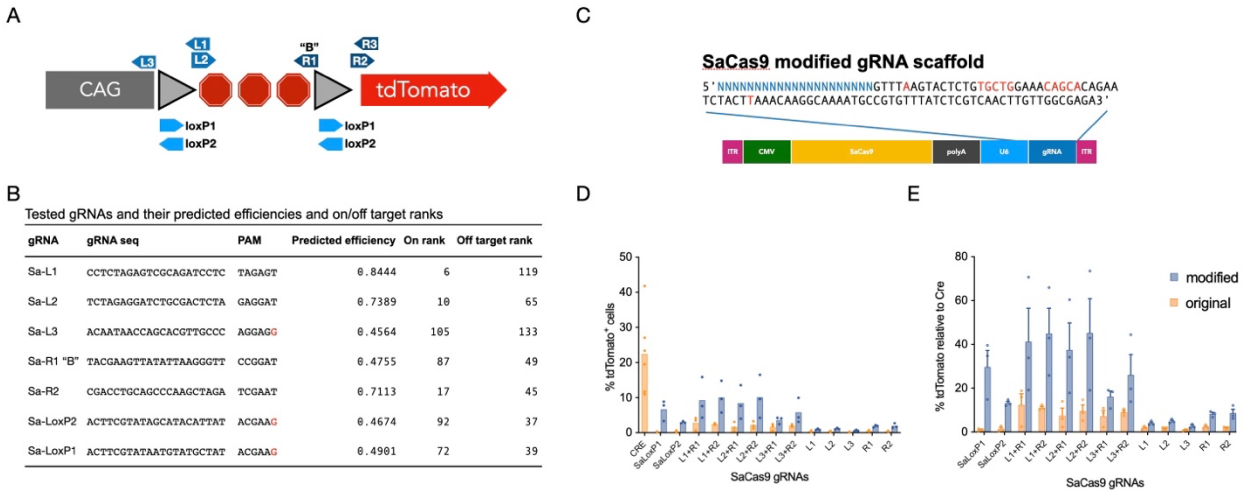

gRNA	gRNA seq	PAM	Predicted efficiency	On rank	Off target rank
Sa-L1	CCTCTAGAGTCGCAGATCCTC	TAGAGT	0.8444	6	119
Sa-L2	TCTAGAGGATCTGCGACTCTA	GAGGAT	0.7389	10	65
Sa-L3	ACAATAACCAGCACGTTGCCC	AGGAGG	0.4564	105	133
Sa-R1 "B"	TACGAAGTTATATTAAGGGTT	CCGGAT	0.4755	87	49
Sa-R2	CGACCTGCAGCCCAAGCTAGA	TCGAAT	0.7113	17	45
Sa-LoxP2	ACTTCGTATAGCATACATTAT	ACGAAG	0.4674	92	37
Sa-LoxP1	ACTTCGTATAATGTATGCTAT	ACGAAG	0.4901	72	39

Figure 1. Improved editing of the Ai9 locus *in vitro* after optimization of gRNAs and modification of the gRNA scaffold. A. The schematic shows the Ai9 lox-stop-lox-tdTomato cassette and the positions of tested gRNA target sequences. **B.** gRNA sequences, PAMs and their predicted efficiency and on- and off-target editing ranks as predicted by the Broad Genetic Perturbation Platform **https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design**. Sequences that deviate from the NNGRRT consensus SaCas9 PAM are highlighted in red. **C.** A schematic of the Cas9-gRNA construct design. Modifications to the gRNA scaffold are highlighted in red. The position of the spacer is shown in blue. **D.** *In vitro* editing efficiency by SaCas9 with single or paired gRNA combinations. Ai9 fibroblasts were transfected with a single or pair of CMV-SaCas9 vectors with a U6-driven gRNA as indicated. The cells were then assessed for tdTomato expression as a marker of editing by flow cytometry. Plasmids harboring modifications to the gRNA scaffold enhance the editing of the Ai9 lox-stop-lox cassette. **E.** Editing efficiency normalized to the recombination efficiency achieved by EF1a-Cre expression, which served as a positive control.

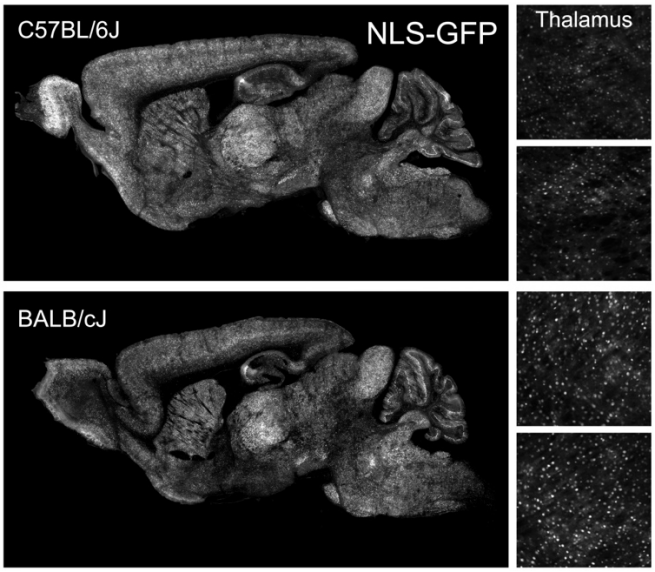

Figure 2. The novel AAV capsid BI28 crosses the adult blood brain and transduces CNS cells throughout the brains of C57BL/6J and BALB/cJ mice. AAV-BI28:CAG-NLS-GFP (1E12 vg/mouse) was administered to adult C57BL/6J (left) or BALB/cJ (right) mice and expression was assessed by imaging native GFP fluorescence 10 days later.

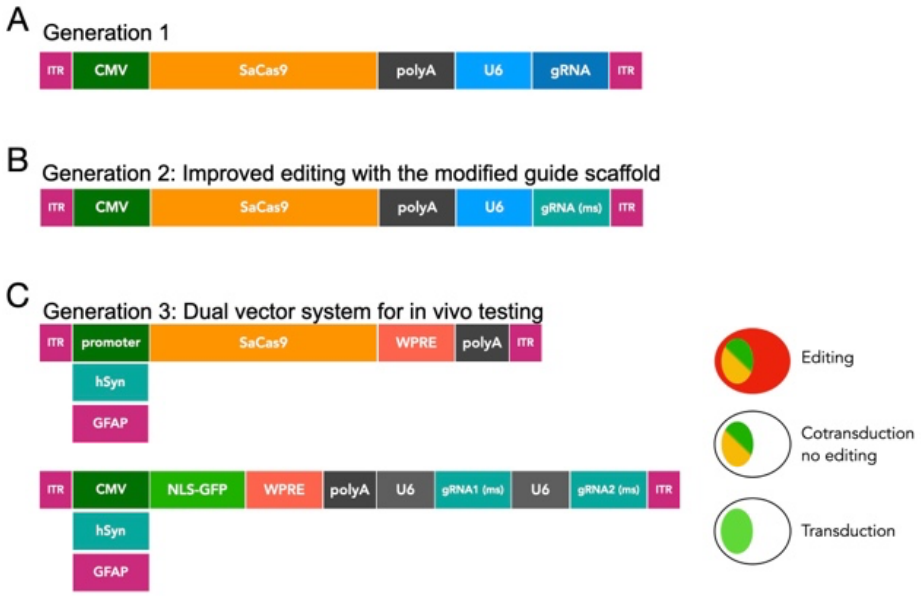

Figure 3. AAV genome editing vector optimization for in vivo CNS editing. A. The schematic shows the original vector design based on Addgene plasmid 61591 from the Feng Zhang laboratory. Editing of the Ai9 locus is achieved through co-delivery of vectors with two different gRNA sequences (left and right ends of the Lox-stop-lox cassette). **B.** The second-generation vector used a modified gRNA scaffold to improve editing efficiency. **C.** The third-generation vector was optimized for *in vivo* delivery and split into two distinct vectors. The first vector contains the SaCas9 cDNA with a WPRE sequence to improve SaCas9 expression. Versions with three different promoters were constructed (CMV, for ubiquitous expression; hSyn for neuron-specific expression; and a truncated GFAP promoter (GfABC1D) for astrocyte-specific expression. The second vector contains an NLS-GFP driven by one of the same three promoters (CMV, hSyn, or GFAP) and two U6-gRNA cassettes. For editing the Ai9 locus in vivo, L1 and R2 (Fig. 1) were used. This dual vector system makes it possible to evaluate editing, transduction, and co-transduction efficiencies (right).

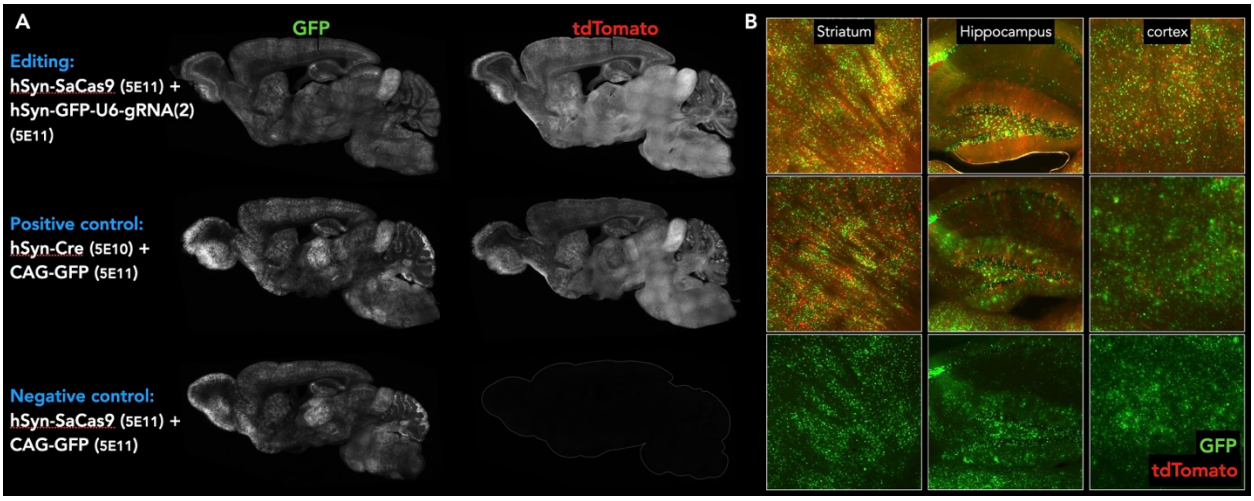

Figure 4. ***In vivo*** **editing with the AAV-BI28 capsid and third generation hSyn-SaCas9 vectors. A-B.** Mice were injected with BI28:hSyn-SaCas9 (5E11vg/mouse) and BI28:hSyn-NLS-GFP-U6-gRNA(L1-R2) (5E11 vg/mouse) (top), BI28:hSyn-Cre (5E10 vg/mouse) and BI28:CAG-GFP at 5E11 vg/mouse per vector (middle), or BI28:hSyn-SaCas9 (5E11 vg/mouse) and BI28:CAG-GFP (5E11 vg/mouse) per vector (bottom) **A.** Whole brain section images of transduction (GFP) and Ai9 locus editing (tdTomato) enabled by AAV-BI28:hSyn-Cas9. **B.** Transduction (GFP, green) and Editing (tdTomato, red) in the striatum, hippocampus and cortex.

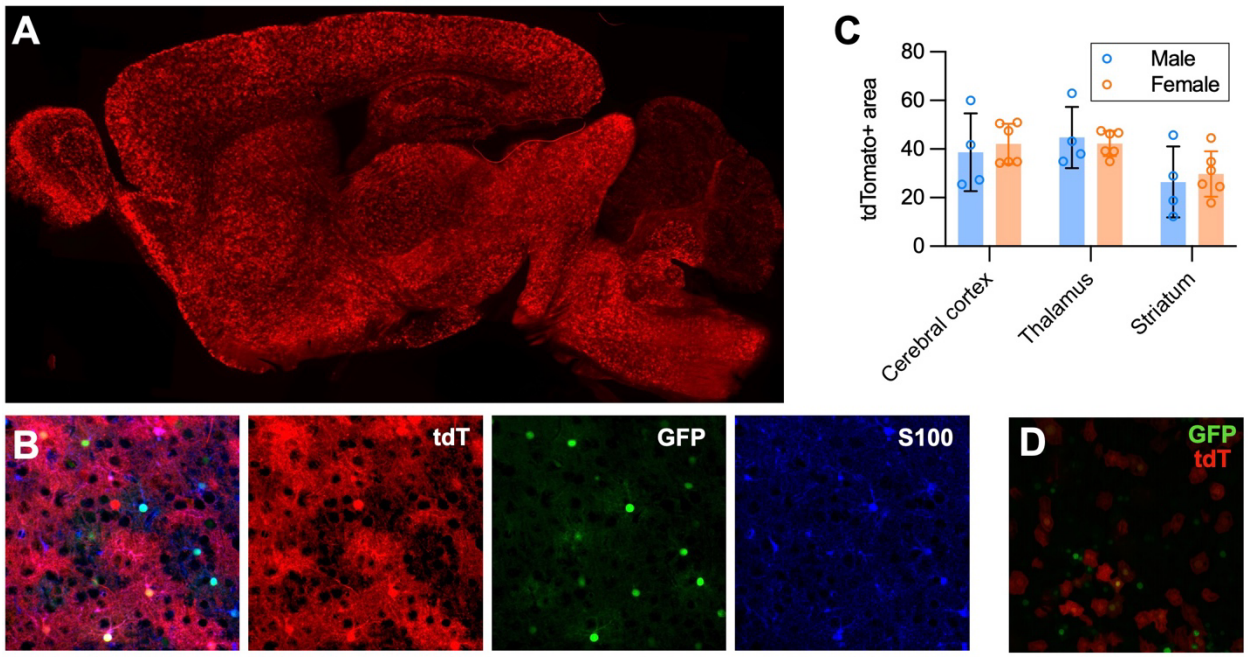

Figure 5. *In vivo* editing with the AAV-BI28 capsid and third generation GFAP-SaCas9 vectors. Ai9 mice were injected with BI28:GFAP-SaCas9 (3E11vg/mouse) and BI28:GFAP-NLS-GFP-U6-gRNA(L1-R2) (3E11 vg/mouse) and expression and editing were assessed 4 weeks later. **A.** Whole brain section and Ai9 locus editing (tdTomato) enabled by AAV-BI28:GFAP-Cas9. **B.** Efficient co-transduction of BI28:GFAP-SaCas9 and BI28:GFAP-NLS-GFP-gRNA-L1-R2 in cortical astrocytes (S100). **C.** The graph shows quantification of CNS astrocyte editing measured by the fraction of tdTomato+ area above threshold within the indicated brain regions (mean ± SD, n=6 females and n=4 males). **D.** Image shows transduction and editing in the liver.

Figure legends for the figures provided as CSV files. These are the same data as shown in Figure 1D, 1E and 5C.

Figure 1D. Improved editing of the Ai9 locus *in vitro* after optimization of gRNAs and modification of the gRNA scaffold. *In vitro* editing efficiency by SaCas9 with single or paired gRNA combinations. Ai9 fibroblasts were transfected with a single or pair of CMV-SaCas9 vectors with a U6-driven gRNA as indicated. The cells were then assessed for tdTomato expression as a marker of editing by flow cytometry. Plasmids harboring modifications to the gRNA scaffold enhance the editing of the Ai9 lox-stop-lox cassette.

X axis title: SaCas9 gRNA
Y axis title: % tdTomato+ cells

Figure 1E. Improved editing of the Ai9 locus *in vitro* after optimization of gRNAs and modification of the gRNA scaffold. *In vitro* editing efficiency by SaCas9 with single or paired gRNA combinations. Ai9 fibroblasts were transfected with a single or pair of CMV-SaCas9 vectors with a U6-driven gRNA as indicated. The cells were then assessed for tdTomato expression as a marker of editing by flow cytometry. Plasmids harboring modifications to the gRNA scaffold enhance the editing of the Ai9 lox-stop-lox cassette. Data shown are normalized to tdTomato expression induced by Cre recombinase, which recombines the lox-stop-lox cassette and served as a positive control.

X axis title: SaCas9 gRNA
y axis title: % tdTomato relative to Cre transfection

Figure 5C. *In vivo* editing with the AAV-BI28 capsid and third generation GFAP-SaCas9 vectors. Ai9 mice were injected with BI28:GFAP-SaCas9 (3E11vg/mouse) and BI28:GFAP-NLS-GFP-U6-gRNA(L1-R2) (3E11 vg/mouse) and expression and editing were assessed 4 weeks later. The graph shows quantification of CNS astrocyte editing measured by the fraction of tdTomato+ area above threshold within the indicated brain regions (mean ± SD, n=6 females and n=4 males).