

**COMPOSITIONS AND METHODS FOR THE TREATMENT OF DISORDERS
RELATED TO FRATAXIN DEFICIENCY**

RELATED APPLICATIONS

[01] This application claims the benefit of and priority to US Provisional Application Serial No. 63/606,275, filed December 5, 2023.

SEQUENCE LISTING

[02] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing file, entitled 14640_0102-00304_SL.xml, was created on September 18, 2024, and is 1,772,400 bytes in size. The information in electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD

[03] Described herein are compositions and methods relating to adeno-associated virus (AAV) viral particles for the delivery of polynucleotides, e.g., polynucleotides encoding a frataxin (FXN) protein for use in the treatment of Friedreich's Ataxia (FA). In some embodiments, compositions described herein may be used to treat a subject in need thereof, such as a human subject diagnosed with FA, or as a research tool in the study of diseases or conditions in cells or animal models of FA.

BACKGROUND

[04] Friedreich's Ataxia (FA) is an autosomal recessive inherited disease that causes progressive damage to the nervous system. See Parkinson et al., Journal of Neurochemistry, 2013, 126 (Suppl. 1), 103-117, the contents of which are herein incorporated by reference in their entirety. FA typically results from the degeneration of nervous tissue in the spinal cord due to reduced expression of the mitochondrial protein frataxin (FXN; also known as, e.g., CyaY, FA, FARR, FRDA, and X25) in sensory neurons that direct muscle movement of the arms and legs. See Koeppen, Arnulf, J Neurol Sci., 2011, April 15; 303(1-2): 1-12. Onset usually occurs at puberty or by age 25. See Campuzano, et al., Science, 271.5254 (Mar 8, 1996): 1423. Initial symptoms of FA include poor coordination such as gait disturbance, poor balance, leg weakness, decreased walking, impaired coordination, dysarthria, nystagmus, impaired sensation, kyphoscoliosis, and foot deformities. See Parkinson et al., Journal of Neurochemistry, 2013, 126 (Suppl. 1), 103-117. FA is also associated with scoliosis, heart disease, and diabetes. The disease generally progresses until a wheelchair is required for mobility. Incidence of FA among Caucasian populations is between about 1 in 20,000 and about 1 in 50,000, with a deduced carrier frequency of about 1 in 120 in European populations. See Nageshwaran and Festenstein, Frontiers in Neurology, Vol. 6, Art. 262 (2015); Campuzano, et al., Science, 271.5254 (Mar 8, 1996): 1423, the contents of which are herein incorporated by reference in their entirety. The expansion of an intronic GAA triplet repeat in the FXN gene is the genetic cause of reduced expression of FXN resulting in FA. See Parkinson et al.,

Journal of Neurochemistry, 2013, 126 (Suppl. 1), 103-117. Over time, the deficiency causes the aforementioned symptoms, as well as frequent fatigue due to effects on cellular metabolism. Currently, omaveloxolone (Skyclarys®) is the only FDA approved treatment for FA. Omaveloxolone is a semisynthetic oleanane triterpenoid that activates Nrf2, a master transcription factor that regulates genes with antioxidative, anti-inflammatory, and mitochondrial bioenergetic properties. See Reisman et al. (2019) Drug Des Devel Ther. 13:1259-1270. While gene therapy constructs for delivering a frataxin protein have been described in the art, there remains a need to develop improved constructs for better targeting of the appropriate tissues in the body.

[05] Adeno-associated viruses (AAVs) have emerged as a widely studied and utilized viral particles for delivery of therapeutically effective polypeptides to mammalian cells. See, e.g., Tratschin et al., Mol. Cell Biol., 5(11):3251-3260 (1985) and Grimm et al., Hum. Gene Ther., 10(15):2445-2450 (1999), the contents of each of which are herein incorporated by reference in their entirety.

[06] There remains a need for effective methods of treatment using AAV capsids that are capable of delivering FXN to a target cell or tissue, e.g., a CNS cell or tissue.

SUMMARY

[07] The present disclosure addresses these challenges by providing AAV-based compositions comprising AAV capsid variants and methods for treating Friedreich's Ataxia (FA) or at least one symptom thereof in subjects. Disclosed herein are compositions and methods directed to AAV-based gene delivery of FXN (e.g., human FXN) to ameliorate loss-of-function and to improve FXN expression (e.g., in the brain, e.g., in neurons). In some embodiments, the compositions and methods can be used to slow, halt, or reverse symptoms of FA.

[08] In some aspects, the present disclosure provides an adeno-associated virus (AAV) particle comprising an AAV capsid variant and a viral genome, wherein the viral genome comprises a frataxin (FXN)-encoding sequence and the AAV capsid variant comprises an amino acid sequence having the formula [N1]-[N2]-[N3] (SEQ ID NO: 4681) in loop VIII, wherein [N2] comprises the amino acid sequence of DWHR (SEQ ID NO: 4682); [N1] comprises amino acids X₁, X₂, X₃, and X₄, wherein X₄ is Q, K, E, S, P, R, N, or H; and/or [N3] comprises amino acids X₅, X₆, and X₇, wherein X₅ is I, V, T, M, S, N, L, or F. In some embodiments, the AAV capsid variant is an AAV9 capsid variant. In some embodiments, the AAV capsid variant comprises an amino acid sequence that is at least 95% identical to positions 203-736 of the amino acid sequence of SEQ ID NO: 981. In some embodiments, loop VIII is present at amino acids comprising those corresponding to positions 580-599 of the amino acid sequence of SEQ ID NO: 981.

[09] In some embodiments, X₁ is T, S, R, A, I, C, N, K, L, or Q; X₂ is N, T, G, V, S, Y, K, I, H, D, or F; and X₃ is T, N, K, D, I, S, P, A, Y, E, V, L, M, R, H, Q, or C. In some embodiments, X₆ is A, Y, P, N, S, T, G, E, V, W, F, or Q; and X₇ is Q, G, N, K, H, R, E, L, P, or M.

[010] In some embodiments, [N1] comprises the amino acid sequence of TNTQ (SEQ ID NO: 4688). In some embodiments, [N3] comprises the amino acid sequence of IAQ. In some embodiments, [N1]-[N2]-[N3] comprises the amino acid sequence of TNTQDWHRIAQ (SEQ ID NO: 343).

[011] In some embodiments, [N1] is present at amino acids comprising those corresponding to positions 580-599 of the amino acid sequence of SEQ ID NO: 981, [N2] is present at amino acids corresponding to positions 586-589 of the amino acid sequence of SEQ ID NO: 981, and [N3] is present at amino acids corresponding to positions 590-592 of the amino acid sequence of SEQ ID NO: 981.

[012] In some embodiments, the AAV capsid variant further comprises [N4] comprising amino acids X_8 , X_9 , X_{10} , and X_{11} , wherein: X_8 is T, S, N, P, A, or I; X_9 is G, N, D, R, V, A, S, or Q; X_{10} is W, S, C, R, L, or G; and/or X_{11} is V, A, S, I, C, G, D, F, L, or T. In some embodiments, [N4] comprises the amino acid sequence of TGVV (SEQ ID NO: 5066). In some embodiments, [N4] is present at amino acids corresponding to positions 593-596 of the amino acid sequence of SEQ ID NO: 981.

[013] In some embodiments, [N1]-[N2]-[N3]-[N4] comprises the amino acid sequence of TNTQDWHRIAQTGVV (SEQ ID NO: 201).

[014] In some aspects, the present disclosure provides an AAV particle comprising a viral genome comprising a FXN-encoding sequence and an AAV capsid variant comprising an amino acid sequence that is at least 95% identical to positions 203-736 of the amino acid sequence of SEQ ID NO: 981, wherein the AAV capsid variant comprises: T at an amino acid corresponding to position 584 of the amino acid sequence of SEQ ID NO: 981; D at an amino acid corresponding to position 586 of the amino acid sequence of SEQ ID NO: 981; W at an amino acid corresponding to position 587 of the amino acid sequence of SEQ ID NO: 981; H at an amino acid corresponding to position 588 of the amino acid sequence of SEQ ID NO: 981; R at an amino acid corresponding to position 589 of the amino acid sequence of SEQ ID NO: 981; and I at an amino acid corresponding to position 590 of the amino acid sequence of SEQ ID NO: 981.

[015] In some aspects, the present disclosure provides an AAV particle comprising a viral genome comprising a FXN-encoding sequence and an AAV capsid variant comprising an amino acid sequence that is at least 95% identical to positions 138-736 of the amino acid sequence of SEQ ID NO: 981. In some aspects, the present disclosure provides an AAV particle comprising a viral genome comprising a FXN-encoding sequence and an AAV capsid variant comprising an amino acid sequence that is at least 95% identical to positions 138-736 of the amino acid sequence of SEQ ID NO: 981, wherein the AAV capsid variant comprises: T at an amino acid corresponding to position 584 of the amino acid sequence of SEQ ID NO: 981; D at an amino acid corresponding to position 586 of the amino acid sequence of SEQ ID NO: 981; W at an amino acid corresponding to position 587 of the amino acid sequence of SEQ ID NO: 981; H at an amino acid corresponding to position 588 of the amino acid sequence of SEQ ID NO: 981; R at an amino acid corresponding to position 589 of the amino acid sequence of SEQ ID NO: 981; and I at an amino acid corresponding to position 590 of the amino acid sequence of SEQ ID NO: 981.

[016] In some aspects, the present disclosure provides an AAV particle comprising a viral genome comprising a FXN-encoding sequence and an AAV capsid variant comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 981. In some aspects, the present disclosure provides an AAV particle comprising a viral genome comprising a FXN-encoding sequence and an AAV capsid variant comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 981, wherein the AAV capsid variant comprises: T at an amino acid corresponding to position 584 of the amino acid sequence of SEQ ID NO: 981; D at an amino acid corresponding to position 586 of the amino acid sequence of SEQ ID NO: 981; W at an amino acid corresponding to position 587 of the amino acid sequence of SEQ ID NO: 981; H at an amino acid corresponding to position 588 of the amino acid sequence of SEQ ID NO: 981; R at an amino acid corresponding to position 589 of the amino acid sequence of SEQ ID NO: 981; and I at an amino acid corresponding to position 590 of the amino acid sequence of SEQ ID NO: 981.

[017] In some aspects, the present disclosure provides an AAV particle comprising a viral genome comprising a FXN-encoding sequence and an AAV capsid variant, wherein the AAV capsid variant comprises: (i) an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 981; (ii) an amino acid sequence that is at least 99% identical to positions 138-736 of the amino acid sequence of SEQ ID NO: 981; and/or (iii) an amino acid sequence that is at least 99% identical to positions 203-736 of the amino acid sequence of SEQ ID NO: 981.

[018] In some embodiments, the AAV capsid variant comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941). In some embodiments, the AAV capsid variant comprises at least 8, at least 9, or at least 10 consecutive amino acids from the amino acid sequence of TNTQDWHRIAQ (SEQ ID NO: 343). In some embodiments, the AAV capsid variant comprises the amino acid sequence of TNTQDWHRIAQ (SEQ ID NO: 343) present at amino acids corresponding to positions 582-592 of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the amino acid sequence of TQDWHRI (SEQ ID NO: 941) or TNTQDWHRIAQ (SEQ ID NO: 343) is present in loop VIII, wherein loop VIII comprises amino acids 580-599 of the amino acid sequence of SEQ ID NO: 981.

[019] In some embodiments, the AAV capsid variant comprises: (i) the amino acid sequence of SEQ ID NO: 981; (ii) the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981; and/or (iii) the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981.

[020] In some embodiments, the FXN protein encoded by the FXN-encoding sequence is not a cynomolgus monkey FXN protein. In some embodiments, the FXN-encoding sequence encodes a human FXN protein. In some embodiments, the human FXN protein comprises the amino acid sequence of any one of SEQ ID NOs: 5124-5126 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the FXN-encoding sequence encodes a human FXN protein. In some embodiments, the human FXN protein comprises the amino acid

sequence of SEQ ID NO: 1825 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the FXN protein is a wildtype human FXN protein.

[021] In some embodiments, the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[022] In some embodiments, the viral genome further comprises a promoter operably linked to the FXN-encoding sequence. In some embodiments, the promoter comprises a human elongation factor 1 α -subunit (EF1 α) promoter, a cytomegalovirus (CMV) immediate-early enhancer and/or promoter, a chicken β -actin (CBA) promoter, a CAG promoter, a β glucuronidase (GUSB) promoter, a ubiquitin C (UBC) promoter, a neuron-specific enolase (NSE) promoter, a platelet-derived growth factor (PDGF) promoter, a platelet-derived growth factor B-chain (PDGF- β) promoter, an intercellular adhesion molecule 2 (ICAM-2) promoter, a synapsin (Syn) promoter, a methyl-CpG binding protein 2 (MeCP2) promoter, a Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) promoter, a metabotropic glutamate receptor 2 (mGluR2) promoter, a neurofilament light chain (NFL) promoter, a neurofilament heavy chain (NFH) promoter, a β -globin minigene n β 2 promoter, a preproenkephalin (PPE) promoter, an enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2) promoter, a glial fibrillary acidic protein (GFAP) promoter, a myelin basic protein (MBP) promoter, a cardiovascular promoter (e.g., α MHC, cTnT, and CMV-MLC2k), a liver promoter (e.g., hAAT, TBG), a skeletal muscle promoter (e.g., desmin, MCK, C512), or a functional fragment or truncation thereof. In some embodiments, the promoter is a CMV promoter or CBA promoter, or a functional fragment or truncation of a CMV promoter or CBA promoter. In some embodiments, the promoter is a truncated CBA promoter. In some embodiments, the truncated CBA promoter is 50-400 nucleotides in length, e.g., 100-332 nucleotides in length. In some embodiments, the promoter comprises (i) the nucleotide sequence SEQ ID NO: 1738 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (ii) the nucleotide sequence of SEQ ID NO: 1740 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or (iii) the nucleotide sequence of SEQ ID NO: 1742 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the promoter is a truncated CMV promoter. In some embodiments, the truncated CMV promoter is 50-300 nucleotides in length. In some embodiments, the promoter comprises the nucleotide sequence of SEQ ID NO: 1750 or a nucleotide sequence that is at least 90% identical (e.g.,

at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[023] In some embodiments, the viral genome further comprises a nucleotide sequence encoding a miRNA (miR) binding site that modulates expression of the encoded FXN protein in a cell or tissue of the liver. In some embodiments, the viral genome encodes 3 copies of the miR binding site. In some embodiments, the 3 copies of the miR binding site are identical. In some embodiments, the miR binding site is a miR122 binding site. In some embodiments, the miR122 binding site is encoded by the nucleotide sequence of SEQ ID NO: 1827 or a nucleotide sequence comprising one, two, three, or up to four substitutions relative thereto; or the viral genome encodes 3 copies of the miR122 binding site, wherein the nucleotide sequence encoding the 3 copies of the miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence comprising one, two, three, four, five, six, seven, eight, nine, or up to ten substitutions relative thereto.

[024] In some embodiments, the viral genome further comprises at least one inverted terminal repeat (ITR) region. In some embodiments, the at least one ITR region comprises an AAV2 ITR. In some embodiments, the viral genome comprises a 5' ITR region and a 3' ITR region. In some embodiments, the 5' ITR region and 3' ITR region are each an AAV2 ITR. In some embodiments, the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[025] In some embodiments, the viral genome further comprises an intron/exon region comprising an intron region and/or an exon region. In some embodiments, the intron/exon region comprises an immediate-early 1 (ic1) intron region and/or a human beta-globin (hBglobin) intron 2 region; and/or an ic1 exon region and/or an hBglobin exon region. In some embodiments, the intron region comprises the nucleotide sequence of SEQ ID NO: 1819 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or comprises the nucleotide sequence of SEQ ID NO: 1820 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the exon region comprises the nucleotide sequence of SEQ ID NO: 1817 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or comprises the nucleotide sequence of SEQ ID NO: 1821 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments,

the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[026] In some embodiments, the viral genome further comprises a polyadenylation (poly A) region. In some embodiments, the poly A region comprises the nucleotide sequence of SEQ ID NO: 1828 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[027] In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[028] In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[029] In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[030] In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[031] In some embodiments, the viral genome comprises: (i) a 5' inverted terminal repeat (ITR) region; (ii) a promoter; (iii) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and (iv) a 3' ITR region.

[032] In some embodiments, the viral genome comprises: (i) a 5' inverted terminal repeat (ITR) region; (ii) a promoter; (iii) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iv) a nucleotide sequence encoding at least one miR122 binding site; and (v) a 3' ITR region.

[033] In some embodiments, the viral genome comprises: (i) a 5' inverted terminal repeat (ITR) region; (ii) a promoter; (iii) an intron/exon region; (iv) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that

is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (v) a nucleotide sequence encoding at least one miR122 binding site; and (vi) a 3' ITR region.

[034] In some embodiments, the viral genome comprises: (i) a 5' inverted terminal repeat (ITR) region; (ii) a promoter; (iii) an intron/exon region; (iv) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (v) a nucleotide sequence encoding at least one miR122 binding site; (vi) a polyadenylation (polyA) region; and (vii) a 3' ITR region.

[035] In some embodiments, the viral genome comprises: (i) a 5' inverted terminal repeat (ITR) region; (ii) a promoter; (iii) an intron/exon region; (iv) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (v) a nucleotide sequence encoding at least one miR122 binding site; (vi) a polyadenylation (polyA) region; (vii) a filler sequence; and (viii) a 3' ITR region.

[036] In some embodiments, (i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1742 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (vi) the poly A region comprises the nucleotide sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or (vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least

92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1841 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR.

[037] In some embodiments, (i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1750 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or (vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1840 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR.

[038] In some embodiments, (i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1738 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least

93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (vi) the poly A region comprises the nucleotide sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or (vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1838 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR.

[039] In some embodiments, (i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1740 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence comprising SEQ ID NO: 1826 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (vi) the poly A region comprises the nucleotide

sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or (vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1839 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR.

[040] In some embodiments, the viral genome comprises: (i) the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (ii) the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iii) the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or (iv) the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, (i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811; (ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1742; (iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816; (iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824; (v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826; (vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828; and (vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812. In some embodiments, the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1841, wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR region.

[041] In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797. In some embodiments, the viral genome consists of the nucleotide sequence of SEQ ID NO: 1797.

[042] In some aspects, the present disclosure provides an AAV particle comprising an AAV capsid variant and a viral genome comprising a frataxin (FXN)-encoding sequence, wherein the AAV capsid variant comprises: (i) the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (ii) the amino acid sequence

according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or (iii) the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and the viral genome comprises: (a) the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (b) the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (c) the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or (d) the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[043] In some aspects, the present disclosure provides an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981, the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981, and/or the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[044] In some embodiments, the viral genome is single-stranded.

[045] In some aspects, the present disclosure provides a cell comprising an AAV particle provided herein. In some embodiments, the cell is a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

[046] In some aspects, the present disclosure provides a method of making an AAV particle provided herein. In some embodiments, the method comprises (i) providing a cell comprising a viral genome comprising a frataxin (FXN)-encoding sequence and a nucleic acid encoding an AAV capsid variant; and (ii) incubating the cell under conditions suitable to encapsulate the viral genome in the AAV capsid variant; thereby making the AAV particle.

[047] In some embodiments of the method of making an AAV particle, the viral genome comprises (i) the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (ii) the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%,

at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iii) the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or (iv) the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and the AAV capsid variant comprises (a) the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (b) the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or (c) the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[048] In some embodiments of the method of making an AAV particle, the viral genome comprises (i) the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (ii) the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (iii) the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or (iv) the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 981, the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981, and/or the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981.

[049] In some embodiments of the method of making an AAV particle, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797 and the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 981, the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981, and/or the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981.

[050] In some embodiments, the method of making an AAV particle further comprises, prior to step (i), introducing a nucleic acid comprising the viral genome into the cell. In some embodiments, the method further comprises, prior to step (i), introducing the nucleic acid encoding the AAV capsid variant into the cell. In some embodiments, the cell comprises a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

[051] In some aspects, the present disclosure provides a pharmaceutical composition comprising an AAV particle provided herein and a pharmaceutically acceptable excipient.

[052] In some aspects, the present disclosure provides a method of delivering an AAV particle encoding a frataxin (FXN) protein to a cell, comprising administering an effective amount of a pharmaceutical composition or AAV particle provided herein. In some embodiments, the cell is in a subject. In some embodiments, the subject has, has been diagnosed with having, or is at risk of having a disorder associated with FXN deficiency or at least one symptom thereof. In some embodiments, the disorder is Friedreich's Ataxia (FA).

[053] In some aspects, the present disclosure provides a method of treating a subject having or diagnosed with having a disorder associated with frataxin (FXN) deficiency or at least one symptom thereof, comprising administering to the subject an effective amount of a pharmaceutical composition or AAV particle provided herein. In some embodiments, the subject has, has been diagnosed with having, or is at risk of having Friedreich's Ataxia (FA) or at least one symptom thereof.

[054] In some aspects, the present disclosure provides a method of treating a subject having or diagnosed with having a disorder or at least one symptom thereof, wherein the disorder is FA, comprising administering to the subject an effective amount of a pharmaceutical composition or AAV particle provided herein.

[055] In some embodiments, the treating results in prevention of progression of the disorder or at least one symptom thereof in the subject. In some embodiments, the treating results in amelioration of at least one symptom of the disorder. In some embodiments, the at least one symptom comprises impaired sensory functions, impaired motor function (e.g., ataxia and/or involuntary movements), fatigue, chronic pain, seizures, impaired speech, sleep disturbances, metabolic disorders (e.g., diabetes), and/or increased spasticity. In some embodiments, the treating stabilizes, slows the progression of, or improves the subject's disorder as determined by the modified Friedreich Ataxia Rating Scale (mFARS), the Scale for the Assessment and Rating of Ataxia (SARA), and/or the International Cooperative Ataxia Rating Scale (ICARS). In some embodiments, the treating slows the subject's progression of the disorder as measured by mFARS, SARA, and/or ICARS relative to an individual with the disorder who has not been administered the pharmaceutical composition or the AAV particle.

[056] In some embodiments, the subject is a human.

[057] In some embodiments, the AAV particle or the pharmaceutical composition is delivered to a cell or tissue of the central nervous system (CNS) in the subject. In some embodiments, the cell or tissue of the CNS is a cell or tissue of the amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje

cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region). In some embodiments, the AAV particle or the pharmaceutical composition is delivered to the subject via intravenous administration.

[058] In some embodiments, a method of delivery or treatment provided herein further comprises evaluating, e.g., measuring, the level of FXN expression, e.g., FXN gene, FXN mRNA, and/or FXN protein expression, in the subject, e.g., in a cell, tissue, or fluid, of the subject. In some embodiments, the level of FXN protein expression is measured by an enzyme-linked immunosorbent assay (ELISA), a Western blot, an immunohistochemistry assay, or a frataxin biofluid assay. In some embodiments, evaluating the subject's level of FXN expression is performed before and/or after administration of the pharmaceutical composition or AAV particle, optionally wherein the subject's level of FXN expression before administration is compared to the subject's level of FXN expression after administration. In some embodiments, the cell or tissue in which the level of FXN expression is evaluated is a cell or tissue of the CNS. In some embodiments, the cell or tissue of the CNS is a cell or tissue of the amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region). In some embodiments, the method of delivery or treatment further comprises evaluating the level of FXN expression, e.g., FXN gene, FXN mRNA, and/or FXN protein expression, in a peripheral cell or tissue of the subject. In some embodiments, the peripheral cell or tissue is a cell or tissue of the heart and/or muscle. In some embodiments, the muscle is skeletal muscle.

[059] In some embodiments, the subject's level of FXN protein expression after administration is increased relative to the subject's level of FXN protein expression before administration.

[060] In some embodiments, the method of delivery or treatment further comprises evaluating, e.g., measuring, the level of FXN protein activity in the subject.

[061] In some embodiments, administration of the pharmaceutical composition or AAV particle results in an increase in: (i) the level of FXN protein or FXN gene expression in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g.,

cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region), or a cell or tissue outside of the CNS, e.g., muscle (e.g., skeletal muscle)), and/or fluid (e.g., CSF and or serum), of the subject relative to baseline and/or relative to the level of FXN protein or FXN gene expression in a cell, tissue, or fluid of an individual with a disorder associated with FXN deficiency who has not been administered the pharmaceutical composition or AAV particle; (ii) the number and/or level of viral genomes (VG) per cell in a CNS tissue (e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region)) or non-CNS tissue (e.g., muscle (e.g., skeletal muscle)) of the subject relative to the number and/or level of VG per cell in a peripheral tissue of the subject; and/or (iii) the level of FXN activity in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region), or a cell or tissue outside of the CNS, e.g., muscle (e.g., skeletal muscle)), and/or fluid (e.g., CSF and or serum) of the subject relative to baseline and/or relative to FXN activity in a cell, tissue, or fluid of an individual with a disorder associated with FXN deficiency who has not been administered the pharmaceutical composition or AAV particle.

[062] In some embodiments, a method of delivery or treatment provided herein further comprises administering to the subject at least one additional agent and/or therapy. In some embodiments, the at least one additional agent and/or therapy comprises an agent and/or therapy suitable for treating the disorder associated with FXN deficiency (e.g., Friedreich's Ataxia). In some embodiments, the at least one additional agent and/or therapy comprises omaveloxolone or idebenone. In some embodiments, a method of delivery or treatment provided herein further comprises administering an immunosuppressant to the subject. In some embodiments, the immunosuppressant comprises a corticosteroid (e.g., prednisone, prednisolone, methylprednisolone, and/or dexamethasone), rapamycin, mycophenolate mofetil, tacrolimus, rituximab, and/or eculizumab hydroxychloroquine.

[063] In some aspects, the present disclosure provides a pharmaceutical composition or AAV particle provided herein for use in a method of treating a disorder provided herein.

[064] In some aspects, the present disclosure provides a pharmaceutical composition or AAV particle provided herein for use in a method of treating a disorder is associated with FXN deficiency, or at least one symptom thereof, in a subject. In some embodiments, the disorder is Friedreich's Ataxia. In

some embodiments, the subject has, has been diagnosed with having, or is at risk of having Friedreich's Ataxia.

[065] In some aspects, the present disclosure provides a use of a pharmaceutical composition or AAV particle provided herein in the manufacture of a medicament for the treatment of a disorder associated with FXN deficiency, or at least one symptom thereof, in a subject. In some embodiments, the disorder is Friedreich's Ataxia. In some embodiments, the subject has, has been diagnosed with having, or is at risk of having Friedreich's Ataxia.

Enumerated Embodiments

1. An adeno-associated virus (AAV) particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant), wherein the AAV capsid variant comprises an amino acid sequence having the following formula: [N1]-[N2]-[N3] (SEQ ID NO: 4681), wherein [N2] comprises the amino acid sequence of DWHR (SEQ ID NO: 4682) and wherein:

- (i) [N1] comprises amino acids X₁, X₂, X₃, and X₄, wherein X₄ is Q, K, E, S, P, R, N, H; and/or
- (ii) [N3] comprises amino acids X₅, X₆, and X₇, wherein X₅ is I, V, T, M, S, N, L, F.

2. The AAV particle of embodiment 1, wherein the AAV capsid variant comprises the amino acid Q at position 585, as numbered according to SEQ ID NO: 138 or 981.

3. The AAV particle of embodiment 1, wherein the AAV capsid variant comprises an amino acid other than Q at position 585, as numbered according to SEQ ID NO: 138 or 981.

4. The AAV particle of embodiment 1 or 3, wherein the AAV capsid variant comprises the amino acid K at position 585, as numbered according to SEQ ID NO: 138 or 981.

5. The AAV particle of any one of embodiments 1-4, wherein the AAV capsid variant comprises one, two, three, or all of:

- (i) an amino acid other than T at position 582 (e.g., S, R, A, I, C, N, K, L, or Q);
- (ii) an amino acid other than N at position 583 (e.g., T, G, V, S, Y, K, I, H, D, or F);
- (iii) an amino acid other than H at position 584 (e.g., T, N, K, D, I, S, P, A, Y, E, V, L, M, R, Q,

or C); and/or

- (iv) an amino acid other than Q at position 585 (e.g., K, E, S, P, R, N, or H);

wherein the amino acids are numbered according to SEQ ID NO: 138.

6. The AAV particle of any one of embodiments 1-5, wherein [N1] comprises amino acids X₁, X₂, X₃, and X₄, wherein X₄ is Q, K, E, S, P, R, N, or H.

7. The AAV particle of embodiment 6, wherein X_4 is Q or K.
8. The AAV particle of any one of embodiment 7, wherein X_4 is Q.
9. The AAV particle of embodiment 7, wherein X_4 is K.
10. The AAV particle of any one of embodiments 1-9, wherein the AAV capsid variant comprises an amino acid other than H at position 584 (e.g., T), as numbered according to SEQ ID NO: 138.
11. The AAV particle of any one of embodiments 1-10, wherein the AAV capsid variant comprises the amino acid T at position 584, as numbered according to SEQ ID NO: 138 or 981.
12. The AAV particle of any one of embodiments 1-11, wherein:
- (i) X_1 is T, S, R, A, I, C, N, K, L, or Q;
 - (ii) X_2 is N, T, G, V, S, Y, K, I, H, D, or F; and/or
 - (iii) X_3 is T, N, K, D, I, S, P, A, Y, E, V, L, M, R, H, Q, or C.
13. The AAV particle of any one of embodiments 1-12, wherein [N1] comprises TN, NT, NK, SN, TT, RN, TG, TV, ST, TS, TY, AN, TK, TI, IN, TH, TD, CN, NN, KN, LN, SG, TF, RT, SY, SS, QN, ND, NP, GK, TA, VK, NY, TE, SK, NI, YN, GT, TL, TM, YT, TR, NS, IT, NA, KT, GN, HT, DT, NE, NH, YI, HN, NQ, FS, NM, NL, SM, NC, VT, KQ, TQ, DQ, IQ, SQ, PS, KE, AQ, YQ, TP, EQ, VQ, LQ, MQ, KS, IE, RQ, IK, AK, PK, NR, HQ, QQ, or CQ.
14. The AAV particle of any one of embodiments 1-13, wherein [N1] comprises TNT, TNK, TNN, SNN, SNK, SNT, TTN, TND, TTI, RNT, TTK, TTS, TTD, TNP, TTT, TGK, TTA, TVK, TNY, STK, TTE, TSK, TNI, TYN, STI, TTV, TGT, TTL, TTM, ANN, SNI, TKN, TYT, TTR, TNS, TST, TIT, INT, TNA, TKT, STN, ANT, RNN, TGN, TSN, THT, TDT, TNE, CNT, INN, NNN, KNN, LNN, TIN, TNH, STT, SNS, STS, TYI, SGT, THN, TNQ, RNI, TFS, RNS, TNM, RTT, KNT, TNL, TSM, SYT, TNC, SST, TVT, QNT, NTK, NNQ, NKQ, NNE, NTQ, NDQ, TIQ, TKQ, TSQ, TDQ, NPS, NKE, TTQ, GKQ, TAQ, VKQ, NYQ, NTP, TEQ, SKQ, NIQ, YNQ, TVQ, GTQ, NTR, TLQ, TMQ, KNQ, YTQ, NKS, NTE, NIE, TRQ, NSQ, YTK, NIK, NNK, NSK, ITK, NAK, KTK, GNQ, SNQ, HTK, DTK, NEQ, NPK,YTE, NNR, INQ, NHQ, YIQ, HNQ, ITQ, STQ, NSN, NQQ, NNP, ITE, NTN, FSQ, NNH, NMQ, NTS, NLQ, SMQ, NCQ, or VTQ
15. The AAV particle of any one of embodiments 1-14, wherein [N1] is or comprises TNTQ (SEQ ID NO: 4688), TNTK (SEQ ID NO: 4689), TNNQ (SEQ ID NO: 4690), SNNQ (SEQ ID NO: 4691), TNKQ

(SEQ ID NO: 4692), TNNE (SEQ ID NO: 4693), SNKQ (SEQ ID NO: 4694), SNTQ (SEQ ID NO: 4695), TTNQ (SEQ ID NO: 4696), TNDQ (SEQ ID NO: 4697), TTIQ (SEQ ID NO: 4698), RNTQ (SEQ ID NO: 4699), TTKQ (SEQ ID NO: 4700), TTSQ (SEQ ID NO: 4701), TTDQ (SEQ ID NO: 4702), TNPS (SEQ ID NO: 4703), TNKE (SEQ ID NO: 4704), TTTQ (SEQ ID NO: 4705), TGKQ (SEQ ID NO: 4706), TTAQ (SEQ ID NO: 4707), TVKQ (SEQ ID NO: 4708), TNYQ (SEQ ID NO: 4709), TNTP (SEQ ID NO: 4710), STKQ (SEQ ID NO: 4711), TTEQ (SEQ ID NO: 4712), TSKQ (SEQ ID NO: 4713), TNIQ (SEQ ID NO: 4714), TYNQ (SEQ ID NO: 4715), STIQ (SEQ ID NO: 4716), TTVQ (SEQ ID NO: 4717), TGTQ (SEQ ID NO: 4718), TNTR (SEQ ID NO: 4719), TTLQ (SEQ ID NO: 4720), TTMQ (SEQ ID NO: 4721), ANNQ (SEQ ID NO: 4722), SNIQ (SEQ ID NO: 4723), TKNQ (SEQ ID NO: 4724), TYTQ (SEQ ID NO: 4725), TNKS (SEQ ID NO: 4726), SNTE (SEQ ID NO: 4727), TNTE (SEQ ID NO: 4728), TNIE (SEQ ID NO: 4729), TTRQ (SEQ ID NO: 4730), TNSQ (SEQ ID NO: 4731), TYTK (SEQ ID NO: 4732), TTTK (SEQ ID NO: 4733), TNIK (SEQ ID NO: 4734), SNTK (SEQ ID NO: 4735), TNNK (SEQ ID NO: 4736), TNSK (SEQ ID NO: 4737), TSTK (SEQ ID NO: 4738), TITK (SEQ ID NO: 4739), INTK (SEQ ID NO: 4740), TNAK (SEQ ID NO: 4741), TKTK (SEQ ID NO: 4742), STNQ (SEQ ID NO: 4743), ANTK (SEQ ID NO: 4744), RNNQ (SEQ ID NO: 4745), TGNQ (SEQ ID NO: 4746), TSNQ (SEQ ID NO: 4747), THTK (SEQ ID NO: 4748), TDTK (SEQ ID NO: 4749), TNEQ (SEQ ID NO: 4750), CNTQ (SEQ ID NO: 4751), TNPQ (SEQ ID NO: 4752), INNQ (SEQ ID NO: 4753), TYTE (SEQ ID NO: 4754), NNNQ (SEQ ID NO: 4755), KNNQ (SEQ ID NO: 4756), TNNR (SEQ ID NO: 4757), LNNQ (SEQ ID NO: 4758), TINQ (SEQ ID NO: 4759), TNHQ (SEQ ID NO: 4760), STTQ (SEQ ID NO: 4761), SNSQ (SEQ ID NO: 4762), STSQ (SEQ ID NO: 4763), TYIQ (SEQ ID NO: 4764), SGTQ (SEQ ID NO: 4765), THNQ (SEQ ID NO: 4766), TITQ (SEQ ID NO: 4767), TSTQ (SEQ ID NO: 4768), TNSN (SEQ ID NO: 4769), TNQQ (SEQ ID NO: 4770), RNIQ (SEQ ID NO: 4771), TNNP (SEQ ID NO: 4772), TITE (SEQ ID NO: 4773), TNTN (SEQ ID NO: 4774), TFSQ (SEQ ID NO: 4775), RNSQ (SEQ ID NO: 4776), INTQ (SEQ ID NO: 4777), RNTE (SEQ ID NO: 4778), TNNH (SEQ ID NO: 4779), TNMQ (SEQ ID NO: 4780), RTTQ (SEQ ID NO: 4781), SNIE (SEQ ID NO: 4782), TNTS (SEQ ID NO: 4783), KNTQ (SEQ ID NO: 4784), TNLQ (SEQ ID NO: 4785), TSMQ (SEQ ID NO: 4786), SYTQ (SEQ ID NO: 4787), TNCQ (SEQ ID NO: 4788), SSTQ (SEQ ID NO: 4789), TVTQ (SEQ ID NO: 4790), or QNTQ (SEQ ID NO: 4791).

16. The AAV particle of embodiment 15, wherein [N1] is or comprises TNTQ (SEQ ID NO: 4688).

17. The AAV particle of embodiment 15, wherein [N1] is or comprises TNTK (SEQ ID NO: 4689).

18. The AAV particle of any one of embodiments 1-17, wherein [N1]-[N2] comprises:

(i) TQDWHR (SEQ ID NO: 4686), TKDWHR (SEQ ID NO: 4792), NQDWHR (SEQ ID NO: 4793), KQDWHR (SEQ ID NO: 4794), NEDWHR (SEQ ID NO: 4795), DQDWHR (SEQ ID NO: 4796), IQDWHR (SEQ ID NO: 4797), SQDWHR (SEQ ID NO: 4798), PSDWHR (SEQ ID NO: 4799),

KEDWHR (SEQ ID NO: 4800), AQDWHR (SEQ ID NO: 4801), YQDWHR (SEQ ID NO: 4802), TPDWHR (SEQ ID NO: 4803), EQDWHR (SEQ ID NO: 4804), VQDWHR (SEQ ID NO: 4805), TRDWHR (SEQ ID NO: 4806), LQDWHR (SEQ ID NO: 4807), MQDWHR (SEQ ID NO: 4808), KSDWHR (SEQ ID NO: 4809), TEDWHR (SEQ ID NO: 4810), IEDWHR (SEQ ID NO: 4811), RQDWHR (SEQ ID NO: 4812), IKDWHR (SEQ ID NO: 4813), NKDWHR (SEQ ID NO: 4814), SKDWHR (SEQ ID NO: 4815), AKDWHR (SEQ ID NO: 4816), PKDWHR (SEQ ID NO: 4817), NRDWHR (SEQ ID NO: 4818), HQDWHR (SEQ ID NO: 4819), SNDWHR (SEQ ID NO: 4820), QQDWHR (SEQ ID NO: 4821), NPDWHR (SEQ ID NO: 4822), TNDWHR (SEQ ID NO: 4823), NHDWHR (SEQ ID NO: 4824), TSDWHR (SEQ ID NO: 4825), or CQDWHR (SEQ ID NO: 4826);

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

19. The AAV particle of any one of embodiments 1-18, wherein [N1]-[N2] comprises:

(i) NTQDWHR (SEQ ID NO: 4827), NTKDWHR (SEQ ID NO: 4828), NNQDWHR (SEQ ID NO: 4829), NKQDWHR (SEQ ID NO: 4830), NNEDWHR (SEQ ID NO: 4831), TNQDWHR (SEQ ID NO: 4832), NDQDWHR (SEQ ID NO: 4833), TIQDWHR (SEQ ID NO: 4834), TKQDWHR (SEQ ID NO: 4835), TSQDWHR (SEQ ID NO: 4836), TDQDWHR (SEQ ID NO: 4837), NPSDWHR (SEQ ID NO: 4838), NKEDWHR (SEQ ID NO: 4839), TTQDWHR (SEQ ID NO: 4840), GKQDWHR (SEQ ID NO: 4841), TAQDWHR (SEQ ID NO: 4842), VKQDWHR (SEQ ID NO: 4843), NYQDWHR (SEQ ID NO: 4844), NTPDWHR (SEQ ID NO: 4845), TEQDWHR (SEQ ID NO: 4846), SKQDWHR (SEQ ID NO: 4847), NIQDWHR (SEQ ID NO: 4848), YNQDWHR (SEQ ID NO: 4849), TVQDWHR (SEQ ID NO: 4850), GTQDWHR (SEQ ID NO: 4851), NTRDWHR (SEQ ID NO: 4852), TLQDWHR (SEQ ID NO: 4853), TMQDWHR (SEQ ID NO: 4854), KNQDWHR (SEQ ID NO: 4855), YTQDWHR (SEQ ID NO: 4856), NKSDWHR (SEQ ID NO: 4857), NTEDWHR (SEQ ID NO: 4858), NIEDWHR (SEQ ID NO: 4859), TRQDWHR (SEQ ID NO: 4860), NSQDWHR (SEQ ID NO: 4861), YTKDWHR (SEQ ID NO: 4862), TTKDWHR (SEQ ID NO: 4863), NIKDWHR (SEQ ID NO: 4864), NNKDWHR (SEQ ID NO: 4865), NSKDWHR (SEQ ID NO: 4866), STKDWHR (SEQ ID NO: 4867), ITKDWHR (SEQ ID NO: 4868), NAKDWHR (SEQ ID NO: 4869), KTKDWHR (SEQ ID NO: 4870), GNQDWHR (SEQ ID NO: 4871), SNQDWHR (SEQ ID NO: 4872), HTKDWHR (SEQ ID NO: 4873), DTKDWHR (SEQ ID NO: 4874), NEQDWHR (SEQ ID NO: 4875), NPKDWHR (SEQ ID NO: 4876), YTEDWHR (SEQ ID NO: 4877), NNRDWHR (SEQ ID NO: 4878), INQDWHR (SEQ ID NO: 4879), NHQDWHR (SEQ ID NO: 4880), YIQDWHR (SEQ ID NO: 4881), HNQDWHR (SEQ ID NO: 4882), ITQDWHR (SEQ ID NO: 4883), STQDWHR (SEQ ID NO: 4884), NSNDWHR (SEQ ID NO: 4885), NQQDWHR (SEQ ID

NO: 4886), NNPDWHR (SEQ ID NO: 4887), ITEDWHR (SEQ ID NO: 4888), NTNDWHR (SEQ ID NO: 4889), FSQDWHR (SEQ ID NO: 4890), NNHDWHR (SEQ ID NO: 4891), NMQDWHR (SEQ ID NO: 4892), NTSDWHR (SEQ ID NO: 4893), NLQDWHR (SEQ ID NO: 4894), SMQDWHR (SEQ ID NO: 4895), NCQDWHR (SEQ ID NO: 4896), or VTQDWHR (SEQ ID NO: 4897);

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, 5, or 6 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

20. The AAV particle of any one of embodiments 1-19, wherein [N1]-[N2] is or comprises:

(i) TNTQDWHR (SEQ ID NO: 4898), TNTKDWHR (SEQ ID NO: 4899), TNNQDWHR (SEQ ID NO: 4900), SNNQDWHR (SEQ ID NO: 4901), TNKQDWHR (SEQ ID NO: 4902), TNNEDWHR (SEQ ID NO: 4903), SNKQDWHR (SEQ ID NO: 4904), SNTQDWHR (SEQ ID NO: 4905), TTNQDWHR (SEQ ID NO: 4906), TNDQDWHR (SEQ ID NO: 4907), TTIQDWHR (SEQ ID NO: 4908), RNTQDWHR (SEQ ID NO: 4909), TTKQDWHR (SEQ ID NO: 4910), TTSQDWHR (SEQ ID NO: 4911), TTDQDWHR (SEQ ID NO: 4912), TNPSDWHR (SEQ ID NO: 4913), TNKEDWHR (SEQ ID NO: 4914), TTTQDWHR (SEQ ID NO: 4915), TGKQDWHR (SEQ ID NO: 4916), TTAQDWHR (SEQ ID NO: 4917), TVKQDWHR (SEQ ID NO: 4918), TNYQDWHR (SEQ ID NO: 4919), TNTPDWHR (SEQ ID NO: 4920), STKQDWHR (SEQ ID NO: 4921), TTEQDWHR (SEQ ID NO: 4922), TSKQDWHR (SEQ ID NO: 4923), TNIQDWHR (SEQ ID NO: 4924), TYNQDWHR (SEQ ID NO: 4925), STIQDWHR (SEQ ID NO: 4926), TTVQDWHR (SEQ ID NO: 4927), TGTQDWHR (SEQ ID NO: 4928), TNTRDWHR (SEQ ID NO: 4929), TTLQDWHR (SEQ ID NO: 4930), TTMQDWHR (SEQ ID NO: 4931), ANNQDWHR (SEQ ID NO: 4932), SNIQDWHR (SEQ ID NO: 4933), TKNQDWHR (SEQ ID NO: 4934), TYTQDWHR (SEQ ID NO: 4935), TNKSDWHR (SEQ ID NO: 4936), SNTEDWHR (SEQ ID NO: 4937), TNTEDWHR (SEQ ID NO: 4938), TNIEDWHR (SEQ ID NO: 4939), TTRQDWHR (SEQ ID NO: 4940), TNSQDWHR (SEQ ID NO: 4941), TYTKDWHR (SEQ ID NO: 4942), TTTKDWHR (SEQ ID NO: 4943), TNIKDWHR (SEQ ID NO: 4944), SNTKDWHR (SEQ ID NO: 4945), TNNKDWHR (SEQ ID NO: 4946), TNSKDWHR (SEQ ID NO: 4947), TSTKDWHR (SEQ ID NO: 4948), TITKDWHR (SEQ ID NO: 4949), INTKDWHR (SEQ ID NO: 4950), TNAKDWHR (SEQ ID NO: 4951), TKTKDWHR (SEQ ID NO: 4952), STNQDWHR (SEQ ID NO: 4953), ANTKDWHR (SEQ ID NO: 4954), RNNQDWHR (SEQ ID NO: 4955), TGNQDWHR (SEQ ID NO: 4956), TSNQDWHR (SEQ ID NO: 4957), THTKDWHR (SEQ ID NO: 4958), TDTKDWHR (SEQ ID NO: 4959), TNEQDWHR (SEQ ID NO: 4960), CNTQDWHR (SEQ ID NO: 4961), TNPQDWHR (SEQ ID NO: 4962), INNQDWHR (SEQ ID NO: 4963), TYTEDWHR (SEQ ID NO: 4964), NNNQDWHR (SEQ ID NO: 4965), KNNQDWHR (SEQ ID NO: 4966), TNNRDWHR

(SEQ ID NO: 4967), LNNQDWHR (SEQ ID NO: 4968), TINQDWHR (SEQ ID NO: 4969), TNHQDWHR (SEQ ID NO: 4970), STTQDWHR (SEQ ID NO: 4971), SNSQDWHR (SEQ ID NO: 4972), STSQDWHR (SEQ ID NO: 4973), TYIQDWHR (SEQ ID NO: 4974), SGTQDWHR (SEQ ID NO: 4975), THNQDWHR (SEQ ID NO: 4976), TITQDWHR (SEQ ID NO: 4977), TSTQDWHR (SEQ ID NO: 4978), TNSNDWHR (SEQ ID NO: 4979), TNQQDWHR (SEQ ID NO: 4980), RNIQDWHR (SEQ ID NO: 4981), TNNPDWHR (SEQ ID NO: 4982), TITEDWHR (SEQ ID NO: 4983), TNTNDWHR (SEQ ID NO: 4984), TFSQDWHR (SEQ ID NO: 4985), RNSQDWHR (SEQ ID NO: 4986), INTQDWHR (SEQ ID NO: 4987), RNTEDWHR (SEQ ID NO: 4988), TNNHDWHR (SEQ ID NO: 4989), TNMQDWHR (SEQ ID NO: 4990), RTTQDWHR (SEQ ID NO: 4991), SNIEDWHR (SEQ ID NO: 4992), TNTSDWHR (SEQ ID NO: 4993), KNTQDWHR (SEQ ID NO: 4994), TNLQDWHR (SEQ ID NO: 4995), TSMQDWHR (SEQ ID NO: 4996), SYTQDWHR (SEQ ID NO: 4997), TNCQDWHR (SEQ ID NO: 4998), SSTQDWHR (SEQ ID NO: 4999), TVTQDWHR (SEQ ID NO: 5000), or QNTQDWHR (SEQ ID NO: 5001);

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, 5, 6, or 7 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions, relative to any one of the amino acid sequences in (i).

21. The AAV particle of embodiment 20, wherein [N1]-[N2] is or comprises TNTQDWHR (SEQ ID NO: 4898).

22. The AAV particle of embodiment 20, wherein [N1]-[N2] is or comprises TNTKDWHR (SEQ ID NO: 4899).

23. The AAV particle of any one of embodiments 1-22, wherein the AAV capsid variant comprises one, two, or all of: an amino acid other than Q at position 590 (e.g., I, V, T, M, S, N, L, or F), an amino acid other than A at position 591 (e.g., Y, P, N, S, T, G, E, V, W, F, Q), and/or an amino acid other than Q at position 592 (e.g., G, N, K, H, R, E, L, P, or M), as numbered according to SEQ ID NO: 138 or 981.

24. The AAV particle of any one of embodiments 1-23, wherein the AAV capsid variant comprises an amino acid other than Q at position 590 (e.g., I, V, T, M, S, N, L, or F), as numbered according to SEQ ID NO: 138 or 981.

25. The AAV particle of any one of embodiments 1-24, wherein the AAV capsid variant comprises the amino acid I at position 590, as numbered according to SEQ ID NO: 138 or 981.

26. The AAV particle of any one of embodiments 1-24, wherein the AAV capsid variant comprises the amino acid V at position 590, as numbered according to SEQ ID NO: 138 or 981.

27. The AAV particle of any one of embodiments 1-26, wherein the AAV capsid variant comprises the amino acid A at position 591 and/or the amino acid Q at position 592, as numbered according to SEQ ID NO: 138 or 981.

28. The AAV particle of any one of embodiments 1-27, wherein [N3] comprises amino acids X₅, X₆, and X₇, wherein X₅ is I, V, T, M, S, N, L, or F.

29. The AAV particle of any one of embodiments 1-28, wherein X₅ is I or V.

30. The AAV particle of embodiment 29, wherein X₅ is I.

31. The AAV particle of any one of embodiments 1-30, wherein:

(i) X₆ is A, Y, P, N, S, T, G, E, V, W, F, or Q; and/or

(ii) X₇ is Q, G, N, K, H, R, E, L, P, or M.

32. The AAV particle of any one of embodiments 1-31, wherein [N3] comprises IA, IY, VP, IN, VN, VY, VA, IS, IT, TA, MA, SA, IG, IE, IV, NA, LA, IP, FA, VS, VT, IW, IF, IQ, VQ, AQ, AG, YQ, PQ, AN, NQ, SG, SQ, TQ, GQ, EQ, AK, AH, AR, AE, AL, AP, TM, SM, WQ, FQ, QQ, FM, AM, or SN.

33. The AAV particle of any one of embodiments 1-32, wherein [N3] is or comprises IAQ, IAG, IYQ, VPQ, IAN, INQ, VNQ, VYQ, VAN, ISG, ISQ, VAQ, ITQ, TAQ, MAQ, SAQ, IGQ, IEQ, IVQ, NAQ, LAQ, IAK, IAH, IPQ, IAR, IAE, IAL, IAP, FAQ, VSQ, VTM, ISM, IWQ, IFQ, IQQ, VQQ, IFM, IAM, or ISN.

34. The AAV particle of any one of embodiments 1-33, wherein [N3] is or comprises IAQ.

35. The AAV particle of any one of embodiments 1-34, wherein [N2]-[N3] comprises:

(i) DWHRIA (SEQ ID NO: 5002), DWHRIV (SEQ ID NO: 5003), DWHRVP (SEQ ID NO: 5004), DWHRIN (SEQ ID NO: 5005), DWHRVN (SEQ ID NO: 5006), DWHRVY (SEQ ID NO: 5007), DWHRVA (SEQ ID NO: 5008), DWHRIS (SEQ ID NO: 5009), DWHRIT (SEQ ID NO: 5010), DWHRTA (SEQ ID NO: 5011), DWHRMA (SEQ ID NO: 5012), DWHRSA (SEQ ID NO: 5013), DWHRIG (SEQ ID NO: 5014), DWHRIE (SEQ ID NO: 5015), DWHRIV (SEQ ID NO: 5016), DWHRNA (SEQ ID NO: 5017), DWHRLA (SEQ ID NO: 5018), DWHRIP (SEQ ID NO: 5019),

DWHRFA (SEQ ID NO: 5020), DWHRVS (SEQ ID NO: 5021), DWHRVT (SEQ ID NO: 5022), DWHRIW (SEQ ID NO: 5023), DWHRIF (SEQ ID NO: 5024), DWHRIQ (SEQ ID NO: 5025), or DWHRVQ (SEQ ID NO: 5026);

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

36. The AAV particle of any one of embodiments 1-35, wherein [N2]-[N3] is or comprises:

(i) DWHRIAQ (SEQ ID NO: 5027), DWHRIAG (SEQ ID NO: 5028), DWHRIYQ (SEQ ID NO: 5029), DWHRVPQ (SEQ ID NO: 5030), DWHRIAN (SEQ ID NO: 5031), DWHRINQ (SEQ ID NO: 5032), DWHRVNQ (SEQ ID NO: 5033), DWHRVYQ (SEQ ID NO: 5034), DWHRVAN (SEQ ID NO: 5035), DWHRISG (SEQ ID NO: 5036), DWHRISQ (SEQ ID NO: 5037), DWHRVAQ (SEQ ID NO: 5038), DWHRITQ (SEQ ID NO: 5039), DWHRTAQ (SEQ ID NO: 5040), DWHRMAQ (SEQ ID NO: 5041), DWHRSAQ (SEQ ID NO: 5042), DWHRIGQ (SEQ ID NO: 5043), DWHRIEQ (SEQ ID NO: 5044), DWHRIVQ (SEQ ID NO: 5045), DWHRNAQ (SEQ ID NO: 5046), DWHRLAQ (SEQ ID NO: 5047), DWHRIAK (SEQ ID NO: 5048), DWHRIAH (SEQ ID NO: 5049), DWHRI PQ (SEQ ID NO: 5050), DWHRIAR (SEQ ID NO: 5051), DWHRIAE (SEQ ID NO: 5052), DWHRIAL (SEQ ID NO: 5053), DWHRIAP (SEQ ID NO: 5054), DWHRFAQ (SEQ ID NO: 5055), DWHRVSQ (SEQ ID NO: 5056), DWHRVTM (SEQ ID NO: 5057), DWHRISM (SEQ ID NO: 5058), DWHRIWQ (SEQ ID NO: 5059), DWHRIFQ (SEQ ID NO: 5060), DWHRIQQ (SEQ ID NO: 5061), DWHRVQQ (SEQ ID NO: 5062), DWHRIFM (SEQ ID NO: 5063), DWHRIAM (SEQ ID NO: 5064), or DWHRISN (SEQ ID NO: 5065);

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, 5, or 6 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

37. The AAV particle of any one of embodiments 1-36, wherein [N2]-[N3] is or comprises DWHRIAQ (SEQ ID NO: 5027).

38. The AAV particle of any one of embodiments 1-37, wherein [N1]-[N2]-[N3] is or comprises:

(i) the amino acid sequence of any one of SEQ ID NOs: 343-538;

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

39. The AAV particle of embodiment 38, wherein [N1]-[N2]-[N3] is or comprises TNTQDWHRIAQ (SEQ ID NO: 343).

40. The AAV particle of embodiment 38, wherein [N1]-[N2]-[N3] is or comprises TNTKDWHRIAQ (SEQ ID NO: 344).

41. The AAV particle of any one of embodiments 1-40, wherein the AAV capsid variant comprises one, two, three, or all of: an amino acid other than T at position 593 (e.g., S, N, P, A, or I), an amino acid other than G at position 594 (e.g., N, D, R, V, A, S, or Q), an amino acid other than W at position 595 (e.g., S, C, R, L, or G), and/or an amino acid other than V at position 596 (e.g., A, S, I, C, G, D, F, L, or T), as numbered according to SEQ ID NO: 138 or 981.

42. The AAV particle of any one of embodiments 1-40, wherein the AAV capsid variant comprises the amino acid T at position 593, the amino acid G at position 594, the amino acid W at position 595, and the amino acid V at position 596, as numbered according to SEQ ID NO: 138 or 981.

43. The AAV particle of any one of embodiments 1-42, wherein the AAV capsid variant further comprises [N4], wherein [N4] comprises amino acids X₈, X₉, X₁₀, and X₁₁, wherein:

(i) X₈ is T, S, N, P, A, or I;

(ii) X₉ is G, N, D, R, V, A, S, or Q;

(iii) X₁₀ is W, S, C, R, L, or G; and/or

(iv) X₁₁ is V, A, S, I, C, G, D, F, L, or T.

44. The AAV particle of embodiment 43, wherein [N4] comprises TG, TN, SN, NN, SG, PG, TD, AG, IG, NG, TR, TV, TA, TS, SV, TQ, WV, WA, WS, WI, WC, WG, CV, RV, LV, GV, WD, WF, WL, WT, GW, NW, GS, DW, GC, GR, GL, GG, RW, VW, AW, SW, or QW.

45. The AAV particle of embodiment 43 or 44, wherein [N4] comprises TGW, TNW, SNW, NNW, SGW, PGW, TGS, TDW, TGC, TGR, TGL, TGG, AGW, IGW, NGW, TRW, TVW, TAW, TSW, SVW,

TQW, GWV, GWA, NWS, NWV, NWI, GWS, GWI, GWC, GWG, GSV, DWV, GCV, GRV, GLV, GGV, GWD, GWF, RWV, VWV, GWL, AWV, SWV, GWT, or QWV.

46. The AAV particle of any one of embodiments 43-45, wherein [N4] is or comprises TGWV (SEQ ID NO: 5066), TGWA (SEQ ID NO: 5067), TNWS (SEQ ID NO: 5068), SNWV (SEQ ID NO: 5069), TNWV (SEQ ID NO: 5070), TNWI (SEQ ID NO: 5071), NNWV (SEQ ID NO: 5072), TGWS (SEQ ID NO: 5073), TGWI (SEQ ID NO: 5074), TGWC (SEQ ID NO: 5075), TGWG (SEQ ID NO: 5076), SGWV (SEQ ID NO: 5077), PGWV (SEQ ID NO: 5078), TGSV (SEQ ID NO: 5079), TDWV (SEQ ID NO: 5080), TGCV (SEQ ID NO: 5081), TGRV (SEQ ID NO: 5082), TGLV (SEQ ID NO: 5083), TGGV (SEQ ID NO: 5084), AGWV (SEQ ID NO: 5085), IGWV (SEQ ID NO: 5086), TGWD (SEQ ID NO: 5087), NGWV (SEQ ID NO: 5088), TGWF (SEQ ID NO: 5089), TRWV (SEQ ID NO: 5090), TVWV (SEQ ID NO: 5091), TGWL (SEQ ID NO: 5092), TAWV (SEQ ID NO: 5093), TSWV (SEQ ID NO: 5094), TGWT (SEQ ID NO: 5095), SVWV (SEQ ID NO: 5096), TQWV (SEQ ID NO: 5097), or PGWG (SEQ ID NO: 5098).

47. The AAV particle of any one of embodiments 43-46, wherein [N4] is or comprises TGWV (SEQ ID NO: 5066).

48. The AAV particle of any one of embodiments 43-47, wherein [N1]-[N2]-[N3]-[N4] is or comprises:

- (i) the amino acid sequence of any one of SEQ ID NOs: 201-245, 247-250, 253-255, 257-265, 268-274, 276-286, 288, 290-297, 299-303, 305-309, 311, 313-319, 323-328, 330-337, 339-342, 539-542, 544, 546, 547, 549-557, 559-589, 592, 593, 595, 596, 598, 599, 601-608, 610-614, 616-622, 625, 628, 630, 631, 633, 636, 638, 639-646, 649, 651-657, 667, 669, 670, 672, 673, 679-683, 685-690, 692, 693, 695, 697, 699-701, 703-705, 708-710, 712-717, 719-723, 728-731, 733-738, 740, and 742;
- (ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids, thereof;
- (iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or
- (iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

49. The AAV particle of embodiment 48, wherein [N1]-[N2]-[N3]-[N4] is or comprises TNTQDWHRIAQTGWV (SEQ ID NO: 201).

50. The AAV particle of any one of embodiment 48, wherein [N1]-[N2]-[N3]-[N4] is or comprises TNTKDWHRIAQTGWV (SEQ ID NO: 202).

51. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising an amino acid sequence having the following formula: [N1]-[N2]-[N3] (SEQ ID NO: 4683), wherein [N2] comprises the amino acid sequence of DWHR (SEQ ID NO: 4682) and wherein:

(i) [N1] comprises amino acids X₁, X₂, X₃, and X₄, wherein X₄ is Q, P, or a conservative substitution thereof; and/or

(ii) [N3] comprises amino acids X₅, X₆, and X₇, wherein X₅ is I, V, or a conservative substitution thereof.

52. The AAV particle of embodiment 51, wherein the AAV capsid variant comprises the amino acid Q at position 585, as numbered according to SEQ ID NO: 138 or 981.

53. The AAV particle of embodiment 51 or 52, wherein the AAV capsid variant comprises one, two, three, or all of:

(i) an amino acid other than T at position 582 (e.g., S);

(ii) an amino acid other than N at position 583 (e.g., T, G, S, I, or V);

(iii) an amino acid other than H at position 584 (e.g., N, I, S, A, V, or L); and/or

(iv) an amino acid other than Q at position 585 (e.g., P),

wherein the amino acids are as numbered according to SEQ ID NO: 138 or 981.

54. The AAV particle of any one of embodiments 51-53, wherein [N1] comprises amino acids X₁, X₂, X₃, and X₄, wherein X₄ is Q or P.

55. The AAV particle of any one of embodiments 51-54, wherein X₄ is Q.

56. The AAV particle of any one of embodiments 51-55, wherein the AAV capsid variant comprises an amino acid other than H at position 584 (e.g., T), as numbered according to SEQ ID NO: 138 or 981.

57. The AAV particle of any one of embodiments 51-56, wherein the AAV capsid variant comprises the amino acid T at position 584, as numbered according to SEQ ID NO: 138 or 981.

58. The AAV particle of any one of embodiments 51-57, wherein:

(i) X₁ is T or S;

(ii) X₂ is N, T, G, S, I, or V; and/or

(iii) X₃ is T, N, I, S, A, V, or L.

59. The AAV particle of any one of embodiments 51-58, wherein [N1] comprises TN, TT, TG, ST, TS, TI, TV, TQ, NQ, IQ, SQ, AQ, VQ, TP, LQ, NT, TA, NI, GT, IT, NN, TL, NS, or VT.

60. The AAV particle of any one of embodiments 51-59, wherein [N1] comprises TNT, TTN, TTI, TTS, TTT, TTA, TNI, TTV, TGT, STT, TST, TIT, TNN, TTL, TNS, TVT, NTQ, TNQ, TIQ, TSQ, TTQ, TAQ, NIQ, TVQ, GTQ, STQ, ITQ, NTP, NNQ, TLQ, NSQ, or VTQ.

61. The AAV particle of any one of embodiments 51-60, wherein [N1] is or comprises TNTQ (SEQ ID NO: 4688), TTNQ (SEQ ID NO: 4696), TTIQ (SEQ ID NO: 4698), TTSQ (SEQ ID NO: 4701), TTTQ (SEQ ID NO: 4705), TTAQ (SEQ ID NO: 4707), TNIQ (SEQ ID NO: 4714), TTVQ (SEQ ID NO: 4717), TGTQ (SEQ ID NO: 4718), STTQ (SEQ ID NO: 4761), TSTQ (SEQ ID NO: 4768), TITQ (SEQ ID NO: 4767), TNTP (SEQ ID NO: 4710), TNNQ (SEQ ID NO: 4690), TTLQ (SEQ ID NO: 4720), TNSQ (SEQ ID NO: 4731), or TVTQ (SEQ ID NO: 4790).

62. The AAV particle of embodiment 61, wherein [N1] is or comprises TNTQ (SEQ ID NO: 4688).

63. The AAV particle of any one of embodiments 51-62, wherein [N1]-[N2] comprises:

(i) TQDWHR (SEQ ID NO: 4686), NQDWHR (SEQ ID NO: 4793), IQDWHR (SEQ ID NO: 4797), SQDWHR (SEQ ID NO: 4798), AQDWHR (SEQ ID NO: 4801), VQDWHR (SEQ ID NO: 4805), TPDWHR (SEQ ID NO: 4803), or LQDWHR (SEQ ID NO: 4807);

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

64. The AAV particle of any one of embodiments 51-63, wherein [N1]-[N2] comprises:

(i) NTQDWHR (SEQ ID NO: 4827), TNQDWHR (SEQ ID NO: 4832), TIQDWHR (SEQ ID NO: 4834), TSQDWHR (SEQ ID NO: 4836), TTQDWHR (SEQ ID NO: 4840), TAQDWHR (SEQ ID NO: 4842), NIQDWHR (SEQ ID NO: 4848), TVQDWHR (SEQ ID NO: 4850), GTQDWHR (SEQ ID NO: 4851), STQDWHR (SEQ ID NO: 4884), ITQDWHR (SEQ ID NO: 4883), NTPDWHR (SEQ ID NO: 4845), NNQDWHR (SEQ ID NO: 4829), TLQDWHR (SEQ ID NO: 4853), NSQDWHR (SEQ ID NO: 4861), VTQDWHR (SEQ ID NO: 4897);

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, 5 or 6 amino acids, e.g., consecutive amino acids, thereof.

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

65. The AAV particle of any one of embodiments 51-64, wherein [N1]-[N2] is or comprises:

(i) TNTQDWHR (SEQ ID NO: 4898), TTNQDWHR (SEQ ID NO: 4906), TTIQDWHR (SEQ ID NO: 4908), TTSQDWHR (SEQ ID NO: 4911), TTTQDWHR (SEQ ID NO: 4915), TTAQDWHR (SEQ ID NO: 4917), TNIQDWHR (SEQ ID NO: 4924), TTVQDWHR (SEQ ID NO: 4927), TGTQDWHR (SEQ ID NO: 4928), STTQDWHR (SEQ ID NO: 4971), TSTQDWHR (SEQ ID NO: 4978), TITQDWHR (SEQ ID NO: 4977), TNTPDWHR (SEQ ID NO: 4920), TNNQDWHR (SEQ ID NO: 4900), TTLQDWHR (SEQ ID NO: 4930), TNSQDWHR (SEQ ID NO: 4941), TVTQDWHR (SEQ ID NO: 5000);

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, 5, 6, or 7 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

66. The AAV particle of embodiment 65, wherein [N1]-[N2] is or comprises TNTQDWHR (SEQ ID NO: 4898).

67. The AAV particle of any one of embodiments 51-66, wherein the AAV capsid variant comprises one, two, or all of: an amino acid other than Q at position 590 (e.g., I or V), an amino acid other than A at position 591 (e.g., P, S, Y, or N), and/or an amino acid other than Q at position 592 (e.g., G or N), as numbered according to SEQ ID NO: 138 or 981.

68. The AAV particle of any one of embodiments 51-67, wherein the AAV capsid variant comprises an amino acid other than Q at position 590 (e.g., I or V), as numbered according to SEQ ID NO: 138 or 981.

69. The AAV particle of any one of embodiments 51-68, wherein the AAV capsid variant comprises the amino acid I at position 590, as numbered according to SEQ ID NO: 138 or 981.

70. The AAV particle of any one of embodiments 51-68, wherein the AAV capsid variant comprises the amino acid V at position 590, as numbered according to SEQ ID NO: 138 or 981.

71. The AAV particle of any one of embodiments 51-70, wherein the AAV capsid variant comprises the amino acid A at position 591 and/or the amino acid Q at position 592, as numbered according to SEQ ID NO: 138 or 981.

72. The AAV particle of any one of embodiments 51-71, wherein [N3] comprises amino acids X₅, X₆, and X₇, wherein X₅ is I or V.

73. The AAV particle of embodiment 72, wherein X₅ is I.

74. The AAV particle of any one of embodiments 51-73, wherein:

(i) X₆ is A, P, S, Y, or N; and/or

(ii) X₇ is Q, G, or N.

75. The AAV particle of any one of embodiments 51-74, wherein [N3] comprises IA, VP, VA, VS, IY, IN, IS, AQ, AG, PQ, SQ, AN, YQ, or NQ.

76. The AAV particle of any one of embodiments 51-75, wherein [N3] is or comprises IAQ, IAG, VPQ, VAQ, VSQ, IAN, IYQ, INQ, or ISQ.

77. The AAV particle of any one of embodiments 51-76, wherein [N3] is or comprises IAQ.

78. The AAV particle of any one of embodiments 51-77, wherein [N2]-[N3] comprises:

(i) DWHRIA (SEQ ID NO: 5002), DWHRVP (SEQ ID NO: 5004), DWHRVA (SEQ ID NO: 5008), DWHRVS (SEQ ID NO: 5021), DWHRIY (SEQ ID NO: 5003), DWHRIN (SEQ ID NO: 5005), or DWHRIS (SEQ ID NO: 5009);

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

79. The AAV particle of any one of embodiments 51-78, wherein [N2]-[N3] is or comprises:

(i) DWHRIAQ (SEQ ID NO: 5027), DWHRIAG (SEQ ID NO: 5028), DWHRVPQ (SEQ ID NO: 5030), DWHRVAQ (SEQ ID NO: 5038), DWHRVSQ (SEQ ID NO: 5056), DWHRIAN (SEQ ID NO: 5031), DWHRIYQ (SEQ ID NO: 5029), DWHRINQ (SEQ ID NO: 5032), or DWHRISQ (SEQ ID NO: 5037);

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, 5, or 6 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

80. The AAV particle of any one of embodiments 51-79, wherein [N2]-[N3] is or comprises DWHRIAQ (SEQ ID NO: 5027).

81. The AAV particle of any one of embodiments 51-80, wherein [N1]-[N2]-[N3] is or comprises:

(i) the amino acid sequence of any one of SEQ ID NOs: 343, 350, 352, 355, 359, 361, 364, 367, 370, 371, 373, 374, 376, 377, 378, 381, 395, 420, 454, 457, 460, 464, 481, 482, 488, 493, 494, 516, 525, 536;

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

82. The AAV particle of any one of embodiments 51-81, wherein [N1]-[N2]-[N3] is or comprises TNTQDWHRIAQ (SEQ ID NO: 343).

83. The AAV particle of any one of embodiments 51-82, wherein the AAV capsid variant comprises the amino acid W at position 595, as numbered according to SEQ ID NO: 138 or 981.

84. The AAV particle of any one of embodiments 51-83, wherein the AAV capsid variant comprises one, two or all of: an amino acid other than T (e.g., S or N) at position 593, an amino acid other than G (e.g., N) at position 594, and/or an amino acid other than V (e.g., A, I, or S) at position 596, as numbered according to SEQ ID NO: 138 or 981.

85. The AAV particle of any one of embodiments 51-84, wherein the AAV capsid variant comprises:

(i) the amino acid T at position 593, the amino acid G at position 594, the amino acid W at position 595, and the amino acid V at position 596, as numbered according to SEQ ID NO: 138 or 981;

(ii) the amino acid T at position 593, the amino acid G at position 594, the amino acid W at position 595, and the amino acid A at position 596, as numbered according to SEQ ID NO: 138 or 981;

(iii) the amino acid S at position 593, the amino acid N at position 594, the amino acid W at position 595, and the amino acid V at position 596, as numbered according to SEQ ID NO: 138 or 981;

(iv) the amino acid N at position 593, the amino acid N at position 594, the amino acid W at position 595, and the amino acid V at position 596, as numbered according to SEQ ID NO: 138 or 981;

(v) the amino acid T at position 593, the amino acid G at position 594, the amino acid W at position 595, and the amino acid I at position 596, as numbered according to SEQ ID NO: 138 or 981; or

(vi) the amino acid T at position 593, the amino acid G at position 594, the amino acid W at position 595, and the amino acid S at position 596, as numbered according to SEQ ID NO: 138 or 981.

86. The AAV particle of any one of embodiments 51-85, wherein the AAV capsid variant comprises the amino acid T at position 593, the amino acid G at position 594, the amino acid W at position 595, and the amino acid V at position 596, as numbered according to SEQ ID NO: 138 or 981.

87. The AAV particle of any one of embodiments 51-86, wherein the AAV capsid variant further comprises [N4], wherein [N4] comprises amino acids X₈, X₉, X₁₀, and X₁₁, wherein X₁₀ is W.

88. The AAV particle of embodiment 87, wherein:

- (i) X₈ is T, S, or N;
- (ii) X₉ is G or N; and/or
- (iv) X₁₁ is V, A, I, or S.

89. The AAV particle of embodiment 87 or 88, wherein [N4] comprises TG, SN, NN, WV, WA, WI, WS, GW, or NW.

90. The AAV particle of any one of embodiments 87-89, wherein [N4] comprises TGW, SNW, NNW, GWV, GWA, NWV, GWI, or GWS.

91. The AAV particle of any one of embodiments 87-90, wherein [N4] is or comprises TGWV (SEQ ID NO: 5066), TGWA (SEQ ID NO: 5067), SNWV (SEQ ID NO: 5069), NNWV (SEQ ID NO: 5072), TGWI (SEQ ID NO: 5074), or TGWS (SEQ ID NO: 5073).

92. The AAV particle of any one of embodiments 87-91, wherein [N4] is or comprises TGWV (SEQ ID NO: 5066).

93. The AAV particle of any one of embodiments 87-92, wherein [N1]-[N2]-[N3]-[N4] is or comprises:

- (i) the amino acid sequence of any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336;

(ii) an amino acid sequence comprising any portion of an amino acid sequence in (i), e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids, thereof;

(iii) an amino acid sequence comprising one, two, or three but no more than four modifications relative to any one of the amino acid sequences in (i); or

(iv) an amino acid sequence comprising one, two, or three but no more than four substitutions relative to any one of the amino acid sequences in (i).

94. The AAV particle of any one of embodiments 87-93, wherein [N1]-[N2]-[N3]-[N4] is or comprises TNTQDWHRIAQTGWV (SEQ ID NO: 201).

95. The AAV particle of any one of embodiments 1-94, wherein [N1]-[N2]-[N3] is present in loop VIII, optionally wherein loop VIII comprises amino acids 580-599 as numbered according to SEQ ID NO: 138 or 981.

96. The AAV particle of any one of embodiments 43-50 and 87-95, wherein [N4] is present in loop VIII, optionally wherein loop VIII comprises amino acids 580-599 as numbered according to SEQ ID NO: 138 or 981.

97. The AAV particle of any one of embodiments 1-96, wherein [N1] replaces amino acids 582-585 (e.g., T582, N583, H584, Q585) of SEQ ID NO: 138.

98. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, and 85-97, wherein [N1] corresponds to amino acids 582-585 (e.g., T582, N583, T584, Q585) of SEQ ID NO: 981.

99. The AAV particle of any one of embodiments 1-98, wherein [N1] is present at amino acids 582-585, as numbered according to SEQ ID NO: 138 or 981.

100. The AAV particle of any one of embodiments 1-99, wherein [N1] corresponds to positions 582-585 (e.g., T582, N583, T584, Q585) of SEQ ID NO: 981.

101. The AAV particle of any one of embodiments 1-100, wherein [N2] replaces amino acids 586-589 (e.g., S586, A587, Q588, A589) of SEQ ID NO: 138.

102. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101, wherein [N2] corresponds to amino acids 586-589 (e.g., D586, W587, H588, R589) of SEQ ID NO: 981.

103. The AAV particle of any one of embodiments 1-102, wherein [N2] is present at amino acids 586-589, as numbered according to SEQ ID NO: 138 or 981.

104. The AAV particle of any one of embodiments 1-103, wherein [N1]-[N2] replaces amino acids 582-589 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589) of SEQ ID NO: 138.

105. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-104, wherein [N1]-[N2] corresponds to amino acids 582-589 (e.g., T582, N583, T584, Q585, D586, W587, H588, R589) of SEQ ID NO: 981.

106. The AAV particle of any one of embodiments 1-105, wherein [N1]-[N2] is present at amino acids 582-589, as numbered according to SEQ ID NO: 138 or 981.

107. The AAV particle of any one of embodiments 1-106, wherein [N3] replaces amino acids 590-592 (e.g., Q590, A591, and Q592) of SEQ ID NO: 138.

108. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-107, wherein [N3] corresponds to amino acids 590-592 (e.g., I590, A591, and Q592) of SEQ ID NO: 981.

109. The AAV particle of any one of embodiments 1-108, wherein [N3] is present at amino acids 590-592, as numbered according to SEQ ID NO: 138 or 981.

110. The AAV particle of any one of embodiments 1-109, wherein [N2]-[N3] replaces amino acids 586-592 (e.g., S586, A587, Q588, A589, Q590, A591, and Q592) of SEQ ID NO: 138.

111. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-110, wherein [N2]-[N3] corresponds to amino acids 586-592 (e.g., D586, W587, H588, R589, I590, A591, and Q592) of SEQ ID NO: 981.

112. The AAV particle of any one of embodiments 1-111, wherein [N2]-[N3] is present at amino acids 586-592, as numbered according to SEQ ID NO: 138 or 981.

113. The AAV particle of any one of embodiments 1-112, wherein [N1]-[N2]-[N3] replaces amino acids 582-592 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, Q590, A591, Q592) of SEQ ID NO: 138.

114. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-113, wherein [N1]-[N2]-[N3] corresponds to amino acids 582-592 (e.g., T582, N583, T584, Q585, D586, W587, H588, R589, I590, A591, Q592) of SEQ ID NO: 981.

115. The AAV particle of any one of embodiments 1-114, wherein [N1]-[N2]-[N3] is present at amino acids 582-592, as numbered according to SEQ ID NO: 138 or 981.

116. The AAV particle of any one of embodiments 43-50 and 87-115, wherein [N4] replaces amino acids 593-596 (e.g., T593, G594, W595, and V596) of SEQ ID NO: 138.

117. The AAV particle of any one of embodiments 43-49 and 87-115, wherein [N4] corresponds to amino acids 593-596 (e.g., T593, G594, W595, and V596) of SEQ ID NO: 138 or 981.

118. The AAV particle of any one of embodiments 43-50 and 87-117, wherein [N4] is present at amino acids 593-596, as numbered according to SEQ ID NO: 138 or 981.

119. The AAV particle of any one of embodiments 43-50 and 87-118, wherein [N2]-[N3]-[N4] replaces amino acids 586-596 (e.g., S586, A587, Q588, A589, Q590, A591, Q592, T593, G594, W595, and V596) of SEQ ID NO: 138.

120. The AAV particle of any one of embodiments 43-49 and 87-119, wherein [N2]-[N3]-[N4] corresponds to amino acids 586-596 (e.g., D586, W587, H588, R589, I590, A591, Q592, T593, G594, W595, and V596) of SEQ ID NO: 981.

121. The AAV particle of any one of embodiments 43-50 and 87-120, wherein [N2]-[N3]-[N4] is present at amino acids 586-596, as numbered according to SEQ ID NO: 138 or 981.

122. The AAV particle of any one of embodiments 43-50 and 87-121, wherein [N1]-[N2]-[N3]-[N4] replaces amino acids 582-596 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, Q590, A591, Q592, T593, G594, W595, and V596), as numbered according to SEQ ID NO: 138.

123. The AAV particle of any one of embodiments 43-49 and 87-122, wherein [N1]-[N2]-[N3]-[N4] corresponds to amino acids 582-596 (e.g., T582, N583, T584, Q585, D586, W587, H588, R589, I590, A591, Q592, T593, G594, W595, and V596) of SEQ ID NO: 981.

124. The AAV particle of any one of embodiments 43-50 and 87-123, wherein [N1]-[N2]-[N3]-[N4] is present at amino acids 582-596, as numbered according to SEQ ID NO: 138 or 981.

125. The AAV particle of any one of embodiments 1-124, wherein [N2] is present immediately subsequent to [N1].

126. The AAV particle of any one of embodiments 1-125, wherein [N3] is present immediately subsequent to [N2].

127. The AAV particle of any one of embodiments 43-50 and 87-126, wherein [N4] is present immediately subsequent to [N3].

128. The AAV particle of any one of embodiments 1-127, wherein the AAV capsid variant comprises, from N-terminus to C-terminus, [N1]-[N2]-[N3].

129. The AAV particle of any one of embodiments 43-50 and 87-128, wherein the AAV capsid variant comprises, from N-terminus to C-terminus, [N1]-[N2]-[N3]-[N4].

130. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising:

(a) the amino acid sequence of any one of the sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26;

(b) an amino acid sequence comprising at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, or at least 14 consecutive amino acids from any one of the sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26; or

(c) an amino acid sequence comprising at least one, at least two, or at least three but no more than four substitutions relative to any one of the sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26; or

(d) an amino acid sequence comprising at least one, at least two, or at least three but no more than four modifications relative to the amino acid sequence of any one of the sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26.

131. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising:

(a) the amino acid sequence of any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336;

(b) an amino acid sequence comprising at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, or at least 14 consecutive amino acids from any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336;

(c) an amino acid sequence comprising at least one, at least two, or at least three but no more than four substitutions, relative to the amino acid sequence of any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336; or

(d) an amino acid sequence comprising at least one, at least two, or at least three but no more than four modifications relative to the amino acid sequence of any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336.

132. The AAV particle of embodiment 130 or 131, wherein the AAV capsid variant does not comprise at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 consecutive amino acids from TNHQAQAQ (SEQ ID NO: 5100), optionally wherein the TNHQAQAQ (SEQ ID NO: 5100) corresponds to amino acids 582-592 of SEQ ID NO: 138.

133. The AAV particle of any one of embodiments 130-132, wherein the AAV capsid variant does not comprise: TNH, TNHQ (SEQ ID NO: 4760), TNHQS (SEQ ID NO: 5101), TNHQA (SEQ ID NO: 5102), TNHQAQ (SEQ ID NO: 5103), TNHQAQA (SEQ ID NO: 5104), TNHQAQAQ (SEQ ID NO: 5100), NHQ, NHQS (SEQ ID NO: 5105), NHQA (SEQ ID NO: 5106), NHQAQ (SEQ ID NO: 5107), NHQAQA (SEQ ID NO: 5108), NHQAQAQ (SEQ ID NO: 5109), HQS, HQSA (SEQ ID NO: 5110), HQSAQ (SEQ ID NO: 5111), HQSAQA (SEQ ID NO: 5112), HQSAQAQ (SEQ ID NO: 5113), QSA, QSAQ (SEQ ID NO: 5114), QSAQA (SEQ ID NO: 5115), QSAQAQ (SEQ ID NO: 5116), SAQA (SEQ ID NO: 5117), or SAQAQ (SEQ ID NO: 5118).

134. The AAV particle of any one of embodiments 130-124, wherein the AAV capsid variant comprises an amino acid sequence comprising at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, or at least 14 consecutive amino acids from any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336.

135. The AAV particle of any one of embodiments 130-134, wherein the at least 3 consecutive amino acids comprise TQD.

136. The AAV particle of any one of embodiments 130-135, wherein the at least 4 consecutive amino acids comprise TQDW (SEQ ID NO: 4684).

137. The AAV particle of any one of embodiments 130-136, wherein the at least 5 consecutive amino acids comprise TQDWH (SEQ ID NO: 4685).

138. The AAV particle of any one of embodiments 130-137, wherein the at least 6 consecutive amino acids comprise TQDWHR (SEQ ID NO: 4686).

139. The AAV particle of any one of embodiments 130-138, wherein the at least 7 consecutive amino acids comprise TQDWHRI (SEQ ID NO: 941).

140. The AAV particle of any one of embodiments 130-134, wherein the at least 3 consecutive amino acids comprise TNT.

141. The AAV particle of any one of embodiments 130-134 and 140, wherein the at least 4 consecutive amino acids comprise TNTQ (SEQ ID NO: 4688).

142. The AAV particle of any one of embodiments 130-134, 140, and 141, wherein the at least 5 consecutive amino acids comprise TNTQD (SEQ ID NO: 5119).

143. The AAV particle of any one of embodiments 130-134 and 140-142, wherein the at least 6 consecutive amino acids comprise TNTQDW (SEQ ID NO: 5120).

144. The AAV particle of any one of embodiments 130-134 and 140-143, wherein the at least 7 consecutive amino acids comprise TNTQDWH (SEQ ID NO: 5121).

145. The AAV particle of any one of embodiments 130-134 and 140-144, wherein the at least 8 consecutive amino acids comprise TNTQDWHR (SEQ ID NO: 4898).

146. The AAV particle of any one of embodiments 130-134 and 140-145, wherein the at least 9 consecutive amino acids comprise TNTQDWHRI (SEQ ID NO: 746).

147. The AAV particle of any one of embodiments 130-146, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three but no more than four modifications relative to the amino acid sequence of any one of SEQ ID NOs: 201, 205-209, 211-214,

216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336.

148. The AAV particle of any one of embodiments 130-147, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three but no more than four modifications relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941).

149. The AAV particle of any one of embodiments 130-147, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three but no more than four modifications relative to the amino acid sequence of TNTQDWHRI (SEQ ID NO: 746).

150. The AAV particle of any one of embodiments 130-149, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three but no more than four modifications relative to the amino acid sequence of ATNTQDWHRIAQT (SEQ ID NO: 744).

151. The AAV particle of any one of embodiments 130-150, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three but no more than four substitutions relative to the amino acid sequence of any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336.

152. The AAV particle of any one of embodiments 130-149 and 151, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three but no more than four substitutions relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941).

153. The AAV particle of any one of embodiments 130-149, 151, and 152, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three but no more than four substitutions relative to the amino acid sequence of TNTQDWHRI (SEQ ID NO: 746).

154. The AAV particle of any one of embodiments 130-153, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three but no more than four substitutions relative to the amino acid sequence of ATNTQDWHRIAQT (SEQ ID NO: 744).

155. The AAV particle of any one of embodiments 1-154, wherein the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336.

156. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-155, wherein the AAV capsid variant comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941), optionally wherein the amino acid sequence replaces amino acids 584-590 of SEQ ID NO: 138.

157. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-156, wherein the AAV capsid variant comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941), optionally wherein the amino acid sequence corresponds to amino acids 584-590 of SEQ ID NO: 981.

158. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, 101-149, 151-153, and 155-157, wherein the AAV capsid variant comprises an amino acid sequence encoded by:

- (i) the nucleotide sequence of SEQ ID NO: 942;
- (ii) a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications, but no more than ten modifications, relative to the nucleotide sequence of SEQ ID NO: 942; or
- (iii) a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven, but no more than ten different nucleotides relative to the nucleotide sequence of SEQ ID NO: 942.

159. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-158, wherein the AAV capsid variant comprises an amino acid sequence encoded by:

- (i) the nucleotide sequence of SEQ ID NO: 747;
- (ii) a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications, but no more than ten modifications, relative to the nucleotide sequence of SEQ ID NO: 747; or
- (iii) a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven, but no more than ten different nucleotides relative to the nucleotide sequence of SEQ ID NO: 747.

160. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-149, 151-153, and 155-158, wherein the nucleotide sequence encoding the AAV capsid variant comprises:

- (i) the nucleotide sequence of SEQ ID NO: 942;

(ii) a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications, but no more than ten modifications, relative to the nucleotide sequence of SEQ ID NO: 942; or

(iii) a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven, but no more than ten different nucleotides relative to the nucleotide sequence of SEQ ID NO: 942.

161. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-160, wherein the nucleotide sequence encoding the AAV capsid variant comprises:

(i) the nucleotide sequence of SEQ ID NO: 747;

(ii) a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications, but no more than ten modifications, relative to the nucleotide sequence of SEQ ID NO: 747; or

(iii) a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven, but no more than ten different nucleotides relative to the nucleotide sequence of SEQ ID NO: 747.

162. The AAV particle of any one of embodiments 130-157, wherein the amino acid sequence provided in Table 1, 2A, 2B, 9, 24, 25, or 26 is present in loop VIII, optionally wherein loop VIII comprises amino acids 580-599, as numbered according to SEQ ID NO: 138 or 981.

163. The AAV particle of any one of embodiments 130-157, wherein the amino acid sequence provided in Table 1, 2A, 2B, 9, 24, 25, or 26 replaces amino acids 584, 585, 586, 587, 588, 589, and/or 590 (e.g., H584, Q585, S586, A587, Q588, A589, and/or Q590), as numbered according to the amino acid sequence of SEQ ID NO: 138.

164. The AAV particle of any one of embodiments 130-157, wherein the amino acid sequence provided in Table 1, 2A, 2B, 9, 24, 25, or 26 is present at amino acids 584, 585, 586, 587, 588, 589, and/or 590, as numbered according to the amino acid sequence of SEQ ID NO: 981.

165. The AAV particle of any one of embodiments 130-157, wherein the amino acid sequence provided in Table 1, 2A, 2B, 9, 24, 25, or 26 corresponds to amino acids 584-590 (e.g., T584, Q585, D586, W587, H588, R589, and/or I590), numbered according to the amino acid sequence of SEQ ID NO: 981.

166. The AAV particle of any one of embodiments 121-157, wherein the amino acid sequence provided in Table 1, 2A, 2B, 9, 24, 25, or 26 replaces amino acids 582, 583, 584, 585, 586, 587, 588, 589, and/or 590 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, and/or Q590) of SEQ ID NO: 138.

167. The AAV particle of any one of embodiments 121-157, wherein the amino acid sequence provided in Table 1, 2A, 2B, 9, 24, 25, or 26 is present at amino acids 582, 583, 584, 585, 586, 587, 588, 589, and/or 590, as numbered according to the amino acid sequence of SEQ ID NO: 981.

168. The AAV particle of any one of embodiments 121-157, wherein the amino acid sequence provided in Table 1, 2A, 2B, 9, 24, 25, or 26 replaces amino acids 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and/or 596 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, Q590, A591, Q592, T593, G594, W595, and/or V5965) of SEQ ID NO: 138.

169. The AAV particle of any one of embodiments 121-157, wherein the amino acid sequence provided in Table 1, 2A, 2B, 9, 24, 25, or 26 is present at amino acids 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and/or 596, as numbered according to the amino acid sequence of SEQ ID NO: 981.

170. The AAV particle of any one of embodiments 1-169, wherein the AAV capsid variant comprises one, two, three, four, five, or all of:

- (i) an amino acid other than H at position 584 (e.g., T);
- (ii) an amino acid other than S at position 586 (e.g., D);
- (iii) an amino acid other than A at position 587 (e.g., W);
- (iv) an amino acid other than Q at position 588 (e.g., H);
- (v) an amino acid other than A at position 589 (e.g., R); and/or
- (vi) an amino acid other than Q at position 590 (e.g., I);

wherein the amino acids in (i)-(vi) are numbered according to SEQ ID NO: 138.

171. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising one, two, three, four, five, or all of:

- (i) an amino acid other than H at position 584 (e.g., T);
- (ii) an amino acid other than S at position 586 (e.g., D);
- (iii) an amino acid other than A at position 587 (e.g., W);
- (iv) an amino acid other than Q at position 588 (e.g., H);
- (v) an amino acid other than A at position 589 (e.g., R); and/or
- (vi) an amino acid other than Q at position 590 (e.g., I);

wherein the amino acids in (i) and (vi) are numbered according to SEQ ID NO: 138.

172. The AAV particle of any one of embodiments 1-171, wherein the AAV capsid variant comprises one, two, three, four, five, or all of: T at position 584, D at position 586, W at position 587, H at position 588, R at position 589, and/or I at position 590, as numbered according to SEQ ID NO: 981.

173. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising one, two, three, four, five, or all of: T at position 584, D at position 586, W at position 587, H at position 588, R at position 589, and/or I at position 590, as numbered according to SEQ ID NO: 981.

174. The AAV particle of any one of embodiments 1-173, wherein the AAV capsid variant comprises one, two, three, four, five, or all of the substitutions H584T, S586D, A587W, Q588H, A589R, and/or Q590I, as numbered according to SEQ ID NO: 138.

175. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising one, two, three, four, five or all of the substitutions H584T, S586D, A587W, Q588H, A589R, and/or Q590I, as numbered according to SEQ ID NO: 138.

176. The AAV particle of any one of embodiments 1-175, wherein the AAV capsid variant comprises an amino acid other than H at position 584 (e.g., T), an amino acid other than S at position 586 (e.g., D), an amino acid other than A at position 587 (e.g., W), an amino acid other than Q at position 588 (e.g., H), an amino acid other than A at position 589 (e.g., R), and an amino acid other than Q at position 590 (e.g., I), as numbered according to SEQ ID NO: 138.

177. The AAV particle of any one of embodiments 1-176, wherein the AAV capsid variant comprises T at position 584, D at position 586, W at position 587, H at position 588, R at position 589, and I at position 590, as numbered according to SEQ ID NO: 981.

178. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-98, and 100-164, wherein the AAV capsid variant comprises an amino acid at each of positions 584, 586, 587, 588, 589, and 590 that is different from the amino acid at the corresponding positions in SEQ ID NO: 138.

179. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-178, wherein the AAV capsid variant comprises an amino acid at each of positions 582, 583, 584, 586, 587, 588, and 589 that is different from the amino acid at the corresponding positions in SEQ ID NO: 138.

180. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, and 101-179, wherein the AAV capsid variant comprises an amino acid at each of positions 582, 583, 584, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and 596 that is different from the amino acid at the corresponding positions in SEQ ID NO: 138.

181. The AAV particle of any one of embodiments 1-181, wherein the AAV capsid variant comprises the substitutions H584T, S586D, A587W, Q588H, A589R, and Q590I, as numbered according to SEQ ID NO: 138.

182. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising T at position 584, D at position 586, W at position 587, H at position 588, R at position 589, and I at position 590, as numbered according to SEQ ID NO: 981.

183. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising the substitutions H584T, S586D, A587W, Q588H, A589R, and Q590I, as numbered according to SEQ ID NO: 138.

184. The AAV particle of any one of embodiments 1-183, wherein the AAV capsid variant further comprises an amino acid other than A position 581, as numbered according to SEQ ID NO: 138 or 981.

185. The AAV particle of any one of embodiments 1-184, wherein the AAV capsid variant further comprises a T at position 581 or a V at position 581, as numbered according to SEQ ID NO: 138 or 981.

186. The AAV particle of any one of embodiments 1-185, wherein the AAV capsid variant comprises the substitutions A581T or A581V, as numbered according to SEQ ID NO: 138 or 981.

187. The AAV particle of any one of embodiments 1, 3-7, 9-15, 17-20, 22-38, 40-48, 50, 130-134, 147, 151, 155, 162, 163, 166-177, and 181-186, wherein the AAV capsid variant comprises an amino acid other than Q at position 585, as numbered according to SEQ ID NO: 138.

188. The AAV particle of any one of embodiments 1, 3-7, 9-15, 17-20, 22-38, 40-48, 50, 130-134, 147, 151, 155, 162, 163, 166-177, and 181-187, wherein the AAV capsid variant comprises the amino acid K at position 585, as numbered according to SEQ ID NO: 138.

189. The AAV particle of any one of embodiments 1-189, wherein the AAV capsid variant further comprises:

(i) a modification in loop I, II, IV, and/or VI; and/or

(ii) a substitution at position K449, e.g., a K449R substitution, as numbered according to SEQ ID NO: 138 or 981.

190. The AAV particle of any one of embodiments 1-189, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three modifications, but not more than 30, not more than 20, or not more than 10 modifications, relative to the amino acid sequence of SEQ ID NO: 138.

191. The AAV particle of any one of embodiments 1-190, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three, but no more than not more than 30, not more than 20, or not more than 10 substitutions relative to the amino acid sequence of SEQ ID NO: 138.

192. The AAV particle of any one of embodiments 1-191, wherein the AAV capsid variant an amino acid sequence with at least 90% (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO: 138.

193. The AAV particle of embodiment 192, wherein the AAV capsid variant an amino acid sequence with at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO: 138.

194. The AAV particle of any one of embodiments 1-193, wherein the AAV capsid variant comprises an amino acid sequence encoded by a sequence with at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO: 137.

195. The AAV particle of embodiment 194, wherein the AAV capsid variant comprises an amino acid sequence encoded by a sequence with at least 90% (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO: 137.

196. The AAV particle of any one of embodiments 1-195, wherein the AAV capsid variant comprises a VP1 protein, a VP2 protein, and a VP3 protein in an about 1:1:10 ratio.

197. The AAV particle of any one of embodiments 1-196, wherein the AAV capsid variant comprises amino acids 138-736, e.g., a VP2, of SEQ ID NO: 981, or a sequence with at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto.

198. The AAV particle of any one of embodiments 1-197, wherein the AAV capsid variant comprises amino acids 203-736, e.g., a VP3, of SEQ ID NO: 981, or a sequence with at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto.

199. The AAV particle of any one of embodiments 1-198, wherein the AAV capsid variant comprises an amino acid sequence that is at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 138-736, e.g., a VP2, of SEQ ID NO: 138.

200. The AAV particle of any one of embodiments 1-199, wherein the AAV capsid variant comprises an amino acid sequence that is at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 203-736, e.g., a VP3, of SEQ ID NO: 138.

201. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, 101-186, and 189-200, wherein the AAV capsid variant comprises an amino acid sequence comprising at least 3, at least 4, at least 5, at least 6, or all 7 consecutive amino acids from the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein:

- (i) the at least 3 consecutive amino acids comprise TQD;
- (ii) the at least 4 consecutive amino acids comprise TQDW (SEQ ID NO: 4684);
- (iii) the at least 5 consecutive amino acids comprise TQDWH (SEQ ID NO: 4685);
- (iv) the at least 6 consecutive amino acids comprise TQDWHR (SEQ ID NO: 4686); or
- (v) the 7 consecutive amino acids comprise TQDWHRI (SEQ ID NO: 941);

wherein the AAV capsid variant comprises: (a) a VPI protein comprising the amino acid sequence of SEQ ID NO: 981; (b) a VP2 protein comprising amino acids 138-736 of SEQ ID NO: 981; (c) a VP3 protein comprising amino acids 203-736 of SEQ ID NO: 981; or (d) an amino acid sequence with at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity to any one of the amino acid sequences in (a)-(c).

202. The AAV particle of embodiment 201, wherein the AAV capsid variant comprises an amino acid sequence with at least 90% (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity to any one of the amino acid sequences in (a)-(c).

203. The AAV particle of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, 101-186, and 189-202, wherein the AAV capsid variant comprises 1, 2, or 3 substitutions relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein the AAV capsid variant comprises an amino acid sequence at least 90% (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981.

204. The AAV particle of any one of embodiments 1-203, wherein the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NO: 981, or an amino acid sequence with at least 90% (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto.

205. The AAV particle of any one of embodiments 1-204, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two, or at least three modifications, but not more than 30, not more than 20 or not more than 10 modifications relative to the amino acid sequence of SEQ ID NO: 981.

206. The AAV particle of any one of embodiments, 1-205, wherein the AAV capsid variant comprises an amino acid sequence comprising at least one, at least two or at least three, but not more than 30, not more than 20 or not more than 10 substitutions, relative to the amino acid sequence of SEQ ID NO: 981.

207. The AAV particle of any one of embodiments 1-206, wherein the AAV capsid variant comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 983, or a nucleotide sequence with at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto.

208. The AAV particle of embodiment 207, wherein the AAV capsid variant comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 983.

209. The AAV particle of embodiment 207, wherein the AAV capsid variant is encoded by a codon-optimized nucleotide sequence.

210. An AAV particle comprising an AAV capsid variant (e.g., an AAV9 capsid variant) comprising the amino acid sequence of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, 101-186, and 189, and further comprising an amino acid sequence at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to SEQ ID NO: 981.

211. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising the amino acid sequence of SEQ ID NO: 981.

212. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 983, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

213. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising amino acids 203-736 of SEQ ID NO: 981, or an amino acid sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto, wherein the AAV capsid variant comprises T at position 584, D at position 586, W at position 587, H at position 588, R at position 589, and I at position 590, as numbered according to SEQ ID NO: 981.

214. The AAV particle of embodiment 213, wherein the AAV capsid variant comprises amino acids 203-736 of SEQ ID NO: 981.

215. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising amino acids 203-736 of SEQ ID NO: 981.

216. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising amino acids 138-736 of SEQ ID NO: 981, or an amino acid sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto, wherein the AAV capsid variant comprises

T at position 584, D at position 586, W at position 587, H at position 588, R at position 589, and I at position 590, as numbered according to SEQ ID NO: 981.

217. The AAV particle any one of embodiments 213-216, wherein the AAV capsid variant comprises amino acids 138-736 of SEQ ID NO: 981.

218. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising amino acids 138-736 of SEQ ID NO: 981.

219. An AAV particle comprising a viral genome comprising a frataxin (FXN)-encoding sequence (e.g., encoding a human FXN protein) and an AAV capsid variant (e.g., an AAV9 capsid variant) comprising the amino acid sequence of SEQ ID NO: 981, or an amino acid sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto, wherein the AAV capsid variant comprises T at position 584, D at position 586, W at position 587, H at position 588, R at position 589, and I at position 590, as numbered according to SEQ ID NO: 981.

220. The AAV particle any one of embodiments 213-219, wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 981.

221. The AAV particle of any one of embodiments 1-220, wherein the AAV capsid variant has increased tropism for a CNS cell or tissue, e.g., a brain cell, brain tissue, spinal cord cell, or spinal cord tissue, relative to the tropism of an AAV particle comprising an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138.

222. The AAV particle of any one of embodiments 1-221, wherein the AAV capsid variant transduces a brain region, e.g., sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, and/or cerebellum; optionally wherein the level of transduction is at least 39, at least 50, at least 100, at least 120, at least 132, at least 146, at least 150, at least 161, at least 174, at least 175, at least 200, at least 225, at least 250, at least 275, at least 283, at least 300, at least 350, at least 400, at least 450, at least 500, at least 525, at least 528, or at least 550-fold greater as compared to an AAV particle comprising an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138, e.g., when measured by an assay, e.g., an immunohistochemistry assay or a qPCR or ddPCR assay, e.g., as described in Example 2.

223. The AAV particle of any one of embodiments 1-222, which is enriched at least 10, at least 14, at least 20, at least 24, at least 50, at least 100, at least 150, at least 200, at least 250, at least 300, at least

350, at least 400, at least 425, at least 450, or at least 460-fold in the brain compared to an AAV particle comprising an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Example 1 or 3.

224. The AAV particle of any one of embodiments 1-223, which is enriched at least 200, at least 300, at least 400, at least 425, at least 450, or at least 460-fold in the brain compared to an AAV particle comprising an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Example 1.

225. The AAV particle of any one of embodiments 1-224, which is enriched in the brain of at least two or at least three species, e.g., a non-human primate and rodent (e.g., mouse), e.g., as compared to an AAV particle comprising an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138.

226. The AAV particle of any one of embodiments 1-225, which is enriched at least 2, at least 3, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 105, at least 115, at least 120, at least 125, at least 130, at least 135, at least 140, at least 145, at least 150, at least 155, at least 160, at least 165, at least 170, at least 175, at least 180, at least 190, at least 200, at least 205, or at least 210-fold in the brain of at least two or at least three species, e.g., a non-human primate and rodent (e.g., mouse), compared to an AAV particle comprising an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Example 1 or 4.

227. The AAV particle of embodiment 225 or 226, wherein the at least two or at least three species comprise *Macaca fascicularis*, *Chlorocebus sabaeus*, *Callithrix jacchus*, and/or mouse (e.g., outbred mice).

228. The AAV particle of any one of embodiments 1-227, which is enriched at least 2, at least 3, at least 4, at least 5, at least 10, at least 15, at least 17, at least 20, at least 50, at least 75, at least 100, at least 103, at least 107, at least 125, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 750, at least 1000, at least 1200-fold in the brain compared to an AAV particle comprising an AAV capsid comprising the amino acid sequence of SEQ ID NO: 981, e.g., when measured by an assay as described in Example 3.

229. The AAV particle of any one of embodiments 1-228, which delivers an increased level of FXN to a brain region, optionally wherein the FXN level is increased by at least 39, at least 50, at least 100, at least 120, at least 132, at least 146, at least 150, at least 161, at least 174, at least 175, at least 200, at least 225,

at least 250, at least 275, at least 283, at least 300, at least 350, at least 400, at least 450, at least 500, at least 525, at least 528, or at least 550-fold as compared to an AAV particle comprising an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138, e.g., when measured by an assay, e.g., a qRT-PCR, a ddPCR, or a qPCR assay (e.g., as described in Example 2).

230. The AAV particle of any one of embodiments 1-229, which delivers an increased level of viral genomes to a brain region, optionally wherein the level of viral genomes is increased by at least 2, at least 5, at least 7, at least 10, at least 15, at least 19, at least 20, at least 22, or at least 25-fold as compared to an AAV particle comprising an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138, e.g., when measured by an assay, e.g., a qRT-PCR or a qPCR assay (e.g., as described in Example 2).

231. The AAV particle of embodiment 229 or 230, wherein the brain region is a sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, and/or cerebellum.

232. The AAV particle of any one of embodiments 1-231, which is enriched at least 5, at least 10, at least 50, at least 100, at least 115, at least 120, at least 150, at least 175, at least 200, at least 207, at least 225, at least 250, or at least 275-fold in the spinal cord compared to an AAV particle comprising an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Example 1 or 2.

[Embodiments 233-311 are intentionally absent.]

312. The AAV particle of any one of embodiments 1-232, wherein the viral genome is single-stranded.

313. The AAV particle of any one of embodiments 1-232, wherein the viral genome is self-complementary.

314. The AAV particle of any one of embodiments 1-232, embodiment 312, or embodiment 313, wherein the viral genome further comprises a nucleotide sequence encoding a Rep protein, e.g., a non-structural protein, wherein the Rep protein comprises a Rep78 protein, a Rep68 protein, a Rep52 protein, and/or a Rep40 protein (e.g., a Rep78 protein and a Rep52 protein).

315. The AAV particle of any one of embodiments 1-232, embodiment 312, or embodiment 313, wherein the AAV particle further comprises a nucleotide sequence encoding a Rep protein, e.g., a non-structural protein, wherein the Rep protein comprises a Rep78 protein, a Rep68 protein, a Rep52 protein, and/or a Rep40 protein (e.g., a Rep78 protein and a Rep52 protein).

316. The AAV particle of embodiment 314 or 315, wherein the Rep78 protein, the Rep68 protein, the Rep52 protein, and/or the Rep40 protein is encoded by at least one Rep gene.

317. The AAV particle of any one of embodiments 1-232 and 312-316, wherein the AAV particle is an isolated AAV particle.

318. The AAV particle of any one of embodiments 1-232 and 312-317, wherein the AAV particle is a recombinant AAV particle.

319. A cell, e.g., a host cell, comprising the AAV particle of any one of embodiments 1-232 and 312-318.

320. The cell of embodiment 319, wherein the cell is a mammalian cell or an insect cell.

321. The cell of embodiment 319 or 320, wherein the cell is a cell of a brain region or a spinal cord region, optionally wherein the cell is a cell of the sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, or cerebellum.

[Embodiments 322-325 are intentionally absent].

326. A pharmaceutical composition comprising the AAV particle of any one of embodiments 1-232 and 312-318, and a pharmaceutically acceptable excipient.

327. A method of delivering FXN to a cell or tissue (e.g., a CNS cell or CNS tissue), comprising administering an effective amount of the pharmaceutical composition of embodiment 326 or the AAV particle of any one of embodiments 1-232 and 312-318.

328. The method of embodiment 327, wherein the cell is a cell of a brain region or a spinal cord region, optionally a cell of the frontal cortex, sensory cortex, motor cortex, caudate, cerebellar cortex, cerebral cortex, brain stem, hippocampus, or thalamus.

329. The method of embodiment 327 or 328, wherein the cell is a neuron, a sensory neuron, and/or a motor neuron.

330. The method of any one of embodiments 327-329, wherein the cell or tissue is within a subject.

331. The method of embodiment 330, wherein the subject has, has been diagnosed with having, or is at risk of having a genetic disorder.

332. The method of embodiment 330 or embodiment 331, wherein the subject has, has been diagnosed with having, or is at risk of having a neurological disorder.

333. The method of embodiment 330 or embodiment 331, wherein the subject has, has been diagnosed with having, or is at risk of having a neurodegenerative disorder.

334. The method of embodiment 330 or embodiment 331, wherein the subject has, has been diagnosed with having, or is at risk of having a muscular disorder or a neuromuscular disorder.

335. A method of treating a subject having or diagnosed with having a genetic disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 326 or the AAV particle of any one of embodiments 1-232 and 312-318.

336. A method of treating a subject having or diagnosed with having a neurological disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 326 or the AAV particle of any one of embodiments 1-232 and 312-318.

337. A method of treating a subject having or diagnosed with having a muscular disorder or a neuromuscular disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 326 or the AAV particle of any one of embodiments 1-232 and 312-318.

338. A method of treating a subject having or diagnosed with having a neurodegenerative disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 326 or the AAV particle of any one of embodiments 1-232 and 312-318.

339. The method of any one of embodiments 327-338, wherein the genetic disorder, neurological disorder, neurodegenerative disorder, muscular disorder, or neuromuscular disorder is a disorder associated with FXN deficiency (e.g., Friedreich's ataxia).

340. The method of any one of embodiments 335-339, where treating comprises prevention of progression of the disorder in the subject.

341. The method of any one of embodiments 330-340, wherein the subject is a human.

342. The method of any one of embodiments 330-341, wherein the AAV particle or the pharmaceutical composition is administered to the subject intravenously, via intra-cisterna magna injection (ICM), intracerebrally, intrathecally, intracerebroventricularly, via intraparenchymal administration, intraarterially, or intramuscularly.

343. The method of any one of embodiments 330-342, wherein the AAV particle or pharmaceutical composition is administered to the subject via focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration.

344. The method of any one of embodiments 330-343, wherein the AAV particle or pharmaceutical composition is administered to the subject intravenously.

345. The method of any one of embodiments 330-344, wherein the AAV particle or pharmaceutical composition is administered to the subject via intra-cisterna magna injection (ICM).

346. The method of any one of embodiments 330-345, wherein the AAV particle or pharmaceutical composition is administered to the subject intraarterially.

347. The method of any one of embodiments 342-346, wherein administration of the AAV particle or pharmaceutical composition results in an increased expression of a FXN gene, mRNA, protein, or combination thereof.

348. The method of any one of embodiments 342-346, wherein administration of the AAV particle or pharmaceutical composition results in an increased activity of a FXN gene, mRNA, protein, or a combination thereof.

349. The pharmaceutical composition of embodiment 326 or the AAV particle of any one of embodiments 1-232 and 312-318, for use in a method of delivering FXN to a cell or tissue.

350. The pharmaceutical composition of embodiment 326 or the AAV particle of any one of embodiments 1-232 and 312-318, for use in a method of treating a genetic disorder, a neurological disorder, a neurodegenerative disorder, a muscular disorder, or a neuromuscular disorder.

351. The pharmaceutical composition of embodiment 326 or the AAV particle of any one of embodiments 1-232 and 312-318, for use in the manufacture of a medicament.

352. Use of the pharmaceutical composition of embodiment 326 or the AAV particle of any one of embodiments 1-232 and 312-318 in the manufacture of a medicament.

353. Use of the pharmaceutical composition of embodiment 326 or the AAV particle of any one of embodiments 1-232 and 312-318 in the manufacture of a medicament for treating a genetic disorder, a neurological disorder, a neurodegenerative disorder, a muscular disorder, or a neuromuscular disorder.

354. An adeno-associated virus (AAV) particle comprising an AAV capsid variant and a viral genome, wherein the viral genome comprises a frataxin (FXN)-encoding sequence and the AAV capsid variant comprises an amino acid sequence having the formula [N1]-[N2]-[N3] (SEQ ID NO: 4681) in loop VIII;

wherein [N2] comprises the amino acid sequence of DWHR (SEQ ID NO: 4682); and

wherein:

(i) [N1] comprises amino acids X₁, X₂, X₃, and X₄, wherein X₄ is Q, K, E, S, P, R, N, or H;

and/or

(ii) [N3] comprises amino acids X₅, X₆, and X₇, wherein X₅ is I, V, T, M, S, N, L, or F.

355. The AAV particle of embodiment 354, wherein the AAV capsid variant is an AAV9 capsid variant.

356. The AAV particle of embodiment 354 or embodiment 355, wherein the AAV capsid variant comprises an amino acid sequence that is at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 203-736 of SEQ ID NO: 981.

357. The AAV particle of any one of embodiments 354-356, wherein loop VIII comprises amino acids 580-599 as numbered according to SEQ ID NO: 981.

358. The AAV particle of any one of embodiments 354-357, wherein:

(i) X₁ is T, S, R, A, I, C, N, K, L, or Q;

(ii) X₂ is N, T, G, V, S, Y, K, I, H, D, or F; and

(iii) X₃ is T, N, K, D, I, S, P, A, Y, E, V, L, M, R, H, Q, or C.

359. The AAV particle of any one of embodiments 354-358, wherein:

(i) X₆ is A, Y, P, N, S, T, G, E, V, W, F, or Q; and

(ii) X₇ is Q, G, N, K, H, R, E, L, P, or M.

360. The AAV particle of any one of embodiments 354-359, wherein [N1] comprises the amino acid sequence of TNTQ (SEQ ID NO: 4688).

361. The AAV particle of any one of embodiments 354-360, wherein [N3] comprises the amino acid sequence of IAQ.

362. The AAV particle of any one of embodiments 354-361, wherein [N1]-[N2]-[N3] comprises the amino acid sequence of TNTQDWHRIAQ (SEQ ID NO: 343).

363. The AAV particle of any one of embodiments 354-362, wherein:

(i) [N1] is present at amino acids 582-585;

(ii) [N2] is present at amino acids 586-589; and

(iii) [N3] is present at amino acids 590-592;

wherein the amino acids are numbered according to SEQ ID NO: 981.

364. The AAV particle of any one of embodiments 354-363, further comprising [N4] comprising amino acids X₈, X₉, X₁₀, and X₁₁, wherein:

(i) X₈ is T, S, N, P, A, or I;

(ii) X₉ is G, N, D, R, V, A, S, or Q;

(iii) X₁₀ is W, S, C, R, L, or G; and/or

(iv) X₁₁ is V, A, S, I, C, G, D, F, L, or T.

365. The AAV particle of embodiment 364, wherein [N4] comprises the amino acid sequence of TGVV (SEQ ID NO: 5066).

366. The AAV particle of embodiment 364 or embodiment 365, wherein [N4] is present at amino acids 593-596 as numbered according to SEQ ID NO: 981.

367. The AAV particle of any one of embodiments 354-366, wherein [N1]-[N2]-[N3]-[N4] comprises the amino acid sequence of TNTQDWHRIAQTGVV (SEQ ID NO: 201).

368. An adeno-associated virus (AAV) particle comprising:

(i) a viral genome comprising a frataxin (FXN)-encoding sequence; and

(ii) an AAV capsid variant comprising an amino acid sequence at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 203-736 of SEQ ID NO:

981, wherein the AAV capsid variant comprises:

T at position 584,

D at position 586,

W position 587,

H at position 588,
R at position 589, and
I at position 590;
wherein the amino acids are numbered according to SEQ ID NO: 981.

369. The AAV particle of any one of embodiments 354-368, wherein the AAV capsid variant comprises an amino acid sequence having at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identity to amino acids 138-736 of SEQ ID NO: 981.

370. An adeno-associated virus (AAV) particle comprising:

- (i) a viral genome comprising a frataxin (FXN)-encoding sequence; and
- (ii) an AAV capsid variant comprising an amino acid sequence at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 138-736 of SEQ ID NO: 981, wherein the AAV capsid variant comprises:

T at position 584,
D at position 586,
W position 587,
H at position 588,
R at position 589, and
I at position 590;
wherein the amino acids are numbered according to SEQ ID NO: 981.

371. The AAV particle of any one of embodiments 354-370, wherein the AAV capsid variant comprises an amino acid sequence having at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identity to SEQ ID NO: 981.

372. An adeno-associated virus (AAV) particle comprising:

- (i) a viral genome comprising a frataxin (FXN)-encoding sequence; and
- (ii) an AAV capsid variant comprising an amino acid sequence that is at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to SEQ ID NO: 981, wherein the AAV capsid variant comprises:

T at position 584,
D at position 586,
W position 587,
H at position 588,
R at position 589, and
I at position 590;

wherein the amino acids are numbered according to SEQ ID NO: 981.

373. The AAV particle of embodiment 372, wherein the AAV capsid variant comprises:

- (i) a VP1 protein comprising an amino acid sequence having at least 99% identity to SEQ ID NO: 981;
- (ii) a VP2 protein comprising an amino acid sequence having at least 99% identity to amino acids 138-736 of SEQ ID NO: 981; and/or
- (iii) a VP3 protein comprising an amino acid sequence having at least 99% identity to amino acids 203-736 of SEQ ID NO: 981.

374. The AAV particle of any one of embodiments 368-373, wherein the AAV capsid variant comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941).

375. The AAV particle of any one of embodiments 368-374, wherein the AAV capsid variant comprises at least 8, at least 9, or at least 10 consecutive amino acids from the amino acid sequence of TNTQDWHRIAQ (SEQ ID NO: 343).

376. The AAV particle of any one of embodiments 368-375, wherein the AAV capsid variant comprises the amino acid sequence of TNTQDWHRIAQ (SEQ ID NO: 343) at amino acids 582-592 as numbered according to SEQ ID NO: 981.

377. The AAV particle of any one of embodiments 374-376, wherein the amino acid sequence of TQDWHRI (SEQ ID NO: 941) or TNTQDWHRIAQ (SEQ ID NO: 343) is present in loop VIII, wherein loop VIII comprises amino acids 580-599 as numbered according to SEQ ID NO: 981.

378. The AAV particle of any one of embodiments 354-377, wherein the AAV capsid variant comprises:

- (i) a VP1 protein comprising or consisting of the amino acid sequence of SEQ ID NO: 981;
- (ii) a VP2 protein comprising or consisting of the amino acid sequence according to positions 138-736 of SEQ ID NO: 981; and/or
- (iii) a VP3 protein comprising or consisting of the amino acid sequence according to positions 203-736 of SEQ ID NO: 981.

379. The AAV particle of any one of embodiments 1-232, 312-318, and 354-378, wherein the FXN-encoding sequence encodes an FXN protein, wherein the FXN protein is not a cynomolgus FXN protein.

380. The AAV particle of any one of embodiments 1-232, 312-318, and 354-379, wherein the FXN-encoding sequence encodes a human FXN protein.

381. The AAV particle of embodiment 380, wherein the human FXN protein comprises the amino acid sequence of SEQ ID NO: 1825, or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to SEQ ID NO: 1825, optionally, wherein the FXN protein is a wildtype human FXN protein.

382. The AAV particle of any one of embodiments 1-232, 312-318, and 354-380, wherein the FXN-encoding sequence comprises SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to SEQ ID NO: 1824.

383. The AAV particle of any one of embodiments 1-232, 312-318, and 354-382, wherein the viral genome further comprises a promoter operably linked to the FXN-encoding sequence.

384. The AAV particle of embodiment 383, wherein the promoter comprises a human elongation factor 1 α -subunit (EF1 α) promoter, a cytomegalovirus (CMV) immediate-early enhancer and/or promoter, a chicken β -actin (CBA) promoter, a CAG promoter, a β glucuronidase (GUSB) promoter, a ubiquitin C (UBC) promoter, a neuron-specific enolase (NSE) promoter, a platelet-derived growth factor (PDGF) promoter, a platelet-derived growth factor B-chain (PDGF- β) promoter, an intercellular adhesion molecule 2 (ICAM-2) promoter, a synapsin (Syn) promoter, a methyl-CpG binding protein 2 (MeCP2) promoter, a Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) promoter, a metabotropic glutamate receptor 2 (mGluR2) promoter, a neurofilament light chain (NFL) or neurofilament heavy chain (NFH) promoter, a β -globin minigene n β 2 promoter, a preproenkephalin (PPE) promoter, an enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2) promoter, a glial fibrillary acidic protein (GFAP) promoter, a myelin basic protein (MBP) promoter, a cardiovascular promoter (e.g., α MHC, cTnT, and CMV-MLC2k), a liver promoter (e.g., hAAT, TBG), a skeletal muscle promoter (e.g., desmin, MCK, C512), or a functional fragment or truncation of any one of the foregoing.

385. The AAV particle of embodiment 383 or embodiment 384, wherein the promoter is a CMV promoter or CBA promoter, or a functional fragment or truncation thereof.

386. The AAV particle of embodiment 385, wherein the promoter is a truncated CBA promoter.

387. The AAV particle of embodiment 386, wherein the truncated CBA promoter is 50-400 nucleotides in length, e.g., 100-332 nucleotides in length.

388. The AAV particle of any one of embodiments 385-387, wherein the promoter comprises or consists of the nucleotide sequence of any one of SEQ ID NOs: 1738, 1740, and 1742 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at

least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to any one of SEQ ID NOs: 1738, 1740, and 1742.

389. The AAV particle of embodiment 385, wherein the promoter is a truncated CMV promoter.

390. The AAV particle of embodiment 389, wherein the truncated CMV promoter is 50-300 nucleotides in length (e.g., 109 nucleotides in length).

391. The AAV particle of embodiment 389 or embodiment 390, wherein the promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 1750 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to SEQ ID NO: 1750.

392. The AAV particle of any one of embodiments 1-232, 312-318, and 354-391, wherein the viral genome further comprises a miRNA (miR) binding site that modulates expression of the encoded FXN protein in a cell or tissue of the liver.

393. The AAV particle of embodiment 392, wherein the viral genome comprises 3 copies of the miR binding site.

394. The AAV particle of embodiment 393, wherein the 3 copies of the miR binding site are identical.

395. The AAV particle of embodiment 393 or embodiment 394, wherein the 3 copies of the miR binding site are continuous.

396. The AAV particle of any one of embodiments 392-395, wherein the miR binding site is a miR122 binding site, optionally wherein:

the miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1827 or a sequence having one, two, three, or at most four substitutions relative to SEQ ID NO: 1827; or

the 3 copies of miR122 binding sites (miR122 binding site series) comprises the nucleotide sequence of SEQ ID NO: 1826 or a sequence having one, two, three, four, five, six, seven, eight, nine, or at most ten substitutions relative to SEQ ID NO: 1826.

397. The AAV particle of any one of embodiments 1-232, 312-318, and 354-396, wherein the viral genome further comprises at least one inverted terminal repeat (ITR) region.

398. The AAV particle of embodiment 397, wherein the at least one ITR region comprises an AAV2 ITR.

399. The AAV particle of embodiment 397 or embodiment 398, wherein the viral genome comprises a 5' ITR region and a 3' ITR region.

400. The AAV particle of embodiment 399, wherein the 5' ITR region and 3' ITR region are each an AAV2 ITR.

401. The AAV particle of embodiment 400, wherein the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

402. The AAV particle of any one of embodiments 1-232, 312-318, and 354-401, wherein the viral genome further comprises an intron/exon region comprising an intron region and/or an exon region.

403. The AAV particle of embodiment 402, wherein the intron/exon region comprises:

an immediate-early 1 (ie1) intron region and/or a human beta-globin (hBglobin) intron region; and/or

an ie1 exon region and/or an hBglobin exon region.

404. The AAV particle of embodiment 402 or embodiment 403, wherein the intron region comprises:

an ie1 intron 1 comprising the nucleotide sequence of SEQ ID NO: 1819 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

a hBglobin intron 2 comprising the nucleotide sequence of SEQ ID NO: 1820 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

405. The AAV particle of embodiment 404, wherein the exon region comprises:

an ie1 exon region comprising the nucleotide sequence of SEQ ID NO: 1817 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

an hBglobin exon region comprising the nucleotide sequence of SEQ ID NO: 1821 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

406. The AAV particle of any one of embodiments 1-232, 312-318, and 354-405, wherein the viral genome further comprises a polyadenylation (polyA) region.

407. The AAV particle of embodiment 406, wherein the polyA region comprises a human growth hormone (hGH) polyA region, optionally wherein the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

408. The AAV particle of any one of embodiments 1-232, 312-318, and 354-380, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1797.

409. The AAV particle of any one of embodiments 1-232, 312-318, and 354-380, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1801.

410. The AAV particle of any one of embodiments 1-232, 312-318, and 354-380, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1808.

411. The AAV particle of any one of embodiments 1-232, 312-318, and 354-380, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1809.

412. The AAV particle of any one of embodiments 1-232, 312-318, and 354-380, wherein the viral genome comprises:

- (i) a 5' inverted terminal repeat (ITR) region;
- (ii) a promoter;
- (iii) a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 1824; and
- (iv) a 3' ITR region.

413. The AAV particle of any one of embodiments 1-232, 312-318, and 354-380, wherein the viral genome comprises:

- (i) a 5' inverted terminal repeat (ITR) region;
- (ii) a promoter;
- (iii) a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 1824;
- (iv) at least one miR122 binding site; and
- (v) a 3' ITR region.

414. The AAV particle of any one of embodiments 1-232, 312-318, and 354-380, wherein the viral genome comprises:

- (i) a 5' inverted terminal repeat (ITR) region;
- (ii) a promoter;
- (iii) an intron and/or exon region;
- (iv) a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 1824;
- (v) at least one miR122 binding site; and
- (vi) a 3' ITR region.

415. The AAV particle of any one of embodiments 1-232, 312-318, and 354-380, wherein the viral genome comprises:

- (i) a 5' inverted terminal repeat (ITR) region;
- (ii) a promoter;

(iii) an intron and/or exon region;

(iv) a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 1824;

(v) at least one miR122 binding site;

(vi) a polyadenylation (polyA) region; and

(vii) a 3' ITR region.

416. The AAV particle of any one of embodiments 1-232, 312-318, and 354-380, wherein the viral genome comprises:

(i) a 5' inverted terminal repeat (ITR) region;

(ii) a promoter;

(iii) an intron and/or exon region;

(iv) a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 1824;

(v) at least one miR122 binding site;

(vi) a polyadenylation (polyA) region;

(vii) a filler sequence; and

(viii) a 3' ITR region.

417. The AAV particle of embodiment 415 or embodiment 416, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 1742 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(v) the at least one miR122 binding site comprises a miR122 binding site series comprising SEQ ID NO: 1826 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

(vii) the 3' ITR region comprises SEQ ID NO: 1812 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

418. The AAV particle of embodiment 417, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1841 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR.

419. The AAV particle of embodiment 415 or embodiment 416, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 1750 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(v) the at least one miR122 binding site comprises a miR122 binding site series comprising SEQ ID NO: 1826 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

(vii) the 3' ITR region comprises SEQ ID NO: 1812 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

420. The AAV particle of embodiment 419, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1840 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the poly A region and 5' to the 3' ITR.

421. The AAV particle of embodiment 415 or embodiment 416, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 1738 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(v) the at least one miR122 binding site comprises a miR122 binding site series comprising SEQ ID NO: 1826 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vi) the poly A region comprises the nucleotide sequence of SEQ ID NO: 1828 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

(vii) the 3' ITR region comprises SEQ ID NO: 1812 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

422. The AAV particle of embodiment 421, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1838 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at

least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the poly A region and 5' to the 3' ITR.

423. The AAV particle of embodiment 415 or embodiment 416, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 1740 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(v) the at least one miR122 binding site comprises a miR122 binding site series comprising SEQ ID NO: 1826 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

(vii) the 3' ITR region comprises SEQ ID NO: 1812 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

424. The AAV particle of embodiment 423, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1839 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR.

425. The AAV particle of any one of embodiments 1-232, 312-318, and 354-378, wherein the viral genome comprises:

(a) the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1797;

(b) the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1801;

(c) the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1808; or

(d) the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1809.

426. The AAV particle of any one of embodiments 354-425, wherein the viral genome is single-stranded.

427. A cell comprising the AAV particle of any one of embodiments 1-232, 312-318, and 354-426, optionally wherein the cell is a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

428. A method of making an AAV particle of any one of embodiments 1-232, 312-318, and 354-426, wherein the method comprises:

(i) providing a cell comprising a viral genome comprising a frataxin (FXN)-encoding sequence and a nucleic acid encoding an AAV capsid variant; and

(ii) incubating the cell under conditions suitable to encapsulate the viral genome in the AAV capsid variant;

thereby making the AAV particle.

429. The method of embodiment 428, wherein the viral genome comprises the nucleotide sequence of any one of SEQ ID NOs: 1797, 1801, 1808, and 1809, or a nucleotide sequence at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to any one of the nucleotide sequences of SEQ ID NOs: 1797, 1801, 1808, and 1809; and wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 981.

430. The method of embodiment 428 or embodiment 429, further comprising, prior to step (i), introducing a nucleic acid comprising the viral genome into the cell.

431. The method of any one of embodiments 428-430, further comprising, prior to step (i), introducing the nucleic acid encoding the AAV capsid variant into the cell.

432. The method of any one of embodiments 428-431, wherein the cell comprises a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

433. A pharmaceutical composition comprising the AAV particle of any one of embodiments 1-232, 312-318, and 354-426 and a pharmaceutically acceptable excipient.

434. A method of delivering a frataxin (FXN) protein to a subject, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 433 or the AAV particle of any one of embodiments 1-232, 312-318, and 354-426, thereby delivering the FXN protein.

435. The method of embodiment 434, wherein the subject has, has been diagnosed with having, or is at risk of having a disorder associated with FXN deficiency.

436. The method of embodiment 435, wherein the disorder associated with FXN deficiency is Friedreich's Ataxia (FA).

437. A method of treating a disorder associated with frataxin (FXN) deficiency in a subject, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 433 or the AAV particle of any one of embodiments 1-232, 312-318, and 354-426, thereby treating the disorder.

438. The method of embodiment 437, wherein the subject has, has been diagnosed with having, or is at risk of having a disorder associated with FXN deficiency.

439. The method of embodiment 437 or embodiment 438, wherein the disorder is Friedreich's Ataxia (FA).

440. The method of any one of embodiments 434-439, wherein the administration results in an increase in the subject's FXN protein level as compared to baseline, optionally wherein the administration results in a 0.5-3x or 1.5-4x increase in FXN levels as compared to baseline.

441. The method of any one of embodiments 437-439, wherein the treatment results in amelioration of at least one symptom of Friedreich's Ataxia (FA).

442. The method of embodiment 441, wherein the at least one symptom of FA comprises impaired sensory functions, impaired motor function (e.g., ataxia and/or involuntary movements), fatigue, chronic pain, seizures, impaired speech, sleep disturbances, metabolic disorders (e.g., diabetes), and/or increased spasticity.

443. The method of any one of embodiments 437-442, wherein the treatment stabilizes, slows the progression of, or improves the subject's FA as determined by the modified Friedreich Ataxia Rating Scale (mFARS), the Scale for the Assessment and Rating of Ataxia (SARA), and/or the International Cooperative Ataxia Rating Scale (ICARS).

444. The method of embodiment 443, wherein the treatment slows the subject's progression of FA as measured by mFARS, SARA, and/or ICARS relative to an individual with the disorder associated with FXN deficiency who has not been administered the pharmaceutical composition or the AAV particle.

445. The method of any one of embodiments 434-444, wherein the subject is a human.

446. The method of any one of embodiments 434-445, wherein the AAV particle or the pharmaceutical composition is delivered to a cell or tissue of the CNS, optionally wherein the AAV particle or the pharmaceutical composition is delivered via intravenous administration.

447. The method of embodiment 446, wherein the cell or tissue of the CNS is a cell or tissue of the amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral pallidum, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region).

448. The method of any one of embodiments 434-447, further comprising evaluating, e.g., measuring, the level of FXN expression, e.g., FXN gene, FXN mRNA, and/or FXN protein expression, in the subject, e.g., in a cell, tissue, or fluid, of the subject.

449. The method of embodiment 448, wherein the level of FXN protein expression is measured by an enzyme-linked immunosorbent assay (ELISA), a Western blot, an immunohistochemistry assay, or a frataxin biofluid assay.

450. The method of embodiment 448 or embodiment 449, wherein the cell or tissue is a cell or tissue of the central nervous system (CNS).

451. The method of embodiment 450, wherein the cell or tissue of the CNS is a cell or tissue of the amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region).

452. The method of embodiment 448 or embodiment 449, wherein the cell or tissue is a peripheral cell or tissue.

453. The method of embodiment 452, wherein the peripheral cell or tissue is a cell or tissue of the liver, heart, and/or muscle.

454. The method of any one of embodiments 434-453, wherein the administration results in an increase in:

(i) the level of FXN protein or FXN gene expression in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region), and/or fluid (e.g., CSF and/or serum), of the subject relative to baseline and/or relative to the level of FXN protein or FXN gene expression in a cell, tissue, or fluid of an individual with a disorder associated with FXN deficiency who has not been administered the pharmaceutical composition or AAV particle, optionally wherein the level of FXN protein or FXN gene expression is increased by 0.5-3x or 1.5-4x as compared to baseline;

(ii) the number and/or level of viral genomes (VG) per cell in a CNS tissue (e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate

nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region)) of the subject relative to the number and/or level of VG per cell in a peripheral tissue of the subject; and/or

(iii) the level of FXN activity in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region)), and/or fluid (e.g., CSF and or serum) of the subject relative to baseline and/or relative to FXN activity in a cell, tissue, or fluid of an individual with a disorder associated with FXN deficiency who has not been administered the pharmaceutical composition or AAV particle.

455. The method of any one of embodiments 437-454, further comprising administering to the subject at least one additional therapeutic agent and/or therapy.

456. The method of embodiment 455, wherein the at least one additional therapeutic agent and/or therapy comprises an agent and/or therapy for treating the disorder associated with FXN deficiency (e.g., Friedreich's Ataxia).

457. The method of embodiment 456, wherein the at least one additional therapeutic agent and/or therapy comprises omaveloxolone or idebenone.

458. The method of any one of embodiments 437-457, further comprising administering an immunosuppressant to the subject.

459. The method of embodiment 458, wherein the immunosuppressant comprises a corticosteroid (e.g., prednisone, prednisolone, methylprednisolone, and/or dexamethasone), rapamycin, mycophenolate mofetil, tacrolimus, rituximab, and/or eculizumab hydroxychloroquine.

460. The pharmaceutical composition of embodiment 433 or the AAV particle of any one of embodiments 1-232, 312-318, and 354-426 for use in a method of treating a disorder according to any one of embodiments 437-459.

461. The pharmaceutical composition of embodiment 433 or the AAV particle of any one of embodiments 1-232, 312-318, and 354-426 for use in the treatment of a disorder associated with FXN deficiency in a subject, optionally wherein the disorder is Friedreich's Ataxia.

462. The pharmaceutical composition or the AAV particle for use of embodiment 461, wherein the subject has, has been diagnosed with having, or is at risk of having Friedreich's Ataxia.

463. Use of an effective amount of the pharmaceutical composition of embodiment 433 or the AAV particle of any one of embodiments 1-232, 312-318, and 354-426 in the manufacture of a medicament for the treatment of a disorder associated with FXN deficiency in a subject, optionally wherein the disorder is Friedreich's Ataxia.

464. The use of embodiment 463, wherein the subject has, has been diagnosed with having, or is at risk of having Friedreich's Ataxia.

[066] The details of various aspects or embodiments of the present disclosure are set forth below. Other features, objects, and advantages of the disclosure will be apparent from the description and the claims. In the description, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art in the field of this disclosure. In the case of conflict, the present description will control.

BRIEF DESCRIPTION OF THE DRAWINGS

[067] FIGS. 1A-1D depict frataxin expression and the number of viral genomes per cell in the heart (FIG. 1A); cerebellum (FIG. 1B); lumbar and DRG (FIG. 1C); and liver (FIG. 1D).

[068] FIG. 2 shows an exemplary ITR-to-ITR construct encoding FXN.

[069] FIG. 3A and FIG. 3B show the ratio of human frataxin (huFXN) mRNA expression to cynomolgus frataxin (cyFXN) mRNA expression in the motor cortex (FIG. 3A) and somatosensory cortex (FIG. 3B) following intravenous administration of AAV particles comprising the TTJ-001 capsid variant and a single stranded viral genome comprising SEQ ID NO: 1797, at the doses indicated on the X-axis (from left to right: 1e13 vg/kg, 3e12 vg/kg, and 1e12 vg/kg) in cynomolgus macaques.

DETAILED DESCRIPTION

Overview

[070] Described herein, *inter alia*, are compositions comprising an AAV capsid variant comprising a sequence encoding a FXN protein, e.g., a human FXN protein. In some embodiments, the present disclosure provides a method for delivering the AAV capsid variant comprising the sequence encoding the FXN protein to a cell or tissue in a subject. In some embodiments, the present disclosure provides a

method for delivering the AAV capsid variant, thereby providing a FXN protein, e.g., a human FXN protein to a cell or tissue in a subject. The AAV capsid variants described herein have enhanced CNS tropism compared to other cells or tissues in the body, e.g., liver and/or the DRG.

[071] AAVs have proven to be useful as a biological tool due to their relatively simple structure, their ability to infect a wide range of cells (including quiescent and dividing cells) without integration into the host genome and without replicating, and their relatively benign immunogenic profile. Engineered AAV capsids with improved brain tropism represent an attractive solution to the limitations of CNS delivery. AAV-derived vectors are promising tools for clinical gene transfer because of their non-pathogenic nature, their low immunogenic profile, low rate of integration into the host genome, and long-term transgene expression in non-dividing cells. However, the transduction efficiency of naturally occurring AAVs in certain organs is too low for clinical applications, and capsid neutralization by pre-existing neutralizing antibodies may prevent treatment of a large proportion of patients. For these reasons, considerable efforts have been devoted to obtaining capsid variants with enhanced properties. Of many approaches tested so far, significant advances have resulted from directed evolution of AAV capsids using in vitro or in vivo selection of capsid variants created by capsid sequence randomization using either error-prone PCR, shuffling of various parent serotypes, or insertion of fully randomized short peptides at defined positions.

[072] The genome of the virus may be modified to contain a minimum of components for the assembly of a functional recombinant virus, or viral particle, which is loaded with or engineered to target a particular tissue and express or deliver FXN. The genome of the virus may encode a FXN protein, and the viral particle comprising said genome may be delivered to a target cell, tissue, or organism. In some embodiments, the genome encodes a human FXN protein, e.g., a wildtype human FXN protein. In some embodiments, the target cell is a CNS cell. In some embodiments, the target tissue is a CNS tissue. The target CNS tissue may be brain tissue. In some embodiments, the CNS tissue comprises amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral pallidum, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region).

[073] Gene therapy presents an alternative approach for treating FA. AAVs are commonly used in gene therapy approaches as a result of a number of advantageous features. Without being bound by theory, it is believed in some embodiments, an AAV particle described herein can be used to administer and/or deliver a gene encoding FXN (e.g., a human FXN) preferentially to the CNS (e.g., the brain or the spinal cord).

[074] Provided herein are compositions and methods which may provide for improved features compared to prior AAV-mediated approaches, including (i) increased biodistribution throughout the CNS

(e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region)), (ii) elevated FXN expression in multiple brain regions (e.g., cortex, thalamus, and brain stem); and (iii) preferential biodistribution in the CNS over the liver and/or DRG, of the subject.

[075] Also provided herein are AAV capsid variants (e.g., AAV9 capsid variants) with improved properties compared to wildtype AAV9, such as (i) increased penetrance through the blood brain barrier following intravenous administration, (ii) wider distribution throughout the multiple brain regions, e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region), (iii) elevated FXN expression in multiple brain regions, (iv) wider distribution in one or more peripheral tissues, e.g., the heart, kidney, muscle (e.g., quadriceps muscle or skeletal muscle), pancreas, and/or liver, and/or (v) elevated FXN expression in one or more peripheral tissues. In some embodiments, the AAV capsids described herein enhance the delivery of FXN to multiple regions of the CNS including, for example, amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region). In some embodiments, the AAV capsids described herein enhance the delivery of FXN to the forebrain. In some embodiments, the AAV capsids described herein enhance the delivery of FXN to the frontal cortex. In some embodiments, the AAV capsids described herein enhance the delivery of FXN to the spinal cord. In some embodiments, the AAV capsids described herein enhance the delivery of FXN to the cerebellum. In some embodiments, the AAV capsids described herein enhance the delivery of FXN to the dentate nucleus. In some embodiments, the AAV capsids described herein enhance the delivery of FXN to the somatosensory cortex. In some embodiments, the AAV capsid variants with enhanced brain tropism described herein are capable of significantly increasing FXN mRNA expression in the brain.

[076] Thus, the compositions and methods described herein can be used in the treatment of Friedreich's Ataxia (FA). In some embodiments, the disclosure provides an AAV particle comprising one

of the AAV capsid variants disclosed herein and an AAV viral genome comprising a nucleotide sequence comprising a truncated promoter region and a sequence encoding a FXN protein (e.g., comprising the nucleotide sequence of any one of SEQ ID NOs: 1797, 1801, 1808, and 1809) for use in treating FA.

I. Compositions

Adeno-associated viral (AAV) Particles

[077] AAVs have a genome of about 5,000 nucleotides in length and which contains two open reading frames encoding the proteins responsible for replication (Rep) and the structural protein of the capsid (Cap). The open reading frames are flanked by two Inverted Terminal Repeat (ITR) sequences, which serve as the origin of replication of the viral genome. The wild-type AAV viral genome comprises nucleotide sequences for two open reading frames, one for the four non-structural Rep proteins (Rep78, Rep68, Rep52, Rep40, encoded by Rep genes) and one for the three capsid, or structural, proteins (VP1, VP2, VP3, encoded by capsid genes or Cap genes). The Rep proteins are important for replication and packaging, while the capsid proteins are assembled to create the protein shell of the AAV, or AAV capsid. Alternative splicing and alternate initiation codons and promoters result in the generation of four different Rep proteins from a single open reading frame and the generation of three capsid proteins from a single open reading frame. Though it varies by AAV serotype, as a non-limiting example, for AAV9/hu.14 (SEQ ID NO: 123 of US 7,906,111, the contents of which are herein incorporated by reference in their entirety), VP1 refers to amino acids 1-736, VP2 refers to amino acids 138-736, and VP3 refers to amino acids 203-736. In some embodiments, with reference to the amino acid sequence of SEQ ID NO: 981, VP1 comprises amino acids 1-736, VP2 comprises amino acids 138-736, and VP3 comprises amino acids 203-736. In other words, VP1 is the full-length capsid protein sequence, while VP2 and VP3 are shorter components of the whole. As a result, changes in the sequence in the VP3 region are also changes to VP1 and VP2, however, the percent difference as compared to the parent sequence will be greatest for VP3 since it is the shortest sequence of the three. Though described here in relation to the amino acid sequence, the nucleic acid sequence encoding these proteins can be similarly described. Together, the three capsid proteins assemble to create the AAV capsid. Without being bound by theory, the AAV capsid typically comprises a molar ratio of 1:1:10 of VP1:VP2:VP3.

[078] The AAV particle typically requires a co-helper (e.g., adenovirus) to undergo productive infection in cells. In the absence of such helper functions, the AAV virions essentially enter host cells but do not integrate into the cells' genome.

[079] AAV particles have been investigated for delivery of gene therapeutics because of several unique features. Non-limiting examples of the features include (i) the ability to infect both dividing and non-dividing cells; (ii) a broad host range for infectivity, including human cells; (iii) wild-type AAV has not been associated with any disease and has not been shown to replicate in infected cells; (iv) the lack of cell-mediated immune response against the particle, and (v) the non-integrative nature in a host chromosome thereby reducing potential for long-term genetic alterations. Moreover, infection with AAV particles has minimal influence on changing the pattern of cellular gene expression (Stilwell and

Samulski *et al.*, Biotechniques, 2003, 34, 148, the contents of which are herein incorporated by reference in their entirety).

[080] Typically, AAV particles for FXN delivery may be recombinant viral particles which are replication defective as they lack sequences encoding functional Rep and Cap proteins within the viral genome. In some cases, the replication defective AAV particles may lack most or all coding sequences and essentially only contain one or two AAV ITR sequences and a nucleic acid sequence encoding a FXN protein (e.g., human FXN protein).

[081] In some embodiments, the AAV particles of the present disclosure may be introduced into mammalian cells.

[082] AAV particles may be modified to enhance the efficiency of delivery. Such modified AAV particles of the present disclosure can be packaged efficiently and can be used to successfully infect the target cells at high frequency and with minimal toxicity.

[083] In other embodiments, AAV particles of the present disclosure may be used to deliver FXN to the central nervous system (see, e.g., U.S. Pat. No. 6,180,613; the contents of which are herein incorporated by reference in their entirety) or to specific tissues of the CNS.

[084] It is understood that the compositions described herein may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on their functions.

[085] In some embodiments, an AAV capsid variant comprises one or more modifications (e.g., one or more substitutions) in loop VIII of AAV9, e.g., at one or more amino acids corresponding to positions 580-599 of VP1 of AAV9. In some embodiments, an AAV capsid variant comprises a substitution at one, two, three, four, five, or all of amino acids corresponding to position(s) 584, 586, 587, 588, 589, and/or 590 of the amino acid sequence of SEQ ID NO: 138 or 981. In some embodiments, the term loop (e.g., loop VIII) is used interchangeably herein with the term variable region (e.g., variable region VIII), or VR (e.g., VR-VIII). In some embodiments, loop VIII comprises amino acids corresponding to positions 580-599 (e.g., the positions of amino acids VATNHQSAQAQAQTGWVQNN (SEQ ID NO: 5122)) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, loop VIII is present (is located) at amino acids comprising those corresponding to positions 580-599 (e.g., the positions of amino acids VATNHQSAQAQAQTGWVQNN (SEQ ID NO: 5122)) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, loop VIII comprises amino acids corresponding to positions 582-593 (e.g., the positions of amino acids TNHQAQAQAQT (SEQ ID NO: 5123)) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, loop VIII comprises amino acids corresponding to positions 587-593 (e.g., the positions of amino acids AQAQAQT (SEQ ID NO: 4687)) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, loop VIII comprises amino acids corresponding to positions 587-590 (e.g., the positions of amino acids AQAQ (SEQ ID NO: 5099)) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, loop VIII is present at amino acids comprising those corresponding to positions 587-590 (e.g., the positions of amino acids AQAQ (SEQ ID NO: 5099)) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, loop VIII or variable region VIII (VR-

VIII) is as described in DiMattia et al. "Structural Insights into the Unique Properties of the Adeno-Associated Virus Serotype 9," *Journal of Virology*, 12(86):6947-6958 (the contents of which are hereby incorporated by reference in their entirety), e.g., comprising amino acids corresponding to positions 581-593 of the amino acid sequence of SEQ ID NO: 138. In some embodiments, loop VIII is present at amino acids comprising those corresponding to positions 581-593 of the amino acid sequence of SEQ ID NO: 138.

[086] The AAV particles and payloads of the disclosure may be delivered to one or more target cells, tissues, organs, or organisms. In some embodiments, the AAV particles demonstrate enhanced tropism for a target cell type, tissue, or organ. As a non-limiting example, the AAV particle may have enhanced tropism for cells and tissues of the central or peripheral nervous systems (CNS and PNS, respectively). In some embodiments, an AAV particle may, in addition, or alternatively, have decreased tropism for a cell-type, tissue or organ.

[087] In some embodiments, AAV particles are used as a biological tool due to a relatively simple structure, their ability to infect a wide range of cells (including quiescent and dividing cells) without integration into the host genome and without replicating, and their relatively benign immunogenic profile. The genome of the virus may be manipulated to contain a minimum of components for the assembly of a functional recombinant virus, or viral particle, which is loaded with or engineered to target a particular tissue and express or deliver a desired payload.

[088] In some embodiments, the AAV particle is a recombinant AAV particle. In some embodiments, the wild-type AAV viral genome is a linear, single-stranded DNA (ssDNA) molecule approximately 5,000 nucleotides (nt) in length. In some embodiments, inverted terminal repeats (ITRs) cap the viral genome at both the 5' and the 3' end, providing origins of replication for the viral genome. In some embodiments, an AAV viral genome comprises two ITR sequences. In some embodiments, the ITRs have a characteristic T-shaped hairpin structure defined by a self-complementary region (145nt in wild-type AAV) at the 5' and 3' ends of the ssDNA which form an energetically stable double stranded region. In some embodiments, the double stranded hairpin structures comprise multiple functions including, but not limited to, acting as an origin for DNA replication by functioning as primers for the endogenous DNA polymerase complex of the host viral replication cell.

[089] AAV particles of the present disclosure may be produced recombinantly and may be based on AAV reference sequences. In addition to single-stranded AAV viral genomes (e.g., ssAAVs), the present disclosure also provides for self-complementary AAV (scAAV) viral genomes. scAAV viral genomes contain DNA strands that anneal together to form double-stranded DNA. By skipping second strand synthesis, scAAVs allow for rapid expression in the transduced cell. In some embodiments, the AAV particle of the present disclosure is an scAAV. In some embodiments, the AAV particle of the present disclosure is an ssAAV.

[090] Methods for producing and/or modifying AAV particles are disclosed in the art such as pseudotyped AAV particles (PCT Patent Publication Nos. WO200028004; WO200123001;

WO2004112727; WO2005005610; and WO2005072364, the contents of each of which are incorporated herein by reference in their entirety).

[091] As described herein, the AAV particles of the disclosure comprising an AAV capsid variant, and a viral genome, have enhanced tropism for a cell-type or a tissue, e.g., a CNS cell-type, region, or tissue.

AAV Capsid Variants

[092] Disclosed herein are AAV particles comprising an AAV capsid variant comprising one or more modifications (e.g., comprising one or more substitutions relative to a wildtype AAV capsid) for enhanced or improved transduction of a target tissue (e.g., cells, regions, and/or tissues of the CNS and/or PNS). In some embodiments, the peptide (e.g., comprising one or more substitutions relative to a wildtype AAV capsid) is present in VP1, VP2, and/or VP3 proteins of the AAV capsid variant. In some embodiments, the modification (e.g., comprising one or more substitutions relative to a wildtype AAV capsid) is present in VP1, VP2, and VP3 proteins of the AAV capsid variant.

[093] In some embodiments, the modification (e.g., comprising the one or more substitutions relative to a wildtype AAV capsid) is in loop VIII. In some embodiments, the AAV capsid variant is an AAV9 capsid variant.

[094] In some embodiments, the one or more substitutions in the AAV capsid may increase distribution of an AAV particle to a cell, region, or tissue of the CNS. The cell of the CNS may be, but is not limited to, neurons (e.g., excitatory, inhibitory, motor, sensory, autonomic, sympathetic, parasympathetic, Purkinje, Betz, etc.), glial cells (e.g., microglia, astrocytes, oligodendrocytes) and/or supporting cells of the brain such as immune cells (e.g., T cells). The tissue of the CNS may be, but is not limited to, amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral pallidum, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region).

[095] In some embodiments, the one or more substitutions may increase distribution of an AAV particle to a cell, region, or tissue of the CNS. In some embodiments, the one or more substitutions may decrease distribution of an AAV particle to the DRG.

[096] In some embodiments, the one or more substitutions may increase distribution of an AAV particle to the CNS (e.g., the cortex) after intravenous administration. In some embodiments, the one or more substitutions may increase distribution of an AAV particle to the CNS (e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral

palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region) following focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration.

[097] In some embodiments, the one or more substitutions may increase distribution of an AAV particle to the PNS (e.g., DRG) after intravenous administration. In some embodiments, the one or more substitutions may increase distribution of an AAV particle to non-DRG cells of the PNS following focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration. In some embodiments, the one or more substitutions may decrease distribution of an AAV particle to the DRG following focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration.

[098] In some embodiments, the one or more modifications, e.g., substitutions, may increase distribution of an AAV particle to a cell, region, or tissue of a heart, e.g., a heart atrium or a heart ventricle. In some embodiments, the one or more substitutions may increase distribution of an AAV particle to a heart cell, region, or tissue after intravenous administration.

[099] In some embodiments, the one or more modifications, e.g., substitutions, may increase distribution of an AAV particle to a cell, region, or tissue of a muscle. In some embodiments, the muscle is a heart muscle (e.g., a heart atrium or a heart ventricle) or a quadriceps muscle. In some embodiments, the muscle is a skeletal muscle. In some embodiments, the one or more substitutions may increase distribution of an AAV particle to a muscle cell, region, or tissue after intravenous administration.

[0100] In some embodiments, the one or more modifications, e.g., substitutions, may increase distribution an AAV particle to a cell, region, or tissue of the kidney. In some embodiments, the one or more substitutions may increase distribution an AAV particle to a cell, region, or tissue of the pancreas.

[0101] In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) a sequence as set forth in Table 1. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) a sequence as set forth in Table 2A. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) a sequence as set forth in Table 2B (e.g., a sequence of any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336). In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) a sequence set forth in Table 9. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) a sequence as set forth in Table 24. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) a sequence as set forth in Table 25. In some embodiments, the AAV capsid variant is an AAV9 capsid variant.

Table 1. Exemplary Sequences

Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:
TNTQDWHRIAQ	343	TTRQDWHRIAQ	392	KNNQDWHRIAQ	441	RNTQDWHRISQ	490
TNTKQDWHRIAQ	344	TNTQDWHRVAN	393	TNNQDWHRIAR	442	TNQDQDWHRIAQ	491
SNNQDWHRIAQ	345	TNTQDWHRISG	394	SNTQDWHRVQP	443	RNIQDWHRIAQ	492
TNKQDWHRIAQ	346	TNSQDWHRIAQ	395	TNNQDWHRIAK	444	TTTQDWHRISQ	493
TNNDQDWHRIAQ	347	TYTKDWHRIAQ	396	TNTKDWHRIAP	445	TSTQDWHRVAQ	494
SNKQDWHRIAQ	348	TTTKDWHRIAQ	397	TNNRDWHRIAQ	446	TSTQDWHRVSQ	495
SNTQDWHRIAQ	349	TNIKDWHRIAQ	398	LNNQDWHRIAQ	447	TNKQDWHRIEQ	496
TTNQDWHRIAQ	350	TNTKDWHRISQ	399	TINQDWHRIAQ	448	TNNPDWHRIAQ	497
TNDQDWHRIAQ	351	SNTKDWHRIAQ	400	TNNQDWHRIAH	449	TITEDWHRIAQ	498
TTIQDWHRIAQ	352	TNNKDWHRIAQ	401	TNNQDWHRIGQ	450	TNTNDWHRIAQ	499
RNTQDWHRIAQ	353	TNSKDWHRIAQ	402	TNNQDWHRFAQ	451	SNTQDWHRIPQ	500
TTKQDWHRIAQ	354	TSTKDWHRIAQ	403	SNNQDWHRMAQ	452	STTQDWHRISQ	501
TTSQDWHRIAQ	355	TNTKDWHRVAQ	404	TNHQDWHRIAQ	453	TFSQDWHRIAQ	502
TTDQDWHRIAQ	356	TITKDWHRIAQ	405	STTQDWHRIAQ	454	RNSQDWHRIAQ	503
TNPSDWHRIAQ	357	INTKDWHRIAQ	406	TNNQDWHRTAQ	455	INTQDWHRIAQ	504
TNKEDWHRIAQ	358	TNAKDWHRIAQ	407	SNSQDWHRIAQ	456	RNTEDWHRIAQ	505
TTTQDWHRIAQ	359	TNTKDWHRITQ	408	TNIQDWHRVAQ	457	TNNHDWHRIAQ	506
TGKQDWHRIAQ	360	TNTKDWHRTAQ	409	SNTQDWHRVAQ	458	SNTQDWHRVSQ	507
TTAQDWHRIAQ	361	TKTKDWHRIAQ	410	TNSQDWHRIAG	459	TNMQDWHRIAQ	508
TVKQDWHRIAQ	362	STNQDWHRIAQ	411	TTTQDWHRVAQ	460	TNSQDWHRVQP	509
TNYQDWHRIAQ	363	TNTKDWHRMAQ	412	SNTQDWHRISQ	461	RTTQDWHRIAQ	510
TNTPDWHRIAQ	364	TNTKDWHRSAQ	413	SNSQDWHRVAQ	462	SNIEDWHRIAQ	511
STKQDWHRIAQ	365	TNTKDWHRIGQ	414	SNTQDWHRIAG	463	TITQDWHRTAQ	512
TNNQDWHRIAQ	366	TNTKDWHRIEQ	415	TNSQDWHRVAQ	464	TNTSDWHRIAQ	513
TNTQDWHRIAG	367	ANTKDWHRIAQ	416	TNNQDWHRSAQ	465	TNTQDWHRVMT	514
TTEQDWHRIAQ	368	RNNQDWHRIAQ	417	STSQDWHRIAQ	466	TNTQDWHRISM	515
TSKQDWHRIAQ	369	TNTKDWHRIVQ	418	TNNQDWHRMAQ	467	TNTQDWHRVSQ	516
TNIQDWHRIAQ	370	TNNQDWHRISQ	419	TNNQDWHRLAQ	468	TNTQDWHRIWQ	517
TNTQDWHRIYQ	371	TNNQDWHRVAQ	420	TNIQDWHRISQ	469	TNTQDWHRIFQ	518
TYNQDWHRIAQ	372	TNTKDWHRNAQ	421	TNTQDWHRIPQ	470	TNTQDWHRIQQ	519
TNTQDWHRVQP	373	TGNQDWHRIAQ	422	TNTQDWHRTAQ	471	KNTQDWHRIAQ	520
TNTQDWHRIAN	374	TNTKDWHRLAQ	423	TYIQDWHRIAQ	472	TNTQDWHRVQQ	521
STIQDWHRIAQ	375	TNNQDWHRIAN	424	TNNQDWHRIVQ	473	TNTQDWHRIFM	522
TTVQDWHRIAQ	376	TNTKDWHRIAK	425	TNNQDWHRNAQ	474	TNLQDWHRIAQ	523
TGTQDWHRIAQ	377	TNTKDWHRIAH	426	SGTQDWHRIAQ	475	TNTQDWHRIAM	524
TNTQDWHRINQ	378	TSNQDWHRIAQ	427	THNQDWHRIAQ	476	TITQDWHRIAQ	525
TNTRDWHRIAQ	379	TNNQDWHRIPQ	428	TITQDWHRVAQ	477	TNTQDWHRIAK	526
TNTQDWHRVNQ	380	THTKDWHRIAQ	429	TNTEDWHRVAQ	478	TSMQDWHRIAQ	527
TTLQDWHRIAQ	381	TDTKDWHRIAQ	430	TTTQDWHRIPQ	479	TNTQDWHRISN	528
TTMQDWHRIAQ	382	TNTKDWHRIAR	431	SNNQDWHRIAE	480	TNTQDWHRIGQ	529
ANNQDWHRIAQ	383	TNEQDWHRIAQ	432	TSTQDWHRIAQ	481	SYTQDWHRIAQ	530
SNIQDWHRIAQ	384	CNTQDWHRIAQ	433	TNTQDWHRVAQ	482	TNCQDWHRIAQ	531
TKNQDWHRIAQ	385	TNPKDWHRIAQ	434	TNIQDWHRIAN	483	TNTQDWHRMAQ	532
TYTQDWHRIAQ	386	TNTKDWHRIAE	435	TNSNDWHRIAQ	484	SSTQDWHRIAQ	533
TNKSQDWHRIAQ	387	TNTKDWHRIAL	436	TNTQDWHRSAQ	485	TYTQDWHRTAQ	534
TNTQDWHRVYQ	388	INNQDWHRIAQ	437	TNIQDWHRIPQ	486	TYTQDWHRVAQ	535
SNTEDWHRIAQ	389	TYTEDWHRIAQ	438	SNTQDWHRIAN	487	TVTQDWHRIAQ	536
TNTEDWHRIAQ	390	TNNQDWHRITQ	439	TNTQDWHRISQ	488	QNTQDWHRIAQ	537
TNIEDWHRIAQ	391	NNNQDWHRIAQ	440	TNNQDWHRIEQ	489	TNSQDWHRIPQ	538
VTNTQDWHRIAQT	748	TTNTQDWHRIAQT	749				

Table 2A. Exemplary Sequences

SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:	Nucleotide Sequence
941	TQDWHRI	942	ACCCAGGACTGGCACAGGATT
744	ATNTQDWHRIAQT	745	GCTACAAACACCCAGGACTGGCACAGGATTGCGCAGACC
746	TNTQDWHRI	747	ACAAACACCCAGGACTGGCACAGGATT

Table 2B. Exemplary Sequences

Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:
TNTQDWHRIAQTGWV	201	TGTQDWHRIAQTGWV	220	TTLQDWHRIAQTGWV	286
TTNQDWHRIAQTGWV	205	STQDWHRIAQTGWV	230	TTTQDWHRIAQTGWV	290
TTIQDWHRIAQTGWV	206	TNTQDWHRVAQTGWV	232	TNSQDWHRIAQTGWA	291
TTSQDWHRIAQTGWV	207	TNTQDWHRVSQTGWV	237	TNSQDWHRVAQTGWV	293
TTTQDWHRIAQTGWV	208	TSTQDWHRIAQTGWV	238	TSTQDWHRIAQTGWA	301
TTAQDWHRIAQTGWV	209	TITQDWHRIAQTGWV	255	TTTQDWHRIAQTGWI	306
TNTQDWHRIAQTGWA	211	TNTQDWHRIANNWV	262	TNTQDWHRIAQTGWS	307
TNTQDWHRIAQTGWV	212	TNTQDWHRIYQTGWV	263	TTTQDWHRISQTGWV	308
TNIQDWHRIAQTGWV	213	TNTPDWHRIAQTGWV	264	TSTQDWHRVAQTGWV	309
TNTQDWHRIAGSNWV	214	TNTQDWHRINQTGWV	265	TNTQDWHRISQTGWA	314
TNTQDWHRVPTGWV	216	TNNQDWHRVAQTGWV	274	TVTQDWHRIAQTGWV	336
TTVQDWHRIAQTGWV	219	TNIQDWHRVAQTGWV	283		

[0102] In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence having the formula [N1]-[N2]-[N3] (SEQ ID NO: 4681), wherein [N2] comprises the amino acid sequence of DWHR (SEQ ID NO: 4682) and wherein [N1] comprises amino acids X₁, X₂, X₃, and X₄, wherein X₄ is Q, K, E, S, P, R, N, H, or a conservative substitution thereof and/or [N3] comprises X₅, X₆, and X₇, wherein X₅ is I, V, T, M, S, N, L, F, or a conservative substitution thereof. In some embodiments, [N2] comprises the amino acid sequence of DWHR (SEQ ID NO: 4682), [N1] comprises X₁, X₂, X₃, and X₄, wherein X₄ is Q, K, E, S, P, R, N, or H, and/or [N3] comprises X₅, X₆, and X₇, wherein X₅ is I, V, T, M, S, N, L, or F. In some embodiments, X₄ of [N1] is Q. In some embodiments, X₄ of [N1] is K. In some embodiments, X₅ of [N3] is I. In some embodiments, X₁ of [N1] is T, S, R, A, I, C, N, K, L, or Q. In some embodiments, X₂ of [N1] is N, T, G, V, S, Y, K, I, H, D, or F. In some embodiments, X₃ of [N1] is T, N, K, D, I, S, P, A, Y, E, V, L, M, R, H, Q, or C. In some embodiments, [N1] is or comprises TNTQ (SEQ ID NO: 4688). In some embodiments, [N1] is or comprises TNTK (SEQ ID NO: 4689). In some embodiments, [N1]-[N2] is or comprises TNTQDWHR (SEQ ID NO: 4898). In some embodiments, [N1]-[N2] is or comprises TNTKDWHR (SEQ ID NO: 4899). In some embodiments, X₆ of [N3] is A, Y, P, N, S, T, G, E, V, W, F, or Q. In some embodiments, X₇ of [N3] is Q, G, N, K, H, R, E, L, P, or M. In some embodiments, [N3] is or comprises IAQ. In some embodiments, [N2]-[N3] is or comprises DWHRIAQ (SEQ ID NO: 5027). In some embodiments, [N1]-[N2]-[N3] is or comprises TNTQDWHRIAQ (SEQ ID NO: 343). In some embodiments, [N1]-[N2]-[N3] is or comprises TNTKDWHRIAQ (SEQ ID NO: 344).

[0103] In some embodiments, the amino acid sequence having the formula [N1]-[N2]-[N3] further comprises [N4], wherein [N4] comprises amino acids X₈, X₉, X₁₀, and X₁₁, wherein X₈ is T, S, N, P, A, or

I; X₉ is G, N, D, R, V, A, S, or Q; X₁₀ is W, S, C, R, L, or G; and/or X₁₁ is V, A, S, I, C, G, D, F, L, or T. In some embodiments, [N4] is or comprises TGWV (SEQ ID NO: 5066). In some embodiments, [N1]-[N2]-[N3]-[N4] is or comprises any one of SEQ ID NOs: 201-245, 247-250, 253-255, 257-265, 268-274, 276-286, 288, 290-297, 299-303, 305-309, 311, 313-319, 323-328, 330-337, 339-342, 539-542, 544, 546, 547, 549-557, 559-589, 592, 593, 595, 596, 598, 599, 601-608, 610-614, 616-622, 625, 628, 630, 631, 633, 636, 638, 639-646, 649, 651-657, 667, 669, 670, 672, 673, 679-683, 685-690, 692, 693, 695, 697, 699-701, 703-705, 708-710, 712-717, 719-723, 728-731, 733-738, 740, and 742. In some embodiments, [N1]-[N2]-[N3]-[N4] is or comprises TNTQDWHRIAQTGWV (SEQ ID NO: 201). In some embodiments, [N1]-[N2]-[N3]-[N4] is or comprises TNTKDWHRIAQTGWV (SEQ ID NO: 202).

[0104] In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence having the formula [N1]-[N2]-[N3] (SEQ ID NO: 4683), wherein [N2] comprises the amino acid sequence of DWHR (SEQ ID NO: 4682) and wherein [N1] comprises amino acids X₁, X₂, X₃, and X₄, wherein X₄ is Q, P, or a conservative substitution thereof; and/or [N3] comprises amino acids X₅, X₆, and X₇, wherein X₅ is I, V, or a conservative substitution thereof. In some embodiments, [N2] comprises the amino acid sequence of DWHR (SEQ ID NO: 4682); [N1] comprises amino acids X₁, X₂, X₃, and X₄, wherein X₄ is Q or P; and/or [N3] comprises amino acids X₅, X₆, and X₇, wherein X₅ is I or V. In some embodiments, X₄ of [N1] is Q. In some embodiments, X₅ of [N3] is I. In some embodiments, X₅ of [N3] is V. In some embodiments, X₁ of [N1] is T or S. In some embodiments, X₂ of [N1] is N, T, G, S, I, or V. In some embodiments, X₃ of [N1] is T, N, I, S, A, V, or L. In some embodiments, [N1] is or comprises TNTQ (SEQ ID NO: 4688). In some embodiments, [N1]-[N2] is or comprises TNTQDWHR (SEQ ID NO: 4898). In some embodiments, X₆ of [N3] is A, P, S, Y, or N. In some embodiments, X₇ of [N3] is Q, G, or N. In some embodiments, [N3] is or comprises IAQ. In some embodiments, [N2]-[N3] is or comprises DWHRIAQ (SEQ ID NO: 5027). In some embodiments, [N1]-[N2]-[N3] is or comprises TNTQDWHRIAQ (SEQ ID NO: 343). In some embodiments, the amino acid sequence having the formula [N1]-[N2]-[N3] further comprises [N4], wherein [N4] comprises amino acids X₈, X₉, X₁₀, and X₁₁, wherein X₁₀ is W. In some embodiments, X₈ of [N4] is T, S, or N. In some embodiments, X₉ of [N4] is G or N. In some embodiments, X₁₁ of [N4] is V, A, I, or S. In some embodiments, [N4] is or comprises TGWV (SEQ ID NO: 5066). In some embodiments, [N1]-[N2]-[N3]-[N4] is or comprises any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336. In some embodiments, [N1]-[N2]-[N3]-[N4] is or comprises TNTQDWHRIAQTGWV (SEQ ID NO: 201).

[0105] In some embodiments, [N1]-[N2]-[N3] is present in loop VIII of the AAV capsid variant. In some embodiments, [N4] is present in loop VIII of the AAV capsid variant. In some embodiments, [N1]-[N2]-[N3]-[N4] is present in loop VIII of the AAV capsid variant. In some embodiments, loop VIII is present at amino acids comprising those corresponding to positions 581-593 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, loop VIII is present at amino acids

comprising those corresponding to positions 580-599 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981.

[0106] In some embodiments, [N1] is present at amino acids corresponding to positions 582-585 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, X₁ of [N1] is present at an amino acid corresponding to position 582 of the amino acid sequence of SEQ ID NO: 138 or 981, X₂ of [N1] is present at an amino acid corresponding to position 583 of the amino acid sequence of SEQ ID NO: 138 or 981, X₃ of [N1] is present at an amino acid corresponding to position 584 of the amino acid sequence of SEQ ID NO: 138 or 981, and X₄ of [N1] is present at an amino acid corresponding to position 585 of the amino acid sequence of SEQ ID NO: 138 or 981. In some embodiments, [N1] replaces amino acids corresponding to positions 582-585 (e.g., T582, N583, H584, and Q585) of the amino acid sequence of SEQ ID NO: 138.

[0107] In some embodiments, [N2] is present at amino acids corresponding to positions 586-589 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, [N2] replaces amino acids corresponding to positions 586-589 of the amino acid sequence of SEQ ID NO: 138 (e.g., S586, A587, Q588, and A589 may be replaced with D586, W587, H588, and R589).

[0108] In some embodiments, [N1]-[N2] is present at amino acids corresponding to positions 582-589 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, [N1]-[N2] replaces amino acids corresponding to positions 582-589 of the amino acid sequence of SEQ ID NO: 138 (e.g., T582, N583, H584, Q585, S586, A587, Q588, and A589 may be replaced with T582, N583, T584, Q585, D586, W587, H588, and R589).

[0109] In some embodiments, [N3] is present at amino acids corresponding to positions 590-592 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, X₅ of [N3] is present at an amino acid corresponding to position 590 of the amino acid sequence of SEQ ID NO: 138 or 981, X₆ of [N3] is present at an amino acid corresponding to position 591 of the amino acid sequence of SEQ ID NO: 138 or 981, and X₇ of [N3] is present at an amino acid corresponding to position 592 of the amino acid sequence of SEQ ID NO: 138 or 981. In some embodiments, [N3] replaces amino acids corresponding to positions 590-592 (e.g., I590, A591, and Q592) of the amino acid sequence of SEQ ID NO: 138.

[0110] In some embodiments, [N2]-[N3] is present at amino acids corresponding to positions 586-592 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, [N2]-[N3] replaces amino acids corresponding to positions 586-592 of the amino acid sequence of SEQ ID NO: 138 (e.g., S586, A587, Q588, A589, Q590, A591, and Q592 may be replaced with D586, W587, H588, R589, I590, A591, and Q592).

[0111] In some embodiments, [N1]-[N2]-[N3] is present at amino acids corresponding to positions 582-592 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, [N1]-[N2]-[N3] replaces amino acids corresponding to positions 582-592 of the amino acid sequence of

SEQ ID NO: 138 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, Q590, A591, and Q592 may be replaced with T582, N583, T584, Q585, D586, W587, H588, R589, I590, A591, and Q592).

[0112] In some embodiments, [N4] is present at amino acids corresponding to positions 593-596 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, X₈ of [N4] is present at an amino acid corresponding to position 593 of the amino acid sequence of SEQ ID NO: 138 or 981, X₉ of [N4] is present at an amino acid corresponding to position 594 of the amino acid sequence of SEQ ID NO: 138 or 981, X₁₀ of [N4] is present at an amino acid corresponding to position 595 of the amino acid sequence of SEQ ID NO: 138 or 981, and X₁₁ of [N4] is present at an amino acid corresponding to position 596 of the amino acid sequence of SEQ ID NO: 138 or 981. In some embodiments, [N4] replaces amino acids corresponding to positions 593-596 (e.g., T593, G594, W595, and V596) of the amino acid sequence of SEQ ID NO: 138.

[0113] In some embodiments, [N2]-[N3]-[N4] is present at amino acids corresponding to positions 586-596 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, [N2]-[N3]-[N4] replaces amino acids corresponding to positions 586-596 of the amino acid sequence of SEQ ID NO: 138 (e.g., S586, A587, Q588, A589, Q590, A591, Q592, T593, G594, W595, and V596 may be replaced with D586, W587, H588, R589, I590, A591, Q592, T593, G594, W595, and V596).

[0114] In some embodiments, [N1]-[N2]-[N3]-[N4] is present at amino acids corresponding to positions 582-596 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, [N1]-[N2]-[N3]-[N4] replaces amino acids corresponding to positions 582-596 of the amino acid sequence of SEQ ID NO: 138 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, Q590, A591, Q592, T593, G594, W595, and V596 may be replaced with T582, N583, T584, Q585, D586, W587, H588, R589, I590, A591, Q592, T593, G594, W595, and V596).

[0115] In some embodiments, [N2] is present immediately subsequent to [N1]. In some embodiments, [N3] is present immediately subsequent to [N2]. In some embodiments, [N4] is present immediately subsequent to [N3]. In some embodiments, loop VIII of the AAV capsid variant comprises an amino acid sequence comprising, from N-terminus to C-terminus, [N1]-[N2]-[N3]. In some embodiments, loop VIII of the AAV capsid variant comprises an amino acid sequence comprising, from N-terminus to C-terminus, [N1]-[N2]-[N3]-[N4].

[0116] In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence comprising at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, or at least 14 consecutive amino acids from any one of the sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence comprising at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, or at least 14 consecutive amino acids from any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336.

[0117] In some embodiments, the at least 3 consecutive amino acids comprise TQD. In some embodiments, the at least 4 consecutive amino acids comprise TQDW (SEQ ID NO: 4684). In some embodiments, the at least 5 consecutive amino acids comprise TQDWH (SEQ ID NO: 4685). In some embodiments, the at least 6 consecutive amino acids comprise TQDWHR (SEQ ID NO: 4686). In some embodiments, the at least 7 consecutive amino acids comprise TQDWHRI (SEQ ID NO: 941).

[0118] In some embodiments, the at least 3 consecutive amino acids comprise TNT. In some embodiments, the at least 4 consecutive amino acids comprise TNTQ (SEQ ID NO: 4688). In some embodiments, the at least 5 consecutive amino acids comprise TNTQD (SEQ ID NO: 5119). In some embodiments, the at least 6 consecutive amino acids comprise TNTQDW (SEQ ID NO: 5120). In some embodiments, the at least 7 consecutive amino acids comprise TNTQDWH (SEQ ID NO: 5121). In some embodiments, the at least 8 consecutive amino acids comprise TNTQDWHR (SEQ ID NO: 4898). In some embodiments, the at least 9 consecutive amino acids comprise TNTQDWHRI (SEQ ID NO: 746).

[0119] In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence comprising at least one, at least two, or at least three modifications, but no more than four modifications, relative to the amino acid sequence of any one of the sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence comprising at least one, at least two, or at least three substitutions, but no more than four substitutions, relative to the amino acid sequence of any one of the sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence comprising at least one, at least two, or at least three modifications, but no more than four modifications, relative to the amino acid sequence of any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence comprising at least one, at least two, or at least three substitutions, but no more than four substitutions, relative to the amino acid sequence of any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219, 220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336.

[0120] In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence comprising at least one, at least two, or at least three modifications, but no more than four modifications, relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941). In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence comprising at least one, at least two, or at least three substitutions, but no more than four substitutions, relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941).

[0121] In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of the sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 201, 205-209, 211-214, 216, 219,

220, 230, 232, 237, 238, 255, 262-265, 274, 283, 286, 290, 291, 293, 301, 306, 307, 308, 309, 314, and 336.

[0122] In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, loop VIII comprises amino acids corresponding to positions 581-593 of the amino acid sequence of SEQ ID NO: 138. In some embodiments, loop VIII comprises amino acids corresponding to positions 580-599 of the amino acid sequence of SEQ ID NO: 138. In some embodiments, loop VIII is present (is located) at amino acids comprising those corresponding to positions 580-599 (e.g., the positions of amino acids VATNHQSAQAQAQTGWVQNNQ (SEQ ID NO: 5122)) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all of amino acids corresponding to position(s) 582 (e.g., T582), 583 (e.g., N583), 584 (e.g., H584), 585 (e.g., Q585), 586 (e.g., S586), 587 (e.g., A587), 588 (e.g., Q588), 589 (e.g., A589), 590 (e.g., Q590), 591 (e.g., A591), 592 (e.g., Q592), 593 (e.g., T593), 594 (e.g., G594), 595 (e.g., W595), and/or 596 (e.g., V596) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence is present at 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all of amino acids corresponding to position(s) 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and/or 596 of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the AAV capsid variant comprises one or more substitutions at amino acids corresponding to position(s) 582 (e.g., T582), 583 (e.g., N583), 584 (e.g., H584), 585 (e.g., Q585), 586 (e.g., S586), 587 (e.g., A587), 588 (e.g., Q588), 589 (e.g., A589), 590 (e.g., Q590), 591 (e.g., A591), 592 (e.g., Q592), 593 (e.g., T593), 594 (e.g., G594), 595 (e.g., W595), and/or 596 (e.g., V596) of the amino acid sequence of SEQ ID NO: 138.

[0123] In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence encoded by a nucleotide sequence described herein, e.g., a nucleotide sequence of Table 2A. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence encoded by a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications, but no more than ten modifications, relative to the nucleotide sequence of SEQ ID NO: 942. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence encoded by a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven different substitutions, but not more than ten substitutions, relative to the nucleotide sequence of SEQ ID NO: 942. In some embodiments, the AAV capsid variant comprises (e.g., in loop VIII) an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence substantially identical (e.g., having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity) thereto.

[0124] In some embodiments, the nucleotide sequence encoding an AAV capsid variant described herein, or a portion thereof that is modified relative to a wildtype AAV capsid (e.g., comprising one or more substitutions relative to a wildtype AAV capsid), comprises a nucleotide sequence described herein, e.g., as described in Table 2A. In some embodiments, the nucleotide sequence encoding an AAV capsid

variant described herein, or a portion thereof that is modified relative to a wildtype AAV capsid (e.g., comprising one or more substitutions relative to a wildtype AAV capsid), is codon optimized. In some embodiments, the nucleotide sequence is an isolated nucleotide sequence. In some embodiments, the nucleotide sequence is a recombinant nucleotide sequence.

[0125] In some embodiments, the nucleotide sequence encoding an AAV capsid variant described herein, or a portion thereof that is modified relative to a wildtype AAV capsid (e.g., comprising one or more substitutions relative to a wildtype AAV capsid), comprises the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications, but no more than ten modifications, relative to the nucleotide sequence of SEQ ID NO: 942. In some embodiments, the nucleotide sequence comprises a nucleotide sequence comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven substitutions, but no more than ten substitutions, relative to the nucleotide sequence of SEQ ID NO: 942. In some embodiments, the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence substantially identical (e.g., having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity) thereto.

[0126] In some embodiments, the AAV capsid variant further comprises an amino acid other than A at a position corresponding to position 581 of the amino acid sequence of SEQ ID NO: 138 or 981. In some embodiments, the AAV capsid variant further comprises the amino acid T at a position corresponding to position 581 of the amino acid sequence of SEQ ID NO: 138 or 981. In some embodiments, the AAV capsid variant further comprises the amino acid V at a position corresponding to position 581 of the amino acid sequence of SEQ ID NO: 138 or 981. In some embodiments, the AAV capsid variant comprises the substitution A581T or A581V, wherein the substitution is at a position that corresponds to position 581 of the amino acid sequence of SEQ ID NO: 138 or 981.

[0127] In some embodiments, an AAV capsid variant described herein comprises one, two, three, four, five, or all of an amino acid other than H at a position corresponding to position 584 (e.g., comprises T at an amino acid corresponding to position 584) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than S at a position corresponding to position 586 (e.g., comprises D at an amino acid corresponding to position 586) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than A at a position corresponding to position 587 (e.g., comprises W at an amino acid corresponding to position 587) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than Q at a position corresponding to position 588 (e.g., comprises H at an amino acid corresponding to position 588) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than A at a position corresponding to position 589 (e.g., comprises R at an amino acid corresponding to position 589) of the amino acid sequence of SEQ ID NO: 138; and/or an amino acid other than Q at a position corresponding to position 590 (e.g., comprises I at an amino acid corresponding to position 590) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises an amino acid

other than H at a position corresponding to position 584 (e.g., comprises T at an amino acid corresponding to position 584) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than S at a position corresponding to position 586 (e.g., comprises D at an amino acid corresponding to position 586) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than A at a position corresponding to position 587 (e.g., comprises W at an amino acid corresponding to position 587) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than Q at a position corresponding to position 588 (e.g., comprises H at an amino acid corresponding to position 588) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than A at a position corresponding to position 589 (e.g., comprises R at an amino acid corresponding to position 589) of the amino acid sequence of SEQ ID NO: 138; and an amino acid other than Q at a position corresponding to position 590 (e.g., comprises I at an amino acid corresponding to position 590) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein comprises the amino acid T at a position corresponding to position 584 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid D at a position corresponding to position 586 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid W at a position corresponding to position 587 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid H at a position corresponding to position 588 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid R at a position corresponding to position 589 of the amino acid sequence of SEQ ID NO: 138 or 981, and amino acid I at a position corresponding to position 590 of the amino acid sequence of SEQ ID NO: 138 or 981.

[0128] In some embodiments, an AAV capsid variant described herein comprises one, two, three, four, five, or all of the amino acid T at a position corresponding to position 584 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid D at a position corresponding to position 586 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid W at a position corresponding to position 587 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid H at a position corresponding to position 588 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid R at a position corresponding to position 589 of the amino acid sequence of SEQ ID NO: 138 or 981, and/or amino acid I at a position corresponding to position 590 of the amino acid sequence of SEQ ID NO: 138 or 981. In some embodiments, the AAV capsid variant comprises the amino acid T at a position corresponding to position 584 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid D at a position corresponding to position 586 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid W at a position corresponding to position 587 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid H at a position corresponding to position 588 of the amino acid sequence of SEQ ID NO: 138 or 981, amino acid R at a position corresponding to position 589 of the amino acid sequence of SEQ ID NO: 138 or 981, and amino acid I at a position corresponding to position 590 of the amino acid sequence of SEQ ID NO: 138 or 981.

[0129] In some embodiments, an AAV capsid variant described herein comprises one, two, three, four, five, or all of the substitutions H584T, S586D, A587W, Q588H, A589R, and/or Q590I, wherein the

substitution(s) is/are at position(s) that correspond to position(s) 584, 586, 587, 588, 589, and/or 590 of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises the substitutions H584T, S586D, A587W, Q588H, A589R, and Q590I, wherein the substitution(s) is/are at position(s) that correspond to position(s) 584, 586, 587, 588, 589, and/or 590 of the amino acid sequence of SEQ ID NO: 138 or 981.

[0130] In some embodiments, an AAV capsid variant described herein comprises the amino acid Q at an amino acid corresponding to position 585 of the amino acid sequence of SEQ ID NO: 138 or 981.

[0131] In some embodiments, an AAV capsid variant described herein comprises an amino acid other than Q at an amino acid corresponding to position 585 of the amino acid sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein comprises the amino acid K at an amino acid corresponding to position 585 of the amino acid sequence of SEQ ID NO: 138.

[0132] In some embodiments, an AAV capsid variant described herein comprises an amino acid other than Q at an amino acid corresponding to position 590 of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises the amino acid I at an amino acid corresponding to position 590 of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises the amino acid V at an amino acid corresponding to position 590 of the amino acid sequence of SEQ ID NO: 138.

[0133] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein TQDWHRI (SEQ ID NO: 941) is present in the AAV capsid variant at amino acids corresponding to positions 584-590 of the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981. In some embodiments, TQDWHRI (SEQ ID NO: 941) is present at amino acids corresponding to positions 584-590 of the amino acid sequence of SEQ ID NO: 138 (e.g., H584, S586, A587, Q588, A589, and Q590 may be replaced with T584, D586, W587, H588, R589, and I590).

[0134] In some embodiments, an AAV capsid variant described herein comprises the amino acid W at an amino acid corresponding to position 595 of the amino acid sequence of SEQ ID NO: 138 or 981.

[0135] In some embodiments, the AAV capsid variant further comprises a substitution corresponding to amino acid K449 (e.g., K449R) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an amino acid other than K at a position corresponding to position 449 (e.g., comprises an R at a position corresponding to position 449) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises an R at amino acid corresponding to position 449 of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a modification in loop I, II, IV, and/or VI.

[0136] In some embodiments, the AAV capsid variant further comprises an amino acid sequence comprising at least one, at least two, or at least three modifications, but not more than 30, not more than 20, or not more than 10 modifications, relative to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence does not comprise more than 10 modifications. In some

embodiments, the AAV capsid variant further comprises an amino acid sequence comprising at least one, at least two, or at least three, but not more than 30, not more than 20, or not more than 10 amino acids that differ from the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises the amino acid sequence of SEQ ID NO: 138, or an amino acid sequence with at least 70% (e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto.

[0137] In some embodiments, the AAV capsid variant comprises (a) a VP1 protein comprising the amino acid sequence of SEQ ID NO: 981 (i.e., comprising amino acids 1-736 of SEQ ID NO: 981); (b) a VP2 protein comprising amino acids 138-736 of SEQ ID NO: 981; (c) a VP3 protein comprising amino acids 203-736 of SEQ ID NO: 981; or (d) an amino acid sequence with at least 70% (e.g., at least 70%, at least 75%, at least 80%, at least 85%, 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 the amino acid sequences in (a)-(c). an amino acid sequence comprising at least one, at least two, or at least three substitutions, but not more than 30, not more than 20, or not more than 10 substitutions relative to any one of the amino acid sequences in (a)-(c), or an amino acid sequence comprising at least one, at least two, or at least three modifications, but not more than 30, not more than 20, or not more than 10 such modifications relative to any one of the amino acid sequences in (a)-(c).

[0138] In some embodiments, the AAV capsid variant comprises (a) a VP1 protein comprising an amino acid sequence that is at least 90% (e.g., at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence of SEQ ID NO: 981; (b) a VP2 protein comprising an amino acid sequence that is at least 90% (e.g., at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 138-736 of SEQ ID NO: 981; and/or (c) a VP3 protein comprising an amino acid sequence that is at least 90% (e.g., at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 203-736 of SEQ ID NO: 981, wherein the AAV capsid variant comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941) or a sequence comprising one, two, or three but not more than three substitutions relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941). In some embodiments, the AAV capsid variant comprises the amino acid sequence comprising one, two, or three but not more than three substitutions relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941) in loop VIII. In some embodiments, the AAV capsid variant comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941) in loop VIII.

[0139] In some embodiments, the AAV capsid variant comprises an amino acid sequence as described herein, e.g., an amino acid sequence of TTJ-001, e.g., as described in Tables 3 and 4A. In some embodiments, the AAV capsid variant is or comprises TTJ-001. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 941 in a loop VIII region.

[0140] In some embodiments, the AAV capsid variant comprises a VP1, VP2, and/or VP3 protein comprising an amino acid sequence described herein, e.g., an amino acid sequence of TTJ-001, e.g., as described in Tables 3 and 4A.

[0141] In some embodiments, the AAV capsid variant comprises an amino acid sequence encoded by a nucleotide sequence as described herein, e.g., a nucleotide sequence encoding TTJ-001, e.g., as described in Tables 3 and 4B.

[0142] In some embodiments, the polynucleotide or nucleic acid encoding the AAV capsid variant comprises a nucleotide sequence described herein, e.g., a nucleotide sequence encoding TTJ-001, e.g., as described in Tables 3 and 4B.

Table 3. Exemplary full length capsid sequences

Name	VP1 DNA SEQ ID NO:	VP1 (amino acid) SEQ ID NO:	Peptide (amino acid) SEQ ID NO:	Peptide DNA SEQ ID NO:
TTJ-001	983	981	941	942

Table 4A. Exemplary full length capsid amino acid sequences

Name and Annotation	SEQ ID NO:	Amino Acid Sequence
TTJ-001 (modifications at amino acids 584, 586, 587, 588, 589, and 590, as numbered relative to SEQ ID NO: 138 underlined)	981	MAADGYLPDWLEDNLSEGITREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLPGP NGLDKGEFVNAADAAALEHDKAYDQQLKAGDNPYLYKNHADAEEFQERLKEEDTSFGG NLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQS PQEPDSSAGIGKSGAQPAK KRLNFGQTDGTEVPDPQPIGEPPAAPSGVGSMTMASGGGAPVADNNEGADGVGSS SGNWHCDSQWLGDVRVITTTSTRTWALPTYNNHLYKQISNSTSGSSNDNAYFGYSTP WGYFDNRFHCHFS PRDWQRLINNNWGFRPKRLNFKLFNIQVKEVDNNGVKTIAN NLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTINDGSQAVGRS SFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPIDQYLYLS KTINGSGQNQQTLLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWP GASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVM TNEEEIKTNPVATESYGVATN <u>TDWHRIA</u> QTGWVQNQILPGMVWQDRDVLQ PIWAKI PHTDGNFHPS PLMGFGMKHPPQILIKNTFPVPADPTAFNKDKLSFIT QYSTGQVSVEIEWELQKENS KRWNPETQYTSNYYKSNNVEFAVNTGQVYSEPRPIG TRYLTRNL

Table 4B. Exemplary full length capsid nucleic acid sequences

Name and Annotation	SEQ ID NO:	Nucleotide Sequence
TTJ-001 (portion of sequence encoding the amino acid sequence of SEQ ID NO: 744 is underlined)	983	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTTAGTGAAGGAAT TCGCGAGTGGTGGGCTTTGAAACCTGGAGCCCCCTCAACCAAGGCAAATCAACAAC ATCAAGACAACGCTCGAGGTCTTGTCCTCCGGGTACAAATACCTTGGACCCGGC AACGGACTCGACAAGGGGGAGCCGGTCAACGCAGCAGACGCGGCGCCCTCGAGCA CGACAAGGCCTACGACCAGCAGCTCAAGGCCGGAGACAACCCGTACCTCAAGTACA ACCACGCCGACGCCGAGTTCAGGAGCGGCTCAAAGAAGATACGTCTTTTGGGGGC AACCTCGGGCGAGCAGTCTCCAGGCCAAAAAGAGGCTTCTTGAACTCTTGGTCT GGTTGAGGAAGCGGCTAAGACGGCTCCTGGAAGAAGAGGCCTGTAGAGCAGTCTC CTCAGGAACCGGACTCCTCCGCGGGTATTGGCAAATCGGGTGCACAGCCCGCTAAA AAGAGACTCAATTCGGTCAGACTGGCGACACAGAGTCAGTCCCAGACCTCAACC AATCGGAGAACCTCCCGCAGCCCCCTCAGGTGTGGGATCTCTTACAATGGCTTCAG GTGGTGGCGCACCAAGTGGCAGACAATAACGAAGGTGCCGATGGAGTGGGTAGTTCC TCGGGAAATTGGCATTGCGATTCCCAATGGCTGGGGGACAGAGTCATCACCACCA CACCCGAACCTGGGCCCTGCCACCTACAACAATCACCCTACAAGCAATCTCCA ACAGCACATCTGGAGGATCTTCAATGACAACGCCTACTTCGGCTACAGCACCCCC

	TGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCA GCGACTCATCAACAACAACACTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCT TCAACATTCAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCATCGCCAAT AACCTTACCAGCACGGTCCAGGTCTTCACGGACTCAGACTATCAGCTCCCGTACGT GCTCGGGTCGGCTCACGAGGGCTGCCTCCCGCGTTCCAGCGGACGTTTTCATGA TTCCTCAGTACGGGTATCTGACGCTTAATGATGGAAGCCAGGCCGTGGGTCGTTCCG TCCTTTTACTGCCTGGAATATTTCCCGTCGCAAATGCTAAGAAGGGTAACAACCTT CCAGTTTACGCTACGAGTTTGAGAACGTACCTTTCCATAGCAGCTACGCTCACAGCC AAAGCCTGGACCGACTAATGAATCCACTCATCGCCAATACTTGACTATCTCTCA AAGACTATTAACGGTTCTGGACAGAATCAACAACGCTAAAATTCAGTGTGGCCGG ACCCAGCAACATGGCTGTCCAGGGAAGAACTACATACCTGGACCCAGCTACCGAC AACAACGTGCTCAACCACTGTGACTCAAAACACAACAGCGAATTTGCTTGGCCT GGAGCTTCTTCTTGGGCTCTCAATGGACGTAATAGCTTGATGAATCCTGGACCTGC TATGGCCAGCCACAAAGAAGGAGAGGACGTTTCTTTCTTTGCTGGATCTTTAA TTTTTGGCAACAAGGAAGTGGAGAGACAACGTGGATCGCGACAAGTCATGATA ACCAACGAAGAAGAAATTAATACTACTACCCGGTAGCAACGGAGTCCATGGACA GGTC<u>GCTACAAACACCCAGGACTGGCACAGGATTGCGCAGACC</u>GGCTGGGTTCAAA ACCAAGGAATACTTCCGGGTATGGTTTGGCAGGACAGAGATTTGTACCTGCAAGGA CCCATTGGGCCAAAATTCCTCACACGGACGGCAACTTTCACCCCTCTCCGCTGAT GGGAGGGTTTGGAAATGAAGCACCCGCCTCTCAGATCCTCATCAAAAACACACCTG TACCTGCCGATCCTCCAACGGCCTTCAACAAGGACAAGCTGAACCTCTTTCATCACC CAGTATTCTACTGGCCAAAGTCAGCGTGGAGATCGAGTGGGAGCTGCAGAAGGAAAA CAGCAAGCGGTGGAACCCGGAGATCCAGTACACTTCCAATATTACAAGTCTAATA ATGTTGAATTTGCTGTTAATACTGAAGGTGTATATAGTGAACCCGCCCATTTGGC ACCAGATACCTGACTCGTAATCTGTAA
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[0143] In some embodiments, the polynucleotide encoding an AAV capsid variant comprises the nucleotide sequence of SEQ ID NO: 983, or a nucleotide sequence with at least 70% (e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto.

[0144] In some embodiments, the nucleotide sequence encoding an AAV capsid variant described herein, comprises a nucleotide sequence comprising at least one, at least two, or at least three modifications but not more than 30, not more than 20, or not more than 10 modifications, relative to the nucleotide sequence of SEQ ID NO: 983. In some embodiments, the nucleotide sequence encoding an AAV capsid variant described herein, comprises a nucleotide sequence comprising at least one, at least two, or at least three substitutions, but not more than 30, not more than 20, or not more than 10 substitutions relative to the amino acid sequence of SEQ ID NO: 983. In some embodiments, the nucleic acid sequence encoding an AAV capsid variant described herein is codon optimized.

[0145] In some embodiments, the AAV capsid variant comprises one or more substitutions in loop VIII and comprises the amino acid sequence of SEQ ID NO: 981, or an amino acid sequence with at least 70% (e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto. In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence comprising at least one, at least two, or at least three modifications, but not more than 30, not more than 20, or not more than 10 modifications, relative to the amino acid sequence of SEQ ID NO: 981. In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence comprising at least one, at least two,

or at least three substitutions, but not more than 30, not more than 20, or not more than 10 substitutions, relative to the amino acid sequence of SEQ ID NO: 981.

[0146] In some embodiments, the AAV capsid variant comprises one or more substitutions in loop VIII and comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 983, or a nucleotide sequence with at least 70% (e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto. In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence encoded by a nucleotide sequence comprising at least one, at least two, or at least three substitutions, but not more than 30, not more than 20, or not more than 10 substitutions, relative to the amino acid sequence of SEQ ID NO: 983. In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence encoded by a nucleotide sequence comprising at least one, at least two, or at least three modifications, but not more than 30, not more than 20, or not more than 10 modifications, relative to the nucleotide sequence of SEQ ID NO: 983.

[0147] In some embodiments, an AAV capsid variant described herein comprises a VP1, VP2, VP3 protein, wherein at least one of the VP1, VP2, and VP3 comprise one or more substitutions in loop VIII. In some embodiments, the AAV capsid variant comprises amino acids 138-736, e.g., a VP2, of the amino acid sequence of SEQ ID NO: 981, or an amino acid sequence with at least 70% (e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto. In some embodiments, the AAV capsid protein comprises amino acids 203-736, e.g., a VP3, of the amino acid sequence of SEQ ID NO: 981, or an amino acid sequence with at least 70% (e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto. In some embodiments, the AAV capsid variant comprises amino acids 1-736, e.g., a VP1, of the amino acid sequence of SEQ ID NO: 981, or an amino acid sequence with at least 70% (e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity thereto.

[0148] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of amino acids 203-736 of SEQ ID NO: 981, or an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of amino acids 138-736 of SEQ ID NO: 981, or an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 981, or an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

[0149] In some embodiments, an AAV capsid variant described herein has an increased tropism for a CNS cell or tissue, e.g., a brain cell, brain tissue, spinal cord cell, or spinal cord tissue, relative to the tropism of an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138.

[0150] In some embodiments, an AAV capsid variant described herein transduces a brain region, e.g., a midbrain region (e.g., the hippocampus, or thalamus) or the brain stem. In some embodiments, the level of transduction is at least 39, at least 50, at least 100, at least 120, at least 132, at least 146, at least 150, at least 161, at least 174, at least 175, at least 200, at least 225, at least 250, at least 275, at least 283, at least 300, at least 350, at least 400, at least 450, at least 500, at least 525, at least 528, or at least 550-fold greater as compared to an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138.

[0151] In some embodiments, an AAV capsid variant described herein is enriched at least 10, at least 14, at least 20, at least 24, at least 50, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 425, at least 450, or at least 460-fold in the brain compared to enrichment in the brain of an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein is enriched at least 200, at least 250, at least 300, at least 350, at least 400, at least 425, at least 450, or at least 460-fold in the brain compared to enrichment in the brain of an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138.

[0152] In some embodiments, an AAV capsid variant described herein is enriched in the brain of at least two to three species, e.g., a non-human primate and rodent (e.g., mouse) species, compared to enrichment in the brain of an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein is enriched at least 2, at least 3, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 105, at least 115, at least 120, at least 125, at least 130, at least 135, at least 140, at least 145, at least 150, at least 155, at least 160, at least 165, at least 170, at least 175, at least 180, at least 190, at least 200, at least 205, or at least 210-fold in the brain of at least two to three species, e.g., a non-human primate and rodent (e.g., mouse) species, compared to enrichment in the brain of an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138. In some embodiments, the at least two to three species are *Macaca fascicularis*, *Chlorocebus sabaeus*, *Callithrix jacchus*, and/or mouse (e.g., outbred mice).

[0153] In some embodiments, an AAV capsid variant described herein is enriched at least 2, at least 3, at least 4, at least 5, at least 10, at least 15, at least 17, at least 20, at least 50, at least 75, at least 100, at least 103, at least 107, at least 125, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 750, at least 1000, or at least 1200-fold in the brain compared to enrichment in the brain of an AAV capsid comprising the amino acid sequence of SEQ ID NO: 981.

[0154] In some embodiments, an AAV capsid variant described herein delivers an increased level of viral genomes to a brain region. In some embodiments, the level of viral genomes is increased by at least 2, at least 5, at least 7, at least 10, at least 15, at least 19, at least 20, at least 22, or at least 25-fold, as compared to the level of viral genomes delivered by an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138. In some embodiments, the brain region comprises a sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, and/or cerebellum.

[0155] In some embodiments, an AAV capsid variant delivers an increased level of a payload to a brain region. In some embodiments, the level of the payload is increased by at least 39, at least 50, at least 100, at least 120, at least 132, at least 146, at least 150, at least 161, at least 174, at least 175, at least 200, at least 225, at least 250, at least 275, at least 283, at least 300, at least 350, at least 400, at least 450, at least 500, at least 525, at least 528, or at least 550-fold, as compared to the level of a payload delivered by an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138. In some embodiments, the brain region comprises a sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, and/or cerebellum.

[0156] In some embodiments, an AAV capsid variant described herein is enriched at least 5, at least 10, at least 50, at least 100, at least 115, at least 120, at least 150, at least 175, at least 200, at least 207, at least 225, at least 250, or at least 275-fold in the spinal cord compared to enrichment in the spinal cord of an AAV capsid comprising the amino acid sequence of SEQ ID NO: 138.

[0157] In some embodiments, an AAV capsid variant of the present disclosure has decreased tropism for the liver. In some embodiments, an AAV capsid variant comprises a modification that results in reduced tropism (e.g., de-targeting) and/or activity in the liver. In some embodiments, the reduced tropism in the liver is compared to an otherwise similar capsid that does not comprise the modification, e.g., a wild-type capsid polypeptide. In some embodiments, an AAV capsid variant comprises a modification that results in one or more of the following properties: (1) reduced tropism in the liver; (2) de-targeted expression in the liver; (3) reduced activity in the liver; and/or (4) reduced binding to galactose. In some embodiments, the reduction in any one or all of properties (1)-(3) is compared to an otherwise similar AAV capsid variant that does not comprise the modification. Exemplary modifications are provided in WO 2018/119330; Pulicherla et al. (2011) *Mol. Ther.* 19(6): 1070-1078; Adachi et al. (2014) *Nature Communications* 5(3075), DOI: 10.1038/ncomms4075; and Bell et al. (2012) *J. Virol.* 86(13): 7326-33; the contents of which are hereby incorporated by reference in their entirety.

[0158] In some embodiments, the AAV capsid variant comprises a modification at an amino acid corresponding to N470 (e.g., comprises N470A at an amino acid corresponding to N470) of the amino acid sequence of SEQ ID NO: 138; an amino acid corresponding to D271 (e.g., comprises D271A at an amino acid corresponding to D271) of the amino acid sequence of SEQ ID NO: 138; an amino acid corresponding to N272 (e.g., comprises N272A at an amino acid corresponding to N272) of the amino acid sequence of SEQ ID NO: 138; an amino acid corresponding to Y446 (e.g., comprises Y446A at an amino acid corresponding to Y446) of the amino acid sequence of SEQ ID NO: 138; an amino acid corresponding to N498 (e.g., comprises N498Y or N498I at an amino acid corresponding to N498) of the amino acid sequence of SEQ ID NO: 138; an amino acid corresponding to W503 (e.g., comprises W503R or W503A at an amino acid corresponding to W503) of the amino acid sequence of SEQ ID NO: 138; an amino acid corresponding to L620 (e.g., comprises L620F at an amino acid corresponding to L620) of the amino acid sequence of SEQ ID NO: 138; or a combination thereof. In some embodiments, the AAV capsid variant comprises one, two, three, four, five, or all of an amino acid other than N at a

position corresponding to position 470 (e.g., comprises A at an amino acid corresponding to position 470) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than D at a position corresponding to position 271 (e.g., comprises A at an amino acid corresponding to position 271) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than N at a position corresponding to position 272 (e.g., comprises A at an amino acid corresponding to position 272) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than Y at a position corresponding to position 446 (e.g., comprises A at an amino acid corresponding to position 446) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than N at a position corresponding to position 498 (e.g., comprises Y or I at an amino acid corresponding to position 498) of the amino acid sequence of SEQ ID NO: 138; an amino acid other than W at a position corresponding to position 503 (e.g., comprises R or A at an amino acid corresponding to position 503) of the amino acid sequence of SEQ ID NO: 138; and an amino acid other than L at a position corresponding to position 620 (e.g., comprises F at an amino acid corresponding to position 620) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises a modification at positions corresponding to amino acid N470 (e.g., comprises N470A at an amino acid corresponding to N470) of the amino acid sequence of SEQ ID NO: 138; corresponding to amino acid D271 (e.g., comprises D271A at an amino acid corresponding to D271) of the amino acid sequence of SEQ ID NO: 138; corresponding to amino acid N272 (e.g., comprises N272A at an amino acid corresponding to N272) of the amino acid sequence of SEQ ID NO: 138; corresponding to amino acid Y446 (e.g., comprises Y446A at an amino acid corresponding to Y446) of the amino acid sequence of SEQ ID NO: 138; and corresponding to amino acid W503 (e.g., comprises W503R or W503A at an amino acid corresponding to W503) of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises a modification corresponding to amino acid N498 (e.g., comprises N498Y at an amino acid corresponding to N498) of the amino acid sequence of SEQ ID NO: 138 and corresponding to amino acid L620 (e.g., comprises L620F at an amino acid corresponding to L620) of the amino acid sequence of SEQ ID NO: 138.

[0159] In some embodiments, the AAV capsid variant comprises a modification as described in Adachi et al. (2014) *Nature Communications* 5(3075), DOI: 10.1038/ncomms4075, the contents of which are hereby incorporated by reference in their entirety. Exemplary modifications that alter or do not alter tissue transduction in at least the brain, liver, heart, lung, and/or kidney can be found in Supplementary Data 2 showing the AAV Barcode-Seq data obtained with AAV9-AA-VBCLib of Adachi et al. (*supra*), the contents of which are hereby incorporated by reference in their entirety.

[0160] In some embodiments, the AAV capsid variant is an isolated capsid variant. In some embodiments, the AAV capsid variant is a recombinant capsid variant. In some embodiments, a polynucleotide encoding an AAV capsid polypeptide, e.g., an AAV capsid variant is an isolated and/or a recombinant AAV capsid polypeptide.

[0161] Also provided herein are polynucleotide sequences encoding any of the AAV capsid variants described above and AAV particles, vectors, and cells comprising the same.

Exemplary Properties of AAV Capsids

[0162] In some embodiments, the AAV particle may comprise a capsid protein or variant of any natural or recombinant AAV serotype. AAV serotypes may differ in characteristics such as, but not limited to, packaging, tropism, transduction and immunogenic profiles. Without being bound by theory, it is believed in some embodiments that the AAV capsid protein, e.g., an AAV capsid variant, can modulate AAV particle tropism in a particular tissue.

[0163] In some embodiments, the AAV capsid variant allows for blood brain barrier penetration following intravenous administration. In some embodiments, the AAV capsid variant allows for blood brain barrier penetration following intravenous administration, focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration. In some embodiments, the AAV capsid variant allows for increased distribution to a CNS region, e.g., a brain region. In some embodiments, the CNS region comprises the amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral pallidum, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region). In some embodiments, the AAV capsid variant allows for preferential transduction in a brain region relative to the transduction in the dorsal root ganglia (DRG). In some embodiments, the AAV capsid variant allows for preferential transduction in a brain region relative to the transduction in the liver. In some embodiments, the AAV capsid variant allows for transduction in neuronal cells. In some embodiments, the AAV capsid variant allows for transduction in a non-neuronal cell, e.g., a glial cell (e.g., an astrocyte, an oligodendrocyte, or a combination thereof). In some embodiments, the AAV capsid variant allows for transduction in both neuronal cells and non-neuronal cell, e.g., a glial cell (e.g., an astrocyte, an oligodendrocyte, or a combination thereof).

[0164] In some embodiments, an AAV capsid variant allows for increased distribution to a spinal cord region. In some embodiments, the spinal region comprises a cervical spinal cord region, thoracic spinal cord region, and/or lumbar spinal cord region.

[0165] In some embodiments, the AAV capsid variant allows for increased distribution to a heart region.

[0166] In some embodiments, the AAV capsid variant is suitable for intramuscular administration and/or transduction of muscle fibers. In some embodiments, the AAV capsid variant allows for increased distribution to a muscle region. In some embodiments, the muscle region comprises a heart muscle, quadriceps muscle, skeletal muscle, a diaphragm muscle region, or a combination thereof. In some embodiments, the muscle region comprises a heart muscle region, e.g., a heart atrium muscle region or a heart ventricle muscle region.

[0167] In some embodiments, the AAV capsid variant is suitable for increased distribution to a kidney. In some embodiments, the AAV capsid variant is suitable for increased distribution to a pancreas.

[0168] In some embodiments, the initiation codon for translation of the AAV VP1 capsid protein, e.g., a capsid variant, described herein may be CTG, TTG, or GTG as described in US Patent No. US8163543, the contents of which are herein incorporated by reference in its entirety.

[0169] The present disclosure refers to structural capsid proteins (including VP1, VP2 and VP3) which are encoded by capsid (Cap) genes. These capsid proteins form an outer protein structural shell (e.g., capsid) of a viral vector such as AAV. VP capsid proteins synthesized from Cap polynucleotides generally include a methionine as the first amino acid in the peptide sequence (Met1), which is associated with the start codon (AUG or ATG) in the corresponding Cap nucleotide sequence. However, it is common for a first-methionine (Met1) residue or generally any first amino acid (AA1) to be cleaved off after or during polypeptide synthesis by protein processing enzymes such as Met-aminopeptidases. This "Met/AA-clipping" process often correlates with a corresponding acetylation of the second amino acid in the polypeptide sequence (e.g., alanine, valine, serine, threonine, etc.). Met-clipping commonly occurs with VP1 and VP3 capsid proteins but can also occur with VP2 capsid proteins.

[0170] Where the Met/AA-clipping is incomplete, a mixture of one or more (one, two or three) VP capsid proteins comprising the viral capsid may be produced, some of which may include a Met1/AA1 amino acid (Met+/AA+) and some of which may lack a Met1/AA1 amino acid as a result of Met/AA-clipping (Met-/AA-). For further discussion regarding Met/AA-clipping in capsid proteins, see Jin, et al. Direct Liquid Chromatography/Mass Spectrometry Analysis for Complete Characterization of Recombinant Adeno-Associated Virus Capsid Proteins. *Hum Gene Ther Methods*. 2017 Oct. 28(5):255-267; Hwang, et al. N-Terminal Acetylation of Cellular Proteins Creates Specific Degradation Signals. *Science*. 2010 February 19. 327(5968): 973-977; the contents of which are each incorporated herein by reference in its entirety.

[0171] According to the present disclosure, references to capsid proteins, e.g., AAV capsid variants, is not limited to either clipped (Met-/AA-) or unclipped (Met+/AA+) and may, in context, refer to independent capsid proteins, viral capsids comprised of a mixture of capsid proteins, and/or polynucleotide sequences (or fragments thereof) which encode, describe, produce or result in capsid proteins of the present disclosure. A direct reference to a capsid protein or capsid polypeptide (such as VP1, VP2 or VP3) may also comprise VP capsid proteins which include a Met1/AA1 amino acid (Met+/AA+) as well as corresponding VP capsid proteins which lack the Met1/AA1 amino acid as a result of Met/AA-clipping (Met-/AA-).

[0172] Further according to the present disclosure, a reference to a specific SEQ ID NO (whether a protein or nucleic acid) which comprises or encodes, respectively, one or more capsid proteins which include a Met1/AA1 amino acid (Met+/AA+) should be understood to teach the VP capsid proteins which lack the Met1/AA1 amino acid as upon review of the sequence, it is readily apparent any sequence which merely lacks the first listed amino acid (whether or not Met1/AA1).

[0173] As a non-limiting example, reference to a VP1 polypeptide sequence which is 736 amino acids in length and which includes a “Met1” amino acid (Met+) encoded by the AUG/ATG start codon may also be understood to teach a VP1 polypeptide sequence which is 735 amino acids in length and which does not include the “Met1” amino acid (Met-) of the 736 amino acid Met+ sequence. As a second non-limiting example, reference to a VP1 polypeptide sequence which is 736 amino acids in length and which includes an “AA1” amino acid (AA1+) encoded by any NNN initiator codon may also be understood to teach a VP1 polypeptide sequence which is 735 amino acids in length and which does not include the “AA1” amino acid (AA1-) of the 736 amino acid AA1+ sequence.

[0174] References to viral capsids formed from VP capsid proteins (such as reference to specific AAV capsid serotypes), can incorporate VP capsid proteins which include a Met1/AA1 amino acid (Met+/AA1+), corresponding VP capsid proteins which lack the Met1/AA1 amino acid as a result of Met/AA1-clipping (Met-/AA1-), and combinations thereof (Met+/AA1+ and Met-/AA1-).

[0175] As a non-limiting example, an AAV capsid serotype can include VP1 (Met+/AA1+), VP1 (Met-/AA1-), or a combination of VP1 (Met+/AA1+) and VP1 (Met-/AA1-). An AAV capsid serotype can also include VP3 (Met+/AA1+), VP3 (Met-/AA1-), or a combination of VP3 (Met+/AA1+) and VP3 (Met-/AA1-); and can also include similar optional combinations of VP2 (Met+/AA1) and VP2 (Met-/AA1-).

Additional AAV Capsid Sequences

[0176] In some embodiments, the AAV capsid variant comprises at amino acids corresponding to positions 582, 583, 584, 585, 586, 587, 588, 589, and/or 590 of the amino acid sequence of SEQ ID NO: 138, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 consecutive amino acids of any one of the amino acid sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26.

[0177] In some embodiments, the AAV capsid variant comprises, immediately subsequent to an amino acid corresponding to position 582, 583, 584, 585, 586, 587, 588, 589, and/or 590 of the amino acid sequence of SEQ ID NO: 138 (e.g., corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987 (the contents of which are hereby incorporated by reference in their entirety))), at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 consecutive amino acids of any one of the amino acid sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26. In some embodiments, the at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 consecutive amino acids of any one of the amino acid sequences provided in Table 1, 2A, 2B, 9, 24, 25, or 26 replaces at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, or all of amino acid(s) corresponding to position(s) 582, 583, 584, 585, 586, 587, 588, 589, and/or 590 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, and/or Q590) of the amino acid

sequence of SEQ ID NO: 138 (e.g., corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987, which is hereby incorporated by reference in its entirety)). In some embodiments, the AAV capsid variant comprises an amino acid other than the wild-type amino acid, at one, two, three, four, five, six, seven, eight, or all of amino acid(s) corresponding to position(s) 582, 583, 584, 585, 586, 587, 588, 589, and/or 590 of the amino acid sequence of SEQ ID NO: 138 (e.g., corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987, which is hereby incorporated by reference in its entirety)). In some embodiments, the AAV capsid variant comprises a modification replacing one, two, three, four, five, six, seven, eight, or all amino acid(s) corresponding to position(s) 582, 583, 584, 585, 586, 587, 588, 589, and/or 590 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, and/or Q590) of the amino acid sequence of SEQ ID NO: 138 (e.g., corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV6, AAV7, AAV8, AAV9, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987, which is hereby incorporated by reference in its entirety)).

[0178] In some embodiments, the AAV capsid variant may comprise a variant of a VOY101 capsid polypeptide, an AAVPHP.B (PHP.B) capsid polypeptide, an AAVPHP.N (PHP.N) capsid polypeptide, an AAV1 capsid polypeptide, an AAV2 capsid polypeptide, an AAV5 capsid polypeptide, an AAV9 capsid polypeptide, an AAV9 K449R capsid polypeptide, or an AAVrh10 capsid polypeptide. In some embodiments, the AAV capsid polypeptide, e.g., AAV capsid variant, comprises an amino acid sequence of any one of the AAV capsid polypeptides in Table 5, or an amino acid sequence substantially identical (e.g., having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity) thereto. In some embodiments, the nucleotide sequence encoding the AAV capsid polypeptide comprises any one of the nucleotide sequences in Table 5, or a nucleotide sequence substantially identical (e.g., having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity) thereto.

Table 5. AAV Sequences

Serotype	SEQ ID NO:	Sequence
AAV9/hu. 14 K449R	11	MAADGYLPDWLEDNLSEGIREWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPNGGLD KGEFVNAAADAAALEHDKAYDQQLKAGDNPYLKYNHADAQERLKEDTSFGGNLGRAVFQ AKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSGNWHCDSQWLGDRI TTSTRTWALPTYNNHLYKQISNSTSGSSNDNAYFGYSTPWGYFDNRFHCHFSRPRDWR LINNNWGFRRPKRLNFKLENIQVKEVTDNNGVKTIANNLTSTVQVFTDSQYQLPYVLGSAH EGCLPPFPADVFMIPQYGYLTLDGSGAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENV PFHSSYAHSQSLDRMLMNPIDQYLYLSRTINGSGQNQQTLEKFSVAGPSNMAVQGRNYIP

		GPSYRQQRVSTTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGS LIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG ILPGMVWQDRDVYLQGGPIWAKIIPHTDGNFHPSPLMGGFGMKHPPQILIKNTPVPADPPT AFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTGEGV YSEERPIGTRYLTRNL
AAV9/hu. 14 WT (amino acid)	138	MAADGYLPDWLEDNLSEGIREWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPNGGLD KGEFVNAADAAALEHDKAYDQQLKAGDNPLYLKYNHADAEFQERLKEEDTSFGGNLGRAVFQ AKKRLLEPLGLVEEAAKTAPEGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVSSSGNWHCDSQWLGDRTVI TTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWRQ LINNNWGFPRKRLNFKLFNIQVKEVTDNNGVKTIANNLSTVQVFTDSDYQLPYVLGSAH EGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENV PFHSSYAHSQSLDRMLNPLIDQYLYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIP GPSYRQQRVSTTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGS LIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG ILPGMVWQDRDVYLQGGPIWAKIIPHTDGNFHPSPLMGGFGMKHPPQILIKNTPVPADPPT AFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTGEGV YSEERPIGTRYLTRNL
AAV9/hu. 14 WT (DNA)	137	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTTAGTGAAGGAATTCGC GAGTGGTGGGCTTTGAAACCTGGAGCCCCCAACCCAAGGCAAATCAACAACATCAGAC AACGCTCGAGGTCTTGTGCTTCCGGGTACAAATACCTTGGACCCGGCAACGGACTCGAC AAGGGGGAGCCGGTCAACGACGACGACGCGCGCGGCTCGAGCACGACAAGGCTACGAC CAGCAGCTCAAGGCCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCCGAGTTC CAGGAGCGGCTCAAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAG GCCAAAAAGAGGCTTCTTGAACCTCTTGGTCTGGTTGAGGAAGCGGCTAAGACGGCTCCT GGAAGAAGAGGGCTGTAGAGCAGTCTCCTCAGGAACCGGACTCCTCCGGGGTATTGGC AAATCGGGTGCACAGCCCCGCTAAAAAGAGACTCAATTTCCGTGAGACTGGCGACACAGAG TCAGTCCCAGACCTCAACCAATCGGAGAACCTCCCGCAGCCCCCTCAGGTGTGGGATCT CTTACAATGGCTTCAGGTGGTGGCGCACCAGTGGCAGACAATAACGAAGGTGCCGATGGA GTGGGTAGTTCTCGGGAATTTGGCATTGCGATTCCCAATGGCTGGGGGACAGAGTCATC ACCACCAGCACCCGAACCTGGGCCCTGCCACCTACAACAATCACCTCTACAAGCAATC TCCAACAGCACATCTGGAGGATCTTCAATGACAACGCCTACTTCGGCTACAGCACCCCC TGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCGA CTCATCAACAACAACCTGGGGATTCCGGCTAAGCGACTCAACTTCAAGCTCTTCAACATT CAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCATCGCCAAATCAACCTTACCAG ACGGTCCAGGTCTTACGGACTCAGACTATCAGCTCCCGTACGTGCTCGGGTCCGGCTCAC GAGGGCTGCCTCCCGCGTTCACAGCGGACGTTTTTCATGATTCCTCAGTACGGGTATCTG ACGCTTAATGATGGAAGCCAGGCCGTGGGTCGTTCTGTCCTTTTACTGCCTGGAATATTT CCGTCGCAATGCTAAGAACGGGTAAACAACCTCCAGTTCAGCTACGAGTTTGAGAACGTA CCTTTCCATAGCAGCTACGCTCACAGCCAAAGCCTGGACCGACTAATGAATCCACTCATC GACCAATACTTGTACTATCTCTCAAAGACTATTAACGGTTCTGGACAGAATCAACAACG CTAAAAATCAGTGTGGCCGGACCCAGCAACATGGCTGTCCAGGGAAGAACTACATACCT GGACCCAGCTACCGACAACAACGTGTCTCAACCACTGTGACTCAAAACAACAACAGCGAA TTTGCTTGGCCTGGAGCTTCTTCTTGGGCTCTCAATGGACGTAATAGCTTGATGAATCCT GGACCTGTATGGCCAGCCACAAAGAAGGAGAGGACCGTTTCTTCTCTTGTCTGGATCT TTAATTTTTGGCAAACAAGGAACCTGGAAGAGACAACGTGGATGCGGACAAGTCATGATA ACCAACGAAGAAGAAATTAAACTACTAACCCTGGTAGCAACGGAGTCTATGGACAAGTG GCCACAAACCACCAGAGTGCCCAAGCACAGGCGCAGACCGGCTGGGTTCAAACCAAGGA ATACTTCCGGGTATGGTTTGGCAGGACAGAGATGTGTACCTGCAAGGACCCATTTGGGCC AAAATTCCTCACACGGACGGCAACTTTCACCTTCTCCGCTGATGGGAGGGTTTGAATG AAGCACCCGCTCCTCAGATCCTCATCAAAAACACACCTGTACCTGCGGATCCTTCAACG GCCTTCAACAAGGACAAGCTGAACCTTTTCATCACCCAGTATTCTACTGGCCAAGTCAGC GTGGAGATCGAGTGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCGGAGATCCAG TACACTTCCAACATTACAAGTCTAATAATGTTGAATTTGCTGTTAATACTGAAGGTGTA TATAGTGAACCCCGCCCATTTGGCACAGATACCTGACTCGTAATCTGTAA

AAV Viral Genome

[0179] In some embodiments, the AAV particle of the present disclosure serves as an expression vector comprising a viral genome that encodes a FXN protein (e.g., a wildtype FXN protein, e.g., a wildtype human FXN protein).

[0180] In some embodiments, an AAV particle, e.g., an AAV particle for the vectorized delivery of a FXN protein described herein, comprises a viral genome, e.g., an AAV viral genome, also referred to as an AAV genome, a vector genome, or an AAV vector genome. In some embodiments, the viral genome, e.g., the AAV viral genome, further comprises an inverted terminal repeat (ITR) region, an enhancer, a promoter, an intron region, an exon region, a nucleic acid encoding a transgene encoding a FXN protein sequence, e.g., any one of the sequences in Table 6G or a sequence that is at least 90% identical to any one of the sequences provided in Table 6G), a nucleotide sequence encoding at least one miR binding site (e.g., at least one miR122 binding site), a polyA region, or a combination thereof.

Viral Genome Component: Inverted Terminal Repeats (ITRs)

[0181] In some embodiments, the viral genome may comprise at least one inverted terminal repeat (ITR) region. The AAV particles of the present disclosure comprise a viral genome with at least one ITR region and a FXN-encoding sequence. In some embodiments, the viral genome has two ITRs. These two ITRs flank the FXN-encoding sequence at the 5' and 3' ends. In some embodiments, the ITR functions as an origin of replication comprising a recognition site for replication. In some embodiments, the ITR comprises a sequence region which can be complementary and symmetrically arranged. In some embodiments, the ITR incorporated into a viral genome described herein may be comprised of a naturally occurring polynucleotide sequence or a recombinantly derived polynucleotide sequence.

[0182] In some embodiments, the AAV viral genome may comprise at least one inverted terminal repeat (ITR) region having a length of 50-250 nucleotides. In some embodiments, the AAV viral genome comprises a 5' ITR region that is 50-250 nucleotides in length and a 3' ITR region that is 50-250 nucleotides in length. In some embodiments, the 5' ITR region and the 3' ITR region may comprise the same length and/or the same sequence. In some embodiments, the 5' ITR region and the 3' ITR region are different in length and/or in sequence.

[0183] Non-limiting examples of ITR sequence regions are described in Table 6A. In some embodiments, the viral genome comprises an ITR comprising the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the viral genome comprises an ITR comprising the nucleotide sequence of SEQ ID NO: 1812 or a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the viral genome comprises a 5' ITR region comprising the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least

92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or the viral genome comprises a 3' ITR region comprising the nucleotide sequence of SEQ ID NO: 1812 or a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

Table 6A. Exemplary Inverted Terminal Repeat (ITR) Sequence Regions

Sequence Region Name	Sequence Length	SEQ ID NO	Sequence
ITR1	141	1811	CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGC CGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGT CGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGG GAGTGGCCAACTCCATCACTAGGGGTTCTT
ITR2	141	1812	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTC TCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGA CCAAAGGTGCGCCGACGCCCGGGCTTTGCCCGGG CGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCT GCAGG

Viral Genome Component: Promoters and Enhancers

[0184] In some embodiments, the viral genome comprises at least one element to enhance the transgene target specificity and expression. See, e.g., Powell *et al.* Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy, 2015; the contents of which are herein incorporated by reference in their entirety. Non-limiting examples of elements to enhance the transgene target specificity and expression include promoters, endogenous miRNAs, post-transcriptional regulatory elements (PREs), polyadenylation (PolyA) region, upstream enhancers (USEs), CMV enhancers, and introns.

[0185] In some embodiments, expression of the polypeptides in a target cell may be driven by a specific promoter, including but not limited to, a promoter that is species specific, inducible, tissue-specific, or cell cycle-specific (Parr *et al.*, *Nat. Med.*3:1145-9 (1997); the contents of which are herein incorporated by reference in their entirety).

[0186] In some embodiments, the viral genome comprises a promoter that is sufficient for expression, e.g., in a target cell, of a FXN protein, e.g., a human FXN protein, encoded by a transgene. In some embodiments, the promoter is deemed to be efficient when it drives expression of the FXN encoded in viral genome of the AAV particle.

[0187] In some embodiments, the promoter is deemed to be efficient when it drives expression in the cell or tissue being targeted.

[0188] Promoters may be naturally occurring or non-naturally occurring. Non-limiting examples of promoters include viral promoters, plant promoters and mammalian promoters. In some embodiments, the promoter may be a human promoter. In some embodiments, the promoter may be truncated.

[0189] Promoters that promote expression in most tissues include, but are not limited to, human elongation factor 1 α -subunit (EF1 α) promoters, immediate-early cytomegalovirus (CMV) promoters, chicken β -actin (CBA) promoters, CAG promoters, CAG derivative promoters, β glucuronidase (GUSB) promoters, ubiquitin C (UBC) promoters, or a fragment of any one of the aforementioned. A CAG promoter typically comprises: (C) the cytomegalovirus early enhancer element; (A) the promoter, the first exon, and the first intron of the chicken beta-actin gene, and (G) the splice acceptor of the rabbit beta-globin gene. In some embodiments, a derivative of a CAG promoter may comprise (i) a CMVie enhancer and a beta-actin promoter or (ii) a beta-actin promoter and an intron sequence.

[0190] Tissue-specific expression elements can be used to restrict expression to certain cell types such as, but not limited to, nervous system promoters which can be used to restrict expression to neurons, astrocytes, or oligodendrocytes. Non-limiting examples of tissue-specific expression elements for neurons include neuron-specific enolase (NSE), platelet-derived growth factor (PDGF), platelet-derived growth factor B-chain (PDGF- β), synapsin (Syn), methyl-CpG binding protein 2 (MeCP2), CaMKII, mGluR2, NFL, NFH, n β 2, PPE, Enk, and EAAT2 promoters. Non-limiting examples of tissue-specific expression elements for astrocytes include the glial fibrillary acidic protein (GFAP) and EAAT2 promoters. A non-limiting example of a tissue-specific expression element for oligodendrocytes include the myelin basic protein (MBP) promoter.

[0191] In some embodiments, the viral genome comprises a ubiquitous promoter. Non-limiting examples of ubiquitous promoters include H1, U6, CMV, CBA (including derivatives CAG, CBh, etc.), EF-1 α , PGK, UBC, GUSB (hGBp), and UCOE (promoter of HNRPA2B1-CBX₃).

[0192] In some embodiments, the viral genome comprises a CBA promoter. In some embodiments, the viral genome comprises a truncated CBA promoter, e.g., a CBA promoter that is 50-400 nucleotides in length, e.g., 100-332 nucleotides in length. In some embodiments, the viral genome comprises a CMV promoter. In some embodiments, the viral genome comprises a truncated CMV promoter, e.g., a CMV promoter that is 50-300 nucleotides in length, e.g., a CMV promoter that is 109 nucleotides in length.

[0193] In some embodiments, the AAV vector comprises an enhancer element, a promoter, and/or a 5'UTR intron. The enhancer may be, but is not limited to, a CMV enhancer; the promoter may be, but is not limited to, a CMV, CBA, FXN, UBC, GUSB, NSE, Synapsin, MeCP2, or GFAP promoter; and the 5'UTR/intron may be, but is not limited to, SV40, and CBA-MVM. In some embodiments, the enhancer, promoter, and/or intron used in combination may be: (1) CMV enhancer, CMV promoter, SV40 5'UTR intron; (2) CMV enhancer, CBA promoter, SV40 5'UTR intron; (3) CMV enhancer, CBA promoter, CBA-MVM 5'UTR intron; (4) UBC promoter; (5) GUSB promoter; (6) NSE promoter; (7) Synapsin promoter; (8) MeCP2 promoter; (9) GFAP promoter; (10) H1 promoter; and/or (11) U6 promoter.

[0194] In some embodiments, the viral genome comprises an engineered promoter.

[0195] In some embodiments, the viral genome comprises an enhancer. In some embodiments, an enhancer may be a separate component of the viral genome than the promoter. In some embodiments, an

enhancer may be 5' to a promoter sequence in a viral genome. In some embodiments, an enhancer may be 3' to a promoter sequence in a viral genome.

[0196] In some embodiments, the viral genome comprises an enhancer, for example an immediate-early “ie” enhancer or a CMV/globin enhancer. In some embodiments, the enhancer comprises ie1 exon 1 and ie1 intron 1 or a fragment thereof. In some embodiments, the enhancer comprises an ie1 exon 1, an ie1 intron 1 or fragment thereof, a human beta-globin intron 2, and a human beta-globin exon 3.

[0197] In some embodiments, an enhancer may comprise at least one intron sequence. In some embodiments, an enhancer may comprise at least one exon sequence. In some embodiments, an enhancer comprises one intron sequence and one exon sequence. In some embodiments, an enhancer sequence comprises two intron sequences. In some embodiments, an enhancer sequence comprises two exon sequences. In some embodiments, an enhancer sequence comprises two intron sequences and two exon sequences.

[0198] Exemplary promoters are provided in Table 6B. In some embodiments, the promoter comprises or consists of any one of the nucleotide sequences provided in Table 6B, or a nucleotide sequence at least 90% (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) identical thereto. In some embodiments, the promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 1738. In some embodiments, the promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 1740. In some embodiments, the promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 1742. In some embodiments, the promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 1745.

[0199] In some embodiments, the promoter comprises a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to the nucleotide sequence of SEQ ID NO: 1742. In some embodiments, the promoter comprises a nucleotide sequence at least 95% identical to SEQ ID NO: 1742. In some embodiments, the promoter comprises a nucleotide sequence at least 98% identical to SEQ ID NO: 1742. In some embodiments, the promoter comprises a nucleotide sequence at least 99% identical to SEQ ID NO: 1742. In some embodiments, the promoter comprises the nucleotide sequence of SEQ ID NO: 1742.

Table 6B. Exemplary Promoters

Promoter	SEQ ID NO:	Sequence
CBA-D4	1738	TACATGACCTTATGGGACTTTCTACTTGCCAGTACATCTACGTATTAGTC ATCGCTATTACCATGTGAGGCCACGTTCTGCTTCACTCTCCCATCTCCC CCCCCTCCCCACCCCAATTTTGTATTTATTTATTTTAAATTATTTTGTG CAGCGATGGGGCGGGGGGGGGGGCGCGGCCAGGCGGGGCGGGGCGGGG CGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAG CGGCGCGCTCCGAAAGTTTCCTTTATGGCGAGGCGGCGGCGGCGGCGCC CTATAAAAAGCGAAGCGCGCGGCGGG
CBA-D6	1740	CCACGTTCTGCTTCACTCTCCCATCTCCCCCTCCCCACCCCAATTT TGTATTTATTTATTTTAAATTATTTTGTGAGCGATGGGGGCGGGGGGGG GGGGCGCGCGCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAG GCGGAGAGGTGCGGCGGCGAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCC

		TTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGG
CBA-D8	1742	GGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGG
CMV-D7	1750	TTTGGCACCAAATCAACGGGACTTTCCAAATGTCGTAACAACCTCCGCCCATTTGACGCAAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATAAGCAGAGCTC

Viral Genome Component: Introns and Exon Sequences

- [0200] In some embodiments, the AAV viral genome comprises at least one intron and/or exon sequence region.
- [0201] In some embodiments, the AAV viral genome may comprise at least one intron sequence region. The intron sequence region(s) may be 10-1200 nucleotides in length. As a non-limiting example, the viral genome comprises an intron sequence region that is about 32 nucleotides in length. As a non-limiting example, the viral genome comprises an intron sequence region that is about 53 nucleotides in length. As a non-limiting example, the viral genome comprises an intron sequence region that is about 134 nucleotides in length. As a non-limiting example, the viral genome comprises an intron sequence region that is about 347 nucleotides in length. As a non-limiting example, the viral genome comprises an intron sequence region that is about 379 nucleotides in length. As a non-limiting example, the viral genome comprises an intron sequence region that is about 566 nucleotides in length. As a non-limiting example, the viral genome comprises an intron sequence region that is about 1016 nucleotides in length. As a non-limiting example, the viral genome comprises an intron sequence region that is more than 1016 nucleotides in length.
- [0202] In some embodiments, the AAV viral genome comprises two intron sequence regions. In some embodiments, the AAV viral genome comprises three intron sequence regions. In some embodiments, the AAV viral genome comprises more than three intron sequence regions.
- [0203] In some embodiments, the AAV viral genome may comprise at least one exon sequence region. In some embodiments, the exon sequence may be 10-1200 nucleotides in length. As a non-limiting example, the viral genome comprises an exon region that is about 32 nucleotides in length. As a non-limiting example, the viral genome comprises an exon sequence region that is about 53 nucleotides in length. As a non-limiting example, the viral genome comprises an exon sequence region that is about 134 nucleotides in length. As a non-limiting example, the viral genome comprises an exon sequence region that is about 347 nucleotides in length. As a non-limiting example, the viral genome comprises an exon sequence region that is about 379 nucleotides in length. As a non-limiting example, the viral genome comprises an exon sequence region that is about 566 nucleotides in length. As a non-limiting example, the viral genome comprises an exon sequence region that is about 1016 nucleotides in length. As a non-limiting example, the viral genome comprises an exon sequence region that is more than 1016 nucleotides in length.

[0204] In some embodiments, the AAV particle viral genome comprises two exon sequence regions. In some embodiments, the AAV particle viral genome comprises three exon sequence regions. In some embodiments, the AAV particle viral genome comprises more than three exon sequence regions.

[0205] In some embodiments, the AAV particle viral genome comprises a hybrid intron/exon sequence region comprising at least one intron and at least one exon. In some embodiments, the hybrid intron/exon sequence region comprises one intron and one exon. In some embodiments, the hybrid intron/exon sequence region comprises two introns and two exons. In some embodiments, an intron or exon sequence may comprise a full-length intron or exon. In some embodiments, an intron or exon sequence may comprise a fragment or variant of an intron or exon sequence.

[0206] The hybrid intron/exon sequence region(s) may, independently, have a length such as, but not limited to, 15-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, and more than 1200 nucleotides. As a non-limiting example, the viral genome comprises a hybrid intron/exon sequence region that is about 379 nucleotides in length. As a non-limiting example, the viral genome comprises a hybrid intron/exon sequence region that is about 566 nucleotides in length. As a non-limiting example, the viral genome comprises a hybrid intron/exon region that is about 379 nucleotides in length.

[0207] In some embodiments, the intron/exon sequence region is an enhancer sequence. In some embodiments, the intron/exon sequence region is not an enhancer sequence.

[0208] In some embodiments, the intron/exon sequence region is a component of a promoter sequence. In some embodiments, the intron/exon sequence region is not a component of a promoter sequence.

[0209] In some embodiments, the AAV particle viral genome comprises at least one intron and/or exon sequence region. Non-limiting examples of intron and exon sequence regions are described in Table 6C. In some embodiments, the intron and/or exon sequence region comprises the nucleotide sequence of SEQ ID NO: 1816, or a nucleotide sequence with at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto. In some embodiments, the exon sequence region comprises the nucleotide sequence of SEQ ID NO: 1817, or a nucleotide sequence with at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto. In some embodiments, the intron sequence region comprises the nucleotide sequence of SEQ ID NO: 1819, or a nucleotide sequence with at least at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto. In some embodiments, the intron sequence region comprises the nucleotide sequence of SEQ ID NO: 1820, or a nucleotide sequence with at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto. In some embodiments, the exon sequence region comprises the nucleotide sequence of SEQ ID NO: 1821, or a nucleotide sequence with at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

Table 6C. Exemplary Intron and Exon Sequence Regions

Sequence Region Name	Sequence Length	SEQ ID NO	Sequence
hBglobin intron/exon	566	1816	TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGCGGATTTCGAATCCCGGCCGG GAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGACGTAA GTACCGCCTATAGAGTCTATAGGCCACAAAAATGCTTTCTTCTT TTAATATACTTTTTTGTATTCTTATTTCTAATACTTTCCCTAATC TCTTTCTTTCAGGGCAATAATGATACAATGTATCATGCCTCTTTGC ACCATTTCTAAAGAATAACAGTGATAATTTCTGGGTTAAGGCAATAG CAATATTTCTGCATATAAATATTTCTGCATATAAATGTAACTGAT GTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCCAGCTACCAT TCTGCTTTTATTTTATGGTTGGGATAAGGCTGGATTATTCTGAGTC CAAGCTAGGCCCTTTTGCATATCATGTTTCATACCTCTTATCTTCTC CCACAGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCAC TTTGGCAAAGAATT
ie1 exon 1	134	1817	TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA AGACACCGGGACCGATCCAGCCTCCGCGGATTTCGAATCCCGGCCGG GAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGAC
ie1 intron 1 (partial)	32	1819	GTAAGTACCGCCTATAGAGTCTATAGGCCAC
hBglobin intron 2	347	1820	AAAAAATGCTTTTCTTTTAAATATACTTTTTTGTATTCTTATTT CTAATACTTTCCCTAATCTCTTTCTTTCAGGGCAATAATGATACAA TGATCATGCCTCTTTGCACCATTTCTAAAGAATAACAGTGATAATT TCTGGGTTAAGGCAATAGCAATATTTCTGCATATAAATATTTCTGC ATATAAATTGTAAGTATGTAAGAGGTTTCATATTTGCTAATAGCAG CTACAATCCAGCTACCATTTCTGCTTTTATTTTATGGTTGGGATAAG GCTGGATTATTTCTGAGTCCAAGCTAGGCCCTTTTGTCTAATCATGTT CATACCTCTTATCTTCTCCACAG
hBglobin exon 3	53	1821	CTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCA AGAATT

Viral Genome Component: Nucleotide Sequence Encoding a miR Binding Site

[0210] Tissue- or cell-specific expression of the AAV viral particles of the disclosure can be enhanced by introducing tissue- or cell-specific regulatory sequences, *e.g.*, promoters, enhancers, microRNA binding sites, *e.g.*, a detargeting site. Without being bound by theory, it is believed that an encoded miR binding site can modulate, *e.g.*, prevent, suppress, or otherwise inhibit, the expression of a gene of interest on the viral genome of the invention, based on the expression of the corresponding endogenous microRNA (miRNA) or a corresponding controlled exogenous miRNA in a tissue or cell, *e.g.*, a non-targeting cell or tissue. In some embodiments, a miR binding site modulates, *e.g.*, reduces, expression of the FXN-encoding region in a viral genome of an AAV particle described herein in a cell or tissue where the corresponding mRNA is expressed. In some embodiments, the miR binding site modulates, *e.g.*, reduces, expression of the encoded FXN protein in a cell or tissue of the DRG or liver.

[0211] In some embodiments, the viral genome of an AAV particle described herein comprises a nucleotide sequence encoding a microRNA binding site, *e.g.*, a detargeting site. In some embodiments, the viral genome of an AAV particle described herein comprises a nucleotide sequence encoding a miR binding site, a microRNA binding site series (miR BSs), or a reverse complement thereof. In some embodiments, the 3' UTR of the viral genome may be engineered to include at least one miRNA binding site.

[0212] In some embodiments, the encoded miR binding site series comprise at least 1-5 copies, *e.g.*, at least 1-3, 2-4, 3-5, or at least 1, at least 2, at least 3, at least 4, at least 5 copies or more of a miR binding site (miR BS). In some embodiments, the encoded miR binding site series comprises 3 copies of a miR binding site. In some embodiments, all copies are identical, *e.g.*, comprise the same miR binding site. In some embodiments, the miR binding sites within the encoded miR binding site series are continuous and not separated by a spacer. In some embodiments, the miR binding sites within an encoded miR binding site series are separated by a spacer, *e.g.*, a non-coding sequence.

[0213] In some embodiments, the viral genome comprises at least one nucleotide sequence encoding a miRNA binding site to reduce the expression of the transgene in a specific tissue. In some embodiments, the viral genome may comprise a nucleotide sequence encoding a miR122 binding site (miR122BS) or tandem copies of the miR122BS to reduce the expression of the viral genome in the liver. In some embodiments, the viral genome may comprise a nucleotide sequence encoding a miR183 miRNA binding site (miR183BS) or tandem copies of the miR183BS to reduce expression of the viral genome in the DRG.

[0214] In some embodiments, the nucleotide sequence encoding the miR binding site and/or the encoded miR binding site may be 20-75 nucleotides in length. In some embodiments, the nucleotide sequence encoding the miR binding site and/or the encoded miR binding site is 23 nucleotides in length. In some embodiments, the nucleotide sequence encoding the miR binding site and/or the encoded miR binding site is 71 nucleotides in length.

[0215] Non-limiting examples of nucleotide sequences encoding a miR binding site or binding site series are shown in Table 6D.

[0216] In some embodiments, the encoded miR binding site is fully complementary to an miR. In some embodiments, the encoded miR binding site is partially complementary to an miR. In some embodiments, the miR is expressed in the liver or in hepatocytes. In some embodiments, the miR is miR122. In some embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR122 binding site sequence. In some embodiments, the nucleotide sequence encoding the miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1827, or a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity, or comprising at least one, at least two, at least three, at least four, at least five, or six modifications, but no more than six modifications, relative to the nucleotide sequence of SEQ ID NO: 1827, wherein the modification(s) can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises a nucleotide sequence encoding a miR122 binding site series, wherein the nucleotide sequence encoding the miR122 binding site series encodes at least 2, at least 3, at least 4, or at least 5 copies of the encoded miR122 binding site. In some embodiments, the viral genome comprises a nucleotide sequence encoding a miR122 binding site series encoding 3 copies of a miR122 binding site. In some embodiments, the nucleotide sequence encoding the miR122 binding site series comprises the nucleotide sequence of SEQ ID NO: 1826, or a

nucleotide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity, or comprising at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications, but no more than ten modifications relative to the nucleotide sequence of SEQ ID NO: 1826, wherein the modification(s) can result in a mismatch between the encoded miR binding site and the corresponding miRNA.

Table 6D. Exemplary Nucleotide Sequences Encoding a miR Binding Site or miR Binding Site Series

Sequence Region Name	Sequence Length	SEQ ID NO	Sequence
miR122 binding site series	71	1826	ACAAACACCATTGTCACACTCCACACAAACACCATTGTCACACTCCA
Single miR122 binding site	23	1827	ACAAACACCATTGTCACACTCCA

Viral Genome Component: Polyadenylation Region

- [0217] In some embodiments, the viral genome of the AAV particles of the present disclosure comprises at least one polyadenylation (polyA) region. In some embodiments, the polyA region is positioned 3' relative to the nucleic acid encoding a FXN protein described herein.
- [0218] In some embodiments, the polyA region comprises a length of about 100 to about 500 nucleotides, e.g., about 477 nucleotides. In some embodiments, the polyA region comprises a length of 477 nucleotides.
- [0219] A non-limiting example of a polyA region is described in Table 6E. In some embodiments, the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828, or a nucleotide sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

Table 6E. Exemplary PolyA Region

Sequence Region Name	Sequence Length	SEQ ID NO	Sequence
hGHpA	477	1828	GGGTGGCATCCCTGTGACCCCTCCCGAGTGCCTCTCCTGGCCCTGG AAGTTGCCACTCCAGTGCCACCAGCCTTGTCCTAATAAAATTAAG TTGCATCATTTTGTCTGACTAGGTGCTCTCTATAATATTATGGGG TGGAGGGGGGTGGTATGGAGCAAGGGGCAAGTTGGGAAGACAACCT GTAGGGCTGCGGGGTCTATTGGGAACCAAGCTGGAGTGCAGTGGC ACAATCTTGGCTCACTGCAATCTCCGCCCTCCTGGGTCAAGCGATT CTCCTGCCTCAGCCTCCCGAGTTGTTGGGATTCAGGCATGCATGA CCAGGCTCAGCTAATTTTGTGTTTTTTGGTAGAGACGGGGTTTCAC CATATTGGCCAGGCTGGTCTCCAACCTCCTAATCTCAGGTGATCTAC CCACCTTGGCCTCCCAAATGCTGGGATTACAGGCGTGAACCACTG CTCCCTTCCCTGTCCTT

Viral Genome Component: Filler (Stuffer) Sequence

[0220] As used herein, the terms “stuffer sequence” and “filler sequence” are used interchangeably. In some embodiments, the AAV particle viral genome comprises at least one filler sequence. In some embodiments, the AAV particle viral genome comprises a filler sequence comprising a human albumin sequence. In some embodiments, the AAV particle viral genome comprises the filler sequence of Alb2034. In some embodiments, the AAV particle viral genome comprises the filler sequence of Alb2106. In some embodiments, the AAV particle viral genome comprises the filler sequence of Alb2264. In some embodiments, the AAV particle viral genome comprises the filler sequence of Alb2266

[0221] Non-limiting examples of filler sequences are described in Table 6F. In some embodiments, the filler sequence comprises the nucleotide sequence of SEQ ID NO: 1838, or a nucleotide sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto. In some embodiments, the filler sequence comprises the nucleotide sequence of SEQ ID NO: 1839, or a nucleotide sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto. In some embodiments, the filler sequence comprises the nucleotide sequence of SEQ ID NO: 1840, or a nucleotide sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto. In some embodiments, the filler sequence comprises the nucleotide sequence of SEQ ID NO: 1841, or a nucleotide sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

Table 6F. Exemplary Filler Sequences

Sequence Region Name	Sequence Length	SEQ ID NO	Sequence
Alb2034	2034	1838	TCTCCTGAACAACACTAGTTAAACTTGGCTTTGAGTTCACCTGTACCACTTGCATAATCTTGGGAAAGTGAGTTGCCTAATTCAGTGACATTAATAAATTTATTAATTTCTTCTTCAATAAAACCTGGAGAGAGCTTCATATGTATCAGCATATGCTAAACTTGAAAGATACAAGTAGAAAATGGAAGGAAATATATCTGACTCAATAGGGATAGTTCAAGGGTTAAATTAAAAGTAGTAAAGTATTATAATTAATCTGACATGGTACCTAATATATAATAATCATGTATTAAGAATGCCAGTCACCATTAAAAGTCAATGTATGACTTTAATCTACTCAGGGAAGAAAATATGCTCTGTTCACTGTTATTATCTCTAAAATCCATAATCAGAAGAGCAATGTGTATGAGCCACACAATAAATATCTACTGTATAATATGTCTCTTCTTGTTTTTAACCTTCATAGATAAGACTCTATTGAATTGGGACATTAGTCCAGCAAGCCATTCTGTCTCTGTCTCTTCTATGGAGGGAAAGGTTTAAACCATCAAAGACTAGGTGCATCTCCCAACAACCTGAATTTAATATTCAAATATGTATCTAAATTCATTTGTTACATTTTGTGTTCAGCTTACATATTACTTTTGTGAGCGACATCTATTCAAGGCCTACTACTTGCTGCTCTACAAAATATTGCATGCTCTATTTGCCCATTAACATTTTCTTAACCTTCAAGGGACATGCTCAGTTCCTGATATACCAAGATTTGGTATTTACCCTCCCAGCCTACATACTTCCAATCTTAAGAGACAATTTTGTAGACTACATTCAAATATAGACCTCTCCACCCCATCAACTATTTTATCTCTCCTCTCCTATCTTTCTTGACAAAGAGTGATTAGAAATATGCAATGTATTTTCATTCCTTAAAAGTTCATGCTAGGTGG

			CTCATATATTAAGTCTTAAATAATTACAAATTGATAAAAAAATCAGTCA ATAAAAAATTACTTACGCATTCTGGAATTTGTACTCTCCAAGCTGCTCAAA AAGCTCACAAATTTTGTGTTGATTAAATTCGAGGCTCTTCCACAAGAGGTT TAAATTCATCGAACTGAAACATAAAAGAATTGTGTTAATAGTATTATGCC TCAGGATCAGATGCAGGTTGTTTTTCACCCCTCTGGTCAGAATGATATCC TTTCTTCTCTCTACTCAAAGCCACATGAATAGATGAATATCAATGCCGCT CTGTCACAATACATTCGAACTATTATTTGGTGAGCTTGCATCATGTAAG TGGTTAAGAATATTCTAGGCTCTGGAGTTCAATGACCAGTATTTGAGCCAA GTCATGTAACCTCATTTCTCAGTTGTGCAATTTTACTTACCATCTCCACAT TTCAGAATCCCTTATCCCTAAAAATAGGGGCAAAGATGGCACTCATCTCATA GAGCTGCTCTGTAAACCAGGTAAGCTAATGAAATTAACAGTGCCCTTGCA TATAATAAAAGTTTAATAAATGATGGCTATGATTGTACATATTATATTATTA TTAGTTACCTTTCCAGTTAGATTCCAGGCTCTCCAAAAATGAGAGATTTT ACGTTGGTCATTGTTTGTGCATGGTGCCCTGGCATACAGTAGCTTCTTGAA AGCAGGAAAAATGAGTTTCTCTGATTCTGAATAGTTCAGAAACCATTAC ATATGCTAGTGGGAACCTAAAAATCCTCCAGAACAGATTTCTCTCCTA ACTTAATTTCTTGTAAACTATTCTTAACATCTCCAAAAAGATTTGAAA ATATTCTAAAGGGATAGGTTCTATAATTTGCCATAAGATACATAATCTAG TACTTGATTAATCTGGAATCAGGTTAACTCACTTTACATCTAAGTTAAA TATCTTCTAATTAACATTTAAATTTAATTTTTTTTTTGTCTCAGGATTGT GAAAAAGAGAAAAAAGATCAAAATTTTTTAGAGATTGCTCTATTTCAGAT CTTCTATTCTAACTAGTCTAAATTTTGTCTCTAG
Alb2106	2106	1839	GATGTTGCTATGTTCCATTCATCATATTATCTCCATCTGCAGAGTAGTGG GTTAGTGGAGGGTAGAAAACATTCTCCTGAACAACCTAGTTAAACTTGGCT TTGAGTTCCACCTGTACCATTGTCATAATCTTGGGAAAGTGAGTTGCCTA ATTCAGTGACATTAATAAATTTATTAATTTCTTCTTTCAATAAACTGG AGAGAGCTTCATATGTATCAGCATATGCTAAACTTGAAAGATACAAGTAG AAAATGGAAGGAAATATATCTGACTCAATAGGGATAGTTCAAGGGTTAAA TTAAAGTAGTAAAGTATTATAATTAATCTGACATGGTACCCTAATATATA ATAATCATGTATTAAAGATGCCAGTCACCATTAAAGTCAATGTATGATC TTAATCTACTCGAGGAAAGAACTATGTCTGTGTTCACTGTTATTATCTCT AAAATCCATAATCAGAAGAGCACCATGTGTATGAGCCACACAATAAATAT CTACTGTATAATATGTCTCTTCTTGTTTTTTAACCTTCATAGATAAGACTC TATTGAATTGGGACATTAGTCCAGCAAGCATTCTGTCTCTGTCTTCTCT ATGGAGGGAAAGGTTTAACCATCAAAGACTAGGTGCATCTCCCAAACAAC CTGAATTTAATATTCAAATATGTATCTAAATTCATTTGTTACATTTTTGT GTTCACTTACATATTACTTTTTTGAGCGACATCTATTCAAGGCCCTACTAC TTGCTGCTCTACAAAATATTGCCATGCTCTATTTGCCATTAACATATTTCT TTAACTTCAAGGGACATGCTCAGTTCTGATATACCAAGATTTGGTATTT ACCTCCAGCCTACATACTTCCAATCTTAAGAGAACAATTTTTAGACTA CATTCAAATATAGACCTCTCCACCCCATCAACTATTTTATCTCTCTCTCTC CTATCTTTCTTGACAAAGAGTGATTAGAAATATGCAATGTATTTTCATTC TTAAAAGTTCATGCTAGGTGGCTCATATATTAAGTCTTAAATAATTACAA AATTGATAAAAAAATCAGTCAATAAAAAATTACTTACGCATTCTGGAATT TGTAATCTCCAAGCTGCTCAAAAAGCTCACAATTTTGTGTTGATTAAATTC TGAGGCTCTTCCACAAGAGGTTTAAATTCATCGAACTGAAACATAAAAGA ATTGTGTTAATAGTATTATGCCTCAGGATCAGATGCAGGTTGTTTTTCAC CCCTCTGGTCAGAATGATATCCTTTCTTCTCTCTACTCAAAGCCACATGA ATAGATGAATATCAATGCCGCTCTGTGACAAATACATTGAACTATTTATT TGGTGAGCTTGCATCATGTAAGTGGTTAAGAATATTCTAGGCTCTGGAGTT CAATGACCAGTATTTGAGCCAAGTCATGTAACCTATTCTCAGTTGTGCA ATTTTACTTACCATCTCCACATTTTCAATCCTTATCCCTAAAAATAGGGG CAAAGATGGCACTCATCTCATAGAGCTGCTCTGTAAACCAGGTAAGCTAA TGAAATTAACAGTGCCCTTGCAATATAATAAAAGTTTAAATAAATGATGGCT ATGATTGTACATATTATTATATTAGTTTACCTTTCCAGTTAGATTCCAG GCTCTCCAAAATGAGAGATTTTACGTTGGTCATTGTTTGTGCATGGTGCC TGGCATACAGTAGCTTCTTGAAAGCAGGAAAAATGAGTTTCTCTGATTCT TGAATAGTTTCAAAACCATTACATATGCTAGTGGGAACCTAAAAATCCT CCAGAACAGATTTCTCTCTTAACCTAATTTCTTGTAACCAATTTCTCTTA ACATCTCTCAAAAAGATTTGAAAAATTTCTAAAGGGATAGGTTCTATAATT TGCCATAAGATACTAATTTAGTACTTGATTAACTCTGGAACTCAGGTTAA CTCACTTTACATCTAAGTTAAATATCTTCTAATTAACATTTAAATTTAAT

			TTTTTTTGTCTCAGGATTGTGAAAAAGAGAAAAAAGATCAAAATTTT TTAGAGATTGCTCTATTTCAGATCTTTCTATTCTAACTAGTCTAAATTTTG TCCTAG
Alb2264	2264	1840	CTAGGTTTCTTGAGACCTCTACAAGAGTTGGAGTTGACACTTGGGGTACT TTCTTGGTGTAACGAACCTAATAGCCTGAAAAAAGAAGTCATGTGTTTTTC AGCAAGGCAAGAACTGTCTAACATAGTAGATAAAACAGAGAACACTTGG CCGGAATCAACTAAGATGTTGCTATGTTCCATTTCATCATATTATCTCCAT CTGCAGAGTAGTGGGTTAGTGGAGGGTAGAAAACATTCTCTGAACAACCT AGTTAACTTTGGCTTTGAGTTCCACCTGTACCCTTGCATAATCTTGGGA AAGTGAGTTGCCCTAATTCAGTGACATTAATAAATTTATTAATTTCTTCTT TCAATAAAACCTGGAGAGAGCTTCATATGTATCAGCATATGCTAAACTTG AAAGATACAAGTAGAAAATGGAAGGAAATATATCTGACTCAATAGGGATA GTTCAAGGGTTAAATTAAAAGTAGTAAAGTATTATAATTAATCTGACATG GTACCTAATATAATAATCATGTATTAAAGAAATGCCAGTCACCATTAATA GTCAATGTATGACTTTAATCTACTCGAGGAAAGAACTATGTCTTGTTC CTGTTATATCTCTAAATCCATAATCAGAAGAGCACCATGTGTATGAGC CACACAATAAATATCTACTGTATAATATGTCTCTTCTGTTTTAACCTT CATAGATAAGACTCTATTGAATTGGGACATTAGTCCAGCAAGCCATTCTG TCTCTGTCTCTCTATGGAGGGAAAGGTTTAACCATCAAGACTAGGTGC ATCTCCCAACAACCTGAATTTAATATTCAAATATGTATCTAAATTCATT TGTTACATTTTTGTGTTTCTGCTTACATATTACTTTTTGAGCGACATCTAT TCAAGGCTACTACTTGTGCTCTACAAAATATTGCCATGCTCTATTGTC CCATTAACATTTCTTAACCTTCAAGGGACATGCTCAGTCTGTATATACC AAGATTTGGTATTTACCTCCAGCCTACATACTTCCAATCTTAAGAGAA CAATTTTTAGACTACATTCAAATATAGACCTCTCCACCCCATCAACTATT TTATCTCTCCTCTCTATCTTTCTTGACAAAGAGTGATTGAAAATATGCA ATGTATTTTCATTCTTAAAAGTTCTATGTAGGTGGCTCATATATTAAGT CTTAAATAATTACAAATTGATAAAAAAATCAGTCAATAAAAATTACTTA CGCATTTGGAATTTGTACTCTCCAAGCTGTCTCAAAAGCTCACAATTTT GTTTGATTAAATCTGAGGCTCTTCCACAAGAGGTTTAAATTCATCGAAC TGAAACATAAAAGAATTGTGTTAATAGTATATGCTCAGGATCAGATGC AGGTTGTTTTTCACCCCTCTGGTCAGAAATGATATCCTTTCTCTTCTTAC TCAAAGCCACATGAATAGATGAATATCAATGCCGCTCTGTCCACAATACAT TCGAACATTTTATTTGGTGAGCTTGCATCATGTAAGTGTTTAAAGATATT CAGGCTCTGGAGTTCAATGACCAGTATTTGAGCCAAAGTCATGTAATCAT TCTTCAGTTGTGCAATTTTACTTACCATCTCCACATTTCAGAATCCTTAT CCCTAAATAGGGGCAAGATGGCACTCATCTCATAGAGCTGCTCTGTAA ACCAGGTAAGCTAATGAAATTAAACAGTGCCTTGCAATATAATAAAGTTT AATAAATGATGGCTATGATTGTACATATATTATTTAGTTTCACTTTCTT CAGTTAGATTCAGGCTCTCCAAAATGAGAGATTTTACGTTGGTCATTGT TTGTGATGGTGCTGGCATAACAGTAGCTTCTTGAAAGCAGGAAAAATGA GTTTTCTCTGATTCTGAATAGTTTCAAAACCATTACATATGCTAGTGGGA ACCTAAAAATCCTCCAGAACAGATTTTCTCTCTTAACTTAATTTCTTGT AAAATATTCCTAAACATCCTCAAAAAGATTTGAAAATATTCTAAAGGGA TAGGTTCTATAATTTGCCATAAGATACTAATTTCTAGTACTTGATTAATCC TGAATCAGGTTAACTCACTTTACATCTAAGTTAAATATCTTCTAATTA CATTTAAATTTAATTTTTTTTTTTGTTCTCAGGATTGTGAAAAGAGAAAA AAGATCAAAATTTTTTATAGAGATTGCTCTATTTCAGATCTTTCTATTCTAAC TAGTCTAAATTTTC
Alb2266	2266	1841	GTTTCTTGAGACCTCTACAAGAGTTGGAGTTGACACTTGGGGTACTTTCT TGGTGTAACGAACCTAATAGCCTGAAAAAAGAAGTCATGTGTTTTTCAGCA AGGCAAGAACTGTCTAACATAGTAGATAAAACAGAGAACACTTGGCCGG AATCAACTAAGATGTTGCTATGTTCCATTTCATCATATTATCTCCATCTGC AGAGTAGTGGGTTAGTGGAGGGTAGAAAACATTCTCTGAACAACCTAGTT AACTTGGCTTTGAGTTCCACCTGTACCCTTGCATAATCTTGGGAAAGT GAGTTGCCCTAATTCAGTGACATTAATAAATTTATTAATTTCTTCTTTCAA TAAACCTGGAGAGAGCTTCATATGTATCAGCATATGCTAACTTGAAAG ATACAAGTAGAAAATGGAAGGAAATATATCTGACTCAATAGGGATAGTTT AAGGGTTAAATTAAAAGTAGTAAAGTATTATAATTAATCTGACATGGTAC CTAATATATAATAATCATGTATTAAAGAAATGCCAGTCACCATTAAGTCA ATGTATGACTTTAATCTACTCGAGGAAAGAACTATGTCTTGTTCCTGT TATTATCTCTAAATCCATAATCAGAAGAGCACCATGTGTATGAGCCACA

			CAATAAATATCTACTGTATAATATGTCTCTTCTTGTGTTTTAACCTTCATA GATAAGACTCTATTGAATTGGGACATTAGTCCAGCAAGCCATTCTGTCTC TGTCTCTTCTATGGAGGGAAAGGTTTAACCATCAAAGACTAGGTGCATCT CCCAAACAACCTGAATTTAATATTCAAATATGTATCTAAATTCATTGTT ACATTTTTGTGTTTCAGCTTACATATTACTTTTTGAGCGACATCTATTCAA GGCTACTACTTGTGCTCTACAAAATATTGCCATGCTCTATTTGCCCAT TAACTATTTCTTAACCTTCAAGGGACATGCTCAGTTCTGATATACCAAGA TTTGGTATTTACCTCCCAGCCTACATACTTCCAATCTTAAGAGAACAAT TTTTAGACTACATTCAAATATAGACCTCTCCACCCCATCAACTATTTTAT CTCTCCTCTCCTATCTTTCTTGACAAAGAGTGATTAGAAATATGCAATGT ATTTTCATCTTTAAAAGTTCTATGCTAGGTGGCTCATATATTAAGTCTTA AATAATTACAAATTGATAAAAAAATCAGTCAATAAAAAATTACTTACGCA TTCTGGAATTTGTACTCTCCAAGCTGCTCAAAAAGCTCACAATTTTGTTT GATTAAATTCTGAGGCTCTTCCACAAGAGGTTTAAATTCATCGAACTGAA ACATAAAAGAATTGTGTTAATAGTATTATGCCTCAGGATCAGATGCAGGT TGTTTTTCAACCTCTGGTCAGAATGATATCCTTTCTTCTCTCTACTCAA AGCCACATGAATAGATGAATATCAATGCCGCTCTGTGACAAATACATTGCA ACTATTTATTTGGTGAGCTTGCAATCATGTAAGTGGTTAAGAAATATTGAG CTCTGGAGTTCAATGACCAGTATTTGAGCCAAGTCATGTAACCTATTCTT CAGTTGTGCAATTTACTTACCATCTCCACATTTTCAAGATCCTTATCCCT AAAATAGGGGCAAGATGGCACTCATCTCATAGAGCTGCTCTGTAAACCA GGTAAGCTAATGAAATTAAACAGTGCCTTGCATATAATAAAAGTTAATA AATGATGGCTATGATTGTACATATTATTATTATTAGTTTACCTTTCCAGT TAGATTCCAGGCTCTCCAAAATGAGAGATTTTACGTTGGTCATTGTTTGT GCATGGTGCCCTGGCATACAGTAGCTTCTTGAAAGCAGGAAAAATGAGTTT TCTCTGATTCTGAATAGTTCAGAAACCATACATATGCTAGTGGGAACCC TAAAAATCCTCCAGAACAGATTTCTCTCTCTAACTTAATTTCTTGTA CTATTCTTAAACATCCTCAAAAAGATTTGAAAAATTTCTAAAGGGATAGG TTCTATAATTTGCCATAAGATACTAATTTCTAGTACTTGATTAATCTGGA ATCAGGTTAACTCACTTTACATCTAAGTTAAATATCTTCTAATTAACATT TAAATTTAATTTTTTTTTGTTCTCAGGATTGTGAAAAAGAGAAAAAAGA TCAAAATTTTTTAGAGATTGCTCTATTTCAGATCTTCTATTCTAACTAGT CTAAATTTTGTCCTAG
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Viral Genome Component: FXN-Encoding Sequences

[0222] In some embodiments, the disclosure provides an AAV particle comprising a viral genome encoding a FXN protein, e.g., a human frataxin protein, wherein the viral genome comprises the polynucleotide sequence of any one of SEQ ID NOs: 1797, 1801, 1808, and 1809, or a nucleotide sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto. In some embodiments, the viral genome comprises the polynucleotide sequence of SEQ ID NO: 1797, or a nucleotide sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto. In some embodiments, the viral genome comprises a promoter operably linked to a polynucleotide sequence encoding a FXN protein. In some embodiments, the viral genome comprises components as arranged in **FIG. 2**.

[0223] In some embodiments, the disclosure herein provides constructs that allow for improved expression of FXN protein (e.g., a human frataxin protein) delivered by gene therapy vectors.

[0224] In some embodiments, the disclosure provides constructs that allow for improved biodistribution of FXN protein (e.g., a human frataxin protein) delivered by gene therapy vectors.

[0225] In some embodiments, the present disclosure relates to a composition containing or comprising a nucleic acid sequence encoding a FXN protein (e.g., a human frataxin protein) or a functional fragment or variant thereof and methods of administering the composition *in vitro* or *in vivo* in a subject, e.g., a human subject and/or an animal model of disease, e.g., Friedreich’s Ataxia.

[0226] In some embodiments, the disclosure provides a nucleotide sequence encoding a FXN protein (e.g., a human frataxin protein) for use in an AAV viral genome, wherein the nucleotide sequence comprises any one of the sequences provided in Table 6G, or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to any one of the sequences provided in Table 6G. In some embodiments, the AAV viral genome further comprises one or more, e.g., all of, a 5’ ITR sequence, a promoter, an intron sequence, a polyA sequence, at least one miR122 binding site, and a 3’ ITR region. In some embodiments, the FXN protein encoded by the viral genome is a human FXN protein.

Table 6G. Exemplary Frataxin Sequences

Type	Species	Description	SEQ ID NO
PRT	<i>Homo sapiens</i>	NP_000135.2	5124
PRT	<i>Homo sapiens</i>	NP_852090.1	5125
PRT	<i>Homo sapiens</i>	NP_001155178.1	5126
DNA	<i>Homo sapiens</i>	NM_000144.4 encodes NP_000135.2	5127
DNA	<i>Homo sapiens</i>	NM_181425.2 encodes NP_852090.1	5128
DNA	<i>Homo sapiens</i>	NM_001161706.1 encodes NP_001155178.1	5129

Table 6H. Exemplary Frataxin Sequences

SEQ ID NO:	Sequence
1824	ATGTGGACTCTCGGGCGCCGCGCAGTAGCCGGCCTCCTGGCGTCACCCAGCCAGCCAGGCCAGAGCCAGACCCGAGTCCCGCGCCGCGCAGAGTTGGCCCCACTCTGCGGCCGCGCTGGCCTGCGCACCCGACATCGATGCGACCTGCACGCCCGCGCGCAAGTTCGAACCAACGTGGCCTCAACCAGATTGGAATGTCAAAAAGCAGAGTGCTATTTGATGAATTTGAGGAAATCTGGAACCTTTGGGCCACCCAGGCTCTCTAGATGAGACCACCTATGAAAGACTAGCAGAGGAAACGCTGGACTCTTTAGCAGAGTTTTTTGAAGACCTTGACAGACAAGCCATACACGTTTGAGGACTATGATGTCTCCTTTGGGAGTGGTGTCTTAACTGTCAACTGGGTGGAGATCTAGGAACCTATGTGATCAACAAGCAGACGCCAAACAAGCAAATCTGGCTATCTTCTCCATCCAGTGGACCTAAGCGTTATGACTGGACTGGGAAAACTGGGTGTACTCCCACGACGCGTGTCCTCCATGAGCTGCTGGCCGCAGAGCTCACTAAAGCCTTAAAAACCAAACTGGACTTGCTCTCCTTGGCCTATTCGGAAAAGATGCTTGA
1825	MWTLGRRVAVGLLASPSPAQAQTLTRVPRPAELAPLCGRRLRTDIDATCTERRASSNQRLNQIWNVKKQSVYLMNLRKSGTLGHFPGSLDETTYERLAEETLDSLAEFFEDLADKPYTFEDYDVVSFSGSVLTVKLGDLGTYVINKQTPNKQIWLSSPSSGPKRYDWTGKNWVYSHDGVSLHELLAELTKALKTKLDLS SLAYSGKDA

[0227] In some embodiments, the FXN protein is encoded by the nucleotide sequence of SEQ ID NO: 1824, or a nucleotide sequence at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0228] In some embodiments, the encoded FXN protein comprises the amino acid sequence of SEQ ID NO: 1825, or an amino acid sequence at least 90% (e.g., at least 90%, at least 91%, at least 92%, at

least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0229] In some embodiments, the AAV viral genome comprises a combination of coding and non-coding nucleic acid sequences.

[0230] In some embodiments, the FXN-encoding sequence encodes a gene therapy product including, but not limited to, a polypeptide, protein, RNA molecule, or other gene product that, when expressed in a target cell, provides a desired therapeutic effect. In some embodiments, a gene therapy product may provide a substitute for a non-functional gene or a gene that is absent, expressed in insufficient amounts, or mutated. In some embodiments, a gene therapy product may comprise a substitute for a non-functional protein or polypeptide or a protein or polypeptide that is absent, expressed in insufficient amounts, misfolded, degraded too rapidly, or mutated. For example, a gene therapy product may be encoded by a polynucleotide encoding a FXN protein to treat FA. In some embodiments, the gene therapy product is encoded by a polynucleotide sequence in an AAV genome, wherein the AAV genome comprises the nucleotide sequence of SEQ ID NO: 1797. In some embodiments, the gene therapy product is encoded by a polynucleotide sequence in an AAV genome, wherein the AAV genome comprises the nucleotide sequence of SEQ ID NO: 1801. In some embodiments, the gene therapy product is encoded by a polynucleotide sequence in an AAV genome, wherein the AAV genome comprises the nucleotide sequence of SEQ ID NO: 1808. In some embodiments, the gene therapy product is encoded by a polynucleotide sequence in an AAV genome, wherein the AAV genome comprises the nucleotide sequence of SEQ ID NO: 1809.

[0231] In some embodiments, the payload encodes a messenger RNA (mRNA). As used herein, the term “messenger RNA” (mRNA) refers to any polynucleotide that encodes a polypeptide of interest and that is capable of being translated to produce the encoded polypeptide of interest *in vitro*, *in vivo*, *in situ*, or *ex vivo*. Certain embodiments provide the mRNA as encoding FXN (e.g., human FXN) or a variant thereof.

[0232] In some embodiments, a FXN-encoding sequence may comprise or encode a selectable marker. In some embodiments, a selectable marker may comprise a gene sequence or a protein or polypeptide encoded by a gene sequence expressed in a host cell that allows for the identification, selection, and/or purification of the host cell from a population of cells that may or may not express the selectable marker. In some embodiments, the selectable marker provides resistance to survive a selection process that would otherwise kill the host cell, such as treatment with an antibiotic. In some embodiments, an antibiotic selectable marker may comprise one or more antibiotic resistance factors, including but not limited to neomycin resistance (*e.g.*, neo), hygromycin resistance, kanamycin resistance, and/or puromycin resistance. In some embodiments, a FXN-encoding sequence may comprise a selectable marker including, but not limited to, β -lactamase, luciferase, β -galactosidase, or any other reporter gene as that term is understood in the art.

[0233] In some embodiments, a FXN-encoding sequence may encode a selectable marker comprising a fluorescent protein. A fluorescent protein as herein described may comprise any fluorescent marker including but not limited to green, yellow, and/or red fluorescent protein (GFP, YFP, and/or RFP). In some embodiments, a FXN-encoding sequence may encode a selectable marker comprising a human influenza hemagglutinin (HA) tag.

[0234] In certain embodiments, a nucleic acid for expression of a FXN protein in a target cell will be incorporated into the viral genome and located between two ITR sequences.

Exemplary FXN AAV Viral Genome Sequence Regions and ITR-to-ITR Sequences

[0235] In some embodiments, a viral genome described herein comprises a promoter operably linked to a transgene encoding a FXN protein (e.g., a human FXN protein). In some embodiments, the viral genome further comprises an inverted terminal repeat (ITR) region, a promoter, an intron/exon region, a miR binding site region, a polyA region, or a combination thereof (e.g., all of these elements).

[0236] Exemplary sequence regions within ITR-to-ITR sequences for viral genomes according to the description are provided in Table 7 and Table 8A and in further disclosures below. An exemplary arrangement of an ITR-to-ITR sequence is also depicted in **FIG. 2**. Exemplary ITR-to-ITR sequences are provided in Table 8B.

Table 7. Exemplary Sequence Regions within ITR-to-ITR Sequences

ITR-to-ITR (5' to 3')	Exemplary Component(s)
5' ITR	AAV2 ITR
Promoter	truncated CBA promoter or truncated CMV promoter
Exon and Intron Region	ie1 exon 1, ie1 intron 1 partial, human beta globin intron 2, and human beta globin intron 3
FXN-encoding sequence	wildtype frataxin-encoding sequence; codon-optimized frataxin-encoding sequence
miR binding site series-encoding sequence	Nucleotide sequence encoding miR122 binding site (3 copies)
Poly(A)	hGH PolyA
Stuffer	albumin stuffer
3' ITR	AAV2 ITR

Table 8A. Exemplary ITR-to-ITR Sequences

Sequence (SEQ ID NO)	Description (5' to 3')	Length
1797	5' ITR (SEQ ID NO: 1811); Promoter (SEQ ID NO: 1742); Intron/Exon (SEQ ID NO: 1816); FXN-encoding sequence (SEQ ID NO: 1824); miR122 binding site series-encoding sequence (SEQ ID NO: 1826); PolyA region (SEQ ID NO: 1828); Stuffer sequence (SEQ ID NO: 1841); 3' ITR (SEQ ID NO: 1812)	4600
1801	5' ITR (SEQ ID NO: 1811);	4600

	Promoter (SEQ ID NO: 1750); Intron/Exon (SEQ ID NO: 1816); FXN-encoding sequence (SEQ ID NO: 1824); miR122 binding site series-encoding sequence (SEQ ID NO: 1826); PolyA region (SEQ ID NO: 1828); Stuffer sequence (SEQ ID NO: 1841); 3' ITR (SEQ ID NO: 1812)	
1808	5' ITR (SEQ ID NO: 1811); Promoter (SEQ ID NO: 1738); Intron/Exon (SEQ ID NO: 1816); FXN-encoding sequence (SEQ ID NO: 1824); miR122 binding site series-encoding sequence (SEQ ID NO: 1826); PolyA region (SEQ ID NO: 1828); Stuffer sequence (SEQ ID NO: 1841); 3' ITR (SEQ ID NO: 1812)	4600
1809	5' ITR (SEQ ID NO: 1811); Promoter (SEQ ID NO: 1740); Intron/Exon (SEQ ID NO: 1816); FXN-encoding sequence (SEQ ID NO: 1824); miR122 binding site series-encoding sequence (SEQ ID NO: 1826); PolyA region (SEQ ID NO: 1828); Stuffer sequence (SEQ ID NO: 1841); 3' ITR (SEQ ID NO: 1812)	4600

Table 8B. Exemplary ITR-to-ITR Sequences

SEQ ID NO:	ITR-to-ITR Sequence
1797	CCTGCAGGCAGCTGCGCGCTCGCTCGTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTGGGCGACCT TTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCCAGAGAGGGAGTGCCAACTCCATCACTAGGGGTT CCTTGTAGTTAATGATTAACCCGCCATGCTACTTATCTACGTAGCCATGCGTCGACATAACGCGTGGTG CGGCGGCAGCCAAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGGCGGCGCGGC CCTATAAAAAGCGAAGCGCGCGCGGGGAGCAAGCTTCGTTTAGTGAACCGTCAGATCGCCTGGAG ACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGATTCTGAATC CCGGCCGGGAACGGTGCATTGGAACGCGGATTTCCCGTGCCAAGAGTGACGTAAGTACCGCCTATAGAG TCTATAGGCCCAAAAAATGCTTCTTCTTTTAATATACTTTTTTGTATTATCTATTTCTAATACTTT CCCTAATCTCTTCTTTCAGGGCAATAATGATACAATGTATCATGCCTCTTTGCACCATTTCTAAGAAT AACAGTGATAATTTCTGGGTTAAGGCAATAGCAATATTTCTGCATATAAATATTTCTGCATATAAATG TAACTGATGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCCAGCTACCATTCTGCTTTTATTTTA TGGTTGGGATAAGGCTGGATTATCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGTTTCATACCTCTT ATCTTCTCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCAAAGAATTG GGATTCGAACCGGTATGTGGACTCTCGGGCGCCGCGCAGTAGCCGGCCTCCTGGCGTCACCCAGCCAG CCCAGGCCAGACCTCACCCGGGTCCGCGGGCGGCAGAGTTGGCCCACTCTGCGGCCCGCGTGGCC TGCGCACCGACATCGATGCGACCTGCACGCCCCGCGCGCAAGTTCGAACCAACGTGGCCTCAACCAGA TTTGGAATGTCAAAAAGCAGAGTGCTTATTTGATGAATTTGAGGAAATCTGGAACCTTTGGGCCACCCAG GCTCTCTAGATGAGACCACCTATGAAAGACTAGCAGAGGAAACGCTGGACTCTTTAGCAGAGTTTTTTG AAGACCTTGCAGACAAGCCATACACGTTTGAGGACTATGATGTCTCTTTTGGGAGTGGTGTCTTAACTG TCAAACCTGGGTGGAGATCTAGGAACCTATGTGATCAACAAGCAGACGCCAAACAGCAAATCTGGCTAT CTTCTCCATCCAGTGGACCTAAGCGTTATGACTGGACTGGGAAAAACTGGGTGTAATCCACGACGGCG TGTCCTCCATGAGCTGCTGGCCGAGAGCTCACTAAAGCCTTAAAAACCAAATGGACTGTCTTCTCCT TGGCCTATTCCGAAAAAGATGCTTGACTCGAGCCATTGACTAGTACAAACACCATTGTCACTCCACA CAAACACCATTGTCACTCCACACAAACACCATTGTCACTCCACTGCAGTCAGGTCTATCCTGAGG ATGGGTGGCATCCCTGTGACCCCTCCCCAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCCA CCAGCCTTGTCCTAATAAAATTAAGTTGCATCATTTTGTCTGACTAGGTGTCTTCTATAATATTATGG

	GGTGGAGGGGGTGGTATGGAGCAAGGGGCAAGTTGGGAAGACAACTGTAGGGCTGCGGGGTCTATT GGGAACCAAGCTGGAGTGCAGTGGCACAATCTTGGCTCACTGCAATCTCCGCCCTCTGGGTTCAAGCGA TTCTCCTGCCTCAGCCTCCCGAGTTGTTGGGATCCAGGCATGCATGACCAGGCTCAGCTAATTTTTGT TTTTTTGGTAGAGACGGGGTTTACCATATTGGCCAGGCTGGTCTCCAACCTCCTAATCTCAGGTGATCT ACCCACCTTGGCTCCCAAATTGCTGGGATTACAGGCGTGAACCACTGCTCCCTTCCCTGTCTTGGCC TAGGTTTCTTGAGACCTCTACAAGAGTTGGAGTTGACACTTGGGGTACTTTCTTGGTGTAAACGAATAA TAGCCTGAAAAAAGAAGTCATGTGTTTTCAGCAAGGCAAGAACTGTCTAACATAGTAGATAAAACAG AGAACACTTGGCCGGAATCAACTAAGATGTTGCTATGTTCCATTCATCATATTATCTCCATCTGCAGAG TAGTGGGTTAGTGGAGGTAGAAAACATCTCTGAACAACCTAGTTAACTTGGCTTTGAGTTCACCT GTACCACCTTGCCATAATCTTGGGAAAGTGAGTTGCCAATTCAGTGACATTAATAAATTTATTAATTTCT TCTTTCAATAAAACCTGGAGAGAGCTTCATATGTATCAGCATATGCTAAACTTGAAAGATACAAGTAGA AAATGGAAGGAAATATATCTGACTCAATAGGGATAGTTCAAGGGTTAAATTTAAAGTAGTAAAGTATTA TAATTAATCTGACATGGTACCTAATATATAATAATCATGTATTAAGAATGCCAGTCACCATTAAGAGTC AATGTATGACTTTAATCTACTCAGGAAAGAACTATGTCTTGTTCAGTGTATTATTTATCTTAAATCCA TAATCAGAAGAGCACCATGTGTATGAGCCACACAATAAATATCTACTGTATAATATGTCTCTCTGTGTT TTTAACCTTCATAGATAAGACTCTATTGAATTGGGACATTAGTCCAGCAAGCCATTCTGTCTCTGTCTC TTCTATGGAGGGAAAGGTTTAACCATCAAAGACTAGGTGCATCTCCAAACAACCTGAATTTAATATTC AAATATGTATCTAAATTCATTTGTACATTTTTGTGTTTGTGTTTGTGTTTGTGTTTGTGTTTGTGTTTGT ATTCAAGGCCCTACTACTTGTCTCTACAAAATATTGCCATGCTCTATTGCCCCATTAACTATTTCTTA ACCTTCAAGGGACATGCTCAGTCTGTATATACCAAGATTGGTATTTACCTCCAGCCTACATACTTC CAATCTTAAGAGAACAATTTTAGACTACATTCAAATATAGACCTCTCCACCCCATCAACTATTTTATC TCTCCTCTCTATCTTTCTTGACAAAGAGTGATTAGAATATGCAATGATTTTTCATCTTAAAGTTCT TATGCTAGGTGGCTCATATATTAAGTCTTAAATAATTACAAATTGATAAAAAAATCAGTCAATAAAAA TTACTTACGCATCTTGAATTTGTACTCTCAAGCTGCTCAAAAAGCTCACAATTTTGTGTTGATTAAAT TCTGAGGCTCTTCCACAAGAGGTTTAAATTCATCGAAGTGAACATAAAAAAGATTGTGTTAATAGTATT ATGCTTCAGGATCAGATGCAGGTTGTTTTTCAACCCCTCTGGTCAGAATGATATCTTCTCTCTAC TCAAAGCCACATGAATAGATGAATATCAATGCCGCTCTGTGCACAATACATTGCAACTATTTATTTGGTG AGCTTGCATCATGTAAGTGGTTAAGAATATTCAGGCTCTGGAGTTCAATGACCAGTATTTGAGCCAGT CATGTAACCTATTCTTCAAGTTGTGCAATTTTACTTACCATCTCCACATTCAGAATCCTTATCCCTAAA ATAGGGGCAAAAGATGGCACTCATCTCATAGAGCTGCTCTGTAAACAGGTAAGCTAATGAAATTAACA GTGCCTTGCATATAATAAAAGTTTAATAAATGATGGCTATGATTGTACATATTATTTATTTAGTTTAC CTTTCCAGTTAGATTCCAGGCTCTCCAAAATGAGAGATTTTACGTTGGTCATTGTTTGTGATGTTGCC TGGCATACAGTAGCTTCTTGAAGCAGGAAAAATGAGTTTCTCTGATTCTGAATAGTTTCAAGAACCAT TACATATGCTAGTGGGAACCTTAAAAATCTCCAGAACAGATTCTCTCTCTCTCTCTCTCTCTCTCTCTCT AACTATTCTTAAACATCTCTCAAAAAGATTGAAAAATTTCTAAAGGGATAGGTTCTATAAATTTGCCATA AGATACTAATTTAGTACTTGTATTAATCTGGAATCAGGTTAACTCACTTTACATCTAAGTTAAATATC TTCTAATTAACATTTAAATTTAATTTTTTTTTTGTCTCAGGATTGTGAAAAAGAGAAAAAAGATCAAAA ATTTTGTAGAGATTGCTTATTCATGCTTTTCTATTCTAAGTCTAATTTTGTCTAGGATGCTGAT GCTACGTAGATAAGTAGCATGGCGGTTAATCATTAACACAGAGGAACCCCTAGTGTAGGAGTTGGCC ACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCAGCGCCGGGCTTT GCCCGGGCGGCTCAGTGAGCGAGCGAGCGCGAGCTGCCTGCAGG
1801	CCTGCAGGCGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAGCCCGGGCGTGGGCGACCT TTGGTCGCCCGGCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAAGCTCATCTACTAGGGGTT CCTTGTAGTTAATGATTAAACCCGCCATGCTACTTATCTACGTAGCCATGCGTCGACATAACCGGTTTTT GGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCGCCCCATTGACGCAATGGGCGGTA GGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCAAGCTTCGTTTAGTGAACCGCTCAGATCGCCTGG AGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGATTGCAA TCCCGGCCGGGAACGGTGCATTGGAACGCGGATTCCCGGTGCCAAGAGTGACGTAAGTACCGCTATAG AGTCTATAGGCCCACAAAAAATGCTTTCTTCTTTAATATACTTTTTTGTATTATCTATTCTAATACT TTCCCTAATCTCTTTCTTTCAGGGCAATAATGATACAATGTATCATGCCCTCTTTGACCATCTTAAAGA ATAACAGTGATAATTTCTGGGTAAAGCAATAGCAATATTTCTGCATATAAATATTTCTGCATATAAAT TGTAACGTATGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCCAGCTACCATTCTGCTTTTTATTT TATGGTTGGGATAAGGCTGGATTATTTCTGAGTCCAAGCTAGGCCCTTTTGTCTAATCATGTTTCATACCTC TTATCTTCTCTCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCAAGAAAT TGGGATTGCAACCGGTATGTGGACTCTCGGGCGCCGCGCAGTAGCCGGCTCTTGGCTCACCAGCCG AGCCCAGGCCAGACCCTCACC CGGGTCCCGCGGCCGCGCAGAGTTGGCCCATCTGCGGCCCGCGTGG CCTGCGCACCGACATCGATGCGACCTGCACGCCCGCCGCGCAAGTTTGAACCAACGTGGCTCAACCA GATTTGGAATGTCAAAAAGCAGAGTGTCTATTTGATGAATTTGAGGAAATCTGGAACCTTTGGGCCACCC GAGTCTCTAGATGAGACCACTATGAAAGACTAGCAGAGGAAACGCTGGACTCTTTGAGCAGAGTTTTT TGAAGACCTTGACAGACAAGCCATACACGTTTGAGGACTATGATGTCTCTTTGGGAGTGGTGTCTTAAC TGTCAAACTGGGTGGAGATCTAGGAACCTATGTGATCAACAAGCAGACGCCAAACAGCAATCTGGCT ATCTTCTCCATCCAGTGGACCTAAGCGTTATGACTGGACTGGGAAAACTGGGTGTACTCCACGACGG

	CGTGTCCCTCCATGAGCTGCTGGCCGCGAGAGCTCACTAAAGCCTTAAAAACCAAAGTGGACTTGTCTTC CTTGGCCTATTCCGGAAGATGCTTGACTCGAGCCATTGACTAGTACAAACACCATTGTCACACTCCA CACAAACACCATTGTCACACTCCACACAAACACCATTGTCACACTCCACTGCAGTCAGGTCTATCCTGA GGATGGGTGGCATCCCTGTGACCCCTCCCCAGTGCCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGCC CACCAGCCTTGCTTAATAAAATTAAGTTGCATCATTTTGTCTGACTAGGTGTCCTTCTATAATATTAT GGGGTGGAGGGGGTGGTATGGAGCAAGGGGCAAGTTGGGAAGACAACCTGTAGGGCCTGCGGGGTCTA TTGGGAACCAAGCTGGAGTGCAGTGGCACAATCTTGGCTCACTGCAATCTCCGCCCTCGGGTTCAAGC GATTCTCCTGCTCAGCCTCCCGAGTTGTTGGGATTCCAGGCATGCATGACCAGGCTCAGCTAATTTTT GTTTTTTTGGTAGAGACGGGGTTTACCATATTGGCCAGGCTGGTCTCCAACTCCTAATCTCAGGTGAT CTACCCACCTTGGCCTCCCAAATTGCTGGGATTACAGGCGTGAACCACTGCTCCCTTCCCTGCTCTTGG CCTAGGTTTCTTGAGACCTCTACAAGAGTTGGAGTTGACACTTGGGGTACTTTCTTGGTGTAAACGAACT AATAGCCTGAAAAAAGAAGTCATGTGTTTTAGCAAGGCAAGAACTGTCTAACATAGTAGATAAAAC AGAGAACACTTGGCCGAATCAACTAAGATGTTGCTATGTTCCATTATCATATTATCTCCATCTGCAG AGTAGTGGGTTAGTGGAGGGTAGAAAAACATTCTCCTGAACAACAGTTAAACTTGGAGTTTCAGTTCCAC CTGTACCCTTGCCATAATCTTGGGAAGTGAGTTGCCTAATTCAGTGACATTAATAAATTTATTAATTT CTTCTTTCAATAAAACCTGGAGAGAGCTTCATATGTATCAGCATATGCTAAACTTGAAAGATACAAAGTA GAAATGGAAGGAAATATATCTGACTCAATAGGGATAGTTCAAGGGTTAAATTTAAAGTAGTAAAGTAT TATAAATTAATCTGACATGGTACCTAATATATAATAATCATGTATTAAAGATGCCAGTCAGTTTAAAG TCAATGTATGACTTTAATCTACTCGAGGAAAGAACTATGTCTTGTTCACTGTTATTATCTCTAAATC CATAATCAGAAAGCACCATGTGTATGAGCCACACAATAAATATCTACTGTATATATGTCTCTCTTG TTTTTAACCTTCATAGATAAGACTCTATTGAATTGGGACATTAGTCCAGCAAGCCATTCTGTCTCTGTC TCTTCTATGGAGGAAAGGTTTAACTCAAGACTAGGTGCATCTCCAAACAACTCAATTTAATAT TCAAATATGTATCTAAATTCATTTGTTACATTTTTGTGTTCACTTACATATTACTTTTTGAGCGACAT CTATTCAAGGCCACTACTTGTGCTCTACAAAATATTGCCATGCTCTATTGGCCATTAACTATTCT TAACCTTCAAGGGACATGCTCAGTTCTGATATACCAAGATTGGTATTTACCTCCAGCCATACACT TCCATGTTTAAAGAGAACAAATTTTGAAGTACATTCAAATATAGACCTCTCCACCCATCACTAATTTA TCTCTCTCTCTCTATCTTTCTTGACAAAGAGTGATTAGAAATATGCAATGTATTTTCACTCTTAAAGT TCTATGCTAGGTGGCTCATATATTAAGTCTTAAATAATTACAAATTGATAAAAAAATCAGTCAATAAA AATTACTTACGCATTCTGGAATTTGACTCTCCAAGCTGCTCAAAAAGCTCACAATTTTGTGTTGATTAA ATTCTTAGGCTCTTCCACAAGAGGTTTAAATTCATCGAAGTGAACATAAAAGATGTGTTAATGAT TTATGCCTCAGGATCAGATGCAGGTTGTTTTTCAACCCCTCTGGTCAGAATGATATCCTTTCTCTTCCT ACTCAAAGCCACATGAATAGATGAATATCAATGCCGCTCTGTCCAAATACATTGAACTATTTATTTGG TGAGCTTGCAATCATGTAAGTGGTTAAGAATATTCAGGCTCTGGAGTTCAATGACCAGTATTTGAGCCAA GTCATGTAAGTCACTCTTCAAGTTGTGCAATTTTACTTACCATCTCCACATTTTCAAGATTCAGAAAC AAATAGGGGCAAGATGGCACTCATCTCATAGAGCTGCTCTGTAACCAGGTAAGCTAATGAAATTTAA CAGTGCCCTTGATATAATAAAAGTTTAAATAAATGATGGCTATGATTGTACATATTATTATTATTAGTTC ACCTTTCCAGTTAGATTCCAGGCTCTCCAAAATGAGAGATTTTACGTTGGTCAATGTTTGTGATGGTG CCTGGCATACAGTAGCTTCTTGAAAGCAGGAAATAGATTTTCTCTGATTCTGAATAGTTTCAAGAAC ATTACATATGCTAGTGGGAACCTAAAAATCTCCAGAACAGATTCTCTCTTAACTTAAATTTCTTGT AAAATATTCTTAAACATCTCTCAAAAAGATTTGAAAATATTCTAAAGGGATAGGTTCTATAATTTGCCA TAAGATACTAATCTTAGTACTTGATTAATCTCTGGAATCAGGTTAACTCACTTTACATCTAAGTTAAATA TCTTCTAATTAACATTTAAATTTAATTTTTTTTTTGTCTCAGGATTGTGAAAAGAGAAAAAAGATCA AAATTTTTTAGAGATTGCTCTATTAGATCTTTCTATTCTAATAGTCTAAATTTTCTTAGGTATCGAT GCTACGTAGATAAGTAGCATGGCGGTTAATCATTAACTACAGAGGAACCCCTAGTGATGGAGTTGGCC ACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGACGCCCGGGCTTT GCCCGGGCGGCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG
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	GGCCCATCACTTTGGCAAAGAATTGGGATTGGAACCGGTATGTGGACTCTCGGGCGCCGCGCAGTAGCC GGCCTCCTGGCGTCACCCAGCCAGCCAGGCCAGACCCCTACCCGGGTCCC GCGGCCGCGCAGAGTTG GCCCCACTCTGCGGCCGCGTGGCTGCGCACCGACATCGATGCGACCTGCACGCCCCGCGCGCAAGT TCGAACCAACGTGGCCTCAACCAGATTTGGAATGTCAAAAAGCAGAGTGTCTATTGTATGAATTTGAGG AAATCTGGAACCTTTGGGCCACCCAGGCTCTCTAGATGAGACCACCTATGAAAGACTAGCAGAGGAAACG CTGGACTCTTTAGCAGAGTTTTTGAAGACCTTGCAGACAAGCCATACACGTTTGAGGACTATGATGTC TCCTTTGGGAGTGGTGTCTTAAGTGTCAAACCTGGGTGGAGATCTAGGAACCTATGTGATCAACAAGCAG ACGCCAAACAAGCAAAATCTGGCTATCTTCTCCATCCAGTGGACCTAAGCGTTATGACTGGACTGGGAAA AACTGGGTGTACTCCCACGACGCGTGTCCCTCCATGAGCTGCTGGCCGCGAGAGCTCACTAAAGCCTTA AAAACCAAACCTGGAAGTGTCTTCTTGGCCTATTCGCGAAAAGATGCTTGACTCGAGCCATTGACTAGT ACAAACACCATTGTCACTCCACACAACACCATTGTCACTCCACACAACACCATTGTCACTC CACTGCAGTCAGTCTATCTGAGGATGGGTGGCATCCCTGTGACCCCTCCCAGTGCCTCTCTCTGGCC CTGGAAGTTGCCACTCCAGTCCCACAGCCTTGTCTTAATAAAATTAAGTTGCATCATTTTGTCTGAC TAGGCTCTCTTCTATAATATTATGGGGTGGAGGGGGTGGTATGGAGCAAGGGGCAAGTTGGGAAGACA ACCTGTAGGGCTGCGGGTCTATTGGGAACCAAGCTGGAGTGCAGTGGCACAATCTTGGCTCACTGCA ATCTCCGCTCTCTGGGTCAAGCGATTCTCTGCTCAGCCTCCCGAGTGTGGGATTCAGGCGATGC ATGACCAGGCTCAGCTAATTTTTGTCTTTTGGTAGAGACGGGTTTACCATATTTGGCCAGGCTGGTC TCCAACCTCTAATCTCAGGTGATCTACCCACCTTGGCTCCCAAATTGCTGGGATACAGGCGTGAACC ACTGCTCCCTTCCCTGCTCTTGGCTAGTCTCTGGAACAACCTAGTTAACTTGGCTTTGAGTTCCACCT GTACCCTTGCATAATCTTGGGAAAGTGAGTTGCCAATTCAGTGACATTAATAAATTTATTAATTTCT TCTTTCAATAAAACCTGGAGAGAGCTTCATATGTATCAGCATATGCTAACTTGAAGATACAAGTAGA AAATGGGAAGAAATATATCTGACTCAATAGGGATAGTTCAAGGGTTAAATTAAGAGTAGTAAAGTATTA TAATTAATCTGACATGGTACCTAATATATAATAATCATGTATTAAGAATGCCAGTCACCATTAAAGTC AATGTATGACTTTAATCTACTCAGGAAAGAACTATGCTTGTTCAGTGTATATCTCTAAATCCA TAATCAGAAGAGCACCATGTGTATGAGCCACACAATAAATATCTACTGTATAATATGCTCTCTCTGT TTTAACCTTCATAGATAAGACTCTATCTGAATTTGGGACATTAGTCCAGCAAGCCATTGCTCTCTCTC TTCTATGGAGGAAAGGTTTAAACCATCAAAGACTAGGTGCATCTCCCAAACAACCTGAATTTAATATTC AAATATGTATCTAAATTCATTTGTACATTTTGTGTTTCTAGCTTACATATTACTTTTGGAGCGACATCT ATTCAGGCTTACTACTTGTCTCTACAAAATATTTGCCATGCTCTATTTGCCCATTAACCTATTTCTTA ACCTTCAAGGGACATGCTCAGTCTCTGATATACCAAGATTGGTATTTACCCTCCAGCTTGGATCTAT CAATCTTAAGAGAACAATTTTAGACTACATTCAAATATAGACCTCTCCACCCCATCAACTATTTTATC TCTCCTCTCTATCTTTCTTGACAAAGAGTGATTAGAAATATGCAATGTATTTTCTATCTTAAAGTTC TATGCTAGGTGGCTCATATATTAAGTCTTAAATAATTACAAATTGATAAAAAAATCAGTCAATAAAAA TTACTTACGCATTTCTGGAATTTGTACTCTCCAAGCTGCTCAAAAAGCTCACAATTTTGTGTGATTAAT TCTGAGGCTCTTCCACAAGAGGTTTAAATTCATCGAAGTGAACACATAAAAGATTGTGTTAATAGTATT ATGCCCTCAGGATCAGATGCAGGTGTTTTTACCCCTCTGGTCAAGATGATATCTTTCTCTCTCTAC TCAAAGCCACATGAATAGATGAATATCAATGCCGCTCTGTCTACAATACATTGCAACTATTTATTTGGTG AGCTTGCATCATGTAAGTGGTTAAGAATATTCAGGCTCTGGAGTTCAATGACCATGATTGAGCAAGT CATGTAACCTATTCTTCTAGTTGTGCAATTTTACTTACCATCTCCACATTCAGAATCCTTATCCCTAAA ATAGGGGCAAAGATGGCACTCATCTCATAGAGCTGCTCTGTAAACCAGGTAAGCTAATGAAATTAACA GTGCCCTGCGATATAATAAAGTTTAAATAAATGATGGCTATGATTGTACATATTATTTATTTAGTTTCA CTTTCTCAGTTAGATTCCAGGCTCTCCAAATGAGAGATTTTACGTTGGTCATTGTTTGTGTCATGGTGCC TGGCATCAGTAGCTTCTTGAAGCAGGAAAAATGAGTTTCTCTGATTCTGAATAGTTTGAAGAACCT TACATATGCTAGTGGGAACCCATAAAATCCTCAGAACAGATTCTCTCTCTAAGTTAATTTCTGTAA AACTATTCCTAAACATCTCAAAAAGATTTGAAAATATCTAAAGGGATAGGTTCTATAATTTGCCATA AGATACTAATTTCTAGTACTTGATTAATCCTGGAATCAGGTTAACTCACTTTACATCTAAGTTAAATATC TCTAATTAACATTTAAATTTAATTTTTTTTTTTTCTCTCAGGATTGTGAAAAAGAGAAAAAAGATCAAA ATTTTTTAGAGATTGCTCTATTAGATCTTTCTATTCTAAGTCTAAATTTTGTCTAGGTATCGAT GCTACGTAGATAAGTAGCATGGCGGGTTAATCATTAACACAGAGGAACCCCTAGTGATGGAGTTGGCC ACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCGCGACGCCCGGGCTTT GCCCGGCGGCTCAGTGAGCGAGCGAGCGCGAGCTGCCTGCAGG
1809	CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCGCGGCAAGCCCGGGCGTGGGCGACCT TTGGTCGCCCCGCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCATCCTAGGGGTT CCTGTAGTTAATGATTAACCCGCTGCTACTTATCTACGTAGCCATGCGTCGACATAACGCGTCCAC GTTCTGCTTCACTCTCCCATCTCCCCCCCCCTCCCAACCCCAATTTTGTATTTATTTATTTTAAAT ATTTTGTGCGAGGATGGGGGCGGGGGGGGGGGCGCGCCAGCGGGGCGGGGCGGGGCGAGGGGCGG GGCGGGGCGAGCGGAGAGGTGCGGCGGCGAGCAATCAGAGCGCGCGCTCCGAAAGTTTCTTTTATG GCGAGGCGGCGGCGGCGGCGGCTATAAAAAGCGAAGCGCGCGGCGGGGCGGGAGCAAGCTTCGTTAG TGAAACCTCAGATCGCTGGAGAGCGCATCCACGCTGTTTTGACCTCCATAGAAAGACCGGACCGGAT CCAGCCTCCGCGGATTGGAATCCGCGCGGGAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGT GACGTAAGTACCGCTATAGAGTCTATAGGCCACAAAAATGCTTTCTCTTTTAAATATACTTTTTTG TTTATCTTATTCTAATACTTTCCCTAATCTCTTTCTTTCAGGCAATAATGATACAATGTATCATGCC

TCTTTGCACCATTCCTAAAGAATAACAGTGATAATTTCTGGGTTAAGGCAATAGCAATATTTCTGCATAT AAATATTTCTGCATATAAATTGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCCAG CTACCATTCTGCTTTTATTTTATGGTTGGGATAAGGCTGGATTATTTCTGAGTCCAAGCTAGGCCCTTTT GCTAATCATGTTTCATACCTCTTATCTTCCTCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTCTGGC CCATCACTTTGGCAAAGAATTGGGATTGGAACCGGTATGTGGAATCTCGGGCGCCGCGCAGTAGCCGGC CTCCTGGCGTCACCCAGCCCAGCCCAGGCCAGACCTCACCCGGGTCGCCGCGCCGCGCAGAGTTGGCC CCTCTGCGGGCGCCGTGGCCTGCGCACCGACATCGATGCGACCTGCGACGCCCCGCGCGCAAGTTCTG AACCAACGTGGCCTCAACCAGATTTGGAATGTCAAAAAGCAGAGTGTCTATTTGATGAATTTGAGGAAA TCTGGAACTTTGGGCCACCCAGGCTCTCTAGATGAGACCACCTATGAAAGACTAGCAGAGGAAACGCTG GACTCTTTAGCAGAGTTTTTGAAGACCTTGACAGACAAGCCATACACGTTTGAGGACTATGATGTCTCC TTTGGGAGTGGTGTCTTAAGTGTCAAACTGGGTGGAGATCTAGGAACCTATGTGATCAACAAGCAGACG CCAAACAGCAAACTCTGGCTATCTTCTCCATCCAGTGGACCTAAGCGTTATGACTGGACTGGGAAAAAC TGGGTGTACTCCACGACGGCGTGTCCCTCCATGAGCTGCTGGCCGCGAGAGCTCACTAAAGCCTTAA ACCAACTGGACTTGTCTTCTGGCCTATTCGGAAAGATGCTTGACTCGAGCCATTGCTGAGCAATACA AACACCATTGTACACTCCACACAACACCATTGTCACTCCACACAACACCATTGTCACTCCAC TGCAGTCAGGTATCTCTGAGGATGGGTGGCATCCCTGTGACCCCTCCCGAGTGCCTCTCTCTGGCCCTG GAAGTTGCCACTCCAGTGGCCACCAGCCTTGTCTAATAAAATTAAGTTGCATCATTTTGTCTGACTAG GTGTCTCTCTATAAATATTTATGGGTGGAGGGGGTGGTATGGAGCAAGGGGCAAGTTGGGAAGACAAC TGTAGGGCCTGCGGGGTCTATTGGGAACCAAGCTGGAGTGCAGTGGCACAATCTTGGCTCACTGCAATC TCCGCTCTCTGGGTTCAGCGATTTCTCTGCTCAGCCTCCCGAGTTGTGGGATTCAGGCATGCATG ACCAGGCTCAGCTAATTTTGTCTTTTGGTAGAGACGGGTTTACCATATTGGCCAGGCTGGTCTCC AACTCTAATCTCAGGTGATCTACCCACCTTGGCCTCCCAAATGTCTGGGATTACAGGCGTGAACCACT GCTCCCTTCCCTGTCTTGGCCTAGGATGTGTCTATGTTCATTATCATATTTATCTCCATCTGCAGAG TAGTGGGTTAGTGGAGGTAGAAAACATCTCTGAAACACTAGTTAACTTGGCTTTGAGTTCCACCT GTACCCTTGCATAATCTTGGGAAAGTGAGTTGCCTAATTCAGTGACATTAATAAATTTATTAATTTCT TCTTTCAATAAAACCTGGAGAGAGCTTATATGTATGACATATGCTAACTTGAAGATACAGATAGAGA AAATGGAAGGAAATATATCTGACTCAATAGGGATAGTTCAAGGGTTAAATTAAGAGTAGTAAAGTATTA TAATTAATCTGACATGTACCTAATATATAAATCATGTATTAAAGATGCCAGTCACCATTAAGAGTC AATGTATGACTTAACTACTCGAGGAAAGAACTATGTCTTGTTCAGTGTATTATCTCTAAATCCA AATCCGAAGAGCACCATGTGTATGAGCCACACAATAAATATCTACTGTATAAATATGCTCTCTGTGTT TTTAACCTTCATAGATAAGACTCTATTGAATTGGGACATTAGTCCAGCAAGCCATTCTGTCTCTGTCTC TTCTATGGAGGAAAGGTTTAAACCATCAAAGACTAGGTGCATCTCCCAACCACTGAATTTAATATTC AAATATGTATCTAAATTCATTTGTACATTTTGTGTTCAGCTTACATATTACTTTTTGAGCGACATCT ATTCAAGGCCTACTACTTGTCTGCTCTACAAAAATATTGCCATGCTCTATTGGCCATTAACTTTCTTA ACCTTCAAGGGACATGCTCAGTTCTGATATACCAAGATTGGTATTTACCCCTCCAGCCTACATACTTC CAATCTTAAGAGAACAATTTTGTAGACTACATTCAAATATAGACCTCTCCACCCCATCAACTATTTTATC TCTCTCTCTCTATCTTTCTTGACAAAGAGTGATTAGAAATATGCAATGTATTTTCATTTCTAAAGTTTC TATGCTAGGTGGCTCATATATTAAGTCTTAAATAATTACAAATGATAAAAAAATCAGTCATAAAAA TTACTTTACGCATTCTGGAATTTGTACTCTCCAAGCTGCTCAAAAAGCTCACAAATTTGTTTGATTAAAT TCTGAGGCTCTTCCACAAGAGGTTTAAATTCATCGAAGTGAACATAAAAGAAATGTGTAAATAGTATT ATGCCCTCAGGATCAGATGCAGGTTGTTTTCACCCCTCTGGTCAAGATGATATCTTTCTCTCTCTAC TCAAAGCCACATGAATAGATGAATATCAATGCCGCTCTGTACAATACATTGAACTATTTATTTGGTG AGCTTGCATCATGTAAGTGGTTAAGAATATTCAGGCTCTGGAGTTCAATGACCAGTATTTGAGCCAAAT CATGTAACCTCATTTCTCAGTTGTGCAATTTTACTTACCATCTCCACATTTCAGAATCCTTATCCCTAAA ATAGGGGCAAAGATGGCACTCATCTCATAGAGCTGCTCTGTAAACCAGGTAAGCTAATGAATTAACA GTGCCTTGCATATAATAAAAGTTTAAATAATGATGGCTATGATTGTACATATTATTTATTTAGTTTAC CTTTCCAGTTAGATTCCAGGCTCTCCAAAATGAGAGATTTACGTTGGTCATTGTTTGTGCATGGTGCC TGGCATAACAGTAGCTTCTTGAAGCAGGAAAAATGAGTTTCTCTGATTCTGAATAGTTTCAGAAACCAT TACATATGCTAGTGGGAACCTTAAAAATCCTCCAGAACAGATTTCCTCTCCTAATTAATTTCTGTAA AACTATTTCTAAACATCTCCTAAAAAGATTTGAAAAATTTCTAAAGGGATAGGTTCTATAATTTGCCATA AGATACTAATTTCTAGTACTTGATTAATCTCGAATCAGGTTAACTCACTTTACATCTAAGTTAAATATC TTCTAATTAACATTTAAATTTAATTTTTTTTGTCTCAGGATTGTGAAAAAGAGAAAAAAGATCAAA ATTTTTTAGAGATTGCTCTATTTCAGATCTTTCTATTCTAAGTCTAATTTTGTCTTAGGTATCGAT GCTACGTAGATAAGTAGCATGGCGGGTTAATCATTAACACAGAGGAACCCCTAGTGATGGAGTTGGCC ACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCGGGCGACCAAAGTCTGCCCCGACGCCGGGCTTT GCCCGGGCGGCTCAGTGAGCGAGCGAGCGCGAGCTGCCTGCAGG

[0237] In some embodiments, the AAV viral genome comprises a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least

94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the nucleotide sequence of SEQ ID NO: 1824. In some embodiments, the AAV viral genome comprises a 5' ITR region comprising the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1811 and/or a 3' ITR region comprising the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1812.

[0238] In some embodiments, the AAV viral genome further comprises or consists of a truncated promoter region of 100-332 nucleotides in length and comprises the nucleotide sequence of any one of SEQ ID NOs: 1738, 1740, 1742, and 1750, or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of any one of SEQ ID NOs: 1738, 1740, 1742, and 1750.

[0239] In some embodiments, the AAV viral genome further comprises an intron/exon region comprising the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1816.

[0240] In some embodiments, the AAV viral genome further comprises a nucleotide sequence encoding a miR122 binding site, wherein the nucleotide sequence encoding the miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1827 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1827. In some embodiments, the AAV viral genome comprises a nucleotide sequence encoding a miR122 binding site series, wherein the nucleotide sequence encoding the miR122 binding site series comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1826.

[0241] In some embodiments, the AAV viral genome further comprises a polyA region comprising the nucleotide sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1828.

[0242] In some embodiments, the AAV viral genome further comprises a filler sequence comprising the nucleotide sequence of any one of SEQ ID NOs: 1838, 1839, 1840, and 1841, or a nucleotide

sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of any one of SEQ ID NOs: 1838, 1839, 1840, and 1841.

[0243] In some embodiments, the AAV viral genome does not comprise a filler sequence.

[0244] In some embodiments, the AAV particle comprises, from 5' to 3', a 5' ITR comprising the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a promoter comprising the nucleotide sequence of SEQ ID NO: 1742 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; an intron/exon region comprising the nucleotide sequence of SEQ ID NO: 1816 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a nucleotide sequence encoding a miR122 binding site series, wherein the nucleotide sequence encoding the miR122 binding site series comprises the nucleotide sequence of SEQ ID NO: 1826 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a polyA region comprising the nucleotide sequence of SEQ ID NO: 1828 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or a 3' ITR comprising the nucleotide sequence of SEQ ID NO: 1812 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the AAV particle comprising the FXN-encoding sequence of SEQ ID NO: 1824 further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1841 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR region.

[0245] In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1809.

[0246] In some embodiments, the viral genome is single-stranded.

[0247] In some embodiments, the viral genome is capable of forming double-stranded DNA. In some embodiments, the viral genome is self-complementary.

[0248] In some embodiments, the viral genome comprises: (i) a 5' inverted terminal repeat (ITR) region; (ii) a promoter; (iii) a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% (e.g., at least 80, at least 85, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1824; and (iv) a 3' ITR region. In some embodiments, features (i), (ii), and/or (iv) may be as described herein.

[0249] In some embodiments, the viral genome comprises: (i) a 5' inverted terminal repeat (ITR) region; (ii) a promoter; (iii) a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the nucleotide sequence of SEQ ID NO: 1824; (iv) a nucleotide sequence at least one miR122 binding site; and (v) a 3' ITR region. In some embodiments, features (i), (ii), (iv), and/or (v) may be as described herein.

[0250] In some embodiments, the viral genome comprises: (i) a 5' inverted terminal repeat (ITR) region; (ii) a promoter; (iii) an intron and/or exon region; (iv) a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the nucleotide sequence of SEQ ID NO: 1824; (v) a nucleotide sequence encoding at least one miR122 binding site; and (vi) a 3' ITR region. In some embodiments, features (i), (ii), (iii), (v), and/or (vi) may be as described herein.

[0251] In some embodiments, the viral genome comprises: (i) a 5' inverted terminal repeat (ITR) region; (ii) a promoter; (iii) an intron and/or exon region; (iv) a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the nucleotide sequence of SEQ ID NO: 1824; (v) a nucleotide sequence encoding at least one miR122 binding site; (vi) a polyadenylation (polyA) region; and (vii) a 3' ITR region. In some embodiments, features (i), (ii), (iii), (v), (vi), and/or (vii) may be as described herein.

[0252] In some embodiments, the viral genome comprises: (i) a 5' inverted terminal repeat (ITR) region; (ii) a promoter; (iii) an intron and/or exon region; (iv) a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the nucleotide sequence of SEQ ID NO: 1824; (v) a nucleotide sequence encoding at least one miR122 binding site; (vi) a polyadenylation (polyA) region; (vii) a filler sequence; and (viii) a 3' ITR region. In some embodiments, features (i), (ii), (iii), (v), (vi), (vii), and/or (viii) may be as described herein.

[0253] In some embodiments, the AAV viral genome comprises the nucleotide sequence of SEQ ID NO: 1797 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1797. In some embodiments, the AAV viral genome comprises a truncated promoter comprising or consisting of the nucleotide sequence of SEQ ID NO: 1742, or a sequence that is at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1742.

[0254] In some embodiments, the AAV particle comprises, from 5' to 3', one or more of, e.g., all of, a 5' ITR comprising the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a promoter comprising the nucleotide sequence of SEQ ID NO: 1750 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; an intron/exon region comprising the nucleotide sequence of SEQ ID NO: 1816 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a nucleotide sequence encoding a miR122 binding site series, wherein the nucleotide sequence encoding the miR122 binding site series comprises the nucleotide sequence of SEQ ID NO: 1826 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a polyA region comprising the nucleotide sequence of SEQ ID NO: 1828 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or a 3' ITR comprising the nucleotide sequence of SEQ ID NO: 1812 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the AAV particle comprising the FXN-encoding sequence of SEQ ID NO: 1824 further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1840 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR region.

[0255] In some embodiments, the AAV viral genome comprises the nucleotide sequence of SEQ ID NO: 1801 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1801. In some embodiments, the AAV viral genome

comprises a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1801. In some embodiments, the AAV viral genome comprises a truncated promoter comprising or consisting of the nucleotide sequence of SEQ ID NO: 1750, or a sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1750, where the truncated promoter region is no more than 109 nucleotides in length.

[0256] In some embodiments, the AAV particle comprises, from 5' to 3', a 5' ITR comprising the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a promoter comprising the nucleotide sequence of SEQ ID NO: 1738 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; an intron/exon region comprising the nucleotide sequence of SEQ ID NO: 1816 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a nucleotide sequence encoding a miR122 binding site series, wherein the nucleotide sequence encoding the miR122 binding site series comprises the nucleotide sequence of SEQ ID NO: 1826 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a polyA region comprising the nucleotide sequence of SEQ ID NO: 1828 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or a 3' ITR comprising the nucleotide sequence of SEQ ID NO: 1812 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the AAV particle comprising the FXN-encoding sequence of SEQ ID NO: 1824 further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1838 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR region.

[0257] In some embodiments, the AAV viral genome comprises the nucleotide sequence of SEQ ID NO: 1808 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1808. In some embodiments, the AAV viral genome

comprises a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1808. In some embodiments, the AAV viral genome comprises a truncated promoter comprising or consisting of the nucleotide sequence of SEQ ID NO: 1738, or a sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1738, where the truncated promoter region is no more than 332 nucleotides in length.

[0258] In some embodiments, the AAV particle comprises, from 5' to 3', a 5' ITR comprising the nucleotide sequence of SEQ ID NO: 1811 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a promoter comprising the nucleotide sequence of SEQ ID NO: 1740 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; an intron/exon region comprising the nucleotide sequence of SEQ ID NO: 1816 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a FXN-encoding sequence comprising the nucleotide sequence of SEQ ID NO: 1824 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a nucleotide sequence encoding a miR122 binding site series, wherein the nucleotide sequence encoding the miR122 binding site series comprises the nucleotide sequence of SEQ ID NO: 1826 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; a polyA region comprising the nucleotide sequence of SEQ ID NO: 1828 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or a 3' ITR comprising the nucleotide sequence of SEQ ID NO: 1812 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the AAV particle comprising the FXN-encoding sequence of SEQ ID NO: 1824 further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1839 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR region.

[0259] In some embodiments, the AAV viral genome comprises the nucleotide sequence of SEQ ID NO: 1809 or a sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1809. In some embodiments, the AAV viral genome

comprises a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1809. In some embodiments, the AAV viral genome comprises a truncated promoter comprising or consisting of the nucleotide sequence of SEQ ID NO: 1740, or a sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 1740, where the truncated promoter region is no more than 260 nucleotides in length.

[0260] Without being bound by theory, the FXN-encoding sequences described herein may provide a desirable level of frataxin expression in disease-relevant cells or tissues. For example, in some embodiments, the FXN-encoding sequences described herein facilitate an intermediate level of expression (e.g., 0.5x-3x or 1.5x-4x relative to baseline) such that the resulting expression alleviates one or more symptoms of FXN deficiency or FA while minimizing or avoiding toxicity. In some embodiments, this result is facilitated at least by a truncated promoter as described herein. In some embodiments, the viral genomes described herein minimize or avoid overexpression of frataxin in the liver. In some embodiments, this result is facilitated at least by a nucleotide sequence encoding a miR binding site or series as described herein. In some embodiments, desirable expression levels or distribution of frataxin are further facilitated by the AAV capsid variants described herein. For example, without being bound by theory, the AAV capsid variants described herein may enhance delivery to the cerebellum. Further, without being bound by theory, the AAV capsid variants described herein may enhance delivery to the dentate nucleus. In some embodiments, the AAV capsids described herein may also enhance delivery to the somatosensory cortex.

[0261] In some embodiments, the AAV particle comprises a viral genome encoding a capsid protein, e.g., a structural protein. In some embodiments, the capsid protein comprises a VP1 polypeptide, a VP2 polypeptide, and/or a VP3 polypeptide. In some embodiments, the VP1 polypeptide, the VP2 polypeptide, and/or the VP3 polypeptide are encoded by at least one Cap gene. In some embodiments, the AAV viral genome further comprises a nucleic acid encoding a Rep protein, e.g., a non-structural protein. In some embodiments, the Rep protein comprises a Rep78 protein, a Rep68 protein, a Rep52 protein, and/or a Rep40 protein. In some embodiments, the Rep78 protein, the Rep68 protein, the Rep52 protein, and/or the Rep40 protein are encoded by at least one Rep gene.

[0262] In some embodiments, the AAV particle comprises a viral genome that is packaged in a capsid comprising an amino acid sequence selected from Table 3 or Table 4A. In some embodiments, the amino acid sequence of TQDWHRI (SEQ ID NO: 941) or TNTQDWHRIAQ (SEQ ID NO: 343) is present in loop VIII, wherein loop VIII comprises amino acids 580-599 of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the AAV capsid variant comprises: (i) a VP1 protein comprising or consisting of the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; (ii) a VP2 protein comprising or consisting of the amino acid sequence according to

positions 138-736 of SEQ ID NO: 981 or an amino acid sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or (iii) a VP3 protein comprising or consisting of the amino acid sequence according to positions 203-736 of SEQ ID NO: 981 or an amino acid sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[0263] In some embodiments, the AAV particle comprises a viral genome that is packaged in a capsid comprising the amino acid T at position 584, D at position 586, W at position 587, H at position 588, R at position 589, and I at position 590, numbered according to SEQ ID NO: 981. In some embodiments, the AAV capsid variant comprises the amino acid sequence of amino acids 203-736 of SEQ ID NO: 981, or an amino acid sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the AAV capsid variant comprises the amino acid sequence of amino acids 138-736 of SEQ ID NO: 981, or an amino acid sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the AAV capsid variant comprises the amino acid sequence SEQ ID NO: 981, or an amino acid sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[0264] In some embodiments, the AAV particle comprises a viral genome that is packaged in a capsid comprising the amino acid T at a position corresponding to position 584 of SEQ ID NO: 981, D at a position corresponding to position 586 of SEQ ID NO: 981, W at a position corresponding to position 587 of SEQ ID NO: 981, H at a position corresponding to position 588 of SEQ ID NO: 981, R at a position corresponding to position 589 of SEQ ID NO: 981, and I at a position corresponding to position 590 of SEQ ID NO: 981. In some embodiments, the AAV capsid variant comprises the amino acid sequence of amino acids 203-736 of SEQ ID NO: 981, or an amino acid sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the AAV capsid variant comprises the amino acid sequence of amino acids 138-736 of SEQ ID NO: 981, or an amino acid sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the AAV capsid variant comprises the amino acid sequence SEQ ID NO: 981, or an amino acid sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

[0265] In some embodiments, the present disclosure provides an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0266] In some embodiments, the present disclosure provides an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant

comprising amino acids 138-736, e.g., a VP2, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0267] In some embodiments, the present disclosure provides an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of amino acids 138-736 of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0268] In some embodiments, the present disclosure provides an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 203-736, e.g., a VP3, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0269] In some embodiments, the present disclosure provides an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of amino acids 203-736 of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0270] The present disclosure provides, in some embodiments, vectors, cells, and/or AAV particles comprising the above-identified viral genomes.

Self-Complementary and Single Stranded Vectors

[0271] In some embodiments, the AAV viral genome used in the present disclosure is single-stranded.

[0272] In some embodiments, the AAV viral genome is capable of forming double-stranded DNA. In some embodiments, the AAV viral genome is self-complementary. *See, e.g.*, US Patent No. 7,465,583. scAAV vectors contain both DNA strands that anneal together to form double stranded DNA. By skipping second strand synthesis, scAAVs allow for rapid expression in the cell.

[0273] Methods for producing and/or modifying AAV viral genome and particles are disclosed in the art such as pseudotyped AAV vectors (International Patent Publication Nos. WO200028004; WO200123001; WO2004112727; WO 2005005610 and WO 2005072364, the content of each of which are incorporated herein by reference in their entirety).

II. AAV Production

[0274] Viral production disclosed herein describes processes and methods for producing AAV particles (with enhanced, improved and/or increased tropism for a target tissue), e.g., an AAV particle comprising an AAV capsid variant that may be used to contact a target cell to deliver FXN.

[0275] In some embodiments, disclosed herein is a method of making an AAV particle of the present disclosure, e.g., an AAV particle comprising an AAV capsid variant disclosed herein, wherein the method comprises: (i) providing a cell comprising a viral genome disclosed herein (e.g., a viral genome comprising a FXN-encoding sequence and a nucleic acid encoding an AAV capsid variant disclosed herein); and (ii) incubating the cell under conditions suitable to encapsulate the viral genome in an AAV capsid variant described herein (e.g., an AAV capsid variant listed in Tables 3, 4A, or 4B); thereby

making the AAV particle. In some embodiments, the viral genome comprises: (a) the nucleotide sequence of SEQ ID NO: 2006 or a nucleotide sequence at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or (b) the nucleotide sequence of SEQ ID NO: 2007 or a nucleotide sequence at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 981, amino acids 138-736 of the amino acid sequence of SEQ ID NO: 981, and/or amino acids 203-736 of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the method comprises, prior to step (i), introducing a nucleic acid comprising the viral genome into the cell. In some embodiments, the method comprises, prior to step (i), introducing the nucleic acid encoding the AAV capsid variant into the cell. In some embodiments, the cell comprises a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell. In some embodiments, the AAV particle described herein is an isolated AAV particle. In some embodiments, the AAV particle described herein is a recombinant AAV particle.

[0276] In some embodiments, the present disclosure provides a method of making an AAV particle that comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0277] In some embodiments, the present disclosure provides a method of making an AAV particle that comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 138-736, e.g., a VP2, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0278] In some embodiments, the present disclosure provides a method of making an AAV particle that comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 203-736, e.g., a VP3, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0279] Any method known in the art may be used for the preparation of AAV particles. In some embodiments, AAV particles are produced in mammalian cells (e.g., HEK293 cells). In some embodiments, AAV particles are produced in insect cells (e.g., Sf9 cells).

[0280] Methods of making AAV particles are well known in the art and are described in e.g., U.S. Patent Nos. US6204059, US5756283, US6258595, US6261551, US6270996, US6281010, US6365394, US6475769, US6482634, US6485966, US6943019, US6953690, US7022519, US7238526, US7291498 and US7491508, US5064764, US6194191, US6566118, US8137948; or International Publication Nos. WO1996039530, WO1998010088, WO1999014354, WO1999015685, WO1999047691, WO2000055342, WO2000075353 and WO2001023597; *Methods In Molecular Biology*, ed. Richard, Humana Press, NJ (1995); O'Reilly et al., *Baculovirus Expression Vectors, A Laboratory Manual*, Oxford Univ. Press (1994); Samulski et al., *J. Vir.* 63:3822-8 (1989); Kajigaya et al., *Proc. Nat'l. Acad.*

Sci. USA 88: 4646-50 (1991); Ruffing et al., *J. Vir.* 66:6922-30 (1992); Kimbauer et al., *Vir.*, 219:37-44 (1996); Zhao et al., *Vir.* 272:382-93 (2000); the contents of each of which are herein incorporated by reference in their entirety. In some embodiments, the AAV particles are made using the methods described in International Patent Publication WO2015191508, the contents of which are herein incorporated by reference in their entirety.

III. Pharmaceutical Compositions

[0281] In some embodiments, the present disclosure provides pharmaceutical compositions of an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0282] In some embodiments, the present disclosure provides pharmaceutical compositions of an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 138-736, e.g., a VP2, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0283] In some embodiments, the present disclosure provides pharmaceutical compositions of an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 203-736, e.g., a VP3, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0284] In some embodiments, a composition described herein comprises an AAV polynucleotide or AAV genome or AAV particle, and at least one excipient.

[0285] Although pharmaceutical compositions provided herein, e.g., comprising AAV particles comprising a FXN-encoding sequence, are principally directed to pharmaceutical compositions that are suitable for administration to humans, it will be understood by the skilled artisan that such compositions may be suitable for administration to any other animal, e.g., to non-human animals, e.g., non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[0286] In some embodiments, compositions are administered to humans, e.g., human patients or human subjects.

[0287] In some embodiments, the AAV particle formulations described herein may contain a nucleic acid comprising at least one FXN-encoding sequence. In some embodiments, the formulations may

contain a nucleic acid comprising 1, 2, 3, 4, or 5 FXN-encoding sequences. In some embodiments, the formulation may contain a FXN-encoding sequence encoding a FXN protein selected from categories such as, but not limited to, human proteins, veterinary proteins, bacterial proteins, biological proteins, antibodies, immunogenic proteins, therapeutic peptides and proteins, secreted proteins, plasma membrane proteins, cytoplasmic proteins, cytoskeletal proteins, intracellular membrane bound proteins, nuclear proteins, proteins associated with human disease, and/or proteins associated with non-human diseases. In some embodiments, the AAV genome comprises at least one sequence encoding human FXN. In some embodiments, the AAV genome comprises at least one sequence encoding wildtype human FXN.

[0288] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a “unit dose” refers to a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

IV. Formulations

[0289] Formulations of the AAV pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0290] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the disclosure will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered.

[0291] For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient. By way of example, the composition may comprise between 0.1% and 100%, e.g., between .5% and 50%, between 1-30%, between 5-80%, or at least 80% (w/w) active ingredient.

[0292] The AAV particles of the disclosure can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection or transduction; (3) permit the sustained or delayed release; (4) alter the biodistribution (e.g., target the viral particle to specific tissues or cell types); (5) increase the translation of encoded protein *in vivo*; (6) alter the release profile of encoded protein *in vivo* and/or (7) allow for regulatable expression of the FXN.

[0293] Formulations of the present disclosure can include, without limitation, saline, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with AAV particles (e.g., for transplantation into a subject), nanoparticle mimics and combinations thereof. Further, the AAV particles of the present disclosure may be formulated using self-assembled nucleic acid nanoparticles.

[0294] In some embodiments, the present disclosure provides formulations of an AAV particle that comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0295] In some embodiments, the present disclosure provides formulations of an AAV particle that comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 138-736, e.g., a VP2, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0296] In some embodiments, the present disclosure provides formulations of an AAV particle that comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 203-736, e.g., a VP3, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0297] In some embodiments, the AAV particles encoding FXN may be formulated to optimize baricity and/or osmolality. In some embodiments, the baricity and/or osmolality of the formulation may be optimized to ensure optimal drug distribution in the central nervous system or a region or component of the central nervous system.

Excipients

[0298] The formulations of the disclosure can include one or more excipients, each in an amount that together increases the stability of the AAV particle, increases cell transfection or transduction by the AAV particle, increases the expression of frataxin protein, and/or alters the release profile of frataxin protein. In some embodiments, a pharmaceutically acceptable excipient may be at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use for humans and for veterinary use. In some embodiments, an excipient may be approved by United States Food and Drug Administration. In some embodiments, an excipient may be of pharmaceutical grade. In some embodiments, an excipient may meet the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[0299] Excipients, which, as used herein, include, but are not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (*see* Remington: The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, Lippincott, Williams & Wilkins, Baltimore, MD, 2006; the contents of which are herein incorporated by reference in their entirety). The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any

undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition.

Inactive Ingredients

[0300] In some embodiments, AAV formulations may comprise at least one excipient which is an inactive ingredient. As used herein, the term “inactive ingredient” refers to one or more agents that do not contribute to the activity of the pharmaceutical composition included in formulations. In some embodiments, all, none, or some of the inactive ingredients which may be used in the formulations of the present disclosure may be approved by the US Food and Drug Administration (FDA).

[0301] Formulations of AAV particles disclosed herein may include cations or anions. In one embodiment, the formulations include metal cations such as, but not limited to, Zn^{2+} , Ca^{2+} , Cu^{2+} , Mg^{+} , or combinations thereof. In some embodiments, formulations may include polymers or polynucleotides complexed with a metal cation (*See, e.g.*, U.S. Pat. Nos. 6,265,389 and 6,555,525, the contents of each of which are herein incorporated by reference in their entirety).

V. Uses and Applications

[0302] The compositions of the disclosure may be administered to a subject, e.g., to deliver a FXN protein, e.g., to a subject who has, has been diagnosed with having, or is at risk of having a disorder associated with FXN deficiency (e.g., Friedrich's Ataxia (FA)). The compositions may similarly be used in the manufacture of a medicament for administration to a subject having a FXN protein deficiency, such as FA.

[0303] In some embodiments, the disclosure provides a method of delivering a FXN protein to a subject comprising administering to the subject an effective amount of a pharmaceutical composition or AAV particle disclosed herein that comprises a viral genome encoding a FXN protein. In some embodiments, the subject has, has been diagnosed with having, or is at risk of having a disorder associated with FXN deficiency (e.g., FA).

[0304] In some embodiments, the disclosure provides a method for treating a disorder associated with FXN deficiency, such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (e.g., FA). In some embodiments, the disclosure provides an AAV particle or pharmaceutical composition according to any one of the embodiments disclosed herein for treating a disorder associated with FXN deficiency, such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (e.g., FA). In some embodiments, the present disclosure provides the pharmaceutical composition or the AAV particle of any one the embodiments disclosed herein for use in a method of treating a disorder as disclosed herein, such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (e.g., FA).

[0305] In certain embodiments, a pharmaceutical composition or AAV particle disclosed herein that comprises a viral genome encoding FXN may be administered to a subject to treat a disorder associated with FXN deficiency such as FA, or for treating at least one symptom associated with the disorder

associated with FXN deficiency (e.g., FA). In some embodiments, the subject has, has been diagnosed with having, or is at risk of having a disorder associated with FXN deficiency such as FA. In some embodiments, the subject has, has been diagnosed with having, or is at risk of having at least one symptom of a disorder associated with FXN deficiency such as FA. In some embodiments, the treatment results in a 0.5-3x increase in the subject's FXN protein level as compared to baseline. In some embodiments, the treatment results in a 1.5-4x increase in the subject's FXN protein level as compared to baseline.

[0306] In some embodiments, the treatment may halt or slow progression of FA. In certain embodiments, the treatment improves at least one symptom of FA, e.g., impaired sensory functions, impaired motor function (e.g., ataxia and/or involuntary movements), fatigue, chronic pain, seizures, impaired speech, sleep disturbances, metabolic disorders (e.g., diabetes), and/or increased spasticity. In some embodiments, the treatment stabilizes, slows the progression of, or improves the subject's FA as determined by the modified Friedreich Ataxia Rating Scale (mFARS), the Scale for the Assessment and Rating of Ataxia (SARA), and/or the International Cooperative Ataxia Rating Scale (ICARS). In some embodiments, the treatment slows the subject's progression of FA as measured by mFARS, SARA, and/or ICARS relative to an individual with the disorder associated with FXN deficiency who has not been administered the pharmaceutical composition or the AAV particle.

[0307] In some embodiments, the treatment results in an increase in: (i) the level of FXN protein or FXN gene expression in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region), or a cell or tissue outside of the CNS, e.g., muscle (e.g., skeletal muscle)), and/or fluid (e.g., CSF and/or serum), of the subject, optionally wherein the level of FXN protein or gene expression is increased by 0.5x-3x or 1.5-4x as compared to baseline, further optionally wherein the level of FXN protein or gene expression is increased by 1.05x-1.55x (e.g., in the cerebellum) as compared to an untreated subject; (ii) the number and/or level of viral genomes (VG) per cell in a CNS tissue (e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region) or non-CNS tissue (e.g., muscle (e.g., skeletal muscle)) of the subject relative to the number and/or level of VG per cell in a peripheral tissue of the subject; and/or (iii) the level of FXN activity in a cell, tissue (e.g., a cell or tissue of the CNS, e.g.,

amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral pallidum, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region), or a cell or tissue outside of the CNS, e.g., muscle (e.g., skeletal muscle)), and/or fluid (e.g., CSF and or serum) of the subject).

[0308] Without being bound by theory, AAV particles comprising AAV capsid variants described herein (e.g., AAV9 variants comprising the amino acid sequence of SEQ ID NO: 941 or SEQ ID NO: 981, e.g., in loop VIII) may be desirable for the alleviation or treatment of frataxin deficiency or Friedreich's Ataxia at least due to their tropism of the dentate nucleus. In some embodiments, the present disclosure encompasses the delivery of pharmaceutical, prophylactic, diagnostic, or imaging compositions in combination with agents that may improve their bioavailability, reduce and/or modify their metabolism, and/or modify their distribution within the body.

[0309] In some embodiments, the present disclosure provides delivery of an AAV particle to a subject for treating a disorder associated with FXN deficiency, such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (e.g., FA), wherein the AAV particle comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0310] In some embodiments, the present disclosure provides delivery of an AAV particle to a subject for treating a disorder associated with FXN deficiency such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (e.g., FA), wherein the AAV particle comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 138-736, e.g., a VP2, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0311] In some embodiments, the present disclosure provides delivery of an AAV particle to a subject for treating a disorder associated with FXN deficiency such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (e.g., FA), wherein the AAV particle comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 203-736, e.g., a VP3, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0312] In certain embodiments, the pharmaceutical compositions described herein are used as research tools, particularly in *in vitro* investigations using human cell lines such as HEK293T and *in vivo* testing in nonhuman primates which will occur prior to human clinical trials.

[0313] The present disclosure provides a method for treating a disease, disorder, and/or condition in a mammalian subject, including a human subject, comprising administering to the subject any of the viral

particles *e.g.*, AAV, AAV particle, or AAV genome that produces FXN protein described herein or administering to the subject a particle comprising said AAV particle or AAV genome, or administering to the subject any of the described compositions, including pharmaceutical compositions.

[0314] In some embodiments, the present disclosure provides a method of treating a disorder associated with FXN deficiency, such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (*e.g.*, FA), wherein the method comprises administering an effective amount of an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0315] In some embodiments, the present disclosure provides a method of treating a disorder associated with FXN deficiency, such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (*e.g.*, FA), wherein the method comprises administering an effective amount of an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 138-736, *e.g.*, a VP2, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0316] In some embodiments, the present disclosure provides a method of treating a disorder associated with FXN deficiency, such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (*e.g.*, FA), wherein the method comprises administering an effective amount of an AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 203-736, *e.g.*, a VP3, of the amino acid sequence of SEQ ID NO: 981. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0317] In some embodiments, AAV particles of the present disclosure, through delivery of a functional FXN that is a therapeutic product comprising a FXN protein or variant thereof can modulate the level or function of FXN in the CNS.

[0318] A functional FXN may alleviate or reduce symptoms that result from abnormal level and/or function of the FXN gene product (*e.g.*, an absence or defect in a FXN protein) in a subject in need thereof or that otherwise confers a benefit to a CNS disorder in a subject in need thereof.

[0319] As non-limiting examples, companion or combination therapeutic products delivered by AAV particles of the present disclosure may include, but are not limited to, growth and trophic factors, cytokines, hormones, neurotransmitters, enzymes, anti-apoptotic factors, angiogenic factors, FXN polypeptides, and any protein known to be mutated in pathological disorders such as FA (*e.g.*, brain specific Mir-128a, *See* Adlakha and Saini, *Molecular cancer*, 2014, 13:33, incorporated herein by reference in its entirety).

[0320] In some embodiments, the neurodegenerative disorder is Friedreich's ataxia, e.g., resulting from expansion of an intronic GAA triplet repeat in the FXN gene, which reduces expression of the mitochondrial protein frataxin causing progressive damage to the nervous system.

[0321] In some embodiments, the AAV particles of the present disclosure may be used to ameliorate at least one symptom of FA, including, but not limited to, impaired sensory functions, impaired motor function, e.g., ataxia and/or involuntary movements, fatigue, chronic pain, seizures, impaired speech, sleep disturbances, metabolic disorders, e.g., diabetes, and increased spasticity.

[0322] In some embodiments, the delivery of the AAV particles may halt or slow the disease progression of Friedreich's ataxia by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more than 95% using a known analysis method and comparator group for Friedreich's ataxia. As a non-limiting example, the delivery of the AAV particles may halt or slow progression of Friedreich's ataxia as measured by mFARS/SARA by 50% relative to a comparator group.

[0323] In some embodiments, the AAV particle encoding FXN may increase the amount of FXN in a tissue by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or more than 100%. In some embodiments, the AAV particle encoding FXN may increase the amount of FXN in a tissue to be comparable to (e.g., approximately the same as) the amount of FXN in the corresponding tissue of a healthy individual. In some embodiments, the AAV particle encoding FXN may increase the amount of FXN in a tissue effective to reduce one or more symptoms of a disease associated with decreased FXN expression or a deficiency in the quantity and/or function of FXN, e.g., FA.

VI. Delivery of AAV Particles

Delivery to Cells

[0324] In some aspects, the present disclosure provides a method of delivering to a cell or tissue any of the above-described AAV particles, comprising contacting the cell or tissue with said AAV particle or contacting the cell or tissue with a formulation comprising said AAV particle, or contacting the cell or tissue with any of the described compositions, including pharmaceutical compositions. The method of delivering the AAV particle to a cell or tissue can be accomplished *in vitro*, *ex vivo*, or *in vivo*.

[0325] In some embodiments, the AAV particles are delivered to a cell, tissue, or region of the CNS. In some embodiments, the AAV particles are delivered to a cell or tissue of the amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region).

Delivery to Subjects

[0326] In some aspects, the present disclosure additionally provides a method of delivering to a subject, including a mammalian subject, any of the above-described AAV particles comprising administering to the subject said AAV particle, or administering to the subject a formulation comprising said AAV particle, or administering to the subject any of the described compositions, including pharmaceutical compositions.

[0327] In some embodiments, the AAV particles may be delivered to bypass anatomical blockages (e.g., the blood brain barrier).

[0328] In some embodiments, the AAV particles may be formulated and delivered to a subject by a route which increases the speed of drug effect as compared to oral delivery.

[0329] In some embodiments, the AAV particles may be delivered using intrathecal infusion.

[0330] In some embodiments, a subject may be administered the AAV particles described herein using a bolus infusion.

[0331] In some embodiments, the AAV particles encoding FXN may be delivered in a continuous and/or bolus infusion. A different dosing regimen may be used for different sites of delivery, or the same dosing regimen may be used for each site of delivery. As a non-limiting example, the sites of delivery may be in the cervical and the lumbar region. As another non-limiting example, the sites of delivery may be in the cervical region. As another non-limiting example, the sites of delivery may be in the lumbar region.

[0332] In some embodiments, the AAV particles may be delivered to a subject via a single route of administration.

[0333] In some embodiments, the AAV particles may be delivered to a subject via a multi-site route of administration. For example, a subject may be administered the AAV particles at 2, 3, 4, 5, or more than 5 sites.

[0334] In some embodiments, a subject may be administered the AAV particles described herein using sustained delivery over a period of minutes, hours, or days. The infusion rate may be changed depending on the subject, distribution, formulation, or another delivery parameter known to those in the art.

[0335] In some embodiments, if continuous delivery (continuous infusion) of the AAV particles is used, the continuous infusion may be for 1 hour, 2, hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, or more than 24 hours.

[0336] In some embodiments, the intracranial pressure may be evaluated prior to administration. The route, volume, AAV particle concentration, infusion duration and/or vector titer may be optimized based on the intracranial pressure of a subject.

[0337] In some embodiments, the AAV particles may be delivered by systemic delivery. In some embodiments, the systemic delivery may be by intravascular administration. In some embodiments, the systemic delivery may be by intravenous administration.

[0338] In some embodiments, the AAV particles may be delivered by injection into the CSF pathway. Non-limiting examples of delivery to the CSF pathway include intrathecal and intracerebroventricular administration.

[0339] In some embodiments, an AAV particle described herein is administered intravenously.

[0340] In some embodiments, the AAV particles may be delivered by direct (intraparenchymal) injection into the substance of an organ. *e.g.*, one or more regions of the brain.

[0341] In some embodiments, the AAV particles may be delivered by subpial injection into the spinal cord. For example, subjects may be placed into a spinal immobilization apparatus. A dorsal laminectomy may be performed to expose the spinal cord. Guiding tubes and XYZ manipulators may be used to assist catheter placement. Subpial catheters may be placed into the subpial space by advancing the catheter from the guiding tube and AAV particles may be injected through the catheter (Miyano *et al.*, *Mol Ther Methods Clin Dev.* 2016; 3: 16046). In some cases, the AAV particles may be injected into the cervical subpial space. In some cases, the AAV particles may be injected into the thoracic subpial space.

[0342] In some embodiments, the present disclosure provides delivery of an AAV particle to a subject for treating a disorder associated with FXN deficiency such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (*e.g.*, FA), wherein the AAV particle comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981, and wherein delivery is by intravenous injection. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0343] In some embodiments, the present disclosure provides delivery of an AAV particle to a subject for treating a disorder associated with FXN deficiency such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (*e.g.*, FA), wherein the AAV particle comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 138-736, *e.g.*, a VP2, of the amino acid sequence of SEQ ID NO: 981, and wherein delivery is by intravenous injection. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0344] In some embodiments, the present disclosure provides delivery of an AAV particle to a subject for treating a disorder associated with FXN deficiency such as FA, or for treating at least one symptom associated with the disorder associated with FXN deficiency (*e.g.*, FA), wherein the AAV particle comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising amino acids 203-736, *e.g.*, a VP3, of the amino acid sequence of SEQ ID NO:

981, and wherein delivery is by intravenous injection. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

[0345] In some embodiments, the AAV particles may be delivered by direct injection to the CNS of a subject. In some embodiments, direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intra-cisterna magna injection, or any combination thereof. In some embodiments, direct injection to the CNS of a subject comprises convection enhanced delivery (CED). In some embodiments, administration comprises peripheral injection. In some embodiments, peripheral injection is intravenous injection.

[0346] In some embodiments, the AAV particles may be delivered to a subject to increase a FXN protein level in the CNS (e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region) as compared to a baseline level in the subject. The increase may be 0.1x to 5x, 0.5x to 5x, 1x to 5x, 2x to 5x, 3x to 5x, 4x to 5x, 0.1x to 4x, 0.5x to 4x, 1x to 4x, 2x to 4x, 3x to 4x, 0.1x to 3x, 0.5x to 3x, 1x to 3x, 2x to 3x, 0.1x to 2x, 0.5x to 2x, 0.1x to 1x, 0.5x to 1x, 0.1x to 0.5x, 1x to 2x, 0.1x, 0.2x, 0.3x, 0.4x, 0.5x, 0.6x, 0.7x, 0.8x, 0.9x, 1.0x, 1.1x, 1.2x, 1.3x, 1.4x, 1.5x, 1.6x, 1.7x, 1.8x, 1.9x, 2.0x, 2.1x, 2.2x, 2.3x, 2.4x, 2.5x, 2.6x, 2.7x, 2.8x, 2.9x, 3.0x, 3.1x, 3.2x, 3.3x, 3.4x, 3.5x, 3.6x, 3.7x, 3.8x, 3.9x, 4.0x, 4.1x, 4.2x, 4.3x, 4.4x, 4.5x, 4.6x, 4.7x, 4.8x, 4.9x or more than 5x as compared to a baseline level. In some embodiments, the increase may be 0.5x-3x as compared to a baseline level. In some embodiments, the increase may be 1.5-4x as compared to a baseline level. In some embodiments, the increase may be about 1.05-1.55x as compared to an untreated subject. In some embodiments, the increase may be 1.05x as compared to an untreated subject. In some embodiments, the increase may be 1.19x as compared to an untreated subject. In some embodiments, the increase may be 1.53x as compared to an untreated subject.

[0347] In some embodiments, the AAV particles may be delivered to a subject to increase a FXN protein level in the CNS (e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region)) by transducing cells in these CNS regions. Transduction may also be referred to as the number of cells that are positive for FXN protein. The transduction may be greater than or equal to 1%, greater than or equal to 5%, greater than or equal to 10%, greater than or equal to 15%, greater than or equal to 20%, greater than or equal to 25%, greater than or equal to 30%, greater than or equal to 35%, greater than or equal to

40%, greater than or equal to 45%, greater than or equal to 50%, greater than or equal to 55%, greater than or equal to 60%, greater than or equal to 65%, greater than or equal to 70%, greater than or equal to 75%, greater than or equal to 80%, greater than or equal to 85%, greater than or equal to 90%, greater than or equal to 95%, or greater than or equal to 99% of cells in these CNS regions.

[0348] In some embodiments, delivery of AAV particles comprising a viral genome encoding FXN as described herein to neurons in the brain (e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, and/or vestibular nucleus) may lead to an increased expression of FXN protein in one or more of those neurons. In some embodiments, the increased FXN protein expression may lead to improved survival and/or function of various cell types in these CNS regions and/or improvement of at least one symptom of Friedrich's Ataxia.

[0349] In particular embodiments, the AAV particles may be delivered to a subject in order to establish widespread distribution of the FXN throughout the CNS by administering the AAV particles to the thalamus of the subject.

[0350] In some embodiments, the increased expression of FXN protein may lead to improved gait, improved ataxia-associated heart conditions, decreased feeling of exhaustion, improved symptoms relating to metabolic disorders such as diabetes, and/or improved quality of life.

Administration

[0351] In some embodiments, the present disclosure provides methods comprising administering AAV particles in accordance with the disclosure to a subject in need thereof. AAV particle pharmaceutical, diagnostic, or prophylactic compositions thereof, may be administered to a subject using any amount and any route of administration effective for treating, or diagnosing a disease, disorder, and/or condition associated with decreased FXN expression or FXN deficiency. In some embodiments, the disease, disorder, and/or condition is FA.

[0352] Compositions in accordance with the disclosure may be formulated in unit dosage form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present disclosure may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific FXN employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[0353] In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used. As used herein, a “split dose” is the division of single unit dose or total daily dose into two or more doses, *e.g.*, two or more administrations of the single unit dose. As used herein, a “single unit dose” is a dose of any therapeutic composition administered in one dose/at one time/single route/single point of contact, *e.g.*, single administration event. In some embodiments, a single unit dose is provided as a discrete dosage form (*e.g.*, a tablet, capsule, patch, loaded syringe, vial, *etc.*). As used herein, a “total daily dose” is an amount given or prescribed in 24-hour period. It may be administered as a single unit dose. The AAV particles may be formulated in buffer only or in a formulation described herein.

[0354] In some embodiments, a pharmaceutical composition described herein can be formulated into a topical, intranasal, pulmonary, intratracheal, or injectable dosage form. In some embodiments, a pharmaceutical composition described herein can be formulated in a dosage form suitable for intravenous, intraocular, intravitreal, intramuscular, intracardiac, intraperitoneal, and/or subcutaneous administration.

[0355] In some embodiments, an AAV particle described herein is administered via intravenous administration. In some embodiments, an AAV particle described herein is formulated for intravenous administration.

[0356] In some embodiments, delivery of the AAV particles described herein results in minimal serious adverse events (SAEs) as a result of the delivery of the AAV particles.

Combinations

[0357] The AAV particles may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. The phrase “in combination with,” is not intended to require that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of pharmaceutical, prophylactic, diagnostic, or imaging compositions in combination with agents that may improve their bioavailability, reduce and/or modify their metabolism, and/or modify their distribution within the body.

[0358] The therapeutic agents may be approved by the US Food and Drug Administration or may be in clinical trial or at the preclinical research stage. The therapeutic agents may utilize any therapeutic modality known in the art, with non-limiting examples including gene silencing or interference (*e.g.*, miRNA, siRNA, RNAi, shRNA), gene editing (*e.g.*, TALEN, CRISPR/Cas9 systems, zinc finger nucleases), and gene, protein, or enzyme replacement.

[0359] In some embodiments, an AAV particle described herein, or a pharmaceutical composition comprising the AAV particle, may be administered in combination with at least one additional agent and/or therapy. In some embodiments, the at least one additional agent and/or therapy comprises an agent and/or therapy suitable for treating the disorder associated with FXN deficiency (e.g., Friedreich's Ataxia) or at least one symptom thereof. In some embodiments, the at least one additional agent and/or therapy comprises omaveloxolone or idebenone. In some embodiments, the at least one additional agent and/or therapy comprises CoQ10, IFN gamma, or a drug to treat comorbidities including diabetes, heart disease, and/or pain.

[0360] In some embodiments, the at least one additional agent and/or therapy comprises an immunosuppressant. In some embodiments, the immunosuppressant may be administered to the subject prior to administration of an AAV particle or pharmaceutical composition described herein. In some embodiments, the immunosuppressant may be administered to the subject simultaneously with administration of an AAV particle or pharmaceutical composition described herein. In some embodiments, the immunosuppressant may be administered to the subject after administration of an AAV particle or pharmaceutical composition described herein. In some embodiments, the AAV particle or pharmaceutical composition is administered to a subject who is receiving or has received an immunosuppressant. In some embodiments, the immunosuppressant comprises a corticosteroid (for example, and without limitation, prednisone, prednisolone, methylprednisolone, and/or dexamethasone), rapamycin, mycophenolate mofetil, tacrolimus, rituximab, and/or eculizumab hydroxychloroquine.

Measurement of Expression

[0361] Expression of FXN from viral genomes may be determined using various methods known in the art such as, but not limited to immunochemistry (e.g., IHC), enzyme-linked immunosorbent assay (ELISA), affinity ELISA, ELISPOT, flow cytometry, immunocytology, surface plasmon resonance analysis, kinetic exclusion assay, liquid chromatography-mass spectrometry (LCMS), high-performance liquid chromatography (HPLC), BCA assay, immunoelectrophoresis, Western blot, SDS-PAGE, protein immunoprecipitation, PCR, and/or in situ hybridization (ISH). In some embodiments, transgenes encoding FXN delivered in different AAV capsids may have different expression levels in dorsal root ganglion (DRG).

[0362] In certain embodiments, the FXN protein is detectable by an enzyme-linked immunosorbent assay (ELISA).

[0363] In certain embodiments, the FXN protein is detectable by an immunohistochemistry assay.

[0364] In certain embodiments, the FXN protein is detectable by Western blot.

[0365] In certain embodiments, the FXN protein is detectable by a frataxin biofluid assay, such as the assay described in WO2021/030303, the contents of which are hereby incorporated in their entirety.

[0366] In certain embodiments, a FXN gene, mRNA, and/or protein expression is measured in a cell or tissue of a subject who is receiving or has received an AAV particle described herein. In certain embodiments, the FXN gene, mRNA, and/or protein expression is measured in a cell or tissue of the

CNS, such as the amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, and/or temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region). In certain embodiments, the FXN gene, mRNA, and/or protein expression is measured in a peripheral cell or tissue, such as the liver, heart, kidney, pancreas, and/or muscle.

VII. Kits and Devices

Kits

[0367] In some aspects, the present disclosure provides a variety of kits for conveniently and/or effectively carrying out methods of the present disclosure. Typically, kits will comprise sufficient amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments.

[0368] Any of the vectors, constructs, or FXN sequences of the present disclosure may be comprised in a kit. In some embodiments, kits may further include reagents and/or instructions for creating and/or synthesizing compounds and/or compositions of the present disclosure. In some embodiments, kits may also include one or more buffers. In some embodiments, kits of the disclosure may include components for making protein or nucleic acid arrays or libraries and thus, may include, for example, solid supports.

[0369] In some embodiments, kit components may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and suitably aliquoted. Where there is more than one kit component, (labeling reagent and label may be packaged together), kits may also generally contain second, third or other additional containers into which additional components may be separately placed. In some embodiments, kits may also comprise second container means for containing sterile, pharmaceutically acceptable buffers and/or other diluents. In some embodiments, various combinations of components may be comprised in one or more vial. Kits of the present disclosure may also typically include means for containing compounds and/or compositions of the present disclosure, e.g., proteins, nucleic acids, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which desired vials are retained.

[0370] In some embodiments, kit components are provided in one and/or more liquid solutions. In some embodiments, liquid solutions are aqueous solutions, with sterile aqueous solutions being particularly used. In some embodiments, kit components may be provided as dried powder(s). When reagents and/or components are provided as dry powders, such powders may be reconstituted by the addition of suitable volumes of solvent. In some embodiments, it is envisioned that solvents may also be provided in another container means. In some embodiments, labeling dyes are provided as dried powders.

In some embodiments, it is contemplated that 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900, 1000 micrograms or at least or at most those amounts of dried dye are provided in kits of the disclosure. In such embodiments, dye may then be resuspended in any suitable solvent, such as DMSO.

[0371] In some embodiments, kits may include instructions for employing kit components as well the use of any other reagent not included in the kit. Instructions may include variations that may be implemented.

Devices

[0372] In some embodiments, compounds and/or compositions of the present disclosure may be combined with, coated onto or embedded in a device. Devices may include, but are not limited to, dental implants, stents, bone replacements, artificial joints, valves, pacemakers and/or other implantable therapeutic device.

[0373] The present disclosure provides for devices which may incorporate viral vectors that encode one or more FXN molecules. These devices contain in a stable formulation the viral vectors which may be immediately delivered to a subject in need thereof, such as a human patient.

[0374] Devices for administration may be employed to deliver the viral vectors encoding FXN of the present disclosure according to single, multi- or split-dosing regimens taught herein.

[0375] Methods and devices known in the art for multi-administration to cells, organs and tissues are contemplated for use in conjunction with the methods and compositions disclosed herein as embodiments of the present disclosure.

VIII. Definitions

[0376] At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual sub-combination of the members of such groups and ranges. The following is a non-limiting list of term definitions.

[0377] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

[0378] The articles “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process.

[0379] The term “comprising” is intended to be open and permits but does not require the inclusion of additional elements or steps.

[0380] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[0381] *Adeno-associated virus*: As used herein, the term “adeno-associated virus” or “AAV” refers to members of the dependovirus genus or a functional variant thereof. Unless stated otherwise, “AAV” may refer to wildtype (i.e., naturally occurring) AAV or recombinant AAV.

[0382] *AAV Particle*: As used herein, an “AAV particle” refers to a particle comprising an AAV capsid, e.g., an AAV capsid variant (such as a parent capsid sequence with at least one peptide insertion and/or with at least one substitution), and a polynucleotide, e.g., a viral genome or a vector genome. The AAV particle may be capable of delivering a FXN polynucleotide to cells. The cells may be mammalian cells, e.g., human cells. In some embodiments, an AAV particle of the present disclosure may be produced recombinantly. An AAV particle may be derived from any serotype, described herein or known in the art, including combinations of serotypes (e.g., “pseudotyped” AAV) or from various genomes (e.g., single stranded or self-complementary). In some embodiments, the AAV particle may be replication defective and/or targeted. In some embodiments, the AAV particle may comprise a peptide present in, e.g., inserted into and/or replacing a wildtype amino acid of, the capsid to enhance tropism for a desired target tissue. It is to be understood that reference to the AAV particle of the disclosure also includes pharmaceutical compositions thereof, even if not explicitly recited.

[0383] *Administering*: As used herein, the term “administering” refers to providing a pharmaceutical agent or composition to a subject.

[0384] *Amelioration*: As used herein, the term “amelioration” or “ameliorating” refers to a lessening of severity of at least one indicator of a condition or disease. For example, in the context of a neurodegenerative disorder, amelioration includes the reduction or stabilization of neuron loss.

[0385] *Approximately*: As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to, i.e., within 10% of, a stated reference value.

[0386] *Baseline*: The term “baseline,” when used to describe a measurement in a subject receiving or about to receive a treatment, refers to a measurement made before starting the treatment.

[0387] *Capsid*: As used herein, the term “capsid” refers to the exterior, e.g., a protein shell, of a virus particle, e.g., an AAV particle, that is substantially (e.g., >50%, >60%, >70%, >80%, >90%, >95%, >99%, or 100%) protein. In some embodiments, the capsid is an AAV capsid comprising an AAV capsid protein described herein, e.g., a VP1, VP2, and/or VP3 polypeptide. The AAV capsid protein can be a wild-type AAV capsid protein or a variant, e.g., a structural and/or functional variant from a wild-type or a reference capsid protein, referred to herein as an “AAV capsid variant.” For example, and without limitation, an AAV capsid variant may refer to at least a VP1 protein, a VP2 protein, or a VP3 protein (e.g., all of the VP1, VP2, and VP3 proteins forming the AAV capsid) as will be clear from context. In

some embodiments, the AAV capsid variant described herein may comprise a peptide and/or amino acid insertion and/or substitution. The terms “substitution” and “replacement” are used interchangeably in this context. In some embodiments, the AAV capsid variant described herein has the ability to encapsulate (i.e., encapsidate) a viral genome and/or is capable of entry into a cell, e.g., a mammalian cell. In some embodiments, the AAV capsid variant described herein may have modified tropism compared to that of a wild-type AAV capsid, e.g., the corresponding wild-type capsid.

[0388] *CNS Cells:* As used herein, “CNS cells” refers to cells of the central nervous system and sub-structures thereof. Non-limiting examples of CNS cells include neurons and sub-types thereof, glia, microglia, oligodendrocytes, ependymal cells, and astrocytes. Non-limiting examples of neurons include sensory neurons, motor neurons, interneurons, unipolar cells, bipolar cells, multipolar cells, pseudounipolar cells, pyramidal cells, basket cells, stellate cells, Purkinje cells, Betz cells, amacrine cells, granule cell, ovoid cell, medium aspiny neurons and large aspiny neurons, GABAergic neurons and/or glutamatergic neurons.

[0389] *Codon optimization:* As used herein, the term “codon optimization” refers to a process of changing codons of a given gene in such a manner that the polypeptide sequence encoded by the gene remains the same.

[0390] *Complementary and substantially complementary:* As used herein, the term “complementary” refers to the ability of polynucleotides to form base pairs with one another. Perfect complementarity or 100% complementarity refers to the situation in which each nucleotide unit of one polynucleotide strand can form a hydrogen bond with a nucleotide unit of a second polynucleotide strand. Less than perfect complementarity refers to the situation in which some, but not all, nucleotide units of two strands can form hydrogen bond with each other. For example, for two 20-mers, if only two base pairs on each strand can form a hydrogen bond with each other, the polynucleotide strands exhibit 10% complementarity. In the same example, if 18 base pairs on each strand can form hydrogen bonds with each other, the polynucleotide strands exhibit 90% complementarity. The term “complementary” as used herein can encompass fully complementary or partially (e.g., substantially) complementary. “Fully complementary”, “perfect complementarity”, or “100% complementarity” refers to the situation in which each nucleotide unit of one polynucleotide or oligonucleotide strand can base-pair with a nucleotide unit of a second polynucleotide or oligonucleotide strand. As used herein, the term “substantially complementary” means that >50% of the nucleotide units of a first polynucleotide strand can base pair with nucleotide units on a second polynucleotide strand. When used in the context of RNA silencing, “substantially complementary” refers to an siRNA that has a sequence (e.g., in the antisense strand) that is sufficient to bind the desired target mRNA and to trigger the RNA silencing of the target mRNA.

[0391] *Conservative substitution:* As used herein, a conservative substitution, as applied to an amino acid sequence, also referred to as a “conservative amino acid substitution,” is one in which the amino acid residue is replaced with an amino acid residue having similar biochemical properties. When used in reference to a nucleic acid sequence, the term “conservative substitution” refers to a nucleotide

replacement that results in an amino acid residue having similar biochemical properties compared to a reference sequence. Families of amino acid residues having similar biochemical properties have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

[0392] *Corresponding to:* As used herein, the phrase “corresponding to” in the context of an amino acid sequence refers to the location of an amino acid in a reference sequence or the equivalent position in a modified sequence when aligned. For example, an amino acid corresponding to position 585 of the amino acid sequence of SEQ ID NO: 138 refers to the amino acid at the 585th position from the N-terminus in the amino acid sequence of SEQ ID NO: 138 or the equivalent position in an aligned sequence. As used herein, an amino acid at a position corresponding to that in a designated sequence may also be referred to as an amino acid at a particular position, numbered according or numbered relative to the designated sequence. For instance, an amino acid corresponding to position 585 of the amino acid sequence of SEQ ID NO: 138 may also be referred to as an amino acid at position 585 (or amino acid 585), numbered according to the amino acid sequence of SEQ ID NO: 138 or as numbered according to a sequence corresponding to the amino acid sequence of SEQ ID NO: 138 (*i.e.*, relative to a reference sequence of the amino acid sequence of SEQ ID NO: 138).

[0393] *Derivative:* As used herein, the term “derivative” refers to a composition (*e.g.*, sequence, compound, formulation, *etc.*) that is derived from, or finds its basis in, a parent composition. Non-limiting examples of a parent composition include a wild-type or original amino acid or nucleic acid sequence, or an undiluted formulation. In some embodiments, a derivative is a variant of a parent composition. A derivative may differ from the parent composition by less than about 1%, less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, or less than about 50%. In certain embodiments, a derivative may differ from a parent composition by more than about 50%. In certain embodiments, a derivative may differ from a parent composition by more than about 75%. In some embodiments, a derivative may be a fragment or truncation of a parent amino acid or nucleotide sequence. As a non-limiting example, a derivative may be a sequence with a nucleotide, amino acid, or peptide substitution and/or insertion as compared to a parent nucleic acid or amino acid sequence (*e.g.*, as compared to AAV9).

[0394] *Effective amount:* As used herein, the term “effective amount” or “therapeutically effective amount” of an agent is that amount sufficient to effect beneficial or desired results. An effective amount is provided in a single dose or multiple doses to treat, improve symptoms of, delay progression of symptoms, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[0395] *Fragment*: A “fragment,” as used herein, refers to a contiguous portion of a reference sequence. A fragment may comprise a functional fragment that retains at least one activity of the reference sequence. For example, fragments of proteins may comprise polypeptides obtained by digesting full-length protein isolated from cultured cells. A fragment may also refer to a truncation (*e.g.*, an N-terminal and/or C-terminal truncation) of a protein or a truncation (*e.g.*, at the 5' and/or 3' end) of a nucleic acid. A protein fragment may be obtained by expression of a truncated nucleic acid, such that the nucleic acid encodes a portion of the full-length protein.

[0396] *Healthy individual*: As used herein, the term “healthy individual” refers to an individual who does not have a disease or disorder associated with FXN protein deficiency. *e.g.*, an individual who does not have Friedreich's Ataxia.

[0397] *Identity*: As used herein, the term “identity” refers to the overall relatedness between polymeric molecules, *e.g.*, between oligonucleotide molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two polynucleotide sequences, for example, may be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using methods such as those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; each of which is incorporated herein by reference in its entirety. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Myers and Miller (CABIOS, 1989, 4:11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix. Methods commonly employed to determine percent identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM J Applied Math., 48:1073 (1988); incorporated herein by reference in its entirety. Techniques for

determining identity are codified in publicly available computer programs. Computer software to determine homology between two sequences include, but are not limited to, GCG program package, Devereux, J., *et al.*, *Nucleic Acids Research*, 12(1), 387 (1984)), the Basic Local Alignment Search Tool (BLAST, which includes, e.g., BLASTP for protein sequences and BLASTN for nucleic acid sequences), and FASTA Altschul, S. F. *et al.*, *J. Molecular Biol.*, 215, 403 (1990)), EMBOSS Needle, Clustal Omega, Benchling, and Geneious. In preferred embodiments, sequence identity may be determined using BLAST, Clustal Omega, or EMBOSS Needle. In some embodiments, sequence identity may be determined using global alignment. In some embodiments, sequence identity may be determined using local alignment.

[0398] *Inverted terminal repeat*: As used herein, the term “inverted terminal repeat” or “ITR” refers to a cis-regulatory element for the packaging of polynucleotide sequences into viral capsids.

[0399] *Isolated*: As used herein, the term “isolated” refers to a substance or entity that is altered or removed from the natural state, e.g., altered or removed from at least some of component with which it is associated in the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not “isolated,” but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is “isolated.” An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of the environment in which it is found in nature. In some embodiments, an isolated nucleic acid is recombinant, e.g., incorporated into a vector.

[0400] *miRNA binding site*: As used herein, a “miRNA binding site” or “miR binding site” refers either to a DNA sequence corresponding to an RNA sequence that is bound by a microRNA, or to the RNA sequence that is bound by the microRNA. The miR binding site is capable of binding, or binds, in whole or in part to a microRNA (miRNA, miR) through complete or partial hybridization. A miR binding site may be encoded or transcribed in series, also referred to as a “miR binding site series” or “miR BSs”, which includes two or more miR binding sites having the same or a different nucleic acid sequence.

[0401] *Modification*: As used herein, the term “modification” or “modified,” refers to any substance, compound, or molecule that has been changed in any way. For example, a modification in an amino acid sequence may comprise a substitution (e.g., a conservative substitution), an insertion, and/or a deletion of one or more amino acids in the sequence.

[0402] *Neurological disease*: As used herein, a “neurological disease” is any disease associated with the central or peripheral nervous system and components thereof (e.g., neurons).

[0403] *Operably linked*: As used herein, the phrase “operably linked” refers to a functional connection between two or more molecules, constructs, transcripts, entities, moieties or the like.

[0404] *Payload*: As used herein, “payload,” “payload sequence,” or “payload region” refers to one or more polynucleotides or polynucleotide regions encoded by or within a viral genome or an expression

product of such polynucleotide or polynucleotide region, *e.g.*, a transgene, a polynucleotide encoding a polypeptide.

[0405] *Pharmaceutical Composition:* As used herein, the term “pharmaceutical composition” or pharmaceutically acceptable composition” comprises AAV polynucleotides, AAV genomes, or AAV particle and one or more pharmaceutically acceptable excipients, solvents, adjuvants, and/or the like.

[0406] *Position:* The term “position,” as used herein in the context of an amino acid sequence, refers to the location of a particular amino acid or set of amino acids relative to a larger sequence. A position or positions of amino acids may interchangeably be referred to by an amino acid number or numbers of a reference sequence. For example, and unless otherwise specified, “positions 1-736, as numbered according to SEQ ID NO: 981” is interchangeable with “amino acids 1-736, as numbered according to SEQ ID NO: 981” and “one or more modifications at one or more of positions 580-599, as numbered according to SEQ ID NO: 138” is interchangeable with “one or more modifications at one or more of amino acids 580-599, as numbered according to SEQ ID NO: 138.” Within a sequence, an amino acid position is counted from the *N*-terminus.

[0407] *Preventing:* As used herein, the term “preventing” refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition. The term “prevention” or “preventing” of an infection, disease, disorder and/or condition may be considered a subset within the meaning with the term “treatment” or “treating” of the infection, disease, disorder and/or condition.

[0408] *Region:* As used herein, the term “region” refers to a zone or general area. In some embodiments, when referring to a protein or protein module, a region may comprise a linear sequence of amino acids along the protein or protein module or may comprise a three-dimensional area. In some embodiments, regions comprise terminal regions. As used herein, the term “terminal region” refers to regions located at the ends or termini of a given agent. When referring to proteins, terminal regions may comprise N- and/or C-termini. N-termini refer to the end of a protein comprising an amino acid with a free amino group. C-termini refer to the end of a protein comprising an amino acid with a free carboxyl group. N- and/or C-terminal regions may comprise the N- and/or C-termini as well as surrounding amino acids. When referring to a polynucleotide, a region may comprise a linear sequence of nucleic acids along the polynucleotide or may comprise a three-dimensional area, secondary structure, or tertiary structure. In some embodiments, regions comprise terminal regions. As used herein, the term “terminal region” refers to regions located at the ends or termini of a given agent. When referring to polynucleotides, terminal regions may comprise 5' and/or 3' termini.

[0409] *Sample*: As used herein, the term “sample” or “biological sample” refers to a subset of tissues, cells, nucleic acids, or a component or part of the body (e.g., a body fluid, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid, and semen).

[0410] *Self-complementary AAV*: As used herein, the term “self-complementary AAV” refers to an AAV comprising at least a protein capsid and a self-complementary viral genome.

[0411] *Serotype*: As used herein, the term “serotype” refers to distinct variations in a capsid of an AAV based on surface antigens which allow epidemiologic classifications of the AAVs at the sub-species level.

[0412] *Silent Mutation*: As used herein, a “silent mutation” or “silent substitution” refers to a nucleotide replacement that results in the same amino acid residue as a reference sequence.

[0413] *Signal Sequence*: As used herein, the phrase “signal sequence” refers to a sequence which can direct the transport or localization.

[0414] *Similarity*: As used herein, the term “similarity” refers to the overall relatedness between polymeric molecules. e.g., between polynucleotide molecules (e.g., DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of percent similarity of polymeric molecules to one another can be performed in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art.

[0415] *Spacer*: As used herein, a “spacer” is generally any selected nucleic acid sequence of, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length, which is located between two or more consecutive miR binding site sequences. In some embodiments, spacers may also be more than 10 nucleotides in length, e.g., 20, 30, 40, or 50 or more than 50 nucleotides.

[0416] *Subject*: As used herein, the term “subject” or “patient” refers to any organism to which a composition in accordance with the disclosure may be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Similarly, “subject” or “patient” refers to an organism who may seek, who may require, who is receiving, or who will receive treatment or who is under care by a trained professional for a particular disease or condition. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans). As used herein, a subject or patient may be susceptible to, suspected of having, or have a deficiency in frataxin protein, e.g., may be susceptible to, suspected of having, or have Friedreich’s Ataxia (FA).

[0417] *Substantially*: As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0418] *Suffering from*: An individual who is “suffering from” a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of a disease, disorder, and/or condition.

[0419] *Susceptible to*: An individual who is “susceptible to” a disease, disorder, and/or condition has not been diagnosed with and/or may not exhibit symptoms of the disease, disorder, and/or condition but harbors a propensity to develop a disease or its symptoms. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein and/or nucleic acid associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, and/or condition; (5) a family history of the disease, disorder, and/or condition; and (6) exposure to and/or infection with a microbe associated with development of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[0420] *Target Cells*: As used herein, “target cells” refers to any one or more cells of interest. The cells may be found *in vitro*, *in vivo*, *in situ* or in the tissue or organ of an organism. The organism may be an animal, preferably a mammal, more preferably a human and most preferably a human patient.

[0421] *Target Tissue*: As used herein, “target tissue” refers to a tissue of interest that may be found *in vitro*, *in situ*, or as part of an animal, preferably a mammal, more preferably a human and most preferably a human patient.

[0422] *Therapeutic Agent*: The term “therapeutic agent” refers to any agent that, when administered to a subject, elicits a desired biological and/or pharmacological effect.

[0423] *Therapeutically Effective Outcome*: As used herein, the term “therapeutically effective outcome” means an outcome that is sufficient in a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, improve symptoms of, delay progression of symptoms, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition.

[0424] *Treating*: As used herein, the term “treating” refers to partially or completely alleviating, ameliorating, improving, relieving, delaying onset of, inhibiting progression of (e.g., stabilizing), reducing severity of, reducing incidence of, and/or preventing one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[0425] *Unmodified*: As used herein, “unmodified” refers to any substance, compound or molecule prior to being changed in any way. Unmodified may, but does not always, refer to the wild-type or native

form of a biomolecule or entity. Molecules or entities may undergo a series of modifications whereby each modified product may serve as the “unmodified” starting molecule or entity for a subsequent modification.

[0426] *Variant*: The term “variant” refers to a polypeptide or polynucleotide that has an amino acid or a nucleotide sequence that has at least 90% (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity to a reference sequence. The variant may be a functional variant. As used herein, the term “functional variant” refers to a polypeptide variant or a polynucleotide variant that has at least one activity of the reference sequence.

[0427] *Vector*: As used herein, a “vector” is any molecule or moiety which transports, transduces, or otherwise acts as a carrier of a heterologous molecule. Vectors of the present disclosure may be produced recombinantly and may be based on and/or may comprise adeno-associated virus (AAV) parent or reference sequence(s).

[0428] *Viral genome*: As used herein, a “viral genome,” “vector genome,” or “VG” is a polynucleotide comprising at least one inverted terminal repeat (ITR) and at least one nucleic acid sequence encoding a payload. A viral genome encodes at least one copy of the payload.

EXAMPLES

[0429] The present disclosure is further illustrated by the following non-limiting examples.

Example 1. High-throughput screen of TRACER AAV library in non-human primates (NHPs)

[0430] A TRACER based method as described in WO2020072683, WO 2021/202651, and WO2021230987, the contents of which are herein incorporated by reference in their entirety, was used to generate the AAV capsid variants described herein. An orthogonal evolution approach was combined with a high throughput screening by NGS. Briefly, the library of AAV capsid variants was generated using a sliding window approach, where amino acid modifications, e.g., substitutions, were randomly introduced into various positions across loop VIII of AAV9, including between positions 581-593, relative to a reference sequence numbered according to SEQ ID NO: 138. The initial library (passage 1) was passed first through cynomolgus macaques (*Macaca fascicularis*, n=2, 2-4 years of age) or human brain microvascular endothelial cells (hBMVECs) followed by cynomolgus macaques (n=2). These first passage libraries were then pooled and screened a second time through cynomolgus macaques (passage 2). After the second passage (e.g., 28 days post injection into two NHPs). RNA was extracted from multiple brain and spinal cord regions. Following RNA recovery and RT-PCR amplification, a systematic NGS enrichment analysis was performed to calculate fold enrichment relative to an AAV9 wild-type control. The top variants were selected for the creation of a synthetic library.

[0431] After creation of the synthetic library with the sub-selected variants, the synthetic library was screened (passage 3) in two NHPs (2-4 years of age). The animals were injected intravenously with the synthetic library. After a period *in vivo*, (e.g., 28-days) RNA was extracted from nervous tissue, e.g., brain, spinal cord, and DRG of the NHPs. Following RNA recovery and RT-PCR amplification, a

systematic NGS enrichment analysis was performed, and the peptides comprised within the variants were identified and the capsid enrichment ratio for each variant compared to the wild-type AAV9 control was calculated (fold enrichment relative to wild-type AAV9). Values above 1 indicate an increase in expression relative to AAV9. All animals were dosed intravenously at 2e13 VG/kg across the screen.

[0432] Following these three passages, approximately 1542 variants were identified with an average fold change greater than wild-type AAV9 in the brain of the NHPs (*Macaca fascicularis*). Of the 1542 variants, 422 demonstrated a fold-change of greater than 5 and 118 demonstrated a fold-change greater than 10 compared to wild-type and were detected across all brain regions investigated. The capsid variant that demonstrated the greatest fold-change in expression relative to AAV9 in the brain of the NHP comprised the amino acid sequence TQDWHRI (SEQ ID NO: 941), resulting in a fold-change in expression of 460 relative to the AAV9 control (Table 9).

Table 9. NGS fold-enrichment of AAV capsid variants in NHPs (*Macaca fascicularis*)

Peptide Sequence	SEQ ID NO:	Fold enrichment over AAV9		
		Brain	Spinal cord	DRG
TQDWHRI	941	460.196	207.400	3.677

[0433] Taken together, these results demonstrate that after 3 rounds of screening of this AAV9 variant library with loop VIII modifications in NHPs (cynomolgus macaques (*Macaca fascicularis*)), many AAV capsid variants outperformed the wild-type AAV9, for example, in penetration of the blood brain barrier (BBB) and spinal cord expression.

Example 2. Individual Capsid Characterization in NHPs

[0434] The goal of these experiments was to determine the transduction level, tropism, ability to cross the blood brain barrier, and overall spatial distribution in the central nervous system (CNS) of a capsid variant selected from the study described in Example 1 relative to AAV9 following intravenous injection in NHPs (cynomolgus macaques (*Macaca fascicularis*)) and marmosets (*Callithrix jacchus*). The capsid variant was TTJ-001 (SEQ ID NO: 981 (amino acid) and 983 (DNA), comprising SEQ ID NO: 941 or 201), as outlined in Table 3 above. The amino acid and DNA sequences of TTJ-001 are provided, e.g., in Tables 4A and 4B, respectively.

A. Evaluation of TTJ-001 in Cynomolgus macaques

[0435] AAV particles were generated with this capsid variant encapsulating a sequence encoding a cynomolgus frataxin protein tagged with hemagglutinin (cyno-FXN-HA) driven by a CBA promoter. The capsid variant and AAV9 control were tested by intravenously administering the AAV particle formulation at 2e13 VG/kg to NHPs (n=2). The in-life period was 14 days and the brain, spinal cord, and peripheral tissues including the heart, liver, and quadriceps were collected for measuring transgene mRNA, transgene protein, and viral DNA (biodistribution). The AAV particles administered to the NHPs comprised single stranded viral genomes.

[0436] The brains isolated from the NHPs injected with the AAV particles encapsulated in the TTJ-001 capsid variant (AAV_TTJ-001) were assayed by ddPCR for the presence of transgene RNA as a

measure of transgene expression, and the presence of viral DNA as a measure of viral genome levels. Data were provided as average mRNA fold change for the transgene relative to a housekeeping gene as well as the fold change relative to the AAV9 control (Table 10). As shown in Table 10, mRNA transgene expression from the TTTJ-001 capsid variant, which is an AAV9 capsid variant, was significantly higher in the brain of NHPs relative to the wild-type AAV9 control. More specifically, mRNA expression was approximately 40-280-fold higher from the TTTJ-001 capsid variant compared to wild-type AAV9 in the brain of NHPs. Additionally, mRNA expression was approximately 3-274-fold higher from the TTTJ-001 capsid variants compared to wild-type AAV9 in the spinal cord of the NHPs. Comparable results were obtained when mRNA levels were measured by qPCR.

Table 10. Transgene mRNA expression with the TTTJ-001 capsid variant in NHPs

Capsid Variant	Tissue	AVG transgene mRNA fold change relative to housekeeping gene	Fold change relative to AAV9
AAV9	Sensory Cortex	0.00197	1.0
	Motor Cortex	0.001188	1.0
	Putamen	0.00077	1.0
	Thalamus	0.005241	1.0
	Caudate	0.000484	1.0
	Hippocampus	0.001214	1.0
	Cerebellum	0.001163	1.0
	SC Cervical	0.0119	1.0
	SC Thoracic	0.00545	1.0
	SC Lumbar	1.941	1.0
	DRG Lumbar	2.137	1.0
	DRG Sacral	2.886	1.0
	DRG Cervical	0.626	1.0
	DRG Thoracic	3.291	1.0
	Heart	3.641	1.0
	Liver	1.329	1.0
	Quadriceps	1.431	1.0
TTTJ-001	Sensory Cortex	0.559	283.804
	Motor Cortex	0.628	528.829
	Putamen	0.124	161.093
	Thalamus	0.695	132.663
	Caudate	0.084	174.627
	Hippocampus	0.178	146.898
	Cerebellum	0.046	39.784
	SC Cervical	1.377	115.923
	SC Thoracic	1.497	274.522
	SC Lumbar	7.339	3.780
	DRG Lumbar	20.446	9.5662
	DRG Sacral	6.5096	2.2552
	DRG Cervical	3.429	5.4731
	DRG Thoracic	15.524	4.7174
	Heart	26.573	7.299
	Liver	8.981	6.757
	Quadriceps	19.011	13.287

[0437] The brains, spinal cord, and peripheral tissues isolated from the NHPs were also assayed for the presence of viral DNA as a measure of viral genome levels by ddPCR. Data are provided in Table 11 as average DNA (viral genome (VG)) copies per diploid genome as well as fold change relative to the AAV9 control. As shown in Table 11, biodistribution of the AAV9 capsid variant, TTJ-001, was significantly higher in the NHP brain relative to the wild-type AAV9 control. Biodistribution of TTJ-001 was lower in the NHP liver relative to the wild-type AAV9 control. Comparable results were obtained when average DNA levels were measured by qPCR.

Table 11. Viral DNA biodistribution with the TTJ-001 capsid variant in NHPs

Capsid Variant	Tissue	AVG DNA copies per diploid genome	Fold change relative to AAV9
AAV9	Sensory Cortex	0.199017	1.0
	Motor Cortex	0.085628	1.0
	Putamen	1.05028	1.0
	Thalamus	0.187792	1.0
	Caudate	0.083988	1.0
	Hippocampus	0.078499	1.0
	Cerebellum	0.049565	1.0
	SC Cervical	0.104	1.0
	SC Thoracic	0.102	1.0
	SC Lumbar	0.521	1.0
	DRG Lumbar	0.345	1.0
	DRG Sacral	1.005	1.0
	DRG Cervical	0.188	1.0
	DRG Thoracic	0.318	1.0
	Heart	0.934	1.0
	Liver	337.290	1.0
	Quadriceps	1.894	1.0
TTJ-001	Sensory Cortex	1.590	7.989
	Motor Cortex	1.889	22.063
	Putamen	2.069	1.970
	Thalamus	1.985	10.570
	Caudate	2.151	25.615
	Hippocampus	1.548	19.725
	Cerebellum	0.952	19.216
	SC Cervical	1.836	17.646
	SC Thoracic	0.747	7.290
	SC Lumbar	0.863	1.658
	DRG Lumbar	0.319	0.926
	DRG Sacral	0.249	0.248
	DRG Cervical	1.179	6.273
	DRG Thoracic	1.189	3.738
	Heart	2.286	2.448
	Liver	191.741	0.568
	Quadriceps	0.901	0.475

[0438] The brain tissues and spinal cords of the mice were also subjected to immunohistochemistry staining to evaluate overall CNS tropism and biodistribution in various regions. Immunohistochemical staining correlated with the qPCR analysis, as TTJ-001 showed significantly stronger staining and more

widespread payload expression in the brain and spinal cord, as compared to the AAV9 control. More specifically, TTJ-001 demonstrated moderately to strongly positive staining in the globus pallidus, thalamus, substantia nigra, oculomotor nucleus, geniculate nucleus, central grey, inferior colliculus, external cuneate nucleus, gracile nucleus, nucleus ambiguus, ventral horn of the spinal cord, vestibular nucleus, inferior olivary complex, deep cerebellar nuclei, and the DRG. TTJ-001 demonstrated weakly positive to moderately positive staining in the ventral pallidum, amygdala, caudate, putamen, hippocampus, temporal cortex, auditory cortex, and somatosensory cortex.

[0439] The peripheral tissues were also subjected to immunohistochemistry staining to evaluate tropism and biodistribution. TTJ-001 resulted in widespread transduction of multiple peripheral tissues, as compared to the AAV9 control. TTJ-001 demonstrated weakly positive to moderately positive staining in the pancreas and quadriceps and moderately to strongly positive staining in the heart, kidney, and liver.

[0440] Taken together, these data demonstrate that TTJ-001 is an enhanced CNS-tropic capsid variant in NHPs. Additionally, this capsid variant was able to successfully penetrate the blood brain barrier following intravenous injection.

B. Evaluation of TTJ-001 in Marmosets (*Callithrix jacchus*)

[0441] AAV particles were generated with the TTJ-001 capsid variant or the AAV9 capsid control which comprised a self-complementary viral genome encoding a histone H2b protein with a VSV tag (TTJ-001 capsid variant) or HA tag (AAV9 control capsid) driven by a ubiquitous CAG promoter. The AAV particles comprising the TTJ-001 capsid variant or the AAV9 capsid control were administered to the marmosets (*Callithrix jacchus*) (n=3) intravenously in a single solution, at the doses indicated in Table 12. The in-life period was 28 days and then various CNS and peripheral tissues were collected for measuring transgene mRNA (expression) by RT-qPCR, protein expression by IHC, and viral DNA (biodistribution) by ddPCR. Data were then normalized to the dose of each viral vector in the dosing solution.

Table 12. Titer of the AAV particles comprising the TTJ-001 or AAV9 capsid in the solution dosed in marmosets

Capsid Variant	Actual Titer Dosed	Ratio of Capsid Variant to AAV9
TTJ-001	2.23 x 10 ¹¹ vg/mL	0.56
AAV9	4.00 x 10 ¹¹ vg/mL	1.0

[0442] As shown in Table 13, the TTJ-001 capsid variant demonstrated increased biodistribution in the caudate and motor cortex in the brain of the marmosets relative to the AAV9 control. The TTJ-001 capsid variant also led to increased transgene expression (Table 14) in the caudate and motor cortex in the brain of the marmosets. In fact, biodistribution and transgene expression were increased over 5-7 fold and 15-17 fold, respectively, for TTJ-001 in the brain of marmosets relative to AAV9. Similar expression and biodistribution was observed by immunohistochemistry. More specifically, staining for TTJ-001 was detected in the mid-brain, caudate, putamen, thalamus, and cerebellum, and this staining was increased

relative to AAV9. Staining for TTJ-001 was also observed in the molecular and granule layer of the cerebellum.

[0443] Biodistribution and transgene expression were also measured in the peripheral tissues of the liver, heart, and quadriceps. In the liver, the TTJ-001 capsid variant exhibited lower biodistribution (Table 13) and transgene expression (Table 14) relative to AAV9, indicating that the TTJ-001 capsid variant was detargeted in the liver relative to AAV9 in marmosets. Additionally, the TTJ-001 capsid variant resulted in increased transduction of the heart and muscle, as compared to the AAV9 control (Table 13 and Table 14).

Table 13. Quantification of viral genome copies per diploid genome (biodistribution) by ddPCR following intravenous administration of AAV particles comprising the TTJ-001 capsid variant normalized to the actual titer of the viral vector in the dosing solution (vg/dg = viral genome copies/diploid genome)

Capsid	Tissue									
	Caudate		Motor Cortex		Heart		Muscle		Liver	
	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9
TTJ-001	0.10	7.23	0.13	5.86	1.41	3.43	0.51	2.67	3.05	0.22
AAV9	0.01	1.00	0.02	1.00	0.48	1.00	0.23	1.00	13.79	1.00

Table 14. Quantification of transgene mRNA by RT-qPCR following intravenous administration of AAV particles comprising the TTJ-001 capsid variant normalized to the actual titer of the viral vector in the dosing solution (mRNA = transgene mRNA fold over housekeeping gene; rel. to AAV9= transgene mRNA fold over housekeeping gene relative to AAV9)

Capsid	Tissue									
	Caudate		Motor Cortex		Heart		Muscle		Liver	
	mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9
TTJ-001	0.54	17.94	0.62	15.65	28.37	2.56	0.42	4.09	0.32	0.18
AAV9	0.03	1.00	0.05	1.00	12.67	1.00	0.15	1.00	1.85	1.00

[0444] These data in marmosets for TTJ-001 were similar to those observed in cynomolgus macaques, demonstrating cross-species compatibility of the TTJ-001 capsid variant.

[0445] Taken together, these data demonstrate that TTJ-001 is an enhanced CNS tropic capsid in marmosets. Additionally, the TTJ-001 capsid variant was able to successfully penetrate the blood brain barrier following intravenous injection in the marmosets.

C. Further Evaluation of TTJ-001 in Cynomolgus Macaques (Macaca fascicularis)

[0446] AAV particles were generated with the TTJ-001 capsid variant comprising a single-stranded viral genome encapsulating codon-optimized nucleotide sequence encoding GBA1, which is tagged with hemagglutinin (GBA1-HA). The GBA1-HA payload is driven by a CBA promoter. The TTJ-001 capsid variant and AAV9 control were tested by intravenously administering the AAV particle formulation at 1e13 vg/kg (n=3) or 3e12 vg/kg (n=3) to the NHPs. The in-life period was 28 days and the brain, spinal cord, and peripheral tissues including the heart, liver, and quadriceps were collected for measuring transgene mRNA, transgene protein, and viral DNA (biodistribution).

[0447] As shown in Tables 15 and 16, the TTJ-001 capsid variant demonstrated increased biodistribution and transduction as well as transgene expression in the brain (putamen, motor cortex, frontal cortex, substantia nigra, and dentate nucleus) and spinal cord (cervical C3 ventral horn) relative to AAV9. When dosed at 1×10^{13} vg/kg, TTJ-001 led to at least a 6-9-fold increase in biodistribution in the brain and at least an 8-fold increase in biodistribution in the spinal cord relative to AAV9. With respect to transgene expression, when dosed at 1×10^{13} kg/vg, TTJ-001 demonstrated at least a 25-61-fold increase in expression in the brain and at least a 20-fold increase in the spinal cord relative to AAV9. TTJ-001 also demonstrated comparable biodistribution and transgene expression in the DRG relative to AAV9 (Tables 15, 16, and 17).

[0448] By immunohistochemistry (IHC), when dosed at 1×10^{13} vg/kg, TTJ-001 demonstrated largely neuronal tropism, as evidenced by positive co-staining for HA/SMI 331 (neuronal marker) at least in the putamen and substantia nigra. No staining was observed in astrocytes in the putamen and substantia nigra. Additionally, by IHC, increased staining and transduction by AAV particles comprising the TTJ-001 capsid variant was also observed in multiple brain regions (substantia nigra, putamen, motor cortex, frontal cortex) and the spinal cord regions (cervical region, thoracic region, Clarke's column) of the NHPs, relative to AAV9, when administered at a dose of 1×10^{13} vg/kg. Staining and therefore transduction was also observed in the brain and spinal cord regions even when the AAV particles comprising the TTJ-001 capsid variant were administered at the lower dose of 3×10^{12} vg/kg. Comparable transduction and expression were observed by IHC in the DRG following administration of AAV particles comprising the TTJ-001 capsid variant and AAV particles comprising the AAV9 control capsid. IHC staining of the payload-HA positive cells of various brain regions following transduction with the AAV particles comprising the TTJ-001 capsid variant or AAV particles comprising an AAV9 control capsid was also quantified (Table 18). At a dose of 1×10^{13} vg/kg, TTJ-001 was capable of transducing greater than 2-9% of cells across the various brain regions investigated (Table 18). Even at the low dose of 3×10^{12} vg/kg, TTJ-001 was capable of transducing greater than 0.9 to 5% cells in the brain regions tested (Table 18). The percent HA+ cells in Table 18 may underestimate true positivity for the TTJ-001 capsid variant, as it demonstrated neuronal tropism.

[0449] Biodistribution and transgene expression were also measured in the peripheral tissues of the liver and heart. In the liver, at a dose of 1×10^{13} vg/kg, the TTJ-001 capsid variant exhibited lower biodistribution (Table 15) and transgene expression (Tables 16 and 17) relative to AAV9, indicating that the TTJ-001 capsid variant was detargeted in the liver relative to AAV9. At the 1×10^{13} vg/kg dose, the TTJ-001 demonstrated comparable biodistribution but decreased transgene expression in the heart relative to AAV9 (Tables 15, 16, and 17). Similar transduction and expression were observed in the heart by IHC following transduction with AAV particles comprising the TTJ-001 capsid variant and AAV particles comprising the AAV9 capsid control at a dose of 1×10^{13} vg/kg.

Table 15. Quantification of viral genome copies per diploid genome (biodistribution) by ddPCR following intravenous administration of AAV particles comprising the TTJ-001 capsid variant (vg/dg = viral genome copies/ diploid genome)

Capsid	Dose (vg/kg)	Tissues					
		Putamen		Motor Cortex		Frontal Cortex	
		vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9
AAV9	1e13	0.23	1.00	0.31	1.00	0.25	1.00
TTJ-001	1e13	1.48	6.54	2.69	8.70	2.03	8.23
TTJ-001	3e12	1.03		1.26		1.02	
Capsid	Dose (vg/kg)	Substantia Nigra		Dentate Nucleus		C3 Ventral Horn	
		vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9
		vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9
AAV9	1e13	0.28	1.00	0.24	1.00	0.45	1.00
TTJ-001	1e13	2.55	9.24	2.76	11.5	3.64	8.14
TTJ-001	3e12	1.07		0.857		1.55	
Capsid	Dose (vg/kg)	DRG (C3)		Liver		Heart	
		vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9
		vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9
AAV9	1e13	0.72	1.00	140.22	1.00	2.38	1.00
TTJ-001	1e13	0.60	0.84	33.51	0.24	2.68	1.13
TTJ-001	3e12	0.15		10.23		0.76	

Table 16. Quantification of transgene mRNA by RT-qPCR following intravenous administration of AAV particles comprising the TTJ-001 capsid variant (mRNA = transgene mRNA fold over housekeeping gene; rel. to AAV9= transgene mRNA fold over housekeeping gene relative to AAV9)

Capsid	Dose (vg/kg)	Tissues					
		Frontal Cortex		Motor Cortex		Putamen	
		mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9
AAV9	1e13	0.04	1.00	0.03	1.00	0.05	1.00
TTJ-001	1e13	1.09	28.14	1.60	61.76	1.79	33.43
TTJ-001	3e12	0.34		0.44		1.07	
Capsid	Dose (vg/kg)	Substantia Nigra		C3 Ventral Horn		DRG (C3)	
		mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9
		mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9
AAV9	1e13	0.14	1.00	1.40	1.00	26.01	1.00
TTJ-001	1e13	3.64	25.33	28.16	20.14	41.63	1.60
TTJ-001	3e12	1.57		2.42		5.81	
Capsid	Dose (vg/kg)	Dentate Nucleus		Heart		Liver	
		mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9
		mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9
AAV9	1e13	0.22	1.0	48.99	1.00	5.39	1.00
TTJ-001	1e13	13.01	59.136	36.31	0.74	1.40	0.26
TTJ-001	3e12	4.497		18.18		0.87	

Table 17. Quantification of transgene mRNA by RT-qPCR following intravenous administration of AAV particles comprising the TTJ-001 capsid variant (mRNA = transgene mRNA fold over endogenous GBA mRNA; rel. to AAV9= transgene mRNA fold change over endogenous GBA mRNA relative to fold change over AAV9)

Capsid	Dose (vg/kg)	Tissues					
		Frontal Cortex		Motor Cortex		Putamen	
		mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9
AAV9	1e13	0.02		0.01		0.02	
TTJ-001	1e13	0.55	23.75	1.09	84.46	0.71	29.82
TTJ-001	3e12	0.17		0.26		0.43	
Capsid		Substantia Nigra		C3 Ventral Horn		DRG (C3)	

	Dose (vg/kg)	mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9
AAV9	1e13	0.10		0.58		14.51	
TTJ-001	1e13	2.87	28.58	14.46	24.89	13.31	0.92
TTJ-001	3e12	0.96		0.99		1.68	
Capsid	Dose (vg/kg)	Dentate Nucleus		Heart		Liver	
		mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9
AAV9	1e13	N.D.		45.82		3.66	
TTJ-001	1e13	N.D.		32.00	0.70	0.88	0.24
TTJ-001	3e12	N.D.		11.11		0.63	

Table 18. Quantification of payload-HA positive cells by immunohistochemistry

Capsid	Dose (vg/kg)	%HA + cells per region			
		Frontal Cortex	Motor Cortex	Putamen	Substantia Nigra
AAV9	1e13	0.5	0.1	0.6	1.3
TTJ-001	1e13	3.6	2	9.2	9.8
TTJ-001	3e12	2.9	0.9	3.9	5.6

[0450] These data further confirm that TTJ-001 is a CNS-tropic capsid, capable of crossing the blood brain barrier following intravenous administration in NHPs.

D. Further Evaluation of TTJ-001 in Cynomolgus Macaques (*Macaca fascicularis*)

[0451] AAV particles were generated with the TTJ-001 capsid variant or the AAV9 capsid control, each of which comprised a self-complementary viral genome encoding a histone H2b protein driven by a ubiquitous CAG promoter. The AAV particles comprising the TTJ-001 capsid control were administered to a first group of male cynomolgus macaques (*Macaca fascicularis*; 4-6 kg body weight; over 2 years old) intravenously at a dose per capsid of 4e12 VG/kg. The AAV particles comprising the AAV9 capsid control were administered to a second group of male cynomolgus macaques (*Macaca fascicularis*; 4-6 kg body weight; over 2 years old) intravenously at a dose per capsid of 4e12 VG/kg. The in-life period was 28 days for both groups, and then various CNS and peripheral tissues were collected for measuring transgene mRNA (expression) by RT-qPCR; protein expression by IHC/chromogenic staining (e.g., DAB staining for percent of DAB+ cells indicating the percent of cells transduced); percent positive cells (e.g., neurons, motor neurons, and astrocytes) in brain and spinal cord regions by immunofluorescence microscopy; and viral DNA (biodistribution) by ddPCR.

[0452] As shown in Table 19, compared to the AAV9 control, TTJ-001 demonstrated increased CNS transduction and/or biodistribution in several regions of the brain (greater than 10-20% of cells transduced observed in several regions) and spinal cord of the cynomolgus macaques after intravenous administration at a relatively low dose of 4e12 vg/kg. More specifically, TTJ-001 was capable of transducing up to 11% of cells in the putamen; up to 19% of cells in the caudate, and up to 23% of cells in the putamen. TTJ-001 also showed improved delivery to the spinal cord relative to AAV9, at a dose of 4e12 vg/kg.

[0453] TTJ-001 also demonstrated increased transduction and/or biodistribution in the peripheral tissues of the muscle, specifically the vastus lateralis muscle and the gastrocnemius muscle relative to

AAV9, and comparable transduction and/or biodistribution in the heart relative to AAV9 (Table 20 and Table 21). TTJ-001 also appeared to exhibit partial de-targeting in the liver (Table 20 and Table 21).

Table 19. Quantification of viral genome copies per diploid genome (vg/dg) (biodistribution) by ddPCR, transgene mRNA by RT-qPCR (mRNA = transgene mRNA fold over housekeeping gene), and percent of DAB+ cells in tissues of the CNS of cynomolgus macaques

Capsid	Putamen		
	vg/dg	mRNA	%DAB+ Cells
AAV9	0.1	0.03	2
TTJ-001	1.1	0.15	11
Capsid	Caudate		
	vg/dg	mRNA	%DAB+ Cells
AAV9	0.04	0.02	2
TTJ-001	0.91	0.34	19
Capsid	Motor Cortex		
	vg/dg	mRNA	%DAB+ Cells
AAV9	0.1	0.02	1
TTJ-001	0.9	0.62	23
Capsid	Cervical (C3) Spinal Cord		
	vg/dg (ventral horn)	mRNA (ventral horn)	%DAB+ Cells (grey matter)
AAV9	0.1	0.4	2
TTJ-001	2.2	5.8	11
Capsid	Cervical Dorsal Root Ganglion		
	vg/dg	mRNA	%DAB+ Cells
AAV9	0.10	3.9	1
TTJ-001	0.17	2.9	1

Table 20. Quantification of viral genome copies per diploid genome (vg/dg) (biodistribution) by ddPCR, transgene mRNA by RT-qPCR (mRNA = transgene mRNA fold over housekeeping gene), and percent of DAB+ cells in peripheral tissues of cynomolgus macaques

Capsid	Liver		
	vg/dg	mRNA	%DAB+ Cells
AAV9	147	8.9	93
TTJ-001	15	0.7	44
Capsid	Heart		
	vg/dg	mRNA	%DAB+ Cells
AAV9	2.2	25.8	27
TTJ-001	0.9	20.1	33
Capsid	Muscle (Vastus Lateralis)		
	vg/dg	mRNA	%DAB+ Cells
AAV9	0.58	1.38	
TTJ-001	1.68	28.72	72
Capsid	Muscle (Gastrocnemius)		
	vg/dg	mRNA	%DAB+ Cells
AAV9	0.56	2.13	
TTJ-001	0.86	3.95	64

Table 21. Ratio of viral genome copies per diploid genome (vg/dg) (biodistribution) or transgene mRNA fold over housekeeping gene (mRNA) in the muscle and motor cortex relative to the liver and/or heart

Capsid	Gastrocnemius to Liver	
	Biodistribution	mRNA
AAV9	0.0038	0.24
TTJ-001	0.06	5.65
Capsid	Vastus Lateralis to Liver	
	Biodistribution	mRNA
AAV9	0.0039	0.16

TTJ-001	0.12	41.59
Capsid	Gastrocnemius to Heart	
	Biodistribution	mRNA
AAV9	0.25	0.083
TTJ-001	0.96	0.20
Capsid	Vastus Lateralis to Heart	
	Biodistribution	mRNA
AAV9	0.26	0.053
TTJ-001	1.87	1.43
Capsid	Motor Cortex to Liver	
	Biodistribution	mRNA
AAV9	0.00068	0.0022
TTJ-001	0.06	0.899

[0454] Taken together, these data demonstrate that TTJ-001 demonstrated increased CNS tropism but also increased tropism in the heart and muscle in cynomolgus macaques.

Example 3. Evaluation of TTJ-001 AAV9 capsid variant in Diverse Primate Species

[0455] This Example evaluates the tropism and cross-species compatibility of the TTJ-001 (SEQ ID NO: 981 (amino acid) and 983 (DNA), comprising SEQ ID NO: 941 or 201) capsid variant in two diverse primate species, marmosets (*Callithrix jacchus*) and African green monkeys (*Chlorocebus sabaues*), as compared to their tropism in cynomolgus macaques (*Macaca fascicularis*) provided in Example 1 and 2.

[0456] To investigate tropism in African green monkeys, AAV particles comprising the TTJ-001 capsid variant or an AAV9 control, under the control of a synapsin promoter, were intravenously injected into NHPs (n=2, 3-12 years of age) at a dose of 2E13 vg/kg. After 14-days in life, the brains and tissues (liver, DRG, quadriceps, and heart) of the NHPs were collected and RNA was extracted. Following RNA recovery and RT-PCR amplification, a systematic NGS enrichment analysis was performed to calculate the fold enrichment ratio relative to the AAV9 wild-type control.

[0457] To investigate tropism in marmoset monkeys, AAV particles comprising the TTJ-001 capsid variant or an AAV9 control, were intravenously injected into the NHPs (n=2, >10 months of age) at a dose of 2E13 vg/kg (8.75E12 vg/mL). After 28-days in life, the brains and tissues (liver quadriceps, and heart) of the NHPs were collected and RNA was extracted. Following RNA recovery and RT-PCR amplification, a systematic NGS enrichment analysis was performed to calculate the fold enrichment ratio relative to the AAV9 wild-type control.

[0458] As provided in Table 22 (African green monkeys) and Table 23 (marmosets), the TTJ-001 capsid variant demonstrated increased CNS tropism in diverse primate species relative to the AAV9 control. The TTJ-001 capsid variant demonstrated a 460.2-fold increase in expression relative to AAV9 in the brain of cynomolgus macaques (Table 9, Example 1), a 14.67-fold increase in expression relative to AAV9 in the brain of African green monkeys, and a 24.2-fold increase in expression relative to AAV9 in the brain of marmosets.

Table 22. NGS fold-enrichment of TTJ-001 AAV capsid variant comprising SEQ ID NO: 941 in African green monkeys

Sequence	SEQ ID NO:	Fold enrichment relative to AAV9					
		Brain	DRG	Heart	Liver DNA	Liver RNA	Muscle DNA
TQDWHRI	941	14.670	0.05099	1.4567	0.1935	0.4688	1.2547

Table 23. NGS-fold enrichment of TTJ-001 (comprises SEQ ID NO: 941) in marmosets

Sequence	SEQ ID NO:	Fold enrichment relative to AAV9				
		Brain	Heart	Liver DNA	Liver RNA	Muscle DNA
TQDWHRI	941	24.2163	1.5775	0.0720	0.1706	3.6998

[0459] Taken together, these data demonstrate that the AAV9 capsid variant TTJ-001 demonstrated increased CNS tropism relative to the AAV9 control in the CNS across three diverse primate species, providing evidence of strong cross-species capacity.

Example 4. Maturation of TTJ-001 Capsid in NHPs and Mice

[0460] This Example describes maturation of the AAV9 capsid variant, TTJ-001 (SEQ ID NO: 981 (amino acid) and 983 (DNA), comprising SEQ ID NO: 941 (encoded by SEQ ID NO: 942)) or 201) in two species of NHPs, cynomolgus macaques (*Macaca fascicularis*) and marmosets (*Callithrix jacchus*) to further enhance their transduction and biodistribution in the central nervous system as well as other tissues, and evolve the AAV capsid variants to provide further cross-species compatibility. The TTJ-001 capsid variant was also matured in mice. Two approaches were used to mature the TTJ-001 capsid sequence in order to randomize and mutate within and around the modifications comprised within loop VIII of the capsid variant. In the first maturation approach, sets of three contiguous amino acids were randomized across the mutagenesis region in the TTJ-001 sequence, which spanned from position 582 to position 593, numbered according to SEQ ID NO: 981. In the second maturation approach, mutagenic primers were used to introduce point mutations at a low frequency, scattered across the mutagenesis region in the TTJ-001 sequence ranging from position 582 to position 593, numbered according to SEQ ID NO: 981.

[0461] The library of pooled matured AAV capsid variants generated from TTJ-001 using the first maturation approach and the library of pooled matured AAV capsid variants generated from TTJ-001 using the second maturation approach were each injected into two cynomolgus macaques (*Macaca fascicularis*), two marmosets (*Callithrix jacchus*), and three outbred mice. After a 14- or 28-day period in life, the brains of the NHPs and mice were isolated and RNA was extracted. Following RNA recovery and RT-PCR amplification, a systematic NGS enrichment analysis was performed to calculate the fold enrichment ratio relative to the TTJ-001 non-matured control or an AAV9 control, and the peptides comprised within the variants were identified.

[0462] Following the RNA recovery and NGS analysis from the second maturation approach, the matured capsid variants were filtered based on their coefficient of variation (CV), which was calculated for each peptide across the brain samples taken from the NHPs and mice. Those that had a CV value <1 were identified, as these were the peptides that were reliably detected in the majority of samples isolated

from the brains of the two NHPs. The peptides were also filtered based on a read per million (RPM) greater than or equal to 1 in the original virus stock.

[0463] Table 24 provides the peptide sequences of the matured capsid variants from the first maturation approach having an RPM greater than or equal to 1 in the original virus stock, a CV of less than 1 for the brain samples isolated, and that also demonstrated a 24-fold or greater fold-increase in expression in the brain relative to the AAV9 control in the brain of cynomolgus macaques (*Macaca fascicularis*) and/or a 50-fold or greater fold-increase in expression in the brain relative to the AAV9 control in the brain of marmosets (*Callithrix jacchus*), that were also detected in the maturation screen in mice, as these variants demonstrate cross-species compatibility. For example, the TTJ-001 capsid variants comprising SEQ ID NOs: 205, 208, 212, 219, 286, or 307 generated using the first maturation approach demonstrated a fold change greater than the non-matured TTJ-001 control and the AAV9 control in both species of NHPs and mice. TTJ-001 capsid variants comprising any one of SEQ ID NOs: 262-265 generated using the first maturation approach demonstrated a fold change greater than the non-matured TTJ-001 control and the AAV9 control in both species of NHPs.

Table 24. NGS fold-enrichment of the TTJ-001 matured AAV capsid variants in the brain of cynomolgus macaques, marmosets, and mice generated using the first maturation approach

Sequence	SEQ ID NO	Brain of cynomolgus macaques		Brain of marmosets		Brain of mice	
		Fold change relative to AAV9	Fold change relative to TTJ-001	Fold change relative to AAV9	Fold change relative to TTJ-001	Fold change relative to AAV9	Fold change relative to TTJ-001
TNHQSAQAQAQTGWV	200	1.000	0.012	1.000	0.009	1.000	0.337
TNTQDWHRIAQTGWV	201	86.808	1.000	107.267	1.000	2.968	1.000
TNTKQDWHRIAQTGWV	202	104073.655	1198.899	103.431	0.964	1.864	0.628
TNNQDWHRIAQTGWV	203	5142.077	59.235	97.137	0.906	1.841	0.620
SNTQDWHRIAQTGWV	204	328.308	3.782	79.847	0.744	1.327	0.447
TTNQDWHRIAQTGWV	205	276.077	3.180	114.308	1.066	5.302	1.786
TTIQDWHRIAQTGWV	206	260.231	2.998	141.163	1.316	3.616	1.218
TTSQDWHRIAQTGWV	207	210.077	2.420	92.842	0.866	5.559	1.873
TTTQDWHRIAQTGWV	208	169.577	1.953	120.786	1.126	4.445	1.498
TTAQDWHRIAQTGWV	209	164.462	1.895	79.735	0.743	5.480	1.846
TVKQDWHRIAQTGWV	210	160.308	1.847	0.994	0.009	1.709	0.576
TNTQDWHRIAQTGWA	211	147.692	1.701	100.449	0.936	6.250	2.106
TNTQDWHRIAQTGWV	212	146.231	1.685	134.751	1.256	3.718	1.253
TNIQDWHRIAQTGWV	213	132.423	1.525	108.511	1.012	3.557	1.198
TNTQDWHRIAGSNV	214	122.115	1.407	130.419	1.216	0.711	0.240
TNTQDWHRIAGTNV	215	122.077	1.406	83.846	0.782	2.332	0.786
TNTQDWHRVPQTGWV	216	110.962	1.278	96.982	0.904	5.602	1.887
TNTQDWHRIAQTGNV	217	106.346	1.225	92.251	0.860	1.014	0.342
STIQDWHRIAQTGWV	218	106.077	1.222	64.835	0.604	3.720	1.253
TTVQDWHRIAQTGWV	219	105.962	1.221	73.066	0.681	4.468	1.505
TGTQDWHRIAQTGWV	220	105.769	1.218	78.978	0.736	6.607	2.226
TNTRDWHRIAQTGWV	221	101.000	1.163	40.015	0.373	0.761	0.257
TNTQDWHRVNQTGWV	222	98.154	1.131	54.952	0.512	1.045	0.352
TNTQDWHRIAQTGWI	223	94.692	1.091	55.884	0.521	1.082	0.364
SNIQDWHRIAQTGWV	224	90.846	1.047	73.408	0.684	1.109	0.374
TYTQDWHRIAQTGWV	225	90.192	1.039	105.558	0.984	3.048	1.027
TNTEDWHRIAQTGWV	226	82.077	0.946	58.641	0.547	2.900	0.977
TNIEDWHRIAQTGWV	227	81.462	0.938	41.710	0.389	4.566	1.538

TNTQDWHRISGTGWV	228	77.077	0.888	95.306	0.888	1.434	0.483
TNSQDWHRIAQTGWV	229	73.231	0.844	95.140	0.887	1.184	0.399
STTQDWHRIAQTGWV	230	71.269	0.821	89.973	0.839	5.816	1.959
TNTSDWHRIAQTGWV	231	68.231	0.786	42.394	0.395	1.789	0.603
TNTQDWHRVAQTGWV	232	67.962	0.783	179.050	1.669	5.439	1.832
TNTQDWHRIAQTGWD	233	67.308	0.775	97.864	0.912	2.755	0.928
TNTQDWHRVMTGWV	234	60.077	0.692	128.642	1.199	3.098	1.044
TNTQDWHRISMTGWV	235	55.808	0.643	64.425	0.601	2.134	0.719
TNTQDWHRISQTGWV	236	55.808	0.643	106.204	0.990	1.941	0.654
TNTQDWHRVSQTGWV	237	53.538	0.617	149.383	1.393	3.516	1.185
TSTQDWHRIAQTGWV	238	52.846	0.609	80.307	0.749	6.952	2.342
TNTQDWHRIANTGWV	239	51.154	0.589	95.615	0.891	2.470	0.832
TNTQDWHRIANSVWV	240	47.808	0.551	68.424	0.638	1.936	0.652
TNTQDWHRIWQTGWV	241	41.577	0.479	127.904	1.192	2.364	0.796
TNTQDWHRIFQTGWV	242	37.654	0.434	62.285	0.581	2.250	0.758
TNTQDWHRIQQTGWV	243	37.154	0.428	52.927	0.493	1.568	0.528
TNTQDWHRIPTQTGWV	244	35.231	0.406	123.655	1.153	2.259	0.761
KNTQDWHRIAQTGWV	245	34.500	0.397	72.759	0.678	1.448	0.488
TNTQVWHRIAQTGWV	246	34.346	0.396	141.787	1.322	1.668	0.562
TNTQDWHRVQQTGWV	247	32.692	0.377	36.503	0.340	1.350	0.455
STSQDWHRIAQTGWV	248	31.308	0.361	64.130	0.598	2.616	0.881
SNSQDWHRIAQTGWV	249	30.769	0.354	39.277	0.366	0.834	0.281
TNTQDWHRIFMTGWV	250	28.462	0.328	179.824	1.676	1.027	0.346
TNTQDWHRIAQTGWV	251	28.154	0.324	71.807	0.669	1.630	0.549
TNTQDWHRIAQTGWV	252	27.500	0.317	47.538	0.443	0.925	0.312
TNLQDWHRIAQTGWV	253	26.500	0.305	48.431	0.451	0.882	0.297
TNTQDWHRIAMTGWV	254	25.885	0.298	53.924	0.503	2.466	0.831
TITQDWHRIAQTGWV	255	24.192	0.279	37.506	0.350	4.073	1.372
TNTQAWHRIAQTGWV	256	17.885	0.206	103.032	0.960521	1.363636	0.459418
TNTQDWHRIANTQWV	257	21.654	0.249	93.59179	0.872514	1.527273	0.514548
TNTQDWHRIAKTGWV	258	23.615	0.272	75.35993	0.702546	1.061364	0.35758
TNTQDWHRIAQTRWV	259	11.769	0.136	65.30475	0.608806	0.713636	0.240429
TSMQDWHRIAQTGWV	260	12.808	0.148	61.10777	0.56968	3.618182	1.218989
TNTQDWHRISNTGWV	261	17.423	0.201	56.85073	0.529993	1.122727	0.378254
TNTQDWHRIANNWV	262	109.038	1.256	131.614	1.227		
TNTQDWHRIYQTGWV	263	117.000	1.348	122.610	1.143		
TNTPDWHRIAQTGWV	264	150.192	1.730	110.406	1.029		
TNTQDWHRINQTGWV	265	104.962	1.209	133.429	1.244		
TTLQDWHRIAQTGWV	286	96.692	1.114	89.011	0.830	5.220	1.759
TNTQDWHRIAQTGWS	307	100.192	1.154	159.377	1.486	3.989	1.344

[0464] Table 25 provides the peptide sequences of the matured capsid variants from the second maturation approach having an RPM greater than or equal to 1 in the original virus stock, a CV of less than 1 for the brain samples isolated, and that also demonstrated a 36-fold or greater fold-increase in expression in the brain relative to the AAV9 control in the brain of cynomolgus macaques (*Macaca fascicularis*) and/or about a 10-fold or greater fold-increase in expression in the brain relative to the AAV9 control in the brain of marmosets (*Callithrix jacchus*), that were also detected in the maturation screen in mice, as these variants demonstrate cross-species compatibility. For example, the TTJ-001 capsid variants comprising SEQ ID NOs: 283, 286, 290, 291, and 293 generated using the second maturation approach demonstrated a fold change greater than the non-matured TTJ-001 control and the AAV9 control in both species of NHPs and mice. The TTJ-001 capsid variants comprising SEQ ID NOs: 205, 208, 212, 213, or 307 generated using the second maturation approach demonstrated a fold change

greater than the non-matured TTJ-001 control in cynomolgus macaques and mice and a fold-change greater than the AAV9 control in both species of NHPs and mice. The TTJ-001 capsid variants comprising SEQ ID NOs: 219 and 265 generated using the second maturation approach demonstrated a fold change greater than the non-matured TTJ-001 control and the AAV9 control in both species of NHPs and the TTJ-001 capsid variant comprising SEQ ID NO: 263 generated using the second maturation approach lead to a fold-change greater than AAV9 in both species of NHPs and a fold-change greater than TTJ-001 in marmosets.

Table 25. NGS fold-enrichment of the TTJ-001 matured AAV capsid variants in the brain of cynomolgus macaques, marmosets, and mice generated using the second maturation approach

Sequence	SEQ ID NO	Brain of cynomolgus macaques		Brain of marmosets (Callithrix jacchus)		Brain of mice	
		Fold change relative to AAV9	Fold change relative to TTJ-001	Fold change relative to AAV9	Fold change relative to TTJ-001	Fold change relative to AAV9	Fold change relative to TTJ-001
TNHQSAQAQQTGWV	200	1.000	0.014	1.000	0.082	1.000	0.552
TNTQDWHRIAQTGWV	201	73.612	1.000	12.159	1.000	1.812	1.000
TNTKDWHRIAQTGWV	202	33375.082	453.390	5.684	0.467	0.419	0.231
TTTKDWHRIAQTGWV	268	25769.367	350.069	5.950	0.489	0.588	0.324
TNIKDWHRIAQTGWV	269	24409.245	331.592	7.741	0.637	0.467	0.258
TSTKDWHRIAQTGWV	270	12410.469	168.592	4.343	0.357	0.544	0.300
SNNQDWHRIAQTGWV	271	2542.510	34.539	3.599	0.296	0.387	0.214
TNNQDWHRIAQTGWV	203	1335.286	18.139	8.723	0.717	0.868	0.479
TNNQDWHRIAQTGWA	272	1313.633	17.845	8.078	0.664	1.935	1.068
TTNQDWHRIAQTGWV	205	1301.776	17.684	12.114	0.996	2.992	1.652
TNNQDWHRISQTGWV	273	1283.327	17.434	7.778	0.640	0.788	0.435
TNNQDWHRVAQTGWV	274	1237.306	16.808	10.524	0.866	2.197	1.213
SNMQTALQLAQTGWV	275	811.857	11.029	1.133	0.093	0.562	0.310
TSNQDWHRIAQTGWV	276	691.816	9.398	1.580	0.130	0.567	0.313
TNNQDWHRIPQTGWV	277	653.020	8.871	7.901	0.650	0.539	0.298
TNNQDWHRIAQTGWD	278	597.592	8.118	4.004	0.329	0.571	0.315
TNNQDWHRIAQTGWF	279	411.490	5.590	3.987	0.328	0.450	0.248
INNQDWHRIAQTGWV	280	342.551	4.653	3.427	0.282	0.391	0.216
TYNQDWHRIAQTGWV	281	299.327	4.066	2.838	0.233	1.226	0.677
TTIQDWHRIAQTGWV	206	168.531	2.289	11.981	0.985	2.141	1.182
TINQDWHRIAQTGWV	282	142.776	1.940	1.775	0.146	0.384	0.212
SNIQDWHRIAQTGWV	224	133.633	1.815	3.791	0.312	0.677	0.374
STTQDWHRIAQTGWV	230	122.041	1.658	8.430	0.693	3.141	1.734
SNTQDWHRIAQTGWV	204	119.367	1.622	7.652	0.629	0.662	0.365
TNIQDWHRVAQTGWV	283	111.469	1.514	15.433	1.269	2.916	1.609
TNIQDWHRIAQTGWA	284	109.082	1.482	8.147	0.670	2.139	1.181
SNTQDWHRVAQTGWV	285	108.653	1.476	9.001	0.740	1.973	1.089
TTLQDWHRIAQTGWV	286	106.367	1.445	12.205	1.004	3.363	1.856
TNTQDWHRTIAHTGWV	287	99.388	1.350	8.489	0.698	0.953	0.526
SNTQDWHRIAQTGWG	288	94.020	1.277	9.090	0.748	0.406	0.224
TTSQDWHRIAQTGWV	207	93.306	1.268	10.701	0.880	2.795	1.543
TNNQDWHKIAQTGWV	289	92.265	1.253	1.508	0.124	0.351	0.194
TTTQDWHRVAQTGWV	290	89.898	1.221	18.615	1.531	2.202	1.216
TNSQDWHRIAQTGWA	291	89.245	1.212	13.447	1.106	2.235	1.234
TNIQDWHRIAQTGWV	213	89.224	1.212	12.043	0.990	2.065	1.139
TNTQDWHRVPTGWV	216	89.041	1.210	10.853	0.893	3.075	1.697
SNTQDWHRISQTGWV	292	88.245	1.199	4.468	0.367	0.402	0.222
TNSQDWHRVAQTGWV	293	86.367	1.173	17.030	1.401	2.246	1.240
TNTQDWHRIAGTGWV	212	84.469	1.147	8.580	0.706	2.620	1.446

TNIQDWHRISQTGWV	294	79.510	1.080	9.123	0.750	1.735	0.958
TYIQDWHRIAQTGWV	295	77.102	1.047	10.317	0.849	1.416	0.781
TTTQDWHRIAQTGWV	208	75.980	1.032	11.968	0.984	2.567	1.417
TNNQDWHRNAQTGWV	296	75.898	1.031	1.070	0.088	0.386	0.213
TITQDWHRVAQTGWV	297	72.612	0.986	15.948	1.312	1.677	0.926
TNNQDSHRIAQTGWV	298	72.286	0.982	0.842	0.069	0.461	0.254
TNTEDWHRVAQTGWV	299	70.939	0.964	6.856	0.564	2.898	1.600
TTTQDWHRIPQTGWV	300	69.959	0.950	14.367	1.182	2.088	1.152
TSTQDWHRIAQTGWA	301	67.816	0.921	10.481	0.862	3.038	1.677
TNTQDWHRVAQTGWV	232	67.265	0.914	15.332	1.261	2.838	1.566
SNSQDWHRIAQTGWV	249	66.347	0.901	3.312	0.272	0.374	0.207
TNTQDWHRIAQTGWC	302	66.163	0.899	10.852	0.893	2.340	1.292
TNTQDWHRSAQTGWA	303	65.796	0.894	5.320	0.438	2.829	1.562
TNNQDQORIAQTGWV	304	65.449	0.889	1.976	0.163	0.498	0.275
TNIQDWHRIPQTGWV	305	64.918	0.882	8.006	0.658	1.939	1.070
TTTQDWHRIAQTGWI	306	64.755	0.880	10.242	0.842	2.323	1.282
TNTQDWHRIAQTGWA	211	64.531	0.877	9.148	0.752	2.256	1.245
TNTQDWHRIAQTNNV	217	64.531	0.877	10.720	0.882	0.547	0.302
TNTQDWHRISQTGWV	236	64.408	0.875	11.776	0.969	1.264	0.697
TNTQDWHRIAQTGWS	307	61.061	0.829	8.875	0.730	2.011	1.110
TTTQDWHRISQTGWV	308	60.571	0.823	12.149	0.999	2.217	1.223
TSTQDWHRVAQTGWV	309	60.020	0.815	14.685	1.208	4.129	2.279
TNSQDWHKIAQTGWA	310	58.653	0.797	0.316	0.026	0.428	0.236
TNIEDWHRIAQTGWV	227	57.184	0.777	6.278	0.516	2.206	1.217
TNIQDWHRIAQTGWD	311	56.224	0.764	6.485	0.533	1.055	0.582
TNTQDWHRIAQTGWI	223	55.306	0.751	7.555	0.621	0.530	0.292
TYTQDWHKIAQTGWV	312	53.878	0.732	8.042	0.661	2.631	1.452
TYTQDWHRIAQTGWA	313	53.633	0.729	12.179	1.002	1.007	0.556
TNSQDWHRIAQTGWV	229	52.959	0.719	8.475	0.697	0.662	0.365
TNTQDWHRISQTGWA	314	52.939	0.719	11.037	0.908	2.278	1.257
TNTEDWHRIAQTGWV	226	52.469	0.713	5.899	0.485	1.515	0.836
TNNQDWHRIAQTGWV	315	52.143	0.708	6.363	0.523	1.473	0.813
TGTQDWHRIAQTGWV	220	51.755	0.703	10.758	0.885	3.423	1.889
TNTQDWHRIAQTGWT	316	50.612	0.688	16.498	1.357	1.282	0.708
TITQDWHRIAQTGWV	317	50.143	0.681	3.892	0.320	3.096	1.709
TNTQDWHRVAQTGWA	318	49.163	0.668	8.373	0.689	2.504	1.382
SNTQDWHRIAQTAWV	319	49.143	0.668	7.360	0.605	0.852	0.470
TNNQDGORIAQTGWV	320	48.980	0.665	0.240	0.020	0.569	0.314
TNNQDGHRIAQTGWV	321	47.755	0.649	1.436	0.118	0.621	0.343
TNTQDWHRVSQTGWV	237	47.245	0.642	17.402	1.431	2.254	1.244
TNNQDLHRIAQTGWV	322	46.837	0.636	1.208	0.099	0.495	0.273
TNTSDWHRIAQTGWV	231	46.388	0.630	5.387	0.443	1.406	0.776
TTTQDWHRIAQTGWA	323	46.347	0.630	8.971	0.738	1.648	0.909
TNTQDWHRIGQTGWA	324	44.796	0.609	8.983	0.739	0.869	0.479
TNNQDWHRIAQTGGV	325	44.306	0.602	1.098	0.090	0.447	0.247
SYTQDWHRIAQTGWV	326	43.694	0.594	6.276	0.516	2.302	1.271
TNTEDWHRIAQTGWA	327	42.000	0.571	1.916	0.158	0.898	0.496
TYTQDWHRIAQTGWG	328	41.959	0.570	6.335	0.521	0.511	0.282
TNNQDWHRIAQTGWV	329	41.939	0.570	1.908	0.157	0.353	0.195
TNCQDWHRIAQTGWV	330	41.837	0.568	5.317	0.437	1.027	0.567
TNTQDWHRMAQTGWA	331	40.816	0.554	3.509	0.289	1.323	0.730
SSTQDWHRIAQTGWV	332	39.796	0.541	1.253	0.103	1.016	0.561
TYTQDWHRIAQTGWV	225	39.714	0.540	6.574	0.541	1.335	0.737
TNTQDWHRIANTGWV	239	39.673	0.539	6.450	0.530	1.253	0.691
TYTQDWHRIAQTGWV	333	39.143	0.532	5.309	0.437	1.614	0.891
TYTQDWHRVAQTGWV	334	38.776	0.527	5.233	0.430	0.616	0.340
SNTQDWHRIAQTGWD	335	37.612	0.511	3.097	0.255	0.394	0.217
TVTQDWHRIAQTGWV	336	36.776	0.500	7.408	0.609	3.253	1.795

QNTQDWHRIAQTGWV	337	25.469	0.346	13.394	1.102	2.073	1.144
TNTQDWHRIPTGTGWV	244	33.735	0.458	12.324	1.014	1.295	0.715
TNSQDWHRIPTGTGWV	339	27.531	0.374	11.380	0.936	0.405	0.223
TNTQDWHRIAQPGWG	340	7.612	0.103	11.170	0.919	0.502	0.277
TNTQDWHRVAQTGWD	341	8.306	0.113	10.221	0.841	1.089	0.601
TNTEDWHRIAQTGWG	342	25.673	0.349	10.085	0.829	0.442	0.244
TTVQDWHRIAQTGWV	219	114.673	1.558	27.218	2.239		
TNTQDWHRIQTGWV	265	91.776	1.247	14.651	1.205		
TNTQDWHRIYQTGWV	263	55.571	0.755	15.477	1.273		
TNTPDWHRIAQTGWV	264	17.286	0.235	5.269	0.433		

[0465] Table 26 provides the sequences of TTJ-001 capsid variants with an RPM greater than or equal to 1 in the original virus stock, a CV value of less than or equal to 1 and a fold-change in the brain of cynomolgus macaques of greater than 50 relative to AAV9. Approximately 59 variants were identified using the first maturation approach with a fold change above the TTJ-001 non-matured control in the brain of cynomolgus macaques. Approximately 181 variants were identified using the second maturation approach with a fold change above the TTJ-001 non-matured control in the brain of cynomolgus macaques. Many of the variants with increased expression in the brain of cynomolgus macaques relative to AAV9 and the non-matured TTJ-001 controls comprised a K at position 585 numbered according to SEQ ID NO: 981 or 138.

Table 26. NGS fold-enrichment of TTJ-001 in the brain of cynomolgus macaques by both maturation approaches

Sequence	SEQ ID NO:	First Maturation Approach	
		Fold change relative to AAV9	Fold change relative to TTJ-001
TNTKQDWHRIAQTGWV	202	104073.7	1198.899
TNNQDWHRIAQTGWV	203	5142.077	59.23527
SNNQDWHRIAQTGWV	271	2083.962	24.00665
TNKQDWHRIAQTGWV	539	2062.769	23.76252
TNNQDWHRIAQTGWV	540	428.3846	4.934869
SNKQDWHRIAQTGWV	541	372.0385	4.285778
SNTQDWHRIAQTGWV	204	328.3077	3.782012
TTNQDWHRIAQTGWV	205	276.0769	3.180328
TNDQDWHRIAQTGWV	542	274.6538	3.163934
TNTKDRHRIAQTGWV	543	265.9615	3.063802
TTIQDWHRIAQTGWV	206	260.2308	2.997785
RNTQDWHRIAQTGWV	544	242.5769	2.794417
TNTKDSHRIAQTGWV	545	238.7308	2.750111
TTKQDWHRIAQTGWV	546	221.8077	2.555162
TTSQDWHRIAQTGWV	207	210.0769	2.420027
TTDQDWHRIAQTGWV	547	204.7308	2.35844
TNTKDLHRIAQTGWV	548	199.8846	2.302614
TNPSDWHRIAQTGWV	549	181.500	2.090829
TNKEDWHRIAQTGWV	550	174.4231	2.009304
TTTQDWHRIAQTGWV	208	169.5769	1.953478
TGKQDWHRIAQTGWV	551	168.9615	1.946389
TTAQDWHRIAQTGWV	209	164.4615	1.89455
TVKQDWHRIAQTGWV	210	160.3077	1.846699
TNNQDWQRIAQTGWV	304	154.0385	1.774479
TNYQDWHRIAQTGWV	552	153.7692	1.771378
TNTPDWHRIAQTGWV	264	150.1923	1.730173
STKQDWHRIAQTGWV	553	148.3846	1.709349
TNTQDWHRIAQTGWA	211	147.6923	1.701374

TNTQDWHRIAQTGWV	212	146.2308	1.684537
TNTQDWHRIAQTNWS	554	137.6923	1.586176
TTEQDWHRIAQTGWV	555	134.2308	1.5463
TSKQDWHRIAQTGWV	556	134.1154	1.544971
TNIQDWHRIAQTGWV	213	132.4231	1.525476
TNTQDWHRIAGSNWV	214	122.1154	1.406735
TNTQDWHRIAGTNWV	215	122.0769	1.406292
TNTQDWHRIAQTNWI	557	120.2692	1.385467
TNTQDWHRIYQTGWV	263	117	1.347807
TYNQDWHRIAQTGWV	281	116.0769	1.337173
TNTQDWHRVPQTGWV	216	110.9615	1.278245
TNTQDWHRIANNWV	262	109.0385	1.256092
TNTQDWHRIAQTNNWV	217	106.3462	1.225078
STIQDWHRIAQTGWV	218	106.0769	1.221976
TTVQDWHRIAQTGWV	219	105.9615	1.220647
TGTQDWHRIAQTGWV	220	105.7692	1.218432
TNTQDWHRIQTGWV	265	104.9615	1.209127
TNNQEWHRIAQTGWV	558	101.1538	1.165264
TNTRDWHRIAQTGWV	221	101	1.163491
TNTQDWHRIAQTGWS	307	100.1923	1.154187
TNTQDWHRVNQTGWV	222	98.15385	1.130704
TTLQDWHRIAQTGWV	286	96.69231	1.113868
TNTQDWHRIANSNWV	559	96.53846	1.112096
TNTQDWHRIAQTGWI	223	94.69231	1.090829
TTMQDWHRIAQTGWV	560	93.11538	1.072663
ANNQDWHRIAQTGWV	561	91.26923	1.051396
SNIQDWHRIAQTGWV	224	90.84615	1.046522
TKNQDWHRIAQTGWV	562	90.69231	1.04475
TYTQDWHRIAQTGWV	225	90.19231	1.03899
TNKSDWHRIAQTGWV	563	89.92308	1.035888
TNTQDWHRVYQTGWV	564	86.96154	1.001772
TNTQDWHRIAQTGWV	201	86.80769	1
TNTQDWHRIAQTGWC	302	84.5	0.973416
SNTEDWHRIAQTGWV	565	82.23077	0.947275
TNTEDWHRIAQTGWV	226	82.07692	0.945503
TNIEDWHRIAQTGWV	227	81.46154	0.938414
TTRQDWHRIAQTGWV	566	81.23077	0.935755
TNTQDWHRVANTGWV	567	78.80769	0.907842
TNTQDWHRISQTGWV	228	77.07692	0.887904
TNSQDWHRIAQTGWV	229	73.23077	0.843598
TNHQSAQAQTGWV	200	1	0.01152
Second Maturation Approach			
Sequence	SEQ ID NO:	Fold change relative to AAV9	Fold change relative to TTJ-001
TYTKDWHRIAQTGWV	568	39542.980	537.179
TNTKDWHRIAQTGWV	202	33375.082	453.390
TTTKDWHRIAQTGWV	268	25769.367	350.069
TNIKDWHRIAQTGWV	269	24409.245	331.592
TNTKDWHRISQTGWV	569	21847.571	296.793
SNTKDWHRIAQTGWV	570	15438.020	209.721
TNNKDWHRIAQTGWV	571	14378.939	195.334
TNSKDWHRIAQTGWV	572	12740.796	173.080
TSTKDWHRIAQTGWV	270	12410.469	168.592
TNTKDWHRVAQTGWV	4680	10573.939	143.644
TITKDWHRIAQTGWV	573	9400.980	127.709
TNTKDWHRIAQTGWG	574	6661.061	90.488
SNKQDWHRIAQTGWV	541	4260.143	57.873
INTKDWHRIAQTGWV	575	3926.816	53.345

TNAKDWHRIAQTGWV	576	3924.163	53.309
TNKQDWHRIAQTGWV	539	3600.776	48.915
TNTKDWHRITQTGWV	577	3002.204	40.784
SNNQDWHRIAQTGWV	271	2542.510	34.539
TNTKDWHRTAQTGWV	578	2383.510	32.379
TKTKDWHRIAQTGWV	579	2025.184	27.512
STNQDWHRIAQTGWV	580	1985.184	26.968
TNTKDWHRIAQSGWV	581	1846.837	25.089
TNTKDWHRIAQPGWV	582	1696.184	23.042
TNTKDWHRMAQTGWV	583	1681.082	22.837
TNTKDWHRSAQTGWV	584	1647.714	22.384
TNTKDWHRIGQTGWV	585	1618.429	21.986
TNTKDWHRIAQTGSV	586	1599.571	21.730
TNTKDWHRIEQTGWV	587	1590.959	21.613
ANTKDWHRIAQTGWV	588	1565.184	21.263
RNNQDWHRIAQTGWV	589	1495.755	20.319
TNTKDWHKIAQTGWV	590	1405.082	19.088
TNTKDWLRIAQTGWV	591	1405.041	19.087
TNTKDWHRIVQTGWV	592	1352.265	18.370
TNNQDWHRIAQTGWV	203	1335.286	18.139
TNNQDWHRIAQTGWA	272	1313.633	17.845
TTNQDWHRIAQTGWV	205	1301.776	17.684
TNNQDWHRISQTGWV	273	1283.327	17.434
TNNQDWHRVAQTGWV	274	1237.306	16.808
TNTKDWHRNAQTGWV	593	1228.306	16.686
TNTKEWHRIAQTGWV	594	1144.388	15.546
TNTKDWHRIAQTDDV	595	1132.163	15.380
TNTKDWHRIAQTGCV	596	1118.531	15.195
TNTKDWQRIAQTGWV	597	1042.082	14.156
TGNQDWHRIAQTGWV	598	1024.367	13.916
TNNEQDWHRIAQTGWV	540	985.776	13.391
TNTKDWHRLAQTGWV	599	903.531	12.274
TNDQDWHRIAQTGWV	542	900.204	12.229
TNTKDWNRRIAQTGWV	600	867.490	11.785
TNYQDWHRIAQTGWV	552	860.408	11.688
TNNQDWHRIAQTGWI	601	857.367	11.647
TNNQDWHRIANTGWV	602	795.592	10.808
TNTKDWHRIAKTGWV	603	728.204	9.892
TNTKDWHRIAHTGWV	604	726.347	9.867
TNTKDWHRIAQTGRV	605	694.367	9.433
TSNQDWHRIAQTGWV	276	691.816	9.398
TNTKDWHRIAQTGLV	606	675.531	9.177
TNTKDWHRIAQTGGV	607	668.612	9.083
TNTKDWHRIAQAGWV	608	653.143	8.873
TNNQDWHRI PQTGWV	277	653.020	8.871
TNTKDWHTIAQTGWV	609	627.694	8.527
THTKDWHRIAQTGWV	610	614.735	8.351
TNTKDWHRIAQIGWV	611	612.653	8.323
TNNQDWHRIAQTGWD	278	597.592	8.118
TDTKDWHRIAQTGWV	612	596.000	8.096
TNTKDWHRIAQNGWV	613	588.612	7.996
TNTKDWHRIARTGWV	614	587.816	7.985
TNTKDWHRIAQTGWV	615	549.510	7.465
TNNQDWHRIAQTGWG	616	537.082	7.296
TNTKDSHRIAQTGWV	545	526.327	7.150
TNEQDWHRIAQTGWV	617	511.755	6.952
CNTQDWHRIAQTGWV	618	511.633	6.950
TTNQDWHRIAQSGWV	619	479.388	6.512

TNPKDWHRIAQTGWV	620	467.224	6.347
TNTKDLHRIAQTGWV	548	433.245	5.886
TNTKDWHRIAETGWV	621	431.939	5.868
TNTKDWHRIALTGWV	622	428.020	5.815
TNNQDWHRIAQTGWF	279	411.490	5.590
TNTKDWHGIAQTGWV	623	381.612	5.184
TNTKDCHRIAQTGWV	624	350.041	4.755
INNQDWHRIAQTGWV	280	342.551	4.653
TYTEDWHRIAQTGWV	625	320.429	4.353
TNNQNWHRIAQTGWV	626	299.531	4.069
TYNQDWHRIAQTGWV	281	299.327	4.066
TNNQHWHRIAQTGWV	627	263.571	3.581
TNNQDWHRITQTGWV	628	238.796	3.244
TNRKTALHLAQTGWV	629	234.857	3.190
NNNQDWHRIAQTGWV	630	227.776	3.094
KNNQDWHRIAQTGWV	631	221.327	3.007
TNTKDGHRIAQTGWV	632	217.143	2.950
TNSQDWHRIAQTGWS	633	205.082	2.786
STIQDWHRIAQTGWV	218	198.000	2.690
TNNQDWSIAHTGWV	634	189.735	2.577
TNTKDWPRIAQTGWV	635	187.939	2.553
TNNQDWHRIARTGWV	636	186.408	2.532
TNTKDRHSIAQTGWV	637	180.449	2.451
TNTKDRHRIAQTGWV	543	174.041	2.364
SNTQDWHRVPQTGWV	638	172.735	2.347
TNNQDWHRIAKTGWV	639	171.694	2.332
TTIQDWHRIAQTGWV	206	168.531	2.289
TNTKDWHRIAPTGWV	640	160.204	2.176
SNTQDWHRIAQTGWA	641	154.224	2.095
TNNRDWHRIAQTGWV	642	150.102	2.039
TNNQDWHRIAQTRWV	643	148.388	2.016
LNNQDWHRIAQTGWV	644	148.204	2.013
TINQDWHRIAQTGWV	282	142.776	1.940
TNNQDWHRIAHTGWV	645	142.551	1.937
TNNQDWHRIGQTGWV	646	141.245	1.919
TNTKDRQRIAQTGWV	647	141.020	1.916
TNNQDWYRIAQTGWV	648	137.347	1.866
TNNQDWHRIAQPGWV	649	135.980	1.847
TNNQGWHRIAQTGWV	650	135.755	1.844
SNNQDWHRIAQSGWV	651	134.653	1.829
SNIQDWHRIAQTGWV	224	133.633	1.815
TNNQDWHRFAQTGWV	652	132.020	1.793
SNNQDWHRMAQTGWV	653	130.939	1.779
TNHQDWHRIAQTGWV	654	129.143	1.754
STTQDWHRIAQTGWV	230	122.041	1.658
TNNQDWHRTAQTGWV	655	121.612	1.652
SNSQDWHRIAQTGWA	656	120.041	1.631
SNTQDWHRIAQTGWV	204	119.367	1.622
TNNQDWHRIAQTGRV	657	118.327	1.607
TNSKDGHRIAQTGWV	658	115.265	1.566
SNNQDWHKIAQTGWV	659	115.061	1.563
TNTKDWDRIAQTGWV	660	114.184	1.551
TNIQDWHRVAQTGWV	283	111.469	1.514
TNNQDWQRIAHTGWV	661	109.755	1.491
TNIQDWHRIAQTGWA	284	109.082	1.482
SNTQDWHRVAQTGWV	285	108.653	1.476
TTLQDWHRIAQTGWV	286	106.367	1.445
SNNQDWQRIAQTGWV	662	106.245	1.443

TNTNDLHRIAQTGWV	663	105.367	1.431
TNTQDWHRTIAHTGWV	664	99.388	1.350
TNNQEWHRRIAQTGWV	558	97.918	1.330
SNNQDWNRIAQTGWV	665	97.122	1.319
TNTQDWHKRIAQTGWA	666	96.469	1.311
TNNQDWHRIAQTSGWV	667	95.388	1.296
SNTQDWHRIAQTGWG	288	94.020	1.277
TNNQDWHRTIAHTGWV	668	93.980	1.277
TNSQDWHRIAQTGWV	669	93.776	1.274
TTSQDWHRIAQTGWV	207	93.306	1.268
TNNQDWHKRIAQTGWV	289	92.265	1.253
TTTQDWHRVAQTGWV	290	89.898	1.221
TNSQDWHRIAQTGWA	291	89.245	1.212
TNIQDWHRIAQTGWV	213	89.224	1.212
TNTQDWHRVPTGWV	216	89.041	1.210
SNTQDWHRTISQTGWV	292	88.245	1.199
SNSQDWHRVAQTGWV	670	88.163	1.198
TNNQDVHRIAQTGWV	671	87.816	1.193
SNTQDWHRIAQTGWV	672	87.041	1.182
TNSQDWHRVAQTGWV	293	86.367	1.173
TNNQDWHRSAQTGWV	673	86.224	1.171
SNNQDGHRIAQTGWV	674	86.163	1.171
ANNQDWQRIAQTGWV	675	86.082	1.169
STSQDWHRIAQTGWV	248	85.939	1.167
TNNQDWLRIAQTGWV	676	85.082	1.156
ANNQDWHRIAQTGWV	561	84.980	1.154
TNTQDWHRTISGTGWV	228	84.633	1.150
TNTQDWHRIAQTGWV	212	84.469	1.147
TNNQDWRRIAQTGWV	677	83.143	1.129
TNNQDWHWIAQTGWV	678	83.102	1.129
TNNQDWHRMAQTGWV	679	82.571	1.122
TNNQDWHRLAQTGWV	680	81.612	1.109
SNTQDWHRIAQTGWI	681	80.469	1.093
TNNQDWHRIAQTGVV	682	80.429	1.093
TNIQDWHRTISQTGWV	294	79.510	1.080
TNTQDWHRTIPQTGWA	683	79.265	1.077
TNKQDGHRIAQTGWV	684	79.041	1.074
TNTQDWHRTAQTGWA	685	77.980	1.059
TNIQDWHRIAQTGWI	686	77.755	1.056
TKNQDWHRIAQTGWV	562	77.694	1.055
TYIQDWHRIAQTGWV	295	77.102	1.047
TNNQDWHRIAQTGCV	687	77.061	1.047
TNNQDWHRTIVQTGWV	688	77.061	1.047
TNNQDWHRIAQTGWL	689	76.776	1.043
TNNQDWHRIAQTAWV	690	76.265	1.036
TTTQDWHRIAQTGWV	208	75.980	1.032
TNNQDWHRNAQTGWV	296	75.898	1.031
TNNQDRHGIAQTGWV	691	75.367	1.024
SGTQDWHRIAQTGWV	692	75.000	1.019
THNQDWHRIAQTGWV	693	74.551	1.013
TNSQDWHRTIAHTGWV	694	74.510	1.012
TNTQDWHRIAQTGWV	201	73.612	1.000
SNNQDWHRIAQTGGV	695	73.429	0.998
TITQDWHRVAQTGWV	297	72.612	0.986
TNNQDSHRIAQTGWV	298	72.286	0.982
TNTEDWHRVAQTGWV	299	70.939	0.964
TNNQDGHRIAQTSGWV	696	70.857	0.963
TNNQDWHRIAQTNGWV	697	70.776	0.961

TNNQDSQRIAQTGWV	698	70.469	0.957
TTTQDWHRI PQTGWV	300	69.959	0.950
RNTQDWHRIAQTGWV	544	69.776	0.948
SNNQDWHRIAQTGSV	699	69.388	0.943
TNSQDWHRIAQTNWV	700	68.469	0.930
SNNQDWHRIAETGWV	701	68.306	0.928
TSTQDWHRIAQTGWA	301	67.816	0.921
TNTQDWHRVAQTGWV	232	67.265	0.914
SNNQDSHRIAQTGWV	702	66.776	0.907
TNIQDWHRIANTGWV	703	66.673	0.906
TNSNDWHRIAQTGWV	704	66.367	0.902
SNSQDWHRIAQTGWV	249	66.347	0.901
TNTQDWHRIAQTGWC	302	66.163	0.899
TNTQDWHRSAQTGWA	303	65.796	0.894
TNNQDWQRIAQTGWV	304	65.449	0.889
TNIQDWHRI PQTGWV	305	64.918	0.882
TTTQDWHRIAQTGWI	306	64.755	0.880
SNTQDWHRIANTGWV	705	64.592	0.877
TNTQDWHRIAQTGWA	211	64.531	0.877
TNTQDWHRIAQTNWV	217	64.531	0.877
TNTQDWHRISQTGWV	236	64.408	0.875
TNNQHWQRIAQTGWV	706	64.306	0.874
TYNQDWQRIAQTGWV	707	63.918	0.868
TNNQDWHRIEQTGWV	708	63.408	0.861
TNNQDWHRIAQTSWV	709	62.551	0.850
RNTQDWHRISQTGWV	710	61.429	0.834
TNTQDWHRTIAQTGWA	711	61.327	0.833
TNTQDWHRIAQTGWS	307	61.061	0.829
TNQDQDWHRIAQTGWV	712	60.796	0.826
RNIQDWHRIAQTGWV	713	60.755	0.825
TTTQDWHRISQTGWV	308	60.571	0.823
TSTQDWHRVAQTGWV	309	60.020	0.815
TSTQDWHRVSTGWV	714	59.490	0.808
TNSQDWHKIAQTGWA	310	58.653	0.797
TNKQDWHRIEQTGWV	715	58.612	0.796
TNNPDWHRIAQTGWV	716	58.143	0.790
SNNQDWHRIAQTGAGV	717	58.041	0.788
TNNQDYHRIAQTGWV	718	57.755	0.785
TITEDWHRIAQTGWV	719	57.694	0.784
TNIEDWHRIAQTGWV	227	57.184	0.777
TNTNDWHRIAQTGWV	720	57.163	0.777
TNNQDWHRIAQTQDVV	721	56.837	0.772
SNTEDWHRIAQTGWV	565	56.306	0.765
TNIQDWHRIAQTGWD	311	56.224	0.764
SNTQDWHRI PQTGWV	722	55.939	0.760
TNNQDWHRIAQTGSV	723	55.571	0.755
SNNQDRHRIAQTGWV	724	55.469	0.754
TNTQDWHRIAQTGWI	223	55.306	0.751
TNNQDWQRISQTGWV	725	55.286	0.751
TNNQDWKRIAQTGWV	726	54.918	0.746
TNNEDRHRIAQTGWV	727	54.429	0.739
STTQDWHRISQTGWV	728	54.102	0.735
TYTQDWHKIAQTGWV	312	53.878	0.732
TFSQDWHRIAQTGWV	729	53.776	0.731
TYTQDWHRIAQTGWA	313	53.633	0.729
RNSQDWHRIAQTGWV	730	53.592	0.728
INTQDWHRIAQTGWA	731	53.571	0.728
TNNQDRHRIAQTGWV	732	53.408	0.726

RNTEDWHRIAQTGWV	733	53.204	0.723
TNNQDWHRIAQIGWV	734	53.143	0.722
TNNHDWHRIAQTGWV	735	53.041	0.721
TNSQDWHRIAQTGWV	229	52.959	0.719
TNTQDWHRISQTGWA	314	52.939	0.719
TNTDWHRIAQTGWV	226	52.469	0.713
SNTQDWHRVSTGWV	736	52.408	0.712
TNMQDWHRIAQTGWV	315	52.143	0.708
TNSQDWHRVPTGWV	737	52.041	0.707
RTTQDWHRIAQTGWV	738	51.939	0.706
TGTQDWHRIAQTGWV	220	51.755	0.703
TNYQDWQRIAQTGWV	739	51.327	0.697
SNIEDWHRIAQTGWV	740	50.776	0.690
TNTQDWHRIAQTGWT	316	50.612	0.688
TNNQDRHSIAQTGWV	741	50.408	0.685
TITQDWHRTAQTGWV	317	50.143	0.681
RNTQDWHRIAQTGWA	742	50.082	0.680
TNNQDGHRIAQTGSV	743	50.061	0.680
TNHQSAQAQAQTGWV	200	1.000	0.014

[0466] These data demonstrate that following two maturation approaches, matured TTJ-001 capsid variants (AAV9 capsid variants) with loop VIII modifications were generated with significantly enhanced CNS tropism over wild-type AAV9 controls in two species of NHPs (cynomolgus macaques (*Macaca fascicularis*) and marmosets (*Callithrix jacchus*)) and mice, as well as cross-species compatibility.

Example 5. Individual Capsid Characterization in Mice

[0467] The goal of these experiments was to determine the transduction level, tropism, ability to cross the blood brain barrier, and overall spatial distribution in the central nervous system (CNS) of a capsid variant selected from the study described in Example 1 relative to AAV9 following intravenous injection in BALB/c mice. The capsid variant was TTJ-001 (SEQ ID NO: 981 (amino acid) and 983 (DNA), comprising SEQ ID NO: 941 or 201), as outlined in Table 3 above. The amino acid and DNA sequences of TTJ-001 are provided, e.g., in Tables 4A and 4B, respectively.

[0468] AAV particles were generated with this capsid variant encapsulating a luciferase-EGFP-HA tagged reporter gene driven by a CBA promoter encoded by a single stranded viral genome. The TTJ-001 capsid variant and AAV9 control were tested by intravenously administering the AAV particle formulation at 2e13 VG/kg (5e11/dose) to mice (n=3). The in-life period was 28 days and the brain and the liver were collected for measuring transgene mRNA, transgene protein, and viral DNA (biodistribution).

[0469] At 27 days post-injection of the AAV particles encapsulated in the TTJ-001 capsid variant, mice were injected with luciferin and imaged on the ventral and dorsal side by the IVIS imager. Robust luciferase signal was observed in mice injected with AAV particles encapsulated in the AAV9 control capsid but less luciferase signal was observed in mice injected with the AAV particles encapsulated in the TTJ-001 capsid variant.

[0470] The brains and livers were isolated on day 28 and subsequently assayed by qPCR for the presence of transgene mRNA, as a measure of transgene expression. Data were provided as average mRNA fold change for the transgene relative to a housekeeping gene as well as the fold change relative to the AAV9 control (Table 27). As shown in Table 27, mRNA expression from the TTJ-001 capsid variant was slightly increased in the brain of the mice relative to the AAV9 control. More specifically, mRNA expression was approximately 1.3-fold higher from the TTJ-001 capsid variant compared to wild-type AAV9 in the brain of the mice. In the liver, mRNA expression from the TTJ-001 capsid variant was decreased relative to the AAV9 control.

Table 27. Transgene mRNA expression with the TTJ-001 capsid variant in the brain and liver of mice

Capsid Variant	Tissue	AVG transgene mRNA fold change relative to housekeeping gene	Fold change relative to AAV9
AAV9	Brain	0.544	1.0
	Liver	8.938	1.0
TTJ-001	Brain	0.734	1.349
	Liver	4.9999	0.559

[0471] The brains and livers isolated from the mice on day 28 were also assayed for the presence of viral DNA by qPCR as a measure of viral genome levels. Data are provided in Table 28 as average DNA (viral genome (VG) copies per diploid genome (dg) as well as fold change relative to the AAV9 control. As shown in Table 28, biodistribution of TTJ-001 was slightly higher in the mouse brain relative to the wild-type AAV9 control. Biodistribution of TTJ-001 was lower in the mouse liver relative to the wild-type AAV9 control.

Table 28. Viral DNA biodistribution with the TTJ-001 capsid variant in the brain and liver of mice

Capsid Variant	Tissue	AVG VG per diploid cell	Fold change relative to AAV9
AAV9	Brain	0.141	1.0
	Liver	32.224	1.0
TTJ-001	Brain	0.375	2.650
	Liver	6.056	0.188

[0472] The brain, heart, liver, and muscle tissue isolated from the mice on day 28 were also subjected to immunohistochemistry staining to evaluate overall tropism in the CNS and peripheral tissues and transgene expression. TTJ-001 showed comparable staining in the brain, muscle, and liver, as compared to the AAV9 control. Additionally, TTJ-001 demonstrated decreased staining in the heart relative to AAV9.

Example 6. Evaluation of additional TTJ-001 capsid variants

[0473] Matured variants of the TTJ-001 capsid variant generated using the methods described in Example 4 were injected into cynomolgus macaques (*Macaca fascicularis*), marmosets (*Callithrix jacchus*), or mice. The matured AAV capsid variants generated from the TTJ-001 non-matured capsid variant (SEQ ID NO: 981 (amino acid) and SEQ ID NO: 983 (DNA), comprising SEQ ID NO: 941 or SEQ ID NO: 201)) were injected into two NHPs (cynomolgus macaques or marmosets) or mice. After a period in life, the brains, heart, muscles, and/or livers of the NHPs or mice were isolated and DNA/RNA

was extracted. Following DNA/RNA recovery and amplification, a systematic NGS enrichment analysis was performed to calculate the fold enrichment ratio relative to the TTJ-001 control or an AAV9 control, and the peptides comprised within the AAV capsid variants were identified.

[0474] Table 29 provides the sequences of the matured capsid variants having a counts per million greater than or equal to 10, a CV of less than or equal to 1, and an average fold-change in cDNA levels in the brain relative to the cDNA levels of TTJ-001 that is greater than or equal to 1 in cynomolgus macaques. These variants demonstrated increased tropism in the brain of cynomolgus macaques.

Table 29. NGS fold-change in cDNA or viral DNA (vDNA) levels of TTJ-001 matured AAV capsid variants in the brains and livers of cynomolgus macaques (changes in the peptide sequence relative to TTJ-001 are bolded and underlined) (Fc rel. to AAV9= fold change relative to AAV9; Fc. rel. to TTJ-001= fold change relative to TTJ-001)

Sequence	SEQ ID NO:	Brain cDNA		Brain vDNA		Liver cDNA	
		Fc rel. to AAV9	Fc rel. to TTJ-001	Fc rel. to AAV9	Fc rel. to TTJ-001	Fc rel. to AAV9	Fc rel. to TTJ-001
TNTQDWHR <u>VP</u> QTGWV	216	146.809	1.031	28.774	0.798	9.141	1.421
TNTQDWHR <u>IAQTGWVH</u>	943	163.563	1.086	52.241	1.413	6.717	1.044

[0475] Table 30 provides the sequences of the matured capsid variants having a counts per million greater than or equal to 10, a CV of less than or equal to 1, and an average fold-change in cDNA levels in the brain relative to the cDNA levels of TTJ-001 that is greater than or equal to 1 in marmosets. These variants demonstrated increased tropism in the marmoset brain. AAV capsid variants comprising SEQ ID NO: 216 or SEQ ID NO: 943, were able to cross the blood brain barrier and demonstrate increased expression in the brains of both cynomolgus macaques (Table 29) and marmosets (Table 30) relative to AAV9, and comparable or increased expression relative to TTJ-001 in the same tissues.

Table 30. NGS fold-change in cDNA levels of TTJ-001 matured AAV capsid variants in the brains, hearts, and muscles of marmosets (changes in the peptide sequence relative to TTJ-001 are bolded and underlined) (Fc rel. to AAV9= fold change relative to AAV9; Fc. rel. to TTJ-001= fold change relative to TTJ-001)

Sequence	SEQ ID NO:	Brain cDNA		Heart cDNA		Muscle cDNA	
		Fc rel. to AAV9	Fc rel. to TTJ-001	Fc rel. to AAV9	Fc rel. to TTJ-001	Fc rel. to AAV9	Fc rel. to TTJ-001
TNTQDWHR <u>VP</u> QTGWV	216	27.944	1.524	0.781	0.801	0.294	0.648
TNTQDWHR <u>IAQTGWVH</u>	943	34.967	1.907	0.817	0.838	0.311	0.686

[0476] Table 31 provides the sequences of additional matured capsid variants having a counts per million greater than or equal to 10, a CV across the brain tissues of less than or equal to 1, and an average fold-change in cDNA levels in the brain relative to the cDNA levels of TTJ-001 that is greater than or equal to 1 in marmosets that were generated using two different maturation approaches. These variants demonstrated increased or comparable tropism in the marmoset brain relative to TTJ-001.

Table 31. NGS fold-change in cDNA levels of TTTJ-001 matured AAV capsid variants in the brains, liver, hearts, and muscles of marmosets (changes in the peptide sequence relative to TTTJ-001 are **bolded and underlined**)

Mutational Approach 1					
Sequence	SEQ ID NO:	Fold change relative to TTTJ-001		Fold change relative to AAV9	
		Brain	Liver	Heart	Muscle
<u>TTT</u> TQDWHRIAQTGWV	208	2.253	0.639	1.172	0.382
TNTQDWHR <u>V</u> AQTGWV	232	3.895	1.267	0.708	0.312
Mutational Approach 2					
Sequence	SEQ ID NO:	Fold change relative to TTTJ-001		Fold change relative to AAV9	
		Brain	Liver	Heart	Muscle
<u>TTT</u> TQDWHRIAQTGWV	208	1.001	0.886	4.025	2.737
TNTQDWHR <u>V</u> AQTGWV	232	3.478	0.576	2.722	3.727

[0477] The AAV capsid variants comprising either SEQ ID NO: 208 or SEQ ID NO: 232 were also isolated from the brains of mice following two maturation approaches, as they demonstrated increased tropism in the brain relative to TTTJ-001 (Table 32). Table 32 provides data that was filtered by a counts per million greater than or equal to 10, a CV across the brain tissues of less than or equal to 1, and an average fold-change in cDNA levels in the brain relative to the cDNA levels of TTTJ-001 that is greater than or equal to 1 in mice.

Table 32. NGS fold-change in cDNA levels of TTTJ-001 matured AAV capsid variants in the brains, hearts, and muscles of mice (changes in the peptide sequence relative to TTTJ-001 are **bolded and underlined**)

Mutational Approach 1				
Sequence	SEQ ID NO:	Fold change relative to TTTJ-001		Fold change relative to AAV9
		Brain	Liver	Heart
<u>TTT</u> TQDWHRIAQTGWV	208	1.903	0.568	0.169
TNTQDWHR <u>V</u> AQTGWV	232	1.127	1.258	0.193
Mutational Approach 2				
Sequence	SEQ ID NO:	Fold change relative to TTTJ-001		Fold change relative to AAV9
		Brain		Heart
<u>TTT</u> TQDWHRIAQTGWV	208	1.954		0.189
TNTQDWHR <u>V</u> AQTGWV	232	1.156		0.271

[0478] Table 33 provides the sequences of the matured capsid variants having a counts per million greater than or equal to 10, a CV of less than or equal to 1, and an average fold-change in cDNA levels in the heart relative to the cDNA levels of AAV9 that is greater than or equal to 2 in cynomolgus macaques. These variants demonstrated increased tropism in the heart and muscles (intercostal, diaphragm, and quadriceps) relative to AAV9 and comparable expression to TTTJ-001 in these same tissues in cynomolgus macaques.

Table 33. NGS fold-change in cDNA levels of TTTJ-001 matured AAV capsid variants in the muscle, heart, and liver of cynomolgus macaques (changes in the peptide sequence relative to TTTJ-001 are bolded and underlined)

Sequence	SEQ ID NO:	Diaphragm		Quadriceps		Intercostal Muscle		Heart		Liver	
		Avg. FC rel. to AAV9	Avg. FC rel. to TTTJ-001	Avg. FC rel. to AAV9	Avg. FC rel. to TTTJ-001	Avg. FC rel. to AAV9	Avg. FC rel. to TTTJ-001	Avg. FC rel. to AAV9	Avg. FC rel. to TTTJ-001	Avg. FC rel. to AAV9	Avg. FC rel. to TTTJ-001
TNTQDWHRIAQTGWV	201	6.912	1.000	22.665	1.000	25.629	1.000	16.404	1.000	6.433	1.000
TNHQSAQAQAQTGWV	200	1.000	0.145	1.000	0.044	1.000	0.039	1.000	0.061	1.000	0.155
TNTQDWHRIWQTGWV	241	8.833	1.278	12.601	0.556	22.872	0.892	14.873	0.907	3.543	0.551
<u>TTQ</u> QDWHRIAQTGWV	984	3.716	0.538	10.270	0.453	18.858	0.736	17.761	1.083	4.472	0.695

[0479] The AAV capsid variants comprising SEQ ID NO: 241 or SEQ ID NO: 984, also demonstrated increased expression in the heart and brain relative to AAV9 and TTTJ-001 and in the muscle relative to TTTJ-001 in marmosets (Table 34). The AAV capsid variants comprising SEQ ID NO: 241 or SEQ ID NO: 984 also demonstrated detargeting in the liver relative to AAV9 and TTTJ-001 (Table 34). Table 34 provides data that was filtered by a counts per million greater than or equal to 10, a CV of less than or equal to 1, and an average fold-change in cDNA levels in the heart relative to the cDNA levels of AAV9 that is greater than or equal to 2 in marmosets.

Table 34. NGS fold-change in cDNA levels of TTTJ-001 matured AAV capsid variants in the brains, hearts, livers, and muscles of marmosets (changes in the peptide sequence relative to TTTJ-001 are bolded and underlined) (Fc rel. to AAV9= fold change relative to AAV9; Fc. rel. to TTTJ-001= fold change relative to TTTJ-001)

Sequence	SEQ ID NO:	Heart cDNA		Muscle cDNA		Brain cDNA		Liver cDNA	
		Fc rel. to AAV9	Fc rel. to TTTJ-001	Fc rel. to AAV9	Fc rel. to TTTJ-001	Fc rel. to AAV9	Fc rel. to TTTJ-001	Fc rel. to AAV9	Fc rel. to TTTJ-001
TNHQSAQAQAQTGWV	200	1.000	1.026	1.000	2.207	1.000	0.055	1.000	9.769
TNTQDWHRIAQTGWV	201	0.975	1.000	0.453	1.000	18.334	1.000	0.102	1.000
TNTQDWHRIWQTGWV	241	2.888	2.963	0.944	2.084	56.054	3.057	0.125	1.221
<u>TTQ</u> QDWHRIAQTGWV	984	2.591	2.658	0.701	1.547	153.641	8.380	0.052	0.509

[0480] These data demonstrate that following maturation, several matured TTTJ-001 capsid variants with loop VIII modifications were generated with enhanced CNS, heart, and/or muscle tropism over wild-type AAV9 controls and/or the non-matured TTTJ-001 capsid in two species of NHPs (cynomolgus macaques (*Macaca fascicularis*) and marmosets (*Callithrix jacchus*)) and mice.

Example 7. In vivo Promoter Selection Studies

[0481] ITR-to-ITR sequences comprising promoters were packaged into AAV6 capsids and delivered by intrastriatal injection to Sprague Dawley rats at a dose of 1x10¹⁰ VG. Tissue samples were collected at 3 weeks or 10 weeks and frataxin protein levels quantified. Frataxin constructs comprising truncated CBA and CMV promoters were packed into an AAV9 capsid variant (VOY201) and

administered by intravenous delivery to Sprague Dawley rats at a dose of either 6.3×10^{12} or 2×10^{13} VG/kg. After 28 or 90 days, tissue samples were collected and processed for quantification of frataxin expression (ng/mg). The sequence for the VOY201 capsid is disclosed in, e.g., WO2020/069461, the contents of which are incorporated herein in their entirety.

[0482] Promoters CMV-D7 (SEQ ID NO: 1750) and CBA-D8 (SEQ ID NO: 1742) demonstrated the target moderate frataxin expression as compared to the other constructs and were therefore selected for further study.

[0483] To test the durability and persistence of frataxin expression driven by the CMV-D7 (SEQ ID NO: 1750) and CBA-D8 (SEQ ID NO: 1742) promoters, a time course study was conducted. Viral genomes comprising a CMV-D7 (SEQ ID NO: 1750) or a CBA-D8 (SEQ ID NO: 1742) promoter with a frataxin payload sequence were packaged into another AAV9-variant (VOY101) capsid to generate AAV particles. The sequence for the VOY101 capsid is disclosed in, e.g., WO2020/069461, the contents of which are incorporated herein in their entirety. These AAV particles were administered by intravenous delivery via the tail vein to male Sprague Dawley rats at one of two doses (6.3×10^{12} or 2×10^{13}). At 28, 90, or 180 days after administration, tissue samples were collected (heart, liver, cerebellum, thoracic and lumbar DRG) and processed for quantification of viral genome per diploid cell and frataxin expression levels based on an Anti-Frataxin SimpleStep ELISA. Data are shown below in Tables 35 and 36, and in **FIGs. 1A, 1B, 1C, and 1D.**

Table 35. Frataxin expression (ng/mg)

Tissue	CMV-D7						CBA-D8					
	6.3e12			2.3e13			6.3e12			2.3e13		
	28d	90d	180d	28d	90d	180d	28d	90d	180d	28d	90d	180d
Heart	14.9	168.7	98.5	37.4	254.9	234.7	20.4	112.1	164.6	77.5	713.8	304.8
Cerebellum	1.0	1.9	4.3	5.8	13.6	23.6	0.9	2.2	3.1	6.9	11.5	21.4
Lumbar DRG	75.2	144.7	64.7	249.0	250	152.1	45.0	98.3	53.2	121.3	212.8	95.9

Table 36. Viral genome/diploid cell (VG/dc)

Tissue	CMV-D7						CBA-D8					
	6.3e12			2.3e13			6.3e12			2.3e13		
	28d	90d	180d	28d	90d	180d	28d	90d	180d	28d	90d	180d
Heart	1.8	2.1	1.8	3.3	8.11	7.2	1.1	2.2	1.8	3.6	9.6	6.1
Liver	0.7	0.6	0.6	4.2	2.4	2.9	0.7	0.5	0.5	3.2	1.4	0.9
Cerebellum	0.0	0.0	0.00	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Thoracic DRG	0.1	0.1	0.1	0.5	0.4	0.1	0.1	0.1	0.1	0.5	0.4	0.3

	CBA (no miR-122)						CMV					
	6.3e12			2.3e13			6.3e12			2.3e13		
	28d	90d	180d	28d	90d	180d	28d	90d	180d	28d	90d	180d
Heart	4.3	7.0	7.0	-	-	-	-	-	-	4.8	-	-
Liver	0.8	0.4	0.6	-	-	-	-	-	-	5.7	-	-
Cerebellum	0.1	0.0	0.0	-	-	-	-	-	-	0.1	-	-
Thoracic DRG	0.5	0.4	0.1	-	-	-	-	-	-	2.2	-	-

[0484] In tissue collected from the heart ventricle, driving frataxin expression using the CMV-D7 (SEQ ID NO: 1750) promoter enhanced frataxin expression 0.2-2.5x, while driving frataxin expression using the CBA-D8 (SEQ ID NO: 1742) promoter enhanced frataxin expression 0.3-7.8x (**FIG. 1A**).

[0485] In tissue collected from the cerebellum, driving frataxin expression using the CMV-D7 (SEQ ID NO: 1750) promoter enhanced frataxin expression 0.01-0.31x, while driving frataxin expression using the CBA-D8 (SEQ ID NO: 1742) promoter enhanced frataxin expression 0.01-0.28x (**FIG. 1B**).

[0486] In tissue collected from the lumbar DRG, driving frataxin expression using the CMV-D7 (SEQ ID NO: 1750) promoter enhanced frataxin expression 1.6-6.2x, while driving frataxin expression using the CBA-D8 (SEQ ID NO: 1742) promoter enhanced frataxin expression 1.1-5.2x (**FIG. 1C**).

[0487] Immunohistochemical analysis was performed on 30 μ m tissue samples collected 28 days after AAV particle administration. An anti-hFXN antibody (1/50,000) was used. Frataxin expression driven by the CMV-D7 (SEQ ID NO: 1750) and CBA-D8 (SEQ ID NO: 1742) promoters was detected in the dentate nucleus of treated rats.

[0488] Each of CMV-D7 (SEQ ID NO: 1750), CBA-D8 (SEQ ID NO: 1742) and CBA (SEQ ID NO: 1734) promoters showed similar distribution and expression patterns in the DRG and brain. In the heart, CMV-D7 and CBA-D8 promoters generated FXN expression approximately 50-260-fold lower than CBA-driven frataxin expression.

[0489] The CMV-D7 (SEQ ID NO: 1750) and CBA-D8 (SEQ ID NO: 1742) promoters both drove frataxin expression in the cerebellum, heart and DRG at 180 days after administration of the AAV particles. At this time point, expression in the cerebellum was approximately 3-fold greater than that achieved using a reference CBA promoter, indicating that the CMV-D7 and CBA-D8 promoters are active in target cells of the cerebellum.

Example 8. Evaluation of AAV Particles comprising the TTJ-001 capsid variant in cynomolgus macaques to deliver a frataxin protein

[0490] The goal of these experiments was to determine the ability of AAV particles comprising TTJ-001 capsid variant (SEQ ID NO: 981 (amino acid) and SEQ ID NO: 983 (DNA), comprising SEQ ID NO: 941 or SEQ ID NO: 201) to deliver a frataxin protein encoded by the viral genome of SEQ ID NO: 1797, following intravenous injection in NHPs (cynomolgus macaques (*Macaca fascicularis*)).

[0491] AAV particles were generated with the TTJ-001 capsid variant encapsulating a single-stranded viral genome encoding a human frataxin (FXN) protein (SEQ ID NO: 1824) driven by a truncated CBA promoter (SEQ ID NO: 1742) and comprising three miR122 binding sites. The single-stranded viral genome comprised the nucleotide sequence of SEQ ID NO: 1797. These AAV particles or a vehicle control (modified PBS) were administered to the NHPs intravenously. The AAV particles were administered at increasing doses of 1×10^{13} , 3×10^{12} , or 1×10^{12} VG/kg to the NHPs (n=12 total; n=3 per group). The in-life period was 92 days. The brain, spinal cord, and peripheral tissues were collected for measuring frataxin expression (mRNA and protein) and viral DNA (biodistribution). The AAV particles encoding the frataxin protein were well tolerated in the NHPs.

[0492] Table 37 provides the AAV biodistribution (viral genomes per diploid genome (vg/dg)) and frataxin protein levels (ng/mg of tissue) quantified in the various regions of the brain and spinal cord, as well as in the peripheral tissues of the NHPs. Viral DNA biodistribution was determined using ddPCR and total frataxin protein expression (both human (exogenous) and cynomolgus (endogenous) frataxin protein) was determined by ELISA. AAV biodistribution was observed in the brain and spinal cord as well as in the peripheral tissues at all three doses tested, with the greatest biodistribution observed at the dose of 1×10^{13} vg/kg. The fold-change in the total frataxin protein (exogenous human frataxin protein and endogenous cynomolgus frataxin protein) was quantified post-intravenous administration of the AAV particles comprising the TTTJ-001 capsid variant encapsulating the viral genome comprising SEQ ID NO: 1797, relative to the vehicle control (Table 37). When these AAV particles were administered at a dose of 1×10^{13} vg/kg, an increase in frataxin protein levels was observed in the cerebellum, dorsal root ganglia (DRG) (cervical, thoracic, and lumbar), and heart relative to the vehicle control. Increases in frataxin protein expression was also observed in some tissues even at the lower doses of 3×10^{12} vg/kg and 1×10^{12} vg/kg (Table 37).

Table 37. Biodistribution and Frataxin Protein Expression in Select Tissues

Group	1			2			3			4		
Dose	1×10^{13} vg/kg			3×10^{12} vg/kg			1×10^{12} vg/kg			Vehicle		
Tissue	DNA vg/dg mean (SD)	FXN ng/mg mean (SD)	FXN fold- over vehicle	DNA vg/dg mean (SD)	FXN ng/mg mean (SD)	FXN fold- over vehicle	DNA vg/dg mean (SD)	FXN ng/mg mean (SD)	FXN fold- over vehicle	DNA vg/dg mean (SD)	FXN ng/mg mean (SD)	FXN fold- over vehicle
Cerebellum	0.39 (0.14)	42.5 (2.6)	1.53	0.09 (0.04)	29.1 (0.1)	1.05	0.11 (0.05)	33.2 (1.7)	1.19	0.0 (0.0)	27.8 (1.5)	--
Dentate Nucleus	0.76 (0.19)	NT	NT	0.88 (0.63)	NT	NT	0.59 (0.36)	NT	NT	0.0 (0.0)	NT	--
Somatosensory Cortex	1.01 (0.21)	NT	NT	0.45 (0.24)	NT	NT	0.36 (0.03)	NT	NT	0.0 (0.0)	NT	--
Motor Cortex	1.27 (0.30)	NT	NT	0.45 (0.21)	NT	NT	0.41 (0.06)	NT	NT	0.0 (0.0)	NT	--
DRG-Cervical	0.24 (0.04)	44.4 (24.1)	5.04	0.04 (0.02)	11.6 (2.5)	1.31	0.03 (0.01)	11.9 (1.9)	1.35	0.0 (0.0)	8.8 (2.4)	--
DRG-Thoracic	0.32 (0.14)	21.3 (9.6)	2.05	0.04 (0.03)	9.1 (1.6)	0.88	0.04 (0.01)	8.4 (1.2)	0.81	0.0 (0.0)	10.4 (1.5)	--
DRG-Lumbar	0.19 (0.06)	34.9 (17.4)	5.39	0.03 (0.01)	10.3 (2.8)	1.59	0.03 (0.0)	8.3 (2.1)	1.28	0.0 (0.0)	6.5 (1.8)	--
Heart	1.20 (0.27)	93.3 (31.5)	2.36	0.34 (0.21)	45.4 (10.6)	1.15	0.22 (0.08)	37.3 (2.4)	0.94	0.0 (0.0)	39.5 (2.2)	--
Liver	23.74 (16.07)	19.1 (4.7)	1.25	1.83 (1.36)	15.9 (4.9)	1.04	0.69 (0.60)	20.8 (2.5)	1.35	0.0 (0.0)	15.3 (3.1)	--
Pancreas	1.82 (2.44)	13.2 (1.4)	1.33	0.03 (0.01)	13.1 (2.3)	1.32	0.02 (0.0)	11.7 (0.8)	1.17	0.0 (0.0)	10.0 (0.9)	--
Testis	0.02 (0.01)	13.5 (1.3)	1.14	NT	NT	NT	NT	NT	NT	0.0 (0.0)	11.8 (0.9)	--

dg=diploid genome; FXN=frataxin protein; NT=not tested; SD=standard deviation; vg=viral genome

Note: As the FXN ELISA assay used to quantify total FXN protein does not distinguish between cynomolgus and human FXN, a comparison is made of the FXN levels in treated animals over vehicle-treated animals (FXN fold-over vehicle).

[0493] Human (exogenous) and cynomolgus (endogenous) frataxin mRNA levels were also quantified by RT-PCR in the motor cortex, somatosensory cortex, and the dentate nucleus post-administration of the AAV particles comprising the TTJ-001 capsid variant encapsulating the viral genome comprising SEQ ID NO: 1797. The ratio of the human frataxin mRNA to cynomolgus frataxin mRNA was calculated as shown in Table 38, FIG. 3A, and FIG. 3B.

Table 38. Ratio of Human Frataxin (huFXN) mRNA to Cynomolgus Frataxin (cyFXN) mRNA Levels in Select Tissues

Tissue	huFXN mRNA:cyFXN mRNA		
	Dose		
	1 × 10 ¹³ vg/kg	3 × 10 ¹² vg/kg	1 × 10 ¹² vg/kg
Motor Cortex	0.34	0.38	0.19
Somatosensory Cortex	0.36	0.22	0.09
Dentate Nucleus	0.74	0.96	0.32

[0494] As provided in Table 39, the percentage of cells that were positive for exogenous, human frataxin (e.g., frataxin protein encoded by the viral genome comprising SEQ ID NO: 1797) was quantified by ISH in the dentate nucleus (large neurons), cervical DRG, thoracic DRG, lumbar DRG, and the heart (cardiomyocytes in the left and right ventricle; intraventricular septum) post-administration of the AAV particles comprising the TTJ-001 capsid variant encapsulating the viral genome comprising SEQ ID NO: 1797 or the vehicle control. Additionally, weak to moderate expression of exogenous human frataxin was observed by ISH in the gracile nucleus post-administration at the doses of 1e13 vg/kg and 3e12 vg/kg; and moderate to strong expression of exogenous human frataxin was observed by ISH in the cuneate nucleus post-administration at the doses of 1e13 vg/kg and 3e12 vg/kg. Cells positive for exogenous human frataxin were also observed in Clarke’s Column of the thoracic spinal cord at all three doses investigated.

Table 39. Percentage of Cells that are Positive for Exogenous Frataxin (eFXN) Expression in Selected Tissues

Tissue	% eFXN+ Cells			Vehicle Control
	Dose			
	1 × 10 ¹³ vg/kg	3 × 10 ¹² vg/kg	1 × 10 ¹² vg/kg	
Large Neurons of the Dentate Nucleus	55.3	41.8	17.6	1.7
Cervical DRG	54.8	12.7	5.6	2.6
Thoracic DRG	51.3	10.2	5.0	3.9
Lumbar DRG	51.9	10.0	4.7	2.5
Cardiomyocytes of the Heart (left and right ventricle; intraventricular septum)	14.3	8.8	8.3	3.7

[0495] Taken together, these data demonstrate that robust frataxin expression was observed in NHPs, particularly in the dentate nucleus, spinal cord, and the heart, following intravenous administration of AAV particles comprising the TTJ-001 capsid variant encapsulating the viral genome comprising SEQ ID NO: 1797 encoding human frataxin protein, at a dose of 1e13 vg/kg.

Equivalents and Scope

[0496] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the Detailed Description provided herein. The scope of the present disclosure is not intended to be limited to the above Detailed Description, but rather is as set forth in the appended claims.

[0497] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[0498] In addition, it is to be understood that any particular embodiment of the present disclosure that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the disclosure (e.g., any, composition, therapeutic or active ingredient; any method of production; any method of use; etc.) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[0499] It is to be understood that the words which have been used are words of description rather than limitation, and that changes may be made within the purview of the appended claims without departing from the true scope and spirit of the disclosure in its broader aspects.

[0500] While the present disclosure has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the disclosure.

[0501] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, section headings, the materials, methods, and examples are illustrative only and not intended to be limiting.

CLAIMS

We claim:

1. An adeno-associated virus (AAV) particle comprising an AAV capsid variant and a viral genome, wherein the viral genome comprises a frataxin (FXN)-encoding sequence and the AAV capsid variant comprises an amino acid sequence having the formula [N1]-[N2]-[N3] (SEQ ID NO: 4681) in loop VIII; wherein [N2] comprises the amino acid sequence of DWHR (SEQ ID NO: 4682); and wherein:
 - (i) [N1] comprises amino acids X₁, X₂, X₃, and X₄, wherein X₄ is Q, K, E, S, P, R, N, or H;and/or
 - (ii) [N3] comprises amino acids X₅, X₆, and X₇, wherein X₅ is I, V, T, M, S, N, L, or F.
2. The AAV particle of claim 1, wherein the AAV capsid variant is an AAV9 capsid variant.
3. The AAV particle of claim 1 or claim 2, wherein the AAV capsid variant comprises an amino acid sequence that is at least 95% identical to positions 203-736 of the amino acid sequence of SEQ ID NO: 981.
4. The AAV particle of any one of claims 1-3, wherein loop VIII is present at amino acids comprising those corresponding to positions 580-599 of the amino acid sequence of SEQ ID NO: 981.
5. The AAV particle of any one of claims 1-4, wherein:
 - (i) X₁ is T, S, R, A, I, C, N, K, L, or Q;
 - (ii) X₂ is N, T, G, V, S, Y, K, I, H, D, or F; and
 - (iii) X₃ is T, N, K, D, I, S, P, A, Y, E, V, L, M, R, H, Q, or C.
6. The AAV particle of any one of claims 1-5, wherein:
 - (i) X₆ is A, Y, P, N, S, T, G, E, V, W, F, or Q; and
 - (ii) X₇ is Q, G, N, K, H, R, E, L, P, or M.
7. The AAV particle of any one of claims 1-6, wherein [N1] comprises the amino acid sequence of TNTQ (SEQ ID NO: 4688).
8. The AAV particle of any one of claims 1-7, wherein [N3] comprises the amino acid sequence of IAQ.
9. The AAV particle of any one of claims 1-8, wherein [N1]-[N2]-[N3] comprises the amino acid sequence of TNTQDWHRIAQ (SEQ ID NO: 343).

10. The AAV particle of any one of claims 1-9, wherein:

(i) [N1] is present at amino acids corresponding to positions 582-585 of the amino acid sequence of SEQ ID NO: 981;

(ii) [N2] is present at amino acids corresponding to positions 586-589 of the amino acid sequence of SEQ ID NO: 981; and

(iii) [N3] is present at amino acids corresponding to positions 590-592 of the amino acid sequence of SEQ ID NO: 981.

11. The AAV particle of any one of claims 1-10, wherein the AAV capsid variant further comprises [N4] comprising amino acids X₈, X₉, X₁₀, and X₁₁, wherein:

(i) X₈ is T, S, N, P, A, or I;

(ii) X₉ is G, N, D, R, V, A, S, or Q;

(iii) X₁₀ is W, S, C, R, L, or G; and/or

(iv) X₁₁ is V, A, S, I, C, G, D, F, L, or T.

12. The AAV particle of claim 11, wherein [N4] comprises the amino acid sequence of TGWV (SEQ ID NO: 5066).

13. The AAV particle of claim 11 or claim 12, wherein [N4] is present at amino acids corresponding to positions 593-596 of the amino acid sequence of SEQ ID NO: 981.

14. The AAV particle of any one of claims 11-13, wherein [N1]-[N2]-[N3]-[N4] comprises the amino acid sequence of TNTQDWHRIAQTGWV (SEQ ID NO: 201).

15. An adeno-associated virus (AAV) particle comprising:

(i) a viral genome comprising a frataxin (FXN)-encoding sequence; and

(ii) an AAV capsid variant comprising an amino acid sequence that is at least 95% identical to positions 203-736 of the amino acid sequence of SEQ ID NO: 981, wherein the AAV capsid variant comprises:

T at an amino acid corresponding to position 584 of the amino acid sequence of SEQ ID NO: 981;

D at an amino acid corresponding to position 586 of the amino acid sequence of SEQ ID NO: 981;

W at an amino acid corresponding to position 587 of the amino acid sequence of SEQ ID NO: 981;

H at an amino acid corresponding to position 588 of the amino acid sequence of SEQ ID NO: 981;

R at an amino acid corresponding to position 589 of the amino acid sequence of SEQ ID NO: 981; and

I at an amino acid corresponding to position 590 of the amino acid sequence of SEQ ID NO: 981.

16. The AAV particle of any one of claims 1-15, wherein the AAV capsid variant comprises an amino acid sequence that is at least 95% identical to positions 138-736 of the amino acid sequence of SEQ ID NO: 981.

17. An adeno-associated virus (AAV) particle comprising:

(i) a viral genome comprising a frataxin (FXN)-encoding sequence; and

(ii) an AAV capsid variant comprising an amino acid sequence that is at least 95% identical to positions 138-736 of the amino acid sequence of SEQ ID NO: 981, wherein the AAV capsid variant comprises:

T at an amino acid corresponding to position 584 of the amino acid sequence of SEQ ID NO: 981;

D at an amino acid corresponding to position 586 of the amino acid sequence of SEQ ID NO: 981;

W at an amino acid corresponding to position 587 of the amino acid sequence of SEQ ID NO: 981;

H at an amino acid corresponding to position 588 of the amino acid sequence of SEQ ID NO: 981;

R at an amino acid corresponding to position 589 of the amino acid sequence of SEQ ID NO: 981; and

I at an amino acid corresponding to position 590 of the amino acid sequence of SEQ ID NO: 981.

18. The AAV particle of any one of claims 1-17, wherein the AAV capsid variant comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 981.

19. An adeno-associated virus (AAV) particle comprising:

(i) a viral genome comprising a frataxin (FXN)-encoding sequence; and

(ii) an AAV capsid variant comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 981, wherein the AAV capsid variant comprises:

T at an amino acid corresponding to position 584 of the amino acid sequence of SEQ ID NO: 981;

D at an amino acid corresponding to position 586 of the amino acid sequence of SEQ ID NO: 981;

W at an amino acid corresponding to position 587 of the amino acid sequence of SEQ ID NO:

981;

H at an amino acid corresponding to position 588 of the amino acid sequence of SEQ ID NO:

981;

R at an amino acid corresponding to position 589 of the amino acid sequence of SEQ ID NO:

981; and

I at an amino acid corresponding to position 590 of the amino acid sequence of SEQ ID NO: 981.

20. The adeno-associated virus (AAV) particle of claim 19, wherein the AAV capsid variant comprises:

(i) an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 981;

(ii) an amino acid sequence that is at least 99% identical to positions 138-736 of the amino acid sequence of SEQ ID NO: 981; and/or

(iii) an amino acid sequence that is at least 99% identical to positions 203-736 of the amino acid sequence of SEQ ID NO: 981.

21. The AAV particle of any one of claims 15-20, wherein the AAV capsid variant comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941).

22. The AAV particle of any one of claims 15-21, wherein the AAV capsid variant comprises at least 8, at least 9, or at least 10 consecutive amino acids from the amino acid sequence of TNTQDWHRIAQ (SEQ ID NO: 343).

23. The AAV particle of any one of claims 15-22, wherein the AAV capsid variant comprises the amino acid sequence of TNTQDWHRIAQ (SEQ ID NO: 343) present at amino acids corresponding to positions 582-592 of the amino acid sequence of SEQ ID NO: 981.

24. The AAV particle of any one of claims 21-23, wherein the amino acid sequence of TQDWHRI (SEQ ID NO: 941) or TNTQDWHRIAQ (SEQ ID NO: 343) is present in loop VIII, wherein loop VIII comprises amino acids 580-599 of the amino acid sequence of SEQ ID NO: 981.

25. The AAV particle of any one of claims 1-24, wherein the AAV capsid variant comprises:

(i) the amino acid sequence of SEQ ID NO: 981;

(ii) the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981; and/or

(iii) the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981.

26. The AAV particle of any one of claims 1-25, wherein the FXN-encoding sequence encodes a FXN protein that is not a cynomolgus monkey FXN protein.

27. The AAV particle of any one of claims 1-26, wherein the FXN-encoding sequence encodes a human FXN protein.

28. The AAV particle of claim 27, wherein the human FXN protein comprises

(a) the amino acid sequence of SEQ ID NO: 1825 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or

(b) the amino acid sequence of any one of SEQ ID NOs: 5124-5126 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

optionally, wherein the FXN protein is a wildtype human FXN protein.

29. The AAV particle of any one of claims 1-27, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

30. The AAV particle of any one of claims 1-29, wherein the viral genome further comprises a promoter operably linked to the FXN-encoding sequence.

31. The AAV particle of claim 30, wherein the promoter comprises a human elongation factor 1 α -subunit (EF1 α) promoter, a cytomegalovirus (CMV) immediate-early enhancer and/or promoter, a chicken β -actin (CBA) promoter, a CAG promoter, a β glucuronidase (GUSB) promoter, a ubiquitin C (UBC) promoter, a neuron-specific enolase (NSE) promoter, a platelet-derived growth factor (PDGF) promoter, a platelet-derived growth factor B-chain (PDGF- β) promoter, an intercellular adhesion molecule 2 (ICAM-2) promoter, a synapsin (Syn) promoter, a methyl-CpG binding protein 2 (MeCP2) promoter, a Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) promoter, a metabotropic glutamate receptor 2 (mGluR2) promoter, a neurofilament light chain (NFL) promoter, a neurofilament heavy chain (NFH) promoter, a β -globin minigene β 2 promoter, a preproenkephalin (PPE) promoter, an enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2) promoter, a glial fibrillary acidic protein (GFAP) promoter, a myelin basic protein (MBP) promoter, a cardiovascular promoter (e.g., α MHC, cTnT, and CMV-MLC2k), a liver promoter (e.g., hAAT, TBG), a skeletal muscle promoter (e.g., desmin, MCK, C512), or a functional fragment or truncation thereof.

32. The AAV particle of claim 30 or claim 31, wherein the promoter is a CMV promoter or CBA promoter, or a functional fragment or truncation of a CMV promoter or CBA promoter.
33. The AAV particle of claim 32, wherein the promoter is a truncated CBA promoter.
34. The AAV particle of claim 33, wherein the truncated CBA promoter is 50-400 nucleotides in length, e.g., 100-332 nucleotides in length.
35. The AAV particle of any one of claims 32-34, wherein the promoter comprises
- (i) the nucleotide sequence SEQ ID NO: 1738 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;
 - (ii) the nucleotide sequence of SEQ ID NO: 1740 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or
 - (iii) the nucleotide sequence of SEQ ID NO: 1742 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.
36. The AAV particle of claim 32, wherein the promoter is a truncated CMV promoter.
37. The AAV particle of claim 36, wherein the truncated CMV promoter is 50-300 nucleotides in length.
38. The AAV particle of claim 36 or claim 37, wherein the promoter comprises the nucleotide sequence of SEQ ID NO: 1750 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.
39. The AAV particle of any one of claims 1-38, wherein the viral genome further comprises a nucleotide sequence encoding a miRNA (miR) binding site that modulates expression of the encoded FXN protein in a cell or tissue of the liver.
40. The AAV particle of claim 39, wherein the viral genome encodes 3 copies of the miR binding site.
41. The AAV particle of claim 40, wherein the 3 copies of the miR binding site are identical.

42. The AAV particle of any one of claims 39-41, wherein the miR binding site is a miR122 binding site.

43. The AAV particle of claim 42, wherein:

the miR122 binding site is encoded by the nucleotide sequence of SEQ ID NO: 1827 or a nucleotide sequence comprising one, two, three, or up to four substitutions relative thereto; or

the viral genome encodes 3 copies of the miR122 binding site, wherein the nucleotide sequence encoding the 3 copies of the miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence comprising one, two, three, four, five, six, seven, eight, nine, or up to ten substitutions relative thereto.

44. The AAV particle of any one of claims 1-43, wherein the viral genome further comprises at least one inverted terminal repeat (ITR) region.

45. The AAV particle of claim 44, wherein the at least one ITR region comprises an AAV2 ITR.

46. The AAV particle of claim 44 or claim 45, wherein the viral genome comprises a 5' ITR region and a 3' ITR region.

47. The AAV particle of claim 46, wherein the 5' ITR region and 3' ITR region are each an AAV2 ITR.

48. The AAV particle of claim 46 or claim 47, wherein:

the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

49. The AAV particle of any one of claims 1-48, wherein the viral genome further comprises an intron/exon region comprising an intron region and/or an exon region.

50. The AAV particle of claim 49, wherein the intron/exon region comprises:

an immediate-early 1 (ie1) intron region and/or a human beta-globin (hBglobin) intron 2 region; and/or

an ie1 exon region and/or an hBglobin exon region.

51. The AAV particle of claim 49 or claim 50, wherein the intron region comprises:

the nucleotide sequence of SEQ ID NO: 1819 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

the nucleotide sequence of SEQ ID NO: 1820 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

52. The AAV particle of any one of claims 49-51, wherein the exon region comprises:

the nucleotide sequence of SEQ ID NO: 1817 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

the nucleotide sequence of SEQ ID NO: 1821 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

53. The AAV particle of any one of claims 49-52, wherein the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

54. The AAV particle of any one of claims 1-53, wherein the viral genome further comprises a polyadenylation (polyA) region.

55. The AAV particle of claim 54, wherein the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

56. The AAV particle of any one of claims 1-35, or 39-55, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

57. The AAV particle of any one of claims 1-55, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

58. The AAV particle of any one of claims 1-55, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

59. The AAV particle of any one of claims 1-55, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

60. The AAV particle of any one of claims 1-27, wherein the viral genome comprises:

- (i) a 5' inverted terminal repeat (ITR) region;
- (ii) a promoter;
- (iii) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and
- (iv) a 3' ITR region.

61. The AAV particle of any one of claims 1-27, wherein the viral genome comprises:

- (i) a 5' inverted terminal repeat (ITR) region;
- (ii) a promoter;
- (iii) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;
- (iv) a nucleotide sequence encoding at least one miR122 binding site; and
- (v) a 3' ITR region.

62. The AAV particle of any one of claims 1-27, wherein the viral genome comprises:

- (i) a 5' inverted terminal repeat (ITR) region;
- (ii) a promoter;
- (iii) an intron/exon region;
- (iv) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%,

at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

- (v) a nucleotide sequence encoding at least one miR122 binding site; and
- (vi) a 3' ITR region.

63. The AAV particle of any one of claims 1-27, wherein the viral genome comprises:

- (i) a 5' inverted terminal repeat (ITR) region;
- (ii) a promoter;
- (iii) an intron/exon region;
- (iv) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;
- (v) a nucleotide sequence encoding at least one miR122 binding site;
- (vi) a polyadenylation (polyA) region; and
- (vii) a 3' ITR region.

64. The AAV particle of any one of claims 1-27, wherein the viral genome comprises:

- (i) a 5' inverted terminal repeat (ITR) region;
- (ii) a promoter;
- (iii) an intron/exon region;
- (iv) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;
- (v) a nucleotide sequence encoding at least one miR122 binding site;
- (vi) a polyadenylation (polyA) region;
- (vii) a filler sequence; and
- (viii) a 3' ITR region.

65. The AAV particle of claim 63 or claim 64, wherein:

- (i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;
- (ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1742 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

(vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

66. The AAV particle of claim 65, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1841 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR.

67. The AAV particle of claim 63 or claim 64, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1750 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

(vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

68. The AAV particle of claim 67, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1840 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR.

69. The AAV particle of claim 63 or claim 64, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1738 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vi) the poly A region comprises the nucleotide sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

(vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

70. The AAV particle of claim 69, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1838 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR.

71. The AAV particle of claim 63 or claim 64, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1740 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

(vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

72. The AAV particle of claim 71, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1839 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR.

73. The AAV particle of any one of claims 1-27, wherein the viral genome comprises:

(i) the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or

(iv) the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

74. The AAV particle of any one of claims 63-65, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811;

(ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1742;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824;

(v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828; and

(vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812.

75. The AAV particle of claim 74, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1841, wherein the filler sequence is positioned 3' to the poly A region and 5' to the 3' ITR region.

76. The AAV particle of any one of claims 1-35, 39-56, 63-66, or 73-75, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

77. The AAV particle of any one of claims 1-35, 39-56, 63-66, or 73-76, wherein the viral genome consists of the nucleotide sequence of SEQ ID NO: 1797.

78. An adeno-associated virus (AAV) particle comprising an AAV capsid variant and a viral genome comprising a frataxin (FXN)-encoding sequence,

wherein the AAV capsid variant comprises:

(i) the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

(iii) the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and

wherein the viral genome comprises:

(a) the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(b) the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(c) the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or

(d) the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

79. An adeno-associated virus (AAV) particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981, the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981, and/or the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981.

80. The AAV particle of claim 78 or claim 79, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

81. The AAV particle of claims 1-80, wherein the viral genome is single-stranded.

82. A cell comprising the AAV particle of any one of claims 1-81, optionally wherein the cell is a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

83. A method of making an AAV particle of any one of claims 1-81, wherein the method comprises:

(i) providing a cell comprising the viral genome comprising a frataxin (FXN)-encoding sequence and a nucleic acid encoding the AAV capsid variant; and

(ii) incubating the cell under conditions suitable to encapsulate the viral genome in the AAV capsid variant;

thereby making the AAV particle.

84. The method of claim 83, wherein the viral genome comprises

(i) the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or

(iv) the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and

wherein the AAV capsid variant comprises

(a) the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(b) the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and/or

(c) the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981 or an amino acid sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

85. The method of claim 83, wherein the viral genome comprises

(i) the nucleotide sequence of SEQ ID NO: 1797 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) the nucleotide sequence of SEQ ID NO: 1801 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) the nucleotide sequence of SEQ ID NO: 1808 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; or

(iv) the nucleotide sequence of SEQ ID NO: 1809 or a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and

wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 981, the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981, and/or the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981.

86. The method of claim 83, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797 and the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 981, the amino acid sequence according to positions 138-736 of the amino acid sequence of SEQ ID NO: 981, and/or the amino acid sequence according to positions 203-736 of the amino acid sequence of SEQ ID NO: 981.

87. The method of any one of claims 83-86, further comprising, prior to step (i), introducing a nucleic acid comprising the viral genome into the cell.

88. The method of any one of claims 83-87, further comprising, prior to step (i), introducing the nucleic acid encoding the AAV capsid variant into the cell.

89. The method of any one of claims 83-88, wherein the cell comprises a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

90. A pharmaceutical composition comprising the AAV particle of any one of claims 1-81 and a pharmaceutically acceptable excipient.

91. A method of delivering an AAV particle encoding a frataxin (FXN) protein to a cell, comprising administering an effective amount of the pharmaceutical composition of claim 90 or the AAV particle of any one of claims 1-81.

92. The method of claim 91, wherein the cell is in a subject.

93. The method of claim 92, wherein the subject has, has been diagnosed with having, or is at risk of having a disorder associated with FXN deficiency or at least one symptom thereof.

94. The method of claim 93, wherein the disorder is Friedreich's Ataxia (FA).

95. A method of treating a subject having or diagnosed with having a disorder associated with frataxin (FXN) deficiency or at least one symptom thereof, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 90 or the AAV particle of any one of claims 1-81.

96. The method of claim 95, wherein the subject has, has been diagnosed with having, or is at risk of having Friedreich's Ataxia (FA) or at least one symptom thereof.

97. A method of treating a subject having or diagnosed with having a disorder or at least one symptom thereof, wherein the disorder is Friedreich's Ataxia (FA), comprising administering to the subject an effective amount of the pharmaceutical composition of claim 90 or the AAV particle of any one of claims 1-81.

98. The method of any one of claims 95-97, wherein the treating results in prevention of progression of the disorder or at least one symptom thereof in the subject.

99. The method of any one of claims 95-98, wherein the treating results in amelioration of at least one symptom of the disorder.

100. The method of any one of claims 93-99, wherein the at least one symptom comprises impaired sensory functions, impaired motor function (e.g., ataxia and/or involuntary movements), fatigue, chronic pain, seizures, impaired speech, sleep disturbances, metabolic disorders (e.g., diabetes), and/or increased spasticity.

101. The method of any one of claims 93-100, wherein the treating stabilizes, slows the progression of, or improves the subject's disorder as determined by the modified Friedreich Ataxia Rating Scale (mFARS), the Scale for the Assessment and Rating of Ataxia (SARA), and/or the International Cooperative Ataxia Rating Scale (ICARS).

102. The method of claim 101, wherein the treating slows the subject's progression of the disorder as measured by mFARS, SARA, and/or ICARS relative to an individual with the disorder who has not been administered the pharmaceutical composition or the AAV particle.

103. The method of any one of claims 92-102, wherein the subject is a human.

104. The method of any one of claims 92-103, wherein the AAV particle or the pharmaceutical composition is delivered to a cell or tissue of the of the central nervous system (CNS) in the subject.

105. The method of claim 104, wherein the cell or tissue of the CNS is a cell or tissue of the amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region).

106. The method of any one of claims 92-105, wherein the AAV particle or the pharmaceutical composition is delivered to the subject via intravenous administration.

107. The method of any one of claims 92-106, further comprising evaluating, e.g., measuring, the level of FXN expression, e.g., FXN gene expression, FXN mRNA expression, and/or FXN protein expression, in the subject, e.g., in a cell, tissue, or fluid of the subject.

108. The method of claim 107, wherein the level of FXN protein expression is measured by an enzyme-linked immunosorbent assay (ELISA), a Western blot, an immunohistochemistry assay, or a frataxin biofluid assay.

109. The method of claim 107 or claim 108, wherein evaluating the subject's level of FXN expression is performed before and/or after administration of the pharmaceutical composition or AAV particle, optionally wherein the subject's level of FXN expression before administration is compared to the subject's level of FXN expression after administration.

110. The method of any one of claims 107-109, comprising evaluating the level of FXN expression in a cell or tissue of the CNS.

111. The method of claim 110, wherein the cell or tissue of the CNS is a cell or tissue of the amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral palladium, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region).

112. The method of any one of claims 107-109, comprising evaluating the level of FXN expression, e.g., FXN gene, FXN mRNA, and/or FXN protein expression, in a peripheral cell or tissue of the subject.

113. The method of claim 112, wherein the peripheral cell or tissue is a cell or tissue of the heart and/or muscle.

114. The method of claim 113, wherein the muscle is skeletal muscle.

115. The method of any one of claims 107-114, wherein the subject's level of FXN protein expression after administration is increased relative to the subject's level of FXN protein expression before administration.

116. The method of any one of claims 107-115, further comprising evaluating, e.g., measuring, the level of FXN protein activity in the subject.

117. The method of any one of claims 92-116, wherein administering the pharmaceutical composition or AAV particle to the subject results in an increase in:

(i) the level of FXN protein or FXN gene expression in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral pallidum, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region), or a cell or tissue outside of the CNS, e.g., muscle (e.g., skeletal muscle)), and/or fluid (e.g., CSF and or serum), of the subject relative to baseline and/or relative to the level of FXN protein or FXN gene expression in a cell, tissue, or fluid of an individual with a disorder associated with FXN deficiency who has not been administered the pharmaceutical composition or AAV particle;

(ii) the number and/or level of viral genomes (VG) per cell in a CNS tissue (e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral pallidum, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region)) or non-CNS tissue (e.g., muscle (e.g., skeletal muscle)) of the subject relative to the number and/or level of VG per cell in a peripheral tissue of the subject; and/or

(iii) the level of FXN activity in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., amygdala, brainstem, caudate, central grey, cerebellum (e.g., Purkinje cell layer and deep cerebellar nuclei), cortex (e.g., frontal cortex, motor cortex, perirhinal cortex, sensory cortex, temporal cortex), external cuneate nucleus, geniculate nucleus, globus pallidus, gracile nucleus, hippocampus, inferior colliculus, inferior olivary complex, nucleus ambiguus, oculomotor nucleus, putamen, substantia nigra, thalamus, ventral pallidum, vestibular nucleus, and/or spinal cord (e.g., cervical spinal cord region, lumbar spinal cord region, or thoracic spinal cord region)), or a cell or tissue outside of the CNS, e.g., muscle (e.g., skeletal muscle)), and/or fluid (e.g., CSF and or serum) of the subject relative to baseline and/or relative to FXN activity in a cell, tissue, or fluid of an individual with a disorder associated with FXN deficiency who has not been administered the pharmaceutical composition or AAV particle.

118. The method of any one of claims 93-117, further comprising administering to the subject at least one additional agent and/or therapy.

119. The method of claim 118, wherein the at least one additional agent and/or therapy comprises an agent and/or therapy suitable for treating the disorder associated with FXN deficiency (e.g., Friedrich's Ataxia).

120. The method of claim 118 or claim 119, wherein the at least one additional agent and/or therapy comprises omaveloxolone or idebenone.

121. The method of any one of claims 92-120, further comprising administering an immunosuppressant to the subject.

122. The method of claim 121, wherein the immunosuppressant comprises a corticosteroid (e.g., prednisone, prednisolone, methylprednisolone, and/or dexamethasone), rapamycin, mycophenolate mofetil, tacrolimus, rituximab, and/or eculizumab hydroxychloroquine.

123. The pharmaceutical composition of claim 90 or the AAV particle of any one of claims 1-81 for use in a method of treating a disorder according to any one of claims 94-122.

124. The pharmaceutical composition of claim 90 or the AAV particle of any one of claims 1-81 for use in the treatment of a disorder associated with FXN deficiency, or at least one symptom thereof, in a subject, optionally wherein the disorder is Friedrich's Ataxia (FA).

125. The pharmaceutical composition or the AAV particle of claim 124, wherein the subject has, has been diagnosed with having, or is at risk of having FA.

126. Use of the pharmaceutical composition of claim 90 or the AAV particle of any one of claims 1-81 in the manufacture of a medicament for the treatment of a disorder associated with FXN deficiency, or at least one symptom thereof, in a subject, optionally wherein the disorder is Friedrich's Ataxia (FA).

127. The use of claim 126, wherein the subject has, has been diagnosed with having, or is at risk of having FA.