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Supplemental Information

dCas9-Based *Scn1a* Gene Activation Restores Inhibitory Interneuron Excitability and Attenuates Seizures in Dravet Syndrome Mice

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Figure S1. Related to Figure 1

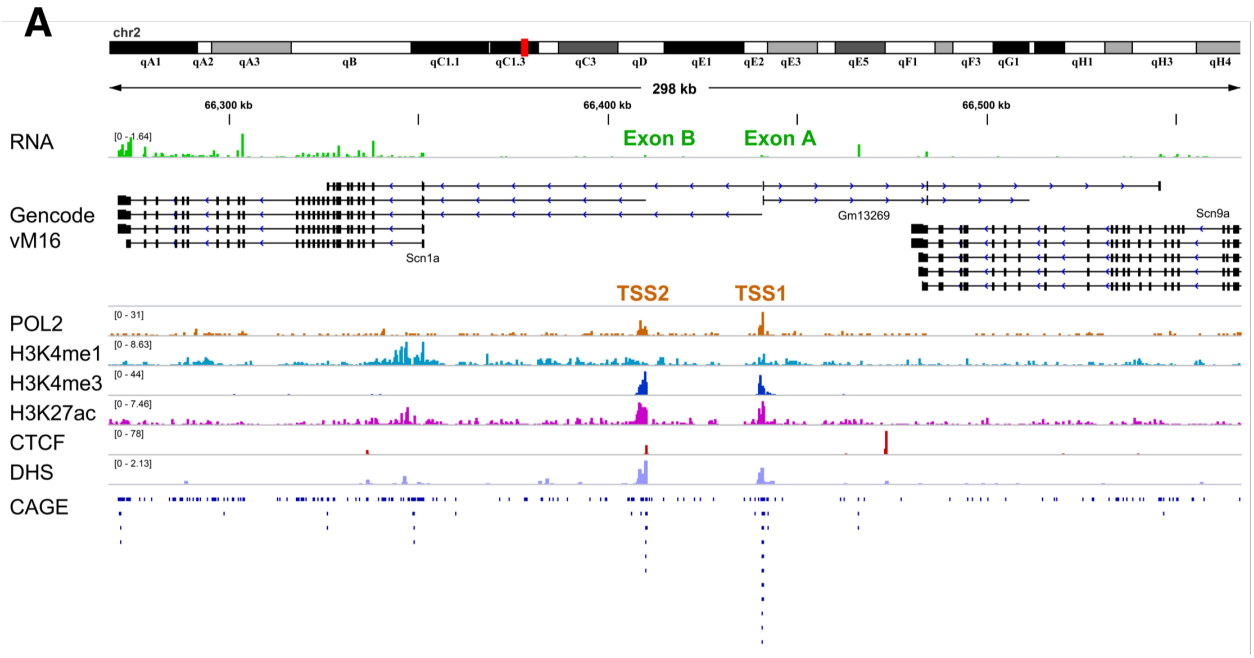
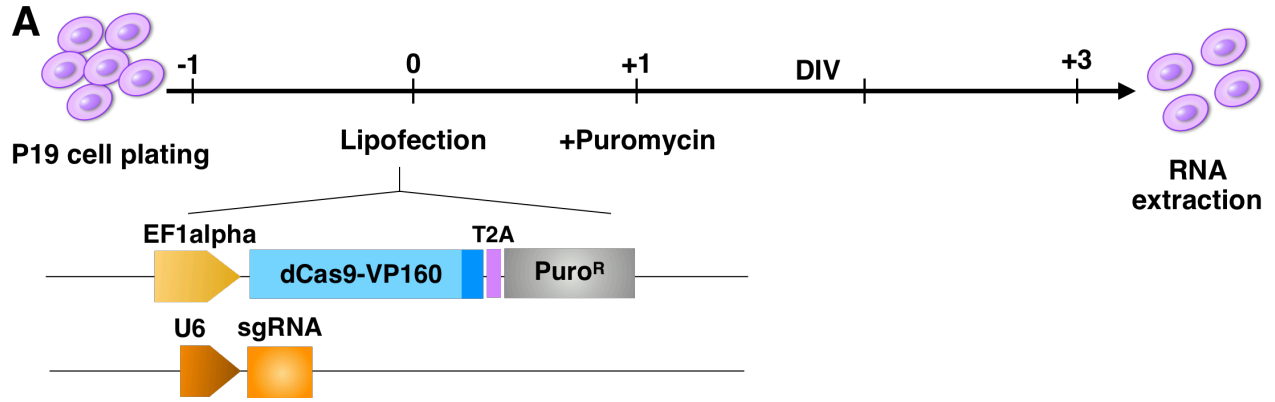
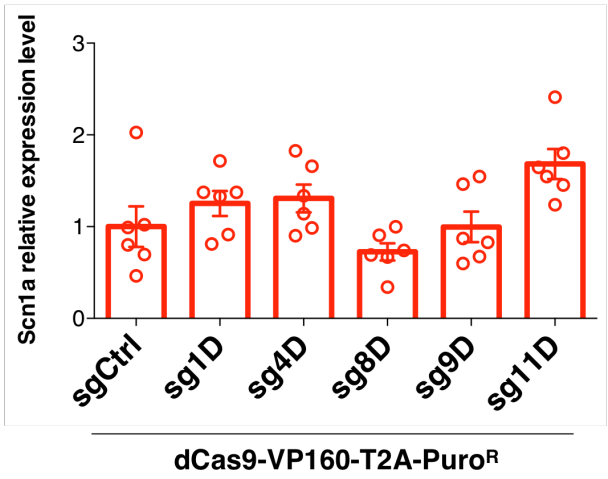
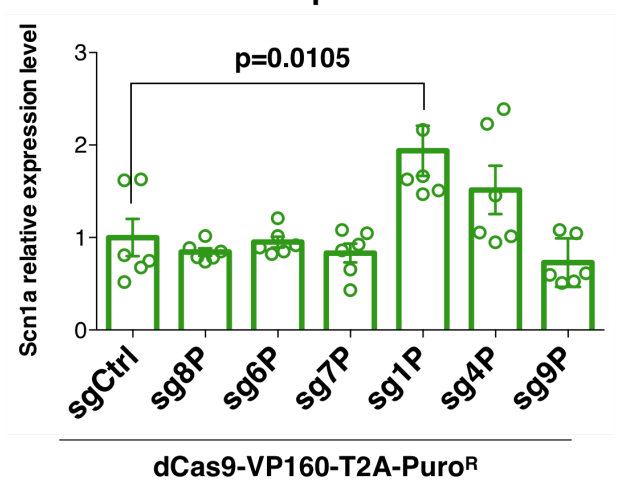


Figure S2. Related to Figure 1



P19 cells





MEFs

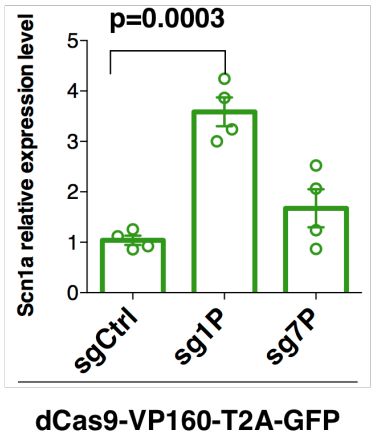


Figure S3. Related to Figure 4

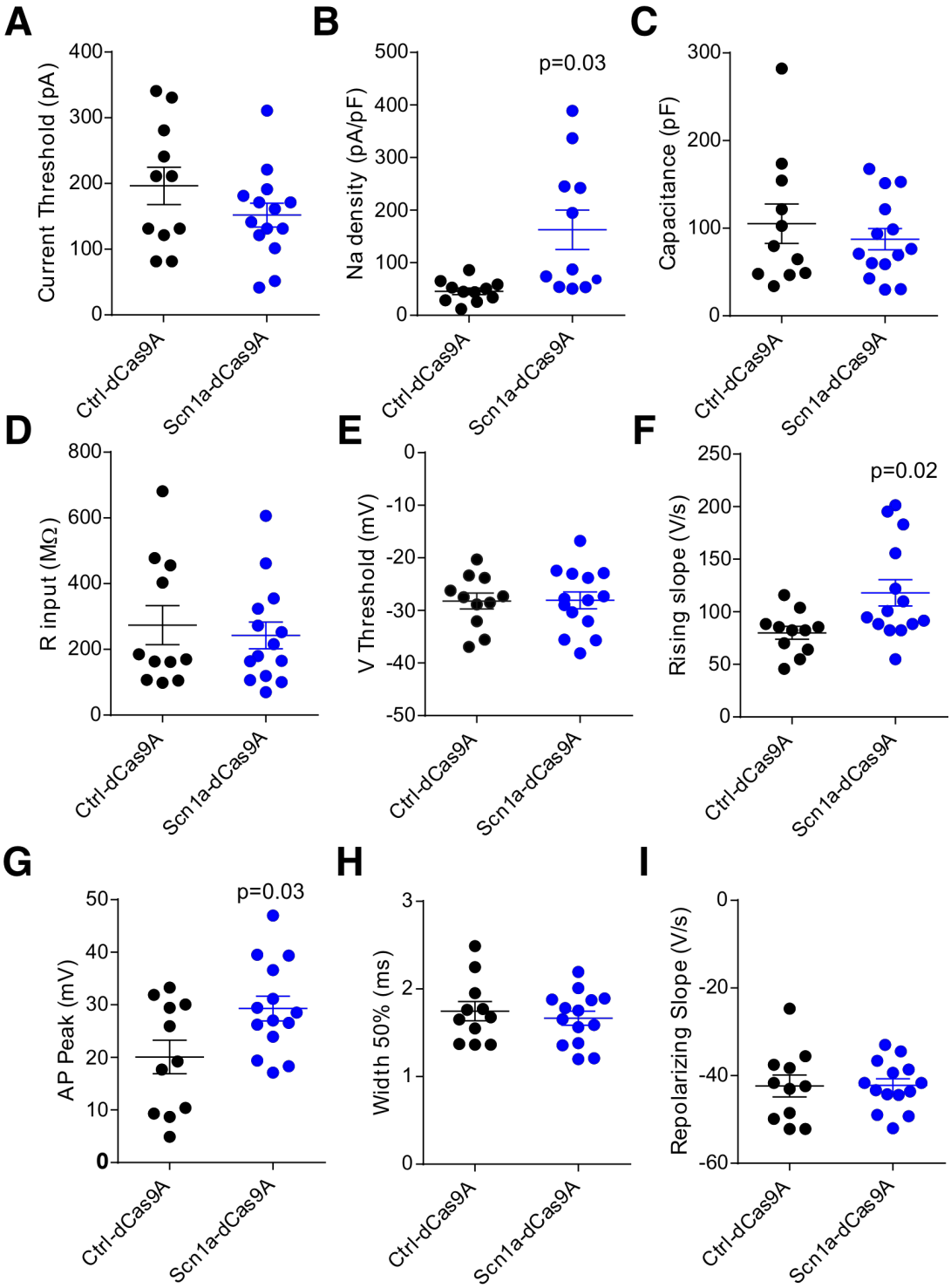
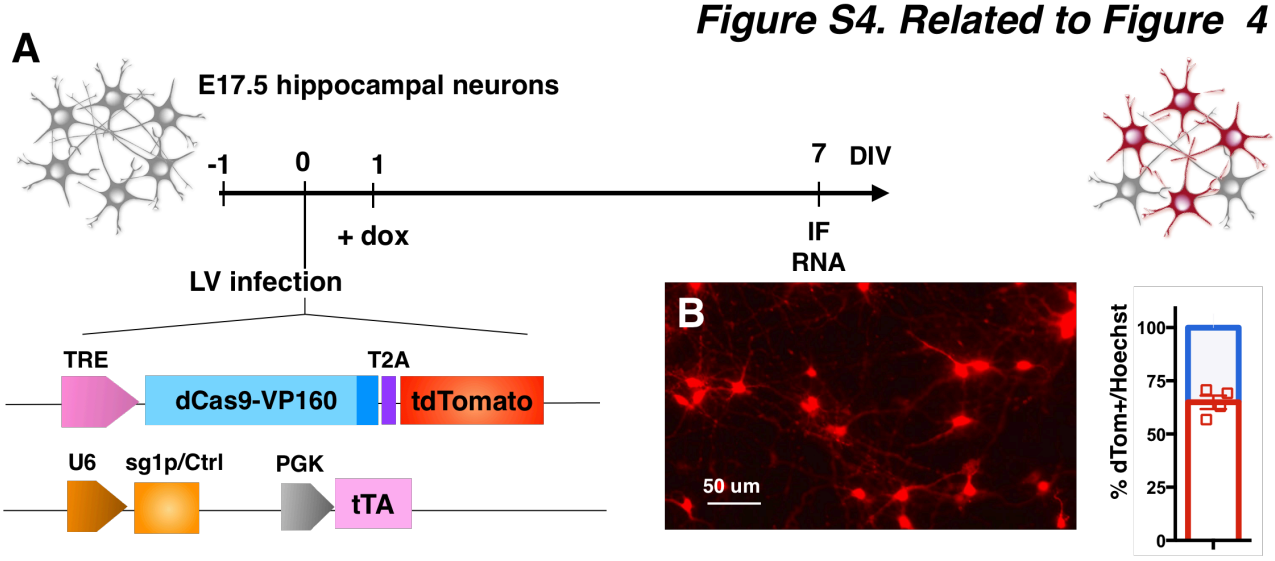
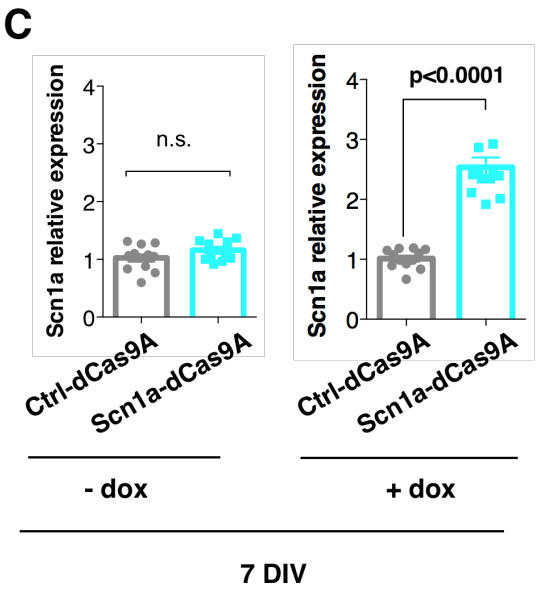
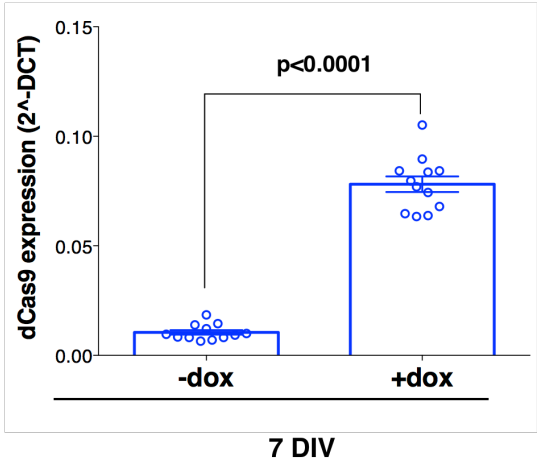


Figure S4. Related to Figure 4







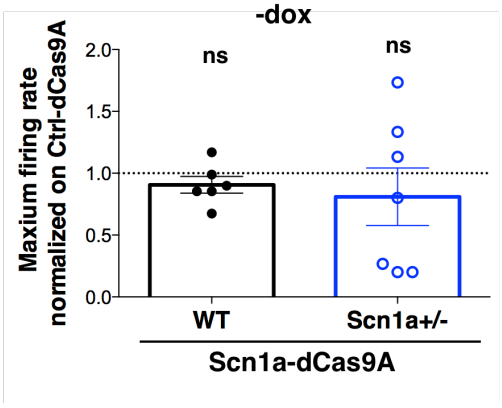


Figure S5. Related to Figure 6

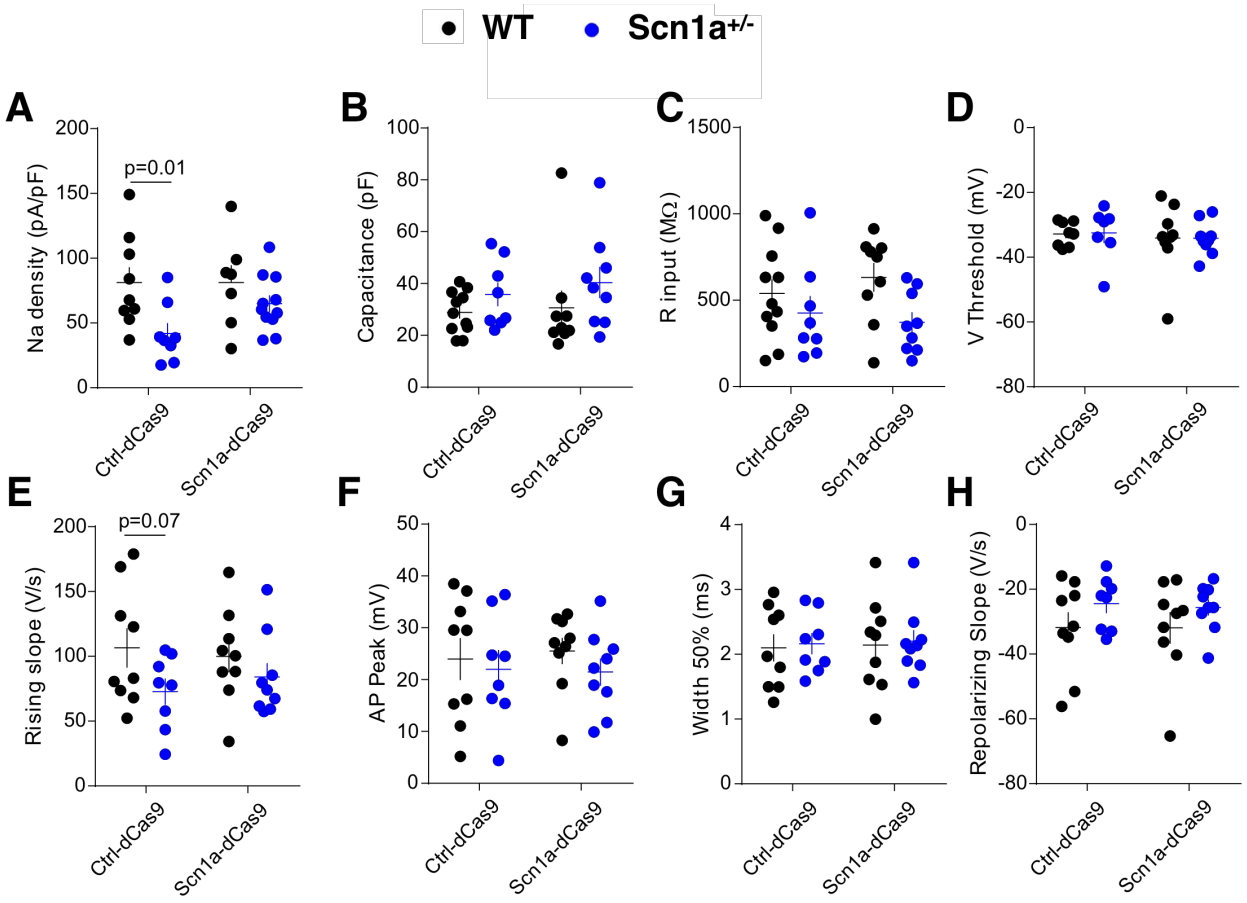


Figure S6. Related to Figure 7

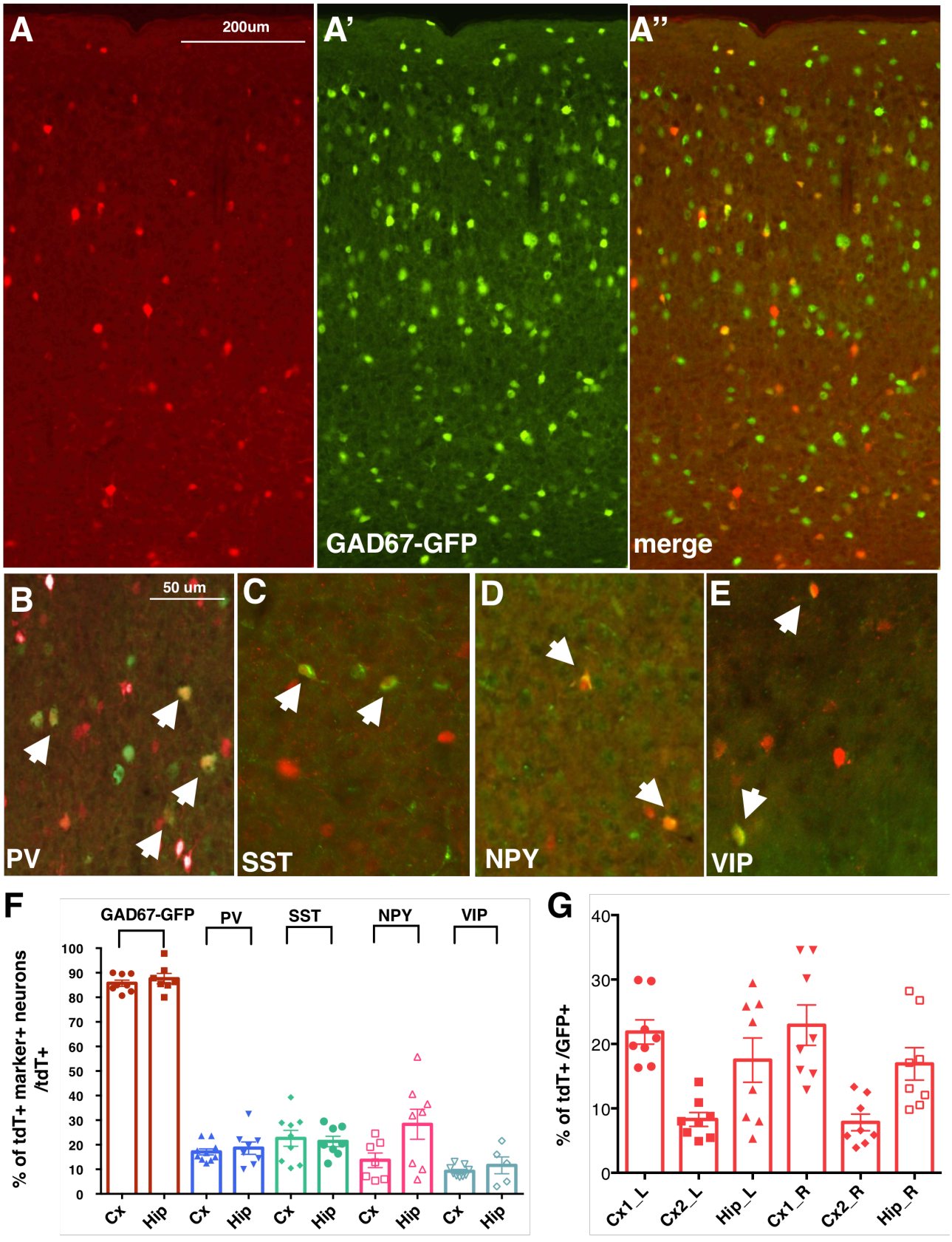


Figure S7. Related to Figure 7

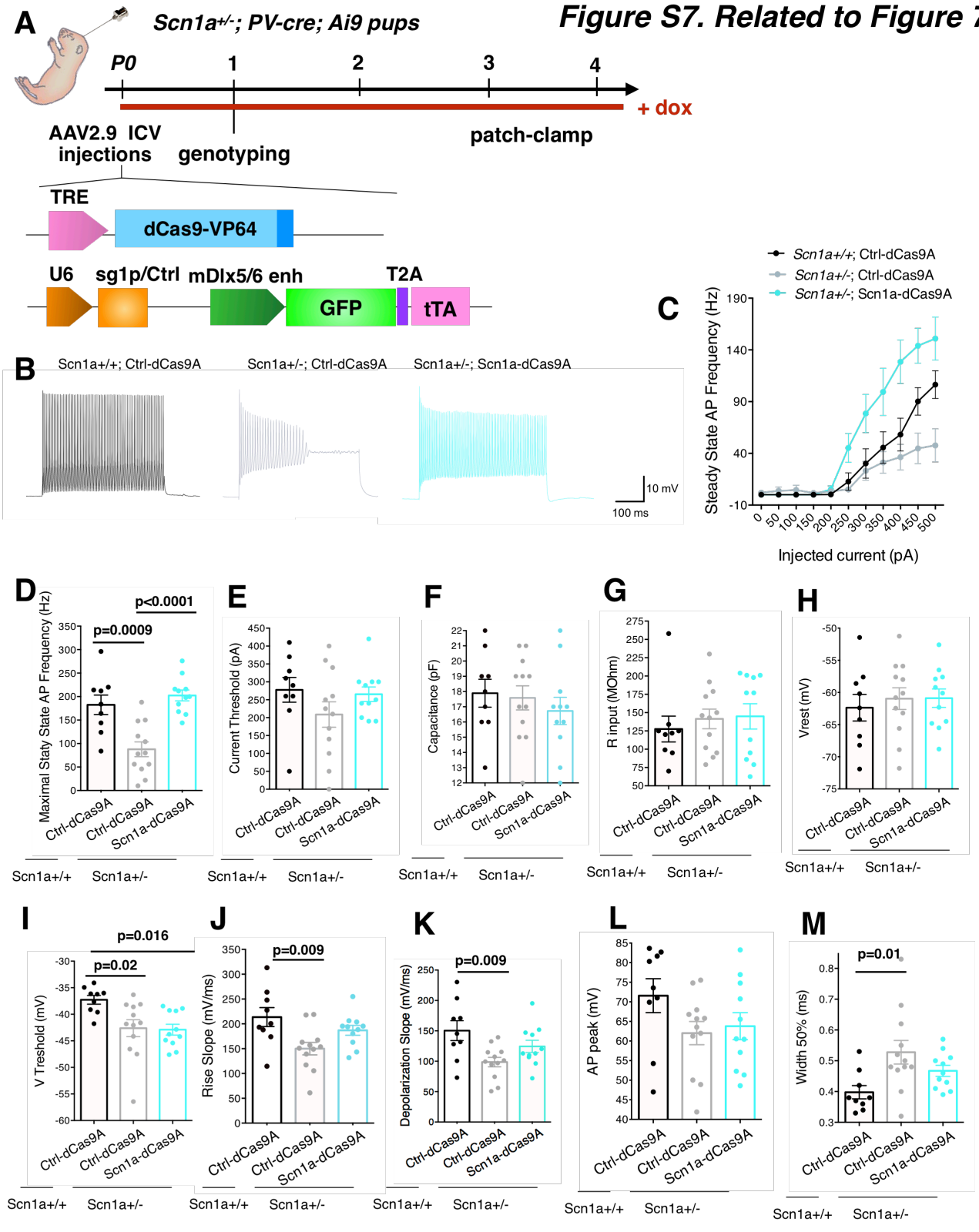


Figure S8. Related to Figure 7

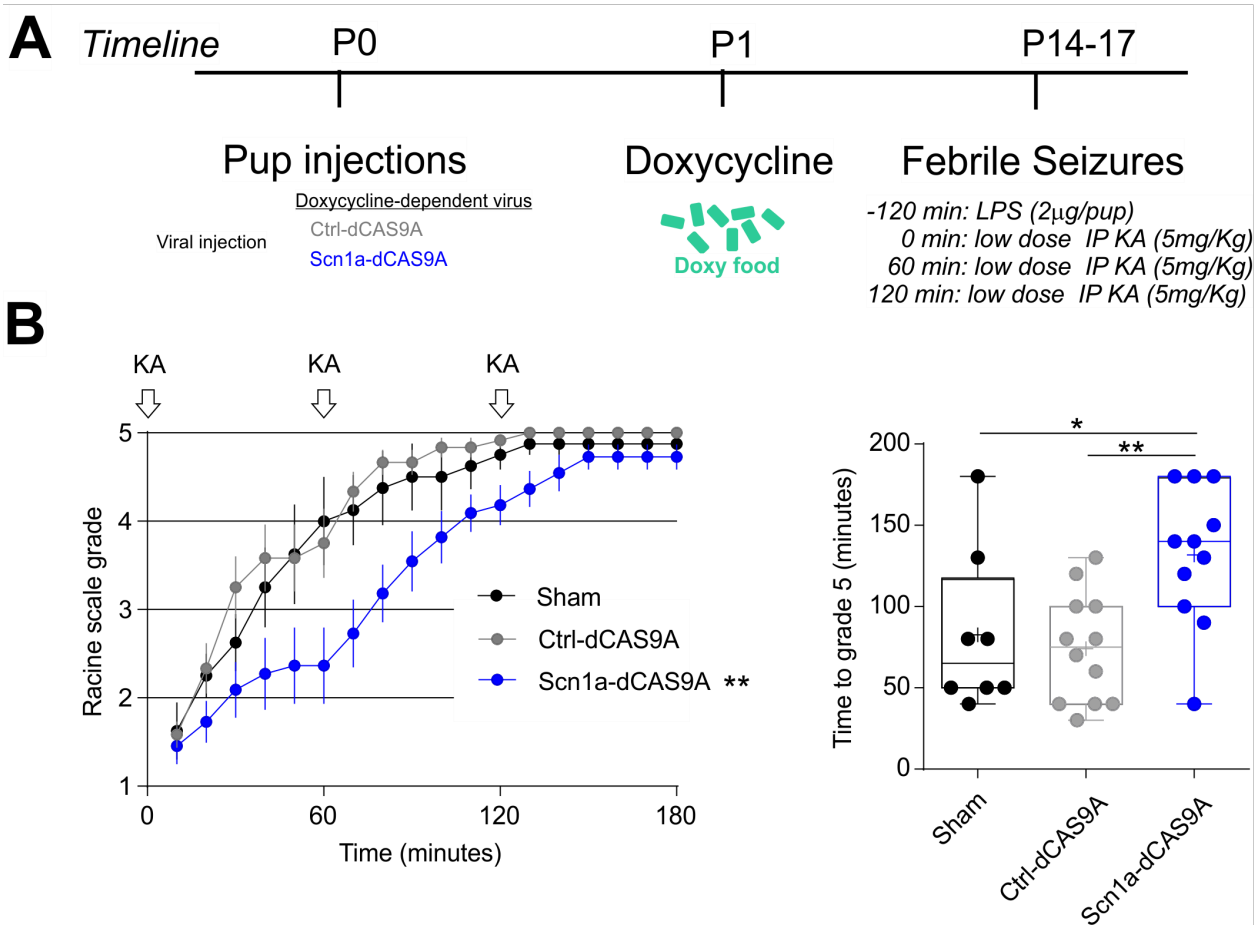


Table S1: Sequences of sgRNAs

sg1D	CGTTTTTGAAACGTTTTGGA AGG
sg4D	AGCATGAAAGCTAAATCTCC TGG
sg8D	ATAGGTCTCATTTTGTGGGT AGG
sg9D	TTGCATGGAAATCATGAACC AGG
sg11D	AAGTATTGGCAGCAGCAAGC AGG
sg8P	AATAAGCAAATTTCATTCAT GGG
sg6P	ATTGTTACTTTTACAGATTA CGG
sg7P	CCCCTTTGCTCTGCCTATCA TGG
sg1P	TAAGTCAATAGTTCCATGAT AGG
sg9P	CCCCTTTGCTCTGCCTATCA TGG
sg4P	CCATGATAGGCAGAGCAAAG TGG
sglacZ	TGCGAATACGCCCACGCGAT

Table S2: Primers for RT-qPCR

18s_F	GGTGAAATTCTTGGACCGGC
18s_R	GACTTTGGTTTCCCGGAAGC
Scn1a_F	CACCAACGCTTCCCTTGAGG
Scn1a_R	TGGACATTGGCCTGCATCAG
Scn1a_exA_F	GGTCCTGGTGGTACAAGCACT
Scn1a_exA_R	GAGGCTGCAGGAAGCTGAG
Scn2a_F	CCTCAGGAGGTCTATGCCAAA
Scn2a_R	GTGTCAGCTGGTTGCGAAAA
Scn3a_F	GCATTGCGTCCACGTAGATAA
Scn3a_R	GGAGCTGAAGACATGGGTCA
Scn4a_F	AGATCCCGCCTCCTGATTTA
Scn4a_R	ATCATGGGGGTGAGAGGAGT
Scn5a_F	TGGGAGAGGAGACAGTGTGG
Scn5a_R	CACGGGGATGATTGGACTTA
Scn7a_F	CCTTACCAACTTGCCTTGGA
Scn7a_R	ACCAACCAACCAACCAAACA
Scn8a_F	GCTTCTGCCATATCCCTCCA
Scn8a_R	GGCAGCTCCATCTTTCCATC
Scn9a_F	ATGGAAGATGCCAAGCAGTG
Scn9a_R	TGGATGTTTTGTGTGGCTCA
Scn10a_F	TCCCACCATCCTATGACAGC
Scn10a_R	ACTGAGGTCCAGGGCTCTTC
Scn11a_F	TTCATGGAGGCCAATCCTTT
Scn11a_R	TGACCTGCCTTTCAGCTTCA
Pde4b_F	TCAGCCAGGTCTAATCTGCCA
Prp4_F	GGTCCATGGTGACCTTCAAGA
Prp4_R	ATGTGGACTGTAGGTGGTGC
BC02_F	AGTGAGTGCAGGGGTCCT
BC02_R	GAAGGATGGTGGTTGGTGGG
Olfr919_F	CCTGGATGGTAGGTGGGGTA
Olfr919_R	GCAGGCAAGCTCCATCAATG
Plrg1_F	AGTTGCTACCGTGAGATGCC
Plrg1_R	TGGTTCGTCAGTGTCACTCG

Supplementary Figure Legends

Figure S1 | Bioinformatics analysis of the *Scn1a* gene locus for promoter regulatory region prediction.

Alignment to the *Scn1a* gene reference sequence of RNA-seq, ChIP-seqs, DNase-seq and CAGE- seq profiles related to adult mouse brains. The enrichment of markers associated with transcriptional activation in the regions upstream of the first two untranslated exons (Exon A and Exon B) of the gene highlights the presence of two TSS (TSS1 and TSS2) and allows to localize a distal promoter in the 200 bp upstream of exon-A and a proximal promoter upstream of the exon-B. POL2, RNA polymerase II ChIP-seq; H3K4me3, tri-methylation of lysine 4 on the histone H3 ChIP-seq; H3K4me1, mono- methylation of lysine 4 on histone H3 ChIP-seq; H3K27ac, acetylation of lysine 27 on histone H3 ChIP-seq; CTCF, factor that binds the CCCTC; DHS, DNase I Hyper Sensitivity mapping; CAGE-seq, Cap Analysis of Gene Expression-sequencing.

Figure S2 | Screening of sgRNAs for *Scn1a* gene activation by targeting its distal or proximal promoter in association with the dCas9-VP160-T2A-GFP in different cell types.

a, Screening of the guides lipofected in P19 cells in association with dCas9VP160-T2A-Puro^R. Quantitative RT-PCRs performed on RNA extracted from P19 cells 3 days after lipofection with dCas9VP160-T2A-Puro^R and sgRNAs targeting distal **(b)** or proximal **(c)** promoters to evaluate levels of *Scn1a* gene transcript. Data are normalized on 18S rRNA and relative to sgCtrl lipofected cells; n = 6, p = 0.0001, one-way ANOVA followed by Bonferroni multi comparison tests. **d**, RT-qPCR on RNA extracted from MEFs infected with sg1P and sg7P in association with dCas9VP160-T2A-Puro^R; n = 4, p = 0.0003, One-way ANOVA followed by Bonferroni's multiple comparison tests. Data are shown as mean ± s.e.m. with dots representing individual samples.

Figure S3 | The *Scn1a*-dCas9A system accelerates functional maturation of primary wild-type hippocampal neurons at 9-11 DIV.

Analysis of passive properties, voltage steps and current threshold (**a-d**), and single AP shape (**e-i**) in 9-11 DIV wild-type primary neurons transduced with either the Ctrl-dCas9A or *Scn1a*-dCas9A system. Student's *t* test was used for statistical analysis.

Figure S4 | Assessing the leakiness of the *Scn1a* gene activation by the *Scn1a*-dCas9A system.

A, Illustration of the dual LV doxycycline (dox) inducible system set for patch-clamp experiments *in vitro:* a first LV carrying dCas9-VP160 regulated by the rtTA responsive element (TRE) and a second

carrying the transactivator rtTA together with the sgRNA. Dox was administered or not at / DIV IF and RNA extraction were performed; **B**, anti-RFP immunofluorescence and quantification of tdTomato^{+} transduced cells over total neurons. **C**, Relative RT-qPCR for Scn1a performed on RNA extracted from either Ctrl-dCas9A or *Scn1a*-dCasA in WT neurons at 7 DIV in the absence or presence of dox. Data are expressed as ratios relative to Ctrl-dCas9A. **D,** RT-qPCR for dCas9 (2^-DCt) in neurons transduced with Scn1a-dCas9A system in the absence or presence of dox (n=12, $p<0.0001$ Student's *t* test. **E,** Histogram plot of maximum firing rate in Scn1a-dCas9A treated wt and Scn1a$^{+/-}$ GAD67-GFP neurons relative to Ctrl-dCas9A in the absence of dox (n=7, Student's *t* test).

Figure S5| The *Scn1a*-dCas9 system corrects some functional impairments in 18-20 DIV primary *Scn1a*$^{+/-}$ neurons while is not altering activity in corresponding wild-type neurons.
A-H, Analysis of passive properties, Na^{+} current density and single AP shape in *Scn1a*$^{+/+}$ (black dots) and *Scn1a*$^{+/-}$ (blue dots) primary neurons transduced with either the Ctrl-dCas9A or the *Scn1a*-dCas9A system.

Figure S6 | AAVs packaged with the Scn1a-dCas9A system controlled by the Dlx5/6 enhancer direct tdTomato expression specifically in cortical interneuron subpopulations *in vivo*.
A-A'', Anti-GFP and anti-RFP dual immunofluorescence in brain sections of P30 GAD67-GFP mice subjected to intracerebroventricular injections at P0 with AAVs carrying *Scn1a*-dCas9A elements, scale bars 200um. **B-E**, Representative cortical areas of P30 mouse brain sections transduced at P0 with the *Scn1a*- dCas9A elements stained for anti-PV, -SST, -NPY and -VIP in association with anti-RFP to reveal transduced neurons, scale bars 50 um. **F,** Quantification of the percentage of tdTomato^{+} cells co-expressing each of the interneuron markers listed above (GAD67-GFP, PV, SST, NPY and VIP) over the total number of tdTomato^{+} cells. **G**, Quantifications of the percentage of tdTomato^{+} cells over the total of GAD67-GFP+ cells in various areas (Cx1, Cx2 and Hip in each brain hemisphere). Data are shown as mean ± s.e.m., with dots representing individual quantifications.

Figure S7 | Scn1a-dCas9A treatment ameliorates firing in Scn1a+/- PV interneurons
A, Schematic illustration showing the experimental setting for ICV injections of Ctrl and Scn1a-dCas9A with GFP reporter into *Scn1a*$^{+/+}$ and *Scn1a*$^{+/-}$; PV-Cre Ai9 P0 pups. Transduced PV interneurons appear GFP^{+} and tdTomato^{+}. Dox was administered in drinking water until the final analysis. **B**, Representative traces recorded from GFP^{+}/tdTomato PV^{+} interneurons in somatosensory cortex (SSC) (P21-28). **C**, I/O plot analysis show impaired functionality in *Scn1a*$^{+/-}$ Ctrl-dCas9A compared to *Scn1a*$^{+/+}$; Ctrl-dCas9A interneurons which is recovered in *Scn1a*$^{+/-}$;Scn1a-dCas9A PV

interneurons (p=0.003, two-way ANOVA/Bonferroni). **D.** Maximal steady state AP frequency and other passive and AP parameters of *Scn1a*$^{+/+}$; Ctrl-dCas9A and *Scn1a*$^{+/-}$; Ctrl-/ Scn1a-dCas9A transduced PV interneurons (one-way ANOVA/Bonferroni's multiple comparison tests).

Figure S8 | Upregulation of Nav1.1 during early development is protective against febrile seizures. A. Timeline of the experimental plan. **B.** *Left.* Racine scale scoring following low dose KA injections every hour over a 3-hour experimental time period with behavioural scoring every 10 minutes. ** $p<0.01$, two-way ANOVA, Sham or Ctrl-dCAS9A vs Scn1a-dCAS9A. *Right.* Box plots of the time taken to reach grade 5. Middle line represents the median, "+" the mean and the box, the 10-90 percentile range. *$p<0.05$, **$p<0.01$, one-way ANOVA followed by Bonferroni multi-comparison test.

Supplementary Table 1: Sequences of sgRNAs.

Supplementary Table 2: Primers for RT-qPCRs.