Title: Comprehensive Methods
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Methods

Plasmids

The CMV-SaCAS9 vector (AAV-CMV::NLS-Sa- Cas9-NLS-3xHA-bGHpA;U6::BsaI-sgRNA) was obtained from Dr. Feng Zhang through Addgene (#61591). The gRNA scaffold sequence was modified as described in Tabebordbar Science 2016 (see Figure 1 of our toolkit submission data). sgRNAs specifically targeting the lox-stop-lox cassette of the Ai9 locus were cloned using NEB HiFi Assembly mix for single gRNA insertions, or through the use of IDT gBlocks to insert a second U6-gRNA-scaffold sequence. The hSyn promoter was cloned by PCR from AAV-hSyn-mRuby (Chan et al 2017, Gradinaru lab Addgene 99126) and the GFAP promoter (GfABC1D) was obtained from M. Brenner and described in Lee et al *GLIA.* Complete annotated sequence maps are provided as supplementary files for the all of the plasmids used in the study.

Editing evaluation in Ai9 fibroblasts

gRNA optimization was performed in Ai9 fibroblasts (a kind gift from Amy Wagers and Sharif Tabebordbar). The vectors contained the original or a modified gRNA scaffold sequence (Fig. 1). The all-in-one SaCas9-gRNA plasmids were tested by transient transfection of Ai9 fibroblasts with a single or co-transfection followed by flow cytometry to quantify the fraction of cells that were tdTomato+. A EF1A-Cre recombinase plasmid obtained from Karl Deisseroth (Addgene #55636) was used as a positive control and to normalize the data in Fig 1E.

AAV production

Recombinant AAV vectors were generated by PEI triple transfection of HEK293 cells and purified using gradient ultracentrifugation as previously described (Challis et al. Nature Protocols 2019). All AAV genomes were packaged into AAV-BI28, which is an engineered variant of AAV9 identified through high-throughput screening (Chan, Huang, Chen, and Deverman unpublished).

AAV administration in mice

All procedures were performed as approved by the Broad Institute IACUC (0213-06-18). Recombinant AAV-BI28 vectors were administered to adult male and female mice obtained from Jackson Laboratory, C57Bl/6J (000664) and Ai9 (007909), via the retro-orbital sinus. No mice were excluded from the analyses. Experimenters were not blinded to sample groups.

Tissue processing and IHC

Mice were anesthetized with Euthasol (Broad) and transcardially perfused with phosphate buffered saline (PBS) at room temperature followed by 4% paraformaldehyde (PFA) in PBS. Tissues were post-fixed overnight in 4% PFA in PBS and sectioned by vibratome. Samples were then washed with PBS three times and blocked with 10% donkey serum for 1 hour. IHC for S100

was performed on floating sections with an S100 antibody (Abcam Ab52642) diluted 1:200 in PBS containing 5% donkey serum, 0.1% Triton X-100 and incubated overnight at 4C. Samples were then washed with PBS three times. A secondary antibody (Abcam 150087) was used at 1:500 and incubated for one hour at room temperature and washed with PBS three times. Images were taken on a Zeiss epifluorescence microscope with an LED illumination source or a Keyence BZ-X. Quantification of the fraction of area occupied by functionally edited astrocytes (tdTomato+) was quantified in the associated protocol:
Deverman_method_for_area_based_quantification_of_editing_efficiency.pdf