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Supplementary Materials for

Development of a CRISPR-SaCas9 system for projection- and function-specific gene editing in the rat brain

Haojie Sun, Su Fu, Shuang Cui, Xiangsha Yin, Xiaoyan Sun, Xuetao Qi, Kun Cui, Jiaxin Wang, Longyu Ma, Feng-Yu Liu, Fei-Fei Liao, Xin-Hong Wang, Ming Yi*, You Wan*

*Corresponding author. Email: ywan@hsc.pku.edu.cn (Y.W.); mingyi@bjmu.edu.cn (M.Y.)

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This PDF file includes:

Supplementary Materials

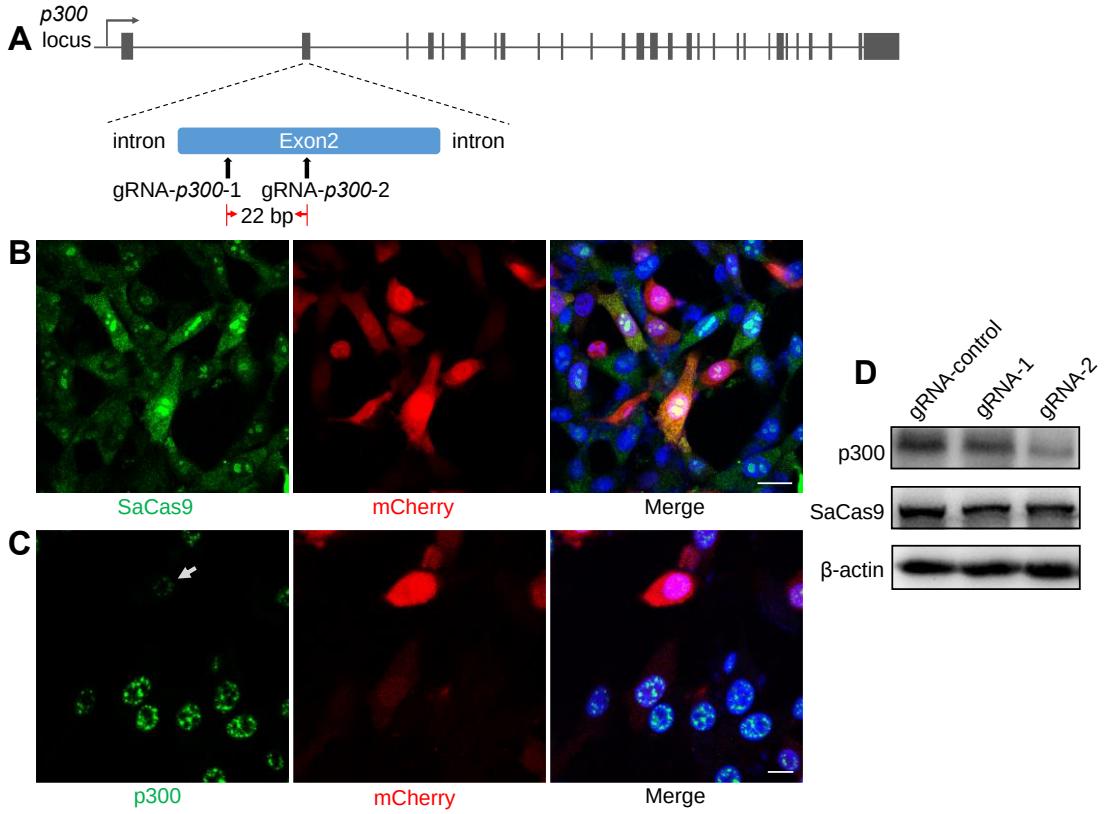

Fig. S1. SaCas9 inactivates the *p300* gene in vitro with high efficiency.
(**A**) Graphical representation of the rat *p300* gene locus showing gRNA target sites. (**B**) Elevated expression of SaCas9 shown by immunofluorescence three days after lentivirus transfection in F98 cells. Scale bars, 20 μm. (**C**) Immunofluorescent staining of p300 one week after CRISPR-SaCas9 targeting of *p300* locus in F98 cells, with white arrows indicating decreased p300 expression in SaCas9- and gRNA-positive cells. Scale bars, 10 μm. (**D**) Western blot analysis showing decreased p300 expression in F98 cells one week after lentivirus transfection.

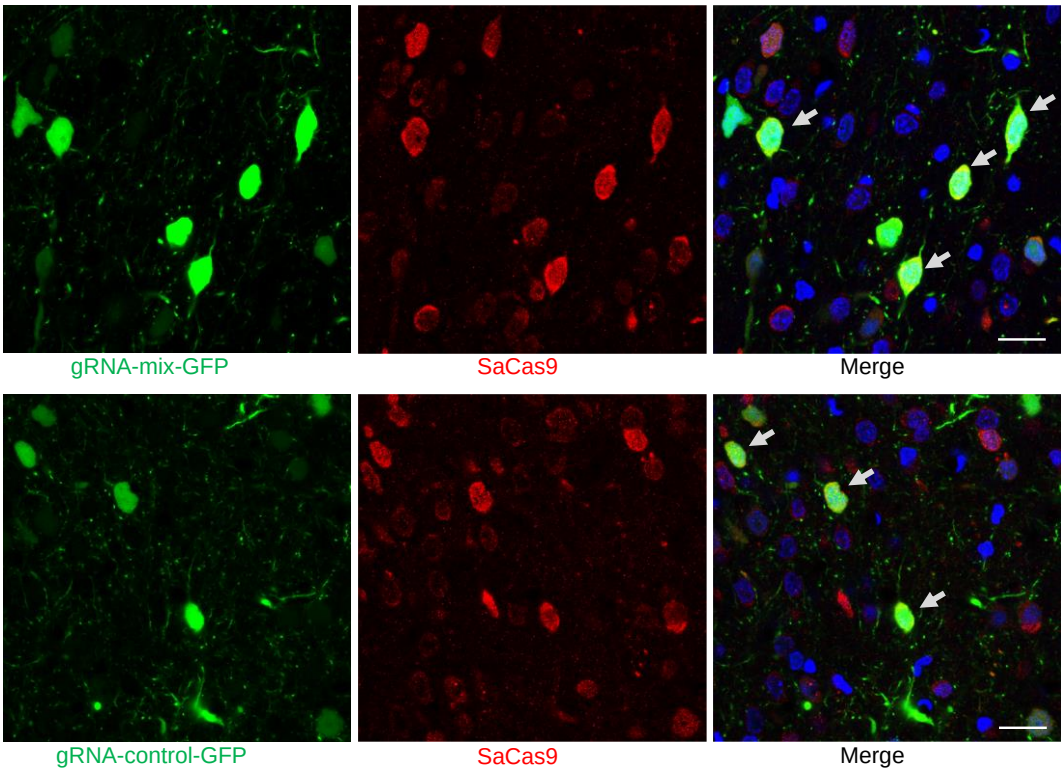

Fig. S2. Neuronal specificity for spine density analysis. The presence of SaCas9 in gRNA-mix-GFP^{+} (upper) and gRNA-control-GFP^{+} (lower) neurons for morphology analysis was verified by immunofluorescence. Scale bars, 20 μm.

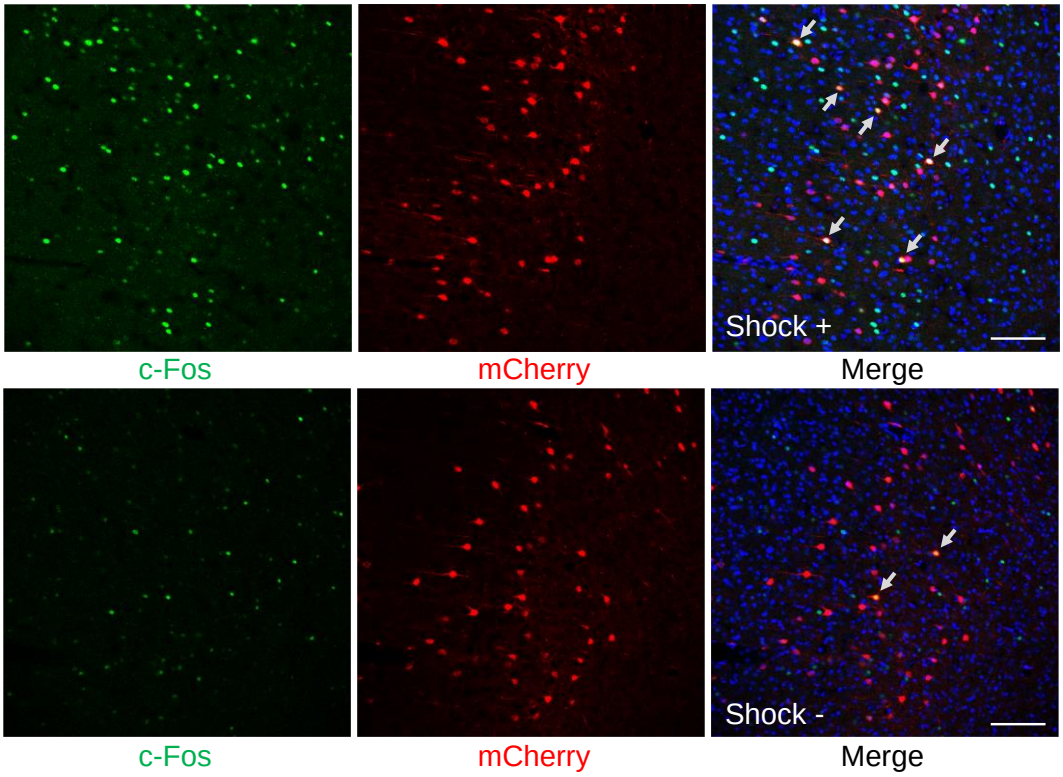

Fig. S3. Amygdala-projecting IL neurons are activated during extinction. Illustration of amygdala-projecting IL neurons (mCherry^{+}) co-labeled with c-Fos 2 h after context exposure in the shock + (with shock during CFC training) and shock - (without shock during CFC training) groups. Scale bars, 100 μm.

Table S1. gRNA sequences targeting *cbp* gene or *p300* gene.

	gRNA sequence	PAM	Exon	Direction
gRNA-control	GGAGACGAATAATGCGTCTCC	/	/	/
CBP-gRNA-1	CTGGCTGCCTGTTTGGGCAGG	CTGGGT	2	-
CBP-gRNA-2	AAATGTCAGCGACGAGAGCAA	GCGAAT	4	+
CBP-gRNA-3	AGCAGCTGTGGAAGCAGGAGG	TGGAGT	14	-
CBP-gRNA-4	ATTCAGTGCTTGGGAGGCAGG	GGGAGT	2	-
CBP-gRNA-5	AGTAACTCTGGCCATAGCTTA	ATGAAT	2	+
p300-gRNA-1	TAGTTCCCCCAACCTCAATAT	GGGAGT	2	+
p300-gRNA-2	GTTATGGCCAGCCAGGCCCAA	CAGAAT	2	+

Table S2. AAV vectors used.

AAV	Titer (v.g./ml)	Serotype
AAV-hSyn-GFP	9.3×10^{12}	9
AAV-CaMKII-Cre	8.1×10^{13}	1
AAV-CaMKII-Cre-P2A-GFP	5.2×10^{13}	1
AAV-hSyn-Cre-P2A-GFP	4.5×10^{13}	1
AAV-hSyn-DIO-mCherry	1.8×10^{13}	9
AAV-hSyn-Cre	6.7×10^{13}	retro
AAV-hSyn-Cre-P2A-GFP	1.7×10^{13}	retro
AAV-hSyn-mCherry	5.4×10^{13}	retro
AAV-hSyn-DIO-SaCas9-3×flag	3.3×10^{13}	9
AAV-hSyn-SaCas9-3×flag	4.1×10^{13}	9
AAV-U6-gRNA1-U6-gRNA4-U6-gRNA5-CMV-GFP	1.2×10^{13}	9
AAV-c-fos-rtTA-U6-gRNA1-U6-gRNA4-U6-gRNA5	1.7×10^{13}	9
AAV-c-fos-rtTA	3.7×10^{13}	9
AAV-TRE^{3G}-SaCas9-3×flag	2.2×10^{13}	9
AAV-TRE^{3G}-DIO-SaCas9-3×flag	3.2×10^{13}	9

Table S3. Primer sequences for T7 endonuclease assays.

gRNA-1-F	GATTTTGGATCATTGTT
gRNA-1-R	CCTGTGTCAAAGTCTCA
gRNA-2-F	GCTGGTGCTGTTTCTGG
gRNA-2-R	CAAATCACCTGCCTTCT
gRNA-3-F	TAAGGTGCCCTGTATGA
gRNA-3-R	GTCGGTAAACTAAGTAAATGTG

gRNA-4-F	TGTGCCGTATTCAGTCC
gRNA-4-R	CTGCGTCCAAACATAAAC
gRNA-5-F	CCAGATGCTGCGTCCAA
gRNA-5-R	CAACAACCCGTTTCTTTC
gRNA-1+4+5-F	CGGGATAGGCAATGTG
gRNA-1+4+5-R	CTGGAGCAGGGTAGGGCATT

Table S4. Primer sequences for off-target analysis.

gRNA-1-F	CTGTGATGTGGCAGGACTAC
gRNA-1-R	CGGGATAGGCAATGTG
off-target 1-F	TTGCTTCAGACTCCACATTG
off-target 1-R	TGAAGAACTTGAGAAGAAGGG
off-target 2-F	AATTATGTCCCACCCAATGA
off-target 2-R	AGACTGATTTTCCCCTAAG
off-target 3-F	GCCCCTTAAATCGAACTC
off-target 3-R	TCACCCGAGACCATGTAGAG
off-target 4-F	ACGTTAAGAGATGGCATCTG
off-target 4-R	AGTTTGCACTCATAGCCAA
off-target 5-F	AGTTAAAAACCAAGGCAC
off-target 5-R	TCAGTTTGATTTAGTCGC
off-target 6-F	TGTTCTGCTAAACTCGAGGC
off-target 6-R	AAGAAGTCAGGCGTGCT
off-target 7-F	ACAGCATGACACCGTGTGAG
off-target 7-R	GGAAAGGAAGGCAGGTAGAC
off-target 8-F	CAGAAAGGAAGTCAGGGTAT
off-target 8-R	AACAGCAGCATTAGCCA
off-target 9-F	TTCTGTGTTGGCATGGTATT
off-target 9-R	TGTTTTCGTGGAGCTATTCA
gRNA-4-F	AAGCTATGGCCAGAGTTACT
gRNA-4-R	GCATGGCCAGTTTAGG
off-target 10-F	AAGCCACTGCCTTGTATTCC
off-target 10-R	CAATGAGCAGTTGGTTCCC
off-target 11-F	GGGACCATGGAAAGCATTGA
off-target 11-R	GCCCTGAGTCTAGAGCATGT
off-target 12-F	AAAATGGAGCATACTTAGAG
off-target 12-R	CCAACATCATGCAAAGTGTC
off-target 13-F	TGATAACCTACTTCCCTAAC
off-target 13-R	GCGAAACAGTAAGGACT
off-target 14-F	CCAAATTAAAATCCAGTTAG
off-target 14-R	GATGACCTGGAACTCTT
off-target 15-F	CTTCCCAAAGAGCAGATTAG
off-target 15-R	GAGGCACATTTAAGTCCC

off-target 16-F	GCTGAAACAGTATAAGTCGG
off-target 16-R	GCCTGCTATTTAAGACCTT
off-target 17-F	GGACCGTTCCTTCAGACC
off-target 17-R	ACCGATAATCCCAGCTCTAC
off-target 18-F	TTAAAGGGTTGCAGCGTTAG
off-target 18-R	TGGTTAAGTGGGGAATGTC
off-target 19-F	CCCTTGAGTTATTGTTGAG
off-target 19-R	CACCAAAGGTTCTGGTTAGA
off-target 20-F	GTCTGTGAGACATACCCTGAGG
off-target 20-R	ATGTGCAGGCAGCCATTCAC
off-target 21-F	AAAACAAGACAACTTGGC
off-target 21-R	TTAGCAAGACTACACGGAGA
off-target 22-F	TCCAATCTGAGGGGAGTGTA
off-target 22-R	TCCCATTTCCAATGATTCAC
off-target 23-F	ACGATATTAAAGGTGGTTAG
off-target 23-R	TGTTAGGACTCCCACATAA
off-target 24-F	AGACTAGCCTCAAACTCAT
off-target 24-R	ATAAAGTGATTTCAGGACTC
off-target 25-F	CGGCCAATGGATGCTA
off-target 25-R	GTCCTCCCCACTTGATCACT
off-target 26-F	AATTAAGAGAAAAGAACCAC
off-target 26-R	ATGGTTTAAATTTAGCAAT
off-target 27-F	CTCGTCAATAAAAGACAGTA
off-target 27-R	AGAAGAGGAAAATCTCAT
off-target 28-F	AAGGGTAAGAAACTTCC
off-target 28-R	AAAACTAAAGGTAACATTTG
off-target 29-F	CTACCAGCTGGAAATCCTC
off-target 29-R	ACTGCTGGAGTGAGCTCTTG
gRNA-5-F	AAGCACTGAATCCGCAAGC
gRNA-5-R	CTGGAGCAGGGTAGGGCATT
off-target 30-F	TCCACCTTCCTGTGAACCC
off-target 30-R	AATTCTAGAGGCTGATATCTGC
off-target 31-F	TAGGGCTTCATACTTGC
off-target 31-R	AGAAAAGTGTGGGACCCTAT
off-target 32-F	GATGGGTGAGGAAGGTGATA
off-target 32-R	GGCTTCTGCTCTATGGC
off-target 33-F	AGATGGCACAACTACTCACCT
off-target 33-R	TTGGGTTCCATCCCTAGTAC