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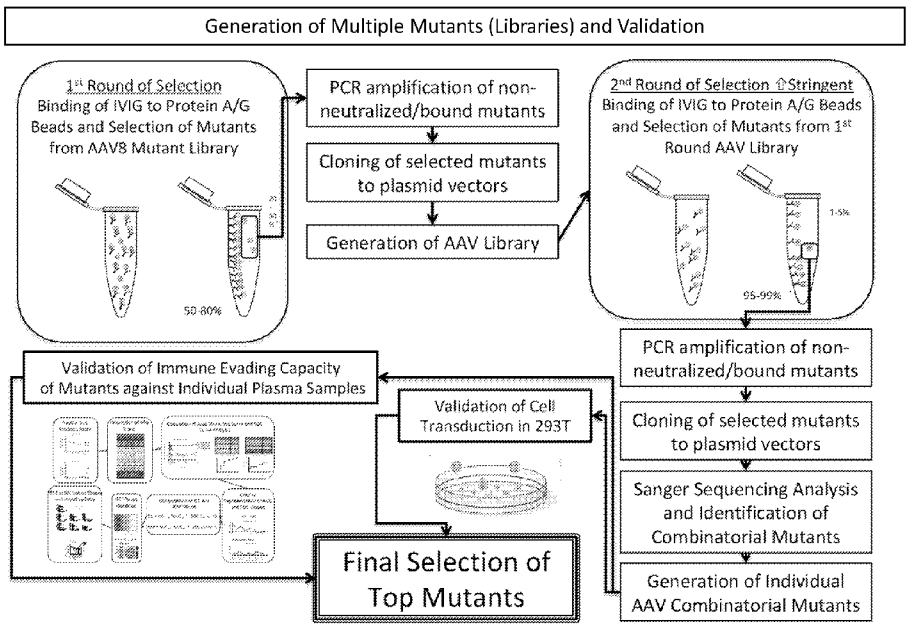
(54) Title: NEW AAV8 BASED IMMUNE ESCAPING VARIANTS

FIG. 1



(57) **Abstract:** The present disclosure provides variant AAV8 capsid polypeptides that exhibit altered capsid properties, *e.g.*, enhanced immune-escaping capacity. Also provided are nucleic acids encoding the variant AAV8 capsid polypeptides, recombinant AAV (rAAV) vectors comprising the variant AAV8 capsid polypeptides, as well as host cells and pharmaceutical compositions comprising the same. Further provided are methods of delivering a gene product to a subject and methods of treatment of a liver-borne blood disorder, the methods generally involving administering an effective amount of the rAAV vectors to a subject in need thereof.

[Continued on next page]

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NEW AAV8 BASED IMMUNE ESCAPING VARIANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims priority to U.S. Provisional Application No. 63/182,337, filed April 30, 2021, the disclosure of which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The disclosure relates to variant AAV8 capsid polypeptides that exhibit altered capsid properties, *e.g.*, enhanced immune-escaping capacity. The present disclosure further relates to nucleic acids encoding the variant AAV8 capsid polypeptides, recombinant AAV (rAAV) vectors comprising the variant AAV8 capsid polypeptides, as well as host cells and pharmaceutical compositions comprising the same. The present disclosure further relates to methods of delivering a gene product to a subject, the methods generally involving administering an effective amount of the rAAV vectors to a subject in need thereof. The present disclosure also relates to methods of treating a liver-borne blood disorder in a human subject in need thereof, the methods generally involving administering an effective amount of the rAAV vectors to a subject in need thereof.

BACKGROUND OF THE INVENTION

[0003] Adeno-associated virus (AAV) is a versatile virus that has been engineered for gene therapy. Recombinant AAV (rAAV), which lacks viral genes in its DNA genome and is used for gene therapy, is primarily a protein-based nanoparticle engineered to cross the cell membrane in order to traffic and deliver its DNA cargo into the nucleus of a cell. The rAAV DNA genome can form circular concatemers persisting as episomes in the nucleus of a transduced cell. As the rAAV DNA rarely integrates into the host genome (Chandler *et al.* (2017) *Hum. Gene Ther.* 28(4):314-322), which contributes to its long-term gene expression and durability, rAAV is ideal for gene therapy.

[0004] Though AAV has not been associated with any human diseases, its presence can be detected in many human tissues. The infected individuals develop an immune response to AAVs. A full spectrum of immune responses to AAV capsids and/or AAV serotypes has been assessed including innate responses, neutralizing antibodies (nAbs) and cytotoxic T cell responses, in human populations. It was estimated that between 50%–90% of the human population has been exposed to AAV infection, of which around 50% of cases lead to the development of nAbs against AAV capsids (Erles *et al.* (1999) *J. Med. Virol.* 59(3):406-411; Calcedo *et al.* (2009) *J.*

Infect. Dis. 199:381-390; Boutin *et al.* (2010) *Hum. Gene Ther.* 21(6):704–12; and Wang *et al.* (2015) *Gene Ther.* 22(12):984–992). Additionally, as there is a high degree of capsid amino acid sequence homology among AAV serotypes, cross-reactivity of nAbs against various AAV serotypes is another factor that limits the usefulness of AAV vectors (Boutin *et al.* (2010) *Hum. Gene Ther.* 21(6):704-12; Kruzik *et al.* (2019) *Mol. Ther. Meth. Clin Dev.* 14:126-133).

[0005] Consequently, patients in need of gene therapy that have pre-existing anti-AAV antibody titers are excluded from clinical trials. The use of alternative serotypes is problematic due to lack of transduction efficiency or other possible side effects. Moreover, there are patient-specific antibodies and currently not a single variant can avoid neutralization by various antibodies.

[0006] There remains a need for new immuno-escaping AAVs, especially those that can escape neutralization by sera from different patients and will hence increase the number of patients included in clinical trials.

SUMMARY OF THE INVENTION

[0007] In one aspect, provided herein is a variant adeno-associated virus 8 (AAV8) capsid polypeptide comprising one or more mutations relative to a wild-type AAV8 capsid polypeptide in one or more regions (VP1 numbering) selected from amino acids 262-274, amino acids 328-333, amino acids 383-391, amino acids 452-471, amino acids 490-507, amino acids 528-545, amino acids 547-564, amino acids 582-597, and amino acids 706-720. In some embodiments, the one or more mutations are one or more amino acid substitutions. In some embodiments, the one or more amino acid substitutions are one or more alanine substitutions and/or glutamic acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises one or more amino acid substitutions at positions G455, G456, S466, N471, V491, T493, N498, K547, Q548, D584, T591, T711, or T719 relative to a wild-type AAV8 capsid polypeptide.

[0008] In some embodiments, the variant AAV8 capsid polypeptide further comprises a peptide insertion selected from the group consisting of NSVRGDG (SEQ ID NO: 122), GNDVRAR (SEQ ID NO: 123), and SRGATVL (SEQ ID NO: 124) after amino acid 590 relative to the wild-type AAV8 capsid polypeptide. In some embodiments, the peptide insertion further comprises a G at the N-terminus and an A at the C-terminus. In some embodiments, the three amino acids preceding the site into which said peptide is inserted into the capsid polypeptide have been changed to GQS or GQR and/or the three amino acids following the site into which said peptide is inserted have been changed to QAA.

[0009] In some embodiments, the variant AAV8 capsid polypeptide comprises one of the following sets of alanine substitution(s) (with the exception of T719E in case of b.):

a. G456A, S466A, D584A, and T591A;

b. G456A, T493A, N498A, D584A, T591A, T711A, and T719E;

c. S466A, V491A, D584A, and T591A;

d. G455A, G456A, S466A, Q548A, and D584A;

e. D584A, T591A, T711A, and T719A;

f. K547A, D584A, and T591A;

g. G456A, T493A, N498A, D584A, and T591A;

h. G456A, S466A, N471A, T711A, and T719A;

i. G456A, N471A, and T493A;

j. T711A, and T719A;

k. T711A; and

l. K547A, Q548A, D584A, and T719A.

[0010] In some embodiments, the variant AAV8 capsid polypeptide comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and one of the following sets of alanine substitution(s):

m. T711A and T719A;

n. G456A, S466A, N471A, T711A, and T719A;

o. T711A; and

p. G456A, N471A, and T493A.

[0011] In some embodiments, the variant AAV8 capsid polypeptide comprises a peptide insertion of GNDVRAR (SEQ ID NO: 123) after amino acid 590, and alanine substitutions T711A and T719A.

[0012] In some embodiments, the variant AAV8 capsid polypeptide comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and one of the following sets of alanine substitution(s) (with the exception of T719E):

q. G456A, T493A, N498A, T591A, T711A, and T719E;

r. G456A, S466A, N471A, T711A, and T719A;

s. G456A, N471A, and T493A; and

t. T711A, and T719A.

[0013] In some embodiments, the one or more mutations result in decreased binding of the variant AAV8 capsid polypeptide to a neutralizing factor, compared to the binding of a wild-type AAV8 capsid polypeptide to the neutralizing factor. In some embodiments, the neutralizing factor is a neutralizing antibody.

[0014] In some embodiments, the one or more mutations do not affect the genome packaging ability and/or transduction efficiency of AAV8.

[0015] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence having at least 80% sequence identity to an amino acid sequence selected from SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 36, 41, 43, 45, 47, and 121.

[0016] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence having at least 80% sequence identity to an amino acid sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, 16, 22, 26, 28, 30, 41, 43, 45, 47, and 121.

[0017] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence selected from SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 36, 41, 43, 45, 47, and 121.

[0018] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, 16, 22, 26, 28, 30, 41, 43, 45, 47, and 121.

[0019] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence of amino acids 138-747 of any one of the variant AAV8 capsid polypeptides described above.

[0020] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence of amino acids 204-747 of any one of the variant AAV8 capsid polypeptides described above.

[0021] In another aspect, provided herein is a nucleic acid encoding a variant adeno-associated virus 8 (AAV8) capsid polypeptide described herein.

[0022] In some embodiments, the nucleic acid comprises a nucleotide sequence having at least 80% sequence identity to a nucleotide sequence selected from SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 35, 40, 42, 44, 46 and 120.

[0023] In some embodiments, the nucleic acid comprises a nucleotide sequence having at least 80% sequence identity to a nucleotide sequence selected from SEQ ID NOs: 5, 7, 9, 11, 13, 15, 21, 25, 27, 29, 40, 42, 44, 46, and 120.

[0024] In some embodiments, the nucleic acid comprises a nucleotide sequence selected from SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 35, 40, 42, 44, 46 and 120.

[0025] In some embodiments, the nucleic acid comprises a nucleotide sequence selected from SEQ ID NOs: 5, 7, 9, 11, 13, 15, 21, 25, 27, 29, 40, 42, 44, 46, and 120.

[0026] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide, comprising a nucleotide sequence of nucleotides 412-2244 of the nucleic acid described above.

[0027] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide, comprising a nucleotide sequence of nucleotides 610-2244 of the nucleic acid described above.

[0028] In another aspect, provided herein is a recombinant DNA comprising the nucleic acid described herein.

[0029] In another aspect, provided herein is an isolated host cell comprising the nucleic acid described herein or the recombinant DNA described herein.

[0030] In another aspect, provided herein is an adeno-associated virus (AAV) vector comprising the variant AAV8 capsid polypeptide described herein. In some embodiments, the AAV vector further comprises a heterologous nucleic acid. In some embodiments, the heterologous nucleic acid comprises a nucleotide sequence encoding a therapeutic protein. In some embodiments, the therapeutic protein is coagulation factor VIII or coagulation factor IX, or a functional fragment or derivative thereof.

[0031] In some embodiments, the AAV vector exhibits an improved immune escaping capacity compared an AAV8 wild-type vector.

[0032] In some embodiments, the AAV vector has an immune escaping capacity score (IECS) of between about 0.01 to about 4.

[0033] In another aspect, provided herein is a pharmaceutical composition comprising the AAV vector described herein, and a pharmaceutically acceptable carrier and/or excipient.

[0034] In another aspect, provided herein is a method of delivering a gene product to a subject in need thereof, said method comprising administering to the subject an effective amount of an adeno-associated virus (AAV) vector described herein or the pharmaceutical composition described herein. In some embodiments, the subject has existing neutralizing antibodies against AAV8 prior to the administration.

[0035] In some embodiments, the AAV vector or the pharmaceutical composition is administered intravenous, intramuscular, subcutaneous, intratumor, intradermal, transdermal, intranodal, intraspinal, intraprostatic, intralymphatic, intraparenchymal, intraportal or intraperitoneal injection.

[0036] In some embodiments, the subject has hemophilia A or hemophilia B.

[0037] In some embodiments, the subject is human. In some embodiments, the subject is a non-human. In some embodiments, the non-human is a mouse, a rat, a rabbit, a dog, a cat, a sheep, a pig, or a non-human primate.

[0038] In another aspect, provided herein are methods of treating a liver-borne blood disorder in a human subject in need thereof, said methods comprising administering to the subject an effective amount of an adeno-associated virus (AAV) vector described herein or a pharmaceutical composition described herein, wherein the AAV vector comprises a heterologous nucleic acid comprising a nucleotide sequence encoding a therapeutic protein, wherein the therapeutic protein is a protein used for the treatment of a liver-borne blood disorder.

[0039] In some embodiments, the adeno-associated virus (AAV) vector administered in this method comprises a variant AAV8 capsid polypeptide that comprises a peptide insertion selected from the group consisting of NSVRGDG (SEQ ID NO: 122), GNDVRAR (SEQ ID NO: 123) and SRGATVL (SEQ ID NO: 124) after amino acid 590 relative to the wild-type AAV8 capsid polypeptide. In some embodiments, the variant AAV8 capsid polypeptide comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and one of the following sets of alanine substitution(s): (i) T711A and T719A; (ii) G456A, S466A, N471A, T711A, and T719A; (iii) T711A; and (iv) G456A, N471A, and T493A. In some embodiments, the variant AAV8 capsid polypeptide comprises a peptide insertion of GNDVRAR (SEQ ID NO: 123) after amino acid 590, and alanine substitutions T711A and T719A. In some embodiments, the variant AAV8 capsid polypeptide comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and one of the following sets of alanine and/or glutamic acid substitution(s): (i) G456A, T493A, N498A, T591A, T711E, and T719A; (ii) G456A, S466A, N471A, T711A, and T719A; (iii) G456A, N471A, and T493A; and (iv) T711A, and T719A. In some embodiments, the peptide insertion further comprises a G at the N-terminus and an A at the C-terminus. In some embodiments, the three amino acids preceding the site into which said peptide is inserted into the AAV8 capsid polypeptide have been changed to GQS or GQR and/or the three amino acids following the site into which said peptide is inserted have been changed to QAA.

[0040] In some embodiments, the therapeutic protein is a blood coagulation factor.

[0041] In some embodiments, the liver-borne blood disorder is a coagulation disorder. In some embodiments, the coagulation disorder is hemophilia. In some embodiments, the hemophilia is hemophilia A or hemophilia B.

[0042] In some embodiments, the AAV vector or the pharmaceutical composition is administered at about $1x10^{11}$ to about $1x10^{14}$ vg/kg. In some embodiments, the AAV vector or the pharmaceutical composition is administered at about $5x10^{11}$ vg/kg.

[0043] In some embodiments, the AAV vector or the pharmaceutical composition is administered via intravenous, intramuscular, subcutaneous, intratumor, intradermal, transdermal, intranodal, intraspinal, intraprostatic, intralymphatic, intraparenchymal, intraportal or intraperitoneal route injection.

[0044] The foregoing summary is not intended to define every aspect of the invention, and additional aspects are described in other sections, such as the following detailed description. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features is not found together in the same sentence, or paragraph, or section of this document. Other features and advantages of the invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, because various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE FIGURES

[0045] **Figure 1** shows the generation of clones containing multiple mutations (combinatorial mutant libraries) and validation.

[0046] **Figure 2A** shows the transduction efficiency of combinatorial mutants (ITR flanked) in 293T cells from the 1st round of nAb screens from 3 experiments (AAV8 CombA, B, C) and cumulative data of fold transduction efficiency to WT.

[0047] **Figure 2B** shows the transduction efficiency of combinatorial mutants (ITR flanked) in 293T cells from the 2nd round of nAb screens from 5 experiments (AAV8 CombA/B, C, D, E, F) and cumulative data of fold transduction efficiency to WT.

[0048] **Figure 3A** shows cumulative Immune Escaping Capacity Score (IECS) of AAV8 combinatorial mutants (ITR flanked) from 1st and 2nd round of nAb screenings sorted by assay (upper panel) and sorted by clone (lower panel).

[0049] **Figure 3B** shows cumulative Immune Escaping Capacity Score (IECS) of AAV8 combinatorial mutants (ITR flanked) from 1st and 2nd round of nAb screenings in standard scale (upper panel) and log scale (lower panel).

[0050] **Figure 4** shows selected immune escape mutants for combinatorial library generation and C-terminal AAV8 Cap fragment used for generation of the combinatorial library, without or with a peptide insertion (PI).

[0051] **Figure 5** shows reduced cumulative response index (RI) of PV27, PV27.QC11, PV27.6A12, PV27.6H2 and PV27.B10 relative to WT. Each shade represents the RI of a donor.

[0052] **Figure 6A** shows the transduction efficiency of combinatorial mutants (not ITR flanked) in 293T cells from the 1st round of nAb screens from 2 experiments (AAV8 CombA, and B) and cumulative data of fold transduction efficiency to WT.

[0053] **Figure 6B** shows the transduction efficiency of combinatorial mutants (not ITR flanked) in 293T cells from the 2nd round of nAb screens from 3 experiments (AAV8 CombA/B, C, D, E) and cumulative data of fold transduction efficiency to WT.

[0054] **Figure 7A** shows cumulative Immune Escaping Capacity Score (IECS) of AAV8 combinatorial mutants (not ITR flanked) from 1st and 2nd round of nAb screenings sorted by assay (upper panel) and sorted by clone (lower panel).

[0055] **Figure 7B** shows cumulative Immune Escaping Capacity Score (IECS) of AAV8 combinatorial mutants (not ITR flanked) from 1st and 2nd round of nAb screenings in standard scale (upper panel) and log scale (lower panel).

DETAILED DESCRIPTION OF THE INVENTION

[0056] The present disclosure provides, among other things, variant adeno-associated virus 8 (AAV8) capsid polypeptides that exhibit altered capsid properties, *e.g.*, enhanced immune-escaping ability. As detailed in the Examples section below, variant AAV8 capsid polypeptides were identified through targeted point mutations or their combinations. Various studies were performed to identify the variant AAV8 capsid polypeptides including (1) optimizations in library selection methodologies, (2) the generation and selection of AAV variant libraries and (3) the identification and validation of selected variants. AAV vectors comprising the variant AAV8 capsid polypeptides have been produced and tested for reduced neutralization by existing antibodies in patient sera.

Definitions

[0057] As used herein, the term "nucleic acid" means a chain of two or more nucleotides such as RNA (ribonucleic acid) and DNA (deoxyribonucleic acid). The terms "nucleic acid", "nucleotide", and "polynucleotide" encompass both DNA and RNA unless specified otherwise. The terms "nucleic acid sequence" or "nucleotide sequence" mean the nucleic acid sequence

encoding an amino acid, and may also refer to the nucleic acid sequence including the portion coding for any amino acids added as an artifact of cloning, including any amino acids coded for by linkers.

[0058] The phrases "cap nucleic acid," "cap gene," and "capsid gene" as used herein mean a nucleic acid that encodes a capsid protein. Examples of cap nucleic acids include "wild-type" (WT) cap-encoding nucleic acid sequences from AAV serotype 8; a native form cap cDNA; a nucleic acid having sequences from which a cap cDNA can be transcribed; and/or allelic variants and homologs of the foregoing.

[0059] As used herein, "protein" or "polypeptide" mean any peptide-linked chain of amino acids, regardless of length or post-translational modification, *e.g.*, glycosylation or phosphorylation.

[0060] The phrases "capsid protein," "capsid polypeptide," "cap protein," or "cap polypeptide" refer to an expression product of a cap nucleic acid from an AAV serotype, such as a wild-type capsid protein from serotypes 8; or a protein that shares at least 50% (alternatively at least 75, 80, 85, 90, 95, 96, 97, 98, or 99%) amino acid sequence identity with a wild-type capsid protein and displays a functional activity of a wild-type capsid protein. The capsid homology of commonly used AAV serotypes is described in, *e.g.*, Daya and Berns (2008) *Clin. Microbiol. Rev.* 21(4):583–593, which is incorporated herein by reference in its entirety. A "functional activity" of a protein is any activity associated with the physiological function of the protein. For example, functional activities of a wild-type capsid protein may include the ability to form a capsid. In some embodiments, the capsid protein is a variant of the wild-type capsid protein (*e.g.*, AAV8 cap protein) with an altered functional activity such as immune-escaping capacity or tissue tropism. In some embodiments, the capsid protein is a variant of an AAV8 wild-type capsid protein with improved immune-escaping capacity. The wild-type AAV genome encodes three capsid proteins: VP1, VP2 and VP3. As used herein, the capsid protein includes VP1, VP2 and VP3. The amino acid positions described herein with reference to an AAV8 capsid protein are denoted according to VP1 numbering, unless noted otherwise.

[0061] A polynucleotide is typically composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); and thymine (T) (uracil (U) for thymine (T) when the polynucleotide is RNA). Thus, the term polynucleotide sequence is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching.

[0062] As defined herein, a nucleotide sequence is intended to refer to a natural or synthetic linear and sequential array of nucleotides and/or nucleosides, and derivatives thereof. The terms "encoding" and "coding" refer to the process by which a nucleotide sequence, through the mechanisms of transcription and translation, provides the information, *e.g.*, to a cell, from which a series of amino acids can be assembled into a specific amino acid sequence to produce a polypeptide.

[0063] An "isolated polynucleotide" molecule is a nucleic acid molecule separate and discrete from the whole organism with which the molecule is found in nature; or a nucleic acid molecule devoid, in whole or part, of sequences normally associated with it in nature; or a sequence, as it exists in nature, but having heterologous sequences in association therewith.

[0064] The term "variant" as used herein refers to a modified or altered form of a wild-type AAV sequence, such as the amino acid sequence of a wild-type AAV8 capsid protein (*e.g.*, SEQ ID NO: 2) or the nucleotide sequence encoding a wild-type AAV8 capsid protein (*e.g.*, SEQ ID NO: 1). The variant may contain an insertion, a deletion, or a substitution of at least one amino acid residue or nucleotide.

[0065] "Heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is being compared. For example, a polynucleotide introduced by genetic engineering techniques into a plasmid or vector derived from a different species is a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked is a heterologous promoter. Thus, for example, an rAAV that includes a heterologous nucleic acid encoding a heterologous gene product is an rAAV that includes a nucleic acid not normally included in a naturally occurring, wild-type AAV, and the encoded heterologous gene product is a gene product not normally encoded by a naturally occurring, wild-type AAV.

[0066] Techniques for determining nucleic acid and amino acid "sequence identity" also are known in the art. Typically, such techniques include determining the nucleotide sequence of the mRNA for a gene and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequence and multiplied by 100. Percent identity may also be determined, for example, by comparing sequence information

using the advanced BLAST computer program, including version 2.2.9, available from the National Institutes of Health. The BLAST program is based on the alignment method of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268 and as discussed in Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410; Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877; and Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389-3402. Briefly, the BLAST program defines identity as the number of identical aligned symbols *(i.e.*, nucleotides or amino acids), divided by the total number of symbols in the shorter of the two sequences. The program may be used to determine percent identity over the entire length of the proteins being compared. Default parameters are provided to optimize searches with short query sequences in, e.g., blastp with the program. The program also allows use of an SEG filter to mask-off segments of the query sequences as determined by the SEG program of Wootton and Federhen (1993) *Comput. Chem.* 17:149-163. Ranges of desired degrees of sequence identity are approximately 70% to 100% and integer values therebetween. Typically, the percent identities between a disclosed sequence and a claimed sequence are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%.

[0067] The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (*e.g.*, a foreign gene) can be introduced into a host cell, so as to promote expression (*e.g.*, transcription and translation) of the introduced sequence. Vectors include plasmids, synthesized RNA and DNA molecules, transposons, phages, viruses, *etc.* In certain embodiments, the vector is a viral vector such as, but not limited to, an adenoviral, adeno-associated, alphaviral, herpes, lentiviral, retroviral, or vaccinia vector. In certain embodiments, the vector is an adeno-associated virus (AAV).

[0068] The term "regulatory element" refers to any cis-acting or trans-acting genetic element that controls some aspect of the expression of a nucleic acid. In some embodiments, the term "promoter" comprises essentially the minimal sequences required to initiate transcription. In some embodiments, some regulatory elements can upregulate or downregulate transcription, commonly termed "enhancer elements" and "repressor elements", respectively.

[0069] The term "operably linked" in reference to a nucleic acid means a nucleic acid is placed in a functional relationship with another nucleic acid. For example, if a coding nucleic acid sequence is operably linked to a promoter nucleic acid sequence, this generally means that the promoter may promote transcription of the coding nucleic acid. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. However, since enhancers may function when

separated from the promoter by several kilobases and intronic sequences may be of variable length, some nucleic acids may be operably linked but not contiguous.

[0070] The term "neutralizing factor" as used herein, refers to any molecule that is capable of blocking the infectivity of a virus. Examples of neutralizing factors include neutralizing antibodies and serum factors. Although not wishing to be bound by any particular theory, neutralization of AAVs is predominately associated with neutralizing antibodies (Rapti et al. (2012) Mol. Ther. 20:73-83, which is incorporated herein by reference in its entirety), however several serum factors, such as Galectin 3 Binding Protein (G3BP) (Denard *et al.* (2012) *J. Virol.* 86(12):6620-31, which is incorporated herein by reference in its entirety), C-Reactive Protein (CRP) (Denard *et al.* (2013) *J. Virol.* 87(19):10784-91, which is incorporated herein by reference in its entirety), or Platelet Factor 4 (PF4) (Denard *et al.* (2018) *Mol. Ther. Meth. Clin. Dev.* 10:291-302, which is incorporated herein by reference in its entirety) could also inhibit transduction by AAVs.

[0071] The term "neutralizing antibody" or "nAb," as used herein, refers to an antibody capable of blocking the infectivity of a virus. A neutralizing antibody can act by blocking attachment of the virus to the host cell, preventing the virus from penetration the host cell membrane, or interfering with uncoating of the virus within the cell, for example. Accordingly, an "AAV neutralizing antibody" refers to an antibody capable of blocking the infectivity of an AAV.

[0072] The term "immune escaping capacity" as used herein refers to the ability of a virus to evade host immune response, such as neutralization by a neutralizing factor (*e.g.*, nAb). In some embodiments of the present disclosure, the immune escaping capacity is quantitatively described using an "immune escaping capacity score" or "IECS". The "immune escaping capacity score" can be calculated as log2(AUCvar/AUCwt) * ROC curve area ^ 3. Specifically, the two curves stemming from the graphical representation of the neutralizing antibody assays from variant and parent vectors are compared. AUCvar and AUCwt are the Area Under the Curve of the nAb Assay for the AAV variant and the parent vector respectively. ROC curve area is a statistical analysis of the specificity and sensitivity of each AAV variant assay, when compared to the parent vector (*e.g.*, AAV8) assay. The ROC curve area values are between 0.5 and 1, with 0.5 being a "weak" assay and 1 a perfect test. The IECS is positive for immune evading variants, whereas it is negative for variants that are neutralized stronger, than the parent vector. Calculation of the IECS is further described in Examples 8 and 11 in the Examples section below.

[0073] The term "library" as used herein refers to a collection of compounds having a set of features in common, however differing in at least one determinable property. For example, in a

virus library, the member viruses are derived from the viruses of the same species but differ, *e.g.*, in a surface-exposed peptide. In the virus library of the present disclosure, specific mutations (*e.g.*, substitutions) in the viral capsid polypeptide, and their combinations, are introduced. In some embodiments, the viral particles of each virus variant in the library comprise a single mutation in the viral capsid polypeptide. In some embodiments, the viral particles of each virus variant in the library comprise one or more amino acid substitutions in the viral capsid polypeptide. In some embodiments, the viral particles of each virus variant in the library comprise one or more amino acid substitutions and a peptide inserted into the viral capsid polypeptide. In certain embodiments, each virus variant in the library comprises exactly one specific combination of amino acid substitution(s) and a peptide inserted into the viral capsid polypeptide.

[0074] The terms "treat" or "treatment" of a state, disorder or condition include: (1) preventing, delaying, or reducing the incidence and/or likelihood of the appearance of at least one clinical or sub-clinical symptom of the state, disorder or condition developing in a subject that may be afflicted with or predisposed to the state, disorder or condition, but does not yet experience or display clinical or subclinical symptoms of the state, disorder or condition; or (2) inhibiting the state, disorder or condition, *i.e.*, arresting, reducing or delaying the development of the disease or a relapse thereof or at least one clinical or sub-clinical symptom thereof; or (3) relieving the disease, *i.e.*, causing regression of the state, disorder or condition or at least one of its clinical or sub-clinical symptoms. The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

[0075] The term "effective" applied to dose or amount refers to that quantity of a compound or pharmaceutical composition that is sufficient to result in a desired activity upon administration to a subject in need thereof. Note that when a combination of active ingredients is administered, the effective amount of the combination may or may not include amounts of each ingredient that would have been effective if administered individually. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition being treated, the particular drug or drugs employed, the mode of administration, and the like.

[0076] The phrase "pharmaceutically acceptable", as used in connection with compositions described herein, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a mammal (*e.g.*, a human). In certain embodiments, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S.

Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans.

[0077] The terms "patient", "individual", "subject", and "animal" are used interchangeably herein and refer to mammals, including, without limitation, human and non-human animals (*e.g.*, cats, dogs, cows, horses, sheep, pigs, *etc.*) and experimental animal models. In a particular embodiment, the subject is a human.

[0078] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions may be employed as carriers, particularly for injectable solutions. Alternatively, the carrier can be a solid dosage form carrier, including but not limited to one or more of a binder (for compressed pills), a glidant, an encapsulating agent, a flavorant, and a colorant. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[0079] Singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (*i.e.*, meaning "including, but not limited to") unless otherwise noted.

[0080] The term "about" or "approximately" includes being within a statistically meaningful range of a value. Such a range can be within an order of magnitude, e.g., within 50%, within 20%, within 10%, and within 5% of a given value or range. The allowable variation encompassed by the term "about" or "approximately" depends on the particular system under study, and can be readily appreciated by one of ordinary skill in the art.

[0081] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range and each endpoint, unless otherwise indicated herein, and each separate value and endpoint is incorporated into the specification as if it were individually recited herein.

[0082] The technology illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein.

[0083] The terms and expressions which have been employed are used as terms of description and not of limitation, and use of such terms and expressions do not exclude any equivalents of

the features shown and described or portions thereof, and various modifications are possible within the scope of the technology claimed.

AAV Capsid Variants

[0084] In one aspect, the present disclosure provides a variant adeno-associated virus 8 (AAV8) capsid polypeptide comprising one or more mutations relative to a wild-type AAV8 capsid polypeptide.

[0085] Although not wishing to be bound by any particular theory, the AAV capsid is formed by 60 protein subunits, VP1, VP2 and VP3, which assemble in a stoichiometric ratio of about 1:1:10 (VP1:VP2:VP3) arranged in T=1 icosahedral symmetry. VP1 (AAV8: 738 amino acids, aa) represents the largest subunit followed by VP2 (AAV8: 601 aa) and the main capsid component VP3 (AAV8: 535 aa).

[0086] AAV8 capsid polypeptides referred to herein include AAV8 capsid proteins VP1, VP2 and VP3, and functional fragments thereof. VP1, VP2 and VP3 are alternative splice variants of the AAV cap protein. In some embodiments, the variant AAV8 capsid polypeptide is VP1. In some embodiments, the variant AAV8 capsid polypeptide is VP2. In some embodiments, the variant AAV8 capsid polypeptide is VP3.

[0087] The amino acid sequence and nucleotide sequence encoding the wild-type AAV8 capsid polypeptide are set forth in SEQ ID NOs: 2 and 1, respectively. The amino acid sequence of AAV8 capsid VP1 comprises amino acids 1 to 738 of SEQ ID NO: 2, AAV8 capsid VP2 comprises amino acids 138 to 738 of SEQ ID NO: 2, and AAV8 capsid VP3 comprises amino acids 204 to 738 of SEQ ID NO: 2 (*See*, *e.g.*, U.S. Patent Nos. 7,790,449; 9,493,788; and 9,677,089, which are incorporated herein by reference in their entirety for all purposes). Accordingly, the nucleotide sequence encoding AAV8 capsid VP1 comprises nucleotides 1 to 2217 of SEQ ID NO: 1, the nucleotide sequence encoding AAV8 capsid VP2 comprises nucleotides 412 to 2217 of SEQ ID NO: 1, and the nucleotide sequence encoding VP3 comprises nucleotides 610 to 2217 of SEQ ID NO: 1.

[0088] In some embodiments, the functional fragments of the capsid polypeptide include Variable Regions (VR). In some embodiments, the functional fragment is one of the nine Variable Regions listed in Table 1. In one embodiment, the functional fragment is VRI (*e.g.*, amino acids 262-274 of SEQ ID NO:2). In one embodiment, the functional fragment is VRII (*e.g.*, amino acids 328-333 of SEQ ID NO:2). In one embodiment, the functional fragment is VRIII (*e.g.*, amino acids 383-391 of SEQ ID NO:2). In one embodiment, the functional fragment is VRIV (*e.g.*, amino acids 452-471 of SEQ ID NO:2). In one embodiment, the functional fragment is VRV (*e.g.*, amino acids 490-507 of SEQ ID NO:2). In one embodiment, the

functional fragment is VRVI (*e.g.*, amino acids 528-545 of SEQ ID NO:2). In one embodiment, the functional fragment is VRVII (*e.g.*, amino acids 547-564 of SEQ ID NO:2). In one embodiment, the functional fragment is VRVIII (*e.g.*, amino acids 582-597 of SEQ ID NO:2). In one embodiment, the functional fragment is VRIX (*e.g.*, amino acids 706-720 of SEQ ID NO:2).

[0089] Other desirable fragments of the capsid protein include the hypervariable regions (HPV) of the variant AAV8 capsid polypeptide. Yet other desirable fragments of the capsid protein include the constant and variable regions, located between HPV regions. Positions of HPV regions, constant and variable regions of an AAV8 capsid protein are as defined in the art (*See*, *e.g.*, U.S. Patent Nos. 7,790,449; 9,493,788; 9,677,089, which are incorporated herein by reference in their entirety for all purposes).

[0090] In some embodiments, the one or more mutations occur in one or more regions (VP1 numbering) selected from amino acids 262-274 of SEQ ID NO:2, amino acids 328-333 of SEQ ID NO:2, amino acids 383-391 of SEQ ID NO:2, amino acids 452-471 of SEQ ID NO:2, amino acids 490-507 of SEQ ID NO:2, amino acids 528-545 of SEQ ID NO:2, amino acids 547-564 of SEQ ID NO:2, amino acids 582-597 of SEQ ID NO:2, and amino acids 706-720 of SEQ ID NO:2, or any combination thereof.

[0091] Any amino acid other than the existing amino acid at the intended position may be used to substitute that amino acid. In some embodiments, the amino acid used for substitution is a natural amino acid, for example, alanine (A), arginine (R), asparagine (N), aspartic acid (D), cysteine (C), glutamine (Q), glutamic acid (E), glycine (G), histidine (H), isoleucine (I), leucine (L), lysine (K), methionine (M), phenylalanine (F), proline (P), serine (S), threonine (T), tryptophan (W), tyrosine (Y), and valine (V). In some embodiments, unnatural amino acids may also be utilized such as, but not limited to, pyrrolysine analogs, tyrosine analogs, and methionine analogs (*See*, Kelemen *et al.* (2018) *Curr. Opin. Chem. Biol.* 46:164–171, which is incorporated herein by reference in its entirety).

[0092] In some embodiments, the one or more mutations are one or more amino acid substitutions. In some embodiments, the one or more amino acid substitutions occur at positions G455, G456, S466, N471, V491, T493, N498, K547, Q548, D584, T591, T711, or T719 relative to a wild-type AAV8 capsid polypeptide (VP1 numbering). Any combination of amino acid substitutions at the listed positions are encompassed herein.

[0093] In some embodiments, the variant AAV8 capsid polypeptide comprises one amino acid substitution. In some embodiments, the variant AAV8 capsid polypeptide comprises two amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises three

amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises four amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises five amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises six amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises seven amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises eight amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises nine amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises ten amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises eleven amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises twelve amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises thirteen amino acid substitutions. In some embodiments, the variant AAV8 capsid polypeptide may comprise more than thirteen amino acid substitutions. In addition to the amino acid substitutions, the AAV8 capsid polypeptide may comprise one or more additional mutations (*e.g.*, an insertion, a deletion, or a substitution).

[0094] In some embodiments, the one or more amino acid substitutions are one or more alanine substitutions and/or glutamic acid substitutions. In some embodiments, the one or more alanine substitutions occur at position G455, G456, S466, N471, V491, T493, N498, K547, Q548, D584, T591, T711, or T719 relative to a wild-type AAV8 capsid polypeptide (VP1 numbering). Any combination of alanine substitutions at the listed positions are encompassed herein.

[0095] In some embodiments, the variant AAV8 capsid polypeptide comprises one alanine substitution. In some embodiments, the variant AAV8 capsid polypeptide comprises two alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises three alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises four alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises five alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises six alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises seven alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises eight alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises nine alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises ten alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises eleven alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide comprises twelve alanine substitutions. In some

embodiments, the variant AAV8 capsid polypeptide comprises thirteen alanine substitutions. In some embodiments, the variant AAV8 capsid polypeptide may comprise more than thirteen alanine substitutions. In addition to the alanine substitutions, the AAV8 capsid polypeptide may comprise one or more additional mutations (*e.g.*, an insertion, a deletion, or a substitution).

[0096] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at G455 relative to a wild-type AAV8 capsid polypeptide.

[0097] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at G456 relative to a wild-type AAV8 capsid polypeptide.

[0098] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at S466 relative to a wild-type AAV8 capsid polypeptide.

[0099] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at N471 relative to a wild-type AAV8 capsid polypeptide.

[00100] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at V491 relative to a wild-type AAV8 capsid polypeptide.

[00101] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at T493 relative to a wild-type AAV8 capsid polypeptide.

[00102] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at N498 relative to a wild-type AAV8 capsid polypeptide.

[00103] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at K547 relative to a wild-type AAV8 capsid polypeptide.

[00104] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at Q548 relative to a wild-type AAV8 capsid polypeptide.

[00105] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at D584 relative to a wild-type AAV8 capsid polypeptide.

[00106] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at T591 relative to a wild-type AAV8 capsid polypeptide.

[00107] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at T711 relative to a wild-type AAV8 capsid polypeptide.

[00108] In one embodiment, the variant AAV8 capsid polypeptide comprises an alanine substitution at T719 relative to a wild-type AAV8 capsid polypeptide.

[00109] In one embodiment, the variant AAV8 capsid polypeptide comprises a glutamic acid substitution at T719 relative to a wild-type AAV8 capsid polypeptide.

[00110] In some embodiments, the variant AAV8 capsid polypeptide comprises a peptide insertion after amino acid 590 of a wild-type AAV8 capsid polypeptide (VP1 numbering).

[00111] The peptide insertion may comprise a short peptide of about 5-12 amino acids. In some embodiments, the peptide insertion comprises a short peptide of about 7-9 amino acids. In some embodiments, the peptide insertion comprises a short peptide of 7 amino acids. In some embodiments, the peptide insertion comprises a short peptide of 8 amino acids. In some embodiments, the peptide insertion comprises a short peptide of 9 amino acids.

[00112] In some embodiments, the peptide insertion comprises an amino acid sequence NSVRGDG (SEQ ID NO: 122), LAGNTIR (SEQ ID NO: 125), GNDVRAR (SEQ ID NO: 123), or SRGATVL(SEQ ID NO: 124), or a variant having at least 85% identity thereto.

[00113] In some embodiments, the peptide insertion comprises an amino acid sequence NSVRGDG (SEQ ID NO: 122). In some embodiments, the peptide insertion comprises an amino acid sequence LAGNTIR (SEQ ID NO: 125). In some embodiments, the peptide insertion comprises an amino acid sequence GNDVRAR (SEQ ID NO: 123). In some embodiments, the peptide insertion comprises an amino acid sequence SRGATVL (SEQ ID NO: 124).

[00114] In some embodiments, the peptide insertion further comprises a glycine (G) at the N-terminus and/or an alanine (A) at the C-terminus. In some embodiments, the peptide insertion further comprises a glycine (G) at the N-terminus. In some embodiments, the peptide insertion further comprises an alanine (A) at the C-terminus. In some embodiments, the peptide insertion further comprises a glycine (G) at the N-terminus and an alanine (A) at the C-terminus.

[00115] In some embodiments, the variant AAV8 capsid polypeptide comprises one or more amino acid substitutions relative to a wild-type AAV8 capsid protein (*e.g.*, SEQ ID NO:2) at amino acid positions (VP1 numbering): (a) glutamine 588; (b) asparagine 590; (c) threonine 591; and/or (d) proline 593.

[00116] In some embodiments, the variant AAV8 capsid polypeptide comprises one or more amino acid substitutions relative to a wild-type AAV8 capsid protein (*e.g.*, of SEQ ID NO:2) at amino acid positions (VP1 numbering): (a) Q588G; (b) N590S or N590R; (c) T591Q; and/or (d) P593A.

[00117] In some embodiments, the variant AAV8 capsid polypeptide comprises amino acid substitutions relative to a wild-type AAV8 capsid protein (*e.g.*, of SEQ ID NO:2) at amino acid positions (VP1 numbering): (a) Q588G; (b) N590S; (c) T591Q; and (d) P593A.

[00118] In some embodiments, the variant AAV8 capsid polypeptide comprises amino acid substitutions relative to a wild-type AAV8 capsid protein (*e.g.*, of SEQ ID NO:2) at amino acid positions (VP1 numbering): (a) Q588G; (b) N590R; (c) T591Q; and (d) P593A.

[00119] In some embodiments, the three amino acids preceding the site into which said peptide is inserted into the capsid polypeptide have been changed to GQS or GQR and/or the

three amino acids following the site into which said peptide is inserted have been changed to QAA.

[00120] In some embodiments, the three amino acids preceding the site into which said peptide is inserted into the capsid polypeptide have been changed to GQS and the three amino acids following the site into which said peptide is inserted have been changed to QAA.

[00121] In some embodiments, the three amino acids preceding the site into which said peptide is inserted into the capsid polypeptide have been changed to GQR and the three amino acids following the site into which said peptide is inserted have been changed to QAA.

[00122] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one of the following sets of alanine and/or glutamic acid substitutions relative to a wild-type AAV8 capsid polypeptide:

a. G456A, S466A, D584A, and T591A;
b. G456A, T493A, N498A, D584A, T591A, T711A, and T719E;
c. S466A, V491A, D584A, and T591A;
d. G455A, G456A, S466A, Q548A, and D584A;
e. D584A, T591A, T711A, and T719A;
f. K547A, D584A, and T591A;
g. G456A, T493A, N498A, D584A, and T591A;
h. G456A, S466A, N471A, T711A, and T719A;
i. G456A, N471A, and T493A;
j. T711A, and T719A;
k. T711A; and
l. K547A, Q548A, D584A, and T719A.

[00123] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one or more of G456A, S466A, D584A, or T591A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises G456A, S466A, D584A, and T591A relative to a wild-type AAV8 capsid polypeptide.

[00124] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one or more of G456A, T493A, N498A, D584A, T591A, T711A, or T719E relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises G456A, T493A, N498A, D584A, T591A, T711A, and T719E relative to a wild-type AAV8 capsid polypeptide.

[00125] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one or more of S466A, V491A, D584A, or T591A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises S466A, V491A, D584A, and T591A relative to a wild-type AAV8 capsid polypeptide.

[00126] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one or more of G455A, G456A, S466A, Q548A, or D584A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises G455A, G456A, S466A, Q548A, and D584A relative to a wild-type AAV8 capsid polypeptide.

[00127] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one or more of D584A, T591A, T711A, or T719A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises D584A, T591A, T711A, and T719A relative to a wild-type AAV8 capsid polypeptide.

[00128] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one or more of K547A, D584A, or T591A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises K547A, D584A, and T591A relative to a wild-type AAV8 capsid polypeptide.

[00129] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one or more of G456A, T493A, N498A, D584A, or T591A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises G456A, T493A, N498A, D584A, and T591A relative to a wild-type AAV8 capsid polypeptide.

[00130] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one or more of G456A, S466A, N471A, T711A, or T719A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises G456A, S466A, N471A, T711A, and T719A relative to a wild-type AAV8 capsid polypeptide.

[00131] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one or more of G456A, N471A, or T493A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises G456A, N471A, and T493A relative to a wild-type AAV8 capsid polypeptide.

[00132] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises one or more of T711A, or T719A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises T711A, and T719A relative to a wild-type AAV8 capsid polypeptide.

[00133] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises T711A relative to a wild-type AAV8 capsid polypeptide.

[00134] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises K547A, Q548A, D584A, and/or T719A relative to a wild-type AAV8 capsid polypeptide. In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises K547A, Q548A, D584A, and T719A relative to a wild-type AAV8 capsid polypeptide.

[00135] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and one of the following sets of alanine substitution(s) relative to a wild-type AAV8 capsid polypeptide:

m. T711A and T719A;

n. G456A, S466A, N471A, T711A, and T719A;

o. T711A; and

p. G456A, N471A, and T493A.

[00136] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and alanine substitution(s) T711A and/or T719A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and alanine substitutions T711A and T719A relative to a wild-type AAV8 capsid polypeptide.

[00137] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and alanine substitution(s) G456A, S466A, N471A, T711A, and/or T719A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and alanine substitutions G456A, S466A, N471A, T711A, and T719A relative to a wild-type AAV8 capsid polypeptide.

[00138] In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and alanine substitution T711A relative to a wild-type AAV8 capsid polypeptide.

[00139] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and alanine substitution(s) G456A, N471A, and/or T493A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and alanine substitutions G456A, N471A, and T493A relative to a wild-type AAV8 capsid polypeptide.

[00140] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of GNDVRAR (SEQ ID NO: 123) after amino acid 590, and alanine substitution(s) T711A and/or T719A relative to a wild-type AAV8 capsid polypeptide. In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of GNDVRAR (SEQ ID NO: 123) after amino acid 590, and alanine substitutions T711A and T719A relative to a wild-type AAV8 capsid polypeptide.

[00141] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and one of the following sets of alanine substitution(s):

q. G456A, T493A, N498A, T591A, T711A, and T719E;
r. G456A, S466A, N471A, T711A, and T719A;
s. G456A, N471A, and T493A; and
t. T711A, and T719A.

[00142] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and alanine substitution(s) G456A, T493A, N498A, T591A, T711A, and/or T719E relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and alanine substitutions G456A, T493A, N498A, T591A, T711A, and T719E relative to a wild-type AAV8 capsid polypeptide.

[00143] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and alanine substitution(s) G456A, S466A, N471A, T711A, and/or T719A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and alanine substitutions G456A, S466A, N471A, T711A, and T719A relative to a wild-type AAV8 capsid polypeptide.

[00144] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and alanine substitution(s) G456A, N471A, and/or T493A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and alanine substitutions G456A, N471A, and T493A relative to a wild-type AAV8 capsid polypeptide.

[00145] In some embodiments, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and alanine substitution(s) T711A, and/or T719A relative to a wild-type AAV8 capsid polypeptide. In one embodiment, a variant AAV8 capsid polypeptide of the present disclosure comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and alanine substitutions T711A, and T719A relative to a wild-type AAV8 capsid polypeptide.

[00146] In some embodiments, the present disclosure provides a variant AAV8 capsid polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 32, 36, 41, 43, 45, 47, or 121, or a functional fragment thereof.

[00147] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 32, 36, 41, 43, 45, 47, or 121, or a functional fragment thereof.

[00148] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to an amino acid sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, 16, 22, 26, 28, 30, 41, 43, 45, 47 , or 121, or a functional fragment thereof.

[00149] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 22, 26, 28, 30, 41, 43, 45, 47, or 121, or a functional fragment thereof.

[00150] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence of SEQ ID NO: 41, or a functional fragment thereof.

[00151] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence of SEQ ID NO: 43, or a functional fragment thereof.

[00152] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence of SEQ ID NO: 45, or a functional fragment thereof.

[00153] In some embodiments, the variant AAV8 capsid polypeptide comprises an amino acid sequence of SEQ ID NO: 47, or a functional fragment thereof.

[00154] In some embodiments, the present disclosure provides a variant AAV8 capsid polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to an amino acid sequence of amino acids 138-747 of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 32, 36, 41, 43, 45, 47, or 121, or a functional fragment thereof.

[00155] In some embodiments, the variant AAV8 capsid polypeptide, comprises an amino acid sequence of amino acids 138-747 of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 32, 36, 41, 43, 45, 47, or 121.

[00156] In some embodiments, the present disclosure provides a variant AAV8 capsid polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to an amino acid sequence of amino acids 204-747 of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 32, 36, 41, 43, 45, 47, or 121, or a functional fragment thereof.

[00157] In some embodiments, the variant AAV8 capsid polypeptide, comprises an amino acid sequence of amino acids 204-747 of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 32, 36, 41, 43, 45, 47, or 121.

[00158] It will be appreciated that conservative amino acid substitutions may be introduced to the polypeptide of any of those described above, to achieve a polypeptide having, for example, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to the referenced sequence and retaining the same or similar activity of that sequence. Conservative amino acid substitutions, as known in the art and as referred to herein, involve substituting amino acids in a protein with amino acids having similar side chains in terms of, for example, structure, size and/or chemical properties. For example, the amino acids within each of the following groups may be interchanged with other amino acids in the same group: amino acids having aliphatic side chains, including glycine, alanine, valine, leucine and isoleucine; amino acids having non-aromatic, hydroxyl-containing side chains, such as serine and threonine; amino acids having acidic side chains, such as aspartic acid and glutamic acid; amino acids having amide side chains, including glutamine and asparagine; basic amino acids, including lysine, arginine and histidine; amino acids having aromatic ring side chains, including phenylalanine, tyrosine and tryptophan; and amino acids having sulfur-containing side chains, including cysteine and methionine. Additionally, amino acids having acidic side chains, such as aspartic acid and glutamic acid, are

considered interchangeable herein with amino acids having amide side chains, such as asparagine and glutamine.

[00159] In some embodiments, the one or more mutations result in decreased binding of the variant AAV8 capsid polypeptide to a neutralizing antibody, compared to the binding of a wild-type AAV8 capsid polypeptide to the neutralizing antibody. In some embodiments, the binding of a variant AAV8 capsid polypeptide of the present disclosure to a neutralizing antibody is decreased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, about 1-20%, about 10-30%, about 20-40%, about 30-50%, about 40-60%, about 50-70%, about 60-80%, about 70-90%, about 80-100%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 98%, or about 99%.

[00160] In some embodiments, the one or more mutations have minimal effect on the genome packaging ability and/or transduction efficiency of AAV. In some embodiments, the one or more mutations result in less than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50% reduction in genome packaging ability of the AAV. In some embodiments, the one or more mutations result in less than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50% reduction in transduction efficiency of AAV. In some embodiments, the one or more mutations do not affect the genome packaging ability and/or transduction efficiency of AAV.

Nucleic Acids and Vectors

[00161] In another aspect, the present disclosure provides a nucleic acid encoding a variant AAV8 capsid polypeptide described herein.

[00162] In some embodiments, the nucleic acid of the present disclosure encodes a variant AAV8 capsid VP1 polypeptide. In some embodiments, the nucleic acid of the present disclosure encodes a variant AAV8 capsid VP2 polypeptide. In some embodiments, the nucleic acid of the present disclosure encodes a variant AAV8 capsid VP3 polypeptide.

[00163] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to a nucleotide sequence of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 31, 35, 40, 42, 44, 46, or 120.

[00164] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide comprises a nucleotide sequence of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 31, 35, 40, 42, 44, 46, or 120.

[00165] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to a nucleotide sequence of SEQ ID NOs: 5, 7, 9, 11, 13, 15, 21, 25, 27, 29, 40, 42, 44, 46, or 120.

[00166] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide comprises a nucleotide sequence of SEQ ID NOs: 5, 7, 9, 11, 13, 15, 21, 25, 27, 29, 40, 42, 44, 46, or 120.

[00167] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to a nucleotide sequence of nucleotides 412-2244 of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 31, 35, 40, 42, 44, 46, or 120.

[00168] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide comprises a nucleotide sequence of nucleotides 412-2244 of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 31, 35, 40, 42, 44, 46, or 120.

[00169] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to a nucleotide sequence of nucleotides 610-2244 of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 31, 35, 40, 42, 44, 46, or 120.

[00170] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide comprises a nucleotide sequence of nucleotides 610-2244 of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 31, 35, 40, 42, 44, 46, or 120.

[00171] Polynucleotides of the present disclosure encompass variants of the specific nucleic acid sequences recited herein. Such variants may represent orthologs, paralogs or other homologs of a polynucleotide of the present disclosure. The polynucleotide variants, *e.g.*, comprise a nucleic acid sequence characterized in that the sequence can be derived from the specific nucleic acid sequences recited herein by at least one nucleotide insertion, deletion and/or substitution, whereby the variant nucleic acid sequence still encodes a capsid polypeptide having the activity as specified above. Variants also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the specific nucleic acid sequences recited herein, *e.g.*, under

stringent hybridization conditions. These stringent conditions are known to a skilled artisan and can be found in for example, Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989) 6.3.1-6.3.6. An example of stringent hybridization conditions are hybridization conditions in 6× sodium chloride/sodium citrate (=SSC) at approximately 45° C., followed by one or more wash steps in 0.2×SSC, 0.1% SDS at 50 to 65° C. It will be understood by a skilled artisan that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under "standard hybridization conditions" the temperature differs depending on the type of nucleic acid between 42° C. and 58° C. in aqueous buffer with a concentration of 0.1 to 5×SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42° C. The hybridization conditions for DNA:DNA hybrids may be, *e.g.*, 0.1×SSC at 20° C to 45° C, *e.g.*, between 30° C and 45° C. The hybridization conditions for DNA:RNA hybrids are, *e.g.*, 0.1×SSC at 30° C to 55° C, *e.g.*, between 45° C and 55° C. Such hybridization temperatures may be determined for example for a nucleic acid with approximately 100 base pairs (bp) in length and a G+C content of 50% in the absence of formamide. The skilled artisan can readily determine the required hybridization conditions by referring to textbooks such as the textbook mentioned above.

[00172] Polynucleotide variants may also be obtained by PCR-based techniques such as mixed oligonucleotide primer-based amplification of DNA, *i.e.*, using degenerated primers against conserved domains of the capsid polypeptides of the present disclosure. Conserved domains of the capsid polypeptides of the present disclosure may be identified by a sequence comparison of the nucleic acid sequence of the polynucleotide or of the amino acid sequence of the capsid polypeptides described herein. Suitable PCR conditions are well known in the art. As a template, DNA or cDNA from AAVs (*e.g.*, AAV8) may be used. Further, variants include polynucleotides comprising nucleic acid sequences which are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the nucleic acid sequences recited herein. Moreover, also encompassed are polynucleotides which comprise nucleic acid sequences encoding amino acid sequences which are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequences referred to above. The percent identity values are, *e.g.*, calculated over the entire amino acid or nucleic acid sequence region. A series of programs based on a variety of algorithms is available to the skilled artisan for comparing different sequences. For example, the algorithms of Needleman and Wunsch or Smith and Waterman may be used. To carry out the

sequence alignments, the program PileUp (Feng and Doolittle (1987) *J. Mol. Evolution* 25:351-360; Higgins *et al.* (1989) *CABIOS* 5:151-153) or the programs Gap and BestFit (Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453) and Smith and Waterman (Smith and Waterman (1981) *Adv. Appl. Math.* 2:482-489), which are part of the GCG software packet (Genetics Computer Group, 575 Science Drive, Madison, Wis., USA 53711 (1991)), may also be used. The sequence identity values recited above in percent (%) may be determined, *e.g.*, using the program GAP over the entire sequence region with the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000, which, unless otherwise specified, can be used as standard settings for sequence alignments.

[00173] A polynucleotide comprising a fragment of any of the nucleic acid sequences described herein is also contemplated as a polynucleotide of the present disclosure. The fragment may encode a capsid polypeptide which still has the biological activity (*e.g.*, improved immune-escaping capacity) as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment may comprise at least 50, at least 60, at least 90, at least 100, at least 150, at least 240, at least 250, at least 300, at least 450, at least 500, at least 600, at least 750, at least 800, at least 900, at least 1000, at least 1200, at least 1500 consecutive nucleotides of any one of the nucleic acid sequences described herein or encodes an amino acid sequence comprising at least 20, at least 30, at least 50, at least 80, at least 100, at least 150, at least 200, at least 250, at least 300, at least 400, or at least 500 consecutive amino acids of any one of the amino acid sequences described herein.

[00174] Polynucleotides of the present disclosure may either consist essentially of the nucleic acid sequences described herein or comprise the nucleic acid sequences described herein. In some embodiments, a polynucleotide of the present disclosure may contain other nucleic acid sequences as well. For example, the polynucleotides of the present invention may encode fusion proteins wherein one partner of the fusion protein is a capsid polypeptide being encoded by a nucleic acid sequence recited above. Such fusion proteins may, *e.g.*, comprise as additional part polypeptides for monitoring expression (*e.g.*, green, yellow, blue or red fluorescent proteins, alkaline phosphatase and the like) or so called "tags" which may serve as a detectable marker or as an auxiliary measure for purification purposes. Tags for the different purposes are well known in the art and examples include FLAG-tags, 6-histidine-tags, MYC-tags and the like.

[00175] Polynucleotides of the present invention may be provided, *e.g.*, either as an isolated polynucleotide (*i.e.*, isolated from its natural context) or in genetically modified form. The polynucleotide, *e.g.*, is DNA including cDNA or RNA. The term encompasses both single and

double stranded polynucleotides. Moreover, also encompassed are chemically modified polynucleotides including naturally occurring modified polynucleotides such as glycosylated or methylated polynucleotides or artificially modified ones such as biotinylated polynucleotides.

[00176] In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide is a DNA. In some embodiments, the nucleic acid encoding a variant AAV8 capsid polypeptide is an RNA.

[00177] The present disclosure further provides vectors generated using the nucleic acid and amino acid sequences of the variant AAV8 capsid polypeptide described herein.

[00178] In one aspect, the present disclosure provides a recombinant vector comprising the nucleic acid encoding a variant AAV8 capsid polypeptide described herein.

[00179] In some embodiments, the recombinant vector is a viral vector. In some embodiments, the recombinant vector is a non-viral vector.

[00180] In some embodiments, the recombinant vector is a recombinant AAV vector. In some embodiments, the recombinant vector is a recombinant AAV8 vector.

[00181] In some embodiments, the recombinant vector is a recombinant DNA. The recombinant DNA of the present disclosure is useful for delivering the nucleic acid of the present disclosure to cells *in vitro*, *ex vivo* or *in vivo* and imparting the ability to express the variant AAV8 capsid protein to the cells. Then, the cell to which the nucleic acid of the present disclosure is delivered is useful for producing AAV particles. The recombinant DNA can be particularly used for delivery or introduction of the nucleic acid of the present disclosure into animal cells, *e.g.*, mammal cells.

[00182] The recombinant DNA of the present disclosure can be prepared by making a DNA used as a vector containing the nucleic acid of the present disclosure. For example, a plasmid DNA, a phage DNA, a transposon, a cosmid DNA, an episomal DNA, or a viral genome can be used.

[00183] In one aspect, the present disclosure provides a recombinant vector comprising a variant AAV8 capsid polypeptide described herein.

[00184] A skilled artisan will readily understand that the variant AAV8 capsid polypeptide described herein can be readily adapted for use in AAV and other viral vector systems for *in vitro*, *ex vivo* or *in vivo* gene delivery. Similarly, one of skill in the art can readily select other fragments of the AAV genome (*e.g.*, rep, ITR) for use in a variety of rAAV and non-rAAV vector systems. Such vectors systems may include, *e.g.*, lentiviruses, retroviruses, poxviruses, vaccinia viruses, and adenoviral systems, among others. Selection of these vector systems is not a limitation of the present invention.

[00185] The vectors of the present disclosure are useful for a variety of purposes, including for delivery of therapeutic molecules to a subject with pre-existing neutralizing antibodies. Particularly desirable for delivery of therapeutic molecules are recombinant AAV vectors comprising the variant AAV8 capsid polypeptides, which are specially described below.

[00186] In some embodiments, the AAV vector comprising a variant AAV8 capsid polypeptide described herein exhibits an improved immune escaping capacity compared an AAV8 wild-type vector.

[00187] In some embodiments, the AAV vector has a positive immune escaping capacity score (IECS). In some embodiments, the AAV vector has an IECS that is between about 0.01 to about 4. In some embodiments, the AAV vector has an IECS that is between about 0.01 to about 0.1, between about 0.01 to about 0.2, between about 0.01 to about 0.3, between about 0.01 to about 0.4, between about 0.01 to about 0.5, between about 0.1 to about 0.2, between about 0.1 to about 0.3, between about 0.1 to about 0.4, between about 0.1 to about 0.5, between about 0.1 to about 0.6, between about 0.1 to about 0.7, between about 0.1 to about 0.8, between about 0.1 to about 0.9, between about 0.1 to about 1, between about 0.2 to about 0.3, between about 0.2 to about 0.4, between about 0.2 to about 0.5, between about 0.2 to about 0.6, between about 0.2 to about 0.7, between about 0.2 to about 0.8, between about 0.2 to about 0.9, between about 0.2 to about 1, between about 0.3 to about 0.4, between about 0.3 to about 0.5, between about 0.3 to about 0.6, between about 0.3 to about 0.7, between about 0.3 to about 0.8, between about 0.3 to about 0.9, between about 0.3 to about 1, between about 0.4 to about 0.5, between about 0.4 to about 0.6, between about 0.4 to about 0.7, between about 0.4 to about 0.8, between about 0.4 to about 0.9, between about 0.4 to about 1, between about 0.5 to about 0.6, between about 0.5 to about 0.7, between about 0.5 to about 0.8, between about 0.5 to about 0.9, between about 0.5 to about 1, between about 0.5 to about 1.1, between about 0.5 to about 1.2, between about 0.5 to about 1.3, between about 0.5 to about 1.4, between about 0.5 to about 1.5, between about 0.6 to about 0.7, between about 0.6 to about 0.8, between about 0.6 to about 0.9, between about 0.6 to about 1, between about 0.6 to about 1.1, between about 0.6 to about 1.2, between about 0.6 to about 1.3, between about 0.6 to about 1.4, between about 0.6 to about 1.5, between about 0.7 to about 0.8, between about 0.7 to about 0.9, between about 0.7 to about 1, between about 0.7 to about 1.1, between about 0.7 to about 1.2, between about 0.7 to about 1.3, between about 0.7 to about 1.4, between about 0.7 to about 1.5, between about 0.8 to about 0.9, between about 0.8 to about 1, between about 0.8 to about 1.1, between about 0.8 to about 1.2, between about 0.8 to about 1.3, between about 0.8 to about 1.4, between about 0.8 to about 1.5, between about 0.9 to about 1, between about 0.9 to about 1.1, between about 0.9 to about 1.2, between about 0.9 to

about 1.3, between about 0.9 to about 1.4, between about 0.9 to about 1.5, between about 1 to about 1.2, between about 1 to about 1.5, between about 1 to about 1.8, between about 1 to about 2, between about 1.5 to about 2, between about 2 to about 2.5, between about 2.5 to about 3, between about 2 to about 3, between about 3 to about 3.5, between about 3.5 to about 4, or between about 3 to about 4. In some embodiments, the AAV vector has an IECS of about 0.01, about 0.02, about 0.03, about 0.04, about 0.05, about 0.06, about 0.07, about 0.08, about 0.09, about 0.1, about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2, about 2.2, about 2.5, about 2.7, about 3, about 3.2, about 3.5, about 3.6, about 3.8, or about 4.

Recombinant AAV vectors

[00188] In one aspect, the present invention provides an AAV vector comprising a variant AAV8 capsid polypeptide described herein. In some embodiments, the AAV vector comprises one or more variant AAV8 VP1 capsid polypeptide, variant AAV8 VP2 capsid polypeptide, and/or variant AAV8 VP3 capsid polypeptide described herein.

[00189] In some embodiments, the AAV vector comprises a variant AAV8 capsid polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 32, 36, 41, 43, 45, 47, or 121, or a functional fragment thereof.

[00190] In some embodiments, the AAV vector comprises a variant AAV8 capsid polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 32, 36, 41, 43, 45, 47, or 121, or a functional fragment thereof.

[00191] In some embodiments, the AAV vector comprises a variant AAV8 capsid polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to an amino acid sequence selected from SEQ ID NOs: 6, 8, 10, 12, 14, 16, 22, 26, 28, 30, 41, 43, 45, 47 , or 121, or a functional fragment thereof.

[00192] In some embodiments, the AAV vector comprises a variant AAV8 capsid polypeptide comprising an amino acid sequence of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 22, 26, 28, 30, 41, 43, 45, 47 , or 121, or a functional fragment thereof.

[00193] In some embodiments, the AAV vector comprises a variant AAV8 capsid polypeptide encoded by a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to a nucleotide sequence of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 31, 35, 40, 42, 44, 46, or 120, or a functional fragment thereof.

[00194] In some embodiments, the AAV vector comprises a variant AAV8 capsid polypeptide encoded by a nucleotide sequence of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 31, 35, 40, 42, 44, 46, or 120, or a functional fragment thereof.

[00195] In some embodiments, the AAV vector comprises a variant AAV8 capsid polypeptide encoded by a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to a nucleotide sequence of SEQ ID NOs: 5, 7, 9, 11, 13, 15, 21, 25, 27, 29, 40, 42, 44, 46, or 120, or a functional fragment thereof.

[00196] In some embodiments, the AAV vector comprises a variant AAV8 capsid polypeptide encoded by a nucleotide sequence of SEQ ID NOs: 5, 7, 9, 11, 13, 15, 21, 25, 27, 29, 40, 42, 44, 46, or 120, a functional fragment thereof.

[00197] One of skill in the art may readily prepare other recombinant AAV viral vectors containing the variant AAV8 capsid polypeptides provided herein using a variety of techniques known to those of skill in the art. One may similarly prepare other chimeric recombinant AAV viral vectors containing a variant AAV8 capsid polypeptide provided herein and AAV capsid protein(s) of another serotype.

[00198] In some embodiments, a recombinant AAV vector comprises, in addition to one or more variant AAV8 capsid polypeptides, wild-type Rep78, Rep68, Rep52, and Rep40 proteins. In other embodiments, a recombinant AAV vector comprises, in addition to one or more variant AAV8 capsid polypeptides, one or more mutations in one or more of Rep78, Rep68, Rep52, and Rep40 proteins.

[00199] The recombinant AAV vectors of the present disclosure may further comprise a heterologous nucleic acid. In some embodiments, the heterologous nucleic acid comprises a nucleotide sequence encoding a gene of interest. The gene of interest can be any gene, including and not limited to, proteins, peptides and RNAs (*e.g.*, mRNA, siRNA, miRNA). Many suitable genes for expression for therapeutic or non-therapeutic purposes are readily identified by a skilled artisan.

[00200] Recombinant AAV vectors can be constructed using known techniques to at least provide as operatively linked components in the direction of transcription, for example, control

elements including a transcriptional initiation region, the gene of interest and a transcriptional termination region. The control elements can be selected to be functional in a mammalian liver cell. The resulting construct which contains the operatively linked components is typically bounded (5′ and 3′) with functional AAV ITR sequences.

[00201] The nucleotide sequences of AAV ITR regions are known. *See*, *e.g.*, Kotin (1994) *Hum. Gene Ther*. 5:793-801; and Berns, "Parvoviridae and their Replication" in Fundamental Virology, 2nd Edition (B. N. Fields and D. M. Knipe, eds.; incorporated herein by reference in its entirety) for the AAV2 sequence. AAV ITRs that can be used in the present disclosure may be derived from any of several AAV serotypes, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV7, AAV8, *etc.* AAV ITRs used in the vectors of the invention may have a wild-type nucleotide sequence, or may be altered, *e.g.*, by the insertion, deletion or substitution of nucleotides. Furthermore, 5′ and 3′ ITRs which flank a selected nucleotide sequence in an AAV expression vector need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended, *i.e.*, to allow for excision and rescue of the sequence of interest from a host cell genome or vector, and to allow integration of the DNA molecule into the recipient cell genome when AAV Rep gene products are present in the cell. ITRs allow replication of the vector sequence in the presence of an appropriate mixture of Rep proteins. ITRs also allow for the incorporation of the vector sequence into the capsid to generate an AAV particle.

[00202] A suitable heterologous nucleic acid for use in a rAAV vector of the present disclosure may generally be less than about 5 kilobases (kb) in size and include, for example, a gene that encodes a protein that is defective or missing from a recipient subject; a gene that encodes a protein having a desired biological or therapeutic effect (*e.g.*, an antibacterial, antiviral or antitumor function); a nucleotide sequence that encodes an RNA that inhibits or reduces production of a deleterious or otherwise undesired protein; a nucleotide sequence that encodes an antigenic protein; or a nucleotide sequence that encodes an RNA that inhibits or reduces production of a protein.

[00203] In some embodiments, the heterologous nucleic acid comprises a nucleotide sequence encoding a therapeutic protein or peptide. The therapeutic protein or peptide can be used to correct or replace a deficient gene, or ameliorate gene deficiencies in a subject, which may include deficiencies in which normal genes are expressed at less than normal levels or deficiencies in which the functional gene product is not expressed. In some embodiments, the heterologous nucleic acid comprises nucleotide sequences encoding multiple transgenes, which can be helpful to correct or replace multiple defective genes, or ameliorate a gene defect caused

by a multi-subunit protein. In certain embodiments, two or more transgenes may be used to encode different subunits of a protein, or to encode different peptides or proteins. This is desirable when the size of the DNA encoding the protein subunit is large, for example, in the cases of an immunoglobulin, the platelet-derived growth factor, or a dystrophin protein. In order for the cell to produce the multi-subunit protein, a cell is infected with a population of recombinant viruses containing each of the different subunits. Alternatively, different subunits of a protein may be encoded by the same transgene. For example, a single transgene may include the DNA encoding each of the subunits, with the DNA for each subunit separated by an internal ribozyme entry site (IRES) or a self-cleaving sequence. This is desirable when the size of the DNA encoding each of the subunits is small, *e.g.*, the total size of the DNA encoding the subunits and the IRES is less than 5 kilobases. As an alternative to an IRES, the DNA may be separated by sequences encoding a self-cleaving sequence such as a 2A peptide. *See*, *e.g.*, Donnelly *et al.* (1997) *J. Gen. Virol.* 78(1):13-21; Furler *et al.* (2001) *Gene Ther.* 8(11):864-873; Klump *et al.* (2001) *Gene Ther.* 8(10):811-817. The 2A peptide is significantly smaller than an IRES, making it well suited for use when space is a limiting factor. In some embodiments, when the transgene is large, consists of multi-subunits, or when two or more transgenes are co-delivered, rAAVs carrying the desired transgene(s) or subunits may be co-administered to allow them to concatamerize *in vivo* to form a single vector genome. In such an embodiment, a first AAV may carry an expression cassette which expresses a single transgene and a second AAV may carry an expression cassette which expresses a different transgene for co-expression in the host cell.

[00204] A variety of therapeutic proteins and peptides, either in single or split into two or more vectors (*See*, *e.g.*, Truong *et al.* (2015) *Nucleic Acids Res.* 43, 6450–6458; Moretti *et al.* (2020) *Nat Med.* 26(2):207-214), are suitable for inclusion in a recombinant AAV vector of the present disclosure. Suitable proteins include, but are not limited to, an antibody (*e.g.*, a monoclonal antibody such as Orthoclone Okt3® (muromonab-CD3), Centoxin® (nebacumab), Panorex® (edrecolomab), Removab® (catumaxomab), Zinbryta® (daclizumab), Reopro® (abciximab), Rituxan® (rituximab), Simulect® (basiliximab), Synagis® (palivizumab), Remicade® (infliximab), Herceptin® (trastuzumab), Humira™ (adalimumab), Xolair® (omalizumab), Bexxar® (tositumomab), Raptiva™ (efalizumab), Erbitux™ (cetuximab), Avastin® (bevacizumab), Tysabri® (natalizumab), Vectibix® (panitumumab), Lucentis® (ranibizumab), Soliris® (eculizumab), Stelara® (ustekinumab), Ilaris® (canakinumab), Simponi® (golimumab), Arzerra® (ofatumumab), RoActemra® (tocilizumab), Prolia® (denosumab), Benlysta® (belimumab), Yervoy® (ipilumumab), Perjeta® (pertuzumab), raxibacumab, Gazyva® (obinutuzumab), Sylvant® (siltuximab), Cyramza® (ramucirumab),

Entyvio® (vedolizumab), Opdivo® (nivolumab), Keytruda® (pembrolizumab), Blincyto® (blinatumomab), Lemtrada® (alemtuzumab), Repatha® (evolocumab), Praxbind® (idarucizumab), Portrazza® (necitumumab), Unituxin® (dinutuximab), Cosentyx® (secukimumab), Nucala® (mepolizumab), Praluent® (alirocumab), Darzalex® (daratumumab), Empliciti® (elotuzumab), Taltz® (ixekizumab), Cinqaero® (reslizumab), Lartruvo® (olaratumab), Zinplava® (bezlotoxumab), Tecentriq® (atezolizumab), Anthim® (obiltoxaximab), Siliq® (brodalumab), Dupixent® (dupilumab), Tremfya® (guselkumab), Kevzara® (sarilumab), Bavencio® (avelumab), Hemlibra® (emicizumab), Ocrevus® (ocrelizumab), Fasenra® (benralizumab), Imfinzi® (durvalumab), Aimovig® (erenumab), Emgality® (galcanezumab), Crysvita® (burosumab), Takhzyro® (lanadelumab), Poteligeo® (mogamulizumab), Ilumya® (tildrakizumab), Ajovy® (fremanezumab), Ultomiris® (ravulizumab), Libtayo® (cemiplimab), Trogarzo® (ibalizumab), Gamifant® (emapalumab), Cablivi® (caplacizumab), Skyrizi® (risankizumab), Polivy® (polazuzumab), Evenity® (romosozumab), Beovu® (brolucizumab), Adakveo® (crizanlizumab), Padcev® (enfortumab), Tepezza® (teprotumumab), Vyepti® (eptinezumab), Sarclisa® (isatuximab), Uplizna® (inebilizumab), Monjuvi® (tafasitamab), Enspryng® (satralizumab), sutimlimab, naxitamab, margetuximab, tanezumab, narsoplimab, evinacumab, aducanumab, tralokinumab, dostarlimab, teplizumab, omburtamab, inolimomb, ansuvimab, bimekizumab, balstilimab, anifrolumab, and the like; antibodies for use in the treatment or prevention of infectious diseases such as, *e.g.*, antibodies binding to the CoV spike (S) glycoprotein of SARS-CoV-2 (*e.g.*, CR3022, see Wrapp *et al.* (2020) *Science* 367:1260-1263) for use in the treatment or prevention of COVID-19), or an antibody fragment (*e.g.*, antigen-binding fragment of a monoclonal antibody, a single chain variable fragment (scFv), a Fab, a Fab', a F(ab')2, and a Fv fragment); an angiogenic agent (*e.g.*, vascular endothelial growth factor (VEGF); an anti-angiogenic agent (*e.g.*, a soluble VEGF receptor); a blood factor (*e.g.*, Activase® (alteplase) tissue plasminogen activator, NovoSeven® (recombinant human factor VIIa), coagulation Factor VIIa, coagulation Factor VIII (*e.g.*, Kogenate®), coagulation Factor IX, β-globin hemoglobin, and the like); a chemokine (*e.g.*, IP-10, Mig, Groa/IL-8, RANTES, MIP-1α, MIP-1β, MCP-1, PF-4, and the like); a cytokine; a colony stimulating factor (*e.g.*, Neupogen® (filgrastim GCSF) Neulasta® (pegfilgrastim); granulocyte colony stimulating factor (G-CSF), granulocyte-monocyte colony stimulating factor, macrophage colony stimulating factor, megakaryocyte colony stimulating factor and the like); an enzyme (*e.g.*, α-glucosidase, Cerazyme® (imiglucarase β-glucocerebrosidase, Ceredase® (alglucerase), an enzyme activator (*e.g.*, tissue plasminogen activator); an erythropoietin ("EPO", *e.g.*, Procrit®, Eprex®, or Epogen® (epoetin-α),

Aranesp® (darbepoietin-α), NeoRecormon®, Epogin® (epoetin-β) and the like); a growth hormone (*e.g.*, a somatotropin, *e.g.*, Genotropin®, Nutropin®, Norditropin®, Saizen®, Serostim®, Humatrope®, *etc.*, a human growth hormone and the like); a growth factor (*e.g.*, Regranex® (beclapermin PDGF), Fiblast® (trafermin bFGF), Stemgen® (ancestim stem cell factor), keratinocyte growth factor, an acidic fibroblast growth factor, a stem cell factor, a basic fibroblast growth factor, a hepatocyte growth factor, and the like); an insulin (*e.g.*, Novolin®, Humulin®, Humalog®, Lantus®, Ultralente, *etc.*); an interferon (*e.g.*, IFN-γ, IFN-α, IFN-β, IFN-ω, IFN-τ); an interleukin (*e.g.*, IL-1, IL-2, including, *e.g.*, Proleukin®, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, *etc.*); a soluble receptor (*e.g.*, a TNF-α-binding receptor such as Enbrel® (etanercept), a VEGF receptor, an interleukin receptor, a γ/δT cell receptor, and the like); a protein vaccine; a neuroactive peptide such as bradykinin, cholecystokinin, gastin, secretin, oxytocin, gonadotropin-releasing hormone, beta-endorphin, enkephalin, substance P, somatostatin, prolactin, galanin, growth hormone-releasing hormone, bombesin, dynorphin, neurotensin, motilin, thyrotropin, neuropeptide Y, luteinizing hormone, calcitonin, insulin, glucagon, vasopressin, angiotensin II, thyrotropin-releasing hormone, vasoactive intestinal peptide, a sleep peptide, *etc.*; other proteins such as a thrombolytic agent, an atrial natriuretic peptide, bone morphogenic protein, thrombopoietin, relaxin, glial fibrillary acidic protein, follicle stimulating hormone, a human alpha-1 antitrypsin, a leukemia inhibitory factor, a transforming growth factor, an insulin-like growth factor, a luteinizing hormone, a macrophage activating factor, tumor necrosis factor, a neutrophil chemotactic factor, a nerve growth factor a tissue inhibitor of metalloproteinases; a vasoactive intestinal peptide, angiogenin, angiotropin, fibrin, hirudin, a leukemia inhibitory factor, an IL-1 receptor antagonist (*e.g.*, Kineret® (anakinra)), an ion channel, *e.g.*, cystic fibrosis transmembrane conductance regulator (CFTR), dystrophin, utrophin, a tumor suppressor, lysosomal enzyme acid a-glucosidase (GAA) and the like. Proteins that can be delivered using a recombinant AAV vector of the present disclosure also include a functional fragment of any of the aforementioned proteins; or functional variants of any of the aforementioned proteins.

[00205] In some embodiments, the heterologous nucleic acid comprises a nucleotide sequence encoding an antigenic protein. Suitable antigenic proteins include, but are not limited to, tumor-associated antigens, autoantigens ("self" antigens), viral antigens, bacterial antigens, protozoal antigens, and allergens; and antigenic fragments thereof. In some embodiments, a cytotoxic T lymphocyte (CTL) response to the rAAV-encoded antigenic protein may be induced in the mammalian host. In other embodiments, a humoral response to the rAAV-encoded antigenic protein may be induced in the mammalian host, such that antibodies specific to the antigenic

protein are generated. In many embodiments, a TH1 immune response to the rAAV-encoded antigenic protein may be induced in the mammalian host. Whether an immune response to the antigenic protein has been generated is readily determined using well-established methods. For example, an enzyme-linked immunosorbent assay can be used to determine whether antibody to an antigenic protein has been generated. Methods of detecting antigen-specific CTL are well known in the art. For example, a detectably labeled target cell expressing the antigenic protein on its surface is used to assay for the presence of antigen-specific CTL in a blood sample.

[00206] In some embodiments, the therapeutic protein encoded by the recombinant AAV vector of the present disclosure is a blood coagulation factor. In some embodiments, the therapeutic protein encoded by the recombinant AAV vector of the present disclosure is Factor V (FV), FVII, FVIII, FIX, FX, FXI, FXIII, FII, Protein C, C1-inhibitor, prekallikrein, high molecular weight kininogen (HMWK) or von Willebrand's factor. In some embodiments, the therapeutic protein encoded by the recombinant AAV vector of the present disclosure is Factor VIII or Factor IX.

[00207] In some embodiments, the therapeutic protein is a protein used for the treatment of a liver-borne blood disorder. Such proteins are, *e.g.*, the blood coagulation factors disclosed in the preceding paragraph.

[00208] In some embodiments, the heterologous nucleic acid may comprise a nucleotide sequence encoding a reporter sequence, which upon expression produces a detectable signal. Such reporter sequences include, without limitation, DNA sequences encoding β-lactamase, β-galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), yellow fluorescent protein (YFP), chloramphenicol acetyltransferase (CAT), luciferase, membrane bound proteins including, for example, CD2, CD4, CD8, the influenza hemagglutinin protein, and others well known in the art, to which high affinity antibodies directed thereto exist or can be produced by conventional means, and fusion proteins comprising a membrane bound protein appropriately fused to an antigen tag domain from, among others, hemagglutinin or Myc.

[00209] In some embodiments, the heterologous nucleic acid may comprise a nucleotide sequence encoding an RNA molecule. Exemplary RNA molecules include tRNA, dsRNA, ribosomal RNA, catalytic RNAs (*e.g.*, ribozymes), siRNA, miRNA, small hairpin RNA (shRNA), trans-splicing RNA, antisense RNAs, and CRISPR guide RNAs (*e.g.*, sgRNA). The RNA sequence may be designed such that it inhibits or eliminates expression of a targeted nucleic acid sequence in the treated subject. Suitable target sequences for the inhibitory RNAs include oncologic targets (*e.g.*, oncogenes such as myb, myc, fyn, and the translocation gene bcr/abl, ras, src, P53, neu, trk and EGRF) and viral targets (*e.g.*, HIV, Hepatitis A, B or C,

influenza virus). Whether a therapeutically effective amount of a non-translated RNA has been delivered to a mammalian host using a subject method is readily determined using any appropriate assay. For example, where the gene product is an siRNA that inhibits HIV, viral load can be measured.

[00210] The recombinant AAV vectors described herein are contemplated for use in methods of expressing a gene of interest in a variety of cells or in a mammal. Transduction into cells lines in addition to the cell lines described herein, for example in Example 4, and other cells lines, particularly stem cells, are contemplated. In terms of *in vivo* use, the method may comprise introducing a recombinant AAV into the mammal, the recombinant AAV encoding the gene of interest and comprising a variant AAV8 capsid polypeptide described herein. The vector expressing a gene of interest is introduced to the mammal, typically by injection, intravenously, subcutaneously, parenterally, or the like. The nucleotide sequence of the gene of interest is typically operably linked to one or more other nucleotide sequences, including but not limited to the gene for a promoter; an enhancer; transcription initiation, termination sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*i.e.*, Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product.

[00211] Various expression control sequences, including promoters which are native, constitutive, inducible and/or tissue-specific, are known in the art and may be utilized in the recombinant AAV vectors of the present disclosure. Non-limiting examples of constitutive promoters include the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer), the **SV40 promoter, the dihydrofolate reductase promoter, the β-actin promoter, the phosphoglycerol** kinase (PGK) promoter, and the EF1 promoter.

[00212] In some embodiments, an inducible promoter is used. Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, *e.g.*, acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. Examples of inducible promoters regulated by exogenously supplied compounds, include, the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter,

the T7 polymerase promoter system; the ecdysone insect promoter (No et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:3346-335, the tetracycline-repressible system (Gossen *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551], the tetracycline-inducible system (Gossen *et al.* (1995) *Science* 268:1766-1769, Harvey *et al.* (1998) *Curr. Opin. Chem. Biol.* 2:512-518], the RU486-inducible system (Wang *et al.*, (1997) *Nat. Biotech.* 15:239-243 and Wang *et al.* (1997) *Gene Ther.* 4:432-441) and the rapamycin-inducible system (Magari *et al.* (1997) *J. Clin. Invest.* 100:2865-2872). Other types of inducible promoters which may be useful in this context are those which are regulated by a specific physiological state, *e.g.*, temperature, acute phase reaction, a particular differentiation state of the cell, or in replicating cells only.

[00213] In some embodiments, the native promoter of the transgene may be used. The native promoter may be preferred when it is desired that expression of the transgene should mimic the native expression. The native promoter may be used when expression of the transgene must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be included to mimic the native expression.

[00214] In some embodiments, the nucleotide sequence encoding a gene of interest is operably linked to a tissue-specific promoter. In some embodiments, the nucleotide sequence encoding a gene of interest is under control of a promoter active in a liver cell. Non-limiting examples of the liver-specific promoter include LP1 promoter, liver albumin promoter, alpha-fetoprotein promoter, alpha 1-antitrypsin promoter (*e.g.*, hAAT), transferrin transthyretin promoter (*e.g.*, hTTR, mTTR), and thyroxine binding globulin (TBG) promoter. *See*, Powell (2015) *Discov. Med.* 19(102):49-57; Kyostio-Moore (2016) *Mol. Ther. Methods Clin. Dev.* 3:16006, which are herein incorporated by reference in their entirety.

[00215] Additionally, other promoters may also be used, including but not limited to, a breast-specific promoter, a brain-specific promoter, a pancreas-specific promoter, a colon-specific promoter, a kidney-specific promoter, a bladder-specific promoter, a lung-specific promoter, a thyroid-specific promoter, a stomach-specific promoter, a prostate-specific promoter, an ovary-specific promoter, or a cervix-specific promoter. Non-limiting examples of the breast-specific promoter include c-erb-B2 promoter, erb-B3 promoter, β-casein, β-lacto-globulin, and WAB (whey acidic protein) promoter. Non-limiting examples of the brain-specific promoter include glial fibrillary acidic protein promoter, mature astrocyte specific protein promoter, myelin promoter, and tyrosine hydroxylase promoter. Non-limiting examples of the pancreas-specific promoter include villin promoter, glucagon promoter, and Insulin Islet amyloid polypeptide

(amylin) promoter. Non-limiting examples of the colon-specific promoter include carbonic anhydrase I promoter and carcinoembryonic antigen promoter (CEA). Non-limiting examples of the kidney-specific promoter include renin promoter, liver/bone/kidney alkaline phosphatase promoter, and erythropoietin (epo) promoter. Non-limiting examples of the lung-specific promoter include surfactant protein C Uroglobin (cc-10, Cllacell 10 kd protein) promoter. Non-limiting examples of the thyroid-specific promoter include thyroglobulin promoter, and calcitonin promoter. Non-limiting examples of the bone-specific promoter include Alpha 1 (I) collagen promoter, osteocalcin promoter, and bone sialoglycoprotein promoter. Non-limiting examples of the skin-specific promoter include K-14-keratin promoter, human keratin 1 or 6 promoter, and loicrin promoter. Non-limiting examples of the prostate-specific promoter include **prostate specific antigen (PSA) promoter and its mutants including ΔPSA, ARR2PB and** probasin (PB) promoters, gp91-phox gene promoter, and prostate-specific kallikrein (hKLK2) promoter. Non-limiting examples of the ovary- or placenta-specific promoter include estrogen-responsive promoter, aromatase cytochrome P450 promoter, cholesterol side chain cleavage P450 promoter, 17 alpha-hydroxylase P450 promoter.

[00216] The AAV vectors comprising a variant AAV8 capsid polypeptide described herein may exhibit an improved immune escaping capacity.

Host Cells

[00217] In one aspect, the present disclosure provides a host cell comprising a recombinant vector comprising a variant AAV8 capsid polypeptide described herein or a recombinant vector comprising a nucleic acid encoding a variant AAV8 capsid polypeptide described herein.

[00218] Host cells may be selected from any biological organism, including prokaryotic (*e.g.*, bacterial) cells, and eukaryotic cells, including and not limited to, insect cells, yeast cells and mammalian cells. Exemplary host cells include those selected from mammalian species, including, without limitation, cells such as A549, WEHI, 3T3, 10T1/2, BHK, MDCK, COS 1, COS 7, C3H10T1/2 fibroblasts, BSC 1, BSC 40, BMT 10, VERO, W138, HeLa, 293 cells (which express functional adenoviral E1), Saos, C2C12, L cells, HT1080, HepG2 and primary fibroblast, hepatocyte and myoblast cells derived from mammals including human, monkey, mouse, rat, rabbit, and hamster. A particular requirement for the cell used is that it not carry any adenovirus gene other than E1, E2a and/or E4 ORF6; it not contain any other virus gene which could result in homologous recombination of a contaminating virus during the production of rAAV; and it is capable of infection or transfection of DNA and expression of the transfected DNA. In one embodiment, the host cell is one that has rep and cap stably transfected in the cell.

[00219] The host cell of the present disclosure can be used for production of the AAV vectors of the present invention. When the host cell of the present invention is used for producing vectors, the host cell may be referred to as a "packaging cell" or "producer cell". The host cell of the present disclosure may comprise the recombinant vector of the present disclosure as described herein integrated into the genome or retain the recombinant vector in the cell so as to transiently express the variant AAV capsid polypeptide(s).

[00220] One host cell useful in the present disclosure is a host cell stably transformed with the sequences encoding rep and cap, and which is transfected with the adenovirus E1, E2a, and E40RF6 DNA and a construct carrying the heterologous nucleic acid as described above. Stable rep and/or cap expressing cell lines, such as B-50 (*See, e.g.*, US Patent No. 7,238,526, incorporated herein by reference in its entirety), or those described in U.S. Patent No. 5,658,785, incorporated herein by reference in its entirety, may also be similarly employed. Another desirable host cell contains the minimum adenoviral DNA which is sufficient to express E4 ORF6. Yet other cell lines can be constructed using the variant AAV8 capsid sequences of the present disclosure.

[00221] The preparation of a host cell according to this disclosure involves techniques such as assembly of selected DNA sequences. This assembly may be accomplished utilizing conventional techniques. Such techniques include cDNA and genomic cloning, which are well known (*See, e.g.*, Sambrook *supra*), use of overlapping oligonucleotide sequences of the adenovirus and AAV genomes, combined with polymerase chain reaction, synthetic methods, and any other suitable methods which provide the desired nucleotide sequence.

[00222] Introduction of the molecules (as plasmids or viruses) into the host cell may also be accomplished using techniques known to the skilled artisan and as discussed throughout the specification. In some embodiment, standard transfection techniques are used, *e.g.*, polyethylenimine (PEI)-mediated transfection, $CaPO_4$ transfection or electroporation, and/or infection by hybrid adenovirus/AAV vectors into cell lines such as the human embryonic kidney cell line HEK 293 (a human kidney cell line containing functional adenovirus E1 genes which provides trans-acting E1 proteins). Other techniques, such as direct microinjection into cells, liposome-mediated gene transfection, or nucleic acid delivery using a high-speed particle gun can also be used.

AAV Production

[00223] AAV vectors of the present disclosure may be prepared using methods and techniques known in the art and as described in the Examples section below. For example, the AAV preparation may be produced by transfected host cells. In certain embodiments, the AAV

preparation represents a supernatant harvested or cell suspension from a cell culture comprising host cells transfected with a triple plasmid system, wherein one plasmid of the system comprises a gene or cDNA of interest, one plasmid encodes a capsid protein. In certain embodiments, the capsid protein is a variant AAV8 capsid protein described herein. Triple plasmid transfection for purposes of rAAV production is known in the art. *See, e.g.*, Qu *et al.* (2015), *Curr Pharm Biotechnol. 16(8): 684–695,* and Mizukami *et al.* (1998) "A Protocol for AAV vector production and purification." PhD dissertation, Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical School; and Kotin *et al.* (2011) *Hum. Mol. Genet.* 20(R1):R2-R6. In certain embodiments, the transfection may be carried out using inorganic compounds, *e.g.*, calcium phosphate, or organic compounds, polyethylenimine (PEI), or non-chemical means, *e.g.*, electroporation.

[00224] In certain embodiments, the host cells are adherent cells. In certain embodiments, the host cells are suspension cells. In certain embodiments, the host cells are HEK293 cells or Sf9 cells (*e.g.*, baculovirus infected Sf9 cells) or HeLa or BHK (Herpes Virus System). In certain embodiments, the cell culture comprises culture medium which is serum and protein free. In certain embodiments, the medium is chemically defined and is free of animal derived components, *e.g.*, hydrolysates.

[00225] In certain embodiments, the preparation comprising recombinant AAV particles represents a preparation comprising HEK293 cells transfected with a triple plasmid system. In certain embodiments, the preparation comprising AAV particles represents a preparation of the harvest after about 2 to about 7 days after transfection of the HEK293 cells or when the cell culture has a cell density of greater than or about $5x10^6$ cells/mL and has a cell viability of greater than or about 50%.

[00226] In certain embodiments, the AAV is prepared by a triple plasmid transfection followed by harvest from one to 7 days later. In certain embodiments, the AAV is prepared from cell disruption.

[00227] In certain embodiments, the AAV is prepared by the following: The HEK293 cells are adherent and grown in a commercially-available culture medium that may be chemically-defined and may be free of animal-derived components, *e.g.*, serum and proteins. The cells are cultured to a cell density of about 3 x 10^6 to about 12 x 10^6 cells/ml, *e.g.*, about 6 x 10^6 to about 10 x 10^6 cells/ml, which leads to a confluence of 50-80% when the cells adhere to the surface. The cells are then split in about a 1:2 ratio such that the cell density is about 3 – 5 x 10^6 cells/ml. After the split and after the cells are allowed sufficient time to adhere firmly to the growth surface, the cells may be transfected with three plasmids that include (1) a helper plasmid capable

of providing one or more helper viral functions essential AAV production, (2) a plasmid that encodes for one or more genes involved in capsid generation, replication and packaging of the virus, and (3) a plasmid comprising a gene of interest (GOI) to be packaged into the resulting rAAV particle. For example, the GOI may be a vector DNA comprising human coagulation Factor IX Padua in a single stranded self-complementary form, with the vector DNA. As another example, the GOI may be a vector DNA comprising human coagulation Factor IX Padua in a double stranded self-complementary form, with the vector DNA having a full length of 4.8 kB. As another example, the GOI may be a vector DNA comprising a B-domain deleted human coagulation Factor VIII in a single stranded self-complementary form, with the vector DNA, included by ITRs, having a full length of 4.8 kB, after rescue from the plasmid vector. Other GOI may be used. Transfection may be carried out in a transient manner, such as by using cationic polymers. Before elution, the HEK293 cell line may be cultivated for at least about 1 days, *e.g.*, 3-5 days, before harvesting.

[00228] In some embodiments, the AAV preparation is a concentrated AAV preparation. In certain embodiments, the AAV preparation comprises at least about 1×10^{10}, about 1×10^{11}, about 1×10^{12}, about 1×10^{13}, about 1×10^{14}, or about 1×10^{15}AAV total particles per mL. In certain embodiments, the AAV preparation comprises at least about 1×10^{12} AAV total particles per mL. The AAV particles may include empty AAV capsids and full AAV capsids.

[00229] In certain embodiments, the preparation comprising AAV particles may be performed as described in U.S. Publication No. US20190365835, which is incorporated herein in its entirety.

[00230] Purification of the AAV vectors of the present disclosure may be carried out using standard techniques known in the art and may optionally be combined with one or more additional steps.

[00231] In exemplary embodiments, purification of the AAV vectors of the present disclosure may comprise an ultracentrifugation step during which a density gradient is formed. Though not wishing to be bound to a theory, it is believed that the ultracentrifugation step allows for full AAV capsids to be partially separated from empty AAV capsids. Examples of ultracentrifugation protocols can be found in, for example, US Patent No. 8,969,533 and U.S. Publication No. US20190365835, each of which are incorporated herein in their entirety for all purposes.

[00232] Purification of the AAV vectors of the present disclosure may comprise yet other additional steps, which may further increase the purity of the AAV and remove other unwanted

components and/or concentrate the preparation and/or condition the preparation for a subsequent step.

[00233] In certain embodiments, purification comprises a depth filtration step. In certain embodiments, purification comprises subjecting a fraction of a transfected HEK293 cell culture supernatant to depth filtration using a filter comprising cellulose and perlites and having a minimum permeability of about 500L/m^2. In certain embodiments, purification further comprises use of a filter having a minimum pore size of about 0.2 μm. In certain embodiments, the depth filtration is followed by filtration through the filter having a minimum pore size of about 0.2 μm. In certain embodiments, one or both of the depth filter and filter having a minimum pore size of about 0.2 μm are washed and the washes are collected. In certain embodiments, the washes are pooled together and combined with the filtrate obtained upon depth filtration and filtration with the filter having a minimum pore size of about 0.2 μm. In certain embodiments, the depth filtration step and other filtration step occurs prior to the ultracentrifugation step described herein.

[00234] In certain embodiments, purification of the AAV vectors of the present disclosure may comprise one or more chromatography steps. In certain embodiments, purification comprises a negative chromatography step whereby unwanted components bind to the chromatography resin and the desired AAV does not bind to the chromatography resin. In certain embodiments, purification comprises a negative anion exchange (AEX) chromatography step, or an AEX chromatography step in the "non-binding mode". Advantages of "non-binding mode" include relative ease of carrying out the procedure and in conducting subsequent assaying. Accordingly, in certain embodiments, the methods of purifying AAV particles comprise performing negative anion exchange (AEX) chromatography on a fraction comprising AAV particles by applying the fraction to an AEX chromatography column or membrane under conditions that allow for the AAV to flow through the AEX chromatography column or membrane and collecting AAV particles. In certain embodiments, the fraction is applied to the AEX chromatography column or membrane with a loading buffer comprising about 100 mM to about 150 mM salt, *e.g.*, NaCl, optionally, wherein the pH of the loading buffer is about 8 to about 9. In certain embodiments, the loading buffer comprises about 115 mM to about 130 mM salt, *e.g.*, NaCl, optionally, wherein the loading buffer comprises about 120 mM to about 125 mM salt, *e.g.*, NaCl. In certain embodiments, the negative AEX step occurs prior to the ultracentrifugation step described herein.

[00235] In certain embodiments, purification of the AAV vectors of the present disclosure may comprise concentrating an AAV fraction using an ultra/diafiltration system. In certain

embodiments, purification methods comprise one more tangential flow filtration (TFF) steps. In certain embodiments, the AAV fraction undergoes ultra/diafiltration. In certain embodiments, the AAV fraction is concentrated with the ultra/diafiltration system before a step comprising performing negative AEX chromatography, after a step comprising performing negative AEX chromatography, or before and after comprising performing negative AEX chromatography.

[00236] In certain embodiments, the methods of the present disclosure comprise treating a fraction comprising AAV particles with a solvent detergent to inactivate lipid enveloped viruses.

[00237] In certain embodiments, purification of the AAV vectors of the present disclosure comprises filtration of a fraction comprising rAAV particles to remove viruses of greater size than the rAAV particles in the fraction. In certain embodiments, purification of the AAV vectors of the present disclosure comprises filtration of a fraction comprising AAV to remove viruses sized greater than or about 35 nm. In certain embodiments, the pore size of the filter is in the nanometer range, and, in certain embodiments, the purification method comprises nanofiltration. In certain embodiments, the purification method of the present disclosure comprises use of a nanofilter of pore size in the range of 35 nanometer ± 2 nanometer, as determined by a water flow method. Classification of the type of filter is dependent on membrane structure, material, and vendor.

[00238] In certain embodiments, during the filtration step, a pressure difference over the filter is maintained. In certain embodiments, the pressure (pressure drop across the filter) is about 0.02 MPa to about 0.1 MPa. In certain embodiments, the pressure (*e.g.*, pressure drop across the filter) is about 0.02 MPa to about 0.08 MPa. In case the filter is run in dead-end mode, the pressure difference can be affected by the feed pressure of the sample applied (*i.e.*, by adjustment of a pump to a specific flow, which affects the feed pressure).

[00239] In certain embodiments, the filtration step for removal of viruses larger than the rAAV particles occurs once during the process of the present disclosure. In certain embodiments, the filtration step occurs twice during the process. In certain embodiments, the filtration step for removal of viruses larger than the rAAV particles occurs after the ultracentrifugation step described herein. In certain embodiments, the filtration step for removal of viruses larger than the rAAV particles occurs after a polish step.

[00240] In certain embodiments, purification of the AAV vectors of the present disclosure comprises a polish step comprising performing AEX chromatography, optionally with a column comprising tentacle gel.

Pharmaceutical Compositions

[00241] In one aspect, the present disclosure provides a pharmaceutical composition comprising a recombinant vector (*e.g.*, recombinant AAV vector) described herein. The pharmaceutical composition containing a recombinant vector (*e.g.*, recombinant AAV vector) of the disclosure, *e.g.*, contains a pharmaceutically acceptable excipient, diluent or carrier. Examples of suitable pharmaceutical carriers and/or excipients are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions *etc.* Such carriers can be formulated by conventional methods and can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, *e.g.*, by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. The route of administration may depend on the kind of vector contained in the pharmaceutical composition. The dosage regimen will be determined by the attending physician and other clinical factors. As is well known in the medical arts, dosages for any one patient depends on many factors, including the patient's size, body surface area, age, sex, the particular compound to be administered, time and route of administration, the kind and stage of infection or disease, general health and other drugs being administered concurrently.

Therapeutic Methods

[00242] In one aspect, the present disclosure provides a method of delivering a gene product to a subject in need thereof, said method comprising administering to the subject an effective amount of the AAV vectors described herein or the pharmaceutical composition described herein. In some embodiments, the subject has existing neutralizing antibodies against AAV8 prior to the administration.

[00243] In some embodiments, for the therapeutic methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1×10^5 vg to about 1×10^{16} vg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1×10^6 vg to about 1×10^9 vg, about 1×10^7 vg to about 1×10^{10} vg, about 1×10^8 vg to about 1×10^{11} vg, about 1×10^9 vg to about 1×10^{12} vg, about 1×10^{10} vg to about 1×10^{13} vg, about 1×10^{11} vg to about 1×10^{14} vg, about 1×10^{12} vg to about 1×10^{15} vg, about 1×10^{13} vg to about 1×10^{16} vg, or about 1×10^{14} vg to about 1×10^{16} vg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1×10^{10} vg to about 1×10^{16} vg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vector vectors administered to the subject is at least about 1×10^6 vg, at least about 1×10^7 vg, at least about 1×10^8 vg, at

least about 1 x 10^9 vg, at least about 1 x 10^{10} vg, at least about 1 x 10^{11} vg, at least about 1 x 10^{12} vg, at least about 5 x 10^{12} vg, at least about 1 x 10^{13} vg, at least about 1 x 10^{14} vg, or at least about 1 x 10^{15} vg. In certain embodiments, the vg is total vector genome per subject.

[00244] In certain embodiments, for the therapeutic methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1 x 10^5 vg/kg to about 1 x 10^{14} vg/kg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1 x 10^6 vg/kg to about 1 x 10^8 vg/kg, about 1 x 10^7 vg/kg to about 1 x 10^9 vg/kg , about 1 x 10^8 vg/kg to about 1 x 10^{10} vg/kg, about 1 x 10^9 vg/kg to about 1 x 10^{11} vg/kg, about 1 x 10^{10} vg/kg to about 1 x 10^{12} vg/kg, about 1 x 10^{11} vg/kg to about 1 x 10^{13} vg/kg, or about 1 x 10^{12} vg/kg to about 1 x 10^{14} vg/kg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1 x 10^{10} vg/kg to about 1 x 10^{16} vg/kg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vector vectors administered to the subject is at least about 1 x 10^6 vg/kg, at least about 1 x 10^7 vg/kg, at least about 1 x 10^8 vg/kg, at least about 1 x 10^9 vg/kg, at least about 1 x 10^{10} vg/kg, at least about 1 x 10^{11} vg/kg, at least about 1 x 10^{12} vg/kg, or at least about 1 x 10^{13} vg/kg. In certain embodiments, the vg/kg is total vector genome per kg of the subject.

[00245] In some embodiments, a therapeutically effective amount of a protein or peptide encoded by the rAAV vectors is produced in the mammalian host. Whether a therapeutically effective amount of a particular protein is produced in the mammalian host using a subject method is readily determined using assays appropriate to the particular protein. For example, where the protein is EPO, hematocrit is measured.

[00246] The AAV vectors or the pharmaceutical compositions described herein may be administered to the subject via any route, for example and without limitation, intravenous, parenteral, intramuscular, subcutaneous, intratumor, intradermal, transdermal, intranodal, intraspinal, intraprostatic, intralymphatic, intraparenchymal, intraportal or intraperitoneal route injection. In one embodiment, the AAV vector(s) or the pharmaceutical composition(s) described herein is administered to the subject via intravenous injection.

[00247] The AAV vectors or the pharmaceutical compositions described herein may be administered at least once in order to treat or ameliorate or prevent a disease or condition described herein. In some embodiments, the AAV vectors or the pharmaceutical compositions may be administered more than one time, for example from one to four times daily up to a non-limited number of days.

[00248] The gene product delivered via the recombinant AAV vectors to a subject may be used for the treatment or prevention of a disease or disorder that arise from or are related to liver cells and/or liver function. For example, the gene product and associated disease states include, but are not limited to: glucose-6-phosphatase, associated with glycogen storage deficiency type 1A; phosphoenolpyruvate-carboxykinase, associated with Pepck deficiency; galactose-1 phosphate uridyl transferase, associated with galactosemia; phenylalanine hydroxylase, associated with phenylketonuria; branched chain alpha-ketoacid dehydrogenase, associated with Maple syrup urine disease; fumarylacetoacetate hydrolase, associated with tyrosinemia type 1; methylmalonyl-CoA mutase, associated with methylmalonic acidemia; medium chain acyl CoA dehydrogenase, associated with medium chain acetyl CoA deficiency; ornithine transcarbamylase, associated with ornithine transcarbamylase deficiency; argininosuccinic acid synthetase, associated with citrullinemia; low density lipoprotein receptor protein, associated with familial hypercholesterolemia; UDP-glucuronosyltransferase, associated with Crigler-Najjar disease; adenosine deaminase, associated with severe combined immunodeficiency disease; hypoxanthine guanine phosphoribosyl transferase, associated with Gout and Lesch-Nyan syndrome; biotinidase, associated with biotinidase deficiency; beta-glucocerebrosidase, associated with Gaucher disease; beta-glucuronidase, associated with Sly syndrome; peroxisome membrane protein 70 kDa, associated with Zellweger syndrome; porphobilinogen deaminase, associated with acute intermittent porphyria; alpha-1 antitrypsin for treatment of alpha-1 antitrypsin deficiency (emphysema); and a tumor suppressor gene such as p53 for the treatment of various cancers.

[00249] In some embodiments, the gene product delivered via the recombinant AAV vectors to a subject may be used for the treatment or prevention of a coagulation disorder. "Coagulation disorders" include bleeding disorders caused by deficient blood coagulation factor activity and deficient platelet activity. Blood coagulation factors include, but are not limited to, Factor V (FV), FVII, FVIII, FIX, FX, FXI, FXIII, FII (responsible for hypoprothrombinemia), Protein C, and von Willebrand's factor. Factor deficiencies are caused by, for instance, a shortened in vivo-half-life of the factor, altered binding properties of the factor, genetic defects of the factor, and a reduced plasma concentration of the factor. Coagulation disorders can be congenital or acquired. Potential genetic defects include deletions, additions and/or substitution within a nucleotide sequence encoding a clotting factor whose absence, presence, and/or substitution, respectively, has a negative impact on the clotting factor's activity. Coagulation disorders also stem from development of inhibitors or autoimmunity (*e.g.*, antibodies) against clotting factors.

In one example, the coagulation disorder is hemophilia A. Alternatively, the coagulation disorder is hemophilia B or hemophilia C.

[00250] Platelet disorders are caused by deficient platelet function or abnormally low platelet number in circulation. Low platelet count may be due to, for instance, underproduction, platelet sequestration, or uncontrolled patent destruction. Thrombocytopenia (platelet deficiencies) may be present for various reasons, including chemotherapy and other drug therapy, radiation therapy, surgery, accidental blood loss, and other disease conditions. Exemplary disease conditions that involve thrombocytopenia are: aplastic anemia; idiopathic or immune thrombocytopenia (ITP), including idiopathic thrombocytopenic purpura associated with breast cancer; HIV-associated ITP and HIV-related thrombotic thrombocytopenic purpura; metastatic tumors which result in thrombocytopenia; systemic lupus erythematosus, including neonatal lupus syndrome splenomegaly; Fanconi's syndrome; vitamin B12 deficiency; folic acid deficiency; May-Hegglin anomaly; Wiskott-Aldrich syndrome; chronic liver disease; myelodysplastic syndrome associated with thrombocytopenia; paroxysmal nocturnal hemoglobinuria; acute profound thrombocytopenia following C7E3 Fab (Abciximab) therapy; alloimmune thrombocytopenia, including maternal alloimmune thrombocytopenia; thrombocytopenia associated with antiphospholipid antibodies and thrombosis; autoimmune thrombocytopenia; drug-induced immune thrombocytopenia, including carboplatin-induced thrombocytopenia and heparin-induced thrombocytopenia; fetal thrombocytopenia; gestational thrombocytopenia; Hughes' syndrome; lupoid thrombocytopenia; accidental and/or massive blood loss; myeloproliferative disorders; thrombocytopenia in patients with malignancies; thrombotic thrombocytopenia purpura, including thrombotic microangiopathy manifesting as thrombotic thrombocytopenic purpura/hemolytic uremic syndrome in cancer patients; post-transfusion purpura (PTP); autoimmune hemolytic anemia; occult jejunal diverticulum perforation; pure red cell aplasia; autoimmune thrombocytopenia; nephropathia epidemica; rifampicin-associated acute renal failure; Paris-Trousseau thrombocytopenia; neonatal alloimmune thrombocytopenia; paroxysmal nocturnal hemoglobinuria; hematologic changes in stomach cancer; hemolytic uremic syndromes (e.g., uremic conditions in childhood); and hematologic manifestations related to viral infection including hepatitis A virus and CMV-associated thrombocytopenia. Platelet disorders also include, but are not limited to, Von Willebrand Disease, paraneoplastic platelet dysfunction, Glanzman's thrombasthenia, and Bernard-Soulier disease. Additional bleeding disorders amenable to treatment with a recombinant AAV vector of the present disclosure include, but are not limited to, hemorrhagic conditions induced by trauma; a deficiency in one or more contact factors, such as FXI, FXII,

prekallikrein, C1-inihibitor and high molecular weight kininogen (HMWK); vitamin K deficiency; a fibrinogen disorder, including afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia; and alpha2-antiplasmin deficiency. All of the above are considered "blood coagulation disorders" in the context of the disclosure.

[00251] Other suitable therapeutic polypeptides and proteins include those which may be useful for treating individuals suffering from autoimmune diseases and disorders by conferring a broad based protective immune response against targets that are associated with autoimmunity including cell receptors and cells which produce "self"-directed antibodies. T cell mediated autoimmune diseases include Rheumatoid arthritis (RA), multiple sclerosis (MS), Sjögren's syndrome, sarcoidosis, insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma, polymyositis, dermatomyositis, psoriasis, vasculitis, Wegener's granulomatosis, Crohn's disease and ulcerative colitis.

[00252] In some embodiments, for all therapeutic methods disclosed herein, the subject is a human or non-human animal (*e.g.*, chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats, rabbit and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, or geese). In some embodiments, the subject is a human. In some embodiments, the subject is a non-human, such as a mouse, a rat, a mouse, a rabbit, a dog, a cat, a sheep, a pig, or a non-human primate.

[00253] In one aspect, provided herein are methods of treating liver-borne blood disorders in a human subject in need thereof, said methods comprising administering to the subject an effective amount of an adeno-associated virus (AAV) vector described herein or a pharmaceutical composition described herein, wherein the AAV vector comprises a heterologous nucleic acid comprising a nucleotide sequence encoding a therapeutic protein, wherein the therapeutic protein is a protein used for the treatment of a liver-borne blood disorder. The adeno-associated virus (AAV) vector or the pharmaceutical composition can for example comprise a variant AAV8 capsid polypeptide according to the present disclosure that comprises a peptide insertion selected from the group consisting of NSVRGDG (SEQ ID NO: 122), GNDVRAR (SEQ ID NO: 123) and SRGATVL (SEQ ID NO: 124) after amino acid 590 relative to the wild-type AAV8 capsid polypeptide. In some embodiments, the peptide insertion further comprises a G at the N-terminus and an A at the C-terminus. In some embodiments, the three amino acids preceding the site into which said peptide is inserted into the AAV8 capsid

polypeptide have been changed to GQS or GQR and/or the three amino acids following the site into which said peptide is inserted have been changed to QAA.

[00254] In some embodiments, the adeno-associated virus (AAV) vector administered in this method comprises a variant AAV8 capsid polypeptide that comprises a peptide insertion selected from the group consisting of NSVRGDG (SEQ ID NO: 122), GNDVRAR (SEQ ID NO: 123) and SRGATVL (SEQ ID NO: 124) after amino acid 590 relative to the wild-type AAV8 capsid polypeptide, and at least one of the alanine substitution(s) as disclosed herein. Thus, in some embodiments, the variant AAV8 capsid polypeptide comprises a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and one of the following sets of alanine substitution(s): (i) T711A and T719A; (ii) G456A, S466A, N471A, T711A, and T719A; (iii) T711A; and (iv) G456A, N471A, and T493A. In some embodiments, the variant AAV8 capsid polypeptide comprises a peptide insertion of GNDVRAR (SEQ ID NO: 123) after amino acid 590, and alanine substitutions T711A and T719A. In some embodiments, the variant AAV8 capsid polypeptide comprises a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and one of the following sets of alanine and/or glutamic acid substitution(s): (i) G456A, T493A, N498A, T591A, T711A, and T719E; (ii) G456A, S466A, N471A, T711A, and T719A; (iii) G456A, N471A, and T493A; and (iv) T711A, and T719A.

[00255] In some embodiments, for the therapeutic methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1×10^5 vg to about 1×10^{16} vg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1×10^6 vg to about 1×10^9 vg, about 1×10^7 vg to about 1×10^{10} vg, about 1×10^8 vg to about 1×10^{11} vg, about 1×10^9 vg to about 1×10^{12} vg, about 1×10^{10} vg to about 1×10^{13} vg, about 1×10^{11} vg to about 1×10^{14} vg, about 1×10^{12} vg to about 1×10^{15} vg, about 1×10^{13} vg to about 1×10^{16} vg, or about 1×10^{14} vg to about 1×10^{16} vg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1×10^{10} vg to about 1×10^{16} vg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vector vectors administered to the subject is at least about 1×10^6 vg, at least about 1×10^7 vg, at least about 1×10^8 vg, at least about 1×10^9 vg, at least about 1×10^{10} vg, at least about 1×10^{11} vg, at least about 1×10^{12} vg, at least about 5×10^{12} vg, at least about 1×10^{13} vg, at least about 1×10^{14} vg, or at least about 1×10^{15} vg. In certain embodiments, the vg is total vector genome per subject.

[00256] In certain embodiments, for the therapeutic methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1×10^5 vg/kg to about 1×10^{14} vg/kg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vectors

administered to the subject is between about 1 x 10^6 vg/kg to about 1 x 10^8 vg/kg, about 1 x 10^7 vg/kg to about 1 x 10^9 vg/kg , about 1 x 10^8 vg/kg to about 1 x 10^{10} vg/kg, about 1 x 10^9 vg/kg to about 1 x 10^{11} vg/kg, about 1 x 10^{10} vg/kg to about 1 x 10^{12} vg/kg, about 1 x 10^{11} vg/kg to about 1 x 10^{13} vg/kg, or about 1 x 10^{12} vg/kg to about 1 x 10^{14} vg/kg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vectors administered to the subject is between about 1 x 10^{10} vg/kg to about 1 x 10^{16} vg/kg. In certain embodiments, for the methods disclosed herein, the dose of the AAV vector vectors administered to the subject is at least about 1 x 10^6 vg/kg, at least about 1 x 10^7 vg/kg, at least about 1 x 10^8 vg/kg, at least about 1 x 10^9 vg/kg, at least about 1 x 10^{10} vg/kg, at least about 1 x 10^{11} vg/kg, at least about 1 x 10^{12} vg/kg, at least about 1 x 10^{13} vg/kg. In certain embodiments, the vg/kg is total vector genome per kg of the subject.

[00257] In some embodiments, for the therapeutic methods disclosed herein, a therapeutically effective amount of a protein or peptide encoded by the rAAV vectors is produced in the mammalian host. Whether a therapeutically effective amount of a particular protein is produced in the mammalian host using a subject method is readily determined using assays appropriate to the particular protein. For example, where the protein is EPO, hematocrit is measured.

[00258] The AAV vectors or the pharmaceutical compositions described herein may be administered to the subject via any route, for example and without limitation, intravenous, parenteral, intramuscular, subcutaneous, intratumor, intradermal, transdermal, intranodal, intraspinal, intraprotatic, intralymphatic, intraparenchymal, intraportal or intraperitoneal route injection. In one embodiment, the AAV vector(s) or the pharmaceutical composition(s) described herein is administered to the subject via intravenous injection.

[00259] The AAV vectors or the pharmaceutical compositions described herein may be administered at least once in order to treat or ameliorate a liver-borne blood disorder. In some embodiments, the AAV vectors or the pharmaceutical compositions may be administered more than one time, for example from one to four times daily up to a non-limited number of days.

[00260] The gene product delivered via the recombinant AAV vectors to a subject may be used for the treatment of a liver-borne blood disorder. A “liver-borne blood disorder” is for example a coagulation disorder or a blood coagulation disorder as disclosed in the next two paragraphs, optionally disclosed therein together with corresponding therapeutic proteins to be used for the treatment of a liver-borne blood disorder.

[00261] In some embodiments, the gene product delivered via the recombinant AAV vectors to a subject may be used for the treatment of a coagulation disorder. “Coagulation disorders” include bleeding disorders caused by deficient blood coagulation factor activity and deficient

platelet activity. Blood coagulation factors include, but are not limited to, Factor V (FV), FVII, FVIII, FIX, FX, FXI, FXIII, FII (responsible for hypoprothrombinemia), Protein C, and von Willebrand's factor. Factor deficiencies are caused by, for instance, a shortened *in vivo*-half-life of the factor, altered binding properties of the factor, genetic defects of the factor, and a reduced plasma concentration of the factor. Coagulation disorders can be congenital or acquired. Potential genetic defects include deletions, additions and/or substitution within a nucleotide sequence encoding a clotting factor whose absence, presence, and/or substitution, respectively, has a negative impact on the clotting factor's activity. Coagulation disorders also stem from development of inhibitors or autoimmunity (*e.g.*, antibodies) against clotting factors. In one example, the coagulation disorder is hemophilia A. Alternatively, the coagulation disorder is hemophilia B or hemophilia C.

[00262] Platelet disorders are caused by deficient platelet function or abnormally low platelet number in circulation. Low platelet count may be due to, for instance, underproduction, platelet sequestration, or uncontrolled patent destruction. Thrombocytopenia (platelet deficiencies) may be present for various reasons, including chemotherapy and other drug therapy, radiation therapy, surgery, accidental blood loss, and other disease conditions. Exemplary disease conditions that involve thrombocytopenia are: aplastic anemia; idiopathic or immune thrombocytopenia (ITP), including idiopathic thrombocytopenic purpura associated with breast cancer; HIV-associated ITP and HIV-related thrombotic thrombocytopenic purpura; metastatic tumors which result in thrombocytopenia; systemic lupus erythematosus, including neonatal lupus syndrome splenomegaly; Fanconi's syndrome; vitamin B12 deficiency; folic acid deficiency; May-Hegglin anomaly; Wiskott-Aldrich syndrome; chronic liver disease; myelodysplastic syndrome associated with thrombocytopenia; paroxysmal nocturnal hemoglobinuria; acute profound thrombocytopenia following C7E3 Fab (Abciximab) therapy; alloimmune thrombocytopenia, including maternal alloimmune thrombocytopenia; thrombocytopenia associated with antiphospholipid antibodies and thrombosis; autoimmune thrombocytopenia; drug-induced immune thrombocytopenia, including carboplatin-induced thrombocytopenia and heparin-induced thrombocytopenia; fetal thrombocytopenia; gestational thrombocytopenia; Hughes' syndrome; lupoid thrombocytopenia; accidental and/or massive blood loss; myeloproliferative disorders; thrombocytopenia in patients with malignancies; thrombotic thrombocytopenia purpura, including thrombotic microangiopathy manifesting as thrombotic thrombocytopenic purpura/hemolytic uremic syndrome in cancer patients; post-transfusion purpura (PTP); autoimmune hemolytic anemia; occult jejunal diverticulum perforation; pure red cell aplasia; autoimmune thrombocytopenia; nephropathia epidemica;

rifampicin-associated acute renal failure; Paris-Trousseau thrombocytopenia; neonatal alloimmune thrombocytopenia; paroxysmal nocturnal hemoglobinuria; hematologic changes in stomach cancer; hemolytic uremic syndromes (*e.g.*, uremic conditions in childhood); and hematologic manifestations related to viral infection including hepatitis A virus and CMV-associated thrombocytopenia. Platelet disorders also include, but are not limited to, Von Willebrand Disease, paraneoplastic platelet dysfunction, Glanzman's thrombasthenia, and Bernard-Soulier disease. Additional bleeding disorders amenable to treatment with a recombinant AAV vector of the present disclosure include, but are not limited to, hemorrhagic conditions induced by trauma; a deficiency in one or more contact factors, such as FXI, FXII, prekallikrein, C1-inihibitor and high molecular weight kininogen (HMWK); vitamin K deficiency; a fibrinogen disorder, including afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia; and alpha2-antiplasmin deficiency. All of the above are considered "blood coagulation disorders" in the context of the disclosure.

Exemplary Embodiments

[00263] Embodiment 1. A variant adeno-associated virus 8 (AAV8) capsid polypeptide comprising one or more mutations relative to a wild-type AAV8 capsid polypeptide in one or more regions (according to VP1 numbering) selected from the group consisting of amino acids 262-274, amino acids 328-333, amino acids 383-391, amino acids 452-471, amino acids 490-507, amino acids 528-545, amino acids 547-564, amino acids 582-597, and amino acids 706-720.

[00264] Embodiment 2. The variant AAV8 capsid polypeptide of embodiment 1, wherein the one or more mutations are one or more amino acid substitutions.

[00265] Embodiment 3. The variant AAV8 capsid polypeptide of embodiment 2, wherein the one or more amino acid substitutions are one or more alanine substitutions and/or glutamic acid substitutions.

[00266] Embodiment 4. The variant AAV8 capsid polypeptide any one of embodiments 1-3, wherein the variant AAV8 capsid polypeptide comprises one or more amino acid substitutions at positions G455, G456, S466, N471, V491, T493, N498, K547, Q548, D584, T591, T711, or T719 relative to a wild-type AAV8 capsid polypeptide.

[00267] Embodiment 5. The variant AAV8 capsid polypeptide of any one of embodiments 1-4, further comprising a peptide insertion selected from the group consisting of NSVRGDG (SEQ ID NO: 122), GNDVRAR (SEQ ID NO: 123), and SRGATVL (SEQ ID NO: 124) after amino acid 590 relative to the wild-type AAV8 capsid polypeptide.

[00268] Embodiment 6. The variant AAV8 capsid polypeptide of embodiment 5, wherein the peptide insertion further comprises a G at the N-terminus and an A at the C-terminus.

[00269] Embodiment 7. The variant AAV8 capsid polypeptide of embodiment 6, wherein the three amino acids preceding the site into which said peptide is inserted into the AAV8 capsid polypeptide have been changed to GQS or GQR and/or the three amino acids following the site into which said peptide is inserted have been changed to QAA.

[00270] Embodiment 8. The variant AAV8 capsid polypeptide of any one of embodiments 1-7, comprising one of the following sets of alanine and/or glutamic acid substitutions:

a. G456A, S466A, D584A, and T591A;

b. G456A, T493A, N498A, D584A, T591A, T711A, and T719E;

c. S466A, V491A, D584A, and T591A;

d. G455A, G456A, S466A, Q548A, and D584A;

e. D584A, T591A, T711A, and T719A;

f. K547A, D584A, and T591A;

g. G456A, T493A, N498A, D584A, and T591A

h. G456A, S466A, N471A, T711A, and T719A;

i. G456A, N471A, and T493A;

j. T711A, and T719A;

k. T711A; and

l. K547A, Q548A, D584A, and T719A.

[00271] Embodiment 9. The variant AAV8 capsid polypeptide of any one of embodiments 5-8, comprising a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and one of the following sets of alanine substitution(s):

m. T711A and T719A;

n. G456A, S466A, N471A, T711A, and T719A;

o. T711A; and

p. G456A, N471A, and T493A.

[00272] Embodiment 10. The variant AAV8 capsid polypeptide of any one of embodiments 5-7, comprising a peptide insertion of GNDVRAR (SEQ ID NO: 123) after amino acid 590, and alanine substitutions T711A and T719A.

[00273] Embodiment 11. The variant AAV8 capsid polypeptide of any one of embodiments 5-7, comprising a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and one of the following sets of alanine and/or glutamic acid substitution(s):

q. G456A, T493A, N498A, T591A, T711A, and T719E;

r. G456A, S466A, N471A, T711A, and T719A;

s. G456A, N471A, and T493A; and

t. T711A, and T719A.

[00274] Embodiment 12. The variant AAV8 capsid polypeptide of any one of embodiments 1-11, wherein the one or more mutations result in decreased binding of the variant AAV8 capsid polypeptide to a neutralizing factor, compared to the binding of a wild-type AAV8 capsid polypeptide to the neutralizing factor.

[00275] Embodiment 13. The variant AAV8 capsid polypeptide of any one of embodiments 1-12, wherein the one or more mutations do not affect the genome packaging ability and/or transduction efficiency of AAV8.

[00276] Embodiment 14. The variant AAV8 capsid polypeptide of any one of embodiments 1-13, comprising an amino acid sequence having at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 36, 41, 43, 45, 47, and 121.

[00277] Embodiment 15. The variant AAV8 capsid polypeptide of embodiment 14, comprising an amino acid sequence having at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 22, 26, 28, 30, 41, 43, 45, 47, and 121.

[00278] Embodiment 16. The variant AAV8 capsid polypeptide of embodiment 14, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 36, 41, 43, 45, 47, and 121.

[00279] Embodiment 17. The variant AAV8 capsid polypeptide of any one of embodiments 14-16, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 22, 26, 28, 30, 41, 43, 45, 47, and 121.

[00280] Embodiment 18. A variant adeno-associated virus 8 (AAV8) capsid polypeptide, comprising an amino acid sequence of amino acids 138-747 of the variant AAV8 capsid polypeptide of any one of embodiments 1-17.

[00281] Embodiment 19. A variant adeno-associated virus 8 (AAV8) capsid polypeptide, comprising an amino acid sequence of amino acids 204-747 of the variant AAV8 capsid polypeptide of any one of embodiments 1-17.

[00282] Embodiment 20. A nucleic acid encoding the variant AAV8 capsid polypeptide of any one of embodiments 1-19.

[00283] Embodiment 21. The nucleic acid of embodiment 20, comprising a nucleotide sequence having at least 80% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 35, 40, 42, 44, 46 and 120.

[00284] Embodiment 22. The nucleic acid of embodiment 21, comprising a nucleotide sequence having at least 80% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5, 7, 9, 11, 13, 15, 21, 25, 27, 29, 40, 42, 44, 46, and 120.

[00285] Embodiment 23. The nucleic acid of embodiment 21, comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 35, 40, 42, 44, 46 and 120.

[00286] Embodiment 24. The nucleic acid of any one of embodiments 21-23, comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5, 7, 9, 11, 13, 15, 21, 25, 27, 29, 40, 42, 44, 46, and 120.

[00287] Embodiment 25. A nucleic acid encoding a variant adeno-associated virus 8 (AAV8) capsid polypeptide, comprising a nucleotide sequence of nucleotides 412-2244 of the nucleic acid of any one of embodiments 21-24.

[00288] Embodiment 26. A nucleic acid encoding a variant adeno-associated virus 8 (AAV8) capsid polypeptide, comprising a nucleotide sequence of nucleotides 610-2244 of the nucleic acid of any one of embodiments 21-24.

[00289] Embodiment 27. A recombinant DNA comprising the nucleic acid of any one of embodiments 21-26.

[00290] Embodiment 28. An isolated host cell comprising the nucleic acid of any one of embodiments 20-26 or the recombinant DNA of embodiment 27.

[00291] Embodiment 29. An adeno-associated virus (AAV) vector comprising the variant AAV8 capsid polypeptide of any one of embodiments 1-19.

[00292] Embodiment 30. The AAV vector of embodiment 29, further comprising a heterologous nucleic acid.

[00293] Embodiment 31. The AAV vector of embodiment 30, wherein the heterologous nucleic acid comprises a nucleotide sequence encoding a therapeutic protein.

[00294] Embodiment 32. The AAV vector of embodiment 31, wherein the therapeutic protein is coagulation factor VIII or coagulation factor IX, or a functional fragment or derivative thereof.

[00295] Embodiment 33. The AAV vector of any one of embodiments 29-32, wherein the AAV vector exhibits an improved immune escaping capacity compared an AAV8 wild-type vector.

[00296] Embodiment 34. The AAV vector of embodiment 33, wherein the AAV vector has an immune escaping capacity score (IECS) of between about 0.01 to about 4.

[00297] Embodiment 35. A pharmaceutical composition comprising the AAV vector of any one of embodiments 29-34, and a pharmaceutically acceptable carrier and/or excipient.

[00298] Embodiment 36. A method of delivering a gene product to a subject in need thereof, said method comprising administering to the subject an effective amount of an adeno-associated virus (AAV) vector of any one of embodiments 29-34 or the pharmaceutical composition of embodiment 35.

[00299] Embodiment 37. The method of embodiment 36, wherein the subject has existing neutralizing antibodies against AAV8 prior to the administration.

[00300] Embodiment 38. The method of embodiments 36 or 37, wherein the AAV vector or the pharmaceutical composition is administered intravenous, intramuscular, subcutaneous, intratumor, intradermal, transdermal, intranodal, intraspinal, intraprostatic, intralymphatic, intraparenchymal, intraportal or intraperitoneal injection.

[00301] Embodiment 39. The method of any one of embodiments 36-38, wherein the subject has hemophilia A or hemophilia B.

[00302] Embodiment 40. The method of any one of embodiments 36-39, wherein the subject is human.

[00303] Embodiment 41. The method of any one of embodiments 36-40, wherein the subject is a non-human.

[00304] Embodiment 42. The method of embodiment 41, wherein the non-human is a mouse, a rat, a rabbit, a dog, a cat, a sheep, a pig, or a non-human primate.

[00305] Embodiment 43. A method of treating a liver-borne blood disorder in a human subject in need thereof, said method comprising administering to the subject an effective amount of the adeno-associated virus (AAV) vector of any one of embodiments 32-33 or a pharmaceutical

composition comprising the AAV vector of any one of embodiments 32-33, and a pharmaceutically acceptable carrier and/or excipient, wherein the therapeutic protein is a protein used for the treatment of a liver-borne blood disorder.

[00306] Embodiment 44. The method of embodiment 43, wherein the therapeutic protein is a blood coagulation factor.

[00307] Embodiment 45. The method of embodiment 43 or 44, wherein the liver-borne blood disorder is a coagulation disorder.

[00308] Embodiment 46. The method of embodiment 45, wherein the coagulation disorder is hemophilia.

[00309] Embodiment 47. The method of embodiment 46, wherein the hemophilia is hemophilia A or hemophilia B.

[00310] Embodiment 48. The method of embodiment 43, wherein the AAV vector or the pharmaceutical composition is administered at about $1x10^{11}$ to about $1x10^{14}$ vg/kg.

[00311] Embodiment 49. The method of embodiment 48, wherein the AAV vector or the pharmaceutical composition is administered at about $5x10^{11}$ vg/kg.

[00312] Embodiment 50. The method of any one of embodiments 43-49, wherein the AAV vector or the pharmaceutical composition is administered via intravenous, intramuscular, subcutaneous, intratumor, intradermal, transdermal, intranodal, intraspinal, intraprostatic, intralymphatic, intraparenchymal, intraportal or intraperitoneal route injection.

[00313] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein, is intended merely to better illuminate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure.

[00314] The embodiments of this disclosure are described herein. Variations of these embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the disclosure to be practiced otherwise than as specifically described herein. Accordingly, this disclosure includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable

law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the disclosure, even if the combination of features is not found together in the same sentence, or paragraph, or section of this document, unless otherwise indicated herein or otherwise clearly contradicted by context. The disclosure also includes, for instance, all embodiments of the disclosure narrower in scope in any way than the variations specifically mentioned above.

[00315] All publications, patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference in its entirety to the extent that it is not inconsistent with the disclosure.

[00316] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

EXAMPLES

[00317] Additional aspects and details of the disclosure will be apparent from the following examples, which are intended to be illustrative rather than limiting.

EXAMPLE 1:

Alanine Exchange Of Variable AAV Surface Regions (Single Mutations)

[00318] A comparison of VP1 peptide sequences from several AAV serotypes revealed 12 hypervariable regions (HVR) with strong divergence in their sequence (Gao *et al.* (2003) *Proc. Natl. Acad. Sci. USA* 100:6081-6086; Rutledge *et al.* (1998) *J. Virol.* 72:309-319; Chiorini *et al.* (1999) *J. Virol.* 73:1309-1319). By another definition, nine variable regions (VR I-IX) have been assigned to VP3, the main capsid component, which coincide with the HVR (Govindasamy *et al.* (2006) *J. Virol.* 80:11556-11570; Tseng and Agbandje-McKenna (2014) *Front. Immunol.*, Jan 30;5:9; Agbandje-McKenna and Kleinschmidt (2011) *Methods Mol Biol.* 2011;807:47-92). To cover as many known and potential antibody binding sites as possible, systematic mutagenesis of all VRs was performed and a total of 133 single alanine mutations within VR I-IX were generated. The amino acid-to-alanine exchange was introduced by PCR mutagenesis using a plasmid template carrying the native AAV8 capsid gene. A proof-reading polymerase (Q5, NEB) was used to reduce the occurrence of spontaneous mutations within the PCR product. Following sequence analysis, the mutated AAV8 capsid genes were cloned into an adequate plasmid backbone for vector production.

EXAMPLE 2:

Test Of Packaging Efficiency And Production Of Surface Variants

[00319] Mutations in the capsid can impair the genome packaging efficiency of AAV when introduced into relevant regions, rendering the vector ineffective for gene transfer. To exclude such capsid variants from further use, a medium-scale test production of the created AAV variants was done and the production efficiency monitored by qPCR. As control, a wild-type AAV8 vector and an already established packaging-competent AAV8 capsid mutant were used. Packaging-competent AAV mutants were then used to test the transduction efficiency and evasion from antibody neutralization *in vitro*.

EXAMPLE 3:

In Vitro Transduction Of Hepatocytes With Mutants In The Presence Of AAV8 Nab-Containing Serum

[00320] Besides retaining packaging competence, capsid mutant AAV8 vectors must also retain their transduction efficiency to levels comparable to wild-type AAV8. To test the transduction efficiency *in vitro*, a CMV promoter-controlled luciferase gene expression cassette was packaged into the AAV mutants and subjected to screening in a 96-well luminometric assay. Validation of the mutants was performed. Human hepatocytes (HuH-7) were used and transduced with AAV mutants or wild-type AAV8 (control).

[00321] To assess the AAV mutants' ability to evade binding by neutralizing antibodies, 293T cells were transduced with AAV8 mutants at a fixed Multiplicity of Infection (MOI) (determined in the pilot test) in the presence or absence of several dilutions of human serum containing nAbs. Wild-type AAV8 and AAV2 "AAV2/AAV8 domain swap" mutants with improved immune evasion characteristics were used as benchmark constructs.

EXAMPLE 4:

Identification of Immunogenic Hotspots by Alanine Scanning of Variable Region

[00322] In order to identify immunogenic hotspots on the capsid of AAV8, an initial screen was performed by mutating each amino acid of the Variable Regions (VRI-IX, see **Table 1**) towards alanine or glycine, if alanine is in a specific position.

Table 1

Variable Regions	Amino Acid Positions (VP1 numbering)
VRI	262-274
VRII	328-333
VRIII	383-391
VRIV	452-471

VRV	490-507
VRVI	528-545
VRVII	547-564
VRVIII	582-597
VRIX	706-720

[00323] The AAV8 mutants were produced as AAVs and validated for the immune escaping property using a cell-based neutralizing antibody assay and a novel quantification methodology (Jungmann *et al.* (2017) *Methods Mol. Biol.* 1521:109-126; Rapti *et al.* (2018) Identification of Immunogenic Epitopes on the Variable Regions of Adeno-Associated Virus 9 and Generation of Novel AAVs with Immune Evading Properties. in XXIVth Annual Meeting of the German Society for Gene Therapy, Freiburg, Germany; Rapti *et al.* (2018) Generation of Novel Immune-Evading AAVs through Identification and Mutation of Immunogenic Epitopes in the Variable Capsid Regions of Adeno-Associated Virus 9. in ESGCT Annual Congress, Lausanne Switzerland). Briefly, plasma samples from 8 patients were screened for seropositivity against AAV8 capsid mutants. Mutants of one variable region together with the control/parent vector were screened as a group. The data from the luciferase assays were analyzed on multiple levels. Analysis typically involved plotting of the nAb assay curve, nAb titer determination and analysis of Area Under the Curve (AUC) and Receiver Operating Characteristic (ROC) curve analysis in Prism. The basic analysis was performed to identify the immune properties of mutants compared to those of the parent vector. The AUC formed from the nAb assay curve of each mutant is compared to the AUC from the nAb assay curve from the parent. If there is an alleviation of neutralization of the mutant, then the AUC is increased. If the neutralization of the mutant is higher, then the AUCmut is lower compared to the AUC of the parent vector. The two nAb assay curves are also compared using the ROC curve analysis. As defined in the Prism software "A truly useless test has an area of 0.5" and "a perfect test has an area of 1.00." The interpretation of the immune escaping properties of mutants based exclusively on the AUC does not take into account the experimental variation of the nAb assay performed in triplicate, which is measured through the ROC curve analysis. In order to interpret the data based on both these data points, the development of one variable that incorporated both of them was pursued. The new variable is termed "Immune Evading Capacity Score" or IEC score. Positive IEC values denote alleviation of nAb binding compared to the parent vector, AAV8. Based on the combinatorial IEC values of each mutation screened against 8 plasma samples, 13 mutants (**Table 2**) with the most positive ones were chosen for further evaluation.

Table 2

Variable Regions	Mutants
VRIV	G455A, G456A, S466A, N471A
VRV	V491A, T493A, N498A
VRVII	K547A, Q548A
VRVIII	D584A, T591A
VRIX	T711A, T719A

[00324] **Table 3** provides the original capsid sequence of VP1 from AAV8 with the position of the mutations highlighted in bold letters with emphasis marks, with or without a peptide variant insertion (italic letters).

Table 3

AAV8 (SEQ ID NO: 2)
MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRY NHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVPDP QPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYS TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADV FMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF **S**QGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYS DVMLTSEEEIKTTNPVATEEYGIVA**D**NLQQQN**T**APQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILI KNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*
AAV8. PV (SEQ ID NO: 48)
MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRY NHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVPDP QPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYS TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADV FMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF **S**QGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYS DVMLTSEEEIKTTNPVATEEYGIVA**D**NLQGQ***RGXXXXXXX***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGL KHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLT RNL*

EXAMPLE 5:

Generation of Combinatorial Immune Escape Libraries

[00325] The 13 immunogenic hotspots (see **Table 2**) identified in the 1st screen were used to generate combinatorial libraries. The C-terminal AAV8 capsid fragment used for generation of the combinatorial library is shown in **Figure 4**.

[00326] A total of four combinatorial AAV8 libraries were generated. These were based on (a) AAV8 wt, and three peptide variants identified as offering enhanced liver tropism, (b) PV2, (c) PV29, and (d) PV35. The amino acid sequences for PV2, PV29, and PV35 are provided in **Table 4** below. The positions of the mutations are highlighted in bold letters with emphasis marks, and the peptide variant insertions (with two N-terminal amino acids RG or SG) are indicated with italic letters.

Table 4

AAV8. PV2 (SEQ ID NO: 18)

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRY
NHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVPDP
QPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADV
FMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTTGGTANTQTLGF
SQGGPNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFGKQNAARDNADYS
DVMLTSEEEIKTTNPVATEEYGIVADNLQGQ*RGNSVRGDG*AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGL
KHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTSVDFAVNTEGVYSEPRPIGTRYLT
RNL*

AAV8. PV29 (SEQ ID NO: 49)

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRY
NHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVPDP
QPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADV
FMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTTGGTANTQTLGF
SQGGPNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFGKQNAARDNADYS
DVMLTSEEEIKTTNPVATEEYGIVADNLQGQ*RGLAGNTIR*AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGL
KHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTSVDFAVNTEGVYSEPRPIGTRYLT
RNL*

AAV8. PV35 (SEQ ID NO: 34)

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRY
NHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVPDP
QPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADV
FMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTTGGTANTQTLGF
SQGGPNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFGKQNAARDNADYS
DVMLTSEEEIKTTNPVATEEYGIVADNLQGQ*SGGNDVRAR*AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGL
KHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTSVDFAVNTEGVYSEPRPIGTRYLT
RNL*

[00327] Gene synthesis of combinatorial immune escape libraries was performed using codon triplets covering the individual 13 Ala-exchange mutants of the *cap* 8 gene. Each codon triplet harbors a 1:1 combination of the *cap* 8 WT codon position and the Ala mutation.

[00328] The amplified library was analyzed via Sanger sequencing, which resulted in a library correctness of 83% (20 out of 24 total clones).

[00329] The initial plasmid libraries generated are shown in **Table 5**.

Table 5

	Id	Library Name	Number of Clones	AAV8 cap
Library 1	4859	pWH-Rep2-Cap8-NIS-CombALib	250000	wt
Library 2	4860	pWH-Rep2-Cap8-NIS-PV2-CombALib	873000	PV2
Library 3	4861	pWH-Rep2-Cap8-NIS-PV29-CombALib	147000	PV29
Library 4	4862	pWH-Rep2-Cap8-NIS-PV35-CombALib	1750000	PV35

[00330] The complexity of library 1 was 8191 and the complexity of libraries 2-4 was 4095. During the cloning and subcloning experiments (pWH to pAAV), these diversities were maintained. The number of colony forming units (CFUs), always exceeded these complexities.

The pAAV libraries 1-4 were analyzed via Sanger sequencing. The respective library correctness was 83%, 79%, 75%, and 75%.

[00331] For production, adherent HEK293 T cells were co-transfected with the pAAV libraries with the helper plasmid, pHelper, using transfection reagent polyethylenimine (PEI). Viral particles harvested 48 hours after transfection and isolated from cell pellets and culture medium.

[00332] Cell pellets were lysed with 0.1% Triton. AAV particles were PEG-precipitated from culture medium. AAV particles were purified by iodixanol gradient centrifugation, concentrated and filter sterilized (*See, e.g.*, Merten (2016) Cell Gene Ther. Insights 2(5):521-551).

[00333] The viral productions were titrated using qPCR and purity was assessed with SDS-PAGE using SYBRO Ruby staining for visualization of Cap proteins (VP1, VP2, and VP3). Volume and vector genome of the final library produced is shown in **Table 6**.

Table 6

Library Name	Lot#	Volume	[VG/ml]	Total VG
AAV8-wt-CombALib	KA18001-AA4948_V_1	0.8 ml	8.0E+12	6.4E+12
AAV8-PV2-CombALib	KA18001-AA4949_V_1	0.9 ml	4.0E+12	3.6E+12
AAV8-PV29-CombALib	KA18001-AA4950_V_1	0.8 ml	5.5E+12	4.4E+12
AAV8-PV35-CombALib	KA18001-AA4951_V_1	0.7 ml	9.0E+12	6.3E+12

EXAMPLE 6:

Selection of Immune Escaping Mutants Combinatorial Libraries

[00334] The immune escaping combinatorial libraries were generated as described in Example 5. The DNA fragments were cloned into the appropriate plasmids, validated by sequencing and packaged to AAVs. The selection was performed based on the protocol found in Paulk *et al.* (2018) *Mol. Ther.* 26(1):289-303, but other published protocols were also taken into consideration Li *et al.* (2012) *J. Virol.* 86(15):7752-9; Maersch *et al.* (2010) *Vir.* 397(1):167-75. The libraries were subjected to 2 rounds of selection (for the 1st round PureProteome Protein A/G and for the 2nd Round Agarose beads were used). The libraries were selected against a mix of IntraVenous ImmunoGlobulins (IVIG) (KioVig (PZN-4668775) and Octapharma (PZN-05373935)). An outline of the methodology applied is shown in **Figure 1**. After the 2 rounds of selection a total of 179 single clones were sequenced by Sanger sequencing (WTLib: 19 Clones, PV35Lib: 8 Clones, PV2Lib:152 Clones). All clones without any or few alanines in the selected positions were excluded. Several clones from the 3 libraries (WTmu, WTmu+PV2 and WTmu+PV35), from the *in silico* analysis, were selected for further validation (**Tables 8, 12**).

The sequence provided in **Tables 7, 11** indicate the amino acids at the 13 mutant positions (in the order of appearance in the VP1 sequence) as identified in **Tables 2 and 3**. "X" means the amino acid is unknown due to low quality sequencing data. "NA" means the peptide identity was not determined due to mixed sequencing data.

Table 7: AAV Production Capacity of Individual Combinatorial Mutants Chosen For Further Studies

Clone	Production Efficiency	Peptide	Sequence	SEQ ID NO
A10	unsuccessful	NA	Mix	-
B8	good	WT	GAANVTNKQAATT	50
B10	good	WT	GASNVAAKQAAAE	51
C1	good	PV2	GGSNVTNKQDGAA	52
C5	unsuccessful	NA	Mix	-
C10	good	WT	GGANATNKQAATT	53
D3	good	WT	AAANVTNKAATTT	54
D5	unsuccessful	WT	GGSNVTNAAATTA	55
D6	good	WT	GGSNVTNKQAAAA	56
6/A12	low	PV2	GAAAVTNKQDGAA	57
5/B4	unsuccessful	WT	GASAVAAKAATTT	58
6/B4	extr. low	WT	GASAVAAKAATTT	59
6/E7	good	PV2	GGSNVTNKQDGAA	60
6/E11	good	PV35	GGSNVTNKQDGAA	61
6/H2	good	PV2	GGSNVTNKQDGAA	62
6/H3	good	WT	GGSNVTNAQAATT	63
6/H12	unsuccessful	WT	XXSAAQKANQETX	64
9/F1	good	PV2	GGSNVTNKQDGAT	65
9/E3	unsuccessful	PV35	AGAAATAKQDGTX	66
Q/C11	low	PV2	GASAVANKQDGTT	67
WT.A3	good	WT	GASNVAAKQAATT	68
PV2.A6	good	PV2	GGSNVTNKQDGTT	69

EXAMPLE 7:

Validation of Selected AAV8 Combinatorial Mutants

[00335] The selected clones, together with the parent vectors (AAV8 capsid polypeptide without variants), were produced as previously described (Jungmann *et al.* (2017) *Hum. Gene Ther. Meth.* 28(5):235-246; Jungmann *et al.* (2017) *Meth. Mol. Biol.* 1521:109-126) and purified by iodixanol gradient (nAb Round 1) or additionally by buffer exchange and concentrator (nAb Round 2). The AAVs were then tested against 6 plasma samples as described in Example 4. 2 Rounds of nAb assays were performed: in the 1st round 3 and in the 2nd round 5 sets/nAb Assays were performed. From the analysis of the nAb assays the following values were determined: 1) transduction efficiency, 2) nAb titers, and 3) Immune Evading Capacity Score (the IECS was determined through the AUC and ROC values as described in Example 4).

[00336] The transduction data (1) and the nAb titers (2) from the experiments are represented in **Figures 2A and 2B** and **Table 8a** and **Table 8b**.

Transduction Efficiency

[00337] All combinatorial mutants without PV had lower transduction compared to WT, whereas the PV insertion enhanced transduction of 293T cells by several fold.

nAb Titers

[00338] **1st Round of nAb Screenings**: three productions (PV2.C11, F1 and H2) were of insufficient quality and they were repeated. Most of the combinatorial mutants have same or lower titers compared to the WT controls. The immune evading capacity of the AAV8 combinatorial mutants was more prominent in the assays against sera with higher titers. Therefore a 3rd set of experiments (nAb CombC) against sera with high titers was performed. Indeed, even with the higher titers, some of the mutants did show lower titer. It is of note, that due to limited packaging capacity, the assays for mutant PV2.A12 were performed using half the vgs of what normally is used in these assays, which could lead to a misleading increased titer. It is also noteworthy, that although the titer is similar between WT and some mutants, the nAb assay curve shows a difference between the two, with the majority of the mutants displaying a higher immune evading capacity compared to WT.

[00339] **2nd Round of nAb Screenings**: in Assay A/B (Assay A: B025 to B013, Assay B: V110-B015) and C the productions of AAV mutants were not as good as the WT production, whereas for the rest of the assays, the production quality was similar. In summary, as with the 1st round, most of the combinatorial mutants show an enhanced immune evading capacity compared to the parent control. Production quality seems to affect the nAb assays, however the final data are the cumulation of 8 individual assays, which should overcome the challenge of AAV production quality.

Table 8a: Titers of Combinatorial Mutants from the 1st Round of nAb Screens from 3 Experiments (AAV8 CombA, B, C)

AAV8 Comb A	B025	B031	V110	B013
WT1	<1:5	<1:5	1:10	1:80
WT2	1:5	1:5	1:5	1:80
WT.B8	1:5	1:5	<1:5	1:40
WT.B10	1:5	1:5	<1:5	1:20
PV2.C1	1:5	1:5	1:5	1:40
WT.C11	<1:5	<1:5	1:5	1:40
WT.D3	<1:5	1:5	1:5	1:80
WT.D6	1:5	1:5	1:5	1:80
PV2.A12	<1:5	<1:5	1:5	1:40

AAV8 Comb B	B025	B031	V110	B013
WT3	1:5	<1:5	1:5	1:80
WT1	<1:5	<1:5	<1:5	1:80
PV2.E7	<1:5	<1:5	1:5	1:40
PV35.E11	<1:5	<1:5	1:5	1:40
PV2.H2	<1:5	<1:5	1:5	1:20
PV2.F1	<1:5	<1:5	<1:5	1:20
PV2.C11	<1:5	<1:5	<1:5	1:40
WT.D6	<1:5	<1:5	1:5	1:40
PV2.C11	<1:5	<1:5	<1:5	1:20
PV2.F1	<1:5	<1:5	<1:5	1:20
PV2.H2	<1:5	<1:5	1:5	1:40
PV2.A12	<1:5	<1:5	1:5	1:80

AAV8 Comb C	V110	B015
WT1	>1:320	>1:320
WT2	>1:320	>1:320
WT.B8	>1:320	>1:320
WT.B10	1:320	>1:320
PV2.C1	1:320	1:320
WT.C11	>1:320	>1:320
WT.D3	>1:320	>1:320
WT.D6	>1:320	>1:320
WT3	>1:320	>1:320
PV2.E7	>1:320	>1:320
PV35.E11	1:320	>1:320
WT.D6	>1:320	>1:320
PV2.C11	>1:320	>1:320
PV2.F1	>1:320	>1:320
PV2.H2	>1:320	>1:320
PV2.A12	>1:320	>1:320

Table 8b: Titers of Combinatorial Mutants from the 2nd Round of nAb Screens from Experiments (AAV8 CombA/B, C, D, E, F)

AAV8 Comb A/B	B025	B031	V110	B013	V110	B015
WT	**<1:5**	**<1:5**	**1:5**	**1:40**	**>1:320**	**>1:320**
WT.B8	<1:5	<1:5	1:10	1:20	>1:320	>1:320
WT.B10	<1:5	<1:5	<1:5	1:40	>1:320	>1:320
WT.A3	<1:5	<1:5	<1:5	1:80	>1:320	>1:320
WT	**<1:5**	**<1:5**	**1:5**	**1:80**	**>1:320**	**>1:320**
WT.C10	<1:5	<1:5	1:5	1:40	>1:320	>1:320
WT.D6	<1:5	<1:5	1:10	1:80	>1:320	>1:320
PV2.QC11	<1:5	<1:5	<1:5	1:40	>1:320	>1:320
PV2.9F1	<1:5	<1:5	1:5	1:40	1:160	>1:320
PV2.6H2	<1:5	<1:5	1:5	1:20	1:160	>1:320
AAV8 Comb C	B025	B031	V110	B013	V110	B015
WT	**1:5**	**<1:5**	**1:20**	**1:160**	**>1:320**	**>1:320**
PV2.A6	<1:5	<1:5	1:20	1:80	>1:320	>1:320
PV2.6E7	<1:5	<1:5	1:20	1:80	>1:320	>1:320
PV35.6E11	<1:5	<1:5	1:10	1:80	>1:320	>1:320
WT.6H3	<1:5	<1:5	1:5	1:80	>1:320	>1:320

PV2	<1:5	1:5	1:10	1:160	>1:320	>1:320
PV35	<1:5	<1:5	1:10	1:160	>1:320	>1:320
PV2.6A12	<1:5	<1:5	1:10	1:80	>1:320	>1:320
AAV8 Comb D	**B025**	**B031**	**V110**	**B013**	**V110**	**B015**
WT	**1:5**	**<1:5**	**1:20**	**1:80**	**>1:320**	**>1:320**
PV2.C1	<1:5	<1:5	1:10	1:20	1:160	>1:320
PV35.6E11	<1:5	<1:5	1:10	1:40	>1:320	>1:320
PV2.QC11	<1:5	<1:5	1:10	1:40	>1:320	>1:320
PV2.9F1	<1:5	<1:5	1:10	1:40	1:160	>1:320
PV2.H2	<1:5	<1:5	1:10	1:40	>1:320	>1:320
WT.6H3	<1:5	<1:5	<1:5	1:40	>1:320	>1:320
PV2.A3	<1:5	<1:5	<1:5	1:40	>1:320	>1:320
AAV8 Comb E	**B025**	**B031**	**V110**	**B013**	**V110**	**B015**
WT	**1:5**	**<1:5**	**1:10**	**1:80**	**>1:320**	**>1:320**
WT.B8	<1:5	<1:5	1:5	1:80	>1:320	>1:320
WT.B10	<1:5	<1:5	<1:5	1:40	1:80	1:160
WT.C10	<1:5	<1:5	1:5	1:40	>1:320	>1:320
WT.D6	<1:5	<1:5	1:5	1:80	>1:320	>1:320
AAV8 Comb F	**B025**	**B031**	**V110**	**B013**	**V110**	**B015**
WT1	**1:5**	**<1:5**	**1:10**	**1:160**	**>1:320**	**>1:320**
WT2	1:5	<1:5	1:5	1:160	>1:320	>1:320
D3	<1:5	<1:5	1:5	1:160	>1:320	>1:320

EXAMPLE 8:

Cumulative Immune Escaping Capacity Score (IECS) of AAV8 Combinatorial Mutants

[00340] Due to the limited sensitivity of the titer determination and in order to quantify the immune evading capacity of the combinatorial mutants, the AUC and ROC curve analysis was performed, as described in Example 4, and the IECS (Immune Escaping Capacity Score) was calculated. As shown in **Tables 9a** and **9b** for nAb rounds 1 and 2 respectively, most of the mutants display higher immune evasion properties compared to the parent vector (values are positive/negative for higher/lower immune evasion capacity compared to the parent vector, WT, respectively). Several mutants were tested 2 or 3 times due to low production quality (measured by AAV viral genome yield) (marked with an asterisk *). It is of note, that the cumulative IECS value was lower when the production quality was low and this highlights the importance of testing AAV mutants with similar production efficiencies/quality. Furthermore, regarding the nAb assay quality and therefore the IECS, the latter was calculated using the internal WT assay control. The PV combinatorial alanine mutants were not directly compared to their corresponding parent PVs, but with the WT parent vector. Additionally, for the combinatorial mutants containing a PV, their scores are more consistent between assays, probably owing to

their higher transduction capacity and therefore assay quality. It is finally noteworthy, that for the 2nd round of nAb assays the PV2 and PV35 vectors without alanine mutations were compared to the WT parent vector, but they did not show enhanced immune escaping capacity.

[00341] In **Table 9a**, IECS_comb values (sum of individual values) that are the sum of 4 assays, compared to 6, and are hence lessened, are in italics. Assays in which the quality of the AAV productions, or the assay itself, is low, are marked with an asterisk (*)

Table 9a: IECS Values and Cumulative IECS Value of the Combinatorial Mutants, as well as Transduction Efficiency Ratio to the Parent Vector from the 1st Round of Screenings

	IECS-B025	IECS-B031	IECS-V110	IECS-B013	IECS-V111	IECS-B015	IECS_comb	IECS_SD	Transduction
WT.B8	-0.01	0.33	0.50	0.27	0.14	0.06	1.287	0.186	0.99
WT.B10	0.03	-0.03	0.91	0.53	0.37	0.09	1.905	0.360	0.69
PV2.C1	0.04	0.03	0.27	0.30	0.22	0.15	1.014	0.116	21.27
WT.C11	0.00	0.05	0.08	0.08	0.10	0.07	0.368	0.037	0.45
WT.D3	0.42	-0.02	0.19	0.11	0.18	0.08	0.966	0.146	0.45
WT.D6	-0.01	0.06	0.09	0.07	0.04	0.04	0.283	0.033	0.36
PV2.A12	0.01	0.05	0.12	0.28			*0.465*	0.116	6.95
PV2.E7	0.04	0.13	0.14	0.14	0.13	0.09	0.673	0.038	10.75
PV35.E11	0.04	0.02	0.22	0.17	0.22	0.12	0.793	0.085	13.87
PV2.C11*	-0.02	*-0.04*	0.20	0.11			*0.258*	0.114	14.24
PV2.F1*	0.03	*-0.04*	0.10	0.29			*0.368*	0.140	20.10
PV2.H2*	0.02	*-0.01*	0.08	0.30			*0.393*	0.141	10.59
WT.D6	0.02	*-0.03*	0.03	0.22	0.40	0.04	0.683	0.163	0.54
PV2.C11	0.10	0.11	0.30	0.69	0.12	0.06	1.381	0.240	12.89
PV2.F1	0.07	0.09	0.38	0.52	0.04	0.01	1.110	0.210	20.87
PV2.H2	0.05	0.05	0.27	0.24	0.08	0.10	0.793	0.098	10.10
PV2.A12	-0.02	0.08	0.50	0.09	0.02	0.03	0.706	0.191	5.66
WT vs WT1			-0.02	0.00	-0.01	-0.01	-0.041	0.008	1.15

[00342] In **Table 9b**, assays in which the quality of the AAV productions, or the assay itself, is low, are marked with an asterisk (*).

Table 9b: IECS Values and Cumulative IECS Value of the Combinatorial Mutants, as well as Transduction Efficiency Ratio to the Parent Vector from the 2nd Round of Screenings

Assay		IECS-B025	IECS-B031	IECS-V1110	IECS-B013	IECS-V111	IECS-B015	IECS_comb	IECS_SD	Transduction
A+B	WT.B8 *	0.00	-0.11	-0.21	0.19	0.08	0.02	-0.032*	0.142	0.441
	WT.B10 *	0.01	-0.03	0.08	0.00	0.19	0.62	0.867*	0.245	0.474
	WT.A3 *	-0.07	-0.03	0.08	-0.06	0.07	0.19	0.179*	0.101	0.316
	WT.C10	0.00	0.01	0.01	0.04	-0.01	0.02	0.068	0.017	0.337
	WT.D6	0.04	-0.05	-0.02	0.13	0.00	0.01	0.110	0.060	0.297
	PV2.QC11 *	0.08*	0.00*	0.10*	0.34*	0.14*	0.05*	0.716*	0.118	9.990
	PV2.9F1 *	0.23*	0.02*	0.07*	0.37*	0.22*	0.06*	0.986*	0.158	8.938
	PV2.6H2	0.71	0.02	0.18	0.50	0.26	0.14	1.808	0.258	7.978
C	PV2.A6	0.05	0.13	0.01	0.02	-0.08	0.01	0.132	0.070	8.836
	PV2.6E7	0.22	-0.01	0.00	0.06	-0.12	0.08	0.228	0.107	9.808
	PV35.6E11	0.29	0.00	0.22	0.16	-0.03	0.07	0.720	0.123	14.037
	WT.6H3	0.02	0.04	0.54	0.11	-0.08	0.24	0.882	0.246	1.074
	PV2	0.03	-0.06	0.18	0.02	-0.11	0.01	0.076	0.099	20.809
	PV35	0.04	-0.01	0.12	0.03	-0.05	0.04	0.179	0.058	15.576
	PV2.6A12	0.21	-0.06	0.31	0.09	-0.06	0.07	0.566	0.148	7.059
	PV2.C1	0.11	0.01	0.07	0.45	0.17	0.19	1.002	0.154	23.566
	PV35.6E11	0.07	0.04	0.07	0.37	0.16	0.16	0.875	0.121	22.979
	PV2.QC11	0.01	-0.01	0.09	0.39	0.10	0.08	0.665	0.145	12.081
	PV2.9F1	0.01	0.06	0.12	0.48	0.17	0.10	0.948	0.169	25.775
	PV2.H2	0.24	0.03	0.07	0.32	0.12	0.11	0.906	0.112	19.294
	WT.6H3	-0.02	0.02	0.41	0.46	0.15	0.14	1.164	0.199	0.495
D	WT.A3	0.25	0.08	0.12	0.14	0.12	0.25	0.947	0.071	0.580
	WT.B8	-0.01	0.21	0.04	0.06	0.05	0.04	0.390	0.074	0.638
	WT.B10	0.05	0.22	1.17	0.59	0.97	0.57	3.569	0.428	0.704
	WT.C10	0.08	0.11	0.24	0.20	0.18	0.18	0.995	0.059	0.465
E	WT.D6	0.07	-0.01	0.12	0.12	0.09	0.25	0.638	0.086	0.317
F	WT.D3	0.01	0.32	0.15	0.16	0.03	0.01	0.678	0.124	0.417
A/B	A/B_WT vs WT1	-0.03	-0.10	-0.03	-0.19	0.05	0.01	-0.288	0.085	1.142
F	F_WT1 vs WT2	0.01	0.04	0.03	-0.01	-0.04	-0.04	0.001	0.034	1.815

[00343] The cumulative IECS of all the assays from both round of screenings is depicted in **Figures 3A-3B**. As shown, all mutants display, on average, higher immune evading capacity

compared to the parent vector, with the following combinatorial mutants being the most promising: WT.B10, WT.D3, WT.6H3, PV2.9F1, PV2.QC11, PV2.6H2 and PV2.6A12.

EXAMPLE 9:

T Cell Proliferation Assay

[00344] The following combinatorial mutants (all resulting in substitutions to alanine at the respective positions except for B10 at the position T719, where the mutation is T719E) identified in example 8 as being most promising were combined with a the peptide insertion SRGATVL (SEQ ID NO: 124) after amino acid 590 relative to the wild-type AAV8 capsid polypeptide (i.e. PV27) in order to arrive at the following combination variants comprising not only the combinatorial mutants (i.e. B10, 6A12, QC11, and 6H2) but also the peptide insertion SRGATVL (SEQ ID NO: 124) (i.e. PV27): (i) B10 in order to arrive at PV27.B10 (in this combinatorial mutant, the D584A mutation is not present, see SEQ ID: 46 and 47); (ii) 6A12 in order to arrive at PV27.6A12; (iii) QC11 in order to arrive at PV27.QC11; and (iv) 6H2 in order to arrive at PV27.6H2. The DNA and protein sequences of PV27 (SEQ ID NO: 38 and 39), PV27.QC11 (SEQ ID NO: 40 and 41), PV27.6A12 (SEQ ID NO: 42 and 43), PV27.6H2 (SEQ ID NO: 44 and 45), and PV27.B10 (SEQ ID NO: 46 and 47) are shown in the sequence listing below.

[00345] Peptide pools of 15 amino acids were designed to represent regions of amino acid differences between WT, PV27, PV27.QC11, PV27.6A12, PV27.6H2 and PV27.B10 (Table 10). A cohort of 50 healthy human donors was selected for PBMC isolation to best represent the number and frequency of HLA-DR and HLA-DQ allotypes expressed in the world population. PBMCs were challenged with individual peptides, KLH (neoantigen) or CEFT (antigen peptide pool) for 6 days, or with PHA (mitogen) for a total of 6 days before adding ^{3}[H]-thymidine to detect proliferating T cells. After 18 hours, ^{3}[H]-thymidine incorporated in proliferating T cells were quantitated using a TomTec Mach III cell harvester and cpm (counts per minute) for each well were determined by Meltilex scintillation counting. Six replicates for each peptide, each donor were used. Stimulation index (SI) is defined as mean cpm of test wells/ mean cpm medium control wells. An empirical threshold of SI > 1.5 (p <0.05, unpaired two sample Student's t-test), where 50% higher proliferation compared to negative (medium) control was considered positive signal. The Response Index (RI) was calculated from percent antigenicity (% of responded donors) multiplied by the strength of response (SI). The combined response index of each donor for the variant peptide pools of PV27, PV27.QC11, PV27.6A12, PV27.6H2 and PV27.B10 were all reduced relative to WT (Figure 5).

Table 10: List of peptides used for T cell proliferation assay along with which were included in the pools of WT, PV27, PV27.QC11, PV27.6A12, PV27.6H2 and PV27.B10

Peptide #	Sequence	SEQ ID NO	WT	PV27	PV27.QC11	PV27-6A12	PV27-6H2	PV27-B10
1	YYLSRTQTTGGTANT	70	1	1			1	
2	TQTTGGTANTQTLGF	71	1	1			1	
3	GTANTQTLGFSQGGP	72	1	1			1	
4	QTLGFSQGGPNTMAN	73	1	1			1	1
5	SQGGPNTMANQAKNW	74	1	1			1	1
6	NTMANQAKNWLPGPC	75	1	1			1	1
7	LPGPCYRQQRVSTTT	76	1	1		1	1	
8	YRQQRVSTTTGQNNN	77	1	1		1	1	
9	VSTTTGQNNNSNFAW	78	1	1		1	1	
10	GQNNNSNFAWTAGTK	79	1	1	1	1	1	
11	FFPSNGILIFGKQNA	80	1	1	1	1	1	1
12	GILIFGKQNAARDNA	81	1	1	1	1	1	1
13	GKQNAARDNADYSDV	82	1	1	1	1	1	1
14	TNPVATEEYGIVADN	83	1	1	1	1	1	
15	TEEYGIVADNLQQQN	84	1					
16	IVADNLQQQNTAPQI	85	1					
17	LQQQNTAPQIGTVNS	86	1					
18	TAPQIGTVNSQGALP	87	1					
19	IQYTSNYYKSTSVDF	88	1	1	1			
20	NYYKSTSVDFAVNTE	89	1	1	1			
21	TSVDFAVNTEGVYSE	90	1	1	1			
22	AVNTEGVYSEPRPIG	91	1	1	1			
23	YYLSRTQTTGATANT	92			1	1		1
24	TQTTGATANTQTLGF	93			1	1		1
25	ATANTQTLGFSQGGP	94			1	1		1
26	QTLGFSQGGPATMAN	95			1			
27	QTLGFAQGGPATMAN	96				1		
28	SQGGPATMANQAKNW	97			1			
29	AQGGPATMANQAKNW	98				1		
30	ATMANQAKNWLPGPC	99			1	1		
31	LPGPCYRQQRVSATT	100			1			1
32	YRQQRVSATTGQNNN	101			1			1
33	VSATTGQNNNSNFAW	102			1			1
34	GQANNSNFAWTAGTK	103						1
35	TNPVATEEYGIVAAN	104						1
36	TEEYGIVAANLQGQR	105						1
37	IVAANLQGQRGSRGA	106						1
38	IQYTSNYYKSASVDF	107				1	1	1
39	NYYKSASVDFAVNAE	108				1	1	
40	NYYKSASVDFAVNEE	109						1
41	ASVDFAVNAEGVYSE	110				1	1	
42	ASVDFAVNEEGVYSE	111						1
43	AVNAEGVYSEPRPIG	112				1	1	
44	AVNEEGVYSEPRPIG	113						1
45	TEEYGIVADNLQGQR	114		1	1	1	1	
46	IVADNLQGQRGSRGA	115		1	1	1	1	

47	LQGQRGSRGATVLAQ	116		1	1	1	1	1
48	GSRGATVLAQAAQIG	117		1	1	1	1	1
49	TVLAQAAQIGTVNSQ	118		1	1	1	1	1
50	AAQIGTVNSQGALPG	119		1	1	1	1	1
	Total peptide #		22	24	24	24	24	24

[00346] For Examples 7 and 8 plasmids were used in which the Rep and Cap genes as well as the reporter gene were flanked by ITRs. This could have resulted in AAV productions that contained a mixture of packaged genomes, Rep2Cap and CMVLuc. The final validation experiments were therefore repeated using plasmids for the Rep and Cap without ITRs (see Examples 10 and 11).

Table 11: AAV Production Capacity of Individual Combinatorial Mutants Chosen for Further Studies

Clone	Production Efficiency	Peptide	Sequence	SEQ ID NO
A10	unsuccessful	NA	Mix	-
B8	good	WT	GAANVTNKQAATT	50
B10	good	WT	GASNVAAKQAAAE	51
C1	good	PV2	GGSNVTNKQDGAA	52
C5	unsuccessful	NA	Mix	-
C10	good	WT	GGANATNKQAATT	53
D3	good	WT	AAANVTNKAATTT	54
D5	low	WT	GGSNVTNAAATTA	55
D6	good	WT	GGSNVTNKQAAAA	56
6/A12	low	PV2	GAAAVTNKQDGAA	57
5/B4	unsuccessful	WT	GASAVAAKAATTT	58
6/B4	extr. low	WT	GASAVAAKAATTT	59
6/E7	good	PV2	GGSNVTNKQDGAA	60
6/E11	good	PV35	GGSNVTNKQDGAA	61
6/H2	good	PV2	GGSNVTNKQDGAA	62
6/H3	good	WT	GGSNVTNAQAATT	63
6/H12	unsuccessful	WT	XXSAAQKANQETX	64
9/F1	good	PV2	GGSNVTNKQDGAT	65
9/E3	unsuccessful	PV35	AGAAATAKQDGTX	66
Q/C11	low	PV2	GASAVANKQDGTT	67
WT.A3	good	WT	GASNVAAKQAATT	68
PV2.A6	good	PV2	GGSNVTNKQDGTT	69

EXAMPLE 10:

Validation of Selected AAV8 Combinatorial Mutants

[00347] The selected clones (subcloned into plasmid vectors without ITRs), together with the parent vectors (AAV8 capsid polypeptide without variants), were produced as previously described (Jungmann *et al.* (2017) *Hum. Gene Ther. Meth.* 28(5):235-246; Jungmann *et al.* (2017) *Meth. Mol. Biol.* 1521:109-126) and purified by iodixanol gradient and additionally by buffer exchange and concentrator. The AAVs were then tested against 6 plasma samples as described in Example 4. 2 Rounds of nAb assays were performed: in the 1st round 2 sets and in the 2nd round 3 sets/nAb Assays were performed. From the analysis of the nAb assays the following values were determined: 1) transduction efficiency, 2) nAb titer, and 3) Immune Evading Capacity Score (the IECS was determined through the AUC and ROC values as described in Example 4).

[00348] The transduction data (1) and the nAb titers (2) from the experiments are represented in **Figures 6A and 6B** and **Table 12a** and **Table 12b**.

Transduction Efficiency

[00349] All combinatorial mutants without PV had lower transduction compared to WT, whereas the PV insertion enhanced transduction of 293T cells by several fold.

nAb Titers

[00350] **1st Round of nAb Screenings**: six productions (PV2, PV2.A6, PV2.9F1, AAV8.D5, PV35 and PV35.6/E11) were of insufficient quality and they were repeated. One production for Set b (WT.D5) was low and the nAb assays showed high evasion properties. However, this was attributed to the low production efficiency (Set b assays marked with asterisk). The production of AAV8.B10 was sufficient; however the viral production was unstable over the course of the experiments. Therefore, the final assays with AAV8.B10 in Set a were removed (in order: Do111, B015, V110, B013, B025, B031, B025, B031) (Set a assays marked with asterisk) and the production and assays were repeated (Set e). Some of the assays in Set a showed inconsistencies with regards to production (PV2 and PV35) or due to errors in the luciferase assay and they were removed from the calculations. Most of the combinatorial mutants have same or lower titers compared to the WT controls. The immune evading capacity of the AAV8 combinatorial mutants was more prominent in the assays against sera with higher titers. Although the titer is similar between WT and some mutants, the nAb assay curve showed a difference between the two, with the majority of the mutants displaying a higher immune evading capacity compared to WT.

[00351] **2nd Round of nAb Screenings**: in Assay Set c and d the peptide display and the WT mutants were tested separately and in Set e the mutant AAV8.B10, that showed the most promising results was retested. In all these assays the productions were of good quality. In summary, as with the 1st round, most of the combinatorial mutants showed an enhanced immune evading capacity compared to the parent control. Production quality seemed to affect the nAb assays, however the final data are the cumulation of 5 or more individual assays, which should overcome the challenge of AAV production quality.

Table 12a: Titers of Combinatorial Mutants from the 1st Round of nAb Screens from 2 Experiments (AAV8 Comb Set a, b)

AAV8 Comb a	**B025**	**B031**	**V110**	**B013**	**Do111**	**B015**	**V110**	**B013**	**B025**	**B031**	**B025**	**B031**
WT	1/5	<1/5	1/10	1/160	>1/320	>1/320	1/10	1/80	1/5	<1/5	1/80	1/10
WT	1/5	<1/5	1/10	1/160	>1/320	>1/320	1/10	1/80	<1/5	<1/5	1/80	1/10
PV2.A6	<1/5	<1/5	1/5	1/80	>1/320	>1/320	1/5	1/80	<1/5	<1/5		
PV2.C1	<1/5	<1/5	1/5	1/80	>1/320	>1/320	1/5	1/40	<1/5	<1/5		
PV2.6/E7	<1/5	<1/5	1/5	1/80	>1/320	>1/320	1/5	1/80	<1/5	1/5		
PV2.QC11	<1/5	<1/5	1/5	1/40	>1/320	>1/320	<1/5	1/40	<1/5	<1/5		
WT.D3	1/5	<1/5	1/10	1/160	>1/320	>1/320	1/10	1/80	<1/5	<1/5		
WT					>1/320	>1/320	1/5	1/40	<1/5	<1/5	1/40	1/5
WT					>1/320	>1/320	1/5	1/80	<1/5	<1/5	1/80	1/5
WT	<1/5	1/5	1/10	1/160	>1/320	>1/320	1/5	1/80	<1/5	1/5		
WT.A3	1/5	1/5	1/5	1/160	>1/320	>1/320	<1/5	1/80	<1/5	<1/5		
WT.B8	<1/5	1/5	1/5	1/160	>1/320	>1/320	1/5	1/160	<1/5	<1/5		
WT.B10*	<1/5	<1/5	1/5	>1/320*								
WT.C10	1/5	1/5	1/10	>1/320	>1/320	>1/320	1/10	1/160	<1/5	<1/5		
WT.D6	<1/5	<1/5	1/10	1/80	>1/320	>1/320	1/10	1/80	<1/5	<1/5		
PV2.9F1	<1/5	<1/5	1/10	1/160	>1/320	>1/320	1/5	1/40	<1/5	<1/5		
WT.D5	1/5	1/5	1/10	>1/320	>1/320	>1/320	1/5	1/160	<1/5	1/20		
WT.C7	1/5	<1/5	1/5	1/160	>1/320	>1/320	1/5	1/80	<1/5	<1/5		
WT	<1/5	<1/5	1/10	1/160	>1/320	>1/320	1/10	1/80	<1/5	<1/5		
PV2.6H2	<1/5	<1/5	1/5	1/80	>1/320	>1/320	1/5	1/40	<1/5	<1/5		
PV2.6A12	<1/5	<1/5	1/5	1/80	>1/320	>1/320	1/5	1/40	<1/5	<1/5		
WT	1/5	<1/5	1/10	1/160	>1/320	>1/320	1/5	1/80	<1/5	1/5		
PV35.6/E11	<1/5	<1/5	1/10	1/80	>1/320	>1/320	1/5	1/40	<1/5	<1/5		
WT.C10	1/5	<1/5	1/10	>1/320	>1/320	>1/320	1/5	1/160	<1/5	<1/5		
AAV8	**B025**	**B031**	**V110**	**B013**	**Do111**	**B015**						
WT1	<1/5	<1/5	1/5	1/80	>1/320	>1/320						
WT2	<1/5	<1/5	1/5	1/80	>1/320	>1/320						
PV2	<1/5	<1/5	<1/5	1/40	>1/320	>1/320						
PV2.A6	<1/5	<1/5	<1/5	1/40	1/160	>1/320						
PV2.9F1	<1/5	<1/5	<1/5	1/40	1/160	>1/320						
WT.D5*	<1/5*	<1/5*	<1/5*	1/5*	1/40*	1/80*						
PV35	<1/5	<1/5	1/5	1/40	>1/320	>1/320						

PV35.6/E11	<1/5	<1/5	1/5	1/40	>1/320	>1/320						

Table 12b: Titers of Combinatorial Mutants from the 1st Round of nAb Screens from 3 Experiments (AAV8 Comb Set c, d, e)

AAV8 Comb c	B025	B031	V110	B013	Do111	B015
WT1	<1/5	<1/5	1/5	1/80	>1/320	>1/320
WT2	<1/5	<1/5	1/5	1/80	>1/320	>1/320
PV2	<1/5	<1/5	<1/5	1/40	>1/320	>1/320
PV2.A6	<1/5	<1/5	<1/5	1/40	>1/320	>1/320
PV2.C1	<1/5	<1/5	1/5	1/40	>1/320	>1/320
PV2.6/E7	<1/5	<1/5	<1/5	1/80	>1/320	>1/320
PV2.QC11	<1/5	<1/5	<1/5	1/40	>1/320	>1/320
PV2.9F1	<1/5	<1/5	<1/5	1/20	>1/320	>1/320
PV2.6H2	<1/5	<1/5	1/5	1/40	>1/320	>1/320
PV2.6A12	<1/5	<1/5	1/5	1/40	>1/320	1/160
PV35	<1/5	<1/5	<1/5	1/40	1/160	>1/320
PV35.6/E11	<1/5	<1/5	1/5	1/40	>1/320	>1/320

AAV8 Comb d	B025	B031	V110	B013	Do111	B015
WT	1/5	<1/5	1/10	1/160	>1/320	>1/320
WT	1/5	<1/5	1/10	1/160	>1/320	>1/320
WT.A3	1/5	<1/5	<1/5	>1/320	>1/320	>1/320
WT.B8	<1/5	<1/5	1/10	1/160	>1/320	>1/320
WT.B10	<1/5	<1/5	<1/5	1/160	>1/320	>1/320
WT.D6	<1/5	<1/5	1/10	1/80	>1/320	>1/320
WT.D5	<1/5	1/10	1/10	>1/320	>1/320	>1/320
WT.C7	<1/5	<1/5	1/10	1/160	>1/320	>1/320
WT.D3	<1/5	<1/5	1/40	1/160	>1/320	>1/320
WT.B10	<1/5	1/5	<1/5	1/160	>1/320	>1/320

AAV8 Comb e	B025	B031	V110	B013	Do111	B015
WT	1/5	<1/5	1/5	1/160	>1/320	>1/320
WT.B10	<1/5	<1/5	<1/5	1/160	>1/320	>1/320
WT	<1/5	1/5	1/10	1/160	>1/320	>1/320
WT.B10	<1/5	<1/5	<1/5	1/160	>1/320	>1/320

EXAMPLE 11:

Cumulative Immune Escaping Capacity Score (IECS) of AAV8 Combinatorial Mutants

[00352] Due to the limited sensitivity of the titer determination and in order to quantify the immune evading capacity of the combinatorial mutants, the AUC and ROC curve analysis was performed, as described in Example 4, and the IECS (Immune Escaping Capacity Score) was calculated. As shown in **Tables 13a** and **13b** for nAb rounds 1 and 2 respectively, most of the mutants display higher immune evasion properties compared to the parent vector (values are

positive/negative for higher/lower immune evasion capacity compared to WT (Figure 7A) or their respective parent vector, WT, PV2 or PV35 (Figure 7B)). Several mutants were tested 2 or 3 times due to low production quality (measured by AAV viral genome yield) or vector stability (marked with an asterisk *) or to mitigate inter-assay variability. The cumulative IECS value was higher (WT.D5) when the production quality was low and this highlights the importance of testing AAV mutants with similar production efficiencies/quality. Furthermore, regarding the nAb assay quality and therefore the IECS, the latter was calculated using the internal WT assay control. The PV combinatorial alanine mutants were directly compared to their corresponding parent PVs when possible, but also with the WT parent vector. Additionally, for the combinatorial mutants containing a PV, their scores are more consistent between assays, probably owing to their higher transduction capacity and therefore assay quality. It is finally noteworthy, that the PV2 and PV35 vectors without alanine mutations were compared to the WT parent vector and showed enhanced immune escaping capacity.

[00353] In **Table 13a**, IECS_comb values (sum of individual values) that are the sum of 4 assays, compared to 6, and are hence lessened, are in italics. Assays in which the quality of the AAV productions or vector, or the assay itself, is low, are marked with an asterisk (*).

[00354] In **Table 13b**, assays in which the quality of the AAV production, or the assay itself, is low, are marked with an asterisk (*).

Table 13a: IECS Values and Cumulative IECS Value of the Combinatorial Mutants, as well as Transduction Efficiency Ratio to the Parent Vector from the 1st Round of Screenings

	IECS-B025	IECS-B031	IECS-V110	IECS-B013	IECS-Do111	IECS-B015	Sum	St.Dev.	No	Average	St.Dev.
WTb/WTa	-0,027	0,007	0,023	-0,004	0,009	-0,001	0,007	0,0167	6		
AAV8_A3/WTa	-0,025	0,014	0,286	0,012	0,155	0,097	0,540	0,1162	6	0,351	0,041
AAV8_B8/WTa	-0,031	0,028	0,043	0,019	0,025	0,006	0,090	0,0255	6	0,663	0,137
AAV8_B10/WTa *	*0,116**	*0,108**	*0,243**	*-0,041**			*0,426*	*0,1161*	*4*	*0,114*	*0,035*
AAV8_D6/WTa	0,128	0,340	-0,039	0,018	0,066	0,018	0,531	0,1354	6	0,680	0,092
AAV8_D5/WTa*	0,067*	-0,081*	0,082*	0,940*	1,977*	1,156*	4,141	0,8110	6	0,893	0,116
AAV8_C7/WTa	-0,030	0,315	0,020	-0,017	0,057	0,011	0,356	0,1289	6	0,760	0,075
AAV8_D3/WTa	0,010	-0,014	0,052	-0,017	0,018	0,015	0,064	0,0249	6	0,743	0,084
AAV8_C10/WTa	0,028	0,249	-0,034	-0,042	0,055	-0,006	0,250	0,1080	6	0,487	0,079
PV2/WTa	0,024	0,000	0,205	0,132	0,130	0,086	0,578	0,0760	6	21,465	3,165
PV2_A6/WTa	0,062	-0,034	0,169	0,055	0,131	0,099	0,481	0,0705	6	19,745	2,492
PV2_C1/WTa	0,144	-0,046	0,104	0,094	0,063	0,117	0,477	0,0667	6	24,673	4,576
PV2_6/E7/WTa	0,154	-0,032	0,144	0,049	0,034	0,116	0,466	0,0725	6	16,558	1,965
PV2_QC11/WTa	0,093	-0,024	0,646	0,162	0,052	0,040	0,970	0,2454	6	15,802	1,219
PV2_9F1/WTa	0,041	0,216	0,010	0,038	0,189	0,084	0,577	0,0860	6	14,504	1,897
PV2_6H2/WTa	0,085	-0,182	0,058	0,129	0,084	0,101	0,275	0,1142	6	22,206	4,220
PV2_6A12/WTa	0,049	-0,142	0,077	0,148	0,115	0,111	0,358	0,1044	6	14,803	1,947
PV35/WTa	0,244	-0,092	0,121	0,140	0,161	0,122	0,695	0,1118	6	17,310	2,064

PV35_6/E11/WTa	0,604	0,019	0,024	0,122	0,090	0,090	0,949	0,2220	6	16,502	2,124
PV2_A6/PV2	-0,029	-0,009	0,004	-0,013	0,025	0,018	-0,004	0,0204	6		
PV2_C1/PV2	0,027	-0,014	-0,022	0,003	-0,005	0,073	0,061	0,0349	6		
PV2_6/E7/PV2	0,016	0,011	-0,031	-0,006	0,000	0,062	0,053	0,0310	6		
PV2_QC11/PV2	-0,009	0,002	0,011	0,012	0,035	0,021	0,072	0,0152	6		
PV2_9F1/PV2	0,015	-0,005	-0,063	-0,031	0,025	0,030	-0,029	0,0363	6		
PV2_6H2/PV2							0,000		0		
PV2_6A12/PV2							0,000		0		
PV35_6/E11/PV35	-0,114	-0,028	-0,058	-0,008	-0,009	0,058	-0,159	0,0573	0		

Table 13b: IECS Values and Cumulative IECS Value of the Combinatorial Mutants, as well as Transduction Efficiency Ratio to the Parent Vector from the 2nd Round of Screenings

							IECS			Tranduction/ Ratio to WT		
	IECS-B025	IECS-B031	IECS-V110	IECS-B013	IECS-Do111	IECS-B015	**Sum**	**St.Dev.**	**No**	**Average**	**St.Dev.**	**No**
WTb/WTa	-0,009	-0,040	-0,010	0,019	-0,016	0,006	-0,052	0,0202	6			
AAV8_A3/WTa	-0,024	0,029	0,196	0,014	0,022	0,072	0,309	0,0772	6	0,130	0,035	6
AAV8_B8/WTa	0,185	-0,005	-0,040	-0,010	-0,028	0,010	0,113	0,0835	6	0,407	0,052	6
AAV8_B10/WTa	0,252	-0,032	0,347	0,001	0,005	0,122	0,694	0,1543	6	0,557	0,059	24
AAV8_D6/WTa	0,570	-0,039	0,058	0,043	0,026	0,037	0,695	0,2250	6	0,568	0,115	6
AAV8_D5/WTa	0,015	-0,198	0,001	-0,047	0,083	0,357	0,211	0,1838	6	0,090	0,013	6
AAV8_C7/WTa	0,006	-0,103	-0,003	0,015	-0,028	0,101	-0,011	0,0660	6	0,358	0,048	6

AAV8_D3/WTa	0,021	-0,081	-0,014	-0,002	-0,021	0,060	-0,037	0,0468	6	0,270	0,033	6
AAV8_C10/WTa							0,000		0			0
PV2/WTa	0,160	-0,004	0,039	0,199	0,049	0,048	0,490	0,0789	6	15,131	2,013	7
PV2_A6/WTa	0,024	0,008	0,053	0,166	0,134	0,080	0,465	0,0621	6	15,410	2,331	7
PV2_C1/WTa	0,294	-0,026	0,018	0,241	0,042	0,153	0,723	0,1295	6	11,543	1,371	7
PV2_6/E7/WTa	0,218	0,008	0,013	0,181	0,049	0,141	0,609	0,0904	6	12,653	1,750	7
PV2_QC11/WTa	0,107	-0,002	0,066	0,295	0,098	0,081	0,646	0,0998	6	14,153	2,373	7
PV2_9F1/WTa	0,008	-0,168	0,043	0,236	0,067	0,097	0,283	0,1315	6	16,826	3,779	7
PV2_6H2/WTa	0,028	0,024	0,012	0,173	0,085	0,057	0,378	0,0600	6	13,271	1,404	7
PV2_6A12/WTa	0,129	-0,170	0,005	0,243	0,097	0,170	0,474	0,1453	6	16,360	1,304	7
PV35/WTa	0,031	-0,055	0,009	0,330	0,155	0,083	0,553	0,1364	6	12,953	2,337	7
PV35_6/E11/WTa	0,065	-0,035	-0,010	0,168	0,102	0,080	0,371	0,0745	6	8,699	1,334	7
PV2_A6/PV2	-0,061	0,015	0,008	-0,018	0,047	0,018	0,009	0,0370	6			
PV2_C1/PV2	0,027	-0,014	-0,022	0,003	-0,005	0,073	0,061	0,0349	6			
PV2_6/E7/PV2	0,016	0,011	-0,031	-0,006	0,000	0,062	0,053	0,0310	6			
PV2_QC11/PV2	-0,009	0,002	0,011	0,012	0,035	0,021	0,072	0,0152	6			
PV2_9F1/PV2	-0,100	-0,134	-0,036	0,001	0,011	0,029	-0,228	0,0654	6			
PV2_6H2/PV2	-0,018	0,090	-0,036	-0,008	0,021	0,008	0,056	0,0443	6			
PV2_6A12/PV2	-0,003	-0,143	-0,044	0,003	0,029	0,075	-0,083	0,0746	6			
PV35_6/E11/PV35	0,017	0,006	-0,013	-0,037	-0,022	-0,005	-0,055	0,0191	6			

[00355] The cumulative IECS of all the assays from both rounds of screening are depicted in **c**. As shown, all mutants display, on average, higher immune evading capacity compared to the parent vector, with the following combinatorial mutants being the most promising: WT_A3, WT_B10, WT_C10, WT_D6, WT_D5, WT_C7, PV2.9F1, PV2.QC11, PV2.6H2 and PV2.6A12.

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[00356] The invention has been described in terms of particular embodiments found or proposed to comprise specific modes for the practice of the invention. Various modifications and variations of the described invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

List of Sequences

[00357] For the DNA sequences listed below, the nucleotide codons coding for the amino acids at the 13 mutant positions (listed in Table 2) are provided before each sequence and also underlined in the sequence. The region corresponding to the peptide insertion and two amino acids, RG or SG, N-terminal to the peptide insertion is indicated with italic letters.

[00358] For the protein sequences listed below, the amino acids at the 13 mutant positions (listed in Table 2) are provided before each sequence and also bolded in the sequence. The region corresponding to the peptide insertion and two amino acids, RG or SG, N-terminal to the peptide insertion is indicated with italic letters.

SEQ ID NO: 1 AAV8_WT DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacaa gat acg aca aca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
agcccaaagccaaccagcaaaagcaggacgacggccggggtctggtgcttcctggctacaagtacctcggacccttcaacggactcgac
aaggggagcccgtcaacgcggcggacgcagcggccctcgagcacgacaaggcctacgaccagcagctgcaggcgggtgacaatcc
gtacctgcggtataaccacgccgacgccgagtttcaggagcgtctgcaagaagatacgtcttttgggggcaacctcgggcgagcagtcttc
caggccaagaagcgggttctcgaacctctcggtctggttgaggaaggcgctaagacggctcctggaaagaagagaccggtagagccatc
accccagcgttctccagactcctctacgggcatcggcaagaaaggccaacagcccgccagaaaaagactcaattttggtcagactggcga
ctcagagtcagttccagaccctcaacctctcggagaacctccagcagcgccctctggtgtgggacctaatacaatggctgcaggcggtggc
gcaccaatggcagacaataacgaaggcgccgacggagtgggtagttcctcgggaaattggcattgcgattccacatggctgggcgacag
agtcatcaccaccagcacccgaacctgggccctgcccacctacaacaaccacctctacaagcaaatctccaacgggacatcgggaggag
ccaccaacgacaacacctacttcggctacagcacccccctgggggtattttgactttaacagattccactgccacttttcaccacgtgactggca
gcgactcatcaacaacaactggggattccggcccaagagactcagcttcaagctcttcaacatccaggtcaaggaggtcacgcagaatga
aggcaccaagaccatcgccaataacctcaccagcaccatccaggtgtttacggactcggagtaccagctgccgtacgttctcggctctgcc
caccagggctgcctgcctccgttcccggcggacgtgttcatgattccccagtacggctacctaacactcaacaacggtagtcaggccgtgg
gacgctcctccttctactgcctggaatactttccttcgcagatgctgagaaccggcaacaacttccagtttacttacaccttcgaggacgtgcct
ttccacagcagctacgcccacagccagagcttggaccggctgatgaatcctctgattgaccagtacctgtactacttgtctcggactcaaaca
aca**ggaggc**acggcaaatacgcagactctgggcttc**agc**caaggtgggcct**aat**acaatggccaatcaggcaaagaactggctgccag
gaccctgttaccgccaacaacgc**gtc**tca**acg**acaaccgggcaa**aac**aacaatagcaactttgcctggactgctgggaccaaataccatc
tgaatggaagaaattcattggctaatcctggcatcgctatggcaacacacaaagacgacgaggagcgttttttttcccagtaacgggatcctga
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ggctacagaggaatacggtatcgtggca**gat**aacttgcagcagcaaaac**acg**gctcctcaaattggaactgtcaacagccaggggcctt
acccggtatggtctggcagaaccgggacgtgtacctgcagggtcccatctgggccaagattcctcacacggacggcaacttccacccgtct
ccgctgatgggcggctttggcctgaaacatcctccgcctcagatcctgatcaagaacacgcctgtacctgcggatcctccgaccaccttcaa
ccagtcaaagctgaactctttcatcacgcaatacagcaccggacaggtcagcgtggaaattgaatgggagctgcagaaggaaaacagcaa
gcgctggaaccccgagatccagtacacctccaactactacaaatct**aca**agtgtggactttgctgttaat**aca**gaaggcgtgtactctgaac
cccgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 2 AAV8_WT Protein sequence

Protein, **GGSNVTNKQDTTT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQQQN**T**APQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 3 AAV8_B8 DNA sequence

DNA, **ggtgca gca aac gtc acg aac aaacaa gca gca aca aca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
agcccaaagccaaccagcaaaagcaggacgacggccggggtctggtgcttcctggctacaagtacctcggacccttcaacggactcgac
aaggggagcccgtcaacgcggcggacgcagcggccctcgagcacgacaaggcctacgaccagcagctgcaggcgggtgacaatcc
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gacgctcctccttctactgcctggaatactttccttcgcagatgctgagaaccggcaacaacttccagtttacttacaccttcgaggacgtgcct
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gaccctgttaccgccaacaacgc**gtc**tca**acg**acaaccgggcaa**aac**aacaatagcaactttgcctggactgctgggaccaaataccatc
tgaatggaagaaattcattggctaatcctggcatcgctatggcaacacacaaagacgacgaggagcgttttttttcccagtaacgggatcctga
tttttggc**aaacaa**aatgctgccagagacaatgcggattacagcgatgtcatgctcaccagcgaggaagaaatcaaaaccactaaccctgt
ggctacagaggaatacggtatcgtggca**gca**aacttgcagcagcaaaac**gca**gctcctcaaattggaactgtcaacagccaggggcctt
acccggtatggtctggcagaaccgggacgtgtacctgcagggtcccatctgggccaagattcctcacacggacggcaacttccacccgtct
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ccagtcaaagctgaactctttcatcacgcaatacagcaccggacaggtcagcgtggaaattgaatgggagctgcagaaggaaaacagcaa
gcgctggaaccccgagatccagtacacctccaactactacaaatct**aca**agtgtggactttgctgttaat**aca**gaaggcgtgtactctgaac
cccgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 4 AAV8_B8 Protein sequence

Protein, **GAANVTNKQAATT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN

LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GA**TANTQTLGF
AQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**A**NLQQQN**A**APQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 5 AAV8_B10 DNA sequence

DNA, **ggtgca tct aac gtt gca gca aaacag gca gca gca gaa** (A1644G, silent mutation)

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
agcccaaagccaaccagcaaaagcaggacgacggccggggtctggtgcttcctggctacaagtacctcggacccttcaacggactcgac
aaggggagcccgtcaacgcggcggacgcagcggccctcgagcacgacaaggcctacgaccagcagctgcaggcgggtgacaatcc
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ctcagagtcagttccagaccctcaacctctcggagaacctccagcagcgccctctggtgtgggacctaatacaatggctgcaggcggtggc
gcaccaatggcagacaataacgaaggcgccgacggagtgggtagttcctcgggaaattggcattgcgattccacatggctgggcgacag
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ccaccaacgacaacacctacttcggctacagcacccccctgggggtattttgactttaacagattccactgccacttttcaccacgtgactggca
gcgactcatcaacaacaactggggattccggcccaagagactcagcttcaagctcttcaacatccaggtcaaggaggtcacgcagaatga
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ccggtatggtctggcagaaccgggacgtgtacctgcagggtcccatctgggccaagattcctcacacggacggcaacttccacccgtctcc
gctgatgggcggctttggcctgaaacatcctccgcctcagatcctgatcaagaacacgcctgtacctgcggatcctccgaccaccttcaacc
agtcaaagctgaactctttcatcacgcaatacagcaccggacaggtcagcgtggaaattgaatgggagctgcagaaggaaaacagcaagc
gctggaaccccgagatccagtacacctccaactactacaaatct**gca**agtgtggactttgctgttaat**gaa**gaaggcgtgtactctgaaccc
cgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 6 AAV8_B10 Protein sequence

Protein, **GASNVAAKQAAAE**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW

HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GA**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**A**TTGQ**A**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**A**NLQQQN**A**APQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKS**A**SVDFAVN**E**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 7 AAV8_C10 DNA sequence

DNA, **ggtggt gca aac gca acc aac aaacag gca gca aca aca** (C1626T and A1644G, both silent mutations)

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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aaggggagcccgtcaacgcggcggacgcagcggccctcgagcacgacaaggcctacgaccagcagctgcaggcgggtgacaatcc
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gcgactcatcaacaacaactggggattccggcccaagagactcagcttcaagctcttcaacatccaggtcaaggaggtcacgcagaatga
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aca**ggtggt**acggcaaatacgcagactctgggcttc**gca**caaggtgggcct**aac**acaatggccaatcaggcaaagaactggctgccag
gaccctgttaccgccaacaacgc**gca**tca**acc**acaaccgggcaa**aac**aacaatagcaactttgcctggactgctgggaccaaataccatc
tgaatggaagaaattcattggctaatcctggcatcgctatggcaacacacaaagacgacgaggagcgttttttttcccagtaacgggat**T**ctg
atttttggc**aaacag**aatgctgccagagacaatgcggattacagcgatgtcatgctcaccagcgaggaagaaatcaaaaccactaaccctg
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SEQ ID NO: 8 AAV8_C10 Protein sequence

Protein, **GGANATNKQAATT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF

NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
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RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
AQGGP**N**TMANQAKNWLPGPCYRQQR**AS**TTTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**A**NLQQQN**A**APQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 9 AAV8_D3 DNA sequence

DNA, **gcagca gca aac gtt acc aac aaagca gca acc aca aca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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SEQ ID NO: 10 AAV8_D3 Protein sequence

Protein, **AAANVTNKAATTT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML

RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**AA**TANTQTLGF
AQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KA**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**A**NLQQQN**T**APQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 11 AAV8_D6 DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacag gca gca gca gca** (A1644G, silent mutation)

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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gcgctggaaccccgagatccagtacacctccaactactacaaatct**gca**agtgtggactttgctgttaat**gca**gaaggcgtgtactctgaac
cccgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 12 AAV8_D6 Protein sequence

Protein, **GGSNVTNKQAAAA**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN

PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**A**NLQQQN**A**APQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKS**A**SVDFAVN**A**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 13 AAV8_6H3 DNA sequence

DNA, **ggaggc agc aat gtc acg aac gcacag gct GCG aca aca** (A1644G, silent mutation)

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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tttttggc**GCACAG**AATGCTGCCAGAGACAATGCGGATTACAGCGATGTCATGCTCACCA
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GCA**GCT**AACTTGCAGGGCAGAGTG**GCG**GTAATGACGTGCGGGCTAGGGCCCAGGC
GGCCCAAATTGGAACTGTCAACAGCCAGGGGGCCTTACCCGGTATGGTCTGGCAGA
ACCGGGACGTGTACCTGCAGGGTCCCATCTGGGCCAAGATTCCTCACACGGACGGC
AACTTCCACCCGTCTCCGCTGATGGGCGGCTTTGGCCTGAAACATCCTCCGCCTCAG
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AAGCTGAACTCTTTCATCACGCAATACAGCACCGGACAGGTCAGCGTGGAAATTGA
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CCAACTACTACAAATCT**ACA**AGTGTGGACTTTGCTGTTAAT**ACA**gaaggcgtgtactctgaaccc
cgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 14 AAV8_6H3 Protein sequence From Sequencing

Protein, **GGSNVTNAQAATT** (INSERTION)

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT

DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**AQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VAA**A**NLQGRV**A**VMTCGLGPRRPKLELSTARGPYPVWSGRTGTCTCRVPSGPRFLTRTAT
STRLR*WAALA*NILRLRS*SRTRLYLRILRPPSTSQS*TLSSRNTAPDRSAWKLNGSCRR
KTASAGTPRSSTPPTTTNLQVWTLLLIQKACTLNPAPLAPVTSPVIC

* stop codons introduced at the cloning step

SEQ ID NO: 15 AAV8_A3 DNA sequence

DNA, **ggtgca tct aac gtt gca gca aaacag gca gca acc acc** (A1644G, silent mutation)

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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SEQ ID NO: 16 AAV8_A3 Protein sequence

Protein, **GASNVAAKQAATT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
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LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT

DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
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SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**A**TTGQ**A**NNSNFAWTAGTKYHLNGRNSLAN
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GFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNP
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SEQ ID NO: 17 PV2 WT DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacaa gat GGC aca aca**

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SEQ ID NO: 18 PV2 WT Protein sequence

Protein, **GGSNVTNKQDGTT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF

SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQGQ***RGNSVRGDG***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG
NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 19 PV2 C1 DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacaa gat GGC gca gca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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attgaatgggagctgcagaaggaaaacagcaagcgctggaaccccgagatccagtacacctccaactactacaaatct**gca**agtgtggac
tttgctgttaat**gca**gaaggcgtgtactctgaaccccgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 20 PV2 C1 Protein sequence

Protein, **GGSNVTNKQDGAA**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI

VA**D**NLQGQ***RG<u>NSVRGDG</u>***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG
NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**A**SVDFAVN**A**EGVYSEPRPIGTRYLTRNL*

<u>SEQ ID NO: 21 PV2_6A12 DNA sequence</u>

DNA, **ggtgca gca gca gtc acg aac aaacaa gat <u>GGC</u> gca gca**

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gcgactcatcaacaacaactggggattccggcccaagagactcagcttcaagctcttcaacatccaggtcaaggaggtcacgcagaatga
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gaccctgttaccgccaacaacgc**<u>gtc</u>**tca**<u>acg</u>**acaaccgggcaa**<u>aac</u>**aacaatagcaactttgcctggactgctgggaccaaataccatc
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ggctacagaggaatacggtatcgtggca**<u>gat</u>**aacttgca*ggg<u>ccagaga</u>**<u>***GGC***</u>*<u>aacagcgttcgcggcgatggcgcccaggcggcc</u>*
caaattggaactgtcaacagccaggggccttacccggtatggtctggcagaaccgggacgtgtacctgcagggtcccatctgggccaag
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gcctgtacctgcggatcctccgaccaccttcaaccagtcaaagctgaactctttcatcacgcaatacagcaccggacaggtcagcgtggaa
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tttgctgttaat**<u>gca</u>**gaaggcgtgtactctgaaccccgccccattggcacccgttacctcacccgtaatctgtaa

<u>SEQ ID NO: 22 PV2_6A12 Protein sequence</u>

Protein, **GAAAVTNKQDGAA**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GA**TANTQTLGF
AQGGP**A**TMANQAKNWLPGPCYRQQR**VST**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQGQ***RG<u>NSVRGDG</u>***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG

NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**A**SVDFAVN**A**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 23 PV2 6E7 DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacaa gat GGC gca gca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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SEQ ID NO: 24 PV2 6E7 Protein sequence

Protein, **GGSNVTNKQDGAA**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
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NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**A**SVDFAVN**A**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 25 PV2_6H2 DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacaa gat GGC gca gca** (G1875A, G1965T, both silent mutations)

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SEQ ID NO: 26 PV2_6H2 Protein sequence

Protein, **GGSNVTNKQDGAA**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQRV**S**T**T**TGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQGQ***RG*NSVRGDG**AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG
NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**A**SVDFAVN**A**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 27 PV2_9F1 DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacaa gat GGC gca aca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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SEQ ID NO: 28 PV2_9F1 Protein sequence

Protein, **GGSNVTNKQDGAT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQGQ***RG**NSVRGDG*AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG
NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**A**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 29 PV2_QC11 DNA sequence

DNA, **ggtgca tct gca gtt gca aac aaacaa gat GGC aca aca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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gaatgggagctgcagaaggaaaacagcaagcgctggaaccccgagatccagtacacctccaactactacaaatct**aca**agtgtggacttt
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SEQ ID NO: 30 PV2_QC11 Protein sequence

Protein, **GASAVANKQDGTT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GA**TANTQTLGF
SQGGP**A**TMANQAKNWLPGPCYRQQR**V**S**A**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQGQ***RGNSVRGDG***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG
NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 31 PV2_A6 DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacaa gat GGC aca aca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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SEQ ID NO: 32 PV2 A6 Protein sequence

Protein, **GGSNVTNKQDGTT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQGQ***RGNSVRGDG***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG
NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 33 PV35 WT DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacaa gat GGC aca aca**

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SEQ ID NO: 34 PV35_WT Protein sequence

Protein, **GGSNVTNKQDGTT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
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SEQ ID NO: 35 PV35_6E11 DNA sequence

DNA, **ggaggc agc aat gtt acc aac aaacaa gat GGC gca gca**

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gacgctcctccttctactgcctggaatactttccttcgcagatgctgagaaccggcaacaacttccagtttacttacaccttcgaggacgtgcct
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tgaatgggagctgcagaaggaaaacagcaagcgctggaaccccgagatccagtacacctccaactactacaaatct**gca**agtgtggactt
tgctgttaat**gca**gaaggcgtgtactctgaaccccgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 36 PV35_6E11 Protein sequence

Protein, **GGSNVTNKQDGAA**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQGQ*SGGNDVRAR*AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG
NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**A**SVDFAVN**A**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 37 AAV8_6H3 Protein sequence (Expected)

Protein, **GGSNVTNAQAATT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF

SQGGPNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFGAQNAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VAANLQQQNAAPQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKSTSVDFAVNTEGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 38 AAV8_PV27 DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacaa gat GGC aca aca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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attgaatgggagctgcagaaggaaaacagcaagcgctggaaccccgagatccagtacacctccaactactacaaatct**aca**agtgtggac
tttgctgttaat**aca**gaaggcgtgtactctgaaccccgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 39 AAV8_PV27 Protein sequence

Protein, **GGSNVTNKQDGTT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGPNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLAN

PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQGQ***RGSRGATVL***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG
NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 40 AAV8_PV27 QC11 DNA sequence

DNA, **ggtgca tct gca gtt gca aac aaacaa gat GGC aca aca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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ctgtacctgcggatcctccgaccaccttcaaccagtcaaagctgaactctttcatcacgcaatacagcaccggacaggtcagcgtggaaatt
gaatgggagctgcagaaggaaaacagcaagcgctggaaccccgagatccagtacacctccaactactacaaatct**aca**agtgtggacttt
gctgttaat**aca**gaaggcgtgtactctgaaccccgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 41 AAV8_PV27 QC11 Protein sequence

Protein, **GASAVANKQDGTT**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GA**TANTQTLGF
SQGGP**A**TMANQAKNWLPGPCYRQQR**V**S**A**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI

VA**D**NLQGQ***RG<u>SRGATVL</u>***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG
NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**T**SVDFAVN**T**EGVYSEPRPIGTRYLTRNL*

<u>SEQ ID NO: 42 AAV8_PV27 6A12 DNA sequence</u>

DNA, **ggtgca gca gca gtc acg aac aaacaa gat <u>GGC</u> gca gca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
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aca**<u>ggtgca</u>**acggcaaatacgcagactctgggcttc**<u>gca</u>**caaggtgggcct**<u>gca</u>**acaatggccaatcaggcaaagaactggctgccag
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tgaatggaagaaattcattggctaatcctggcatcgctatggcaacacacaaagacgacgaggagcgttttttttcccagtaacgggatcctga
ttttggc**<u>aaacaa</u>**aatgctgccagagacaatgcggattacagcgatgtcatgctcaccagcgaggaagaaatcaaaaccactaaccctgt
ggctacagaggaatacggtatcgtggca**<u>gat</u>**aacttgcagg*gccagaga**<u>GGC</u>tcgcgcggggctacggtcctggcccaggcggcc*
caaattggaactgtcaacagccaggggccttacccggtatggtctggcagaaccgggacgtgtacctgcagggtcccatctgggccaag
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tttgctgttaat**<u>gca</u>**gaaggcgtgtactctgaaccccgccccattggcacccgttacctcacccgtaatctgtaa

<u>SEQ ID NO: 43 AAV8_PV27 6A12 Protein sequence</u>

Protein, **GAAAVTNKQDGAA**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GA**TANTQTLGF
AQGGP**A**TMANQAKNWLPGPCYRQQR**VST**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQGQ***RG<u>SRGATVL</u>***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG

NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**A**SVDFAVN**A**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 44 PV2_6H2 DNA sequence

DNA, **ggaggc agc aat gtc acg aac aaacaa gat GGC gca gca** (G1875A, *G1965T, both silent mutations)*

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gattcctcacacggacggcaacttccacccgtctccgctgatgggcggctttggcctgaaacatcctcc**T**cctcagatcctgatcaagaaca
cgcctgtacctgcggatcctccgaccaccttcaaccagtcaaagctgaactctttcatcacgcaatacagcaccggacaggtcagcgtgga
aattgaatgggagctgcagaaggaaaacagcaagcgctggaaccccgagatccagtacacctccaactactacaaatct**gca**agtgtgga
ctttgctgttaat**gca**gaaggcgtgtactctgaaccccgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 45 PV27_6H2 Protein sequence

Protein, **GGSNVTNKQDGAA**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQRV**S**T**T**TGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**D**NLQGQ***RGSRGATVL***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG

NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**A**SVDFAVN**A**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 46 AAV8_PV27 B10 DNA sequence

DNA, **ggtgca tct aac gtt gca gca aaacag gat GGC gca gaa** (A1644G, a silent mutation)

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
agcccaaagccaaccagcaaaagcaggacgacggccggggtctggtgcttcctggctacaagtacctcggacccttcaacggactcgac
aaggggagcccgtcaacgcggcggacgcagcggccctcgagcacgacaaggcctacgaccagcagctgcaggcgggtgacaatcc
gtacctgcggtataaccacgccgacgccgagtttcaggagcgtctgcaagaagatacgtcttttgggggcaacctcgggcgagcagtcttc
caggccaagaagcgggttctcgaacctctcggtctggttgaggaaggcgctaagacggctcctggaaagaagagaccggtagagccatc
accccagcgttctccagactcctctacgggcatcggcaagaaaggccaacagcccgccagaaaaagactcaattttggtcagactggcga
ctcagagtcagttccagaccctcaacctctcggagaacctccagcagcgccctctggtgtgggacctaatacaatggctgcaggcggtggc
gcaccaatggcagacaataacgaaggcgccgacggagtgggtagttcctcgggaaattggcattgcgattccacatggctgggcgacag
agtcatcaccaccagcacccgaacctgggccctgcccacctacaacaaccacctctacaagcaaatctccaacgggacatcgggaggag
ccaccaacgacaacacctacttcggctacagcacccccctgggggtattttgactttaacagattccactgccacttttcaccacgtgactggca
gcgactcatcaacaacaactggggattccggcccaagagactcagcttcaagctcttcaacatccaggtcaaggaggtcacgcagaatga
aggcaccaagaccatcgccaataacctcaccagcaccatccaggtgtttacggactcggagtaccagctgccgtacgttctcggctctgcc
caccagggctgcctgcctccgttcccggcggacgtgttcatgattccccagtacggctacctaacactcaacaacggtagtcaggccgtgg
gacgctcctccttctactgcctggaatactttccttcgcagatgctgagaaccggcaacaacttccagtttacttacaccttcgaggacgtgcct
ttccacagcagctacgcccacagccagagcttggaccggctgatgaatcctctgattgaccagtacctgtactacttgtctcggactcaaaca
aca**ggtgca**acggcaaatacgcagactctgggcttc**tct**caaggtgggcct**aac**acaatggccaatcaggcaaagaactggctgccagg
accctgttaccgccaacaacgc**gtt**tca**gca**acaaccgggcaa**gca**aacaatagcaactttgcctggactgctgggaccaaataccatctg
aatggaagaaattcattggctaatcctggcatcgctatggcaacacacaaagacgacgaggagcgttttttttcccagtaacgggatcctgattt
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ctacagaggaatacggtatcgtggca**gat**aacttgcag***ggccagaga*GGC***tcgcgcggggctacggtcctggcccaggcggcc***caa
attggaactgtcaacagccaggggggccttacccggtatggtctggcagaaccgggacgtgtacctgcagggtcccatctgggccaagatt
cctcacacggacggcaacttccacccgtctccgctgatgggcggctttggcctgaaacatcctccgcctcagatcctgatcaagaacacgc
ctgtacctgcggatcctccgaccaccttcaaccagtcaaagctgaactctttcatcacgcaatacagcaccggacaggtcagcgtggaaatt
gaatgggagctgca**A**aaggaaaacagcaagcgctggaaccccgagatccagtacacctccaactactacaaatct**gca**agtgtggacttt
gctgttaat**gaa**gaaggcgtgtactctgaaccccgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 47 AAV8 PV27_B10 Protein sequence

*Protein, **GASNVAAKQDGAE***

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GA**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**A**TTGQ**A**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**KQ**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VADNLQGQ***RGSRGATVL***AQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDG

NFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ
KENSKRWNPEIQYTSNYYKS**A**SVDFAVN**E**EGVYSEPRPIGTRYLTRNL*

SEQ ID NO: 120 AAV8_D5 DNA sequence

DNA, **ggaggc agc aat gtc acg aac gcagca gca acg aca gca**

atggctgccgatggttatcttccagattggctcgaggacaacctctctgagggcattcgcgagtggtgggcgctgaaacctggagccccga
agcccaaagccaaccagcaaaagcaggacgacggccggggtctggtgcttcctggctacaagtacctcggacccttcaacggactcgac
aaggggagcccgtcaacgcggcggacgcagcggccctcgagcacgacaaggcctacgaccagcagctgcaggcgggtgacaatcc
gtacctgcggtataaccacgccgacgccgagtttcaggagcgtctgcaagaagatacgtcttttgggggcaacctcgggcgagcagtcttc
caggccaagaagcgggttctcgaacctctcggtctggttgaggaaggcgctaagacggctcctggaaagaagagaccggtagagccatc
accccagcgttctccagactcctctacgggcatcggcaagaaaggccaacagcccgccagaaaaagactcaattttggtcagactggcga
ctcagagtcagttccagaccctcaacctctcggagaacctccagcagcgccctctggtgtgggacctaatacaatggctgcaggcggtggc
gcaccaatggcagacaataacgaaggcgccgacggagtgggtagttcctcgggaaattggcattgcgattccacatggctgggcgacag
agtcatcaccaccagcacccgaacctgggccctgcccacctacaacaaccacctctacaagcaaatctccaacgggacatcgggaggag
ccaccaacgacaacacctacttcggctacagcacccctgggggtattttgactttaacagattccactgccacttttcaccacgtgactggca
gcgactcatcaacaacaactggggattccggcccaagagactcagcttcaagctcttcaacatccaggtcaaggaggtcacgcagaatga
aggcaccaagaccatcgccaataacctcaccagcaccatccaggtgtttacggactcggagtaccagctgccgtacgttctcggctctgcc
caccagggctgcctgcctccgttcccggcggacgtgttcatgattccccagtacggctacctaacactcaacaacggtagtcaggccgtgg
gacgctcctccttctactgcctggaatactttccttcgcagatgctgagaaccggcaacaacttccagtttacttacaccttcgaggacgtgcct
ttccacagcagctacgcccacagccagagcttggaccggctgatgaatcctctgattgaccagtacctgtactacttgtctcggactcaaaca
aca**ggaggc**acggcaaatacgcagactctgggcttc**agc**caaggtgggcct**aat**acaatggccaatcaggcaaagaactggctgccag
gaccctgttaccgccaacaacgc**gtc**tca**acg**acaaccgggcaa**aac**aacaatagcaactttgcctggactgctgggaccaaataccatc
tgaatggaagaaattcattggctaatcctggcatcgctatggcaacacacaaagacgacgaggagcgttttttttcccagtaacgggatcctga
tttttggc**gcagca**aatgctgccagagacaatgcggattacagcgatgtcatgctcaccagcgaggaagaaatcaaaaccactaaccctgt
ggctacagaggaatacggtatcgtggca**gca**aacttgcagcagcaaaac**acg**gctcctcaaattggaactgtcaacagccaggggggcctt
acccggtatggtctggcagaaccgggacgtgtacctgcagggtcccatctgggccaagattcctcacacggacggcaacttccacccgtct
ccgctgatgggcggctttggcctgaaacatcctccgcctcagatcctgatcaagaacacgcctgtacctgcggatcctccgaccaccttcaa
ccagtcaaagctgaactctttcatcacgcaatacagcaccggacaggtcagcgtggaaattgaatgggagctgcagaaggaaaacagcaa
gcgctggaaccccgagatccagtacacctccaactactacaaatct**aca**agtgtggactttgctgttaat**gca**gaaggcgtgtactctgaac
cccgccccattggcacccgttacctcacccgtaatctgtaa

SEQ ID NO: 121 AAV8_D5_Protein sequence

Protein, **GGSNVTNAAATTA**

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRL
NFGQTGDSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSSSGNW
HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDF
NRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT
DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQML
RTGNNFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT**GG**TANTQTLGF
SQGGP**N**TMANQAKNWLPGPCYRQQR**V**S**T**TTGQ**N**NNSNFAWTAGTKYHLNGRNSLAN
PGIAMATHKDDEERFFPSNGILIFG**AA**NAARDNADYSDVMLTSEEEIKTTNPVATEEYGI
VA**A**NLQQQN**T**APQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKS**T**SVDFAVN**A**EGVYSEPRPIGTRYLTRNL*

CLAIMS

WHAT IS CLAIMED IS:

1. A variant adeno-associated virus 8 (AAV8) capsid polypeptide comprising one or more mutations relative to a wild-type AAV8 capsid polypeptide in one or more regions (according to VP1 numbering) selected from the group consisting of amino acids 262-274, amino acids 328-333, amino acids 383-391, amino acids 452-471, amino acids 490-507, amino acids 528-545, amino acids 547-564, amino acids 582-597, and amino acids 706-720.
2. The variant AAV8 capsid polypeptide of claim 1, wherein the one or more mutations are one or more amino acid substitutions.
3. The variant AAV8 capsid polypeptide of claim 2, wherein the one or more amino acid substitutions are one or more alanine substitutions and/or glutamic acid substitutions.
4. The variant AAV8 capsid polypeptide any one of claims 1-3, wherein the variant AAV8 capsid polypeptide comprises one or more amino acid substitutions at positions G455, G456, S466, N471, V491, T493, N498, K547, Q548, D584, T591, T711, or T719 relative to a wild-type AAV8 capsid polypeptide.
5. The variant AAV8 capsid polypeptide of any one of claims 1-4, further comprising a peptide insertion selected from the group consisting of NSVRGDG (SEQ ID NO: 122), GNDVRAR (SEQ ID NO: 123), and SRGATVL (SEQ ID NO: 124) after amino acid 590 relative to the wild-type AAV8 capsid polypeptide.
6. The variant AAV8 capsid polypeptide of claim 5, wherein the peptide insertion further comprises a G at the N-terminus and an A at the C-terminus.
7. The variant AAV8 capsid polypeptide of claim 6, wherein the three amino acids preceding the site into which said peptide is inserted into the AAV8 capsid polypeptide have been changed to GQS or GQR and/or the three amino acids following the site into which said peptide is inserted have been changed to QAA.
8. The variant AAV8 capsid polypeptide of any one of claims 1-7, comprising one of the following sets of alanine and/or glutamic acid substitution(s):
    a. G456A, S466A, D584A, and T591A;

b. G456A, T493A, N498A, D584A, T591A, T711A, and T719E;

c. S466A, V491A, D584A, and T591A;

d. G455A, G456A, S466A, Q548A, and D584A;

e. D584A, T591A, T711A, and T719A;

f. K547A, D584A, and T591A;

g. G456A, T493A, N498A, D584A, and T591A;

h. G456A, S466A, N471A, T711A, and T719A;

i. G456A, N471A, and T493A;

j. T711A, and T719A;

k. T711A; and

l. K547A, Q548A, D584A, and T719A.

9. The variant AAV8 capsid polypeptide of any one of claims 5-8, comprising a peptide insertion of NSVRGDG (SEQ ID NO: 122) after amino acid 590 and one of the following sets of alanine substitution(s):

m. T711A and T719A;

n. G456A, S466A, N471A, T711A, and T719A;

o. T711A; and

p. G456A, N471A, and T493A.

10. The variant AAV8 capsid polypeptide of any one of claims 5-8, comprising a peptide insertion of GNDVRAR (SEQ ID NO: 123) after amino acid 590, and alanine substitutions T711A and T719A.

11. The variant AAV8 capsid polypeptide of any one of claims 5-8, comprising a peptide insertion of SRGATVL (SEQ ID NO: 124) after amino acid 590 and one of the following sets of alanine and/or glutamic acid substitution(s):

q. G456A, T493A, N498A, T591A, T711A, and T719E;

r. G456A, S466A, N471A, T711A, and T719A;

s. G456A, N471A, and T493A; and

t. T711A, and T719A.

12. The variant AAV8 capsid polypeptide of any one of claims 1-11, wherein the one or more mutations result in decreased binding of the variant AAV8 capsid polypeptide to a neutralizing factor, compared to the binding of a wild-type AAV8 capsid polypeptide to the neutralizing factor.

13. The variant AAV8 capsid polypeptide of any one of claims 1-12, wherein the one or more mutations do not affect the genome packaging ability and/or transduction efficiency of AAV8.

14. The variant AAV8 capsid polypeptide of any one of claims 1-13, comprising an amino acid sequence having at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 36, 41, 43, 45, 47, and 121.

15. The variant AAV8 capsid polypeptide of claim 14, comprising an amino acid sequence having at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 22, 26, 28, 30, 41, 43, 45, 47, and 121.

16. The variant AAV8 capsid polypeptide of claim 14, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 36, 41, 43, 45, 47, and 121.

17. The variant AAV8 capsid polypeptide of any one of claims 14-16, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 22, 26, 28, 30, 41, 43, 45, 47, and 121.

18. A variant adeno-associated virus 8 (AAV8) capsid polypeptide, comprising an amino acid sequence of amino acids 138-747 of the variant AAV8 capsid polypeptide of any one of claims 1-17.

19. A variant adeno-associated virus 8 (AAV8) capsid polypeptide, comprising an amino acid sequence of amino acids 204-747 of the variant AAV8 capsid polypeptide of any one of claims 1-17.

20. A nucleic acid encoding the variant AAV8 capsid polypeptide of any one of claims 1-19.

21. The nucleic acid of claim 20, comprising a nucleotide sequence having at least 80% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 35, 40, 42, 44, 46, and 120.

22. The nucleic acid of claim 21, comprising a nucleotide sequence having at least 80% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5, 7, 9, 11, 13, 15, 21, 25, 27, 29, 40, 42, 44, 46, and 120.

23. The nucleic acid of claim 21, comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 19, 21, 23, 25, 27, 29, 35, 40, 42, 44, 46, and 120.

24. The nucleic acid of any one of claims 21-23, comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5, 7, 9, 11, 13, 15, 21, 25, 27, 29, 40, 42, 44, 46, and 120.

25. A nucleic acid encoding a variant adeno-associated virus 8 (AAV8) capsid polypeptide, comprising a nucleotide sequence of nucleotides 412-2244 of the nucleic acid of any one of claims 21-24.

26. A nucleic acid encoding a variant adeno-associated virus 8 (AAV8) capsid polypeptide, comprising a nucleotide sequence of nucleotides 610-2244 of the nucleic acid of any one of claims 21-24.

27. A recombinant DNA comprising the nucleic acid of any one of claims 21-26.

28. An isolated host cell comprising the nucleic acid of any one of claims 20-26 or the recombinant DNA of claim 27.

29. An adeno-associated virus (AAV) vector comprising the variant AAV8 capsid polypeptide of any one of claims 1-19.

30. The AAV vector of claim 29, further comprising a heterologous nucleic acid.

31. The AAV vector of claim 30, wherein the heterologous nucleic acid comprises a nucleotide sequence encoding a therapeutic protein.

32. The AAV vector of claim 31, wherein the therapeutic protein is coagulation factor VIII or coagulation factor IX, or a functional fragment or derivative thereof.

33. The AAV vector of any one of claims 29-32, wherein the AAV vector exhibits an improved immune escaping capacity compared an AAV8 wild-type vector.

34. The AAV vector of claim 33, wherein the AAV vector has an immune escaping capacity score (IECS) of between about 0.01 to about 4.

35. A pharmaceutical composition comprising the AAV vector of any one of claims 29-34, and a pharmaceutically acceptable carrier and/or excipient.

36. A method of delivering a gene product to a subject in need thereof, said method comprising administering to the subject an effective amount of an adeno-associated virus (AAV) vector of any one of claims 29-34 or the pharmaceutical composition of claim 35.

37. The method of claim 36, wherein the subject has existing neutralizing antibodies against AAV8 prior to the administration.

38. The method of claims 36 or 37, wherein the AAV vector or the pharmaceutical composition is administered intravenous, intramuscular, subcutaneous, intratumor, intradermal, transdermal, intranodal, intraspinal, intraprostatic, intralymphatic, intraparenchymal, intraportal or intraperitoneal injection.

39. The method of any one of claims 36-38, wherein the subject has hemophilia A or hemophilia B.

40. The method of any one of claims 36-39, wherein the subject is human.

41. The method of any one of claims 36-40, wherein the subject is a non-human.

42. The method of claim 41, wherein the non-human is a mouse, a rat, a rabbit, a dog, a cat, a sheep, a pig, or a non-human primate.

43. A method of treating a liver-borne blood disorder in a human subject in need thereof, said method comprising administering to the subject an effective amount of the adeno-associated virus (AAV) vector of any one of claims 31-32 or a pharmaceutical composition comprising the AAV vector of any one of claims 31-32, and a pharmaceutically acceptable carrier and/or

excipient, wherein the therapeutic protein is a protein used for the treatment of a liver-borne blood disorder.

44. The method of claim 43, wherein the therapeutic protein is a blood coagulation factor.

45. The method of claim 43 or 44, wherein the liver-borne blood disorder is a coagulation disorder.

46. The method of claim 45, wherein the coagulation disorder is hemophilia.

47. The method of claim 46, wherein the hemophilia is hemophilia A or hemophilia B.

48. The method of claim 43, wherein the AAV vector or the pharmaceutical composition is administered at about $1x10^{11}$ to about $1x10^{14}$ vg/kg.

49. The method of claim 48, wherein the AAV vector or the pharmaceutical composition is administered at about $5x10^{11}$ vg/kg.

50. The method of any one of claims 43-49, wherein the AAV vector or the pharmaceutical composition is administered via intravenous, intramuscular, subcutaneous, intratumor, intradermal, transdermal, intranodal, intraspinal, intraprostatic, intralymphatic, intraparenchymal, intraportal or intraperitoneal route injection.

FIG. 1

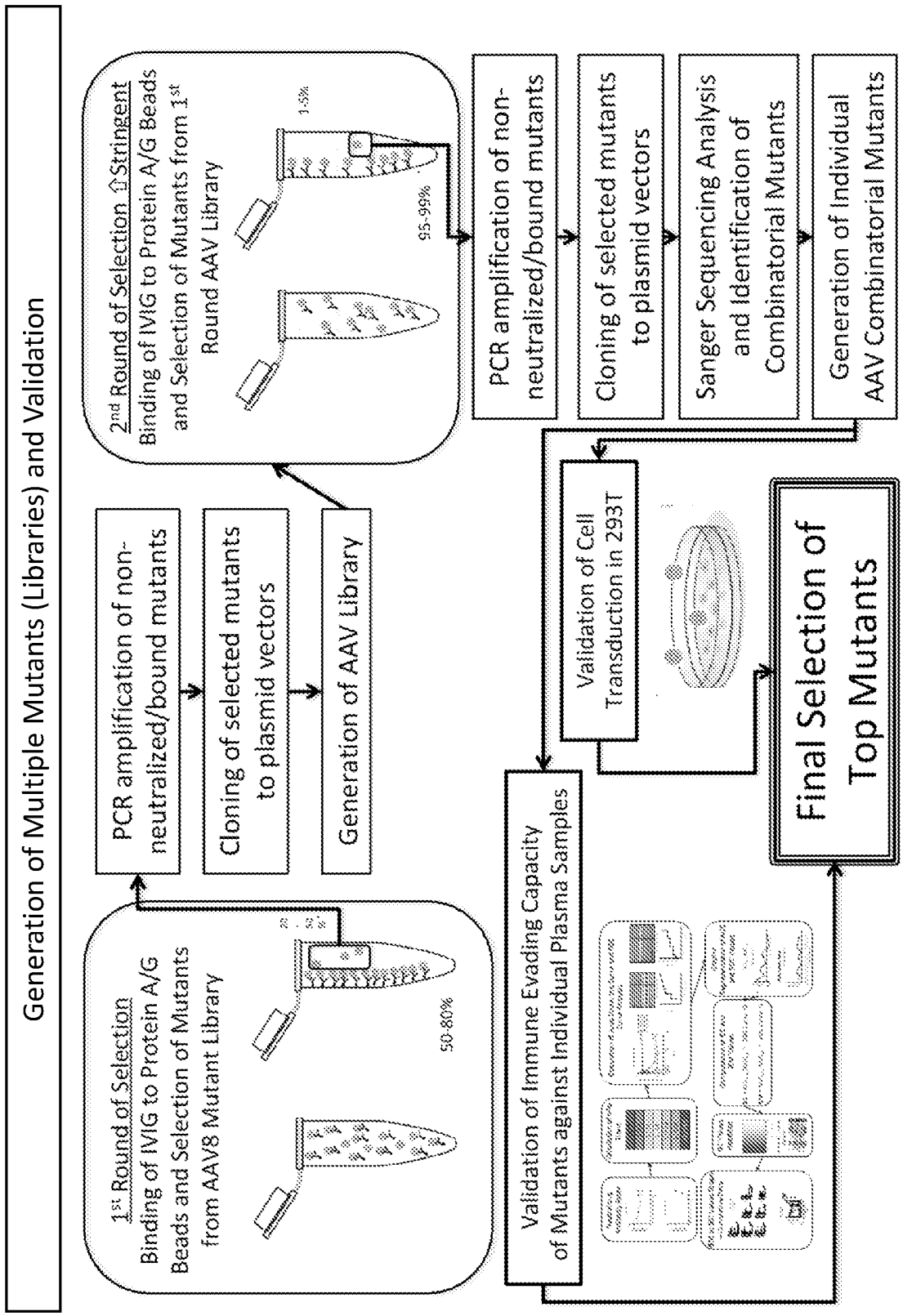

Generation of Multiple Mutants (Libraries) and Validation
1st Round of Selection
Binding of IVIG to Protein A/G Beads and Selection of Mutants from AAV8 Mutant Library
50-80%
PCR amplification of non-neutralized/bound mutants
Cloning of selected mutants to plasmid vectors
Generation of AAV Library
2nd Round of Selection ⇧Stringent
Binding of IVIG to Protein A/G Beads and Selection of Mutants from 1st Round AAV Library
95-99%
1-5%
PCR amplification of non-neutralized/bound mutants
Cloning of selected mutants to plasmid vectors
Sanger Sequencing Analysis and Identification of Combinatorial Mutants
Generation of Individual AAV Combinatorial Mutants
Validation of Cell Transduction in 293T
Validation of Immune Evading Capacity of Mutants against Individual Plasma Samples
Final Selection of Top Mutants

FIG. 2A

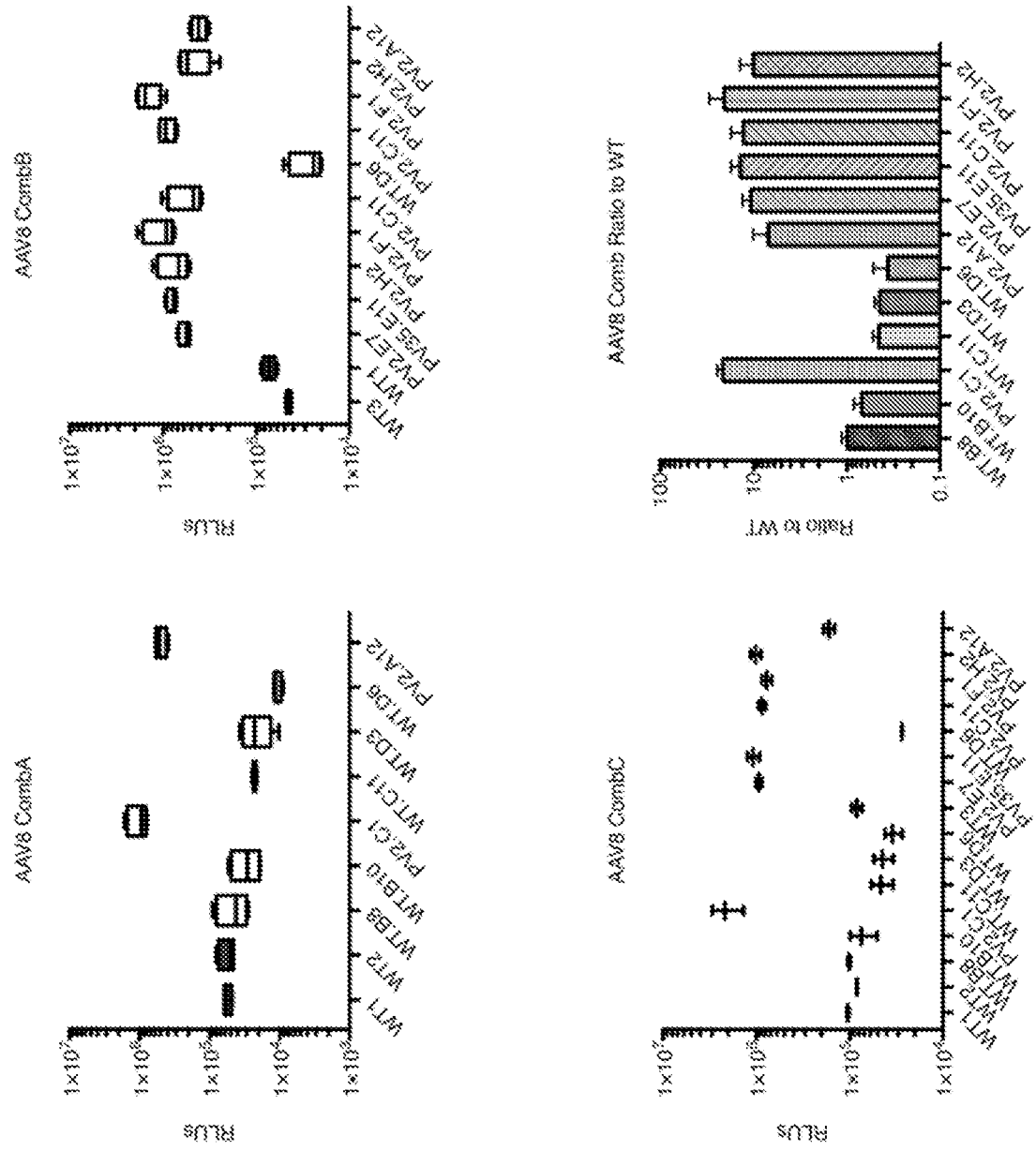

AAV8 CombA
AAV8 CombB
AAV8 CombC
AAV8 Comb Ratio to WT
RLUs
Ratio to WT
WT1
WT2
WT B9
WT B10
PV2 C1
WT C1
WT D3
WT D6
PV2 A12
PV2 E7
PV35 E11
PV2 C11
PV2 F1
PV2 H2

FIG. 2B

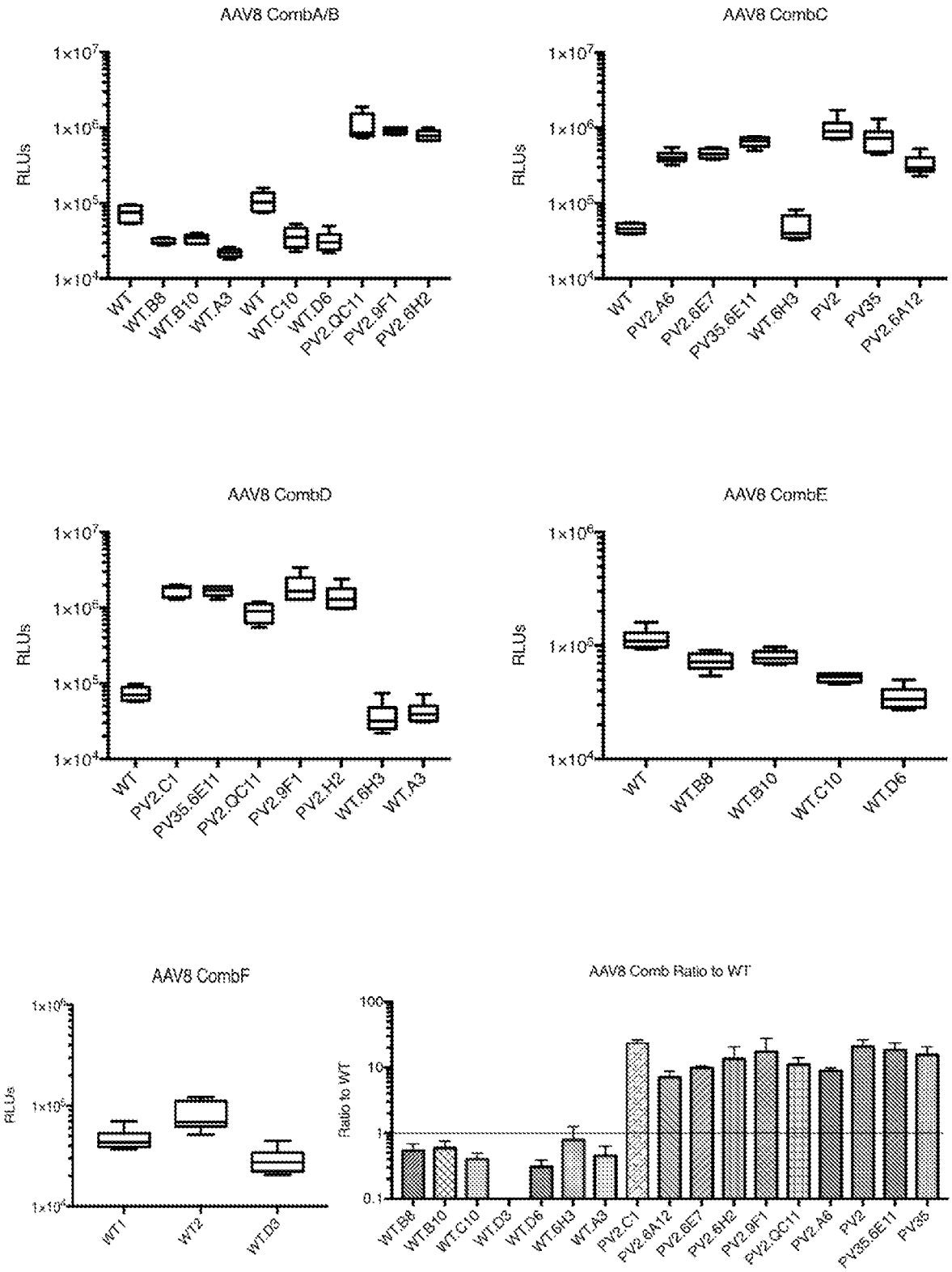
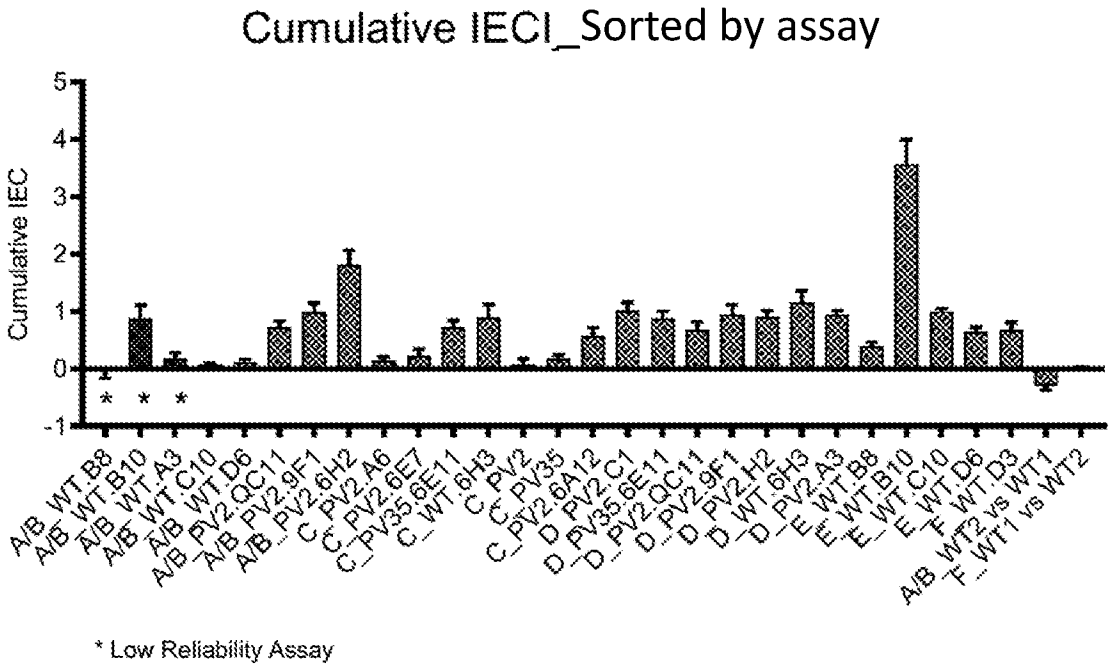

AAV8 CombA/B
RLUs
1x10^7
1x10^6
1x10^5
1x10^4
WT
WT.B8
WT.B10
WT.A3
WT
WT.C10
WT.D6
PV2.QC11
PV2.9F1
PV2.6H2
AAV8 CombC
RLUs
1x10^7
1x10^6
1x10^5
1x10^4
WT
PV2.A6
PV2.6E7
PV35.6E11
WT.6H3
PV2
PV35
PV2.6A12
AAV8 CombD
RLUs
1x10^7
1x10^6
1x10^5
1x10^4
WT
PV2.C1
PV35.6E11
PV2.QC11
PV2.9F1
PV2.H2
WT.6H3
WT.A3
AAV8 CombE
RLUs
1x10^6
1x10^5
1x10^4
WT
WT.B8
WT.B10
WT.C10
WT.D6
AAV8 CombF
RLUs
1x10^6
1x10^5
1x10^4
WT1
WT2
WT.D3
AAV8 Comb Ratio to WT
Ratio to WT
100
10
1
0.1
WT.B8
WT.B10
WT.C10
WT.D3
WT.D6
WT.6H3
WT.A3
PV2.C1
PV2.6A12
PV2.6E7
PV2.6H2
PV2.9F1
PV2.QC11
PV2.A6
PV2
PV35.6E11
PV35

FIG. 3A



* Low Reliability Assay

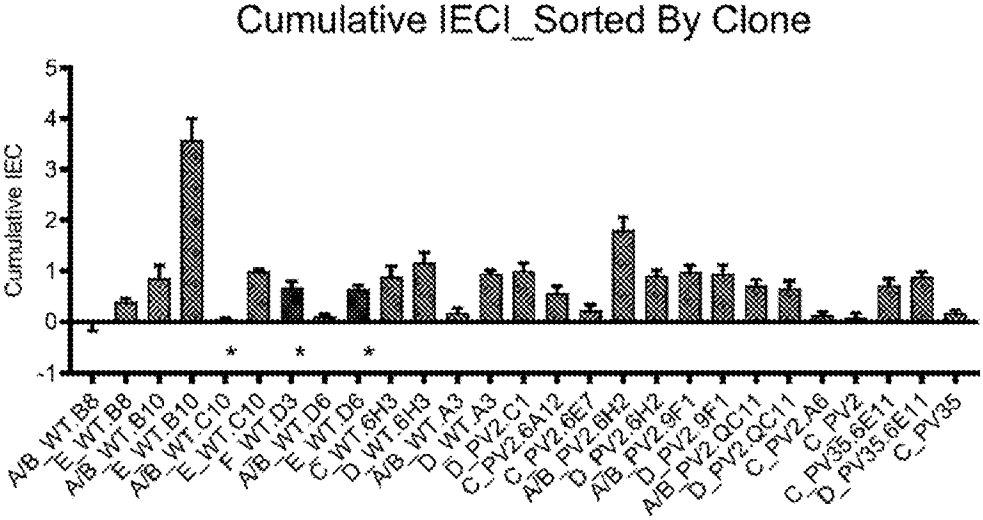


FIG. 3B

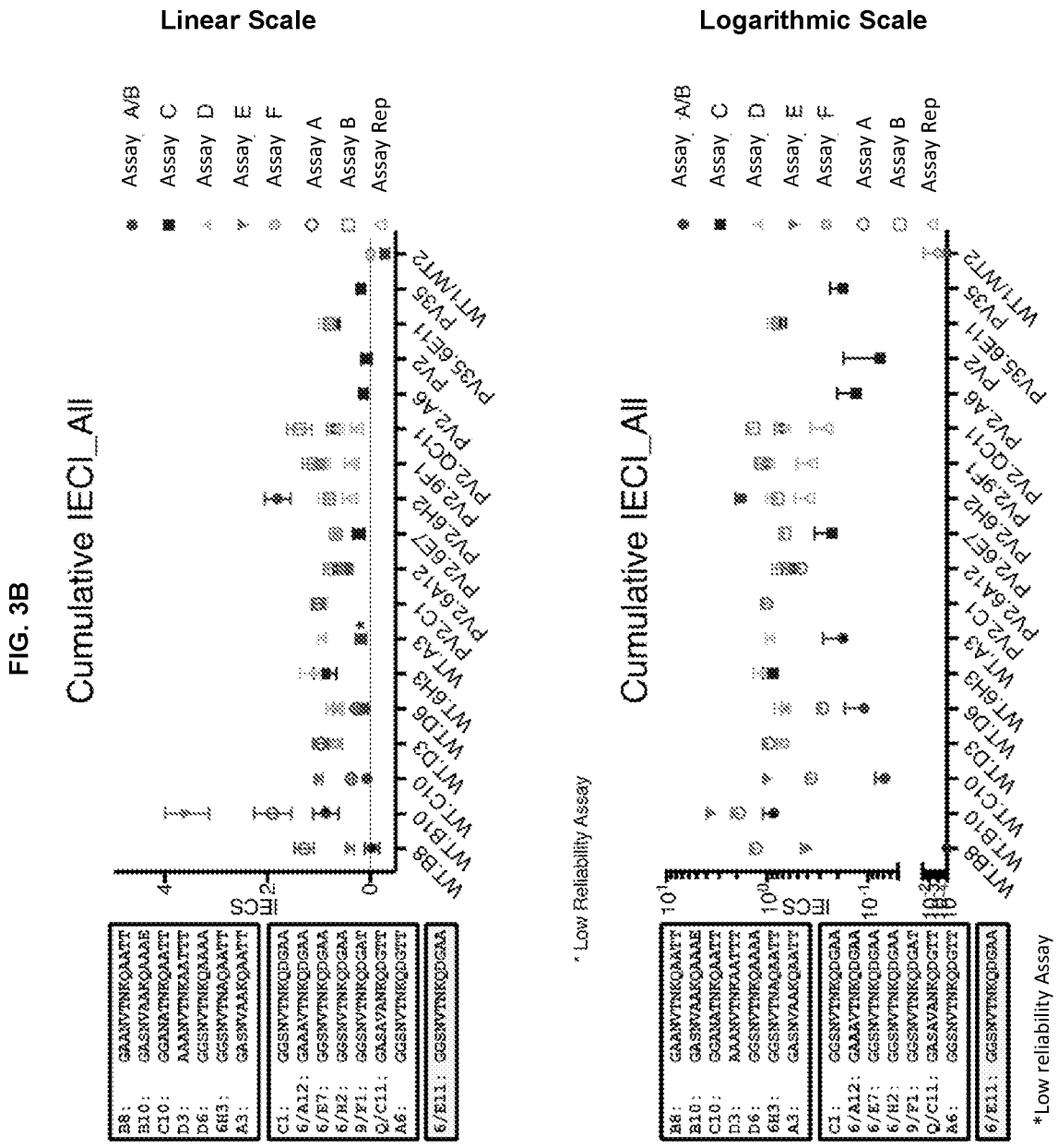

Linear Scale
Logarithmic Scale
Cumulative IECI_All
IECS
Assay A/B
Assay C
Assay D
Assay E
Assay F
Assay A
Assay B
Assay Rep
WT.B8
WT.B10
WT.C10
WT.D3
WT.D6
WT.6H3
WT.A3
PV2.C1
PV2.6A12
PV2.6E7
PV2.6H2
PV2.9F1
PV2.QC11
PV2.A6
PV2
PV35.6E11
PV35
WT1/MT2
B8: GAAWVTNKQAATT
B10: GASWVAAKQAAAE
C10: GGAWATNKQAATT
D3: AAAWVTNKAATTT
D6: GGSWVTNKQAAAA
6H3: GGSWVTNAQAATT
A3: GASWVAAKQAATT
C1: GGSWVTNKQDGAA
6/A12: GAAAVTNKQDGAA
6/E7: GGSWVTNKQDGAA
6/H2: GGSWVTNKQDGAA
9/F1: GGSWVTNKQDGAT
Q/C11: GASAVANKQDGTT
A6: GGSWVTNKQDGTT
6/E11: GGSWVTNKQDGAA
* Low Reliability Assay
*Low reliability Assay

FIG. 4

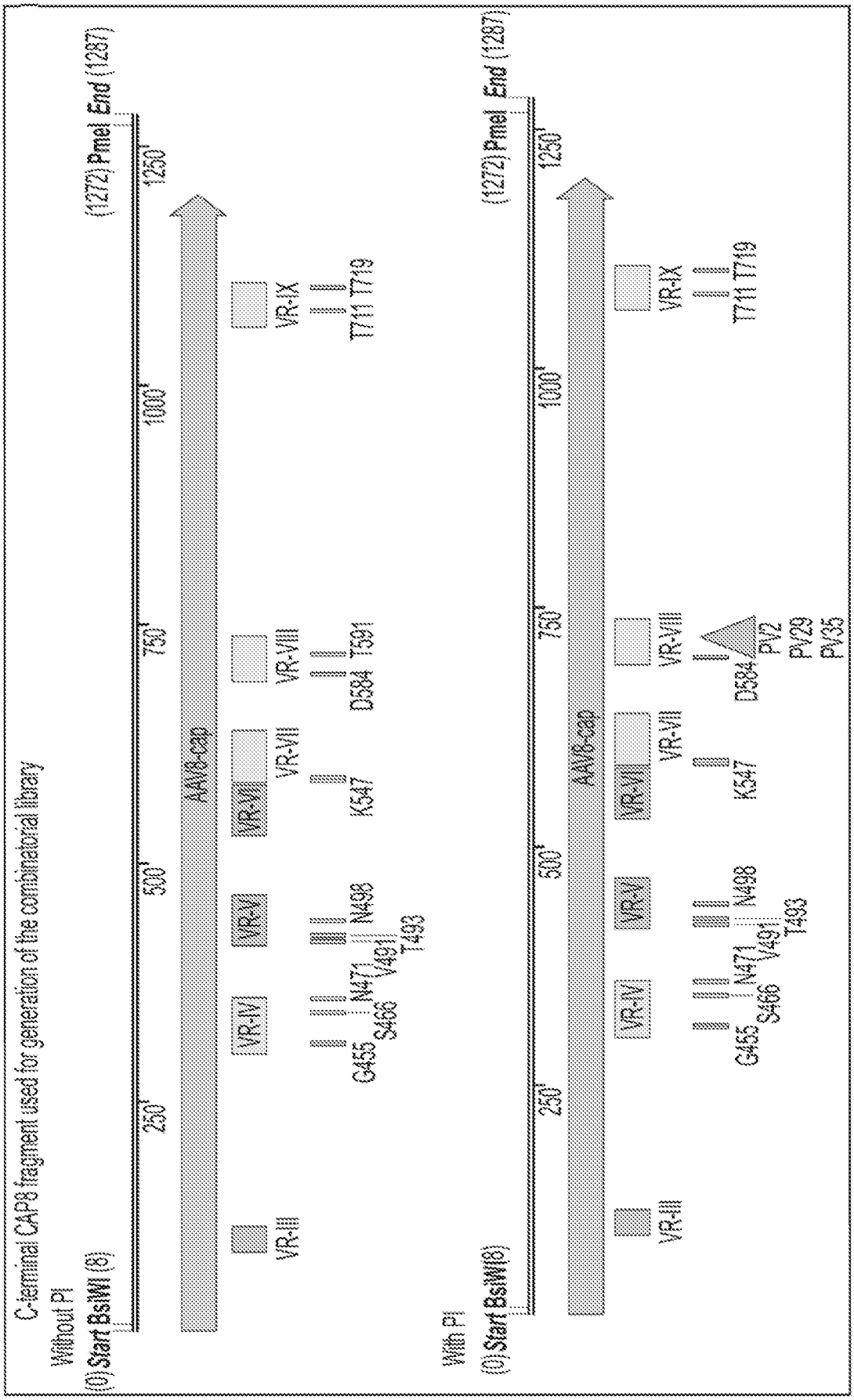

C-terminal CAP8 fragment used for generation of the combinatorial library
Without PI
(0) Start BsiWI (8)
(1272) PmeI End (1287)
250'
500'
750'
1000'
1250'
AAV8-cap
VR-III
VR-IV
VR-V
VR-VII
VR-VIII
VR-IX
G455
S466
N471
V491
N498
T493
K547
D584
T591
T711
T719
With PI
(0) Start BsiWI(8)
(1272) PmeI End (1287)
PV2
PV29
PV35

FIG. 5

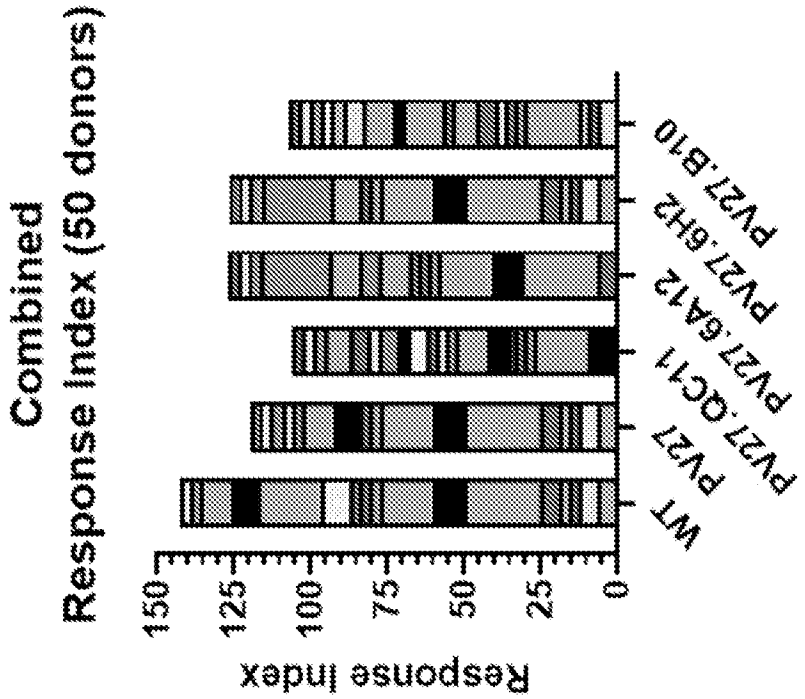

Combined
Response Index (50 donors)
Response index
150
125
100
75
50
25
0
WT
PV27
PV27.QC11
PV27.6A12
PV27.6H2
PV27.B10

FIG. 6A

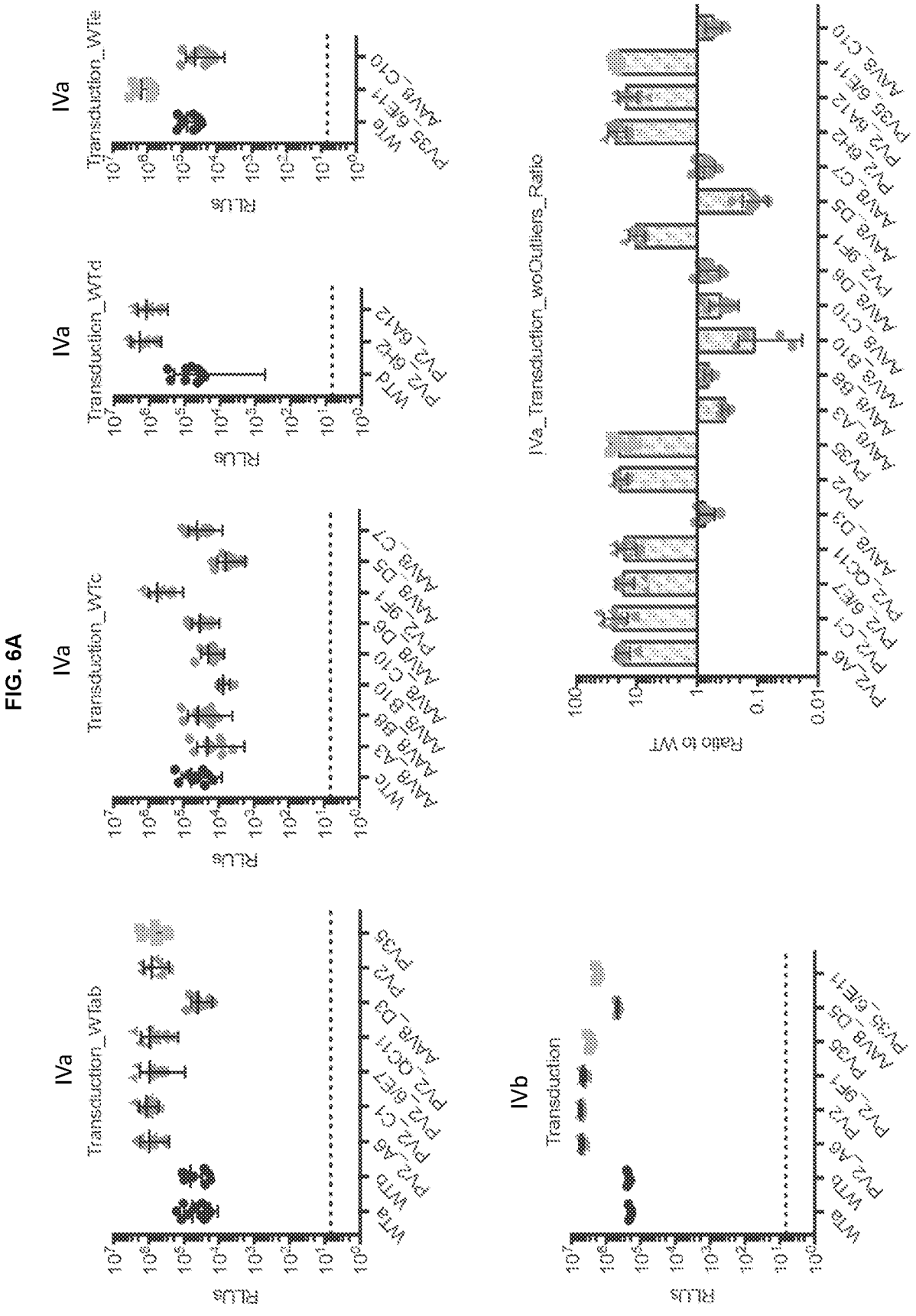

IVa
Transduction_WTab
IVa
Transduction_WTc
IVa
Transduction_WTd
IVa
Transduction_WTa
IVb
Transduction
IVa_Transduction_woOutliers_Ratio
RLUs
Ratio to WT

FIG. 6B

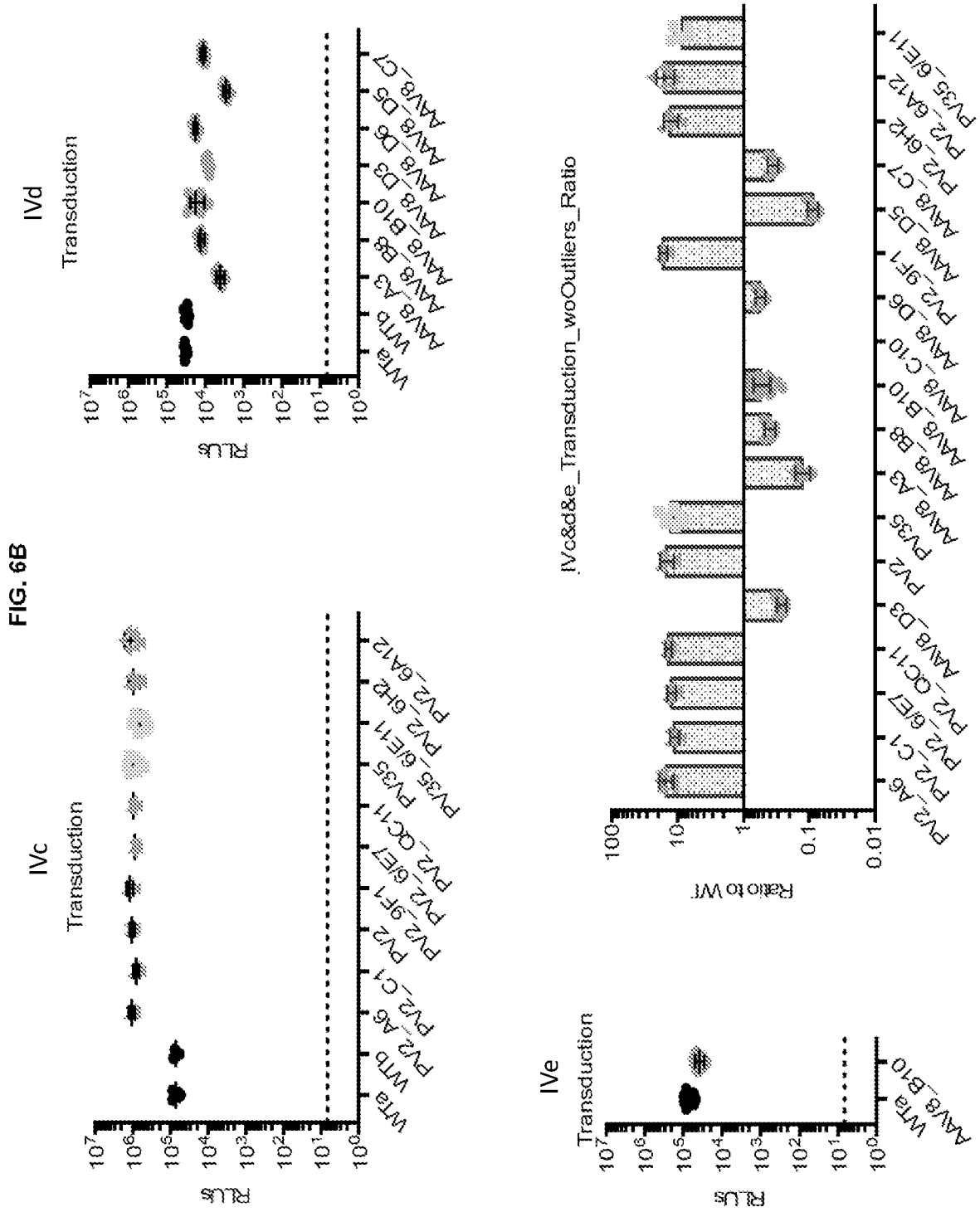
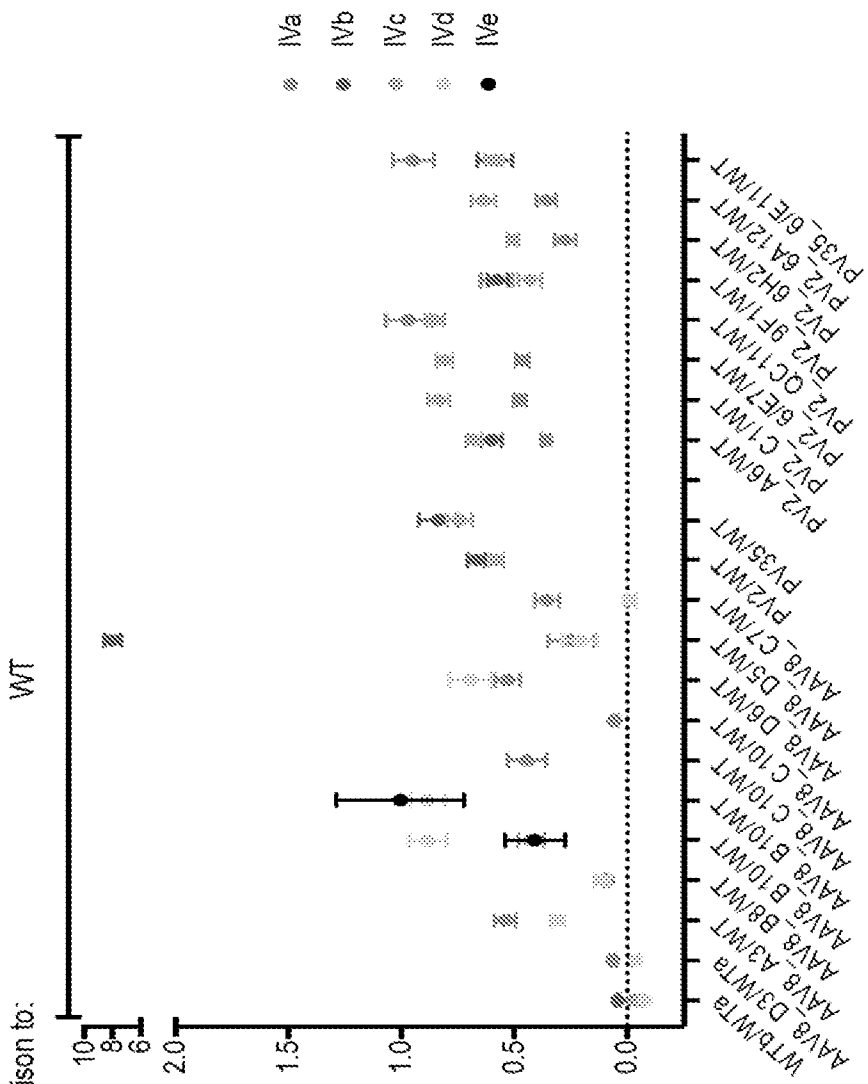
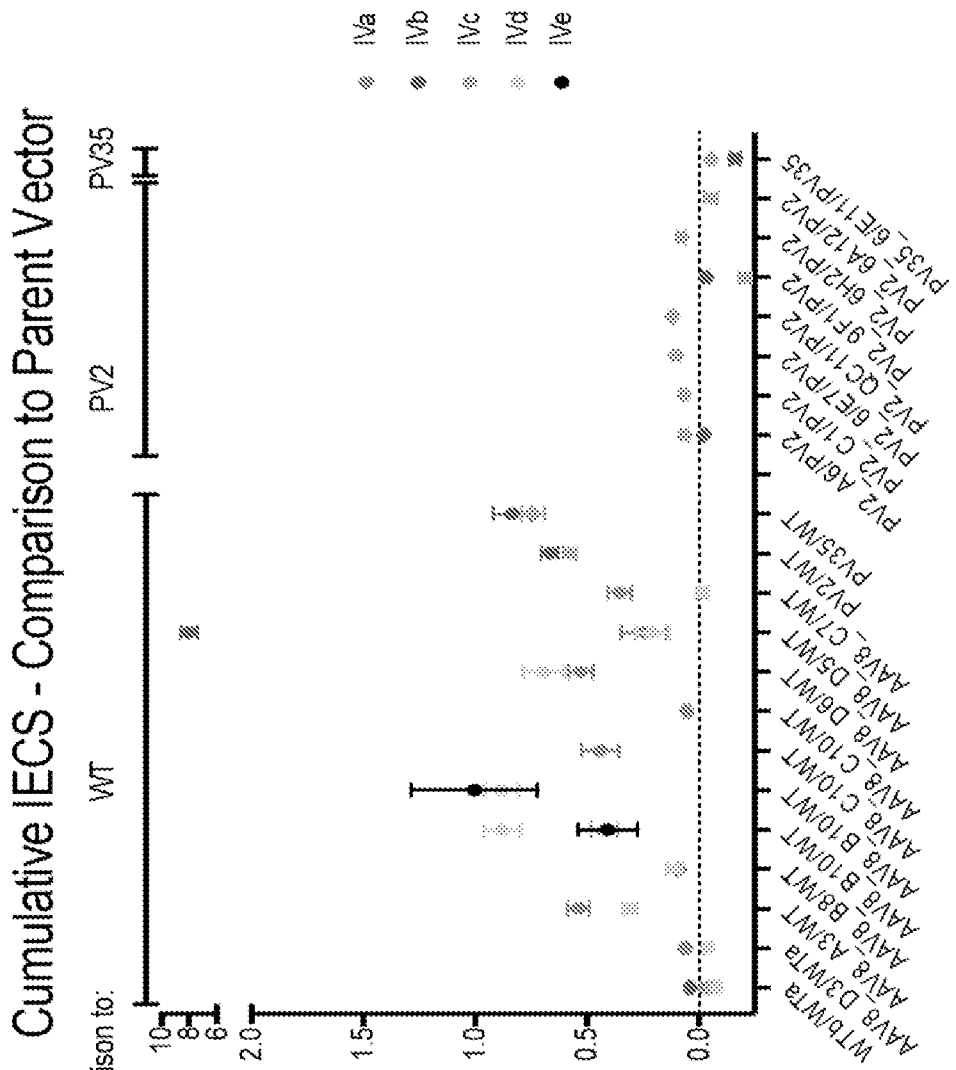

IVc
Transduction
RLUs
WTa
WTb
PV2_A6
PV2_C1
PV2_9F1
PV2_6/E7
PV2_QC11
PV35_6/E11
PV2_6H2
PV2_6A12
IVd
Transduction
RLUs
WTa
WTb
AAV8_A3
AAV8_B8
AAV8_B10
AAV8_D3
AAV8_D6
AAV8_D5
AAV8_C7
IVe
Transduction
RLUs
WTa
AAV8_B10
IVc&d&e_Transduction_woOutliers_Ratio
Ratio to WT
PV2_A6
PV2_C1
PV2_6/E7
PV2_QC11
AAV8_D3
PV2
PV35
AAV8_A3
AAV8_B8
AAV8_B10
AAV8_C10
AAV8_D6
PV2_9F1
AAV8_D5
AAV8_C7
PV2_6H2
PV2_6A12
PV35_6/E11

FIG. 7A



Amino acid sequence	Clone	Parent
GASK VAA KQ AA TT	A3	WT
GAAK VTN KQ AA TY	B8	WT
GASK VAA KQ AA AE	B10	WT
GGSK VTN KQ AA AA	D6	WT
GGSK HAL AA AT TA	D5	WT
GGSA VAM AQ AA TT	C7	WT
AAAK VTN KA AT TT	D3	WT
GGAK ATN KQ AA TT	C10	WT
	WT	PV2
	WT	PV35
GGSK VTN KQ DG TT	A6	PV2
GGSK VTN KQ DG AA	C1	PV2
GGSK VTN KQ DG AA	6/E7	PV2
GASA VAM KQ DG TT	6C11	PV2
GGSK VTN KQ DG AT	9F1	PV2
GGSK VTN KQ DG AA	6H2	PV2
GAAA VTN KQ DG AA	6A12	PV2
GGSK VTN KQ DG AA	6/E11	PV35

FIG. 7B



Amino acid sequence	Clone	Parent
GASM VAA KQ AA TT	A3	WT
GAAM VTM KQ AA TY	B8	WT
GASM VAA KQ AA AE	B10	WT
GGSM VTM KQ AA AA	B6	WT
GGSN HAL AA AT TA	B5	WT
GGSA VAM AQ AA TT	C7	WT
AAAM VTM KA AT TT	B3	WT
GGAM ATM KQ AA TT	C10	WT
	WT	PV2
	WT	PV35
GGSM VTM KQ BG TT	A6	PV2
GGSM VTM KQ DG AA	C1	PV2
GGSM VTM KQ DG AA	6/E7	PV2
GASA VAM KQ BG TT	QC11	PV2
GGSM VTM KQ BG AT	9F1	PV2
GGSM VTM KQ DG AA	6H2	PV2
GAAA VTM KQ BG AA	6A12	PV2
GGSM VTM KQ BG AA	6/E11	PV35