

IMPROVED ADENO-ASSOCIATED VIRUS VECTORS

Cross-Reference to Related Applications

[0001] The present application claims priority to U.S. Provisional Application No. 63/524,265, filed June 30, 2023, which is incorporated herein by reference in its entirety.

Sequence Listing

[0002] The text of the computer readable sequence listing filed herewith, titled "CATA_41926_601_SequenceListing.xml", created June 27, 2024, having a file size of 168,432 bytes, is hereby incorporated by reference in its entirety.

Field

[0003] The present invention relates to AAV genomes, vectors and nucleic acids containing variant capsid protein sequences that allow for increased titer during recombinant AAV vector particle production.

Background

[0004] Adeno-associated virus (AAV) is one of the most actively investigated gene therapy vehicles. It was initially discovered as a contaminant of adenovirus preparations. AAV is a protein shell surrounding and protecting a small, single-stranded DNA genome of approximately 4.8 kilobases (kb). AAV belongs to the parvovirus family and is dependent on co-infection with other viruses, mainly adenoviruses, in order to replicate. Initially distinguished serologically, molecular cloning of AAV genes has identified hundreds of unique AAV strains in numerous species. Its single-stranded genome contains three genes, *Rep* (Replication), *Cap* (Capsid), and *aap* (Assembly). These three genes give rise to at least nine gene products through the use of three promoters, alternative translation start sites, and differential splicing. These coding sequences are flanked by inverted terminal repeats (ITRs) that are required for genome replication and packaging. These include four Rep gene encoded proteins (Rep78, Rep68, Rep52, and Rep40), which are required for viral genome replication and packaging, while *Cap* expression genes gives rise to the viral capsid proteins (VP; VP1/VP2/VP3), which form the outer capsid shell that protects the viral genome, as well as being actively involved in cell binding and internalization. It is estimated that the viral coat is comprised of 60 proteins arranged into an icosahedral structure with the capsid proteins in a molar ratio of 1:1:10 (VP1:VP2:VP3)

The *aap* gene encodes the assembly-activating protein (AAP) in an alternate reading frame overlapping the cap gene. This nuclear protein is thought to provide a scaffolding function for capsid assembly. While AAP is essential for nucleolar localization of VP proteins and capsid assembly in AAV2, the subnuclear localization of AAP varies among 11 other serotypes recently examined, and is nonessential in AAV4, AAV5, and AAV11. Another protein named membrane-associated accessory protein (MAAP), has been recently identified by Ogden *et. al.*, *Science* 366,1139-1143 (2019) which is encoded from an alternative ORF within the AAV *Cap* gene.

[0005] A variety of AAV genomes and production systems for producing recombinant AAV vector particles for use in gene therapies are known in the art. However, there is a need for systems which provide for higher titer and thus more efficient production of recombinant AAV vector particles.

Summary

[0006] The present invention relates to AAV genomes, vectors and nucleic acids containing variant capsid protein sequences that allow for increased titer, without an increase in encapsidated residual host cell DNA during recombinant AAV vector particle production.

[0007] Accordingly, in some preferred embodiments, the present invention provides an adeno-associated virus (AAV) genome, comprising a variant membrane associated accessory protein (MAAP) nucleic acid sequence having a mutation selected from the group consisting of a stop codon substitution at a codon position corresponding to positions W19, C20, S27, S57 and S88, an amino acid substitution at position L1, an amino acid substitution at position C20, an amino acid substitution at position C21, and combinations thereof, wherein the positions correspond to consensus sequence SEQ ID NO:1.

[0008] In some preferred embodiments, the genome comprises a substitution mutation in at least one of amino acid positions L1, C20 and C21 and/or a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88. In some preferred embodiments, the genome comprises a substitution mutation at amino acid position C20 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88. In some preferred embodiments, the genome comprises a substitution mutation at amino acid position C21 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88. In some preferred embodiments, the genome comprises a substitution mutation at amino acid position L1 and a

stop codon substitution at a position selected from the group consisting of amino acid positions W19, C20, S27, S57 and S88. In some preferred embodiments, the genome comprises a substitution mutation at one of amino acid positions C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of S27, S57 and S88. In some preferred embodiments, the genome comprises a substitution mutation at both of amino acid positions C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of S27, S57 and S88. In some preferred embodiments, the substitution mutation at position L1 is an L1P substitution mutation. In some preferred embodiments, the substitution mutation at position C20 is selected from the group consisting of C20S, C20Y and C20F substitution mutations. In some preferred embodiments, the substitution mutation at amino acid position C21 is a C21S substitution mutation. In some preferred embodiments, the stop codon substitution is at position W19. In some preferred embodiments, the stop codon substitution is at position C20. In some preferred embodiments, the stop codon substitution is at position S27. In some preferred embodiments, the stop codon substitution is at position S57. In some preferred embodiments, the stop codon substitution is at position S88.

[0009] In some preferred embodiments, the AAV genome is selected from the group consisting of a naturally-occurring serotype and a non-naturally-occurring serotype. In some preferred embodiments, the AAV genome is a non-naturally-occurring serotype. In some preferred embodiments, is a naturally-occurring serotype. In some preferred embodiments, the AAV genome comprises a serotype 6 genome. In some preferred embodiments, the AAV genome comprises a serotype 2 genome. In some preferred embodiments, the AAV genome is selected from a serotype 1, serotype 3, serotype 4, serotype 5, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh8 and serotype rh10 genome. In some preferred embodiments, the AAV genome is not an AAV serotype 2 genome.

[0010] In some preferred embodiments, the AAV genome comprises a VP1 nucleic sequence, and wherein the MAAP and VP1 nucleotide sequences encode MAAP and VP1 peptide sequences that each have at least 80% homology to wild type, with the proviso that the MAAP nucleotide sequence consists of at least one mutation as defined above. In some preferred embodiments, the MAAP and VP1 peptide sequences each have at least 90% homology to wild type. In some preferred embodiments, the MAAP and VP1 peptide sequences each have at least 95% homology to wild type.

[0011] In some preferred embodiments, the present invention provides a producer cell that produces adeno-associated virus, the producer cell comprising the adeno-associated virus genome as described above. In some preferred embodiments, the producer cell is eukaryotic. In some preferred embodiments, the producer cell is mammalian. In some preferred embodiments, the producer cell is a human cell. In some preferred embodiments, the producer cell is selected from yeast cells and insect cells.

[0012] In some preferred embodiments, the present invention provides methods for producing adeno-associated virus, the method comprising: introducing the AAV genome as described above a cell to create a producer cell and then culturing the producer cell whereby said producer cell produces adeno-associated virus. In some preferred embodiments, the methods further comprise harvesting said adeno-associated virus, where said harvested adeno-associated virus comprises a transgene. In some preferred embodiments, the titer of the produced adeno-associated virus is greater than the titer achieved with an adeno-associated virus genome comprising a corresponding wild-type MAAP sequence.

[0013] In some preferred embodiments, the present invention provides adeno-associated virus produced by the process described above.

[0014] In some preferred embodiments, the present invention provides a vector, comprising a variant AAV capsid gene sequence, the variant AAV capsid gene sequence comprising a mutated membrane associated accessory protein (MAAP) nucleic acid sequence having a mutation selected from the group consisting of a stop codon substitution at a codon position corresponding to positions W19, C20, S27, S57 and S88, an amino acid substitution at position L1, an amino acid substitution at position C20, an amino acid substitution at position C21, and combinations thereof, wherein the positions align with consensus sequence SEQ ID NO:1.

[0015] In some preferred embodiments, the variant AAV capsid gene sequence comprises a substitution mutation in at least one of amino acid positions L1, C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88. In some preferred embodiments, the variant AAV capsid gene sequence comprises a substitution mutation at amino acid position C20 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88. In some preferred embodiments, the variant AAV capsid gene sequence comprises a substitution mutation at amino acid position C21 and a stop codon substitution at an amino acid position selected from the group

consisting of W19, C20, S27, S57 and S88. In some preferred embodiments, the variant AAV capsid gene sequence comprises a substitution mutation at amino acid position L1 and a stop codon substitution at a position selected from the group consisting of amino acid positions W19, C20, S27, S57 and S88. In some preferred embodiments, the variant AAV capsid gene sequence comprises a substitution mutation at one of amino acid positions C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of S27, S57 and S88. In some preferred embodiments, the variant AAV capsid gene sequence comprises a substitution mutation at both of amino acid positions C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of S27, S57 and S88. In some preferred embodiments, the substitution mutation at position L1 is an L1P substitution mutation. In some preferred embodiments, the substitution mutation at position C20 is selected from the group consisting of C20S, C20Y and C20F substitution mutations. In some preferred embodiments, the substitution mutation at amino acid position C21 is a C21S substitution mutation. In some preferred embodiments, the stop codon substitution is at position W19. In some preferred embodiments, the stop codon substitution is at position C20. In some preferred embodiments, the stop codon substitution is at position S27. In some preferred embodiments, the stop codon substitution is at position S57. In some preferred embodiments, the stop codon substitution is at position S88.

[0016] In some preferred embodiments, the variant capsid gene sequence is selected from the group consisting of a naturally-occurring serotype and a non-naturally-occurring serotype. In some preferred embodiments, the variant capsid gene sequence is a non-naturally-occurring serotype. In some preferred embodiments, the variant capsid gene sequence is a naturally-occurring serotype. In some preferred embodiments, the variant capsid gene sequence is a serotype 6 capsid gene sequence. In some preferred embodiments, the variant capsid gene sequence is a serotype 2 capsid gene sequence. In some preferred embodiments, the variant capsid gene sequence is selected from a serotype 1, serotype 3, serotype 4, serotype 5, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh8 and serotype rh10 and AAVRH.8 capsid gene sequences.

[0017] In some preferred embodiments, the vector comprises a VP1 nucleic sequence, and wherein the MAAP and VP1 nucleotide sequences encode MAAP and VP1 peptide sequences that each have at least 80% homology to wild type, with the proviso that the modified capsid

nucleotide sequence consists of at least one mutation as defined above. In some preferred embodiments, the MAAP and VP1 peptide sequences each have at least 90% homology to wild type. In some preferred embodiments, the MAAP and VP1 peptide sequences each have at least 95% homology to wild type.

[0018] In some preferred embodiments, the vector further comprises a nucleic acid sequence encoding a *rep* gene.

[0019] In some preferred embodiments, the vector is a plasmid vector.

[0020] In some preferred embodiments, the present invention provides a vector system comprising the vector as described above and a second vector comprising a transgene flanked by ITR sequences. In some preferred embodiments, the system further comprises a third vector comprising adenovirus helper sequences. In some preferred embodiments, the second and third vectors are plasmid vectors.

[0021] In some preferred embodiments, the present invention provides a producer cell line comprising the vector or vector system as described above. In some preferred embodiments, the producer cell is eukaryotic. In some preferred embodiments, the producer cell is mammalian. In some preferred embodiments, the producer cell is a human cell. In some preferred embodiments, the producer cell is selected from of yeast cells and insect cells.

[0022] In some preferred embodiments, the present invention provides methods of for producing adeno-associated virus, the method comprising: introducing the vector or vector system of any of as described above into a cell to create a producer cell and then culturing the producer cell whereby the producer cell produces adeno-associated virus. In some preferred embodiments, the titer of the produced adeno-associated virus is greater than the titer achieved with an adeno-associated virus genome comprising a corresponding wild-type MAAP sequence.

Brief Description of the Drawings

[0023] Fig. 1 provides a process flow diagram for viral particle production.

Detailed Description

[0024] The present invention relates to AAV genomes, vectors and nucleic acids containing variant capsid protein sequences that allow for several fold increased titer during recombinant AAV vector particle production, without an increase in encapsidated residual host cell DNA. The packaging efficiency measured by AUC for full capsid maintained at a similar or higher level as

the standard capsid. A number of vector backbones for the production of recombinant AAV vector particles are known. However, production of recombinant AAV vector particles can be inefficient. The present invention addresses this problem by providing AAV capsid protein sequences containing variant MAAP sequences. As shown in the examples using AAV6 as a model, the variant capsid polypeptides described herein allow for higher-titer production of recombinant AAV particles.

[0025] Section headings as used in this section and the entire disclosure herein are merely for organizational purposes and are not intended to be limiting.

1. Definitions

[0026] The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms “a,” “and” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments “comprising,” “consisting of” and “consisting essentially of,” the embodiments or elements presented herein, whether explicitly set forth or not.

[0027] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0028] Unless otherwise defined herein, scientific, and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear; in the event, however of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0029] As used herein, the term “genome” refers to the genetic material (e.g., chromosomes) of an organism.

[0030] As used herein, the term “vector” refers to any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc., which is capable of replication when

associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

[0031] As used herein, the term "viral vector" refers to a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral particle. The viral vector can contain a nucleic acid (e.g., a payload) encoding a polypeptide as described herein in place of non-essential viral genes. The vector and/or particle may be utilized for the purpose of transferring nucleic acids into cells either in vitro or in vivo. Numerous forms of viral vectors are known in the art.

[0032] [00123] As used herein, an "AAV virus" or "AAV viral particle" refers to a viral particle composed of at least one AAV capsid protein such as VP1 (typically by all of the capsid proteins of a wild-type AAV) and an encapsidated polynucleotide rAAV vector. If the particle comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome, such as a transgene to be delivered to a mammalian cell), it is typically referred to as a "recombinant AAV vector particle" or simply a "rAAV vector". Thus, production of a rAAV particle necessarily includes production of a rAAV vector, as such a vector is contained within a rAAV particle.

[0033] As used herein "membrane-associated accessory protein" or "MAAP" refers to an AAV MAAP protein of any AAV serotype. As used herein, "wild-type MAAP" refers to naturally occurring, AAV MAAP.

[0034] As used herein reference to a specific amino acid residue or residues preferably refers to the residue with the corresponding number in the sequence of the MAAP sequences of SEQ ID NOs 1 to 16 and may be referred to a "position" within the designated sequence. When reference is made herein to a residue or residues or a position or positions in consensus SEQ ID NO: 1 or to a residue or residues or a position or positions aligning with MAAP polypeptide consensus sequence SEQ ID NO: 1 residue number, the residue or residues in one of the MAAP amino acids sequences of AAV serotypes (as depicted in SEQ ID NOs 2 to 16) that correspond to the indicated residue or residues in the consensus sequence of SEQ ID NO: 1 are also encompassed. Hence, as used herein the term "a mutation at a polypeptide residue (or position) aligning with (or corresponding to) MAAP polypeptide consensus sequence SEQ ID NO: 1. Residue (or position) number X" is defined as a mutation in a MAAP polypeptide at a residues or position corresponding to amino acid residue or position number X of the MAAP polypeptide

consensus sequence SEQ ID NO. 1. This can be either the indicated residue number in the sequence of SEQ ID NO: 1 or a corresponding residue number in any AAV MAAP, in particular a corresponding residue number in a MAAP sequence having a sequence of any of SEQ ID NOs: 2-16. A skilled person is well capable of determining the residue in any MAAP or a MAAP having a sequence of any of SEQ ID NOs: 2 to 16 corresponding to a particular residue in SEQ ID NO: 1, e.g., by performing an alignment of the MAAP sequence and the sequence of SEQ ID NO: 1. For example, in one embodiment, a C to S mutation of the MAAP polypeptide is introduced at a polypeptide residue aligning with MAAP polypeptide consensus sequence SEQ ID NO. 1 residue number 20. As is clear to a skilled person, this refers to a mutation in a MAAP polypeptide at a position corresponding to amino acid residue number 20 of the MAAP polypeptide consensus sequence SEQ ID NO. 1, as well as sequence aligned with the consensus sequence, for example SEQ ID NO:7 where the C to S mutation would also be at residue number 20.

[0035] As used herein the term “adeno-associated virus genome” refers to a polynucleotide molecule comprising at least one polynucleotide sequence encoding AAV MAAP. The AAV genome may be present in a vector. In preferred embodiments, the AAV genome or AAV vector comprises at least a gene encoding a variant (*i.e.*, mutant) MAAP. The AAV genome or AAV vector preferably further comprises one or more polynucleotides sequences encoding one or more further AAV genes. In particular, the genes other than the gene encoding MAAP may be wild-type or contain one or more mutations. In preferred embodiments, the AAV genome comprises a polynucleotide molecule comprising an AAV polynucleotide sequence flanked at both ends by AAV Inverted Terminal Repeats (ITRs). In preferred embodiments the AAV genome is encompassed by an AAV expression vector or is an AAV expression vector. The AAV genome or AAV expression vector thus preferably comprises at least one AAV polynucleotide, either wild-type or containing one or more mutations, flanked by AAV ITRs, as long as it contains a variant MAAP. In preferred embodiments, the AAV genome of the invention comprises a polynucleotide molecule comprising an AAV polynucleotide sequence that allows the production of AAV when introduced into a suitable host cell. Alternatively, the AAV genome of the invention is combined with one or more further polynucleotide molecules comprising AAV polynucleotide sequences, such as an AAV helper construct comprising a polynucleotide sequence encoding AAV capsid proteins and other AAV helper functions and/or

an AAV gene of interest (GOI, also referred to as a transgene) construct comprising a gene of interest flanked by AAV ITRs, so that the combined AAV genome and one or more further polynucleotide molecules allows the production of AAV when introduced into a suitable host cell. An example of a transgene is a therapeutic gene.

[0036] The AAV genome or AAV vector may be of any AAV serotype, either a naturally occurring serotype or a non-naturally-occurring serotype. The cap genes encoding the MAAP are well known for each AAV serotype and a skilled person is therefore well capable of preparing a mutant AAV genome as described herein of any AAV serotype MAAP.

[0037] Mutations in MAAP can for instance be introduced by site-directed mutagenesis. Site-directed mutagenesis is well known in the art and can be used to introduce one or more point mutations, including a mutation according to the invention (including substitution, insertion or deletion) into a viral polynucleotide or genome. A skilled person is well capable of introducing a mutation according to the invention. Suitable techniques for site-directed mutagenesis are described in Sambrook's et al. *Molecular Cloning A Laboratory Manual*, second edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), Ausubel et al. *Current Protocols in Molecular Biology* (Greene Publishing Associates (1992)) and Bachman et al. (*J. Methods Enzymol.* 2013; 529:241-248), the content of which references is incorporated herein by reference.

[0038] As used herein, the term "viral capsid polypeptide" refers to the proteinaceous shell or coat of a viral particle. At a minimum, a viral capsid polypeptide as described herein permits packaging or assembly of the capsid polypeptide into a viral particle that is competent for delivery of nucleic acid to the host cell. Capsids function to encapsidate, protect, transport, and release into a host cell a viral genome. Capsids are generally comprised of oligomeric structural subunits of a polypeptide of the viral capsid polypeptides. As used herein, the term "encapsidated" means enclosed within a viral capsid. As an example, the AAV genome comprises three overlapping sequences which encode capsid proteins, VP1, VP2 and VP3, which start from one promoter, p40. The AAV capsid is composed of a mixture of VP1, VP2, and VP3 totaling 60 monomers arranged in icosahedral symmetry in a ratio of 1 : 1 : 10.

[0039] As used herein, "packaging" refers to a series of intracellular events that result in the assembly and encapsidation of an AAV particle.

[0040] As used herein, "payload" refers to a nucleic acid which is encapsidated within a viral vector, e.g., an AAV vector. A payload nucleic acid can encode, e.g., a polypeptide, an inhibitory RNA, an antibody or antibody reagent, an oligonucleotide, or a miRNA.

[0041] As used herein, the term "producer cell" refers to any cell line suitable for the production of AAV vectors.

[0042] The term " polypeptide " as used herein refers to a polymer of amino acids. The terms "protein" and "polypeptide" are used interchangeably herein. A peptide is a relatively short polypeptide, typically between about 2 and 60 amino acids in length. Polypeptides used herein typically contain amino acids such as the 20 L-amino acids that are most commonly found in proteins. However, other amino acids and/or amino acid analogs known in the art can be used. One or more of the amino acids in a polypeptide may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a phosphate group, a fatty acid group, a linker for conjugation, functionalization, etc. A polypeptide that has a nonpolypeptide moiety covalently or noncovalently associated therewith is still considered a "polypeptide." Exemplary modifications include glycosylation and palmitoylation. Polypeptides can be purified from natural sources, produced using recombinant DNA technology or synthesized through chemical means such as conventional solid phase peptide synthesis, etc. The term "polypeptide sequence" or "amino acid sequence" as used herein can refer to the polypeptide material itself and/or to the sequence information (i.e., the succession of letters or three letter codes used as abbreviations for amino acid names) that biochemically characterizes a polypeptide. A polypeptide sequence presented herein is presented in an N-terminal to C- terminal direction unless otherwise indicated.

[0043] A given amino acid can be replaced by a residue having similar physicochemical characteristics, e.g., substituting one aliphatic residue for another (such as Ile, Val, Leu, or Ala for one another), or substitution of one polar residue for another (such as between Lys and Arg; Glu and Asp; or Gln and Asn). Other such conservative substitutions, e.g., substitutions of entire regions having similar hydrophobicity characteristics, are well known. Polypeptides comprising conservative amino acid substitutions can be tested in any one of the assays described herein to confirm that a desired activity, e.g. ligand-mediated receptor activity and specificity of a native or reference polypeptide is retained.

[0044] Amino acids can be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); (4) basic: Lys (K), Arg (R), His (H). Alternatively, naturally occurring residues can be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Particular conservative substitutions include, for example; Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into His; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

[0045] In some embodiments, a polypeptide described herein (or a nucleic acid encoding such a polypeptide) can be a functional fragment of one of the amino acid sequences described herein. As used herein, a "functional fragment" is a fragment or segment of a peptide which retains at least 50% of the wild-type reference polypeptide's activity according to an assay known in the art or described below herein. For example, a functional fragment described herein would retain at least 50% of the VP1 capsid function. One skilled in the art can assess the function of VP1 using standard techniques, for example those described herein below. A functional fragment can comprise conservative substitutions of the sequences disclosed herein.

[0046] In some embodiments, a polypeptide described herein can be a variant of a polypeptide or molecule as described herein. In some embodiments, the variant is a conservatively modified variant. Conservative substitution variants can be obtained by mutations of native nucleotide sequences, for example. A "variant," as referred to herein, is a polypeptide substantially homologous to a native or reference polypeptide, e.g., SEQ ID NO: 1, but which has an amino acid sequence different from that of the native or reference polypeptide because of one or a plurality of deletions, insertions or substitutions. A variant capsid polypeptide as the term is used herein retains the capacity for assembly into a viral capsid. Variant polypeptide-

encoding DNA sequences encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to a native or reference DNA sequence, e.g., SEQ ID NO: 1, but that encode a variant protein or fragment thereof that retains activity of the non-variant polypeptide. A wide variety of PCR-based site-specific mutagenesis approaches are known in the art and can be applied by the ordinarily skilled artisan.

[0047] A variant amino acid or DNA sequence can be at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, identical to a native or reference sequence. The degree of homology (percent identity) between a native and a mutant sequence can be determined, for example, by comparing the two sequences using computer programs commonly employed for this purpose, e.g., that are freely available on the world wide web (e.g. BLASTp or BLASTn with default settings). Alterations of the native amino acid sequence can be accomplished by any of a number of techniques known to one of skill in the art. Mutations can be introduced, for example, at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites permitting ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion. Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered nucleotide sequence having particular codons altered according to the substitution, deletion, or insertion required. Techniques for making such alterations are well established and include, for example, those disclosed by Walder et al. (Gene 42:133, 1986); Bauer et al. (Gene 37:73, 1985); Craik (BioTechniques, January 1985, 12-19); Smith et al. (Genetic Engineering: Principles and Methods, Plenum Press, 1981); and U.S. Pat. Nos. 4,518,584 and 4,737,462, which are herein incorporated by reference in their entireties. Any cysteine residue not involved in maintaining the proper conformation of a polypeptide also can be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) can be added to a polypeptide to improve its stability or facilitate oligomerization.

[0048] As used herein, the term "DNA" is defined as deoxyribonucleic acid. The term "polynucleotide" is used herein interchangeably with "nucleic acid" to indicate a polymer of nucleosides. Typically, a polynucleotide is composed of nucleosides that are naturally found in DNA or RNA (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine,

deoxythymidine, deoxy guanosine, and deoxy cytidine) joined by phosphodiester bonds. However, the term encompasses molecules comprising nucleosides or nucleoside analogs containing chemically or biologically modified bases, modified backbones, etc., whether or not found in naturally occurring nucleic acids, and such molecules may be preferred for certain applications. Where this specification refers to a polynucleotide it is understood that both DNA, RNA, and in each case both single- and double-stranded forms (and complements of each single-stranded molecule) are provided. "Polynucleotide sequence" as used herein can refer to the polynucleotide material itself and/or to the sequence information (i.e. the succession of letters used as abbreviations for bases) that biochemically characterizes a specific nucleic acid. A polynucleotide sequence presented herein is presented in a 5' to 3' direction unless otherwise indicated.

[0049] As used herein, the term "corresponding to," when used in reference to an amino acid or polynucleotide sequence means that a given amino acid or polynucleotide sequence in one polypeptide or polynucleotide molecule has structural properties, functional properties, or both that are similar relative to an amino acid or polynucleotide sequence in a similar location in another polypeptide or polynucleotide molecule. Homologues of a given polypeptide in different species "correspond to" each other, as do regions or domains of homologous polypeptides from different species. Similarly, capsid polypeptides of different serotypes of viral vectors, including but not limited to adeno-associated virus (AAV) vectors, "correspond to" each other, as do regions of such polypeptides, defined, for example by alignment of their amino acid sequences. While other alignment parameters can be used to define such regions, for the avoidance of doubt, alignment can be performed using BLAST® (Basic Local Alignment Search Tool) using default parameters of version BLAST+ 2.8.0 released March 28, 2018.

[0050] As used herein, a "transgene" is a polynucleotide that is delivered to a cell by a vector.

[0051] A "gene" refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular gene product after being transcribed, and sometimes also translated. The term "gene" or "coding sequence" refers to a nucleotide sequence in vitro or in vivo that encodes a gene product. In some instances, the gene consists or consists essentially of coding sequence, that is, sequence that encodes the gene product. In other instances, the gene comprises additional, non-coding, sequence. For example, the gene may or may not include regions preceding and following the coding region, e.g. 5' untranslated (5' UTR) or "leader"

sequences and 3' UTR or "trailer" sequences, as well as intervening sequences (introns) between individual coding segments (exons).

[0052] A "gene product" is a molecule resulting from expression of a particular gene. Gene products include, e.g., a polypeptide, an aptamer, an interfering RNA, an mRNA, and the like. In particular embodiments, a "gene product" is a polypeptide, peptide, protein or interfering RNA including short interfering RNA (siRNA), miRNA or small hairpin RNA (shRNA). In particular embodiments, a gene product is a therapeutic gene product, e.g., a therapeutic protein.

[0053] As used herein, a "therapeutic gene" refers to a gene that, when expressed, produces a therapeutic gene product that confers a beneficial effect on the cell or tissue in which it is present, or on a mammal in which the gene is expressed. Examples of beneficial effects include amelioration of a sign or symptom of a condition or disease, prevention or inhibition of a condition or disease, or conferral of a desired characteristic. Therapeutic genes include, but are not limited to, genes that correct a genetic deficiency in a cell or mammal.

[0054] As used herein, the term "host cell" refers to any eukaryotic cell (e.g., mammalian cells, avian cells, amphibian cells, plant cells, fish cells, and insect cells), whether located in vitro or in vivo.

[0055] As used herein, the term "cell culture" refers to any in vitro culture of cells. Included within this term are continuous cell lines (e.g., with an immortal phenotype), primary cell cultures, finite cell lines (e.g., non-transformed cells), and any other cell population maintained in vitro, including oocytes and embryos.

[0056] The term "gene of interest" refers to any nucleotide sequence (e.g., RNA or DNA), the manipulation of which may be deemed desirable for any reason (e.g., treat disease, confer improved qualities, expression of a protein of interest in a host cell, expression of a ribozyme, etc.), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (e.g., reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.).

[0057] As used herein, the term "protein of interest" refers to a protein encoded by a gene of interest.

[0058] As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," "DNA encoding," "RNA sequence encoding," and "RNA encoding" refer to the order or sequence of deoxyribonucleotides or ribonucleotides along a strand of deoxyribonucleic acid or ribonucleic acid. The order of these deoxyribonucleotides or ribonucleotides determines the order of amino acids along the polypeptide (protein) chain. The DNA or RNA sequence thus codes for the amino acid sequence.

[0059] The term "promoter," "promoter element," or "promoter sequence" as used herein, refers to a DNA sequence which when ligated to a nucleotide sequence of interest is capable of controlling the transcription of the nucleotide sequence of interest into mRNA. A promoter is typically, though not necessarily, located 5' (i.e., upstream) of a nucleotide sequence of interest whose transcription into mRNA it controls, and provides a site for specific binding by RNA polymerase and other transcription factors for initiation of transcription.

[0060] Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription (Maniatis et al., *Science* 236:1237 [1987]). Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect and mammalian cells, and viruses (analogous control elements, i.e., promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset of cell types (for review see, Voss et al., *Trends Biochem. Sci.*, 11:287 [1986]; and Maniatis et al., *supra*). For example, the SV40 early gene enhancer is very active in a wide variety of cell types from many mammalian species and has been widely used for the expression of proteins in mammalian cells (Dijkema et al., *EMBO J.* 4:761 [1985]). Two other examples of promoter/enhancer elements active in a broad range of mammalian cell types are those from the human elongation factor 1 α gene (Uetsuki et al., *J. Biol. Chem.*, 264:5791 [1989]; Kim et al., *Gene* 91:217 [1990]; and Mizushima and Nagata, *Nuc. Acids. Res.*, 18:5322 [1990]) and the long terminal repeats of the Rous sarcoma virus (Gorman et al., *Proc. Natl. Acad. Sci. USA* 79:6777 [1982]) and the human cytomegalovirus (Boshart et al., *Cell* 41:521 [1985]).

[0061] As used herein, the term "promoter/enhancer" denotes a segment of DNA which contains sequences capable of providing both promoter and enhancer functions (i.e., the

functions provided by a promoter element and an enhancer element, see above for a discussion of these functions). For example, the long terminal repeats of retroviruses contain both promoter and enhancer functions. The enhancer/promoter may be "endogenous" or "exogenous" or "heterologous." An "endogenous" enhancer/promoter is one that is naturally linked with a given gene in the genome. An "exogenous" or "heterologous" enhancer/promoter is one that is placed in juxtaposition to a gene by means of genetic manipulation (i.e., molecular biological techniques such as cloning and recombination) such that transcription of that gene is directed by the linked enhancer/promoter.

[0062] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (i.e., a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "5'-A-G-T-3'," is complementary to the sequence "3'-T-C-A-5'." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

[0063] As used herein, the percentage of identity of an amino acid sequence or nucleic acid sequence, or the term "% sequence identity," is defined herein as the percentage of residues of the full length of an amino acid sequence or nucleic acid sequence that is identical with the residues in a reference amino acid sequence or nucleic acid sequence after aligning the two sequences and introducing gaps, if necessary, to achieve the maximum percent identity. The percentage homology of an amino acid sequence or the term "% homology to" is defined herein as the percentage of amino acid residues in a particular sequence that are homologous with the amino acid residues in a reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence homology. This method takes into account conservative amino acid substitutions. Conservative substitutions are substitutions of an amino acid to be substituted with a similar amino acid. Amino acids can be similar in several characteristics, for example, size, shape, hydrophobicity, hydrophilicity, charge, isoelectric point, polarity, aromaticity, etc. Preferably, a conservative substitution is an exchange of one amino acid within a group for another amino acid within the same group, whereby the groups are the

following: (1) alanine, valine, leucine, isoleucine, methionine, and phenylalanine; (2) histidine, arginine, lysine, glutamine, and asparagine; (3) aspartate and glutamate; (4) serine, threonine, alanine, tyrosine, phenylalanine, tryptophan, and cysteine; and (5) glycine, proline, and alanine. Methods and computer programs for the alignment are well known in the art, for example "Align 2". Programs for determining nucleotide sequence identity are also well known in the art, for example, the BESTFIT, FASTA and GAP programs. These programs are readily utilized with the default parameters recommended by the manufacturer.

[0064] The terms "in operable combination," "in operable order," and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of amino acid sequences in such a manner so that a functional protein is produced.

[0065] Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present disclosure. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

2. Variant Capsid Polypeptides

[0066] The present invention provides AAV sequences, genomes and vectors comprising variant MAAP sequences. In some preferred embodiments, the variants comprise, for example, a stop codon substitution at a codon position corresponding to positions W19, C20, S27, S57 and S88, an amino acid substitution at position L1, an amino acid substitution at position C20, an amino acid substitution at position C21, and combinations thereof, wherein the positions correspond or are aligned to consensus sequence SEQ ID NO:1. In some preferred embodiments, the MAAP sequence comprises a substitution mutation at position L1 and a stop codon substitution at a position selected from the group consisting of W19 (or L19 of AAV2 MAAP), C20, S27, S57 and S88. In some preferred embodiments, the MAAP sequence comprises a substitution mutation at position C20 and/or C21 and a stop codon substitution at a position selected from the group consisting of W19, S27, S57 and S88. In some preferred embodiments, the MAAP sequence comprises a substitution mutation at both of positions C20 and C21 and a stop codon substitution at a position selected from the group consisting of S27, S57 and S88. In

some preferred embodiments, the substitution mutation at position L1 is an L1P substitution mutation. In some preferred embodiments, the substitution mutation at position C20 is selected from the group consisting of C20S, C20Y and C20F substitution mutations. In some preferred embodiments, the substitution mutation at position C20 is a C20S substitution mutation.

[0067] In some preferred embodiments, the stop codon substitution is at position W19 (or L19 of AAV2 MAAP). In some preferred embodiments, the variant with a stop codon substitution at position W19 (or L19 of AAV2 MAAP) further comprises a substitution mutation at position L1. In some preferred embodiments, the stop codon substitution is at position C20. In some preferred embodiments, the variant with a stop codon substitution at position C20 further comprises a substitution mutation as described at position L1. In some preferred embodiments, the stop codon substitution is at position S27. In some preferred embodiments, the variant with a stop codon substitution at position S27 further comprises a substitution mutation as described at position L1 and/or C20 and/or C21. In some preferred embodiments, the stop codon substitution is at position S57. In some preferred embodiments, the variant with a stop codon substitution at position S57 further comprises a substitution mutation as described at position L1 and/or C20 and/or C21. In some preferred embodiments, the stop codon substitution is at position S88. In some preferred embodiments, the variant with a stop codon substitution at position S88 further comprises a substitution mutation as described at position L1 and/or C20 and/or C21.

[0068] Exemplary sequences are provided in Table 1. SEQ ID NO:1 is a MAAP consensus sequence. SEQ ID NOs: 2-16 are wild-type MAAP sequences for AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAVrh8 and AAVrh10. SEQ ID NOs:17-30 are exemplary variant MAAP polypeptide sequences for AAV6 MAAP. SEQ ID NOs:31-44 are exemplary variant MAAP polypeptide sequences for AAV2 MAAP. SEQ ID NOs:73-87 are exemplary variant MAAP polypeptide sequences for AAV5 MAAP. SEQ ID NOs:103-114 are exemplary variant MAAP polypeptide sequences for AAV8 MAAP. SEQ ID NOs:127-140 are exemplary variant MAAP polypeptide sequences for AAVrh10 MAAP. It will be understood that the same mutations may be introduced at the corresponding sites in other MAAP polypeptide sequences, e.g., in MAAPs from AAV1, AAV3, AAV4, AAV7, AAV9, AAV10, AAV11, AAV12, AAV13, or AAVrh8. In some preferred embodiments, the AAV genome is not an AAV serotype 2 genome. SEQ ID NOs: 45-50 are nucleic acid sequences for the variant AAV6 MAAP polypeptides (i.e., SEQ ID NOs: 17-30).

SEQ ID NOs: 59-72 are nucleic acid sequences for the variant AAV2 MAAP polypeptides (i.e., SEQ ID NOs: 31-44). SEQ ID NOs: 88-102 are nucleic acid sequences for the variant AAV5 MAAP polypeptides (i.e., SEQ ID NOs: 73-87). SEQ ID NOs: 115-126 are nucleic acid sequences for the variant AAV8 MAAP polypeptides (i.e., SEQ ID NOs: 103-114). SEQ ID NOs: 141-154 are nucleic acid sequences for the variant AAVrh10 MAAP polypeptides (i.e., SEQ ID NOs: 127-140).

[0069] With reference to the amino acid sequences provided in Table 1, where applicable the “-” in the sequence represents the amino acid position at which a stop codon has been substituted in the corresponding nucleic acid coding sequence. In the accompanying sequence ID listing, the amino acid position corresponding to a substituted stop codon in the coding nucleic acid sequence, if present, is indicated by an X. It will be understood by those of ordinary skill in the art that since the MAAP polypeptide is expressed in an alternative reading frame of the wild-type AAV capsid protein sequence, a stop codon that is substituted into a MAAP sequence (e.g., SEQ ID NO:1) will not introduce a stop codon in the reading frame for the capsid protein. Thus, it will further be understood that any MAAP polypeptide that is translated where a stop codon is inserted into the reading frame of the MAAP polypeptide coding sequence will be truncated (i.e., translation will stop) at the amino acid position immediately upstream (or 5’) to the position at which the stop codon is introduced (e.g., at the amino acid just before position W19 (L19), C20, S27, S57 or S88). Because the mutations are introduced into the reading frame of the MAAP polypeptide coding sequence and the genome of the AAV or vector will still comprise functional capsid polypeptides, the variant amino acids sequences in Table 1 are all presented in relation to the full length MAAP polypeptide coding sequence even though in some instances a stop codon has been introduced at a position within the MAAP polypeptide coding sequence.

[0070] Table 1. MAAP wild-type and variant protein and nucleic acid sequences

Sequence name	SEQ ID NO.	Sequence
MAAP consensus sequence	SEQ ID NO:1	LEPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPSTRRTQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKKIRLLGATSGEQSSRPRRGFSNLSVWLRKALRRLLERRDR

AAV1 MAAP Wild-type	SEQ ID NO:2	LEPRSPKPTSKSRRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSTTRPTTSSSKRVITRTCGITTPPSFRSVCKK IRLLGATSGEQSSRPGRGFSNLSVWLRKALRRLLENNVR
AAV2 MAAP Wild-type	SEQ ID NO:3	LAHHHQSPQSGIRTTAGVLCFLGTSTSDPSTDSTRESRSR QTPRPSSTTKPTTGSSTAETTRTSSTTTPTPSFRSALKK IRLLGATSGEQSSRRKRGFLNLWAWLRNLLRRLLEKRG
AAV3 MAAP Wild-type	SEQ ID NO:4	LESLNPKRTNNRRTTVGVLCFRVTNTSDPVTDSSTKESRST RRTQRPSTNTKLTTSSSRPVTTTSSTTTPTPSFRSVFCKK TRLLGATLAEQSSRPKRGSLSLVWLRKQLKRLLEERRGL
AAV4 MAAP Wild-type	SEQ ID NO:5	LEPLNPRQINNIRTTAGVLCFRVTNTSDPATDSTRGNPST QRTQRPSTTRPTTSSSRPVTTPTSSTTTPTPSFRSSSGFRA THRLGATSAEQSSRPKRGFLNLLVWLSKRVRRLLERRDR
AAV5 MAAP Wild-type	SEQ ID NO:6	RAHRNQNPISIKIKPVVLCCLVITISDPETVSIEESLS TGQTRSRETTSTRTSSSRRETTPTSSTTTPTPSFRSS PTTHPSGETSERQSFPRKGFNLLAWLKRVLRRPLPESG
AAV6 MAAP Wild-type	SEQ ID NO:7	LEPRNPKPTSKSRRTTAGVWCFLATSTSDPSTDSTRGSPST RRMQRPSTTRPTTSSSKRVITRTCGITTPPSFRSVCKK IRLLGATSGEQSSRPGRGFSNLLVWLRKVLRRLLLENNVR
AAV7 MAAP Wild-type	SEQ ID NO:8	LEPRNPKPTSKSRRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSTTRPTTSSSKRVITRTCGITTPPSFRSVCKK IRHLGATSGEQSSRPGRGFSNLSVWLRKALRRLLEQRDR
AAV8 MAAP Wild-type	SEQ ID NO:9	LEPRSPKPTSKSRRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSTTRPTTSSCRVTITRTCGITTPPSFRSVCKK IRLLGATSGEQSSRPGRGFSNLSVWLRKALRRLLERRDR
AAV9 MAAP Wild-type	SEQ ID NO:10	LEPLNPRQINNIRTTLEVLCFRVTNTLDPATDSTRGSRST QQTRRPSSTTRPTTSSSRPETTRTSSTTTPTPSRSGSKK IRLLGATSGEQSSRPKRGFLNLLVWLRKRLRRLLEERRGL
AAV10 MAAP Wild-type	SEQ ID NO:11	LEPPSPRPTSRRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSTTRPTTSSSKRVITRTCGITTPPSFRSVCKK IRLLGATSGEQSSRPGRGFSNLSVWLRKLLRRLLERRDR
AAV11 MAAP Wild-type	SEQ ID NO:12	LEPRSPRPTSRRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSTTRPTTSSSKRVITRTCGITTPPSFRSVCKK IRLLGATSGEQSSRPGRGYSNLSVWLRKVLKRLLERRDR
AAV12 MAAP Wild-type	SEQ ID NO:13	LELHNPRPTNSIRTTAGVLCFLGTSTSDPSTDSTRESRST RQTPRPSSTTRPTTSSSRGTTRISSTTTPTPSSSAWRP TPLLGATSGEQSSRPKRGFSSLVWLRKALKRLLENNAH
AAV13 MAAP Wild-type	SEQ ID NO:14	LEPLNPRQINNIRTTAGVLCFRVTNTSDPATDLTRGNPST QRTQRPSTTRPTTSSSRPVTTPTSSTTTPTPSFRSVFCKK IRLLGATSGEQSSRPKRGSLSLVWLRKRLRRLLEKRD

AAVrh8 MAAP Wild-type	SEQ ID NO:15	LEPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSSSTTKPTTSSSKRV TIRTCGIITPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLERRDR
AAVrh10 MAAP Wild-type	SEQ ID NO:16	LEPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSSSTTRPTTSSSKRV TIRTCGIITPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLERRDR
AAV6 Variant MAAP6-L1P- T2C	SEQ ID NO:17	PEPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPST RRMQRPSSSTTRPTTSSSKRV TIRTCGIITPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant MAAP6- W19stop- G56A	SEQ ID NO:18	LEPRNPKPTSKSRTTAGV-CFLATSTSDPSTDSTRGSPST RRMQRPSSSTTRPTTSSSKRV TIRTCGIITPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant MAAP6- C20S-G59C	SEQ ID NO:19	LEPRNPKPTSKSRTTAGVWSFLATSTSDPSTDSTRGSPST RRMQRPSSSTTRPTTSSSKRV TIRTCGIITPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant MAAP6- C20stop- C60A	SEQ ID NO:20	LEPRNPKPTSKSRTTAGVW-FLATSTSDPSTDSTRGSPST RRMQRPSSSTTRPTTSSSKRV TIRTCGIITPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant MAAP6- S27stop-C80A	SEQ ID NO:21	LEPRNPKPTSKSRTTAGVWCFLATST-DPSTDSTRGSPST RRMQRPSSSTTRPTTSSSKRV TIRTCGIITPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant MAAP6- S57stop- C170A	SEQ ID NO:22	LEPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPST RRMQRPSSSTTRPTTSS-KRV TIRTCGIITPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant	SEQ ID NO:23	LEPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPST RRMQRPSSSTTRPTTSSSKRV TIRTCGIITPTPSFRSVCKK

MAAP6-S88stop-C263A		IRLLGAT-GEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant MAAP6-L1P-T2C / S57stop-C170A	SEQ ID NO:24	PEPRNPKPTSKSRRTTAGVWCFLATSTSDPSTDSTRGSPST RRMQRPSSSTRPTTSS-KRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant C20S-G59C / S27stop-C80A	SEQ ID NO:25	LEPRNPKPTSKSRRTTAGVWSFLATST-DPSTDSTRGSPST RRMQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant C20S-G59C / S57stop-C170A	SEQ ID NO:26	LEPRNPKPTSKSRRTTAGVWSFLATSTSDPSTDSTRGSPST RRMQRPSSSTRPTTSS-KRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant C20S-G59C / S88stop-C263A	SEQ ID NO:27	LEPRNPKPTSKSRRTTAGVWSFLATSTSDPSTDSTRGSPST RRMQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGAT-GEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant C20Y-G59A	SEQ ID NO:28	LEPRNPKPTSKSRRTTAGVWYFLATSTSDPSTDSTRGSPST RRMQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant C20F-G59T	SEQ ID NO:29	LEPRNPKPTSKSRRTTAGVWFFLATSTSDPSTDSTRGSPST RRMQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV6 Variant L1P-T2C / C20S-G59C	SEQ ID NO:30	PEPRNPKPTSKSRRTTAGVWSFLATSTSDPSTDSTRGSPST RRMQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRRGFSNLLVWLRKVLRRLLERNVR
AAV2 Variant L19stop-T56A	SEQ ID NO:31	LAHHHQSPQSGIRTTAGV-CFLGTSTSDPSTDSTRESRST RQTPRPSSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGATSDQSSRRKRGFLNLWAWLRNLLRRLREKGR

AAV2 Variant C20S-G59C	SEQ ID NO:32	LAHHHQSPQSGIRTTAGVLSFLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant C20stop- C60A	SEQ ID NO:33	LAHHHQSPQSGIRTTAGVL-FLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant S27stop-C80A	SEQ ID NO:34	LAHHHQSPQSGIRTTAGVLCFLGTST-DPSTDSTRESRST RQTPRPSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant S57stop- C170A	SEQ ID NO:35	LAHHHQSPQSGIRTTAGVLCFLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGS-TAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant S88stop- C263A	SEQ ID NO:36	LAHHHQSPQSGIRTTAGVLCFLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGAT-DEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant L1P-T2C	SEQ ID NO:37	PAHHHQSPQSGIRTTAGVLCFLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant L1P-T2C / S57stop- C170A	SEQ ID NO:38	PAHHHQSPQSGIRTTAGVLCFLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGS-TAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant C20S-G59C / S27stop-C80A	SEQ ID NO:39	LAHHHQSPQSGIRTTAGVLSFLGTST-DPSTDSTRESRST RQTPRPSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant C20S-G59C / S57stop- C170A	SEQ ID NO:40	LAHHHQSPQSGIRTTAGVLSFLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGS-TAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant C20S-G59C /	SEQ ID NO:41	LAHHHQSPQSGIRTTAGVLSFLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGAT-DEQSSRRKRGFLNLWAWLRNLLRRLREKRGR

S88stop- C263A		
AAV2 Variant C20Y-G59A	SEQ ID NO:42	LAHHHQSPQSGIRTTAGVLYFLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant C20F-G59T	SEQ ID NO:43	LAHHHQSPQSGIRTTAGVLFLLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV2 Variant L1P-T2C / C20S-G59C	SEQ ID NO:44	PAHHHQSPQSGIRTTAGVLSFLGTSTSDPSTDSTRESRST RQTPRPSSTTKPTTGSSTAETTRTSSTTTPTRSFRSALKK IRLLGATSDEQSSRRKRGFLNLWAWLRNLLRRLREKRGR
AAV6 Variant MAAP6-L1P- T2C	SEQ ID NO:45	CCGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGA CGACGGCCGGGTCTGGTGCTTCCTGGCTACAAGTACCTCG GACCCCTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGA CCAGCAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTAT AACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAG GCCAAGAAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGA AGGTGCTAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant MAAP6- W19stop- G56A	SEQ ID NO:46	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGTCTAGTGCTTCCTGGCTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant MAAP6- C20S-G59C	SEQ ID NO:47	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG

		CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant MAAP6- C20stop- C60A	SEQ ID NO:48	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTGATTCTGGCTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant MAAP6- S27stop-C80A	SEQ ID NO:49	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTAGG ACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant MAAP6- S57stop- C170A	SEQ ID NO:50	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTAAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG

AAV6 Variant MAAP6- S88stop- C263A	SEQ ID NO:51	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGG ACCCCTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTAGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant MAAP6-L1P- T2C / S57stop- C170A	SEQ ID NO:52	CCGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGG ACCCCTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTAAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant C20S-G59C / S27stop-C80A	SEQ ID NO:53	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTCCTTCCTGGCTACAAGTACCTAGG ACCCCTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant C20S-G59C / S57stop- C170A	SEQ ID NO:54	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTCCTTCCTGGCTACAAGTACCTCGG ACCCCTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTAAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG

		AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant C20S-G59C / S88stop- C263A	SEQ ID NO:55	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTCCTTCCTGGCTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTAGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant C20Y-G59A	SEQ ID NO:56	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTACTTCCTGGCTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV6 Variant C20F-G59T	SEQ ID NO:57	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTTCTTCCTGGCTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG

AAV6 Variant L1P-T2C / C20S-G59C	SEQ ID NO:58	CCGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGAC GACGGCCGGGGTCTGGTCCTTCCTGGCTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGG CGGATGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAG CAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCA CGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAG AAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGGAAGGTGC TAAGACGGCTCCTGGAAAGAAACGTCCGGTAG
AAV2 Variant L19stop-T56A	SEQ ID NO:59	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTAGTGCTTCCTGGGTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant C20S-G59C	SEQ ID NO:60	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTCTTCCTGGGTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant C20stop- C60A	SEQ ID NO:61	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTGATTCTGGGTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT

		CTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant S27stop-C80A	SEQ ID NO:62	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTAGG ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant S57stop- C170A	SEQ ID NO:63	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTAGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant S88stop- C263A	SEQ ID NO:64	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTTGGGGGCAACCTAGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant L1P-T2C	SEQ ID NO:65	CCGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG

		CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant L1P-T2C / S57stop- C170A	SEQ ID NO:66	CCGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTCGG ACCCCTCAACGGA CTGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTAGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant C20S-G59C / S27stop-C80A	SEQ ID NO:67	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTCTTCCTGGGTACAAGTACCTAGG ACCCCTCAACGGA CTGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant C20S-G59C / S57stop- C170A	SEQ ID NO:68	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTCTTCCTGGGTACAAGTACCTCGG ACCCCTCAACGGA CTGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTAGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant	SEQ ID NO:69	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTCTTCCTGGGTACAAGTACCTCGG

C20S-G59C / S88stop- C263A		ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTGGGGGCAACCTAGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant C20Y-G59A	SEQ ID NO:70	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTACTTCCTGGGTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant C20F-G59T	SEQ ID NO:71	CTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTCTTCCTGGGTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV2 Variant L1P-T2C / C20S-G59C	SEQ ID NO:72	CCGGCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGAC GACAGCAGGGGTCTTGTCTTCCTGGGTACAAGTACCTCGG ACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGG CAGACGCCGCGGCCCTCGAGCACGACAAAGCCTACGACCGG CAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCA CGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT CTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAA AAGAGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGT

		TAAGACGGCTCCGGGAAAAAAGAGGCCGGTAG
AAV5 Variant MAAP L19- stop-T56A	SEQ ID NO:73	RAHRNQNPISSIKIKPVV-CCLVITISDPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFRPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant MAAP C20S- G59C	SEQ ID NO:74	RAHRNQNPISSIKIKPVVL S CCLVITISDPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFRPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant MAAP C20S- G59C / C21S- G62C	SEQ ID NO:75	RAHRNQNPISSIKIKPVVL SS LVITISDPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFRPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant MAAP S27stop- C80A	SEQ ID NO:76	RAHRNQNPISSIKIKPVVLCCLVITI-DPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFRPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant MAAP L57stop- T170A	SEQ ID NO:77	RAHRNQNPISSIKIKPVVLCCLVITISDPETVSIEESLS TGQTRSRESTTSRTTSS-RRETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFRPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant MAAP S88stop- C263A	SEQ ID NO:78	RAHRNQNPISSIKIKPVVLCCLVITISDPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGET-ERQSFRPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant MAAP C21S- G62C	SEQ ID NO:79	RAHRNQNPISSIKIKPVVLC S LVITISDPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFRPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant	SEQ ID NO:80	RAHRNQNPISSIKIKPVVL S CCLVITI-DPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFRPRKGF SNLLAWLKRVLRRPLPESG

C20S-G59C / S27stop- C80A		
AAV5 Variant C20S-G59C / L57stop- T170A	SEQ ID NO:81	RAHRNQNPISSIKIKPVVL S CLVITISDPETVSIEESLS TGQTRSRESTTSRTTSS- R RETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant C20S-G59C / S88stop- C263A	SEQ ID NO:82	RAHRNQNPISSIKIKPVVL S CLVITISDPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGET- E RQSFPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant C20Y-G59A	SEQ ID NO:83	RAHRNQNPISSIKIKPVVL Y CLVITISDPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant C20F-G59T	SEQ ID NO:84	RAHRNQNPISSIKIKPVVL F CLVITISDPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant C20S-G59C / C21S-G62C / S27stop- C80A	SEQ ID NO:85	RAHRNQNPISSIKIKPVVL SS LVITI- D PETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant C20S-G59C / C21S-G62C / L57stop- T170A	SEQ ID NO:86	RAHRNQNPISSIKIKPVVL SS LVITISDPETVSIEESLS TGQTRSRESTTSRTTSS- R RETTPTSSTTTTRTPSFRRSS PTTHPSGETSERQSFPRKGF SNLLAWLKRVLRRPLPESG
AAV5 Variant C20S-G59C / C21S-G62C / S88stop- C263A	SEQ ID NO:87	RAHRNQNPISSIKIKPVVL SS LVITISDPETVSIEESLS TGQTRSRESTTSRTTSSLRRETTPTSSTTTTRTPSFRRSS PTTHPSGET- E RQSFPRKGF SNLLAWLKRVLRRPLPESG

AAV5 Variant MAAP C20S- G59C	SEQ ID NO:88	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTCC TGCCTGGTTATAACTATCTCGGACCCGGAACGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCACGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGAAAGCGGATAG
AAV5 Variant MAAP L19- stop-T56A	SEQ ID NO:89	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTAGTGC TGCCTGGTTATAACTATCTCGGACCCGGAACGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCACGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGAAAGCGGATAG
AAV5 Variant MAAP C20S- G59C / C21S- G62C	SEQ ID NO:90	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTCC TCCCTGGTTATAACTATCTCGGACCCGGAACGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCACGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGAAAGCGGATAG
AAV5 Variant MAAP S27stop- C80A	SEQ ID NO:91	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTGC TGCCTGGTTATAACTATCTAGGACCCGGAACGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCACGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGAAAGCGGATAG
AAV5 Variant MAAP L57stop- T170A	SEQ ID NO:92	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTGC TGCCTGGTTATAACTATCTCGGACCCGGAACGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCACGACATCTCGTACAACGAGCAGCTAGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGAAAGCGGATAG
AAV5 Variant MAAP S88stop- C263A	SEQ ID NO:93	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTGC TGCCTGGTTATAACTATCTCGGACCCGGAACGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCACGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTAGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGAAAGCGGATAG

AAV5 Variant MAAP C21S- G62C	SEQ ID NO:94	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTGC TCCCTGGTTATAACTATCTCGGACCCGGAACCGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCAGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGGAAAGCGGATAG
AAV5 Variant C20S-G59C / S27stop- C80A	SEQ ID NO:95	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTCC TGCCTGGTTATAACTATCTAGGACCCGGAACCGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCAGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGGAAAGCGGATAG
AAV5 Variant C20S-G59C / L57stop- T170A	SEQ ID NO:96	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTCC TGCCTGGTTATAACTATCTCGGACCCGGAACCGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCAGACATCTCGTACAACGAGCAGCTAGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGGAAAGCGGATAG
AAV5 Variant C20S-G59C / S88stop- C263A	SEQ ID NO:97	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTCC TGCCTGGTTATAACTATCTCGGACCCGGAACCGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCAGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTAGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGGAAAGCGGATAG
AAV5 Variant C20Y-G59A	SEQ ID NO:98	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTAC TGCCTGGTTATAACTATCTCGGACCCGGAACCGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCAGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGGAAAGCGGATAG
AAV5 Variant C20F-G59T	SEQ ID NO:99	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTTC TGCCTGGTTATAACTATCTCGGACCCGGAACCGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCAGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGGAAAGCGGATAG
AAV5 Variant	SEQ ID NO:100	CGGGCCCACCGAAACCAAAACCCAATCAGCAGCATCAAGATCAAGCCCGTGGTCTTGTCC TCCCTGGTTATAACTATCTAGGACCCGGAACCGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTCGCGCGAGAGCAGACATCTCGTACAACGAGCAGCTTGAGGCGGGAG

C20S-G59C / C21S-G62C / S27stop- C80A		ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGGAAAGCGGATAG
AAV5 Variant C20S-G59C / C21S-G62C / L57stop- T170A	SEQ ID NO:101	CGGGCCCACCGAAACCAAAACCAATCAGCAGCATCAAGATCAAGCCCCTGGTCTTGTCC TCCCTGGTTATAACTATCTCGGACCCGAAACGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTGCGCGAGAGCACGACATCTCGTACAACGAGCAGCTAGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGGAAAGCGGATAG
AAV5 Variant C20S-G59C / C21S-G62C / S88stop- C263A	SEQ ID NO:102	CGGGCCCACCGAAACCAAAACCAATCAGCAGCATCAAGATCAAGCCCCTGGTCTTGTCC TCCCTGGTTATAACTATCTCGGACCCGAAACGGTCTCGATCGAGGAGAGCCTGTCAACA GGGCAGACGAGGTGCGCGAGAGCACGACATCTCGTACAACGAGCAGCTTAGAGGCGGGAG ACAACCCCTACCTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACG ACACATCCTTCGGGGGAAACCTAGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCG AACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCTACCGGAAAGCGGATAG
AAV8 Variant MAAP8- W19stop- G56A	SEQ ID NO:103	LEPRSPKPTSKSRTTAGV-CFLATSTSDPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGATSGEQSSRPR SGFSNLSVWLRKALRRLLERRDR
AAV8 Variant MAAP8-C20S- G59C	SEQ ID NO:104	LEPRSPKPTSKSRTTAGVW S F L ATSTSDPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGATSGEQSSRPR SGFSNLSVWLRKALRRLLERRDR
AAV8 Variant MAAP8- MAAP8-L1P- T2C	SEQ ID NO:105	P EPRSPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGATSGEQSSRPR SGFSNLSVWLRKALRRLLERRDR
AAV8 Variant	SEQ ID NO:106	LEPRSPKPTSKSRTTAGVWCFLATST-DPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGATSGEQSSRPR SGFSNLSVWLRKALRRLLERRDR

MAAP8-S27stop-C80A		
AAV8 Variant MAAP8- L1P-T2C / C20S-G59C	SEQ ID NO:107	PEPRSPKPTSKSRRTTAGVW S FLATSTSDPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGATSGEQSSRP SGFSNLSVWLRKALRRLLEERRDR
AAV8 Variant MAAP8-S88stop-C263A	SEQ ID NO:108	LEPRSPKPTSKSRRTTAGVW S FLATSTSDPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGAT-GEQSSRP SGFSNLSVWLRKALRRLLEERRDR
AAV8 Variant MAAP8- L1P-T2C / S27stop-C80A	SEQ ID NO:109	PEPRSPKPTSKSRRTTAGVW S FLATST-DPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGATSGEQSSRP SGFSNLSVWLRKALRRLLEERRDR
AAV8 Variant C20S-G59C / S27stop-C80A	SEQ ID NO:110	LEPRSPKPTSKSRRTTAGVW S FLATST-DPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGATSGEQSSRP SGFSNLSVWLRKALRRLLEERRDR
AAV8 Variant L1P-T2C / S88stop-C263A	SEQ ID NO:111	PEPRSPKPTSKSRRTTAGVW S FLATSTSDPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGAT-GEQSSRP SGFSNLSVWLRKALRRLLEERRDR
AAV8 Variant C20S-G59C / S88stop-C263A	SEQ ID NO:112	LEPRSPKPTSKSRRTTAGVW S FLATSTSDPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGAT-GEQSSRP SGFSNLSVWLRKALRRLLEERRDR
AAV8 Variant C20Y-G59A	SEQ ID NO:113	LEPRSPKPTSKSRRTTAGVW Y FLATSTSDPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGATSGEQSSRP SGFSNLSVWLRKALRRLLEERRDR

AAV8 Variant C20F-G59T	SEQ ID NO:114	LEPRSPKPTSKSRRTTAGVWFFLATSTSDPSTDSTRGSPSTRRTQRPSS TTRPTTSSCRRVTIRTCGITTPTPSFRSVCKKIRLLGATSGEQSSRPR SGFSNLSVWLRKALRRLRLERDR
AAV8 Variant MAAP8-L1P- T2C	SEQ ID NO:115	CCGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTG TTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTGGGGGCAACCTCGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGAAAGAAGAGACCGGTAG
AAV8 Variant MAAP8- W19stop- G56A	SEQ ID NO:116	CTGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTAGTG TTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTGGGGGCAACCTCGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGAAAGAAGAGACCGGTAG
AAV8 Variant MAAP8-C20S- G59C	SEQ ID NO:117	CTGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTCC TTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTGGGGGCAACCTCGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGAAAGAAGAGACCGGTAG
AAV8 Variant MAAP8- S27stop- C80A	SEQ ID NO:118	CTGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTG TTCCTGGCTACAAGTACCTAGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTGGGGGCAACCTCGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGAAAGAAGAGACCGGTAG
AAV8 Variant MAAP8- S88stop- C263A	SEQ ID NO:119	CTGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTG TTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTGGGGGCAACCTAGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGAAAGAAGAGACCGGTAG
AAV8 Variant MAAP8-L1P- T2C /	SEQ ID NO:120	CCGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTG TTCCTGGCTACAAGTACCTAGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTGGGGGCAACCTCGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGAAAGAAGAGACCGGTAG

S27stop- C80A		
AAV8 Variant C20S-G59C / S27stop- C80A	SEQ ID NO:121	CTGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTCC TTCCTGGCTACAAGTACCTAGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAV8 Variant L1P-T2C / S88stop- C263A	SEQ ID NO:122	CCGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTAGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAV8 Variant C20S-G59C / S88stop- C263A	SEQ ID NO:123	CTGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTCC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTAGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAV8 Variant C20Y-G59A	SEQ ID NO:124	CTGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTAC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAV8 Variant C20F-G59T	SEQ ID NO:125	CTGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTTC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAV8 Variant L1P-T2C / C20S-G59C	SEQ ID NO:126	CCGGAGCCCCGAAGCCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTCC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGACAAGGCCTACGACCAGCAGCTGCAGGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG

AAVRH10 Variant MAAP W19stop- G56A	SEQ ID NO:127	LEPRNPKPTSKSRTTAGV-CFLATSTSDPSTDSTRGSPST RRTQRPSSSTTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant MAAP C20S- G59C	SEQ ID NO:128	LEPRNPKPTSKSRTTAGVW S F L ATSTSDPSTDSTRGSPST RRTQRPSSSTTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant MAAP L1P- T2C	SEQ ID NO:129	P EPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSSSTTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant MAAP S27stop- C80A	SEQ ID NO:130	LEPRNPKPTSKSRTTAGVWCFLATST-DPSTDSTRGSPST RRTQRPSSSTTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant MAAP S57stop- C170A	SEQ ID NO:131	LEPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSSSTTRPTTSS-KRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant MAAP S88stop- C263A	SEQ ID NO:132	LEPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSSSTTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGAT-GEQSSRPRSGFSNLSVWLRKALRRLLEERRDR

AAVRH10 Variant MAAP L1P- T2C / S57stop- C170A	SEQ ID NO:133	PEPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSSSTRPTTSS-KRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant C20S-G59C / S27stop- C80A	SEQ ID NO:134	LEPRNPKPTSKSRTTAGVW S FLATST-DPSTDSTRGSPST RRTQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant C20S-G59C / S57stop- C170A	SEQ ID NO:135	LEPRNPKPTSKSRTTAGVW S FLATSTSDPSTDSTRGSPST RRTQRPSSSTRPTTSS-KRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant C20S-G59C / S88stop- C263A	SEQ ID NO:136	LEPRNPKPTSKSRTTAGVW S FLATSTSDPSTDSTRGSPST RRTQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGAT-GEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant C20Y-G59A	SEQ ID NO:137	LEPRNPKPTSKSRTTAGVW Y FLATSTSDPSTDSTRGSPST RRTQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant C20F-G59T	SEQ ID NO:138	LEPRNPKPTSKSRTTAGVW F FLATSTSDPSTDSTRGSPST RRTQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR
AAVRH10 Variant L1P-T2C / C20S-G59C	SEQ ID NO:139	PEPRNPKPTSKSRTTAGVW S FLATSTSDPSTDSTRGSPST RRTQRPSSSTRPTTSSSKRVTIRTCGITTPTPSFRSVCKK IRLLGATSGEQSSRPRSGFSNLSVWLRKALRRLLEERRDR

AAVRH10 Variant L1P-T2C / S88stop- C263A	SEQ ID NO:140	PEPRNPKPTSKSRTTAGVWCFLATSTSDPSTDSTRGSPST RRTQRPSSSTTRPTTSSSKRVTIIRTCGITTPTPSFRSVCKK IRLLGAT-GEQSSRPRSGFSNLSVWLRKALRRLLERRDR
AAVRH10 Variant MAAP L1P- T2C	SEQ ID NO:141	CCGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGCACAAGGCCTACGACCAGCAGCTCAAAGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAGAAGAGACCGGTAG
AAVRH10 Variant MAAP W19stop- G56A	SEQ ID NO:142	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTAGTGC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGCACAAGGCCTACGACCAGCAGCTCAAAGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAGAAGAGACCGGTAG
AAVRH10 Variant MAAP C20S- G59C	SEQ ID NO:143	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTCC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGCACAAGGCCTACGACCAGCAGCTCAAAGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAGAAGAGACCGGTAG
AAVRH10 Variant MAAP L1P- T2C / S88stop- C263A	SEQ ID NO:144	CCGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGCACAAGGCCTACGACCAGCAGCTCAAAGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTAGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAGAAGAGACCGGTAG
AAVRH10 Variant	SEQ ID NO:145	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGC TTCCTGGCTACAAGTACCTAGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGCACAAGGCCTACGACCAGCAGCTCAAAGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTCCAGGCCAAGAAGCGGGTTCTCG

MAAP S27stop- C80A		AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAVRH10 Variant MAAP S57stop- C170A	SEQ ID NO:146	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTAAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAVRH10 Variant MAAP S88stop- C263A	SEQ ID NO:147	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTAGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAVRH10 Variant MAAP L1P- T2C / S57stop- C170A	SEQ ID NO:148	CCGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTAAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAVRH10 Variant C20S-G59C / S27stop- C80A	SEQ ID NO:149	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTCCCTTCCTGGCTACAAGTACCTAGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAVRH10 Variant C20S-G59C / S57stop- C170A	SEQ ID NO:150	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTCCCTTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGACGCAGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTAAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCGAACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG

AAVRH10 Variant C20S-G59C / S88stop- C263A	SEQ ID NO:151	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTCC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGACAAGGCCTACGACCAGCAGCTCAAAGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTAGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAVRH10 Variant C20Y-G59A	SEQ ID NO:152	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTAC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGACAAGGCCTACGACCAGCAGCTCAAAGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAVRH10 Variant C20F-G59T	SEQ ID NO:153	CTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTCC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGACAAGGCCTACGACCAGCAGCTCAAAGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG
AAVRH10 Variant L1P-T2C / C20S-G59C	SEQ ID NO:154	CCGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGTCTGGTCC TTCCTGGCTACAAGTACCTCGGACCCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACG CGGCGGACGCAGCGGCCCTCGAGCAGACAAGGCCTACGACCAGCAGCTCAAAGCGGGTG ACAATCCGTACCTGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAG ATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAGAGACCGGTAG

[0071] Mutations at corresponding locations in, for example homologous MAAP polypeptides (which can be located in a AAV capsid polypeptide), would be expected to have similar effects on viral function. Corresponding locations can include, for example, a location relative to the consensus MAAP polypeptide sequence (e.g., SEQ ID NO: 1) or indeed any of SEQ ID NOs:2-16). In one aspect, a variant MAAP polypeptide or nucleic acid sequence encoding the variant MAAP polypeptide is provided herein that bears a mutation that corresponds to a mutation of the polypeptide of any of SEQ ID NOs:17-44, 73-87, 103-114 or 127-140, as described herein. In one embodiment, a homologous MAAP polypeptide or nucleic acid sequence encoding a MAAP polypeptide that is encoded in or included in an AAV genome or vector of the present invention has at least 70%, at least 75%, at least 80%, at least 85%, at

least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, or at least 98%, sequence identity to a consensus MAAP sequence (i.e., SEQ ID NO:1) or AAV MAAP wild-type sequence (i.e., SEQ ID NOs:2-16), with the proviso that the MAAP polypeptide sequence or nucleic acid sequence encoding the variant MAAP polypeptide comprises or consists of a one or more substitutions or mutations as described above. In another embodiment, the MAAP polypeptides of AAV serotypes 1, 3-5 and 7-13 are homologous to an AAV6 or AAV2 capsid polypeptide. In still further embodiments, the present invention provides viral genomes and vectors comprising a variant MAAP nucleic acid sequence that has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the variant nucleic acid sequences listed in Table 1, with the proviso that the variant sequences encode, comprise or consist of one or more of the mutations described above (i.e., a stop codon substitution at a codon position corresponding to amino positions W19, C20, S27, S57 and S88, an amino acid substitution at position L1, an amino acid substitution at position C20, an amino acid substitution at position C21, and combinations thereof).

[0072] One aspect described herein is a viral capsid polypeptide comprising a region corresponding to the amino acid sequence of SEQ ID NO: 1, 3, 6, 7, 9 or 16, wherein the region corresponding to the amino acid sequence of SEQ ID NO: 1, 3, 6, 7, 9 or 16 comprises a mutation relative to SEQ ID NO: 1, 3, 6, 7, 9 or 16 as described above. In one embodiment, a homologous viral capsid polypeptide has a region with at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more sequence identity to SEQ ID NO: 1, 3, 6, 7, 9 or 16, with the proviso that region comprises one or more substitutions as described above.

[0073] As noted elsewhere herein, the identification of amino acid sites or regions of amino acid sequence in the AAV2, AAV5, AAV6, AAV8 and AAVrh10 capsid polypeptides that tolerate change in general, i.e., that permit functions of the viral vector can be used to guide changes in the capsid polypeptides of other AAV serotypes to similarly influence function or provide for modified properties. Thus, through use of sequence alignment, a mutation or set of mutations that provides a desired change in function for AAV2, AAV5, AAV6, AAV8 or

AAVrh10 can be introduced to the corresponding location of the capsid polypeptide of another AAV serotype to similarly influence those properties in that serotype.

[0074] As used herein, “mutation” refers to any change in the amino acid sequence, e.g., a substitution, insertion, or deletion of at least one amino acid. Site-directed mutations in the nucleic acid sequence can be generated by one skilled in the art using techniques known in the art or described herein.

[0075] Mutations that are at equivalent positions in other homologous viruses can be made. Examples of other homologous viruses include any of AAV serotypes 1 to 13, as well as other natural isolates or synthetic sequences. Corresponding positions in homologous viruses can be inferred from sequence homology to AAV2, AAV5, AAV6, AAV8 or AAVrh10. In one embodiment, the mutations described herein are introduced into the corresponding amino acid sequence of an AAV1, AAV3, AAV3B, AAV4, AAV7, AAV9, AAV10, AAV11, AAV 12, AAV 13, or AAVrh8 capsid polypeptide.

[0076] One aspect of the technology described herein is a nucleic acid, e.g., a polynucleotide encoding any of the variant viral capsid polypeptides described herein. One skilled in the art can alter the wild-type MAAP sequence (e.g., SEQ ID NOs. 2-16), e.g., by introducing at least one base pair substitution, such that the altered sequence encodes a variant amino acid sequence. The nucleic acid sequence for each codon is known in the art. For example, to alter an amino acid from an isoleucine (which is translated by the nucleotide sequence AUU) to valine (which is translated by the nucleotide sequence GUU), one would change the first nucleotide from an A to G. As another example, the nucleic acid sequence may be altered to introduce a stop codon by substitution of nucleotides in a codon to produce the stop codon. Site-directed mutagenesis is known in the art and can be used to introduce a point mutation(s) (e.g., amino acid substitutions, insertions, or deletions) or other mutations or combinations thereof to a viral capsid polypeptide. Site-directed mutagenesis is further described in, e.g., Li B, et al. Hum Gene Ther Methods. 2015 Dec; 26(6):211-20, and Bachman, J. Methods Enzymol. 2013; 529:241-248, which are incorporated herein by reference in their entireties.

[0077] In some embodiments, a polynucleotide encoding a variant viral capsid polypeptide described herein is operably linked to a promoter sequence. For example, a promoter sequence that drives expression of the polynucleotide in a cell. In some embodiments, the polynucleotide further comprises a sequence that encodes a REP protein, e.g., an AAV rep protein. In some

embodiments, the polynucleotide further comprises a sequence that encodes a CAP protein, e.g., an AAV CAP protein.

[0078] Another aspect of the technology described herein is a viral particle comprising any of the viral capsid polypeptides described herein. It is noted that a viral particle comprising a variant capsid polypeptides described herein can be replication-competent or replication-incompetent. Thus, in some embodiments, the viral particle comprising a variant capsid polypeptides described herein is replication competent. As used herein, the term “replication competent” refers to a virus or viral particle that is infectious, and is also capable of being replicated in an infected cell (i.e., in the presence of a helper virus or helper virus functions). In the case of AAV, replication competence generally requires the presence of functional AAV packaging genes.

[0079] In some other embodiments, the viral particle comprising a variant capsid polypeptide described herein is replication incompetent or replication defective. As used herein, the terms “replication incompetent” and “replication defective” refer to a virus or viral particle that cannot independently replicate and package its genome. For example, when a cell is infected with the virus or viral particle, the heterologous gene is expressed in the infected cells, however, the virus or the viral particle is not able to replicate further.

[0080] In some embodiments, the viral particle further comprises a polynucleotide encoding a transgene, e.g., a polynucleotide comprising a sequence that encodes a gene product such as a therapeutic gene product. In some embodiments, the polynucleotide is flanked on the 5' and 3' ends by functional AAV inverted terminal repeat (ITR) sequences. By “functional AAV ITR sequences” is meant that the ITR sequences function as intended for the rescue, replication, and packaging of the AAV virion. Hence, AAV ITRs for use in the gene delivery vectors need not have a wild-type nucleotide sequence, and can be altered by the insertion, deletion, or substitution of nucleotides or the AAV ITRs can be derived from any of several AAV serotypes, e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, etc. In some embodiments, the viral particles have the wild type *Rep* and *Cap* genes deleted in whole or part, but retain functional flanking ITR sequences.

[0081] The transgene may be any suitable gene or GOI. If the vector plasmid is for use in gene therapy, the transgene may be any gene that comprises or encodes a protein or nucleotide sequence that can be used to treat a disease. For example, the transgene may encode an enzyme,

a metabolic protein, a signaling protein, an antibody, an antibody fragment, an antibody like protein, an antigen, or a non-translated RNA such as an miRNA, siRNA, snRNA, or antisense RNA. In some embodiments, the GOI may encode all or a portion of a gene editing system, such as Cas9, an sgRNA and donor template DNA. In some preferred embodiments, a dual AAV system is utilized for CRISPR gene editing where CAS9 is included as the GOI in one GOI plasmid and sgRNA and donor template DNA as the GOI in a second GOI plasmid.

[0082] One skilled in the art can make viral particles comprising a variant capsid polypeptide described herein using standard methods. For example, an AAV expression vector comprising a polynucleotide cassette can be introduced into a producer cell, followed by introduction of an AAV helper construct comprising a polynucleotide sequence encoding a variant capsid polypeptide described herein, and where the helper construct includes AAV coding regions capable of being expressed in the producer cell and which complement AAV helper functions absent in the AAV vector. This is followed by introduction of helper virus and/or additional vectors into the producer cell, wherein the helper virus and/or additional vectors provide accessory functions capable of supporting efficient rAAV virus production. The producer cells are then cultured to produce rAAV. Some exemplary methods for producing viral particles and amenable to the present invention are described, for example, in U.S. Pat. Nos. 5,436,146; 5,753,500, 6,040,183, 6,093,570 and 6,548,286, contents of each of which is incorporated herein by reference in their entireties.

[0083] In one example, viral particles comprising a variant polypeptide described herein can be produced by first generating a DNA library of AAV capsid variants. By way of non-limiting example, libraries of AAV6 capsid gene sequence variants are cloned into a plasmid containing the AAV Inverted Terminal Repeat regions (ITRs). The final ITR plasmids contain, e.g., a cytomegalovirus (CMV) promoter upstream of the *Cap* gene. AAV virus libraries are produced from the DNA libraries. The capsid library plasmids, AAV pHelper plasmids, and plasmids containing, for example, the AAV2 *Rep* gene are, for example, co-transfected into HEK-293T cells using PEI. Capsids are purified using standard techniques for cell lysis (freeze-thaw or addition of 5 M NaCl), treatment with benzonase to remove unpackaged genomes, and purification and concentration by iodixanol ultra centrifugation.

[0084] In preparing the viral particles, any host cells for producing recombinant viral particles can be employed, including, for example, mammalian cells, insect cells, plant cells,

microorganisms and yeast. Host cells can also be packaging cells in which the AAV rep and cap genes are stably maintained in the host cell or producer cells in which the AAV vector genome is stably maintained and packaged. Exemplary packaging and producer cells include but are not limited to HeLa cell, COS cell, COS-1 cell, COS-7 cell, HEK293 cell, A549 cell, BHK cell, BSC-1 cell, BSC-40 cell, Vero cell, Sfc9 cell, Sf -21 cell, Tn-368 cell, BTI-Tn-5B1- 4 (High-Five) cell, Saos cell, C2C12 cell, L cell, HT1080 cell, HepG2 cell, WEHI cell, 3T3 cell, 10T1/2 cell, MDCK cell, BMT-10 cell, WI38 cell, or primary fibroblast, hepatocyte or myoblast cell derived from mammals.

[0085] Also provided herein is a cell comprising a variant viral capsid polypeptide described herein or a polynucleotide encoding a variant viral capsid polypeptide described herein. The cell can be prokaryotic cell or eukaryotic cell. For example, the cell can be a mammalian cell, insect cell, plant cell, bacterial cell, or yeast cell. Some exemplary cells include, but are not limited to, alveolar cells, basophils, cardiac smooth muscle cells), cardiomyocyte, collecting duct intercalated cells, collecting duct principal cells, ectodermal cells, endocardial cells, endoderm cells, eosinophils, epithelial cells, hepatic stellate cells, interstitial kidney cells, intrahepatic lymphocytes, kidney distal tubule cells, kidney glomerulus parietal cells, kidney glomerulus podocytes, kidney proximal tubule brush border cells, loop of Henle thin segment cells, lung epithelial cells, lung smooth muscle cells, lymphocytes, monocytes, muscle cells, neutrophils, non-parenchymal cells, parenchymal cells, phagocytic Kupffer cells, platelets, red blood cells, sinusoidal endothelial cells, splenic endothelial cells, splenic fibroblasts, splenocytes, and thick ascending limb cells.

[0086] In some embodiments, the cell can be a cell used for producing a viral particle, e.g., a producer cell.

[0087] In some embodiments, the cell can be a cell which has been transduced, infected, transfected or transformed with a viral vector described herein. Typically, a cell is referred to as “transduced”, “infected”; “transfected” or “transformed” dependent on the means used for administration, introduction or insertion of heterologous DNA (i.e., the viral vector) into the cell.

[0088] Also provided herein are pharmaceutical compositions comprising a variant capsid polypeptide described herein or a polynucleotide encoding same and one or more pharmaceutically acceptable diluent, carrier, or excipient. For example, the composition can comprise a viral particle comprising a variant capsid polypeptide described herein. In some

embodiments, the composition can comprise a cell, wherein the cell comprises a variant capsid polypeptide described herein or a polynucleotide encoding same.

[0089] For example, the variant capsid polypeptide or a polynucleotide encoding same (as is or as encompassed in a viral particle or cell) can be combined with pharmaceutically- acceptable carriers, diluents, and reagents useful in preparing a formulation that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for primate use. Such excipients can be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous. Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Supplementary active compounds can also be incorporated into the formulations. Solutions or suspensions used for the formulations can include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates; detergents such as Tween 20 to prevent aggregation; and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. In particular embodiments, the pharmaceutical compositions are sterile. For instances in which ocular cells are to be contacted in vivo, the subject polynucleotide cassettes or gene delivery vectors comprising the subject polynucleotide cassette can be treated as appropriate for delivery to the eye.

[0090] Pharmaceutical compositions can further include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, or phosphate buffered saline (PBS). In some cases, the composition is sterile and should be fluid to the extent that easy syringability exists. In certain embodiments, it is stable under the conditions of manufacture and storage and is preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be, e.g., a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of

surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the internal compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0091] Provided herein is a method of delivering a payload to a cell comprising contacting a cell with an AAV virus or viral particle comprising any of the viral capsid polypeptides described herein. In one embodiment, the contacting occurs *in vitro*. In one embodiment, the contacting occurs *ex vivo*.

[0092] The term “contacting” or “contact” as used herein in connection with contacting a cell with an AAV virus or viral particle includes subjecting the cell to an appropriate culture medium which comprises the AAV virus or viral particle. Where the cell is *in vivo*, “contacting” or “contact” includes administering the AAV virus or viral particle in a pharmaceutical composition to a subject via an appropriate administration route such that the virus or viral particle contacts the cell *in vivo*.

[0093] Further provided herein is a method of delivering a payload *in vivo* to a target cell, comprising administering an AAV virus or viral particle comprising any of the viral capsid polypeptides described herein to a subject. In one embodiment, *in vivo* is systemic delivery.

[0094] Exemplary target or host cells include blood cells (e.g., red blood cells, platelets, neutrophils, eosinophils, basophils, lymphocytes, or monocytes); heart cells (e.g., cardiomyocyte, endocardial cells, or cardiac smooth muscle cells); muscle cells; epithelial cells; endoderm cells; ectodermal cells; kidney cells (e.g., kidney glomerulus parietal cells, kidney glomerulus podocytes, kidney proximal tubule brush border cells, loop of Henle thin segment cells, thick ascending limb cells, kidney distal tubule cells, collecting duct principal cells, collecting duct intercalated cells, and interstitial kidney cells); liver cells (e.g., parenchymal cells, non-parenchymal cells, sinusoidal endothelial cells, phagocytic Kupffer cells, hepatic stellate cells, and intrahepatic lymphocytes); lung cells (e.g., lung epithelial cells, lung smooth muscle cells, and alveolar cells); and spleen cells (e.g., splenocytes, splenic endothelial cells and splenic fibroblasts).

[0095] *In vivo* delivery of the AAV virus or viral particle can be, for example, by injection, infusion, instillation, inhalation, or ingestion. "Injection" includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, intrasternal injection and infusion. The AAV virus or viral can be administered as a single bolus or multiple boluses, as a continuous infusion, or a combination thereof. In some embodiments, the AAV virus or viral particle can be administration into the blood stream of the subject.

[0096] The dose of AAV virions or viral particles required to achieve a particular "therapeutic effect," e.g., the units of dose in vector genomes/per kilogram of body weight (vg/kg), will vary based on several factors including, but not limited to: the route of AAV virus or viral particle administration, the level of gene expression required to achieve a therapeutic effect, the specific disease or disorder being treated, a host immune response to the AAV virus or viral particle, a host immune response to the gene expression product, and the stability of the gene product. One of skill in the art can readily determine a AAV virus or viral particle dose range to treat a patient having a particular disease or disorder based on the aforementioned factors, as well as other factors that are well known in the art. Generally speaking, by "therapeutic effect" is meant a level of expression of one or more transgenes in the AAV virus or viral particle sufficient to alter a component of a disease (or disorder) toward a desired outcome or clinical endpoint, such that a patient's disease or disorder shows clinical improvement, often reflected by the amelioration of a clinical sign or symptom relating to the disease or disorder. Exemplary doses for achieving therapeutic effects are AAV virus or viral particle titers of at least about 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , 10^{14} , 10^{15} , 10^{16} transducing units or more. For example, from about 10^8 to about 10^{13} transducing units. It is noted that more than one administration (e.g., two, three, four, or more administrations) can be employed to achieve desired (e.g. therapeutic) levels of gene expression.

Examples

[0097] The following are examples of the present invention and are not to be construed as limiting.

Example 1

[0098] This example provides a comparison of viral particle titer and cell growth for different mutations in the AAV6 MAAP polypeptide.

[0099] **Materials and Methods.**

[0100] **Reagents**

- Production Cell-line 1: Catalent Clonal HEK-293 C135
- Production Cell-line 2: ThermoFisher HEK-293
- AAV plasmids (CAT pRepCap, CAT pHelper, CAT pGOI (gene of interest), CAPEXCEL MAAP CAPSID variants)
- Gibco™ Viral Production Medium (Cat #A4817901, Thermofisher)
- Gibco™ AAV-MAX Transfection Kit (Cat #A50515, Thermofisher)
- Gibco™Expi293™ Expression Medium (Cat #A1435102, Thermofisher)
- PEI-Pro(Cat # 101000033, Polypus)
- POROS™ CaptureSelect™ AAVX Affinity Resin (Cat# A36740, Thermofisher)
- 10 % Lysis Buffer (10% Tween 20, 500 mM Tris Base, 20 mM MgCl₂, pH 8.0 ± 0.1)\
- Benzonase endonuclease (Cat#1016970001, Millipore Sigma)
- 5M Sodium Chloride (NaCl) Solution

[0101] **Upstream AAV viral particles production.** Production cell lines were thawed and expanded at least 5 passages in their respective culture medium. One day prior transfection, cells were passaged to half the target cell densities required for the transfection. On the day of transfection, cells reached their target cell densities for transfection as per the manufacturer's protocol depending on the transfection reagent used for the experiment. Transfections were setup in Erlenmeyer shake flasks. For transfection, a molar ratio of 1:1:1 of pRepCap : pHelper : pGOI plasmid DNAs were used and transfection was performed as per the manufacturer's protocol. Post transfection, cells were put in 37 °C incubator with shaking at 130 rpm to produce AAV viral particles. Transfected cells were sampled everyday post transfection for Cell density, cell viability, and metabolites. Viral particles were harvested 3 days post transfection with 10%v/v lysis buffer in presence of 100 U/mL of Benzonase for 2h, followed by the 30 minutes treatment with 0.5M NaCl 37 °C incubator with shaking at 130 rpm. Lysed cells were then pelleted through centrifugation at 3500 rpm for 15 minutes. Pelleted cells were discarded. Samples were

collected for ddPCR and Capsid ELISA. Rest of the clarified material was further processed to batch purify AAV particles.

[0102] **Downstream Batch purification of AAV viral particles.** For purification of AAV clarified lysate, samples were buffer adjusted to a final NaCl concentration of 0.6M. Samples were then loaded onto equilibrated POROS CaptureSelect AAVX resin. Once bound, samples were washed with equilibration buffer, then wash buffer to remove impurities, then eluted from the AAVX resin and rapidly neutralized. Next, samples were buffer exchanged using molecular weight cutoff filtration, and purified samples were aliquoted and stored at -80°C. Purified aliquots were subsequently submitted for analytical testing for percent full particles using analytical ultracentrifuge (AUC), host cell DNA (hcDNA), CE-SDS capsid protein purity, and TCID50 infectivity assays.

Results and Discussion.

[0103] Viral titer following production in two different production cell lines (Production Cell Line 1 and 2) of vectors comprising the different MAAP variants was compared with production of vectors with Control AAV6 MAAP (CAPSID-STD-AAV6 (STD)). Analysis was performed for viral genome by ddPCR, and viral particles by ELISA. See Tables 2, 3 and 4. As can be seen in Tables 2 and 3, the variant capsids provided for an approximately 4 to 5-fold increase in titer as compared to control. Furthermore, the % of full viral particles was similar by Analytical Ultracentrifugation (AUC) between the mutant and wild-type particles demonstrating that a similar particle quality was maintained.

Table 2. Evaluation of AAV6 MAAP mutants in Production Cell Line 1

Capsid ID	AVG Vector Genome ddPCR (Vg/mL)	Vector Genome Fold Increase	AVG Capsid ELISA (Particles/mL)	Particle Number Fold Increase	% Full Particles By ddPCR/ELISA ratio
AAV6-RepCAPEXCEL-W19 (EX1)	1.49E+11	2.9	3.99E+11	3.2	37
AAV6-RepCAPEXCEL-S27 (EX2)	2.19E+11	4.2	5.35E+11	4.2	41
AAV6-RepCAPEXCEL-S57 (EX3)	1.92E+11	3.7	3.74E+11	3	51
AAV6-RepCAPEXCEL-S88 (EX4)	2.11E+11	4.1	6.50E+11	5.2	32

AAV6-RepCAPEXCEL-C20S (EX5)	1.90E+11	3.7	5.37E+11	4.3	35
AAV6-RepCAPEXCEL-L1P (EX7)	1.12E+11	2.2	2.89E+11	2.3	38.7
AAV6-RepCAPEXCEL-L1P + S57 (EX8)	1.48E+11	2.9	4.69E+11	3.7	31.7
CAPSID-STD-AAV6 (Control)	0.52E+11	1	1.26E+11	1	41.5

Table 3. Evaluation of AAV6 MAAP mutants in Production Cell Line 2

Capsid ID	AVG Vector Genome ddPCR (Vg/mL)	Vector Genome Fold Increase	AVG Capsid ELISA (Particles/mL)	Particle Number Fold Increase	% Full Particles By ddPCR/ ELISA ratio
AAV6-RepCAPEXCEL-W19 (EX1)	5.3E+11	3.3	1.4E+12	3.6	36.9
AAV6-RepCAPEXCEL-S27 (EX2)	6.4E+11	4.0	2.2E+12	5.4	29.6
AAV6-RepCAPEXCEL-S57 (EX3)	6.3E+11	3.9	2.5E+12	6.3	25.2
AAV6-RepCAPEXCEL-S88 (EX4)	6.5E+11	4.0	2.6E+12	6.6	24.8
AAV6-RepCAPEXCEL-C20S (EX5)	6.5E+11	4.1	1.2E+12	3.0	54.2
AAV6-RepCAPEXCEL-L1P (EX7)	6.0E+11	3.7	1.3E+12	3.4	29.8
AAV6-RepCAPEXCEL-L1P + S57 (EX8)	7.1E+11	4.4	2.7E+12	6.9	26.3

CAPSID-STD-AAV6 (Control)	1.6E+11	1.0	4.0E+11	1.0	42.8
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Table 4. Purified AAV data for production of AAV6 in Production Cell Line 1

Mutation Number	% Full by AUC	Upstream ddPCR	Batch Purified ddPCR (vg/mL)	hcDNA (ng/mL)	hcDNA (ng/E13vg)
EX1	-	1.06E+11	3.7E+12	196.22	530.3
EX2	70.2	1.13E+11	4E+12	237.37	593.43
EX3	61.6	1.82E+11	4.42E+12	131.76	298.1
EX4	60.6	1.38E+11	5.05E+12	200.38	396.8
EX5	71.9	1.18E+11	3.03E+12	203.77	672.5
EX7	72.6	1.12E+11	4.29E+12	273.52	637.6
EX8	56.6	1.48E+11	6.59E+11	<6	NA

Example 2

[0104] This example provides data on production of AAV particles of additional serotypes with mutations in the MAAP sequence. The production of viral particles with the mutant MAAP sequences was compared to the production of viral particles with wild-type MAAP sequences. A process flow diagram for viral particle production is provided as FIG. 1.

[0105] The following materials and reagents were utilized:

- AAV plasmids (CAT pRepCap, CAT pHelper, CAT pGOI (gene of interest), CAPEXCEL MAAP CAPSID mutants)
- Catalent Clonal HEK 293 cells (Clone 135 cells) and Gibco™ Viral Production Cells 2.0 (Cat #A51218, Thermofisher)
 - Gibco™ Viral Production Medium (Cat #A4817901, Thermofisher)
 - Gibco™ AAV-MAX Transfection Kit (Cat #A50515, Thermofisher)
 - Gibco™Expi293™ Expression Medium (Cat #A1435102, Thermofisher)
 - PEI-Pro (Cat # 101000033, Polypus)
 - POROS™ CaptureSelect™ AAVX Affinity Resin (Cat# A36740, Thermofisher)

- 10 % Lysis Buffer (10% Tween 20, 500 mM Tris Base, 20 mM MgCl₂, pH 8.0 ± 0.1)
- Benzonase endonuclease (Cat#1016970001, Millipore Sigma)
- 5M Sodium Chloride (NaCl) Solution

Upstream AAV viral particles production. Clone 135 cells or VP 2.0 cells were thawed and expanded at least 5 passages in their respective culture medium (Clone 135 cells in Expi 293 medium and VP2.0 cells in Viral Production Medium). One day prior transfection, cells were passaged to half the target cell densities required for the transfection. On the day of transfection, cells reached their target cell densities for transfection as per the manufacturer's protocol depending on the transfection reagent used for the experiment. Transfections were setup in Erlenmeyer shake flasks. For transfection, a molar ratio of 1:1:1 of RepCap : pHelper : GOI plasmid DNAs were used and transfection was performed as per the manufacturer's protocol. Post transfection, cells were put in 37 °C incubator with shaking at 130 rpm to produce AAV viral particles. Transfected cells were sampled everyday post transfection for Cell density, viability, and metabolites. Viral particles were harvested 3 days post transfection with 10%v/v lysis buffer in presence of 100 U/mL of Benzonase for 2h, followed by the 30 minutes treatment with 0.5M NaCl 37 °C incubator with shaking at 130 rpm. Lysed cells were then pelleted through centrifugation at 3500 rpm for 15 minutes. Pelleted cells were discarded. Samples were collected for ddPCR and Capsid ELISA. Rest of the clarified material was further processed to batch purify AAV particles.

[0106] Downstream Batch purification of AAV viral particles. For purification of AAV clarified lysate, samples were buffer adjusted to a final NaCl concentration of 0.6M. Samples were then loaded onto equilibrated POROS CaptureSelect AAVX resin. Once bound, samples were washed with equilibration buffer, then wash buffer to remove impurities, then eluted from the AAVX resin and rapidly neutralized. Next, samples were buffer exchanged using molecular weight cutoff filtration, and purified samples were aliquoted and stored at -80°C. Purified aliquots were subsequently submitted for analytical testing for percent full particles using analytical ultracentrifuge (AUC), host cell DNA (hcDNA) and TCID₅₀ infectivity assays.

[0107] A flow-diagram of the viral particle production process is provided in FIG. 1.

[0108] Table 5 provides a summary of the mutations that were evaluated in this example.

Table 5: List of CAPEXCEL Mutations for various AAV serotypes

NO.	RepCapexel ID	RepCapexel ID cell ID	VARIANT	Mutation type	SEROTYPE							
					AAV6 [13]	AAV5 [15]	AAV8 [13]	AAVrh10 [14]	AAV2 [13]			
1.	RepCapexel1	Ex1	W19 / I19	Stop codon	Plasmid-1 W19	Plasmid-51 I19	Plasmid-51 W19	Plasmid-101 W19	Plasmid-21 I19			
2.	RepCapexel2	Ex2	S27	Stop codon	Plasmid-2 S27	Plasmid-64 S27	Plasmid-82 S27	Plasmid-102 S27	Plasmid-24 S27			
3.	RepCapexel3	Ex3	S57	Stop codon	Plasmid-3 S57	Plasmid-55 S57		Plasmid-103 S57	Plasmid-25 S57			
4.	RepCapexel4	Ex4	S88	Stop codon	Plasmid-4 S88	Plasmid-56 S88	Plasmid-83 S88	Plasmid-104 S88	Plasmid-26 S88			
5.	RepCapexel5	Ex5	C205	Point mutation	Plasmid-5 C205	Plasmid-52 C205	Plasmid-84 C205	Plasmid-105 C205	Plasmid-23 C205			
6.	RepCapexel7	Ex7	I1P	Start codon	Plasmid-7 I1P		Plasmid-87 I1P	Plasmid-108 I1P	Plasmid-32 I1P			
7.	RepCapexel8	Ex8	I1P+S27	Combination	Plasmid-8 I1P+S27			Plasmid-109 I1P+S27	Plasmid-33 I1P+S27			
8.	RepCapexel9	Ex9	C205+S27	Combination	Plasmid-9 C205+S27	Plasmid-60 C205+S27	Plasmid-91 C205+S27	Plasmid-112 C205+S27	Plasmid-29 C205+S27			
9.	RepCapexel10	Ex10	C205+S57	Combination	Plasmid-10 C205+S57	Plasmid-61 C205+S57		Plasmid-113 C205+S57	Plasmid-30 C205+S57			
10.	RepCapexel11	Ex11	C205+S88	Combination	Plasmid-11 C205+S88	Plasmid-62 C205+S88	Plasmid-92 C205+S88	Plasmid-114 C205+S88	Plasmid-31 C205+S88			
11.	RepCapexel12	Ex12	C20F	Point mutation	Plasmid-12 C20F	Plasmid-58 C20F	Plasmid-85 C20F	Plasmid-106 C20F	Plasmid-27 C20F			
12.	RepCapexel13	Ex13	C20Y	Point mutation	Plasmid-13 C20Y	Plasmid-59 C20Y	Plasmid-86 C20Y	Plasmid-107 C20Y	Plasmid-28 C20Y			
13.	RepCapexel14	Ex14	I1P+C205	Combination	Plasmid-14 I1P+C205		Plasmid-90 I1P+C205	Plasmid-111 I1P+C205	Plasmid-34 I1P+C205			
14.	RepCapexel15	Ex15	C205/C215	Combination		Plasmid-53 C205/C215						
15.	RepCapexel16	Ex16	C215	Point mutation		Plasmid-57 C215						
16.	RepCapexel17	Ex17	C205/C215+S27	Combination		Plasmid-63 C205/C215+S27						
17.	RepCapexel18	Ex18	C205/C215+I1P	Combination		Plasmid-64 C205/C215+I1P						
18.	RepCapexel19	Ex19	C205/C215+S88	Combination		Plasmid-65 C205/C215+S88						
19.	RepCapexel20	Ex20	I1P+S88	Combination			Plasmid-89 I1P+S88	Plasmid-110 I1P+S88				
20.	RepCapexel21	Ex21	I1P+S27	Combination			Plasmid-88 I1P+S27					

Upstream and downstream analytical results for the tested variants are provided in the following tables. These data demonstrate that MAAP variants of the present invention exhibit several-fold higher titers, while maintaining similar packaging efficiency with similar %full capsid, and without increasing residual contaminants such as host cell DNA. Further, the purity of the AAV particles by Viral protein content and ratios is consistent with the wild-type standards (controls).

Table 6. AAV6 Variant upstream summary data.

CAPSID-ID	AVG Vector Genome titer (vg/mL)	Vector Genome fold increase compared to Genescript AAV6 STD	Vector Genome fold increase compared to Fermentation product AAV6 STD	AVG Capsid ELISA (Particulates/mL)	Vector Genome fold increase compared to Genescript AAV6	% Full Particles by ddPCR/ELISA ratio
AAV6-RepCAPEXCEL-W19 (EX1)	2.07E+11	3.7	2.6	6.09E+11	5.2	34
AAV6-RepCAPEXCEL-S27 (EX2)	2.81E+11	5.0	3.5	6.43E+11	5.5	43.7
AAV6-RepCAPEXCEL-S57 (EX3)	2.56E+11	4.6	3.2	7.26E+11	6.2	35.3
AAV6-RepCAPEXCEL-S88 (EX4)	2.95E+11	5.3	3.7	6.48E+11	5.5	45.5
AAV6-RepCAPEXCEL-C20S (EX5)	2.40E+11	4.3	3.0	6.46E+11	5.5	37.2
AAV6-RepCAPEXCEL-L1P (EX7)	1.15E+11	2.1	1.4	2.98E+11	2.5	38.8
AAV6-RepCAPEXCEL-L1P+S57 (EX8)	1.63E+11	2.9	1.8	5.15E+11	4.4	31.6
AAV6-RepCAPEXCEL-C20S+S27 (EX9)	1.72E+11	3.1	2.1	4.62E+11	3.9	37.2
AAV6-RepCAPEXCEL-C20S+S57 (EX10)	3.10E+11	5.6	3.8	7.17E+11	6.1	43.2
AAV6-RepCAPEXCEL-C20S+S88 (EX11)	1.92E+11	3.4	2.4	5.12E+11	4.4	37.5
AAV6-RepCAPEXCEL-C20F (EX12)	9.21E+10	1.7	1.1	2.87E+11	2.5	32.1
AAV6-RepCAPEXCEL-C20Y (EX13)	1.07E+11	1.9	1.3	3.65E+11	3.1	29.3
AAV6-RepCAPEXCEL-L1P+C20S (EX14)	2.25E+11	4.0	2.8	5.84E+11	5.0	38.5
CAPSID-STD-AAV6 (STD)-fermentation	8.07E+10	NA	1.0	1.50E+11	NA	53.8
AAV6-STD-Genescript	5.58E+10	1.0	NA	1.17E+11	1.0	47.7

*Fold-change was compared with similar plasmid source for Mutants and Control, both used synthesized plasmids.

Table 7a. AAV6 variant downstream summary data.

Sample	Upstream Clarified lysate d dPCR (vg/mL)	Batch Purified ddPCR (vg/ mL)	hcDNA (ng/mL) (L)	hcDNA Norm- alized, ng/1E 13vg	AUC			CE-SDS (% Purity)	Infectivity Ratio of infectivity vg/IU
					%Empty Capsid	%Interme- diate	%full capsid		
AAV6-RepCAPEXCEL-W13 (EX1)	2.07E+11	3.70E+12	196.22	530.32	16.6	8.7	61	97.2	166
AAV6-RepCAPEXCEL-S27 (EX2)	2.81E+11	4.00E+12	237.37	593.43	11.1	11.7	70.2	97	43
AAV6-RepCAPEXCEL-S57 (EX3)	2.56E+11	4.42E+12	131.76	298.10	14.4	14.4	61.6	97.6	68
AAV6-RepCAPEXCEL-S88 (EX4)	2.95E+11	5.05E+12	200.38	396.79	15.4	14.2	60.6	97.2	161
AAV6-RepCAPEXCEL-C205 (EX5)	2.40E+11	3.03E+12	203.77	672.51	10.2	9	71.9	97.8	45
AAV6-RepCAPEXCEL-L1P (EX7)	1.12E+11	4.29E+12	273.52	637.58	9.6	10.7	72.6	97	100
AAV6-RepCAPEXCEL-L1P+S57 (EX8)	1.48E+11	5.71E+12	<6 {Below LOQ}	NA	3.4	22.8	56.6	97	112
AAV6-RepCAPEXCEL-C265+S27 (EX9)	1.72E+11	1.97E+12	131.04	665.2	16.1	12.8	56	98	97
AAV6-RepCAPEXCEL-C265+S57 (EX10)	3.10E+11	3.89E+12	42.24	108.6	16.1	10	59.8	98.5	55
AAV6-RepCAPEXCEL-C265+S88 (EX11)	1.92E+11	2.28E+12	24.59	107.9	16.1	12	60.2	97.7	98
AAV6-RepCAPEXCEL-C20F (EX12)	9.21E+10								
AAV6-RepCAPEXCEL-C20Y (EX13)	1.07E+11								
AAV6-RepCAPEXCEL- L1P+C20S (EX14)	2.25E+11								
CAPSID-STD-AAV6 (STD)-Farm	8.07E+10	1.06E+12	47.4	447.2	15.6	16	56.2	97.1	25
CAPSID-STD-AAV6 (STD)- Generatrix	5.58E+10	NA	NA	NA	NA	NA	NA	NA	NA

Table 7b. AAV6 variant downstream summary data of capsid purity.

Sample	VP1	VP2	VP3	CE-SDS Purity (%)
AAV6-RepCAPEXCEL-W19 (EX1)	1.0	1.0	8.2	97.2
AAV6-RepCAPEXCEL-S27 (EX2)	1.0	0.9	8.4	97.0
AAV6-RepCAPEXCEL-S57 (EX3)	1.0	1.2	7.8	97.6
AAV6-RepCAPEXCEL-S88 (EX4)	1.0	0.7	8.5	97.2
AAV6-RepCAPEXCEL-C20S (EX5)	1.0	1.0	8.3	97.8
AAV6-RepCAPEXCEL-L1P (EX7)	1.0	1.0	9.0	97.0
AAV6-RepCAPEXCEL-L1P+S57 (EX8)	1.0	0.8	8.8	97.0
AAV6-RepCAPEXCEL-C20S+S27 (EX9)	1.0	1.0	8.1	98.0
AAV6-RepCAPEXCEL-C20S+S57 (EX10)	1.0	1.2	8.0	98.5
AAV6-RepCAPEXCEL-C20S+S88 (EX11)	1.0	0.9	7.6	97.7
AAV6-RepCAPEXCEL-C20F (EX12)				
AAV6-RepCAPEXCEL-C20Y (EX13)				
AAV6-RepCAPEXCEL-L1P+C20S (EX14)				
CAPSID-STD-AAV6 (STD)-Ferm	1.0	0.9	8.1	97.1
CAPSID-STD-AAV6 (STD)-Genescript	NA	NA	NA	NA

Table 8. AAV2 variant upstream summary data.

CAPSID ID	AVG Vector Genome ddP CR (VG/ml.)	Vector Genome fold increase compared to GenescriptAAV6	Vector Genome fold increase compared to Fermentation product AAV6	AVG Capsid ELISA (Particulates /ml.)	Vector Genome fold increase compared to GenescriptAAV6	Vector Genome fold increase compared to Fermentation product AAV6	% Full Particles by ddPCR/ELISA A ratio
AAV2-RepCAPEXCEL-L19(EX1)	3.70E+10	1.3	1.2	3.64E+11	1.7	1.2	9.7
AAV2-RepCAPEXCEL-S27(EX2)	2.68E+10	1.0	1	2.67E+11	1.2	0.9	10.6
AAV2-RepCAPEXCEL-S57(EX3)	2.68E+10	1.0	1	3.78E+11	1.7	1.2	8
AAV2-RepCAPEXCEL-S88(EX4)	3.41E+10	1.2	1.1	3.41E+11	1.5	1.2	9.1
AAV2-RepCAPEXCEL-C205(EX5)	3.68E+10	1.3	1.2	3.55E+11	1.6	1.2	10.4
AAV2-RepCAPEXCEL-L1P(EX7)	3.53E+10	1.3	1.2	2.29E+11	1.0	0.7	16.4
AAV2-RepCAPEXCEL-L1P+S57(EX8)	3.85E+10	1.4	1.3	2.46E+11	1.1	0.8	15.2
AAV2-RepCAPEXCEL-C205+S27(EX9)	2.52E+10	0.9	0.8	1.81E+11	0.8	0.6	13.9
AAV2-RepCAPEXCEL-C205+S57(EX10)	3.98E+10	1.4	1.3	2.37E+11	1.0	0.8	17.2
AAV2-RepCAPEXCEL-C205+S68(EX11)	3.02E+10	1.1	1	3.09E+11	1.4	1	9.1
AAV2-RepCAPEXCEL-C20F(EX12)	3.47E+10	1.3	1.1	2.46E+11	1.1	0.8	13.9
AAV2-RepCAPEXCEL-C20W(EX13)	2.50E+10	0.9	0.8	3.71E+11	1.6	1.2	6.7
AAV2-RepCAPEXCEL-L1P+C205(EX14)	4.15E+10	1.5	1.4	2.89E+11	1.3	0.9	14.1
AAV2-STD-Ferm	3.75E+10	NA	1.2	2.69E+11	1.2	1	12.2
AAV2-STD-Genescript	2.76E+10	1.0	NA	2.28E+11	1.0	NA	13.3

Table 9. AAV5 variant upstream summary data.

CAPSID-ID	AVG Vector Genome ddPCR (VG/mL)	Vector Genome fold increase compared to Fermentation in AAV5-STD	Vector Genome fold increase compared to GeneScript AAV5-STD	AVG Capsid ELISA (Particles /mL)	Particle num ber fold increase	% Full Particles by ddPCR/ELISA ratio
AAV5-RepCAPEXCEL-L19 (EX1)	4.90E+11	1.05	3.8	1.50E+12	2.4	32.7
AAV5-RepCAPEXCEL-S27 (EX2)	7.20E+11	1.54	5.5	2.70E+12	4.3	26.7
AAV5-RepCAPEXCEL-L57 (EX3)	6.30E+11	1.35	4.8	2.10E+12	3.4	30.0
AAV5-RepCAPEXCEL-S88 (EX4)	5.70E+11	1.22	4.4	2.10E+12	3.4	27.1
AAV5-RepCAPEXCEL-C20S (EX5)	8.10E+11	1.73	6.2	2.50E+12	4.0	32.4
AAV5-RepCAPEXCEL-C20S+S27 (EX9)	6.50E+11	1.39	5.0	2.50E+12	4.0	26.0
AAV5-RepCAPEXCEL-C20S+L57 (EX10)	6.20E+11	1.32	4.8	2.06E+12	3.3	30.2
AAV5-RepCAPEXCEL-C20S+S88 (EX11)	5.64E+11	1.21	4.3	2.26E+12	3.6	25.0
AAV5-RepCAPEXCEL-C20F (EX12)	6.59E+11	1.41	5.1	2.15E+12	3.4	30.7
AAV5-RepCAPEXCEL-C20Y (EX13)	5.04E+11	1.08	3.9	2.00E+12	3.2	25.2
AAV5-RepCAPEXCEL- C20C21+S20S21 (EX15)	9.55E+11	2.04	7.3	2.80E+12	4.5	34.1
AAV5-RepCAPEXCEL-C21S (EX16)	6.40E+11	1.37	4.9	2.31E+12	3.7	27.7
AAV5-RepCAPEXCEL- C20SC21S+S27 (EX17)	5.52E+11	1.18	4.2	1.87E+12	3.0	29.5
AAV5-RepCAPEXCEL-C20SC21S + L57 (EX18)	6.19E+11	1.32	4.8	1.85E+12	2.9	33.6
AAV5-RepCAPEXCEL-C20SC21S + S88 (EX19)	7.16E+11	1.53	5.5	2.17E+12	3.5	33.0
AAV5-CAPSID (STD)-Ferm	4.68E+11	1.0	NA	1.40E+12	NA	33.6
AAV5-CAPSID (STD)-GeneScript	1.34E+11	NA	1	6.26E+11	1	21.4

Table 10a. AAV5 downstream summary data.

AAV5- CAPSID VARIANT	Upstream ddPCR (vg/mL)	hcDNA (ng/mL)	hcDNA (Normalized, ng/1E13vg)	AUC			CE-SDS (% Purity)	Infectivity	
				% Empty	% Intermediate	%full		Ratio of infectivity vg/1U	
AAV5-RepCAPEXCEL-L19 (EX1)	4.99E+11	70.51	186.5	34.3	23.9	33	97.3	142	
AAV5-RepCAPEXCEL-S27 (EX2)	7.29E+11	73.06	172.7	30.7	28.9	30.3	95.8	120	
AAV5-RepCAPEXCEL-L57 (EX3)	6.39E+11	98.55	233.0	30.1	30	33.2	96.9	191	
AAV5-RepCAPEXCEL-S88 (EX4)	5.79E+11	84.58	264.3	27.9	28.7	36.7	95.9	150	
AAV5-RepCAPEXCEL-C20S (EX5)	8.19E+11	119.27	233.9	30.5	20.5	38.8	97.0	89	
AAV5-RepCAPEXCEL-C21S+S27 (EX9)	6.59E+11	63.60	218.79	30.4	26.6	33.7	96.9	614	
AAV5-RepCAPEXCEL-C20S+L57 (EX10)	6.29E+11	63.83	217.02	30.4	25.3	35.0	96.8	261	
AAV5-RepCAPEXCEL-C20S+S88 (EX11)	5.64E+11	64.12	176.33	27.8	22.7	39.3	96.6	136	
AAV5-RepCAPEXCEL-C20F (EX12)	6.59E+11	40.25	167.03	Spillage during AUC run.			94.8	214	
AAV5-RepCAPEXCEL-C20Y (EX13)	5.04E+11	57.91	162.72	29.2	34.5	33.4	96.5	316	
AAV5-RepCAPEXCEL-C20C21+S20S21	9.55E+11								
AAV5-RepCAPEXCEL-C21S	6.40E+11	57.40	235.34	27.9	29.6	31.8	96.3	91	
AAV5-RepCAPEXCEL-C20SC21S+S27	5.52E+11	77.90	232.921	29.2	36.1	36.6	97.0	293	
AAV5-RepCAPEXCEL-C20SC21S+L57	6.19E+11	69.03	197.42	30.2	25.4	38.1	96.9	415	
AAV5-RepCAPEXCEL-C20SC21S+S88	7.16E+11	74.42	288.74	27.1	23.5	44.1	96.9	306	
AAV5- CAPSID (STD)-Ferm	3.89E+11	76.86	247.9	26.5	30.4	36	96.1	207	
AAV5- CAPSID (STD)-Genescript	1.34E+11	NA	NA	NA	NA	NA	NA	NA	

Table 10b. AAV5 downstream summary data of capsid purity.

AAV5- CAPSID VARIANT	VP1	VP2	VP3	CE-SDS (% Purity)
AAV5-RepCAPEXCEL-L19 (EX1)	1.0	3.0	14.5	97.3
AAV5-RepCAPEXCEL-S27 (EX2)	1.0	3.0	14.0	95.8
AAV5-RepCAPEXCEL-L57 (EX3)	1.0	3.5	16.0	96.9
AAV5-RepCAPEXCEL-S88 (EX4)	1.0	3.4	15.9	95.9
AAV5-RepCAPEXCEL-C20S (EX5)	1.0	3.9	17.3	97.0
AAV5-RepCAPEXCEL-C20S+S27 (EX9)	1.0	3.7	16.8	96.9
AAV5-RepCAPEXCEL-C20S+L57 (EX10)	1.0	3.8	17.4	96.8
AAV5-RepCAPEXCEL-C20S+S88 (EX11)	1.0	3.6	17.1	96.6
AAV5-RepCAPEXCEL-C20F (EX12)	1.0	3.8	17.6	94.8
AAV5-RepCAPEXCEL-C20Y (EX13)	1.0	3.6	17.4	96.5
AAV5-RepCAPEXCEL-C20C21+S20S21	1.0	4.0	17.0	
AAV5-RepCAPEXCEL-C21S	1.0	3.6	16.7	96.3
AAV5-RepCAPEXCEL-C20SC21S+S27	1.0	3.6	16.4	97.0
AAV5-RepCAPEXCEL-C20SC21S + L57	1.1	4.0	18.0	96.9
AAV5-RepCAPEXCEL-C20SC21S + S88	1.1	3.8	17.9	96.9
AAV5-CAPSID (STD)-Ferm	1.0	3.5	15.8	96.1
AAV5-CAPSID (STD)-Genescript	NA	NA	NA	NA

Table 11. AAVRh10 upstream summary data.

CAPSID-ID	Vector Genome ddPCR (VG/mL)	Vector Genome fold increase	AVG Capsid ELISA (Particulates/mL)	Particle number fold increase	% Full Particles by ddPCR/ELISA ratio
AAVRh10-RepCAPEXCEL-L19 (EX1)	1.12E+11	1.1	6.09E+11	1.3	18.4
AAVRh10-RepCAPEXCEL-S27 (EX2)	1.28E+11	1.3	6.62E+11	1.5	19.3
AAVRh10-RepCAPEXCEL-L57 (EX3)	2.68E+11	2.6	1.42E+12	3.1	18.9
AAVRh10-RepCAPEXCEL-S88 (EX4)	2.51E+11	2.5	1.17E+12	2.6	21.5
AAVRh10-RepCAPEXCEL-C20S (EX5)	2.78E+11	2.7	1.42E+12	3.1	19.6
AAVRh10-RepCAPEXCEL-L1P (EX7)	2.93E+11	2.9	1.42E+12	3.1	20.6
AAVRh10-RepCAPEXCEL-L1P+S57 (EX8)	1.48E+11	1.3	1.06E+12	2.3	14.0
AAVRh10-RepCAPEXCEL-C20S+S27 (EX9)	1.75E+11	1.8	9.32E+11	2.1	18.8
AAVRh10-RepCAPEXCEL-C20S+S57 (EX10)	1.35E+11	2.0	9.22E+11	2.0	14.6
AAVRh10-RepCAPEXCEL-C20S+S88 (EX11)	1.63E+11	1.7	1.11E+12	2.5	14.6
AAVRh10-RepCAPEXCEL-C20F (EX12)	1.37E+11	1.3	5.20E+11	1.1	26.3
AAVRh10-RepCAPEXCEL-C20Y (EX13)	1.55E+11	1.5	8.87E+11	2.0	17.5
AAVRh10-RepCAPEXCEL-CLIP + C20S (EX14)	1.74E+11	1.7	1.31E+12	2.9	13.3
AAVRh10-RepCAPEXCEL-L1P+S88 (EX20)	1.76E+11	1.5	1.355E+12	3.0	13.0
AAVRh10-CAPSID (STD)-Genescript	1.02E+11	1.0	4.53E+11	1.0	22.5

Table 12a. AAVRh10 downstream summary data.

AAVRh10- CAPSID VARIANT	Upstream ddPCR (vg/mL)	Batch Purified R (vg/mL)	hcDNA (ng/mL)	hcDNA Nor- malized (ng/1E8 3vg)	AUC			CE-SPS (%) Purity	Infectivity Ratio of Infectivity vg/1U
					% Empty	% Intermediate	% full		
AAVRh10-RepCAPEXCEL-L19 (EX1)	1.13E+11	1.37E+12	28.98	211.55	18.8	22.3	47.2	98.2	1624
AAVRh10-RepCAPEXCEL-S27 (EX2)	1.28E+11	2.38E+12	27.19	121.81	18.6	22.8	50.9	97.2	1487
AAVRh10-RepCAPEXCEL-L57 (EX3)	2.68E+11	2.47E+12	41.52	188.56	17	26.1	45.9	98.2	2196
AAVRh10-RepCAPEXCEL-S88 (EX4)	2.51E+11	3.08E+12	36.39	119.80	20.8	23.6	43.2	97.5	1159
AAVRh10-RepCAPEXCEL- C20S (EX5)	2.78E+11	2.74E+12	28.20	106.58	16.7	23.5	49.9	96.8	1370
AAVRh10-RepCAPEXCEL-L1P (EX7)	2.93E+11	2.97E+12	34.76	117.14	19.8	24	47.4	97.1	352
AAVRh10-RepCAPEXCEL-L1P+S57 (EX8)	1.48E+11		86.49	361.9	55.5	20.8	17.6	97.3	672
AAVRh10-RepCAPEXCEL- C20S+S27 (EX9)	1.75E+11	2.38E+12	65.55	255.1	48.4	24.9	18.8	97.6	723
AAVRh10-RepCAPEXCEL- C20S+S57 (EX10)	1.35E+11	2.57E+12	49.84	311.5	56.0	15.9	21.8	96.7	607
AAVRh10-RepCAPEXCEL- C20S+S88 (EX11)	1.63E+11	2.60E+12	67.55	259.8	54.3	18.1	21.8	96.6	731
AAVRh10-RepCAPEXCEL-C20F (EX12)	1.37E+11	2.2E+12	16.98	75.69	12.6	21.6	55.8	95.9	463
AAVRh10-RepCAPEXCEL-C20Y (EX13)	1.58E+11	2.59E+12	32.75	127.07	17.4	26	44.3	97.0	725
AAVRh10-RepCAPEXCEL-CLIP + C20S (EX14)	1.74E+11	2.10E+12	92.42	440.1	53.4	24.8	13.6	97.5	443
AAVRh10-RepCAPEXCEL- L1P+S88 (EX20)	1.76E+11	2.47E+12	83.59	338.4	62.3	16.8	16.0	96.6	694
AAVRh10-CAPSID (STD)- Genescript	1.03E+11	1.98E+12	22.22		17.8	21.2	49.1	97.1	1320

Table 12b. AAVRh10 downstream summary data of capsid purity by CE-SDS.

AAVRh10- CAPSID VARIANT	VP1	VP2	VP3	CE-SDS (% Purity)
AAVRh10-RepCAPEXCEL-L19 (EX1)	1.0	2.4	7.5	98.2
AAVRh10-RepCAPEXCEL-S27 (EX2)	1.0	2.1	8.1	97.2
AAVRh10-RepCAPEXCEL-L57 (EX3)	1.0	2.1	7.4	98.2
AAVRh10-RepCAPEXCEL-S88 (EX4)	1.0	1.9	7.7	97.5
AAVRh10-RepCAPEXCEL-C20S (EX5)	1.0	2.0	8.6	96.8
AAVRh10-RepCAPEXCEL-L1P (EX7)	1.0	2.2	8.5	97.1
AAV6-RepCAPEXCEL-L1P+S57 (EX8)	1.0	2.3	8.3	97.3
AAV6-RepCAPEXCEL-C20S+S27 (EX9)	1.0	2.5	9.2	97.6
AAV6-RepCAPEXCEL-C20S+S57 (EX10)	1.0	2.0	7.8	96.7
AAV6-RepCAPEXCEL-C20S+S88 (EX11)	1.0	1.5	5.4	96.6
AAVRh10-RepCAPEXCEL-C20F (EX12)	1.0	2.1	8.9	95.9
AAVRh10-RepCAPEXCEL-C20Y (EX13)	1.0	2.1	8.4	97.0
AAVRh10-RepCAPEXCEL-CLIP + C20S (EX14)	1.0	2.4	9.5	97.5
AAVRh10-RepCAPEXCEL-L1P+S88 (EX20)	1.0	1.8	7.5	96.6
AAVRh10-CAPSID (STD)-Genescript	1.0	2.1	7.8	97.1

Table 13. AAV8 variant upstream summary data.

CAPSID-ID	AVG Vector Genome fold increase compared to standard	AVG Capsid ELISA (Particles/mL)	Particle fold increase compared to standard	% Full Particles by ddPCR/ELISA ratio
AAV8-RepCAPEXCEL-L19 (EX1)	3.04E+11	2.4	2.5	23.2
AAV8-RepCAPEXCEL-S27 (EX2)	3.86E+11	3.0	3.0	24.2
AAV8-RepCAPEXCEL-S88 (EX4)	4.14E+11	3.3	3.3	24.2
AAV8-RepCAPEXCEL-C20S (EX5)	4.23E+11	3.3	3.5	23.1
AAV8-RepCAPEXCEL-L1P (EX7)	NA	Not detected	NA	NA
AAV8-RepCAPEXCEL-C20S+S27 (EX9)	2.50E+11	2.0	2.4	19.9
AAV8-RepCAPEXCEL-C20S+S88 (EX11)	2.65E+11	2.1	2.1	24.2
AAV8-RepCAPEXCEL-C20F (EX12)	1.71E+11	1.3	1.3	24.2
AAV8-RepCAPEXCEL-C20Y (EX13)	2.27E+11	1.8	1.8	24.2
AAV8-RepCAPEXCEL-L1P+C20S(EX14)	2.50E+11	2.0	2.1	22.2
AAV8-RepCAPEXCEL-L1P+S88 (EX20)	2.86E+11	2.3	2.3	24.2
AAV8-RepCAPEXCEL-L1P+S27 (EX21)	2.63E+11	2.1	2.3	21.7
AAV8-STD-Genescript (Control)	1.27E+11	1.0	1.0	24.2

Table 14a. AAV8 variant downstream summary data.

AAV8- CAPSID VARIANT	Upstream ddPCR (vg/ml) LJ	Batch Purified PCR (vg/ml)	hcDNA (ng/mL)	hcDNA Normalize d, ng/1E1 3vg	AUC			CE-SDS (% Purity)	Infectivity Ratio of infectivity vg/IU
					% Empty	% Intermediate	%full		
AAV8-RepCAPEXCEL-L19 (EX1)	3.04E+11	2.71E+12	17.14	63.418	54.5	17.2	23.0	97.3	1355
AAV8-RepCAPEXCEL- S27 (EX2)	3.86E+11	5.07E+12	41.56	153.772	78.3	12.8	7.4	95.8	1425
AAV8-RepCAPEXCEL- S88 (EX4)	4.14E+11	5.18E+12	41.09	152.033	66.6	17.4	15.6	96.9	460
AAV8-RepCAPEXCEL- C205 (EX5)	4.23E+11	4.79E+12	36.37	134.569	61.3	19.6	16.6	95.9	1796
AAV8-RepCAPEXCEL- L1P (EX7)	1.93E+11	NA	NA	NA	NA	NA	NA	NA	NA
AAV8-RepCAPEXCEL- C205+S27 (EX9)	2.50E+11	3.99E+12	51.83	191.771	63.7	11.6	18.4	96.9	1995
AAV8-RepCAPEXCEL- C205+S88 (EX11)	2.65E+11	4.20E+12	51.7	191.29	63.6	16.9	17.0	96.8	886
AAV8-RepCAPEXCEL-C20F (EX12)	1.71E+11	2.64E+12	17.99	66.563	63.4	20.1	25.1	96.6	742
AAV8-RepCAPEXCEL-C20Y (EX13)	2.27E+11	3.28E+12	20.96	77.552	63.7	19.6	26.6	94.8	922
AAV8-RepCAPEXCEL- L1P+C205(EX14)	2.50E+11	4.10E+12	76.96	284.752	63.4	17.6	8.7	96.5	648
AAV8-RepCAPEXCEL- L1P+S88 (EX20)	2.86E+11	4.71E+12	33.17	122.729	63.3	18.3	21.5		993
AAV8-RepCAPEXCEL- L1P+S27 (EX21)	2.63E+11	4.21E+12	33.88	125.356	NA	NA	NA	96.3	1184
AAV8-STD-Genescript (control)	1.27E+11	2.04E+12	77.90	232.921	52.3	15.0	25.5	97.0	293

Table 14b. AAV8 variant downstream summary data of capsid purity by CE-SDS

AAV8- CAPSID VARIANT	VP1	VP2	VP3	CE-SDS (% Purity)
AAV8-RepCAPEXCEL-L19 (EX1)	1.0	2.0	8.5	97.3
AAV8-RepCAPEXCEL-S27 (EX2)	1.0	1.9	7.9	95.8
AAV8-RepCAPEXCEL-S88 (EX4)	1.0	1.6	8.0	96.9
AAV8-RepCAPEXCEL-C20S (EX5)	1.0	1.9	8.5	95.9
AAV8-RepCAPEXCEL-L1P (EX7)	NA	NA	NA	NA
AAV8-RepCAPEXCEL-C20S+S27 (EX9)	1.0	1.9	8.1	96.9
AAV8-RepCAPEXCEL-C20S+S88 (EX11)	1.0	1.6	7.8	96.8
AAV8-RepCAPEXCEL-C20F (EX12)	1.0	1.9	9.0	96.6
AAV8-RepCAPEXCEL-C20Y (EX13)	1.0	1.9	8.6	94.8
AAV8-RepCAPEXCEL- L1P+C20S(EX14)	1.0	2.0	8.2	96.5
AAV8-RepCAPEXCEL-L1P+S88 (EX20)	1.0	1.7	8.5	
AAV8-RepCAPEXCEL-L1P+S27 (EX21)	1.0	2.0	8.4	96.3
AAV8-STD-Genescript	1.0	1.7	8.6	97.0

[0109] The scope of the present invention is not limited by what has been specifically shown and described herein above. Those skilled in the art will recognize that there are suitable alternatives to the depicted examples of materials, configurations, constructions, and dimensions. Variations, modifications, and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the invention.

[0110] Numerous references, including patents and various publications, are cited and discussed in the description of this invention. The citation and discussion of such references is provided merely to clarify the description of the present invention and is not an admission that any reference is prior art to the invention described herein. All references cited and discussed in this specification are incorporated herein by reference in their entirety.

Claims

What is claimed is:

1. An adeno-associated virus (AAV) genome comprising a variant membrane associated accessory protein (MAAP) nucleic acid sequence having a mutation selected from the group consisting of a stop codon substitution at a codon position corresponding to amino acid positions W19, C20, S27, S57 and S88, an amino acid substitution at position L1, an amino acid substitution at position C20, an amino acid substitution at position C21, and combinations thereof, wherein the codon positions correspond to the amino acid positions in consensus MAAP amino acid sequence SEQ ID NO:1.
2. The AAV genome of claim 1, wherein the genome comprises a substitution mutation in at least one of amino acid positions L1, C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88.
3. The AAV genome of any one of claims 1 to 2, wherein the genome comprises a substitution mutation at amino acid position C20 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88.
4. The AAV genome of any one of claims 1 to 2, wherein the genome comprises a substitution mutation at amino acid position C21 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88.
5. The AAV genome of any one of claims 1 to 2, wherein the genome comprises a substitution mutation at amino acid position L1 and a stop codon substitution at a position selected from the group consisting of amino acid positions W19, C20, S27, S57 and S88.
6. The AAV genome of any one of claims 1 to 5, wherein the substitution mutation at amino acid position L1 is an L1P substitution mutation.

7. The AAV genome of any one of claims 1 to 6, wherein the genome comprises a substitution mutation at one of amino acid positions C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of S27, S57 and S88.
8. The AAV genome of any one of claims 1 to 6, wherein the genome comprises a substitution mutation at both of amino acid positions C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of S27, S57 and S88.
9. The AAV genome of any one of claims 1 to 8, wherein the substitution mutation at amino acid position C20 is selected from the group consisting of C20S, C20Y and C20F substitution mutations.
10. The AAV genome of any one of claims 1 to 8, wherein the substitution mutation at amino acid position C21 is a C21S substitution mutation.
11. The AAV genome of any one of claims 1 to 10, wherein the stop codon substitution is at amino acid position W19.
12. The AAV genome of any one of claims 1 to 10, wherein the stop codon substitution is at amino acid position C20.
13. The AAV genome of any one of claims 1 to 10, wherein the stop codon substitution is at amino acid position S27.
14. The AAV genome of any one of claims 1 to 10, wherein the stop codon substitution is at amino acid position S57.
15. The AAV genome of any one of claims 1 to 10, wherein the stop codon substitution is at amino acid position S88.

16. The AAV genome of any one of claims 1 to 15, wherein the AAV genome is selected from the group consisting of a naturally-occurring serotype and a non-naturally-occurring serotype.
17. The AAV genome of claim 16, wherein the AAV genome is a non-naturally-occurring serotype.
18. The AAV genome of claim 16, wherein the genome is a naturally-occurring serotype.
19. The AAV genome of claim 18, wherein the AAV genome comprises a serotype 6 genome.
20. The AAV genome of claim 18, wherein the AAV genome comprises a serotype 2 genome.
21. The AAV genome of claim 18, wherein the AAV genome is selected from a serotype 1, serotype 3, serotype 4, serotype 5, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh8 and serotype rh10 genome.
22. The AAV genome of any one of claims 1 to 21, wherein the AAV genome comprises a VP1 nucleic sequence, and wherein the MAAP and VP1 nucleotide sequences encode MAAP and VP1 peptide sequences that each have at least 80% homology to wild type, with the proviso that the MAAP nucleotide sequence consists of at least one mutation as defined in any one of claims 1 to 9.
23. The AAV genome of claim 22, wherein the MAAP and VP1 peptide sequences each have at least 90% homology to wild type.
24. The AAV genome of claim 22, wherein the MAAP and VP1 peptide sequences each have at least 95% homology to wild type.

25. A producer cell that produces adeno-associated virus, the producer cell comprising the adeno-associated virus genome of any one of claims 1 to 24.
26. The producer cell of claim 25, wherein the producer cell is eukaryotic.
27. The producer cell of claim 25, wherein the producer cell is mammalian.
28. The producer cell of claim 25, wherein the producer cell is a human cell.
29. The producer cell of claim 26, wherein the producer cell is selected from of yeast cells and insect cells.
30. A method for producing adeno-associated virus, the method comprising: introducing the AAV genome of any of claims 1 to 24 into a cell to create a producer cell and then culturing the producer cell whereby said producer cell produces adeno-associated virus.
31. The method of claim 30, further comprising harvesting said adeno-associated virus, where said harvested adeno-associated virus comprises a transgene.
32. The method of any one of claims 30 to 31, wherein the titer of the produced adeno-associated virus is greater than the titer achieved with an adeno-associated virus genome comprising a corresponding wild-type MAAP sequence.
33. Adeno-associated virus produced by the process of any of claims 30 to 32.
34. A vector comprising a variant AAV capsid gene sequence, the variant AAV capsid gene sequence comprising a mutated membrane associated accessory protein (MAAP) nucleic acid sequence having a mutation selected from the group consisting of a stop codon substitution at a codon position corresponding to amino acid positions W19, C20, S27, S57 and S88, an amino acid substitution at amino acid position L1, an amino acid substitution at amino acid position C20, and an amino acid substitution at amino acid position C21, and combinations thereof,

wherein the codon positions align with the amino acid positions in consensus MAAP amino acid sequence SEQ ID NO:1.

35. The vector of claim 34, wherein the variant AAV capsid gene sequence comprises a substitution mutation of at least one of amino acid positions L1, C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88.
36. The vector of any one of claims 34 to 35, wherein the variant AAV capsid gene sequence comprises a substitution mutation at amino acid position C20 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88.
37. The vector of any one of claims 34 to 35, wherein the variant AAV capsid gene sequence comprises a substitution mutation at amino acid position C21 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88.
38. The vector of any one of claims 34 to 35, wherein the variant AAV capsid gene sequence comprises a substitution mutation at amino acid position L1 and a stop codon substitution at an amino acid position selected from the group consisting of W19, C20, S27, S57 and S88.
39. The vector of any one of claims 34 to 29, wherein the substitution mutation at amino acid position L1 is an L1P substitution mutation.
40. The vector of any one of claims 34 to 39, wherein the variant AAV capsid gene sequence comprises a substitution mutation at one of amino acid positions C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of S27, S57 and S88.
41. The vector of any one of claims 34 to 39, wherein the variant AAV capsid gene sequence comprises a substitution mutation at both of amino acid positions C20 and C21 and a stop codon substitution at an amino acid position selected from the group consisting of S27, S57 and S88.

42. The vector of any one of claims 34 to 41, wherein the substitution mutation at amino acid position C20 is selected from the group consisting of C20S, C20Y and C20F substitution mutations.
43. The vector of any one of claims 34 to 41, wherein the substitution mutation at amino acid position C21 is a C21S substitution mutation.
44. The vector of any one of claims 34 to 43, wherein the stop codon substitution is at amino acid position W19.
45. The vector of any one of claims 34 to 43, wherein the stop codon substitution is at amino acid position C20.
46. The vector of any one of claims 34 to 43, wherein the stop codon substitution is at amino acid position S27.
47. The vector of any one of claims 34 to 43, wherein the stop codon substitution is at amino acid position S57.
48. The vector of any one of claims 34 to 43, wherein the stop codon substitution is at amino acid position S88.
49. The vector of any one of claims 34 to 48, where the variant capsid gene sequence is selected from the group consisting of a naturally-occurring serotype and a non-naturally-occurring serotype.
50. The vector of claim 49, wherein the variant capsid gene sequence is a non-naturally-occurring serotype.
51. The vector of claim 49, wherein the variant capsid gene sequence is a naturally-occurring serotype.

52. The vector of claim 51, wherein the variant capsid gene sequence is a serotype 6 capsid gene sequence.
53. The vector of claim 51, wherein the variant capsid gene sequence is a serotype 2 capsid gene sequence.
54. The vector of claim 51, wherein the variant capsid gene sequence is selected from a serotype 1, serotype 3, serotype 4, serotype 5, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype 13, serotype rh8 and serotype rh10 capsid gene sequences.
55. The vector of any one of claims 34 to 54, wherein the vector comprises a VP1 nucleic sequence, and wherein the MAAP and VP1 nucleotide sequences encode MAAP and VP1 peptide sequences that each have at least 80% homology to wild type, with the proviso that the modified capsid nucleotide sequence consists of at least one mutation as defined in any one of claims 28 to 36.
56. The vector of claim 55, wherein the MAAP and VP1 peptide sequences each have at least 90% homology to wild type.
57. The vector of claim 55, wherein the MAAP and VP1 peptide sequences each have at least 95% homology to wild type.
58. The vector of any one of claims 34 to 57, wherein the vector further comprises a nucleic acid sequence encoding a *rep* gene.
59. The vector of any one of claims 34 to 58, wherein the vector is a plasmid vector.
60. A vector system comprising the vector of any one of claims 34 to 59 and a second vector comprising a transgene flanked by ITR sequences.

61. The vector system of claim 60, further comprising a third vector comprising adenovirus helper sequences.
62. The vector system of any one of claims 60 to 51, wherein the second and third vectors are plasmid vectors.
63. A producer cell line comprising the vector of any one of claims 34 to 59 or vector system of any one of claims 60 to 62.
64. The producer cell of claim 63, wherein the producer cell is eukaryotic.
65. The producer cell of claim 63, wherein the producer cell is mammalian.
66. The producer cell of claim 65, wherein the producer cell is a human cell.
67. The producer cell of claim 63, wherein the producer cell is selected from of yeast cells and insect cells.
68. A method of for producing adeno-associated virus, the method comprising: introducing the vector or vector system of any of claims 34 to 62 into a cell to create a producer cell and then culturing the producer cell whereby the producer cell produces adeno-associated virus.
69. The method of claim 68, wherein the titer of the produced adeno-associated virus is greater than the titer achieved with an adeno-associated virus genome comprising a corresponding wild-type MAAP sequence.