

ADENO-ASSOCIATED VIRAL VECTORS FOR TREATMENT OF NIEMANN-PICK DISEASE TYPE C

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/082,899, filed on September 24, 2020, U.S. Provisional Application No. 63/082,425, filed on September 23, 2020, U.S. Provisional Application No. 62/923,253, filed on October 18, 2019, and U.S. Provisional Application No. 62/916,749, filed on October 17, 2019, each of which is incorporated by reference herein in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0001] The contents of the text file submitted electronically herewith are incorporated by reference in their entirety: a computer readable format copy of the Sequence Listing (filename: STRD_021_02WO_SeqList_ST25.txt, date recorded October 14, 2020, file size ~425 kilobytes).

TECHNICAL FIELD

[0002] This application relates to recombinant adeno-associated virus (AAV) vectors. In some embodiments, the recombinant AAV vectors evade neutralizing antibodies without decreased transduction efficiency.

BACKGROUND

[0003] Niemann-Pick Disease, type C1 (NPC1) is a neurodegenerative disorder characterized by cholesterol accumulation in endolysosomal compartments. It is caused by mutations in the gene encoding NPC1, an endolysosomal protein mediating intracellular cholesterol trafficking.

[0004] NPC1 can present in infants, children, or adults. Neonates can present with ascites and severe liver disease from infiltration of the liver and/or respiratory failure from infiltration of the lungs. Other infants, without liver or pulmonary disease, have hypotonia and developmental delay. The classic presentation occurs in mid-to-late childhood with the insidious onset of ataxia, vertical supranuclear gaze palsy (VSGP), and dementia.

Dystonia and seizures are common. Dysarthria and dysphagia eventually become disabling, making oral feeding impossible; death usually occurs in the late second or third decade from aspiration pneumonia. Adults are more likely to present with dementia or psychiatric symptoms.

[0005] 2-hydroxypropyl- β -cyclodextrin (HPBCD) has been shown to reduce the cholesterol and lipid accumulation and prolongs survival in NPC1 animal models. However, there are no therapies for NPC1 approved by the Food and Drug Administration (FDA). Accordingly, there is an urgent need for compositions and methods for treating, curing, and/or preventing NPC1.

BRIEF SUMMARY

[0006] Provided herein are gene therapy compositions and methods for treating, preventing, and/or curing NPC1. More specifically, the disclosure provides Adeno-associated virus (AAV) vectors and nucleic acids (including nucleic acids comprising AAV transfer cassettes) for treating, preventing, and/or curing NPC1.

[0007] In some embodiments, an adeno-associated virus (AAV) vector comprises: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180; and (ii) a nucleic acid encapsidated by the protein capsid; wherein the nucleic acid comprises a transfer cassette; wherein the transfer cassette comprises, from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene that encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR.

[0008] In some embodiments, an adeno-associated virus (AAV) vector comprises: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180, or a sequence comprising about 1 to about 25 amino acid mutations relative to SEQ ID NO: 180; and (ii) a nucleic acid encapsidated by the protein capsid; wherein the nucleic acid comprises a transfer cassette; wherein the transfer cassette comprises from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene which encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR.

[0009] In some embodiments, the transfer cassette comprises an intronic sequence. In some embodiments, the intronic sequence comprises the sequence of SEQ ID NO: 10.

In some embodiments, the intronic sequence may be located between the promoter and the transgene.

[0010] In some embodiments, the 5' ITR comprises the sequence of SEQ ID NO: 3003. In some embodiments, the 3' ITR comprises the sequence of SEQ ID NO: 3004.

[0011] In some embodiments, the promoter is the CBA promoter. In some embodiments, the promoter comprises the sequence of SEQ ID NO: 3005.

[0012] In some embodiments, the NPC1 protein is the human NPC1 protein. In some embodiments, the NPC1 protein comprises the sequence of SEQ ID NO: 3001. In some embodiments, the transgene comprises the sequence of SEQ ID NO: 3002.

[0013] In some embodiments, the polyadenylation signal is the SV40 polyadenylation signal. In some embodiments, the polyadenylation signal comprises the sequence of SEQ ID NO: 3012.

[0014] In some embodiments, the transfer cassette comprises an enhancer.

[0015] In some embodiments, the transfer cassette comprises the sequence of SEQ ID NO: 3014. In some embodiments, the transfer cassette comprises the sequence of any one of SEQ ID NO: 3015-3019.

[0016] Also provided herein are compositions comprising an AAV vector of the disclosure. Also provided herein are cells comprising an AAV vector of the disclosure.

[0017] Also provided here in are methods for treating a subject in need thereof comprising administering to the subject an effective amount of an AAV vector, a nucleic acid, a composition, or a cell of the disclosure. In some embodiments, the subject has Neimann-Pick Disease Type C. In some embodiments, the subject is a human subject

[0018] These and other embodiments are described in more detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIG. 1A-1C. Bubble plots showing analysis of library diversity, directed evolution and enrichment of novel antigenic footprints. Parental (FIG. 1A) and evolved libraries from a first round (FIG. 1B) and a second round (FIG. 1C) of evolution were subjected to high-throughput sequencing using the Illumina MiSeq platform. Following analysis with a custom Perl script, enriched amino acid sequences were plotted. Each bubble represents a distinct capsid protein subunit amino acid sequence with the radius of the bubble

proportional to the number of reads for that variant in the respective library. The y-axis represents the percentage of total reads from the sequencing run. Data are spread along the x-axis for ease of visualization. The percent reduction in unique clones (96.5%) directly demonstrates that numerous “un-fit” sequences were removed after a first and second round of evolution. Dominant isolates were selected for further analysis.

[0020] FIG. 2. Volumetric yield of AAV vectors comprising protein capsids comprising capsid protein subunit variants STRD.101 and STRD.102, as compared to wildtype AAV9. Bars represent mean $\pm$ 95% confidence interval.

[0021] FIG. 3. Infectivity values of AAV-STRD.101 and wildtype AAV9 determined using a standard TCID₅₀ assay. Data are graphed as the natural log of the number of particles required to generate an infectious unit (P:I Ratio). Error bars represent standard deviation.

[0022] FIG. 4A-4D. Transduction of U87 cells (FIG. 4A), N2A cells (FIG. 4B), Sy5Y cells (FIG. 4C), and U2OS cells (FIG. 4D) by recombinant AAV vectors comprising the STRD.101 capsid protein subunit and packaging a luciferase transgene, as compared to wildtype AAV9 vectors similarly packaging a luciferase sequence. Error bars represent standard error.

[0023] FIG. 5. Representative fluorescent microscopy images showing tdTomato expression in coronal vibratome sections 24 hours post-fixation with 4% PFA. Each section is 25 μ m thick. Top panel shows images obtained using a 4X objective lens with native tdTomato fluorescence. The bottom panel shows images obtained using a 10X objective lens with native tdTomato fluorescence.

[0024] FIG. 6. Representative immunohistochemistry images showing tdTomato expression in coronal vibratome sections 24 hours post-fixation with 4% PFA. Each section is 25 μ m thick.

[0025] FIG. 7. Representative fluorescent microscopy images showing TdTomato expression in vibratome liver sections 24 hours post-fixation with 4% PFA. Each section is 25 μ m in thick. Panels show native tdTomato fluorescence with DAPI counterstain.

[0026] FIG. 8. Representative fluorescent microscopy images showing TdTomato expression in vibratome heart sections 24 hours post-fixation with 4% PFA. Each section is 50 μ m in thick. Panels show native tdTomato fluorescence with DAPI counterstain.

[0027] FIG. 9. Biodistribution of recombinant AAVs in non-human primates. Horizontal line shows limit of detection.

[0028] FIG. 10A is a graph that shows lysosomal phenotype, as determined by measuring LysoTracker® accumulation, in wildtype U2OS cells, NPC1-deficient (NPC1^{-/-}) U2OS cells, and NPC1^{-/-} cells transduced with AAV2-hNPC at a Multiplicity of Infection (MOI) of either 5×10^3 or 10×10^3 . Statistical significance determined using one-way ANOVA. Error bars represent standard error of the mean (SEM).

[0029] FIG. 10B is a graph that shows cholesterol accumulation, as determined using filipin staining, in wildtype U2OS cells, NPC1-deficient (NPC1^{-/-}) U2OS cells, and NPC1^{-/-} cells transduced with AAV2-hNPC at a Multiplicity of Infection (MOI) of either 5×10^3 or 10×10^3 . Statistical significance determined using one-way ANOVA. Error bars represent SEM.

[0030] FIG. 11 is a Kaplan-Meier survival curve, showing survival of NPC1^{-/-} mice after retro-orbital injection with saline or with AAV9-hNPC1. All AAV9-hNPC1-injected animals survived through the duration of the experiment, and were sacrificed around 100 days of age for histological analysis.

[0031] FIG. 12 shows behavioral phenotype score at about 10 weeks (70 days) of age in wildtype mice, saline-treated NPC1^{-/-} mice, or NPC1^{-/-} mice injected with AAV9-hNPC1. Statistical significance was determined using an unpaired T-test, and error bars represent SEM.

[0032] FIG. 13 shows number of slips in a balance beam walking test at about 8 weeks (56 days) of age in wildtype mice, saline-treated NPC1^{-/-} mice, or NPC1^{-/-} mice treated with AAV9-hNPC1. Error bars represent standard deviation.

DETAILED DESCRIPTION

[0033] Provided herein are recombinant AAV vectors which evade antibody recognition and/or selectively target tissues of the CNS. These AAV vectors may be useful for treating, preventing, and/or curing diseases such as NPC1.

[0034] AAVs are useful as gene delivery agents, and are powerful tools for human gene therapy. Using AAVs, high-frequency DNA delivery and stable expression may be

achieved in a variety of cells, both *in vivo* and *in vitro*. Unlike some other viral vector systems, AAV does not require active cell division for stable integration in target cells.

[0035] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The terminology used in the detailed description herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0036] All publications, patent applications, patents, GenBank or other accession numbers and other references mentioned herein are incorporated by reference in their entirety for all purposes.

[0037] The designation of amino acid positions in the AAV capsid protein subunits in the disclosure and the appended claims is with respect to VP1 numbering. It will be understood by those skilled in the art that the modifications described herein if inserted into the AAV cap gene may result in modifications in the VP1, VP2 and/or VP3 regions. Alternatively, the VP1, VP2, and/or VP3 can be expressed independently to achieve modification in only one or two of these regions (VP1, VP2, VP3, VP1 + VP2, VP1 + VP3, or VP2 +VP3).

Definitions

[0038] The following terms are used in the description herein and the appended claims.

[0039] The singular forms “a,” “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0040] Furthermore, the term “about” as used herein when referring to a measurable value such as an amount of the length of a polynucleotide or polypeptide sequence, dose, time, temperature, and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

[0041] Also as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[0042] Unless the context indicates otherwise, it is specifically intended that the various features described herein can be used in any combination. Moreover, in some embodiments, any feature or combination of features set forth herein can be excluded or

omitted. To illustrate further, if, for example, the specification indicates that a particular amino acid can be selected from A, G, I, L and/or V, this language also indicates that the amino acid can be selected from any subset of these amino acid(s) for example A, G, I or L; A, G, I or V; A or G; only L; etc., as if each such subcombination is expressly set forth herein. Moreover, such language also indicates that one or more of the specified amino acids can be disclaimed. For example, in some embodiments the amino acid is not A, G or I; is not A; is not G or V; etc., as if each such possible disclaimer is expressly set forth herein.

[0043] As used herein, the terms “reduce,” “reduces,” “reduction” and similar terms mean a decrease of at least about 10%, about 15%, about 20%, about 25%, about 35%, about 50%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97% or more.

[0044] As used herein, the terms “increase,” “improve,” “enhance,” “enhances,” “enhancement” and similar terms indicate an increase of at least about 10%, about 15%, about 20%, about 25%, about 50%, about 75%, about 100%, about 150%, about 200%, about 300%, about 400%, about 500% or more.

[0045] The term “parvovirus” as used herein encompasses the family Parvoviridae, including autonomously replicating parvoviruses and dependoviruses. The autonomous parvoviruses include members of the genera *Protoparvovirus*, *Erythroparvovirus*, *Bocaparvovirus*, and *Densovirus* subfamily. Exemplary autonomous parvoviruses include, but are not limited to, minute virus of mouse, bovine parvovirus, canine parvovirus, chicken parvovirus, feline panleukopenia virus, feline parvovirus, goose parvovirus, H1 parvovirus, muscovy duck parvovirus, B19 virus, and any other autonomous parvovirus now known or later discovered. Other autonomous parvoviruses are known to those skilled in the art. See, e.g., BERNARD N. FIELDS et al, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers; Cotmore et al. Archives of Virology DOI 10.1007/s00705-013-1914-I).

[0046] As used herein, the term “adeno-associated virus” (AAV), includes but is not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, AAV type rh32.33, AAV type rh8, AAV type rh10, AAV type rh74, AAV type hu.68, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, snake

AAV, bearded dragon AAV, AAV2i8, AAV2g9, AAV-LK03, AAV7m8, AAV Anc80, AAV PHP.B, and any other AAV now known or later discovered. See, e.g., BERNARD N. FIELDS et al., *VIROLOGY*, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). A number of AAV serotypes and clades have been identified (see, e.g., Gao et al, (2004) *J. Virology* 78:6381-6388; Moris et al, (2004) *Virology* 33-:375-383; and Table 2). Exemplary AAV capsid protein subunit sequences for AAV1-9, AAVrh.10 and AAV11 are provided in SEQ ID NO: 1-11.

[0047] As used herein, the term “chimeric AAV” refers to an AAV comprising a protein capsid comprising capsid protein subunits with regions, domains, individual amino acids that are derived from two or more different serotypes of AAV. In some embodiments, a chimeric AAV comprises a capsid protein subunit comprised of a first region that is derived from a first AAV serotype and a second region that is derived from a second AAV serotype. In some embodiments, a chimeric AAV comprises a capsid protein subunit comprised of a first region that is derived from a first AAV serotype, a second region that is derived from a second AAV serotype, and a third region that is derived from a third AAV serotype. In some embodiments, the chimeric AAV may comprise regions, domains, individual amino acids derived from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and/or AAV12. For example, the chimeric AAV may include regions, domains, and/or individual amino acids from a first and a second AAV serotype as shown below (Table 1), wherein AAVX+Y indicates a chimeric AAV including sequences derived from AAVX and AAVY:

TABLE 1: Chimeric AAVs

Second AAV Serotype													
	AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9	AAV10	AAV11	AAV12	
First AAV Serotype	AAV1	x	AAV1+2	AAV1+3	AAV1+4	AAV1+5	AAV1+6	AAV1+7	AAV1+8	AAV1+9	AAV1+10	AAV1+11	AAV1+12
	AAV2	AAV2+1	x	AAV2+3	AAV2+4	AAV2+5	AAV2+6	AAV2+7	AAV2+8	AAV2+9	AAV2+10	AAV2+11	AAV2+12
	AAV3	AAV3+1	AAV3+2	x	AAV3+4	AAV3+5	AAV3+6	AAV3+7	AAV3+8	AAV3+9	AAV3+10	AAV3+11	AAV3+12
	AAV4	AAV4+1	AAV4+2	AAV4+3	x	AAV4+5	AAV4+6	AAV4+7	AAV4+8	AAV4+9	AAV4+10	AAV4+11	AAV4+12
	AAV5	AAV5+1	AAV5+2	AAV5+3	AAV5+4	x	AAV5+6	AAV5+7	AAV5+8	AAV5+9	AAV5+10	AAV5+11	AAV5+12
	AAV6	AAV6+1	AAV6+2	AAV6+3	AAV6+4	AAV6+5	x	AAV6+7	AAV6+8	AAV6+9	AAV6+10	AAV6+11	AAV6+12
	AAV7	AAV7+1	AAV7+2	AAV7+3	AAV7+4	AAV7+5	AAV7+6	x	AAV7+8	AAV7+9	AAV7+10	AAV7+11	AAV7+12
	AAV8	AAV8+1	AAV8+2	AAV8+3	AAV8+4	AAV8+5	AAV8+6	AAV8+7	x	AAV8+9	AAV8+10	AAV8+11	AAV8+12
	AAV9	AAV9+1	AAV9+2	AAV9+3	AAV9+4	AAV9+5	AAV9+6	AAV9+7	AAV9+8	x	AAV9+10	AAV9+11	AAV9+12
	AAV10	AAV10+1	AAV10+2	AAV10+3	AAV10+4	AAV10+5	AAV10+6	AAV10+7	AAV10+8	AAV10+9	x	AAV10+11	AAV10+12
	AAV11	AAV11+1	AAV11+2	AAV11+3	AAV11+4	AAV11+5	AAV11+6	AAV11+7	AAV11+8	AAV11+9	AAV11+10	x	AAV11+12
	AAV12	AAV12+1	AAV12+2	AAV12+3	AAV12+4	AAV12+5	AAV12+6	AAV12+7	AAV12+8	AAV12+9	AAV12+10	AAV12+11	x

[0048] By including individual amino acids or regions from multiple AAV serotypes in one capsid protein subunit, capsid protein subunits that have multiple desired properties that are separately derived from the multiple AAV serotypes may be obtained.

[0049] The genomic sequences of various serotypes of AAV and the autonomous parvoviruses, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid protein subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC_002077, NC_001401, NC_001729, NC_001863, NC_001829, NC_001862, NC_000883, NC_001701, NC_001510, NC_006152, NC_006261, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226, AY028223, NC_001358, NC_001540, AF513851, AF513852, AY530579; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. See also, e.g., Srivistava et al., (1983) J. Virology 45:555; Chiorini et al., (1998) J Virology 71:6823; Chiorini et al., (1999) J. Virology 73: 1309; Bantel-Schaal et al., (1999) J Virology 73:939; Xiao et al., (1999) J Virology 73:3994; Muramatsu et al., (1996) Virology 221:208; Shade et al., (1986) J. Virol. 58:921; Gao et al., (2002) Proc. Nat. Acad. Sci. USA 99:11854; Moris et al., (2004) Virology 33:375-383; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Patent No. 6,156,303; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. See also Table 2. The protein capsid structures of autonomous parvoviruses and AAV are described in more detail in BERNARD N. FIELDS et al., VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers). See also, description of the crystal structure of AAV2 (Xie et al., (2002) Proc. Nat. Acad. Sci. 99: 10405-10), AAV9 (DiMattia et al., (2012) J. Virol. 86:6947-6958), AAV8 (Nam et al., (2007) J. Virol. 81: 12260-12271), AAV6 (Ng et al., (2010) J. Virol. 84:12945-12957), AAV5 (Govindasamy et al. (2013) J. Virol. 87, 11187-11199), AAV4 (Govindasamy et al. (2006) J. Virol. 80:11556-11570), AAV3B (Lerch et al., (2010) Virology 403:26-36), BPV (Kailasan et al., (2015) J. Virol. 89:2603-2614) and CPV (Xie et al., (1996) J. Mol. Biol. 6:497-520 and Tsao et al., (1991) Science 251:1456-64).

TABLE 2: AAV Serotypes and Clades

	GenBank Accession Number		GenBank Accession Number		GenBank Accession Number
Complete Genomes		Clade C		Rh57	AY530569
Adeno-associated virus 1	NC_002077, AF063497	Hu9	AY530629	Rh50	AY530563
Adeno-associated virus 2	NC_001401	Hu10	AY530576	Rh49	AY530562
Adeno-associated virus 3	NC_001729	Hu11	AY530577	Hu39	AY530601
Adeno-associated virus 3B	NC_001863	Hu53	AY530615	Rh58	AY530570
Adeno-associated virus 4	NC_001829	Hu55	AY530617	Rh61	AY530572
Adeno-associated virus 5	Y18065, AF085716	Hu54	AY530616	Rh52	AY530565
Adeno-associated virus 6	NC_001862	Hu7	AY530628	Rh53	AY530566
Avian AAV ATCC VR-865	AY186198, AY629583, NC_004828	Hu18	AY530583	Rh51	AY530564
Avian AAV strain DA-1	NC_006263, AY629583	Hu15	AY530580	Rh64	AY530574
Bovine AAV	NC_005889, AY388617, AAR26465	Hu16	AY530581	Rh43	AY530560
AAV11	AAT46339, AY631966	Hu25	AY530591	AAV8	AF513852
AAV12	ABI16639, DQ813647	Hu60	AY530622	Rh8	AY242997
Clade A		Ch5	AY243021	Rh1	AY530556
AAV1	NC_002077, AF063497	Hu3	AY530595	Clade F	
AAV6	NC_001862	Hu1	AY530575	Hu14 (AAV9)	AY530579
Hu.48	AY530611	Hu4	AY530602	Hu31	AY530596
Hu 43	AY530606	Hu2	AY530585	Hu32	AY530597

Hu 44	AY530607	Hu61	AY530623	HSC1	MI332400.1
Hu 46	AY530609	Clade D		HSC2	MI332401.1
Clade B		Rh62	AY530573	HSC3	MI332402.1
Hu. 19	AY530584	Rh48	AY530561	HSC4	MI332403.1
Hu. 20	AY530586	Rh54	AY530567	HSC5	MI332405.1
Hu 23	AY530589	Rh55	AY530568	HSC6	MI332404.1
Hu22	AY530588	Cy2	AY243020	HSC7	MI332407.1
Hu24	AY530590	AAV7	AF513851	HSC8	MI332408.1
Hu21	AY530587	Rh35	AY243000	HSC9	MI332409.1
Hu27	AY530592	Rh37	AY242998	HSC11	MI332406.1
Hu28	AY530593	Rh36	AY242999	HSC12	MI332410.1
Hu 29	AY530594	Cy6	AY243016	HSC13	MI332411.1
Hu63	AY530624	Cy4	AY243018	HSC14	MI332412.1
Hu64	AY530625	Cy3	AY243019	HSC15	MI332413.1
Hu13	AY530578	Cy5	AY243017	HSC16	MI332414.1
Hu56	AY530618	Rh13	AY243013	HSC17	MI332415.1
Hu57	AY530619	Clade E		Hu68	
Hu49	AY530612	Rh38	AY530558	Clonal Isolate	
Hu58	AY530620	Hu66	AY530626	AAV5	Y18065, AF085716
Hu34	AY530598	Hu42	AY530605	AAV 3	NC_001729
Hu35	AY530599	Hu67	AY530627	AAV 3B	NC_001863
AAV2	NC_001401	Hu40	AY530603	AAV4	NC_001829
Hu45	AY530608	Hu41	AY530604	Rh34	AY243001
Hu47	AY530610	Hu37	AY530600	Rh33	AY243002
Hu51	AY530613	Rh40	AY530559	Rh32	AY243003
Hu52	AY530614	Rh2	AY243007	Others	
Hu T41	AY695378	Bb1	AY243023	Rh74	
Hu S17	AY695376	Bb2	AY243022	Bearded Dragon AAV	
Hu T88	AY695375	Rh10	AY243015	Snake AAV	NC_006148.1
Hu T71	AY695374	Hu17	AY530582		

Hu T70	AY695373	Hu6	AY530621		
Hu T40	AY695372	Rh25	AY530557		
Hu T32	AY695371	Pi2	AY530554		
Hu T17	AY695370	Pi1	AY530553		
Hu LG15	AY695377	Pi3	AY530555		

[0050] Recombinant AAV (rAAV) vectors can be produced in culture using viral production cell lines. The terms “viral production cell”, “viral production cell line,” or “viral producer cell” refer to cells used to produce viral vectors. HEK293 and 239T cells are common viral production cell lines. Table 3, below, lists exemplary viral production cell lines for various viral vectors. Production of rAAVs typically requires the presence of three elements in the cells: 1) a transgene flanked by AAV inverted terminal repeat (ITR) sequences, 2) AAV rep and cap genes, and 3) helper virus protein sequences. These three elements may be provided on one or more plasmids, and transfected or transduced into the cells.

Table 3: Exemplary viral production cell lines

Virus Vector	Exemplary Viral Production Cell Line(s)
Adenovirus	HEK293, 911, pTG6559, PER.C6, GH329, N52.E6, HeLa-E1, UR, VLI-293
Adeno-Associated Virus (AAV)	HEK293, Sf9
Retrovirus	HEK293
Lentivirus	293T

[0051] “HEK293” refers to a cell line originally derived from human embryonic kidney cells grown in tissue culture. The HEK293 cell line grows readily in culture, and is commonly used for viral production. As used herein, “HEK293” may also refer to one or more variant HEK293 cell lines, i.e., cell lines derived from the original HEK293 cell line that additionally comprise one or more genetic alterations. Many variant HEK293 lines have been developed and optimized for one or more particular applications. For example, the 293T cell line contains the SV40 large T-antigen that allows for episomal replication of

transfected plasmids containing the SV40 origin of replication, leading to increased expression of desired gene products.

[0052] "Sf9" refers to an insect cell line that is a clonal isolate derived from the parental *Spodoptera frugiperda* cell line IPLB-Sf-21-AE. Sf9 cells can be grown in the absence of serum and can be cultured attached or in suspension.

[0053] A "transfection reagent" means a composition that enhances the transfer of nucleic acid into cells. Some transfection reagents commonly used in the art include one or more lipids that bind to nucleic acids and to the cell surface (e.g., Lipofectamine™).

[0054] As used herein, the term "multiplicity of infection" or "MOI" refers to number of virions contacted with a cell. For example, cultured cells may be contacted with AAVs at an MOI in the range of 1×10^2 to 1×10^5 virions per cell.

[0055] The term "self-complementary AAV" or "scAAV" refers to a recombinant AAV vector comprising a nucleic acid (i.e., a DNA) which forms a dimeric inverted repeat molecule that spontaneously anneals, resulting in earlier and more robust transgene expression compared with conventional single-strand (ss) AAV genomes. See, e.g., McCarty, D.M., et al., *Gene Therapy* 8, 1248-1254 (2001). Unlike conventional ssAAV, scAAV can bypass second-strand synthesis, the rate-limiting step for gene expression. Moreover, double-stranded scAAV is less prone to DNA degradation after viral transduction, thereby increasing the number of copies of stable episomes. Notably, scAAV can typically only hold a genome that is about 2.4kb, half the size of a conventional AAV vector. In some embodiments, the AAV vectors described herein are self-complementary AAVs.

[0056] As used herein, the term "peptide" refers to a short amino acid sequence. The term peptide may be used to refer to portion or region of an AAV capsid protein subunit amino acid sequence. The peptide may be a peptide that naturally occurs in a native AAV capsid protein, or a peptide that does not naturally occur in a native AAV capsid protein. Naturally occurring AAV peptides in an AAV capsid protein may be substituted by non-naturally occurring peptides. For example, a non-naturally occurring peptide may be substituted into an AAV capsid protein to provide a modified capsid protein, such that the naturally-occurring peptide is replaced by the non-naturally occurring peptide.

[0057] The term "tropism" as used herein refers to preferential entry of the virus into certain cells or tissues, optionally followed by expression (e.g., transcription and,

optionally, translation) of a sequence(s) carried by the viral genome in the cell, e.g., for a recombinant virus, expression of a transgene of interest.

[0058] As used here, "systemic tropism" and "systemic transduction" (and equivalent terms) indicate that the virus vector or a virus-like particle as described herein exhibits tropism for or transduces, respectively, tissues throughout the body (e.g., brain, lung, skeletal muscle, heart, liver, kidney and/or pancreas). In some embodiments, systemic transduction of muscle tissues (e.g., skeletal muscle, diaphragm and cardiac muscle) is achieved. In some embodiments, systemic transduction of skeletal muscle tissues is achieved. For example, in some embodiments, essentially all skeletal muscles throughout the body are transduced (although the efficiency of transduction may vary by muscle type). In some embodiments, systemic transduction of limb muscles, cardiac muscle and diaphragm muscle is achieved. Optionally, the virus vector or virus-like particle is administered via a systemic route (e.g., systemic route such as intravenously, intra-articularly or intra-lymphatically).

[0059] Alternatively, in some embodiments, the virus vector or virus-like particle is delivered locally (e.g., to the footpad, intramuscularly, intradermally, subcutaneously, topically). In some embodiments, the virus vector or virus-like particle is delivered locally to a tissue of the central nervous system (CNS), such as the brain or the spinal cord. In some embodiments, the virus vector or virus-like particle is administered by intrathecal, intracerebral or intracerebroventricular injection.

[0060] Unless indicated otherwise, "efficient transduction" or "efficient tropism," or similar terms, can be determined by reference to a suitable control (e.g., at least about 50%, about 60%, about 70%, about 80%, about 85%, about 90%, about 95% or more of the transduction or tropism, respectively, of the control). In some embodiments, the virus vector (e.g., the AVV vector) efficiently transduces or has efficient tropism for skeletal muscle, cardiac muscle, diaphragm muscle, pancreas (including β -islet cells), spleen, the gastrointestinal tract (e.g., epithelium and/or smooth muscle), cells of the central nervous system, lung, joint cells, and/or kidney. Suitable controls will depend on a variety of factors including the desired tropism profile. For example, AAV8 and AAV9 are highly efficient in transducing skeletal muscle, cardiac muscle and diaphragm muscle, but have the disadvantage of also transducing liver with high efficiency. Thus, viral vectors can be

identified that demonstrate the efficient transduction of skeletal, cardiac and/or diaphragm muscle of AAV8 or AAV9, but with a much lower transduction efficiency for liver. Further, because the tropism profile of interest may reflect tropism toward multiple target tissues, it will be appreciated that a suitable virus vector may represent some tradeoffs. To illustrate, a virus vector may be less efficient than AAV8 or AAV9 in transducing skeletal muscle, cardiac muscle and/or diaphragm muscle, but because of low level transduction of liver, may nonetheless be very desirable.

[0061] Similarly, it can be determined if a virus “does not efficiently transduce” or “does not have efficient tropism” for a target tissue, or similar terms, by reference to a suitable control. In some embodiments, the virus vector does not efficiently transduce (*i.e.*, does not have efficient tropism) for liver, kidney, gonads and/or germ cells. In some embodiments, undesirable transduction of tissue(s) (*e.g.*, liver) is about 20% or less, about 10% or less, about 5% or less, about 1% or less, about 0.1% or less of the level of transduction of the desired target tissue(s) (*e.g.*, skeletal muscle, diaphragm muscle, cardiac muscle and/or cells of the central nervous system).

[0062] As used herein in connection with an AAV vector (or a protein capsid, capsid protein subunit, or peptide thereof), the terms “selectively binds,” “selective binding” and similar terms, refer to binding of the AAV vector (or a protein capsid, capsid protein subunit, or peptide thereof) to a target in a manner dependent upon the presence of a particular molecular structure. In some embodiments, selective binding refers to binding of the AAV predominantly to a specific target, without substantial or significant binding to other targets. In some embodiments, an AAV vector (or a protein capsid, capsid protein subunit, or peptide thereof) specifically binds to a receptor in a cell or tissue of interest, but does not exhibit substantial or significant binding to other receptors.

[0063] A “polynucleotide” is a sequence of nucleotide bases, and may be RNA, DNA or DNA-RNA hybrid sequences (including both naturally occurring and non-naturally occurring nucleotide). In some embodiments, a polynucleotide is either a single or double stranded DNA sequence.

[0064] As used herein, an “isolated” polynucleotide (*e.g.*, an “isolated DNA” or an “isolated RNA”) means a polynucleotide at least partially separated from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral

structural components or other polypeptides or nucleic acids commonly found associated with the polynucleotide. In some embodiments an “isolated” nucleotide is enriched by at least about 10-fold, about 100-fold, about 1000-fold, about 10,000-fold or more as compared with the starting material.

[0065] Likewise, an “isolated” polypeptide means a polypeptide that is at least partially separated from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide. In some embodiments an “isolated” polypeptide is enriched by at least about 10-fold, about 100-fold, about 1000-fold, about 10,000-fold or more as compared with the starting material.

[0066] As used herein, by “isolate” or “purify” (or grammatical equivalents) a virus vector, it is meant that the virus vector is at least partially separated from at least some of the other components in the starting material. In some embodiments an “isolated” or “purified” virus vector is enriched by at least about 10-fold, about 100-fold, about 1000-fold, about 10,000-fold or more as compared with the starting material.

[0067] A “therapeutic” polypeptide or protein is one that can alleviate, reduce, prevent, delay and/or stabilize symptoms that result from an absence or defect in a protein in a cell or subject and/or is a polypeptide that otherwise confers a benefit to a subject, e.g., anti-cancer effects or improvement in transplant survivability.

[0068] By the terms “treat,” “treating” or “treatment of” (and grammatical variations thereof) it is meant that the severity of the subject's condition is reduced, at least partially improved or stabilized and/or that some alleviation, mitigation, decrease or stabilization in at least one clinical symptom is achieved and/or there is a delay in the progression of the disease or disorder.

[0069] The terms “prevent,” “preventing” and “prevention” (and grammatical variations thereof) refer to prevention and/or delay of the onset of a disease, disorder and/or a clinical symptom(s) in a subject and/or a reduction in the severity of the onset of the disease, disorder and/or clinical symptom(s) relative to what would occur in the absence of the compositions and/or methods described herein. The prevention can be complete, e.g., the total absence of the disease, disorder and/or clinical symptom(s). The prevention can also be partial, such that the occurrence of the disease, disorder and/or clinical

symptom(s) in the subject and/or the severity of onset is less than what would occur in the absence of the compositions and/or methods described herein.

[0070] As used herein, an “effective amount” is the amount of an AAV vector, nucleic acid, or other agent provided herein that is effective to treat or prevent a disease or disorder in a subject or to ameliorate a sign or symptom thereof. The “effective amount” may vary depending, for example, on the disease and/or symptoms of the disease, severity of the disease and/or symptoms of the disease or disorder, the age, weight, and/or health of the patient to be treated, and the judgment of the prescribing physician. An appropriate amount in any given instance may be ascertained by those skilled in the art or may be capable of determination by routine experimentation.

[0071] As used herein, the terms “virus vector,” “vector” refer to a virus (e.g., AAV) particle that functions as a nucleic acid delivery vehicle, and which comprises a vector genome (e.g., a nucleic acid comprising a transgene) packaged within a virion or virus-like particle.

[0072] An “adeno-associated virus vector” or “AAV vector” typically comprises a protein capsid, and a nucleic acid (e.g., a nucleic acid comprising a transgene) encapsidated by the protein capsid. The “protein capsid” is a near-spherical protein shell that comprises individual “capsid protein subunits” (e.g., about 60 capsid protein subunits) associated and arranged with T=1 icosahedral symmetry. The protein capsids of the AAV vectors described herein comprise a plurality of capsid protein subunits. When an AAV vector is described as comprising an AAV capsid protein subunit, it will be understood that the AAV vector comprises a protein capsid, wherein the protein capsid comprises one or more AAV capsid protein subunits. As used herein, the term “capsid protein” is sometimes used to refer to a capsid protein subunit. The term “viral-like particle” or “virus-like particle” refers to a protein capsid that does not comprise any vector genome or nucleic acid comprising a transfer cassette or transgene.

[0073] In some embodiments, an AAV vector may comprise a nucleic acid comprising a “transfer cassette,” i.e., a nucleic acid comprising one or more sequences which can be delivered by the AAV to a cell. In some embodiments, the nucleic acid is self-complementary (i.e., double stranded). In some embodiments, the nucleic acid is not self-complementary (i.e., single stranded).

[0074] A “rAAV vector genome” or “rAAV genome” is an AAV genome (*i.e.*, vDNA) that comprises one or more heterologous nucleic acid sequences. rAAV vectors generally require only the inverted terminal repeat(s) (ITR(s)) in *cis* to promote nucleic acid replication. All other viral sequences are dispensable and may be supplied in *trans* (Muzyczka, (1992) *Curr. Topics Microbiol. Immunol.* 158:97). Typically, the rAAV vector genome will only retain the one or two ITR sequences so as to maximize the size of the transgene that can be efficiently packaged by the AAV vector. The structural and non-structural protein coding sequences may be provided in *trans* (*e.g.*, from a plasmid, or by stably integrating the sequences into a packaging cell). In some embodiments, the rAAV vector genome comprises at least one ITR sequence (*e.g.*, AAV ITR sequence), optionally two ITRs (*e.g.*, two AAV ITRs), which typically will be at the 5' and 3' ends of the vector genome (*i.e.*, the 5' ITR and the 3' ITR) and flank the heterologous nucleic acid, but need not be contiguous thereto.

[0075] The virus vectors described herein can further be “targeted” virus vectors (*e.g.*, having a directed tropism) and/or “hybrid” virus vectors (*i.e.*, in which the viral ITRs and viral protein capsid are from different viruses) as described in international patent publication WO00/28004 and Chao et al, (2000) *Molecular Therapy* 2:619. In some embodiments, the virus vectors are targeted to a cell and/or tissue of the CNS.

[0076] The virus vectors described herein can further be duplexed virus particles as described in international patent publication WO 01/92551 (the disclosure of which is incorporated herein by reference in its entirety). Thus, in some embodiments, double stranded (duplex) genomes can be packaged into the virus protein capsids described herein. Further, the protein capsid, protein capsid subunits, or genomic elements can contain other modifications, including insertions, deletions and/or substitutions.

[0077] As used herein, the term “amino acid” encompasses any naturally occurring amino acid, modified forms thereof, and synthetic amino acids. Naturally occurring, levorotatory (L-) amino acids are shown in Table 4.

TABLE 4: Amino acid residues and abbreviations.

Amino Acid Residue	Abbreviation	
	Three-Letter Code	One-Letter Code

Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid (Aspartate)	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid (Glutamate)	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0078] Alternatively, the amino acid can be a modified amino acid residue (nonlimiting examples are shown in Table 5) and/or can be an amino acid that is modified by post-translation modification (e.g., acetylation, amidation, formylation, hydroxylation, methylation, phosphorylation or sulfatation). Methods of chemically modifying amino acids are known in the art (see, e.g., Greg T. Hermanson, *Bioconjugate Techniques*, 1st edition, Academic Press, 1996).

TABLE 5: Modified Amino Acid Residues

Modified Amino Acid Residue	Abbreviation
Amino Acid Residue Derivatives	
2-Aminoadipic acid	Aad
3-Aminoadipic acid	bAad
beta-Alanine, beta-Aminopropionic acid	bAla
2-Aminobutyric acid	Abu
4-Aminobutyric acid, Piperidinic acid	4Abu
6-Aminocaproic acid	Acp
2-Aminoheptanoic acid	Ahe

2-Aminoisobutyric acid	Aib
3-Aminoisobutyric acid	bAib
2-Aminopimelic acid	Apm
t-butylalanine	t-BuA
Citrulline	Cit
Cyclohexylalanine	Cha
2,4-Diaminobutyric acid	Dbu
Desmosine	Des
2,21-Diaminopimelic acid	Dpm
2,3-Diaminopropionic acid	Dpr
N-Ethylglycine	EtGly
N-Ethylasparagine	EtAsn
Homoarginine	hArg
Homocysteine	hCys
Homoserine	hSer
Hydroxylysine	Hyl
Allo-Hydroxylysine	aHyl
3-Hydroxyproline	3Hyp
4-Hydroxyproline	4Hyp
Isodesmosine	Ide
allo-Isoleucine	alle
Methionine sulfoxide	MSO
N-Methylglycine, sarcosine	MeGly
N-Methyl isoleucine	Melle
6-N-Methyllysine	MeLys
N-Methylvaline	MeVal
2-Naphthylalanine	2-Nal
Norvaline	Nva
Norleucine	Nle
Ornithine	Orn
4-Chlorophenylalanine	Phe(4-C1)
2-Fluorophenylalanine	Phe(2-F)
3-Fluorophenylalanine	Phe(3-F)
4-Fluorophenylalanine	Phe(4-F)
Phenylglycine	Phg
Beta-2-thienylalanine	Thi

[0079] Further, the non-naturally occurring amino acid can be an "unnatural" amino acid (as described by Wang et al., Annu Rev Biophys Biomol Struct. 35:225-49 (2006)). These unnatural amino acids can advantageously be used to chemically link molecules of interest to the AAV protein capsid or capsid protein subunit.

Modified AAV Protein Capsid Subunits, Protein Capsids, and AAV Vectors Comprising the Same

AAV Vectors

[0080] Additionally provided herein are adeno-associated virus (AAV) vectors comprising (i) a protein capsid comprising recombinant capsid protein subunits and (ii) a transfer cassette encapsidated by the protein capsid. In some embodiments, the recombinant capsid protein subunits (including VP1, VP2 and/or VP3 regions) may comprise a peptide in their amino acid sequence that does not occur in any native AAV capsid protein subunit sequence. Capsid protein subunits comprising the peptides described herein can confer one or more desirable properties to virus vectors including, without limitation, the ability to evade neutralizing antibodies. Thus, AAV vectors described herein address the limitations associated with conventional AAV vectors.

[0081] Accordingly, in some embodiments, the present disclosure provides adeno-associated virus (AAV) vectors comprising (i) one or more recombinant capsid proteins and (ii) a transfer cassette encapsidated by the protein capsid; wherein the capsid protein comprises a peptide having the sequence of any one of SEQ ID NO: 12-20. In some embodiments, the transfer cassette comprises 5' and 3' AAV inverted terminal repeats. In some embodiments, the transfer cassette comprises a transgene (e.g., a NPC1 transgene). In some embodiments, the transfer cassette is double stranded. In some embodiments, the transfer cassette is single stranded. In some embodiments, the transgene encodes a therapeutic protein or RNA. In some embodiments, the recombinant capsid protein has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the native sequence of the AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.8, AAVrh.10, AAVrh32.33, AAVrh74, bovine AAV or avian AAV capsid protein. In some embodiments, the recombinant capsid protein has at least 90% sequence identity to the native sequence of the AAV9 capsid protein.

[0082] In some embodiments, the peptide is located at the amino acid positions corresponding to amino acids 451-458 of the native AAV9 capsid protein subunit, or the equivalent amino acid residues in AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAVrh.8, AAVrh.10, AAVrh32.33, AAVrh74, bovine AAV or avian AAV, and the peptide is selected from any one of SEQ ID NO: 12-18. In some embodiments, the peptide is located at the amino acid positions corresponding to amino acids 587-594 of the native AAV9 capsid protein subunit, or the equivalent amino acid

residues in AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAVrh.8, AAVrh.10, AAVrh32.33, AAVrh74, bovine AAV or avian AAV, and the peptide is selected from SEQ ID NO: 19 or 20.

[0083] In some embodiments, a recombinant capsid protein subunit comprises a) a first peptide having a sequence of any one of SEQ ID NO: 12-18; and b) a second peptide having a sequence of any one of SEQ ID NO: 19-20. In some embodiments, the first peptide is at amino acid positions 451-458, and the second peptide is at amino acids 587-594, wherein the amino acid numbering is based on the native AAV9 capsid protein subunit, or the equivalent amino acid residues in AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAVrh.8, AAVrh.10, AAVrh32.33, AAVrh74, bovine AAV or avian AAV.

[0084] In some embodiments, the peptide inhibits binding of at least one antibody to the protein capsid or a capsid protein subunit thereof. In some embodiments, the peptide inhibits neutralization of infectivity of the AAV vector by the antibody.

[0085] In some embodiments, the peptide selectively binds to a receptor expressed on the surface of a cell in the central nervous system (CNS). In some embodiments, the cell is in the premotor cortex, the thalamus, the cerebellar cortex, the dentate nucleus, the spinal cord, or the dorsal root ganglion. In some embodiments, the peptide selectively binds to a receptor expressed on the surface of a cell in the heart.

[0086] In some embodiments, an adeno-associated virus (AAV) vector comprises (i) a protein capsid comprising a mutant AAV9 capsid protein subunit and (ii) a transfer cassette encapsidated by the protein capsid, wherein the capsid protein subunit comprises a peptide having the sequence X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 (SEQ ID NO: 158) at amino acids 451-458 of the native AAV9 capsid protein subunit sequence, wherein the peptide does not occur in the native AAV9 capsid protein subunit sequence. In some embodiments, X^1 is not I, X^2 is not N, X^3 is not G, X^4 is not S, X^5 is not G, X^6 is not Q, X^7 is not N, and /or X^8 is not Q. In some embodiments, X^1 is S, F, Q, G, K, or R. In some embodiments, X^2 is C, G, R, D, T, or Q. In some embodiments, X^3 is Q, V, G, Y, R, F, or D. In some embodiments, X^4 is P, Q, A, or R. In some embodiments, X^5 is T, N, A, P, or I. In some embodiments, X^6 is V, Q, A, or I. In some embodiments, X^7 is M, P, R, Q, or N. In some embodiments, X^8 is N, L, F, E, H, or A. In some embodiments, X^1 is S, X^2 is

C, X³ is Q, X⁴ is P, X⁵ is T, X⁶ is V, X⁷ is M, and X⁸ is N. In some embodiments, X¹ is F, X² is G, X³ is V, X⁴ is P, X⁵ is N, X⁶ is Q, X⁷ is P, and X⁸ is L. In some embodiments, X¹ is Q, X² is R, X³ is G, X⁴ is Q, X⁵ is A, X⁶ is A, X⁷ is P, and X⁸ is F. In some embodiments, X¹ is G, X² is D, X³ is Y, X⁴ is A, X⁵ is P, X⁶ is I, X⁷ is R, and X⁸ is E. In some embodiments, X¹ is K, X² is T, X³ is R, X⁴ is R, X⁵ is I, X⁶ is V, X⁷ is Q, and X⁸ is H. In some embodiments, X¹ is F, X² is G, X³ is F, X⁴ is P, X⁵ is N, X⁶ is Q, X⁷ is P, and X⁸ is L. In some embodiments, X¹ is R, X² is Q, X³ is D, X⁴ is Q, X⁵ is P, X⁶ is I, X⁷ is N, and X⁸ is A.

[0087] In some embodiments, an adeno-associated virus (AAV) vector comprises (i) a protein capsid comprising a mutant AAV9 capsid protein subunit and (ii) a transfer cassette encapsidated by the protein capsid, wherein the capsid protein subunit comprises a peptide having the sequence X¹-X²-X³-X⁴-X⁵-X⁶-X⁷-X⁸ (SEQ ID NO: 158) at amino acids 587-594 of the native AAV9 capsid protein subunit sequence, wherein the peptide does not occur in the native AAV9 capsid protein subunit sequence. In some embodiments, X¹ is not A, X² is not Q, X³ is not A, X⁴ is not Q, X⁵ is not A, X⁶ is not Q, X⁷ is not T, and/or X⁸ is not G. In some embodiments, X¹ is S. In some embodiments, X² is K or T. In some embodiments, X³ is V. In some embodiments, X⁴ is E or D. In some embodiments, X⁵ is S. In some embodiments, X⁶ is W or I. In some embodiments, X⁷ is T or A. In some embodiments, X⁸ is E or I. In some embodiments, X¹ is S, X² is K, X³ is V, X⁴ is E, X⁵ is S, X⁶ is W, X⁷ is T, and X⁸ is E. In some embodiments, X¹ is S, X² is T, X³ is V, X⁴ is D, X⁵ is S, X⁶ is I, X⁷ is A, and X⁸ is I.

[0088] In some embodiments, an adeno-associated virus (AAV) vector comprises (i) a protein capsid comprising a recombinant capsid protein subunit and (ii) a transfer cassette encapsidated by the protein capsid, wherein the capsid protein subunit comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any one of SEQ ID NO: 165-187. In some embodiments, the capsid protein subunit comprises the amino acid sequence of any one of SEQ ID NO: 165-187. In some embodiments, the capsid protein subunit comprises the amino acid sequence of SEQ ID NO: 175. In some embodiments, the capsid protein subunit comprises the amino acid sequence of SEQ ID NO: 180.

[0089] In some embodiments, an AAV vector selectively delivers the transfer cassette to a cell or tissue of the central nervous system. In some embodiments, the tissue of the

central nervous system is the premotor cortex, the thalamus, the cerebellar cortex, the dentate nucleus, the spinal cord, or the dorsal root ganglion. In some embodiments, the AAV vector delivers the transfer cassette to the brain, but does not deliver the AAV vector to the heart. In some embodiments, the AAV vector delivers the transfer cassette to the brain and to the heart. In some embodiments, delivery of the transfer cassette is greater to the brain than to the heart. In some embodiments, delivery of the transfer cassette is approximately equal in the brain and in the heart.

AAV Capsid Protein Subunits and Protein Capsids Comprising the Same

[0090] In some embodiments, the disclosure provides an adeno-associated virus (AAV) capsid protein subunit comprising one or more amino acid modifications (e.g., substitutions and/or deletions) compared to a native AAV capsid protein subunit, wherein the one or more modifications modify one or more antigenic sites on the AAV capsid protein subunit. The modification of the one or more antigenic sites results in reduced recognition by an antibody of the one or more antigenic sites and/or inhibition of neutralization of infectivity of a virus particle comprising the AAV capsid protein subunit. The one or more amino acid modifications (e.g., substitutions and/or deletions) can be in one or more antigenic footprints identified by peptide epitope mapping and/or cryo-electron microscopy studies of AAV-antibody complexes containing AAV capsid protein subunits. In some embodiments, the one or more antigenic sites are common antigenic motifs or CAMs as described in WO 2017/058892, which is incorporated herein by reference in its entirety. In some embodiments, the antigenic sites are in a variable region (VR) of the AAV capsid protein subunit, such as VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII, VR-IX. In some embodiments, one or more antigenic sites is in the HI loop of the AAV capsid protein subunit.

[0091] In some embodiments, an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAV10, AAV11, AAV12, AAVrh32.22, bovine AAV, or Avian AAV capsid protein subunit comprises an amino acid modification (e.g., a substitution or deletion) in one or more of the regions identified in Table 6, below.

TABLE 6: Exemplary antigenic or other regions on various AAV capsid protein subunits that may be partially or fully substituted/replaced. Respective VP1 numbering of residues in the native AAV capsid protein subunit sequence is shown.

AAV1 Sequence (amino acid numbers)	SEQ ID NO	AAV2 Sequence (amino acid numbers)	SEQ ID NO	AAV3 Sequence (amino acid numbers)	SEQ ID NO	AAV4 Sequence (amino acid numbers)	SEQ ID NO	AAV5 Sequence (amino acid numbers)	SEQ ID NO
SASTGAS (262-268)	2591	SQSGAS (262-267)	2601	SQSGAS (262-267)	2611	RLGESL QS (253-260)	2621	EIKSGSVD GS (249- 258)	2631
VFMIPQY GYL (370- 379)	2592	VFMVPQY GYL (369- 378)	2602	VFMVPQ GYL (369-378)	2612	VFMVPQ GYC (360-369)	2622	VFTLPQY GYA (360- 369)	2632
NQSGSA QNK (451-459)	2593	TPSGTTT QS (450- 458)	2603	TTSGETT QS (451- 459)	2613	GTTLNA GTA (445-453)	2623	STNNTGG VQ (440- 448)	2633
SV (472- 473)	2594	RD (471- 472)	2604	SL (472- 473)	2614	SN (466- 467)	2624	AN (458- 459)	2634
KTDNNN SN (493- 500)	2595	SADNNNS E (492- 499)	2605	ANDNNN SN (493- 500)	2615	ANQNYKI PATGS (487-498)	2625	SGVNRAS (479-485)	2635
KDDEDK F (528- 534)	2596	KDDEEKF (527-533)	2606	KDDEEK F (528- 534)	2616	GPADSK F (527- 533)	2626	LQGSNTY (515-521)	2636
SAGASN (547-552)	2597	GSEKTN (546-551)	2607	GTTASN (547-552)	2617	QNGNTA (545-560)	2627	ANPGTTA T (534- 541)	2637
STDPATG DVH (588- 597)	2598	NRQAATA DVN (587- 596)	2608	NTAPTGG TVN (588- 597)	2618	SNLPTV DRLT (583-595)	2628	TTAPATG TVN (577- 586)	2638
AN (709- 710)	2599	VN (708- 709)	2609	VN (709- 710)	2619	NS (707- 708)	2629	QF (697- 698)	2639
DNNGLY T (716- 722)	2600	DTNGVYS (715-721)	2610	DTNGVY S (716- 722)	2620	DAAGKY T (714- 720)	2630	DSTGEYR (704-710)	2640

AAV6 (amino acid numbers)	SEQ ID NO	AAV7 (amino acid numbers)	SEQ ID NO	AAV8 (amino acid numbers)	SEQ ID NO	AAV9 (amino acid numbers)	SEQ ID NO	AAVrh8 (amino acid numbers)	SEQ ID NO
SASTGAS (262-268)	2641	SETAGST (263-269)	2651	NGTSGG AT (263- 270)	2661	NSTSGG SS (262- 269)	2671	NGTSGGS T (262- 269)	2681
VFMIPQY GYL (370- 379)	2642	VFMIPQY GYL (371- 380)	2652	VFMIPQY GYL (372- 381)	2662	VFMIPQY GYL (371-380)	2672	VFMVPQY GYL (371- 380)	2682
NQSGSA QNK (451-459)	2643	NPGGTA GNR (453- 461)	2653	TTGGTA NTQ (453-461)	2663	INGSGQ NQ (451-459)	2673	QTTGTGG TQ (451- 459)	2683
SV (472- 473)	2644	AN (474- 475)	2654	AN (474- 475)	2664	AV (472- 473)	2674	AN (472- 473)	2684
KTDNNN SN (493- 500)	2645	LDQNNNS N (495- 502)	2655	TGQNNN SN (495- 502)	2665	VTQNNN SE (493- 500)	2675	TNQNNNS N (493- 500)	2685
KDDKDK F (528- 534)	2646	KDDEDRF (530-536)	2656	KDEER F (530- 536)	2666	KEGEDR F (528- 534)	2676	KDDDDRF (528-534)	2686
SAGASN (547-552)	2647	GATNKT (549-554)	2657	NAARDN (549-554)	2667	GTGRDN (547-552)	2677	GAGNDG (547-552)	2687
STDPATG DVH (588- 897)	2648	NTAAQTQ VVN (589- 598)	2658	NTAPQIG TVNS (590-600)	2668	QAQAQT GWVQ (588-597)	2678	NTQAQTG LVH (588- 597)	2688
AN (709- 710)	2649	TG (710- 711)	2659	TS (711- 712)	2669	NN (709- 710)	2679	TN (709- 710)	2689
DNNGLY T (716- 722)	2650	DSQGVY S (717- 723)	2660	NTEGVY S (718- 724)	2670	NTEGVY S (716- 722)	2680	NTEGVYS (716-722)	2690

AAVrh10 (amino acid numbers)	SEQ ID NO	AAV10 (amino acid numbers)	SEQ ID NO	AAV11 (amino acid numbers)	SEQ ID NO	AAV12 (amino acid numbers)	SEQ ID NO	AAVrh32.33 (amino acid numbers)	SEQ ID NO
NGTSGGS T (263- 270)	2691	NGTSGG ST (263- 270)	2701	RLGTTSS S (253- 260)	2711	RIGTTAN S (262- 269)	2721	RLGTTSNS (253-260)	2731
VFMIPQY GYL (372- 381)	2692	VFMIPQY GYL (372- 381)	2702	VFMVPQ YGYC (360-369)	2712	VFMVPQ YGYC (369-378)	2722	VFMVPQYG YC (360-369)	2732
STGGTAG TQ (453- 461)	2693	STGGTQ GTQ (453- 461)	2703	GETLNQ GNA (444-452)	2713	GNSLNQ GTA (453-461)	2723	GETLNQGN A (444-452)	2733
SA (474- 475)	2694	SA (474- 475)	2704	AF (465- 466)	2714	AY (474- 475)	2724	AF (465-466)	2734
LSQNNNS N (495- 502)	2695	LSQNNNS N (495- 502)	2705	ASQNYKI PASGG (486-497)	2715	ANQNYKI PASGG (495-506)	2725	ASQNYKIPA SGG (486- 497)	2735
KDDEERF (530-536)	2696	KDDEERF (530-536)	2706	GPSDGD F (526- 532)	2716	GAGDSD F (535- 541)	2726	GPSDGD (526-532)	2736
GAGKDN (549-554)	2697	GAGRDN (549-554)	2707	VTGNNT (544-549)	2717	PSGNNT (553-558)	2727	VTGNNT (544-549)	2737
NAAPVIG AVN (590- 599)	2698	NTGPVIG NVN (590- 599)	2708	TTAPITG NVT (585- 594)	2718	TTAPHIA NLD (594-503)	2728	TTAPITGNV T (585-594)	2738
TN (711- 712)	2699	TN (711- 712)	2709	SS (706- 707)	2719	NS (715- 716)	2729	SS (706-707)	2739
NTDGTYS (718-724)	2700	NTEGTYS (718-724)	2710	DTTGKYT (713-719)	2720	DNAGNY H (722- 728)	2730	DTTGKYT (713-719)	2740

Bovine AAV (amino acid numbers)	SEQ ID NO	Avian AAV (amino acid numbers)	SEQ ID NO
RLGSSN AS (255- 262)	2741	RIQGPSG G (265- 272)	2751
VFMVPQ YGYC (362-371)	2742	IYTIPQYG YC (375- 384)	2752
GGTLNQ GNS (447-455)	2743	VSQAGS SGR (454- 462)	2753
SG (468- 469)	2744	AA (475- 476)	2754
ASQNYKI PQGRN (489-500)	2745	ASNITKN NVFSV (496-507)	2755
ANDATDF (529-535)	2746	FSGEPDR (533-539)	2756
ITGNIT (547-552)	2747	VYDQTTA T (552- 559)	2757
TTVPTVD DVD (588- 597)	2748	VTPGTRA AVN (595- 604)	2758
DS (709- 710)	2749	AD (716- 717)	2759
DNAGAY K (716- 722)	2750	SDTGSYS (723-729)	2760

[0092] In some embodiments, the amino acid substitution replaces any eight amino acids in an AAV capsid protein subunit from any one of the following serotypes: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAV10, AAV11, AAV12, AAVrh32.22, bovine AAV, or Avian AAV. For example, the amino acid substitution may replace the following amino acids (VP1 numbering): 355-362, 363-370, 371-378, 379-386, 387-394, 395-402, 403-410, 411-418, 419-426, 427-434, 435-442, 443-450, 451-458, 459-466, 467-474, 475-482, 483-490, 491-498, 499-506, 507-514, 515-522, 523-530, 531-538, 539-546, 547-554, 555-562, 563-570, 571-578, 579-586, 587-594, 595-602, 603-610, 611-618, 619-626, 627-634, 635-642, 643-650, 651-658, 659-666, 667-674, 675-682, 683-690, 691-698, 699-706, 707-714, 715-722 in any of the above-listed AAV serotypes.

[0093] In some embodiments, the amino acid substitution is selected from any one of SEQ ID NO: 19-20. In some embodiments, the amino acid substitution has at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with any one of SEQ ID NO: 12-18. In some embodiments, the substitution is at the amino acids corresponding to amino acids 587-594 of the wildtype AAV9 capsid protein subunit. In some embodiments, the substitution is at the amino acids corresponding to amino acids 587-594 of the wildtype AAV1 capsid protein subunit. In some embodiments, the substitution is at the amino acids corresponding to amino acids 587-594 of the wildtype AAV6 capsid protein subunit. In some embodiments, the substitution is at the amino acids corresponding to amino acids 589-596 of the wildtype AAV8 capsid protein subunit. In some embodiments, the substitution is at the amino acids corresponding to amino acids 587-594 of the wildtype AAVrh8 capsid protein subunit. In some embodiments, the substitution is at the amino acids corresponding to amino acids 589-596 of the wildtype AAVrh10 capsid protein subunit.

[0094] In some embodiments, the amino acid substitution is selected from any one of SEQ ID NO: 18-20. In some embodiments, the amino acid substitution has at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with any one of SEQ ID NO: 18-20. In some embodiments, the substitution is at the amino acids corresponding to amino acids 451-458 of the wildtype AAV9 capsid protein subunit.

[0095] In some embodiments, an amino acid deletion comprises a deletion of at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten amino acids compared to the wildtype capsid protein subunit.

[0096] In some embodiments, an AAV capsid protein subunit comprises one or more amino acid substitutions and one or more amino acid deletions. In some embodiments, a capsid protein subunit comprises at least one amino acid substitution and at least one amino acid deletion. In some embodiments, a capsid protein subunit comprises at least one amino acid substitution and at least one amino acid deletion, wherein the at least one amino acid substitution and the at least one amino acid deletion are immediately adjacent to one another in the capsid protein subunit amino acid sequence.

[0097] In some embodiments, the capsid protein subunits are modified to produce an AAV capsid protein subunit that, when present in an AAV virus particle or AAV virus vector, has a phenotype of selectively targeting the CNS (e.g., the brain, the spinal cord). In some embodiments, the capsid protein subunits are modified to produce an AAV capsid protein subunit that, when present in an AAV virus particle or AAV virus vector, has a phenotype of evading neutralizing antibodies. The AAV virus-like particle or AAV vector can also have a phenotype of enhanced or maintained transduction efficiency in addition to the phenotype of evading neutralizing antibodies and/or targeting the CNS.

[0098] In some embodiments, the one or more substitutions can introduce one or more sequences from a capsid protein subunit of a first AAV serotype into the capsid protein subunit of a second AAV serotype that is different from the first AAV serotype.

[0099] The base AAV capsid protein subunit to which modifications are added can be a capsid protein subunit of an AAV serotype selected from AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh.8, AAVrh.10, AAVrh.32.33, AAVrh74, bovine AAV, avian AAV or any other AAV now known or later identified. In some embodiments, the base AAV capsid protein subunit is of the AAV9 serotype. In some embodiments, the base AAV capsid protein subunit is chimeric. In some embodiments, the base AAV capsid protein subunit is an AAV8/9 chimera.

[0100] Several examples of a modified AAV capsid protein subunit are provided herein. In the following examples, the capsid protein subunit can comprise the specific

substitutions described and, in some embodiments, can comprise fewer or more substitutions than those described. As used herein, “substitution” may refer to a single amino acid substitution, or a substitution of more than one contiguous amino acid. For example in some embodiments, a capsid protein subunit can comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc., single amino acid substitutions. In some embodiments, a capsid protein subunit can comprise one or more substitutions of multiple contiguous amino acids, such as one or more substitutions of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 contiguous amino acids.

[0101] Furthermore, in some embodiments described herein wherein an amino acid residue is substituted by any amino acid residue other than the amino acid residue present in the wildtype or native amino acid sequence, the any other amino acid residue can be any natural or non-natural amino acid residue known in the art (see, e.g., Tables 2 and 4). In some embodiments, the substitution can be a conservative substitution and in some embodiments, the substitution can be a nonconservative substitution. In some embodiments, an AAV capsid protein subunit comprises one or more amino acid substitutions, wherein the amino acid substitutions are each individually selected from SEQ ID NO: 12-18 as shown in Table 7.1.

TABLE 7.1: AMINO ACID SUBSTITUTIONS

Amino Acid Substitution	SEQ ID NO.
SCQPTVMN	12
FGVPNQPL	13
QRGQAAPF	14
GDYAPIRE	15
KTRRIVQH	16
FGFPNQPL	17
RQDQPINA	18

[0102] In some embodiments, an AAV capsid protein subunit comprises one or more amino acid substitutions, wherein the amino acid substitutions are each selected from SEQ ID NO: 19-20 as shown in Table 7.2.

TABLE 7.2: AMINO ACID SUBSTITUTIONS

Amino Acid Substitution	SEQ ID NO.
SKVESWTE	19
STVDSIAI	20

[0103] In some embodiments, an AAV capsid protein subunit may comprise a first substitution selected from the sequences listed in Table 7.1 and a second substitution selected from the sequences listed in Table 7.2. In some embodiments, an AAV capsid protein subunit may comprise a first substitution, a second substitution as shown in Tables 7.3 and 7.4.

TABLE 7.3: COMBINATIONS OF AMINO ACID SUBSTITUTIONS

First Substitution (SEQ ID NO)	Second Substitution (SEQ ID NO)
12, 13, 14, 15, 16, 17, or 18	19 or 20

TABLE 7.4 COMBINATIONS OF AMINO ACID SUBSTITUTIONS

First Substitution (SEQ ID NO)	Second Substitution (SEQ ID NO)
12	19
12	20
13	19
13	20
14	19
14	20
15	19
15	20
16	19
16	20
17	19

17	20
18	19
18	20

[0104] In some embodiments, an AAV capsid protein subunit comprises an amino acid modification (e.g., substitution and/or deletion), wherein the amino acid modification modifies one or more surface-exposed regions, such as an antigenic region, on the AAV capsid protein subunit.

[0105] In some embodiments, an AAV capsid protein subunit comprises one or more amino acid substitutions, wherein at least one of the amino acid substitutions comprises one of SEQ ID NOs: 19-20. In some embodiments, the substitution replaces the amino acids corresponding to amino acids 587-594 of the wildtype AAV9 capsid protein subunit.

[0106] In some embodiments, an AAV capsid protein subunit comprises one or more amino acid substitutions, wherein at least one of the amino acid substitutions comprises one of SEQ ID NOs: 12-18. In some embodiments, the substitution replaces the amino acids corresponding to amino acids 451-458 of the wildtype AAV9 capsid protein subunit.

[0107] In some embodiments, an AAV capsid protein subunit comprises a substitution comprising a sequence of eight amino acids (X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8) (SEQ ID NO: 158) that does not occur in the native capsid protein subunit sequence. In some embodiments, X^1 is not I, X^2 is not N, X^3 is not G, X^4 is not S, X^5 is not G, X^6 is not Q, X^7 is not N, and/or X^8 is not Q. In some embodiments, X^1 is S, F, Q, G, K, or R. In some embodiments, X^2 is C, G, R, D, T, or Q. In some embodiments, X^3 is Q, V, G, Y, R, F, or D. In some embodiments, X^4 is P, Q, A, or R. In some embodiments, X^5 is T, N, A, P, or I. In some embodiments, X^6 is V, Q, A, or I. In some embodiments, X^7 is M, P, R, Q, or N. In some embodiments, X^8 is N, L, F, E, H, or A. In some embodiments, X^1 is S, X^2 is C, X^3 is Q, X^4 is P, X^5 is T, X^6 is V, X^7 is M, and X^8 is N. In some embodiments, X^1 is F, X^2 is G, X^3 is V, X^4 is P, X^5 is N, X^6 is Q, X^7 is P, and X^8 is L. In some embodiments, X^1 is Q, X^2 is R, X^3 is G, X^4 is Q, X^5 is A, X^6 is A, X^7 is P, and X^8 is F. In some embodiments, X^1 is G, X^2 is D, X^3 is Y, X^4 is A, X^5 is P, X^6 is I, X^7 is R, and X^8 is E. In some embodiments, X^1 is K, X^2 is T, X^3 is R, X^4 is R, X^5 is I, X^6 is V, X^7 is Q, and X^8 is H. In some embodiments, X^1 is F, X^2 is G, X^3 is F, X^4 is P, X^5 is N, X^6 is Q, X^7 is P, and X^8 is L. In some embodiments, X^1 is R, X^2 is Q, X^3 is D, X^4 is Q, X^5 is P, X^6 is I, X^7 is N, and X^8 is A.

[0108] In some embodiments, X¹ is not A, X² is not Q, X³ is not A, X⁴ is not Q, X⁵ is not A, X⁶ is not Q, X⁷ is not T, and/or X⁸ is not G. In some embodiments, X¹ is S. In some embodiments, X² is K or T. In some embodiments, X³ is V. In some embodiments, X⁴ is E or D. In some embodiments, X⁵ is S. In some embodiments, X⁶ is W or I. In some embodiments, X⁷ is T or A. In some embodiments, X⁸ is E or I. In some embodiments, X¹ is S, X² is K, X³ is V, X⁴ is E, X⁵ is S, X⁶ is W, X⁷ is T, and X⁸ is E. In some embodiments, X¹ is S, X² is T, X³ is V, X⁴ is D, X⁵ is S, X⁶ is I, X⁷ is A, and X⁸ is I.

[0109] In some embodiments, an AAV subunit protein comprises one or more amino acid deletions, wherein the amino acid deletion comprises a deletion of at least six or at least eight amino acids compared to the wildtype AAV capsid protein subunit. In some embodiments, an AAV capsid protein subunit comprises a deletion of eight consecutive amino acids compared to the native capsid protein subunit sequence. In some embodiments, an AAV capsid protein subunit comprises a deletion of six consecutive amino acids compared to the native capsid protein subunit sequence.

[0110] In some embodiments, an AAV capsid protein subunit comprises the sequence LSKTQTLK (SEQ ID NO: 1374) or the sequence LSKTDPQTLK (SEQ ID NO: 1375). In some embodiments, the AAV capsid protein subunit comprising SEQ ID NO: 1374 or 1375 is of a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV and Bovine AAV.

[0111] In some embodiments, an AAV capsid protein subunit comprises a first substitution comprising a sequence selected from SEQ ID NO: 12-18; and a second substitution comprising a sequence selected from SEQ ID NO: 19-20.

[0112] In some embodiments, an AAV capsid protein subunit comprises an amino acid deletion and a substitution, wherein the substitution comprises a sequence selected from SEQ ID NO: 12-20.

[0113] In some embodiments, a recombinant capsid protein subunit has a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 9 (AAV9) and comprises one or more of the following amino acid substitutions: I451S, I451F, I451Q, I451G, I451K, I451R, N452C, N452G, N452R, N452D, N452T, N452Q, G453Q, G453V, G453Y, G453R, G453F, G453D,

S454P, S454Q, S454A, S454R, G455T, G455N, G455A, G455P, G455I, Q456V, Q456A, Q456I, N457M, N457P, N457R, N457Q, Q458N, Q458L, Q458F, Q458E, Q458H, Q458A, A587S, Q588K, Q588T, A589V, Q590E, Q590D, A591S, Q592W, Q592I, T593A, G594E, G594I.

[0114] Any of the AAV capsid protein subunits described herein may further comprise a modification (e.g., a substitution or a deletion) in the HI loop. The HI loop is a prominent domain on the AAV capsid protein subunit surface, between β strands β H and β I, that extends from each viral protein (VP) subunit overlapping the neighboring fivefold VP. In some embodiments, an AAV capsid protein subunit comprises one, two, three, four, five, six, seven, or eight amino acid substitutions in the HI loop. In some embodiments, the AAV capsid protein subunit comprises one or more of the following substitutions in the HI loop: P661R, T662S, Q666G, S667D, wherein the numbering corresponds to the wildtype AAV8 capsid protein subunit (SEQ ID NO: 8). In some embodiments, the AAV capsid protein subunit comprises one or more of the following substitutions in the HI loop: P659R, T660S, A661T, K664G, wherein the numbering corresponds to the wildtype AAV9 capsid protein subunit (SEQ ID NO: 9).

[0115] In some embodiments, an AAV capsid protein subunit comprises one, two, three, or four amino acid substitutions, wherein each substitution modifies a different antigenic site on the AAV capsid protein subunit, and wherein at least one of the amino acid substitutions modifies the HI loop of the capsid protein subunit.

[0116] In some embodiments, an AAV capsid protein subunit comprises a first, a second, a third, and a fourth amino acid substitution. In some embodiments, at least one of the substitutions modifies the HI Loop of the capsid protein subunit. In some embodiments, the AAV capsid protein subunit comprises one or more of the following substitutions in the HI loop: P661R, T662S, Q666G, S667D, wherein the numbering corresponds to the wildtype AAV8 capsid protein subunit (SEQ ID NO: 8); or P659R, T660S, A661T, K664G, wherein the numbering corresponds to the wildtype AAV9 capsid protein subunit (SEQ ID NO: 9). In some embodiments, an AAV capsid protein subunit comprises the amino acid sequence of any one of SEQ ID NO: 185-187. In some embodiments, an AAV capsid protein subunit comprises an amino acid sequence sharing at least 90%, at least 95%, at

least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NO: 165-187.

[0117] Also provided herein is a nucleic acid, or a plasmid comprising the same that encodes one or more of the AAV capsid protein subunits described herein. The nucleotide sequence may be a DNA sequence or an RNA sequence. In some embodiments, cell comprises one or more nucleic acids or plasmids described herein.

[0118] In some embodiments, an AAV protein capsid comprises an AAV capsid protein subunit as described herein. Further provided herein is a viral vector comprising an AAV protein capsid as well as a composition comprising the AAV protein capsid, AAV capsid protein subunit and/or viral vector in a pharmaceutically acceptable carrier.

[0119] In some embodiments, modification of one or more antigenic sites results in reduced binding by an antibody to the one or more antigenic sites. In some embodiments, modification of the one or more antigenic sites results in inhibition of neutralization of infectivity of a virus particle comprising the AAV capsid protein subunit.

[0120] As described herein, the nucleic acid and amino acid sequences of the capsid protein subunits from a number of AAV are known in the art. Thus, the amino acids "corresponding" to amino acid positions of the native AAV capsid protein subunits can be readily determined for any other AAV (e.g., by using sequence alignments).

[0121] The modified capsid protein subunits can be produced by modifying the capsid protein subunit of any AAV now known or later discovered. Further, the base AAV capsid protein subunit that is to be modified can be a naturally occurring AAV capsid protein subunit (e.g., an AAV2, AAV3a or 3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 or AAV11 capsid protein subunit or any of the AAV shown in Table 2) but is not so limited. Those skilled in the art will understand that a variety of manipulations to the AAV capsid protein subunits are known in the art and the disclosure is not limited to modifications of naturally occurring AAV capsid protein subunits. For example, the capsid protein to be modified may already have alterations as compared with naturally occurring AAV (e.g., is derived from a naturally occurring AAV capsid protein subunit, e.g., AAV2, AAV3a, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 or any other AAV now known or later discovered). In some embodiments, the capsid protein subunit may be a chimeric capsid protein subunit. In some embodiments, the capsid protein

subunit may be an engineered AAV, such as AAV2i8, AAV2g9, AAV-LK03, AAV7m8, AAV Anc80, AAV PHP.B.

[0122] Thus, in some embodiments, the AAV capsid protein subunit to be modified can be derived from a naturally occurring AAV but further comprises one or more foreign sequences (e.g., that are exogenous to the native virus) that are inserted and/or substituted into the capsid protein subunit and/or has been altered by deletion of one or more amino acids.

[0123] Accordingly, when referring herein to a specific AAV capsid protein subunit (e.g., an AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 or AAV11 capsid protein subunit or a capsid protein subunit from any of the AAV shown in Table 2, etc.), it is intended to encompass the native capsid protein subunit as well as capsid protein subunits that have alterations other than the modifications described herein. Such alterations include substitutions, insertions and/or deletions. In some embodiments, the capsid protein subunit comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, less than 20, less than 30, less than 40, less than 50, less than 60, or less than 70 amino acids inserted therein (other than the insertions described herein) as compared with the native AAV capsid protein subunit sequence. In some embodiments, the capsid protein subunit comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, less than 20, less than 30, less than 40, less than 50, less than 60, or less than 70 amino acid substitutions (other than the amino acid substitutions described herein) as compared with the native AAV capsid protein subunit sequence, in some embodiments, the capsid protein subunit comprises a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, less than 20, less than 30, less than 40, less than 50, less than 60, or less than 70 amino acids as compared with the native AAV capsid protein subunit sequence.

[0124] In some embodiments, the AAV capsid protein subunit has an amino acid sequence that is at least about 90%, about 95%, about 97%, about 98% or about 99% similar or identical to a native AAV capsid protein subunit sequence.

[0125] Methods of determining sequence similarity or identity between two or more amino acid sequences are known in the art. Sequence similarity or identity may be determined for an entire length of a nucleic acid or for an indicated portion of a nucleic acid. Sequence

similarity or identity may be determined using standard techniques, including, but not limited to, the local sequence identity algorithm of Smith & Waterman, *Adv. Appl. Math.* 2, 482 (1981), by the sequence identity alignment algorithm of Needleman & Wunsch, *J Mol. Biol.* 48,443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 85, 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux et al., *Nucl. Acid Res.* 12, 387-395 (1984), or by inspection.

[0126] Another suitable algorithm is the BLAST algorithm, described in Altschul et al., *J Mol. Biol.* 215, 403-410, (1990) and Karlin et al., *Proc. Natl. Acad. Sci. USA* 90, 5873-5877 (1993). A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul et al., *Methods in Enzymology*, 266, 460-480 (1996); <http://blast.wustl.edu/blast/README.html>. WU-BLAST-2 uses several search parameters, which are optionally set to the default values. The parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

[0127] Further, an additional useful algorithm is gapped BLAST as reported by Altschul et al., (1997) *Nucleic Acids Res.* 25, 3389-3402.

[0128] For purposes of the instant disclosure, unless otherwise indicated, percent identity is calculated using the Basic Local Alignment Search Tool (BLAST) available online at blast.ncbi.nlm.nih.gov/Blast.cgi. The skilled artisan will understand that other algorithms may be substituted as appropriate.

[0129] In some embodiments, a protein capsid comprises a modified AAV capsid protein subunit as described herein. In some embodiments, the protein capsid is a parvovirus capsid, which may further be an autonomous parvovirus capsid or a dependovirus capsid. Optionally, the protein capsid is an AAV protein capsid. In some embodiments, the AAV protein capsid is an AAV1, AAV2, AAV3a, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, bovine AAV protein capsid, avian AAV protein capsid or any other AAV now known or later identified. A

nonlimiting list of AAV serotypes is shown in Table 2. An AAV protein capsid can be any AAV serotype listed in Table 2 or derived from any of the foregoing by one or more insertions, substitutions and/or deletions. Molecules that can be packaged by the modified virus protein capsid and transferred into a cell include transfer cassettes (e.g., heterologous DNA or RNA), polypeptides, small organic molecules, metals, or combinations of the same.

[0130] Heterologous molecules are defined as those that are not naturally found in an AAV infection, e.g., those not encoded by a wild-type AAV genome. Further, therapeutically useful molecules can be associated with the outside of the chimeric protein capsid for transfer of the molecules into host target cells. Such associated molecules can include DNA, RNA, small organic molecules, metals, carbohydrates, lipids and/or polypeptides. In some embodiments the therapeutically useful molecule is covalently linked (*i.e.*, conjugated or chemically coupled) to the protein capsid or a capsid protein thereof. Methods of covalently linking molecules are known by those skilled in the art.

[0131] The modified protein capsids also find use in raising antibodies against the novel protein capsid structures. As a further alternative, an exogenous amino acid sequence may be inserted into the modified protein capsid or capsid protein subunit thereof for antigen presentation to a cell, e.g., for administration to a subject to produce an immune response to the exogenous amino acid sequence.

[0132] In some embodiments, the protein capsids can be administered to block certain cellular sites prior to and/or concurrently with (e.g., within minutes or hours of each other) administration of a virus vector delivering a nucleic acid encoding a polypeptide or functional RNA of interest. For example, the inventive protein capsids can be delivered to block cellular receptors on liver cells and a delivery vector (e.g., an AAV vector) can be administered subsequently or concurrently, which may reduce transduction of liver cells, and enhance transduction of other targets (e.g., skeletal, cardiac and/or diaphragm muscle).

[0133] According to some embodiments, modified protein capsids can be administered to a subject prior to and/or concurrently with a modified virus vector as described herein. Further, the disclosure provides compositions and pharmaceutical formulations

comprising the inventive modified protein capsids or capsid protein subunit thereof; optionally, the composition also comprises a modified virus vector as described herein.

[0134] In some embodiments, a nucleic acid (optionally, an isolated nucleic acid) encodes the modified protein capsid subunits described herein. Further provided are nucleic acids, and cells (*in vivo* or in culture) comprising the nucleic acids and/or virus vectors described herein. As one example, a virus vector may comprise: (a) a protein capsid comprising a modified AAV capsid protein subunit as described herein; and (b) a nucleic acid comprising at least one terminal repeat sequence, wherein the nucleic acid is encapsidated by the AAV protein capsid.

[0135] Suitable viral vectors include, for example, adenovirus, AAV, herpesvirus, vaccinia, poxviruses, baculovirus, lentivirus, coronavirus, and the like. Suitable nucleic acids include, but are not limited to, plasmids, phage, YACs, BACs, and the like. Such nucleic acids and cells can be used, for example, as reagents (e.g., helper packaging constructs or packaging cells) for the production of modified virus protein capsids, protein capsid subunits, or virus vectors as described herein.

[0136] Protein capsids and capsid protein subunits described herein can be produced using any method known in the art, e.g., by using a baculovirus system (Brown et al., (1994) *Virology* 198:477-488).

[0137] The modifications to the AAV capsid protein subunit as described herein are "selective" modifications. This approach is in contrast to previous work with whole subunit or large domain swaps between AAV serotypes (see, e.g., international patent publication WO 00/28004 and Hauck et al., (2003) *J. Virology* 77:2768-2774). In some embodiments, a "selective" modification results in the insertion and/or substitution and/or deletion of less than or equal to about 20, 18, 15, 12, 10, 9, 8, 7, 6, 5, 4 or 3 contiguous amino acids.

[0138] The modified capsid protein subunits and protein capsids described herein can further comprise any other modification, now known or later identified. For example, the AAV capsid protein subunits and protein capsids can be chimeric in that they can comprise all or a portion of a capsid protein subunit from another virus, optionally another parvovirus or AAV, e.g., as described in international patent publication WO 00/28004.

[0139] In some embodiments, the protein capsid or capsid protein subunit can be a targeted protein capsid or capsid protein subunit, comprising a targeting sequence (e.g.,

substituted or inserted in the protein capsid or capsid protein subunit) that directs the protein capsid or capsid protein subunit to interact with cell-surface molecules present on desired target tissue(s) (see, e.g., International patent publication WO 00/28004 and Hauck *et al.*, (2003) J. Virology 77:2768-2774); Shi *et al.*, Human Gene Therapy 17:353-361 (2006) [describing insertion of the integrin receptor binding motif RGD at positions 520 and/or 584 of the AAV capsid protein subunit]; and U.S. Patent No. 7,314,912 [describing insertion of the PI peptide containing an RGD motif following amino acid positions 447, 534, 573 and 587 of the AAV2 capsid protein subunit]). Other positions within the AAV capsid protein subunit that tolerate insertions are known in the art (e.g., positions 449 and 588 described by Grifman *et al.*, Molecular Therapy 3:964-975 (2001)).

[0140] For example, a protein capsid or capsid protein subunit as described herein may have relatively inefficient tropism toward certain target tissues of interest (e.g., liver, skeletal muscle, heart, diaphragm muscle, kidney, brain, stomach, intestines, skin, endothelial cells, and/or lungs). A targeting sequence can advantageously be incorporated into these low-transduction vectors to thereby confer to the protein capsid (or a capsid protein subunit thereof) a desired tropism and, optionally, selective tropism for particular tissue(s). AAV capsid protein subunits, protein capsids and AAV vectors comprising targeting sequences are described, for example in international patent publication WO 00/28004. As another example, one or more non-naturally occurring amino acids as described by Wang *et al.*, Annu Rev Biophys Biomol Struct. 35:225-49 (2006)) can be incorporated into an AAV capsid protein subunit as described herein at an orthogonal site as a means of redirecting a low-transduction vector to desired target tissue(s). These unnatural amino acids can advantageously be used to chemically link molecules of interest to the AAV capsid protein subunit including without limitation: glycans (mannose - dendritic cell targeting); RGD, bombesin or a neuropeptide for targeted delivery to specific cancer cell types; RNA aptamers or peptides selected from phage display targeted to specific cell surface receptors such as growth factor receptors, integrins, and the like.

[0141] In some embodiments, the targeting sequence may be a capsid protein subunit sequence (e.g., an autonomous parvovirus capsid sequence, AAV capsid protein subunit

sequence, or any other viral capsid sequence) that directs infection to a particular cell type(s).

[0142] As another nonlimiting example, a heparin or heparan sulfate binding domain (e.g., the respiratory syncytial virus heparin binding domain) may be inserted or substituted into a capsid protein subunit that does not typically bind HS receptors (e.g., AAV4, AAV5) to confer heparin and/or heparan sulfate binding to the resulting mutant.

[0143] B19 infects primary erythroid progenitor cells using globoside as its receptor (Brown et al, (1993) Science 262: 114). The structure of B19 has been determined to 8 Å resolution (Agbandje-McKenna et al, (1994) Virology 203: 106). The region of the B19 capsid that binds to globoside has been mapped between amino acids 399-406 (Chapman et al, (1993) Virology 194:419), a looped out region between β -barrel structures E and F (Chipman et al, (1996) Proc. Nat. Acad. Sci. USA 93:7502). Accordingly, the globoside receptor binding domain of the B19 capsid may be substituted into an AAV capsid protein subunit to target a protein capsid or virus vector comprising the same to erythroid cells.

[0144] In some embodiments, the exogenous targeting sequence may be any amino acid sequence encoding a peptide that alters the tropism of a protein capsid or virus vector comprising the modified AAV capsid protein subunit. In some embodiments, the targeting peptide or protein may be naturally occurring or, alternately, completely or partially synthetic. Exemplary targeting sequences include ligands and other peptides that bind to cell surface receptors and glycoproteins, such as ROD peptide sequences, bradykinin, hormones, peptide growth factors (e.g., epidermal growth factor, nerve growth factor, fibroblast growth factor, platelet-derived growth factor, insulin-like growth factors I and II, etc.), cytokines, melanocyte stimulating hormone (e.g., α , β or γ), neuropeptides and endorphins, and the like, and fragments thereof that retain the ability to target cells to their cognate receptors. Other illustrative peptides and proteins include substance P, keratinocyte growth factor, neuropeptide Y, gastrin releasing peptide, interleukin 2, hen egg white lysozyme, erythropoietin, gonadolibcrin, corticostatin, β -endorphin, leu-enkephalin, rimorphin, α -neo-enkephalin, angiotensin, pneumadin, vasoactive intestinal peptide, neurotensin, motilin, and fragments thereof as described above. As yet a further alternative, the binding domain from a toxin (e.g., tetanus toxin or snake toxins,

such as alpha-bungarotoxin, and the like) can be substituted into the capsid protein subunit as a targeting sequence. In some embodiments, the AAV capsid protein subunit can be modified by substitution of a "nonclassical" import/export signal peptide (e.g., fibroblast growth factor-1 and -2, interleukin 1, HIV- 1 Tat protein, herpes virus VP22 protein, and the like) as described by Cleves (Current Biology 7:R318 (1997)) into the AAV capsid protein subunit. Also encompassed are peptide motifs that direct uptake by specific cells, e.g., a FVFLP (SEQ ID NO: 22) peptide motif triggers uptake by liver cells.

[0145] Phage display techniques, as well as other techniques known in the art, may be used to identify peptides that recognize any cell type of interest.

[0146] The targeting sequence may encode any peptide that targets to a cell surface binding site, including receptors (e.g., protein, carbohydrate, glycoprotein or proteoglycan). Examples of cell surface binding sites include, but are not limited to, heparan sulfate, chondroitin sulfate, and other glycosaminoglycans, sialic acid moieties found on mucins, glycoproteins, and gangliosides, MHC 1 glycoproteins, carbohydrate components found on membrane glycoproteins, including, mannose, N-acetyl-galactosamine, N-acetyl-glucosamine, fucose, galactose, and the like.

[0147] In some embodiments, a heparan sulfate (HS) or heparin binding domain is substituted into the capsid protein subunit (for example, in an AAV protein capsid subunit that otherwise does not bind to HS or heparin). It is known in the art that HS/heparin binding is mediated by a "basic patch" that is rich in arginines and/or lysines. In some embodiments, a sequence following the motif BXXB (SEQ ID NO: 23), where "B" is a basic residue and X is neutral and/or hydrophobic residue can be employed. As a nonlimiting example, BXXB can be RGNR (SEQ ID NO: 24). As another nonlimiting example, BXXB is substituted for amino acid positions 262 through 265 in the native AAV2 capsid protein subunit or at the corresponding position(s) in the capsid protein subunit of another AAV serotype.

[0148] Table 8 shows other non-limiting examples of suitable targeting sequences.

TABLE 8: TARGETING SEQUENCES

Sequence	SEQ ID NO	Reference
NSVRDL(G/S)	25	Muller et al., Nature Biotechnology 21: 1040-1046 (2003)
PRSVTVP	26	Muller et al., Nature Biotechnology 21: 1040-1046 (2003)
NSVSSX(S/A)	27	Muller et al., Nature Biotechnology 21: 1040-1046 (2003)
NGR, NGRAHA	28	Grifman et al., Molecular Therapy 3:964-975 (2001)
QPEHSST	29	Work et al., Molecular Therapy 13:683-693 (2006)
VNTANST	30	Work et al., Molecular Therapy 13:683-693 (2006)
HGPMQS	31	Work et al., Molecular Therapy 13:683-693 (2006)
PHKPPLA	32	Work et al., Molecular Therapy 13:683-693 (2006)
IKNNEMW	33	Work et al., Molecular Therapy 13:683-693 (2006)
RNLDTPM	34	Work et al., Molecular Therapy 13:683-693 (2006)
VDSHRQS	35	Work et al., Molecular Therapy 13:683-693 (2006)
YDSKTKT	36	Work et al., Molecular Therapy 13:683-693 (2006)
SQLPHQK	37	Work et al., Molecular Therapy 13:683-693 (2006)
STMQQNT	38	Work et al., Molecular Therapy 13:683-693 (2006)
TERYMTQ	39	Work et al., Molecular Therapy 13:683-693 (2006)
QPEHSST	40	Work et al., Molecular Therapy 13:683-693 (2006)
DASLSTS	41	Work et al., Molecular Therapy 13:683-693 (2006)
DLPNKT	42	Work et al., Molecular Therapy 13:683-693 (2006)
DLTAARL	43	Work et al., Molecular Therapy 13:683-693 (2006)
EPHQFNY	44	Work et al., Molecular Therapy 13:683-693 (2006)
EPQSNHT	45	Work et al., Molecular Therapy 13:683-693 (2006)
MSSWPSQ	46	Work et al., Molecular Therapy 13:683-693 (2006)
NPKHNAT	47	Work et al., Molecular Therapy 13:683-693 (2006)
PDGMRTT	48	Work et al., Molecular Therapy 13:683-693 (2006)
PNNKTT	49	Work et al., Molecular Therapy 13:683-693 (2006)
QSTTHDS	50	Work et al., Molecular Therapy 13:683-693 (2006)
TGSKQKQ	51	Work et al., Molecular Therapy 13:683-693 (2006)
SLKHQAL	52	Work et al., Molecular Therapy 13:683-693 (2006)
SPIDGEQ	53	Work et al., Molecular Therapy 13:683-693 (2006)
WIFPWIQL	54	Hajitou et al., TCM 16:80-88 (2006)
CDCRGDCFC	55	Hajitou et al., TCM 16:80-88 (2006)
CNGRC	56	Hajitou et al., TCM 16:80-88 (2006)
CPRECES	57	Hajitou et al., TCM 16:80-88 (2006)
CTTHWGFTLC	58	Hajitou et al., TCM 16:80-88 (2006)
CGRRAGGSC	59	Hajitou et al., TCM 16:80-88 (2006)
CKGGRAKDC	60	Hajitou et al., TCM 16:80-88 (2006)
CVPELGHEC	61	Hajitou et al., TCM 16:80-88 (2006)
CRRETAWAK	62	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)

Sequence	SEQ ID NO	Reference
VSWFSHRYSPFAVS	63	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
GYRDGYAGPILYN	64	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
XXXY*XXX	65	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
Y*E/MNW	66	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
RPLPLP	67	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
APPLPPR	68	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
DVFYPYPYASGS	69	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
MYWYPY	70	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
DITWDQLWDLMK	71	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
CWDD(G/L)WLC	72	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
EWCEYLGGYLRCYA	73	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
YXCXXGPXTWXCXP	74	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
IEGPTLRQWLAARA	75	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
LWXX(Y/W/F/H)	76	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
XFXXYLW	77	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
RWGLCD	78	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
MSRPACPPNDKYE	79	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
CLRSGRGC	80	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
CHWMFSPWC	81	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
WXXF	82	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
CSSRLDAC	83	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
CLPVASC	84	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
CGFECVRQCPCERC	85	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
CVALCREACGEGC	86	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
SWCEPGWCR	87	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
YSGWGW	88	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
GLSGGRS	89	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
LMLPRAD	90	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
CSCFRDVCC	91	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
CRDVVSVIC	92	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
CNGRC	93	Koivunen et al., J. Nucl. Med. 40:883-888 (1999)
MARSGL	94	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
MARAKE	95	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
MSRTMS	96	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)

Sequence	SEQ ID NO	Reference
KCCYSL	97	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
MYWGDSHWLQYWYE	98	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
MQLPLAT	99	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
EWLS	100	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
SNEW	101	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
TNYL	102	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
WIFPWIQL	103	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
WDLAWMFRLPVG	104	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CTVALPGGYVRVC	105	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CVPELGHEC	106	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CGRRAGGSC	107	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CVAYCIEHHCWTC	108	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CVFAHNYDYLC	109	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CVFTSNYAFC	110	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)

Sequence	SEQ ID NO	Reference
VHSPNKK	111	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CDCRGDCFC	112	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CRGDGWC	113	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
XRGCDX	114	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
PXX(S/T)	115	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CTTHWGFTLC	116	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
SGKGPRQITAL	117	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
A(A/Q)(N/A)(L/Y)(T/V/M/R)(R/K)	118	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
VYMSPF	119	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
MQLPLAT	120	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
ATWLPPR	121	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
HTMYHHYQHHL	122	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
SEVGCRAGPLQWL CEKYFG	123	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CGLLPVGRPDRNV WRWLC	124	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)

Sequence	SEQ ID NO	Reference
CKGQCDFKGLPW EC	125	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
SGRSA	126	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
WGFP	127	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
LWXXAr	128	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
XFXXYLW	129	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
AEPMPHSLNFSQYL WYT	130	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
WAY(W/F)SP	131	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
IELLQAR	132	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
DITWDQLWDLMK	133	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
AYTKCSRQWRTCM TTH	134	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
PQNSKIPGPTFLDP H	135	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
SMEPALPDWWWK MFK	136	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
ANTPCGPYTHDCP VKR	137	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
TACHQHVRMVRP	138	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)

Sequence	SEQ ID NO	Reference
VPWMEPAYQRFL	139	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
DPRATPGS	140	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
FRPNRAQDYNTN	141	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CTKNSYLMC	142	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
C(R/Q)L/RT(G/N)XX G(A/V)GC	143	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
CPIEDRPMC	144	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
HEWSYLAPYPWF	145	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
MCPKHPLGC	146	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
RMWPSSTVNLSAG RR	147	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
SAKTAVSQRVWLP SHRGGEF	148	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
KSREHVNNSACPS KRITAAL	149	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
EGFR	150	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
AGLGVR	151	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
GTRQGHTMRLGVS DG	152	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)

Sequence	SEQ ID NO	Reference
IAGLATPGWSHWLA L	153	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
SMSIARL	154	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
HTFEPGV	155	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
NTSLKRISNKR1RR K	156	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)
LRIKRKRKRKKTR K	157	Newton & Deutscher, Phage Peptide Display in Handbook of Experimental Pharmacology, pages 145-163, Springer-Verlag, Berlin (2008)

Y* is phospho-Tyr

[0149] In some embodiments, the targeting sequence may be a peptide that can be used for chemical coupling (e.g., can comprise arginine and/or lysine residues that can be chemically coupled through their R groups) to another molecule that targets entry into a cell.

[0150] In some embodiments, the AAV capsid protein subunit or protein capsid can comprise a mutation as described in WO 2006/066066. For example, the capsid protein subunit can comprise a selective amino acid substitution at amino acid position 263, 705, 708 and/or 716 of the native AAV2 capsid protein subunit or a corresponding change(s) in a capsid protein subunit from another AAV serotype.

[0151] Additionally, or alternatively, in some embodiments, the capsid protein subunit, protein capsid or viral vector comprises a selective amino acid insertion directly following amino acid position 264 of the AAV2 capsid protein subunit or a corresponding change in the capsid protein subunit from other AAV. By "directly following amino acid position X" it is intended that the insertion immediately follows the indicated amino acid position (for example, "following amino acid position 264" indicates a point insertion at position 265 or a larger insertion, e.g., from positions 265 to 268, etc.).

[0152] Furthermore, in some embodiments, the capsid protein subunit, protein capsid or viral vector can comprise amino acid modifications such as described in PCT Publication

No. WO 2010/093784 (e.g., 2i8) and/or in PCT Publication No. WO 2014/144229 (e.g., dual glycan).

[0153] In some embodiments, the capsid protein subunit, protein capsid or viral vector can have equivalent or enhanced transduction efficiency relative to the transduction efficiency of the AAV serotype from which the capsid protein subunit, protein capsid or viral vector originated. In some embodiments, the capsid protein subunit, protein capsid or viral vector can have reduced transduction efficiency relative to the transduction efficiency of the AAV serotype from which the capsid protein subunit, protein capsid or viral vector originated. In some embodiments, the capsid protein subunit, protein capsid or viral vector can have equivalent or enhanced tropism relative to the tropism of the AAV serotype from which the capsid protein subunit, protein capsid or viral vector originated. In some embodiments, the capsid protein subunit, protein capsid or viral vector can have an altered or different tropism relative to the tropism of the AAV serotype from which the capsid protein subunit, protein capsid or viral vector originated. In some embodiments, the capsid protein subunit, protein capsid or viral vector can have or be engineered to have tropism for brain tissue. In some embodiments, the capsid protein subunit, protein capsid or viral vector can have or be engineered to have tropism for liver tissue.

[0154] The AAV vectors described herein can be used to deliver a heterologous nucleic acid to a cell or subject. For example, the modified vector can be used to treat a lysosomal storage disorder such as a mucopolysaccharidosis disorder (e.g., Sly syndrome [β -glucuronidase], Hurler Syndrome [α -L-iduronidase], Scheie Syndrome [α -L-iduronidase], Hurler-Scheie Syndrome [α -L-iduronidase], Hunter's Syndrome [iduronate sulfatase], Sanfilippo Syndrome (A [heparan sulfamidase], B [N-acetylglucosaminidase], C [acetyl-CoA: α -glucosaminide acetyltransferase], D [N-acetylglucosamine 6-sulfatase]), Morquio Syndrome (A [galactose-6-sulfate sulfatase], B [β -galactosidase]), Maroteaux-Lamy Syndrome [N-acetylgalactosamine-4-sulfatase], etc.), Fabry disease (α -galactosidase), Gaucher's disease (glucocerebrosidase), or a glycogen storage disorder (e.g., Pompe disease; lysosomal acid α -glucosidase) as described herein.

[0155] Those skilled in the art will appreciate that for some AAV capsid protein subunits, the corresponding modification will be an insertion and/or a substitution, depending on

whether the corresponding amino acid positions are partially or completely present in the virus or, alternatively, are completely absent.

[0156] In some embodiments, virus vectors comprise the modified capsid protein subunits and protein capsids described herein. In some embodiments, the virus vector is a parvovirus vector (e.g., comprising a parvovirus protein capsid and/or vector genome), for example, an AAV vector (e.g., comprising an AAV protein capsid and/or vector genome). In some embodiments, the virus vector comprises a modified AAV protein capsid comprising a modified capsid protein subunit as described herein and a vector genome.

[0157] For example, in some embodiments, the virus vector comprises: (a) a modified protein capsid (e.g., a modified AAV protein capsid) comprising a modified capsid protein subunit described herein; and (b) a nucleic acid comprising a terminal repeat sequence (e.g., an AAV TR), wherein the nucleic acid comprising the terminal repeat sequence is encapsidated by the modified protein capsid. The nucleic acid can optionally comprise two terminal repeats (e.g., two AAV TRs).

[0158] In some embodiments, the virus vector is a recombinant virus vector comprising a heterologous nucleic acid encoding a polypeptide or functional RNA of interest. Recombinant virus vectors are described in more detail below.

[0159] In some embodiments, the virus vectors (i) have reduced transduction of liver as compared with the level of transduction by a virus vector without the modified capsid protein subunit; (ii) exhibit enhanced systemic transduction by the virus vector in an animal subject as compared with the level observed by a virus vector without the modified capsid protein subunit; (iii) demonstrate enhanced movement across endothelial cells as compared with the level of movement by a virus vector without the modified capsid protein subunit, and/or (iv) exhibit a selective enhancement in transduction of muscle tissue (e.g., skeletal muscle, cardiac muscle and/or diaphragm muscle), (v) exhibit a selective enhancement in transduction of liver tissue, and/or (vi) reduced transduction of brain tissues (e.g., neurons) as compared with the level of transduction by a virus vector without the modified capsid protein subunit. In some embodiments, the virus vector has systemic transduction toward liver.

[0160] In some embodiments, an adeno-associated virus (AAV) vector comprises: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180 or 175; and (ii) a transfer cassette encapsidated by the protein capsid; wherein the transfer cassette comprises from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene which encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR. In some embodiments, the capsid protein subunit comprises the sequence of SEQ ID NO: 180. In some embodiments, the capsid protein subunit comprises the sequence of SEQ ID NO: 175.

[0161] In some embodiments, an adeno-associated virus (AAV) vector comprises: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180 or 175, or a sequence comprising about 1 to about 25 amino acid mutations relative to SEQ ID NO: 180 or 175; and (ii) a transfer cassette encapsidated by the protein capsid; wherein the transfer cassette comprises from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene which encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR. In some embodiments, the capsid protein subunit comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more amino acid mutations relative to SEQ ID NO: 180 or 175.

[0162] In some embodiments, at least one of the 5' ITR and the 3' ITR is about 110 to about 160 nucleotides in length. In some embodiments, the 5' ITR is the same length as the 3' ITR. In some embodiments the 5' ITR and the 3' ITR have different lengths. In some embodiments, at least one of the 5' ITR and the 3' ITR is isolated or derived from the genome of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV or Bovine AAV. In some embodiments, the 5' ITR comprises the sequence of SEQ ID NO: 3003. In some embodiments, the 3' ITR comprises the sequence of SEQ ID NO: 3004.

[0163] In some embodiments, the promoter is a constitutive promoter. In some embodiments, the promoter is an inducible promoter. In some embodiments, the promoter is a tissue-specific promoter. In some embodiments, the promoter is selected from the group consisting of the CBA promoter, the GUSB240 promoter, the GUSB379 promoter, the HSVTK promoter, the CMV promoter, the SV40 early promoter, the SV40 late promoter, the metallothionein promoter, the murine mammary tumor virus (MMTV)

promoter, the Rous sarcoma virus (RSV) promoter, the polyhedrin promoter, the chicken β -actin (CBA) promoter, the EF-1 alpha promoter, the dihydrofolate reductase (DHFR) promoter, and the phosphoglycerol kinase (PGK) promoter. In some embodiments, the promoter is selected from the group consisting of the CBA promoter, the GUSB240 promoter, the GUSB379 promoter, and the HSVTK promoter. In some embodiments, the promoter comprises a sequence at least 95% or 100% identical to any one of SEQ ID NO: 3005, SEQ ID NO: 3006, SEQ ID NO: 3007, or SEQ ID NO: 3008.

[0164] In some embodiments, the NPC1 protein is the human NPC1 protein. In some embodiments, the NPC1 protein has a sequence that is at least 90% identical to the sequence of the human NPC1 protein. In some embodiments, the NPC1 protein has a sequence that is at least 95% identical to the sequence of the human NPC1 protein. In some embodiments, the NPC1 protein has a sequence that is at least 98% identical to the sequence of the human NPC1 protein. In some embodiments, the NPC1 protein comprises the sequence of SEQ ID NO: 3001.

[0165] In some embodiments, the transgene comprises the sequence of SEQ ID NO: 3002.

[0166] In some embodiments, the polyadenylation signal is selected from simian virus 40 (SV40), rBG, α -globin, β -globin, human collagen, human growth hormone (hGH), polyoma virus, human growth hormone (hGH) and bovine growth hormone (bGH). In some embodiments, the polyadenylation signal is the SV40 polyadenylation signal. In some embodiments, the polyadenylation signal is the rBG polyadenylation signal.

[0167] In some embodiments, the polyadenylation signal comprises the sequence at least 95% or 100% identical to SEQ ID NO: 3012 or to SEQ ID NO: 3013.

[0168] In some embodiments, the transfer cassette further comprises an enhancer. In some embodiments, the enhancer is the CMV enhancer. In some embodiments, the enhancer comprises the sequence of SEQ ID NO: 3009, or a sequence at least 95% identical thereto.

[0169] In some embodiments, the transfer cassette further comprises an intronic sequence. In some embodiments, the intronic sequence is a chimeric sequence.

[0170] In some embodiments, the intronic sequence is a hybrid sequence. In some embodiments, the intronic sequence comprises a sequence isolated or derived from

SV40. In some embodiments, the intronic sequence comprises the sequence of any one of SEQ ID NO: 3010-3011. In some embodiments, the AAV transfer cassette comprises the sequence of any one of SEQ ID NO: 3014-3019.

[0171] It will be understood by those skilled in the art that the modified capsid protein subunits, protein capsids and virus vectors described herein exclude those capsid protein subunits, protein capsids and virus vectors that have the indicated amino acids at the specified positions in their native state (i.e., are not mutants).

AAV Transfer Cassettes

[0172] Described herein are AAV transfer cassettes, nucleic acids and plasmids used in the production of recombinant adeno-associated viral (rAAV) vectors. The disclosed cassettes, nucleic acids and plasmids comprise sequences that may be used to express one or more transgenes having therapeutic efficacy in the amelioration, treatment and/or prevention of one or more diseases or disorders.

[0173] In some embodiments, the AAV transfer cassettes comprise a 5' inverted terminal repeat (ITR); a transgene; and a 3' ITR. In some embodiments, the AAV transfer cassettes comprise a 5' ITR, a promoter, a transgene, and a 3' ITR. In some embodiments, the AAV transfer cassettes comprise a 5' ITR, a promoter, a transgene, a polyadenylation sequence and a 3' ITR. In some embodiments, the AAV transfer cassettes comprise a 5' ITR, a promoter, a transgene, a polyadenylation sequence and a 3' ITR; wherein the transfer cassette comprises an intronic sequence. In some embodiments, the AAV transfer cassettes comprise a 5' ITR, a promoter, an intronic sequence, a transgene, a polyadenylation sequence and a 3' ITR. In some embodiments, wherein the transgene encodes the NPC1 protein, or a fragment or variant thereof.

Inverted Terminal Repeat

[0174] Inverted Terminal Repeat or ITR sequences are sequences that mediate AAV proviral integration and for packaging of AAV DNA into virions. ITRs are involved in a variety of activities in the AAV life cycle. For example, the ITR sequences, which can form

a hairpin structure, play roles in excision from the plasmid after transfection, replication of the vector genome, and integration and rescue from a host cell genome.

[0175] The AAV transfer cassettes of the disclosure may comprise a 5' ITR and a 3' ITR. The ITR sequences may be about 110 to about 160 nucleotides in length, for example 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159 or 160 nucleotides in length. In some embodiments, the ITR sequences may be about 141 nucleotides in length. In some embodiments, the 5' ITR is the same length as the 3' ITR. In some embodiments, the 5' ITR and the 3' ITR have different lengths. In some embodiments, the 5' ITR is longer than the 3' ITR, and in other embodiments, the 3' ITR is longer than the 5' ITR.

[0176] The ITRs may be isolated or derived from the genome of any AAV, for example the AAVs listed in Table 1. In some embodiments, at least one of the 5' ITR and the 3' ITR is isolated or derived from the genome of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV or Bovine AAV. In some embodiments, at least one of the 5' ITR and the 3' ITR may be a wildtype or mutated ITR isolated derived from a member of another parvovirus species besides AAV. For example, in some embodiments, an ITR may be a wildtype or mutant ITR isolated or derived from bocavirus or parvovirus B19.

[0177] In some embodiments, the ITR comprises a modification to promote production of a scAAV. In some embodiments, the modification to promote production of a scAAV is deletion of the terminal resolution sequence (TRS) from the ITR. In some embodiments, the 5' ITR is a wildtype ITR, and the 3' ITR is a mutated ITR lacking the terminal resolution sequence. In some embodiments, the 3' ITR is a wildtype ITR, and the 5' ITR is a mutated ITR lacking the terminal resolution sequence. In some embodiments, the terminal resolution sequence is absent from both the 5' ITR and the 3' ITR. In other embodiments, the modification to promote production of a scAAV is replacement of an ITR with a different hairpin-forming sequence, such as a shRNA-forming sequence.

[0178] In some embodiments, the 5' ITR may comprise the sequence of SEQ ID NO: 3003, or a sequence at least 95% identical thereto. In some embodiments, the 3' ITR

may comprise the sequence of SEQ ID NO: 3004, or a sequence at least 95% identical thereto. In some embodiments, the 5' ITR comprises the sequence of SEQ ID NO: 3003, and the 3' ITR comprises the sequence of SEQ ID NO: 3004.

[0179] In some embodiments, the AAV transfer cassettes comprise one or more "surrogate" ITRs, i.e., non-ITR sequences that serve the same function as ITRs. See, e.g., Xie, J. et al., *Mol. Ther.*, 25(6): 1363-1374 (2017). In some embodiments, an ITR in an AAV transfer cassette is replaced by a surrogate ITR. In some embodiments, the surrogate ITR comprises a hairpin-forming sequence. In some embodiments, the surrogate ITR is a short hairpin (sh)RNA-forming sequence.

Promoters, Enhancers, Repressors and Other Regulatory Sequences

[0180] Gene expression may be controlled by nucleotide sequences called promoters and enhancers that flank the coding region for a given protein.

[0181] As used herein, the term "promoter" refers to one or more nucleic acid control sequences that direct transcription of an operably linked nucleic acid. Promoters may include nucleic acid sequences near the start site of transcription, such as a TATA element. Promoters may also include cis-acting polynucleotide sequences that can be bound by transcription factors.

[0182] A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[0183] Gene expression may also be controlled by one or more distal "enhancer" or "repressor" elements, which can be located as much as several thousand base pairs from the start site of transcription. Enhancer or repressor elements regulate transcription in an analogous manner to cis-acting elements near the start site of transcription, with the exception that enhancer elements can act from a distance from the start site of transcription.

[0184] In some embodiments, the AAV transfer cassettes described herein comprise a promoter. The promoter may be, for example, a constitutive promoter or an inducible promoter. In some embodiments, the promoter is a tissue-specific promoter.

[0185] Exemplary promoters that may be used in the AAV transfer cassettes described herein include the CMV promoter, the SV40 early promoter, the SV40 late promoter, the metallothionein promoter, the murine mammary tumor virus (MMTV) promoter, the Rous sarcoma virus (RSV) promoter, the polyhedrin promoter, the chicken β -actin (CBA) promoter, the dihydrofolate reductase (DHFR) promoter, and the phosphoglycerol kinase (PGK) promoter. In some embodiments, the promoter is selected from the group consisting of the chicken β -actin (CBA) promoter, the EF-1 alpha promoter, and the EF-1 alpha short promoter. In some embodiments, the promoter comprises a sequence selected from any one of SEQ ID NO: 3005-3008, or a sequence at least 95% identical thereto.

[0186] In some embodiments, the AAV transfer cassettes described herein comprise an enhancer. The enhancer may be, for example, the CMV enhancer. In some embodiments, the enhancer comprises the sequence of SEQ ID NO: 3009, or a sequence at least 95% identical thereto.

[0187] A non-limiting list of exemplary tissue-specific promoters and enhancers that may be used in the AAV transfer cassettes described herein includes: HMG-CoA reductase promoter; sterol regulatory element 1 (SRE-1); phosphoenol pyruvate carboxy kinase (PEPCK) promoter; human C-reactive protein (CRP) promoter; human glucokinase promoter; cholesterol 7-alpha hydroxylase (CYP-7) promoter; beta-galactosidase alpha-2,6 sialyltransferase promoter; insulin-like growth factor binding protein (IGFBP-1) promoter; aldolase B promoter; human transferrin promoter; collagen type I promoter; prostatic acid phosphatase (PAP) promoter; prostatic secretory protein of 94 (PSP 94) promoter; prostate specific antigen complex promoter; human glandular kallikrein gene promoter (hgt-1); the myocyte-specific enhancer binding factor MEF-2; muscle creatine kinase promoter; pancreatitis associated protein promoter (PAP); elastase 1 transcriptional enhancer; pancreas specific amylase and elastase enhancer promoter; pancreatic cholesterol esterase gene promoter; uteroglobin promoter; cholesterol side-chain cleavage (SCC) promoter; gamma-gamma enolase (neuron-specific enolase, NSE)

promoter; neurofilament heavy chain (NF-H) promoter; human CGL-1/granzyme B promoter; the terminal deoxy transferase (TdT), lambda 5, VpreB, and Ick (lymphocyte specific tyrosine protein kinase p56^{lck}) promoter; the humans CD2 promoter and its 3' transcriptional enhancer; the human NK and T cell specific activation (NKG5) promoter; pp60c-src tyrosine kinase promoter; organ-specific neoantigens (OSNs), mw 40 kDa (p40) promoter; colon specific antigen-P promoter; human alpha-lactalbumin promoter; phosphoenolpyruvate carboxykinase (PEPCK) promoter, HER2/neu promoter, casein promoter, IgG promoter, Chorionic Embryonic Antigen promoter, elastase promoter, porphobilinogen deaminase promoter, insulin promoter, growth hormone factor promoter, tyrosine hydroxylase promoter, albumin promoter, alphafetoprotein promoter, acetylcholine receptor promoter, alcohol dehydrogenase promoter, alpha or beta globin promoter, T-cell receptor promoter, the osteocalcin promoter the IL-2 promoter, IL-2 receptor promoter, whey (wap) promoter, and the MHC Class II promoter.

Transgene

[0188] The AAV transfer cassettes described herein comprise a transgene for expression in a target cell.

[0189] The transgene may be any heterologous nucleic acid sequence(s) of interest. Such nucleic acids may include nucleic acids encoding polypeptides, including therapeutic (e.g., for medical or veterinary uses) or immunogenic (e.g., for vaccines) polypeptides or RNAs. Alternatively, the nucleic acid may encode an antisense nucleic acid, a ribozyme, RNAs that effect spliceosome-mediated/ram-splicing, interfering RNAs (RNAi) including siRNA, shRNA or miRNA that mediate gene silencing, and other non-translated RNAs. In some embodiments, the nucleic acid sequence may direct gene editing. For example, the nucleic acid may encode a gene-editing molecule such as a guide RNA or a nuclease. In some embodiments, the nucleic acid may encode a zinc-finger nuclease, a homing endonuclease, a TALEN (transcription activator-like effector nuclease), a NgAgo (agronaute endonuclease), a SGN (structure-guided endonuclease), or a RGN (RNA-guided nuclease) such as a Cas9 nuclease or a Cpf1 nuclease. In some embodiments, the nucleic acid may share homology with and recombine with a locus on a host

chromosome. This approach can be utilized, for example, to correct a genetic defect in the host cell.

[0190] The virus vectors according to the present disclosure provide a means for delivering transgenes into a broad range of cells, including dividing and non-dividing cells. The virus vectors can be employed to deliver a transgene to a cell *in vitro*, e.g., to produce a polypeptide *in vitro* or for *ex vivo* gene therapy. The virus vectors are additionally useful in a method of delivering a transgene to a subject in need thereof, e.g., to express an immunogenic or therapeutic polypeptide or a functional RNA. In this manner, the polypeptide or functional RNA can be produced *in vivo* in the subject. The subject can be in need of the polypeptide because the subject has a deficiency of the polypeptide. Further, the method can be practiced because the production of the polypeptide or functional RNA in the subject may impart some beneficial effect. As used herein, the term “functional RNA” refers to any non-coding RNA sequence that has one or more functions in a cell, such as those described in the preceding paragraph.

[0191] The virus vectors can also be used to deliver nucleic acids for the production of a polypeptide of interest or functional RNA in cultured cells or in a subject (e.g., using the subject as a bioreactor to produce the polypeptide or to observe the effects of the functional RNA on the subject, for example, in connection with screening methods).

[0192] In general, the virus vectors of the present disclosure can be employed to deliver a transgene encoding a polypeptide or functional RNA to treat and/or prevent any disease state for which it is beneficial to deliver a therapeutic polypeptide or functional RNA.

[0193] In some embodiments, the transgene is useful for treating NPC1. In some embodiments, the transgene encodes the NPC1 protein. The NPC1 protein may be, for example, the human NPC1 protein. In some embodiments, the NPC1 protein has a sequence that is at least 90% identical, at least 95% identical, or at least 98% identical to the sequence of the human NPC1 protein. In some embodiments, the NPC1 protein comprises one or more of the single nucleotide changes listed in the Table 9 (numbering based on SEQ ID NO: 3001 or 3020). In some embodiments, the NPC1 protein is a truncated form of the human NPC1 protein. In some embodiments, the NPC1 protein comprises the sequence of SEQ ID NO: 3001, or a sequence at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical,

or at least 99% identical thereto. In some embodiments, the NPC1 protein comprises the sequence of SEQ ID NO: 3020, or a sequence at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical thereto. In some embodiments, the NPC1 protein comprises the sequence of SEQ ID NO: 3001 or 3020, with one or more of the single nucleotide changes listed in Table 9. In some embodiments, the NPC1 protein has a sequence as shown in UniProt Accession No. O15118, incorporated herein by reference in its entirety.

[0194] In some embodiments, the transgene comprises the sequence of SEQ ID NO: 3002, or a sequence at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical thereto. In some embodiments, the transgene comprises the sequence of SEQ ID NO: 3002, or a sequence with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more nucleic acid changes relative thereto. In some embodiments, the transgene encodes the amino acid sequence of SEQ ID NO: 3001. In some embodiments, the transgene encodes the amino acid sequence of SEQ ID NO: 3020.

Table 9: NPC1 Variant Sequences

Position numbering based on SEQ ID NO: 3001 or SEQ ID NO: 3020.

Position	Mutation	Position	Mutation	Position	Mutation
63	C→R	691	P→L	1012	G→D
74	C→Y	695	L→V	1015	G→V
92	Q→R	700	D→N	1016	H→R
113	C→R	703	F→S	1023	V→G
137	T→M	724	L→P	1034	G→R
151	S→G	727	V→F	1035	A→V
166	P→S	734	S→I	1036	T→K
177	C→G	742	E→K	1036	T→M
177	C→Y	745	A→E	1049	A→V
215	H→R	754	M→K	1054	A→T
222	N→S	757	V→A	1059	R→Q
231	V→G	763	F→L	1061	I→T
237	P→S	767	A→V	1062	A→V
242	D→H	775	Q→P	1066	T→N
242	D→N	789	R→C	1087	F→L
247	C→Y	789	R→G	1088	Y→C
248	G→V	825	Y→C	1089	E→K
272	M→R	849	S→I	1094	I→T
333	G→D	858	I→V	1097	D→N

372	R→W	862	Q→L	1137	N→I
378	V→A	865	S→L	1140	G→V
380	L→F	871	Y→C	1142	M→T
381	W→C	873	V→A	1150	N→K
388	A→P	874	D→V	1156	N→I
389	R→C	888	P→S	1156	N→S
401	P→T	889	V→M	1165	V→M
404	R→P	890	Y→C	1167	F→L
404	R→Q	899	Y→D	1168	C→Y
404	R→W	910	G→S	1174	A→V
433	P→L	917	D→Y	1186	R→H
434	P→L	926	A→T	1189	E→G
434	P→S	927	A→V	1205	T→K
451	E→K	928	Q→P	1205	T→R
472	L→P	929	L→P	1212	V→L
473	S→P	934	R→Q	1213	L→F
474	P→L	940	S→L	1213	L→V
479	C→Y	942	W→C	1216	A→V
509	Y→S	943	I→M	1220	I→T
510	H→P	944	D→N	1224	F→L
511	T→M	945	D→N	1236	G→E
512	H→R	948	D→H	1240	G→R
518	R→Q	948	D→N	1249	S→G
518	R→W	948	D→Y	1266	R→Q
521	A→S	950	V→M		
537	F→L	954	S→L		
543	P→L	956	C→Y		
574	T→K	958	R→L		
576	K→R	958	R→Q		
605	A→V	959	V→E		
612	E→D	961-966	NITDQF→S		
615	R→C	961	N→S		
615	R→L	968	N→S		
631	M→R	971	V→G		
640	G→R	976	C→R		
642	M→I	978	R→C		
652	S→W	986	G→S		
660	G→S	992	G→A		
664	V→M	992	G→R		
666	S→N	992	G→W		
670	C→W	996	M→R		
673	G→V	1004	S→L		
684	L→F	1007	P→A		

Polyadenylation (PolyA) Signal

[0195] Polyadenylation signals are nucleotide sequences found in nearly all mammalian genes and control the addition of a string of approximately 200 adenosine residues (the poly(A) tail) to the 3' end of the gene transcript. The poly(A) tail contributes to mRNA stability, and mRNAs lacking the poly(A) tail are rapidly degraded. There is also evidence

that the presence of the poly(A) tail positively contributes to the translatability of mRNA by affecting the initiation of translation.

[0196] In some embodiments, the AAV transfer cassettes of the disclosure comprise a polyadenylation signal. The polyadenylation signal may be selected from the polyadenylation signal of simian virus 40 (SV40), α -globin, β -globin, human collagen, human growth hormone (hGH), polyoma virus, human growth hormone (hGH) and bovine growth hormone (bGH). In some embodiments, the polyadenylation signal is the SV40 polyadenylation signal. In some embodiments, the polyadenylation signal is the rBG polyadenylation signal. In some embodiments, the polyadenylation signal comprises the sequence of SEQ ID NO: 3012 or SEQ ID NO: 3013. In some embodiments, the polyadenylation signal comprises a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence of SEQ ID NO: 3012 or SEQ ID NO: 3013.

Stuffer Sequences

[0197] AAV vectors typically accept inserts of DNA having a defined size range which is generally about 4 kb to about 5.2 kb, or slightly more. Thus, for shorter transgene sequences, it may be necessary to include additional nucleic acid in the insert fragment in order to achieve the required length which is acceptable for the AAV vector. Accordingly, in some embodiments, the AAV transfer cassettes of the disclosure may comprise a stuffer sequence. The stuffer sequence may be for example, a sequence between 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-75, 75-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-750, 750-1,000, 1,000-1,500, 1,500-2,000, 2,000-2,500, 2,500-3,000, 3,000-3,500, 3,500-4,000, 4,000-4,500, to 4,500-5,000 nucleotides in length. The stuffer sequence can be located in the cassette at any desired position such that it does not prevent a function or activity of the vector.

Intronic Sequences

[0198] In some embodiments, the AAV transfer cassettes of the disclosure may comprise an intronic sequence. Inclusion of an intronic sequence may enhance expression

compared with expression in the absence of the intronic sequence. In some the intronic sequence can increase gene expression without functioning as a binding site for transcription factors. For example, the intronic sequence can increase transcript levels by affecting the rate of transcription, nuclear export, and transcript stability. In some embodiments, the intronic sequence increases the efficiency of mRNA translation.

[0199] In some embodiments, the intronic sequence is a hybrid or chimeric sequence. In some embodiments, the intronic sequence is isolated or derived from an intronic sequence of one or more of SV40, β -globin, chicken beta-actin, minute virus of mice (MVM), factor IX, and/or human IgG (heavy or light chain). In some embodiments, the intronic sequence is isolated or derived from SV40. In some embodiments, the intronic sequence is chimeric. In some embodiments, the intronic sequence comprises the sequence of SEQ ID NO: 3010 or SEQ ID NO: 3011, or a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

[0200] The intronic sequence may be located anywhere in the transfer cassette where it doesn't interfere with production of the AAV vector. For example, in some embodiments, the intronic sequence may be located between the promoter and the transgene.

Illustrative AAV Transfer Cassettes

[0201] In some embodiments, an adeno-associated virus (AAV) transfer cassette comprises a 5' inverted terminal repeat (ITR), a promoter, a transgene, a polyadenylation signal, and a 3' ITR. In some embodiments, an adeno-associated virus (AAV) transfer cassette comprises a 5' inverted terminal repeat (ITR), a promoter, an intronic sequence, a transgene, a polyadenylation signal, and a 3' ITR. In some embodiments, the transgene encodes the NPC1 protein. In some embodiments, the AAV transfer cassette further comprises an enhancer. In some embodiments, the AAV transfer cassette further comprises an intronic sequence.

[0202] In some embodiments, the 5' ITR comprises the sequence of SEQ ID NO: 3003 and the 3' ITR comprises the sequence of SEQ ID NO: 3004. In some embodiments, the enhancer comprises the sequence of SEQ ID NO: 3009. In some embodiments, the promoter comprises the sequence of any one of SEQ ID NO: 3005-3008. In some embodiments, the intronic sequence comprises the sequence of SEQ ID NO: 3010 or

3011. In some embodiments, the transgene comprises the sequence of SEQ ID NO: 3002. In some embodiments, the polyA signal comprises the sequence of SEQ ID NO: 3012 or 3013. In some embodiments, the AAV transfer cassette comprises the sequence of any one of SEQ ID NO: 3014-3019.

[0203] In some embodiments, an AAV transfer cassette comprises a 5' ITR, a CBA promoter, a SV40 intron, a transgene encoding the NPC1 protein, a SV40 polyadenylation signal, and a 3' ITR. In some embodiments, an AAV transfer cassette comprises a 5' ITR, a GUSB240 promoter, a chimeric intron, a transgene encoding the NPC1 protein, a rBG polyadenylation signal, and a 3' ITR. In some embodiments, an AAV transfer cassette comprises a 5' ITR, a GUSB379 promoter, a SV40 intron, a transgene encoding the NPC1 protein, a rBG polyadenylation signal, and a 3' ITR. In some embodiments, an AAV transfer cassette comprises a 5' ITR, a GUSB240 promoter, a chimeric intron, a transgene encoding the NPC1 protein, a SV40 polyadenylation signal, and a 3' ITR. In some embodiments, an AAV transfer cassette comprises a 5' ITR, a GUSB240 promoter, a SV40 intron, a transgene encoding the NPC1 protein, a SV40 polyadenylation signal, and a 3' ITR. In some embodiments, an AAV transfer cassette comprises a 5' ITR, a CMV enhancer, a HSVTK promoter, a transgene encoding the NPC1 protein, a rBG polyadenylation signal, and a 3' ITR.

[0204] In some embodiments, an AAV transfer cassette comprises a 5' ITR comprising the sequence of SEQ ID NO: 3003, a CBA promoter comprising the sequence of SEQ ID NO: 3005, a SV40 intron comprising the sequence of SEQ ID NO: 3010, a transgene encoding the NPC1 protein (SEQ ID NO: 3001), a SV40 polyadenylation signal comprising SEQ ID NO: 3012, and a 3' ITR comprising the sequence of SEQ ID NO: 3004.

[0205] In some embodiments, an AAV transfer cassette comprises a 5' ITR comprising the sequence of SEQ ID NO: 3003, a GUSB240 promoter comprising the sequence of SEQ ID NO: 3006, a chimeric intron comprising SEQ ID NO: 3011, a transgene encoding the NPC1 protein (SEQ ID NO: 3001), a rBG polyadenylation signal comprising SEQ ID NO: 3013, and a 3' ITR comprising the sequence of SEQ ID NO: 3004.

[0206] In some embodiments, an AAV transfer cassette comprises a 5' ITR comprising the sequence of SEQ ID NO: 3003, a GUSB379 promoter comprising SEQ ID NO: 3006,

a SV40 intron comprising the sequence of SEQ ID NO: 3010, a transgene encoding the NPC1 protein (SEQ ID NO: 3001), a rBG polyadenylation signal comprising SEQ ID NO: 3013, and a 3' ITR comprising the sequence of SEQ ID NO: 3004.

[0207] In some embodiments, an AAV transfer cassette comprises a 5' ITR comprising the sequence of SEQ ID NO: 3003, a GUSB240 promoter comprising SEQ ID NO: 3007, a chimeric intron comprising the sequence of SEQ ID NO: 3011, a transgene encoding the NPC1 protein (SEQ ID NO: 3001), a SV40 polyadenylation signal comprising SEQ ID NO: 3012, and a 3' ITR comprising the sequence of SEQ ID NO: 3004.

[0208] In some embodiments, an AAV transfer cassette comprises a 5' ITR comprising the sequence of SEQ ID NO: 3003, a GUSB240 promoter comprising SEQ ID NO: 3006, a SV40 intron comprising the sequence of SEQ ID NO: 3010, a transgene encoding the NPC1 protein (SEQ ID NO: 3001), a SV40 polyadenylation signal comprising SEQ ID NO: 3012, and a 3' ITR comprising the sequence of SEQ ID NO: 3004.

[0209] In some embodiments, an AAV transfer cassette comprises a 5' ITR comprising the sequence of SEQ ID NO: 3003, a CMV enhancer, a HSVTK promoter comprising SEQ ID NO: 3008, a transgene encoding the NPC1 protein (SEQ ID NO: 3001), a rBG polyadenylation signal comprising SEQ ID NO: 3013, and a 3' ITR comprising the sequence of SEQ ID NO: 3004.

[0210] In some embodiments, a nucleic acid comprises an AAV transfer cassette. In some embodiments, a nucleic acid comprises a transgene, wherein the transgene encodes the amino acid sequence of SEQ ID NO: 3001. In some embodiments, a nucleic acid comprises a transgene, wherein the transgene encodes the amino acid sequence of SEQ ID NO: 3020. In some embodiments, a nucleic acid comprises, from 5' to 3', a 5' inverted terminal repeat (ITR); a promoter; a transgene; a polyadenylation signal; and a 3' ITR; wherein the transgene encodes the amino acid sequence of SEQ ID NO: 3001 or SEQ ID NO: 3020. In some embodiments, a nucleic acid comprises, from 5' to 3', a 5' inverted terminal repeat (ITR); a promoter; a transgene; a polyadenylation signal; and a 3' ITR; wherein the nucleic acid comprises an intronic sequence; wherein the transgene encodes the amino acid sequence of SEQ ID NO: 3001 or SEQ ID NO: 3020. In some embodiments, a nucleic acid comprises, from 5' to 3', a 5' inverted terminal repeat (ITR); a chicken beta-actin promoter; a transgene; a polyadenylation signal; and a 3' ITR;

wherein the transfer cassette comprises an intronic sequence; wherein the transgene encodes the amino acid sequence of SEQ ID NO: 3001 or SEQ ID NO: 3020. In some embodiments, a nucleic acid comprises, from 5' to 3', a 5' inverted terminal repeat (ITR); a promoter; an intronic sequence; a transgene; a polyadenylation signal; and a 3' ITR; wherein the transgene encodes the amino acid sequence of SEQ ID NO: 3001 or SEQ ID NO: 3020. In some embodiments, a nucleic acid comprises, from 5' to 3', a 5' inverted terminal repeat (ITR); a chicken beta-actin promoter; an intronic sequence; a transgene; a polyadenylation signal; and a 3' ITR; wherein the transgene encodes the amino acid sequence of SEQ ID NO: 3001 or SEQ ID NO: 3020. The AAV transfer cassettes described herein may be incorporated into a vector (e.g., a plasmid or a bacmid) using standard molecular biology techniques. The vector (e.g., plasmid or bacmid) may further comprise one or more genetic elements used during production of AAV, including, for example, AAV rep and cap genes, and helper virus protein sequences.

Methods for Producing Virus Vectors

[0211] Also provided herein are methods of producing virus vectors. In some embodiments, a method of producing an AAV vector that evades neutralizing antibodies, comprises: a) identifying contact amino acid residues that form a three dimensional antigenic footprint on an AAV capsid protein subunit or protein capsid; b) generating a library of AAV capsid protein subunits comprising amino acid substitutions of the contact amino acid residues identified in (a); c) producing AAV particles comprising capsid protein subunits from the library of AAV capsid protein subunits of (b); d) contacting the AAV particles of (c) with cells under conditions whereby infection and replication can occur; e) selecting AAV particles that can complete at least one infectious cycle and replicate to titers similar to control AAV particles: 1) contacting the AAV particles selected in (e) with neutralizing antibodies and cells under conditions whereby infection and replication can occur; and g) selecting AAV particles that are not neutralized by the neutralizing antibodies of (f). Nonlimiting examples of methods for identifying contact amino acid residues include peptide epitope mapping and/or cryo-electron microscopy.

[0212] Resolution and identification of the antibody contact residues within the three dimensional antigenic footprint allows for their subsequent modification through random,

rational and/or degenerate mutagenesis to generate antibody-evading AAV protein capsids and/or capsid protein subunits that can be identified through further selection and/or screening.

[0213] Thus, in some embodiments, a method of producing an AAV vector that evades neutralizing antibodies comprises: a) identifying contact amino acid residues that form a three dimensional antigenic footprint on an AAV capsid protein subunits and/or protein capsids; b) generating AAV capsid protein subunits comprising amino acid substitutions of the contact amino acid residues identified in (a) by random, rational and/or degenerate mutagenesis; c) producing AAV particles comprising capsid protein subunits from the AAV capsid protein subunits of (b); d) contacting the AAV particles of (c) with cells under conditions whereby infection and replication can occur; e) selecting AAV particles that can complete at least one infectious cycle and replicate to titers similar to control AAV particles; f) contacting the AAV particles selected in (e) with neutralizing antibodies and cells under conditions whereby infection and replication can occur; and g) selecting AAV particles that are not neutralized by the neutralizing antibodies of (f).

[0214] Nonlimiting examples of methods for identifying contact amino acid residues include peptide epitope mapping and/or cryo-electron microscopy. Methods of generating AAV capsid protein subunits comprising amino acid substitutions of contact amino acid residues by random, rational and/or degenerate mutagenesis are known in the art.

[0215] This comprehensive approach presents a platform technology that can be applied to modifying any AAV protein capsid and/or capsid protein subunit. Application of this platform technology yields AAV antigenic variants derived from the original AAV capsid protein subunit template without loss of transduction efficiency. As one advantage and benefit, application of this technology will expand the cohort of patients eligible for gene therapy with AAV vectors.

[0216] In some embodiments, a method of producing a virus vector comprises providing to a cell: (a) a nucleic acid template comprising at least one TR sequence (e.g., AAV TR sequence), and (b) AAV sequences sufficient for replication of the nucleic acid template and encapsidation into AAV protein capsids (e.g., AAV rep sequences and AAV cap sequences encoding the AAV capsid subunits). Optionally, the nucleic acid template further comprises at least one heterologous nucleic acid sequence. In some

embodiments, the nucleic acid template comprises two AAV ITR sequences, which are located 5' and 3' to the heterologous nucleic acid sequence (if present), although they need not be directly contiguous thereto.

[0217] The nucleic acid template and AAV rep and cap sequences are provided under conditions such that virus vector comprising the nucleic acid template packaged within the AAV protein capsid is produced in the cell. The method can further comprise the step of collecting the virus vector from the cell. The virus vector can be collected from the medium and/or by lysing the cells.

[0218] The cell can be a cell that is permissive for AAV viral replication. Any suitable cell known in the art may be employed. In some embodiments, the cell is a mammalian cell. As another option, the cell can be a trans-complementing packaging cell line that provides functions deleted from a replication-defective helper virus, e.g., 293 cells or other E1a trans-complementing cells.

[0219] The AAV replication and capsid protein subunit sequences may be provided by any method known in the art. Current protocols typically express the AAV rep/cap genes on a single plasmid. The AAV replication and packaging sequences need not be provided together, although it may be convenient to do so. The AAV rep and/or cap sequences may be provided by any viral or non-viral vector. For example, the rep/cap sequences may be provided by a hybrid adenovirus or herpesvirus vector (e.g., inserted into the E1a or E3 regions of a deleted adenovirus vector). EBV vectors may also be employed to express the AAV cap and rep genes. One advantage of this method is that EBV vectors are episomal, yet will maintain a high copy number throughout successive cell divisions (i.e., are stably integrated into the cell as extra-chromosomal elements, designated as an "EBV based nuclear episome," see Margolski, (1992) Curr. Top. Microbiol. Immun. 158:67).

[0220] As a further alternative, the rep/ cap sequences may be stably incorporated into a cell.

[0221] Typically the AAV rep/ cap sequences will not be flanked by the TRs, to prevent rescue and/or packaging of these sequences.

[0222] The nucleic acid template can be provided to the cell using any method known in the art. For example, the template can be supplied by a plasmid or viral vector. In some

embodiments, the nucleic acid template is supplied by a herpesvirus or adenovirus vector (e.g., inserted into the E1a or E3 regions of a deleted adenovirus). As another illustration, Palombo et al., (1998) J. Virology 72:5025, describes a baculovirus vector carrying a reporter gene flanked by the AAV TRs. EBV vectors may also be employed to deliver the template, as described above with respect to the rep/cap genes.

[0223] In some embodiments, the nucleic acid template is provided by a replicating rAAV virus. In some embodiments, an AAV provirus comprising the nucleic acid template is stably integrated into the nucleus of the cell.

[0224] To enhance virus titers, helper virus functions (e.g., adenovirus or herpesvirus) that promote a productive AAV infection can be provided to the cell. Helper virus sequences necessary for AAV replication are known in the art. Typically, these sequences will be provided by a helper adenovirus or herpesvirus vector. Alternatively, the adenovirus or herpesvirus sequences can be provided by another non-viral or viral vector, e.g., as a noninfectious adenovirus miniplasmid that carries all of the helper genes that promote efficient AAV production as described by Ferrari et al., (1997) Nature Med. 3: 1295, and U.S. Patent Nos. 6,040,183 and 6,093,570.

[0225] Further, the helper virus functions may be provided by a packaging cell with the helper sequences embedded in the chromosome or maintained as a stable extrachromosomal element. Generally, the helper virus sequences cannot be packaged into AAV virions, e.g., are not flanked by ITRs.

[0226] Those skilled in the art will appreciate that it may be advantageous to provide the AAV replication and capsid protein subunit sequences and the helper virus sequences (e.g., adenovirus sequences) on a single helper construct. This helper construct may be a non-viral or viral construct. As one nonlimiting illustration, the helper construct can be a hybrid adenovirus or hybrid herpesvirus comprising the AAV rep/cap genes.

[0227] In some embodiments, the AAV rep/cap sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. This vector further can further comprise the nucleic acid template. The AAV rep/cap sequences and/or the rAAV template can be inserted into a deleted region (e.g., the E1a or E3 regions) of the adenovirus.

[0228] In some embodiments, the AAV rep/cap sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. According to this embodiment, the rAAV template can be provided as a plasmid template.

[0229] In some embodiments, the AAV rep/cap sequences and adenovirus helper sequences are provided by a single adenovirus helper vector, and the rAAV template is integrated into the cell as a provirus. Alternatively, the rAAV template is provided by an EBV vector that is maintained within the cell as an extrachromosomal element (e.g., as an EBV based nuclear episome).

[0230] In some embodiments, the AAV rep/cap sequences and adenovirus helper sequences are provided by a single adenovirus helper. The rAAV template can be provided as a separate replicating viral vector. For example, the rAAV template can be provided by a rAAV particle or a second recombinant adenovirus particle.

[0231] According to the foregoing methods, the hybrid adenovirus vector typically comprises the adenovirus 5' and 3' *cis* sequences sufficient for adenovirus replication and packaging (i.e., the adenovirus terminal repeats and PAC sequence). The AAV rep/cap sequences and, if present, the rAAV template are embedded in the adenovirus backbone and are flanked by the 5' and 3' *cis* sequences, so that these sequences may be packaged into adenovirus protein capsids. As described above, the adenovirus helper sequences and the AAV rep/cap sequences are generally not flanked by ITRs so that these sequences are not packaged into the AAV virions. Zhang et al., ((2001) Gene Ther. 18:704-12) describe a chimeric helper comprising both adenovirus and the AAV rep and cap genes.

[0232] Herpesvirus may also be used as a helper virus in AAV packaging methods. Hybrid herpesviruses encoding the AAV Rep protein(s) may advantageously facilitate scalable AAV vector production schemes. A hybrid herpes simplex virus type I (HSV-1) vector expressing the AAV-2 rep and cap genes has been described (Conway et al., (1999) Gene Therapy 6:986 and WO 00/17377).

[0233] As a further alternative, virus vectors can be produced in insect cells using baculovirus vectors to deliver the rep/cap genes and rAAV template as described, for example, by Urabe et al., (2002) Human Gene Therapy 13: 1935-43.

[0234] AAV vector stocks free of contaminating helper virus may be obtained by any method known in the art. For example, AAV and helper virus may be readily differentiated based on size. AAV may also be separated away from helper virus based on affinity for a heparin substrate (Zolotukhin et al. (1999) *Gene Therapy* 6:973). Deleted replication-defective helper viruses can be used so that any contaminating helper virus is not replication competent. As a further alternative, an adenovirus helper lacking late gene expression may be employed, as only adenovirus early gene expression is required to mediate packaging of AAV virus. Adenovirus mutants defective for late gene expression are known in the art (e.g., ts100K and ts149 adenovirus mutants).

Recombinant Virus Vectors

[0235] The virus vectors described herein are useful for the delivery of nucleic acids to cells *in vitro*, *ex vivo*, and *in vivo*. In particular, the virus vectors can be advantageously employed to deliver or transfer nucleic acids to animal, including mammalian, cells. Thus, in some embodiments, a nucleic acid may be encapsidated by a protein capsid described herein. In some embodiments, the nucleic acid is a transfer cassette. In some embodiments, the transfer cassette comprises a vector genome (e.g., 5' ITR, transgene, and 3' ITR). In some embodiments, the nucleic acid is an AAV transfer cassette.

[0236] The transfer cassette sequence delivered by the virus vectors may be any heterologous nucleic acid sequence(s) of interest. Nucleic acids of interest include nucleic acids encoding polypeptides, including therapeutic (e.g., for medical or veterinary uses) or immunogenic (e.g., for vaccines) polypeptides or RNAs. In some embodiments, the transfer cassette comprises a 5' ITR and a 3' ITR. In some embodiments, the transfer cassette comprises a 5' ITR, a transgene, and a 3' ITR. In some embodiments, the transgene encodes a therapeutic protein or RNA.

[0237] Therapeutic polypeptides include, but are not limited to, cystic fibrosis transmembrane regulator protein (CFTR), dystrophin (including mini- and micro-dystrophins, see, e.g., Vincent et al, (1993) *Nature Genetics* 5: 130; U.S. Patent Publication No. 2003/017131; International publication WO/2008/088895, Wang et al., *Proc. Natl. Acad. Sci. USA* 97: 1 3714-13719 (2000); and Gregorevic et al., *Mol. Ther.* 16:657-64 (2008)), myostatin propeptide, follistatin, activin type 11 soluble receptor, IGF-

1, apolipoproteins such as apoA (apoA1, apoA2, apoA4, apoA-V), apoB (apoB100, ApoB48), apoC (apoCI, apoCII, apoCIII, apoCIV), apoD, apoE, apoH, apoL, apo(a), anti-inflammatory polypeptides such as the Ikappa B dominant mutant, amyloid beta, tau, sarcospan, utrophin (Tinsley et al, (1996) Nature 384:349), mini-utrophin, clotting factors (e.g., Factor VIII, Factor IX, Factor X, etc.), erythropoietin, angiostatin, endostatin, catalase, tyrosine hydroxylase, superoxide dismutase, leptin, the LDL receptor, lipoprotein lipase, progranulin, ornithine transcarbamylase, β -globin, α -globin, spectrin, alpha-1-antitrypsin, adenosine deaminase, hypoxanthine guanine phosphoribosyl transferase, β -glucocerebrosidase, battenin, sphingomyelinase, lysosomal hexosaminidase A, branched-chain keto acid dehydrogenase, frataxin, RP65 protein, cytokines (e.g., alpha-interferon, beta-interferon, gamma-interferon, interleukin-2, interleukin-4, alpha synuclein, parkin, granulocyte-macrophage colony stimulating factor, lymphotoxin, and the like), peptide growth factors, neurotrophic factors and hormones (e.g., somatotropin, insulin, insulin-like growth factors 1 and 2, platelet derived growth factor, epidermal growth factor, fibroblast growth factor, nerve growth factor, neurotrophic factor -3 and -4, brain-derived neurotrophic factor, bone morphogenic proteins [including RANKL and VEGF], glial derived growth factor, transforming growth factor - α and - β , and the like), huntingin, lysosomal acid alpha-glucosidase, iduronate-2-sulfatase, N-sulfoglucosamine sulfohydrolase, alpha-galactosidase A, receptors (e.g., the tumor necrosis growth factor soluble receptor), S100A1, ubiquitin protein ligase E3, parvalbumin, adenylyl cyclase type 6, a molecule that modulates calcium handling (e.g., SERCA_{2A}, Inhibitor 1 of PP1 and fragments thereof [e.g., WO 2006/029319 and WO 2007/100465]), a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct, anti-inflammatory factors such as IRAP, anti-myostatin proteins, aspartoacylase, monoclonal antibodies (including single chain monoclonal antibodies; an exemplary Mab is the Herceptin® Mab), neuropeptides and fragments thereof (e.g., galanin, Neuropeptide Y (see, U.S. 7,071,172)), angiogenesis inhibitors such as Vasohibins and other VEGF inhibitors (e.g., Vasohibin 2 [see, WO JP2006/073052]). Other illustrative heterologous nucleic acid sequences encode suicide gene products (e.g., thymidine kinase, cytosine deaminase, diphtheria toxin, and tumor necrosis factor), proteins that enhance or inhibit transcription

of host factors (e.g., nuclease-dead Cas9 linked to a transcription enhancer or inhibitor element, zinc-finger proteins linked to a transcription enhancer or inhibitor element, transcription activator-like (TAL) effectors linked to a transcription enhancer or inhibitor element), proteins conferring resistance to a drug used in cancer therapy, tumor suppressor gene products (e.g., p53, Rb, Wt-1), TRAIL, FAS-ligand, and any other polypeptide that has a therapeutic effect in a subject in need thereof. AAV vectors can also be used to deliver monoclonal antibodies and antibody fragments, for example, an antibody or antibody fragment directed against myostatin (see, e.g., Fang et al., *Nature Biotechnology* 23:584-590 (2005)). Heterologous nucleic acid sequences encoding polypeptides include those encoding reporter polypeptides (e.g., an enzyme). Reporter polypeptides are known in the art and include, but are not limited to, Green Fluorescent Protein, β -galactosidase, alkaline phosphatase, luciferase, and chloramphenicol acetyltransferase gene.

[0238] Optionally, the heterologous nucleic acid encodes a secreted polypeptide (e.g., a polypeptide that is a secreted polypeptide in its native state or that has been engineered to be secreted, for example, by operable association with a secretory signal sequence as is known in the art).

[0239] Alternatively, in some embodiments, the heterologous nucleic acid may encode an antisense nucleic acid, a ribozyme (e.g., as described in U.S. Patent No. 5,877,022), RNAs that effect spliceosome-mediated/ram-splicing (see, Puttaraju et al, (1999) *Nature Biotech.* 17:246; U.S. Patent No. 6,013,487; U.S. Patent No. 6,083,702), interfering RNAs (RNAi) including siRNA, shRNA or miRNA that mediate gene silencing (see, Sharp et al, (2000) *Science* 287:2431), and other non-translated RNAs, such as "guide" RNAs (Gorman et al., (1998) *Proc. Nat. Acad. Sci. USA* 95 :4929; U.S. Patent No. 5,869,248 to Yuan et al.), and the like. Exemplary untranslated RNAs include RNAi against a multiple drug resistance (MDR) gene product (e.g., to treat and/or prevent tumors and/or for administration to the heart to prevent damage by chemotherapy), RNAi against myostatin (e.g., for Duchenne muscular dystrophy), RNAi against VEGF (e.g., to treat and/or prevent tumors), RNAi against phospholamban (e.g., to treat cardiovascular disease, see, e.g., Andino et al., *J. Gene Med.* 10: 132-142 (2008) and Li et al., *Acta Pharmacol Sin.* 26:51-55 (2005)); phospholamban inhibitory or dominant-negative molecules such as

phospholamban S16E (e.g., to treat cardiovascular disease, see, e.g., Hoshijima et al. Nat. Med. 8:864-871 (2002)), RNAi to adenosine kinase (e.g., for epilepsy), and RNAi directed against pathogenic organisms and viruses (e.g., hepatitis B and/or C virus, human immunodeficiency virus, CMV, herpes simplex virus, human papilloma virus, etc.).

[0240] Further, a nucleic acid sequence that directs alternative splicing can be delivered. To illustrate, an antisense sequence (or other inhibitory sequence) complementary to the 5' and/or 3' splice site of dystrophin exon 51 can be delivered in conjunction with a U1 or U7 small nuclear (sn) RNA promoter to induce skipping of this exon. For example, a DNA sequence comprising a U1 or U7 snRNA promoter located 5' to the antisense/inhibitory sequence(s) can be packaged and delivered in a modified protein capsid.

[0241] In some embodiments, a nucleic acid sequence that directs gene editing can be delivered. For example, the nucleic acid may encode a guide RNA. In some embodiments, the guide RNA is a single guide RNA (sgRNA) comprising a crRNA sequence and a tracrRNA sequence. In some embodiments, the nucleic acid may encode a nuclease. In some embodiments, the nuclease is a zinc-finger nuclease, a homing endonuclease, a TALEN (transcription activator-like effector nuclease), a NgAgo (agronaute endonuclease), a SGN (structure-guided endonuclease), a RGN (RNA-guided nuclease), or modified or truncated variants thereof. In some embodiments, the RNA-guided nuclease is a Cas9 nuclease, a Cas12(a) nuclease (Cpf1), a Cas12b nuclease, a Cas12c nuclease, a TrpB-like nuclease, a Cas13a nuclease (C2c2), a Cas13b nuclease, or modified or truncated variants thereof. In some embodiments, the Cas9 nuclease is isolated or derived from *S. pyogenes* or *S. aureus*.

[0242] In some embodiments, a nucleic acid sequence that directs gene knockdown can be delivered. For example, the nucleic acid sequence may encode a siRNA, an shRNA, a microRNA, or an antisense nucleic acid. The virus vector may also comprise a heterologous nucleic acid that shares homology with and recombines with a locus on a host chromosome. This approach can be utilized, for example, to correct a genetic defect in the host cell.

[0243] Also provided are virus vectors that express an immunogenic polypeptide, e.g., for vaccination. The nucleic acid may encode any immunogen of interest known in the art including, but not limited to, immunogens from human immunodeficiency virus (HIV),

simian immunodeficiency virus (SIV), influenza virus, HIV or SIV gag proteins, tumor antigens, cancer antigens, bacterial antigens, viral antigens, and the like.

[0244] The use of parvoviruses as vaccine vectors is known in the art (see, e.g., Miyamura et al, (1994) Proc. Nat. Acad. Sci USA 91:8507; U.S. Patent No. 5,916,563 to Young et al, U.S. Patent No. 5,905,040 to Mazzara et al, U.S. Patent No. 5,882,652, U.S. Patent No. 5,863,541 to Samulski et al). The antigen may be presented in the parvovirus capsid.

[0245] Alternatively, the antigen may be expressed from a heterologous nucleic acid introduced into a recombinant vector genome. In some embodiments, any immunogen of interest as described herein and/or as is known in the art can be provided by the virus vectors described herein.

[0246] An immunogenic polypeptide can be any polypeptide suitable for eliciting an immune response and/or protecting the subject against an infection and/or disease, including, but not limited to, microbial, bacterial, protozoal, parasitic, fungal and/or viral infections and diseases. For example, the immunogenic polypeptide can be an orthomyxovirus immunogen (e.g., an influenza virus immunogen, such as the influenza virus hemagglutinin (HA) surface protein or the influenza virus nucleoprotein, or an equine influenza virus immunogen) or a lentivirus immunogen (e.g., an equine infectious anemia virus immunogen, a Simian Immunodeficiency Virus (SIV) immunogen, or a Human Immunodeficiency Virus (HIV) immunogen, such as the HIV or SIV envelope GP 160 protein, the HIV or SIV matrix/capsid proteins, and the HIV or SIV gag, pol and env genes products). The immunogenic polypeptide can also be an arenavirus immunogen (e.g., Lassa fever virus immunogen, such as the Lassa fever virus nucleocapsid protein and the Lassa fever envelope glycoprotein), a poxvirus immunogen (e.g., a vaccinia virus immunogen, such as the vaccinia LI or L8 gene products), a flavivirus immunogen (e.g., a yellow fever virus immunogen or a Japanese encephalitis virus immunogen), a filovirus immunogen (e.g., an Ebola virus immunogen, or a Marburg virus immunogen, such as NP and GP gene products), a bunyavirus immunogen (e.g., RVFV, CCHF, and/or SFS virus immunogens), or a coronavirus immunogen (e.g., an infectious human coronavirus immunogen, such as the human coronavirus envelope glycoprotein, or a porcine transmissible gastroenteritis virus immunogen, or an avian infectious bronchitis virus immunogen). The immunogenic polypeptide can further be a polio immunogen, a herpes

immunogen (e.g., CMV, EBV, HSV immunogens), a mumps immunogen, a measles immunogen, a rubella immunogen, a diphtheria toxin or other diphtheria immunogen, a pertussis antigen, a hepatitis (e.g., hepatitis A, hepatitis B, hepatitis C, etc.) immunogen, and/or any other vaccine immunogen now known in the art or later identified as an immunogen.

[0247] Alternatively, the immunogenic polypeptide can be any tumor or cancer cell antigen. Optionally, the tumor or cancer antigen is expressed on the surface of the cancer cell.

[0248] Exemplary cancer and tumor cell antigens are described in S.A. Rosenberg (Immunity 10:281 (1991)). Other illustrative cancer and tumor antigens include, but are not limited to: BRCA1 gene product, BRCA2 gene product, gp100, tyrosinase, GAGE-1/2, BAGE, RAGE, LAGE, NY-ESO-1, CDK-4, β -catenin, MUM-1, Caspase-8, KIAA0205, HPVE, SART-1, FRAME, p15, melanoma tumor antigens (Kawakami et al., (1994) Proc. Natl. Acad. Sci. USA 91:3515; Kawakami et al., (1994) J. Exp. Med., 180:347; Kawakami et al., (1994) Cancer Res. 54:3124), MART-1, gp100, MAGE-1, MAGE-2, MAGE-3, CEA, TRP-1, TRP-2, P-15, tyrosinase (Brichard et al., (1993) J Exp. Med. 178:489); HER-2/neu gene product (U.S. Pat. No. 4,968,603), CA 125, LK26, FB5 (endosialin), TAG 72, AFP, CA 19-9, NSE, DU-PAN-2, CA50, SPan-1, CA72-4, HCG, STN (sialyl Tn antigen), c-erbB-2 proteins, PSA, L-CanAg, estrogen receptor, milk fat globulin, p53 tumor suppressor protein (Levine, (1993) Ann. Rev. Biochem. 62:623); mucin antigens (International Patent Publication No. WO 90/05142); telomerases; nuclear matrix proteins; prostatic acid phosphatase; papilloma virus antigens; and/or antigens now known or later discovered to be associated with the following cancers: melanoma, adenocarcinoma, thymoma, lymphoma (e.g., non-Hodgkin's lymphoma, Hodgkin's lymphoma), sarcoma, lung cancer, liver cancer, colon cancer, leukemia, uterine cancer, breast cancer, prostate cancer, ovarian cancer, cervical cancer, bladder cancer, kidney cancer, pancreatic cancer, brain cancer and any other cancer or malignant condition or metastasis thereof now known or later identified (see, e.g., Rosenberg, (1996) Ann. Rev. Med. 47:481-91).

[0249] As a further alternative, the heterologous nucleic acid can encode any polypeptide that is desirably produced in a cell in vitro, ex vivo, or in vivo. For example, the virus

vectors may be introduced into cultured cells and the expressed gene product isolated therefrom.

[0250] It will be understood by those skilled in the art that the heterologous nucleic acid(s) of interest can be operably associated with appropriate control sequences. For example, the heterologous nucleic acid can be operably associated with expression control elements, such as transcription/translation control signals, origins of replication, polyadenylation signals, internal ribosome entry sites (IRES), promoters, and/or enhancers, and the like.

[0251] Further, regulated expression of the heterologous nucleic acid(s) of interest can be achieved at the post-transcriptional level, e.g., by regulating selective splicing of different introns by the presence or absence of an oligonucleotide, small molecule and/or other compound that selectively blocks splicing activity at specific sites (e.g., as described in WO 2006/119137).

[0252] Those skilled in the art will appreciate that a variety of promoter/enhancer elements can be used depending on the level and tissue-specific expression desired. The promoter/enhancer can be constitutive or inducible, depending on the pattern of expression desired. The promoter/enhancer can be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced.

[0253] In some embodiments, the promoter/enhancer elements can be native to the target cell or subject to be treated. In some embodiments, the promoters/enhancer element can be native to the heterologous nucleic acid sequence. The promoter/enhancer element is generally chosen so that it functions in the target cell(s) of interest. Further, in some embodiments the promoter/enhancer element is a mammalian promoter/enhancer element. The promoter/enhancer element may be constitutive or inducible.

[0254] Inducible expression control elements are typically advantageous in those applications in which it is desirable to provide regulation over expression of the heterologous nucleic acid sequence(s). Inducible promoters/enhancer elements for gene delivery can be tissue-specific or -preferred promoter/enhancer elements, and include muscle specific or preferred (including cardiac, skeletal and/or smooth muscle specific or

preferred), neural tissue specific or preferred (including brain-specific or preferred), eye specific or preferred (including retina-specific and cornea-specific), liver specific or preferred, bone marrow specific or preferred, pancreatic specific or preferred, spleen specific or preferred, and lung specific or preferred promoter/enhancer elements. Other inducible promoter/enhancer elements include hormone-inducible and metal-inducible elements. Exemplary inducible promoters/enhancer elements include, but are not limited to, a Tet on/off element, a RU486-inducible promoter, an ecdysone-inducible promoter, a rapamycin-inducible promoter, and a metallothionein promoter.

[0255] In some embodiments wherein the heterologous nucleic acid sequence(s) is transcribed and then translated in the target cells, specific initiation signals are generally included for efficient translation of inserted protein coding sequences. These exogenous translational control sequences, which may include the ATG initiation codon and adjacent sequences, can be of a variety of origins, both natural and synthetic.

[0256] The virus vectors described herein provide a means for delivering heterologous nucleic acids into a broad range of cells, including dividing and non-dividing cells. The virus vectors can be employed to deliver a nucleic acid of interest to a cell *in vitro*, e.g., to produce a polypeptide *in vitro* or for *ex vivo* gene therapy. The virus vectors are additionally useful in a method of delivering a nucleic acid to a subject in need thereof e.g., to express an immunogenic or therapeutic polypeptide or a functional RNA. In this manner, the polypeptide or functional RNA can be produced *in vivo* in the subject. The subject can be in need of the polypeptide because the subject has a deficiency of the polypeptide. Further, the method can be practiced because the production of the polypeptide or functional RNA in the subject may impart some beneficial effect.

[0257] The virus vectors can also be used to produce a polypeptide of interest or functional RNA in cultured cells or in a subject (e.g., using the subject as a bioreactor to produce the polypeptide or to observe the effects of the functional RNA on the subject, for example, in connection with screening methods).

[0258] In general, the virus vectors of the described herein can be employed to deliver a heterologous nucleic acid encoding a polypeptide or functional RNA to treat and/or prevent any disease state for which it is beneficial to deliver a therapeutic polypeptide or functional RNA. Illustrative disease states include, but are not limited to: cystic fibrosis

(cystic fibrosis transmembrane regulator protein) and other diseases of the lung, hemophilia A (Factor VIII), hemophilia B (Factor IX), thalassemia (β -globin), anemia (erythropoietin) and other blood disorders. Alzheimer's disease (GDF; neprilysin), multiple sclerosis (β -interferon), Parkinson's disease (glial-cell line derived neurotrophic factor [GDNF]), Huntington's disease (RNAi to remove repeats), Canavan's disease, amyotrophic lateral sclerosis, epilepsy (galanin, neurotrophic factors), and other neurological disorders, cancer (endostatin, angiostatin, TRAIL, FAS-ligand, cytokines including interferons; RNAi including RNAi against VEGF or the multiple drug resistance gene product, mir-26a [e.g., for hepatocellular carcinoma]), diabetes mellitus (insulin), muscular dystrophies including Duchenne (dystrophin, mini-dystrophin, insulin-like growth factor I, a sarcoglycan [e.g., α , β , γ], RNAi against myostatic myostatin propeptide, follistatin, activin type II soluble receptor, anti-inflammatory polypeptides such as the Ikappa B dominant mutant, sarcospan, utrophin, mini-utrophin, antisense or RNAi against splice junctions in the dystrophin gene to induce exon skipping [see, e.g., WO/2003/095647], antisense against U7 snRNAs to induce exon skipping [see, e.g., WO/2006/021724], and antibodies or antibody fragments against myostatin or myostatin propeptide) and Becker, Myotonic dystrophy 1 or 2, facioscapulohumeral muscular dystrophy (FSHD), Gaucher disease (glucocerebrosidase), Hurler's disease (α -L-iduronidase), adenosine deaminase deficiency (adenosine deaminase), glycogen storage diseases (e.g., Fabry disease [α -galactosidase] and Pompe disease [lysosomal acid alpha-glucosidase]) and other metabolic disorders, congenital emphysema (α -1-antitrypsin), Lesch-Nyhan Syndrome (hypoxanthine guanine phosphoribosyl transferase), Niemann-Pick disease (sphingomyelinase), Tay-Sachs disease (lysosomal hexosaminidase A), frontotemporal dementia, Maple Syrup Urine Disease (branched-chain keto acid dehydrogenase), retinal degenerative diseases (and other diseases of the eye and retina; e.g., PDGF for macular degeneration and/or vasohibin or other inhibitors of VEGF or other angiogenesis inhibitors to treat/prevent retinal disorders, e.g., in Type I diabetes), diseases of solid organs such as brain (including Parkinson's Disease [GDNF], astrocytomas [endostatin, angiostatin and/or RNAi against VEGF], glioblastomas [endostatin, angiostatin and/or RNAi against VEGF]), liver, kidney, heart including congestive heart failure or peripheral artery disease (PAD) (e.g., by delivering protein

phosphatase inhibitor I (I-1) and fragments thereof (e.g., IIC), serca2a, zinc finger proteins that regulate the phospholamban gene, Barkct, [32-adrenergic receptor, 2-adrenergic receptor kinase (BARK), phosphoinositide-3 kinase (PI3 kinase), S100A1, parvalbumin, adenylyl cyclase type 6, a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct; calsarcin, RNAi against phospholamban; phospholamban inhibitory or dominant-negative molecules such as phospholamban S16E, etc.), arthritis (insulin-like growth factors), joint disorders (insulin-like growth factor 1 and/or 2), intimal hyperplasia (e.g., by delivering enos, inos), improve survival of heart transplants (superoxide dismutase), AIDS (soluble CD4), muscle wasting (insulin-like growth factor I), kidney deficiency (erythropoietin), anemia (erythropoietin), arthritis (anti-inflammatory factors such as I RAP and TNFa soluble receptor), hepatitis (a-interferon), LDL receptor deficiency (LDL receptor), hyperammonemia (ornithine transcarbamylase), Krabbe's disease (galactocerebrosidase), Batten's disease, spinal cerebral ataxias including SCA1, SCA2 and SCA3, phenylketonuria (phenylalanine hydroxylase), autoimmune diseases, and the like. The compositions and methods disclosed herein can further be used following organ transplantation to increase the success of the transplant and/or to reduce the negative side effects of organ transplantation or adjunct therapies (e.g., by administering immunosuppressant agents or inhibitory nucleic acids to block cytokine production). As another example, bone morphogenic proteins (including BNP 2, 7, etc., RANKL and/or VEGF) can be administered with a bone allograft, for example, following a break or surgical removal in a cancer patient.

[0259] In some embodiments, the virus vectors described herein can be employed to deliver a heterologous nucleic acid encoding a polypeptide or functional RNA to treat and/or prevent a liver disease or disorder. The liver disease or disorder may be, for example, primary biliary cirrhosis, nonalcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), autoimmune hepatitis, hepatitis B, hepatitis C, alcoholic liver disease, fibrosis, jaundice, primary sclerosing cholangitis (PSC), Budd-Chiari syndrome, hemochromatosis, Wilson's disease, alcoholic fibrosis, non-alcoholic fibrosis, liver steatosis, Gilbert's syndrome, biliary atresia, alpha-1-antitrypsin deficiency, alagille syndrome, progressive familial intrahepatic cholestasis, Hemophilia B, Hereditary

Angioedema (HAE), Homozygous Familial Hypercholesterolemia (HoFH), Heterozygous Familial Hypercholesterolemia (HeFH), Von Gierke's Disease (GSD I), Hemophilia A, Methylmalonic Acidemia, Propionic Acidemia, Homocystinuria, Phenylketonuria (PKU), Tyrosinemia Type 1, Arginase 1 Deficiency, Argininosuccinate Lyase Deficiency, Carbamoyl-phosphate synthetase 1 deficiency, Citrullinemia Type 1, Citrin Deficiency, Crigler-Najjar Syndrome Type 1, Cystinosis, Fabry Disease, Glycogen Storage Disease 1b, LPL Deficiency, N-Acetylglutamate Synthetase Deficiency, Ornithine Transcarbamylase Deficiency, Ornithine Translocase Deficiency, Primary Hyperoxaluria Type 1, or ADA SCID.

[0260] The compositions and methods described herein can also be used to produce induced pluripotent stem cells (iPS). For example, a virus vector described herein can be used to deliver stem cell associated nucleic acid(s) into a non-pluripotent cell, such as adult fibroblasts, skin cells, liver cells, renal cells, adipose cells, cardiac cells, neural cells, epithelial cells, endothelial cells, and the like.

[0261] Nucleic acids encoding factors associated with stem cells are known in the art. Nonlimiting examples of such factors associated with stem cells and pluripotency include Oct-3/4, the SOX family (e.g., SOX 1, SOX2, SOX3 and/or SOX 15), the Klf family (e.g., Klf1, Klf4 and/or Klf5), the Myc family (e.g., C-myc, L-myc and/or N-myc), NANOG and/or LIN28.

[0262] The methods described herein can also be practiced to treat and/or prevent a metabolic disorder such as diabetes (e.g., insulin), hemophilia (e.g., Factor IX or Factor VIII), a lysosomal storage disorder such as a mucopolysaccharidosis disorder (e.g., Sly syndrome [β -glucuronidase], Hurler Syndrome [α -L-iduronidase], Scheie Syndrome [α -L-iduronidase], Hurler-Scheie Syndrome [α -L-iduronidase], Hunter's Syndrome [iduronate sulfatase], Sanfilippo Syndrome A [heparan sulfamidase], B [N-acetylglucosaminidase], C [acetyl-CoA: α -glucosaminide acetyltransferase], D [N-acetylglucosamine 6-sulfatase], Morquio Syndrome A [galactoses-sulfate sulfatase], B [β -galactosidase], Maroteaux-Lamy Syndrome [N-acetylgalactosamine-4-sulfatase], etc.), Fabry disease (α -galactosidase), Gaucher's disease (glucocerebrosidase), or a glycogen storage disorder (e.g., Pompe disease; lysosomal acid α -glucosidase).

[0263] Gene transfer has substantial use for understanding and providing therapy for disease states. There are a number of inherited diseases in which defective genes are known and have been cloned. In general, the above disease states fall into two classes: deficiency states, usually of enzymes, which are generally inherited in a recessive manner, and unbalanced states, which may involve regulatory or structural proteins, and which are typically inherited in a dominant manner. For deficiency state diseases, gene transfer can be used to bring a normal gene into affected tissues for replacement therapy, as well as to create animal models for the disease using antisense mutations. For unbalanced disease states, gene transfer can be used to create a disease state in a model system, which can then be used in efforts to counteract the disease state. Thus, virus vectors as described herein permit the treatment and/or prevention of genetic diseases.

[0264] The virus vectors described herein may also be employed to provide a functional RNA to a cell *in vitro* or *in vivo*. The functional RNA may be, for example, a non-coding RNA. In some embodiments, expression of the functional RNA in the cell can diminish expression of a particular target protein by the cell. Accordingly, functional RNA can be administered to decrease expression of a particular protein in a subject in need thereof. In some embodiments, expression of the functional RNA in the cell can increase expression of a particular target protein by the cell. Accordingly, functional RNA can be administered to increase expression of a particular protein in a subject in need thereof. In some embodiments, expression of the functional RNA can regulate splicing of a particular target RNA in a cell. Accordingly, functional RNA can be administered to regulate splicing a particular RNA in a subject in need thereof. In some embodiments, expression of the functional RNA in the cell can regulate the function of a particular target protein by the cell. Accordingly, functional RNA can be administered to regulate the function of a particular protein in a subject in need thereof. Functional RNA can also be administered to cells *in vitro* to regulate gene expression and/or cell physiology, e.g., to optimize cell or tissue culture systems or in screening methods.

[0265] In addition, virus vectors as described herein find use in diagnostic and screening methods, whereby a nucleic acid of interest is transiently or stably expressed in a cell culture system, or alternatively, a transgenic animal model.

[0266] The virus vectors can also be used for various non-therapeutic purposes, including but not limited to use in protocols to assess gene targeting, clearance, transcription, translation, etc., as would be apparent to one skilled in the art. The virus vectors can also be used for the purpose of evaluating safety (spread, toxicity, immunogenicity, etc.). Such data, for example, are considered by the United States Food and Drug Administration as part of the regulatory approval process prior to evaluation of clinical efficacy.

[0267] In some embodiments, the virus vectors may be used to produce an immune response in a subject. According to this embodiment, a virus vector comprising a heterologous nucleic acid sequence encoding an immunogenic polypeptide can be administered to a subject, and an active immune response is mounted by the subject against the immunogenic polypeptide. Immunogenic polypeptides are as described hereinabove. In some embodiments, a protective immune response is elicited.

[0268] Alternatively, the virus vector may be administered to a cell *ex vivo* and the altered cell is administered to the subject. The virus vector comprising the heterologous nucleic acid is introduced into the cell, and the cell is administered to the subject, where the heterologous nucleic acid encoding the immunogen can be expressed and induce an immune response in the subject against the immunogen. In some embodiments, the cell is an antigen-presenting cell (e.g., a dendritic cell).

[0269] An “active immune response” or “active immunity” is characterized by “participation of host tissues and cells after an encounter with the immunogen. It involves differentiation and proliferation of immunocompetent cells in lymphoreticular tissues, which lead to synthesis of antibody or the development of cell-mediated reactivity, or both.” Herbert B. Herscovitz, *Immunophysiology: Cell Function and Cellular Interactions in Antibody Formation*, in *IMMUNOLOGY: BASIC PROCESSES* 117 (Joseph A. Bellanti ed., 1985). Alternatively stated, an active immune response is mounted by the host after exposure to an immunogen by infection or by vaccination. Active immunity can be contrasted with passive immunity, which is acquired through the transfer of preformed substances (antibody, transfer factor, thymic graft, interleukin-2) from an actively immunized host to a non-immune host.

[0270] A “protective” immune response or “protective” immunity as used herein indicates that the immune response confers some benefit to the subject in that it prevents or

reduces the incidence of disease. Alternatively, a protective immune response or protective immunity may be useful in the treatment and/or prevention of disease, in particular cancer or tumors (e.g., by preventing cancer or tumor formation, by causing regression of a cancer or tumor and/or by preventing metastasis and/or by preventing growth of metastatic nodules). The protective effects may be complete or partial, as long as the benefits of the treatment outweigh any disadvantages thereof.

[0271] In some embodiments, the virus vector or cell comprising the heterologous nucleic acid can be administered in an immunogenically effective amount, as described below.

[0272] In some embodiments, the virus vectors can be administered for cancer immunotherapy by administration of a virus vector expressing one or more cancer cell antigens (or an immunologically similar molecule) or any other immunogen that produces an immune response against a cancer cell. To illustrate, an immune response can be produced against a cancer cell antigen in a subject by administering a virus vector comprising a heterologous nucleic acid encoding the cancer cell antigen, for example to treat a patient with cancer and/or to prevent cancer from developing in the subject. The virus vector may be administered to a subject *in vivo* or by using *ex vivo* methods, as described herein.

[0273] Alternatively, the cancer antigen can be expressed as part of the capsid protein subunit, or be otherwise associated with the protein capsid (e.g., as described above).

[0274] As another alternative, any other therapeutic nucleic acid (e.g., RNAi) or polypeptide (e.g., cytokine) known in the art can be administered to treat and/or prevent cancer.

[0275] As used herein, the term “cancer” encompasses tumor-forming cancers. Likewise, the term “cancerous tissue” encompasses tumors. A “cancer cell antigen” encompasses tumor antigens.

[0276] The term “cancer” has its understood meaning in the art, for example, an uncontrolled growth of tissue that has the potential to spread to distant sites of the body (*i.e.*, metastasize). Exemplary cancers include, but are not limited to melanoma, adenocarcinoma, thymoma, lymphoma (e.g., non-Hodgkin's lymphoma, Hodgkin's lymphoma), sarcoma, lung cancer, liver cancer, colon cancer, leukemia, uterine cancer, breast cancer, prostate cancer, ovarian cancer, cervical cancer, bladder cancer, kidney

cancer, pancreatic cancer, brain cancer and any other cancer or malignant condition now known or later identified. In some embodiments, a method of treating and/or preventing tumor-forming cancers is provided.

[0277] The term "tumor" is also understood in the art, for example, as an abnormal mass of undifferentiated cells within a multicellular organism. Tumors can be malignant or benign. In some embodiments, the methods disclosed herein are used to prevent and treat malignant tumors.

[0278] By the terms "treating cancer," "treatment of cancer" and equivalent terms it is intended that the severity of the cancer is reduced or at least partially eliminated and/or the progression of the disease is slowed and/or controlled and/or the disease is stabilized. In some embodiments, these terms indicate that metastasis of the cancer is prevented or reduced or at least partially eliminated and/or that growth of metastatic nodules is prevented or reduced or at least partially eliminated.

[0279] By the terms "prevention of cancer" or "preventing cancer" and equivalent terms it is intended that the methods at least partially eliminate or reduce and/or delay the incidence and/or severity of the onset of cancer. Alternatively stated, the onset of cancer in the subject may be reduced in likelihood or probability and/or delayed.

[0280] In some embodiments, cells may be removed from a subject with cancer and contacted with a virus vector expressing a cancer cell antigen as described herein. The modified cell is then administered to the subject, whereby an immune response against the cancer cell antigen is elicited. This method can be advantageously employed with immunocompromised subjects that cannot mount a sufficient immune response in vivo (i.e., cannot produce enhancing antibodies in sufficient quantities).

[0281] It is known in the art that immune responses may be enhanced by immunomodulatory cytokines (e.g., alpha-interferon, beta-interferon, gamma-interferon, omega-interferon, tau-interferon, interleukin-1-alpha, interleukin-1 β , interleukin-2, interleukin-3, interleukin-4, interleukin 5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, interleukin-14, interleukin-18, B cell Growth factor, CD40 Ligand, tumor necrosis factor-alpha, tumor necrosis factor- β , monocyte chemoattractant protein-1, granulocyte-macrophage colony stimulating factor, and lymphotoxin). Accordingly, immunomodulatory cytokines

(preferably, CTL inductive cytokines) may be administered to a subject in conjunction with the virus vector. Cytokines may be administered by any method known in the art. Exogenous cytokines may be administered to the subject, or alternatively, a nucleic acid encoding a cytokine may be delivered to the subject using a suitable vector, and the cytokine produced *in vivo*.

Subjects, Pharmaceutical Formulations, and Modes of Administration

[0282] Virus vectors and viral-like particles as described herein find use in both veterinary and medical applications. Suitable subjects include both avians and mammals. The term “avian” as used herein includes, but is not limited to, chickens, ducks, geese, quail, turkeys, pheasant, parrots, parakeets, and the like. The term “mammals” as used herein includes, but is not limited to, humans, non-human primates, bovines, ovines, caprines, equines, felines, canines, lagomorphs, etc. Human subjects include neonates, infants, juveniles, adults and geriatric subjects.

[0283] In some embodiments, the subject is “in need” of the methods described herein.

[0284] In some embodiments, a pharmaceutical composition is provided comprising a virus vector and/or virus-like particle in a pharmaceutically acceptable carrier and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, etc. For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be respirable, and optionally can be in solid or liquid particulate form.

[0285] By “pharmaceutically acceptable” it is meant a material that is not toxic or otherwise undesirable, i.e., the material may be administered to a subject without causing any undesirable biological effects.

[0286] Also provided herein are method of transferring a nucleic acid to a cell *in vitro*. The virus vector may be introduced into the cells at the appropriate multiplicity of infection according to standard transduction methods suitable for the particular target cells. Titers of virus vector to administer can vary, depending upon the target cell type and number, and the particular virus vector, and can be determined by those of skill in the art without

undue experimentation. In some embodiments, at least about 10^3 infectious units, optionally at least about 10^5 infectious units are introduced to the cell.

[0287] The cell(s) into which the virus vector is introduced can be of any type, including but not limited to neural cells (including cells of the peripheral and central nervous systems, in particular, brain cells such as neurons and oligodendrocytes), lung cells, cells of the eye (including retinal cells, retinal pigment epithelium, and corneal cells), epithelial cells (e.g., gut and respiratory epithelial cells), muscle cells (e.g., skeletal muscle cells, cardiac muscle cells, smooth muscle cells and/or diaphragm muscle cells), dendritic cells, pancreatic cells (including islet cells), hepatic cells, myocardial cells, bone cells (e.g., bone marrow stem cells), hematopoietic stem cells, spleen cells, keratinocytes, fibroblasts, endothelial cells, prostate cells, germ cells, and the like. In some embodiments, the cell can be any progenitor cell. As a further possibility, the cell can be a stem cell (e.g., neural stem cell, liver stem cell). As still a further alternative, the cell can be a cancer or tumor cell. Moreover, the cell can be from any species of origin, as indicated above.

[0288] The virus vector can be introduced into cells *in vitro* for the purpose of administering the modified cell to a subject. In some embodiments, the cells have been removed from a subject, the virus vector is introduced therein, and the cells are then administered back into the subject. Methods of removing cells from the subject for manipulation *ex vivo*, followed by introduction back into the subject are known in the art (see, e.g., U.S. patent No. 5,399,346). Alternatively, the recombinant virus vector can be introduced into cells from a donor subject, into cultured cells, or into cells from any other suitable source, and the cells are administered to a subject in need thereof (*i.e.*, a “recipient” subject).

[0289] Suitable cells for *ex vivo* nucleic acid delivery are as described above. Dosages of the cells to administer to a subject will vary upon the age, condition and species of the subject, the type of cell, the nucleic acid being expressed by the cell, the mode of administration, and the like. Typically, at least about 10^2 to about 10^8 cells or at least about 10^3 to about 10^6 cells will be administered per dose in a pharmaceutically acceptable carrier. In some embodiments, the cells transduced with the virus vector are

administered to the subject in an effective amount in combination with a pharmaceutical carrier.

[0290] In some embodiments, the virus vector is introduced into a cell and the cell can be administered to a subject to elicit an immunogenic response against the delivered polypeptide (e.g., expressed as a transgene or in the protein capsid). Typically, a quantity of cells expressing an immunogenically effective amount of the polypeptide in combination with a pharmaceutically acceptable carrier is administered. An “immunogenically effective amount” is an amount of the expressed polypeptide that is sufficient to evoke an active immune response against the polypeptide in the subject to which the pharmaceutical formulation is administered. In some embodiments, the dosage is sufficient to produce a protective immune response (as defined above). The degree of protection conferred need not be complete or permanent, as long as the benefits of administering the immunogenic polypeptide outweigh any disadvantages thereof.

[0291] Thus, in some embodiments, a method of administering a nucleic acid to a cell comprises contacting the cell with the virus vector, virus particle and/or composition as described herein.

[0292] Also provided herein is a method of administering the virus vector, virus particle and/or virus-like particle as described herein to a subject. In some embodiments, a method of delivering a nucleic acid to a subject comprises administering to the subject a virus particle, virus vector and/or composition as described herein. Administration of the virus vectors, virus particles and/or viral-like particles to a human subject or an animal in need thereof can be by any means known in the art. Optionally, the virus vector, virus particle and/or viral-like particle is delivered in an effective dose in a pharmaceutically acceptable carrier. In some embodiments, an effective amount of the virus vector, virus particle and/or viral-like particle is delivered.

[0293] The virus vectors and/or viral-like particles described herein can further be administered to elicit an immunogenic response (e.g., as a vaccine). Typically, immunogenic compositions comprise an immunogenically effective amount of virus vector and/or viral-like particle in combination with a pharmaceutically acceptable carrier. Optionally, the dosage is sufficient to produce a protective immune response (as defined above). The degree of protection conferred need not be complete or permanent, as long

as the benefits of administering the immunogenic polypeptide outweigh any disadvantages thereof. Subjects and immunogens are as described above.

[0294] Dosages of the virus vector and/or viral-like particle to be administered to a subject depend upon the mode of administration, the disease or condition to be treated and/or prevented, the individual subject's condition, the particular virus vector or protein capsid, and the nucleic acid to be delivered, and the like, and can be determined in a routine manner. In some embodiments, the dose of recombinant AAV is an effective dose. Exemplary effective doses may be, for example, a dose of at least about 10^5 , about 10^6 , about 10^7 , about 10^8 , about 10^9 , about 10^{10} , about 10^{11} , about 10^{12} , about 10^{13} , about 10^{14} , about 10^{15} transducing units, optionally about 10^8 to about 10^{13} transducing units. In some embodiments, an effective dose of recombinant AAV is a dose in the range of about 1×10^{11} to about 1×10^{15} vector genomes per kilogram body weight of the subject. For example, the effective dose may be about 1×10^{11} , about 5×10^{11} , about 1×10^{12} , about 5×10^{12} , about 1×10^{13} , about 5×10^{13} , about 1×10^{14} , about 5×10^{14} , or about 1×10^{15} vector genomes per kilogram (vg/kg) body weight of the subject. In some embodiments, the dose of AAV administered may be 2.8×10^{13} vg/kg or 2.9×10^{13} vg/kg. In some embodiments, the dose may be 2.1×10^{13} vg or 3.0×10^{13} vg.

[0295] In some embodiments, more than one administration (e.g., two, three, four or more administrations) may be employed to achieve the desired level of gene expression over a period of various intervals, e.g., daily, weekly, monthly, yearly, etc.

[0296] Exemplary modes of administration include oral, rectal, transmucosal, intranasal, inhalation (e.g., via an aerosol), buccal (e.g., sublingual), vaginal, intrathecal, intraocular, transdermal, in utero (or in ovo), parenteral (e.g., intravenous, subcutaneous, intradermal, intramuscular [including administration to skeletal, diaphragm and/or cardiac muscle], intradermal, intrapleural, intracerebral, and intraarticular), topical (e.g., to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), intralymphatic, and the like, as well as direct tissue or organ injection (e.g., to liver, skeletal muscle, cardiac muscle, diaphragm muscle or brain). Administration can also be to a tumor (e.g., in or near a tumor or a lymph node). The most suitable route in any given case will depend on the nature and severity of the condition being treated and/or prevented and on the nature of the particular vector that is being used.

[0297] Administration to skeletal muscle includes but is not limited to administration to skeletal muscle in the limbs (e.g., upper arm, lower arm, upper leg, and/or lower leg), back, neck, head (e.g., tongue), thorax, abdomen, pelvis/perineum, and/or digits. Suitable skeletal muscles include but are not limited to abductor digiti minimi (in the hand), abductor digiti minimi (in the foot), abductor hallucis, abductor ossis metatarsi quinti, abductor pollicis brevis, abductor pollicis longus, adductor brevis, adductor hallucis, adductor longus, adductor magnus, adductor pollicis, anconeus, anterior scalene, articularis genus, biceps brachii, biceps femoris, brachialis, brachioradialis, buccinator, coracobrachialis, corrugator supercilii, deltoid, depressor anguli oris, depressor labii inferioris, digastric, dorsal interossei (in the hand), dorsal interossei (in the foot), extensor carpi radialis brevis, extensor carpi radialis longus, extensor carpi ulnaris, extensor digiti minimi, extensor digitorum, extensor digitorum brevis, extensor digitorum longus, extensor hallucis brevis, extensor hallucis longus, extensor indicis, extensor pollicis brevis, extensor pollicis longus, flexor carpi radialis, flexor carpi ulnaris, flexor digiti minimi brevis (in the hand), flexor digiti minimi brevis (in the foot), flexor digitorum brevis, flexor digitorum longus, flexor digitorum profundus, flexor digitorum superficialis, flexor hallucis brevis, flexor hallucis longus, flexor pollicis brevis, flexor pollicis longus, frontalis, gastrocnemius, geniohyoid, gluteus maximus, gluteus medius, gluteus minimus, gracilis, iliocostalis cervicis, iliocostalis lumborum, iliocostalis thoracis, iliopsoas, inferior gemellus, inferior oblique, inferior rectus, infraspinatus, interspinalis, intertransversi, lateral pterygoid, lateral rectus, latissimus dorsi, levator anguli oris, levator labii superioris, levator labii superioris alaeque nasi, levator palpebrae superioris, levator scapulae, long rotators, longissimus capitis, longissimus cervicis, longissimus thoracis, longus capitis, longus colli, lumbricals (in the hand), lumbricals (in the foot), masseter, medial pterygoid, medial rectus, middle scalene, multifidus, mylohyoid, obliquus capitis inferior, obliquus capitis superior, obturator externus, obturator internus, occipitalis, omohyoid, opponens digiti minimi, opponens pollicis, orbicularis oculi, orbicularis oris, palmar interossei, palmaris brevis, palmaris longus, pectineus, pectoralis major, pectoralis minor, peroneus brevis, peroneus longus, peroneus tertius, piriformis, plantar interossei, plantaris, platysma, popliteus, posterior scalene, pronator quadratus, pronator teres, psoas major, quadratus femoris, quadratus plantae, rectus capitis anterior, rectus capitis lateralis,

rectus capitis posterior major, rectus capitis posterior minor, rectus femoris, rhomboid major, rhomboid minor, risorius, sartorius, scalenus minimus, semimembranosus, semispinalis capitis, semispinalis cervicis, semispinalis thoracis, semitendinosus, serratus anterior, short rotators, soleus, spinalis capitis, spinalis cervicis, spinalis thoracis, splenius capitis, splenius cervicis, sternocleidomastoid, sternohyoid, sternothyroid, stylohyoid, subclavius, subscapularis, superior gemellus, superior oblique, superior rectus, supinator, supraspinatus, temporalis, tensor fascia lata, teres major, teres minor, thoracis, thyrohyoid, tibialis anterior, tibialis posterior, trapezius, triceps brachii, vastus intermedius, vastus lateralis, vastus medialis, zygomaticus major, and zygomaticus minor, and any other suitable skeletal muscle as known in the art.

[0298] The virus vector and/or viral-like particle can be delivered to skeletal muscle by intravenous administration, intra-arterial administration, intraperitoneal administration, limb perfusion, (optionally, isolated limb perfusion of a leg and/or arm; see, e.g. Arruda et al., (2005) Blood 105: 3458-3464), and/or direct intramuscular injection. In some embodiments, the virus vector and/or viral-like particle is administered to a limb (arm and/or leg) of a subject (e.g., a subject with muscular dystrophy such as Duchenne muscular dystrophy (DMD) or limb-girdle muscular dystrophy (LGMD)) by limb perfusion, optionally isolated limb perfusion (e.g., by intravenous or intra-articular administration). In some embodiments, the virus vectors and/or viral-like particles can advantageously be administered without employing "hydrodynamic" techniques. Tissue delivery (e.g., to muscle) of prior art vectors is often enhanced by hydrodynamic techniques (e.g., intravenous/intravenous administration in a large volume), which increase pressure in the vasculature and facilitate the ability of the vector to cross the endothelial cell barrier. In some embodiments, the viral vectors and/or viral-like particles can be administered in the absence of hydrodynamic techniques such as high volume infusions and/or elevated intravascular pressure (e.g., greater than normal systolic pressure, for example, less than or equal to a 5%, 10%, 15%, 20%, 25% increase in intravascular pressure over normal systolic pressure). Such methods may reduce or avoid the side effects associated with hydrodynamic techniques such as edema, nerve damage and/or compartment syndrome. Administration to cardiac muscle includes administration to the left atrium, right atrium, left ventricle, right ventricle and/or septum. The virus vector and/or viral-like particle can

be delivered to cardiac muscle by intravenous administration, intra-arterial administration such as intra-aortic administration, direct cardiac injection (e.g., into left atrium, right atrium, left ventricle, right ventricle), and/or coronary artery perfusion.

[0299] Administration to diaphragm muscle can be by any suitable method including intravenous administration, intra-arterial administration, and/or intra-peritoneal administration.

[0300] Delivery to a target tissue can also be achieved by delivering a depot comprising the virus vector and/or viral-like particle. As described herein, delivery of a “depot” refers to administration of a sustained-action formulation that allows slow release and/or gradual dissemination of the virus, so that the virus can act for longer periods than is possible with standard injections. In some embodiments, a depot comprising the virus vector and/or viral-like particle is implanted into skeletal, cardiac and/or diaphragm muscle tissue or the tissue can be contacted with a film or other matrix comprising the virus vector and/or viral-like particle. Such implantable matrices or substrates are described in U.S. Patent No. 7,201,898.

[0301] In some embodiments, a virus vector and/or viral-like particle according is administered to skeletal muscle, diaphragm muscle and/or cardiac muscle (e.g., to treat and/or prevent muscular dystrophy, heart disease [for example, PAD or congestive heart failure]).

[0302] In some embodiments, the compositions and methods described herein are used to treat and/or prevent diseases or disorders of skeletal, cardiac and/or diaphragm muscle. The diseases or disorders of the muscle may be, for example, muscular dystrophy, myopathy, motor neuron disease, and cardiomyopathy. The diseases or disorders of the muscle may be, for example, dystrophinopathies, Duchenne muscular dystrophy, Becker muscular dystrophy, myotonic dystrophies (e.g., myotonic dystrophy 1 and 2), facioscapulohumeral muscular dystrophy (FDHD), Eimery-Dreifuss muscular dystrophy, limb-girdle disease, facioscapulohumeral muscular dystrophy, oculopharyngeal muscular dystrophy, distal muscular dystrophy, congenital muscular dystrophy, juvenile macular dystrophy, centronuclear myopathy, central core myopathy, and inclusion body myositis.

[0303] In some embodiments, a method of treating and/or preventing muscular dystrophy in a subject in need thereof is provided, the method comprising: administering a treatment or prevention effective amount of a virus vector to a mammalian subject, wherein the virus vector comprises a heterologous nucleic acid encoding dystrophin, a mini-dystrophin, a micro-dystrophin, myostatin propeptide, follistatin, activin type II soluble receptor, IGF-1, anti-inflammatory polypeptides such as the Ikappa B dominant mutant, sarcospan, utrophin, a micro-dystrophin, laminin-a2, alpha-sarcoglycan, beta-sarcoglycan, gamma-sarcoglycan, delta-sarcoglycan, IGF-1, an antibody or antibody fragment against myostatin or myostatin propeptide, and/or RNAi against myostatin. In some embodiments, the virus vector can be administered to skeletal, diaphragm and/or cardiac muscle as described elsewhere herein.

[0304] Alternatively, methods described herein can be practiced to deliver a nucleic acid to skeletal, cardiac or diaphragm muscle, which is used as a platform for production of a polypeptide (e.g., an enzyme) or functional RNA (e.g., RNAi, micro RNA, antisense RNA) that normally circulates in the blood or for systemic delivery to other tissues to treat and/or prevent a disorder (e.g., a metabolic disorder, such as diabetes [e.g., insulin], hemophilia [e.g., Factor IX or Factor VIII], a mucopolysaccharide disorder [e.g., Sly syndrome, Hurler Syndrome, Scheie Syndrome, Hurler-Scheie Syndrome, Hunter's Syndrome, Sanfilippo Syndrome A, B, C, D, Morquio Syndrome, Maroteaux-Lamy Syndrome, etc.] or a lysosomal storage disorder such as Gaucher's disease [glucocerebrosidase] or Fabry disease [a-galactosidase A] or a glycogen storage disorder such as Pompe disease [lysosomal acid alpha glucosidase]). Other suitable proteins for treating and/or preventing metabolic disorders are described herein. The use of muscle as a platform to express a nucleic acid of interest is described in U.S. Patent publication US 2002/0192189.

[0305] In some embodiments, a method of treating and/or preventing a metabolic disorder in a subject in need thereof comprises administering a treatment or prevention effective amount of a virus vector to skeletal muscle of a subject, wherein the virus vector comprises a heterologous nucleic acid encoding a polypeptide, wherein the metabolic disorder is a result of a deficiency and/or defect in the polypeptide. Illustrative metabolic disorders and heterologous nucleic acids encoding polypeptides are described herein. Optionally, the polypeptide is secreted (e.g., a polypeptide that is a secreted polypeptide

in its native state or that has been engineered to be secreted, for example, by operable association with a secretory signal sequence as is known in the art). Without being limited by any particular theory, according to this embodiment, administration to the skeletal muscle can result in secretion of the polypeptide into the systemic circulation and delivery to target tissue(s). Methods of delivering virus vectors to skeletal muscle is described in more detail herein.

[0306] The methods described herein can also be practiced to produce noncoding RNA, such as antisense RNA, RNAi or other functional RNA (e.g., a ribozyme) for systemic delivery.

[0307] In some embodiments, a method of treating and/or preventing congenital heart failure or PAD in a subject in need thereof comprises administering a treatment or prevention effective amount of a virus vector to a mammalian subject, wherein the virus vector comprises a heterologous nucleic acid encoding, for example, a sarcoplasmic endoreticulum Ca^{2+} -ATPase (SERCA2a), an angiogenic factor, phosphatase inhibitor I (I-1) and fragments thereof (e.g., I1C), RNAi against phospholamban; a phospholamban inhibitory or dominant-negative molecule such as phospholamban S16E, a zinc finger protein that regulates the phospholamban gene, beta-2-adrenergic receptor, beta-2-adrenergic receptor kinase (BARK), PI3 kinase, calsarcin, a β -adrenergic receptor kinase inhibitor (PARKct), inhibitor 1 of protein phosphatase 1 and fragments thereof (e.g., I1 C), S100A1, parvalbumin, adenylyl cyclase type 6, a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct, Pim-1, PGC-I α , SOD-1, SOD-2, EC-SOD, kallikrein, HIF, thymosin-p4, mir-1, mir-133, mir-206, mir-208 and/or mir-26a.

[0308] Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Alternatively, one may administer the virus vector and/or viral-like particle in a local rather than systemic manner, for example, in a depot or sustained-release formulation. Further, the virus vector and/or viral-like particle can be delivered adhered to a surgically implantable matrix (e.g., as described in U.S. Patent Publication No. US-2004-0013645-A1).

[0309] The virus vectors and/or virus-like particles disclosed herein can be administered to the lungs of a subject by any suitable means, optionally by administering an aerosol suspension of respirable particles comprised of the virus vectors and/or virus-like particles, which the subject inhales. The respirable particles can be liquid or solid. Aerosols of liquid particles comprising the virus vectors and/or virus-like particles may be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. See, e.g., U.S. Patent No. 4,501,729. Aerosols of solid particles comprising the virus vectors and/or virus-like particles may likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

[0310] The virus vectors and virus-like particles can be administered to tissues of the CNS (e.g., brain, eye) and may advantageously result in broader distribution of the virus vector or virus-like particles than would be observed in the absence of the compositions and methods described herein.

[0311] In some embodiments, the virus vectors described herein may be administered to treat diseases of the CNS, including genetic disorders, neurodegenerative disorders, psychiatric disorders and tumors. Illustrative diseases of the CNS include, but are not limited to Adrenomyeloneuropathy (AMN), Alzheimer's disease, Angelman Syndrome, Frontotemporal Dementia, Parkinson's disease, Huntington's disease, Fragile X syndrome, Canavan disease, Leigh's disease, Refsum disease, Tourette syndrome, primary lateral sclerosis, amyotrophic lateral sclerosis, progressive muscular atrophy, Pick's disease, muscular dystrophy, multiple sclerosis, myasthenia gravis, Binswanger's disease, trauma due to spinal cord or head injury, Tay Sachs disease (GM2 Gangliosidosis), Lesch-Nyhan disease, MC4R Obesity, Metachromatic Leukodystrophy (MLD), MPS I (Hurler/Scheie), MPS IIIA (Sanfilippo A), Niemann Pick C1, Rett Syndrome, Spinal Muscular Atrophy (SMA), AADC Deficiency, Monogenic Amyotrophic Lateral Sclerosis (ALS), Alpha mannosidosis, Aspartylglucosaminuria, Dravet Syndrome, Giant Axonal Neuropathy, Globoid Cell Leukodystrophy (Krabbe), Glut 1 Deficiency, GM1 Gangliosidosis, Infantile Neuronal Ceroid Lipofuscinosis (INCL, Batten), Juvenile Neuronal Ceroid Lipofuscinosis (JNCL, Batten), Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL, Batten), MPS II (Hunter), MPS IIIB (Sanfilippo B), MPS IIIC (Sanfilippo C), MPS IVA

(Morquio Syndrome), MPS VI (Maroteaux-Lamy), Peroxisome Biogenesis Disorders (Zellweger Syndrome Spectrum), Sandhoff Disease (GM2 Gangliosidosis), epilepsy, cerebral infarcts, psychiatric disorders including mood disorders (e.g., depression, bipolar affective disorder, persistent affective disorder, secondary mood disorder), schizophrenia, drug dependency (e.g., alcoholism and other substance dependencies), neuroses (e.g., anxiety, obsessional disorder, somatoform disorder, dissociative disorder, grief, post-partum depression), psychosis (e.g., hallucinations and delusions), dementia, paranoia, attention deficit disorder, psychosexual disorders, sleeping disorders, pain disorders, eating or weight disorders (e.g., obesity, cachexia, anorexia nervosa, and bulimia) and cancers and tumors (e.g., pituitary tumors) of the CNS.

[0312] Disorders of the CNS include ophthalmic disorders involving the retina, posterior tract, and optic nerve (e.g., retinitis pigmentosa, diabetic retinopathy and other retinal degenerative diseases, uveitis, age-related macular degeneration, glaucoma).

[0313] Most, if not all, ophthalmic diseases and disorders are associated with one or more of three types of indications: (1) angiogenesis, (2) inflammation, and (3) degeneration. The viral vectors described herein can be employed to deliver anti-angiogenic factors; anti-inflammatory factors; factors that retard cell degeneration, promote cell sparing, or promote cell growth and combinations of the foregoing.

[0314] Diabetic retinopathy, for example, is characterized by angiogenesis. Diabetic retinopathy can be treated by delivering one or more anti-angiogenic factors either intraocularly (e.g., in the vitreous) or periorcularly (e.g., in the sub-Tenon's region). One or more neurotrophic factors may also be co-delivered, either intraocularly (e.g., intravitreally) or periorcularly.

[0315] Uveitis involves inflammation. One or more anti-inflammatory factors can be administered by intraocular (e.g., vitreous or anterior chamber) administration of a viral vector.

[0316] Retinitis pigmentosa, by comparison, is characterized by retinal degeneration. In some embodiments, retinitis pigmentosa can be treated by intraocular (e.g., vitreal administration) of a viral vector encoding one or more neurotrophic factors.

[0317] Age-related macular degeneration involves both angiogenesis and retinal degeneration. This disorder can be treated by administering the inventive viral vectors

encoding one or more neurotrophic factors intraocularly (e.g., vitreous) and/or one or more anti-angiogenic factors intraocularly or periorcularly (e.g., in the sub-Tenon's region).

[0318] Glaucoma is characterized by increased ocular pressure and loss of retinal ganglion cells. Treatments for glaucoma include administration of one or more neuroprotective agents that protect cells from excitotoxic damage using the inventive viral vectors. Such agents include N-methyl-D-aspartate (NMDA) antagonists, cytokines, and neurotrophic factors, delivered intraocularly, optionally intravitreally.

[0319] In some embodiments, the compositions and methods described herein may be used to treat seizures, e.g., to reduce the onset, incidence or severity of seizures. The efficacy of a therapeutic treatment for seizures can be assessed by behavioral (e.g., shaking, ticks of the eye or mouth) and/or electrographic means (most seizures have signature electrographic abnormalities). Thus, epilepsy, which is marked by multiple seizures over time, may also be treated.

[0320] In some embodiments, a method of treating a subject in need thereof comprises administering to the subject an AAV vector comprising a protein capsid comprising capsid protein subunit, wherein the capsid protein subunit comprises the amino acid sequence of any one of SEQ ID NO: 165-187. In some embodiments, the AAV vector comprises a protein capsid comprising a capsid protein subunit comprising the amino acid sequence of SEQ ID NO: 175, or a sequence at least 95% identical thereto. In some embodiments, the AAV vector comprises a protein capsid comprising a capsid protein subunit comprising the amino acid sequence of SEQ ID NO: 175, or a sequence at least 95% identical thereto. In some embodiments, the subject has Dravet syndrome. In some embodiments, the subject has Rett syndrome. In some embodiments, the subject has Angelman syndrome. In some embodiments, the subject has Niemann-Pick disease. In some embodiments, the subject has Fragile X syndrome. In some embodiments, the subject has Alzheimer's disease. In some embodiments, the subject has Gaucher's disease. In some embodiments, the subject has Huntington's disease. In some embodiments, the subject has Parkinson's disease. In some embodiments, the subject has Friedrich's ataxia. In some embodiments, the AAV vector is administered to the subject by intracerebroventricular (ICV) injection. In some embodiments, the AAV vector

is administered to the subject by intrathecal (IT) injection. In some embodiments, the AAV vector is administered to the subject by intravenous (IV) injection.

[0321] In some embodiments, a method of treating a subject in need thereof comprises administering to the subject an AAV vector comprising a protein capsid comprising a capsid protein subunit, wherein the capsid protein subunit comprises the amino acid sequence of SEQ ID NO: 175 or 180, wherein the subject has Dravet syndrome, Rett syndrome, Angelman syndrome, Niemann-Pick disease, or Fragile X syndrome, and wherein the AAV vector is administered to the subject by ICV or IT injection.

[0322] In some embodiments, a method of treating a subject in need thereof comprises administering to the subject an AAV vector comprising a protein capsid comprising a capsid protein subunit, wherein the capsid protein subunit comprises the amino acid sequence of SEQ ID NO: 175 or 180, wherein the subject has Gaucher's disease, Huntington's disease, Parkinson's disease, or Friedrich's ataxia, and wherein the AAV vector is administered to the subject by ICV or IT injection.

[0323] In some embodiments, somatostatin (or an active fragment thereof) is administered to the brain using a viral vector to treat a pituitary tumor. According to this embodiment, the viral vector encoding somatostatin (or an active fragment thereof) is administered by microinfusion into the pituitary. Likewise, such treatment can be used to treat acromegaly (abnormal growth hormone secretion from the pituitary). The nucleic acid (e.g., GenBank Accession No. J00306) and amino acid (e.g., GenBank Accession No. P01166; contains processed active peptides somatostatin-28 and somatostatin-14) sequences of somatostatins are known in the art.

[0324] In some embodiments, the virus vector can comprise a secretory signal as described in U.S. Patent No. 7,071,172.

[0325] In some embodiments, the virus vector and/or viral-like particle is administered to the CNS (e.g., to the brain or to the eye). The virus vector and/or viral-like particle may be introduced into the spinal cord, brainstem (medulla oblongata, pons), midbrain (hypothalamus, thalamus, epithalamus, pituitary gland, substantia nigra, pineal gland), cerebellum, telencephalon (corpus striatum, cerebrum including the occipital, temporal, parietal and frontal lobes, cortex, basal ganglia, hippocampus and portaamygdala), limbic system, neocortex, corpus striatum, cerebrum, and inferior colliculus. The virus vector

and/or viral-like particle may also be administered to different regions of the eye such as the retina, cornea and/or optic nerve.

[0326] The virus vector and/or viral-like particle may be delivered into the cerebrospinal fluid (e.g., by lumbar puncture) for more disperse administration of the vector. The virus vector and/or viral-like particle may further be administered intravascularly to the CNS in situations in which the blood-brain barrier has been perturbed (e.g., brain tumor or cerebral infarct).

[0327] The virus vector and/or viral-like particle can be administered to the desired region(s) of the CNS by any route known in the art, including but not limited to, intrathecal, intra-ocular, intracerebral, intraventricular, intravenous (e.g., in the presence of a sugar such as mannitol), intranasal, intra-aural, intra-ocular (e.g., intra-vitreous, sub-retinal, anterior chamber) and peri-ocular (e.g., sub-Tenon's region) delivery as well as intramuscular delivery with retrograde delivery to motor neurons. In some embodiments, the virus vector and/or viral-like particle is administered in a liquid formulation by direct injection (e.g., stereotactic injection) to the desired region or compartment in the CNS. In some embodiments, the virus vector and/or viral-like particle may be provided by topical application to the desired region or by intra-nasal administration of an aerosol formulation. Administration to the eye, may be by topical application of liquid droplets. As a further alternative, the virus vector and/or viral-like particle may be administered as a solid, slow-release formulation (see, e.g., U.S. Patent No. 7,201,898).

[0328] In some embodiments, the virus vector can be used for retrograde transport to treat and/or prevent diseases and disorders involving motor neurons (e.g., amyotrophic lateral sclerosis (ALS); spinal muscular atrophy (SMA), etc.). For example, the virus vector can be delivered to muscle tissue from which it can migrate into neurons.

EXAMPLES

[0329] The following examples, which are included herein for illustration purposes only, are not intended to be limiting. As used herein, the terms STRD.101 and STRD.102 are used to describe capsid protein subunit sequences, and AAV-STRD.101 and AAV-STRD.102 are used to describe AAV vectors comprising recombinant or modified capsid protein subunit sequences. However, the terms STRD.101 and STRD.102 may be used in some contexts to describe AAV vectors comprising a protein capsid comprising the named capsid protein subunits, as will be apparent to the skilled artisan.

EXAMPLE 1. Combinatorial engineering and selection of antibody-evading AAV vectors

[0330] Antibody evading AAV mutants are prepared according to the following method. The first step involves identification of conformational 3D antigenic epitopes on the AAV capsid protein capsid surface, for example using cryo-electron microscopy. Selected residues within antigenic motifs are then subjected to mutagenesis using degenerate primers with each codon substituted by nucleotides NNK and gene fragments combined together by Gibson assembly and/or multistep PCR. Capsid protein subunit-encoding genes containing a degenerate library of mutated antigenic motifs are cloned into a wild type AAV genome to replace the original Cap encoding DNA sequence, yielding a plasmid library. Plasmid libraries are then transfected into 293 producer cell lines with an adenoviral helper plasmid to generate AAV capsid protein subunit libraries, which can then be subjected to selection. Successful generation of AAV libraries is confirmed via DNA sequencing.

[0331] In order to select for new AAV strains that can escape neutralizing antibodies (NAbs) and/or target the central nervous system (CNS), AAV libraries are subjected to multiple rounds of infection in non-human primates. At each stage, tissues of interest are isolated from animal subjects. Cell lysates harvested from the tissues of interest are sequenced to identify AAV isolates escaping antibody neutralization. After multiple rounds of infection in non-human primates, the isolated sequences from each mutagenized region are combined in all permutations and combinations.

[0332] As a specific example, a common antigenic motif on an AAV capsid protein subunit (VP1) was subjected to mutagenesis as described above. The degenerate libraries (**FIG. 1A**) were then subjected to a first round of infection in a non-human primate (intravenous injection). Tissues were harvested at day 7 post-infection and sequenced to identify single AAV isolates.

[0333] Various recombinant AAV isolates were identified in tissue samples, including the spinal cord, dorsal root ganglion, frontal lobe, temporal lobe, occipital lobe, putamen, globus pallidus, thalamus, amygdala, hippocampus, substantia nigra, pons, cerebellum, medulla. Results from this first round of evolution are shown in **FIG. 1B**.

[0334] The recombinant AAVs isolated during the first round of evolution (**FIG. 1B**) were then reintroduced into a second non-human primate. Tissues were harvested at day 7 post-infection and sequenced to identify single AAV isolates. The results from this second round of evolution are shown in **FIG. 1C**.

[0335] Recombinant AAVs with the highest frequency were sequenced. Substitutions present in these AAVs are shown in Tables 7.1 and 7.2. These data demonstrate that recombinant AAV virions having capsid protein subunits comprising the substitutions listed in Tables 7.1 and 7.2 evade neutralizing antibodies *in vivo* in non-human primates and have a tropism for the desired target tissues.

EXAMPLE 2: Manufacturability of recombinant AAV vectors

[0336] To determine whether various recombinant AAVs identified in Example 1 may be manufactured in large-scale systems, the AAVs were produced according to standard methods, and yield was compared to that of wildtype AAV vectors.

[0337] AAVs were produced in HEK293 cells according to a standard triple transfection protocol. Briefly, the cells were transfected with (i) a plasmid comprising either the wildtype AAV9 capsid protein subunit sequence, the STRD.101 capsid protein subunit variant sequence (SEQ ID NO: 180), or the STRD.102 capsid protein subunit variant sequence (SEQ ID NO: 175), (ii) a plasmid comprising a 5' ITR, a transgene, and a 3' ITR sequence, and (iii) a plasmid comprising helper genes necessary for AAV production. Two different transgenes were used with each capsid protein subunit, in self-complementary constructs. The cells were subsequently lysed and the virions were purified using an

affinity column, CsCl density ultracentrifugation, and dialysis. Subsequently, yield of each AAV was measured using a PCR-based quantification approach.

[0338] As shown in **FIG. 2**, recombinant AAV vectors comprising the STRD.101 and STRD.102 capsid protein subunits had a yield that was similar to the yield of wildtype AAV9. This data confirms that recombinant AAVs comprising the recombinant capsid protein subunits are suitable for commercial manufacturing.

EXAMPLE 3: *In vitro* transduction using recombinant AAV viral vectors

[0339] To confirm whether the recombinant AAV vectors of Example 1 are generally infective and able to transduce cells in culture, various AAV vectors were prepared according to a standard protocol.

[0340] The infectivity of the recombinant AAVs was tested using a standard TCID₅₀ assay. Briefly, HeLaRC32 cells were infected with recombinant AAV particles in the presence of Adenovirus (Ad5) at doses spanning 5 orders of magnitude. After 72 hours, DNA was extracted and vector genome replication was quantified by qPCR.

[0341] The particle to infectivity ratio was calculated to determine infectivity. As shown in **FIG. 3**, the infectivity ratio of an AAV-STRD.101 vector was lower compared to that of wildtype AAV9. Because a lower infectivity ratio translates to a higher potency, AAV-STRD.101 is more infectious than wildtype AAV9.

[0342] Separately, infectivity was also determined in various cell lines. Recombinant AAVs packaging a luciferase transgene were generated and contacted with the cells in culture at a dose of 10,000 vector genomes (vg) per cell. 48-hours post infection, cells were lysed. The lysate was contacted with a bioluminescent substrate, and relative fluorescence units (RFUs) were measured. As shown in **FIG. 4A-4D**, AAV-STRD.101 vectors infected U87 cells (human glioblastoma cell line, **FIG. 4A**), N2A cells (mouse neural crest-derived cell line, **FIG. 4B**), SY5Y cells (human neuroblastoma cell line, **FIG. 4C**), and U2OS cells (human osteosarcoma cell line, **FIG. 4D**) at levels comparable to wildtype AAV9.

[0343] Accordingly, this data demonstrates that the recombinant AAV vectors of Example 1 can effectively transduce cells in culture.

EXAMPLE 4: *In Vivo* Characterization of Recombinant AAVs Targeting the Central Nervous System

[0344] Recombinant capsid protein subunits STRD.101 and STRD.102 were selected for *in vivo* characterization. Recombinant AAVs comprising these capsid protein subunits and packaging a native tdTomato fluorescent transgene were generated. The recombinant AAVs were administered to neonatal mice by intracerebroventricular injection at day 0. At three weeks post-injection, brain tissues were harvested and fixed to evaluate the expression by visual assessment of the tdTomato fluorescence. **FIG. 5** provides representative images showing tdTomato expression in coronal vibratome sections after 24 hours post-fixation with 4% PFA. These same sections were also visualized using immunohistochemistry (**FIG. 6**). As shown in the images of **FIG. 5** and **FIG. 6**, AAV9, AAV-STRD.102 and AAV-STRD.101 vectors each had different distribution in the brain tissues, with the highest transgene expression localized near the site of injection. Taken together, this data shows that the recombinant AAVs tested successfully deliver a transgene to target cells *in vivo* after intracerebroventricular injection.

[0345] The AAV-STRD.101 and AAV-STRD.102 vectors packaging tdTomato were also administered to four adult mice by intravenous injection at a dose of 5.5×10^{13} vg/kg. Three weeks post-injection, liver and heart were harvested and fixed to evaluate the expression profile by visual assessment of tdTomato fluorescence.

[0346] Representative images from one mouse showing TdTomato expression in vibratome liver sections after 24 hours post-fixation with 4% PFA are provided in **FIG. 7**. Notably, the AAV-STRD.102 and AAV-STRD.101 vectors were detargeted to the liver compared to wildtype AAV9. This desirable property was unexpected, as no counter screen in the liver was performed during evolution.

[0347] Representative images from one mouse showing TdTomato expression in vibratome heart sections after 24 hours post-fixation with 4% PFA are provided in **FIG. 8**. Notably, the vectors tested had different tropism for the heart. Specifically, the AAV-STRD.102 vector was less infective in heart compared to AAV-STRD.101. Because no heart screen was performed during evolution, this differential transduction was wholly unexpected.

[0348] Taken together, this data indicates that the AAV-STRD.102 and AAV-STRD.101 vectors can be successfully used to target CNS tissues *in vivo*, avoid clearance by the liver, and are powerful tools for gene therapy. Given their different tropisms (*i.e.*, AAV-STRD.101 was more infective in the heart than AAV-STRD.102), these vectors will be powerful tools for targeting gene therapy treatments to specifically desired tissues.

EXAMPLE 5: Biodistribution of recombinant AAVs in non-human primates

[0349] Recombinant AAVs were administered to non-human primates, in order to determine biodistribution. Recombinant AAVs were administered by intravenous (IV) and intracerebrovascular (ICV) injection (**FIG. 9**). AAV-STRD.101 was administered at a dose of 2.9×10^{13} vector genomes per kilogram (vg/kg) by IV injection, and 2.1×10^{13} vg by ICV injection (black dots). AAV-STRD.102 was administered at a dose of 2.8×10^{13} vg/kg by IV injection, and 3.0×10^{13} vg by ICV injection (white dots). After 30 days, the animals were sacrificed, and viral load in various CNS tissues was measured by qPCR.

[0350] As shown in **FIG. 9**, both AAV-STRD.102 and AAV-STRD.101 infected various CNS tissues. Additionally, because the AAVs showed high levels of transduction, this data suggest that these AAVs likely avoid neutralizing antibodies *in vivo*.

EXAMPLE 6: Cell therapy method for treating a subject in need thereof

[0351] Cells are transduced using an AAV vector *ex vivo*. For some purposes, the cells may be autologous (*i.e.*, derived from the subject to be treated) or allogenic (*i.e.*, derived from a different subject/donor). After transduction of the cells using an AAV, and after expression of a transgene has been verified, the cells are administered to the subject using standard clinical methods.

[0352] Cells may be administered to the subject once, or administration may be repeated multiple times. The number of cells administered varies depending on, for example, the disease or condition to be treated, the severity of the subject's disease/condition, and the subject's height and weight.

EXAMPLE 7: Gene therapy method for treating a subject in need thereof

[0353] An AAV vector described herein (e.g., an AAV vector comprising a capsid protein subunit having the sequence of SEQ ID NO: 175 or 180) is administered to a subject in need thereof, wherein the subject has a disease or disorder of the CNS. The AAV vector is administered to the subject once, or administration may be repeated multiple times. The administration is by one or more routes, such as intravenous (IV), intracerebroventricular (ICV), or intrathecal (IT) injection. The dose of AAV vector varies depending on, for example, the disease or condition to be treated, the severity of the subject's disease/condition, and the subject's height and weight. For example, the dose of AAV administered to the subject may be 2.8×10^{13} vg/kg or 2.9×10^{13} vg/kg when the AAV vector is administered by IV injection. When the AAV vector is administered by ICV injection, the dose may be 2.1×10^{13} vg or 3.0×10^{13} vg. In some protocols, the AAV vector may be administered to the subject by both IV and ICV injection.

EXAMPLE 8: Preparation of a recombinant AAV vector in mammalian cells

[0354] Three plasmids are provided. The first plasmid comprises a transfer cassette comprising a transgene (SEQ ID NO: 3002) encoding NPC1 flanked by two ITRs (SEQ ID NO: 3003 and 3004). The first plasmid comprises the sequence of any one of SEQ ID NO: 3014-3019. The second plasmid comprises sequences encoding the Rep and Cap genes. The third plasmid comprises various "helper" sequences required for AAV production (E4, E2a, and VA).

[0355] The three plasmids are transfected into viral production cells (e.g., HEK293) using an appropriate transfection reagent (e.g., Lipofectamine™). After incubation at 37°C for a predetermined period of time, AAV particles are collected from the media or the cells are lysed to release the AAV particles. The AAV particles are then purified and titered using either quantitative PCR (qPCR) or droplet digital PCR (ddPCR) according to standard methods. The AAV particles may be stored at -80°C for later use.

EXAMPLE 9: Preparation of a recombinant AAV vector in insect cells

[0356] A first recombinant baculoviral vector is provided. The first recombinant baculoviral vector comprises a transfer cassette sequence comprising a transgene (SEQ

ID NO: 3002) encoding NPC1 flanked by two ITRs (SEQ ID NO: 3003 and 3004). The transfer cassette comprises the sequence of any one of SEQ ID NO: 3014-3019.

[0357] Insect cells (e.g., Sf9) are co-infected in suspension culture with the first recombinant baculoviral vector and a least one additional recombinant baculoviral vector comprising sequences encoding the AAV Rep and Cap proteins (e.g., the STRD.101 or STRD.012 capsid protein subunit). After incubation at 28°C for a predetermined period of time, AAV particles are collected from the media or the cells are lysed to release the AAV particles. The AAV particles are then purified and titered using either quantitative PCR (qPCR) or droplet digital PCR (ddPCR) according to standard methods. The AAV particles may be stored at -80°C for later use.

EXAMPLE 10: *In vitro* potency assay

[0358] To determine whether the AAV transfer cassettes described herein are able to rescue the NPC1 lysosomal phenotype in cultured cells, a recombinant AAV2 vector packaging a hNPC1 transfer cassette (SEQ ID NO: 3014) was prepared in HEK293 cells using a triple-transfection protocol (See, e.g., Example 1). The AAV2-hNPC1 vector was then used to transduce wildtype U2OS cells (osteosarcoma), and U2OS cells which do not express NPC1 (NPC^{-/-}) *in vitro* at a multiplicity of infection (MOI) of either 5 x 10³ (5K) or 10 x 10³ (10K). Cells were then incubated at 37°C in a 5% CO₂ atmosphere.

[0359] NPC1 cells exhibit a characteristic accumulation of cholesterol in lysosomes, which can be monitored by observing the size and number of lysosomes in a cell. In this assay, lysosomal phenotype was monitored by measuring accumulation of a fluorescent organelle dye, LysoTracker® (ThermoFisher Scientific®), in the cells. 72 hours after transduction with the AAV2-hNPC1 vector, 50 mM of LysoTracker® was added to the cells. After 2 hours, the cells were fixed and LysoTracker® fluorescence was measured.

[0360] Results are shown in **FIG. 10A**. As expected, wildtype U2OS cells did not show significant accumulation of LysoTracker® fluorescence in lysosomes, whereas the NPC1^{-/-} cells did. Cells transduced with AAV2-hNPC1 at a MOI of either 5K or 10K had significantly reduced accumulation of LysoTracker® fluorescence in lysosomes.

[0361] In a separate assay, cells transduced with hNPC1 were fixed and stained using filipin, a histochemical stain for cholesterol. The filipin stain, derived from *Streptomyces*

filipinensis, was purchased from Polysciences, and was used at a final concentration of 50 µg/mL. The cells were visualized using a Pico Automated Cell Imaging System (ImageXpress®), and filipin stain was quantified. Results are shown in **FIG 10B**. As expected, wildtype U2OS cells did not show significant cholesterol accumulation, whereas the NPC1^{-/-} cells did. Cells transduced with AAV2-hNPC1 at a MOI of either 5K or 10K had significantly reduced cholesterol accumulation.

[0362] Taken together, these data show that transduction of cells using AAV2-hNPC1 successfully rescued lysosomal phenotype in NPC1-deficient U2OS cells.

EXAMPLE 11: *In vivo* potency assay

[0363] To determine whether the AAV transfer cassettes described herein are able to rescue the NPC1 phenotype *in vivo*, a recombinant AAV9 vector packaging a hNPC1 transfer cassette (SEQ ID NO: 3014) was prepared in HEK293 cells using a triple-transfection protocol (See, e.g., Example 1). Mice deficient for NPC1 (i.e., NPC1^{-/-} mice) were injected intravenously at a dose of 3.0×10^{14} vector genomes per kilogram (vg/kg), by retro-orbital injection, with either saline or with the AAV9-hNPC1 vector around the age of 24-28 days. Results are shown in **FIG. 11**. All saline-treated mice died by the age of about 80 days. However, all AAV9-hNPC1-injected animals survived through the duration of experiment. The AAV9-hNPC1-injected mice were sacrificed around 100 days of age for analysis.

[0364] Mice were also challenged in a balance beam walking test, wherein number of slips were measured as mice walked across a balance beam. The test was performed at about 8 weeks (56 days) of age. As shown in **FIG. 13**, wildtype mice did not slip off the balance beam,. Although there was no statistically significant difference in the number of slips between NPC1^{-/-} mice treated with AAV9-hNPC1 and saline-treated NPC1^{-/-} mice, the average number of slips observed in the AAV9-hNPC1 group was less.

[0365] Behavioral phenotype score of the mice was also assessed at about 10 weeks (70 days) of age. The behavioral phenotype score is a composite score measuring various disease symptoms, including grooming, gait, kyphosis, ledge test, hindlimb clasp, and tremor. (See Alam et al, Sci Transl Med, 2016; Guyenet et al, J Vis Exp, 2010). As shown

in FIG. 12, NPC1^{-/-} mice treated with AAV9-hNPC1 had a significantly reduced score as compared to saline-treated NPC1^{-/-} mice.

[0366] Taken together, these data demonstrate that AAV9-hNPC1 can at least partially rescue the disease phenotype of NPC1 deficient mice.

EXAMPLE 12: Testing a STRD.101 vector packaging a cassette encoding NPC1 *in vitro* and *in vivo*

[0367] An AAV-STRD.101 vector comprising a nucleic acid comprising a transfer cassette encoding human NPC1 (e.g., the transfer cassette of SEQ ID NO: 14) is prepared according to the method of Example 8 or 9. This vector is referred to herein as AAV-STRD.101-hNPC1

[0368] To determine whether the AAV-STRD.101 vector is able to rescue the NPC1 lysosomal phenotype in cultured cells, the AAV-STRD.101-hNPC1 vector is then used to transduce wildtype U2OS cells (osteosarcoma), and U2OS cells which do not express NPC1 (NPC1^{-/-}) *in vitro* at a multiplicity of infection (MOI) of either 5×10^3 (5K) or 10×10^3 (10K). Cells are then incubated at 37°C in a 5% CO₂ atmosphere.

[0369] NPC1 cells exhibit a characteristic accumulation of cholesterol in lysosomes, which can be monitored by observing the size and number of lysosomes in a cell. Accordingly, lysosomal phenotype is monitored by measuring accumulation of a fluorescent organelle dye, LysoTracker® (ThermoFisher Scientific®), in the cells. 72 hours after transduction with the AAV2-hNPC1 vector, 50 mM of LysoTracker® is added to the cells. After 2 hours, the cells are fixed and LysoTracker® fluorescence is measured.

[0370] In a separate assay, cells transduced with the AAV-STRD.101-hNPC1 vector are fixed and stained using filipin, a histochemical stain for cholesterol. The filipin stain, derived from *Streptomyces filipinensis*, is used at a final concentration of 50 µg/mL. The cells are visualized using a Pico Automated Cell Imaging System (ImageXpress®), and filipin stain is quantified.

[0371] The AAV-STRD.101-hNPC1 vector is also tested to determine whether it can rescue the NPC1 phenotype *in vivo*. Mice deficient for NPC1 (i.e., NPC1^{-/-} mice) are injected intravenously at a dose of 3.0×10^{14} vg/kg, by retro-orbital injection, with either

saline or with the AAV9-hNPC1 vector around the age of 24-28 days. Survival is monitored until at least 100 days of age.

[0372] Mice are also challenged in a balance beam walking test, wherein number of slips are measured as mice walked across a balance beam. The test is performed at about 8 weeks (56 days) of age.

[0373] Behavioral phenotype score of the mice is also assessed at about 10 weeks (70 days) of age. The behavioral phenotype score is a composite score measuring various disease symptoms, including grooming, gait, kyphosis, ledge test, hindlimb clasp, and tremor. (See Alam et al, Sci Transl Med, 2016; Guyenet et al, J Vis Exp, 2010).

[0374] The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

NUMBERED EMBODIMENTS

[0375] Notwithstanding the appended claims, the disclosure sets forth the following numbered embodiments:

[0376] 1. An adeno-associated virus (AAV) vector comprising: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180 or 175; and (ii) a nucleic acid encapsidated by the protein capsid; wherein the nucleic acid comprises a transfer cassette; wherein the transfer cassette comprises from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene sequence which encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR.

[0377] 2. The AAV vector of embodiment 1, wherein at least one of the 5' ITR and the 3' ITR is about 110 to about 160 nucleotides in length.

[0378] 3. The AAV vector of embodiment 1 or 2, wherein the 5' ITR is the same length as the 3' ITR.

[0379] 4. The AAV vector of embodiment 1 or 2, wherein the 5' ITR and the 3' ITR have different lengths.

[0380] 5. The AAV vector of any one of embodiments 1-4, wherein at least one of the 5' ITR and the 3' ITR is isolated or derived from the genome of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV or Bovine AAV.

[0381] 6. The AAV vector of embodiment 1, wherein the 5' ITR comprises the sequence of SEQ ID NO: 3003.

[0382] 7. The AAV vector of embodiment 1, wherein the 3' ITR comprises the sequence of SEQ ID NO: 3004.

[0383] 8. The AAV vector of any one of embodiments 1-7, wherein the promoter is a constitutive promoter.

[0384] 9. The AAV vector of any one of embodiments 1-7, wherein the promoter is an inducible promoter.

[0385] 10. The AAV vector of any one of embodiments 1-9, wherein the promoter is a tissue-specific promoter.

[0386] 11. The AAV vector of any one of embodiments 1-7, wherein the promoter is selected from the group consisting of the CBA promoter, the GUSB240 promoter, the

GUSB379 promoter, the HSVTK promoter, the CMV promoter, the SV40 early promoter, the SV40 late promoter, the metallothionein promoter, the murine mammary tumor virus (MMTV) promoter, the Rous sarcoma virus (RSV) promoter, the polyhedrin promoter, the chicken β -actin (CBA) promoter, the EF-1 alpha promoter, the dihydrofolate reductase (DHFR) promoter, and the phosphoglycerol kinase (PGK) promoter.

[0387] 12. The AAV vector of embodiment 11, wherein the promoter is selected from the group consisting of the CBA promoter, the GUSB240 promoter, the GUSB379 promoter, and the HSVTK promoter.

[0388] 13. The AAV vector of any one of embodiments 1-7, wherein the promoter comprises a sequence at least 95% or 100% identical to any one of SEQ ID NO: 3005, SEQ ID NO: 3006, SEQ ID NO: 3007, or SEQ ID NO: 3008.

[0389] 14. The AAV vector of any one of embodiments 1-13, wherein the NPC1 protein is the human NPC1 protein.

[0390] 15. The AAV vector of any one of embodiments 1-13, wherein the NPC1 protein has a sequence that is at least 90% identical to the sequence of the human NPC1 protein.

[0391] 16. The AAV vector of embodiment 15, wherein the NPC1 protein has a sequence that is at least 95% identical to the sequence of the human NPC1 protein.

[0392] 17. The AAV vector of embodiment 16, wherein the NPC1 protein has a sequence that is at least 98% identical to the sequence of the human NPC1 protein.

[0393] 18. The AAV vector of any one of embodiments 1-13, wherein the NPC1 protein comprises the sequence of SEQ ID NO: 3001.

[0394] 19. The AAV vector of any one of embodiments 1-13, wherein the transgene comprises the sequence of SEQ ID NO: 3002.

[0395] 20. The AAV vector of any one of embodiments 1-18, wherein the polyadenylation signal is selected from simian virus 40 (SV40), rBG, α -globin, β -globin, human collagen, human growth hormone (hGH), polyoma virus, human growth hormone (hGH) and bovine growth hormone (bGH).

[0396] 21. The AAV vector of embodiment 20, wherein the polyadenylation signal is the SV40 polyadenylation signal.

[0397] 22. The AAV vector of embodiment 20, wherein the polyadenylation signal is the rBG polyadenylation signal.

- [0398]** 23. The AAV vector of any one of embodiments 1-19, wherein the polyadenylation signal comprises the sequence at least 95% or 100% identical to SEQ ID NO: 3012 or to SEQ ID NO: 3013.
- [0399]** 24. The AAV vector of any one of embodiments 1-23, wherein the cassette further comprises an enhancer.
- [0400]** 25. The AAV vector of embodiment 24, wherein the enhancer is the CMV enhancer.
- [0401]** 26. The AAV vector of embodiment 24, wherein the enhancer comprises the sequence of SEQ ID NO: 3009, or a sequence at least 95% identical thereto.
- [0402]** 27. The AAV vector of any one of embodiments 1-26, wherein the cassette further comprises an intronic sequence.
- [0403]** 28. The AAV vector of embodiment 27, wherein the intronic sequence is a chimeric sequence.
- [0404]** 29. The AAV vector of embodiment 27, wherein the intronic sequence is a hybrid sequence.
- [0405]** 30. The AAV vector of embodiment 27, wherein the intronic sequence comprises a sequence isolated or derived from SV40.
- [0406]** 31. The AAV vector of embodiment 27, wherein the intronic sequence comprises the sequence of any one of SEQ ID NO: 3010-3011.
- [0407]** 32. The AAV vector of embodiment 1, wherein the AAV transfer cassette comprises the sequence of any one of SEQ ID NO: 3014-3019.
- [0408]** 33. An adeno-associated virus (AAV) vector comprising: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180 or 175, or a sequence comprising about 1 to about 25 amino acid mutations relative to SEQ ID NO: 180 or 175; and (ii) a transfer cassette encapsidated by the protein capsid; wherein the transfer cassette comprises from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene sequence which encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR.
- [0409]** 34. A composition comprising the AAV vector of any one of embodiments 1-33.
- [0410]** 35. The composition of embodiment 34, wherein the composition comprises a pharmaceutically acceptable carrier or excipient.

[0411]36. A method for treating a subject in need thereof comprising administering to the subject a therapeutically effective amount of the AAV vector of any one of embodiments 1-33, or the composition of any one of embodiments 34-35.

[0412]37. The method of embodiment 36, wherein the subject has Neimann-Pick Disease Type C.

[0413]38. The method of embodiment 36 or 37, wherein the subject is a human subject.

[0414]39. An adeno-associated virus (AAV) vector comprising: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180 or 175; and (ii) a transfer cassette encapsidated by the protein capsid; wherein the transfer cassette comprises from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene sequence comprising the sequence of SEQ ID NO: 3002; a polyadenylation signal; and a 3' ITR.

[0415]40. An adeno-associated virus (AAV) vector comprising: (i) a protein capsid comprising a capsid protein comprising the sequence of SEQ ID NO: 180 or 175; and (ii) a nucleic acid encapsidated by the protein capsid; wherein the nucleic acid comprises a transfer cassette; wherein the transfer cassette comprises from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene sequence which encodes the NPC1 protein, wherein the NPC1 protein comprises the sequence of SEQ ID NO: 3001; a polyadenylation signal; and a 3' ITR.

[0416]41. The AAV vector of any one of embodiments 1-33, 39 and 40, wherein the AAV vector selectively delivers the transfer cassette to a cell or tissue of the central nervous system.

[0417]42. The AAV vector of embodiment 41, wherein the tissue of the central nervous system is the premotor cortex, the thalamus, the cerebellar cortex, the dentate nucleus, the spinal cord, or the dorsal root ganglion.

[0418]43. The AAV vector of any one of embodiments 1-33, 39 and 40, wherein the AAV vector delivers the transfer cassette to the brain, but does not deliver the AAV vector to the heart.

[0419]44. The AAV vector of any one of embodiments 1-33, 39 and 40, wherein the AAV vector delivers the transfer cassette to the brain and to the heart.

[0420] 45. The AAV vector of embodiment 44, wherein delivery of the transfer cassette is greater to the brain than to the heart.

[0421] 46. The AAV vector of embodiment 44, wherein delivery of the transfer cassette is approximately equal in the brain in the heart.

[0422] 47. A cell comprising the AAV vector of any one of embodiments 1-33 and 39-46.

[0423] 48. An *in vitro* method of introducing a transfer cassette into a cell, comprising contacting the cell with the AAV vector of any one of embodiments 1-33 and 39-46.

[0424] 49. An AAV vector of any one of embodiments 1-33 and 39-46 for use as a medicament.

[0425] 50. An AAV vector of any one of embodiments 1-33 and 39-46 for use in a method of treating or preventing Neimann-Pick Disease Type C in a subject in need thereof.

[0426] 51. The AAV vector of any one of embodiments 1-33 and 39-46, wherein the capsid protein subunit comprises the sequence of SEQ ID NO: 180.

[0427] 52. The AAV vector of any one of embodiments 1-33 and 39-46, wherein the capsid protein subunit comprises the sequence of SEQ ID NO: 175.

[0428] 53. An Adeno-Associated Virus (AAV) transfer cassette comprising, from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene; a polyadenylation signal; and a 3' ITR; wherein the transgene encodes the NPC1 protein.

[0429] 54. The AAV transfer cassette of embodiment 53, wherein at least one of the 5' ITR and the 3' ITR is about 110 to about 160 nucleotides in length.

[0430] 55. The AAV transfer cassette of embodiment 53 or 54, wherein the 5' ITR is the same length as the 3' ITR.

[0431] 56. The AAV transfer cassette of embodiment 53 or 54, wherein the 5' ITR and the 3' ITR have different lengths.

[0432] 57. The AAV transfer cassette of any one of embodiments 53-56, wherein at least one of the 5' ITR and the 3' ITR is isolated or derived from the genome of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV or Bovine AAV.

[0433] 58. The AAV transfer cassette of embodiment 53 wherein the 5' ITR comprises the sequence of SEQ ID NO: 3003.

[0434]59. The AAV transfer cassette of embodiment 53, wherein the 3' ITR comprises the sequence of SEQ ID NO: 3004.

[0435]60. The AAV transfer cassette of any one of embodiments 53-59, wherein the promoter is a constitutive promoter.

[0436]61. The AAV transfer cassette of any one of embodiments 53-59, wherein the promoter is an inducible promoter.

[0437]62. The AAV transfer cassette of any one of embodiments 53-59, wherein the promoter is a tissue-specific promoter.

[0438]63. The AAV transfer cassette of any one of embodiments 53-59, wherein the promoter is selected from the group consisting of the CBA promoter, the GUSB240 promoter, the GUSB379 promoter, the HSVTK promoter, the CMV promoter, the SV40 early promoter, the SV40 late promoter, the metallothionein promoter, the murine mammary tumor virus (MMTV) promoter, the Rous sarcoma virus (RSV) promoter, the polyhedrin promoter, the chicken β -actin (CBA) promoter, the EF-1 alpha promoter, the dihydrofolate reductase (DHFR) promoter, and the phosphoglycerol kinase (PGK) promoter.

[0439]64. The AAV transfer cassette of embodiment 63, wherein the promoter is selected from the group consisting of the CBA promoter, the GUSB240 promoter, the GUSB379 promoter, and the HSVTK promoter.

[0440]65. The AAV transfer cassette of any one of embodiments 53-59, wherein the promoter comprises a sequence at least 95% or 100% identical to any one of SEQ ID NO: 3005, SEQ ID NO: 3006, SEQ ID NO: 3007, or SEQ ID NO: 3008.

[0441]66. The AAV transfer cassette of any one of embodiments 53-65, wherein the NPC1 protein is the human NPC1 protein.

[0442]67. The AAV transfer cassette of any one of embodiments 53-65, wherein the NPC1 protein has a sequence that is at least 90% identical to the sequence of the human NPC1 protein.

[0443]68. The AAV transfer cassette of embodiment 67, wherein the NPC1 protein has a sequence that is at least 95% identical to the sequence of the human NPC1 protein.

[0444]69. The AAV transfer cassette of embodiment 68, wherein the NPC1 protein has a sequence that is at least 98% identical to the sequence of the human NPC1 protein.

[0445] 70. The AAV transfer cassette of any one of embodiments 53-65, wherein the NPC1 protein comprises the sequence of SEQ ID NO: 3001.

[0446] 71. The AAV transfer cassette of any one of embodiments 53-65, wherein the transgene comprises the sequence of SEQ ID NO: 3002.

[0447] 72. The AAV transfer cassette of any one of embodiments 53-71, wherein the polyadenylation signal is selected from simian virus 40 (SV40), rBG, α -globin, β -globin, human collagen, human growth hormone (hGH), polyoma virus, human growth hormone (hGH) and bovine growth hormone (bGH).

[0448] 73. The AAV transfer cassette of embodiment 72, wherein the polyadenylation signal is the SV40 polyadenylation signal.

[0449] 74. The AAV transfer cassette of embodiment 72, wherein the polyadenylation signal is the rBG polyadenylation signal.

[0450] 75. The AAV transfer cassette of any one of embodiments 53-71, wherein the polyadenylation signal comprises the sequence at least 95% or 100% identical to SEQ ID NO: 3012 or to SEQ ID NO: 3013.

[0451] 76. The AAV transfer cassette of any one of embodiments 53-75, wherein the cassette further comprises an enhancer.

[0452] 77. The AAV transfer cassette of embodiment 76, wherein the enhancer is the CMV enhancer.

[0453] 78. The AAV transfer cassette of embodiment 76, wherein the enhancer comprises the sequence of SEQ ID NO: 3009, or a sequence at least 95% identical thereto.

[0454] 79. The AAV transfer cassette of any one of embodiments 53-78, wherein the cassette further comprises an intronic sequence.

[0455] 80. The AAV transfer cassette of embodiment 79, wherein the intronic sequence is a chimeric sequence.

[0456] 81. The AAV transfer cassette of embodiment 79, wherein the intronic sequence is a hybrid sequence.

[0457] 82. The AAV transfer cassette of embodiment 79, wherein the intronic sequence comprises sequences isolated or derived from SV40.

[0458] 83. The AAV transfer cassette of embodiment 79, wherein the intronic sequence comprises the sequence of any one of SEQ ID NO: 3010-3011.

[0459]84. The AAV transfer cassette of embodiment 53, wherein the AAV transfer cassette comprises the sequence of any one of SEQ ID NO: 3014-3019.

[0460]85. A plasmid comprising the AAV transfer cassette of any one of embodiments 53-84.

[0461]86. A cell comprising the AAV transfer cassette of any one of embodiments 53-84 or the plasmid of embodiment 85.

[0462]87. A method of producing a recombinant AAV vector, the method comprising contacting an AAV producer cell with the AAV transfer cassette of any one of embodiments 53-84 or the plasmid of embodiment 85.

[0463]88. A recombinant AAV vector produced by the method of embodiment 87.

[0464]89. The recombinant AAV vector of embodiment 88, wherein the vector is of a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV and Bovine AAV.

[0465]90. A composition comprising the AAV transfer cassette of any one of embodiments 53-84, the plasmid of embodiment 85, the cell of embodiment 86, or the recombinant AAV vector of embodiment 88 or 89.

[0466]91. A method for treating a subject in need thereof comprising administering to the subject an effective amount of the AAV transfer cassette of any one of embodiments 53-84, the plasmid of embodiment 85, the cell of embodiment 86, or the recombinant AAV vector of embodiment 88 or 89.

[0467]92. The method of embodiment 91, wherein the subject suffers from the disease NPC1.

[0468]93. The method of embodiment 91 or 92, wherein the subject is a human subject.

[0469]94. An adeno-associated virus (AAV) vector comprising: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180; and (ii) a nucleic acid encapsidated by the protein capsid; wherein the nucleic acid comprises a transfer cassette; wherein the transfer cassette comprises, from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene that encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR.

[0470] 95. An adeno-associated virus (AAV) vector comprising: (i) a protein capsid comprising a capsid protein subunit comprising the sequence of SEQ ID NO: 180, or a sequence comprising about 1 to about 25 amino acid mutations relative to SEQ ID NO: 180; and (ii) a nucleic acid encapsidated by the protein capsid; wherein the nucleic acid comprises a transfer cassette; wherein the transfer cassette comprises from 5' to 3': a 5' inverted terminal repeat (ITR); a promoter; a transgene which encodes the NPC1 protein; a polyadenylation signal; and a 3' ITR.

[0471] 96. The AAV vector of embodiment 94 of 95, wherein the transfer cassette comprises an intronic sequence.

[0472] 97. The AAV vector of any one of embodiments 94-96, wherein the intronic sequence comprises the sequence of SEQ ID NO: 10.

[0473] 98. The AAV vector of any one of embodiments 94-97, wherein the 5' ITR comprises the sequence of SEQ ID NO: 3003.

[0474] 99. The AAV vector of any one of embodiments 94-98, wherein the 3' ITR comprises the sequence of SEQ ID NO: 3004.

[0475] 100. The AAV vector of any one of embodiments 94-99, wherein the promoter is the CBA promoter.

[0476] 101. The AAV vector of any one of embodiments 94-99, wherein the promoter comprises the sequence of SEQ ID NO: 3005.

[0477] 102. The AAV vector of any one of embodiments 94-101, wherein the NPC1 protein is the human NPC1 protein.

[0478] 103. The AAV vector of any one of embodiments 94-101, wherein the NPC1 protein comprises the sequence of SEQ ID NO: 3001.

[0479] 104. The AAV vector of any one of embodiments 94-101, wherein the transgene comprises the sequence of SEQ ID NO: 3002.

[0480] 105. The AAV vector of any one of embodiments 94-104, wherein the polyadenylation signal is the SV40 polyadenylation signal.

[0481] 106. The AAV vector of any one of embodiments 94-104, wherein the polyadenylation signal comprises the sequence of SEQ ID NO: 3012.

[0482] 107. The AAV vector of any one of embodiments 94-106, wherein the cassette comprises an enhancer.

[0483] 108. The AAV vector of embodiment 94, wherein the AAV transfer cassette comprises the sequence of SEQ ID NO: 3014

[0484] 109. The AAV vector of embodiment 94, wherein the AAV transfer cassette comprises the sequence of any one of SEQ ID NO: 3015-3019.

[0485] 110. A composition comprising the AAV vector of any one of embodiments 94-109.

[0486] 111. A cell comprising the AAV vector of any one of embodiments 94-109.

[0487] 112. A method for treating a subject in need thereof comprising administering to the subject an effective amount of the AAV vector of any one of embodiments 94-109, the composition of embodiment 110, or the cell of embodiment 111.

[0488] 113. The method of embodiment 112, wherein the subject has Neimann-Pick Disease Type C.

[0489] 114. The method of embodiment 112 or 113, wherein the subject is a human subject.