VARIANT RNAi

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application No. 62/561,843, filed September 22, 2017, which is hereby incorporated by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 159792014740SeqList.txt, date recorded: September 20, 2018, size:19 KB).

FIELD OF THE INVENTION

[0003] The present invention relates to variant RNAi molecules. In some aspects, the invention relates to variant RNAi to treat Huntington's disease.

BACKGROUND

[0004] RNA interference (RNAi) has been shown to be a useful tool for gene silencing in basic research of gene function and shows great promise as a therapeutic agent to suppress genes associated with the development of a number of diseases. In nature, gene regulation by RNAi occurs through small RNAs known as microRNAs (miRNAs) (Ambros, (2004) *Nature* 431:350-355; Krol *et al.*, (2010) *Nat. Rev. Genet.* 11:597-610). MicroRNAs have emerged as powerful regulators of diverse cellular processes, and when delivered by viral vectors, artificial miRNAs are continually expressed, resulting in a robust and sustained suppression of target genes. The elucidation of the mechanisms involved in miRNA processing has allowed scientists to co-opt the endogenous cellular RNAi machinery and direct the degradation of a target gene product with the use of artificial miRNAs (see, *e.g.*, US PG Pub. 2014/0163214 and Davidson *et al.*, (2012) *Cell* 150:873-875).

[0005] A hurdle to the clinical development of RNAi is the potential for off-target silencing where the seed region of the RNAi (typically nucleotides 1-7 or 1-8) pairs with sequences in non-target mRNAs in the 3' untranslated region (UTR) leading to transcript

destabilization. Attempts to reduce off-target silencing include the use of algorithms to identify candidate seed sequences with high specificity for the target mRNA with minimal off-target potential (Boudreau RL *et al.*, (2012) *Nucl. Acids Res.* 41(1):e9) and placing an internal bulge in the guide region of the RNAi (Terasawa *et al.*, (2011) *Journal of nucleic acids* 2011:131579).

[0006] RNAi has been investigated as a therapeutic to treat Huntington's disease (HD). HD is an inherited neurodegenerative disease caused by an expansion of the CAG repeat in exon 1 of the huntingtin gene (HTT). The resulting extension of the polyglutamine tract in the N-terminal region confers a toxic gain-of-function to the mutant huntingtin protein (mHtt). The potential of silencing mHtt expression as a therapeutic strategy for HD was first demonstrated in a conditional mouse model of the disease (Yamamoto *et al.*, (2000) *Cell* 101:57-66.). When the expression of mHtt was induced in these mice, pathological and behavioral aberrations became apparent. Subsequent tetracycline-mediated repression of the mHtt transgene reversed these abnormalities, indicating that a reduction of mHtt levels allowed protein clearance mechanisms within neurons to normalize mHtt-induced changes. Hence, therapeutic strategies that reduce mHtt levels could potentially halt disease progression and alleviate HD symptoms. miRNAs that target Htt are provided in WO 2016/130589, incorporated herein in its entirety.

[0007] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

[0008] In some aspects, the invention provides an RNAi comprising a first strand and a second strand, wherein a) the first strand and the second strand form a duplex; b) the first strand comprises a guide region, wherein the guide region comprises the nucleic acid sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1) or 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7); and c) the second strand comprises a non-guide region. In some embodiments, the nucleic the guide region comprises the nucleic acid sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1) and the non-guide region comprises the sequence 5'- CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2). In some embodiments, the first strand comprises a nucleic acid sequence having

about 90% identity to SEQ ID NO:1 or about 90% identity to SEQ ID NO:2. In other embodiments, the nucleic the guide region comprises the nucleic acid sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7) and the non-guide region comprises the sequence 5'- UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8). In some embodiments, the second strand comprises a nucleic acid sequence having about 90% identity to SEQ ID NO:7 or about 90% identity to SEQ ID NO:8. In some embodiments of the above embodiments, the first strand and the second strand are linked by means of RNA linker capable of forming a loop structure. In some embodiments, the RNA linker comprises from 4 to 50 nucleotides. In some embodiments, the loop structure comprises 4 to 20 nucleotides. In some embodiments, the RNAi comprises 5' to 3' the second strand, the RNA linker, and the first strand. In some embodiments, the RNAi comprises 5' to 3' the first strand, the RNA linker, and the second strand. In some embodiments, the RNAi comprises the nucleic acid sequence of SEQ ID NO:4 or SEQ ID NO:10. In some embodiments, the RNAi comprises a nucleotide sequence about 90% identical to the nucleotide sequence of SEQ ID NO:4 or SEQ ID NO:10. In some embodiments, the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA). In some embodiments, the RNAi targets RNA encoding a polypeptide associated with Huntington's disease. In some embodiments, the polypeptide is huntingtin. In some embodiments, the huntingtin comprises a mutation associated with Huntington's disease.

[0009] In some embodiments of the above aspects and embodiments, the invention provides an expression construct comprising nucleic acid encoding the RNAi of any one of claims 1-16. In some embodiments, the nucleic acid encoding the RNAi comprises a miRNA scaffold. In some embodiments, the nucleic acid encoding the RNAi is operably linked to a promoter. In some embodiments, the promoter is selected from a cytomegalovirus (CMV) immediate early promoter, an RSV LTR, a MoMLV LTR, a phosphoglycerate kinase- 1 (PGK) promoter, a simian virus 40 (SV40) promoter, a CK6 promoter, a transthyretin promoter (TTR), a TK promoter, a tetracycline responsive promoter (TRE), an HBV promoter, an hAAT promoter, a LSP promoter, a chimeric liver-specific promoter (LSP), an E2F promoter, a telomerase (hTERT) promoter; a cytomegalovirus enhancer/chicken beta-actin/Rabbit β-globin promoter (CAG) promoter, an elongation factor 1-alpha promoter (EFl-alpha) promoter, a human β-glucuronidase promoter, a chicken β-actin (CBA) promoter, a retroviral Rous sarcoma virus (RSV) LTR promoter, a dihydrofolate reductase promoter, and a 13-actin promoter. In some embodiments, the expression construct further comprises a

polyadenylation signal. In some embodiments, the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA.

[0010] In some embodiments, the invention provides a vector comprising the expression construct of any one of the embodiments described herein. In some embodiments, the vector is a recombinant adeno-associated virus (rAAV) vector, a recombinant adenoviral vector, a recombinant lentiviral vector or a recombinant herpes simplex virus (HSV) vector. In some embodiments, the vector is a recombinant adenoviral vector. In some embodiments, the recombinant adenoviral vector is derived from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3. In some embodiments, the recombinant adenoviral vector is derived from adenovirus serotype 2 or a variant of adenoviral serotype 5. In some embodiments, the vector is a recombinant lentiviral vector. In some embodiments, the recombinant lentiviral vector is derived from a lentivirus pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein. In some embodiments, the vector is a rHSV vector. In some embodiments, the rHSV vector is derived from rHSV-1 or rHSV-2.

[0011] In some embodiments of the above aspects and embodiments, the vector is a rAAV vector. In some embodiments, the expression construct is flanked by one or more AAV inverted terminal repeat (ITR) sequences. In some embodiments, the expression construct is flanked by two AAV ITRs. In some embodiments, the AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV serotype ITRs. In some embodiments, the AAV ITRs are AAV2 ITRs. In some embodiments, the vector further comprises a stuffer nucleic acid. In some embodiments, the stuffer nucleic acid is located upstream or downstream of the nucleic acid encoding the RNAi. In some embodiments, the vector is a self-complementary rAAV vector. In some embodiments, the vector comprises first nucleic acid sequence encoding the RNAi and a second nucleic acid sequence encoding a complement of the RNAi, wherein the first nucleic acid sequence can form intrastrand base pairs with the second nucleic acid sequence along

most or all of its length. In some embodiments, the first nucleic acid sequence and the second nucleic acid sequence are linked by a mutated AAV ITR, wherein the mutated AAV ITR comprises a deletion of the D region and comprises a mutation of the terminal resolution sequence. In some embodiments, the invention provides a cell comprising any of vectors (*e.g.*, rAAV vectors) described herein.

[0012] In some embodiments of the above aspects and embodiments, the invention provides a viral particle comprising any of the vectors described herein, wherein the viral particle is an AAV particle encapsidating the rAAV vector, an adenovirus particle encapsidating the recombinant adenoviral vector, a lentiviral particle encapsidating the recombinant lentiviral vector or an HSV particle encapsidating the recombinant HSV vector. In some embodiments, the viral particle is an adenovirus particle encapsidating the recombinant adenoviral vector. In some embodiments, the adenovirus particle comprises a capsid from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3. In some embodiments, the adenovirus particle comprises an adenovirus serotype 2 capsid or a variant of an adenoviral serotype 5 capsid. In some embodiments, the viral particle is a lentiviral particle encapsidating the recombinant lentiviral vector. In some embodiments, the lentiviral particle comprises a capsid pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein. In some embodiments, the viral particle is a HSV particle. In some embodiments, the HSV particle is a rHSV-1 particle or a rHSV-2 particle.

[0013] In some embodiments, the invention provides a recombinant AAV particle comprising any of the rAAV vectors described herein. In some embodiments, the AAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, a goat AAV, AAV1/AAV2 chimeric, bovine AAV, or mouse AAV capsid, rAAV2-HBKO capsid (see WO 2015/168666, which is incorporated herein by reference). In some embodiments, the ITR and the capsid of the rAAV viral particle are derived from the same AAV serotype. In

some embodiments, the ITR and the capsid of the rAAV viral particle are derived from different AAV serotypes. In some embodiments, the ITR is derived from AAV2 and the capsid of the rAAV particle is derived from AAV1. In some embodiments, the rAAV vector comprises 5' to 3' an AAV2 ITR, a promoter, nucleic acid encoding the RNAi, a polyadenylation signal, and an AAV2 ITR. In some embodiments, the promoter is a CBA promoter. In some embodiments, the polyadenylation signal is a bovine growth hormone polyadenylation signal. In some embodiments, the rAAV vector comprises 5' to 3' all or a portion (e.g., a functional portion) of an AAV2 ITR, the CBA promoter, an intron (e.g., a chimeric intron), nucleic acid encoding the RNAi, a bovine growth hormone polyadenylation signal, and an AAV2 ITR. In some embodiments, the vector further comprises a stuffer nucleic acid. In some embodiments, the stuffer nucleic acid further comprises nucleic acid encoding a reporter polypeptide (*e.g.*, green fluorescent protein (GFP)). In some embodiments, the stuffer nucleic acid is located upstream or downstream of the nucleic acid encoding the RNAi.

[0014] In some embodiments, the invention provides a composition (*e.g.*, a pharmaceutical composition) comprising any of the viral particles (*e.g.*, rAAV particles) described herein. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

[0015] In some aspects, the invention provides a kit comprising any of the RNAi described herein. In some embodiments, the kit comprises any of the viral particles (*e.g.*, rAAV particles) described herein. In some embodiments, the kit comprises any of the compositions described herein. In some embodiments, the kit further comprises instructions for use.

[0016] In some aspects, the invention provides methods for treating Huntington's disease in a mammal comprising administering to the mammal an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-UGGCCGUCCAUCUUGGACCCG -3' (SEQ ID NO:1) and a second strand comprising a second nucleic acid comprising the sequence 5'- CGGGUCCAAGAUGGACGGCCA -3' (SEQ ID NO:2) or a first strand comprising a first nucleic acid comprising the sequence 5'-AGUCGGUGUGGUUGACAAGCA -3' (SEQ ID NO:7) and a second strand comprising a second nucleic acid comprising the sequence 5'- UGCUUGUCAACCACACCGACU -3' (SEQ ID NO:8). In some aspects, the invention provides methods for inhibiting the

expression of htt in a mammal with Huntington's disease comprising administering to the mammal an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'- UGGCCGUCCAUCUUGGACCCG -3' (SEQ ID NO:1) and a second strand comprising a second nucleic acid comprising the sequence 5'- CGGGUCCAAGAUGGACGGCCA -3' (SEQ ID NO:2) or a first strand comprising a first nucleic acid comprising the sequence 5'- AGUCGGUGUGGUUGACAAGCA -3' (SEQ ID NO:7) and a second strand comprising a second nucleic acid comprising the sequence 5'- UGCUUGUCAACCACACCGACU -3' (SEQ ID NO:10). In some aspects, the invention provides methods for inhibiting the accumulation of htt in a cell of a mammal with Huntington's disease comprising administering to the mammal an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'- UGGCCGUCCAUCUUGGACCCG -3' (SEQ ID NO:1) and a second strand comprising a second nucleic acid comprising the sequence 5'- CGGGUCCAAGAUGGACGGCCA -3' (SEQ ID NO:2) or a first strand comprising a first nucleic acid comprising the sequence 5'- AGUCGGUGUGGUUGACAAGCA -3' (SEQ ID NO:7) and a second strand comprising a second nucleic acid comprising the sequence 5'- UGCUUGUCAACCACACCGACU -3' (SEQ ID NO:8).

[0017] In some embodiments of the above methods, the first strand comprises a nucleic acid sequence having about 90% identity to SEQ ID NO:1 or about 90% identity to SEQ ID NO:7. In some embodiments, the second strand comprises a nucleic acid sequence having about 90% identity to SEQ ID NO:2 or about 90% identity to SEQ ID NO:8. In some embodiments, the first strand and the second strand are linked by means of RNA linker capable of forming a loop structure. In some embodiments, the RNA linker comprises from 4 to 50 nucleotides. In some embodiments, the loop structure comprises 4 to 20 nucleotides. In some embodiments, the RNAi comprises 5' to 3' the second strand, the RNA linker, and the first strand. In some embodiments, the RNAi comprises 5' to 3' the first strand, the RNA linker, and the second strand. In some embodiments, the RNAi comprises the nucleic acid sequence of SEQ ID NO:4 or SEQ ID NO:10. In some embodiments, the RNAi comprises a nucleotide sequence about 90% identical to the nucleotide sequence of SEQ ID NO:4 or SEQ ID NO:10.

[0018] In some embodiments of the above methods, the RNAi is encoded on an expression construct. In some embodiments, the nucleic acid encoding the RNAi comprises

a miRNA scaffold. In some embodiments, the nucleic acid encoding the RNAi is operably linked to a promoter. In some embodiments, the promoter is capable of expressing the RNAi in the brain of a mammal. In some embodiments, the promoter is selected from a cytomegalovirus (CMV) immediate early promoter, a RSV LTR, a MoMLV LTR, a phosphoglycerate kinase- 1 (PGK) promoter, a simian virus 40 (SV40) promoter, a CK6 promoter, a transthyretin promoter (TTR), a TK promoter, a tetracycline responsive promoter (TRE), an HBV promoter, an hAAT promoter, a LSP promoter, a chimeric liver-specific promoter (LSP), a E2F promoter, a telomerase (hTERT) promoter; a cytomegalovirus enhancer/chicken beta-actin/Rabbit β-globin (CAG) promoter, an elongation factor 1-alpha promoter (EFl-alpha) promoter and a human β-glucuronidase promoter. In some embodiments, the promoter is a hybrid chicken β-actin promoter. In some embodiments, the nucleic acid further comprises all or a portion (e.g., functional portion) of an intron and a polyadenylation signal. In some embodiments, the polyadenylation signal is a bovine growth hormone polyadenylation signal, and the intron is a chimeric intron.

[0019] In some embodiments of the above methods, the RNAi is encoded on a vector comprising the expression construct of any one of the embodiments described herein. In some embodiments, the vector is a recombinant adeno-associated virus (rAAV) vector, a recombinant adenoviral vector, a recombinant lentiviral vector or a recombinant herpes simplex virus (HSV) vector. In some embodiments, the vector is a recombinant adenoviral vector. In some embodiments, the recombinant adenoviral vector is derived from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3. In some embodiments, the recombinant adenoviral vector is derived from adenovirus serotype 2 or a variant of adenoviral serotype 5. In some embodiments, the vector is a recombinant lentiviral vector. In some embodiments, the recombinant lentiviral vector is derived from a lentivirus pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein. In some embodiments, the vector is a rHSV vector. In some embodiments, the rHSV vector is derived from rHSV-1 or rHSV-2.

[0020] In some embodiments of the above methods, the vector is a rAAV vector. In some embodiments, the expression construct is flanked by one or more AAV inverted terminal repeat (ITR) sequences. In some embodiments, the expression construct is flanked by two AAV ITRs. In some embodiments, the AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV serotype ITRs. In some embodiments, the AAV ITRs are AAV2 ITRs. In some embodiments, the vector further comprises a stuffer nucleic acid. In some embodiments, the stuffer nucleic acid is located between the promoter and the nucleic acid encoding the RNAi. In some embodiments, the vector is a self-complementary rAAV vector. In some embodiments, the vector comprises first nucleic acid sequence encoding the RNAi and a second nucleic acid sequence encoding a complement of the RNAi, wherein the first nucleic acid sequence can form intrastrand base pairs with the second nucleic acid sequence along most or all of its length. In some embodiments, the first nucleic acid sequence and the second nucleic acid sequence are linked by a mutated AAV ITR, wherein the mutated AAV ITR comprises a deletion of the D region and comprises a mutation of the terminal resolution sequence. In some embodiments, the invention provides a cell comprising any of vectors (*e.g.*, rAAV vectors) described herein.

[0021] In some embodiments of the above methods, vector encoding the RNAi is in a viral particle, wherein the viral particle is an AAV particle encapsidating the rAAV vector, an adenovirus particle encapsidating the recombinant adenoviral vector, a lentiviral particle encapsidating the recombinant lentiviral vector or an HSV particle encapsidating the recombinant HSV vector. In some embodiments, the viral particle is an adenovirus particle encapsidating the recombinant adenoviral vector. In some embodiments, the adenovirus particle comprises a capsid from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3. In some embodiments, the adenovirus particle comprises an adenovirus serotype 2 capsid or a variant of an adenoviral serotype 5 capsid. In some embodiments, the viral particle is a lentiviral particle encapsidating the recombinant lentiviral vector. In some embodiments, the lentiviral particle comprises a capsid pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola

virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein. In some embodiments, the viral particle is a HSV particle. In some embodiments, the HSV particle is a rHSV-1 particle or a rHSV-2 particle.

[0022] In some embodiments of the above methods, the invention provides a recombinant AAV particle comprising any of the rAAV vectors described herein. In some embodiments, the AAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, a goat AAV, AAV1/AAV2 chimeric, bovine AAV, or mouse AAV capsid rAAV2/HBoV1 serotype capsid. In some embodiments, the ITR and the capsid of the rAAV viral particle are derived from the same AAV serotype. In some embodiments, the ITR and the capsid of the rAAV viral particle are derived from different AAV serotypes. In some embodiments, the ITR is derived from AAV2 and the capsid of the rAAV particle is derived from AAV1. The invention provides a vector comprising the expression construct of any one of the embodiments described herein. In some embodiments, the vector is a recombinant adeno-associated virus (rAAV) vector, a recombinant adenoviral vector, a recombinant lentiviral vector or a recombinant herpes simplex virus (HSV) vector. In some embodiments, the vector is a recombinant adenoviral vector. In some embodiments, the recombinant adenoviral vector is derived from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3. In some embodiments, the recombinant adenoviral vector is derived from adenovirus serotype 2 or a variant of adenoviral serotype 5. In some embodiments, the vector is a recombinant lentiviral vector. In some embodiments, the recombinant lentiviral vector is derived from a lentivirus pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein. In some embodiments, the vector is a rHSV vector. In some embodiments, the rHSV vector is derived from rHSV-1 or rHSV-2.

[0023] In some embodiments of the above aspects and embodiments, the vector is a rAAV vector. In some embodiments, the expression construct is flanked by one or more

AAV inverted terminal repeat (ITR) sequences. In some embodiments, the expression construct is flanked by two AAV ITRs. In some embodiments, the AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV serotype ITRs. In some embodiments, the AAV ITRs are AAV2 ITRs. In some embodiments, the vector further comprises a stuffer nucleic acid. In some embodiments, the stuffer nucleic acid is located between the promoter and the nucleic acid encoding the RNAi. In some embodiments, the vector is a self-complementary rAAV vector. In some embodiments, the vector comprises first nucleic acid sequence encoding the RNAi and a second nucleic acid sequence encoding a complement of the RNAi, wherein the first nucleic acid sequence can form intrastrand base pairs with the second nucleic acid sequence along most or all of its length. In some embodiments, the first nucleic acid sequence and the second nucleic acid sequence are linked by a mutated AAV ITR, wherein the mutated AAV ITR comprises a deletion of the D region and comprises a mutation of the terminal resolution sequence. In some embodiments, the invention provides a cell comprising any of vectors (*e.g.*, rAAV vectors) described herein.

[0024] In some embodiments of the above aspects and embodiments, the invention provides a viral particle comprising any of the vectors described herein, wherein the viral particle is an AAV particle encapsidating the rAAV vector, an adenovirus particle encapsidating the recombinant adenoviral vector, a lentiviral particle encapsidating the recombinant lentiviral vector or an HSV particle encapsidating the recombinant HSV vector. In some embodiments, the viral particle is an adenovirus particle encapsidating the recombinant adenoviral vector. In some embodiments, the adenovirus particle comprises a capsid from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3. In some embodiments, the adenovirus particle comprises an adenovirus serotype 2 capsid or a variant of an adenoviral serotype 5 capsid. In some embodiments, the viral particle is a lentiviral particle encapsidating the recombinant lentiviral vector. In some embodiments, the lentiviral particle comprises a capsid pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein. In some embodiments, the

viral particle is a HSV particle. In some embodiments, the HSV particle is a rHSV-1 particle or a rHSV-2 particle.

[0025] In some embodiments, the invention provides a recombinant AAV particle comprising any of the rAAV vectors described herein. In some embodiments, the AAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, a goat AAV, AAV1/AAV2 chimeric, bovine AAV, or mouse AAV capsid rAAV2/HBoV1 serotype capsid. In some embodiments, the ITR and the capsid of the rAAV viral particle are derived from the same AAV serotype. In some embodiments, the ITR and the capsid of the rAAV viral particle are derived from different AAV serotypes. In some embodiments, the ITR is derived from AAV2 and the capsid of the rAAV particle is derived from AAV1. In some embodiments, the rAAV vector comprises 5' to 3' an AAV2 ITR, a promoter, nucleic acid encoding the RNAi, a polyadenylation signal, and an AAV2 ITR. In some embodiments, the promoter is a CBA promoter. In some embodiments, the polyadenylation signal is a bovine growth hormone polyadenylation signal. In some embodiments, the rAAV vector comprises 5' to 3' an AAV2 ITR, the CBA promoter, an intron, nucleic acid encoding the RNAi, a bovine growth hormone polyadenylation signal, and an AAV2 ITR. In some embodiments, the vector further comprises a stuffer nucleic acid. In some embodiments, the stuffer nucleic acid comprises nucleic acid encoding a green fluorescent protein (GFP). In some embodiments, the stuffer nucleic acid is located between the promoter and the nucleic acid encoding the RNAi.

[0026] In some embodiments of the above methods, the viral particle (*e.g.*, the rAAV particle) is in a composition (*e.g.*, a pharmaceutical composition). In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] **Fig. 1A** shows a DNA sequence for Htt miRNA 206 (SEQ ID NO:22) and Htt miRNA 207 (SEQ ID NO:9). **Fig. 1B** shows a map of ssAAV2/1miRHtt.de. **Fig. 1C** shows the sequence of the coding strand of ssAAV2/1miRHtt.de (SEQ ID NO: 16) and the noncoding strand of ssAAV2/1miRHtt.de (SEQ ID NO:19).

[0028] **Fig. 2** shows the ability of Htt miRNA 170XA, Htt miRNA 206 and Htt miRNA 207 to mediate Htt reduction in vitro. Values are given as the means ± SEM.

[0029] **Figs. 3A and 3B** show the ability of AAV2/1-Htt miRNA 206 and AAV2/1-Htt miRNA 207 to mediate Htt reduction as measured by protein (**Fig. 3A**) or mRNA (**Fig. 3B**). CTL-3 is a noncoding miRNA control. Values are given as the means ± SEM. * indicates significantly different from CTL3 mice, $p<0.05$; ANOVA followed by Tukey's post-hoc test.

[0030] **Figs. 4A and 4B** show body weight (**Fig. 4A**) and brain weight (**Fig. 4B**) one month after administration of AAV2/1-Htt miRNA 206 and AAV2/1-Htt miRNA 207. CTL-3 is a noncoding miRNA control. *Significantly different from CTL3 control mice, $p<0.05$; ANOVA followed by Tukey's post-hoc test.

[0031] **Figs. 5A-5D** show human Htt was significantly reduced in the striatum of AAV2/1-miRNA-Htt-207 injected YAC128 and FVB wild-type littermate mice. Human HTT protein levels are shown in **Fig. 5A**. Mouse HTT protein levels are shown in **Fig. 5B**. Human HTT mRNA levels are shown in **Fig. 5C**. Mouse HTT mRNA levels are shown in **Fig. 5D**.

[0032] **FIGS. 6A and 6B** show that treatment with AAV2/1-miRNA-Htt-207 can correct motor coordination deficits in YAC128 mice as determined by rotarod test (**FIG. 6A**) and a depressive phenotype in YAC128 mice as determined using the Porsolt swim test (**FIG. 6B**). Mice were either wild type (WT also referred to as FVB) or YAC128 (YAC) treated with a non-coding RNA control (CTL3) or AAV2/1-miRNA-Htt-207 (207) .figs.6 * indicate a significant deficiency in CTL3 noncoding miRNA control mice, $p<0.05$; ANOVA followed by Tukey's post-hoc test compared to wild type mice, wild type mice treated with AAV2/1-miRNA-Htt-207, and YAC128 mice treated with AAV2/1-miRNA-Htt-207.

[0033] **FIGS. 7A and 7B** show body weights (FIG. 7A) and brain weights (FIG. 7B) three months post infection. Mice were either wild type (WT) or YAC128 (YAC) treated with a non-coding RNA control (CTL3) or AAV2/1-miRNA-Htt-207 (207).

[0034] **FIG. 8** shows a map of a self-complementary miRHtt 207 vector genome. CMV enh/CBA promoter is the CMV enhancer/chicken beta actin promoter. Δ chimeric intron is an abbreviated chimeric intron. BGH is the bovine growth hormone polyadenlyation signal. ΔITR is an AAV ITR lacking the terminal resolution sequence.

[0035] **FIG. 9** shows a map of an alternative self-complementary miRHtt 207 vector genome. CBA promoter is the chicken beta actin promoter. Δ chimeric intron is an abbreviated chimeric intron. BGH is the bovine growth hormone polyadenlyation signal.

DETAILED DESCRIPTION

[0036] In some aspects, the invention provides RNAi for treating Huntington's disease, wherein the RNAi comprises a first strand comprising a first nucleic acid comprising the sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1) and a second strand comprising a second nucleic acid comprising the sequence 5'-CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2), where the first strand and second strand form a duplex. In some aspects, the invention provides RNAi for treating Huntington's disease, wherein the RNAi comprises a first strand comprising a first nucleic acid comprising the sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7) and a second strand comprising a second nucleic acid comprising the sequence 5'-UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8), where the first strand and second strand form a duplex. In some aspects, the invention provides expression cassettes, vectors (*e.g.*, recombinant AAV, adenoviral, lentiviral, or HSV vectors), cells, viral particles (*e.g.*, AAV, adenoviral, lentiviral, or HSV viral particles), and pharmaceutical compositions comprising an RNAi of the present disclosure. In further aspects, the invention provides methods for treating Huntington's disease, inhibiting the expression of htt, and inhibiting the accumulation of htt in a cell in a mammal comprising administering to the mammal a pharmaceutical composition comprising an RNAi of the present disclosure. In still further aspects, the invention provides for the use of a pharmaceutical composition comprising an RNAi of the present disclosure to treat Huntington's disease (*e.g.*, ameliorate the symptoms of Huntington's disease), inhibit the expression of htt, or inhibit the accumulation of htt in a cell in a mammal with Huntington's disease. In yet further aspects, the invention provides kits for treating Huntington's disease in a mammal comprising an RNAi of the present disclosure.

I. General Techniques

[0037] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the

art, such as, for example, the widely utilized methodologies described in *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2012); *Current Protocols in Molecular Biology* (F.M. Ausubel, *et al.* eds., 2003); the series *Methods in Enzymology* (Academic Press, Inc.); *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds., 1995); *Antibodies, A Laboratory Manual* (Harlow and Lane, eds., 1988); *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications* (R.I. Freshney, 6th ed., J. Wiley and Sons, 2010); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., Academic Press, 1998); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, Plenum Press, 1998); *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., J. Wiley and Sons, 1993-8); *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds., 1996); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis *et al.*, eds., 1994); *Current Protocols in Immunology* (J.E. Coligan *et al.*, eds., 1991); *Short Protocols in Molecular Biology* (Ausubel *et al.*, eds., J. Wiley and Sons, 2002); *Immunobiology* (C.A. Janeway *et al.*, 2004); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow and D. Lane, Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita *et al.*, eds., J.B. Lippincott Company, 2011).

II. Definitions

[0038] A "vector," as used herein, refers to a recombinant plasmid or virus that comprises a nucleic acid to be delivered into a host cell, either in vitro or in vivo.

[0039] The term "polynucleotide" or "nucleic acid" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double- or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases, or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups (as

may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups. Alternatively, the backbone of the polynucleotide can comprise a polymer of synthetic subunits such as phosphoramidates and thus can be an oligodeoxynucleoside phosphoramidate (P-NH_2) or a mixed phosphoramidate- phosphodiester oligomer. In addition, a double-stranded polynucleotide can be obtained from the single stranded polynucleotide product of chemical synthesis either by synthesizing the complementary strand and annealing the strands under appropriate conditions, or by synthesizing the complementary strand de novo using a DNA polymerase with an appropriate primer.

[0040] The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present invention, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0041] A "recombinant viral vector" refers to a recombinant polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of viral origin). In the case of recombinant AAV vectors, the recombinant nucleic acid is flanked by at least one, and in some embodiments two, inverted terminal repeat sequences (ITRs).

[0042] A "recombinant AAV vector (rAAV vector)" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of AAV origin) that are flanked by at least one, and in some embodiments two, AAV inverted terminal repeat sequences (ITRs). Such rAAV vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper virus (or that is expressing suitable helper functions) and that is expressing AAV rep and cap gene products (*i.e.* AAV Rep and Cap proteins). When a rAAV vector is

incorporated into a larger polynucleotide (*e.g.*, in a chromosome or in another vector such as a plasmid used for cloning or transfection), then the rAAV vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of AAV packaging functions and suitable helper functions. A rAAV vector can be in any of a number of forms, including, but not limited to, plasmids, linear artificial chromosomes, complexed with lipids, encapsulated within liposomes, and encapsidated in a viral particle, particularly an AAV particle. A rAAV vector can be packaged into an AAV virus capsid to generate a "recombinant adeno-associated viral particle (rAAV particle)".

[0043] A "recombinant adenoviral vector" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of adenovirus origin) that are flanked by at least one adenovirus inverted terminal repeat sequence (ITR). In some embodiments, the recombinant nucleic acid is flanked by two inverted terminal repeat sequences (ITRs). Such recombinant viral vectors can be replicated and packaged into infectious viral particles when present in a host cell that is expressing essential adenovirus genes deleted from the recombinant viral genome (*e.g.*, E1 genes, E2 genes, E4 genes, *etc.*). When a recombinant viral vector is incorporated into a larger polynucleotide (*e.g.*, in a chromosome or in another vector such as a plasmid used for cloning or transfection), then the recombinant viral vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of adenovirus packaging functions. A recombinant viral vector can be in any of a number of forms, including, but not limited to, plasmids, linear artificial chromosomes, complexed with lipids, encapsulated within liposomes, and encapsidated in a viral particle, for example, an adenovirus particle. A recombinant viral vector can be packaged into an adenovirus virus capsid to generate a "recombinant adenoviral particle."

[0044] A "recombinant lentivirus vector" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of lentivirus origin) that are flanked by at least one lentivirus terminal repeat sequences (LTRs). In some embodiments, the recombinant nucleic acid is flanked by two lentiviral terminal repeat sequences (LTRs). Such recombinant viral vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper functions. A recombinant lentiviral vector can be packaged into a lentivirus capsid to generate a "recombinant lentiviral particle."

[0045] A "recombinant herpes simplex vector (recombinant HSV vector)" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of HSV origin) that are flanked by HSV terminal repeat sequences. Such recombinant viral vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper functions. When a recombinant viral vector is incorporated into a larger polynucleotide (*e.g.*, in a chromosome or in another vector such as a plasmid used for cloning or transfection), then the recombinant viral vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of HSV packaging functions. A recombinant viral vector can be in any of a number of forms, including, but not limited to, plasmids, linear artificial chromosomes, complexed with lipids, encapsulated within liposomes, and encapsidated in a viral particle, for example, an HSV particle. A recombinant viral vector can be packaged into an HSV capsid to generate a "recombinant herpes simplex viral particle."

[0046] "Heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is compared or into which it is introduced or incorporated. For example, a polynucleotide introduced by genetic engineering techniques into a different cell type is a heterologous polynucleotide (and, when expressed, can encode a heterologous polypeptide). Similarly, a cellular sequence (*e.g.*, a gene or portion thereof) that is incorporated into a viral vector is a heterologous nucleotide sequence with respect to the vector.

[0047] The term "transgene" refers to a polynucleotide that is introduced into a cell and is capable of being transcribed into RNA and optionally, translated and/or expressed under appropriate conditions. In aspects, it confers a desired property to a cell into which it was introduced, or otherwise leads to a desired therapeutic or diagnostic outcome. In another aspect, it may be transcribed into a molecule that mediates RNA interference, such as miRNA, siRNA, or shRNA.

[0048] "Chicken β-actin (CBA) promoter" refers to a polynucleotide sequence derived from a chicken β-actin gene (*e.g.*, *Gallus gallus* beta actin, represented by GenBank Entrez Gene ID 396526). As used herein, "chicken β-actin promoter" may refer to a promoter containing a cytomegalovirus (CMV) early enhancer element, the promoter and first exon and intron of the chicken β-actin gene, and the splice acceptor of the rabbit beta-globin gene, such as the sequences described in Miyazaki, J. *et al.* (1989) *Gene* 79(2):269-77. As used

herein, the term "CAG promoter" may be used interchangeably. As used herein, the term "CMV early enhancer/chicken beta actin (CAG) promoter" may be used interchangeably.

[0049] The terms "genome particles (gp)," "genome equivalents," or "genome copies" as used in reference to a viral titer, refer to the number of virions containing the recombinant AAV DNA genome, regardless of infectivity or functionality. The number of genome particles in a particular vector preparation can be measured by procedures such as described in the Examples herein, or for example, in Clark *et al.* (1999) *Hum. Gene Ther.*, 10:1031-1039; Veldwijk *et al.* (2002) *Mol. Ther.*, 6:272-278.

[0050] The term "vector genome (vg)" as used herein may refer to one or more polynucleotides comprising a set of the polynucleotide sequences of a vector, *e.g.*, a viral vector. A vector genome may be encapsidated in a viral particle. Depending on the particular viral vector, a vector genome may comprise single-stranded DNA, double-stranded DNA, or single-stranded RNA, or double-stranded RNA. A vector genome may include endogenous sequences associated with a particular viral vector and/or any heterologous sequences inserted into a particular viral vector through recombinant techniques. For example, a recombinant AAV vector genome may include at least one ITR sequence flanking a promoter, a stuffer, a sequence of interest (*e.g.*, an RNAi), and a polyadenylation sequence. A complete vector genome may include a complete set of the polynucleotide sequences of a vector. In some embodiments, the nucleic acid titer of a viral vector may be measured in terms of vg/mL. Methods suitable for measuring this titer are known in the art (*e.g.*, quantitative PCR).

[0051] As used herein, the term "inhibit" may refer to the act of blocking, reducing, eliminating, or otherwise antagonizing the presence, or an activity of, a particular target. Inhibition may refer to partial inhibition or complete inhibition. For example, inhibiting the expression of a gene may refer to any act leading to a blockade, reduction, elimination, or any other antagonism of expression of the gene, including reduction of mRNA abundance (*e.g.*, silencing mRNA transcription), degradation of mRNA, inhibition of mRNA translation, and so forth. In some embodiments, inhibiting the expression of HTT may refer a blockade, reduction, elimination, or any other antagonism of expression of HTT, including reduction of *HTT* mRNA abundance (*e.g.*, silencing *HTT* mRNA transcription), degradation of *HTT* mRNA, inhibition of *HTT* mRNA translation, and so forth. As another example, inhibiting the accumulation of a protein in a cell may refer to any act leading to a blockade, reduction,

elimination, or other antagonism of expression of the protein, including reduction of mRNA abundance (*e.g.*, silencing mRNA transcription), degradation of mRNA, inhibition of mRNA translation, degradation of the protein, and so forth. In some embodiments, inhibiting the accumulation of HTT protein in a cell refers to a blockade, reduction, elimination, or other antagonism of expression of the HTT protein in a cell, including reduction of *HTT* mRNA abundance (*e.g.*, silencing *HTT* mRNA transcription), degradation of *HTT* mRNA, inhibition of *HTT* mRNA translation, degradation of the HTT protein, and so forth

[0052] The terms "infection unit (iu)," "infectious particle," or "replication unit," as used in reference to a viral titer, refer to the number of infectious and replication-competent recombinant AAV vector particles as measured by the infectious center assay, also known as replication center assay, as described, for example, in McLaughlin *et al.* (1988) *J. Virol.*, 62:1963-1973.

[0053] The term "transducing unit (tu)" as used in reference to a viral titer, refers to the number of infectious recombinant AAV vector particles that result in the production of a functional transgene product as measured in functional assays such as described in Examples herein, or for example, in Xiao *et al.* (1997) *Exp. Neurobiol.*, 144:113-124; or in Fisher *et al.* (1996) *J. Virol.*, 70:520-532 (LFU assay).

[0054] An "inverted terminal repeat" or "ITR" sequence is a term well understood in the art and refers to relatively short sequences found at the termini of viral genomes which are in opposite orientation.

[0055] An "AAV inverted terminal repeat (ITR)" sequence, a term well-understood in the art, is an approximately 145-nucleotide sequence that is present at both termini of the native single-stranded AAV genome. The outermost 125 nucleotides of the ITR can be present in either of two alternative orientations, leading to heterogeneity between different AAV genomes and between the two ends of a single AAV genome. The outermost 125 nucleotides also contains several shorter regions of self-complementarity (designated A, A', B, B', C, C' and D regions), allowing intrastrand base-pairing to occur within this portion of the ITR.

[0056] A "terminal resolution sequence" or "trs" is a sequence in the D region of the AAV ITR that is cleaved by AAV rep proteins during viral DNA replication. A mutant terminal resolution sequence is refractory to cleavage by AAV rep proteins.

[0057] "AAV helper functions" refer to functions that allow AAV to be replicated and packaged by a host cell. AAV helper functions can be provided in any of a number of forms, including, but not limited to, helper virus or helper virus genes which aid in AAV replication and packaging. Other AAV helper functions are known in the art such as genotoxic agents.

[0058] A "helper virus" for AAV refers to a virus that allows AAV (which is a defective parvovirus) to be replicated and packaged by a host cell. A helper virus provides "helper functions" which allow for the replication of AAV. A number of such helper viruses have been identified, including adenoviruses, herpesviruses and, poxviruses such as vaccinia and baculovirus. The adenoviruses encompass a number of different subgroups, although Adenovirus type 5 of subgroup C (Ad5) is most commonly used. Numerous adenoviruses of human, non-human mammalian and avian origin are known and are available from depositories such as the ATCC. Viruses of the herpes family, which are also available from depositories such as ATCC, include, for example, herpes simplex viruses (HSV), Epstein-Barr viruses (EBV), cytomegaloviruses (CMV) and pseudorabies viruses (PRV). Examples of adenovirus helper functions for the replication of AAV include E1A functions, E1B functions, E2A functions, VA functions and E4orf6 functions. Baculoviruses available from depositories include *Autographa californica* nuclear polyhedrosis virus.

[0059] A preparation of rAAV is said to be "substantially free" of helper virus if the ratio of infectious AAV particles to infectious helper virus particles is at least about 10^2:1; at least about 10^4:1, at least about 10^6:1; or at least about 10^8:1 or more. In some embodiments, preparations are also free of equivalent amounts of helper virus proteins (*i.e.*, proteins as would be present as a result of such a level of helper virus if the helper virus particle impurities noted above were present in disrupted form). Viral and/or cellular protein contamination can generally be observed as the presence of Coomassie staining bands on SDS gels (*e.g.*, the appearance of bands other than those corresponding to the AAV capsid proteins VP1, VP2 and VP3).

[0060] "Percent (%) sequence identity" with respect to a reference polypeptide or nucleic acid sequence is defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues or nucleotides in the reference polypeptide or nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of

determining percent amino acid or nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software programs, for example, those described in Current Protocols in Molecular Biology (Ausubel *et al.*, eds., 1987), Supp. 30, section 7.7.18, Table 7.7.1, and including BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. A preferred alignment program is ALIGN Plus (Scientific and Educational Software, Pennsylvania). Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y, where X is the number of amino acid residues scored as identical matches by the sequence alignment program in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. For purposes herein, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows: 100 times the fraction W/Z, where W is the number of nucleotides scored as identical matches by the sequence alignment program in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

[0061] An "isolated" molecule (*e.g.*, nucleic acid or protein) or cell means it has been identified and separated and/or recovered from a component of its natural environment.

[0062] An "effective amount" is an amount sufficient to effect beneficial or desired results, including clinical results (*e.g.*, amelioration of symptoms, achievement of clinical endpoints, and the like). An effective amount can be administered in one or more

administrations. In terms of a disease state, an effective amount is an amount sufficient to ameliorate, stabilize, or delay development of a disease.

[0063] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (*e.g.*, cows, sheep, cats, dogs, and horses), primates (*e.g.*, humans and non-human primates such as monkeys), rabbits, and rodents (*e.g.*, mice and rats). In certain embodiments, the individual or subject is a human.

[0064] As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*e.g.*, not worsening) state of disease, preventing spread (*e.g.*, metastasis) of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

[0065] As used herein, the term "prophylactic treatment" refers to treatment, wherein an individual is known or suspected to have or be at risk for having a disorder but has displayed no symptoms or minimal symptoms of the disorder. An individual undergoing prophylactic treatment may be treated prior to onset of symptoms.

[0066] "Huntington's disease (HD)" refers to the progressive brain disorder typically caused by mutations in the *HTT* gene (aka huntingtin, *HD* or *IT15*). It may be characterized by symptoms including abnormal movements (termed chorea), gradual loss of motor function, emotional or psychiatric illnesses, and progressively impaired cognition. Although most symptoms appear in the 30s and 40s, juvenile forms of the disease have also been observed. For further description of HD, see OMIM Entry No. 143100.

[0067] "Huntingtin (*HTT*)" may refer either to the gene or to a polypeptide product thereof associated with most cases of Huntington's disease. The normal function of huntingtin is not fully understood. However, mutations in the huntingtin gene are known to cause HD. These mutations are typically inherited in an autosomal dominant fashion and involve expansion of trinucleotide CAG repeats in the *HTT* gene, leading to a polyglutamine (polyQ) tract in the Htt protein.

[0068] As used herein, an "RNAi" may refer to any RNA molecule that induces RNA interference in a cell. Examples of RNAi include without limitation small inhibitory RNAs (siRNAs), microRNAs (miRNAs), and small hairpin RNAs (shRNAs).

[0069] "miRNA scaffold" may refer to a polynucleotide containing (i) a double-stranded sequence targeting a gene of interest for knockdown by RNAi and (ii) additional sequences that form a stem-loop structure resembling that of endogenous miRNAs. A sequence targeting a gene of interest for RNAi (*e.g.*, a short, ~20-nt sequence) may be ligated to sequences that create a miRNA-like stem-loop and a sequence that base pairs with the sequence of interest to form a duplex when the polynucleotide is assembled into the miRNA-like secondary structure. As described herein, this duplex may hybridize imperfectly, *e.g.*, it may contain one or more unpaired or mispaired bases. Upon cleavage of this polynucleotide by Dicer, this duplex containing the sequence targeting a gene of interest may be unwound and incorporated into the RISC complex. A miRNA scaffold may refer to the miRNA itself or to a DNA polynucleotide encoding the miRNA. An example of a miRNA scaffold is the miR-155 sequence (Lagos-Quintana, M. *et al.* (2002) *Curr. Biol.* 12:735-9). Commercially available kits for cloning a sequence into a miRNA scaffold are known in the art (*e.g.*, the Invitrogen™ BLOCK-iT™ Pol II miR RNAi expression vector kit from Life Technologies, Thermo Fisher Scientific; Waltham, MA).

[0070] As used herein, a "bulge" refers to a region of nucleic acid that is non-complementary to nucleic acid opposite it in a duplex nucleic acid. For example, a bulge may refer to a nucleic acid sequence that is noncomplementary to nucleic acid opposite in a duplex nucleic acid where the bulge is flanked by regions of nucleic acid that are complementary to nucleic acid opposite in a duplex nucleic acid. In some examples, the bulge may be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or greater than 10 bases in length. In some examples, the bulge may be the result of mispairing (*e.g.*, the opposite strand contains a base that is noncomplementary) or the bulge may be the result of nonpairing (*e.g.*, the opposite strand comprises nucleic acid complementary to nucleic acid flanking the bulge but the opposite strand does not contain nucleic acid opposite the bulge).

[0071] As used herein, the term "sense" nucleic acid is a nucleic acid comprising a sequence that encodes all or a part of a transgene. In some examples, mRNA for a transgene is a sense nucleic acid.

[0072] As used herein, "antisense" nucleic acid is a sequence of nucleic acid that is complementary to a "sense" nucleic acid. For example, an antisense nucleic acid may be complementary to a mRNA encoding a transgene.

[0073] As used herein, the "guide region" of an RNAi is the strand of the RNAi that binds the target mRNA, typically on the basis of complementarity. The region of complementarity may encompass the all or a portion of the guide region. Typically, the region of complementarity includes at least the seed region. In many cases, the antisense region of a RNAi is the guide region.

[0074] As used herein, the "passenger region," or "non-guide region," used interchangeably herein, of an RNAi is the region of the RNAi that is complementary to the guide region. In many cases, the sense region of a RNAi is the passenger region.

[0075] As used herein, the "seed region" of a RNAi (*e.g.*, miRNA) is a region of about 1–8 nucleotides in length of a microRNA. In some examples, the seed region and the 3′-UTR of its target mRNA may be a key determinant in RNAi recognition.

[0076] As used herein, "off-target gene silencing" refers to the pairing of a seed region of an RNAi with sequences in 3′-UTRs of unintended mRNAs and directs translational repression and destabilization of those transcripts (*e.g.*, reduces expression of the unintended mRNAs).

[0077] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. For example, description referring to "about X" includes description of "X."

[0078] As used herein, the singular form of the articles "a," "an," and "the" includes plural references unless indicated otherwise.

[0079] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and/or "consisting essentially of" aspects and embodiments.

III. RNAi

[0080] In some aspects, the invention provides improved RNAi targeting htt RNA for the treatment of Huntington's disease. In some embodiments, the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA). A small inhibitory or interfering RNA (siRNA) is known in the art as a double-stranded RNA molecule of approximately 19-25 (*e.g.*, 19-23) base pairs in length that induces RNAi in a cell. A small hairpin RNA (shRNA) is known in the art as an RNA molecule comprising approximately 19-25 (*e.g.*, 19-23) base pairs of double stranded RNA linked by a short loop (*e.g.*, ~4-11 nucleotides) that induces RNAi in a cell. In some embodiments, the RNAi comprises a first strand and a second strand, wherein a) the first strand and the second strand form a duplex; b) the first strand comprises a guide region, wherein the guide region comprises the nucleic acid sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1) or 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7); and c) the second strand comprises a non-guide region. In some embodiments, the nucleic the guide region comprises the nucleic acid sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1) and the non-guide region comprises the sequence 5'- CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2). In other embodiments, the nucleic the guide region comprises the nucleic acid sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7) and the non-guide region comprises the sequence 5'- UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8).

[0081] In some embodiments, the first strand comprises a guide region, wherein the guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1). In some embodiments, the first strand comprises a guide region, wherein the guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1) but maintains at least one CpG motif. In some embodiments, the first strand comprises a guide region, wherein the guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7). In some embodiments, the first strand comprises a guide region, wherein the guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7) but maintains at least one CpG

motif. In some embodiments, the second strand comprises a non-guide region, wherein the non-guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'- CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2). In some embodiments, the second strand comprises a non-guide region, wherein the non-guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'-CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2) but maintains at least one CpG motif. In some embodiments, the second strand comprises a non-guide region, wherein the non-guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'- UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8). In some embodiments, the second strand comprises a non-guide region, wherein the non-guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'-UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8) but maintains at least one CpG motif.

[0082] In some embodiments, the RNAi comprises the nucleic acid sequence of SEQ ID NO:4. In some embodiments, the RNAi comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:4. In some embodiments, the RNAi comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:4 but maintains at least one sequence (*e.g.*, in a seed sequence). In some embodiments, the RNAi is miRNA-207. In other embodiments, the RNAi is miRNA-206.

[0083] In some embodiments, the RNAi comprises the nucleic acid sequence of SEQ ID NO:10. In some embodiments, the RNAi comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:10. In some embodiments, the RNAi comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:10 but maintains at least one CpG sequence (*e.g.*, in a seed sequence). In some embodiments, the RNAi is miRNA-207. In some embodiments, the RNAi is miRNA-206.

[0084] A microRNA (miRNA) is known in the art as an RNA molecule that induces RNAi in a cell comprising a short (*e.g.*, 19-25 base pairs) sequence of double-stranded RNA linked by a loop and containing one or more additional sequences of double-stranded RNA

comprising one or more bulges (*e.g.,* mispaired or unpaired base pairs). As used herein, the term "miRNA" encompasses endogenous miRNAs as well as exogenous or heterologous miRNAs. In some embodiments, "miRNA" may refer to a pri-miRNA or a pre-miRNA. During miRNA processing, a pri-miRNA transcript is produced. The pri-miRNA is processed by Drosha-DGCR8 to produce a pre-miRNA by excising one or more sequences to leave a pre-miRNA with a 5'flanking region, a guide strand, a loop region, a non-guide strand, and a 3'flanking region; or a 5'flanking region, a non-guide strand, a loop region, a guide strand, and a 3'flanking region. The pre-miRNA is then exported to the cytoplasm and processed by Dicer to yield a siRNA with a guide strand and a non-guide (or passenger) strand. The guide strand is then used by the RISC complex to catalyze gene silencing, *e.g.,* by recognizing a target RNA sequence complementary to the guide strand. Further description of miRNAs may be found, *e.g.,* in WO 2008/150897. The recognition of a target sequence by a miRNA is primarily determined by pairing between the target and the miRNA seed sequence, *e.g.,* nucleotides 1-8 (5' to 3') of the guide strand (see, *e.g.,* Boudreau, R.L. *et al.* (2013) *Nucleic Acids Res.* 41:e9).

[0085] In the pri/pre-miRNA structure, the guide strand:non-guide strand interface in a duplex is formed in part through complementary base pairing (*e.g.,* Watson-Crick base pairing). However, in some embodiments, this complementary base pairing does not extend through the entire duplex. In some embodiments, a bulge in the interface may exist at one or more nucleotide positions. As used herein, the term "bulge" may refer to a region of nucleic acid that is non- complementary to the nucleic acid opposite it in a duplex. In some embodiments, the bulge is formed when the regions of complementary nucleic acids bind to each other, whereas the regions of central non-complementary region do not bind. In some embodiments, the bulge is formed when the two strands of nucleic acid positioned between the two complementary regions are of different lengths. As described below, a bulge may comprise 1 or more nucleotides.

[0086] During miRNA processing, the miRNA is cleaved at a cleavage site adjacent to the guide strand:non-guide strand interface, thus releasing the siRNA duplex of the guide and non-guide strands. In some embodiments, the miRNA comprises a bulge in the sense or antisense strand adjacent to the cleavage site. To state another way, in some embodiments, the miRNA comprises a bulge in the guide or non-guide strand adjacent to the seed sequence. See **FIG. 1A**.

[0087] In some embodiments, the miRNA comprises a bulge in the guide strand opposite the 5' cleavage site of the mature non-guide strand. In some embodiments, the miRNA comprises a bulge opposite the 5' nucleotide of the non-guide strand. In some embodiments, the miRNA comprises a bulge in the sense strand opposite the 3' cleavage site of the mature guide strand. In some embodiments, the miRNA comprises a bulge opposite the 3' nucleotide of the guide strand.

[0088] In some embodiments, the RNAi comprises a first strand and a second strand, wherein a) the first strand and the second form a duplex; b) the first strand comprises a guide region of at least 11 bases, wherein the guide region comprises a seed region comprising bases 1-N of the guide strand, wherein N=7 or N=8; and c) the second strand comprises a non-guide region of at least 11 bases, wherein the non-guide region comprises a bulge sequence opposite of any one or more of bases 1-(N+2) of the guide region in the duplex. In some embodiments, wherein N=7 and the bulge is opposite base 1, 2, 3, 4, 5, 6, 7, 8, or 9 of the guide region. In other embodiments, N=8 and the bulge is opposite base 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the guide region.

[0089] In some embodiments, the RNAi comprises a first strand and a second strand, wherein a) the first strand and the second form a duplex; b) the first strand comprises a guide region of at least 10 bases, wherein the guide region comprises a seed region comprising bases 1-N of the guide strand, wherein N=7 or N=8; and c) the second strand comprises a non-guide region of at least 10 bases, wherein the non-guide region comprises a bulge sequence opposite of any one or more of bases 1-(N+1) of the guide region in the duplex. In some embodiments, wherein N=7 and the bulge is opposite base 1, 2, 3, 4, 5, 6, 7, or 8 of the guide region. In other embodiments, N=8 and the bulge is opposite base 1, 2, 3, 4, 5, 6, 7, 8, or 9 of the guide region.

[0090] In some embodiments, the non-guide region comprises a bulge sequence opposite of any one or more of bases 1-N of the guide region in the duplex. In some embodiments, N=7 and the bulge is opposite base 1, 2, 3, 4, 5, 6 or 7 of the guide region. In other embodiments, N=8 and the bulge is opposite base 1, 2, 3, 4, 5, 6, 7 or 8 of the guide region.

[0091] In some embodiments, the RNAi comprises a first strand and a second strand, wherein a) the first strand and the second form a duplex, b) the first strand comprises a guide region of at least 9 bases, wherein the guide region comprises a seed region comprising bases

2-7 or 2-8 of the guide strand, and c) the second strand comprises a non-guide region of at least 9 bases, wherein the non-guide region comprises a bulge sequence opposite of base 1 or base 9 of the guide region in the duplex.

[0092] In some embodiments, the RNAi comprises a first strand and a second strand, wherein a) the first strand and the second form a duplex, b) the first strand comprises a guide region of at least 9 bases, wherein the guide region comprises a seed region comprising bases 2-7 or 2-8 of the guide strand, and c) the second strand comprises a non-guide region of at least 9 bases, wherein the non-guide region comprises a bulge sequence opposite of base 1 of the guide region in the duplex.

[0093] In some embodiments, the bulge is formed by one or more bases of the non-guide strand in the duplex that lack a complementary base on the guide region, wherein the bulge is flanked by bases that do basepair with the guide strand. In some embodiments, the bulge sequence has about 1-10 nucleotides. In some embodiments, the bulge sequence has about 2-15 nucleotides. In some embodiments, the bulge sequence has about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15 nucleotides.

[0094] The safety of RNAi-based therapies can be hampered by the ability of small inhibitory RNAs (siRNAs) to bind to unintended mRNAs and reduce their expression, an effect known as off-target gene silencing. Off-targeting primarily occurs when the seed region (nucleotides 2–8 of the small RNA) pairs with sequences in 3′-UTRs of unintended mRNAs and directs translational repression and destabilization of those transcripts. Reduced off-targeting RNAi may be designed by substituting bases within the guide and nonguide sequences; *e.g.*, by creating CpG motifs. Potential substitutions that may result in a significantly lower off-target score can be evaluated using the SiSPOTR algorithm, a specificity-focused siRNA design algorithm which identifies candidate sequences with minimal off-targeting potentials and potent silencing capacities (Boudreau *et al, Nucleic Acids Res.* 2013 Jan; 41(1) e9. A reduced SiSPOTR score predicts sequences that have a lower number of potential human off targets compared parent RNAi molecules. In some embodiments of the invention, the RNAi is improved to reduce off-target gene silencing. In some embodiments, the RNAi comprises one or more CpG motifs. In some embodiments, the RNAi comprises one or more CpG motifs in a seed region.

[0095] In some embodiments, the first strand and the second strand are linked by means of a RNA (*e.g.*, a RNA linker) capable of forming a loop structure. As is commonly known in the art, an RNA loop structure (*e.g.*, a stem-loop or hairpin) is formed when an RNA molecule comprises two sequences of RNA that basepair together separated by a sequence of RNA that does not base pair together. For example, a loop structure may form in the RNA molecule A-B-C if sequences A and C are complementary or partially complementary such that they base pair together, but the bases in sequence B do not base pair together.

[0096] In some embodiments, the RNA capable of forming a loop structure comprises from 4 to 50 nucleotides. In certain embodiments, the RNA capable of forming a loop structure comprises 13 nucleotides. In some embodiments, the number of nucleotides in the RNA capable of forming a loop is from 4 to 50 nucleotides or any integer therebetween. In some embodiments, from 0-50% of the loop can be complementary to another portion of the loop. As used herein, the term "loop structure" is a sequence that joins two complementary strands of nucleic acid. In some embodiments, 1-3 nucleotides of the loop structure are contiguous to the complementary strands of nucleic acid and may be complementary to 1-3 nucleotides of the distal portion of the loop structure. For example, the three nucleotides at the 5' end of the loop structure may be complementary to the three nucleotides at the 3' end of the loop structure.

[0097] In some embodiments, nucleic acid encoding an RNAi of the present disclosure comprises a heterologous miRNA scaffold. In some embodiments, use of a heterologous miRNA scaffold is used to modulate miRNA expression; for example, to increase miRNA expression or to decrease miRNA expression. Any miRNA scaffold known in the art may be used. In some embodiments, the miRNA scaffold is derived from a miR-155 scaffold (see, *e.g.*, Lagos-Quintana, M. *et al.* (2002) *Curr. Biol.* 12:735-9 and the Invitrogen™ BLOCK-iT™ Pol II miR RNAi expression vector kit from Life Technologies, Thermo Fisher Scientific; Waltham, MA).

IV. Huntington's disease and experimental models thereof

[0098] Huntington's disease (HD) is an inherited neurodegenerative disease caused by an expansion of the CAG repeat in exon 1 of the huntingtin gene (HTT). The resulting extension of the polyglutamine tract in the N-terminal region confers a toxic gain-of-function to the mutant huntingtin protein (mHtt). mHtt toxicity may arise from the formation of insoluble

mHtt-containing aggregates, transcriptional dysregulation, and perturbations in protein homeostasis, all of which can lead to neuronal death (Saudou *et al.* (1998) *Cell*, 95:55-66; Zuccato *et al.* (2003) *Nat. Genet.* 35:76-83; Schaffar *et al.* (2004) *Mol.Cell.* 15:95-105; Benn *et al.*, (2008) *J. Neurosci.* 28:10720-10733). Pathological findings in patients with HD include cortical thinning and a striking progressive loss of striatal neurons (Rosas *et al.*, (2002) *Neurology* 58:695-701). Disease onset typically occurs during the third to fourth decade of life; symptoms include choreiform movements, impaired coordination, progressive dementia, and other psychiatric disturbances (Vonsattel *et al.*, (1985) *J. Neuropathol. Exp. Neurol.* 44:559-577). In most cases, symptoms begin to appear between 30 and 40 years of age with subtle disruptions in motor skills, cognition, and personality. Over time, these progress into jerky, uncontrollable movements and loss of muscle control, dementia, and psychiatric illnesses such as depression, aggression, anxiety, and obsessive-compulsive behaviors. Death typically occurs 10-15 years after the onset of symptoms. Less than 10% of HD cases involve a juvenile-onset form of the disease, characterized by a faster disease progression. It is thought that approximately 1 in 10,000 Americans has HD.

[0099] Although the genetic basis of HD has been known for almost 20 years, current therapies are largely palliative and do not address the underlying cause of the disease. This is likely due in part to the fact that the etiology of this disease is complex, with detrimental effects observed in a wide variety of cellular processes. Hence, the focus of drug development has been directed at addressing the primary offending trigger, namely, the mutant HTT gene itself.

[0100] Most cases of HD are associated with a trinucleotide CAG repeat expansion in the *HTT* gene. The number of CAG repeats in the *HTT* gene is strongly correlated with the manifestation of HD. For example, individuals with 35 or fewer repeats typically do not develop HD, but individuals with between 27 and 35 repeats have a greater risk of having offspring with HD. Individuals with between 36 and 40-42 repeats have an incomplete penetrance of HD, whereas individuals with more than 40-42 repeats show complete penetrance. Cases of juvenile-onset HD may be associated with CAG repeat sizes of 60 or more.

[0101] The polyQ-expanded Htt protein resulting from this CAG repeat expansion is associated with cellular aggregates or inclusion bodies, perturbations to protein homeostasis, and transcriptional dysregulation. While these toxic phenotypes may be seen thoughout the

body, they are most typically associated with neuronal cell death in the CNS. HD patients often display cortical thinning and a striking, progressive loss of striatal neurons. The striatum appears to be the most vulnerable region of the brain in HD (particularly the striatal medium spiny neurons), with early effects seen in the putamen and caudate nucleus. Cell death in the striatal spiny neurons, increased numbers of astrocytes, and activation of microglia are observed in the brains of HD patients. HD may also affect certain regions of the hippocampus, cerebral cortex, thalamus, hypothalamus, and cerebellum.

[0102] Proposed approaches to blocking Htt expression include the use of antisense oligonucleotides (ASOs) as well as RNA interference (RNAi) that uses either duplex RNAs (dsRNAs) or chemically modified single-stranded RNAs (ssRNAs) (Harper *et al.*, (2005) *Proc. Natl. Acad. Sci. USA* 102:5820-5825; DiFiglia *et al.*, (2007) *Proc. Natl. Acad. Sci. USA* 104:17204-17209; Boudreau *et al.*, (2009b) *Mol. Ther.* 17:1053-1063; Drouet *et al.*, (2009) *Ann. Neurol.*65:276-285; Sah *et al.*, (2011) *J. Clin. Invest.* 121:500-507; Matsui *et al.*, (2012) *Drug Discov. Today* 17:443-450; Yu *et al.*, (2012) *Cell* 150:895-908). However, hurdles to translating an ASO approach into the clinic may include the need to incorporate a device to facilitate repeated and chronic infusions of ASO into the CNS, and to the need to adequately distribute the drug to target regions in a large brain.

[0103] To circumvent these potential issues with ASO, employing AAV-mediated expression of an RNAi (*e.g.*, siRNA), which offers the potential for increased safety, increased efficiency, and longer-lasting efficacy, may be advantageous. As HD patients express both mutant and wild-type Htt alleles, a majority of siRNA targeting sequences will likely degrade both alleles. However, non-allele-specific Htt silencing in HD mice has been shown to be well tolerated and can afford the same benefit as reducing mutant Htt alone (Boudreau *et al.*, (2009b) *Mol. Ther.* 17:1053-1063; Drouet *et al.*, (2009) *Ann. Neurol.* 65:276-285; Kordasiewicz *et al.*, (2012) *Neuron* 74(6):1031-1044). Moreover, the partial and sustained suppression of wild-type Htt in the putamen of non-human primates following AAV-mediated RNAi reportedly did not have any untoward effects, which suggests that the adult brain can tolerate reduced levels of wild-type Htt (McBride *et al.*, (2011) *Mol. Ther.* 19:2152-2162; Grondin *et al.*, (2012) *Brain* 135:1197-1209).

[0104] Animal models of HD may be used to test potential therapeutic strategies, such as the compositions and methods of the present disclosure. Mouse models for HD are known in the art. These include mouse models with fragments of mutant *HTT* such as the R6/1 and

N171-82Q HD mice (Harper *et al.*, (2005) *Proc. Natl. Acad. Sci. USA* 102:5820-5825, Rodriguez-Lebron *et al.*, (2005) *Mol. Ther.* 12:618-633, Machida *et al.*, (2006) *Biochem. Biophys. Res. Commun.* 343:190-197). Another example of a mouse HD model described herein is the YAC128 mouse model. This model bears a yeast artificial chromosome (YAC) expressing a mutant human *HTT* gene with 128 CAG repeats, and YAC128 mice exhibit significant and widespread accumulation of Htt aggregates in the striatum by 12 months of age (Slow *et al.*, (2003) *Hum. Mol. Genet.* 12:1555-1567, Pouladi *et al.*, (2012) *Hum. Mol. Genet.* 21:2219-2232).

[0105] Other animal models for HD may also be used. For example, transgenic rat (von Horsten, S. *et al.* (2003) *Hum. Mol. Genet.* 12:617-24) and rhesus monkey (Yang, S.H. *et al.* (2008) *Nature* 453:921-4) models have been described. Non-genetic models are also known. These most often involve the use of excitotoxic compounds (such as quinolinic acid or kainic acid) or mitochondrial toxins (such as 3-nitropropionic acid and malonic acid) to induce striatal neuron cell death in rodents or non-human primates (for more description and references, see Ramaswamy, S. *et al.* (2007) *ILAR J.* 48:356-73).

V. Methods to treat Huntington's disease

[0106] In some aspects, the invention provides methods and compositions for treating Huntington's disease in a mammal comprising administering to the mammal a pharmaceutical composition of the present disclosure (*e.g.*, a pharmaceutical composition comprising a viral particle of the present disclosure). In some aspects, the invention provides methods and compositions for inhibiting the expression of htt in a mammal with Huntington's disease comprising administering to the mammal a pharmaceutical composition of the present disclosure (*e.g.*, a pharmaceutical composition comprising a viral particle of the present disclosure). In some aspects, the invention provides methods and compositions for inhibiting the accumulation of htt in a cell of a mammal with Huntington's disease comprising administering to the mammal a pharmaceutical composition of the present disclosure (*e.g.*, a pharmaceutical composition comprising a viral particle of the present disclosure).

[0107] In some aspects, the invention provides methods and compositions for ameliorating a symptom of HD, comprising administration of an effective amount of recombinant viral particles comprising a vector encoding an RNAi of the present disclosure

to the brain of a mammal. In some embodiments, the symptoms of HD include, but are not limited to, chorea, rigidity, uncontrollable body movements, loss of muscle control, lack of coordination, restlessness, slowed eye movements, abnormal posturing, instability, ataxic gait, abnormal facial expression, speech problems, difficulties chewing and/or swallowing, disturbance of sleep, seizures, dementia, cognitive deficits (*e.g.,* diminished abilities related to planning, abstract thought, flexibility, rule acquisition, interpersonal sensitivity, self-control, attention, learning, and memory), depression, anxiety, changes in personality, aggression, compulsive behavior, obsessive-compulsive behavior, hypersexuality, psychosis, apathy, irritability, suicidal thoughts, weight loss, muscle atrophy, heart failure, reduced glucose tolerance, testicular atrophy, and osteoporosis.

[0108] In some aspects, the invention provides methods to prevent or delay progression of HD. Autosomal dominant HD is a genetic disease that can be genotyped. For example, the number of CAG repeats in *HTT* may be determined by PCR-based repeat sizing. This type of diagnosis may be performed at any stage of life through directly testing juveniles or adults (*e.g.,* along with presentation of clinical symptoms), prenatal screening or prenatal exclusion testing (*e.g.,* by chorionic villus sampling or amniocentesis), or preimplantation screening of embryos. As such, the methods described herein may be used as a prophylactic treatment of HD since diagnosis may occur before symptom onset. For example, HD may be diagnosed by genetic testing (prenatal testing, testing at birth, *etc.*) and treated prophylactically (*e.g.,* using a rAAV particle described herein) prior to symptom onset (*e.g.,* CNS cell loss) to prevent HD symptom onset and/or progression. HD patients may display shrinkage of the caudate nuclei and/or putamen and/or cortex and/or enlarged ventricles as seen by brain imaging. These symptoms, combined with a family history of HD and/or clinical symptoms, may indicate HD.

[0109] Means for determining amelioration of the symptoms of HD are known in the art. For example, the Unified Huntington's Disease Rating Scale (UHDRS) may be used to assess motor function, cognitive function, behavioral abnormalities, and functional capacity (see, *e.g.,* Huntington Study Group (1996) *Movement Disorders* 11:136-42). This rating scale was developed to provide a uniform, comprehensive test for multiple facets of the disease pathology, incorporating elements from tests such as the HD Activities and Daily Living Scale, Marsden and Quinn's chorea severity scale, the Physical Disability and Independence scales, the HD motor rating scale (HDMRS), the HD functional capacity scale

(HDFCS), and the quantitated neurological exam (QNE). Other test useful for determining amelioration of HD symptoms may include without limitation the Montreal Cognitive Assessment, brain imaging (*e.g.,* MRI), Category Fluency Test, Trail Making Test, Map Search, Stroop Word Reading Test, Speeded Tapping Task, and the Symbol Digit Modalities Test.

[0110] In some aspects of the invention, the methods and compositions are used for the treatment of humans with HD. As described above, HD is inherited in an autosomal dominant manner and caused by CAG repeat expansion in the *HTT* gene. Juvenile-onset HD is most often inherited from the paternal side. Huntington disease-like phenotypes have also been correlated with other genetic loci, such as *HDL1, PRNP, HDL2, HDL3,* and *HDL4*. It is thought that other genetic loci may modify the manifestation of HD symptoms, including mutations in the *GRIN2A, GRIN2B, MSX1, GRIK2,* and *APOE* genes.

[0111] In some aspects, the invention provides an improved RNAi for targeting htt mRNA in a mammal with Huntington's disease. In some embodiments, the RNAi comprises a first strand comprising a first nucleic acid comprising the sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1) and a second strand comprising a second nucleic acid comprising the sequence5'- CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2). An RNAi described herein (*e.g.,* as part of a rAAV vector) may find use, *inter alia*, in treating Huntington's disease.

[0112] In some aspects, the invention provides an improved RNAi for targeting htt mRNA in a mammal with Huntington's disease. In some embodiments, the RNAi comprises a first strand comprising a first nucleic acid comprising the sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7)and a second strand comprising a second nucleic acid comprising the sequence 5'- UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8). An RNAi described herein (*e.g.,* as part of a rAAV vector) may find use, *inter alia*, in treating Huntington's disease.

[0113] In some embodiments of the invention, the RNAi is improved to reduce off-target gene silencing. In some embodiments, the RNAi comprises one or more CpG motifs. In some embodiments, the RNAi comprises one or more CpG motifs in a seed region.

[0114] In some embodiments, the first strand comprises a nucleic acid sequence having more than about any of 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ

ID NO:1 but maintains the CpG motif. In some embodiments, the second strand comprises a nucleic acid sequence having more than about any of 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:2 but maintains the CpG motif.

[0115] In some embodiments, the first strand comprises a nucleic acid sequence having more than about any of 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:7 but maintains the CpG motif. In some embodiments, the second strand comprises a nucleic acid sequence having more than about any of 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:8 but maintains the CpG motif.

[0116] In some embodiments, the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA). A small inhibitory or interfering RNA (siRNA) is known in the art as a double-stranded RNA molecule of approximately 19-25 (*e.g.*, 19-23) base pairs in length that induces RNAi in a cell. A small hairpin RNA (shRNA) is known in the art as an RNA molecule comprising approximately 19-25 (*e.g.*, 19-23) base pairs of double stranded RNA linked by a short loop (*e.g.*, ~4-11 nucleotides) that induces RNAi in a cell.

[0117] In some embodiments, the miRNA comprises a guide sequence that is about 90% identical to SEQ ID NO:1. In some embodiments, the miRNA comprises a guide sequence that is about any of 90% identical, 91% identical, 92% identical, 93% identical, 94% identical, 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 100% identical to SEQ ID NO:1.

[0118] In some embodiments, the miRNA comprises a non-guide sequence that is about 90% identical to SEQ ID NO:2. In some embodiments, the miRNA comprises a non-guide sequence that is about any of 90% identical, 91% identical, 92% identical, 93% identical, 94% identical, 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 100% identical to SEQ ID NO:2.

[0119] In some embodiments, the miRNA comprises a guide sequence that is about 90% identical to SEQ ID NO:7. In some embodiments, the miRNA comprises a guide sequence that is about any of 90% identical, 91% identical, 92% identical, 93% identical, 94% identical, 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 100% identical to SEQ ID NO:7.

[0120] In some embodiments, the miRNA comprises a non-guide sequence that is about 90% identical to SEQ ID NO:8. In some embodiments, the miRNA comprises a non-guide sequence that is about any of 90% identical, 91% identical, 92% identical, 93% identical, 94% identical, 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 100% identical to SEQ ID NO:8.

[0121] In some embodiments, the first strand and the second strand are linked by means of RNA capable of forming a loop structure. As is commonly known in the art, an RNA loop structure (*e.g.*, a stem-loop or hairpin) is formed when an RNA molecule comprises two sequences of RNA that basepair together separated by a sequence of RNA that does not base pair together. For example, a loop structure may form in the RNA molecule A-B-C if sequences A and C are complementary or partially complementary such that they base pair together, but the bases in sequence B do not base pair together.

[0122] In some embodiments, the RNA capable of forming a loop structure comprises from 4 to 50 nucleotides. In certain embodiments, the RNA capable of forming a loop structure comprises 13 nucleotides. In certain embodiments, the RNA capable of forming a loop structure comprises the nucleotide sequence GUUUUGGCCACUGACUGAC (SEQ ID NO:13). In some embodiments, the vector genome comprises a nucleotide sequence that is at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:13.

[0123] In some aspects, the invention provides methods comprising administering to a mammal (*e.g.*, a mammal with HD) an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1) and a second strand comprising a second nucleic acid comprising the sequence5'-CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2). In some embodiments, a recombinant viral particle comprises the RNAi. In some embodiments, the recombinant viral particle is an AAV particle encapsidating a rAAV vector, an adenovirus particle encapsidating a recombinant adenoviral vector, a lentiviral particle encapsidating a recombinant lentiviral vector or an HSV particle encapsidating a recombinant HSV vector wherein the rAAV vector, the adenoviral vector, the lentiviral vector or the HSV vector encodes the RNAi.

[0124] In some aspects, the invention provides methods comprising administering to a mammal (*e.g.*, a mammal with HD) an RNAi comprising a first nucleic acid comprising the sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7)and a second strand comprising a second nucleic acid comprising the sequence 5'-UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8). In some embodiments, a recombinant viral particle comprises the RNAi. In some embodiments, the recombinant viral particle is an AAV particle encapsidating a rAAV vector, an adenovirus particle encapsidating a recombinant adenoviral vector, a lentiviral particle encapsidating a recombinant lentiviral vector or an HSV particle encapsidating a recombinant HSV vector wherein the rAAV vector, the adenoviral vector, the lentiviral vector or the HSV vector encodes the RNAi.

[0125] In some embodiments, delivery of recombinant viral particles is by injection of viral particles to the brain. In some embodiments, delivery of recombinant viral particles is by injection of viral particles to the striatum. Intrastriatal administration delivers recombinant viral particles to an area of the brain, the striatum (including the putamen and caudate nucleus), that is highly affected by HD. In addition, and without wishing to be bound to theory, it is thought that recombinant viral particles (*e.g.*, rAAV particles) injected into the striatum may be also dispersed (*e.g.*, through retrograde transport) to other areas of the brain, including without limitation projection areas (*e.g.*, the cortex). In some embodiments, the recombinant viral particles are delivered by convection enhanced delivery (*e.g.*, convection enhanced delivery to the striatum).

[0126] In some aspects, the invention provides methods for treating Huntington's disease in a mammal comprising administering to the mammal the pharmaceutical composition of the present disclosure. In some aspects, the invention provides methods for inhibiting the accumulation of htt in a cell of a mammal with Huntington's disease comprising administering to the mammal the pharmaceutical composition of the present disclosure. In some aspects, the invention provides methods for inhibiting the expression of htt in a mammal with Huntington's disease comprising administering to the mammal the pharmaceutical composition of the present disclosure. In some embodiments, the htt is a mutant htt (*e.g.*, an htt comprising greater than 35, greater than 36, greater than 37, greater than 38, greater than 39, greater than 40, greater than 41, or greater than 42 CAG repeats). In some embodiments, expression and/or accumulation of a wild-type htt is also inhibited. As

described herein, and without wishing to be bound to theory, it is thought that inhibition of expression and/or accumulation of mutant htt in a mammal with HD is highly beneficial, but the inhibition of expression and/or accumulation of wild-type htt in the same mammal as a side effect (*e.g.*, of an RNAi of the present disclosure) may be well tolerated (*e.g.*, produces few or no unintended side effects).

[0127] In some embodiments, a cell comprises a vector (*e.g.*, a vector comprising an expression construct encoding an RNAi of the present disclosure). In some embodiments, the vector is a rAAV vector. In some embodiments, the vector is a recombinant adenoviral vector, a recombinant lentiviral vector or a recombinant herpes simplex virus (HSV) vector. In some embodiments, the cell is a central nervous system (CNS) cell.

[0128] In some embodiments, the administration of an effective amount of recombinant viral particles comprising a vector encoding an RNAi of the present disclosure transduces neurons (*e.g.*, striatal neurons, such as spiny neurons) at or near the site of administration. In some embodiments, more than about any of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or 100% of neurons are transduced. In some embodiments, about 5% to about 100%, about 10% to about 50%, about 10% to about 30%, about 25% to about 75%, about 25% to about 50%, or about 30% to about 50% of the neurons are transduced. Methods to identify neurons transduced by recombinant viral particles expressing miRNA are known in the art; for example, immunohistochemistry, RNA detection (*e.g.*, qPCR, Northern blotting, RNA-seq, in situ hybridization, and the like) or the use of a co-expressed marker such as enhanced green fluorescent protein can be used to detect expression.

[0129] In some embodiments of the invention, the methods comprise administration to the brain of a mammal an effective amount of recombinant viral particles comprising a vector encoding an RNAi of the present disclosure for treating a mammal, *e.g.*, a human, with HD. In some embodiments, the composition is injected to one or more locations in the brain to allow expression of an RNAi of the present disclosure in at least the neurons. In some embodiments, the composition is injected into any one of one, two, three, four, five, six, seven, eight, nine, ten or more than ten locations in the brain. In some embodiments, the composition is injected into the striatum. In some embodiments, the composition is injected into the dorsal striatum. In some embodiments, the composition is injected into the putamen.

In some embodiments, the composition is injected into the caudate nucleus. In some embodiments, the composition is injected into the putamen and into the caudate nucleus.

[0130] In some embodiments, the recombinant viral particles are administered to one hemisphere of the brain. In some embodiments, the recombinant viral particles are administered to both hemispheres of the brain.

[0131] In some embodiments the recombinant viral particles are administered to more than one location simultaneously or sequentially. In some embodiment, multiple injections of recombinant viral particles are no more than one hour, two hours, three hours, four hours, five hours, six hours, nine hours, twelve hours or 24 hours apart.

[0132] In some embodiments, the invention provides a method for treating a human with HD by administering an effective amount of a pharmaceutical composition comprising a recombinant viral vector encoding an RNAi of the present disclosure to suppress the activity of a mutant *HTT*. In some embodiments, the pharmaceutical composition comprises one or more pharmaceutically acceptable excipients.

[0133] In some embodiments, the methods comprise administering an effective amount of a pharmaceutical composition comprising a recombinant viral vector encoding an RNAi of the present disclosure to suppress the activity of a mutant *HTT*. In some embodiments, the viral titer of the viral particles (*e.g.*, rAAV particles) is at least about any of 5×10^{12}, 6×10^{12}, 7×10^{12}, 8×10^{12}, 9×10^{12}, 10×10^{12}, 11×10^{12}, 15×10^{12}, 20×10^{12}, 25×10^{12}, 30×10^{12}, or 50×10^{12} genome copies/mL. In some embodiments, the viral titer of the viral particles (*e.g.*, rAAV particles) is about any of 5×10^{12} to 6×10^{12}, 6×10^{12} to 7×10^{12}, 7×10^{12} to 8×10^{12}, 8×10^{12} to 9×10^{12}, 9×10^{12} to 10×10^{12}, 10×10^{12} to 11×10^{12}, 11×10^{12} to 15×10^{12}, 15×10^{12} to 20×10^{12}, 20×10^{12} to 25×10^{12}, 25×10^{12} to 30×10^{12}, 30×10^{12} to 50×10^{12} , or 50×10^{12} to 100×10^{12} genome copies/mL. In some embodiments, the viral titer of the viral particles (*e.g.*, rAAV particles) is about any of 5×10^{12} to 10×10^{12}, 10×10^{12} to 25×10^{12}, or 25×10^{12} to 50×10^{12}genome copies/mL. In some embodiments, the viral titer of the viral particles (*e.g.*, rAAV particles) is at least about any of 5×10^{9}, 6×10^{9}, 7×10^{9}, 8×10^{9}, 9×10^{9}, 10×10^{9}, 11×10^{9}, 15×10^{9}, 20×10^{9}, 25×10^{9}, 30×10^{9}, or 50×10^{9} transducing units /mL. In some embodiments, the viral titer of the viral particles (*e.g.*, rAAV particles) is about any of 5×10^{9} to 6×10^{9}, 6×10^{9} to 7×10^{9}, 7×10^{9} to 8×10^{9}, 8×10^{9} to 9×10^{9}, 9×10^{9} to 10×10^{9}, 10×10^{9} to 11×10^{9}, 11×10^{9} to 15×10^{9}, 15×10^{9} to 20

$\times 10^9$, 20×10^9 to 25×10^9, 25×10^9 to 30×10^9, 30×10^9 to 50×10^9 or 50×10^9 to 100×10^9 transducing units /mL. In some embodiments, the viral titer of the viral particles (*e.g.*, rAAV particles) is about any of 5×10^9 to 10×10^9, 10×10^9 to 15×10^9, 15×10^9 to 25×10^9, or 25×10^9 to 50×10^9 transducing units /mL. In some embodiments, the viral titer of the viral particles (*e.g.*, rAAV particles) is at least any of about 5×10^{10}, 6×10^{10}, 7×10^{10}, 8×10^{10}, 9×10^{10}, 10×10^{10}, 11×10^{10}, 15×10^{10}, 20×10^{10}, 25×10^{10}, 30×10^{10}, 40×10^{10}, or 50×10^{10} infectious units/mL. In some embodiments, the viral titer of the viral particles (*e.g.*, rAAV particles) is at least any of about 5×10^{10} to 6×10^{10}, 6×10^{10} to 7×10^{10}, 7×10^{10} to 8×10^{10}, 8×10^{10} to 9×10^{10}, 9×10^{10} to 10×10^{10}, 10×10^{10} to 11×10^{10}, 11×10^{10} to 15×10^{10}, 15×10^{10} to 20×10^{10}, 20×10^{10} to 25×10^{10}, 25×10^{10} to 30×10^{10}, 30×10^{10} to 40×10^{10}, 40×10^{10} to 50×10^{10}, or 50×10^{10} to 100×10^{10} infectious units/mL. In some embodiments, the viral titer of the viral particles (*e.g.*, rAAV particles) is at least any of about 5×10^{10} to 10×10^{10}, 10×10^{10} to 15×10^{10}, 15×10^{10} to 25×10^{10}, or 25×10^{10} to 50×10^{10} infectious units/mL.

[0134] In some embodiments, the dose of viral particles administered to the individual is at least about any of 1×10^8 to about 1×10^{13} genome copies/kg of body weight. In some embodiments, the dose of viral particles administered to the individual is about any of 1×10^8 to about 1×10^{13} genome copies/kg of body weight.

[0135] In some embodiments, the total amount of viral particles administered to the individual is at least about any of 1×10^9 to about 1×10^{14} genome copies. In some embodiments, the total amount of viral particles administered to the individual is about any of 1×10^9 to about 1×10^{14} genome copies.

[0136] In some embodiments of the invention, the volume of the composition injected to the striatum is more than about any one of 1 µl, 2 µl, 3 µl, 4 µl, 5 µl, 6 µl, 7 µl, 8 µl, 9 µl, 10 µl, 15 µl, 20 µl, 25 µl, 50 µl, 75 µl, 100 µl, 200 µl, 300 µl, 400 µl, 500 µl, 600 µl, 700 µl, 800 µl, 900 µl, or 1 mL, or any amount therebetween.

[0137] In some embodiments, a first volume of the composition is injected into a first region of the brain, and a second volume of the composition is injected into a second region of the brain. For example, in some embodiments, a first volume of the composition is injected into the caudate nucleus, and a second volume of the composition is injected into the putamen. In some embodiments, a 1X volume of the composition is injected into the caudate

nucleus, and a 1.5X, 2X, 2.5X, 3X, 3.5X, or 4X volume of the composition is injected into the putamen, where X is a volume that is more than about any one of 1 µl, 2 µl, 3 µl, 4 µl, 5 µl, 6 µl, 7 µl, 8 µl, 9 µl, 10 µl, 15 µl, 20 µl, 25 µl, 50 µl, 75 µl, 100 µl, 200 µl, 300 µl, 400 µl, 500 µl, 600 µl, 700 µl, 800 µl, 900 µl, or 1 mL, or any amount therebetween.

[0138] Compositions of the invention (*e.g.*, recombinant viral particles comprising a vector encoding an RNAi of the present disclosure) can be used either alone or in combination with one or more additional therapeutic agents for treating HD. The interval between sequential administration can be in terms of at least (or, alternatively, less than) minutes, hours, or days.

V. RNAi Expression Constructs and Vectors

[0139] The invention provides expression constructs, vectors and viral particles for expression of the RNAi described herein.

[0140] In some embodiments, nucleic acid encoding an RNAi of the present disclosure comprises a heterologous miRNA scaffold. In some embodiments, use of a heterologous miRNA scaffold is used to modulate miRNA expression; for example, to increase miRNA expression or to decrease miRNA expression. Any miRNA scaffold known in the art may be used. In some embodiments, the miRNA scaffold is derived from a miR-155 scaffold (see, *e.g.*, Lagos-Quintana, M. *et al.* (2002) *Curr. Biol.* 12:735-9 and the Invitrogen™ BLOCK-iT™ Pol II miR RNAi expression vector kit from Life Technologies, Thermo Fisher Scientific; Waltham, MA). In some embodiments, nucleic acid encoding an RNAi of the present disclosure comprises a miRNA scaffold. In some embodiments, miRNA scaffold is provided by SEQ ID NO:14.

[0141] In some embodiments, the RNAi targets RNA encoding a polypeptide associated with Huntington's disease (*e.g.*, mutant *HTT*). Without wishing to be bound to theory, it is thought that an RNAi may be used to reduce or eliminate the expression and/or activity of a polypeptide whose gain-of-function has been associated with Huntington's disease (*e.g.*, mutant *HTT*).

[0142] In some embodiments, the transgene (*e.g.*, an RNAi of the present disclosure) is operably linked to a promoter. Exemplary promoters include, but are not limited to, the cytomegalovirus (CMV) immediate early promoter, the RSV LTR, the MoMLV LTR, the

phosphoglycerate kinase- 1 (PGK) promoter, a simian virus 40 (SV40) promoter and a CK6 promoter, a transthyretin promoter (TTR), a TK promoter, a tetracycline responsive promoter (TRE), an HBV promoter, an hAAT promoter, a LSP promoter, chimeric liver-specific promoters (LSPs), the E2F promoter, the telomerase (hTERT) promoter; the cytomegalovirus enhancer/chicken beta-actin/Rabbit β-globin promoter (CAG promoter; Niwa *et al.*, *Gene*, 1991, 108(2):193-9) and the elongation factor 1-alpha promoter (EFl-alpha) promoter (Kim *et al.*, *Gene*, 1990, 91(2):217-23 and Guo *et al.*, *Gene Ther.*, 1996, 3(9):802-10). In some embodiments, the promoter comprises a human β-glucuronidase promoter or a cytomegalovirus enhancer linked to a chicken β-actin (CBA) promoter. The promoter can be a constitutive, inducible or repressible promoter. In some embodiments, the invention provides a recombinant vector comprising nucleic acid encoding a heterologous transgene of the present disclosure operably linked to a CBA promoter. Exemplary promoters and descriptions may be found, *e.g.,* in U.S. PG Pub. 20140335054. In some embodiments, the promoter is a CBA promoter, a minimum CBA promoter, a CMV promoter or a GUSB promoter.

[0143] Examples of constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) [see, *e.g.*, Boshart *et al*, *Cell*, 41:521-530 (1985)], the SV40 promoter, the dihydrofolate reductase promoter, the 13-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EFla promoter [Invitrogen].

[0144] Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, *e.g.*, acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. Examples of inducible promoters regulated by exogenously supplied promoters include the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system (WO 98/10088); the ecdysone insect promoter (No *et al*, *Proc. Natl. Acad. Sci. USA*, 93:3346-3351 (1996)), 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)). Still other types of inducible promoters which may be useful in this context are those which are regulated by a specific physiological state, *e.g.*, temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only.

[0145] In another embodiment, the native promoter, or fragment thereof, for the transgene will be used. The native promoter may be preferred when it is desired that expression of the transgene should mimic the native expression. The native promoter may be used when expression of the transgene must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression.

[0146] In some embodiments, the regulatory sequences impart tissue-specific gene expression capabilities. In some cases, the tissue-specific regulatory sequences bind tissue-specific transcription factors that induce transcription in a tissue specific manner. Such tissue-specific regulatory sequences (*e.g.*, promoters, enhancers, *etc.*) are well known in the art. Exemplary tissue-specific regulatory sequences include, but are not limited to the following tissue specific promoters: neuronal such as neuron-specific enolase (NSE) promoter (Andersen *et al., Cell. Mol. Neurobiol.*, 13:503-15 (1993)), neurofilament light-chain gene promoter (Piccioli *et al., Proc. Natl. Acad. Sci. USA*, 88:5611-5 (1991)), and the neuron-specific vgf gene promoter (Piccioli *et al., Neuron*, 15:373-84 (1995)). In some embodiments, the tissue-specific promoter is a promoter of a gene selected from: neuronal nuclei (NeuN), glial fibrillary acidic protein (GFAP), adenomatous polyposis coli (APC), and ionized calcium-binding adapter molecule 1 (Iba-1). Other appropriate tissue specific promoters will be apparent to the skilled artisan. In some embodiments, the promoter is a chicken Beta-actin (CBA) promoter.

[0147] In some embodiments, the promoter expresses the heterologous nucleic acid in a cell of the CNS. As such, in some embodiments, a therapeutic polypeptide or a therapeutic

nucleic acid of the invention may be used to treat a disorder of the CNS. In some embodiments, the promoter expresses the heterologous nucleic acid in a brain cell. A brain cell may refer to any brain cell known in the art, including without limitation a neuron (such as a sensory neuron, motor neuron, interneuron, dopaminergic neuron, medium spiny neuron, cholinergic neuron, GABAergic neuron, pyramidal neuron, *etc.*), a glial cell (such as microglia, macroglia, astrocytes, oligodendrocytes, ependymal cells, radial glia, *etc.*), a brain parenchyma cell, microglial cell, ependemal cell, and/or a Purkinje cell. In some embodiments, the promoter expresses the heterologous nucleic acid in a neuron and/or glial cell. In some embodiments, the neuron is a medium spiny neuron of the caudate nucleus, a medium spiny neuron of the putamen, a neuron of the cortex layer IV and/or a neuron of the cortex layer V.

[0148] Various promoters that express transcripts (*e.g.*, a heterologous transgene) in CNS cells, brain cells, neurons, and glial cells are known in the art and described herein. Such promoters can comprise control sequences normally associated with the selected gene or heterologous control sequences. Often, useful heterologous control sequences include those derived from sequences encoding mammalian or viral genes. Examples include, without limitation, the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a Rous sarcoma virus (RSV) promoter, synthetic promoters, hybrid promoters, and the like. In addition, sequences derived from nonviral genes, such as the murine metallothionein gene, may also be used. Such promoter sequences are commercially available from, *e.g.*, Stratagene (San Diego, CA). CNS-specific promoters and inducible promoters may be used. Examples of CNS-specific promoters include without limitation those isolated from CNS-specific genes such as myelin basic protein (MBP), glial fibrillary acid protein (GFAP), and neuron specific enolase (NSE). Examples of inducible promoters include DNA responsive elements for ecdysone, tetracycline, metallothionein, and hypoxia, *inter alia*.

[0149] The present invention contemplates the use of a recombinant viral genome for introduction of one or more nucleic acid sequences encoding for a RNAi as described herein or packaging into an AAV viral particle. The recombinant viral genome may include any element to establish the expression of a RNAi, for example, all or a functional portion of a

promoter, an intron (*e.g.*, a chimeric intron)), a heterologous nucleic acid, an ITR, a ribosome binding element, terminator, enhancer, selection marker, intron, polyA signal, and/or origin of replication. In some embodiments, the rAAV vector comprises one or more of an enhancer, an intron (*e.g.*, a splice donor/splice acceptor pair), a matrix attachment site, or a polyadenylation signal. A variety of introns for use in the invention are known to those of skill in the art, and include the MVM intron, the F IX truncated intron 1, the β-globin SD/immunoglobin heavy chain SA, the adenovirus SD/immunoglobin SA, the SV40 late SD/SA (19S/16S), and the hybrid adenovirus SD/IgG SA. (Wu *et al.* 2008, Kurachi *et al.*, 1995, Choi *et al.* 2014), Wong *et al.* 1985, Yew *et al.* 1997, Huang and Gorman (1990).

[0150] In some embodiments, the administration of an effective amount of rAAV particles comprising a vector encoding a RNAi transduces cells (*e.g.*, CNS cells, brain cells, neurons, and/or glial cells) at or near the site of administration (*e.g.*, the striatum and/or cortex) or more distal to the site of administration. In some embodiments, more than about any of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or 100% of neurons are transduced. In some embodiments, about 5% to about 100%, about 10% to about 50%, about 10% to about 30%, about 25% to about 75%, about 25% to about 50%, or about 30% to about 50% of the neurons are transduced. Methods to identify neurons transduced by recombinant viral particles expressing miRNA are known in the art; for example, immunohistochemistry, RNA detection (*e.g.*, qPCR, Northern blotting, RNA-seq, in situ hybridization, and the like) or the use of a co-expressed marker such as enhanced green fluorescent protein can be used to detect expression.

[0151] In some aspects, the invention provides viral particles comprising a recombinant self-complementing genome (*e.g.*, a self-complementary rAAV vector). AAV viral particles with self-complementing vector genomes and methods of use of self-complementing AAV genomes are described in US Patent Nos. 6,596,535; 7,125,717; 7,465,583; 7,785,888; 7,790,154; 7,846,729; 8,093,054; and 8,361,457; and Wang Z., *et al.*, (2003) *Gene Ther* 10:2105-2111, each of which are incorporated herein by reference in its entirety. A rAAV comprising a self-complementing genome will quickly form a double stranded DNA molecule by virtue of its partially complementing sequences (*e.g.*, complementing coding and non-coding strands of a heterologous nucleic acid). In some embodiments, the vector comprises first nucleic acid sequence encoding the heterologous nucleic acid and a second nucleic acid sequence encoding a complement of the nucleic acid, where the first nucleic acid

sequence can form intrastrand base pairs with the second nucleic acid sequence along most or all of its length.

[0152] In some embodiments, the first heterologous nucleic acid sequence encoding a RNAi and a second heterologous nucleic acid sequence encoding the complement of the RNAi are linked by a mutated ITR (*e.g.*, the right ITR). In some embodiments, the ITR comprises the polynucleotide sequence 5'-CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA-3 (SEQ ID NO:15). The mutated ITR comprises a deletion of the D region comprising the terminal resolution sequence. As a result, on replicating an AAV viral genome, the rep proteins will not cleave the viral genome at the mutated ITR and as such, a recombinant viral genome comprising the following in 5' to 3' order will be packaged in a viral capsid: an AAV ITR, the first heterologous polynucleotide sequence including regulatory sequences, the mutated AAV ITR, the second heterologous polynucleotide in reverse orientation to the first heterologous polynucleotide and a third AAV ITR.

VI. Viral particles and methods of producing viral particles

[0153] The invention provides, *inter alia*, recombinant viral particles comprising a nucleic acid encoding an RNAi of the present disclosure, as well as methods of use thereof to treat a disease or disorder in a mammal; *e.g.*, Huntington's disease.

Viral particles

[0154] The invention provides viral particles comprising the RNAi as disclosed herein. In some embodiments, the invention provides viral particles for delivering the RNAi of the invention as disclosed herein. For example, the invention provides methods of using recombinant viral particles to deliver RNAi to treat a disease or disorder in a mammal; *e.g.*, rAAV particles comprising RNAi to treat HD. In some embodiments, the recombinant viral particle is a recombinant AAV particle. In some embodiments, the viral particle is a recombinant AAV particle comprising a nucleic acid comprising a sequence an RNAi of the present disclosure flanked by one or two ITRs. The nucleic acid is encapsidated in the AAV particle. The AAV particle also comprises capsid proteins. In some embodiments, the nucleic acid comprises the coding sequence(s) of interest (*e.g.*, nucleic acid an RNAi of the present disclosure) operatively linked components in the direction of transcription, control

sequences including transcription initiation and termination sequences, thereby forming an expression cassette. The expression cassette is flanked on the 5' and 3' end by at least one functional AAV ITR sequences. By "functional AAV ITR sequences" it is meant that the ITR sequences function as intended for the rescue, replication and packaging of the AAV virion. See Davidson *et al.*, *PNAS*, 2000, 97(7)3428-32; Passini *et al.*, *J. Virol.*, 2003, 77(12):7034-40; and Pechan *et al.*, *Gene Ther.*, 2009, 16:10-16, all of which are incorporated herein in their entirety by reference. For practicing some aspects of the invention, the recombinant vectors comprise at least all of the sequences of AAV essential for encapsidation and the physical structures for infection by the rAAV. AAV ITRs for use in the vectors of the invention need not have a wild-type nucleotide sequence (*e.g.*, as described in Kotin, *Hum. Gene Ther.*, 1994, 5:793-801), and may be altered by the insertion, deletion or substitution of nucleotides or the AAV ITRs may be derived from any of several AAV serotypes. More than 40 serotypes of AAV are currently known, and new serotypes and variants of existing serotypes continue to be identified. See Gao *et al.*, *PNAS*, 2002, 99(18): 11854-6; Gao *et al.*, *PNAS*, 2003, 100(10):6081-6; and Bossis *et al.*, *J. Virol.*, 2003, 77(12):6799-810. Use of any AAV serotype is considered within the scope of the present invention. In some embodiments, a rAAV vector is a vector derived from an AAV serotype, including without limitation, AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV capsid serotype ITRs or the like. In some embodiments, the nucleic acid in the AAV comprises an ITR of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV capsid serotype ITRs or the like. In some embodiments, the nucleic acid in the AAV further encodes an RNAi as described herein. For example, the nucleic acid in the AAV can comprise at least one ITR of any AAV serotype contemplated herein and can further encode an RNAi comprising one strand that comprises a guide region and another strand that comprises a non-guide region. In one embodiment, the nucleic acid in the AAV can comprise at least one ITR of any AAV serotype and can further encode an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1) and a second strand comprising a second nucleic acid comprising the sequence 5'- CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a promoter, a nucleic acid

encoding an RNAi as disclosed herein, a polyadenylation signal, and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a promoter, a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1) and a second strand comprising a second nucleic acid comprising the sequence 5'-CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2), a polyadenylation signal, and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a CBA promoter, a nucleic acid encoding an RNAi as disclosed herein, a polyadenylation signal (*e.g.*, a bovine growth hormone polyA), and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: all or a functional portion of an ITR (*e.g.*, an AAV2 ITR), a CBA promoter, an intron (*e.g.*, a chimeric intron), a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1), and a second strand comprising a second nucleic acid comprising the sequence 5'- CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2), a polyadenylation signal (*e.g.*, a bovine growth hormone polyA), and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the first strand and second strand form a duplex. In some embodiments, the first strand is linked to the second strand by a linker. In some embodiments, the linker comprises the nucleic acid sequence of SEQ ID NO:13.

[0155] In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a CBA promoter, a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'- CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2), and a second strand comprising a second nucleic acid comprising the sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1), a polyadenylation signal (*e.g.*, a bovine growth hormone polyA), and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the first strand and second strand form a duplex. In some embodiments, the first strand is linked to the second strand by a linker. In some embodiments, the linker comprises the nucleic acid sequence of SEQ ID NO:13.

[0156] In another embodiment, the nucleic acid in the AAV can comprise at least one ITR of any AAV serotype contemplated herein and can further encode an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7) and a second strand comprising a second nucleic acid comprising the sequence5'- UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a promoter, a nucleic acid encoding an RNAi as disclosed herein, a polyadenylation signal, and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a promoter, a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7) and a second strand comprising a second nucleic acid comprising the sequence 5'-UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8), a polyadenylation signal, and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a CBA promoter, a chimeric intron, a nucleic acid encoding an RNAi as disclosed herein, a polyadenylation signal (*e.g.*, a bovine growth hormone polyA), and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a CBA promoter, a chimeric intron, a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7), and a second strand comprising a second nucleic acid comprising the sequence 5'-UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8), a polyadenylation signal (*e.g.*, a bovine growth hormone polyA), and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the first strand and second strand form a duplex. In some embodiments, the first strand is linked to the second strand by a linker. In some embodiments, the linker comprises the nucleic acid sequence of SEQ ID NO:13.

[0157] In another embodiment, the nucleic acid in the AAV can comprise at least one ITR of any AAV serotype contemplated herein and can further encode an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8) and a second strand comprising a second nucleic acid comprising the sequence AGUCGGUGUGGUUGACAAGCA-3' (SEQ

ID NO:7). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a promoter, a nucleic acid encoding an RNAi as disclosed herein, a polyadenylation signal, and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a promoter, an intron, a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'- UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8) and a second strand comprising a second nucleic acid comprising the sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7), a polyadenylation signal, and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a CBA promoter, a nucleic acid encoding an RNAi as disclosed herein, a polyadenylation signal (*e.g.*, a bovine growth hormone polyA), and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a CBA promoter, an intron, a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'- UGCUUGUCAACCACACCGACU-3', and a second strand comprising a second nucleic acid comprising the sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7), a polyadenylation signal (*e.g.*, a bovine growth hormone polyA), and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the first strand and second strand form a duplex. In some embodiments, the first strand is linked to the second strand by a linker. In some embodiments, the linker comprises the nucleic acid sequence of SEQ ID NO:13.

[0158] In some embodiments, a vector may include a (one or more) stuffer nucleic acid. In some embodiments, the stuffer nucleic acid may comprise a sequence that encodes a reporter polypeptide. As will be appreciated by those of skill in the art, the stuffer nucleic acid may be located in a variety of regions within the vector, and may be comprised of a continuous sequence (*e.g.*, a single stuffer nucleic acid in a single location) or multiple sequences (*e.g.*, more than one stuffer nucleic acid in more than one location (*e.g.*, 2 locations, 3 locations, etc.) within the vector. In some embodiments, the stuffer nucleic acid may be located downstream of the RNAi sequence. In embodiments, the stuffer nucleic acid may be located upstream of the RNAi sequence (*e.g.*, between the promoter and the nucleic acid encoding the RNAi). As will also be appreciated by those of skill in the art a variety of nucleic acids may be used as a stuffer nucleic acid. In some embodiments, the stuffer nucleic

acid comprises all or a portion of a human alpha-1-antitrypsin (AAT) stuffer sequence or a C16 P1 chromosome 16 P1 clone (human C16) stuffer sequence. In some embodiments, the stuffer sequence comprises all or a portion of a gene. For example, the stuffer sequence comprises a portion of the human AAT sequence. One skilled in the art would recognize that different portions of a gene (*e.g.*, the human AAT sequence) can be used as a stuffer fragment. For example, the stuffer fragment may be from the 5' end of the gene, the 3' end of the gene, the middle of a gene, a non-coding portion of the gene (*e.g.*, an intron), a coding region of the gene (*e.g.* an exon), or a mixture of non-coding and coding portions of a gene. One skilled in the art would also recognize that all or a portion of stuffer sequence may be used as a stuffer sequence. In some embodiments, the stuffer sequence comprises the nucleotide sequence of SEQ ID NO:18.

[0159] In further embodiments, the rAAV particle comprises capsid proteins of AAV1, AAV2, AAV3, AAV4, AAV5, AA6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh8R, AAVrh.10, AAV11, AAV12, or mutants of these capsid proteins. In some embodiments, a mutant capsid protein maintains the ability to form an AAV capsid. In some embodiments, the rAAV particle comprises AAV5 tyrosine mutant capsid (Zhong L. *et al.*, (2008) *Proc Natl Acad Sci U S A* 105(22):7827-7832. In further embodiments, the rAAV particle comprises capsid proteins of an AAV serotype from Clades A-F (Gao, *et al., J. Virol.* 2004, 78(12):6381).

[0160] Different AAV serotypes are used to optimize transduction of particular target cells or to target specific cell types within a particular target tissue (*e.g.*, a diseased tissue). A rAAV particle can comprise viral proteins and viral nucleic acids of the same serotype or a mixed serotype. For example, in some embodiments a rAAV particle can comprise AAV1 capsid proteins and at least one AAV2 ITR or it can comprise AAV2 capsid proteins and at least one AAV1 ITR. Any combination of AAV serotypes for production of a rAAV particle is provided herein as if each combination had been expressly stated herein. In some embodiments, the invention provides rAAV particles comprising an AAV1 capsid and a rAAV vector of the present disclosure (*e.g.*, an expression cassette comprising nucleic acid encoding an RNAi of the present disclosure), flanked by at least one AAV2 ITR. In some embodiments, the invention provides rAAV particles comprising an AAV2 capsid.

[0161] In some aspects, the invention provides viral particles comprising a recombinant self-complementing genome. AAV viral particles with self-complementing genomes and

methods of use of self-complementing AAV genomes are described in US Patent Nos. 6,596,535; 7,125,717; 7,465,583; 7,785,888; 7,790,154; 7,846,729; 8,093,054; and 8,361,457; and Wang Z., *et al.*, (2003) *Gene Ther* 10:2105-2111, each of which are incorporated herein by reference in its entirety. A rAAV comprising a self-complementing genome will quickly form a double stranded DNA molecule by virtue of its partially complementing sequences (*e.g.*, complementing coding and non-coding strands of a transgene). In some embodiments, the invention provides an AAV viral particle comprising an AAV genome, wherein the rAAV genome comprises a first heterologous polynucleotide sequence (*e.g.*, an RNAi of the present disclosure) and a second heterologous polynucleotide sequence (*e.g.*, antisense strand of an RNAi of the present disclosure) wherein the first heterologous polynucleotide sequence can form intrastrand base pairs with the second polynucleotide sequence along most or all of its length. In some embodiments, the first heterologous polynucleotide sequence and a second heterologous polynucleotide sequence are linked by a sequence that facilitates intrastrand basepairing; *e.g.*, a hairpin DNA structure. Hairpin structures are known in the art, for example in miRNA or siRNA molecules. In some embodiments, the first heterologous polynucleotide sequence and a second heterologous polynucleotide sequence are linked by a mutated ITR (*e.g.*, the right ITR). In some embodiments, the ITR comprises the polynucleotide sequence 5'- CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC GCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGA GAGGGA-3 (SEQ ID NO:15). The mutated ITR comprises a deletion of the D region comprising the terminal resolution sequence. As a result, on replicating an AAV viral genome, the rep proteins will not cleave the viral genome at the mutated ITR and as such, a recombinant viral genome comprising the following in 5' to 3' order will be packaged in a viral capsid: an AAV ITR, the first heterologous polynucleotide sequence including regulatory sequences, the mutated AAV ITR, the second heterologous polynucleotide in reverse orientation to the first heterologous polynucleotide and a third AAV ITR. In some embodiments, the invention provides AAV viral particles comprising a recombinant viral genome comprising a functional AAV2 ITR, a first polynucleotide sequence encoding an RNAi of the present disclosure, a mutated AAV2 ITR comprising a deletion of the D region and lacking a functional terminal resolution sequence, a second polynucleotide sequence comprising the complementary sequence to the sequence encoding an RNAi of the present disclosure, of the first polynucleotide sequence and a functional AAV2 ITR.

[0162] In some embodiments, the viral particle is an adenoviral particle. In some embodiments, the adenoviral particle is a recombinant adenoviral particle, *e.g.*, a polynucleotide vector comprising an RNAi of the present disclosure between two ITRs. In some embodiments, the adenoviral particle lacks or contains a defective copy of one or more E1 genes, which renders the adenovirus replication-defective. Adenoviruses include a linear, double-stranded DNA genome within a large (~950Å), non-enveloped icosahedral capsid. Adenoviruses have a large genome that can incorporate more than 30kb of heterologous sequence (*e.g.*, in place of the E1 and/or E3 region), making them uniquely suited for use with larger heterologous genes. They are also known to infect dividing and non-dividing cells and do not naturally integrate into the host genome (although hybrid variants may possess this ability). In some embodiments, the adenoviral vector may be a first generation adenoviral vector with a heterologous sequence in place of E1. In some embodiments, the adenoviral vector may be a second generation adenoviral vector with additional mutations or deletions in E2A, E2B, and/or E4. In some embodiments, the adenoviral vector may be a third generation or gutted adenoviral vector that lacks all viral coding genes, retaining only the ITRs and packaging signal and requiring a helper adenovirus *in trans* for replication, and packaging. Adenoviral particles have been investigated for use as vectors for transient transfection of mammalian cells as well as gene therapy vectors. For further description, see, *e.g.*, Danthinne, X. and Imperiale, M.J. (2000) *Gene Ther.* 7:1707-14 and Tatsis, N. and Ertl, H.C. (2004) *Mol. Ther.* 10:616-29.

[0163] In some embodiments, the viral particle is a recombinant adenoviral particle comprising a nucleic acid encoding an RNAi of the present disclosure. Use of any adenovirus serotype is considered within the scope of the present invention. In some embodiments, the recombinant adenoviral vector is a vector derived from an adenovirus serotype, including without limitation, AdHu2, AdHu 3, AdHu4, AdHu5, AdHu7, AdHu11, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, and porcine Ad type 3. The adenoviral particle also comprises capsid proteins. In some embodiments, the recombinant viral particles comprise an adenoviral particle in combination with one or more foreign viral capsid proteins. Such combinations may be referred to as pseudotyped recombinant adenoviral particles. In some embodiments, foreign viral capsid proteins used in pseudotyped recombinant adenoviral particles are derived from a foreign virus or from another adenovirus serotype. In some embodiments, the foreign viral capsid proteins are

derived from, including without limitation, reovirus type 3. Examples of vector and capsid protein combinations used in pseudotyped adenovirus particles can be found in the following references (Tatsis, N. *et al.* (2004) *Mol. Ther.* 10(4):616-629 and Ahi, Y. *et al.* (2011) *Curr. Gene Ther.* 11(4):307-320). Different adenovirus serotypes can be used to optimize transduction of particular target cells or to target specific cell types within a particular target tissue (*e.g.*, a diseased tissue). Tissues or cells targeted by specific adenovirus serotypes, include without limitation, lung (*e.g.* HuAd3), spleen and liver (*e.g.* HuAd37), smooth muscle, synoviocytes, dendritic cells, cardiovascular cells, tumor cell lines (*e.g.* HuAd11), and dendritic cells (*e.g.* HuAd5 pseudotyped with *reovirus type 3, HuAd30, or HuAd35*). For further description, see Ahi, Y. *et al.* (2011) *Curr. Gene Ther.* 11(4):307-320, Kay, M. *et al.* (2001) *Nat. Med.* 7(1):33-40, and Tatsis, N. *et al.* (2004) *Mol. Ther.* 10(4):616-629. Adenoviral vectors have been administered by intrastriatal administration (see, *e.g.,* Mittoux, V. *et al.* (2002) *J. Neurosci.* 22:4478-86).

[0164] In some embodiments, the viral particle is a lentiviral particle. In some embodiments, the lentiviral particle is a recombinant lentiviral particle, *e.g.,* a polynucleotide vector encoding an RNAi of the present disclosure between two LTRs. Lentiviruses are positive-sense, ssRNA retroviruses with a genome of approximately 10 kb. Lentiviruses are known to integrate into the genome of dividing and non-dividing cells. Lentiviral particles may be produced, for example, by transfecting multiple plasmids (typically the lentiviral genome and the genes required for replication and/or packaging are separated to prevent viral replication) into a packaging cell line, which packages the modified lentiviral genome into lentiviral particles. In some embodiments, a lentiviral particle may refer to a first generation vector that lacks the envelope protein. In some embodiments, a lentiviral particle may refer to a second generation vector that lacks all genes except the gag/pol and tat/rev regions. In some embodiments, a lentiviral particle may refer to a third generation vector that only contains the endogenous rev, gag, and pol genes and has a chimeric LTR for transduction without the tat gene (see Dull, T. *et al.* (1998) *J. Virol.* 72:8463-71). For further description, see Durand, S. and Cimarelli, A. (2011) *Viruses* 3:132-59.

[0165] In some embodiments, the viral particle is a recombinant lentiviral particle comprising a nucleic acid encoding an RNAi of the present disclosure. Use of any lentiviral vector is considered within the scope of the present invention. In some embodiments, the lentiviral vector is derived from a lentivirus including, without limitation, human

immunodeficiency virus-1 (HIV-1), human immunodeficiency virus-2 (HIV-2), simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), equine infectious anemia virus (EIAV), bovine immunodeficiency virus (BIV), Jembrana disease virus (JDV), visna virus (VV), and caprine arthritis encephalitis virus (CAEV). The lentiviral particle also comprises capsid proteins. In some embodiments, the recombinant viral particles comprise a lentivirus vector in combination with one or more foreign viral capsid proteins. Such combinations may be referred to as pseudotyped recombinant lentiviral particles. In some embodiments, foreign viral capsid proteins used in pseudotyped recombinant lentiviral particles are derived from a foreign virus. In some embodiments, the foreign viral capsid protein used in pseudotyped recombinant lentiviral particles is Vesicular stomatitis virus glycoprotein (VSV-GP). VSV-GP interacts with a ubiquitous cell receptor, providing broad tissue tropism to pseudotyped recombinant lentiviral particles. In addition, VSV-GP is thought to provide higher stability to pseudotyped recombinant lentiviral particles. In other embodiments, the foreign viral capsid proteins are derived from, including without limitation, Chandipura virus, Rabies virus, Mokola virus, Lymphocytic choriomeningitis virus (LCMV), Ross River virus (RRV), Sindbis virus, Semliki Forest virus (SFV), Venezuelan equine encephalitis virus, Ebola virus Reston, Ebola virus Zaire, Marburg virus, Lassa virus, Avian leukosis virus (ALV), Jaagsiekte sheep retrovirus (JSRV), Moloney Murine leukemia virus (MLV), Gibbon ape leukemia virus (GALV), Feline endogenous retrovirus (RD114), Human T-lymphotropic virus 1 (HTLV-1), Human foamy virus, Maedi-visna virus (MVV), SARS-CoV, Sendai virus, Respiratory syncytia virus (RSV), Human parainfluenza virus type 3, Hepatitis C virus (HCV), Influenza virus, Fowl plague virus (FPV), or *Autographa californica* multiple nucleopolyhedro virus (AcMNPV). Examples of vector and capsid protein combinations used in pseudotyped Lentivirus particles can be found, for example, in Cronin, J. *et al.* (2005). *Curr. Gene Ther.* 5(4):387-398. Different pseudotyped recombinant lentiviral particles can be used to optimize transduction of particular target cells or to target specific cell types within a particular target tissue (*e.g.*, a diseased tissue). For example, tissues targeted by specific pseudotyped recombinant lentiviral particles, include without limitation, liver (*e.g.* pseudotyped with a VSV-G, LCMV, RRV, or SeV F protein), lung (*e.g.* pseudotyped with an Ebola, Marburg, SeV F and HN, or JSRV protein), pancreatic islet cells (*e.g.* pseudotyped with an LCMV protein), central nervous system (*e.g.* pseudotyped with a VSV-G, LCMV, Rabies, or Mokola protein), retina (*e.g.* pseudotyped with a VSV-G or Mokola protein), monocytes or muscle (*e.g.* pseudotyped with a Mokola or Ebola protein), hematopoietic system (*e.g.* pseudotyped with an RD114 or GALV protein), or cancer cells

(*e.g.* pseudotyped with a GALV or LCMV protein). For further description, see Cronin, J. *et al.* (2005). *Curr. Gene Ther.* 5(4):387-398 and Kay, M. *et al.* (2001) *Nat. Med.* 7(1):33-40.

[0166] In some embodiments, the viral particle is a herpes simplex virus (HSV) particle. In some embodiments, the HSV particle is a rHSV particle, *e.g.,* a polynucleotide vector encoding an RNAi of the present disclosure between two TRs. HSV is an enveloped, double-stranded DNA virus with a genome of approximately 152 kb. Advantageously, approximately half of its genes are nonessential and may be deleted to accommodate heterologous sequence. HSV particles infect non-dividing cells. In addition, they naturally establish latency in neurons, travel by retrograde transport, and can be transferred across synapses, making them advantageous for transfection of neurons and/or gene therapy approaches involving the nervous system. In some embodiments, the HSV particle may be replication-defective or replication-competent (*e.g.,* competent for a single replication cycle through inactivation of one or more late genes). For further description, see Manservigi, R. *et al.* (2010) *Open Virol. J.* 4:123-56.

[0167] In some embodiments, the viral particle is a rHSV particle comprising a nucleic acid encoding an RNAi of the present disclosure. Use of any HSV vector is considered within the scope of the present invention. In some embodiments, the HSV vector is derived from a HSV serotype, including without limitation, HSV-1 and HSV-2. The HSV particle also comprises capsid proteins. In some embodiments, the recombinant viral particles comprise a HSV vector in combination with one or more foreign viral capsid proteins. Such combinations may be referred to as pseudotyped rHSV particles. In some embodiments, foreign viral capsid proteins used in pseudotyped rHSV particles are derived from a foreign virus or from another HSV serotype. In some embodiments, the foreign viral capsid protein used in a pseudotyped rHSV particle is a Vesicular stomatitis virus glycoprotein (VSV-GP). VSV-GP interacts with a ubiquitous cell receptor, providing broad tissue tropism to pseudotyped rHSV particles. In addition, VSV-GP is thought to provide higher stability to pseudotyped rHSV particles. In other embodiments, the foreign viral capsid protein may be from a different HSV serotype. For example, an HSV-1 vector may contain one or more HSV-2 capsid proteins. Different HSV serotypes can be used to optimize transduction of particular target cells or to target specific cell types within a particular target tissue (*e.g.,* a diseased tissue). Tissues or cells targeted by specific adenovirus serotypes include without limitation, central nervous system and neurons (*e.g.* HSV-1). For further description, see

Manservigi, R. *et al.* (2010) *Open Virol J* 4:123-156, Kay, M. *et al.* (2001) *Nat. Med.* 7(1):33-40, and Meignier, B. *et al.* (1987) *J. Infect. Dis.* 155(5):921-930.

Production of viral particles

[0168] rAAV particles can be produced using methods known in the art. See, *e.g.*, U.S. Pat. Nos. 6,566,118; 6,989,264; and 6,995,006. In practicing the invention, host cells for producing rAAV particles include mammalian cells, insect cells, plant cells, microorganisms and yeast. Host cells can also be packaging cells in which the AAV rep and cap genes are stably maintained in the host cell or producer cells in which the AAV vector genome is stably maintained. Exemplary packaging and producer cells are derived from 293, A549 or HeLa cells. AAV vectors are purified and formulated using standard techniques known in the art.

[0169] Methods known in the art for production of rAAV vectors include but are not limited to transfection, stable cell line production, and infectious hybrid virus production systems which include adenovirus-AAV hybrids, herpesvirus-AAV hybrids (Conway, JE *et al.*, (1997) *J. Virology* 71(11):8780-8789) and baculovirus-AAV hybrids. rAAV production cultures for the production of rAAV virus particles all require; 1) suitable host cells, including, for example, human-derived cell lines such as HeLa, A549, or 293 cells, or insect-derived cell lines such as SF-9, in the case of baculovirus production systems; 2) suitable helper virus function, provided by wild-type or mutant adenovirus (such as temperature sensitive adenovirus), herpes virus, baculovirus, or a plasmid construct providing helper functions; 3) AAV rep and cap genes and gene products; 4) a nucleic acid (such as a therapeutic nucleic acid) flanked by at least one AAV ITR sequences ; and 5) suitable media and media components to support rAAV production. In some embodiments, the AAV rep and cap gene products may be from any AAV serotype. In general, but not obligatory, the AAV rep gene product is of the same serotype as the ITRs of the rAAV vector genome as long as the rep gene products may function to replicated and package the rAAV genome. Suitable media known in the art may be used for the production of rAAV vectors. These media include, without limitation, media produced by Hyclone Laboratories and JRH including Modified Eagle Medium (MEM), Dulbecco's Modified Eagle Medium (DMEM), custom formulations such as those described in U.S. Patent No. 6,566,118, and Sf-900 II SFM media as described in U.S. Patent No. 6,723,551, each of which is incorporated herein by reference in its entirety, particularly with respect to custom media formulations for use in production of recombinant AAV vectors. In some embodiments, the AAV helper functions

are provided by adenovirus or HSV. In some embodiments, the AAV helper functions are provided by baculovirus and the host cell is an insect cell (*e.g.*, *Spodoptera frugiperda* (Sf9) cells).

[0170] In some embodiments, rAAV particles may be produced by a triple transfection method, such as the exemplary triple transfection method provided infra. Briefly, a plasmid containing a rep gene and a capsid gene, along with a helper adenoviral plasmid, may be transfected (*e.g.*, using the calcium phosphate method) into a cell line (*e.g.*, HEK-293 cells), and virus may be collected and optionally purified. As such, in some embodiments, the rAAV particle was produced by triple transfection of a nucleic acid encoding the rAAV vector, a nucleic acid encoding AAV rep and cap, and a nucleic acid encoding AAV helper virus functions into a host cell, wherein the transfection of the nucleic acids to the host cells generates a host cell capable of producing rAAV particles.

[0171] In some embodiments, rAAV particles may be produced by a producer cell line method, such as the exemplary producer cell line method provided infra (see also (referenced in Martin *et al.*, (2013) *Human Gene Therapy Methods* 24:253-269). Briefly, a cell line (*e.g.*, a HeLa cell line) may be stably transfected with a plasmid containing a rep gene, a capsid gene, and a promoter-heterologous nucleic acid sequence. Cell lines may be screened to select a lead clone for rAAV production, which may then be expanded to a production bioreactor and infected with an adenovirus (*e.g.*, a wild-type adenovirus) as helper to initiate rAAV production. Virus may subsequently be harvested, adenovirus may be inactivated (*e.g.*, by heat) and/or removed, and the rAAV particles may be purified. As such, in some embodiments, the rAAV particle was produced by a producer cell line comprising one or more of nucleic acid encoding the rAAV vector, a nucleic acid encoding AAV rep and cap, and a nucleic acid encoding AAV helper virus functions.

[0172] In some aspects, a method is provided for producing any rAAV particle as disclosed herein comprising (a) culturing a host cell under a condition that rAAV particles are produced, wherein the host cell comprises (i) one or more AAV package genes, wherein each said AAV packaging gene encodes an AAV replication and/or encapsidation protein; (ii) an rAAV pro-vector comprising a nucleic acid encoding an RNAi of the present disclosure as described herein flanked by at least one AAV ITR, and (iii) an AAV helper function; and (b) recovering the rAAV particles produced by the host cell. In some embodiments, the RNAi comprises the nucleotide sequence of SEQ ID NO:7. In some

embodiments, said at least one AAV ITR is selected from the group consisting of AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV capsid serotype ITRs or the like. In some embodiments, said encapsidation protein is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6 (*e.g.*, a wild-type AAV6 capsid, or a variant AAV6 capsid such as ShH10, as described in U.S. PG Pub. 2012/0164106), AAV7, AAV8, AAVrh8, AAVrh8R, AAV9 (*e.g.*, a wild-type AAV9 capsid, or a modified AAV9 capsid as described in U.S. PG Pub. 2013/0323226), AAV10, AAVrh10, AAV11, AAV12, a tyrosine capsid mutant, a heparin binding capsid mutant, an AAV2R471A capsid, an AAVAAV2/2-7m8 capsid, an AAV DJ capsid (*e.g.*, an AAV-DJ/8 capsid, an AAV-DJ/9 capsid, or any other of the capsids described in U.S. PG Pub. 2012/0066783), AAV2 N587A capsid, AAV2 E548A capsid, AAV2 N708A capsid, AAV V708K capsid, goat AAV capsid, AAV1/AAV2 chimeric capsid, bovine AAV capsid, mouse AAV capsid, rAAV2/HBoV1 capsid, or an AAV capsid described in U.S. Pat. No. 8,283,151 or International Publication No. WO/2003/042397. In some embodiments, a mutant capsid protein maintains the ability to form an AAV capsid. In some embodiments, the encapsidation protein is an AAV5 tyrosine mutant capsid protein. In further embodiments, the rAAV particle comprises capsid proteins of an AAV serotype from Clades A-F. In some embodiments, the rAAV particles comprise an AAV1 capsid and a recombinant genome comprising AAV2 ITRs, a mutant AAV2 ITR and nucleic acid encoding an RNAi of the present disclosure. In a further embodiment, the rAAV particles are purified. The term "purified" as used herein includes a preparation of rAAV particles devoid of at least some of the other components that may also be present where the rAAV particles naturally occur or are initially prepared from. Thus, for example, isolated rAAV particles may be prepared using a purification technique to enrich it from a source mixture, such as a culture lysate or production culture supernatant. Enrichment can be measured in a variety of ways, such as, for example, by the proportion of DNase-resistant particles (DRPs) or genome copies (gc) present in a solution, or by infectivity, or it can be measured in relation to a second, potentially interfering substance present in the source mixture, such as contaminants, including production culture contaminants or in-process contaminants, including helper virus, media components, and the like.

[0173] Numerous methods are known in the art for production of adenoviral vector particles. For example, for a gutted adenoviral vector, the adenoviral vector genome and a

helper adenovirus genome may be transfected into a packaging cell line (*e.g.*, a 293 cell line). In some embodiments, the helper adenovirus genome may contain recombination sites flanking its packaging signal, and both genomes may be transfected into a packaging cell line that expresses a recombinase (*e.g.*, the Cre/loxP system may be used), such that the adenoviral vector of interest is packaged more efficiently than the helper adenovirus (see, *e.g.*, Alba, R. *et al.* (2005) *Gene Ther.* 12 Suppl 1:S18-27). Adenoviral vectors may be harvested and purified using standard methods, such as those described herein.

[0174] Numerous methods are known in the art for production of lentiviral vector particles. For example, for a third-generation lentiviral vector, a vector containing the lentiviral genome of interest with gag and pol genes may be co-transfected into a packaging cell line (*e.g.*, a 293 cell line) along with a vector containing a rev gene. The lentiviral genome of interest also contains a chimeric LTR that promotes transcription in the absence of Tat (see Dull, T. *et al.* (1998) *J. Virol.* 72:8463-71). Lentiviral vectors may be harvested and purified using methods (*e.g.*, Segura MM, *et al.*, (2013) *Expert Opin Biol Ther.* 13(7):987-1011) described herein.

[0175] Numerous methods are known in the art for production of HSV particles. HSV vectors may be harvested and purified using standard methods, such as those described herein. For example, for a replication-defective HSV vector, an HSV genome of interest that lacks all of the immediate early (IE) genes may be transfected into a complementing cell line that provides genes required for virus production, such as ICP4, ICP27, and ICP0 (see, *e.g.*, Samaniego, L.A. *et al.* (1998) *J. Virol.* 72:3307-20). HSV vectors may be harvested and purified using methods described (*e.g.*, Goins, WF *et al.*, (2014) Herpes Simplex Virus Methods in Molecular Biology 1144:63-79).

[0176] Also provided herein are pharmaceutical compositions comprising a recombinant viral particle comprising a transgene encoding an RNAi of the present disclosure and a pharmaceutically acceptable carrier. The pharmaceutical compositions may be suitable for any mode of administration described herein. A pharmaceutical composition of a recombinant viral particle comprising a nucleic acid encoding an RNAi of the present disclosure can be introduced to the brain. For example, a recombinant viral particle comprising a nucleic acid encoding an RNAi of the present disclosure can be administered intrastriatally. Any of the recombinant viral particles of the present disclosure may be used, including rAAV, adenoviral, lentiviral, and HSV particles.

[0177] In some embodiments, the pharmaceutical compositions comprising a recombinant viral particle comprising a transgene encoding an RNAi of the present disclosure described herein and a pharmaceutically acceptable carrier is suitable for administration to human. Such carriers are well known in the art (see, *e.g.*, Remington's Pharmaceutical Sciences, 15th Edition, pp. 1035-1038 and 1570-1580). In some embodiments, the pharmaceutical compositions comprising a rAAV described herein and a pharmaceutically acceptable carrier is suitable for injection into the brain of a mammal (*e.g.*, intrastriatal administration). In some embodiments, the pharmaceutical compositions comprising a recombinant lentiviral particle described herein and a pharmaceutically acceptable carrier is suitable for injection into the brain of a mammal (*e.g.*, intrastriatal administration). In some embodiments, the pharmaceutical compositions comprising a recombinant adenoviral particle described herein and a pharmaceutically acceptable carrier is suitable for injection into the brain of a mammal (*e.g.*, intrastriatal administration). In some embodiments, the pharmaceutical compositions comprising a recombinant HSV particle described herein and a pharmaceutically acceptable carrier is suitable for injection into the brain of a mammal (*e.g.*, intrastriatal administration).

[0178] Such pharmaceutically acceptable carriers can be sterile liquids, such as water and oil, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and the like. Saline solutions and aqueous dextrose, polyethylene glycol (PEG) and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. The pharmaceutical composition may further comprise additional ingredients, for example preservatives, buffers, tonicity agents, antioxidants and stabilizers, nonionic wetting or clarifying agents, viscosity-increasing agents, and the like. The pharmaceutical compositions described herein can be packaged in single unit dosages or in multidosage forms. The compositions are generally formulated as sterile and substantially isotonic solution.

VII. Articles of Manufacture and Kits

[0179] Also provided are kits or articles of manufacture for use in the methods described herein. In aspects, the kits comprise the compositions described herein (*e.g.*, a recombinant viral particle of the present disclosure, such as a rAAV particle comprising nucleic acid encoding an RNAi of the present disclosure) in suitable packaging. Suitable packaging for compositions (such as intrastriatal compositions) described herein are known in the art, and

include, for example, vials (such as sealed vials), vessels, ampules, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

[0180] The present invention also provides kits comprising compositions described herein and may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein. For example, in some embodiments, the kit comprises a composition of recombinant viral particles comprising a transgene encoding an RNAi of the present disclosure for delivery of at least 1×10^9 genome copies into the brain of a mammal (*e.g.*, through intrastriatal administration) to a primate as described herein, a pharmaceutically acceptable carrier suitable for injection into the brain of a primate, and one or more of: a buffer, a diluent, a filter, a needle, a syringe, and a package insert with instructions for performing injections into the brain of a primate (*e.g.*, intrastriatal administration). In some embodiments, the kit comprising instructions for treating Huntington's disease with the recombinant viral particles described herein. In some embodiments, the kit comprising instructions for using the recombinant viral particles described herein according to any one of the methods described herein.

EXAMPLES

[0181] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: AAV2/1-miRNA-Htt reduces Htt expression *in vitro*.

[0182] RNA interference (RNAi) provides an approach for the treatment of many human diseases. However, the safety of RNAi-based therapies can be hampered by the ability of small inhibitory RNAs (siRNAs) to bind to unintended mRNAs and reduce their expression, an effect known as off-target gene silencing. Off-targeting primarily occurs when the seed

region (nucleotides 2–8 of the small RNA) pairs with sequences in 3′-UTRs of unintended mRNAs and directs translational repression and destabilization of those transcripts. To date, most therapeutic RNAi sequences are selected primarily for gene silencing efficacy, and later evaluated for safety. Two siRNAs were generated to treat Huntington's disease (HD), a dominant neurodegenerative disorder, with minimal off-targeting potential (*i.e.*, those with a scarcity of seed complements within all known human and rhesus monkey 3′-UTRs) which demonstrates potent huntingtin silencing in the mouse brain with a low in silico off-target profile (Table 1, Fig. 1A). One sequence (207) was tested for its ability to rescue behavioral phenotypes in the YAC128 mouse model of HD. Striatal delivery of AAV2/1-miRNA-Htt-207 not only reduces Htt mRNA and protein levels in the brain, but also corrects the aberrant behavioral profiles in YAC128 mice and demonstrates high guide strand activity and precise 5' processing, minimizing the potential for off target effects.

TABLE 1. miRNA and reverse complement (target) sequences for 206 and 207 as well as the top and bottom sequences for cloning, including restriction site overhangs.

miRNA ID	Component	Sequence	SEQ ID NO:
206	miRNA sequence (anti-sense, 5'→3')	uGGCCGUCCAUCUUGGACCCG	1
206	reverse complement (sense, 5'→3')	CGGGUCCAAGAUGGACGGCCa	2
206	DNA sequence encoding miRNA duplex	GTGGCCGTCCATCTTGGACC CGGTTTTGGCCACTGACTGA CCGGGTCCAATGGACGGCCA	3
206	RNA sequence of miRNA duplex	GUGGCCGUCCAUCUUGGACC CGGUUUUGGCCACUGACUGA CCGGGUCCAAUGGACGGCCA	4
206	top sequence for cloning (5'→3') stem loop that contains the actual miRNA sequence, including restriction site overhangs for cloning*	TGCT**GTGGCCGTCCATCTTG GACCCG**GTTTTGGCCACTGA CTGAC***CGGGTCC****AATGGACG GCCA*	5
206	bottom sequence for cloning (5'→3') reverse complement of sequence in column to the left, including restriction site overhangs for cloning*	CCTG*TGGCCGTCCAT****TGGAC CCG***GTCAGTCAGTGGCCAAA AC**CGGGTCCAAGATGGACGG CCA**C	6
207	miRNA sequence (anti-sense, 5'→3')	AGUCGGUGUGGUUGACAAGCA	7
207	reverse complement (sense, 5'→3')	UGCUUGUCAACCACACCGACU	8
207	DNA sequence encoding miRNA duplex	AGTCGGTGTGGTTGACAAGCA GTTTTGGCCACTGACTGACTG	9

		CTTGTCCCACACCGACT	
207	RNA sequence of miRNA duplex	AGUCGGUGUGGUUGACAAGCA GUUUUGGCCACUGACUGACUG CUUGUCCCACACCGACU	10
207	top sequence for cloning (5'→3') stem loop that contains the actual miRNA sequence, including restriction site overhangs for cloning*	TGCTG**AGTCGGTGTGGTTGA CAAGCA**GTTTTGGCCACTGA CTGAC***TGCTTGTC***CCACACC *GACT*	11
207	bottom sequence for cloning (5'→3') reverse complement of sequence in column to the left, including restriction site overhangs for cloning*	CCTG*AGTCGGTGTGG***GACAA *GCA***GTCAGTCAGTGGCCAAA AC**TGCTTGTCAACCACACCG ACT**C	12

*For sequences for cloning-Restriction site overhangs for cloning are underlined; miRNA sequences in bold; loop sequence in plain text; bases 1-8 of miRNA reverse complement in bold, italics; bases 11-21 (11-20 for 170XX) of miRNA reverse complement in italics.

[0183] The ability of AAV2/1-miRNA-Htt 206 and 207 to mediate human huntingtin mRNA reduction was tested in vitro using human embryonic kidney (HEK293) cells. AAV2/1-miRNA-206 and 207 expression plasmids, as well as a positive control plasmid (170XA) containing a miRNA sequence previously shown to reduce Htt levels by approximately 50%, were transfected HEK293 cells (8 replicates per treatment). Cells were transfected using Fugene transfection reagent and harvested 48 hours later. Total RNA was isolated using the TaqMan® Cells-to-CT™ Kit (Ambion). RNA levels were measured by quantitative real-time RT-PCR (conducted and analyzed on an ABI Prism 7500 Sequence Detector (Applied Biosystems)). Expression levels were normalized to human PPIA (peptidylprolyl isomerase). As shown in Fig. 2, human Htt mRNA levels were reduced following tranfection with both 206 and 207 plasmids compared to untreated untreated controls. Level of Htt reduction were nearly equivalent compared the 170XA positive control.

Example 2: AAV2/1-miRNA-Htt reduces Htt expression *in vivo*.

[0184] The ability of AAV2/1-miRNA- 206 and 207 to reduce HTT protein levels in the striatum of YAC128 HD mice was tested. Adult YAC128 mice received bilateral intra-striatal injections of AAV2/1-miRNA-Htt 206 (1e10 vgs/site) or AAV2/1-miRNA-Htt 207 (1e10 vgs/site), or AAV2/1-CTL3 (a noncoding miRNA control) (1e10 vgs/site). One month following AAV injection, animals were sacrificed and perfused with PBS. Brains were collected for histology and biochemical analyses. For biochemical analyses the striatal region of one hemisphere was micro-dissected and snap frozen in liquid nitrogen. Striatal

levels of mutant human and mouse Htt mRNA and HTT protein were evaluated by QPCR and Western blot respectively. Mutant human Htt and mouse Htt mRNA was significantly reduced in AAV2/1-miRNA-Htt 206 and AAV2/1-miRNA-Htt 207 injected mice when compared to CTL3 control animals (Fig. 3A). PPIA served as a normalization control gene for all QPCR assays. Mutant human and mouse HTT protein was significantly reduced in all AAV2/1-miRNA-Htt-injected mice when compared to CTL3 control animals and an equivalent extent of reduction (approximately 50%, $p<0.05$) was noted across all treatments (Fig. 3B). Beta-tubulin served as a normalization control gene for all western blots.

[0185] The effect of AAV2/1-miRNA-Htt 206 and 207 on brain and body weights of YAC128 mice was evaluated. Animal body weights on the day of surgery were compared to body weights taken on the day of sacrifice, 1 month post injection (Fig. 4A). There were no differences between AAV2/1-miRNA-Htt 206 and 207 compared to CTL3 controls. All mice appeared healthy, alert, and responsive one month post treatment and no weight loss was observed in any treatment group. Wet brain wets were recorded after PBS perfusion and brain dissection. A statistically significant increase in brain weights of YAC128 mice treated with AAV2/1-miRNA-Htt 206 and 207 was observed compared on CTL3 treated controls (Fig. 4B).

Example 3: AAV2/1-miRNA-Htt corrects behavioral and coordination deficits in YAC128 mice

[0186] The ability of striatal delivery of AAV2/1-miRNA-Htt-207 to correct the aberrant behavioral phenotypes in YAC128 mice was evaluated. The impact of the AAV2/1-miRNA-Htt 207 mediated reduction of mutant Htt levels on the well-characterized phenotypic deficits that are present in the YAC128 mouse model of HD was also examined. Age-matched (3 month old) YAC128 and FVB wild-type littermate mice received bilateral intra-striatal injections of either AAV2/1-miRNA-Htt-207 (2e10vg/site) or AAV2/1-CTL3 control vector (2e10 vgs/site). Mice received behavioral testing and were sacrificed 3 months after treatment. Western blot analysis of brain homogenates showed the levels of mutant human HTT protein was significantly reduced in the striatum of AAV2/1-miRNA-Htt-207 injected YAC128 and FVB wild-type littermate mice (approximately 50% reduction, $p<0.01$) when compared to AAV2/1-CTL3-treated controls. Mouse HTT protein levels were not significantly reduced in this study (Figs. 5A and 5B). Real-time quantitative PCR analysis indicated a commensurate reduction in mRNA levels (Figs. 5C and 5D).

[0187] YAC128 mice have been reported to exhibit motor coordination deficits (which can be revealed using the rotarod test) and a depressive phenotype (which can be revealed using the Porsolt swim test) beginning at 3 months of age (Slow *et al.*, 2003, Van Raamsdonk *et al.*, 2007). Rotarod testing of AAV2/1-CTL3-treated YAC128 mice at 3 months post-injection showed significant motor coordination deficits when compared to AAV2/1-CTL3-treated wild-type littermates (ANOVA, $p<0.05$) (Fig. 6A). However, YAC128 mice that had been treated with AAV2/1-miRNA-Htt-207 showed performance levels that were indistinguishable from those of wild-type mice (ANOVA, Tukey's post-hoc; WT 207 vs. YAC128 207, p=NS; WT CTL3 vs. YAC128 CTL3, $p<0.05$). Hence, partial lowering of mutant Htt levels was sufficient to correct the motor deficits of YAC128 mice. There were no significant differences in rotarod performance between wild-type mice that received AAV2/1-miRNA-Htt-207 and wild-type mice that received AAV2/1-CTL3. Previous reports indicated that YAC128 mice exhibit a depressive phenotype that can be detected using the Porsolt swim test (Pouladi *et al.*, 2009). Animals are deemed to exhibit a depressive state if they are immobile for an extended period when placed into a container of water. Using a basic swim speed test (where swim latency to reach a platform was measured) researchers have demonstrated that this depressive phenotype in the Porsolt swim test is unrelated to the swimming ability of YAC128 mice and is independent of the well documented motor coordination deficits observed in this model (Pouladi *et al.*, 200). Three-month-old YAC128 and WT littermate mice were injected with AAV2/1-miRNA-Htt-207 - or AAV2/1-CTL3-vectors and tested 3 months later in the Porsolt swim test. CTL3 treated YAC128 mice displayed an increased period of time in an immobile state when compared to either AAV2/1-miRNA-Htt-207 -treated YAC mice or AAV2/1-CTL3-treated wild-type animals (Fig. 6B; ANOVA $p<0.05$). Again, there were no significant differences in the performance of wild-type mice that received either AAV2/1-miRNA-Htt or AAV2/1-CTL3. YAC128 mice that had been injected with AAV2/1-miRNA-Htt-207 spent significantly less time in an immobile state than AAV2/1-CTL3-treated controls. Indeed, the performance of AAV2/1-miRNA-Htt-207 treated YAC128 mice was similar to that of their wild-type littermates, suggesting a near-complete correction of this aberrant phenotype (ANOVA, Tukey's post-hoc; YAC 207 vs. YAC CTL3, $p<0.05$).

[0188] The effect of AAV2/1-miRNA-Htt 207 on brain and body weights of YAC128 mice was evaluated. Animal body weights on the day of surgery were compared to body weights taken on the day of sacrifice, 3 months post injection. There were no differences in

body weight between AAV2/1-miRNA-Htt 207 treated mice compared to CTL3 treated controls (Fig. 7A). All mice appeared healthy, alert, and responsive three months post treatment and no weight loss was observed in any treatment group. Wet brain wets were recorded after PBS perfusion and brain dissection. There were no differences in brain weights of YAC128 mice treated with AAV2/1-miRNA-Htt 207 compared on CTL3 treated controls (Fig. 7B).

Example 4. miRNA's demonstrate high guide activity and precise 5' processing following in vivo delivery

[0189] YAC128 mice were treated with AAV2/1-miRNA-Htt 206 or AAV2/1-miRNA-Htt 207 via intracranial injection. Post-treatment, the striatum was removed, and total RNA was isolated. Small RNA sequencing libraries were constructed using the NEBNext Small RNA Library Prep Set (New England Biolabs), and sequencing was performed on the Illumina MiSeq instrument. Samples from 2 separate mice were analyzed for each treatment. Here the total of all miRNA reads including endogenous sequences are shown as well as the total guide and passenger reads for each treatment vector. The AAV2/1-miRNA-Htt 202T vector treatment was included in this experiment as a control since it had been previously sequenced. The percent expected start position for each guide and passenger strand was > 99%, and the 207 vector had high guide: passenger strand ratios of 76.1% and 79.3%.

Table 2. Guide activity and 5' processing

Vector	**202T#**	**206**		**207**	
Sample ID #	202	23	28	33	34
Total Reads*	1,898,745	3,184,602	3,307,273	3,386,131	2,599,808
# Total Reads (guide)	47,001	196	186	11,801	39,177
% within expected start position	99.1	100	99.5	97.9	97.6
# Total Reads (passenger)	465,981	554	719	3,075	12,327
% within expected start position	99.2	99.1	99.4	99.5	99.2
% Guide	0.2	26.1	20.6	79.3	79.1

Example 5. Self-complementary miRHtt207 vector

[0190] The 207 miRHtt expression cassette can be packaged as a self - complementary vector genome. To achieve this, the ITR plasmid is designed to be only 2.3kb in size, this facilitates packaging of a 4.6kb dimeric vector; 4.6 kb is the packaging capacity of an AAV vector. The ITR plasmid can be designed to have a 5'WT ITR and a mutated D deleted, truncated 3 'ITR (Δ ITR), as depicted in Fig. 8. The predicted vector genomes that could be packaged are the self-complementary vector genome, which would be 3165 bp, and would contain a 5'and 3' WT ITR and a third, internal, delta ITR (*e.g.*, a chimeric intron). Additionally, it is expected that some monomeric vector genomes would be packaged, and these would be 1656bp in size.

[0191] An alternative approach to generating a self - complementary AAV miRHtt 207 vector *i.e.*, packaging two vector genomes per capsid, would be to make a small, single stranded, *i.e.*, 1755bp vector genome, so that two copies of the vector genome are packaged as a replication intermediate species, 3365 bp, (Fig. 9). In this example the ITR plasmid would have a 5' and 3' WT ITR and the replication intermediate, 3365bp, would have three WT ITRs, one 5' and 3' and one internal ITR. The single stranded vector gnome species, 1755bp, could also be packaged.

ADDITIONAL SEQUENCES

All polypeptide sequences are presented as N-terminal to C-terminal unless indicated otherwise. All nucleic acid sequences are presented as 5' to 3' unless indicated otherwise.

miRNA scaffold DNA sequence

ctggaggcttgctgaaggctgtatgctgttagacaatgattcacacggtgttttggccactgactgacaccgtgt
gtcattgtctaacaggacacaaggcctgttactagcactcacatggaacaaatggcc (SEQ ID NO:14)

Variant AAV ITR for scAAV vectors

CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCC
GGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA (SEQ ID NO:15).

ssAAV2/1miRHtt.de

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTT
GGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTCTATA
TTACCCTGCTAGGCAATTGGATCCCGGACCGTCGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGG
GGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGC
CCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGAC
GTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCC
CTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT
GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCA
TCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGG
GGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGG
CAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCCCTATAAAAAGC
GAAGCGCGCGGCGGGCGGGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCGCCTCGCGCCGCC
CGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATT
AGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTT
TGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCTCCGCGCTGC
CCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCG
GGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGT
GAGCAGGGGGTGTGGGCGCGTCGGTCGGGCTGCAACCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCC
CGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGG
GGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCG
GCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTT
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*CACACACCATCCCCACAGTTACGTACTAGTTCGAAGCCACGCGG*ACCGTTATAGTTACGAGGAACCCCTAGTGAT
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CTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAAAGATCT
(SEQ ID NO:16)

mIR207 DNA sequence shown in bold (SEQ ID NO:17)

Stuffer sequence shown in italics (SEQ ID NO:18)

Portion of A1AT gene

aattcgccctttggc**ctaggcaattggatcc**ggcggcagagaaaaacatcccagggatttacagatcacatgcagg
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attttaacccctcaggggcaggaaggcagcctgagatacagaagagtccatcacctgctgtatgccacacaccat
ccccacagttacgtactagttcgaagccacgcgtccgaagggcgaatt

(SEQ ID NO:20)

Stuffer sequence used in some embodiments is underlined

Delta chimeric intron sequence

ggagtcgctgcgcgctgccttcgccccgtgccccgctccgccgccgcctcgcgccgcccgccccggctctgactg
accgcgttactcccacaggtgagcgggcgggacggcccttctcctccgggctgtaattagcgcttggtttaatga
cggcttgtttcttttctgtggctgcgtgaaagccttgaggggctccgggagctagagcctctgctaaccatgttc
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tcctcgaagatccggtacccaattccggggccccacgctgcgcatccgcg

(SEQ ID NO:21)

CLAIMS

What is claimed is:

1. An RNAi comprising a first strand and a second strand, wherein

a) the first strand and the second strand form a duplex;

b) the first strand comprises a guide region, wherein the guide region comprises the nucleic acid sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1), or 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7); and

c) the second strand comprises a non-guide region.

2. The RNAi of claim 1, wherein:
(i) the guide region comprises the nucleic acid sequence 5'-UGGCCGUCCAUCUUGGACCCG-3' (SEQ ID NO:1); and the non-guide region comprises the sequence 5'- CGGGUCCAAGAUGGACGGCCA-3' (SEQ ID NO:2), or a nucleic acid sequence having more than 90% identity to SEQ ID NO: 2; or
(ii) the guide region comprises the nucleic acid sequence 5'-AGUCGGUGUGGUUGACAAGCA-3' (SEQ ID NO:7); and the non-guide region comprises the sequence 5'- UGCUUGUCAACCACACCGACU-3' (SEQ ID NO:8).

3. The RNAi of claim 1 or 2, wherein the first strand and the second strand are linked by means of a RNA linker capable of forming a loop structure.

4. The RNAi of claim 3, wherein:
(i) the RNA linker comprises from 4 to 50 nucleotides; and/or
(ii) the loop structure comprises 4 to 20 nucleotides; and/or
(iii) the RNAi comprises 5' to 3' the second strand, the RNA linker, and the first strand; or
(iv) the RNAi comprises 5' to 3' the first strand, the RNA linker, and the second strand

5. The RNAi of claim 3, wherein the RNAi comprises 5' to 3' the first strand, the RNA linker, and the second strand, and wherein the RNAi comprises the nucleic acid sequence of SEQ ID NO:4 or SEQ ID NO:10.

6. The RNAi of any one of claims 1-5, wherein the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA).

7. The RNAi of any one of claims 1-6, wherein the RNAi targets RNA encoding a polypeptide associated with Huntington's disease.

8. The RNAi of any one of claims 1-7, wherein the polypeptide is huntingtin.

9. The RNAi of claim 8, huntingtin comprises a mutation associated with Huntington's disease.

10. An expression construct comprising nucleic acid encoding the RNAi of any one of claims 1-7.

11. The expression construct of claim 10, wherein:
(i) the nucleic acid encoding the RNAi comprises a miRNA scaffold; and/or
(ii) the nucleic acid encoding the RNAi is operably linked to a promoter; and/or
(ii) the expression construct further comprises an intron; and/or
(iii) the expression construct further comprises a polyadenylation signal.

12. The RNAi of claim 11, wherein the promoter is selected from a cytomegalovirus (CMV) immediate early promoter, an RSV LTR, a MoMLV LTR, a phosphoglycerate kinase-1 (PGK) promoter, a simian virus 40 (SV40) promoter, a CK6 promoter, a transthyretin promoter (TTR), a TK promoter, a tetracycline responsive promoter (TRE), an HBV promoter, an hAAT promoter, a LSP promoter, a chimeric liver-specific promoter (LSP), an E2F promoter, a telomerase (hTERT) promoter; a cytomegalovirus enhancer/chicken beta-actin/Rabbit β-globin promoter (CAG) promoter, an elongation factor 1-alpha promoter (EFl-alpha) promoter, a human β-glucuronidase promoter, a chicken β-actin (CBA) promoter, a retroviral Rous sarcoma virus (RSV) LTR promoter, a dihydrofolate reductase promoter, and a 13-actin promoter.

13. The RNAi of claim 11, wherein the intron is a chimeric intron.

14. The RNAi of claim 11, wherein the expression vector is a self-complementary vector and the intron is a delta chimeric intron.

15. The RNAi of claim 11, wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA.

16. A vector comprising the expression construct of any one of claims 10 or 11.

17. The vector of claim 16, wherein:
(i) the vector is a recombinant adenoviral vector; or
(ii) the vector is a recombinant lentiviral vector; or
(iii) the vector is a recombinant herpes simplex virus (HSV) vector.

18. The vector of claim 17, wherein:
(i) the vector is a recombinant adenoviral vector, wherein the recombinant adenoviral vector is derived from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3
(ii) the vector is a recombinant lentiviral vector, wherein the recombinant lentiviral vector is derived from a lentivirus pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein; or
(iii) the vector is a recombinant herpes simplex virus (HSV) vector, wherein the rHSV vector is derived from rHSV-1 or rHSV-2.

19. The vector of claim 16, wherein the vector is a rAAV vector.

20. The rAAV vector of claim 19, wherein the expression construct is flanked by one or more AAV inverted terminal repeat (ITR) sequences.

21. The rAAV vector of claim 20, wherein:
(i) the expression construct is flanked by two AAV ITRs; and/or
(ii) the AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV serotype ITRs.

22. The rAAV vector of claim 19 or 20, wherein the vector further comprises a stuffer nucleic acid, wherein the stuffer nucleic acid is located upstream or downstream of the nucleic acid encoding the RNAi.

23. The rAAV vector of any one of claims 19-22, wherein the vector is a self-complementary rAAV vector.

24. The rAAV vector of claim 23, wherein the vector comprises first nucleic acid sequence encoding the RNAi and a second nucleic acid sequence encoding a complement of the RNAi, wherein the first nucleic acid sequence can form intrastrand base pairs with the second nucleic acid sequence along most or all of its length, and optionally wherein the first nucleic acid sequence and the second nucleic acid sequence are linked by a mutated AAV ITR, wherein the mutated AAV ITR comprises a deletion of the D region and comprises a mutation of the terminal resolution sequence.

25. A cell comprising the vector of claim 16 or 17 or the rAAV vector of any one of claims 19-23.

26. A viral particle comprising the vector of claim 16, wherein:
(i) the viral particle is an AAV particle encapsidating the rAAV vector; or
(ii) the viral particle is an adenovirus particle encapsidating the recombinant adenoviral vector,; or
(iii) the viral particle is a lentiviral particle encapsidating the recombinant lentiviral vector; or
(iv) the viral particle is an HSV particle encapsidating the recombinant HSV vector.

27. The viral particle of claim 26, wherein:
(i) the viral particle is an adenoviral particle encapsidating the recombinant adenoviral vector, wherein the adenovirus particle comprises a capsid from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3; or
(ii) the viral particle is an adenovirus particle encapsidating the recombinant adenoviral vector, wherein the adenovirus particle comprises an adenovirus serotype 2 capsid or a variant of an adenoviral serotype 5 capsid; or
(iii) the viral particle is a lentiviral particle encapsidating the recombinant lentiviral vector, wherein the lentiviral particle comprises a capsid pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein; or
(iv) the viral particle is an HSV particle encapsidating the recombinant HSV vector, wherein the HSV particle is a rHSV-1 particle or a rHSV-2 particle.

28. A recombinant AAV particle comprising the rAAV vector of any one of claims 19-23.

29. The recombinant AAV particle of claim 28, wherein:
(i) the AAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, AAV2-HBKO, AAVDJ8, AAVPHP.B, AAVPHP.eB, AAVBR1, AAVHSC15, AAVHSC17, a goat AAV, AAV1/AAV2 chimeric, bovine AAV, or mouse AAV capsid rAAV2/HBoV1 serotype capsid; and/or
(ii) the ITR and the capsid of the rAAV viral particle are derived from the same AAV serotype; or
(iii) the ITR and the capsid of the rAAV viral particle are derived from different AAV serotypes, optionally wherein the ITR is derived from AAV2 and the capsid of the rAAV particle is derived from AAV1.

30. A composition comprising the viral particle of claim 26 or the rAAV particle of claim 28, wherein the composition further comprises a pharmaceutically acceptable carrier.

31. A kit comprising the RNAi of any one of claims 1-6, the viral particle of claim 26, the AAV particle of claim 28, or the composition of claim 30.

32. Use of an RNAi of any one of claims 2-6, a viral particle of claim 26, an AAV particle of claim 28, or a composition of claim 30 in the preparation of a medicament for treating Huntington's disease in a mammal.

33. Use of an RNAi of any one of claims 2-6, a viral particle of claim 26, an AAV particle of claim 28, or a composition of claim 30 in the preparation of a medicament for inhibiting the expression of htt or inhibiting the accumulation of htt in a cell in a mammal with Huntington's disease.