ONLINE METHODS

Mutagenesis and vector production. Domain swapping studies with the AAV2 capsid protein subunit were carried out using the Quik-change site-directed mutagenesis kit using primers (IDT) designed as per manufacturer's instructions (**Supplementary Table 3**). All AAV vectors packaging the cytomegalovirus or CBA (chicken beta actin)-driven luciferase transgene cassette were produced and purified using previously published procedures[21]. Vector genome titers were determined through Q-PCR using primers specific for the firefly luciferase transgene (**Supplementary Table 3**).

Vector characterization. Heparin binding studies with AAV2i mutants were carried out as described earlier with some modifications[10,11]. Briefly, heparin-agarose beads (Sigma) packed in disposable microspin columns (Bio-Rad) were loaded with different AAV vectors and subjected to four wash cycles using phosphate buffer containing 15 mM NaCl followed by elution with varying NaCl concentrations in phosphate buffer. Vector genomic DNA was extracted from fractions collected from each step using a DNeasy kit (Qiagen) and subjected to dot blot analysis using a ^{32}P-labeled probe specific for the luciferase transgene.

The potential role of cell surface heparan sulfate or sialylated glycans in AAV infection was characterized in CHOpgsD and HEK293 cell lines, respectively. Briefly, 2×10^5 HEK293 cells were left untreated or treated for 2 h at 37 °C with 50 mU/ml neuraminidase type III (sialidase) from *Vibrio cholerae* in culture media without serum. At 24 h after infection with AAV2i mutants (multiplicity of infection: 1,000), cell lysates were obtained using passive lysis buffer and subjected to a luminometric assay (Promega). Studies with CHOpgsD cells were carried out using similar conditions.

Vector administration and animal studies. Housing and handling of BALB/c mice used in the current study were carried out in compliance with National Institutes of Health guidelines and approved by IACUC at University of North Carolina-Chapel Hill (protocols #06–300 and #09–117). AAV2i mutants or parental AAV serotype vectors were administered through the intramuscular (right hind limb; gastrocnemius muscle) in a volume of 50 µl PBS or intravenous route (tail vein) in a volume of 200 µl PBS. Luciferase expression in animals was imaged using a Xenogen IVIS100 imaging system (Caliper Lifesciences) after intraperitoneal injection of luciferin substrate (120 mg/kg; Nanolight). Image analysis was carried out using the Living Image software. Luciferase transgene expression in various tissue types was determined as described earlier using a luminometric assay (Promega). Vector genome copy numbers were determined after extraction of genomic DNA at different time intervals from whole blood (10 µl collected from tail vein in heparinized capillary tubes) and various tissue types using a DNeasy kit (Qiagen).

Isolated limb perfusion studies were carried out in BALB/c mice as described earlier[19]. Mice were anesthetized with 1–2% isoflurane throughout the procedure. Prior to injection of AAV vectors at three different doses (1×10^9, 1×10^{10}, or 1×10^{11} vg), a tourniquet was placed on the upper hind limb to restrict blood flow into and out of the hind limb. AAV2i8 or AAV8 vectors were injected into the saphenous vein at a rate of 8 ml/min using a needle catheter connected to a programmable Harvard PHD 2000 syringe pump (Harvard Instruments). Mice received acetaminophen (100 mg/kg/day, in drinking water) for the first 48 h after each surgical procedure. Mice were euthanized at 2 weeks post-injection and limb muscles harvested and separated into six groups (quadriceps, biceps, hamstring, gastrocnemius, shin and foot). Luciferase activity from each muscle group was determined using a luminometric assay and total level of luciferase expression per gram of muscle tissue was determined.

Molecular modeling. Structural models of the Vp3 protein subunit of AAV2i mutants were generated using the SWISS-MODEL online three-dimensional (3D) model building server with the crystal structure of AAV2 supplied as template (PDB ID: 1lp3a)[9]. **Figure 1a** showing 3D capsid surface topology was generated using the programs Pymol (http://www.pymol.org/) and Roadmap[13].

21. Grieger, J.C. *et al.* Production and characterization of AAV vectors. *Nat. Protoc.* **1**, 1412–1428 (2006).

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