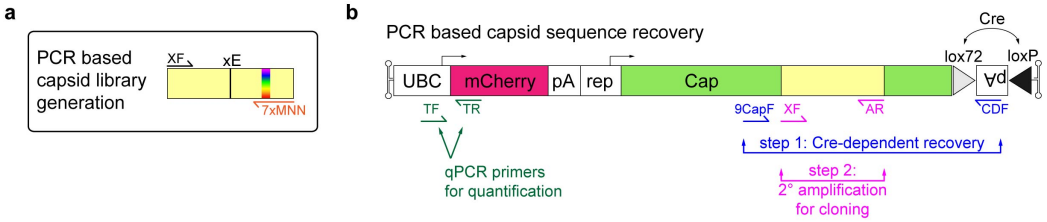


c

Primer sequences for library generation and recovery

Primer	Purpose	Sequence
9CapF	Step 1: forward	CAGGTCTTCACGGACTCAGACTATCAG
CDF	Step 1: reversed by Cre	CAAGTAAAACCTCTACAAATGTGGTAAAATCG
XF	Step 2 forward	ACTCATCGACCAATACTTGTACTATCTCTCTAGAAC
AR	Step 2 reverse	GGAAGTATTCCTTGGTTTTGAACCCA
TF	qPCR forward	GGTCGCGGTTCTTGTTTGTGGAT
TR	qPCR reverse	GCACCTTGAAGCGCATGAACTCCT
7xMNN	588i library generation reverse	GTATTCCTTGGTTTTGAACCCAACCGGTCTGCGCCTGTGCMNN MNNMNNMNNMNNMNNMNNTTGGGCACTCTGGTGGTTTGTG

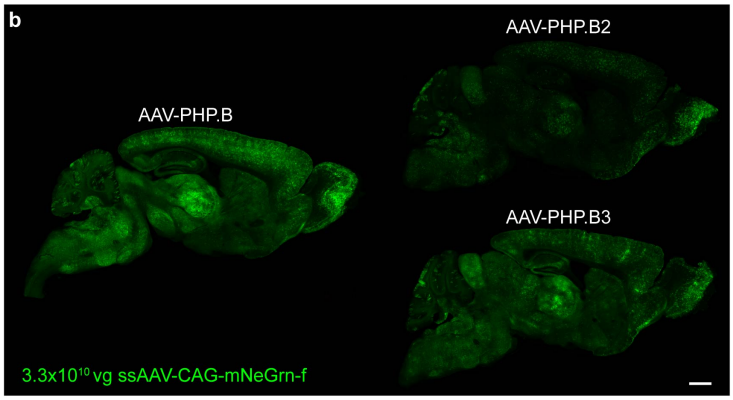
Supplementary Figure 1

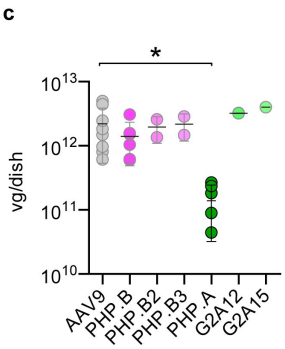
Capsid library fragment generation and Cre-dependent capsid sequence recovery.

(**a**) Schematic shows PCR products (yellow bar) with 7AA of randomized sequence (represented by the full spectrum vertical bar) inserted after amino acid 588. The primers used to generate the library are indicated by name and half arrows. The PCR template was modified to eliminate a naturally occurring EarI restriction site within the capsid gene fragment (xE) (See Online Methods for details). (**b**) The schematic shows the rAAV-Cap-in-cis genome and the primers used to quantify vector genomes (left) and recover sequences that have transduced Cre expressing cells (right). The PCR-based recovery is performed in two steps. Step 1 (blue) provides target cell-specific sequence recovery by selectively amplifying *Cap* sequences from genomes that have undergone Cre-dependent inversion of the downstream polyadenylation (pA) sequence. For step 1, 9CapF functions as a forward primer and the CDF primer functions as the reverse primer on templates recombined by Cre. Step 2 (magenta) uses primers XF and AR to generate the PCR product that is cloned into rAAV-ΔCap-in-cis plasmid (library regeneration) or to clone into an AAV2/9 rep-cap trans plasmid (variant characterization). (**c**) The table provides the sequences for the primers shown in **a** and **b**.

a **AAV capsid variants identified through selection in GFAP-Cre mice**

Variant	AA sequence	nucleotide sequence	% of total	in vivo characteristics	production
AAV-PHP.B	TLAVPFK	ACTTTGGCGGTGCCTTTTAAG	25.00%	Broad CNS transduction	good
AAV-PHP.B2	SVSKPFL	AGTGTGAGTAAGCCTTTTTTG	11.76%	Broad CNS transduction	good
AAV-PHP.B3	FTLTTPK	TTTACGTTGACGACGCCTAAG	7.35%	Broad CNS transduction	good
AAV-PHP.A	YTLSQGW	TATACTTTGTCGCAGGGTTGG	40.00%	increased astrocyte selectivity	poor
G2A12	QAVRTSL	CAGGCGGTTAGGACGTCTTTG	33.33%	similar to AAV9 in the brain	excellent
G2A15	LAKERLS	CTTGCGAAGGAGCGGCTTTCG	20.00%	similar to AAV9 in the brain	excellent

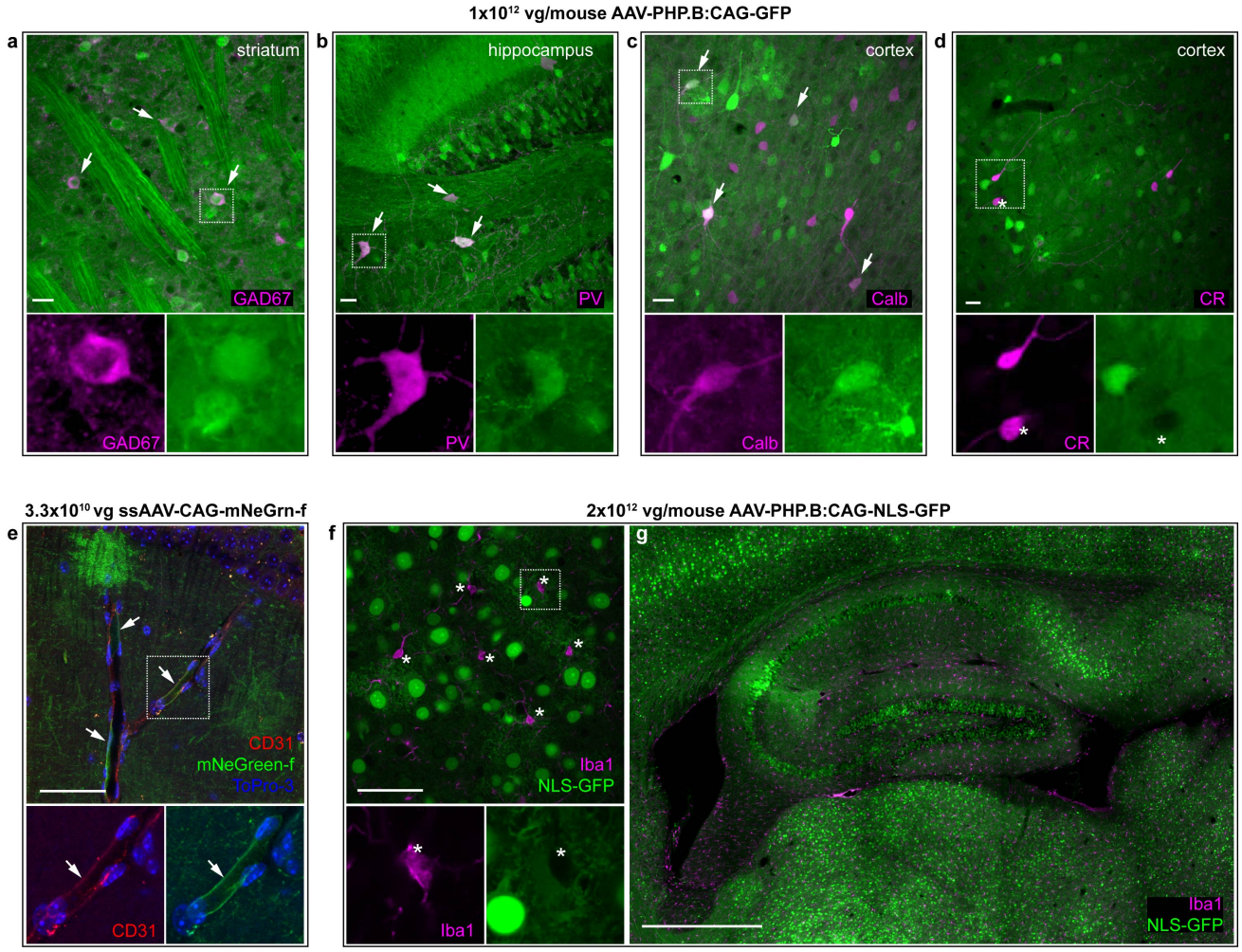




Supplementary Figure 2

The most enriched variants recovered from *in vivo* selections in GFAP-Cre mice.

(**a**) The table provides the 7-mer AA and nucleic acid sequences, percentage enrichment (% of total variants sequenced), capsid characteristics, and production efficiencies of the three most enriched variants from each selection. (**b**) Images of representative sagittal brain sections from mice assessed 2 weeks after injection of $3.3x10^{10}$ vg/mouse of ssAAV-CAG-mNeonGreen-farnesylated (mNeGreen-f) packaged into AAV-PHP.B or the second or third most enriched variants, AAV-PHP.B2 and AAV-PHP.B3. Data are representative of 2 (AAV-PHP.B) and 3 (AAV-PHP.B2 and AAV-PHP.B3) mice per group. (**c**) DNase-resistant vg obtained from preparations of the individual variants recovered from GFAP-Cre selections. Yields are given as the number of purified vector genome (vg) copies per 150 mm dish of producer cells; mean ± s.d. *$p<0.05$, one-way ANOVA and Tukey's multiple comparison test. The number of independent preparations for each capsid is shown within the bar.



Supplementary Figure 3

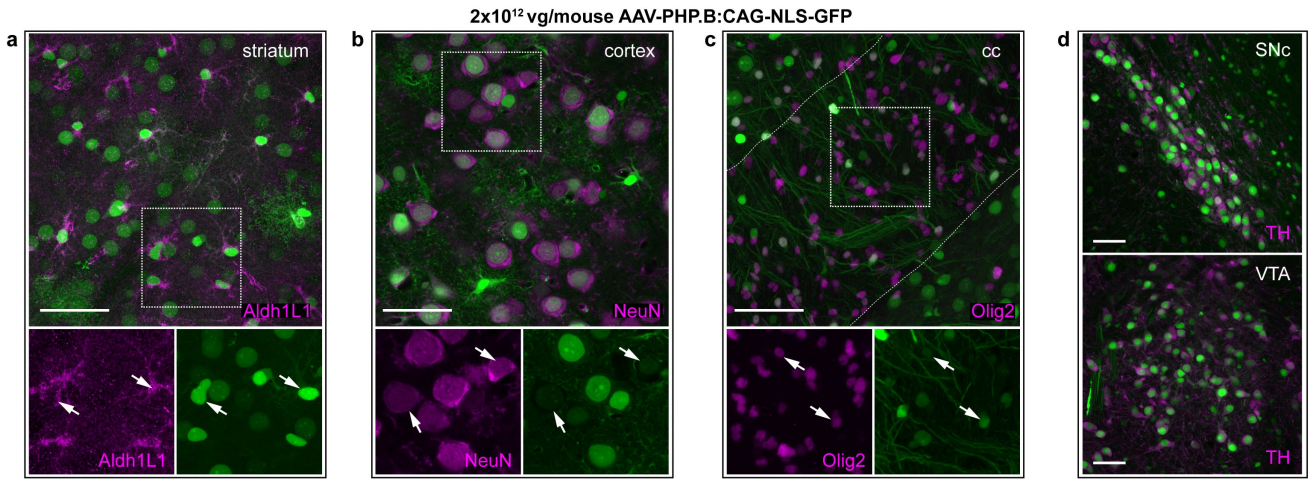
AAV-PHP.B transduces several interneuron cell types and endothelial cells but does not appear to transduce microglia.

(**a-d**) Adult mice were injected with $1x10^{12}$ vg of AAV-PHP.B:CAG-GFP and assessed for GFP expression 3 weeks later. Representative images show IHC for GFP (**a-c**) or native GFP fluorescence (**d**) in green together with IHC for the indicated antigen (magenta) and brain region. (**e**) Adult mice were injected with $3.3x10^{10}$ vg of ssAAV-PHP.B:CAG-mNeGrn-f and assessed at 2 weeks post injection. Native fluorescence from mNeGrn-f co-localizes with some endothelial cells expressing CD31. (**f**, **g**) Adult mice were injected with $2x10^{12}$ vg of ssAAV-PHP.B:CAG-NLS-GFP and assessed at 3 weeks post injection. Images show native NLS-GFP expression along with Iba1. Asterisks indicate cells that express the indicated antigen, but not detectable GFP. Parvalbumin (PV), Calbindin (Calb) and Calretinin (CR). Scale bars: 20 μm (**a-d**); 50 μm (**e**, **f**) and 500 μm (**g**).

Supplementary Figure 4

Long-term eGFP expression in the brain following gene transfer with AAV-PHP.B.

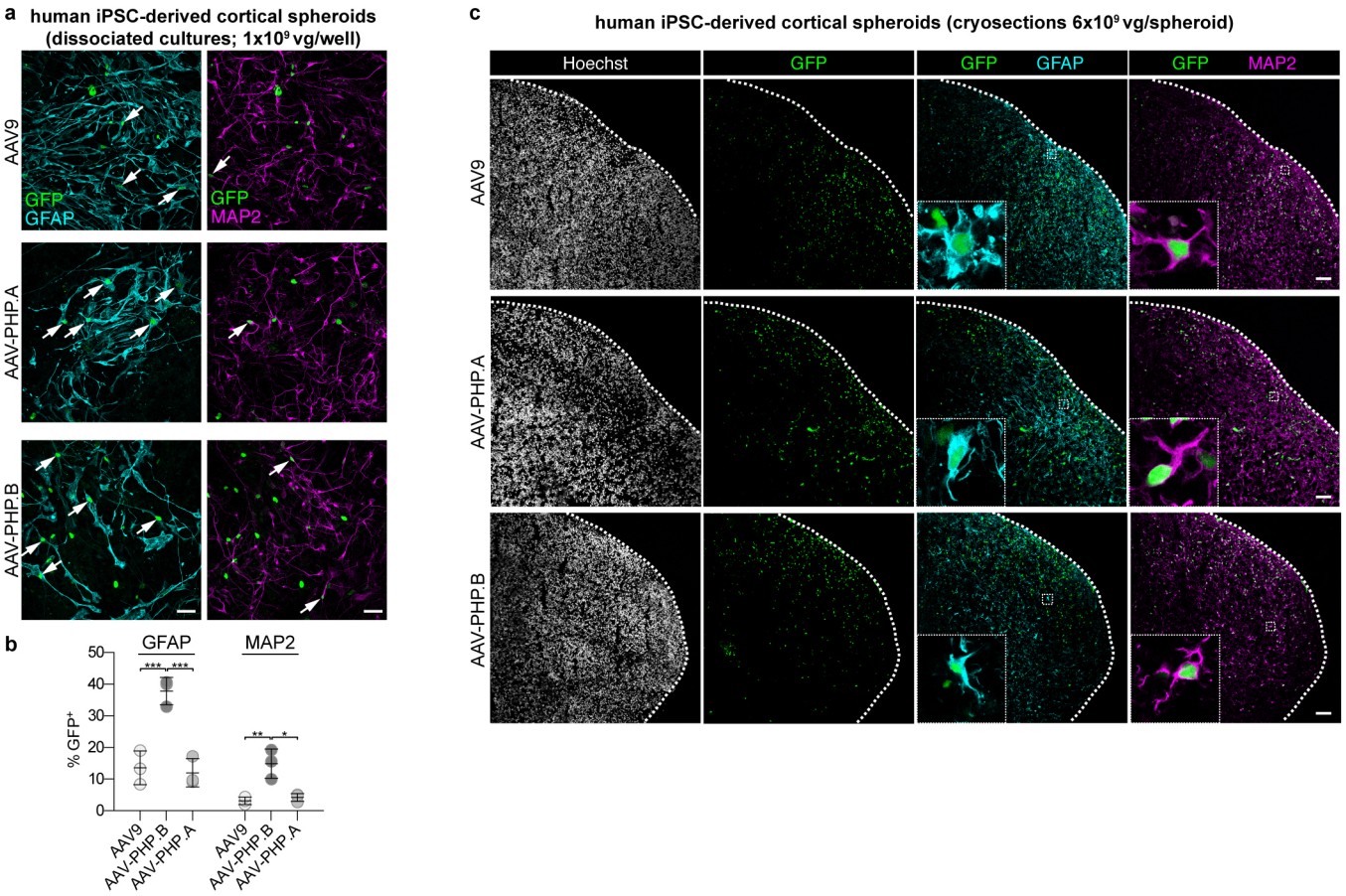
Adult mice were intravenously injected with the indicated dose of ssAAV-CAG-GFP packaged into AAV9 or AAV-PHP.B and were assessed for native eGFP fluorescence 377 Days later. *N*=1 per vector/dose.



Supplementary Figure 5

Representative images of native GFP fluorescence and IHC for several neuron and glial cell types following transduction by AAV-PHP.B:CAG-NLS-GFP.

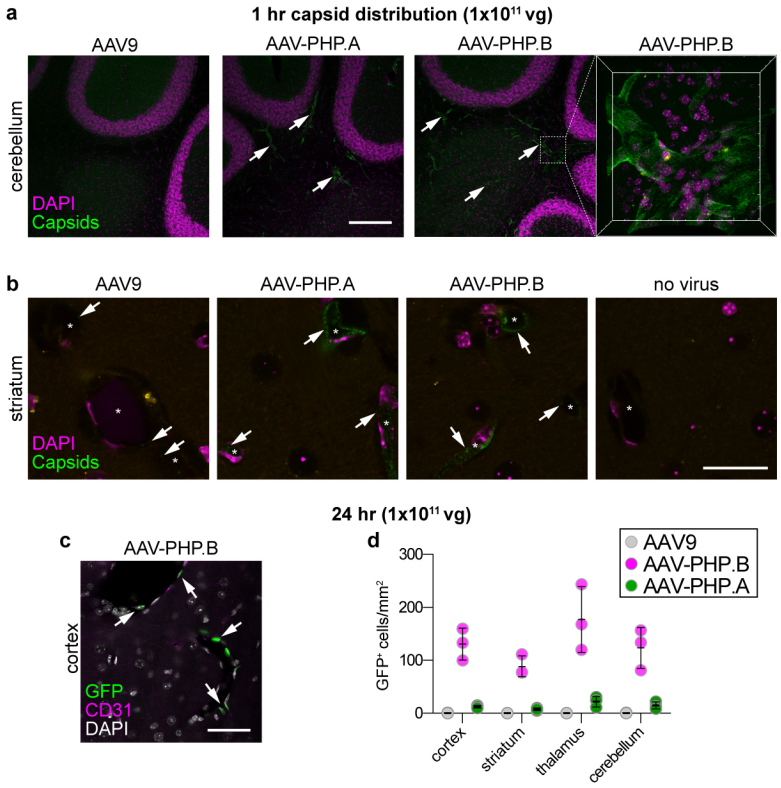
(**a-d**) Adult mice were injected with $2x10^{12}$ vg of ssAAV-PHP.B:CAG-NLS-GFP and assessed at 3 weeks post injection. Images show native NLS-GFP expression along with IHC for the indicated antigen in the indicated brain region. In all panels, arrows indicate co-localization of GFP expression with IHC for the indicated antigen. (**b, c**) Single-plane confocal images; (**a, d**) MIP. Corpus callosum (cc), substantia nigra pars compacta (SNc), ventral tegmental area (VTA). Scale bars: 50 μm.



Supplementary Figure 6

AAV9, AAV-PHP.A and AAV-PHP.B transduce human iPSC-derived cortical neurons and astrocytes in dissociated cultures and intact 3D cortical cultures.

(**a**) AAV-PHP.B provides higher transduction of human neurons and astrocytes in dissociated monolayer cultures. Representative images show GFP expression at five days after viral transduction (ssAAV-CAG-NLS-GFP packaged in AAV9, AAV-PHP.A or AAV-PHP.B; $1x10^9$ vg/well) of dissociated iPSC-derived human cortical spheroids differentiated *in vitro*. GFP expressing cells (green) co-localize with astrocytes immunostained for GFAP (cyan) or neurons immunostained for MAP2 (magenta) as indicated by white arrows. (**b**) Quantification of the percentage of $GFAP^+$ or $MAP2^+$ cells transduced by AAV9, AAV-PHP.A or AAV-PHP.B (n=3 differentiations into cortical spheroids of two human iPSC lines derived from two subjects; two-way ANOVA, Tukey's multiple comparison test; mean ± s.d). (**c**) AAV9, AAV-PHP.A and AAV-PHP.B transduce intact human 3D cortical cultures (cortical spheroids differentiated from human iPSCs). Images of human iPSC-derived cortical spheroid cryosections (day 205 of *in vitro* differentiation) transduced with ssAAV-CAG-NLS-GFP packaged in AAV9, AAV-PHP.A or AAV-PHP.B show native GFP fluorescence together with immunostaining for GFAP and MAP2. Insets show co-labeling of GFP fluorescence with $GFAP^+$ astrocytes (cyan) or $MAP2^+$ (magenta) neurons. Scale bars: 40 μm (**a**); 100 μm (**c**).



Supplementary Figure 7

AAV-PHP.B and AAV-PHP.A capsids localize to the brain vasculature after intravenous injection and transduce cells along the vasculature by 24 hours post-administration.

Adult mice were injected with $1x10^{11}$ vg of ssAAV-CAG-NLS-GFP packaged into AAV9, AAV-PHP.A or AAV-PHP.B as indicated. (**a**, **b**) Representative images of capsid immunostaining (green) using the B1 anti-AAV VP3 antibody that recognizes a shared internal epitope in the cerebellum (**a**) or striatum (**b**) in the brains of mice injected intravenously one hour prior to fixation by cardiac perfusion. Cell nuclei are labeled with DAPI (magenta). Lipofuscin autofluorescence (yellow) can be distinguished from capsid staining by its presence in both green and red channels. The inset (right) shows a 3D MIP image of the area highlighted in the AAV-PHP.B image. Arrows highlight capsid IHC signal; asterisks indicate vascular lumens. Data are representative of 2 (no virus and AAV-PHP.A) or 3 (AAV9 and AAV-PHP.B) mice per group. (**c**) Representative image of GFP expression (green) with DAPI (white) and CD31 (magenta) 24 hours post-administration of AAV-PHP.B. Arrows highlight GFP-expressing cells. (**d**) Quantification of the number of GFP expressing cells present along the vasculature in the indicated brain regions. *N*=3 per group; mean ± s.d; AAV-PHP.B vs AAV9 and AAV-PHP.A, ***$p<0.001$ for all regions; AAV9 vs AAV-PHP.A, not significant; two-way ANOVA. Scale bars: 200 μm (**a**); 50 μm (**b, c**); Major tick marks are 50 μm in the high magnification inset (**a**).