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(54) Title: AAV CAPSID VARIANTS AND USES THEREOF

(57) Abstract: The disclosure relates to compositions and methods for the preparation, use, and/or formulation of adeno-associated virus capsid protein variants.

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AAV CAPSID VARIANTS AND USES THEREOF

RELATED APPLICATIONS

[01] This application claims priority to U.S. Provisional Application No. 63/307,742 filed on February 8, 2022 and U.S. Provisional Application No. 63/339,574 filed on May 9, 2022; the entire contents of each of which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[02] This 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 January 24, 2023, is named V2071-1111PCT_SL.xml and is 2,233,613 bytes in size.

FIELD OF THE DISCLOSURE

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

BACKGROUND

[04] Gene delivery to the 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.

[05] 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.

[06] Attempts at providing AAV capsids with improved properties, e.g., improved tropism to a target cell or tissue upon systemic administration, have had 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, or a muscle cell or tissue.

SUMMARY OF THE DISCLOSURE

[07] 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 (e.g., an AAV5 capsid variant). In some embodiments, the AAV capsid variant has an enhanced tropism for a tissue or a cell, e.g., a CNS tissue, a CNS cell, a heart cell, a heart tissue, a muscle cell, a muscle tissue, a liver cell, or a liver tissue. 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.

[08] Accordingly, in one aspect, the present disclosure provides an AAV capsid variant, e.g., an AAV5 capsid variant, which comprises an amino acid other than T at position 577 (e.g., Y, N, or C), numbered relative to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises a Y at position 577, numbered relative to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises a N at position 577, numbered relative to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises a C at position 577, numbered relative to SEQ ID NO: 138.

[09] In another aspect, the present disclosure provides an AAV capsid variant, e.g., a variant of the wild-type AAV5 capsid, which comprises more than one amino acid that replaces the threonine (T) at position 577, numbered relative to SEQ ID NO: 138. In some embodiments, an insert of two, three, four, five, six, seven, eight, nine, or ten amino acids replaces the T at position 577, numbered relative to SEQ ID NO: 138. In some embodiments an insert of eight amino acids replaces the T at position 577, numbered relative to SEQ ID NO: 138.

[010] In another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising the formula [N2]-[N3], wherein: (i) [N2] comprises positions X1, X2, X3, X4, and X5, wherein: (a) position X1 is Y, N, or C; (b) position X2 is P, K, T, or Q; (c) position X3 is A or P; (d) position X4 is E, S, or A; and (e) position X5 is V, L, or E; and (ii) the amino acid sequence of [N3] comprises the amino acid sequence of VQK, EQK, VKK, VHK, VQQ, or LQK; and optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii); optionally wherein [N2]-[N3] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138 and replaces position 577, numbered relative to SEQ ID NO: 138. In some embodiments, [N2] comprises Y at position X1. In some embodiments, [N2] comprises P at position X2. In some embodiments, [N2] comprises A at position X3. In some embodiments, [N2] comprises E at position X4. In some embodiments, [N2] comprises V at position X5. In some embodiments, the amino acid sequence of [N3] is VQK. In some embodiments, X1 of [N2] is present at position 577, X2 of [N2] is present at position 578, X3 of [N2] is present at position 579, X4 of [N2] is present at position 580, and of X5 of [N2] is present at position 581, numbered according to SEQ ID NO: 982. In some embodiments, [N2] is present at positions 577-581, numbered according to SEQ ID NO: 982. In some embodiments, [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982. In some embodiments, [N2]-[N3] is present at positions 577-584, numbered according to SEQ ID NO: 982.

[011] In some embodiments, the amino acid sequence of [N2] consists of YPAEV (SEQ ID NO: 1). In some embodiments, the amino acid sequence of [N3] consists of VQK. In some embodiments, [N2]-[N3] replaces the threonine (T) at position 577 of wild-type AAV5, e.g., SEQ ID NO: 138. In some embodiments, [N2]-[N3] replaces the threonine (T) at position 577 of wild-type AAV5, e.g., SEQ ID NO: 138, and the amino acid sequence of [N2] consists of YPAEV (SEQ ID NO: 1), and the amino acid sequence of [N3] consists of VQK. In some embodiments, [N2] is present at positions 577-581, numbered according to SEQ ID NO: 982. In some embodiments, [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982. In some embodiments, [N2]-[N3] is present at positions 577-584, numbered according to SEQ ID NO: 982.

[012] In another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising one, two, three, or all of: (i) an [N0], wherein the amino acid sequence of [N0] comprises TNN, TNT, INN, TNS, NNN, or TNK; (ii) an [N1], wherein the amino acid sequence of [N1] comprises QSS, QSK, TSL, SSS, QSR, AGA, IGS, QAS, ASS, LGS, QST, HSS, LSS, or QRS; (iii) an [N2], wherein the amino acid sequence of [N2] comprises YPAEV (SEQ ID NO: 1), YPPSL (SEQ ID NO: 2), NKAEV (SEQ ID NO: 3), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), YQAEV (SEQ ID NO: 6), YTPSL (SEQ ID NO: 7), YPAAV (SEQ ID NO: 8), NPAEV (SEQ ID NO: 9), CPAEV (SEQ ID NO: 10), or YQAEE (SEQ ID NO: 11); (iv) an [N3], wherein the amino acid sequence of [N3] comprises VQK, EQK, VKK, VHK, VQQ, or LQK; and/or (v) an [N4], wherein the amino acid sequence of [N4] comprises TA, PA, or NA; and optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i)-(v); optionally wherein [N0] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In some embodiments, the amino acid sequence of [N0] is TNN. In some embodiments, the amino acid sequence of [N1] is QSS. In some embodiments, the amino acid sequence of [N2] is YPAEV (SEQ ID NO: 1). In some embodiments, the amino acid sequence of [N3] is VQK. In some embodiments, the amino acid sequence of [N4] is TA. In some embodiments, the amino acid sequence of [N0] is TNN, the amino acid sequence of [N1] is QSS, the amino acid sequence of [N2] is YPAEV (SEQ ID NO: 1), the amino acid sequence of [N3] is VQK, and/or the amino acid sequence of [N4] is TA. In some embodiments, the amino acid sequence of [N0] is TNN, the amino acid sequence of [N1] is QSS, the amino acid sequence of [N2] is YPAEV (SEQ ID NO: 1), the amino acid sequence of [N3] is VQK, and the amino acid sequence of [N4] is TA. In any of these embodiments, [N0] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In any of these embodiments, [N0] replaces positions 571-573 (e.g., T571, N572, and N573), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N0] is present immediately subsequent to position 570, and [N0] positions 571-573 (e.g., T571, N572, and N573), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N1] is present immediately subsequent to position 573, numbered relative to SEQ ID NO: 138. In any of these embodiments, [N1] replaces positions 574-576 (e.g., Q574, S575, and S576), numbered

relative to SEQ ID NO: 138. In any of these embodiments, [N1] is present immediately subsequent to position 573, and [N1] replaces positions 574-576 (e.g., Q574, S575, and S576), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2] is present immediately subsequent to position 576, and [N2] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2]-[N3] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2]-[N3] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2]-[N3] is present immediately subsequent to position 576, and [N2]-[N3] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2]-[N3]-[N4] replaces positions 577-579 (e.g., T577, T578, and A579), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2]-[N3]-[N4] is present immediately subsequent to position 576, and [N2]-[N3]-[N4] replaces positions 577-579 (e.g., T577, T578, and A579), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 570, and [N0]-[N1]-[N2]-[N3]-[N4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. For example, in some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533) and is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138, wherein [N2]-[N3] (YPAEVVQK (SEQ ID NO: 943)) replaces position 577 (e.g., replaces T577), numbered relative to SEQ ID NO: 138. In some embodiments, [N0] is present at positions 571-573, numbered according to SEQ ID NO: 982. In some embodiments, [N1] is present at positions 574-576, numbered according to SEQ ID NO: 982. In some embodiments, [N2] is present at positions 577-581, numbered according to SEQ ID NO: 982. In some embodiments, [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982. In some embodiments, [N4] is present at positions 585-586, numbered according to SEQ ID NO: 982. In some embodiments, [N2]-[N3] is present at positions 577-584, numbered according to SEQ ID NO: 982. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present at positions 571-586, numbered according to SEQ ID NO: 982.

[013] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising the formula [B]-[C], wherein: (i) [B] comprises positions X1, X2, and X3, wherein: (a) position X1 is Q, T, S, A, I, L, or H; (b) position X2 is S, G, or A; and (c) position X3 is S, K, L, R, or A; and (ii) [C] comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943); and optionally wherein the AAV capsid variant comprises an amino acid modification,

e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii); and further optionally wherein [B] is present immediately subsequent to position 573, numbered relative to SEQ ID NO: 138. In some embodiments, [B] comprises Q at position X1. In some embodiments, [B] comprises S at position X2. In some embodiments, [B] comprises S at position X3. In some embodiments, the amino acid sequence of [B] is QSS. In some embodiments, [B] is present immediately subsequent to position 573, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B] is present immediately subsequent to position 573, and [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C] is present immediately subsequent to position 576, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C] is present immediately subsequent to position 576, and [C] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B]-[C] is present immediately subsequent to position 573, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B]-[C] is present immediately subsequent to position 573, and [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, X1 of [B] is present at position 574, X2 of [B] is present at position 575, and X3 of [B] is present at position 576, numbered according to SEQ ID NO: 982. In some embodiments, [B] is present at positions 574-576, numbered according to SEQ ID NO: 982. In some embodiments, [C] is present at positions 577-584, numbered according to SEQ ID NO: 982. In some embodiments, [B]-[C] is present at positions 574-584, numbered according to SEQ ID NO: 982.

[014] In another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising one, two, three, or all of (i) an [A], wherein the amino acid sequence of [A] comprises TNN, TNT, INN, NNN, TNS, or TNK; (ii) a [B], wherein the amino acid sequence of [B] comprises QSS, TSL, SSS, QSR, QSK, AGA, IGS, QAS, ASS, LGS, or HSS; (iii) a [C], wherein [C] comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943); and (iv) a [D], wherein [D] comprises the amino acid sequence of TA or PA; and optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i)-(v); optionally wherein [A] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138 and [C] replaces position 577, numbered relative to SEQ ID NO: 138. In some embodiments, the amino acid sequence of [A] is TNN. In some embodiments, the amino acid sequence of [B] is QSS. In some embodiments, the amino acid sequence of [A] is TNN and the amino

acid sequence of [B] is QSS. In some embodiments, the amino acid sequence of [A] is TNN, the amino acid sequence of [B] is QSS, and the amino acid sequence of [C] is YPAEVVQK (SEQ ID NO: 943). In some embodiments, the amino acid sequence of [A] is TNN, the amino acid sequence of [B] is QSS, the amino acid sequence of [C] is YPAEVVQK (SEQ ID NO: 943), and the amino acid sequence of [D] is TA. In any of these embodiments, [A] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In any of these embodiments, [A] replaces positions 571-573 (e.g., T571, N572, and N573) numbered relative to SEQ ID NO: 138. In any of these embodiments, [A] is present immediately subsequent to position 570, and [A] replaces positions 571-573 (e.g., T571, N572, and N573) numbered relative to SEQ ID NO: 138. In any of these embodiments, [B] is present immediately subsequent to position 573, relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [B] replaces positions 574-576 (e.g., Q574, S575, and S576), numbered relative to SEQ ID NO: 138. In any of these embodiments, [B] is present immediately subsequent to position 573, and [B] replaces positions 574-576 (e.g., Q574, S575, and S576), numbered relative to SEQ ID NO: 138. In any of these embodiments, [C] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138. In any of these embodiments, [C] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [C] is present immediately subsequent to position 576, and [C] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [B]-[C] is present immediately subsequent to position 573, numbered relative to SEQ ID NO: 138. In any of these embodiments, [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [B]-[C] is present immediately subsequent to position 573, and [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [C]-[D] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138. In any of these embodiments, [C]-[D] replaces positions 577-579 (e.g., T577, T578, and A579), numbered relative to SEQ ID NO: 138. In any of these embodiments, [A]-[B]-[C]-[D] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In any of these embodiments, [A]-[B]-[C]-[D] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In any of these embodiments, [A]-[B]-[C]-[D] is present immediately subsequent to position 570, and [A]-[B]-[C]-[D] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. For example, in some embodiments, [A]-[B]-[C]-[D] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533) and is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138, wherein [C] (YPAEVVQK (SEQ ID NO: 943)) replaces position 577 (e.g., replaces T577) numbered relative to SEQ ID NO: 138. In some embodiments, [A] is present at positions 571-573, numbered according to SEQ ID NO: 982. In some embodiments, [B] is present at positions 574-576, numbered according to SEQ ID NO: 982. In some embodiments, [C]

is present at positions 577-584, numbered according to SEQ ID NO: 982. In some embodiments, [D] is present at positions 585-586, numbered according to SEQ ID NO: 982. In some embodiments, [A]-[B]-[C]-[D] is present at positions 571-586, numbered according to SEQ ID NO: 982.

[015] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising the formula [N2]-[N3], wherein: (i) [N2] comprises positions X1, X2, X3, X4, and X5, wherein: (a) position X1 is Y or T; (b) position X2 is Q, T, P, or E; (c) position X3 is A; (d) position X4 is E or D; and (e) position X5 is V or E; and (ii) [N3] comprises the amino acid sequence of VQK or VQN; and optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii); and further optionally wherein [N2]-[N3] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138 and replaces position 577, numbered relative to SEQ ID NO: 138. In some embodiments, the amino acid sequence of [N2] consists of YPAEV (SEQ ID NO: 1). In some embodiments, the amino acid sequence of [N3] consists of VQK. In some embodiments, [N2]-[N3] replaces position 577 (e.g., T577) of wild-type AAV5, e.g., SEQ ID NO: 138. In some embodiments, [N2]-[N3] replaces the threonine (T) at position 577 of wild-type AAV5, e.g., SEQ ID NO: 138, and the amino acid sequence of [N2] consists of YPAEV (SEQ ID NO: 1), and the amino acid sequence of [N3] consists of VQK. In some embodiments, X1 of [N2] is present at position 577, X2 of [N2] is present at position 578, X3 of [N2] is present at position 579, X4 of [N2] is present at position 580, and of X5 of [N2] is present at position 581, numbered according to SEQ ID NO: 982. In some embodiments, [N2] is present at positions 577-581, numbered according to SEQ ID NO: 982. In some embodiments, [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982. In some embodiments, [N2]-[N3] is present at positions 577-584, numbered according to SEQ ID NO: 982.

[016] In another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising one, two, three, four, or all of: (i) an [N0], wherein [N0] comprises the amino acid sequence of TNN, TNS, TNT, or TNK; (ii) an [N1], wherein [N1] comprises the amino acid of QSS, SLS, SLY, SAT, or QTS; (iii) an [N2] wherein [N2] comprises the amino acid sequence of YPAEV (SEQ ID NO: 1), YQAEV (SEQ ID NO: 6), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), TEAEV (SEQ ID NO: 12), or YPADV (SEQ ID NO: 13); (iv) an [N3] wherein [N3] comprises the amino acid sequence of VQK or VQN; and/or (v) an [N4] wherein [N4] comprises the amino acid sequence of TA, PA, TD, NA, or PA; and optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i)-(v); and further optionally wherein [N0] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138 and [N2]-[N3] replaces position 577, numbered relative to SEQ ID NO: 138. In any of these embodiments, [N0] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In any of these embodiments, [N0] replaces positions 571-573 (e.g., T571, N572, and N573), numbered relative to SEQ ID NO: 138. In any of these

embodiments, [N0] is present immediately subsequent to position 570, and [N0] replaces positions 571-573 (e.g., T571, N572, and N573), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N1] is present immediately subsequent to position 573, numbered relative to SEQ ID NO: 138. In any of these embodiments, [N1] replaces positions 574-576 (e.g., Q574, S575, and S576), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N1] is present immediately subsequent to position 573, and [N1] replaces positions 574-576 (e.g., Q574, S575, and S576), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2] is present immediately subsequent to position 576, and [N2] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2]-[N3] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2]-[N3] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2]-[N3] is present immediately subsequent to position 576, and [N2]-[N3] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2]-[N3]-[N4] replaces positions 577-579 (e.g., T577, T578, and A579), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N2]-[N3]-[N4] is present immediately subsequent to position 576, and [N2]-[N3]-[N4] replaces positions 577-579 (e.g., T577, T578, and A579), numbered relative to SEQ ID NO: 138. In any of these embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In any of these embodiments, [N0]-[N1]-[N2]-[N3]-[N4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 570, and [N0]-[N1]-[N2]-[N3]-[N4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [N0] is present at positions 571-573, numbered according to SEQ ID NO: 982. In some embodiments, [N1] is present at positions 574-576, numbered according to SEQ ID NO: 982. In some embodiments, [N2] is present at positions 577-581, numbered according to SEQ ID NO: 982. In some embodiments, [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982. In some embodiments, [N4] is present at positions 585-586, numbered according to SEQ ID NO: 982. In some embodiments, [N2]-[N3] is present at positions 577-584, numbered according to SEQ ID NO: 982. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present at positions 571-586, numbered according to SEQ ID NO: 982.

[017] In some embodiments, the amino acid sequence of [N0] is TNN, the amino acid sequence of [N1] is QSS, the amino acid sequence of [N2] is YPAEV (SEQ ID NO: 1), the amino acid sequence of [N3] is VQK, and/or the amino acid sequence of [N4] is TA. In some embodiments, the amino acid sequence of [N0] is TNN, the amino acid sequence of [N1] is QSS, the amino acid

sequence of [N2] is YPAEV (SEQ ID NO: 1), the amino acid sequence of [N3] is VQK, and the amino acid sequence of [N4] is TA. In any of these embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In any of these embodiments, the amino acid sequence of [N2]-[N3] replaces position 577, numbered relative to SEQ ID NO: 138. For example, in some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533) and is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138, wherein [N2]-[N3] (YPAEVVQK (SEQ ID NO: 943)) replaces position 577 (e.g., replaces T577) numbered relative to SEQ ID NO: 138. In some embodiments, [N0] is present at positions 571-573, numbered according to SEQ ID NO: 982. In some embodiments, [N1] is present at positions 574-576, numbered according to SEQ ID NO: 982. In some embodiments, [N2] is present at positions 577-581, numbered according to SEQ ID NO: 982. In some embodiments, [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982. In some embodiments, [N4] is present at positions 585-586, numbered according to SEQ ID NO: 982. In some embodiments, [N2]-[N3] is present at positions 577-584, numbered according to SEQ ID NO: 982. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present at positions 571-586, numbered according to SEQ ID NO: 982.

[018] In another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising [B]-[C], wherein: (i) [B] comprises positions X1, X2, and X3, wherein (a) position X1 is Q or S; (b) position X2 is S, L, or A; and (c) position X3 is S, Y, or T; and (ii) [C] comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943); and optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii); and further optionally wherein [B] is present immediately subsequent to position 573, numbered relative to SEQ ID NO: 138, and [C] replaces position 577, numbered relative to SEQ ID NO: 138. In some embodiments, [B] is QSS. In some embodiments, [C] consists of YPAEVVQK (SEQ ID NO: 943), is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138, and replaces position 577, numbered relative to SEQ ID NO: 138. In some embodiments, [B] is present immediately subsequent to position 573, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B] is present immediately subsequent to position 573, and [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C] is present immediately subsequent to position 576, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C] is present immediately subsequent to position 576, and [C] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B]-[C] is present

immediately subsequent to position 573, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B]-[C] is present immediately subsequent to position 573, and [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, X1 of [B] is present at position 574, X2 of [B] is present at position 575, and X3 of [B] is present at position 576, numbered according to SEQ ID NO: 982. In some embodiments, [B] is present at positions 574-576, numbered according to SEQ ID NO: 982. In some embodiments, [C] is present at positions 577-584, numbered according to SEQ ID NO: 982. In some embodiments, [B]-[C] is present at positions 574-584, numbered according to SEQ ID NO: 982.

[019] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising one, two, three, four, or all of: (i) an [A], wherein [A] comprises the amino acid sequence of TNN, TNS, TNT, or TNK; (ii) a [B], wherein [B] comprises the amino acid sequence of QSS, SLY, SAT, or SLS; (iii) a [C], wherein [C] comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943); and (iv) a [D], wherein [D] comprises the amino acid sequence of TA, TD, NA, or PA; and optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i)-(v); and further optionally wherein [A] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138, and [C] replaces position 577, numbered relative to SEQ ID NO: 138. In any of these embodiments, [A] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In any of these embodiments, [A] replaces positions 571-573 (e.g., T571, N572, and N573) numbered relative to SEQ ID NO: 138. In any of these embodiments, [A] is present immediately subsequent to position 570, and [A] replaces positions 571-573 (e.g., T571, N572, and N573) numbered relative to SEQ ID NO: 138. In any of these embodiments, [B] is present immediately subsequent to position 573, relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [B] is present immediately subsequent to position 573, and [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [C] is present immediately subsequent to position 576, relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [C] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [C] is present immediately subsequent to position 576, and [C] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [B]-[C] is present immediately subsequent to position 573, relative to a reference sequence numbered according to SEQ ID NO: 138.

In any of these embodiments, [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [B]-[C] is present immediately subsequent to position 573, and [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [C]-[D] is present immediately subsequent to position 576, relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [C]-[D] replaces positions 577-579 (e.g., T577, T578, and A579), relative to a reference sequence numbered according to SEQ ID NO: 138. In any of these embodiments, [C]-[D] is present immediately subsequent to position 576, and [C]-[D] replaces positions 577-579 (e.g., T577, T578, and A579), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [A] is present at positions 571-573, numbered according to SEQ ID NO: 982. In some embodiments, [B] is present at positions 574-576, numbered according to SEQ ID NO: 982. In some embodiments, [C] is present at positions 577-584, numbered according to SEQ ID NO: 982. In some embodiments, [D] is present at positions 585-586, numbered according to SEQ ID NO: 982. In some embodiments, [A]-[B]-[C]-[D] is present at positions 571-586, numbered according to SEQ ID NO: 982.

[020] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising [K1]-[K2], wherein: (i) [K1] comprises LSY or LYY, and (ii) [K2] comprises positions X1, X2, X3, and X4, wherein (a) position X1 is Q, T, or P; (b) position X2 is A; (c) position X3 is E or D; and (d) position X4 is V or E; and optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii). In some embodiments, [K1]-[K2] is present immediately subsequent to position 574, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1]-[K2] replaces positions 575-577 (e.g., S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1]-[K2] is present immediately subsequent to position 574, and [K1]-[K2] replaces positions 575-577 (e.g., S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138.

[021] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising one, two, three, four, or all of: (i) a [K0], which comprises TNNS (SEQ ID NO: 14); (ii) a [K1], which comprises LSY or LYY; (iii) a [K2], which comprises QAEV (SEQ ID NO: 15), TAEV (SEQ ID NO: 16), PAEV (SEQ ID NO: 17), PAEE (SEQ ID NO: 18), or PADV (SEQ ID NO: 19); (iv) a [K3], which comprise VQK or VQN; and (v) a [K4], which comprises TA, TD, NA, or PA; and optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i)-(v). In some embodiments, [K0] is present immediately subsequent to position 570, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K0] replaces positions 571-574 (e.g., T571, N572, N573, and Q574), relative to a reference sequence numbered according to

SEQ ID NO: 138. In some embodiments, [K0] is present immediately subsequent to position 570, and [K0] replaces positions 571-574 (e.g., T571, N572, N573, and Q574), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1] is present immediately subsequent to position 574, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1] replaces positions 575-577 (e.g., S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1] is present immediately subsequent to position 574, and [K1] replaces positions 575-577 (e.g., S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3] is present immediately subsequent to position 574, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3] replaces positions 575-577 (e.g., S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3] is present immediately subsequent to position 574, and [K1]-[K2]-[K3] replaces positions 575-577 (e.g., S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 574, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3]-[K4] replaces positions 575-579 (e.g., S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 574, and [K1]-[K2]-[K3]-[K4] replaces positions 575-579 (e.g., S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [K0]-[K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 570, relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, [K0]-[K1]-[K2]-[K3]-[K4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, [K0]-[K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 570, and [K0]-[K1]-[K2]-[K3]-[K4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138.

[022] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising: (a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20; (b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20; (c) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or (d) an amino sequence comprising 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 amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20.

[023] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising: (a) the amino acid sequence of any of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624; (b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624; (c) an amino acid sequence comprising 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: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624; or (d) an amino sequence comprising 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: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624.

[024] In another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising (a) the amino acid sequence of any of SEQ ID NOs: 943 or 2064-2080; (b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 943 or 2064-2080; (c) an amino acid sequence comprising 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: 943 or 2064-2080; or (d) an amino sequence comprising 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: 943 or 2064-2080.

[025] In yet another aspect, present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising the amino acid sequence of SEQ ID NO: 943. In some embodiments, the amino acid sequence of SEQ ID NO: 943 is present immediately subsequent to position 576, relative to a reference sequence numbered according to SEQ ID NO: 138 or 982. In some embodiments, the amino acid sequence of SEQ ID NO: 943 replaces position 577 (e.g., replaces T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence of SEQ ID NO: 943 is present immediately subsequent to position 576, wherein the amino acid sequence of SEQ ID NO: 943 replaces position 577 (e.g., replaces T577), relative to a reference sequence numbered according to SEQ ID NO: 138.

[026] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising YPAEVVQK (SEQ ID NO: 943), which is present immediately

subsequent to position 576, numbered relative to SEQ ID NO: 138 or 982. In some embodiments, the amino acid sequence of YPAEVVQK (SEQ ID NO: 943) replaces position 577 (e.g., replaces T577), numbered relative to SEQ ID NO: 138.

[027] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising YPAEVVQK (SEQ ID NO: 943), wherein YPAEVVQK (SEQ ID NO: 943) replaces position 577 (e.g., replaces T577), numbered relative to SEQ ID NO: 138.

[028] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising YPAEVVQK (SEQ ID NO: 943), which is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138, and wherein YPAEVVQK (SEQ ID NO: 943) replaces position 577 (e.g., replaces T577), numbered relative to SEQ ID NO: 138.

[029] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) which comprises the amino acid Y at position 577, and further comprises the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577, numbered relative to SEQ ID NO: 138.

[030] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) which comprises the amino acid Y at position 577, and comprises the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577, numbered relative to SEQ ID NO: 982. In some embodiments, the AAV capsid variant further comprises the amino acid sequence of SEQ ID NO: 739, or an amino acid sequence at least 95% (e.g., at least 96, 97, 98, or 99%) identical thereto. In some embodiments, the AAV capsid variant further comprises the amino acid sequence of SEQ ID NO: 738, or an amino acid sequence at least 95% (e.g., at least 96, 97, 98, or 99%) identical thereto. In some embodiments, the AAV capsid variant further comprises the amino acid sequence of SEQ ID NO: 982, or an amino acid sequence at least 95% (e.g., at least 96, 97, 98, or 99%) identical thereto.

[031] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising the amino acid Y at position 577 and the amino acid sequence of PAEVVQK (SEQ ID NO: 20) at positions 578-584, numbered relative to SEQ ID NO: 982. In some embodiments, the AAV capsid variant further comprises the amino acid sequence of SEQ ID NO: 739, or an amino acid sequence at least 95% (e.g., at least 96, 97, 98, or 99%) identical thereto. In some embodiments, the AAV capsid variant further comprises the amino acid sequence of SEQ ID NO: 738, or an amino acid sequence at least 95% (e.g., at least 96, 97, 98, or 99%) identical thereto. In some embodiments, the AAV capsid variant further comprises the amino acid sequence of SEQ ID NO: 982, or an amino acid sequence at least 95% (e.g., at least 96, 97, 98, or 99%) identical thereto.

[032] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), which is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138 or 982. In some

embodiments, the amino acid sequence of YPAEVVQK (SEQ ID NO: 943), replaces position 577 (e.g., replaces T577) numbered relative to SEQ ID NO: 138.

[033] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), wherein YPAEVVQK (SEQ ID NO: 943) replaces position 577 (e.g., replaces T577), numbered relative to SEQ ID NO: 138.

[034] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), which is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138, wherein YPAEVVQK (SEQ ID NO: 943) replaces position 577 (e.g., replaces T577), numbered relative to SEQ ID NO: 138.

[035] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), which is present at positions 571-586, numbered according to SEQ ID NO: 982.

[036] In another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising the amino acid sequence of SEQ ID NO: 982. In another aspect, the present disclosure provides an AAV capsid variant that consists of the amino acid sequence of SEQ ID NO: 982.

[037] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 984. In another aspect, the present disclosure provides an AAV capsid variant comprising an amino acid sequence encoded by a nucleotide sequence having at least 95% identity to SEQ ID NO: 984.

[038] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of positions 193-731 of SEQ ID NO: 982, wherein the AAV capsid variant comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943). In some embodiments, the AAV capsid variant comprises the amino acid sequence of positions 193-731 of SEQ ID NO: 982.

[039] In yet another aspect, the present disclosure provides an AAV capsid variant (e.g., an AAV5 capsid variant) comprising an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 739, wherein the AAV capsid variant comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943). In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 739.

[040] 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, or 15-20; (b) an amino acid

sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or (c) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or (d) an amino sequence comprising 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 amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20.

[041] In yet another aspect, the present disclosure provides a peptide comprising the amino acid sequence of YPAEVVQK (SEQ ID NO: 943). In yet another aspect, the present disclosure provides a peptide comprising (i) an amino acid sequence comprising one, two, or three, but no more than four different amino acids relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943); (ii) an amino acid sequence comprising 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 YPAEVVQK (SEQ ID NO: 943); or (iii) at least 3, 4, 5, 6, or 7 consecutive amino acids from the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

[042] In yet another aspect, the present disclosure provides a peptide encoded by the nucleotide sequence of SEQ ID NO: 944, 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 yet another aspect, the present disclosure provides a peptide encoded by (i) a nucleotide sequence comprising one, two, three, four, five, six, or seven, but no more than ten different nucleotides, relative to the nucleotide sequence of SEQ ID NO: 944; or (ii) a nucleotide sequence comprising 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: 944.

[043] In yet another aspect, the present disclosure provides a peptide, wherein the nucleotide sequence encoding the peptide comprises (i) the nucleotide sequence of SEQ ID NO: 944, 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 one, two, three, four, five, six, or seven, but no more than ten different nucleotides relative to the nucleotide sequence of SEQ ID NO: 944; or (iii) a nucleotide sequence comprising 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: 944.

[044] In another aspect, the present disclosure provides a polynucleotide encoding an AAV capsid variant (e.g., an AAV5 capsid variant), wherein the encoded AAV capsid variant comprises:

(a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20; (b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or (c) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or (d) an amino sequence comprising 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 amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20.

[045] In yet another aspect, the present disclosure provides a polynucleotide encoding an AAV capsid variant (e.g., an AAV5 capsid variant), wherein the encoded AAV capsid variant comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943). In yet another aspect, the present disclosure provides a polynucleotide encoding an AAV capsid variant, wherein the encoded AAV capsid variant comprises (i) an amino acid sequence comprising one, two, or three, but no more than four different amino acids relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943); (ii) an amino acid sequence comprising 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 YPAEVVQK (SEQ ID NO: 943); or (iii) at least 3, 4, 5, 6, or 7 consecutive amino acids from the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

[046] In yet another aspect, the present disclosure provides an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant (e.g., an AAV5 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 sequence encoding the payload.

[047] In yet another aspect, the present disclosure provides a method of making an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant (e.g., an AAV5 capsid variant), described herein. In some embodiments, 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.

[048] In yet another aspect, the present disclosure provides a method of delivering a payload to a cell or tissue (e.g., a CNS cell, a CNS tissue, a muscle cell, or a muscle tissue). The method comprising administering an effective amount of an AAV particle comprising an AAV capsid variant described herein (e.g., an AAV5 capsid variant).

[049] 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 an effective amount of an AAV particle comprising an AAV capsid variant described herein (e.g., an AAV5 capsid variant).

[050] In yet another aspect, the present disclosure provides a method of treating a subject having or diagnosed with having a neurological, e.g., a neurodegenerative, disorder. The method comprising administering an effective amount of an AAV particle comprising an AAV capsid variant described herein (e.g., an AAV5 capsid variant).

[051] In yet another aspect, the present disclosure provides a method of treating a subject having or diagnosed with having a muscular disorder or a neuromuscular disorder. The method comprising administering an effective amount of an AAV particle comprising an AAV capsid variant described herein (e.g., an AAV5 capsid variant).

[052] In yet another aspect, the present disclosure provides a method of treating a subject having or diagnosed with having a cardiac disorder, e.g., a cardiac disorder as described herein (e.g., 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). The method comprising administering an effective amount of an AAV particle comprising an AAV capsid variant described herein (e.g., an AAV5 capsid variant).

[053] 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 (e.g., an AAV5 capsid variant).

[054] 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 AAV5 capsid variant), comprising an amino sequence comprising the following formula:

[N2]-[N3], wherein:

(i) [N2] comprises positions X1, X2, X3, X4, and X5, wherein:

(a) position X1 is Y, N, C, or T;

(b) position X2 is P, E, K, T, or Q;

(c) position X3 is A or P;

(d) position X4 is E, S, D, or A; and

(e) position X5 is V, L, or E; and

(ii) [N3] comprises the amino acid sequence of VQK, VQN, EQK, VKK, VHK, VQQ, or LQK; or

wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii).

2. An AAV capsid variant (e.g., an AAV5 capsid variant), comprising an amino sequence comprising the following formula:
[N2]-[N3], wherein:

(i) [N2] comprises positions X1, X2, X3, X4, and X5, wherein:

(a) position X1 is Y, N, or C;

(b) position X2 is P, K, T, or Q;

(c) position X3 is A or P;

(d) position X4 is E, S, or A; and

(e) position X5 is V, L, or E; and

(ii) [N3] comprises the amino acid sequence of VQK, EQK, VKK, VHK, VQQ, or LQK; or

wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii).

3. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising one, two, three, four, or all of:

(i) an [N0] comprising TNN, TNT, INN, TNS, NNN, or TNK;

(ii) an [N1] comprising QSS, QSK, TSL, SSS, QSR, AGA, IGS, QAS, ASS, LGS, QST, HSS, LSS, or QRS;

(iii) an [N2] comprising YPAEV (SEQ ID NO: 1), YPPSL (SEQ ID NO: 2), NKAEV (SEQ ID NO: 3), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), YQAEV (SEQ ID NO: 6), YTPSL (SEQ ID NO: 7), YPAAV (SEQ ID NO: 8), NPAEV (SEQ ID NO: 9), CPAEV (SEQ ID NO: 10), or YQAEE (SEQ ID NO: 11);

(iv) an [N3] comprising VQK, EQK, VKK, VHK, VQQ, or LQK; and

(v) an [N4] comprising TA, PA, or NA; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i)-(v).

4. The AAV capsid variant of embodiment 1 or 2, wherein:

(a) position X1 is Y or N;

(b) position X2 is P, T or Q;

(c) position X3 is A;

(d) position X4 is E or S; and/or

(e) position X5 is V or L.

5. The AAV capsid variant of any one of embodiments 1, 2, or 4, wherein [N2] comprises YP, NK, YT, YQ, NP, CP, TH, AE, PS, AA, AS, PA, PP, KA, TA, QA, TP, HA, EV, SL, EE, AV, or SH.

6. The AAV capsid variant of any one of embodiments 1, 2, 4, or 5, wherein [N2] comprises YPA, YPP, NKA, YTA, YQA, YTP, NPA, CPA, THA, PAE, PPS, KAE, TAE, QAE, TPS, PAA, HAS, AEV, PSL, AEE, or AAV.

7. The AAV capsid variant of any one of embodiments 1-2 or 4-6, wherein [N2] comprises YPAE (SEQ ID NO: 21), YPPS (SEQ ID NO: 22), NKAE (SEQ ID NO: 23), YTAE (SEQ ID NO: 24), YQAE (SEQ ID NO: 25), YTPS (SEQ ID NO: 26), YPAA (SEQ ID NO: 27), NPAE (SEQ ID NO: 28), CPAE (SEQ ID NO: 29), THAS (SEQ ID NO: 30), PAEV (SEQ ID NO: 17), PPSL (SEQ ID NO: 31), KAEV (SEQ ID NO: 32), TAEV (SEQ ID NO: 16), PAEE (SEQ ID NO: 18), QAEV (SEQ ID NO: 15), TPSL (SEQ ID NO: 33), PAAV (SEQ ID NO: 34), or QAEE (SEQ ID NO: 35).

8. The AAV capsid variant of any one of embodiments 1-7, wherein [N2] is or comprises YPAEV (SEQ ID NO: 1), YPPSL (SEQ ID NO: 2), NKAEV (SEQ ID NO: 3), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), YQAEV (SEQ ID NO: 6), YTPSL (SEQ ID NO: 7), YPAAV (SEQ ID NO: 8), NPAEV (SEQ ID NO: 9), CPAEV (SEQ ID NO: 10), or YQAEE (SEQ ID NO: 11).

9. The AAV capsid variant of any one of embodiments 1-2 or 4-8, wherein [N3] comprises the amino acid sequence of VQK, EQK, or VKK.

10. The AAV capsid variant of any one of embodiments 1-2 or 4-9, wherein [N3] comprises VQK.

11. The AAV capsid variant of any one of embodiments 1-2 or 4-9, wherein [N3] comprises EQK.

12. The AAV capsid variant of any one of embodiments 1-2 or 4-9, wherein [N3] comprises VKK

13. The AAV capsid variant of any one of embodiments 1-12, wherein [N2] is or comprises the amino acid sequence of YPAEV (SEQ ID NO: 1) and [N3] is or comprises the amino acid sequence of VQK.

14. The AAV capsid variant of any one of embodiments 1-12, wherein:

(i) [N2] is or comprises the amino acid sequence of YTPSL (SEQ ID NO: 7) and [N3] is or comprises the amino acid sequence of VQK;

(ii) [N2] is or comprises the amino acid sequence of YPPSL (SEQ ID NO: 2) and [N3] is or comprises the amino acid sequence of VQK;

(iii) [N2] is or comprises the amino acid sequence of YPPS (SEQ ID NO: 2)L and [N3] is or comprises the amino acid sequence of EQK; or

(iv) [N2] is or comprises the amino acid sequence of YPPSL (SEQ ID NO: 2) and [N3] is or comprises the amino acid sequence of VKK.

15. The AAV capsid variant of any one of embodiments 1-2 or 4-14, wherein [N2]-[N3] comprises:

(i) AEVVQK (SEQ ID NO: 36), PSLVQK (SEQ ID NO: 37), AEVEQK (SEQ ID NO: 38), AEEVQK (SEQ ID NO: 39), PSLEQK (SEQ ID NO: 40), PSLVKK (SEQ ID NO: 41), AEVVKK (SEQ ID NO: 42), AEVVHK (SEQ ID NO: 43), AAVVQK (SEQ ID NO: 44), AEVVQQ (SEQ ID NO: 45), or AEVLQK (SEQ ID NO: 46);

(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).

16. The AAV capsid variant of any one of embodiments 1-2 or 4-15, wherein [N2]-[N3] comprises:

(i) PAEVVQK (SEQ ID NO: 20), PPSLVQK (SEQ ID NO: 47), KAEVVQK (SEQ ID NO: 48), TAEVVQK (SEQ ID NO: 49), PAEVEQK (SEQ ID NO: 50), PAEEVQK (SEQ ID NO: 51), QAEVVQK (SEQ ID NO: 52), TPSLVQK (SEQ ID NO: 53), PPSLEQK (SEQ ID NO: 54), PPSLVKK (SEQ ID NO: 55), PAEVVKK (SEQ ID NO: 56), PAEVVHK (SEQ ID NO: 57), PAAVVQK (SEQ ID NO: 58), PAEVVQQ (SEQ ID NO: 59), TAEVVKK (SEQ ID NO: 60), PAEVLQK (SEQ ID NO: 61), or QAEEVQK (SEQ ID NO: 62);

(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).

17. The AAV capsid variant of any one of embodiments 1-16, wherein [N2]-[N3] is or comprises:

(i) YPAEVVQK (SEQ ID NO: 943), YPPSLVQK (SEQ ID NO: 946), NKAEVVQK (SEQ ID NO: 947), YTAEVVQK (SEQ ID NO: 948), YPAEVEQK (SEQ ID NO: 949), YPAEEVQK (SEQ ID NO: 950), YQAEVVQK (SEQ ID NO: 951), YTPSLVQK (SEQ ID NO: 952), YPPSLEQK

(SEQ ID NO: 953), YPPSLVKK (SEQ ID NO: 954), YPAEVVKK (SEQ ID NO: 955), YPAEVVHK (SEQ ID NO: 956), YPAAVVQK (SEQ ID NO: 957), NPAEVVQK (SEQ ID NO: 958), YPAEVVQQ (SEQ ID NO: 959), CPAEVVQK (SEQ ID NO: 960), YTAEVVKK (SEQ ID NO: 961), YPAEVLQK (SEQ ID NO: 962), or YQAEEVQK (SEQ ID NO: 963);

(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).

18. The AAV capsid variant of any one of embodiments 1-17, which further comprises one, two, three or all of an amino acid other than Q at position 574 (e.g., T, S, A, I, L, or H), an amino acid other than S at position 575 (e.g., G, A, or R), and/or an amino acid other than S at position 576 (e.g., K, L, R, A, or T), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

19. The AAV capsid variant of any one of embodiments 1-17, which further comprises:

(i) a Q at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(ii) a T at position 574, an S at position 575, and/or a L at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(iii) an S at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(iv) a Q at position 574, an S at position 575, and/or an R at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(v) a Q at position 574, an S at position 575, and/or a K at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(vi) an A at position 574, a G at position 575, and/or an A at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(vii) an I at position 574, a G at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(viii) a Q at position 574, an A at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(ix) an A at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(x) an L at position 574, a G at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(xi) a Q at position 574, an S at position 575, and/or a T at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(xii) an H at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(xiii) an L at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982; or

(xiv) a Q at position 574, an R at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

20. The AAV capsid variant of any one of embodiments 1-19, which further comprises [N1], wherein [N1] comprises positions X_D, X_E, and X_F, wherein:

(a) position X_D is Q, T, S, A, I, L, or H;

(b) position X_E is S, G, A, or R; and

(c) position X_F is S, K, L, R, A, or T; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a)-(c).

21. The AAV capsid variant of embodiment 20, wherein [N1] comprises SK, SL, SS, SR, GA, GS, AS, ST, RS, QS, TS, AG, IG, QA, LG, HS, LS, or QR.

22. The AAV capsid variant of any one of embodiments 3, 20, or 21, wherein [N1] is or comprises QSS, QSK, TSL, SSS, QSR, AGA, IGS, QAS, ASS, LGS, QST, HSS, LSS, or QRS.

23. The AAV capsid variant of any one of embodiments 20-22, wherein [N1]-[N2] comprises:

(i) SSYPA (SEQ ID NO: 63), SKYPA (SEQ ID NO: 64), SLYPA (SEQ ID NO: 65), SRYPA (SEQ ID NO: 66), SSYPP (SEQ ID NO: 67), GAYPA (SEQ ID NO: 68), GSYPA (SEQ ID NO: 69), ASYPA (SEQ ID NO: 70), STNKA (SEQ ID NO: 71), SSYTA (SEQ ID NO: 72), SSYQA (SEQ ID NO: 73), SSYTP (SEQ ID NO: 74), SSNPA (SEQ ID NO: 75), SLCPA (SEQ ID NO: 76), RSYTA (SEQ ID NO: 77), or SSTHA (SEQ ID NO: 78);

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

24. The AAV capsid variant of any one of embodiments 20-23, wherein [N1]-[N2] comprises:

(i) SSYPAE (SEQ ID NO: 79), SKYPAE (SEQ ID NO: 80), SLYPAE (SEQ ID NO: 81), SRYPAE (SEQ ID NO: 82), SSYPPS (SEQ ID NO: 83), GAYPAE (SEQ ID NO: 84), GSYPAE (SEQ ID NO: 85), ASYPAE (SEQ ID NO: 86), STNKAE (SEQ ID NO: 87), SSYTAE (SEQ ID NO: 88), SSYQAE (SEQ ID NO: 89), SSYTPS (SEQ ID NO: 90), SSYPAA (SEQ ID NO: 91), SSNPAE (SEQ ID NO: 92), SLCPAE (SEQ ID NO: 93), RSYTAE (SEQ ID NO: 94), SSTHAS (SEQ ID NO: 95);

(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).

25. The AAV capsid variant of any one of embodiments 3 or 20-24, wherein [N1]-[N2] is or comprises:

(i) QSSYPAEV (SEQ ID NO: 96), QSKYPAEV (SEQ ID NO: 97), TSLYPAEV (SEQ ID NO: 98), SSSYPAEV (SEQ ID NO: 99), QSRYPAEV (SEQ ID NO: 100), QSSYPPSL (SEQ ID NO: 101), AGAYPAEV (SEQ ID NO: 102), IGSYPAEV (SEQ ID NO: 103), QASYPAEV (SEQ ID NO: 104), ASSYPAEV (SEQ ID NO: 105), LGSYPAEV (SEQ ID NO: 106), QSTNKAEV (SEQ ID NO: 107), HSSYPAEV (SEQ ID NO: 108), SSSYTAEV (SEQ ID NO: 109), TSLYPAEE (SEQ ID NO: 110), ASSYQAEV (SEQ ID NO: 111), QSSYTPSL (SEQ ID NO: 112), QSRYPAEE (SEQ ID NO: 113), LSSYQAEV (SEQ ID NO: 114), HSSYPAAV (SEQ ID NO: 115), QSSNPAEV (SEQ ID NO: 116), QSSYTAEV (SEQ ID NO: 117), TSLCPAEV (SEQ ID NO: 118), QRSYTAEV (SEQ ID NO: 119), or QSSYQAEE (SEQ ID NO: 120);

(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).

26. The AAV capsid variant of any one of embodiments 20-25, wherein [N1]-[N2]-[N3] comprises:

(i) SSYPAEVVQ (SEQ ID NO: 121), SKYPAEVVQ (SEQ ID NO: 122), SLYPAEVVQ (SEQ ID NO: 123), SRYPAEVVQ (SEQ ID NO: 124), SSYPPSLVQ (SEQ ID NO: 125), GAYPAEVVQ (SEQ ID NO: 126), GSYPAEVVQ (SEQ ID NO: 127), ASYPAEVVQ (SEQ ID NO: 128), STNKAEVVQ (SEQ ID NO: 129), SSYTAEVVQ (SEQ ID NO: 130), SKYPAEVEQ (SEQ ID NO: 131), SLYPAEEVQ (SEQ ID NO: 132), SSYQAEVVQ (SEQ ID NO: 133), SSYTPSLVQ (SEQ ID NO: 134), SRYPAEEVQ (SEQ ID NO: 135), SSYPPSLEQ (SEQ ID NO: 136), SSYPPSLVK (SEQ ID NO: 140), SSYPAEVVK (SEQ ID NO: 141), SKYPAEVVH (SEQ ID NO: 142), SSYPAAVVQ (SEQ ID NO: 143), SSNPAEVVQ (SEQ ID NO: 144), SLCPAEVVQ (SEQ ID NO: 145), RSYTAEVVQ (SEQ ID NO: 146), SSYTAEVVK (SEQ ID NO: 147), SSYPAEVLQ (SEQ ID NO: 148), or SSYQAEEVQ (SEQ ID NO: 149);

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

27. The AAV capsid variant of any one of embodiments 3 or 20-26, wherein [N1]-[N2]-[N3] is or comprises:

(i) QSSYPAEVVQK (SEQ ID NO: 150), QSKYPAEVVQK (SEQ ID NO: 151), TSLYPAEVVQK (SEQ ID NO: 152), SSSYPAEVVQK (SEQ ID NO: 153), QSRYPAEVVQK (SEQ ID NO: 154), QSSYPPSLVQK (SEQ ID NO: 155), AGAYPAEVVQK (SEQ ID NO: 156), IGSYPAEVVQK (SEQ ID NO: 157), QASYPAEVVQK (SEQ ID NO: 158), ASSYPAEVVQK (SEQ ID NO: 159), LGSYPAEVVQK (SEQ ID NO: 160), QSTNKAEVVQK (SEQ ID NO: 161), HSSYPAEVVQK (SEQ ID NO: 162), SSSYTAEVVQK (SEQ ID NO: 163), QSKYPAEVEQK (SEQ ID NO: 164), TSLYPAEEVQK (SEQ ID NO: 165), ASSYQAEVVQK (SEQ ID NO: 166), QSSYTPSLVQK (SEQ ID NO: 167), QSRYPAEEVQK (SEQ ID NO: 168), QSSYPPSLEQK (SEQ ID NO: 169), QSSYPPSLVKK (SEQ ID NO: 170), LSSYQAEVVQK (SEQ ID NO: 171), SSSYPAEVVKK (SEQ ID NO: 172), QSKYPAEVVHK (SEQ ID NO: 173), HSSYPAAVVQK (SEQ ID NO: 174), QSSNPAEVVQK (SEQ ID NO: 175), SSSYPAEVVQQ (SEQ ID NO: 176), QSSYTAEVVQK (SEQ ID NO: 177), TSLCPAEVVQK (SEQ ID NO: 178), QRSYTAEVVQK (SEQ ID NO: 179), QSSYTAEVVKK (SEQ ID NO: 180), HSSYPAEVLQK (SEQ ID NO: 181), or QSSYQAEEVQK (SEQ ID NO: 182);

(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).

28. The AAV capsid variant of any one of embodiments 1-27, which further comprises [N0], wherein [N0] comprises positions X_A, X_B, and X_C, wherein:

(a) position X_A is T, I, or N;

(b) position X_B is N;

(c) position X_C is N, T, S, or K; and

optionally, wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a)-(c).

29. The AAV capsid variant of embodiment 28, wherein [N0] comprises TN, IN, NN, NT, NS, or NK.

30. The AAV capsid variant of embodiment 3, 28, or 29, wherein [N0] is or comprises TNN, TNT, INN, TNS, NNN, or TNK.

31. The AAV capsid variant of any one of embodiments 3 or 28-30, wherein [N0]-[N1] is or comprises:

(i) TNNQSS (SEQ ID NO: 183), TNNQSK (SEQ ID NO: 184), TNNTSL (SEQ ID NO: 185), TNNSSS (SEQ ID NO: 186), TNNQSR (SEQ ID NO: 187), TNNAGA (SEQ ID NO: 188), TNNIGS (SEQ ID NO: 189), TNNQAS (SEQ ID NO: 190), TNTASS (SEQ ID NO: 191), TNNLGS (SEQ ID NO: 192), TNNQST (SEQ ID NO: 193), TNNHSS (SEQ ID NO: 194), TNNQSK (SEQ ID NO: 184), TNNLSS (SEQ ID NO: 195), INNQSS (SEQ ID NO: 196), TNSQSS (SEQ ID NO: 197), NNNQSR (SEQ ID NO: 198), TNSTSL (SEQ ID NO: 199), TNNQRS (SEQ ID NO: 200), or TNKQAS (SEQ ID NO: 201);

(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).

32. The AAV capsid variant of any one of embodiments 3 or 28-31, wherein [N0]-[N1]-[N2]-[N3] is or comprises:

(i) TNNQSSYPAEVVQK (SEQ ID NO: 500), TNNQSKYPAEVVQK (SEQ ID NO: 503), TNNTSLYPAEVVQK (SEQ ID NO: 506), TNNSSSYPAEVVQK (SEQ ID NO: 508), TNNQSRYPAEVVQK (SEQ ID NO: 510), TNNQSSYPPSLVQK (SEQ ID NO: 512), TNNAGAYPAEVVQK (SEQ ID NO: 513), TNNIGSYPAEVVQK (SEQ ID NO: 514), TNNQASYPAEVVQK (SEQ ID NO: 517), TNTASSYPAEVVQK (SEQ ID NO: 520), TNNLGSYPAEVVQK (SEQ ID NO: 523), TNNQSTNKAEVVQK (SEQ ID NO: 524), TNNHSSYPAEVVQK (SEQ ID NO: 525), TNNSSSYTAEVVQK (SEQ ID NO: 526), TNNQSKYPAEVEQK (SEQ ID NO: 529), TNNTSLYPAEEVQK (SEQ ID NO: 530), TNTASSYQAEVVQK (SEQ ID NO: 531), TNNQSSYTPSLVQK (SEQ ID NO: 533), TNNQSRYPAEEVQK (SEQ ID NO: 534), TNNQSSYPPSLEQK (SEQ ID NO: 535), TNNQSSYPPSLVKK (SEQ ID NO: 536), TNNLSSYQAEVVQK (SEQ ID NO: 539), TNNSSSYPAEVVKK (SEQ ID NO: 540), TNNQSKYPAEVVHK (SEQ ID NO: 542), INNQSSYPAEVVQK (SEQ ID NO: 543), TNNHSSYPAAVVQK (SEQ ID NO: 545), TNSQSSNPAEVVQK (SEQ ID NO: 548), TNNSSSYPAEVVQQ (SEQ ID NO: 551), NNNQSRYPAEVVQK (SEQ ID NO: 552), TNNQSSYTAEVVQK (SEQ ID NO: 553), TNNTSLCPAEVVQK (SEQ ID NO: 554), TNSTSLYPAEVVQK (SEQ ID NO: 556), TNNQRSYTAEVVQK (SEQ ID NO: 557), TNNQSSYTAEVVKK (SEQ ID NO: 558), TNNHSSYPAEVLQK (SEQ ID NO: 560), TNNQSSYQAEEVQK (SEQ ID NO: 562), or TNKQASYPAEVVQK (SEQ ID NO: 563);

(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).

33. The AAV capsid variant of embodiment 3 or 32, wherein [N0]-[N1]-[N2]-[N3] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500).

34. The AAV capsid variant of embodiment 3 or 32, wherein [N0]-[N1]-[N2]-[N3] is or comprises TNNAGAYPAEVVQK (SEQ ID NO: 513), TNNTSLYPAEVVQK (SEQ ID NO: 506), TNNQSKYPAEVVQK (SEQ ID NO: 503), TNNQSSYTPSLVQK (SEQ ID NO: 533), TNNQSSYPPSLVQK (SEQ ID NO: 512), TNNQSRYPAEVVQK (SEQ ID NO: 510),

TNNQSSYPPSLEQK (SEQ ID NO: 535), TNNQSSYPPSLVKK (SEQ ID NO: 536), or INNQSSYPAEVVQK (SEQ ID NO: 543).

35. The AAV capsid variant of any one of embodiments 1-34, which further comprises [N4], wherein [N4] comprises positions X_G and X_H, wherein:

(a) position X_G is T, P, or N; and

(b) position X_H is A; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a) or (b).

36. The AAV capsid variant of embodiment 35, wherein [N4] is or comprises TA, PA, or NA.

37. The AAV capsid variant of any one of embodiments 3, 35, or 36, wherein [N3]-[N4] is or comprises:

(i) VQKTA (SEQ ID NO: 564), EQKTA (SEQ ID NO: 565), VKKTA (SEQ ID NO: 566), VQKPA (SEQ ID NO: 567), VHKTA (SEQ ID NO: 568), VQQTA (SEQ ID NO: 569), VQKNA (SEQ ID NO: 570), or LQKTA (SEQ ID NO: 571);

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

38. The AAV capsid variant of any one of embodiments 3 or 35-37, wherein [N0]-[N1]-[N2]-[N3]-[N4] is or comprises:

(i) TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNTSLYPAEVVQKTA (SEQ ID NO: 1232), TNNSSSYPAEVVQKTA (SEQ ID NO: 1539), TNNQSRYPAEVVQKTA (SEQ ID NO: 1327), TNNQSSYPPSLVQKTA (SEQ ID NO: 1300), TNNAGAYPAEVVQKTA (SEQ ID NO: 1021), TNNIGSYPAEVVQKTA (SEQ ID NO: 1112), TNNQASYPAEVVQKTA (SEQ ID NO: 1194), TNTASSYPAEVVQKTA (SEQ ID NO: 1575), TNNLGSYPAEVVQKTA (SEQ ID NO: 1027), TNNQSTNKAEVVQKTA (SEQ ID NO: 1578), TNNHSSYPAEVVQKTA (SEQ ID NO: 1310), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNSSSYTAEVVQKTA (SEQ ID NO: 1214), TNNQSKYPAEVEQKTA (SEQ ID NO: 1254), TNNTSLYPAEEVQKTA (SEQ ID NO: 1583), TNTASSYQAEVVQKTA (SEQ ID NO: 1584), TNNQSSYTPSLVQKTA (SEQ ID NO: 1585), TNNQSRYPAEEVQKTA (SEQ ID NO:

1342), TNNQSSYPPSLEQKTA (SEQ ID NO: 1590), TNNQSSYPPSLVKKTA (SEQ ID NO: 1591), TNNLSSYQAEVVQKTA (SEQ ID NO: 1592), TNNQSSYPPSLVQKPA (SEQ ID NO: 1593), TNNSSSYPAEVVKKTA (SEQ ID NO: 1331), TNNQSKYPAEVVHKTA (SEQ ID NO: 1453), TNNSSSYPAEVVQKPA (SEQ ID NO: 1142), INNQSSYPAEVVQKTA (SEQ ID NO: 1024), TNNHSSYPAAVVQKTA (SEQ ID NO: 1598), TNSQSSNPAEVVQKTA (SEQ ID NO: 1599), TNNSSSYPAEVVQQTA (SEQ ID NO: 1419), NNNQSRYPAEVVQKTA (SEQ ID NO: 1601), TNNQSSYTAEVVQKNA (SEQ ID NO: 1602), TNNTSLCPAEVVQKTA (SEQ ID NO: 1603), TNSTSLYPAEVVQKTA (SEQ ID NO: 1605), TNNQRSYTAEVVQKTA (SEQ ID NO: 1604), TNNQSSYTAEVVKKTA (SEQ ID NO: 1606), TNNHSSYPAEVLQKTA (SEQ ID NO: 1607), TNNQSSYQAEEVQKTA (SEQ ID NO: 1608), or TNKQASYPAEVVQKTA (SEQ ID NO: 1587);

(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, 13, 14, or 15 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 embodiment 3 or 35-38, wherein [N0]-[N1]-[N2]-[N3]-[N4] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

40. The AAV capsid variant of embodiment 3 or 35-38, wherein [N0]-[N1]-[N2]-[N3]-[N4] is or comprises TNNAGAYPAEVVQKTA (SEQ ID NO: 1021), TNNTSLYPAEVVQKTA (SEQ ID NO: 1232), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNQSSYTPSLVQKTA (SEQ ID NO: 1585), TNNQSSYPPSLVQKTA (SEQ ID NO: 1300), TNNQSRYPAEVVQKTA (SEQ ID NO: 1327), TNNQSSYPPSLEQKTA (SEQ ID NO: 1590), TNNQSSYPPSLVKKTA (SEQ ID NO: 1591), or INNQSSYPAEVVQKTA (SEQ ID NO: 1024).

41. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising an amino sequence comprising the following formula: [B]-[C], wherein

(i) [B] comprises positions X1, X2, and X3, wherein:

(a) position X1 is Q, T, S, A, I, L, or H;

(b) position X2 is S, G, or A; and

(c) position X3 is S, K, L, R, or A; and

(ii) [C] comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943); and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii).

42. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising one, two, three, four, or all of:

(i) an [A], wherein [A] comprises the amino acid sequence of TNN, TNT, INN, NNN, TNS, or TNK;

(ii) a [B], wherein [B] comprises the amino acid sequence of QSS, TSL, SSS, QSR, QSK, AGA, IGS, QAS, ASS, LGS, or HSS;

(iii) a [C], wherein [C] comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943); and

(iv) a [D], wherein [D] comprises the amino acid sequence of TA or PA; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i)-(v); and

further optionally wherein [C] replaces position 577 relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

43. The AAV capsid variant of embodiment 41, wherein:

(a) position X1 is Q, T, S, A, or H;

(b) position X2 is S or G; and

(c) position X3 is S, K, L, or R.

44. The AAV capsid variant of embodiment 41 or 43, wherein [B] comprises QS, TS, SS, AG, IG, QA, AS, LG, HS, SK, SL, SR, GA, or GS.

45. The AAV capsid variant of any one of embodiments 41-44, wherein [B] is or comprises QSS, TSL, SSS, QSR, QSK, AGA, IGS, QAS, ASS, LGS, or HSS.

46. The AAV capsid variant of any one of embodiments 41 or 43-45, wherein [B]-[C] comprises:

(i) SSYPAEVVQK (SEQ ID NO: 572), SKYPAEVVQK (SEQ ID NO: 573), SLYPAEVVQK (SEQ ID NO: 574), SRYPAEVVQK (SEQ ID NO: 575), GAYPAEVVQK (SEQ ID NO: 576), GSYPAEVVQK (SEQ ID NO: 580), or ASYPAEVVQK (SEQ ID NO: 582);

(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, or 9 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).

47. The AAV capsid variant of any one of embodiments 41-46, wherein [B]-[C] is or comprises:

(i) QSSYPAEVVQK (SEQ ID NO: 150), QSKYPAEVVQK (SEQ ID NO: 151), TSLYPAEVVQK (SEQ ID NO: 152), SSSYPAEVVQK (SEQ ID NO: 153), QSRYPAEVVQK (SEQ ID NO: 154), AGAYPAEVVQK (SEQ ID NO: 156), IGSYPAEVVQK (SEQ ID NO: 157), QASYPAEVVQK (SEQ ID NO: 158), ASSYPAEVVQK (SEQ ID NO: 159), LGSYPAEVVQK (SEQ ID NO: 160), or HSSYPAEVVQK (SEQ ID NO: 162);

(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).

48. The AAV capsid variant of any one of embodiments 41-47, wherein [B]-[C] is or comprises QSSYPAEVVQK (SEQ ID NO: 150).

49. The AAV capsid variant of any one of embodiments embodiment 41-47, wherein [B]-[C] is or comprises AGAYPAEVVQK (SEQ ID NO: 156), TSLYPAEVVQK (SEQ ID NO: 152), QSKYPAEVVQK (SEQ ID NO: 151), or QSRYPAEVVQK (SEQ ID NO: 154).

50. The AAV capsid variant of any one of embodiments 1-49, which further comprises one or both of an amino acid other than T at position 571 (e.g., I or N), and/or an amino acid other than N at position 573 (e.g., T, S, or K), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

51. The AAV capsid variant of any one of embodiments 1-49, which further comprises:

(i) a T at position 571, an N at position 572, and/or an N at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(ii) a T at position 571, an N at position 572, and/or a T at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(iii) an I at position 571, an N at position 572, and/or an N at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(iv) a T at position 571, an N at position 572, and/or an S at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(v) an N at position 571, an N at position 572, and/or an N at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982; or

(vi) a T at position 571, an N at position 572, and/or a K at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

52. The AAV capsid variant of any one of embodiments 41-51, which further comprises [A], wherein [A] comprises positions X_A, X_B, and X_C, wherein:

(a) position X_A is T, I, or N;

(b) position X_B is N; and

(c) position X_C is N, T, S, or K; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a)-(c).

53. The AAV capsid variant of embodiment 52, wherein [A] comprises TN, IN, NN, NT, NS, or NK.

54. The AAV capsid variant of any one of embodiments 42, 52, or 53, wherein [A] is or comprises TNN, TNT, INN, NNN, TNS, or TNK.

55. The AAV capsid variant of any one of embodiments 42 or 52-54, wherein [A]-[B] is or comprises:

(i) TNNQSS (SEQ ID NO: 183), TNNQSK (SEQ ID NO: 184), TNNTSL (SEQ ID NO: 185), TNNSSS (SEQ ID NO: 186), TNNQSR (SEQ ID NO: 187), TNNAGA (SEQ ID NO: 188), TNNIGS (SEQ ID NO: 189), TNNQAS (SEQ ID NO: 190), TNTASS (SEQ ID NO: 191), TNNLGS (SEQ ID NO: 192), TNNHSS (SEQ ID NO: 194), INNQSS (SEQ ID NO: 196), NNNQSR (SEQ ID NO: 198), TNSTSL (SEQ ID NO: 199), or TNKQAS (SEQ ID NO: 201);

(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).

56. The AAV capsid variant of any one of embodiments 42 or 52-55, wherein [A]-[B]-[C] is or comprises:

(i) TNNQSSYPAEVVQK (SEQ ID NO: 500), TNNQSKYPAEVVQK (SEQ ID NO: 503), TNNTSLYPAEVVQK (SEQ ID NO: 506), TNNSSSYPAEVVQK (SEQ ID NO: 508), TNNQSRYPAEVVQK (SEQ ID NO: 510), TNNAGAYPAEVVQK (SEQ ID NO: 513), TNNIGSYPAEVVQK (SEQ ID NO: 514), TNNQASYPAEVVQK (SEQ ID NO: 517), TNTASSYPAEVVQK (SEQ ID NO: 520), TNNLGSYPAEVVQK (SEQ ID NO: 523),

TNNHSSYPAEVVQK (SEQ ID NO: 525), INNQSSYPAEVVQK (SEQ ID NO: 543), NNNQSRYPAEVVQK (SEQ ID NO: 552), TNSTSLYPAEVVQK (SEQ ID NO: 556), or TNKQASYPAEVVQK (SEQ ID NO: 563);

(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).

57. The AAV capsid variant of any one of embodiments 42 or 52-54, wherein [A]-[B]-[C] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500).

58. The AAV capsid variant of any one of embodiments 42 or 52-54, wherein [A]-[B]-[C] is or comprises TNNAGAYPAEVVQK (SEQ ID NO: 513), TNNTSLYPAEVVQK (SEQ ID NO: 506), TNNQSKYPAEVVQK (SEQ ID NO: 503), TNNQSRYPAEVVQK (SEQ ID NO: 510), or INNQSSYPAEVVQK (SEQ ID NO: 543).

59. The AAV capsid variant of any one of embodiments 1-58, which further comprises:

(i) an amino acid other than T at position 578 (e.g., P or N), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138; or an

(ii) an amino acid other than T at position 585 (e.g., P or N), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

60. The AAV capsid variant of any one of embodiments 1-58, which further comprises:

(i) a T at position 578 and/or an A at position 579, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138; or a T at position 585 and/or an A at position 586 relative to a reference sequence numbered according to SEQ ID NO: 982;

(ii) a P at position 578 and/or an A at position 579, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138; or a P at position 585 and/or an A at position 586 relative to a reference sequence numbered according to SEQ ID NO: 982; or

(iii) an N at position 578 and/or an A at position 579, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138; or an N at position 585 and/or an A at position 586 relative to a reference sequence numbered according to SEQ ID NO: 982.

61. The AAV capsid variant of any one of embodiments 41-60, which further comprises [D], wherein [D] comprises positions X4 and X5, wherein:

(a) position X4 is T or N; and

(b) position X5 is A; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a) or (b).

62. The AAV capsid variant of embodiment 42 or 61, wherein [D] is or comprises TA or PA.

63. The AAV capsid variant of any one of embodiments 42, 61, or 62, wherein [C]-[D] is or comprises:

(i) YPAEVVQKTA (SEQ ID NO: 584) or YPAEVVQKPA (SEQ ID NO: 586);

(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, or 9 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 42 or 61-63, wherein [A]-[B]-[C]-[D] is or comprises:

(i) TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNTSLYPAEVVQKTA (SEQ ID NO: 1232), TNNSSSYPAEVVQKTA (SEQ ID NO: 1539), TNNQSRYPAEVVQKTA (SEQ ID NO: 1327), TNNAGAYPAEVVQKTA (SEQ ID NO: 1021), TNNIGSYPAEVVQKTA (SEQ ID NO: 1112), TNNQASYPAEVVQKTA (SEQ ID NO: 1194), TNTASSYPAEVVQKTA (SEQ ID NO: 1575), TNNLGSYPAEVVQKTA (SEQ ID NO: 1027), TNNHSSYPAEVVQKTA (SEQ ID NO: 1310), TNNSSSYPAEVVQKPA (SEQ ID NO: 1142), INNQSSYPAEVVQKTA (SEQ ID NO: 1024), NNNQSRYPAEVVQKTA (SEQ ID NO: 1601), TNSTSLYPAEVVQKTA (SEQ ID NO: 1605), or TNKQASYPAEVVQKTA (SEQ ID NO: 1587);

(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, 13, 14, or 15 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 42 or 61-64, wherein [A]-[B]-[C]-[D] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

66. The AAV capsid variant of any one of embodiments 42 or 61-64, wherein [A]-[B]-[C]-[D] is or comprises TNNAGAYPAEVVQKTA (SEQ ID NO: 1021), TNNTSLYPAEVVQKTA (SEQ ID NO: 1232), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNQSRYPAEVVQKTA (SEQ ID NO: 1327), or INNQSSYPAEVVQKTA (SEQ ID NO: 1024).

67. An AAV capsid variant (e.g., an AAV5 capsid variant), comprising an amino sequence comprising the formula
[N2]-[N3], wherein:

(i) [N2] comprises positions X1, X2, X3, X4, and X5, wherein:

(a) position X1 is Y or T;

(b) position X2 is Q, T, P, or E;

(c) position X3 is A;

(d) position X4 is E or D; and

(e) position X5 is V or E; and

(ii) [N3] comprises the amino acid sequence of VQK or VQN; or

wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii).

68. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising one, two, three, four, or all of:

(i) an [N0] comprising TNN, TNS, TNT, or TNK;

(ii) an [N1] comprising QSS, SLS, SLY, SAT, or QTS;

(iii) an [N2] comprising YPAEV (SEQ ID NO: 1), YQAEV (SEQ ID NO: 6), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), TEAEV (SEQ ID NO: 12), or YPADV (SEQ ID NO: 13);

(iv) an [N3] comprising VQK or VQN; and

(v) an [N4] comprising TA, PA, TD, NA, or PA; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i)-(v).

69. The AAV capsid variant of embodiment 1 or 67, wherein [N2] comprises YP, YQ, YT, TE, QA, TA, PA, EA, EV, EE, DV, AE, or AD.

70. The AAV capsid variant of any one of embodiments 1, 67 or 69, wherein [N2] comprises YPA, YQA, YTA, TEA, QAE, TAE, PAE, EAE, PAD, AEV, AEE, or ADV.

71. The AAV capsid variant of any one of embodiments 1, 67, 69, or 70, wherein [N2] comprises YPAE (SEQ ID NO: 21), YQAE (SEQ ID NO: 25), YTAE (SEQ ID NO: 24), TEAE (SEQ ID NO: 587), YPAD (SEQ ID NO: 588), QAEV (SEQ ID NO: 15), TAEV (SEQ ID NO: 16), PAEV (SEQ ID NO: 17), PAEE (SEQ ID NO: 18), EAEV (SEQ ID NO: 590), or PADV (SEQ ID NO: 19).

72. The AAV capsid variant of any one of embodiments 1 or 67-71, wherein [N2] is or comprises YPAEV (SEQ ID NO: 1), YQAEV (SEQ ID NO: 6), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), TEAEV (SEQ ID NO: 12), or YPADV (SEQ ID NO: 13).

73. The AAV capsid variant of any one of embodiments 1 or 67-72, wherein [N3] comprises the amino acid sequence of VQK.

74. The AAV capsid variant of any one of embodiments 1, 67, or 69-73, wherein [N2]-[N3] comprises:

(i) AEVVQK (SEQ ID NO: 36), AEEVQK (SEQ ID NO: 39), AEVVQN (SEQ ID NO: 591), or ADVVQK (SEQ ID NO: 593);

(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).

75. The AAV capsid variant of any one of embodiments 1, 67, or 69-74, wherein [N2]-[N3] comprises:

(i) PAEVVQN (SEQ ID NO: 594), QAEVVQK (SEQ ID NO: 52), TAEVVQK (SEQ ID NO: 49), PAEVVQK (SEQ ID NO: 20), PAEEVQK (SEQ ID NO: 51), EAEVVQK (SEQ ID NO: 595), or PADVVQK (SEQ ID NO: 596);

(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).

76. The AAV capsid variant of any one of embodiments 1 or 57-75, wherein [N2]-[N3] is or comprises:

(i) YPAEVVQK (SEQ ID NO: 943), YQAEVVQK (SEQ ID NO: 951), YTAEVVQK (SEQ ID NO: 948), YPAEEVQK (SEQ ID NO: 950), YPAEVVQN (SEQ ID NO: 964), TEAEVVQK (SEQ ID NO: 965), or YPADVVQK (SEQ ID NO: 966);

(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).

77. The AAV capsid variant of any one of embodiments 1 or 67-76, wherein [N2]-[N3] is or comprises YPAEVVQK (SEQ ID NO: 943).

78. The AAV capsid variant of any one of embodiments 1-77, wherein [N2]-[N3] replaces position 577, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

79. The AAV capsid variant of any one of embodiments 1 or 67-78, which further comprises one, two, three or all of an amino acid other than Q at position 574 (e.g., S), an amino acid other than S at position 575 (e.g., L, A, or T), and/or an amino acid other than S at position 576 (e.g., Y or T), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

80. The AAV capsid variant of any one of embodiments 1 or 67-78, which further comprises:

(i) a Q at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(ii) an S at position 574, an L at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(iii) an S at position 574, an L at position 575, and/or a Y at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982;

(iv) an S at position 574, an A at position 575, and/or a T at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982; or

(v) a Q at position 574, a T at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

81. The AAV capsid variant of any one of embodiments 1 or 67-80, which further comprises [N1], wherein [N1] comprises positions X_D, X_E, and X_F, wherein:

(a) position X_D is Q or S;

(b) position X_E is S, L, A, or T; and

(c) position X_F is S, Y, or T; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a)-(c).

82. The AAV capsid variant of embodiment 81, wherein [N1] comprises QS, SL, SA, QT, LS, LY, AT, TS, or SS.

83. The AAV capsid variant of any one of embodiment 68, 81, 82, wherein [N1] is or comprises QSS, SLS, SLY, SAT, or QTS.

84. The AAV capsid variant of any one of embodiments 81-83, wherein [N1]-[N2] comprises:

(i) SSYPA (SEQ ID NO: 63), LSYQA (SEQ ID NO: 597), LSYTA (SEQ ID NO: 598), LYYPA (SEQ ID NO: 600), ATYPA (SEQ ID NO: 601), LSYPA (SEQ ID NO: 603), or TSTEA (SEQ ID NO: 605);

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

85. The AAV capsid variant of any one of embodiments 81-84, wherein [N1]-[N2] comprises:

(i) SSYPAE (SEQ ID NO: 79), LSYQAE (SEQ ID NO: 607), LSYTAE (SEQ ID NO: 610), LYYPAE (SEQ ID NO: 611), ATYPAE (SEQ ID NO: 613), LSYPAE (SEQ ID NO: 616), TSTEAE (SEQ ID NO: 619), or LSYPAD (SEQ ID NO: 621);

(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).

86. The AAV capsid variant of any one of embodiments 68 or 81-85, wherein [N1]-[N2] is or comprises:

(i) QSSYPAEV (SEQ ID NO: 96), SLSYQAEV (SEQ ID NO: 622), SLSYTAEV (SEQ ID NO: 623), SLYYPAEV (SEQ ID NO: 624), SATYPAEV (SEQ ID NO: 625), SLSYPAEV (SEQ ID NO: 629), SLSYPAEE (SEQ ID NO: 632), QTSTEAEV (SEQ ID NO: 633), or SLSYPADV (SEQ ID NO: 634);

(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).

87. The AAV capsid variant of any one of embodiments 68 or 81-86, wherein [N1]-[N2]-[N3] is or comprises:

(i) QSSYPAEVVQK (SEQ ID NO: 150), SLSYQAEVVQK (SEQ ID NO: 635), SLSYTAEVVQK (SEQ ID NO: 637), SLYYPAEVVQK (SEQ ID NO: 639), SATYPAEVVQK (SEQ ID NO: 641), SLSYPAEVVQK (SEQ ID NO: 642), SLSYPAEEVQK (SEQ ID NO: 643), SLSYPAEVVQN (SEQ ID NO: 644), QTSTEAEVVQK (SEQ ID NO: 645), or SLSYPADVVQK (SEQ ID NO: 646);

(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, or 12 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).

88. The AAV capsid variant of any one of embodiments 68 or 81-87, wherein [N1]-[N2]-[N3] is or comprises QSSYPAEVVQK (SEQ ID NO: 150).

89. The AAV capsid variant of any one of embodiments 1 or 67-88, which further comprises [N0], wherein [N0] comprises positions X_A, X_B, and X_C, wherein:

(a) position X_A is T;

(b) position X_B is N; and

(c) position X_C is N, T, S, or K; and

optionally wherein the AAV capsid comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a)-(c).

90. The AAV capsid variant of embodiment 89, wherein [N0]:

(i) comprises TN, NS, NT, NN, or NK; and/or

(ii) is or comprises TNS, TNT, TNN, or TNK.

91. The AAV capsid variant of any one of embodiments 68, 89, or 90, wherein [N0]-[N1] is or comprises:

(i) TNNQSS (SEQ ID NO: 183), TNSSLS (SEQ ID NO: 647), TNSSLY (SEQ ID NO: 648), TNTSAT (SEQ ID NO: 649), TNNQTS (SEQ ID NO: 650), or TNKSAT (SEQ ID NO: 651;

(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).

92. The AAV capsid variant of any one of embodiments 68 or 89-91, wherein [N0]-[N1]-[N2]-[N3] is or comprises:

(i) TNNQSSYPAEVVQK (SEQ ID NO: 500), TNSSLSYQAEVVQK (SEQ ID NO: 652), TNSSLSYTAEVVQK (SEQ ID NO: 654), TNSSLYYPAEVVQK (SEQ ID NO: 655), TNTSATYPAEVVQK (SEQ ID NO: 656), TNSSLSYPAEVVQK (SEQ ID NO: 657), TNSSLSYPAEEVQK (SEQ ID NO: 658), TNSSLSYPAEVVQN (SEQ ID NO: 660), TNNQTSTEAEVVQK (SEQ ID NO: 662), TNKSATYPAEVVQK (SEQ ID NO: 663), or TNSSLSYPADVVQK (SEQ ID NO: 665);

(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).

93. The AAV capsid variant of any one of embodiments 68 or 89-92, wherein [N0]-[N1]-[N2]-[N3] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500).

94. The AAV capsid variant of any one of embodiments 1 or 67-93, which further comprises [N4], wherein [N4] comprises positions X_G and X_H, wherein:

(a) position X_G is T, P, or N; and

(b) position X_H is A or D; and

optionally wherein the AAV capsid variant comprise an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a) or (b).

95. The AAV capsid variant of embodiment 94, wherein [N4] is or comprises TA, TD, PA, or NA.

96. The AAV capsid variant of embodiment 68, 94, or 95, wherein [N3]-[N4] is or comprises:

(i) VQKTA (SEQ ID NO: 564), EQKTA (SEQ ID NO: 565), VKKTA (SEQ ID NO: 566), VQKPA (SEQ ID NO: 567), VHKTA (SEQ ID NO: 568), VQQTA (SEQ ID NO: 569), VQKNA (SEQ ID NO: 570), or LQKTA (SEQ ID NO: 571);

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

97. The AAV capsid variant of any one of embodiments 68 or 94-96, wherein [N0]-[N1]-[N2]-[N3]-[N4] is or comprises:

(i) TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNSSLSYQAEVVQKTA (SEQ ID NO: 2064), TNSSLSYTAEVVQKTA (SEQ ID NO: 2065), TNSSLYYPAEVVQKTA (SEQ ID NO: 2066), TNTSATYPAEVVQKTA (SEQ ID NO: 2067), TNSSLSYPAEVVQKTA (SEQ ID NO: 2068), TNSSLSYPAEEVQKTA (SEQ ID NO: 2069), TNSSLSYPAEVVQKTD (SEQ ID NO: 2070), TNSSLSYPAEVVQNTA (SEQ ID NO: 2071), TNSSLSYPAEVVQKNA (SEQ ID NO: 2072), TNSSLSYPAEVVQKPA (SEQ ID NO: 2073), TNNQTSTEAEVVQKTA (SEQ ID NO: 2074), TNKSATYPAEVVQKTA (SEQ ID NO: 2075), or TNSSLSYPADVVQKTA (SEQ ID NO: 2076);

(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, 13, 14, or 15 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).

98. The AAV capsid variant of any one of embodiments 68 or 94-97, wherein [N0]-[N1]-[N2]-[N3]-[N4] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

99. An AAV capsid variant (e.g., an AAV5 capsid variant) comprises the formula [K1]-[K2], wherein:

(i) [K1] comprises LSY or LYY; and

(ii) [K2] comprises positions X1, X2, X3, and X4, wherein:

(a) position X1 is Q, T, or P;

(b) position X2 is A;

(c) position X3 is E or D; and

(d) position X4 is V or E; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii).

100. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising one, two, three, four, or all of:

(i) an [K0], which comprises TNNS (SEQ ID NO: 14);

(ii) an [K1], which comprises LSY or LYY;

(iii) an [K2], which comprises QAEV (SEQ ID NO: 15), TAEV (SEQ ID NO: 16), PAEV (SEQ ID NO: 17), PAEE (SEQ ID NO: 18), or PADV (SEQ ID NO: 19);

(iv) an [K3], which comprise VQK or VQN; and

(v) an [K4], which comprises TA, TD, NA, or PA; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i)-(v).

101. The AAV capsid variant of embodiment 99 or 100, wherein [K1] comprises LSY.

102. The AAV capsid variant of embodiment 99 or 101, wherein [K2] comprises QA, TA, PA, EV, EE, DV, AE, or AD.

103. The AAV capsid variant of any one of embodiments 99, 101, or 102, wherein [K2] comprises QAE, TAE, PAE, PAD, AEV, AEE, or ADV.

104. The AAV capsid variant of any one of embodiments 99-103, wherein [K2] is or comprises QAEV (SEQ ID NO: 15), TAEV (SEQ ID NO: 16), PAEV (SEQ ID NO: 17), PAEE (SEQ ID NO: 18), or PADV (SEQ ID NO: 19).

105. The AAV capsid variant of any one of embodiments 99 or 101-104, wherein [K1]-[K2] comprises LSYQA (SEQ ID NO: 597), LSYTA (SEQ ID NO: 598), LYYPA (SEQ ID NO: 600), or LSYPA (SEQ ID NO: 603).

106. The AAV capsid variant of any one of embodiments 99 or 101-105, wherein [K1]-[K2] comprises:

(i) LSYQAE (SEQ ID NO: 607), LSYTAE (SEQ ID NO: 610), LYYPAE (SEQ ID NO: 611), LSYPAE (SEQ ID NO: 616), or LSYPAD (SEQ ID NO: 621);

(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).

107. The AAV capsid variant of any one of embodiments 99-106, wherein [K1]-[K2] is or comprises:

(i) LSYQAEV (SEQ ID NO: 667), LSYTAEV (SEQ ID NO: 668), LYYPAEV (SEQ ID NO: 669), LSYPAEV (SEQ ID NO: 671), LSYPAEE (SEQ ID NO: 673), or LSYPADV (SEQ ID NO: 674);

(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).

108. The AAV capsid variant of any one of embodiments 99-107, which further comprises an amino acid other than Q at position 574 (e.g., S), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

109. The AAV capsid variant of any one of embodiments 99-108, which further comprises S at position 574, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

110. The AAV capsid variant of any one of embodiments 99-109, which further comprises [K0], wherein [K0] is or comprises TNNS (SEQ ID NO: 14); an amino acid sequence comprising any portion of an amino acid sequence, e.g., any 2 or 3 amino acids, e.g., consecutive amino acids, thereof; 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 the amino acid sequence of TNNS (SEQ ID NO: 14); or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of TNNS (SEQ ID NO: 14).

111. The AAV capsid variant of embodiment 110, wherein [K0]-[K1] comprises:

(i) TNSSLS (SEQ ID NO: 647) or TNSSLY (SEQ ID NO: 648);

(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).

112. The AAV capsid variant of any one of embodiments 100, 110 or 111, wherein [K0]-[K1] is or comprises:

(i) TNSSLSY (SEQ ID NO: 676) or TNSSLYY (SEQ ID NO: 678);

(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).

113. The AAV capsid variant of any one of embodiments 110-112, wherein [K0]-[K1]-[K2] comprises:

(i) TNSSLSYQA (SEQ ID NO: 679), TNSSLSYTA (SEQ ID NO: 681), TNSSLYYPA (SEQ ID NO: 682), or TNSSLSYPA (SEQ ID NO: 683);

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

114. The AAV capsid variant of any one of embodiments 110-113, wherein [K0]-[K1]-[K2] comprises:

(i) TNSSLSYQAE (SEQ ID NO: 684), TNSSLSYTAE (SEQ ID NO: 685), TNSSLYYPAE (SEQ ID NO: 686), TNSSLSYPAE (SEQ ID NO: 687), or TNSSLSYPAD (SEQ ID NO: 689);

(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, or 9 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).

115. The AAV capsid variant of any one of embodiments 100 or 110-114, wherein [K0]-[K1]-[K2] is or comprises:

(i) TNSSLSYQAEV (SEQ ID NO: 692), TNSSLSYTAEV (SEQ ID NO: 693), TNSSLYYPAEV (SEQ ID NO: 696), TNSSLSYPAEV (SEQ ID NO: 697), TNSSLSYPAEE (SEQ ID NO: 698), or TNSSLSYPADV (SEQ ID NO: 699);

(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).

116. The AAV capsid variant of any one of embodiments 99-115, which further comprises [K3], wherein [K3] comprises positions X_A, X_B, and X_C, wherein:

(a) position X_A is V;

(b) position X_B is Q; and

(c) position X_C is K or N; and

optionally wherein the AAV capsid comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a)-(c).

117. The AAV capsid variant of embodiment 116, wherein [K3]:

(i) comprises VQ, QK, or QN;

(ii) is or comprises VQK or VQN.

118. The AAV capsid variant of embodiment 100, 116, or 117, wherein [K2]-[K3] is or comprises:

(i) QAEVVQK (SEQ ID NO: 52), TAEVVQK (SEQ ID NO: 49), PAEVVQK (SEQ ID NO: 20), PAEEVQK (SEQ ID NO: 51), PAEVVQN (SEQ ID NO: 594), or PADVVQK (SEQ ID NO: 596);

(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).

119. The AAV capsid variant of embodiment 100 or 116-118, wherein [K1]-[K2]-[K3] is or comprises:

(i) LSYQAEVVQK (SEQ ID NO: 700), LSYTAEVVQK (SEQ ID NO: 701), LYYPAEVVQK (SEQ ID NO: 702), LSYPAEVVQK (SEQ ID NO: 703), LSYPAEEVQK (SEQ ID NO: 704), LSYPAEVVQN (SEQ ID NO: 706), or LSYPADVVQK (SEQ ID NO: 708);

(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, or 9 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).

120. The AAV capsid variant of embodiment 100 or 116-119, wherein [K0]-[K1]-[K2]-[K3] is or comprises:

(i) TNSSLSYQAEVVQK (SEQ ID NO: 652), TNSSLSYTAEVVQK (SEQ ID NO: 654), TNSSLYYPAEVVQK (SEQ ID NO: 655), TNSSLSYPAEVVQK (SEQ ID NO: 657), TNSSLSYPAEEVQK (SEQ ID NO: 658), TNSSLSYPAEVVQN (SEQ ID NO: 660), or TNSSLSYPADVVQK (SEQ ID NO: 665);

(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).

121. The AAV capsid variant of any one of embodiments 99-120, which further comprises [K4], wherein [K4] comprises positions X_D and X_E, wherein:

(a) position X_D is T, P, or N; and

(b) position X_E is A or D; and

optionally wherein the AAV capsid variant comprise an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a) or (b).

122. The AAV capsid variant of embodiment 100 or 121, wherein [K4] is or comprises TA, TD, PA, or NA.

123. The AAV capsid variant of any one of embodiments 100, 121, or 122, wherein [K3]-[K4] is or comprises:

(i) VQKTA (SEQ ID NO: 564), VQKTD (SEQ ID NO: 714), VQNTA (SEQ ID NO: 715), VQKNA (SEQ ID NO: 570), or VQKPA (SEQ ID NO: 567);

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

124. The AAV capsid variant of any one of embodiments 100 or 121-123, wherein [K0]-[K1]-[K2]-[K3]-[K4] is or comprises:

(i) TNSSLSYQAEVVQKTA (SEQ ID NO: 2064), TNSSLSYTAEVVQKTA (SEQ ID NO: 2065), TNSSLYYPAEVVQKTA (SEQ ID NO: 2066), TNSSLSYPAEVVQKTA (SEQ ID NO: 2068), TNSSLSYPAEEVQKTA (SEQ ID NO: 2069), TNSSLSYPAEVVQKTD (SEQ ID NO: 2070), TNSSLSYPAEVVQNTA (SEQ ID NO: 2071), TNSSLSYPAEVVQKNA (SEQ ID NO: 2072), TNSSLSYPAEVVQKPA (SEQ ID NO: 2073), or TNSSLSYPADVVQKTA (SEQ ID NO: 2076);

(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, 13, 14, or 15 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).

125. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising [B]-[C], wherein:

(i) [B] comprises positions X1, X2, and X3, wherein:

(a) position X1 is Q or S;

(b) position X2 is S, L, or A; and

(c) position X3 is S, Y, or T; and

(ii) [C] comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943); and

optionally wherein there AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i) and/or (ii).

126. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising one, two, three, four, or all of:

(i) an [A] comprising TNN, TNS, TNT, or TNK;

(ii) a [B] comprising QSS, SLY, SAT, or SLS;

(iii) a [C] comprising YPAEVVQK (SEQ ID NO: 943); and

(iv) a [D] comprising TA, TD, NA, or PA; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (i)-(v), and

further optionally wherein [C] replaces position 577 relative to reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

127. The AAV capsid variant of embodiment 125, comprising wherein [B] comprises QS, SL, SA, LY, AT, LS, or SS.

128. The AAV capsid variant of any one of embodiments 125-127, comprising wherein [B] is or comprises QSS, SLY, SAT, or SLS.

129. The AAV capsid variant of any one of embodiments 125, 127, or 128, wherein [B]-[C] comprises:

(i) SSYPAEVVQK (SEQ ID NO: 572), LYYPAEVVQK (SEQ ID NO: 702), ATYPAEVVQK (SEQ ID NO: 718), or LSYPAEVVQK (SEQ ID NO: 703);

(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, or 9 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).

130. The AAV capsid variant of any one of embodiments 125-129, wherein [B]-[C] is or comprises:

(i) QSSYPAEVVQK (SEQ ID NO: 150), SLYYPAEVVQK (SEQ ID NO: 639), SATYPAEVVQK (SEQ ID NO: 641), or SLSYPAEVVQK (SEQ ID NO: 642);

(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).

131. The AAV capsid variant of embodiment 126 or 130, wherein [B]-[C] is or comprises QSSYPAEVVQK (SEQ ID NO: 150).

132. The AAV capsid variant of any one of embodiments 67-98 or 125-131, which further comprises an amino acid other than N at position 573 (e.g., T, S, or K), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

133. The AAV capsid variant of any one of embodiments 67-98 or 125-131, which further comprises:

(i) a T at position 571, an N at position 572, and/or an N at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138;

(ii) a T at position 571, an N at position 572, and/or a T at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138;

(iii) a T at position 571, an N at position 572, and/or a S at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138; or

(iv) a T at position 571, an N at position 572, and/or a K at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

134. The AAV capsid variant of any one of embodiments 125-133, which further comprises [A], wherein [A] comprises positions X_A, X_B, and X_C, wherein:

(a) position X_A is T;

(b) position X_B is N; and

(c) position X_C is N, T, S, or K; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a)-(c).

135. The AAV capsid variant of embodiment 126 or 134, wherein [A]:

(i) comprises TN, NS, NT, NK, or NN;

(ii) is or comprises TNN, TNS, TNT, or TNK.

136. The AAV capsid variant of any one of embodiments 126, 134, or 135, wherein [A]-[B] is or comprises:

(i) TNNQSS (SEQ ID NO: 183), TNSSLY (SEQ ID NO: 648), TNTSAT (SEQ ID NO: 649), TNSSLS (SEQ ID NO: 647), or TNKSAT (SEQ ID NO: 651);

(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).

137. The AAV capsid variant of any one of embodiments 126 or 134-136, wherein [A]-[B]-[C] is or comprises:

(i) TNNQSSYPAEVVQK (SEQ ID NO: 500), TNSSLYYPAEVVQK (SEQ ID NO: 655), TNTSATYPAEVVQK (SEQ ID NO: 656), TNSSLSYPAEVVQK (SEQ ID NO: 657), or TNKSATYPAEVVQK (SEQ ID NO: 663);

(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).

138. The AAV capsid variant of any one of embodiments 126 or 134-137, wherein [A]-[B]-[C] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500).

139. The AAV capsid variant of any one of embodiments 67-98 or 125-138, which further comprises one or both of an amino acid other than T at position 578 (e.g., P or N) and/or an amino acid other than A at position 579 (e.g., D), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

140. The AAV capsid variant of any one of embodiments 67-98 or 125-138, which further comprises:

(i) a T at position 578 and/or an A at position 579, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138;

(ii) a T at position 578 and/or a D at position 579, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138;

(iii) a P at position 578 and/or an A at position 579, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138; or

(iv) an N at position 578 and/or an A at position 579, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

141. The AAV capsid variant of any one of embodiments 125-140, which further comprises [D], wherein [D] comprises positions X4 and X5, wherein:

(a) position X4 is T, N, or P; and

(b) position X5 is A or D; and

optionally wherein the AAV capsid variant comprises an amino acid modification, e.g., a conservative substitution, of any of the aforesaid amino acids in (a) or (b).

142. The AAV capsid variant of embodiment 141, wherein [D] is or comprises TA, TD, NA, or PA.

143. The AAV capsid variant of embodiment 126, 141, or 142, wherein [C]-[D] is or comprises:

(i) YPAEVVQKTA (SEQ ID NO: 584), YPAEVVQKTD (SEQ ID NO: 719), YPAEVVQKNA (SEQ ID NO: 724), or YPAEVVQKPA (SEQ ID NO: 586);

(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, or 9 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).

144. The AAV capsid variant of any one of embodiments 126 or 141-143, wherein [A]-[B]-[C]-[D] is or comprises:

(i) TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNSSLYYPAEVVQKTA (SEQ ID NO: 2066), TNTSATYPAEVVQKTA (SEQ ID NO: 2067), TNSSLSYPAEVVQKTA (SEQ ID NO: 2068), TNSSLSYPAEVVQKTD (SEQ ID NO: 2070), TNSSLSYPAEVVQKNA (SEQ ID NO: 2072), TNSSLSYPAEVVQKPA (SEQ ID NO: 2073), or TNKSATYPAEVVQKTA (SEQ ID NO: 2075);

(ii) (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, 13, 14, or 15 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).

145. The AAV capsid variant of any one of embodiments 126 or 141-144, wherein [A]-[B]-[C]-[D] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

146. The AAV capsid variant of any one of the embodiments 1-40, 59, 60, 67-98, 139, or 140, wherein [N2]-[N3] is present in loop VIII, optionally wherein loop VIII comprises positions 571-592 (e.g., amino acids TNNQSSTTAPATGTYNLQEIVP (SEQ ID NO: 736)) numbered according to SEQ ID NO: 138, or positions 571-599, numbered according to SEQ ID NO: 982.

147. The AAV capsid variant of any one of embodiments 2, 15-40, 59, 60, 68, 79-98, 139, 137, or 146 wherein [N0], [N1], and/or [N4] is present in loop VIII, optionally wherein loop VIII comprises

positions 571-592 (e.g., amino acids TNNQSSTTAPATGTYNLQEIVP (SEQ ID NO: 736)) numbered according to SEQ ID NO: 138, or positions 571-599, numbered according to SEQ ID NO: 982.

148. The AAV capsid variant of any one of embodiments 2, 15-40, 59, 60, 68, 79-98, 139, 140, 146, or 147, wherein [N0]-[N1]-[N2]-[N3]-[N4] is present in loop VIII, optionally wherein loop VIII comprises positions 571-592 (e.g., amino acids TNNQSSTTAPATGTYNLQEIVP (SEQ ID NO: 736)) numbered according to SEQ ID NO: 138, or positions 571-599, numbered according to SEQ ID NO: 982.

149. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-148, wherein [N2] is present immediately subsequent to position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

150. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-149, wherein [N2] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

151. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-150, wherein [N2] is present immediately subsequent to position 576, and wherein [N2] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

152. The AAV capsid variant of any one of 1-40, 59, 60, 67-98, 139, 140, or 146-151, wherein [N2] corresponds to positions 577-581 (e.g., Y577, P578, A579, E580, V581) of SEQ ID NO: 982.

153. The AAV capsid variant of any one of embodiments 1-152, which comprises an amino acid other than T at position 577, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

154. The AAV capsid variant of any one of embodiments 1-153, which comprises a Y at position 577, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

155. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-154, wherein X1 of [N2] is present at position 577 (e.g., T577), and positions X2-X5 of [N2] are present

immediately subsequent to position 577, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

156. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-155, wherein X1 of [N2] corresponds to position 577 (e.g., Y577), X2 of [N2] corresponds to position 578 (e.g., P588), X3 of [N2] corresponds to position 579 (e.g., A579), X4 of [N2] corresponds to position 580 (e.g., E580), and X5 of [N2] corresponds to position 581 (e.g., V581) of SEQ ID NO: 982.

157. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-156, wherein [N2]-[N3] is present immediately subsequent to position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

158. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-157, wherein [N2]-[N3] replaces position 577 relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

159. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-158, wherein [N2]-[N3] is present immediately subsequent to position 576, and wherein [N2]-[N3] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

160. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-159, wherein [N2]-[N3] corresponds to positions 577-584 (e.g., Y577, P578, A579, E580, V581, V582, Q583, K584) of SEQ ID NO: 982.

161. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-160, wherein [N2]-[N3]-[N4] is present immediately subsequent to position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

162. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-161, wherein [N2]-[N3]-[N4] replaces positions 577-579 (e.g., T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

163. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-162, wherein [N2]-[N3]-[N4] is present immediately subsequent to position 576, and wherein [N2]-[N3]-[N4] replaces positions 577-579 (e.g., T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

164. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-163, wherein [N2]-[N3]-[N4] corresponds to positions 577-586 (e.g., Y577, P578, A579, E580, V581, V582, Q583, K584, T585, A586) of SEQ ID NO: 982.

165. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-164, wherein [N1] is present immediately subsequent to position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

166. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-165, wherein [N1] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

167. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-166, wherein [N1] is present immediately subsequent to position 573, and wherein [N1] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

168. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-167, wherein [N1] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

169. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-168, wherein [N1] is present immediately subsequent to position 573, and wherein [N1] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

170. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-169, wherein [N1] corresponds to positions 574-576 (e.g., Q574, S575, and S576) of SEQ ID NO: 982.

171. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-170, wherein [N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

172. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-171, wherein [N1]-[N2]-[N3]-[N4] replaces positions 574-579 (e.g., Q574, S575, S576, T577,

T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

173. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-171, wherein [N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 573, and wherein [N1]-[N2]-[N3]-[N4] replaces positions 574-579 (e.g., Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

174. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-173, wherein [N1]-[N2]-[N3]-[N4] corresponds to positions 574-586 (e.g., Q574, S575, S576, Y577, P578, A579, E580, V581, V582, Q583, K584, T585, A586) of SEQ ID NO: 982.

175. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-174 wherein [N0] is present immediately subsequent to position 570, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

176. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-175, wherein [N0] replaces positions 571-573 (e.g., T571, N572, and N573), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

177. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-176, wherein [N0] is present immediately subsequent to position 570, and wherein [N0] replaces positions 571-573 (e.g., T571, N572, and N573), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

178. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-177 wherein [N0] is present immediately subsequent to position 570, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

179. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-178, wherein [N0] replaces positions 571-573 (e.g., T571, N572, and N573), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

180. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-179, wherein [N0] is present immediately subsequent to position 570, and wherein [N0] replaces

positions 571-573 (e.g., T571, N572, and N573), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

181. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-181, wherein [N0] corresponds to positions 571-573 (e.g., T571, N572, and N573) of SEQ ID NO: 982.

182. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-181, wherein [N0]-[N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 570, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

183. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-182, wherein [N0]-[N1]-[N2]-[N3]-[N4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

184. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-183, wherein [N0]-[N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 570, and wherein [N0]-[N1]-[N2]-[N3]-[N4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

185. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-184, wherein [N0]-[N1]-[N2]-[N3]-[N4] corresponds to positions 571-586 (e.g., T571, N572, N573, Q574, S575, S576, Y577, P578, A579, E580, V581, V582, Q583, K584, T585, A586), of SEQ ID NO: 982.

186. The AAV capsid variant of any one of embodiments 3, 31-40, 68, 94-98, 139, 140, or 146-185, wherein [N4] is present immediately subsequent to position 584, numbered according to SEQ ID NO: 982.

187. The AAV capsid variant of any one of embodiments 3, 31-40, 68, 94-98, 139, 140, or 146-186, wherein [N4] replaces position 578 and 579, numbered according to SEQ ID NO: 138; or positions 585 and 586 numbered according to SEQ ID NO: 982.

188. The AAV capsid variant of any one of embodiments 3, 31-40, 68, 94-98, 139, 140, or 146-187, wherein [N4] is present immediately subsequent to position 584 and replaces positions 585 and 586 numbered according to SEQ ID NO: 982.

189. The AAV capsid variant of any one of embodiments 3, 31-40, 68, 94-98, 139, 140, or 146-188, wherein:

(i) X_A of [N0] is present at position 571, X_B of [N0] is present at position 572, and X_C of [N0] is present at position 573, numbered according to SEQ ID NO: 982;

(ii) X_D of [N1] is present at position 574, X_E of [N1] is present at position 575, and X_F of [N1] is present at position 576, numbered according to SEQ ID NO: 982;

(iii) X1 of [N2] is present at position 577, X2 of [N2] is present at position 578, X3 of [N2] is present at position 579, X4 of [N2] is present at position 580, and X5 of [N2] is present at position 581, numbered according to SEQ ID NO: 982;

(iv) [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982; and/or

(v) X_G of [N4] is present at position 585 and X_H of [N4] is present at position 586, numbered according to SEQ ID NO: 982.

190. The AAV capsid variant of any one of embodiments 3, 31-40, 68, 94-98, 139, 140, or 146-189, wherein:

(i) [N0] is present at positions 571-573, numbered according to SEQ ID NO: 982;

(ii) [N1] is present at positions 574-576, numbered according to SEQ ID NO: 982;

(iii) [N2] is present at positions 577-581, numbered according to SEQ ID NO: 982;

(iv) [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982;

(v) [N4] is present at positions 585-586, numbered according to SEQ ID NO: 982;

(vi) [N2]-[N3] is present at positions 577-584, numbered according to SEQ ID NO: 982; and/or

(vii) [N0]-[N1]-[N2]-[N3]-[N4] is present at positions 571-586, numbered according to SEQ ID NO: 982.

191. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-190, wherein [N3] is present immediately subsequent to [N2].

192. The AAV capsid variant of any one of embodiments 3, 31-40, 68, 94-98, 139, 140, or 146-191, wherein [N4] is present immediately subsequent to [N3].

193. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-192, which comprises from N-terminus to C-terminus [N2]-[N3].

194. The AAV capsid variant of any one of embodiments 1-40, 59, 60, 67-98, 139, 140, or 146-193, which comprises from N-terminus to C-terminus [N1]-[N2]-[N3].

195. The AAV capsid variant of any one of embodiments 3, 18, 19, 28-40, 59, 60, 68, 89-98, 139, 140, or 146-194, which comprises from N-terminus to C-terminus [N0]-[N1]-[N2]-[N3].

196. The AAV capsid variant of any one of embodiments 3, 18-40, 59, 60, 68, 79-98, 139, 140, or 146-195, which comprises from N-terminus to C-terminus [N1]-[N2]-[N3]-[N4].

197. The AAV capsid variant of any one of embodiments 3, 18, 19, 28-40, 59, 60, 68, 89-98, 139, 140, or 146-196, which comprises from N-terminus to C-terminus [N0]-[N1]-[N2]-[N3]-[N4].

198. The AAV capsid variant of any one of embodiments 41-66 or 125-145, wherein [B]-[C] is present in loop VIII, optionally wherein loop VIII comprises positions 571-592 (e.g., amino acids TNNQSSTTAPATGTYNLQEIVP (SEQ ID NO: 736)) numbered according to SEQ ID NO: 138, or positions 571-599, numbered according to SEQ ID NO: 982.

199. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198, wherein [A] and/or [D] is present in loop VIII, optionally wherein loop VIII comprises positions 571-592 (e.g., amino acids TNNQSSTTAPATGTYNLQEIVP (SEQ ID NO: 736)) numbered according to SEQ ID NO: 138, or positions 571-599, numbered according to SEQ ID NO: 982.

200. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, 198, or 199, wherein [A]-[B]-[C]-[D] is present in loop VIII, optionally wherein loop VIII comprises positions 571-592 (e.g., amino acids TNNQSSTTAPATGTYNLQEIVP (SEQ ID NO: 736)) numbered according to SEQ ID NO: 138, or positions 571-599, numbered according to SEQ ID NO: 982.

201. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-200, wherein [B] is present immediately subsequent to position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

202. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-201, wherein [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

203. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-202, wherein [B] is present immediately subsequent to position 573, and wherein [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

204. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-203, wherein [B] is present immediately subsequent to position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

205. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-204, wherein [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

206. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-205, wherein [B] is present immediately subsequent to position 573, and wherein [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

207. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-206, wherein [B] corresponds to positions 574-576 (e.g., Q574, S575, and S576) of SEQ ID NO: 982.

208. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-207, wherein [B]-[C] is present immediately subsequent to position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

209. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-208, wherein [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

210. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-209, wherein [B]-[C] is present immediately subsequent to position 573, and wherein [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

211. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-210, wherein [B]-[C] corresponds to positions 574-584 (e.g., Q574, S575, S576, Y577, P578, A579, E580, V581, V582, Q583, K584) of SEQ ID NO: 982.

212. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-211, wherein [B]-[C]-[D] is present immediately subsequent to position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

213. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-212, wherein [B]-[C]-[D] replaces positions 574-579 (e.g., Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

214. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-213, wherein [B]-[C]-[D] is present immediately subsequent to position 573, and wherein [B]-[C]-[D] replaces positions 574-579 (e.g., Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

215. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-214, wherein [B]-[C]-[D] corresponds to positions 574-586 (e.g., Q574, S575, S576, Y577, P578, A579, E580, V581, V582, Q583, K584, T585, A586) of SEQ ID NO: 982.

216. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-215, wherein [C] is present immediately subsequent to position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

217. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-216, wherein [C] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

218. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-217, wherein [C] is present immediately subsequent to position 576, and wherein [C] replaces 577 (e.g., T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

219. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-218, wherein [C] corresponds to positions 577-584 (e.g., Y577, P578, A579, E580, V581, V582, Q583, K584) of SEQ ID NO: 982.

220. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-219, wherein [A] is present immediately subsequent to position 570, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

221. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-220, wherein [A] replaces positions 571-573 (e.g., T571, N572, and N573), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

222. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-221, wherein [A] is present immediately subsequent to position 570, and wherein [A] replaces positions 571-573 (e.g., T571, N572, and N573), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

223. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-222, wherein [A] is present immediately subsequent to position 570, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

224. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-223, wherein [A] replaces positions 571-573 (e.g., T571, N572, and N573), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

225. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-224, wherein [A] is present immediately subsequent to position 570, and wherein [A] replaces positions 571-573 (e.g., T571, N572, and N573), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

226. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-225, wherein [A] corresponds to positions 571-573 (e.g., T571, N572, and N573) of SEQ ID NO: 982.

227. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-226, wherein [D] is present immediately subsequent to position 584, numbered according to SEQ ID NO: 982.

228. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-227, wherein [D] replaces positions 578 and 579 numbered according to SEQ ID NO: 138; or positions 585 and 586, numbered according to SEQ ID NO: 982.

229. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-228, wherein [D] is present immediately subsequent to position 584 and replaces positions 585 and 586, numbered according to SEQ ID NO: 982.

230. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-229, wherein [A]-[B]-[C]-[D] is present immediately subsequent to position 570, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

231. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-230, wherein [A]-[B]-[C]-[D] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

232. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-231, wherein [A]-[B]-[C]-[D] is present immediately subsequent to position 570, and wherein [A]-[B]-[C]-[D] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

233. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-232, wherein [A]-[B]-[C]-[D] corresponds to positions 571-586 (e.g., T571, N572, N573, Q574, S575, S576, Y577, P578, A579, E580, V581, V582, Q583, K584, T585, A586) of SEQ ID NO: 982.

234. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-233, wherein:

(i) X_A of [A] is present at position 571, X_B of [A] is present at position 572, and X_C of [A] is present at position 573, numbered according to SEQ ID NO: 982;

(ii) X1 of [B] is present at position 574, X2 of [B] is present at position 575, and X3 of [B] is present at position 576, numbered according to SEQ ID NO: 982;

(iii) [C] is present at positions 577-584, numbered according to SEQ ID NO: 982; and/or

(iv) X4 of [D] is present at position 585 and position X5 of [D] is present at position 586, numbered according to SEQ ID NO: 982.

235. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-234, wherein:

(i) [A] is present at positions 571-573, numbered according to SEQ ID NO: 982;

(ii) [B] is present at positions 574-576, numbered according to SEQ ID NO: 982;

(iii) [C] is present at positions 577-584, numbered according to SEQ ID NO: 982;

(iv) [D] is present at positions 585-586, numbered according to SEQ ID NO: 982; and/or

(v) [A]-[B]-[C]-[D] is present at positions 571-586, numbered according to SEQ ID NO: 982.

236. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-235, wherein [C] is present immediately subsequent to [B].

237. The AAV capsid variant of any one of embodiments 42, 61-66, 126, 141-145, or 198-236 wherein [D] is present immediately subsequent to [C].

238. The AAV capsid variant of any one of embodiments 41-66, 125-145, or 198-237, which comprises from N-terminus to C-terminus [B]-[C].

239. The AAV capsid variant of any one of embodiments 42, 52-66, 126, 134-145, or 198-238, which comprises from N-terminus to C-terminus [A]-[B]-[C].

240. The AAV capsid variant of any one of embodiments 42, 61-66, 126, 141-145, or 198-239, which comprises from N-terminus to C-terminus [B]-[C]-[D].

241. The AAV capsid variant of any one of embodiments 42, 61-66, 126, 141-145, or 198-240, which comprises from N-terminus to C-terminus [A]-[B]-[C]-[D].

242. The AAV capsid variant of any one of the embodiments 99-124, wherein [K1]-[K2] is present in loop VIII.

243. The AAV capsid variant of any one of embodiments 100, 92-124, or 242, wherein [K0], [K3], and/or [K4] is present in loop VIII, optionally wherein loop VIII comprises positions 571-592 (e.g., amino acids TNNQSSTTAPATGTYNLQEIVP (SEQ ID NO: 736)) numbered according to SEQ ID NO: 138, or positions 571-599, numbered according to SEQ ID NO: 982.

244. The AAV capsid variant of any one of embodiments 100, 92-124, 242, or 243, wherein [K0]-[K1]-[K2]-[K3]-[K4] is present in loop VIII, optionally wherein loop VIII comprises positions 571-592 (e.g., amino acids TNNQSSTTAPATGTYNLQEIVP (SEQ ID NO: 736)) numbered according to SEQ ID NO: 138, or positions 571-599, numbered according to SEQ ID NO: 982.

245. The AAV capsid variant of any one of embodiments 99-124 or 242-244, wherein [K1] is present immediately subsequent to position 574, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

246. The AAV capsid variant of any one of embodiments 99-124 or 242-245, wherein [K1] replaces positions 575-577 (e.g., S575, S576, and T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

247. The AAV capsid variant of any one of embodiments 99-124 or 242-246, wherein [K1] is present immediately subsequent to position 574, and wherein [K1] replaces positions 575-577 (e.g., S575, S576, and T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

248. The AAV capsid variant of any one of embodiments 100, 116-124, or 242-247, wherein [K1]-[K2]-[K3] is present immediately subsequent to position 574, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

249. The AAV capsid variant of any one of embodiments 100, 116-124, or 242-248, wherein [K1]-[K2]-[K3] replaces positions 575-577 (e.g., S575, S576, and T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

250. The AAV capsid variant of any one of embodiments 100, 116-124, or 242-249, wherein [K1]-[K2]-[K3] is present immediately subsequent to position 574, and wherein [K1]-[K2]-[K3] replaces positions 575-577 (e.g., S575, S576, and T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

251. The AAV capsid variant of any one of embodiments 100, 121-124, or 242-250, wherein [K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 574, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

252. The AAV capsid variant of any one of embodiments 100, 121-124, or 242-251, wherein [K1]-[K2]-[K3]-[K4] replaces positions 575-579 (e.g., S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

253. The AAV capsid variant of any one of embodiments 100, 121-124, or 242-252, wherein [K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 574, and wherein [K1]-[K2]-[K3]-[K4] replaces positions 575-579 (e.g., S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

254. The AAV capsid variant of any one of embodiments 100, 110-124, or 242-253, wherein [K0] is present immediately subsequent to position 570, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

255. The AAV capsid variant of any one of embodiments 100, 110-124, or 242-254, wherein [K0] replaces positions 571 to 574 (e.g., T571, N572, N573, and Q574), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

256. The AAV capsid variant of any one of embodiments 100, 110-124, or 242-255, wherein [K0] is present immediately subsequent to position 570, and wherein [K0] replaces positions 571 to 574 (e.g., T571, N572, N573, and Q574), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

257. The AAV capsid variant of any one of embodiments 100, 121-124, or 242-256, wherein [K0]-[K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 570, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

258. The AAV capsid variant of any one of embodiments 100, 121-124, or 242-257, wherein [K0]-[K1]-[K2]-[K3]-[K4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

259. The AAV capsid variant of any one of embodiments 100, 121-124, or 242-258, wherein [K0]-[K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 570, and wherein [K0]-[K1]-[K2]-[K3]-[K4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

260. The AAV capsid variant of any one of embodiments 100, 116-124, or 242-259, wherein [K3] is present immediately subsequent [K2].

261. The AAV capsid variant of any one of embodiments 100, 121-124, or 242-260, wherein [K4] is present immediately subsequent [K3].

262. The AAV capsid variant of any one of embodiments 100, 121-124, or 242-261, which comprises from N-terminus to C-terminus [K1]-[K2].

263. The AAV capsid variant of any one of embodiments 100, 116-124, or 242-262, which comprises from N-terminus to C-terminus [K1]-[K2]-[K3].

264. The AAV capsid variant of any one of embodiments 100, 116-124, or 242-263, which comprises from N-terminus to C-terminus [K0]-[K1]-[K2]-[K3].

265. The AAV capsid variant of any one of embodiments 100, 121-124, or 242-264, which comprises from N-terminus to C-terminus [K1]-[K2]-[K3]-[K4].

266. The AAV capsid variant of any one of embodiments 100, 121-124, or 242-265, which comprises from N-terminus to C-terminus [K0]-[K1]-[K2]-[K3]-[K4].

267. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising:

(a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20;

(c) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or

(d) an amino sequence comprising 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 amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20.

268. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising:

(a) the amino acid sequence of any of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583-1587, 1590, 1591-1593, 1598-1608, or 1610-1624;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583-1587, 1590, 1591-1593, 1598-1608, or 1610-1624;

(c) an amino acid sequence comprising 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: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583-1587, 1590, 1591-1593, 1598-1608, or 1610-1624; or

(d) an amino sequence comprising 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: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583-1587, 1590, 1591-1593, 1598-1608, or 1610-1624.

269. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising:

(a) the amino acid sequence of any of SEQ ID NOs: 943 or 2064-2080;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 943 or 2064-2080;

(c) an amino acid sequence comprising 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: 943 or 2064-2080; or

(d) an amino sequence comprising 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: 943 or 2064-2080.

270. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising:

(a) the amino acid sequence of any of SEQ ID NOs: 1021, 1024, 1232, 1300, 1327, 1533, 1538, 1585, 1590, or 1591;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 1021, 1024, 1232, 1300, 1327, 1533, 1538, 1585, 1590, or 1591;

(c) an amino acid sequence comprising 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: 1021, 1024, 1232, 1300, 1327, 1533, 1538, 1585, 1590, or 1591; or

(d) an amino sequence comprising 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: 1021, 1024, 1232, 1300, 1327, 1533, 1538, 1585, 1590, or 1591.

271. The AAV capsid variant of any one of embodiments 267-270, which comprises at least 3, 4, 5, 6, or 7 consecutive amino acids from of any one of SEQ ID NOs: 943 or 946-966.

272. The AAV capsid variant of embodiment 267-271, wherein the 3 consecutive amino acids comprise YPA.

273. The AAV capsid variant of embodiment 267-272, wherein the 4 consecutive amino acids comprise YPAE (SEQ ID NO: 21).

274. The AAV capsid variant of embodiment 267-273, wherein the 5 consecutive amino acids comprise YPAEV (SEQ ID NO: 1).

275. The AAV capsid variant of embodiment 267-274, wherein the 6 consecutive amino acids comprise YPAEVV (SEQ ID NO: 725).

276. The AAV capsid variant of embodiment 267-275, wherein the 7 consecutive amino acids comprise YPAEVVQ (SEQ ID NO: 726).

277. The AAV capsid variant of embodiment 267-276, wherein the amino acid sequence comprises YPAEVVQK (SEQ ID NO: 943).

278. The AAV capsid variant of any one of embodiments 267, 268, or 270, wherein:

(i) the 3 consecutive amino acids comprise YTP;

(ii) the 4 consecutive amino acids comprise YTPS (SEQ ID NO: 26);

(iii) the 5 consecutive amino acids comprise YTPSL (SEQ ID NO: 7);

(iv) the 6 consecutive amino acids comprise YTPSLV (SEQ ID NO: 727);

(v) the 7 consecutive amino acids comprise YTPSLVQ (SEQ ID NO: 728); and/or

(vi) the amino acid sequence comprises YTPSLVQK (SEQ ID NO: 952).

279. The AAV capsid variant of any one of embodiments 267, 268, or 270, wherein:

(i) the 3 consecutive amino acids comprise YPP;

(ii) the 4 consecutive amino acids comprise YPPS (SEQ ID NO: 22);

(iii) the 5 consecutive amino acids comprise YPPSL (SEQ ID NO: 2);

(iv) the 6 consecutive amino acids comprise YPPSLV (SEQ ID NO: 729);

(v) the 7 consecutive amino acids comprise YPPSLVQ (SEQ ID NO: 732); and/or

(vi) the amino acid sequence comprises YPPSLVQK (SEQ ID NO: 946).

280. The AAV capsid variant of any one of embodiments 267, 268, or 270, wherein:

(i) the 3 consecutive amino acids comprise YPP;

(ii) the 4 consecutive amino acids comprise YPPS (SEQ ID NO: 22);

(iii) the 5 consecutive amino acids comprise YPPSL (SEQ ID NO: 2);

(iv) the 6 consecutive amino acids comprise YPPSLE (SEQ ID NO: 733);

(v) the 7 consecutive amino acids comprise YPPSLEQ (SEQ ID NO: 734); and/or

(vi) the amino acid sequence comprises YPPSLEQK (SEQ ID NO: 953).

281. The AAV capsid variant of any one of embodiments 267, 268, or 270, wherein:

(i) the 3 consecutive amino acids comprise YPP;

(ii) the 4 consecutive amino acids comprise YPPS (SEQ ID NO: 22);

(iii) the 5 consecutive amino acids comprise YPPSL (SEQ ID NO: 2);

(iv) the 6 consecutive amino acids comprise YPPSLV (SEQ ID NO: 729);

(v) the 7 consecutive amino acids comprise YPPSLVK (SEQ ID NO: 735); and/or

(vi) the amino acid sequence comprises YPPSLVKK (SEQ ID NO: 954).

282. The AAV capsid variant of any one of embodiments 267-277, which comprises an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

283. The AAV capsid variant of any one of embodiments 267, 268, 270, or 278-281, which comprises an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any of SEQ ID NOs: 1021, 1024, 1232, 1300, 1327, 1533, 1538, 1585, 1590, or 1591.

284. The AAV capsid variant of any one of embodiments 267, 268, 270, or 378-281, which comprises an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any of SEQ ID NOs: 946, 952, 953, or 954.

285. The AAV capsid variant of any one of embodiments 267-277 or 282, which comprises 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 the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

286. The AAV capsid variant of any one of embodiments 267, 268, 270, 278-281, or 283, which comprises 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 the amino acid sequence of any one of SEQ ID NOs: 1021, 1024, 1232, 1300, 1327, 1533, 1538, 1585, 1590, or 1591.

287. The AAV capsid variant of any one of embodiments 267, 268, 270, 278-281, or 284, which comprises 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 the amino acid sequence of any one of SEQ ID NOs: 946, 952, 953, or 954.

288. The AAV capsid variant of any one of embodiments 267-277, 282, or 285, which comprises an amino acid sequence encoded by:

(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 sequence of SEQ ID NO: 944; 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: 944.

289. The AAV capsid variant of any one of embodiments 267-277, 282, 285, or 289, wherein the nucleotide sequence encoding the amino acid sequence 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 sequence of SEQ ID NO: 944; 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: 944.

290. The AAV capsid variant of embodiment 267, which comprises:

(a) the amino acid sequence of any of SEQ ID NOs: 2024-2063;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 2024-2063;

(c) an amino acid sequence comprising 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: 2024-2063; or

(d) an amino sequence comprising 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: 2024-2063.

291. The AAV capsid variant of embodiment 267 or 290, wherein the different amino acids of the amino acid sequence comprising 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: 2024-2063, are present at one or more of the following positions:

(i) position 1, wherein the different amino acid is T or L;

(ii) position 2, wherein the different amino acid is N, L, K, A, T, or P;

(iii) position 3, wherein the different amino acid is N, K, L, A, Y, or S;

(iv) position 4, wherein the different amino acid is Q, L, T, S, F, Y, K, or A;

(v) position 5, wherein the different amino acid is S, H, A, M, Q, T, V, or F;

(vi) position 6, wherein the different amino acid is S, P, V, A, Q, L, T, N, or M;

(vii) position 7, wherein the different amino acid is Y, H, S, V, A, L, or T;

(viii) position 8, wherein the different amino acid is D, P, A, Q, F, L, S, H, or M;

(ix) position 9, wherein the different amino acid is F, A, L, D, or Q;

(x) position 10, wherein the different amino acid is T, E, I, or S;

(xi) position 11, wherein the different amino acid is V, A, N, or S;

(xii) position 12, wherein the different amino acid is V, L, or P;

(xiii) position 13, wherein the different amino acid is Q, E, or P;

(xiv) position 14, wherein the different amino acid is K, N, S, or L;

(xv) position 15, wherein the different amino acid is T, V, M, or L; and/or

(xvi) position 16, wherein the different amino acid is A, G, or R.

292. The AAV capsid variant of embodiment 267, which comprises:

(a) the amino acid sequence of any one of SEQ ID NOs: 1632-2023;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 1632-2023;

(c) an amino acid sequence comprising 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: 1632-2023; or

(d) an amino sequence comprising 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: 1632-2023.

293. The AAV capsid variant of embodiment 268 or 292, wherein the different amino acids of the amino acid sequence comprising 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: 1632-2023, are present at one or more of the following positions:

(i) position 1, wherein the different amino acid is T, G, N, S, E, L, Y, V, or I;

(ii) position 2, wherein the different amino acid is D, N, K, E, V, G, R, L, H, F, P, T, A, S, I, or Y;

(iii) position 3, wherein the different amino acid is Y, N, K, T, W, Q, M, V, C, A, L, F, H, G, R, S, or P;

(iv) position 4, wherein the different amino acid is H, Q, P, E, R, K, A, S, V, L, T, D, I, G, M, or N;

(v) position 5, wherein the different amino acid is R, S, K, N, H, G, W, A, P, V, Q, Y, L, or F;

(vi) position 6, wherein the different amino acid is G, S, F, R, W, H, I, C, M, A, Y, K, N, Q, V, P, E, D, T, or L;

(vii) position 7, wherein the different amino acid is D, Y, S, I, H, F, P, K, R, G, L, Q, A, M, T, N, V, W, C, or E;

(viii) position 8, wherein the different amino acid is P, L, Q, T, W, V, G, K, I, Y, N, H, R, D, S, M, A, F, or E;

(ix) position 9, wherein the different amino acid is A, R, T, Q, S, M, L, E, K, V, G, D, N, H, F, P, or I;

(x) position 10, wherein the different amino acid is K, E, Q, H, V, G, R, S, P, I, N, M, A, L, D, or T;

(xi) position 11, wherein the different amino acid is V, A, E, N, R, L, M, T, Q, S, K, C, G, D, Y, P, H, F, or I;

(xii) position 12, wherein the different amino acid is V, P, L, S, T, N, A, G, K, R, I, H, E, Q, or M;

(xiii) position 13, wherein the different amino acid is Q, K, N, A, H, R, T, V, E, I, P, G, S, or L;

(xiv) position 14, wherein the different amino acid is K, E, I, Y, Q, R, G, D, L, N, or S;

(xv) position 15, wherein the different amino acid is S, T, N, Q, I, P, E, G, K, M, or H; and/or

(xvi) position 16, wherein the different amino acid is A, D, L, Y, Q, or T.

294. The AAV capsid variant of any one of embodiments 267-293, wherein the amino acid sequence is present in loop VIII.

295. The AAV capsid variant of any one of embodiments 267-294, wherein the amino acid sequence is present immediately subsequent to position 570, 571, 572, 573, 574, 575, or 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

296. The AAV capsid variant of any one of embodiments 267-295, wherein the amino acid sequence replaces one, two, three, four, five or all of positions 571, 572, 573, 574, 575, and/or 576 (e.g., positions T571, N572, N573, Q574, S575, S576, T577, T578, and/or A579), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

297. The AAV capsid variant of any one of embodiments 267-296, wherein the amino acid sequence is present immediately subsequent to position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

298. The AAV capsid variant of any one of embodiments 267-297, which comprises an amino acid residue other than T at position 577, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

299. The AAV capsid variant of any one of embodiments 267-298, which comprises the amino acid Y at position 577, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

300. The AAV capsid variant of any one of embodiments 267-299, which comprises the substitution T577Y, numbered according to SEQ ID NO: 138.

301. The AAV capsid variant of embodiment 298 or 299, wherein the amino acid sequence is or comprises YPAEVVQK (SEQ ID NO: 943), and starts at position 577, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138 or 982.

302. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-301, which comprises an amino acid other than T at position 577, and further comprises the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577, numbered relative to SEQ ID NO: 138.

303. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-302, which comprises the amino acid Y at position 577, and further comprises the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577, numbered relative to SEQ ID NO: 138.

304. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-303, which comprises the amino acid Y at position 577, and further comprises the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577 (e.g., at positions 578-584), numbered relative to SEQ ID NO: 982.

305. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-304, which comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943), wherein the amino acid sequence replaces position 577 (e.g., T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

306. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-305, which comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943), wherein the amino acid sequence:

(i) is present immediately subsequent to position 576; and

(ii) replaces position 577 (e.g., T577),

wherein in (i) and (ii) are numbered relative to SEQ ID NO: 138.

307. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-306, which further comprises an amino acid other than T at position 571 (e.g., I), numbered relative to SEQ ID NO: 138 or SEQ ID NO: 982.

308. The AAV capsid variant of any one of embodiments 267-277, 272, 275, 278, 289, or 294-307, which further comprises I at position 571, numbered relative to SEQ ID NO: 138 or SEQ ID NO: 982.

309. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-308, which further comprises one, two, or all of an amino acid other than Q at position 574 (e.g., A or T), S at position 575 (e.g., G), and/or S (e.g., A, L, K, or R) at position 576, numbered relative to SEQ ID NO: 138 or SEQ ID NO: 982.

310. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-309, which further comprises:

(i) A at position 574, G at position 575, and A at position 576, numbered relative to SEQ ID NO: 138 or 982; or

(ii) T at position 574 and L at position 576, numbered relative to SEQ ID NO: 138 or 982.

311. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-309, which further comprises:

(i) K at position 576, numbered relative to SEQ ID NO: 138 or 982; or

(ii) R at position 576, numbered relative to SEQ ID NO: 138 or 982.

312. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-310, which comprises:

(i) A at position 574, G at position 575, A at position 576, and Y at position 577, numbered relative to SEQ ID NO: 138 or 982; and

(ii) the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577, numbered according to SEQ ID NO: 138 or 982.

313. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, or 294-310, which comprises:

(i) T at position 574, L at position 576, and Y at position 577, numbered relative to SEQ ID NO: 138 or 982; and

(ii) the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577, numbered according to SEQ ID NO: 138 or 982.

314. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, 294-309, or 311, which comprises:

(i) K at position 576 and Y at position 577, numbered relative to SEQ ID NO: 138 or 982; and

(ii) the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577, numbered according to SEQ ID NO: 138 or 982.

315. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, 295-309, or 311, which comprises:

(i) R at position 576 and Y at position 577, numbered relative to SEQ ID NO: 138 or 982; and

(ii) the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577, numbered according to SEQ ID NO: 138 or 982.

316. The AAV capsid variant of any one of embodiments 267-277, 282, 285, 288, 289, 294-308, which comprises:

(i) I at position 571 and Y at position 577, numbered relative to SEQ ID NO: 138 or 982; and

(ii) the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577, numbered according to SEQ ID NO: 138 or 982.

317. The AAV capsid variant of any one of embodiments 267, 268, 270, 278, 283, 284, 286, or 287, which comprises Y at position 577 and the amino acid sequence TPSLVQK (SEQ ID NO: 53), which is present immediately subsequent to position 577, all numbered according to SEQ ID NO: 138 or 982.

318. The AAV capsid variant of any one of embodiments 267, 268, 270, 279, 283, 284, 286, or 287, which comprises Y at position 577 and the amino acid sequence PPSLVQK (SEQ ID NO: 47), which is present immediately subsequent to position 577, all numbered according to SEQ ID NO: 138 or 982.

319. The AAV capsid variant of any one of embodiments 267, 268, 270, 280, 283, 284, 286, or 287, which comprises Y at position 577 and the amino acid sequence PPSLEQK (SEQ ID NO: 54), which is present immediately subsequent to position 577, all numbered according to SEQ ID NO: 138 or 982.

320. The AAV capsid variant of any one of embodiments 267, 268, 270, 281, 283, 284, 286, or 287, which comprises Y at position 577 and the amino acid sequence PPSLVKK (SEQ ID NO: 55), which is present immediately subsequent to position 577, all numbered according to SEQ ID NO: 138 or 982.

321. The AAV capsid variant, of any one of the preceding embodiments, which further comprises a modification, e.g., an insertion, substitution (e.g., conservative substitution), and/or deletion, in loop I, II, IV, and/or VI.

322. 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), 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: 138.

323. 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.

324. 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 about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

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

326. 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 about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

327. 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 about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

328. 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.

329. The AAV capsid variant, of any one of embodiments 1-98, 125-242, 267-277, 282, 285, 288, 289, 294-306, or 321-328, which comprises the amino acid sequence corresponding to positions 137-731, e.g., a VP2, of SEQ ID NO: 982, or a sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

330. The AAV capsid variant, of any one of embodiments 1-98, 125-242, 267-277, 282, 285, 288, 289, 294-306, or 321-329, which comprises the amino acid sequence corresponding to positions 193-731, e.g., a VP3, of SEQ ID NO: 982, or a sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

331. The AAV capsid variant, of any one of embodiments 1-321, which comprises the amino acid sequence corresponding to positions 137-724, e.g., a VP2, of SEQ ID NO: 138, or a sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

332. The AAV capsid variant, of any one of embodiments 1-321, which comprises the amino acid sequence corresponding to positions 193-724, e.g., a VP3, of SEQ ID NO: 138, or a sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

333. The AAV capsid variant, of any one of embodiments 267-277, 282, 285, 288, 289, 294-306, or 321-328, comprising an amino acid sequence comprising at least 3, 4, 5, or 6 consecutive amino acids from the amino acid sequence of YPAEVVQK (SEQ ID NO: 943), wherein:

(i) the 3 consecutive amino acids comprise YPA;

(ii) the 4 consecutive amino acids comprise YPAE (SEQ ID NO: 21);

(iii) the 5 consecutive amino acids comprise YPAEV (SEQ ID NO: 1);

(iv) the 6 consecutive amino acids comprise YPAEVV (SEQ ID NO: 725);

(v) the 7 consecutive amino acids comprise YPAEVVQ (SEQ ID NO: 726);

wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 739, or an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 739.

334. The AAV capsid variant, of any one of embodiments 267-277, 282, 285, 288, 289, 294-306, 321-328, or 333, comprising an amino acid sequence comprising at least 3, 4, 5, or 6 consecutive amino acids from the amino acid sequence of YPAEVVQK (SEQ ID NO: 943), wherein:

(i) the 3 consecutive amino acids comprise YPA;

(ii) the 4 consecutive amino acids comprise YPAE (SEQ ID NO: 21);

(iii) the 5 consecutive amino acids comprise YPAEV (SEQ ID NO: 1);

(iv) the 6 consecutive amino acids comprise YPAEVV (SEQ ID NO: 725);

(v) the 7 consecutive amino acids comprise YPAEVVQ (SEQ ID NO: 726);

wherein the AAV capsid variant comprises: (a) a VP1 protein comprising the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 982; (b) a VP2 protein comprising the amino acid sequence of positions 137-724 of SEQ ID NO: 138 or positions 137-731 of SEQ ID NO: 982; (c) a VP3 protein comprising the amino acid sequence of positions 193-724 of SEQ ID NO: 138 or

positions 193-731 of SEQ ID NO: 982; or (d) an amino acid sequence with at least 90% (e.g., at least about 95, 96, 97, 98, or 99%) sequence identity to any of the amino acid sequences in (a)-(c).

335. The AAV capsid variant, of any one of embodiments 267-277, 282, 285, 288, 289, 294-306, 321-328, or 334, comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943), wherein the AAV capsid variant comprises:

(a) a VP1 protein comprising the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 982;

(b) a VP2 protein comprising the amino acid sequence of positions 137-724 of SEQ ID NO: 138 or positions 137-731 of SEQ ID NO: 982;

(c) a VP3 protein comprising the amino acid sequence of positions 193-724 of SEQ ID NO: 138 or positions 193-731 of SEQ ID NO: 982; or

(d) an amino acid sequence with at least 90% (e.g., at least about 95, 96, 97, 98, or 99%) sequence identity to any of the amino acid sequences in (a)-(c).

336. The AAV capsid variant, of any one of embodiments 267-277, 282, 285, 288, 289, 294-306, 321-328, or 333-335, comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943), wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 739; or an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 739.

337. The AAV capsid variant, of any one of embodiments 267-277, 282, 285, 288, 289, 294-306, 321-328, or 333-336, comprising one or two, but no more than three substitutions relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943), wherein the AAV capsid variant comprises an amino acid sequence at least 90% (e.g., at least about 95, 96, 97, 98, or 99%) identical to the amino acid sequence of SEQ ID NO: 982.

338. The AAV capsid variant, of any one of embodiments 1-98, 125-241, 267-277, 282, 285, 288, 289, 294-306, 321-328, or 333-337, which comprises the amino acid sequence of SEQ ID NO: 982, or an amino acid sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

339. The AAV capsid variant, of any one of embodiments 1-98, 125-241, 267-277, 282, 285, 288, 289, 294-306, 321-328, or 333-338, which comprises the amino acid sequence of SEQ ID NO: 982 or an amino acid sequence with at least 95% identity thereto.

340. The AAV capsid variant, of any one of embodiments 1-98, 125-241, 267-277, 282, 285, 288, 289, 294-306, 321-328, or 333-339, which comprises the amino acid sequence of SEQ ID NO: 982 or an amino acid sequence with at least 98% identity thereto.

341. The AAV capsid variant of any one of embodiments 1-98, 125-241, 267-277, 282, 285, 288, 289, 294-306, 321-328, or 333-340, which 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: 982.

342. The AAV capsid variant, of any one of embodiments 1-98, 125-241, 267-277, 282, 285, 288, 289, 294-306, 321-328, or 333-341, 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: 982.

343. The AAV capsid variant, of any one of the preceding embodiments 1-98, 125-241, 267-277, 282, 285, 288, 289, 294-306, 321-328, or 333-342, wherein the nucleotide sequence encoding the capsid variant comprises the nucleotide sequence of SEQ ID NO: 984, or a nucleotide sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

344. The AAV capsid variant, of any one of the preceding embodiments, wherein the nucleotide sequence encoding the capsid variant is codon optimized.

345. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising the amino acid sequence of any one of embodiments 267-277, 282, 285, 288, 289, 294-306, 321-328, or 333-342, and further comprising an amino acid sequence at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) identical to SEQ ID NO: 982.

346. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 739, wherein the AAV capsid variant comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

347. The AAV capsid variant of embodiment 346, wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 739.

348. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising the amino acid sequence of SEQ ID NO: 982.

349. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 984, or a nucleotide sequence at least 95% identical thereto.

350. The AAV capsid variant of any one embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-349, which 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.

351. The AAV capsid variant of any one embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-350, which 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: 982.

352. The AAV capsid variant of any one embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-351, which 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: 139.

353. The AAV capsid variant of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-352, which transduces a brain region, e.g., a temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, and/or cerebellum, optionally wherein the level of transduction is at least 1.5, 2.2, 2.4, 2.5, 2.6, 2.7, 3.0, 3.2, 3.5, 3.7, 4.0, 4.2, 4.5, 4.7, 4.9, 5, 10, 15, 20, 25, 30, 35-fold greater as compared to a reference sequence of SEQ ID NO: 139, e.g., when measured by an assay, e.g., an immunohistochemistry assay or a qPCR assay, e.g., as described in Example 2.

354. The AAV capsid variant of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-353, which is enriched at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 25, 30, 35, 40, 45, 50, 55, 60, or 65-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.

355. The AAV capsid variant of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-354, which is enriched at least about 10, 12, 15, 17, 20, 25, 30, 35, 40, 45, 50, 55, 60, 61, 62,

63, 64, or 65-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.

356. The AAV capsid variant of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-355, which is enriched in the brain of at least two to three species, e.g., a non-human primate and rodent (e.g., rat and/or mouse), e.g., as compared to a reference sequence of SEQ ID NO: 138.

357. The AAV capsid variant, of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-356, which is enriched at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 25, 30, 35, 40, 45-fold, in the brain of at least two to three species, e.g., a non-human primate and rodent (e.g., rat and/or mouse), compared to a reference sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Examples 1, 2, 4, and 5.

358. The AAV capsid variant of embodiment 356 or 357, wherein the at least two to three species are *Macaca fascicularis*, *Chlorocebus sabaeus*, *Callithrix jacchus*, rat, and/or mouse (e.g., BALB/c mice).

359. The AAV capsid variant, of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-358, which is enriched at least about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 125, 150, 175, 200, or 225-fold, in the brain compared to a reference sequence of SEQ ID NO: 982, e.g., when measured by an assay as described in Example 4.

360. The AAV capsid variant of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-359, which delivers an increased level of a payload to a brain region, optionally wherein the level of the payload is increased by at least 20, 25, 30, 35-fold, as compared to a reference sequence of SEQ ID NO: 139, e.g., when measured by an assay, e.g., a qRT-PCR or a qPCR assay (e.g., as described in Example 2).

361. The AAV capsid variant of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-360, which delivers an increased level of viral genomes to a brain region, optionally wherein the level of viral genomes is increased by at least 1.5, 2.2, 2.4, 2.5, 2.6, 2.7, 3.0, 3.2, 3.5, 3.7, 4.0, 4.2, 4.5, 4.7, 4.9, or 5-fold, as compared to a reference sequence of SEQ ID NO: 139, e.g., when measured by an assay, e.g., a qRT-PCR or a qPCR assay (e.g., as described in Example 2).

362. The AAV capsid variant of any one of embodiments 350-361, wherein the brain region is a temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, cerebellum, or a combination thereof.

363. The AAV capsid variant of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-362, which is enriched at least about 3, 3.5, 4.0, 4.5, 5, 5.0, 6.0, or 6.5-fold, in a spinal cord region compared to a reference sequence of SEQ ID NO: 139, e.g., when measured by an assay as described in Example 2.

364. The AAV capsid variant of any one of embodiments 363, wherein the spinal cord region is a cervical region, a lumbar region, a thoracic region, or a combination thereof.

365. The AAV capsid variant of any one of the preceding embodiments, which shows preferential transduction in a brain region relative to the transduction in the dorsal root ganglia (DRG).

366. The AAV capsid variant of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-365, which is capable of transducing neuronal cells.

367. The AAV capsid variant of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-366, which is capable of transducing non-neuronal cells, e.g., glial cells (e.g., oligodendrocytes).

368. The AAV capsid variant of any one of embodiments 1-66, 139, 140, 146-241, 267, 268, 270-289, or 294-367, which shows preferential transduction in a brain region relative to the transduction in the liver.

369. The AAV capsid variant of any one embodiments 67-267, 269, 271-277, 282, 285, 288, 289, 294-306, or 321-349, which has an increased tropism for a heart cell or heart tissue, e.g., a heart ventricle or heart atrium, relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 138.

370. The AAV capsid variant of any one embodiments 67-267, 269, 271-277, 282, 285, 288, 289, 294-306, 321-349, or 369, which has an increased tropism for a heart cell or tissue, e.g., a heart ventricle or heart atrium, relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 139.

371. The AAV capsid variant of any one of embodiments 67-267, 269, 271-277, 282, 285, 288, 289, 294-306, 321-349, 369, or 370, which delivers an increased level of a payload to a heart region, optionally wherein the level of the payload is increased by at least 1.5, 2, or 2.5-fold, as compared to a reference sequence of SEQ ID NO: 139, e.g., when measured by an assay, e.g., an IHC assay or a RT-ddPCR assay (e.g., as described in Example 2).

372. The AAV capsid variant of any one embodiments 67-267, 269, 271-277, 282, 285, 288, 289, 294-306, 321-349, or 369-371, which has an increased tropism for a heart cell or tissue, e.g., a heart ventricle or heart atrium, relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 982.

373. The AAV capsid variant, of any one of embodiments 67-267, 269, 271-277, 282, 285, 288, 289, 294-306, 321-349, or 369-372, which is enriched at least about 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50-fold, in the heart compared to a reference sequence of SEQ ID NO: 982, e.g., when measured by an assay as described in Example 4.

374. The AAV capsid variant of any one embodiments 267, 290, 291, 321-328, 331, 332, or 344, which has an increased tropism for a muscle cell or tissue, relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 982.

375. The AAV capsid variant, of any one of embodiments 267, 290, 291, 321-328, 331, 332, 344, or 374, which is enriched at least about 2, 3, 4, 5, 10, 15, 20, 25, 30, or 35-fold, in the muscle compared to a reference sequence of SEQ ID NO: 982, e.g., when measured by an assay as described in Example 4.

376. The AAV capsid variant embodiment 374 or 375, wherein the muscle cell or tissue is a heart muscle (e.g., a heart ventricle or a heart atrium, or both), a quadriceps muscle, or both.

377. The AAV capsid variant of any one embodiments 267, 292, 293, 321-328, 331, 332, or 344, which has an increased tropism for a liver cell or tissue, relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 982.

378. The AAV capsid variant, of any one of embodiments 267, 292, 293, 321-328, 331, 332, 344, or 377, which is enriched at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 115, 120, 130, 140, 150, 160, 170, 180, 185, or 190-fold, in the liver compared to a reference sequence of SEQ ID NO: 982, e.g., when measured by an assay as described in Example 4.

379. A polynucleotide encoding the AAV capsid variant of any one of embodiments 1-378.

380. The polynucleotide of embodiment 379, 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: 944;

(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: 944; or

(iii) the nucleotide sequence of SEQ ID NO: 944, or nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto.

381. The polynucleotide of embodiment 379 or 380, which comprises the nucleotide sequence of SEQ ID NO: 984, or a nucleotide sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

382. A polynucleotide encoding an AAV capsid variant (e.g., an AAV5 capsid variant), wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 984.

383. A polynucleotide encoding an AAV capsid variant (e.g., an AAV5 capsid variant), wherein the encoded AAV capsid variant comprise the amino acid sequence of SEQ ID NO: 982.

384. A polynucleotide encoding an AAV capsid variant (e.g., an AAV5 capsid variant), wherein the encoded AAV capsid variant comprises:

(a) the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or

(c) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or

(d) an amino sequence comprising 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 amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20.

385. A polynucleotide encoding an AAV capsid variant (e.g., an AAV5 capsid variant), wherein the encoded AAV capsid variant comprises:

(i) the amino acid sequence of YPAEVVQK (SEQ ID NO: 943);

(ii) an amino acid sequence comprising one, two, or three, but no more than four different amino acids relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943);

(iii) an amino acid sequence comprising 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 YPAEVVQK (SEQ ID NO: 943); or

(iv) at least 3, 4, 5, 6, or 7 consecutive amino acids from the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

386. The polynucleotide of any one of embodiments 384 or 385, which comprises:

(i) the nucleotide sequence of SEQ ID NO: 944, 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: 944; or

(iii) 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: 944.

387. The polynucleotide of any one of embodiments 384-386, wherein the AAV capsid variant comprises:

(i) the amino acid sequence of SEQ ID NO: 982, or an amino acid sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto;

(ii) an amino acid sequence comprising one, two or three, but no more than four different amino acids, relative to the amino acid sequence of SEQ ID NO: 982; or

(iii) an amino acid sequence comprising 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: 982.

388. The polynucleotide of any one of embodiments 384-387, comprising the nucleotide sequence of SEQ ID NO: 984, or a nucleotide sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

389. The polynucleotide of any one of embodiments 384-388, which comprises a nucleotide sequence that is codon optimized.

390. A peptide comprising:

(a) the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or

(c) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or

(d) an amino sequence comprising 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 amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20.

391. A peptide comprising:

(i) the amino acid sequence of YPAEVVQK (SEQ ID NO: 943);

(ii) an amino acid sequence comprising one, two, or three, but no more than four different amino acids relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943);

(iii) an amino acid sequence comprising 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 YPAEVVQK (SEQ ID NO: 943); or

(iv) at least 3, 4, 5, 6, or 7 consecutive amino acids from the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

392. A peptide comprising the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

393. A peptide encoded by:

(i) the nucleotide sequence of SEQ ID NO: 944, 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: 944;

(iii) 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: 944.

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

(i) the nucleotide sequence of SEQ ID NO: 944, 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: 944; or

(iii) 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: 944.

395. An AAV capsid variant (e.g., an AAV5 capsid variant) comprising the peptide of any one of embodiments 390-394.

396. An AAV capsid variant (e.g., an AAV5 capsid variant) encoded by the polynucleotide of any one of embodiments 382-389.

397. An AAV particle comprising the AAV capsid variant of any one of embodiments 1-378, 395, or 396.

398. The AAV particle of embodiment 399, which comprises a nucleotide sequence encoding a payload.

399. The AAV particle of embodiment 398, 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.

400. The AAV particle of embodiment 399, 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.

401. The AAV particle of embodiment 399 or 400, 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.

402. The AAV particle of embodiment 399, 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).

403. The AAV particle of embodiment 399, wherein the enzyme comprises a meganuclease, a zinc finger nuclease, a TALEN, a recombinase, integrase, a base editor, a Cas9, or a fragment thereof.

404. The AAV particle of embodiment 399, wherein the component of a gene editing system comprises one or more components of a CRISPR-Cas system.

405. The AAV particle of embodiment 404, 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.

406. The AAV particle of embodiment 399, 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.

407. The AAV particle of embodiment 406, 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.

408. The AAV particle of any one of embodiments 397-407, which comprises a viral genome comprising a promoter operably linked to the nucleic acid sequence encoding the payload.

409. The AAV particle of embodiment 408, wherein the promoter is chosen from human elongation factor 1α-subunit (EF1α), 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 neurofilament heavy chain (NFH), β-globin minigene nβ2, 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., αMHC, cTnT, and CMV-MLC2k), a liver promoter (e.g., hAAT, TBG), a skeletal muscle promoter (e.g., desmin, MCK, C512) or a functional fragment, e.g., a truncation, or a functional variant thereof.

410. The AAV particle of embodiment 408 or 409, wherein the promoter is an EF-1a promoter variant, e.g., a truncated EF-1a promoter.

411. The AAV particle of any one of embodiments 408-410, wherein the promoter comprises the nucleotide sequence of any one of SEQ ID NOs: 2100, 2101, 2103, 2104, 2108, 2109, 2111-2120, or any one of the nucleotide sequences provided in Table 8, a nucleotide sequence comprising at least one, two, three, four, five, six, or seven but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NOs: 2100, 2101, 2103, 2104, 2108, 2109, 2111-2120, or any one of the nucleotide sequences 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: 2100, 2101, 2103, 2104, 2108, 2109, 2111-2120, or any one of the nucleotide sequences provided in Table 8.

412. The AAV particle of any one of embodiments 408-411 wherein the viral genome further comprises a polyA signal sequence.

413. The AAV particle of any one of embodiments 408-412, wherein the viral genome further comprises an inverted terminal repeat (ITR) sequence.

414. The AAV particle of any one of embodiments 408-413, wherein the viral genome comprises an ITR sequence positioned 5' relative to the nucleic acid sequence encoding the payload.

415. The AAV particle of any one of embodiments 408-414, wherein the viral genome comprises an ITR sequence positioned 3' relative to the nucleic acid sequence encoding the payload.

416. The AAV particle of any one of embodiments 408-415, wherein the viral genome comprises an ITR sequence positioned 5' relative to the payload and an ITR sequence positioned 3' relative to the nucleic acid sequence encoding the payload.

417. The AAV particle of any one of embodiments 408-416, wherein the viral genome further comprises an enhancer, a Kozak sequence, an intron region, and/or an exon region.

418. The AAV particle of any one of embodiments 408-417, 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 payload encoded by the viral genome in a cell or tissue where the corresponding miRNA is expressed.

419. The AAV particle of embodiment 418, 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.

420. The AAV particle of embodiment 418 or 419, 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.

421. The AAV particle of any one of embodiments 408-420, 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.

422. The AAV particle of any one of embodiments 408-421, 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.

423. The AAV particle of embodiment 422, wherein the 3 copies of the encoded miR binding sites are continuous (e.g., not separated by a spacer).

424. The AAV particle of embodiment 422, wherein the 3 copies of the encoded miR binding sites are separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence comprising 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 GATAGTTA.

425. The AAV particle of any one of embodiments 408-424, 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.

426. The AAV particle of embodiment 425, wherein the 4 copies of the encoded miR binding sites are continuous (e.g., not separated by a spacer).

427. The AAV particle of embodiment 425, wherein the 4 copies of the encoded miR binding sites are separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence comprising at 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 GATAGTTA.

428. The AAV particle of any one of embodiments 408-427, wherein the encoded miR binding site comprises a miR122 binding site, a miR183 binding site, a miR-1 binding site, a miR-142-3p, 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 miR-1 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 miR-142-3p 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.

429. The AAV particle of any one of embodiments 408-428, wherein the viral genome comprises an encoded miR122 binding site.

430. The AAV particle of any one of embodiments 408-429, 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 comprising 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 GATAGTTA.

431. The AAV particle of embodiment 429 or 430, 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 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.

432. The AAV particle of any one of embodiments 408-431, 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 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) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence comprising 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; 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 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; 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 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) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence comprising 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;

(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 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;

(iv) a second spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence comprising 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; 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 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.

433. The AAV particle of any one of embodiments 408-432, wherein the viral genome comprises an encoded miR183 binding site.

434. The AAV particle of any one of embodiments 408- 433, 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 comprising 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 GATAGTTA.

435. The AAV particle of embodiment 433 or 434, 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 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.

436. The AAV particle of any one of embodiments 408-435, 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 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;

(ii) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence comprising 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 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 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; 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 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;

(ii) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence comprising 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 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 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;

(iv) a second spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence comprising 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 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 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.

437. The AAV particle of any one of embodiments 408-436, wherein the viral genome comprises an encoded miR122 binding site and a miR-1 binding site.

438. The AAV particle of any one of embodiments 408-437, wherein the viral genome is single stranded or self-complementary.

439. The AAV particle of any one of embodiments 408-438, 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).

440. The AAV particle of any one of embodiments 397-439, 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).

441. The AAV particle of embodiment 439 or 440, wherein the Rep78 protein, the Rep68 protein, the Rep52 protein, and/or the Rep40 protein are encoded by at least one Rep gene.

442. The AAV particle of any one of embodiments 408-441, wherein the viral genome further comprises a nucleotide sequence encoding the AAV capsid variant of any one of embodiments 1-364, 381, or 382.

443. The AAV particle of any one of embodiments 397-442, wherein the AAV particle further comprises a nucleotide sequence encoding the AAV capsid variant of any one of embodiments 1-364, 381, or 382.

444. The AAV capsid variant, polynucleotide, peptide, or AAV particle of any one of the preceding embodiments, which is isolated, e.g., recombinant.

445. A vector comprising a polynucleotide encoding the AAV capsid variant of any one of embodiments 1-378, 395, 396, or 444, the polynucleotide of any one of embodiments 379-389 or 444, or a polynucleotide encoding the peptide of any one of embodiments 390-394 or 444.

446. A cell, e.g., a host cell, comprising the AAV capsid variant of any one of embodiments 1-378, 395, 396, or 444, the polynucleotide of any one of embodiments 379-389 or 444, the peptide of any one of embodiments 390-394 or 444, the AAV particle of any one of embodiments 397-444, or the vector of embodiment 445.

447. The cell of embodiment 446, wherein the cell is a mammalian cell or an insect cell.

448. The cell of embodiment 446 or 447, wherein the cell is a cell of a brain region 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, thoracic spinal cord, lumbar spinal cord, or a combination thereof.

449. The cell of any one of embodiments 446-448, wherein 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).

450. 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 1-378, 395, 396, or 444, or an AAV capsid variant encoded by the polynucleotide of any one of embodiments 379-389 or 444;

thereby making the AAV particle.

451. The method of embodiment 450, further comprising, prior to step (i), introducing a first nucleic acid molecule comprising the viral genome into the host cell.

452. The method of embodiment 450 or 451, wherein the host cell comprises a second nucleic acid encoding the capsid variant.

453. The method of embodiment 452, wherein the second nucleic acid molecule is introduced into the host cell prior to, concurrently with, or after the first nucleic acid molecule.

454. A pharmaceutical composition comprising the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444, and a pharmaceutically acceptable excipient.

455. A method of delivering a payload to a cell or tissue (e.g., a CNS cell, a CNS tissue, a heart cell, a heart tissue, a muscle cell, a muscle tissue, a liver cell, or a liver tissue), comprising administering an effective amount of the pharmaceutical composition of embodiment 454, the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444.

456. The method of embodiment 455, wherein the cell is a cell of a brain region 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.

457. The method of embodiment 455, wherein the cell is a cell of the heart, e.g., a heart atrium or a heart ventricle.

458. The method of embodiment 455, wherein the cell is a cell of the muscle (e.g., a cell of the quadriceps) or liver.

459. The method of embodiment 455 or 456, wherein the cell is a neuron, a sensory neuron, a motor neuron, an astrocyte, a glial cell, or an oligodendrocyte.

460. The method of any one of embodiments 455-459, wherein the cell or tissue is within a subject.

461. The method of embodiment 460, 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.

462. The method of embodiment 460 or 461, wherein the subject has, has been diagnosed with having, or is at risk of having a neurological, e.g., a neurodegenerative disorder.

463. The method of embodiment 460 or 461, wherein the subject has, has been diagnosed with having, or is at risk of having a muscular disorder or a neuromuscular disorder.

464. The method of embodiment 460 or 461, wherein the subject has, has been diagnosed with having, or is at risk of having a neuro-oncological disorder.

465. 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 454, the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444.

466. 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 454, the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444.

467. 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 454, the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444.

468. 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 454, the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444.

469. The method of any one of embodiments 465-468, 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).

470. The method of any one of embodiments 465-469, wherein treating comprises prevention of progression of the disease or disorder in the subject.

471. The method of any one of embodiments 460-470, wherein the subject is a human.

472. The method of any one of embodiments 465-471, wherein the AAV particle is administered to the subject intravenously, via intra-cisterna magna injection (ICM), intracerebrally, intrathecally, intracerebroventricularly, via intraparenchymal administration, or intramuscularly.

473. The method of any one of embodiments 465-472, wherein 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.

474. The method of any one of embodiments 465-472, wherein the AAV particle is administered to the subject intravenously.

475. The method of any one of embodiments 465-475, wherein administration of the AAV particle results in a decreased presence, level, and/or activity of a gene, mRNA, protein, or combination thereof.

476. The method of any one of embodiments 465-475, wherein administration of the AAV particle results in an increased presence, level, and/or activity of a gene, mRNA, protein, or a combination thereof.

477. The pharmaceutical composition of embodiment 454, the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444, for use in a method of delivering a payload to a cell or tissue.

478. The pharmaceutical composition of embodiment 454, the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444, 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.

479. The pharmaceutical composition of embodiment 454, the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444, an AAV particle comprising the peptide of any one of embodiments 376-380 or 430, for use in the manufacture of a medicament.

480. Use of the pharmaceutical composition of embodiment 454, the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444, in the manufacture of a medicament.

481. Use of the pharmaceutical composition of embodiment 454, the AAV particle of any one of embodiments 397-444, an AAV particle comprising the capsid variant of any one of embodiments 1-378, 395, 396, or 444, or an AAV particle comprising the peptide of any one of embodiments 390-394 or 444, 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.

[055] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[056] **FIGs. 1A-1D** show immunohistochemistry images from various CNS and peripheral tissues isolated from NHPs (cynomolgus macaques) at 28 days post intravenous administration of AAV particles comprising the TTN-002 capsid variant (top panels) or AAV9 control capsid (bottom panels) and a self-complementary genome encoding a payload fused to an HA tag driven by a heterologous constitutive promoter. **FIG 1A** shows, from left to right, the cerebellum (Purkinje layer), spinal cord (cervical), cortex (temporal), and the brainstem. **FIG. 1B** shows, from left to right, the globus pallidus, the hippocampus, the thalamus, the putamen, and the dentate. **FIG. 1C** shows, from left to right, the whole brain (level H), the whole brain (level K), and the cerebellum. **FIG. 1D** shows, from left to right, the spinal cord (thoracic), the DRG (thoracic), the liver, and the heart.

DETAILED DESCRIPTION OF THE DISCLOSURE

[057] 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, a CNS cell, a heart cell, a heart tissue, a muscle cell, a muscle tissue, a liver cell, or a liver tissue.

[058] As demonstrated in the Examples herein below, certain AAV capsid variants described herein show multiple advantages over wild-type AAV5 and/or 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, (iii) elevated payload expression in multiple brain regions, (iv) wider distribution in one or more peripheral tissues, e.g., the heart, muscle, and/or liver, and/or (v) elevated payload expression in one or more peripheral tissues. Without wishing to be being 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. In some embodiments, the AAV capsids described herein enhance the delivery of a payload to the heart, the muscle, and/or the liver.

[059] In some embodiments, AAV capsid variants disclosed herein comprise a modification in loop VIII of AAV5, e.g., at positions between 571-579, e.g., at position 577, numbered relative to SEQ ID NO: 138. Without wishing to be bound by theory, it is believed in some embodiments that the aforesaid region (e.g., positions between 571-579, e.g., at position 577) of the AAV5 capsid protrudes above the 3-fold axis of symmetry, e.g., is a surface-exposed location in the AAV5 capsid, e.g., as described in Govindasamy et al. "Structural Insights into Adeno-Associated Virus Serotype 5," *Journal of Virology*, 2013, 87(20):11187-11199 (the contents of which are hereby incorporated by reference in their entirety). 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 (e.g., VR-VIII) comprises positions 571-592 (e.g., amino acids TNNQSSTTAPATGTYNLQEIVP (SEQ ID NO: 736)), numbered according to SEQ ID NO: 138. In some embodiments, loop VIII (e.g., VR-VIII) comprises positions 571-599 (e.g., amino acids TNNQSSYPAEVVQKTAPATGTYNLQEIVP (SEQ ID NO: 756)), numbered according to SEQ ID NO: 982. In some embodiments, loop VIII or variable region VIII (VR-VIII) is as described in Govindasamy et al. (*supra)* (the contents of which are hereby incorporated by reference in their entirety).

[060] Several approaches have been used 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) 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) 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; which is herein incorporated by reference) 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.

[061] 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 capabilities 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.

[062] 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 AAV capsid variants generated using the TRACER method which demonstrate enhance tropism in for example a CNS cell, a CNS tissue, a muscle cell, or a muscle tissue.

[063] 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.

[064] In some embodiments, an AAV particle is 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.

[065] In some embodiments, the AAV particle is a naturally occurring (e.g., wild-type) AAV or a recombinant AAV. In some embodiments, the wild-type AAV viral genome is a linear, single-stranded DNA (ssDNA) molecule approximately 5,000 nucleotides (nt) in length. In some embodiments, inverted terminal repeats (ITRs) cap the viral genome at both the 5' and the 3' end, providing origins of replication for the viral genome. In some embodiments, an AAV viral genome 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.

[066] 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 AAV5 (SEQ ID NO: 138 and 137) VP1 refers to amino acids 1-724, VP2 refers to amino acids 137-724, and VP3 refers to amino acids 193-724. In some embodiments, for the amino acid sequence of SEQ ID NO: 982, VP1 comprises amino acids 1-731, VP2 comprises amino acids 137-731, and VP3 comprises amino acids 193-731. 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.

[067] AAV particles 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 viral 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.

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

[069] 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

[070] 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.

[071] 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 temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, cerebellum, cervical spinal cord, thoracic spinal cord, or lumbar spinal cord.

[072] 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).

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

[074] 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.

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

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

[077] In some embodiments, the peptide may increase distribution of an AAV particle to a cell, region, or tissue of a liver. In some embodiments, the peptide may increase distribution of an AAV particle to a liver cell, region, or tissue after intravenous administration.

[078] 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 6 to 12 amino acids in length, e.g., about 9 amino acids in length. In some embodiments, a peptide comprises about 7 to 11 amino acids in length, e.g., about 8 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. In some embodiments, a peptide comprises about 4 to 9 amino acids in length, e.g., about 6 amino acids in length.

[079] In some embodiments a peptide may comprise a sequence as set forth in Table 1 (e.g., comprising the amino acid sequence of any of SEQ ID NOs: 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583-1587,

1590-1593, 1598-1624, or 2064-2079). In some embodiments a peptide may comprise a sequence as set forth in Table 2A or 2B. In some embodiments, the peptide may comprise a sequence set forth in Tables 9 or 15-20. 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:
TNNQSSYPAEVVQKTA	1533	TNNQSSYPPSLEQKTA	1590	TNNQSSYPAEVVQKNA	1619
TNNAGAYPAEVVQKTA	1021	TNNQSSYPPSLVKKTA	1591	TNNQRKYPAEVVQKTA	1620
INNQSSYPAEVVQKTA	1024	TNNLSSYQAEVVQKTA	1592	TNNQSSNPAEEVQKTA	1621
TNNLGSYPAEVVQKTA	1027	TNNQSSYPPSLVQKPA	1593	NNNQSSYPAEVVQKTA	1622
TNNIGSYPAEVVQKTA	1112	TNNHSSYPAAVVQKTA	1598	TNNQSYYPAEEVQKTA	1623
TNNSSSYPAEVVQKPA	1142	TNSQSSNPAEVVQKTA	1599	TKNHSSYPAEVVQKTA	1624
TNNSSSYTAEVVQKTA	1214	NNNQSRYPAEVVQKTA	1601	TNSSLSYQAEVVQKTA	2064
TNNTSLYPAEVVQKTA	1232	TNNQSSYTAEVVQKNA	1602	TNSSLSYTAEVVQKTA	2065
TNNQSKYPAEVEQKTA	1254	TNNTSLCPAEVVQKTA	1603	TNSSLYYPAEVVQKTA	2066
TNNQSSYPPSLVQKTA	1300	TNNQRSYTAEVVQKTA	1604	TNTSATYPAEVVQKTA	2067
TNNHSSYPAEVVQKTA	1310	TNSTSLYPAEVVQKTA	1605	TNSSLSYPAEVVQKTA	2068
TNNQSRYPAEVVQKTA	1327	TNNQSSYTAEVVKKTA	1606	TNSSLSYPAEEVQKTA	2069
TNNSSSYPAEVVKKTA	1331	TNNHSSYPAEVLQKTA	1607	TNSSLSYPAEVVQKTD	2070
TNNQSRYPAEEVQKTA	1342	TNNQSSYQAEEVQKTA	1608	TNSSLSYPAEVVQNTA	2071
TNNSSSYPAEVVQQTA	1419	TNNKSKYPAEVVQKTA	1610	TNSSLSYPAEVVQKNA	2072
TNNQSKYPAEVVHKTA	1453	TNNQSRYPAEVMQKTA	1611	TNSSLSYPAEVVQKPA	2073
TNNQSKYPAEVVQKTA	1538	TNSTSPYPAEVVQKTA	1612	TNNQTSTEAEVVQKTA	2074
TNNSSSYPAEVVQKTA	1539	TNNQSSYPAWLIQKTA	1613	TNKSATYPAEVVQKTA	2075
TNTASSYPAEVVQKTA	1575	TNNQSSYPAEATKKTA	1614	TNSSLSYPADVVQKTA	2076
TNNQSTNKAEVVQKTA	1578	TNKQSSYTAEVVQKTA	1615	TNNQSSYPAEVVQKTA	2077
TNNTSLYPAEEVQKTA	1583	TNKIGSYPAEVVQKTA	1616	TNNQSSYPAEVVQKNA	2078
TNTASSYQAEVVQKTA	1584	TNNKSRYPAEVVQKTA	1617	TNNQSSYPSTDVQKTA	2079
TNNQSSYTPSLVQKTA	1585	TNNQSSYPAEVVQKTD	1618	TNNKGLYPAEVVQKTA	2080
TNNQASYPAEVVQKTA	1586	TNKQASYPAEVVQKTA	1587	TNSTHSYPAEVVQKTA	751

Table 2A. Exemplary Peptide Sequences

Peptide	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:	Nucleotide Sequence
2	943	YPAEVVQK	944	TATCCGGCGGAGGTGGTGCAGAAG

Table 2B. Exemplary Peptide Sequences

Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:
YPAEVVQK	943	YPPSLEQK	953	CPAEVVQK	960
YPPSLVQK	946	YPPSLVKK	954	YTAEVVKK	961
NKAEVVQK	947	YPAEVVKK	955	YPAEVLQK	962
YTAEVVQK	948	YPAEVVHK	956	YQAEEVQK	963
YPAEVEQK	949	YPAAVVQK	957	YPAEVVQN	964
YPAEEVQK	950	NPAEVVQK	958	TEAEVVQK	965
YQAEVVQK	951	YPAEVVQQ	959	YPADVVQK	966
YTPSLVQK	952				

[080] In some embodiments, a peptide described herein comprises an amino acid sequence having the formula [N2]-[N3], wherein [N2] comprises positions X1, X2, X3, X4, and X5 and [N3]

comprises the amino acid sequence of VQK, VQN, EQK, VKK, VHK, VQQ, or LQK. In some embodiments, position X1 of [N2] is Y, N, C, or T. In some embodiments, position X2 of [N2] is P, E, K, T, or Q. In some embodiments, position X3 of [N2] is A or P. In some embodiments, position X4 of [N2] is E, S, D, or A. In some embodiments, position X5 of [N2] is V, L, or E. In some embodiments, [N2] comprises Y at position X1. In some embodiments, [N2] comprises P at position X2. In some embodiments, [N2] comprises A at position X3. In some embodiments, [N2] comprises E at position X4. In some embodiments, [N2] comprises V at position X5. In some embodiments, the amino acid sequence of [N3] comprises VQK. In some embodiments, the amino acid sequence of [N3] consists of VQK.

[081] In some embodiments, a peptide described herein comprises an amino acid sequence having the formula [N2]-[N3], wherein [N2] comprises positions X1, X2, X3, X4, and X5 and [N3] comprises the amino acid sequence of VQK, EQK, VKK, VHK, VQQ, or LQK. In some embodiments [N3] comprises the amino acid sequence of VQK, EQK, or VKK. In some embodiments [N3] comprises the amino acid sequence VQK. In some embodiments [N3] consists of the amino acid sequence VQK. In some embodiments, position X1 of [N2] is Y, N, or C. In some embodiments position X1 of [N2] is Y or N. In some embodiments, position X2 of [N2] is P, K, T, or Q. In some embodiments position X2 of [N2] is P, T, or Q. In some embodiments, position X3 of [N2] is A or P. In some embodiments, position X3 of [N2] is A. In some embodiments, position X4 of [N2] is E, S, or A. In some embodiments, position X5 of [N2] is V, L, or E. In some embodiments, position X5 of [N2] is V or L. In some embodiments, [N2] comprises Y at position X1. In some embodiments, [N2] comprises P at position X2. In some embodiments, [N2] comprises A at position X3. In some embodiments, [N2] comprises E at position X4. In some embodiments, [N2] comprises V at position X5. In some embodiments, [N2] comprises YPA, YPP, NKA, YTA, YQA, YTP, NPA, CPA, THA, PAE, PPS, KAE, TAE, QAE, TPS, PAA, HAS, AEV, PSL, AEE, or AAV. In some embodiments, [N2] comprises YPAE (SEQ ID NO: 21), YPPS (SEQ ID NO: 22), NKAE (SEQ ID NO: 23), YTAE (SEQ ID NO: 24), YQAE (SEQ ID NO: 25), YTPS (SEQ ID NO: 26), YPAA (SEQ ID NO: 27), NPAE (SEQ ID NO: 28), CPAE (SEQ ID NO: 29), THAS (SEQ ID NO: 30), PAEV (SEQ ID NO: 17), PPSL (SEQ ID NO: 31), KAEV (SEQ ID NO: 32), TAEV (SEQ ID NO: 16), PAEE (SEQ ID NO: 18), QAEV (SEQ ID NO: 15), TPSL (SEQ ID NO: 33), PAAV (SEQ ID NO: 34), or QAEE (SEQ ID NO: 35). In some embodiments [N2] is or comprises YPAEV (SEQ ID NO: 1), YPPSL (SEQ ID NO: 2), NKAEV (SEQ ID NO: 3), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), YQAEV (SEQ ID NO: 6), YTPSL (SEQ ID NO: 7), YPAAV (SEQ ID NO: 8), NPAEV (SEQ ID NO: 9), CPAEV (SEQ ID NO: 10), or YQAEE (SEQ ID NO: 11). In some embodiments, [N2] comprises the amino acid sequence of YPAEV (SEQ ID NO: 1). In some embodiments, the amino acid sequence of [N2] consists of YPAEV (SEQ ID NO: 1). In some embodiments, [N2]-[N3] comprises the amino acid sequence of AEVVQK (SEQ ID NO: 36), PSLVQK (SEQ ID NO: 37), AEVEQK (SEQ ID NO: 38), AEEVQK (SEQ ID NO: 39), PSLEQK

(SEQ ID NO: 40), PSLVKK (SEQ ID NO: 41), AEVVKK (SEQ ID NO: 42), AEVVHK (SEQ ID NO: 43), AAVVQK (SEQ ID NO: 44), AEVVQQ (SEQ ID NO: 45), or AEVLQK (SEQ ID NO: 46). In some embodiments [N2]-[N3] comprises the amino acid sequence PAEVVQK (SEQ ID NO: 20), PPSLVQK (SEQ ID NO: 47), KAEVVQK (SEQ ID NO: 48), TAEVVQK (SEQ ID NO: 49), PAEVEQK (SEQ ID NO: 50), PAEEVQK (SEQ ID NO: 51), QAEVVQK (SEQ ID NO: 52), TPSLVQK (SEQ ID NO: 53), PPSLEQK (SEQ ID NO: 54), PPSLVKK (SEQ ID NO: 55), PAEVVKK (SEQ ID NO: 56), PAEVVHK (SEQ ID NO: 57), PAAVVQK (SEQ ID NO: 58), PAEVVQQ (SEQ ID NO: 59), TAEVVKK (SEQ ID NO: 60), PAEVLQK (SEQ ID NO: 61), or QAEEVQK (SEQ ID NO: 62). In some embodiments [N2]-[N3] is or comprises YPAEVVQK (SEQ ID NO: 943), YPPSLVQK (SEQ ID NO: 946), NKAEVVQK (SEQ ID NO: 947), YTAEVVQK (SEQ ID NO: 948), YPAEVEQK (SEQ ID NO: 949), YPAEEVQK (SEQ ID NO: 950), YQAEVVQK (SEQ ID NO: 951), YTPSLVQK (SEQ ID NO: 952), YPPSLEQK (SEQ ID NO: 953), YPPSLVKK (SEQ ID NO: 954), YPAEVVKK (SEQ ID NO: 955), YPAEVVHK (SEQ ID NO: 956), YPAAVVQK (SEQ ID NO: 957), NPAEVVQK (SEQ ID NO: 958), YPAEVVQQ (SEQ ID NO: 959), CPAEVVQK (SEQ ID NO: 960), YTAEVVKK (SEQ ID NO: 961), YPAEVLQK (SEQ ID NO: 962), or YQAEEVQK (SEQ ID NO: 963); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, or 7 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N2]-[N3] is YPAEVVQK (SEQ ID NO: 943). In some embodiments, [N2]-[N3] comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943).

[082] In some embodiments, the peptide comprising the amino acid sequence comprising the formula of [N2]-[N3], further comprises [N1], which comprises positions X_D, X_E, and X_F. In some embodiments, position X_D of [N1] is Q, T, S, A, I, L, or H. In some embodiments, position X_E of [N1] is S, G, A, or R. In some embodiments, position X_F of [N1] is S, K, L, R, A, or T. In some embodiments, [N1] comprises SK, SL, SS, SR, GA, GS, AS, ST, RS, QS, TS, AG, IG, QA, LG, HS, LS, or QR. In some embodiments, [N1] is or comprises QSS, QSK, TSL, SSS, QSR, AGA, IGS, QAS, ASS, LGS, QST, HSS, LSS, or QRS. In some embodiments, the amino acid sequence of [N1] is QSS. In some embodiments, [N1]-[N2] comprises SSYPA (SEQ ID NO: 63), SKYPA (SEQ ID NO: 64), SLYPA (SEQ ID NO: 65), SRYPA (SEQ ID NO: 66), SSYPP (SEQ ID NO: 67), GAYPA (SEQ ID NO: 68), GSYPA (SEQ ID NO: 69), ASYPA (SEQ ID NO: 70), STNKA (SEQ ID NO: 71), SSYTA (SEQ ID NO: 72), SSYQA (SEQ ID NO: 73), SSYTP (SEQ ID NO: 74), SSNPA (SEQ ID NO: 75), SLCPA (SEQ ID NO: 76), RSYTA (SEQ ID NO: 77), or SSTHA (SEQ ID NO: 78). In some embodiments, [N1]-[N2] comprises SSYPAE (SEQ ID NO: 79), SKYPAE (SEQ ID NO: 80), SLYPAE (SEQ ID NO: 81), SRYPAE (SEQ ID NO: 82), SSYPPS (SEQ ID NO: 83), GAYPAE

(SEQ ID NO: 84), GSYPAE (SEQ ID NO: 85), ASYPAE (SEQ ID NO: 86), STNKAE (SEQ ID NO: 87), SSYTAE (SEQ ID NO: 88), SSYQAE (SEQ ID NO: 89), SSYTPS (SEQ ID NO: 90), SSYPAA (SEQ ID NO: 91), SSNPAE (SEQ ID NO: 92), SLCPAE (SEQ ID NO: 93), RSYTAE (SEQ ID NO: 94), SSTHAS (SEQ ID NO: 95). In some embodiments, [N1]-[N2] is or comprises QSSYPAEV (SEQ ID NO: 96), QSKYPAEV (SEQ ID NO: 97), TSLYPAEV (SEQ ID NO: 98), SSSYPAEV (SEQ ID NO: 99), QSRYPAEV (SEQ ID NO: 100), QSSYPPSL (SEQ ID NO: 101), AGAYPAEV (SEQ ID NO: 102), IGSYPAEV (SEQ ID NO: 103), QASYPAEV (SEQ ID NO: 104), ASSYPAEV (SEQ ID NO: 105), LGSYPAEV (SEQ ID NO: 106), QSTNKAEV (SEQ ID NO: 107), HSSYPAEV (SEQ ID NO: 108), SSSYTAEV (SEQ ID NO: 109), TSLYPAEE (SEQ ID NO: 110), ASSYQAEV (SEQ ID NO: 111), QSSYTPSL (SEQ ID NO: 112), QSRYPAEE (SEQ ID NO: 113), LSSYQAEV (SEQ ID NO: 114), HSSYPAAV (SEQ ID NO: 115), QSSNPAEV (SEQ ID NO: 116), QSSYTAEV (SEQ ID NO: 117), TSLCPAEV (SEQ ID NO: 118), QRSYTAEV (SEQ ID NO: 119), or QSSYQAEE (SEQ ID NO: 120); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, or 7 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, the amino acid sequence of [N1]-[N2] is QSSYPAEV (SEQ ID NO: 96). In some embodiments, [N1]-[N2]-[N3] comprises SSYPAEVVQ (SEQ ID NO: 121), SKYPAEVVQ (SEQ ID NO: 122), SLYPAEVVQ (SEQ ID NO: 123), SRYPAEVVQ (SEQ ID NO: 124), SSYPPSLVQ (SEQ ID NO: 125), GAYPAEVVQ (SEQ ID NO: 126), GSYPAEVVQ (SEQ ID NO: 127), ASYPAEVVQ (SEQ ID NO: 128), STNKAEVVQ (SEQ ID NO: 129), SSYTAEVVQ (SEQ ID NO: 130), SKYPAEVEQ (SEQ ID NO: 131), SLYPAEEVQ (SEQ ID NO: 132), SSYQAEVVQ (SEQ ID NO: 133), SSYTPSLVQ (SEQ ID NO: 134), SRYPAEEVQ (SEQ ID NO: 135), SSYPPSLEQ (SEQ ID NO: 136), SSYPPSLVK (SEQ ID NO: 140), SSYPAEVVK (SEQ ID NO: 141), SKYPAEVVH (SEQ ID NO: 142), SSYPAAVVQ (SEQ ID NO: 143), SSNPAEVVQ (SEQ ID NO: 144), SLCPAEVVQ (SEQ ID NO: 145), RSYTAEVVQ (SEQ ID NO: 146), SSYTAEVVK (SEQ ID NO: 147), SSYPAEVLQ (SEQ ID NO: 148), or SSYQAEEVQ (SEQ ID NO: 149). In some embodiments, [N1]-[N2]-[N3] is or comprises QSSYPAEVVQK (SEQ ID NO: 150), QSKYPAEVVQK (SEQ ID NO: 151), TSLYPAEVVQK (SEQ ID NO: 152), SSSYPAEVVQK (SEQ ID NO: 153), QSRYPAEVVQK (SEQ ID NO: 154), QSSYPPSLVQK (SEQ ID NO: 155), AGAYPAEVVQK (SEQ ID NO: 156), IGSYPAEVVQK (SEQ ID NO: 157), QASYPAEVVQK (SEQ ID NO: 158), ASSYPAEVVQK (SEQ ID NO: 159), LGSYPAEVVQK (SEQ ID NO: 160), QSTNKAEVVQK (SEQ ID NO: 161), HSSYPAEVVQK (SEQ ID NO: 162), SSSYTAEVVQK (SEQ ID NO: 163), QSKYPAEVEQK (SEQ ID NO: 164), TSLYPAEEVQK (SEQ ID NO: 165), ASSYQAEVVQK (SEQ ID NO: 166), QSSYTPSLVQK (SEQ ID NO: 167), QSRYPAEEVQK

(SEQ ID NO: 168), QSSYPPSLEQK (SEQ ID NO: 169), QSSYPPSLVKK (SEQ ID NO: 170), LSSYQAEVVQK (SEQ ID NO: 171), SSSYPAEVVKK (SEQ ID NO: 172), QSKYPAEVVHK (SEQ ID NO: 173), HSSYPAAVVQK (SEQ ID NO: 174), QSSNPAEVVQK (SEQ ID NO: 175), SSSYPAEVVQQ (SEQ ID NO: 176), QSSYTAEVVQK (SEQ ID NO: 177), TSLCPAEVVQK (SEQ ID NO: 178), QRSYTAEVVQK (SEQ ID NO: 179), QSSYTAEVVKK (SEQ ID NO: 180), HSSYPAEVLQK (SEQ ID NO: 181), or QSSYQAEEVQK (SEQ ID NO: 182); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, the amino acid sequence of [N1]-[N2]-[N3] is QSSYPAEVVQK (SEQ ID NO: 150).

[083] In some embodiments, the peptide comprising the amino acid sequence comprising the formula [N2]-[N3], further comprises [N0], wherein [N0] comprises positions X_A, X_B, and X_C. In some embodiments, position X_A of [N0] is T, I, or N. In some embodiments, positions X_B of [N0] is N. In some embodiments, position X_C of [N0] is N, T, S, or K. In some embodiments, [N0] comprises TN, IN, NN, NT, NS, or NK. In some embodiments, [N0] is or comprises TNN, TNT, INN, TNS, NNN, or TNK. In some embodiments, the amino acid sequence of [N0] is TNN. In some embodiments, [N0]-[N1] is or comprises TNNQSS (SEQ ID NO: 183), TNNQSK (SEQ ID NO: 184), TNNTSL (SEQ ID NO: 185), TNNSSS (SEQ ID NO: 186), TNNQSR (SEQ ID NO: 187), TNNAGA (SEQ ID NO: 188), TNNIGS (SEQ ID NO: 189), TNNQAS (SEQ ID NO: 190), TNTASS (SEQ ID NO: 191), TNNLGS (SEQ ID NO: 192), TNNQST (SEQ ID NO: 193), TNNHSS (SEQ ID NO: 194), TNNQSK (SEQ ID NO: 184), TNNLSS (SEQ ID NO: 195), INNQSS (SEQ ID NO: 196), TNSQSS (SEQ ID NO: 197), NNNQSR (SEQ ID NO: 198), TNSTSL (SEQ ID NO: 199), TNNQRS (SEQ ID NO: 200), or TNKQAS (SEQ ID NO: 201); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1] is TNNQSS (SEQ ID NO: 183). In some embodiments, [N0]-[N1]-[N2]-[N3] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500), TNNQSKYPAEVVQK (SEQ ID NO: 503), TNNTSLYPAEVVQK (SEQ ID NO: 506), TNNSSSYPAEVVQK (SEQ ID NO: 508), TNNQSRYPAEVVQK (SEQ ID NO: 510), TNNQSSYPPSLVQK (SEQ ID NO: 512), TNNAGAYPAEVVQK (SEQ ID NO: 513), TNNIGSYPAEVVQK (SEQ ID NO: 514), TNNQASYPAEVVQK (SEQ ID NO: 517), TNTASSYPAEVVQK (SEQ ID NO: 520),

TNNLGSYPAEVVQK (SEQ ID NO: 523), TNNQSTNKAEVVQK (SEQ ID NO: 524), TNNHSSYPAEVVQK (SEQ ID NO: 525), TNNSSSYTAEVVQK (SEQ ID NO: 526), TNNQSKYPAEVEQK (SEQ ID NO: 529), TNNTSLYPAEEVQK (SEQ ID NO: 530), TNTASSYQAEVVQK (SEQ ID NO: 531), TNNQSSYTPSLVQK (SEQ ID NO: 533), TNNQSRYPAEEVQK (SEQ ID NO: 534), TNNQSSYPPSLEQK (SEQ ID NO: 535), TNNQSSYPPSLVKK (SEQ ID NO: 536), TNNLSSYQAEVVQK (SEQ ID NO: 539), TNNSSSYPAEVVKK (SEQ ID NO: 540), TNNQSKYPAEVVHK (SEQ ID NO: 542), INNQSSYPAEVVQK (SEQ ID NO: 543), TNNHSSYPAAVVQK (SEQ ID NO: 545), TNSQSSNPAEVVQK (SEQ ID NO: 548), TNNSSSYPAEVVQQ (SEQ ID NO: 551), NNNQSRYPAEVVQK (SEQ ID NO: 552), TNNQSSYTAEVVQK (SEQ ID NO: 553), TNNTSLCPAEVVQK (SEQ ID NO: 554), TNSTSLYPAEVVQK (SEQ ID NO: 556), TNNQRSYTAEVVQK (SEQ ID NO: 557), TNNQSSYTAEVVKK (SEQ ID NO: 558), TNNHSSYPAEVLQK (SEQ ID NO: 560), TNNQSSYQAEEVQK (SEQ ID NO: 562) or TNKQASYPAEVVQK (SEQ ID NO: 563); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1]-[N2]-[N3] is TNNQSSYPAEVVQK (SEQ ID NO: 500).

[084] In some embodiments, the peptide comprising the amino acid sequence comprising the formula [N2]-[N3], further comprises [N4], which comprises positions X_G and X_H. In some embodiments, position X_G of [N4] is T, P, or N. In some embodiments, position X_G of [N4] is T. In some embodiments, position X_H of [N4] is A. In some embodiments, [N4] is or comprises TA, PA, or NA. In some embodiments, [N4] is TA. In some embodiments, [N3]-[N4] is or comprises VQKTA (SEQ ID NO: 564), EQKTA (SEQ ID NO: 565), VKKTA (SEQ ID NO: 566), VQKPA (SEQ ID NO: 567), VHKTA (SEQ ID NO: 568), VQQTA (SEQ ID NO: 569), VQKNA (SEQ ID NO: 570), or LQKTA (SEQ ID NO: 571); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, or 4 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N3]-[N4] is VQKTA (SEQ ID NO: 564). In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNTSLYPAEVVQKTA (SEQ ID NO: 1232),

TNNSSSYPAEVVQKTA (SEQ ID NO: 1539), TNNQSRYPAEVVQKTA (SEQ ID NO: 1327), TNNQSSYPPSLVQKTA (SEQ ID NO: 1300), TNNAGAYPAEVVQKTA (SEQ ID NO: 1021), TNNIGSYPAEVVQKTA (SEQ ID NO: 1112), TNNQASYPAEVVQKTA (SEQ ID NO: 1194), TNTASSYPAEVVQKTA (SEQ ID NO: 1575), TNNLGSYPAEVVQKTA (SEQ ID NO: 1027), TNNQSTNKAEVVQKTA (SEQ ID NO: 1578), TNNHSSYPAEVVQKTA (SEQ ID NO: 1310), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNSSSYTAEVVQKTA (SEQ ID NO: 1214), TNNQSKYPAEVEQKTA (SEQ ID NO: 1254), TNNTSLYPAEEVQKTA (SEQ ID NO: 1583), TNTASSYQAEVVQKTA (SEQ ID NO: 1584), TNNQSSYTPSLVQKTA (SEQ ID NO: 1585), TNNQSRYPAEEVQKTA (SEQ ID NO: 1342), TNNQSSYPPSLEQKTA (SEQ ID NO: 1590), TNNQSSYPPSLVKKTA (SEQ ID NO: 1591), TNNLSSYQAEVVQKTA (SEQ ID NO: 1592), TNNQSSYPPSLVQKPA (SEQ ID NO: 1593), TNNSSSYPAEVVKKTA (SEQ ID NO: 1331), TNNQSKYPAEVVHKTA (SEQ ID NO: 1453), TNNSSSYPAEVVQKPA (SEQ ID NO: 1142), INNQSSYPAEVVQKTA (SEQ ID NO: 1024), TNNHSSYPAAVVQKTA (SEQ ID NO: 1598), TNSQSSNPAEVVQKTA (SEQ ID NO: 1599), TNNSSSYPAEVVQQTA (SEQ ID NO: 1419), NNNQSRYPAEVVQKTA (SEQ ID NO: 1601), TNNQSSYTAEVVQKNA (SEQ ID NO: 1602), TNNTSLCPAEVVQKTA (SEQ ID NO: 1603), TNSTSLYPAEVVQKTA (SEQ ID NO: 1605), TNNQRSYTAEVVQKTA (SEQ ID NO: 1604), TNNQSSYTAEVVKKTA (SEQ ID NO: 1606), TNNHSSYPAEVLQKTA (SEQ ID NO: 1607), TNNQSSYQAEEVQKTA (SEQ ID NO: 1608), or TNKQASYPAEVVQKTA (SEQ ID NO: 1587); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

[085] In some embodiments, a peptide described herein comprises the formula [N2]-[N3], wherein [N2] comprises positions X1, X2, X3, X4, and X5 and [N3] comprises the amino acid sequence of VQK or VQN. In some embodiments, [N3] comprises the amino acid sequence VQK. In some embodiments, position X1 of [N2] is Y or T. In some embodiments, position X2 of [N2] is Q, T, P, or E. In some embodiments, position X3 of [N2] is A. In some embodiments, position X4 of [N2] is E or D. In some embodiments, position X4 of [N2] is E or D. In some embodiments, position X5 of [N2] is V or E. In some embodiments, [N2] comprises Y at position X1. In some embodiments, [N2] comprises P at position X2. In some embodiments, [N2] comprises A at position X3. In some embodiments, [N2] comprises E at position X4. In some embodiments, [N2] comprises V at position X5. In some embodiments, [N2] comprises YP, YQ, YT, TE, QA, TA, PA, EA, EV, EE, DV, AE, or AD. In some embodiments, [N2] comprises YPA, YQA, YTA, TEA, QAE, TAE, PAE, EAE, PAD,

AEV, AEE, or ADV. In some embodiments, [N2] comprises YPAE (SEQ ID NO: 21), YQAE (SEQ ID NO: 25), YTAE (SEQ ID NO: 24), TEAE (SEQ ID NO: 587), YPAD (SEQ ID NO: 588), QAEV (SEQ ID NO: 15), TAEV (SEQ ID NO: 16), PAEV (SEQ ID NO: 17), PAEE (SEQ ID NO: 18), EAEV (SEQ ID NO: 590), or PADV (SEQ ID NO: 19). In some embodiments, [N2] is or comprises YPAEV (SEQ ID NO: 1), YQAEV (SEQ ID NO: 6), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), TEAEV (SEQ ID NO: 12), or YPADV (SEQ ID NO: 13). In some embodiments, [N2] is YPAEV (SEQ ID NO: 1). In some embodiments, [N2]-[N3] comprises AEVVQK (SEQ ID NO: 36), AEEVQK (SEQ ID NO: 39), AEVVQN (SEQ ID NO: 591), or ADVVQK (SEQ ID NO: 593). In some embodiments, [N2]-[N3] comprises PAEVVQN (SEQ ID NO: 594), QAEVVQK (SEQ ID NO: 52), TAEVVQK (SEQ ID NO: 49), PAEVVQK (SEQ ID NO: 20), PAEEVQK (SEQ ID NO: 51), EAEVVQK (SEQ ID NO: 595), or PADVVQK (SEQ ID NO: 596). In some embodiments, [N2]-[N3] is or comprises YPAEVVQK (SEQ ID NO: 943), YQAEVVQK (SEQ ID NO: 951), YTAEVVQK (SEQ ID NO: 948), YPAEEVQK (SEQ ID NO: 950), YPAEVVQN (SEQ ID NO: 964), TEAEVVQK (SEQ ID NO: 965), or YPADVVQK (SEQ ID NO: 966); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, or 7 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N2]-[N3] is YPAEVVQK (SEQ ID NO: 943).

[086] In some embodiments, the peptide comprising the amino acid sequence comprising the formula of [N2]-[N3], further comprises [N1], which comprises positions X_D, X_E, and X_F. In some embodiments, position X_D of [N1] is Q or S. In some embodiments, position X_E of [N1] is S, L, A, or T. In some embodiments, position X_F of [N1] is S, Y, or T. In some embodiments, [N1] comprises QS, SL, SA, QT, LS, LY, AT, TS, or SS. In some embodiments, [N1] is or comprises QSS, SLS, SLY, SAT, or QTS. In some embodiments, [N1] is QSS. In some embodiments, [N1]-[N2] comprises SSYPA (SEQ ID NO: 63), LSYQA (SEQ ID NO: 597), LSYTA (SEQ ID NO: 598), LYYPA (SEQ ID NO: 600), ATYPA (SEQ ID NO: 601), LSYPA (SEQ ID NO: 603), or TSTEA (SEQ ID NO: 605). In some embodiments, [N1]-[N2] comprises SSYPAE (SEQ ID NO: 79), LSYQAE (SEQ ID NO: 607), LSYTAE (SEQ ID NO: 610), LYYPAE (SEQ ID NO: 611), ATYPAE (SEQ ID NO: 613), LSYPAE (SEQ ID NO: 616), TSTEAE (SEQ ID NO: 619), or LSYPAD (SEQ ID NO: 621). In some embodiments, [N1]-[N2] is or comprises QSSYPAEV (SEQ ID NO: 96), SLSYQAEV (SEQ ID NO: 622), SLSYTAEV (SEQ ID NO: 623), SLYYPAEV (SEQ ID NO: 624), SATYPAEV (SEQ ID NO: 625), SLSYPAEV (SEQ ID NO: 629), SLSYPAEE (SEQ ID NO: 632), QTSTEAEV (SEQ ID NO: 633), or SLSYPADV (SEQ ID NO: 634); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, or 7 amino acids, e.g., consecutive amino

acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N1]-[N2] is QSSYPAEV (SEQ ID NO: 96). In some embodiments, [N1]-[N2]-[N3] is or comprises QSSYPAEVVQK (SEQ ID NO: 150), SLSYQAEVVQK (SEQ ID NO: 635), SLSYTAEVVQK (SEQ ID NO: 637), SLYYPAEVVQK (SEQ ID NO: 639), SATYPAEVVQK (SEQ ID NO: 641), SLSYPAEVVQK (SEQ ID NO: 642), SLSYPAEEVQK (SEQ ID NO: 643), SLSYPAEVVQN (SEQ ID NO: 644), QTSTEAEVVQK (SEQ ID NO: 645), or SLSYPADVVQK (SEQ ID NO: 646); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N1]-[N2]-[N3] is QSSYPAEVVQK (SEQ ID NO: 150).

[087] In some embodiments, the peptide comprising the amino acid sequence comprising the formula [N2]-[N3], further comprises [N0], wherein [N0] comprises positions X_A, X_B, and X_C. In some embodiments, position X_A of [N0] is T. In some embodiments, positions X_B of [N0] is N. In some embodiments, position X_C of [N0] is N, T, S, or K. In some embodiments [N0] comprises TN, NS, NT, NN, or NK. In some embodiments, [N0] is or comprises TNS, TNT, TNN, or TNK. In some embodiment, [N0] is TNN. In some embodiments, [N0]-[N1] is or comprises TNNQSS (SEQ ID NO: 183), TNSSLS (SEQ ID NO: 647), TNSSLY (SEQ ID NO: 648), TNTSAT (SEQ ID NO: 649), TNNQTS (SEQ ID NO: 650), or TNKSAT (SEQ ID NO: 651); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1] is TNNQSS (SEQ ID NO: 183). In some embodiments, [N0]-[N1]-[N2]-[N3] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500), TNSSLSYQAEVVQK (SEQ ID NO: 652), TNSSLSYTAEVVQK (SEQ ID NO: 654), TNSSLYYPAEVVQK (SEQ ID NO: 655), TNTSATYPAEVVQK (SEQ ID NO: 656), TNSSLSYPAEVVQK (SEQ ID NO: 657), TNSSLSYPAEEVQK (SEQ ID NO: 658), TNSSLSYPAEVVQN (SEQ ID NO: 660), TNNQTSTEAEVVQK (SEQ ID NO: 662), TNKSATYPAEVVQK (SEQ ID NO: 663), or TNSSLSYPADVVQK (SEQ ID NO: 665); an amino

acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1]-[N2]-[N3] is TNNQSSYPAEVVQK (SEQ ID NO: 500).

[088] In some embodiments, the peptide comprising the amino acid sequence comprising the formula [N2]-[N3], further comprises [N4], which comprises positions X_G and X_H. In some embodiments, position X_G of [N4] is T, P, or N. In some embodiments, position X_H of [N4] is A or D. In some embodiments, [N4] is or comprises TA, TD, PA, or NA. In some embodiments, [N4] is TA. In some embodiments, [N3]-[N4] is or comprises VQKTA (SEQ ID NO: 564), EQKTA (SEQ ID NO: 565), VKKTA (SEQ ID NO: 566), VQKPA (SEQ ID NO: 567), VHKTA (SEQ ID NO: 568), VQQTA (SEQ ID NO: 569), VQKNA (SEQ ID NO: 570), or LQKTA (SEQ ID NO: 571); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, or 4 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N3]-[N4] is VQKTA (SEQ ID NO: 564). In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNSSLSYQAEVVQKTA (SEQ ID NO: 2064), TNSSLSYTAEVVQKTA (SEQ ID NO: 2065), TNSSLYYPAEVVQKTA (SEQ ID NO: 2066), TNTSATYPAEVVQKTA (SEQ ID NO: 2067), TNSSLSYPAEVVQKTA (SEQ ID NO: 2068), TNSSLSYPAEEVQKTA (SEQ ID NO: 2069), TNSSLSYPAEVVQKTD (SEQ ID NO: 2070), TNSSLSYPAEVVQNTA (SEQ ID NO: 2071), TNSSLSYPAEVVQKNA (SEQ ID NO: 2072), TNSSLSYPAEVVQKPA (SEQ ID NO: 2073), TNNQTSTEAEVVQKTA (SEQ ID NO: 2074), TNKSATYPAEVVQKTA (SEQ ID NO: 2075), or TNSSLSYPADVVQKTA (SEQ ID NO: 2076); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

[089] In some embodiments, [N1] is present immediately subsequent to [N0]. In some embodiments, [N2] is present immediately subsequent to [N1]. In some embodiments, [N3] is present

immediately subsequent to [N2]. In some embodiments, [N4] is present immediately subsequent to [N3]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [N2]-[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]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [N0]-[N1]-[N2]-[N3]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [N0]-[N1]-[N2]-[N3]-[N4].

[090] In some embodiments, a peptide described herein comprises an amino acid sequence having the formula [B]-[C], wherein [B] comprises positions X1, X2, and X3, and [C] comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943). In some embodiments, position X1 of [B] X1 is Q, T, S, A, I, L, or H. In some embodiments, position X1 of [B] X1 is Q, T, S, A, or H. In some embodiments, position X2 of [B] is S, G, or A. In some embodiments, position X2 of [B] is S or G. In some embodiments, position X3 of [B] is S, K, L, R, or A. In some embodiments, position X3 of [B] is S, K, L, or R. In some embodiments, [B] comprises Q at position X1. In some embodiments, [B] comprises S at position X2. In some embodiments, [B] comprises S at position X3. In some embodiments, [B] comprises QS, TS, SS, AG, IG, QA, AS, LG, HS, SK, SL, SR, GA, or GS. In some embodiments, [B] is or comprises QSS, TSL, SSS, QSR, QSK, AGA, IGS, QAS, ASS, LGS, or HSS. In some embodiments, the amino acid sequence of [B] is QSS. In some embodiments, [B]-[C] comprises SSYPAEVVQK (SEQ ID NO: 572), SKYPAEVVQK (SEQ ID NO: 573), SLYPAEVVQK (SEQ ID NO: 574), SRYPAEVVQK (SEQ ID NO: 575), GAYPAEVVQK (SEQ ID NO: 576), GSYPAEVVQK (SEQ ID NO: 580), or ASYPAEVVQK (SEQ ID NO: 582). In some embodiments, [B]-[C] is or comprises QSSYPAEVVQK (SEQ ID NO: 150), QSKYPAEVVQK (SEQ ID NO: 151), TSLYPAEVVQK (SEQ ID NO: 152), SSSYPAEVVQK (SEQ ID NO: 153), QSRYPAEVVQK (SEQ ID NO: 154), AGAYPAEVVQK (SEQ ID NO: 156), IGSYPAEVVQK (SEQ ID NO: 157), QASYPAEVVQK (SEQ ID NO: 158), ASSYPAEVVQK (SEQ ID NO: 159), LGSYPAEVVQK (SEQ ID NO: 160), or HSSYPAEVVQK (SEQ ID NO: 162); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [B]-[C] is QSSYPAEVVQK (SEQ ID NO: 150).

[091] In some embodiments, a peptide comprising the formula [B]-[C], further comprises [A], which comprises positions X_A, X_B, and X_C. In some embodiments, position X_A of [A] is T, I, or N. In some embodiments, position X_B of [A] is N. In some embodiments, position X_C of [A] is N, T, S, or K. In some embodiments, [A] comprises TN, IN, NN, NT, NS, or NK. In some embodiments, [A] is or comprises TNN, TNT, INN, NNN, TNS, or TNK. In some embodiments, [A] is TNN. In some

embodiments, [A]-[B] is or comprises TNNQSS (SEQ ID NO: 183), TNNQSK (SEQ ID NO: 184), TNNTSL (SEQ ID NO: 185), TNNSSS (SEQ ID NO: 186), TNNQSR (SEQ ID NO: 187), TNNAGA (SEQ ID NO: 188), TNNIGS (SEQ ID NO: 189), TNNQAS (SEQ ID NO: 190), TNTASS (SEQ ID NO: 191), TNNLGS (SEQ ID NO: 192), TNNHSS (SEQ ID NO: 194), INNQSS (SEQ ID NO: 196), NNNQSR (SEQ ID NO: 198), TNSTSL (SEQ ID NO: 199), or TNKQAS (SEQ ID NO: 201); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, the amino acid sequence of [A]-[B] is TNNQSS (SEQ ID NO: 183). In some embodiments, [A]-[B]-[C] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500), TNNQSKYPAEVVQK (SEQ ID NO: 503), TNNTSLYPAEVVQK (SEQ ID NO: 506), TNNSSSYPAEVVQK (SEQ ID NO: 508), TNNQSRYPAEVVQK (SEQ ID NO: 510), TNNAGAYPAEVVQK (SEQ ID NO: 513), TNNIGSYPAEVVQK (SEQ ID NO: 514), TNNQASYPAEVVQK (SEQ ID NO: 517), TNTASSYPAEVVQK (SEQ ID NO: 520), TNNLGSYPAEVVQK (SEQ ID NO: 523), TNNHSSYPAEVVQK (SEQ ID NO: 525), INNQSSYPAEVVQK (SEQ ID NO: 543), NNNQSRYPAEVVQK (SEQ ID NO: 552), TNSTSLYPAEVVQK (SEQ ID NO: 556), or TNKQASYPAEVVQK (SEQ ID NO: 563); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [A]-[B] is TNNQSS (SEQ ID NO: 183). In some embodiments, [A]-[B]-[C] is TNNQSSYPAEVVQK (SEQ ID NO: 500).

[092] In some embodiments, a peptide comprising the formula [B]-[C], further comprises [D], wherein [D] comprises position X4 and X5. In some embodiments, position X4 of [D] is T or N. In some embodiments, position X5 of [D] is A. In some embodiments [D] is or comprises TA or PA. In some embodiments, the amino acid sequence of [D] is TA. In some embodiments, [C]-[D] is or comprises YPAEVVQKTA (SEQ ID NO: 584) or YPAEVVQKPA (SEQ ID NO: 586); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, or 9 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different

amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, the amino acid sequence of [C]-[D] is YPAEVVQKTA (SEQ ID NO: 584). In some embodiments, [A]-[B]-[C]-[D] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNTSLYPAEVVQKTA (SEQ ID NO: 1232), TNNSSSYPAEVVQKTA (SEQ ID NO: 1539), TNNQSRYPAEVVQKTA (SEQ ID NO: 1327), TNNAGAYPAEVVQKTA (SEQ ID NO: 1021), TNNIGSYPAEVVQKTA (SEQ ID NO: 1112), TNNQASYPAEVVQKTA (SEQ ID NO: 1194), TNTASSYPAEVVQKTA (SEQ ID NO: 1575), TNNLGSYPAEVVQKTA (SEQ ID NO: 1027), TNNHSSYPAEVVQKTA (SEQ ID NO: 1310), TNNSSSYPAEVVQKPA (SEQ ID NO: 1142), INNQSSYPAEVVQKTA (SEQ ID NO: 1024), NNNQSRYPAEVVQKTA (SEQ ID NO: 1601), TNSTSLYPAEVVQKTA (SEQ ID NO: 1605), or TNKQASYPAEVVQKTA (SEQ ID NO: 1587); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, or 15 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [C]-[D] is YPAEVVQKTA (SEQ ID NO: 584). In some embodiments, [A]-[B]-[C]-[D] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

[093] In some embodiments, a peptide described herein comprises an amino acid sequence having the formula [B]-[C], wherein [B] comprises positions X1, X2, and X3, and [C] comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943). In some embodiments, position X1 of [B] is Q or S. In some embodiments, position X2 of [B] is S, L, or A. In some embodiments, position X3 of [B] is S, Y, or T. In some embodiments, [B] comprises Q at position X1. In some embodiments, [B] comprises S at position X2. In some embodiments, [B] comprises S at position X3. In some embodiments, [B] comprises QS, SL, SA, LY, AT, LS, or SS. In some embodiments, [B] is or comprises QSS, SLY, SAT, or SLS. In some embodiments, [B] is QSS. In some embodiments, [B]-[C] comprises SSYPAEVVQK (SEQ ID NO: 572), LYYPAEVVQK (SEQ ID NO: 702), ATYPAEVVQK (SEQ ID NO: 718), or LSYPAEVVQK (SEQ ID NO: 703). In some embodiments, [B]-[C] is or comprises QSSYPAEVVQK (SEQ ID NO: 150), SLYYPAEVVQK (SEQ ID NO: 639), SATYPAEVVQK (SEQ ID NO: 641), or SLSYPAEVVQK (SEQ ID NO: 642); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [B]-[C] is QSSYPAEVVQK (SEQ ID NO: 150).

[094] In some embodiments, a peptide comprising the formula [B]-[C], further comprises [A], which comprises positions X_A, X_B, and X_C. In some embodiments, position X_A of [A] is T. In some embodiments, position X_B of [A] is N. In some embodiments, position X_C of [A] is N, T, S, or K. In some embodiments, [A] comprises TN, NS, NT, NK, or NN. In some embodiments, [A] is or comprises TNN, TNS, TNT, or TNK. In some embodiments, the amino acid sequence of [A] is TNN. In some embodiments, [A]-[B] is or comprises TNNQSS (SEQ ID NO: 183), TNSSLY (SEQ ID NO: 648), TNTSAT (SEQ ID NO: 649), TNSSLS (SEQ ID NO: 647), or TNKSAT (SEQ ID NO: 651); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [A]-[B] is TNNQSS (SEQ ID NO: 183). In some embodiments, [A]-[B]-[C] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500), TNSSLYYPAEVVQK (SEQ ID NO: 655), TNTSATYPAEVVQK (SEQ ID NO: 656), TNSSLSYPAEVVQK (SEQ ID NO: 657), or TNKSATYPAEVVQK (SEQ ID NO: 663); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [A]-[B] is TNNQSS (SEQ ID NO: 183). In some embodiments, [A]-[B]-[C] is TNNQSSYPAEVVQK (SEQ ID NO: 500).

[095] In some embodiments, a peptide comprising the formula [B]-[C], further comprises [D], wherein [D] comprises position X4 and X5. In some embodiments, position X4 of [D] is T, N, or P. In some embodiments, position X5 of [D] is A or D. In some embodiments [D] is or comprises TA, TD, NA, or PA. In some embodiments, the amino acid sequence of [D] is TA. In some embodiments, [C]-[D] is or comprises YPAEVVQKTA (SEQ ID NO: 584), YPAEVVQKTD (SEQ ID NO: 719), YPAEVVQKNA (SEQ ID NO: 724), or YPAEVVQKPA (SEQ ID NO: 586); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, or 9 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [C]-[D] is YPAEVVQKTA (SEQ ID NO: 584). In some embodiments, [A]-[B]-[C]-[D] is or comprises TNNQSSYPAEVVQKTA

(SEQ ID NO: 1533), TNSSLYYPAEVVQKTA (SEQ ID NO: 2066), TNTSATYPAEVVQKTA (SEQ ID NO: 2067), TNSSLSYPAEVVQKTA (SEQ ID NO: 2068), TNSSLSYPAEVVQKTD (SEQ ID NO: 2070), TNSSLSYPAEVVQKNA (SEQ ID NO: 2072), TNSSLSYPAEVVQKPA (SEQ ID NO: 2073), or TNKSATYPAEVVQKTA (SEQ ID NO: 2075); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, or 15 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [A]-[B]-[C]-[D] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

[096] In some embodiments, [B] is present immediately subsequent to [A]. In some embodiments, [C] is present immediately subsequent to [B]. In some embodiments, [D] is present immediately subsequent to [C]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [B]-[C]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [B]-[C]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [A]-[B]-[C]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [B]-[C]-[D]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [A]-[B]-[C]-[D].

[097] In some embodiments, a peptide described herein comprises the formula [K1]-[K2], wherein, [K1] comprises LSY or LYY, and [K2] comprises positions X1, X2, X3, and X4. In some embodiments, [K1] comprises LSY. In some embodiments, position X1 of [K2] is Q, T or P. In some embodiments, position X2 of [K2] is A, in some embodiments, position X3 of [K2] is E or D. In some embodiments, position X4 of [K2] is V or E. In some embodiments, [K2] comprises QA, TA, PA, EV, EE, DV, AE, or AD. In some embodiments, [K2] comprises QAE, TAE, PAE, PAD, AEV, AEE, or ADV. In some embodiments, [K2] is or comprises QAEV (SEQ ID NO: 15), TAEV (SEQ ID NO: 16), PAEV (SEQ ID NO: 17), PAEE (SEQ ID NO: 18), or PADV (SEQ ID NO: 19). In some embodiments, [K1]-[K2] comprises LSYQA (SEQ ID NO: 597), LSYTA (SEQ ID NO: 598), LYYPA (SEQ ID NO: 600), or LSYPA (SEQ ID NO: 603). In some embodiments, [K1]-[K2] comprises LSYQAE (SEQ ID NO: 607), LSYTAE (SEQ ID NO: 610), LYYPAE (SEQ ID NO: 611), LSYPAE (SEQ ID NO: 616), or LSYPAD (SEQ ID NO: 621). In some embodiments, [K1]-[K2] is or comprises LSYQAEV (SEQ ID NO: 667), LSYTAEV (SEQ ID NO: 668), LYYPAEV (SEQ ID NO: 669), LSYPAEV (SEQ ID NO: 671), LSYPAEE (SEQ ID NO: 673), or LSYPADV (SEQ ID NO: 674); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, or 6 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid

sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences.

[098] In some embodiments, the peptide comprising the amino acid sequence comprising the formula of [K1]-[K2], further comprises [K0], which comprises TNNS (SEQ ID NO: 14). In some embodiments, [K0]-[K1] comprises TNSSLS (SEQ ID NO: 647) or TNSSLY (SEQ ID NO: 648). In some embodiments, [K0]-[K1] is or comprises TNSSLSY (SEQ ID NO: 676) or TNSSLYY (SEQ ID NO: 678); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, or 6 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [K0]-[K1]-[K2] comprises TNSSLSYQA (SEQ ID NO: 679), TNSSLSYTA (SEQ ID NO: 681), TNSSLYYPA (SEQ ID NO: 682), or TNSSLSYPA (SEQ ID NO: 683). In some embodiments, [K0]-[K1]-[K2] comprises TNSSLSYQAE (SEQ ID NO: 684), TNSSLSYTAE (SEQ ID NO: 685), TNSSLYYPAE (SEQ ID NO: 686), TNSSLSYPAE (SEQ ID NO: 687), or TNSSLSYPAD (SEQ ID NO: 689). In some embodiments, [K0]-[K1]-[K2] is or comprises TNSSLSYQAEV (SEQ ID NO: 692), TNSSLSYTAEV (SEQ ID NO: 693), TNSSLYYPAEV (SEQ ID NO: 696), TNSSLSYPAEV (SEQ ID NO: 697), TNSSLSYPAEE (SEQ ID NO: 698), or TNSSLSYPADV (SEQ ID NO: 699); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences.

[099] In some embodiments, peptide comprising the amino acid sequence comprising the formula of [K1]-[K2], further comprises [K3], wherein [K3] comprises positions X_A, X_B, and X_C. In some embodiments, position X_A of [K3] is V. In some embodiments, position X_B of [K3] is Q. In some embodiments, position X_C of [K3] is K or N. In some embodiments [K3] comprises VQ, QK, or QN. In some embodiments, [K3] is or comprises VQK or VQN. In some embodiments, K2]-[K3] is or comprises QAEVVQK (SEQ ID NO: 52), TAEVVQK (SEQ ID NO: 49), PAEVVQK (SEQ ID NO: 20), PAEEVQK (SEQ ID NO: 51) ,PAEVVQN (SEQ ID NO: 594), or PADVVQK (SEQ ID NO: 596); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, or 6 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different

amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [K1]-[K2]-[K3] is or comprises LSYQAEVVQK (SEQ ID NO: 700), LSYTAEVVQK (SEQ ID NO: 701), LYYPAEVVQK (SEQ ID NO: 702), LSYPAEVVQK (SEQ ID NO: 703), LSYPAEEVQK (SEQ ID NO: 704), LSYPAEVVQN (SEQ ID NO: 706), or LSYPADVVQK (SEQ ID NO: 708); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, or 9 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [K0]-[K1]-[K2]-[K3] is or comprises TNSSLSYQAEVVQK (SEQ ID NO: 652), TNSSLSYTAEVVQK (SEQ ID NO: 654), TNSSLYYPAEVVQK (SEQ ID NO: 655), TNSSLSYPAEVVQK (SEQ ID NO: 657), TNSSLSYPAEEVQK (SEQ ID NO: 658), TNSSLSYPAEVVQN (SEQ ID NO: 660), or TNSSLSYPADVVQK (SEQ ID NO: 665); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences.

[0100] In some embodiments, the peptide comprising the amino acid sequence comprising the formula of [K1]-[K2], further comprises [K4], wherein [K4] comprises positions X_D and X_E. In some embodiments, position X_D of [K4] is T, P, or N. In some embodiments, position X_E of [K4] is A or D. In some embodiments, [K4] is or comprises TA, TD, PA, or NA. In some embodiments, [K3]-[K4] is or comprises VQKTA (SEQ ID NO: 564), VQKTD (SEQ ID NO: 714), VQNTA (SEQ ID NO: 715), VQKNA (SEQ ID NO: 570), or VQKPA (SEQ ID NO: 567); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, or 4 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is or comprises TNSSLSYQAEVVQKTA (SEQ ID NO: 2064), TNSSLSYTAEVVQKTA (SEQ ID NO: 2065), TNSSLYYPAEVVQKTA (SEQ ID NO: 2066), TNSSLSYPAEVVQKTA (SEQ ID NO: 2068), TNSSLSYPAEEVQKTA (SEQ ID NO: 2069), TNSSLSYPAEVVQKTD (SEQ ID NO: 2070), TNSSLSYPAEVVQNTA (SEQ ID NO: 2071), TNSSLSYPAEVVQKNA (SEQ ID NO: 2072), TNSSLSYPAEVVQKPA (SEQ ID NO: 2073), or TNSSLSYPADVVQKTA (SEQ ID NO: 2076); an

amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences.

[0101] In some embodiments, [K2] is present immediately subsequent to [K1]. In some embodiments, [K1] is present immediately subsequent to [K0]. In some embodiments, [K3] is present immediately subsequent to [K2]. In some embodiments, [K4] is present immediately subsequent to [K3]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [K1]-[K2]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [K0]-[K1]-[K2]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [K1]-[K2]-[K3]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [K0]-[K1]-[K2]-[K3]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [K1]-[K2]-[K3]-[K4]. In some embodiments, the peptide comprises from N-terminus to C-terminus, [K0]-[K1]-[K2]-[K3]-[K4].

[0102] In some embodiments, a peptide described herein comprises an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, and 15-20. In some embodiments, the peptide comprises an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624. In some embodiments, the peptide comprises an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 943 or 2064-2080. In some embodiments, the peptide comprises at least 3, 4, 5, 6, or 7 consecutive amino acids from any one of SEQ ID NOs: 943 or 946-966.

[0103] In some embodiments, the 3 consecutive amino acids comprise YPA. In some embodiments, the 4 consecutive amino acids comprise YPAE (SEQ ID NO: 21). In some embodiments, the 5 consecutive amino acids comprise YPAEV (SEQ ID NO: 1). In some embodiments, the 6 consecutive amino acids comprise YPAEVV (SEQ ID NO: 725). In some embodiments, the 7 consecutive amino acids comprise YPAEVVQ (SEQ ID NO: 726). In some embodiments, the amino acid sequence comprises YPAEVVQK (SEQ ID NO: 943). In some embodiments, the amino acid sequence consists of YPAEVVQK (SEQ ID NO: 943).

[0104] In some embodiments, a peptide described herein comprises 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 the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, and 15-20. In some embodiments, the peptide

comprises an amino acid sequence comprising 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, and 15-20. In some embodiments, the peptide comprises 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 the amino acid sequence of any one of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624. In some embodiments, the peptide comprises an amino acid sequence comprising 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: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624. In some embodiments, the peptide comprises 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 the amino acid sequence of any one of SEQ ID NOs: 943 or 2064-2080. In some embodiments, the peptide comprises an amino acid sequence comprising 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: 943 or 2064-2080.

[0105] In some embodiments, the peptide comprises 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 the amino acid sequence of YPAEVVQK (SEQ ID NO: 943). In some embodiments, the peptide comprises an amino acid sequence comprising one, two, or three but no more than four different amino acids relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

[0106] In some embodiments, the peptide comprises an amino sequence comprising 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: 2024-2063. In some embodiments, the peptide comprises an amino acid sequence comprising 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: 2024-2063. In some embodiments, the different amino acids of the amino acid sequence comprising 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: 2024-2063, are present at one or more of the following positions: (i) position 1, wherein the different amino acid is T or L; (ii) position 2, wherein the different amino acid is N, L, K, A, T, or P; (iii) position 3, wherein the different amino acid is N, K, L, A, Y, or S; (iv) position 4, wherein the different amino acid is Q, L, T, S, F, Y, K, or A; (v) position 5, wherein the different amino acid is S, H, A, M, Q, T, V, or F; (vi) position 6, wherein the different amino acid is S, P, V, A, Q, L, T, N, or M; (vii) position 7, wherein the different amino acid is Y, H, S, V, A, L, or T; (viii) position 8, wherein the different amino acid is D, P, A, Q, F, L, S, H,

or M; (ix) position 9, wherein the different amino acid is F, A, L, D, or Q; (x) position 10, wherein the different amino acid is T, E, I, or S; (xi) position 11, wherein the different amino acid is V, A, N, or S; (xii) position 12, wherein the different amino acid is V, L, or P; (xiii) position 13, wherein the different amino acid is Q, E, or P; (xiv) position 14, wherein the different amino acid is K, N, S, or L; (xv) position 15, wherein the different amino acid is T, V, M, or L; and/or (xvi) position 16, wherein the different amino acid is A, G, or R.

[0107] In some embodiments, the peptide comprises an amino sequence comprising 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: 1632-2023. In some embodiments, the peptide comprises an amino acid sequence comprising 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: 1632-2023. In some embodiments, the different amino acids of the amino acid sequence comprising 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: 1623-2023, are present at one or more of the following positions: (i) position 1, wherein the different amino acid is T, G, N, S, E, L, Y, V, or I; (ii) position 2, wherein the different amino acid is D, N, K, E, V, G, R, L, H, F, P, T, A, S, I, or Y; (iii) position 3, wherein the different amino acid is Y, N, K, T, W, Q, M, V, C, A, L, F, H, G, R, S, or P; (iv) position 4, wherein the different amino acid is H, Q, P, E, R, K, A, S, V, L, T, D, I, G, M, or N; (v) position 5, wherein the different amino acid is R, S, K, N, H, G, W, A, P, V, Q, Y, L, or F; (vi) position 6, wherein the different amino acid is G, S, F, R, W, H, I, C, M, A, Y, K, N, Q, V, P, E, D, T, or L; (vii) position 7, wherein the different amino acid is D, Y, S, I, H, F, P, K, R, G, L, Q, A, M, T, N, V, W, C, or E; (viii) position 8, wherein the different amino acid is P, L, Q, T, W, V, G, K, I, Y, N, H, R, D, S, M, A, F, or E; (ix) position 9, wherein the different amino acid is A, R, T, Q, S, M, L, E, K, V, G, D, N, H, F, P, or I; (x) position 10, wherein the different amino acid is K, E, Q, H, V, G, R, S, P, I, N, M, A, L, D, or T; (xi) position 11, wherein the different amino acid is V, A, E, N, R, L, M, T, Q, S, K, C, G, D, Y, P, H, F, or I; (xii) position 12, wherein the different amino acid is V, P, L, S, T, N, A, G, K, R, I, H, E, Q, or M; (xiii) position 13, wherein the different amino acid is Q, K, N, A, H, R, T, V, E, I, P, G, S, or L; (xiv) position 14, wherein the different amino acid is K, E, I, Y, Q, R, G, D, L, N, or S; (xv) position 15, wherein the different amino acid is S, T, N, Q, I, P, E, G, K, M, or H; and/or (xvi) position 16, wherein the different amino acid is A, D, L, Y, Q, or T.

[0108] In some embodiments, the peptide comprises the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, and 15-20. In some embodiments, the peptide comprises the amino acid sequence of any of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624. In some embodiments, the peptide comprises the amino acid sequence of any of SEQ ID NOs: 943 or 2064-2080. In some embodiments, the peptide comprises the amino acid sequence of any of SEQ ID NOs: 2024-2063. In some embodiments, the peptide

comprises the amino acid sequence of any of SEQ ID NOs: 1632-2023. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 943.

[0109] 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: 944. 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: 944. In some embodiments, the peptide comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 944, 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.

[0110] 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.

[0111] In some embodiments, the nucleotide sequence encoding a peptide described herein comprises the nucleotide sequence of SEQ ID NO: 944, 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: 944. 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: 944. In some embodiments the nucleic acid encoding a peptide described herein comprises a nucleotide sequence comprising the nucleotide sequence of SEQ ID NO: 944, 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.

[0112] 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.

[0113] 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.

[0114] 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.

[0115] 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.

[0116] 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.

[0117] 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

[0118] In some embodiments, an AAV capsid variant described herein (e.g., an AAV5 capsid variant) comprises an amino acid other than T at position 577 (e.g., Y, N, or C), numbered relative to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises Y at position 577, numbered relative to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises N at position 577, numbered relative to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises C at position 577, numbered relative to SEQ ID NO: 138.

[0119] In some embodiments, an AAV capsid variant described herein (e.g., an AAV5 capsid variant) comprises more than one amino acid that replaces the threonine (T) at position 577, numbered relative to SEQ ID NO: 138. In some embodiments, an insert of two, three, four, five, six, seven, eight, nine, or ten amino acids replaces the T at position 577, numbered relative to SEQ ID NO: 138. In some embodiments an insert of eight amino acids replaces the T at position 577, numbered relative to SEQ ID NO: 138.

[0120] 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 or an amino acid sequence described herein). In some embodiments, an AAV capsid variant comprises a peptide as set forth in any of Tables 1, 2A, 2B, 9, or 15-20.

[0121] In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence having the formula [N2]-[N3], wherein [N2] comprises positions X1, X2, X3, X4, and X5 and [N3] comprises the amino acid sequence of VQK, VQN, EQK, VKK, VHK, VQQ, or LQK. In some embodiments, position X1 of [N2] is Y, N, C, or T. In some embodiments, position X2 of [N2] is P, E, K, T, or Q. In some embodiments, position X3 of [N2] is A or P. In some embodiments, position X4 of [N2] is E, S, D, or A. In some embodiments, position X5 of [N2] is V, L, or E. In some embodiments, [N2] comprises Y at position X1. In some embodiments, [N2] comprises P at position X2. In some embodiments, [N2] comprises A at position X3. In some embodiments, [N2] comprises E at position X4. In some embodiments, [N2] comprises V at position X5. In some embodiments, the amino acid sequence of [N3] comprises VQK. In some embodiments, the amino acid sequence of [N3] is VQK.

[0122] In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence having the formula [N2]-[N3], wherein [N2] comprises positions X1, X2, X3, X4, and X5 and [N3] comprises the amino acid sequence of VQK, EQK, VKK, VHK, VQQ, or LQK. In some embodiments [N3] comprises the amino acid sequence of VQK, EQK, or VKK. In some embodiments [N3] comprises the amino acid sequence VQK. In some embodiments, position X1 of [N2] is Y, N, or C. In some embodiments position X1 of [N2] is Y or N. In some embodiments, position X2 of [N2] is P, K, T, or Q. In some embodiments position X2 of [N2] is P, T, or Q. In some embodiments, position X3 of [N2] is A or P. In some embodiments, position X3 of [N2] is A. In some embodiments, position X4 of [N2] is E, S, or A. In some embodiments, position X5 of [N2] is V, L, or E. In some embodiments, position X5 of [N2] is V or L. In some embodiments, [N2] comprises Y at position X1. In some embodiments, [N2] comprises P at position X2. In some embodiments, [N2] comprises A at position X3. In some embodiments, [N2] comprises E at position X4. In some embodiments, [N2] comprises V at position X5. In some embodiments, [N2] comprises YPA, YPP, NKA, YTA, YQA, YTP, NPA, CPA, THA, PAE, PPS, KAE, TAE, QAE, TPS, PAA, HAS, AEV, PSL, AEE, or AAV. In some embodiments, [N2] comprises YPAE (SEQ ID NO: 21), YPPS (SEQ ID NO: 22), NKAE (SEQ ID NO: 23), YTAE (SEQ ID NO: 24), YQAE (SEQ ID NO: 25), YTPS (SEQ ID NO: 26), YPAA (SEQ ID NO: 27), NPAE (SEQ ID NO: 28), CPAE (SEQ ID NO: 29), THAS (SEQ ID NO: 30), PAEV (SEQ ID NO: 17), PPSL (SEQ ID NO: 31), KAEV (SEQ ID NO: 32), TAEV (SEQ ID NO: 16), PAEE (SEQ ID NO: 18), QAEV (SEQ ID NO: 15), TPSL (SEQ ID NO: 33), PAAV (SEQ ID NO: 34), or QAEE (SEQ ID NO: 35). In some embodiments [N2] is or comprises YPAEV (SEQ ID NO: 1), YPPSL (SEQ ID NO: 2), NKAEV (SEQ ID NO: 3), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), YQAEV (SEQ ID NO: 6), YTPSL (SEQ ID NO: 7), YPAAV (SEQ ID NO: 8), NPAEV (SEQ ID NO: 9), CPAEV (SEQ ID NO: 10), or YQAEE (SEQ ID NO: 11). In some embodiments [N2] is YPAEV (SEQ ID NO: 1). In some embodiments, [N2]-[N3] comprises the amino acid sequence of AEVVQK (SEQ ID NO: 36), PSLVQK (SEQ ID NO: 37), AEVEQK (SEQ ID NO: 38), AEEVQK (SEQ ID NO: 39), PSLEQK (SEQ ID NO: 40),

PSLVKK (SEQ ID NO: 41), AEVVKK (SEQ ID NO: 42), AEVVHK (SEQ ID NO: 43), AAVVQK (SEQ ID NO: 44), AEVVQQ (SEQ ID NO: 45), or AEVLQK (SEQ ID NO: 46). In some embodiments [N2]-[N3] comprises the amino acid sequence PAEVVQK (SEQ ID NO: 20), PPSLVQK (SEQ ID NO: 47), KAEVVQK (SEQ ID NO: 48), TAEVVQK (SEQ ID NO: 49), PAEVEQK (SEQ ID NO: 50), PAEEVQK (SEQ ID NO: 51), QAEVVQK (SEQ ID NO: 52), TPSLVQK (SEQ ID NO: 53), PPSLEQK (SEQ ID NO: 54), PPSLVKK (SEQ ID NO: 55), PAEVVKK (SEQ ID NO: 56), PAEVVHK (SEQ ID NO: 57), PAAVVQK (SEQ ID NO: 58), PAEVVQQ (SEQ ID NO: 59), TAEVVKK (SEQ ID NO: 60), PAEVLQK (SEQ ID NO: 61), or QAEEVQK (SEQ ID NO: 62). In some embodiments [N2]-[N3] is or comprises YPAEVVQK (SEQ ID NO: 943), YPPSLVQK (SEQ ID NO: 946), NKAEVVQK (SEQ ID NO: 947), YTAEVVQK (SEQ ID NO: 948), YPAEVEQK (SEQ ID NO: 949), YPAEEVQK (SEQ ID NO: 950), YQAEVVQK (SEQ ID NO: 951), YTPSLVQK (SEQ ID NO: 952), YPPSLEQK (SEQ ID NO: 953), YPPSLVKK (SEQ ID NO: 954), YPAEVVKK (SEQ ID NO: 955), YPAEVVHK (SEQ ID NO: 956), YPAAVVQK (SEQ ID NO: 957), NPAEVVQK (SEQ ID NO: 958), YPAEVVQQ (SEQ ID NO: 959), CPAEVVQK (SEQ ID NO: 960), YTAEVVKK (SEQ ID NO: 961), YPAEVLQK (SEQ ID NO: 962), or YQAEEVQK (SEQ ID NO: 963); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, or 7 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments [N2]-[N3] is YPAEVVQK (SEQ ID NO: 943).

[0123] In some embodiments, the AAV capsid variant comprising the amino acid sequence comprising the formula of [N2]-[N3], further comprises [N1], which comprises positions X_D, X_E, and X_F. In some embodiments, position X_D of [N1] is Q, T, S, A, I, L, or H. In some embodiments, position X_E of [N1] is S, G, A, or R. In some embodiments, position X_F of [N1] is S, K, L, R, A, or T. In some embodiments, [N1] comprises SK, SL, SS, SR, GA, GS, AS, ST, RS, QS, TS, AG, IG, QA, LG, HS, LS, or QR. In some embodiments, [N1] is or comprises QSS, QSK, TSL, SSS, QSR, AGA, IGS, QAS, ASS, LGS, QST, HSS, LSS, or QRS. In some embodiments, the amino acid sequence of [N1] is QSS. In some embodiments, [N1]-[N2] comprises SSYPA (SEQ ID NO: 63), SKYPA (SEQ ID NO: 64), SLYPA (SEQ ID NO: 65), SRYPA (SEQ ID NO: 66), SSYPP (SEQ ID NO: 67), GAYPA (SEQ ID NO: 68), GSYPA (SEQ ID NO: 69), ASYPA (SEQ ID NO: 70), STNKA (SEQ ID NO: 71), SSYTA (SEQ ID NO: 72), SSYQA (SEQ ID NO: 73), SSYTP (SEQ ID NO: 74), SSNPA (SEQ ID NO: 75), SLCPA (SEQ ID NO: 76), RSYTA (SEQ ID NO: 77), or SSTHA (SEQ ID NO: 78). In some embodiments, [N1]-[N2] comprises SSYPAE (SEQ ID NO: 79), SKYPAE (SEQ ID NO: 80), SLYPAE (SEQ ID NO: 81), SRYPAE (SEQ ID NO: 82), SSYPPS (SEQ ID NO: 83), GAYPAE (SEQ ID NO: 84), GSYPAE (SEQ ID NO: 85), ASYPAE (SEQ ID NO: 86), STNKAE

(SEQ ID NO: 87), SSYTAE (SEQ ID NO: 88), SSYQAE (SEQ ID NO: 89), SSYTPS (SEQ ID NO: 90), SSYPAA (SEQ ID NO: 91), SSNPAE (SEQ ID NO: 92), SLCPAE (SEQ ID NO: 93), RSYTAE (SEQ ID NO: 94), SSTHAS (SEQ ID NO: 95). In some embodiments, [N1]-[N2] is or comprises QSSYPAEV (SEQ ID NO: 96), QSKYPAEV (SEQ ID NO: 97), TSLYPAEV (SEQ ID NO: 98), SSSYPAEV (SEQ ID NO: 99), QSRYPAEV (SEQ ID NO: 100), QSSYPPSL (SEQ ID NO: 101), AGAYPAEV (SEQ ID NO: 102), IGSYPAEV (SEQ ID NO: 103), QASYPAEV (SEQ ID NO: 104), ASSYPAEV (SEQ ID NO: 105), LGSYPAEV (SEQ ID NO: 106), QSTNKAEV (SEQ ID NO: 107), HSSYPAEV (SEQ ID NO: 108), SSSYTAEV (SEQ ID NO: 109), TSLYPAEE (SEQ ID NO: 110), ASSYQAEV (SEQ ID NO: 111), QSSYTPSL (SEQ ID NO: 112), QSRYPAEE (SEQ ID NO: 113), LSSYQAEV (SEQ ID NO: 114), HSSYPAAV (SEQ ID NO: 115), QSSNPAEV (SEQ ID NO: 116), QSSYTAEV (SEQ ID NO: 117), TSLCPAEV (SEQ ID NO: 118), QRSYTAEV (SEQ ID NO: 119), or QSSYQAEE (SEQ ID NO: 120); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, or 7 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N1]-[N2] is QSSYPAEV (SEQ ID NO: 96). In some embodiments, [N1]-[N2]-[N3] comprises SSYPAEVVQ (SEQ ID NO: 121), SKYPAEVVQ (SEQ ID NO: 122), SLYPAEVVQ (SEQ ID NO: 123), SRYPAEVVQ (SEQ ID NO: 124), SSYPPSLVQ (SEQ ID NO: 125), GAYPAEVVQ (SEQ ID NO: 126), GSYPAEVVQ (SEQ ID NO: 127), ASYPAEVVQ (SEQ ID NO: 128), STNKAEVVQ (SEQ ID NO: 129), SSYTAEVVQ (SEQ ID NO: 130), SKYPAEVEQ (SEQ ID NO: 131), SLYPAEEVQ (SEQ ID NO: 132), SSYQAEVVQ (SEQ ID NO: 133), SSYTPSLVQ (SEQ ID NO: 134), SRYPAEEVQ (SEQ ID NO: 135), SSYPPSLEQ (SEQ ID NO: 136), SSYPPSLVK (SEQ ID NO: 140), SSYPAEVVK (SEQ ID NO: 141), SKYPAEVVH (SEQ ID NO: 142), SSYPAAVVQ (SEQ ID NO: 143), SSNPAEVVQ (SEQ ID NO: 144), SLCPAEVVQ (SEQ ID NO: 145), RSYTAEVVQ (SEQ ID NO: 146), SSYTAEVVK (SEQ ID NO: 147), SSYPAEVLQ (SEQ ID NO: 148), or SSYQAEEVQ (SEQ ID NO: 149). In some embodiments, [N1]-[N2]-[N3] is or comprises QSSYPAEVVQK (SEQ ID NO: 150), QSKYPAEVVQK (SEQ ID NO: 151), TSLYPAEVVQK (SEQ ID NO: 152), SSSYPAEVVQK (SEQ ID NO: 153), QSRYPAEVVQK (SEQ ID NO: 154), QSSYPPSLVQK (SEQ ID NO: 155), AGAYPAEVVQK (SEQ ID NO: 156), IGSYPAEVVQK (SEQ ID NO: 157), QASYPAEVVQK (SEQ ID NO: 158), ASSYPAEVVQK (SEQ ID NO: 159), LGSYPAEVVQK (SEQ ID NO: 160), QSTNKAEVVQK (SEQ ID NO: 161), HSSYPAEVVQK (SEQ ID NO: 162), SSSYTAEVVQK (SEQ ID NO: 163), QSKYPAEVEQK (SEQ ID NO: 164), TSLYPAEEVQK (SEQ ID NO: 165), ASSYQAEVVQK (SEQ ID NO: 166), QSSYTPSLVQK (SEQ ID NO: 167), QSRYPAEEVQK (SEQ ID NO: 168), QSSYPPSLEQK (SEQ ID NO: 169), QSSYPPSLVKK (SEQ ID NO: 170), LSSYQAEVVQK (SEQ

ID NO: 171), SSSYPAEVVKK (SEQ ID NO: 172), QSKYPAEVVHK (SEQ ID NO: 173), HSSYPAAVVQK (SEQ ID NO: 174), QSSNPAEVVQK (SEQ ID NO: 175), SSSYPAEVVQQ (SEQ ID NO: 176), QSSYTAEVVQK (SEQ ID NO: 177), TSLCPAEVVQK (SEQ ID NO: 178), QRSYTAEVVQK (SEQ ID NO: 179), QSSYTAEVVKK (SEQ ID NO: 180), HSSYPAEVLQK (SEQ ID NO: 181), or QSSYQAEEVQK (SEQ ID NO: 182); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N1]-[N2]-[N3] is QSSYPAEVVQK (SEQ ID NO: 150).

[0124] In some embodiments, the AAV capsid variant comprising the amino acid sequence comprising the formula [N2]-[N3], further comprises [N0], wherein [N0] comprises positions X_A, X_B, and X_C. In some embodiments, position X_A of [N0] is T, I, or N. In some embodiments, positions X_B of [N0] is N. In some embodiments, position X_C of [N0] is N, T, S, or K. In some embodiments, [N0] comprises TN, IN, NN, NT, NS, or NK. In some embodiments, [N0] is or comprises TNN, TNT, INN, TNS, NNN, or TNK. In some embodiments, [N0] is TNN. In some embodiments, [N0]-[N1] is or comprises TNNQSS (SEQ ID NO: 183), TNNQSK (SEQ ID NO: 184), TNNTSL (SEQ ID NO: 185), TNNSSS (SEQ ID NO: 186), TNNQSR (SEQ ID NO: 187), TNNAGA (SEQ ID NO: 188), TNNIGS (SEQ ID NO: 189), TNNQAS (SEQ ID NO: 190), TNTASS (SEQ ID NO: 191), TNNLGS (SEQ ID NO: 192), TNNQST (SEQ ID NO: 193), TNNHSS (SEQ ID NO: 194), TNNQSK (SEQ ID NO: 184), TNNLSS (SEQ ID NO: 195), INNQSS (SEQ ID NO: 196), TNSQSS (SEQ ID NO: 197), NNNQSR (SEQ ID NO: 198), TNSTSL (SEQ ID NO: 199), TNNQRS (SEQ ID NO: 200), or TNKQAS (SEQ ID NO: 201); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1] is TNNQSS (SEQ ID NO: 183). In some embodiments, [N0]-[N1]-[N2]-[N3] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500), TNNQSKYPAEVVQK (SEQ ID NO: 503), TNNTSLYPAEVVQK (SEQ ID NO: 506), TNNSSSYPAEVVQK (SEQ ID NO: 508), TNNQSRYPAEVVQK (SEQ ID NO: 510), TNNQSSYPPSLVQK (SEQ ID NO: 512), TNNAGAYPAEVVQK (SEQ ID NO: 513), TNNIGSYPAEVVQK (SEQ ID NO: 514), TNNQASYPAEVVQK (SEQ ID NO: 517), TNTASSYPAEVVQK (SEQ ID NO: 520), TNNLGSYPAEVVQK (SEQ ID NO: 523), TNNQSTNKAEVVQK (SEQ ID NO: 524),

TNNHSSYPAEVVQK (SEQ ID NO: 525), TNNSSSYTAEVVQK (SEQ ID NO: 526), TNNQSKYPAEVEQK (SEQ ID NO: 529), TNNTSLYPAEEVQK (SEQ ID NO: 530), TNTASSYQAEVVQK (SEQ ID NO: 531), TNNQSSYTPSLVQK (SEQ ID NO: 533), TNNQSRYPAEEVQK (SEQ ID NO: 534), TNNQSSYPPSLEQK (SEQ ID NO: 535), TNNQSSYPPSLVKK (SEQ ID NO: 536), TNNLSSYQAEVVQK (SEQ ID NO: 539), TNNSSSYPAEVVKK (SEQ ID NO: 540), TNNQSKYPAEVVHK (SEQ ID NO: 542), INNQSSYPAEVVQK (SEQ ID NO: 543), TNNHSSYPAAVVQK (SEQ ID NO: 545), TNSQSSNPAEVVQK (SEQ ID NO: 548), TNNSSSYPAEVVQQ (SEQ ID NO: 551), NNNQSRYPAEVVQK (SEQ ID NO: 552), TNNQSSYTAEVVQK (SEQ ID NO: 553), TNNTSLCPAEVVQK (SEQ ID NO: 554), TNSTSLYPAEVVQK (SEQ ID NO: 556), TNNQRSYTAEVVQK (SEQ ID NO: 557), TNNQSSYTAEVVKK (SEQ ID NO: 558), TNNHSSYPAEVLQK (SEQ ID NO: 560), TNNQSSYQAEEVQK (SEQ ID NO: 562) or TNKQASYPAEVVQK (SEQ ID NO: 563); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1]-[N2]-[N3] is TNNQSSYPAEVVQK (SEQ ID NO: 500).

[0125] In some embodiments, the AAV capsid variant comprising the amino acid sequence comprising the formula [N2]-[N3], further comprises [N4], which comprises positions X_G and X_H. In some embodiments, position X_G of [N4] is T, P, or N. In some embodiments, position X_H of [N4] is A. In some embodiments, [N4] is or comprises TA, PA, or NA. In some embodiments, [N4] is TA. In some embodiments, [N3]-[N4] is or comprises VQKTA (SEQ ID NO: 564), EQKTA (SEQ ID NO: 565), VKKTA (SEQ ID NO: 566), VQKPA (SEQ ID NO: 567), VHKTA (SEQ ID NO: 568), VQQTA (SEQ ID NO: 569), VQKNA (SEQ ID NO: 570), or LQKTA (SEQ ID NO: 571); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, or 4 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N3]-[N4] is VQKTA (SEQ ID NO: 564). In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNTSLYPAEVVQKTA (SEQ ID NO: 1232), TNNSSSYPAEVVQKTA (SEQ ID NO: 1539), TNNQSRYPAEVVQKTA (SEQ ID NO: 1327), TNNQSSYPPSLVQKTA (SEQ ID NO: 1300),

TNNAGAYPAEVVQKTA (SEQ ID NO: 1021), TNNIGSYPAEVVQKTA (SEQ ID NO: 1112), TNNQASYPAEVVQKTA (SEQ ID NO: 1194), TNTASSYPAEVVQKTA (SEQ ID NO: 1575), TNNLGSYPAEVVQKTA (SEQ ID NO: 1027), TNNQSTNKAEVVQKTA (SEQ ID NO: 1578), TNNHSSYPAEVVQKTA (SEQ ID NO: 1310), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNSSSYTAEVVQKTA (SEQ ID NO: 1214), TNNQSKYPAEVEQKTA (SEQ ID NO: 1254), TNNTSLYPAEEVQKTA (SEQ ID NO: 1583), TNTASSYQAEVVQKTA (SEQ ID NO: 1584), TNNQSSYTPSLVQKTA (SEQ ID NO: 1585), TNNQSRYPAEEVQKTA (SEQ ID NO: 1342), TNNQSSYPPSLEQKTA (SEQ ID NO: 1590), TNNQSSYPPSLVKKTA (SEQ ID NO: 1591), TNNLSSYQAEVVQKTA (SEQ ID NO: 1592), TNNQSSYPPSLVQKPA (SEQ ID NO: 1593), TNNSSSYPAEVVKKTA (SEQ ID NO: 1331), TNNQSKYPAEVVHKTA (SEQ ID NO: 1453), TNNSSSYPAEVVQKPA (SEQ ID NO: 1142), INNQSSYPAEVVQKTA (SEQ ID NO: 1024), TNNHSSYPAAVVQKTA (SEQ ID NO: 1598), TNSQSSNPAEVVQKTA (SEQ ID NO: 1599), TNNSSSYPAEVVQQTA (SEQ ID NO: 1419), NNNQSRYPAEVVQKTA (SEQ ID NO: 1601), TNNQSSYTAEVVQKNA (SEQ ID NO: 1602), TNNTSLCPAEVVQKTA (SEQ ID NO: 1603), TNSTSLYPAEVVQKTA (SEQ ID NO: 1605), TNNQRSYTAEVVQKTA (SEQ ID NO: 1604), TNNQSSYTAEVVKKTA (SEQ ID NO: 1606), TNNHSSYPAEVLQKTA (SEQ ID NO: 1607), TNNQSSYQAEEVQKTA (SEQ ID NO: 1608), or TNKQASYPAEVVQKTA (SEQ ID NO: 1587); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

[0126] In some embodiments, the AAV capsid variant described herein comprises the formula [N2]-[N3], wherein [N2] comprises positions X1, X2, X3, X4, and X5 and [N3] comprises the amino acid sequence of VQK or VQN. In some embodiments, [N3] comprises the amino acid sequence VQK. In some embodiments, [N3] is the amino acid sequence VQK. In some embodiments, position X1 of [N2] is Y or T. In some embodiments, position X2 of [N2] is Q, T, P, or E. In some embodiments, position X3 of [N2] is A. In some embodiments, position X4 of [N2] is E or D. In some embodiments, position X4 of [N2] is E or D. In some embodiments, position X5 of [N2] is V or E. In some embodiments, position X2 of [N2] is P, E, K, T, or Q. In some embodiments, position X3 of [N2] is A or P. In some embodiments, position X4 of [N2] is E, S, D, or A. In some embodiments, position X5 of [N2] is V, L, or E. In some embodiments, [N2] comprises Y at position X1. In some embodiments, [N2] comprises P at position X2. In some embodiments, [N2] comprises A at position X3. In some embodiments, [N2] comprises E at position X4. In some embodiments, [N2] comprises V at position X5. In some embodiments, [N2] comprises YP, YQ, YT, TE, QA, TA, PA, EA, EV, EE,

DV, AE, or AD. In some embodiments, [N2] comprises YPA, YQA, YTA, TEA, QAE, TAE, PAE, EAE, PAD, AEV, AEE, or ADV. In some embodiments, [N2] comprises YPAE (SEQ ID NO: 21), YQAE (SEQ ID NO: 25), YTAE (SEQ ID NO: 24), TEAE (SEQ ID NO: 587), YPAD (SEQ ID NO: 588), QAEV (SEQ ID NO: 15), TAEV (SEQ ID NO: 16), PAEV (SEQ ID NO: 17), PAEE (SEQ ID NO: 18), EAEV (SEQ ID NO: 590), or PADV (SEQ ID NO: 19). In some embodiments, [N2] is or comprises YPAEV (SEQ ID NO: 1), YQAEV (SEQ ID NO: 6), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), TEAEV (SEQ ID NO: 12), or YPADV (SEQ ID NO: 13). In some embodiments, [N2] is YPAEV (SEQ ID NO: 1). In some embodiments, [N2]-[N3] comprises AEVVQK (SEQ ID NO: 36), AEEVQK (SEQ ID NO: 39), AEVVQN (SEQ ID NO: 591), or ADVVQK (SEQ ID NO: 593). In some embodiments, [N2]-[N3] comprises PAEVVQN (SEQ ID NO: 594), QAEVVQK (SEQ ID NO: 52), TAEVVQK (SEQ ID NO: 49), PAEVVQK (SEQ ID NO: 20), PAEEVQK (SEQ ID NO: 51), EAEVVQK (SEQ ID NO: 595), or PADVVQK (SEQ ID NO: 596). In some embodiments, [N2]-[N3] is or comprises YPAEVVQK (SEQ ID NO: 943), YQAEVVQK (SEQ ID NO: 951), YTAEVVQK (SEQ ID NO: 948), YPAEEVQK (SEQ ID NO: 950), YPAEVVQN (SEQ ID NO: 964), TEAEVVQK (SEQ ID NO: 965), or YPADVVQK (SEQ ID NO: 966); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, or 7 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N2]-[N3] is YPAEVVQK (SEQ ID NO: 943).

[0127] In some embodiments, the AAV capsid variant comprising the amino acid sequence comprising the formula of [N2]-[N3], further comprises [N1], which comprises positions X_D, X_E, and X_F. In some embodiments, position X_D of [N1] is Q or S. In some embodiments, position X_E of [N1] is S, L, A, or T. In some embodiments, position X_F of [N1] is S, Y, or T. In some embodiments, [N1] comprises QS, SL, SA, QT, LS, LY, AT, TS, or SS. In some embodiments, [N1] is or comprises QSS, SLS, SLY, SAT, or QTS. In some embodiments, [N1] is QSS. In some embodiments, [N1]-[N2] comprises SSYPA (SEQ ID NO: 63), LSYQA (SEQ ID NO: 597), LSYTA (SEQ ID NO: 598), LYYPA (SEQ ID NO: 600), ATYPA (SEQ ID NO: 601), LSYPA (SEQ ID NO: 603), or TSTEA (SEQ ID NO: 605). In some embodiments, [N1]-[N2] comprises SSYPAE (SEQ ID NO: 79), LSYQAE (SEQ ID NO: 607), LSYTAE (SEQ ID NO: 610), LYYPAE (SEQ ID NO: 611), ATYPAE (SEQ ID NO: 613), LSYPAE (SEQ ID NO: 616), TSTEAE (SEQ ID NO: 619), or LSYPAD (SEQ ID NO: 621). In some embodiments, [N1]-[N2] is or comprises QSSYPAEV (SEQ ID NO: 96), SLSYQAEV (SEQ ID NO: 622), SLSYTAEV (SEQ ID NO: 623), SLYYPAEV (SEQ ID NO: 624), SATYPAEV (SEQ ID NO: 625), SLSYPAEV (SEQ ID NO: 629), SLSYPAEE (SEQ ID NO: 632), QTSTEAEV (SEQ ID NO: 633), or SLSYPADV (SEQ ID NO: 634); an amino acid sequence

comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, or 7 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N1]-[N2] is QSSYPAEV (SEQ ID NO: 96). In some embodiments, [N1]-[N2]-[N3] is or comprises QSSYPAEVVQK (SEQ ID NO: 150), SLSYQAEVVQK (SEQ ID NO: 635), SLSYTAEVVQK (SEQ ID NO: 637), SLYYPAEVVQK (SEQ ID NO: 639), SATYPAEVVQK (SEQ ID NO: 641), SLSYPAEVVQK (SEQ ID NO: 642), SLSYPAEEVQK (SEQ ID NO: 643), SLSYPAEVVQN (SEQ ID NO: 644), QTSTEAEVVQK (SEQ ID NO: 645), or SLSYPADVVQK (SEQ ID NO: 646); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N1]-[N2]-[N3] is QSSYPAEVVQK (SEQ ID NO: 150).

[0128] In some embodiments, the AAV capsid variant comprising the amino acid sequence comprising the formula [N2]-[N3], further comprises [N0], wherein [N0] comprises positions X_A, X_B, and X_C. In some embodiments, position X_A of [N0] is T. In some embodiments, positions X_B of [N0] is N. In some embodiments, position X_C of [N0] is N, T, S, or K. In some embodiments [N0] comprises TN, NS, NT, NN, or NK. In some embodiments, [N0] is or comprises TNS, TNT, TNN, or TNK. In some embodiments, [N0] is TNN. In some embodiments, [N0]-[N1] is or comprises TNNQSS (SEQ ID NO: 183), TNSSLS (SEQ ID NO: 647), TNSSLY (SEQ ID NO: 648), TNTSAT (SEQ ID NO: 649), TNNQTS (SEQ ID NO: 650), or TNKSAT (SEQ ID NO: 651); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1] is TNNQSS (SEQ ID NO: 183). In some embodiments, [N0]-[N1]-[N2]-[N3] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500), TNSSLSYQAEVVQK (SEQ ID NO: 652), TNSSLSYTAEVVQK (SEQ ID NO: 654), TNSSLYYPAEVVQK (SEQ ID NO: 655), TNTSATYPAEVVQK (SEQ ID NO: 656), TNSSLSYPAEVVQK (SEQ ID NO: 657), TNSSLSYPAEEVQK (SEQ ID NO: 658), TNSSLSYPAEVVQN (SEQ ID NO: 660), TNNQTSTEAEVVQK (SEQ ID NO: 662),

TNKSATYPAEVVQK (SEQ ID NO: 663), or TNSSLSYPADVVQK (SEQ ID NO: 665); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1]-[N2]-[N3] is TNNQSSYPAEVVQK (SEQ ID NO: 500).

[0129] In some embodiments, the AAV capsid variant comprising the amino acid sequence comprising the formula [N2]-[N3], further comprises [N4], which comprises positions X_G and X_H. In some embodiments, position X_G of [N4] is T, P, or N. In some embodiments, position X_H of [N4] is A or D. In some embodiments, [N4] is or comprises TA, TD, PA, or NA. In some embodiments, [N4] is TA. In some embodiments, [N3]-[N4] is or comprises VQKTA (SEQ ID NO: 564), EQKTA (SEQ ID NO: 565), VKKTA (SEQ ID NO: 566), VQKPA (SEQ ID NO: 567), VHKTA (SEQ ID NO: 568), VQQTA (SEQ ID NO: 569), VQKNA (SEQ ID NO: 570), or LQKTA (SEQ ID NO: 571); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, or 4 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or 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 aforesaid amino acid sequences. In some embodiments, [N3]-[N4] is VQKTA (SEQ ID NO: 564). In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNSSLSYQAEVVQKTA (SEQ ID NO: 2064), TNSSLSYTAEVVQKTA (SEQ ID NO: 2065), TNSSLYYPAEVVQKTA (SEQ ID NO: 2066), TNTSATYPAEVVQKTA (SEQ ID NO: 2067), TNSSLSYPAEVVQKTA (SEQ ID NO: 2068), TNSSLSYPAEEVQKTA (SEQ ID NO: 2069), TNSSLSYPAEVVQKTD (SEQ ID NO: 2070), TNSSLSYPAEVVQNTA (SEQ ID NO: 2071), TNSSLSYPAEVVQKNA (SEQ ID NO: 2072), TNSSLSYPAEVVQKPA (SEQ ID NO: 2073), TNNQTSTEAEVVQKTA (SEQ ID NO: 2074), TNKSATYPAEVVQKTA (SEQ ID NO: 2075), or TNSSLSYPADVVQKTA (SEQ ID NO: 2076); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is TNNQSSYPAEVVQKT (SEQ ID NO: 737).

[0130] In some embodiments, [N2]-[N3] is present in loop VIII of the AAV capsid variant. In some embodiments [N0], [N1], and/or [N4] are present in loop VIII of the AAV capsid variant. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present in loop VIII of the AAV capsid variant. In some embodiments, loop VIII comprises positions 571-592 numbered according to SEQ ID NO: 138, or positions 571-599, numbered according to SEQ ID NO: 982.

[0131] In some embodiments, [N0] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In some embodiments, [N0] replaces positions 571-573 (e.g., T571, N572, and N573), numbered relative to SEQ ID NO: 138. In some embodiments, [N0] is present immediately subsequent to position 570, and [N0] replaces positions 571-573 (e.g., amino acids T571, N572, and N573), numbered relative to SEQ ID NO: 138. In some embodiments, [N1] is present immediately subsequent to position 573, numbered relative to SEQ ID NO: 138. In some embodiments, [N1] replaces positions 574-576 (e.g., Q574, S575, and S576), numbered relative to SEQ ID NO: 138. In some embodiments, [N1] is present immediately subsequent to position 573, and [N1] replaces positions 574-576 (e.g., Q574, S575, and S576), numbered relative to SEQ ID NO: 138. In some embodiments, [N2] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138. In some embodiments, [N2] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In some embodiments, [N2] is present immediately subsequent to position 576, and [N2] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In some embodiments, [N2]-[N3] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138. In some embodiments, [N2]-[N3] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In some embodiments, [N2]-[N3] is present immediately subsequent to position 576, and [N2]-[N3] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In some embodiments, [N2]-[N3]-[N4] is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138. In some embodiments, [N2]-[N3]-[N4] replaces positions 577-579 (e.g., T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments, [N2]-[N3]-[N4] is present immediately subsequent to position 576, and [N2]-[N3]-[N4] replaces positions 577-579 (e.g., T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments, [N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 573, numbered relative to SEQ ID NO: 138. In some embodiments, [N1]-[N2]-[N3]-[N4] replaces positions 574-579 (e.g., Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments, [N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 573, and [N1]-[N2]-[N3]-[N4] replaces positions 574-579 (e.g., Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 570. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 570, and [N0]-[N1]-[N2]-[N3]-[N4] replaces positions 571-579

(e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138, wherein [N2]-[N3] replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138.

[0132] In some embodiments, the AAV capsid variant comprises an amino acid other than T at position 577, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises Y at position 577, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, X1 of [N2] is present at position 577 (e.g., T577), and positions X2 and X3 of [N2] are present immediately subsequent to position 577, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [N3] is present immediately subsequent to [N2].

[0133] In some embodiments, X_A of [N0] is present at position 571, X_B of [N0] is present at position 572, and X_C of [N0] is present at position 573, numbered according to SEQ ID NO: 982. In some embodiments, X_D of [N1] is present at position 574, X_E of [N1] is present at position 575, and X_F of [N1] is present at position 576, numbered according to SEQ ID NO: 982. In some embodiments, X1 of [N2] is present at position 577, X2 of [N2] is present at position 578, X3 of [N2] is present at position 579, X4 of [N2] is present at position 580, and of X5 of [N2] is present at position 581, numbered according to SEQ ID NO: 982. In some embodiments, [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982. In some embodiments, X_G of [N4] is present at position 585 and X_H of [N4] is present at position 586, numbered according to SEQ ID NO: 982.

[0134] In some embodiments, [N0] is present at positions 571-573, numbered according to SEQ ID NO: 982. In some embodiments, [N1] is present at positions 574-576, numbered according to SEQ ID NO: 982. In some embodiments, [N2] is present at positions 577-581, numbered according to SEQ ID NO: 982. In some embodiments, [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982. In some embodiments, [N4] is present at positions 585-586, numbered according to SEQ ID NO: 982. In some embodiments, [N2]-[N3] is present at positions 577-584, numbered according to SEQ ID NO: 982. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is present at positions 571-586, numbered according to SEQ ID NO: 982.

[0135] In some embodiments, [N1] is present immediately subsequent to [N0]. In some embodiments, [N2] is present immediately subsequent to [N1]. In some embodiments, [N3] is present immediately subsequent to [N2]. In some embodiments, [N4] is present immediately subsequent to [N3].

[0136] In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [N2]-[N3]. 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]. In some embodiments, the AAV capsid variant comprises from N-

terminus to C-terminus, [N0]-[N1]-[N2]-[N3]. In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [N0]-[N1]-[N2]-[N3]-[N4].

[0137] In some embodiments, [N2]-[N3] is YPAEVVQK (SEQ ID NO: 943), wherein YPAEVVQK (SEQ ID NO: 943) replaces position 577, numbered relative to SEQ ID NO: 138. In some embodiments, [N0]-[N1]-[N2]-[N3]-[N4] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533) and is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138, wherein [N2]-[N3] (YPAEVVQK (SEQ ID NO: 943)) replaces position 577 (e.g., replaces T577) numbered relative to SEQ ID NO: 138. In some embodiments, [N2]-[N3] is YPAEVVQK, wherein [N2]-[N3] is present at positions 577-584, numbered according to SEQ ID NO: 982.

[0138] In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence having the formula [B]-[C], wherein [B] comprises positions X1, X2, and X3, and [C] comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943). In some embodiments, position X1 of [B] is Q, T, S, A, I, L, or H. In some embodiments, position X1 of [B] is Q, T, S, A, or H. In some embodiments, position X2 of [B] is S, G, or A. In some embodiments, position X2 of [B] is S or G. In some embodiments, position X3 of [B] is S, K, L, R, or A. In some embodiments, position X3 of [B] is S, K, L, or R. In some embodiments, [B] comprises Q at position X1. In some embodiments, [B] comprises S at position X2. In some embodiments, [B] comprises S at position X3. In some embodiments, [B] comprises QS, TS, SS, AG, IG, QA, AS, LG, HS, SK, SL, SR, GA, or GS. In some embodiments, [B] is or comprises QSS, TSL, SSS, QSR, QSK, AGA, IGS, QAS, ASS, LGS, or HSS. In some embodiments, [B] is QSS. In some embodiments, [B]-[C] comprises SSYPAEVVQK (SEQ ID NO: 572), SKYPAEVVQK (SEQ ID NO: 573), SLYPAEVVQK (SEQ ID NO: 574), SRYPAEVVQK (SEQ ID NO: 575), GAYPAEVVQK (SEQ ID NO: 576), GSYPAEVVQK (SEQ ID NO: 580), or ASYPAEVVQK (SEQ ID NO: 582). In some embodiments, [B]-[C] is or comprises QSSYPAEVVQK (SEQ ID NO: 150), QSKYPAEVVQK (SEQ ID NO: 151), TSLYPAEVVQK (SEQ ID NO: 152), SSSYPAEVVQK (SEQ ID NO: 153), QSRYPAEVVQK (SEQ ID NO: 154), AGAYPAEVVQK (SEQ ID NO: 156), IGSYPAEVVQK (SEQ ID NO: 157), QASYPAEVVQK (SEQ ID NO: 158), ASSYPAEVVQK (SEQ ID NO: 159), LGSYPAEVVQK (SEQ ID NO: 160), or HSSYPAEVVQK (SEQ ID NO: 162); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [B]-[C] is QSSYPAEVVQK (SEQ ID NO: 150).

[0139] In some embodiments, an AAV capsid variant comprising the formula [B]-[C], further comprises [A], which comprises positions X_A, X_B, and X_C. In some embodiments, position X_A of [A] is T, I, or N. In some embodiments, position X_B of [A] is N. In some embodiments, position X_C of [A] is N, T, S, or K. In some embodiments, [A] comprises TN, IN, NN, NT, NS, or NK. In some embodiments, [A] is or comprises TNN, TNT, INN, NNN, TNS, or TNK. In some embodiments, [A] is TNN. In some embodiments, [A]-[B] is or comprises TNNQSS (SEQ ID NO: 183), TNNQSK (SEQ ID NO: 184), TNNTSL (SEQ ID NO: 185), TNNSSS (SEQ ID NO: 186), TNNQSR (SEQ ID NO: 187), TNNAGA (SEQ ID NO: 188), TNNIGS (SEQ ID NO: 189), TNNQAS (SEQ ID NO: 190), TNTASS (SEQ ID NO: 191), TNNLGS (SEQ ID NO: 192), TNNHSS (SEQ ID NO: 194), INNQSS (SEQ ID NO: 196), NNNQSR (SEQ ID NO: 198), TNSTSL (SEQ ID NO: 199), or TNKQAS (SEQ ID NO: 201); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [A]-[B] is TNNQSS (SEQ ID NO: 183). In some embodiments, [A]-[B]-[C] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500), TNNQSKYPAEVVQK (SEQ ID NO: 503), TNNTSLYPAEVVQK (SEQ ID NO: 506), TNNSSSYPAEVVQK (SEQ ID NO: 508), TNNQSRYPAEVVQK (SEQ ID NO: 510), TNNAGAYPAEVVQK (SEQ ID NO: 513), TNNIGSYPAEVVQK (SEQ ID NO: 514), TNNQASYPAEVVQK (SEQ ID NO: 517), TNTASSYPAEVVQK (SEQ ID NO: 520), TNNLGSYPAEVVQK (SEQ ID NO: 523), TNNHSSYPAEVVQK (SEQ ID NO: 525), INNQSSYPAEVVQK (SEQ ID NO: 543), NNNQSRYPAEVVQK (SEQ ID NO: 552), TNSTSLYPAEVVQK (SEQ ID NO: 556), or TNKQASYPAEVVQK (SEQ ID NO: 563); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [A]-[B]-[C] is TNNQSSYPAEVVQK (SEQ ID NO: 500).

[0140] In some embodiments, an AAV capsid variant comprising the formula [B]-[C], further comprises [D], wherein [D] comprises position X4 and X5. In some embodiments, position X4 of [D] is T or N. In some embodiments, position X5 of [D] is A. In some embodiments [D] is or comprises TA or PA. In some embodiments, [D] is TA. In some embodiments, [C]-[D] is or comprises YPAEVVQKTA (SEQ ID NO: 584) or YPAEVVQKPA (SEQ ID NO: 586); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, or 9

amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [C]-[D] is YPAEVVQKTA (SEQ ID NO: 584). In some embodiments, [A]-[B]-[C]-[D] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNTSLYPAEVVQKTA (SEQ ID NO: 1232), TNNSSSYPAEVVQKTA (SEQ ID NO: 1539), TNNQSRYPAEVVQKTA (SEQ ID NO: 1327), TNNAGAYPAEVVQKTA (SEQ ID NO: 1021), TNNIGSYPAEVVQKTA (SEQ ID NO: 1112), TNNQASYPAEVVQKTA (SEQ ID NO: 1194), TNTASSYPAEVVQKTA (SEQ ID NO: 1575), TNNLGSYPAEVVQKTA (SEQ ID NO: 1027), TNNHSSYPAEVVQKTA (SEQ ID NO: 1310), TNNSSSYPAEVVQKPA (SEQ ID NO: 1142), INNQSSYPAEVVQKTA (SEQ ID NO: 1024), NNNQSRYPAEVVQKTA (SEQ ID NO: 1601), TNSTSLYPAEVVQKTA (SEQ ID NO: 1605), or TNKQASYPAEVVQKTA (SEQ ID NO: 1587); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, or 15 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [A]-[B]-[C]-[D] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

[0141] In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence having the formula [B]-[C], wherein [B] comprises positions X1, X2, and X3, and [C] comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943). In some embodiments, position X1 of [B] is Q or S. In some embodiments, position X2 of [B] is S, L, or A. In some embodiments, position X3 of [B] is S, Y, or T. In some embodiments, [B] comprises Q at position X1. In some embodiments, [B] comprises S at position X2. In some embodiments, [B] comprises S at position X3. In some embodiments, [B] comprises QS, SL, SA, LY, AT, LS, or SS. In some embodiments, [B] is or comprises QSS, SLY, SAT, or SLS. In some embodiments, [B] is QSS. In some embodiments, [B]-[C] comprises SSYPAEVVQK (SEQ ID NO: 572), LYYPAEVVQK (SEQ ID NO: 702), ATYPAEVVQK (SEQ ID NO: 718), or LSYPAEVVQK (SEQ ID NO: 703). In some [B]-[C] is or comprises QSSYPAEVVQK (SEQ ID NO: 150), SLYYPAEVVQK (SEQ ID NO: 639), SATYPAEVVQK (SEQ ID NO: 641), or SLSYPAEVVQK (SEQ ID NO: 642); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an

amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some [B]-[C] is QSSYPAEVVQK (SEQ ID NO: 150).

[0142] In some embodiments, an AAV capsid variant comprising the formula [B]-[C], further comprises [A], which comprises positions X_A, X_B, and X_C. In some embodiments, position X_A of [A] is T. In some embodiments, position X_B of [A] is N. In some embodiments, position X_C of [A] is N, T, S, or K. In some embodiments, [A] comprises TN, NS, NT, NK, or NN. In some embodiments, [A] is or comprises TNN, TNS, TNT, or TNK. In some embodiments, [A] is TNN. In some embodiments, [A]-[B] is or comprises TNNQSS (SEQ ID NO: 183), TNSSLY (SEQ ID NO: 648), TNTSAT (SEQ ID NO: 649), TNSSLS (SEQ ID NO: 647), or TNKSAT (SEQ ID NO: 651); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, or 5 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [A]-[B] is TNNQSS (SEQ ID NO: 183). In some embodiments, [A]-[B]-[C] is or comprises TNNQSSYPAEVVQK (SEQ ID NO: 500), TNSSLYYPAEVVQK (SEQ ID NO: 655), TNTSATYPAEVVQK (SEQ ID NO: 656), TNSSLSYPAEVVQK (SEQ ID NO: 657), or TNKSATYPAEVVQK (SEQ ID NO: 663); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [A]-[B]-[C] is TNNQSSYPAEVVQK (SEQ ID NO: 500).

[0143] In some embodiments, an AAV capsid variant comprising the formula [B]-[C], further comprises [D], wherein [D] comprises position X4 and X5. In some embodiments, position X4 of [D] is T, N, or P. In some embodiments, position X5 of [D] is A or D. In some embodiments, [D] is or comprises TA, TD, NA, or PA. In some embodiments, [D] is TA. In some embodiments, [C]-[D] is or comprises YPAEVVQKTA (SEQ ID NO: 584), YPAEVVQKTD (SEQ ID NO: 719), YPAEVVQKNA (SEQ ID NO: 724), or YPAEVVQKPA (SEQ ID NO: 586); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, or 9 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any

one of the aforesaid amino acid sequences. In some embodiments, [C]-[D] is YPAEVVQKTA (SEQ ID NO: 584). In some embodiments, [A]-[B]-[C]-[D] is or comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNSSLYYPAEVVQKTA (SEQ ID NO: 2066), TNTSATYPAEVVQKTA (SEQ ID NO: 2067), TNSSLSYPAEVVQKTA (SEQ ID NO: 2068), TNSSLSYPAEVVQKTD (SEQ ID NO: 2070), TNSSLSYPAEVVQKNA (SEQ ID NO: 2072), TNSSLSYPAEVVQKPA (SEQ ID NO: 2073), or TNKSATYPAEVVQKTA (SEQ ID NO: 2075); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, or 15 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [A]-[B]-[C]-[D] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

[0144] In some embodiments, [B]-[C] is present in loop VIII of the AAV capsid variant. In some embodiments, [A] and/or [D] is present in loop VIII of the AAV capsid variant. In some embodiments, [A]-[B]-[C]-[D] is present in loop VIII of the AAV capsid variant. In some embodiments, loop VIII comprises positions 571-592 numbered according to SEQ ID NO: 138. In some embodiments, loop VIII comprises positions 571-599, numbered according to SEQ ID NO: 982.

[0145] In some embodiments, [A] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In any of these embodiments, [A] replaces positions 571-573 (e.g., T571, N572, and N573) numbered relative to SEQ ID NO: 138. In some embodiments, [A] is present immediately subsequent to position 570, and [A] replaces positions 571-573 (e.g., T571, N572, and N573) numbered relative to SEQ ID NO: 138. In some embodiments, [B] is present immediately subsequent to position 573, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B] is present immediately subsequent to position 573, and [B] replaces positions 574-576 (e.g., Q574, S575, and S576), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C] is present immediately subsequent to position 576, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C] is present immediately subsequent to position 576, wherein [C] replaces position 577 (e.g., T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B]-[C] is present immediately subsequent to position 573, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B]-[C] is present immediately subsequent to position 573,

and [B]-[C] replaces positions 574-577 (e.g., Q574, S575, S576, and T577), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C]-[D] is present immediately subsequent to position 576, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C]-[D] replaces positions 577-579 (e.g., T577, T578, and A579), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [C]-[D] is present immediately subsequent to position 576, and [C]-[D] replaces positions 577-579 (e.g., T577, T578, and A579), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, [B]-[C]-[D] is present immediately subsequent to position 573, numbered relative to SEQ ID NO: 138. In some embodiments, [B]-[C]-[D] replaces positions 574-579 (e.g., Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments [B]-[C]-[D] is present immediately subsequent to position 573, and [B]-[C]-[D] replaces positions 574-579 (e.g., Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments [A]-[B]-[C]-[D] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In some embodiments, [A]-[B]-[C]-[D] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments [A]-[B]-[C]-[D] is present immediately subsequent to position 570, and [A]-[B]-[C]-[D] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138.

[0146] In some embodiments, X_A of [A] is present at position 571, X_B of [A] is present at position 572, and X_C of [A] is present at position 573, numbered according to SEQ ID NO: 982. In some embodiments, X1 of [B] is present at position 574, X2 of [B] is present at position 575, and X3 of [B] is present at position 576, numbered according to SEQ ID NO: 982. In some embodiments, [C] is present at positions 577-584, numbered according to SEQ ID NO: 982. In some embodiments, X4 of [D] is present at position 585 and position X5 of [D] is present at position 586, numbered according to SEQ ID NO: 982.

[0147] In some embodiments, [A] is present at positions 571-573, numbered according to SEQ ID NO: 982. In some embodiments, [B] is present at positions 574-576, numbered according to SEQ ID NO: 982. In some embodiments, [C] is present at positions 577-584, numbered according to SEQ ID NO: 982. In some embodiments, [D] is present at positions 585-586, numbered according to SEQ ID NO: 982. In some embodiments, [A]-[B]-[C]-[D] is present at positions 571-586, numbered according to SEQ ID NO: 982.

[0148] In some embodiments, [B] is present immediately subsequent to [A]. In some embodiments, [C] is present immediately subsequent to [B]. In some embodiments, [D] is present immediately subsequent to [C]. In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [B]-[C]. In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [B]-[C]. In some embodiments, the AAV capsid variant comprises from N-

terminus to C-terminus, [A]-[B]-[C]. In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [B]-[C]-[D]. In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [A]-[B]-[C]-[D].

[0149] In some embodiments, [C] is YPAEVVQK (SEQ ID NO: 943), wherein YPAEVVQK (SEQ ID NO: 943) replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In some embodiments, [A]-[B]-[C]-[D] is TNNQSSYPAEVVQKTA (SEQ ID NO: 1533) and is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138, wherein [C] (YPAEVVQK (SEQ ID NO: 943)) replaces position 577 (e.g., replaces T577) numbered relative to SEQ ID NO: 138. In some embodiments, [C] is YPAEVVQK, wherein [C] is present at positions 577-584, numbered according to SEQ ID NO: 982.

[0150] In some embodiments, an AAV capsid variant described herein comprises the formula [K1]-[K2], wherein, [K1] comprises LSY or LYY, and [K2] comprises positions X1, X2, X3, and X4. In some embodiments, [K1] comprises LSY. In some embodiments, position X1 of [K2] is Q, T or P. In some embodiments, position X2 of [K2] is A, in some embodiments, position X3 of [K2] is E or D. In some embodiments, position X4 of [K2] is V or E. In some embodiments, [K2] comprises QA, TA, PA, EV, EE, DV, AE, or AD. In some embodiments, [K2] comprises QAE, TAE, PAE, PAD, AEV, AEE, or ADV. In some embodiments, [K2] is or comprises QAEV (SEQ ID NO: 15), TAEV (SEQ ID NO: 16), PAEV (SEQ ID NO: 17), PAEE (SEQ ID NO: 18), or PADV (SEQ ID NO: 19). In some embodiments, [K1]-[K2] comprises LSYQA (SEQ ID NO: 597), LSYTA (SEQ ID NO: 598), LYYPA (SEQ ID NO: 600), or LSYPA (SEQ ID NO: 603). In some embodiments, [K1]-[K2] comprises LSYQAE (SEQ ID NO: 607), LSYTAE (SEQ ID NO: 610), LYYPAE (SEQ ID NO: 611), LSYPAE (SEQ ID NO: 616), or LSYPAD (SEQ ID NO: 621). In some embodiments, [K1]-[K2] is or comprises LSYQAEV (SEQ ID NO: 667), LSYTAEV (SEQ ID NO: 668), LYYPAEV (SEQ ID NO: 669), LSYPAEV (SEQ ID NO: 671), LSYPAEE (SEQ ID NO: 673), or LSYPADV (SEQ ID NO: 674); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, or 6 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences.

[0151] In some embodiments, the AAV capsid variant comprising the amino acid sequence comprising the formula of [K1]-[K2], further comprises [K0], which comprises TNNS (SEQ ID NO: 14). In some embodiments, [K0]-[K1] comprises TNSSLS (SEQ ID NO: 647) or TNSSLY (SEQ ID NO: 648). In some embodiments, [K0]-[K1] is or comprises TNSSLSY (SEQ ID NO: 676) or TNSSLYY (SEQ ID NO: 678); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, or 6 amino acids, e.g., consecutive amino acids)

thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [K0]-[K1]-[K2] comprises TNSSLSYQA (SEQ ID NO: 679), TNSSLSYTA (SEQ ID NO: 681), TNSSLYYPA (SEQ ID NO: 682), or TNSSLSYPA (SEQ ID NO: 683). In some embodiments, [K0]-[K1]-[K2] comprises TNSSLSYQAE (SEQ ID NO: 684), TNSSLSYTAE (SEQ ID NO: 685), TNSSLYYPAE (SEQ ID NO: 686), TNSSLSYPAE (SEQ ID NO: 687), or TNSSLSYPAD (SEQ ID NO: 689). In some embodiments, [K0]-[K1]-[K2] is or comprises TNSSLSYQAEV (SEQ ID NO: 692), TNSSLSYTAEV (SEQ ID NO: 693), TNSSLYYPAEV (SEQ ID NO: 696), TNSSLSYPAEV (SEQ ID NO: 697), TNSSLSYPAEE (SEQ ID NO: 698), or TNSSLSYPADV (SEQ ID NO: 699); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences.

[0152] In some embodiments, AAV capsid variant comprising the amino acid sequence comprising the formula of [K1]-[K2], further comprises [K3], wherein [K3] comprises positions X_A, X_B, and X_C. In some embodiments, position X_A of [K3] is V. In some embodiments, position X_B of [K3] is Q. In some embodiments, position X_C of [K3] is K or N. In some embodiments [K3] comprises VQ, QK, or QN. In some embodiments, [K3] is or comprises VQK or VQN. In some embodiments, K2]-[K3] is or comprises QAEVVQK (SEQ ID NO: 52), TAEVVQK (SEQ ID NO: 49), PAEVVQK (SEQ ID NO: 20), PAEEVQK (SEQ ID NO: 51) ,PAEVVQN (SEQ ID NO: 594), or PADVVQK (SEQ ID NO: 596); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, or 6 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [K1]-[K2]-[K3] is or comprises LSYQAEVVQK (SEQ ID NO: 700), LSYTAEVVQK (SEQ ID NO: 701), LYYPAEVVQK (SEQ ID NO: 702), LSYPAEVVQK (SEQ ID NO: 703), LSYPAEEVQK (SEQ ID NO: 704), LSYPAEVVQN (SEQ ID NO: 706), or LSYPADVVQK (SEQ ID NO: 708); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, or 9 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [K0]-[K1]-[K2]-[K3] is or comprises TNSSLSYQAEVVQK (SEQ ID NO: 652), TNSSLSYTAEVVQK (SEQ ID NO: 654), TNSSLYYPAEVVQK (SEQ ID NO: 655), TNSSLSYPAEVVQK (SEQ ID NO: 657), TNSSLSYPAEEVQK (SEQ ID NO: 658), TNSSLSYPAEVVQN (SEQ ID NO: 660), or TNSSLSYPADVVQK (SEQ ID NO: 665); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences.

[0153] In some embodiments, the AAV capsid variant comprising the amino acid sequence comprising the formula of [K1]-[K2], further comprises [K4], wherein [K4] comprises positions X_D and X_E. In some embodiments, position X_D of [K4] is T, P, or N. In some embodiments, position X_E of [K4] is A or D. In some embodiments, [K4] is or comprises TA, TD, PA, or NA. In some embodiments, [K3]-[K4] is or comprises VQKTA (SEQ ID NO: 564), VQKTD (SEQ ID NO: 714), VQNTA (SEQ ID NO: 715), VQKNA (SEQ ID NO: 570), or VQKPA (SEQ ID NO: 567); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, or 4 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences. In some embodiments, [K0]-[K1]-[K2]-[K3]-[K4] is or comprises TNSSLSYQAEVVQKTA (SEQ ID NO: 2064), TNSSLSYTAEVVQKTA (SEQ ID NO: 2065), TNSSLYYPAEVVQKTA (SEQ ID NO: 2066), TNSSLSYPAEVVQKTA (SEQ ID NO: 2068), TNSSLSYPAEEVQKTA (SEQ ID NO: 2069), TNSSLSYPAEVVQKTD (SEQ ID NO: 2070), TNSSLSYPAEVVQNTA (SEQ ID NO: 2071), TNSSLSYPAEVVQKNA (SEQ ID NO: 2072), TNSSLSYPAEVVQKPA (SEQ ID NO: 2073), or TNSSLSYPADVVQKTA (SEQ ID NO: 2076); an amino acid sequence comprising any portion of any of the aforesaid amino acid sequences (e.g., any 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids, e.g., consecutive amino acids) thereof; 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 aforesaid amino acid sequences; or an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to any one of the aforesaid amino acid sequences.

[0154] In some embodiments, [K1]-[K2] is present in loop VIII of the AAV capsid variant. In some embodiments, [K0], [K3], and/or [K4] are present in loop VIII of the AAV capsid variant. In some embodiments, [K0]-[K1]-[K2]-[K3]-[K4] is present in loop VIII of the AAV capsid variant. In some embodiments, loop VIII comprises positions 571-592 numbered according to SEQ ID NO: 138. In some embodiments, loop VIII comprises positions 571-599, numbered according to SEQ ID NO: 982.

[0155] In some embodiments, [K0] is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138. In some embodiments, [K0] replaces positions 571-574 (e.g., T571, N572, N573, and Q574), numbered relative to SEQ ID NO: 138. In some embodiments, [K0] is present immediately subsequent to position 570, and [K0] replaces positions 571-574 (e.g., T571, N572, N573, and Q574), numbered relative to SEQ ID NO: 138. In some embodiments, [K1] is present immediately subsequent to position 574, numbered relative to SEQ ID NO: 138. In some embodiments, [K1] replaces positions 575-577 (e.g., S575, S576, and T577), sequence relative to SEQ ID NO: 138. In some embodiments, [K1] is present immediately subsequent to position 574, wherein [K1] replaces positions 575-577 (e.g., S575, S576, and T577), numbered relative to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3] is present immediately subsequent to position 574, numbered relative to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3] replaces positions 575-577 (e.g., S575, S576, and T577), numbered relative to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3] is present immediately subsequent to position 574, wherein [K1]-[K2]-[K3] replaces positions 575-577 (e.g., S575, S576, and T577), numbered relative to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 574, numbered relative to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3]-[K4] replaces positions 575-579 (e.g., S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments, [K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 574, wherein [K1]-[K2]-[K3]-[K4] replaces positions 575-579 (e.g., S575, S576, T577, T578, and A579), numbered relative to SEQ ID NO: 138. In some embodiments, [K0]-[K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 570, numbered relative to SEQ ID NO 138. In some embodiments, [K0]-[K1]-[K2]-[K3]-[K4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and T579), numbered relative to SEQ ID NO 138. In some embodiments, [K0]-[K1]-[K2]-[K3]-[K4] is present immediately subsequent to position 570, and [K0]-[K1]-[K2]-[K3]-[K4] replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and T579), numbered relative to SEQ ID NO 138.

[0156] In some embodiments, AAV capsid variant comprises from N-terminus to C-terminus, [K1]-[K2]. In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [K0]-[K1]-[K2]. In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [K1]-[K2]-[K3]. In some embodiments, the AAV capsid variant comprises from N-

terminus to C-terminus, [K0]-[K1]-[K2]-[K3]. In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [K1]-[K2]-[K3]-[K4]. In some embodiments, the AAV capsid variant comprises from N-terminus to C-terminus, [K0]-[K1]-[K2]-[K3]-[K4].

[0157] 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, 14, or 15 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, and 15-20. 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, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624. 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, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 943 or 2064-2080. In some embodiments, the AAV capsid variant comprises at least 3, 4, 5, 6, or 7 consecutive amino acids from any one of SEQ ID NOs: 943 or 946-966. In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, the amino acid sequence is present immediately subsequent to position 570, 571, 572, 573, 574, 575, or 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

[0158] In some embodiments, the 3 consecutive amino acids comprise YPA. In some embodiments, the 4 consecutive amino acids comprise YPAE (SEQ ID NO: 21). In some embodiments, the 5 consecutive amino acids comprise YPAEV (SEQ ID NO: 1). In some embodiments, the 6 consecutive amino acids comprise YPAEVV (SEQ ID NO: 725). In some embodiments, the 7 consecutive amino acids comprise YPAEVVQ (SEQ ID NO: 726). In some embodiments, the amino acid sequence comprises YPAEVVQK (SEQ ID NO: 943). In some embodiments, the amino acid sequence consists of YPAEVVQK (SEQ ID NO: 943).

[0159] In In some embodiments, an AAV capsid variant described herein comprises 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 the amino acid sequence of any one of the sequences provided in Tables 1, 2A, 2B, 9, and 15-20. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising 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, and 15-20. In some embodiments, the AAV capsid variant comprises 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 the amino acid sequence of any one of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising

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: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624. In some embodiments, the AAV capsid variant comprises 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 the amino acid sequence of any one of SEQ ID NOs: 943 or 2064-2080. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising 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: 943 or 2064-2080. In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, loop VIII comprises positions 571-592 numbered according to SEQ ID NO: 138. In some embodiments, loop VIII comprises positions 571-599, numbered according to SEQ ID NO: 982. In some embodiments, the amino acid sequence is present immediately subsequent to position 570, 571, 572, 573, 574, 575, or 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces position 577 (e.g., T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

[0160] In some embodiments, the AAV capsid variant comprises 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 the amino acid sequence of YPAEVVQK (SEQ ID NO: 943). In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising one, two, or three, but no more than four different amino acids that relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

[0161] In some embodiments, the AAV capsid variant comprises an amino sequence comprising 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: 2024-2063. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising 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: 2024-2063. In some embodiments, the different amino acids of the amino acid sequence comprising 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: 2024-2063, are present at one or more of the following positions: (i) position 1, wherein the different amino acid is T or L; (ii) position 2, wherein the different amino acid is N, L, K, A, T, or P; (iii) position 3, wherein the different amino acid N, K, L, A, Y, or S; (iv) position 4, wherein the different amino acid is Q, L, T, S, F, Y, K, or A; (v) position 5, wherein the different amino acid is S, H, A, M, Q, T, V, or F; (vi) position 6, wherein the different amino acid is S, P, V, A, Q, L, T, N, or M; (vii) position 7, wherein the different amino acid is Y, H, S, V, A, L, or T; (viii) position 8, wherein the different amino acid is D, P, A, Q, F, L, S, H, or M; (ix) position 9, wherein the different amino acid is F, A, L, D, or Q; (x) position 10, wherein

the different amino acid is T, E, I, or S; (xi) position 11, wherein the different amino acid is V, A, N, or S; (xii) position 12, wherein the different amino acid is V, L, or P; (xiii) position 13, wherein the different amino acid is Q, E, or P; (xiv) position 14, wherein the different amino acid is K, N, S, or L; (xv) position 15, wherein the different amino acid is T, V, M, or L; and/or (xvi) position 16, wherein the different amino acid is A, G, or R. In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, the amino acid sequence is present immediately subsequent to position 570, 571, 572, 573, 574, 575, or 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

[0162] In some embodiments, the AAV capsid variant comprises an amino sequence comprising 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: 1632-2023. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising 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: 1632-2023. In some embodiments, the different amino acids of the amino acid sequence comprising 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: 1623-2023, are present at one or more of the following positions: (i) position 1, wherein the different amino acid is T, G, N, S, E, L, Y, V, or I; (ii) position 2, wherein the different amino acid is D, N, K, E, V, G, R, L, H, F, P, T, A, S, I, or Y; (iii) position 3, wherein the different amino acid is Y, N, K, T, W, Q, M, V, C, A, L, F, H, G, R, S, or P; (iv) position 4, wherein the different amino acid is H, Q, P, E, R, K, A, S, V, L, T, D, I, G, M, or N; (v) position 5, wherein the different amino acid is R, S, K, N, H, G, W, A, P, V, Q, Y, L, or F; (vi) position 6, wherein the different amino acid is G, S, F, R, W, H, I, C, M, A, Y, K, N, Q, V, P, E, D, T, or L; (vii) position 7, wherein the different amino acid is D, Y, S, I, H, F, P, K, R, G, L, Q, A, M, T, N, V, W, C, or E; (viii) position 8, wherein the different amino acid is P, L, Q, T, W, V, G, K, I, Y, N, H, R, D, S, M, A, F, or E; (ix) position 9, wherein the different amino acid is A, R, T, Q, S, M, L, E, K, V, G, D, N, H, F, P, or I; (x) position 10, wherein the different amino acid is K, E, Q, H, V, G, R, S, P, I, N, M, A, L, D, or T; (xi) position 11, wherein the different amino acid is V, A, E, N, R, L, M, T, Q, S, K, C, G, D, Y, P, H, F, or I; (xii) position 12, wherein the different amino acid is V, P, L, S, T, N, A, G, K, R, I, H, E, Q, or M; (xiii) position 13, wherein the different amino acid is Q, K, N, A, H, R, T, V, E, I, P, G, S, or L; (xiv) position 14, wherein the different amino acid is K, E, I, Y, Q, R, G, D, L, N, or S; (xv) position 15, wherein the different amino acid is S, T, N, Q, I, P, E, G, K, M, or H; and/or (xvi) position 16, wherein the different amino acid is A, D, L, Y, Q, or T. In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, the amino acid sequence is present immediately subsequent to position 570, 571, 572, 573, 574, 575, or 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

[0163] In some embodiments, the AAV capsid variant comprises the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, and 15-20. In some embodiments, the AAV capsid

variant comprises the amino acid sequence of any of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any of SEQ ID NOs: 943 or 2064-2080. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any of SEQ ID NOs: 2024-2063. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any of SEQ ID NOs: 1632-2023. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 943. In some embodiments, the amino acid sequence is present in loop VIII. In some embodiments, the amino acid sequence is present immediately subsequent to position 570, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 570, and the amino acid sequence replaces positions 571-579 (e.g., T571, N572, N573, Q574, S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 571, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces positions 572-579 (e.g., N572, N573, Q574, S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 571, and the amino acid sequence replaces positions 572-579 (e.g., N572, N573, Q574, S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 572, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces positions 573-579 (e.g., N573, Q574, S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 572, and the amino acid sequence replaces positions 573-579 (e.g., N573, Q574, S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces positions 574-579 (e.g., Q574, S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 573, and the amino acid sequence replaces positions 574-579 (e.g., Q574, S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the

amino acid sequence is present immediately subsequent to position 574, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces positions 575-579 (e.g., S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 574, and the amino acid sequence replaces positions 575-579 (e.g., S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 575, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces positions 576-579 (e.g., S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 575, and the amino acid sequence replaces positions 576-579 (e.g., S575, S576, T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In some embodiments, the amino acid sequence replaces positions 577-579 (e.g., T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 576, and the amino acid sequence replaces position 577 (e.g., T577), relative to a reference sequence numbered according to SEQ ID NO 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 576, and the amino acid sequence replaces positions 577-579 (e.g., T577, T578, and T579), relative to a reference sequence numbered according to SEQ ID NO 138.

[0164] In some embodiments, the AAV capsid variant comprises the amino acid sequence of any of SEQ ID NOs: 943 or 946-966, wherein the amino acid sequence replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any of SEQ ID NOs: 943 or 946-966, wherein the amino acid sequence is present immediately subsequent to position 576, numbered relative to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any of SEQ ID NOs: 943 or 946-966, wherein the amino acid sequence is present immediately subsequent to position 576, and wherein the amino acid sequence replaces position 577 (e.g., T577), numbered relative to SEQ ID NO: 138.

[0165] 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: 944, 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: 944, 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: 944. 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: 944.

[0166] In some embodiments, the nucleotide sequence encoding the AAV capsid variant (e.g., an AAV capsid variant described herein), comprises the nucleotide sequence of SEQ ID NO: 944, 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 nucleic acid sequence encoding the AAV capsid variant comprises 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 sequences of SEQ ID NO: 944. In some embodiments, the nucleotide sequence encoding an AAV capsid variant 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: 944.

[0167] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943), wherein the amino acid sequence is present in loop VIII. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of YPAEVVQK (SEQ ID NO: 943), wherein the amino acid sequence is present immediately subsequent to position 576, relative to a reference sequence 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 YPAEVVQK (SEQ ID NO: 943), wherein the amino acid sequence of YPAEVVQK (SEQ ID NO: 943) replaces position 577 (e.g., T577), relative to a reference sequence 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 YPAEVVQK (SEQ ID NO: 943), wherein the amino acid sequence of YPAEVVQK (SEQ ID NO: 943) is present immediately subsequent to position 576, and wherein the amino acid sequence of YPAEVVQK (SEQ ID NO: 943) replaces position 577 (e.g., T577), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

[0168] In some embodiments, an AAV capsid variant described herein comprises the amino acid Y at position 577, and further comprises the amino acid sequence of PAEVVQK (SEQ ID NO: 20), which is present immediately subsequent to position 577, numbered relative to SEQ ID NO: 138.

[0169] In some embodiments, an AAV capsid variant described herein comprises the amino acid Y at position 577 and the amino acid sequence of PAEVVQK (SEQ ID NO: 20) at positions 578-584, numbered relative to SEQ ID NO: 982.

[0170] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), wherein the amino acid sequence is present in loop VIII. In some embodiments, the AAV capsid variant comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533) and is present immediately subsequent to position 570, numbered relative to SEQ ID NO: 138, wherein YPAEVVQK (SEQ ID NO: 943) replaces position 577 (e.g., replaces T577) numbered relative to SEQ ID NO: 138.

[0171] In some embodiments, the AAV capsid variant further one, two, three or all of an amino acid other than Q at position 574 (e.g., T, S, A, I, L, or H), an amino acid other than S at position 575 (e.g., G, A, L, T, or R), and/or an amino acid other than S at position 576 (e.g., K, L, R, A, Y, or T), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a Q at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a T at position 574, an S at position 575, and/or a L at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an S at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a Q at position 574, an S at position 575, and/or an R at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises Q at position 574, an S at position 575, and/or a K at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an A at position 574, a G at position 575, and/or an A at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an I at position 574, a G at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a Q at position 574, an A at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an A at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an L at position 574, a G at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the

amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a Q at position 574, an S at position 575, and/or a T at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an H at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an L at position 574, an S at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a Q at position 574, an R at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an S at position 574, an L at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an S at position 574, an L at position 575, and/or a Y at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an S at position 574, an A at position 575, and/or a T at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a Q at position 574, a T at position 575, and/or an S at position 576, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

[0172] In some embodiments, the AAV capsid variant comprises amino acid other than Q at position 574 (e.g., S), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises S at position 574, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

[0173] In some embodiments, the AAV capsid variant further comprises one or both of an amino acid other than T at position 571 (e.g., I or N), and/or an amino acid other than N at position 573 (e.g., T, S, or K), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an R at position 456, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a T at position 571, an N at position 572, and/or an N at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a T at position 571, an N at position 572, and/or a T at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an I at position 571, an N at position 572, and/or an N at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a T at position 571, an N at

position 572, and/or an S at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an N at position 571, an N at position 572, and/or an N at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a T at position 571, an N at position 572, and/or a K at position 573, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

[0174] In some embodiments, the AAV capsid variant further comprises an amino acid other than T at position 578 (e.g., P or N), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises one or both of an amino acid other than T at position 578 (e.g., P or N), and/or an amino acid other than A at position 589 (e.g., D), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a T at position 578 and/or an A at position 588, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a P at position 578 and/or an A at position 588, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises an N at position 578 and/or an A at position 588, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a T at position 578 and/or a D at position 579, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

[0175] In some embodiments, the AAV capsid variant further comprises an amino acid other than T (e.g., Y) at position 577, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises Y at position 577, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138.

[0176] 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. In some embodiments, loop I, II, IV, VI, and VIII can be identified as described in Govindasamy et al. Structurally Mapping the Diverse Phenotype of Adeno-Associated Virus Serotype 4. *Journal of Virology*. 2006 Dec. 80(23):11556-11570; and Govindasamy et al. Structural Insights into Adeno-Associated Virus Serotype 5. *Journal of Virology*. 2013 Oct. 87(20):11187-11199; the contents of which are each hereby incorporated by reference in their entirety.

[0177] In some embodiments, additional modifications, e.g., substitutions (e.g., conservative substitutions), insertions, and/or deletions can be introduced into an AAV capsid variant described herein at positions determined using a structural map of wild-type AAV5, e.g., a structural map

described and generated by Govindasamy et al. et al. Structural Insights into Adeno-Associated Virus Serotype 5. *Journal of Virology*. 2013 Oct. 87(20):11187-11199 (the contents of which are hereby incorporated herein by reference in their entirety) or Walters et al. "Structure of Adeno-Associated Virus Serotype 5," *Journal of Virology*, 2004, 78(7):3361-3371 (the contents of which are hereby incorporated by reference in their entirety).

[0178] In some embodiments, an AAV capsid variant described herein comprises a modification as described in Jose et al. "High-Resolution Structural Characterization of a New Adenoassociated Virus Serotype 5 Antibody Epitope toward Engineering Antibody-Resistant Recombinant Gene Delivery Vectors," *Journal of Virology*, 2020, 93(1): e01394-18; Qian et al. "Directed Evolution of AAV Serotype 5 for Increased Hepatocyte Transduction and Retained Low Humoral Seroreactivity," *Molecular Therapy: Methods and Clinical Development*, 2021, 20:122-132; Afione et al. "Identification and Mutagenesis of the Adeno-Associated Virus 5 Sialic Acid Binding Region," *Journal of Virology*, 2015, 89(3):1660-1672; and/or Wang et al. "Directed evolution of adeno-associated virus 5 capsid enables specific liver tropism," *Mol Ther Nucleic Acids*, 2022, 28:293-306; the contents of each of which are hereby incorporated by reference in their entirety.

[0179] 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 different amino acids relative to 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 about 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

[0180] 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 about 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.

[0181] 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

about 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.

[0182] 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 TTN-002, TTN-003, TTN-004, TTN-005, or TTN-006, e.g., as described in Tables 3 and 4. 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 TTN-002, e.g., as described in Tables 3 and 4.

[0183] 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 TTN-002, TTN-003, TTN-004, TTN-005, or TTN-006, e.g., as described in Tables 3 and 4. 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 TTN-002, e.g., as described in Tables 3 and 4.

[0184] 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 an AAV capsid variant of TTN-002, e.g., as described in Tables 3 and 5.

[0185] 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 an AAV capsid variant of TTN-002, 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 SEQ ID NO:	DNA encoding Peptide SEQ ID NO:
TTN-002	984	982	943	944

Table 4. Exemplary full length capsid amino acid sequences

Name and Annotation	SEQ ID NO:	Amino Acid Sequence
TTN-002 8mer peptide underlined, starts at	982	MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGN GLDRGEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGN LGKAVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDA EAGPSGSQQLQIPAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTW MGDRVVTKSTRTWVLPSYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHS

position 577 (immediately subsequent to position 576)		HWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTD DDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFP SKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQ FNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQV PPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNR VAYNVGGQMATNNQSS**YPAEVVQK**TAPATGTYNLQEIVPGSVWMERDVYLQGPIWA KIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTG QVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLT RPL
TTN-002 VP2 (positions 137-731 of SEQ ID NO: 982; 8mer peptide underlined)	738	TAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQIPAQPASSLGADT MSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYR EIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRV KIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQV FTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSS FAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRT QGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMI FNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSS**YPAEVVQK** TAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPP PMMLIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQY TNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLTRPL
TTN-002 VP3 (positions 193-731 of SEQ ID NO: 982; 8mer peptide underlined)	739	MSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYR EIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRV KIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQV FTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSS FAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRT QGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMI FNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSS**YPAEVVQK** TAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPP PMMLIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQY TNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLTRPL
TTN-003 Modifications at positions 574-577, and 7-mer insert present immediately subsequent to position 577 underlined	740	MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGN GLDRGEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGN LGKAVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDA EAGPSGSQQLQIPAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTW MGDRVVTKSTRTWVLPSYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHS HWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTD DDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFP SKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQ FNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQV PPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNR VAYNVGGQMATNN**AGAYPAEVVQK**TAPATGTYNLQEIVPGSVWMERDVYLQGPIWA KIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTG QVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLT RPL
TTN-004 Modifications at positions 573-575, 577, and 7-mer insert present immediately subsequent to position 577 underlined	741	MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGN GLDRGEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGN LGKAVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDA EAGPSGSQQLQIPAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTW MGDRVVTKSTRTWVLPSYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHS HWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTD DDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFP SKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQ FNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQV PPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNR VAYNVGGQMATN**STH**S**YPAEVVQK**TAPATGTYNLQEIVPGSVWMERDVYLQGPIWA KIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTG QVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLT RPL
TTN-005 Modifications at positions	742	MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGN GLDRGEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGN LGKAVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDA

574, 575, 577, and 7-mer insert present immediately subsequent to position 577 underlined		EAGPSGSQQLQIPAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTW MGDRVVTKSTRTWVLPSYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHS HWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTD DDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFP SKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQ FNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQV PPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNR VAYNVGGQMATNN**IG**S**YPAEVVQK**TAPATGTYNLQEIVPGSVWMERDVYLQGPIWA KIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTG QVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLT RPL
TTN-006 Modification at position 577 and 7-mer insert present immediately subsequent to position 577 underlined	743	MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGN GLDRGEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGN LGKAVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDA EAGPSGSQQLQIPAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTW MGDRVVTKSTRTWVLPSYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHS HWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTD DDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFP SKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQ FNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQV PPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNR VAYNVGGQMATNNQSS**YPAERSHK**TAPATGTYNLQEIVPGSVWMERDVYLQGPIWA KIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTG QVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLT RPL

Table 5. Exemplary full length capsid nucleic acid sequences

Name and Annotation	**SEQ ID NO:**	**NT Sequence**
TTN-002 Nucleotide sequence encoding 8mer peptide underlined	984	ATGTCTTTTGTTGATCACCCTCCAGATTGGTTGGAAGAAGTTGGTGAAGGTCTTCG CGAGTTTTTGGGCCTTGAAGCGGGCCCACCGAAACCAAAACCCAATCAGCAGCATC AAGATCAAGCCCGTGGTCTTGTGCTGCCTGGTTATAACTATCTCGGACCCGGAAAC GGTCTCGATCGAGGAGAGCCTGTCAACAGGGCAGACGAGGTCGCGCGAGAGCACGA CATCTCGTACAACGAGCAGCTTGAGGCGGGAGACAACCCCTACCTCAAGTACAACC ACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACGACACATCCTTCGGGGGAAAC CTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCGAACCTTTTGGCCTGGT TGAAGAGGGTGCTAAGACGGCCCCTACCGGAAAGCGGATAGACGACCACTTTCCAA AAAGAAAGAAGGCCCGGACCGAAGAGGACTCCAAGCCTTCCACCTCGTCAGACGCC GAAGCTGGACCCAGCGGATCCCAGCAGCTGCAAATCCCAGCCCAACCAGCCTCAAG TTTGGGAGCTGATACAATGTCTGCGGGAGGTGGCGGCCCATTGGGCGACAATAACC AAGGTGCCGATGGAGTGGGCAATGCCTCGGGAGATTGGCATTGCGATTCCACGTGG ATGGGGGACAGAGTCGTCACCAAGTCCACCCGAACCTGGGTGCTGCCCAGCTACAA CAACCACCAGTACCGAGAGATCAAAAGCGGCTCCGTCGACGGAAGCAACGCCAACG CCTACTTTGGATACAGCACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGC CACTGGAGCCCCCGAGACTGGCAAAGACTCATCAACAACTACTGGGGCTTCAGACC CCGGTCCCTCAGAGTCAAAATCTTCAACATTCAAGTCAAAGAGGTCACGGTGCAGG ACTCCACCACCACCATCGCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGAC GACGACTACCAGCTGCCCTACGTCGTCGGCAACGGGACCGAGGGATGCCTGCCGGC CTTCCCTCCGCAGGTCTTTACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGCG ACAACACAGAAAATCCCACCGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCC AGCAAGATGCTGAGAACGGGCAACAACTTTGAGTTTACCTACAACTTTGAGGAGGT GCCCTTCCACTCCAGCTTCGCTCCCAGTCAGAACCTCTTCAAGCTGGCCAACCCGC TGGTGGACCAGTACTTGTACCGCTTCGTGAGCACAAATAACACTGGCGGAGTCCAG TTCAACAAGAACCTGGCCGGGAGATACGCCAACACCTACAAAAACTGGTTCCCGGG GCCCATGGGCCGAACCCAGGGCTGGAACCTGGGCTCCGGGGTCAACCGCGCCAGTG TCAGCGCCTTCGCCACGACCAATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTG CCCCCGCAGCCGAACGGCATGACCAACAACCTCCAGGGCAGCAACACCTATGCCCT GGAGAACACTATGATCTTCAACAGCCAGCCGGCGAACCCGGGCACCACCGCCACGT ACCTCGAGGGCAACATGCTCATCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGC

		GTGGCGTACAACGTCGGCGGGCAGATGGCCACCAACAACCAGAGCTCT**TATCCGGC** **GGAGGTGGTGCAGAAG**actgcccccgcgaccggcaCGTACAACCTCCAGGAAATCG TGCCCGGCAGCGTGTGGATGGAGAGGGACGTGTACCTCCAAGGACCCATCTGGGCC AAGATCCCAGAGACGGGGGCGCACTTTCACCCCTCTCCGGCtATGGGCGGATTCGG ACTCAAACACCCACCGCCCATGATGCTCATCAAGAACACGCCTGTGCCCGGAAATA TCACCAGCTTCTCGGACGTGCCCGTCAGCAGCTTCATCACCCAGTACAGCACCGGG CAGGTCACCGTGGAGATGGAGTGGGAGCTCAAGAAGGAAAACTCCAAGAGGTGGAA CCCAGAGATCCAGTACACAAACAACTACAACGACCCCCAGTTTGTGGACTTTGCCC CGGACAGCACCGGGGAATACAGAACCACCAGACCTATCGGAACCCGATACCTTACC CGACCCCTTTAA

[0186] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 982, or an amino acid sequence with at least 70% (e.g., at least about 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 982 or an amino acid sequence with at least 90% sequence identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 982 or an amino acid sequence with at least 95% sequence identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 982 or an amino acid sequence with at least 96% sequence identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 982 or an amino acid sequence with at least 97% sequence identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 982 or an amino acid sequence with at least 98% sequence identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 982 or an amino acid sequence with at least 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: 982. In some embodiments, the AAV capsid variant, 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: 982.

[0187] In some embodiments, In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of any one of SEQ ID NOs: 740-743, or an amino acid sequence with at least 70% (e.g., at least about 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 any one of SEQ ID NOs: 740-743. In some embodiments, the AAV capsid variant, 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 any one of SEQ ID NOs: 740-743.

[0188] In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 984, or a nucleotide sequence with at least 70% (e.g., at least about 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: 984. 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: 984.

[0189] In some embodiments, the nucleotide sequence encoding an AAV capsid variant, described herein comprises the nucleotide sequence of SEQ ID NO: 984, or a nucleotide sequence with at least 70% (e.g., at least about 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 the nucleotide sequence of SEQ ID NO: 984, or a nucleotide sequence with at least 70% (e.g., at least about 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: 984. 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: 984. In some embodiments, the nucleic acid sequence encoding an AAV capsid variant described herein is codon optimized.

[0190] 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 137-731, e.g., a VP2, of SEQ ID NO: 982, or a sequence with at least 70% (e.g., at least about 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 193-731, e.g., a VP3, of SEQ ID NO: 982, or a sequence with at least 70% (e.g., at least about 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 1-731, e.g., a VP1, of SEQ ID NO: 982, or an amino acid sequence with at least 70% (e.g., at least about 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

[0191] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 739, or an amino acid sequence at least 95% (e.g., at least 96, 97, 98, or 99%) identical thereto. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 738, or an amino acid sequence at least 95% (e.g., at least 96, 97, 98, or 99%) identical thereto. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 982, or an amino acid sequence at least 95% (e.g., at least 96, 97, 98, or 99%) identical thereto.

[0192] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 739 (e.g., VP3). In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 738 (e.g., VP2). In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 982 (e.g., VP1).

[0193] 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.

[0194] 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: 982.

[0195] 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: 139.

[0196] In some embodiments, an AAV capsid variant described herein transduces a brain region, e.g., a temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, and/or cerebellum. In some embodiments, the level of transduction is at least .5, 2.2, 2.4, 2.5, 2.6, 2.7, 3.0, 3.2, 3.5, 3.7, 4.0, 4.2, 4.5, 4.7, 4.9, 5, 10, 15, 20, 25, 30, or 35-fold greater as compared to a reference sequence of SEQ ID NO: 139.

[0197] In some embodiments, an AAV capsid variant described herein is enriched at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 25, 30, 35, 40, 45, 50, 55, 60, or 65-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 10, 12, 15, 17, 20, 25, 30, 35, 40, 45, 50, 55, 60, 61, 62, 63, 64, or 65-fold in the brain compared to a reference sequence of SEQ ID NO: 138.

[0198] 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 and/or rat) 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, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, or 100-fold in the brain of at least two to three species, e.g., a non-human primate and rodent (e.g., mouse and/or rat) species, compared to a reference sequence of SEQ ID NO:

138 or 982. In some embodiments, the at least two to three species are *Macaca fascicularis*, *Chlorocebus sabaeus*, *Callithrix jacchus*, rat and/or mouse (e.g., BALB/c mice).

[0199] In some embodiments, an AAV capsid variant described herein is enriched about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 125, 150, 175, 200, or 225-fold in the brain compared to a reference sequence of SEQ ID NO: 982.

[0200] 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 1.5, 2.2, 2.4, 2.5, 2.6, 2.7, 3.0, 3.2, 3.5, 3.7, 4.0, 4.2, 4.5, 4.7, 4.9, or 5-fold, as compared to a reference sequence of SEQ ID NO: 139. In some embodiments, the brain region comprises a midbrain region (e.g., the hippocampus or thalamus) and/or the brainstem.

[0201] 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 20, 25, 30, 35-fold, as compared to a reference sequence of SEQ ID NO: 139. In some embodiments, the brain region comprises a temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, cerebellum, or a combination thereof.

[0202] In some embodiments an AAV capsid variant described herein is enriched at least about 3, 3.5, 4.0, 4.5, 5, 5.0, 6.0, or 6.5-fold, in a spinal cord region compared to a reference sequence of SEQ ID NO: 139. In some embodiments, the spinal cord region comprises a cervical spinal cord region, a lumbar spinal cord region, a thoracic spinal cord region, or a combination thereof

[0203] In some embodiments an AAV capsid variant described herein shows preferential transduction in a brain region relative to the transduction in the dorsal root ganglia (DRG). In some embodiments an AAV capsid variant described herein shows preferential transduction in a brain region relative to the transduction in the liver.

[0204] In some embodiments, an AAV capsid variant described herein is capable of transducing neuronal cells.

[0205] In some embodiments, an AAV capsid variant described herein is capable of transducing non-neuronal cells, e.g., glial cells (e.g., oligodendrocytes or astrocytes). In some embodiments, the AAV capsid variant is capable of transducing neuronal cells and non-neuronal cells, e.g., glial cells (e.g., oligodendrocytes or astrocytes). In some embodiments, the non-neuronal cells are glial cells (e.g., oligodendrocytes or astrocytes).

[0206] In some embodiments, an AAV capsid variant described herein has an increased tropism for a heart cell or heart tissue, e.g., a heart cell or a heart tissue of a heart atrium or a heart ventricle relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein has an increased tropism for a

heart cell or heart tissue, e.g., a heart cell or a heart tissue of a heart atrium or a heart ventricle relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 139.

[0207] In some embodiments, an AAV capsid variant described herein delivers an increased level of a payload to a heart region. In some embodiments, the level of the payload is increased by at least 1.5, 2, or 2.5-fold, as compared to a reference sequence of SEQ ID NO: 139.

[0208] In some embodiments, an AAV capsid variant described herein has an increased tropism for a heart cell or heart tissue, e.g., a heart cell or a heart tissue of a heart atrium or a heart ventricle relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 982. In some embodiments, the AAV capsid variant is enriched about 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50-fold, in the heart compared to a reference sequence of SEQ ID NO: 982.

[0209] In some embodiments an AAV capsid variant described herein has an increased tropism for a muscle cell or tissue, relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 982. In some embodiments, the muscle region comprises a quadriceps muscle, and/or a diaphragm muscle region. In some embodiments, the AAV capsid variant is enriched at least about 2, 3, 4, 5, 10, 15, 20, 25, 30, or 35-fold, in the muscle compared to a reference sequence of SEQ ID NO: 982.

[0210] In some embodiments, an AAV capsid variant described herein has increased tropism for a liver cell or liver tissue, relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 982. In some embodiments, the AAV capsid variant is enriched at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 115, 120, 130, 140, 150, 160, 170, 180, 185, or 190-fold, in the liver, compared to a reference sequence of SEQ ID NO: 982.

[0211] In some embodiments, an AAV capsid variant described herein has decreased tropism for the liver. In some embodiments, an AAV capsid variant comprises a modification, e.g., 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, 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. In some embodiments, the AAV capsid variant, e.g., the AAV capsid variant having reduced tropism in the liver, comprises one or more of: an amino acid other than A, G, K, M, N, Q, R, S, and/or T at position 581; an amino acid other than A, C, H, I, K, S, T, and/or V at position 582; an amino acid other than A, G, H, K, M, N, Q, R, S, T, and/or V at position 583; an amino acid other than L, M, P, Q, R. T and/or W at position 584;

an amino acid other than F, H, I, K, M, T and/or Y at position 585; an amino acid other than E, G, H, L, M, N, Q, T, and/or W at position 586; an amino acid other than A, C, G, H, L, M, R, and/or S at position 587; an amino acid other than A, C, D, F, G, H, M, Q, S, V, W, and/or Y at position 588; and/or an amino acid other than A, C, E, G, H, M, N, P, Q, S, V, and/or W at position 589, all numbered relative to SEQ ID NO: 138.

[0212] 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.

[0213] 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

[0214] 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 AAV particle tropism in a particular tissue.

[0215] 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 temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, cerebellum, frontal cortex, sensory cortex, motor cortex, dentate nucleus, cerebellar cortex, cerebral cortex, brain stem, 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). In some embodiments, the AAV capsid variant allows for preferential transduction in a brain region relative to the transduction in the liver. In some embodiments, the AAV capsid variant allows for transduction in neuronal cells. In some embodiments, the AAV capsid variant allows for transduction in a non-neuronal cell, e.g., a glial cell (e.g., an astrocyte, an oligodendrocyte, or a combination thereof). In some embodiments, the AAV capsid variant allows for transduction in both neuronal cells and non-neuronal cell, e.g., a glial cell (e.g., an astrocyte, an oligodendrocyte, or a combination thereof).

[0216] 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.

[0217] In some embodiments the AAV capsid variant, allows for increased distribution to a heart region.

[0218] 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.

[0219] In some embodiments the AAV capsid variant, allows for increased distribution to a liver region.

[0220] In some embodiments, an AAV capsid described herein comprises a modification as described in Jose et al. High-Resolution Structural Characterization of a New Adenoassociated Virus Serotype 5 Antibody Epitope toward Engineering Antibody-Resistant Recombinant Gene Delivery Vectors. *Journal of Virology*. 2019 Jan. 93(1):e01394-18; Qian et al. Directed Evolution of AAV Serotype 5 for Increased Hepatocyte Transduction and Retained Low Humoral Seroreactivity. *Molecular Therapy: Methods & Clinical Development*. 2020 Oct. 20:122-132; Afione et al. Identification and Mutagenesis of the Adeno-Associated Virus 5 Sialic Binding Region. *Journal of Virology*. 2015 Feb. 89(3):1660-1672; the contents of which are each hereby incorporated by reference in their entirety.

[0221] 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.

[0222] 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.

[0223] 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.

[0224] 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-).

[0225] 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).

[0226] 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.

[0227] 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-).

[0228] 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

[0229] In some embodiments, the AAV capsid variant, comprises immediately subsequent to position 570, 571, 572, 573, 574, 575, or 576, numbered relative to SEQ ID NO: 138, at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 consecutive amino acids of any of amino acid sequence provided in Tables 1, 2A, 2B, 9, or 15-20.

[0230] In some embodiments, the AAV capsid variant, comprises immediately subsequent to position 570, 571, 572, 573, 574, 575, 576, or 577, 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, SEQ ID NO: 139, 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, 15, or 16 consecutive amino acids of any of amino acid sequence provided in Tables 1, 2A, 2B, 9, or 15-22. In some embodiments, the at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 consecutive amino acids of any of amino acid sequence provided in Tables 1, 2A, 2B, 9, or 15-22 replaces at least one, two, three, four, five, six, seven, eight, or all of positions T571, N572, N573, Q574, S575, S576, T577, T578, and/or A579, 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, SEQ ID NO: 139, 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 at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 consecutive amino acids of any of amino acid sequence provided in Tables 1, 2A, 2B, 9, or 15-22 replaces positions T577, 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, SEQ ID NO: 139, 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 T571, N572, N573, Q574, S575, S576, T577, T578, and/or A579, 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, SEQ ID NO: 139, 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 position T577, 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, SEQ ID NO: 139, 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 T571, N572, N573, Q574, S575, S576, T577, T578, and/or A579, 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, SEQ ID NO: 139, 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 position T577, 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, SEQ ID NO: 139, 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).

[0231] In some embodiments, 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, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence having at least 90% identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence having at least 95% identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence having at least 96% identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence having at least 97% identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence having at least 98% identity thereto. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence having at least 99% 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 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. 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.

[0232] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence or is encoded by the nucleotide sequence of any of SEQ ID NO: 1 or 2 of US7427396; SEQ ID NO: 114 of US20030138772; SEQ ID NO: 199 of US20150315612; or SEQ ID NOs: 13, 14, 16, 17, 19, 20, 22, 23, 25, 26, 28, 29, 31, 32, 34, 35, 37, 38, 40, 41, 43, or 44 of US20160289275A1 (the contents of each are incorporated herein by reference in their entirety), or a sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto.

[0233] In some embodiments, the AAV capsid variant described herein comprises a modification, e.g., substitution, at position 569 (e.g., M569V), 652 (e.g., D652A), 362 (e.g., T362M), 359 (e.g., Q359D), 350 (e.g., E350Q), 533 (e.g., P533S), 585 (e.g., Y585V), 587 (e.g., L587T), 581 (e.g., A581T), 582 (e.g., T582A), 584 (e.g., T584A), or a combination thereof, all numbered relative to SEQ ID NO: 138.

[0234] In some embodiments, an AAV capsid variant described herein comprises an amino acid from a wild-type AAV5 sequence, e.g., the amino acid sequence of SEQ ID NO: 138, at one or more of positions 581 to 589, numbered relative to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises 1, 2, 3, 4, 5, 6, 7, 8, or all of: the amino acid from a wild-type AAV5 sequence (e.g., SEQ ID NO: 138) at position 581 (e.g., comprises the amino acid A at position 581); the amino acid from a wild-type AAV5 sequence (e.g., SEQ ID NO: 138) at position 582 (e.g., comprises the amino acid T at position 582); the amino acid from a wild-type AAV5 sequence (e.g., SEQ ID NO: 138) at position 583 (e.g., comprises the amino acid G at position 583); the amino acid from a wild-type AAV5 sequence (e.g., SEQ ID NO: 138) at position 584 (e.g., comprises the amino acid T at position 584); the amino acid from a wild-type AAV5 sequence (e.g., SEQ ID NO: 138) at position 585 (e.g., comprises the amino acid Y at position 585); the amino acid from a wild-type AAV5 sequence (e.g., SEQ ID NO: 138) at position 586 (e.g., comprises the amino acid N at position 586); the amino acid from a wild-type AAV5 sequence (e.g., SEQ ID NO: 138) at position 587 (e.g., comprises the amino acid L at position 587); the amino acid from a wild-type AAV5 sequence (e.g., SEQ ID NO: 138) at position 588 (e.g., comprises the amino acid Q at position 588); and/or the amino acid from a wild-type AAV5 sequence (e.g., SEQ ID NO: 138) at position 589 (e.g., comprises the amino acid E at position 589).

[0235] In certain embodiments, an AAV capsid described herein does not comprise a T at position 581, an A at position 582, an A at position 584, a V at position 585, a T at position 585, a V

at position 569, an A at position 652, an M at position 362, a Q at position 359, a Q at position 350, an S at position 533, or a combination thereof, all numbered relative to SEQ ID NO: 138.

[0236] In some embodiments, an AAV capsid described herein does not comprise a modification, e.g., substitution, at positions 581-589 (numbered according to SEQ ID NO: 138), wherein the modification has the amino acid sequence of any of the sequences provided in Tables 2, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, or 71-86 of WO 2021/242909.

[0237] In any of the embodiments described herein, a position numbered relative to SEQ ID NO: 138 can be identified by providing an alignment of a reference sequence and a query sequence, wherein the reference sequence is SEQ ID NO: 138, and identifying the residues corresponding to the positions in the query sequence that correspond to positions in the reference sequence.

Table 6. AAV Sequences

Serotype	SEQ ID NO:	Sequence
AAV5 WT (amino acid)	138	MSFVDHPPDWLEEVGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDR GEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGKAVFQA KKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQI PAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLP SYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPR SLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQV FTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPS QNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSG VNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTA TYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERD VYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFIT QYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYL TRPL
AAV5 WT VP2 (amino acid; positions 137-724 of SEQ ID NO: 138)	744	TAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQIPAQPASSLGADTMSAG GGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKSGSVD GSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTV QDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNT ENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLY RFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRME LEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQ PVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGA HFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELK KENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLTRPL
AAV5 WT VP3 (amino acid; positions 193-724 of SEQ ID NO: 138)	750	MSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKS GSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRVKIFNIQVK EVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLN RDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPLVD QYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATT NRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSE SETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIP ETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEME WELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLTRPL
AAV5 WT (DNA)	137	atgtcttttgttgatcaccctccagattggttggaagaagttggtgaaggtcttcgcgag ttttgggccttgaagcgggcccaccgaaaccaaaacccaatcagcagcatcaagatcaa gcccgtggtcttgtgctgcctggttataactatctcggacccggaaacggtctcgatcga ggagagcctgtcaacagggcagacgaggtcgcgcgagagcacgacatctcgtacaacgag cagcttgaggcgggagacaaccctacctcaagtacaaccacgcggacgccgagtttcag

		gagaagctcgccgacgacacatccttcgggggaaacctcggaaaggcagtctttcaggcc aagaaaagggttctcgaacctttggcctggttgaagagggtgctaagacggcccctacc ggaaagcggatagacgaccactttccaaaaagaaagaaggctcggaccgaagaggactcc aagccttccacctcgtcagacgccgaagctggacccagcggatcccagcagctgcaaatc ccagcccaaccagcctcaagtttgggagctgatacaatgtctgcgggaggtggcggccca ttgggcgacaataaccaaggtgccgatggagtgggcaatgcctcgggagattggcattgc gattccacgtggatgggggacagagtcgtcaccaagtccacccgaacctgggtgctgccc agctacaacaaccaccagtaccgagagatcaaaagcggctccgtcgacggaagcaacgcc aacgcctactttggatacagcacccctgggggtactttgactttaaccgcttccacagc cactggagcccccgagactggcaaagactcatcaacaactactggggcttcagaccccgg tccctcagagtcaaaatcttcaacattcaagtcaaagaggtcacggtgcaggactccacc accaccatcgccaacaacctcacctccaccgtccaagtgtttacggacgacgactaccag ctgccctacgtcgtcggcaacgggaccgagggatgcctgccggccttccctccgcaggtc tttacgctgccgcagtacggttacgcgacgctgaaccgcgacaacacagaaaatcccacc gagaggagcagcttcttctgcctagagtactttcccagcaagatgctgagaacgggcaac aactttgagtttacctacaactttgaggaggtgcccttccactccagcttcgctcccagt cagaacctgttcaagctggccaacccgctggtggaccagtacttgtaccgcttcgtgagc acaaataacactggcggagtccagttcaacaagaacctggccgggagatacgccaacacc tacaaaaactggttcccggggcccatgggccgaacccagggctggaacctgggctccggg gtcaaccgcgccagtgtcagcgccttcgccacgaccaataggatggagctcgagggcgcg agttaccaggtgcccccgcagccgaacggcatgaccaacaacctccagggcagcaacacc tatgccctggagaacactatgatcttcaacagccagccggcgaacccgggcaccaccgcc acgtacctcgagggcaacatgctcatcaccagcgagagcgagacgcagccggtgaaccgc gtggcgtacaacgtcggcgggcagatggccaccaacaaccagagctcTACTACTGCCCCC GCGACCGGCACGTACAACCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGAC GTGTACCTCCAAGGACCCATCTGGGCCAAGATCCCAGAGACGGGGGCGCACTTTCACCCC TCTCCGGCCATGGGCGGATTCGGACTCAAACACCCACCGCCCATGATGCTCATCAAGAAC ACGCCTGTGCCCGGAAATATCACCAGCTTCTCGGACGTGCCCGTCAGCAGCTTCATCACC CAGTACAGCACCGGGCAGGTCACCGTGGAGATGGAGTGGGAGCTCAAGAAGGAAAACTCC AAGAGGTGGAACCCAGAGATCCAGTACACAAACAACTACAACGACCCCCAGTTTGTGGAC TTTGCCCCGGACAGCACCGGGGAATACAGAACCACCAGACCTATCGGAACCCGATACCTT ACCCGACCCCTTTAA
AAV9/hu.14 WT (amino acid)	139	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ AKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVI TTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAH EGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENV PFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIP GPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGS LIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG ILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPT AFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGV YSEPRPIGTRYLTRNL

Viral Genome of the AAV particle

[0238] 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, heart, liver, 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, heart, liver, 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.

[0239] 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 heart, 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. In some embodiments, the AAV particles described herein are used to deliver a payload to cells of a liver after intravenous delivery.

[0240] 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.

[0241] In some embodiments, the viral genome components are selected and/or engineered for expression of the payload in a target tissue (e.g., CNS (e.g., brain, spinal cord, or both), heart, liver, and/or muscle tissue).

Viral Genome Component: Inverted Terminal Repeats (ITRs)

[0242] 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.

[0243] 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 some embodiments, both ITRs of the viral genome of the AAV particle are AAV2 ITRs.

[0244] Independently, each ITR may be about 100 to about 150 nucleotides in length. An ITR may be about 100-105 nucleotides in length, 106-110 nucleotides in length, 111-115 nucleotides in length, 116-120 nucleotides in length, 121-125 nucleotides in length, 126-130 nucleotides in length, 131-135 nucleotides in length, 136-140 nucleotides in length, 141-145 nucleotides in length or 146-150 nucleotides in length. In some embodiments, the ITRs are 140-142 nucleotides in length. Non-limiting examples of ITR length are 102, 105, 130, 140, 141, 142, 145 nucleotides in length.

Viral Genome Component: Promoters

[0245] 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.

[0246] 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).

[0247] 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.

[0248] 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 1α-subunit (EF1α) 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.

[0249] 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., αMHC, cTnT, and CMV-MLC2k).

[0250] 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 Na_V 1.8 promoter, tyrosine hydroxylase (TH) promoter, choline acetyltransferase (ChaT) promoter, methyl-CpG binding protein 2 (MeCP2) promoter, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) promoter, metabotropic glutamate receptor 2 (mGluR2) promoter, neurofilament light chain (NFL) or heavy chain (NFH) promoter, neuron-specific enolase (NSE) promoter, β-globin minigene nβ2 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.

[0251] 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.

[0252] In some embodiments, the promoter comprises an insulin promoter or a fragment thereof.

[0253] 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-1α promoter or variant thereof, e.g., as provided in Table 40. In some embodiments, the EF-1α promoter comprises the nucleotide sequence of any one of SEQ ID NOs: 2100, 2101, 2103, 2104, 2108, 2109, 2111-2120, or any one of the nucleotide sequences provided in Table 8, a nucleotide sequence comprising at least one, two, three, four, five, six or seven but no more than ten modifications, e.g., substitutions, insertions, or deletions, relative to the nucleotide sequence of SEQ ID NOs: 2100, 2101, 2103, 2104, 2108, 2109, 2111-2120, or any one of the nucleotide sequences 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: 2100, 2101, 2103, 2104, 2108, 2109, 2111-2120, or any one of the nucleotide sequences provided in Table 8.

Table 8. Exemplary Promoter Variants

Description	Sequences	SEQ ID NO:
EF1a Promoter (intron underlined)	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCG AGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCG GGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTG GGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACG GGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCC TCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGT ACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGC CTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGC GCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTT TCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTT TCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGG TTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGG CGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAG CTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCT GGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGC TTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGC GGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCG CTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCT CGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGG AGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGA TGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCA AGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGA	2100
miniEF1a	GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGA GGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTAT ATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGA ACACGCGTAAG	2101
Promoter Variant 1	GCATG	
Promoter Variant 2	GGTGGAGAAGAGCATG	2103
Promoter Variant 3	GTCATCACTGAGGTGGAGAAGAGCATG	2104
Promoter Variant 4	CGTGAG	
Promoter Variant 5	GT	
Promoter Variant 6	GCTCCGGT	
Promoter Variant 19	GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGA GGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTAT ATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGA ACACAG	2108
Promoter Variant 20	GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGA GGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTAT ATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGA ACACGC	2109
Promoter Variant 7	GTAAG	

Promoter Variant 8	GTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGG GAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGG AAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGT ATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCA GAACACGCGTAAG	2111
Promoter Variant 9	GCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGT TGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAA ACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAG AACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTG CCGCCAGAACACGCGTAAG	2112
Promoter Variant 10	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCG AGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCG GGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTG GGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACG GGTTTGCCGCCAGAACACGCGTAAG	2113
Promoter Variant 11	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCG AGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCG GGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTG GGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACG GGTTTGCCGCCAGAACACAG	2114
Promoter Variant 12	GCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGT CCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTG GCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGA GGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCG CAACGGGTTTGCCGCCAGAACACGCGTAAG	2115
Promoter Variant 13	GCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGT CCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTG GCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGA GGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCG CAACGGGTTTGCCGCCAGAACACAG	2116
Promoter Variant 14	GGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACA TCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCC TAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGC CTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAAC GTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACGCGTAAG	2117
Promoter Variant 15	GGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACA TCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCC TAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGC CTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAAC GTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAG	2118
Promoter Variant 16	GTCATCACTGAGGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGG CAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATT GAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGT ACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAG TCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACGCGTAAG	2119
Promoter Variant 18	GTCATCACTGAGGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGG CAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATT GAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGT ACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAG TCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAG	2120

Viral Genome Component: Untranslated Regions (UTRs)

[0254] 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.

[0255] 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.

[0256] 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'.

[0257] In one embodiment, the 5'UTR in the viral genome includes a Kozak sequence.

[0258] In one embodiment, the 5'UTR in the viral genome does not include a Kozak sequence.

[0259] 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-a, 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*.

[0260] 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.

[0261] In one embodiment, the 3' UTR of the viral genome may include an oligo(dT) sequence for templated addition of a poly-A tail.

[0262] 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.

[0263] 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.

[0264] 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.

[0265] 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.

[0266] 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

[0267] 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

[0268] 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/immunoglobin splice acceptor (500 bps), SV40 late splice donor/splice acceptor (19S/16S) (180 bps) and hybrid adenovirus splice donor/IgG splice acceptor (230 bps).

[0269]

Viral Genome Component: Stuffer sequences

[0270] In some embodiments, the viral genome of an AAV particle described herein 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.

[0271] In some embodiments, the stuffer or filler sequence may be from about 100-3500 nucleotides in length. The stuffer sequence may have a length of about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, or 3000 nucleotides.

Viral Genome Component: miRNA

[0272] In some embodiments, 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 miR-122 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 miR-142-3p, 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 miR-1, 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

[0273] 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.

[0274] 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.

[0275] 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.

[0276] 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.

[0277] 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.

[0278] 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.

[0279] 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, to 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.

[0280] In some embodiments, an encoded miR binding site or sequence region is at least about 10 to about 125 nucleotides in length, e.g., at least about 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 about 7 to about 28 nucleotides in length, e.g., at least about 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).

[0281] 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 ACAAACACCATTGTCACACTCCA (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 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.,

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: ACAAACACCATTGTCACACTCCACACAAACACCATTGTCACACTCCACACAAACACCATTGTCACACT CCA (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 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: 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 comprising 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.

[0282] 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 miR-1 binding site. In some embodiments, the encoded miR-1 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 (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: 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 miR-1 binding site, e.g., an encoded miR-1 binding site series. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR-1 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 comprising 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.

[0283] 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.

[0284] In embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR-142-3p binding site sequence. In some embodiments, the encoded miR-142-3p 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 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: 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 miR-142-3p binding site, e.g., an encoded miR-142-3p binding site series. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR-142-3p 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 comprising 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.

[0285] 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.

[0286] 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 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 (e.g., conservative 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 miR-183 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 comprising 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).

[0287] 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 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: 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 comprising 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).

[0288] 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 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: 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 comprising 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).

[0289] In some embodiments, the encoded miR binding site series comprises a miR122 binding site, a miR-1, 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 comprising 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.

[0290] 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 miR-1, 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 comprising 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.

[0291] 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 miR-122 binding site and a miR-1 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 comprising 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

[0292] 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.

[0293] In one embodiment, the viral genome may be a small single stranded viral genome. A small single stranded viral genome may be 2.1 to 3.5 kb in size such as, but not limited to, about 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, and 3.5 kb in size.

[0294] In one embodiment, the viral genome may be a small double stranded viral genome. A small double stranded viral genome may be 1.3 to 1.7 kb in size such as, but not limited to, about 1.3, 1.4, 1.5, 1.6, and 1.7 kb in size.

[0295] In one embodiment, the viral genome may be a medium single stranded viral genome. A medium single stranded viral genome may be 3.6 to 4.3 kb in size such as, but not limited to, about 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2 and 4.3 kb in size.

[0296] In one embodiment, the viral genome may be a medium double stranded viral genome. A medium double stranded viral genome may be 1.8 to 2.1 kb in size such as, but not limited to, about 1.8, 1.9, 2.0, and 2.1 kb in size.

[0297] In one embodiment, the viral genome may be a large single stranded viral genome. A large single stranded viral genome may be 4.4 to 6.0 kb in size such as, but not limited to, about 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 and 6.0 kb in size.

[0298] In one embodiment, the viral genome may be a large double stranded viral genome. A large double stranded viral genome may be 2.2 to 3.0 kb in size such as, but not limited to, about 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 and 3.0 kb in size.

Payloads

[0299] 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.

[0300] 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.

[0301] 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

[0302] In some embodiments, the encoded payload of the 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.

[0303] 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

[0304] 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.

[0305] 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.

[0306] 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.

[0307] 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).

[0308] 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.

[0309] 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).

[0310] 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

[0311] 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

[0312] 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.

[0313] 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.

[0314] 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.

[0315] 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.

[0316] 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.

[0317] 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.

[0318] In some embodiments, the encoded RNAi agent is an siRNA.

[0319] 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%.

[0320] 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 of any disease associated with the central or peripheral nervous systems.

Design of siRNA

[0321] 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.

[0322] 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.

[0323] 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.

[0324] 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.

[0325] 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.

[0326] 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.

[0327] 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.

[0328] 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.

[0329] 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.

[0330] 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.

[0331] 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.

[0332] 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

[0333] In some embodiments, the siRNA molecules may be encoded in a modulatory polynucleotide which also comprises a molecular scaffold.

[0334] 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.

[0335] 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.

[0336] In one embodiment, the molecular scaffold comprises a dual-function targeting modulatory polynucleotide. 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.

[0337] 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

[0338] 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.

[0339] 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.

[0340] 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).

[0341] 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

[0342] 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.

[0343] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant described herein, e.g., an AAV5 capsid variant) is 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) is 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.

[0344] 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.

[0345] 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.

[0346] 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.

[0347] 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.

[0348] 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.

[0349] 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.

[0350] 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.

[0351] 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 (ALS). 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.

[0352] 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.

[0353] 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.

[0354] 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.

[0355] 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).

[0356] 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), adrenoleukodystrophy (ALD), 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.

[0357] 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.

[0358] 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.

[0359] 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.

[0360] 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.

[0361] 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.

[0362] 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.

[0363] 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.

[0364] 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.

[0365] 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.

[0366] 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.

[0367] 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.

[0368] 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.

[0369] 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.

[0370] 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.

[0371] 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.

[0372] 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.

[0373] 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.

[0374] 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.

[0375] 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.

[0376] 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.

[0377] 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.

[0378] 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.

[0379] 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.

[0380] 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.

[0381] 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.

[0382] 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 neuro-oncological disorder in a subject. 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.

[0383] 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.

[0384] 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 muscular disorder and/or neuromuscular disorder in a subject. In some embodiments, treatment of a muscular disorder and/or neuromuscular disorder comprises prevention of said muscular disorder and/or neuromuscular disorder.

[0385] 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.

[0386] 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, MIH7, BAG3, MIPN, TNNT2, SCN5A, RBN20, TNPO, LAMA4, VCL, LDB3, TCAP, PSEN1/2, ACTN2, CRYAB, TPM1, ABCC9, ACTC1, PDLIM3, ILK, TNNC1, TNNI3, PLN, DES, SGCD, CSRP3, MIH6, 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.

[0387] 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.

[0388] 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

[0389] 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.

[0390] 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.

[0391] 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.

[0392] 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.

[0393] 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

[0394] In some embodiments, an AAV particle disclosed herein (e.g., an AAV particle comprising an AAV capsid variant) may be administered by a to a subject by a delivery route, e.g., a localized delivery route or a systemic delivery route.

[0395] 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.

[0396] In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered intramuscularly, intravenously, intracerebrally, intrathecally, intracerebroventricularly, via intraparenchymal administration, or via intra-cisterna magna injection (ICM).

[0397] 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.

[0398] 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.

[0399] 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

[0400] In some embodiments, an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., 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, 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, e.g., an AAV particle described herein, is administered to the subject intravenously. In some embodiments, the subject is a human.

Administration to the CNS

[0401] 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.

[0402] 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.

[0403] 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

[0404] 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.

[0405] 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.

[0406] 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.

[0407] In some embodiments, an AAV particle pf 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 particles can be transported to the central nervous system, for example, to the motor neurons (e.g., U.S. Patent Publication Nos. 20100240739 and 20100130594; the content of each of which is incorporated herein by reference in their entirety).

[0408] 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

[0409] 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 polypeptide, e.g., an AAV capsid variant) are formulated in depots for extended release. Generally, specific organs or tissues are targeted for administration.

[0410] In some embodiments, a pharmaceutical composition and/or an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., 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.

[0411] 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 polypeptide, e.g., 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

[0412] 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 described herein) 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 embodiments, 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 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).

[0413] 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.

[0414] 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.

[0415] 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.

[0416] 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.

[0417] 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).

[0418] 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.

[0419] 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 $1x10^4$ VG per subject. As a non-limiting example, the AAV particles are administered via IV injection at a dose of $2x10^{13}$ VG per subject.

[0420] 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).

[0421] 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.

[0422] 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.

[0423] 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.

[0424] 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.

[0425] 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).

[0426] 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 brains stem.

[0427] 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.

[0428] 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.

[0429] 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.

[0430] 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.

[0431] 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.

[0432] 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.

[0433] 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.

[0434] 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.

[0435] 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).

[0436] 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.

[0437] 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.

[0438] 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.

[0439] 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.

[0440] 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.

[0441] 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.

[0442] 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.

[0443] 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.

[0444] 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 or an antibody or antibody fragment, 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.

[0445] 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.

[0446] 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.

[0447] 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.

[0448] 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.

[0449] 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.

[0450] 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.

[0451] 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 GSK3β (lithium) or PP2A, immunization with Aβ 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).

[0452] 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.

[0453] 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).

[0454] 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

[0455] 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.

[0456] 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.

[0457] 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.

[0458] 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.

[0459] *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.

[0460] *AAV Particle*: As used herein, an "AAV particle" refers to an AAV capsid, e.g., an AAV capsid variant, and a polynucleotide, e.g., a viral 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.

[0461] *Amelioration*: As used herein, the term "amelioration" or "ameliorating" refers to a lessening of severity of at least one indicator of a condition or disease. For example, in the context of a neurodegeneration disorder, amelioration includes the reduction of neuron loss.

[0462] *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.

[0463] *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. When referring to a measurable value such as an amount, a temporal duration, and the like, the term is meant to encompass is meant to encompass variations of ±20% or in some instances ±10%, or in some instances ±5%, or in some instances ±1%, or in some instances ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0464] *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%, >90%, 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.

[0465] *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.

[0466] *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% 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.

[0467] *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.

[0468] *Expression*: As used herein, "expression" of a nucleic acid sequence refers to production of an RNA template from a DNA sequence (*e.g.*, by transcription). In some embodiments, expression further comprises one or more of: (1) processing of an RNA transcript (*e.g.*, by splicing, editing, 5′ cap formation, and/or 3′ end processing); (2) translation of an RNA into a polypeptide or protein; and (3) post-translational modification of a polypeptide or protein.

[0469] *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. In some embodiments, a fragment is a nucleic acid fragment.

[0470] *Identity*: As used herein, "identity" refers to the subunit sequence identity between two polymeric molecules, e.g., between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; e.g., if a position in each of two DNA molecules is occupied by adenine, then they are identical at that position. The identity between two sequences is a direct function of the number of matching positions; e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two sequences are identical, the two sequences are 50% identical; if 90% of the positions (e.g., 9 of 10), are matched, the two sequences are 90% identical. 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)).

[0471] *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.

[0472] *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 the components 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 or may be incorporated into a vector.

[0473] *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).

[0474] *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, e.g., 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.

[0475] *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.

[0476] *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. 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.

[0477] *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.

[0478] *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.

[0479] *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.

[0480] *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.

[0481] *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.

[0482] 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.

[0483] *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.

[0484] *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).

[0485] *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.

[0486] *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), e.g., 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 viral genome encoding the miR binding site.

[0487] 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.

[0488] *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.

[0489] *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).

[0490] *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.

[0491] *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.

[0492] *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.

[0493] *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.

[0494] *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.

[0495] *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.

[0496] *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.

[0497] *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.

[0498] *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).

[0499] *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.

[0500] *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.

[0501] *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. Vectors of the present disclosure may be produced recombinantly. The heterologous molecule may be a polynucleotide and/or a polypeptide.

[0502] *Viral Genome:* As used herein, the term "viral genome" refers 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

[0503] 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.

[0504] 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.

[0505] 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.

[0506] 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.

[0507] The present disclosure is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1. High-throughput screen of TRACER AAV library in NHP and Rats

[0508] A TRACER based method as described in WO 2020/072683, WO 2021/202651, and WO 2021/230987, 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 high throughput screening by NGS. Briefly, the library of AAV capsid variants was generated using a mutagenesis approach, where sequences of 7 to 8 amino acids in length were inserted into different positions across loop VIII of AAV5, including between residues 570-584, relative to a reference sequence numbered according to SEQ ID NO: 138. The initial library was passed three times through non-human primates (NHP), specifically cynomolgus macaques (*Macaca fascicularis*), rats, or human brain microvascular endothelial cells (hBMVECs). Following the third passage in each system, 572 variants from the NHPs, 80 variants from the rats, and 99 variants from the hBMVECs were pooled into a passage 3 synthetic library of 747 total variants. This library was then passaged in NHPs and rats. After this passage (e.g., one-month post injection into two NHPs and the rats), RNA was extracted from three brain regions. Following RNA recovery and RT-PCR amplification, a systematic NGS enrichment analysis was performed to calculate fold enrichment relative to an AAV5 wild-type control in NHPs (**Table 9,** left column) and rats (**Table 9,** right

column) and the peptides comprised within the variants were identified in both animals. Fold enrichment values above 1 indicate an increase in expression relative to AAV5. All libraries of AAV5 variants generated and passaged in the different hosts were under the control of the synapsin promoter.

[0509] As shown in **Table 9**, approximately 288 variants were identified with an average fold change greater than wild-type AAV5 in the brain of NHPs. Of the 288 NHP variants, 27 demonstrated a fold-change of greater than 5 compared to wild-type, with 1 variant demonstrating a fold change of greater than 60. For instance, the variant comprising YPAEVVQK (SEQ ID NO: 943) demonstrated a 64.9-fold enrichment in the brain of NHPs.

[0510] As shown in **Table 9**, approximately 98 variants were identified with an average fold change greater than wild-type AAV5 in the brain of rats. Of the 98 variants, 33 demonstrated a fold-change of greater than 2 compared to wild-type, with one variant demonstrating a fold change of greater than 40. For instance, the variant comprising YPAEVVQK (SEQ ID NO: 943) demonstrated a 41.1-fold enrichment in the brain of rats.

[0511] The variant comprising YPAEVVQK (SEQ ID NO: 943) which demonstrated a high fold enrichment in the brains of NHPs relative to wild-type AAV5 (64.9-fold enrichment), also demonstrated a high fold-change in the brains of rats (41.1-fold enrichment). This indicates that this AAV capsid variant comprising SEQ ID NO: 943 is able to cross species, as evidenced by increased expression and tropism in both the NHP and rat brain.

Table 9. NGS fold-enrichment of AAV capsid variants in NHPs and rats

Peptide Sequence	SEQ ID NO:	Fold enrichment over AAV5 in the brain of NHPs	Peptide Sequence	SEQ ID NO:	Fold enrichment over AAV5 in the brain of Rats
YPAEVVQK	943	64.910	YPAEVVQK	943	41.054
RNQKVQGG	202	23.240	NHVVVISG	432	25.930
PDNRVKPV	203	17.843	TYAKDTSM	659	16.868
ARVEVSAG	204	12.970	TVLAQSTP	547	7.098
MLKRVITI	205	10.323	TFQTANKV	235	6.518
ESTHVELI	206	10.126	RAQTVSVK	638	5.209
GRVHVNMA	207	9.652	TMLNSAQQ	666	4.636
TSQSSTKS	208	9.586	RLAKVTQP	223	4.558
AKQVVKPP	209	8.506	TIPNKVTA	779	3.480
STMDVNKR	210	8.284	TPEATNHK	271	3.471
KKSQVANA	211	8.264	TTLSAKDR	383	3.334
TNAVSTKQ	212	8.071	PSPNVTKN	295	3.285
TLATHENM	213	7.093	TRPNPSPK	850	3.255
VKGKVSAG	214	6.637	TANSVKSQ	319	3.003
IRQKVPAS	215	6.467	SDNLVKHD	664	2.973
GRSEVLKG	216	6.139	LRGQVKSN	248	2.932
TPIKTAVR	217	6.088	TLKHSDLS	264	2.904
CKLQVDCQ	218	5.842	EVKMVSNV	380	2.787

TKLKPAGP	219	5.830	RNQKVQGG	202	2.769
NTMHVELR	220	5.721	RLVTVKSP	332	2.690
TKTKLSSP	221	5.686	IIAEVGKQ	528	2.519
DNMKVLGG	222	5.585	TKQMSDHG	336	2.455
RLAKVTQP	223	5.454	TIGGMKGK	390	2.435
GSNMVASK	224	5.325	SHSDVSVR	776	2.383
TMAPTKNP	225	5.241	TKTLQTSP	631	2.364
TAQHTTLS	226	5.040	VMSNVTQR	483	2.216
TQPSGKTQ	227	5.024	TKLSAVTK	755	2.213
MNSKVSSV	228	4.985	TLASHENI	329	2.198
TEATVKSN	229	4.971	TKLKPAGP	219	2.161
TSEIQGAK	230	4.969	TTAKPTVE	516	2.133
LSSNVTKA	231	4.952	TSEPPSTE	930	2.078
TPLCGMAL	232	4.830	ALNSVRRE	781	2.053
NNTAVSNK	233	4.789	LHNKVNDV	680	2.014
NKNRVRDL	234	4.770	LSSNVTKA	231	1.953
TFQTANKV	235	4.669	TSNAVRQQ	765	1.948
TTKQPKAL	236	4.662	QGANVVKQ	320	1.901
GSAEVKMR	237	4.650	KKPTVATI	300	1.892
SLLQVKLE	238	4.515	KMAGVARE	615	1.872
THAVSTKP	239	4.431	TMASQEHM	661	1.857
DINSVRRG	240	4.425	GGQGVNQS	821	1.812
QSKTVPAK	241	4.181	RPQKVSSA	386	1.741
TGKPDKSY	242	4.163	NLNIVRAP	869	1.728
TKIPSTSR	243	4.055	GMNSVRNA	854	1.726
TPAATNPK	244	4.036	LSSKVTDS	1596	1.622
TITKPPAP	245	4.007	NPTVVKSP	303	1.576
GRLIVTDG	246	4.003	NSPNVSRQ	589	1.563
TPKKSTSV	247	3.994	MLNEVSRR	872	1.562
LRGQVKSN	248	3.979	ITSSVARS	891	1.561
GLPDVIKQ	249	3.920	TKIPSTSR	243	1.533
TREQTSTA	250	3.892	TKVSGTPD	267	1.516
TRNGQPVN	251	3.847	ALDAVSNK	283	1.514
TRASLSIK	252	3.807	SRENVSRQ	478	1.464
SFSEVKNM	253	3.748	EAMMVDRK	608	1.401
TQSRSGGP	254	3.666	THTRIIAT	784	1.397
TSTPAVSQ	255	3.650	LSTKVSDQ	721	1.396
TASVPATS	256	3.626	TSSITPNP	268	1.387
TLASHENK	257	3.600	TATPSQSK	335	1.373
TQSSDHKV	258	3.590	RSKSVNPM	327	1.367
TLASQEHM	259	3.590	RYMTVKTQ	2083	1.363
TKGLPTQM	260	3.581	TTMSATSP	324	1.354
SSVVVDKM	261	3.542	TSTPAVSQ	255	1.348
TLDSHEHK	262	3.516	PLKNVPPG	577	1.329
TNQKVTGS	263	3.511	TPIKTAVR	217	1.318
TLKHSDLS	264	3.463	TNANQVTR	691	1.296
TTSTVSSP	265	3.431	TSSSPSNP	354	1.280
TKASHEHM	266	3.359	TNGATLGS	705	1.261
TKVSGTPD	267	3.330	KLLKVGSS	316	1.249

TSSITPNP	268	3.328	PSNNVKAL	469	1.222
AAETVKPN	269	3.279	TPLCGMAL	232	1.213
TNTQSKLS	270	3.271	YANSVKAM	477	1.207
TPEATNHK	271	3.229	KRLNVESR	448	1.195
TGGRNPGG	272	3.227	IKSGVQQK	348	1.193
TGGRVQGP	273	3.224	TTDLTTVE	431	1.173
KMSGVVGK	274	3.194	KPSKVGGV	423	1.170
KGGVVKTV	275	3.190	IKPPVPKA	883	1.161
TNGASSKL	276	3.143	PFQAVLGS	604	1.160
SSGDVKLW	277	3.108	SSPNVSRN	284	1.135
TSGTPNNF	278	3.012	NKLGVVTK	439	1.130
KRTTVSSP	279	3.010	KKSQVANA	211	1.116
TLASNENM	280	2.997	KEESVDTS	886	1.110
TKQKDLPR	281	2.987	TPVSVNTH	967	1.098
ATNVVSSK	282	2.984	TRETPSTE	507	1.095
ALDAVSNK	283	2.971	LGSNVSAR	414	1.093
SSPNVSRN	284	2.955	KAVSVDTS	579	1.088
THAASNKS	285	2.933	TPMSTSDL	688	1.069
TSSSTKSI	286	2.925	PVNVVRAS	797	1.059
EGKLVGTQ	287	2.923	VSGNVSRS	677	1.047
PNKLVGSV	288	2.903	AIEKVSVN	498	1.046
THASLPKP	289	2.868	TLGHSAKQ	511	1.046
TPGNSARS	290	2.856	QQDSVPGQ	515	1.033
TAPKVGNM	291	2.846	DNMKVLGG	222	1.030
TRALVKPP	292	2.846	TPGNSARS	290	1.030
GLEDVKGR	293	2.823	THAVTTKQ	406	1.030
QSEKVHMG	294	2.812	NCTVVQCR	326	1.024
PSPNVTKN	295	2.783	TSNRTTLQ	561	1.013
THAQSTKP	296	2.736	TITKPPAP	245	1.010
RPKTVSTF	297	2.722	TPTPPSAQ	436	1.007
EPSEVHKM	298	2.720	TLRTNESV	801	1.000
TNQKPTLQ	299	2.665	TTAPATGT	489	1.000
KKPTVATI	300	2.661	TTRAQTTK	858	0.991
TATVQSNP	301	2.652	KSRSVSGV	896	0.977
YQSDVPNR	302	2.646	TAGTTVTP	463	0.976
NPTVVKSP	303	2.624	WSNSVSTR	546	0.961
TVGSESRP	304	2.595	TGKLPSGQ	391	0.960
TSKSAAVR	305	2.591	NSNRVGGN	313	0.957
TLANHEHM	306	2.582	TVSSPSGG	709	0.955
TLASHENM	307	2.582	DVASVSSK	602	0.955
RKPSVQTV	308	2.574	TQPSGKTQ	227	0.944
TGRDVPKL	309	2.507	IRQKVPAS	215	0.939
SQSSVKTP	310	2.492	DANSVKVV	522	0.938
ATSVVSQA	311	2.477	QAHRVPVE	2082	0.932
TGTKVESY	312	2.474	KRTTVSSP	279	0.922
NSNRVGGN	313	2.470	TSLQVSKI	559	0.921
TMTRVGDG	314	2.455	AGNSVRVS	537	0.920
NNGNVSAK	315	2.413	AKQVVKPP	209	0.919
KLLKVGSS	316	2.402	LAKTVNQT	442	0.912

SQPKVSTA	317	2.402	TPLDKRIR	895	0.912
MNDKVLTN	318	2.395	RQNKVTSG	863	0.897
TANSVKSQ	319	2.373	ARVEVSAG	204	0.894
QGANVVKQ	320	2.367	THAASNKS	285	0.888
TSSVSKQP	321	2.363	KMSGVVGK	274	0.873
SQGNVAKA	322	2.354	ISNMVRSS	848	0.872
IYVAVPAA	323	2.324	TTSTVSSP	265	0.863
TTMSATSP	324	2.321	TAVKSQMP	339	0.844
TFTATSNP	325	2.315	MNSKVSSV	228	0.841
NCTVVQCR	326	2.289	TSSSTKSI	286	0.840
RSKSVNPM	327	2.267	TAKILSTE	2084	0.836
GQVDVPKL	328	2.266	TDRNTPKL	351	0.834
TLASHENI	329	2.266	MVNVVHQS	813	0.829
THNSVRDR	330	2.233	TEATVKSN	229	0.825
GQSTVGQM	331	2.231	TSQSSTKS	208	0.822
RLVTVKSP	332	2.229	LVNAVKSF	811	0.821
THEVSTKQ	333	2.218	KIKVVNTP	342	0.817
TDKKQTTS	334	2.208	VLSEVKLQ	640	0.810
TATPSQSK	335	2.206	VKGKVSAG	214	0.809
TKQMSDHG	336	2.109	ATLSVSTN	618	0.806
TLASNEHK	337	2.108	VNSKVISD	549	0.793
NMSPVPKL	338	2.106	NAVGVAHG	754	0.791
TAVKSQMP	339	2.100	TSASKVSH	357	0.788
SNGAVGKH	340	2.092	AKDAVSGL	617	0.788
SNSKVNTG	341	2.081	TSGTVGTK	447	0.787
KIKVVNTP	342	2.068	TGLSVSTN	636	0.777
LLETVATK	343	2.060	MSNKVLRD	532	0.777
DSAKVMPQ	344	2.045	YMNSVKSF	876	0.777
TTKEVLSG	345	2.042	TLASNENM	280	0.776
ISSNVARG	346	2.037	ATNVVSSK	282	0.769
TGSTQSDL	347	2.027	TNQSGPGS	922	0.766
IKSGVQQK	348	2.023	QLNKVVIR	479	0.758
TATATQSK	349	2.017	LSNSVTPR	509	0.754
DGSQVHNR	350	1.996	TTKEVLSG	345	0.748
TDRNTPKL	351	1.984	TQSSDHKV	258	0.741
TTSGSPQP	352	1.966	YSNNVVKS	672	0.740
THEVSTKP	353	1.958	EGLKVLVG	497	0.739
TSSSPSNP	354	1.933	TTTSVNRP	746	0.739
LVAPVKQV	355	1.924	TDNRVRSY	880	0.732
LELLVPKL	356	1.922	TSSPGMKL	753	0.731
TSASKVSH	357	1.903	TTKQPKAL	236	0.726
LNAGVSSP	358	1.901	LGNRVSAL	722	0.722
TVTNGGKG	359	1.886	KLRQVNAI	481	0.715
TLGKVSLE	360	1.879	TMLSSAEV	793	0.713
NQEAVVKP	361	1.874	AVVGVGQA	936	0.711
AAMTVQKV	362	1.851	TTSKNRPD	900	0.709
KPQDVSSR	363	1.843	TKLNVTAS	502	0.705
YESKVQHT	364	1.837	TATSSHQQ	627	0.703
NMRGVEVK	365	1.835	TNMASSGK	373	0.699

TREKPSTA	366	1.816	PSSDVVSQ	374	0.696
VERSVTSN	367	1.805	IGPSVGTD	606	0.693
VGDLVHTG	368	1.803	TLPTPRQH	620	0.692
KNDHVSLS	369	1.796	STNTVRVN	487	0.688
SNEKVSMS	370	1.793	VGNRVTGN	758	0.688
TRRAQESP	371	1.782	NKNRVRDL	234	0.687
TTVKSDQS	372	1.773	TPEETNPK	711	0.686
TNMASSGK	373	1.759	YVSNVAKA	499	0.680
PSSDVVSQ	374	1.757	RSNKVRET	467	0.668
TLSSHENM	375	1.755	KSLDVSIS	518	0.666
TNPSMQSP	376	1.741	TPTPKNSH	614	0.660
GVSLVKTG	377	1.703	TSETPSTA	486	0.648
TPRNGAQP	378	1.701	TRALVKPP	292	0.647
TVSDKSMS	379	1.689	SNSKVNTG	341	0.643
EVKMVSNV	380	1.678	TLTQTVRD	918	0.641
VSAGVSKN	381	1.676	NNGNVSAK	315	0.637
LSKGVGHE	382	1.673	SRENVSRS	445	0.636
TTLSAKDR	383	1.668	ISNSVRQS	745	0.630
TMASHEHK	384	1.639	TLATQEHM	433	0.629
TKALTSGP	385	1.638	TDKQASGN	495	0.629
RPQKVSSA	386	1.636	THNSVRDR	330	0.628
TPQGVSSP	387	1.601	RSTGVHSD	550	0.625
TKGTTSSH	388	1.590	TTALHTEE	849	0.624
TMTSVVSP	389	1.576	TMASHEHK	384	0.622
TIGGMKGK	390	1.564	QRNLVKSA	609	0.622
TGKLPSGQ	391	1.555	QDSTVSKQ	519	0.618
AQDAVSAR	392	1.551	TSPNRNQS	830	0.617
TKVPSVSP	393	1.551	TGGRNPGG	272	0.616
DKGTVERR	394	1.538	KAATVQEP	527	0.612
SKLDVNQR	395	1.538	TKALTSGP	385	0.611
VSGKVVHQ	396	1.528	TSPLANKS	496	0.609
ASNKVGTL	397	1.525	SANKVKPD	541	0.608
TKPPGQQL	398	1.523	NNTAVSNK	233	0.607
TLASQEHK	399	1.516	HTNSVRTP	599	0.599
SIGKVGVG	400	1.511	GSNDVRNT	628	0.598
TRGASNSP	401	1.510	PSTGVVNP	822	0.596
TNAVSTKP	402	1.492	RKPSVQTV	308	0.594
TVSSSPGT	403	1.481	SQRGVSNP	690	0.591
KAETVDTS	404	1.461	YFTGVKSE	979	0.581
TLAYHEHK	405	1.459	PLVGVGSP	716	0.581
THAVTTKQ	406	1.456	TMRTAQSK	2086	0.580
TLATHEHK	407	1.455	HSNMVRNA	844	0.578
TSLNDARR	408	1.445	LNAGVSSP	358	0.577
TNVQSSGK	409	1.433	KPQDVSSR	363	0.575
APKPVMSG	410	1.423	SGNMVRAA	795	0.573
TSVSNPSK	411	1.408	TVQNSAST	592	0.572
TTSQTSAP	412	1.390	VLGSVSSN	416	0.569
TRQPSVTR	413	1.388	TPKKSTSV	247	0.568
LGSNVSAR	414	1.379	TGSTQSDL	347	0.567

TNPGVSNS	415	1.378	SANMVKHD	675	0.566
VLGSVSSN	416	1.378	GHRVVNNE	712	0.566
TLTSTGGN	417	1.366	SSSYVTVD	491	0.565
MIAKVRTE	418	1.359	GVSLVKTG	377	0.564
NGKQVSVH	419	1.349	GLPDVIKQ	249	0.564
TLLKSDRS	420	1.348	TTETASRG	975	0.563
TAGQVSSS	421	1.343	SQVVVSEK	452	0.561
TTMLAGGP	422	1.320	LRANVSSS	626	0.560
KPSKVGGV	423	1.313	LSSNVSRA	462	0.559
LSNNVRIK	424	1.309	GRLIVTDG	246	0.558
THAHVKLE	425	1.308	TTLSVSTP	905	0.551
VTKHVDLL	426	1.306	THSGVVIR	2085	0.547
SRAEVAQR	427	1.301	SITGVAKM	752	0.547
SCTNVASC	428	1.292	GGNLVRSV	730	0.546
TNSRKENI	429	1.288	SQGNVAKA	322	0.542
QQVSVPGP	430	1.278	NTDAVNQA	2081	0.541
TTDLTTVE	431	1.271	SKLDVNQR	395	0.536
NHVVVISG	432	1.254	THTTIKSP	446	0.536
TLATQEHM	433	1.246	GGGKVTDT	583	0.535
TPRSKPSP	434	1.238	TVNTTRMA	867	0.534
TTRKPQTT	435	1.229	TTSGSPQP	352	0.534
TPTPPSAQ	436	1.225	HLSNVSKV	504	0.529
TQKPHPQG	437	1.213	TTPANSRV	538	0.526
TQLSMKAN	438	1.203	TLATHEHK	407	0.522
NKLGVVTK	439	1.202	SSKEVSRV	749	0.517
NQTDVPPR	440	1.200	HYSKVADP	695	0.515
THAETTKP	441	1.186	TFTATSNP	325	0.515
LAKTVNQT	442	1.185	TSVDKARV	492	0.514
TRPGVSDR	443	1.182	STNAVRSS	842	0.512
LMNRVTQY	444	1.181	KQRQVSVQ	747	0.511
SRENVSRS	445	1.177	RKTGVMSS	748	0.504
THTTIKSP	446	1.171	NHTTVGER	521	0.501
TSGTVGTK	447	1.165	LPSQVVKT	720	0.499
KRLNVESR	448	1.164	SGTTVDSM	454	0.499
TMAPPGKI	449	1.162	TRQPSVTR	413	0.497
TVHKASMQ	450	1.153	ANLKVIVK	723	0.492
TATASQTK	451	1.153	SGTEVASL	799	0.491
SQVVVSEK	452	1.149	TNGASSKL	276	0.490
GTATVTTR	453	1.147	DINSVRRG	240	0.487
SGTTVDSM	454	1.144	TKGTTSSH	388	0.481
ASTVVLEK	455	1.143	TERASSSL	694	0.481
TASATQTK	456	1.141	ADTRVSNP	544	0.480
SVLAVNKS	457	1.138	EPSEVHKM	298	0.477
SANLVKND	458	1.134	PDNRVKPV	203	0.477
RDAQVPKL	459	1.129	RGKAVANN	501	0.477
PQRAVQEV	460	1.124	TRGASNSP	401	0.476
TGKSPDQN	461	1.123	TAVVVNSV	578	0.475
LSSNVSRA	462	1.122	SLKSVSLD	630	0.474
TAGTTVTP	463	1.117	TMNPSRNP	707	0.471

TGSSPTGS	464	1.115	KSRNVPTL	653	0.467
KRNEVISK	465	1.112	TRASLSIK	252	0.467
TLASHEHK	466	1.105	SGSKVPTL	505	0.464
RSNKVRET	467	1.101	STLDVARR	710	0.462
TSEKIQVM	468	1.100	THAESTKQ	762	0.462
PSNNVKAL	469	1.098	VERSVTSN	367	0.457
TLATLSTV	470	1.098	KFAAVPGI	1595	0.453
THLTPKGN	471	1.097	TLATHENM	213	0.450
TPASVKGT	472	1.091	TSTRSSGN	731	0.449
TPGTPSAG	473	1.091	TREKPSTA	366	0.447
RKNTVVNS	474	1.090	ILSNVSSR	670	0.447
PQKSVSNL	475	1.088	TLTKTTMG	555	0.447
TRLSSSGS	476	1.083	TTRKPQTT	435	0.446
YANSVKAM	477	1.074	FLNAVRGN	717	0.443
SRENVSRQ	478	1.067	SVLAVNKS	457	0.442
QLNKVVIR	479	1.046	AAMTVQKV	362	0.440
TVKEKGMQ	480	1.043	TSNSVRLG	612	0.440
KLRQVNAI	481	1.039	TVSSSPGT	403	0.434
NASMVANK	482	1.038	ANTNVTRP	847	0.428
VMSNVTQR	483	1.026	TPEPPTTA	929	0.426
GSGSVSKA	484	1.025	LSNNVRIK	424	0.426
TAVKVDHS	485	1.025	TTAMHTVE	713	0.424
TSETPSTA	486	1.015	SKLVVSDR	585	0.423
STNTVRVN	487	1.009	LLNKVPLN	928	0.422
LSNQVSAR	488	1.005	SQPKVSTA	317	0.419
TTAPATGT	489	1.000	RKQAVSVA	887	0.419
TVLQKSTG	490	0.993	TVSDKSMS	379	0.415
SSSYVTVD	491	0.982	SGLNVTTR	798	0.415
TSVDKARV	492	0.978	TGSSPTGS	464	0.409
RRATVTTP	493	0.978	TREQTSTA	250	0.408
TGGRTPGG	494	0.971	MPNTVRAH	852	0.408
TDKQASGN	495	0.969	SSGDVKLW	277	0.406
TSPLANKS	496	0.963	VNNRVHQS	581	0.404
EGLKVLVG	497	0.949	SDNLVKTD	774	0.403
AIEKVSVN	498	0.943	TSPNRVSY	864	0.401
YVSNVAKA	499	0.942	LGNRVSST	807	0.399

[0512] Taken together, these results demonstrate that after 3 rounds of screening of this AAV5 variant library with loop VIII modifications in NHP and rats, many AAV capsid variants outperformed the wild-type AAV5, for example, in penetration of the blood brain barrier (BBB) and expression in the brain. Also, capsid variants were identified that could infect both rats and NHPs, indicating cross-species tropism and compatibility.

Example 2. Individual capsid characterization in NHPs, rats, and mice

[0513] This Example describes the transduction level, tropism, ability to cross the blood brain barrier, and overall spatial distribution in the central nervous system (CNS) of an AAV capsid variant selected from the study described in Example 1, relative to wild-type AAV5, following intravenous

injection in cynomolgus macaques (*Macaca fascicularis*), Norway rats, and BALB/c mice. The capsid variant was TTN-002 (SEQ ID NO: 982 (amino acid) and 984 (DNA), comprising SEQ ID NO: 943 (encoded by SEQ ID NO: 944)), as outlined in Table 3 above. The amino acid and DNA sequence of TTN-002 is provided, e.g., in Tables 4 and 5, respectively.

[0514] AAV particles were generated with this capsid variant encapsulating a luciferase-EGFP transgene or a payload-HA tag driven by a heterologous constitutive promoter. Each capsid variant and control AAV5 and/or AAV9 capsids were tested by intravenously administering the AAV particle formulation at 5e13 VG/kg to NHPs (n=2), 1e13 VG per rat (n=3; 3e13 VG/kg), and/or 5e11 VG per mouse (n=3; 2e13 VG/kg). The in-life period was 28 days for NHPs and mice, and 25 days for rats. Various CNS and peripheral tissues were then collected for measuring transgene mRNA, transgene protein, and/or viral DNA (biodistribution). The AAV particles administered to the NHPs and rats comprised self-complementary viral genomes and the AAV particles administered to mice comprised a single-stranded viral genome.

A. Individual capsid characterization in NHPs

[0515] The brains, spinal cord, and peripheral tissues including the heart, liver, and quadriceps, were isolated from NHPs (cynomolgus macaques (*Macaca fascicularis*)) at 28 days post intravenous administration of the AAV particles comprising the TTN-002 capsid variant and were assayed by qPCR for the presence of transgene RNA as a measure of transgene expression and compared to the AAV9 control. 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 TTN-002 capsid variant, which is an AAV5 capsid variant, was significantly higher in the brain of NHPs relative to the wild-type AAV9 control. More specifically, mRNA expression was approximately 20-25-fold higher from the TTN-002 capsid variant compared to wild-type AAV9 in the brain of NHPs. Additionally, mRNA expression was approximately 4-5-fold higher from the TTN-002 capsid variants compared to wild-type AAV9 in the spinal cord of the NHPs. TTN-002 also demonstrated lower mRNA expression in the liver and DRG relative the AAV9 control (**Table 10**).

[0516] 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. 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 AAV5 capsid variant, TTN-002, was significantly higher in the NHP brain relative to the wild-type AAV9 control. Biodistribution of TTN-002 was lower in the NHP liver relative to the wild-type AAV9 control.

[0517] The brain tissues and spinal cords of the NHPs were also subjected to immunohistochemistry staining to evaluate overall CNS tropism and biodistribution in various regions (**FIGs. 1A-1D**). Immunohistochemical staining correlated with the qPCR analysis, as TTN-002

showed significantly stronger staining and payload expression in the brain (e.g., across the entire cerebrum and cerebellum, **FIG. 1A-1C**) and spinal cord (**FIG. 1A** and **1D**), as compared to the AAV9 control. More specifically, TTN-002 demonstrated localization, strong payload expression, and transduction in both neurons and glial cells in the temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, dentate nucleus, brainstem, and cerebellum (**FIGs. 1A-1C**). Payload expression was also observed in astrocytes in the dentate nucleus. Additionally, quantification of co-expression of the payload and the pan-neuronal marker SMI-311 showed payload expression in approximately 73.4% of the large neurons in the dentate nucleus of the deep cerebral nucleus in the cerebellum at 28-days post intravenous administration the AAV particles comprising the TTN-002 capsid variant. In the spinal cord, TTN-002 demonstrated localization, strong payload expression, and transduction in the cervical region (e.g., C2), thoracic region (e.g., T10), and lumbar region (e.g., L2) (**FIG. 1A** and **1D**). Additionally, TTN-002 showed less staining in the DRG relative to the wild-type AAV9 control (approximately 2-fold less) (**FIG. 1D**). Both TTN-002 and AAV9 appeared to transduce the liver and heart with similarly high efficiency, by IHC analysis (**FIG. 1D**). Additionally, the histopathology of these samples isolated from the NHPs showed no signs of toxicity in the NHPs, following intravenous administration of AAV particles comprising the TTN-002 capsid variant at a dose of 5e13 VG/kg with a self-complementary viral genome.

Table 10. Transgene mRNA expression with the TTN-002 capsid variant in NHPs

Capsid Variant	Tissue	AVG transgene mRNA fold change relative to housekeeping gene			Fold change relative to AAV9
		NHP1	NHP2	Average	
AAV9	Cortex	0.219	0.018	0.118	1.0
	Thalamus	0.141	0.090	0.116	1.0
	Cerebellum	0.677	0.098	0.388	1.0
	SC Cervical	0.610	2.040	1.325	1.0
	SC Thoracic	0.796	1.238	1.017	1.0
	SC Lumbar	4.950	0.890	2.920	1.0
	DRG Cervical	32.413	63.331	47.872	1.0
	DRG Thoracic	27.509	57.666	42.588	1.0
	DRG Lumbar	115.851	69.920	92.885	1.0
	Liver	21.591	9.198	15.394	1.0
	Heart	231.804	20.067	125.936	1.0
	Quadriceps	37.732	3.686	20.709	1.0
TTN-002	Cortex	1.277	4.632	2.954	24.932
	Thalamus	3.618	1.194	2.406	20.818
	Cerebellum	3.839	14.762	9.301	23.998
	SC Cervical	3.683	9.095	6.389	4.822
	SC Thoracic	5.759	6.639	6.199	6.096
	SC Lumbar	7.544	16.550	12.047	4.126
	DRG Cervical	31.162	22.002	26.582	0.555
	DRG Thoracic	25.228	14.915	20.071	0.471
	DRG Lumbar	39.386	16.611	27.998	0.301

	Liver	13.599	16.140	14.869	0.966
	Heart	380.487	143.202	261.844	2.079
	Quadriceps	45.659	4.207	24.933	1.204

Table 11. Viral DNA biodistribution with the TTN-002 capsid variant in NHPs

Capsid Variant	Tissue	AVG DNA copies per diploid genome			Fold change relative to AAV9
		NHP1	NHP2	Average	
AAV9	Cortex	0.213	0.074	0.1435	1.0
	Thalamus	0.225	0.107	0.166	1.0
	Cerebellum	0.199	0.079	0.139	1.0
	SC Cervical	0.688	0.103	0.396	1.0
	SC Thoracic	0.385	0.192	0.288	1.0
	SC Lumbar	0.214	0.21	0.212	1.0
	DRG Cervical	0.320	0.32	0.320	1.0
	DRG Thoracic	0.303	0.733	0.518	1.0
	DRG Lumbar	0.240	0.246	0.243	1.0
	Liver	274.545	268.553	271.549	1.0
	Heart	2.367	2.833	2.600	1.0
	Quadriceps	1.380	0.768	1.074	1.0
TTN-002	Cortex	0.600	0.031	0.3155	2.203734
	Thalamus	0.388	0.417	0.402	2.425078
	Cerebellum	0.492	0.886	0.689	4.95212
	SC Cervical	0.008	0.298	0.153	0.386545
	SC Thoracic	0.679	0.481	0.580	2.010803
	SC Lumbar	0.801	0.194	0.497	2.347401
	DRG Cervical	0.711	0.636	0.674	2.106828
	DRG Thoracic	0.000	0.305	0.153	0.294746
	DRG Lumbar	0.356	0.517	0.437	1.795147
	Liver	21.177	156.337	88.757	0.326855
	Heart	2.078	2.380	2.229	0.857412
	Quadriceps	0.641	0.486	0.564	0.524939

B. Individual capsid characterization in rats

[0518] The brains and spinal cords were isolated from rats at 25 days post intravenous administration of AAV particles comprising the TTN-002 capsid variant (AAV5 variant) and assayed for the presence of transgene RNA as a measure of transgene expression, relative to a wild-type AAV5 control capsid or a wild-type AAV9 control capsid (**Table 12**). 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 AAV5 and AAV9 controls (**Table 12**). As shown in **Table 12**, mRNA transgene expression from the TTN-002 capsid variant was higher in both the brains and spinal cords relative to the AAV5 and AAV9 controls. More specifically, transgene mRNA expression was approximately 40-67-fold higher from the TTN-002 variant in the rat brain and spinal cord regions (cervical, thoracic and lumbar) compared to wild-type AAV5 and transgene mRNA expression was approximately 5-7-

fold higher in the rat brain and spinal cord regions (cervical, thoracic and lumbar) compared to wild-type AAV9.

[0519] The brain and spinal cord tissues, as well as the heart peripheral tissue were subject to immunohistochemistry staining to evaluate overall tropism and biodistribution. Immunohistochemical staining correlated with the qPCR analysis, as TTN-002 showed increased staining relative to both AAV9 and AAV5 in the cortex, hippocampus, cerebellum, and spinal cord of the rat. TTN-002 showed increased staining in the heart of the rat relative to AAV5 but decreased staining relative to AAV9.

Table 12. Transgene mRNA expression with TTN-002 capsid variant in rats

Tissue	AVG (n=3 rats) transgene mRNA fold change relative to housekeeping gene			Fold change in mRNA expression relative to AAV5		Fold change in mRNA expression relative to AAV9	
	AAV5	AAV9	TTN-002	AAV9	TTN-002	AAV5	TTN-002
Brain	0.051	0.381	2.051	7.533	40.510	0.133	5.378
Spinal cord C1	0.121	1.045	8.209	8.629	67.796	0.116	7.856
Spinal cord L1	0.118	1.647	4.901	13.947	41.499	0.072	2.975
Spinal cord T1	0.133	1.066	6.952	8.015	52.291	0.125	6.524

C. Individual capsid characterization in mice

[0520] The brains and livers were isolated from BALB/c mice 28 days post intravenous injection of following intravenous administration of AAV particles comprising the TTN-002 capsid variant and were assayed by qPCR for the presence of transgene RNA as a measure of transgene expression and compared to an AAV9 and AAV5 control. Data were provided as average mRNA fold change for the transgene relative to a housekeeping gene (**Table 13**) and as fold change in transgene mRNA expression relative AAV9 and AAV5 controls (**Table 14**). As shown in **Table 13** and **Table 14**, the AAV5 capsid variant TTN-002 demonstrated similar levels of transgene expression relative to AAV9 in the brain and higher expression than wild-type AAV5. Transgene mRNA expression in the mouse brain was 265.9-fold higher with the TTN-002 capsid variant as compared to wild-type AAV5 (**Table 14**). Additionally, wild-type AAV5 and the AAV5 capsid variant, TTN-002 both resulted in lower transgene expression in the liver, as compared to wild-type AAV9.

[0521] The brains and livers isolated from the mice were also assayed for the presence of viral DNA as a measure of viral genome levels. Data are provided in **Table 13** as average DNA (viral genome (VG)) copies per diploid genome and in **Table 14** as fold change in DNA copies per diploid genome relative AAV9 and AAV5 controls. The AAV5 capsid variant TTN-002 demonstrated comparable biodistribution relative to AAV9 in the mouse brain and increased biodistribution and viral genome levels than wild-type AAV5. More specifically, in the brain, the TTN-002 capsid variant led to 9-fold higher DNA (viral genome (VG)) copies per diploid genome relative to the AAV5 control (**Table 14**). Furthermore, wild-type AAV5 and the AAV5 capsid variant, TTN-002 resulted in decreased biodistribution and DNA (viral genome (VG)) copies per diploid genome in the liver relative to AAV9 (**Table 14**).

Table 13. Transgene mRNA expression with the TTN-002 capsid variant in mice

Tissue	Capsid Variant	Mouse	AVG transgene mRNA fold change relative to housekeeping gene	AVG Transgene DNA per diploid genome
Brain	AAV5	Mouse 1	0.002	0.0152
		Mouse 2	0.004	0.0000
		Mouse 3	0.004	0.0114
		Average	**0.003**	**0.0089**
	AAV9	Mouse 1	0.553	0.2000
		Mouse 2	0.235	0.1027
		Mouse 3	0.844	0.1213
		Average	**0.544**	**0.1414**
	TTN-002	Mouse 1	0.179	0.1334
		Mouse 2	0.462	0.0555
		Mouse 3	1.828	0.0608
		Average	**0.823**	**0.0832**
Liver	AAV5	Mouse 1	0.395	0.9669
		Mouse 2	0.566	1.4158
		Mouse 3	0.882	1.4627
		Average	**0.614**	**1.2818**
	AAV9	Mouse 1	9.164	34.1673
		Mouse 2	12.460	38.4415
		Mouse 3	5.190	24.0644
		Average	**8.938**	**32.2244**
	TTN-002	Mouse 1	1.106	1.8292
		Mouse 2	1.305	1.6726
		Mouse 3	2.292	2.8985
		Average	**1.568**	**2.1334**

Table 14. Fold-change in transgene mRNA expression and DNA copies per diploid genome relative to AAV9 and AAV5 in the brain and liver of mice

Tissue	Fold change in mRNA expression relative to AAV5		Fold change in mRNA expression relative to AAV9	
	AAV9	TTN-002	AAV5	TTN-002
Brain	175.796	265.874	0.006	1.512
Liver	14.549	2.552	0.069	0.175

Tissue	Fold change in DNA copies per diploid genome relative to AAV5		Fold change in DNA copies per diploid genome relative to AAV9	
	AAV9	TTN-002	AAV5	TTN-002
Brain	15.910	9.367	0.063	0.589
Liver	25.140	1.664	0.040	0.066

Conclusions

[0522] Taken together, these data demonstrate that TTN-002, which is an AAV5 capsid variant, is an enhanced CNS tropic capsid, in both NHPs and rodents (e.g., rats and mice), and was able to successfully penetrate the blood brain barrier. More specifically, TTN-002 demonstrated the ability to cross species, demonstrating enhanced CNS tropism and biodistribution in both NHPs and rats, and

improvement relative to AAV5 in mice. Additionally, the TTN-002 capsid variant was able to successfully penetrate the blood brain barrier.

Example 3. Maturation of the TTN-002 capsid in mice

[0523] This Example describes maturation of the TTN-002 (SEQ ID NO: 982 (amino acid) and 984 (DNA), comprising SEQ ID NO: 943) capsid variant in mice to further enhance its transduction and biodistribution in the central nervous system, evolve the AAV capsid variant further, and to provide cross-species compatibility. Two approaches were used to mature the TTN-002 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, mutagenic primers were used to introduce point mutations at a low frequency, scattered across the mutagenesis region in the TTN-002 sequence ranging from approximately position 571 to position 586, numbered according to SEQ ID NO: 982. In the second maturation approach, sets of three contiguous amino acids were randomized across the mutagenesis region in the TTN-002 sequence, which spanned from approximately position 571 to position 586, numbered according to SEQ ID NO: 982. AAV capsid variants arising from each maturation approach for TTN-002 were pooled together, for subsequent testing and characterization in mice.

[0524] The library of pooled matured AAV capsid variants generated from TTN-002 using the first maturation approach and the library of pooled matured AAV capsid variants generated from TTN-002 using the second maturation approach were each injected into three CD-1 Outbred mice. After a period in life, the brains of the 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 TTN-002 control, and the peptides comprised within the matured variants were identified. The data from the first maturation approach are provided in **Table 15** and the data from the second maturation approach is provided in **Table 16**.

[0525] Following the RNA recovery and NGS analysis from the first maturation approach, the matured capsid variants were filtered based on their coefficient of variance (CV), which was calculated for each peptide across six brain samples taken (two per mouse). Those that had a CV value <1 were identified, as these were the peptides that were reliably detected in 5/6 or 6/6 brain samples isolated from the three mice. **Table 15** provides the peptide sequences of these matured capsid variants and the fold enrichment of the matured capsid variant relative to the non-matured TTN-002 control. As shown in **Table 15**, approximately 28 TTN-002 matured capsid variants demonstrated an increase in expression relative to the non-matured TTN-002 control, with approximately 16 variants demonstrating at least a 2-fold increase in expression. Several variants demonstrated at least an 8-fold to 15-fold increase in expression relative to the non-matured TTN-002 control.

Table 15. NGS fold-enrichment of TTN-002 matured AAV capsid variants in the brain of outbred mice following first mutagenesis approach

Sequence	SEQ ID NO:	Fold enrichment over TTN-002
TNSVSSYPAEVVQKTA	1537	15.404
TNNQSKYPAEVVQKTA	1538	10.261
TNNSSSYPAEVVQKTA	1539	8.539
TNNQSSYPATVVQKTA	1540	4.641
TNNSGSYPAEVVQKTA	1541	4.553
TNNLSKYPAEVVQKTA	1542	4.329
TNNQSSYPASVVQKTA	1543	3.849
TNNMSRYPAEVVQKTA	1544	3.586
TNNLSRYPAEVVQKTA	1545	3.580
TNNQSSYPPSIVQKTA	1546	3.529
TNNASKYPAEVVQKTA	1547	3.008
TNNQSSYPPSVVQKTA	1548	2.927
TNNLSSYPAEVVQKTA	1549	2.912
TNNQSSYPATLVQKTA	1550	2.688
TNSLSSYPAEVVQKTA	1551	2.243
TNQVSSYPAEVVQKTA	1552	2.157
TNNVSLYPAEVVQKTA	1553	2.075
TNNQSSYPVTVVQKTA	1554	1.857
TNNTSTYPAEVVQKTA	1555	1.753
TNNQSSLTAEVVQKTA	1556	1.612
TNNTSLYPAEVVQKTA	1232	1.484
TNNQSSYPAPVVQKTA	1558	1.478
TNNQSSYPASLIQKTA	1559	1.365
TNTISSYPAEVVQKTA	1560	1.263
TNNQSSYPAPLQQKTA	1561	1.261
TNNQSSYPAPLVQKTA	1562	1.149
TNNQSSYPPSLVQKTA	1300	1.141
TNNPSRYPAEVVQKTA	1564	1.106
TNNQSSYPAEVVQKTA	1533	1.000
TNNSARYPAEVVQKTA	1566	0.980

[0526] Following the RNA recovery and NGS analysis from the second maturation approach, the matured capsid variants were filtered for those that were detectable in all samples from all mice injected with the matured capsid variants. **Table 16** provides the peptide sequences of these matured capsid variants, the fold enrichment of the matured capsid variant relative to the non-matured TTN-002 control, as well as the predicted capsid of origin from which the variant was matured. As shown in **Table 16**, approximately 526 TTN-002 matured capsid variants demonstrated an increase in expression relative to the non-matured TTN-002 control, with approximately 358 variants demonstrated at least a 2-fold increase in expression. Several variants demonstrated a 20-711-fold or greater increase in expression relative to the non-matured TTN-002 control.

Table 16. NGS fold-enrichment of TTN-002 matured AAV capsid variants in the brain of outbred mice following second mutagenesis approach

Sequence	SEQ ID NO:	Fold enrichment over TTN-002	Sequence	SEQ ID NO:	Fold enrichment over TTN-002
TNNFFMYPAEVVQKTA	1000	711.864	TNNQSSYPAELVQKTA	1269	3.158
TNNQSSYPAEALLKTA	1001	107.670	TNSLWSYPAEVVQKTA	1270	3.150
TNNQGTYPAEVVQKTA	1002	87.226	TNNQSSYPAETVSKTA	1271	3.149
TISASSYPAEVVQKTA	1003	79.340	TNNISLYPAEVVQKTA	1272	3.114
TNGVHSYPAEVVQKTA	1004	37.131	TNNQSSYPAEVVQKST	1273	3.103
TNNHSAYPAEVVQKTA	1005	35.420	TNNQSSYPAELVSKTA	1274	3.082
TNNMAPYPAEVVQKTA	1006	34.417	TNNTGRYPAEVVQKTA	1275	3.067
TNNIATYPAEVVQKTA	1007	32.786	TYSTSSYPAEVVQKTA	1276	3.065
TNQISSYPAEVVQKTA	1008	30.799	TNTLSSYTAEVVQKTA	1277	3.040
TNNQSSYPAEVVQKLS	1009	30.539	TNSTSSYPAEVVQKTA	1278	3.033
TNNLGQYPAEVVQKTA	1010	28.520	TNAVFSYPAEVVQKTA	1279	3.025
TNNPGAYPAEVVQKTA	1011	27.637	TNSTYSYPAEVVQKTA	1280	3.025
TNNQSSYPAEVVQKLK	1012	27.076	TNNLSMYPAEVVQKTA	1281	2.997
TNNQSSYPAAIIQKTA	1013	26.618	TNNQSSYPATLVQKPA	1282	2.973
TNNQSSYNTKVVQKTA	1014	22.549	TNNISVYPAEVVQKTA	1283	2.969
TNKITSYPAEVVQKTA	1015	22.308	TNNQSSYPAPVVQKTA	1284	2.937
TNNIGRYPAEVVQKTA	1016	22.159	TNTQSSYPAEVVQKTA	1285	2.929
TNLIASYPAEVVQKTA	1017	22.050	TNNQSSYPQSVVQKTA	1286	2.922
TNNTAGYPAEVVQKTA	1018	21.846	TNNSSVYPAEVVQKPA	1287	2.920
TNNMAKYPAEVVQKTA	1019	21.371	TNNQSSYPANQSQKTA	1288	2.889
TNNMAQYPAEVVQKTA	1020	20.203	TNNLAVYPAEVVQKTA	1289	2.888
TNNAGAYPAEVVQKTA	1021	19.342	TNNPSRYPAEVVQKTA	1290	2.877
TMSASSYPAEVVQKTA	1022	19.003	TNTTSSYPAEVVQKPA	1291	2.873
TNNAAVYPAEVVQKTA	1023	18.655	TNNQSSYPTNTVQKTA	1292	2.866
INNQSSYPAEVVQKTA	1024	18.426	TNNTGLYPAEVVQKTA	1293	2.862
TNNNSKYPAEVVQKTA	1025	18.030	TLLNSSYPAEVVQKTA	1294	2.847
TNNTSHYPAEVVQKTA	1026	17.574	TNNSALYPAEVVQKTA	1295	2.797
TNNLGSYPAEVVQKTA	1027	17.430	TNNLSLYPAEVVQKTA	1296	2.777
TNNQSSYPAEVISKTA	1028	17.172	TNNQSSYPAEVKSRTA	1297	2.760
TNLIDSYPAEVVQKTA	1029	16.804	TNNQSTATAEVVQKTA	1298	2.745
TNNTSGYPAEVVQKTA	1030	16.556	TNNQSSYPRDLVQKTA	1299	2.726
TNNQSSYPANDTQKTA	1031	15.769	TNNQSSYPPSLVQKTA	1300	2.719
TNNTAQYPAEVVQKTA	1032	15.607	TNSVSSYPAEVVQNTA	1301	2.703
TNLVSSYPAEVVQKTA	1033	15.329	TNNTAMYPAEVVQKTA	1302	2.700
TNNVSQYPAEVVQKTA	1034	15.269	TNNQGAYPAEVVQKTA	1303	2.678
TNNMSMYPAEVVQKTA	1035	15.241	TNNQSSYPPTVVQKTA	1304	2.665
TNSVSSYPAEVVQKTA	1036	14.066	TNNSAKYPAEVVQKTA	1305	2.645
TNSIGSYPAEVVQKTA	1037	13.549	TNNQSLYPAEVVQKTA	1306	2.630
TNNVSYYPAEVVQKTA	1038	13.380	TNNVSSYPAEVVQKPA	1307	2.622
TNNAASYPAEVVQKTA	1039	13.290	TNNLAPYPAEVVQKTA	1308	2.619
TNNMSIYPAEVVQKTA	1040	13.095	TNSPSSYPAEVVQKTA	1309	2.608
TNNVSGYPAEVVQKTA	1041	12.734	TNNHSSYPAEVVQKTA	1310	2.600
TNNMASYPAEVVQKTA	1042	12.673	TNNQSSYPAPVIQKTA	1311	2.599
TNNQSSYPAQIVQKTA	1043	12.669	TNNLSLYPAEVVQKPA	1312	2.598
TNKISSYPAEVVQKTA	1044	12.551	TNNVSKYPAEVVQKTA	1313	2.593
TNRVSSYPAEVVQKTA	1045	12.551	TNNQSYNPAEVVQKTA	1314	2.582
TNNVGYYPAEVVQKTA	1046	12.544	TNNPSVYPAEVVQKTA	1315	2.559
TNNLGAYPAEVVQKTA	1047	12.445	TNNIAPYPAEVVQKTA	1316	2.552
TNNTSQYPAEVVQKTA	1048	12.264	TNNQLTTPAEVVQKTA	1317	2.486
TNNQSSYPPKVVQKTA	1049	12.245	TNNLGKYPAEVVQKTA	1318	2.484
TNNVTMYPAEVVQKTA	1050	12.226	TNNQSSYPAHLIQKTA	1319	2.480

TNNQSSNPTEVVQKTA	1051	12.190	TNNMSTYPAEVVQKTA	1320	2.467
TNNIAWYPAEVVQKTA	1052	12.018	TNNQSSYPASVIQKTA	1321	2.457
TNNAAAYPAEVVQKTA	1053	11.885	TNNQSSYPAEVESKLA	1322	2.455
TNNTASYPAEVVQKTA	1054	11.862	TNSVSSYPAEVVKKTA	1323	2.451
TNKVASYPAEVVQKTA	1055	11.641	TNNVSPYPAEVVQKTA	1324	2.437
TNNIGTYPAEVVQKTA	1056	11.597	TNNQSSYPPNMVQKTA	1325	2.413
TNNLSKYPAEVVQKTA	1057	11.521	TNNQSSYPANQTQKTA	1326	2.412
TNNLAKYPAEVVQKTA	1058	11.498	TNNQSRYPAEVVQKTA	1327	2.409
TNNQARYPAEVVQKTA	1059	11.473	TNTQASYPAEVVQKTA	1328	2.390
TNNQSSYPATIIQKTA	1060	11.443	TNNQSSYPMPLVQKTA	1329	2.388
TNNISHYPAEVVQKTA	1061	11.290	TNNQSSYPREVVQKTA	1330	2.364
TKTTSSYPAEVVQKTA	1062	11.194	TNNSSSYPAEVVKKTA	1331	2.340
TNNQSSYPAEVVSKLA	1063	11.081	TNNQSSYPAPLQQKTA	1332	2.339
TNFVASYPAEVVQKTA	1064	11.061	TNLSGSYPAEVVQKTA	1333	2.326
TNNTSDYPAEVVQKTA	1065	11.043	TNNKSMYPAEVVQKTA	1334	2.319
TNNQSSYPPELVQKTA	1066	11.019	TNNQSSYPVTVVQKTA	1335	2.315
TNNMSLYPAEVVQKTA	1067	10.882	TNKQSSYPASVVQKTA	1336	2.312
TNNQEKYPAEVVQKTA	1068	10.869	TNNLSYYPAEVVQKTA	1337	2.311
TNNQSSYPADIVQKTA	1069	10.822	TNNSSLYPAEVVQKTA	1338	2.307
TNNMAMYPAEVVQKTA	1070	10.807	TNNQSSYPPNTVQKTA	1339	2.307
TNNQSSYPAELIEKTA	1071	10.660	TNNQSSYPALVIQKTA	1340	2.289
TNNQSSYPPDVVQKTA	1072	10.538	TNNQGVYPAEVVQKTA	1341	2.275
TNNLSAYPAEVVQKTA	1073	10.500	TNNQSRYPAEEVQKTA	1342	2.233
TNNIAHYPAEVVQKTA	1074	10.408	TNNQSSYPPHVVQKTA	1343	2.231
TNNHAKYPAEVVQKTA	1075	10.319	TNVVSSYPAEVVQKTA	1344	2.225
TNNMSRYPAEVVQKTA	1076	10.233	TNNQGTIPAEVVQKTA	1345	2.218
TNNTSSYPAEVVQKTA	1077	10.000	TNNQSSYPATLVQKTA	1346	2.200
TNNPAAYPAEVVQKTA	1078	9.842	TNNLSSYPAEVVQKTA	1347	2.190
TNSTFSYPAEVVQKTA	1079	9.812	TTLTSSYPAEVVQKTA	1348	2.129
TNNQSSYPAEVVQKTS	1080	9.758	TNLGGSYPAEVVQKTA	1349	2.125
TNNQSSYPAEVVQKSS	1081	9.758	TNNQSSYPAEVVNKLA	1350	2.107
TNNTSIYPAEVVQKTA	1082	9.739	TNNVGLYPAEVVQKTA	1351	2.106
TNNLAYYPAEVVQKTA	1083	9.674	TNNQSTPPAEVVQKTA	1352	2.094
TNNMGYYPAEVVQKTA	1085	9.584	TNNSTIYPAEVVQKTA	1353	2.089
TNLVSSYPAEVVQKPA	1086	9.577	TNNSAMYPAEVVQKTA	1354	2.073
TNNQAIYPAEVVQKTA	1087	9.556	TNTISSYPAEVVQKTA	1355	2.069
TNNPNSYPAEVVQKTA	1088	9.484	TNNQSSYPAALIQKTA	1356	2.069
TKTISSYPAEVVQKTA	1089	9.438	TNLGSSYPAEVVQKTA	1357	2.061
TNNIASYPAEVVQKTA	1090	9.423	TNKMSSYPAEVVQKTA	1358	2.056
TNMIASYPAEVVQKTA	1091	9.410	TNNQSSYPAEEVQKLK	1359	2.032
TNNMGAYPAEVVQKTA	1092	9.403	NNNQSSYPASVVQKTA	1360	2.032
TNAVHSYPAEVVQKTA	1093	9.332	TNNISPYPAEVVQKTA	1361	2.008
TNNISNYPAEVVQKTA	1094	9.304	TNNQSSYPPPVVQKTA	1362	2.000
TKSASSYPAEVVQKTA	1095	9.240	TNSVASYPAEVVQKTA	1363	1.983
TNNVGRYPAEVVQKTA	1096	9.183	TNNQSSYPAEVIRKTA	1364	1.974
TNNMSVYPAEVVQKTA	1097	9.158	TNNSSKYPAEVVQKTA	1365	1.972
TNNVSTYPAEVVQKTA	1098	9.126	TNNMALYPAEVVQKTA	1366	1.970
TNNTSAYPAEVVQKTA	1099	9.074	TNKLSSYPAEVVQKTA	1367	1.960
TNNQSSYPAEVVNKTA	1100	8.893	TNNQSSYPAPVVQKPA	1368	1.959
TNNQAMYPAEVVQKTA	1101	8.788	TNNQSSSVAEVVQKTA	1369	1.945
TNNQSSYPAEVIQKTA	1102	8.727	TNSIWSYPAEVVQKTA	1370	1.937
TNNASAYPAEVVQKTA	1103	8.711	TNNQRSYPATLVQKTA	1371	1.936
TNNLAAYPAEVVQKTA	1104	8.670	TNNQSSRDAEVVQKTA	1372	1.933
TNKVSSYPAEVVQKTA	1105	8.280	TNKVTSYPAEVVQKTA	1373	1.932
TNNQSSYPANLIQKTA	1106	8.247	TNNQAYYPAEVVQKTA	1374	1.928
TNNQSSYPSDIVQKTA	1107	8.206	TNNQSSYPATVVQKTA	1375	1.916
TNNQSSRESEVVQKTA	1108	8.198	TNNQSGISAEVVQKTA	1376	1.915
TNNISRYPAEVVQKTA	1109	8.130	TNNLALYPAEVVQKTA	1377	1.904

TNNTAPYPAEVVQKTA	1110	8.050	TNNQAVYPAEVVQKTA	1378	1.889
TNNQSQYPAEVVQKTA	1111	7.947	TNNQSSNQAEVVQKTA	1379	1.864
TNNIGSYPAEVVQKTA	1112	7.845	TNNMSRYPAEVEQKTA	1380	1.857
TNNIGVYPAEVVQKTA	1113	7.821	TKTLSSYPAEVVQKTA	1381	1.855
TNNTNSYPAEVVQKTA	1114	7.794	TNNAWSYPAEVVQKTA	1382	1.848
TNNIARYPAEVVQKTA	1115	7.751	TNNQSSYPASMSQKTA	1384	1.832
TNNLSRYPAEVVQKTA	1116	7.723	TNLTPSYPAEVVQKTA	1385	1.828
TNNQSSYPEQVVQKTA	1117	7.705	TNLTSSYPAEVVQKTA	1386	1.818
TNKNASYPAEVVQKTA	1118	7.686	TNNLSSYPAEVEQKTA	1387	1.793
TNNTSTYPAEVVQKTA	1119	7.650	TNNQSNVTAEVVQKTA	1388	1.792
TNNSSIYPAEVVQKTA	1120	7.645	TNNQSSYPPQVVQKTA	1389	1.779
TNNQSSYPAEVVQKTT	1121	7.641	TNNQSSVSAEVVQKTA	1390	1.762
TNSGWSYPAEVVQKTA	1122	7.631	TNNQSSYPPNTVQKPA	1391	1.730
TNFIASYPAEVVQKTA	1123	7.624	TNLESSYPAEVVQKTA	1392	1.728
TNSTWSYPAEVVQKTA	1124	7.604	TNMVASYPAEVVQKTA	1394	1.715
TNNLGNYPAEVVQKTA	1125	7.585	TNNQSSTPSEVVQKTA	1395	1.709
TNNQSSYPPSIVQKTA	1126	7.576	TNNLSSYPAEVVQKPA	1396	1.698
TVASSSYPAEVVQKTA	1127	7.500	TNNQSPPRAEVVQKTA	1397	1.692
TNNQSSYPAELIAKTA	1128	7.498	TNSLGSYPAEVVQKTA	1398	1.680
TNNLARYPAEVVQKTA	1129	7.477	TNSTWSYPAEVVQKPA	1399	1.674
TNNQSSYPAEVVQKLN	1130	7.430	TNNQSSYPASVVQKPA	1400	1.672
TNNLSQYPAEVVQKTA	1131	7.420	TNNQSSYPAEVASKTA	1401	1.669
TNNISYYPAEVVQKTA	1132	7.337	TNNQSSYPAEVLHKTA	1402	1.664
TNNSSVYPAEVVQKTA	1133	7.278	TNTQSSYPASVVQKTA	1403	1.636
TNNQSSYPAEIVHKTA	1134	7.273	TNNQSSYPPSVEQKTA	1404	1.635
TNNLSVYPAEVVQKTA	1135	7.251	TNNQRSYPPSVVQKTA	1405	1.632
TNITGSYPAEVVQKTA	1136	7.246	TNNQSSYPAELVNKTA	1407	1.624
TNNMSKYPAEVVQKTA	1137	7.233	TNNLSGYPAEVVQKTA	1408	1.618
TNNCGSYPAEVVQKTA	1138	7.143	TNNQSSYPAEVVSKTA	1409	1.617
TNNVSSYPAEVVQKTA	1139	7.074	TNTVSSYTAEVVQKTA	1410	1.614
TNNIALYPAEVVQKTA	1140	7.043	TNSTSSYPAEVVQKPA	1411	1.614
TNNQLSIPAEVVQKTA	1141	7.017	TNNTSHYPAEEVQKTA	1412	1.608
TNNSSSYPAEVVQKPA	1142	6.889	TNNPSIYPAEVVQKTA	1413	1.605
TNNQSSYPAAVIQKTA	1143	6.870	TNTMSSYPAEVVQKTA	1414	1.602
TNNQSSYPAEIISKTA	1144	6.669	TNNQSHNPAEVVQKTA	1415	1.597
TNCVSSYPAEVVQKTA	1145	6.662	TNNQSSYPAELIHKTA	1416	1.592
TNNTARYPAEVVQKTA	1146	6.649	TNNQSSYPPSVVQKTA	1417	1.587
TNNQSSYPAEVINKTA	1147	6.627	TNNQSFYPAEVVQKTA	1418	1.574
TNSIASYPAEVVQKTA	1148	6.607	TNNSSSYPAEVVQQTA	1419	1.571
TNNQSSREAEVVQKTA	1149	6.499	TNTVSSYPAEVVQKTA	1420	1.570
TNNITRYPAEVVQKTA	1150	6.373	TNNQSPPSAEVVQKTA	1421	1.563
TNNTSVYPAEVVQKTA	1151	6.372	TNNQSTAPAEVVQKTA	1422	1.552
TNNTGSYPAEVVQKTA	1152	6.357	TNNQSSYPAEVVHKTA	1423	1.534
TNNMSQYPAEVVQKTA	1153	6.346	TNNQSSYPAMVVQKTA	1424	1.532
TNNISSYPAEVVQKTA	1154	6.335	TNNQATNPAEVVQKTA	1425	1.530
TNNSASYPAEVVQKTA	1155	6.319	TNNVSSYQAEVVQKTA	1426	1.530
TNSISSYPAEVVQKTA	1156	6.294	TNHVSSYPAEVVQKTA	1427	1.523
TNNISMYPAEVVQKTA	1157	6.235	TNNVSWYPAEVVQKTA	1428	1.520
TNNTATYPAEVVQKTA	1158	6.215	TNSLSSYPAEVVQKTA	1429	1.504
TNNISTYPAEVVQKTA	1159	6.201	TNSVYSYPAEVVQKTA	1430	1.498
TNNVSHYPAEVVQKTA	1160	6.141	TNHTSSYPAEVVQKTA	1431	1.493
TNNSGSYPAEVVQKTA	1161	6.123	TNNQSSYPAPLVQKPA	1432	1.490
TNNLASYPAEVVQKTA	1162	6.091	TNSTTSYPAEVVQKTA	1433	1.483
TNNVSFYPAEVVQKTA	1163	6.088	TNNQSSYPAEVGSKLA	1434	1.482
TNNQSSYPVQVVQKTA	1164	6.075	TNNQSSYPVNTVQKTA	1435	1.477
TNNQAAYPAEVVQKTA	1165	6.059	TNNQSQSPAEVVQKTA	1436	1.471
TNQVSSYPAEVVQKTA	1166	6.058	TNNQSSYPPPLVQKTA	1437	1.468
TNSVGSYPAEVVQKTA	1167	6.020	TNNTSGYPAEVVQKPA	1438	1.463

TNTPASYPAEVVQKTA	1168	5.992	TNFSSSYPAEVVQKTA	1439	1.460
TNNLAMYPAEVVQKTA	1169	5.970	TNGVYSYPAEVVQKTA	1440	1.457
TNTTSSYPAEVVQKTA	1170	5.964	TNNQSSYPAPVVQQTA	1441	1.455
TNNSSSYPAEVVQKTA	1539	5.903	TNSLFSYPAEVVQKTA	1442	1.450
TNNQSSYPAEVVQNLK	1172	5.868	TNNQSSYPAEVIKKTA	1443	1.449
TNNLSIYPAEVVQKTA	1173	5.815	TNNQSSYPASMIQKTA	1444	1.446
TNNQSSYPAQVVQKTA	1174	5.773	TNNSVSYPAEVVQKTA	1445	1.445
TNTSASYPAEVVQKTA	1175	5.741	TNFVSSYPAEVVQKTA	1446	1.444
TNNTSKYPAEVVQKTA	1176	5.652	TNLISSYPAEVVQKTA	1447	1.427
TNNTTVYPAEVVQKTA	1177	5.625	TNNSAVYPAEVVQKTA	1448	1.418
TNNQSSYPPEVVQKTA	1178	5.541	TLSASSYPAEVVQKTA	1449	1.417
TNNQSSYPAEVMSRTA	1179	5.539	TNAVSSYPAEVVQKTA	1450	1.417
TNNASQYPAEVVQKTA	1180	5.532	TNNQSSTLIEVVQKTA	1451	1.411
TNNTSYYPAEVVQKTA	1181	5.525	TNNQSAYPAEVVQKTA	1452	1.404
TNNVSMYPAEVVQKTA	1182	5.522	TNNQSKYPAEVVHKTA	1453	1.402
TNNLGRYPAEVVQKTA	1183	5.509	TNNLSIYPAEVVQKPA	1454	1.402
TNNVAPYPAEVVQKTA	1184	5.492	TNPISSYPAEVVQKTA	1455	1.396
TNNQSIYPAEVVQKTA	1185	5.491	TNNQSSYPVAVVQKTA	1456	1.394
TNNQSKYPAEVVQKTA	1538	5.468	TNNSNMYPAEVVQKTA	1457	1.383
TNKIASYPAEVVQKTA	1187	5.445	TNNSNSYPAEVVQKTA	1458	1.379
TNNSSRYPAEVVQKTA	1188	5.231	TNNSSPYPAEVVQKTA	1459	1.362
TNNQSSYPAAVVQKTA	1189	5.228	SLVQSSYPAEVVQKTA	1460	1.358
TNNQSSYPAEVVQKLT	1190	5.112	TNNQSSYPAELKSKTA	1461	1.357
TNTIASYPAEVVQKTA	1191	5.112	TNSISSYTAEVVQKTA	1462	1.357
TNTLSSYPAEVVQKTA	1192	5.055	TNNQSSYPTNSVQKTA	1463	1.344
TNNQSSYPAKLIQKTA	1193	5.051	TNNSSQYPAEVVQKTA	1464	1.340
TNNQASYPAEVVQKTA	1194	5.004	TSLQSSYPAEVVQKTA	1465	1.339
TNNVGSYPAEVVQKTA	1195	4.953	TNNQSPYPAEVVQKTA	1466	1.325
HTRQSSYPAEVVQKTA	1196	4.804	TNNQSYYPASVVQKTA	1467	1.318
TNNMGSYPAEVVQKTA	1197	4.785	TNNLSAYTAEVVQKTA	1468	1.313
TNNVASYPAEVVQKTA	1198	4.765	TNNVSAYPAEVVQKTA	1469	1.307
TNNASGYPAEVVQKTA	1199	4.760	TNTVSSYPAEVVQKPA	1470	1.299
TNSPWSYPAEVVQKTA	1200	4.674	TNNNSVYPAEVVQKTA	1471	1.289
TNNVSNYPAEVVQKTA	1201	4.611	TNNQSSYPAPLVQKTA	1472	1.284
TNNQAKYPAEVVQKTA	1202	4.606	TNNQNSPPAEVVQKTA	1473	1.263
TNNQTTPPAEVVQKTA	1204	4.480	TNSVVSYPAEVVQKTA	1474	1.261
TSSASSYPAEVVQKTA	1205	4.465	TNNISKYPAEVVQKTA	1475	1.258
TNNTSFYPAEVVQKTA	1206	4.427	TNSPFSYPAEVVQKTA	1476	1.252
TNNLANYPAEVVQKTA	1207	4.415	TNNQSSYPAELLSKTA	1477	1.252
TNNTSRYPAEVVQKTA	1208	4.396	TNNQSSTRSEVVQKTA	1478	1.250
TNNQSSYPASVVKKTA	1209	4.347	TNMVSSYPAEVVQKTA	1479	1.249
TLQQSSYPAEVVQKTA	1210	4.309	TNNQLPIPAEVVQKTA	1480	1.244
TNNQSSYPAGVIQKTA	1211	4.272	TNNTSMYPAEVVQKTA	1481	1.241
TNNQSSYPAETMSKTA	1212	4.266	TNNQSSYPSTVVQKTA	1482	1.240
TYTLSSYPAEVVQKTA	1213	4.256	TNNQSSYPATVVKKTA	1483	1.238
TNNSSSYTAEVVQKTA	1214	4.204	TNNQSSLTAEVVQKTA	1484	1.237
TNNTALYPAEVVQKTA	1215	4.200	TNNQSSYPPSVVQKPA	1485	1.224
TNNLTYYPAEVVQKTA	1216	4.198	TNNVTKYPAEVVQKTA	1486	1.210
TNNQSSYPAMVIQKTA	1217	4.192	TNNQSSYPPNVVQKTA	1487	1.210
TNNVTRYPAEVVQKTA	1218	4.189	TNNQSSYPAELMSKTA	1488	1.205
TNNMSAYPAEVVQKTA	1219	4.186	TNALSSYPAEVVQKTA	1489	1.202
TNNQSSYPAEVIAKTA	1220	4.186	TNNQSSYPANVVQKTA	1490	1.201
TNNLAQYPAEVVQKTA	1221	4.176	TNNSALYPAEVVQKPA	1491	1.201
TNNTGGYPAEVVQKTA	1222	4.111	TVTQSSYPAEVVQKTA	1492	1.197
TNNQSSYPAEIVQKTA	1223	4.093	TNNQSSVTLEVVQKTA	1493	1.191
TNNISAYPAEVVQKTA	1224	4.078	TNNVSVYPAEVVQKTA	1494	1.190
TNNQSMYPAEVVQKTA	1225	4.058	TNNQSSYPQELVQKTA	1495	1.188
TNTLGSYPAEVVQKTA	1226	4.043	TNNQSSYPASVNQKTA	1496	1.180

TNNQSSYPAESVQKTA	1227	4.042	TNNQSSYPASIIQKTA	1497	1.171
TNNPSGYPAEVVQKTA	1228	3.980	TNNQSSYPAPVVKKTA	1498	1.163
TNNQDNMPAEVVQKTA	1229	3.917	TNNLAFYPAEVVQKTA	1499	1.158
TNNVSLYPAEVVQKTA	1230	3.868	TNNQSSYPAEAVSKTA	1500	1.157
TNNASSYPAEVVQKTA	1231	3.832	TNNQSSYPAPLEQKTA	1501	1.156
TNNTSLYPAEVVQKTA	1232	3.828	TNNLSKYPAEVVQKTD	1502	1.154
TNNQSSYPASVVQKTA	1233	3.809	TNNQSSYPAPLIQKTA	1503	1.143
TNNQSSYPAELISKTA	1234	3.772	TNTLASYPAEVVQKTA	1504	1.140
TNSVHSYPAEVVQKTA	1235	3.770	TNNQSSYPPGVVQKTA	1505	1.139
TNSVWSYPAEVVQKTA	1236	3.762	TNNQSTQRAEVVQKTA	1506	1.125
TTTPSSYPAEVVQKTA	1237	3.732	TNNLSRYTAEVVQKTA	1507	1.122
TTTTSSYPAEVVQKTA	1238	3.728	TNTVSSYPAEEVQKTA	1508	1.122
TNNQSSYPESNVQKTA	1240	3.683	TNNQSSVAAEVVQKTA	1509	1.106
TNNQSSYPPDLVQKTA	1241	3.672	TNNHSRYPAEVVQKTA	1510	1.101
TNLVASYPAEVVQKTA	1242	3.621	TNNQSSYPPAVVQKTA	1511	1.088
TNLVSSYQAEVVQKTA	1243	3.575	TNNVSRYPAEVVQKTA	1512	1.078
TNNQSVYPAEVVQKTA	1244	3.568	TNKQSSYPAPVVQKTA	1513	1.074
TNNQGYYPAEVVQKTA	1245	3.550	TNNQSSYPAEVSSKTA	1514	1.072
TNSPRSYPAEVVQKTA	1248	3.508	TNNSSHYPAEVVQKTA	1515	1.072
TNNQSSYPASLIQKTA	1249	3.504	TNNSPSYPAEVVQKTA	1516	1.070
TNNSTRYPAEVVQKTA	1250	3.500	TNNQSSYPSTIVQKTA	1517	1.070
TNNSGAYPAEVVQKTA	1251	3.497	TNATSSYPAEVVQKTA	1518	1.069
TNNLAIYPAEVVQKTA	1252	3.450	TNVSLSYPAEVVQKTA	1519	1.068
TNHVASYPAEVVQKTA	1253	3.436	TNNQSSYPASMVQKTA	1520	1.067
TNNQSKYPAEVEQKTA	1254	3.434	TNLVSSYPAEVLQKTA	1521	1.058
TNNHASYPAEVVQKTA	1255	3.413	TNNQSSLNAEVVQKTA	1522	1.057
TNSESSYPAEVVQKTA	1256	3.392	TNNPSKYPAEVVQKTA	1523	1.050
TNNQSSYPANSVQKTA	1257	3.344	TNNQSSYPASIVQKTA	1524	1.046
TNNQSSYPAELVHKTA	1258	3.344	TNNTVSYPAEVVQKTA	1525	1.045
TNNQSSYPPNSVQKTA	1259	3.313	TNNVALYPAEVVQKTA	1526	1.045
TNNTAKYPAEVVQKTA	1260	3.304	TNNQSSYPPVVVQKTA	1527	1.014
TNNKTSYPAEVVQKTA	1261	3.271	TNNQSSYPSSVVKKTA	1528	1.014
TNNNSNYPAEVVQKTA	1262	3.220	TNNQSSYPAEVLKKTA	1529	1.011
TNTLSSYQAEVVQKTA	1263	3.212	TNNQSTLTAEVVQKTA	1530	1.011
TNRISSYPAEVVQKTA	1264	3.210	TNNQSSYPAPLVQQTA	1531	1.005
TNNQSSYPATLIQKTA	1265	3.200	TNNLSPYPAEVVQKTA	1532	1.001
TNNGVSYPAEVVQKTA	1266	3.191	TNNQSSYPAEVVQKTA	1533	1.000
TNNTSSYPAEVVQKPA	1267	3.173	TNNQLTSPAEVVQKTA	1534	0.995
TNNQSSYPAEVVQKTP	1268	3.165	TNNLSHYPAEVVQKTA	1535	0.991

[0527] These data demonstrate that following two maturation approaches, matured TTN-002 capsid variants with loop VIII modifications were generated with significantly enhanced CNS tropism in mice compared to the corresponding non-matured capsid variants, which already exhibited a significant fold enrichment over AAV5 and/or AAV9 in the brain of mice, rats, and/or NHPs.

Example 4. Maturation of the TTN-002 capsid in NHPs

[0528] This Example describes maturation of TTN-002 (SEQ ID NO: 982 (amino acid) and 984 (DNA), comprising SEQ ID NO: 943 (encoded by SEQ ID NO: 944)) capsid variant in NHPs, specifically cynomolgus macaques (*Macaca fascicularis*), to further enhance its transduction and biodistribution in the central nervous system and peripheral tissues and to evolve the AAV capsid variant further. Two approaches were used to mature the TTN-002 capsid sequences 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, mutagenic primers were used to introduce point mutations at a low frequency, scattered across the mutagenesis region in the TTN-002 sequence ranging from approximately position 571 to position 586, numbered according to SEQ ID NO: 982. In the second maturation approach, sets of three contiguous amino acids were randomized across the mutagenesis region in the TTN-002 sequence, which spanned from approximately position 571 to position 586, numbered according to SEQ ID NO: 982. AAV capsid variants arising from each maturation approach for TTN-002 were pooled together, for subsequent testing and characterization in NHPs.

[0529] The library of pooled matured AAV capsid variants generated from TTN-002 using the first maturation approach and the library of pooled matured AAV capsid variants generated from TTN-002 using the second maturation approach were each injected into two NHPs. After a period in life, the brains, heart, liver, muscle, and DRG of the NHPs 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 TTN-002 control, and the peptides comprised within the variants were identified.

[0530] Following the RNA recovery and NGS analysis from the second maturation approach, the matured capsid variants were filtered based on their coefficient of variance (CV), which was calculated for each peptide across the brain, heart, liver, muscle and DRG samples taken from the two NHPs. Those that had a CV value <2 were identified, as these were the peptides that were reliably detected in the majority of samples isolated from the brains of the two NHPs.

[0531] **Table 17** provides the peptide sequences of these matured capsid variants and the fold enrichment of the matured capsid variant relative to the non-matured TTN-002 control that demonstrated the greatest fold-change in expression relative to the non-matured TTN-002 capsid variant in the brain of NHPs, following the first maturation approach and the second maturation approach. As shown in **Table 17**, following the first maturation approach, approximately 5 TTN-002 matured capsid variants demonstrated an increase in expression relative to the non-matured TTN-002 control, which demonstrated at least a 5-fold to 53-fold increase in expression in the NHP brain relative to the non-matured TTN-002 control. Following the second maturation approach, approximately 38 TTN-002 matured capsid variants demonstrated an increase in expression in the NHP brain relative to the non-matured TTN-002 control, with at least 27 demonstrating at least a 2-fold increase in expression (**Table 17**). Several variants demonstrated at least a 12-fold to 222-fold increase in expression in the NHP brain relative to the non-matured TTN-002 control (**Table 17**).

[0532] Fold-change in expression for the TTN-002 matured variants in **Table 17** were also calculated for the DRG, muscle, liver (RNA and DNA), and heart of the NHPs following each maturation approach. Several variants that led to increased expression relative to the non-matured TTN-002 variant in the brain of the NHP also led to increased expression in other tissues. For instance, the matured TTN-002 capsid variant comprising the amino acid sequence

TNNQSSYTPSLVQKTA (SEQ ID NO: 1585) demonstrated increased expression in the brain, heart, and liver relative to the non-matured TTN-002 control. Similarly, the matured TTN-002 capsid variants comprising the amino acid sequence TNNQSSYPPSLVKKTA (SEQ ID NO: 1591) and TNNQSSYPPSLVQKPA (SEQ ID NO: 1593), demonstrated increased expression in the brain and heart relative to the non-matured TTN-002 control. Additionally, the matured TTN-002 capsid variant comprising the amino acid sequence INNQSSYPAEVVQKTA (SEQ ID NO: 1024) demonstrated increased expression in the brain and the muscle relative to the non-matured TTN-002 control. Also, as shown in **Table 17**, many of the TTN-002 capsid variants that had increased expression in the brain, were de-targeted in the DRG. Therefore, several matured variants demonstrated increased tropism in more than one tissue type in the NHPs, with many showing reduced expression in the DRG.

Table 17. NGS fold-enrichment of the TTN-002 matured AAV capsid variants in the brain of NHPs following first and second mutagenesis approaches as compared to other NHP and mouse tissues

Sequence	SEQ ID NO:	Fold Enrichment relative to TTN-002						
		Brain (NHP)	DRG (NHP)	Liver RNA (NHP)	Liver DNA (NHP)	Heart (NHP)	Muscle (NHP)	Brain (Mouse)
First Maturation Approach								
TNNQSKYPAEVVQKTA	1538	53.751	0.000	2.270	0.518	0.000	0.000	10.261
TNNTSLYPAEVVQKTA	1232	22.903	0.000	2.824	0.000	0.000	0.000	1.484
TNNSSSYPAEVVQKTA	1539	19.002	0.000	1.240	0.000	0.000	0.000	8.539
TNNQSRYPAEVVQKTA	1327	8.499	0.000	0.846	0.516	0.000	0.000	1.704
TNNQSSYPPSLVQKTA	1300	5.458	0.000	0.966	0.000	5.100	0.000	1.141
TNNQSSYPAEVVQKTA	1533	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Second Maturation Approach								
TNNAGAYPAEVVQKTA	1021	222.785	0.000	0.000	0.000	0.000	0.000	19.342
TNNIGSYPAEVVQKTA	1112	93.586	0.000	0.000	0.142	0.000	0.000	7.845
TNTASSYPAEVVQKTA	1575	46.343	0.000	0.019	2.353	0.234	0.000	0.012
TNNTSLYPAEVVQKTA	1232	45.861	0.000	5.118	0.432	0.059	0.087	3.828
TNNLGSYPAEVVQKTA	1027	44.023	0.000	0.000	0.156	0.000	0.000	17.430
TNNQSTNKAEVVQKTA	1578	42.633	0.000	0.268	4.032	0.000	0.000	0.000
TNNHSSYPAEVVQKTA	1310	38.584	0.000	0.275	0.430	0.076	0.000	2.600
TNNQSKYPAEVVQKTA	1538	20.857	0.000	2.180	1.568	0.001	0.000	5.468
TNNSSSYTAEVVQKTA	1214	19.625	0.000	0.000	2.152	0.000	0.000	4.204
TNNQSKYPAEVEQKTA	1254	15.501	0.000	3.630	0.000	0.000	0.000	3.434
TNNTSLYPAEEVQKTA	1583	14.603	0.000	1.276	0.000	0.000	0.000	1.249
TNTASSYQAEVVQKTA	1584	13.180	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSSYTPSLVQKTA	1585	12.552	0.000	5.700	2.591	24.396	0.000	1.831
TNNQSSYPPSLVQKTA	1300	9.123	0.000	2.178	1.093	20.059	0.052	2.719
TNNQSRYPAEVVQKTA	1327	8.503	0.000	1.887	1.125	0.002	0.000	2.409
TNNSSSYPAEVVQKTA	1539	8.092	0.267	0.854	0.904	0.003	0.000	5.903
TNNQSRYPAEEVQKTA	1342	7.267	0.000	0.879	0.783	0.000	0.000	2.233
TNNQSSYPPSLEQKTA	1590	6.924	0.000	1.616	0.000	8.962	0.000	1.550
TNNQSSYPPSLVKKTA	1591	6.625	0.000	0.000	0.891	10.953	0.000	1.783
TNNLSSYQAEVVQKTA	1592	5.913	276.643	0.000	0.000	0.000	0.000	1.280
TNNQSSYPPSLVQKPA	1593	5.789	0.000	2.977	1.161	25.392	0.000	3.704
TNNSSSYPAEVVKKTA	1331	4.191	0.000	0.766	0.521	0.000	0.000	2.340
TNNQSKYPAEVVHKTA	1453	3.201	0.000	0.766	0.000	0.000	0.000	1.402
TNNSSSYPAEVVQKPA	1142	2.748	0.000	0.794	0.000	0.000	0.000	6.889

INNQSSYPAEVVQKTA	1024	2.472	0.000	0.992	0.695	0.275	75.541	18.426
TNNHSSYPAAVVQKTA	1598	2.007	0.000	0.000	0.000	0.000	0.000	0.587
TNSQSSNPAEVVQKTA	1599	1.829	0.000	0.000	0.000	0.000	0.000	0.116
TNNSSSYPAEVVQQTA	1419	1.645	0.000	0.510	0.000	0.000	0.000	1.571
NNNQSRYPAEVVQKTA	1601	1.513	0.000	0.565	0.000	0.000	0.000	0.480
TNNQSSYTAEVVQKNA	1602	1.350	0.000	0.000	0.891	0.000	0.000	0.159
TNNTSLCPAEVVQKTA	1603	1.307	0.000	0.851	0.000	0.000	0.000	0.132
TNNQRSYTAEVVQKTA	1604	1.186	0.000	2.836	2.348	0.000	0.000	0.407
TNSTSLYPAEVVQKTA	1605	1.186	0.000	0.000	0.000	0.000	0.000	0.196
TNNQSSYTAEVVKKTA	1606	1.143	0.000	0.839	2.325	0.000	0.000	0.697
TNNHSSYPAEVLQKTA	1607	1.136	0.000	0.000	0.000	0.000	0.000	0.163
TNNQSSYQAEEVQKTA	1608	1.113	0.000	0.000	0.000	0.000	0.000	0.561
TNNQSSYPAEVVQKTA	1533	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TNNKSKYPAEVVQKTA	1610	0.980	0.000	0.000	2.953	0.000	0.000	0.600
TNNQSRYPAEVMQKTA	1611	0.967	0.000	0.000	0.000	0.000	0.000	0.085
TNSTSPYPAEVVQKTA	1612	0.880	0.000	0.000	0.000	0.000	0.000	0.211
TNNQSSYPAWLIQKTA	1613	0.713	0.000	0.000	0.000	0.000	0.000	0.091
TNNQSSYPAEATKKTA	1614	0.693	0.000	0.408	6.529	0.000	0.000	0.000
TNKQSSYTAEVVQKTA	1615	0.678	0.000	0.000	1.151	0.000	0.000	0.240
TNKIGSYPAEVVQKTA	1616	0.660	0.000	0.000	0.812	0.000	0.000	0.046
TNNKSRYPAEVVQKTA	1617	0.648	0.000	5.889	2.080	3.490	0.000	0.467
TNNQSSYPAEVVQKTD	1618	0.646	0.000	0.756	1.247	0.945	3.270	0.530
TNNQSSYPAEVVQKNA	1619	0.640	4.200	3.327	3.647	0.622	3.655	0.505
TNNQRKYPAEVVQKTA	1620	0.629	0.000	6.158	2.709	0.000	0.000	0.171
TNNQSSNPAEEVQKTA	1621	0.617	0.000	0.000	0.989	0.000	0.000	0.449
NNNQSSYPAEVVQKTA	1622	0.546	0.000	1.197	1.528	1.025	58.686	0.750
TNNQSYYPAEEVQKTA	1623	0.537	0.000	0.000	0.000	0.000	0.000	0.365
TKNHSSYPAEVVQKTA	1624	0.522	0.000	0.000	0.845	0.000	0.000	0.062
TNNQASYPAEVVQKTA	1586	88.651						
TNKQASYPAEVVQKTA	1587	1.100						

[0533] Furthermore, several of the TTN-002 matured capsid variants demonstrating an increase in expression relative to the non-matured TTN-002 control following the first and second maturation approaches in the brain of NHPs as shown in **Table 17,** also demonstrated an increase in expression in the brain of mice following the first and second maturation approach in mice. For instance, the matured TTN-002 capsid variant comprising the amino acid sequence TNNQSKYPAEVVQKTA (SEQ ID NO: 1538) demonstrated a 53.7-fold increase in expression relative to the non-matured TTN-002 following the first maturation approach in brain of NHPs, demonstrated a 20.86-fold increase in expression relative to the non-matured TTN-002 following the second maturation approach in brain of NHPs, a 10.26-fold increase in expression relative to the non-matured TTN-002 following the first maturation approach in brain of mice, and a 5.47-fold increase in increase in expression relative to the non-matured TTN-002 following the second maturation approach in brain of mice. Similarly, the matured TTN-002 capsid variant comprising the amino acid sequence TNNSSSYPAEVVQKTA (SEQ ID NO: 1539) demonstrated an 18.997-fold increase in expression relative to the non-matured TTN-002 following the first maturation approach in brain of NHPs, an 8.093-fold increase in expression relative to the non-matured TTN-002 following the second maturation approach in brain of NHPs, an 8.539-fold increase in expression relative to the non-matured TTN-002 following the first maturation approach in brain of mice, and a 5.903-fold increase

in increase in expression relative to the non-matured TTN-002 following the second maturation approach in brain of mice. Matured TTN-002 capsid variants comprising the amino acid sequences of SEQ ID NOs: 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, and 1593 also demonstrated an increase in expression in the brain of both NHPs and mice, relative to the non-matured TTN-002 control. Therefore, several matured variants demonstrated increased expression relative to the non-matured controls in at least two different species, indicating cross-species tropism.

[0534] **Table 18** provides the peptide sequences of these matured capsid variants, and the fold enrichment of the matured capsid variant relative to the non-matured TTN-002 control that demonstrated increased expression in the heart of NHPs following the second maturation approach. As shown in **Table 18**, approximately 17 TTN-002 matured capsid variants demonstrated an increase in expression in the heart relative to the non-matured TTN-002 control, with at least 13 demonstrating at least a 2-fold increase in expression. Several variants demonstrated at least a 10-fold to 47-fold increase in expression in the heart relative to the non-matured TTN-002 control. Fold-change in expression for the TTN-002 matured variants in **Table 18** were also calculated for the brain, DRG, muscle, and liver (RNA and DNA), of the NHPs and in the brains of mice.

Table 18. NGS fold-enrichment of the TTN-002 matured AAV capsid variants in the heart of NHPs following the second mutagenesis approach as compared to other NHP and mouse tissues

Sequence	SEQ ID NO	Fold Enrichment relative to TTN-002						
		Heart (NHP)	Brain (NHP)	DRG (NHP)	Liver RNA (NHP)	Liver DNA (NHP)	Muscle (NHP)	Brain (Mouse)
TNSSLSYQAEVVQKTA	2064	47.380	0.000	0.000	0.000	0.000	0.000	0.000
TNSSLSYTAEVVQKTA	2065	45.827	0.066	0.000	4.885	8.710	0.000	0.000
TNSSLYYPAEVVQKTA	2066	41.710	0.000	0.000	0.000	0.000	0.000	0.000
TNTSATYPAEVVQKTA	2067	35.136	0.000	0.000	0.000	0.756	0.000	0.476
TNSSLSYPAEVVQKTA	2068	32.805	0.015	0.000	0.744	2.122	0.000	0.002
TNSSLSYPAEEVQKTA	2069	23.357	0.000	0.000	0.000	0.000	0.000	0.000
TNSSLSYPAEVVQKTD	2070	18.293	0.000	0.000	0.000	0.000	0.000	0.000
TNSSLSYPAEVVQNTA	2071	17.081	0.000	0.000	0.000	0.000	0.000	0.000
TNSSLSYPAEVVQKNA	2072	13.129	0.000	0.000	0.000	0.000	0.000	0.000
TNSSLSYPAEVVQKPA	2073	13.115	0.000	0.000	0.000	0.521	0.000	0.000
TNNQTSTEAEVVQKTA	2074	10.056	0.000	0.000	0.000	0.000	0.000	0.000
TNKSATYPAEVVQKTA	2075	9.090	0.000	0.000	0.000	0.000	0.000	0.000
TNSSLSYPADVVQKTA	2076	4.692	0.000	0.000	0.000	0.384	0.000	0.000
TNNQSSYPAEVVQKTA	1533	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TNNQSSYPAEVVQKNA	2078	0.622	0.640	4.200	3.327	3.647	3.655	0.505
TNNQSSYPSTDVQKTA	2079	0.572	0.020	0.000	0.000	3.216	0.000	0.000
TNNKGLYPAEVVQKTA	2080	0.506	0.000	0.000	0.000	5.411	0.000	0.002

[0535] **Table 19** provides the peptide sequences of these matured capsid variants, and the fold enrichment of the matured capsid variant relative to the non-matured TTN-002 control that demonstrated increased expression in the muscle of NHPs following the second maturation approach. As shown in **Table 19**, approximately 33 TTN-002 matured capsid variants demonstrated an increase in expression in the muscle relative to the non-matured TTN-002 control, with at least 19

demonstrating at least a 2-fold increase in expression. Several variants demonstrated at least a 7-fold to 38-fold increase in expression in the muscle relative to the non-matured TTN-002 control. Fold-change in expression for the TTN-002 matured variants in **Table 19** were also calculated for the brain, DRG, heart, and liver (RNA and DNA), of the NHPs and in the brains of mice.

Table 19. NGS fold-enrichment of the TTN-002 matured AAV capsid variants in the muscle of NHPs following the second mutagenesis approach

Sequence	SEQ ID NO	Fold Enrichment relative to TTN-002						
		Muscle (NHP)	Brain (NHP)	DRG (NHP)	Liver RNA (NHP)	Liver DNA (NHP)	Heart (NHP)	Brain (Mouse)
TNNQSSYDFTVVQKTA	2024	38.128	0.000	267911.1	0.000	4.209	0.032	0.000
TNNQSSYPAEVVQNVG	2025	20.373	0.000	82501.777	7.770	0.000	0.015	0.000
TNNQSPHAAEVVQKTA	2026	9.977	0.000	85036.503	2.478	0.841	0.009	0.003
TNNLHVYPAEVVQKTA	2027	9.969	0.000	59488.293	0.000	0.934	0.006	0.000
TLKTSSYPAEVVQKTA	2028	9.646	0.000	69692.245	0.032	1.906	0.155	0.000
TNNQAASPAEVVQKTA	2029	7.483	0.000	147630.5	0.000	1.848	0.023	0.000
TNLSMSYPAEVVQKTA	2030	5.960	0.000	67462.229	0.220	1.697	0.014	0.005
TKAFSSYPAEVVQKTA	2031	5.743	0.000	42730.728	0.000	0.919	0.000	0.000
TAYQSSYPAEVVQKTA	2032	4.703	0.000	61779.917	0.022	1.908	0.000	0.000
TNNQSQHQAEVVQKTA	2033	4.510	127.1	107992.3	3.186	4.569	0.015	0.000
TNNQSSVQLEVVQKTA	2034	3.846	0.000	54250.016	7.862	4.173	0.013	0.000
TNNQSSAFAEVVQKTA	2035	3.503	32.731	67253.425	3.207	5.348	0.004	0.000
TNNQSAVLAEVVQKTA	2036	3.351	0.000	28296.228	2.069	3.784	0.007	0.000
LNAQSSYPAEVVQKTA	2037	3.311	0.000	76314.798	0.000	0.476	0.000	0.000
TNNQSQSSAEVVQKTA	2038	3.310	0.000	198372.9	2.579	0.766	0.007	0.003
TNNQSSLHAEVVQKTA	2039	2.685	0.013	23403.089	4.165	4.578	0.005	0.001
TNNFQLYPAEVVQKTA	2040	2.460	0.000	28791.699	0.000	0.053	0.000	0.000
TNNQSSYPAEVVQSMR	2041	2.099	0.000	176004.5	0.000	0.000	0.000	0.000
TLLYSSYPAEVVQKTA	2042	2.044	0.000	8688.765	0.000	2.362	0.006	0.000
TNNQSSTLDEVVQKTA	2043	1.947	0.000	0.000	0.000	0.043	397.2	0.001
TNNQTTTPAEVVQKTA	2044	1.839	0.000	59308.623	1.658	2.863	0.003	0.004
TNNQSSYPAEVVELLA	2045	1.800	0.000	12934.481	0.000	0.175	0.004	0.000
TNNQVNYPAEVVQKTA	2046	1.694	0.000	0.000	12.363	2.400	0.000	0.000
TTSTSSYPAEVVQKTA	2047	1.576	0.002	42212.609	8.264	2.618	0.000	0.003
TNNQSSYPQIAVQKTA	2048	1.506	0.000	0.000	28.933	3.364	0.000	0.000
TNNQSSYPAENLPKTA	2049	1.327	0.000	56068.064	0.000	2.002	0.000	0.008
TNNQSSYPASSPQKTA	2050	1.304	0.000	0.000	1.637	1.814	466.4	0.000
TNNQFMVPAEVVQKTA	2051	1.151	0.000	63928.318	0.000	2.409	0.000	0.000
TNNTTTYPAEVVQKTA	2052	1.121	0.073	12941.562	0.719	0.808	0.065	0.008
TPSKSSYPAEVVQKTA	2053	1.083	0.000	1.366	7.344	1.632	0.000	0.000
TNYLMSYPAEVVQKTA	2054	1.078	0.000	24113.689	0.000	1.271	0.382	0.000
TNLAMSYPAEVVQKTA	2055	1.074	0.000	77469.008	0.701	1.184	0.000	0.006
TNNQSSLMQEVVQKTA	2056	1.057	0.000	0.000	21.968	3.563	0.000	0.000
TNNQSSYPNAIVQKTA	2057	0.960	0.000	0.000	0.077	0.929	0.019	0.000
TNNQSSYPAEVRTATA	2058	0.915	0.000	0.000	30.158	1.431	0.000	0.000
TNNQSSSMTEVVQKTA	2059	0.788	66.451	33379.460	1.383	3.930	0.000	0.000
TNLSPSYPAEVVQKTA	2060	0.778	0.008	11925.049	2.716	1.300	0.000	0.000
TNNQSSYPAESSRKTA	2061	0.657	0.000	0.000	7.755	3.289	377.3	0.000
TNNPLTYPAEVVQKTA	2062	0.641	0.000	18890.829	0.000	1.060	0.000	0.000
TNNLSTYPAEVVQKTA	2063	0.595	0.029	32495.446	0.000	1.113	0.065	0.013

[0536] **Table 20** provides the peptide sequences of these matured capsid variants, and the fold enrichment of the matured capsid variant relative to the non-matured TTN-002 control that

demonstrated increased expression in the liver of NHPs following the first and second maturation approach. As shown in **Table 20**, following the first maturation approach, approximately 7 TTN-002 matured capsid variants demonstrated an 11-fold to 189-fold increase in expression in the liver relative to the non-matured TTN-002 control. Following the second maturation approach, approximately 395 TTN-002 matured capsid variants demonstrated an increase in expression in the liver of at least 9-fold relative to the non-matured TTN-002 control (**Table 20**). Several variants demonstrated at least a 50-fold to 114-fold increase in expression in the liver relative to the non-matured TTN-002 control (**Table 20**). Fold-change in expression for the TTN-002 matured variants in **Table 20** were also calculated for the brain, DRG, heart, and muscle of the NHPs and in the brains of mice.

Table 20. NGS fold-enrichment of the TTN-002 matured AAV capsid variants in the liver of NHPs following both mutagenesis approaches

Sequence	SEQ ID NO	Fold Enrichment relative to TTN-002						
		Liver RNA (NHP)	Liver DNA (NHP)	Brain (NHP)	DRG (NHP)	Heart (NHP)	Muscle (NHP)	Brain (Mouse)
First Maturation Approach								
TDYHRGDPAKVVQKSA	1625	189.324	0.000	0.000	0.000	0.000	0.000	0.000
TNKQKFSLTEELQKNA	1626	101.263	5.032	0.000	0.000	0.000	0.000	0.000
GKTPRHFTQQVVQENA	1627	84.647	1.661	0.000	0.000	0.000	0.000	0.000
SENERSDTSVVVAQIL	1628	56.534	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSSIRLEVVQKTA	1629	13.829	2.357	0.000	0.000	0.000	0.000	0.000
TNNQSSSLAEVVQKTA	1630	11.832	4.625	29.220	0.000	0.000	0.000	0.000
TNNQSGPTAEVVQKTA	1631	11.391	0.000	0.000	0.000	0.000	0.000	1.678
Second Maturation Approach								
TNNQSSYPAEAPKKTA	1632	114.219	5.207	419.144	0.000	0.000	0.000	0.000
TNNQSSYPRKVVQKTA	1633	104.291	0.000	0.000	0.000	0.000	0.000	0.045
TNNQSRIQAEVVQKTA	1634	94.646	4.673	0.000	0.000	0.000	0.182	0.001
TNNQSWHQAEVVQKTA	1635	90.698	8.068	0.000	0.000	0.000	0.000	0.000
TNNQSGPTAEVVQKTA	1631	78.597	5.825	0.000	0.000	0.005	0.000	5.645
NEWQSSYPAEVVQKTA	1636	73.960	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSIKWAEVVQKTA	1637	73.487	1.668	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVVNIQA	1638	71.285	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAHNSQKTA	1639	71.153	1.854	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVVQYQD	1640	67.017	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSSRQQEVVQKTA	1641	63.063	7.092	0.002	0.000	0.000	0.660	0.000
TNNQNCSPAEVVQKTA	1642	59.544	0.951	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAERSHKTA	1643	53.889	4.190	0.000	0.000	0.000	0.000	0.004
TVNRSSYPAEVVQKTA	1644	53.711	6.381	0.000	0.000	0.000	0.000	0.000
TNNQHGGPAEVVQKTA	1645	53.380	0.565	0.000	0.000	0.000	0.000	0.000
TNNQSMIWAEVVQKTA	1646	52.362	1.443	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEASRKTA	1647	49.543	3.268	0.000	0.000	0.040	0.000	0.000
TNNQSSYPAHLTQKTA	1648	49.237	4.942	0.000	0.000	0.000	0.000	0.000
TNNRRGYPAEVVQKTA	1649	48.153	0.000	0.000	0.000	0.000	0.000	0.000
TNNKRIYPAEVVQKTA	1650	48.033	0.000	0.000	0.000	0.000	0.000	0.000
TNNQGALPAEVVQKTA	1651	47.761	1.178	0.000	0.000	0.000	0.000	0.036
TNNQSSYPAEASKKTA	1652	44.984	6.761	0.010	0.000	0.000	0.000	0.000
TGQHSSYPAEVVQKTA	1653	43.610	2.355	0.000	0.000	0.000	0.000	35.110
TNNQSSIVQEVVQKTA	1654	43.603	5.228	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAERNTKTA	1655	43.470	5.264	0.000	0.000	0.007	0.000	0.000
TNNQSSYPAQALQKTA	1656	43.207	2.932	0.000	0.000	0.000	0.000	0.000

TNNQSRRGAEVVQKTA	1657	42.384	2.205	0.000	0.000	0.000	0.000	0.000
TNNQSSYPQVMVQKTA	1658	42.160	0.019	0.000	0.000	0.000	0.000	0.000
TNNQSSQKREVVQKTA	1659	42.065	5.253	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAENARKTA	1660	42.025	4.024	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAGTGQKTA	1661	41.566	3.734	0.000	0.000	0.000	0.000	0.000
TRMPSSYPAEVVQKTA	1662	40.545	2.585	0.000	0.000	0.000	0.000	0.002
TNNQSSPISEVVQKTA	1663	39.647	1.460	0.000	0.000	0.000	0.000	0.000
TNNQSSYGAVVVQKTA	1664	39.126	1.251	0.000	0.000	0.000	0.385	0.000
TNNQSSYPAEQPVKTA	1665	38.694	1.536	0.000	0.000	0.000	0.000	0.000
TNNQSSGYREVVQKTA	1666	37.888	3.088	0.000	0.000	0.003	0.000	0.000
TNNQSGAQAEVVQKTA	1667	37.624	5.498	0.000	0.000	0.000	0.000	0.000
TNNQSSKGREVVQKTA	1668	37.172	2.348	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAESKHKTA	1669	36.198	1.594	0.000	0.000	0.000	0.000	0.000
TNNQSSYNMRVVQKTA	1670	35.863	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEKNTKTA	1671	34.039	2.322	0.000	0.000	0.000	0.000	0.083
TNNQSSYPAERREKTA	1672	33.598	12.673	0.000	0.000	0.000	0.000	0.000
TNNQWWMPAEVVQKTA	1673	33.472	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSYTHAEVVQKTA	1674	33.128	4.433	0.000	0.000	0.000	0.000	0.004
TNNAAKYPAEVVQKTA	1675	32.786	3.029	0.009	0.000	0.000	0.330	0.007
TNNQSSYPLVCVQKTA	1676	32.659	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSNAYAEVVQKTA	1677	32.331	0.978	0.000	0.000	0.000	0.000	0.000
TGVKSSYPAEVVQKTA	1678	32.102	0.884	0.000	0.000	0.000	0.000	0.000
TNNQSSKKTEVVQKTA	1679	31.801	10.223	0.000	0.000	0.012	0.000	0.000
TNNQSSYPAEMANKTA	1680	31.613	3.467	0.000	0.000	0.000	0.000	0.000
TNNQSSYPASMGQKTA	1681	30.875	3.131	0.002	0.000	0.000	0.000	0.000
TNNQSSYPAPGIQKTA	1682	30.648	1.520	0.000	0.000	0.000	0.000	0.015
TNNQSSYPAQDKQKTA	1683	30.569	0.444	0.000	0.000	0.000	0.000	0.000
TNNQSSYPERQVQKTA	1684	30.436	6.881	0.000	0.000	0.000	0.000	0.000
TNNQSSYPASQSQKPA	1685	30.284	0.810	0.000	0.000	0.000	0.000	0.000
TNNQSQGTAEVVQKTA	1686	30.072	2.154	0.002	0.000	0.000	0.000	0.014
TNNQPRLPAEVVQKTA	1687	29.628	2.410	0.000	0.000	0.014	0.000	0.003
TNNQSSYPAELGKKTA	1688	29.441	5.305	0.000	0.000	0.000	0.000	0.009
TNNQSSLTKEVVQKTA	1689	29.297	2.313	0.000	0.000	0.000	0.000	0.001
ELCQSSYPAEVVQKTA	1690	29.051	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSSYPVSRVQKTA	1691	28.960	1.001	0.000	0.000	0.000	0.000	0.000
TNNQSSHHQEVVQKTA	1692	28.825	3.732	0.000	0.000	0.000	0.000	0.000
TNNQWCSPAEVVQKTA	1693	28.739	0.354	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVVQREY	1694	28.521	0.000	0.000	0.000	0.000	0.000	0.000
THKSSSYPAEVVQKTA	1695	28.280	1.162	0.000	0.000	0.000	0.000	0.010
TGNVSSYPAEVVQKTA	1696	28.028	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSSYPTSDVQKTA	1697	27.923	3.549	0.000	0.000	0.130	0.479	0.009
TNNQSSYPAINTQKTA	1698	27.590	5.013	0.000	0.000	0.000	0.000	0.000
TNAPRSYPAEVVQKTA	1699	27.525	2.321	0.000	0.000	0.000	0.000	0.002
TNNQAHNPAEVVQKTA	1700	27.459	2.355	0.000	0.000	0.000	0.000	0.000
TNNQSARIAEVVQKTA	1701	27.305	5.056	0.000	0.000	0.000	0.000	0.000
TNNQSSIHQEVVQKTA	1702	27.261	3.709	0.000	0.000	0.000	0.000	0.011
TNNQSSYPGPKVQKTA	1703	26.890	4.998	0.000	0.000	0.000	0.000	12.243
TNNQSSYPASQSQKTA	1704	26.085	3.225	0.000	0.000	0.001	0.000	0.000
TNNQSVAKAEVVQKTA	1705	25.379	2.456	0.004	0.000	0.000	0.000	0.000
TGLLSSYPAEVVQKTA	1706	25.248	1.073	0.000	17.422	0.000	0.000	0.866
TNNQSSYPAEMNKKTA	1707	25.179	11.154	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVVIRGA	1708	24.885	0.454	0.000	0.000	0.000	0.000	0.000
TNNQSSYVLEVVQKTA	1709	24.760	3.119	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAERSAKTA	1710	24.694	4.869	0.001	0.000	0.000	0.000	0.000
TNNQSSYRTNVVQKTA	1711	24.382	0.619	0.000	0.000	0.000	0.000	0.000
TNFTPSYPAEVVQKTA	1712	24.209	0.528	0.000	1.558	0.000	0.000	0.005
TNNQSSTTTEVVQKTA	1713	24.072	0.872	0.005	0.000	0.058	0.000	0.001
TNNQSFLDAEVVQKTA	1714	24.054	0.885	0.008	0.000	0.000	0.000	0.006

TNNQSSYPTSNVQKTA	1715	23.530	3.586	0.000	0.000	0.000	0.622	0.000
TNNQSSRHDEVVQKTA	1716	23.512	1.200	0.000	0.000	0.000	0.000	0.000
TNNQSSYPQKSVQKTA	1717	23.414	2.234	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAERHPKTA	1718	23.276	4.332	0.000	0.000	0.000	0.000	0.000
TNNQSSKVQEVVQKTA	1719	23.231	2.813	0.000	0.000	0.000	0.000	0.000
LGVQSSYPAEVVQKTA	1720	23.175	2.605	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEARQKTA	1721	22.889	4.006	0.000	0.000	0.000	0.000	0.000
TNNQSSYPDSRVQKTA	1722	22.614	4.720	0.000	0.000	0.000	0.000	0.000
TNNQSSYPQMDVQKTA	1723	22.445	2.469	0.000	0.000	0.000	0.000	0.000
TNNQSVQGAEVVQKTA	1724	22.409	0.161	0.000	0.000	0.000	0.000	0.000
TNNQSSYSEAVVQKTA	1725	22.354	0.911	0.000	0.000	0.000	1.593	0.000
TNNQSSYTALVSQKTA	1726	22.345	8.610	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVRAKTA	1727	22.283	5.669	0.013	0.000	0.000	0.000	0.002
TNNQSSPGTEVVQKTA	1728	22.240	5.707	0.000	0.000	0.000	0.000	0.000
TNNQSMMKAEVVQKTA	1729	22.043	2.468	0.000	0.000	0.000	0.000	0.000
TNNQSSYPADNNQKTA	1730	21.899	0.254	0.000	0.000	0.000	0.000	0.074
TNNQSSQNLEVVQKTA	1731	21.884	4.064	0.000	0.000	0.000	0.000	0.000
TNNKPPYPAEVVQKTA	1732	21.774	1.524	0.000	0.000	0.000	0.000	0.002
TNNQSNTYAEVVQKTA	1733	21.680	4.281	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAERPGKTA	1734	21.642	4.857	0.000	0.000	0.015	0.000	0.002
TNNQSSYPAESVRKTA	1735	21.641	5.644	0.000	0.000	0.000	0.784	0.002
TNNQSSYSVGVVQKTA	1736	21.595	2.004	0.000	0.000	0.000	0.000	0.000
TNNQSSYPLDRVQKTA	1737	21.582	4.140	0.000	0.000	0.005	0.000	0.000
TNNQSQIKAEVVQKTA	1738	21.553	5.308	0.000	0.000	0.000	0.000	0.000
TNNQSSNMVEVVQKTA	1739	21.435	2.898	0.000	0.000	0.000	0.000	0.000
TNNQSSYSSHVVQKTA	1740	21.301	2.342	0.000	0.000	0.000	0.000	0.000
TNNQGSSPAEVVQKTA	1741	21.255	2.814	0.000	0.000	0.000	0.000	0.006
TNNQSSKYSEVVQKTA	1742	21.233	2.717	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEARAKTA	1743	21.084	3.135	0.000	0.000	0.000	0.000	0.000
TNNQSSYTASLTQKTA	1744	21.040	14.386	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEGPQKTA	1745	21.024	1.251	0.000	0.000	0.000	0.000	0.000
TNNQSQPKAEVVQKTA	1746	20.892	1.972	0.000	1.841	0.000	0.000	0.000
TNNQSQVIAEVVQKTA	1747	20.571	0.344	0.000	0.000	0.000	0.000	0.007
TNNQSSYPAERPNKTA	1748	20.524	5.620	0.000	0.000	0.000	0.000	0.000
TNNQSWTVAEVVQKTA	1749	20.134	3.768	0.000	0.000	0.000	0.000	0.000
TNNQSRVIAEVVQKTA	1750	20.128	2.855	0.000	0.000	0.000	0.506	0.000
TNNQSSYPAEYERKTA	1751	19.648	1.217	0.000	0.000	0.000	0.000	0.000
TFHKSSYPAEVVQKTA	1752	19.633	8.119	0.000	0.000	0.000	0.000	0.000
TNNQRERPAEVVQKTA	1753	19.581	2.717	0.000	0.000	0.000	0.000	0.022
TNNQSSYPAERQGKTA	1754	19.516	6.304	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEMTKKTA	1755	19.434	2.047	0.000	0.000	0.000	0.000	0.016
TNNQSWNMAEVVQKTA	1756	19.403	5.718	0.000	0.000	0.000	0.000	0.000
TNNQSSLKDEVVQKTA	1757	19.297	1.734	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAGPSQKTA	1758	18.941	3.244	0.000	0.000	0.000	0.000	0.000
TNNQSSKVNEVVQKTA	1759	18.822	1.309	0.000	0.000	0.000	0.000	0.000
TNNQSSYPMRDVQKTA	1760	18.821	3.804	0.000	0.000	0.000	0.000	0.000
TNNQSSYPALTNQKTA	1761	18.740	2.259	0.000	0.000	0.000	0.000	0.001
TNNQSSNALEVVQKTA	1762	18.623	3.784	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVVQKSQ	1763	18.240	2.245	0.000	0.000	0.000	0.000	0.000
TPYKSSYPAEVVQKTA	1764	18.238	0.798	0.000	0.000	0.000	0.000	0.000
TTTPSSYPAEVVQKTA	1765	18.196	2.669	0.007	1.392	0.000	0.000	3.732
TNNQSSYKDAVVQKTA	1766	18.131	6.014	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEDTRKTA	1767	17.933	5.009	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAERTQKTA	1768	17.912	4.907	0.000	0.000	0.003	0.000	0.000
TNNQSSYPAEHAQKTA	1769	17.800	0.929	0.000	0.000	0.000	0.000	0.011
TNNQSSYPAASPQKTA	1770	17.783	2.261	0.000	0.000	0.083	0.000	0.000
TNNQSSKNREVVQKTA	1771	17.772	7.501	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVTTKTA	1772	17.474	2.148	0.000	0.000	0.000	0.000	0.004

TNNQSSYPAEVVSKQA	1773	17.439	2.945	0.000	0.000	0.000	0.000	0.041
TNNQRNQPAEVVQKTA	1774	17.352	5.416	0.000	0.000	0.000	0.000	0.005
TNNQSSYPAEKVSKTA	1775	17.300	3.161	0.000	0.000	0.000	0.000	0.008
TNNQSSYPAEFTKKTA	1776	17.287	4.123	0.000	0.000	0.000	0.000	0.000
TAMDSSYPAEVVQKTA	1777	17.278	6.714	0.000	0.000	0.000	0.000	0.000
YLKQSSYPAEVVQKTA	1778	17.185	2.032	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEKLTKTA	1779	17.174	4.714	0.005	5.527	0.000	0.000	0.000
TNNQSSYPAEVVNGKA	1780	17.143	1.029	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEKGSKTA	1781	17.038	5.983	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAHINQKTA	1782	16.961	7.399	0.000	0.000	0.000	0.000	0.000
TNNQSSYPVQNVQKTA	1783	16.911	3.355	0.000	0.000	0.000	0.000	0.000
TNNQSSYPKDTVQKTA	1784	16.890	1.297	0.000	0.000	0.000	0.000	0.000
TNNQVIWPAEVVQKTA	1785	16.816	2.555	0.000	0.000	0.000	0.000	0.000
TNNQSWVAAEVVQKTA	1786	16.775	0.627	0.000	0.000	0.000	0.000	0.000
TDGISSYPAEVVQKTA	1787	16.708	0.150	0.000	0.000	0.000	0.000	0.000
TNNQSSRNGEVVQKTA	1788	16.667	0.367	0.000	0.000	0.000	0.000	0.000
TNNQSSHPAEVVQKTA	1789	16.653	3.700	6.125	0.000	0.006	0.000	0.897
TNNQSSMQMEVVQKTA	1790	16.616	6.837	0.000	0.000	0.000	0.000	0.000
TNNQSSVSHEVVQKTA	1791	16.522	4.597	0.000	0.000	0.000	12.027	0.004
TNNQSSTFTEVVQKTA	1792	16.482	2.661	0.000	0.000	0.000	0.000	0.000
TNNQSSYTATASQKTA	1793	16.475	5.560	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAESPKKTA	1794	16.426	3.157	0.023	0.000	0.000	0.000	0.000
TNNQSSYPAEPRGKTA	1795	16.249	6.345	0.000	0.000	0.000	0.000	0.000
TNNQSSIRLEVVQKTA	1796	16.197	3.887	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAKDLQKTA	1797	16.197	0.255	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEHVSKTA	1798	16.196	5.228	0.000	0.000	0.000	0.000	0.032
TNNQSSYPAEGKAKTA	1799	16.075	3.013	0.000	0.000	0.000	0.000	0.026
TNNQSRMKAEVVQKTA	1800	15.914	1.084	0.000	0.000	1.069	0.000	0.000
TNNQSWLQAEVVQKTA	1801	15.911	3.287	0.000	0.000	0.009	0.000	0.000
TNNQSHDRAEVVQKTA	1802	15.847	3.669	0.000	0.000	0.000	0.000	0.000
TNNQSSGSTEVVQKTA	1803	15.778	5.237	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEPAKKTA	1804	15.763	3.448	0.000	0.000	0.000	0.000	0.005
TNNQSSYPAERSNKTA	1805	15.735	3.609	0.000	0.000	0.000	0.000	0.000
TFNKSSYPAEVVQKTA	1806	15.700	0.476	0.000	0.000	0.000	0.000	0.000
TNNQSCLYAEVVQKTA	1807	15.679	2.156	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAERVLKTA	1808	15.674	3.436	0.000	0.000	0.000	0.000	0.000
TFRRSSYPAEVVQKTA	1809	15.621	10.989	0.000	0.000	0.000	0.000	0.000
TNNRHNYPAEVVQKTA	1810	15.620	1.758	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAETEGKTA	1811	15.607	1.076	0.000	0.000	0.000	0.000	0.000
TNHPSSYPAEVVQKTA	1812	15.593	1.681	0.000	0.000	0.000	0.000	0.002
TTGISSYPAEVVQKTA	1813	15.532	1.301	0.000	0.000	0.000	0.000	0.000
TNAAQSYPAEVVQKTA	1814	15.531	1.512	0.000	0.000	0.000	0.000	0.000
TNNQSSGSMEVVQKTA	1815	15.459	5.283	0.000	0.000	0.000	0.000	0.000
TNNARGYPAEVVQKTA	1816	15.450	4.233	0.000	0.000	0.000	0.000	0.000
TNNQSSYPFRSVQKTA	1817	15.429	0.000	0.000	0.000	0.000	0.000	0.000
TNLTGSYPAEVVQKTA	1818	15.427	1.420	0.000	0.000	0.000	0.000	0.001
TNNQSSTYLEVVQKTA	1819	15.377	2.983	0.004	0.000	0.000	0.000	0.000
TNNQSSYPPAQVQKTA	1820	15.332	3.214	0.000	0.000	0.005	0.000	0.000
THYGSSYPAEVVQKTA	1821	15.294	0.251	0.000	0.000	0.000	0.000	0.000
TNNQSRFGAEVVQKTA	1822	15.279	2.490	0.000	0.000	0.000	0.000	0.000
TNNVKHYPAEVVQKTA	1823	15.239	0.208	0.000	0.000	0.000	0.000	0.000
TNNQSSYPTKSVQKTA	1824	15.221	3.402	0.010	0.000	0.000	0.000	0.047
TNNQSSYSQHVVQKTA	1825	15.207	2.302	0.000	0.000	0.000	0.000	0.003
TNNQSSGHTEVVQKTA	1826	15.202	7.933	0.000	0.000	0.000	0.000	0.005
TNNQSSYPAQTVQKTA	1827	15.159	3.586	0.000	0.000	0.077	0.000	0.002
TNNQSSYPAHFPQKTA	1828	15.145	1.610	0.000	0.000	0.000	0.000	0.000
TNNQSSYPLAKVQKTA	1829	15.138	3.309	0.000	0.000	0.000	0.000	0.000
TNNQSSYTALTVQKTA	1830	15.057	10.012	0.000	0.000	0.000	0.000	0.000

TNQRSSYPAEVVQKTA	1831	15.029	3.694	0.000	0.000	0.000	0.000	1.493
TNNQSSYPAEVVSKKA	1832	14.986	1.378	0.000	0.000	0.000	0.000	0.003
TNNQSSYPASRSQKTA	1833	14.966	1.741	0.000	0.000	0.000	0.000	0.005
TNNQSDIRAEVVQKTA	1834	14.861	9.186	0.000	0.000	0.000	0.000	0.005
TNNQYSRPAEVVQKTA	1835	14.818	6.222	0.000	0.000	0.000	0.000	0.672
TNNQSGLNAEVVQKTA	1836	14.713	4.785	0.005	0.000	0.000	0.000	0.000
TNNQSRMMAEVVQKTA	1837	14.700	4.917	0.000	0.000	113.3	0.387	0.000
TSAKSSYPAEVVQKTA	1838	14.673	1.418	0.000	0.000	0.000	0.000	0.009
TNNQSSYPANGPQKTA	1839	14.649	2.642	0.000	0.000	0.000	0.000	0.000
VFYQSSYPAEVVQKTA	1840	14.578	0.183	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAERTSKTA	1841	14.574	3.973	0.000	0.000	0.002	0.000	0.000
TNSTPSYPAEVVQKTA	1842	14.527	2.179	0.072	0.000	0.000	0.000	1.229
TNNQSSYPAEHAVKTA	1843	14.486	2.272	0.000	0.000	0.000	0.000	0.000
TNNQSRGHAEVVQKTA	1844	14.451	3.993	0.000	0.000	0.000	0.185	0.000
TNNRAGYPAEVVQKTA	1845	14.424	4.286	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEQRLKTA	1846	14.420	6.535	0.000	0.000	0.002	0.000	0.000
TNNQSRIMAEVVQKTA	1847	14.362	4.485	0.000	0.000	0.086	0.000	0.000
TNNQSTTLAEVVQKTA	1848	14.334	4.654	0.000	0.000	0.004	0.109	0.004
TNNQSSYPAEVANKTA	1849	14.333	5.878	0.000	0.000	0.000	0.000	0.004
TNNQSSSVKEVVQKTA	1850	14.316	3.803	0.019	0.000	0.000	0.000	0.000
TNNQSSYTTSLVQKTA	1851	14.263	9.339	0.000	0.000	0.000	0.000	0.053
TNNQSSYPAHNMQKTA	1852	14.168	2.792	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAQTTQKTA	1853	14.112	3.444	0.000	0.000	0.000	0.000	0.003
TNNQSSYPFNSVQKTA	1854	14.027	1.747	0.001	0.000	0.000	0.000	0.002
TNNQSIIRAEVVQKTA	1855	13.962	3.872	0.000	0.000	0.000	0.000	0.000
TNNQSLRIAEVVQKTA	1856	13.958	2.105	0.001	0.000	0.000	0.000	0.000
TNNQSCCLAEVVQKTA	1857	13.938	3.967	0.000	0.000	0.000	1.090	0.000
TNNQSSALLEVVQKTA	1858	13.926	4.607	0.017	0.000	0.000	0.000	0.001
TNNQSSYPAEVVQRNT	1859	13.901	0.859	0.000	0.000	0.000	0.000	0.000
TNNQLNLPAEVVQKTA	1860	13.886	1.557	0.000	0.000	0.000	0.000	0.003
TNNQSRMYAEVVQKTA	1861	13.872	4.626	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVVLDMA	1862	13.847	0.023	0.000	0.000	0.000	0.000	0.000
TNNQSKVMAEVVQKTA	1863	13.836	4.270	0.000	0.000	0.006	0.000	0.000
TNNQSSVRQEVVQKTA	1864	13.782	5.311	0.032	0.000	0.005	224130.7	0.001
TNNQSSYVSKVVQKTA	1865	13.778	5.007	0.000	0.000	0.000	1.049	0.000
TNNQSSYPAETHVKTA	1866	13.750	4.737	0.000	0.000	0.000	0.000	0.000
TNNQSSHGIEVVQKTA	1867	13.712	4.717	0.000	0.000	0.000	0.000	0.000
THSQSSYPAEVVQKTA	1868	13.670	1.734	0.001	0.000	0.000	0.000	2.202
TNNQSSYPGNAVQKTA	1869	13.658	3.408	0.000	0.000	0.000	0.000	0.001
TNNQSSMNLEVVQKTA	1870	13.627	3.094	0.000	0.000	0.000	0.000	0.001
TNNQSSYTARVVQKTA	1871	13.624	1.418	0.039	0.000	0.000	0.000	0.000
TNNQSQGAAEVVQKTA	1872	13.582	4.669	0.000	0.000	0.000	0.000	0.009
TNNQSSYPAERGAKTA	1873	13.571	4.712	0.000	0.000	0.000	0.000	0.000
TNNQSSPILEVVQKTA	1874	13.557	6.002	0.000	0.000	0.000	0.000	0.005
TNNQSSYPAETHSKTA	1875	13.530	6.586	0.000	0.000	0.000	0.000	0.000
TAFRSSYPAEVVQKTA	1876	13.525	2.361	0.000	0.000	0.000	0.000	0.000
TNNVRSYPAEVVQKTA	1877	13.465	3.993	0.000	0.000	0.003	0.000	0.010
TNNQSATPAEVVQKTA	1878	13.413	2.222	0.000	0.270	0.000	0.000	0.003
TNNQSLTYAEVVQKTA	1879	13.296	1.688	0.000	0.000	0.000	0.000	0.000
TNNQKRGPAEVVQKTA	1880	13.274	5.012	0.000	0.000	0.000	0.000	0.000
TNNQSSYPMPAVQKTA	1881	13.125	3.693	0.000	0.000	0.000	0.000	0.001
TNNQSRNPAEVVQKTA	1882	13.084	2.666	0.019	0.000	0.000	0.222	0.004
TNNQSNVRAEVVQKTA	1883	13.033	4.034	0.000	0.000	0.000	0.000	0.005
TNNQSKLLAEVVQKTA	1884	13.004	3.829	0.000	0.000	0.000	0.000	0.000
TNNQSYMIAEVVQKTA	1885	12.960	2.494	0.000	0.000	0.000	0.000	0.000
TNNQSSYPEPKVQKTA	1886	12.959	5.548	0.000	0.000	0.000	0.646	0.000
TNNQSSYPAESMLKTA	1887	12.928	2.621	0.000	0.000	0.002	0.144	0.001
TNNQSSYPAEILRKTA	1888	12.921	5.048	0.000	0.000	0.000	0.000	0.001

TNNQSFMVAEVVQKTA	1889	12.921	1.794	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEKLVKTA	1890	12.911	4.402	0.005	0.000	0.000	0.168	0.004
TNNQSSYPAHSGQKTA	1891	12.828	3.582	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAENRGKTA	1892	12.790	4.245	0.000	0.000	0.023	0.000	0.003
TNNQSHLFAEVVQKTA	1893	12.741	2.798	0.000	0.000	0.000	0.000	0.000
TNNQSMTYAEVVQKTA	1894	12.738	0.925	0.000	0.000	0.000	0.000	0.000
TNNQSGRQAEVVQKTA	1895	12.598	2.184	0.000	0.000	0.000	0.000	2.796
IAPQSSYPAEVVQKTA	1896	12.595	0.000	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEKTGKTA	1897	12.539	4.257	0.000	0.000	0.000	0.000	0.000
TNNQSWALAEVVQKTA	1898	12.529	1.420	0.000	0.000	0.000	0.000	0.000
TNNQSSAPLEVVQKTA	1899	12.517	3.246	0.000	0.000	0.000	0.000	0.000
TNNQSFVKAEVVQKTA	1900	12.515	5.062	0.078	0.000	0.005	0.000	0.000
TNNQSSYPAEVGKKTA	1901	12.494	2.992	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVRKLTA	1902	12.486	3.952	0.000	0.000	0.000	0.000	0.000
TNNQYQSPAEVVQKTA	1903	12.288	2.134	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAERQNKTA	1904	12.283	7.027	0.008	0.000	0.000	0.000	0.000
TNNQSSYTAESMLKTA	1905	12.250	0.891	0.000	0.000	0.000	0.000	0.000
TNLTSSYPAEVVQKTA	1906	12.093	3.006	0.000	0.000	0.000	0.000	1.818
TNNQSTVKAEVVQKTA	1907	12.085	3.357	0.009	0.000	0.000	0.000	0.000
TNNQSSPHTEVVQKTA	1908	11.974	1.811	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAVVMQKTA	1909	11.964	1.683	0.000	0.000	0.000	0.000	0.000
TNNQSSPGREVVQKTA	1910	11.961	1.945	0.000	0.000	0.000	0.000	0.000
TIQTSSYPAEVVQKTA	1911	11.943	1.273	0.055	0.000	0.033	0.000	0.000
TNNQSSYPSHDVQKTA	1912	11.932	2.338	0.000	0.000	0.000	0.000	0.000
TNNQSSTIFEVVQKTA	1913	11.924	0.277	0.000	0.000	0.000	0.000	0.000
TNNQSGKRAEVVQKTA	1914	11.869	1.710	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVSAKTA	1915	11.755	2.942	3.684	0.000	0.000	0.000	0.549
TNNQSTAPAEVVQKTA	1916	11.701	2.848	0.020	0.000	0.000	0.000	1.552
TNNQSVRNAEVVQKTA	1917	11.700	5.299	0.004	0.000	0.000	0.000	0.000
TNNQSSYPAVNVQKTA	1918	11.683	2.855	0.000	0.000	0.003	0.624	0.000
TNNQSVFRAEVVQKTA	1919	11.636	3.423	0.000	0.000	0.010	0.000	0.000
TNNQSSYPARDLQKTA	1920	11.635	2.649	0.000	0.000	0.000	0.000	0.000
TNNRVTYPAEVVQKTA	1921	11.604	4.162	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAGASQKTA	1922	11.587	2.692	0.000	0.000	0.000	0.000	0.001
TNNQRLTPAEVVQKTA	1923	11.563	0.939	0.000	0.000	0.000	0.000	0.000
TNNQSKMFAEVVQKTA	1924	11.548	5.878	0.004	0.000	0.000	0.000	0.000
TNNQSSYTTMVVQKTA	1925	11.545	2.022	0.000	0.000	0.000	0.000	0.001
TNNQHSPPAEVVQKTA	1926	11.524	1.602	0.090	0.000	0.000	0.000	0.001
TNNQSSYPADHQQKTA	1927	11.518	2.922	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVVKNHA	1928	11.518	2.302	0.000	0.000	0.000	0.000	0.000
TNNQSKGIAEVVQKTA	1929	11.494	2.897	0.000	0.000	0.000	0.000	0.000
TNNPFVYPAEVVQKTA	1930	11.477	0.766	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAEVNQRTA	1931	11.467	5.421	0.000	0.000	0.000	0.000	0.017
TNNQSQARAEVVQKTA	1932	11.462	3.052	0.000	1.765	0.000	0.000	0.000
TNAMRSYPAEVVQKTA	1933	11.435	0.717	0.297	0.000	0.000	0.000	0.008
TNNQSSYPAEMRTKTA	1934	11.414	6.798	0.000	0.000	0.000	0.000	0.000
TNNQSSYPMGTVQKTA	1935	11.385	1.645	0.000	0.000	0.000	0.000	0.000
TNNQSKLLAEVVQKPA	1936	11.342	2.915	0.000	0.000	0.000	0.000	0.000
TNNGHSYPAEVVQKTA	1937	11.323	1.066	0.000	0.000	0.123	0.000	0.006
TNNQSSYPAEKASKTA	1938	11.283	3.164	0.000	0.000	0.005	0.000	0.000
TNNQSYLPAEVVQKTA	1939	11.281	3.598	0.000	0.000	0.000	0.000	0.000
TNNQSSYPATASQKTA	1940	11.239	4.205	0.000	0.000	0.006	0.104	0.000
TNNQSSYPAEVGKSTA	1941	11.236	5.856	0.000	0.000	0.000	0.000	0.000
TNNQSSFNVEVVQKTA	1942	11.231	1.252	0.000	0.000	0.000	0.000	0.008
TNNQSSRYQEVVQKTA	1943	11.191	2.650	0.000	0.000	0.000	0.000	0.000
TNNQQDLPAEVVQKTA	1944	11.184	0.000	0.000	0.000	0.000	0.000	0.000
TTSQSSYPAEVVQKTA	1945	11.183	1.308	0.000	2.070	0.000	0.000	0.000
TNNQSMGLAEVVQKTA	1946	11.182	3.666	0.000	0.000	0.000	0.000	1.522

TNNQSSILAEVVQKTA	1947	11.168	4.373	0.011	0.000	0.000	0.000	0.003
TNNQSSYPARESQKTA	1948	11.167	5.693	0.000	0.000	0.000	0.000	0.002
TFFKSSYPAEVVQKTA	1949	11.161	6.543	0.000	0.000	0.000	0.000	0.000
TNNQSYLQAEVVQKTA	1950	11.149	5.709	0.000	0.000	0.000	0.000	0.000
TNNQSSYPLKSVQKTA	1951	11.144	2.721	0.000	3.887	1.099	0.000	0.000
TNNQVSNPAEVVQKPA	1952	11.144	0.756	0.000	0.000	0.000	0.000	0.000
TNNQSSYPASSVKKTA	1953	11.121	2.694	0.000	0.000	0.000	0.000	0.000
SRSQSSYPAEVVQKTA	1954	11.116	3.092	0.000	0.000	0.000	0.000	0.000
TNNQSSYPATANQKTA	1955	11.110	1.662	0.006	0.000	0.000	0.000	0.008
TNNQRVSPAEVVQKTA	1956	11.107	2.954	0.000	0.491	0.000	0.000	0.006
TNNQSWTLAEVVQKTA	1957	11.074	5.721	0.000	0.000	0.000	0.000	0.000
TNNQSSTSQEVVQKTA	1958	11.070	5.925	0.000	0.000	0.000	0.000	0.004
TNNQSSTWSEVVQKTA	1959	11.024	4.711	0.104	0.000	0.000	0.000	1.778
TNNQSFRLAEVVQKTA	1960	11.023	3.075	0.000	0.000	0.000	0.000	0.000
TNNQSSYQREVVQKTA	1961	11.009	1.955	0.000	0.000	0.000	0.000	0.006
TNNQSSYPAEMTRKTA	1962	11.004	2.855	0.000	0.000	0.000	0.000	0.000
TNNQSSYPVHNVQKTA	1963	10.987	2.473	0.000	0.000	0.000	0.000	0.000
TNNQSSLHREVVQKTA	1964	10.972	2.605	0.000	0.000	0.000	0.000	0.000
TNNQSSFTREVVQKTA	1965	10.958	2.447	0.000	0.000	0.000	0.000	0.000
TNNQSSRVNEVVQKTA	1966	10.934	2.861	0.000	0.000	0.000	0.000	0.000
TNNQSSYPRGTVQKTA	1967	10.909	2.381	0.000	0.000	0.499	1.353	0.021
TPRPSSYPAEVVQKTA	1968	10.907	2.676	0.000	14.902	0.000	0.000	0.011
TNNQSRTYAEVVQKTA	1969	10.896	3.655	0.000	0.000	0.000	0.000	0.000
TNNQSSYTALVTQKTA	1970	10.889	7.450	0.000	0.000	0.000	0.000	0.000
TNNQSFNLAEVVQKTA	1971	10.863	5.748	0.036	0.000	0.000	0.000	0.002
TNWQSSYPAEVVQKTA	1972	10.804	3.863	0.000	0.000	0.000	0.000	0.000
TLQTSSYPAEVVQKTA	1973	10.800	3.298	0.012	77.694	374.6	0.000	0.000
TNNQSSYPAERLQKTA	1974	10.798	4.850	0.000	0.000	0.000	0.000	0.000
TNNQSSMLPEVVQKTA	1975	10.792	3.735	0.001	0.000	0.000	0.000	0.001
TNNQSSYPESGVQKTA	1976	10.772	2.301	0.000	0.000	0.000	0.000	0.000
TNNQSSYPSTNVQKTA	1977	10.723	3.581	0.000	0.000	0.000	0.000	0.005
TNNQSSYPFSQVQKTA	1978	10.655	4.068	0.000	0.000	0.000	0.000	0.000
TNNQSGQLAEVVQKTA	1979	10.620	2.718	0.000	0.000	0.000	0.000	0.003
TNNQSSRWQEVVQKTA	1980	10.619	0.039	0.000	0.000	0.000	0.000	0.000
TNNQSSSPAEVVQKPA	1981	10.609	4.216	0.000	0.000	0.000	0.000	0.299
TNNQSSIALEVVQKTA	1982	10.601	4.298	0.000	0.000	0.000	0.000	0.000
TNNQSSARSEVVQKTA	1983	10.585	4.080	0.009	0.000	0.000	0.000	0.003
TNNQSSSVREVVQKTA	1984	10.563	2.732	0.000	0.000	0.000	0.000	0.000
TNNQSMSHAEVVQKTA	1985	10.559	2.887	0.000	0.000	0.000	0.000	0.000
TNNQSSKTQEVVQKTA	1986	10.559	1.692	0.000	1.914	0.000	0.000	0.002
TNNQSSIGTEVVQKTA	1987	10.505	2.615	0.000	0.000	0.000	0.000	0.009
TNNQSSYPASAMQKTA	1988	10.505	3.785	0.001	0.000	0.000	0.000	0.000
TNNQSSYPAQHTQKTA	1989	10.502	3.440	0.000	0.000	0.000	0.000	0.000
TNNQSSYPPKQVQKTA	1990	10.501	1.690	0.000	0.000	0.000	0.000	0.006
TNSKNSYPAEVVQKTA	1991	10.499	2.291	0.033	0.000	0.000	0.000	0.000
TNNQRSMPAEVVQKTA	1992	10.485	3.812	0.050	0.000	0.000	0.000	0.000
TNNQSSSNLEVVQKTA	1993	10.475	3.045	0.000	0.000	0.000	0.000	0.000
TNNQVLYPAEVVQKTA	1994	10.467	2.334	0.005	0.000	0.016	0.000	0.001
TNNQSIHPAEVVQKTA	1995	10.463	2.236	0.000	0.000	0.000	0.248	0.003
TNNQSYNMAEVVQKTA	1996	10.429	5.146	0.000	0.000	0.000	0.000	0.000
TNNQSSYTASSAQKTA	1997	10.427	3.158	0.000	0.000	0.000	0.000	0.000
TNNQSSNGVEVVQKTA	1998	10.415	2.651	0.000	0.000	0.000	0.000	0.000
TNNQSSYPDRLVQKTA	1999	10.409	2.906	0.001	0.000	0.000	0.000	0.000
TNNQSSLLAEVVQKTA	2000	10.367	3.838	0.000	23.928	0.011	2.016	0.090
TNNQSPTAAEVVQKTA	2001	10.307	1.949	0.000	0.000	0.000	0.000	0.001
TNNQSSQLAEVVQKTA	2002	10.294	3.792	0.008	0.000	0.005	0.000	0.000
TNNQSSYPTTEVQKTA	2003	10.277	5.539	0.000	0.000	0.000	0.000	0.004
TNNQSSYPAEASQKTA	2004	10.233	3.979	0.007	0.000	0.000	0.000	0.000

TNNQSWVLAEVVQKTA	2005	10.223	2.827	0.000	0.000	0.000	0.000	0.000
TNNQSSIVREVVQKTA	2006	10.214	4.402	0.000	0.000	0.000	0.178	0.002
TNNQWTRPAEVVQKTA	2007	10.195	1.655	0.000	0.000	0.000	0.000	0.000
TNNQSSYERVVVQKTA	2008	10.189	0.652	0.000	0.000	0.000	0.000	0.000
TNNQSSVRSEVVQKTA	2009	10.180	3.489	0.001	0.000	0.000	0.000	0.000
TNNQSSELREVVQKTA	2010	10.174	2.995	0.000	0.000	0.015	0.000	0.000
TNNQSSYVKSVVQKTA	2011	10.171	2.873	0.000	0.000	0.000	0.000	0.000
TNNQSSYPKLQVQKTA	2012	10.161	3.023	0.000	0.000	0.000	0.000	0.000
TNNQSSYDVMVVQKTA	2013	10.132	6.923	0.000	0.000	0.000	0.000	0.000
TNSNVSYPAEVVQKTA	2014	10.114	2.151	0.000	0.000	0.008	0.000	0.000
TNNQWSMPAEVVQKTA	2015	10.109	2.452	0.026	0.000	0.000	0.000	0.000
TNNQSSSQLEVVQKTA	2016	10.102	3.295	0.000	0.000	0.000	0.309	1.567
TNNQSTANAEVVQKTA	2017	10.084	5.631	0.000	0.000	0.000	0.000	0.000
TNNQSSYPATGSQKTA	2018	10.069	5.184	0.000	0.000	0.000	0.000	0.001
TNNQSSPSLEVVQKTA	2019	10.046	7.144	0.000	0.000	0.000	0.000	0.002
TNNAYTYPAEVVQKTA	2020	10.027	0.424	0.000	0.000	0.000	0.000	0.000
TNNQSSYPASVVKKTA	2021	10.026	0.746	7.839	0.000	0.000	0.000	4.347
TYAKSSYPAEVVQKTA	2022	10.011	1.568	0.000	0.000	0.000	0.000	0.000
TNNQSSYPAVGGQKTA	2023	9.968	2.899	0.000	0.000	0.000	0.000	0.000

[0537] These data demonstrate that following two maturation approaches, matured TTN-002 capsid variants (AAV5 capsid variants) with loop VIII modifications were generated with significantly enhanced CNS, heart, muscle, and liver tropism in NHPs compared to the corresponding non-matured capsid variants, which already exhibited a significant fold enrichment over AAV5 and/or AAV9 in the brain of mice, rats, and/or NHPs. Also, several of the resulting matured variants demonstrated cross-species CNS tropism in both NHPs and mice.

Example 5. Evaluation of TTN-002 AAV capsid variant in diverse primate species

[0538] This Example evaluates the tropism and cross-species compatibility of the TTN-002 (SEQ ID NO: 982 (amino acid) and 984 (DNA), comprising SEQ ID NO: 943) capsid variant in two diverse primate species, marmosets (*Callithrix jacchus*) and African green monkeys (*Chlorocebus sabaeus*), as compared to their tropism in cynomolgus macaques (*Macaca fascicularis*) provided in Example 1 and 2. The amino acid and DNA sequences of the TTN-002 capsid variant are provided, e.g., in Tables 4 and 5, respectively.

[0539] To investigate tropism in African green monkeys, AAV particles comprising the TTN-002 capsid variant or an AAV5 control under the control of a synapsin promoter, were intravenously injected into the African green monkeys (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 AAV5 wild-type control.

[0540] To investigate tropism in marmoset monkeys, AAV particles comprising the TTN-002 capsid variant, or an AAV5 control, were intravenously injected into marmosets (n=2, >10 months of age) at a dose of 2E13 vg/kg. 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 AAV5 wild-type control.

[0541] As provided in **Table 21** (African green monkeys) and **Table 22** (marmosets), the TTN-002 capsid variant demonstrated increased CNS tropism in diverse primate species. The TTN-002 capsid variant demonstrated a 64.9-fold increase in expression relative to AAV5 in the brain of cynomolgus macaques (**Table 9**, Example 1), a 7.5-fold increase in expression relative to AAV5 in the brain of African green monkeys, and a 40.4-fold increase in expression relative to AAV5 in the brain of marmosets. Furthermore, TTN-002 also resulted in increased expression in the brain of rats (**Table 9**, Example 1), demonstrating an average fold change in expression relative to AAV5 of 41.1.

Table 21. NGS-Fold Enrichment of TTN-002 in African green monkeys

Sequence	SEQ ID NO:	Fold Enrichment relative to AAV9					
		Brain	DRG	Heart	Liver DNA	Liver RNA	Muscle
YPAEVVQK	943	7.462	3.9674	0.3592	0.0543	0.0227	0.6982

Table 22. NGS-Fold Enrichment of TTN-002 in Marmosets

Sequence	SEQ ID NO:	Fold Enrichment relative to AAV9				
		Brain	Heart	Liver DNA	Liver RNA	Muscle
YPAEVVQK	943	40.381	3.1981	0.0321	0.0328	2.0631

[0542] Taken together, these data demonstrate that the AAV5 capsid variant TTN-002 demonstrated increased CNS tropism relative to the AAV5 control in the CNS across three diverse primate species and rats, providing evidence of strong cross-species capacity.

We claim:

1. An AAV capsid variant, comprising an amino acid sequence having the following formula: [N2]-[N3], wherein:

(i) [N2] comprises positions X1, X2, X3, X4, and X5, wherein:

(a) position X1 is Y, N, or C;

(b) position X2 is P, K, T, or Q;

(c) position X3 is A or P;

(d) position X4 is E, S, or A; and

(e) position X5 is V, L, or E; and

(ii) [N3] comprises the amino acid sequence of VQK, EQK, VKK, VHK, VQQ, or LQK; and

wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 739, or an amino acid sequence at least 95% identical thereto.

2. The AAV capsid variant of claim 1, wherein:

(a) [N2] comprises YP, NK, YT, YQ, NP, CP, TH, AE, PS, AA, AS, PA, PP, KA, TA, QA, TP, HA, EV, SL, EE, AV, or SH;

(b) [N2] comprises YPA, YPP, NKA, YTA, YQA, YTP, NPA, CPA, THA, PAE, PPS, KAE, TAE, QAE, TPS, PAA, HAS, AEV, PSL, AEE, or AAV;

(c) [N2] comprises YPAE (SEQ ID NO: 21), YPPS (SEQ ID NO: 22), NKAE (SEQ ID NO: 23), YTAE (SEQ ID NO: 24), YQAE (SEQ ID NO: 25), YTPS (SEQ ID NO: 26), YPAA (SEQ ID NO: 27), NPAE (SEQ ID NO: 28), CPAE (SEQ ID NO: 29), THAS (SEQ ID NO: 30), PAEV (SEQ ID NO: 17), PPSL (SEQ ID NO: 31), KAEV (SEQ ID NO: 32), TAEV (SEQ ID NO: 16), PAEE (SEQ ID NO: 18), QAEV (SEQ ID NO: 15), TPSL (SEQ ID NO: 33), PAAV (SEQ ID NO: 34), or QAEE (SEQ ID NO: 35); and/or

(d) [N2] comprises YPAEV (SEQ ID NO: 1), YPPSL (SEQ ID NO: 2), NKAEV (SEQ ID NO: 3), YTAEV (SEQ ID NO: 4), YPAEE (SEQ ID NO: 5), YQAEV (SEQ ID NO: 6), YTPSL (SEQ ID NO: 7), YPAAV (SEQ ID NO: 8), NPAEV (SEQ ID NO: 9), CPAEV (SEQ ID NO: 10), or YQAEE (SEQ ID NO: 11).

3. The AAV capsid variant of claim 1 or 2, wherein [N2]-[N3] comprises:

(i) the amino acid sequence of AEVVQK (SEQ ID NO: 36), PSLVQK (SEQ ID NO: 37), AEVEQK (SEQ ID NO: 38), AEEVQK (SEQ ID NO: 39), PSLEQK (SEQ ID NO: 40), PSLVKK (SEQ ID NO: 41), AEVVKK (SEQ ID NO: 42), AEVVHK (SEQ ID NO: 43), AAVVQK (SEQ ID NO: 44), AEVVQQ (SEQ ID NO: 45), or AEVLQK (SEQ ID NO: 46)

(ii) the amino acid sequence of PAEVVQK (SEQ ID NO: 20), PPSLVQK (SEQ ID NO: 47), KAEVVQK (SEQ ID NO: 48), TAEVVQK (SEQ ID NO: 49), PAEVEQK (SEQ ID NO: 50), PAEEVQK (SEQ ID NO: 51), QAEVVQK (SEQ ID NO: 52), TPSLVQK (SEQ ID NO: 53),

PPSLEQK (SEQ ID NO: 54), PPSLVKK (SEQ ID NO: 55), PAEVVKK (SEQ ID NO: 56), PAEVVHK (SEQ ID NO: 57), PAAVVQK (SEQ ID NO: 58), PAEVVQQ (SEQ ID NO: 59), TAEVVKK (SEQ ID NO: 60), PAEVLQK (SEQ ID NO: 61), or QAEEVQK (SEQ ID NO: 62).

4. The AAV capsid variant of any one of claims 1-3, wherein [N2]-[N3] comprises YPAEVVQK (SEQ ID NO: 943), YPPSLVQK (SEQ ID NO: 946), NKAEVVQK (SEQ ID NO: 947), YTAEVVQK (SEQ ID NO: 948), YPAEVEQK (SEQ ID NO: 949), YPAEEVQK (SEQ ID NO: 950), YQAEVVQK (SEQ ID NO: 951), YTPSLVQK (SEQ ID NO: 952), YPPSLEQK (SEQ ID NO: 953), YPPSLVKK (SEQ ID NO: 954), YPAEVVKK (SEQ ID NO: 955), YPAEVVHK (SEQ ID NO: 956), YPAAVVQK (SEQ ID NO: 957), NPAEVVQK (SEQ ID NO: 958), YPAEVVQQ (SEQ ID NO: 959), CPAEVVQK (SEQ ID NO: 960), YTAEVVKK (SEQ ID NO: 961), YPAEVLQK (SEQ ID NO: 962), or YQAEEVQK (SEQ ID NO: 963).

5. The AAV capsid variant of any one of claims 1-4, which further comprises [N1], wherein [N1] comprises positions X_D, X_E, and X_F, wherein:

(a) position X_D is Q, T, S, A, I, L, or H;

(b) position X_E is S, G, A, or R; and

(c) position X_F is S, K, L, R, A, or T.

6. The AAV capsid variant of claim 5, wherein [N1]:

(a) comprises SK, SL, SS, SR, GA, GS, AS, ST, RS, QS, TS, AG, IG, QA, LG, HS, LS, or QR; and/or

(b) comprises QSS, QSK, TSL, SSS, QSR, AGA, IGS, QAS, ASS, LGS, QST, HSS, LSS, or QRS.

7. The AAV capsid variant of claim 5 or 6, wherein [N1]-[N2] comprises QSSYPAEV (SEQ ID NO: 96), QSKYPAEV (SEQ ID NO: 97), TSLYPAEV (SEQ ID NO: 98), SSSYPAEV (SEQ ID NO: 99), QSRYPAEV (SEQ ID NO: 100), QSSYPPSL (SEQ ID NO: 101), AGAYPAEV (SEQ ID NO: 102), IGSYPAEV (SEQ ID NO: 103), QASYPAEV (SEQ ID NO: 104), ASSYPAEV (SEQ ID NO: 105), LGSYPAEV (SEQ ID NO: 106), QSTNKAEV (SEQ ID NO: 107), HSSYPAEV (SEQ ID NO: 108), SSSYTAEV (SEQ ID NO: 109), TSLYPAEE (SEQ ID NO: 110), ASSYQAEV (SEQ ID NO: 111), QSSYTPSL (SEQ ID NO: 112), QSRYPAEE (SEQ ID NO: 113), LSSYQAEV (SEQ ID NO: 114), HSSYPAAV (SEQ ID NO: 115), QSSNPAEV (SEQ ID NO: 116), QSSYTAEV (SEQ ID NO: 117), TSLCPAEV (SEQ ID NO: 118), QRSYTAEV (SEQ ID NO: 119), or QSSYQAEE (SEQ ID NO: 120).

8. The AAV capsid variant of any one of claims 5-7, wherein:

(a) [N1]-[N2]-[N3] comprises SSYPAEVVQ (SEQ ID NO: 121), SKYPAEVVQ (SEQ ID NO: 122), SLYPAEVVQ (SEQ ID NO: 123), SRYPAEVVQ (SEQ ID NO: 124), SSYPPSLVQ (SEQ ID NO: 125), GAYPAEVVQ (SEQ ID NO: 126), GSYPAEVVQ (SEQ ID NO: 127), ASYPAEVVQ (SEQ ID NO: 128), STNKAEVVQ (SEQ ID NO: 129), SSYTAEVVQ (SEQ ID NO: 130), SKYPAEVEQ (SEQ ID NO: 131), SLYPAEEVQ (SEQ ID NO: 132), SSYQAEVVQ (SEQ ID NO: 133), SSYTPSLVQ (SEQ ID NO: 134), SRYPAEEVQ (SEQ ID NO: 135), SSYPPSLEQ (SEQ ID NO: 136), SSYPPSLVK (SEQ ID NO: 140), SSYPAEVVK (SEQ ID NO: 141), SKYPAEVVH (SEQ ID NO: 142), SSYPAAVVQ (SEQ ID NO: 143), SSNPAEVVQ (SEQ ID NO: 144), SLCPAEVVQ (SEQ ID NO: 145), RSYTAEVVQ (SEQ ID NO: 146), SSYTAEVVK (SEQ ID NO: 147), SSYPAEVLQ (SEQ ID NO: 148), or SSYQAEEVQ (SEQ ID NO: 149); or

(b) [N1]-[N2]-[N3] comprises QSSYPAEVVQK (SEQ ID NO: 150), QSKYPAEVVQK (SEQ ID NO: 151), TSLYPAEVVQK (SEQ ID NO: 152), SSSYPAEVVQK (SEQ ID NO: 153), QSRYPAEVVQK (SEQ ID NO: 154), QSSYPPSLVQK (SEQ ID NO: 155), AGAYPAEVVQK (SEQ ID NO: 156), IGSYPAEVVQK (SEQ ID NO: 157), QASYPAEVVQK (SEQ ID NO: 158), ASSYPAEVVQK (SEQ ID NO: 159), LGSYPAEVVQK (SEQ ID NO: 160), QSTNKAEVVQK (SEQ ID NO: 161), HSSYPAEVVQK (SEQ ID NO: 162), SSSYTAEVVQK (SEQ ID NO: 163), QSKYPAEVEQK (SEQ ID NO: 164), TSLYPAEEVQK (SEQ ID NO: 165), ASSYQAEVVQK (SEQ ID NO: 166), QSSYTPSLVQK (SEQ ID NO: 167), QSRYPAEEVQK (SEQ ID NO: 168), QSSYPPSLEQK (SEQ ID NO: 169), QSSYPPSLVKK (SEQ ID NO: 170), LSSYQAEVVQK (SEQ ID NO: 171), SSSYPAEVVKK (SEQ ID NO: 172), QSKYPAEVVHK (SEQ ID NO: 173), HSSYPAAVVQK (SEQ ID NO: 174), QSSNPAEVVQK (SEQ ID NO: 175), SSSYPAEVVQQ (SEQ ID NO: 176), QSSYTAEVVQK (SEQ ID NO: 177), TSLCPAEVVQK (SEQ ID NO: 178), QRSYTAEVVQK (SEQ ID NO: 179), QSSYTAEVVKK (SEQ ID NO: 180), HSSYPAEVLQK (SEQ ID NO: 181), or QSSYQAEEVQK (SEQ ID NO: 182).

9. The AAV capsid variant of any one of claims 1-8, which further comprises [N0], wherein [N0] comprises positions X_A, X_B, and X_C, wherein:

(a) position X_A is T, I, or N;

(b) position X_B is N; and

(c) position X_C is N, T, S, or K.

10. The AAV capsid variant of claim 9, wherein [N0]:

(a) comprises TN, IN, NN, NT, NS, or NK; or

(b) comprises TNN, TNT, INN, TNS, NNN, or TNK.

11. The AAV capsid variant of claim 9 or 10, wherein [N0]-[N1] comprises TNNQSS (SEQ ID NO: 183), TNNQSK (SEQ ID NO: 184), TNNTSL (SEQ ID NO: 185), TNNSSS (SEQ ID NO: 186),

TNNQSR (SEQ ID NO: 187), TNNAGA (SEQ ID NO: 188), TNNIGS (SEQ ID NO: 189), TNNQAS (SEQ ID NO: 190), TNTASS (SEQ ID NO: 191), TNNLGS (SEQ ID NO: 192), TNNQST (SEQ ID NO: 193), TNNHSS (SEQ ID NO: 194), TNNQSK (SEQ ID NO: 184), TNNLSS (SEQ ID NO: 195), INNQSS (SEQ ID NO: 196), TNSQSS (SEQ ID NO: 197), NNNQSR (SEQ ID NO: 198), TNSTSL (SEQ ID NO: 199), TNNQRS (SEQ ID NO: 200), or TNKQAS (SEQ ID NO: 201).

12. The AAV capsid variant of any one of claims 9-11, wherein [N0]-[N1]-[N2]-[N3] comprises TNNQSSYPAEVVQK (SEQ ID NO: 500), TNNQSKYPAEVVQK (SEQ ID NO: 503), TNNTSLYPAEVVQK (SEQ ID NO: 506), TNNSSSYPAEVVQK (SEQ ID NO: 508), TNNQSRYPAEVVQK (SEQ ID NO: 510), TNNQSSYPPSLVQK (SEQ ID NO: 512), TNNAGAYPAEVVQK (SEQ ID NO: 513), TNNIGSYPAEVVQK (SEQ ID NO: 514), TNNQASYPAEVVQK (SEQ ID NO: 517), TNTASSYPAEVVQK (SEQ ID NO: 520), TNNLGSYPAEVVQK (SEQ ID NO: 523), TNNQSTNKAEVVQK (SEQ ID NO: 524), TNNHSSYPAEVVQK (SEQ ID NO: 525), TNNSSSYTAEVVQK (SEQ ID NO: 526), TNNQSKYPAEVEQK (SEQ ID NO: 529), TNNTSLYPAEEVQK (SEQ ID NO: 530), TNTASSYQAEVVQK (SEQ ID NO: 531), TNNQSSYTPSLVQK (SEQ ID NO: 533), TNNQSRYPAEEVQK (SEQ ID NO: 534), TNNQSSYPPSLEQK (SEQ ID NO: 535), TNNQSSYPPSLVKK (SEQ ID NO: 536), TNNLSSYQAEVVQK (SEQ ID NO: 539), TNNSSSYPAEVVKK (SEQ ID NO: 540), TNNQSKYPAEVVHK (SEQ ID NO: 542), INNQSSYPAEVVQK (SEQ ID NO: 543), TNNHSSYPAAVVQK (SEQ ID NO: 545), TNSQSSNPAEVVQK (SEQ ID NO: 548), TNNSSSYPAEVVQQ (SEQ ID NO: 551), NNNQSRYPAEVVQK (SEQ ID NO: 552), TNNQSSYTAEVVQK (SEQ ID NO: 553), TNNTSLCPAEVVQK (SEQ ID NO: 554), TNSTSLYPAEVVQK (SEQ ID NO: 556), TNNQRSYTAEVVQK (SEQ ID NO: 557), TNNQSSYTAEVVKK (SEQ ID NO: 558), TNNHSSYPAEVLQK (SEQ ID NO: 560), TNNQSSYQAEEVQK (SEQ ID NO: 562), or TNKQASYPAEVVQK (SEQ ID NO: 563).

13. The AAV capsid variant of any one of claims 1-12, which further comprises [N4], wherein [N4] comprises positions X_G and X_H, wherein:

(a) position X_G is T, P, or N; and

(b) position X_H is A.

14. The AAV capsid variant of claim 13, wherein [N4] comprises TA, PA, or NA.

15. The AAV capsid variant of claims 13 or 14, wherein [N3]-[N4] comprises VQKTA (SEQ ID NO: 564), EQKTA (SEQ ID NO: 565), VKKTA (SEQ ID NO: 566), VQKPA (SEQ ID NO: 567),

VHKTA (SEQ ID NO: 568), VQQTA (SEQ ID NO: 569), VQKNA (SEQ ID NO: 570), or LQKTA (SEQ ID NO: 571).

16. The AAV capsid variant of any one of claims 13-15, wherein [N0]-[N1]-[N2]-[N3]-[N4] comprises TNNQSSYPAEVVQKTA (SEQ ID NO: 1533), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNTSLYPAEVVQKTA (SEQ ID NO: 1232), TNNSSSYPAEVVQKTA (SEQ ID NO: 1539), TNNQSRYPAEVVQKTA (SEQ ID NO: 1327), TNNQSSYPPSLVQKTA (SEQ ID NO: 1300), TNNAGAYPAEVVQKTA (SEQ ID NO: 1021), TNNIGSYPAEVVQKTA (SEQ ID NO: 1112), TNNQASYPAEVVQKTA (SEQ ID NO: 1194), TNTASSYPAEVVQKTA (SEQ ID NO: 1575), TNNLGSYPAEVVQKTA (SEQ ID NO: 1027), TNNQSTNKAEVVQKTA (SEQ ID NO: 1578), TNNHSSYPAEVVQKTA (SEQ ID NO: 1310), TNNQSKYPAEVVQKTA (SEQ ID NO: 1538), TNNSSSYTAEVVQKTA (SEQ ID NO: 1214), TNNQSKYPAEVEQKTA (SEQ ID NO: 1254), TNNTSLYPAEEVQKTA (SEQ ID NO: 1583), TNTASSYQAEVVQKTA (SEQ ID NO: 1584), TNNQSSYTPSLVQKTA (SEQ ID NO: 1585), TNNQSRYPAEEVQKTA (SEQ ID NO: 1342), TNNQSSYPPSLEQKTA (SEQ ID NO: 1590), TNNQSSYPPSLVKKTA (SEQ ID NO: 1591), TNNLSSYQAEVVQKTA (SEQ ID NO: 1592), TNNQSSYPPSLVQKPA (SEQ ID NO: 1593), TNNSSSYPAEVVKKTA (SEQ ID NO: 1331), TNNQSKYPAEVVHKTA (SEQ ID NO: 1453), TNNSSSYPAEVVQKPA (SEQ ID NO: 1142), INNQSSYPAEVVQKTA (SEQ ID NO: 1024), TNNHSSYPAAVVQKTA (SEQ ID NO: 1598), TNSQSSNPAEVVQKTA (SEQ ID NO: 1599), TNNSSSYPAEVVQQTA (SEQ ID NO: 1419), NNNQSRYPAEVVQKTA (SEQ ID NO: 1601), TNNQSSYTAEVVQKNA (SEQ ID NO: 1602), TNNTSLCPAEVVQKTA (SEQ ID NO: 1603), TNSTSLYPAEVVQKTA (SEQ ID NO: 1605), TNNQRSYTAEVVQKTA (SEQ ID NO: 1604), TNNQSSYTAEVVKKTA (SEQ ID NO: 1606), TNNHSSYPAEVLQKTA (SEQ ID NO: 1607), TNNQSSYQAEEVQKTA (SEQ ID NO: 1608), or TNKQASYPAEVVQKTA (SEQ ID NO: 1587).

17. The AAV capsid variant of any one of claims 1-16, wherein:

(a) [N2]-[N3] is present in loop VIII; and/or

(b) [N0], [N1], and [N4] are present in loop VIII;

wherein loop VIII comprises positions 571-599 of SEQ ID NO: 982.

18. The AAV capsid variant of any one of claims 1-17, wherein:

(i) X_A of [N0] is present at position 571, X_B of [N0] is present at position 572, and X_C of [N0] is present at position 573, numbered according to SEQ ID NO: 982;

(ii) X_D of [N1] is present at position 574, X_E of [N1] is present at position 575, and X_F of [N1] is present at position 576, numbered according to SEQ ID NO: 982;

(iii) X1 of [N2] is present at position 577, X2 of [N2] is present at position 578, X3 of [N2] is present at position 579, X4 of [N2] is present at position 580, and X5 of [N2] is present at position 581, numbered according to SEQ ID NO: 982;

(iv) [N3] is present at positions 582-584, numbered according to SEQ ID NO: 982; and/or

(v) X_G of [N4] is present at position 585 and X_H of [N4] is present at position 586, numbered according to SEQ ID NO: 982.

19. An AAV capsid variant comprising:

(a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or

(c) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20.

20. The AAV capsid variant of any one of claims 1-19, comprising:

(a) the amino acid sequence of any of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583-1587, 1590, 1591-1593, 1598-1608, or 1610-1624;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of SEQ ID NOs: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624; or

(c) an amino acid sequence comprising 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: 943, 1021, 1024, 1027, 1112, 1142, 1214, 1232, 1254, 1300, 1310, 1327, 1331, 1342, 1419, 1453, 1533, 1538, 1539, 1575, 1578, 1583- 1587, 1590, 1591-1593, 1598-1608, or 1610-1624.

21. The AAV capsid variant of claim 19 or 20, wherein:

(i) the 3 consecutive amino acids comprise YPA;

(ii) the 4 consecutive amino acids comprise YPAE (SEQ ID NO: 21);

(iii) the 5 consecutive amino acids comprise YPAEV (SEQ ID NO: 1);

(iv) the 6 consecutive amino acids comprise YPAEVV (SEQ ID NO: 725);

(v) the 7 consecutive amino acids comprise YPAEVVQ (SEQ ID NO: 726); and/or

(vi) the amino acid sequence comprises YPAEVVQK (SEQ ID NO: 943).

22. The AAV capsid variant of any one of claims 19-21, which comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943) or an amino acid sequence comprising one, two, or three but no more than four different amino acids relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

23. The AAV capsid variant of any one of claims 1-22, wherein:

(i) the AAV capsid variant comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 944; 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: 944; and/or

(ii) the nucleotide sequence encoding the capsid variant comprises the nucleotide sequence of SEQ ID NO: 944; 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: 944.

24. The AAV capsid variant of any one of claims 1-23, wherein the amino acid Y is present at position 577, and the amino acid sequence of PAEVVQK (SEQ ID NO: 20) is present at positions 578-584, numbered relative to SEQ ID NO: 982.

25. The AAV capsid variant of any one of the preceding claims, which comprises the amino acid sequence of SEQ ID NO: 739.

26. The AAV capsid variant of any one of the preceding claims, which comprises the amino acid sequence of SEQ ID NO: 738, or an amino acid sequence with at least 95% sequence identity thereto, optionally wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 738.

27. The AAV capsid variant, of any one of claims 1-26, wherein:

(i) the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 982, or an amino acid sequence with at least 95% sequence identity thereto, optionally wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 982; and/or

(ii) the nucleotide sequence encoding the AAV capsid variant comprises the nucleotide sequence of SEQ ID NO: 984, or a nucleotide sequence with at least 90% sequence identity thereto, optionally wherein the nucleotide sequence is codon optimized.

28. An AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 982.

29. The AAV capsid variant, of any one of claims 1-28, wherein the AAV capsid variant

(i) which 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, 139, or 982;

(ii) transduces a brain region, e.g., a temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, and/or cerebellum, optionally wherein the level of transduction is at least 1.5, 2.2, 2.4, 2.5, 2.6, 2.7, 3.0, 3.2, 3.5, 3.7, 4.0, 4.2, 4.5, 4.7, 4.9, 5, 10, 15, 20, 25, 30, 35-fold greater as compared to a reference sequence of SEQ ID NO: 139 , e.g., when measured by an assay, e.g., an immunohistochemistry assay or a qPCR assay, e.g., as described in Example 2;

(iii) is enriched at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 25, 30, 35, 40, 45, 50, 55, 60, or 65-fold, in the brain compared to a reference sequence of SEQ ID N O: 138, e.g., when measured by an assay as described in Example 1;

(iv) is enriched at least about 10, 12, 15, 17, 20, 25, 30, 35, 40, 45, 50, 55, 60, 61, 62, 63, 64, or 65-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, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 25, 30, 35, 40, 45-fold, in the brain of at least two to three species, e.g., a non-human primate and rodent (e.g., rat or mouse), compared to a reference sequence of SEQ ID NO: 138, e.g., when measured by an assay as described in Examples 1, 2, and 4;

(vi) is enriched at least about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 125, 150, 175, 200, or 225-fold, in the brain compared to a reference sequence of SEQ ID NO: 982, e.g., when measured by an assay as described in Example 4;

(vii) delivers an increased level of a payload to a brain region, optionally wherein the level of the payload is increased by at least 20, 25, 30, 35-fold, as compared to a reference sequence of SEQ ID NO: 139, e.g., when measured by an assay, e.g., a qRT-PCR or a qPCR assay (e.g., as described in Example 2), optionally wherein the brain region is a temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, and/or cerebellum;

(viii) delivers an increased level of viral genomes to a brain region, optionally wherein the level of viral genomes is increased by at least 1.5, 2.2, 2.4, 2.5, 2.6, 2.7, 3.0, 3.2, 3.5, 3.7, 4.0, 4.2, 4.5, 4.7, 4.9, or 5-fold, as compared to a reference sequence of SEQ ID NO: 139, e.g., when measured by an assay, e.g., a qRT-PCR or a qPCR assay (e.g., as described in Example 2), optionally wherein the brain region is a temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje Layer, deep cerebellar nuclei, and/or cerebellum;

(ix) enriched at least about 3, 3.5, 4.0, 4.5, 5, 5.0, 6.0, or 6.5-fold, in a spinal cord region compared to a reference sequence of SEQ ID NO: 139, e.g., when measured by an assay as described

in Example 2, optionally wherein the spinal cord region is a cervical region, a lumbar region, a thoracic region, or a combination thereof;

(x) shows preferential transduction in a brain region relative to the transduction in the dorsal root ganglia (DRG);

(xi) shows preferential transduction in a brain region relative to the transduction in the liver; and/or

(xii) is capable of transducing neuronal cell and non-neuronal cells, e.g., glial cells (e.g., oligodendrocytes or astrocytes).

30. A polynucleotide encoding the AAV capsid variant of any one of claims 1-29.

31. The polynucleotide of claim 30, which 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: 944;

(iii) the nucleotide sequence of SEQ ID NO: 944, or nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; and/or

(iv) the nucleotide sequence of SEQ ID NO: 984, or a nucleotide sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto,

optionally wherein the polynucleotide comprises a nucleotide sequence that is codon optimized.

32. A peptide comprising:

(a) the amino acid sequence of any of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20;

(b) an amino acid sequence comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive amino acids from any one of the sequences provided in Tables 1, 2A, 2B, 9, or 15-20; or

(c) an amino acid sequence comprising one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the amino acid sequences provided in Tables 1, 2A, 2B, 9, or 15-20.

33. A peptide comprising:

(i) the amino acid sequence of YPAEVVQK (SEQ ID NO: 943);

(ii) an amino acid sequence comprising one, two, or three, but no more than four different amino acids relative to the amino acid sequence of YPAEVVQK (SEQ ID NO: 943); or

(iii) at least 3, 4, 5, 6, or 7 consecutive amino acids from the amino acid sequence of YPAEVVQK (SEQ ID NO: 943).

34. An AAV particle comprising the AAV capsid variant of any one of claims 1-29, an AAV capsid variant encoded by the polynucleotide of claim 30 or 31, or an AAV capsid variant comprising the peptide of claim 32 or 33.

35. The AAV particle of claim 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 34-36, 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 1α-subunit (EF1α), 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 nβ2, 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., αMHC, 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: 987-1007, a nucleotide sequence comprising at least one, two, three, four, five, six, or seven but no more than four modifications, e.g., substitutions, relative to the nucleotide sequence of SEQ ID NOs: 987-1007, 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: 987-1007.

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 miR-1 binding site, a miR-142-3p, 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 miR-1 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 miR-142-3p 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, polynucleotide, peptide, or AAV particle of any one of the preceding claims which is isolated, e.g., recombinant.

43. A vector comprising a polynucleotide encoding the AAV capsid variant of any one of claims 1-29 or 42, the polynucleotide of any one of claims 30, 31, or 42, or a polynucleotide encoding the peptide of any one of claims 32, 33, or 42.

44. A cell, e.g., a host cell, comprising the AAV capsid variant of any one of claims 1-29 or 42, the polynucleotide of any one of claims 30, 31, or 42, a polynucleotide encoding the peptide of any one of claims 32, 33, or 42, the AAV particle of any one of claims 34-42, 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 1-29 or 42, or an AAV capsid variant encoded by the polynucleotide of any one of claims 30, 31, or 32;

thereby making the AAV particle.

46. A pharmaceutical composition comprising the AAV particle of any one of claims 34-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-29 or 42, an AAV particle comprising the peptide of any one of claims 32, 33, 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 34-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-29 or 42, or an AAV particle comprising the peptide of any one of claims 32, 33, 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;

(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 34-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-29 or 42, or an AAV particle comprising the peptide of any one of claims 32, 33, 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 34-42, an AAV particle comprising

the AAV capsid variant of any one of claims 1-29 or 42, or an AAV particle comprising the peptide of any one of claims 32, 33, 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 34-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-29 or 42, an AAV particle comprising the peptide of any one of claims 32, 33, 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 34-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-29 or 42, or an AAV particle comprising the peptide of any one of claims 32, 33, 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 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 34-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-29 or 42, or an AAV particle comprising the peptide of any one of claims 32, 33, 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 34-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-29 or 42, or an AAV particle comprising the peptide of any one of claims 32, 33, 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 34-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-29 or 42, or an AAV particle comprising the peptide of any one of claims 32, 33, 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 34-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-29 or 42, or an AAV particle comprising the peptide of any one of claims 32, 33, 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 34-42, an AAV particle comprising the AAV capsid variant of any one of claims 1-29 or 42, or an AAV particle comprising the peptide of any one of claims 32, 33, 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.

FIG. 1A

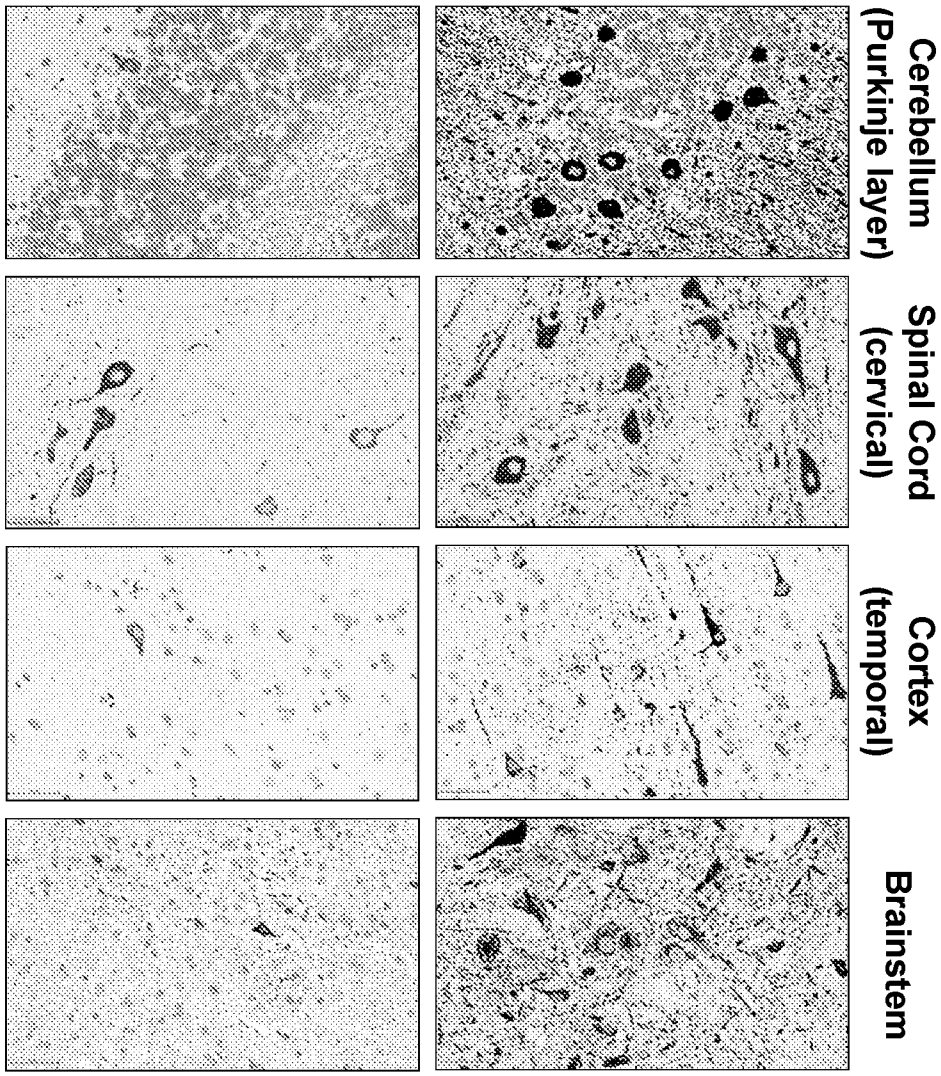

Cerebellum (Purkinje layer)
Spinal Cord (cervical)
Cortex (temporal)
Brainstem
TTN-002 scAAV
AAV9 scAAV

FIG. 1B

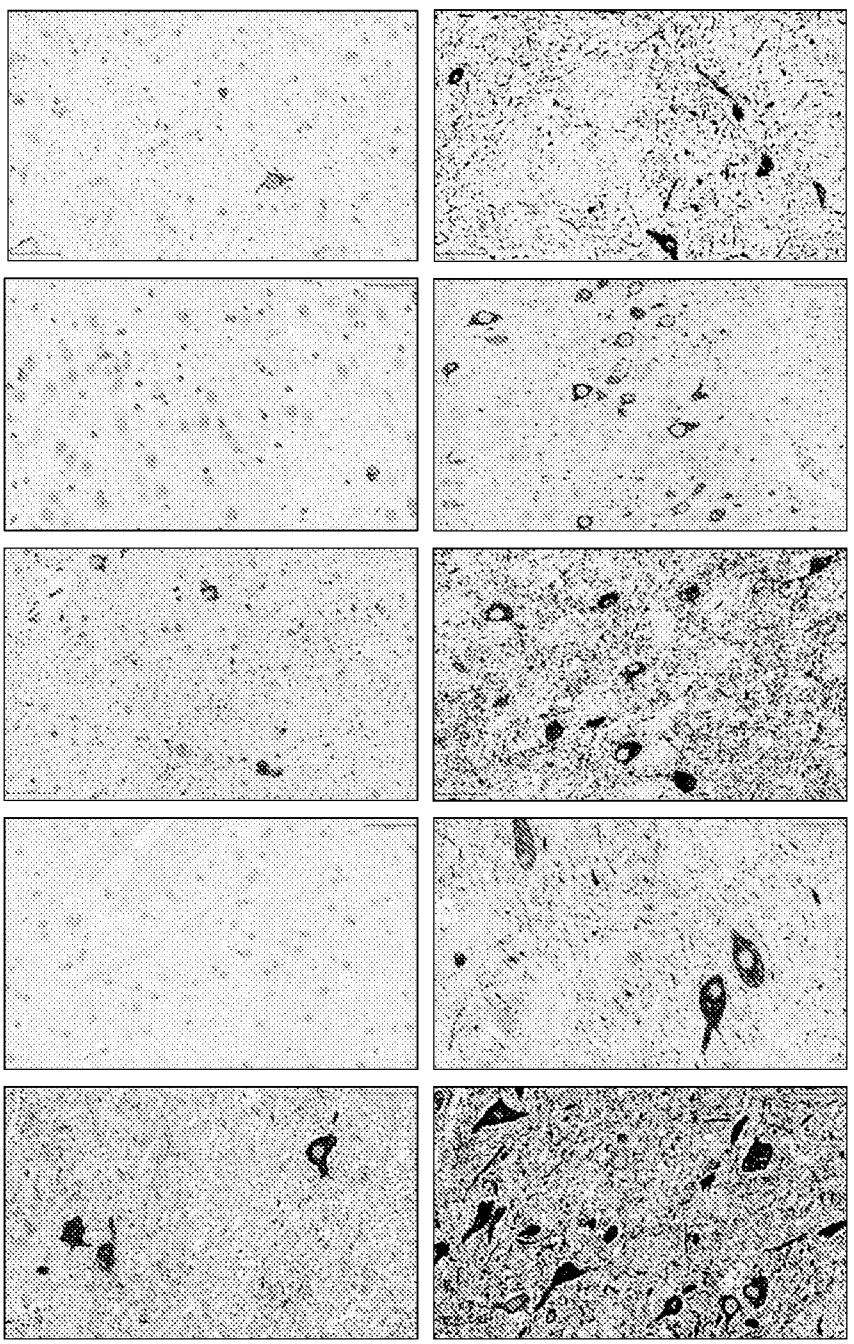

Globus Pallidus
Hippocampus
Thalamus
Putamen
Dentate
TTN-002 scAAV
AAV9 scAAV

FIG. 1C

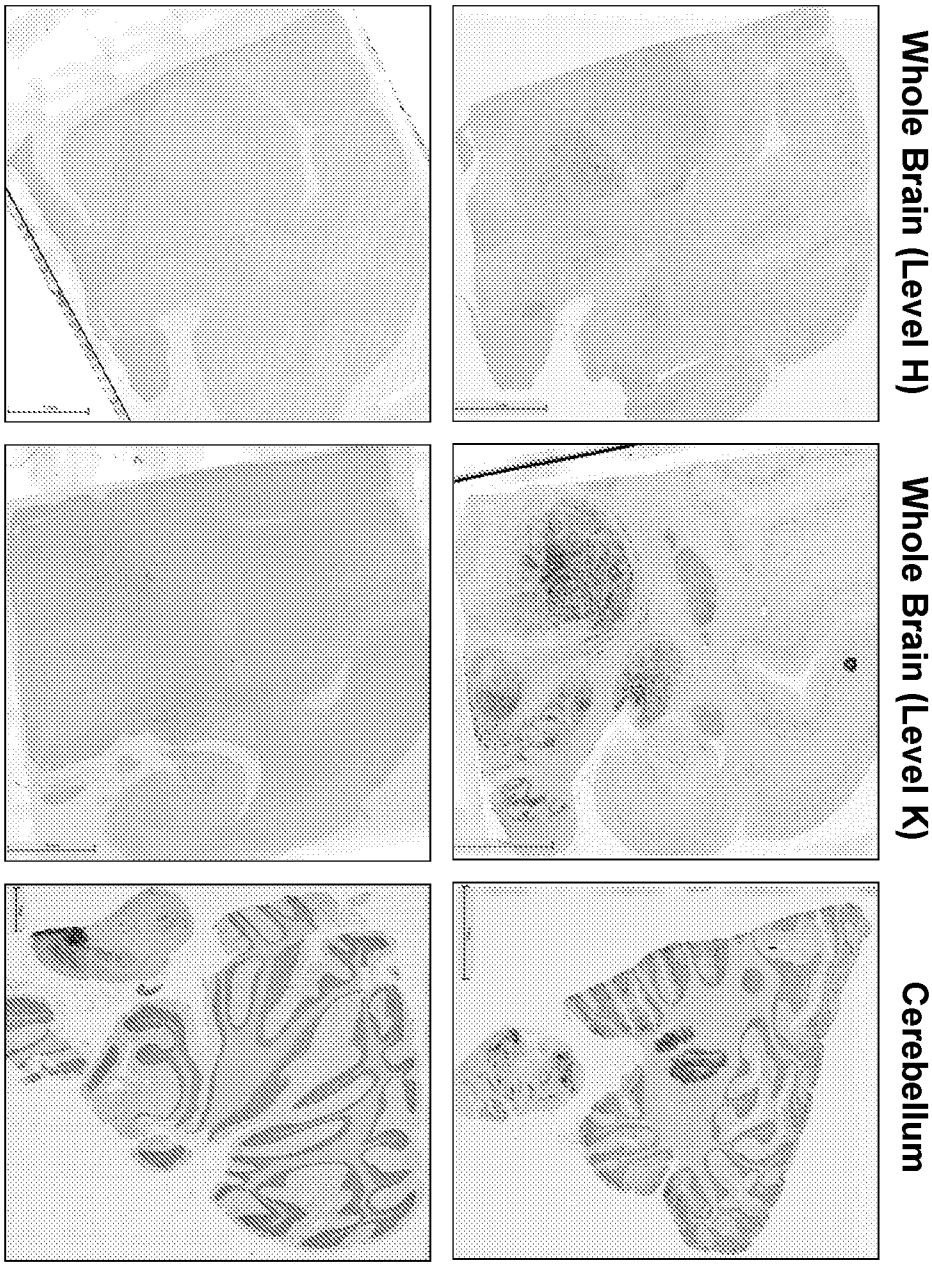
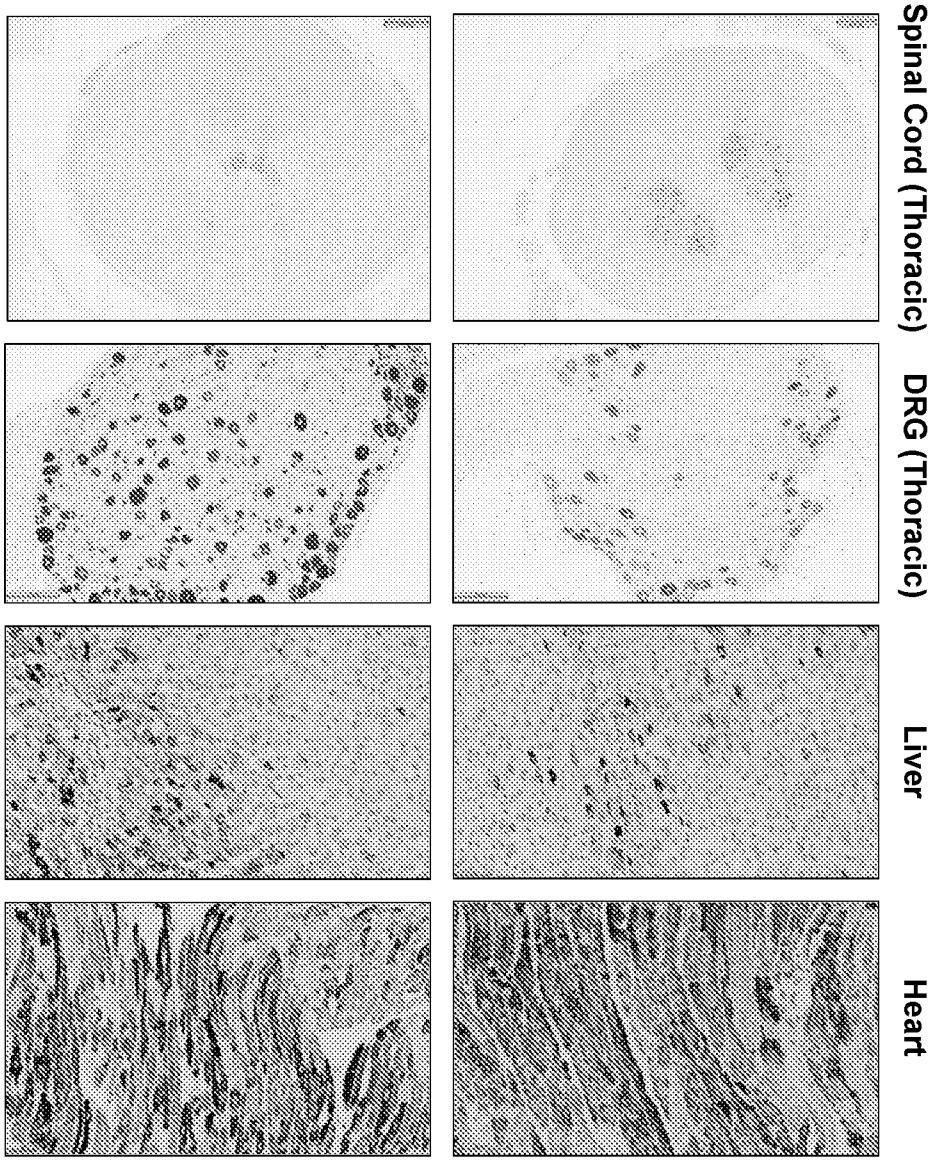

Whole Brain (Level H)
Whole Brain (Level K)
Cerebellum
TTN-002 scAAV
AAV9 scAAV

FIG. 1D


Spinal Cord (Thoracic)
DRG (Thoracic)
Liver
Heart
TTN-002 scAAV
AAV9 scAAV

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/062101

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/015 C12N15/35 C07K7/04 C12N15/863 A61K48/00
C40B40/08 C12N15/10
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2021/230987 A1 (VOYAGER THERAPEUTICS INC [US]) 18 November 2021 (2021-11-18) cited in the application figures 2,3; example 4; table 7	1-61
A	WO 2021/202651 A1 (VOYAGER THERAPEUTICS INC [US]) 7 October 2021 (2021-10-07) cited in the application figures 12,29; example 17	1-61

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 May 2023	24/07/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Brenz Verca, Stefano

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/062101

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/062101

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 19, 21, 23, 27, 29-61(all partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
21-24, 27, 28, 31, 33(completely); 1-20, 25, 26, 29, 30, 32
34-61(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 21-24, 27, 28, 31, 33(completely); 1-20, 25, 26, 29, 30, 32, 34-61(partially)

1. An AAV capsid variant, comprising an amino acid sequence having the following formula:[N2]-[N3], wherein: (i) [N2] comprises positions X1, X2, X3, X4, and X5, wherein: (a) position X1 is Y, N, or C;(b) position X2 is P, K, T, or Q;(c) position X3 is A or P;(d) position X4 is E, S, or A; and(e) position X5 is V, L, or E; and (ii) [N3] comprises the amino acid sequence of VQK, EQK, VKK, VHK, VQQ, or LQK; and wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 739, or an amino acid sequence at least 95% identical thereto.
19. An AAV capsid variant comprising: (a) the amino acid sequence of any of the sequences provided in Tables 1 (partially), 2A, 2B, SEQ ID No: 943 in Table 9, or Tables 15-17;
28. An AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 982.
30. A polynucleotide encoding said AAV capsid variant;
32. A peptide comprising: (a) the amino acid sequence of any of the sequences provided in Tables 1 (partially), 2A, 2B, SEQ ID No: 943 in Table 9, or Tables 15-17;
33. A peptide comprising: (i) the amino acid sequence of YPAEVVQK (SEQ ID NO: 943);
34. An AAV particle comprising said AAV capsid variant;
43. A vector comprising a polynucleotide encoding said AAV capsid variant;
44. A cell comprising said AAV capsid variant, said polynucleotide, said AAV particle, or said vector;
45. A method of making an AAV particle involving the use of said AAV capsid variant;
46. A pharmaceutical composition comprising said AAV particle and a pharmaceutically acceptable excipient;
47. A method of delivering a payload to a cell or tissue comprising administering an effective amount of said pharmaceutical composition or said AAV particle.
49/50/51/52/57-61: Medical uses of said pharmaceutical composition or said AAV particle.

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2. claims: 19, 20, 25, 26, 29, 30, 32, 34-61(all partially)

19. An AAV capsid variant comprising: (a) the amino acid sequence of SEQ ID No: 202 provided in Table 9;
30. A polynucleotide encoding said AAV capsid variant;
32. A peptide comprising: (a) the amino acid sequence of SEQ ID No: 202 provided in Table 9;
34. An AAV particle comprising said AAV capsid variant;
43. A vector comprising a polynucleotide encoding said AAV capsid variant;
44. A cell comprising said AAV capsid variant, said

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

polynucleotide, said AAV particle, or said vector;
45. A method of making an AAV particle involving the use of said AAV capsid variant;
46. A pharmaceutical composition comprising said AAV particle and a pharmaceutically acceptable excipient;
47. A method of delivering a payload to a cell or tissue comprising administering an effective amount of said pharmaceutical composition or said AAV particle.
49/50/51/52/57-61: Medical uses of said pharmaceutical composition or said AAV particle.

3-299. claims: 19, 20, 25, 26, 29, 30, 32, 34-61(all partially)

The same subject matter of invention 2, however, with the difference that each SEQ ID No in the second column of Table 9 represents a separate invention, e.g.:
Invention 3: SEQ ID No: 203
Invention 4: SEQ ID No: 204
etc
Invention 299: SEQ ID No: 499

300-448. claims: 19, 20, 25, 26, 29, 30, 32, 34-61(all partially)

The same subject matter of invention 2, however, with the difference that each SEQ ID No in the fifth column of Table 9 whose numbering is higher than 499 but not 943 represents a separate invention, e.g.:
Invention 300: SEQ ID No: 659
Invention 301: SEQ ID No: 547
etc
Invention 448: SEQ ID No: 807

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449. claims: 1-20, 25, 26, 29, 30, 32, 34-61(all partially)

1. An AAV capsid variant, comprising an amino acid sequence having the following formula:[N2]-[N3], wherein: (i) [N2] comprises positions X1, X2, X3, X4, and X5, wherein: (a) position X1 is Y, N, or C;(b) position X2 is P, K, T, or Q;(c) position X3 is A or P;(d) position X4 is E, S, or A; and(e) position X5 is V, L, or E; and (ii) [N3] comprises the amino acid sequence of VQK, EQK, VKK, VHK, VQQ, or LQK; and wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 739, or an amino acid sequence at least 95% identical thereto.
19. An AAV capsid variant comprising: (a) the amino acid sequence of any of the sequences provided in Table 18;
30. A polynucleotide encoding said AAV capsid variant;
32. A peptide comprising: (a) the amino acid sequence of any of the sequences provided in Table 18;
34. An AAV particle comprising said AAV capsid variant;
43. A vector comprising a polynucleotide encoding said AAV capsid variant;

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

44. A cell comprising said AAV capsid variant, said polynucleotide, said AAV particle, or said vector;
45. A method of making an AAV particle involving the use of said AAV capsid variant;
46. A pharmaceutical composition comprising said AAV particle and a pharmaceutically acceptable excipient;
47. A method of delivering a payload to a cell or tissue comprising administering an effective amount of said pharmaceutical composition or said AAV particle.
49/50/51/52/57-61: Medical uses of said pharmaceutical composition or said AAV particle.

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450. claims: 1-20, 25, 26, 29, 30, 32, 34-61(all partially)

1. An AAV capsid variant, comprising an amino acid sequence having the following formula:[N2]-[N3], wherein: (i) [N2] comprises positions X1, X2, X3, X4, and X5, wherein: (a) position X1 is Y, N, or C;(b) position X2 is P, K, T, or Q;(c) position X3 is A or P;(d) position X4 is E, S, or A; and(e) position X5 is V, L, or E; and (ii) [N3] comprises the amino acid sequence of VQK, EQK, VKK, VHK, VQQ, or LQK; and wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 739, or an amino acid sequence at least 95% identical thereto.
19. An AAV capsid variant comprising: (a) the amino acid sequence of any of the sequences provided in Table 19;
30. A polynucleotide encoding said AAV capsid variant;
32. A peptide comprising: (a) the amino acid sequence of any of the sequences provided in Table 19;
34. An AAV particle comprising said AAV capsid variant;
43. A vector comprising a polynucleotide encoding said AAV capsid variant;
44. A cell comprising said AAV capsid variant, said polynucleotide, said AAV particle, or said vector;
45. A method of making an AAV particle involving the use of said AAV capsid variant;
46. A pharmaceutical composition comprising said AAV particle and a pharmaceutically acceptable excipient;
47. A method of delivering a payload to a cell or tissue comprising administering an effective amount of said pharmaceutical composition or said AAV particle.
49/50/51/52/57-61: Medical uses of said pharmaceutical composition or said AAV particle.

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451. claims: 1-20, 25, 26, 29, 30, 32, 34-61(all partially)

1. An AAV capsid variant, comprising an amino acid sequence having the following formula:[N2]-[N3], wherein: (i) [N2] comprises positions X1, X2, X3, X4, and X5, wherein: (a) position X1 is Y, N, or C;(b) position X2 is P, K, T, or Q;(c) position X3 is A or P;(d) position X4 is E, S, or A; and(e) position X5 is V, L, or E; and (ii) [N3] comprises the amino acid sequence of VQK, EQK, VKK, VHK, VQQ, or LQK;

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

and wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 739, or an amino acid sequence at least 95% identical thereto.
19. An AAV capsid variant comprising: (a) the amino acid sequence of any of the sequences provided in Table 20;
30. A polynucleotide encoding said AAV capsid variant;
32. A peptide comprising: (a) the amino acid sequence of any of the sequences provided in Table 20;
34. An AAV particle comprising said AAV capsid variant;
43. A vector comprising a polynucleotide encoding said AAV capsid variant;
44. A cell comprising said AAV capsid variant, said polynucleotide, said AAV particle, or said vector;
45. A method of making an AAV particle involving the use of said AAV capsid variant;
46. A pharmaceutical composition comprising said AAV particle and a pharmaceutically acceptable excipient;
47. A method of delivering a payload to a cell or tissue comprising administering an effective amount of said pharmaceutical composition or said AAV particle.
49/50/51/52/57-61: Medical uses of said pharmaceutical composition or said AAV particle.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 19, 21, 23, 27, 29-61(all partially)

1) Independent claim 19 options (b) and (c) embrace under invention 1 capsid variants comprising only partial fragments of the peptide sequences, or mutations to not nearly specified residues at not nearly defined positions of the peptide sequences recited in Tables 1 (partially, as SEQ ID No: 2064-2080 pertain to invention 449), 2A, 2B, SEQ ID No: 943 in Table 9, or 15-17 in the background of any kind of AAV capsid. Dependent claims embrace inter alia capsid variants comprising amino acid sequences encoded by not nearly defined variants of the nucleotide sequence of SEQ ID No: 944. This embraces a plethora of possible products lacking tangible support and disclosure in the sense of Article 6 PCT and Article 5 PCT. Even in the presence of a currently absent functional limitation as encompassed by dep. claim 29 it would require an equally unquantifiable and thus unreasonable amount of experimentation, imposing a severe and undue burden on all those wishing to ascertain the scope of the claim, which is not in compliance with the clarity requirement of Article 6 PCT. The non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of claim 19 and dep. claims 20, 21, 23 and 25-27 (PCT Guidelines 9.19, 9.23 and 9.24) and was limited to AAV capsid variants comprising the exact amino acid sequences recited in said tables or encoded by a peptide coding sequence of SEQ ID No: 944.

2) For the same reasons the search under invention 1 of the polynucleotide of independent claim 30 and dep. claim 31 was limited to polynucleotides encoding AAV capsid variants comprising the exact peptide sequences of Tables 1 (partially, as SEQ ID No: 2064-2080 pertain to invention 449), 2A, 2B, SEQ ID No: 943 in Table 9, or 15-17 and a polynucleotide comprising SEQ ID No: 944, respectively

3) Independent claim 32 options (b) and (c) embrace under invention 1 peptides which are only partial fragments of, or mutated to not nearly specified residues at not nearly defined positions of the peptide sequences recited in Tables 1 (partially, as SEQ ID No: 2064-2080 pertain to invention 449), 2A, 2B, SEQ ID No: 943 in Table 9, or in Tables 15-17. Independent claim 33 (ii) and (iii) embraces the mutation of up to 4 amino acids in the sequence of SEQ ID No: 943 and fragments thereof. All together, this embraces again a plethora of possible peptides lacking tangible support and disclosure in the sense of Article 6 PCT and Article 5 PCT. There is no data in the application in support of the notion that a peptide comprising e.g. only 3 consecutive amino acids from the peptides of the above mentioned tables are capable of achieving the same technical effect(s). Even in the presence of a possible functional limitation it would require an equally unquantifiable and thus unreasonable amount of experimentation, imposing a severe and undue burden on all those wishing to ascertain the scope of the claim, which is not in compliance with the clarity requirement of Article 6 PCT. The non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of claims 32

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

and 33 (PCT Guidelines 9.19, 9.23 and 9.24) and was limited to the exact peptide sequences recited in the above mentioned tables insofar pertaining to invention 1.

4) The search of the derived subject matter of claims 34 to 61 was carried out only insofar pertaining to the subject matter searched as described under the preceding points.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) PCT declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2023/062101

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