridol were also monitored in real-time using MRI. Compared to Study 005-010, the transition from targeting putamen with a transfrontal trajectory to an occipital trajectory coupled to a reduced infusate volume resulted in similar coverage of the structure (~63%). Despite the infusate volume being unchanged, targeting of the caudate with a parietal instead of a transfrontal trajectory resulted in nearly doubling coverage increase of the structure (~41% vs ~22% in Study 005-010). Animals were injected with increasing doses of rAAV-miR21:  $1 \times 10^{10}$ ,  $1 \times$

$10^{11}$ , and  $1 \times 10^{12}$  vg/brain. Target structure coverage robustly correlated with rAAV-miR21 biodistribution and dose-dependent reduction of HTT mRNA and HTT protein in the caudate and putamen. The study showed that rAAV-miR21 safely induced 60% to 70% HTT protein reduction in both target structures employing doses up to  $1 \times 10^{12}$  vg/brain with minimal localized damage at the site of administration.

**[00396]** Study 005-017 confirmed the safety and efficacy of the rAAV-miR21 at different doses. FIG. 11 illustrates the effect of different doses on HTT protein. Doses between  $1 \times 10^{10}$  and  $1 \times 10^{12}$  vg/brain lowered the total HTT protein in a dose-dependent manner in NHP three and twelve months after rAAV-miR21 administration with no significant difference across different doses at the same timepoint, indicating durability of treatment. The 25% and 50% dotted lines are relative to the 0 vg/brain control. Converting vg/mm<sup>3</sup> for each target brain structure (putamen or caudate), using volumetric data from T1-weighted real-time MRIs at dosing of study cohort 1 (3M), apportioning the putamen with 67% of the total dose per hemisphere and caudate at 33% of the total, for the  $1 \times 10^{12}$  vg/brain dose, provides on average  $6.8 \times 10^8$  vg/mm<sup>3</sup> and  $4.8 \times 10^8$  vg/mm<sup>3</sup> for the putamen and caudate, respectively.

**[00397]** Study 005-017 analysis of tissue samples 3 and 12 months after injection confirmed the durability of rAAV-miR21 vector genome distribution at different doses in both caudate (FIG. 14A) and putamen (FIG. 14B) and the minimal spread of viral genome to the periphery. Most abundant vector levels were associated consistently with the injection sites (caudate and putamen) and the area adjacent to the injection sites, such as external and internal globus pallidus, and VL thalamus. Other brain tissues, spinal cords (sacral, cervical, thoracic, lumbar), dorsal root ganglia (sacral, cervical, thoracic, lumbar), spleen and liver contained much less vector levels than that in the injection sites or nearby area. Peripheral tissues, except the spleen and liver, had limited exposure to rAAV-miR21. All sciatic nerve, ovary, and testis tissues collected from the vector-dosed animals were negative.

**[00398]** FIGs. 15A and 15B illustrate results from Study 005-017 measuring HTT knockdown. FIG. 15A illustrates HTT knockdown in the caudate. HTT mean protein levels quantified in animals injected with either  $1 \times 10^{11}$  or  $1 \times 10^{12}$  vg/brain of rAAV-miR21 were significantly lower than HTT protein mean levels quantified in diluent-injected animals. HTT mean protein levels quantified in animals dosed at  $1 \times 10^{12}$  vg/brain were also significantly lower than HTT protein levels in animals injected at lower doses.

**[00399]** FIG. 15B illustrates HTT knockdown in the caudate. HTT mean protein levels quantified in animals injected with either  $1 \times 10^{10}$ ,  $1 \times 10^{11}$ , or  $1 \times 10^{12}$  vg/brain of rAAV-miR21 were significantly lower than HTT protein mean levels quantified in diluent-injected animals. HTT mean protein levels quantified in animals dosed at  $1 \times 10^{11}$  vg/brain were also

significantly lower than HTT protein levels in animals injected with either  $1 \times 10^9$  or  $1 \times 10^{10}$  vg/brain.

**[00400]** Durability of transgene expression deriving from rAAV-miR21 transduction was confirmed in brain tissue and also by measuring mature miR21 in the CSF collected from animals 3 and 12-months after injection. FIG. 16A and FIG. 16B illustrate results measuring brain miR21 and NF-L.

**[00401]** Neurofilament light chain (NF-L) is a neuronal cytoplasmatic protein highly expressed in myelinated axons and an established biomarker used to monitor the course of neurological diseases and traumatic events like IP surgery. Data reported in FIG. 16C and FIG. 16D illustrate elevation of NF-L levels in the CSF of all animals 3 months after IP injections performed in Study 005-017 (Cohort 1) compared to the baseline levels (pre-injection), confirming that increased NF-L levels in the CSF were due to the surgical procedure alone rather than rAAV-miR21. Levels of circulating NF-L 12 months after surgery (Cohort 2) were generally comparable to those measured at baseline, indicating that IP surgery induces acute and not chronic changes to the brain parenchyma homeostasis.

**[00402]** Safety of rAAV-miR21 IP delivery was confirmed by the acquisition and analysis of longitudinal brain MRI scans of all animals treated in Study 005-017. Volumetric measurements of caudate (FIG. 16D), putamen (FIG. 16E), and lateral ventricle (FIG. 16E) of both hemispheres did not highlight any significant change between pre-treatment and terminal phase at either 3 or 12 months after surgery.

**[00403]** Bilateral intraparenchymal (caudate and putamen) infusion of rAAV-miR21 of up to  $1 \times 10^{12}$  vg/brain in cynomolgus monkeys treated in Study 005-017 did not reveal obvious treatment-related histopathology changes in the nervous system (hemi-brain, meninges/dura mater [injection sites], spinal cord, ganglia [dorsal root and trigeminal], spinal nerve roots, and nerves) and select protocol-directed systemic tissues evaluated on day  $92 \pm 4$  post-dosing. Loss of neural tissue was limited to target injection sites (caudate and putamen) and cannula tracks (adjacent white matter tracts and/or adjacent cerebral cortices) that are commonly noted as procedural effects seen with direct intraparenchymal routes of administration. Test article-related changes 3 and 12 months post injection were limited to slight increases in perivascular cuffing by mononuclear cell infiltrates (lymphocytes and/or macrophages) at the target sites that were documented in animals treated with rAAV-miR21 at the highest dose.

**[00404]** The protocol and results of the different studies are summarized in Tables 14-18.

[00405] Table 14

| Study 005-001                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| A 90 and 180 days route of administration study of rAAV-miR21 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Test System (N)                                               | Dose (vg/brain) and Delivery                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Duration       | Objective(s) and Results                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Male and Female Rhesus Monkey<br>N=4/group                    | $1 \times 10^{12}$ (IP)<br>$3 \times 10^{12}$ (IP)<br>$6 \times 10^{12}$ (IP)<br>$1 \times 10^{13}$ (IT)<br>$3 \times 10^{13}$ (IT)<br>$6 \times 10^{13}$ (IT)<br>$1 \times 10^{13}$ (ICV)<br>$3 \times 10^{13}$ (ICV)<br>$6 \times 10^{13}$ (ICV)<br>$3 \times 10^{12}$ (IP) + $1 \times 10^{13}$ (IT)<br>$3 \times 10^{12}$ (IP) + $3 \times 10^{13}$ (IT)<br>$3 \times 10^{12}$ (IP) + $6 \times 10^{13}$ (IT)<br>$3 \times 10^{12}$ (IP) + $1 \times 10^{13}$ (ICV)<br>$3 \times 10^{12}$ (IP) + $3 \times 10^{13}$ (ICV)<br>$3 \times 10^{12}$ (IP) + $6 \times 10^{13}$ (ICV) | 3 and 6 Months | <p>Objective: safety and biodistribution evaluation of rAAV-miR21 in the caudate and putamen following different routes of administration</p> <p>Results:</p> <ul style="list-style-type: none"> <li>• IP delivery resulted in effective transduction of caudate and putamen at lower AAV doses than IT or ICV.</li> <li>• IP delivery resulted in increased safety in terms of less transduction of non-target tissues and lower anti-AAV humoral immune response</li> </ul> |

[00406] Table 15

| Study 005-004                                           |                                                                                                                                                                                                             |                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 12-month safety and biodistribution study of rAAV-miR21 |                                                                                                                                                                                                             |                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Test System (N)                                         | Dose (vg/brain) and Delivery                                                                                                                                                                                | Duration        | Objective(s) and Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Male and Female Rhesus Monkey<br>N=5-6/group            | $3.17 \times 10^9$ (IP)<br>$1.00 \times 10^{10}$ (IP)<br>$3.17 \times 10^{10}$ (IP)<br>$1.00 \times 10^{11}$ (IP)<br>$3.17 \times 10^{11}$ (IP)<br>$1.00 \times 10^{12}$ (IP)<br>$3.17 \times 10^{12}$ (IP) | 3 and 12 Months | <p>Objective: safety and biodistribution evaluation of rAAV-miR21 following bilateral intraparenchymal administration into the caudate/putamen</p> <p>Results:</p> <ul style="list-style-type: none"> <li>• Good dose-dependent transduction of caudate and putamen, resulting in a robust reduction of Huntingtin mRNA and protein up to 12-M post-injection.</li> <li>• Doses up to <math>3.17 \times 10^{12}</math> vg/brain were not associated with any effects on animal physiology.</li> <li>• Doses above <math>1 \times 10^{10}</math> vg/brain were associated with transient clinical signs (tremors, convulsions, and limb weakness/misplacement) in 3-M cohort animals.</li> <li>• Doses above <math>1 \times 10^{10}</math> vg/brain were associated with neurological findings that affected general sensory and motor function and spinal reflexes in 3-M cohort animals.</li> <li>• 12-M cohort animals were not associated with neurological findings.</li> <li>• Clinical and neurological findings were consistent with histopathology, in that procedural-related</li> </ul> |

|  |  |  |                                                                            |
|--|--|--|----------------------------------------------------------------------------|
|  |  |  | trauma was enhanced in rAAV-miR21 dosed animals at the higher dose levels. |
|--|--|--|----------------------------------------------------------------------------|

[00407] Table 16

| Study 005-010<br>A 90-day delivery procedure evaluation of rAAV-miR21 |                                                    |          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------------------------------------------------------------|----------------------------------------------------|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Test System (N)                                                       | Dose (vg/brain) and Delivery                       | Duration | Objective(s) and Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Male and Female Cynomolgus Monkey<br>N=3-4/group                      | $1 \times 10^{11}$ (IP)<br>$1 \times 10^{12}$ (IP) | 3 Months | <p>Objective: evaluation of delivery procedure to improve safety and biodistribution of rAAV-miR21 following bilateral intraparenchymal administration into the caudate/putamen.</p> <p>Results:</p> <ul style="list-style-type: none"> <li>• Tested infusion speed rates did not affect rAAV-miR21 pharmacology and led to comparable results.</li> <li>• Caudate and putamen structures were infused at an average of 22% and 67% respectively, of their total volume.</li> <li>• MRI evaluation of injected areas demonstrated that identified change was procedural-related.</li> <li>• NF-L increased levels 3-M post-surgery were attributed to surgery.</li> <li>• No rAAV-miR21 related clinical signs were noted in any of the animals over the course of the study.</li> <li>• No test article effects associated with animal physiology.</li> <li>• Infusion speed rate did affect histopathology assessment.</li> <li>• Although delivery at a steady speed rate resulted in wider biodistribution of rAAV-miR21 across the target structures, it was also more detrimental to tissue integrity.</li> </ul> |

[00408] Table 17

| Study 005-016<br>Seven-week safety and efficacy study to evaluate intraparenchymal administration by 2 devices of rAAV-21 to the caudate and putamen |                                                                               |            |                                                                                                                                                                                                                                                                                                                                                                          |
|------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Test System (N)                                                                                                                                      | Dose (vg/brain) and Delivery                                                  | Duration   | Objective(s) and Results                                                                                                                                                                                                                                                                                                                                                 |
| Male Cynomolgus Monkeys<br>N= 3-4/group                                                                                                              | $1 \times 10^{10}$ (IP)<br>$1 \times 10^{11}$ (IP)<br>$1 \times 10^{12}$ (IP) | 1-5 Months | <p>Objective: evaluation of delivery trajectories by 2 devices to improve safety and biodistribution of rAAV-miR21 following bilateral intraparenchymal administration.</p> <p>Results:</p> <ul style="list-style-type: none"> <li>• Caudate and putamen were infused with ramp-up speed and targeted with parietal and occipital trajectories, respectively.</li> </ul> |

|  |  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--|--|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  |  | <ul style="list-style-type: none"> <li>• Dose administration was performed either by monitoring in real-time 90- and 180-days MRI injections (ClearPoint®-like) or according to pre-planned trajectories (Neurochase).</li> <li>• Caudate and putamen structures were infused by the ClearPoint®-like device at an average of 41% and 63%, respectively, of their total volume.</li> <li>• Caudate and putamen structures were infused by Neurochase device at an average of 55% and 75%, respectively, of their total volume.</li> <li>• Caudate structure coverage was doubled using both devices compared to a previous study (005-010).</li> <li>• MRI evaluation of injected areas demonstrated that any identified change was procedural-related.</li> <li>• NF-L increased levels 1.5-M post-surgery were attributed to surgery.</li> <li>• Any clinical signs noted in any of the animals immediately after surgery were considered procedure-related.</li> <li>• No test article effects associated with animal physiology.</li> <li>• Both the ClearPoint®-like and Neurochase-injected animals presented a mild to moderate dose-dependent increase in the severity of histopathology findings. Severity of changes was clearly increased for the groups injected using the Neurochase device compared with animals injected with the ClearPoint®-like system at all doses, including the diluent Group.</li> </ul> |
|--|--|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

[00409] Table 18

| Study 005-017                                                                                                 |                                                                                                       |                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|---------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Fifty two-week safety, efficacy, and biodistribution study of rAAV-miR21 delivered to the caudate and putamen |                                                                                                       |                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Test System (N)                                                                                               | Dose (vg/brain) and Delivery                                                                          | Duration        | Objective(s) and Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Male and Female Cynomolgus Monkey<br>N= 1-4/group                                                             | $1 \times 10^9$ (IP)<br>$1 \times 10^{10}$ (IP)<br>$1 \times 10^{11}$ (IP)<br>$1 \times 10^{12}$ (IP) | 3 and 12 Months | <p>Objective: evaluation of safety, efficacy, and biodistribution of rAAV-miR21 after bilateral intraparenchymal administration into the caudate and putamen</p> <p>3-M and 12-M post infusion results:</p> <ul style="list-style-type: none"> <li>• Good dose-dependent transduction of caudate and putamen, resulting in a robust reduction of HTT mRNA and protein.</li> <li>• Caudate and putamen were infused with ramp-up speed and targeted with parietal and occipital trajectories, respectively.</li> <li>• Caudate and putamen structures were infused at an average of 34% and 50%, respectively, of their total volume.</li> <li>• MRI evaluation of injected areas demonstrated that any identified change was procedural-related.</li> </ul> |



|  |  |  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|--|--|--|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  |  |  | <ul style="list-style-type: none"> <li>• NF-L increased levels 3-M post-surgery were attributed to surgery and were reduced to baseline 12-M post-surgery independent from dose</li> <li>• miR21 was detectable in CSF of animals injected at and above <math>1 \times 10^{11}</math> vg/brain 3 and 12 months post injection.</li> <li>• Any clinical signs noted in any animals immediately after surgery were considered procedure-related.</li> <li>• No test article effects associated with animal physiology.</li> <li>• Test article-related changes were limited to a slight increase in perivascular cuffing by mononuclear cells and/or macrophages at the target site compared to sham and diluent controls, suggesting a mild immunomodulatory effect at the highest dose.</li> </ul> |
|--|--|--|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**[00410]** The percent of dose-dependent lowering of HTT protein in NHPs for different studies is summarized in Table 19.

**[00411]** Table 19

| Study No. | N | Time M | Dose (vg/brain)     |                     |                      |                       |                      |                       |                     |                       |                    |
|-----------|---|--------|---------------------|---------------------|----------------------|-----------------------|----------------------|-----------------------|---------------------|-----------------------|--------------------|
|           |   |        | $1 \times 10^9$     | $3.17 \times 10^9$  | $1 \times 10^{10}$   | $3.17 \times 10^{10}$ | $1 \times 10^{11}$   | $3.17 \times 10^{11}$ | $1 \times 10^{12}$  | $3.17 \times 10^{12}$ | $6 \times 10^{12}$ |
| 005-001   | 4 | 3      | NT                  | NT                  | NT                   | NT                    | NT                   | NT                    | 76.94<br>$\pm 7.1$  | 64.84<br>$\pm 14.5$   | 83.71<br>$\pm 7.2$ |
|           |   | 6      | NT                  | NT                  | NT                   | NT                    | NT                   | NT                    | NT                  | 76.76<br>$\pm 3.2$    | NT                 |
| 005-004   | 6 | 3      | NT                  | NT                  | 23.23<br>$\pm 11.2$  | 28.37<br>$\pm 14.5$   | 61.88<br>$\pm 11.6$  | 66.14<br>$\pm 8.9$    | 80.69<br>$\pm 12.7$ | 84.18<br>$\pm 7.9$    | NT                 |
|           |   | 12     | NT                  | 15.80<br>$\pm 13.7$ | 11.85<br>$\pm 14.5$  | 35.99<br>$\pm 6.3$    | 48.79<br>$\pm 9.9$   | 59.50<br>$\pm 16.1$   | 83.67<br>$\pm 5.5$  | NT                    | NT                 |
| 005-010   | 4 | 3      | NT                  | NT                  | NT                   | NT                    | 27.67<br>$\pm 12.1$  | NT                    | 55.28<br>$\pm 5.2$  | NT                    | NT                 |
| 005-016   | 4 | 1.5    | NT                  | NT                  | 30.53<br>$\pm 29.70$ | NT                    | 35.32<br>$\pm 18.26$ | NT                    | 64.3<br>$\pm 18.26$ | NT                    | NT                 |
| 005-017   | 4 | 3      | 11.62<br>$\pm 6.50$ | NT                  | 21.94<br>$\pm 5.60$  | NT                    | 43.61<br>$\pm 6.8$   | NT                    | 69.33<br>$\pm 4.0$  | NT                    | NT                 |
|           |   | 12     | NT                  | NT                  | 25.10<br>$\pm 7.63$  | NT                    | 33.25<br>$\pm 10.25$ | NT                    | 73.04<br>$\pm 5.19$ | NT                    | NT                 |

**[00412]** Observation from the different studies include the reduction of HTT protein driven by rAAV-miR21 was generally dose-dependent and durable, with  $1 \times 10^{12}$  vg/brain achieving a maximum HTT protein knockdown of up to 62-64% and 69-73% in the caudate and putamen, respectively, 3 and 12 months post injection. HTT protein knockdown was up to 77-59% and 50-45% in the brain structures of external and internal globus pallidus, respectively, which are adjacent to the injection sites. The cerebellar cortex was the only tested region with no HTT protein reduction at 3 months post injection. About a 10% average reduction of HTT was observed in the cerebellum at 12-months post injection.

**[00413]** A single, bilateral IP (caudate and putamen) infusion of rAAV-miR21 of up to  $1 \times 10^{12}$  vg/brain did not reveal significant treatment-related histopathology microscopic changes in the nervous system (hemi-brain, spinal cord, ganglia [sensory and autonomic], nerve roots, and nerves) and selected systemic tissues (heart, kidney, liver, lung, lymph nodes) evaluated at 3 months and 12 months after dosing.

**[00414]** Collectively, the results demonstrate that administration of rAAV-miR21 at doses up to  $1 \times 10^{12}$  vg/brain was not associated with test article-related adverse clinical or neurologic changes, using optimized surgical procedures.

**[00415]** Using the 3-month NHP data from Study 005-017 on efficacy of various doses of rAAV-miR21 expressed as vg/mm<sup>3</sup> per putamen, FIG. 17 illustrates an estimated dose-response curve with 95% confidence intervals throughout the dose range. For clinical low and high dose extrapolations, the lower bands of the 95% confidence interval are associated with 25% and 50% HTT lowering in the putamen, respectively.

**[00416]** miR21 was detectable in the CSF of Cynomolgus Macaques 3- and 12-months post rAAV IP injection in the caudate and putamen along parietal and occipital trajectories, illustrating that miR21 detection in CSF provides a valuable biomarker to assess therapy durability. Absolute quantification of miR21 was carried out in CSF samples using reverse transcription and quantitative polymerase chain reaction (RT-qPCR) and synthesized miRNA oligonucleotide standards, using the Enhanced Assay procedure described in the Example 12.

**[00417]** Longitudinal analysis of whole brain MR-scans revealed that surgery-driven injury was already resolving two months post injection and that volumes of the lateral ventricles and target structures were unchanged up to 12-months post injection. Surgical injury resolution and lack of AAV-driven toxicity were also monitored by neurofilament-light chain (NFL) protein readouts in CSF and histopathology analysis of the brain, confirming minimal to no neuronal damage and anatomical changes at  $1 \times 10^{12}$  vg/brain.

**[00418]** Example 10: Clinical Trials

**[00419]** Information from the NHP studies can be used to guide human treatment and clinical trial administration. In NHP a bilateral IP infusion of rAAV-miR21 at doses up to  $1.00 \times 10^{12}$  vg/brain was not associated with any effects on body weights, heart rate, body temperature, or respiration, ophthalmological endpoints, changes in hematology, coagulation, serum chemistry parameters, or CSF total cell.

**[00420]** The putamen and caudate volumes of different Huntington patients can show a large degree of variability within a particular disease stage and between different disease stages. Patients can receive individualized treatment based on putamen and caudate volumes, determined, for example, by vMRI (volumetric magnetic resonance imaging). Patients can be

dosed in an exposure-based manner where the vector genome (vg)/mm<sup>3</sup> of target tissue is kept constant, but the total IP infusion volume (and total vg/brain) is adjusted to accommodate various putamen and caudate volumes. Although the interindividual volume variability can be large, the within-subject volume variability between left and right hemisphere are negligible (typically within 5%).

**[00421]** Based on a putamen to caudate ratio about of 67% putamen and 33% caudate upon administration, a dose of  $1 \times 10^{12}$  vg/brain represents  $6.8 \times 10^8$  vg/mm<sup>3</sup> in the putamen (unilateral) and  $4.8 \times 10^8$  vg/mm<sup>3</sup> in caudate (unilateral). The lower bands of the 95% confidence intervals for the estimated doses reflecting 25% and 50% reduction of HTT levels in the putamen were  $2.0 \times 10^7$  vg/mm<sup>3</sup> and  $2.0 \times 10^8$  vg/mm<sup>3</sup>, respectively (FIG. 17).

**[00422]** The infusion volume can be adjusted to correspond to particular putamen and caudate volumes (e.g., based on baseline vMRI) to provide coverage preferably of at least 50% in the targeted area. Coverage can be measured using an imaging agent, such as gadoteridol. The targeted area for putamen is the whole putamen, and for the caudate the targeted area is the precommissural caudate.

**[00423]** Clinical trials currently plan for two ascending doses of rAAV-mi21 to be administered bilaterally into the putamen and caudate. The low dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> represents an estimated dose to provide a 25% to 35% reduction (95% confidence interval) in putamen HTT levels in NHPs. The high dose of  $2.0 \times 10^8$  vg/mm<sup>3</sup> represents an estimated dose to provide a 50% to 60% reduction (95% confidence interval) in putamen HTT levels in NHPs.

**[00424]** Table 20 provides an example of a high dosing calculation for different size putamen and caudate volumes using a dose formulation of  $6.2 \times 10^{11}$  vg/ml and the MRI contrast agent gadoteridol. The parameters can vary, for example, different dosages, formulation concentrations, and infusion volumes could be used; putamen and caudate volumes are expected to vary among patients; and other imaging agents could be used.

**[00425]** Table 20

|                                                    | Mean example         | Min example          | Max example          |
|----------------------------------------------------|----------------------|----------------------|----------------------|
| Putamen volume per hemisphere (mm <sup>3</sup> )   | 3000                 | 1400                 | 5600                 |
| Caudate volume per hemisphere (mm <sup>3</sup> )   | 2000                 | 500                  | 4400                 |
| IP administration in putamen per hemisphere        |                      |                      |                      |
| Target gadoteridol distribution (mm <sup>3</sup> ) | 1930                 | 910                  | 3640                 |
| Infusion volume (mL)                               | 1.0                  | 0.5                  | 1.8                  |
| Target vg                                          | $6.0 \times 10^{11}$ | $2.8 \times 10^{11}$ | $1.1 \times 10^{12}$ |
| IP administration in caudate per hemisphere        |                      |                      |                      |
| Target gadoteridol distribution (mm <sup>3</sup> ) | 1300                 | 325                  | 2860                 |
| Infusion volume (mL)                               | 0.7                  | 0.2                  | 1.4                  |
| Target vg                                          | $4.0 \times 10^{11}$ | $1.0 \times 10^{11}$ | $8.9 \times 10^{11}$ |

IP= intraparenchymal; Max=maximum; Min=minimum; vg=vector genome.

[00426] A formulation for vector administration may contain, for example, 10 mM sodium phosphate, 150 mM sodium chloride, 0.001% Kolliphor, pH 7.3.

[00427] The study will measure standard biomarkers for Huntington disease, and current plans are to also include the following three potential biomarkers as potential surrogate endpoints: (1) changes in the volume of the caudate or putamen measured by vMRI; (2) a Huntington disease motor assessment (HDDMS), which provides a composite score to sensitively assess motor symptom progression using smartphone-based motor tasks assessing fine motor control (speeded tapping task), chorea of the upper extremities (chorea task), chorea of the trunk (balance task), and gait (2-minute walking); and (3) changes in Huntington disease neuropathology measured by neurofilament light chain protein (NfL).

[00428] Clinical studies are planned to evaluate the initial efficacy of a one-time IP infusion of rAAV-miR-21 into the caudate and putamen of adults with Huntington disease. Initial efficacy and pharmacodynamics can be assessed in an exploratory manner by evaluating changes in selected biomarkers (MRI, HDDMS, and NfL as potential surrogate endpoints) and clinical or participant-reported outcome measures (assessments of physical activity and sleep, clinical assessments, participant-reported outcomes, neuroimaging parameters, and biofluid biomarkers). Well-established clinical measures will also be assessed.

[00429] Administration of rAAV-miR21 can be achieved, for example, via IP infusions to the caudate and putamen along the long axis of both structures, using convection-enhanced delivery. Administration can be conducted under intraoperative MRI guidance to visualize and optimize coverage of the target regions, and using the SmartFlow® neuro ventricular cannula and ClearPoint® Neuro Navigation System.

**[00430] Example 11: Long term efficacy of rAAV.EF1 $\alpha$ .S26.miR18**

[00431] The efficacy of rAAV.EF1 $\alpha$ .S26.miR18 was assessed in a 12-month study in YAC128 HD mice. Three doses of vector ( $6 \times 10^8$ ,  $6 \times 10^9$  and  $6 \times 10^{10}$  vg/brain) per animal were delivered directly to the striatum via stereotactic injection. Motor function was assessed using the accelerating rotarod test at baseline and every 2 months post treatment.

[00432] A restricted maximum likelihood model was fit to the data set and showed significant effects of Sex, Time, and Group as well as multiple factor interactions (Table 21). Female YAC128 mice did not show any significant differences between dilutant and rAAV.EF1 $\alpha$ .S26.miR18 treated mice, but males showed significantly improved motor performance at  $6 \times 10^8$  and  $6 \times 10^9$  vg/brain doses in YAC128 mice (Table 22).

[00433] Table 21

| Source     | # of Parameters | F Ratio | Prob > F |
|------------|-----------------|---------|----------|
| Sex        | 1               | 11.2566 | 0.0015*  |
| Time       | 6               | 21.3055 | <.0001*  |
| Group      | 5               | 9.8690  | <.0001*  |
| Sex*Group  | 5               | 3.6190  | 0.0067*  |
| Time*Sex   | 6               | 4.4124  | 0.0003*  |
| Group*Time | 30              | 2.2881  | 0.0002*  |

[00434] Table 22

| Sex | Group             | Difference | Std Error | t Ratio | Prob> t |
|-----|-------------------|------------|-----------|---------|---------|
| M   | Dilutant vs 6e8vg | -79.901    | 21.32803  | -3.75   | 0.0201* |
| M   | Dilutant vs 6e9vg | -94.664    | 21.40582  | -4.42   | 0.0025* |
| M   | Dilutant vs WT    | -88.975    | 21.40593  | -4.16   | 0.0059* |

[00435] FIGs. 18A, 18B and 18C illustrate average time on a rotarod. FIG. 18A, depicts the time in seconds male mice remained on an accelerating rotarod for an average of 3 trials at each timepoint. There was a significant rescue of the motor phenotype in male mice starting at 4 months post injection (mice at age 6 months) and lasting for the duration of the study. FIG. 18B depicts the average time in seconds on a rotarod across all timepoints in male mice. FIG. 18C shows the average time on rotarod in seconds across all timepoints for female mice. Female mice in the high dose group trended towards improved motor function, but there were no significant differences between dilutant and treatment groups. These results indicate that rAAV.EF1 $\alpha$ .S26.miR18 is well tolerated and has a durable therapeutic effect in the YAC128 HD mouse model.

#### [00436] Example 12: Detection and Quantification of miR21 in Cerebrospinal Fluid

[00437] Enhancement of procedures for detecting and quantifying miR21 in cynomolgus monkeys CSF were carried out. The general procedure involves miRNA purification from CSF, followed by Reverse Transcription (RT)-quantitative Polymerase Chain Reaction (qPCR) analysis. Purification was performed using a Norgen Urine microRNA purification kit and adjusting the conditions. RT-qPCR was performed using primers targeting the miRNA scaffold. Assay evaluation involved generation of calibrations curves obtained by spiking known amounts of miR39 (a synthetic miRNA derived from cel-miR-39 found in *C. elegans*) and miR21 into monkey CSF, purification, and analysis by RT-qPCR.

#### [00438] Enhanced Assay

[00439] An enhanced assay was identified by varying different reaction conditions such as RNA volume and incubation conditions. The enhanced assay involved the following reagents, purification procedure, and RT-qPCR.

[00440] **Reagents:** Reagent sources are summarized in Table 23

Table 23

| Product Name                                 | Vendor Name              | Catalog Number |
|----------------------------------------------|--------------------------|----------------|
| RNase Away                                   | Thermo Fisher Scientific | 7003           |
| Nuclease-free Water                          | Invitrogen               | AM9937         |
| TE Buffer pH 7.4                             | Thermo Fisher Scientific | J75893         |
| beta-Mercaptoethanol                         | Sigma-Aldrich            | 444203         |
| Urine microRNA Purification Kit              | Norgen                   | 29000          |
| Lysis Buffer A                               | Norgen                   | 90056          |
| Wash Solution A                              | Norgen                   | 90026          |
| Elution Solution A                           | Norgen                   | 90023          |
| Spin Column                                  | Norgen                   | 20045          |
| Ethyl alcohol, 200 proof                     | Sigma-Aldrich            | E7023          |
| Nuclease-Free Water                          | Invitrogen               | AM9937         |
| 100mM dNTPs (with dTTP)                      | Applied Biosystems       | 4366596        |
| MultiScribe™ Reverse Transcriptase, 50U/μL   | Applied Biosystems       | 4366596        |
| 10X Reverse Transcription Buffer             | Applied Biosystems       | 4366596        |
| RNase Inhibitor (20 U/μL)                    | Applied Biosystems       | 4366596        |
| TaqMan® Small RNA assay, ID: CTDJXRN (miR21) | Applied Biosystems       | 4398988        |
| TaqMan® miRNA Assay, ID: 000200 (miR39)      | Applied Biosystems       | 4440887        |
| Carrier RNA                                  | Ambion                   | 432878         |
| TaqMan® universal master mix                 | Applied Biosystems       | 4318157        |
| THE RNA Storage Solution                     | Invitrogen               | AM7001         |

[00441] Spiking mixes and QC Controls (HQC, MQC and LQC) are shown in Table 24. miRNA standards were prepared in 20 ng/μL carrier RNA solution, spiking miRNA controls were prepared in 400 ng/μL carrier RNA solution. The miRNA standard mix and QC controls for HQC, MQC and LQC contain both miR21 and miR39 at the same copy numbers. The copy numbers for the assay reagents are shown in Table 24:

Table 24

| Reagent name | Conc. (copies/μL) | Diluent | Amount/PCR (copies/PCR) |
|--------------|-------------------|---------|-------------------------|
|--------------|-------------------|---------|-------------------------|

|               |         |                                      |         |
|---------------|---------|--------------------------------------|---------|
| miRNA Std mix | 2.5E+06 | 20 ng/ $\mu$ L carrier RNA solution  | 1.0E+07 |
| HQC           | 2.5E+05 | 20 ng/ $\mu$ L carrier RNA solution  | 1.0E+06 |
| MQC           | 2.5E+03 | 20 ng/ $\mu$ L carrier RNA solution  | 1.0E+04 |
| LQC           | 2.5E+02 | 20 ng/ $\mu$ L carrier RNA solution  | 1.0E+03 |
| ULQC          | 2.5E+01 | 20 ng/ $\mu$ L carrier RNA solution  | 1.0E+02 |
| miR39 spiking | 5.0E+05 | 400 ng/ $\mu$ L carrier RNA solution | 2.0E+05 |
| miR21 spiking | 5.0E+05 | 400 ng/ $\mu$ L carrier RNA solution | 2.0E+05 |

**[00442] CSF Sample Processing and miRNA Purification**

- i. Dilute spiking miR21 at 1:100 to low spiking miR21 solution (5E3 CN/5  $\mu$ L) with Std. carrier RNA solution.
- ii. Add 42 mL of 96-100% ethanol to concentrated wash Solution A and mix by inversion.
- iii. Prepare completed Lysis Buffer A by adding 25  $\mu$ L of  $\beta$ -mercaptoethanol to each 2.5 mL of the Lysis Buffer A.
- iv. Transfer 200  $\mu$ L of CSF to a new 1.5-mL tube.
- v. Add 300  $\mu$ L of the completed lysis buffer A to the CSF and vortex for 15 seconds.
- vi. Spike 5  $\mu$ L of miR39 spiking stock and miR21 spiking stock or diluted miR21 as illustrated in Table 25.

Table 25

| Sample Nr. | CSF ( $\mu$ L) | lysis buffer A ( $\mu$ L) | miR39 (CN/Sample) | miR21 (CN/Sample) | Ethanol ( $\mu$ L) | Elution Buffer ( $\mu$ L) |
|------------|----------------|---------------------------|-------------------|-------------------|--------------------|---------------------------|
| sample 1   | 200            | 300                       | 2.50E+06          | 2.50E+06          | 300                | 50                        |
| sample 2   | 200            | 300                       | 2.50E+06          | 2.50E+06          | 300                | 50                        |
| sample 3   | 200            | 300                       | 2.50E+06          | 2.50E+06          | 300                | 50                        |
| sample 4   | 200            | 300                       | 2.50E+06          | 2.50E+04          | 300                | 50                        |
| sample 5   | 200            | 300                       | 2.50E+06          | 2.50E+04          | 300                | 50                        |
| sample 6   | 200            | 300                       | 2.50E+06          | 2.50E+04          | 300                | 50                        |
| sample 7   | 200            | 300                       | 2.50E+06          | NA                | 300                | 50                        |
| sample 8   | 200            | 300                       | 5.00E+06          | NA                | 300                | 50                        |

- vii. Add 300  $\mu$ L of ethanol and immediately mix by pipetting up and down 5 times.
- viii. Incubate the sample tube for 5 minutes at room temperature.

- ix. Apply 500  $\mu$ L of the CSF lysate onto the provided column with a 2 mL collection tube and centrifuge for 1 minute at 8000 rpm. Discard the flowthrough.
- x. Place the column onto a new 2 mL collection tube, apply the remaining lysate to the column, centrifuge for 1 minute at 8000 rpm, and discard the flowthrough.
- xi. Place the column onto a new 2 mL collection tube, add 400  $\mu$ L of Wash Solution A to the column, centrifuge for 1 minute at 14,000 rpm, and discard the flowthrough.
- xii. Repeat the wash step twice. Discard the flowthrough and place the column on to a new 2-mL collection tube each wash.
- xiii. Spin the column at 14,000 rpm for 2 minutes.
- xiv. Place the column in a fresh 1.7-mL elution tube. Add 50  $\mu$ L of Elution Solution A to the center of the film on the bottom of the column.
- xv. Incubate the column for at least 5 minutes at room temperature.
- xvi. Centrifuge for 2 minutes at 2,000 rpm and then for additional 2 minutes at 14,000 rpm.
- xvii. Store the miRNA samples at -80°C, or on ice if the RT is performed immediately.

#### [00443] Reverse Transcription Protocol

- i. Prepare RT Master Mix according to Table 26.

Table 26

| Reagent                                         | Vol ( $\mu$ L) /<br>Rxn | Vol ( $\mu$ L) For<br>30 Rxns |
|-------------------------------------------------|-------------------------|-------------------------------|
| 100mM dNTPs (with dTTP)                         | 0.15                    | 4.5                           |
| MultiScribe Reverse Transcriptase, 50U/ $\mu$ L | 1.0                     | 30                            |
| 10X Reverse Transcription Buffer                | 1.5                     | 45                            |
| RNase Inhibitor (20 U/ $\mu$ L)                 | 0.19                    | 5.7                           |
| Primer Mix (10X) (miR21 & miR39)                | 1.5                     | 45                            |
| Nuclease-Free Water                             | 5.66                    | 169.8                         |
| Total                                           | 10                      | 300                           |

- ii. Pipette 20  $\mu$ L of RT Master Mix into the wells of a PCR strip.
- iii. Standard RNA dilution:
  1. Transfer 50  $\mu$ L of the miRNA standard mix (2.5E6 CN/ $\mu$ L each miRNA) in Tube 1 of the standard 8-well strip.
  2. Serially dilute the standard (Table 27) to construct a 7-point standard curve. Adjust the quantities in Table 27 as needed.



Table 27

| Tube.  | Standard Name | Vol (μL) of miRNA | From Tube | Vol (μL) of Matrix RNA | CN / RT / 20uL of RNA |
|--------|---------------|-------------------|-----------|------------------------|-----------------------|
| Tube 1 | Standard 1    | 50                | Std mix   | 0                      | 5.00E+07              |
| Tube 2 | Standard 2    | 5                 | Tube 1    | 45                     | 5.00E+06              |
| Tube 3 | Standard 3    | 5                 | Tube 2    | 45                     | 5.00E+05              |
| Tube 4 | Standard 4    | 5                 | Tube 3    | 45                     | 5.00E+04              |
| Tube 5 | Standard 5    | 5                 | Tube 4    | 45                     | 5.00E+03              |
| Tube 6 | Standard 6    | 5                 | Tube 5    | 40                     | 5.00E+02              |
| Tube 7 | Standard 7    | 5                 | Tube 6    | 20                     | 5.00E+01              |
| Tube 8 | NTC           | 0                 | NA        | 45                     | 0                     |
|        |               |                   |           | 285                    |                       |

## iv. RT reaction set up:

- Pipette 20 μL of RNA sample miRNA, QC samples, standards, and reagent controls into the PCR strips containing RT Master Mix solution according to the RT strip layout in Table 28:

Table 28

| Strip 1 | Strip 2 | Strip 3 |
|---------|---------|---------|
| Std1    | S1      | HQC1    |
| Std2    | S2      | MQC1    |
| Std3    | S3      | LQC1    |
| Std4    | S4      | ULQC1   |
| Std5    | S5      | HQC2    |
| Std6    | S6      | MQC2    |
| Std7    | S7      | LQC2    |
| MC      | RNA-RC  | ULQC2   |

- Briefly centrifuge to collect sample and reagents in the bottom of the well using a mini centrifuge.
- Load strips into a PCR Thermal Cycler and run according to the parameters provided in Table 29.

Table 29

| Step | Step Type | Time (minutes) | Temperature (°C) |
|------|-----------|----------------|------------------|
| 1    | Hold      | 30             | 16               |
| 2    | Hold      | 30             | 42               |
| 3    | Hold      | 5              | 85               |
| 4    | Hold      |                | 4                |

vii. Add 10  $\mu\text{L}$  of TE buffer to each well after RT thermal cycle. Mix by pipetting at least 8 times.

viii. Store the remaining RNA back in the  $-80^{\circ}\text{C}$  freezer.

**[00444] qPCR Protocol**

**[00445]** Prepare master mix A (MMA) for miR21 qPCR as provided in Table 29 and prepare master mix B (MMB) for miR39 qPCR as provided in Table 30.

Table 29

| Components       | 1 Rxn ( $\mu\text{L}$ ) | 60 x Rxns ( $\mu\text{L}$ ) |
|------------------|-------------------------|-----------------------------|
| 2x master mix    | 12.5                    | 750                         |
| 20x miR21 mix    | 1.25                    | 75                          |
| water            | 1.25                    | 75                          |
| Total master mix | 15                      | 900                         |

Table 30

| Components       | 1 RxN ( $\mu\text{L}$ ) | 60 x RxN ( $\mu\text{L}$ ) |
|------------------|-------------------------|----------------------------|
| 2x master mix    | 12.5                    | 750                        |
| 20x miR39 mix    | 1.25                    | 75                         |
| water            | 1.25                    | 75                         |
| Total master mix | 15                      | 900                        |

**[00446]** Load 15  $\mu\text{L}$  of MMA into columns 1 to 6, 15  $\mu\text{L}$  of MMB into columns 7-12, and 10  $\mu\text{L}$  of cDNA of standard, as provided in Table 31. Centrifuge at 2500 rpm for 5 minutes.

Table 31

| Column | 1 & 2 | 3&4    | 5&6   | 7&8    | 9&10   | 11&12 |
|--------|-------|--------|-------|--------|--------|-------|
| A      | Std1  | S1     | HQC1  | Std1   | S1     | HQC1  |
| B      | Std2  | S2     | MQC1  | Std2   | S2     | MQC1  |
| C      | Std3  | S3     | LQC1  | Std3   | S3     | LQC1  |
| D      | Std4  | S4     | ULQC1 | Std4   | S4     | ULQC1 |
| E      | Std5  | S5     | HQC2  | Std5   | S5     | HQC2  |
| F      | Std6  | S6     | MQC2  | Std6   | S6     | MQC2  |
| G      | Std7  | S7     | LQC2  | Std7   | S7     | LQC2  |
| H      | MC    | RNA-RC | ULQC2 | MC     | RNA-RC | ULQC2 |
| MM for | miR21 |        |       | miR-39 |        |       |

**[00447]** Thermal cycling and analysis was carried out as provided in Tables 32 and 33.

Table 32

| Stages  | Temp ( $^{\circ}\text{C}$ ) | Time (mm:ss) | Repeat |
|---------|-----------------------------|--------------|--------|
| Stage-1 | 50                          | 2:00         | x1     |

|         |    |       |     |
|---------|----|-------|-----|
| Stage-2 | 95 | 10:00 | x1  |
| Stage-3 | 95 | 0:15  | x40 |
|         | 60 | 1:00  |     |

Table 33

|                       |                                       |
|-----------------------|---------------------------------------|
| Instrument Type       | QuantStudio™ 7 Flex System            |
| Block                 | 96-Well                               |
| Type of Experiment    | Standard Curve                        |
| Reagents              | TaqMan Reagents                       |
| Properties            | Standard                              |
| Target 1:             | miR21; FAM Reporter, NFQ-MGB Quencher |
| Target 2:             | miR39, FAM Reporter, NFQ-MGB Quencher |
| Passive Reference:    | ROX                                   |
| Reaction Vol Per Well | 25 µL                                 |

**[00448] Results with Different Conditions**

**[00449]** Results of experiments quantifying miR21 and miR39 from NHP CSF fluid samples spiked with miRNA are described in Run-3, Run-4 and Run 5.

**[00450] Run-3:**

**[00451]** Table 34 summarizes the experimental protocol for Run 3. Run 3 comprised 200 µL CSF + 300 µL lysis buffer spiked with (a) 5e6 CN of miR39 in each sample; (b) 5e6 CN of miR21 in sample 1, 2, 5, and 6; and (c) 5e4 CN in sample 3, 4, 7, and 8.

Table 34

| Sample | Vol of CSF (µL) | lysis buffer A (µL) | Spiked miR39 (CN) | Spiked miR21 (CN) | Add Ethanol (µl) | Ethanol Incubation (minutes) | Elution (µL) | Incubation for Elution |
|--------|-----------------|---------------------|-------------------|-------------------|------------------|------------------------------|--------------|------------------------|
| 1      | 200             | 300                 | 5.00E+06          | 5.00E+06          | 300              | No                           | 50           | No                     |
| 2      | 200             | 300                 | 5.00E+06          | 5.00E+06          | 300              | No                           | 50           | No                     |
| 3      | 200             | 300                 | 5.00E+06          | 5.00E+04          | 300              | No                           | 50           | No                     |
| 4      | 200             | 300                 | 5.00E+06          | 5.00E+04          | 300              | No                           | 50           | No                     |
| 5      | 200             | 300                 | 5.00E+06          | 5.00E+06          | 300              | 5 min                        | 50           | 5 min                  |
| 6      | 200             | 300                 | 5.00E+06          | 5.00E+06          | 300              | 5 min                        | 50           | 5 min                  |
| 7      | 200             | 300                 | 5.00E+06          | 5.00E+04          | 300              | 5 min                        | 50           | 5 min                  |
| 8      | 200             | 300                 | 5.00E+06          | 5.00E+04          | 300              | 5 min                        | 50           | 5 min                  |

**[00452]** Reverse transcriptase reaction were carried using either: (a) 15 µL RT: 5 µL of RNA; (b) 25 µL RT: 10 µL of RNA; or (c) 40 µL RT: 20 µL of RNA. RNA standards for 15 µL RT, qPCR reads: 1e7, 1e6, 1e5, 1e4, 1e3, 1e2, 1e1, 0. RNA standards for 25 µL RT, qPCR reads were 2 times the quantity of the 15 µL of RNA. RNA standards for 40 µL RT, qPCR reads were 4 times the quantity of the 15 µL of RNA. TE buffer was added to obtain a final reaction volume of 50 µL.

[00453] Recovery and detection of miR21 and miR39 using various volumes of RT products is summarized in Table 35.

Table 35

| Std Curve | RNA (μL) | RT (μL) | Slope   | Intercept | R2     | E      |
|-----------|----------|---------|---------|-----------|--------|--------|
| miR21-1   | 5        | 15      | -3.378  | 30.432    | 0.9997 | 0.9773 |
| miR21-2   | 10       | 25      | -3.4071 | 30.626    | 0.9999 | 0.9656 |
| miR21-3   | 20       | 40      | -3.3897 | 30.457    | 0.9998 | 0.9725 |
| miR39-1   | 5        | 15      | -3.3269 | 40.699    | 1      | 0.998  |
| miR39-2   | 10       | 25      | -3.3645 | 40.828    | 0.9999 | 0.983  |
| miR39-3   | 20       | 40      | -3.3505 | 40.691    | 1      | 0.988  |

[00454] The standard curves derived from different amount of RNA and RT were close, thus higher amount of RT can be used to improve detection sensitivity. The slopes for both miR21 and miR39 were comparable.

[00455] In all groups, recovery rates for both miR21 and miR39 ranged from > 50% to < 110%. The recovery rates for higher conc. RNA (20 μL) and RT (40 μL) were highest and are shown Tables 36 and 37.

Table 36

| miR21    | Ct mean | Ct SD | Ct %CV | Qty mean | Qty SD | Qty %CV | miR21 Recovery |
|----------|---------|-------|--------|----------|--------|---------|----------------|
| Sample 1 | 11.74   | 0.03  | 0.29   | 331,714  | 7,688  | 2.32    | 82.93          |
| Sample 2 | 12.09   | 0.09  | 0.73   | 261,843  | 15,612 | 5.96    | 65.46          |
| Sample 3 | 18.44   | 0.06  | 0.34   | 3,508    | 149    | 4.26    | 87.70          |
| Sample 4 | 18.51   | 0.06  | 0.31   | 3,344    | 132    | 3.93    | 83.60          |
| Sample 5 | 11.67   | 0.06  | 0.50   | 349,237  | 13,884 | 3.98    | 87.31          |
| Sample 6 | 11.71   | 0.07  | 0.61   | 339,722  | 16,418 | 4.83    | 84.93          |
| Sample 7 | 18.29   | 0.06  | 0.33   | 3,886    | 158    | 4.06    | 97.16          |
| Sample 8 | 18.19   | 0.06  | 0.32   | 4,169    | 163    | 3.91    | 104.21         |

Table 37

| miR39    | Ct mean | Ct SD | Ct %CV | Qty mean | Qty SD | Qty %CV | miR39 Recovery |
|----------|---------|-------|--------|----------|--------|---------|----------------|
| Sample 1 | 22.56   | 0.02  | 0.08   | 258,669  | 3,256  | 1.26    | 64.67          |
| Sample 2 | 23.76   | 0.02  | 0.09   | 113,151  | 1,742  | 1.54    | 28.29          |
| Sample 3 | 22.72   | 0.12  | 0.55   | 230,992  | 19,812 | 8.58    | 57.75          |
| Sample 4 | 22.71   | 0.06  | 0.28   | 232,190  | 9,982  | 4.30    | 58.05          |
| Sample 5 | 22.53   | 0.02  | 0.08   | 263,391  | 3,274  | 1.24    | 65.85          |
| Sample 6 | 22.57   | 0.03  | 0.13   | 255,482  | 5,158  | 2.02    | 63.87          |
| Sample 7 | 22.49   | 0.00  | 0.02   | 270,583  | 901    | 0.33    | 67.65          |
| Sample 8 | 22.52   | 0.03  | 0.12   | 264,912  | 5,076  | 1.92    | 66.23          |

[00456] For Run-3, for the samples analyzed with the three RT reaction volumes, the average recovery rate for miR39 was 60.71% from the samples purified without two incubation steps

(ethanol incubation and incubation for elution), and 70.07% with the additional incubation steps. The average recovery rates for miR21 were 77.75% from the samples without the two incubation steps and 88.23% with the two incubation steps.

**[00457] Run-4:**

**[00458]** In Run-4, the RT-qPCR detection sensitivity was studied using an RNA standard curve from  $1 \times 10^7$  to  $10 \text{ CN/reaction}$  and various RT reaction volumes including 15, 25 and  $40 \mu\text{L}$  RT products.

**[00459]** In summary for Run-4, 10 copies of miRNA standards for both miR21 and miR39 were detected as 2 copies/ $\mu\text{L}$ , 1 copy/ $\mu\text{L}$  and 0.5 copy/ $\mu\text{L}$  present in various RT products correspondingly. Thus, a low concentration of miRNA can be detected with more RT volume. (Data not shown.)

**[00460] Run-5:**

**[00461]** The miRNA standards, QC controls including HQC, MQC and LQC and the protocol were analyzed using the Enhanced Assay described above. The following results were obtained for miR21 and QC controls (underlying data not shown):

- Standard curve :  $Y = -3.4243X + 41.238$ ,  $R^2 = 0.9997$ ,  $E = 0.9590$ . The Ct value mean for 10 copies/PCR was 38
- QC recovery rates were 95.79% for HQC, 92.07% for MQC and 82.97% for LQC

The following results were obtained for miR39 and QC controls (underlying data not shown):

- Standard curve :  $Y = -3.3401X + 41.245$ ,  $R^2 = 0.9992$ ,  $E = 0.9925$ . The Ct value mean for 10 copies/PCR was 37.54.
- QC recovery rates were 105.50% for HQC, 93.807% for MQC and 80.99% for LQC

**[00462]** While the invention has been described and illustrated with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various adaptations, changes, modifications, substitutions, deletions, or additions of procedures and protocols may be made without departing from the spirit and scope of the invention.

## CLAIMS

I/we claim:

1. An RNA polynucleotide comprising a targeting RNA sequence at least 80% identical to the sequence of any of SEQ ID NOs: 1-19; provided that if the targeting sequence is at least 80% identical to SEQ ID NO: 18, then the RNA polynucleotide is either (a) a pri-miRNA comprising the targeting sequence embedded in a scaffold selected from an S155e scaffold, a S-26a scaffold, and S-33 scaffold; or (b) the RNA polynucleotide comprises the sequence of SEQ ID NOs: 117 or 118.
2. The RNA polynucleotide of claim 1, wherein the targeting RNA sequence is at least 90% identical with any of SEQ ID NOs: 1-3, 5, 6, and 16-19.
3. The RNA polynucleotide of claim 2, wherein the targeting RNA sequence comprises the sequence of any of SEQ ID NOs: 1-3, 5, 6, and 16-19.
4. The RNA polynucleotide of claims 2 or 3, wherein the RNA polynucleotide further comprises a second RNA sequence, wherein the second RNA sequence is substantially complementary to the targeting RNA sequence.
5. The RNA polynucleotide of claim 4, wherein the RNA polynucleotide is a pri-miRNA comprising a pri-miRNA scaffold, a guide sequence and a passenger sequence, wherein the guide sequence comprises the targeting sequence and the passenger sequence comprises the second RNA sequence.
6. The RNA polynucleotide of claim 5, wherein the scaffold is an S155e scaffold, S26a scaffold, a S33 scaffold or S155 scaffold.
7. The RNA polynucleotide of claim 5, wherein the RNA polynucleotide comprises a sequence at least 90% identical to any of SEQ ID NOs: 51-62, 64-68, and 78-87.
8. The RNA polynucleotide of claim 1, wherein the RNA polynucleotide comprises the sequence of any of SEQ ID NOs: 51-62, 64-68, and 78-87.
9. The RNA polynucleotide of claim 1, wherein the RNA polynucleotide consists of the sequence of any of SEQ ID NOs: 51-62, 64-68, and 78-87.
10. The RNA polynucleotide of claim 4, wherein the RNA polynucleotide is a pre-miRNA comprising a guide sequence and a passenger sequence, wherein the guide sequence comprises the targeting sequence and the passenger sequence comprises the second RNA sequence.
11. The RNA polynucleotide of claim 4, wherein the RNA polynucleotide is a shRNA comprising a guide sequence and a passenger sequence, wherein the guide sequence comprises the targeting sequence and the passenger sequence comprises the second RNA sequence.
12. The RNA polynucleotide of claim 1, wherein the RNA polynucleotide comprises a sequence at least 90% identical to any of SEQ ID NOs: 88-96, 98-101, and 111-118.

13. The RNA polynucleotide of claim 12 wherein the RNA polynucleotide comprises the sequence of any of SEQ ID NOs: 88-96, 98-101, and 111-118.

14. The RNA polynucleotide of claim 4, wherein the RNA polynucleotide is an inhibitory RNA duplex comprising a guide sequence and a passenger sequence, wherein the guide sequence comprises the targeting sequence and the passenger sequence comprises the second RNA sequence.

15. The RNA polynucleotide of claim 14, wherein the inhibitory RNA duplex has a guide stand to passenger strand combination selected from group consisting of:

a) a guide strand comprising the sequence of SEQ ID NO: 1 and a passenger strand comprising a sequence at least 80% identical to the sequence of any of SEQ ID NOs: 20, 21, and 22;

b) a guide strand comprising the sequence of SEQ ID NO: 2 and a passenger strand comprising a sequence at least 80% identical to the sequence of any of SEQ ID NOs: 23, 24 and 25;

c) a guide strand comprising the sequence of SEQ ID NO: 3 and a passenger strand comprising a sequence at least 80% identical to the sequence of any of SEQ ID NOs: 26, 27 and 28;

d) a guide strand comprising the sequence of SEQ ID NO: 4 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 29;

e) a guide strand comprising the sequence of SEQ ID NO: 5 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 30;

f) a guide strand comprising the sequence of SEQ ID NO: 6 and a passenger strand comprising a sequence at least 80% identical to the sequence of any of SEQ ID NOs: 31, 32 and 33;

g) a guide strand comprising the sequence of SEQ ID NO: 7 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 34;

h) a guide strand comprising the sequence of SEQ ID NO: 8 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 35;

i) a guide strand comprising the sequence of SEQ ID NO: 9 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 36;

j) a guide strand comprising the sequence of SEQ ID NO: 10 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 37;

k) a guide strand comprising the sequence of SEQ ID NO: 11 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 38;

l) a guide strand comprising the sequence of SEQ ID NO: 12 and a passenger strand

comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 39;

m) a guide strand comprising the sequence of SEQ ID NO: 13 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 40;

n) a guide strand comprising the sequence of SEQ ID NO: 14 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 41;

o) a guide strand comprising the sequence of SEQ ID NO: 15 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 42;

p) a guide strand comprising the sequence of SEQ ID NO: 16 and a passenger strand comprising a sequence at least 80% identical to the sequence of any of SEQ ID NOs: 43, 44 and 45.

q) a guide strand comprising the sequence of SEQ ID NO: 17 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 46;

r) a guide strand comprising the sequence of SEQ ID NO: 19 and a passenger strand comprising a sequence at least 80% identical to the sequence of SEQ ID NO: 47; and

s) a guide strand comprising the sequence of SEQ ID NO: 18 and a passenger strand comprising a sequence at least 80% identical to the sequence of any of SEQ ID NOs: 48, 49, and 50.

16. The RNA polynucleotide of claim 1, wherein the targeting sequence is a guide sequence at least 90% identical to SEQ ID NO: 18 and the guide sequence is embedded in a S26a scaffold or a S33 scaffold.

17. The RNA polynucleotide of claim 1, wherein the RNA polynucleotide comprises the sequence of SEQ ID NOs: 117 or 118.

18. The RNA polynucleotide of claim 17, wherein the RNA polynucleotide comprises the sequence of SEQ ID NO: 86 or 87.

19. An optionally modified inhibitory RNA comprising (a) a guide strand able to hybridize to the target sequence of any of SEQ ID NOs: 119-137; and (b) a substantially complementary passenger sequence; wherein one or more nucleotides of the guide strand and the passenger strand are optionally modified RNA.

20. A polynucleotide comprising a nucleic acid sequence encoding the RNA polynucleotide of any one of claims 1-19.

21. An expression cassette comprising a nucleic acid sequence encoding the RNA polynucleotide of any one of claims 1-19, and one or more expression control elements operably linked to the nucleic acid sequence encoding the RNA polynucleotide.



22. The expression cassette of claim 21, wherein the expression cassette comprises an upstream promoter and a downstream polyadenylation signal, operably linked to the nucleic acid sequence encoding the RNA polynucleotide.

23. The expression cassette of claim 22, wherein the expression cassette comprises 5' to 3', operably linked to the nucleic acid sequence encoding the RNA polynucleotide, a promoter or promoter/enhancer, the nucleic acid sequence encoding the RNA polynucleotide, and a polyadenylation signal.

24. The expression cassette of claims 22 or 23, wherein the promoter provides for high CNS expression.

25. The expression cassette of claims 22 or 23, wherein the promoter is an EF-1 $\alpha$  promoter.

26. The expression cassette of any one of claims 21-25, wherein the expression cassette is DNA.

27. The expression cassette of claim 26, wherein the expression cassette comprises a sequence at least 90% identical with any one of SEQ ID NOs:188-199, 201-205, and 215-224.

28. The expression cassette of claim 27, comprising the sequence of SEQ ID NOs: 223 or 224.

29. A recombinant viral vector nucleic acid comprising the expression cassette of any one of claims 1-28, and 5' and/or 3' viral elements providing for viral packaging and/or replication.

30. The recombinant viral vector nucleic acid of claim 29, wherein the recombinant viral vector nucleic acid is a recombinant DNA and comprises an adeno-associated virus (AAV) inverted repeat (ITR) flanking the 5' terminus of the recombinant viral vector nucleic acid and an AAV ITR flanking the 3' terminus of the recombinant viral vector nucleic acid.

31. The recombinant viral vector nucleic acid of claim 30, wherein the 5' ITR and 3' ITR are selected from the 5' ITR and the 3' ITR of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.10, AAVrh.74 and AAV3B.

32. The recombinant viral vector nucleic acid of claim 30, wherein the recombinant viral vector nucleic acid is self-complementary.

33. The recombinant viral vector nucleic acid of claims 30 or 32, wherein the 5' ITR comprises a sequence at least 95% identical to SEQ ID NO: 254, and the 3' ITR comprises a sequence with a at least 95% identical to SEQ ID NO: 253.

34. The recombinant viral vector nucleic acid of claim 30, comprising a sequence at least 90% identical to the sequence of any of SEQ ID NOs: 233-235 and 237-242.

35. A recombinant viral nucleotide comprising a sequence of any of SEQ ID NOs: 237-242.

36. A delivery vehicle comprising a viral or a non-viral vector and the inhibitory RNA of claim 19, the polynucleotide of claim 20, the expression cassette of any one of claims 21-28 or

the recombinant viral vector nucleic acid of any one of claims 29-35.

37. The delivery vehicle of claim 36, wherein the delivery vehicle is the viral vector.

38. The delivery vehicle of claim 37, wherein the viral vector is a recombinant AAV, a recombinant lentivirus vector, or a recombinant adenovirus vector.

39. The delivery vehicle of claim 38, wherein the viral vector is a recombinant AAV.

40. The delivery vehicle of claim 39, wherein the recombinant AAV vector comprises a capsid comprising a VP1, VP2 or VP3 having a sequence at least 90% identical to a VP1, VP2 or VP3 of any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.74, AAV3B, AAV-2i8, AAVrh.10, AAVrh.8, AAVHSC, AAV-B1, AAV-AS, or AAV1/rh.10; or VP1 of SEQ ID NO: 257 or SEQ ID NO: 260.

41. The delivery vehicle of claim 40, wherein the capsid is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.74, AAV3B, AAV-2i8, AAVrh.10, AAVrh.8, AAVHSC, AAV-B1, AAV-AS, or AAV1/rh.10 capsid; or the capsid comprises VP1 of SEQ ID NO: 257 or SEQ ID NO: 260.

42. The delivery vehicle of claim 36, wherein the delivery vehicle is a nanoparticle selected from the group consisting of a lipid nanoparticle (LNP), a polymeric nanoparticle, a lipid polymer nanoparticle (LPNP), a protein or peptide-based nanoparticle, a DNA dendrimer or DNA-based nanocarrier, a carbon nanotube, a microparticle, a microcapsule, an inorganic nanoparticle, a peptide cage nanoparticle, and an exosome.

43. The delivery of vehicle of claim 42, wherein the delivery vehicle is an LNP or LPNP.

44. A pharmaceutical composition comprising the inhibitory RNA of claim 19, the polynucleotide of claim 20, the expression cassette of any one of claims 21-28, the recombinant viral vector nucleic acid of any one of claims 29-35, or the delivery vehicle of any one of claims 36-43 and a pharmaceutically acceptable carrier.

45. A method of reducing huntingtin expression in a cell or subject comprising administering to the cell or subject the inhibitory RNA of claim 19, the polynucleotide of claim 20, the expression cassette of any one of claims 21-28, the recombinant viral vector nucleic acid of any one of claims 29-35, the delivery vehicle of any one of claims 36-43, or the pharmaceutical composition of claim 44.

46. A method of treating a subject for Huntington disease comprising administering to a subject the inhibitory RNA of claim 19, the polynucleotide of claim 20, the expression cassette of any one of claims 21-28, the recombinant viral vector nucleic acid of any one of claims 29-35, the delivery vehicle of any one of claims 36-43, or the pharmaceutical composition of claim 44.

47. The method of claims 45 or 46, wherein administering comprises direct

intraparenchymal, intracisternal or intraventricular administration.

48. The method of claims 45 or 46, wherein initial administration is outside the CNS.

49. The method of any one of claims 45-48, wherein the subject is a human.

50. An AAV vector genome plasmid comprising the recombinant viral nucleic acid of any one of claims 30-35.

51. The AAV genome plasmid of claim 50, wherein the plasmid lacks *rep* and *cap* genes.

52. A method of producing a rAAV vector comprising the step of culturing a rAAV packaging cell line comprising a rAAV helper virus activity, wherein the genome of said production cell comprises the nucleic acid of any one of claims 30-35, a *rep* gene and a *cap* gene, wherein said rAAV vector is produced.

53. A method of producing a rAAV vector comprising the step of culturing a rAAV permissive cell comprising the rAAV genome plasmid of claim 51, wherein the rAAV permissive cell further comprises (a) *rep* and *cap* genes provided either as part of the cell genome and/or by one or more separate plasmids, and (b) helper virus activity provided by the cell genome and/or provided by one or more separate plasmids.

54. The method of claim 53, wherein the rAAV permissive cell is a packaging cell, wherein the genome of said packaging cell comprises a *cap* gene and a *rep* gene.

55. The method of claim 53, wherein either (a) said *rep* gene, said *cap* gene and said helper activity is provided in a single plasmid or (b) said *rep* gene and said *cap* gene is provided in a *rep/cap* plasmid and said helper activity is provided by a helper plasmid.

56. A method of obtaining a rAAV vector comprising the steps of (a) producing the rAAV vector using the method of any one of claims 52-55 and (b) purifying the rAAV vector.

57. A polynucleotide comprising the RNA sequence of SEQ ID NO: 255 wherein N<sub>01</sub> to N<sub>42</sub> are ribonucleotides, N<sub>01</sub> is complementary to N<sub>42</sub>, N<sub>02</sub> is not complementary to N<sub>41</sub>, N<sub>03</sub>-N<sub>10</sub> is complementary to N<sub>33</sub>-N<sub>40</sub>, N<sub>11</sub> is not complementary to N<sub>32</sub>, and N<sub>12</sub>-N<sub>21</sub> is complementary to N<sub>22</sub>-N<sub>31</sub>; or the corresponding DNA.

58. The polynucleotide of claim 57, further comprising a 5' flanking region and a 3' flanking region, wherein the polynucleotide comprises the RNA sequence of SEQ ID NO: 256; or the corresponding DNA sequence.

59. A DNA polynucleotide comprising in a 5' to 3' direction:

- (a) a 5' inverted terminal repeat (ITR) sequence comprising the sequence of SEQ ID NO: 262;
- (b) a CAG promoter;

(c) a pre-miRNA encoding sequence comprising the sequence of SEQ ID NO: 261, wherein the CAG promoter is operably linked to the pre-miRNA encoding sequence and a polyadenylation signal; and

(d) a 3' inverted terminal repeat (ITR) sequence comprising the sequence of SEQ ID NO: 263.

60. The polynucleotide of claim 59, wherein the polyadenylation signal comprises the sequence of SEQ ID NOs: 264 or 252 and the CAG promoter comprises the sequence of the SEQ ID NO: 250 or 265.

61. The polynucleotide of claims 59 or 60, wherein the end of the 5' ITR to the end 3' ITR is up to about 2.5 kb.

62. The polynucleotide of claim 59, comprising the sequence of SEQ ID NO: 266.

63. The polynucleotide of any one of claims 59-62, wherein the polynucleotide is plasmid further comprising an origin of replication and a selectable marker.

64. The polynucleotide of any one of claims 59-62, wherein the polynucleotide is a recombinant adeno-associated viral (rAAV) nucleic acid comprising the 5' ITR at the 5' terminus and 3' ITR at the 3' terminus.

65. A recombinant adeno-associated viral (rAAV) vector comprising:

(a) the rAAV nucleic acid of claim 64; and

(b) a rAAV capsid comprising: VP1 comprising the amino acid sequence of SEQ ID NO: 257, VP2 comprising the amino acid sequence of SEQ ID NO: 258, and VP3 comprising the amino acid sequence of SEQ ID NO: 259.

66. A recombinant adeno-associated viral (rAAV) vector comprising:

(a) an rAAV nucleic acid comprising a pre-miRNA encoding sequence comprising the sequence of SEQ ID NO: 261 operably linked to an upstream promoter and to a downstream polyA, and

(b) a rAAV capsid comprising: VP1 comprising the amino acid sequence of SEQ ID NO: 257, VP2 comprising the amino acid sequence of SEQ ID NO: 258, and VP3 comprising the amino acid sequence of SEQ ID NO: 259.

67. A pharmaceutical composition comprising about  $1.0 \times 10^{10}$  vg to about  $1.0 \times 10^{13}$  vg of the rAAV vector of claims 65 or 66, and a pharmaceutically acceptable carrier.

68. The pharmaceutical composition of claim 67, comprising about  $1.0 \times 10^{11}$  vg to about  $1.0 \times 10^{12}$  vg.

69. The composition of claims 67 or 68, further comprising a MRI imaging agent.

70. The composition of claim 69, wherein the MRI imaging agent is gadoteridol.

71. A pharmaceutical composition comprising a sufficient amount of the rAAV vector of any one of claims 39-41, 65 and 66 to provide a 20% to 90% reduction in total huntingtin protein, and a pharmaceutically acceptable carrier.

72. The pharmaceutical composition of claim 71, where the composition comprises a sufficient amount of the rAAV vector to provide a 20% to 65% reduction in total huntingtin protein.

73. The pharmaceutical composition of claim 72, wherein the composition comprises a sufficient amount of the rAAV vector to provide a 25% to 40% reduction in total huntingtin protein.

74. A method of treating Huntington disease in a subject comprising intraparenchymal administration to the subject the rAAV vector of any one of claims 39-41, 65 and 66, or the pharmaceutical composition of any one of claims 67-73.

75. A method of treating Huntington disease in a subject comprising;

(a) determining in the subject, the putamen and/or caudate volume in the right hemisphere and/or left hemisphere; and

(b) intraparenchymally administering to the subject a recombinant adeno-associated viral (rAAV) vector at a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> to the right hemisphere and/or a dose of about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup> to the left hemisphere; wherein said dose is based on the volume determined in step (a).

76. The method of claim 75, wherein administration to the right hemisphere comprises direct administration to the right hemisphere putamen and caudate; and administration to the left hemisphere comprises direct administration to the left hemisphere putamen and caudate.

77. The method of claim 76, wherein the putamen and caudate volume of the right hemisphere is measured and the putamen and caudate volume of the left hemisphere is measured, and each hemisphere independently receives a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> based on the measured volume.

78. The method of any one of claims 75-77, wherein a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the right hemisphere putamen and caudate in approximate proportion to the volume of the putamen and caudate of the right hemisphere.

79. The method of any one of claims 75-78, wherein a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the right hemisphere putamen and caudate in a ratio of about 67% putamen to about 33% caudate.

80. The method of any one of claims 75-79, wherein for the left hemisphere a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the putamen and to the caudate of the left

hemisphere in approximate proportion to the volume of the putamen and caudate of the left hemisphere.

81. The method of any one of claims 75-80, wherein a dose of  $2.0 \times 10^7$  vg/mm<sup>3</sup> to  $2.0 \times 10^8$  vg/mm<sup>3</sup> is administered to the left hemisphere putamen and caudate in a ratio of about 67% putamen to about 33% caudate.

82. The method of any one of claims 75-81, wherein the same dose is administered to the right hemisphere and left hemisphere.

83. The method of any one of claims 75-82, wherein administration to the caudate comprises a parietal trajectory.

84. The method of any one of claims 75-83, wherein administration to the putamen comprises an occipital trajectory.

85. The method of any one of claims 75-84, wherein each hemisphere is administered  $1.0 \times 10^{10}$  vg to  $1.0 \times 10^{13}$  vg of rAAV vector.

86. The method of claim 85, wherein each hemisphere is administered  $1.0 \times 10^{11}$  vg to  $1.0 \times 10^{12}$  vg of rAAV vector.

87. The method of any one of claims 75-86, wherein the rAAV vector is the rAAV vector of any one of claims 39-41, 65 and 66.

88. The method of any one of claims 75-87, wherein administration comprises convection enhanced delivery.

89. The method of any one of claims 75-88, comprising:

(e) determining the putamen and/or caudate volume in mm<sup>3</sup> in the right hemisphere and/or the left hemisphere; and

(f) multiplying said volume obtained in step (a) by a desired dose in vg/mm<sup>3</sup>, further obtaining said desired dose in vg per putamen and caudate; and

(g) manipulating said dose obtained in the step (b) using a given drug concentration in vg/ml to obtain a required drug dose volume; and

(h) intraparenchymally administering to the subject a dose of the rAAV vector, wherein said dose in ml is obtained according to the steps (b) and (c).

90. The method of claim 89, wherein the desired drug concentration is about  $6.2 \times 10^{11}$  vg/ml.

91. A method of treating Huntington disease in a subject comprising:

(e) determining the putamen and/or caudate volume in mm<sup>3</sup> the right hemisphere and/or the left hemisphere; and

(f) multiplying said volume obtained in step (a) by a desired dose in vg/mm<sup>3</sup>, further obtaining said desired dose in vg per putamen and/or caudate; and

(g) manipulating said dose obtained in the step (b) using a given drug concentration in vg/ml to obtain a required drug dose volume; and

(h) intraparenchymally administering to the subject a dose of a recombinant adeno-associated viral (rAAV) vector, wherein said dose in ml is obtained according to said steps (b) and (c).

92. The method of claim 91, wherein the desired dose is about  $2.0 \times 10^7$  vg/mm<sup>3</sup> to about  $2.0 \times 10^8$  vg/mm<sup>3</sup>.

93. The method of claim 92, wherein the desired drug concentration is about  $6.2 \times 10^{11}$  vg/ml.

94. The method of any one of claims 89-93, wherein step (a) measures the putamen and caudate in one or both hemispheres and step (b) obtains a dose for both the putamen and caudate.

95. The method of any one of claims 74-94, wherein the rAAV nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 261, and expressed inhibitory RNA is measured in cerebrospinal fluid by detecting an miR155 scaffold.

96. The method of claim 95, wherein detection of expressed inhibitory nucleic acid comprises the steps of: (a) RNA purification from cerebrospinal fluid; (b) reverse transcriptase; and (c) quantitative Polymerase Chain Reaction (qPCR).

97. The method of claim 96, wherein step (a) comprises incubation of cerebrospinal fluid in lysis buffer and ethanol for about 5 minutes.

98. The method of claims 96 or 97, wherein said step (b) comprises about 20 µL RNA and about 40 µL RT.

99. The method of any one of claims 74-98, wherein said subject is a human.