Supplementary Materials

CRISPR activation rescues abnormalities in *SCN2A* haploinsufficiency-associated autism spectrum disorder

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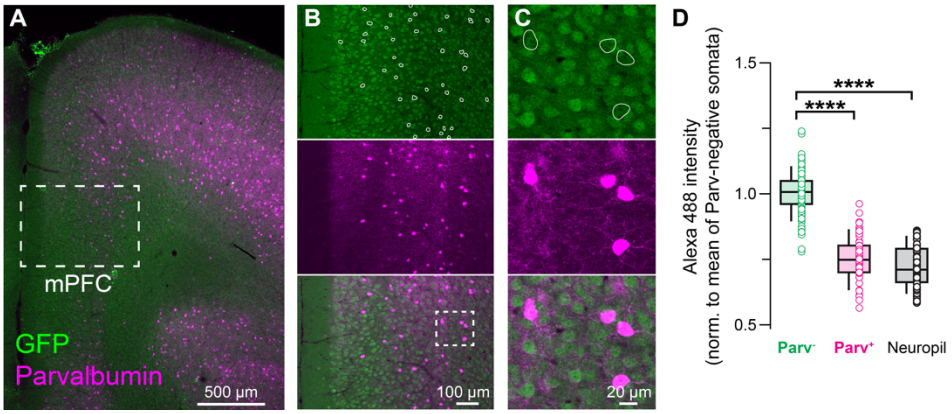

Figure S1: Excitatory pyramidal neurons in the mPFC are GFP+ in Cre-negative *Scn2a*$^{+/KI}$ animals.
A-C: Coronal brain sections from P60 *Scn2a*$^{+/KI}$ mouse (Cre-) immunostained with anti-GFP and anti-parvalbumin (PV).
D: Quantification of mean fluorescence intensify of GFP in PV-negative cells, PV-positive cells, and neuropil (area without somata as a measure of background fluorescence). Data are from 2 mice. Parv-: 1.0 ± 0.01, n = 67 cells; Parv+ 0.7 ± 0.01, n = 37 cells; neuropil: 0.75 ± 0.01. Parv- vs. Parv+: ****p < 0.0001. Parv- vs. neuropil: ****p < 0.0001. Holm-Šídák multiple comparisons test.

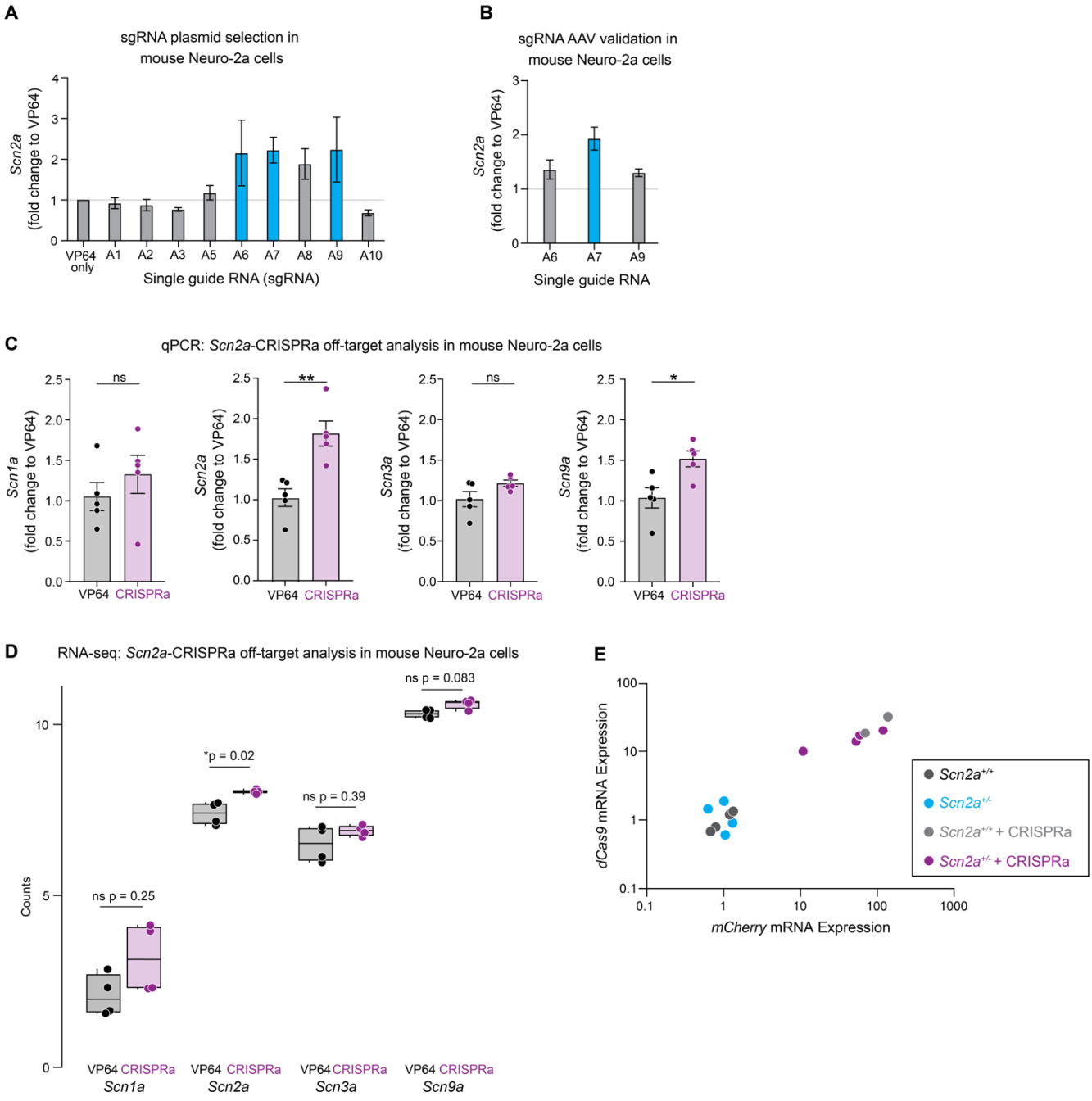

Figure S2: *In vitro* optimization of CRISPRa constructs in mouse Neuroblastoma-2A (Neuro-2a) cells

A: Fold change of *Scn2a* expression in Neuro-2a cells transfected with plasmids containing sgRNAs targeting the promoter of mouse *Scn2a* compared to a no-sgRNA VP64 control. Blue bars represent plasmids with largest increase in *Scn2a* expression.

B: Fold change of *Scn2a* transduced with rAAV-DJ virus in Neuro-2a cells.

C: qPCR off-target analysis of transcripts encoding other sodium channels in the topologically associated domain (TAD). Mann-Whitney test.

D: RNA-seq of other sodium channel subtypes from *Scn2a*-rAAV-CRISPRA treated Neuro-2a cells compared to VP64-only. Significance noted above data. Wald-log test.

E: qPCR analysis of *dCas9* and *mCherry* mRNA within the mPFC of tail vein injected *Scn2a*$^{+/+}$ + CRISPRa (light gray) and *Scn2a*$^{+/-}$ + CRISPRa (purple) versus uninjected controls *Scn2a*$^{+/+}$ (dark gray) or *Scn2a*$^{+/-}$ (cyan). Injected animals with at least a 10-fold increase in expression levels of both *dCas9* and *mCherry* to the average *Scn2a*$^{+/+}$ uninjected controls were included in EEG datasets in Fig 3.

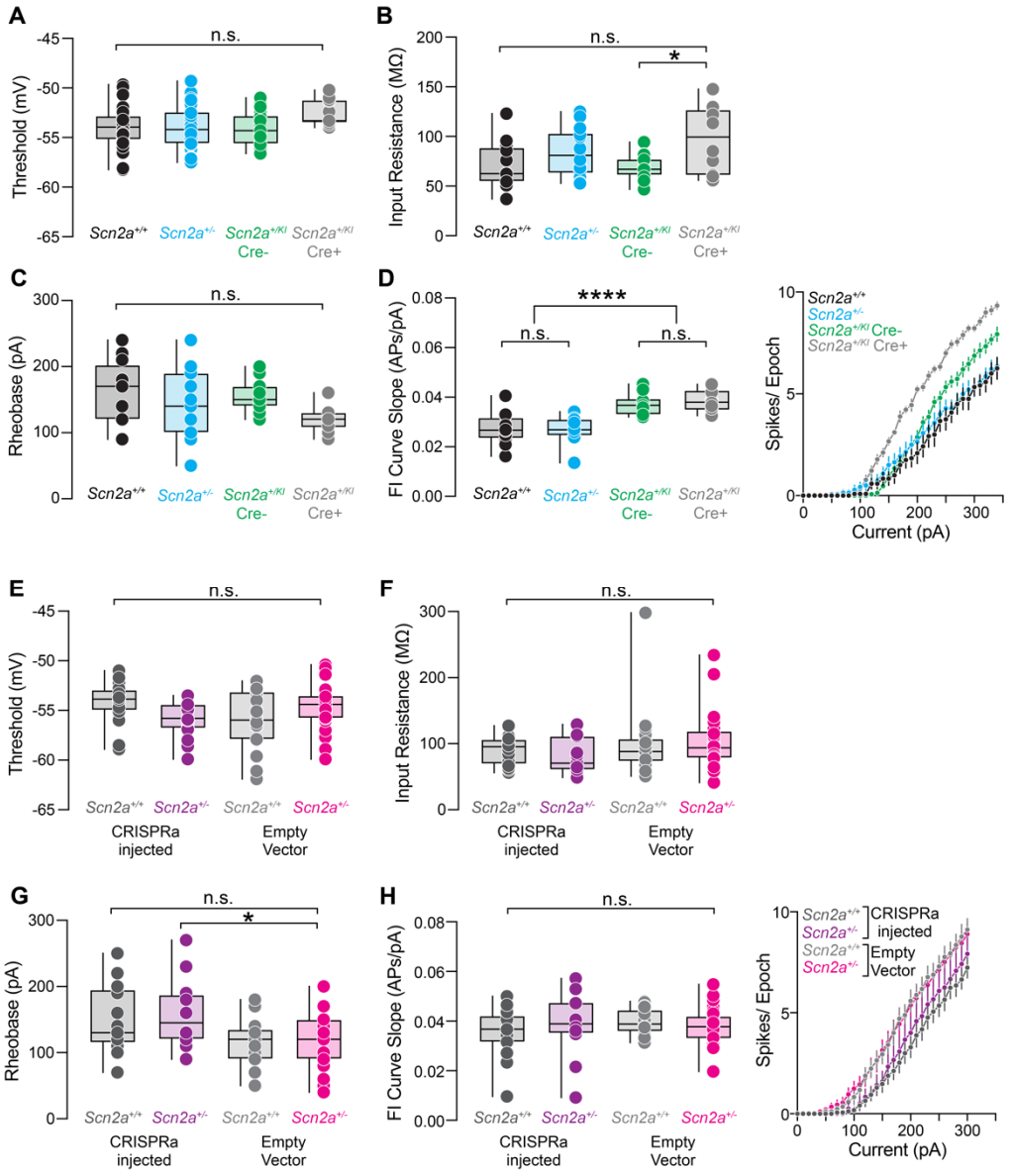

Figure S3: Additional intrinsic electrophysiological measurements in the *Scn2a*$^{+/KI}$ conditional mouse model and CRISPRa treated neurons.

A: Summary of AP threshold from P60-70 *Scn2a*$^{+/+}$ (black), *Scn2a*$^{+/-}$ (cyan), *Scn2a*$^{+/KI}$ Cre- (green) and *Scn2a*$^{+/KI}$ Cre+ (gray) neurons. Threshold of the first AP evoked by a near-rheobase current. Box plots are median and quartiles with min. and max. tails. Circles represent single cells. *Scn2a*$^{+/+}$: -54.0 ± 0.3, n = 44 cells; *Scn2a*$^{+/-}$: -54.0 ± 0.4, n = 37 cells, *Scn2a*$^{+/KI}$ Cre-: -54.1 ± 0.4 n = 18 cells; *Scn2a*$^{+/KI}$ Cre+: -52.5 ± 0.4, n = 11 cells. No significant differences. Holm-Šídák multiple comparisons test.

B: Summary of input resistance (MΩ). *Scn2a*$^{+/+}$: 71.3 ± 6.9, n = 12 cells; *Scn2a*$^{+/-}$: 83.8 ± 6.5, n = 14 cells, *Scn2a*$^{+/KI}$ Cre-: -69.6 ± 3.1 n = 15 cells; *Scn2a*$^{+/KI}$ Cre+: 97.7 ± 10.7, n = 10 cells. No significant differences. Holm-Šídák multiple comparisons test.

C: Summary of rheobase current (pA) to generate first spike. *Scn2a*$^{+/+}$: 163.3 ± 13.3, n = 12 cells; *Scn2a*$^{+/-}$: 144.3 ± 13.6, n = 14 cells, *Scn2a*$^{+/KI}$ Cre-: 153.3 ± 5.6 n = 15 cells; *Scn2a*$^{+/KI}$ Cre+: 119.0 ± 6.1, n = 10 cells. No significant differences. Holm-Šídák multiple comparisons test.

D: APs per 300 ms stimulation epoch for each current amplitude. Left: Quantification of firing rate slope of data on Right. *Scn2a*$^{+/+}$: 0.03 ± 0.002, n = 12 cells; *Scn2a*$^{+/-}$: 0.03 ± 0.002, n = 14 cells, *Scn2a*$^{+/KI}$ Cre-: 0.04 ± 0.001, n = 15 cells; *Scn2a*$^{+/KI}$ Cre+: 0.04 ± 0.001, n = 10 cells. *Scn2a*$^{+/+}$ vs. *Scn2a*$^{+/KI}$ Cre-: ****p < 0.0001, *Scn2a*$^{+/+}$ vs. *Scn2a*$^{+/KI}$ Cre+: ****p < 0.0001, *Scn2a*$^{+/-}$ vs. *Scn2a*$^{+/KI}$ Cre-: ****p < 0.0001. *Scn2a*$^{+/-}$ vs. *Scn2a*$^{+/KI}$ Cre+: ****p < 0.0001. Holm-Šídák multiple comparisons test. Right: Number of APs versus current amplitude injected.

E: Summary of AP threshold from P57-85 *Scn2a*-rAAV-CRISPRa treated *Scn2a*$^{+/+}$ (dark gray) and *Scn2a*$^{+/-}$ (purple) neurons and *Scn2a*-rAAV-empty transduced *Scn2a*$^{+/+}$ (light gray) and *Scn2a*$^{+/-}$ (magenta) neurons. Threshold of the first AP evoked by a near-rheobase current. represent single cells. *Scn2a*$^{+/+}$ + CRISPRa: -54.2 ± 0.4, n = 24 cells; *Scn2a*$^{+/-}$

+ CRISPRa: -55.7 ± 0.4, n = 19 cells, *Scn2a*$^{+/+}$ + empty: -56 ± 0.7 n = 18 cells; *Scn2a*$^{+/-}$ + empty: -55.6 ± 0.4, n = 29 cells. No significant differences. Holm-Šídák multiple comparisons test.

F: Summary of input resistance (MΩ) from *Scn2a*-rAAV-CRISPRa or *Scn2a*-rAAV-empty neurons. *Scn2a*$^{+/+}$ + CRISPRa: 89.5 ± 5.0, n = 17 cells; *Scn2a*$^{+/-}$ + CRISPRa: 80.5 ± 8.0, n = 11 cells, *Scn2a*$^{+/+}$ + empty: 99.3 ± 12.7, n = 18 cells; *Scn2a*$^{+/-}$ + empty: 97.7 ± 10.7, n = 29 cells. No significant differences. Holm-Šídák multiple comparisons test.

G: Summary of rheobase current (pA) to generate first spike from *Scn2a*-rAAV-CRISPRa or *Scn2a*-rAAV-empty neurons. *Scn2a*$^{+/+}$ + CRISPRa: 148.2 ± 11.6, n = 17 cells; *Scn2a*$^{+/-}$ + CRISPRa: 157.5 ± 15.2, n = 12 cells, *Scn2a*$^{+/+}$ + empty: 116.5 ± 8.5, n = 17 cells; *Scn2a*$^{+/-}$ + empty: 115.9 ± 7.8, n = 29 cells. *Scn2a*$^{+/-}$ + CRISPRa vs. *Scn2a*$^{+/-}$ + empty: *p = 0.04. Holm-Šídák multiple comparisons test.

H: APs per 300 ms stimulation epoch for each current amplitude. Left: Quantification of firing rate slope of data on Right. *Scn2a*$^{+/+}$ + CRISPRa: 0.04 ± 0.002, n = 17 cells; *Scn2a*$^{+/-}$ + CRISPRa: 0.04 ± 0.004, n = 12 cells, *Scn2a*$^{+/+}$ + empty: 0.04 ± 0.001, n = 17 cells; *Scn2a*$^{+/-}$ + empty: 0.04 ± 0.001, n = 29 cells. No significant differences. Holm-Šídák multiple comparisons test. Right: Number of APs versus current amplitude injected.

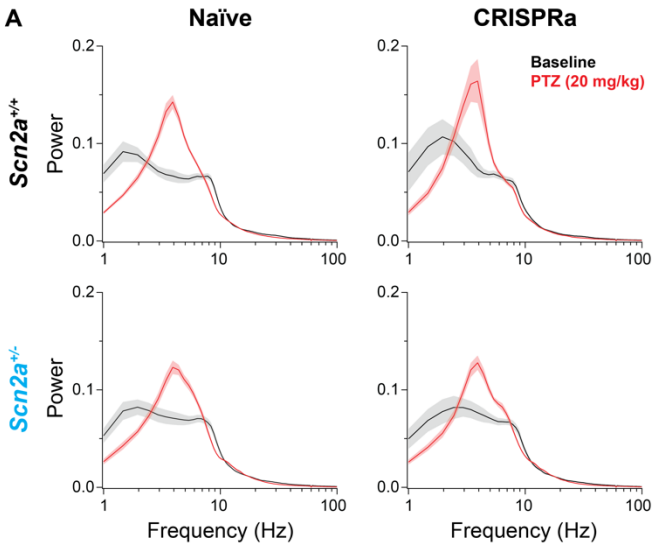

Figure S4: Power spectral density (PSD) during baseline and 20 mg/kg PTZ administration

A: Average PSD across *Scn2a*$^{+/+}$: n = 24 mice; *Scn2a*$^{+/-}$: n = 16 mice; *Scn2a*$^{+/+}$ + CRISPR: n = 7 mice; *Scn2a*$^{+/-}$ + CRISPRa: n = 12 mice during baseline (black) and 20 mg/kg PTZ (red). Note marked increase in PSD in 3-5 Hz observed across all conditions.

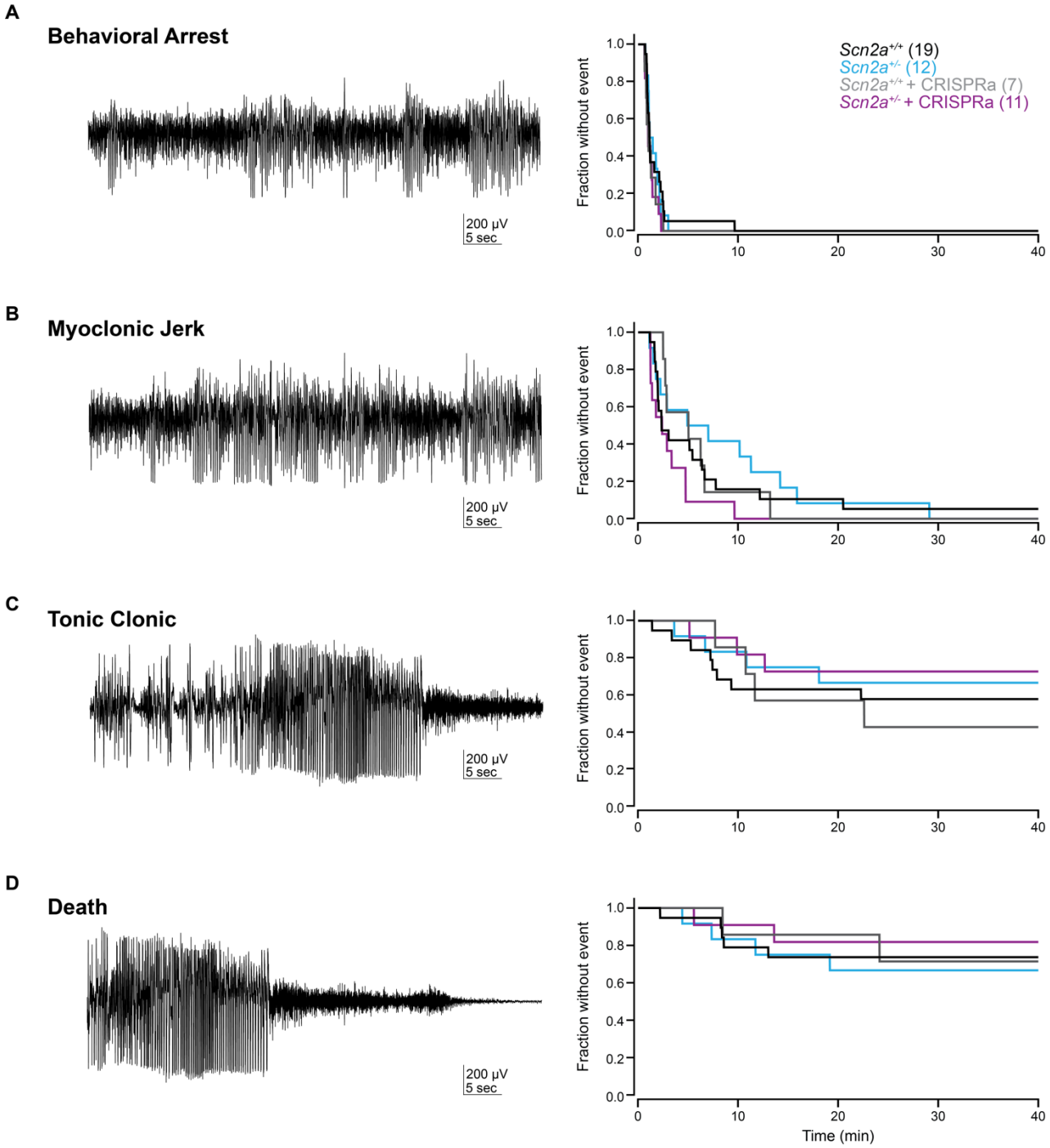

Figure S5: Detection of 50 mg/kg PTZ-induced seizures

A-D: Left: Example EEG trace of behavioral arrest (p = 0.51), myoclonic jerk (p = 0.14), tonic clonic seizure (p = 0.69; reprinted data from Main Fig. 3), and death (p = 0.65, Mantel log-rank test). Right: Survival curves over 40 minutes across *Scn2a*$^{+/+}$ (black): n = 19 mice; *Scn2a*$^{+/-}$ (cyan): n = 12 mice; *Scn2a*$^{+/+}$ + CRISPRa (gray): n = 7 mice; *Scn2a*$^{+/-}$ + CRISPRa (purple): n = 11.

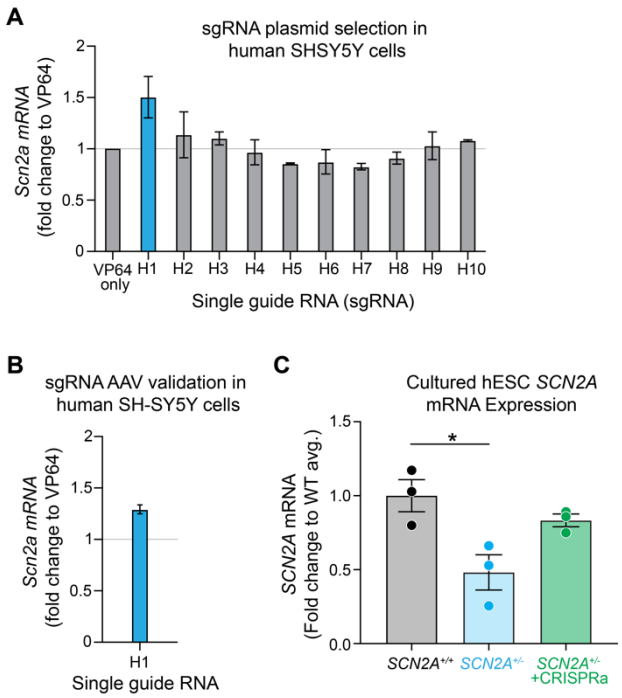

Figure S6: *In vitro* optimization of CRISPRa constructs in human SH-SY5Ycells

A: Fold change of *Scn2a* expression in SH-SY5Y cells transfected with plasmids containing sgRNAs targeting the promoter of human *Scn2a* compared to a no sgRNA VP64 control.

B: Fold change of *Scn2a* transduced with rAAV-DJ virus in human SH-SY5Y cells.

C: *SCN2A* mRNA expression from *SCN2A*$^{+/+}$ (black), *SCN2A*$^{+/-}$ (cyan), and *SCN2A*-rAAV-CRISPRa treated *SCN2A*$^{+/-}$ (purple) hESC-derived neurons normalized to wild type average. *SCN2A*$^{+/+}$: 1.0 ± 0.1, n = 3 dishes; *SCN2A*$^{+/-}$: 0.48 ± 0.1, n = 3 dishes; *SCN2A*$^{+/-}$ + CRISPRa: 0.8 ± 0.04, n = 3 dishes. *SCN2A*$^{+/+}$ vs. *SCN2A*$^{+/-}$: *p = 0.03. Holm-Šídák multiple comparisons test.

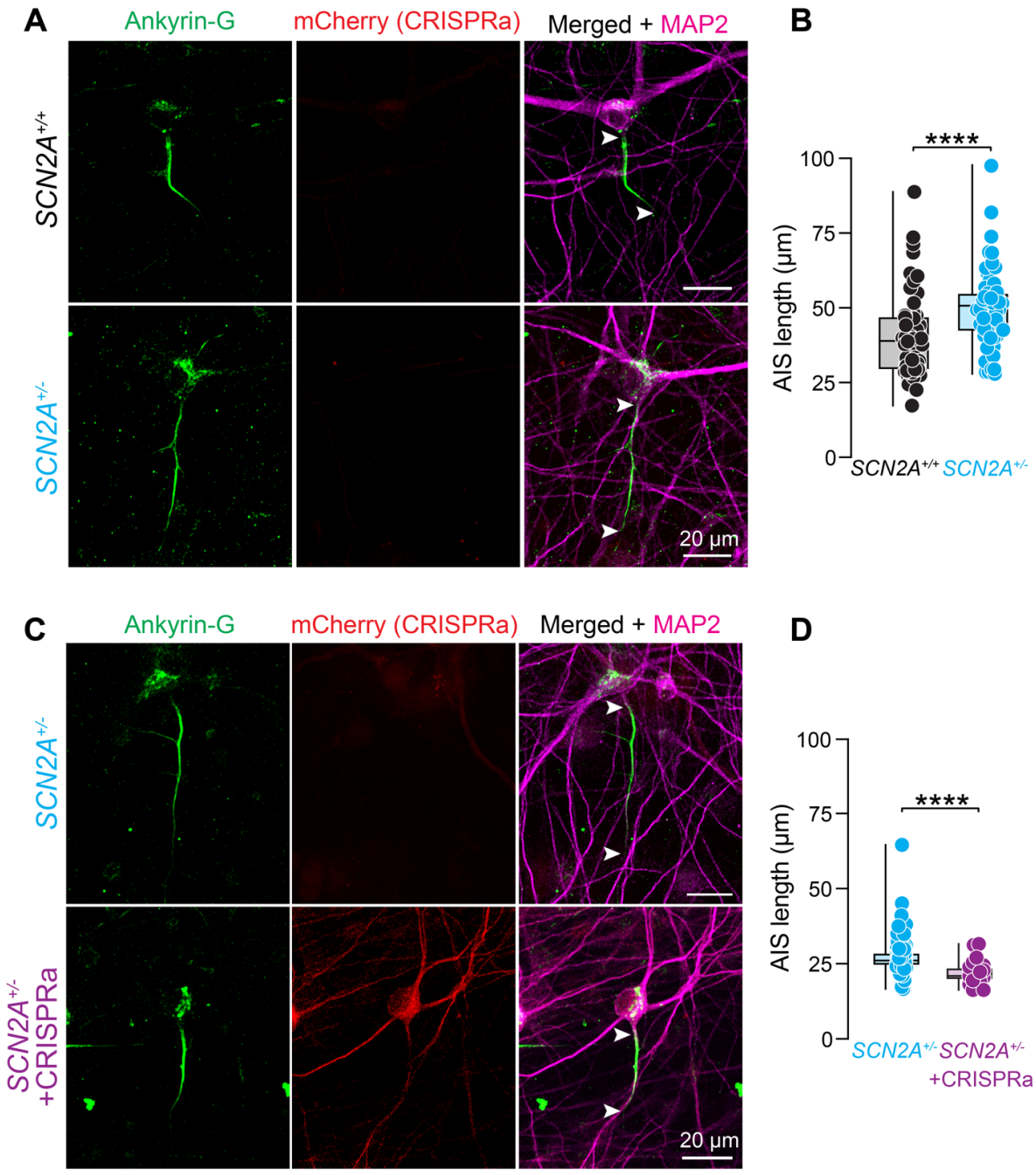

Figure S7: Axon initial segment structural plasticity in *SCN2A*$^{+/-}$ neurons is rescued by CRISPRa.

A: Representative images of *SCN2A*$^{+/+}$ (black) and *SCN2A*$^{+/-}$ (cyan) human stem-cell-derived neurons immunostained with antibodies against ankyrin-G (green) and MAP2 (magenta). Arrows denote start and end points used to quantify AIS length.

B: Quantification of AIS length. *SCN2A*$^{+/+}$: 40.5 ± 1.9 µm, n = 56 cells, 3 dishes. *SCN2A*$^{+/-}$: 50.2 ± 1.8 µm, n = 56 cells, 3 dishes. ****p < 0.0001. Mann-Whitney test.

C: Representative images of *SCN2A*$^{+/-}$ neurons expressing *Scn2a*-rAAV-CRISPRa-mCherry (purple) and mCherry-negative internal *SCN2A*$^{+/-}$ controls (cyan). Immunostaining against ankyrin-G and MAP2.

D: Quantification of AIS length. *SCN2A*$^{+/-}$: 27.1 ± 0.5 µm, n = 122 cells, 3 dishes. *SCN2A*$^{+/-}$ + CRISPRa: 22.0 ± 0.8 µm, n = 23 cells, 3 dishes. ****p < 0.0001. Mann-Whitney test.

Supplemental Table 1 (Attached): ASD risk genes (Fu et al., 2021) and haploinsufficiency likelihood.
All genes shown with gene IDs, cDNA length, presence in ASD72 list (genes with genome-wide significance, Fu et al., 2021), and pLI (probability of being loss-of-function intolerance) and LOEUF (loss-of-function observed/expected upper bound fraction) scores. Genes in blue have cDNA > 3000 BPs with a LOEUF score of < 0.273.

Supplemental Table 2: sgRNA sequences tested per species, and primer sequences used for qPCR.

Primers	Forward	Reverse	
qPCR-Mouse_Scn2a	ATTTTCGGCTCATTCTTCACACT	GGGCGAGGTATCGGTTTTTGT	
qPCR-Mouse_Bactin	GACGATGCTCCCCGGGCTGTATTC	TCTCTTGCTCTGGGCCTCGTCACC	
qPCR-sadCas9-VP64	ATCACCCCCCACCAGATCAAGC	GTCCTTGTCGTACAGGCCGTTCA	
qPCR-Human_*SCN2A*	CGCTTCTTTACCAGGGAATCC	TCCTGTTTGGGTCTCTTAGCTTT	
qPCR-Human_Bactin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT	
genotyping-Mouse_Scn2a	TGCGAGGAGCTAAACAGTGATTAAAG	GGCTCCATTCCCTTATCAGACCTACCC	
tested sgRNA			
mouse_*Scn2a*	sgRNA sequence		
A1	ACAGAATCAGTAACGCACTGT		
A2	CGGGTAAGCCAAGTTTAGTCA		
A3	AAGCACTTGCCTCACATAAAT		
A5	CTAGGTCATAGAAAGGAAACC		
A6	TTTATTGGACCCCAGATATTC		
A7	AGAAAATTAACTTAGTGCATA		
A8	AAGCCGCCAGGGACCCGAGCA		
A9	TATAACTGCCACTAGAGGGCT		
A10	GACCCTCCTCCGGGCTCCACC		
human_*SCN2A*	sgRNA sequence		
H1	TGCTGACTGCTACATAGCCAA		
H2	GTGCTGACTGCTACATAGCCA		
H3	CTGCTACATAGCCAAAGGAAC		
H4	GCTCCATCTCCTGGTCAAAAG		
H5	CAGCCCATAATTCCACTCTAT		
H6	AGTAGTTGATTTCAAATAGAG		
H7	ATTAAAGTAGTTGATTTCAAA		
H8	GATTTCAAATAGAGTGGAATT		
H9	AAGTAGTTGATTTCAAATAGA		
H10	AGCTCCATCTCCTGGTCAAAA		

Supplemental Table 3: AAV titers for all viruses used

Sequence	Serotype	Plasmid	Genomic Titer (vg/mL)
Mouse sgRNA	DJ	pU6-sasgRNA-CMV-mCherry	4.40E+13
Human sgRNA	DJ	pU6-sasgRNA-CMV-mCherry	1.33E+14
mCherry	DJ	pAAV-CMV-mCherry	3.20E+13
sadCas9VP64	DJ	pCMV-sadCas9-VP64-pA	4.82E+13
Mouse sgRNA	PhP.eb	pU6-sasgRNA-CMV-mCherry	5.01E+13
mCherry	PhP.eb	pAAV-CMV-mCherry	5.00E+13
sadCas9VP64	PhP.eb	pCMV-sadCas9-VP64-pA	5.02E+13