

SUPPLEMENTAL MATERIAL

Neonatal but not Juvenile Gene Therapy Reduces Seizures and Prolongs Lifespan in *SCN1B*-Dravet Syndrome Mice

Chunling Chen^{†1}, Yukun Yuan^{†1}, Heather A. O'Malley¹, Robert Duba-Kiss², Yan Chen¹, Karl Habig¹, Yosuke Niibori³, Samantha L. Hodges¹, David R. Hampson^{2,3}, and Lori L. Isom^{*1}

[†]These authors contributed equally to this work.

Author affiliations:

¹Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109, USA

²Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada

³Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada

Correspondence to: Lori L. Isom, Ph.D.

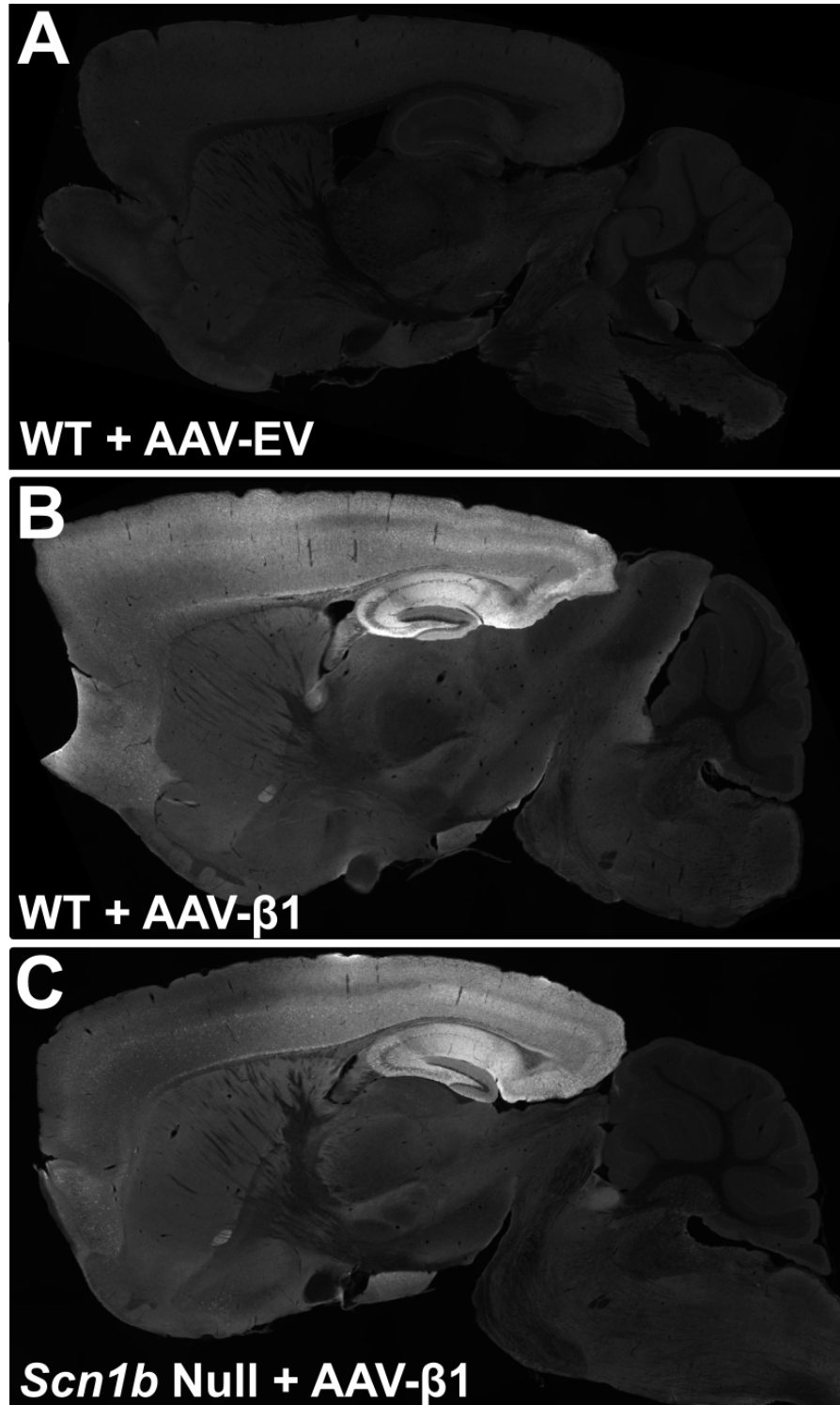
Department of Pharmacology, University of Michigan, 2301 E MSRB III, Ann Arbor, MI 48109-5632, USA

E-mail: lisom@umich.edu

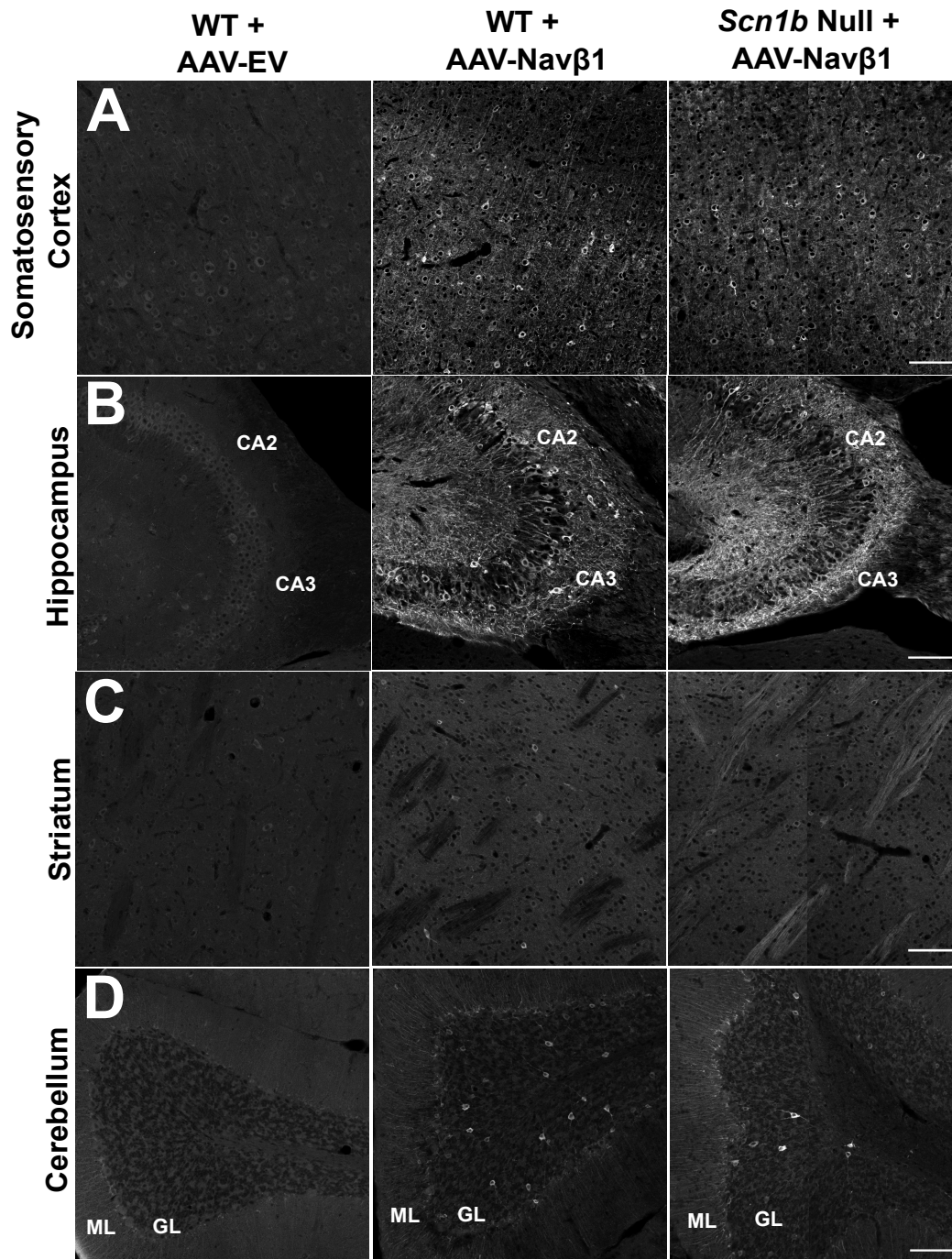
Running title: Gene Therapy for Epilepsy

Keywords: sodium channel, β subunit, epilepsy, Dravet syndrome, AAV9

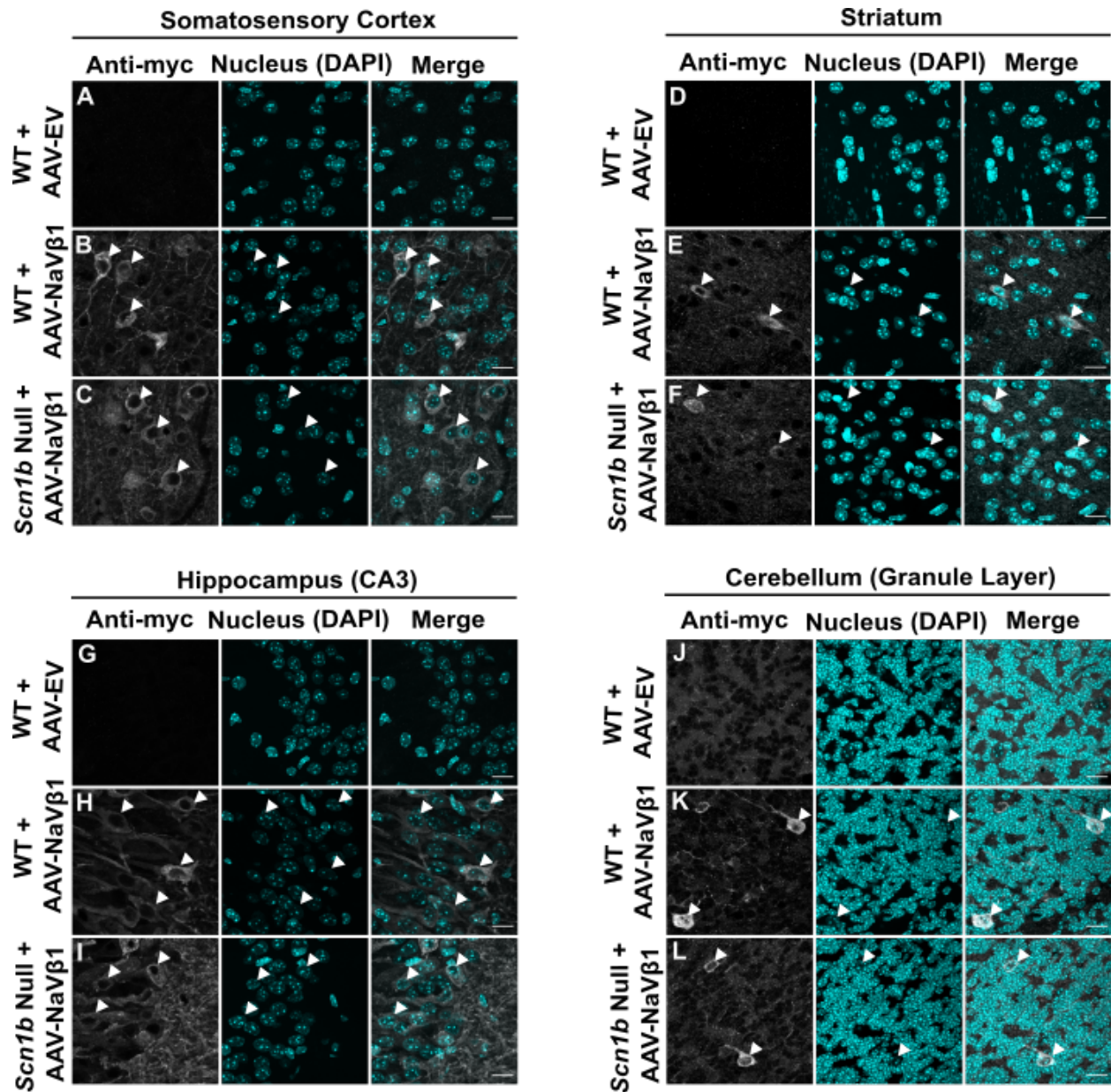
Conflict of Interest Statement: The authors have declared that no conflict of interest exists.



Supplemental Figure 1. Distribution of expression of Navβ1-myc in mouse brain following neonatal AAV administration. Immunofluorescence staining using anti-myc antibody was used to detect transgenic Navβ1-myc in P30 WT mice treated with AAV-EV (A) or with AAV-Navβ1 (B), and in P30 *Scn1b* null mice treated with AAV-Navβ1 (C) at P2. Image acquisition was performed using 4x magnification on a Cytation5 slide scanner.



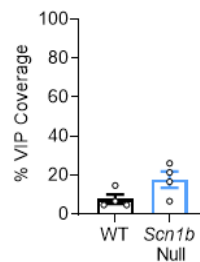
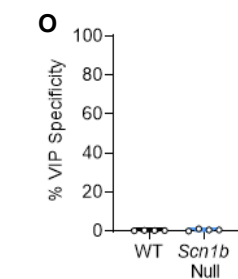
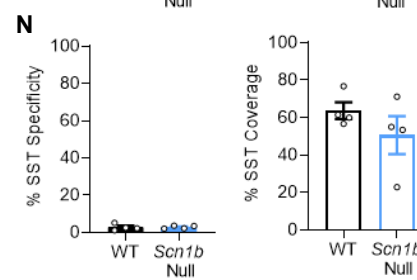
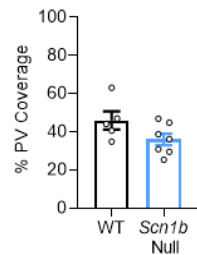
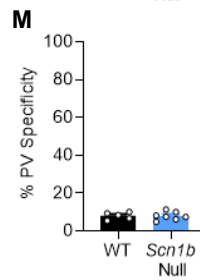
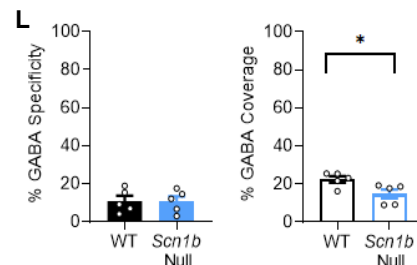
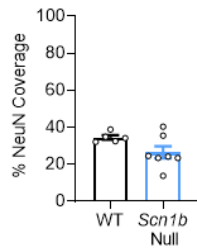
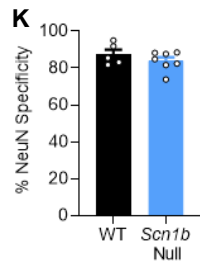
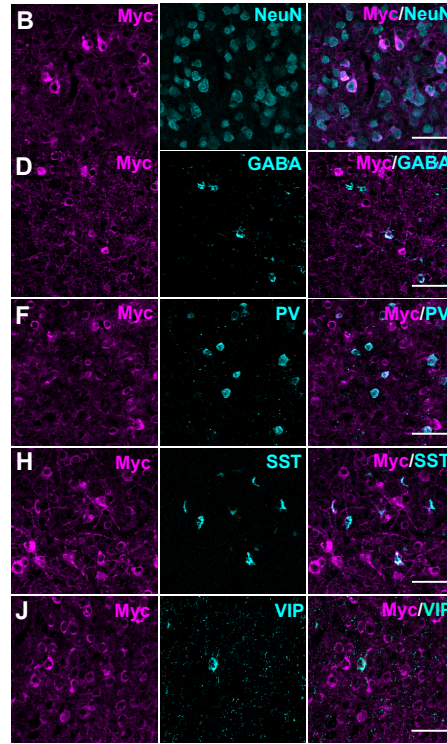
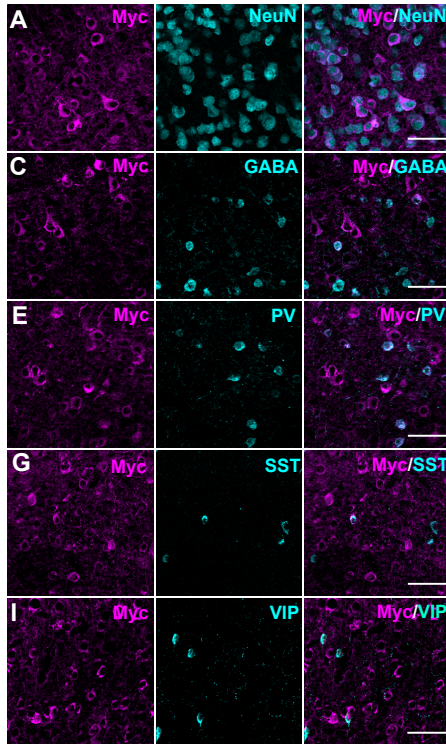
Supplemental Figure 2. Expression of Nav β 1-myc in (A) the somatosensory cortex, (B) the CA2 and CA3 regions of the hippocampus, (C) the caudoputamen of the striatum, and (D) the cerebellum of P30 WT mice treated with AAV-EV or AAV-Nav β 1, and P30 *Scn1b* null mice treated with AAV-Nav β 1 at P2. Nav β 1-myc expression was detected by immunofluorescence staining with anti-myc antibody. Subdivisions of the cerebellar cortex are indicated by ML (molecular layer) and GL (granule cell layer). Scale bars: 100 μ m.



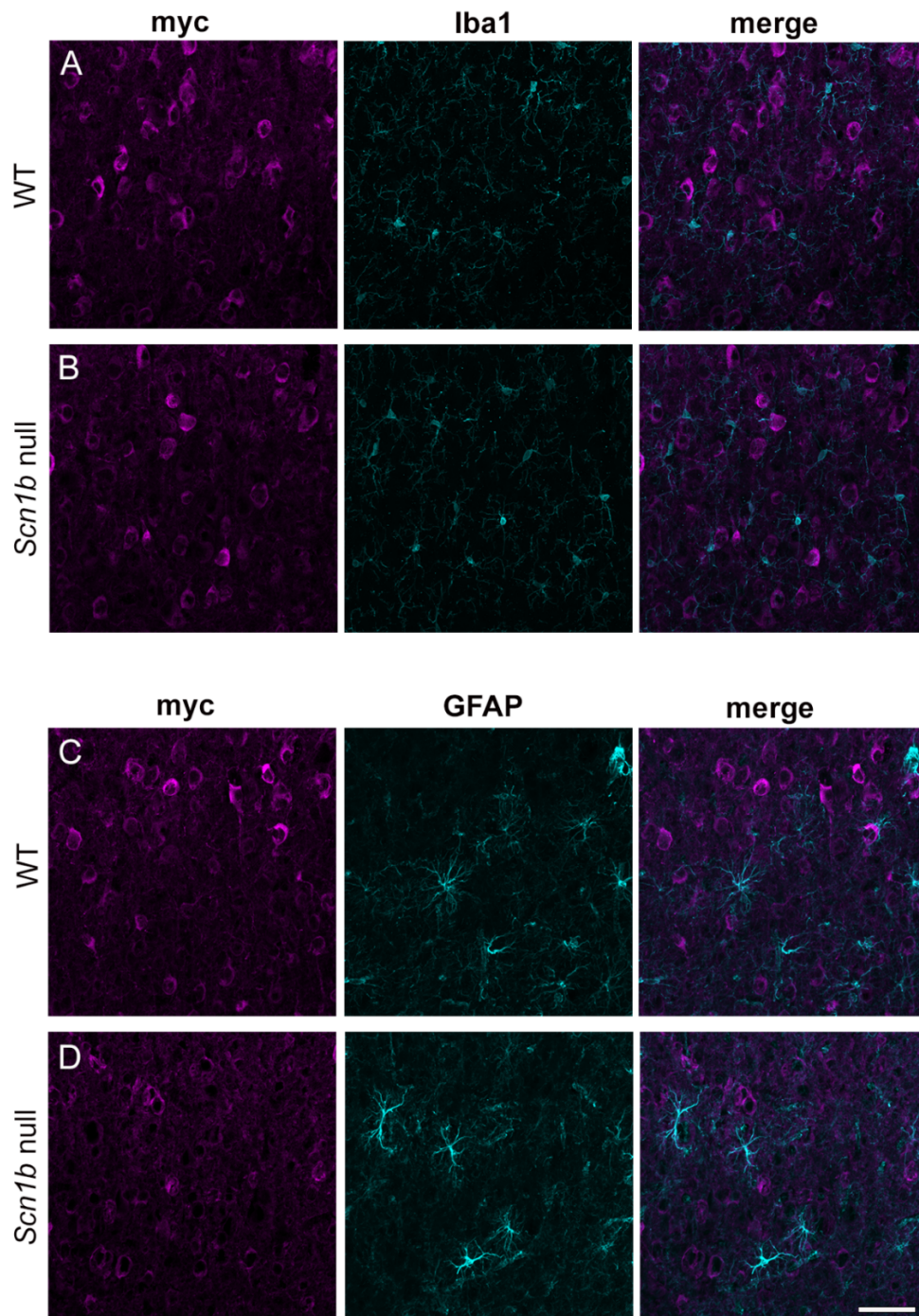
Supplemental Figure 3. High magnification images of Navβ1-myc immunopositive cells (grey) in the somatosensory cortex (A-C), the caudoputamen of the striatum (D-F), the pyramidal cell layer of the hippocampal CA3 region (G-I), and the granule layer of the cerebellum (J-L) of P30 WT mice treated with AAV-EV or AAV-β1, and P30 *Scn1b* null mice treated with AAV-Navβ1 at P2. DAPI (cyan) was used as a nuclear marker. Arrows indicate overlap of select Navβ1-myc positive cells with DAPI signal. Scale bars: 15 μm.

WT

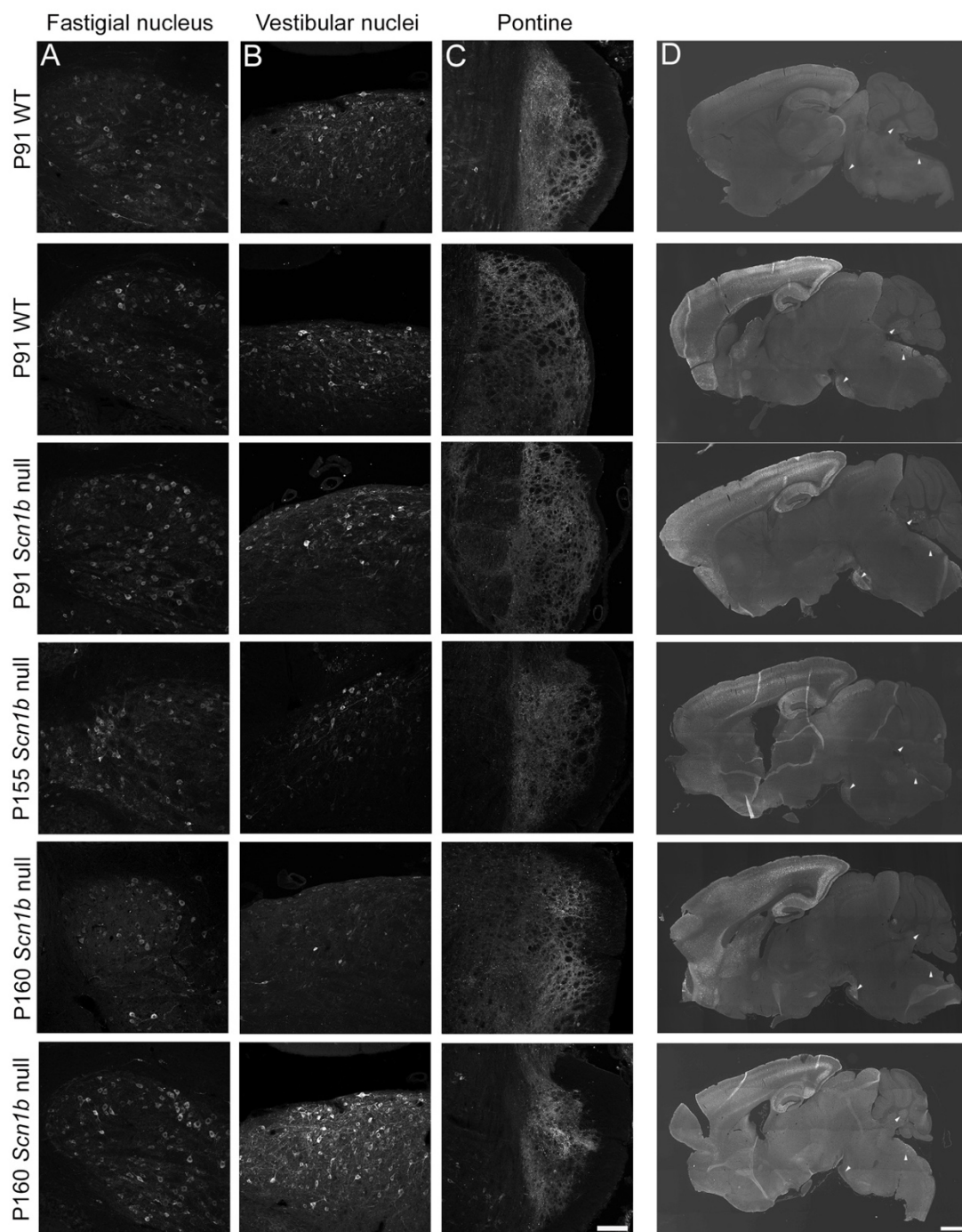
Scn1b Null



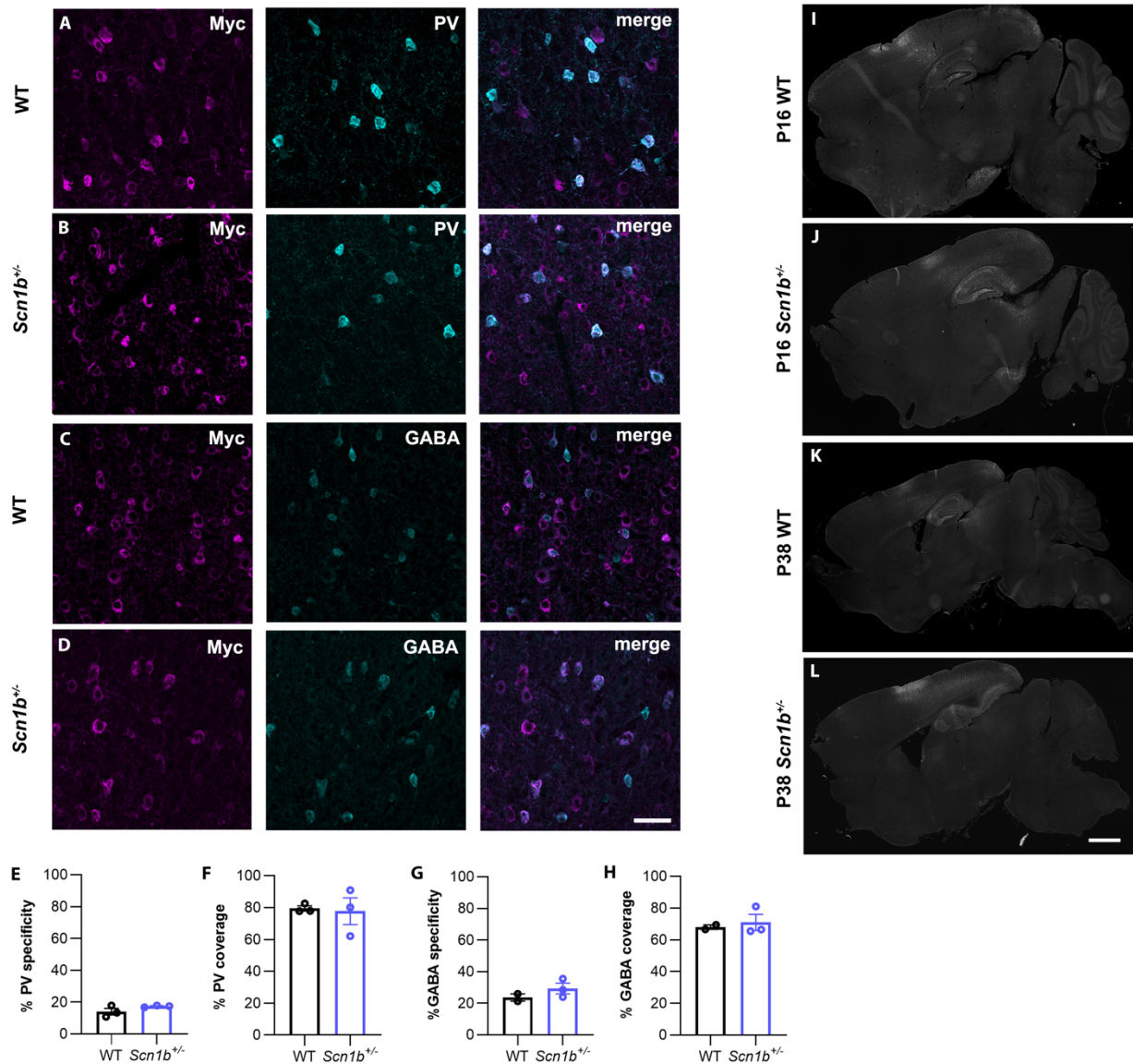
Supplemental Figure 4. Neuronal selectivity of AAV-Nav β 1 in somatosensory cortex of WT and *Scn1b* null mice. Expression of Nav β 1-myc (magenta) in various cell classes (cyan) in P30 WT (**A, C, E, G, I**) and *Scn1b* null mice (**B, D, F, H, J**). In panels **K - O**, cell-type specificity was calculated as the number of cells double positive for myc and the cell-type marker divided by the total number of myc positive cells, multiplied by 100%. Cell-type coverage was calculated as the number of cells double positive for myc and the cell-type marker divided by the total number of cell-type marker positive cells, multiplied by 100%. Anti-NeuN was used to label neurons; anti-GABA was used to label GABAergic neurons; anti-PV was used to label fast-spiking PV interneurons; anti-SST was used to label SST expressing interneurons; anti-VIP was used to label VIP expressing interneurons. Scale bars: 50 μ m. Quantitative results of NeuN (**K**), GABA (**L**), PV (**M**), SST (**N**) and VIP (**O**) specificity and coverage; N = 4-7 mice in each analytical cohort. * $p < 0.05$, unpaired t-test.



Supplemental Figure 5. Glial expression of Navβ1-myc in somatosensory cortex of P30 WT and *Scn1b* null mice. Navβ1-myc expression is not detected in Iba1+ microglia or GFAP+ astroglia. Navβ1-myc (magenta) expression is not detected in Iba1+ microglia (cyan) in either (A) WT or (B) *Scn1b* null somatosensory cortex. Navβ1-myc (magenta) expression is not detected in GFAP+ astroglia (cyan) in either (C) WT or (D) *Scn1b* null somatosensory cortex. Right column: merged images. Scale bar: 50 μm.

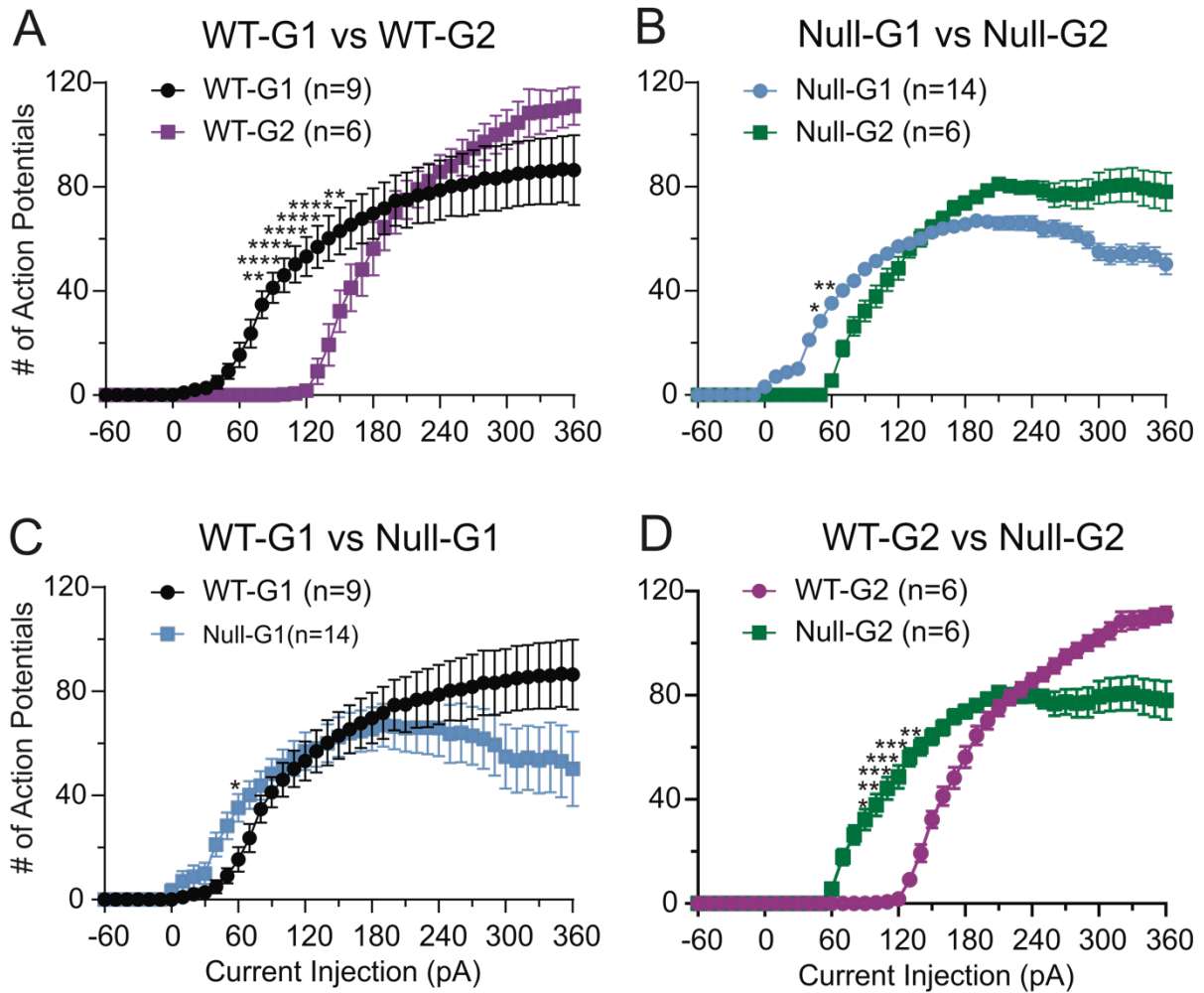


Supplemental Figure 6. Nav β 1-myc expression in multiple regions of AAV-Nav β 1 injected mice after P90. (A) Expression of Nav β 1-myc in cerebellar fastigial nucleus. **(B)** Expression of myc- β 1 in brainstem vestibular nuclei. **(C)** Expression of Nav β 1-myc in brainstem pontine grey. **(D)** Low magnification images showing whole-brain expression of Nav β 1-myc, with arrowheads indicating the regions imaged at higher magnification in columns A-C. Scale bar (columns A-C): 100 μ m. Scale bar (column D): 1 mm. Genotype and age are indicated on the left side of the figure.

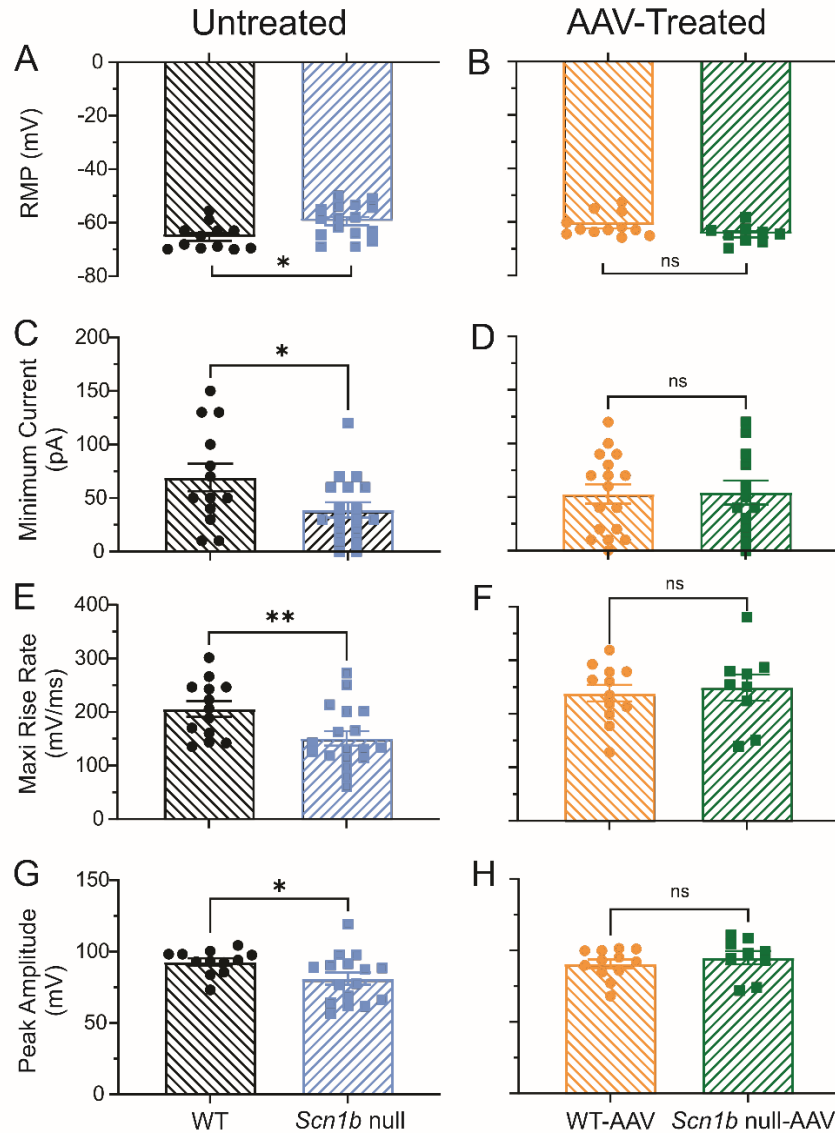


Supplemental Figure 7. Expression of Navβ1-myc in *Scn1b* mice injected at P10. (A-D)

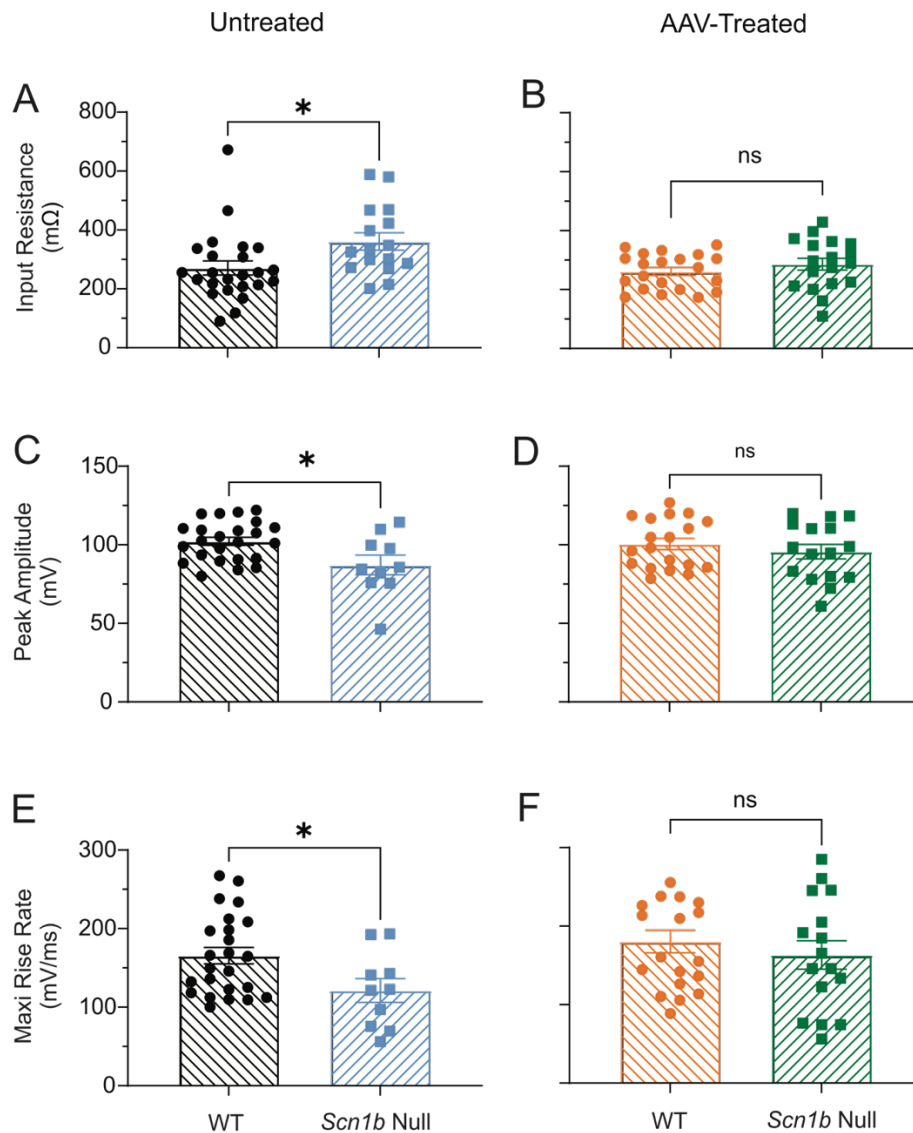
Neuronal selectivity of AAV-Navβ1 in somatosensory cortex of P38 WT and *Scn1b*^{+/-} mice treated with AAV-Navβ1 at P10. Expression of Navβ1-myc (magenta) in PV+ (A, B) or GABA+ (C, D) interneurons (cyan) in WT (A, C) and *Scn1b*^{+/-} mice (B, D). Scale bar: 50 μm. (E-H) Quantitative results of PV (E, F) and GABA (G, H) specificity and coverage expressed as a percentage, where specificity = number of cells double positive for myc and PV or GABA divided by the number of PV+ or GABA+ cells, and coverage = number of double positive cells divided by the number of myc+ cells. N = 3 mice per genotype. (I-L) Navβ1-myc expression in whole brain sections. Navβ1-myc expression is primarily restricted to cortex and hippocampus at P16 in WT (I) and *Scn1b* null mice (J), and at P38 in WT (K) and *Scn1b*^{+/-} mice (L). Scale bar: 1 mm.



Supplemental Figure 8. WT and *Scn1b* null mice have two subpopulations of PV+ interneurons in terms of sensitivity to depolarizing current injection. (A) Comparison of I-O curves for AP firing of untreated WT subgroup 1 (black: WT-G1, lower threshold group) and subgroup 2 (purple: WT-G2, higher threshold group). (B) Comparison of I-O curves for AP firing of untreated null subgroup 1 (blue: Null-G1, lower threshold group) and subgroup 2 (green: Null-G2, higher threshold group). (C) Comparison of I-O curves for AP firing of untreated WT subgroup 1 (black: WT-G1) and null subgroup 1 (blue: Null-G1). (D) Comparison of I-O curves for AP firing of untreated WT subgroup 2 (purple: WT-G2) and null subgroup 2 (green: Null-G2). Values are mean \pm SEM of 6-14 cells (as indicated in the parenthesis) from 7 untreated WT mice or 8 untreated *Scn1b* null mice, respectively.



Supplemental Figure 9. AP properties of P16-18 WT vs. *Scn1b* null untreated and AAV-Navβ1-treated PV+ interneurons. (A, B) Comparison of RMP of untreated or AAV-Navβ1-treated WT or *Scn1b* null P16-18 PV+ interneurons, respectively. (C, D) Comparison of minimum current required to fire an AP in untreated or AAV-Navβ1-treated WT or *Scn1b* null P16-18 PV+ interneurons, respectively. (E, F) Comparison of maximum rise rates of APs of untreated or AAV-Navβ1-treated WT or *Scn1b* null P16-18 PV+ interneurons, respectively. (G, H) Comparison of peak AP amplitude of untreated or AAV-Navβ1-treated WT or *Scn1b* null P16-18 PV+ interneurons, respectively. Values are mean \pm SEM of 12-13 cells from 6-7 untreated WT mice, 12-14 cells from 4-6 AAV-treated WT mice, 16-18 cells from 7 untreated *Scn1b* null mice, or 9-12 cells from 4-6 AAV-treated *Scn1b* null mice. Untreated WT: black; untreated *Scn1b* null: blue; AAV-Navβ1-treated WT: orange; AAV-Navβ1-treated *Scn1b* null: green. * $p < 0.05$, ** $p < 0.01$. Results are tabulated in Supplemental Table 1.



Supplemental Figure 10. AP properties of P16-18 WT vs. *Scn1b* null untreated and AAV-Navβ1-treated cortical layer 2-6 pyramidal neurons. (A, B) Comparison of input resistance of untreated or AAV-Navβ1-treated WT or *Scn1b* null P16-18 pyramidal neurons, respectively. (C, D) Comparison of peak AP amplitude in untreated or AAV-Navβ1-treated WT or *Scn1b* null P16-18 pyramidal neurons, respectively. (E, F) Comparison of maximum rise rates of APs of untreated or AAV-Navβ1-treated WT or *Scn1b* null P16-18 pyramidal neurons, respectively. Values are mean ± SEM of 22-24 cells from 6-7 untreated WT mice, 12-21 cells from 4-6 AAV-treated WT mice, 9-12 cells from 7 untreated *Scn1b* null mice, or 14-18 cells from 4-6 AAV-treated *Scn1b* null mice. Untreated WT: black; untreated *Scn1b* null: blue; AAV-Navβ1-treated WT: orange; AAV-Navβ1-treated *Scn1b* null: green. * $p < 0.05$, ** $p < 0.01$. Results are tabulated in Supplemental Table 2.

Supplemental Tables

Parameters	WT untreated	<i>Scn1b</i> null untreated	WT-AAV	<i>Scn1b</i> null-AAV
Resting Membrane Potential mV	-78.38 ± 1.38 (12)	-72.44 ± 1.56* (25)	-73.73 ± 1.12 (17)	-77.36 ± 0.93 (11)
Capacitance pF	16.66 ± 1.37 (14)	15.61 ± 2.36 (15)	13.64 ± 1.17 (12)	13.56 ± 1.23 (9)
Input Resistance mΩ	194.32 ± 11.35 (14)	241.44 ± 17.04* (16)	265.97 ± 26.43 (13)	243.19 ± 20.46 (12)
Threshold Potential mV	-55.84 ± 2.67 (11)	-49.08 ± 3.09 (15)	-50.73 ± 2.64 (12)	-58.16 ± 3.02 (9)
Peak AP Amplitude mV	92.8 ± 2.45 (12)	80.51 ± 4.53* (15)	90.68 ± 2.98 (12)	94.97 ± 4.61 (9)
Half-Width ms	1.39 ± 0.17 (12)	1.44 ± 0.15 (15)	0.93 ± 0.05 (12)	1.00 ± 0.07 (9)
Maximum Rise Rate mV/ms	210.83 ± 15.10 (12)	157.47 ± 15.38** (15)	238.65 ± 15.63 (12)	249.02 ± 24.38 (9)
Maximum Decay Rate mV/ms	-75.94 ± 6.59 (12)	-67.50 ± 5.43 (15)	-113.98 ± 6.64 (12)	-107.08 ± 10.85 (9)

Supplemental Table 1. Comparisons of passive and active membrane electrical properties of cortical layer 2-6 WT and *Scn1b* null PV+ interneurons with or without AAV-Navβ1 treatment. All values are expressed as mean ± SEM (n). * $p < 0.05$; ** $p < 0.01$, compared with untreated WT, Student's unpaired t test or Mann-Whitney test.

Parameters	WT untreated	<i>Scn1b</i> null untreated	WT-AAV	<i>Scn1b</i> null-AAV
Resting Membrane Potential mV	-75.98 ± 1.26 (24)	-75.35 ± 2.12 (10)	-73.43 ± 1.72 (18)	-76.00 ± 1.57 (16)
Capacitance (pF)	15.00 ± 1.59 (23)	12.92 ± 1.75 (12)	14.91 ± 1.88 (12)	12.45 ± 1.41 (16)
Input Resistance (mΩ)	254.43 ± 18.12 (22)	341.07 ± 28.61* (14)	261.58 ± 13.09 (21)	286.31 ± 20.21 (18)
Threshold Potential (mV)	-44.26 ± 1.12 (24)	-44.17 ± 1.56 (9)	-46.42 ± 1.84 (19)	-44.24 ± 1.53 (16)
Peak AP Amplitude (mV)	102.8 ± 2.56 (24)	87.21 ± 6.23* (10)	100.59 ± 3.60 (19)	95.69 ± 4.61 (16)
Half-Width (ms)	3.54 ± 0.27 (24)	3.73 ± 0.42 (10)	2.87 ± 0.23 (18)	2.90 ± 0.07 (14)
Maximum Rise Rate (mV/ms)	165.66 ± 10.46 (24)	121.22 ± 15.16* (10)	180.47 ± 14.39 (19)	163.35 ± 18.13 (16)
Maximum Decay Rate (mV/ms)	-33.52 ± 2.31 (24)	-29.91 ± 2.67 (10)	-39.48 ± 3.84 (19)	-38.95 ± 5.19 (14)

Supplemental Table 2: Comparisons of passive and active membrane electrical properties of cortical layer 2-6 WT and *Scn1b* null pyramidal cells with or without AAV-Navβ1 treatment. All values are expressed as mean ± SEM (n). * $p < 0.05$; ** $p < 0.01$, compared with untreated WT, Student's unpaired *t* test or Mann-Whitney test.

Supplemental Movie

Supplemental Movie 5: This movie consists of 2 video clips. The first video clip shows 3 P100 AAV-Nav β 1-treated *Scn1b* null mice. The second video clip shows 2 P101 AAV-Nav β 1-treated *Scn1b* null mice plus 1 AAV-Nav β 1-treated P101 WT mouse from the same litter for size comparison.