

[006] Thus, there is a need for rAAVs capable of achieving high specificity to target specific cell types or organs and low specificity for unimpacted (off-target) organs and cell types in a subject when delivered to the subject systemically (*e.g.*, intravenously, intranasally).

SUMMARY

[007] Disclosed herein are recombinant AAVs (rAAVs) comprising capsids with specificity engineered into the capsid structure through iterative rounds of positive and negative selection. This engineering yields rAAV variants with altered specificity for certain tissues or organs relative to the unmodified parental capsid from which they were derived. In some [embodimentexamples](#), the rAAV variant comprises an AAV capsid protein having a 7-mer peptide substitution at the residues corresponding to amino acids 452-458 of the AAV9 capsid protein VP1. In certain [embodimentexamples](#), the substitution is of amino acids 452-458 of AAV9 VP1. In certain other [embodimentexamples](#), the substitution is of amino acids 452-458 of AAV-PHP.B VP1. In yet other [embodimentexamples](#), the substitution is of amino acids 452-458 of AAV-PHP.eB VP1. In some [embodimentexamples](#), the substitution reduces the tropism of the rAAV capsid for a cell or tissue, enabling the rAAV variant to reduce the off-target effects of therapeutic viral transduction, thereby obviating a need for larger viral dosage amounts. In some [embodimentexamples](#), the substitution increases the tropism of the rAAV capsid for a cell or tissue, enabling the rAAV variant to achieve widespread transduction to a target environment (*e.g.*, target cell types or tissues) in a subject upon systemic delivery (*e.g.*, intravenous injection). Typically, the 7-mer peptide substitution is not ILGTGTS (SEQ ID NO: 45479), QSSQTPR (SEQ ID NO: 45479), or TLAVPFK (SEQ ID NO: 45477).

The rAAVs described herein are identified using a modified version of the Cre recombination-based AAV targeted evolution (CREATE) method. The Multiplexed-CREATE (M-CREATE) method generates enhanced transduction efficiency and/or specificity by introducing variations in the capsid protein sequence, unbiased *in vivo* selection and recovery of only those variants that travel to defined cell populations, cross the cell membrane, travel to the nucleus, and unpackage and express their genetic payload. Variant capsids exhibiting the most desirable tropism (*e.g.*, enhanced efficiency and specificity for a particular *in vivo* environment) are recovered and identified by deep sequencing. Strategies for unbiased selection and analysis include determining variants' enrichment score (by normalizing the target tissue library to starting virus library) and unbiased propagation between rounds of selections through a synthetic library construction (where each variant is represented equally). The detailed characterizations of the resultant libraries from sequencing data is also described herein, which provide useful insights on the selection of variants towards a target.

[008] Also disclosed herein are AAV capsid libraries comprising AAV capsid proteins having 7-mer peptide substitutions at the residues corresponding to amino acids 452-458 of AAV9 VP1. In some [embodimentexamples](#), the library is a library of capsids comprising 7-mer peptide substitutions at amino acids 452-458 of the AAV9 variant AAV-PHP.eB or AAV-PHP.B. Such libraries find particular use in *in*

in vivo positive selections across different brain cell types (*e.g.*, endothelial cells, neurons, and astrocytes) and negative selections across the liver, such libraries having been shown herein to yield a large pool of AAV9 variants with enhanced ability to cross the BBB, broadly transduce the central nervous system (CNS), and de-target off-target *in vivo* environments, *e.g.*, the liver. In other [embodiment examples](#), the library is an AAV capsid library comprising AAV capsid proteins having 7-mer peptide substitutions at amino acids 452-458 of AAV9. Such libraries find particular use in *in vivo* selections across different organs, such as the spinal cord, brain, a liver, a stomach, an intestine, a lung, a heart, teste, spleen, adipose (fat), and muscle.

[009] Also disclosed herein are methods and kits for producing therapeutic recombinant AAV (rAAV) particles, as well as methods and pharmaceutical compositions or formulations comprising the rAAV particles for the treatment of a disease or condition affecting, for *e.g.*, the CNS, PNS, or target *in vivo* environment in a subject in need thereof (*e.g.*, lung).

[0010] [In one Aspect](#), disclosed herein provide recombinant AAV (rAAVs), the rAAV comprising: (a) a variant AAV capsid comprising a variant AAV capsid protein comprising an amino acid substitution of three or more amino acids within a 7-mer peptide sequence at a 3-fold axis of symmetry of a corresponding parental AAV capsid protein, wherein the 7-mer peptide sequence is at an amino acid position that corresponds to amino acid residues 452-458 of AAV9 VP1 (SEQ ID NO:1), and wherein the amino acid substitution is not ILGTGTS (SEQ ID NO: 45479), QSSQTPR (SEQ ID NO: 45479), or TLAVPFK (SEQ ID NO: 45477); and (b) a heterologous polynucleotide comprising a nucleotide sequence encoding a gene product. In some [embodiment examples](#), the variant AAV capsid has an increased tropism for a target tissue or a target cell, when measured in a subject, relative to a tropism of the corresponding parental AAV capsid. In some [embodiment examples](#), the target tissue or the target cell comprises a tissue or a cell of a central nervous system (CNS) or a peripheral nervous system (PNS), or a combination thereof. In some [embodiment examples](#), the variant AAV capsid has a decreased tropism for an off-target tissue comprising liver tissue or an off-target cell comprising a liver cell, when measured in a subject, relative to the tropism of the corresponding parental AAV capsid. In some [embodiment examples](#), the three or more amino acids comprise three contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L Q, S, T, and V at amino acid positions in the parental AAV capsid protein corresponding to amino acid residues 452-454, 453-455, 454-456, 455-457, or 456-458 of AAV9 VP1 (SEQ ID NO: 1). In some [embodiment examples](#), the three or more amino acids comprise four contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L Q, S, T, and V at amino acid positions in the parental AAV capsid protein corresponding to amino acid residues 452-455, 453-456, 454-457, or 455-458 of AAV9 VP1 (SEQ ID NO: 1). In some [embodiment examples](#), three or more amino acids comprise at least five contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L Q, S, T, and V at amino acid positions in the parental AAV capsid protein corresponding to amino acid residues 452-456, 453-457, or 454-458 of AAV9 VP1

(SEQ ID NO: 1). In some embodimentexamples, the three or more amino acids comprise three or more of: (a) A, D, E, G, H, M, N, Q, S, T, or V substituted at an amino acid position corresponding to amino acid residue 452 of AAV9 VP1 (SEQ ID NO: 1); (b) A, D, E, G, K, N, Q, S, T, or V substituted at an amino acid position corresponding to amino acid residue 453 of AAV9 VP1 (SEQ ID NO: 1); (c) A, D, E, G, K, N, Q, S, T, or V substituted at amino acid position corresponding to amino acid residue 454 of AAV9 VP1(SEQ ID NO: 1); (d) A, D, E, G, K, N, P, Q, S, or T substituted at an amino acid position corresponding to amino acid residue 455 of AAV9 VP1(SEQ ID NO: 1); (e) A, D, E, G, H, K, N, P, Q, S, or T substituted at an amino acid position corresponding to amino acid residue 456 of AAV9 VP1(SEQ ID NO: 1); (f) A, D, E, G, K, N, P, S, T, or V substituted at an amino acid position corresponding to amino acid residue 457 of AAV9 VP1(SEQ ID NO: 1); and (g) A, E, G, H, K, L, N, Q, S, T, or V substituted at amino acid position corresponding to amino acid residue 458 of AAV9 VP1(SEQ ID NO: 1). In some embodimentexamples, the three or more amino acids comprise three or more of: (a) A, D, E, G, H, K, L, M, N, Q, S, T, or V substituted at an amino acid position corresponding to amino acid residue 452 of AAV9 VP1 (SEQ ID NO: 1); (b) A, D, G, H, M, N, P, Q, S, T, or V substituted at an amino acid position corresponding to amino acid residue 453 of AAV9 VP1 (SEQ ID NO: 1); (c) A, D, E, G, K, N, Q, S, T, or V substituted at amino acid position corresponding to amino acid residue 454 of AAV9 VP1(SEQ ID NO: 1); (d) A, D, E, G, K, N, P, Q, S, or T substituted at an amino acid position corresponding to amino acid residue 455 of AAV9 VP1(SEQ ID NO: 1); (e) A, D, G, K, N, P, Q, S, or T substituted at an amino acid position corresponding to amino acid residue 456 of AAV9 VP1(SEQ ID NO: 1); (f) A, G, H, L, M, N, P, Q, S, T, or V substituted at an amino acid position corresponding to amino acid residue 457 of AAV9 VP1(SEQ ID NO: 1); and (g) A, D, E, G, H, I, K, L, N, Q, R, S, T, or V substituted at amino acid position corresponding to amino acid residue 458 of AAV9 VP1(SEQ ID NO: 1). In some embodimentexamples, the amino acid substitution comprises an amino acid sequence selected from DGAATKN (SEQ ID NO: 3943), and DGQSSKS (SEQ ID NO: 2764). In some embodimentexamples, the three or more amino acids comprises three or more of: (a) A, D, G, L, N, Q, S, or T substituted at an amino acid at a position corresponding to amino acid residue 452 of AAV9 VP1 (SEQ ID NO: 1); (b) A, G, N, P, Q, R, S, or substituted at an amino acid at a position corresponding to amino acid residue 453 of AAV9 VP1 (SEQ ID NO: 1); (c) A, D, G, N, S, or T substituted at an amino acid at a position corresponding to amino acid residue 454 of AAV9 VP1 (SEQ ID NO: 1); (d) A, D, G, K, N, P, Q, S, or T substituted at an amino acid at a position corresponding to amino acid residue 455 of AAV9 VP1 (SEQ ID NO: 1); (e) A, G, K, N, P, R, S, or T substituted at an amino acid at a position corresponding to amino acid residue 456 of AAV9 VP (SEQ ID NO: 1); (f) A, G, K, N, P, R, S, T, or V substituted at an amino acid at a position corresponding to amino acid residue 457 of AAV9 VP1 (SEQ ID NO: 1); and (g) A, G, K, L, R, S, T, or V substituted at an amino acid at a position corresponding to amino acid residue 458 of AAV9 VP1 (SEQ ID NO: 1). In some embodimentexamples, the amino acid substitution comprises an amino acid sequence selected from the group consisting of LQTSSPG (SEQ ID NO: 2933), QQGKQSV (SEQ ID NO: 79),

SINTKTN (SEQ ID NO: 45475), SNGTKQT (SEQ ID NO: 442), GSGKTAA (SEQ ID NO: 88), MGDKPTR (SEQ ID NO: 2466), QPSGGNT (SEQ ID NO: 2672), ERGANTK (SEQ ID NO: 5192), TTGGHSS (SEQ ID NO: 2743), GTTKTSE (SEQ ID NO: 3064), GTGTSVL (SEQ ID NO: 11958), NQSGTKG (SEQ ID NO: 780), KGPGQMG (SEQ ID NO: 45476), and GTPSKAG (SEQ ID NO: 2741). In some [embodimentexamples](#), the target tissue or the target cell in the tissue is selected from the group consisting of lung, intestine, kidney, heart, and stomach. In some [embodimentexamples](#), the three or more amino acids comprise three contiguous amino acids at an amino acid position corresponding to amino acid residues 452-454, 453-455, 454-456, 455-457, or 456-458 of AAV9 VP1, wherein: (a) 452 is selected from the group consisting of N, K, R, and T; (b) 453 is selected from the group consisting of L, N, P, and S; (c) 454 is selected from the group consisting of A, D, G, N, S, and T; (d) 455 is selected from the group consisting of L, P, S, and T; (e) 456 is selected from the group consisting of P, R, and S; (f) 457 is selected from the group consisting of G, N, S, and T; and (g) 458 is selected from the group consisting of I, L, and R. In some [embodimentexamples](#), the three or more amino acids comprise: (a) four contiguous amino acids at an amino acid position corresponding to amino acid residues 452-455, 453-456, 454-457, or 455-458 of AAV9 VP1 (SEQ ID NO: 1); (b) five contiguous amino acids at an amino acid position corresponding to amino acid residues 452-456, 453-457, or 453-458 of AAV9 VP1 (SEQ ID NO: 1); (c) six contiguous amino acids at an amino acid position corresponding to amino acid residues 452-457 or 453-458 of AAV9 VP1 (SEQ ID NO: 1); and (d) seven contiguous amino acids at an amino acid position corresponding to amino acid residues 452-458 of AAV9 VP1 (SEQ ID NO: 1). In some [embodimentexamples](#), the target tissue is lung, and the three or more amino acids are provided in an amino acid sequence comprising KDNTPGR (SEQ ID NO: 32538),>NNLPRNL (SEQ ID NO: 32867), or any amino acid sequence provided in FIG. 13. In some [embodimentexamples](#), the target tissue is intestine, and the three or more amino acids are provided in an amino acid sequence comprising RESSPSL (SEQ ID NO: 26474), KDNTPGR (SEQ ID NO: 26584), or any amino acid sequence provided in FIG. 5. In some [embodimentexamples](#), the tissue is kidney, and the three or more amino acids are provided in an amino acid sequence comprising RVPLSTI (SEQ ID NO: 26933),>NNLPRNL (SEQ ID NO: 27530), KDNTPGR (SEQ ID NO: 28509), or any amino acid sequence provided in FIG. 6. In some [embodimentexamples](#), the tissue is heart, and the three or more amino acids are provided in an amino acid sequence comprising KDNTPGR (SEQ ID NO: 25633), or any amino acid sequence provided in FIG. 4. In some [embodimentexamples](#), the tissue is stomach, and the three or more amino acids are provided in an amino acid sequence comprising RESSPSL (SEQ ID NO: 31904) or any amino acid sequence of FIG. 12. In some [embodimentexamples](#), the corresponding parental AAV capsid protein is AAV9 VP1 or a variant thereof. In some [embodimentexamples](#), the AAV9 VP1 variant has a sequences identity of 90% or more to SEQ ID NO:1. In some [embodimentexamples](#), the AAV9 VP1 variant has a sequences identity of 95% or more to SEQ ID NO:1. In some [embodimentexamples](#), the corresponding parental AAV capsid protein further comprises an insertion of an amino acid sequence selected from the group consisting of TLAXPFK (SEQ ID NO: 46424), TLAX (SEQ ID NO: 46425),

LAVX (SEQ ID NO: 46426), AVPX (SEQ ID NO: 46427), and VPFX (SEQ ID NO: 46428), at an amino acid position corresponding to 588_589 of the AAV9 VP1 (SEQ ID NO: 1), wherein X is any amino acid other than V. In some embodimentexamples, the parental AAV capsid protein is from AAV-PHP.B or AAV-PHP.eB. In some embodimentexamples, the rAAV is isolated and purified. [0011] In another aspect, disclosed herein provide pharmaceutical formulations comprising the rAAV of the present disclosure and a pharmaceutical excipient. In some embodimentexamples, the pharmaceutical formulation is formulated for intravenous, intraarterial, intranasal, intrathecal, intracisternae magna, or subcutaneous injection.

[0012] In yet another aspect, the present disclosure provides a use of the RAAV as described herein, or the pharmaceutical formulation as described herein, in the manufacture of a medicament for treating a disease, wherein the disease or the condition is selected from the group consisting of pulmonary fibrosis, surfactant protein disorders, peroxisome biogenesis disorders, or chronic obstructive pulmonary disease (COPD). AspectExample disclosed herein provide methods of treating a disease or condition in a subject, the method comprising administering to the subject a therapeutically effective amount the rAAV of the present disclosure, or the pharmaceutical formulation of the present disclosure, wherein the gene product is a therapeutic gene product. In some embodimentexamples, the administering is by intravenous, intraarterial, intranasal, intrathecal, intracisternae magna, or subcutaneous injection. In some embodimentexamples, the disease or the condition is selected from the group consisting of pulmonary fibrosis, surfactant protein disorders, peroxisome biogenesis disorders, or COPD. In some embodimentexamples, the disease of the condition is a central nervous system (CNS) peripheral nervous system (PNS) disorder.

[0013] In a further aspect, the present disclosure provides aA method of manufacturing an rAAV of the present disclosure, the method comprising: (a) introducing into a cell a nucleic acid comprising: (i) a first nucleic acid sequence encoding a therapeutic gene expression product enclosed by a 5' and a 3' inverted terminal repeat (ITR) sequence; (ii) a second nucleic acid sequence encoding a viral genome comprising a 5' ITR sequence, a Replication (Rep) gene, Capsid (Cap) gene, and a 3' ITR, wherein the Cap gene encodes a rAAV capsid protein; and (iii) a third nucleic acid sequence encoding a first helper virus protein selected from the group consisting of E4orf6, E2a, and VA RNA, and optionally, a second helper virus protein comprising E1a or E1b55k; (b) expressing in the cell the AAV capsid protein; (c) assembling the rAAV of the present disclosure; and (d) packaging the first nucleic acid sequence in the rAAV. In a further aspect, disclosed herein provide variant adeno-associated virus (AAV) capsids comprising: (a) at least one of a decreased specificity and a decreased transduction efficiency, as measured in a liver of a primate subject when delivered to the primate subject intravenously, relative to a corresponding parental AAV capsid with an AAV capsid protein of SEQ ID NO: 1; and (b) at least one of an increased specificity and an increased transduction efficiency, as measured in a central nervous system (CNS) of the primate subject when delivered to the primate subject intravenously, relative to the corresponding parental AAV capsid. In some embodimentexamples, the

variant AAV capsids further comprising a variant AAV capsid protein comprising an amino acid substitution of three or more amino acids within a 7-mer peptide sequence at an amino acid position that corresponds to amino acid residues 452-458 of AAV9 VP1 (SEQ ID NO:1). In some embodiment examples, the three or more amino acids comprise three contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L, Q, S, T, and V at amino acid positions in the parental AAV

capsid protein corresponding to amino acid residues 452-454, 453-455, 454-456, 455-457, or 456-458 of AAV9 VP1 (SEQ ID NO: 1). In some [embodimentexamples](#), the 7-mer peptide sequence is DGAATKN (SEQ ID NO: 3943). In some [embodimentexamples](#), the variant AAV capsid protein further comprises an amino acid sequence that is at least 96% identical to an amino acid sequence between amino acid 217 to amino acid 736 of SEQ ID NO: 1. In some [embodimentexamples](#), the amino acid substitution is not ILGTGTS (SEQ ID NO: 45479), QSSQTPR (SEQ ID NO: 45479), or TLAVPFK (SEQ ID NO: 45477). In some [embodimentexamples](#), the variant AAV capsid protein further comprises an insertion of an amino acid sequence selected from the group consisting of TLAXPFK (SEQ ID NO: 46424), TLAX (SEQ ID NO: 46425), LAVX (SEQ ID NO: 46426), AVPX (SEQ ID NO: 46427), and VPFX (SEQ ID NO: 46428), at an amino acid position corresponding to 588_589 of the AAV9 VP1 (SEQ ID NO: 1), wherein X is any amino acid other than V. [In yet another aspects](#), disclosed herein provide variant adeno-associated virus (AAV) capsids comprising: (a) a variant AAV capsid protein comprising an amino acid sequence with an amino acid substitution of three or more amino acids within a 7-mer peptide sequence at an amino acid position that corresponds to amino acid residues 452-458 of AAV9 VP1 (SEQ ID NO: 1); and (b) at least one of an increased specificity and increased transduction efficiency in a target tissue as measured in the target tissue of a subject when delivered to the subject intranasally or intravenously, relative to a corresponding parental AAV capsid protein of SEQ ID NO: 1 or SEQ ID NO: 2, wherein the target tissue is selected from the group consisting of lung, intestine, kidney, heart, and stomach. In some [embodimentexamples](#), the three or more amino acids comprise three contiguous amino acids at an amino acid position corresponding to amino acid residues 452-454, 453-455, 454-456, 455-457, or 456-458 of AAV9 VP1, wherein: (a) 452 is selected from the group consisting of N, K, R, and T; (b) 453 is selected from the group consisting of L, N, P, and S; (c) 454 is selected from the group consisting of A, D, G, N, S, and T; (d) 455 is selected from the group consisting of L, P, S, and T; (e) 456 is selected from the group consisting of P, R, and S; (f) 457 is selected from the group consisting of G, N, S, and T; and (g) 458 is selected from the group consisting of I, L, and R. In some [embodimentexamples](#), the target tissue is the lung, and the three or more amino acids are provided in an amino acid sequence comprising KDNTPGR (SEQ ID NO: 32538),>NNLPRNL (SEQ ID NO: 32867), or any amino acid sequence provided in FIG. 13. In some [embodimentexamples](#), the target tissue is the intestine, and the three or more amino acids are provided in an amino acid sequence comprising RESSPSL (SEQ ID NO: 26474), KDNTPGR (SEQ ID NO: 26584), or any amino acid sequence provided in FIG. 5. In some [embodimentexamples](#), the tissue is the kidney, and the three or more amino acids are provided in an amino acid sequence comprising RVPLSTI (SEQ ID NO: 26933),>NNLPRNL (SEQ ID NO: 27530), KDNTPGR (SEQ ID NO: 28509), or any amino acid sequence provided in FIG. 6. In some [embodimentexamples](#), the tissue is the heart, and the three or more amino acids are provided in an amino acid sequence comprising KDNTPGR (SEQ ID NO: 25633), or any amino acid sequence provided in FIG. 4. In some [embodimentexamples](#), the tissue is the stomach, and the three or more amino acids are provided in an amino acid sequence comprising RESSPSL (SEQ ID NO: 31904) or any amino acid sequence of

FIG. 12. In some [embodimentexamples](#), the variant AAV capsid protein further comprises an amino acid sequence that is at least 96% identical to an amino acid sequence between amino acid 217 to amino acid 736 of SEQ ID NO: 1. In some [embodimentexamples](#), the variant AAV capsid of the present disclosure is isolated and purified. In some [embodimentexamples](#), the variant AAV capsid of the present disclosure is formulated as a pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some [embodimentexample](#), the pharmaceutical formulation is formulated for intranasal or intravenous administration.

[0014] [AspectExamples](#) disclosed provide methods of treating a disease or condition in a subject, the method comprising administering to the subject a therapeutically effective amount the rAAV of the present disclosure or the pharmaceutical formulation of the present disclosure. In some [embodimentexamples](#), the disease or the condition is selected from the group consisting of pulmonary fibrosis, surfactant protein disorders, peroxisome biogenesis disorders, chronic obstructive pulmonary disease (COPD), a CNS disease or condition, and a PNS disease or condition.

[0015] [AspectExamples](#) disclosed herein provide recombinant adeno-associated virus (rAAV) capsids comprising variant AAV capsid proteins, the rAAV capsids having at least one of an increased specificity and an increased transduction efficiency when measured in a target tissue in a primate subject relative to the corresponding parental AAV capsid, and at least one of a decreased specificity and a decreased transduction efficiency when measured in an off-target tissue in the primate subject relative to the corresponding parental AAV capsid. In some instances, the specificity or the transduction efficiency is measured following administration of the rAAV capsid to the subject intravenously, intraarterially, intrathecally, or subcutaneously. In some instances, the rAAV capsid is chimeric. In some instances, the rAAV, or variant AAV protein comprises therein, confer an increase in a localization of the rAAV within the target tissue, as compared to the parental AAV capsid or capsid protein.

[0016] In some instances, the variant AAV capsid protein (and rAAV capsid) comprises an amino acid substitution to an amino acid selected from the group consisting of A, D, E, G, K, H, M, N, P, L Q, S, T, and V at one or more amino acid positions corresponding to one or more of residues 452-458 in an amino acid sequence of the AAV9 VP1 capsid protein provided in SEQ ID NO: 1. In some instances, the amino acid sequence of the AAV9 capsid protein is provided in amino acid 217-736 within SEQ ID NO: 1. In some instances, the target tissue is the central nervous system (CNS). In some instances, the CNS comprises a region selected from the neocortex, the basal ganglia, the hippocampus, the thalamus, the cerebellum, the brain stem, and the spinal cord. In some [embodimentexamples](#), the target tissue is the peripheral nervous system (PNS). In some instances, the PNS comprises a ganglion. In some instances, the ganglion comprises a trigeminal or dorsal root ganglion. In some instances, the target tissue is a non-dividing cell. In some instances, the non-dividing cell is selected from the group consisting of a neuron, an astrocyte, a microglial cell, an oligodendrocyte, and a Schwann cell. In some instances, the off-target tissue is a liver.. In some instances, VP1, VP2, and

VP3 of the AAV capsid comprise the amino acid substitution. In some instances, the amino acid substitution is at a three (3)-fold axis of symmetry of a corresponding parental AAV capsid protein. In some instances, the amino acid substitution comprises at least three contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L, Q, S, T, and V at amino acid positions corresponding to residues 452-454, 453-455, 454-456, 455-457, or 456-458 of the amino acid sequence of the AAV9 VP1 capsid protein. In some instances, the amino acid substitution comprises at least four contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L, Q, S, T, and V at amino acid positions corresponding to residues 452-455, 453-456, 454-457, or 455-458 of the amino acid sequence of the AAV9 VP1 capsid protein. In some instances, the amino acid substitution comprises at least five contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L, Q, S, T, and V at amino acid positions corresponding to residues 452-456, 453-457, or 454-458 of the amino acid sequence the AAV9 VP1 capsid protein. In some instances, the corresponding parental AAV capsid (or AAV capsid protein) is a native AAV9 capsid. In some instances, the corresponding parental AAV capsid is a variant of AAV9 comprising an amino acid sequence provided in any one of SEQ ID NOS: 3-6.

[0017] In some instances, the amino acid substitution at the amino acid position corresponding to residue 452 in AAV9 VP1 is selected from the group consisting of N452A, N452D, N452E, N452G, N452H, N452M, N452N, N452Q, N452S, N452T, and N452V. In some instances, the amino acid substitution at the amino acid position corresponding to residue 453 in AAV9 VP1 is selected from the group consisting of G453A, G453D, G453E, G453G, G453K, G453N, G453Q, G453S, G453T, and G453V. In some instances, the amino acid substitution at the amino acid position corresponding to residue 454 in AAV9 VP1 is selected from the group consisting of S454A, S454D, S454E, S454G, S454K, S454N, S454Q, S454S, S454T, and S454V. In some instances, the amino acid substitution at the amino acid position corresponding to residue 455 in AAV9 VP1 is selected from the group consisting of G455A, G455D, G455E, G455G, G455K, G455N, G455P, G455Q, G455S, and G455T. In some instances, the amino acid substitution at the amino acid position corresponding to residue 456 in AAV9 VP1 is selected from the group consisting Q456A, Q456D, Q456E, Q456G, Q456H, Q456K, Q456N, Q456P, Q456Q, Q456S, and Q456T. In some instances, the amino acid substitution at the amino acid position corresponding to residue 457 in AAV9 VP1 is selected from the group consisting N457A, N457D, N457E, N457G, N457K, N457N, N457P, N457S, N457T, and N457V. In some instances, the amino acid substitution at the amino acid position corresponding to residue 458 in AAV9 VP1 is selected from the group consisting Q458A, Q458E, Q458G, Q458H, Q458K, Q458L, Q458N, Q458Q, Q458S, Q458T, and Q458V. In some instances, the amino acid substitution at amino acid positions corresponding to residues 452-458 in AAV9 VP1 is DGAATKN (SEQ ID NO: 3943). In some instances, the amino acid substitution comprises an amino acid sequence selected from **FIG. 3** or **Table 1**, or a combination thereof.

[0018] In some instances, the variant AAV capsid protein further comprises an insertion of an amino acid sequence selected from the group consisting of TLAXPFK (SEQ ID NO: 46424), TLAX (SEQ ID NO: 46425), LAVX (SEQ ID NO: 46426), AVPX (SEQ ID NO: 46427), and VPFX (SEQ ID NO: 46428), following the amino acid at the position corresponding to residue 588 in the amino acid sequence of the AAV9 VP1, wherein X is any amino acid other than V. In some instances, the substitution comprises an amino acid sequence provided in **FIG. 3**.

[0019] In some instances, the target tissue is a brain, and the rAAV capsids, or variant AAV capsid proteins comprised therein, have the increased specificity for the brain, as compared to the parental AAV capsid or capsid protein (*e.g.*, AAV9). In some instances, the off-target tissue is a liver, and the rAAV capsids, or variant AAV capsid proteins, have a decreased specificity for the liver, as compared to the parental AAV capsid or capsid protein (*e.g.*, AAV9). In some instances, the target tissue is a central nervous system (CNS), and the rAAV capsids, or variant AAV capsid proteins, have an increased efficiency of viral transduction of at least 12-fold in the central nervous system (CNS), as compared to the corresponding parental AAV capsid or capsid protein (*e.g.*, AAV9). In some instances, the target tissue is a spinal cord, and the rAAV capsids, or variant AAV capsid proteins, have an increased efficiency of viral transduction of at least 20-fold in the spinal cord, as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the target tissue is a neuron, and the rAAV capsids, or variant AAV capsid proteins, have an increased specificity for the neuron, as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the off-target tissue is a liver, and the rAAV capsids, or variant AAV capsid proteins, have a decreased efficiency of viral transduction of at least 50-fold in the liver, as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the off-target tissue is a Purkinje cell, and the rAAV capsids, or variant AAV capsid proteins, have a decreased efficiency of viral transduction of at least 4-fold in the Purkinje cell, as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the off-target tissue is a liver, and the rAAV capsids, or variant AAV capsid proteins, have a decreased efficiency of viral transduction of at least 100-fold in the liver, as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the AAV further comprises a heterologous nucleic acid encoding a therapeutic gene expression product. In some instances, the AAV capsid is isolated and purified. In some instances, the AAV capsids described herein are formulated as a pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some instances, the pharmaceutical formulation further comprises a heterologous nucleic acid encoding a therapeutic gene expression product. In some instances, the pharmaceutical formulation further comprises a pharmaceutical excipient. In some instances, the pharmaceutical formulation is formulated for intravenous, intraarterial, intrathecal, or subcutaneous administration to treat a disease or a condition of the CNS or PNS.

[0020] Aspect Examples disclosed herein provide rAAV capsids comprising variant AAV capsid proteins with an amino acid substitution to an amino acid selected from the group consisting of A, D, E, G, K, H,

M, N, P, L Q, S, T, and V, at an amino acid position corresponding to residues selected from 452-458 within an amino acid sequence of the AAV9 VP1, the rAAV capsids, and variant AAV capsid proteins, having at least one of a decreased specificity and a decreased transduction efficiency for an off-target tissue in a subject, relative to a corresponding parental AAV capsid or capsid protein, and at least one of an increased specificity and an increased transduction efficiency for a target tissue in a subject, relative to the corresponding parental AAV capsid or capsid protein. In some instances, the rAAV capsid is chimeric. In some instances, the amino acid substitution is at a three (3)-fold axis of symmetry of the rAAV capsid. In some instances, the rAAV, or variant AAV protein comprises therein, confer an increase in a localization of the rAAV within the target tissue, as compared to the corresponding parental AAV capsid or capsid protein.

[0021] In some instances, the target tissue is the central nervous system (CNS) or the peripheral nervous system (PNS). In some instances, the CNS comprises a region selected from the neocortex, the basal ganglia, the hippocampus, the thalamus, the cerebellum, the brain stem, and the spinal cord. In some instances, the PNS comprises a ganglion. In some instances, the ganglion comprises a trigeminal or dorsal root ganglion. In some instances, the target tissue is a non-dividing cell. In some instances, the CNS comprises a cell selected from the group consisting of a neuron, a spinal cord, an astrocyte, an oligodendrocyte, and Schwann cell. In some instances, the off-target tissue is a liver.

[0022] In some instances, the amino acid substitution comprises at least three contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L Q, S, T, and V at amino acid positions corresponding to residues 452-454, 453-455, 454-456, 455-457, or 456-458, in the amino acid sequence of the AAV9 capsid protein. In some instances, the amino acid substitution comprises at least four contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L Q, S, T, and V at amino acid positions corresponding to residues 452-455, 453-456, 454-457, or 455-458, in the amino acid sequence of the AAV9 capsid protein. In some instances, the amino acid substitution comprises at least five contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L Q, S, T, and V at amino acid positions corresponding to residues 452-456, 453-457, or 454-458, in the amino acid sequence of the AAV9 capsid protein. In some instances, the AAV capsid protein is an AAV9 capsid protein.

[0023] In some instances, the amino acid substitution at the amino acid position corresponding to residue 452 in AAV9 VP1 is selected from the group consisting of N452A, N452D, N452E, N452G, N452H, N452M, N452N, N452Q, N452S, N452T, and N452V. In some instances, the amino acid substitution at the amino acid position corresponding to residue 453 in AAV9 VP1 is selected from the group consisting of G453A, G453D, G453E, G453G, G453K, G453N, G453Q, G453S, G453T, and G453V. In some instances, the amino acid substitution at the amino acid position corresponding to residue 454 in AAV9 VP1 is selected from the group consisting of S454A, S454D, S454E, S454G, S454K, S454N, S454Q, S454S, S454T, and S454V. In some instances, the amino acid substitution at

the amino acid position corresponding to residue 455 in AAV9 VP1 is selected from the group consisting of G455A, G455D, G455E, G455G, G455K, G455N, G455P, G455Q, G455S, and G455T. In some instances, the amino acid substitution at the amino acid position corresponding to residue 456 in AAV9 VP1 is selected from the group consisting Q456A, Q456D, Q456E, Q456H, Q456K, Q456N, Q456P, Q456Q, Q456S, and Q456T. In some instances, the amino acid substitution at the amino acid position corresponding to residue 457 in AAV9 VP1 is selected from the group consisting N457A, N457D, N457E, N457G, N457K, N457N, N457P, N457S, N457T, and N457V. In some instances, the amino acid substitution at the amino acid position corresponding to residue 458 in AAV9 VP1 is selected from the group consisting Q458A, Q458E, Q458G, Q458H, Q458K, Q458L, Q458N, Q458Q, Q458S, Q458T, and Q458V. In some instances, the amino acid substitution comprises at least three contiguous amino acids in an amino acid sequence selected from the group consisting of DGAATKN (SEQ ID NO: 3943), DGQSSKS (SEQ ID NO: 2764), and an amino acid sequence provided in **Table 1**. In some instances, an amino acid sequence of the AAV9 VP1 is provided in SEQ ID NO: 1. In some instances, the amino acid sequence of the AAV9 VP1 is provided in amino acid 217-736 within SEQ ID NO: 1. In some instances, VP1, VP2, and VP3 of the AAV capsid protein comprise the amino acid substitution. In some instances, the corresponding parental AAV capsid or capsid protein is a native AAV9 VP1 capsid. In some instances, the native AAV capsid protein comprises an amino acid sequence provided in SEQ ID NO: 1. In some instances, the corresponding parental AAV capsid or capsid protein is a variant of AAV9 comprising an amino acid sequence provided in any one of SEQ ID NOS: 3-6.

[0024] In some instances, the rAAV capsids, or variant AAV capsid proteins, further comprise an insertion of an amino acid sequence selected from the group consisting of TLAXPFK (SEQ ID NO: 46424), TLAX (SEQ ID NO: 46425), LAVX (SEQ ID NO: 46426), AVPX (SEQ ID NO: 46427), and VPFX (SEQ ID NO: 46428), at an amino acid position 588_589 within an amino acid sequence of the AAV capsid protein, wherein X is any amino acid other than V.

[0025] In some instances, the target tissue is a brain, and the rAAV capsids, or variant AVV capsid proteins, have the increased specificity for the brain, as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the off-target tissue is a liver, and the rAAV capsid s, or variant AVV capsid proteins, have a decreased specificity for the liver, as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the target tissue is a central nervous system (CNS), and the rAAV capsid s, or variant AVV capsid proteins, have an increased efficiency of viral transduction of at least 12-fold in the CNS, as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the target tissue is a spinal cord, and the rAAV capsid s, or variant AVV capsid proteins, have an increased efficiency of viral transduction of at least 20-fold in the spinal cord, as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the target tissue is a neuron, and the rAAV capsid s, or variant AVV capsid proteins, have an increased specificity for the neuron, as compared to the corresponding

parental AAV capsid or capsid protein. In some instances, the off-target tissue is a liver, and the rAAV capsid s, or variant AAV capsid proteins, have a decreased efficiency of viral transduction of at least 50-fold in the liver, as compared to the corresponding parental AAV capsid or capsid protein . In some instances, the off-target tissue is a Purkinje cell, and the rAAV capsid s, or variant AAV capsid proteins, have a decreased efficiency of viral transduction of at least 4-fold in the Purkinje cell, as compared to the r corresponding parental AAV capsid or capsid protein. In some instances, the off-target tissue is a liver, and the rAAV capsid s, or variant AAV capsid proteins, have a decreased efficiency of viral transduction of at least 100-fold in the liver, as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the rAAV capsids, or variant AAV capsid proteins, have described herein are isolated and purified. In some instances, the rAAV capsids or variant capsid proteins described herein are formulated as a pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some instances, the pharmaceutical formulation further comprises a heterologous nucleic acid encoding a therapeutic gene expression product. In some instances, the pharmaceutical formulation further comprises a pharmaceutical excipient. In some instances, the pharmaceutical formulation is formulated for intravenous, intraarterial, intrathecal, or subcutaneous administration to treat a disease or a condition of the CNS or PNS.

[0026] AspectExamples disclosed herein provide recombinant AAV (rAAV) capsids comprising an variant AAV capsid proteins comprising at least four contiguous amino acids at amino acid positions corresponding to residues selected from the group consisting of 452-455, 453-456, 454-457, and 455-458 of an amino acid sequence of the AAV9 capsid protein according to VP1 numbering, conferring at least one of an increased specificity and an increased transduction efficiency for a target tissue in a subject, wherein: (a) an amino acid at amino acid position corresponding to residue 452 is selected from the group consisting of A, D, G, L, N, Q, S, and T; (b) an amino acid at amino acid position corresponding to residue 453 is selected from the group consisting of A, G, N, P, Q, R, S, and T; (c) an amino acid at amino acid position corresponding to residue 454 is selected from the group consisting of A, D, G, N, S, and T; (d) an amino acid at amino acid position corresponding to residue 455 is selected from the group consisting of A, D, G, K, N, P, Q, S, and T; (e) an amino acid at amino acid position corresponding to residue 456 is selected from the group consisting of A, G, K, N, P, R, S, and T; (f) an amino acid at amino acid position corresponding to residue 457 is selected from the group consisting of A, G, K, N, P, R, S, T, and V; and (g) an amino acid at amino acid position corresponding to residue 458 is selected from the group consisting of A, G, K, L, R, S, T, and V. In some instances, the AAV capsid protein does not contain at amino acid positions corresponding to residues selected from the group consisting of 452-455, 453-456, 454-457, and 455-458 of AAV9 VP1 an amino acid sequence ILGTGTS (SEQ ID NO: 45479), QSSQTPR (SEQ ID NO: 45479), or TLAVPFK (SEQ ID NO: 45477). In some instances, the amino acid substitution is at a three (3)-fold axis of symmetry of the rAAV capsid. In some instances, the specificity or the transduction efficiency is measured following systemically administration of the rAAV capsid to the subject. In some

instances, systemic administration comprises intraarterial, intravenous, or subcutaneous, administration. In some instances, the rAAV capsid, or variant AAV capsid protein therein, is from AAV9. In some instances, the rAAV, or variant AAV protein comprises therein, confer an increase in a localization of the rAAV within the target tissue, as compared to the corresponding parental AAV capsid or capsid protein.

[0027] In some instances, the substitution comprises an amino acid sequence provided in **FIG. 3**. In some such instances, the at least four contiguous amino acids are provided in an amino acid sequence selected from the group consisting of LQTSSPG (SEQ ID NO: 2933), SINTKTN (SEQ ID NO: 45475), ERGANTK (SEQ ID NO: 5192), TTGGHSS (SEQ ID NO: 2743), GTTKTSE (SEQ ID NO: 3064), GTGTSVL (SEQ ID NO: 11958), and GTPSKAG (SEQ ID NO: 2741). In some instances, the substitution comprises an amino acid sequence provided in **FIG. 2**. In some such instances the at least four contiguous amino acids are provided in an amino acid sequence selected from the group consisting of QQGKQSV (SEQ ID NO: 79), SNGTKQT (SEQ ID NO: 442), GSGKTAA (SEQ ID NO: 88), MGDKPTR (SEQ ID NO: 2466), QPSGGNT (SEQ ID NO: 2672), and NQSGTKG (SEQ ID NO: 780). In some instances, the at least four contiguous amino acids are provided in an amino acid sequence selected from the group consisting of LQTSSPG (SEQ ID NO: 2933), QQGKQSV (SEQ ID NO: 79), SINTKTN (SEQ ID NO: 45475), SNGTKQT (SEQ ID NO: 442), GSGKTAA (SEQ ID NO: 88), MGDKPTR (SEQ ID NO: 2466), QPSGGNT (SEQ ID NO: 2672), ERGANTK (SEQ ID NO: 5192), TTGGHSS (SEQ ID NO: 2743), GTTKTSE (SEQ ID NO: 3064), GTGTSVL (SEQ ID NO: 11958), NQSGTKG (SEQ ID NO: 780), and GTPSKAG (SEQ ID NO: 2741). In some instances, the at least four contiguous amino acids are provided in an amino acid sequence provided in **Table 2**. In some instances, the target tissue is a central nervous system (CNS). In some instances, the CNS comprises a region selected from the neocortex, the basal ganglia, the hippocampus, the thalamus, the cerebellum, the brain stem, and the spinal cord. In some **embodiment examples**, the target tissue is the peripheral nervous system (PNS). In some instances, the PNS comprises a ganglion. In some instances, the ganglion is a trigeminal or dorsal root ganglion. In some instances, the target tissue is a non-dividing cell. In some instances, the CNS comprises a cell selected from the group consisting of a neuron, an astrocyte, a microglial cell, an oligodendrocyte, and a Schwann cell. In some instances, the target tissue is a CNS, and the AAV capsid has an increased efficiency of viral transduction of at least 12-fold in the CNS, as compared to the parental AAV capsid or AAV capsid protein. In some instances, the target tissue is a brain, and the AAV capsid has the increased specificity for the brain, as compared to the parental AAV capsid or AAV capsid protein. In some instances, the target tissue is a spinal cord, and the AAV capsid has an increased efficiency of viral transduction of at least 20-fold in the spinal cord, as compared to the parental AAV capsid or AAV capsid protein. In some instances, the target tissue is a neuron, and the AAV capsid has an increased specificity for the neuron, as compared to the parental AAV capsid or AAV capsid protein. In some instances, the rAAV capsids, or variant AAV capsid proteins, described herein are isolated and purified. In some instances, the

rAAV capsids, or variant AAV proteins, described herein are formulated as a pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some instances, the pharmaceutical formulation further comprises a heterologous nucleic acid encoding a therapeutic gene expression product. In some instances, the pharmaceutical formulation further comprises a pharmaceutical excipient. In some instances, the pharmaceutical formulation is formulated for intravenous, intraarterial, intrathecal, or subcutaneous administration to treat a disease or a condition of the CNS or PNS.

[0028] **Aspect**Examples disclosed herein provide rAAV capsids comprising AAV capsid proteins comprising at least four contiguous amino acids from an amino acid sequence provided in **FIG. 2** or **Table 2** at an amino acid position corresponding to residues selected from the group consisting of 452-455, 453-456, 454-457, and 455-458 of AAV9 VP1 conferring at least one of an increased specificity and an increased transduction efficiency when measured in a target tissue in a subject, as compared to a parental AAV capsid or capsid protein. In some instances, the at least four contiguous amino acids are at a three (3)-fold axis of symmetry of the rAAV capsid. In some instances, the AAV capsid protein does not contain at amino acid positions corresponding to residues selected from the group consisting of 452-455, 453-456, 454-457, and 455-458 of AAV9 VP1 an amino acid sequence ILGTGTS (SEQ ID NO: 45479), QSSQTPR (SEQ ID NO: 45479), or TLAVPFK (SEQ ID NO: 45477). In some instances, the specificity or the transduction efficiency is measured following systemically administration of the AAV capsid to the subject. In some instances, the rAAV capsid is chimeric. In some instances, the rAAV, or variant AAV protein comprises therein, confer an increase in a localization of the rAAV within the target tissue, as compared to the parental AAV capsid or capsid protein.

[0029] In some instances, the at least four contiguous amino acids comprise at least four, five, six, or seven contiguous amino acids in an amino acid sequence selected from the group consisting of LQTSSPG (SEQ ID NO: 2933), QQGKQSV (SEQ ID NO: 79), SINTKTN (SEQ ID NO: 45475), SNGTKQT (SEQ ID NO: 442), GSGKTAA (SEQ ID NO: 88), MGDKPTR (SEQ ID NO: 2466), QPSGGNT (SEQ ID NO: 2672), ERGANTK (SEQ ID NO: 5192), TTGGHSS (SEQ ID NO: 2743), GTTKTSE (SEQ ID NO: 3064), GTGTSVL (SEQ ID NO: 11958), NQSGTKG (SEQ ID NO: 780), and GTPSKAG (SEQ ID NO: 2741)

[0030] In some instances, the target tissue is a **central nervous system (CNS)**. In some instances, the CNS comprises a region selected from the neocortex, the basal ganglia, the hippocampus, the thalamus, the cerebellum, the brain stem, and the spinal cord. In some embodimentexamples, the target tissue is the **peripheral nervous system (PNS)**. In some instances, the PNS comprises a ganglion. In some instances, the ganglion is a trigeminal or dorsal root ganglion. In some instances, the target tissue is a non-dividing cell. In some instances, the CNS comprises a cell selected from the group consisting of a neuron, an astrocyte, a microglial cell, an oligodendrocyte, and a Schwann cell.

[0031] In some instances, the rAAV capsid, or variant AAV capsid protein comprised therein, further comprises an insertion of an amino acid sequence selected from the group consisting of TLAXPFK (SEQ ID NO: 46424), TLAX (SEQ ID NO: 46425), LAVX (SEQ ID NO: 46426), AVPX (SEQ ID NO: 46427), and VPFX (SEQ ID NO: 46428), at an amino acid position corresponding to residues 588_589 in the amino acid sequence of AAV9 VP1, wherein X is any amino acid other than V. In some instances, the parental AAV capsid or capsid protein is AAV9. In some instances, the amino acid sequence of a AAV9 capsid protein is provided in SEQ ID NO: 1. In some instances, the amino acid sequence of the AAV9 capsid protein is provided in amino acid 217-736 within SEQ ID NO: 1

[0032] In some instances, the target tissue is a brain, and the rAAV capsid, or variant AAV capsid protein, has the increased specificity for the brain, as compared to the parental AAV capsid or capsid protein. In some instances, the target tissue is the CNS, and the rAAV capsid, or variant AAV capsid protein, has an increased efficiency of viral transduction of at least 12-fold in the CNS, as compared to the parental AAV capsid or capsid protein. In some instances, the target tissue is a spinal cord, and the rAAV capsid, or variant AAV capsid protein, has an increased efficiency of viral transduction of at least 20-fold in the spinal cord, as compared to the parental AAV capsid or capsid protein. In some instances, the target tissue is a neuron, and the rAAV capsid, or variant AAV capsid protein, has an increased specificity for the neuron, as compared to the parental AAV capsid or capsid protein. In some instances, the rAAV capsids, or variant AAV capsid proteins, described herein are isolated and purified. In some instances, the rAAV capsids, or variant AAV capsid proteins, described herein are formulated as a pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some instances, the pharmaceutical formulation further comprises a heterologous nucleic acid encoding a therapeutic gene expression product. In some instances, the pharmaceutical formulation further comprises a pharmaceutical excipient.

[0033] AspectExamples provided herein provide rAAV capsids comprising variant AAV capsid proteins, the rAAV capsids having at least one of an increased specificity and increased transduction efficiency when measured in a target tissue of a subject relative to a corresponding parental AAV capsid or capsid protein, the AAV capsid protein comprising three amino acids (X1-X2-X3) at an amino acid position corresponding to residues selected from the group consisting of 452-454, 453-455, 454-456, 455-457, and 456-458 in AAV9 VP1, wherein: (a) X1 is selected from the group consisting of N, K, R, L, and T; (b) X2 is selected from the group consisting of N, S, L, E, D, and P; and (c) X3 is selected from the group consisting of N, S, A, D, G, L, and T. In some instances, the target tissue is selected from the group consisting of a lung, a heart, an intestine, a kidney, and a stomach. In some instances, the target tissue is a non-dividing cell. In some instances, the tissue is the lung and the amino acid sequence is KDNTPGR (SEQ ID NO: 32538), or any amino acid sequence provided in **FIG. 13**. In some instances, the tissue is the lung and the amino acid sequence is>NNLPRNL (SEQ ID NO: 32867), or any amino acid sequence provided in **FIG. 13**. In some instances, the tissue is an intestine and the amino acid sequence is RESSPSL (SEQ ID NO: 29065), or any amino acid sequence provided in **FIG.**

5. In some instances, the tissue is the kidney and the amino acid sequence is RVPLSTI (SEQ ID NO: 26933), or any amino acid sequence provided in **FIG. 6**. In some instances, the AAV capsid protein is an AAV9 capsid protein. In some instances, the AAV9 capsid protein is provided in SEQ ID NO: 1. In some instances, the AAV capsid protein further comprises four amino acids at an amino acid position selected from the group consisting of 452-455, 453-456, 454-457, and 455-458, wherein X4 is selected from the group consisting of T, S, P, and L. In some instances, the AAV capsid protein further comprises five amino acids at an amino acid position selected from the group consisting of 452-456, 453-457, and 453-458, wherein X5 is selected from the group consisting of P, R, and S. In some instances, the AAV capsid protein further comprises six amino acids at an amino acid position 452-457 or 453-458, wherein X6 is selected from the group consisting of N, G, S, and T. In some instances, the AAV capsid protein further comprises seven amino acids at an amino acid position 452-458, wherein X7 is selected from the group consisting of R, L, and I. In some instances, VP1, VP2, and VP3 of the AAV capsid protein comprise the amino acid sequence. In some instances, the corresponding parental AAV capsid is a native AAV9 capsid. In some instances, an amino acid sequence of the VP1 protein of the native AAV9 capsid is provided in SEQ ID NO: 1. In some instances, the corresponding parental AAV is a native AAV5 capsid. In some instances, an amino acid sequence of the VP1 protein of the native AAV5 capsid is provided in SEQ ID NO: 2.

[0034] In some instances, the rAAV, or variant AAV capsid proteins described herein, have at least one of the increased specificity and increased transduction efficiency for a target tissue when measured in a subject as compared to the corresponding parental AAV capsid or capsid protein. In some instances, the target tissue is a lung cell-type that is an alveolar type II epithelial (ATII) cell, and the rAAV capsid, or variant AAV capsid, has an increased efficiency of viral transduction of at least 60-fold in the ATII cell, as compared to a parental AAV capsid or capsid protein. In some instances, the target tissue is a lung cell-type that is an ATII cell, and the rAAV capsid, or variant AAV capsid, has an increased efficiency of viral transduction of at least 60-fold in the ATII cell, as compared to the parental AAV capsid or capsid protein. In some instances, the rAAV capsid, or variant AAV capsid, has an increased efficiency of viral transduction of at least 15-fold in the lung, as compared to the parental AAV capsid or capsid protein. In some instances, the rAAV capsid, or variant AAV capsid, has an increased efficiency of viral transduction of at least 30-fold in the lung, as compared to the parental AAV capsid or capsid protein. Also envisioned are AAV capsids comprising these AAV capsid proteins. In some instances, the rAAV capsids, or variant AAV capsids, described herein are isolated and purified. In some instances, the rAAV capsids, or variant AAV capsids, described herein are formulated as a pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some instances, the pharmaceutical formulation further comprises a heterologous nucleic acid encoding a therapeutic gene expression product. In some instances, the pharmaceutical formulation further comprises a pharmaceutical excipient. In some instances, the pharmaceutical

formulation is formulated for intravenous, intraarterial, subcutaneous, or intranasal administration to treat a disease or a condition of the target tissue.

[0035] AspectExamples disclosed herein provide rAAV capsids comprising variant AAV capsid proteins comprising three amino acids at an amino acid position corresponding to residues selected from the group consisting of 452-454, 453-455, 454-456, 455-457, and 456-458 in AAV9 VP1, wherein the three amino acids are provided in an amino acid sequence is provided in one or more of **FIG. 4-FIG. 14**. In some instances, the rAAV capsid or variant AAV capsid proteins, have at least one of an increased specificity and increased transduction efficiency when measured in a target tissue in a subject. In some instances, the target tissue is selected from the group consisting of a lung, an intestine, a liver, a stomach, a heart, a muscle, an adipose tissue, a spleen, a kidney, or reproductive organs (e.g., testis, ovaries). In some instances, the target tissue is a non-dividing cell. In some instances, the amino acid sequence is provided in **FIG. 4**. In some instances, the amino acid sequence is provided in **FIG. 5**. In some instances, the amino acid sequence is provided in **FIG. 6**. In some instances, the amino acid sequence is provided in **FIG. 7**. In some instances, the amino acid sequence is provided in **FIG. 8**. In some instances, the amino acid sequence is provided in **FIG. 9**. In some instances, the amino acid sequence is provided in **FIG. 10**. In some instances, the amino acid sequence is provided in **FIG. 11**. In some instances, the amino acid sequence is provided in **FIG. 12**. In some instances, the amino acid sequence is provided in **FIG. 13**. In some instances, the amino acid sequence is provided in **FIG. 14**. In some instances, the rAAV, or variant AAV protein comprises therein, confer an increase in a localization of the rAAV within the target tissue, as compared to the parental AAV capsid or capsid protein.

[0036] In some instances, the parental AAV capsid a native AAV9. In some instances, an amino acid sequence of a VP1 protein of the native AAV9 capsid protein is provided in SEQ ID NO: 1.

[0037] In some instances, the three amino acids further comprises four amino acids at an amino acid position corresponding to residues selected from the group consisting of 452-455, 453-456, 454-457, and 455-458 of AAV9 VP1, wherein the amino acid sequence is provided in one or more of **FIG. 4-FIG. 14**. In some instances, the three amino acids comprises five amino acids at an amino acid position corresponding to residues selected from the group consisting of 452-456, 453-457, and 453-458 of AAV9 VP1, wherein the amino acid sequence is provided in one or more of **FIG. 4-FIG. 14**. In some instances, the three amino acids further comprises six amino acids at an amino acid position corresponding to residues 452-457 or 453-458, wherein the amino acid sequence is provided in one or more of **FIG. 4-FIG. 14**. In some instances, the three amino acids further comprises seven amino acids at an amino acid position corresponding to residues 452-458, wherein the amino acid sequence is provided in one or more of **FIG. 4-FIG. 14**. In some instances, VP1, VP2, and VP3 of the rAAV capsid comprise the amino acid sequence. In some instances, the rAAV capsid, or variant AAV capsid proteins, described herein are isolated and purified. In some instances, the AAV capsids, or variant AAV capsid proteins described herein, are formulated as a pharmaceutical formulation further

comprising a pharmaceutically acceptable carrier. In some instances, the pharmaceutical formulation further comprises a heterologous nucleic acid encoding a therapeutic gene expression product. In some instances, the pharmaceutical formulation further comprises a pharmaceutical excipient. In some instances, the pharmaceutical formulation is formulated for intravenous, intraarterial, subcutaneous, or intranasal administration to treat a disease or a condition of the target tissue.

[0038] **AspectExample** disclosed herein provide methods of treating a disease or condition in a subject comprising administering to the subject a therapeutically effective amount of a composition comprising the rAAV capsids disclosed herein, the rAAV capsid encapsidating a nucleic acid sequence encoding a therapeutic gene expression product. In some instances, the mammalian subject is a primate. In some instances, the mammalian subject is a non-human primate. In some instances, the mammalian subject is a human. In some instances, the therapeutic gene expression product is a protein. In other instances, the therapeutic gene expression product is an RNA, e.g. a small interfering RNA (siRNA), a short hairpin RNA (shRNA), or a microRNA (miRNA). In some instances, the administering comprises intravenous or intranasal administration. In some instances, the disease or condition is a disease or condition of the central nervous system or peripheral nervous system. In some instances, the disease or condition is a disease or condition of the CNS or PNS. In some instances, the disease or condition is selected from the group consisting of Adrenoleukodystrophy, Alzheimer's disease, Amyotrophic lateral sclerosis, Angelman syndrome, Ataxia telangiectasia, Charcot-Marie-Tooth syndrome, classical rhizomelic chondrodysplasia punctata (RCDP), Cockayne syndrome, Deafness, Dravet Syndrome, Duchenne muscular dystrophy, Epilepsy, Essential tremor, Fragile X syndrome, Friedreich's ataxia, Frontotemporal dementia (FTD), Gaucher disease, glioblastoma, Huntington disease, infantile Refsum disease (IRD), Lesch-Nyhan syndrome, Maple syrup urine disease, Menkes syndrome, Myotonic dystrophy, Narcolepsy, Neurofibromatosis, Niemann-Pick disease, Parkinson disease, Phenylketonuria, Prader-Willi syndrome, Refsum disease, Rett syndrome, Spinal muscular atrophy, Spinocerebellar ataxia, Tangier disease, Tay-Sachs disease, Tuberous sclerosis, Von Hippel-Lindau syndrome, Williams syndrome, Wilson's disease, and Zellweger syndrome. In some instances, the therapeutic gene expression product is effective to modulate an activity or an expression of a target gene or gene expression product selected from the group consisting of Sarcoglycan Alpha (SGCA), glutamic acid decarboxylase 65 (GAD65), glutamic acid decarboxylase 67 (GAD67), CLN2, Nerve Growth Factor (NGF), Survival Of Motor Neuron 1, Telomeric (SMN1), Factor X (FIX), Retinoid Isomerohydrolase (RPE65), sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA2a), β -Glucocerebrosidase (GCase), Frataxin (FXN), Huntingtin (HTN), methyl-CpG binding protein 2 (MECP2), a peroxisomal biogenesis factor (PEX), progranulin (GRN), an antitubulin agent, copper-zinc superoxide dismutase (SOD1), Glucosylceramidase Beta (GBA), NPC Intracellular Cholesterol Transporter 1 (NPC1), and a NLRP3 inflammasome. In some instances, the therapeutic gene expression product comprises gene editing components. In some instances, the gene editing components are selected from the group consisting of, an artificial site-

specific RNA endonuclease (ASRE), a zinc finger endonuclease (ZFN), a transcription factor like effector nuclease (TALEN), a clustered regularly interspaced short palindromic repeats (CRISPR)/Cas enzyme, and a CRISPR)/Cas guide RNA.

[0039] **AspectExample** disclosed herein comprise plasmid vectors comprising a nucleic acid sequence encoding the AAV capsids described herein. In some instances, the plasmid vector is bacterial. In some instances, the plasmid vector is derived from *Escherichia coli*. In some instances, the nucleic acid sequence comprises, in a 5' to 3' direction: (1) a 5' inverted terminal repeat (ITR) sequence, (2) a Replication (*Rep*) gene, (3) a Capsid (*Cap*) gene, and (4) a 3' ITR, wherein the *Cap* gene encodes the AAV capsid protein described herein. In some instances, the plasmid vector encodes a pseudotyped AAV capsid protein. In some instances, the *Cap* gene is derived from the deoxyribose nucleic acid (DNA) provided in any one of SEQ ID NOs: 6-10. In some instances, the nucleic acid sequence comprising the *Cap* gene is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of the DNA sequences provided in SEQ ID NOS: 46364-46383. In some instances, the 5' ITR and the 3' ITR are derived from an AAV2 serotype. In some instances, the 5' ITR and the 3' ITR are derived from an AAV5 serotype. In some instances, the 5' ITR and the 3' ITR are derived from an AAV9 serotype.

[0040] **AspectExample** disclosed herein provide methods of manufacturing comprising: (a) introducing into a cell a nucleic acid comprising: (i) a first nucleic acid sequence encoding a therapeutic gene expression product, flanked by at its 5' and 3' ends by inverted terminal repeat (ITR) sequences; (ii) a second nucleic acid sequence encoding a viral genome comprising a 5' ITR sequence, a Replication (*Rep*) gene, Capsid (*Cap*) gene, and a 3' ITR, wherein the *Cap* gene encodes the AAV capsid protein described herein; and (iii) a third nucleic acid sequence encoding a first helper virus protein selected from the group consisting of E4orf6, E2a, and VA RNA, and optionally, a second helper virus protein comprising E1a or E1b55k; (b) expressing in the cell the AAV capsid protein described herein; (c) assembling an AAV particle comprising the AAV capsid proteins disclosed herein; and (d) packaging the first nucleic acid sequence in the AAV particle. In some instances, the nucleic acid is comprised by a plasmid and the cell is mammalian. In some instances, the cell is immortalized. In some instances, the immortalized cell is an embryonic stem cell. In some instances, the embryonic stem cell is a human embryonic stem cell. In certain instances, the human embryonic stem cell is a human embryonic kidney 293 (HEK-293) cell. In other instances, the nucleic acid is comprised by a virus, e.g. a baculovirus. In some such instances, the cell is an insect cell. In certain such instances, the cell is an SF9 cell. In some instances, the *Cap* gene is derived from the deoxyribose nucleic acid (DNA) provided in any one of SEQ ID NOs: 6-10. In some instances, the nucleic acid sequence comprising the *Cap* gene is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of the DNA sequences provided in SEQ ID NOS: 46364-46383. In some instances, the 5' ITR and the 3' ITR are derived from an AAV2 serotype. In some instances, the 5' ITR and the 3' ITR are derived from an AAV5 serotype. In some instances, the 5' ITR and the 3' ITR are derived from an

AAV9 serotype. In some instances, the first nucleic acid sequence (encoding a therapeutic gene expression product) and the second nucleic acid sequence (encoding the cap gene) are in *trans*. In some instances, the first nucleic acid sequence and the second nucleic acid sequence are in *cis*. In some instances, the first nucleic acid sequence, the second nucleic acid sequence and the third nucleic acid sequence (encoding the helper virus), are in *trans*.

[0041] AspectExample disclosed herein provide kits comprising: (a) a first vector comprising a first nucleic acid sequence encoding a viral genome comprising in a 5' to 3' direction: (i) a 5' inverted terminal repeat (ITR) sequence; (ii) a Replication (*Rep*) gene; (iii) a Capsid (*Cap*) gene encoding the AAV capsid proteins described herein, and (iv) a 3' ITR; and (b) optionally, a second vector comprising a second nucleic acid sequence encoding a helper virus protein comprising at least one of E4orf6, E2a, VA RNA, E1a and E1b55k. In some instances, the kit further comprises a cell. In some instances, the cell is mammalian. In some instances, the cell is immortalized. In some instances, the immortalized cell is an embryonic stem cell. In some instances, the embryonic stem cell is a human embryonic stem cell. In some instances, the human embryonic stem cell is a human embryonic kidney 293 (HEK-293) cell. In some instances, the kit further comprises a vector comprising a heterologous nucleic acid encoding a therapeutic gene expression product. In some instances, the vector is an episome.

INCORPORATE BY REFERENCE

[0042] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

[0043] The embodiments are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures:

[0044] **FIG. 1A-1D** show a viral engineering overview according to the present embodiments. **FIG. 1A** shows a surface model of three monomers comprising the 3-fold symmetry of AAV9 (circled), illustrating the location of the 7 amino acid substitution introduced in this library (red) at amino acids 452-458, and the 2 amino acid substitution and 7 amino acid insertion introduced by AAV-PHP.eB

CLAIMS

1. A recombinant AAV (rAAV), the rAAV comprising:
 - a) a variant AAV capsid comprising a variant AAV capsid protein comprising an amino acid substitution of three or more amino acids within a 7-mer peptide sequence at a 3-fold axis of symmetry of a corresponding parental AAV capsid protein, wherein the 7-mer peptide sequence is at an amino acid position that corresponds to amino acid residues 452-458 of AAV9 VP1 (SEQ ID NO: 1), and wherein the amino acid substitution is not ILGTGTS (SEQ ID NO: 45479), QSSQTPR (SEQ ID NO: 45479), or TLAVPFK (SEQ ID NO: 45477); and
 - b) a heterologous polynucleotide comprising a nucleotide sequence encoding a gene product.
2. The rAAV of claim 1, wherein the variant AAV capsid has an increased tropism for a target tissue or a target cell, when measured in a subject, relative to a tropism of the corresponding parental AAV capsid.
3. The rAAV of any of the previous claims, wherein the target tissue or the target cell comprises a tissue or a cell of a central nervous system (CNS) or a peripheral nervous system (PNS), or a combination thereof.
4. The rAAV of any of the previous claims, wherein the variant AAV capsid has a decreased tropism for an off-target tissue comprising liver tissue or an off-target cell comprising a liver cell, when measured in a subject, relative to the tropism of the corresponding parental AAV capsid.
5. The rAAV of any of the previous claims, wherein the three or more amino acids comprise three contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L, Q, S, T, and V at amino acid positions in the parental AAV capsid protein corresponding to amino acid residues 452-454, 453-455, 454-456, 455-457, or 456-458 of AAV9 VP1 (SEQ ID NO: 1).

6. The rAAV of any of the previous claims, wherein the three or more amino acids comprise four contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L Q, S, T, and V at amino acid positions in the parental AAV capsid protein corresponding to amino acid residues 452-455, 453-456, 454-457, or 455-458 of AAV9 VP1 (SEQ ID NO: 1).

7. The rAAV of any of the previous claims, wherein three or more amino acids comprise at least five contiguous amino acids, each amino acid independently selected from the group consisting of A, D, E, G, K, H, M, N, P, L Q, S, T, and V at amino acid positions in the parental AAV capsid protein corresponding to amino acid residues 452-456, 453-457, or 454-458 of AAV9 VP1 (SEQ ID NO: 1).

8. The rAAV of any of the previous claims, wherein the three or more amino acids comprise three or more of:

- a) A, D, E, G, H, M, N, Q, S, T, or V substituted at an amino acid position corresponding to amino acid residue 452 of AAV9 VP1 (SEQ ID NO: 1);
- b) A, D, E, G, K, N, Q, S, T, or V substituted at an amino acid position corresponding to amino acid residue 453 of AAV9 VP1 (SEQ ID NO: 1);
- c) A, D, E, G, K, N, Q, S, T, or V substituted at amino acid position corresponding to amino acid residue 454 of AAV9 VPI(SEQ ID NO: 1);
- d) A, D, E, G, K, N, P, Q, S, or T substituted at an amino acid position corresponding to amino acid residue 455 of AAV9 VPI(SEQ ID NO: 1);
- e) A, D, E, G, H, K, N, P, Q, S, or T substituted at an amino acid position corresponding to amino acid residue 456 of AAV9 VPI(SEQ ID NO: 1);
- f) A, D, E, G, K, N, P, S, T, or V substituted at an amino acid position corresponding to amino acid residue 457 of AAV9 VPI(SEQ ID NO: 1); and
- g) A, E, G, H, K, L, N, Q, S, T, or V substituted at amino acid position corresponding to amino acid residue 458 of AAV9 VPI(SEQ ID NO: 1).

9. The rAAV of any of the previous claims, wherein the amino acid substitution comprises an amino acid sequence selected from DGAATKN (SEQ ID NO: 3943), and DGQSSKS (SEQ ID NO: 2764).

10. The rAAV of claim 4 wherein the three or more amino acids comprises three or more of:

- a) A, D, G, L, N, Q, S, or T substituted at an amino acid at a position corresponding to amino acid residue 452 of AAV9 VP1 (SEQ ID NO: 1);
- b) A, G, N, P, Q, R, S, or substituted at an amino acid at a position corresponding to amino acid residue 453 of AAV9 VP1 (SEQ ID NO: 1);
- c) A, D, G, N, S, or T substituted at an amino acid at a position corresponding to amino acid residue 454 of AAV9 VP1 (SEQ ID NO: 1);
- d) A, D, G, K, N, P, Q, S, or T substituted at an amino acid at a position corresponding to amino acid residue 455 of AAV9 VP1 (SEQ ID NO: 1);
- e) A, G, K, N, P, R, S, or T substituted at an amino acid at a position corresponding to amino acid residue 456 of AAV9 VP (SEQ ID NO: 1); f) A, G, K, N, P, R, S, T, or V substituted at an amino acid at a position corresponding to amino acid residue 457 of AAV9 VP1 (SEQ ID NO: 1); and
- g) A, G, K, L, R, S, T, or V substituted at an amino acid at a position corresponding to amino acid residue 458 of AAV9 VP1 (SEQ ID NO: 1).

11. The rAAV of any of the previous claims, wherein the amino acid substitution comprises an amino acid sequence selected from the group consisting of LQTSSPG (SEQ ID NO: 2933), QQGKQSV (SEQ ID NO: 79), SINTKTN (SEQ ID NO: 45475), SNGTKQT (SEQ ID NO: 442), GSGKTAA (SEQ ID NO: 88), MGDKPTR (SEQ ID NO: 2466), QPSGGNT (SEQ ID NO: 2672), ERGANTK (SEQ ID NO: 5192), TTGGHSS (SEQ ID NO: 2743), GTTKTSE (SEQ ID NO: 3064), GTGTSLV (SEQ ID NO: 11958), NQSGTKG (SEQ ID NO: 780), KGPGQMG (SEQ ID NO: 45476), and GTPSKAG (SEQ ID NO: 2741).

12. The rAAV of any one of claims 2-11 wherein the target tissue or the target cell in the tissue is selected from the group consisting of lung, intestine, kidney, heart, and stomach.

13. The rAAV of any of the previous claims, wherein the three or more amino acids comprise three contiguous amino acids at an amino acid position corresponding to amino acid residues 452-454, 453- 455, 454-456, 455-457, or 456-458 of AAV9 VP1, wherein:

- a) 452 is selected from the group consisting of N, K, R, and T;
- b) 453 is selected from the group consisting of L, N, P, and S;
- c) 454 is selected from the group consisting of A, D, G, N, S, and T;
- d) 455 is selected from the group consisting of L, P, S, and T;
- e) 456 is selected from the group consisting of P, R, and S;
- f) 457 is selected from the group consisting of G, N, S, and T; and
- g) 458 is selected from the group consisting of I, L, and R.

14. The rAAV of any of the previous claims, wherein the three or more amino acids comprise:

- a) four contiguous amino acids at an amino acid position corresponding to amino acid residues 452-455, 453-456, 454-457, or 455-458 of AAV9 VP1 (SEQ ID NO: 1);
- b) five contiguous amino acids at an amino acid position corresponding to amino acid residues 452-456, 453-457, or 453-458 of AAV9 VP1 (SEQ ID NO: 1);
- c) six contiguous amino acids at an amino acid position corresponding to amino acid residues 452-457 or 453-458 of AAV9 VP1 (SEQ ID NO: 1); and
- d) seven contiguous amino acids at an amino acid position corresponding to amino acid residues 452-458 of AAV9 VP1 (SEQ ID NO: 1).

15. The rAAV of any one of claims 12-14, wherein the target tissue is lung, and the three or more amino acids are provided in an amino acid sequence comprising KDNTPGR (SEQ ID NO: 32538),>NNLPRNL (SEQ ID NO: 32867), or any amino acid sequence provided in FIG. 13.

16. The rAAV of any one of claims 12-14, wherein the target tissue is intestine, and the three or more amino acids are provided in an amino acid sequence comprising RESSPSL (SEQ ID NO: 26474), KDNTPGR (SEQ ID NO: 26584), or any amino acid sequence provided in FIG. 5.

17. The rAAV of any one of claims 12-14, wherein the tissue is kidney, and the three or more amino acids are provided in an amino acid sequence comprising RVPLSTI (SEQ ID NO: 26933),>NNLPRNL (SEQ ID NO: 27530), KDNTPGR (SEQ ID NO: 28509), or any amino acid sequence provided in FIG. 6.

18. The rAAV of any one of claims 12-14 wherein the tissue is heart, and the three or more amino acids are provided in an amino acid sequence comprising KDNTPGR (SEQ ID NO: 25633), or any amino acid sequence provided in FIG. 4.

19. The rAAV of any one of claims 12-14, wherein the tissue is stomach, and the three or more amino acids are provided in an amino acid sequence comprising RESSPSL (SEQ ID NO: 31904) or any amino acid sequence of FIG. 12.

20. The rAAV of any of the previous claims, wherein the corresponding parental AAV capsid protein is AAV9 VP 1 or a variant thereof.

21. The rAAV of claim 20, wherein the AAV9 VP 1 variant has a sequences identity of 90% or more to SEQ ID NO: 1.

22. The rAAV of claim 20, wherein the AAV9 VP1 variant has a sequences identity of 95% or more to SEQ ID NO: 1.

23. The rAAV of any of the previous claims, wherein the corresponding parental AAV capsid protein further comprises an insertion of an amino acid sequence selected from the group consisting of TLAXPFK (SEQ ID NO: 46424), TLAX (SEQ ID NO: 46425), LAVX (SEQ ID NO: 46426), AVPX (SEQ ID NO: 46427), and VPFX (SEQ ID NO: 46428), at an amino acid position corresponding to 588 _ 589 of the AAV9 VP1 (SEQ ID NO: 1), wherein X is any amino acid other than V.

24. The rAAV of any of the previous claims, wherein the parental AAV capsid protein is from AAV- PHP.B or AAV-PHP.eB.

25. The rAAV of any of the previous claims, wherein the rAAV is isolated and purified.

26. A pharmaceutical formulation comprising the rAAV of any of the previous claims and a pharmaceutical excipient.

27. The pharmaceutical formulation of claim 26, formulated for intravenous, intraarterial, intranasal, intrathecal, intracisternae magna, or subcutaneous injection.

28. Use of A method of treating a disease or condition in a subject, the method comprising administering to the subject a therapeutically effective amount the rAAV of any one of claims 1-25, or the pharmaceutical formulation of any one of claims 26-27, wherein the gene product is a therapeutic gene product in the manufacture of a medicament for treating a disease, wherein the disease or the condition is selected from the group consisting of pulmonary fibrosis, surfactant protein disorders, peroxisome biogenesis disorders, or chronic obstructive pulmonary disease (COPD).

29. The method-use according to claim 28, wherein the medicament is to be administered ~~administering is~~ by intravenous, intraarterial, intranasal, intrathecal, intracisternae magna, or subcutaneous injection.

~~30. The method of claim 28, wherein the disease or the condition is selected from the group consisting of pulmonary fibrosis, surfactant protein disorders, peroxisome biogenesis disorders, or chronic obstructive pulmonary disease (COPD).~~

~~31~~30. The method-use of claim 28 or claim 29, wherein the disease or the condition is a central nervous system (CNS) or peripheral nervous system (PNS) disorder.

~~32~~31. A method of manufacturing an rAAV of any one of claims 1-25, the method comprising:

- a) introducing into a cell a nucleic acid comprising:
 - a) a first nucleic acid sequence encoding a therapeutic gene expression product enclosed by a 5' and a 3' inverted terminal repeat (ITR) sequence;
 - b) a second nucleic acid sequence encoding a viral genome comprising a 5' ITR sequence, a Replication (Rep) gene, Capsid (Cap) gene, and a 3' ITR, wherein the Cap gene encodes a rAAV capsid protein; and
 - c) a third nucleic acid sequence encoding a first helper vims protein selected from the group consisting of E4orf6, E2a, and VA RNA, and optionally, a second helper vims protein comprising Ela or Elb55k;
- b) expressing in the cell the AAV capsid protein;
- c) assembling the rAAV of any one of claims 1-23; and
- d) packaging the first nucleic acid sequence in the rAAV.

3332. A variant adeno-associated virus (AAV) capsid comprising:

- a) at least one of a decreased specificity and a decreased transduction efficiency, as measured in a liver of a primate subject when delivered to the primate subject intravenously, relative to a corresponding parental AAV capsid with an AAV capsid protein of SEQ ID NO: 1; and
- b) at least one of an increased specificity and an increased transduction efficiency, as measured in a central nervous system (CNS) of the primate subject when delivered to the primate subject intravenously, relative to the corresponding parental AAV capsid.

3433. A variant adeno-associated virus (AAV) capsid comprising:

- a) a variant AAV capsid protein comprising an amino acid sequence with an amino acid substitution of three or more amino acids within a 7-mer peptide sequence at an amino acid position that corresponds to amino acid residues 452-458 of AAV9 VP1 (SEQ ID NO: 1); and
- b) at least one of an increased specificity and increased transduction efficiency in a target tissue as measured in the target tissue of a subject when delivered to the subject intranasally or intravenously, relative to a corresponding parental AAV capsid protein of

SEQ ID NO: 1 or SEQ ID NO: 2, wherein the target tissue is selected from the group consisting of lung, intestine, kidney, heart, and stomach.