Materials and Methods

Generation of in capsid plasmid libraries. AAV-CMVc-Cas9 was a gift from Juan Belmonte (Addgene plasmid # 106431). pCAG-Cre-IRES2-GFP was a gift from Anjen Chenn (Addgene plasmid # 26646). pUCmini-iCAP-PHP.eB was a gift from Viviana Gradinaru (Addgene plasmid # 103005). pHelper plasmid was a gift from the Cancer Research United Kingdom viral core facility. We PCR amplified the appropriate sequences to assemble a plasmid containing AAV2 ITRs flanking a CMV-Capsid-T2A-GFP-pA construct with NEBuilder HiFi DNA assembly. The random library was synthesised by Sigma and directly assembled during plasmid assembly. We transformed the plasmid library into NEB stable E.coli, which we cultured overnight in liquid culture. DNA was isolated using the Qiagen Miniprep kit. We introduced stop codons into the RepCap plasmid pUCmini-iCAP-PHP.eB according to the method described by Deverman and colleagues (1). The modified sequence containing stop codons was synthesised by IDT and introduced into pUCmini-iCAP-PHP.eB using NEBuilder HiFi DNA assembly.

Virus production protocol and *in vivo* transfection. AAV production and purification followed the previously described protocol (1). In brief, HEK293 cells were grown in 15mm plastic dishes and triple transfected with the pHelper plasmid, the silenced PHP.eB capsid plasmid, and the transgene plasmid. Five days later cells were lysed and virus was isolated using Optiprep density gradient medium (Sigma; D1556) and ultra-centrifuged at 350,000g. The viral layer was isolated and concentrated using Amicon Ultra-15 Centrifugal Filter Units (Sigma; Z648043-24EA). AAV titer was determined using SYBR green qPCR. For in vivo administration of the virus, 8-week old mice were restrained and $1x10^{12}$ viral genomes per virus were injected into the tail vein.

DTA and shRNA expression in vivo. 8-week old WT mice were injected with AAVs encoding for diphtheria toxin A (DTA). CMV-eGFP mice were injected with AAVs containing shRNA targeting GFP. Both were driven by a human Cd11b promoter. The animals were sacrificed after 28 days and the density of $Iba1^{+}$ microglia in the CNS was determined. Cd11b-DTA animals were compared to control animals that were injected with a construct encoding for the mCherry gene under control of the same human Cd11b promoter. CD11b-shRNA GFP animals were compared to control animals that were injected with a scrambled shRNA construct under control of the same human Cd11b promoter.

Immunofluorescence for tissue sections. Mice received a lethal dose of pento-barbitol and were transcardially perfused with 4% paraformaldehyde (PFA) in PBS. The brains were removed and post-fixed for 2h at RT with 4% PFA. After a rinse in PBS the tissue was incubated in 20% sucrose solution

(in PBS) overnight. The tissue was then embedded in OCT- medium (TissueTek) and stored at −80°C. 12 µm sections were prepared using a cryostat. Tissue sections were air-dried and stored at −80°C. Cryostat cut sections were dried for 45 min at RT. For antigen-retrieval the slides were submerged in preheated citrate buffer pH 6.0 (Sigma) in a water bath at 95°C for 15 min. The slides were washed three times with PBS (5min, RT) and blocked in 0.3% PBST with 10%NDS for 1h at RT. Primary antibodies: anti-Iba-1 (1:1000, Wako), anti-GFP (1:500, Thermofisher), anti-P2RY12 (1:500, Thermofisher), anti-CD163 (1:500, Thermofisher), NeuN (1:500, abcam), Olig2 (1:500, Thermofisher), CD31 (1:500, Thermofischer) and GFAP (1:500, abcam) were diluted in 0.1% PBST with 5%NDS and incubated overnight at 4°C. The slides were washed 3 times for 10 min with PBS. Next, secondary antibodies in blocking solution were applied at a concentration of 1:500 for 2h at RT. Slides were washed 3 times with PBS for 10 min each, whereby the first wash containing Hoechst 33342 nuclear stain (2 µg/ml,). The slides were mounted with coverslips using FluoSave (CalBiochem). Image acquisition was performed using a Leica-SP5 microscope (Leica) and LAS software (Leica) or a Zeiss Observer A1 inverted microscope (Zeiss) and Zeiss Axivision software. Further image processing and analysis was performed using the ImageJ software package.

Isolation of single cell suspension. Adult male and female mice (8 weeks) were decapitated after lethal injection with phenobarbital. The brains were removed quickly and placed into an ice-cold isolation medium. The telencephalon and cerebellum were dissected in isolation medium; meninges, and the olfactory bulb were mechanically removed and the brain tissue was mechanically minced into 1 mm^3 pieces. The tissue pieces were spun down at 100 g for 1 min at RT and the tissue was washed in $HBSS^-$ (no $Mg2^+$ and $Ca2^+$, GIBCO). Each brain was mixed with 5 ml of dissociation solution (34 U/ml papain (Worthington), 20 µg/ml DNase Type IV (GIBCO) in isolation medium). The brain tissue was dissociated on a shaker (50 rpm) for 30 min at 35°C. The digestion was stopped by addition of ice cold $HBSS^-$. The tissue was centrifuged (200 g, 3 min, RT), the supernatant completely aspirated and the tissue resuspended in isolation medium supplemented with 2% B27 and 2 mM sodium-pyruvate (trituration solution). The tissue was allowed to sit in this solution for 5 min. To obtain a single cell suspension the tissue suspension was triturated 10 times using first a 5 ml serological pipette and subsequently three fire polished glass pipettes (opening diameter > 0.5 mm). After each trituration step the tissue suspension was allowed to sediment (approximately 1-2 min) and the supernatant (approximately 2 ml), containing the cells, was transferred into a fresh tube. After each round of trituration 2 ml of fresh trituration solution were added. To remove accidentally transferred undigested tissue bits, the collected supernatant was filtered through 70 µm cell strainers into tubes that contained 90% isotonic Percoll (GE Healthcare, 17-0891-01, in 10xPBS pH7.2 (Lifetech). The final

volume was topped up with phenol-red free DMEM/F12 with HEPES (GIBCO) and mixed to yield a homogeneous suspension with a final Percoll concentration of 22.5%. The single cell suspension was separated from remaining debris particles by gradient density centrifugation (800 g, 20 min, RT, without break). The myelin debris and all layers without cells were discarded and the brain cell containing phase (last 2 ml) and cell pellet were resuspended in $HBSS^+$ and combined in a fresh 15 ml tubes and centrifuged (300 g, 5 min, RT). The cell pellet was resuspended in red blood cell lysis buffer (Sigma, R7757) and incubated for 1 min at RT to remove red blood cells. 10 ml of $HBSS^+$ were added to this cell suspension and spun down (300 g, 5 min, RT). The cell pellets were resuspended in 0.5 ml modified Milteny washing buffer (MWB, 2 mM EDTA, 2 mM Na-Pyruvate, 0.5% BSA in PBS, pH 7.3) supplemented with 10 ng/ml human recombinant insulin (GIBCO).

Fluorescence Activated Cell Sorting. Freshly isolated cells from the brain were stained with primary antibodies (Anti-CD11b-PE and appropriate isotype controls; BioLegend) for 15 min at 4°C. Cells were washed and resuspended in FACS buffer. Cells were analysed using an Attune-NXT (Thermo Scientific) equipped with 405, 488 and 561 lasers. For compensation, beads (OneComp) were used for single stains for the fluorophore. The compensation matrix was automatically calculated and applied by the Attune software. Gates for the quantification of Cd11b and GFP positive cells were set according to appropriate FMOs. A minimum of 100,000 cell singlets were recorded and used for quantification with FlowJo software (v10).

References

1. Challis, R. C. et al. Systemic AAV vectors for widespread and targeted gene delivery in rodents. *Nat Protoc* **14**, 379–414 (2019).