Vectors and Sequences

Adeno-associated viral vectors were pseudotyped with either extant or ancestral viral capsids. Extant capsids include AAV1 (Genbank [GB] AAD27757.1), AAV2 (GB AAC03780.1), AAV5 (GB AAD13756.1), AAV6.2 (GB EU368910) , Rh.10 (gb AAO88201.1), AAV8 (GB AAN03857.1), AAV9 (GB AAS99264.1), and Rh32.33 (GB EU368926). Ancestral AAV capsids include Anc80L65, Anc81, Anc82, Anc83, Anc84, Anc110, Anc113, Anc126, and Anc127 (KT235804-KT235812). Vector transgene cassettes included CMV.eGFP.T2A.ffLuciferase.SVPA, CMV.ffLucifease.SVPA.(in vitro studies), TBG.LacZ.RBG (liver), TBG.eGFP.WPRE.bGH (liver and muscle immunization study), CASI.hA1AT.FF2A.eGFP.RBG (liver, muscle), and CMV.eGFP.WPRE (retina).

Sequence-structure analysis

A pseudoatomic model of Anc80L65 VP3 was generated with the SWISS-MODEL structure homology modeling server (Biasini et al., 2014), using AAV8 crystal structure (PDB 2QA0) as a template. AAV2 (PDB 1LP3), AAV8 (PDB 2QA0) and Anc80 VP3 structures were further superimposed and color-coded according to residue conservation, using the UCSF Chimera package (Pettersen et al., 2004). A structural alignment of Anc80, AAV2 and AAV8 VP3 was then generated and completed by a non-structural alignment of the VP1/2 domains of these three serotypes, generated with the T-coffee alignment package (Notredame et al., 2000). The spatial distribution of the mutations separating Anc80L65 and AAV8 was also visualized at the inner and outer surface of AAV8 trimer structure, where the variable residues in the structural alignment of Anc80L65 and AAV8 VP3 were represented in blue, and polymorphic residues in red.

Transmission electron microscopy of AAV vectors

Anc80L65 particle morphology was assessed by transmission electron microscopy loading 5 µL of a purified preparation of Anc80L65 vector onto formvar coated 400-mesh copper grids and staining with uranyl acetate. Electron micrographs were obtained under 160,000x magnification at a voltage of 120 kV.

Analytical ultracentrifugation of AAV vectors

Empty/Full particle ratios were determined through analytical ultracentrifugation. Briefly, 500 µL samples of high-titer, purified Anc80L65 pseudotyped vector were analyzed using the Beckman Coulter ProteomeLab XL-I analytical ultracentrifuge available at the MIT Biophysical Instrumentation Facility. The samples were spun at 15000rpm in an eight-hole (50 Ti) rotor at 20°C. Refractive index optical measurements were obtained at regular time points throughout the spin to assess the relative sedimentation rates of all molecular species present in the sample. To interpret and analyze the data obtained, the Lamm equation was solved using SEDFIT (Schuck et al., 2002), and a sedimentation coefficient distribution analysis was run to identify the different species contained in our AAV sample.

Thermal stability assessment

The thermal stability of Anc80L0065 was studied by SDS-Page using the NuPAGE® Bis-Tris Mini Gels kit (1.0 mm, 4-12% polyacrylamide, NP0335BOX, Life Technologies). A 45µL master mix containing 3.0×10^{10} GC of AAV, supplemented with 1X NuPAGE® Lithium Dodecyl Sulfate (LDS) Sample Buffer, was prepared. This mix was then split into three 15 µL aliquots, which were incubated for 10 min at room temperature (RT), 70°C and 99°C, respectively. 10 µL of each sample were further loaded into the gel, which was run in NuPAGE® 1X 3-(N-morpholino)propanesulfonic acid (MOPS) SDS Running Buffer for 2h under constant voltage (90V). The gel was subsequently stained using the SYPRO® Ruby Protein Gel Stain (S-12000, Life Technologies), according to the manufacturer instructions. Briefly, the gel was fixed by incubation in 100mL 50% methanol-7% acetic acid (2x15 min), stained in 60 mL protein gel stain (microwave 30s-agitation 30s-microwave 30s-agitation 5min-microwave 30s-agitation 23min) and washed in 100mL 10% methanol-7% acetic acid (30 min). Finally, the gel was imaged using the ChemiDoc™ MP imaging system (BIORAD) available in the labFor differential scanning fluorescence (Rayaprolu et al., 2013), 25 µL aliquots of each AAV were mixed with 5X SYPRO® Orange (S6651, Life Technologies) prior to loading into a 96-well PCR plate (C18480-96, DENVILLE SCIENTIFIC INC.). Samples were subsequently heated from 30°C to 99°C (at a rate of 0.1°C/6s) while measuring the fluorescence of the SYPRO® Orange dye, using a Realplex 2S MasterCycler Real-Time PCR machine (Eppendorf) (excitation: 450 nm; emission: 550 nm). In each experiment, 25 µL of DPBS (21-031, CORNING) and 25uL of a 0.25 mg/mL lysozyme solution (L6876, SIGMA-ALDRICH) were used as negative and positive controls, respectively. The fluorescence of 25 µL AAV vectors was also monitored in the absence of the dye for fluorescence background subtraction. Fluorescence intensity was further normalized between 0 and 100% and plotted as a function of the temperature.

<u>Tissue Histology</u>

Liver tissue with LacZ was 4% PFA fixed, sectioned at 10 µm, was washed with PBS to remove residual fixative, and was stained at 37C using commercial staining solutions (400 mM Potassium ferricyanide, 400 mM Potassium ferrocyanide, 200 mM magnesium chloride, X-gal 95-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) for 0.5-2 h. Tissues were then mounted onto slides and visualized via light microscopy. To visualize eGFP expression in liver, tissues were fixed overnight in 4% Para-formaldehyde (PFA), washed in phosphate-buffered saline (PBS) for 30 min, sequentially incubated in 10%, 20% and 30% sucrose gradients before freezing in O.C.T compound (Sakura Finetek USA, Torrance, CA). To visualize eGFP expression in muscle, tissues were mounted on cork disks holding 10% Gum Tragacanth (Sigma-Aldrich Cat. No. G1128) and flash frozen using liquid nitrogen cooled Isopentane

(Sigma-Aldrich 27,034-2). Muscle cryosections were prepared at 10 μm. Enucleated eyes were fixed in 4% PFA for 1 hour on ice and then embedded in OCT and frozen prior to cryosectioning. Retinal sections were stained with DAPI (1 μg/ml) for 10 minutes. Sections were imaged on a Leica TCS SP5 confocal microscope.

Tissue DNA Biodistribution and RNA quantitation

Snap frozen tissue was proteinase K digested and genomic DNA (gDNA) was extracted using Blood & Cell Culture DNA Mini kit (Qiagen, Cat. No. 69506) as indicated. Isolated gDNA was quantified using the BioTek plate reading spectrophotometer (Biotek Instruments, Inc. Winooski, VT). Viral genome (vg) distribution in diploid cells were detected and quantified by QPCR using Applied Biosystems® 7500 Real-Time PCR Systems with TaqMan ®PCR master mix reagents (Applied Biosystems ®) and transgene-specific primer/probes as previously described (Wang et al., 2010).

Total RNA was isolated from harvested liver using Qiagen RNeasy mini kit (Qiagen, Cat. No. 74106). Total RNA (1μg) was Dnase treated and reverse-transcribed into cDNA using Qiagen QuantiTect Reverse Transcription Kit (Qiagen, Cat. No. 205311). mRNA was detected and quantified using an Applied Biosystems® 7500 Real-Time PCR System with TaqMan ®PCR master mix reagents with specific primer/probe reaction mixtures: GAPDH (Rh02621745_g1), Rhesus Chorionic Gonadotropin (Rh02821983_g1). TaqMan custom primer/probe suggested reaction conditions were used. In addition, viral genomes within liver tissue were quantified as in murine experiments above.

Quantification of human alpha1-antitrypsin (hA1AT)

The expression level of hA1AT in the serum samples was quantified using the Sandwich Enzyme-linked Immunosorbent Assay (ELISA). High-binding 96-well plates (Thermo Cat. No.

Scientific-Fisher 15051) were coated with primary coating rabbit anti-alpha1-antitrypsin antibody (Sigma Cat. No. A0409) and incubated at 4°C overnight. The coating antibody was diluted in 1X coating buffer to a final concentration of 1000 ng/well. On the next day, plates were washed with PBS + 0.05% Tween 20 (Fisher Cat. No. BP337), and blocked for 2h at room temperature using 10% BSA Blocking Solution (VWR Cat. No. 50-61-01). Alpha1-antitrypsin standards were prepared from a 10mg/ml stock and diluted in 1X blocking solution to form a dilution series. Serum samples were diluted in 1X blocking solution and incubated at 4◦C overnight. Following extensive washing, HRP-conjugated goat anti-human alpha1-antitrypsin antibody (Abcam Cat. No. 7635) was added at 500ng/well and incubated for 2h at room temperature. Plates were washed eight times before adding 100 µL of ABTS peroxidase substrate (VWR Cat. No. 50-66-18) to the wells. OD_{405} values were measured using a Biotek plate-reader.

<u>Neutralizing antibody assay</u>

NABs were assessed *in vitro* as previously described (Calcedo et al., 2009) with the following modifications. Serum from rabbits pre-immunized with AAV1, AAV2, AAV5, AAV6, AAV8, AAV9, rh.10 and rh32.33 was obtained from James M. Wilson, University of Pennsylvania. (Gao et al., 2004). Mouse, rabbit, or rhesus sermu was serially diluted 1:40 to 1:20,971,520 and incubated with 10^9 GC particles of AAV.CMV.*luciferase2*.SVPA transgene for 1 h at 37^{o}C. The mixture was then added to HEK-293 cells on a 96-well plate pre-infected with human adenovirus 5 (hAd5) 24 h prior at a multiplicity of infection of 20 particles/cell. The cells were incubated for 48 h after which D-luciferin containing buffer was added and luminescence was measured using Synergy H1 microplate reader (BioTek; Winooski, VT). Luminescence was normalized against control cells infected with AAV incubated without serum. A neutralizing titer was determined at the dilution at which luminescence was <50% compared with control wells.

Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, T., Kiefer, F., Cassarino, T.G., Bertoni, M., Bordoli, L., *et al.* (2014). SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. Nucleic Acids Res *42*, W252-258.

Calcedo, R., Vandenberghe, L.H., Gao, G., Lin, J., and Wilson, J.M. (2009). Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. J Infect Dis *199*, 381-390.

Gao, G., Vandenberghe, L.H., Alvira, M.R., Lu, Y., Calcedo, R., Zhou, X., and Wilson, J.M. (2004). Clades of Adeno-associated viruses are widely disseminated in human tissues. J Virol *78*, 6381-6388.

Notredame, C., Higgins, D.G., and Heringa, J. (2000). T-Coffee: A novel method for fast and accurate multiple sequence alignment. Journal of molecular biology *302*, 205-217.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. Journal of computational chemistry *25*, 1605-1612.

Rayaprolu, V., Kruse, S., Kant, R., Venkatakrishnan, B., Movahed, N., Brooke, D., Lins, B., Bennett, A., Potter, T., McKenna, R., *et al.* (2013). Comparative analysis of adeno-associated virus capsid stability and dynamics. J Virol *87*, 13150-13160.

Schuck, P., Perugini, M.A., Gonzales, N.R., Howlett, G.J., and Schubert, D. (2002). Size-distribution analysis of proteins by analytical ultracentrifugation: strategies and application to model systems. Biophys J *82*, 1096-1111.

Wang, L., Wang, H., Bell, P., McCarter, R.J., He, J., Calcedo, R., Vandenberghe, L.H., Morizono, H., Batshaw, M.L., and Wilson, J.M. (2010). Systematic evaluation of AAV vectors for liver directed gene transfer in murine models. Mol Ther *18*, 118-125.

Supplemental Figure Legends

Figure S1 eGFP Expression after Viral Vector Intramuscular Injection, related to Figure 4. For muscle-targeted eGFP experiments, mice received a single injection in the gastrocnemius muscle. eGFP expression is observed in transversal and longitudinal muscle sections (first and second columns). Blue staining marks nuclei (DAPI). The morphology of muscle is unchanged as seen in haematoxylin and eosin (H&E) stained sections (third column).

Figure S2 Multiple Sequence Alignment of AAV Isolates used in Ancestral Sequence Reconstruction, related to Figure 1 and 6.

Figure S3 Full Phylogeny and Reconstructed Nodes of AAV Evolutionary Lineage, related to Figure 1. Maximum-likelihood phylogeny relating 75 isolates of AAV (see materials and methods for details regarding generation and validation). Red circles represent evolutionary intermediates reconstructed through ASR. Blue circle represents library of probabilistic space built around Anc80.

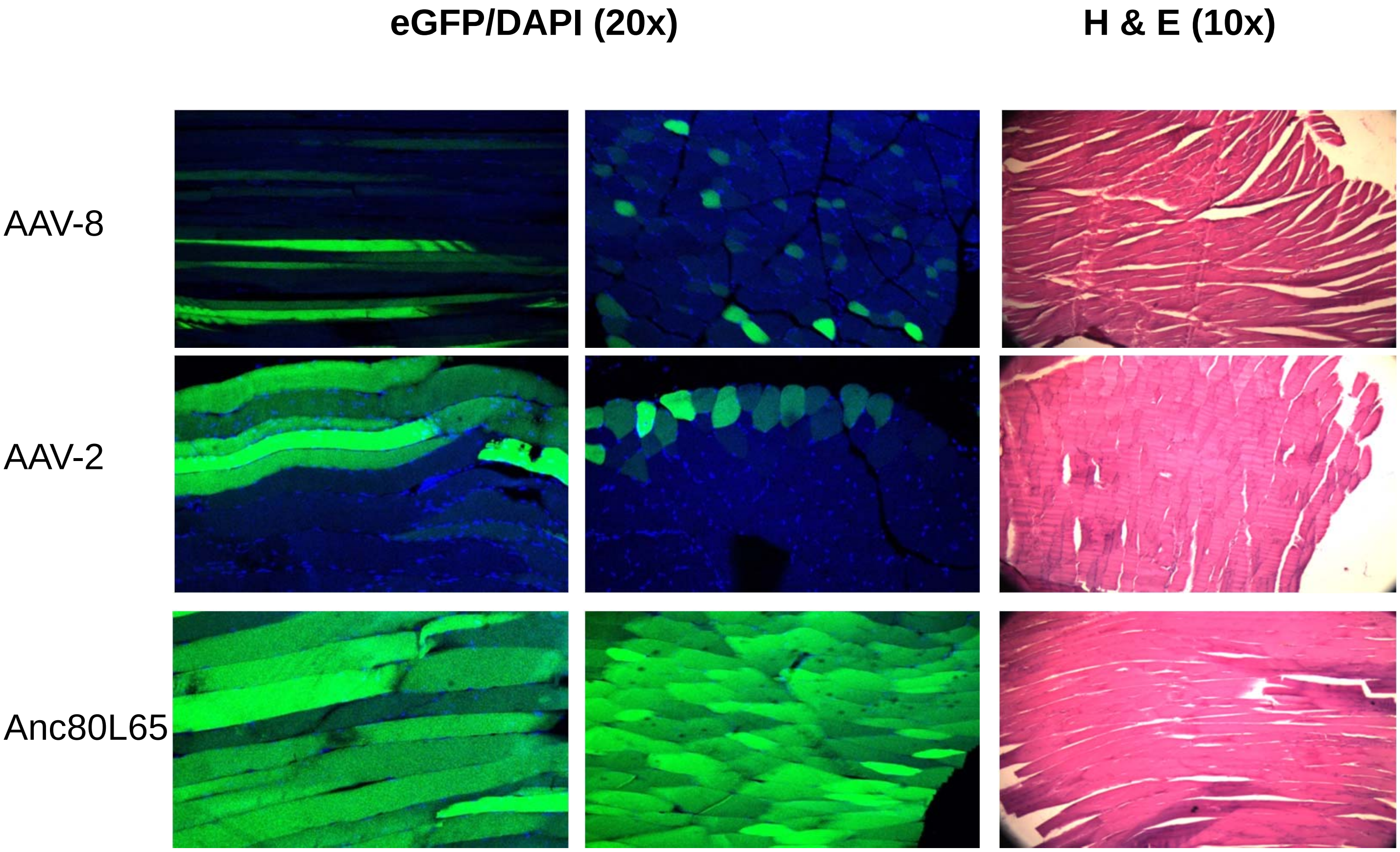
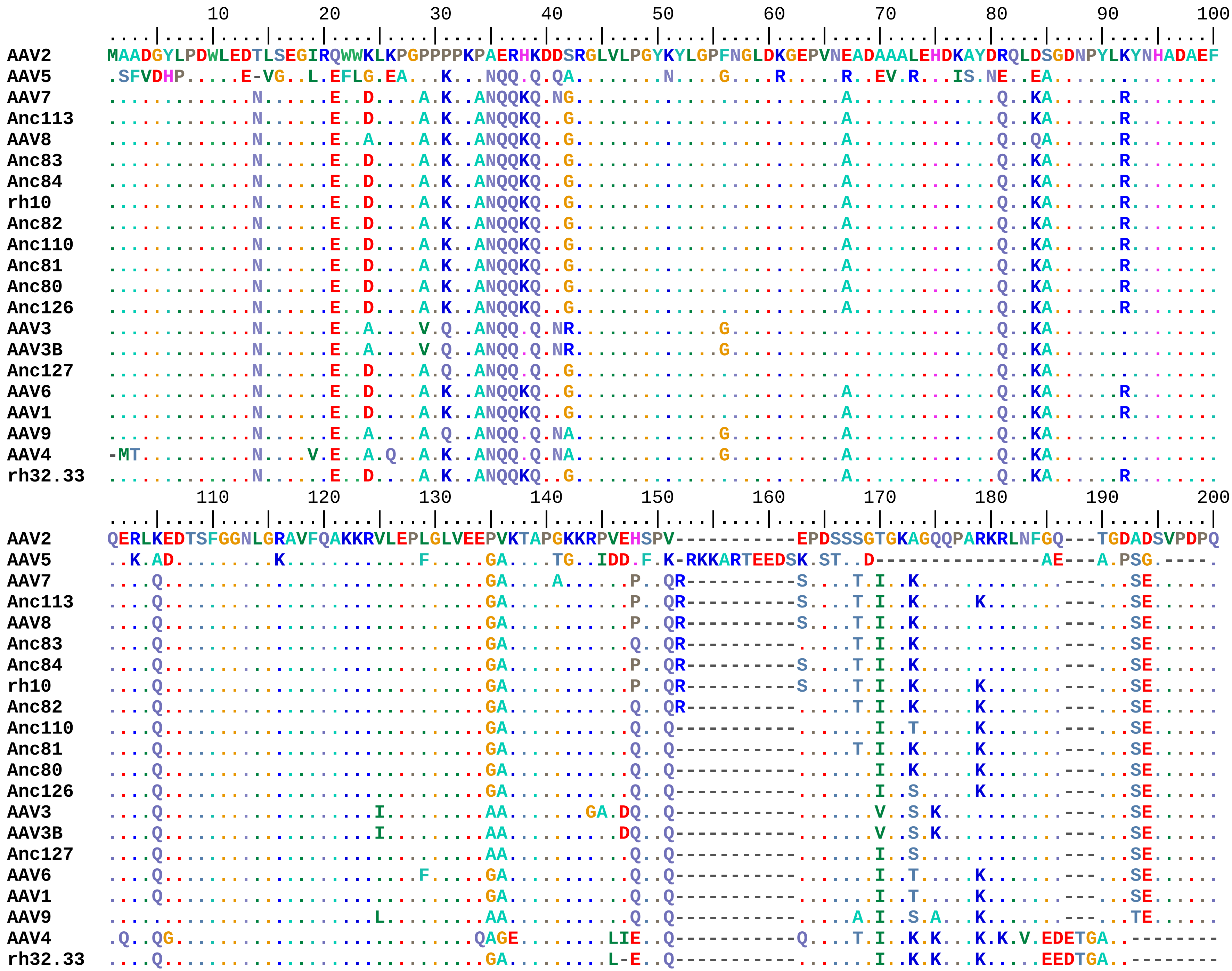


Figure S1


10 20 30 40 50 60 70 80 90 100
AAV2
AAV5
AAV7
Anc113
AAV8
Anc83
Anc84
rh10
Anc82
Anc110
Anc81
Anc80
Anc126
AAV3
AAV3B
Anc127
AAV6
AAV1
AAV9
AAV4
rh32.33
110 120 130 140 150 160 170 180 190 200

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                 210       220       230       240       250       260       270       280       290       300
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAV2      PLGQPPAAPSGLGTNTM----ATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQIS--SQS-GAS-NDNHYFGYSTP
AAV5      Q.QI.AQPA.S..AD..----SA.G.G.LG...Q.......A..D............V.K.....V..S....Q.RE.K--.G.VDG.-.A.A.......
AAV7      ...E......SV.SG.V----.A.G................A..........L..........................--.ET-AG.T...T.......
Anc113    ...E.......V.SG..----.A.G................A..........L..........................--...-AG.T...T.......
AAV8      ...E.......V.P...----.A.G...............S...........L..........................N-GT.-.GAT...T.......
Anc83     ...E.......V.S...----.A.G...............S...........L..........................N-GT.-.G.T...T.......
Anc84     .I.E.......V.SG..----.A.G...............S...........L..........................N-GT.-.G.T...T.......
rh10      .I.E...G.....SG..----.A.G...............S...........L..........................N-GT.-.G.T...T.......
Anc82     ...E.......V.S...----.A.G...........................L..........................N-GT.-.G.T...T.......
Anc110    ...E.......V.S...----.S.G...........................L..........................N-GT.-.G.T...T.......
Anc81     ...E.......V.S...----.A.G................A..........L..........................N-...-.G.T...T.......
Anc80     ...E.......V.S...----.A.G................A..........L..........................--...-...T...T.......
Anc126    ...E.......V.S...----.S.G................A..........L..........................--...-...-...........
AAV3      ...E.....TS..S...----.S.G.........................Q.L..........................--...-...-...........
AAV3B     ...E.....TS..S...----.S.G.........................Q.L..........................--...-...-...........
Anc127    ...E.......V.S...----.S.G...........................L..........................--...-...-...........
AAV6      ...E...T.AAV.PT..----.S.G................A..........L..........................--.A.T...-...........
AAV1      ...E...T.AAV.PT..----.S.G................A..........L..........................--.A.T...-...........
AAV9      .I.E.......V.SL..----.S.G...V...........S.........Q.L..........................-N.T.G.S.-...A.......
AAV4      --.P.EGST..AMSDDSEMRA.A.GA.--VEGGQ.......A..D.......SEGH.T.......V..........RL------GESL-QS.T.N.F...
rh32.33   --.P.EGSDTSAMSSDIEMRA.P.GN.--V.AGQ.S.....A..D.......SEGK.T.......V.........LRL------GTT.-.S.T.N.F...
                 310       320       330       340       350       360       370       380       390       400
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAV2      WGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGY-
AAV5      ..........S.W...........Y.....RS.RV.I.........VQ.S.................DD......V.NGTE....A..PQ..TL.....-
AAV7      ...............................K.R............T...V..........I...S...........................I.....-
Anc113    ...............................K.R............T...V..............S...........................I.....-
AAV8      .................................S..............E..K.........I...............................I.....-
Anc83     ................................................E..K.........I...............................I.....-
Anc84     ................................................E..K.........I...............................I.....-
rh10      ................................................E..K.........I...............................I.....-
Anc82     ..............................................T.E..K.........................................I.....-
Anc110    ..............................................T.E..K.........................................I.....-
Anc81     ..............................................T..............................................I.....-
Anc80     ..............................................T..............................................I.....-
Anc126    ..............................................T..............................................I.....-
AAV3      ...............................K.S........RG.......................................................-
AAV3B     ...............................K.S.................................................................-
Anc127    ...............................K.............................................................I.....-
AAV6      ..............................................T...V..............S...........................I.....-
AAV1      ..............................................T...V..............S...........................I.....-
AAV9      ..............................................D.N.VK................D...........E............I.....-
AAV4      ...........................M...AMRV.I.........TSN.E..V.........I.A..S.E....MDAGQE.S.....N..........C
rh32.33   ...........................L...AMRV.I.........TSN.E..V.........I.A..S.E....MDAGQE.S.....N..........C
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                410       420       430       440       450       460       470       480       490       500
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAV2        --LTLN--NGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN--TPSGTTTQS---RLQFSQAGAS
AAV5        --A...RD.TENPTE....F.......K........E.T.N..E......F.P..N.FK.A...V.....RFVS..--NTG.---------V..NKNL.G
AAV7        --....--....S.......................E...S...............................A..QS-N.G..AGNR---E...Y.G.P.
Anc113      --....--....S.......................E...................................A..QS-.TG..AGNR---E...Y...P.
AAV8        --....--............................Q.T....................................Q--.TG..ANTQ---T.G...G.PN
Anc83       --....--............................Q......................................Q--.TG..AGTQ---T.......P.
Anc84       --....--............................E......................................Q--STG..AGTQ---Q.L.....P.
rh10        --....--............................E...Q..................................Q--STG..AGTQ---Q.L.....PN
Anc82       --....--............................Q......................................Q--.TG..AGTQ---T.......P.
Anc110      --....--............................Q......................................Q--.TG-.AGTQ---T.......P.
Anc81       --....--............................E......................................Q--.TG..AGNR---T.......P.
Anc80       --....--............................E......................................Q--.T...AGNR---T.......P.
Anc126      --....--...................................................................Q--.T...AQNR---E.......P.
AAV3        --....--............................Q...................................N..Q-G.T....N..---..L.....PQ
AAV3B       --....--............................Q...................................N..Q-G.T....N..---..L.....PQ
Anc127      --....--...................................................................Q--.T....Q..---........P.
AAV6        --....--................................................................N..Q--NQ..SAQNK---D.L..RGSPA
AAV1        --....--...................................E............................N..Q--NQ..SAQNK---D.L..RGSPA
AAV9        --....--D...........................Q...E..N.............................K.I--NG..QNQ-Q---T.K..V..P.
AAV4        GLV.G.--TSQ.QTD.NA..................EIT.S..K.....M...................WG.QS.----TT...LNAGTATTN.TKLRPT
rh32.33     GIV.GE---NQNQTD.NA..................EMA.N..K.....M..............L....WH.QS.----T..E.LNQGNAATT.GKIRSG
                510       520       530       540       550       560       570       580       590       600
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAV2        DIRDQSRNWLPGPCYRQQRVSKTSADN-----NNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIF-GKQGSEKTNVDI---EKVMI
AAV5        RYANTYK..F...MG.T.GWNLG.GV.-----RA.VSAFATTNRME.E.ASYQ.P.Q.NGMTNNLQGSNTYALENTM..-NS.PANPGTTATYLEGNML.
AAV7        TMAE.AK.......F........LDQ.-----...NFA............N......V...T.....DR...S......-..T.ATN-KTTL---.N.LM
Anc113      TMAE.AK................LDQ.-----...NFA............N......V...T.....DR...S......-..T.AAN-KTTL---.N.LM
AAV8        TMAN.AK..............T.TGQ.-----...NFA..AG........N..A...I...T......R...SN.I...-...NAARD.A.Y---SD..L
Anc83       NMAN.AK..............T.TSQ.-----...NFA...................V...T.....DR...S..I...-....AG.D...Y---SN..L
Anc84       NMSA.AK..............T.LSQ.-----...NFA...................V...T.....DR...S..I.M.-....AG.D...Y---SN..L
rh10        NMSA.AK..............T.LSQ.-----...NFA...................V...T......R...S....M.-....AG.D...Y---SS..L
Anc82       SMAN.AK..............T.TNQ.-----...NFA...................V...T.....DR...S......-....AGND...Y---SN...
Anc110      SMAN.A...V...........T.TNQ.-----...NFA...............M...V.........DR...S......-....AGND...Y---SQ...
Anc81       SMAN.AK................TNQ.-----...NFA...................V...T.....DR...S......-....AGND...L---DN...
Anc80       SMAN.AK................ANQ.-----...NFA.......................T.....D....M......-....AGNS...L---DN...
Anc126      SMAN.AK................AN..-----...NFA.............................D....M......-....AGAS...L---DN...
AAV3        SMSL.A.............L...AN..-----...NFP..A.S.............................MH.N...-..E.TTAS.AEL---DN...
AAV3B       SMSL.A.............L...AN..-----...NFP..A.S.............................MH.N...-..E.TTAS.AEL---DN...
Anc127      SMAQ.AK................AN..-----...NFA..................................MH.....-....TGAS...L---DN...
AAV6        GMSV.PK................KT..-----...NFT....S..N....E.II...T........KD....M...M..-..ESAGAS.TAL---DN...
AAV1        GMSV.PK................KT..-----...NFT....S..N....E.II...T.........D....M...M..-..ESAGAS.TAL---DN...
AAV9        NMAV.G..YI...S.......T.VTQ.-----....FA.P..SSWA....N..M..........EG.DR...L..S...-....TGRD...A---D....
AAV4        NFSNFKK......SIK..GF...ANQ.YKIPATG.DSLIKYE.HST.D..W.ALT...P..TAGPADS..S--NSQ...A.PKQNGN.ATVP---GTLIF
rh32.33     .FAFYRK.......VK...F...ASQ.YKIPASGGNALLKYD.H.T..N.W.NIA...P..TAGPSDGD.S--NAQ...P.PSVTGN.TTSA---NNLLF
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                  610       620       630       640       650       660       670       680       690       700
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAV2      TDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPST
AAV5      .S.S.TQPV.R..YNVG.QMA..N.SSTTAP..GTY.L.EIV..S..ME..............E.GA......A...........MM........G.-I.
AAV7      .N.....P.......E..I..S...AA.TA.Q.QV..N..A.......N..................N..............................PE
Anc113    .N....K........E..V..S...SA.TAPQ.QT..S..A.......N..................N..............................PE
AAV8      .S....K........E..I.AD...QQ.TAPQIGT..S..A.......N..................N............................D.P.
Anc83     .S....K........E..V.AD...QQ.TAPQIGT..S..A.......N..................N............................D.P.
Anc84     .S....K...........V.AD...QQ.TAPIVGA..S..A.......N..................N............................D.P.
rh10      .S....K...........V.AD...QQ.AAPIVGA..S..A.......N..................N............................D.P.
Anc82     .S....K........E..V.A....SA.TAPQ.GT..S..A.......N..................N............................D.P.
Anc110    .N....K........E..A.A..N.SA.T..Q.GL.HN..........N..................N............................D.P.
Anc81     .S....K........E..V.A....SA.TAPQ.GT..S..A.......N..................N..............................P.
Anc80     .S....K...........T.A....SS.TAP..GT..S..A.......N.................................................P.
Anc126    ......K...........T.A....SS.TAP..GT..S..A.........................................................P.
AAV3      ..................T.AN...SS.TAPT.GT..H..A..............................................M..........P.
AAV3B     ..................T.AN...SS.TAPT.RT..D..A..............................................M..........P.
Anc127    ..................T.A....SS.TAP..GT..S..A.........................................................P.
AAV6      ......KA.......RF.T.AV...SSSTDP..G..HVM.A.........................................................PA
AAV1      ......KA.......RF.T.AV.F.SSSTDP..G..HAM.A........................................N................PA
AAV9      .N....K........S..Q.A..H.SAQA..Q.GW.QN..I..........................N...........M................D.P.
AAV4      .S...LAA..ATD.DMW.NLPGGD.SNSNLPTVDRLTAL.AV......N..I.Y....................I............F..........A.
rh32.33   .S....AA...RD.DMF.QIAD.N.NATTAPI.GN.TAM.........N..I.Y..........A.........I............F..........A.
                  710       720       730       740       750       760       770
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
AAV2      TFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
AAV5      S..DVPVS...........T..M....K..............N...DPQF...AP.ST.E.RTT..........P.
AAV7      V.TP........................................FE.QTG...A..SQ..................
Anc113    V.TP.........................................D..T....A..SE..................
AAV8      ..NQS.LN.....................................Y..TS...A.N.E..................
Anc83     ..NQ..LN.....................................Y..T....A.N.E..................
Anc84     ..NQ..LN.....................................Y..T....A.N.E..................
rh10      ...Q..L......................................Y..T....A.N.D.T................
Anc82     ..NQ..LN.....................................Y..T....A.N.E..................
Anc110    ..NQ..LN.....................................Y..T....A.N.E..................
Anc81     ...P............................................T....A...E..................
Anc80     ...P............................................T....A......................
Anc126    ...P............................................A...........................
AAV3      ...P........................................................................
AAV3B     ...P........................................................................
Anc127    ...P........................................................................
AAV6      E...T.................................V......A..A.......N..L.T............P.
AAV1      E...T.................................V......A..A.......N..L.T............P.
AAV9      A.NKD.LN.....................................Y..N..E.A.N.E..................
AAV4      ...STPVN.............Q.D..I...R.......V.F....GQQNSLLWAP.AA.K.T...A.......HH.
rh32.33   ..T..RVD...........A.Q....IE..R.......V.F....GNQSSMLWAP..T.K.T...V..S....NH.
```

Figure S2

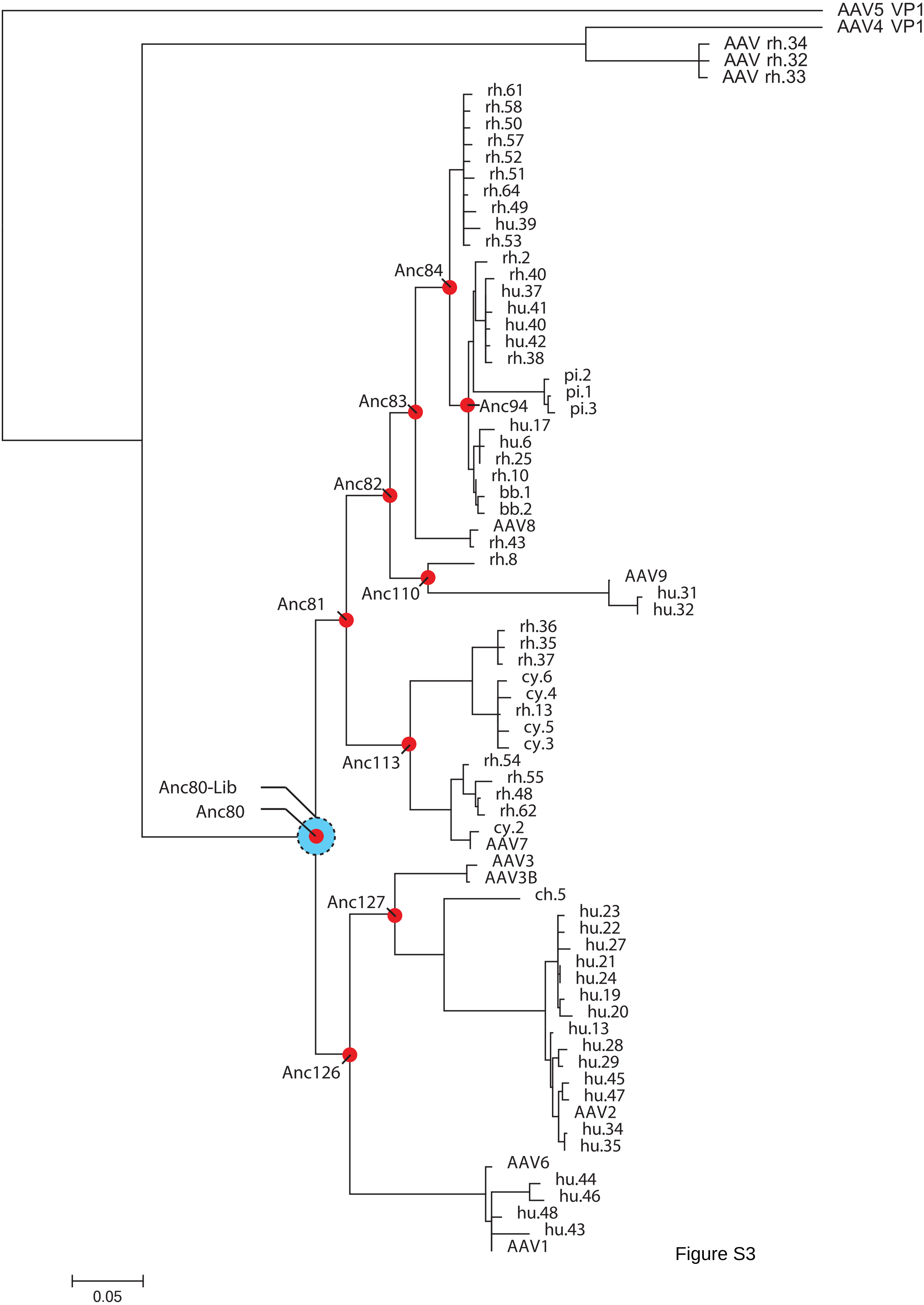
AAV5 VP1
AAV4 VP1
AAV rh.34
AAV rh.32
AAV rh.33
rh.61
rh.58
rh.50
rh.57
rh.52
rh.51
rh.64
rh.49
hu.39
rh.53
rh.2
Anc84
rh.40
hu.37
hu.41
hu.40
hu.42
rh.38
pi.2
pi.1
Anc83
Anc94
pi.3
hu.17
hu.6
rh.25
Anc82
rh.10
bb.1
bb.2
AAV8
rh.43
rh.8
AAV9
Anc110
hu.31
Anc81
hu.32
rh.36
rh.35
rh.37
cy.6
cy.4
rh.13
cy.5
cy.3
Anc113
rh.54
rh.55
Anc80-Lib
rh.48
Anc80
rh.62
cy.2
AAV7
AAV3
AAV3B
ch.5
Anc127
hu.23
hu.22
hu.27
hu.21
hu.24
hu.19
hu.20
hu.13
hu.28
hu.29
Anc126
hu.45
hu.47
AAV2
hu.34
hu.35
AAV6
hu.44
hu.46
hu.48
hu.43
AAV1
0.05

Figure S3

Table S1: Vector Genome Distribution in Mouse Liver, Heart, Spleen, Kidney and Lung, related to Figure 4. Tissue was obtained 7 and 28 days after viral vector injection. Titers were measured using qPCR from isolated mRNA.

	7dpi		28dpi	
	AAV8	Anc80L65	AAV8	Anc80L65
Liver	31.04±7.04	24.19±0.51	8.59±3.1	8.47±1.35
Lung	0.77±0.07	2.2±0.46	0.16±0.04	1.32±0.78
Kidney	0.63±0.06	1.2±0.16	0.22±0.06	0.86±0.26
Heart	0.17±0.06	0.53±0.04	0.1±0.04	0.7±0.32
Spleen	0.02±0	0.19±0.12	0.02±0.01	0.21±0.15

Table S2 Characteristic and Previous Clinical History of Rhesus Macaques Treated with Viral Vectors Injected Via Saphenous Vein, related to figure 4.

Animal ID	Age	Sex	Weight (kg)	Experiment (days)	Previous History	Treatment
AP19	13.5	Female	7.8	71	Inoculated with MVA-HIV vaccine In 2011 diagnosed with early endometriosis	IV Anc80
AP18	9.5	Female	7.2	71	Inoculated with CMV Received anti-CD4 antibody	IV Anc80
AP17	18.5	Female	8.3	71	Inoculated with MVA-HIV vaccine	IV Anc80
AP16	15.5	Female	6.3	70	Inoculated with MVA-HIV vaccine	IV AAV8
AP15	5	Female	5	70	Inoculated with CMV. Received anti-CD4 antibody	IV AAV8
AP14	5.5	Female	5.2	70	Inoculated with CMV Recent weight loss	IV AAV8

Table S3 Complete Blood Count Values for Mice Injected with AAV8 and Anc80L65, related to figure 4. The values outside the references range were highlighted in red (above) and yellow (below).

Species	Test Name	control 2h	AAV2/8 2h	AAV2/Anc80L65 2h	control 24h	AAV2/8 24h	AAV2/Anc80L65 24h	control 72h	AAV2/8 72h	AAV2/Anc80L65 72h	control 7 days	AAV2/8 7 days	AAV2/Anc80L65 7 days	control 14 days	AAV2/8 14 days	AAV2/Anc80L65 14 days	control 28 days	AAV2/8 28 days	AAV2/Anc80L65 28 days	Test Units	Ref Range Low	Ref Range High
Mouse	WBC	4.2	5.2	5.1	7.2	5.7	7.2	5.9	3.9	5	8.4	5.5	5.8	7	8.2	7.3	6.5	5.5	8.1	10^3/µl	2.6	12
Mouse	LYM	3.3	3.8	3.9	6.2	5	6.2	5.3	3.4	4.4	7.4	4.9	5.1	6.2	6.7	6.5	5.8	4.5	7.3	10^3/µl	1.3	9
Mouse	MONO	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.5	0.3	0.2	0.3	0.3	10^3/µl	0.1	0.5
Mouse	GRAN	0.6	1.1	0.9	0.7	0.5	0.7	0.4	0.3	0.3	0.8	0.4	0.5	0.5	1	0.5	0.5	0.7	0.5	10^3/µl	0.4	2.5
Mouse	LYM %	79.1	73.4	77.6	87	86.9	86	90	86.7	88.7	87.4	89.5	87.5	88.2	82.6	88.9	89.4	81.8	90.6	%	0	99.9
Mouse	MONO %	4.5	4.4	3.5	3.3	3.3	3.1	2.8	3.4	3.5	2.9	2.9	3.5	3.5	4.2	3.4	2.9	4.8	2.4	%	0	99.9
Mouse	GRAN %	16.4	22.2	18.9	9.7	9.8	10.9	7.2	9.9	7.8	9.7	7.6	9	8.3	13.2	7.7	7.7	13.4	7	%	0	99.9
Mouse	HCT	49.5	47.7	50.3	46.9	45.2	47.7	43	36.5	42.5	44.7	45.7	44.6	46.1	45.2	47.3	47.2	47.3	46.9	%	32	48
Mouse	MCV	44.6	44.9	44.8	44.3	44.2	44.2	44.7	45.3	44.9	44.7	45.3	44.3	45.1	46.1	45.3	44.9	45.7	45	fl	42	55
Mouse	RDWa	30.7	32	31.2	30.8	30.4	30.6	31.2	31.9	30.9	31.1	32.5	30.6	31.6	32.4	31.7	30.9	32.1	30.8	fl	0	99.9
Mouse	RDW %	16.8	17.8	17	17.2	17.2	17.1	17.5	17.8	17.1	17.4	17.8	17.2	17.3	17.1	17.1	17	17.1	17	%	0	99.9
Mouse	HGB	16.7	16	16.9	16.1	15.4	16.1	14.8	12.5	14.5	15	15.3	15.1	15.6	15.1	15.7	15.7	16	15.8	g/dl	10.1	16.1
Mouse	MCHC	33.8	33.6	33.5	34.3	34.2	33.8	34.5	34.3	34.3	33.6	33.5	33.8	33.8	33.5	33.3	33.2	33.8	33.8	g/dl	29	35
Mouse	MCH	15.1	15.1	15	15.2	15.1	14.9	15.4	15.5	15.4	15	15.2	15	15.2	15.4	15.1	14.9	15.4	15.2	pg	13	18.1
Mouse	RBC	11.07	10.61	11.23	10.57	10.23	10.78	9.62	8.07	9.45	10.01	10.08	10.05	10.2	9.8	10.42	10.51	10.33	10.4	10^6/µl	6.5	10.1
Mouse	PLT	216	423	364	410	208	430	498	205	334	407	175	216	333	342	283	367	476	620	10^3/µl	300	1500
Mouse	MPV	6	5.6	5.6	5.4	5.5	5.6	5.6	6	5.6	5.6	5.6	5.4	5.9	5.5	5.7	5.7	5.7	5.6	fl	0	99.9

Table S4 Serum Biochemistry Values for Mice Injected with AAV8 and Anc80L65, related to Figure 4. The values outside the references range were highlighted in red (above) and yellow (below).

Species	Test Name	control 2h	AAV2/8 2h	AAV2/Anc80L65 2h		control 24h	AAV2/8 24h	AAV2/Anc80L65 24h		control 72h	AAV2/8 72h	AAV2/Anc80L65 72h		control 7 days	AAV2/8 7 days	AAV2/Anc80L65 7 days		control 14 days	AAV2/8 14 days	AAV2/Anc80L65 14 days		control 28 days	AAV2/8 28 days	AAV2/Anc80L65 28 days		Test Units	Ref Range Low	Ref Range High
Mouse	Phosphorus	10.4	8.9	9.9		7.1	7.5	8.5		8	7.2	7.2		6.7	7.2	7.4		6.6	6.1	7.1		5.7	6.4	7.3		mg/dl	5.6	9.2
Mouse	ALT (GPT)	24	28	20		16	21	20		21	18	21		24	17	62		18	26	12		17	24	16		U/l	10	190
Mouse	Total Bilirubin	0.8	0.5	0.4		0.4	0.6	0.3		0.3	0.5	0.8		0.4	0.8	0.6		0.4	0.4	0.4		0.6	0.4	0.3		mg/dl	0.2	0.8
Mouse	ALP	130	114	137		114	102	127		105	94	85		104	95	90		69	95	101		75	76	93		U/l	0	260
Mouse	Albumin	2.8	2.4	2.6		2.6	2.5	2.8		2.7	2.6	2.6		2.6	2.9	2.7		2.2	2.3	2.4		1.7	2.2	2.2		g/dl	3	4
Mouse	GGT	< 10	< 10	< 10		< 10	< 10	< 10		< 10	< 10	< 10		< 10	< 10	<10		<10	<10	<10		<10	<10	<10		U/l	0	<10
Mouse	Creatinine					0.2	0.4			0.2				0.2	0.2			0.2	0.2	0.4		0.2	0.2	0.2		mg/dl	0.5	1.6
Mouse	BUN	24.5	21.4	23.7		27.8	26.8	23		33.3	24.8	26.2		26.4	32	27.3		29.4	27.7	32.6		29.6	28.4	30.6		mg/dl	20	26
Mouse	Cholesterol	*	116	119		113	75	124		104	71	64		111	110	78		116	81	84		97	81	141		mg/dl	28	110
Mouse	Total Protein	*	5.4	6		5.5	5.1	5.7		5.6	4.7	4.8		5.7	5.9	5.4		5.3	5.3	5.4		5.7	5.2	5.4		g/dl	5	7
Mouse	Glucose	*	212	228		171	221	184		183	150	180		143	171	169		176	167	161		162	159	138		mg/dl	190	280
Mouse	Calcium	*	10.4	10.8		8.3	9.9	10.7		10	9.3	9.2		9.6	*	8.5		8.4	10	8.3		9.8	9.3	9.3		mg/dl	7.9	10.5
Mouse	Hemolysis	Mod	slight	slight		slight	Mod	slight		slight	slight	slight		slight	slight	slight		slight	slight	slight		slight	slight	slight				

Hemolysis may cause interference with the following results: false increase in Tbili, TP; false decrease in, ALP.

* not enough serum to do test

blank: below detection limit

Table S5 Levels of Serum Cytokines Measured at Different Timepoints in Mice Injected with Saline, AAV8 and Anc80L65, related to Figure 4. The values within the saline reference range were highlighted in green.

Cytokines (control)	2 hr	24 hr	3 day	7 day
IL-1alpha	215.5	226	135	176.5
IL-1beta	244	213	227.5	222
IL-2	152	73	143	100
IL-3	119	142	115	124
IL-4	198	218	189	200.5
IL-5	94	126	99.5	107
IL-6	228.5	146	124.5	130.5
IL-9	239	264.5	277	225
IL-10	175	234.5	135	156
IL-12p40	764	707	671	641
IL-12p70	280	331.5	284	271.5
IL-13	97.5	117	109	102.5
IL-17A	813	729	605.5	660
Eotaxin	171.5	193	177	178
G-CSF	339	186	171	303
GM-CSF	263	244	272	236
IFN-gamma	271	278	220	248
KC	594	293	288	243.5
MCP-1	123	148	120.5	115
MP-1alpha	511.5	531.5	527	504
MP-1beta	121	144.5	129.5	126
RANTES	576.5	653	505.5	531
TNF-alpha	193	211	189	188

Cytokines (AAV8)	2 hr	24 hr	3 day	7 day
IL-1alpha	255	227	248	188
IL-1beta	252	220	256	227
IL-2	265	106	216.5	149
IL-3	161	120	132	127
IL-4	257	198	217.5	204
IL-5	153.5	108	122.5	121
IL-6	198	144	141	161
IL-9	278	200.5	275.5	239
IL-10	226	203	182	210
IL-12p40	772	794	726	708
IL-12p70	380	286	280	289
IL-13	144.5	117	126	121
IL-17A	1033	703	678	761
Eotaxin	210	185	173	178
G-CSF	331	184.5	191	177
GM-CSF	284.5	234.5	207	258
IFN-gamma	334	240	258	260
KC	339	394	309	324.5
MCP-1	175	131	137.5	141.5
MP-1alpha	555	504.5	505	511
MP-1beta	196	123	133.5	137
RANTES	602	690	571.5	638
TNF-alpha	304	194	207	205

Cytokines (Anc80L65)	2 hr	24 hr	3 day	7 day
IL-1alpha	206	172	271	214
IL-1beta	247	200.5	253	204
IL-2	280	218	212	143
IL-3	149	158.5	133	111
IL-4	232	216	218.5	190
IL-5	130	130	129	105
IL-6	204	134	154	112
IL-9	287	259	283	236
IL-10	211	206	196	174
IL-12p40	716	765	685.5	697
IL-12p70	364.5	320	316.5	255
IL-13	127	125	125	91.5
IL-17A	962	651	814	650
Eotaxin	183	188.5	193	167.5
G-CSF	205	212.5	219	153
GM-CSF	223	250	257	223
IFN-gamma	310	252	284	234.5
KC	321	290	337.5	280
MCP-1	143.5	136	154	109
MP-1alpha	533.5	529	543	453.5
MP-1beta	173.5	160	134.5	115
RANTES	643	804	562	673
TNF-alpha	260.5	220	215	187

Table S6 Complete Blood Count Values for Non-Human Primates Injected with AAV8 and Anc80L65, related to Figure 4. The values outside the references range were highlighted in red (above) and yellow (below).

WBC reference values 3.4-11.2 K/ul

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	5.44	7.78	5.02	5.52	10.9	5.14	5.72	5.86
AP18	5.92	7.22	5.2	4.02	7.06	6.8	7.86	7.14
AP17	8.04	8.04	6.76	6.36	8.32	7.66	8.86	9.38
AP16	6.36	5.64	5	8.3	4.96	4.9	4.92	6.26
AP15	5.52	6.78	6.6	5.94	6.62	7.32	9.2	7.42
AP14	7.86	10.94	8.32	8.76	7.82	9.06	14.62	8.2

Neutrophils reference values 40-68%

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	63.23	56.42	56.28	51.31	37.85	55.71	44.64	46.82
AP18	79.98	72.41	67.16	56.11	58.26	61.49	0.22	59.89
AP17	74.45	68.29	57.87	61.93	73.13	67.32	57.06	73.59
AP16	80.42	64.45	58.41	70.9	58	57.49	45.03	51.27
AP15	68.37	57.44	57.53	57.43	57.84	53.19	32.26	60.72
AP14	72.63	78.34	72.48	72.35	60.34	64.01	59.89	55.86

Lymphocytes reference values 31-64%

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	29.4	29.79	35.48	42.86	55.27	39.68	48.99	46.69
AP18	17.28	19.62	27.05	34.33	35.82	31.97	97.6	34.84
AP17	17.52	16.51	33.76	28.17	23.65	28.59	35.94	21.43
AP16	15.38	27.71	37.5	21.39	30.16	33.34	39.13	35.63
AP15	31.41	34.02	36.92	37.8	38.29	43.49	52.11	28.04
AP14	23.82	18.08	25.95	25.19	35.81	32.78	27.26	39.33

Monocytes reference values 1.5-4%

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	2.81	6.91	4.27	3.34	3.32	2.25	3.1	3.96
AP18	0.77	4.08	3.26	3.64	2.06	2.62	1.47	2.57
AP17	5.02	8.73	4.97	5.21	1.86	2.01	2.16	1.9
AP16	2.04	3.3	1.02	3.06	2.29	2.3	6.63	6.26
AP15	2.15	4.31	2.85	2.43	1.88	1.47	6.23	3.26
AP14	1.77	1.94	0.37	1.52	1.42	0.79	3.84	2.39

RBC reference values 4.98-6.42 M/ul

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	6.52	6.68	6.3	6.57	6.69	6.54	6.89	7.1
AP18	5.24	5.57	5.35	5.47	5.51	5.71	6.69	6.17
AP17	6.46	6.94	6.34	5.39	6.28	6.62	7.01	6.69
AP16	5.35	5.9	5.37	5.18	4.94	5.45	5.7	5.91
AP15	5.78	5.51	5.35	4.89	4.84	5.81	5.53	5.03
AP14	4.92	5.69	5.21	5.5	5.17	5.13	5.47	5.78

HGB reference values 11.7-14.7 g/dl

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	15.7	16.9	15.7	15.9	16.1	16.1	17	16.8
AP18	12.2	12.8	11.8	12.1	12.4	12.2	13.8	13.8
AP17	14.9	16.1	13.9	13.1	14.3	15.2	15.7	15.5
AP16	13.1	13.7	12.4	12.2	11.4	13.4	13.3	13.4
AP15	13.3	13.7	12.8	11.8	12.2	13.9	13.2	12.2
AP14	11.8	12.5	11.6	12.1	12.4	11.3	12.2	12.7

HCT reference values 37.2-47.1 %

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	48.3	49.3	47.2	48.7	49.4	49.2	51.7	53.2
AP18	35.5	37.7	35.9	36.5	37.2	39.3	41.9	41.3
AP17	44.4	47.6	43.5	36.9	43.7	46.1	49.1	47.4
AP16	38.2	42.4	38	36.6	35.1	38.3	40.3	42.1
AP15	42.1	39.8	38.9	35.8	35.2	42.8	40.4	36.4
AP14	33.3	38.6	35.5	36.8	34.8	34.6	36.9	38.6

MCV reference values 69-79 fl

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	74.1	73.8	74.9	74.2	73.9	75.2	75.1	75
AP18	67.7	67.6	67.1	66.7	67.5	68.8	62.7	66.9
AP17	68.8	68.6	68.6	68.5	69.6	69.7	70	70.8
AP16	71.4	71.8	70.7	70.7	71	70.2	71.1	71.3
AP15	72.8	72.3	72.7	73.2	72.7	73.6	73	72.4
AP14	67.7	67.8	68.1	67	67.4	67.5	67.5	66.7

PLT reference values 190-536 K/ul

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	360	396	417	646	541	403	435	432
AP18	409	442	432	765	780	560	644	625
AP17	515	535	554	737	724	525	614	474
AP16	485	509	427	708	721	494	504	545
AP15	351	385	326	613	665	454	505	456
AP14	549	595	561	875	797	586	725	713

MPV referen Normal 8.9-16.1

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	14.1	12.9	12.8	12.2	13.6	13.1	12.2	12.2
AP18	9.6	10.9	8.4	9.7	8.1	9	8.7	10.2
AP17	8.4	8.7	8.9	9.9	9.7	7.7	8	11.4
AP16	9.3	10.4	8.8	10.1	10.4	9.5	9.6	11.1
AP15	13.7	15.4	15.1	11.6	9.7	12.4	11.3	11.3
AP14	10.8	11.1	10.7	10.2	8.9	9.3	9	10.1

Table S7 Serum Biochemistry Values for Non-Human Primates Injected with AAV8 and Anc80L65, related to Figure 4. The values outside the references range were highlighted in red (above) and yellow (below).

ALT reference values 0-59 U/l

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	49	33	45	42	25	38	40	33
AP18	34		34	23	22	16	22	17
AP17	59	38	57	34	25	51	80	44
AP16	45	35	49	37	40	40	73	58
AP15	55	33	65	28	21	37	28	25
AP14	32	19	38	23	23	21	22	17

AST reference values 0-46 U/l

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	59	24	36	31	27	28	24	24
AP18	48		26	19	21	10	17	14
AP17	62	33	38	26	41	25	34	23
AP16	55	31	38	28	29	38	31	22
AP15	72	30	40	22	22	34	24	20
AP14	47	26	24	18	21	20	21	25

GGT reference values 0-69.9 U/l

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	40	41	40	39	38	43	43	48
AP18	35		33	32	33	27	34	34
AP17	42	45	38	36	38	38	44	41
AP16	41	44	38	38	40	42	43	42
AP15	59	62	59	60	55	56	56	57
AP14	60	64	58	59	65	53	53	55

Total bilurrubin reference values 0-0.39 mg/dl

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
AP18	0.2		0.1	0.1	0.1	0.1	0.1	0.1
AP17	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1
AP16	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
AP15	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
AP14	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1

Albumin reference values 3.3-4.7 g/dl

Albumin(g/	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	3.8	4.0	3.8	3.8	4.0	4.0	4.1	4.2
AP18	4.3		4.2	4.1	4.1	2.3	4.2	3.9
AP17	4.0	4.1	3.8	3.7	4.1	2.9	3.9	3.3
AP16	4.1	4.2	4.0	3.9	4.0	4.3	4.2	3.0
AP15	4.2	4.3	4.2	3.9	4.1	3.9	3.8	4.1
AP14	4.3	4.6	4.4	4.4	4.6	4.0	4.0	4.1

BUN reference values 9-23 mg/dl

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	11	17	11	11	11	10	10	10
AP18	14		12	14	13	7	13	11
AP17	15	12	14	10	11	10	12	8
AP16	20	15	12	12	12	13	15	10
AP15	21	21	15	19	16	24	10	13
AP14	18	22	15	17	20	13	13	15

Total protein reference values 6-7.8 g/dl

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	6.8	7.0	7.0	6.8	6.8	6.8	6.8	7.2
AP18	6.9		6.8	6.7	6.7	4.4	6.9	6.6
AP17	6.9	7.0	6.5	6.3	6.7	5.8	6.7	6.1
AP16	7.3	7.4	7.0	6.8	7.0	7.2	7.2	5.8
AP15	6.8	7.0	6.9	6.3	6.5	6.4	6.2	6.7
AP14	6.7	7.1	6.7	6.9	6.9	6.1	6.5	6.6

Amylase reference values 18-612 U/l

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	200	209	407	226	419	177	257	575
AP18	228		277	263	298	198	261	255
AP17	170	159	222	138	126	123	117	115
AP16	560	491	461	435	302	468	461	427
AP15	353	348	425	384	382	418	400	386
AP14	200	227	211	248	259	196	195	215

CK reference values 0-1596 U/l

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	1807	534	1312	1727	643	1174	431	448
AP18	3660		238	290	141	143	165	146
AP17	4346	784	1180	796	1455	794	304	401
AP16	2231	496	940	601	386	770	600	237
AP15	1241	571	221	181	330	380	139	181
AP14	1099	779	292	71	176	239	198	566

LDH reference values 0-785 IU/l

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	430	176	326	249	326	405	181	197
AP18	508		257	189	233	184	183	141
AP17	560	259	277	211	479	307	204	228
AP16	366	227	267	196	290	613	153	160
AP15	329	297	375	174	189	308	203	164
AP14	350	338	253	179	217	232	224	365

ALP reference values 0-704 U/l

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	177	195	174	196	108	173	176	153
AP18	116		103	100	110	79	106	95
AP17	205	194	184	248	193	215	219	156
AP16	100	107	104	96	175	89	117	92
AP15	140	148	151	141	163	155	150	154
AP14	97	111	101	105	111	89	110	97

Creatinine reference values 0.7-1.3 mg/dl

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	0.8	0.8	0.8	0.8	0.8	0.8	0.9	0.8
AP18	0.8		0.7	0.7	0.8	0.5	0.7	0.8
AP17	0.9	0.8	0.8	0.7	0.6	0.8	0.8	0.8
AP16	0.9	0.8	0.8	0.8	0.8	0.8	0.9	0.7
AP15	1.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9
AP14	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7

Glucose reference values 33-95 mg/dl

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	80	87	78	63	63	67	77	88
AP18	68		82	77	43	60	67	88
AP17	92	96	71	93	64	79	106	69
AP16	80	36	74	84	76	53	72	66
AP15	79	35	53	58	83	55	55	67
AP14	71	61	73	75	68	56	83	66

Cholesterol reference values 69-205 mg/dl

	Baseline	1 day	3 day	7 day	15 day	30 day	60 Day	Final
AP19	111	114	119	133	161	121	139	148
AP18	143		135	135	156	108	161	159
AP17	127	155	112	148	129	125	148	119
AP16	169	191	164	166	153	176	189	171
AP15	215	224	204	194	207	216	177	205
AP14	105	115	110	114	127	103	120	125