

DESCRIPTION

ADENO-ASSOCIATED VIRAL VECTOR VARIANTS

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority to U.S. Provisional Application
5 Serial Nos. 62/939,315, filed November 22, 2019, and 63/084,709, filed September 29, 2020,
the entire contents of both applications being hereby incorporated by reference in their
entirety.

REFERENCE TO A SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing, which has been submitted
10 in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said
ASCII copy, created on November 19, 2020, is named CHOPP0038WO_ST25.txt and is 63.8
kilobytes in size.

BACKGROUND

1. Field

15 [0003] The present invention relates generally to the fields of medicine, virology, and
neurology. More particularly, it concerns targeting peptides that target delivery of viral
vectors to distinct structures in the brain.

2. Description of Related Art

[0004] Different strategies have been developed to generate AAV vector variants
20 including rational design and directed evolution. The rational design approach utilizes
knowledge of AAV capsids to make targeted changes to the capsid to alter transduction
efficiency or specificity, such as tyrosine mutations on the capsid surface for increasing
transduction efficiency. The directed evolution approach does not require any knowledge of
capsid structure and is done through random mutagenesis, capsid shuffling, or random
25 peptide insertions. These strategies generally use in vitro systems or mice, which are ideal for
cell-based or mouse studies, but do not imply translation to the clinic. In fact, no AAV
variants target distinct brain structures specifically or efficiently. As such, AAV variants that
are able to target distinct primate brain structures are needed.

SUMMARY

[0005] Provided herein are viral vectors each comprising a modified capsid, wherein the modified capsid comprises at least one amino acid sequence that targets the viral vector to a distinct brain structure.

5 [0006] In one embodiment, provided are modified adeno-associated virus (AAV) capsid proteins comprising a targeting peptide that targets a viral vector comprising the modified AAV capsid protein to a distinct organ or brain structure, and the targeting peptide is three to ten amino acids in length. In some aspects, the modified AAV capsid proteins are modified AAV1 capsid proteins, modified AAV2 capsid proteins, or modified AAV9 capsid
10 proteins.

 [0007] In some aspects, the modified AAV capsid proteins are derived from an AAV1 capsid protein (see SEQ ID NO: 138), and the targeting peptide is inserted after residue 590 of the AAV1 capsid protein. In some aspects, the targeting peptide is flanked by linker sequences, and the linker sequences on each side of the targeting peptides are two or
15 three amino acids long. In some aspects, the linker sequences are SSA on the N-terminal side of the targeting peptide and AS on the C-terminal side of the targeting peptide. In some aspects, the modified AAV1 capsid proteins have a sequence at least 95% identical to SEQ ID NO: 141.

 [0008] In some aspects, the modified AAV capsid proteins are derived from an
20 AAV2 capsid protein (see SEQ ID NO: 139), and the targeting peptide is inserted after residue 587 of the AAV2 capsid protein. In some aspects, the targeting peptide is flanked by linker sequences, and the linker sequences on each side of the targeting peptides are two or three amino acids long. In some aspects, the linker sequences are AAA on the N-terminal side of the targeting peptide and AA on the C-terminal side of the targeting peptide. In some
25 aspects, the modified AAV2 capsid proteins have a sequence at least 95% identical to SEQ ID NO: 142.

 [0009] In some aspects, the modified AAV capsid proteins are derived from an AAV9 capsid protein (see SEQ ID NO: 140), and the targeting peptide is inserted after residue 588 of the AAV9 capsid protein. In some aspects, the targeting peptide is flanked by
30 linker sequences, and the linker sequences on each side of the targeting peptides are two or three amino acids long. In some aspects, the linker sequences are AAA on the N-terminal

side of the targeting peptide and AS on the C-terminal side of the targeting peptide. In some aspects, the modified AAV9 capsid proteins have a sequence at least 95% identical to SEQ ID NO: 143.

5 **[0010]** In some aspects, the target peptide comprises a sequence up to ten amino acids in length having therein an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-137 and 144. In some aspects, the targeting peptide is seven amino acids in length.

10 **[0011]** In some aspects, the distinct brain structure is the brainstem, caudate, cerebellar cortex, cerebral cortex, ependyma, globus pallidus, hippocampus, meninges, optic nerve, putamen, spinal cord, substantia nigra, subthalamic nuclei, or thalamus. In certain aspects, the modified AAV capsid protein is a modified AAV1 capsid protein, and the targeting peptide is selected from those listed in Table 1 in order to target a corresponding brain structure. In certain aspects, the modified AAV capsid protein is a modified AAV2 capsid protein, and the targeting peptide is selected from those listed in Table 2 in order to target a corresponding brain structure. In certain aspects, the modified AAV capsid protein is
15 a modified AAV9 capsid protein, and the targeting peptide is selected from those listed in Table 3 in order to target a corresponding brain structure.

20 **[0012]** In some aspects, the distinct organ is the brain, kidney, heart, liver, gonad, spleen, or liver. In certain aspects, the modified AAV capsid protein is a modified AAV1 capsid protein, and the targeting peptide is selected from those listed in Table 4 in order to target a corresponding organ. In certain aspects, the modified AAV capsid protein is a modified AAV2 capsid protein, and the targeting peptide is selected from those listed in Table 5 in order to target a corresponding organ. In certain aspects, the modified AAV capsid protein is a modified AAV9 capsid protein, and the targeting peptide is selected from those listed in Table 6 in order to target a corresponding organ.

25 **[0013]** In one embodiment, provided herein are nucleic acids comprising a sequence encoding the modified capsid protein of any one of the present embodiments.

30 **[0014]** In one embodiment, provided herein are recombinant adeno-associated viruses (rAAV) comprising the modified capsid protein of any one of the present embodiments. In some aspects, combinations of rAAVs are provided. For example, the combination of an rAAV having a modified AAV1 capsid protein and a targeting peptide of SEQ ID NO: 21, an rAAV having a modified AAV2 capsid protein and a targeting peptide of SEQ ID NO: 53, an

rAAV having a modified AAV2 capsid protein and a targeting peptide of SEQ ID NO: 80, and an rAAV having a modified AAV9capsid protein and a targeting peptide of SEQ ID NO: 113 is provided.

5 **[0015]** In one embodiment, provided herein are viral vectors comprising a nucleic acid encoding the modified capsid protein of any one of the present embodiments. In some aspects, the viral vectors further comprise a nucleic acid sequence encoding a nucleic acid of interest. In some aspects, the nucleic acid of interest is a therapeutic agent. In some aspects, the therapeutic agent is an enzyme or an RNAi molecule.

10 **[0016]** In one embodiment, provided herein are cells comprising the viral vector of any one of the present embodiments. In some aspects, the cell is a mammalian cell, such as a human cell. In some aspects, the cell is in vitro or in vivo.

[0017] In one embodiment, provided herein are pharmaceutical compositions comprising the viral vector of the present embodiments and a pharmaceutically acceptable carrier.

15 **[0018]** In one embodiment, provided herein are methods to deliver an agent to a distinct brain structure of a subject, comprising administering a virus of the present embodiments to the subject. In some aspects, the distinct brain structure is the brainstem, caudate, cerebellar cortex, cerebral cortex, ependyma, globus pallidus, hippocampus, meninges, optic nerve, putamen, spinal cord, substantia nigra, subthalamic nuclei, or
20 thalamus. In certain aspects, an rAAV having a modified AAV1 capsid protein is used, and the targeting peptide is selected from those listed in Table 1 in order to target a corresponding brain structure. In certain aspects, an rAAV having a modified AAV2 capsid protein is used, and the targeting peptide is selected from those listed in Table 2 in order to target a corresponding brain structure. In certain aspects, an rAAV having a modified AAV9 capsid
25 protein is used, and the targeting peptide is selected from those listed in Table 3 in order to target a corresponding brain structure. In various aspects, combinations of any of the rAAVs are used. For example, the combination of an rAAV having a modified AAV1 capsid protein and a targeting peptide of SEQ ID NO: 21, an rAAV having a modified AAV2 capsid protein and a targeting peptide of SEQ ID NO: 53, an rAAV having a modified AAV2 capsid protein and a targeting peptide of SEQ ID NO: 80, and an rAAV having a modified AAV9capsid
30 protein and a targeting peptide of SEQ ID NO: 113 is used.

[0019] In one embodiment, provided herein are methods to deliver an agent to a distinct organ of a subject, comprising administering a virus of the present embodiments to the subject. In some aspects, the organ is the brain, kidney, heart, liver, gonad, spleen, or liver. In certain aspects, an rAAV having a modified AAV1 capsid protein is used, and the targeting peptide is selected from those listed in Table 4 in order to target a corresponding organ. In certain aspects, an rAAV having a modified AAV2 capsid protein is used, and the targeting peptide is selected from those listed in Table 5 in order to target a corresponding organ. In certain aspects, an rAAV having a modified AAV9 capsid protein is used, and the targeting peptide is selected from those listed in Table 6 in order to target a corresponding organ. In various aspects, combinations of any of the rAAVs are used.

[0020] In some aspects, the agent is an siRNA, shRNA, miRNA, non-coding RNA, lncRNA, therapeutic protein, or CRISPR system. In some aspects, the administration is to the central nervous system. In some aspects, the administration is to a cisterna magna, an intraventricular space, an ependyma, a brain ventricle, a subarachnoid space, and/or an intrathecal space. In some aspects, the brain ventricle is the rostral lateral ventricle, and/or the caudal lateral ventricle, and/or the right lateral ventricle, and/or the left lateral ventricle, and/or the right rostral lateral ventricle, and/or the left rostral lateral ventricle, and/or the right caudal lateral ventricle, and/or the left caudal lateral ventricle.

[0021] In some aspects, a plurality of viral particles is administered. In some aspects, the virus is administered at a dose of about 1×10^6 to about 1×10^{18} vector genomes per kilogram (vg/kg). In some aspects, the virus is administered at a dose from about 1×10^7 - 1×10^{17} , about 1×10^8 - 1×10^{16} , about 1×10^9 - 1×10^{15} , about 1×10^{10} - 1×10^{14} , about 1×10^{10} - 1×10^{13} , about 1×10^{10} - 1×10^{13} , about 1×10^{10} - 1×10^{11} , about 1×10^{11} - 1×10^{12} , about 1×10^{12} - 1×10^{13} , or about 1×10^{13} - 1×10^{14} vg/kg of the patient. In some aspects, the subject is human.

[0022] In one embodiment, provided herein are methods of treating a disease in a mammal comprising administering the virus of the present embodiments to the mammal. In some aspects, the disease is a neurodegenerative disease. In some aspects, the neurodegenerative disease is Huntington's disease, ALS, hereditary spastic hemiplegia, primary lateral sclerosis, spinal muscular atrophy, Kennedy's disease, Alzheimer's disease, polyglutamine repeat disease, or Parkinson's disease. In some aspects, the mammal is human.

[0023] As used herein, “essentially free,” in terms of a specified component, is used herein to mean that none of the specified component has been purposefully formulated into a composition and/or is present only as a contaminant or in trace amounts. The total amount of the specified component resulting from any unintended contamination of a composition is therefore well below 0.05%, preferably below 0.01%. Most preferred is a composition in which no amount of the specified component can be detected with standard analytical methods.

[0024] As used herein the specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising,” the words “a” or “an” may mean one or more than one.

[0025] The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” As used herein “another” may mean at least a second or more.

[0026] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, the variation that exists among the study subjects, or a value that is within 10% of a stated value.

[0027] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0029] **FIG. 1.** AAV peptide display library schematic.

[0030] **FIG. 2** In vivo screen strategy schematic.

[0031] **FIG. 3.** Graphical representation of input library diversity. Diversity of the input viral library measured from aliquots of AAV1, AAV2, and AAV9 viral vector prior to Round1 ICV injection.

[0032] **FIG. 4.** Graphical representation of round-over-round barcode enrichment. The total number of unique barcodes recovered after Round 1 and Round 2 enrichment in Rhesus macaque for each tissue collected. Round 2 values for DNA and RNA are shown.

[0033] **FIG. 5.** Illustration of round-over-round enrichment of barcodes in AAV1, AAV2, and AAV9 serotypes for cerebellar cortex.

[0034] **FIG. 6.** Illustration of enrichment for AAV9 1999. Heatmap depictions of barcode enrichment from AAV9, cells are colored by the percentage of barcodes detected from the indicated tissue. Barcodes recovered from DNA are shown on the left while barcodes recovered from RNA are on the right.

[0035] **FIGS. 7A-C.** Heatmap depictions of opool barcode enrichment from AAV1 (FIG. 7A), AAV2 (FIG. 7B), and AAV9 (FIG. 7C).

[0036] **FIG. 8.** AAV9 1999 *in vivo* Rhesus macaque validation. An eGFP expression construct was packaged into AAV9 1999 driven by the CAG promoter. 1.5E13 vg of AAV9 1999 was delivered to a 5 year old, female Rhesus macaque by ICV injection. Representative images of H&E stained cerebellum depicting the transduction pattern of AAV9 1999 are shown.

[0037] **FIGS. 9A-D.** AAV9 1999 *in vivo* mouse validation. An eGFP expression construct was packaged into AAV9 1999 driven by the CAG promoter. AAV9 1999 and

AAV9 capsids containing the eGFP construct were delivered to C57BL/6 p0 mouse pups by ICV injection at 1E10 vg. Representative images of eGFP fluorescence signal is the whole brain (FIG. 9A), whole brain sagittal section (FIG. 9B), S1 cortex sections (FIG. 9C, left), hippocampus sections (FIG. 9C, middle), cerebellum sagittal section (FIG. 9C, right), and
5 lumbar spinal cord coronal section (FIG. 9D).

[0038] FIGS. 10A-C. Fluorescent images of AAV mixture *in vivo* Rhesus macaque lateral ventricle (A), fourth ventricle (B), and meninges (C).

[0039] FIGS. 11A-C. Fluorescent images of cochlear turns (A), inner hair cells (B), organ of corti (C), and distal modiolus (C) following cochlear administration of AAV9 1999
10 capsids containing the eGFP construct to mice.

DETAILED DESCRIPTION

[0040] Provided herein are viral vectors each comprising a modified capsid, wherein the modified capsid comprises at least one amino acid sequence that targets the viral vector to a distinct brain structure. In certain embodiments, the brain structure is the brainstem, caudate, cerebellar cortex, cerebral cortex, ependyma, globus pallidus, hippocampus, meninges, optic nerve, putamen, spinal cord, substantia nigra, subthalamic nuclei, or thalamus. The targeting peptides for each brain structure are provided in Tables 1-3.

[0041] In certain embodiments, the viral vector is an adeno associated viral vector (AAV). In certain embodiments, the AAV is AAV1, AAV2, or AAV9. An exemplary wildtype reference AAV1 capsid protein sequence is provided in SEQ ID NO: 138. An exemplary wildtype reference AAV2 capsid protein sequence is provided in SEQ ID NO: 139. An exemplary wildtype reference AAV9 capsid protein sequence is provided in SEQ ID NO: 140. In certain aspects, the targeting peptide is inserted at position 590 of the AAV1 capsid, position 587 of the AAV2 capsid, or position 588 of the AAV9 capsid. An exemplary modified AAV1 capsid protein sequence is provided in SEQ ID NO: 141, which shows the targeting peptide insertion after position 590 as SSAX₇AS, where the leading SSA and the trailing AS are linker sequences and X₇ represents the targeting peptide. An exemplary modified AAV2 capsid protein sequence is provided in SEQ ID NO: 142, which shows the targeting peptide insertion after position 587 as AAAX₇AA, where the leading AAA and the trailing AA are linker sequences and X₇ represents the targeting peptide. An exemplary modified AAV9 capsid protein sequence is provided in SEQ ID NO: 143, which shows the targeting peptide insertion after position 588 as AAAX₇AS, where the leading AAA and the trailing AS are linker sequences and X₇ represents the targeting peptide.

Table 1. AAV1 targeting peptides for each brain structure.

Region	Peptide	SEQ ID NO
Brainstem	RPGREQA	1
	RGVLVTT	2
	RPGRESA	3
	NESLKKK	4
	DKTRAGS	5
	TAKSKQA	6
	PVKKKDA	7
	GRETLLG	8
	PIPAKK	9

Region	Peptide	SEQ ID NO
Caudate	RPGRESA	3
	PVKKKDA	7
	RPGREQA	1
	NVVRAGT	10
	KATANTR	11
	RDATRSS	12
	VPTKSPK	13
	AGVARSK	14
	RSRSEVL	15
	EVKGK GK	16
	RPGREAS	144
	RDSTRQL	26
	SGVLVQR	32
	DKTRAGS	5
	TAPKSLK	25
Cerebellar Cortex	AKLNKSS	17
	NESLK KK	4
	NSVHNTA	18
	NVVRGGA	19
	NRLVAGG	20
	RPGRESA	3
	ERDRTRG	21
	PI PAGKK	9
	RPGREQA	1
Cerebellar Cortex; Vermis	RPGREAS	144
	DKTRAGS	5
	NSVRPLT	23
	RDSTRQL	26
	RDATRSS	12
Cerebellum; right upper vermis	NSVRPLT	23
	RDATRSS	12
	DKTRAGS	5
	TAPKSLK	25
	RPGREAS	144
Cerebellum; lateral to brainstem (right)	NSVRPLT	23
	TAPKSLK	25
	RDSTRQL	26
	RPGREAS	144
	PI PAGKK	9
	RDATRSS	12
Cerebellum; Left hemisphere adjacent to upper vermis	RPGREAS	144
	DKTRAGS	5
	RDATRSS	12

Region	Peptide	SEQ ID NO
	RDSTRQL	26
	TAPKSLK	25
Cerebellum; Left hemisphere, Most lateral point	RPGREAS	144
	RDSTRQL	26
	DKTRAGS	5
	TAPKSLK	25
	NSVRPLT	23
	RDATRSS	12
	NSVRPLT	23
Cerebellum; Left hemisphere, Adjacent to brain stem	RPGREAS	144
	RDATRSS	12
	DKTRAGS	5
	NSVHNTA	18
	TAPKSLK	25
	VQGSKMK	22
Cerebral Cortex	RDATRSS	12
	NSVRPLT	23
	RPGRESA	3
	NKIHANP	24
	ERDRTRG	21
	DKTRAGS	5
	TAPKSLK	25
	RDSTRQL	26
	RPGREQA	1
	RDATRSS	12
Cochlea (ear)	RPGREAS	144
	TAPKSLK	25
	DKTRAGS	5
	NSVRPLT	23
	TATPRKG	49
	RPGREAS	144
Deep Cerebellar Nuclei	TAPKSLK	25
	RDATRSS	12
	RDSTRQL	26
	DKTRAGS	5
	RPGREAS	144
Dorsal Root Ganglia, Lumbar	RDATRSS	12
	NSVRPLT	23
	DKTRAGS	5
	TAPKSLK	25
	RPGREAS	144
Dorsal Root Ganglia, Cervical	DKTRAGS	5
	TAPKSLK	25

Region	Peptide	SEQ ID NO
	RDATRSS	12
	NSVHNTA	18
	NSVRPLT	23
Dorsal Root Ganglia, Thoracic	RDATRSS	12
	RPGREAS	144
	DKTRAGS	5
	TAPKSLK	25
	RDSTRQL	26
Ependyma	ERDRTRG	21
	RGVLVTT	2
	RPGRESA	3
	PIPAGKK	9
	NESLKKK	4
	NSVKSVL	27
	PVKKKDA	7
	VQGSKMK	22
	NVTIKSK	28
Ependyma; 3rd Ventricular	DKTRAGS	5
	TAPKSLK	25
	RPGREAS	144
	RDATRSS	12
	DRLKGIV	31
Ependyma; 4th Ventricular	TAPKSLK	25
	NSVRPLT	23
	RPGREAS	144
	KAAGRTV	46
	RDATRSS	12
	DKTRAGS	5
Ependyma; Lt Ventricular	RPGREAS	144
	NSVRPLT	23
	RDATRSS	12
	TAPKSLK	25
	DETSRLV	30
	DKTRAGS	5
Globus Pallidus	RPGRESA	3
	ERDRTRG	21
	VSLKERV	29
	VQGSKMK	22
	DKTRAGS	5
	DETSRLV	30
	RDATRSS	12
	AGVARSK	14
	EVKGKGG	16

Region	Peptide	SEQ ID NO
	DRLKGIV	31
Globus Pallidus External	RGVLVTT	2
	SGTFVKA	33
	EVKGK GK	16
	SVASAKK	48
	VSLKERV	29
	TATPRKG	49
Globus Pallidus Internal	RGVLVTT	2
	TAPKSLK	25
	TKTGLKL	50
	ERDRTRG	21
	SGVLVQR	32
	TATPRKG	49
	RPGREAS	144
Hippocampus	RGVLVTT	2
	RPGRESA	3
	SGVLVQR	32
	PVKKKDA	7
	NESLKKK	4
	SGTFVKA	33
	RPGREQA	1
	NSIARPV	34
Hippocampus; CA1	RGVLVTT	2
	SGVLVQR	32
	SVASAKK	48
	RDATRSS	12
	DKTRAGS	5
	TAPKSLK	25
Hippocampus; CA3	RPGREAS	144
	DETSRLV	30
	RDATRSS	12
	DKTRAGS	5
	ERDRTRG	21
	RDSTRQL	26
Hippocampus; DG	NSVRPLT	23
	RDATRSS	12
	TAPKSLK	25
	RDSTRQL	26
	RPGREAS	144
Inferior Olive	RPGREAS	144
	DKTRAGS	5
	TAPKSLK	25
	NDVRAKG	40

Region	Peptide	SEQ ID NO
	RDATRSS	12
	NSVRPLT	23
Lateral Geniculate Nuclei	NSVRPLT	23
	TAPKSLK	25
	RPGREAS	144
	DKTRAGS	5
	RDATRSS	12
Meninges	RPGRESA	3
	ERDRTRG	21
	RDATRSS	12
	DKTRAGS	5
	PIPAGKK	9
	NRARAGE	35
	ARHALGG	36
	HSSRPVA	37
	PVKKKDA	7
	RPGREAS	144
	TAPKSLK	25
	NSVRPLT	23
Motor Cortex	RPGREAS	144
	RGVLVTT	2
	NSVRPLT	23
	TAPKSLK	25
	DKTRAGS	5
	RDATRSS	12
Optic Nerve	AGVARSK	14
	RSRSEVL	15
	EVKGK GK	16
	DRLKGIV	31
	KTGTARL	38
	RPGRESA	3
	PVKKKDA	7
	RGVLVTT	2
	ERDRTRG	21
	RPGREAS	144
	NSVRPLT	23
	TAPKSLK	25
	DKTRAGS	5
	NDVRAKG	40
	RDATRSS	12
Prefrontal Cortex	RPGREAS	144
	NSVRPLT	23
	TAPKSLK	25

Region	Peptide	SEQ ID NO
	DKTRAGS	5
	RDATRSS	12
Pons	RPGREAS	144
	NSVRPLT	23
	TAPKSLK	25
	RDATRSS	12
	DKTRAGS	5
Putamen	DETSRLV	30
	VKALGRP	39
	NDVRAKG	40
	NESLKKK	4
	ERDRTRG	21
	RPGREAS	144
	QGVLVVR	41
	KQYAGSQ	42
	RDATRSS	12
	VPTKSPK	13
	DKTRAGS	5
	KAAGRTV	46
	TAPKSLK	25
Reticular Formation	RPGREAS	144
	RDSTRQL	26
	KGLRTPT	51
	RDATRSS	12
	NSVRPLT	23
	DKTRAGS	5
Spinal Cord	PVKKKDA	7
	RGVLVTT	2
	PIPAGKK	9
	SGVLVQR	32
	NESLKKK	4
	RPGRESA	3
	TNRMALS	43
	ERDRTRG	21
	SGTFVKA	33
Spinal Cord, Thoracic	NSVRPLT	23
	SGTFVKA	33
	RPGREAS	144
	RGVLVTT	2
	RDATRSS	12
	TAPKSLK	25
	DKTRAGS	5
Spinal Cord, Lumbar	RPGREAS	144

Region	Peptide	SEQ ID NO
	TATPRKG	49
	TAPKSLK	25
	RDATRSS	12
	KAAGRTV	46
Spinal Cord, Cervical	SGVLVQR	32
	NSVRPLT	23
	RPGREAS	144
	RGVLVTT	2
	SGTFVKA	33
	TAPKSLK	25
	RDATRSS	12
	DKTRAGS	5
Substantia Nigra	RGVLVTT	2
	GITLGRL	44
	RPGRESA	3
	PIPAGKK	9
	AGIMVRV	45
	SGVLVQR	32
	VSLKERV	29
	RDSTRQL	26
	RPGREAS	144
	RDATRSS	12
Subthalamic Nuclei	KAAGRTV	46
	RDATRSS	12
	RGVLVTT	2
	RPGRESA	3
	HVIRLPS	47
	EVKGKGGK	16
	NESLKKK	4
	DETSRLV	30
	NSVRPLT	23
	SGTFVKA	33
	PIPAGKK	9
	VKALGRP	39
Temporal Cortex	NSVRPLT	23
	RPGREAS	144
	TAPKSLK	25
	RDATRSS	12
	DKTRAGS	5
Thalamus	GRETLLKG	8
	RPGREQA	1
	ERDRTRG	21
	RGVLVTT	2

Region	Peptide	SEQ ID NO
	SVASAKK	48
	TATPRKG	49
	RDATRSS	12
	NVTIKSK	28
	TKTGLKL	50
	KGLRTPPT	51
Thalamus, Anterior	SGVLVQR	32
	NSVHNTA	18
	PIPAGKK	9
	DETSRLV	30
	AKLNKSS	17
	RPGREAS	144
VA/VL Thalamus	RGVLVTT	2
	DKTRAGS	5
	DETSRLV	30
	RDSTRQL	26
	NKIHANP	24
	TAPKSLK	25
Visual Cortex	RPGREAS	144
	NSVRPLT	23
	TAPKSLK	25
	RDATRSS	12
	NSVHNTA	18
	DKTRAGS	5

Table 2. AAV2 targeting peptides for each brain structure.

Region	Peptide	SEQ ID NO
Brainstem	LTSRTSP	52
	DDPSARR	53
	GEQDLRR	54
	VSTALPR	55
	RDDVPLR	56
	TRVGTAG	57
	SSSKTGS	58
	SLSTGPK	59
	VQGRQGG	60
	RGASGAV	61
Caudate	NARAQGV	62
	TSNRQGV	63
	AVRGGMA	64
	RGLDKGT	65
	KGVDLKP	66

Region	Peptide	SEQ ID NO
	TAVREER	67
	GNAGITK	68
	SLSTGPK	59
	SARAGAP	69
	GSRAGVG	105
	NARPVSA	76
	HDGGASR	103
	VTQSKGA	74
	KAQGVGG	79
	ESTGRER	73
Cerebellar Cortex	SGEFVGR	70
	SGRKLEV	71
	SARSGSV	72
	ESTGRER	73
	SSSKTGS	58
	RDDVPLR	56
	VQGRQGG	60
	VTQSKGA	74
	RGSGSAV	75
Cerebellar Cortex; Vermis	HDGGASR	103
	EAQSHPR	91
	ESTGRER	73
	GRSTGMT	95
	GSRAGVG	105
Cerebellum; right upper vermis	HDGGASR	103
	RAVPAGG	84
	RSNAPQT	90
	TKSLSSG	92
	ESTGRER	73
	RGSTQVG	94
Cerebellum; lateral to brainstem (right)	HDGGASR	103
	ESTGRER	73
	KAQGVGG	79
	EAQSHPR	91
	RAVPAGG	84
	RDDVPLR	56
Cerebellar Left hemisphere adjacent to upper vermis	HDGGASR	103
	ESTGRER	73
	EAQSHPR	91
	NARAQGV	62
	LTSRTSP	52
	KAQGVGG	79
Cerebellar Left	HDGGASR	103

Region	Peptide	SEQ ID NO
hemisphere Most lateral point	ESTGRER	73
	EAQSHPR	91
	KAQGVGG	79
	GSRAGVG	105
Cerebellar Left hemisphere Adjacent to brain stem	ESTGRER	73
	HDGGASR	103
	EAQSHPR	91
	GSRAGVG	105
	KAQGVGG	79
	GRGGAAL	100
Cerebral Cortex	DDPSARR	53
	NARPVSA	76
	TSNRQV	63
	NARAQGV	62
	TARGGGG	77
	KGVDLKP	66
	GRSASGS	78
	SSSKTGS	58
	KAQGVGG	79
	VQGRQGG	60
Cochlea (ear)	HDGGASR	103
	KAQGVGG	79
	RSNAPQT	90
	LTSRTSP	52
	EAQSHPR	91
Deep Cerebellar Nuclei	GSRAGVG	105
	RAVPAGG	84
	EAQSHPR	91
	AVRGGMA	64
	VPGRTAG	81
	VTQSKGA	74
Dorsal Root Ganglia Lumbar	HDGGASR	103
	TKSLSSG	92
	KAQGVGG	79
	GSRAGVG	105
	VPGRTAG	81
Dorsal Root Ganglia Cervical	GRSTGMT	95
	AVRGGMA	64
	HDGGASR	103
	TAAGGQR	99
	KAQGVGG	79
Dorsal Root GangliaThoracic	HDGGASR	103
	GSRAGVG	105

Region	Peptide	SEQ ID NO
	ARGSGVN	82
	RAVPAGG	84
	KAQGVGG	79
Ependyma	GRGAPGG	80
	DDPSARR	53
	TSNRGQV	63
	RGSGSAV	75
	VQGRQGG	60
	VTQSKGA	74
	NARPVSA	76
	KGVDLKP	66
	NARAQGV	62
	TARGGGG	77
Ependyma; 4th Ventricular	GSRAGVG	105
	RDDVPLR	56
	RGSTQVG	94
	KAQGVGG	79
	GRGGAAL	100
Ependyma; Lt Ventricular	HDGGASR	103
	ESTGRER	73
	EAQSHPR	91
	NARPVSA	76
	KAQGVGG	79
Ependyma; 3rd Ventricular	GRSTGMT	95
	LTSRTSP	52
	KAQGVGG	79
	GRGGAAL	100
	EAQSHPR	91
	ARGSGVN	82
Globus Pallidus	VQGRQGG	60
	VPGRTAG	81
	ARGSGVN	82
	SVRVGGQ	83
	RGSGSAV	75
	RAVPAGG	84
	VMSSGKP	85
	STPAPKS	86
	RGGAQVV	87
Globus Pallidus External	EAQSHPR	91
	GSRAGVG	105
	VQGRQGG	60
	ESTGRER	73
	GRGGAAL	100

Region	Peptide	SEQ ID NO
	KASGAGG	88
Globus Pallidus Internal	RDDVPLR	56
	GSRAGVG	105
	GAVGGVK	107
	DDPSARR	53
	RGSTQVG	94
	KAQGVGG	79
Hippocampus	DDPSARR	53
	KASGAGG	88
	KAQGVGG	79
	TSNRGQV	63
	VQGRQGG	60
	VSTALPR	55
	TGTAGLK	89
	NARPVSA	76
	SSSKTGS	58
Hippocampus; CA1	VAPISKs	101
	HDGGASR	103
	LTSRTSP	52
	ESTGRER	73
	KAQGVGG	79
Hippocampus; CA3	VQGRQGG	60
	GRSTGMT	95
	GRGGAAL	100
	KAQGVGG	79
	RGSTQVG	94
	TSNRGQV	63
Hippocampus; DG	HDGGASR	103
	ESTGRER	73
	RGSTQVG	94
	EAQSHPR	91
	KAQGVGG	79
	GSRAGVG	105
Inferior Olive	VPGRTAG	81
	HDGGASR	103
	VQGRQGG	60
	GSRAGVG	105
	RDDVPLR	56
	KAQGVGG	79
Lateral geniculate nuclei	HDGGASR	103
	ESTGRER	73
	GSRAGVG	105
	KAQGVGG	79

Region	Peptide	SEQ ID NO
	EAQSHPR	91
	VPGRTAG	81
Meninges	SSSKTGS	58
	NARPVSA	76
	RSNAPQT	90
	EAQSHPR	91
	TKSLSSG	92
	GRGAPGG	80
	AAGAKVM	93
	ESTGRER	73
	KGVDLKP	66
	VQGRQGG	60
	HDGGASR	103
	KAQGVGG	79
	RGSTQVG	94
	VPGRTAG	81
	GSRAGVG	105
Optic Nerve	DDPSARR	53
	RGGAQVV	87
	KASGAGG	88
	RGSGSAV	75
	VQGRQGG	60
	KAQGVGG	79
	TRVG TAG	57
	GEQDLRR	54
	RGSTQVG	94
	SSSKTGS	58
	GSRAGVG	105
	ESTGRER	73
	HDGGASR	103
	RDDVPLR	56
Pons	ESTGRER	73
	GSRAGVG	105
	HDGGASR	103
	KAQGVGG	79
	RDDVPLR	56
	GRGGAAL	100
Prefrontal Cortex	HDGGASR	103
	GSRAGVG	105
	ESTGRER	73
	KAQGVGG	79
	RGSTQVG	94
Primary motor cortex	GSRAGVG	105

Region	Peptide	SEQ ID NO
	VPGRTAG	81
	ESTGRER	73
	RGSTQVG	94
	GRGGAAL	100
	KAQGVGG	79
Primary Visual Cortex	HDGGASR	103
	ESTGRER	73
	EAQSHPR	91
	TKSLSSG	92
	GSRAGVG	105
	KAQGVGG	79
Putamen	GRSTGMT	95
	RATSQST	96
	VGRSVGA	97
	VQGRQGG	60
	GEGGGGR	98
	VSTALPR	55
	RGASGAV	61
	TAAGGQR	99
	SLSTGPK	59
	GRGGAAL	100
	RAVPAGG	84
	KAQGVGG	79
	GSRAGVG	105
	VPGRTAG	81
	HDGGASR	103
	RDDVPLR	56
Reticular Formation	VTQSKGA	74
	DDPSARR	53
	TGTAGLK	89
	ARGSGVN	82
	RGSTQVG	94
	RDDVPLR	56
Spinal Cord	SLSTGPK	59
	GRSTGMT	95
	RGASGAV	61
	TARGGGG	77
	KASGAGG	88
	VAPISK	101
	DDPSARR	53
	TSNRGQV	63
	SSSKTGS	58
	VQGRQGG	60

Region	Peptide	SEQ ID NO
Spinal Cord Thoracic	HDGGASR	103
	ESTGRER	73
	EAQSHPR	91
	GSRAGVG	105
	VQGRQGG	60
	KAQGVGG	79
Spinal Cord Lumbar	HDGGASR	103
	EAQSHPR	91
	DDPSARR	53
	KLSISGN	106
	VMSSGKP	85
	KAQGVGG	79
	TSNRQV	63
Spinal Cord Cervical	ESTGRER	73
	HDGGASR	103
	EAQSHPR	91
	GSRAGVG	105
	RGSTQVG	94
	KAQGVGG	79
Substantia Nigra	LTSRTSP	52
	NARPVSA	76
	SSSKTGS	58
	RGGAQVV	87
	APPVKLS	102
	DDPSARR	53
	RGSGSAV	75
	HDGGASR	103
	TRVGTAG	57
	GSRAGVG	105
	KLSISGN	106
	KAQGVGG	79
	TGTAGLK	89
	ARGSGVN	82
Subthalamic Nuclei	VQGRQGG	60
	RSGGAAV	104
	KASGAGG	88
	SSSKTGS	58
	KAQGVGG	79
	GSRAGVG	105
	RGGAQVV	87
	APPVKLS	102
	RGSGSAV	75
	TRVGTAG	57

Region	Peptide	SEQ ID NO
	RGSTQVG	94
	KLSISGN	106
	RDDVPLR	56
	GRGGAAL	100
	TSNRGQV	63
Temporal Cortex	ESTGRER	73
	HDGGASR	103
	GSRAGVG	105
	KAQGVGG	79
	RDDVPLR	56
Thalamus	KASGAGG	88
	RDDVPLR	56
	LTSRTSP	52
	KLSISGN	106
	VSTALPR	55
	VMSSGKP	85
	GAVGGVK	107
	KNESGKV	108
	VTQSKGA	74
Thalamus, Anterior	AGQLAGR	109
	RGSTQVG	94
	KAQGVGG	79
	TKSLSSG	92
	RAVPAGG	84
	TSNRGQV	63
VA/VL Thalamus	RDDVPLR	56
	TRVGTAG	57
	ARGSGVN	82
	GRGGAAL	100
	NARPVSA	76
	VQGRQGG	60
	GSRAGVG	105

Table 3. AAV9 targeting peptides for each brain structure.

Region	Peptide	SEQ ID NO
Brainstem	KGGGFHG	110
	RAKPGME	111
	GRDVTRS	112
	RGDLQWV	113
	GGDRTRG	114
	RGDLASV	115
	RDTTRNL	116

Region	Peptide	SEQ ID NO
Caudate	KGGGVHGH	117
	KGGGFHGH	110
	RGDLQWV	113
	RGDMYRV	118
	RGDRPVS	119
	RSDVGSL	120
	RGDLASV	115
	RDTRNL	116
	AGVKPGR	121
	KGGGVHGH	117
	RAKPGME	111
	GADRTRG	127
	GRDVTRS	112
	ARGDGWR	132
	RGGGVYGH	126
	GGDRTRG	114
	GRDYTRL	133
Cortex, Cerebellar	KGGGFHGH	110
	RGDLQWV	113
	RAKPGME	111
	RGDWPRG	122
	RGDRPVS	119
	GGRPGSW	123
	RGDYPRS	124
	RGDLRFI	125
Cortex, Cerebral	KGGGFHGH	110
	RAKPGME	111
	RDTRNL	116
	RGGGVYGH	126
	GGDRTRG	114
	GADRTRG	127
	RGDLQWV	113
	RGDLRFI	125
Cortex, Prefrontal	KGGGFHGH	110
	MMGRPGR	136
	AGVKPGR	121
	RGGGVYGH	126
	RGDLRFI	125
	RGDWPRG	122
	RGDLQWV	113
	RGDRPVS	119
Cortex, Motor	MMGRPGR	136
	KGGGFHGH	110

Region	Peptide	SEQ ID NO
	AGVKPGR	121
	RGGGVYG	126
	RGDLRFI	125
	RGDWPRG	122
	RGDLQWV	113
Cortex, Temporal	KGGGFHG	110
	RGGGVYG	126
	MMGRPGR	136
	RGDLRFI	125
	RGDLQWV	113
	RGDWPRG	122
Cortex, Visual	AGVKPGR	121
	MMGRPGR	136
	RGDLRFI	125
	RGDLQWV	113
	RGDFMGL	128
	RGGGVYG	126
	KGGGFHG	110
	AWDGTRV	131
Cochlea (ear)	MMGRPGR	136
	KGGGFHG	110
	RGDLASV	115
	AGVKPGR	121
	GGRPGSW	123
	RGGGVYG	126
	GGDRTRG	114
Deep Cerebellar Nuclei	RSDVGSL	120
	KGGGFHG	110
	RGDLQWV	113
	GRDVTRS	112
	RGDLRFI	125
	RGGGVYG	126
	RAKPGME	111
Dorsal Root Ganglia Lumbar	MMGRPGR	136
	AGVKPGR	121
	GGRPGSW	123
	GRDYTRL	133
	RGGGVYG	126
	GGDRTRG	114
Dorsal Root Ganglia Cervical	RGDLQWV	113
	KGGGFHG	110
	RRDETRT	129
	RGGGVYG	126

Region	Peptide	SEQ ID NO
	GADRTRG	127
	RGDRPVS	119
	GGDRTRG	114
Dorsal Root Ganglia Thoracic	MMGRPGR	136
	AGVKPGR	121
	GGRPGSW	123
	GRDYTRL	133
	RGDFMGL	128
	RGGGVYG	126
	GGDRTRG	114
	RAKPGME	111
	AWDGTRV	131
Ependyma	KGGGFHG	110
	RGDLQWV	113
	RAKPGME	111
	RGDMYRV	118
	RSDVGSL	120
	RGDFMGL	128
	RGDRPVS	119
Ependyma; 4th Ventricular	RGDLQWV	113
	RGDLASV	115
	RSDVGSL	120
	RGDLRFI	125
	MDLTKAV	135
	RGGGVYG	126
	RAKPGME	111
	RGDRPVS	119
	GGDRTRG	114
Ependyma; Lt Ventricular	MMGRPGR	136
	RSDVGSL	120
	AGVKPGR	121
	GGRPGSW	123
	RAKPGME	111
	RGGGVYG	126
	GRDVTRS	112
	RGDLQWV	113
Ependyma; 3rd Ventricular	KGGGVHG	117
	GADRTRG	127
	GGDRTRG	114
	MMGRPGR	136
	RGDLQWV	113
	RGGGVYG	126
	GRDVTRS	112

Region	Peptide	SEQ ID NO
Globus Pallidus	KGGGFHG	110
	RRDETRT	129
	RAKPGME	111
	GGDRTRG	114
	GRDVTRS	112
	RSDVGSL	120
	RGDRPVS	119
Globus Pallidus External	RAKPGME	111
	RRGDAWS	134
	RGDMYRV	118
	RGDLQWV	113
	GRDVTRS	112
	RGGGVYG	126
Globus Pallidus Internal	MDLTKAV	135
	RDTRNL	116
	AWDGTRV	131
	RGGGVYG	126
	KGGGVHG	117
	RGDLQWV	113
Hippocampus	KGGGFHG	110
	AESPWER	130
	RGDLQWV	113
	RAKPGME	111
	RRDETRT	129
	RGDLRFI	125
	GGRPGSW	123
	RDTRNL	116
Hippocampus CA1	KGGGFHG	110
	ARGDGWR	132
	RGGGVYG	126
	AWDGTRV	131
	GADRTRG	127
	RAKPGME	111
Hippocampus CA3	RAKPGME	111
	RRDETRT	129
	MDLTKAV	135
	AWDGTRV	131
	RDTRNL	116
	GGDRTRG	114
	GRDVTRS	112
	RGGGVYG	126
Hippocampus; DG	KGGGFHG	110
	RGDFMGL	128

Region	Peptide	SEQ ID NO
	RGGGVYG	126
	ARGDGWR	132
	RGDLRFI	125
	RGDLQWV	113
	GRDYTRL	133
Inferior Olive	RRGDAWS	134
	GGRPGSW	123
	KGGGFHG	110
	KGGGVHG	117
	RGDWPRG	122
	RGGGVYG	126
	GRDYTRL	133
	RGDRPVS	119
Meninges	KGGGFHG	110
	RGDLQWV	113
	RAKPGME	111
	RGDWPRG	122
	GGDRTRG	114
	AWDGTRV	131
	RGDMYRV	118
	RGDRPVS	119
	RSDVGSL	120
	KGGGVHG	117
	GRDVTRS	112
	AGVKPGR	121
	RGGGVYG	126
	KGGGFHG	110
	RAKPGME	111
Optic Nerve	RRDETRT	129
	RDTRNL	116
	GGDRTRG	114
	ARGDGWR	132
	KGGGVHG	117
	RGDLRFI	125
	RGGGVYG	126
	AGVKPGR	121
	RGDLQWV	113
	KGGGFHG	110
	RGGGVYG	126
Pons	RRDETRT	129
	AGVKPGR	121
	KGGGVHG	117
	RAKPGME	111

Region	Peptide	SEQ ID NO
	RGDLQWV	113
	GRDYTRL	133
Putamen	KGGGFHG	110
	GRDVTRS	112
	GADRTRG	127
	GRDYTRL	133
	RDTRNL	116
	RGDLQWV	113
	RRGDAWS	134
	GGRPGSW	123
	KGGGVHG	117
	RGDMYRV	118
	RAKPGME	111
	RGGGVYG	126
	GGDRTRG	114
	RGDRPVS	119
Reticular Formation	MMGRPGR	136
	GRDVTRS	112
	GADRTRG	127
	RAKPGME	111
	RSDVGSL	120
	RGGGVYG	126
	AWDGTRV	131
Spinal Cord	KGGGFHG	110
	RGDLQWV	113
	RGDRPVS	119
	RGDWPRG	122
	RSDVGSL	120
	GGRPGSW	123
	RRGDAWS	134
	RGDFMGL	128
Spinal Cord; Thoracic	KGGGFHG	110
	RGGGVYG	126
	RGDLQWV	113
	RGDLRFI	125
	RRGDAWS	134
	RGDRPVS	119
	GRDYTRL	133
Spinal Cord; Lumbar	RGDLASV	115
	KGGGFHG	110
	RGGGVYG	126
	MMGRPGR	136
	RGDLQWV	113

Region	Peptide	SEQ ID NO
	AWDGTRV	131
	RAKPGME	111
Spinal Cord; Cervical	KGGGFHG	110
	RGDLRFI	125
	RGGGVYG	126
	RRGDAWS	134
	RGDLQWV	113
	RGDWPRG	122
Substantia Nigra	KGGGFHG	110
	RAKPGME	111
	GRDVTRS	112
	GGDRTRG	114
	RRDETRT	129
	RGDLQWV	113
	KGGGVHG	117
	MDLTKAV	135
	RRGDAWS	134
	GGRPGSW	123
	GADRTRG	127
	RGGGVYG	126
Subthalamic Nuclei	KGGGFHG	110
	RAKPGME	111
	MDLTKAV	135
	RGDRPVS	119
	RGDWPRG	122
	ARGDGWR	132
	RGDLQWV	113
	RSDVGSL	120
	RGDLASV	115
	RGGGVYG	126
Thalamus	KGGGFHG	110
	GRDVTRS	112
	GGDRTRG	114
	RGDLQWV	113
	GRDYTRL	133
	RGDLRFI	125
	MMGRPGR	136
	TGRPGVW	137
Thalamus, Anterior	MDLTKAV	135
	GGRPGSW	123
	RGDLRFI	125
	RDTTRNL	116
	RAKPGME	111

Region	Peptide	SEQ ID NO
	RGGGVYG	126
	GRDYTRL	133
	RGDWPRG	122
VA/VL Thalamus	RGDWPRG	122
	GGDRTRG	114
	RRGDAWS	134
	RGDLRFI	125
	RGDYPRS	124
	RGDRPVS	119
	RGGGVYG	126

Table 4. AAV1 targeting peptides for various organs.

Organ	Peptide	SEQ ID NO
Brain	RPGREAS	144
	TAPKSLK	25
	RDATRSS	12
	DKTRAGS	5
	NSVRPLT	23
	RDSTRQL	26
	RGVLVTT	2

Table 5. AAV2 targeting peptides for various organs.

Organ	Peptide	SEQ ID NO
Brain	HDGGASR	103
	KAQGVGG	79
	GSRAGVG	105
	ESTGREER	73
	RDDVPLR	56
	RGSTQVG	94
	EAQSHPR	91
Kidney	GSRAGVG	105
	TSNRGQV	63
	GRSTGMT	95
	GRGAPGG	80
	GAVGGVK	107
	KAQGVGG	79
	RDDVPLR	56
Heart	HDGGASR	103
	SLSTGPK	59
	GSRAGVG	105
	RGSTQVG	94

Organ	Peptide	SEQ ID NO
	NARAQGV	62
	ESTGRER	73
Liver	KAQGVGG	79
	GSRAGVG	105
	RDDVPLR	56
	HDGGASR	103
	NARPVSA	76
Gonad	VPGRTAG	81
	KAQGVGG	79
	RGSTQVG	94
	HDGGASR	103
	GAVGGVK	107
	RDDVPLR	56
Spleen	KAQGVGG	79
	GSRAGVG	105
	GRGGAAL	100
	VPGRTAG	81
	ESTGRER	73
Lung	ESTGRER	73
	TKSLSSG	92
	RGSTQVG	94
	KAQGVGG	79
	NARPVSA	76

Table 6. AAV9 targeting peptides for various organs.

Organ	Peptide	SEQ ID NO
Brain	RGGGVYG	126
	RAKPGME	111
	KGGGFHG	110
	GGDRTRG	114
	MMGRPGR	136
	RGDLQWV	113
	MDLTKAV	135
	GGRPGSW	123
Kidney	MMGRPGR	136
	RGDLQWV	113
	RAKPGME	111
	RSDVGSL	120
	KGGGFHG	110
	RGGGVYG	126
Heart	GGRPGSW	123
	RSDVGSL	120

Organ	Peptide	SEQ ID NO
	RGDLASV	115
	KGGGFHG	110
	GRDYTRL	133
	RGGGVYG	126
	RAKPGME	111
Liver	GGDRTRG	114
	GRDVTRS	112
	RDTRNL	116
	RRDETRT	129
	RSDVGSL	120
	RGGGVYG	126
	RAKPGME	111
	GRDYTRL	133
Gonad	KGGGVHG	117
	KGGGFHG	110
	RSDVGSL	120
	GGRPGSW	123
	RRDETRT	129
	RGGGVYG	126
	GGDRTRG	114
	RGDRPVS	119
	RAKPGME	111

I. Adeno-Associated Virus (AAV) Vectors

[0042] Adeno-associated virus (AAV) is a small nonpathogenic virus of the parvoviridae family. To date, numerous serologically distinct AAVs have been identified, and more than a dozen have been isolated from humans or primates. AAV is distinct from other members of this family by its dependence upon a helper virus for replication.

[0043] AAV genomes can exist in an extrachromosomal state without integrating into host cellular genomes; possess a broad host range; transduce both dividing and non-dividing cells *in vitro* and *in vivo* and maintain high levels of expression of the transduced genes. AAV viral particles are heat stable; resistant to solvents, detergents, changes in pH, and temperature; and can be column purified and/or concentrated on CsCl gradients or by other means. The AAV genome comprises a single-stranded deoxyribonucleic acid (ssDNA), either positive- or negative-sensed. The approximately 4.7 kb genome of AAV consists of one segment of single stranded DNA of either plus or minus polarity. The ends of the genome are

short inverted terminal repeats (ITRs) that can fold into hairpin structures and serve as the origin of viral DNA replication.

[0044] An AAV “genome” refers to a recombinant nucleic acid sequence that is ultimately packaged or encapsulated to form an AAV particle. An AAV particle often comprises an AAV genome packaged with AAV capsid proteins. In cases where recombinant plasmids are used to construct or manufacture recombinant vectors, the AAV vector genome does not include the portion of the “plasmid” that does not correspond to the vector genome sequence of the recombinant plasmid. This non vector genome portion of the recombinant plasmid is referred to as the “plasmid backbone,” which is important for cloning and amplification of the plasmid, a process that is needed for plasmid propagation and production, but is not itself packaged or encapsulated into viral particles. Thus, an AAV vector “genome” refers to nucleic acid that is packaged or encapsulated by AAV capsid proteins.

[0045] The AAV virion (particle) is a non-enveloped, icosahedral particle approximately 25 nm in diameter that comprises an AAV capsid. The AAV particle comprises an icosahedral symmetry comprised of three related capsid proteins, VP1, VP2 and VP3, which interact together to form the capsid. The genome of most native AAVs often contain two open reading frames (ORFs), sometimes referred to as a left ORF and a right ORF. The right ORF often encodes the capsid proteins VP1, VP2, and VP3. These proteins are often found in a ratio of 1:1:10 respectively, but may be in varied ratios, and are all derived from the right-hand ORF. The VP1, VP2 and VP3 capsid proteins differ from each other by the use of alternative splicing and an unusual start codon. Deletion analysis has shown that removal or alteration of VP1 which is translated from an alternatively spliced message results in a reduced yield of infectious particles. Mutations within the VP3 coding region result in the failure to produce any single-stranded progeny DNA or infectious particles. In certain embodiments, the genome of an AAV particle encodes one, two or all three VP1, VP2 and VP3 polypeptides.

[0046] The left ORF often encodes the non-structural Rep proteins, Rep 40, Rep 52, Rep 68 and Rep 78, which are involved in regulation of replication and transcription in addition to the production of single-stranded progeny genomes. Two of the Rep proteins have been associated with the preferential integration of AAV genomes into a region of the q arm of human chromosome 19. Rep68/78 have been shown to possess NTP binding activity as

well as DNA and RNA helicase activities. Some Rep proteins possess a nuclear localization signal as well as several potential phosphorylation sites. In certain embodiments the genome of an AAV (*e.g.*, an rAAV) encodes some or all of the Rep proteins. In certain embodiments the genome of an AAV (*e.g.*, an rAAV) does not encode the Rep proteins. In certain
5 embodiments one or more of the Rep proteins can be delivered in trans and are therefore not included in an AAV particle comprising a nucleic acid encoding a polypeptide.

[0047] The ends of the AAV genome comprise short inverted terminal repeats (ITR) which have the potential to fold into T-shaped hairpin structures that serve as the origin of viral DNA replication. Accordingly, the genome of an AAV comprises one or more (*e.g.*, a
10 pair of) ITR sequences that flank a single stranded viral DNA genome. The ITR sequences often have a length of about 145 bases each. Within the ITR region, two elements have been described which are believed to be central to the function of the ITR, a GAGC repeat motif and the terminal resolution site (*trs*). The repeat motif has been shown to bind Rep when the ITR is in either a linear or hairpin conformation. This binding is thought to position Rep68/78
15 for cleavage at the *trs* which occurs in a site- and strand-specific manner. In addition to their role in replication, these two elements appear to be central to viral integration. Contained within the chromosome 19 integration locus is a Rep binding site with an adjacent *trs*. These elements have been shown to be functional and necessary for locus specific integration.

[0048] The term “recombinant,” as a modifier of vector, such as recombinant viral,
20 *e.g.*, lenti- or parvo-virus (*e.g.*, AAV) vectors, as well as a modifier of sequences such as recombinant nucleic acid sequences and polypeptides, means that the compositions have been manipulated (*i.e.*, engineered) in a fashion that generally does not occur in nature. A particular example of a recombinant vector, such as an AAV, retroviral, or lentiviral vector would be where a nucleic acid sequence that is not normally present in the wild-type viral
25 genome is inserted within the viral genome. An example of a recombinant nucleic acid sequence would be where a nucleic acid (*e.g.*, gene) encodes an inhibitory RNA cloned into a vector, with or without 5', 3' and/or intron regions that the gene is normally associated within the viral genome. Although the term “recombinant” is not always used herein in reference to vectors, such as viral vectors, as well as sequences such as polynucleotides, “recombinant”
30 forms including nucleic acid sequences, polynucleotides, transgenes, etc. are expressly included in spite of any such omission.

[0049] A recombinant viral “vector” is derived from the wild type genome of a virus by using molecular methods to remove part of the wild type genome from the virus, and replacing with a non-native nucleic acid, such as a nucleic acid sequence. Typically, for example, for AAV, one or both inverted terminal repeat (ITR) sequences of the AAV genome are retained in the recombinant AAV vector. A “recombinant” viral vector (*e.g.*, rAAV) is distinguished from a viral (*e.g.*, AAV) genome, since part of the viral genome has been replaced with a non-native sequence with respect to the viral genomic nucleic acid such a nucleic acid encoding a transactivator or nucleic acid encoding an inhibitory RNA or nucleic acid encoding a therapeutic protein. Incorporation of such non-native nucleic acid sequences therefore defines the viral vector as a “recombinant” vector, which in the case of AAV can be referred to as a “rAAV vector.”

[0050] In certain embodiments, an AAV (*e.g.*, a rAAV) comprises two ITRs. In certain embodiments, an AAV (*e.g.*, a rAAV) comprises a pair of ITRs. In certain embodiments, an AAV (*e.g.*, a rAAV) comprises a pair of ITRs that flank (*i.e.*, are at each 5' and 3' end) of a nucleic acid sequence that at least encodes a polypeptide having function or activity.

[0051] An AAV vector (*e.g.*, rAAV vector) can be packaged and is referred to herein as an “AAV particle” for subsequent infection (transduction) of a cell, *ex vivo*, *in vitro* or *in vivo*. Where a recombinant AAV vector is encapsulated or packaged into an AAV particle, the particle can also be referred to as a “rAAV particle.” In certain embodiments, an AAV particle is a rAAV particle. A rAAV particle often comprises a rAAV vector, or a portion thereof. A rAAV particle can be one or more rAAV particles (*e.g.*, a plurality of AAV particles). rAAV particles typically comprise proteins that encapsulate or package the rAAV vector genome (*e.g.*, capsid proteins). It is noted that reference to a rAAV vector can also be used to reference a rAAV particle.

[0052] Any suitable AAV particle (*e.g.*, rAAV particle) can be used for a method or use herein. A rAAV particle, and/or genome comprised therein, can be derived from any suitable serotype or strain of AAV. A rAAV particle, and/or genome comprised therein, can be derived from two or more serotypes or strains of AAV. Accordingly, a rAAV can comprise proteins and/or nucleic acids, or portions thereof, of any serotype or strain of AAV, wherein the AAV particle is suitable for infection and/or transduction of a mammalian cell. Non-limiting examples of AAV serotypes include AAV1, AAV2, AAV3, AAV4, AAV5,

AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-rh74, AAV-rh10 and AAV-2i8.

[0053] In certain embodiments a plurality of rAAV particles comprises particles of, or derived from, the same strain or serotype (or subgroup or variant). In certain embodiments a plurality of rAAV particles comprise a mixture of two or more different rAAV particles (*e.g.*, of different serotypes and/or strains).

[0054] As used herein, the term “serotype” is a distinction used to refer to an AAV having a capsid that is serologically distinct from other AAV serotypes. Serologic distinctiveness is determined on the basis of the lack of cross-reactivity between antibodies to one AAV as compared to another AAV. Such cross-reactivity differences are usually due to differences in capsid protein sequences/antigenic determinants (*e.g.*, due to VP1, VP2, and/or VP3 sequence differences of AAV serotypes). Despite the possibility that AAV variants including capsid variants may not be serologically distinct from a reference AAV or other AAV serotype, they differ by at least one nucleotide or amino acid residue compared to the reference or other AAV serotype.

[0055] In certain embodiments, a rAAV vector based upon a first serotype genome corresponds to the serotype of one or more of the capsid proteins that package the vector. For example, the serotype of one or more AAV nucleic acids (*e.g.*, ITRs) that comprises the AAV vector genome corresponds to the serotype of a capsid that comprises the rAAV particle.

[0056] In certain embodiments, a rAAV vector genome can be based upon an AAV (*e.g.*, AAV2) serotype genome distinct from the serotype of one or more of the AAV capsid proteins that package the vector. For example, a rAAV vector genome can comprise AAV2 derived nucleic acids (*e.g.*, ITRs), whereas at least one or more of the three capsid proteins are derived from a different serotype, *e.g.*, an AAV1, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, Rh10, Rh74 or AAV-2i8 serotype or variant thereof.

[0057] In certain embodiments, a rAAV particle or a vector genome thereof related to a reference serotype has a polynucleotide, polypeptide or subsequence thereof that comprises or consists of a sequence at least 60% or more (*e.g.*, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, etc.) identical to a polynucleotide, polypeptide or subsequence of an AAV1, AAV2, AAV3, AAV4, AAV5,

AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, Rh10, Rh74 or AAV-2i8 particle. In particular embodiments, a rAAV particle or a vector genome thereof related to a reference serotype has a capsid or ITR sequence that comprises or consists of a sequence at least 60% or more (*e.g.*, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%,
 5 99.3%, 99.4%, 99.5%, etc.) identical to a capsid or ITR sequence of an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, Rh10, Rh74 or AAV-2i8 serotype.

[0058] In certain embodiments, a method herein comprises use, administration or delivery of an rAAV1, rAAV2, rAAV3, rAAV4, rAAV5, rAAV6, rAAV7, rAAV8, rAAV9,
 10 rAAV10, rAAV11, rAAV12, rRh10, rRh74 or rAAV-2i8 particle.

[0059] In certain embodiments, a method herein comprises use, administration or delivery of a rAAV2 particle. In certain embodiments a rAAV2 particle comprises an AAV2 capsid. In certain embodiments a rAAV2 particle comprises one or more capsid proteins (*e.g.*, VP1, VP2 and/or VP3) that are at least 60%, 65%, 70%, 75% or more identical, *e.g.*,
 15 80%, 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, etc., up to 100% identical to a corresponding capsid protein of a native or wild-type AAV2 particle. In certain embodiments a rAAV2 particle comprises VP1, VP2 and VP3 capsid proteins that are at least 75% or more identical, *e.g.*,
 20 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, etc., up to 100% identical to a corresponding capsid protein of a native or wild-type AAV2 particle. In certain embodiments, a rAAV2 particle is a variant of a native or wild-type AAV2 particle. In some aspects, one or more capsid proteins of an AAV2 variant have 1, 2, 3, 4, 5, 5-10, 10-15, 15-20 or more amino acid substitutions compared to capsid protein(s) of a native or wild-type AAV2 particle.

[0060] In certain embodiments a rAAV9 particle comprises an AAV9 capsid. In certain embodiments a rAAV9 particle comprises one or more capsid proteins (*e.g.*, VP1, VP2 and/or VP3) that are at least 60%, 65%, 70%, 75% or more identical, *e.g.*, 80%, 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, etc., up to 100% identical to a corresponding capsid protein of
 30 a native or wild-type AAV9 particle. In certain embodiments a rAAV9 particle comprises VP1, VP2 and VP3 capsid proteins that are at least 75% or more identical, *e.g.*, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%,

99.2%, 99.3%, 99.4%, 99.5%, etc., up to 100% identical to a corresponding capsid protein of a native or wild-type AAV9 particle. In certain embodiments, a rAAV9 particle is a variant of a native or wild-type AAV9 particle. In some aspects, one or more capsid proteins of an AAV9 variant have 1, 2, 3, 4, 5, 5-10, 10-15, 15-20 or more amino acid substitutions compared to capsid protein(s) of a native or wild-type AAV9 particle.

[0061] In certain embodiments, a rAAV particle comprises one or two ITRs (*e.g.*, a pair of ITRs) that are at least 75% or more identical, *e.g.*, 80%, 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, etc., up to 100% identical to corresponding ITRs of a native or wild-type AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-rh74, AAV-rh10 or AAV-2i8, as long as they retain one or more desired ITR functions (*e.g.*, ability to form a hairpin, which allows DNA replication; integration of the AAV DNA into a host cell genome; and/or packaging, if desired).

[0062] In certain embodiments, a rAAV2 particle comprises one or two ITRs (*e.g.*, a pair of ITRs) that are at least 75% or more identical, *e.g.*, 80%, 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, etc., up to 100% identical to corresponding ITRs of a native or wild-type AAV2 particle, as long as they retain one or more desired ITR functions (*e.g.*, ability to form a hairpin, which allows DNA replication; integration of the AAV DNA into a host cell genome; and/or packaging, if desired).

[0063] In certain embodiments, a rAAV9 particle comprises one or two ITRs (*e.g.*, a pair of ITRs) that are at least 75% or more identical, *e.g.*, 80%, 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, etc., up to 100% identical to corresponding ITRs of a native or wild-type AAV2 particle, as long as they retain one or more desired ITR functions (*e.g.*, ability to form a hairpin, which allows DNA replication; integration of the AAV DNA into a host cell genome; and/or packaging, if desired).

[0064] A rAAV particle can comprise an ITR having any suitable number of “GAGC” repeats. In certain embodiments an ITR of an AAV2 particle comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more “GAGC” repeats. In certain embodiments a rAAV2 particle comprises an ITR comprising three “GAGC” repeats. In certain embodiments a rAAV2

particle comprises an ITR which has less than four “GAGC” repeats. In certain embodiments a rAAV2 particle comprises an ITR which has more than four “GAGC” repeats. In certain embodiments an ITR of a rAAV2 particle comprises a Rep binding site wherein the fourth nucleotide in the first two “GAGC” repeats is a C rather than a T.

5 **[0065]** Exemplary suitable length of DNA can be incorporated in rAAV vectors for packaging/encapsidation into a rAAV particle can about 5 kilobases (kb) or less. In particular, embodiments, length of DNA is less than about 5kb, less than about 4.5 kb, less than about 4 kb, less than about 3.5 kb, less than about 3 kb, or less than about 2.5 kb.

10 **[0066]** rAAV vectors that include a nucleic acid sequence that directs the expression of an RNAi or polypeptide can be generated using suitable recombinant techniques known in the art (*e.g.*, see Sambrook *et al.*, 1989). Recombinant AAV vectors are typically packaged into transduction-competent AAV particles and propagated using an AAV viral packaging system. A transduction-competent AAV particle is capable of binding to and entering a mammalian cell and subsequently delivering a nucleic acid cargo (*e.g.*, a heterologous gene)
15 to the nucleus of the cell. Thus, an intact rAAV particle that is transduction-competent is configured to transduce a mammalian cell. A rAAV particle configured to transduce a mammalian cell is often not replication competent, and requires additional protein machinery to self-replicate. Thus, a rAAV particle that is configured to transduce a mammalian cell is engineered to bind and enter a mammalian cell and deliver a nucleic acid to the cell, wherein
20 the nucleic acid for delivery is often positioned between a pair of AAV ITRs in the rAAV genome.

[0067] Suitable host cells for producing transduction-competent AAV particles include but are not limited to microorganisms, yeast cells, insect cells, and mammalian cells that can be, or have been, used as recipients of a heterologous rAAV vectors. Cells from the
25 stable human cell line, HEK293 (readily available through, *e.g.*, the American Type Culture Collection under Accession Number ATCC CRL1573) can be used. In certain embodiments a modified human embryonic kidney cell line (*e.g.*, HEK293), which is transformed with adenovirus type-5 DNA fragments, and expresses the adenoviral E1a and E1b genes is used to generate recombinant AAV particles. The modified HEK293 cell line is readily
30 transfected, and provides a particularly convenient platform in which to produce rAAV particles. Methods of generating high titer AAV particles capable of transducing mammalian

cells are known in the art. For example, AAV particle can be made as set forth in Wright, 2008 and Wright, 2009.

[0068] In certain embodiments, AAV helper functions are introduced into the host cell by transfecting the host cell with an AAV helper construct either prior to, or concurrently with, the transfection of an AAV expression vector. AAV helper constructs are thus sometimes used to provide at least transient expression of AAV rep and/or cap genes to complement missing AAV functions necessary for productive AAV transduction. AAV helper constructs often lack AAV ITRs and can neither replicate nor package themselves. These constructs can be in the form of a plasmid, phage, transposon, cosmid, virus, or virion. A number of AAV helper constructs have been described, such as the commonly used plasmids pAAV/Ad and pIM29+45 which encode both Rep and Cap expression products. A number of other vectors are known which encode Rep and/or Cap expression products.

[0069] An “expression vector” is a specialized vector that contains a gene or nucleic acid sequence with the necessary regulatory regions needed for expression in a host cell. An expression vector may contain at least an origin of replication for propagation in a cell and optionally additional elements, such as a heterologous nucleic acid sequence, expression control element (*e.g.*, a promoter, enhancer), intron, ITR(s), and polyadenylation signal.

II. Therapeutic Agents

[0070] In some embodiments, viral gene transfer methods can be used to introduce nucleic acids in mammalian cells or target tissues. Such methods can be used to administer nucleic acids encoding inhibitory RNAs, non-coding RNAs, and/or therapeutic proteins to cells in culture or in a host organism.

A. Inhibitory RNAs

[0071] “RNA interference (RNAi)” is the process of sequence-specific, post-transcriptional gene silencing initiated by siRNA. During RNAi, siRNA induces degradation of target mRNA with consequent sequence-specific inhibition of gene expression.

[0072] An “inhibitory RNA,” “RNAi,” “small interfering RNA” or “short interfering RNA” or “siRNA” molecule, “short hairpin RNA” or “shRNA” molecule, or “miRNA” is an RNA duplex of nucleotides that is targeted to a nucleic acid sequence of interest. As used herein, the term “siRNA” is a generic term that encompasses the subset of shRNAs and

miRNAs. An “RNA duplex” refers to the structure formed by the complementary pairing between two regions of an RNA molecule. siRNA is “targeted” to a gene in that the nucleotide sequence of the duplex portion of the siRNA is complementary to a nucleotide sequence of the targeted gene. In certain embodiments, the siRNAs are targeted to the sequence encoding huntingtin. In some embodiments, the length of the duplex of siRNAs is less than 30 base pairs. In some embodiments, the duplex can be 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 base pairs in length. In some embodiments, the length of the duplex is 19 to 25 base pairs in length. In certain embodiment, the length of the duplex is 19 or 21 base pairs in length. The RNA duplex portion of the siRNA can be part of a hairpin structure. In addition to the duplex portion, the hairpin structure may contain a loop portion positioned between the two sequences that form the duplex. The loop can vary in length. In some embodiments the loop is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 nucleotides in length. In certain embodiments, the loop is 18 nucleotides in length. The hairpin structure can also contain 3' and/or 5' overhang portions. In some embodiments, the overhang is a 3' and/or a 5' overhang 0, 1, 2, 3, 4 or 5 nucleotides in length.

[0073] shRNAs are comprised of stem-loop structures which are designed to contain a 5' flanking region, siRNA region segments, a loop region, a 3' siRNA region and a 3' flanking region. Most RNAi expression strategies have utilized short-hairpin RNAs (shRNAs) driven by strong polIII-based promoters. Many shRNAs have demonstrated effective knock down of the target sequences in vitro as well as in vivo, however, some shRNAs which demonstrated effective knock down of the target gene were also found to have toxicity in vivo.

[0074] miRNAs are small cellular RNAs (~22 nt) that are processed from precursor stem loop transcripts. Known miRNA stem loops can be modified to contain RNAi sequences specific for genes of interest. miRNA molecules can be preferable over shRNA molecules because miRNAs are endogenously expressed. Therefore, miRNA molecules are unlikely to induce dsRNA-responsive interferon pathways, they are processed more efficiently than shRNAs, and they have been shown to silence 80% more effectively.

[0075] A recently discovered alternative approach is the use of artificial miRNAs (pri-miRNA scaffolds shuttling siRNA sequences) as RNAi vectors. Artificial miRNAs more naturally resemble endogenous RNAi substrates and are more amenable to Pol-II

transcription (*e.g.*, allowing tissue-specific expression of RNAi) and polycistronic strategies (*e.g.*, allowing delivery of multiple siRNA sequences). See U.S. Pat. No. 10,093,927, which is incorporated by reference.

[0076] The transcriptional unit of a “shRNA” is comprised of sense and antisense
5 sequences connected by a loop of unpaired nucleotides. shRNAs are exported from the nucleus by Exportin-5, and once in the cytoplasm, are processed by Dicer to generate functional siRNAs. “miRNAs” stem-loops are comprised of sense and antisense sequences connected by a loop of unpaired nucleotides typically expressed as part of larger primary transcripts (pri-miRNAs), which are excised by the Drosha-DGCR8 complex generating
10 intermediates known as pre-miRNAs, which are subsequently exported from the nucleus by Exportin-5, and once in the cytoplasm, are processed by Dicer to generate functional siRNAs. “Artificial miRNA” or an “artificial miRNA shuttle vector”, as used herein interchangeably, refers to a primary miRNA transcript that has had a region of the duplex stem loop (at least about 9-20 nucleotides) which is excised via Drosha and Dicer processing replaced with
15 the siRNA sequences for the target gene while retaining the structural elements within the stem loop necessary for effective Drosha processing. The term “artificial” arises from the fact the flanking sequences (~35 nucleotides upstream and ~40 nucleotides downstream) arise from restriction enzyme sites within the multiple cloning site of the siRNA. As used herein the term “miRNA” encompasses both the naturally occurring miRNA sequences as well as
20 artificially generated miRNA shuttle vectors.

[0077] The siRNA can be encoded by a nucleic acid sequence, and the nucleic acid sequence can also include a promoter. The nucleic acid sequence can also include a polyadenylation signal. In some embodiments, the polyadenylation signal is a synthetic minimal polyadenylation signal or a sequence of six Ts.

25 [0078] In designing RNAi there are several factors that need to be considered, such as the nature of the siRNA, the durability of the silencing effect, and the choice of delivery system. To produce an RNAi effect, the siRNA that is introduced into the organism will typically contain exonic sequences. Furthermore, the RNAi process is homology dependent, so the sequences must be carefully selected so as to maximize gene specificity, while
30 minimizing the possibility of cross-interference between homologous, but not gene-specific sequences. Preferably the siRNA exhibits greater than 80%, 85%, 90%, 95%, 98%, or even 100% identity between the sequence of the siRNA and the gene to be inhibited. Sequences

less than about 80% identical to the target gene are substantially less effective. Thus, the greater homology between the siRNA and the gene to be inhibited, the less likely expression of unrelated genes will be affected.

[0079] In addition, the size of the siRNA is an important consideration. In some
5 embodiments, the present invention relates to siRNA molecules that include at least about 19-25 nucleotides and are able to modulate gene expression. In the context of the present invention, the siRNA is preferably less than 500, 200, 100, 50, or 25 nucleotides in length. More preferably, the siRNA is from about 19 nucleotides to about 25 nucleotides in length.

[0080] A siRNA target generally means a polynucleotide comprising a region that
10 encodes a polypeptide, or a polynucleotide region that regulates replication, transcription, or translation or other processes important to expression of the polypeptide, or a polynucleotide comprising both a region that encodes a polypeptide and a region operably linked thereto that regulates expression. Any gene being expressed in a cell can be targeted. Preferably, a target gene is one involved in or associated with the progression of cellular activities important to
15 disease or of particular interest as a research object.

B. Non-Coding RNAs

[0081] As evidenced by cDNA cloning projects and genomic tiling arrays, more than 90% of the human genome undergoes transcription but does not code for proteins. These transcriptional products are referred to as non-protein coding RNAs (ncRNAs). A variety of
20 ncRNA transcripts, such as ribosomal RNAs, transfer RNAs, competing endogenous RNA (ceRNA), small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA), are essential for cell function. Similarly, a large number of short ncRNAs such as micro-RNAs (miRNAs), endogenous short interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), and small nucleolar RNAs (snoRNAs) are also known to play important regulatory roles in eukaryotic
25 cells. Recent studies have demonstrated a group of long ncRNA (lncRNA) transcripts that exhibit cell type-specific expression and localize into specific subcellular compartments. lncRNAs are also known to play important roles during cellular development and differentiation supporting the view that they have been selected during the evolutionary process.

[0082] lncRNAs appear to have many different functions. In many cases, they seem
30 to play a role in regulating the activity or localization of proteins, or serve as organizational

frameworks for subcellular structures. In other cases, lncRNAs are processed to yield multiple small RNAs or they may modulate how other RNAs are processed. The latest edition of data produced by the public research consortium GenCode (version #27) catalogs just under 16,000 lncRNAs in the human genome, producing nearly 28,000 transcripts; when
5 other databases are included, more than 40,000 lncRNAs are known.

[0083] Interestingly, lncRNAs can influence the expression of specific target proteins at specific genomic loci, modulate the activity of protein binding partners, direct chromatin-modifying complexes to their sites of action, and are post-transcriptionally processed to produce numerous 5'-capped small RNAs. Epigenetic pathways can also regulate the
10 differential expression of lncRNAs.

[0084] A growing body of evidence also suggests that aberrantly expressed lncRNAs play important roles in normal physiological processes as well as multiple disease states. lncRNAs are misregulated in various diseases, including ischaemia, heart disease, Alzheimer's disease, psoriasis, and spinocerebellar ataxia type 8. This misregulation has also
15 been shown in various types of cancers, such as breast cancer, colon cancer, prostate cancer, hepatocellular carcinoma and leukemia. Several lncRNAs, *e.g.* gadd74 and lncRNA-RoR5, modulate cell cycle regulators such as cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors and p53 and thus provide an additional layer of flexibility and robustness to cell cycle progression. In addition, some lncRNAs are linked to mitotic processes such as
20 centromeric satellite RNA, which is essential for kinetochore formation and thus crucial for chromosome segregation during mitosis in humans and flies. Another nuclear lncRNA, MA-linc1, regulates M phase exit by functioning in cis to repress the expression of its neighbouring gene Pura, a regulator of cell proliferation.

[0085] lncRNAs are a group that is commonly defined as transcripts of more than 200
25 nucleotides (*e.g.* about 200 to about 1200 nt, about 2500 nt, or more) that lack an extended open reading frame (ORF). The term "non-coding RNA" (ncRNA) includes lncRNA as well as shorter transcripts of, *e.g.*, less than about 200 nt, such as about 30 to 200 nt.

[0086] Thus, in some embodiments, delivery of a ncRNA, such as to a specific brain structure of interest, corrects aberrant RNA expression levels or modulates levels of disease-causing lncRNA. Accordingly, in some embodiments, the present invention provides an
30 rAAV, wherein the viral genome is engineered to encode a therapeutic non-coding RNA

(ncRNA). In some embodiments, the ncRNA is a long non-coding RNA (lncRNA) of about 200 nucleotides (nt) in length or greater. In some embodiments, the therapeutic is a ncRNA of about 25 nt or about 30 nt to about 200 nt in length. In some embodiments, the lncRNA is about 200 nt to about 1,200 nt in length. In some embodiments, the lncRNA is about 200 nt to about 1,100, about 1,000, about 900, about 800, about 700, about 600, about 500, about 400, or about 300 nt in length.

C. CRISPR Systems

[0087] Gene editing is a technology that allows for the modification of target genes within living cells. Recently, harnessing the bacterial immune system of CRISPR to perform on demand gene editing revolutionized the way scientists approach genomic editing. The Cas9 protein of the CRISPR system, which is an RNA guided DNA endonuclease, can be engineered to target new sites with relative ease by altering its guide RNA sequence. This discovery has made sequence specific gene editing functionally effective.

[0088] In general, “CRISPR system” refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated (“Cas”) genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (*e.g.* tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a “direct repeat” and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a “spacer” in the context of an endogenous CRISPR system), and/or other sequences and transcripts from a CRISPR locus.

[0089] The CRISPR/Cas nuclease or CRISPR/Cas nuclease system can include a non-coding RNA molecule (guide) RNA, which sequence-specifically binds to DNA, and a Cas protein (*e.g.*, Cas9), with nuclease functionality (*e.g.*, two nuclease domains). One or more elements of a CRISPR system can derive from a type I, type II, or type III CRISPR system, *e.g.*, derived from a particular organism comprising an endogenous CRISPR system, such as *Streptococcus pyogenes*.

[0090] The CRISPR system can induce double stranded breaks (DSBs) at the target site, followed by disruptions as discussed herein. In other embodiments, Cas9 variants, deemed “nickases,” are used to nick a single strand at the target site. Paired nickases can be used, *e.g.*, to improve specificity, each directed by a pair of different gRNAs targeting sequences such that upon introduction of the nicks simultaneously, a 5' overhang is

introduced. In other embodiments, catalytically inactive Cas9 is fused to a heterologous effector domain such as a transcriptional repressor (*e.g.*, KRAB) or activator, to affect gene expression. Alternatively, a CRISPR system with a catalytically inactivate Cas9 further comprises a transcriptional repressor or activator fused to a ribosomal binding protein.

5 **[0091]** In some aspects, a Cas nuclease and gRNA (including a fusion of crRNA specific for the target sequence and fixed tracrRNA) are introduced into the cell. In general, target sites at the 5' end of the gRNA target the Cas nuclease to the target site, *e.g.*, the gene, using complementary base pairing. The target site may be selected based on its location immediately 5' of a protospacer adjacent motif (PAM) sequence, such as typically NGG, or
10 NAG. In this respect, the gRNA is targeted to the desired sequence by modifying the first 20, 19, 18, 17, 16, 15, 14, 14, 12, 11, or 10 nucleotides of the guide RNA to correspond to the target DNA sequence. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence. Typically, "target sequence" generally refers to a sequence to which a guide sequence is designed to
15 have complementarity, where hybridization between the target sequence and a guide sequence promotes the formation of a CRISPR complex. Full complementarity is not necessarily required, provided there is sufficient complementarity to cause hybridization and promote formation of a CRISPR complex.

[0092] The target sequence may comprise any polynucleotide, such as DNA or RNA
20 polynucleotides. The target sequence may be located in the nucleus or cytoplasm of the cell, such as within an organelle of the cell. Generally, a sequence or template that may be used for recombination into the targeted locus comprising the target sequences is referred to as an "editing template" or "editing polynucleotide" or "editing sequence." In some aspects, an exogenous template polynucleotide may be referred to as an editing template. In some
25 aspects, the recombination is homologous recombination.

[0093] Typically, in the context of an endogenous CRISPR system, formation of the CRISPR complex (comprising the guide sequence hybridized to the target sequence and complexed with one or more Cas proteins) results in cleavage of one or both strands in or near (*e.g.* within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the target
30 sequence. The tracr sequence, which may comprise or consist of all or a portion of a wild-type tracr sequence (*e.g.* about or more than about 20, 26, 32, 45, 48, 54, 63, 67, 85, or more nucleotides of a wild-type tracr sequence), may also form part of the CRISPR complex, such

as by hybridization along at least a portion of the tracr sequence to all or a portion of a tracr mate sequence that is operably linked to the guide sequence. The tracr sequence has sufficient complementarity to a tracr mate sequence to hybridize and participate in formation of the CRISPR complex, such as at least 50%, 60%, 70%, 80%, 90%, 95% or 99% of sequence complementarity along the length of the tracr mate sequence when optimally aligned.

[0094] One or more vectors driving expression of one or more elements of the CRISPR system can be introduced into the cell such that expression of the elements of the CRISPR system direct formation of the CRISPR complex at one or more target sites. Components can also be delivered to cells as proteins and/or RNA. For example, a Cas enzyme, a guide sequence linked to a tracr-mate sequence, and a tracr sequence could each be operably linked to separate regulatory elements on separate vectors. The Cas enzyme may be a target gene under the control of a regulated alternative splicing event, as disclosed herein, either as a chimeric target gene minigene or as a target gene for a chimeric minigene transactivator. The gRNA may be under the control of a constitutive promoter.

[0095] Alternatively, two or more of the elements expressed from the same or different regulatory elements, may be combined in a single vector, with one or more additional vectors providing any components of the CRISPR system not included in the first vector. The vector may comprise one or more insertion sites, such as a restriction endonuclease recognition sequence (also referred to as a “cloning site”). In some embodiments, one or more insertion sites are located upstream and/or downstream of one or more sequence elements of one or more vectors. When multiple different guide sequences are used, a single expression construct may be used to target CRISPR activity to multiple different, corresponding target sequences within a cell.

[0096] A vector may comprise a regulatory element operably linked to an enzyme-coding sequence encoding the CRISPR enzyme, such as a Cas protein. Non-limiting examples of Cas proteins include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, homologs thereof, or modified versions thereof. These enzymes are known; for example, the amino acid sequence of *S. pyogenes* Cas9 protein may be found in the SwissProt database under accession number Q99ZW2.

[0097] The CRISPR enzyme can be Cas9 (*e.g.*, from *S. pyogenes* or *S. pneumonia*). The CRISPR enzyme can direct cleavage of one or both strands at the location of a target sequence, such as within the target sequence and/or within the complement of the target sequence. The vector can encode a CRISPR enzyme that is mutated with respect to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence. For example, an aspartate-to-alanine substitution (D10A) in the RuvC I catalytic domain of Cas9 from *S. pyogenes* converts Cas9 from a nuclease that cleaves both strands to a nickase (cleaves a single strand). In some embodiments, a Cas9 nickase may be used in combination with guide sequence(s), *e.g.*, two guide sequences, which target respectively sense and antisense strands of the DNA target. This combination allows both strands to be nicked and used to induce NHEJ or HDR.

[0098] In some embodiments, an enzyme coding sequence encoding the CRISPR enzyme is codon optimized for expression in particular cells, such as eukaryotic cells. The eukaryotic cells may be those of or derived from a particular organism, such as a mammal, including but not limited to human, mouse, rat, rabbit, dog, or non-human primate. In general, codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression in the host cells of interest by replacing at least one codon of the native sequence with codons that are more frequently or most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Various species exhibit particular bias for certain codons of a particular amino acid. Codon bias (differences in codon usage between organisms) often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, among other things, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization.

[0099] In general, a guide sequence is any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of the CRISPR complex to the target sequence. In some embodiments, the degree of complementarity between a guide sequence and its

corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more.

[00100] Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (*e.g.* the Burrows Wheeler Aligner), Clustal W, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net).

[00101] The CRISPR enzyme may be part of a fusion protein comprising one or more heterologous protein domains. A CRISPR enzyme fusion protein may comprise any additional protein sequence, and optionally a linker sequence between any two domains. Examples of protein domains that may be fused to a CRISPR enzyme include, without limitation, epitope tags, reporter gene sequences, and protein domains having one or more of the following activities: methylase activity, demethylase activity, transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, RNA cleavage activity and nucleic acid binding activity. Non-limiting examples of epitope tags include histidine (His) tags, V5 tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Examples of reporter genes include, but are not limited to, glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT) beta galactosidase, beta-glucuronidase, luciferase, green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and autofluorescent proteins including blue fluorescent protein (BFP). A CRISPR enzyme may be fused to a gene sequence encoding a protein or a fragment of a protein that bind DNA molecules or bind other cellular molecules, including but not limited to maltose binding protein (MBP), S-tag, Lex A DNA binding domain (DBD) fusions, GAL4A DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. Additional domains that may form part of a fusion protein comprising a CRISPR enzyme are described in US 20110059502, incorporated herein by reference.

30 D. Therapeutic Proteins

[00102] Some embodiments concern expression of recombinant proteins and polypeptides. In some aspects, the protein or polypeptide may be modified to increase serum

stability. Thus, when the present application refers to the function or activity of “modified protein” or a “modified polypeptide,” one of ordinary skill in the art would understand that this includes, for example, a protein or polypeptide that possesses an additional advantage over the unmodified protein or polypeptide. It is specifically contemplated that embodiments
5 concerning a “modified protein” may be implemented with respect to a “modified polypeptide,” and vice versa.

[00103] Recombinant proteins may possess deletions and/or substitutions of amino acids; thus, a protein with a deletion, a protein with a substitution, and a protein with a deletion and a substitution are modified proteins. In some embodiments, these proteins may
10 further include insertions or added amino acids, such as with fusion proteins or proteins with linkers, for example. A “modified deleted protein” lacks one or more residues of the native protein, but may possess the specificity and/or activity of the native protein. A “modified deleted protein” may also have reduced immunogenicity or antigenicity. An example of a modified deleted protein is one that has an amino acid residue deleted from at least one
15 antigenic region, *i.e.* a region of the protein determined to be antigenic in a particular organism, such as the organism to which the modified protein is being administered.

[00104] Substitution or replacement variants typically contain the exchange of one amino acid for another at one or more sites within the protein and may be designed to modulate one or more properties of the polypeptide, particularly its effector functions and/or
20 bioavailability. Substitutions may or may not be conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine;
25 isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine, or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

[00105] In addition to a deletion or substitution, a modified protein may
30 possess an insertion of residues, which typically involves the addition of at least one residue in the polypeptide. This may include the insertion of a targeting peptide or polypeptide or simply a single residue. Terminal additions, called fusion proteins, are discussed below.

[00106] The term “biologically functional equivalent” is well understood in the art and is further defined in detail herein. Accordingly, sequences that have between about 70% and about 80%, or between about 81% and about 90%, or even between about 91% and about 99% of amino acids that are identical or functionally equivalent to the amino acids of a control polypeptide are included, provided the biological activity of the protein is maintained. A recombinant protein may be biologically functionally equivalent to its native counterpart in certain aspects.

[00107] It also will be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids or 5' or 3' sequences, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region or may include various internal sequences, *i.e.*, introns, which are known to occur within genes.

[00108] As used herein, a protein or peptide generally refers, but is not limited to, a protein of greater than about 200 amino acids, up to a full-length sequence translated from a gene; a polypeptide of greater than about 100 amino acids; and/or a peptide of from about 3 to about 100 amino acids. For convenience, the terms “protein,” “polypeptide,” and “peptide are used interchangeably herein.

[00109] As used herein, an “amino acid residue” refers to any naturally occurring amino acid, any amino acid derivative, or any amino acid mimic known in the art. In certain embodiments, the residues of the protein or peptide are sequential, without any non-amino acids interrupting the sequence of amino acid residues. In other embodiments, the sequence may comprise one or more non-amino acid moieties. In particular embodiments, the sequence of residues of the protein or peptide may be interrupted by one or more non-amino acid moieties.

[00110] Accordingly, the term “protein or peptide” encompasses amino acid sequences comprising at least one of the 20 common amino acids found in naturally occurring proteins, or at least one modified or unusual amino acid.

[00111] Certain embodiments of the present invention concern fusion proteins. These molecules may have a therapeutic protein linked at the N- or C-terminus to a heterologous domain. For example, fusions may also employ leader sequences from other species to permit the recombinant expression of a protein in a heterologous host. Another
5 useful fusion includes the addition of a protein affinity tag, such as a serum albumin affinity tag or six histidine residues, or an immunologically active domain, such as an antibody epitope, preferably cleavable, to facilitate purification of the fusion protein. Non-limiting affinity tags include polyhistidine, chitin binding protein (CBP), maltose binding protein (MBP), and glutathione-S-transferase (GST).

10 [00112] Methods of generating fusion proteins are well known to those of skill in the art. Such proteins can be produced, for example, by *de novo* synthesis of the complete fusion protein, or by attachment of the DNA sequence encoding the heterologous domain, followed by expression of the intact fusion protein.

[00113] Production of fusion proteins that recover the functional activities of
15 the parent proteins may be facilitated by connecting genes with a bridging DNA segment encoding a peptide linker that is spliced between the polypeptides connected in tandem. The linker would be of sufficient length to allow proper folding of the resulting fusion protein.

III. Methods of Administration

[00114] Viral vectors in some aspects may be administered directly to patients
20 (*in vivo*) or they can be used to treat cells *in vitro* or *ex vivo*, and then administered to patients. The term “vector” refers to small carrier nucleic acid molecule, a plasmid, virus (*e.g.*, AAV vector, retroviral vector, lentiviral vector), or other vehicle that can be manipulated by insertion or incorporation of a nucleic acid. Vectors, such as viral vectors, can be used to introduce/transfer nucleic acid sequences into cells, such that the nucleic acid
25 sequence therein is transcribed and, if encoding a protein, subsequently translated by the cells.

[00115] Any suitable cell or mammal can be administered or treated by a method or use described herein. Typically, a mammal in need of a method described herein is suspected of having or expressing an abnormal or aberrant protein that is associated with a
30 disease state. Alternative, the mammalian recipient may have a condition that is amenable to gene replacement therapy. As used herein, “gene replacement therapy” refers to

administration to the recipient of exogenous genetic material encoding a therapeutic agent and subsequent expression of the administered genetic material in situ. Thus, the phrase “condition amenable to gene replacement therapy” embraces conditions such as genetic diseases (*i.e.*, a disease condition that is attributable to one or more gene defects), acquired pathologies (*i.e.*, a pathological condition which is not attributable to an inborn defect), cancers and prophylactic processes (*i.e.*, prevention of a disease or of an undesired medical condition). Accordingly, as used herein, the term “therapeutic agent” refers to any agent or material, which has a beneficial effect on the mammalian recipient. Thus, “therapeutic agent” embraces both therapeutic and prophylactic molecules having nucleic acid or protein components.

[00116] Non-limiting examples of mammals include humans, non-human primates (*e.g.*, apes, gibbons, chimpanzees, orangutans, monkeys, macaques, and the like), domestic animals (*e.g.*, dogs and cats), farm animals (*e.g.*, horses, cows, goats, sheep, pigs) and experimental animals (*e.g.*, mouse, rat, rabbit, guinea pig). In certain embodiments a mammal is a human. In certain embodiments a mammal is a non-rodent mammal (*e.g.*, human, pig, goat, sheep, horse, dog, or the like). In certain embodiments a non-rodent mammal is a human. A mammal can be any age or at any stage of development (*e.g.*, an adult, teen, child, infant, or a mammal in utero). A mammal can be male or female. In certain embodiments a mammal can be an animal disease model, for example, animal models having or expressing an abnormal or aberrant protein that is associated with a disease state or animal models with insufficient expression of a protein, which causes a disease state.

[00117] Mammals (subjects) treated by a method or composition described herein include adults (18 years or older) and children (less than 18 years of age). Adults include the elderly. Representative adults are 50 years or older. Children range in age from 1-2 years old, or from 2-4, 4-6, 6-18, 8-10, 10-12, 12-15 and 15-18 years old. Children also include infants. Infants typically range from 1-12 months of age.

[00118] In certain embodiments, a method includes administering a plurality of viral particles to a mammal as set forth herein, where severity, frequency, progression or time of onset of one or more symptoms of a disease state, such as a neuro-degenerative disease, decreased, reduced, prevented, inhibited or delayed. In certain embodiments, a method includes administering a plurality of viral particles to a mammal to treat an adverse symptom of a disease state, such as a neuro-degenerative disease. In certain embodiments, a method

includes administering a plurality of viral particles to a mammal to stabilize, delay or prevent worsening, or progression, or reverse and adverse symptom of a disease state, such as a neuro-degenerative disease.

5 **[00119]** In certain embodiments a method includes administering a plurality of viral particles to the central nervous system, or portion thereof as set forth herein, of a mammal and severity, frequency, progression or time of onset of one or more symptoms of a disease state, such as a neuro-degenerative disease, are decreased, reduced, prevented, inhibited or delayed by at least about 5 to about 10, about 10 to about 25, about 25 to about 50, or about 50 to about 100 days.

10 **[00120]** In certain embodiments, a symptom or adverse effect comprises an early stage, middle or late stage symptom; a behavior, personality or language symptom; swallowing, movement, seizure, tremor or fidgeting symptom; ataxia; and/or a cognitive symptom such as memory, ability to organize.

IV. Pharmaceutical Compositions

15 **[00121]** As used herein the term “pharmaceutically acceptable” and “physiologically acceptable” mean a biologically acceptable composition, formulation, liquid or solid, or mixture thereof, which is suitable for one or more routes of administration, *in vivo* delivery or contact. A “pharmaceutically acceptable” or “physiologically acceptable” composition is a material that is not biologically or otherwise undesirable, *e.g.*, the material
20 may be administered to a subject without causing substantial undesirable biological effects. Such composition, “pharmaceutically acceptable” and “physiologically acceptable” formulations and compositions can be sterile. Such pharmaceutical formulations and compositions may be used, for example in administering a viral particle to a subject.

[00122] Such formulations and compositions include solvents (aqueous or
25 non-aqueous), solutions (aqueous or non-aqueous), emulsions (*e.g.*, oil-in-water or water-in-oil), suspensions, syrups, elixirs, dispersion and suspension media, coatings, isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration or *in vivo* contact or delivery. Aqueous and non-aqueous solvents, solutions and suspensions may include suspending agents and thickening agents. Supplementary active compounds
30 (*e.g.*, preservatives, antibacterial, antiviral and antifungal agents) can also be incorporated into the formulations and compositions.

[00123] Pharmaceutical compositions typically contain a pharmaceutically acceptable excipient. Such excipients include any pharmaceutical agent that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, sorbitol, Tween80, and liquids such as water, saline, glycerol and ethanol. Pharmaceutically acceptable salts can be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Additionally, auxiliary substances, such as surfactants, wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

[00124] Pharmaceutical compositions can be formulated to be compatible with a particular route of administration or delivery, as set forth herein or known to one of skill in the art. Thus, pharmaceutical compositions include carriers, diluents, or excipients suitable for administration or delivery by various routes.

[00125] Pharmaceutical forms suitable for injection or infusion of viral particles can include sterile aqueous solutions or dispersions which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate form should be a sterile fluid and stable under the conditions of manufacture, use and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. Isotonic agents, for example, sugars, buffers or salts (*e.g.*, sodium chloride) can be included. Prolonged absorption of injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00126] Solutions or suspensions of viral particles can optionally include one or more of the following components: a sterile diluent such as water for injection, saline solution, such as phosphate buffered saline (PBS), artificial CSF, a surfactants, fixed oils, a polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), glycerin, or other synthetic solvents; antibacterial and antifungal agents such as parabens,

chlorobutanol, phenol, ascorbic acid, and the like; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose.

5 **[00127]** Pharmaceutical formulations, compositions and delivery systems appropriate for the compositions, methods and uses of the invention are known in the art (*see, e.g.,* Remington: The Science and Practice of Pharmacy (2003) 20th ed., Mack Publishing Co., Easton, PA; Remington's Pharmaceutical Sciences (1990) 18th ed., Mack Publishing Co., Easton, PA; The Merck Index (1996) 12th ed., Merck Publishing Group, Whitehouse, 10 NJ; Pharmaceutical Principles of Solid Dosage Forms (1993), Technomic Publishing Co., Inc., Lancaster, Pa.; Ansel and Stoklosa, Pharmaceutical Calculations (2001) 11th ed., Lippincott Williams & Wilkins, Baltimore, MD; and Poznansky *et al.*, Drug Delivery Systems (1980), R. L. Juliano, ed., Oxford, N.Y., pp. 253-315).

[00128] Viral particles and their compositions may be formulated in dosage 15 unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for an individual to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The dosage unit forms are dependent upon the number of viral particles believed necessary to 20 produce the desired effect(s). The amount necessary can be formulated in a single dose, or can be formulated in multiple dosage units. The dose may be adjusted to a suitable viral particle concentration, optionally combined with an anti-inflammatory agent, and packaged for use.

[00129] In one embodiment, pharmaceutical compositions will include 25 sufficient genetic material to provide a therapeutically effective amount, *i.e.*, an amount sufficient to reduce or ameliorate symptoms or an adverse effect of a disease state in question or an amount sufficient to confer the desired benefit.

[00130] A "unit dosage form" as used herein refers to physically discrete units 30 suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity optionally in association with a pharmaceutical carrier (excipient, diluent, vehicle or filling agent) which, when administered in one or more doses, is calculated to produce a

desired effect (*e.g.*, prophylactic or therapeutic effect). Unit dosage forms may be within, for example, ampules and vials, which may include a liquid composition, or a composition in a freeze-dried or lyophilized state; a sterile liquid carrier, for example, can be added prior to administration or delivery *in vivo*. Individual unit dosage forms can be included in multi-dose kits or containers. Thus, for example, viral particles, and pharmaceutical compositions thereof, can be packaged in single or multiple unit dosage form for ease of administration and uniformity of dosage.

[00131] Formulations containing viral particles typically contain an effective amount, the effective amount being readily determined by one skilled in the art. The viral particles may typically range from about 1% to about 95% (w/w) of the composition, or even higher if suitable. The quantity to be administered depends upon factors such as the age, weight and physical condition of the mammal or the human subject considered for treatment. Effective dosages can be established by one of ordinary skill in the art through routine trials establishing dose response curves.

V. Definitions

[00132] The terms “polynucleotide,” “nucleic acid” and “transgene” are used interchangeably herein to refer to all forms of nucleic acid, oligonucleotides, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and polymers thereof. Polynucleotides include genomic DNA, cDNA and antisense DNA, and spliced or unspliced mRNA, rRNA, tRNA and inhibitory DNA or RNA (RNAi, *e.g.*, small or short hairpin (sh)RNA, microRNA (miRNA), small or short interfering (si)RNA, trans-splicing RNA, or antisense RNA). Polynucleotides can include naturally occurring, synthetic, and intentionally modified or altered polynucleotides (*e.g.*, variant nucleic acid). Polynucleotides can be single stranded, double stranded, or triplex, linear or circular, and can be of any suitable length. In discussing polynucleotides, a sequence or structure of a particular polynucleotide may be described herein according to the convention of providing the sequence in the 5' to 3' direction.

[00133] A nucleic acid encoding a polypeptide often comprises an open reading frame that encodes the polypeptide. Unless otherwise indicated, a particular nucleic acid sequence also includes degenerate codon substitutions.

[00134] Nucleic acids can include one or more expression control or regulatory elements operably linked to the open reading frame, where the one or more regulatory elements are configured to direct the transcription and translation of the polypeptide encoded by the open reading frame in a mammalian cell. Non-limiting examples of expression control/regulatory elements include transcription initiation sequences (*e.g.*, promoters, enhancers, a TATA box, and the like), translation initiation sequences, mRNA stability sequences, poly A sequences, secretory sequences, and the like. Expression control/regulatory elements can be obtained from the genome of any suitable organism.

[00135] A “promoter” refers to a nucleotide sequence, usually upstream (5') of a coding sequence, which directs and/or controls the expression of the coding sequence by providing the recognition for RNA polymerase and other factors required for proper transcription. A pol II promoter includes a minimal promoter that is a short DNA sequence comprised of a TATA-box and optionally other sequences that serve to specify the site of transcription initiation, to which regulatory elements are added for control of expression. A type 1 pol III promoter includes three cis-acting sequence elements downstream of the transcriptional start site: a) 5' sequence element (A block); b) an intermediate sequence element (I block); c) 3' sequence element (C block). A type 2 pol III promoter includes two essential cis-acting sequence elements downstream of the transcription start site: a) an A box (5' sequence element); and b) a B box (3' sequence element). A type 3 pol III promoter includes several cis-acting promoter elements upstream of the transcription start site, such as a traditional TATA box, proximal sequence element (PSE), and a distal sequence element (DSE).

[00136] An “enhancer” is a DNA sequence that can stimulate transcription activity and may be an innate element of the promoter or a heterologous element that enhances the level or tissue specificity of expression. It is capable of operating in either orientation (5'→3' or 3'→5'), and may be capable of functioning even when positioned either upstream or downstream of the promoter.

[00137] Promoters and/or enhancers may be derived in their entirety from a native gene, or be composed of different elements derived from different elements found in nature, or even be comprised of synthetic DNA segments. A promoter or enhancer may comprise DNA sequences that are involved in the binding of protein factors that

modulate/control effectiveness of transcription initiation in response to stimuli, physiological or developmental conditions.

[00138] Non-limiting examples of promoters include 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, pol II promoters, pol III promoters, synthetic promoters, hybrid promoters, and the like. In addition, sequences derived from non-viral genes, such as the murine metallothionein gene, will also find use herein. Exemplary constitutive promoters include the promoters for the following genes which encode certain constitutive or “housekeeping” functions: hypoxanthine phosphoribosyl transferase (HPRT), dihydrofolate reductase (DHFR), adenosine deaminase, phosphoglycerol kinase (PGK), pyruvate kinase, phosphoglycerol mutase, actin promoter, U6, and other constitutive promoters known to those of skill in the art. In addition, many viral promoters function constitutively in eukaryotic cells. These include: the early and late promoters of SV40; the long terminal repeats (LTRs) of Moloney Leukemia Virus and other retroviruses; and the thymidine kinase promoter of Herpes Simplex Virus, among many others. In addition, sequences derived from intronic miRNA promoters, such as, for example, the miR107, miR206, miR208b, miR548f-2, miR569, miR590, miR566, and miR128 promoter, will also find use herein (see, *e.g.*, Monteys *et al.*, 2010). Accordingly, any of the above-referenced constitutive promoters can be used to control transcription of a heterologous gene insert.

[00139] A “transgene” is used herein to conveniently refer to a nucleic acid sequence/polynucleotide that is intended or has been introduced into a cell or organism. Transgenes include any nucleic acid, such as a gene that encodes an inhibitory RNA or polypeptide or protein, and are generally heterologous with respect to naturally occurring AAV genomic sequences.

[00140] The term “transduce” refers to introduction of a nucleic acid sequence into a cell or host organism by way of a vector (*e.g.*, a viral particle). Introduction of a transgene into a cell by a viral particle is can therefore be referred to as “transduction” of the cell. The transgene may or may not be integrated into genomic nucleic acid of a transduced cell. If an introduced transgene becomes integrated into the nucleic acid (genomic DNA) of the recipient cell or organism it can be stably maintained in that cell or organism and further

passed on to or inherited by progeny cells or organisms of the recipient cell or organism. Finally, the introduced transgene may exist in the recipient cell or host organism extra chromosomally, or only transiently. A “transduced cell” is therefore a cell into which the transgene has been introduced by way of transduction. Thus, a “transduced” cell is a cell into which, or a progeny thereof in which a transgene has been introduced. A transduced cell can be propagated, transgene transcribed and the encoded inhibitory RNA or protein expressed. For gene therapy uses and methods, a transduced cell can be in a mammal.

[00141] Transgenes under control of inducible promoters are expressed only or to a greater degree, in the presence of an inducing agent, (*e.g.*, transcription under control of the metallothionein promoter is greatly increased in presence of certain metal ions). Inducible promoters include responsive elements (REs) which stimulate transcription when their inducing factors are bound. For example, there are REs for serum factors, steroid hormones, retinoic acid and cyclic AMP. Promoters containing a particular RE can be chosen in order to obtain an inducible response and in some cases, the RE itself may be attached to a different promoter, thereby conferring inducibility to the recombinant gene. Thus, by selecting a suitable promoter (constitutive versus inducible; strong versus weak), it is possible to control both the existence and level of expression of a polypeptide in the genetically modified cell. If the gene encoding the polypeptide is under the control of an inducible promoter, delivery of the polypeptide *in situ* is triggered by exposing the genetically modified cell *in situ* to conditions for permitting transcription of the polypeptide, *e.g.*, by intraperitoneal injection of specific inducers of the inducible promoters which control transcription of the agent. For example, *in situ* expression by genetically modified cells of a polypeptide encoded by a gene under the control of the metallothionein promoter, is enhanced by contacting the genetically modified cells with a solution containing the appropriate (*i.e.*, inducing) metal ions *in situ*.

[00142] A nucleic acid/transgene is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. A nucleic acid/transgene encoding and RNAi or a polypeptide, or a nucleic acid directing expression of a polypeptide may include an inducible promoter, or a tissue-specific promoter for controlling transcription of the encoded polypeptide. A nucleic acid operably linked to an expression control element can also be referred to as an expression cassette.

[00143] In certain embodiments, CNS-specific or inducible promoters, enhancers and the like, are employed in the methods and uses described herein. Non-limiting

examples of CNS-specific promoters include those isolated from the genes from myelin basic protein (MBP), glial fibrillary acid protein (GFAP), and neuron specific enolase (NSE). Non-limiting examples of inducible promoters include DNA responsive elements for ecdysone, tetracycline, hypoxia and IFN.

5 **[00144]** In certain embodiments, an expression control element comprises a CMV enhancer. In certain embodiments, an expression control element comprises a beta actin promoter. In certain embodiments, an expression control element comprises a chicken beta actin promoter. In certain embodiments, an expression control element comprises a CMV enhancer and a chicken beta actin promoter.

10 **[00145]** As used herein, the terms “modify” or “variant” and grammatical variations thereof, mean that a nucleic acid, polypeptide or subsequence thereof deviates from a reference sequence. Modified and variant sequences may therefore have substantially the same, greater or less expression, activity or function than a reference sequence, but at least retain partial activity or function of the reference sequence. A particular type of variant is a
15 mutant protein, which refers to a protein encoded by a gene having a mutation, *e.g.*, a missense or nonsense mutation.

[00146] A “nucleic acid” or “polynucleotide” variant refers to a modified sequence which has been genetically altered compared to wild-type. The sequence may be genetically modified without altering the encoded protein sequence. Alternatively, the
20 sequence may be genetically modified to encode a variant protein. A nucleic acid or polynucleotide variant can also refer to a combination sequence which has been codon modified to encode a protein that still retains at least partial sequence identity to a reference sequence, such as wild-type protein sequence, and also has been codon-modified to encode a variant protein. For example, some codons of such a nucleic acid variant will be changed
25 without altering the amino acids of a protein encoded thereby, and some codons of the nucleic acid variant will be changed which in turn changes the amino acids of a protein encoded thereby.

[00147] The terms “protein” and “polypeptide” are used interchangeably herein. The “polypeptides” encoded by a “nucleic acid” or “polynucleotide” or “transgene”
30 disclosed herein include partial or full-length native sequences, as with naturally occurring wild-type and functional polymorphic proteins, functional subsequences (fragments) thereof,

and sequence variants thereof, so long as the polypeptide retains some degree of function or activity. Accordingly, in methods and uses of the invention, such polypeptides encoded by nucleic acid sequences are not required to be identical to the endogenous protein that is defective, or whose activity, function, or expression is insufficient, deficient or absent in a
5 treated mammal.

[00148] Non-limiting examples of modifications include one or more nucleotide or amino acid substitutions (*e.g.*, about 1 to about 3, about 3 to about 5, about 5 to about 10, about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 40, about 40 to about 50, about 50 to about 100, about 100 to about
10 150, about 150 to about 200, about 200 to about 250, about 250 to about 500, about 500 to about 750, about 750 to about 1000 or more nucleotides or residues).

[00149] An example of an amino acid modification is a conservative amino acid substitution or a deletion. In particular embodiments, a modified or variant sequence retains at least part of a function or activity of the unmodified sequence (*e.g.*, wild-type
15 sequence).

[00150] Another example of an amino acid modification is a targeting peptide introduced into a capsid protein of a viral particle. Peptides have been identified that target recombinant viral vectors, to the central nervous system, such as to distinct brain regions.

[00151] A recombinant virus so modified may preferentially bind to one type of tissue (*e.g.*, CNS tissue) over another type of tissue (*e.g.*, liver tissue). In certain
20 embodiments, a recombinant virus bearing a modified capsid protein may “target” brain vascular epithelia tissue by binding at level higher than a comparable, unmodified capsid protein. For example, a recombinant virus having a modified capsid protein may bind to brain vascular epithelia tissue at a level 50% to 100% greater than an unmodified recombinant
25 virus.

[00152] A “nucleic acid fragment” is a portion of a given nucleic acid molecule. Deoxyribonucleic acid (DNA) in the majority of organisms is the genetic material while ribonucleic acid (RNA) is involved in the transfer of information contained within DNA into proteins. Fragments and variants of the disclosed nucleotide sequences and
30 proteins or partial-length proteins encoded thereby are also encompassed by the present invention. By “fragment” or “portion” is meant a full length or less than full length of the

nucleotide sequence encoding, or the amino acid sequence of, a polypeptide or protein. In certain embodiments, the fragment or portion is biologically functional (*i.e.*, retains 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of activity or function of wild-type).

5 **[00153]** A “variant” of a molecule is a sequence that is substantially similar to the sequence of the native molecule. For nucleotide sequences, variants include those sequences that, because of the degeneracy of the genetic code, encode the identical amino acid sequence of the native protein. Naturally occurring allelic variants such as these can be identified with the use of molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques. Variant nucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis, which encode the native protein, as well as those that encode a polypeptide having amino acid substitutions. Generally, nucleotide sequence variants of the invention will have at least 40%, 50%, 60%, to 70%, *e.g.*, 71%, 72%, 73%, 74%, 75%, 76%,
10 77%, 78%, to 79%, generally at least 80%, *e.g.*, 81%-84%, at least 85%, *e.g.*, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, to 98%, sequence identity to the native (endogenous) nucleotide sequence. In certain embodiments, the variant is biologically functional (*i.e.*, retains 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of activity or function of wild-type).

20 **[00154]** “Conservative variations” of a particular nucleic acid sequence refers to those nucleic acid sequences that encode identical or essentially identical amino acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons CGT, CGC, CGA, CGG, AGA and AGG all encode the amino acid arginine. Thus, at every position
25 where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded protein. Such nucleic acid variations are “silent variations,” which are one species of “conservatively modified variations.” Every nucleic acid sequence described herein that encodes a polypeptide also describes every possible silent variation, except where otherwise noted. One of skill in the art
30 will recognize that each codon in a nucleic acid (except ATG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard

techniques. Accordingly, each “silent variation” of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[00155] The term “substantial identity” of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%,
5 76%, 77%, 78%, or 79%, or at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, or at least 90%, 91%, 92%, 93%, or 94%, or even at least 95%, 96%, 97%, 98%, or 99% sequence identity, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill in the art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded
10 by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning, and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 70%, at least 80%, 90%, or even at least 95%.

[00156] The term “substantial identity” in the context of a polypeptide indicates
15 that a polypeptide comprises a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, or 79%, or 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, or at least 90%, 91%, 92%, 93%, or 94%, or even, 95%, 96%, 97%, 98% or 99%, sequence identity to the reference sequence over a specified comparison window. An indication that two polypeptide sequences are identical is that one polypeptide is immunologically reactive with
20 antibodies raised against the second polypeptide. Thus, a polypeptide is identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution.

[00157] The terms “treat” and “treatment” refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent, inhibit, reduce, or decrease an undesired physiological change or disorder, such as the development,
25 progression or worsening of the disorder. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilizing a (*i.e.*, not worsening or progressing) symptom or adverse effect 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.
30 “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those predisposed (*e.g.*, as determined by a genetic assay).

VI. Kits

[00158] The invention provides kits with packaging material and one or more components therein. A kit typically includes a label or packaging insert including a description of the components or instructions for use *in vitro*, *in vivo*, or *ex vivo*, of the components therein. A kit can contain a collection of such components, *e.g.*, a nucleic acid, recombinant vector, and/or viral particles.

[00159] A kit refers to a physical structure housing one or more components of the kit. Packaging material can maintain the components sterily, and can be made of material commonly used for such purposes (*e.g.*, paper, corrugated fiber, glass, plastic, foil, ampules, vials, tubes, etc.).

[00160] Labels or inserts can include identifying information of one or more components therein, dose amounts, clinical pharmacology of the active ingredient(s) including mechanism of action, pharmacokinetics and pharmacodynamics. Labels or inserts can include information identifying manufacturer, lot numbers, manufacture location and date, expiration dates. Labels or inserts can include information identifying manufacturer information, lot numbers, manufacturer location and date. Labels or inserts can include information on a disease for which a kit component may be used. Labels or inserts can include instructions for the clinician or subject for using one or more of the kit components in a method, use, or treatment protocol or therapeutic regimen. Instructions can include dosage amounts, frequency or duration, and instructions for practicing any of the methods, uses, treatment protocols or prophylactic or therapeutic regimes described herein.

[00161] Labels or inserts can include information on any benefit that a component may provide, such as a prophylactic or therapeutic benefit. Labels or inserts can include information on potential adverse side effects, complications or reactions, such as warnings to the subject or clinician regarding situations where it would not be appropriate to use a particular composition. Adverse side effects or complications could also occur when the subject has, will be or is currently taking one or more other medications that may be incompatible with the composition, or the subject has, will be or is currently undergoing another treatment protocol or therapeutic regimen which would be incompatible with the composition and, therefore, instructions could include information regarding such incompatibilities.

[00162] Labels or inserts include “printed matter,” *e.g.*, paper or cardboard, or separate or affixed to a component, a kit or packing material (*e.g.*, a box), or attached to an ampule, tube or vial containing a kit component. Labels or inserts can additionally include a computer readable medium, such as a bar-coded printed label, a disk, optical disk such as CD- or DVD-ROM/RAM, DVD, MP3, or an electrical storage media such as RAM and ROM or hybrids of these such as magnetic/optical storage media, FLASH memory, hybrids and memory type cards.

VII. Examples

[00163] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Identification of AAV Variants that Target Brain Parenchyma

[00164] Advanced bar-coded AAV libraries were developed using AAV1, AAV2 and AAV9 capsids as starting platforms. AAV1, AAV2, and AAV9 peptide display libraries were generated by insertion of random sequences at position 590 of AAV1 capsid, position 587 of AAV2 capsid, and position 588 of AAV9 capsid, respectively (FIG. 1). The library had a diversity of 1×10^7 unique clones (FIG. 3).

[00165] To test the utility of the libraries, pilot studies were performed with bench-grade (low titer, low purity) capsid modified AAV2. The AAV2 library was injected intravenously into two C57BL/6 mice at 8×10^{10} vector genomes per animal. After 72 hours, the cerebral cortex, cerebellum, and spinal cord were dissected. Of note, heart, skeletal muscle, and diaphragm were separately harvested in order to identify the muscle tropism. Viral genomic DNA was isolated, and the recovered random oligonucleotide sequences were amplified by PCR. The PCR products from brain were pooled to generate the second-round library, which was injected into two mice at 4×10^{10} vector genomes per animal. After the second injection, vector genomes were recovered as before and were subjected to NexGen

sequencing along with the starting library and 1st round tissues. To test if sequences showing enrichment in brain tissues could indeed extend AAV2s reach to the brain, individual hits were cloned into the AAV2 capsid packaging plasmid, and eGFP-expressing AAV2s were generated. Bench-grade vectors were made, and 3×10^{10} vector genomes of the AAV2-based capsid modified viruses were injected into mice. Four weeks later, eGFP fluorescence was seen in the brain, even for these low titer variants.

[00166] Using these advanced bar-coded AAV libraries, AAV variants that can target distinct primate brain structures in non-human primates were identified. The AAV1, AAV2, and AAV9 libraries were delivered via intracerebroventricular injection to one non-human primate (FIG. 2). Seventy-two hours post-infusion, brain regions were microdissected for viral DNA isolation and AAV DNA amplified by PCR. Products were pooled and used for packaging the 2nd round library, which was infused into an additional NHP. Brain regions were then microdissected 12 days after infusion. After two rounds of panning, vector genomes were recovered and subjected to next generation sequencing. Specifically, genomic DNA extracted from the round1 and round2 tissues was PCR amplified to generate Illumina amplicon sequencing libraries at the position of the vector barcode. The resulting libraries were pooled and run on a single lane of an Illumina HiSeq 4000 using 100bp single end read chemistry. To illustrate the utility of the approach, several target regions were tested as examples: the ependyma, the meninges and the cerebellum. In general, the sequences directing the AAVx to the ependyma, meninges and cerebellum were different, and different for the various serotypes.

[00167] Round-over-Round enrichment graphs (FIG. 4) and heatmaps (FIGS. 5 and 6) were generated for the following tissues: brainstem, caudate, cerebellar cortex (FIG. 5), cerebral cortex, ependyma, globus pallidus, hippocampus, meninges, optic nerve, putamen, spinal cord, substantia nigra, subthalamic nuclei, and thalamus. These illustrate the enrichment of indicated barcodes at baseline (round 0), and after rounds one and two of in vivo passaging through rhesus macaque. To generate these, the fastq results files for each tissue and round combination were processed using a custom Python script designed to extract and quantify unique barcode configurations observed at the DNA level. A custom R script was used to calculate the percentages of barcodes present in each sample and convert DNA barcodes to amino acid barcodes. Table 1 corresponds to samples treated with the AAV1-derived library; Table 2 represents tissues treated with the AAV2-derived library;

Table 3 corresponds to samples treated with the AAV9-derived library. Top hits were selected from these three libraries and were assembled and generated into a validation library containing 50 (AAV1), 58 (AAV2), and 30 (AAV9) derived barcodes. This validation library was delivered into an additional Rhesus macaque by ICV injection. Tissues were again collected and processed to facilitate recovery of barcode abundance by deep sequencing. Barcode abundance was evaluated in recovered tissues and the input viral library. Enrichment values for each barcode were calculated relative to their abundance in the input viral library. The resulting relative enrichment values are a robust indicator of vector performance amongst the various tissues evaluated, facilitating identification of broad and specific AAV vector variants (FIGS. 7A-C).

[00168] In order to validate the identified cell-type specificity, AAV9-1999 (having a targeting peptide sequence of KGGGFHG; SEQ ID NO: 110) was selected for *in vivo* validation. An eGFP expression construct was packaged into AAV9-1999 driven by the CAG promoter. A 5-year-old, female Rhesus macaque was administered 1.5E13 vg of AAV9-1999 by ICV injection to the left lateral ventricle. Brain was collected 30 days post-injection for histological analysis. Cerebellum slices were H&E stained to depict the transduction pattern of AAV9-1999 (FIG. 8). The cochlea were also collected from this animal and surprisingly had strong transduction of hair cells. In addition, AAV9-1999 and AAV9 capsids containing the eGFP construct were delivered to C57BL/6 p0 mouse pups by ICV injection at 1E10 vg per hemisphere. After 21 days, the mice were perfused. Whole mount brains (FIG. 9A), 40 μ m whole brain sagittal sections (FIG. 9B), 40 μ m S1 cortex sections (FIG. 9C, left), 40 μ m hippocampus sections (FIG. 9C, middle), 40 μ m cerebellum sagittal sections (FIG. 9C, right), and 40 μ m lumbar spinal cord coronal sections (FIG. 9D) were imaged for eGFP fluorescence signal. AAV9-1999 injected into BL/6 neonate mouse pups showed ubiquitous expression greater than dose-matched injections of AAV9.

[00169] One adult rhesus macaque was injected with a mixture of four modified AAVs: AAV9 with a RGDQLQWV (SEQ ID NO: 113) targeting peptide sequence and a mTAGBFP2 tag; AAV1 with a ERDRTRG (SEQ ID NO: 21) targeting peptide sequence as a mTFP1 tag; AAV2 with a GRGAPGG (SEQ ID NO: 80) targeting peptide sequence and a mNG tag; and AAV2 with a DDPSARR (SEQ ID NO: 53) targeting peptide sequence and a mRuby3 tag. The viruses were mixed straight at equal volumes to achieve the final total doses for each as follows:

AAV9.RGDL mTagBFP2	6.13E12 total vg
AAV1.ERDR mTFP1	1.23E13 total vg
AAV2.GRGA mNG	8.8E12 total vg
AAV2.DDPS mRuby3	1.32E13 total vg

5 **[00170]** Brain was collected 30 days post-injection for fluorescence imaging. Lateral ventricle sections (FIG. 10A), fourth ventricle sections (FIG. 10B), and meninges sections (FIG. 10C) were imaged for mTagBFP2, mTFP2, mNG, and mRuby3 fluorescence signals.

10 **[00171]** Additional experiments were performed by injecting the AAV9-1999 into the cochlea of rhesus macaques. Based on the results of cochlear transduction, an animal received AAV9-1999 to their lateral ventricle. A single animal received 3E11 vg of AAV9-1999 injected directly to their round window with canal fenestrations (FIGS. 11A-C).

* * *

15 **[00172]** All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain
20 agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

5

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WHAT IS CLAIMED IS:

1. A modified adeno-associated virus (AAV) capsid protein comprising a targeting peptide that targets a viral vector comprising the modified AAV capsid protein to a distinct organ or brain structure, wherein the targeting peptide is three to ten amino acids in length.
- 5 2. The modified AAV capsid protein of claim 1, wherein the modified AAV capsid protein is a modified AAV9 capsid protein having a sequence at least 95% identical to SEQ ID NO: 143, wherein the targeting peptide is SEQ ID NO: 110, wherein the distinct brain structure is the brainstem, caudate, cerebellar, cochlea (ear), cortex, cerebral cortex, deep cerebellar nuclei, ependyma, globus pallidus, hippocampus, meninges, motor cortex, optic
10 nerve, prefrontal cortex, putamen, spinal cord, substantia nigra, subthalamic nuclei, temporal cortex, thalamus, or visual cortex.
3. The modified AAV capsid protein of claim 1, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, a modified AAV2 capsid protein, or a modified AAV9 capsid protein.
- 15 4. The modified AAV capsid protein of claim 1, wherein the modified AAV capsid protein is derived from an AAV1 capsid protein (see SEQ ID NO: 138), wherein the targeting peptide is inserted after residue 590 of the AAV1 capsid protein.
5. The modified AAV capsid protein of claim 4, wherein the targeting peptide is flanked by linker sequences, wherein the linker sequences on each side of the targeting peptides are
20 two or three amino acids long.
6. The modified AAV capsid protein of claim 5, wherein the linker sequences are SSA on the N-terminal side of the targeting peptide and AS on the C-terminal side of the targeting peptide.
7. The modified AAV capsid protein of claim 6, wherein the modified AAV1 capsid
25 protein has a sequence at least 95% identical to SEQ ID NO: 141.
8. The modified AAV capsid protein of claim 1, wherein the modified AAV capsid protein is derived from an AAV2 capsid protein (see SEQ ID NO: 139), wherein the targeting peptide is inserted after residue 587 of the AAV2 capsid protein.

9. The modified AAV capsid protein of claim 8, wherein the targeting peptide is flanked by linker sequences, wherein the linker sequences on each side of the targeting peptides are two or three amino acids long.
10. The modified AAV capsid protein of claim 9, wherein the linker sequences are AAA on the N-terminal side of the targeting peptide and AA on the C-terminal side of the targeting peptide.
11. The modified AAV capsid protein of claim 10, wherein the modified AAV2 capsid protein has a sequence at least 95% identical to SEQ ID NO: 142.
12. The modified AAV capsid protein of claim 1, wherein the modified AAV capsid protein is derived from an AAV9 capsid protein (see SEQ ID NO: 140), wherein the targeting peptide is inserted after residue 588 of the AAV9 capsid protein.
13. The modified AAV capsid protein of claim 12, wherein the targeting peptide is flanked by linker sequences, wherein the linker sequences on each side of the targeting peptides are two or three amino acids long.
14. The modified AAV capsid protein of claim 13, wherein the linker sequences are AAA on the N-terminal side of the targeting peptide and AS on the C-terminal side of the targeting peptide.
15. The modified AAV capsid protein of any claim 14, wherein the modified AAV9 capsid protein has a sequence at least 95% identical to SEQ ID NO: 143.
16. The modified AAV capsid protein of claim 1, wherein the target peptide comprises a sequence up to ten amino acids in length having therein an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-137 or 144.
17. The modified AAV capsid protein of claim 16, wherein the targeting peptide is seven amino acids in length.
18. The modified AAV capsid protein of any one of claims 1-17, wherein the distinct brain structure is the brainstem, caudate, cerebellar cortex, cerebral cortex, ependyma, globus pallidus, hippocampus, meninges, optic nerve, putamen, spinal cord, substantia nigra, subthalamic nuclei, or thalamus.

19. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the brainstem, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 1-9.
20. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the caudate, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 1, 3, 5, 7, 10-16, 25, 26, 32, and 144.
21. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the cerebellar cortex, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 1, 3, 4, 9, and 17-21.
22. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the cerebral cortex, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 1, 3, 5, 12, and 21-26.
23. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the ependyma, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 2-4, 7, 9, 21, 22, 27, and 28.
24. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the globus pallidus, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 3, 5, 12, 14, 16, 21, 22, and 29-31.
25. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the hippocampus, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 1-4, 7, and 32-34.
26. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the meninges, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 3, 5, 7, 9, 12, 21, and 35-37.
27. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the optic nerve, wherein the modified AAV capsid protein is a modified AAV1 capsid

protein, wherein the targeting peptide is selected from SEQ ID NOs: 2, 3, 7, 14-16, 21, 31, and 38.

28. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the putamen, wherein the modified AAV capsid protein is a modified AAV1 capsid protein,
5 wherein the targeting peptide is selected from SEQ ID NOs: 3, 4, 12, 13, 21, 30, and 39-42.

29. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the spinal cord, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 2-4, 7, 9, 21, 32, 33, and 43.

10 30. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the substantia nigra, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 2, 3, 9, 44, and 45.

31. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the subthalamic nuclei, wherein the modified AAV capsid protein is a modified AAV1 capsid
15 protein, wherein the targeting peptide is selected from SEQ ID NOs: 2-4, 12, 16, 30, 46, and 47.

32. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the thalamus, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 1, 2, 8, 12, 21, 28, and 48-51.

20 33. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the brainstem, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 52-60.

34. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the caudate, wherein the modified AAV capsid protein is a modified AAV2 capsid protein,
25 wherein the targeting peptide is selected from SEQ ID NOs: 59 and 61-69.

35. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the cerebellar cortex, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 56, 58, 60, and 70-75.

36. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the cerebral cortex, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 53, 58, 60, 62, 63, 66, and 76-79.
- 5 37. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the ependyma, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 53, 60, 62, 63, 66, 74-77, and 80.
38. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the globus pallidus, wherein the modified AAV capsid protein is a modified AAV2 capsid
10 protein, wherein the targeting peptide is selected from SEQ ID NOs: 60, 75, and 81-87.
39. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the hippocampus, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 53, 55, 58, 60, 63, 76, 79, 88, and 89.
- 15 40. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the meninges, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 58, 60, 66, 73, 76, 80, and 90-93.
41. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the optic nerve, wherein the modified AAV capsid protein is a modified AAV2 capsid
20 protein, wherein the targeting peptide is selected from SEQ ID NOs: 53, 54, 57, 58, 60, 75, 79, 87, 88, and 94.
42. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the putamen, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 55, 59, 60, 61, and 95-100.
- 25 43. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the spinal cord, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 53, 58-61, 63, 77, 88, 95, and 101.

44. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the substantia nigra, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 52, 53, 57, 58, 75, 76, 87, 102, and 103.
- 5 45. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the subthalamic nuclei, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 57, 58, 60, 75, 79, 87, 88, 102, 104, and 105.
46. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is
10 the thalamus, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 52, 55, 56, 74, 85, 88, and 106-109.
47. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the brainstem, wherein the modified AAV capsid protein is a modified AAV9 capsid protein,
15 wherein the targeting peptide is selected from SEQ ID NOs: 110-117.
48. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the caudate, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 110, 113, 115, 116, and 118-121.
49. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is
20 the cerebellar cortex, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 110, 111, 113, 119, and 122-125.
50. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the cerebral cortex, wherein the modified AAV capsid protein is a modified AAV9 capsid
25 protein, wherein the targeting peptide is selected from SEQ ID NOs: 110, 111, 113, 114, 116, and 125-127.
51. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the ependyma, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 110, 111, 113, 118-120, and 128.

52. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the globus pallidus, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 110-112, 114, 119, 120, and 129.

5 53. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the hippocampus, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 110, 111, 113, 116, 123, 125, 129, and 130.

54. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is
10 the meninges, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 110, 111, 113, 114, 118, 119, 122, and 131.

55. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the optic nerve, wherein the modified AAV capsid protein is a modified AAV9 capsid
15 protein, wherein the targeting peptide is selected from SEQ ID NOs: 110, 111, 114, 115, 117, 129, and 132.

56. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the putamen, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 110, 112, 113, 116, 123, 127,
20 133, and 134.

57. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the spinal cord, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 110, 113, 119, 120, 122, 123, 128, and 134.

25 58. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the substantia nigra, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is selected from SEQ ID NOs: 110-114, 117, and 129.

59. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the subthalamic nuclei, wherein the modified AAV capsid protein is a modified AAV9 capsid

protein, wherein the targeting peptide is selected from SEQ ID NOs: 110, 111, 113, 119, 120, 122, 132, and 135.

60. The modified AAV capsid protein of claim 18, wherein the distinct brain structure is the thalamus, wherein the modified AAV capsid protein is a modified AAV9 capsid protein,
5 wherein the targeting peptide is selected from SEQ ID NOs: 110, 112-114, 125, 133, 136, and 137.

61. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 1, wherein the distinct brain structure is the brainstem, caudate, cerebellar cortex, cerebral
10 cortex, hippocampus, or thalamus.

62. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 2, wherein the distinct brain structure is the brainstem, ependyma, hippocampus, optic nerve, spinal cord, substantia nigra, subthalamic nuclei, or thalamus.

15 63. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 3, wherein the distinct brain structure is the brainstem, caudate, cerebellar cortex, cerebral cortex, ependyma, globus pallidus, hippocampus, meninges, optic nerve, putamen, spinal cord, substantia nigra, or subthalamic nuclei.

20 64. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 4, wherein the distinct brain structure is the brainstem, cerebellar cortex, ependyma, hippocampus, putamen, spinal cord, or subthalamic nuclei.

25 65. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 5, wherein the distinct brain structure is the brainstem, cerebral cortex, globus pallidus, or meninges.

66. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 6, wherein the distinct brain structure is the brainstem.
67. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 7, wherein the distinct brain structure is the brainstem, caudate, ependyma, hippocampus, meninges, optic nerve, or spinal cord.
68. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 8, wherein the distinct brain structure is the brainstem or thalamus.
69. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 9, wherein the distinct brain structure is the brainstem, cerebellar cortex, ependyma, meninges, spinal cord, or substantia nigra.
70. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 10 or 11, wherein the distinct brain structure is the caudate.
71. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 12, wherein the distinct brain structure is the caudate, cerebral cortex, globus pallidus, meninges, putamen, subthalamic nuclei, or thalamus.
72. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 13, wherein the distinct brain structure is the caudate or putamen.
73. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 14, wherein the distinct brain structure is the caudate, globus pallidus, or optic nerve.

74. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 15, wherein the distinct brain structure is the caudate or optic nerve.
75. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 16, wherein the distinct brain structure is the caudate, globus pallidus, optic nerve, or subthalamic nuclei.
76. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is any one of SEQ ID NOs: 17-20, wherein the distinct brain structure is the cerebellar cortex.
77. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 21, wherein the distinct brain structure is the cerebellar cortex, cerebral cortex, ependyma, globus pallidus, meninges, optic nerve, putamen, spinal cord, or thalamus.
78. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 22, wherein the distinct brain structure is the cerebral cortex, ependyma, or globus pallidus.
79. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is any one of SEQ ID NOs: 23-26, wherein the distinct brain structure is the cerebral cortex.
80. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 27, wherein the distinct brain structure is the ependyma.
81. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 28, wherein the distinct brain structure is the ependyma or thalamus.
82. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 29, wherein the distinct brain structure is the globus pallidus.

83. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 30, wherein the distinct brain structure is the globus pallidus, putamen, or subthalamic nuclei.
84. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 31, wherein the distinct brain structure is the globus pallidus or optic nerve.
85. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 32 or 33, wherein the distinct brain structure is the hippocampus or spinal cord.
86. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 34, wherein the distinct brain structure is the hippocampus.
87. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is any one of SEQ ID NOs: 35-37, wherein the distinct brain structure is the meninges.
88. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 38, wherein the distinct brain structure is the optic nerve.
89. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is any one of SEQ ID NOs: 39-42, wherein the distinct brain structure is the putamen.
90. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 43, wherein the distinct brain structure is the spinal cord.
91. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 44 or 45, wherein the distinct brain structure is the substantia nigra.

92. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is SEQ ID NO: 46 or 47, wherein the distinct brain structure is the subthalamic nuclei.
93. The modified AAV capsid protein of claim 1 or 7, wherein the modified AAV capsid protein is a modified AAV1 capsid protein, wherein the targeting peptide is any one of SEQ ID NOs: 48-51, wherein the distinct brain structure is the thalamus.
94. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 52, wherein the distinct brain structure is the brainstem, substantia nigra, or thalamus.
95. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 53, wherein the distinct brain structure is the brainstem, cerebral cortex, ependyma, hippocampus, meninges, optic nerve, spinal cord, or substantia nigra.
96. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 54, wherein the distinct brain structure is the brainstem or optic nerve.
97. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 55, wherein the distinct brain structure is the brainstem, hippocampus, putamen, or thalamus.
98. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 56, wherein the distinct brain structure is the brainstem, cerebellar cortex, or thalamus.
99. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 57, wherein the distinct brain structure is the brainstem, optic nerve, substantia nigra, or subthalamic nuclei.
100. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID

NO: 58, wherein the distinct brain structure is the brainstem, cerebellar cortex, cerebral cortex, hippocampus, meninges, optic nerve, spinal cord, substantia nigra, or subthalamic nuclei.

101. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
5 capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 59, wherein the distinct brain structure is the brainstem, caudate, putamen, or spinal cord.

102. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID
10 NO: 60, wherein the distinct brain structure is the brainstem, cerebellar cortex, cerebral cortex, ependyma, globus pallidus, hippocampus, meninges, optic nerve, putamen, spinal cord, or subthalamic nuclei.

103. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID
15 NO: 61, wherein the distinct brain structure is the caudate, putamen, or spinal cord.

104. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID
NO: 62, wherein the distinct brain structure is the caudate, cerebral cortex, or ependyma.

105. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
20 capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 63, wherein the distinct brain structure is the caudate, cerebral cortex, ependyma, hippocampus, or spinal cord.

106. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is any one of
25 SEQ ID NOs: 64, 65, and 67-69, wherein the distinct brain structure is the caudate.

107. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID
NO: 66, wherein the distinct brain structure is the caudate, cerebral cortex, ependyma, or
meninges.

108. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is any one of SEQ ID NOs: 70-72, wherein the distinct brain structure is the cerebellar cortex.

109. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
5 capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 73, wherein the distinct brain structure is the cerebellar cortex or meninges.

110. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 74, wherein the distinct brain structure is the cerebellar cortex, ependyma, or thalamus.

10 111. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 75, wherein the distinct brain structure is the cerebellar cortex, ependyma, globus pallidus, optic nerve, substantia nigra or subthalamic nuclei.

112. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
15 capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 76, wherein the distinct brain structure is the cerebral cortex, ependyma, hippocampus, meninges or substantia nigra.

113. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID
20 NO: 77, wherein the distinct brain structure is the cerebral cortex, ependyma or spinal cord.

114. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 78, wherein the distinct brain structure is the cerebral cortex.

115. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
25 capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 79, wherein the distinct brain structure is the cerebral cortex, hippocampus, optic nerve or subthalamic nuclei.

116. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 80, wherein the distinct brain structure is the ependyma, hippocampus, or meninges.

117. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
5 capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is any one of SEQ ID NOs: 81-84 and 86, wherein the distinct brain structure is the globus pallidus.

118. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 85, wherein the distinct brain structure is the globus pallidus or thalamus.

10 119. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 87, wherein the distinct brain structure is the globus pallidus, optic nerve, substantia nigra or subthalamic nuclei.

120. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
15 capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 88, wherein the distinct brain structure is the hippocampus, optic nerve, spinal cord, subthalamic nuclei, or thalamus.

121. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID
20 NO: 89, wherein the distinct brain structure is the hippocampus.

122. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is any one of SEQ ID NOs: 90-93, wherein the distinct brain structure is the meninges.

123. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
25 capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 94, wherein the distinct brain structure is the optic nerve.

124. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 95, wherein the distinct brain structure is the putamen or spinal cord.

125. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is any one of SEQ ID NOs: 96-100, wherein the distinct brain structure is the putamen.

126. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV
5 capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 101, wherein the distinct brain structure is the spinal cord.

127. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 102, wherein the distinct brain structure is the substantia nigra or subthalamic nuclei.

10 128. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID NO: 103, wherein the distinct brain structure is the substantia nigra.

129. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is SEQ ID
15 NO: 104 or 105, wherein the distinct brain structure is the subthalamic nuclei.

130. The modified AAV capsid protein of claim 1 or 11, wherein the modified AAV capsid protein is a modified AAV2 capsid protein, wherein the targeting peptide is any one of SEQ ID NOs: 106-109, wherein the distinct brain structure is the thalamus.

131. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV
20 capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 110, wherein the distinct brain structure is the brainstem, caudate, cerebellar cortex, cerebral cortex, ependyma, globus pallidus, hippocampus, meninges, optic nerve, putamen, spinal cord, substantia nigra, subthalamic nuclei, or thalamus.

132. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV
25 capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 111, wherein the distinct brain structure is the brainstem, cerebellar cortex, cerebral cortex, ependyma, globus pallidus, hippocampus, meninges, optic nerve, substantia nigra, or subthalamic nuclei.

133. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 112, wherein the distinct brain structure is the brainstem, globus pallidus, putamen, substantia nigra, or thalamus.
- 5 134. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 113, wherein the distinct brain structure is the brainstem, caudate, cerebellar cortex, cerebral cortex, ependyma, hippocampus, meninges, putamen, spinal cord, substantia nigra, subthalamic nuclei, or thalamus.
- 10 135. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 114, wherein the distinct brain structure is the brainstem, cerebral cortex, globus pallidus, meninges, optic nerve, substantia nigra, or thalamus.
136. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV
15 capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 115, wherein the distinct brain structure is the brainstem or caudate.
137. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 116, wherein the distinct brain structure is the brainstem, caudate, cerebral cortex,
20 hippocampus, optic nerve, or putamen.
138. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 117, wherein the distinct brain structure is the brain stem, optic nerve, or substantia nigra.
- 25 139. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 118, wherein the distinct brain structure is the caudate, ependyma, or meninges.
140. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID

NO: 119, wherein the distinct brain structure is the caudate, cerebellar cortex, ependyma, globus pallidus, meninges, spinal cord, or subthalamic nuclei.

141. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID
5 NO: 120, wherein the distinct brain structure is the caudate, ependyma, globus pallidus, meninges, spinal cord, or subthalamic nuclei.

142. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID
NO: 121, wherein the distinct brain structure is the caudate.

10 143. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID
NO: 122, wherein the distinct brain structure is the cerebellar cortex, meninges, spinal cord, or subthalamic nuclei.

144. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV
15 capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID
NO: 123, wherein the distinct brain structure is the cerebellar cortex, hippocampus, putamen, or spinal cord.

145. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID
20 NO: 124, wherein the distinct brain structure is the cerebellar cortex.

146. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID
NO: 125, wherein the distinct brain structure is the cerebellar cortex, cerebral cortex, hippocampus, or thalamus.

25 147. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID
NO: 126, wherein the distinct brain structure is the cerebral cortex.

148. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 127, wherein the distinct brain structure is the cerebral cortex or putamen.

149. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 128, wherein the distinct brain structure is the ependyma or spinal cord.

150. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 129, wherein the distinct brain structure is the globus pallidus, hippocampus, optic nerve, or substantia nigra.

151. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 130, wherein the distinct brain structure is the hippocampus.

152. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 131, wherein the distinct brain structure is the meninges.

153. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 132, wherein the distinct brain structure is the optic nerve or subthalamic nuclei.

154. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 133, wherein the distinct brain structure is the putamen or thalamus.

155. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 134, wherein the distinct brain structure is the putamen or spinal cord.

156. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 135, wherein the distinct brain structure is the subthalamic nuclei.

157. The modified AAV capsid protein of claim 1 or 15, wherein the modified AAV capsid protein is a modified AAV9 capsid protein, wherein the targeting peptide is SEQ ID NO: 136 or 137, wherein the distinct brain structure is the thalamus.

158. A nucleic acid comprising a sequence encoding the modified capsid protein of any one of claims 1-157.

159. A recombinant adeno-associated virus (rAAV) virus comprising the modified capsid protein of any one of claims 1-157.

160. A viral vector comprising a nucleic acid encoding the modified capsid protein of any one of claims 1-157.

161. The viral vector of claim 160, further comprising a nucleic acid sequence encoding a nucleic acid of interest.

162. The viral vector of claim 161, wherein the nucleic acid of interest is a therapeutic agent.

163. The viral vector of claim 162, wherein the therapeutic agent is an enzyme or an RNAi molecule.

164. A cell comprising the viral vector of any one of claims 160-163.

165. The cell of claim 164, wherein the cell is a mammalian cell.

166. The cell of claim 164, wherein the cell is a human cell.

167. The cell of claim 164, wherein the cell is in vitro.

168. The cell of claim 164, wherein the cell is in vivo.

169. A pharmaceutical composition comprising the viral vector of claim 159 and a pharmaceutically acceptable carrier.

170. A method to deliver an agent to a distinct brain structure of a subject, comprising administering the virus of claim 159 to the subject.

171. The method of claim 170, wherein the method is a method to deliver an agent to the brainstem of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 1-9, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 52-60, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110-117.

172. The method of claim 170, wherein the method is a method to deliver an agent to the caudate of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 1, 3, 7, and 10-16, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 59 and 61-69, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110, 113, 115, 116, and 118-121.

173. The method of claim 170, wherein the method is a method to deliver an agent to the cerebellar cortex of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 1, 3, 4, 9, and 17-21, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 56, 58, 60, and 70-75, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110, 111, 113, 119, and 122-125.

174. The method of claim 170, wherein the method is a method to deliver an agent to the cerebral cortex of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 1, 3, 5, 12, and 21-26, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 53, 58, 60, 62, 63, 66, and 76-79, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110, 111, 113, 114, 116, and 125-127.

175. The method of claim 170, wherein the method is a method to deliver an agent to the ependyma of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 2-4, 7, 9, 21, 22, 27, and 28, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 53, 60, 62, 63, 66, 74-77, and 80, or an AAV9 virus that

comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110, 111, 113, 118-120, and 128.

176. The method of claim 170, wherein the method is a method to deliver an agent to the globus pallidus of a subject, comprising administering an AAV1 virus that comprises a
5 modified capsid protein having a targeting peptide selected from SEQ ID NOs: 3, 5, 12, 14, 16, 21, 22, and 29-31, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 60, 75, and 81-87, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110-112, 114, 119, 120, and 129.

10 177. The method of claim 170, wherein the method is a method to deliver an agent to the hippocampus of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 1-4, 7, and 32-34, and 28, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 53, 55, 58, 60, 63, 76, 79, 80, 88, and 89, or an AAV9
15 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110, 111, 113, 116, 123, 125, 129, and 130.

178. The method of claim 170, wherein the method is a method to deliver an agent to the meninges of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 3, 5, 7, 9, 12, 21, and
20 35-37, and 28, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 53, 58, 60, 66, 73, 76, 80, and 90-93, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110, 111, 113, 114, 118, 119, 122, and 131.

179. The method of claim 170, wherein the method is a method to deliver an agent to the
25 optic nerve of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 2, 3, 7, 14-16, 21, 31, and 38, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 53, 54, 57, 58, 60, 75, 79, 87, 88, and 94, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs:
30 110, 111, 114, 115, 117, 129, and 132.

180. The method of claim 170, wherein the method is a method to deliver an agent to the putamen of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 3, 4, 12, 13, 21, 30, and 39-42, an AAV2 virus that comprises a modified capsid protein having a targeting peptide
5 selected from SEQ ID NOs: 55, 59, 60, 61, and 95-100, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110, 112, 113, 116, 123, 127, 133, and 134.

181. The method of claim 170, wherein the method is a method to deliver an agent to the spinal cord of a subject, comprising administering an AAV1 virus that comprises a modified
10 capsid protein having a targeting peptide selected from SEQ ID NOs: 2-4, 7, 9, 21, 32, 33, and 43, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 53, 58-61, 63, 77, 88, 95, and 101, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110, 113, 119, 120, 122, 123, 128, and 134.

182. The method of claim 170, wherein the method is a method to deliver an agent to the substantia nigra of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 2, 3, 9, 44, and 45, and 28, an AAV2 virus that comprises a modified capsid protein having a targeting
15 peptide selected from SEQ ID NOs: 52, 53, 57, 58, 75, 76, 87, 102, and 103, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110-114, 117, and 129.

183. The method of claim 170, wherein the method is a method to deliver an agent to the subthalamic nuclei of a subject, comprising administering an AAV1 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 2-4, 12, 16,
25 30, 46, and 47, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 57, 58, 60, 75, 79, 87, 88, 102, 104, and 105, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110, 111, 113, 119, 120, 122, 132, and 135.

184. The method of claim 170, wherein the method is a method to deliver an agent to the thalamus of a subject, comprising administering an AAV1 virus that comprises a modified
30 capsid protein having a targeting peptide selected from SEQ ID NOs: 1, 2, 8, 12, 21, 28, and

48-51, an AAV2 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 52, 55, 56, 74, 85, 88, and 106-109, or an AAV9 virus that comprises a modified capsid protein having a targeting peptide selected from SEQ ID NOs: 110, 112-114, 125, 133, 136, and 137.

- 5 185. The method of any one of claims 170-184, wherein the agent is an siRNA, shRNA, miRNA, non-coding RNA, lncRNA, therapeutic protein, or CRISPR system.

186. The method of any one of claims 170-184, wherein the administration is to the central nervous system.

187. The method of claim 186, wherein the administration is to a cisterna magna, an intraventricular space, an ependyma, a brain ventricle, a subarachnoid space, a cochlea, and/or an intrathecal space.

188. The method of claim 187, wherein the brain ventricle is the rostral lateral ventricle, and/or the caudal lateral ventricle, and/or the right lateral ventricle, and/or the left lateral ventricle, and/or the right rostral lateral ventricle, and/or the left rostral lateral ventricle, and/or the right caudal lateral ventricle, and/or the left caudal lateral ventricle.

189. The method of any one of claims 170-188, wherein a plurality of viral particles are administered.

190. The method of claim 189, wherein the virus is administered at a dose of about 1×10^6 to about 1×10^{18} vector genomes per kilogram (vg/kg).

- 20 191. The method of claim 189, wherein the virus is administered at a dose from about 1×10^7 - 1×10^{17} , about 1×10^8 - 1×10^{16} , about 1×10^9 - 1×10^{15} , about 1×10^{10} - 1×10^{14} , about 1×10^{10} - 1×10^{13} , about 1×10^{10} - 1×10^{11} , about 1×10^{11} - 1×10^{12} , about 1×10^{12} - 1×10^{13} , or about 1×10^{13} - 1×10^{14} vg/kg of the patient.

192. The method of any one of claims 170-191, wherein the subject is human.

- 25 193. A method of treating a disease in a mammal comprising administering the virus of claim 159 to the mammal.

194. The method of claim 193, wherein the disease is a neurodegenerative disease.

195. The method of claim 194, wherein the neurodegenerative disease is Huntington's disease, ALS, hereditary spastic hemiplegia, primary lateral sclerosis, spinal muscular atrophy, Kennedy's disease, Alzheimer's disease, a polyglutamine repeat disease, or Parkinson's disease.

- 5 196. The method of claim 193, wherein the mammal is human.