Supplemental Material

Trafficking of AAV vectors across a model of the blood-brain barrier; a comparative study of transcytosis and transduction using primary human brain endothelial cells.

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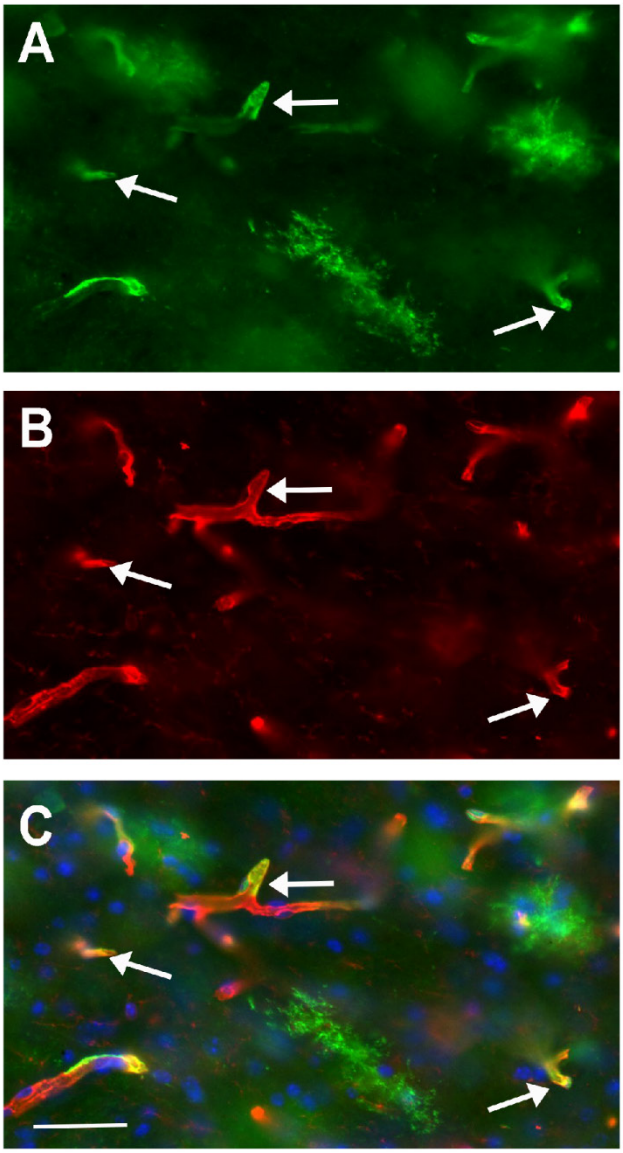
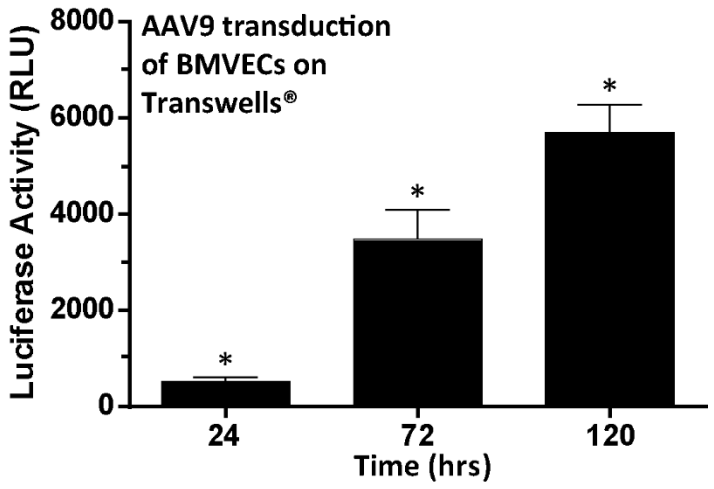
Figure S1



Supplemental Figure 1: *In vivo* transduction of brain endothelium after peripheral intravenous injection of AAV9 vector encoding eGFP. A double immunostaining was performed in order to determine if a single intravenous infusion of AAV9-eGFP could transduce brain endothelium *in vivo* (analysis 4 weeks after injection into the lateral tail vain). Representative images of GFP positive cells (astrocytes and endothelium) transduced by AAV9-eGFP **(A),** and cerebral endothelial cells identified by the marker Tomato-lectin **(B)** in the cortex. The MERGE image **(C)** demonstrates the presence of GFP expressing vessels in the neural tissue (white arrows). Scale bar: 50µm

Figure S2



Supplemental Figure 2: AAV9 transgene expression in primary human BMVEC monolayers grown on Transwells®. BMVEC cultures exhibiting monolayer formation indicative of barrier properties were prepared on collagen-coated cell culture inserts and incubated with AAV9-Fluc vectors ($2x10^5$ gc/cell) for 24, 72, and 120 hours. At the indicated times, viral-mediated transgene expression was assessed using a luciferase assay. Data were normalized to 0-hour controls and are presented as mean luciferase activity in relative light units (RLU) for treatments performed in quadruplicate + SEM. Statistical significance was analyzed as a function of the 24-hour read-outs for each group. Asterisks denote $p < 0.05$.

Movie 1: Nuclear accumulation of intracellular AAV2 in primary human BMVEC cultures. BMVEC cultures were prepared in MatTek dishes, expanded, and washed to remove heparin as previously described. Cultures were incubated with $2.5x10^6$ gc/cell DyLight™-488 conjugated AAV2 (green signal). BMVECs were washed to remove unbound virus and stained with CMTPX (red signal), and imaged using multiphoton live cell microscopy. Images were acquired 24 hours post introduction of the viral vectors. Video was made from a volume-rendered Z-stack taken under 2-photon microscopy. Scale bar is shown in the bottom left corner.

Movie 2: Basolateral trafficking of AAV9 across primary human BMVECs. BMVEC cultures were prepared in MatTek dishes as previously described. Cultures were then incubated with $2.5x10^6$ gc/cell DyLight™-488 conjugated AAV9 (green signal). After 24 hours, BMVECs were washed to remove unbound virus, stained with CMTPX (red signal), and imaged using multiphoton live cell microscopy. Images were acquired 24 hours post introduction of the viral vectors. Video was made from a volume-rendered Z-stack taken under 2-photon microscopy. Scale bar is shown in the bottom left corner.

SUPPLEMENTAL MATERIALS AND METHODS

AAV9 vector for *in vivo* use

For immunofluorescence analysis of transduced cell types, we used a self-complementary (sc) AAV construct encoding eGFP driven by the hybrid CMV-enhancer/chicken beta actin (CBA) promoter.

Animals and tail vein injection

All animal experiments were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care following guidelines set forth by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Female BALB/c mice aged 6-8 weeks were purchased from Jackson Laboratory (Bar Harbor, ME). For tail vein injections of AAV9-CBA-eGFP, 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 200 µl volume of vector ($7.5x10^{12}$ gc/kg, diluted in PBS) was slowly injected into a lateral tail vein before gently finger-clamping the injection site until bleeding stopped.

Histology and GFP expression for *in vivo* transduction analysis

At four weeks post-injection of AAV9-CBA-eGFP, mice were given an overdose of anesthesia and transcardially perfused with PBS and 4% formaldehyde in 1 x PBS. The brain was removed, post-fixed in 4% formaldehyde in PBS for 48 h, cryoprotected for three days in 30% sucrose and then frozen in a dry ice/2-methylbutane bath for immunohistological analysis of tissue sections. Brains were cut on the coronal plane in 40 µm sections using a cryostat microtome. Immunofluorescence was performed in free-floating sections with a primary antibody for GFP (chicken anti-GFP, Aves, 1:500), altogether with a DyLight 594 labeled Lycopersicon Esculentum (Tomato) Lectin (Vector Laboratories) in order to identify blood vessels in the cortical mantel. Briefly, the sections were permeabilized in PBS with Triton-X 0.5% for 2 h, blocked in 5% normal goat serum for 1 h, and incubated with the primary antibody overnight at 4°C. The sections were thoroughly washed in PBS, incubated with 1:1000 goat anti-chicken Alex Fluor 488-conjugated antibody (Cell Signaling, Danvers, MA) for 1 h at room temperature, washed again in PBS, and coverslipped with fluorescent mounting media containing DAPI (Dako, Carpinteria, CA).