

AAV CAPSID VARIANTS AND USES THEREOF

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/348,154 filed on June 2, 2022 and U.S. Provisional Application No. 63/501,935 filed on May 12, 2023; the entire contents of each of which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on March 21, 2023, is named V2071-1110PCT_SL.xml and is 1,725,870 bytes in size.

FIELD OF THE DISCLOSURE

[0003] The disclosure relates to compositions and methods for the preparation, use, and/or formulation of adeno-associated virus capsid proteins and variants thereof.

BACKGROUND

[0004] Gene delivery to the adult central nervous system (CNS) remains a significant challenge in gene therapy. Engineered adeno-associated virus (AAV) capsids with improved brain tropism represent an attractive solution to the limitations of CNS delivery.

[0005] 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 AAV natural variants 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.

[0006] Attempts at providing AAV capsids with improved properties, e.g., improved tropism to a target cell or tissue upon systemic administration, have met with limited success. As such, there is a need for improved methods of producing AAV capsids and resulting AAV capsids for delivery of a payload of interest to a target cell or tissue, e.g., a CNS cell or tissue.

SUMMARY OF THE DISCLOSURE

[0007] The present disclosure pertains at least in part, to compositions and methods for the production and use of an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid

variant. In some embodiments, the AAV capsid variant has an enhanced tropism for a tissue or a cell, e.g., a CNS tissue or a CNS cell. Said tropism can be useful for delivery of a payload, e.g., a payload described herein to a cell or tissue, for the treatment of a disorder, e.g., a neurological or a neurodegenerative disorder, a muscular or a neuromuscular disorder, or a neuro-oncological disorder.

[0008] Accordingly, in one aspect, the present disclosure provides an AAV capsid variant, comprising 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 positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q, K, E, S, P, R, N, H, or a conservative substitution thereof; and/or (ii) [N3] comprises positions X₅, X₆, and X₇, wherein position X₅ is I, V, T, M, S, N, L, F, or a conservative substitution thereof. In some embodiments, position X₄ of [N1] is Q. In some embodiments, position X₄ of [N1] is K. In some embodiments, position X₅ of [N3] is I. In some embodiments, [N1] replaces positions 582-585 (e.g., T582, N583, H584, Q585), numbered according to SEQ ID NO: 138. In some embodiments, [N1] corresponds to positions 582-585 (e.g., T582, N583, T584, Q585) of SEQ ID NO: 981. In some embodiments, [N1] is present at positions 582-585, numbered according to SEQ ID NO: 981. In some embodiments, X₁ of [N1] is present at position 582, X₂ of [N1] is present at position 583, X₃ of [N1] is present at position 584, and X₄ of [N1] is present at position 585, numbered according to SEQ ID NO: 981. In some embodiments, [N2] replaces positions 586-589 (e.g., S586, A587, Q588, A589), numbered according to SEQ ID NO: 138. In some embodiments [N2] corresponds to positions 586-589 (e.g., D586, W587, H588, and R589) of SEQ ID NO: 981. In some embodiments, [N2] is present at positions 586-589, numbered according to SEQ ID NO: 981. In some embodiments [N3] replaces positions 590-592 (e.g., Q590, A591, Q592), numbered according to SEQ ID NO: 138. In some embodiments, [N3] corresponds to positions 590-592 (e.g., I590, A591, Q592) of SEQ ID NO: 981. In some, embodiments, [N3] is present at positions 590-592, numbered according to SEQ ID NO: 981. In some embodiments, X₅ of [N3] is present at position 590, X₆ of [N3] is present at position 591, and X₇ of [N3] is present at position 592, numbered according to SEQ ID NO: 981. In some embodiments, [N1]-[N2] is present at positions 582-589, numbered according to SEQ ID NO: 981. In some embodiments, [N2]-[N3] is present at positions 586-592, numbered according to SEQ ID NO: 981. In some embodiments, [N1]-[N2]-[N3] is present at positions 582-592, numbered according to SEQ ID NO: 981.

[0009] In another aspect, the present disclosure provides an AAV 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 positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q, P, or a conservative substitution thereof; and/or (ii) [N3] comprises positions X₅, X₆, and X₇, wherein position X₅ is I, V, or a conservative substitution thereof. In some embodiments, position X₄ of [N1] is Q. In some embodiments, position X₅ of [N3] is I. In some embodiments, [N1] replaces positions 582-585 (e.g.,

T582, N583, H584, Q585), numbered according to SEQ ID NO: 138. In some embodiments, [N1] corresponds to positions 582-585 (e.g., T582, N583, T584, Q585) of SEQ ID NO: 981. In some embodiments, [N1] is present at positions 582-585, numbered according to SEQ ID NO: 981. In some embodiments, X₁ of [N1] is present at position 582, X₂ of [N1] is present at position 583, X₃ of [N1] is present at position 584, and X₄ of [N1] is present at position 585, numbered according to SEQ ID NO: 981. In some embodiments, [N2] replaces positions 586-589 (e.g., S586, A587, Q588, A589), numbered according to SEQ ID NO: 138. In some embodiments [N2] corresponds to positions 586-589 (e.g., D586, W587, H588, and R589) of SEQ ID NO: 981. In some embodiments, [N2] is present at positions 586-589, numbered according to SEQ ID NO: 981. In some embodiments [N3] replaces positions 590-592 (e.g., Q590, A591, Q592), numbered according to SEQ ID NO: 138. In some embodiments, [N3] corresponds to positions 590-592 (e.g., I590, A591, Q592) of SEQ ID NO: 981. In some, embodiments, [N3] is present at positions 590-592, numbered according to SEQ ID NO: 981. In some embodiments, X₅ of [N3] is present at position 590, X₆ of [N3] is present at position 591, and X₇ of [N3] is present at position 592, numbered according to SEQ ID NO: 981. In some embodiments, [N1]-[N2] is present at positions 582-589, numbered according to SEQ ID NO: 981. In some embodiments, [N2]-[N3] is present at positions 586-592, numbered according to SEQ ID NO: 981. In some embodiments, [N1]-[N2]-[N3] is present at positions 582-592, numbered according to SEQ ID NO: 981.

[0010] In another aspect, the present disclosure provides an AAV capsid variant comprising (a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; (b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, consecutive amino acids from any one of the sequences provided in Tables 11, 2A, 2B, 9, 14, 15, or 16; (c) an amino acid sequence comprising at least one, two, or three, but no more than four different amino acids, relative to any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; or (d) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, the amino acid sequence replaces positions 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 V596), numbered according to SEQ ID NO: 138.

[0011] In yet another aspect, the present disclosure provides an AAV capsid variant comprising (a) the amino acid sequence of any 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, or 336; (b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 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, or 336; (c) an amino acid

sequence comprising at least one, two, or three but no more than four different amino acids, 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, or 336; (d) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative 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, or 336. In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, the amino acid sequence replaces positions 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 V596), numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces positions 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and/or 596 (e.g., A581, T582, N583, H584, Q585, S586, A587, Q588, A589, Q590, A591, Q592, T593, G594, W595, and/or V596), numbered according to SEQ ID NO: 138.

[0012] In yet another aspect, the present disclosure provides a polynucleotide encoding an AAV capsid variant described herein. In some embodiments, the polynucleotide comprises (i) a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequences of SEQ ID NO: 942; (ii) a nucleotide sequence comprising at least one, two, three, four, five, six, or seven, but no more than ten different nucleotides, relative to the nucleotide sequences of SEQ ID NO: 942; or (iii) the nucleotide sequence of SEQ ID NOs: 942, or nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto.

[0013] In yet another aspect, present disclosure provides an AAV capsid variant comprising an amino acid sequence comprising at least 3, 4, 5, 6, or 7 consecutive amino acids from the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein: (i) the 3 consecutive amino acids comprise TQD; (ii) the 4 consecutive amino acids comprise TQDW (SEQ ID NO: 4684); (iii) the 5 consecutive amino acids comprise TQDWH (SEQ ID NO: 4685); or (iv) the 6 consecutive amino acids comprise TQDWHR (SEQ ID NO: 4686); wherein the AAV capsid variant comprises: (a) a VP1 protein comprising the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981; (b) a VP2 protein comprising the amino acid sequence of positions 138-736 of SEQ ID NO: 138 or positions 138-736 of SEQ ID NO: 981; (c) a VP3 protein comprising the amino acid sequence of positions 203-736 of SEQ ID NO: 138 or positions 203-736 of SEQ ID NO: 981; or (d) an amino acid sequence with at least 70% (e.g., at least 75, 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity to any of the amino acid sequences in (a)-(c).

[0014] In yet another aspect, the present disclosure provides an AAV capsid variant comprising one or two, but no more than three substitutions relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein the AAV capsid variant comprises: (a) a VP1 protein comprising the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981; (b) a VP2 protein comprising the amino acid sequence of positions 138-736 of SEQ ID NO: 138 or positions 138-736 of SEQ ID NO: 981; (c) a VP3 protein comprising the amino acid sequence of positions 203-736 of SEQ ID NO: 138 or positions 203-736 of SEQ ID NO: 981; or (d) an amino acid sequence with at least 70% (e.g., at least 75, 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity to any of the amino acid sequences in (a)-(c).

[0015] In yet another aspect, the present disclosure provides an AAV capsid variant comprising one, two, three, four, five or all of an amino acid other than H at position 584 (e.g., T), S at position 586 (e.g., D), A at position 587 (e.g., W), Q at position 588 (e.g., H), A at position 589 (e.g., R), and/or Q at position 590 (e.g., I), numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises an amino acid other than H at position 584 (e.g., T), S at position 586 (e.g., D), A at position 587 (e.g., W), Q at position 588 (e.g., H), A at position 589 (e.g., R), and/or Q at position 590 (e.g., I), numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises one, two, three, four, five or all of the amino acid 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, numbered according to SEQ ID NO: 138 or 981. In some embodiments, the AAV capsid variant comprises 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: 138 or 981.

[0016] In yet another aspect, the present disclosure provides comprising one, two, three, four, five or all of the amino acid 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, numbered according to SEQ ID NO: 138 or 981. In some embodiments, the AAV capsid variant comprises 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: 138 or 981.

[0017] In yet another aspect, the present disclosure provides an AAV capsid variant comprising one, two, three, four, five or all of the substitutions H584T, S586D, A587W, Q588H, A589R, and/or Q590I, numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises the substitutions H584T, S586D, A587W, Q588H, A589R, and Q590I, numbered according to SEQ ID NO: 138.

[0018] In yet another aspect, the present disclosure provides an AAV capsid variant 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: 138 or 981.

[0019] In another aspect, the present disclosure provides an AAV capsid variant comprising the substitutions H584T, S586D, A587W, Q588H, A589R, and Q590I, numbered according to SEQ ID NO: 138.

[0020] In yet another aspect, the present disclosure provides an AAV capsid variant comprising the amino acid sequence of positions 203-736 of SEQ ID NO: 981, or an amino acid sequence at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) thereto, wherein the AAV capsid variant comprises 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 positions 203-736 of SEQ ID NO: 981. In some embodiments, the AAV capsid variant comprises the amino acid sequence of positions 138-736 of SEQ ID NO: 981. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 981.

[0021] In yet another aspect, the present disclosure provides an AAV capsid variant comprising the amino acid sequence of positions 138-736 of SEQ ID NO: 981, or an amino acid sequence at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) thereto, wherein the AAV capsid variant comprises 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 positions 138-736 of SEQ ID NO: 981. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 981.

[0022] In yet another aspect, the present disclosure provides an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981, or an amino acid sequence at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) thereto, wherein the AAV capsid variant comprises 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 an amino acid sequence comprising 30, 20, or 10 modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of SEQ ID NO: 981. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 981.

[0023] In yet another aspect, the present disclosure provides an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981.

[0024] In yet another aspect, the present disclosure provides an AAV 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 thereto.

[0025] In yet another aspect, the present disclosure provides a peptide comprising: (a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; (b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; (c) an amino acid sequence

comprising at least one, two, or three, but no more than four different amino acids relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; or (d) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16.

[0026] In another aspect, the present disclosure provides a peptide comprising (a) the amino acid sequence of any 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, or 336; (b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 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, or 336; (c) an amino acid sequence comprising at least one, two, or three, but no more than four different amino acids 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, or 336; or (d) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., 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, or 336.

[0027] In yet another aspect, the present disclosure provides a peptide comprising: (i) the amino acid sequence of TQDWHRI (SEQ ID NO: 941); (ii) an amino acid sequence comprising at least one, two, or three, but no more than four different amino acids relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941); (iii) an amino acid sequence comprising at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941); or (iv) at least 3, 4, or 5 consecutive amino acids from the amino acid sequence of TQDWHRI (SEQ ID NO: 941).

[0028] In yet another aspect, the present disclosure provides a peptide encoded by (i) the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or (ii) a nucleotide sequence comprising at least one, two, three, four, five, six, or seven, but no more than ten different nucleotides relative to the nucleotide sequence of SEQ ID NO: 942; or (iii) a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to the nucleotide sequence of SEQ ID NO: 942.

[0029] In yet another aspect, the present disclosure provides a nucleotide sequence encoding a peptide comprising (i) the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence

substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; (ii) a nucleotide sequence comprising at least one, two, three, four, five, six, or seven, but not more than 10 different nucleotides, relative to the nucleotide sequence of SEQ ID NO: 942; or (iii) a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to the nucleotide sequence of SEQ ID NO: 942.

[0030] In yet another aspect, the present disclosure provides a polynucleotide encoding an AAV capsid variant comprising: (a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; (b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; (c) an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; or (d) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (conservative substitutions), relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the amino acid sequence of (a), (b), (c), and/or (d) replaces position 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 V596), numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence of (a), (b), (c), and/or (d) corresponds to positions 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and/or 596 (e.g., T582, N583, T584, Q585, D586, W587, H588, R589, I590, A591, Q592, T593, G594, W595, and/or V596) of SEQ ID NO: 981.

[0031] In yet another aspect, the present disclosure provides a polynucleotide encoding an AAV capsid variant, wherein the AAV capsid variant comprises: (i) the amino acid sequence of TQDWHRI (SEQ ID NO: 941); (ii) an amino acid sequence comprising at least one, two, or three, but no more than four different amino acids, relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941); (iii) an amino acid sequence comprising at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941); or (iv) at least 3, 4, 5, 6, 7, 8, or 9 consecutive amino acids from the amino acid sequence of TQDWHRI (SEQ ID NO: 941). In some embodiments, the amino acid sequence of (i), (ii), (iii), and/or (iv) replaces positions 584, 585, 586, 587, 588, 589, and/or 590 (e.g., H584, Q585, S586, A587, Q588, A589, and/or Q590), numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence of (i), (ii), (iii), and/or (iv) corresponds to positions 584, 585, 586, 587, 588, 589, and/or 590 (e.g., T584, Q585, D586, W587, H588, R589, and/or I590) of SEQ ID NO: 981.

[0032] In yet another aspect, the present disclosure provides an AAV particle comprising an AAV capsid variant, described herein. In some embodiments, the AAV particle comprises a nucleic acid sequence encoding a payload. In some embodiments, the AAV particle further comprises a viral genome comprising a promoter operably linked to the nucleic acid encoding the payload.

[0033] In yet another aspect, the present disclosure provides a method of making an AAV particle comprising an AAV capsid variant described herein. The method comprises providing a host cell comprising a viral genome and incubating the host cell under conditions suitable to enclose the viral genome in the AAV capsid variant, e.g., an AAV capsid variant described herein, thereby making the AAV particle.

[0034] In yet another aspect, the present disclosure provides a method of delivering a payload to a cell or tissue (e.g., a CNS cell or a CNS tissue). The method comprising administering an effective amount of an AAV particle comprising an AAV capsid variant described herein.

[0035] In yet another aspect, the present disclosure provides a method of treating a subject having or diagnosed with having a genetic disorder, e.g., a monogenic disorder or a polygenic disorder. The method comprising administering to the subject an effective amount an AAV particle comprising an AAV capsid variant described herein.

[0036] In yet another aspect, the present disclosure provides a method of treating a subject having or diagnosed with having neurological, e.g., a neurodegenerative, disorder. The method comprising administering an effective amount of an AAV particle comprising an AAV capsid variant described herein.

[0037] In yet another aspect, the present disclosure provides a method of treating a subject having or diagnosed with having a neuro-oncological disorder. The method comprising administering an effective amount of an AAV particle comprising an AAV capsid variant described herein.

[0038] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following enumerated embodiments.

Enumerated Embodiments

1. 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: 4681), wherein [N2] comprises the amino acid sequence of DWHR (SEQ ID NO: 4682) and wherein:

(i) [N1] comprises positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q, K, E, S, P, R, N, H, or a conservative substitution thereof; and/or

(ii) [N3] comprises positions X₅, X₆, and X₇, wherein position X₅ is I, V, T, M, S, N, L, F, or a conservative substitution thereof.

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

3. The AAV capsid variant of embodiment 1, which comprises an amino acid other than Q at position 585, numbered according to SEQ ID NO: 138 or 981.
4. The AAV capsid variant of embodiment 1 or 3, which comprises the amino acid K at position 585, numbered according to SEQ ID NO: 138 or 981.
5. The AAV capsid variant of any one of embodiments 1-4, which comprises one, two, three, or all of an amino acid other than T at position 582 (e.g., S, R, A, I, C, N, K, L, or Q), an amino acid other than N at position 583 (e.g., T, G, V, S, Y, K, I, H, D, or F), 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 an amino acid other than Q at position 585 (e.g., K, E, S, P, R, N, H), numbered according to SEQ ID NO: 138.
6. The AAV capsid variant of any one of embodiments 1-5, wherein [N1] comprises positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q, K, E, S, P, R, N, or H.
7. The AAV capsid variant of any one of embodiments 1-6, wherein position X₄ is Q or K.
8. The AAV capsid variant of any one of embodiments 1, 2, or 5-7, wherein position X₄ is Q.
9. The AAV capsid variant of any one of embodiments 1 or 3-7, wherein position X₄ is K.
10. The AAV capsid variant of any one of embodiments 1-9, which comprises an amino acid other than H at position 584 (e.g., T), numbered according to SEQ ID NO: 138.
11. The AAV capsid variant of any one of embodiments 1-10, which comprises the amino acid T at position 584, numbered according to SEQ ID NO: 138 or 981.
12. The AAV capsid variant of any one of embodiments 1-11, wherein:
 - (i) position X₁ is T, S, R, A, I, C, N, K, L, or Q;
 - (ii) position X₂ is N, T, G, V, S, Y, K, I, H, D, or F; and/or
 - (iii) position X₃ is T, N, K, D, I, S, P, A, Y, E, V, L, M, R, H, Q, or C.
13. The AAV capsid variant 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 capsid variant 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 capsid variant 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 capsid variant of any one of embodiments 1-15, wherein [N1] is or comprises TNTQ (SEQ ID NO: 4688).

17. The AAV capsid variant of any one of embodiments 1-15, wherein [N1] is or comprises TNTK (SEQ ID NO: 4689).

18. The AAV capsid variant 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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

19. The AAV capsid variant 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), 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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

20. The AAV capsid variant 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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

21. The AAV capsid variant of any one of embodiments 1-20, wherein [N1]-[N2] is or comprises TNTQDWHR (SEQ ID NO: 4898).

22. The AAV capsid variant of any one of embodiments 1-20, wherein [N1]-[N2] is or comprises TNTKDWHR (SEQ ID NO: 4899).

23. The AAV capsid variant of any one of embodiments 1-22, which 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), A at position 591 (e.g., Y, P, N, S, T, G, E, V, W, F, Q), and/or Q at position 592 (e.g., G, N, K, H, R, E, L, P, or M), numbered according to SEQ ID NO: 138.

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

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

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

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

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

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

30. The AAV capsid variant of any one of embodiments 1-25 or 27-29, wherein position X₅ is I.
31. The AAV capsid variant of any one of embodiments 1-30, wherein:
- (i) position X₆ is A, Y, P, N, S, T, G, E, V, W, F, or Q; and/or
 - (ii) position X₇ is Q, G, N, K, H, R, E, L, P, or M.
32. The AAV capsid variant 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 capsid variant 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 capsid variant of any one of embodiments 1-33, wherein [N3] is or comprises IAQ.
35. The AAV capsid variant of any one of embodiments 1-34, wherein [N2]-[N3] comprises:
- (i) DWHRIA (SEQ ID NO: 5002), DWHRIY (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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or
 - (iv) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the amino acid sequences in (i).
36. The AAV capsid variant 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), DWHRIPQ (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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

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

38. The AAV capsid variant 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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

39. The AAV capsid variant of any one of embodiments 1-38, wherein [N1]-[N2]-[N3] is or comprises TNTQDWHRIAQ (SEQ ID NO: 343).
40. The AAV capsid variant of any one of embodiments 1-38, wherein [N1]-[N2]-[N3] is or comprises TNTKDWHRIAQ (SEQ ID NO: 344).
41. The AAV capsid variant of any one of embodiments 1-40, which comprises one, two, three, or all of an amino acid other than T at position 593 (e.g., S, N, P, A, or I), a G at position 594 (e.g., N, D, R, V, A, S, or Q), a W at position 595 (e.g., S, C, R, L, or G), and/or a V at position 596 (e.g., A, S, I, C, G, D, F, L, or T), numbered according to SEQ ID NO: 138 or 981.
42. The AAV capsid variant of any one of embodiments 1-40, which 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, numbered according to SEQ ID NO: 138 or 981.
43. The AAV capsid variant of any one of embodiments 1-42, which further comprises [N4], wherein [N4] comprises positions X₈, X₉, X₁₀, and X₁₁, wherein:
- (i) position X₈ is T, S, N, P, A, or I;
 - (ii) position X₉ is G, N, D, R, V, A, S, or Q;
 - (iii) position X₁₀ is W, S, C, R, L, or G; and/or
 - (iv) position X₁₁ is V, A, S, I, C, G, D, F, L, or T.
44. The AAV capsid variant 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 capsid variant 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 capsid variant 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 capsid variant of any one of embodiments 43-46, wherein [N4] is or comprises TGWV (SEQ ID NO: 5066).

48. The AAV capsid variant 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, or 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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

49. The AAV capsid variant of any one of embodiments 43-48, wherein [N1]-[N2]-[N3]-[N4] is or comprises TNTQDWHRIAQTGWV (SEQ ID NO: 201).

50. The AAV capsid variant of any one of embodiments 43-48, wherein [N1]-[N2]-[N3]-[N4] is or comprises TNTKDWHRIAQTGWV (SEQ ID NO: 202).

51. 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 positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q, P, or a conservative substitution thereof; and/or

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

52. The AAV capsid variant of embodiment 51, which comprises the amino acid Q at position 585, numbered according to SEQ ID NO: 138 or 981.
53. The AAV capsid variant of embodiment 51 or 52, which comprises one, two, three, or all of an amino acid other than T at position 582 (e.g., S), an amino acid other than N at position 583 (e.g., T, G, S, I, or V), an amino acid other than H at position 584 (e.g., N, I, S, A, V, or L), and/or an amino acid other than Q at position 585 (e.g., P), numbered according to SEQ ID NO: 138.
54. The AAV capsid variant of any one of embodiments 51-53, wherein [N1] comprises positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q or P.
55. The AAV capsid variant of any one of embodiments 51, 52, or 54, wherein position X₄ is Q.
56. The AAV capsid variant of any one of embodiments 51-55, which comprises an amino acid other than H at position 584 (e.g., T), numbered according to SEQ ID NO: 138.
57. The AAV capsid variant of any one of embodiments 51-56, which comprises the amino acid T at position 584, numbered according to SEQ ID NO: 138 or 981.
58. The AAV capsid variant of any one of embodiments 51-57, wherein:
- (i) position X₁ is T or S;
 - (ii) position X₂ is N, T, G, S, I, or V; and/or
 - (iii) position X₃ is T, N, I, S, A, V, or L.
59. The AAV capsid variant 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 capsid variant 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 capsid variant 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 capsid variant of any one of embodiments 51-61, wherein [N1] is or comprises TNTQ (SEQ ID NO: 4688).

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

(i) TQDWH (SEQ ID NO: 4686), NQDWH (SEQ ID NO: 4793), IQDWH (SEQ ID NO: 4797), SQDWH (SEQ ID NO: 4798), AQDWH (SEQ ID NO: 4801), VQDWH (SEQ ID NO: 4805), TPDWH (SEQ ID NO: 4803), or LQDWH (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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

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

(i) NTQDWH (SEQ ID NO: 4827), TNQDWH (SEQ ID NO: 4832), TIQDWH (SEQ ID NO: 4834), TSQDWH (SEQ ID NO: 4836), TTQDWH (SEQ ID NO: 4840), TAQDWH (SEQ ID NO: 4842), NIQDWH (SEQ ID NO: 4848), TVQDWH (SEQ ID NO: 4850), GTQDWH (SEQ ID NO: 4851), STQDWH (SEQ ID NO: 4884), ITQDWH (SEQ ID NO: 4883), NTPDWH (SEQ ID NO: 4845), NNQDWH (SEQ ID NO: 4829), TLQDWH (SEQ ID NO: 4853), NSQDWH (SEQ ID NO: 4861), VTQDWH (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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

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

(i) TNTQDWH (SEQ ID NO: 4898), TTNQDWH (SEQ ID NO: 4906), TTIQDWH (SEQ ID NO: 4908), TTSQDWH (SEQ ID NO: 4911), TTTQDWH (SEQ ID NO: 4915), TTAQDWH (SEQ ID NO: 4917), TNIQDWH (SEQ ID NO: 4924), TTVQDWH (SEQ ID NO: 4927), TGTQDWH (SEQ ID NO: 4928), STTQDWH (SEQ ID NO: 4971), TSTQDWH (SEQ ID NO: 4978), TITQDWH (SEQ ID NO: 4977), TNPQDWH (SEQ ID NO: 4920), TNNQDWH

(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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

66. The AAV capsid variant of any one of embodiments 51-65, wherein [N1]-[N2] is or comprises TNTQDWHR (SEQ ID NO: 4898).

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

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

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

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

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

72. The AAV capsid variant of any one of embodiments 51-71, [N3] comprises positions X₅, X₆, and X₇, wherein position X₅ is I or V.

73. The AAV capsid variant of any one of embodiments 51-72, wherein position X₅ is I.

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

(i) position X₆ is A, P, S, Y, or N; and/or

(ii) position X₇ is Q, G, or N.

75. The AAV capsid variant 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 capsid variant 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 capsid variant of any one of embodiments 51-76, wherein [N3] is or comprises IAQ.

78. The AAV capsid variant 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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

79. The AAV capsid variant 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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or

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

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

81. The AAV capsid variant 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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or
- (iv) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the amino acid sequences in (i).

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

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

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

85. The AAV capsid variant of any one of embodiments 51-84 which 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, 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, 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, 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, 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, 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, numbered according to SEQ ID NO: 138 or 981;.

86. The AAV capsid variant of any one of embodiments 51-85 which 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, numbered according to SEQ ID NO: 138 or 981;

87. The AAV capsid variant of any one of embodiments 51-86, which further comprises [N4], wherein [N4] comprises positions X₈, X₉, X₁₀, and X₁₁, wherein position X₁₀ is W.

88. The AAV capsid variant of embodiment 87, wherein:

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

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

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

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

93. The AAV capsid variant 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, or 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, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (i); or
- (iv) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the amino acid sequences in (i).

94. The AAV capsid variant of any one of embodiments 87-93, wherein [N1]-[N2]-[N3]-[N4] is or comprises TNTQDWHRIAQTGWV (SEQ ID NO: 201).
95. The AAV capsid variant of any one of embodiments 1-94, wherein [N1]-[N2]-[N3] is present in loop VIII, optionally wherein loop VIII comprises positions 580-599, numbered according to SEQ ID NO: 138 or 981.
96. The AAV capsid variant of any one of embodiments 43-50 or 87-95, wherein [N4] is present in loop VIII, optionally wherein loop VIII comprises positions 580-599, numbered according to SEQ ID NO: 138 or 981.
97. The AAV capsid variant of any one of embodiments 1-96, wherein [N1] replaces positions 582-585 (e.g., T582, N583, H584, Q585), numbered according to SEQ ID NO: 138.
98. The AAV capsid variant of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, or 85-97, wherein [N1] corresponds to positions 582-585 (e.g., T582, N583, T584, Q585) of SEQ ID NO: 981.
99. The AAV capsid variant of any one of embodiments 1-98, wherein [N1] is present at positions 582-585, numbered according to SEQ ID NO: 138 or 981.
100. The AAV capsid variant of any one of embodiments 1-99, wherein [N1] corresponds to positions 582-585 (e.g., T582, N583, T584, Q585) of SEQ ID NO: 138.
101. The AAV capsid variant of any one of embodiments 1-100, wherein [N2] replaces positions 586-589 (e.g., S586, A587, Q588, A589), numbered according to the amino acid sequence of SEQ ID NO: 138.
102. The AAV capsid variant of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, or 101, wherein [N2] corresponds to positions 586-589 (e.g., D586, W587, H588, R589) of SEQ ID NO: 981.
103. The AAV capsid variant of any one of embodiments 1-102, wherein [N2] is present at positions 586-589, numbered according to SEQ ID NO: 138 or 981.

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

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

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

107. The AAV capsid variant of any one of embodiments 1-106, wherein [N3] replaces positions 590-592 (e.g., Q590, A591, and Q592), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

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

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

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

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

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

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

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

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

116. The AAV capsid variant of any one of embodiments 43-50 or 87-115, wherein [N4] replaces positions 593-596 (e.g., T593, G594, W595, and V596), numbered according to SEQ ID NO: 138.

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

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

119. The AAV capsid variant of any one of embodiments 43-50 or 87-118, wherein [N2]-[N3]-[N4] replaces positions 586-596 (e.g., S586, A587, Q588, A589, Q590, A591, Q592, T593, G594, W595, and V596), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

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

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

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

123. The AAV capsid variant of any one of embodiments 43-49 or 87-122, wherein [N1]-[N2]-[N3]-[N4] corresponds to positions 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 capsid variant of any one of embodiments 43-50 or 87-123, wherein [N1]-[N2]-[N3]-[N4] is present at positions 582-596, numbered according to the amino acid sequence of SEQ ID NO: 138 or 981.
125. The AAV capsid variant of any one of embodiments 1-124, wherein [N2] is present immediately subsequent to [N1].
126. The AAV capsid variant of any one of embodiments 1-125, wherein [N3] is present immediately subsequent to [N2].
127. The AAV capsid variant of any one of embodiments 43-50 or 87-126, wherein [N3] is present immediately subsequent to [N4].
128. The AAV capsid variant of any one of embodiments 1-127, which comprises from N-terminus to C-terminus, [N1]-[N2]-[N3].
129. The AAV capsid variant of any one of embodiments 43-50 or 87-128, which comprises from N-terminus to C-terminus, [N1]-[N2]-[N3]-[N4].
130. An AAV capsid variant (e.g., an AAV9 capsid variant) comprising:
- (a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16;
 - (b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; or
 - (c) an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; or
 - (d) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16.
131. An AAV capsid variant (e.g., an AAV9 capsid variant) comprising:
- (a) the amino acid sequence of any 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, or 336;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 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, or 336;

(c) an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, 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, or 336; or

(d) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative 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, or 336.

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

133. The AAV capsid variant of any one of embodiments 130-132, wherein the AAV capsid variant does not comprise: TNH, TNHQA (SEQ ID NO: 4760), TNHQS (SEQ ID NO: 5101), TNHQA (SEQ ID NO: 5102), TNHQAQA (SEQ ID NO: 5103), TNHQAQA (SEQ ID NO: 5104), TNHQAQAQA (SEQ ID NO: 5100), NHQA, NHQS (SEQ ID NO: 5105), NHQA (SEQ ID NO: 5106), NHQAQA (SEQ ID NO: 5107), NHQAQA (SEQ ID NO: 5108), NHQAQAQA (SEQ ID NO: 5109), HQA, HQA (SEQ ID NO: 5110), HQAQA (SEQ ID NO: 5111), HQAQA (SEQ ID NO: 5112), HQAQAQA (SEQ ID NO: 5113), QA, QAQA (SEQ ID NO: 5114), QAQA (SEQ ID NO: 5115), QAQAQA (SEQ ID NO: 5116), QAQA (SEQ ID NO: 5117), or QAQAQA (SEQ ID NO: 5118).

134. The AAV capsid variant of any one of embodiments 130-134, which comprises an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 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, or 336.

135. The AAV capsid variant of any one of embodiments 130-134, wherein the 3 consecutive amino acids comprise TQA.

136. The AAV capsid variant of any one of embodiments 130-135, wherein the 4 consecutive amino acids comprise TQDA (SEQ ID NO: 4684).

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

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

139. The AAV capsid variant of embodiment 130-138, wherein the 7 consecutive amino acids comprise TQDWHRI (SEQ ID NO: 941).

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

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

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

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

144. The AAV capsid variant of embodiment 130-134 or 140-143, wherein the 7 consecutive amino acids comprise TNTQDWH (SEQ ID NO: 5121).

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

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

147. The AAV capsid variant of any one of embodiments 130-146, which comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative 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, or 336.

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

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

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

151. The AAV capsid variant of any one of embodiments 130-150, which comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids 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, or 336.

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

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

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

155. The AAV capsid variant of any one of embodiments 1-154, which comprises the amino acid sequence of any 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, or 336.

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

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

158. The AAV capsid variant of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, or 101-149, 151-153, or 155-157, comprising an amino acid sequence encoded by: the nucleotide sequence of SEQ ID NO: 942; a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to the nucleotide sequence of SEQ ID NO: 942; or a nucleotide sequence comprising at least one, two, three, four, five, six, or seven, but no more than ten different nucleotides relative to the nucleotide sequence of SEQ ID NO: 942.

159. The AAV capsid variant of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, or 101-158, comprising an amino acid sequence encoded by: the nucleotide sequence of SEQ ID NO: 747; a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to the nucleotide sequence of SEQ ID NO: 747; or a nucleotide sequence comprising at least one, two, three, four, five, six, or seven, but no more than ten different nucleotides relative to the nucleotide sequence of SEQ ID NO: 747.

160. The AAV capsid variant of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, or 101-149, 151-153, or 155-158, wherein the nucleotide sequence encoding the capsid variant comprises the nucleotide sequence of SEQ ID NO: 942; a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to the nucleotide sequence of SEQ ID NO: 942; or a nucleotide sequence comprising at least one, two, three, four, five, six, or seven, but no more than ten different nucleotides relative to the nucleotide sequence of SEQ ID NO: 942.

161. The AAV capsid variant of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, or 101-160, wherein the nucleotide sequence encoding the capsid variant comprises the nucleotide sequence of SEQ ID NO: 747; a nucleotide sequence comprising at least one, two, three,

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

162. The AAV capsid variant of any one of embodiments 130-161, wherein the amino acid sequence is present in loop VIII, optionally wherein loop VIII comprises positions 580-599, numbered according to SEQ ID NO: 138 or 981.

163. The AAV capsid variant of any one of embodiments 130-153, wherein the amino acid sequence replaces positions 584, 585, 586, 587, 588, 589, and/or 590 (e.g., H584, Q585, S586, A587, Q588, A589, and/or Q590), numbered according to the amino acid sequence of SEQ ID NO: 138.

164. The AAV capsid variant of any one of embodiments 130-163, wherein the amino acid sequence is present at positions 584, 585, 586, 587, 588, 589, and/or 590, numbered according to the amino acid sequence of SEQ ID NO: 138 or 981.

165. The AAV capsid variant of any one of embodiments 130-164, wherein the amino acid sequence corresponds to positions 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 capsid variant of any one of embodiments 121-165, wherein the amino acid sequence replaces positions 582, 583, 584, 585, 586, 587, 588, 589, and/or 590 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, and/or Q590), numbered according to the amino acid sequence of SEQ ID NO: 138.

167. The AAV capsid variant of any one of embodiments 121-166, wherein the amino acid sequence is present at positions 582, 583, 584, 585, 586, 587, 588, 589, and/or 590, numbered according to the amino acid sequence of SEQ ID NO: 138 or 981.

168. The AAV capsid variant of any one of embodiments 121-167, wherein the amino acid sequence replaces positions 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 V596), numbered according to the amino acid sequence of SEQ ID NO: 138.

169. The AAV capsid variant of any one of embodiments 121-168, wherein the amino acid sequence is present at positions 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and/or 596, numbered according to the amino acid sequence of SEQ ID NO: 138 or 981.

170. The AAV capsid variant of any one of embodiments 1-169, which comprises one, two, three, four, five or all of an amino acid other than H at position 584 (e.g., T), S at position 586 (e.g., D), A at position 587 (e.g., W), Q at position 588 (e.g., H), A at position 589 (e.g., R), and/or Q at position 590 (e.g., I), numbered according to SEQ ID NO: 138.

171. An AAV capsid variant (e.g., an AAV9 capsid variant) comprising one, two, three, four, five or all of an amino acid other than H at position 584 (e.g., T), S at position 586 (e.g., D), A at position 587 (e.g., W), Q at position 588 (e.g., H), A at position 589 (e.g., R), and/or Q at position 590 (e.g., I), numbered according to SEQ ID NO: 138.

172. The AAV capsid variant of any one of embodiments 1-171, which comprises one, two, three, four, five or all of the amino acid 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, numbered according to SEQ ID NO: 138 or 981.

173. An AAV capsid variant (e.g., an AAV9 capsid variant) comprising one, two, three, four, five or all of the amino acid 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, numbered according to SEQ ID NO: 138 or 981.

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

175. 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, numbered according to SEQ ID NO: 138.

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

177. The AAV capsid variant of any one of embodiments 1-176, which comprises 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: 138 or 981.

178. The AAV capsid variant of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-98, or 100-164, which corresponds to positions 584, 586, 587, 588, 589, and 590 of SEQ ID NO: 981.

179. The AAV capsid variant of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, or 101-178, which corresponds to positions 582, 583, 584, 586, 587, 588, 589, and 590 of SEQ ID NO: 981.

180. The AAV capsid variant of any one of embodiments 1-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, or 101-179, which corresponds to positions 582, 583, 584, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and 596 of SEQ ID NO: 981.

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

182. An AAV capsid variant (e.g., an AAV9 capsid variant) 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: 138 or 981.

183. An AAV capsid variant (e.g., an AAV9 capsid variant) comprising the substitutions H584T, S586D, A587W, Q588H, A589R, and Q590I, numbered according to SEQ ID NO: 138.

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

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

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

187. The AAV capsid variant 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, or 181-186, which comprises an amino acid other than Q at position 585, numbered according to SEQ ID NO: 138.

188. The AAV capsid variant 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, 181-187, which comprises the amino acid K at position 585, numbered according to SEQ ID NO: 138.

189. The AAV capsid variant of any one of the preceding embodiments, which further comprises:

- (i) a modification, e.g., an insertion, substitution (e.g., conservative substitution), and/or deletion, in loop I, II, IV, and/or VI; and/or
- (ii) a substitution at position K449, e.g., a K449R substitution, numbered according to SEQ ID NO: 138.

190. The AAV capsid variant of any one of the preceding embodiments, which comprises an amino acid sequence comprising at least one, two or three modifications, e.g., substitutions (e.g., conservative substitutions), but not more than 30, 20 or 10 modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of SEQ ID NO: 138.

191. The AAV capsid variant of any one of the preceding embodiments, which comprises an amino acid sequence comprising at least one, two or three, but no more than 30, 20 or 10 different amino acids relative to the amino acid sequence of SEQ ID NO: 138.

192. The AAV capsid variant of any one of the preceding embodiments, which comprises the amino acid sequence of SEQ ID NO: 138, or an amino acid sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

193. The AAV capsid variant of any one of the preceding embodiments, which comprises the amino acid sequence of SEQ ID NO: 138.

194. The AAV capsid variant of any one of the preceding embodiments, which comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 137, or a sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

195. The AAV capsid variant of any one of the preceding embodiments, wherein the nucleotide sequence encoding the capsid variant comprises the nucleotide sequence of SEQ ID NO: 137, or a sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

196. The AAV capsid variant of any one of the preceding embodiments, which comprises a VP1 protein, a VP2 protein, a VP3 protein, or a combination thereof.

197. The AAV capsid variant of any one of embodiments 1-196, which comprises the amino acid sequence corresponding to positions 138-736, e.g., a VP2, of SEQ ID NO: 981, or a sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

198. The AAV capsid variant of any one of embodiments 1-197, which comprises the amino acid sequence corresponding to positions 203-736, e.g., a VP3, of SEQ ID NO: 981, or a sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

199. The AAV capsid variant of any one of embodiments 1-198, which comprises the amino acid sequence corresponding to positions 138-736, e.g., a VP2, of SEQ ID NO: 138, or a sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

200. The AAV capsid variant of any one of embodiments 1-199, which comprises the amino acid sequence corresponding to positions 203-736, e.g., a VP3, of SEQ ID NO: 138, or a sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

201. The AAV capsid variant 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, or 189-200, comprising an amino acid sequence comprising at least 3, 4, 5, 6, or 7 consecutive amino acids from the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein:

- (i) the 3 consecutive amino acids comprise TQD;
- (ii) the 4 consecutive amino acids comprise TQDW (SEQ ID NO: 4684);
- (iii) the 5 consecutive amino acids comprise TQDWH (SEQ ID NO: 4685);
- (iv) the 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 VP1 protein comprising the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981; (b) a VP2 protein comprising the amino acid sequence of positions 138-736 of SEQ ID NO: 138 or positions 138-736 of SEQ ID NO: 981; (c) a VP3 protein comprising the amino acid sequence of positions 203-736 of SEQ ID NO: 138 or positions 203-736 of SEQ ID NO: 981; or (d) an amino acid sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity to any of the amino acid sequences in (a)-(c).

202. The AAV capsid variant 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, or 189-201, comprising an amino acid sequence comprising at least 3, 4, 5, 6,

3, 4, 5, 6, or 7 consecutive amino acids from the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein:

- (i) the 3 consecutive amino acids comprise TQD;
- (ii) the 4 consecutive amino acids comprise TQDW (SEQ ID NO: 4684);
- (iii) the 5 consecutive amino acids comprise TQDWH (SEQ ID NO: 4685);
- (iv) the 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 an amino acid sequence at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) identical to the amino acid sequence of SEQ ID NO: 981.

203. The AAV capsid variant of any one of embodiments 11-3, 5-8, 10-16, 18-21, 23-39, 42-49, 51-69, 71-83, 85-99, 101-186, or 189-202, comprising one or two, but no more than three 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 95, 96, 97, 98, or 99%) identical to the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 981.

204. The AAV capsid variant of any one of embodiments 1-203, which comprises the amino acid sequence of any one of SEQ ID NO: 981, or an amino acid sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

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

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

207. The AAV capsid variant of any one of embodiments 1-206, which 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 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

208. The AAV capsid variant of any one of the preceding embodiments 1-207, wherein the nucleotide sequence encoding the capsid variant comprises the nucleotide sequence of SEQ ID NO: 983, or a nucleotide sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

209. The AAV capsid variant of any one of the preceding embodiments, wherein the nucleotide sequence encoding the capsid variant is codon optimized.
210. 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, or 189, and further comprising an amino acid sequence at least 95% identical to SEQ ID NO: 981.
211. An AAV capsid variant (e.g., an AAV9 capsid variant) comprising the amino acid sequence of SEQ ID NO: 981.
212. 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 thereto.
213. An AAV capsid variant (e.g., an AAV9 capsid variant) comprising the amino acid sequence of positions 203-736 of SEQ ID NO: 981, or an amino acid sequence at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) thereto, wherein the AAV capsid variant comprises 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.
214. The AAV capsid variant of embodiment 213, which comprises the amino acid sequence of positions 203-736 of SEQ ID NO: 981.
215. An AAV capsid variant (e.g., an AAV9 capsid variant) comprising the amino acid sequence of positions 203-736 of SEQ ID NO: 981.
216. An AAV capsid variant (e.g., an AAV9 capsid variant) comprising the amino acid sequence of positions 138-736 of SEQ ID NO: 981, or an amino acid sequence at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) thereto, wherein the AAV capsid variant comprises 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.
217. The AAV capsid any one of embodiments 213-216, wherein the AAV capsid variant comprises the amino acid sequence of positions 138-736 of SEQ ID NO: 981.
218. An AAV capsid variant (e.g., an AAV9 capsid variant) comprising the amino acid sequence of positions 138-736 of SEQ ID NO: 981.

219. 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 85, 90, 95, 96, 97, 98, or 99%) thereto, wherein the AAV capsid variant comprises 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.

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

221. The AAV capsid variant of any one of embodiments 1-220, which 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 a reference sequence comprising the amino acid sequence of SEQ ID NO: 138.

222. The AAV capsid variant of any one of embodiments 1-221, which transduces a brain region, e.g., sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, cerebellum optionally wherein the level of transduction is at least 39, 50, 100, 120, 132, 146, 150, 161, 174, 175, 200, 225, 250, 275, 283, 300, 350, 400, 450, 500, 525, 528, or 550-fold greater as compared to a reference 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 capsid variant of any one of embodiments 1-222, which is enriched at least about 10, 14, 20, 24, 50, 100, 150, 200, 250, 300, 350, 400, 425, 450, or 460-fold, in the brain compared to a reference sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Example 1 or 3.

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

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

226. The AAV capsid variant of any one of embodiments 1-225, which is enriched at least about 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 190, 200, 205, or 210-fold, in the brain of at least

two to three species, e.g., a non-human primate and rodent (e.g., mouse), compared to a reference sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Example 1 or 4.

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

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

229. The AAV capsid variant of any one of embodiments 1-228, which delivers an increased level of a payload to a brain region, optionally wherein the level of the payload is increased by at least 39, 50, 100, 120, 132, 146, 150, 161, 174, 175, 200, 225, 250, 275, 283, 300, 350, 400, 450, 500, 525, 528, or 550-fold, as compared to a reference 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 capsid variant 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, 5, 7, 10, 15, 19, 20, 22, or 25-fold, as compared to a reference 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 capsid variant 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 capsid variant of any one of embodiments 1-231, which is enriched at least about 5, 10, 50, 100, 115, 120, 150, 175, 200, 207, 225, 250, or 275-fold, in the spinal cord compared to a reference sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Example 1 or 2.

233. The AAV capsid variant of any one of the preceding embodiments, which is isolated, e.g., recombinant.

234. A polynucleotide encoding the AAV capsid variant of any one of embodiments 1-233.

235. The polynucleotide of embodiment 234, which comprises:

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

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

(iii) the nucleotide sequence of SEQ ID NO: 942, or nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto.

236. The polynucleotide of embodiment 234 or 235, which comprises the nucleotide sequence of SEQ ID NO: 983, or a nucleotide sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

237. The polynucleotide of any one of embodiments 234-236, which comprises a nucleotide sequence that is codon optimized.

238. A peptide comprising:

(a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 14, 15, or 16;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 14, 15, or 16;

(c) an amino acid sequence comprising at least one, two, or three but no more than four different amino acids relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 14, 15, or 16; or

(d) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 14, 15, or 16.

239. A peptide comprising:

(a) the amino acid sequence of any 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, or 336;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 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, or 336;

(c) an amino acid sequence comprising at least one, two, or three but no more than four different amino acids 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, or 336; or

(d) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative 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, or 336.

240. A peptide comprising:

(i) the amino acid sequence of TQDWHRI (SEQ ID NO: 941);

(ii) an amino acid sequence comprising at least one, two, or three, but no more than four different amino acids relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941);

(iii) an amino acid sequence comprising at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941); or

(iv) at least 3, 4, 5, or 6 consecutive amino acids from the amino acid sequence of TQDWHRI (SEQ ID NO: 941).

241. A peptide encoded by:

(i) the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto;

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

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

242. A peptide wherein the nucleotide sequence encoding the peptide comprises:

(i) the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto;

(ii) a nucleotide sequence comprising at least one, two, three, four, five, six, or seven, but not more than 10 different nucleotides, relative to the nucleotide sequence of SEQ ID NO: 942; or

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

243. A peptide comprising an amino acid sequence having the following formula: [N1]-[N2]-[N3] according to any one of embodiments 1-4, 7-22, 28-40, 43-51, 54, 55, 58-66, 72-82, 87-94, or 125-129.
244. The peptide of any one of embodiments 238-243, which is fused or coupled, e.g., conjugated, to an active agent, e.g., a therapeutic agent or a diagnostic agent.
245. The peptide of any one of embodiments 238-244, wherein at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides are fused or coupled, e.g., conjugated, to an active agent, e.g., a therapeutic agent or a diagnostic agent.
246. The peptide of embodiment 245, wherein the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides comprise the same amino acid sequence.
247. The peptide of embodiment 245, wherein the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides comprise different amino acid sequences.
248. The peptide of any one of embodiments 245-247, wherein the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides are present in tandem (e.g., connected directly or indirectly via a linker) or in a multimeric configuration.
249. The peptide of any one of embodiments 238-248, wherein the peptide comprises an amino acid sequence of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, 25, 30, or 35 amino acids in length.
250. The peptide of any one of embodiments 244-249, wherein the active agent is or comprises a therapeutic agent chosen from a protein (e.g., an enzyme), an antibody molecule, a nucleic acid molecule (e.g., an RNAi agent), or a small molecule.
251. The peptide of any one of embodiments 244-249, wherein the active agent is or comprises a ribonucleic acid complex (e.g., a Cas9/gRNA complex), a plasmid, a closed-end DNA, a circ-RNA, or an mRNA.
252. The peptide of any one of embodiments 244-249, wherein the active agent is an RNAi agent.
253. The peptide of embodiment 252, wherein the RNAi agent is a dsRNA, a siRNA, a shRNA, a pre-miRNA, a pri-miRNA, a miRNA, a stRNA, a lncRNA, a piRNA, an antisense oligonucleotide agent

(ASO), or a snoRNA, optionally wherein the RNAi agent is an siRNA or an ASO, which further optionally comprises at least one modified nucleotide.

254. The peptide of any one of embodiments 244-253, wherein the active agent modulates, e.g., inhibits, decreases or increases, expression of, a CNS related gene, mRNA, and/or protein.

255. The peptide of any one of embodiments 244-249, wherein the active agent is a diagnostic agent is or comprises an imaging agent (e.g., a protein or small molecule compound coupled to a detectable moiety).

256. The peptide of any one of embodiments 244-255, wherein the peptide covalently linked, e.g., directly or indirectly via a linker, to the active agent.

257. The peptide of any one of embodiments 244-256, wherein the peptide is conjugated to the active agent via a linker.

258. The peptide of embodiment 257, wherein the linker is a cleavable linker or a non-cleavable linker.

259. The peptide of embodiment 258, wherein the cleavable linker is a pH sensitive linker or an enzyme sensitive linker.

260. The peptide of embodiment 258 or 259, wherein:

- (i) the pH sensitive linker comprises a hydrazine/hydrazone linker or a disulfide linker;
- (ii) the enzyme sensitive linker comprises a peptide based linker, e.g., a peptide linker sensitive to a protease (e.g., a lysosomal protease); or a beta-glucuronide linker; or
- (iii) the non-cleavable linker is a linker comprising a thioether group or a maleimidocaproyl group.

261. The peptide of any one of embodiments 244-260, wherein:

- (i) the peptide and the active agent are fused or coupled post-translationally, e.g., using click chemistry; or
- (ii) the peptide and the active agent are fused or couple via chemically induced dimerization.

262. The peptide of any one of embodiments 244-261, wherein the peptide is present N-terminal relative to the active agent.

263. The peptide of any one of embodiments 244-261, wherein the peptide is present C-terminal relative to the active agent.

264. The peptide of any one of embodiment 244-249, 254, or 256-263, wherein the peptide is present or coupled to a carrier, e.g., an exosome, a microvesicle, or a lipid nanoparticle (LNP), optionally, wherein the carrier comprises a therapeutic agent (e.g., an RNAi agent (e.g., an dsRNA, a siRNA, a shRNA, a pre-miRNA, a pri-miRNA, a miRNA, a stRNA, a lncRNA, a piRNA, an antisense oligonucleotide agent (ASO), or a snoRNA), an mRNA, a ribonucleoprotein complex (e.g., a Cas9/gRNA complex), or a circRNA).

265. The peptide of embodiment 264, wherein the peptide is present on the surface of the carrier, optionally wherein at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the surface of the carrier comprises at least 1-5, e.g., at least 1, 2, 3, 4, or 5 peptides according to any one of embodiments 422-436.

266. An AAV capsid variant comprising the peptide of any one of embodiments 238-243.

267. A polynucleotide encoding an AAV capsid variant (e.g., an AAV9 capsid variant) comprising:

(a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16;

(c) an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; or

(d) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16;

optionally wherein:

(i) the amino acid sequence of (a), (b), (c), and/or (d) replaces positions T582, N583, H584, Q585, S586, A587, Q588, A589, Q590, A591, Q592, T593, G594, W595, and/or V596, numbered according to the amino acid sequence of SEQ ID NO: 138; or

(ii) the amino acid sequence of (a), (b), (c), and/or (d) corresponds to positions T582, N583, T584, Q585, D586, W587, H588, R589, I590, A591, Q592, T593, G594, W595, and/or V596 of SEQ ID NO: 981.

268. A polynucleotide encoding an AAV capsid variant (e.g., an AAV9 capsid variant), wherein the AAV capsid variant comprises:

- (i) the amino acid sequence of TQDWHRI (SEQ ID NO: 941);
- (ii) an amino acid sequence comprising at least one, two, or three, but no more than four different amino acids, relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941);
- (iii) an amino acid sequence comprising at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941); or
- (iv) at least 3, 4, 5, or 6 consecutive amino acids from the amino acid sequence of TQDWHRI (SEQ ID NO: 941);

optionally wherein:

- (a) the amino acid sequence of (i), (ii), (iii), and/or (iv) replaces positions H584, Q585, S586, A587, Q588, A589, and/or Q590, numbered according to the amino acid sequence of SEQ ID NO: 138;
- (b) the amino acid sequence of (i), (ii), (iii), and/or (iv) corresponds to positions T584, Q585, D586, W587, H588, R589, and/or I590 of SEQ ID NO: 981.

269. The polynucleotide of embodiment 267 or 268, which comprises:

- (i) the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto;
- (ii) a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to the nucleotide sequence of SEQ ID NO: 942;
- (iii) a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to the nucleotide sequence of SEQ ID NO: 942.

270. The polynucleotide of any one of embodiments 267-269, wherein the AAV capsid variant comprises:

- (i) the amino acid sequence of SEQ ID NO: 981, or an amino acid sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto;
- (ii) an amino acid sequence having at least one, two or three, but no more than four different amino acids, relative to the amino acid sequence of SEQ ID NO: 981; or

(iii) an amino acid sequence having at least one, two or three modifications, e.g., substitutions (e.g., conservative substitutions), but not more than 30, 20 or 10 modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of SEQ ID NO: 981.

271. The polynucleotide of any one of embodiments 267-270, comprising the nucleotide sequence of SEQ ID NO: 983, or a nucleotide sequence with at least 80% (e.g., at least 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

272. The polynucleotide, peptide, or AAV capsid variant, of any one of embodiments 1-272, which is isolated, e.g., recombinant.

273. An AAV particle comprising the AAV capsid variant of any one of embodiments 1-233, 266, or 272, an AAV capsid variant comprising the peptide of any one of embodiments 238-243 or 272, or an AAV capsid variant encoded by the polynucleotide of any one of embodiments 234-237 or 267-272.

274. The AAV particle of embodiment 273, which comprises a nucleotide sequence encoding a payload.

275. The AAV particle of embodiment 274, wherein the encoded payload comprises a therapeutic protein or functional variant thereof; an antibody or antibody fragment; an enzyme; a component of a gene editing system; an RNAi agent (e.g., a dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA); or a combination thereof.

276. The AAV particle of embodiment 275, wherein the therapeutic protein or functional variant thereof, e.g., a recombinant protein, is associated with (e.g., aberrantly expressed in) a neurological or neurodegenerative disorder, a muscular or neuromuscular disorder, or a neuro-oncological disorder.

277. The AAV particle of embodiment 275 or 276, the therapeutic protein or functional variant thereof is chosen from apolipoprotein E (APOE) (e.g., ApoE2, ApoE3 and/or ApoE4); human survival of motor neuron (SMN) 1 or SMN2; glucocerebrosidase (GBA1); aromatic L-amino acid decarboxylase (AADC); aspartoacylase (ASPA); tripeptidyl peptidase I (CLN2); beta-galactosidase (GLB1); N-sulphoglucosamine sulphohydrolase (SGSH); N-acetyl-alpha-glucosaminidase (NAGLU); iduronate 2-sulfatase (IDS); intracellular cholesterol transporter (NPC1); gigaxonin (GAN); or a combination thereof.

278. The AAV particle of embodiment 275, wherein the antibody or antibody binding fragment binds to:

- (i) a CNS related target, e.g., an antigen associated with a neurological or neurodegenerative disorder, e.g., β -amyloid, APOE, tau, SOD1, TDP-43, huntingtin (HTT), and/or synuclein;
- (ii) a muscular or neuromuscular related target, e.g., an antigen associated with a muscular or neuromuscular disorder; or
- (iii) a neuro-oncology related target, e.g., an antigen associated with a neuro-oncological disorder, e.g., HER2, or EGFR (e.g., EGFRvIII).

279. The AAV particle of embodiment 275, wherein the enzyme comprises a meganuclease, a zinc finger nuclease, a TALEN, a recombinase, integrase, a base editor, a Cas9, or a fragment thereof.

280. The AAV particle of embodiment 275, wherein the component of a gene editing system comprises one or more components of a CRISPR-Cas system.

281. The AAV particle of embodiment 275 or 280, wherein the one or more components of the CRISPR-Cas system comprises a Cas9, e.g., a Cas9 ortholog or a Cpf1, and a single guide RNA (sgRNA), optionally wherein:

- (i) the sgRNA is located upstream (5') of the cas9 enzyme; or
- (ii) the sgRNA is located downstream (3') of the cas9 enzyme.

282. The AAV particle of embodiment 275, wherein the RNAi agent (e.g., a dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA), modulates, e.g., inhibits, expression of, a CNS related gene, mRNA, and/or protein.

283. The AAV particle of embodiment 282, wherein the CNS related gene is chosen from SOD1, MAPT, APOE, HTT, C9ORF72, TDP-43, APP, BACE, SNCA, ATXN1, ATXN3, ATXN7, SCN1A-SCN5A, SCN8A-SCN11A, or a combination thereof.

284. The AAV particle of any one of embodiments 273-283, which comprises a viral genome comprising a promoter operably linked to the nucleic acid sequence encoding the payload.

285. The AAV particle of embodiment 284, wherein the promoter is chosen from human elongation factor 1 α -subunit (EF1 α), cytomegalovirus (CMV) immediate-early enhancer and/or promoter, chicken β -actin (CBA) and its derivative CAG, β glucuronidase (GUSB), or ubiquitin C (UBC), neuron-specific enolase (NSE), platelet-derived growth factor (PDGF), platelet-derived growth factor B-chain (PDGF- β), intercellular adhesion molecule 2 (ICAM-2), synapsin (Syn), methyl-CpG binding protein 2 (MeCP2), Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), metabotropic glutamate receptor 2 (mGluR2), neurofilament light (NFL) or heavy (NFH), β -globin minigene n β 2, preproenkephalin (PPE), enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2), glial

fibrillary acidic protein (GFAP), myelin basic protein (MBP), 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 fragment, e.g., a truncation, or a functional variant thereof.

286. The AAV particle of embodiment 284 or 285, wherein the promoter is an EF-1a promoter variant, e.g., a truncated EF-1a promoter.

287. The AAV particle of any one of embodiments 284-286, wherein the promoter comprises the nucleotide sequence of any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998, 999-1007, or any one of the nucleotide sequence provided in Table 8; a nucleotide sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998, 999-1007, or any one of the nucleotide sequence provided in Table 8; or a nucleotide sequence with at least 80% (e.g., 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998, 999-1007, or any one of the nucleotide sequence provided in Table 8.

288. The AAV particle of any one of embodiments 284-287 wherein the viral genome further comprises a polyA signal sequence.

289. The AAV particle of any one of embodiments 284-288, wherein the viral genome further comprises an inverted terminal repeat (ITR) sequence.

290. The AAV particle of any one of embodiments 284-289, wherein the viral genome comprises an ITR sequence positioned 5' relative to the encoded payload.

291. The AAV particle of any one of embodiments 284-290, wherein the viral genome comprises an ITR sequence positioned 3' relative to the encoded payload.

292. The AAV particle of any one of embodiments 284-291, wherein the viral genome comprises an ITR sequence positioned 5' relative to the encoded payload and an ITR sequence positioned 3' relative to the encoded payload.

293. The AAV particle of any one of embodiments 284-292, wherein the viral genome further comprises an enhancer, a Kozak sequence, an intron region, and/or an exon region.

294. The AAV particle of any one of embodiments 284-293, wherein the viral genome further comprises a nucleotide sequence encoding a miR binding site, e.g., a miR binding site that modulates,

e.g., reduces, expression of the antibody molecule encoded by the viral genome in a cell or tissue where the corresponding miRNA is expressed.

295. The AAV particle of embodiment 294, wherein the encoded miRNA binding site is complementary, e.g., fully complementary or partially complementary, to a miRNA expressed in a cell or tissue of the DRG, liver, heart, hematopoietic, or a combination thereof.

296. The AAV particle of embodiment 294 or 295, wherein the encoded miR binding site modulates, e.g., reduces, expression of the encoded antibody molecule in a cell or tissue of the DRG, liver, heart, hematopoietic lineage, or a combination thereof.

297. The AAV particle of any one of embodiments 284-296, wherein the viral genome comprises at least 1-5 copies of the encoded miR binding site, e.g., at least 1, 2, 3, 4, or 5 copies.

298. The AAV particle of any one of embodiments 284-297, wherein the viral genome comprises at least 3 copies of an encoded miR binding sites, optionally wherein all three copies comprise the same miR binding site, or at least one, two, three, or all of the copies comprise a different miR binding site.

299. The AAV particle of embodiment 298, wherein the 3 copies of the encoded miR binding sites are continuous (e.g., not separated by a spacer), or are separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to GATAGTTA.

300. The AAV particle of any one of embodiments 284-299, wherein the viral genome comprises at least 4 copies of an encoded miR binding site, optionally wherein all four copies comprise the same miR binding site, or at least one, two, three, or all of the copies comprise a different miR binding site.

301. The AAV particle of embodiment 300, wherein the 4 copies of the encoded miR binding sites are continuous (e.g., not separated by a spacer), or are separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, but no more than four modifications, e.g., substitutions, relative to GATAGTTA.

302. The AAV particle of any one of embodiments 294-301, wherein the encoded miR binding site comprises a miR122 binding site, a miR183 binding site, a miR1 binding site, a miR142-3p, or a combination thereof, optionally wherein:

(i) the encoded miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4673;

(ii) the encoded miR183 binding site comprises the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4676;

(iii) the encoded miR1 binding site comprises the nucleotide sequence of SEQ ID NO: 4679, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4679; and/or

(iv) the encoded miR142-3p binding site comprises the nucleotide sequence of SEQ ID NO: 4675, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4675.

303. The AAV particle of any one of embodiments 284-2302, wherein the viral genome comprises an encoded miR122 binding site.

304. The AAV particle of any one of embodiments 284-303, wherein the viral genome comprises at least 1-5 copies, e.g., 1, 2, or 3 copies of a miR122 binding site, optionally wherein each copy is continuous (e.g., not separated by a spacer), or each copy is separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, but no more than four modifications, e.g., substitutions, relative to GATAGTTA.

305. The AAV particle of embodiment 303 or 304, wherein the encoded miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4673.

306. The AAV particle of any one of embodiments 284-305, wherein the viral genome comprises:

(A) (i) a first encoded miR122 binding site comprising the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4673;

(ii) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, but no more than four modifications, e.g., substitutions, relative to GATAGTTA; and

(iii) a second encoded miR122 binding site comprising the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4673; or

(B) (i) a first encoded miR122 binding site comprising the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4673;

(ii) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, but no more than four modifications, e.g., substitutions, relative to GATAGTTA;

(iii) a second encoded miR122 binding site comprising the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4673;

(iv) a second spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, but no more than four modifications, e.g., substitutions, relative to GATAGTTA; and

(v) a third encoded miR122 binding site comprising the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4673.

307. The AAV particle of any one of embodiments 284-306, wherein the viral genome comprises an encoded miR183 binding site.

308. The AAV particle of any one of embodiments 284-307, wherein the viral genome comprises at least 1-5 copies, e.g., 1, 2, or 3 copies of a miR183 binding site, optionally wherein each copy is continuous (e.g., not separated by a spacer), or each copy is separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, but no more than four modifications, e.g., substitutions, relative to GATAGTTA.

309. The AAV particle of embodiment 307 or 308, wherein the encoded miR183 binding site comprises the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4673.

310. The AAV particle of any one of embodiments 284-309, wherein the viral genome comprises:

(A) (i) a first encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4676;

(ii) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, but no more than four modifications, e.g., substitutions, relative to GATAGTTA; and

(iii) a second encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4676; or

(B) (i) a first encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4676;

(ii) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, but no more than four modifications, e.g., substitutions, relative to GATAGTTA;

(iii) a second encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4676;

(iv) a second spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, but no more than four modifications, e.g., substitutions, relative to GATAGTTA; and

(v) a third encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, but no more than ten modifications, e.g., substitutions, relative to SEQ ID NO: 4676.

311. The AAV particle of any one of embodiments 284-310, wherein the viral genome comprises an encoded miR122 binding site and a miR1 binding site.

312. The AAV particle of any one of embodiments 284-311, wherein the viral genome is single stranded or self-complementary.

313. The AAV particle of any one of embodiments 284-312, 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, Rep52 protein, and/or a Rep40 protein (e.g., a Rep78 and a Rep52 protein).

314. The AAV particle of any one of embodiments 284-313, 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, Rep52 protein, and/or a Rep40 protein (e.g., a Rep78 and a Rep52 protein).

315. The AAV particle of embodiment 313 or 314, wherein the Rep78 protein, the Rep68 protein, the Rep52 protein, and/or the Rep40 protein are encoded by at least one Rep gene.

316. The AAV particle of any one of embodiments 284-315, wherein the viral genome further comprises a nucleic acid sequence encoding the AAV capsid variant of any one of embodiments 1-212, 222, or 228, an AAV capsid variant comprising the peptide of any one of embodiments 217-221 or 228, or an AAV capsid variant encoded by the polynucleotide of any one of embodiments 213-216 or 223-228.

317. The AAV particle of any one of embodiments 273-316, which is isolated, e.g., recombinant.

318. A vector comprising a polynucleotide encoding the AAV capsid variant of any one of embodiments 1-233, 266, or 272, the polynucleotide of any one of embodiments 234-237 or 267-272, or a polynucleotide encoding the peptide of any one of embodiments 238-243 or 272.

319. A cell, e.g., a host cell, comprising the AAV capsid variant of any one of embodiments 1-233, 266, or 272, the polynucleotide of any one of embodiments 234-237 or 267-272, the peptide of any one of embodiments 238-243 or 272, the AAV particle of any one of embodiments 273-317, or the vector of embodiment 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 a cell of the sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, cerebellum.

322. A method of making an AAV particle, comprising

(i) providing a host cell comprising a viral genome; and

(ii) incubating the host cell under conditions suitable to enclose the viral genome in the AAV capsid variant of any one of embodiments 1-233, 266, or 272, an AAV capsid variant comprising the peptide of any one of embodiments 238-243 or 272, or an AAV capsid variant encoded by the polynucleotide of any one of embodiments 234-237 or 267-272;

thereby making the AAV particle.

323. The method of embodiment 322, further comprising, prior to step (i), introducing a first nucleic acid molecule comprising the viral genome into the host cell.

324. The method of embodiment 323, wherein the host cell comprises a second nucleic acid encoding the capsid variant.

325. The method of embodiment 324, wherein the second nucleic acid molecule is introduced into the host cell prior to, concurrently with, or after the first nucleic acid molecule.

326. A pharmaceutical composition comprising the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, an AAV particle comprising the peptide of any one of embodiments 238-243 or 272, and a pharmaceutically acceptable excipient.

327. A method of delivering a payload 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, the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, or an AAV particle comprising the peptide of any one of embodiments 238-243 or 272.

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, e.g., a monogenic disorder or a polygenic disorder.

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

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

334. The method of embodiment 330 or 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, e.g., a monogenic disorder or a polygenic disorder, comprising administering to the subject an effective

amount of the pharmaceutical composition of embodiment 326, the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, or an AAV particle comprising the peptide of any one of embodiments 238-243 or 272.

336. A method of treating a subject having or diagnosed with having a neurological disorder, e.g., a neurodegenerative disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 326, the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, or an AAV particle comprising the peptide of any one of embodiments 238-243 or 272.

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, the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, or an AAV particle comprising the peptide of any one of embodiments 238-243 or 272.

338. A method of treating a subject having or diagnosed with having a neuro-oncological disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 326, the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, or an AAV particle comprising the peptide of any one of embodiments 238-243 or 272.

339. The method of any one of embodiments 287-294, wherein the genetic disorder, neurological disorder, neurodegenerative disorder, muscular disorder, neuromuscular disorder, or neuro-oncological disorder is Huntington's Disease, Amyotrophic Lateral Sclerosis (ALS), Gaucher Disease, Dementia with Lewy Bodies, Parkinson's disease, Spinal Muscular Atrophy, Alzheimer's Disease, a leukodystrophy (e.g., Alexander disease, autosomal dominant leukodystrophy with autonomic diseases (ADLD), Canavan disease, cerebrotendinous xanthomatosis (CTX), metachromatic leukodystrophy (MLD), Pelizaeus-Merzbacher disease, or Refsum disease), or a cancer (e.g., a HER2/neu positive cancer or a glioblastoma).

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

341. The method of embodiment 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 a decreased presence, level, and/or activity of a 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 presence, level, and/or activity of a gene, mRNA, protein, or a combination thereof.

349. The pharmaceutical composition of embodiment 326, the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, or an AAV particle comprising the peptide of any one of embodiments 238-243 or 272, for use in a method of delivering a payload to a cell or tissue.

350. The pharmaceutical composition of embodiment 326, the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, or an AAV particle comprising the peptide of any one of embodiments 238-243 or 272, for use in a method of treating a genetic disorder, a neurological disorder, a neurodegenerative disorder, a muscular disorder, a neuromuscular disorder, or a neuro-oncological disorder.

351. The pharmaceutical composition of embodiment 326, the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, or an AAV particle comprising the peptide of any one of embodiments 238-243 or 272, for use in the manufacture of a medicament.

352. Use of the pharmaceutical composition of embodiment 326, the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, or an AAV particle comprising the peptide of any one of embodiments 238-243 or 272, in the manufacture of a medicament.

353. Use of the pharmaceutical composition of embodiment 326, the AAV particle of any one of embodiments 273-317, an AAV particle comprising the capsid variant of any one of embodiments 1-233, 266, or 272, or an AAV particle comprising the peptide of any one of embodiments 238-243 or 272, in the manufacture of a medicament for treating a genetic disorder, a neurological disorder, a neurodegenerative disorder, a muscular disorder, a neuromuscular disorder, or a neuro-oncological disorder.

[0039] The details of one or more embodiments of the disclosure are set forth in the accompanying description below. Other features, objects and advantages of the disclosure will be apparent from the description. In the description, the singular forms also include the plural unless the context clearly dictates otherwise. Certain terms are defined in the Definition section and throughout.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0040] Described herein, *inter alia*, are compositions comprising an AAV capsid variant, e.g., an AAV capsid variant described herein, and methods of making and using the same. Generally, the AAV capsid variant has enhanced tropism for a cell or tissue, e.g., for the delivery of a payload to said cell or tissue, for example a CNS tissue or a CNS cell or a liver cell or liver tissue.

[0041] As demonstrated in the Examples herein below, certain AAV capsid variants described herein show multiple advantages over wild-type AAV9, including (i) increased penetrance through the blood brain barrier following intravenous administration, (ii) wider distribution throughout the multiple brain regions, e.g., frontal cortex, sensory cortex, motor cortex, putamen, thalamus, cerebellar cortex, dentate nucleus, caudate, and/or hippocampus, and/or (iii) elevated payload expression in multiple brain regions. Without wishing to be bound by theory, it is believed that these advantages may be due, in part, to the dissemination of the AAV capsid variants through the brain vasculature. In some embodiments, the AAV capsids described herein enhance the delivery of a payload to multiple regions of the brain including for example, the frontal cortex, sensory cortex, motor cortex, putamen, thalamus, cerebellar cortex, dentate nucleus, caudate, and/or hippocampus.

[0042] Several approaches have been used previously to produce AAV capsids with enhanced tropism for a cell or tissue, e.g., a CNS cell or tissue. One approach used co-infection of cultured cells (Grimm et al. In vitro and in vivo gene therapy vector evolution via multispecies interbreeding and retargeting of adeno-associated viruses. J. Virol. 2008 June 82(12):5887–5911, the contents of which are herein incorporated by reference in its entirety) or *in situ* animal tissue (Lisowski et al. Selection and evaluation of clinically relevant AAV variants in a xenograft liver model. Nature 2014 506:382–386, the contents of which are herein incorporated by reference in its entirety) with adenovirus, in order to trigger exponential replication of infectious AAV DNA. Another approach involved the use of cell-specific CRE transgenic mice (Deverman et al. Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain. Nat Biotechnol. 2016 Feb. 34(2)204–209; the contents of which are herein incorporated by reference in its entirety) allowing viral DNA recombination specifically in astrocytes, followed by recovery of CRE-recombined capsid variants. Other approaches apply high throughput DNA synthesis, multiplexing, sequencing technologies, and machine learning to evaluate sequencing reads of viral DNA in different tissues to engineer variant capsids. These approaches are different from the approach disclosed herein.

[0043] There are some limitations to the art-known capsid generation methods. For example, the transgenic CRE system used by Deverman et al. (2016) has limited tractability in other animal species and AAV variants selected by directed evolution in mouse tissue do not show similar properties in large animals. Previously described transduction-specific approaches are not amenable to large animal studies because: 1) many tissues of interest (e.g., CNS) are not readily accessible to adenovirus co-infection, 2) the specific adenovirus tropism itself would bias the library distribution, and 3) large animals are typically not amenable to transgenesis or genetic engineering to express CRE recombinase in defined cell types.

[0044] To address these limitations, a broadly-applicable functional AAV capsid library screening platform for cell type-specific biopanning in non-transgenic animals has been developed and is described in the appended Examples. In the TRACER (Tropism Redirection of AAV by Cell type-specific Expression of RNA) platform system, the capsid gene is placed under the control of a cell type-specific promoter to drive capsid mRNA expression in the absence of helper virus co-infection. Without wishing to be bound by theory, it is believed that this RNA-driven screen increases the selective pressure in favor of capsid variants which transduce a specific cell type. The TRACER platform allows for generation of AAV capsid libraries whereby specific recovery and subcloning of capsid mRNA expressed in transduced cells is achieved with no need for transgenic animals or helper virus co-infection. Without wishing to be bound by theory, it is believed that since mRNA transcription is a hallmark of full transduction, the methods disclosed herein allow identification of fully infectious AAV capsid mutants, and in addition to its higher stringency, this method allows identification of capsids with high tropism for particular cell types using libraries designed to express

CAP mRNA under the control of any cell-specific promoter such as, but not limited to, synapsin-1 promoter (neurons), GFAP promoter (astrocytes), TBG promoter (liver), CAMK promoter (skeletal muscle), MYH6 promoter (cardiomyocytes). Described herein are novel AAV capsid variants generated using the TRACER method which demonstrate enhance tropism in for example a CNS cell, a CNS tissue, a liver cell, a liver tissue, a muscle cell, or a muscle tissue.

[0045] In some embodiments, an AAV capsid variant disclosed herein comprises a modification in loop VIII of AAV9, e.g., at positions between 580-599, e.g., at one, two, three, four, five or all of positions 584, 586, 587, 588, 589, and/or 590, numbered relative to SEQ ID NO: 138 or 981. In some embodiments, 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 positions 580-599 (e.g., amino acids VATNHQSAQAQAQTGWVQNQ (SEQ ID NO: 5122)), numbered according to SEQ ID NO: 138. In some embodiments loop VIII comprises positions 582-593 (e.g., amino acids TNHQSAQAQAQT (SEQ ID NO: 5123)), numbered according to SEQ ID NO: 138. In some embodiments loop VIII comprises positions 587-593 (e.g., amino acids AQAQAQT (SEQ ID NO: 4687)), numbered according to SEQ ID NO: 138. In some embodiments loop VIII comprises positions 587-590 (e.g., amino acids AQAQ (SEQ ID NO: 5099)), numbered according to 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 positions 581-593, numbered according to SEQ ID NO: 138.

[0046] 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 of the disclosure 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 of the disclosure may, in addition, or alternatively, have decreased tropism for a cell-type, tissue or organ.

[0047] In some embodiments, an AAV comprises a small non-enveloped icosahedral capsid virus of the Parvoviridae family and is characterized by a single stranded DNA viral genome. Parvoviridae family viruses consist of two subfamilies: Parvovirinae, which infect vertebrates, and Densovirinae, which infect invertebrates. The Parvoviridae family comprises the Dependovirus genus which includes AAV, capable of replication in vertebrate hosts including, but not limited to, human, primate, bovine, canine, equine, and ovine species.

[0048] The parvoviruses and other members of the Parvoviridae family are generally described in Kenneth I. Berns, "Parvoviridae: The Viruses and Their Replication," Chapter 69 in *FIELDS VIROLOGY* (3d Ed. 1996), the contents of which are incorporated by reference in their entirety.

[0049] In some embodiments, AAV 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.

[0050] In some embodiments, the AAV, is a naturally occurring (e.g., wild-type) AAV or a recombinant AAV. In some embodiments, the wild-type AAV vector 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 typically comprises two ITR sequences. These 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. 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.

[0051] In some embodiments, the wild-type AAV viral genome further 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 used for replication and packaging, while the capsid proteins are assembled to create the protein shell of the AAV, or AAV capsid polypeptide, e.g., an AAV capsid variant. 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, for any one of the amino acid sequences 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 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 protein. While not wishing to be bound by theory, the AAV capsid protein typically comprises a molar ratio of 1:1:10 of VP1:VP2:VP3.

[0052] AAV vectors of the present disclosure may be produced recombinantly and may be based on adeno-associated virus (AAV) reference sequences. In addition to single stranded AAV viral genomes (e.g., ssAAVs), the present disclosure also provides for self-complementary AAV (scAAVs) viral genomes. scAAV vector genomes contain DNA strands which 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.

[0053] Methods for producing and/or modifying AAV particles are disclosed in the art such as pseudotyped AAV vectors (PCT Patent Publication Nos. WO200028004; WO200123001; WO2004112727; WO2005005610; and WO2005072364, the content of each of which is incorporated herein by reference in its entirety).

[0054] 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.

Peptides

[0055] Disclosed herein are peptides, and associated AAV particles comprising an AAV capsid variant and a peptide for enhanced or improved transduction of a target tissue (e.g., cells of the CNS or PNS). In some, embodiments, the peptide is an isolated, e.g., recombinant, peptide. In some embodiments, the nucleic acid encoding the peptide, is an isolated, e.g., recombinant nucleic acid.

[0056] In some embodiments, the peptide 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, the cortex (e.g., frontal, parietal, occipital, temporal), thalamus, hypothalamus, striatum, putamen, caudate nucleus, hippocampus, entorhinal cortex, basal ganglia, or deep cerebellar nuclei. In some embodiments, the tissue of the CNS is a sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, cerebellum, or a combination thereof.

[0057] In some embodiments, the peptide may increase distribution of an AAV particle to a cell, region, or tissue of the PNS. The cell or tissue of the PNS may be, but is not limited to, a dorsal root ganglion (DRG).

[0058] In some embodiments, the peptide may increase distribution of an AAV particle to the CNS (e.g., sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, and/or cerebellum) after intravenous administration. In some embodiments, the peptide may increase distribution of an AAV particle to the CNS (e.g., the sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, and/or cerebellum) following focused ultrasound (FUS), e.g., coupled with the

intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration.

[0059] In some embodiments, the peptide may increase distribution of an AAV particle to the PNS (e.g., DRG) after intravenous administration. In some embodiments, the peptide may increase distribution of an AAV particle to the PNS (e.g., DRG) following focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration.

[0060] A peptide may vary in length. In some embodiments, the peptide is about 3 to about 20 amino acids in length. As non-limiting examples, the peptide may be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 3-5, 3-8, 3-10, 3-12, 3-15, 3-18, 3-20, 5-10, 5-15, 5-20, 10-12, 10-15, 10-20, 12-20, or 15-20 amino acids in length. In some embodiments, a peptide comprises about 10 to 16 amino acids in length, e.g., about 15 amino acids in length. In some embodiments, a peptide comprises about 5 to 10 amino acids in length, e.g., about 7 amino acids in length.

[0061] In some embodiments a peptide may comprise a sequence as set forth in Table 1. In some embodiments a peptide may comprise a sequence as set forth in Table 2A. In some embodiments, a peptide may comprise a sequence as set forth in Table 2B (e.g., a sequence of any 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, or 336). In some embodiments, the peptide may comprise a sequence set forth in Table 9. In some embodiments, the peptide may comprise a sequence as set forth in Table 14. In some embodiments, the peptide may comprise a sequence as set forth in Table 15. In some embodiments, the peptide may comprise a sequence as set forth in Table 16. In some embodiments, the peptide is isolated, e.g., recombinant.

Table 1. Exemplary Peptide Sequences

Peptide Sequence	SEQ ID NO:	Peptide Sequence	SEQ ID NO:	Peptide Sequence	SEQ ID NO:	Peptide Sequence	SEQ ID NO:
TNTQDWHRIAQ	343	TTRQDWHRIAQ	392	KNNQDWHRIAQ	441	RNTQDWHRISQ	490
TNTKDWHRIAQ	344	TNTQDWHRVAN	393	TNNQDWHRIAR	442	TNQDQDWHRIAQ	491
SNNQDWHRIAQ	345	TNTQDWHRISG	394	SNTQDWHRVPQ	443	RNIQDWHRIAQ	492
TNKQDWHRIAQ	346	TNSQDWHRIAQ	395	TNNQDWHRIAK	444	TTTQDWHRISQ	493
TNNQDWHRIAQ	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
TNPQDWHRIAQ	357	INTKDWHRIAQ	406	TNNQDWHRTAQ	455	INTQDWHRIAQ	504
TNKQDWHRIAQ	358	TNAKDWHRIAQ	407	SNSQDWHRIAQ	456	RNTQDWHRIAQ	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	TNSQDWHRVPQ	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
TNTQDWHRIAQ	367	ANTKDWHRIAQ	416	TNNQDWHRSAQ	465	TNTQDWHRVTM	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
TNTQDWHRVPQ	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
TNKSDWHRIAQ	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 Peptide 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 Peptide Sequences

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

TTVQDWHRIAQTGWV	219	TNIQDWHRVAQTGWV	283		
-----------------	-----	-----------------	-----	--	--

[0062] In some embodiments, a peptide described herein comprises 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 positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q, K, E, S, P, R, N, H, or a conservative substitution thereof and/or [N3] comprises positions X₅, X₆, and X₇, wherein position X₅ is I, V, T, M, S, N, L, F, or a conservative substitution thereof. In some embodiments, position X₄ of [N1] is Q. In some embodiments, position X₄ of [N1] is K. In some embodiments, position X₅ of [N3] is I. In some embodiments, position X₁ of [N1] is T, S, R, A, I, C, N, K, L, or Q. In some embodiments, position X₂ of [N1] is N, T, G, V, S, Y, K, I, H, D, or F. In some embodiments, position 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, position X₆ of [N3] is A, Y, P, N, S, T, G, E, V, W, F, or Q. In some embodiments, position 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). TNTKDWHRIAQ (SEQ ID NO: 344). In some embodiments, the peptide further comprises [N4], wherein [N4] comprises positions X₈, X₉, X₁₀, and X₁₁, wherein position X₈ is T, S, N, P, A, or I; position X₉ is G, N, D, R, V, A, S, or Q; position X₁₀ is W, S, C, R, L, or G; and/or position X₁₁ is V, A, S, I, C, G, D, F, L, or T. In some embodiments, [N4] is or comprises TGVV (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, or 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).

[0063] In some embodiments, a peptide described herein a peptide described herein comprises 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 positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q, P, or a conservative substitution thereof; and/or [N3] comprises positions X₅, X₆, and X₇, wherein position X₅ is I, V, or a conservative substitution thereof. In some embodiments, position X₄ of [N1] is Q. In some embodiments, position X₅ of [N3] is I. In some embodiments, position X₅ of [N3] is V. In some embodiments, position X₁ of

[N1] is T or S. In some embodiments, position X₂ of [N1] is N, T, G, S, I, or V. In some embodiments, position 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, position X₆ of [N3] is A, P, S, Y, or N. In some embodiments, position 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 peptide further comprises [N4], wherein [N4] comprises positions X₈, X₉, X₁₀, and X₁₁, wherein position X₁₀ is W. In some embodiments, position X₈ of [N4] is T, S, or N. In some embodiments, position X₉ of [N4] is G, or N. In some embodiments, position X₁₁ of [N4] is V, A, I or S. In some embodiments, [N4] is or comprises TGVV (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, or 336. In some embodiments, [N1]-[N2]-[N3]-[N4] is or comprises TNTQDWHRIAQTGVV (SEQ ID NO: 201).

[0064] In some embodiments, [N1] is present immediately subsequent to [N2]. In some embodiments, [N3] is present immediately subsequent to [N2]. In some embodiments, [N4] is present immediately subsequent to [N3]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [N1]-[N2]-[N3]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [N1]-[N2]-[N3]-[N4].

[0065] In some embodiments, the peptide comprises an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the peptide comprises an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 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, or 336.

[0066] In some embodiments, the 3 consecutive amino acids comprise TQD. In some embodiments, the 4 consecutive amino acids comprise TQDW (SEQ ID NO: 4684). In some embodiments, the 5 consecutive amino acids comprise TQDWH (SEQ ID NO: 4685). In some embodiments, the 6 consecutive amino acids comprise TQDWHR (SEQ ID NO: 4686). In some embodiments, the 7 consecutive amino acids comprise TQDWHRI (SEQ ID NO: 941).

[0067] In some embodiments, 3 consecutive amino acids comprise TNT. In some embodiments, the 4 consecutive amino acids comprise TNTQ (SEQ ID NO: 4688). In some embodiments, the 5 consecutive amino acids comprise TNTQD (SEQ ID NO: 5119). In some embodiments, the 6 consecutive amino acids comprise TNTQDW (SEQ ID NO: 5120). In some embodiments, the 7 consecutive amino acids comprise TNTQDWH (SEQ ID NO: 5121). In some embodiments, the 8

consecutive amino acids comprise TNTQDWHR (SEQ ID NO: 4898). In some embodiments, the 9 consecutive amino acids comprise TNTQDWHRI (SEQ ID NO: 746).

[0068] In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, 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, or 336. In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, 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, or 336.

[0069] In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941). In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941).

[0070] In some embodiments, the peptide comprises the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the peptide comprises the amino acid sequence of any 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, or 336.

[0071] In some embodiments, the peptide comprises an amino acid sequence encoded by a nucleotide sequence described herein, e.g., a nucleotide sequence of Table 2A. In some embodiments, the peptide comprises an amino acid sequence encoded by a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 942. In some embodiments, the peptide comprises an amino acid sequence encoded by a nucleotide sequence comprising at least one, two, three, four, five, six, or seven, but no more than ten different nucleotides, relative to the nucleotide sequence of SEQ ID NO: 942. In some embodiments, the peptide comprises 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%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto.

[0072] In some embodiments, the nucleotide sequence encoding a peptide described herein comprises a nucleotide sequence described herein, e.g., as described in Table 2A. In some embodiments, the nucleotide sequence encoding a peptide described herein is codon optimized. In some embodiments, the nucleotide sequence encoding a peptide described herein is isolated, e.g., recombinant.

[0073] In some embodiments the nucleotide sequence encoding a peptide described herein comprises the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 942. In some embodiments, the nucleotide sequence encoding a peptide described herein comprises a nucleotide sequence comprising at least one, two, three, four, five, six, or seven, but no more than ten different nucleotides, relative to the nucleotide sequence of SEQ ID NO: 942. In some embodiments the nucleic acid sequence encoding a peptide described herein comprises a nucleotide sequence comprising the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto.

[0074] In some embodiments, a peptide described herein is fused or coupled, e.g., conjugated, to an active agent. In some embodiments, the active agent is a therapeutic agent. In some embodiments, the active agent comprises a therapeutic protein, an antibody molecule, an enzyme, one or more components of a genome editing system, an Fc polypeptide fused or coupled (e.g., covalently or non covalently) to a therapeutic agent, and/or an RNAi agent (e.g., a dsRNA, antisense oligonucleotide (ASO), siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA). In some embodiments, the therapeutic agent is an antibody. In some embodiments, a peptide described herein is fused or coupled, e.g., conjugated (e.g., directly or indirectly) to the Fc region of the antibody, e.g., at the C-terminus of the Fc region or the N-terminus of the Fc region. In some embodiments, the therapeutic agent is an RNAi agent. In some embodiments, the RNAi agent is a siRNA or an ASO. In some embodiments, the ASO or siRNA comprises at least one (e.g., one or more or all) modified nucleotides. In some embodiments, a peptide described herein is fused or coupled, e.g., conjugated (e.g., directly or indirectly via a linker), to at least one strand of the RNAi agent. In some embodiments, a peptide described herein is conjugated, e.g., directly or indirectly via a linker, to the C-terminus of at least one strand of the RNAi agent. In some embodiments, a peptide described herein is conjugated, e.g., directly or indirectly via a linker, to an internal nucleotide of at least one strand of the RNAi agent. In some embodiments, the at least one strand is the sense strand.

In some embodiments, the therapeutic agent modulates, e.g., inhibits, decreases, or increases, expression of a CNS related gene, mRNA, and/or protein.

[0075] In some embodiments, the active agent is a diagnostic agent. In some embodiments, the diagnostic agent is or comprises an imaging agent (e.g., a protein or small molecule compound coupled to a detectable moiety). In some embodiments, the imaging agent comprises a PET or MRI ligand, or an antibody molecule coupled to a detectable moiety. In some embodiments, the detectable moiety is or comprises a radiolabel, a fluorophore, a chromophore, or an affinity tag. In some embodiments, the radiolabel is or comprises tc99m, iodine-123, a spin label, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen- 15, oxygen-17, gadolinium, manganese, or iron. In some embodiments, the active agent is a small molecule. In some embodiments, the active agent is a ribonucleic acid complex (e.g., a Cas9/gRNA complex), a plasmid, a closed-end DNA, a circ-RNA, or an mRNA.

[0076] In some embodiments, at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides are fused or coupled, e.g., conjugated, to an active agent, e.g., a therapeutic agent or a diagnostic agent. In some embodiments, the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides comprise the same amino acid sequence. In some embodiments, the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides comprise different amino acid sequences. In some embodiments, the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides are present in tandem (e.g., connected directly or indirectly via a linker) or in a multimeric configuration. In some embodiments, the peptide comprises an amino acid sequence of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, 25, 30, or 35 amino acids in length.

[0077] In some embodiments, the peptide covalently linked, e.g., directly or indirectly via a linker, to the active agent. In some embodiments, the peptide is conjugated to the active agent via a linker. In some embodiments, the linker is a cleavable linker or a non-cleavable linker. In some embodiments, the cleavable linker is a pH sensitive linker or an enzyme sensitive linker. In some embodiments, the pH sensitive linker comprises a hydrazine/hydrazone linker or a disulfide linker. In some embodiments, the enzyme sensitive linker comprises a peptide based linker, e.g., a peptide linker sensitive to a protease (e.g., a lysosomal protease); or a beta-glucuronide linker. In some embodiments, the non-cleavable linker is a linker comprising a thioether group or a maleimidocaproyl group. In some embodiments, the peptide and the active agent are fused or coupled post-translationally, e.g., using click chemistry. In some embodiments, the peptide and the active agent are fused or couple via chemically induced dimerization. In some embodiments, the peptide is present N-terminal relative to the active agent. In some embodiments, the peptide is present C-terminal relative to the active agent.

[0078] In some embodiments, the peptide is present or coupled to a carrier. In some embodiments, the carrier comprises an exosome, a microvesicle, or a lipid nanoparticle (LNP). In some embodiments, the carrier comprises a therapeutic agent (e.g., an RNAi agent (e.g., an dsRNA, a

siRNA, a shRNA, a pre-miRNA, a pri-miRNA, a miRNA, a stRNA, a lncRNA, a piRNA, an antisense oligonucleotide agent (ASO), or a snoRNA), an mRNA, a ribonucleoprotein complex (e.g., a Cas9/gRNA complex), or a circRNA). In some embodiments, the peptide is present on the surface of the carrier. In some embodiments, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the surface of the carrier comprises at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides described herein.

[0079] The present disclosure also provides a nucleic acid or polynucleotide encoding any of the peptides described herein and AAV capsid variants, AAV particles, vectors, and cells comprising the same.

AAV Capsid Variant

[0080] In some embodiments, an AAV particle described herein comprises an AAV capsid variant, e.g., an AAV capsid variant described herein (e.g., an AAV capsid variant comprising a peptide described herein). In some embodiments, an AAV capsid variant comprises a peptide as set forth in any of Tables 1, 2A, 2B, 9, 14, 15, or 16.

[0081] In some embodiments, an AAV capsid variant described herein comprises 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 positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q, K, E, S, P, R, N, H, or a conservative substitution thereof and/or [N3] comprises positions X₅, X₆, and X₇, wherein position X₅ is I, V, T, M, S, N, L, F, or a conservative substitution thereof. In some embodiments, position X₄ of [N1] is Q. In some embodiments, position X₄ of [N1] is K. In some embodiments, position X₅ of [N3] is I. In some embodiments, position X₁ of [N1] is T, S, R, A, I, C, N, K, L, or Q. In some embodiments, position X₂ of [N1] is N, T, G, V, S, Y, K, I, H, D, or F. In some embodiments, position 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, position X₆ of [N3] is A, Y, P, N, S, T, G, E, V, W, F, or Q. In some embodiments, position 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). TNTKDWHRIAQ (SEQ ID NO: 344). In some embodiments, the AAV capsid variant further comprises [N4], wherein [N4] comprises positions X₈, X₉, X₁₀, and X₁₁, wherein position X₈ is T, S, N, P, A, or I; position X₉ is G, N, D, R, V, A, S, or Q; position X₁₀ is W, S, C, R, L, or G; and/or position 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, or 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).

[0082] In some embodiments, an AAV capsid variant described herein a peptide described herein comprises 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 positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q, P, or a conservative substitution thereof; and/or [N3] comprises positions X₅, X₆, and X₇, wherein position X₅ is I, V, or a conservative substitution thereof. In some embodiments, position X₄ of [N1] is Q. In some embodiments, position X₅ of [N3] is I. In some embodiments, position X₅ of [N3] is V. In some embodiments, position X₁ of [N1] is T or S. In some embodiments, position X₂ of [N1] is N, T, G, S, I, or V. In some embodiments, position 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, position X₆ of [N3] is A, P, S, Y, or N. In some embodiments, position 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 AAV capsid variant further comprises [N4], wherein [N4] comprises positions X₈, X₉, X₁₀, and X₁₁, wherein position X₁₀ is W. In some embodiments, position X₈ of [N4] is T, S, or N. In some embodiments, position X₉ of [N4] is G, or N. In some embodiments, position 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, or 336. In some embodiments, [N1]-[N2]-[N3]-[N4] is or comprises TNTQDWHRIAQTGWV (SEQ ID NO: 201).

[0083] 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 comprises positions 581-593, numbered according to SEQ ID NO: 138. In some embodiments, loop VIII comprises positions 580-599, numbered according to SEQ ID NO: 138.

[0084] In some embodiments, [N1] replaces positions 582-585 (e.g., T582, N583, H584, and Q585), numbered according to SEQ ID NO: 138. In some embodiments, [N1] corresponds to positions 582-585 of SEQ ID NO: 981. In some embodiments, [N1] corresponds to positions 582-585

(e.g., T582, N583, H584, Q585) of SEQ ID NO: 138. In some embodiments, [N1] is present at positions 582-585, numbered according to SEQ ID NO: 981. In some embodiments, X₁ of [N1] is present at position 582, X₂ of [N1] is present at position 583, X₃ of [N1] is present at position 584, and X₄ of [N1] is present at position 585, numbered according to SEQ ID NO: 981. In some embodiments, [N2] replaces positions 586-589 (e.g., S586, A587, Q588, and A589), numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, [N2] corresponds to positions 586-589 (e.g., D586, W587, H588, and R589) of SEQ ID NO: 981. In some embodiments, [N2] is present at positions 586-589, numbered according to SEQ ID NO: 981. In some embodiments, [N1]-[N2] replaces to positions 582-589 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589), numbered according to SEQ ID NO: 138. In some embodiments, [N1]-[N2] corresponds to positions 582-589 (e.g., T582, N583, T584, Q585, D586, W587, H588, R589) of SEQ ID NO: 981. In some embodiments, [N1]-[N2] is present at positions 582-589, numbered according to SEQ ID NO: 981. In some embodiments, [N3] replaces positions 590-592 (e.g., Q590, A591, and Q592), numbered according to SEQ ID NO: 138. In some embodiments, [N3] corresponds to positions 590-592 (e.g., I590, A591, and Q592) of SEQ ID NO: 981. In some, embodiments, [N3] is present at positions 590-592, numbered according to SEQ ID NO: 981. In some embodiments, X₅ of [N3] is present at position 590, X₆ of [N3] is present at position 591, and X₇ of [N3] is present at position 592, numbered according to SEQ ID NO: 981. In some embodiments, [N2]-[N3] replaces positions 586-592 (e.g., S586, A587, Q588, A589, Q590, A591, and Q592), numbered according to SEQ ID NO: 138. In some embodiments, [N2]-[N3] corresponds to positions 586-592 (e.g., D586, W587, H588, R589, I590, A591, and Q592) of SEQ ID NO: 981. In some embodiments, [N2]-[N3] is present at positions 586-592, numbered according to SEQ ID NO: 981. In some embodiments, [N1]-[N2]-[N3] replaces positions 582-592 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, Q590, A591, Q592), numbered according to SEQ ID NO: 138. In some embodiments, [N1]-[N2]-[N3] corresponds to positions 582-592 (e.g., T582, N583, T584, Q585, D586, W587, H588, R589, I590, A591, Q592) of SEQ ID NO: 981. In some embodiments, [N1]-[N2]-[N3] is present at positions 582-592, numbered according to SEQ ID NO: 981. In some embodiments, [N4] replaces positions 593-596 (e.g., T593, G594, W595, and V596), numbered according to SEQ ID NO: 138. In some embodiments, [N4] corresponds to positions 593-596 (e.g., T593, G594, W595, and V596) of SEQ ID NO: 138 or 981. In some embodiments, [N4] is present at positions 593-596, numbered according to SEQ ID NO: 981. In some embodiments, X₈ of [N4] is present at position 593, X₉ of [N4] is present at position 594, X₁₀ of [N4] is present at position 595, and X₁₁ of [N4] is present at position 596, numbered according to SEQ ID NO: 981. In some embodiments, [N2]-[N3]-[N4] replaces positions 586-596 (e.g., S586, A587, Q588, A589, Q590, A591, Q592, T593, G594, W595, and V596), numbered according to SEQ ID NO: 138. In some embodiments, [N2]-[N3]-[N4] corresponds to positions 586-596 (e.g., D586, W587, H588, R589, I590, A591, Q592, T593, G594, W595, and V596) of SEQ ID NO: 981. In some embodiments, [N2]-[N3]-[N4] is present at positions

586-596, numbered according to SEQ ID NO: 981. In some embodiments, [N1]-[N2]-[N3]-[N4] replaces positions 582-596 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, Q590, A591, Q592, T593, G594, W595, and V596), numbered according to SEQ ID NO: 138. In some embodiments, [N1]-[N2]-[N3]-[N4] corresponds to positions 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. In some embodiments, [N1]-[N2]-[N3]-[N4] is present at positions 582-596, numbered according to the SEQ ID NO: 981.

[0085] In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [N1]-[N2]-[N3]. In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [N1]-[N2]-[N3]-[N4].

[0086] In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 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, or 336. In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, loop VIII comprises positions 581-593, numbered according to SEQ ID NO: 138. In some embodiments, loop VIII comprises positions 580-599, numbered according to 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 positions 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), numbered according to 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 positions 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and/or 596, numbered according to SEQ ID NO: 981. In some embodiments, the AAV capsid variant comprises one or more amino acid substitutions at positions 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), numbered according to SEQ ID NO: 138.

[0087] In some embodiments, the 3 consecutive amino acids comprise TQD. In some embodiments, the 4 consecutive amino acids comprise TQDW (SEQ ID NO: 4684). In some embodiments, the 5 consecutive amino acids comprise TQDWH (SEQ ID NO: 4685). In some embodiments, the 6 consecutive amino acids comprise TQDWHR (SEQ ID NO: 4686). In some embodiments, the 7 consecutive amino acids comprise TQDWHRI (SEQ ID NO: 941).

[0088] In some embodiments, 3 consecutive amino acids comprise TNT. In some embodiments, the 4 consecutive amino acids comprise TNTQ (SEQ ID NO: 4688). In some embodiments, the 5 consecutive amino acids comprise TNTQD (SEQ ID NO: 5119). In some embodiments, the 6 consecutive amino acids comprise TNTQDW (SEQ ID NO: 5120). In some embodiments, the 7 consecutive amino acids comprise TNTQDWH (SEQ ID NO: 5121). In some embodiments, the 8 consecutive amino acids comprise TNTQDWHR (SEQ ID NO: 4898). In some embodiments, the 9 consecutive amino acids comprise TNTQDWHRI (SEQ ID NO: 746).

[0089] In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of any one of SEQ ID NOs: 945-980 or 985-986. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, 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, or 336. In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, loop VIII comprises positions 581-593, numbered according to SEQ ID NO: 138. In some embodiments, loop VIII comprises positions 580-599, numbered according to 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 positions 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), numbered according to 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 positions 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and/or 596, numbered according to SEQ ID NO: 981.

[0090] In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941). In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three, but no more than four different amino acids from the amino acid sequence of TQDWHRI (SEQ ID NO: 941).

[0091] In some embodiments, the AAV capsid variant, comprises the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the peptide comprises the amino acid sequence of any 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, or 336. In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, loop VIII comprises positions 581-593, numbered according to SEQ ID NO: 138. In some embodiments, loop VIII comprises positions 580-599, numbered according to 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 positions 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), numbered according to 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 positions 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and/or 596, numbered according to SEQ ID NO: 981. In some embodiments, the amino acid sequence is present at 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all of positions 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, and/or 596, numbered according to SEQ ID NO: 981. In some embodiments, the AAV capsid variant comprises one or more amino acid substitutions at positions 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), numbered according to SEQ ID NO: 138.

[0092] In some embodiments, the AAV capsid variant (e.g., an AAV capsid variant described herein), comprises 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%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments, the AAV capsid variant described herein, comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 942, or a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 942. In some embodiments, the AAV capsid variant comprises an amino acid sequence encoded by a nucleotide sequence comprising at least one, two, three, four, five, six, or seven, but no more than ten different nucleotides relative to the nucleotide sequence of SEQ ID NO: 942.

[0093] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein the amino acid sequence replaces positions 584-590 (e.g., H584, Q585, S586, A587, Q588, A589, Q590), numbered according to the amino acid

sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein the amino acid sequence corresponds to positions 584-590 (e.g., T584, Q585, D586, W587, H588, R589, I590) of SEQ ID NO: 981. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein the amino acid sequence is present at positions 584-590, numbered according to SEQ ID NO: 981. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein the amino acid sequence is present at positions 584-590, numbered according to SEQ ID NO: 138.

[0094] In some embodiments, the AAV capsid variant further comprises an amino acid other than A at position 581, numbered according to SEQ ID NO: 138 or 981. In some embodiments, the AAV capsid variant further comprises the amino acid T at position 581, numbered according to SEQ ID NO: 138 or 981. In some embodiments, the AAV capsid variant further comprises the amino acid V at position 581, numbered according to SEQ ID NO: 138 or 981. In some embodiments, the AAV capsid variant comprises the substitutions A581T or A581V, numbered according to SEQ ID NO: 138 or 981.

[0095] 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 position 584 (e.g., T), S at position 586 (e.g., D), A at position 587 (e.g., W), Q at position 588 (e.g., H), A at position 589 (e.g., R), and/or Q at position 590 (e.g., I), numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises an amino acid other than H at position 584 (e.g., T), S at position 586 (e.g., D), A at position 587 (e.g., W), Q at position 588 (e.g., H), A at position 589 (e.g., R), and Q at position 590 (e.g., I), numbered according to SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein comprises 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: 138 or 981.

[0096] In some embodiments, an AAV capsid variant described herein comprises one, two, three, four, five, or all of the amino acid 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, numbered according to SEQ ID NO: 138 or 981. In some embodiments, the AAV capsid variant comprises 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: 138 or 981.

[0097] 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, numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises

the substitutions H584T, S586D, A587W, Q588H, A589R, and Q590I, numbered according to SEQ ID NO: 138 or 981.

[0098] In some embodiments, an AAV capsid variant described herein comprises the amino acid Q at position 585, numbered according to SEQ ID NO: 138 or 981.

[0099] In some embodiments, an AAV capsid variant described herein comprises an amino acid other than Q at position 585, numbered according to SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein comprises the amino acid K at position 585, numbered according to SEQ ID NO: 138.

[0100] In some embodiments, an AAV capsid variant described herein comprises an amino acid other than Q at position 590, numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises the amino acid I at position 590, numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises the amino acid V at position 590, numbered according to SEQ ID NO: 138.

[0101] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein the amino acid sequence of TQDWHRI (SEQ ID NO: 941) is present in the AAV capsid variant at positions 584-590, numbered according to SEQ ID NO: 981. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of TQDWHRI (SEQ ID NO: 941), wherein the amino acid sequence of TQDWHRI (SEQ ID NO: 941) is present in the AAV capsid variant at positions 584-590, numbered according to SEQ ID NO: 138.

[0102] In some embodiments, an AAV capsid variant described herein comprises the amino acid W at position 595, numbered according to SEQ ID NO: 138 or 981.

[0103] In some embodiments, the AAV capsid variant, further comprises a substitution at position K449, e.g., a K449R substitution, numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant, further comprises an amino acid other than K at position 449 (e.g., R), numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises an R at position 449, numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a modification, e.g., an insertion, substitution, and/or deletion in loop I, II, IV, and/or VI.

[0104] In some embodiments, the AAV capsid variant, further comprises an amino acid sequence comprising at least one, two or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant, further comprises an amino acid sequence comprising at least one, two or three, but not more than 30, 20 or 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 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

[0105] In some embodiments, the AAV capsid variant further comprises (a) a VP1 protein comprising the amino acid sequence of SEQ ID NO: 138 or 981; (b) a VP2 protein comprising the amino acid sequence of positions 138-736 of SEQ ID NO: 138 or 981; (c) a VP3 protein comprising the amino acid sequence of positions 203-736 of SEQ ID NO: 138 or 981; or (d) an amino acid sequence with at least 70% (e.g., at least 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity to any of the amino acid sequences in (a)-(c), an amino acid sequence comprising at least one, two or three, but not more than 30, 20 or 10 different amino acids relative to any of the amino acid sequences in (a)-(c), or an amino acid sequence comprising at least one, two or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (a)-(c).

[0106] In some embodiments, the AAV capsid variant further comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 137, or a sequence with at least 70% (e.g., at least 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto. In some embodiments, the AAV capsid variant further comprises an amino acid sequence encoded by a nucleotide sequence comprising at least one, two or three modifications, e.g., substitutions, insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 137. In some embodiments, the AAV capsid variant further comprises an amino acid sequence encoded by a nucleotide sequence comprising at least one, two or three, but not more than 30, 20 or 10 different nucleotides, relative to the amino acid sequence of SEQ ID NO: 137.

[0107] In some embodiments, the nucleotide sequence encoding the AAV capsid variant further comprises the nucleotide sequence of SEQ ID NO: 137, or a sequence with at least 70% (e.g., at least 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto. In some embodiments, the nucleotide sequence encoding the AAV capsid variant further comprises a nucleotide sequence comprising at least one, two or three modifications, e.g., substitutions, insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 137. In some embodiments, the nucleotide sequence encoding the AAV capsid variant further comprises a nucleotide sequence comprising at least one, two or three, but not more than 30, 20 or 10 different nucleotides, relative to the amino acid sequence of SEQ ID NO: 137.

[0108] In some embodiments, an AAV capsid variant of the present disclosure comprises an amino acid sequence as described herein, e.g., an amino acid sequence of an AAV capsid variant of TTJ-001, e.g., as described in Tables 3 and 4.

[0109] In some embodiments, an AAV capsid variant described herein comprises a VP1, VP2, and/or VP3 protein comprising an amino acid sequence described herein, e.g., an amino acid sequence of an AAV capsid variant of TTJ-001, e.g., as described in Tables 3 and 4.

[0110] In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence encoded by a nucleotide sequence as described herein, e.g., a nucleotide sequence of the AAV capsid variant of TTJ-001, e.g., as described in Tables 3 and 5.

[0111] In some embodiments, a polynucleotide or nucleic acid encoding an AAV capsid variant, of the present disclosure comprises a nucleotide sequence described herein, e.g., a nucleotide sequence of the AAV capsid variant of TTJ-001, e.g., as described in Tables 3 and 5.

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 4. Exemplary full length capsid amino acid sequences

Name and Annotation	SEQ ID NO:	Amino Acid Sequence
TTJ-001 (modifications at positions 584, 586, 587, 588, 589, and 590, numbered relative to SEQ ID NO: 138 underlined)	981	MAADGYLPDWLEDNLSEGIREWALKPGAPQPKANQQHQDNARGLVLPGYKYLPGP NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADADEFQERLKEDTSFGG NLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAK KRLNFGQTGDTSVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSS SGNWHCDSQWLGDVRVITTSRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTP WGYFDNFRFHCHFSRPRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTDNNGVKTIAN NLSTVQVFTDSYQLPYVLGSAHEGCLPFPADVFMIPIQGYLTLNDGSQAVGRS SFYCLEYFPPSMLRGTGNFQFSYEFENVFPFHSSYAHSQSLDRLMNPLIDQYLYL KTINGSGQNQQTLKF SVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWP GASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVM TNEEEIKTTNPVATESYGGQVATN <u>TQDWHRI</u> AQTGWVQNQGILPGMVWQDRDVLQ PIWAKIPHTDGNFHPSPLMGGFGMKHPPPIILIKNTVPADPPTAFNKDKLNSFIT QYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSNNVEFAVNTGEGVYSEPRPIG TRYLTRNL

Table 5. Exemplary full length capsid nucleic acid sequences

Name and Annotation	SEQ ID NO:	NT Sequence
TTJ-001 (portion of sequence encoding the amino acid sequence of SEQ ID NO: 744 is underlined)	983	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTTAGTGAAGGAAT TCGCGAGTGGTGGGCTTTGAAACCTGGAGCCCCTCAACCCAAGGCAAATCAACAAC ATCAAGACAACGCTCGAGGTCTTGTGCTTCCGGGTACAAATACCTTGGACCCGGC AACGGACTCGACAAGGGGGAGCCGGTCAACGCAGCAGACGCGGCGGCCCTCGAGCA CGACAAGGCCTACGACCAGCAGCTCAAGGCCGAGACAACCCGTACCTCAAGTACA ACCACGCCGACGCCGAGTTCAGGAGCGGCTCAAAGAAGATACGTCTTTTGGGGGC AACCTCGGGCGAGCAGTCTTCCAGGCCAAAAGAGGCTTCTTGAACCTCTTGGTCT GGTTGAGGAAGCGGCTAAGACGGCTCCTGGAAGAAGAGGCCTGTAGAGCAGTCTC CTCAGGAACCGGACTCTCCGCGGGTATTGGCAAATCGGGTGACAGCCCGCTAAA AAGAGACTCAATTCGGTCAGACTGGCGACACAGAGTCAGTCCCAGACCCTCAACC AATCGGAGAACCCTCCCGCAGCCCCCTCAGGTGTGGGATCTCTTACAATGGCTTCAG GTGGTGGCGCACCAGTGGCAGACAATAACGAAGGTGCCGATGGAGTGGGTAGTTCC TCGGGAAATTGGCATTGCGATTCCCAATGGCTGGGGGACAGAGTCATCACCACCAG

		CACCCGAACCTGGGCCCTGCCACCTACAACAATCACCTCTACAAGCAAATCTCCA ACAGCACATCTGGAGGATCTTCAAATGACAACGCCTACTTCGGCTACAGCACCCCC TGGGGGTATTTTGAATCAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCA GCGACTCATCAACAACAACCTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCT TCAACATTCAAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCATCGCCAAAT AACCTTACCAGCACGGTCCAGGTCTTCACGGACTCAGACTATCAGTCCCCGTACGT GCTCGGGTCGGCTCACGAGGGCTGCCTCCCGCCGTTCACAGCGGACGTTTTTCATGA TTCTCAGTACGGGTATCTGACGCTTAATGATGGAAGCCAGGCCGTGGGTCTGTCG TCCTTTTACTGCCTGGAATATTTCCCGTCGCAAATGCTAAGAACGGGTAACAACCTT CCAGTTCAGCTACGAGTTTGAGAACGTACCTTTCCATAGCAGCTACGCTCACAGCC AAAGCCTGGACCGACTAATGAATCCACTCATCGACCAATACTTGTACTATCTCTCA AAGACTATTAACGGTCTTGGACAGAATCAACAAACGCTAAAATTCAGTGTGGCCGG ACCCAGCAACATGGCTGTCCAGGGAAGAACTACATACCTGGACCCAGCTACCGAC AACAACGTGTCTCAACCACTGTGACTCAAAACAACAACAGCGAATTTGCTTGGCCT GGAGCTTCTTCTTGGGCTCTCAATGGACGTAATAGCTTGATGAATCCTGGACCTGC TATGGCCAGCCACAAAGAAGGAGAGGACCGTTTCTTTCTTTGTCTGGATCTTTAA TTTTTGGCAAACAAGGAAGTGAAGAGACAACGTGGATGCGGACAAAGTCATGATA ACCAACGAAGAAGAAATTAATACTACTAACCCTGAGCAACGGAGTCTTATGGACA GGTCT GCTACAAACACCCAGGACTGGCACAGGATTGCGCAGACC GGCTGGGTTCAAA ACCAAGGAATACTTCCGGGTATGGTTTGGCAGGACAGAGATGTGTACCTGCAAGGA CCCATTTGGGCCAAAATTCCTCACACGGACGGCAACTTTCACCTTCTCCGCTGAT GGGAGGGTTTGAATGAAGCACCCGCTCCTCAGATCCTCATAAAAACACACCTG TACCTGCCGATCCTCAACGGCCTTCAACAAGGACAAGCTGAACCTTTTCATCACC CAGTATTCTACTGGCCAAGTCAGCGTGGAGATCGAGTGGGAGCTGCAGAAGGAAAA CAGCAAGCGGTGGAACCCGGAGATCCAGTACACTTCCAATATTACAAGTCTAATA ATGTTGAATTTGCTGTTAATACTGAAGGTGTATATAGTGAACCCGCCCATTTGGC ACCAGATACCTGACTCGTAATCTGTAA
--	--	--

[0112] In some embodiments, the polynucleotide encoding an AAV capsid variant, described herein comprises the nucleotide sequence of SEQ ID NO: 983, or a nucleotide sequence with at least 70% (e.g., at least 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

[0113] In some embodiments, the polynucleotide encoding an AAV capsid variant described herein comprises the nucleotide sequence of SEQ ID NO: 983, or a nucleotide sequence with at least 70% (e.g., at least 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto. In some embodiments, the nucleotide sequence encoding an AAV capsid variant described herein, comprises a nucleotide sequence comprising at least one, two or three modifications, e.g., substitutions, insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions, insertions, or deletions, 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, two or three, but not more than 30, 20 or 10 different nucleotides 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.

[0114] In some embodiments, an AAV capsid variant described herein, comprises the amino acid sequence of SEQ ID NO: 981, or an amino acid sequence with at least 70% (e.g., at least 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto. In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence comprising at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions (e.g., conservative substitutions), insertions,

or deletions, 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, two or three, but not more than 30, 20 or 10 different amino acids, relative to the amino acid sequence of SEQ ID NO: 981.

[0115] In some embodiments, an AAV capsid variant described herein 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 80, 85, 90, 95, 96, 97, 98, or 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, two or three, but not more than 30, 20 or 10 different nucleotides, 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, two or three modifications, e.g., substitutions, insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 983.

[0116] In some embodiments, an AAV capsid variant described herein comprises a VP1, VP2, VP3 protein, or a combination thereof. In some embodiments, an AAV capsid variant comprises the amino acid sequence corresponding to positions 138-736, e.g., a VP2, of SEQ ID NO: 981, or a sequence with at least 70% (e.g., at least 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto. In some embodiments, the AAV capsid protein comprises the amino acid sequence corresponding to positions 203-736, e.g., a VP3, of SEQ ID NO: 981, or a sequence with at least 70% (e.g., at least 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto. In some embodiments, the AAV capsid variant comprises the amino acid sequence corresponding to positions 1-736, e.g., a VP1, of SEQ ID NO: 981, or an amino acid sequence with at least 70% (e.g., at least 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

[0117] 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 a reference sequence comprising the amino acid sequence of SEQ ID NO: 138.

[0118] 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, 50, 100, 120, 132, 146, 150, 161, 174, 175, 200, 225, 250, 275, 283, 300, 350, 400, 450, 500, 525, 528, or 550-fold greater as compared to a reference sequence of SEQ ID NO: 138.

[0119] In some embodiments, an AAV capsid variant described herein is enriched at least about 10, 14, 20, 24, 50, 100, 150, 200, 250, 300, 350, 400, 425, 450, or 460-fold in the brain compared to a reference sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described

herein is enriched at least about 200, 250, 300, 350, 400, 425, 450, or 460-fold in the brain compared to a reference sequence of SEQ ID NO: 138.

[0120] 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 a reference sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein is enriched at least about 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 190, 200, 205, or 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 a reference 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).

[0121] In some embodiments, an AAV capsid variant described herein is enriched at least about 2, 3, 4, 5, 10, 15, 17, 20, 50, 75, 100, 103, 107, 125, 150, 200, 250, 300, 350, 400, 450, 500, 750, 1000, 1200-fold, in the brain compared to a reference sequence of SEQ ID NO: 981.

[0122] 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, 5, 7, 10, 15, 19, 20, 22, or 25-fold, as compared to a reference 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.

[0123] In some embodiments, an AAV capsid variant described herein 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, 50, 100, 120, 132, 146, 150, 161, 174, 175, 200, 225, 250, 275, 283, 300, 350, 400, 450, 500, 525, 528, or 550-fold, as compared to a reference 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.

[0124] In some embodiments, an AAV capsid variant described herein is enriched at least about 5, 10, 50, 100, 115, 120, 150, 175, 200, 207, 225, 250, or 275-fold, in the spinal cord compared to a reference sequence of SEQ ID NO: 138.

[0125] 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, e.g., substitution (e.g., conservative substitution), insertion, or deletion, 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 described comprises a modification, e.g., substitution (e.g., conservative substitution), insertion, or deletion, 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. In some embodiments, the AAV capsid variant comprises a modification e.g., substitution (e.g., conservative substitution), insertion, or deletion, at position N470 (e.g., N470A), D271 (e.g., D271A), N272 (e.g., N272A), Y446 (e.g., Y446A), N498 (e.g., N498Y or N498I), W503 (e.g., W503R or W503A), L620 (e.g., L620F), or a combination thereof, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises one, two, three, four, five or all of an amino acid other than N at position 470 (e.g., A), an amino acid other than D at position 271 (e.g., A), an amino acid other than N at position 272 (e.g., A), an amino acid other than Y at position 446 (e.g., A), and amino acid other than N at position 498/ (e.g., Y or I), and amino acid other than W at position 503 (e.g., R or A), and amino acid other than L at position 620 (e.g., F), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises a modification e.g., substitution (e.g., conservative substitution), insertion, or deletion, at position N470 (e.g., N470A), D271 (e.g., D271A), N272 (e.g., N272A), Y446 (e.g., Y446A), and W503 (e.g., W503R or W503A), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises a modification e.g., substitution (e.g., conservative substitution), insertion, or deletion, at N498 (e.g., N498Y) and L620 (e.g., L620F).

[0126] In some embodiments, an AAV capsid variant comprised herein 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 its 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 its entirety.

[0127] In some embodiments, an AAV capsid variant of the present disclosure is isolated, e.g., recombinant. In some embodiments, a polynucleotide encoding an AAV capsid polypeptide, e.g., an AAV capsid variant, of the present disclosure is isolated, e.g., recombinant.

[0128] Also provided herein are polynucleotide sequences encoding any of the AAV capsid variants described above and AAV particles, vectors, and cells comprising the same.

AAV serotypes and capsids

[0129] In some embodiments, an AAV particle of the present disclosure may comprise a capsid protein or variant thereof any natural or recombinant AAV serotype. AAV serotypes may differ in characteristics such as, but not limited to, packaging, tropism, transduction and immunogenic profiles. While not wishing to be bound by theory, it is believed in some embodiments, that the AAV capsid protein, e.g., an AAV capsid variant, can modulate, e.g., direct, AAV particle tropism to a particular tissue.

[0130] In some embodiments, an AAV capsid variant described herein 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 brain region. In some embodiments, the brain region comprises a frontal cortex, sensory cortex, motor cortex, caudate, dentate nucleus, cerebellar cortex, cerebral cortex, brain stem, hippocampus, thalamus, putamen, or a combination thereof. 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).

[0131] 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.

[0132] 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, 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.

[0133] 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.

[0134] 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.

[0135] 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.

[0136] 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 VP2) 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-).

[0137] 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).

[0138] 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.

[0139] 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-).

[0140] 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 Sequences

[0141] In some embodiments, the AAV capsid variant, comprises at positions 582, 583, 584, 585, 586, 587, 588, 589, and/or 590, numbered relative to SEQ ID NO: 138, at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids of any of amino acid sequence provided in Tables 1, 2A, 2B, 9, 14, 15, or 16.

[0142] In some embodiments, the AAV capsid variant, comprises immediately subsequent to position at positions 582, 583, 584, 585, 586, 587, 588, 589, and/or 590, numbered relative to SEQ ID NO: 138 or 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, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids of any of amino acid sequence provided in Tables 1, 2A, 2B, 9, 14, 15, or 16. In some embodiments, the at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids of any of amino acid sequence provided in Tables 1, 2A, 2B, 9, 14, 15, or 16 replaces at least one, two, three, four, five, six, seven, eight, or all of positions replaces positions 582, 583, 584, 585, 586, 587, 588, 589, and/or 590 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, and/or Q590), numbered according to the amino acid sequence of SEQ ID NO: 138, numbered according to SEQ ID NO: 138 or 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)). In some embodiments, the AAV capsid variant comprises an amino acid other than the wild-type, e.g., native, amino acid, at one, two, three, four, five, six, seven, eight, or all of positions replaces positions 582, 583, 584, 585, 586, 587, 588, 589, and/or 590, numbered according to the amino acid sequence of SEQ ID NO: 138, numbered according to SEQ ID NO: 138 or 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)). In some embodiments, the AAV capsid variant comprises a modification, e.g., substitution, at one, two, three, four, five, six, seven, eight, or all of positions replaces positions 582, 583, 584, 585, 586, 587, 588, 589, and/or 590 (e.g., T582, N583, H584, Q585, S586, A587, Q588, A589, and/or Q590), numbered according to the amino acid sequence of SEQ ID NO: 138, numbered according to SEQ ID NO: 138 or 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)).

[0143] In some embodiments, an AAV capsid polypeptide or AAV capsid variant described herein may comprise a VOY101 capsid polypeptide, an AAVPHP.B (PHP.B) capsid polypeptide, a 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, an AAVrh10 capsid polypeptide, or a functional variant thereof. In some embodiments, the AAV capsid polypeptide, e.g., AAV capsid variant, comprises an amino acid sequence of any of the AAV capsid polypeptides in Table 6, or an amino acid sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments, the nucleotide sequence encoding the AAV capsid polypeptide comprises any one of the nucleotide sequences in Table 6, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto.

[0144] In some embodiments, an AAV capsid polypeptide or an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments the AAV capsid polypeptide or the AAV capsid variant, comprises an amino acid sequence comprising at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than 30, 20, or 10 modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid polypeptide or the AAV capsid variant, comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 137 or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments, the nucleotide sequence encoding the AAV capsid polypeptide or the AAV capsid variant comprises the nucleotide sequence of SEQ ID NO: 137 or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments, the AAV capsid polypeptide or the AAV capsid variant, comprises substitution at position K449, e.g., a K449R substitution, numbered according to SEQ ID NO: 138.

[0145] In some embodiments, the AAV capsid polypeptide or the AAV capsid variant, comprises a peptide comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 1262). In some embodiments, the peptide is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, the capsid polypeptide comprises the amino acid substitutions of A587D and Q588G, numbered according to SEQ ID NO: 138.

[0146] In some embodiments, the AAV capsid polypeptide or the AAV capsid variant comprises the amino acid substitution of K449R, numbered according to SEQ ID NO: 138; and a peptide comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 1262), wherein the peptide is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO: 138.

[0147] In some embodiments, the AAV capsid polypeptide or the AAV capsid variant comprises the amino acid substitution of K449R, numbered according to SEQ ID NO: 138; an peptide comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 1262), wherein the insert is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO: 138; and the amino acid substitutions of A587D and Q588G, numbered according to SEQ ID NO: 138.

[0148] In some embodiments, the AAV capsid polypeptide or the AAV capsid variant comprises a peptide comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 1262), wherein the insert is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO: 138; and the amino acid substitutions of A587D and Q588G, numbered according to SEQ ID NO: 138.

[0149] In some embodiments, the AAV capsid polypeptide or the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 11 or an amino acid sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments the AAV capsid polypeptide or the AAV capsid variant, comprises an amino acid sequence comprising at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than 30, 20, or 10 modifications, e.g., substitutions (conservative substitutions), relative to the amino acid sequence of SEQ ID NO: 11, optionally wherein position 449 is not R.

[0150] In some embodiments, the AAV capsid polypeptide or AAV capsid variant, comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments the AAV capsid polypeptide or the AAV capsid variant, comprises an amino acid sequence comprising at least one, two, or three modifications, e.g., substitutions (e.g., conservative

substitutions), but no more than 30, 20, or 10 modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of SEQ ID NO: 1.

Table 6. AAV Sequences

Serotype	SEQ ID NO:	Sequence
VOY101	1	MAADGYLPDWLEDNLSEGIREWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPNGGLD KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADADEFQERLKEDTSFGGNLGRAVFQ AKKRLLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVPDPQPIGEPPAAPSGVGSMTMASGGGAPVADNNEGADGVGSSSGNWHCDSQLGDRVI TTSTRTWALPTYNNHLYKQISNSTSGSSSNDNAYFGYSTPWGYDFNRFHCHFSRDPWQR LINNNWGFPRKRLNFKLFNIQVKEVTDNNGVKTIANNLSTSTVQVFTDSQYQLPYVLGSAH EGCLPPFPADVFMIPQYGYLTLDNGSQAVGRSSFYCLEYFPPSOMLRTGNFQFSYEFENV PFHSSYAHSQSLDRLMNPIDQYLYLSKTINGSGQNQQTLKFVAGPSNMAVQGRNYIP GPSYRQQRVSTTVTQNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGS LIFGKQGTGRDNVDADKVMITNEEEIKTNPVATESYQVATNHQSDGTLAVPFKAQAQT GWVQNGGILPGMVWQDRDVYLQGPWAKIPHTDGNFHPSPMLGGFGMKHPPQILIKNT VPADPPTAFNKDKLSNFIITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSNNVEF AVNTEGVYSEPRPIGTRYLTRNL
AAV9/hu. 14 K449R	11	MAADGYLPDWLEDNLSEGIREWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPNGGLD KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADADEFQERLKEDTSFGGNLGRAVFQ AKKRLLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVPDPQPIGEPPAAPSGVGSMTMASGGGAPVADNNEGADGVGSSSGNWHCDSQLGDRVI TTSTRTWALPTYNNHLYKQISNSTSGSSSNDNAYFGYSTPWGYDFNRFHCHFSRDPWQR LINNNWGFPRKRLNFKLFNIQVKEVTDNNGVKTIANNLSTSTVQVFTDSQYQLPYVLGSAH EGCLPPFPADVFMIPQYGYLTLDNGSQAVGRSSFYCLEYFPPSOMLRTGNFQFSYEFENV PFHSSYAHSQSLDRLMNPIDQYLYLSRTINGSGQNQQTLKFVAGPSNMAVQGRNYIP GPSYRQQRVSTTVTQNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGS LIFGKQGTGRDNVDADKVMITNEEEIKTNPVATESYQVATNHQSAQAQAQTGWVQNGG ILPGMVWQDRDVYLQGPWAKIPHTDGNFHPSPMLGGFGMKHPPQILIKNTVPADPPT AFNKDKLSNFIITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSNNVEFAVNTEGV YSEPRPIGTRYLTRNL
AAV9/hu. 14 WT (amino acid)	138	MAADGYLPDWLEDNLSEGIREWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPNGGLD KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADADEFQERLKEDTSFGGNLGRAVFQ AKKRLLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVPDPQPIGEPPAAPSGVGSMTMASGGGAPVADNNEGADGVGSSSGNWHCDSQLGDRVI TTSTRTWALPTYNNHLYKQISNSTSGSSSNDNAYFGYSTPWGYDFNRFHCHFSRDPWQR LINNNWGFPRKRLNFKLFNIQVKEVTDNNGVKTIANNLSTSTVQVFTDSQYQLPYVLGSAH EGCLPPFPADVFMIPQYGYLTLDNGSQAVGRSSFYCLEYFPPSOMLRTGNFQFSYEFENV PFHSSYAHSQSLDRLMNPIDQYLYLSKTINGSGQNQQTLKFVAGPSNMAVQGRNYIP GPSYRQQRVSTTVTQNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGS LIFGKQGTGRDNVDADKVMITNEEEIKTNPVATESYQVATNHQSAQAQAQTGWVQNGG ILPGMVWQDRDVYLQGPWAKIPHTDGNFHPSPMLGGFGMKHPPQILIKNTVPADPPT AFNKDKLSNFIITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSNNVEFAVNTEGV YSEPRPIGTRYLTRNL
AAV9/hu. 14 WT (DNA)	137	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTTAGTGAAGGAATTCGC GAGTGGTGGGCTTTGAAACCTGGAGCCCTCAACCAAGGCAATCAACAACATCAAGAC AACGCTCGAGGTCTTGTGCTTCCGGGTACAAATACCTTGGACCGGCAACGGACTCGAC AAGGGGGAGCCGGTCAACGCAGCAGACGCGCGGCCCTCGAGCAGCAAGGCCTACGAC CAGCAGCTCAAGGCCGAGACAACCGTACCTCAAGTACAACACGCGGACGCGGAGTTC CAGGAGCGGCTCAAAGAAGATACGTCTTTTGGGGCAACCTCGGCGAGCAGTCTTCCAG GCCAAAAGAGGCTTCTTGAACCTCTTGGTCTGGTTGAGGAAGCGGCTAAGACGGCTCCT GGAAAGAAGAGGCTGTAGAGCAGTCTCCTCAGGAACCGGACTCCTCCGCGGTATTGGC AAATCGGGTGACAGCCGCTAAAAAGAGACTCAATTTCCGTCAGACTGGCGACACAGAG TCAGTCCCAGACCTCAACCAATCGGAGAACCTCCCGCAGCCCCCTCAGGTGTGGGATCT CTTACAATGGCTTCAGGTGGTGGCGCACCAAGTGGCAGACAATAACGAAGGTGCCGATGGA GTGGGTAGTTCCTCGGGAAATTGGCATTGCGATTCCCAATGGCTGGGGGACAGAGTCATC ACCACCAGACCCGAACCTGGGCCCTGCCACCTACAACAATCACCTCTACAAGCAAATC

		TCCAACAGCACATCTGGAGGATCTTCAAATGACAACGCCTACTTCGGCTACAGCACCCCC TGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCGA CTCATCAACAACAACCTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCTTCAACATT CAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCATCGCCAATAACCTTACCAGC ACGGTCCAGGTCTTACGGACTCAGACTATCAGCTCCCGTACGTGCTCGGGTCGGGCTCAC GAGGGCTGCCTCCCGCCGTTCCAGCGGACGTTTTTCATGATTCTCAGTACGGGTATCTG ACGCTTAATGATGGAAGCCAGGCCGTGGGTCTGTCCTTTTACTGCCTGGAATATTTT CCGTCGCAAATGCTAAGAACGGGTAACTTCCAGTTCAGTACGAGTTTGAGAACGTA CCTTTCCATAGCAGCTACGCTCACAGCCAAAGCCTGGACCGACTAATGAATCCACTCATC GACCAATACTTGTACTATCTCTCAAAGACTATTAACGGTTCTGGACAGAATCAACAAACG CTAAAATTCAGTGTGGCCGGACCCAGCAACATGGCTGTCCAGGGAAGAACTACATACCT GGACCCAGCTACCGACAACAACGTGTCTCAACCACTGTGACTCAAAACAACAACAGCGAA TTTGCTTGGCCTGGAGCTTCTTCTTGGGCTCTCAATGGACGTAATAGCTTGATGAATCCT GGACCTGCTATGGCCAGCCACAAAGAAGGAGAGGACCGTTTCTTCTTTGTCTGGATCT TTAATTTTGGCAAACAAGGAACCTGGAAGAGACAACGTGGATGCGGACAAAGTCATGATA ACCAACGAAGAAGAAATTAATACTACTAACCCTAGCAACGGAGTCTATGGACAAGTG GCCACAACACCAGAGTGCCCAAGCACAGGCGCAGACCGGCTGGGTTCAAACCAAGGA ATACTTCCGGGTATGGTTTGGCAGGACAGAGATGTGTACCTGCAAGGACCCATTTGGGCC AAAATTCCTCACACGGACGGCAACTTTCACCCTTCTCCGCTGATGGGAGGGTTTGGAAATG AAGCACCCGCTCTCAGATCCTCATCAAAAACACACCTGTACCTGCGGATCTCCAACG GCCTTCAACAAGGACAAGCTGAACCTTTTCATCACCCAGTATTTACTGGCCAGTCCAGC GTGGAGATCGAGTGGGAGCTGCAGAAAGGAAAACAGCAAGCGCTGGAACCCGGAGATCCAG TACACTTCCAACCTATTACAAGTCTAATAATGTTGAATTTGCTGTTAATACTGAAGGTGTA TATAGTGAACCCCGCCCATTTGGCACCAGATACCTGACTCGTAATCTGTAA
--	--	--

Viral Genome of the AAV particle

[0151] In some embodiments, an AAV particle as described herein comprising an AAV capsid variant described herein, may be used for the delivery of a viral genome to a tissue (e.g., CNS, DRG, and/or muscle). In some embodiments, an AAV particle comprising an AAV capsid variant described herein can be used for delivery of a viral genome to a tissue or cell, e.g., CNS, DRG, or muscle cell or tissue. In some embodiments, an AAV particle of the present disclosure is a recombinant AAV particle. In some embodiments, an AAV particle of the present disclosure is an isolated AAV particle.

[0152] The viral genome may encode any payload, such as but not limited to a polypeptide (e.g., a therapeutic polypeptide), an antibody, an enzyme, an RNAi agent and/or components of a gene editing system. In one embodiment, the AAV particles described herein are used to deliver a payload to cells of the CNS, after intravenous delivery. In another embodiment, the AAV particles described herein are used to deliver a payload to cells of the DRG, after intravenous delivery. In some embodiments, the AAV particles described herein are used to deliver a payload to cells of a muscle, e.g., a heart muscle, after intravenous delivery.

[0153] In some embodiments, a viral genome of an AAV particle comprising an AAV capsid variant, as described herein, comprises a nucleotide sequence comprising a transgene encoding a payload. In some embodiments, the viral genome comprises an inverted terminal repeat sequence (ITR). In some embodiments, the viral genome comprises two ITR sequences, one at the 5' end of the viral genome (e.g., 5' relative to the encoded payload) and one at the 3' end of the viral genome (e.g., 3' relative to the encoded payload). In some embodiments, a viral genome of an AAV particle, e.g.,

an AAV particle comprising an AAV capsid variant described herein, may comprise a regulatory element (e.g., promoter), untranslated regions (UTR), a miR binding site, a polyadenylation sequence (polyA), a filler or stuffer sequence, an intron, and/or a linker sequence, e.g., for enhancing transgene expression.

[0154] In some embodiments, the viral genome components are selected and/or engineered for expression of the payload in a target tissue (e.g., CNS, muscle, or DRG).

Viral Genome Component: Inverted Terminal Repeats (ITRs)

[0155] In some embodiments, the AAV particle comprising an AAV capsid variant described herein comprises a viral genome comprising an ITR and a transgene encoding a payload. In some embodiments, the viral genome comprises two ITRs. In some embodiments, the two ITRs flank the nucleotide sequence encoding the payload at the 5' and 3' ends. In some embodiments, the ITRs function as origins of replication comprising recognition sites for replication. In some embodiments, the ITRs comprise sequence regions which can be complementary and symmetrically arranged. In some embodiments, the ITRs incorporated into viral genomes as described herein may be comprised of naturally occurring polynucleotide sequences or recombinantly derived polynucleotide sequences.

[0156] In some embodiments, the ITR may be from the same serotype as the capsid polypeptide, e.g., capsid variant, selected from any of the known serotypes, or a variant thereof. In some embodiments, the ITR may be of a different serotype than the capsid. In some embodiments, the viral genome comprises two ITR sequence regions, wherein the ITRs are of the same serotype as one another. In some embodiments, the viral genome comprises two ITR sequence regions, wherein the ITRs are of different serotypes. Non-limiting examples include zero, one or both of the ITRs having the same serotype as the capsid. In one embodiment both ITRs of the viral genome of the AAV particle are AAV2 ITRs.

Viral Genome Component: Promoters

[0157] In some embodiments, viral genome of an AAV particle described herein comprises at least one element to enhance the payload 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 payload target specificity and expression include promoters, endogenous miRNAs, post-transcriptional regulatory elements (PREs), polyadenylation (PolyA) signal sequences and upstream enhancers (USEs), CMV enhancers and introns.

[0158] In some embodiments, an AAV particle comprising an AAV capsid variant described herein comprises a viral genome comprising a nucleic acid comprising a transgene encoding a payload, wherein the transgene is operably linked to a promoter. In some embodiments, the promoter is a species specific promoter, an inducible promoter, a tissue-specific promoter, or a cell cycle-

specific promoter (e.g., a promoter as described in Parr et al., *Nat. Med.* 3:1145-9 (1997); the contents of which are herein incorporated by reference in their entirety).

[0159] In some embodiments, the Promoter may be naturally occurring or non-naturally occurring. Non-limiting examples of promoters include those derived from viruses, plants, mammals, or humans. In some embodiments, the promoters may be those derived from human cells or systems. In some embodiments, the promoter may be truncated or mutated, e.g., a promoter variant.

[0160] In some embodiments, the promoter is a ubiquitous promoter, e.g., capable of expression in multiple tissues. In some embodiments the promoter is a human elongation factor 1 α -subunit (EF1 α) promoter, the cytomegalovirus (CMV) immediate-early enhancer and/or promoter, the chicken β -actin (CBA) promoter and its derivative CAG, β glucuronidase (GUSB) promoter, or ubiquitin C (UBC) promoter. In some embodiments, the promoter is a cell or tissue specific promoter, e.g., capable of expression in tissues or cells of the central or peripheral nervous systems, targeted regions within (e.g., frontal cortex), and/or sub-sets of cells therein (e.g., excitatory neurons). In some embodiments, the promoter is a cell-type specific promoters capable of expression of a payload in excitatory neurons (e.g., glutamatergic), inhibitory neurons (e.g., GABA-ergic), neurons of the sympathetic or parasympathetic nervous system, sensory neurons, neurons of the dorsal root ganglia, motor neurons, or supportive cells of the nervous systems such as microglia, glial cells, astrocytes, oligodendrocytes, and/or Schwann cells.

[0161] In some embodiments, the promoter is a liver specific promoter (e.g., hAAT, TBG), skeletal muscle specific promoter (e.g., desmin, MCK, C512), B cell promoter, monocyte promoter, leukocyte promoter, macrophage promoter, pancreatic acinar cell promoter, endothelial cell promoter, lung tissue promoter, and/or cardiac or cardiovascular promoter (e.g., α MHC, cTnT, and CMV-MLC2k).

[0162] In some embodiments, the promoter is a tissue-specific promoter for payload expression in a tissue or cell of the central nervous system. In some embodiments, the promoter is a synapsin (Syn) promoter, glutamate vesicular transporter (VGLUT) promoter, vesicular GABA transporter (VGAT) promoter, parvalbumin (PV) promoter, sodium channel Nav 1.8 promoter, tyrosine hydroxylase (TH) promoter, choline acetyltransferase (ChaT) promoter, methyl-CpG binding protein 2 (MeCP2) promoter, Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) promoter, metabotropic glutamate receptor 2 (mGluR2) promoter, neurofilament light (NFL) or heavy (NFH) promoter, neuron-specific enolase (NSE) promoter, β -globin minigene n β 2 promoter, preproenkephalin (PPE) promoter, enkephalin (Enk) promoter, and excitatory amino acid transporter 2 (EAAT2) promoter, or a fragment thereof. In some embodiments, the promoter is a cell-type specific promoter capable of expression in an astrocyte, e.g., a glial fibrillary acidic protein (GFAP) promoter and a EAAT2 promoter, or a fragment thereof. In some embodiments, the promoter is a cell-type specific promoter capable of expression in an oligodendrocyte, e.g., a myelin basic protein (MBP) promoter or a fragment thereof.

[0163] In some embodiments, the promoter is a GFAP promoter. In some embodiments, the promoter is a synapsin (syn or syn1) promoter, or a fragment thereof.

[0164] In some embodiments, the promoter comprises an insulin promoter or a fragment thereof.

[0165] In some embodiments, the promoter of the viral genome described herein (e.g., comprised within an AAV particle comprising an AAV capsid variant described herein) comprises an EF-1 α promoter or variant thereof, e.g., as provided in Table 8. In some embodiments, the EF-1 α promoter comprises the nucleotide sequence of any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998, 999-1007, or any one of the nucleotide sequence provided in Table 8; a nucleotide sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions, relative to the nucleotide sequence of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998, 999-1007, or any one of the nucleotide sequence provided in Table 8; or a nucleotide sequence with at least 70% (e.g., 80, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998, 999-1007, or any one of the nucleotide sequence provided in Table 8.

Table 8. Exemplary Promoter Variants

Description	Sequences	SEQ ID NO:
EF1 α Promoter (intron underlined)	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGA GAAGTTGGGGGAGGGGTGCGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGG GTAAACTGGGAAAGTGATGTCGTGTAAGTGGCTCCGCCTTTTCCCGAGGGTGGGG GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTCGCAACGGGTT TGCCGCCAGAACACAGGTAAGTGGCGTGTGTGGTTCGCCGGGGCCTGGCCCTCTTT ACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGA TTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCTTTCGCG TTAAGGAGCCCTTCGCTCGTGTGAGTTGAGGCTGGCCTGGGCGCTGGGGC CGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTTCGATAAGT CTCTAGCCATTTAAATTTTGTAGACCTGCTGCGACGCTTTTCTGGCAAGA TAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCC GCGGGCGCGACGGGGCCCGTGCCTCCAGCGCACATGTTTCGCGAGGCGGGGCC TGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGC TCTGGTGCCTGGCCTCGCGCCGCGTGTATCGCCCGCCCTGGGCGGAAGGCTG GCGCGGTCGGCACCAAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTG CAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACC CACACAAAGGAAAAGGGCCTTTCCGTCTCAGCCGTGCTTCATGTGACTCCACG GAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGT CGTCTTTAGGTTGGGGGGAGGGGTTTATGCGATGGAGTTTCCCACTGAGTG GGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGTATGTAATCTCCTTGAATTT GCCCTTTTGTAGTTTGGATCTTGGTTCAATCTCAAGCCTCAGACAGTGGTTCAAA GTTTTTTCTTCCATTCAGGTGTCGTGA	987
miniEF1 α	GCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAG GGGTCGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGTAAACTGGGAAAG TGATGTCGTGTAAGTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATATA AGTGCAGTAGTCGCCGTGAACGTTCTTTTCGCAACGGGTTTCCCGCCAGAACAC GCGTAAG	988
Promoter Variant 1	GCATG	
Promoter Variant 2	GGTGGAGAAGAGCATG	990
Promoter Variant 3	GTCATCACTGAGGTGGAGAAGAGCATG	991
Promoter Variant 4	CGTGAG	

Promoter Variant 5	GT	
Promoter Variant 6	GCTCCGGT	
Promoter Variant 19	GCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAG GGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAG TGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATATA AGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACAC AG	995
Promoter Variant 20	GCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAG GGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAG TGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATATA AGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACAC GC	996
Promoter Variant 7	GTAAG	
Promoter Variant 8	GTGCCCCTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGG AGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATA TAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAAC ACGCGTAAG	998
Promoter Variant 9	GCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTT GGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAAC TGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAAC CGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGC CAGAACACGCGTAAG	999
Promoter Variant 10	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGA GAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGG GTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGG GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTT TGCCGCCAGAACACGCGTAAG	1000
Promoter Variant 11	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGA GAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGG GTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGG GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTT TGCCGCCAGAACACAG	1001
Promoter Variant 12	GCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTC CCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGC GCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGG TGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAAC GGGTTTGCCGCCAGAACACGCGTAAG	1002
Promoter Variant 13	GCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTC CCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGC GCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGG TGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAAC GGGTTTGCCGCCAGAACACAG	1003
Promoter Variant 14	GGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACAT CGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTA GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTT TTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTT TTTTTCGCAACGGGTTTGCCGCCAGAACACGCGTAAG	1004
Promoter Variant 15	GGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACAT CGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTA GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTT TTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTT TTTTTCGCAACGGGTTTGCCGCCAGAACACAG	1005
Promoter Variant 16	GTCATCACTGAGGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGC AGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGA ACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACT	1006

	GGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGC CGTGAACGTTCTTTTCGCAACGGGTTTGCCGCCAGAACACGCGTAAG	
Promoter Variant 18	GTCATCACTGAGGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGC AGAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGGTGGCAATTGA ACCGGTGCCTAGAGAAGGTGGCGCGGGGTAACTGGGAAAGTGATGTCGTGTACT GGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGC CGTGAACGTTCTTTTCGCAACGGGTTTGCCGCCAGAACACAG	1007

Viral Genome Component: Untranslated Regions (UTRs)

[0166] In some embodiments, wild type untranslated regions (UTRs) of a gene are transcribed but not translated. Generally, the 5' UTR starts at the transcription start site and ends at the start codon and the 3' UTR starts immediately following the stop codon and continues until the termination signal for transcription.

[0167] Features typically found in abundantly expressed genes of specific target organs (e.g., CNS tissue, muscle, or DRG) may be engineered into UTRs to enhance stability and protein production. As a non-limiting example, a 5' UTR from mRNA normally expressed in the brain (e.g., huntingtin) may be used in the viral genomes of the AAV particles described herein to enhance expression in neuronal cells or other cells of the central nervous system.

[0168] While not wishing to be bound by theory, wild-type 5' untranslated regions (UTRs) include features which play roles in translation initiation. Kozak sequences, which are commonly known to be involved in the process by which the ribosome initiates translation of many genes, are usually included in 5' UTRs. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (ATG), which is followed by another 'G'.

[0169] In one embodiment, the 5'UTR in the viral genome includes a Kozak sequence.

[0170] In one embodiment, the 5'UTR in the viral genome does not include a Kozak sequence.

[0171] While not wishing to be bound by theory, wild-type 3' UTRs are known to have stretches of Adenosines and Uridines embedded therein. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995, the contents of which are herein incorporated by reference in its entirety): Class I AREs, such as, but not limited to, c-Myc and MyoD, contain several dispersed copies of an AUUUA motif within U-rich regions. Class II AREs, such as, but not limited to, GM-CSF and TNF- α , possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Class III AREs, such as, but not limited to, c-Jun and Myogenin, are less well defined. These U rich regions do not contain an AUUUA motif. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3' UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo*.

[0172] Introduction, removal or modification of 3' UTR AU rich elements (AREs) can be used to modulate the stability of a polynucleotide. When engineering specific polynucleotides, e.g., payload regions of viral genomes, one or more copies of an ARE can be introduced to make polynucleotides less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein.

[0173] In one embodiment, the 3' UTR of the viral genome may include an oligo(dT) sequence for templated addition of a poly-A tail.

[0174] In one embodiment, the viral genome may include at least one miRNA seed, binding site or full sequence. microRNAs (or miRNA or miR) are 19-25 nucleotide noncoding RNAs that bind to the sites of nucleic acid targets and down-regulate gene expression either by reducing nucleic acid molecule stability or by inhibiting translation. In some embodiments, a microRNA sequence comprises a seed region, e.g., a sequence in the region of positions 2-8 of the mature microRNA, which has Watson-Crick sequence fully or partially complementarity to the miRNA target sequence of the nucleic acid.

[0175] In one embodiment, the viral genome may be engineered to include, alter or remove at least one miRNA binding site, full sequence or seed region.

[0176] Any UTR from any gene known in the art may be incorporated into the viral genome of the AAV particle. These UTRs, or portions thereof, may be placed in the same orientation as in the gene from which they were selected or they may be altered in orientation or location. In one embodiment, the UTR used in the viral genome of the AAV particle may be inverted, shortened, lengthened, made with one or more other 5' UTRs or 3' UTRs known in the art. As used herein, the term "altered" as it relates to a UTR, means that the UTR has been changed in some way in relation to a reference sequence. For example, a 3' or 5' UTR may be altered relative to a wild type or native UTR by the change in orientation or location as taught above or may be altered by the inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides.

[0177] In one embodiment, the viral genome of the AAV particle comprises at least one artificial UTR which is not a variant of a wild type UTR.

[0178] In one embodiment, the viral genome of the AAV particle comprises UTRs which have been selected from a family of transcripts whose proteins share a common function, structure, feature or property.

Viral Genome Component: Polyadenylation Sequence

[0179] The viral genome of the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant, described herein) may comprise a polyadenylation sequence. In some embodiments, the viral genome of the AAV particle (e.g., an AAV particle comprising an AAV

capsid variant, described herein) comprises a polyadenylation sequence between the 3' end of the nucleotide sequence encoding the payload and the 5' end of the 3' ITR.

Viral Genome Component: Introns

[0180] In some embodiments, the viral genome of the AAV particle as described herein (e.g., an AAV particle comprising an AAV capsid variant), comprises an element to enhance the payload target specificity and expression (See e.g., Powell et al. *Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy*, Discov. Med, 2015, 19(102): 49-57; the contents of which are herein incorporated by reference in their entirety), such as an intron. Non-limiting examples of introns include, MVM (67-97 bps), F.IX truncated intron 1 (300 bps), β -globin SD/immunoglobulin heavy chain splice acceptor (250 bps), adenovirus splice donor/immunoglobulin splice acceptor (500 bps), SV40 late splice donor/splice acceptor (19S/16S) (180 bps) and hybrid adenovirus splice donor/IgG splice acceptor (230 bps).

Viral Genome Component: Stuffer sequences

[0181] In some embodiments, the viral genome of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant), comprises an element to improve packaging efficiency and expression, such as a stuffer or filler sequence. Non-limiting examples of stuffer sequences include albumin and/or alpha-1 antitrypsin. Any known viral, mammalian, or plant sequence may be manipulated for use as a stuffer sequence.

Viral Genome Component: miRNA

[0182] In one embodiment, the viral genome comprises a sequence encoding a miRNA to reduce the expression of the payload in a tissue or cell, e.g., the DRG (dorsal root ganglion), or neurons of other ganglia, such as those of the sympathetic or parasympathetic nervous system. In some embodiments, a miRNA, e.g., a miR183, a miR182, and/or miR96, may be encoded in the viral genome to modulate, e.g., reduce the expression, of the viral genome in a DRG neuron. As another non-limiting example, a miR122 miRNA may be encoded in the viral genome to modulate, e.g., reduce, the expression of the viral genome in the liver. In some embodiments, a miRNA, e.g., a miR142-3p, may be encoded in the viral genome to modulate, e.g., reduce, the expression, of the viral genome in a cell or tissue of the hematopoietic lineage, including for example immune cells (e.g., antigen presenting cells or APC, including dendritic cells (DCs), macrophages, and B-lymphocytes). In some embodiments, a miRNA, e.g., a miR1, may be encoded in the viral genome to modulate, e.g., reduce, the expression, of the viral genome in a cell or tissue of the heart.

Viral Genome Component: miR Binding Site

[0183] Tissue- or cell-specific expression of the AAV viral particles disclosed herein can be enhanced by introducing tissue- or cell-specific regulatory sequences, e.g., promoters, enhancers,

microRNA binding sites, e.g., a detargeting site. Without wishing to be 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 disclosed herein, 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 payload encoded by a viral genome of an AAV particle described herein in a cell or tissue where the corresponding mRNA is expressed.

[0184] 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.

[0185] In some embodiments, the nucleotide sequence encoding the miR binding site series or the miR binding site is located in the 3'-UTR region of the viral genome (e.g., 3' relative to the nucleotide sequence encoding a payload), e.g., before the polyA sequence, 5'-UTR region of the viral genome (e.g., 5' relative to the nucleotide sequence encoding a payload), or both.

[0186] 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, 1, 2, 3, 4, 5 or more copies of a miR binding site (miR BS). 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. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides, nucleotides in length. In some embodiments, the spacer coding sequence or reverse complement thereof comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions relative to the nucleotide sequence of GATAGTTA.

[0187] 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, 1, 2, 3, 4, 5 or more copies of a miR binding site (miR BS). In some embodiments, at least 1, 2, 3, 4, 5, or all of the copies are different, e.g., comprise a different 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. In some

embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides, in length. In some embodiments, the spacer comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions relative to the nucleotide sequence of GATAGTTA.

[0188] In some embodiments, the encoded miR binding site is substantially identical (e.g., at least 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% identical), to the miR in the host cell. In some embodiments, the encoded miR binding site comprises at least 1, 2, 3, 4, or 5 mismatches or no more than 6, 7, 8, 9, or 10 mismatches to a miR in the host cell. In some embodiments, the mismatched nucleotides are contiguous. In some embodiments, the mismatched nucleotides are non-contiguous. In some embodiments, the mismatched nucleotides occur outside the seed region-binding sequence of the miR binding site, such as at one or both ends of the miR binding site. In some embodiments, the miR binding site is 100% identical to the miR in the host cell.

[0189] In some embodiments, the nucleotide sequence encoding the miR binding site is substantially complementary (e.g., at least 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% complementary), to the miR in the host cell. In some embodiments, the complementary sequence of the nucleotide sequence encoding the miR binding site comprises at least 1, 2, 3, 4, or 5 mismatches or no more than 6, 7, 8, 9, or 10 mismatches to a miR in the host cell. In some embodiments, the mismatched nucleotides are contiguous. In some embodiments, the mismatched nucleotides are non-contiguous. In some embodiments, the mismatched nucleotides occur outside the seed region-binding sequence of the miR binding site, such as at one or both ends of the miR binding site. In some embodiments, the encoded miR binding site is 100% complementary to the miR in the host cell.

[0190] In some embodiments, an encoded miR binding site or sequence region is at least 10 to about 125 nucleotides in length, e.g., at least 10 to 50 nucleotides, 10 to 100 nucleotides, 50 to 100 nucleotides, 50 to 125 nucleotides, or 100 to 125 nucleotides in length. In some embodiments, an encoded miR binding site or sequence region is at least 7 to 28 nucleotides in length, e.g., at least 8-28 nucleotides, 7-28 nucleotides, 8-18 nucleotides, 12-28 nucleotides, 20-26 nucleotides, 22 nucleotides, 24 nucleotides, or 26 nucleotides in length, and optionally comprises at least one consecutive region (e.g., 7 or 8 nucleotides) complementary (e.g., fully or partially complementary) to the seed sequence of a miRNA (e.g., a miR122, a miR142, a miR183, or a miR1).

[0191] In some embodiments, the encoded miR binding site is complementary (e.g., fully or partially complementary) to a miR expressed in liver or hepatocytes, such as 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 encoded miR122 binding site comprises the

nucleotide sequence of ACAAACACCATTTGTCACACTCCA (SEQ ID NO: 4673), or a nucleotide sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., insertions, deletions, or substitutions, relative to the nucleotide sequence of SEQ ID NO: 4673, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 2, 3, 4, or 5 copies of the encoded miR122 binding site, e.g., an encoded miR122 binding site series, optionally wherein the encoded miR122 binding site series comprises the nucleotide sequence of:

ACAAACACCATTTGTCACACTCCACACAAACACCATTTGTCACACTCCACACAAACACCATTTGTCACACTCCA (SEQ ID NO: 4674), or a nucleotide sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4674, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, at least two of the encoded miR122 binding sites are connected directly, e.g., without a spacer. In other embodiments, at least two of the encoded miR122 binding sites are separated by a spacer, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length, which is located between two or more consecutive encoded miR122 binding site sequences. In embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8, in length. In some embodiments, the spacer coding sequence or reverse complement thereof comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, an encoded miR binding site series comprises at least 3-5 copies (e.g., 4 copies) of a miR122 binding site, with or without a spacer, wherein the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions relative to the nucleotide sequence of GATAGTTA.

[0192] In some embodiments, the encoded miR binding site is complementary (e.g., fully or partially complementary) to a miR expressed in the heart. In embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR1 binding site. In some embodiments, the encoded miR1 binding site comprises the nucleotide sequence of ATACATACTTCTTTACATTCCA (SEQ ID NO: 4679), a nucleotide sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three,

four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4679, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 2, 3, 4, or 5 copies of the encoded miR1 binding site, e.g., an encoded miR1 binding site series. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR1 binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, insertions, or deletions, but no more than four modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of GATAGTTA.

[0193] In some embodiments, the encoded miR binding site is complementary (e.g., fully or partially complementary) to a miR expressed in hematopoietic lineage, including immune cells (e.g., antigen presenting cells or APC, including dendritic cells (DCs), macrophages, and B-lymphocytes). In some embodiments, the encoded miR binding site complementary to a miR expressed in hematopoietic lineage comprises a nucleotide sequence disclosed, e.g., in US 2018/0066279, the contents of which are incorporated by reference herein in its entirety.

[0194] In embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR142-3p binding site sequence. In some embodiments, the encoded miR142-3p binding site comprises the nucleotide sequence of TCCATAAAGTAGGAAACACTACA (SEQ ID NO: 4675), a nucleotide sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4675, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 2, 3, 4, or 5 copies of the encoded miR142-3p binding site, e.g., an encoded miR142-3p binding site series. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR142-3p binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at

least one, two, or three modifications, e.g., substitutions, insertions, or deletions, but no more than four modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of GATAGTTA.

[0195] In some embodiments, the encoded miR binding site is complementary (e.g., fully complementary or partially complementary) to a miR expressed in a DRG (dorsal root ganglion) neuron, e.g., a miR183, a miR182, and/or miR96 binding site. In some embodiments, the encoded miR binding site is complementary to a miR expressed in expressed in a DRG neuron comprises a nucleotide sequence disclosed, e.g., in WO2020/132455, the contents of which are incorporated by reference herein in its entirety.

[0196] In some embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR183 binding site sequence. In some embodiments, the encoded miR183 binding site comprises the nucleotide sequence of AGTGAATTCTACCAGTGCCATA (SEQ ID NO: 4676), or a nucleotide sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4676, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the sequence complementary to the seed sequence corresponds to the double underlined of the encoded miR183 binding site sequence. In some embodiments, the viral genome comprises at least comprises at least 2, 3, 4, or 5 copies (e.g., at least 2 or 3 copies) of the encoded miR183 binding site, e.g., an encoded miR183 binding site. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR183 binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, insertions, or deletions, but no more than four modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of GATAGTTA. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii).

[0197] In some embodiments, the encoded miR binding site or the encoded miR binding site series comprises a miR182 binding site sequence. In some embodiments, the encoded miR182 binding site comprises, the nucleotide sequence of AGTGTGAGTTCTACCATTGCCAAA (SEQ ID NO: 4677), a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g.,

substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4677, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 2, 3, 4, or 5 copies of the encoded miR182 binding site, e.g., an encoded miR182 binding site series. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR182 binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, insertions, or deletions, but no more than four modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of GATAGTTA. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii).

[0198] In certain embodiments, the encoded miR binding site or the encoded miR binding site series comprises a miR96 binding site sequence. In some embodiments, the encoded miR96 binding site comprises the nucleotide sequence of AGCAAAAATGTGCTAGTGCCAAA (SEQ ID NO: 4678), a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4678, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 2, 3, 4, or 5 copies of the encoded miR96 binding site, e.g., an encoded miR96 binding site series. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR96 binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, insertions, or deletions, but no more than four modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of GATAGTTA. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii).

[0199] In some embodiments, the encoded miR binding site series comprises a miR122 binding site, a miR1, a miR142 binding site, a miR183 binding site, a miR182 binding site, a miR 96 binding site, or a combination thereof. In some embodiments, the encoded miR binding site series comprises at least 2, 3, 4, or 5 copies of a miR122 binding site, a miR142 binding site, a miR183 binding site, a

miR182 binding site, a miR 96 binding site, or a combination thereof. In some embodiments, at least two of the encoded miR binding sites are connected directly, e.g., without a spacer. In other embodiments, at least two of the encoded miR binding sites are separated by a spacer, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length, which is located between two or more consecutive encoded miR binding site sequences. In embodiments, the spacer is at least about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer coding sequence or reverse complement thereof comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of GATAGTTA.

[0200] In some embodiments, an encoded miR binding site series comprises at least 2-5 copies (e.g., 2 or 3 copies) of a combination of at least two, three, four, five, or all of a miR1, miR122 binding site, a miR142 binding site, a miR183 binding site, a miR182 binding site, a miR96 binding site, wherein each of the miR binding sites within the series are continuous (e.g., not separated by a spacer) or are separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, insertions, or deletions, but no more than four modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of GATAGTTA.

[0201] In some embodiments, an encoded miR binding site series comprises at least 2-5 copies (e.g., 2 or 3 copies) of a combination of a miR122 binding site and a miR1 binding site, wherein each of the miR binding sites within the series are continuous (e.g., not separated by a spacer) or are separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions, insertions, or deletions, but no more than four modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of GATAGTTA.

Genome Size

[0202] In one embodiment, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant), may comprise a single-stranded or double-stranded viral genome. The size of

the viral genome may be small, medium, large or the maximum size. As described above, the viral genome may comprise a promoter and a polyA tail.

[0203] In one embodiment, the viral genome may be a small single stranded viral genome. A small single stranded viral genome may be about 2.1 to about 3.5 kb in size such as, but not limited to, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, or 3.5 kb in size.

[0204] In one embodiment, the viral genome may be a small double stranded viral genome. A small double stranded viral genome may be about 1.3 to about 1.7 kb in size such as, but not limited to, 1.3, 1.4, 1.5, 1.6, or 1.7 kb in size.

[0205] In one embodiment, the viral genome may be a medium single stranded viral genome. A medium single stranded viral genome may be about 3.6 to about 4.3 kb in size such as, but not limited to, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2 or 4.3 kb in size.

[0206] In one embodiment, the viral genome may be a medium double stranded viral genome. A medium double stranded viral genome may be about 1.8 to about 2.1 kb in size such as, but not limited to, 1.8, 1.9, 2.0, or 2.1 kb in size.

[0207] In one embodiment, the viral genome may be a large single stranded viral genome. A large single stranded viral genome may be about 4.4 to about 6.0 kb in size such as, but not limited to, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9 or 6.0 kb in size.

[0208] In one embodiment, the viral genome may be a large double stranded viral genome. A large double stranded viral genome may be about 2.2 to about 3.0 kb in size such as, but not limited to, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3.0 kb in size.

Payloads

[0209] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant described herein) comprises a viral genome comprising a nucleic acid encoding a payload. In some embodiments, the encoded payload is an RNAi agent or a polypeptide. A payload of the present disclosure may be, but is not limited to, a peptide, a polypeptide, a protein, an antibody, an RNAi agent, etc.

[0210] In some embodiments, the nucleotide sequence encoding a payload may comprise a combination of coding and non-coding nucleic acid sequences. In some embodiments, the nucleotide sequence encoding the payload may encode a coding or non-coding RNA.

[0211] In some embodiments, the AAV particles described herein, e.g., an AAV particle comprising an AAV capsid variant, comprises a nucleic acid encoding a payload. In some embodiments, the encoded payload comprises a therapeutic protein, an antibody, an enzyme, one or more components of a genome editing system, and/or an RNAi agent (e.g., a dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA). In some embodiments, the encoded payload modulates, e.g., increases or decreases, the presence, level, and/or activity of a gene, mRNA, protein, or a combination thereof, e.g., in a cell or a tissue.

Polypeptides

[0212] In some embodiments, the encoded payload of AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant, described herein comprises a polypeptide, protein, or peptide, e.g., a polypeptide, protein, or peptide described herein. The nucleic acid encoding the payload, may encode a product of any known gene and/or a recombinant version thereof. In some embodiments, the nucleic acid encoding the payload may encode at least one allele of apolipoprotein E (APOE) such as, but not limited to ApoE2, ApoE3 and/or ApoE4. In one embodiment, the nucleic acid encoding the payload encodes ApoE2 (cys112, cys158) protein or a fragment or variant thereof. In one embodiment, the nucleic acid encoding the payload encodes an ApoE3 (cys112, arg158) protein or fragment or variant thereof. In one embodiment, the nucleic acid encoding the payload encodes ApoE4 (arg112, arg158). As another non-limiting example, the encoded payload comprises an aromatic L-amin acid decarboxylase (AADC) protein. As another non-limiting example, the encoded payload comprises an antibody, or a fragment thereof. As another non-limiting example, the encoded payload comprises a human survival of motor neuron (SMN) 1 or SMN2 protein, or fragments or variants thereof. As another non-limiting example, the encoded payload region comprises a glucocerebrosidase (GBA1) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises a granulin precursor or progranulin (GRN) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises an aspartoacylase (ASPA) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises a tripeptidyl peptidase I (CLN2) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises a beta-galactosidase (GLB1) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises a N-sulphoglucosamine sulphohydrolase (SGSH) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises an N-acetyl-alpha-glucosaminidase (NAGLU) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises an iduronate 2-sulfatase (IDS) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises an intracellular cholesterol transporter (NPC1) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises a gigaxonin (GAN) protein, or a fragment or variant thereof. The AAV viral genomes encoding polypeptides described herein may be useful in the fields of human disease, viruses, infections veterinary applications and a variety of *in vivo* and *in vitro* settings.

[0213] Amino acid sequences of a payload polypeptide encoded by a viral genome described herein, may be translated as a whole polypeptide, a plurality of polypeptides or fragments of polypeptides, which independently may be encoded by one or more nucleic acids, fragments of nucleic acids or variants of any of the aforementioned.

Antibodies and Antibody Binding Fragments

[0214] In some embodiments, the encoded payload of AAV particle comprising an AAV capsid variant described herein comprises an antibody or antibody binding fragment. In some embodiments, the antibody may be a full antibody, a fragment, or any functional variant thereof. As non-limiting examples, an antibody may be a native antibody (e.g., with two heavy and two light chains), a heavy chain variable region, a light chain variable region, a heavy chain constant region, a light chain constant region, Fab, Fab', F(ab')₂, Fv, or scFv fragments, a diabody, a linear antibody, a single-chain antibody, a multi-specific antibody, an intrabody, one or more heavy chain complementarity determining regions (CDR), one or more light chain CDRs, a bi-specific antibody, a monoclonal antibody, a polyclonal antibody, a humanized antibody, an antibody mimetic, an antibody variant, a miniaturized antibody, a unibody, a maxibody, and/or a chimeric antigen receptor. The encoded antibody or antibody binding fragment may be useful in the treatment of a neurological disease, a neurodegenerative disorder, a muscular disease, a neuromuscular disorder, a neuro-oncological disorder, or any disorder associated with the central and/or peripheral nervous systems.

[0215] In some embodiments, the viral genome of the AAV particle (e.g., an AAV particle comprising an AAV capsid variant described herein) may comprise a nucleic acid which has been engineered to enable or enhance the expression of an antibody, or antibody binding fragment thereof.

[0216] In some embodiments, the encoded antibody of the payload of an AAV particle comprising an AAV capsid variant, described herein comprises at least one immunoglobulin variable domain sequence. An antibody may include, for example, full-length, mature antibodies and antigen-binding fragments of an antibody. For example, an antibody can include a heavy (H) chain variable domain sequence (VH), and a light (L) chain variable domain sequence (VL). In another example, an antibody includes two heavy (H) chain variable domain sequences and two light (L) chain variable domain sequence, thereby forming two antigen binding sites, such as Fab, Fab', F(ab')₂, Fc, Fd, Fd', Fv, single chain antibodies (scFv for example), single variable domain antibodies, diabodies (Dab) (bivalent and bispecific), and chimeric (e.g., humanized) antibodies, which may be produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. These functional antibody fragments, e.g., an antibody binding fragments, retain the ability to selectively bind with their respective antigen or receptor.

[0217] In some embodiments, the antibody binding fragment comprises at least one portion of an intact antibody, or recombinant variants thereof, and refers to the antigen binding domain, for example, an antigenic determining variable region of an intact antibody, that is sufficient to confer recognition and specific binding of the antibody fragment to a target, such as an antigen. Examples of antigen binding fragments include: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1

domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a diabody (dAb) fragment, which consists of a VH domain; (vi) a camelid or camelized variable domain; (vii) a single chain Fv (scFv), see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883; and (viii) a single domain antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. An antibody fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, for example, Hollinger and Hudson, Nature Biotechnology 23:1126-1136, 2005).

[0218] In some embodiments, the encoded antibody of the payload of an AAV particle described herein comprises a multispecific antibody, e.g., it comprises a plurality of immunoglobulin variable domains sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In some embodiments, the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In some embodiments, the first and second epitopes overlap. In some embodiments, the first and second epitopes do not overlap. In some embodiments, the first and second epitopes are on different antigens, e.g., the different proteins (or different subunits of a multimeric protein). In some embodiments, a multispecific antibody comprises a third, fourth or fifth immunoglobulin variable domain. In some embodiments, a multispecific antibody is a bispecific antibody, a trispecific antibody, or tetraspecific antibody.

[0219] In some embodiments, an encoded multispecific antibody of the payload of an AAV particle described herein is an encoded bispecific antibody. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In some embodiments, the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In some embodiments, the first and second epitopes overlap. In some embodiments, the first and second epitopes do not overlap. In some embodiments, the first and second epitopes are on different antigens, e.g., the different proteins (or different subunits of a multimeric protein).

[0220] An antibody or an antibody binding fragment encoded by a viral genome of an AAV particle described herein, may be, but is not limited to, an antibody or antibody fragment that binds to β -amyloid, APOE, tau, SOD1, TDP-43, huntingtin, and/or synuclein. In some embodiments, the encoded payload comprises an antibody or antibody fragment that binds to a neuro-oncology related target, e.g., HER2, EGFR (e.g., EGFRvIII). In some embodiments, the encoded payload comprises an

antibody that binds to HER2/neu. In some embodiments, the encoded payload comprises an antibody that binds to β -amyloid. In some embodiments, the encoded payload comprises an antibody that binds to tau.

Gene Editing System

[0221] In some embodiments, the encoded payload of AAV particle comprising an AAV capsid variant described herein comprises a gene editing system or one or more components thereof. In some embodiments, the gene editing system comprises nucleic acid sequences that encode proteins having enzymatic activity to (i) selectively induce double or single stranded breaks in a DNA or RNA sequence, or (ii) substitute, insert or delete a particular base or set of bases of a DNA or RNA sequence in the absence of a double or single stranded break in the DNA or RNA. In some embodiments, the gene editing system includes, but is not limited to a CRISPR-Cas system (including different Cas or Cas-related nucleases), a Zinc finger nuclease, a meganuclease, a TALEN or a base editors. In some embodiments, the gene editing system comprises a chromosomal integration of a transgene, e.g., introduced by a parvovirus vector in the absence of an exogenous nuclease or an enzymatic entity.

RNAi agents

[0222] In some embodiments, the encoded payload of AAV particle comprising an AAV capsid variant described herein comprises an RNAi agent, e.g., an RNAi agent described herein. In some embodiments, the encoded payload of a viral genome of an AAV particle comprising an AAV capsid variant described herein comprises an RNAi agent, the RNAi, such as but not limited to, a dsRNA, a siRNA, a shRNA, a pre-miRNA, a pri-miRNA, a miRNA, a stRNA, a lncRNA, a piRNA, or a snoRNA. In some embodiments, the encoded payload comprises an RNAi agent for inhibiting expression of a SOD1, MAPT, APOE, HTT, C9ORF72, TDP-43, APP, BACE, SNCA, ATXN1, ATXN3, ATXN7, SCN1A-SCN5A, or SCN8A-SCN11A gene, protein, and/or mRNA. In some embodiments, the RNAi agent encoded by a viral genome described herein inhibits SOD1, MAPT, APOE, HTT, C9ORF72, TDP-43, APP, BACE, SNCA, ATXN1, ATXN3, ATXN7, SCN1A-SCN5A, or SCN8A-SCN11A.

[0223] An AAV particle comprising an AAV capsid variant described herein may comprise a viral genome encoding an RNAi agent, which targets the mRNA of a gene to modulate, e.g., interfere with gene expression and/or protein production.

[0224] In some embodiments, the RNAi agent may target a gene at the location of a single-nucleotide polymorphism (SNP) or variant within the nucleotide sequence of the gene.

[0225] The RNAi agent may be an siRNA duplex, wherein the siRNA duplex contains an antisense strand (guide strand) and a sense strand (passenger strand) hybridized together forming a duplex structure, wherein the antisense strand is complementary to the nucleic acid sequence of the

targeted gene, and wherein the sense strand is homologous to the nucleic acid sequence of the targeted gene. In some aspects, the 5' end of the antisense strand has a 5' phosphate group and the 3' end of the sense strand contains a 3' hydroxyl group. In other aspects, there are none, one or 2 nucleotide overhangs at the 3' end of each strand.

[0226] Each strand of an siRNA duplex targeting a gene of interest may be about 19 to 25, 19 to 24 or 19 to 21 nucleotides in length, preferably about 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, or 25 nucleotides in length.

[0227] In one embodiment, an siRNA or dsRNA includes at least two sequences that are complementary to each other. The dsRNA includes a sense strand having a first sequence and an antisense strand having a second sequence. The antisense strand includes a nucleotide sequence that is substantially complementary to at least part of an mRNA encoding the target gene, and the region of complementarity is 30 nucleotides or less, and at least 15 nucleotides in length. Generally, the dsRNA is 19 to 25, 19 to 24 or 19 to 21 nucleotides in length. In some embodiments, the dsRNA is from about 15 to about 25 nucleotides in length, and in other embodiments the dsRNA is from about 25 to about 30 nucleotides in length. In some embodiments, the dsRNA is about 15 nucleotides in length, 16 nucleotides in length, 17 nucleotides in length, 18 nucleotides in length, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides in length, 26 nucleotides in length, 27 nucleotides in length, 28 nucleotides in length, 29 nucleotides in length, or 30 nucleotides in length.

[0228] In some embodiments, the encoded RNAi agent is an siRNA.

[0229] In some embodiments, the RNAi agent, e.g., an RNAi agent described herein inhibits the expression of the gene, mRNA, and/or protein by at least 10%, at least 20%, at least 25%, at least 30%, at least 35% or at least 40% or more, such as when assayed by a method known in the art. In some embodiments, the RNAi agent inhibits expression of a gene, mRNA, and protein by 50-100%, e.g., by 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%.

[0230] In some embodiments, the AAV particle described herein, comprising a viral genome encoding an RNAi agent targeting a gene of interest is administered to a subject in need for treating and/or ameliorating a disease, e.g., a neurological disorder or any disease associated with the central or peripheral nervous systems.

Design of siRNA

[0231] An AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) may comprise a viral genome encoding a siRNA molecule (e.g., siRNA duplex or encoded dsRNA) that target a gene of interest and suppress target gene expression, mRNA expression, and protein production. In some aspects, the siRNA molecules are designed and used to knock out target gene variants in cells, e.g., transcripts that are identified in neurological disease. In some aspects, the siRNA molecules are designed and used to knock down target gene variants in cells.

[0232] Some guidelines for designing siRNAs (for insertion into a viral genome of the AAV particles described herein) have been proposed in the art. These guidelines generally recommend generating a 19-nucleotide duplexed region, symmetric 2-3 nucleotide 3' overhangs, 5-phosphate and 3-hydroxyl groups targeting a region in the gene to be silenced. Other rules that may govern siRNA sequence preference include, but are not limited to, (i) A/U at the 5' end of the antisense strand; (ii) G/C at the 5' end of the sense strand; (iii) at least five A/U residues in the 5' terminal one-third of the antisense strand; and (iv) the absence of any GC stretch of more than 9 nucleotides in length. In accordance with such considerations, together with the specific sequence of a target gene, highly effective siRNA molecules essential for suppressing mammalian target gene expression may be readily designed.

[0233] In one embodiment, the sense and/or antisense strand is designed based on the method and rules outlined in European Patent Publication No. EP1752536, the contents of which are herein incorporated by reference in their entirety. As a non-limiting example, the 3'-terminal base of the sequence is adenine, thymine or uracil. As a non-limiting example, the 5'-terminal base of the sequence is guanine or cytosine. As a non-limiting example, the 3'-terminal sequence comprises seven bases rich in one or more bases of adenine, thymine and uracil.

[0234] In one embodiment, an siRNA molecule comprises a sense strand and a complementary antisense strand in which both strands are hybridized together to form a duplex structure. The antisense strand has sufficient complementarity to the target mRNA sequence to direct target-specific RNAi, e.g., the siRNA molecule has a sequence sufficient to trigger the destruction of the target mRNA by the RNAi machinery or process.

[0235] In some embodiments, the antisense strand and target mRNA sequences have 100% complementarity. The antisense strand may be complementary to any part of the target mRNA sequence. Neither the identity of the sense sequence nor the homology of the antisense sequence need be 100% complementary to the target.

[0236] In other embodiments, the antisense strand and target mRNA sequences comprise at least one mismatch. As a non-limiting example, the antisense strand and the target mRNA sequence have at least 50-90%, 50-95%, 50-99%, 60-70%, 60-80%, 60-90%, 60-95%, 60-99%, 70-80%, 70-90%, 70-95%, 70-99%, 80-90%, 80-95%, 80-99%, 90-95%, 90-99% or 95-99% complementary.

[0237] The siRNA molecule may have a length from about 10-50 or more nucleotides, e.g., each strand comprising 10-50 nucleotides (or nucleotide analogs). Preferably, the siRNA molecule has a length from about 15-30, e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in each strand, wherein one of the strands is sufficiently complementary to a target region. In one embodiment, the siRNA molecule has a length from about 19 to 25, 19 to 24 or 19 to 21 nucleotides.

[0238] In some embodiments, the siRNA molecule can be a synthetic RNA duplex comprising about 19 nucleotides to about 25 nucleotides, and two overhanging nucleotides at the 3'-end.

[0239] The siRNA molecule may comprise an antisense sequence and a sense sequence, or a fragment or variant thereof. As a non-limiting example, the antisense sequence and the sense sequence have at least 50-90%, 50-95%, 50-99%, 60-70%, 60-80%, 60-90%, 60-95%, 60-99%, 70-80%, 70-90%, 70-95%, 70-99%, 80-90%, 80-95%, 80-99%, 90-95%, 90-99% or 95-99% complementary.

[0240] The sense and antisense sequences may be completely complementary across a substantial portion of their length. In other embodiments, the sense sequence and antisense sequence may be at least 70, 80, 90, 95 or 99% complementary across independently at least 50, 60, 70, 80, 85, 90, 95, or 99% of the length of the strands.

[0241] In some embodiments, the sense and antisense strands of a siRNA duplex are linked by a short spacer sequence leading to the expression of a stem-loop structure termed short hairpin RNA (shRNA). The hairpin is recognized and cleaved by Dicer, thus generating mature siRNA molecules.

[0242] In some embodiments, the siRNA molecules, as well as associated spacer and/or flanking regions once designed, can be encoded by the viral genome of the AAV particles described herein, for delivery to a cell.

Molecular Scaffold

[0243] In some embodiments, the siRNA molecules may be encoded in a modulatory polynucleotide which also comprises a molecular scaffold.

[0244] In some embodiments, the modulatory polynucleotide which comprises the payload (e.g., siRNA, miRNA or other RNAi agent described herein) includes a molecular scaffold which comprises a 5' flanking sequence, a loop region, and/or a 3' flanking region. In some embodiments a 5' or 3' flanking region may be of any length and may a wild type microRNA sequence or a portion thereof, or may be completely artificial. A 3' flanking sequence may mirror the 5' flanking sequence in size and origin. Either flanking sequence may be absent. In one embodiment, both the 5' and 3' flanking sequences are absent. The 3' flanking sequence may optionally contain one or more CNNC motifs, where "N" represents any nucleotide. In some embodiments, the loop comprises at least one UGUG motif. In some embodiments, the UGUG motif is located at the 5' terminus of the loop. In some embodiments the 5' and 3' flanking sequences are the same sequence. In some embodiments they differ by 2%, 3%, 4%, 5%, 10%, 20% or more than 30% when aligned to each other.

[0245] In some embodiments, modulatory polynucleotide comprises a stem loop structure. In some embodiments, the modulatory polynucleotide comprises in 5' to 3' order: a 5' flanking sequence, a guide strand sequence, a loop region, a passenger strand sequence, and a 3' flanking sequence. In some embodiments, the modulatory polynucleotide comprises in 5' to 3' order: a 5'

flanking sequence, a passenger strand sequence, a loop region, a guide strand sequence, and a 3' flanking sequence.

[0246] In one embodiment, the molecular scaffold comprises a dual-function targeting modulatory polynucleotide.

[0247] In one embodiment, the molecular scaffold may comprise one or more linkers known in the art. The linkers may separate regions or one molecular scaffold from another. As a non-limiting example, the molecular scaffold may be polycistronic.

[0248] In one embodiment, the modulatory polynucleotide is designed using at least one of the following properties: loop variant, seed mismatch/bulge/wobble variant, stem mismatch, loop variant and basal stem mismatch variant, seed mismatch and basal stem mismatch variant, stem mismatch and basal stem mismatch variant, seed wobble and basal stem wobble variant, or a stem sequence variant.

AAV production

[0249] 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 a payload.

[0250] In some embodiments, disclosed herein is a method of making AAV particle of the present disclosure, e.g., an AAV particle comprising an AAV capsid variant the method comprising: (i) providing a host cell comprising a viral genome described herein and (ii) incubating the host cell under conditions suitable to enclose the viral genome in an AAV capsid variant, e.g., an AAV capsid variant described herein (e.g., an AAV capsid variant listed in Tables 3, 4, or 5), thereby making the AAV particle. In some embodiments, the method comprises prior to step (i), introducing a first nucleic acid comprising the viral genome into a cell. In some embodiments, the host cell comprises a second nucleic acid encoding the AAV capsid variant. In some embodiments, the second nucleic acid is introduced into the host cell prior to, concurrently with, or after the first nucleic acid molecule. 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.

[0251] 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). In another embodiment, AAV particles are produced in insect cells (e.g., Sf9).

[0252] 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.

Therapeutic Applications

[0253] The present disclosure provides a method for treating a disease, disorder and/or condition in a subject, including a human subject, comprising administering to the subject an AAV particle described herein, e.g., an AAV particle comprising an AAV capsid variant (e.g., an AAV capsid variant described herein), or administering to the subject any of the described compositions, including a pharmaceutical composition, described herein.

[0254] In some embodiments, the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) are administered to a subject prophylactically, to prevent on-set of disease. In another embodiment, the AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of the present disclosure are administered to treat (e.g., lessen the effects of) a disease or symptoms thereof. In yet another embodiment, the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) are administered to cure (eliminate) a disease. In another embodiment, the AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of the present disclosure are administered to prevent or slow progression of disease. In yet another embodiment, the AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of the present disclosure are used to reverse the deleterious effects of a disease. Disease status and/or progression may be determined or monitored by standard methods known in the art.

[0255] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for treatment, prophylaxis, palliation or amelioration of a genetic disorder, e.g., an autosomal dominant genetic disorder, an autosomal recessive disorder, X-linked dominant genetic disorder, an X-linked recessive genetic disorder, or a Y-linked genetic disorder. In some embodiments, the genetic disorder is a monogenetic disorder or a polygenic disorder. In some embodiments, treatment of a genetic disorder, e.g., a monogenic disorder, comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy.

[0256] In some embodiments, provided herein is method for treating a neurological disorder and/or neurodegenerative disorder in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition described herein or an AAV particle, e.g., a plurality of particles, comprising an AAV capsid variant described herein. In some embodiments, treatment of a

neurological disorder and/or neurodegenerative disorder comprises prevention of said neurological disorder and/or neurological disorder.

[0257] In some embodiments, the AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of the disclosure is useful for the treatment, prophylaxis, palliation or amelioration of neurological diseases and/or disorders. In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of tauopathy.

[0258] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is for the treatment, prophylaxis, palliation or amelioration of Alzheimer's Disease. In some embodiments, treatment of Alzheimer's Disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ApoE2 protein, ApoE4 protein, an ApoE3 protein, BDNF protein, CYP46A1 protein, Klotho protein, fractalkine (FKN) protein, neprilysin protein (NEP), CD74 protein, caveolin-1, or a combination or variant thereof. In some embodiments, treatment of Alzheimer's Disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a reduction in the expression of a tau gene and/or protein, a synuclein gene and/or protein, or a combination or variant thereof. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an antibody that binds to tau or synuclein, an RNAi agent for inhibiting tau or synuclein, a gene editing system (e.g., a CRISPR-Cas system) for altering tau or synuclein expression, or a combination thereof.

[0259] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is for the treatment, prophylaxis, palliation or amelioration of frontal temporal dementia. In some embodiments, treatment of frontal temporal dementia comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a progranulin protein or variant thereof.

[0260] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Parkinson's Disease. In some embodiments, treatment of Parkinson's disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an AADC protein, GAD protein, GDNF protein, TH-GCH1 protein, GBA protein, AIMP2-DX2 protein, or a combination or variant

thereof. In some embodiments, treatment of Parkinson's disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene knock-down therapy or a gene editing therapy (e.g., knock-out, repression, or correction). In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a modulator, e.g., an RNAi agent or a CRISPR-Cas system, for altering expression of an alpha-synuclein gene, mRNA, and/or protein, or variant thereof. In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of an AADC deficiency. In some embodiments, treatment of AADC deficiency comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an AADC protein or variant thereof.

[0261] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Amyotrophic lateral sclerosis. In some embodiments, treatment of ALS comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an TDP-43 protein, UPF1 protein, C9orf72 protein, CCNF protein, HSF1 protein, Factor H protein, NGF protein, ADAR2 protein, GDNF protein, VEGF protein, HGF protein, NRTN protein, AIMP2-DX2 protein, or a combination or variant thereof. In some embodiments, treatment of ALS comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene knock-down therapy or a gene editing therapy (e.g., knock-out, repression, or correction). In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a modulator, e.g., an RNAi agent or a CRISPR-Cas system, for altering expression of a SOD1 or C9ORF72 gene, mRNA, and/or protein, or a combination or variant thereof.

[0262] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Huntington's Disease. In some embodiments, treatment of ALS comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene knock-down (e.g., knock-out) therapy or a gene editing therapy (e.g., knock-out, repression, or correction). In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a modulator, e.g., an RNAi agent or a CRISPR-Cas system, for altering expression of an HTT gene, mRNA, and/or protein, or a variant thereof.

[0263] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of spinal muscular atrophy. In some embodiments, treatment of spinal muscular atrophy comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an SMN1 protein, an SMN2 protein, or a combination or variant thereof.

[0264] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of multiple system atrophy. In some embodiments, treatment of multiple system atrophy comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy.

[0265] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Gaucher disease (GD) (e.g., Type 1 GD, Type 2 GD, or Type 3 GD). In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Parkinson's disease associated with a GBA mutation. In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of dementia with Lewy Bodies (DLB).

[0266] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for treatment, prophylaxis, palliation or amelioration of a leukodystrophy, e.g., Alexander disease, autosomal dominant leukodystrophy with autonomic diseases (ADLD), Canavan disease, cerebrotendinous xanthomatosis (CTX), metachromatic leukodystrophy (MLD), Pelizaeus-Merzbacher disease, or Refsum disease. In some embodiments, treatment of MLD comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ARSA protein or variant thereof. In some embodiments, treatment of ALD comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ABCD-1 protein or variant thereof.

[0267] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of megalencephalic leukoencephalopathy (MLC). In some embodiments, treatment of MLC

comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an MLC1 protein or variant thereof.

[0268] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Krabbe disease. In some embodiments, treatment of Krabbe disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a GALC protein or variant thereof.

[0269] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Mucopolysaccharidosis, e.g., a Type I (MPS I), Type II (MPS II), Type IIIA (MPS IIIA), Type IIIB (MPS IIIB), or Type IIIC (MPS IIIC). In some embodiments, treatment of Mucopolysaccharidosis comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy or a gene editing therapy (e.g., enhancement or correction). In some embodiments, the payload encoded or corrected by an AAV particle comprising a capsid variant described herein comprises an IDUA protein, IDS protein, SGSH protein, NAGLU protein, HGSNAT protein, or a combination or variant thereof.

[0270] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Batten/NCL. In some embodiments, treatment of Batten/NCL comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a CLN1 protein, CLN2 protein, CLN3 protein, CLN5 protein, CLN6 protein, CLN7 protein, CLN8 protein, or a combination or variant thereof.

[0271] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Rett Syndrome. In some embodiments, treatment of Rett Syndrome comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an MeCP2 protein or variant thereof.

[0272] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Angelman Syndrome. In some embodiments, treatment of Angelman Syndrome comprises the use

of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a UBE3A protein or variant thereof.

[0273] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Fragile X Syndrome. In some embodiments, treatment of Fragile X Syndrome comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a Reelin protein, a DgkK protein, a FMR1 protein, or a combination or variant thereof.

[0274] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Canavan Disease. In some embodiments, treatment of Canavan Disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ASPA protein or variant thereof.

[0275] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Gangliosidosis, e.g., a GM1 Gangliosidosis or a GM2 Gangliosidosis (e.g., Tay Sachs Sandhoff). In some embodiments, treatment of a Gangliosidosis, e.g., a GM1 Gangliosidosis or a GM2 Gangliosidosis (e.g., Tay Sachs Sandhoff), comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a GLB1 protein, a HEXA protein, a HEXB protein, a GM2A protein, or a combination or variant thereof.

[0276] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of GM3 Synthase Deficiency. In some embodiments, treatment of GM3 Synthase Deficiency comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ST3GAL5 protein or variant thereof.

[0277] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Niemann-Pick disorder, e.g., a Niemann-Pick A or a Niemann-Pick C1 (NPC-1). In some

embodiments, treatment of a Niemann-Pick disorder, e.g., a Niemann-Pick A or a Niemann-Pick C1 (NPC-1) comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ASM protein, an NPC1 protein, or variant thereof.

[0278] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Schwannoma (e.g., Neuroma). In some embodiments, treatment of Schwannoma (e.g., Neuroma) comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a Caspase-1 protein or variant thereof.

[0279] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Tuberous Sclerosis, e.g., Tuberous Sclerosis Type 1 or Tuberous Sclerosis Type 2. In some embodiments, treatment of Tuberous Sclerosis, e.g., Tuberous Sclerosis Type 1 or Tuberous Sclerosis Type 2 comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a TSC1 protein, a TSC2 protein, or variant thereof.

[0280] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a CDKL5 Deficiency. In some embodiments, treatment of a CDKL5 Deficiency comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a CDKL5 protein or variant thereof.

[0281] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Charcot-Marie-Tooth disorder, e.g., a Charcot-Marie-Tooth Type 1X (CMT1X) disorder, a Charcot-Marie-Tooth Type 2A (CMT2A) disorder, or a Charcot-Marie-Tooth Type 4J (CMT4J) disorder. In some embodiments, treatment of a Charcot-Marie-Tooth disorder, e.g., a Charcot-Marie-Tooth Type 1X (CMT1X) disorder, a Charcot-Marie-Tooth Type 2A (CMT2A) disorder, or a Charcot-Marie-Tooth Type 4J (CMT4J) disorder, comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a

capsid variant described herein comprises a GJB1 protein, a MFN2 protein, a FIG4 protein, or variant thereof.

[0282] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of an Aspartylglucosaminuria (AGU). In some embodiments, treatment of an AGU comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an AGA protein or variant thereof.

[0283] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Leigh Syndrome. In some embodiments, treatment of a Leigh Syndrome comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a SURF1 protein or variant thereof.

[0284] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of epilepsy. In some embodiments, treatment of epilepsy comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an NPY/Y2 protein, a Galanin protein, a Dynorphin protein, an AIMP2-DX2 protein, an SLC6A1 protein, an SLC13A5 protein, a KCNQ2 protein, or variant thereof.

[0285] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Dravet Syndrome. In some embodiments, treatment of Dravet Syndrome comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an SCN1a protein, or variant thereof.

[0286] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Duchenne muscular dystrophy (DMD). In some embodiments, treatment of DMD comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy or enhancement (e.g., correction of exon-skipping), or a gene editing therapy (e.g., enhancement or correction). In some embodiments, the payload encoded or corrected by an AAV particle comprising a capsid variant described herein comprises a

Dystrophin gene and/or protein, a Utrophin gene and/or protein, or a GALGT2 gene and/or protein, or a Follistatin gene and/or protein, or a combination or variant thereof.

[0287] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Pompe Disease. In some embodiments, treatment of Pompe Disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a GAA protein, or variant thereof.

[0288] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Limb-Girdle Muscular Dystrophy (LGMD2A). In some embodiments, treatment of LGMD2A comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a CAPN-3 protein, DYSF protein, a SGCG protein, a SGCA protein, a SGCB protein, a FKRP protein, a ANO5 protein, or a combination or variant thereof.

[0289] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of chronic or neuropathic pain.

[0290] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising AAV capsid variant) is useful for treatment, prophylaxis, palliation or amelioration of a disease associated with the central nervous system.

[0291] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for treatment, prophylaxis, palliation or amelioration of a disease associated with the peripheral nervous system.

[0292] In some embodiments, provided herein is a method for treating a neuro-oncological disorder in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition described herein or an AAV particle, e.g., a plurality of particles, comprising an AAV capsid variant described herein. In some embodiments, treatment of a neuro-oncological disorder comprises prevention of said neuro-oncological disorder. In some embodiments, a neuro-oncological disorder comprises a cancer of a primary CNS origin (e.g., a CNS cell, a tissue, or a region), or a metastatic cancer in a CNS cell, tissue, or region. Examples of primary CNS cancers could be gliomas (which may include glioblastoma (also known as glioblastoma multiforme), astrocytomas, oligodendrogliomas, and ependymomas, and mixed gliomas), meningiomas, medulloblastomas, neuromas, and primary CNS lymphoma (in the brain, spinal cord, or meninges), among others. Examples of metastatic cancers include those originating in another tissue or organ, e.g., breast, lung,

lymphoma, leukemia, melanoma (skin cancer), colon, kidney, prostate, or other types that metastasize to brain.

[0293] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of a disease associated with expression of HER2, e.g., a disease associated with overexpression of HER2. In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of a HER2-positive cancer. In some embodiments, the HER2-positive cancer is a HER2-positive solid tumor. Additionally, or alternatively, the HER2-positive cancer may be a locally advanced or metastatic HER2-positive cancer. In some instances, the HER2-positive cancer is a HER2-positive breast cancer or a HER2-positive gastric cancer. In some embodiments, the HER2-positive cancer is selected from the group consisting of a HER2-positive gastroesophageal junction cancer, a HER2-positive colorectal cancer, a HER2-positive lung cancer (e.g., a HER2-positive non-small cell lung carcinoma), a HER2-positive pancreatic cancer, a HER2-positive colorectal cancer, a HER2-positive bladder cancer, a HER2-positive salivary duct cancer, a HER2-positive ovarian cancer (e.g., a HER2-positive epithelial ovarian cancer), or a HER2-positive endometrial cancer. In some instances, the HER2-positive cancer is prostate cancer. In some embodiments, the HER2-positive cancer has metastasized to the central nervous system (CNS). In some instances, the metastasized HER2-cancer has formed CNS neoplasms.

[0294] In some embodiments, the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) is administered to a subject having at least one of the diseases or symptoms described herein. In some embodiments, an AAV particle of the present disclosure is administered to a subject having or diagnosed with having a disease or disorder described herein.

[0295] In some embodiments, provided herein is a method for treating a muscular disorder and/or neuromuscular disorder in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition described herein or an AAV particle, e.g., a plurality of particles, comprising an AAV capsid variant described herein. In some embodiments, treatment of a muscular disorder and/or neuromuscular disorder comprises prevention of said muscular disorder and/or neuromuscular disorder.

[0296] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for treatment, prophylaxis, palliation or amelioration of a cardiac disease or heart disease and/or method of improving (e.g., enhancing) cardiac function in a subject. In some embodiments, the cardiac disease is a cardiomyopathy (e.g., arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, or hypertrophic cardiomyopathy), congestive heart failure, tachycardia (e.g., catecholaminergic polymorphic ventricular tachycardia), ischemic

heart disease, and/or myocardial infarction. In some embodiments, the cardiac disease is a disease associated with expression, e.g., aberrant expression, of LAMP2B, MYBPC3, TNNI3, LMNA, BAG3, DWORF, PKP2, Cx43, TAZ, CASQ2, SERCA2a, I-1c, S100A1 and/or ARC, S100A1, ASCL1, miR133, Mydelta3, Sav, or a combination or variant thereof. In some embodiments, treatment of a cardiac disorder described herein comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy.

[0297] In some embodiments, the cardiac disease is a genetic disorder, e.g., an autosomal dominant genetic disorder, an autosomal recessive disorder, or an X-linked recessive genetic disorder. In some embodiments, the cardiomyopathy is a genetic disorder, e.g., a genetic disorder associated with an abnormality (e.g., mutation, insertion, rearrangement and/or deletion) in a gene chosen from TTN, LMNA, MYH7, MYH6, SCN5A, TNNT2, RBM20, TNNI3, MYL2, MYL3, PKP2, DSP, DSG2, DSC2, JUP, or a combination thereof. In some embodiments, the cardiac disorder is a dilated cardiomyopathy, e.g., a dilated cardiomyopathy associated with an abnormality (e.g., mutation, insertion, rearrangement and/or deletion) in a gene chosen from TTN, LMNA, MYH7, BAG3, MIPN, TNNT2, SCN5A, RBM20, TNPO, LAMA4, VCL, LDB3, TCAP, PSEN1/2, ACTN2, CRYAB, TPM1, ABCC9, ACTC1, PDLIM3, ILK, TNNC1, TNNI3, PLN, DES, SGCD, CSRP3, MYH6, EYA4, ANKRD1, DMD, GATAD1, TAZ/G4.5, or combination thereof. In some embodiments, the cardiac disorder is a hypertrophic cardiomyopathy, e.g., a hypertrophic cardiomyopathy associated with an abnormality (e.g., mutation, insertion, rearrangement and/or deletion) in a gene chosen from MYH7, TNNT2, TNNI3, TPM1, MYL2, MYL3, ACTC1, CSRP3, TTN, ACTN2, MYH6, TCAP, TNNC1, or a combination thereof. In some embodiments, the cardiac disorder is an arrhythmogenic ventricular cardiomyopathy, e.g., an arrhythmogenic ventricular cardiomyopathy associated with an abnormality (e.g., mutation, insertion, rearrangement and/or deletion) in a gene chosen from PKP2, DSG2, DSP, RYR2, DSC2, TGFB3, TMEM43, DES, TTN, LMNA, or a combination thereof.

[0298] In some embodiments, the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) is administered to a subject having at least one of the diseases or symptoms described herein. In some embodiments, an AAV particle of the present disclosure is administered to a subject having or diagnosed with having a disease or disorder described herein.

[0299] Any neurological disease or disorder, neurodegenerative disorder, muscular disorder, neuromuscular disorder, and/or neuro-oncological disorder may be treated with the AAV particles of the disclosure, or pharmaceutical compositions thereof.

Pharmaceutical Composition and Formulations

[0300] According to the present disclosure, an AAV particle comprising an AAV capsid variant described herein may be prepared as a pharmaceutical composition. In some embodiments, the

pharmaceutical composition comprises at least one active ingredients. In some embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable excipient.

[0301] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) can be formulated using an excipient to: (1) increase stability; (2) increase cell transfection or transduction; (3) permit the sustained or delayed expression of the payload; (4) alter the biodistribution (e.g., target the viral particle to specific tissues or cell types); (5) increase the translation of encoded protein; (6) alter the release profile of encoded protein; and/or (7) allow for regulatable expression of the payload. Formulations of the present disclosure can include, without limitation, saline, liposomes, lipid nanoparticles, polymers, peptides, proteins, cells transfected with viral vectors (e.g., for transfer or transplantation into a subject) and combinations thereof.

[0302] In some embodiments, the relative amount of the active ingredient (e.g. an AAV particle comprising an AAV capsid variant described herein), a pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. 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%, at least 80% (w/w) active ingredient.

[0303] In some embodiments, the pharmaceutical composition comprising an AAV particle described herein may comprise an AAV capsid variant and a viral genome encoding a payload, e.g., a payload described herein, with or without a pharmaceutically acceptable excipient.

[0304] The present disclosure also provides in some embodiments, a pharmaceutical composition suitable for administration to a subject, e.g., a human. In some embodiments, the pharmaceutical composition is administered to a subject, e.g., a human.

Administration

[0305] In some embodiments, an AAV particle disclosed herein (e.g., an AAV particle comprising an AAV capsid variant) may be administered to a subject by a delivery route, e.g., a localized delivery route or a systemic delivery route.

[0306] In some embodiments, an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) may be administered via such a route that it is able to cross the blood-brain barrier, vascular barrier, or other epithelial barrier. In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be administered in any suitable form, either as a liquid solution or suspension, as a solid form suitable for liquid solution or suspension in a liquid solution. In some embodiments, an AAV particle (e.g., an

AAV particle comprising an AAV capsid variant) may be formulated with any appropriate and pharmaceutically acceptable excipient.

[0307] In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered intramuscularly, intravenously, intracerebrally, intrathecally, intratumorally, intracerebroventricularly, via intraparenchymal administration, or via intra-cisterna magna injection (ICM). In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered intravenously. In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered via intra-cisterna magna injection (ICM). In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered intratumorally. In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered intraarterially.

[0308] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be delivered to a subject via a single route administration. In some embodiments, an AAV particle of the present disclosure may be delivered to a subject via a multi-site route of administration. In some embodiments, a subject may be administered at 2, 3, 4, 5, or more than 5 sites.

[0309] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered via a bolus infusion. In some embodiments, an AAV particle of the present disclosure is administered via sustained delivery over a period of minutes, hours, or days. In some embodiments, the infusion rate may be changed depending on the subject, distribution, formulation, and/or another delivery parameter. In some embodiments, an AAV particle of the present disclosure is administered using a controlled release. In some embodiments, an AAV particle of the present disclosure is administered using a sustained release, e.g., a release profile that conforms to a release rate over a specific period of time.

[0310] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant) may be delivered by more than one route of administration. As non-limiting examples of combination administrations, an AAV particle may be delivered by intrathecal and intracerebroventricular, or by intravenous and intraparenchymal administration.

Intravenous administration

[0311] In some embodiments, an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) may be administered to a subject by systemic administration. In some embodiments, the systemic administration is intravenous administration. In another embodiment, the systemic administration is intraarterial administration. In some embodiments, an AAV particle of the present disclosure may be administered to a subject by intravenous administration. In some embodiments, the intravenous administration may be achieved by

subcutaneous delivery. In some embodiments, the AAV particle 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, e.g., as described in Terstappen et al. (Nat Rev Drug Discovery, <https://doi.org/10.1038/s41573-021-00139-y> (2021)), the contents of which are incorporated herein by reference in its entirety. In some embodiments, the AAV particle is administered to the subject intravenously. In some embodiments, the subject is a human.

Administration to the CNS

[0312] In some embodiments, an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) may be delivered by direct injection into the brain. As a non-limiting example, the brain delivery may be by intrahippocampal administration. In some embodiments, an AAV particle of the present disclosure may be administered to a subject by intraparenchymal administration. In some embodiments, the intraparenchymal administration is to tissue of the central nervous system. In some embodiments, an AAV particle of the present disclosure may be administered to a subject by intracranial delivery (See, e.g., US Pat. No. 8119611; the content of which is incorporated herein by reference in its entirety). In some embodiments, an AAV particle described herein may be delivered by injection into the CSF pathway. Non-limiting examples of delivery to the CSF pathway include intrathecal and intracerebroventricular administration. In some embodiments, an AAV particle described herein may be administered via intracisternal magna (ICM) injection.

[0313] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be delivered to the brain by systemic delivery. As a non-limiting example, the systemic delivery may be by intravascular administration. As a non-limiting example, the systemic or intravascular administration may be intravenous.

[0314] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of the present disclosure may be delivered by an intraocular delivery route. A non-limiting example of an intraocular administration includes an intravitreal injection.

Intramuscular administration

[0315] In some embodiments, an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) may be delivered by intramuscular administration. Without wishing to be bound by theory, it is believed in some embodiments, that the multi-nucleated nature of muscle cells provides an advantage to gene transduction subsequent to AAV delivery. In some embodiments, cells of the muscle are capable of expressing recombinant proteins with the appropriate post-translational modifications. Without wishing to be bound by theory, it is believed in some embodiments, the enrichment of muscle tissue with vascular structures allows for transfer to the blood stream and whole-body delivery. Examples of intramuscular administration include systemic (e.g.,

intravenous), subcutaneous or directly into the muscle. In some embodiments, more than one injection is administered. In some embodiments, an AAV particle of the present disclosure may be delivered by an intramuscular delivery route. (See, e.g., U. S. Pat. No. 6506379; the content of which is incorporated herein by reference in its entirety). Non-limiting examples of intramuscular administration include an intravenous injection or a subcutaneous injection.

[0316] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to a subject and transduces the muscle of a subject. As a non-limiting example, an AAV particle is administered by intramuscular administration. In some embodiments, an AAV particle of the present disclosure may be administered to a subject by subcutaneous administration. In some embodiments, the intramuscular administration is via systemic delivery. In some embodiments, the intramuscular administration is via intravenous delivery. In some embodiments, the intramuscular administration is via direct injection to the muscle.

[0317] In some embodiments, the muscle is transduced by administration, e.g., intramuscular administration. In some embodiments, an intramuscular delivery comprises administration at one site. In some embodiments, an intramuscular delivery comprises administration at more than one site. In some embodiments, an intramuscular delivery comprises administration at two, three, four, or more sites. In some embodiments, intramuscular delivery is combined with at least one other method of administration.

[0318] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be administered to a subject by peripheral injections. Non-limiting examples of peripheral injections include intraperitoneal, intramuscular, intravenous, conjunctival, or joint injection. It was disclosed in the art that the peripheral administration of AAV vectors can be transported to the central nervous system, for example, to the motor neurons (e.g., U. S. Patent Publication Nos. US20100240739 and US20100130594; the content of each of which is incorporated herein by reference in their entirety).

[0319] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be administered to a subject by intraparenchymal administration. In some embodiments, the intraparenchymal administration is to muscle tissue. In some embodiments, an AAV particle of the present disclosure is delivered as described in Bright et al 2015 (Neurobiol Aging. 36(2):693-709), the contents of which are herein incorporated by reference in their entirety. In some embodiments, an AAV particle of the present disclosure is administered to the gastrocnemius muscle of a subject. In some embodiments, an AAV particle of the present disclosure is administered to the bicep femorii of the subject. In some embodiments, an AAV particles of the present disclosure is administered to the tibialis anterior muscles. In some embodiments, an AAV particle of the present disclosure is administered to the soleus muscle.

Depot administration

[0320] As described herein, in some embodiments, a pharmaceutical composition and/or an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) are formulated in depots for extended release. Generally, specific organs or tissues are targeted for administration.

[0321] In some embodiments, a pharmaceutical composition and/or an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) are spatially retained within or proximal to target tissues. Provided are methods of providing a pharmaceutical composition, an AAV particle, to target tissues of mammalian subjects by contacting target tissues (which comprise one or more target cells) with the pharmaceutical composition and/or the AAV particle, under conditions such that they are substantially retained in target tissues, e.g., such that at least 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the composition is retained in the target tissues. In some embodiments, retention is determined by measuring the amount of pharmaceutical composition and/or AAV particle that enter a target cell or a plurality of target cells. For example, at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, or greater than 99.99% of a pharmaceutical composition and/or an AAV particle, administered to a subject are present intracellularly at a period of time following administration. For example, intramuscular injection to a subject may be performed using aqueous compositions comprising a pharmaceutical composition and/or an AAV particle of the present disclosure and a transfection reagent, and retention is determined by measuring the amount of the pharmaceutical composition and/or the AAV particle, present in the muscle cell or plurality of muscle cells.

[0322] In some embodiments, disclosed herein are methods of providing a pharmaceutical composition and/or an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) to a tissue of a subject, by contacting the tissue (comprising a cell, e.g., a plurality of cells) with the pharmaceutical composition and/or the AAV particle under conditions such that they are substantially retained in the tissue. In some embodiments, a pharmaceutical composition and/or AAV particle described herein comprise a sufficient amount of an active ingredient such that the effect of interest is produced in at least one cell. In some embodiments, a pharmaceutical composition and/or an AAV particle generally comprise one or more cell penetration agents. In some embodiments, the disclosure provides a naked formulations (such as without cell penetration agents or other agents), with or without pharmaceutically acceptable carriers.

Methods of Treatment

[0323] Provided in the present disclosure are methods for introducing (e.g., delivering) an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) into cells. In some embodiments, the method comprises introducing into said cells an AAV particle or vector

described herein in an amount sufficient to modulate, e.g., increase, the production of a target gene, mRNA, and/or protein. In some embodiments, the method comprises introducing into said cells an AAV particle or vector described herein in an amount sufficient to modulate, e.g., decrease, expression of a target gene, mRNA, and/or protein. In some aspects, the cells may be neurons such as but not limited to, motor, hippocampal, entorhinal, thalamic, cortical, sensory, sympathetic, or parasympathetic neurons, and glial cells such as astrocytes, microglia, and/or oligodendrocytes. In other aspects, the cells may be a muscle cell, e.g., a cell of a diaphragm, a quadriceps, or a heart (e.g., a heart atrium or a heart ventricle). In other embodiments, the cells may be a muscle cell (e.g., a cell of a diaphragm, a quadriceps, or a heart (e.g., a heart atrium or a heart ventricle)) or a liver cell. In some embodiments, the cell may be a heart cell (e.g., a cell of a heart atrium or a cell of a heart ventricle).

[0324] Disclosed in the present disclosure are methods for treating a neurological disease/disorder or a neurodegenerative disorder, a muscular or neuromuscular disorder, or a neuro-oncological disorder associated with aberrant, e.g., insufficient or increased, function/presence of a protein, e.g., a target protein in a subject in need of treatment.

[0325] In some embodiments, the method comprises administering to the subject a therapeutically effective amount of a composition comprising AAV particles of the present disclosure. As a non-limiting example, the AAV particles can increase target gene expression, increase target protein production, and thus reduce one or more symptoms of neurological disease in the subject such that the subject is therapeutically treated.

[0326] In other embodiments, the method comprises administering to the subject a therapeutically effective amount of a composition comprising AAV particles (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) comprising a viral genome with a nucleic acid sequence encoding one or more siRNA molecules. As a non-limiting example, the siRNA molecules can silence target gene expression, inhibit target protein production, and reduce one or more symptoms of neurological disease in the subject such that the subject is therapeutically treated.

[0327] In some embodiments, the composition comprising the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant described herein) is administered to the central nervous system of the subject via systemic administration. In some embodiments, the systemic administration is intravenous (IV) injection. In some embodiments, the AAV particle described herein or a pharmaceutical composition comprising an AAV particle described herein is administered by focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB) or MRI-guided FUS coupled with intravenous administration.

[0328] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via intraventricular administration. In some embodiments, the

composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered via intra-cisterna magna injection (ICM).

[0329] In some embodiments, the composition comprising an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via intraventricular injection and intravenous injection.

[0330] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via ICM injection and intravenous injection at a specific dose per subject. As a non-limiting example, the AAV particles are administered via ICM injection at a dose of 1×10^4 VG per subject. As a non-limiting example, the AAV particles are administered via IV injection at a dose of 2×10^{13} VG per subject.

[0331] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject. In other embodiments, the composition comprising the AAV particles of the present disclosure is administered to a CNS tissue of a subject (e.g., putamen, hippocampus, thalamus, or cortex of the subject).

[0332] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via intraparenchymal injection. Non-limiting examples of intraparenchymal injections include intraputamenal, intracortical, intrathalamic, intrastriatal, intrahippocampal or into the entorhinal cortex.

[0333] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via intraparenchymal injection and intravenous injection.

[0334] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via intraventricular injection, intraparenchymal injection and intravenous injection.

[0335] In some embodiments, the composition comprising an AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of a plurality of particles of the present disclosure is administered to a muscle of the subject via intravenous injection. In some embodiments, the composition comprising an AAV particle of a plurality of particles of the present disclosure is administered to a muscle of the subject via intramuscular injection.

[0336] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be delivered into specific types of cells, including, but not limited to, thalamic, hippocampal, entorhinal, cortical, motor, sensory, excitatory, inhibitory,

sympathetic, or parasympathetic neurons; glial cells including oligodendrocytes, astrocytes and microglia; and/or other cells surrounding neurons such as T cells. In some embodiments, an AAV particle of the present disclosure may be delivered into a muscle cell, e.g., a cell of the quadriceps, diaphragm, liver, and/or heart (e.g., heart atrium or heart ventricle).

[0337] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be delivered to a cell or region of the midbrain. In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be delivered to a cell or region of the brain's stem.

[0338] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be delivered to neurons in the putamen, hippocampus, thalamus and/or cortex.

[0339] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a genetic disorder, e.g., an autosomal dominant genetic disorder, an autosomal recessive disorder, X-linked dominant genetic disorder, an X-linked recessive genetic disorder, or a Y-linked genetic disorder. In some embodiments, the genetic disorder is a monogenetic disorder or a polygenic disorder. In some embodiments, treatment of a genetic disorder, e.g., a monogenic disorder, comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy.

[0340] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a neurological disease.

[0341] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for tauopathies.

[0342] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Alzheimer's Disease.

[0343] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Amyotrophic Lateral Sclerosis.

[0344] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Huntington's Disease.

[0345] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Parkinson's Disease.

[0346] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Gaucher disease (GD) (e.g., Type 1 GD, Type 2 GD, or Type 3 GD). In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Parkinson's disease associated with a GBA mutation. In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for dementia with Lewy Bodies (DLB).

[0347] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for spinal muscular atrophy.

[0348] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a leukodystrophy, e.g., Alexander disease, autosomal dominant leukodystrophy with autonomic diseases (ADLD), Canavan disease, cerebrotendinous xanthomatosis (CTX), metachromatic leukodystrophy (MLD), Pelizaeus-Merzbacher disease, or Refsum disease.

[0349] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for chronic or neuropathic pain.

[0350] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a muscular disorder or a neuromuscular disorder.

[0351] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a cardiac disease or heart disease and/or method of improving (e.g., enhancing) cardiac function in a subject. In some embodiments, the cardiac disease is a cardiomyopathy (e.g., arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, or hypertrophic cardiomyopathy), congestive heart failure, tachycardia (e.g., catecholaminergic polymorphic ventricular tachycardia), ischemic heart disease, and/or myocardial infarction.

[0352] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a disease associated with expression of HER2, e.g., a disease associated with overexpression of HER2. In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid

variant) is useful for the treatment, prophylaxis, palliation or amelioration of a HER2-positive cancer. In some embodiments, the HER2-positive cancer is a HER2-positive solid tumor. Additionally, or alternatively, the HER2-positive cancer may be a locally advanced or metastatic HER2-positive cancer. In some instances, the HER2-positive cancer is a HER2-positive breast cancer or a HER2-positive gastric cancer. In some embodiments, the HER2-positive cancer is selected from the group consisting of a HER2-positive gastroesophageal junction cancer, a HER2-positive colorectal cancer, a HER2-positive lung cancer (e.g., a HER2-positive non-small cell lung carcinoma), a HER2-positive pancreatic cancer, a HER2-positive colorectal cancer, a HER2-positive bladder cancer, a HER2-positive salivary duct cancer, a HER2-positive ovarian cancer (e.g., a HER2-positive epithelial ovarian cancer), or a HER2-positive endometrial cancer. In some instances, the HER2-positive cancer is prostate cancer. In some embodiments, the HER2-positive cancer has metastasized to the central nervous system (CNS). In some instances, the metastasized HER2-cancer has formed CNS neoplasms.

[0353] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant) e.g., a plurality of particles, of the present disclosure may be used as a therapy for a neuro-oncological disorder. In some embodiments, the neuro-oncological disorder is a cancer of primary CNS origin (e.g., a cancer of a CNS cell and/or CNS tissue). In some embodiments, the neuro-oncological disorder is metastatic cancer in a CNS cell, CNS region, and/or a CNS tissue. Examples of primary CNS cancers could be gliomas (which may include glioblastoma (also known as glioblastoma multiforme), astrocytomas, oligodendrogliomas, and ependymomas, and mixed gliomas), meningiomas, medulloblastomas, neuromas, and primary CNS lymphoma (in the brain, spinal cord, or meninges), among others. Examples of metastatic cancers include those originating in another tissue or organ, e.g., breast, lung, lymphoma, leukemia, melanoma (skin cancer), colon, kidney, prostate, or other types that metastasize to brain.

[0354] In some embodiments, administration of the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) to a subject may increase target gene, mRNA, and/or protein levels in a subject, relative to a control, e.g., the gene, mRNA, and/or mRNA levels in the subject prior to receiving AAV particle. The target gene, mRNA, and/or protein levels may be increased by about 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100% in a subject such as, but not limited to, the CNS, a region of the CNS, or a specific cell of the CNS, or a muscle, a region of a muscle, or a cell of a muscle, of a subject. In some embodiments, cell of the CNS comprises an astrocyte, microglia, cortical neuron, hippocampal neuron, DRG and/or sympathetic neuron, sensory neuron,

oligodendrocyte, motor neuron, or combination thereof. As a non-limiting example, the AAV particles may increase the gene, mRNA, and/or protein levels of a target protein by fold increases over baseline. In some embodiments, AAV particles lead to 5-6 times higher levels of a target gene, mRNA, or protein.

[0355] In some embodiments, administration of the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant), e.g., an AAV particle comprising a nucleic acid encoding a siRNA molecule, to a subject may decrease target gene, mRNA, and/or protein levels in a subject, relative to a control, e.g., the gene, mRNA, and/or mRNA levels in the subject prior to receiving AAV particle. The target gene, mRNA, and/or protein levels may be decreased by about 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100% in a subject such as, but not limited to, the CNS, a region of the CNS, or a specific cell of the CNS, or a muscle, a region of a muscle, or a cell of a muscle, of a subject. In some embodiments, cell of the CNS comprises an astrocyte, microglia, cortical neuron, hippocampal neuron, DRG and/or sympathetic neuron, sensory neuron, oligodendrocyte, motor neuron, or combination thereof. As a non-limiting example, the AAV particles may decrease the gene, mRNA, and/or protein levels of a target protein by fold decreases over baseline.

[0356] In some embodiments, the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) may be used to increase target protein and reduce symptoms of neurological disease in a subject. In some embodiments, the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) may be used to decrease target protein and reduce symptoms of neurological disease in a subject.

[0357] In some embodiments, the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) may be used to reduce the decline of functional capacity and activities of daily living as measured by a standard evaluation system such as, but not limited to, the total functional capacity (TFC) scale.

[0358] In some embodiments, the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) may be used to improve performance on any assessment used to measure symptoms of neurological disease. Such assessments include, but are not limited to ADAS-cog (Alzheimer Disease Assessment Scale – cognitive), MMSE (Mini-Mental State Examination), GDS (Geriatric Depression Scale), FAQ (Functional Activities Questionnaire), ADL (Activities of Daily Living), GPCOG (General

Practitioner Assessment of Cognition), Mini-Cog, AMTS (Abbreviated Mental Test Score), Clock-drawing test, 6-CIT (6-item Cognitive Impairment Test), TYM (Test Your Memory), MoCa (Montreal Cognitive Assessment), ACE-R (Addenbrookes Cognitive Assessment), MIS (Memory Impairment Screen), BADLS (Bristol Activities of Daily Living Scale), Barthel Index, Functional Independence Measure, Instrumental Activities of Daily Living, IQCODE (Informant Questionnaire on Cognitive Decline in the Elderly), Neuropsychiatric Inventory, The Cohen-Mansfield Agitation Inventory, BEHAVE-AD, EuroQol, Short Form-36 and/or MBR Caregiver Strain Instrument, or any of the other tests as described in Sheehan B (Ther Adv Neurol Disord. 5(6):349-358 (2012)), the contents of which are herein incorporated by reference in their entirety.

[0359] In some embodiments, the present composition is administered as a solo therapeutic or as combination therapeutic for the treatment of a neurological disease/disorder or a neurodegenerative disorder, a muscular disorder or neuromuscular disorder, and/or a neuro-oncological disorder.

[0360] The AAV particles (e.g., an AAV particle comprising an AAV capsid variant) encoding the target protein may be used in combination with one or more other therapeutic agents. In some embodiments, compositions can be administered concurrently with, prior to, or subsequent to, additional therapeutic or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent.

[0361] Therapeutic agents that may be used in combination with the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) can be small molecule compounds which are antioxidants, anti-inflammatory agents, anti-apoptosis agents, calcium regulators, anti-glutamatergic agents, structural protein inhibitors, compounds involved in muscle function, and compounds involved in metal ion regulation. As a non-limiting example, the combination therapy may be in combination with one or more neuroprotective agents such as small molecule compounds, growth factors and hormones which have been tested for their neuroprotective effect on motor neuron degeneration.

[0362] Compounds tested for treating neurological disease which may be used in combination with the AAV particles described herein include, but are not limited to, cholinesterase inhibitors (donepezil, rivastigmine, galantamine), NMDA receptor antagonists such as memantine, anti-psychotics, anti-depressants, anti-convulsants (e.g., sodium valproate and levetiracetam for myoclonus), secretase inhibitors, amyloid aggregation inhibitors, copper or zinc modulators, BACE inhibitors, inhibitors of tau aggregation, such as Methylene blue, phenothiazines, anthraquinones, n-phenylamines or rhodamines, microtubule stabilizers such as NAP, taxol or paclitaxel, kinase or phosphatase inhibitors such as those targeting GSK3 β (lithium) or PP2A, immunization with A β peptides or tau phospho-epitopes, anti-tau or anti-amyloid antibodies, dopamine-depleting agents (e.g., tetrabenazine for chorea), benzodiazepines (e.g., clonazepam for myoclonus, chorea, dystonia, rigidity, and/or spasticity), , amino acid precursors of dopamine (e.g., levodopa for rigidity), skeletal

muscle relaxants (e.g., baclofen, tizanidine for rigidity and/or spasticity), inhibitors for acetylcholine release at the neuromuscular junction to cause muscle paralysis (e.g., botulinum toxin for bruxism and/or dystonia), atypical neuroleptics (e.g., olanzapine and quetiapine for psychosis and/or irritability, risperidone, sulpiride and haloperidol for psychosis, chorea and/or irritability, clozapine for treatment-resistant psychosis, aripiprazole for psychosis with prominent negative symptoms), selective serotonin reuptake inhibitors (SSRIs) (e.g., citalopram, fluoxetine, paroxetine, sertraline, mirtazapine, venlafaxine for depression, anxiety, obsessive compulsive behavior and/or irritability), hypnotics (e.g., xopiclone and/or zolpidem for altered sleep-wake cycle), anticonvulsants (e.g., sodium valproate and carbamazepine for mania or hypomania) and mood stabilizers (e.g., lithium for mania or hypomania).

[0363] Neurotrophic factors may be used in combination therapy with the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) for treating neurological disease. Generally, a neurotrophic factor is defined as a substance that promotes survival, growth, differentiation, proliferation and/or maturation of a neuron, or stimulates increased activity of a neuron. In some embodiments, the present methods further comprise delivery of one or more trophic factors into the subject in need of treatment. Trophic factors may include, but are not limited to, IGF-I, GDNF, BDNF, CTNF, VEGF, Colivelin, Xaliproden, Thyrotrophin-releasing hormone and ADNF, and variants thereof.

[0364] In one aspect, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) may be co-administered with AAV particles expressing neurotrophic factors such as AAV-IGF-I (See e.g., Vincent et al., *Neuromolecular medicine*, 2004, 6, 79-85; the contents of which are incorporated herein by reference in their entirety) and AAV-GDNF (See e.g., Wang et al., *J Neurosci.*, 2002, 22, 6920-6928; the contents of which are incorporated herein by reference in their entirety).

[0365] In some embodiments, administration of the AAV particles (e.g., an AAV particle comprising an AAV capsid variant) to a subject will modulate, e.g., increase or decrease, the expression of a target protein in a subject and the modulation, e.g., increase or decrease of the presence, level, activity, and/or expression of the target protein will reduce the effects and/or symptoms of a neurological disease/disorder or a neurodegenerative disorder, a muscular disorder or neuromuscular disorder, and/or a neuro-oncological disorder in a subject.

DEFINITIONS

[0366] 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.

[0367] Articles such as “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.

[0368] It is also noted that the term “comprising” is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term “comprising” is used herein, the term “consisting of” and “consisting essentially thereof” is thus also encompassed and disclosed.

[0369] 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.

[0370] *Adeno-associated virus:* As used herein, the term “adeno-associated virus” or “AAV” refers to members of the dependovirus genus or a variant, e.g., a functional variant, thereof. In some embodiments, the AAV is wildtype, or naturally occurring. In some embodiments, the AAV is recombinant.

[0371] *AAV Particle:* As used herein, an “AAV particle” refers to a particle or a virion comprising an AAV capsid, e.g., an AAV capsid variant, and a polynucleotide, e.g., a viral genome or a vector genome. In some embodiments, the viral genome of the AAV particle comprises at least one payload region and at least one ITR. In some embodiments, an AAV particle of the disclosure is an AAV particle comprising an AAV variant. In some embodiments, the AAV particle is capable of delivering a nucleic acid, e.g., a payload region, encoding a payload to cells, typically, mammalian, e.g., human, cells. In some embodiments, an AAV particle of the present disclosure may be produced recombinantly. In some embodiments, 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. It is to be understood that reference to the AAV particle of the disclosure also includes pharmaceutical compositions thereof, even if not explicitly recited.

[0372] *Administering:* As used herein, the term “administering” refers to providing a pharmaceutical agent or composition to a subject.

[0373] *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 neurodegeneration disorder, amelioration includes the reduction of neuron loss.

[0374] *Amplicon:* As used herein, “amplicon” may refer to any piece of RNA or DNA formed as the product of amplification events, e.g. PCR. In some embodiments, full-length capsid amplicons may be used as templates for next generation sequencing (NGS) library generation. Full-length capsid amplicons may be used for cloning into a DNA library for any number of additional rounds of AAV selection as described herein.

[0375] *Animal:* As used herein, the term “animal” refers to any member of the animal kingdom. In some embodiments, “animal” refers to humans at any stage of development. In some embodiments, “animal” refers to non-human animals at any stage of development. In certain embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, and worms. In some embodiments, the animal is a transgenic animal, genetically engineered animal, or a clone.

[0376] *Antisense strand:* As used herein, the term “the antisense strand” or “the first strand” or “the guide strand” of a siRNA molecule refers to a strand that is substantially complementary to a section of about 10-50 nucleotides, e.g., about 15-30, 16-25, 18-23 or 19-22 nucleotides of the mRNA of a gene targeted for silencing. The antisense strand or first strand has sequence sufficiently complementary to the desired target mRNA sequence to direct target-specific silencing, e.g., complementarity sufficient to trigger the destruction of the desired target mRNA by the RNAi machinery or process.

[0377] *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 a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0378] *Biopanning:* As used herein, the term “biopanning” refers to an AAV capsid library selection process comprising administration of an AAV particle with enhanced tissue- and/or cell type-specific transduction to a cell and/or subject; extraction of nucleotides encoded by said AAV particle from said transduced tissue- and/or cell type-specific; and, use of the extracted nucleotides for cloning into a nucleotide library for the generation of AAV particles for subsequent rounds of the same.

[0379] *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.” In some embodiments, the AAV capsid variant described herein has the ability to enclose, e.g., encapsulate, 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.

[0380] *Complementary and substantially complementary:* As used herein, the term “complementary” refers to the ability of polynucleotides to form base pairs with one another. Base pairs are typically formed by hydrogen bonds between nucleotide units in antiparallel polynucleotide strands. Complementary polynucleotide strands can form base pairs in the Watson-Crick manner (e.g., A to T, A to U, C to G), or in any other manner that allows for the formation of duplexes. As persons skilled in the art are aware, when using RNA as opposed to DNA, uracil rather than thymine is the base that is considered to be complementary to adenine. However, when a U is denoted in the context of the present disclosure, the ability to substitute a T is implied, unless otherwise stated. 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, partially complementary, or substantially complementary. As used herein, the term “substantially complementary” means that the siRNA has a sequence (e.g., in the antisense strand) which is sufficient to bind the desired target mRNA, and to trigger the RNA silencing of the target mRNA. “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.

[0381] *Control Elements:* As used herein, “control elements”, “regulatory control elements” or “regulatory sequences” refers to promoter regions, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites (“IRES”), enhancers, and the like, which provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these control elements need always be present as long as the selected coding sequence is capable of being replicated, transcribed and/or translated in an appropriate host cell.

[0382] *Delivery*: As used herein, “delivery” refers to the act or manner of delivering an AAV particle, a compound, substance, entity, moiety, cargo or payload.

[0383] *Element*: As used herein, the term “element” refers to a distinct portion of an entity. In some embodiments, an element may be a polynucleotide sequence with a specific purpose, incorporated into a longer polynucleotide sequence.

[0384] *Encapsulate*: As used herein, the term “encapsulate” means to enclose, surround or encase. As an example, a capsid protein, e.g., an AAV capsid variant, often encapsulates a viral genome. In some embodiments, encapsulate within a capsid, e.g., an AAV capsid variant, encompasses 100% coverage by a capsid, as well as less than 100% coverage, e.g., 95%, 90%, 85%, 80%, 70%, 60% or less. For example, gaps or discontinuities may be present in the capsid so long as the viral genome is retained in the capsid, e.g., prior to entry into a cell.

[0385] *Effective Amount*: As used herein, the term “effective amount” of an agent is that amount sufficient to effect beneficial or desired results, for example, clinical results, and, as such, an “effective amount” depends upon the context in which it is being applied. For example, in the context of administering an agent that treats cancer, an effective amount of an agent is, for example, an amount sufficient to achieve treatment, as defined herein, of cancer, as compared to the response obtained without administration of the agent.

[0386] *Expression*: As used herein, “expression” of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end processing); (3) translation of an RNA into a polypeptide or protein; and (4) post-translational modification of a polypeptide or protein.

[0387] *Formulation*: As used herein, a “formulation” includes at least one AAV particle (active ingredient) and an excipient, and/or an inactive ingredient.

[0388] *Fragment*: A “fragment,” as used herein, refers to a portion. For example, an antibody fragment may comprise a CDR, or a heavy chain variable region, or a scFv, etc.

[0389] *Homology*: As used herein, the term “homology” 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. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical or similar. The term “homologous” necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences). In accordance with the disclosure, two polynucleotide sequences are considered to be homologous if the polypeptides they encode are at least about 50%, 60%, 70%, 80%, 90%, 95%, or even 99% for at least one stretch of at least about 20 amino acids. In some embodiments, homologous polynucleotide sequences are characterized by the ability to encode a

stretch of at least 4–5 uniquely specified amino acids. For polynucleotide sequences less than 60 nucleotides in length, homology is determined by the ability to encode a stretch of at least 4–5 uniquely specified amino acids. In accordance with the disclosure, two protein sequences are considered to be homologous if the proteins are at least about 50%, 60%, 70%, 80%, or 90% identical for at least one stretch of at least about 20 amino acids.

[0390] *Identity:* As used herein, the term “identity” 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 the percent identity of two polynucleotide sequences, for example, can 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). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the length of the reference sequence. 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; the contents of each of which are incorporated herein by reference in their entirety. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers 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. Techniques for determining identity are codified in publicly available computer programs. Exemplary 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)), BLASTP, BLASTN, and FASTA Altschul, S. F. *et al.*, *J. Molec. Biol.*, 215, 403 (1990)).

[0391] *Inhibit expression of a gene:* As used herein, the phrase “inhibit expression of a gene” means to cause a reduction in the amount of an expression product of the gene. The expression product can be an RNA transcribed from the gene (*e.g.*, an mRNA) or a polypeptide translated from an mRNA transcribed from the gene. Typically, a reduction in the level of an mRNA results in a reduction in the level of a polypeptide translated therefrom. The level of expression may be determined using standard techniques for measuring mRNA or protein.

[0392] *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.

[0393] *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.

[0394] *Library:* As used herein, the term “library” refers to a diverse collection of linear polypeptides, polynucleotides, viral particles, or viral vectors. As examples, a library may be a DNA library or an AAV capsid library.

[0395] *Molecular scaffold:* As used herein a “molecular scaffold” is a framework or starting molecule that forms the sequence or structural basis against which to design or make a subsequent molecule.

[0396] *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).

[0397] *Orthogonal evolution:* As used herein, the term “orthogonal evolution” refers to a method wherein AAV particles are administered for a first round of AAV selection as described herein across a set of any number of cell- and/or subject-types that may be from different species and/or strains, and wherein any number of additional, *i.e.*, subsequent, AAV selection rounds are performed either across a set of any number of cell- and/or subject-types that may be from different species and/or strains, or across a set of any number of cell- and/or subject-types that may be from the same species and/or strain.

[0398] *Open reading frame:* As used herein, “open reading frame” or “ORF” refers to a sequence which does not contain a stop codon in a given reading frame.

[0399] *Particle:* As used herein, a “particle” is a virus comprised of at least two components, a protein capsid and a polynucleotide sequence enclosed within the capsid.

[0400] *Payload region:* As used herein, a “payload region” is any nucleic acid sequence (e.g., within the viral genome) which encodes one or more “payloads” of the disclosure. As non-limiting examples, a payload region may be a nucleic acid sequence within the viral genome of an AAV particle, which encodes a payload, wherein the payload is an RNAi agent or a polypeptide. Payloads of the present disclosure may be, but are not limited to, peptides, polypeptides, proteins, antibodies, RNAi agents, etc.

[0401] *Polypeptide:* As used herein, “polypeptide” means a polymer of amino acid residues (natural or unnatural) linked together most often by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. In some instances, the polypeptide encoded is smaller than about 50 amino acids and the polypeptide is then termed a peptide. If the polypeptide is a peptide, it will be at least about 2, 3, 4, or at least 5 amino acid residues long. Thus, polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide may be a single molecule or may be a multi-molecular complex such as a dimer, trimer or tetramer. They may also comprise single chain or multichain polypeptides and may be associated or linked. The term polypeptide may also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid.

[0402] *Polypeptide variant:* The term “polypeptide variant” refers to molecules which differ in their amino acid sequence from a native or reference sequence. The amino acid sequence variants may possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. In some embodiments, a variant comprises a sequence having at least about 50%, at least about 80%, or at least about 90%, identical (homologous) to a native or a reference sequence.

[0403] *Peptide:* As used herein, “peptide” is less than or equal to 50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[0404] *Pharmaceutically acceptable:* The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0405] *Preventing*: As used herein, the term “preventing” or “prevention” 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.

[0406] *Prophylactic*: As used herein, “prophylactic” refers to a therapeutic or course of action used to prevent the spread of disease.

[0407] *Prophylaxis*: As used herein, a “prophylaxis” refers to a measure taken to maintain health and prevent the spread of disease.

[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, an epitope and/or a cluster of epitopes. 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.

[0409] In some embodiments, 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.

[0410] *RNA or RNA molecule*: As used herein, the term “RNA” or “RNA molecule” or “ribonucleic acid molecule” refers to a polymer of ribonucleotides; the term “DNA” or “DNA molecule” or “deoxyribonucleic acid molecule” refers to a polymer of deoxyribonucleotides. DNA and RNA can be synthesized naturally, e.g., by DNA replication and transcription of DNA, respectively; or be chemically synthesized. DNA and RNA can be single-stranded (i.e., ssRNA or ssDNA, respectively) or multi-stranded (e.g., double stranded, i.e., dsRNA and dsDNA, respectively). The term “mRNA” or “messenger RNA”, as used herein, refers to a single stranded RNA that encodes the amino acid sequence of one or more polypeptide chains.

[0411] *RNA interfering or RNAi*: As used herein, the term “RNA interfering” or “RNAi” refers to a sequence specific regulatory mechanism mediated by RNA molecules which results in the inhibition or interfering or “silencing” of the expression of a corresponding protein-coding gene. RNAi has been observed in many types of organisms, including plants, animals and fungi. RNAi occurs in cells naturally to remove foreign RNAs (e.g., viral RNAs). Natural RNAi proceeds via fragments cleaved

from free dsRNA which direct the degradative mechanism to other similar RNA sequences. RNAi is controlled by the RNA-induced silencing complex (RISC) and is initiated by short/small dsRNA molecules in cell cytoplasm, where they interact with the catalytic RISC component argonaute. The dsRNA molecules can be introduced into cells exogenously. Exogenous dsRNA initiates RNAi by activating the ribonuclease protein Dicer, which binds and cleaves dsRNAs to produce double-stranded fragments of 21-25 base pairs with a few unpaired overhang bases on each end. These short double stranded fragments are called small interfering RNAs (siRNAs).

[0412] *RNAi agent*: As used herein, the term “RNAi agent” refers to an RNA molecule, or its derivative, that can induce inhibition, interfering, or “silencing” of the expression of a target gene and/or its protein product. An RNAi agent may knock-out (virtually eliminate or eliminate) expression, or knock-down (lessen or decrease) expression. The RNAi agent may be, but is not limited to, dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA.

[0413] *miR binding site*: As used herein, a “miR binding site” comprises a nucleic acid sequence (whether RNA or DNA, e.g., differ by “U” of RNA or “T” in DNA) that is capable of binding, or binds, in whole or in part to a microRNA (miR) through complete or partial hybridization. Typically, such binding occurs between the miR and the miR binding site in the reverse complement orientation. In some embodiments, the miR binding site is transcribed from the AAV vector genome encoding the miR binding site.

[0414] In some embodiments, a miR binding site may be encoded or transcribed in series. Such a “miR binding site series” or “miR BSs” may include two or more miR binding sites having the same or different nucleic acid sequence.

[0415] *Spacer*: As used here, 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. Spacers may also be more than 10 nucleotides in length, e.g., 20, 30, 40, or 50 or more than 50 nucleotides.

[0416] *Sample*: As used herein, the term “sample” or “biological sample” refers to a subset of its tissues, cells, nucleic acids, or component parts (e.g. body fluids, including but not limited to blood, serum, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen).

[0417] *Self-complementary viral particle*: As used herein, a “self-complementary viral particle” is a particle comprised of at least two components, a protein capsid and a self-complementary viral genome enclosed within the capsid.

[0418] *Sense Strand*: As used herein, the term “the sense strand” or “the second strand” or “the passenger strand” of a siRNA molecule refers to a strand that is complementary to the antisense strand or first strand. The antisense and sense strands of a siRNA molecule are hybridized to form a duplex

structure. As used herein, a “siRNA duplex” includes a siRNA strand having sufficient complementarity to a section of about 10-50 nucleotides of the mRNA of the gene targeted for silencing and a siRNA strand having sufficient complementarity to form a duplex with the other siRNA strand.

[0419] *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.

[0420] *Short interfering RNA or siRNA:* As used herein, the terms “short interfering RNA,” “small interfering RNA” or “siRNA” refer to an RNA molecule (or RNA analog) comprising between about 5-60 nucleotides (or nucleotide analogs) which is capable of directing or mediating RNAi. Preferably, a siRNA molecule comprises between about 15-30 nucleotides or nucleotide analogs, such as between about 16-25 nucleotides (or nucleotide analogs), between about 18-23 nucleotides (or nucleotide analogs), between about 19-22 nucleotides (or nucleotide analogs) (*e.g.*, 19, 20, 21 or 22 nucleotides or nucleotide analogs), between about 19-25 nucleotides (or nucleotide analogs), and between about 19-24 nucleotides (or nucleotide analogs). The term “short” siRNA refers to a siRNA comprising 5-23 nucleotides, preferably 21 nucleotides (or nucleotide analogs), for example, 19, 20, 21 or 22 nucleotides. The term “long” siRNA refers to a siRNA comprising 24-60 nucleotides, preferably about 24-25 nucleotides, for example, 23, 24, 25 or 26 nucleotides. Short siRNAs may, in some instances, include fewer than 19 nucleotides, *e.g.*, 16, 17 or 18 nucleotides, or as few as 5 nucleotides, provided that the shorter siRNA retains the ability to mediate RNAi. Likewise, long siRNAs may, in some instances, include more than 26 nucleotides, *e.g.*, 27, 28, 29, 30, 35, 40, 45, 50, 55, or even 60 nucleotides, provided that the longer siRNA retains the ability to mediate RNAi or translational repression absent further processing, *e.g.*, enzymatic processing, to a short siRNA. siRNAs can be single stranded RNA molecules (ss-siRNAs) or double stranded RNA molecules (ds-siRNAs) comprising a sense strand and an antisense strand which hybridized to form a duplex structure called an siRNA duplex.

[0421] *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. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants.

[0422] *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.

[0423] *Target Cells:* As used herein, “target cells” or “target tissue” 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 patient.

[0424] *Therapeutic Agent:* The term “therapeutic agent” refers to any agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect.

[0425] *Therapeutically effective amount:* As used herein, the term “therapeutically effective amount” means an amount of an agent to be delivered (*e.g.*, nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, *etc.*) that is sufficient, when administered to a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is provided in a single dose.

[0426] *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 an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[0427] *Treating:* As used herein, the term “treating” refers to partially or completely alleviating, ameliorating, improving, relieving, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular infection, disease, disorder, and/or condition. For example, “treating” cancer may refer to inhibiting survival, growth, and/or spread of a tumor. 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.

[0428] *Conservative amino acid substitution:* As used herein, a “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains 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).

[0429] *Variant*: As used herein, the term “variant” refers to a polypeptide or polynucleotide that has an amino acid or a nucleotide sequence that is substantially identical, e.g., having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to a reference sequence. In some embodiments, the variant is a functional variant.

[0430] *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.

[0431] *Insertional Variant*: “Insertional variants” when referring to polypeptides are those with one or more amino acids inserted, e.g., immediately adjacent or subsequent, to a position in an amino acid sequence. “Immediately adjacent” or “immediately subsequent” to an amino acid means connected to either the alpha-carboxy or alpha-amino functional group of the amino acid.

[0432] *Deletional Variant*: “Deletional variants” when referring to polypeptides, are those with one or more amino acids in deleted from a reference protein.

[0433] *Vector*: As used herein, the term “vector” refers to any molecule or moiety which transports, transduces or otherwise acts as a carrier of a heterologous molecule. In some embodiments, vectors may be plasmids. In some embodiments, vectors may be viruses. An AAV particle is an example of a vector. 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 sequences. The heterologous molecule may be a polynucleotide and/or a polypeptide.

[0434] *Viral Genome*: As used herein, the terms “viral genome” or “vector genome” refer to the nucleic acid sequence(s) encapsulated in an AAV particle. A viral genome comprises a nucleic acid sequence with at least one payload region encoding a payload and at least one ITR.

Equivalents and Scope

[0435] The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. 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 disclosure described herein. The scope of the present disclosure is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[0436] 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 antibiotic, 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.

[0437] 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.

[0438] 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.

[0439] The present disclosure is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1. High-throughput screen of TRACER AAV library in non-human primates (NHPs)

[0440] 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.

[0441] 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.

[0442] 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

[0443] 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

[0444] 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 4 and 5, respectively.

A. Evaluation of TTJ-001 in Cynomolgus macaques

[0445] AAV particles were generated with this capsid variant encapsulating a payload-HA tag driven by a heterologous constitutive promoter. The capsid variant and AAV9 control were tested by intravenously administering the AAV particle formulation at 2×10^{13} 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.

[0446] 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 TTJ-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 TTJ-001 capsid variant compared to wild-type AAV9 in the brain of NHPs. Additionally, mRNA expression was approximately 3-274-fold higher from the TTJ-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 TTJ-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
TTJ-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

[0447] 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

[0448] 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.

[0449] 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.

[0450] Taken together, these data demonstrate that TTJ-001 is an enhanced CNS tropic capsids 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*)

[0451] 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 17**. 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 17. 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 vg/mL	0.56
AAV9	4.00 vg/mL	1.0

[0452] As shown in **Table 18**, 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 19**) 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.

[0453] 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 18**) and transgene expression (**Table 19**) 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 18** and **Table 19**).

Table 18. 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 19. 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

[0454] 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.

[0455] 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 Cynomolgus macaques

[0456] AAV particles were generated with the TTJ-001 capsid variant comprising a single stranded viral genome encapsulating a payload-HA tag driven by a heterologous constitutive 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).

[0457] As shown in **Tables 20** and **21**, 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 20** and **21**).

[0458] 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 was 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 22**). 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 22**). 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 22**). The percent HA+ cells in **Table 22** may underestimate true positivity for the TTJ-001 capsid variant, as it demonstrated neuronal tropism.

[0459] 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 20**) and transgene expression (**Table 21**) 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 20** and **21**). Similar transduction and expression was 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 20. 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 21. 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 22. 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

[0460] These data further confirm that TTJ-001 is a CNS tropic capsid, capable of crossing the blood brain barrier following intravenous administration in NHPs.

Example 3. Evaluation of TTJ-001 AAV9 capsid variant in Diverse Primate Species

[0461] 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.

[0462] 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.

[0463] 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.

[0464] As provided in **Table 12** (African green monkeys) and **Table 13** (marmosets), the TTJ-001 capsid variant demonstrated increased CNS tropism in diverse primate species. 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 12. 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 13. 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

[0465] 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

[0466] 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 peptide insert 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.

[0467] 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.

[0468] 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.

[0469] **Table 14** 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, and 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 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 14. 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
TNTKDWHRRIAQTGWV	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
TNTQDWHRVPTGWV	216	110.962	1.278	96.982	0.904	5.602	1.887
TNTQDWHRIAQTNV	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
TNTQDWHRVSTQTGWV	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
TNTQHWHRIAQTGWV	251	28.154	0.324	71.807	0.669	1.630	0.549
TNTQNWHRRIAQTGWV	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
TNTQAWHRRIAQTGWV	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		
TNTQDWHRIINQTGWV	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

[0470] Table 15 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 15. 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
TNHQSAQAQAQTGWV	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
TNTKDWHRRIAQTGWV	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
TSTKDWHRRIAQTGWV	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
TNTQDWHRVPQTGWV	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
TNTQDWHRIAQTGWV	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
TNNQDSHRRIAQTGWV	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
TNNQDQWRIAQTGWV	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
TNTQDWHRIAQTINWV	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
TNSQDWHRIAQTGWA	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
TNTQDWHRIAQTGWV	226	52.469	0.713	5.899	0.485	1.515	0.836
TNMQDWHRIAQTGWV	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
TITQDWHRTAQTGWV	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
TNNQDQGRIAQTGWV	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
TNTQDWHRIAQTGWA	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
TNNQDWSIAQTGWV	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
TYTQDWHRTAQTGWV	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
TNTQDWHRIPQTGWV	244	33.735	0.458	12.324	1.014	1.295	0.715
TNSQDWHRIPQTGWV	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
TNTQDWHRIAQTGWG	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		
TNTPDWHRIQTGWV	264	17.286	0.235	5.269	0.433		

[0471] Table 16 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 16. NGS fold-enrichment of TTJ-001 in the brain of cynomolgus macaques by both maturation approaches

First Maturation Approach			
Sequence	SEQ ID NO:	Fold change relative to AAV9	Fold change relative to TTJ-001
TNTKQDWHRIQTGWV	202	104073.7	1198.899
TNNQDWHRIQTGWV	203	5142.077	59.23527
SNNQDWHRIQTGWV	271	2083.962	24.00665
TNKQDWHRIQTGWV	539	2062.769	23.76252
TNNQDWHRIQTGWV	540	428.3846	4.934869
SNKQDWHRIQTGWV	541	372.0385	4.285778
SNTQDWHRIQTGWV	204	328.3077	3.782012
TTNQDWHRIQTGWV	205	276.0769	3.180328
TNDQDWHRIQTGWV	542	274.6538	3.163934
TNTKDRHRIQTGWV	543	265.9615	3.063802
TTIQDWHRIQTGWV	206	260.2308	2.997785
RNTQDWHRIQTGWV	544	242.5769	2.794417
TNTKDSHRIQTGWV	545	238.7308	2.750111
TTKQDWHRIQTGWV	546	221.8077	2.555162
TTSQDWHRIQTGWV	207	210.0769	2.420027
TTDQDWHRIQTGWV	547	204.7308	2.35844
TNTKDLHRIQTGWV	548	199.8846	2.302614
TNPQDWHRIQTGWV	549	181.500	2.090829
TNKQDWHRIQTGWV	550	174.4231	2.009304
TTTQDWHRIQTGWV	208	169.5769	1.953478
TGKQDWHRIQTGWV	551	168.9615	1.946389
TTAQDWHRIQTGWV	209	164.4615	1.89455
TVKQDWHRIQTGWV	210	160.3077	1.846699
TNNQDQRIQTGWV	304	154.0385	1.774479
TNYQDWHRIQTGWV	552	153.7692	1.771378
TNTPDWHRIQTGWV	264	150.1923	1.730173
STKQDWHRIQTGWV	553	148.3846	1.709349
TNTQDWHRIQTGWA	211	147.6923	1.701374
TNTQDWHRIQTGWV	212	146.2308	1.684537
TNTQDWHRIQTNWS	554	137.6923	1.586176
TTEQDWHRIQTGWV	555	134.2308	1.5463
TSKQDWHRIQTGWV	556	134.1154	1.544971
TNIQDWHRIQTGWV	213	132.4231	1.525476

TNTQDWHRIAGSNWV	214	122.1154	1.406735
TNTQDWHRIAGTNWV	215	122.0769	1.406292
TNTQDWHRIAQTNIWI	557	120.2692	1.385467
TNTQDWHRIYQTGWV	263	117	1.347807
TYNQDWHRIAQTGWV	281	116.0769	1.337173
TNTQDWHRVPTGWV	216	110.9615	1.278245
TNTQDWHRIANNWV	262	109.0385	1.256092
TNTQDWHRIAQTNIWV	217	106.3462	1.225078
STIQDWHRIAQTGWV	218	106.0769	1.221976
TTVQDWHRIAQTGWV	219	105.9615	1.220647
TGTQDWHRIAQTGWV	220	105.7692	1.218432
TNTQDWHRIINQTGWV	265	104.9615	1.209127
TNNQEWHRRIAQTGWV	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
TNTQDWHRISGTGWV	228	77.07692	0.887904
TNSQDWHRIAQTGWV	229	73.23077	0.843598
TNHQSAQAQAQTGWV	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
TNFKDWHRIAQTGWV	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
TNTKDWHRVQAQTGWV	4680	10573.939	143.644
TIITKDWHRIAQTGWV	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
TNTKDWHRKIAQTGWV	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
TNTKDWHRIAQTDWV	595	1132.163	15.380
TNTKDWHRIAQTGCV	596	1118.531	15.195
TNTKDWQRIAQTGWV	597	1042.082	14.156
TGNQDWHRIAQTGWV	598	1024.367	13.916
TNNEDWHRIAQTGWV	540	985.776	13.391
TNTKDWHRLAQTGWV	599	903.531	12.274
TNDQDWHRIAQTGWV	542	900.204	12.229
TNTKDWNRIAQTGWV	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
TNNQDWHRIPQTGWV	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
TNTKDWSIAQTGWV	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
TNTKDCRHIAQTGWV	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
TNNQDWHRIAHTGWV	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
STQDWHRIAQTGWV	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
TNNQEWHRIAQTGWV	558	97.918	1.330
SNNQDWNRIAQTGWV	665	97.122	1.319
TNTQDWHKIAQTGWA	666	96.469	1.311

TNNQDWHRIAQSGWV	667	95.388	1.296
SNTQDWHRIAQTGWG	288	94.020	1.277
TNNQDWHRIAQTGWV	668	93.980	1.277
TNSQDWHRIAQTGWV	669	93.776	1.274
TTSQDWHRIAQTGWV	207	93.306	1.268
TNNQDWHKIAQTGWV	289	92.265	1.253
TTTQDWHRVAQTGWV	290	89.898	1.221
TNSQDWHRIAQTGWA	291	89.245	1.212
TNIQDWHRIAQTGWV	213	89.224	1.212
TNTQDWHRVPQTGWV	216	89.041	1.210
SNTQDWHRISQTGWV	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
TNNQDWLRRIAQTGWV	676	85.082	1.156
ANNQDWHRIAQTGWV	561	84.980	1.154
TNTQDWHRISGTGWV	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
TNNQDWHRIAQTVWV	682	80.429	1.093
TNIQDWHRISQTGWV	294	79.510	1.080
TNTQDWHRIPQTGWA	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
TNNQDWHRIVQTGWV	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
TNSQDWHRTAHTGWV	694	74.510	1.012
TNTQDWHRIAQTGWV	201	73.612	1.000
SNNQDWHRIAQTGGV	695	73.429	0.998
TTTQDWHRVAQTGWV	297	72.612	0.986
TNNQDSHRIAQTGWV	298	72.286	0.982
TNTEDWHRVAQTGWV	299	70.939	0.964
TNNQDGHRIAQSGWV	696	70.857	0.963
TNNQDWHRIAQNGWV	697	70.776	0.961
TNNQDSQRIAQTGWV	698	70.469	0.957
TTTQDWHRIPQTGWV	300	69.959	0.950
RNTQDWHRIAQTGWV	544	69.776	0.948
SNNQDWHRIAQTGSV	699	69.388	0.943
TNSQDWHRIAQTNWV	700	68.469	0.930

SNNQDWHRIAETG WV	701	68.306	0.928
TSTQDWHRIAQTGWA	301	67.816	0.921
TNTQDWHRVAQTG WV	232	67.265	0.914
SNNQDSHRIAQTG WV	702	66.776	0.907
TNIQDWHRIANTG WV	703	66.673	0.906
TNSNDWHRIAQTG WV	704	66.367	0.902
SNSQDWHRIAQTG WV	249	66.347	0.901
TNTQDWHRIAQTGWC	302	66.163	0.899
TNTQDWHRSAQTGWA	303	65.796	0.894
TNNQDWQRIAQTG WV	304	65.449	0.889
TNIQDWHRIPQTG WV	305	64.918	0.882
TTTQDWHRIAQTGWI	306	64.755	0.880
SNTQDWHRIANTG WV	705	64.592	0.877
TNTQDWHRIAQTGWA	211	64.531	0.877
TNTQDWHRIAQTNWV	217	64.531	0.877
TNTQDWHRISQTG WV	236	64.408	0.875
TNNQHWQRIAQTG WV	706	64.306	0.874
TYNQDWQRIAQTG WV	707	63.918	0.868
TNNQDWHRIEQTG WV	708	63.408	0.861
TNNQDWHRIAQT SWV	709	62.551	0.850
RNTQDWHRISQTG WV	710	61.429	0.834
TNTQDWHRIAQTGWA	711	61.327	0.833
TNTQDWHRIAQTGWS	307	61.061	0.829
TNQDQDWHRIAQTG WV	712	60.796	0.826
RNIQDWHRIAQTG WV	713	60.755	0.825
TTTQDWHRISQTG WV	308	60.571	0.823
TSTQDWHRVAQTG WV	309	60.020	0.815
TSTQDWHRVSQTG WV	714	59.490	0.808
TNSQDWHKIAQTGWA	310	58.653	0.797
TNKQDWHRIEQTG WV	715	58.612	0.796
TNNPDWHRIAQTG WV	716	58.143	0.790
SNNQDWHRIAQTAG WV	717	58.041	0.788
TNNQDYHRIAQTG WV	718	57.755	0.785
TITEDWHRIAQTG WV	719	57.694	0.784
TNIEDWHRIAQTG WV	227	57.184	0.777
TNTNDWHRIAQTG WV	720	57.163	0.777
TNNQDWHRIAQT DWV	721	56.837	0.772
SNTEDWHRIAQTG WV	565	56.306	0.765
TNIQDWHRIAQTGWD	311	56.224	0.764
SNTQDWHRIPQTG WV	722	55.939	0.760
TNNQDWHRIAQTG SV	723	55.571	0.755
SNNQDRHRIAQTG WV	724	55.469	0.754
TNTQDWHRIAQTGWI	223	55.306	0.751
TNNQDWQRISQTG WV	725	55.286	0.751
TNNQDWKRIAQTG WV	726	54.918	0.746
TNNEDRHRIAQTG WV	727	54.429	0.739
STTQDWHRISQTG WV	728	54.102	0.735
TYTQDWHKIAQTG WV	312	53.878	0.732
TFSQDWHRIAQTG WV	729	53.776	0.731
TYTQDWHRIAQTGWA	313	53.633	0.729
RNSQDWHRIAQTG WV	730	53.592	0.728
INTQDWHRIAQTGWA	731	53.571	0.728
TNNQDRHRIAQTG WV	732	53.408	0.726
RNTEDWHRIAQTG WV	733	53.204	0.723
TNNQDWHRIAQTIG WV	734	53.143	0.722
TNNHDWHRIAQTG WV	735	53.041	0.721
TNSQDWHRIAQTG WV	229	52.959	0.719
TNTQDWHRISQTGWA	314	52.939	0.719

TNTEDWHRIAQTGWV	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

[0472] 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

[0473] 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 4 and 5, respectively.

[0474] 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).

[0475] 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.

[0476] 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 24**). As shown in **Table 24**, 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 24. 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

[0477] 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 25** 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 25**, 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 25. 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

[0478] 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.

We claim:

1. An AAV capsid variant, comprising 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 positions X₁, X₂, X₃, and X₄, wherein position X₄ is Q, K, E, S, P, R, N, or H; and/or

(ii) [N3] comprises positions X₅, X₆, and X₇, wherein position X₅ is I, V, T, M, S, N, L, or F; wherein the AAV capsid variant comprises the amino acid sequence of positions 203-736 of SEQ ID NO: 981, or an amino acid sequence at least 95% identical to positions 203-736 of SEQ ID NO: 981.

2. The AAV capsid variant of claim 1, wherein [N1] comprises positions X₁, X₂, X₃, and X₄, wherein:

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

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

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

(iv) position X₄ is Q, K, E, S, P, R, N, or H.

3. The AAV capsid variant of claim 1 or 2, wherein [N1] 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), TNPK (SEQ ID NO: 4752), INNQ (SEQ ID NO: 4753), TYTE (SEQ ID NO: 4754), NNNQ (SEQ ID NO: 4755),

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).

4. The AAV capsid variant of any one of claims 1-3, wherein [N1] comprises TNTQ (SEQ ID NO: 4688) or TNTK (SEQ ID NO: 4689).

5. The AAV capsid variant of any one of claims 1-4, wherein [N1]-[N2] comprises TNTQDWHR (SEQ ID NO: 4898), TNTKQDWHR (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), TNPKDWHR (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).

6. The AAV capsid variant of any one of claims 1-5, wherein [N1]-[N2] comprises TNTQDWHR (SEQ ID NO: 4898) or TNTKDWHR (SEQ ID NO: 4899).

7. The AAV capsid variant of any one of claims 1-6, [N3] comprises positions X₅, X₆, and X₇, wherein:

- (i) position X₆ is A, Y, P, N, S, T, G, E, V, W, F, or Q;
- (ii) position X₇ is Q, G, N, K, H, R, E, L, P, or M; and
- (iii) position X₅ is I, V, T, M, S, N, L, or F.

8. The AAV capsid variant of any one of claims 1-7, wherein [N3] 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.

9. The AAV capsid variant of any one of claims 1-8, wherein [N2]-[N3] comprises 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), DWHRIPQ (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).

10. The AAV capsid variant of any one of claims 1-9, wherein [N2]-[N3] comprises DWHRIAQ (SEQ ID NO: 5027).

11. The AAV capsid variant of any one of claims 1-10, wherein [N1]-[N2]-[N3] comprises the amino acid sequence of any one of SEQ ID NOs: 343-538.

12. The AAV capsid variant of any one of claims 1-11, wherein [N1]-[N2]-[N3] comprises TNTQDWHRIAQ (SEQ ID NO: 343) or TNTKDWHRIAQ (SEQ ID NO: 344).

13. The AAV capsid variant of any one of claims 1-12, which further comprises [N4], wherein [N4] comprises positions X₈, X₉, X₁₀, and X₁₁, wherein:

- (i) position X₈ is T, S, N, P, A, or I;
- (ii) position X₉ is G, N, D, R, V, A, S, or Q;
- (iii) position X₁₀ is W, S, C, R, L, or G; and/or
- (iv) position X₁₁ is V, A, S, I, C, G, D, F, L, or T.

14. The AAV capsid variant of claim 13, wherein [N4] 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).

15. The AAV capsid variant of claim 13 or 14, wherein [N1]-[N2]-[N3]-[N4] 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, or 742;
- (ii) TNTQDWHRIAQTGWV (SEQ ID NO: 201); or
- (iii) TNTKDWHRIAQTGWV (SEQ ID NO: 202).

16. The AAV capsid variant of any one of claims 13-15, wherein:

- (a) [N1]-[N2]-[N3] is present in loop VIII; and/or
- (b) [N4] is present in loop VIII;

wherein loop VIII comprises positions 580-599 of SEQ ID NO: 981.

17. The AAV capsid variant of any one of claims 13-16, wherein:

- (i) X₁ of [N1] is present at position 582, X₂ of [N1] is present at position 583, X₃ of [N1] is present at position 584, and X₄ of [N1] is present at position 585, numbered according to SEQ ID NO: 981;
- (ii) [N2] is present at positions 586-589, numbered according to SEQ ID NO: 981;
- (iii) X₅ of [N3] is present at position 590, X₆ of [N3] is present at position 591, and X₇ of [N3] is present at position 592, numbered according to SEQ ID NO: 981; and
- (iv) X₈ of [N4] is present at position 593, X₉ of [N4] is present at position 594, X₁₀ of [N4] is present at position 595, and X₁₁ of [N4] is present at position 596, numbered according to SEQ ID NO: 981.

18. An AAV capsid variant comprising:

- (a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15 or 16;
- (b) an amino acid sequence comprising at least 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; or
- (c) an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16; or

(d) an amino acid sequence comprising at least one, two, or three but no more than four substitutions, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, 14, 15, or 16;

wherein the AAV capsid variant comprises the amino acid sequence of positions 203-736 of SEQ ID NO: 981, or an amino acid sequence at least 95% identical to positions 203-736 of SEQ ID NO: 981.

19. The AAV capsid variant of claim 18, which comprises:

(a) 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, 742, or 941;

(b) an amino acid sequence comprising at least 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acids from 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, 742, or 941;

(c) an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to 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, 742, or 941; or

(d) an amino acid sequence comprising at least one, two, or three but no more than four substitutions, relative to 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, 742, or 941.

20. An AAV capsid variant comprising an amino acid sequence with at least 95% sequence identity to positions 203-736 of SEQ ID NO: 981, wherein the AAV capsid variant comprises 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.

21. An AAV capsid variant comprising an amino acid sequence with at least 95% sequence identity to positions 138-736 of SEQ ID NO: 981, wherein the AAV capsid variant comprises 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.

22. An AAV capsid variant comprising an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 981, wherein the AAV capsid variant comprises 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.

23. The AAV capsid variant of any one of claims 1-20, which comprises the amino acid sequence of positions 203-736 of SEQ ID NO: 981.

24. The AAV capsid variant of any one of claims 1-21 or 23, which comprises the amino acid sequence of positions 138-736 of SEQ ID NO: 981.

25. The AAV capsid variant of any one of the preceding claims, which comprises the amino acid sequence of SEQ ID NO: 981.

26. An AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 981.

27. The AAV capsid variant of any one of the preceding claims, which comprises one, two, three, four, five, six, or all of the following properties:

(i) 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 a reference sequence comprising the amino acid sequence of SEQ ID NO: 138;

(ii) transduces a brain region, e.g., sensory cortex, motor cortex, putamen, thalamus, caudate, hippocampus, cerebellum optionally wherein the level of transduction is at least 39, 50, 100, 120, 132, 146, 150, 161, 174, 175, 200, 225, 250, 275, 283, 300, 350, 400, 450, 500, 525, 528, or 550-fold greater as compared to a reference 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;

(iii) is enriched at least about 10, 14, 20, 24, 50, 100, 150, 200, 250, 300, 350, 400, 425, 450, or 460-fold, in the brain compared to a reference sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Example 1 or 3;

(iv) is enriched at least about 200, 300, 400, 425, 450, or 460-fold, in the brain compared to a reference sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Example 1;

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

(vi) is enriched at least about 2, 3, 4, 5, 10, 15, 17, 20, 50, 75, 100, 103, 107, 125, 150, 200, 250, 300, 350, 400, 450, 500, 750, 1000, 1200-fold, in the brain compared to a reference sequence of SEQ ID NO: 981, e.g., when measured by an assay as described in Example 3;

(vi) delivers an increased level of a payload to a brain region, optionally wherein the level of the payload is increased by at least 39, 50, 100, 120, 132, 146, 150, 161, 174, 175, 200, 225, 250, 275, 283, 300, 350, 400, 450, 500, 525, 528, or 550-fold, as compared to a reference 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);

(v) delivers an increased level of viral genomes to a brain region, optionally wherein the level of viral genomes is increased by at least 2, 5, 7, 10, 15, 19, 20, 22, or 25-fold, as compared to a reference 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); and/or

(vi) is enriched at least about 5, 10, 50, 100, 115, 120, 150, 175, 200, 207, 225, 250, or 275-fold, in the spinal cord compared to a reference sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Example 1 or 2.

28. A polynucleotide encoding the AAV capsid variant of any one of claims 1-27.

29. A polynucleotide encoding an AAV capsid variant, wherein the polynucleotide comprises:

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

(iii) the nucleotide sequence of SEQ ID NO: 942, or nucleotide sequence having at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity thereto; and/or

(iv) the nucleotide sequence of SEQ ID NO: 983, or a nucleotide sequence with at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity thereto,

optionally wherein the polynucleotide comprises a nucleotide sequence that is codon optimized.

30. A peptide comprising:

- (a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 14, 15, or 16;
- (b) an amino acid sequence comprising at least 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 14, 15, or 16;
- (c) an amino acid sequence comprising at least one, two, or three but no more than four different amino acids relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 14, 15, or 16; or
- (d) an amino acid sequence comprising at least one, two, or three but no more than four substitutions, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 14, 15, or 16.

31. A peptide comprising:

- (i) the amino acid sequence of TQDWHRI (SEQ ID NO: 941);
- (ii) an amino acid sequence comprising at least one, two, or three, but no more than four different amino acids relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941);
- (iii) an amino acid sequence comprising at least one, two, or three substitutions, but no more than four substitutions, relative to the amino acid sequence of TQDWHRI (SEQ ID NO: 941); or
- (iv) at least 3, 4, 5, or 6 consecutive amino acids from the amino acid sequence of TQDWHRI (SEQ ID NO: 941).

32. An AAV capsid variant comprising the peptide of claim 31, wherein the peptide is present at positions 584-590, numbered according to SEQ ID NO: 981 or 138.

33. An AAV particle comprising the AAV capsid variant of claim 32.

34. An AAV particle comprising the AAV capsid variant of any one of claims 1-27, an AAV capsid variant encoded by the polynucleotide of claim 28 or 29, or an AAV capsid variant comprising the peptide of claim 30 or 31.

35. The AAV particle of claim 33 or 34, which comprises a nucleotide sequence encoding a payload, optionally wherein the encoded payload comprises a therapeutic protein or functional variant thereof; an antibody or antibody fragment; an enzyme; a component of a gene editing system; an RNAi agent (e.g., a dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA); or a combination thereof.

36. The AAV particle of claim 35, wherein:

(i) the therapeutic protein or functional variant thereof, e.g., a recombinant protein, is associated with (e.g., aberrantly expressed in) a neurological or neurodegenerative disorder, a muscular or neuromuscular disorder, or a neuro-oncological disorder, optionally wherein the therapeutic protein or functional variant thereof is chosen from apolipoprotein E (APOE) (e.g., ApoE2, ApoE3 and/or ApoE4); human survival of motor neuron (SMN) 1 or SMN2; glucocerebrosidase (GBA1); aromatic L-amino acid decarboxylase (AADC); aspartoacylase (ASPA); tripeptidyl peptidase I (CLN2); beta-galactosidase (GLB1); N-sulphoglucosamine sulphohydrolase (SGSH); N-acetyl-alpha-glucosaminidase (NAGLU); iduronate 2-sulfatase (IDS); intracellular cholesterol transporter (NPC1); gigaxonin (GAN); or a combination thereof;

(ii) the antibody or antibody binding fragment binds to

(a) a CNS related target, e.g., an antigen associated with a neurological or neurodegenerative disorder, e.g., β -amyloid, APOE, tau, SOD1, TDP-43, huntingtin (HTT), and/or synuclein;

(b) a muscular or neuromuscular related target, e.g., an antigen associated with a muscular or neuromuscular disorder; or

(c) a neuro-oncology related target, e.g., an antigen associated with a neuro-oncological disorder, e.g., HER2, or EGFR (e.g., EGFRvIII);

(iii) the enzyme comprises a meganuclease, a zinc finger nuclease, a TALEN, a recombinase, integrase, a base editor, a Cas9, or a fragment thereof;

(iv) the component of a gene editing system comprises one or more components of a CRISPR-Cas system, optionally wherein the one or more components of the CRISPR-Cas system comprises a Cas9, e.g., a Cas9 ortholog or a Cpf1, and a single guide RNA (sgRNA), wherein:

(a) the sgRNA is located upstream (5') of the cas9 enzyme; and/or

(b) the sgRNA is located downstream (3') of the cas9 enzyme; and/or

(v) the RNAi agent (e.g., a dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA), modulates, e.g., inhibits, expression of, a CNS related gene, mRNA, and/or protein, optionally wherein the CNS related gene is chosen from SOD1, MAPT, APOE, HTT, C9ORF72, TDP-43, APP, BACE, SNCA, ATXN1, ATXN3, ATXN7, SCN1A-SCN5A, SCN8A-SCN11A, or a combination thereof.

37. The AAV particle of any one of claims 33-36, which comprises a viral genome comprising a promoter operably linked to the nucleic acid sequence encoding the payload, optionally wherein:

(i) the promoter is chosen from human elongation factor 1 α -subunit (EF1 α), cytomegalovirus (CMV) immediate-early enhancer and/or promoter, chicken β -actin (CBA) and its derivative CAG, β glucuronidase (GUSB), or ubiquitin C (UBC), neuron-specific enolase (NSE), platelet-derived growth factor (PDGF), platelet-derived growth factor B-chain (PDGF- β), intercellular adhesion molecule 2 (ICAM-2), synapsin (Syn), methyl-CpG binding protein 2 (MeCP2), Ca²⁺/calmodulin-dependent

protein kinase II (CaMKII), metabotropic glutamate receptor 2 (mGluR2), neurofilament light chain (NFL) or heavy chain (NFH), β -globin minigene β 2, preproenkephalin (PPE), enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), 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 fragment, e.g., a truncation, or a functional variant thereof;

(ii) the promoter is an EF-1a promoter variant, e.g., a truncated EF-1a promoter; or

(iii) the promoter comprises the nucleotide sequence of any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998, 999-1007, or any one of the nucleotide sequence provided in Table 8; a nucleotide sequence comprising at least one, two, three, four, five, six, or seven but no more than four substitutions, relative to the nucleotide sequence of any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998, 999-1007, or any one of the nucleotide sequence provided in Table 8; or a nucleotide sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998, 999-1007, or any one of the nucleotide sequence provided in Table 8.

38. The AAV particle of claim 37, wherein the viral genome further comprises:

(i) a polyA signal sequence;

(ii) an inverted terminal repeat (ITR) sequence, optionally wherein the ITR sequence is positioned 5' relative to the encoded payload and/or the ITR sequence is positioned 3' relative to the encoded payload;

(iii) an enhancer, a Kozak sequence, an intron region, and/or an exon region; and/or

(iv) a nucleotide sequence encoding a miR binding site, e.g., a miR binding site that modulates, e.g., reduces, expression of the antibody molecule encoded by the viral genome in a cell or tissue where the corresponding miRNA is expressed, optionally wherein the encoded miR binding site modulates, e.g., reduces, expression of the encoded antibody molecule in a cell or tissue of the DRG, liver, heart, hematopoietic lineage, or a combination thereof.

39. The AAV particle of claim 38, wherein the viral genome comprises:

(i) at least 1-5 copies of the encoded miR binding site, e.g., at least 1, 2, 3, 4, or 5 copies;

(ii) at least 3 copies of an encoded miR binding sites, optionally wherein:

(a) all three copies comprise the same miR binding site, or at least one, two, three, or all of the copies comprise a different miR binding site; and/or

(b) the 3 copies of the encoded miR binding sites are continuous (e.g., not separated by a spacer), or are separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than

four modifications, e.g., substitutions (e.g., conservative substitutions), relative to GATAGTTA; or

(iii) at least 4 copies of an encoded miR binding site, optionally wherein

(a) all four copies comprise the same miR binding site, or at least one, two, three, or all of the copies comprise a different miR binding site; and/or

(b) the 4 copies of the encoded miR binding sites are continuous (e.g., not separated by a spacer), or are separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to GATAGTTA.

40. The AAV particle of claim 38 or 39, wherein the encoded miR binding site comprises a miR122 binding site, a miR183 binding site, a miR1 binding site, a miR142-3p, or a combination thereof, optionally wherein:

(i) the encoded miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to SEQ ID NO: 4673;

(ii) the encoded miR183 binding site comprises the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to SEQ ID NO: 4676;

(iii) the encoded miR1 binding site comprises the nucleotide sequence of SEQ ID NO: 4679, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or deletions, but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to SEQ ID NO: 4679; and/or

(iv) the encoded miR142-3p binding site comprises the nucleotide sequence of SEQ ID NO: 4675, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence comprising at least one, two, three, four, five, six, or seven modifications, e.g., substitutions, insertions, or

deletions, but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to SEQ ID NO: 4675.

41. The AAV particle of any one of claims 37-40, wherein the viral genome is:

- (i) single stranded; or
- (ii) self-complementary.

42. The AAV capsid variant of any one of claims 1-27 or 32, the polynucleotide of claim 28 or 29, the peptide of claim 30 or 31, or the AAV particle of any one of claims 33-41, which is isolated, e.g., recombinant.

43. A vector comprising a polynucleotide encoding the AAV capsid variant of any one of claims 1-27, 32, or 42, the polynucleotide of any one of claims 28, 29, or 42, or a polynucleotide encoding the peptide of any one of claims 30, 31, or 42.

44. A cell, e.g., a host cell, comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, the polynucleotide of any one of claims 28, 29, or 42, a polynucleotide encoding the peptide of any one of claims 30, 31, or 42, the AAV particle of any one of claims 33-42, or the vector of claim 43, optionally wherein:

- (i) the cell is a mammalian cell or an insect cell;
- (ii) the cell is a cell of a brain region or a spinal cord region, optionally a cell of the brain stem, hippocampus, or thalamus; and/or
- (iii) the cell is a neuron, a sensory neuron, a motor neuron, an astrocyte, a glial cell, oligodendrocyte, or a muscle cell (e.g., a cell of the heart, diaphragm, or quadriceps).

45. A method of making an AAV particle, comprising

- (i) providing a host cell comprising a viral genome; and
 - (ii) incubating the host cell under conditions suitable to enclose the viral genome in the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV capsid variant encoded by the polynucleotide of any one of claims 28, 29, or 42;
- thereby making the AAV particle.

46. A pharmaceutical composition comprising the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42, and a pharmaceutically acceptable excipient.

47. A method of delivering a payload to a cell or tissue (e.g., a CNS cell or a CNS tissue), comprising administering an effective amount of the pharmaceutical composition of claim 46, the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42.

48. The method of claim 47, wherein the cell is:

(i) a cell of a brain region or a or a spinal cord region, optionally a cell of the temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, cerebellum, cervical spinal cord region, thoracic spinal cord region, lumbar spinal cord region, or a combination thereof;

(ii) is a cell of the heart, e.g., a heart atrium or a heart ventricle;

(iii) is a cell of the muscle (e.g., a cell of the quadriceps) or liver;

(iv) a neuron, a sensory neuron, a motor neuron, an astrocyte, a glial cell, or an oligodendrocyte; or

(v) within a subject, optionally wherein the subject has, has been diagnosed with having, or is at risk of having a genetic disorder (e.g., a monogenic disorder or a polygenic disorder), a neurological disorder (e.g., a neurodegenerative disorder), a neuro-oncological disorder, a muscular disorder, or a neuromuscular disorder.

49. A method of treating a subject having or diagnosed with having a genetic disorder, e.g., a monogenic disorder or a polygenic disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 46, the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42.

50. A method of treating a subject having or diagnosed with having a neurological disorder, e.g., a neurodegenerative disorder, a neuro-oncological disorder, a muscular disorder, or a neuromuscular disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 46, the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42.

51. 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 claim 46, the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42.

52. A method of treating a subject having or diagnosed with having a neuro-oncological disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 46, the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42.

53. The method of any one of claims 48-52, wherein the genetic disorder, neurological disorder, neurodegenerative disorder, muscular disorder, neuromuscular disorder, or neuro-oncological disorder is Huntington's Disease, Amyotrophic Lateral Sclerosis (ALS), Gaucher Disease, Dementia with Lewy Bodies, Parkinson's disease, Spinal Muscular Atrophy, Alzheimer's Disease, a leukodystrophy (e.g., Alexander disease, autosomal dominant leukodystrophy with autonomic diseases (ADLD), Canavan disease, cerebrotendinous xanthomatosis (CTX), metachromatic leukodystrophy (MLD), Pelizaeus-Merzbacher disease, or Refsum disease), or a cancer (e.g., a HER2/neu positive cancer or a glioblastoma).

54. The method of any one of claims 49-53, wherein treating comprises prevention of progression of the disease or disorder in the subject.

55. The method of any one of claims 48-54, wherein the subject is a human.

56. The method of any one of claims 48-55, wherein the AAV particle or the pharmaceutical composition is administered to the subject:

(i) intravenously, via intra-cisterna magna injection (ICM), intracerebrally, intrathecally, intracerebroventricularly, via intraparenchymal administration, or intramuscularly;

(ii) via focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration; or

(iii) intravenously.

57. The pharmaceutical composition of claim 46, the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42, for use in a method of delivering a payload to a cell or tissue.

58. The pharmaceutical composition of claim 46, the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42, for use in a method of treating a

genetic disorder, a neurological disorder, a neurodegenerative disorder, a muscular disorder, a neuromuscular disorder, or a neuro-oncological disorder.

59. The pharmaceutical composition of claim 46, the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42, for use in the manufacture of a medicament.

60. Use of the pharmaceutical composition of claim 46, the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42, in the manufacture of a medicament.

61. Use of the pharmaceutical composition of claim 46, the AAV particle of any one of claims 33-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-27, 32, or 42, or an AAV particle comprising the peptide of any one of claims 30, 31, or 42, in the manufacture of a medicament for treating a genetic disorder, a neurological disorder, a neurodegenerative disorder, a muscular disorder, a neuromuscular disorder, or a neuro-oncological disorder.