Supplementary Methods

Cell culture. Human 293T cells were obtained from American Type Culture Collection (Manassas, VA). Cells were cultured in high glucose Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO) and 100 U/ml penicillin, 100 µg/ml streptomycin (Invitrogen) in a humidified atmosphere supplemented with 5% CO2 at 37 °C.

Vector constructs and production. Briefly, 293T were transfected with AAV (AAV9 rep/cap and AAV-ITR containing transgene expression plasmid [single stranded genome]) and helper plasmid (Fd6) by the calcium phosphate method. 72 h post-transfection cells were harvested and vector purified using a standard iodixanol density gradient and ultracentrifugation protocol. Iodixanol was removed and vector concentrated in PBS by diafiltration using Amicon Ultra 100 kDa MWCO centrifugal devices (Millipore, Billerica, MA). Vector was stored at -80°C until use.

Animals. Animal experiments were approved by the Institutional Animal Care and Use Committee at the Massachusetts General Hospital (MGH), and performed in accordance with their guidelines and regulations. Nude athymic nu/nu mice (age 6-8 weeks) were ordered from the MGH in-house COX-7 facility. C57BL/6N mice (age 6-8 weeks) were purchased from Charles River Laboratories (Wilmington, MA). For tail vein injections of AAV vectors, mice were placed into a restrainer, (Braintree Scientific, Inc., Braintree, MA). Next the tail was warmed in 40°C water for 30 seconds, before wiping the tail with 70% isopropyl alcohol pads. A 100-300 µl volume of vector (in PBS) was slowly injected into a lateral tail vein, before gently clamping the injection site until bleeding stopped.

Bioluminescence imaging. All imaging experiments were performed using the IVIS Spectrum imager outfitted with an XGI-8 gas anesthesia system (Caliper Lifesciences, Hopkinton, MA). Mice were anesthetized and then injected intraperitoneally with 4.5 mg of $_{D}$-luciferin resuspended in 150 µl of PBS. Five minutes post-substrate injection, mice were imaged for luciferase expression using auto-acquisition. Region of interest were selected and imaging were analyzed using LivingImage software (v4.17,Caliper Life Sciences).

Ex-vivo luciferase assay and quantitative PCR for vector genomes. Four weeks post-vector injection, mice were sacrificed and organs (liver, whole brain or cerebellum, and olfactory bulb)

were harvested for analysis of Fluc levels as well as virus genome copies (g.c.). Tissues were quickly removed from the animals, and immediately frozen on liquid nitrogen and stored at -80 °C. For Fluc assay, 50 mg of each tissue was lysed in a 96-deepwell plate (Nunc/Thermo Fisher Scientific, PA, USA) using a stainless steel bead (Qiagen Inc., CA, USA) and 500 µL of Mammalian Protein Extraction Reagent (M-PER; Pierce Biotechnology, IL, USA). Tissues were homogenized in a TissueLyser II (Qiagen Inc.) at 7.0 Hz 4x three minutes. Next, the plate was centrifuged for 5 minutes at 1200 rpm and 20 µL of tissue homogenate was transferred to 96-well white bottom plate and analyzed using 100 µL of Bright-Glo™ Fluc substrate reagent (Promega, WI, USA) and a plate luminometer (Dynex Technologies, VA, USA). A Bradford assay was performed to normalize each Fluc value to the total amount of protein in the sample.
For the quantitative PCR assay to detect AAV genomes in organs, 25 mg of each organ was cut into small pieces using a sterile razor blade. Isolation of mouse genomic and AAV DNA was performed using the DNeasy® Blood and Tissue Handbook (Qiagen, Valencia, CA). Next 100 ng of total DNA was used as a template for a quantitative TaqMan PCR that detects AAV genomes (Poly A region of the transgene cassette).

Histology and GFP expression. At two weeks post-injection, mice were given an overdose of anesthesia and transcardially perfused with PBS. The liver and brain were removed. The brain was separated into two hemispheres. One hemisphere was snap frozen in the vapor phase of liquid nitrogen and stored at -80°C for subsequent QPCR analysis and the other hemisphere was fixed in 4% formaldehyde in PBS and cryoprotected in 30% sucrose for immunohistological analysis of tissue sections. These hemispheres were cut on the sagittal plane in 40 µm sections using a sledging freezing microtome. Double immunohistochemistry was performed in free-floating sections with primary antibodies for GFP (chicken polyclonal, 1:500, Aves Labs, Cat#GFP-1010) and either GFAP (rabbit polyclonal, 1:1000, Sigma-Aldrich, Cat#G9269), or glutamine synthetase (mouse monoclonal, clone GS-6, 1:1000, Millipore, Cat#MAB302). Briefly, the sections were permeabilized in Tris-buffered saline (TBS) with Triton-X 0.5% for 1 h, blocked in 10% normal goat serum for 1 h, and incubated with the primary antibodies overnight at 4ºC. Next day, the sections were thoroughly washed in TBS with Triton-X 0.1%, incubated with the appropriate fluorescently-labeled secondary antibodies for 1 h at room temperature, washed again in TBS with Triton-X 0.1%, and coverslipped with Vectashield mounting media with DAPI (Vector Labs, Cat#H-1200). The number of cortical GFP-positive astrocytes and neurons was counted in every 10^{th} section under the 4x objective of a BX51 Olympus epifluorescence microscope (Olympus, Tokyo, Japan), equipped with a motorized stage and a

DP70 camera that are coupled to a computer through the image analysis software CAST. The region of interest (cortex) was outlined under the 1.25x objective and the number of GFP-positive astrocytes and neurons were counted under the 4x objective using the appropriate tools of the software. Neurons and astrocytes were easily distinguished from each other using morphological criteria. The volume of interest corresponding to the cortex from every 10^{th} section was estimated with the CAST software. Total numbers of GFP-positive neurons and astrocytes in the whole brain cortical mantle were then estimated based on the fractionator principle.