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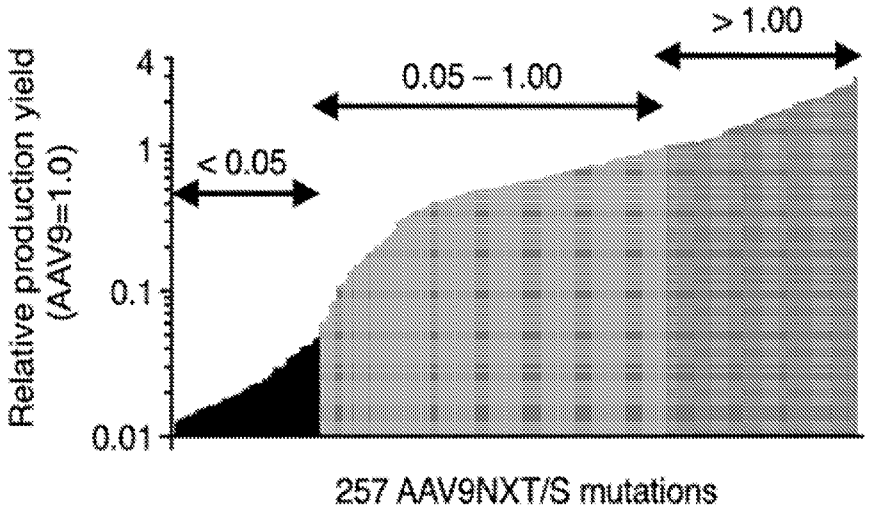
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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

(54) Title: MUTANT ADENO-ASSOCIATED VIRUS (AAV) CAPSIDS

FIG. 3



(57) Abstract: A library of mutant adeno-associated virus (AAV) 9 capsid proteins and compositions thereof are described. The mutant AAV9 capsid proteins can be selected to target expression in the liver, lungs, skeletal muscle, pancreas, kidney, brain, heart, intestine, spleen, and/or testis; detarget the liver; improve vector yield; and/or prolong the half-life. Methods of using the mutant AAV9 capsids for expression of a transgene in a subject are also provided.

[Continued on next page]

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MUTANT ADENO-ASSOCIATED VIRUS (AAV) CAPSIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/349,861 filed June 7, 2022, which is incorporated herein by reference in its entirety as if fully set forth herein.

REFERENCE TO SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in XML format in lieu of a paper copy and is hereby incorporated by reference into the specification. The name of the file containing the Sequence Listing is O046-0062PCT.xml. The file is 2.73 KB, was created on May 26, 2023, and is being submitted electronically via Patent Center.

FIELD OF THE DISCLOSURE

[0003] The current disclosure describes a library of mutant adeno-associated virus (AAV) 9 capsid proteins. The present disclosure also provides compositions and methods of using the mutant AAV9 capsids for expression in a subject to produce administration benefits, such as selective cell delivery, reduced hepatic uptake, extended *in vivo* half-life, and/or improved production yields.

BACKGROUND OF THE DISCLOSURE

[0004] Adeno-associated virus (AAV), a member of the Parvovirus family, is a small nonenveloped, icosahedral virus with a single-stranded linear DNA genome of 4.7 kilobases (kb). AAV is assigned to the genus, Dependoparvovirus, because the virus was discovered as a contaminant in purified adenovirus stocks. AAVs life cycle includes a latent phase at which AAV genomes, after infection, are integrated into host genomes or persist as extrachromosomal genomes, and an infectious phase in which, following either adenovirus or herpes simplex virus infection, the integrated or extrachromosomal AAV genomes are subsequently rescued, replicated, and packaged into infectious viruses. The use of recombinant AAV (rAAV) has become a promising tool for gene delivery because of several properties: 1) the wild-type virus is not associated with any pathologic human condition; 2) the recombinant form does not contain native viral coding sequences; and 3) persistent transgenic expression has been observed in many applications.

[0005] There are several naturally-occurring serotypes and subtypes of AAV and different

serotypes can be useful in targeting different tissues. AAV serotype 8 (AAV8) and AAV serotype 9 (AAV9) have gained attention because AAV vectors derived from these two serotypes can transduce a variety of organs including the liver, heart, skeletal muscles and central nervous system with high efficiency following systemic administration (Ghosh *et al.*, Mol Ther 15, 750-755 (2007); Pacak *et al.*, Circ Res 99, 3-9 (2006); Inagaki *et al.*, Mol Ther A, 45-53 (2006); Zhu *et al.*, Circulation 112, 2650-2659 (2005); Wang *et al.*, Nat Biotechnol 23, 321 -328 (2005); Nakai *et al.*, J Virol 79, 214-224 (2005); and Foust *et al.*, Nature Biotechnol 23, 321 -328 (2009)). This robust transduction by AAV8 and AAV9 vectors has been ascribed to strong tropism for these cell types, efficient cellular uptake of vectors, and/or rapid uncoating of virion shells in cells (Thomas *et al.*, J Virol 78, 3110-3122 (2004)). The different subtypes of AAV capsids can deliver nucleic acids to a wide variety of cells and tissues. This is due to the organization of the icosahedron-shaped capsid that interacts with specific molecules on target cells via protruding spike structures on the capsid surface.

[0006] A challenge in many gene therapies is the absorption of AAV viruses into cells that are not targeted for therapeutic intervention. This can decrease virus efficiency at intended target sites and cause undesired responses in other non-targeted cell and tissue types. To overcome this challenge, capsid engineering can be used to alter natural AAV capsid targets for improved cell specificity (tropism).

SUMMARY OF THE DISCLOSURE

[0007] The present disclosure describes a library of mutant adeno-associated virus (AAV) 9 capsid proteins with targeted transduction, detargeted transduction, more efficient transduction compared to the wild-type AAV9 capsid proteins, higher vector yield, and/or prolonged half-life.

[0008] In particular embodiments, a mutant AAV9 capsid for hepatic gene delivery includes mutations D554N/D556T. A mutant AAV9 capsid including mutations D554N/D556T includes the following biological properties: 1) rapid blood clearance following intravenous injection, 2) ability to bind albumin, and 3) more efficient transduction of hepatocytes in the liver compared to a wild-type AAV9 capsid.

[0009] In particular embodiments, a reference wild-type AAV9 capsid is mutated (or includes mutations) to create a mutant AAV9 capsid. In particular embodiments, the reference wild-type AAV9 capsid includes the sequence as set forth in SEQ ID NO: 1.

[00010] In particular embodiments, an AAV9 capsid mutant includes a mutation or set of mutations selected from: S16T, Q37T, R43T, L59T, A68T, Y90T, A96T, E106N, D107N, G115T, A136N, E147N, P153N, D154N, G160N, G174T, G174N, G177N, T179N, A194N, V198N,

S200N, M203N, E216T, G217T, P250N/Y252T, T251N/N253T, Y252N/N254T, H255T, L256T, H255N/Y257T, L256N/K258T, Y257N/Q259T, K258N/I260T, Q259N/S261T, I260N/N262T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T, G266N/S268T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, D271N/A273T, Y274T, A273N/F275T, Y274N/G276T, S278N/P280T, R288N/H290T, H290N/H292T, E324N, V325N/D327T, T326N/N328T, D327N/N329T, G330T, V331T, G330N/K332T, V331N, K332N/I334T, T333N/A335T, 334N/N336T, A335N/N337T, L338T, E361N/C363T, G362N/L364T, C363N/P365T, L364N/P366T, P365N/F367T, P366N/P368T, D370N/F372T, F372N/I374T, Q376N/G378T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, P438N/I440T, L439N/D441T, I440N/Q442T, D441N/Y443T, Q442N/L444T, Y443N/Y445T, L444N/Y446T, Y445N/ L447T, Y446N/S448T, L447N/K449T, S448N, K449N/I451T, T450N/N452T, I451N/G453T, S454T, G453N/G455T, S454N/Q456T, G455N/N457T, Q456N/Q458T, Q459T, Q458N, Q459N/L461T, T460N/K462T, L461N/F463T, K462N/S464T, F463N/V465T, S464N/A466T, V465N/G467T, A466N/P468T, G467N/S469T, P468N/N470T, S469N/M471T, A472T, M471N/V473T, A472N/Q474T, V473N/G475T, R485N/Q487T, R488N/S490T, T491N/V493T, T492N, V493N/Q495T, T494N/N496T, Q495N/N497T, N498T, S499T, E500T, S499N/F501T, E500N/A502T, F501N/W503T, A502N/P504T, W503N/G505T, P504N/A506T, G505N/S507T, A510N/N512T, L511N/G513T, R514T, G513N/N515T, R514N/S516T, L517T, S516N/M518T, L517N/N519T, S526N/K528T, H527N/E529T, K528N/G530T, E529N/E531T, G530N/D532T, E531N/R533T, D532N/F534T, R533N/F535T, F534N/P536T, F535N/L537T, F543N/K545T, G544N/Q546T, K545N/G547T, Q546N, G547N/G549T, T548N/R550T, G549N/D551T, R550N/N552T, D551N/V553T, D554T, V553N/A555T, D554N/D556T, A555N/K557T, D556N/V558T, K557N/M559T, V558N/I560T, M559N, I560N/N562T, T561N/E563T, E564T, E563N/E565T, E564N/I566T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q579N/A581T, V580N, A581N/N583T, T582N/H584T, Q585T, H584N/S586T, Q585N/A587T, S586N/Q588T, A587N/A589T, Q588N/Q590T, A589N/A591T, Q590N/Q592T, A591N, Q592N/G594T, T593N/W595T, G594N/V596T, W595N/Q597T, V596N/N598T, Q597N/Q599T, G600T, D611N/Y613T, V612N/L614T, Y613N/Q615T, L614N/G616T, Q615N/P617T, A656N/P658T, D657N/P659T, P658N, P659N/A661T, T660N/F662T, A661N/N663T, F662N/K664T, D665T, K664N/K666T, D665N/L667T, K666N/N668T, L667N/S669T, F670T, S669N/I671T, F670N, I671N/Q673T, T672N/Y674T, Q673N/S675T, Y674N, Y701N/S703T, T702N/N704T, S703N/Y705T, Y706T, Y705N/K707T, Y706N/S708T, K707N/N709T, S708N/N710T, V711T, E712T, V711N/F713T, E712N/A714T, F713N/V715T, A714N/N716T,

V715N, E718T, T717N/G719T, E718N/V720T, G719N/Y721T, V720N/S722T, Y721N/E723T, S722N/P724T, E723N/R725T, P724N/P726T, R725N/I727T, P726N/G728T, I727N, G728N/R730T, T729N/Y731T, R730N/L732T, and Y731N.

[00011] In particular embodiments, a mutant AAV9 capsid that has higher vector yields compared to a wild-type AAV9 capsid includes mutations S16T, Q37T, R43T, L59T, A68T, Y90T, A96T, E106N, D107N, G115T, A136N, P153N, G174N, T179N, A194N, Q259N/S261T, S263N/S265T, T264N/G266T, S269N/D271T, D271N/A273T, D327N/N329T, G330T, V331T, T333N/A335T, T450N/N452T, I451N/G453T, S454T, S454N/Q456T, G455N/N457T, Q456N/Q458T, Q459T, Q458N, Q459N/L461T, A466N/P468T, G467N/S469T, P468N/N470T, R488N/S490T, T491N/V493T, N498T, S499T, G505N/S507T, A510N/N512T, R514T, G513N/N515T, R514N/S516T, L517T, L517N/N519T, S526N/K528T, K528N/G530T, E529N/E531T, R533N/F535T, F535N/L537T, G544N/Q546T, K545N/G547T, Q546N, G547N/G549T, Q579N/A581T, V580N, A581N/N583T, T582N/H584T, Q585T, H584N/S586T, Q585N/A587T, S586N/Q588T, A587N/A589T, Q588N/Q590T, A589N/A591T, Q590N/Q592T, A591N, Q592N/G594T, T593N/W595T, or E723N/R725T.

[0012] In particular embodiments, a mutant AAV9 capsid that yields more than two times higher titers compared to a wild-type AAV9 capsid includes mutations S16T, Q37T, R43T, L59T, A68T, Y90T, A96T, E106N, D107N, S269N/D271T, D327N/N329T, G505N/S507T, A510N/N512T, R514T, G513N/N515T, L517T, S526N/K528T, Q585N/A587T, Q588N/Q590T, Q590N/Q592T, Q592N/G594T, or T593N/W595T.

[0013] In particular embodiments, a mutant AAV9 capsid that detargets the liver includes mutations L59T, Y90T, A96T, G115T, L256T, L256N/K258T, Q259N/S261T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, Y274T, A273N/F275T, L338T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, Q442N/L444T, V473N/G475T, S499N/F501T, F501N/W503T, P504N/A506T, R514T, R514N/S516T, S516N/M518T, L517N/N519T, H527N/E529T, K528N/G530T, T561N/E563T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q588N/Q590T, G600T, D611N/Y613T, Y613N/Q615T, F662N/K664T, F670T, F670N, Y705N/K707T, K707N/N709T, V711T, E712N/A714T, E723N/R725T, R725N/I727T, P726N/G728T, A68T, G217T, Y443N/Y445T, L444N/Y446T, L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, L511N/G513T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, E563N/E565T, Q590N/Q592T, A591N, S703N/Y705T, Y706T, Y706N/S708T, P724N/P726T, F543N/K545T, or K545N/G547T.

[0014] In particular embodiments, a mutant AAV9 capsid that targets the lungs while detargeting the liver includes mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T.

[0015] In particular embodiments, a mutant AAV9 capsid that targets the skeletal muscle while detargeting the liver includes mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, F534N/P536T, K545N/G547T, A591N, or Y706T.

[0016] In particular embodiments, a mutant AAV9 capsid that targets the pancreas while detargeting the liver includes a mutations G453N/G455T, Q456N/Q458T, L511N/G513T, S526N/K528T, F543N/K545T, Y706T, Y706N/S708T, or P724N/P726T.

[0017] In particular embodiments, a mutant AAV9 capsid that targets the kidney while detargeting the liver includes mutations Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T.

[0018] In particular embodiments, a mutant AAV9 capsid that targets the brain while detargeting the liver includes mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, K545N/G547T, Q590N/Q592T, A591N, Y706T, Y706N/S708T, G467N/S469T, Y443N/Y445T, or L444N/Y446T.

[0019] In particular embodiments, a mutant AAV9 capsid that targets the heart while detargeting the liver includes mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, S526N/K528T, R533N/F535T, F534N/P536T, K545N/G547T, A591N, Y706T, or Y706N/S708T.

[0020] In particular embodiments, a mutant AAV9 capsid that targets the intestine while detargeting the liver includes mutations L447N/K449T, G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, F534N/P536T, F543N/K545T, K545N/G547T, A591N, Y706T, or Y706N/S708T.

[0021] In particular embodiments, a mutant AAV9 capsid that targets the spleen while detargeting the liver includes mutations K545N/G547T.

[0022] In particular embodiments, a mutant AAV9 capsid that targets the testis includes mutations G330N/K332T or N14T.

[0023] In particular embodiments, a mutant AAV9 capsid that targets the intestine and pancreas includes mutations F543N/K545T.

[0024] In particular embodiments, a mutant AAV9 capsid for brain gene delivery includes mutation H255T.

[0025] In particular embodiments, a mutant AAV9 capsid with a prolonged half-life includes mutations A68T, E563N/E565T, S703N/Y705T, G217T, or G330N/K332T.

[0026] In particular embodiments, mutant AAV9 capsids can be associated with a viral vector. In particular embodiments, compositions include mutant AAV9 capsids and/or a viral vector.

BRIEF DESCRIPTION OF THE FIGURES

[0027] Some of the drawings submitted herewith may be better understood in color. Applicant considers the color versions of the drawings as part of the original submission and reserves the right to present color images of the drawings in later proceedings.

[0028] FIG. 1. A map of the double-stranded (ds) adeno-associated virus (AAV)-U6-VBC vector genome. A pair of 12 nucleotide-long viral barcodes (VBCs) (lt-VBC and rt VBC) are placed downstream of the human U6 snRNA gene promoter to drive the expression of the two barcodes shown as lt-VBC and rt-VBC. The right inverted terminal repeat (ITR) has a deletion to produce double-stranded (ds) AAV vector.

[0029] FIG. 2. A map of the single-stranded (ss) AAV-CAG-nlsGFP-VBCLib viral genome. A pair of 12 nucleotide-long VBCs are placed downstream of the CAG promoter to drive the expression of the two barcodes shown as lt-VBC and rt-VBC.

[0030] FIG. 3. Vector yields of AAV9NXT/S mutants assessed by AAV Barcode-Seq. dsAAV-U6-VBC vector was produced in HEK293 cells using 257 AAV9NXT/S mutant capsids and the benchmark AAV9 capsid. Relative vector yields of all 257 AAV9NXT/S mutants were then determined by the AAV Barcode-Seq analysis. Vector yields are shown as bars and sorted from the lowest to the highest.

[0031] FIGs. 4A-4C. Topological locations of the NXT/S mutations that lead to viable and nonviable virion formation. (4A) The AAV9 capsid viewed down the 3-fold symmetry axis. (4B) The locations of the NXT/S mutations that lead to nonviable virion formation are indicated with dark gray. (4C) The locations of the NXT/S mutations that lead to viable virion formation are indicated with light gray. 3f, 3-fold symmetry axis; 5f, 5-fold symmetry axis. Images are generated by Pymol.

[0032] FIG. 5. Table of vector yields of AAV9NXT/S mutants.

[0033] FIG. 6. Vector yields of AAV9NXT/S mutants assessed by a quantitative dot blot assay. dsAAV-CMV-GFP vector was produced in HEK293 cells using 5 select AAV9NXT/S mutant capsids and the benchmark AAV9 capsid. Vector yields were then determined by a quantitative dot blot assay. Y-axis indicates PD values. A bar for AAV9 showing PD value of 1 is omitted in the presentation.

[0034] FIG. 7. Table of relative transduction efficiency of AAV9NXT/S mutants in mouse tissues following intravenous administration.

[0035] FIG. 8. Uniform Manifold Approximation and Projection (UMAP) plotting of AAV capsids based on their *in vivo* tropism and transduction efficiencies in mice. The 9 groups identified by the x-means clustering are visualized in a UMAP plot generated using the AAV capsid biological feature data shown in FIG. 7. Each group uses a different grayscale. To generate the UMAP plot, the following parameters were used: number of neighboring points=9, minimal distance=0.4 and number of components=2.

[0036] FIG. 9. *In vivo* transduction profiles of AAV9NXT/S mutants in each categorical group. Relative transduction efficiency (*i.e.*, phenotypic difference (PD) values obtained by the AAV Barcode-Seq analysis) in each tissue of a set of AAV capsids in each group are averaged and shown as stacked bars. The Y-axis values are arbitrary units (AU) where AAV9 shows a value of 10, which is the sum of the 10 PD values of 1.0 in each tissue. The profile of the Groups 4 and 6 AAV capsids represents that of a single AAV capsid (see FIG. 8), AAV9NXT330 and AAV9NXT554, respectively. The grey-scale bars indicate the tissue where the lung is indicated by black bars at the top of the stacked bar chart and the brain is indicated by the black bar at the bottom of the stacked bar chart.

[0037] FIG. 10. Histological assessment of hepatic transduction with AAV9 and three AAV9NXT mutant vectors in mice. Three weeks post-injection of ssAAV-CAG-nlsGFP vectors (ss is single stranded), liver transduction efficiencies were assessed with GFP marker gene expression. Liver tissues were fixed with 4% paraformaldehyde (PFA) and native GFP fluorescence was visualized by fluorescence microscopy. Representative images of mouse livers from each group are shown. Black and white negative images are used for data presentation. Low, $3.0x10^{11}$ vg/mouse; high, $1.0x10^{12}$ vg/mouse.

[0038] FIG. 11. GFP protein quantification in the AAV vector-transduced mouse liver by an enzyme-linked immunosorbent assay (ELISA). Total proteins were extracted from the mice injected with 3.0×10^{11} vg/mouse of each of the 4 ssAAV-CAG-nlsGFP vectors as indicated in the figure. GFP antigen levels in the liver extracts were determined by a GFP-specific ELISA, normalized by the quantity of total protein in samples, and expressed as ng/mg protein. A one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was used for a statistical comparison of each AAV9NXT mutant and the control AAV9 (n=4 to 5). P values shown in the figure are adjusted P values.

[0039] FIG. 12. Histological assessment of brain transduction with AAV9 and three AAV9NXT mutant vectors in mice. Three weeks post-injection of ssAAV-CAG-nlsGFP vectors, brain transduction efficiencies were assessed with GFP marker gene expression. Brain tissues were fixed with 4% PFA, cryosectioned in the sagittal plane at a thickness of 40 μm, and stained with

a chicken anti-GFP antibody (ab13970). Images were acquired using Zeiss Axioscan with 10X objective. Brain sections of all the mice are shown together with the brain section obtained from an uninjected control. Black and white negative images are used for data presentation. Low, $3.0x10^{11}$ vg/mouse; high, $1.0x10^{12}$ vg/mouse.

[0040] FIG. 13. Histological assessment of heart transduction with AAV9 and AAV9NXT554 vectors in mice. Three weeks post-injection of ssAAV-CAG-nlsGFP vectors, cardiac transduction efficiencies were assessed with GFP marker gene expression. Heart tissues were fixed with 4% PFA, cryosectioned at a thickness of 10 μm, and stained with a chicken anti-GFP antibody (ab13970). Images were acquired using Zeiss Axioscan with 10X objective. Representative images of the heart from the mice injected with $3.0x10^{11}$ vg/mouse of each AAV vector are shown. Black and white negative images are used for data presentation.

[0041] FIG. 14. AAV RNA Barcode-Seq analysis of tissues of mice injected with a low-diversity barcoded library. Three C57BL/6J mice were intravenously injected with a barcoded ssAAV-CAG-nlsGFP-VBC library containing only the following 4 AAV capsids, AAV9, AAV9NXT253, AAV9NXT330 and AAV9NXT554. Three weeks post-injection, RNAs extracted from the tissues shown in the figure were subjected to the AAV RNA Barcode-Seq analysis. A two-tailed one sample t-test was used for statistical comparisons between the values in each group and a hypothetical value of 1 (*i.e.*, AAV9=1.0). **, $P<0.01$, ***, $P<0.001$ and ****, $P<0.0001$.

[0042] FIG. 15. Table of relative vector concentration of AAV9NXT/S mutants in mice following intravenous administration.

[0043] FIG. 16. Blood vector concentration-time curves following intravenous injection of AAV9NXT/S mutants in mice. The two barcoded dsAAV-U6-VBC libraries (Library A and Library B) were injected into mice via the tail vein in bolus at a dose of 1.0×10^{13} vg/kg (n=2 per library). Blood vector concentrations of each AAV mutant relative to the concentration of the reference control AAV9 at each time point were determined by AAV DNA Barcode-Seq. AAV9NXT554 showed an immediate decline of the blood concentration following injection, and rapidly disappeared from the bloodstream during the distribution phase (1 to 30 min post-injection). In the elimination phase (1 h or after following injection), the clearance of AAV9NXT554 from the blood circulation was relatively slow compared to other mutants.

[0044] FIG. 17. Blood vector concentration-time curves following intravenous injection of AAV9 or AAV9NXT554 vector in mice. ssAAV9-CAG-nlsGFP-VBC vector or ssAAV9-CAG-nlsGFP-VBC vector was injected intravenously into C57BL/6J mice at a dose of 1.0×10^{13} vg/kg (n=3 per group), and blood vector concentrations were determined by qPCR. Concentrations of AAV vector particles in the blood are plotted as a function of time after injection. (17A) The first 60 minutes

following vector administration. (17B) Up to 72 hours post-injection. The data for FIGs. 17A and 17B were obtained from the same experiment. A mixed model analysis of variance (ANOVA) and a two-way repeated-measured ANOVA were performed to statistically compare the blood clearance between the AAV9 and AAV9NXT554 groups, demonstrating significant differences in the blood vector concentrations between the two groups (P=0.0006 and P=0.001, respectively). Vertical bars represent standard errors.

[0045] FIG. 18. Table of *in vitro* cell surface binding and transduction efficiencies of AAV9NXT/S mutants.

[0046] FIGs. 19A-19D. Scatter plots showing the relationship between two sets of AAV DNA/RNA Barcode-Seq data. dsAAV-U6-VBC Library A and Library B were applied on CHO-Lec2 cells and HepG2 cells in a triplicated set of experiments, and cell surface binding and transduction efficiency in each experimental setting were assessed by AAV DNA Barcode-Seq and AAV RNA Barcode-Seq, respectively. PD values of the 43 AAV9NXT/S mutants contained in both Library A and Library B are plotted in an XY plots. Pearson's correlation coefficient (r) is indicated in each (19A) CHO Lec2 (binding), (19B) HepG2 (binding), (19C) CHO Lec2 (Transduction), and (19D) HepG2 (transduction).

[0047] FIGs. 20A-20D. Transduction efficiencies of AAV9 and AAV9NXT554 vectors in HepG2 cells. HepG2 cells were infected with ssAAV9-CAG-nlsGFP-VBC vector or ssAAV9NXT-CAG-nlsGFP-VBC vector at a multiplicity of infection (MOI) of 10^6. Three days post-infection, GFP expression was assessed by fluorescence microscopy. (20A and 20B) GFP fluorescence signals observed under a fluorescence microscope. (20C and 20D) Phase contrast images of the same areas shown in FIGs. 20A and 20B. FIGs. 20A and 20C, AAV9 vector-injected cells; FIGs. 20B and 20D, AAV9NXT554 vector-infected cells. Black and white negative images are used for data presentation. HepG2 cells (MOI = 10^6).

[0048] FIG. 21. Table of AAV9NXT mutants showing enhanced Erythrina Cristagalli Lectin (ECL) Agarose binding or Control Agarose binding. Top 20 mutants showing enhanced binding to ECL Agarose or Control Agarose are listed. Fifteen of 20 mutants show enhanced binding to both types of agarose beads, which are indicated with asterisks.

[0049] FIGs. 22A, 22B. Scatter plots showing the relationship between ECL and Control Agarose beads binding ability of AAV9NXT/S mutants in two different conditions. dsAAV-U6-VBC Library was mixed with ECL Agarose beads or Control Agarose beads pretreated (22A) or untreated (22B) with bovine serum albumin (BSA). Vector genome DNA was recovered from agarose bead-bound AAV vector particles and subjected to AAV DNA Barcode-Seq analysis to determine relative quantity of the control AAV9 and each of the AAV9NXT/S mutant vector particles bound

to the beads. Two AAV9NXT mutants indicated with arrows in FIG. 22A, AAV9NXT543 and AAV9NXT554 were identified as outliers that do not follow the binding profile expected from the varying degrees of non-specific binding to ECL Agarose and Control Agarose observed in other AAV9NXT/S mutants.

[0050] FIGs. 23A-23C. A negatively-charged patch on the surface of the capsid introduced by the NXT554 mutation. (23A) The AAV9 capsid viewed down the 3-fold symmetry axis showing the topological locations of the D554/A555/D556, the target of the NXT554 mutation. (23B and 23C) Electrostatic potentials of AAV9 capsid (23B) and AAV9NXT554 capsid (23C) are shown. The images are magnified views of the region indicated with a dotted square in FIG. 23A. The dotted circles indicate the target of the NXT554 mutation. The Adaptive Poisson-Boltzmann Solver (APBS) method was used to visualize electrostatic potentials. The NXT554 mutation significantly increased negative charges (an increase in the darkness) at the mutagenized region in FIG. 23C compared to that in FIG. 23B. 2f, 2-fold symmetry axis 3f, 3-fold symmetry axis; 5f, 5-fold symmetry axis. Images are generated by Pymol.

[0051] FIG. 24. Table of relative quantity of viral genome DNA of AAV9NXT/S mutants in mouse tissues following intravenous administration.

[0052] FIG. 25. Table of AAV9 capsid amino acids that were identified as those important for CHO-Lec2 cell binding by two different mutagenesis approaches.

[0053] FIGs. 26A, 26B. Newly identified AAV9 capsid amino acids that might be responsible for galactose binding. (26A) A three-dimensional map of AAV9 capsid showing the galactose binding motif. (26B) A close up view of the capsid region indicated with a dotted rectangle in FIG. 26A. In both FIGs. 26A and 26B, the galactose binding motif that has previously determined are shown with light gray and the amino acids that the NXT/S scanning mutagenesis study has newly identified as those potentially contributing to galactose binding are shown with dark gray. The latter amino acids reside in the two regions, acid positions 388-392 and 543-545. V389 and R391 are surface exposed in the 388-392 region and K545 is surface exposed in the 543-545 region; thus, these three amino acids might play a role in galactose binding. 2f and an oval, 2-fold symmetry axis; 3f and a triangle, 3-fold symmetry axis; 5f, 5-fold symmetry axis. Images are generated by Pymol.

[0054] FIG. 27. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of purified AAV9 and AAV9NXT virus-like particles (VLPs) treated or untreated with Peptide - N - Glycosidase F (PNGase F). A half μg of AAV9 and AAV9NXT554 VLPs were treated with PNGase F (+) or untreated (-) and separated by PAGE using an 8% gel. The protein bands were stained by a Coomassie Brilliant Blue dye.

[0055] FIG. 28. Table of mutation names and mutations.

DETAILED DESCRIPTION

[0056] The present disclosure describes a library of mutant adeno-associated virus (AAV) 9 capsid proteins with targeted transduction, detargeted transduction, more efficient transduction compared to wild-type AAV9 capsid proteins, higher vector yield, and/or prolonged half-life.

[0057] In particular embodiments, a mutant AAV9 capsid for hepatic gene delivery includes mutations D554N/D556T. A mutant AAV9 capsid including mutations D554N/D556T includes the following biological properties: 1) rapid blood clearance following intravenous injection, 2) ability to bind albumin, and 3) more efficient transduction of hepatocytes in the liver compared to a wild-type AAV9 capsid.

[0058] In particular embodiments, a reference wild-type AAV9 capsid is mutated to create a mutant AAV9 capsid. In particular embodiments, the reference wild-type AAV9 capsid includes the sequence as set forth in SEQ ID NO: 1.

[0059] In particular embodiments, an AAV9 capsid mutant includes a mutation or set of mutations selected from: S16T, Q37T, R43T, L59T, A68T, Y90T, A96T, E106N, D107N, G115T, A136N, E147N, P153N, D154N, G160N, G174T, G174N, G177N, T179N, A194N, V198N, S200N, M203N, E216T, G217T, P250N/Y252T, T251N/N253T, Y252N/N254T, H255T, L256T, H255N/Y257T, L256N/K258T, Y257N/Q259T, K258N/I260T, Q259N/S261T, I260N/N262T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T, G266N/S268T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, D271N/A273T, Y274T, A273N/F275T, Y274N/G276T, S278N/P280T, R288N/H290T, H290N/H292T, E324N, V325N/D327T, T326N/N328T, D327N/N329T, G330T, V331T, G330N/K332T, V331N, K332N/I334T, T333N/A335T, 334N/N336T, A335N/N337T, L338T, E361N/C363T, G362N/L364T, C363N/P365T, L364N/P366T, P365N/F367T, P366N/P368T, D370N/F372T, F372N/I374T, Q376N/G378T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, P438N/I440T, L439N/D441T, I440N/Q442T, D441N/Y443T, Q442N/L444T, Y443N/Y445T, L444N/Y446T, Y445N/ L447T, Y446N/S448T, L447N/K449T, S448N, K449N/I451T, T450N/N452T, I451N/G453T, S454T, G453N/G455T, S454N/Q456T, G455N/N457T, Q456N/Q458T, Q459T, Q458N, Q459N/L461T, T460N/K462T, L461N/F463T, K462N/S464T, F463N/V465T, S464N/A466T, V465N/G467T, A466N/P468T, G467N/S469T, P468N/N470T, S469N/M471T, A472T, M471N/V473T, A472N/Q474T, V473N/G475T, R485N/Q487T, R488N/S490T, T491N/V493T, T492N, V493N/Q495T, T494N/N496T, Q495N/N497T, N498T, S499T, E500T, S499N/F501T,

E500N/A502T, F501N/W503T, A502N/P504T, W503N/G505T, P504N/A506T, G505N/S507T, A510N/N512T, L511N/G513T, R514T, G513N/N515T, R514N/S516T, L517T, S516N/M518T, L517N/N519T, S526N/K528T, H527N/E529T, K528N/G530T, E529N/E531T, G530N/D532T, E531N/R533T, D532N/F534T, R533N/F535T, F534N/P536T, F535N/L537T, F543N/K545T, G544N/Q546T, K545N/G547T, Q546N, G547N/G549T, T548N/R550T, G549N/D551T, R550N/N552T, D551N/V553T, D554T, V553N/A555T, D554N/D556T, A555N/K557T, D556N/V558T, K557N/M559T, V558N/I560T, M559N, I560N/N562T, T561N/E563T, E564T, E563N/E565T, E564N/I566T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q579N/A581T, V580N, A581N/N583T, T582N/H584T, Q585T, H584N/S586T, Q585N/A587T, S586N/Q588T, A587N/A589T, Q588N/Q590T, A589N/A591T, Q590N/Q592T, A591N, Q592N/G594T, T593N/W595T, G594N/V596T, W595N/Q597T, V596N/N598T, Q597N/Q599T, G600T, D611N/Y613T, V612N/L614T, Y613N/Q615T, L614N/G616T, Q615N/P617T, A656N/P658T, D657N/P659T, P658N, P659N/A661T, T660N/F662T, A661N/N663T, F662N/K664T, D665T, K664N/K666T, D665N/L667T, K666N/N668T, L667N/S669T, F670T, S669N/I671T, F670N, I671N/Q673T, T672N/Y674T, Q673N/S675T, Y674N, Y701N/S703T, T702N/N704T, S703N/Y705T, Y706T, Y705N/K707T, Y706N/S708T, K707N/N709T, S708N/N710T, V711T, E712T, V711N/F713T, E712N/A714T, F713N/V715T, A714N/N716T, V715N, E718T, T717N/G719T, E718N/V720T, G719N/Y721T, V720N/S722T, Y721N/E723T, S722N/P724T, E723N/R725T, P724N/P726T, R725N/I727T, P726N/G728T, I727N, G728N/R730T, T729N/Y731T, R730N/L732T, and Y731N.

[0060] In particular embodiments, a mutant AAV9 capsid that has higher vector yields compared to a wild-type AAV9 capsid includes mutations S16T, Q37T, R43T, L59T, A68T, Y90T, A96T, E106N, D107N, G115T, A136N, P153N, G174N, T179N, A194N, Q259N/S261T, S263N/S265T, T264N/G266T, S269N/D271T, D271N/A273T, D327N/N329T, G330T, V331T, T333N/A335T, T450N/N452T, I451N/G453T, S454T, S454N/Q456T, G455N/N457T, Q456N/Q458T, Q459T, Q458N, Q459N/L461T, A466N/P468T, G467N/S469T, P468N/N470T, R488N/S490T, T491N/V493T, N498T, S499T, G505N/S507T, A510N/N512T, R514T, G513N/N515T, R514N/S516T, L517T, L517N/N519T, S526N/K528T, K528N/G530T, E529N/E531T, R533N/F535T, F535N/L537T, G544N/Q546T, K545N/G547T, Q546N, G547N/G549T, Q579N/A581T, V580N, A581N/N583T, T582N/H584T, Q585T, H584N/S586T, Q585N/A587T, S586N/Q588T, A587N/A589T, Q588N/Q590T, A589N/A591T, Q590N/Q592T, A591N, Q592N/G594T, T593N/W595T, or E723N/R725T.

[0061] In particular embodiments, a mutant AAV9 capsid that yields more than two times higher titers compared to a wild-type AAV9 capsid includes mutations S16T, Q37T, R43T, L59T, A68T,

Y90T, A96T, E106N, D107N, S269N/D271T, D327N/N329T, G505N/S507T, A510N/N512T, R514T, G513N/N515T, L517T, S526N/K528T, Q585N/A587T, Q588N/Q590T, Q590N/Q592T, Q592N/G594T, or T593N/W595T.

[0062] In particular embodiments, a mutant AAV9 capsid that detargets the liver includes mutations L59T, Y90T, A96T, G115T, L256T, L256N/K258T, Q259N/S261T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, Y274T, A273N/F275T, L338T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, Q442N/L444T, V473N/G475T, S499N/F501T, F501N/W503T, P504N/A506T, R514T, R514N/S516T, S516N/M518T, L517N/N519T, H527N/E529T, K528N/G530T, T561N/E563T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q588N/Q590T, G600T, D611N/Y613T, Y613N/Q615T, F662N/K664T, F670T, F670N, Y705N/K707T, K707N/N709T, V711T, E712N/A714T, E723N/R725T, R725N/I727T, P726N/G728T, A68T, G217T, Y443N/Y445T, L444N/Y446T, L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, L511N/G513T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, E563N/E565T, Q590N/Q592T, A591N, S703N/Y705T, Y706T, Y706N/S708T, P724N/P726T, F543N/K545T, or K545N/G547T.

[0063] In particular embodiments, a mutant AAV9 capsid that targets the lungs while detargeting the liver includes mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T.

[0064] In particular embodiments, a mutant AAV9 capsid that targets the skeletal muscle while detargeting the liver includes mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, F534N/P536T, K545N/G547T, A591N, or Y706T.

[0065] In particular embodiments, a mutant AAV9 capsid that targets the pancreas while detargeting the liver includes a mutations G453N/G455T, Q456N/Q458T, L511N/G513T, S526N/K528T, F543N/K545T, Y706T, Y706N/S708T, or P724N/P726T.

[0066] In particular embodiments, a mutant AAV9 capsid that targets the kidney while detargeting the liver includes mutations Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T.

[0067] In particular embodiments, a mutant AAV9 capsid that targets the brain while detargeting the liver includes mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, K545N/G547T, Q590N/Q592T, A591N, Y706T, Y706N/S708T, G467N/S469T, Y443N/Y445T, or L444N/Y446T. In particular embodiments, a mutant AAV9 capsid including mutations Y443N/Y445T or L444N/Y446T can be further engineered to target

the brain and detarget the liver.

[0068] In particular embodiments, a mutant AAV9 capsid that targets the heart while detargeting the liver includes mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, S526N/K528T, R533N/F535T, F534N/P536T, K545N/G547T, A591N, Y706T, or Y706N/S708T.

[0069] In particular embodiments, a mutant AAV9 capsid that targets the intestine while detargeting the liver includes mutations L447N/K449T, G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, F534N/P536T, F543N/K545T, K545N/G547T, A591N, Y706T, or Y706N/S708T.

[0070] In particular embodiments, a mutant AAV9 capsid that targets the spleen while detargeting the liver includes mutations K545N/G547T.

[0071] In particular embodiments, a mutant AAV9 capsid that targets the testis includes mutations G330N/K332T or N14T. In particular embodiments, a mutant AAV9 capsid including mutation N14T targets the brain, liver, heart, intestine, spleen, skeletal muscle, pancreas, kidney, testis, and lung.

[0072] In particular embodiments, a mutant AAV9 capsid that targets the intestine and pancreas includes mutation F543N/K545T. In particular embodiments, a mutant AAV9 capsid including the mutation F543N/K545T targets the intestine and pancreas and detargets other organs including the liver.

[0073] In particular embodiments, a mutant AAV9 capsid for brain gene delivery includes mutation H255T.

[0074] In particular embodiments, a mutant AAV9 capsid with a prolonged half-life includes mutations A68T, E563N/E565T, S703N/Y705T, G217T, or G330N/K332T. In particular embodiments, a prolonged half-life includes prolonged persistence of the AAV9 capsid in the blood circulation compared to a wild-type AAV9 capsid.

[0075] In particular embodiments, mutant AAV9 capsids can be associated with a viral vector. In particular embodiments, compositions include mutant AAV9 capsids and/or a viral vector.

[0076] Aspects of the current disclosure are now described in more supporting detail as follows: (i) Recombinant Viral Vectors; (i-a) Mutant AAV9 Capsids; (i-b) Gene Products; (i-c) Regulatory Elements; (i-d) Production of Recombinant Viral Vector; (ii) Compositions for Administration; (iii) Methods of Use; (iv) Administration Benefits; (v) Kits; (vi) Exemplary Embodiments; (vii) Experimental Examples; and (viii) Closing Paragraphs. These headings are provided for organizational purposes only and do not limit the scope or interpretation of the disclosure.

[0077] (i) Recombinant Viral Vectors. AAV has a linear single-stranded DNA (ssDNA) genome of

4.7 kilobases (kb), with two 145 nucleotide-long inverted terminal repeats (ITR) at the termini. The virus does not encode a polymerase and therefore relies on cellular polymerases for genome replication. The inverted terminal repeats (ITRs) flank two viral genes, *rep* (replicase) and *cap* (capsid), which encode non-structural and structural proteins, respectively.

[0078] The *rep* gene, through the use of promoters and alternative splicing, encodes regulatory proteins involved in AAV genome replication. The *cap* gene, through alternative splicing and initiation of translation, gives rise to three capsid proteins, VP1 (virion protein 1), VP2, and VP3 and two non-structural proteins, assembly-activating protein (AAP) and membrane-associated accessory protein (MAAP). The capsid proteins assemble into a shell referred to herein as a capsid. The AAP and MAAP play roles in promoting AAV capsid assembly and cellular exit of AAV virions, respectively.

[0079] In particular embodiments, AAV virion formation involves entry of unassembled AAV capsid proteins into the nucleus followed by assembly into capsids in an AAP-dependent process. AAV viral genome DNA replication and encapsidation are directed by protein-protein and protein-viral genome interactions between (1) large Rep proteins (*e.g.*, Rep78 or Rep68) responsible for viral genome replication, (2) small rep proteins (*e.g.*, Rep52 and Rep40) responsible for transferring the AAV genome DNA into empty capsids through the pores and (3) the pre-formed empty capsids.

[0080] A "vector," as used herein, refers to a recombinant plasmid or virus that includes a polynucleotide to be delivered into a host cell, either *in vitro* or *in vivo*. The polynucleotide to be delivered, sometimes referred to as an "expression cassette," "heterologous sequence," "target polynucleotide," "transgene," or "gene of interest" can include a sequence of interest in gene therapy (such as a gene encoding a protein or RNA transcript, such as an antisense transcript or a ribozyme of therapeutic interest) and/or a selectable or detectable marker. A "gene delivery vector" refers to a vector used to deliver a gene or transgene. In particular embodiments, a capsid is associated with a vector such that the expression cassette is encapsidated within the capsid.

[0081] "Recombinant" refers to a genetic entity distinct from that generally found in nature. As applied to a polynucleotide or gene, this means that the polynucleotide is the product of various combinations of cloning, restriction and/or ligation steps, and other procedures that result in the production of a construct that is distinct from a polynucleotide found in nature.

[0082] A "recombinant viral vector" refers to a recombinant polynucleotide vector including one or more heterologous sequences (*i.e.*, polynucleotide sequence not of viral origin). In the case of recombinant viral vectors, the recombinant polynucleotide is flanked by at least one, preferably two, inverted terminal repeat sequences (ITRs).

[0083] A "recombinant AAV vector (rAAV vector)" refers to a polynucleotide vector including one or more heterologous sequences (*i.e.*, polynucleotide sequence not of AAV origin) that are flanked by at least one, preferably two, AAV inverted terminal repeat sequences (ITRs). Such rAAV vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper virus (or that is expressing suitable helper functions) and that is expressing AAV *rep* and *cap* gene products (*i.e.*, AAV Rep and Cap proteins). When a rAAV vector is incorporated into a larger polynucleotide (*e.g.*, in a chromosome or in another vector such as a plasmid used for cloning or transfection), then the rAAV vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of AAV packaging functions and suitable helper functions. An rAAV can be in any of a number of forms, including plasmids, linear artificial chromosomes, complexed with liposomes, encapsulated within liposomes, and encapsidated in a viral particle. In particular embodiments, an rAAV is encapsidated in a mutant AAV9 capsid.

[0084] Herein, the term "viral vector" includes a "recombinant viral vector", a "recombinant AAV vector", and other vectors having viral components including a mutant AAV9 capsid disclosed herein.

[0085] An rAAV vector can be packaged into an AAV virus particle to generate a "recombinant adeno-associated virus" (rAAV). In particular embodiments, the maximum size vector that can be packaged to yield an infectious viral particle is 5.2 kb.

[0086] "Heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is compared or into which it is introduced or incorporated. For example, a polynucleotide introduced by genetic engineering techniques into a different cell type is a heterologous polynucleotide (and, when expressed, can encode a heterologous polypeptide). Similarly, a cellular sequence (*e.g.*, a gene or portion thereof) that is incorporated into a viral vector, is a heterologous nucleotide sequence with respect to the vector. For purposes of this disclosure, "heterologous" means heterologous with respect to a virus which is the basis of a recombinant viral vector.

[0087] In particular embodiments, a recombinant viral vector disclosed herein includes a mutant AAV9 capsid, one or two ITR sequences, and an expression cassette wherein the expression cassette includes a sequence encoding a gene product and regulatory elements. In particular embodiments, a recombinant viral vector includes an expression cassette encapsidated by a mutant AAV9 capsid. In particular embodiments, the recombinant viral vector is infectious. In particular embodiments, the recombinant viral vector is replication-competent. In particular embodiments, the recombinant viral vector is replication-incompetent.

[0088] In particular embodiments, the expression cassette is flanked on the 5′ and 3′ ends by functional AAV inverted terminal repeat (ITR) sequences. An "AAV inverted terminal repeat (ITR)" sequence, a term well-understood in the art, is generally a 145-nucleotide sequence that is present at both termini of the native single-stranded AAV genome. The outermost 125 nucleotides of the ITR can be present in either of two alternative orientations, leading to heterogeneity between different AAV genomes and between the two ends of a single AAV genome. The outermost 125 nucleotides also contain several shorter regions of self-complementarity, allowing intrastrand base-pairing to occur within this portion of the ITR. A "functional AAV ITR sequence" means that the ITR sequence functions as intended for the rescue, replication, and packaging of the AAV virion. The term "rescue" refers to the process by which the AAV genome is released from a chromosomal DNA or an extrachromosomal DNA upon subsequent infection with a helper virus or by nonviral delivery (*e.g.,* transfection) of genetic elements that supply the helper virus functions. Hence, AAV ITRs for use in gene delivery vectors need not have a wild-type sequence, but can be altered by the insertion, deletion or substitution of nucleotides. The AAV ITRs may also be derived from any of several AAV serotypes, *e.g.*, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10. In particular embodiments, AAV vectors have the wild-type *rep* and *cap* genes deleted in whole or in part but retain functional flanking ITR sequences.

[0089] (i-a) Mutant AAV9 Capsids. As used herein, the term "capsid protein" or "capsid" means a protein which is encoded by the *cap* gene present in a viral genome and constitutes the outer shell of a virus. The wild-type AAV genome or *cap* gene encodes three capsid proteins (VP1, VP2, and VP3). In particular embodiments, VP1, VP2, and VP3 are included in the capsid.

[0090] The mutant AAV capsids as described herein are derived from the AAV9 capsid. Other serotypes of AAV capsids from primates include AAV type 1 (AAV1), AAV type 2 (AAV2), AAV type 3 (AAV3a and AAV3b), AAV type 4 (AAV4), AAV type 5 (AAV5), AAV type 6 (AAV6), AAV type 7 (AAV7), AAV type 8 (AAV8), AAV type 9 (AAV9), AAV type 10 (AAV10), AAV type 11 (AAV11), AAV type 12 (AAV12), and AAV type 13 (AAV13), and AAV from non-primate animals such as avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, and goat AAV.

[0091] The mutant of an AAV9 capsid as disclosed herein is produced by replacing at least one amino acid with another amino acid in the amino acid sequence of an AAV9 capsid. Use of the AAV9 capsid mutant enables more efficient introduction of a gene into a target cell or tissue, enhanced vector yields, and/or prolonged half-life. In particular embodiments, one amino acid is replaced with another amino acid in the amino acid sequence of an AAV9 capsid. In particular embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,

26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids are replaced with other amino acids in the amino acid sequence of an AAV9 capsid. In particular embodiments, more than 50 amino acids are replaced with other amino acids in the amino acid sequence of an AAV9 capsid.

[0092] In particular embodiments, the mutant AAV9 capsid shell is a different serotype than the rAAV genome, wherein the rAAV genome includes an expression cassette and at least one AAV ITR sequence. The rAAV genome can be encapsidated by a mutant AAV9 capsid shell as described herein, whereas the ITR sequences contained in that recombinant viral vector may be any AAV serotype.

[0093] Replication-defective AAV virions including mutant AAV capsids can be made by standard techniques known in the art using AAV packaging cells and packaging technology. Examples of these methods may be found, for example, in U.S. Pat. Nos. 5,436,146; 5,753,500, 6,040,183, 6,093,570 and 6,548,286.

[0094] As described herein the chosen mutant AAV9 capsid will determine the tropism of a viral vector to target or detarget a select cell type or tissue.

[0095] In particular embodiments, a mutant AAV9 capsid can be selected to target expression in the liver, lungs, skeletal muscle, pancreas, kidney, brain, heart, intestine, spleen, and/or testis; detarget the liver; improve vector yield; and/or prolong the half-life.

[0096] In particular embodiments, a reference wild-type AAV9 capsid includes the sequence: MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLDK GEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKK RLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDP QPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRT WALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWG FRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADV FMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLD RLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQN NNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADK VMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDRDVYLQGPIW AKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIE WELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 1).

[0097] In particular embodiments, a mutant AAV9 capsid includes mutations of the reference wild-type sequence (SEQ ID NO: 1) including: D554N /D556T, S16T, Q37T, R43T, L59T, A68T, Y90T, A96T, E106N, D107N, G115T, A136N, E147N, P153N, D154N, G160N, G174T, G174N, G177N,

T179N, A194N, V198N, S200N, M203N, E216T, G217T, P250N/Y252T, T251N/N253T, Y252N/N254T, H255T, L256T, H255N/Y257T, L256N/K258T, Y257N/Q259T, K258N/I260T, Q259N/S261T, I260N/N262T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T, G266N/S268T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, D271N/A273T, Y274T, A273N/F275T, Y274N/G276T, S278N/P280T, R288N/H290T, H290N/H292T, E324N, V325N/D327T, T326N/N328T, D327N/N329T, G330T, V331T, G330N/K332T, V331N, K332N/I334T, T333N/A335T, 334N/N336T, A335N/N337T, L338T, E361N/C363T, G362N/L364T, C363N/P365T, L364N/P366T, P365N/F367T, P366N/P368T, D370N/F372T, F372N/I374T, Q376N/G378T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, P438N/I440T, L439N/D441T, I440N/Q442T, D441N/Y443T, Q442N/L444T, Y443N/Y445T, L444N/Y446T, Y445N/L447T, Y446N/S448T, L447N/K449T, S448N, K449N/I451T, T450N/N452T, I451N/G453T, S454T, G453N/G455T, S454N/Q456T, G455N/N457T, Q456N/Q458T, Q459T, Q458N, Q459N/L461T, T460N/K462T, L461N/F463T, K462N/S464T, F463N/V465T, S464N/A466T, V465N/G467T, A466N/P468T, G467N/S469T, P468N/N470T, S469N/M471T, A472T, M471N/V473T, A472N/Q474T, V473N/G475T, R485N/Q487T, R488N/S490T, T491N/V493T, T492N, V493N/Q495T, T494N/N496T, Q495N/N497T, N498T, S499T, E500T, S499N/F501T, E500N/A502T, F501N/W503T, A502N/P504T, W503N/G505T, P504N/A506T, G505N/S507T, A510N/N512T, L511N/G513T, R514T, G513N/N515T, R514N/S516T, L517T, S516N/M518T, L517N/N519T, S526N/K528T, H527N/E529T, K528N/G530T, E529N/E531T, G530N/D532T, E531N/R533T, D532N/F534T, R533N/F535T, F534N/P536T, F535N/L537T, F543N/K545T, G544N/Q546T, K545N/G547T, Q546N, G547N/G549T, T548N/R550T, G549N/D551T, R550N/N552T, D551N/V553T, D554T, V553N/A555T, D554N/D556T, A555N/K557T, D556N/V558T, K557N/M559T, V558N/I560T, M559N, I560N/N562T, T561N/E563T, E564T, E563N/E565T, E564N/I566T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q579N/A581T, V580N, A581N/N583T, T582N/H584T, Q585T, H584N/S586T, Q585N/A587T, S586N/Q588T, A587N/A589T, Q588N/Q590T, A589N/A591T, Q590N/Q592T, A591N, Q592N/G594T, T593N/W595T, G594N/V596T, W595N/Q597T, V596N/N598T, Q597N/Q599T, G600T, D611N/Y613T, V612N/L614T, Y613N/Q615T, L614N/G616T, Q615N/P617T, A656N/P658T, D657N/P659T, P658N, P659N/A661T, T660N/F662T, A661N/N663T, F662N/K664T, D665T, K664N/K666T, D665N/L667T, K666N/N668T, L667N/S669T, F670T, S669N/I671T, F670N, I671N/Q673T, T672N/Y674T, Q673N/S675T, Y674N, Y701N/S703T, T702N/N704T, S703N/Y705T, Y706T, Y705N/K707T, Y706N/S708T, K707N/N709T, S708N/N710T, V711T, E712T, V711N/F713T, E712N/A714T,

F713N/V715T, A714N/N716T, V715N, E718T, T717N/G719T, E718N/V720T, G719N/Y721T, V720N/S722T, Y721N/E723T, S722N/P724T, E723N/R725T, P724N/P726T, R725N/I727T, P726N/G728T, I727N, G728N/R730T, T729N/Y731T, R730N/L732T, Y731N, Q37T, or N14T.

[0098] (i-b) Gene Products. In particular embodiments, the expression cassette includes a sequence encoding a gene product. In particular embodiments, the gene product elicits a therapeutic effect. In particular embodiments, the gene product elicits a therapeutic effect within the liver, lungs, skeletal muscle, pancreas, kidney, brain, heart, intestine, spleen, and/or testis. In particular embodiments, the gene product has an extended *in vivo* half-life. In particular embodiments, the gene product is expressed with increased production yields.

[0099] In particular embodiments, the gene product is heterologous to the AAV ITRs, which encodes a polypeptide, protein, or other product, of interest. The sequence encoding the gene product is operatively linked to regulatory components in a manner which permits transgene transcription, translation, and/or expression in a target cell. In particular embodiments, the gene product includes a therapeutic molecule, an enzyme, a cytokine, a hormone, a receptor, a receptor ligand, an antibody, a reporter gene/protein, or an antisense oligonucleotide.

[0100] A therapeutic molecule can be any gene product that is useful. A therapeutic molecule can be a protein, molecule, coding sequence, or non-coding sequence. A therapeutic molecule can include an enzyme, a cytokine, a hormone, a receptor, a receptor ligand, an antibody, a reporter gene/protein, an antisense oligonucleotide or a repair template. Examples of a therapeutic molecule include pramlinitide acetate, growth hormone (GH), insulin-like growth factor, protein C, α1-proteinase inhibitor, erythropoietin, granulocyte colony-stimulating factor (G-CSF), Interleukin 11, human follicle-stimulating hormone (FSH), human chorionic gonadotropin (HCG), Lutropin-α, Interleukin 2 (IL2), Denileukin diftitox (fusion of IL2 and Diphtheria toxin), Interferon-α2a, Interferon-α2b, Interferon-αn3, Interferon-β1a, Interferon-β1b, Interferon-γ1b, human parathyroid hormone, glucagon-like peptide 1, somatostatin, bone morphogenic protein 2, bone morphogenic protein 7, gonadotropin-releasing hormone (GnRH), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF), B-type natriuretic peptide, hirudin or fragments or mimetics thereof.

[0101] An enzyme deficiency can lead to several disorders including clotting disorders, diabetes, lysosomal storage diseases (*e.g.*, Gaucher disease (GD), Fabry disease, mucopolysaccharidosis (MPS) type I, MPS type II, MPS type VI, and Pompe disease), and other disorders. Diseases or disorders that include an enzyme deficiency include (disease / enzyme): lactose intolerance/lactase, hemolytic anemia/glucose-6-phosphate dehydrogenase (G6PD) or pyruvate kinase; Gaucher disease/glucocerebrosidase; Wilson disease/ATPase7B; galactosemia/galactose-1-phosphate uridyl transferase (GALT); alpha-1 antitrypsin (A1AT)

deficiency/alpha-1 antitrypsin; maple syrup urine disease/branched-chain α-ketoacid dehydrogenase (BCKD) complex; phenylketonuria (PKU)/phenylalanine hydroxylase (PAH); glycogen storage disease Type I (Gierke disease)/glucose-6-phosphatase (G6Pase); glycogen storage disease Type III (Cori disease, or Forbes disease)/debranching enzyme; glycogen storage disease Type IV (Andersen disease)/glycogen-branching enzyme; mitochondrial dysfunction and production of the toxins glutaric acid and 3-OH-glutaric acid/glutaryl-CoA dehydrogenase; Friedreich ataxia (FRDA)/frataxin; Zellweger spectrum disorders (ZSD)/peroxisome biogenesis disorders (PBDs) or peroxisomes; abnormal masculinity/5α-reductase; glucose phosphate isomerase deficiency/glucose phosphate isomerase; Tay-Sachs disease/hexosaminidase A; hemophilia B/factor IX; hemophilia A/factor VIII; hemophilia C/ factor XI; or fibrodysplasia ossificans progressive (FOP)/activin receptor type-1 (ACVR1) (also known as Activin receptor-like kinase-2 (ALK2)).

[0102] Cytokines are small proteins secreted by certain cells of the immune system that play a role in controlling the growth, activity, and communication of other cells. Cytokines can include, for example, interferons such as interferon-alpha, beta, and - gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte- macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL- 1 alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, scIL12, IL-15, IL18, DR-IL18, IL21, IL36γ, or a tumor necrosis factor such as TNF-α or TNF-β.

[0103] Hormones are chemicals that coordinate different functions in the body by carrying signals to organs and tissues. Example hormones include corticotrophin-releasing hormone, dopamine, gonadotrophin-releasing hormone, growth hormone-releasing hormone, oxytocin, somatostatin, thyrotropin-releasing hormone, adrenocorticotropic hormone (ACTH or corticotropin), follicle-stimulating hormone (FSH), growth hormone (GH), luteinizing hormone (LH), prolactin, thyroid-stimulating hormone (TSH), antidiuretic hormone (ADH, or vasopressin), thyroxine (T4), triiodothyronine (T3), reverse triiodothyronine (RT3), calcitonin, cortisol, aldosterone, DHEA and androgens, adrenaline (epinephrine), noradrenaline (norepinephrine), insulin, glucagon, estrogen, progesterone, testosterone, leptin, adiponectin, plasminogen activator inhibitor-1, angiotensin, erythropoietin, renin, insulin-like growth factor 1 (IGF-1), angiotensinogen, glucagon-like peptide 1 (GLP-1), or ghrelin.

[0104] Receptors are proteins either inside a cell or on its surface that receive a signal by binding to intracellular or extracellular molecules. Example receptors include Erb (*e.g.*, epidermal growth factor receptor), glial cell-derived neurotrophic factor (GDNF) receptor, natriuretic peptide receptor (NPR), trk neurotrophin receptor, toll-like receptor, platelet-derived growth factor

receptor, fibroblast growth factor receptor, hepatocyte growth factor receptor, nerve growth factor receptor, transforming growth factor receptor beta 1, vascular endothelial growth factor receptor, or interleukin 8 receptor alpha.

[0105] The intracellular or extracellular molecule that binds a receptor can include a receptor ligand. Example receptor ligands include epidermal growth factor (EGF), amphiregulin, betacellulin, GDNF, neurturin, atrial natriurtetic peptide, C-type natriuretic peptide, nerve growth factor, brain-derived neurotrophic factor, peptidoglycan, LPS, flagellin, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), nerve growth factor (NGFR), transforming growth factor (TGF), vascular endothelial growth factor, or interleukin 8. In particular embodiments, a receptor ligand can be naturally occurring or synthetic.

[0106] An antibody is a protein used in the immune system to identify specific epitopes. Herein the term "antibody" includes antibody or antigen binding fragments thereof including all or a portion of polyclonal antibodies, monoclonal antibodies, human antibodies, humanized antibodies, synthetic antibodies, non-human antibodies, recombinant antibodies, chimeric antibodies, bispecific antibodies, mini bodies, nanobodies, single chain variable fragments (scFv), and linear antibodies. Example antibodies include anti-CD52 antibodies (*e.g.*, alemtuzumab), anti-CD20 antibodies (*e.g.*, rituximab), anti-VEGF antibodies (*e.g.*, bevacizumab), anti-HER2 antibodies (*e.g.*, pertuzumab or trastuzumab), anti-CD19 antibodies, anti-CD22 antibodies, anti-ROR1 antibodies, anti-CD33 antibodies, anti-CD56 antibodies, anti-CLL-1 antibodies, anti-WT-1 antibodies, anti-CD123 antibodies, anti-PD-L1 antibodies, anti-EFGR antibodies, anti-B-cell maturation antigen (BCMA) antibodies, anti-GD2 antibodies, anti-mesothelin antibodies, anti-MUC16 antibodies, anti-folate receptor (FOLR) antibodies, anti-CEA antibodies, anti-carboxy-anhydrase-IX (CAIX) antibodies, anti-L1-CAM antibodies, or anti-Lewis Y antibodies.

[0107] When a therapeutic molecule does not fit within the packaging capacity of AAV vectors due to its size, the molecule can be split into two or more molecules packaged into multiple AAV vectors each of which carries a different portion of the oversized molecule. A set of split AAV vectors can be delivered to target cells simultaneously to allow the split molecules to be reconstituted in a functional molecule in cells. Such technologies are known in the art as split AAV vector technologies that exploit AAV vector genome recombination, trans-splicing of mRNA or intein-based protein splicing.

[0108] In particular embodiments, the gene product includes a reporter gene/protein, which upon expression produces a detectable signal. In particular embodiments, the gene product can be a reporter gene or reporter protein. Exemplary reporter genes particularly can include those which encode an expressible fluorescent protein, or expressible biotin; blue fluorescent proteins (*e.g.*,

eBFP, eBFP2, Azurite, mKalama1, GFPuv, Sapphire, T-sapphire); cyan fluorescent proteins (*e.g.,* eCFP, Cerulean, CyPet, AmCyanl, Midoriishi-Cyan, mTurquoise); green fluorescent proteins (*e.g.,* GFP, GFP-2, tagGFP, turboGFP, EGFP, Emerald, Azami Green, Monomeric Azami Green (mAzamigreen), CopGFP, AceGFP, avGFP, ZsGreenl, Oregon Green™(Thermo Fisher Scientific)); Luciferase; orange fluorescent proteins (mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato, dTomato); red fluorescent proteins (mKate, mKate2, mPlum, DsRed monomer, mCherry, mRuby, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRedl, AsRed2, eqFP611, mRaspberry, mStrawberry, Jred, Texas Red™ (Thermo Fisher Scientific)); far red fluorescent proteins (*e.g.*, mPlum and mNeptune); yellow fluorescent proteins (*e.g.*, YFP, eYFP, Citrine, SYFP2, Venus, YPet, PhiYFP, ZsYellowl); and tandem conjugates.

[0109] In particular embodiments, an antisense oligonucleotide includes interfering RNA including small interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA), and/or guide RNA.

[0110] (i-c) Regulatory Elements. In particular embodiments, the expression cassette includes regulatory elements in addition to the sequence encoding a gene product. The regulatory elements are operably linked to the sequence encoding the gene product in a manner which permits its transcription, translation and/or expression in a cell transfected with the vector containing the expression cassette. As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest.

[0111] "Operably linked" refers to an arrangement of two or more components, wherein the components so described are in a relationship permitting them to function in a coordinated manner. By way of illustration, a transcriptional regulatory sequence or a promoter is operably linked to a coding sequence if the transcriptional regulatory sequence or promoter facilitates some aspect of the transcription of the coding sequence. Aspects of the transcription process include initiation, elongation, attenuation and termination. An operably linked transcriptional regulatory sequence is generally joined in cis with the coding sequence, but it is not necessarily directly adjacent to it.

[0112] Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; introns, 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. A

great number of expression control sequences, including promoters which are native, constitutive, regulatable and/or tissue-specific, are known in the art and may be utilized.

[0113] Examples of constitutive RNA polymerase II 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 hybrid promoters comprising the CMV enhancer and the chicken β-actin promoter (*e.g.*, CB and CAG promoters)), the phosphoglycerol kinase (PGK) promoter, and the EF1 promoter. Examples of constitutive RNA polymerase III promoters include U6 small nuclear (sn) RNA gene promoter and H1 RNA gene promoter. Regulatable 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. Examples of regulatable 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, the tetracycline-repressible system, the tetracycline-inducible system, the RU486-inducible system, and the rapamycin-inducible system. Other types of regulatable promoters which may be useful are those which are regulated by a specific physiological state, *e.g.*, temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only.

[0114] Tissue-specific promoters are known in the art. Examples of liver-specific promoters include albumin promoter, apolipoprotein A-II (APOA2) promoter, serpin peptidase inhibitor clade A member 1 (SERPINA1) promoter, transthyretin (TTR) promoter, apolipoprotein E (apoE) promoter, apoE hepatic control region (HCR)/human alpha1-antitrypsin hybrid promoter, cytochrome P450 family 3 subfamily A polypeptide 4 (CYP3A4) promoter, thyroxine binding globulin (TBG) promoter and microRNA 122 (mIR122) promoter. Examples of pancreas-specific promoters include insulin (INS) promoter, insulin receptor substrate 2 (IRS2) promoter, glucagon promoter, pancreatic and duodenal homeobox 1 (Pdx1) promoter, aristaless-like homeobox 3 (Alx3) promoter, and pancreatic polypeptide (Ppy) promoter. Examples of cardiac-specific promoters include myosin heavy chain 6 (Myh6) promoter, myosin light chain 2 (MYL2) promoter, troponin I type 3 (TNNI3) promoter, natriuretic peptide precursor A (NPPA) promoter, and solute carrier family 8 (Slc8a1) promoter. Examples of central nervous system (CNS)-specific promoters include synapsin I (SYN1) promoter, glial fibrillary acidic protein (GFAP) promoter, internexin neuronal intermediate filament protein alpha (INA) promoter, nestin (NES) promoter, myelin-

associated oligodendrocyte basic protein (MOBP) promoter, myelin basic protein (MBP) promoter, tyrosine hydroxylase (TH) promoter, and forkhead box A2 (FOXA2) promoter. An example of an immune system cell-specific promoter includes integrin alpha M (ITGAM) promoter. An example of a lung-specific promoter includes surfactant protein B promoter. An example of an endothelial cell-specific promoter includes endoglin (ENG) promoter. Examples of urogenital cell-specific promoters include probasin (Pbsn) promoter, uroplakin 2 (Upk2) promoter, spermine binding protein (Sbp) promoter, and Fer-1-like 4 (Fer1l4) promoter. Examples of intestine-specific promoters include human intestinal fatty acid binding protein (HIFABP) promoter, rat intestinal fatty acid binding protein (RIFABP) promoter, human mucin-2 (MNUC2) promoter, and human lysozyme (HLY) promoter. An example of muscle specific promoter includes the muscle creatine kinase (MCK) promoter. An example of a testis-specific promoter includes the Map2k7 promoter and the TSP50 promoter. An example of a kidney-specific promoter include podocin (NPHS2) promoter, nephrin (NPHS1) promoter, synaptopodin promoter, WT1 promoter, podocalyxin prompter, gamma-glutamyltransferase (GGT) promoter, sodium-glucose cotransporter-2 (SGLT2) promoter, kidney androgen-regulated protein (KAP) promoter, megalin promoter, carbonic anhydrase II (CAII) promoter, sodium-phosphate cotransporter 2a (NPT2a) promoter, Na-K-Cl cotransporter 2 (NKCC2, SLC12A1) promoter, epithelial sodium channel (ENaC) promoter, uromodulin promoter, aquaporin 1 (AQP1) promoter, and aquaporin 2 (AQP2) promoter.

[0115] In particular embodiments, the native promoter for the gene product will be used. The native promoter may be used when the 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 particular embodiments, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression.

[0116] The term “enhancer” refers to a sequence that increases transcription of, for example, a nucleic acid sequence to which it is operably linked. Enhancers can be located many kilobases away from the coding region of the nucleic acid sequence and can mediate the binding of regulatory factors, patterns of DNA methylation, or changes in DNA structure. A large number of enhancers from a variety of different sources are well known in the art and are available as or within cloned polynucleotides (from, *e.g.*, depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides including promoters (such as the commonly-used CMV promoter) also include enhancer sequences. Enhancers can be located

upstream, within, or downstream of coding sequences.

[0117] In addition to promoters and enhancers, other regulatory elements include RNA processing signals such as splicing and polyadenylation (poly A) signals; sequences that stabilize cytoplasmic mRNA; sequences encoding signal peptides; 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.

[0118] (i-d) Production of Recombinant Viral Vectors. Recombinant viral vectors (*e.g.*, rAAV virions) including mutant AAV capsids described herein, and optionally encapsidated expression cassettes, may be produced using standard methodology. The recombinant viral vector including an AAV9 mutant capsid, expression cassette, and AAV genome ITRs, is produced using methods known in the art, for example using a mammalian rAAV production system or an insect cell rAAV production system. Methods known in the art are for example described in Pan *et al.* (J. of Virology (1999) 73: 3410-3417), Clark *et al.* (Human Gene Therapy (1999) 10: 1031 -1039), Wang *et al.* (Methods Mol. Biol. (201 1) 807: 361 -404), Grimm (Methods (2002) 28(2): 146-157), and the insect cell system based on Urabe *et al* (Human Gene Therapy (2002) 13: 1935-1943), Kohlbrenner *et al* (Molecular Therapy (2005) 12(6): 1217-1225), International Patent publication WO 2007/046703, International Patent publication WO 2007/148971, International Patent publication WO 2009/014445, International Patent publication WO 2009/104964, International Patent publication WO 2009/154452, International Patent publication WO 2011/112089, International Patent publication WO 2013/036118, and US patent No. 6,723,551.

[0119] For example, in the case of rAAV virions, an AAV vector including an expression cassette can be introduced into a producer cell, followed by introduction of an AAV helper construct including a polynucleotide sequence encoding a mutant AAV capsid disclosed herein, and where the helper construct includes AAV coding regions capable of being expressed in the producer cell and which complement AAV helper functions absent in the AAV vector. This is followed by introduction of helper virus and/or additional vectors into the producer cell, wherein the helper virus and/or additional vectors provide accessory functions capable of supporting efficient rAAV virus production. The producer cells are then cultured to produce rAAV. These steps are carried out using standard methodology.

[0120] Since AAV ITRs contain the cis-acting elements involved in genome rescue, replication, and packaging, and AAV ITRs are segregated from the viral encoding regions, *i.e.*, *rep* and *cap* gene regions, recombinant AAV vector design can follow the gene-removal or “gutless” vector design rationale, similar to other virus systems (*e.g.*, retrovirus system). Typically, rAAV particles are generated by transfecting host cells with a plasmid (AAV cis-plasmid) containing a cloned

recombinant AAV genome composed of the expression cassette flanked by the AAV ITRs, and a separate construct expressing in trans the viral *rep* and *cap* genes. The adenovirus helper factors, such as E1A, E1B, E2A, E4ORF6 and viral associated (VA) RNAs, would be provided by either adenovirus infection or transfecting into production cells a third plasmid that provides these adenovirus helper factors.

[0121] The host cell can be selected from prokaryotic or eukaryotic cells, including, insect cells, yeast cells and mammalian cells. In particular embodiments, host cells are selected from the mammalian cells including A549, WEHI, 3T3, 10T1/2, BHK, MDCK, COS 1, COS 7, BSC 1, BSC 40, BMT 10, VERO, WI38, HeLa, HEK293 cells, Saos, C2C12, L cells, HT1080, HepG2 and primary fibroblast, hepatocyte and myoblast cells. HEK293 cells, for example, are commonly used AAV production cells and already contain the E1A/E1b gene, so the only helper factors that need to be provided are E2A, E4ORF6 and VA RNAs.

[0122] In particular embodiments, the host cell is stably transformed with the sequences encoding *rep* and *cap*, the helper factors, and an expression cassette. In particular embodiments, cells can be transfected using electroporation or infected by a hybrid adenovirus/AAV vector.

[0123] All functions for rAAV vector replication and packaging need to be present, to achieve replication and packaging of the rAAV genome into rAAV vectors. The introduction into the host cell can be carried out using standard molecular biology techniques and can be introduced simultaneously or sequentially. The host cells are cultured to produce rAAV vectors which are then purified using standard techniques such as CsCl gradients (Xiao *et al.* 1996, J. Virol. 70: 8098-8108) or Iodixanol purification. The purified rAAV vector is then typically ready for use (*e.g.*, administration to a subject). High titers of more than 10^{12} particles per ml and high purity (free of detectable helper and wild-type viruses) can be achieved (see for example Clark *et al.* supra and Flotte *et al.* 1995, Gene Ther. 2: 29-37). Further compositions and methods for packaging are described in Wang *et al.* (US 2002/0168342).

[0124] (ii) Compositions for Administration. Mutant AAV9 capsids associated with a vector can be formulated into compositions for administration to subjects. Compositions can include vectors including mutant AAV9 capsids disclosed herein. In particular embodiments, the vector is a gene delivery vector.

[0125] Exemplary generally used pharmaceutically acceptable carriers include any and all absorption delaying agents, antioxidants (*e.g.*, ascorbic acid, methionine, vitamin E), binders, buffering agents, bulking agents or fillers, chelating agents (*e.g.*, EDTA), coatings, disintegration agents, dispersion media, gels, isotonic agents, lubricants, preservatives, salts, solvents or co-solvents, stabilizers, surfactants, and/or delivery vehicles.

[0126] Exemplary antioxidants include ascorbic acid, methionine, and vitamin E.

[0127] Exemplary buffering agents include citrate buffers, succinate buffers, tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, acetate buffers, phosphate buffers, histidine buffers, and/or trimethylamine salts.

[0128] An exemplary chelating agent is EDTA.

[0129] Exemplary isotonic agents include polyhydric sugar alcohols including trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol, or mannitol.

[0130] Exemplary preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides, hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

[0131] Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the mutant AAV9 capsids or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can include polyhydric sugar alcohols; amino acids, such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, and threonine; organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol, and cyclitols, such as inositol; PEG; amino acid polymers; sulfur-containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, alpha-monothioglycerol, and sodium thiosulfate; low molecular weight polypeptides (*i.e.*, <10 residues); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides such as xylose, mannose, fructose and glucose; disaccharides such as lactose, maltose and sucrose; trisaccharides such as raffinose, and polysaccharides such as dextran. Stabilizers are typically present in the range of from 0.1 to 10,000 parts by weight based on therapeutic weight.

[0132] The compositions disclosed herein can be formulated for administration by, for example, injection. For injection, formulation can be formulated as aqueous solutions, such as in buffers including Hanks' solution, Ringer's solution, or physiological saline, or in culture media, such as Iscove's Modified Dulbecco's Medium (IMDM). The aqueous solutions can include formulatory agents such as suspending, stabilizing, and/or dispersing agents. Alternatively, the formulation can be in lyophilized and/or powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[0133] Compositions can be formulated as an aerosol. In particular embodiments, the aerosol is provided as part of an anhydrous, liquid or dry powder inhaler. Aerosol sprays from pressurized

packs or nebulizers can also be used with a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, a dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of gelatin for use in an inhaler or insufflator may also be formulated including a powder mix of mutant AAV9 capsids and a suitable powder base such as lactose or starch.

[0134] Compositions can also be formulated as depot preparations. Depot preparations can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0135] Additionally, compositions can be formulated as sustained-release systems utilizing semipermeable matrices of solid polymers including at least one mutant AAV9 capsid. Various sustained-release materials have been established and are well known by those of ordinary skill in the art. Sustained-release systems may, depending on their chemical nature, release mutant AAV9 capsids following administration for a few weeks up to over 100 days. Depot preparations can be administered by injection; parenteral injection; instillation; or implantation into soft tissues, a body cavity, or occasionally into a blood vessel with injection through fine needles.

[0136] Depot formulations can include a variety of bioerodible polymers including poly(lactide), poly(glycolide), poly(caprolactone) and poly(lactide)-co(glycolide) (PLG) of desirable lactide:glycolide ratios, average molecular weights, polydispersities, and terminal group chemistries. Blending different polymer types in different ratios using various grades can result in characteristics that borrow from each of the contributing polymers.

[0137] The use of different solvents (for example, dichloromethane, chloroform, ethyl acetate, triacetin, N-methyl pyrrolidone, tetrahydrofuran, phenol, or combinations thereof) can alter microparticle size and structure in order to modulate release characteristics. Other useful solvents include water, ethanol, dimethyl sulfoxide (DMSO), N-methyl-2-pyrrolidone (NMP), acetone, methanol, isopropyl alcohol (IPA), ethyl benzoate, and benzyl benzoate.

[0138] Exemplary release modifiers can include surfactants, detergents, internal phase viscosity enhancers, complexing agents, surface active molecules, co-solvents, chelators, stabilizers, derivatives of cellulose, (hydroxypropyl)methyl cellulose (HPMC), HPMC acetate, cellulose acetate, pluronics (*e.g.*, F68/F127), polysorbates, Span® (Croda Americas, Wilmington, Delaware), poly(vinyl alcohol) (PVA), Brij® (Croda Americas, Wilmington, Delaware), sucrose acetate isobutyrate (SAIB), salts, and buffers.

[0139] Excipients that partition into the external phase boundary of microparticles such as

surfactants including polysorbates, dioctylsulfosuccinates, poloxamers, PVA, can also alter properties including particle stability and erosion rates, hydration and channel structure, interfacial transport, and kinetics in a favorable manner.

[0140] Additional processing of the disclosed sustained release depot formulations can utilize stabilizing excipients including mannitol, sucrose, trehalose, and glycine with other components such as polysorbates, PVAs, and dioctylsulfosuccinates in buffers such as Tris, citrate, or histidine. A freeze-dry cycle can also be used to produce very low moisture powders that reconstitute to similar size and performance characteristics of the original suspension.

[0141] Particular embodiments include formulation of mutant AAV9 capsids within hydrogels. Exemplary hydrogels include collagen hydrogels; type I collagen, fibrin, or a mixture thereof cross-linked, as the cross-linked state of these molecules *in vivo*; type I collagen hydrogels naturally cross-linked by lysyl oxidase-derived aldimine bonds (Sabeh *et al.*, (2009) J Cell Biol 185:11-19); or other synthetic hydrogels as described in, for example, Rowe & Weiss (2008) Trends Cell Biol 18:560-574; Rowe & Weiss (2009) Annu Rev Cell Dev Biol 25:567-595; Egeblad *et al.*, (2010) Curr Opin Cell Biol 22:697-706; Harunaga & Yamada (2011) Matrix Biol 30:363-368; Willis *et al.*, (2013) J Microsc 251:250-260; and Gill *et al.* (2012) Cancer Res 72:6013-6023. In particular embodiments, a hydrogel refers to a network of polymer chains that are hydrophilic in which water or an aqueous medium is the dispersion medium. Particular embodiments may utilize a zwitterionic polymer as described in WO2016/040489.

[0142] Any composition disclosed herein can advantageously include any other pharmaceutically acceptable carriers which include those that do not produce significantly adverse, allergic, or other untoward reactions that outweigh the benefit of administration. Exemplary pharmaceutically acceptable carriers and formulations are disclosed in Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990. Moreover, formulations can be prepared to meet sterility, pyrogenicity, general safety, and purity standards as required by U.S. FDA Office of Biological Standards and/or other relevant foreign regulatory agencies.

[0143] In particular embodiments, the compositions include mutant AAV9 capsids of at least 0.1% w/v or w/w of the composition; at least 1% w/v or w/w of composition; at least 10% w/v or w/w of composition; at least 20% w/v or w/w of composition; at least 30% w/v or w/w of composition; at least 40% w/v or w/w of composition; at least 50% w/v or w/w of composition; at least 60% w/v or w/w of composition; at least 70% w/v or w/w of composition; at least 80% w/v or w/w of composition; at least 90% w/v or w/w of composition; at least 95% w/v or w/w of composition; or at least 99% w/v or w/w of composition.

[0144] In particular embodiments, mutant AAV9 capsids within a combination therapy are

formulated into separate individual compositions. In particular embodiments, combinations of mutant AAV9 capsids may be formulated into compositions together. When formulated together, the mutant AAV9 capsids may be included in the same amounts or in different amounts or ratios. For example, if a first type of mutant AAV9 capsid and a second type of mutant AAV9 capsid are provided, these mutant AAV9 capsids could be included in the following exemplary ratios: 1:1, 2:1, 1:2, 5:1, 1:5, 10:1, 1:10, etc. If three types of mutant AAV9 capsids are provided, these mutant AAV9 capsids could be included in the following exemplary ratios: a 1:1:1 ratio, 2:1:1 ratio, 1:2:1 ratio, 1:1:2 ratio, 5:1:1 ratio, 1:5:1 ratio, 1:1:5 ratio, 10:1:1 ratio, 1:10:1 ratio, 1:1:10 ratio, 2:2:1 ratio, 1:2:2 ratio, 2:1:2 ratio, 5:5:1 ratio, 1:5:5 ratio, 5:1:5 ratio, 10:10:1 ratio, 1:10:10 ratio, 10:1:10 ratio, etc. In particular embodiments, a combination of mutant AAV9 capsids targets the heart, skeletal muscle, and liver for metabolic diseases. In particular embodiments, a combination of mutant AAV9 capsids targets the heart and skeletal muscle while detargeting the liver for metabolic diseases. In particular embodiments, a combination of mutant AAV9 capsids targets the central nervous system, skeletal muscle, and heart for mitochondrial diseases.

[0145] Compositions disclosed herein can be formulated for administration by, for example, injection, infusion, perfusion, or lavage. The compositions disclosed herein can further be formulated for intravenous, intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesicular, oral and/or subcutaneous administration and more particularly by intravenous, intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, intrathecal, intratumoral, intramuscular, intravesicular, and/or subcutaneous injection.

[0146] (iii) Methods of Use. Vectors described herein including a mutant AAV9 capsid can be used to deliver a transgene or expression cassette to a target tissue. Vectors including a mutant AAV9 capsid can be used to treat subjects or can be used in research, *e.g.*, to determine the effect that the gene has on cell viability and/or function. In particular embodiments, the expression of a gene in cells includes contacting the cells with a composition of the present disclosure. In particular embodiments, contacting occurs *in vivo*, *i.e.*, the composition is administered to a subject. In particular embodiments, contacting occurs *in vitro*.

[0147] In particular embodiments, methods disclosed herein include treating subjects (*e.g.*, humans, veterinary animals (dogs, cats, reptiles, birds) livestock (*e.g.*, horses, cattle, goats, pigs, chickens) and research animals (*e.g.*, monkeys, rats, mice, fish) with compositions disclosed herein. Treating subjects includes delivering therapeutically effective amounts. Therapeutically effective amounts include those that provide effective amounts, prophylactic treatments and/or

therapeutic treatments.

[0148] An "effective amount" is the amount of a composition necessary to result in a desired physiological change in the subject. Effective amounts are often administered for research purposes. Effective amounts disclosed herein can cause a statistically-significant effect in an animal model or *in vitro* assay relevant to the assessment of a disease's development, progression, and/or resolution.

[0149] A "prophylactic treatment" includes a treatment administered to a subject who does not display signs or symptoms of a disease or displays only early signs or symptoms of a disease such that treatment is administered for the purpose of diminishing or decreasing the risk of developing the disease further. Thus, a prophylactic treatment functions as a preventative treatment against a disease. In particular embodiments, prophylactic treatments reduce, delay, or prevent the worsening of a disease.

[0150] A "therapeutic treatment" includes a treatment administered to a subject who displays symptoms or signs of a disease and is administered to the subject for the purpose of diminishing or eliminating those signs or symptoms of the disease. The therapeutic treatment can reduce, control, or eliminate the presence or activity of the disease and/or reduce control or eliminate side effects of the disease.

[0151] Function as an effective amount, prophylactic treatment or therapeutic treatment are not mutually exclusive, and in particular embodiments, administered dosages may accomplish more than one treatment type.

[0152] For administration, therapeutically effective amounts (also referred to herein as doses) can be initially estimated based on results from *in vitro* assays and/or animal model studies. Such information can be used to determine useful doses more accurately in subjects of interest. The actual dose amount administered to a particular subject can be determined by a physician, veterinarian, or researcher taking into account parameters such as physical and physiological factors including target, body weight, severity of condition, type of disease, stage of disease, previous or concurrent therapeutic interventions, idiopathy of the subject and route of administration.

[0153] Useful doses can range from 5×10^3 to 5×10^{20} AAV genomes per kg, 5×10^5 to 5×10^{15} AAV genomes per kg, or 5×10^{10} to 5×10^{13} AAV genomes per kg.

[0154] Therapeutically effective amounts can be achieved by administering single or multiple doses during the course of a treatment regimen (*e.g.*, daily, every other day, every 3 days, every 4 days, every 5 days, every 6 days, weekly, every 2 weeks, every 3 weeks, monthly, every 2 months, every 3 months, every 4 months, every 5 months, every 6 months, every 7 months, every

8 months, every 9 months, every 10 months, every 11 months or yearly).

[0155] The pharmaceutical compositions described herein can be administered by, for example, injection, inhalation, infusion, perfusion, lavage, or ingestion. Routes of administration can include intravenous, intradermal, intraarterial, intraparenteral, intranasal, intralesional, intramuscular, oral, subcutaneous, and/or sublingual administration.

[0156] The methods and compositions of the present disclosure find use in the treatment of any condition that can be addressed, at least in part, by gene therapy. In particular embodiments, the AAV9 capsid mutants described herein target a specific tissue. Specific tissue types targeted by the mutant AAV9 capsids include the liver, the lungs, skeletal muscle, pancreas, kidney, brain, heart, intestine, spleen, and testis. In particular embodiments, the mutant AAV9 capsids detarget a specific tissue. In particular embodiments, mutant AAV9 capsids detarget the liver. In particular embodiments, mutant AAV9 capsids shorten the *in vivo* half-life of compositions. In particular embodiments, mutant AAV9 capsids extend the *in vivo* half-life of compositions. In particular embodiments, mutant AAV9 capsids improve production yields of compositions.

[0157] (iv) Administration Benefits. Methods of producing a viral vector with an administration benefit, wherein the administration benefit is gene delivery to a selected cell type or selected tissue, are provided herein. In particular embodiments, when a heterologous coding sequence is selectively expressed in "selected" or "targeted" cells/tissue, it leads to expression of the administered heterologous coding sequence in the intended cell type or intended tissue and is not substantially expressed in other cell types or tissues, as explained in additional detail below.

[0158] In particular embodiments, not substantially expressed in other cell types/tissues is less than 60% expression in a reference cell type/tissue as compared to a targeted cell type/tissue; less than 50% expression in a reference cell type/tissue as compared to a targeted cell type/tissue; less than 40% expression in a reference cell type/tissue as compared to a targeted cell type/tissue; less than 30% expression in a reference cell type/tissue as compared to a targeted cell type/tissue; less than 20% expression in a reference cell type/tissue as compared to a targeted cell type/tissue; or less than 10% expression in a reference cell type/tissue as compared to a targeted cell type/tissue. In particular embodiments, a reference cell type refers to non-targeted cells. The non-targeted cells can be within the same anatomical structure as the targeted cells and/or can project to a common anatomical area. In particular embodiments, a reference cell type is within an anatomical structure that is adjacent to an anatomical structure that includes the targeted cell type. In particular embodiments, a reference cell type is a non-targeted cell with a different gene expression profile than the targeted cells.

[0159] In particular embodiments, the product of the coding sequence may be expressed at low

levels in non-selected cell types, for example at less than 1% or 1%, 2%, 3%, 5%, 10%, 15% or 20% of the levels at which the product is expressed in targeted cells. Thus, in particular embodiments, expression occurs exclusively within the targeted cell type.

[0160] Methods of producing a viral vector with an administration benefit, wherein the administration benefit is gene delivery to a selected cell type or selected tissue while detargeting a selected cell type or selected tissue. In particular embodiments, "detargeting" refers to little to no delivery of the heterologous DNA to the detargeted cell type or detargeted tissue. In particular embodiments, "detargeting" refers to little to no expression of the heterologous coding sequence in the detargeted cell type or detargeted tissue. In particular embodiments, heterologous DNA or its expression in detargeted cell types/tissues is less than 30% gene delivery or expression in a detargeted cell type/tissue as compared to a reference cell type/tissue; less than 20% gene delivery or expression in a detargeted cell type/tissue as compared to a reference cell type/tissue; or less than 10% gene delivery or expression in a detargeted cell type/tissue as compared to a reference cell type/tissue. In particular embodiments, gene delivery or expression of the heterologous coding sequence in a detargeted cell type/tissue is less than 1% or 1%, 2%, 3%, 5%, 10%, 15% or 20% of the levels at which the heterologous coding sequence is delivered or expressed in a reference cell type or reference tissue. In particular embodiments a reference cell type or reference tissue includes a non-selected cell type or non-selected tissue, respectively. In particular embodiments a reference cell type or reference tissue includes a targeted cell type or targeted tissue, respectively.

[0161] In particular embodiments, a mutant AAV9 capsid that targets gene delivery in hepatocytes expresses a heterologous sequence in hepatocytes in the liver 1-times, 2-times, 3-times, 4-times, 5-times, 6-times, 7-times, 8-times, 9-times, 10-times, 11-times, 12-times, 13-times, 14-times, 15-times, or 16-times, more efficiently than a wild-type AAV9 capsid expresses the heterologous sequence in hepatocytes. In particular embodiments, a mutant AAV9 capsid that targets gene delivery in hepatocytes does not substantially express a heterologous sequence in other cell types.

[0162] In particular embodiments, a mutant AAV9 capsid that detargets the liver expresses the heterologous sequence in the liver 0.01-times, 0.05-times, 0.1-times, 0.15-times, 0.2-times, 0.25-times, 0.3-times, 0.35-times, 0.4-times, 0.5-times, 0.7-times, 0.75-times, 0.8-times, or 0.9-times that of a wild-type AAV9 capsid expresses the heterologous sequence in the liver.

[0163] In particular embodiments, a mutant AAV9 capsid that targets the lungs while detargeting the liver expresses the heterologous sequence in the lungs 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times more than a wild-type AAV9 capsid expresses

the heterologous sequence in the lungs. In particular embodiments, a mutant AAV9 capsid that targets the lungs while detargeting the liver expresses a heterologous sequence in the liver 0.01-times, 0.05-times, 0.1-times, 0.15-times, 0.2-times, 0.25-times, 0.3-times, 0.35-times, 0.4-times, 0.5-times, 0.7-times, 0.75-times, 0.8-times, or 0.9-times a wild-type AAV9 capsid expresses the heterologous sequence in the liver.

[0164] In particular embodiments, a mutant AAV9 capsid that targets skeletal muscle while detargeting the liver expresses a heterologous sequence in the skeletal muscle 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times more than a wild-type AAV9 capsid expresses the heterologous sequence in the skeletal muscle. In particular embodiments, a mutant AAV9 capsid that targets skeletal muscle while detargeting the liver expresses a heterologous sequence in the liver 0.01-times, 0.05-times, 0.1-times, 0.15-times, 0.2-times, 0.25-times, 0.3-times, 0.35-times, 0.4-times, 0.5-times, 0.7-times, 0.75-times, 0.8-times, or 0.9-times a wild-type AAV9 capsid expresses the heterologous sequence in the liver.

[0165] In particular embodiments, a mutant AAV9 capsid that targets the pancreas while detargeting the liver expresses the heterologous sequence in the pancreas 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times more than a wild-type AAV9 capsid expresses the heterologous sequence in the pancreas. In particular embodiments, a mutant AAV9 capsid that targets the pancreas while detargeting the liver expresses a heterologous sequence in the liver 0.01-times, 0.05-times, 0.1-times, 0.15-times, 0.2-times, 0.25-times, 0.3-times, 0.35-times, 0.4-times, 0.5-times, 0.7-times, 0.75-times, 0.8-times, or 0.9-times a wild-type AAV9 capsid expresses the heterologous sequence in the liver.

[0166] In particular embodiments, a mutant AAV9 capsid that targets the kidney while detargeting the liver expresses the heterologous sequence in the kidney 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times more than a wild-type AAV9 capsid expresses the heterologous sequence in the kidney. In particular embodiments, a mutant AAV9 capsid that targets the kidney while detargeting the liver expresses a heterologous sequence in the liver 0.01-times, 0.05-times, 0.1-times, 0.15-times, 0.2-times, 0.25-times, 0.3-times, 0.35-times, 0.4-times, 0.5-times, 0.7-times, 0.75-times, 0.8-times, or 0.9-times a wild-type AAV9 capsid expresses the heterologous sequence in the liver.

[0167] In particular embodiments, a mutant AAV9 capsid that targets the brain while detargeting the liver expresses the heterologous sequence in the brain 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times a wild-type AAV9 capsid expresses the heterologous sequence in the brain. In particular embodiments, a mutant AAV9 capsid that targets the brain while detargeting the liver expresses a heterologous sequence in the liver 0.01-times,

0.05-times, 0.1-times, 0.15-times, 0.2-times, 0.25-times, 0.3-times, 0.35-times, 0.4-times, 0.5-times, 0.7-times, 0.75-times, 0.8-times, or 0.9-times a wild-type AAV9 capsid expresses the heterologous sequence in the liver.

[0168] In particular embodiments, a mutant AAV9 capsid that targets the heart while detargeting the liver expresses the heterologous sequence in the heart 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times more than a wild-type AAV9 capsid expresses the heterologous sequence in the heart. In particular embodiments, a mutant AAV9 capsid that targets the heart while detargeting the liver expresses a heterologous sequence in the liver 0.01-times, 0.05-times, 0.1-times, 0.15-times, 0.2-times, 0.25-times, 0.3-times, 0.35-times, 0.4-times, 0.5-times, 0.7-times, 0.75-times, 0.8-times, or 0.9-times a wild-type AAV9 capsid expresses the heterologous sequence in the liver.

[0169] In particular embodiments, a mutant AAV9 capsid that targets the intestine while detargeting the liver expresses the heterologous sequence in the intestine 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times more than a wild-type AAV9 capsid expresses the heterologous sequence in the intestine. In particular embodiments, a mutant AAV9 capsid that targets the intestine while detargeting the liver expresses a heterologous sequence in the liver 0.01-times, 0.05-times, 0.1-times, 0.15-times, 0.2-times, 0.25-times, 0.3-times, 0.35-times, 0.4-times, 0.5-times, 0.7-times, 0.75-times, 0.8-times, or 0.9-times a wild-type AAV9 capsid expresses the heterologous sequence in the liver.

[0170] In particular embodiments, a mutant AAV9 capsid that targets the spleen while detargeting the liver expresses the heterologous sequence in the spleen 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times more than a wild-type AAV9 capsid expresses the heterologous sequence in the spleen. In particular embodiments, a mutant AAV9 capsid that targets the spleen while detargeting the liver expresses a heterologous sequence in the liver 0.01-times, 0.05-times, 0.1-times, 0.15-times, 0.2-times, 0.24-times, 0.25-times, 0.3-times, 0.35-times, 0.4-times, 0.5-times, 0.7-times, 0.75-times, 0.8-times, or 0.9-times a wild-type AAV9 capsid expresses the heterologous sequence in the liver.

[0171] In particular embodiments, a mutant AAV9 capsid that targets the testis expresses the heterologous sequence in the testis 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times a wild-type AAV9 capsid expresses the heterologous sequence in the testis.

[0172] In particular embodiments, a mutant AAV9 capsid that targets the intestine and pancreas expresses the heterologous sequence in the intestine and pancreas 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, 3-times, 5-times, 7-times, 10-times, or 15-times

a wild-type AAV9 capsid expresses the heterologous sequence in the intestine and pancreas.

[0173] In particular embodiments, a mutant AAV9 capsid that targets brain gene delivery expresses the heterologous sequence in the brain 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, 3-times, 4-times, 5-times, 7-times, 10-times, or 15-times a wild-type AAV9 capsid expresses the heterologous sequence in the brain.

[0174] In particular embodiments, a mutant AAV9 capsid with a high-yield produces vectors at a level equal to or higher that the wild-type AAV9 vector. In particular embodiments, a mutant AAV9 capsid with a high-yield produces vectors at a level equal to 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, 3-times, or 4-times a wild-type AAV9 vector.

[0175] In particular embodiments, a mutant AAV9 capsid with a prolonged half-life persists in blood circulation 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times longer than a wild-type AAV9 capsid.

[0176] In particular embodiments, a mutant AAV9 capsid with rapid blood clearance is cleared from the blood 0.1-times, 0.15-times, 0.2-times, 0.25-times, 0.3-times, 0.35-times, 0.4-times, 0.5-times, 0.7-times, 0.75-times, 0.8-times, or 0.9-times longer than a wild-type AAV9 capsid is cleared from the blood.

[0177] In particular embodiments, a mutant AAV9 capsid with an increased ability to bind albumin binds albumin 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times better than a wild-type AAV9 capsid.

[0178] In particular embodiments, a mutant AAV9 capsid with enhanced transduction results in 1.25-times, 1.5-times, 1.75-times, 2-times, 2.25-times, 2.5-times, 2.75-times, or 3-times more transduced *in vitro* or *in vivo* cells compared to the number of *in vitro* or *in vivo* cells transduced by a wild-type AAV9 capsid.

[0179]

[0180] (v) Kits. The current disclosure also includes kits. Kits can include various components to practice methods disclosed herein. For example, depending on the aspect of the methods practiced, kits could include an AAV9 capsid mutant; one or more of nucleic acids encoding an AAV9 capsid; one or more nucleic acids encoding an AAV9 capsid mutant; nucleic acids encoding a gene product (*e.g.*, a therapeutic molecule, an enzyme, a cytokine, a hormone, a receptor, a receptor ligand, an antibody, a reporter gene/protein, or an antisense oligonucleotide); one or more of nucleic acids encoding regulatory elements (*e.g.*, promoters, enhancers, polyA sequences, Kozak consensus sequence); a viral vector associated with an AAV9 capsid mutant; a pharmaceutically acceptable carrier; an animal model; host cells (*e.g.*, HEK293 cells); tissue samples; cell formulation or culture components (*e.g.*, saline, buffered saline, phosphate buffered

saline (PBS); biocompatible buffers such as, Ca^{2+}/Mg^{2+} free PBS; physiological saline, water, Hanks' solution, Ringer's solution, RPMI, non-essential amino acids sodium pyruvate penicillin/streptomycin, human serum albumin (HSA) or other human serum components or fetal bovine serum, dextrose, stabilizers, preservatives); culture vessels; GAPDH; enzyme-linked immunosorbent assay (ELISA); culture plates; etc.

[0181] The Exemplary Embodiments and Examples below are included to demonstrate particular embodiments of the disclosure. Those of ordinary skill in the art should recognize in light of the present disclosure that many changes can be made to the specific embodiments disclosed herein and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

[0182] (vi) Exemplary Embodiments.

1. An AAV9 capsid mutant of a wild-type AAV9 capsid having the sequence as set forth in SEQ ID NO: 1, wherein the AAV9 capsid mutant includes a mutation or set of mutations selected from: S16T, Q37T, R43T, L59T, A68T, Y90T, A96T, E106N, D107N, G115T, A136N, E147N, P153N, D154N, G160N, G174T, G174N, G177N, T179N, A194N, V198N, S200N, M203N, E216T, G217T, P250N/Y252T, T251N/N253T, Y252N/N254T, H255T, L256T, H255N/Y257T, L256N/K258T, Y257N/Q259T, K258N/I260T, Q259N/S261T, I260N/N262T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T,G266N/S268T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, D271N/A273T, Y274T, A273N/F275T, Y274N/G276T, S278N/P280T, R288N/H290T, H290N/H292T, E324N, V325N/D327T, T326N/N328T, D327N/N329T, G330T, V331T, G330N/K332T, V331N, K332N/I334T, T333N/A335T, 334N/N336T, A335N/N337T, L338T, E361N/C363T, G362N/L364T, C363N/P365T, L364N/P366T, P365N/F367T, P366N/P368T, D370N/F372T, F372N/I374T, Q376N/G378T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, P438N/I440T, L439N/D441T, I440N/Q442T, D441N/Y443T, Q442N/L444T, Y443N/Y445T, L444N/Y446T, Y445N/ L447T, Y446N/S448T, L447N/K449T, S448N, K449N/I451T, T450N/N452T, I451N/G453T, S454T, G453N/G455T, S454N/Q456T, G455N/N457T, Q456N/Q458T, Q459T, Q458N, Q459N/L461T, T460N/K462T, L461N/F463T, K462N/S464T, F463N/V465T, S464N/A466T, V465N/G467T, A466N/P468T, G467N/S469T, P468N/N470T, S469N/M471T, A472T, M471N/V473T, A472N/Q474T, V473N/G475T, R485N/Q487T, R488N/S490T, T491N/V493T, T492N, V493N/Q495T, T494N/N496T, Q495N/N497T, N498T, S499T, E500T, S499N/F501T, E500N/A502T, F501N/W503T, A502N/P504T, W503N/G505T, P504N/A506T, G505N/S507T, A510N/N512T, L511N/G513T, R514T, G513N/N515T, R514N/S516T, L517T, S516N/M518T, L517N/N519T, S526N/K528T, H527N/E529T,K528N/G530T, E529N/E531T, G530N/D532T, E531N/R533T,

D532N/F534T, R533N/F535T, F534N/P536T, F535N/L537T, F543N/K545T, G544N/Q546T, K545N/G547T, Q546N, G547N/G549T, T548N/R550T, G549N/D551T, R550N/N552T, D551N/V553T, D554T, V553N/A555T, D554N/D556T, A555N/K557T, D556N/V558T, K557N/M559T,V558N/I560T, M559N, I560N/N562T, T561N/E563T, E564T, E563N/E565T, E564N/I566T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q579N/A581T, V580N, A581N/N583T, T582N/H584T, Q585T, H584N/S586T, Q585N/A587T, S586N/Q588T, A587N/A589T, Q588N/Q590T, A589N/A591T, Q590N/Q592T, A591N, Q592N/G594T, T593N/W595T, G594N/V596T, W595N/Q597T, V596N/N598T, Q597N/Q599T, G600T, D611N/Y613T, V612N/L614T, Y613N/Q615T, L614N/G616T, Q615N/P617T, A656N/P658T, D657N/P659T, P658N, P659N/A661T, T660N/F662T, A661N/N663T, F662N/K664T, D665T, K664N/K666T, D665N/L667T, K666N/N668T, L667N/S669T, F670T, S669N/I671T, F670N, I671N/Q673T, T672N/Y674T, Q673N/S675T, Y674N, Y701N/S703T, T702N/N704T, S703N/Y705T, Y706T, Y705N/K707T, Y706N/S708T, K707N/N709T, S708N/N710T, V711T, E712T, V711N/F713T, E712N/A714T, F713N/V715T, A714N/N716T, V715N, E718T, T717N/G719T, E718N/V720T, G719N/Y721T, V720N/S722T, Y721N/E723T, S722N/P724T, E723N/R725T, P724N/P726T, R725N/I727T, P726N/G728T, I727N, G728N/R730T, T729N/Y731T, R730N/L732T, and Y731N.

2. A method including expressing an AAV vector including the AAV9 capsid mutant of embodiment 1.

3. A method of producing a viral vector with an administration benefit including expressing the viral vector with the AAV9 capsid mutant of embodiment 1, thereby producing the viral vector with the administration benefit, wherein

the administration benefit is to detarget the liver and the AAV9 capsid mutant of embodiment 1 has mutations L59T, Y90T, A96T, G115T, L256T, L256N/K258T, Q259N/S261T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, Y274T, A273N/F275T, L338T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, Q442N/L444T, V473N/G475T, S499N/F501T, F501N/W503T, P504N/A506T, R514T, R514N/S516T, S516N/M518T, L517N/N519T, H527N/E529T, K528N/G530T, T561N/E563T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q588N/Q590T, G600T, D611N/Y613T, Y613N/Q615T, F662N/K664T, F670T, F670N, Y705N/K707T, K707N/N709T, V711T, E712N/A714T, E723N/R725T, R725N/I727T, P726N/G728T, A68T, G217T, Y443N/Y445T, L444N/Y446T, L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, L511N/G513T,

S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, E563N/E565T, Q590N/Q592T, A591N, S703N/Y705T, Y706T, Y706N/S708T, P724N/P726T, F543N/K545T, or K545N/G547T;
the administration benefit is targeted gene delivery to the lungs while detargeting the liver and the AAV9 capsid mutant of embodiment 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;
the administration benefit is targeted gene delivery to the skeletal muscle while detargeting the liver and the AAV9 capsid mutant of embodiment 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, F534N/P536T, K545N/G547T, A591N, or Y706T;
the administration benefit is targeted gene delivery to the pancreas while detargeting the liver and the AAV9 capsid mutant of embodiment 1 has mutations G453N/G455T, Q456N/Q458T, L511N/G513T, S526N/K528T, F543N/K545T, Y706T, Y706N/S708T, or P724N/P726T;
the administration benefit is targeted gene delivery to the kidney while detargeting the liver and the AAV9 capsid mutant of embodiment 1 has mutations Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;
the administration benefit is targeted gene delivery to the brain while detargeting the liver and the AAV9 capsid mutant of embodiment 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, K545N/G547T, Q590N/Q592T, A591N, Y706T, Y706N/S708T, G467N/S469T, Y443N/Y445T, or L444N/Y446T;
the administration benefit is targeted gene delivery to the heart while detargeting the liver and the AAV9 capsid mutant of embodiment 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, S526N/K528T, R533N/F535T, F534N/P536T, K545N/G547T, A591N, Y706T, or Y706N/S708T;
the administration benefit is targeted gene delivery to the intestine while detargeting the liver and the AAV9 capsid mutant of embodiment 1 has mutations L447N/K449T, G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, F534N/P536T, F543N/K545T, K545N/G547T, A591N, Y706T, or Y706N/S708T;
the administration benefit is targeted gene delivery to the spleen while detargeting the liver and the AAV9 capsid mutant of embodiment 1 has mutations K545N/G547T;
the administration benefit is targeted gene delivery to the testis and the AAV9 capsid of mutant embodiment 1 has mutations G330N/K332T or N14T;
the administration benefit is targeted gene delivery to the intestine and pancreas and the AAV9 capsid mutant of embodiment 1 has mutation F543N/K545T;
the administration benefit is targeted gene delivery to the brain and the AAV9 capsid mutant of

embodiment 1 has mutation H255T;

the administration benefit is targeted hepatic gene delivery and the AAV9 capsid mutant of embodiment 1 has mutations D554N/D556T; or

the administration benefit is prolonged half-life and the AAV9 capsid mutant of embodiment 1 has mutations A68T, E563N/E565T, S703N/Y705T, G217T, or G330N/K332T.

4. A method of providing targeted gene expression in a selected cell type in a subject including: administering a composition including a viral vector associated with a AAV9 capsid mutant of embodiment 1 to the subject, thereby providing targeted gene expression in the selected cell type in the subject, wherein

the selected cell type is in the lungs and the AAV9 capsid mutant of embodiment 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;

the selected cell type is in the skeletal muscle and the AAV9 capsid mutant of embodiment 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, F534N/P536T, K545N/G547T, A591N, or Y706T;

the selected cell type is in the pancreas and the AAV9 capsid mutant of embodiment 1 has mutations G453N/G455T, Q456N/Q458T, L511N/G513T, S526N/K528T, F543N/K545T, Y706T, Y706N/S708T, or P724N/P726T;

the selected cell type is in the kidney and the AAV9 capsid mutant of embodiment 1 has mutations Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;

the selected cell type is in the brain and the AAV9 capsid mutant of embodiment 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, K545N/G547T, Q590N/Q592T, A591N, Y706T, Y706N/S708T, G467N/S469T, Y443N/Y445T, or L444N/Y446T;

the selected cell type is in the heart and the AAV9 capsid mutant of embodiment 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, S526N/K528T, R533N/F535T, F534N/P536T, K545N/G547T, A591N, Y706T, or Y706N/S708T;

the selected cell type is in the intestine and the AAV9 capsid mutant of embodiment 1 has mutations L447N/K449T, G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, F534N/P536T, F543N/K545T, K545N/G547T, A591N, Y706T, or Y706N/S708T;

the selected cell type is in the spleen and the AAV9 capsid mutant of embodiment 1 has mutations K545N/G547T;

the selected cell type is in the testis and the AAV9 capsid mutant of embodiment 1 has mutations G330N/K332T or N14T;
the selected cell type is in the intestine and pancreas and the AAV9 capsid mutant of embodiment 1 has mutations F543N/K545T

the selected cell type is in the brain and the AAV9 capsid mutant of embodiment 1 has mutation H255T;
the selected cell type is in the liver and the AAV9 capsid mutant of embodiment 1 has mutations D554N/D556T.

5. The method of embodiment 4, wherein the viral vector encodes a gene product.

6. The method of embodiment 5, wherein the gene product includes a therapeutic molecule, an enzyme, a cytokine, a hormone, a receptor, a receptor ligand, an antibody, a reporter gene/protein, or an antisense oligonucleotide.

7. The method of any of embodiments 4-6, wherein the subject has a disease.

8. A method of providing a viral vector-based therapy with reduced hepatic uptake to a subject in need thereof, the method including administering a composition including a viral vector associated with a AAV9 capsid mutant of embodiment 1 to the subject in need thereof, thereby providing the viral vector-based therapy with reduced hepatic uptake to the subject in need thereof, wherein
the AAV9 capsid mutant of embodiment 1 has mutations L59T, Y90T, A96T, G115T, L256T, L256N/K258T, Q259N/S261T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, Y274T, A273N/F275T, L338T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, Q442N/L444T, V473N/G475T, S499N/F501T, F501N/W503T, P504N/A506T, R514T, R514N/S516T, S516N/M518T, L517N/N519T, H527N/E529T, K528N/G530T, T561N/E563T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q588N/Q590T, G600T, D611N/Y613T, Y613N/Q615T, F662N/K664T, F670T, F670N, Y705N/K707T, K707N/N709T, V711T, E712N/A714T, E723N/R725T, R725N/I727T, P726N/G728T, A68T, G217T, Y443N/Y445T, L444N/Y446T, L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, L511N/G513T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, E563N/E565T, Q590N/Q592T, A591N, S703N/Y705T, Y706T, Y706N/S708T, P724N/P726T, F543N/K545T, or K545N/G547T.

9. The method of embodiment 8, wherein the method further includes providing targeted gene expression in a selected cell type in the subject in need thereof, wherein

the selected cell type is in the lungs and the AAV9 capsid mutant of embodiment 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;
the selected cell type is in the skeletal muscle and the AAV9 capsid mutant of embodiment 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, F534N/P536T, K545N/G547T, A591N, or Y706T;
the selected cell type is in the pancreas and the AAV9 capsid mutant of embodiment 1 has mutations G453N/G455T, Q456N/Q458T, L511N/G513T, S526N/K528T, F543N/K545T, Y706T, Y706N/S708T, or P724N/P726T;
the selected cell type is in the kidney and the AAV9 capsid mutant of embodiment 1 has mutations Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;
the selected cell type is in the brain and the AAV9 capsid mutant of embodiment 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, K545N/G547T, Q590N/Q592T, A591N, Y706T, Y706N/S708T, G467N/S469T, Y443N/Y445T, or L444N/Y446T;
the selected cell type is in the heart and the AAV9 capsid mutant of embodiment 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, S526N/K528T, R533N/F535T, F534N/P536T, K545N/G547T, A591N, Y706T, or Y706N/S708T;
the selected cell type is in the intestines and the AAV9 capsid mutant of embodiment 1 has mutations L447N/K449T, G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, F534N/P536T, F543N/K545T, K545N/G547T, A591N, Y706T, or Y706N/S708T; or
the selected cell type is in the spleen and the AAV9 capsid mutant of embodiment 1 has mutations K545N/G547T.

10. The method of embodiments 8 or 9, wherein the viral vector encodes a therapeutic molecule.

11. The method of embodiment 10, wherein the therapeutic molecule is a protein or RNA.

12. A method of providing a viral vector-based therapy with extended *in vivo* half-life to a subject in need thereof, the method including administering a composition including a viral vector associated with the AAV9 capsid mutant of embodiment 1 to the subject in need thereof thereby providing the viral vector-based therapy with extended *in vivo* half-life to the subject in need thereof, wherein

the AAV9 capsid mutant of embodiment 1 has mutations A68T, E563N/E565T, S703N/Y705T,

G217T, or G330N/K332T.

13. A composition including the AAV9 capsid mutant of embodiment 1 and a carrier.

14. The composition of embodiment 13, wherein the carrier is a pharmaceutically acceptable carrier.

15. A composition including a viral vector associated with the AAV9 capsid mutant of embodiment 1.

16. A kit including the AAV9 capsid mutant of embodiment 1, the composition of embodiments 13 or 14, and/or the composition of embodiment 15.

[0183] (vii) Experimental Examples. Example 1. Comprehensive asparagine-X-threonine/serine (NXT/S) scanning of AAV9 capsid and phenotype collection of novel mutants in mice and in vitro. AAV DNA/RNA Barcode-Seq, a next-generation sequencing (NGS)-based technology that allows comprehensive characterization of multifaceted biological phenotypes of hundreds of AAV capsid mutants in a high-throughput manner using only a small number of replicates was developed (Adachi, K. et al., Nat Commun 5, 3075, doi:10.1038/ncomms4075 (2014); and Adachi, K., et al., Mol Ther 22 S111 (2014)). With this technology, biological properties were determined in vivo and in vitro for a number of AAV capsid mutants that have been created through systematic mutagenesis approaches including alanine scanning, domain swapping, short peptide scanning and motif scanning. One of the projects in this direction of research is comprehensive asparagine-X-threonine/serine (NXT/S) scanning of AAV9 capsid, making a set of AAV9NXT and AAV9NXS mutants, followed by phenotypic characterization of each mutant in mice and in vitro. In this NXT/S scanning mutagenesis approach, the NXT/S motif (i.e., "sequons" for N-linked glycosylation) was introduced into AAV9 capsid protein at various locations from the N-terminus to the C-terminus in a systematic manner to create potentially N-glycosylated amino acid residues at non-native sites. It was then investigated how N-linked glycosylation at different locations (if N-linked glycosylation at non-native sites indeed occurs) or NXT/S mutagenesis itself (if N-linked glycosylation does not occur) in the AAV capsids could alter biological phenotypes of AAV capsids, and sought to find clues to developing and identifying novel AAV capsids with altered biological properties more suitable for human gene therapy (e.g., prolonged half-lives for better transduction in target organs, accelerated half-lives for minimal vector dissemination to non-target organs, altered tropism, enhanced transduction in target organs, and resistance to neutralization by anti-AAV capsid antibodies). Although N-glycans are attached exclusively to the nitrogen atoms of the side chain of the asparagine (N) residue within the consensus NXT/S sequence (i.e., sequon), the generation and attachment of N-glycans is a complex biological process and occurs in a context dependent manner, which is influenced by multiple factors. Therefore, one should acknowledge that creation

of the NXT/S consensus sequence would not necessarily lead to N-linked glycosylation. In addition, glycosylation of capsid proteins of non-enveloped viruses is much less common than glycosylation of envelope proteins of enveloped viruses such as gp120 of human immunodeficiency virus type 1 (HIV-1). Therefore, one can assume that only a few or possibly none of the AAV9NXT and AAV9NXS mutants would acquire N-linked glycans at non-native sequon introduced into the AAV9 capsid by the NXT/S mutagenesis. Nonetheless, it is important to note that significant alterations of biological phenotypes could result from amino acid substitutions on their own regardless of the presence or the absence of changes of the glycosylation pattern of the AAV capsids. Therefore, even though it turns out that the NXT/S capsid modifications have no effects on N-linked glycosylation of the AAV9 capsid, the experimental outcomes should significantly enhance knowledge about the amino acid sequence-AAV capsid biological phenotype relationships and provide insights into how to develop novel AAV capsids with biological properties attractive for clinical use. This disclosure identifies novel AAV9 capsid mutants with attractive biological phenotypes that are worthy of immediate clinical implementation and offers new platforms for AAV capsid engineering to enhance the utility of AAV vectors.

[0184] Selection of the amino acid positions within the AAV9 capsid where the N-linked glycosylation NXT or NXS motif is introduced. A total of 257 positions for NXT/S mutagenesis was selected. In the AAV9 capsid region spanning between amino acid positions 1 (*i.e.*, the position of the methionine residue at the N-terminus) and position 218, a total of 25 positions were selected where a single amino acid substitution can create the NXT or NXS motif. There are 16 and 9 sites that fulfill this criterion for the NXT and NXS motifs, respectively. In the AAV9 capsid region spanning between amino acid positions 219 and 736 (*i.e.*, the position of the residue at the C-terminus), a total of 231 positions were selected that are exposed to the outer surface of the assembled AAV9 capsid and changed the native amino acid triplets to NXT (X indicates the native amino acid) by introducing one or two amino acid substitutions. AAV9N14T mutant in which the native NXS N-linked glycosylation motif is disrupted was also created by being inspired by the observation reported by Mary *et al.*, (Mol Pharm 16, 4738-4750, doi:10.1021/acs.molpharmaceut.9b00959 (2019)). Mary *et al.* has shown that a T14N mutation of the AAV2 capsid, which changes the capsid amino sequence from 14-TLS-16 to 14-NLS-16 making a new N-linked glycosylation sequon, can modestly enhance *in vitro* and *in vivo* transduction efficiency and its enhancement is associated with the newly acquired N-linked glycosylation at N14 in this AAV2 mutant capsid (*Mary, B. et al., Mol Pharm 16, 4738-4750, (2019)*). Thus, it was assumed that AAV9N14 might be naturally N-glycosylated and the

AAV9N14T mutation that abolishes N-linked glycosylation at the amino acid position 14 might affect the biological property of the capsid. A randomly selected AAV9NXS266 was also created. Consequently, a total of 258 AAV9 mutants (247 AAV9NXT mutants, 10 AAV9NXS mutants and AAV9N14T) were created and their biological phenotypes were determined as described below.

[0185] Production of DNA/RNA-barcoded AAVNXT/S mutant libraries. 258 AAV9NXT/S mutant vectors and the wild-type AAV9 vector were produced in HEK293 cells by a standard adenovirus-free plasmid transfection method using the DNA/RNA-barcoded double-stranded (ds) AAV-U6-VBC vector genome (Adachi, K., *et al.*, Mol Ther 22 S111 (2014); and Earley, LF *et al.*, J Virol 91, doi:10.1128/JVI.01980-16 (2017)) as the transgene (FIG. 1). Because the mutations introduced into the AAV9 capsid open reading frame (ORF) may affect the expression of the assembly-activating protein (AAP) or introduce a detrimental mutation in the AAP, HEK293 cells were also co-transfected with an AAP9-expressing plasmid at vector production. The dsAAV-U6-VBC vector genomes harbor a human U6 small nuclear (sn) RNA promoter-driven nonfunctional noncoding RNA expression cassette of 0.6 kb containing a pair of 12 nucleotide-long viral DNA barcodes (lt-VBC and rt-VBC). The vector genomes also contain stuffer DNA derived from the bacterial lacZ gene ORF. The dsAAV-U6-VBC vector genomes were designed to express a defined set of DNA barcodes as RNA barcodes ubiquitously in any AAV vector transduced cells.

[0186] To determine the biological phenotypes of all the 258 mutants in comparison to the phenotype of the wild-type AAV9, two libraries were designed as summarized in Table 1. Each viral clone was produced in separate 10 cm dishes. Virus production yield of each AAV9 mutant relative to that of AAV9 was determined by AAV DNA Barcode-Seq as previously reported (Adachi, K. *et al.*, Nat Commun 5, 3075, (2014)). Briefly, in each library group, an equal small fraction of AAV virions produced in a 10 cm dish was collected from all the viral clones and pooled. Viral genome DNA was then extracted from the pooled sample and subjected to the AAV DNA Barcode-Seq analysis. Titers of a limited number of AAV capsid clones were also determined by a standard quantitative dot blot assay (Powers, JM *et al.*, J Vis Exp, doi:10.3791/56766 (2018)). The AAV9 capsid mutants that yielded the titers less than 0.03-fold of the AAV9 control determined by AAV DNA Barcode-Seq were all excluded from the libraries that were used for various downstream experiments for *in vitro* and *in vivo* phenotype determination. All the other mutants were polled in the Group A or Group B library or both, and purified by 2 cycles of cesium chloride (CsCl) gradient ultracentrifugation. The following 4 low-yielding mutants showing 0.03-fold to 0.06-fold yields compared to AAV9, AAV9NXT364, AAV9NXT365, AAV9NXT366 and AAV9NXT370, in the Group A library were excluded from the downstream analysis due to the low stock reads in the AAV DNA Barcode-Seq analysis. Consequently, *in vitro* and *in vivo* biological

phenotypes of mutants were determined relative to the phenotype of AAV9 for a total of 206 AAV9NXT/S mutants.

[0187] Table 1. DNA/RNA-barcoded AAV9NXT/S mutant virus libraries.

Library name	AAV clones produced for each library: AAV capsid	No. of AAV capsids	No. of clones	Total no. of AAV capsids (clones) excluded from the final library stock	Total no. of capsids (clones) included in the final library stock[a]
AAV9NXT/S Library Group A					
	AAV9	control	15	0 (0)	1 (15)
	AAV9NXS mutants	10	20	1(2)	9 (18)
	AAV9NXT mutants	141	282	28 (56)	113 (226)
	AAV9N14T	1	2	0 (0)	1 (2)
	Total	153	319	29 (58)	124 (261)
AAV9NXT/S Library Group B					
	AAV9	control	15	0 (0)	1 (15)
	AAV9NXT mutants	148	296	23 (46)	125 (250)
	AAV9N14T	1	2	0 (0)	1 (2)
	Total	150	313	23 (46)	127 (267)

[a] Each library contains a total of 44 overlapping AAV capsids including AAV9. Therefore, by combining the Group A and Group B libraries, virus production yield data was obtained from 258 AAV9 mutants and biological phenotype data from 206 AAV9 mutant vectors by AAV DNA/RNA Barcode-Seq.

[0188] Comprehensive phenotype characterization of AAV9NXT mutants by AAV DNA/RNA Barcode-Seq. Using the DNA/RNA-barcoded libraries described above, the following biological properties of AAV9NXT mutants were determined by AAV DNA/RNA Barcode-Seq, which are expressed as Phenotypic Difference (PD) values (Adachi, K. *et al.*, Nat Commun 5, 3075, (2014)): 1) virus production yields, 2) transduction efficiency assessed by expression of vector genome transcripts in various organs in mice following intravenous administration of the vector, 3) biodistribution of vector genome DNA in various organs in mice following intravenous administration of the vector, 4) pharmacokinetic profiles following intravenous administration of the vector, 5) the ability to bind surface of HepG2 cells and CHO-Lec2 cells, 6) transduction efficiency in HepG2 cells and CHO-Lec2, and 7) the ability to bind BCL Agarose Bead Standard and ECL-coated agarose beads.

[0189] Single vector validation of the biological phenotypes of select mutants in mice. Based on the data obtained by the AAV DNA/RNA Barcode-Seq analysis of tissue samples harvested from DNA/RNA-barcoded library-injected mice, AAV9NXT253, AAV9NXT330 and AAV9NXT554 mutants were selected for validation. Single-stranded (ss) AAV-CAG-nlsGFP-VBC vectors

packaged with each individual AAV9NXT mutant capsid or the wild-type AAV9 capsid were produced. The AAV-CAG-nlsGFP-VBC vector genome contains the nuclear localization signal (nls)-containing GFP (nlsGFP) expressed under the control of the ubiquitous CAG enhancer-promoter. A pair of 12 nucleotide-long viral DNA barcodes (lt-VBC and rt-VBC) were placed downstream of the nlsGFP so that the viral DNA barcodes can be expressed as viral RNA barcodes in transduced cells (FIG. 2). ssAAV9-CAG-nlsGFP-VBC, ssAAV9NXT253-CAG-nlsGFP-VBC, ssAAV9NXT330-CAG-nlsGFP-VBC and ssAAV9NXT554-CAG-nlsGFP-VBC were produced and purified as described above. Each vector preparation contains 5 unique barcode clones. However, this feature was not used for the single vector validation study described here. Eight-week-old C57BL/6J male mice were intravenously injected with one of the four vectors described above at a dose of either 3.0×10^{11} vector genomes (vg)/mouse or 1.0×10^{12} vg/mouse (n=4 or 5) as summarized in Table 2. Three weeks post-injection, tissues were collected after the animals were perfused intracardially with heparinized phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for histological analyses or rapidly frozen with dry ice for molecular analyses.

[0190] Table 2. The experimental design of the validation study and a summary of brain transduction efficiency.

Group	Mouse[a]	Vector[b]	Dose (vg/mouse)	Route of vector administration[c]	Number of animals per group	Brain transduction score in each animal (Score[d])
1	C57BL/6J (M)	AAV9	3.0×10^{11}	IV	4	1, 1, 1, 1
2	C57BL/6J (M)	AAV9NXT253	3.0×10^{11}	IV	5	1, 1, 2, 2, 2
3	C57BL/6J (M)	AAV9NXT330	3.0×10^{11}	IV	4	1, 1, 1, 1
4	C57BL/6J (M)	AAV9NXT554	3.0×10^{11}	IV	4	0, 0, 0, 0
5	C57BL/6J (M)	AAV9	1.0×10^{12}	IV	4	2, 2, 3, 3
6	C57BL/6J (M)	AAV9NXT253	1.0×10^{12}	IV	4	2, 3, 3, 3

[a] 8-week-old C57BL/6J make mice were used for all the groups.
[b] All the vectors contained the ssAAV-CAG-nlsGFP-VBC vector genome.
[c] IV, intravenous.
[d] GFP signal intensities in the brain sections were visually semiquantified by two independent scientists in a blinded fashion, providing exactly the same results presented in this table. Score 0 (the lowest transduction) < Score 1 < Score 2 < Score 3 (the highest transduction).

[0191] Head-to-head comparison of the biological phenotypes of select mutants in mice using AAV DNA/RNA Barcode-Seq. AAV Barcode-Seq was also used to validate the biological

phenotypes of the select three AAV9NXT mutants. To this end, a DNA/RNA-barcoded library was produced containing equal amounts of ssAAV9-CAG-nlsGFP-VBC, ssAAV9NXT253-CAG-nlsGFP-VBC, ssAAV9NXT330-CAG-nlsGFP-VBC and ssAAV9NXT554-CAG-nlsGFP-VBC and 8-week-old C57BL/6J male mice were injected with the library at a dose of 2.0×10^{13} vg/kg (n=3). Three weeks post-injection, cerebrum, brain stem, heart, lung, liver, intestine, spleen, muscle, pancreas, kidney, and testis were harvested and subjected to AAV RNA Barcode-Seq analysis. This approach can minimize individual-to-individual variations of the data because a head-to-head comparison of biological phenotypes between the four AAV vectors in the same animal is possible using AAV Barcode-Seq.

[0192] AAV DNA/RNA Barcode-Seq. The AAV Barcode-Seq method described herein (Adachi, K. *et al.*, Nat Commun 5, 3075, (2014); and Adachi, K., *et al.*, Mol Ther 22 S111 (2014)) is a powerful approach to characterize various biological phenotypes of multiple different AAV strains (serotypes and mutants) in a high-throughput manner. The method first used only DNA barcodes and quantified viral DNA genome copy numbers in samples with no capability of quantifying transgene expression, an important readout for gene delivery (Adachi, K. *et al.*, Nat Commun 5, 3075, (2014)). To overcome this drawback, a series of novel AAV Barcode-Seq systems were devised that express RNA barcodes under the control of the RNA polymerase II or III promoters, termed AAV DNA/RNA Barcode-Seq. In AAV DNA/RNA Barcode-Seq, AAV libraries were produced in which each viral particle contains a DNA genome that is devoid of the *rep* and *cap* genes but is transcribed into an RNA barcode unique to its own capsid. For the production of DNA/RNA-barcoded AAV libraries (FIG. 1 and FIG. 2), HEK293 cells were transfected with the following three plasmids, pAAV plasmid supplying the viral genome containing ITRs and a barcode expression cassette, pHLP-AAVx supplying AAV2 Rep and AAVx Cap proteins (x=serotype or mutant ID), and pHelper supplying adenoviral component necessary for AAV vector production. A plasmid was also supplied expressing the corresponding AAP protein, pCMV-FLAG-cmAAPx (x=serotype) (Dalwadi, DA *et al.*, Mol Ther, doi:10.1016/j.ymthe.2021.08.031 (2021)) depending on the experimental settings and particularly when capsid gene mutations were introduced within the AAP-VP overlapping ORFs. In AAV DNA Barcode-Seq, clone-specific 12 nucleotide-long DNA barcodes were PCR-amplified from sample DNA (*i.e.*, DNA-PCR barcode amplicons). In AAV RNA Barcode-Seq, clone-specific 12 ribonucleotide-long RNA barcodes transcribed from the corresponding DNA barcode under the control of a defined promoter (*i.e.*, the U6 snRNA gene promoter (FIG. 1) and the CAG promoter (FIG. 2)) were PCR-amplified from sample RNA by means of reverse-transcription (RT)-PCR (*i.e.*, RT-PCR barcode amplicons). DNA-PCR barcode amplicons were also obtained from the input

AAV libraries. The resulting DNA-PCR barcode amplicons and RT-PCR barcode amplicons were then subjected to Illumina barcode sequencing and the obtained data was bioinformatically analyzed to determine the PD values that indicate relative enhancement of a phenotype of interest of each AAV serotype or mutant compared to that of AAV9, the benchmark AAV capsid (Adachi, K. *et al.*, Nat Commun 5, 3075, (2014)). For AAV RNA Barcode-Seq, correction factors were used that had been experimentally determined for each barcode. The correction factors were used to correct barcode sequence-derived biases that could originate from various steps including transcription in cells, reverse-transcription and PCR amplification. The correction factors were determined by injecting mice intravenously with each DNA/RNA-barcoded AAV9 library containing a set of AAV9 barcode clones used for the creation of each of the dsAAV-U6-VBC library and ssAAV-CAG-nlsGFP-VBC libraries.

[0193] Experimental Results. *In silico* validation of the selection of the amino acid positions within the AAV9 capsid where the N-linked glycosylation NXT or NXS motif was introduced. In the wild-type AAV9 capsid VP1 amino acid sequence, there are a total of 5 sequons for N-linked glycosylation, 14-NLS-16, 262-NGT-264, 337-NLT-339, 452-NGS-454 and 497-NNS-499. Knowledge of AAV capsid glycosylation is currently very limited (Mary, B *et al.*, Mol Pharm 16, 4738-4750, doi:10.1021/acs.molpharmaceut.9b00959 (2019); Aloor, A *et al.*, Viruses 10, doi:10.3390/v10110644 (2018); Jin, X *et al.*, Hum Gene Ther Methods 28, 255-267, doi:10.1089/hgtb.2016.178 (2017); Mary, B *et al.*, FEBS J 286, 4964-4981, doi:10.1111/febs.15013 (2019); and Murray, S *et al.*, J Virol 80, 6171-6176, doi:10.1128/JVI.02417-05 (2006)), and none of these five sequons in the AAV9 capsids have been found to be N-glycosylated (Mary, B *et al.*, FEBS J 286, 4964-4981, doi:10.1111/febs.15013 (2019)). The *in silico* prediction of glycosylation sites and the level of glycosylation has been challenging because a variety of factors besides the local amino acid sequences represented by the NXT/S sequons can influence the glycosylation pathways in a context-dependent manner. The glycosylation reaction involves a series of biomolecules including oligosaccharyltransferase and dolichol-linked donor substrates. To overcome this challenge, prediction algorithms have been developed that utilize both structural information provided by the Protein Data Bank (PDB, https://www.rcsb.org) and the pattens of amino acids residues. For the prediction of N-linked glycan chains at the NXT/S sequons, NGlycPred (Chuang, GY *et al.*, Bioinformatics 28, 2249-2255, doi:10.1093/bioinformatics/bts426 (2012)) has been developed and is avai*lab*le for free to academic uses. NGlycPred uses a variety of the features of a protein of interest including surface accessibility and secondary structures, which are the structural properties that can be extracted from PDB files. NGlycPred shows improved accuracy of the prediction of N-linked glycosylation

at the NXT/S sequons and provides convenient prediction scores from 0 to 1. In the 736 amino acids of the AAV9 VP1 protein, the atomic structure has been determined from the amino acid positions of 219 to the C-terminus by X-ray crystallography, while the N-terminus including the first 218 amino acids is disordered and invisible by X-ray crystallography and therefore its structure has not been determined. Within the AAV9 capsid region whose structure is known, the systematic *in silico* NXT scanning mutagenesis with an offset of 1 creates a total of 514 AAV9NXT mutants from AAV9NXT219 to AAV9NXT734 (please note that AAV9NXT262 and AAV9NXT337 are the same as the wild-type AAV9). Thus, the *in silico* prediction of the AAV9 and AAV9NXT/S mutants was performed for a total of 515 AAV9NXT/S mutants (*i.e.*, 514 AAV9NXT mutants and AAV9NXS266) and the wild-type AAV9. The 9 AAV9NXS mutants that fall in the N-terminus including the first 218 amino acids were not included in the analysis.

[0194] With the NGlycPred algorithm, the 4 NXT/S sequons found in the wild-type AAV9 capsid, 262-NGT-264, 337-NLT-339, 452-NGS-454 and 497-NNS-499, are all predicted to be N-glycosylated (Table 3). Among the 515 NXT/S sequons introduced in the AAV9 capsid in silico, 365 AAV9NXT/S mutants (365 out of 515, 70.8%) were predicted to be N-glycosylated by NGlycPred at the non-native sequons introduced by the NXT/S mutagenesis. This analysis revealed that the 232 AAV9NXT/S mutants created by the NXT/S mutagenesis using the criteria described above contain more NXT/S mutants that are predicted to acquire N-linked glycans at the non-native sequons introduced by the NXT/S mutagenesis (200 out of 232, 86.2%) than the 283 AAV9NXT/S mutants that were not selected (165 out of 283, 58.3%) ($P<1 \times 10^{-11}$, a two-sided Boschloo's exact unconditional test, Table 4). These observations support the approach and indicate that many of the AAV9NXT/S mutants have the potential to be subject to N-linked glycosylation at the non-native NXT/S sequons.

[0195] Table 3. NGlycPred decision scores of the four NXT/S sequons in the AAV9 capsid.

NXT/S sequon	Number of AAV capsids analyzed[a]	NGlycPred decision score[b] (average)
262-NGT-264	513	0.868
337-NLT-339	513	1.000
452-NGS-454	513	0.587
497-NNS-499	513	0.512

[a] In addition to the wild-type AAV9, these 4 sequons are preserved in a total of 512 AAV9NXT/S mutants. Therefore, the decision scores were also obtained in the 512 AAV9NXT/S mutants and included to determine average decision scores.
[b] The sequon is predicted to be glycosylated when the score is >0.5.

[0196] Table 4. Prediction of N-linked glycosylation at the non-native sequons introduced by the NXT/S mutagenesis in the AAV9NXT/S mutant capsids.

AAV9NXT/S mutants	Number of mutants	Total

	Score[a] >0.5	Score =<0.5	
Selected for the capsid mutagenesis study[b]	200	32	232
Not selected for the study[c]	165	118	283
Total	365	150	515

[a] The sequon is predicted to be glycosylated when the score is >0.5.
[b] The 232 AAV9NXT/S mutants were created and used for the study.
[c] The 283 AAV9NXT/S mutants were analyzed only in silico and were not created for the study.

[0197] The asparagine-X-threonine/serine (NXT/S) mutations introduced in the protruding surface of the AAV9 capsid could be tolerated in virion formation. The AAV Barcode-Seq analysis of the viral genomes extracted from the AAV9NXT/S libraries (*i.e.*, AAV9NXT/S Libraries Group A and Group B, see Table 1) revealed that 175 out of the 257 AAV9NXT/S mutants (68%) were produced at titers that are more than 0.25-fold of the wild-type AAV9 titer. Seventy-two mutants (28%) were produced at higher titers than AAV9 while 55 mutants were produced at titers that are less than 5% of the AAV9 titer (FIG. 3). The 257 AAV9NXT/S mutants were then categorized into two groups, 55 non-viable mutants showing the vector yields less than 0.05-fold of the wild-type AAV9 titer and 202 viable mutants showing the vector yields that are 0.05-fold or more than the wild-type AAV9 titer, and investigated topological locations of the mutated amino acids in the AAV9 capsid. The positions of the AAV9NXT/S mutations that led to the viable AAV9NXT/S mutants are located primarily on the protruding surface of the capsid while those for the non-viable ones are on the depressed surface around the 5-fold axis (FIG. 4). Among the 25 NXT/S mutants near the N-terminus (from AAV9NXT14 to AAV9NXT215, only AAV9NXS203 could not produce virions, indicating that the AAV9 VP1 unique region (amino acid positions 1 to 202) are tolerant to the NXT/S mutagenesis.

[0198] The asparagine-X-threonine/serine (NXT/S) mutations that may lead to enhanced vector production. The AAV Barcode-Seq analysis identified a total of 72 AAV9NXT/S mutants that produce virions better than AAV9 (FIG. 5). Among these 72 mutants, 32 mutants showed vector yield 2-times or more than AAV9. In order to validate this data, dsAAV-CMV-GFP vectors were produced using the following 5 mutants selected from the 32 mutants in HEK293 cells, AAV9-NXT14, AAV9-NXT35, AAV9-NXT41, AAV9-NXT588 and AAV9-NXT593. The experiment was performed in triplicate and vector yields relative to the yield of AAV9 was determined by a quantitative dot blot assay. Although there is no statistically significant increase in the yields, AAV9-NXT14 and AAV9-NXT35 showed a modest increase of the vector yields, which warrants further investigation (FIG. 6). AAV9-NXT14 and AAV9-NXT35 retain the ability to transduce various organs at decent levels compared to those of AAV9 with a range of PD values between

0.44 and 0.95 in mouse organs (FIG. 7).

[0199] Tropism and transduction efficiency of 206 AAV9NXT/S mutants in mice following intravenous administration. Eight-week-old C57BL/6J male mice were injected intravenously with AAV9NXT/S Library Group A or AAV9NXT/S Library Group B at a dose of 2.0×10^{13} vg/kg (n=3 per group). Three weeks post injection, brain, liver, heart, lung, intestine, spleen, skeletal muscle, pancreas, kidney and testis were harvested. RNA was then extracted from each tissue and subjected to AAV RNA Barcode-Seq. DNA was extracted from each tissue and subjected to AAV DNA Barcode-Seq. The PD values determined by the AAV RNA Barcode-Seq analysis is summarized in FIG. 7.

[0200] In the k-means clustering analysis of the PD values of a total of 207 AAV capsids (*i.e.*, 206 AAV9NXT/S mutants and the wild-type AAV9) obtained from the 10 different tissues, the total within-cluster sum of square (WSS) values and k values were plotted on a graph but failed to identify an elbow point. Next, x-means clustering was performed using the same dataset using the PyClustering library implemented by Python. The x-means clustering was repeated 100,000 times and the optimal number of clusters was determined to be 9. The Uniform Manifold Approximation and Projection (UMAP) plotting showed that the 9 clusters are clearly visualized and separated from each other in a two-dimensional space (FIG. 8). The wild-type AAV9 belongs to Group 3. The AAV9NXT/S mutants in Group 9 are primarily non-infectious resulting in substantially impaired transduction in all the tissues although they could form virions (FIG. 9). AAV9NXT/S mutants in other groups showed different tissue tropism and varying degrees of transduction efficiencies (FIG. 9). AAV9NXT330 and AAV9NXT554 formed two solitary groups, Group 4 and Group 6, respectively (FIG. 8), showing distinct biological features than other groups (FIG. 9). AAV9NXT330 showed modest increase of transduction efficiency in all the tissues up to 2-times and AAV9NXT554 transduced the liver exclusively and 1.4-times more than AAV9. AAV9NXT253 transduced the brain best among the mutants tested, showing 2.1-times more than AAV9. These three mutants showing unique features were selected for further validation as described in detail below. As for AAV9NXT330, *a validation* study described below was not able to reproduce the global transduction enhancement nature of AAV9NXT330.

[0201] AAV9NXT554 transduce the liver exclusively and better than AAV9 with minimal off-target transduction and biodistribution. To validate the data obtained from the AAV Barcode-Seq analysis described above, C57BL/6J mice were intravenously injected with ssAAV-CAG-nlsGFP-VBC vector packaged with the wild-type AAV9, AAV9NXT253, AAV9NXT330 or AAV9NXT554 capsid at two different doses as summarized in Table 2. Three weeks post-injection, transduction efficiencies in the liver, heart and brain were determined histologically. The AAV9NXT554 vector

transduced-liver showed the highest GFP signals among the 4 AAV vectors, consistent with the AAV Barcode-Seq data (FIG. 10). The livers transduced with the higher dose did not show increased transduction compared to those receiving the lower dose, which is indicative of a saturation effect; therefore, the higher dose group was excluded from the comparative assessment of hepatic transduction. AAV vector-mediated liver transduction was also assessed by a GFP-specific ELISA (GFP ELISA Kit, ab171581) using total proteins extracted from the liver tissues, which corroborated the histological data (FIG. 11). Although there was no statistically significant difference in liver transduction efficiency between AAV9NXT253 or AAV9NXT330 and the control AAV9, AAV9NXT554 could transduce the liver 1.9-times more than AAV9 with statistical significance with $\alpha = 0.1$.

[0202] Next, histological analyses of the brain and the heart were performed in the vector-injected mice to assess GFP expression in non-hepatic tissues. In line with the AAV Barcode-Seq data, AAV9NXT554 transduction in the brain and the heart was found significantly impaired (FIG. 12 and FIG. 13).

[0203] To investigate further the unique biological properties of AAV9NXT554, C57BL/6J mice were injected with a low-diversity DNA/RNA-barcoded ssAAV-CAG-nlsGFP-VBC library that contained 5 viral clones each of the AAV9, AAV9NXT253, AAV9NXT330 and AAV9NXT554 vectors at a dose of 2.0×10^{13} vg/kg (n=3). The AAV RNA Barcode-Seq analysis of various mouse tissues harvested three weeks post-injection further confirmed the exclusivity of AAV9NXT554 for liver transduction (FIG. 14).

[0204] AAV9NXT253 can transduce the brain 1.5-times more than AAV9. The AAV Barcode-Seq approach using dsAAV-U6-VBC library showed that the AAV9NXT253 can transduce the brain 2.1-times more than AAV9 (FIG. 7). This was also confirmed in the AAV Barcode-Seq analysis using ssAAV-CAG-nlsGFP-VBC library (FIG. 14). Histological assessment of the brain sections was also performed in mice injected with ssAAV-CAG-nlsGFP-VBC vectors packaged with AAV9, AAV9NXT253, AAV9NXT330 or AAV9NXT554 at two different doses, a higher dose (1.0×10^{12} vg/mouse) and a lower dose (3.0×10^{11} vg/mouse) (FIG. 8). To this end, a total of 26 brain images shown in FIG. 8 were visually assessed by two independent experienced scientists semiquantitatively using Scores 0, 1, 2, and 3 in a blinded fashion. In this scoring system, Score 0 brains show the lowest transduction, Score 3 brains show the highest transduction, and Score 1 and 2 brains show transduction at levels between Score 0 and Score 3 with Score 2 being better than Score 1. In this assessment, the brain sections transduced with ssAAV9NXT554-CAG-nlsGFP-VBC were consistently scored better than those transduced with ssAAV9-CAG-nlsGFP-VBC (Table 2). Thus, AAV9NXT253 transduces the brain better than AAV9 with the degree of

enhancement of 2 times.

[0205] Very fast blood clearance of AAV9NXT554 following intravenous administration. Pharmacokinetic profiles of a total of 207 AAV9NXT/S mutants were determined by AAV Barcode-Seq as described previously (Adachi, K. *et al.*, Nat Commun 5, 3075, (2014)). In brief, the barcode AAV libraries (Table 1) were intravenously injected into mice in bolus, and vector concentration of each mutant relative to that of AAV9 were determined by AAV DNA Barcode-Seq at 1, 10, 30 min, 1, 4, 8, 24 and 72 h post-injection. The relative vector concentrations of each mutant expressed as PD values are summarized in FIG. 15. The AAV Barcode-Seq-based pharmacokinetic analysis of intravenously injected AAV9NXT/S mutants revealed that AAV9NXT554 exhibits very rapid disappearance from the blood circulation immediately after injection, which is quite distinct from any other AAV9NXT mutants and other AAV capsids tested in mice (Adachi, K. *et al.*, Nat Commun 5, 3075, (2014); and Kotchey, NM *et al.*, Mol Ther 19, 1079-1089, doi:10.1038/mt.2011.3 (2011)) (FIG. 16).

[0206] To validate the AAV Barcode-Seq data showing very rapid blood clearance of AAV9NXT554 compared to AAV9, 8-week-old C57BL/6J male mice were intravenously injected with ssAAV9-CAG-nlsGFP-VBC vector or ssAAV9-CAG-nlsGFP-VBC vector at a dose of 1.0 x 10^{13} vg/kg (n=3 per group), and the whole blood samples were collected at 1 min, 10 min, 30 min, 1 h, 4 h, 8 h, 24 h and 72 h post injection. DNA was extracted from the whole blood samples using Extract-N-Amp Blood PCR kit (Sigma Aldrich) and the AAV vector genome copy numbers were quantified by real-time quantitative PCR (qPCR). In concordance with the pharmacokinetic profiles determined by the AAV Barcode-Seq analysis, the AAV9NXT554 vector was cleared much more rapidly than AAV9 with a statistical significance (FIG. 17). AAV vector distribution half-lives ($t_{1/2d:1\text{-}30\ min}$) of AAV9 and AAV9NXT554 were 37 min and 5 min, respectively; and vector elimination half-lives ($t_{1/2e:1\text{-}24h}$) of AAV9 and AAV9NXT554 were 10.4 h and 12.6 h, respectively. Two types of half-lives, distribution half-lives for the first 30 minutes, $t_{1/2d:1\text{-}30\ min}$, and elimination half-lives between 1 and 24 hours post-injection, $t_{1/2e:1\text{-}24h}$) were determined due to the biphasic nature of the blood vector concentration-time curves (Kotchey, NM *et al.*, Mol Ther 19, 1079-1089, doi:10.1038/mt.2011.3 (2011)). Previously, distribution half-lives of common serotypes and a set of mutants were determined, showing a range of 0.2-3.4 hours (Kotchey, NM *et al.*, Mol Ther 19, 1079-1089, doi:10.1038/mt.2011.3 (2011)). Thus, the $t_{1/2d:1\text{-}30\ min}$ of 5 min is exceptionally rapid in AAV vectors. Since there was no substantial difference in AAV vector elimination half-lives between AAV9 and AAV9NXT554, the rapid blood clearance of AAV9NXT554 was primarily due to its very short distribution half-life. It is likely that AAV9NXT554 vector particles immediately bind to and get sequestered by various components in the body including cell surface membranes, in

particular those of hepatocytes, and extracellular matrix, rapidly decreasing the bioavailability of the vector particles in the blood. The much slower clearance of AAV9NXT554 after one-hour post-injection following the distribution phase indicates that a small portion of AAV9NXT554 could bind to serum components, which could prolong the half-life of AAV9NXT554 in the elimination phase. Based on the experimental observations described below, AAV9NXT554 binds to serum albumin which facilitates the hepatic update of vector particles resulting in a rapid loss of blood vector concentrations in the elimination phase and prolongs vector elimination half-life in the elimination phase. The interaction between AAV9NXT554 vector particles and body components most likely involve both specific and non-specific mechanisms.

[0207] AAV9NXT554 binds cell surface better than AAV9 by up to 3.8 times and transduces a human hepatocarcinoma cell line (HepG2 cells) more than an order of magnitude better than AAV9. The dsAAV-U6-VBC Library A and Library B were applied to Chinese hamster ovary (CHO)-Lec2 cells and human hepatocarcinoma HepG2 cells to investigate the ability to bind cell surface and transduce cells compared to that of AAV9 by AAV DNA/RNA Barcode-Seq (FIG. 18). Library A and Library B shared 43 AAV9NXT/S mutants; therefore, data reproducibility could be addressed by comparing the AAV Barcode-Seq data for the 43 mutants obtained from two independent experiments using Library A and Library B. Good data reproducibility was observed for the following three experimental settings, CHO Lec2 cell binding, HepG2 cell binding and CHO Lec2 cell transduction efficiency with Pearson's correlation coefficient of 0.64 to 0.99, while such data reproducibility was not observed for HepG2 cell transduction showing Pearson's correlation coefficient of 0.08 (FIG. 19). AAV9 vectors transduce HepG2 cells poorly resulting in a failure of recovery of vector-derived transcripts at a level sufficient for yielding reliable Barcode-Seq data. It has been well documented that stochastic amplification of low copy number templates can lead to skewed representation of the abundance of each molecule in the next-generation sequencing (Kebschull, JM & AM Zador, Nucleic Acids Res 43, e143, doi:10.1093/nar/gkv717 (2015)). Even with this issue, it was concluded that AAV9NXT554 transduced HepG2 cells substantially better than AAV9 based on the following reasons. In brief, the dsAAV-U6-VBC Library A contained a total of 313 AAV clones among which 2 clones (representing 0.6%) are AAV9NXT554 clones. Although AAV9NXT554 clones represented only a small fraction of the library, Illumina RNA barcode sequencing revealed that AAV9NXT554 clone-derived transcript sequence reads substantially dominated over other AAV-derived sequence reads representing 14% of the total reads. Therefore, despite the inability to accurately quantify transduction efficiency of each AAV9NXT/S mutants in HepG2 cells by the AAV Barcode-Seq analysis, it is unambiguous that AAV9NXT554 can transduce substantially better than AAV9. AAV9NXT554 also binds to the cell

surface of HepG2 cells 3.8-times more than AAV9 (FIG. 18). The substantially enhanced transduction in HepG2 cells with AAV9NXT554 compared to AAV9 has been confirmed in a validation study in which HepG2 cells were infected with ssAAV9-CAG-nlsGFP-VBC or ssAAV9NXT-CAG-nlsGFP-VBC vector at a multiplicity of infection (MOI) of 10^6 (FIG. 20). AAV9NXT554 also binds to the CHO-Lec2 cell surface and transduced CHO-Lec2 cells 2.6-times and 11-times more than AAV9, respectively. However, none of the other AAV9NXT/S mutants could transduce CHO-Lec2 cells substantially better than AAV9. Only the following 4 mutants, AAV9NXT448, AAV9NXT325, AAV9NXT462 and AAV9NXT552, showed modest enhancement of transduction (more than 2 times but up to 2.7 times) compared to AAV9 (FIG. 18).

[0208] AAV9NXT554 potentially exhibits an increased affinity to albumin and AAV9N543 potentially acquires a new glycan chain that binds ECL. To assess the potential ability for AAV9NXT/S mutants to bind to glycan chains, AAV Barcode-Seq-based binding assay was performed using Erythrina Cristagalli Lectin (ECL)-coated agarose beads (ECL, agarose-bound, AL-1143, called "ECL Agarose" in this disclosure), and control beads (4% BCL Agarose Bead Standard, A-1041S-10, called "Control Agarose" in this disclosure) in test tubes. ECL binds galactose, N-acetylgalactosamine and lactose; therefore, an observation that an AAN9NXT/S mutant binds ECL Agarose more efficiently than AAV9 while showing no enhanced binding to Control Agarose is indicative of glycosylation on the AAV capsid. Due to the hepato-specific nature of AAV9NXT554, it was originally hypothesized that AAV9NXT554 utilizes the asialoglycoprotein receptor (ASGPR) that is highly expressed on the surface of hepatocytes and has successfully been exploited for liver-targeted drug delivery (D'Souza, AA & PV Devarajan, J Control Release 203, 126-139, doi:10.1016/j.jconrel.2015.02.022 (2015)).

[0209] In the experiment, a method similar to the immunoprecipitation (IP)-Seq method described in U.S. Patent No.10,746,742 was followed. In brief, after washing of the beads with PBS, the beads were blocked with 2% bovine serum albumin (BSA) at 37°C for 1 hour. After washing with PBS, the beads were incubated with 1.0×10^9 vg of the barcoded library (dsAAV-U6-VBC Library A or dsAAV-U6-VBC Library B) in PBS at 37°C for one hour on a tube rotor. After washing with PBS three times, the beads were resuspended in PBS, treated with Proteinase K, and viral genome DNA was extracted from the bead-bound AAV vector particles, and subjected to the AAV DNA Barcode-Seq analysis. This analysis revealed that 30 and 43 AAV9NXT/S mutants could bind to Control Agarose and ECL Agarose better than AAV9 by more than 2 times, respectively. Top 20 mutants showing enhanced binding to Control Agarose and ECL Agarose are shown in FIG. 21. There is a strong positive correlation of the degrees of binding between ECL Agarose and Control Agarose with Pearson's correlation coefficient of 0.64 ($P<0.0001$) (FIG. 22A). Fifteen

of the top 20 mutants show enhanced binding to both types of agarose beads, which cannot be explained in a random binomial model ($P=5 \times 10^{-66}$). None of the AAV9NXT/S mutants show a substantial loss of binding with the lowest PD values of 0.71 and 0.85 for Control Agarose binding and ECL Agarose binding, respectively. These observations indicate that the enhanced ability for a subset of AAV9NXT/S mutants to bind beads is mediated by interactions between AAV capsids and agarose beads and not through specific interactions between AAV capsids and ECL. However, AAV9NXT543 and AAV9NXT554 were exceptions (FIG. 22A).

[0210] As for AAV9NXT543, the $\log_2$-transforemd ECL Agarose-to-Control Agarose PD value ratio of AAV9NXT543 was 2.71 ($\log_2(29.84/4.56)$) with a z-score 5.54 (two-tailed $P<0.0001$), indicating that the binding of AAV9NXT543 to ECL Agarose is most likely mediated by a specific interaction between AAV9NXT543 and ECL possibly through the acquisition of a glycan chain at the position of N543 in the AAV9NXT543 capsid. Based on the AAV RNA Barcode-Seq data, AAV9NXT543 exhibits an intriguing unique tropism showing that it can transduce the intestine and pancreas at levels comparable to AAV9 while detargeting other organs, although this observation has yet to be validated using individually packaged AAV vectors.

[0211] As for AAV9NXT554, conversely, the $\log_2$-transformed ECL Agarose-to-Control Agarose PD value ratio of AAV9NXT554 was -3.75 ($\log_2(1.20/16.0)$) with a z-score of -8.76 (two-tailed $P<0.0001$). The $\log_2$-transformed ECL Agarose-to-Control Agarose PD value ratio of the control AAV9 was 0 ($\log_2(1/1)$) with a z-score of -0.46 (two-tailed $P=0.64$). When the agarose beads were incubated with BSA, more BSA molecules should attach to Control Agarose than ECL Agarose because the surface of ECL Agarose beads had been precoated by ECL protein molecules. BSA protein molecules can therefore bind only to the residual surface of the beads that have the non-specific capacity to bind proteins. Thus, AAV9NXT554 has an increased *affinity* to BSA and relative inhibition of ECL Agarose binding of AAV9NXT554 compared to AAV9 is attributed to the less occupancy of the BSA molecules on the surface of the ECL Agarose than Control Agarose.

[0212] Subsequently, it was investigated how efficiently AAV9 and AAV9NXT/S mutants bind agarose beads directly that have not been pre-treated with BSA. To this end, an experiment that is similar to that described above except that the BSA blocking step was omitted was performed. In this experimental condition, the degree of enhancement was *significantly* attenuated (FIG. 22B). This also supports that enhanced binding of AAV9NXT554 to Control Agarose is mediated by an increase affinity to the albumin used for a blocking agent. Wang *et al.* has reported that human serum albumin can interact with AAV capsids of various serotype origins and enhance liver transduction, and this is particularly the case for AAV8 *in vivo* (Wang, M *et al.*, Gene Ther 24, 49-59, doi:10.1038/gt.2016.75 (2017)). Meanwhile, Jin *et al.* reported that the wild-type AAV9

does not bind human serum albumin but an AAV9 capsid mutant that was engineered to harbor a non-natural albumin-binding consensus (ABDCon) domain derived from the Streptococcus species can indeed bind human albumin and transduce the liver better than AAV9 (Jin, Q *et al.*, Gene Ther 27, 237-244, doi:10.1038/s41434-019-0107-2 (2020)). All of these observations and previous reports support the proposed model described below.

[0213] A hypothetical model that explains the unique biological features of AAV9NXT554 showing enhanced hepatic transduction with unprecedently high hepatic exclusivity. As detailed above, AAV9NXT554 has a very unique set of the following biological features a combination of which has never been reported in previous studies: (1) enhanced transduction in hepatocytes, (2) substantially suppressed transduction in non-hepatic tissues, exhibiting an excellent off-target profile, (3) very rapid blood clearance immediately following intravenous injection during the distribution phase, (4) slow blood clearance during the elimination phase, and (5) potential ability to bind albumin. These features are very attractive for clinical use for hepatic gene transfer with substantially increased safety because of the enhanced potency in hepatic transduction and substantially reduced off-target biodistribution. The AAV9NXT554 mutant carries a 554-DAD-556 to 554-NAT-556 mutation, resulting in a loss of 2 negatively charged amino acids that are replaced with 2 neutral amino acids. At this point, although the mechanism underlying this unique set of biological features has not yet been definitively determined, a model in which the NXT554 mutation makes a basic amino acid patch on the surface of the AAV9 capsid that does not present in the wild type (FIG. 23) is proposed. Without being bound by theory, this positively-charged patch interacts with negatively-charged albumin or other unidentified cellular receptor on the surface of hepatocytes through electrostatic and conformation-specific interactions, and such interactions mediate rapid sequestration of AAV vector particles by hepatocytes resulting in quick disappearance of vector particles from the bloodstream and minimal dissemination to non-hepatic organs. The unique property of the liver sinusoidal endothelial cells with fenestrae allows AAV vector particles to reach hepatocytes immediately following intravenous administration while, in non-hepatic organs, AAV vector particles cannot traverse freely across the endothelial cells and need to utilize a transcellular transport mechanism to reach parenchymal cells, which takes hours. In this model, the difference in the anatomical structure of endothelial cells between the liver and non-hepatic organs, very rapid blood vector clearance, the enhanced interaction between AAV vector particles and albumin, and possibly the direct interaction between AAV vector particles and hepatocytes, all play pivotal roles in enhanced hepatocyte-specific transduction of AAV9NXT554.

[0214] A set of AAV9NXT/S mutants can detarget the liver while retaining the ability to transduce other organs. AAV vector genome biodistribution in mice following intravenous vector

administration was assessed by the AAV DNA Barcode-Seq analysis using the DNAs extracted from the 10 tissues described above (FIG. 24). The following 86 AAV9NXT/S mutants were found (*i.e.*, AAV9NXT57, AAV9NXT66, AAV9NXT88, AAV9NXT94, AAV9NXT113, AAV9NXT215, AAV9NXT254, AAV9NXT256, AAV9NXT259, AAV9NXT261, AAV9NXT263, AAV9NXT264, AAV9NXT265, AAV9NXS266, AAV9NXT267, AAV9NXT268, AAV9NXT269, AAV9NXT270, AAV9NXT272, AAV9NXT273, AAV9NXT336, AAV9NXT383, AAV9NXT384, AAV9NXT385, AAV9NXT386, AAV9NXT387, AAV9NXT388, AAV9NXT389, AAV9NXT390, AAV9NXT391, AAV9NXT442, AAV9NXT443, AAV9NXT444, AAV9NXT447, AAV9NXT453, AAV9NXT455, AAV9NXT456, AAV9NXT467, AAV9NXT469, AAV9NXT470, AAV9NXT473, AAV9NXT488, AAV9NXT495, AAV9NXT499, AAV9NXT501, AAV9NXT504, AAV9NXT511, AAV9NXT512, AAV9NXT514, AAV9NXT516, AAV9NXT517, AAV9NXT526, AAV9NXT527, AAV9NXT528, AAV9NXT531, AAV9NXT533, AAV9NXT534, AAV9NXT543, AAV9NXT545, AAV9NXT561, AAV9NXT563, AAV9NXT565, AAV9NXT566, AAV9NXT567, AAV9NXT575, AAV9NXT576, AAV9NXT588, AAV9NXT590, AAV9NXT591, AAV9NXT598, AAV9NXT611, AAV9NXT613, AAV9NXT662, AAV9NXT668, AAV9NXT670, AAV9NXT703, AAV9NXT704, AAV9NXT705, AAV9NXT706, AAV9NXT707, AAV9NXT709, AAV9NXT712, AAV9NXT723, AAV9NXT724, AAV9NXT725, and AAV9NXT726) that detarget the liver, showing <0.25-times of the AAV9 vector genome DNA genome copy number. The Group 9 AAV9NXT/S mutants showing loss of the capability of transduction in any tissues (42 mutants, FIG. 9) are all contained in the above 86 mutants. The 42 mutants in Group 9 are: AAV9NXT66, AAV9NXT88, AAV9NXT94, AAV9NXT113, AAV9NXT259, AAV9NXT261, AAV9NXT264, AAV9NXT265, AAV9NXT267, AAV9NXT268, AAV9NXT269, AAV9NXT270, AAV9NXT273, AAV9NXT336, AAV9NXT383, AAV9NXT384, AAV9NXT385, AAV9NXT386, AAV9NXT387, AAV9NXT388, AAV9NXT389, AAV9NXT390, AAV9NXT391, AAV9NXT442, AAV9NXT443, AAV9NXT444, AAV9NXT501, AAV9NXT514, AAV9NXT561, AAV9NXT563, AAV9NXT565, AAV9NXT567, AAV9NXT575, AAV9NXT611, AAV9NXT668, AAV9NXT670, AAV9NXT703, AAV9NXT705, AAV9NXT709, AAV9NXT712, AAV9NXT725, and AAV9NXT726. The other 46 mutants are those that can detarget the liver while retaining the ability to transduce non-hepatic tissue to varying degrees. These 46 mutants are: AAV9NXT66, AAV9NXT88, AAV9NXT94, AAV9NXT113, AAV9NXT259, AAV9NXT261, AAV9NXT264, AAV9NXT265, AAV9NXT267, AAV9NXT268, AAV9NXT269, AAV9NXT270, AAV9NXT273, AAV9NXT336, AAV9NXT383, AAV9NXT384, AAV9NXT385, AAV9NXT386, AAV9NXT387, AAV9NXT388, AAV9NXT389, AAV9NXT390, AAV9NXT391, AAV9NXT442, AAV9NXT443, AAV9NXT444, AAV9NXT501, AAV9NXT514, AAV9NXT561, AAV9NXT563, AAV9NXT565, AAV9NXT567, AAV9NXT575, AAV9NXT611, AAV9NXT668,

AAV9NXT670, AAV9NXT703, AAV9NXT705, AAV9NXT709, AAV9NXT712, AAV9NXT725 and AAV9NXT726. Among the 46 mutants, 9 mutants (AAV9NXT453, AAV9NXT456, AAV9NXT467, AAV9NXT470, AAV9NXT495, AAV9NXT533, AAV9NXT545, AAV9NXT591 and AAV9NXT704) and 9 mutants (AAV9NXT447, AAV9NXT455, AAV9NXT488, AAV9NXT526, AAV9NXT531, AAV9NXT534, AAV9NXT543, AAV9NXT662 and AAV9NXT706) belong to Group 2 and Group 5, respectively, that show one to two-thirds of overall transduction efficiency in mouse organs compared to the transduction levels that AAV9 can attain (FIG. 9). These 46 mutants, in particular the 18 mutants that belong to Group 2 and Group 5 mutants provide novel AAV9-based platforms to engineer liver-detargeting AAV vectors.

[0215] AAV9NXT/S mutants exhibiting other interesting biological features.

[0216] Brain-targeting, liver-detargeting mutants. AAV9NXT447, AAV9NXT453, AAV9NXT455, AAV9NXT456, AAV9NXT467, AAV9NXT469, AAV9NXT470, AAV9NXT488, AAV9NXT495, AAV9NXT526, AAV9NXT531, AAV9NXT533, AAV9NXT534, AAV9NXT545, AAV9NXT590, AAV9NXT591, AAV9NXT704, and AAV9NXT706 transduce the brain by 0.5-times or more than AAV9 while detargeting the liver (<0.25-times the AAV9 vector genome DNA copy number) following intravenous injection in mice.

[0217] Heart-targeting, liver-detargeting mutants. AAV9NXT447, AAV9NXT453, AAV9NXT455, AAV9NXT456, AAV9NXT467, AAV9NXT470, AAV9NXT495, AAV9NXT526, AAV9NXT533, AAV9NXT534, AAV9NXT545, AAV9NXT591, AAV9NXT704, and AAV9NXT706 transduce the heart by 0.5-times or more than AAV9 while detargeting the liver (<0.25-times the AAV9 vector genome DNA copy number) following intravenous injection in mice.

[0218] Intestine-targeting, liver-detargeting mutants. AAV9NXT447, AAV9NXT453, AAV9NXT456, AAV9NXT467, AAV9NXT470, AAV9NXT495, AAV9NXT533, AAV9NXT534, AAV9NXT543, AAV9NXT545, AAV9NXT591, AAV9NXT704 and AAV9NXT706 transduce the intestine by 0.5-times or more than AAV9 while detargeting the liver (<0.25-times the AAV9 vector genome DNA copy number) following intravenous injection in mice.

[0219] Spleen targeting, liver-detargeting mutant. AAV9NXT545 transduces the spleen (0.5-times or more than AAV9) while detargeting the liver (<0.25-times the AAV9 vector genome DNA copy number) following intravenous injection in mice.

[0220] Skeletal muscle, liver-detargeting mutants. AAV9NXT453, AAV9NXT456, AAV9NXT467, AAV9NXT470, AAV9NXT495, AAV9NXT534, AAV9NXT545, AAV9NXT591 and AAV9NXT704 transduce the skeletal muscle by 0.5-times or more than AAV9 while detargeting the liver (<0.25-times the AAV9 vector genome DNA copy number) following intravenous injection in mice.

[0221] Pancreas-targeting, liver-detargeting mutants. AAV9NXT453, AAV9NXT456,

AAV9NXT511, AAV9NXT526, AAV9NXT543, AAV9NXT704, AAV9NXT706 and AAV9NXT724 transduce the pancreas by 0.5-times or more than AAV9 while detargeting the liver (<0.25-times the AAV9 vector genome DNA copy number) following intravenous injection in mice.

[0222] Kidney-targeting, liver-detargeting mutants. AAV9NXT495, AAV9NXT533, AAV9NXT545, AAV9NXT591 and AAV9NXT704 transduce the kidney by 0.5-times or more than AAV9 while detargeting the liver (<0.25-times the AAV9 vector genome DNA copy number) following intravenous injection in mice.

[0223] Lung-targeting, liver-detargeting mutants. AAV9NXT453, AAV9NXT456, AAV9NXT467, AAV9NXT470, AAV9NXT495, AAV9NXT533, AAV9NXT545, AAV9NXT591 and AAV9NXT704 transduce the lung by 0.5-times or more than AAV9 while detargeting the liver (<0.25-times the AAV9 vector genome DNA copy number) following intravenous injection in mice.

[0224] Mutants with a prolonged half-life. AAV9NXT66, AAV9NXT563, AAV9NXT703, AAV9NXT215 and AAV9NXT330 show prolonged persistence in the blood circulation better than AAV9 by 1.5 to 2.0 times when assessed at 72 hours following intravenous injection in mice.

[0225] Mutants showing enhanced gene delivery to the brain while detargeting the liver. AAV9NXT443 and AAV9NXT444 show enhanced binding to HepG2 cells by 15 times and 30 times, respectively. These two mutants can deliver vector genomes to the brain 3.0-times and 1.2-times more than AAV9 while detargeting the liver (0.02-times and 0.01-times the AAV9 vector genome copy number) following intravenous vector injection into mice. Although these two mutants do not mediate transgene expression in the brain, the mechanism of which has yet to be determined, they may offer attractive delivery vehicle platforms for AAV capsid engineering to create novel brain-targeted, liver-detargeted AAV capsids.

[0226] New insights into amino acids in the AAV9 capsid responsible for galactose binding. Amino acid residues that constitute the galactose binding motif in the AAV9 capsid by a systematic double alanine scanning approach have been previously identified (Adachi, K. *et al.*, Nat Commun 5, 3075, (2014) and WO2018/119330). In this approach, the following 16 AAV9 double alanine mutants were identified as those that show significantly impaired binding to CHO-Lec2 cells (*i.e.*, less than 0.2-fold binding compared to the wild-type AAV9) while retaining the ability to bind CHO-Pro5 cells at levels comparable to the level of AAV9: AAV9AA270, AAV9AA272, AAV9AA380, AAV9AA382, AAV9AA440, AAV9AA446, AAV9AA450, AAV9AA464, AAV9AA468, AAV9AA470, AAV9AA474, AAV9AA484, AAV9AA500, AAV9AA502, AAV9AA514 and AAV9AA516. Therefore, the amino acids that are mutated in these AAV9AA mutants are responsible for the ability for AAV9 capsid to bind terminal galactose in the glycan chains, the AAV9 receptor, on the surface of CHO-Lec2 cells. In the current example using AAV9NXT/S mutants and CHO-Lec2 cells, the

following 30 mutants were identified as those that show significantly impaired binding to CHO-Lec2 cells (*i.e.*, less than 0.2-fold binding compared to the wild-type AAV9): AAV9NXT267, AAV9NXT268, AAV9NXT269, AAV9NXT270, AAV9NXT271, AAV9NXT272, AAV9NXT273, AAV9NXT388, AAV9NXT390, AAV9NXT446, AAV9NXT468, AAV9NXT469, AAV9NXT470, AAV9NXT472, AAV9NXT473, AAV9NXT500, AAV9NXT501, AAV9NXT502, AAV9NXT503, AAV9NXT504, AAV9NXT505, AAV9NXT512, AAV9NXT514, AAV9NXT515, AAV9NXT516, AAV9NXT517, AAV9NXT543 and AAV9NXT576 (FIG. 18). The amino acids that could be identified as those responsible for CHO-Lec2 cell binding, hence potentially galactose binding, in these two different approaches are mostly concordant. However, amino acids were also found that could be identified only by one of the two approaches with the cut-off PD value of 0.2 (FIG. 25). Interestingly, it was found that the amino acid positions 388-392 and 543-545 might also contribute to galactose binding to some degree. These positions are not a part of the previously identified as a footprint of galactose binding (Adachi, K. *et al.*, Nat Commun 5, 3075, (2014); and Shen, S *et al.*, J Biol Chem 288, 28814-28823, doi:10.1074/jbc.M113.482380 (2013)). V389 and R391 are surface exposed in the 388-392 region and K545 is surface exposed in the 543-545 region (FIG. 26). Thus, a mutation introduced in this vicinity might have a negative impact in AAV capsid-receptor interactions.

[0227] No evidence of N-linked glycosylation of the wild-type AAV9 and AAV9NXT554.An investigation on whether the wild-type AAV9 and AAV9NXT554 capsids are N-glycosylated by a biochemical method using PNGase F enzyme was performed. PNGase F is an enzyme that effectively removes almost all N-linked oligosaccharides from glycoproteins; therefore, the N-linked glycosylation status of a protein can be addressed by comparing the molecular mass of a protein treated with PNGase F with that of its untreated control. To this end, AAV9 and AAV9NXT554 virus-like particles (VLPs) were produced in HEK293 cells and purified by 2 cycles of CsCl ultracentrifugation using a standard protocol. One µg of purified VLPs was treated with of 500 units of PNGase F at 37°C for 1 h. A half of the treated materials (*i.e.*, 0.5 µg of the PNGase F-treated VLPs) and the same amount of PNGase F-untreated VLPs were separated in an 8% gel by SDS-PAGE and the protein bands were visualized by Coomassie Brilliant Blue G250 staining (Bio-Safe™ Coomassie, Bio-Rad) (FIG. 27). The result indicated that there was no detectable N-linked glycans attached to the wild-type AAV9 or AAV9NXT554 capsid. The N-linked glycosylation status of AAV9NXS266, AAV9NXT329, AAV9NXT330, and AAV9NXT454 were also analyzed by western blot following PNGase F digestion. In all of these mutants, the non-native N-linked glycosylation sequons introduced by mutagenesis are predicted to be N-glycosylated by NGlycPred; however, the result showed no evidence of appreciable N-linked

glycosylation. Although a possibility still remains that a small level of N-linked glycosylation of AAV9 and its mutant capsid proteins could occur, these observations suggest that not many of AAV9NXT/S mutants acquire N-linked glycan chains at the non-native sequons introduced by the NXT/S mutagenesis. One exception found is AAV9NXT543. This mutant showed a strong affinity to ECL Agarose and a high ECL Agarose-to-Control Agarose binding ratio that deviates substantially from the ratios of other mutants. This observation suggests that the AAV9NXT543 capsid protein might be N-glycosylated at N543 in the non-native sequon introduced by mutagenesis. Nonetheless, further studies including mass-spectrometric analysis are warranted to elucidate the effects of the NXT/S mutagenesis on N-linked glycosylation of AAV9NXT/S mutants and its biological consequences.

[0228] Conclusion. Here, a comprehensive NXT/S mutagenesis study was performed where non-native N-linked glycosylation sequons were introduced in the AAV capsids and multifaceted biological phenotypes were collected in test tubes, *in vitro* culture cells and in mice. Introduction of non-native NXT/S sequons did not appear to substantially change the status of N-linked glycosylation of the AAV capsids. However, the comprehensive NXT/S mutagenesis approach by itself, whether or not the mutagenesis can alter the glycosylation status, has led to a set of discoveries including the AAV9NXT554, a novel AAV capsid that has features very attractive for hepatic gene transfer and has an immediate commercial value for clinical translation. The features discovered in AAV9NXT554 are unique. Other discoveries are also disclosed herein that will be useful for engineering novel AAV vectors for human gene therapy.

[0229] Example 2.

[0230] AAV9NXT554 transduces hepatocytes in the liver at a 2-times enhanced efficiency compared to AAV9, and detargets non-hepatic organs, following intravenous injection in mice. This provides an attractive feature for AAV vector-mediated gene therapy that targets the liver. The potential diseases that can be benefited by this novel AAV9 capsid mutant include, hemophilia, inborn errors of metabolism, viral hepatitis, liver fibrosis, a number of diseases for which the liver serves as a factory of therapeutic molecules including antibodies against pathogens, and autoimmune diseases where the liver exerts its function as a tolerogenic organ.

[0231] AAV9NXT253 transduces the brain 2-times more than AAV9 following intravenous injection in mice. This provides an attractive feature for AAV vector-mediated gene therapy for the central nervous system. There are a number of diseases that affect the central nervous system that can be treated by AAV vector-mediated gene therapy.

[0232] AAV9NXT330 transduces the testis 1.6-times more than AAV9 following intravenous injection in mice. This provides an attractive feature for AAV vector-mediated gene delivery to the

testis. The potential diseases and conditions that can be benefited by this novel AAV9 capsid mutant include infertility due to genetic causes and cancer treatment that impair spermatogenesis such as Leydig cell failure.

[0233] AAV9N14T transduces various organs including the brain, liver, heart, intestine, spleen, skeletal muscle, pancreas, kidney, testis and lung, better than AAV9 by up to 2 times following intravenous injection in mice. This provides an attractive feature for AAV vector-mediated systemic gene delivery.

[0234] Liver-detargeting AAV9NXT/S mutants. A set of AAV9 mutants were discovered that detarget the liver following intravenous injection in mice. These mutants offer attractive AAV-based gene delivery vehicle platforms for AAV capsid engineering to create novel AAV capsids that detarget the liver. The necessity of liver detargeting has become critical in particular when therapy requires high-dose injection of an AAV vector. In human clinical trials, high-dose systemic injection of AAV9 or AAV8 vector for the treatment of spinal muscular atrophy (SMA) type 1 and X-linked myotubular myopathy (XLMTM) resulted in acute and subacute hepatotoxicity leading to the death of at least three patients in the XLMTM trial (Mendell, *et al., Mol Ther* 29, 464-488, 2021; and Chand, *et al., J Hepatol* 74, 560-566, 2021). In addition, the risk of the development of liver cancer due to AAV vector-mediated insertional mutagenesis remains a concern (Nguyen, Nat Biotechnol 39, 47-55, 2021; Dalwadi, *et al.*, *Mol Ther,* 2021; La Bella, *et al., Gut* 69, 737-747, 2020; and Chandler, *et al., J Clin Invest* 125, 870-880, 2015).

[0235] AAV9NXT/S mutants that may yield titers higher than the wild-type AAV9 include those that have been identified by AAV Barcode-Seq as AAV9NXT/S mutants that may yield titers higher than that of the wild-type AAV9. Some of these mutants have been validated by producing individual mutant vectors in HEK293 cells. AAV9NXT14 and AAV9NXT35 appear to produce higher titers than that of the wild-type AAV9 by up to 1.5 times.

[0236] There are other AAV9NXT/S mutants that show novel and interesting biological properties that are different from the parental AAV9 capsid (wild type).

[0237] (viii) Closing Paragraphs. The nucleic acid and amino acid sequences provided herein are shown using letter abbreviations for nucleotide bases and amino acid residues, as defined in 37 C.F.R. §1.831-1.835 and set forth in WIPO Standard ST.26 (implemented on July 1, 2022). Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included in embodiments where it would be appropriate.

[0238] Variants of the sequences disclosed and referenced herein are also included. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological activity can be found using computer programs well known in the art, such

as DNASTAR™ (Madison, Wisconsin) software. Preferably, amino acid changes in the protein variants disclosed herein are conservative amino acid changes, *i.e.*, substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains.

[0239] In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and generally can be made without altering a biological activity of a resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, *e.g.*, Watson *et al*. Molecular Biology of the Gene, 4th Edition, 1987, The Benjamin/Cummings Pub. Co., p. 224). Naturally occurring amino acids are generally divided into conservative substitution families as follows: Group 1: Alanine (Ala), Glycine (Gly), Serine (Ser), and Threonine (Thr); Group 2: (acidic): Aspartic acid (Asp), and Glutamic acid (Glu); Group 3: (acidic; also classified as polar, negatively charged residues and their amides): Asparagine (Asn), Glutamine (Gln), Asp, and Glu; Group 4: Gln and Asn; Group 5: (basic; also classified as polar, positively charged residues): Arginine (Arg), Lysine (Lys), and Histidine (His); Group 6 (large aliphatic, nonpolar residues): Isoleucine (Ile), Leucine (Leu), Methionine (Met), Valine (Val) and Cysteine (Cys); Group 7 (uncharged polar): Tyrosine (Tyr), Gly, Asn, Gln, Cys, Ser, and Thr; Group 8 (large aromatic residues): Phenylalanine (Phe), Tryptophan (Trp), and Tyr; Group 9 (non-polar): Proline (Pro), Ala, Val, Leu, Ile, Phe, Met, and Trp; Group 11 (aliphatic): Gly, Ala, Val, Leu, and Ile; Group 10 (small aliphatic, nonpolar or slightly polar residues): Ala, Ser, Thr, Pro, and Gly; and Group 12 (sulfur-containing): Met and Cys. Additional information can be found in Creighton (1984) Proteins, W.H. Freeman and Company.

[0240] In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, J. Mol. Biol. 157(1), 105-32). Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are: Ile (+4.5); Val (+4.2); Leu (+3.8); Phe (+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (−0.4); Thr (−0.7); Ser (−0.8); Trp (−0.9); Tyr (−1.3); Pro (−1.6); His (−3.2); Glutamate (−3.5); Gln (−3.5); aspartate (−3.5); Asn (−3.5); Lys (−3.9); and Arg (−4.5).

[0241] It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.*, still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those within ±1

are particularly preferred, and those within ±0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity.

[0242] As detailed in US 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: Arg (+3.0); Lys (+3.0); aspartate (+3.0±1); glutamate (+3.0±1); Ser (+0.3); Asn (+0.2); Gln (+0.2); Gly (0); Thr (−0.4); Pro (−0.5±1); Ala (−0.5); His (−0.5); Cys (−1.0); Met (−1.3); Val (−1.5); Leu (−1.8); Ile (−1.8); Tyr (−2.3); Phe (−2.5); Trp (−3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

[0243] As outlined above, amino acid substitutions may be based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. As indicated elsewhere, variants of gene sequences can include codon optimized variants, sequence polymorphisms, splice variants, and/or mutations that do not affect the function of an encoded product to a statistically-significant degree.

[0244] Variants of the protein, nucleic acid, and gene sequences disclosed herein also include sequences with at least 70% sequence identity, 80% sequence identity, 85% sequence, 90% sequence identity, 95% sequence identity, 96% sequence identity, 97% sequence identity, 98% sequence identity, or 99% sequence identity to the protein, nucleic acid, or gene sequences disclosed herein.

[0245] "% sequence identity" refers to a relationship between two or more sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between protein, nucleic acid, or gene sequences as determined by the match between strings of such sequences. "Identity" (often referred to as "similarity") can be readily calculated by known methods, including those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1994); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular Biology (Von Heijne, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Oxford University Press, NY (1992). Methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments

and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR, Inc., Madison, Wisconsin). Multiple alignment of the sequences can also be performed using the Clustal method of alignment (Higgins and Sharp CABIOS, 5, 151-153 (1989) with default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Relevant programs also include the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin); BLASTP, BLASTN, BLASTX (Altschul, *et al.*, J. Mol. Biol. 215:403-410 (1990); DNASTAR (DNASTAR, Inc., Madison, Wisconsin); and the FASTA program incorporating the Smith-Waterman algorithm (Pearson, Comput. Methods Genome Res., [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Publisher: Plenum, New York, N.Y.. Within the context of this disclosure, it will be understood that where sequence analysis software is used for analysis, the results of the analysis are based on the "default values" of the program referenced. As used herein "default values" will mean any set of values or parameters, which originally load with the software when first initialized.

[0246] Variants also include nucleic acid molecules that hybridize under stringent hybridization conditions to a sequence disclosed herein and provide the same function as the reference sequence. Exemplary stringent hybridization conditions include an overnight incubation at 42 °C in a solution including 50% formamide, 5XSSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5XDenhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1XSSC at 50 °C. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, moderately high stringency conditions include an overnight incubation at 37°C in a solution including 6XSSPE (20XSSPE=3M NaCl; 0.2M NaH2PO4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50 °C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (*e.g.,* 5XSSC). Variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

[0247] Unless otherwise indicated, the practice of the present disclosure can employ conventional

techniques of immunology, molecular biology, microbiology, cell biology and recombinant DNA. These methods are described in the following publications. See, *e.g.*, Sambrook, *et al.* Molecular Cloning: A Laboratory Manual, 2nd Edition (1989); F. M. Ausubel, *et al.* eds., Current Protocols in Molecular Biology, (1987); the series Methods IN Enzymology (Academic Press, Inc.); M. MacPherson, *et al.*, PCR: A Practical Approach, IRL Press at Oxford University Press (1991); MacPherson *et al.*, eds. PCR 2: Practical Approach, (1995); Harlow and Lane, eds. Antibodies, A Laboratory Manual, (1988); and R. I. Freshney, ed. Animal Cell Culture (1987).

[0248] As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms "include" or "including" should be interpreted to recite: "comprise, consist of, or consist essentially of." The transition term "comprise" or "comprises" means has, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase "consisting of" excludes any element, step, ingredient or component not specified. The transition phrase "consisting essentially of" limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. A material effect would cause a statistically significant increase in gene delivery to a desired tissue or cell type, as described herein.

[0249] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, the term "about" has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, *i.e.* denoting somewhat more or somewhat less than the stated value or range, to within a range of ±20% of the stated value; ±19% of the stated value; ±18% of the stated value; ±17% of the stated value; ±16% of the stated value; ±15% of the stated value; ±14% of the stated value; ±13% of the stated value; ±12% of the stated value; ±11% of the stated value; ±10% of the stated value; ±9% of the stated value; ±8% of the stated value; ±7% of the stated value; ±6% of the stated value; ±5% of the stated value; ±4% of the stated value; ±3% of the stated value; ±2% of

the stated value; or ±1% of the stated value.

[0250] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0251] The terms "a," "an," "the" and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. 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 invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0252] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0253] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention 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 invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0254] Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

[0255] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

[0256] The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0257] Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Eds. Attwood *et al.*, Oxford University Press, Oxford, 2006).

CLAIMS

What is claimed is:

1. An AAV9 capsid mutant of a wild-type AAV9 capsid having the sequence as set forth in SEQ ID NO: 1, wherein the AAV9 capsid mutant comprises a mutation or set of mutations selected from: S16T, Q37T, R43T, L59T, A68T, Y90T, A96T, E106N, D107N, G115T, A136N, E147N, P153N, D154N, G160N, G174T, G174N, G177N, T179N, A194N, V198N, S200N, M203N, E216T, G217T, P250N/Y252T, T251N/N253T, Y252N/N254T, H255T, L256T, H255N/Y257T, L256N/K258T, Y257N/Q259T, K258N/I260T, Q259N/S261T, I260N/N262T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T,G266N/S268T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, D271N/A273T, Y274T, A273N/F275T, Y274N/G276T, S278N/P280T, R288N/H290T, H290N/H292T, E324N, V325N/D327T, T326N/N328T, D327N/N329T, G330T, V331T, G330N/K332T, V331N, K332N/I334T, T333N/A335T, 334N/N336T, A335N/N337T, L338T, E361N/C363T, G362N/L364T, C363N/P365T, L364N/P366T, P365N/F367T, P366N/P368T, D370N/F372T, F372N/I374T, Q376N/G378T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, P438N/I440T, L439N/D441T, I440N/Q442T, D441N/Y443T, Q442N/L444T, Y443N/Y445T, L444N/Y446T, Y445N/ L447T, Y446N/S448T, L447N/K449T, S448N, K449N/I451T, T450N/N452T, I451N/G453T, S454T, G453N/G455T, S454N/Q456T, G455N/N457T, Q456N/Q458T, Q459T, Q458N, Q459N/L461T, T460N/K462T, L461N/F463T, K462N/S464T, F463N/V465T, S464N/A466T, V465N/G467T, A466N/P468T, G467N/S469T, P468N/N470T, S469N/M471T, A472T, M471N/V473T, A472N/Q474T, V473N/G475T, R485N/Q487T, R488N/S490T, T491N/V493T, T492N, V493N/Q495T, T494N/N496T, Q495N/N497T, N498T, S499T, E500T, S499N/F501T, E500N/A502T, F501N/W503T, A502N/P504T, W503N/G505T, P504N/A506T, G505N/S507T, A510N/N512T, L511N/G513T, R514T, G513N/N515T, R514N/S516T, L517T, S516N/M518T, L517N/N519T, S526N/K528T, H527N/E529T,K528N/G530T, E529N/E531T, G530N/D532T, E531N/R533T, D532N/F534T, R533N/F535T, F534N/P536T, F535N/L537T, F543N/K545T, G544N/Q546T, K545N/G547T, Q546N, G547N/G549T, T548N/R550T, G549N/D551T, R550N/N552T, D551N/V553T, D554T, V553N/A555T, D554N/D556T, A555N/K557T, D556N/V558T, K557N/M559T, V558N/I560T, M559N, I560N/N562T, T561N/E563T, E564T, E563N/E565T, E564N/I566T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q579N/A581T, V580N, A581N/N583T, T582N/H584T, Q585T, H584N/S586T, Q585N/A587T, S586N/Q588T, A587N/A589T, Q588N/Q590T, A589N/A591T, Q590N/Q592T, A591N, Q592N/G594T, T593N/W595T, G594N/V596T, W595N/Q597T, V596N/N598T, Q597N/Q599T,

G600T, D611N/Y613T, V612N/L614T, Y613N/Q615T, L614N/G616T, Q615N/P617T, A656N/P658T, D657N/P659T, P658N, P659N/A661T, T660N/F662T, A661N/N663T, F662N/K664T, D665T, K664N/K666T, D665N/L667T, K666N/N668T, L667N/S669T, F670T, S669N/I671T, F670N, I671N/Q673T, T672N/Y674T, Q673N/S675T, Y674N, Y701N/S703T, T702N/N704T, S703N/Y705T, Y706T, Y705N/K707T, Y706N/S708T, K707N/N709T, S708N/N710T, V711T, E712T, V711N/F713T, E712N/A714T, F713N/V715T, A714N/N716T, V715N, E718T, T717N/G719T, E718N/V720T, G719N/Y721T, V720N/S722T, Y721N/E723T, S722N/P724T, E723N/R725T, P724N/P726T, R725N/I727T, P726N/G728T, I727N, G728N/R730T, T729N/Y731T, R730N/L732T, and Y731N.

2. A method comprising expressing an AAV vector comprising the AAV9 capsid mutant of claim 1.

3. A method of producing a viral vector with an administration benefit comprising expressing the viral vector with the AAV9 capsid mutant of claim 1, thereby producing the viral vector with the administration benefit, wherein

the administration benefit is to detarget the liver and the AAV9 capsid mutant of claim 1 has mutations L59T, Y90T, A96T, G115T, L256T, L256N/K258T, Q259N/S261T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, Y274T, A273N/F275T, L338T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, Q442N/L444T, V473N/G475T, S499N/F501T, F501N/W503T, P504N/A506T, R514T, R514N/S516T, S516N/M518T, L517N/N519T, H527N/E529T, K528N/G530T, T561N/E563T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q588N/Q590T, G600T, D611N/Y613T, Y613N/Q615T, F662N/K664T, F670T, F670N, Y705N/K707T, K707N/N709T, V711T, E712N/A714T, E723N/R725T, R725N/I727T, P726N/G728T, A68T, G217T, Y443N/Y445T, L444N/Y446T, L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, L511N/G513T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, E563N/E565T, Q590N/Q592T, A591N, S703N/Y705T, Y706T, Y706N/S708T, P724N/P726T, F543N/K545T, or K545N/G547T;

the administration benefit is targeted gene delivery to the lungs while detargeting the liver and the AAV9 capsid mutant of claim 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;

the administration benefit is targeted gene delivery to the skeletal muscle while detargeting the liver and the AAV9 capsid mutant of claim 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, F534N/P536T, K545N/G547T, A591N, or Y706T;

the administration benefit is targeted gene delivery to the pancreas while detargeting the liver and the AAV9 capsid mutant of claim 1 has mutations G453N/G455T, Q456N/Q458T, L511N/G513T, S526N/K528T, F543N/K545T, Y706T, Y706N/S708T, or P724N/P726T;
the administration benefit is targeted gene delivery to the kidney while detargeting the liver and the AAV9 capsid mutant of claim 1 has mutations Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;
the administration benefit is targeted gene delivery to the brain while detargeting the liver and the AAV9 capsid mutant of claim 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, K545N/G547T, Q590N/Q592T, A591N, Y706T, Y706N/S708T, G467N/S469T, Y443N/Y445T, or L444N/Y446T;
the administration benefit is targeted gene delivery to the heart while detargeting the liver and the AAV9 capsid mutant of claim 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, S526N/K528T, R533N/F535T, F534N/P536T, K545N/G547T, A591N, Y706T, or Y706N/S708T;
the administration benefit is targeted gene delivery to the intestine while detargeting the liver and the AAV9 capsid mutant of claim 1 has mutations L447N/K449T, G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, F534N/P536T, F543N/K545T, K545N/G547T, A591N, Y706T, or Y706N/S708T;
the administration benefit is targeted gene delivery to the spleen while detargeting the liver and the AAV9 capsid mutant of claim 1 has mutations K545N/G547T;
the administration benefit is targeted gene delivery to the testis and the AAV9 capsid mutant of claim 1 has mutations G330N/K332T or N14T;
the administration benefit is targeted gene delivery to the intestine and pancreas and the AAV9 capsid mutant of claim 1 has mutation F543N/K545T;
the administration benefit is targeted gene delivery to the brain and the AAV9 capsid mutant of claim 1 has mutation H255T;
the administration benefit is targeted hepatic gene delivery and the AAV9 capsid mutant of claim 1 has mutations D554N/D556T; or
the administration benefit is prolonged half-life and the AAV9 capsid mutant of claim 1 has mutations A68T, E563N/E565T, S703N/Y705T, G217T, or G330N/K332T.

4. A method of providing targeted gene expression in a selected cell type in a subject comprising:
administering a composition comprising a viral vector associated with a AAV9 capsid mutant of

claim 1 to the subject thereby providing targeted gene expression in the selected cell type in the subject, wherein

the selected cell type is in the lungs and the AAV9 capsid mutant of claim 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;

the selected cell type is in the skeletal muscle and the AAV9 capsid mutant of claim 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, F534N/P536T, K545N/G547T, A591N, or Y706T;

the selected cell type is in the pancreas and the AAV9 capsid mutant of claim 1 has mutations G453N/G455T, Q456N/Q458T, L511N/G513T, S526N/K528T, F543N/K545T, Y706T, Y706N/S708T, or P724N/P726T;

the selected cell type is in the kidney and the AAV9 capsid mutant of claim 1 has mutations Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;

the selected cell type is in the brain and the AAV9 capsid mutant of claim 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, K545N/G547T, Q590N/Q592T, A591N, Y706T, Y706N/S708T, G467N/S469T, Y443N/Y445T, or L444N/Y446T;

the selected cell type is in the heart and the AAV9 capsid mutant of claim 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, S526N/K528T, R533N/F535T, F534N/P536T, K545N/G547T, A591N, Y706T, or Y706N/S708T;

the selected cell type is in the intestine and the AAV9 capsid mutant of claim 1 has mutations L447N/K449T, G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, F534N/P536T, F543N/K545T, K545N/G547T, A591N, Y706T, or Y706N/S708T;

the selected cell type is in the spleen and the AAV9 capsid mutant of claim 1 has mutations K545N/G547T;

the selected cell type is in the testis and the AAV9 capsid mutant of claim 1 has mutations G330N/K332T or N14T;

the selected cell type is in the intestine and pancreas and the AAV9 capsid mutant of claim 1 has mutations F543N/K545T

the selected cell type is in the brain and the AAV9 capsid mutant of claim 1 has mutation H255T;

the selected cell type is in the liver and the AAV9 capsid mutant of claim 1 has mutations

D554N/D556T.

5. The method of claim 4, wherein the viral vector encodes a gene product.

6. The method of claim 5, wherein the gene product comprises a therapeutic molecule, an enzyme, a cytokine, a hormone, a receptor, a receptor ligand, an antibody, a reporter gene/protein, or an antisense oligonucleotide.

7. The method of claim 4, wherein the subject has a disease.

8. A method of providing a viral vector-based therapy with reduced hepatic uptake to a subject in need thereof, the method comprising administering a composition comprising a viral vector associated with a AAV9 capsid mutant of claim 1 to the subject in need thereof thereby providing the viral vector-based therapy with reduced hepatic uptake to the subject in need thereof, wherein

the AAV9 capsid mutant of claim 1 has mutations L59T, Y90T, A96T, G115T, L256T, L256N/K258T, Q259N/S261T, S261N/S263T, S263N/S265T, T264N/G266T, S265N/G267T, G266N, G267N/S269T, S268N/N270T, S269N/D271T, N272T, Y274T, A273N/F275T, L338T, G385T, D384N/S386T, G385N/Q387T, S386N/A388T, Q387N/V389T, A388N/G390T, V389N/R391T, G390N/S392T, R391N/S393T, Q442N/L444T, V473N/G475T, S499N/F501T, F501N/W503T, P504N/A506T, R514T, R514N/S516T, S516N/M518T, L517N/N519T, H527N/E529T, K528N/G530T, T561N/E563T, E565N/K567T, I566N, K567N, E575N/Y577T, S576N/G578T, Q588N/Q590T, G600T, D611N/Y613T, Y613N/Q615T, F662N/K664T, F670T, F670N, Y705N/K707T, K707N/N709T, V711T, E712N/A714T, E723N/R725T, R725N/I727T, P726N/G728T, A68T, G217T, Y443N/Y445T, L444N/Y446T, L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, L511N/G513T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, E563N/E565T, Q590N/Q592T, A591N, S703N/Y705T, Y706T, Y706N/S708T, P724N/P726T, F543N/K545T, or K545N/G547T.

9. The method of claim 8, wherein the method further comprises providing targeted gene expression in a selected cell type in the subject in need thereof, wherein

the selected cell type is in the lungs and the AAV9 capsid mutant of claim 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;

the selected cell type is in the skeletal muscle and the AAV9 capsid mutant of claim 1 has mutations G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, F534N/P536T, K545N/G547T, A591N, or Y706T;

the selected cell type is in the pancreas and the AAV9 capsid mutant of claim 1 has mutations

G453N/G455T, Q456N/Q458T, L511N/G513T, S526N/K528T, F543N/K545T, Y706T, Y706N/S708T, or P724N/P726T;
the selected cell type is in the kidney and the AAV9 capsid mutant of claim 1 has mutations Q495N/N497T, R533N/F535T, K545N/G547T, A591N, or Y706T;
the selected cell type is in the brain and the AAV9 capsid mutant of claim 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, S469N/M471T, A472T, R488N/S490T, Q495N/N497T, S526N/K528T, E531N/R533T, R533N/F535T, F534N/P536T, K545N/G547T, Q590N/Q592T, A591N, Y706T, Y706N/S708T, G467N/S469T, Y443N/Y445T, or L444N/Y446T;
the selected cell type is in the heart and the AAV9 capsid mutant of claim 1 has mutations L447N/K449T, G453N/G455T, G455N/N457T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, S526N/K528T, R533N/F535T, F534N/P536T, K545N/G547T, A591N, Y706T, or Y706N/S708T;
the selected cell type is in the intestines and the AAV9 capsid mutant of claim 1 has mutations L447N/K449T, G453N/G455T, Q456N/Q458T, G467N/S469T, A472T, Q495N/N497T, R533N/F535T, F534N/P536T, F543N/K545T, K545N/G547T, A591N, Y706T, or Y706N/S708T; or
the selected cell type is in the spleen and the AAV9 capsid mutant of claim 1 has mutations K545N/G547T.

10. The method of claim 8, wherein the viral vector encodes a therapeutic molecule.

11. The method of claim 10, wherein the therapeutic molecule is a protein or RNA.

12. A method of providing a viral vector-based therapy with extended *in vivo* half-life to a subject in need thereof, the method comprising administering a composition comprising a viral vector associated with the AAV9 capsid mutant of claim 1 to the subject in need thereof thereby providing the viral vector-based therapy with extended *in vivo* half-life to the subject in need thereof, wherein

the AAV9 capsid mutant of claim 1 has mutations A68T, E563N/E565T, S703N/Y705T, G217T, or G330N/K332T.

13. A composition comprising the AAV9 capsid mutant of claim 1 and a carrier.

14. The composition of claim 13, wherein the carrier is a pharmaceutically acceptable carrier.

15. A composition comprising a viral vector associated with the AAV9 capsid mutant of claim 1.

16. A kit comprising the AAV9 capsid mutant of claim 1, the composition of claim 13, and/or the composition of claim 15.

FIG. 1

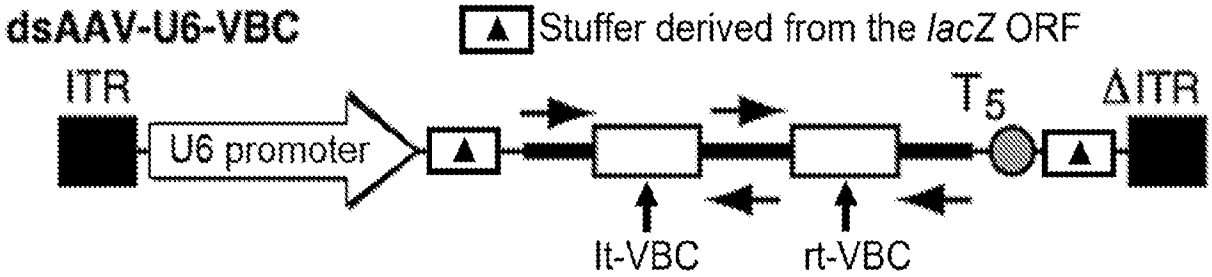


FIG. 2

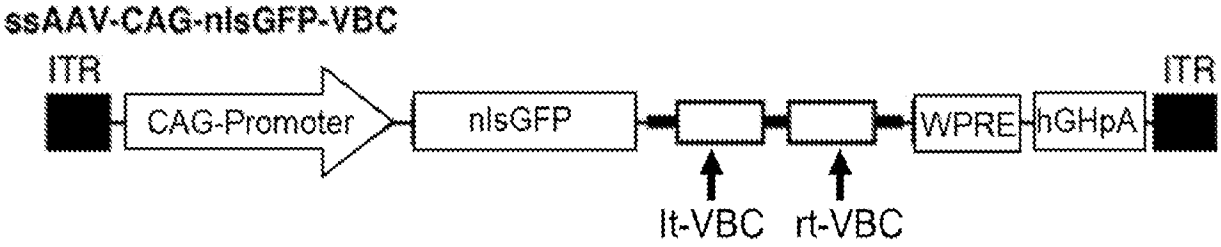


FIG. 3

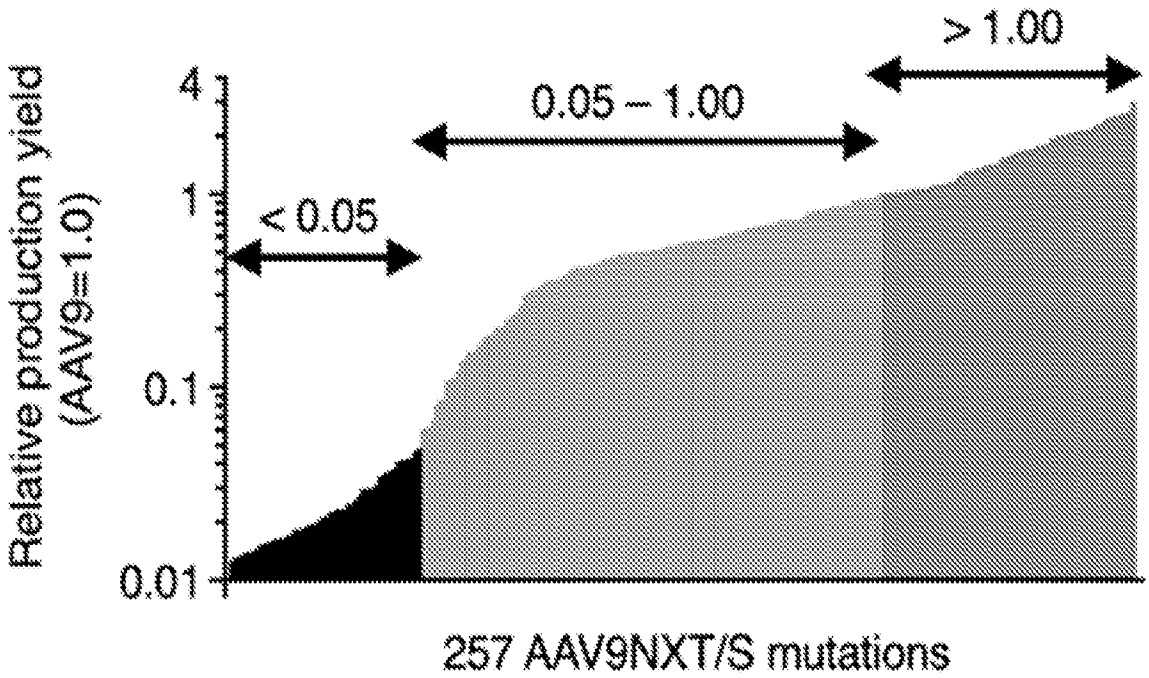


FIG. 4A

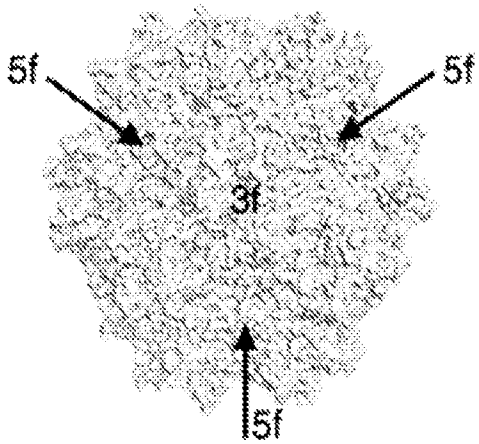


FIG. 4B

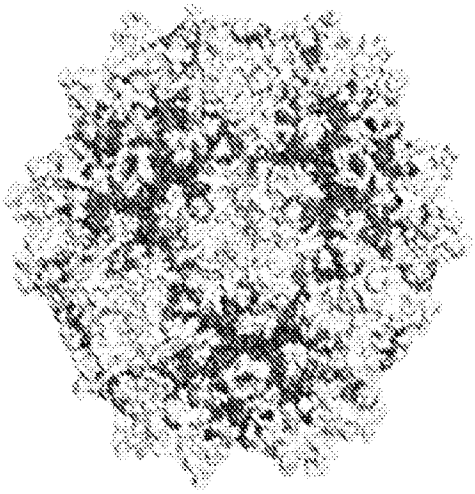


FIG. 4C

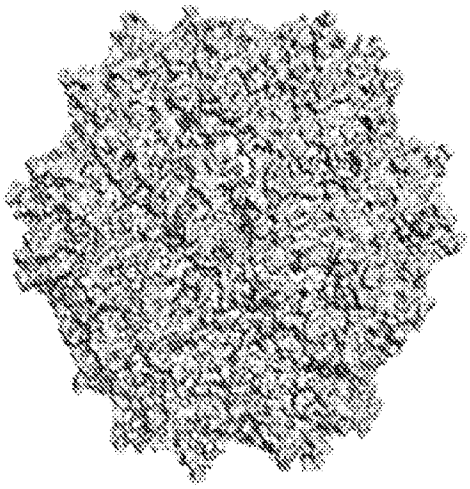


FIG. 5

Vector yields of AAV9NXT/S mutants				
AAV9NXT/S mutants	Vector yield[a]		AAV9NXT/S mutants	Vector yield[a]
AAV9NXT14	3.02		AAV9NXT455	1.43
AAV9NXT35	2.60		AAV9NXT456	1.43
AAV9NXT41	2.58		AAV9NXT457	1.41
AAV9NXT57	2.51		AAV9NXT458	1.40
AAV9NXT66	2.40		AAV9NXT459	1.37
AAV9NXT88	2.13		AAV9NXT466	1.35
AAV9NXT94	2.11		AAV9NXT467	1.33
AAV9NXT106	2.18		AAV9NXT468	1.33
AAV9NXS107	2.05		AAV9NXT488	1.32
AAV9NXT269	2.05		AAV9NXT491	1.26
AAV9NXT327	2.55		AAV9NXT496	1.26
AAV9NXT505	2.08		AAV9NXT497	1.20
AAV9NXT510	2.15		AAV9NXT514	1.17
AAV9NXT512	2.28		AAV9NXT517	1.14
AAV9NXT513	2.48		AAV9NXT528	1.13
AAV9NXT515	2.13		AAV9NXT529	1.13
AAV9NXT526	2.01		AAV9NXT533	1.12
AAV9NXT585	2.01		AAV9NXT535	1.11
AAV9NXT588	2.59		AAV9NXT544	1.10
AAV9NXT590	2.24		AAV9NXT545	1.07
AAV9NXT592	2.30		AAV9NXT546	1.07
AAV9NXT593	2.65		AAV9NXT547	1.07
AAV9NXT113	1.95		AAV9NXT579	1.06
AAV9NXT136	1.95		AAV9NXT580	1.06
AAV9NXS153	1.83		AAV9NXT581	1.05
AAV9NXT174	1.80		AAV9NXT582	1.04
AAV9NXS179	1.79		AAV9NXT583	1.04
AAV9NXS194	1.76		AAV9NXT584	1.04
AAV9NXT259	1.72		AAV9NXT586	1.03
AAV9NXT263	1.71		AAV9NXT587	1.02
AAV9NXT264	1.70		AAV9NXT589	1.02
AAV9NXT271	1.68		AAV9NXT591	1.02
AAV9NXT328	1.67		AAV9NXT723	1.01
AAV9NXT329	1.62			
AAV9NXT333	1.62			
AAV9NXT450	1.57			
AAV9NXT451	1.55			
AAV9NXT452	1.54			
AAV9NXT454	1.44			

[a] Vector yields relative to the yield of the AAV9 determined by AAV Barcode-Seq. Those showing the values of >1.00 are shown. The first 32 mutants are those showing >2.00

FIG. 6

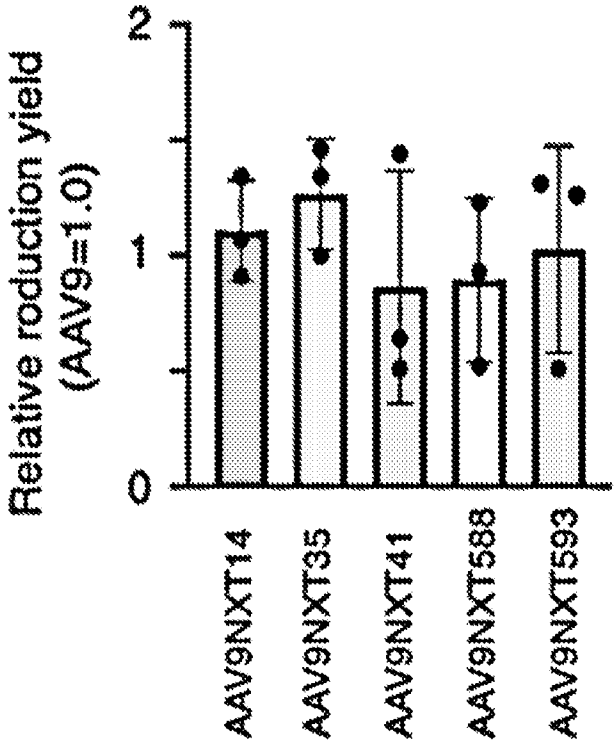

Relative roduction yield
(AAV9=1.0)
2
1
0
AAV9NXT14
AAV9NXT35
AAV9NXT41
AAV9NXT588
AAV9NXT593

FIG. 7

Relative transduction efficiency of AAV9NXT/S mutants in mouse tissues following intravenous administration										
AAV capsid	Relative transduction efficiency (PD values determined by AAV RNA Barcode-Seq)									
	Brain	Liver	Heart	Intestine	Spleen	Muscle	Pancreas	Kidney	Testis	Lung
AAV9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAV9NXS107	0.73	0.77	0.87	0.67	0.43	0.70	0.70	0.64	0.65	0.51
AAV9NXS147	0.26	0.38	0.39	0.28	0.37	0.42	0.30	0.42	0.50	0.45
AAV9NXS153	1.12	1.18	1.11	1.19	1.18	1.21	1.37	1.13	1.10	1.12
AAV9NXS154	0.79	0.69	0.84	0.72	0.74	0.75	0.29	0.70	0.84	0.82
AAV9NXS160	0.67	0.70	0.63	0.53	0.60	0.55	0.36	0.74	0.64	0.68
AAV9NXS179	0.83	0.88	0.99	0.89	0.84	1.12	1.18	0.86	0.82	0.94
AAV9NXS194	0.97	1.05	1.06	1.06	1.09	1.03	1.45	1.03	0.91	1.03
AAV9NXS198	0.82	0.81	0.76	0.82	0.81	0.85	0.66	0.84	0.77	0.84
AAV9NXS266	0.14	0.01	0.17	0.09	0.04	0.14	0.12	0.07	0.03	0.13
AAV9NXT14	0.58	0.59	0.68	0.44	0.57	0.70	0.70	0.67	0.64	0.60
AAV9NXT35	0.95	0.68	0.93	0.55	0.61	0.88	0.57	0.77	0.74	0.74
AAV9NXT41	0.65	0.72	0.70	0.55	0.29	0.65	0.32	0.51	0.57	0.36
AAV9NXT57	0.24	0.19	0.43	0.17	0.04	0.39	0.20	0.13	0.24	0.05
AAV9NXT66	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
AAV9NXT88	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
AAV9NXT94	0.04	0.02	0.10	0.03	0.01	0.05	0.01	0.02	0.02	0.01
AAV9NXT106	0.71	0.77	0.69	0.79	0.62	0.71	0.52	0.64	0.66	0.61
AAV9NXT113	0.01	0.01	0.02	0.01	0.01	0.04	0.01	0.01	0.01	0.01
AAV9NXT136	0.84	0.74	0.84	0.80	0.67	0.86	0.81	0.80	0.84	0.82
AAV9NXT172	0.69	0.72	0.76	0.68	0.74	0.85	0.74	0.74	0.78	0.76
AAV9NXT174	0.59	0.66	0.74	0.61	0.71	0.67	0.53	0.65	0.76	0.72
AAV9NXT177	0.87	0.81	0.89	0.54	0.86	0.89	0.32	0.96	0.83	0.91
AAV9NXT200	0.82	0.86	0.96	0.68	0.79	0.87	0.66	0.89	0.88	0.86
AAV9NXT214	0.38	0.50	0.45	0.51	0.39	0.56	0.24	0.42	0.43	0.35
AAV9NXT215	0.08	0.10	0.12	0.12	0.08	0.10	0.27	0.12	0.09	0.08
AAV9NXT253	2.11	0.66	1.36	1.11	0.86	1.24	0.52	1.13	0.65	1.16
AAV9NXT254	0.20	0.17	0.25	0.17	0.16	0.18	0.01	0.19	0.18	0.22
AAV9NXT256	0.14	0.13	0.25	0.18	0.17	0.23	0.37	0.17	0.14	0.23
AAV9NXT259	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

SUBSTITUTE SHEET (RULE 26)

FIG. 7 cont'd

AAV9NXT261	0.02	0.01	0.05	0.01	0.01	0.04	0.07	0.01	0.01	0.02
AAV9NXT263	0.41	0.02	0.30	0.23	0.08	0.24	0.13	0.13	0.06	0.17
AAV9NXT264	0.02	0.00	0.06	0.02	0.01	0.06	0.00	0.01	0.01	0.02
AAV9NXT265	0.01	0.00	0.02	0.01	0.01	0.02	0.04	0.01	0.01	0.01
AAV9NXT267	0.02	0.01	0.05	0.01	0.01	0.04	0.01	0.01	0.01	0.01
AAV9NXT268	0.01	0.00	0.04	0.01	0.01	0.04	0.03	0.01	0.00	0.01
AAV9NXT269	0.01	0.00	0.02	0.00	0.00	0.01	0.00	0.01	0.00	0.01
AAV9NXT270	0.02	0.01	0.04	0.01	0.01	0.02	0.01	0.02	0.01	0.01
AAV9NXT271	0.35	1.35	0.47	0.25	0.50	0.63	0.18	0.64	0.58	0.57
AAV9NXT272	0.35	0.02	0.29	0.11	0.05	0.21	0.00	0.11	0.05	0.11
AAV9NXT273	0.02	0.01	0.02	0.03	0.01	0.02	0.01	0.01	0.01	0.01
AAV9NXT325	0.10	0.17	0.30	0.13	0.13	0.14	0.18	0.17	0.20	0.19
AAV9NXT326	0.66	0.74	0.79	0.83	0.98	0.68	0.65	0.80	0.95	0.83
AAV9NXT327	0.15	0.20	0.24	0.14	0.13	0.15	0.09	0.16	0.24	0.17
AAV9NXT328	0.95	0.80	1.05	0.91	0.99	0.97	0.81	1.03	1.10	1.07
AAV9NXT329	0.85	1.06	0.89	0.77	0.67	0.90	0.78	0.86	0.84	0.80
AAV9NXT330	1.54	1.52	1.57	1.70	2.00	1.62	2.02	1.81	1.52	1.85
AAV9NXT331	0.84	0.89	0.86	0.96	0.93	0.94	0.65	0.88	0.88	0.91
AAV9NXT333	0.36	0.41	0.52	0.42	0.43	0.44	0.67	0.47	0.52	0.48
AAV9NXT334	0.14	0.23	0.28	0.18	0.24	0.20	0.00	0.28	0.31	0.29
AAV9NXT335	0.66	0.92	0.78	0.79	0.76	0.91	0.76	0.84	0.76	0.82
AAV9NXT336	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.02	0.01	0.02
AAV9NXT361	0.65	0.68	0.66	0.56	0.55	0.67	0.33	0.68	0.65	0.68
AAV9NXT383	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AAV9NXT384	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01
AAV9NXT385	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
AAV9NXT386	0.01	0.01	0.03	0.01	0.01	0.04	0.01	0.01	0.01	0.01
AAV9NXT387	0.02	0.01	0.03	0.02	0.01	0.02	0.00	0.02	0.01	0.02
AAV9NXT388	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
AAV9NXT389	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
AAV9NXT390	0.02	0.01	0.02	0.04	0.02	0.02	0.02	0.02	0.02	0.02
AAV9NXT391	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
AAV9NXT439	0.53	0.58	0.61	0.68	0.62	0.53	0.99	0.54	0.62	0.53

FIG. 7 cont'd

AAV9NXT440	0.36	0.44	0.42	0.34	0.48	0.55	0.44	0.43	0.44	0.47
AAV9NXT442	0.01	0.09	0.03	0.02	0.02	0.00	0.00	0.00	0.04	0.00
AAV9NXT443	0.03	0.02	0.03	0.02	0.03	0.03	0.01	0.04	0.02	0.03
AAV9NXT444	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00
AAV9NXT446	1.31	0.40	0.94	0.83	0.48	1.00	0.33	0.56	0.33	0.69
AAV9NXT447	1.08	0.10	0.55	0.60	0.23	0.38	0.18	0.38	0.14	0.37
AAV9NXT448	1.07	0.86	0.92	1.48	0.95	1.09	1.33	0.96	0.98	0.91
AAV9NXT449	1.15	0.29	0.71	0.75	0.60	0.71	0.36	0.65	0.39	0.65
AAV9NXT450	1.59	0.51	1.20	1.06	0.92	1.01	0.89	0.95	0.63	1.05
AAV9NXT451	1.00	0.86	1.12	0.94	0.71	0.91	0.62	0.82	0.90	0.80
AAV9NXT452	0.85	0.51	0.69	0.71	0.58	0.61	0.55	0.68	0.54	0.69
AAV9NXT453	1.12	0.20	0.72	0.60	0.35	0.55	0.53	0.43	0.23	0.54
AAV9NXT454	1.17	0.29	0.82	0.84	0.63	0.65	0.79	0.73	0.39	0.79
AAV9NXT455	0.88	0.15	0.62	0.45	0.30	0.45	0.22	0.48	0.19	0.48
AAV9NXT456	0.96	0.18	0.72	0.68	0.47	0.63	0.55	0.50	0.26	0.59
AAV9NXT457	1.27	0.39	0.81	0.94	0.82	0.66	0.64	0.79	0.59	0.76
AAV9NXT458	0.62	0.92	0.78	0.56	0.61	0.77	0.40	0.78	0.76	0.65
AAV9NXT459	1.07	0.31	0.88	0.64	0.62	0.67	0.47	0.78	0.41	0.79
AAV9NXT460	1.19	0.40	0.96	0.94	0.93	0.95	1.02	0.89	0.55	0.95
AAV9NXT462	0.94	0.63	0.94	0.93	0.82	0.99	0.78	1.01	0.77	0.92
AAV9NXT464	0.63	1.11	0.62	0.79	0.82	0.94	1.11	0.71	0.83	0.72
AAV9NXT465	0.94	0.49	0.71	0.62	0.46	0.62	0.10	0.61	0.48	0.60
AAV9NXT466	0.97	0.39	0.83	0.70	0.56	0.67	0.37	0.60	0.43	0.64
AAV9NXT467	1.10	0.18	0.69	0.65	0.31	0.62	0.41	0.46	0.22	0.51
AAV9NXT468	1.32	0.40	0.91	0.65	0.55	0.68	0.50	0.65	0.33	0.69
AAV9NXT469	0.55	0.02	0.36	0.19	0.06	0.24	0.02	0.18	0.05	0.20
AAV9NXT470	1.26	0.15	0.83	0.62	0.27	0.73	0.44	0.47	0.20	0.50
AAV9NXT472	0.23	0.36	0.28	0.13	0.24	0.30	0.04	0.31	0.30	0.27
AAV9NXT473	0.43	0.03	0.34	0.19	0.07	0.22	0.00	0.13	0.05	0.15
AAV9NXT485	1.05	0.26	0.60	0.69	0.31	0.45	0.15	0.44	0.27	0.38
AAV9NXT488	0.81	0.04	0.48	0.33	0.16	0.37	0.45	0.32	0.10	0.30
AAV9NXT491	0.75	1.06	0.86	0.76	0.77	1.01	0.71	0.87	0.87	0.81
AAV9NXT492	0.37	1.14	0.43	0.43	0.48	0.72	0.47	0.59	0.58	0.47

FIG. 7 cont'd

AAV9NXT493	1.59	0.32	1.08	1.03	0.53	1.06	0.80	0.77	0.37	0.84
AAV9NXT494	1.74	0.23	0.85	0.64	0.40	0.74	0.12	0.50	0.44	0.56
AAV9NXT495	1.20	0.20	0.90	0.74	0.38	0.76	0.13	0.60	0.24	0.66
AAV9NXT496	0.94	0.26	0.80	0.90	0.66	0.74	0.59	0.71	0.35	0.79
AAV9NXT497	1.31	0.33	1.02	0.86	0.52	1.03	1.45	0.72	0.37	0.81
AAV9NXT498	0.17	0.56	0.30	0.13	0.38	0.38	0.19	0.67	0.33	0.53
AAV9NXT499	0.35	0.01	0.23	0.11	0.03	0.14	0.02	0.06	0.03	0.10
AAV9NXT500	0.48	1.08	0.44	0.19	0.33	0.36	0.47	0.31	0.55	0.37
AAV9NXT501	0.02	0.00	0.04	0.00	0.00	0.01	0.00	0.01	0.00	0.01
AAV9NXT502	0.54	1.35	0.68	0.52	0.65	0.90	0.63	0.67	0.79	0.60
AAV9NXT503	0.95	0.43	0.75	0.60	0.43	0.61	0.50	0.56	0.61	0.61
AAV9NXT504	0.45	0.01	0.32	0.17	0.05	0.32	0.00	0.10	0.05	0.14
AAV9NXT505	0.61	0.86	0.65	0.40	0.51	0.61	0.52	0.65	0.69	0.58
AAV9NXT510	1.41	0.59	1.14	0.77	0.77	1.00	0.50	0.90	0.74	0.91
AAV9NXT511	0.03	0.03	0.02	0.21	0.01	0.02	0.50	0.02	0.02	0.02
AAV9NXT512	0.24	0.01	0.18	0.18	0.05	0.17	0.08	0.09	0.03	0.12
AAV9NXT513	0.92	0.37	0.84	0.67	0.55	0.61	0.52	0.62	0.43	0.63
AAV9NXT514	0.02	0.00	0.02	0.01	0.01	0.03	0.00	0.01	0.01	0.02
AAV9NXT515	0.69	0.90	0.74	0.47	0.74	0.62	0.29	0.71	0.80	0.75
AAV9NXT516	0.03	0.02	0.02	0.07	0.01	0.01	0.37	0.02	0.02	0.02
AAV9NXT517	0.16	0.19	0.19	0.17	0.16	0.15	0.40	0.18	0.17	0.14
AAV9NXT526	0.68	0.08	0.55	0.45	0.29	0.43	0.51	0.42	0.14	0.43
AAV9NXT527	0.16	0.15	0.20	0.18	0.11	0.15	0.24	0.15	0.16	0.13
AAV9NXT528	0.39	0.02	0.23	0.24	0.09	0.22	0.23	0.19	0.06	0.22
AAV9NXT529	1.18	0.29	1.12	1.15	0.40	0.96	1.18	0.66	0.39	0.84
AAV9NXT530	0.92	0.44	0.83	0.63	0.27	0.53	0.44	0.49	0.46	0.57
AAV9NXT531	0.74	0.07	0.47	0.50	0.19	0.35	0.17	0.33	0.11	0.31
AAV9NXT532	0.83	0.73	1.02	0.73	0.49	0.79	0.54	0.69	0.65	0.75
AAV9NXT533	0.94	0.09	0.68	0.59	0.37	0.49	0.45	0.57	0.18	0.52
AAV9NXT534	0.87	0.07	0.65	0.54	0.21	0.56	0.29	0.39	0.13	0.41
AAV9NXT535	0.39	1.29	0.64	0.66	0.53	0.68	0.90	0.60	0.70	0.55
AAV9NXT543	0.18	0.08	0.11	0.86	0.08	0.10	1.45	0.14	0.12	0.11
AAV9NXT544	0.76	1.04	0.77	0.81	0.81	0.91	0.63	0.83	0.75	0.80

FIG. 7 cont'd

AAV9NXT545	1.25	0.24	0.86	0.70	0.53	0.69	0.43	0.68	0.28	0.69
AAV9NXT546	1.07	1.03	1.00	0.72	0.84	0.88	0.42	1.00	0.95	1.01
AAV9NXT547	0.93	1.07	1.03	0.73	0.95	1.05	0.73	0.99	0.93	0.94
AAV9NXT548	0.99	0.65	0.81	0.85	0.87	0.81	0.85	0.88	0.71	0.87
AAV9NXT549	0.45	1.42	0.62	0.39	0.52	0.71	0.40	0.57	0.48	0.60
AAV9NXT550	1.12	1.02	1.53	1.27	1.10	1.21	0.58	1.41	1.03	1.19
AAV9NXT551	0.46	1.33	0.53	0.34	0.54	0.56	0.19	0.51	0.67	0.51
AAV9NXT552	0.47	1.30	0.57	0.52	0.40	0.55	0.38	0.51	0.77	0.52
AAV9NXT553	0.43	1.01	0.63	0.54	0.54	0.59	0.27	0.65	0.65	0.62
AAV9NXT554	0.01	1.39	0.15	0.01	0.02	0.02	0.00	0.04	0.05	0.04
AAV9NXT555	0.75	0.79	0.78	0.82	0.92	0.88	0.44	0.84	0.61	0.93
AAV9NXT556	0.22	0.73	0.37	0.22	0.26	0.58	0.24	0.34	0.47	0.35
AAV9NXT557	0.86	0.32	0.82	0.54	0.63	0.55	0.22	0.77	0.40	0.73
AAV9NXT559	0.93	0.44	0.77	0.58	0.55	0.67	0.47	0.71	0.52	0.71
AAV9NXT560	1.10	0.48	0.96	0.76	0.93	0.92	0.85	0.89	0.71	1.02
AAV9NXT561	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
AAV9NXT562	0.54	0.59	0.73	0.63	0.60	0.63	0.45	0.54	0.59	0.66
AAV9NXT563	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AAV9NXT564	1.25	0.72	1.12	1.10	1.00	0.98	0.86	0.85	0.90	1.06
AAV9NXT565	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00
AAV9NXT566	0.09	0.05	0.09	0.06	0.05	0.07	0.11	0.07	0.06	0.06
AAV9NXT567	0.01	0.01	0.02	0.02	0.00	0.01	0.01	0.02	0.00	0.01
AAV9NXT575	0.03	0.01	0.01	0.03	0.01	0.02	0.10	0.01	0.01	0.01
AAV9NXT576	0.04	0.02	0.02	0.22	0.02	0.02	0.19	0.02	0.02	0.02
AAV9NXT579	1.34	0.40	1.10	1.08	0.62	0.96	0.50	0.80	0.45	0.92
AAV9NXT580	0.72	0.87	0.78	0.68	0.79	0.74	0.66	0.84	0.97	0.86
AAV9NXT581	1.15	0.66	0.99	0.87	0.67	0.79	0.72	0.81	0.71	0.80
AAV9NXT582	0.93	0.98	0.95	0.85	0.90	1.06	0.62	0.95	0.93	0.91
AAV9NXT583	0.90	0.72	0.77	0.64	0.71	0.69	0.50	0.76	0.76	0.79
AAV9NXT584	0.62	1.03	0.70	0.62	0.74	0.87	0.55	0.80	0.84	0.77
AAV9NXT585	1.15	0.84	1.06	0.86	0.84	0.95	1.02	1.00	0.96	0.97
AAV9NXT586	0.90	0.67	0.80	0.67	0.77	0.71	0.57	0.83	0.78	0.85
AAV9NXT587	1.10	0.92	0.99	0.95	1.00	1.03	0.70	1.07	1.02	1.09

FIG. 7 cont'd

AAV9NXT588	0.41	0.02	0.41	0.31	0.07	0.35	0.17	0.16	0.05	0.21
AAV9NXT589	1.00	0.47	0.79	0.75	0.57	0.62	0.40	0.69	0.49	0.68
AAV9NXT590	0.56	0.03	0.45	0.31	0.09	0.38	0.26	0.21	0.06	0.26
AAV9NXT591	1.43	0.20	1.05	0.85	0.42	0.67	0.49	0.70	0.32	0.70
AAV9NXT592	0.75	0.71	0.69	0.66	0.63	0.55	0.58	0.66	0.65	0.69
AAV9NXT593	0.76	0.56	0.74	0.50	0.53	0.52	0.43	0.62	0.56	0.59
AAV9NXT594	0.82	1.10	0.97	0.85	0.79	0.90	0.50	0.90	0.91	0.85
AAV9NXT595	0.75	1.13	0.81	0.77	0.79	0.94	0.42	0.89	0.87	0.85
AAV9NXT596	0.89	0.92	0.80	0.81	0.73	0.79	0.65	0.81	0.85	0.74
AAV9NXT597	0.84	0.81	0.83	0.93	0.98	0.77	0.52	0.78	0.75	0.86
AAV9NXT598	0.10	0.05	0.09	0.06	0.06	0.08	0.15	0.08	0.06	0.07
AAV9NXT611	0.01	0.00	0.01	0.02	0.00	0.03	0.00	0.00	0.00	0.00
AAV9NXT613	0.33	0.12	0.32	0.14	0.40	0.36	0.12	0.33	0.06	0.34
AAV9NXT615	0.86	0.49	0.83	0.50	0.64	0.63	0.36	0.72	0.72	0.85
AAV9NXT657	0.13	0.22	0.26	0.31	0.27	0.26	0.30	0.21	0.27	0.24
AAV9NXT658	0.13	0.19	0.25	0.17	0.23	0.22	0.16	0.21	0.22	0.25
AAV9NXT659	0.55	0.62	0.54	0.39	0.46	0.51	0.31	0.57	0.61	0.57
AAV9NXT660	0.19	0.19	0.29	0.17	0.19	0.23	0.04	0.22	0.26	0.25
AAV9NXT661	0.68	0.77	0.74	0.63	0.65	0.78	0.49	0.72	0.70	0.67
AAV9NXT662	0.23	0.19	0.33	0.24	0.36	0.22	0.41	0.28	0.25	0.34
AAV9NXT663	0.71	1.02	0.97	0.85	0.86	0.85	0.97	0.82	0.99	0.96
AAV9NXT664	0.61	0.57	0.64	0.45	0.64	0.66	0.43	0.72	0.49	0.64
AAV9NXT665	0.50	0.68	0.57	0.45	0.64	0.60	0.37	0.54	0.74	0.62
AAV9NXT666	0.33	0.43	0.48	0.44	0.55	0.46	0.75	0.48	0.52	0.45
AAV9NXT667	0.09	0.18	0.24	0.15	0.20	0.14	0.31	0.15	0.17	0.20
AAV9NXT668	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AAV9NXT670	0.01	0.02	0.03	0.00	0.01	0.00	0.00	0.01	0.01	0.02
AAV9NXT703	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AAV9NXT704	0.96	0.19	0.88	0.73	0.38	0.65	0.66	0.58	0.21	0.66
AAV9NXT705	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AAV9NXT706	0.79	0.10	0.53	0.83	0.23	0.47	0.89	0.37	0.14	0.46
AAV9NXT707	0.05	0.01	0.05	0.03	0.02	0.02	0.34	0.03	0.02	0.03
AAV9NXT708	0.52	0.24	0.51	0.43	0.34	0.38	0.47	0.39	0.25	0.39

SUBSTITUTE SHEET (RULE 26)

FIG. 7 cont'd

AAV9NXT709	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.01	0.01
AAV9NXT710	0.16	0.77	0.23	0.26	0.26	0.35	0.34	0.25	0.33	0.26
AAV9NXT712	0.01	0.00	0.01	0.04	0.00	0.00	0.00	0.01	0.01	0.01
AAV9NXT716	0.93	0.73	0.90	0.67	0.64	0.81	0.39	0.74	0.70	0.75
AAV9NXT718	0.91	0.37	0.93	0.59	1.25	0.76	0.63	0.68	0.39	0.68
AAV9NXT720	1.26	0.65	0.92	0.98	0.72	0.94	0.74	0.89	0.71	0.97
AAV9NXT722	0.76	0.67	0.63	0.44	0.49	0.55	0.36	0.68	0.49	0.67
AAV9NXT723	0.12	0.02	0.11	0.10	0.06	0.08	0.19	0.08	0.03	0.10
AAV9NXT724	0.24	0.22	0.19	0.21	0.13	0.11	0.54	0.20	0.13	0.17
AAV9NXT725	0.02	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00
AAV9NXT726	0.02	0.00	0.01	0.06	0.00	0.01	0.00	0.01	0.01	0.01
AAV9N14T	1.01	1.41	1.01	0.95	0.94	0.97	0.96	1.12	1.65	1.07

FIG. 8

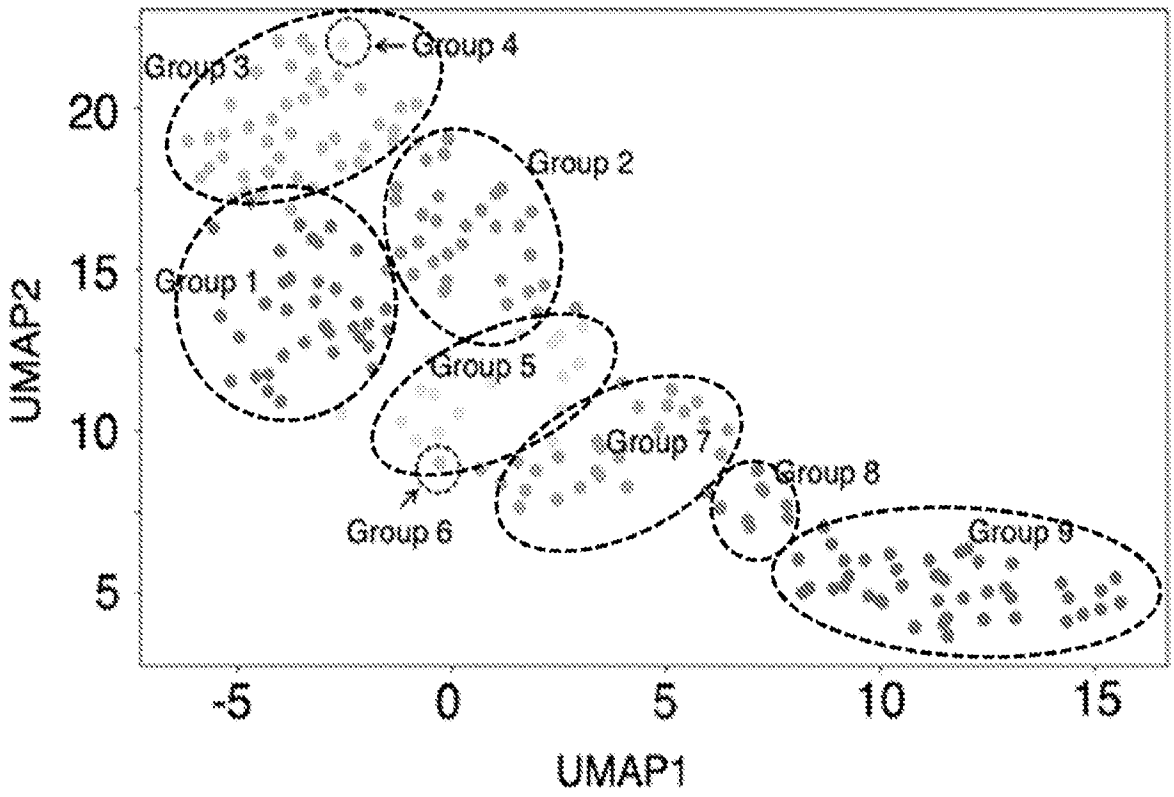

Group 3
Group 4
Group 2
Group 1
Group 5
Group 7
Group 8
Group 6
Group 9
UMAP2
UMAP1
20
15
10
5
-5
0
5
10
15

FIG. 9

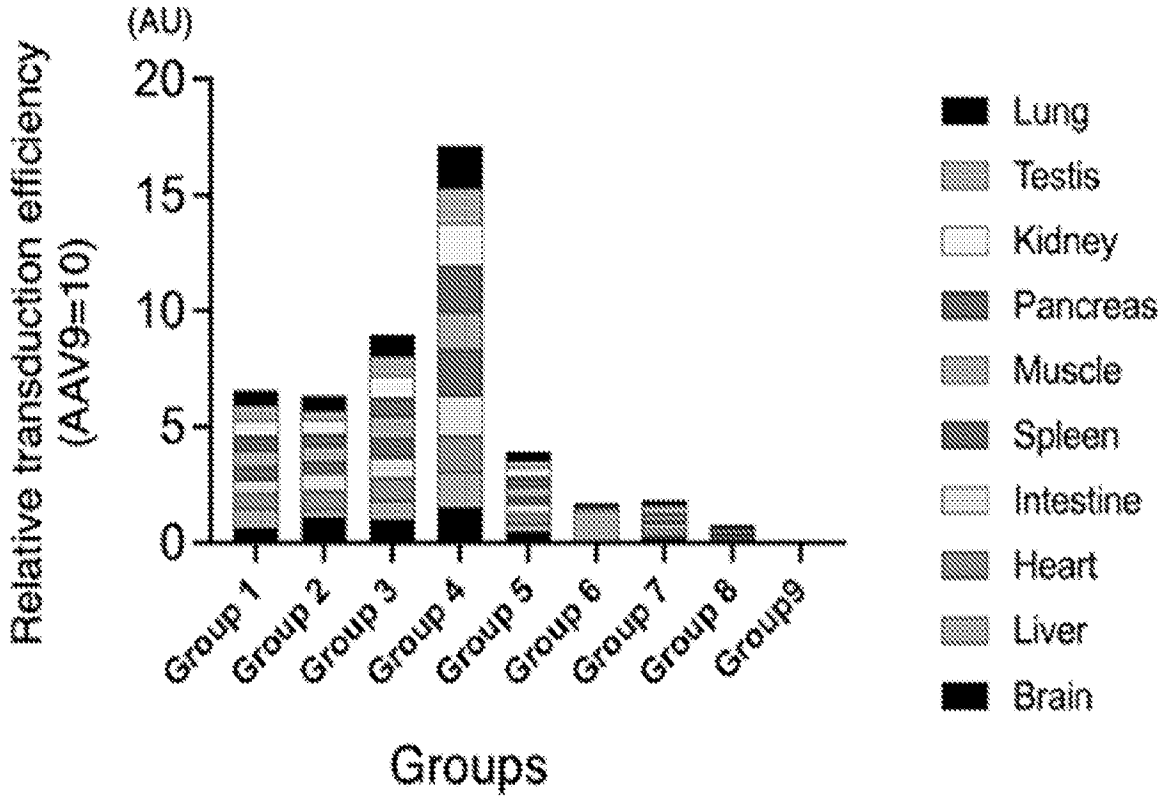

(AU)
Relative transduction efficiency (AAV9=10)
20
15
10
5
0
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
Group 8
Group9
Groups
Lung
Testis
Kidney
Pancreas
Muscle
Spleen
Intestine
Heart
Liver
Brain

FIG. 10

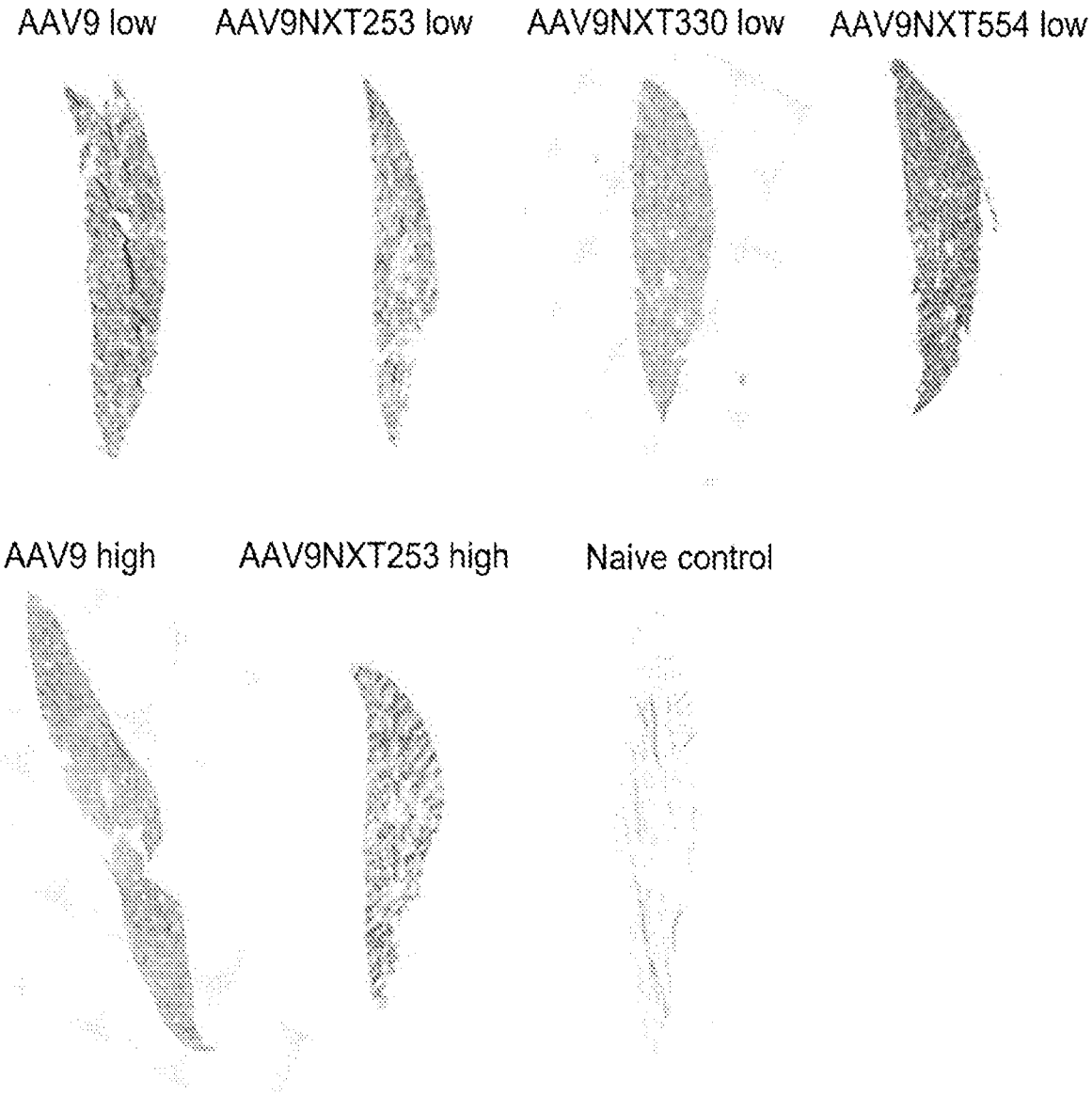

AAV9 low
AAV9NXT253 low
AAV9NXT330 low
AAV9NXT554 low
AAV9 high
AAV9NXT253 high
Naive control

FIG. 11

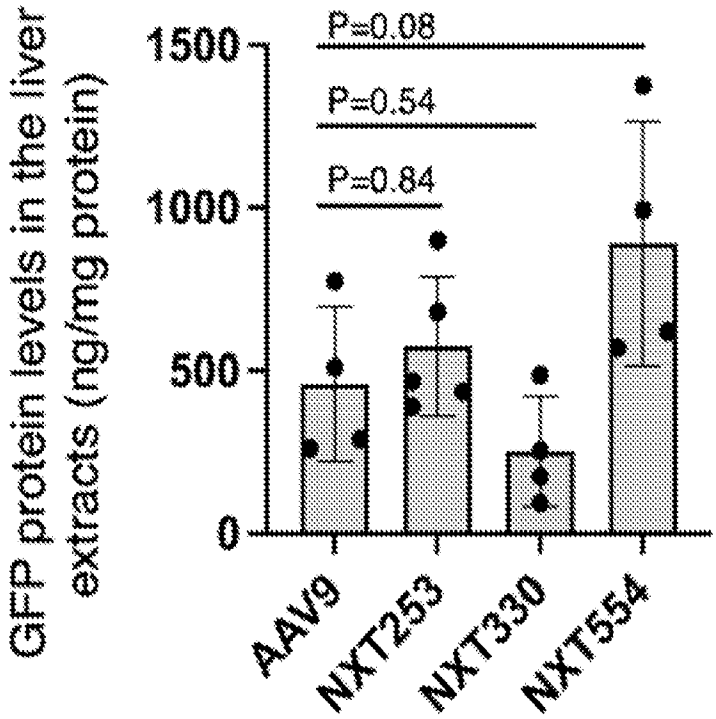

GFP protein levels in the liver extracts (ng/mg protein)
1500
1000
500
0
P=0.08
P=0.54
P=0.84
AAV9
NXT253
NXT330
NXT554

FIG. 12

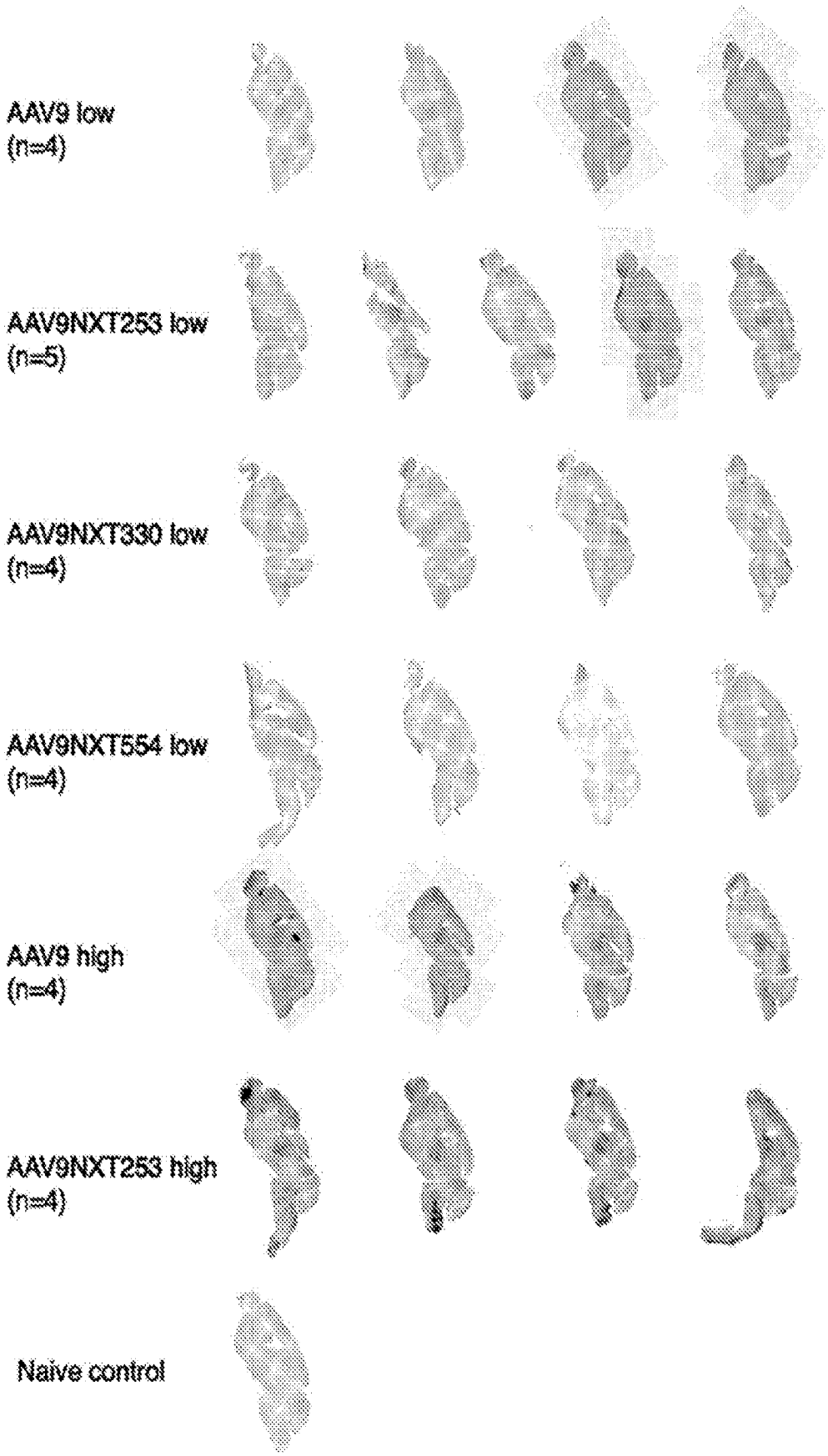

AAV9 low
(n=4)
AAV9NXT253 low
(n=5)
AAV9NXT330 low
(n=4)
AAV9NXT554 low
(n=4)
AAV9 high
(n=4)
AAV9NXT253 high
(n=4)
Naive control

FIG. 13

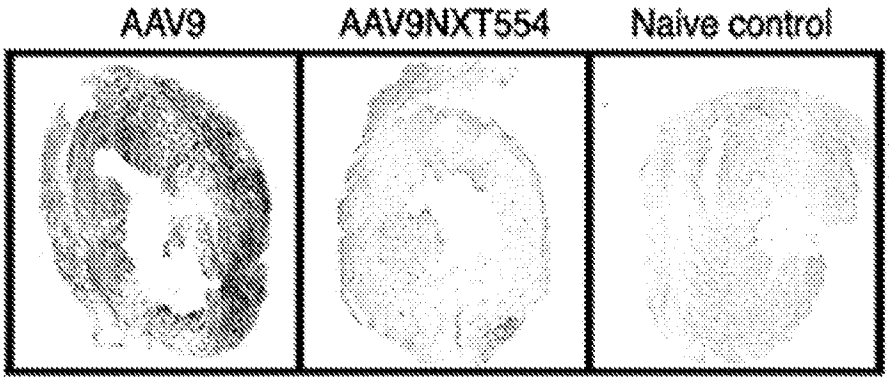

AAV9
AAV9NXT554
Naive control

FIG. 14

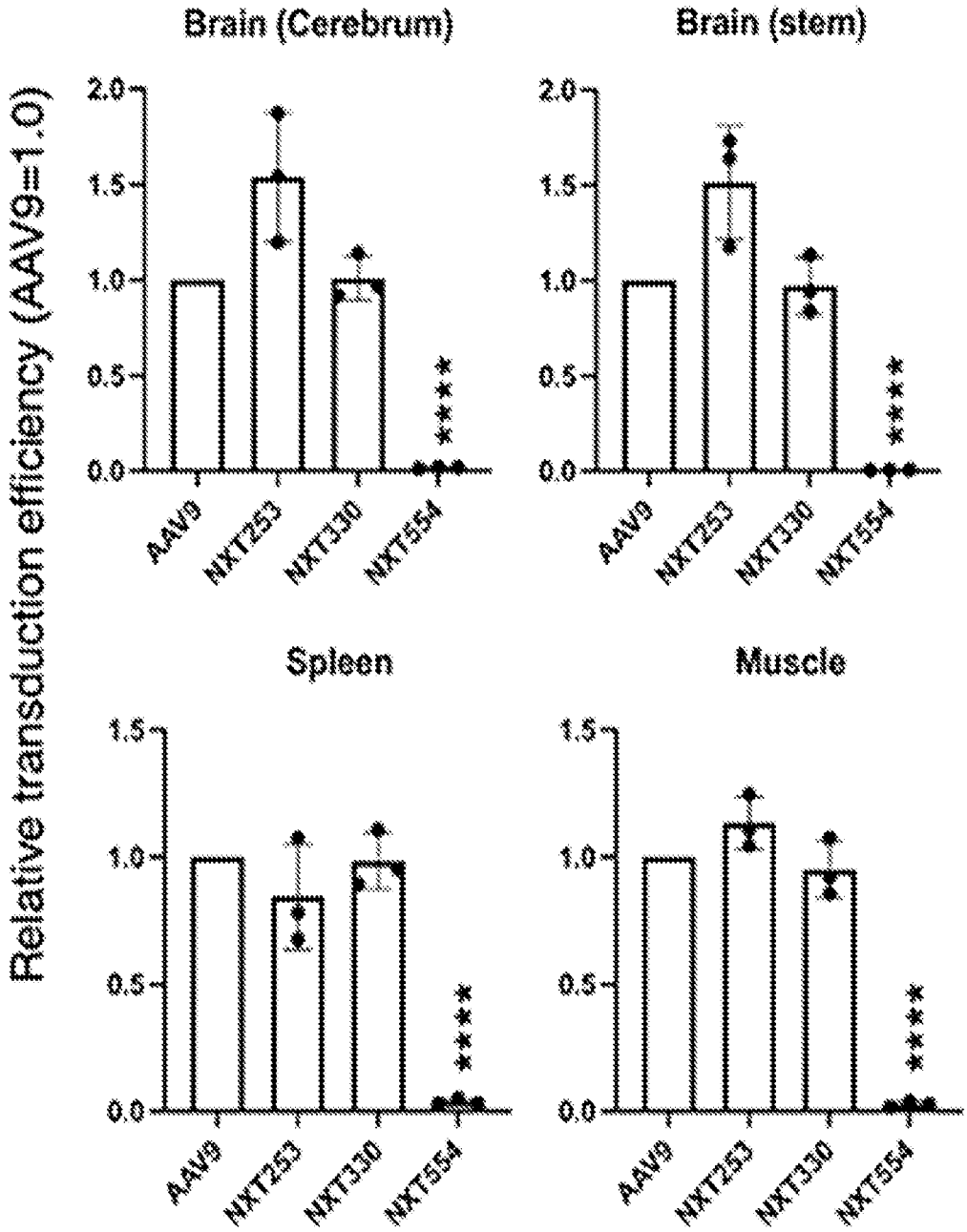

Brain (Cerebrum)
Brain (stem)
Spleen
Muscle
Relative transduction efficiency (AAV9=1.0)
2.0
1.5
1.0
0.5
0.0
AAV9
NXT253
NXT330
NXT554
****

FIG. 14 cont'd

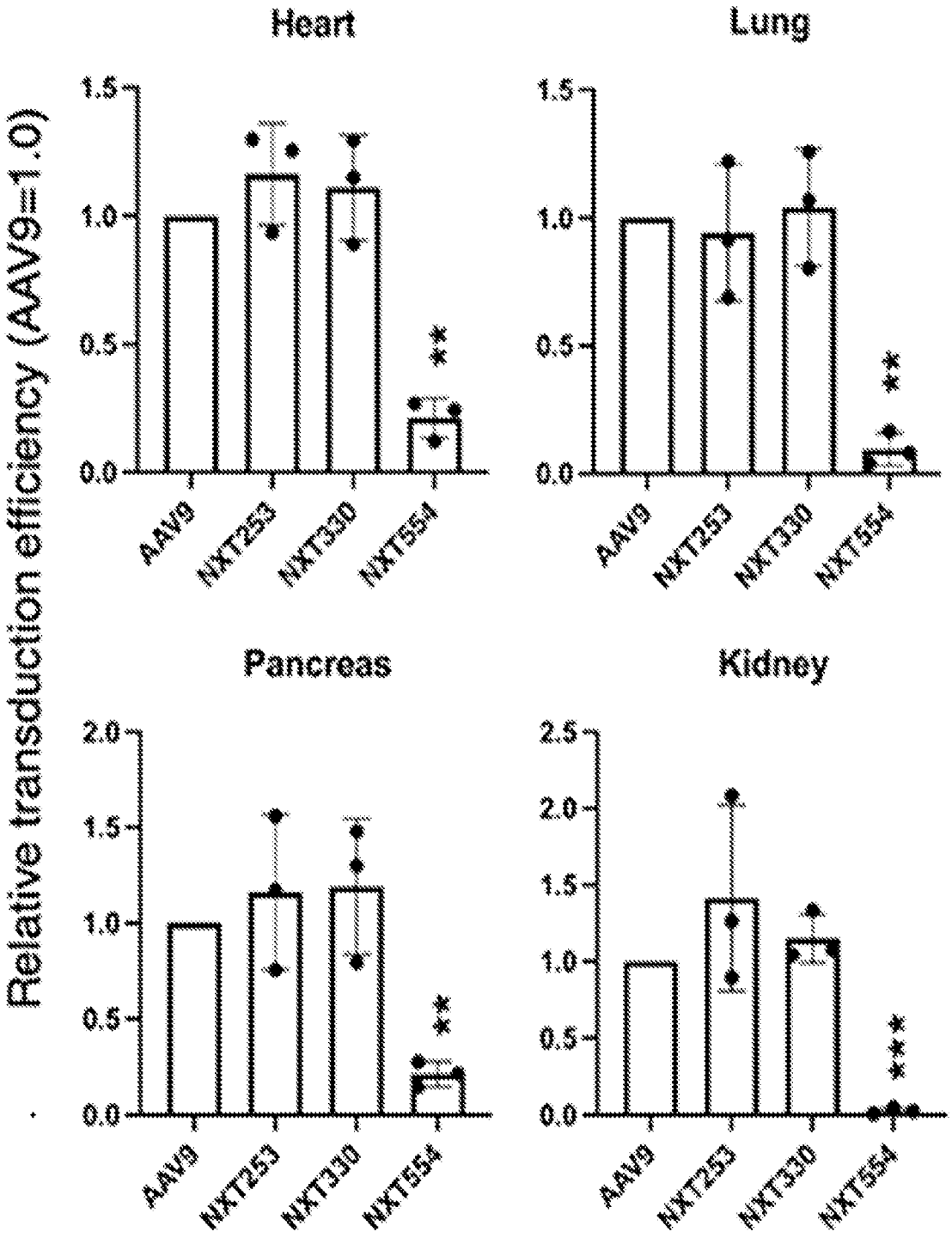

Heart
Lung
Pancreas
Kidney
Relative transduction efficiency (AAV9=1.0)
AAV9
NXT253
NXT330
NXT554

FIG. 14 cont'd

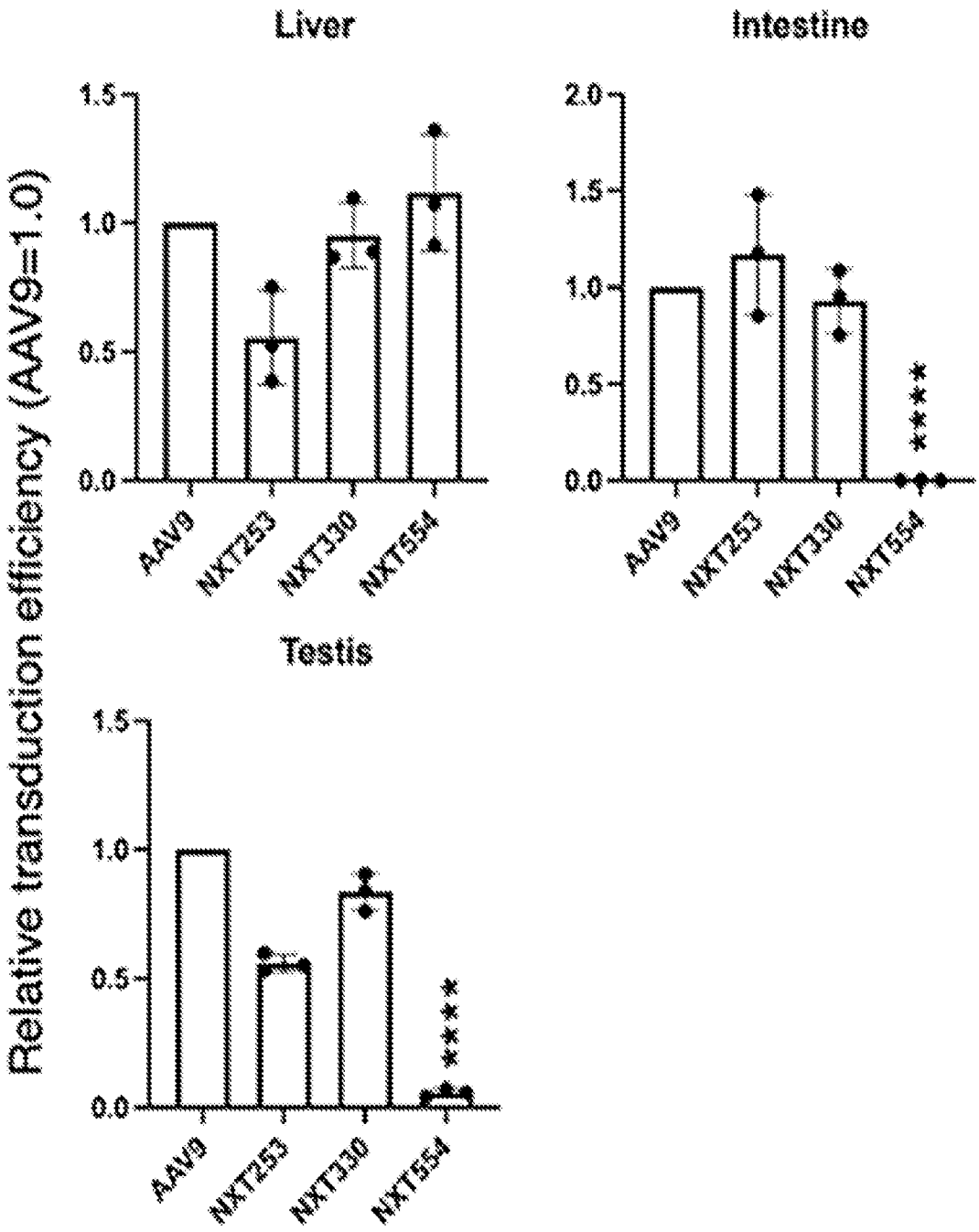

Liver
Intestine
Testis
Relative transduction efficiency (AAV9=1.0)
1.5
1.0
0.5
0.0
2.0
1.5
1.0
0.5
0.0
****
****
AAV9
NXT253
NXT330
NXT554

FIG. 15

Relative vector concentration of AAV9NXT/S mutants in mice following intravenous administration

AAV capsid	Relative vector concentration in the blood (PD values determined by AAV DNA Barcode-Seq)							
	1 m	10 m	30 m	1 h	4 h	8 h	24 h	72 h
AAV9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAV9NXS107	1.13	1.24	1.14	1.10	1.21	1.19	1.21	1.09
AAV9NXS147	1.04	1.07	1.01	0.98	0.98	0.90	0.87	1.10
AAV9NXS153	1.04	1.13	1.11	1.06	1.09	1.08	1.01	1.01
AAV9NXS154	0.93	0.88	0.93	0.94	0.89	0.91	0.94	0.93
AAV9NXS160	0.93	0.95	0.94	0.93	0.98	0.97	1.00	1.00
AAV9NXS179	1.06	1.13	1.12	1.08	1.12	1.11	1.10	1.04
AAV9NXS194	1.10	1.15	1.17	1.18	1.16	1.19	1.23	1.09
AAV9NXS198	1.05	1.10	1.08	1.08	1.16	1.20	1.29	1.05
AAV9NXS266	0.99	1.07	1.14	1.23	1.69	2.39	4.68	0.06
AAV9NXT14	1.17	1.26	1.18	1.17	1.21	1.23	1.33	1.19
AAV9NXT35	1.06	1.06	1.07	1.10	1.07	1.10	1.16	1.04
AAV9NXT41	1.15	1.17	1.17	1.18	1.14	1.14	1.19	1.08
AAV9NXT57	1.01	1.02	1.02	1.05	1.06	1.10	1.23	0.98
AAV9NXT66	1.01	0.99	1.06	1.11	1.14	1.42	2.10	2.00
AAV9NXT88	1.07	1.03	1.08	1.12	1.06	1.10	1.20	1.06
AAV9NXT94	1.06	1.02	1.06	1.11	1.04	1.10	1.19	0.97
AAV9NXT106	1.02	1.02	1.04	1.05	1.00	1.01	1.00	0.90
AAV9NXT113	1.11	1.16	1.15	1.12	1.18	1.24	1.53	1.06
AAV9NXT136	1.02	1.02	1.03	1.10	1.00	1.01	0.93	0.88
AAV9NXT172	1.11	1.11	1.11	1.13	1.15	1.14	1.19	1.07
AAV9NXT174	1.07	1.13	1.09	1.15	1.10	1.12	1.21	1.08
AAV9NXT177	1.08	1.12	1.09	1.14	1.13	1.16	1.22	1.21
AAV9NXT200	1.07	1.12	1.12	1.14	1.11	1.16	1.25	1.07
AAV9NXT214	1.08	1.20	1.10	1.05	1.23	1.33	1.52	1.22
AAV9NXT215	1.13	1.16	1.15	1.12	1.35	1.65	2.23	1.54
AAV9NXT253	1.03	0.99	1.06	1.07	1.40	1.70	2.29	1.00
AAV9NXT254	1.01	1.06	1.11	1.16	1.18	1.37	1.61	1.00
AAV9NXT256	1.13	1.25	1.22	1.38	1.59	2.05	2.98	1.02
AAV9NXT259	1.14	1.29	1.37	1.45	1.91	2.82	5.37	0.05
AAV9NXT261	0.93	0.99	1.04	1.15	1.55	2.12	3.68	0.04
AAV9NXT263	1.05	1.14	1.21	1.28	1.76	2.46	4.53	0.11
AAV9NXT264	1.06	1.02	0.94	0.99	1.28	1.89	3.56	0.03
AAV9NXT265	1.04	1.13	1.21	1.20	1.65	2.35	4.28	0.04
AAV9NXT267	0.92	0.97	1.07	1.09	1.46	2.18	3.65	0.05
AAV9NXT268	1.03	1.09	1.20	1.28	1.75	2.58	4.90	0.03
AAV9NXT269	0.97	1.02	1.12	1.15	1.58	2.25	4.24	0.03
AAV9NXT270	1.06	1.18	1.21	1.29	1.89	2.71	5.04	0.03
AAV9NXT271	1.13	1.16	0.95	0.71	0.24	0.16	0.22	0.60
AAV9NXT272	1.18	1.31	1.32	1.37	1.83	2.31	3.23	0.08
AAV9NXT273	1.17	1.29	1.31	1.39	1.91	2.69	4.55	0.17
AAV9NXT325	0.91	0.90	0.90	0.85	0.93	0.98	1.15	1.49
AAV9NXT326	0.95	0.93	0.94	0.93	0.94	0.97	0.97	1.22
AAV9NXT327	1.04	1.06	1.01	1.01	0.97	0.89	0.89	1.06

FIG. 15 cont'd

AAV9NXT328	1.00	1.01	1.01	0.97	1.00	1.03	1.09	1.19
AAV9NXT329	1.08	1.07	1.13	1.07	1.05	1.13	1.17	1.13
AAV9NXT330	1.09	1.10	1.11	1.04	1.15	1.27	1.35	1.50
AAV9NXT331	0.97	0.99	0.94	0.96	1.00	1.00	1.04	1.22
AAV9NXT333	1.04	1.07	1.09	1.05	1.11	1.19	1.35	1.08
AAV9NXT334	0.92	0.91	0.91	0.91	1.02	1.11	1.16	1.47
AAV9NXT335	1.11	1.04	1.02	1.09	1.15	1.15	1.09	1.26
AAV9NXT336	1.08	1.11	1.11	1.16	1.45	1.63	1.99	0.79
AAV9NXT361	1.21	1.27	1.26	1.17	1.28	1.35	1.36	0.97
AAV9NXT383	1.03	1.10	1.11	1.17	1.72	2.55	4.38	0.02
AAV9NXT384	0.99	1.09	1.14	1.25	1.67	2.46	4.61	0.04
AAV9NXT385	1.02	1.11	1.16	1.30	1.64	2.36	4.52	0.03
AAV9NXT386	1.04	1.13	1.23	1.34	1.71	2.61	5.08	0.03
AAV9NXT387	0.93	1.02	1.08	1.20	1.54	2.28	3.91	0.17
AAV9NXT388	1.05	1.18	1.19	1.23	1.70	2.49	4.68	0.03
AAV9NXT389	1.07	1.27	1.31	1.37	1.78	2.67	4.95	0.08
AAV9NXT390	1.16	1.15	1.18	1.27	1.73	2.41	4.18	0.05
AAV9NXT391	1.04	1.08	1.12	1.25	1.57	2.20	3.73	0.39
AAV9NXT439	1.14	1.15	1.11	1.06	1.07	1.14	1.17	0.70
AAV9NXT440	1.07	1.13	1.07	1.12	1.12	1.17	1.31	0.84
AAV9NXT442	0.79	0.60	0.61	0.62	0.66	0.90	1.22	0.04
AAV9NXT443	0.98	0.53	0.36	0.19	0.23	0.26	0.29	0.12
AAV9NXT444	0.67	0.52	0.50	0.51	0.61	0.66	0.94	0.00
AAV9NXT446	1.19	1.18	1.25	1.33	1.73	2.24	3.16	0.41
AAV9NXT447	1.14	1.27	1.24	1.31	1.83	2.61	4.40	0.09
AAV9NXT448	1.06	1.15	1.20	1.14	1.20	1.31	1.35	0.86
AAV9NXT449	1.08	1.17	1.23	1.32	1.68	2.22	3.49	0.69
AAV9NXT450	1.13	1.12	1.26	1.25	1.48	2.01	2.71	0.33
AAV9NXT451	0.97	0.97	0.95	0.98	1.06	1.08	1.17	0.92
AAV9NXT452	1.07	1.11	1.13	1.15	1.36	1.56	1.78	0.94
AAV9NXT453	1.03	1.09	1.15	1.16	1.50	2.01	3.04	0.45
AAV9NXT454	1.09	1.17	1.23	1.30	1.63	2.18	3.49	0.73
AAV9NXT455	0.96	0.98	1.03	1.16	1.53	2.06	3.69	0.41
AAV9NXT456	1.03	1.09	1.14	1.22	1.64	2.18	3.80	0.53
AAV9NXT457	1.21	1.27	1.28	1.38	1.69	2.06	3.01	0.83
AAV9NXT458	1.05	1.06	1.02	0.99	1.03	1.00	1.14	0.89
AAV9NXT459	1.13	1.15	1.25	1.30	1.64	2.21	3.11	0.28
AAV9NXT460	1.06	1.12	1.11	1.21	1.52	1.90	2.83	0.60
AAV9NXT462	1.15	1.14	1.23	1.39	1.42	1.65	2.06	0.87
AAV9NXT464	1.05	1.11	1.06	1.02	0.85	0.73	0.69	0.38
AAV9NXT465	1.11	1.14	1.14	1.18	1.37	1.63	1.83	0.60
AAV9NXT466	1.12	1.19	1.23	1.34	1.51	2.04	2.70	0.61
AAV9NXT467	1.17	1.21	1.26	1.39	1.82	2.49	3.83	0.46
AAV9NXT468	1.24	1.39	1.38	1.45	1.89	2.50	3.58	0.74
AAV9NXT469	1.06	1.06	1.11	1.20	1.74	2.49	4.34	0.09
AAV9NXT470	1.09	1.17	1.17	1.27	1.79	2.45	3.84	0.36
AAV9NXT472	1.07	1.01	0.96	0.91	0.75	0.62	0.70	0.65
AAV9NXT473	1.11	1.18	1.21	1.30	1.82	2.58	4.21	0.16
AAV9NXT485	1.12	1.15	1.25	1.31	1.56	2.06	2.87	0.02

FIG. 15 cont'd

AAV9NXT488	1.22	1.34	1.33	1.46	2.11	2.95	4.76	0.04
AAV9NXT491	1.20	1.31	1.33	1.26	1.12	1.01	0.99	0.93
AAV9NXT492	1.07	1.11	1.06	0.95	0.64	0.44	0.41	0.64
AAV9NXT493	1.19	1.31	1.32	1.47	1.83	2.46	3.87	0.32
AAV9NXT494	1.00	1.06	1.06	1.13	1.61	1.80	2.75	0.11
AAV9NXT495	1.12	1.29	1.28	1.37	1.78	2.49	4.27	0.39
AAV9NXT496	1.18	1.28	1.30	1.32	1.80	2.53	4.03	0.69
AAV9NXT497	1.22	1.41	1.38	1.45	2.03	2.64	3.76	0.67
AAV9NXT498	1.07	0.91	0.65	0.43	0.08	0.06	0.09	0.18
AAV9NXT499	1.09	1.14	1.26	1.34	1.79	2.60	5.11	0.02
AAV9NXT500	1.10	1.00	0.85	0.70	0.40	0.30	0.41	0.32
AAV9NXT501	1.13	1.18	1.26	1.36	1.87	2.70	5.17	0.01
AAV9NXT502	1.08	1.05	1.05	0.96	0.69	0.53	0.62	0.83
AAV9NXT503	1.23	1.32	1.31	1.41	1.62	1.94	2.48	0.16
AAV9NXT504	1.03	1.10	1.10	1.22	1.63	2.12	2.86	0.03
AAV9NXT505	1.28	1.27	1.26	1.23	1.23	1.17	1.14	0.54
AAV9NXT510	1.12	1.10	1.11	1.18	1.35	1.53	1.81	0.85
AAV9NXT511	0.87	0.77	0.63	0.54	0.39	0.26	0.12	0.04
AAV9NXT512	0.99	1.05	1.10	1.27	1.62	2.23	4.47	0.05
AAV9NXT513	1.06	1.02	1.06	1.13	1.35	1.71	2.30	0.73
AAV9NXT514	1.06	1.06	1.19	1.27	1.64	2.32	4.28	0.03
AAV9NXT515	1.06	1.02	0.99	0.98	0.82	0.74	0.68	0.75
AAV9NXT516	0.85	0.77	0.80	0.86	1.06	1.23	1.80	0.03
AAV9NXT517	1.08	1.10	1.04	1.03	1.07	1.03	1.44	0.36
AAV9NXT526	1.15	1.17	1.30	1.36	1.79	2.43	4.32	0.27
AAV9NXT527	0.94	0.94	0.96	0.97	1.06	1.25	1.65	0.93
AAV9NXT528	1.06	1.13	1.21	1.33	1.77	2.41	4.81	0.08
AAV9NXT529	1.12	1.11	1.21	1.27	1.55	1.87	2.84	0.61
AAV9NXT530	0.87	0.88	0.89	0.92	0.97	1.04	0.95	0.80
AAV9NXT531	0.91	0.96	1.02	1.12	1.45	1.97	3.78	0.19
AAV9NXT532	1.09	1.13	1.08	1.12	1.11	1.06	0.83	0.66
AAV9NXT533	1.13	1.20	1.28	1.38	1.82	2.52	4.85	0.18
AAV9NXT534	1.09	1.17	1.25	1.33	1.76	2.32	4.37	0.07
AAV9NXT535	1.13	1.09	0.96	0.88	0.60	0.39	0.34	0.10
AAV9NXT543	1.25	0.74	0.72	0.51	0.14	0.14	0.14	0.06
AAV9NXT544	1.09	1.09	1.08	1.06	1.01	0.95	0.85	0.41
AAV9NXT545	1.12	1.11	1.25	1.26	1.64	2.20	3.78	0.38
AAV9NXT546	1.14	1.06	1.16	1.04	1.07	1.14	1.11	0.89
AAV9NXT547	1.18	1.19	1.12	1.11	1.09	0.95	0.87	0.81
AAV9NXT548	1.11	1.15	1.22	1.20	1.39	1.58	1.88	0.86
AAV9NXT549	1.12	1.08	0.99	0.87	0.50	0.37	0.43	0.58
AAV9NXT550	0.97	1.04	1.04	1.05	1.16	1.21	1.39	0.86
AAV9NXT551	1.06	0.99	0.86	0.76	0.37	0.22	0.25	0.31
AAV9NXT552	0.94	0.85	0.75	0.67	0.35	0.23	0.25	0.55
AAV9NXT553	1.08	1.02	1.00	0.91	0.70	0.58	0.48	0.33
AAV9NXT554	0.31	0.01	0.01	0.01	0.01	0.01	0.01	0.01
AAV9NXT555	1.08	1.05	1.12	1.12	1.26	1.49	1.64	0.39
AAV9NXT556	1.18	1.13	0.94	0.81	0.38	0.22	0.24	0.21
AAV9NXT557	1.09	1.16	1.19	1.32	1.61	1.99	3.31	0.63

FIG. 15 cont'd

AAV9NXT559	1.00	1.03	1.03	1.05	1.27	1.53	2.11	0.93
AAV9NXT560	1.30	1.30	1.37	1.31	1.51	1.82	2.62	0.87
AAV9NXT561	1.05	1.06	0.98	1.05	1.35	1.59	2.52	0.79
AAV9NXT562	1.14	1.13	1.15	1.03	1.22	1.27	1.40	1.14
AAV9NXT563	1.04	1.07	1.04	1.09	1.26	1.41	1.99	1.80
AAV9NXT564	1.10	1.05	1.11	1.11	1.19	1.29	1.60	1.13
AAV9NXT565	1.01	1.04	1.08	1.24	1.55	1.94	3.71	0.65
AAV9NXT566	0.96	0.92	0.93	0.99	1.20	1.42	2.26	0.42
AAV9NXT567	0.95	0.95	1.05	1.18	1.55	1.99	3.99	0.23
AAV9NXT575	0.94	0.85	0.82	0.86	0.84	0.79	0.48	0.04
AAV9NXT576	1.03	0.96	0.81	0.85	0.43	0.26	0.07	0.04
AAV9NXT579	1.03	1.05	1.09	1.22	1.47	1.82	2.63	0.43
AAV9NXT580	1.15	1.10	1.09	1.10	0.99	0.92	0.82	0.77
AAV9NXT581	1.07	1.06	1.09	1.10	1.19	1.29	1.36	0.05
AAV9NXT582	1.11	1.10	1.17	1.05	1.00	1.03	0.94	0.82
AAV9NXT583	1.00	0.99	1.01	1.03	1.03	1.05	1.13	0.19
AAV9NXT584	1.02	1.02	0.94	0.94	0.72	0.55	0.51	0.63
AAV9NXT585	1.19	1.19	1.18	1.21	1.29	1.32	1.32	0.43
AAV9NXT586	1.06	1.07	1.08	1.08	1.15	1.20	1.32	0.99
AAV9NXT587	1.12	1.09	1.13	1.12	1.17	1.18	1.25	1.06
AAV9NXT588	1.10	1.22	1.30	1.36	1.87	2.65	4.88	0.11
AAV9NXT589	1.05	1.07	1.12	1.15	1.34	1.66	2.04	0.68
AAV9NXT590	1.17	1.30	1.32	1.40	2.03	2.81	5.10	0.12
AAV9NXT591	1.20	1.30	1.40	1.44	1.85	2.51	4.15	0.24
AAV9NXT592	1.07	1.05	1.05	1.02	1.02	1.01	0.99	0.90
AAV9NXT593	1.01	1.03	1.05	1.08	1.17	1.31	1.46	0.77
AAV9NXT594	1.09	1.05	1.08	1.05	1.04	0.97	0.96	0.71
AAV9NXT595	1.22	1.20	1.25	1.13	1.08	1.02	0.95	0.80
AAV9NXT596	1.15	1.15	1.13	1.11	1.13	1.10	1.06	0.68
AAV9NXT597	0.99	0.99	0.98	1.03	1.04	1.07	1.10	1.00
AAV9NXT598	1.01	1.01	0.99	1.03	1.28	1.60	2.26	0.06
AAV9NXT611	1.08	1.12	1.17	1.24	1.65	2.16	3.91	0.80
AAV9NXT613	1.10	1.05	1.19	1.12	1.44	1.94	3.00	1.07
AAV9NXT615	1.14	1.15	1.10	1.25	1.34	1.45	1.80	1.00
AAV9NXT657	1.05	1.03	1.04	1.01	1.04	1.01	1.06	0.96
AAV9NXT658	1.10	1.11	1.11	1.09	1.23	1.40	1.78	1.05
AAV9NXT659	1.07	1.03	1.06	0.98	1.00	1.07	0.99	0.84
AAV9NXT660	1.13	1.10	1.12	1.11	1.25	1.48	1.89	0.97
AAV9NXT661	1.00	1.03	1.02	0.98	1.03	1.05	1.12	0.93
AAV9NXT662	1.13	1.16	1.21	1.11	1.45	1.66	2.31	1.04
AAV9NXT663	1.18	1.14	1.17	1.07	0.95	0.88	0.80	0.94
AAV9NXT664	0.95	1.01	1.05	1.02	1.16	1.38	1.65	0.88
AAV9NXT665	1.13	1.09	1.06	1.02	0.94	0.85	0.79	0.82
AAV9NXT666	1.16	1.16	1.15	1.14	1.19	1.19	1.29	1.15
AAV9NXT667	0.96	1.04	0.97	1.00	1.05	1.00	1.08	1.25
AAV9NXT668	1.12	1.19	1.11	1.15	1.38	1.58	2.41	1.26
AAV9NXT670	1.05	1.05	1.02	1.08	1.19	1.34	1.70	0.92
AAV9NXT703	1.01	1.00	1.02	1.05	1.24	1.58	2.27	1.60
AAV9NXT704	1.08	1.11	1.23	1.24	1.56	2.09	3.33	0.46

FIG. 15 cont'd

AAV9NXT705	0.97	1.03	1.13	1.28	1.66	2.29	4.66	0.03
AAV9NXT706	1.04	1.06	1.16	1.26	1.64	2.13	3.70	0.28
AAV9NXT707	0.99	1.05	1.10	1.18	1.50	2.10	4.10	0.05
AAV9NXT708	1.03	1.02	1.11	1.11	1.36	1.84	2.73	0.80
AAV9NXT709	1.04	1.07	1.10	1.25	1.56	2.02	3.29	0.66
AAV9NXT710	1.19	1.14	1.05	0.93	0.59	0.43	0.46	0.77
AAV9NXT712	1.01	1.01	0.99	1.05	1.13	1.19	1.28	0.51
AAV9NXT716	1.13	1.09	1.16	1.08	1.16	1.27	1.41	1.06
AAV9NXT718	1.24	1.23	1.27	1.24	1.40	1.66	1.82	0.78
AAV9NXT720	1.05	1.05	1.05	1.02	1.15	1.27	1.49	0.80
AAV9NXT722	1.06	1.07	1.11	1.07	1.27	1.40	1.81	1.02
AAV9NXT723	1.16	1.28	1.26	1.40	1.83	2.40	4.47	0.32
AAV9NXT724	1.16	1.14	1.17	1.20	1.41	1.64	2.44	1.32
AAV9NXT725	1.08	1.13	1.16	1.39	1.70	2.27	4.41	0.22
AAV9NXT726	1.02	1.00	1.01	1.09	1.43	1.76	3.26	0.40
AAV9N14T	1.12	1.13	1.14	1.05	1.07	1.02	0.86	1.13

FIG. 16

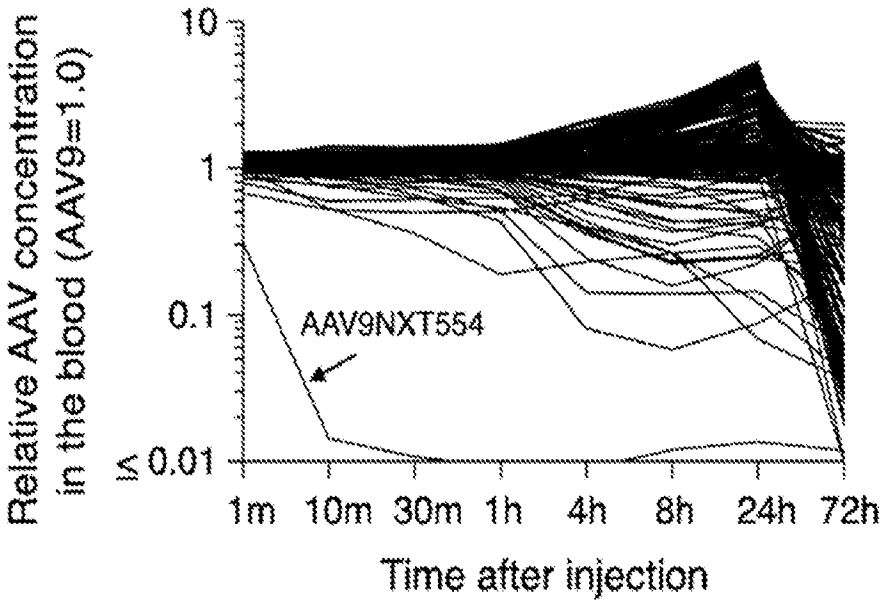


FIG. 17A

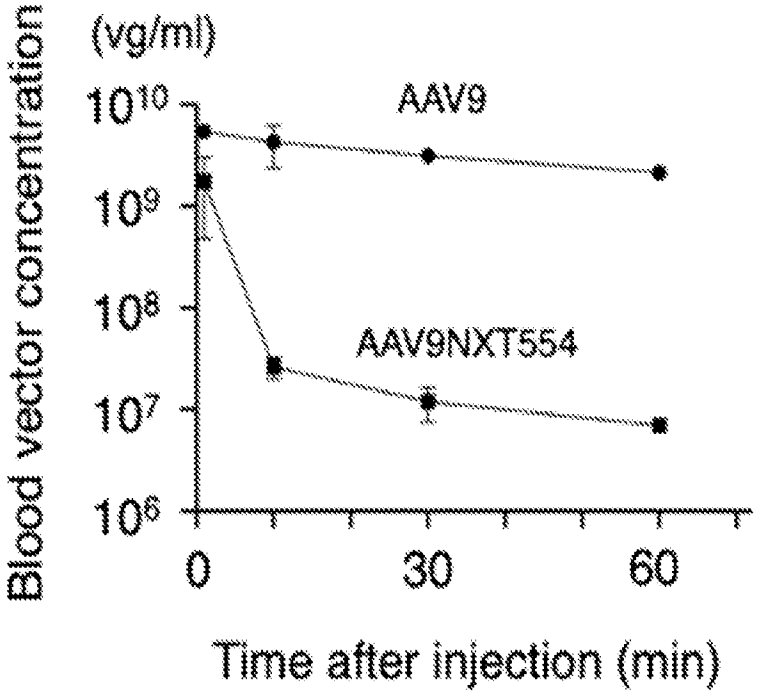


FIG. 17B

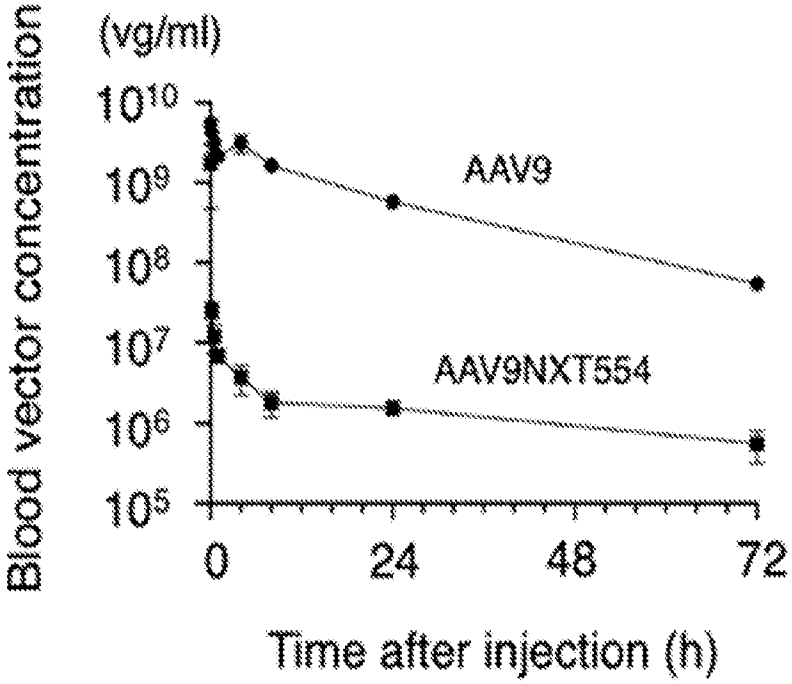


FIG. 18

In vitro cell surface binding and transduction efficiencies of AAV9NXT/S mutants

AAV capsid	PD values (AAV9 = 1.0)		
	CHO-Lec2 binding	CHO-Lec2 transduction	HepG2 binding
AAV9	1.00	1.00	1.00
AAV9NXS107	1.12	0.49	1.25
AAV9NXS147	0.92	0.42	0.80
AAV9NXS153	1.04	0.94	1.23
AAV9NXS154	0.99	1.05	1.08
AAV9NXS160	0.93	1.45	0.80
AAV9NXS179	1.12	0.66	0.94
AAV9NXS194	1.12	0.97	1.06
AAV9NXS198	0.92	0.49	0.89
AAV9NXS266	0.96	0.05	1.13
AAV9NXT14	1.02	0.58	0.92
AAV9NXT35	1.01	0.54	0.87
AAV9NXT41	1.09	0.37	0.89
AAV9NXT57	0.98	0.11	0.92
AAV9NXT66	1.05	0.01	0.39
AAV9NXT88	1.05	0.00	0.97
AAV9NXT94	1.08	0.15	0.87
AAV9NXT106	1.01	0.75	0.85
AAV9NXT113	1.08	0.00	1.04
AAV9NXT136	1.00	1.26	1.12
AAV9NXT172	1.05	0.59	0.92
AAV9NXT174	0.94	0.41	0.80
AAV9NXT177	1.07	0.95	0.94
AAV9NXT200	0.96	0.84	0.84
AAV9NXT214	0.91	0.35	1.73
AAV9NXT215	0.81	0.01	2.64
AAV9NXT253	0.68	1.27	0.42
AAV9NXT254	0.95	0.78	1.50
AAV9NXT256	0.91	0.17	0.89
AAV9NXT259	0.60	0.00	3.02
AAV9NXT261	0.85	0.01	0.69
AAV9NXT263	0.83	0.05	0.93
AAV9NXT264	0.66	0.00	0.82
AAV9NXT265	0.95	0.01	0.95
AAV9NXT267	0.14	0.00	0.86
AAV9NXT268	0.06	0.00	1.07
AAV9NXT269	0.02	0.00	1.56
AAV9NXT270	0.02	0.01	0.91
AAV9NXT271	0.01	0.28	1.15
AAV9NXT272	0.02	0.01	2.14
AAV9NXT273	0.04	0.01	2.56
AAV9NXT325	0.77	2.51	1.38
AAV9NXT326	0.82	1.10	0.92
AAV9NXT327	1.12	1.60	1.08

FIG. 18 cont'd

AAV9NXT328	0.89	1.43	1.09
AAV9NXT329	0.95	1.14	1.02
AAV9NXT330	0.84	1.28	0.88
AAV9NXT331	0.91	0.69	0.91
AAV9NXT333	0.95	1.13	1.48
AAV9NXT334	0.77	0.98	0.64
AAV9NXT335	1.04	0.96	0.40
AAV9NXT336	0.55	0.02	3.75
AAV9NXT361	0.70	0.69	1.23
AAV9NXT383	0.23	0.01	3.02
AAV9NXT384	0.88	0.01	2.26
AAV9NXT385	0.23	0.01	3.14
AAV9NXT386	0.83	0.01	0.53
AAV9NXT387	0.66	0.01	2.35
AAV9NXT388	0.12	0.01	3.21
AAV9NXT389	0.55	0.01	3.46
AAV9NXT390	0.12	0.03	4.29
AAV9NXT391	0.27	0.01	2.39
AAV9NXT439	0.20	0.60	0.95
AAV9NXT440	0.57	0.34	1.69
AAV9NXT442	0.63	0.05	8.61
AAV9NXT443	0.48	0.28	29.85
AAV9NXT444	0.21	0.12	15.48
AAV9NXT446	0.01	0.00	1.32
AAV9NXT447	1.03	0.27	1.53
AAV9NXT448	2.57	2.71	1.12
AAV9NXT449	1.33	0.44	0.90
AAV9NXT450	0.34	0.21	1.35
AAV9NXT451	0.25	0.49	1.13
AAV9NXT452	0.74	0.71	0.94
AAV9NXT453	0.76	0.18	0.98
AAV9NXT454	0.68	0.53	0.90
AAV9NXT455	0.23	0.07	0.77
AAV9NXT456	0.31	0.14	1.00
AAV9NXT457	0.67	0.31	1.09
AAV9NXT458	0.70	0.61	0.94
AAV9NXT459	0.27	0.18	0.98
AAV9NXT460	0.87	0.38	0.99
AAV9NXT462	3.14	2.47	1.00
AAV9NXT464	0.36	0.33	1.12
AAV9NXT465	0.98	0.34	1.23
AAV9NXT466	0.39	0.46	0.96
AAV9NXT467	0.75	0.24	1.08
AAV9NXT468	0.02	0.02	1.17
AAV9NXT469	0.01	0.00	1.31
AAV9NXT470	0.01	0.00	0.89
AAV9NXT472	0.02	0.00	2.69
AAV9NXT473	0.02	0.00	2.36
AAV9NXT485	0.50	0.22	2.97

FIG. 18 cont'd

AAV9NXT488	1.00	0.06	1.94
AAV9NXT491	1.64	1.21	1.02
AAV9NXT492	1.29	0.83	0.67
AAV9NXT493	2.40	0.79	1.22
AAV9NXT494	0.62	0.04	2.34
AAV9NXT495	2.32	0.47	0.99
AAV9NXT496	0.36	0.21	0.77
AAV9NXT497	0.98	0.27	0.96
AAV9NXT498	1.95	1.63	1.49
AAV9NXT499	0.38	0.01	1.62
AAV9NXT500	0.04	0.03	1.40
AAV9NXT501	0.02	0.00	2.36
AAV9NXT502	0.01	0.39	1.16
AAV9NXT503	0.01	0.01	1.34
AAV9NXT504	0.02	0.00	0.90
AAV9NXT505	0.02	0.11	1.29
AAV9NXT510	0.71	0.64	1.81
AAV9NXT511	0.26	0.02	6.56
AAV9NXT512	0.03	0.00	1.34
AAV9NXT513	0.68	0.46	0.96
AAV9NXT514	0.04	0.01	2.54
AAV9NXT515	0.07	0.29	0.89
AAV9NXT516	0.11	0.01	4.66
AAV9NXT517	0.04	0.01	2.85
AAV9NXT526	0.73	0.19	0.88
AAV9NXT527	0.47	0.12	2.09
AAV9NXT528	0.55	0.05	0.87
AAV9NXT529	1.83	0.83	1.37
AAV9NXT530	1.50	1.17	0.83
AAV9NXT531	0.77	0.25	0.90
AAV9NXT532	1.86	1.71	1.48
AAV9NXT533	0.27	0.19	1.18
AAV9NXT534	1.49	0.26	1.35
AAV9NXT535	0.83	0.69	1.57
AAV9NXT543	0.18	0.05	9.50
AAV9NXT544	1.05	0.78	1.04
AAV9NXT545	0.61	0.33	1.08
AAV9NXT546	1.02	0.64	1.37
AAV9NXT547	1.38	0.62	1.25
AAV9NXT548	0.85	0.39	1.05
AAV9NXT549	0.47	0.60	0.93
AAV9NXT550	1.22	0.96	0.97
AAV9NXT551	0.85	1.40	0.99
AAV9NXT552	1.39	2.38	1.14
AAV9NXT553	1.87	1.88	1.02
AAV9NXT554	2.63	11.09	3.79
AAV9NXT555	0.85	0.76	0.78
AAV9NXT556	1.52	1.20	2.21
AAV9NXT557	0.58	0.25	1.68

FIG. 18 cont'd

AAV9NXT559	0.73	1.21	1.18
AAV9NXT560	0.74	0.56	1.48
AAV9NXT561	1.02	0.01	1.83
AAV9NXT562	1.09	0.68	1.32
AAV9NXT563	1.15	0.00	1.13
AAV9NXT564	1.46	1.26	1.20
AAV9NXT565	0.58	0.01	2.53
AAV9NXT566	0.83	0.05	3.51
AAV9NXT567	0.54	0.01	2.74
AAV9NXT575	0.67	0.01	4.87
AAV9NXT576	0.08	0.02	6.40
AAV9NXT579	0.78	0.27	1.34
AAV9NXT580	1.46	1.24	1.37
AAV9NXT581	0.69	0.75	1.65
AAV9NXT582	0.87	0.63	1.07
AAV9NXT583	1.16	1.47	1.29
AAV9NXT584	0.83	0.83	0.96
AAV9NXT585	1.41	1.44	1.14
AAV9NXT586	0.66	0.90	0.73
AAV9NXT587	1.20	0.67	0.99
AAV9NXT588	0.94	0.02	0.95
AAV9NXT589	0.64	0.50	1.02
AAV9NXT590	0.69	0.01	0.96
AAV9NXT591	0.46	0.16	1.31
AAV9NXT592	1.15	0.97	0.95
AAV9NXT593	0.39	0.23	0.85
AAV9NXT594	1.40	1.04	1.30
AAV9NXT595	0.70	0.70	1.10
AAV9NXT596	1.26	0.99	1.16
AAV9NXT597	0.90	0.57	0.95
AAV9NXT598	0.70	0.01	2.57
AAV9NXT611	0.73	0.00	1.50
AAV9NXT613	1.04	0.00	1.66
AAV9NXT615	1.12	0.94	1.14
AAV9NXT657	0.97	1.24	0.97
AAV9NXT658	0.76	0.62	0.95
AAV9NXT659	1.00	0.91	0.68
AAV9NXT660	0.64	0.68	1.39
AAV9NXT661	0.99	1.11	0.90
AAV9NXT662	0.63	0.10	1.70
AAV9NXT663	1.22	1.44	0.97
AAV9NXT664	0.60	0.57	1.17
AAV9NXT665	1.08	0.79	0.87
AAV9NXT666	0.93	0.56	1.04
AAV9NXT667	0.69	1.41	1.25
AAV9NXT668	0.71	0.01	3.18
AAV9NXT670	0.80	0.69	1.98
AAV9NXT703	1.03	0.01	0.74
AAV9NXT704	0.86	0.34	0.68

FIG. 18 cont'd

AAV9NXT705	0.61	0.00	0.62
AAV9NXT706	0.93	0.15	0.93
AAV9NXT707	0.42	0.01	2.01
AAV9NXT708	0.84	0.53	1.98
AAV9NXT709	0.55	0.01	2.35
AAV9NXT710	0.96	0.32	2.13
AAV9NXT712	0.30	0.00	2.00
AAV9NXT716	1.15	1.28	1.32
AAV9NXT718	1.10	1.09	1.29
AAV9NXT720	0.56	0.75	2.32
AAV9NXT722	0.69	0.43	1.33
AAV9NXT723	0.59	0.01	2.11
AAV9NXT724	0.60	0.03	2.69
AAV9NXT725	0.58	0.01	1.08
AAV9NXT726	0.55	0.02	2.16
AAV9N14T	1.06	1.37	1.13

FIG. 19A

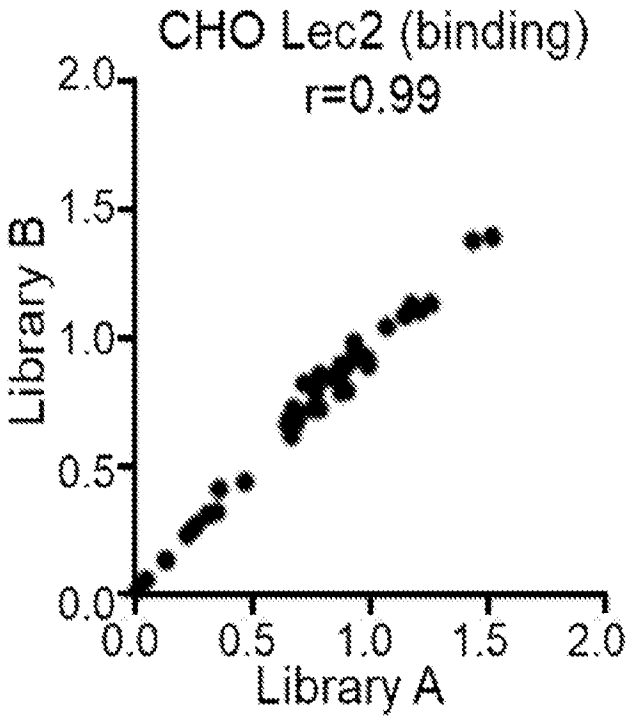


FIG. 19B

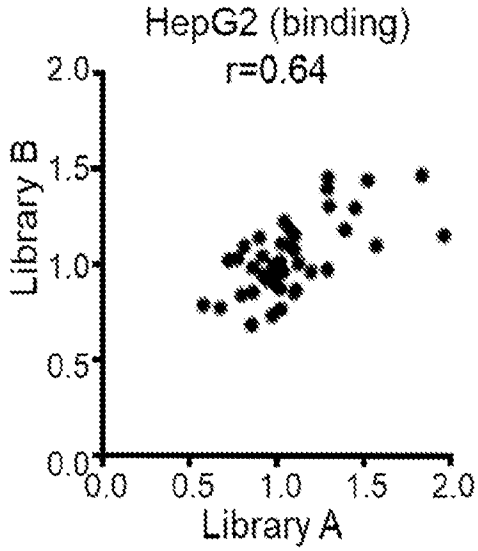


FIG. 19C

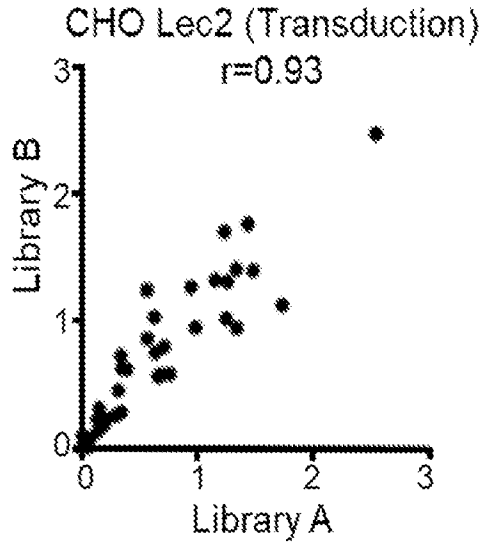


FIG. 19D

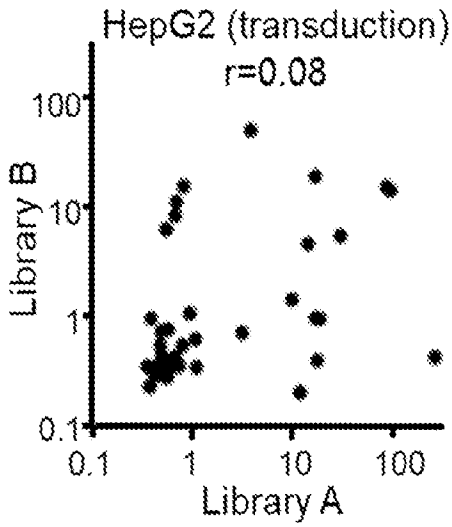


FIG. 20A

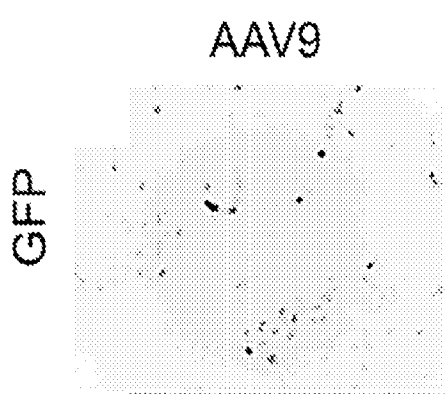


FIG. 20B

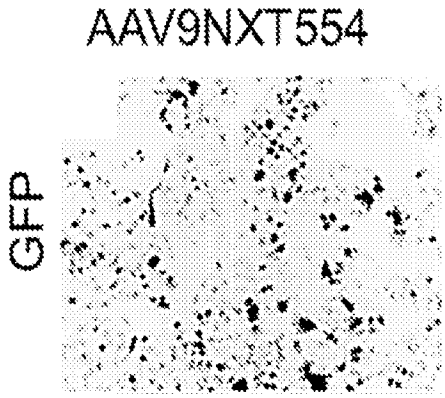


FIG. 20C

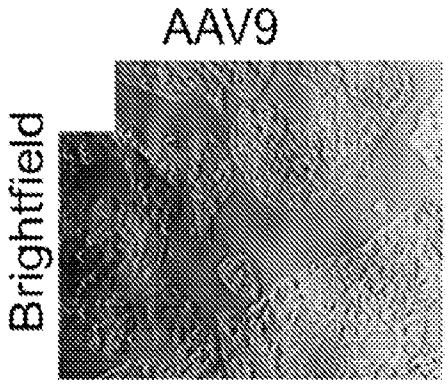


FIG. 20D

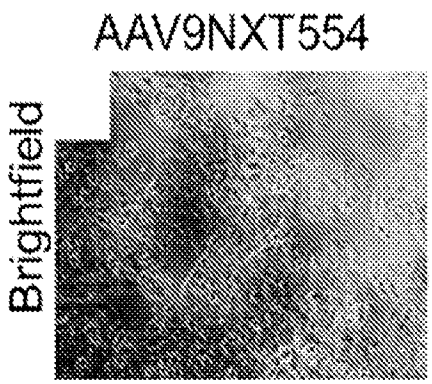


FIG. 21

AAV9NXT mutants showing enhanced ECL Agarose binding or Control Agarose binding[a]

Top 20 AAV9NXT/S mutants with enhanced binding to ECL Agarose				Top 20 AAV9NXT/S mutants with enhanced binding to Control Agarose			
Rank	AAV9NXT Mutants	Binding efficiency (PD values)		Rank	AAV9NXT Mutants	Binding efficiency (PD values)	
		ECL Agarose	Control Agarose			ECL Agarose	Control Agarose
1	AAV9NXT543*	29.84	4.56	1	AAV9NXT554	1.20	16.06
2	AAV9NXT443*	28.93	12.21	2	AAV9NXT443*	28.93	12.21
3	AAV9NXT444*	13.39	7.78	3	AAV9NXT444*	13.39	7.78
4	AAV9NXT442*	11.29	6.89	4	AAV9NXT442*	11.29	6.89
5	AAV9NXT511*	10.33	5.24	5	AAV9NXT511*	10.33	5.24
6	AAV9NXT576*	8.49	3.98	6	AAV9NXT543*	29.84	4.56
7	AAV9NXT388*	6.90	2.51	7	AAV9NXT575*	6.49	4.06
8	AAV9NXT390*	6.57	3.54	8	AAV9NXT576*	8.49	3.98
9	AAV9NXT575*	6.49	4.06	9	AAV9NXT516*	5.99	3.68
10	AAV9NXT336*	6.43	2.55	10	AAV9NXT390*	6.57	3.54
11	AAV9NXT516*	5.99	3.68	11	AAV9NXT720	3.29	3.50
12	AAV9NXT712	5.25	2.08	12	AAV9NXT517	2.88	2.73
13	AAV9NXT385*	5.21	2.31	13	AAV9NXT336*	6.43	2.55
14	AAV9NXT391*	5.06	2.25	14	AAV9NXT388*	6.90	2.51
15	AAV9NXT383	4.66	2.13	15	AAV9NXT598*	4.28	2.51
16	AAV9NXT668*	4.41	2.28	16	AAV9NXT215	2.63	2.44
17	AAV9NXT598*	4.28	2.51	17	AAV9NXT385*	5.21	2.31
18	AAV9NXT566	4.08	2.23	18	AAV9NXT668*	4.41	2.28
19	AAV9NXT565	3.40	2.09	19	AAV9NXT567	3.25	2.27
20	AAV9NXT389	3.29	2.04	20	AAV9NXT391*	5.06	2.25

a Top 20 mutants showing enhanced binding to ECL Agarose or Control Agarose are listed. Fifteen of 20 mutants show enhanced binding to both types of agarose beads, which are indicated with asterisks.

FIG. 22A

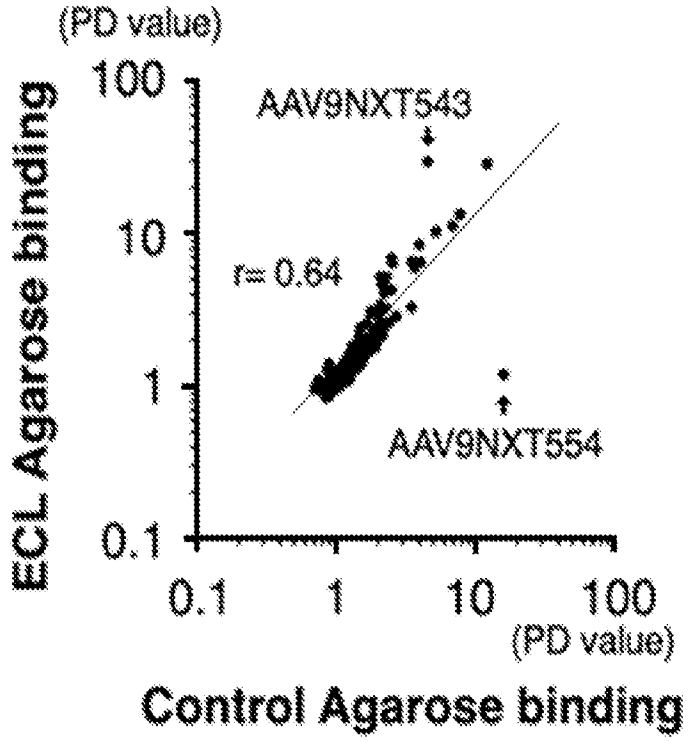


FIG. 22B

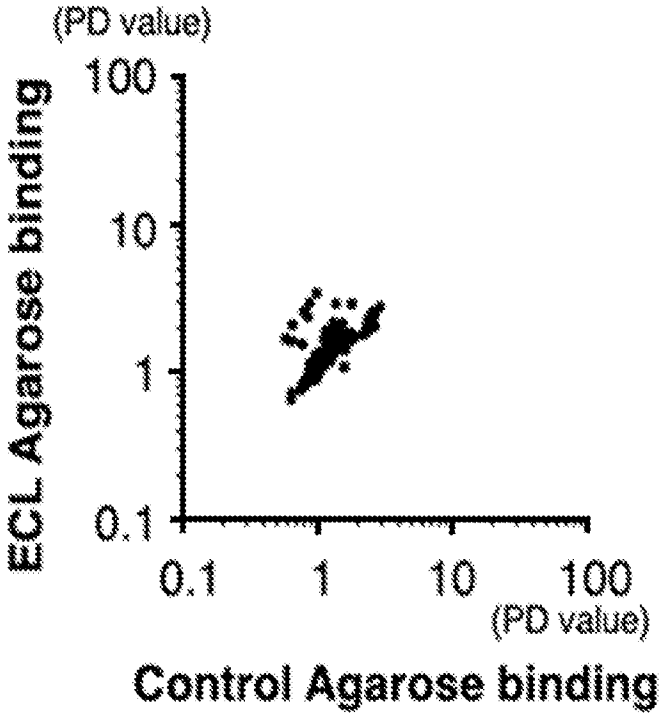


FIG. 23A

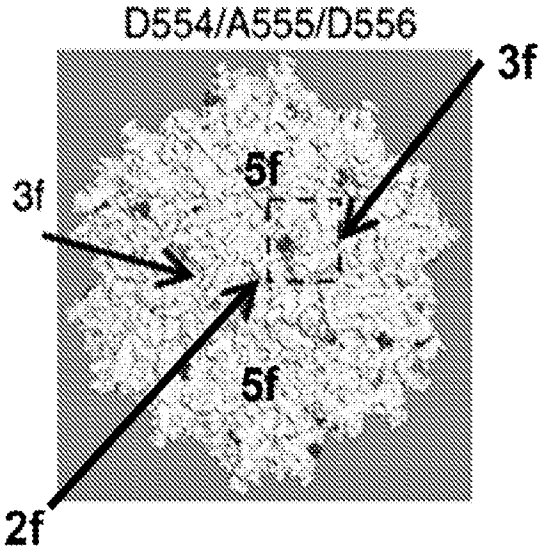


FIG. 23B

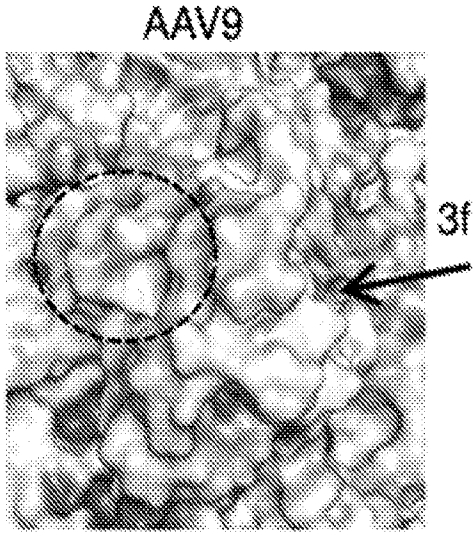


FIG. 23C

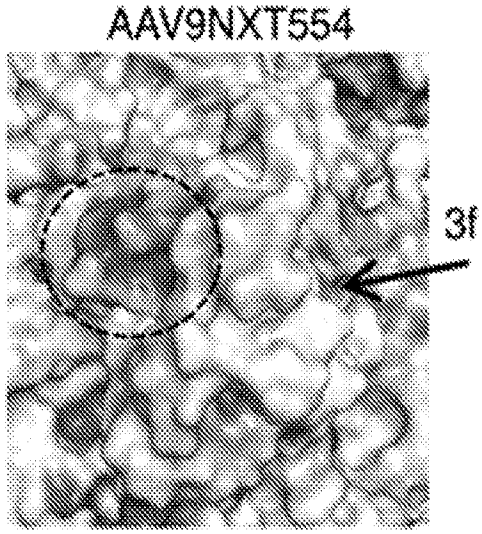


FIG. 24

Relative quantify of viral genome DNA of AAV9NXT/S mutants in mouse tissues following intravenous administration

AAV capsid	Relative quantity of viral genome DNA (PD values determined by AAV DNA Barcode-Seq)									
	Brain	Liver	Heart	Intestine	Spleen	Muscle	Pancreas	Kidney	Testis	Lung
AAV9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAV9NXS107	0.71	1.02	0.89	0.55	0.81	0.68	0.83	0.78	0.82	0.64
AAV9NXS147	0.56	0.60	0.52	0.68	0.68	0.60	0.63	0.61	0.76	0.60
AAV9NXS153	1.22	1.20	1.22	1.12	1.10	1.12	0.96	1.12	1.04	0.99
AAV9NXS154	0.78	0.79	0.84	0.82	0.97	0.75	0.87	0.88	0.97	0.84
AAV9NXS160	0.92	0.89	0.97	0.90	1.02	0.92	0.95	0.90	0.93	0.81
AAV9NXS179	0.90	0.88	0.90	0.91	0.82	1.05	0.90	0.94	1.07	0.83
AAV9NXS194	0.65	1.02	0.99	1.06	1.00	0.88	0.97	0.94	0.72	1.04
AAV9NXS198	1.05	0.83	0.92	0.90	1.10	1.03	0.86	1.02	1.24	1.15
AAV9NXS266	0.34	0.01	0.38	0.17	3.09	0.37	0.70	1.11	1.19	0.18
AAV9NXT14	0.95	0.81	0.87	0.91	0.90	0.93	0.85	0.86	0.80	0.79
AAV9NXT35	1.00	0.75	0.86	0.90	0.88	0.78	0.83	0.82	1.02	0.80
AAV9NXT41	0.30	0.80	0.70	0.53	0.86	0.59	0.69	0.61	0.65	0.59
AAV9NXT57	0.29	0.24	0.41	0.18	0.81	0.30	0.31	0.38	0.75	0.24
AAV9NXT66	0.44	0.01	0.11	0.18	0.83	0.30	0.40	0.66	0.69	0.30
AAV9NXT88	0.21	0.01	0.05	0.02	0.71	0.12	0.21	0.29	0.59	0.11
AAV9NXT94	0.27	0.03	0.14	0.10	0.79	0.23	0.24	0.29	0.68	0.22
AAV9NXT106	0.54	0.74	0.71	0.69	0.80	0.55	0.70	0.70	0.59	0.69
AAV9NXT113	0.43	0.01	0.11	0.11	0.72	0.25	0.26	0.35	0.69	0.26
AAV9NXT136	0.77	0.85	0.71	0.78	0.73	0.81	0.77	0.72	0.53	0.75
AAV9NXT172	1.01	0.86	0.81	0.98	0.96	0.93	0.84	0.96	0.86	0.95
AAV9NXT174	0.75	0.79	0.76	0.79	0.86	0.87	0.83	0.81	0.87	0.89
AAV9NXT177	0.91	1.05	0.97	1.05	1.03	1.00	1.02	1.07	0.88	0.91
AAV9NXT200	1.07	0.88	0.93	1.02	0.91	1.04	0.97	1.02	1.10	1.01
AAV9NXT214	0.75	0.37	0.43	0.47	0.80	0.71	0.54	0.73	0.95	0.60
AAV9NXT215	1.49	0.13	0.33	0.65	1.05	0.85	0.75	0.94	1.72	0.84
AAV9NXT253	1.21	0.56	1.20	0.84	1.32	0.88	1.11	1.32	1.22	0.79
AAV9NXT254	0.48	0.22	0.32	0.39	0.66	0.41	0.48	0.53	0.90	0.46
AAV9NXT256	0.48	0.10	0.32	0.31	0.85	0.47	0.58	0.83	0.92	0.44
AAV9NXT259	0.14	0.00	0.15	0.11	1.66	0.24	0.77	0.84	1.18	0.08
AAV9NXT261	0.17	0.01	0.69	0.20	2.90	0.67	0.89	2.58	3.00	0.12

SUBSTITUTE SHEET (RULE 26)

FIG. 24 cont'd

AAV9NXT263	0.43	0.02	0.46	0.26	2.26	0.39	0.61	1.02	1.30	0.24
AAV9NXT264	0.19	0.00	0.27	0.17	3.36	0.37	0.57	2.82	1.77	0.09
AAV9NXT265	0.21	0.00	0.25	0.11	3.21	0.38	0.73	1.24	1.17	0.12
AAV9NXT267	0.16	0.01	0.54	0.17	4.50	0.53	0.88	3.48	2.29	0.13
AAV9NXT268	0.18	0.00	0.23	0.11	3.73	0.33	0.83	1.00	1.28	0.10
AAV9NXT269	0.14	0.00	0.24	0.11	2.62	0.35	0.81	0.63	1.09	0.08
AAV9NXT270	0.45	0.01	0.14	0.22	1.30	0.41	0.97	1.09	1.44	0.19
AAV9NXT271	0.75	1.38	0.62	0.57	0.51	0.83	0.48	0.61	0.72	0.82
AAV9NXT272	0.26	0.02	0.25	0.18	2.13	0.34	0.83	0.71	0.64	0.14
AAV9NXT273	0.83	0.01	0.18	0.32	1.17	0.54	0.73	0.76	1.21	0.31
AAV9NXT325	0.23	0.31	0.35	0.27	0.78	0.29	0.41	0.38	0.46	0.41
AAV9NXT326	1.06	1.11	1.09	1.28	1.29	1.15	1.18	1.13	1.16	1.09
AAV9NXT327	0.26	0.29	0.31	0.26	0.58	0.27	0.35	0.31	0.40	0.34
AAV9NXT328	1.17	1.08	1.06	1.12	1.07	1.14	1.05	1.04	1.05	1.02
AAV9NXT329	1.10	0.99	0.99	1.03	1.06	0.98	1.04	1.00	1.08	0.86
AAV9NXT330	1.73	1.47	1.73	1.87	1.62	1.70	1.80	1.72	1.74	1.61
AAV9NXT331	1.00	1.13	1.12	1.16	1.14	1.15	1.10	1.14	1.23	1.08
AAV9NXT333	0.59	0.56	0.59	0.55	0.93	0.52	0.66	0.70	0.79	0.59
AAV9NXT334	0.31	0.42	0.42	0.43	0.73	0.49	0.55	0.50	0.49	0.47
AAV9NXT335	0.45	0.76	0.74	0.80	0.98	0.75	0.85	0.79	0.63	0.64
AAV9NXT336	0.62	0.02	0.13	0.28	0.52	0.46	0.33	0.52	1.04	0.49
AAV9NXT361	1.18	0.72	0.76	0.80	0.74	0.78	0.75	0.89	1.17	1.08
AAV9NXT383	0.18	0.00	0.16	0.09	1.97	0.25	0.72	0.79	1.21	0.08
AAV9NXT384	0.43	0.01	0.19	0.19	2.17	0.59	0.89	1.07	1.33	0.18
AAV9NXT385	0.56	0.01	0.27	0.14	1.81	0.53	0.69	1.02	1.35	0.21
AAV9NXT386	0.45	0.01	0.19	0.20	1.85	0.47	1.08	1.36	1.57	0.26
AAV9NXT387	0.31	0.01	0.14	0.13	1.27	0.28	0.61	0.69	1.02	0.15
AAV9NXT388	0.53	0.01	0.17	0.26	1.55	0.39	0.62	0.86	1.22	0.32
AAV9NXT389	0.35	0.01	0.10	0.17	1.60	0.49	0.64	0.89	0.94	0.16
AAV9NXT390	1.06	0.02	0.20	0.37	1.65	0.68	0.59	0.80	1.44	0.41
AAV9NXT391	0.47	0.01	0.14	0.17	1.21	0.36	0.54	0.86	1.27	0.29
AAV9NXT439	0.82	0.71	0.66	0.62	0.76	0.82	0.78	0.79	0.99	0.74
AAV9NXT440	0.62	0.53	0.49	0.62	0.63	0.46	0.69	0.68	0.99	0.40
AAV9NXT442	0.00	0.02	0.00	0.00	0.01	0.00	0.03	0.10	0.00	0.00

FIG. 24 cont'd

AAV9NXT443	3.04	0.02	0.06	0.38	0.22	1.43	0.05	0.61	1.37	0.46
AAV9NXT444	1.19	0.01	0.00	0.00	0.21	0.00	0.00	1.02	0.00	0.00
AAV9NXT446	0.49	0.33	0.88	0.50	1.20	0.57	0.80	0.87	1.10	0.49
AAV9NXT447	0.31	0.09	0.50	0.40	1.09	0.35	0.59	0.64	0.72	0.25
AAV9NXT448	0.71	0.84	0.87	1.02	0.88	0.85	1.55	0.89	0.61	0.61
AAV9NXT449	0.69	0.27	0.74	0.57	1.12	0.52	0.84	0.93	0.72	0.48
AAV9NXT450	1.07	0.46	0.97	0.62	1.53	0.75	0.79	1.26	1.17	0.68
AAV9NXT451	0.91	0.85	0.89	0.75	0.87	0.79	0.77	0.78	0.82	0.87
AAV9NXT452	0.87	0.61	0.99	0.76	1.33	0.69	0.71	1.34	1.13	0.67
AAV9NXT453	0.81	0.23	0.96	0.60	2.36	0.73	0.84	5.96	2.04	0.61
AAV9NXT454	0.98	0.33	0.90	0.56	1.89	0.65	0.84	1.18	1.14	0.81
AAV9NXT455	0.76	0.18	0.70	0.37	1.50	0.42	0.60	0.87	0.91	0.53
AAV9NXT456	0.66	0.21	0.71	0.57	2.16	0.50	0.84	1.47	1.25	0.56
AAV9NXT457	0.98	0.44	0.86	0.76	1.13	0.56	0.87	0.92	1.06	0.59
AAV9NXT458	0.80	0.95	0.86	0.94	0.94	0.80	0.89	0.96	0.83	0.89
AAV9NXT459	0.83	0.33	0.82	0.61	1.53	0.63	0.82	0.96	1.15	0.62
AAV9NXT460	1.08	0.42	0.99	0.77	1.58	0.85	1.06	2.16	1.20	0.80
AAV9NXT462	1.19	0.58	0.84	1.19	1.04	0.81	0.99	1.16	1.43	0.99
AAV9NXT464	0.70	0.99	0.63	0.69	0.70	0.81	0.63	0.64	0.56	0.75
AAV9NXT465	0.69	0.53	0.70	0.71	0.81	0.60	0.78	0.72	0.70	0.40
AAV9NXT466	0.82	0.46	0.93	0.77	1.12	0.80	0.70	0.85	1.07	0.71
AAV9NXT467	0.52	0.18	0.77	0.48	1.40	0.51	0.92	0.98	1.28	0.41
AAV9NXT468	0.95	0.33	0.99	0.33	2.05	0.62	1.09	1.40	1.41	0.57
AAV9NXT469	0.43	0.02	0.55	0.22	2.17	0.66	1.08	1.17	1.52	0.26
AAV9NXT470	0.65	0.14	0.63	0.34	1.39	0.42	0.82	0.92	1.31	0.37
AAV9NXT472	0.17	0.50	0.38	0.31	0.42	0.34	0.30	0.30	0.25	0.38
AAV9NXT473	0.19	0.03	0.37	0.19	2.08	0.35	0.93	1.14	1.08	0.22
AAV9NXT485	0.99	0.27	0.62	0.59	0.68	0.47	0.60	0.59	0.98	0.58
AAV9NXT488	0.34	0.04	0.50	0.41	1.17	0.42	0.57	0.69	0.92	0.16
AAV9NXT491	1.06	1.11	0.96	0.98	0.82	1.06	0.71	0.97	1.11	1.16
AAV9NXT492	0.39	1.03	0.64	0.72	0.46	0.47	0.56	0.52	0.50	0.53
AAV9NXT493	0.81	0.26	0.93	0.73	1.24	0.67	0.87	0.95	1.32	0.57
AAV9NXT494	0.43	0.26	0.71	0.28	0.74	0.42	0.62	0.67	0.86	0.36
AAV9NXT495	0.68	0.17	0.90	0.41	1.21	0.61	0.84	0.97	1.34	0.49

FIG. 24 cont'd

AAV9NXT496	0.70	0.26	0.64	0.62	1.31	0.57	0.72	1.23	0.83	0.64
AAV9NXT497	0.99	0.31	0.92	0.63	1.54	0.77	1.16	1.39	1.47	0.48
AAV9NXT498	0.39	0.79	0.37	0.39	0.43	0.49	0.25	0.50	0.21	0.40
AAV9NXT499	0.28	0.01	0.26	0.12	1.13	0.28	0.56	0.59	1.11	0.12
AAV9NXT500	0.21	1.10	0.46	0.34	0.39	0.35	0.32	0.45	0.40	0.59
AAV9NXT501	0.09	0.00	0.13	0.05	1.08	0.11	0.53	0.39	0.50	0.04
AAV9NXT502	0.45	1.09	0.76	0.65	0.71	0.68	0.64	0.70	0.51	0.68
AAV9NXT503	0.49	0.52	0.53	0.37	1.00	0.54	0.57	0.60	0.73	0.44
AAV9NXT504	0.24	0.01	0.38	0.22	1.29	0.38	0.71	0.87	0.96	0.09
AAV9NXT505	0.60	0.84	0.70	0.68	0.58	0.71	0.57	0.71	0.71	0.74
AAV9NXT510	0.81	0.59	0.91	0.72	1.13	0.61	0.76	0.87	0.91	0.77
AAV9NXT511	0.36	0.02	0.03	0.03	0.06	0.09	0.04	0.05	0.47	0.05
AAV9NXT512	0.18	0.01	0.32	0.09	2.93	0.24	0.58	0.59	1.20	0.20
AAV9NXT513	0.88	0.36	0.73	0.45	1.56	0.56	0.68	0.75	0.90	0.63
AAV9NXT514	0.11	0.00	0.17	0.03	1.76	0.18	0.38	0.37	0.75	0.09
AAV9NXT515	0.66	0.97	0.72	0.65	0.56	0.71	0.77	0.69	0.73	0.72
AAV9NXT516	0.40	0.02	0.07	0.02	0.32	0.08	0.10	0.10	0.22	0.06
AAV9NXT517	0.22	0.23	0.25	0.21	0.72	0.25	0.20	0.27	0.51	0.29
AAV9NXT526	0.57	0.07	0.61	0.30	3.02	0.44	0.70	0.74	1.12	0.33
AAV9NXT527	0.24	0.18	0.23	0.18	0.94	0.27	0.30	0.31	0.49	0.27
AAV9NXT528	0.45	0.02	0.42	0.26	2.95	0.33	0.58	0.57	1.11	0.37
AAV9NXT529	0.87	0.31	1.09	0.62	1.57	0.54	0.86	0.90	1.17	0.69
AAV9NXT530	0.76	0.58	0.75	0.42	0.54	0.54	0.51	0.59	0.74	0.66
AAV9NXT531	0.76	0.09	0.73	0.37	2.70	0.47	0.79	0.93	1.41	0.71
AAV9NXT532	0.76	0.71	0.80	0.79	0.39	0.63	0.63	0.69	0.67	0.74
AAV9NXT533	0.71	0.09	0.71	0.39	2.51	0.41	0.73	0.86	1.14	0.49
AAV9NXT534	0.64	0.07	0.67	0.35	2.09	0.44	0.66	0.80	1.20	0.44
AAV9NXT535	0.44	1.12	0.60	0.69	0.38	0.73	0.57	0.53	0.67	0.53
AAV9NXT543	0.43	0.06	0.17	0.08	0.37	0.09	0.11	0.22	1.28	0.31
AAV9NXT544	0.75	0.83	0.73	0.85	0.56	0.76	0.65	0.73	0.71	0.72
AAV9NXT545	0.91	0.19	0.84	0.51	2.19	0.58	0.79	1.08	1.25	0.63
AAV9NXT546	0.60	0.83	0.87	0.87	0.78	0.59	0.78	0.80	0.63	0.73
AAV9NXT547	0.82	0.92	0.76	0.82	0.63	0.77	0.81	0.78	0.88	0.70
AAV9NXT548	0.88	0.56	0.78	0.72	0.98	0.68	0.97	0.92	0.78	0.76

FIG. 24 cont'd

AAV9NXT549	0.44	1.21	0.65	0.64	0.47	0.79	1.25	0.51	0.52	0.61
AAV9NXT550	0.95	0.77	0.89	0.90	0.81	0.60	1.00	1.01	0.78	0.94
AAV9NXT551	0.41	1.39	0.62	0.75	0.35	0.76	0.53	0.55	0.41	0.64
AAV9NXT552	0.67	1.34	0.67	0.73	0.32	0.81	0.72	0.60	0.63	0.79
AAV9NXT553	0.55	1.15	0.74	0.84	0.44	0.84	0.81	0.73	0.63	0.70
AAV9NXT554	0.00	1.65	0.60	0.13	0.02	0.02	0.27	0.06	0.07	0.07
AAV9NXT555	1.00	0.61	0.76	0.90	0.95	0.84	1.04	0.99	1.25	1.16
AAV9NXT556	0.30	0.97	0.39	0.56	0.19	0.35	0.33	0.36	0.39	0.28
AAV9NXT557	1.34	0.33	0.96	0.73	2.55	0.65	0.97	1.17	1.40	0.90
AAV9NXT559	0.74	0.48	0.81	0.58	1.60	0.63	0.86	0.89	0.91	0.65
AAV9NXT560	0.89	0.55	0.86	0.60	1.28	0.83	0.72	0.97	0.96	0.95
AAV9NXT561	0.05	0.00	0.09	0.05	1.11	0.06	0.24	0.23	0.32	0.14
AAV9NXT562	0.84	0.66	0.71	0.67	0.77	0.65	0.80	0.62	0.77	0.52
AAV9NXT563	0.07	0.00	0.08	0.03	1.06	0.12	0.20	0.26	0.38	0.24
AAV9NXT564	0.73	0.81	1.08	0.95	1.17	0.84	1.09	0.89	0.67	0.77
AAV9NXT565	0.00	0.00	0.16	0.05	2.22	0.08	0.31	0.40	0.52	0.16
AAV9NXT566	0.18	0.05	0.13	0.09	0.95	0.07	0.23	0.26	0.54	0.12
AAV9NXT567	0.05	0.00	0.20	0.05	2.68	0.15	0.42	0.53	0.81	0.08
AAV9NXT575	0.42	0.01	0.03	0.01	0.05	0.06	0.02	0.02	0.40	0.09
AAV9NXT576	0.28	0.01	0.04	0.04	0.07	0.10	0.03	0.06	0.48	0.06
AAV9NXT579	1.09	0.43	0.94	0.74	1.35	0.73	0.82	0.91	1.31	0.72
AAV9NXT580	0.92	1.02	0.82	0.99	0.65	0.79	0.83	0.78	0.74	0.78
AAV9NXT581	0.76	0.74	0.86	0.75	0.73	0.78	0.74	0.76	1.00	0.70
AAV9NXT582	0.83	0.93	0.84	0.82	0.78	0.81	0.79	0.86	0.80	0.78
AAV9NXT583	0.73	0.83	0.81	0.87	0.65	0.82	0.81	0.75	0.79	0.74
AAV9NXT584	0.71	1.11	0.70	0.91	0.59	0.88	0.72	0.69	0.63	0.78
AAV9NXT585	1.03	0.96	1.01	1.10	0.91	0.96	0.99	0.98	0.91	0.86
AAV9NXT586	0.87	0.83	0.81	0.87	0.78	0.85	0.87	0.83	0.95	0.83
AAV9NXT587	1.09	0.85	0.97	0.85	0.90	0.86	0.93	0.90	1.05	0.93
AAV9NXT588	0.47	0.02	0.53	0.28	2.75	0.50	1.13	1.33	1.60	0.28
AAV9NXT589	1.01	0.58	0.90	0.72	1.09	0.68	0.86	1.12	1.13	0.76
AAV9NXT590	0.57	0.03	0.52	0.33	2.42	0.54	0.92	1.03	1.42	0.33
AAV9NXT591	0.90	0.23	0.88	0.62	1.75	0.61	0.73	1.00	1.17	0.54
AAV9NXT592	0.82	0.88	0.81	0.74	0.75	0.78	0.76	0.79	0.78	0.78

FIG. 24 cont'd

AAV9NXT593	0.76	0.59	0.71	0.61	0.76	0.56	0.67	0.70	0.71	0.68
AAV9NXT594	0.60	0.92	0.85	0.88	0.70	0.81	0.84	0.78	0.68	0.74
AAV9NXT595	0.79	0.95	0.79	0.98	0.53	0.65	0.80	0.82	1.00	0.75
AAV9NXT596	0.75	0.87	0.78	0.71	0.60	0.86	0.84	0.78	0.74	0.68
AAV9NXT597	0.99	0.80	0.78	0.82	0.95	0.74	0.77	0.83	1.01	0.86
AAV9NXT598	0.03	0.06	0.14	0.09	0.61	0.11	0.14	0.18	0.31	0.04
AAV9NXT611	0.00	0.00	0.19	0.07	2.22	0.20	0.34	0.49	0.69	0.09
AAV9NXT613	0.31	0.13	0.41	0.26	2.03	0.49	0.38	0.75	0.70	0.40
AAV9NXT615	0.69	0.69	0.77	0.70	1.10	0.73	0.85	0.91	0.94	0.85
AAV9NXT657	0.26	0.33	0.36	0.31	0.71	0.36	0.39	0.39	0.49	0.33
AAV9NXT658	0.30	0.25	0.31	0.25	1.12	0.36	0.44	0.46	0.70	0.47
AAV9NXT659	0.90	0.73	0.68	0.66	0.76	0.55	0.78	0.77	0.79	0.78
AAV9NXT660	0.16	0.29	0.45	0.31	1.31	0.31	0.50	0.83	0.58	0.39
AAV9NXT661	0.65	0.83	0.74	0.78	0.96	0.68	0.92	0.83	0.85	0.57
AAV9NXT662	0.40	0.21	0.36	0.39	1.37	0.34	0.50	0.66	0.66	0.44
AAV9NXT663	0.96	0.99	0.86	0.87	0.78	0.67	0.76	0.82	0.59	0.81
AAV9NXT664	0.82	0.47	0.69	0.56	1.27	0.56	1.06	0.84	1.00	0.85
AAV9NXT665	0.46	0.76	0.60	0.61	0.71	0.60	0.61	0.60	0.46	0.56
AAV9NXT666	0.46	0.49	0.53	0.65	0.82	0.49	0.55	0.55	0.65	0.66
AAV9NXT667	0.30	0.29	0.38	0.29	0.85	0.47	0.32	0.39	0.59	0.41
AAV9NXT668	0.00	0.00	0.12	0.03	1.24	0.07	0.23	0.26	0.34	0.11
AAV9NXT670	0.01	0.03	0.11	0.05	1.07	0.12	0.22	0.24	0.29	0.23
AAV9NXT703	0.04	0.00	0.12	0.05	1.50	0.10	0.23	0.27	0.37	0.16
AAV9NXT704	1.08	0.20	0.85	0.52	2.48	0.48	0.67	0.93	1.20	0.58
AAV9NXT705	0.00	0.00	0.26	0.07	3.63	0.18	0.58	0.87	1.16	0.05
AAV9NXT706	0.85	0.09	0.80	0.41	3.09	0.59	0.71	0.86	1.16	0.64
AAV9NXT707	0.15	0.01	0.30	0.15	3.71	0.32	0.45	1.55	1.62	0.17
AAV9NXT708	0.62	0.30	0.73	0.58	2.33	0.39	0.71	0.78	0.97	0.61
AAV9NXT709	0.12	0.01	0.14	0.05	1.85	0.11	0.34	0.33	0.55	0.09
AAV9NXT710	0.23	0.65	0.31	0.48	0.36	0.30	0.36	0.28	0.44	0.34
AAV9NXT712	0.06	0.00	0.06	0.03	0.47	0.07	0.06	0.12	0.30	0.07
AAV9NXT716	0.82	0.83	1.01	0.88	1.01	0.82	0.91	0.92	1.17	0.84
AAV9NXT718	0.75	0.37	0.80	0.73	1.28	0.57	3.10	0.80	0.87	0.69
AAV9NXT720	1.06	0.64	0.82	0.68	1.02	0.92	0.70	0.78	0.89	0.71

FIG. 24 cont'd

AAV9NXT722	0.79	0.62	0.72	0.60	1.27	0.65	0.79	0.83	0.98	0.75
AAV9NXT723	0.18	0.03	0.27	0.14	2.49	0.28	0.41	0.51	0.85	0.11
AAV9NXT724	0.27	0.21	0.35	0.29	1.79	0.27	0.38	0.45	0.74	0.27
AAV9NXT725	0.11	0.00	0.19	0.07	2.99	0.23	0.47	0.62	0.82	0.10
AAV9NXT726	0.31	0.00	0.14	0.02	1.53	0.18	0.41	0.35	0.77	0.14
AAV9N14T	1.34	1.62	1.38	1.60	1.24	1.21	1.33	1.38	1.46	1.30

FIG. 25

AAV9 capsid amino acids that were identified as those important for CHO-Lec2 cell binding by two different mutagenesis approaches

Amino acid positions[c]	Mutations leading to a substantial loss of binding[a]			Mutations retaining binding[a, b]		
	Type of mutations	Mutations	PD value	Type of mutations	Mutations	PD value
267, 269	NXT267	G267N/S269T	0.14	AA266	G266A/G267A	1.01
267, 269	NXT267	G267N/S269T	0.14	AA268	S268A/S269A	1.07
388, 390	NXT388	A388N/G390T	0.12	AA388	V389A	0.37
390, 392	NXT390	G390N/S392T	0.12	AA390	G390A/R391A	0.23
440, 441	AA440	I440A/D441A	0.12	NXT440	I440N/Q442T	0.57
450, 451	AA450	T450A/I451A	0.11	NXT450	T450N/N452T	0.34
464, 465	AA464	S464A/V465A	0.19	NXT464	S464N/A466T	0.36
484, 485	AA484	Y484A/R485A	0.15	NXT485	R485N/Q487T	0.50
504, 506	NXT504	P504N/A506T	0.02	AA504	P504A/G505A	0.76
505, 507	NXT505	G505N/S507T	0.02	AA504	P504A/G505A	0.76
543, 545	NXT543	F543N/K545T	0.18	AA544	G544A/K545A	0.77

[a]The levels of AAV capsid bind CHO-Lec2 binding were categorized into two groups: a group showing substantial loss of binding (PD values of <0.2) and a group retaining the ability to bind (PD values of $\geq$ 0.2).

[b]These are mutations that do not result in a substantial loss of binding even though the positions have been identified as those responsible by CHO-Lec2 cell binding when mutated in an alternative manner.

[c]These positions are the positions that, when mutated by either AA or MXT mutation, result in a substantial loss of binding.

FIG. 26A

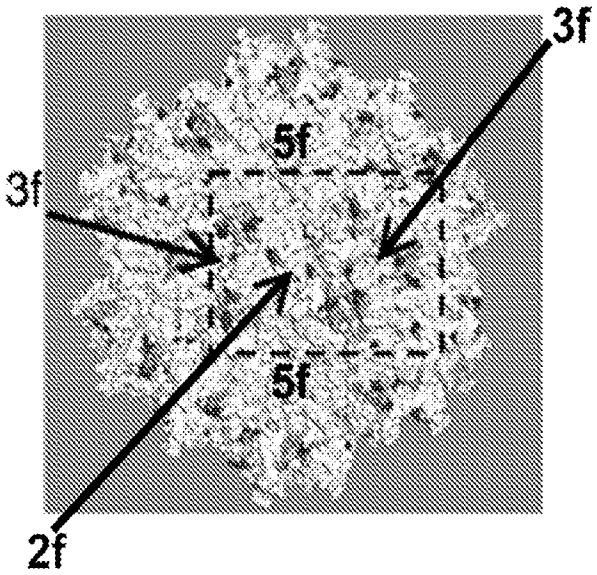


FIG. 26B

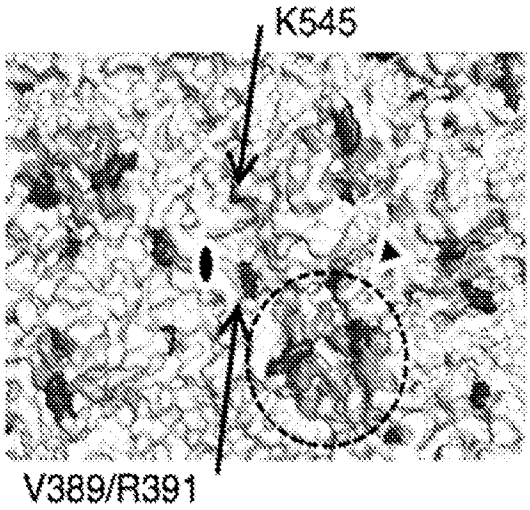


FIG. 27

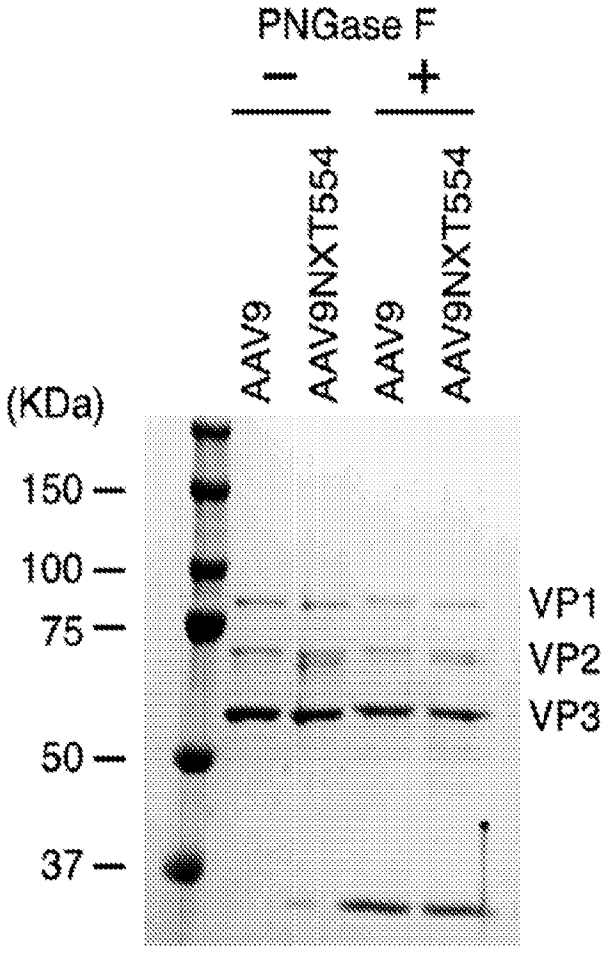


FIG. 28

Name of Mutant	Mutation
AAV9NXT14	S16T
AAV9NXT35	Q37T
AAV9NXT41	R43T
AAV9NXT57	L59T
AAV9NXT66	A68T
AAV9NXT88	Y90T
AAV9NXT94	A96T
AAV9NXT106	E106N
AAV9NXS107	D107N
AAV9NXT113	G115T
AAV9NXT136	A136N
AAV9NXS147	E147N
AAV9NXS153	P153N
AAV9NXS154	D154N
AAV9NXS160	G160N
AAV9NXT172	G174T
AAV9NXT174	G174N
AAV9NXT177	G177N
AAV9NXS179	T179N
AAV9NXS194	A194N
AAV9NXS198	V198N
AAV9NXT200	S200N
AAV9NXS203	M203N
AAV9NXT214	E216T
AAV9NXT215	G217T
AAV9NXT250	P250N/Y252T
AAV9NXT251	T251N/N253T
AAV9NXT252	Y252N/N254T
AAV9NXT253	H255T
AAV9NXT254	L256T
AAV9NXT255	H255N/Y257T
AAV9NXT256	L256N/K258T
AAV9NXT257	Y257N/Q259T
AAV9NXT258	K258N/I260T
AAV9NXT259	Q259N/S261T
AAV9NXT260	I260N/N262T
AAV9NXT261	S261N/S263T
AAV9NXT263	S263N/S265T
AAV9NXT264	T264N/G266T

Name of Mutant	Mutation
AAV9NXT265	S265N/G267T
AAV9NXT266	G266N/S268T
AAV9NXS266	G266N
AAV9NXT267	G267N/S269T
AAV9NXT268	S268N/N270T
AAV9NXT269	S269N/D271T
AAV9NXT270	N272T
AAV9NXT271	D271N/A273T
AAV9NXT272	Y274T
AAV9NXT273	A273N/F275T
AAV9NXT274	Y274N/G276T
AAV9NXT278	S278N/P280T
AAV9NXT288	R288N/H290T
AAV9NXT290	H290N/H292T
AAV9NXT324	E324N
AAV9NXT325	V325N/D327T
AAV9NXT326	T326N/N328T
AAV9NXT327	D327N/N329T
AAV9NXT328	G330T
AAV9NXT329	V331T
AAV9NXT330	G330N/K332T
AAV9NXT331	V331N
AAV9NXT332	K332N/I334T
AAV9NXT333	T333N/A335T
AAV9NXT334	I334N/N336T
AAV9NXT335	A335N/N337T
AAV9NXT336	L338T
AAV9NXT361	E361N/C363T
AAV9NXT362	G362N/L364T
AAV9NXT363	C363N/P365T
AAV9NXT364	L364N/P366T
AAV9NXT365	P365N/F367T
AAV9NXT366	P366N/P368T
AAV9NXT370	D370N/F372T
AAV9NXT372	F372N/I374T
AAV9NXT376	Q376N/G378T
AAV9NXT383	G385T
AAV9NXT384	D384N/S386T
AAV9NXT385	G385N/Q387T

FIG. 28 cont'd

Name of Mutant	Mutation
AAV9NXT386	S386N/A388T
AAV9NXT387	Q387N/V389T
AAV9NXT388	A388N/G390T
AAV9NXT389	V389N/R391T
AAV9NXT390	G390N/S392T
AAV9NXT391	R391N/S393T
AAV9NXT438	P438N/I440T
AAV9NXT439	L439N/D441T
AAV9NXT440	I440N/Q442T
AAV9NXT441	D441N/Y443T
AAV9NXT442	Q442N/L444T
AAV9NXT443	Y443N/Y445T
AAV9NXT444	L444N/Y446T
AAV9NXT445	Y445N/L447T
AAV9NXT446	Y446N/S448T
AAV9NXT447	L447N/K449T
AAV9NXT448	S448N
AAV9NXT449	K449N/I451T
AAV9NXT450	T450N/N452T
AAV9NXT451	I451N/G453T
AAV9NXT452	S454T
AAV9NXT453	G453N/G455T
AAV9NXT454	S454N/Q456T
AAV9NXT455	G455N/N457T
AAV9NXT456	Q456N/Q458T
AAV9NXT457	Q459T
AAV9NXT458	Q458N
AAV9NXT459	Q459N/L461T
AAV9NXT460	T460N/K462T
AAV9NXT461	L461N/F463T
AAV9NXT462	K462N/S464T
AAV9NXT463	F463N/V465T
AAV9NXT464	S464N/A466T
AAV9NXT465	V465N/G467T
AAV9NXT466	A466N/P468T
AAV9NXT467	G467N/S469T
AAV9NXT468	P468N/N470T
AAV9NXT469	S469N/M471T
AAV9NXT470	A472T

Name of Mutant	Mutation
AAV9NXT471	M471N/V473T
AAV9NXT472	A472N/Q474T
AAV9NXT473	V473N/G475T
AAV9NXT485	R485N/Q487T
AAV9NXT488	R488N/S490T
AAV9NXT491	T491N/V493T
AAV9NXT492	T492N
AAV9NXT493	V493N/Q495T
AAV9NXT494	T494N/N496T
AAV9NXT495	Q495N/N497T
AAV9NXT496	N498T
AAV9NXT497	S499T
AAV9NXT498	E500T
AAV9NXT499	S499N/F501T
AAV9NXT500	E500N/A502T
AAV9NXT501	F501N/W503T
AAV9NXT502	A502N/P504T
AAV9NXT503	W503N/G505T
AAV9NXT504	P504N/A506T
AAV9NXT505	G505N/S507T
AAV9NXT510	A510N/N512T
AAV9NXT511	L511N/G513T
AAV9NXT512	R514T
AAV9NXT513	G513N/N515T
AAV9NXT514	R514N/S516T
AAV9NXT515	L517T
AAV9NXT516	S516N/M518T
AAV9NXT517	L517N/N519T
AAV9NXT526	S526N/K528T
AAV9NXT527	H527N/E529T
AAV9NXT528	K528N/G530T
AAV9NXT529	E529N/E531T
AAV9NXT530	G530N/D532T
AAV9NXT531	E531N/R533T
AAV9NXT532	D532N/F534T
AAV9NXT533	R533N/F535T
AAV9NXT534	F534N/P536T
AAV9NXT535	F535N/L537T
AAV9NXT543	F543N/K545T

FIG. 28 cont'd

Name of Mutant	Mutation
AAV9NXT544	G544N/Q546T
AAV9NXT545	K545N/G547T
AAV9NXT546	Q546N
AAV9NXT547	G547N/G549T
AAV9NXT548	T548N/R550T
AAV9NXT549	G549N/D551T
AAV9NXT550	R550N/N552T
AAV9NXT551	D551N/V553T
AAV9NXT552	D554T
AAV9NXT553	V553N/A555T
AAV9NXT554	D554N/D556T
AAV9NXT555	A555N/K557T
AAV9NXT556	D556N/V558T
AAV9NXT557	K557N/M559T
AAV9NXT558	V558N/I560T
AAV9NXT559	M559N
AAV9NXT560	I560N/N562T
AAV9NXT561	T561N/E563T
AAV9NXT562	E564T
AAV9NXT563	E563N/E565T
AAV9NXT564	E564N/I566T
AAV9NXT565	E565N/K567T
AAV9NXT566	I566N
AAV9NXT567	K567N
AAV9NXT575	E575N/Y577T
AAV9NXT576	S576N/G578T
AAV9NXT579	Q579N/A581T
AAV9NXT580	V580N
AAV9NXT581	A581N/N583T
AAV9NXT582	T582N/H584T
AAV9NXT583	Q585T
AAV9NXT584	H584N/S586T
AAV9NXT585	Q585N/A587T
AAV9NXT586	S586N/Q588T
AAV9NXT587	A587N/A589T
AAV9NXT588	Q588N/Q590T
AAV9NXT589	A589N/A591T
AAV9NXT590	Q590N/Q592T
AAV9NXT591	A591N

Name of Mutant	Mutation
AAV9NXT592	Q592N/G594T
AAV9NXT593	T593N/W595T
AAV9NXT594	G594N/V596T
AAV9NXT595	W595N/Q597T
AAV9NXT596	V596N/N598T
AAV9NXT597	Q597N/Q599T
AAV9NXT598	G600T
AAV9NXT611	D611N/Y613T
AAV9NXT612	V612N/L614T
AAV9NXT613	Y613N/Q615T
AAV9NXT614	L614N/G616T
AAV9NXT615	Q615N/P617T
AAV9NXT656	A656N/P658T
AAV9NXT657	D657N/P659T
AAV9NXT658	P658N
AAV9NXT659	P659N/A661T
AAV9NXT660	T660N/F662T
AAV9NXT661	A661N/N663T
AAV9NXT662	F662N/K664T
AAV9NXT663	D665T
AAV9NXT664	K664N/K666T
AAV9NXT665	D665N/L667T
AAV9NXT666	K666N/N668T
AAV9NXT667	L667N/S669T
AAV9NXT668	F670T
AAV9NXT669	S669N/I671T
AAV9NXT670	F670N
AAV9NXT671	I671N/Q673T
AAV9NXT672	T672N/Y674T
AAV9NXT673	Q673N/S675T
AAV9NXT674	Y674N
AAV9NXT701	Y701N/S703T
AAV9NXT702	T702N/N704T
AAV9NXT703	S703N/Y705T
AAV9NXT704	Y706T
AAV9NXT705	Y705N/K707T
AAV9NXT706	Y706N/S708T
AAV9NXT707	K707N/N709T
AAV9NXT708	S708N/N710T

FIG. 28 cont'd

Name of Mutant	Mutation
AAV9NXT709	V711T
AAV9NXT710	E712T
AAV9NXT711	V711N/F713T
AAV9NXT712	E712N/A714T
AAV9NXT713	F713N/V715T
AAV9NXT714	A714N/N716T
AAV9NXT715	V715N
AAV9NXT716	E718T
AAV9NXT717	T717N/G719T
AAV9NXT718	E718N/V720T
AAV9NXT719	G719N/Y721T
AAV9NXT720	V720N/S722T
AAV9NXT721	Y721N/E723T

Name of Mutant	Mutation
AAV9NXT722	S722N/P724T
AAV9NXT723	E723N/R725T
AAV9NXT724	P724N/P726T
AAV9NXT725	R725N/I727T
AAV9NXT726	P726N/G728T
AAV9NXT727	I727N
AAV9NXT728	G728N/R730T
AAV9NXT729	T729N/Y731T
AAV9NXT730	R730N/L732T
AAV9NXT731	Y731N