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Supplemental information

CRISPR-mediated rapid generation of neural cell-specific knockout mice facilitates research in neurophysiology and pathology

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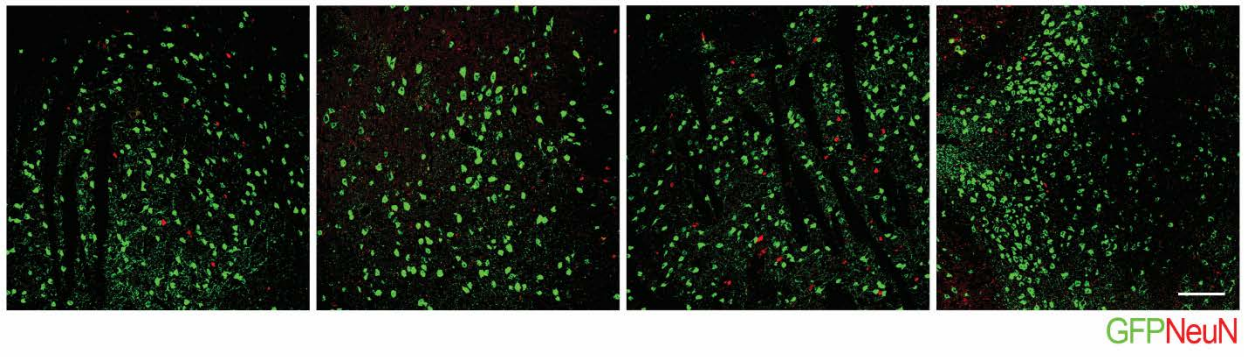

Figure S1. Neuron-specific NeuN knockout in thalamus. PHP.eB-sgNeuN-hSYN1-Cre or PHP.eB-sgScram-hSYN1-Cre was i.v. injected into naïve adult LSL-Cas9 mice at 5×10^{11} vg per mouse. Brains were harvested two weeks later and analyzed by immunostaining. Representative images of four separate locations in the thalamus are shown. Scale bar: 100 μm.

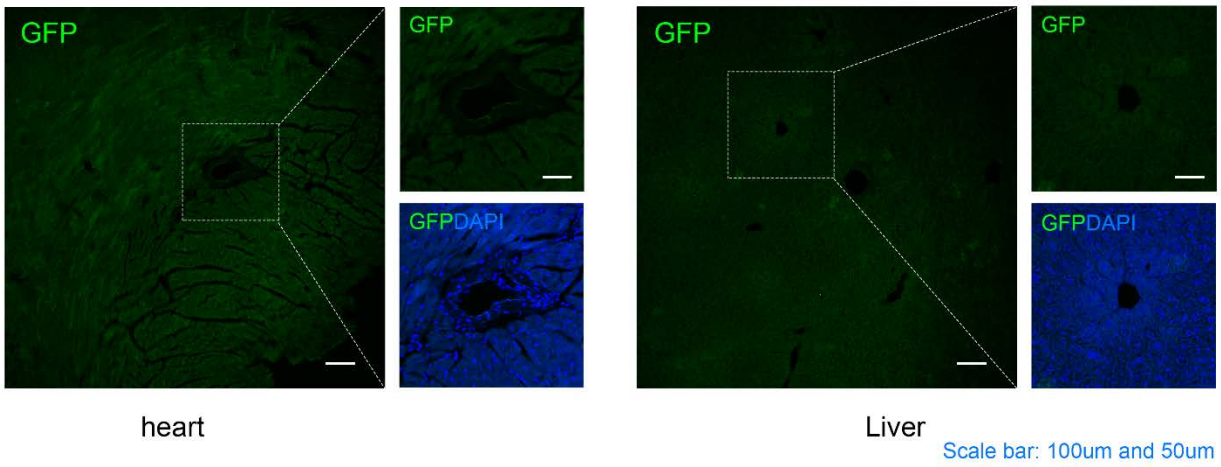

Figure S2. Immunostaining of heart and liver of PHP.eB-sgScram-hSYN1-Cre i.v. injected mice. One representative image of 3 mice was shown. Scale bar: 100 μm, scale bar of zoomed images: 50 μm.

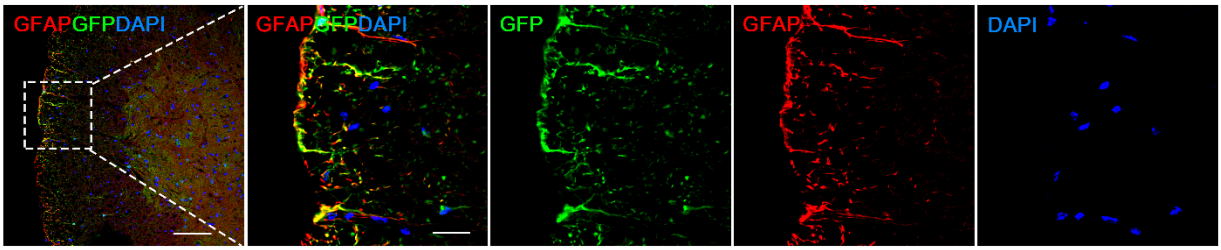

Figure S3. Immunostaining of the PHP.eB-sgScram-GFAP-Cre infected astrocytes in spinal cord of naïve LSL-Cas9 mice. One representative image of 2 mice was shown. Scale bar: 100 μm, Scale bar of zoomed image: 20 μm.

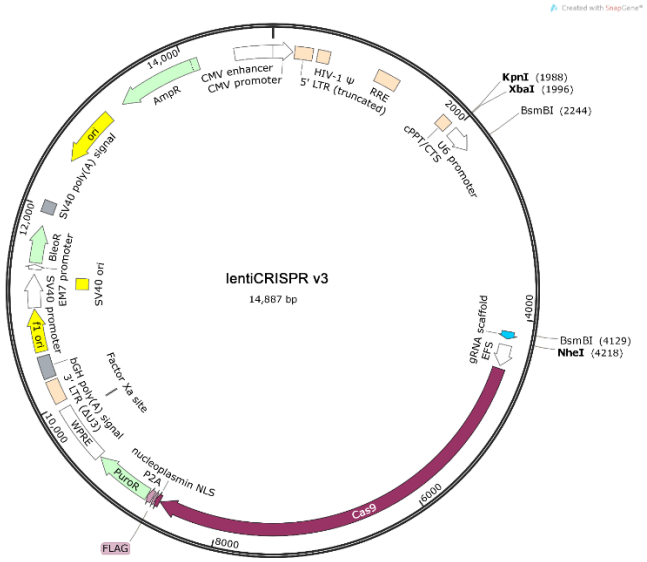

Figure S4. Schematic of lentiCRISPR v3 plasmid.

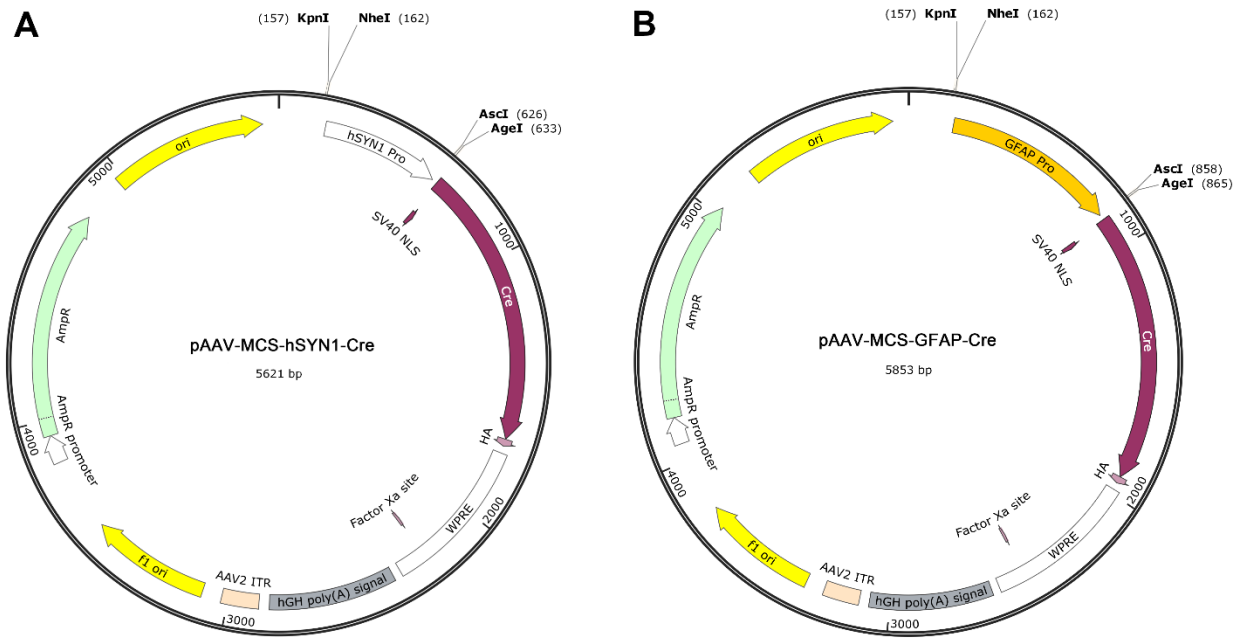

Figure S5. Schematic of AAV transfer plasmids for sgRNA delivery. (A) pAAV-MCS-hSYN1-Cre structure. (B) pAAV-MCS-GFAP-Cre structure.

Table S1. Comparison of Cre-ER-Loxp and AAV-CRISPR methods.

Method for KO	Efficiency	Feasibility	Time to generate	Cost	Labor
CreER-Loxp	70-80%	**Low** Limited by the availability of mouse lines with loxp sites inserted into the gene to be knocked out	Half year to one and a half years	High	High
AAV-CRISPR	**Neurons**: 82% in brain; 65% in SC* **Astrocytes**: 46% in brain 79% in SC	**High** Any gene of interest can be knocked out	Three to four weeks	Low	Low

* SC: spinal cord

Table S2. List of primers used in this paper.

Name of primer	Sequence of primer
mNeuN DP for	agggtagaggggatgagtgg
mNeuN DP reverse	gaggcagacttcaccaaacc
mNeuN sgRNA1 for	CACCgtcggggtccctgaaccgga
mNeuN sgRNA1 reverse	AAACtccggttcagggaccccgac
mNeuN sgRNA2 for	CACCgactccacccttccgacccca
mNeuN sgRNA2 reverse	AAACtggggtcggaagggtggagtc
mNeuN sgRNA3 for	CACCgtgggctgctgcttctccgtg
mNeuN sgRNA3 reverse	AAACcacggagaagcagcagcccac
mGFAP DP for	gtaacagcagcctcgtttcc
mGFAP DP reverse	tctctctgggcaagactggt
mGFAP sgRNA1 for	CACCggcccaacagcaggtccacg
mGFAP sgRNA1 reverse	AAACcgtggacctgctgttgggcc
mGFAP sgRNA2 for	CACCgagagattcgcactcaatacg
mGFAP sgRNA2 reverse	AAACcgtattgagtgcgaatctctc
mGFAP sgRNA3 for	CACCgtggccacatccatctccacg
mGFAP sgRNA3 reverse	AAACcgtggagatggatgtggccac
mGFAP sgRNA4 for	CACCgtctctctcagggccgctgtg
mGFAP sgRNA4 reverse	AAACcacagcggccctgagagagac
mAct1 DP for	ctgggatctcagctttcagc
mAct1 DP reverse	agtctctggacgttggcagt
mAct1 sgRNA1 for	CACCgtagtactgacagttccatg
mAct1 sgRNA1 reverse	AAACcatggaactgtcagtactac

mAct1 sgRNA2 for	CACCgaggtcctgcaggtaacacg
mAct1 sgRNA2 reverse	AAACcgtgttacctgcaggacctc
mAct1 sgRNA3 for	CACCgatgtgcccacgatagacac
mAct1 sgRNA3 reverse	AAACgtgtctatcgtgggcacatc
mAct1 sgRNA4 for	CACCgtggccaagagatgatgccc
mAct1 sgRNA4 reverse	AAACgggcatcatctcttggccac
Scramble sgRNA for	caccgcactcacatcgctacatca
Scramble sgRNA reverse	aaactgatgtagcgatgtgagtgc
U6 KpnI for	TTAATTAAGGTACCATCGATTCTAgagggcctatttcccatga
U6-insert SfuI reverse	CAAAAGCATTCGAAGTTTCTGAAGCAAT
MPAA linker for	cgcgattaattaaggtacctttgctagctttggcgcgcca
MPAA linker reverse	ccggtggcgcgccaaagctagcaaaggtaccttaattaat
GFAP promoter NheI for	GCTAGCCCTGCAGGGAACATATCCTGGTGTGGAGTAG
GFAP promoter AscI reverse	TTCGAAGGCGCGCCGCGAGCAGCGGAGGTGATGC
hSYN1 promoter NheI for	GCTAGCCCTGCAGGgagtgcaagtgggttttag
hSYN1 promoter AscI reverse	TTCGAAGGCGCGCCctgcgctctcaggcacgac