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Supplemental Methods

Animals

Nae-floxed (*Nae1^{ff}*) (38), *PV-Cre* (39) and *Rosa-LSL-tdTomato* (*Ai9*) (40) mice were described previously. Mice were housed under 12-hour light/dark cycle and 22 ± 2 °C. All animal experiments were approved by the Institutional Animal Care and Use Committee of Augusta University and Case Western Reserve University.

Reagents and antibodies

Chemicals were purchased from Sigma-Aldrich unless otherwise indicated. MLN4924 was purchased from Active Biochem. 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), DL-2-amino-5-phosphonopentanoic acid (DL-AP5), and bicuculline were purchased from Tocris Bioscience. Information of primary antibodies are as follows: mouse anti-PV (cat. #235, Swant, 1:1,000 for staining); rabbit anti-PV (cat. #PV 25, Swant, 1:1,000 for staining); mouse anti-NeuN (cat. MAB377, Millipore, 1:1,000 for staining); chicken anti-GFP (cat. GFP-1020, Aves Labs, 1:1,000 for staining); rabbit anti-CCK (cat. 20078, Immunostar, 1:150 for staining); rabbit anti-CUX1 (cat. sc-13024, Santa Cruz, 1:500 for staining); mouse anti-Foxp2 (cat. sc-517261, Santa Cruz, 1:200 for staining); rabbit anti-Nae1 (cat. 14321, Cell Signaling, 1:1,000 for WB, 1:200 for staining); rabbit anti-K48 polyubiquitin (cat.05-1307, Millipore, 1:1,000 for WB); mouse anti-Myc (cat. MA1-980, Thermo Fisher Scientific, 1:1,000 for WB, 1:200 for staining); rabbit anti-Nav1.1 (cat. AB5204, Millipore, 1:500 for WB); rabbit anti-Nav1.6 (cat. ASC-009, Alomone Labs, 1:500 for WB); rabbit anti-Nedd8 (cat. 2754, Cell Signaling, 1:1,000 for WB); mouse anti-ATP1A1 (cat. MA1-16731, Thermo Fisher Scientific, 1:1,000 for WB); anti- β -actin (cat. MABT523, Millipore, 1:5,000 for WB); rabbit-anti-HA (cat. H69081, Sigma, 1:1,000 for WB); mouse anti-Transferrin receptor (cat. 13-6890, Thermo Fisher Scientific, 1: 1,000 for WB).

Plasmids and mutagenesis

The cDNA for human voltage-gated sodium channel type 1 alpha subunit (hNav1.1, Cat. EX-Z9157-M98) was purchased from GeneCopoeia. SCN1B-Myc (Cat. RC209565) and SCN2B-Myc (Cat. RC207778) were purchased from OriGene. pCALNL-GFP (Cat.13770) was purchased from Addgene. The epileptogenic mutation K245N and K1936E of hNav1.1 were generated with Site-Directed Mutagenesis Kits according to user's manual (QuikChange II, Agilent, 200521). The mutagenesis primers were: 5'-CTGAT CCAGT CCGTG AATAA GCTGT CTGAT GTG-3' and 5'-CACAT CAGAC AGCTT ATTCA CGGAC TGGAT CAG-3' for K245N, and 5'-GAGGC CTGTT CCACT GTCCT CTTCA G-3' and 5'-CTTCA CCTAT AACAA GAATAAGATC AAG-3' for K1936E.

Cell Culture and DNA Transfection

Human tsA-201 cells, human embryonic kidney 293 cells stably expressing the simian virus 40 large T antigen (gift from Dr. Qing K. Wang), were transfected with SCN1A-WT, K245N or K1936E plasmids, along with the accessory β 1 and β 2 subunits (70). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (CORNING) supplemented with 10% fetal bovine serum (FBS) (GEMINI). Transient transfection was performed as described previously (108). Briefly, cells were cultured in 100 mm dishes and, at ~70% confluence, were incubated with precipitates formed by 5 μ g of plasmid DNA and 10 μ l of 1mg/ml Milli-Q water dissolved polyethyleneimine 40000 (Polysciences, Inc. Cat # 24765). Cells were harvested 24~48 h post transfection.

Surface expression

Human tsA-201 cells were transfected with SCN1A wt or mt plasmids, along with accessory β 1 and β 2 subunits. 48 hr later, 2 ml of ice-cold PBS containing 0.1 mM Ca^{2+} , 1 mM Mg^{2+} (PBS (+Ca/Mg)) were added to wash cells. Then, 2 ml of freshly prepared Sulfo-NHS-LC-Biotin (Pierce, Cat # 21335) (final

concentration is 0.25 mg/ml) that was dissolved in ice-cold PBS (+Ca/Mg) and incubation for 45 min at 4°C. Afterward, 1 ml of 10 mM glycine buffer was added into the dishes and incubated for 20 min at 4°C. After that, the glycine buffer was discarded, and the dishes were washed 3 times with 2 ml of ice-cold PBS (+Ca/Mg) on ice. Subsequently, 1 ml of RIPA (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM EDTA, 5 mM NaF, 2 mM Na₃VO₄, and cOmplete™ protease inhibitors) lysis solution was added and incubated for 30 min at 4°C on shaker. Lysis solution was then centrifuged at 14,000 rpm for 15 min at 4°C. Supernatants were incubated with 30 µl of Streptavidin-Agarose (Pierce 20347) at 4°C overnight. Precipitated proteins were subjected to western blotting analysis.

Immunohistochemistry

Immunohistochemistry was performed as described previously (109). Briefly, anesthetized mice were perfused transcardially with 4% paraformaldehyde (PFA) in PBS, pH 7.4. After postfixation in 4% PFA overnight, brains were sectioned into 40-µm-thick slices by VT1000S vibratome (Leica Microsystems, Germany). Slices were blocked with PBS containing 0.3% Triton X-100 and 10% donkey serum for 2 hr at room temperature (RT) and incubated with primary antibodies overnight at 4°C. After washing with PBS for 3 times, samples were incubated with secondary antibodies for 2 hr at RT and washed with PBS for 3 times. Finally, slices were mounted with SlowFade Diamond Antifade Mountant (catalog #S36972; Thermo Fisher).

For staining of individual neurons, hippocampi tissues were dissociated using the Papain dissociation system (Worthington) following the manufacturer's instructions. After incubation for 30 min, samples were triturated with fire-polished Pasteur pipettes and filtered through a 30 µm cell strainer (CellTrics, Sysmex). Neurons were pelleted by centrifugation at 100 g for 5 min and resuspended in DMEM culture medium supplemented with 20% FBS. Neurons were plated onto 18-mm coverslips (pre-coated with poly-D-lysine),

and cultured in a 12-well plate for 3 hr. Neurons were dissociated and fixed with 4% PFA for 15 min at RT and subjected to staining as described above.

Western blot analysis

Western blotting analysis was performed as previously described (110). Briefly, samples were separated by SDS-PAGE, transferred onto nitrocellulose membrane, probed with specific primary antibodies, and visualized with enhanced chemiluminescence (Thermo Fisher Scientific) or IRDye® secondary antibodies (LI-COR). The immunoreactive bands were imaged and quantified from 3 to 5 independent experiments using LI-COR Odyssey Fc imaging system. Lysates were prepared by incubating tissues with RIPA buffer.

Ubiquitination and Neddylation assay were performed as previously described with minor modifications (36). Transfected tsA-201 cells or cerebellum dissected from mouse brain were lysed in buffer containing 2% SDS, 150 mM NaCl, 10 mM Tris-HCl (pH 8.0), and protease inhibitors including 1 mM PMSF and cOmplete™ protease inhibitor. After sonication, samples were diluted by adding 9 vol of the dilution buffer containing 10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 2 mM EDTA, and 1% Triton. Supernatants were incubated with respective antibodies overnight at 4°C and then with protein G agarose beads or specific Myc antibody-conjugated beads (Sigma) for 4 hrs at 4°C. Precipitated proteins and 5–10% total lysates were then subjected to western blotting. For experiment involving MLN-4924 treatment, tsA-201 cells were treated with 4 µM MLN-4924 for 8 hrs before lysis.

Quantitative real-time PCR

Total RNA was isolated from mouse cerebral cortex, hippocampus and cerebellum using TRIzol reagent (Invitrogen). cDNA was synthesized using a cDNA synthesis kit (GoScript™ Reverse Transcription System, Promega). The real-time PCR was performed with SYBR Green qPCR mastermix (Qiagen) on StepOnePlus real-time PCR system (Applied Biosystems). Glyceraldehyde 3-

phosphate dehydrogenase (*Gapdh*) was used as internal control. The primers used were as follows: *Nae1* (F: 5'-GCCAG GGAAG ATACT CAAGG A-3'; R: 5'-CGGTT CTTGC CAATA CTGCT TT -3'); *Bcan* (F: 5'-TGCGC GTCAA GGTAACGAA-3'; R: 5'-GACCC CGGAA TCATT GGGC-3'); *Er81* (F: 5'-GGAGG CCATG AAAAG CCAA-3'; R: 5'-TTAAG TGCAG GCGTC TTCTT C-3'); *Hcn4* (F: 5'-GGCGG ACACC GCTAT CAAA -3'; R: 5'-TGCCG AACAT CCTTAGGGAG A -3'); *Kcna1* (F: 5'-CACCG CATCG ACAAC ACCA -3'; R: 5'-CACCA CCAGC TCAAAAGAGA A -3'); *Kcnc1* (F: 5'-AGAGA TTGGC ACTCA GTGAC T -3'; R: 5'-TTGTT CACGA TGGGG TTGAA G -3'); *Kcnc2* (F: 5'-AGGCT ATGGG GATAT GTACC C-3'; R: 5'-TGCAA AATGT AGGCG AGCTT G-3'); *Kcnc3* (F: 5'-GGGCT TCTGG GGCAT AGAC-3'; R: 5'-GTCCT GAAAA CACAG ACGCT T-3'); *Kcnq2* (F: 5'-TATAC ACAGA GAATG GGCAT CCC-3'; R: 5'-CAGTC GCAGG ACCAG TGAG-3'); *Kcnq3* (F: 5'-GAGCC GACAA AGACG GGAC-3'; R: 5'-TTGGC GTTGT TCCTC TTGAC T-3'); *Scn1a* (F: 5'-CAGAG GGAAG CACAG TAGAC-3'; R: 5'-TTCCA CGCTG ATTTG ACAGCA-3'); *Scn3a* (F: 5'-CAGAC CATGT GCCTT ATTGT GT-3'; R: 5'-CCGCG ATCTG GAGGT TGTT-3'); *Scn8a* (F: 5'-ATGGG GTAGG CTCTC CGAG-3'; R: 5'-CCGAC TCTGA CTTAA ACACC TTC-3'); *Scn1b* (F: 5'-GGATT CCGAT ACCGA GGCTG-3'; R: 5'-GGTCC ACTCC GTGAA GGTTT-3'). *Scn2b* (F: 5'-CGGAG CATGG AAGTC ACAG-3'; R: 5'-CTGCT TGTGG TTCAC GGTGT A-3'); *Nedd4l* (F: 5'-CACGG GTGGT GAGGA ATCC-3'; R: 5'-GCCGA GTCCA AGTTG TGGT-3'); *Rack1* (F: 5'-TCCTC TGATG GTCAG TTTGC CC-3'; R: 5'-CACGC TCAAC ACATC CTTGG TG-3'); *FGF14* (F: 5'-TTCTC AGGGT GTCTAAGCTG C-3'; R: 5'-GGGGA TCAGT TGGGT TCTTG TT-3'); *Cacna1a* (F: 5'-CACCG AGTTT GGGAA TAACT TCA-3'; R: 5'-ATTGT GCTCC GTGAT TTGGA A-3'); *Cacna1c* (F: 5'-ATGAA AACAC GAGGA TGTAC GTT-3'; R: 5'-ACTGA CGGTA GAGAT GGTTG C-3'); *Cacna1g* (F: 5'-TGTCT CCGCA CGGTC TGTA-3'; R: 5'-AGATA CCCAA AGCGA CCATC TT-3'); *Cacna1s* (F: 5'-TCAGC ATCGT GGAAT GGAAA C-3'; R: 5'-GTTCA GAGTG TTGTT GTCAT CCT-3'); *Gapdh* (F: 5'-AAGGT CATCC CAGAG CTGAA-3'; R: 5'-CTGCT TCACC ACCTT

CTTGA-3').

Electrophysiological recording

To measure voltage-gated sodium channel currents of tsA-201 cells that were cotransfected with the plasmids of $\alpha 1$, $\beta 1$, $\beta 2$, and GFP (molar ratio 10:1:1:1). After 24 hrs, transfected tsA-201 cells were digested with 0.05% trypsin (thermo fisher) for 3 min, and divided into 3×10^4 cells into PDL- (Sigma, A003E) coated coverslips in 12-well plates. Electrophysiological recording was performed 4 hrs later. Only GFP fluorescence positive cells were recorded. The resistance of glass pipettes was in the range of 1-1.5 M Ω . The intracellular solution containing (in mM): 35 NaCl, 105 CsCH₃SO₃, 10 HEPES, and 10 EGTA, at pH 7.3 adjusted with CsOH. The bath solution containing (in mM): 150 NaCl, 2 KCl, 1 MgCl₂, 1.5 CaCl₂, 10 HEPES and 10 glucose at pH 7.3 adjusted with NaOH. Recording of Na⁺ currents was performed at RT. Cells were held at -120 mV with 50-ms depolarizing steps (from -80 to +80 mV in 5 mV increments) to assess the current-voltage relationship. Value of reflecting peak current was normalized to the capacitance. For measurement of steady-state inactivation, cells were held at -120 mV with 50-ms pre-pulses (from -140 to +5 mV in 5 mV increments), followed by -20 mV pulses (20 ms). Series resistance was compensated to 75 ~ 85%. P/4 subtraction was applied to subtract the leak currents. Currents were sampled at 50 kHz with a 5 kHz filter. The conductance (G_{Na}) of different depolarizing steps are calculated following the formula: $G = (\text{Peak current})/[(\text{Clamping potential})-(\text{Reversal potential})]$. Normalization of G_{Na} was based on the maximal conductance between -80 and +20 mV and fitted to the two-state Boltzmann equation.

Mice (4-5-week-old) were anesthetized with isoflurane and subjected to cardiac perfusion with ice-cold Choline Chloride-based cutting solution containing (in mM): 120 Choline Chloride, 2.5 KCl, 7 MgCl₂, 0.5 CaCl₂, 1.25 NaH₂PO₄, 5 Sodium ascorbate, 3 Sodium pyruvate, 26 NaHCO₃, and 25

glucose. Mice were rapidly decapitated. The brain was removed quickly and placed in cutting solution. Lamellar 300 μm slices of hippocampus was obtained as previously described (111, 112). After sectioning on the VT1200S Vibratome (Leica Microsystems, Germany), brain slices were transferred to a storage chamber containing cutting solution and incubated at 34°C for 15 min. Slices were then transferred and stored in artificial cerebrospinal fluid (ACSF) (in mM): 124 NaCl, 2.5 KCl, 2 MgSO_4 , 2.5 CaCl_2 , 1.25 NaH_2PO_4 , 26 NaHCO_3 , and 10 glucose) at RT for at least 1 hr before recording. Cutting solution and ACSF were saturated with oxygen (95% O_2 /5% CO_2).

Slices were transferred to the recording chamber superfused (2 ml/min) with regular ACSF at 32-34°C. Slice was visualized with a 40x water-immersion lens (BX51WI, Olympus) and CCD monochrome video camera (C2400-75, Hamamatsu). PV+ interneurons were identified with the expression of tdTomato. The borosilicate glass electrodes were pulled by a micropipette puller (P-1000, Sutter Instrument) with resistance in the range of 3-5 $\text{M}\Omega$. Series resistance was below 20 $\text{M}\Omega$ and monitored throughout the experiments. Recordings were performed with MultiClamp 700B amplifier and 1550B digitizer (Molecular Devices). Data were sampled at 10 kHz and low pass-filtered at 2 kHz.

For sEPSCs recording, neurons were held at -70 mV in the presence of 20 μM bicuculline, with the pipette solution containing (in mM): 125 K-gluconate, 5 KCl, 10 HEPES, 0.2 EGTA, 1 MgCl_2 , 4 Mg-ATP , 0.3 Na-GTP and 10 phosphocreatine, at pH 7.3 adjusted with KOH, (290-295 mOsm). 20 μM bicuculline was added into bath solution to blocked GABAA receptors. For sIPSCs recording, the pipette solution contained 125 mM K-gluconate and the 5 mM KCl were replaced by 130 mM CsCl, and pH was adjusted to 7.3 with CsOH. 20 μM CNQX and 50 μM DL-AP5 were added into bath solution to blocked AMPA and NMDA receptors. Action potential frequency was analyzed from current clamp recording and measured by injecting a series of depolarizing pulses in the presence of 20 μM CNQX, 50 μM DL-AP5 and 20 μM bicuculline. The pipette solution contained (in mM): 125 K-gluconate, 5 KCl, 10 HEPES, 0.2

EGTA, 1 MgCl₂, 4 Mg-ATP, 0.3 Na-GTP and 10 phosphocreatine, at pH 7.3 adjusted with KOH, (290-295 mOsm). To measure neuronal excitability in CA1 CCK+ neurons, the pipettes were filled with K-gluconate internal solution containing 125 mM K-gluconate and 0.5% biocytin. Neurons labeled with biocytin were stained with anti-CCK antibody. Astrocytes were labeled with sulforhodamine 101 (SR-101) and recorded for rest membrane potential and characterization of the current-voltage relations (113).

To measure voltage-gated sodium channel currents of PVINs, the patch pipettes were coated with Sylgard and filled with solution containing (in mM): 100 CsCH₃SO₃, 10 tetraethylammonium chloride (TEA-Cl), 5 4-aminopyridine (4-AP), 10 HEPES, 10 EGTA, 1 CaCl₂, 4 Mg-ATP, 0.3 Na-GTP, 4 Na₂-phosphocreatine and 4 NaCl, at pH 7.3 adjusted with CsOH, (290-295 mOsm). 100 μM CdCl₂, 20 μM CNQX, 50 μM DL-AP5 and 20 μM Bicuculline were added to the bath solution containing (in mM): 124 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2 MgCl₂, 2.5 CaCl₂, 10 glucose and 26 NaHCO₃. Series resistance (Rs) was compensated by > 80%. To induce the activation of uncontrolled axial Na⁺ currents, neurons were evoked with a 4-ms depolarizing pulse (-20 mV) from a holding potential of -80 mV, followed by a brief (0.5 ms) step to -65 mV and 5 mV step from -80 to +60 mV (54). After recording, Na⁺ current was confirmed by 1 μM TTX. To measure hyperpolarization-activated HCN channel currents of PVINs, the pipettes were filled with 125 mM K-gluconate internal solution. PVINs were held at -60 mV, HCN channel currents were generated at voltages from -130 mV to -50 mV for 1 s, with 10 mV command voltage increments, followed by a -120 mV step to measure tail currents. The ACSF contained 20 μM CNQX, 50 μM DL-AP5, 20 μM Bicuculline, 1 μM TTX, 5 mM TEA-Cl, 100 μM CdCl₂ and 200 μM BaCl₂. To measure voltage-gated calcium channel currents of PVINs, the pipettes were filled with 110 mM CsMeSO₄ internal solution. PVINs were held at -70 mV, calcium channel currents were generated at voltages from -60 mV to +30 mV, with 10 mV command voltage increments. The ACSF contained 20 μM CNQX, 50 μM DL-AP5, 20 μM

Bicuculline, 1 μ M TTX and 5 mM TEA-Cl.

Behavioral tests

Mice (5-6-week-old) were used in behavioral tests. To assess the motor balance and coordination of mice, beam-walk (0.6-meter length) assay was performed as previously described (114). Video records of the mouse walking, the latency and movement distance of mouse falling from the balance beam was calculated. Each mouse was tested three times. To assess the motor reflex and reaching motion of mice, tail suspension test was performed as previously described with minor modifications (41). Briefly, the tail of mouse was taped ~1 cm above the wire cagetop and allowed to attempt to reach for the wire bars for 10 s. The time during which mouse intentionally reaching for the wire bar was calculated.

Electroencephalography (EEG) recording

EEGs were recorded in freely behaving mice for analysis of epileptic seizure in the *PV-Cre* and *PV-Nae1^{-/-}* mice, according to a previous study with minor modification (115). A Teflon-coated silver wire was implanted in the cortex (1.5 mm posterior and 1.2 mm lateral to the bregma) for signal capture, with the other electrode implanted above the cerebellum being used as the reference. The electrode implantation surgery was conducted under 2% isoflurane anesthesia (vol/vol). Mice were moved to the recording chamber after a recovery period of one week. A USB video camera synchronized with the EEG signal was used to observe the epileptic seizure-like behavior. EEG signals were amplified using a preamplifier and primary amplifier (EX1, Dagan) and sampled at 10000 Hz (gain, 1000; band-pass filter, 0.1 to 500 Hz). Epileptic seizure was identified by checking both the EEG signal and the behaviors of animals.

In Vivo electroporation

Newborn pups (P0-P2) were anesthetized by hypothermia (less than 5 min on

ice). Plasmid DNA of 50-200 nl was injected into the somatosensory cortex through a capillary glass tube under pressure. Two trains of ten electric pulses were applied (99 V, 50 ms, of 950-ms interval, and 3-s pause between the two trains, using a square-pulse electroporator ECM830 (BTX)). The pups were placed on a warm pad at 37°C and were expected to fully recover within 10 min. After that, pups were returned to home cages with their mother.

Proteomic analysis

Frozen cerebellar tissues were homogenized in the buffer containing 8 M urea, 50 mM NH_4HCO_3 , in an ultrasonic homogenizer (Thermo Fisher Scientific, Waltham). Samples (100 μg protein) were incubated with 5 mM DTT and alkylated by 10 mM iodoacetamide, and digested in 2 μg trypsin (Promega, Madison, WI) in 1 M urea and 50 mM NH_4HCO_3 , at 37°C overnight. Digested peptides were desalted using MonoSpin C18 desalination column (GL Sciences, Tokyo), 2 μg of which of each sample were analyzed on Q Exactive HF-X (Thermo Fisher Scientific, Waltham, MA).

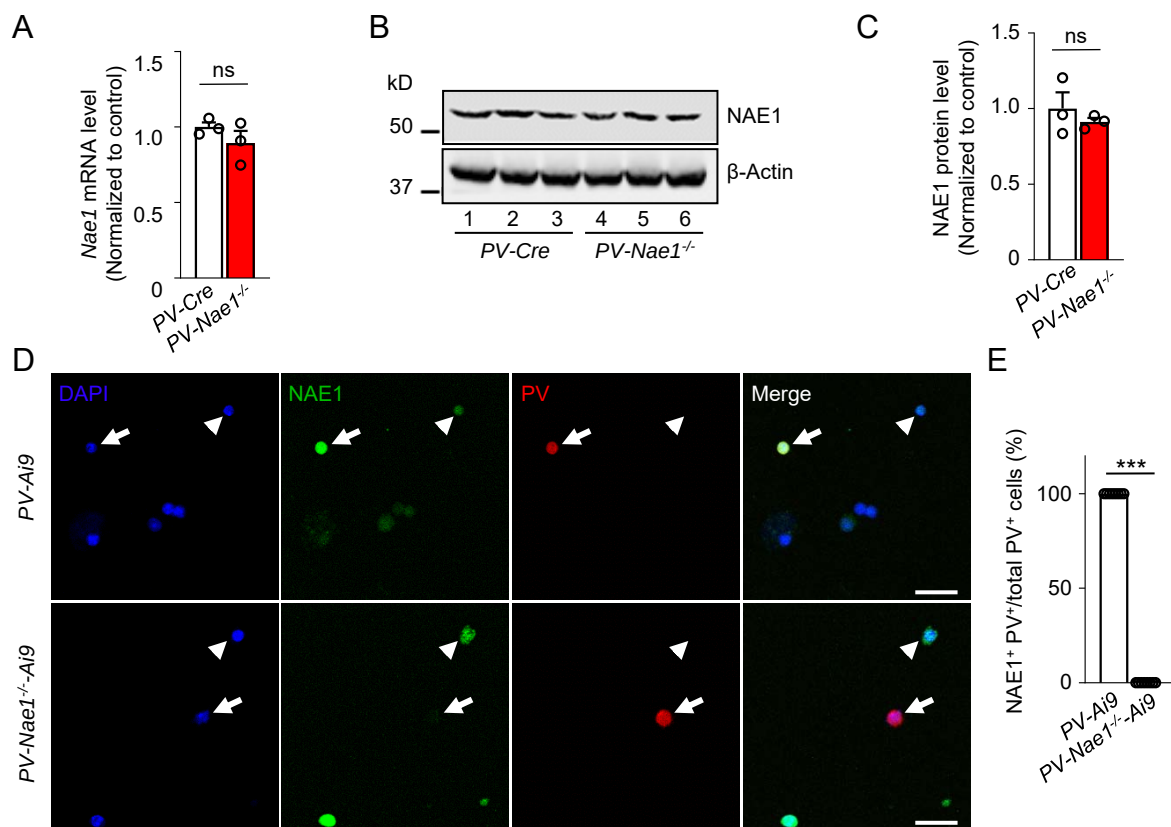
Raw MS data acquired were imported into MaxQuant version 1.5.4.1 for protein and peptide identification and quantitation were conducted by using the stabilize large LFQ ratios with false discovery rate, $\text{FDR} < 0.01$. The MS/MS spectra were run against the human Uniport FASTA database (release 2016_07, 49863 sequences) with trypsin/P set as the enzyme and allowance of two missed cleavage sites of trypsin. Mass errors were set to 5 ppm for precursor ions and 0.02 Da for fragment ions. Carbamido-methylation on Cys was specified as the fixed modification with modification variables as oxidation (M), deamidation (NQ), acetylation (Protein N-term). Minimum peptide length was set at 7. The options of “Second peptides”, “Match between runs” and “Dependent peptides” were enabled. All other parameters were set to default values. Total peptide amounts on channels average were used for normalization and scaling. A total of 5,167 proteins were identified in the fractions at 1% FDR. Relative protein abundance ratios between groups were

calculated using the average of normalized reporter ion intensities of the group and subjected to a two-tailed Student t test with the threshold of significance at $P < 0.05$. Volcano plots and heatmap were generated by GraphPad and Tbtools softwares (116), respectively. The Database for Annotation, Visualization and Integrated Discovery database (<https://david.ncifcrf.gov/>; DAVID v6.8) was used to perform GO of biological processes and KEGG protein enrichment analyses (117). The enriched GO biological processes and KEGG pathway were identified and listed according to their enrichment P -value ($P < 0.05$).

Supplemental References

36. Li L, et al. Enzymatic Activity of the Scaffold Protein Rapsyn for Synapse Formation. *Neuron*. 2016;92(5):1007-1019.
38. Zou J, et al. Neddylation mediates ventricular chamber maturation through repression of Hippo signaling. *Proc Natl Acad Sci U S A*. 2018;115(17):E4101-E4110.
39. Hippenmeyer S, et al. A developmental switch in the response of DRG neurons to ETS transcription factor signaling. *PLoS Biol*. 2005;3(5):e159.
40. Madisen L, et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci*. 2010;13(1):133-140.
41. Kalume F, et al. Reduced sodium current in Purkinje neurons from Nav1.1 mutant mice: implications for ataxia in severe myoclonic epilepsy in infancy. *J Neurosci*. 2007;27(41):11065-11074.
54. Milesco LS, et al. Isolation of somatic Na⁺ currents by selective inactivation of axonal channels with a voltage prepulse. *J Neurosci*. 2010;30(22):7740-7748.
70. Lossin C, et al. Molecular basis of an inherited epilepsy. *Neuron*. 2002;34(6):877-884.
108. Tao Y, et al. Erbin interacts with TARP gamma-2 for surface expression of AMPA receptors in cortical interneurons. *Nat Neurosci*. 2013;16(3):290-299.
109. Wang H, et al. Genetic recovery of ErbB4 in adulthood partially restores brain functions in null mice. *Proc Natl Acad Sci U S A*. 2018;115(51):13105-13110.
110. Barik A, et al. LRP4 is critical for neuromuscular junction maintenance. *J Neurosci*. 2014;34(42):13892-13905.
111. Bischofberger J, et al. Patch-clamp recording from mossy fiber terminals in hippocampal slices. *Nat Protoc*. 2006;1(4):2075-2081.
112. Dong Z, et al. CUL3 Deficiency Causes Social Deficits and Anxiety-like Behaviors by Impairing Excitation-Inhibition Balance through the Promotion of Cap-Dependent Translation. *Neuron*. 2020;105(3):475-490.
113. Schnell C, et al. Active sulforhodamine 101 uptake into hippocampal astrocytes. *PLoS One*. 2012;7(11):e49398.

114. Sathyamurthy A, et al. ERBB3-mediated regulation of Bergmann glia proliferation in cerebellar lamination. *Development*. 2015;142(3):522-532.
115. Lin TW, et al. Regulation of Synapse Development by Vgat Deletion from ErbB4-Positive Interneurons. *J Neurosci*. 2018;38(10):2533-2550.
116. Chen C, et al. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol Plant*. 2020;13(8):1194-1202.
117. Huang da W, et al. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4(1):44-57.



Supplemental Figure 1 No change in NAE1 mRNA or protein level in the hippocampus of PV-Nae1^{-/-} mice.

(A) No alteration in mRNA level of NAE1 in the hippocampus of PV-Nae1^{-/-} mice. n = 3 mice for per genotype.

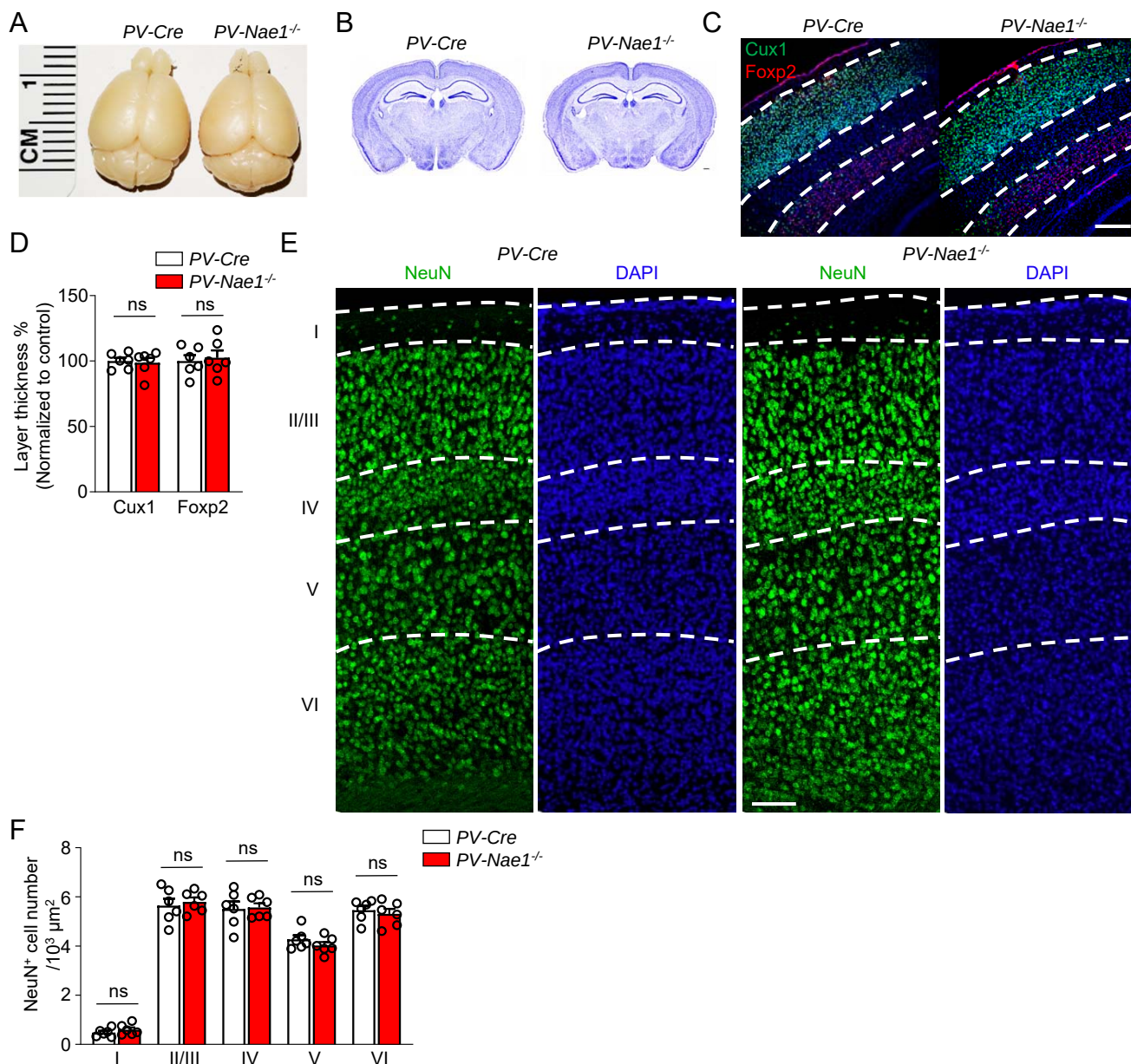
(B) Immunoblot of NAE1 in the hippocampus of PV-Cre and PV-Nae1^{-/-} at P35.

(C) Quantification of data in B showing no alteration in NAE1 protein level. n = 3 mice for per genotype; β-Actin was used as loading control.

(D) Dissociated cortical cells of PV-Ai9 (top) and PV-Nae1^{-/-}-Ai9 (bottom) mice were stained with anti-Nae1 (green) antibody, PV positive neurons were shown in red (tdTomato) and nuclei were shown in blue (DAPI staining). PV positive cells (arrow) and PV negative cells (arrowhead). Scale bar: 30 μm.

(E) Quantification of data in D. The percentage of NAE1⁺PV⁺ double positive cells among total PV⁺ cells in dissociated cortical cells.

Data represent mean ± SEM. ***P < 0.001; ns, no significant difference. unpaired two-tailed Student's t-test.



Supplemental Figure 2 Characterization of brain gross morphology and neuron numbers in *PV-Nae1^{-/-}* mice.

(A) Images of P35 mouse brains.

(B) Nissl staining showing comparative brain gross morphology between *PV-Cre* and *PV-Nae1^{-/-}* mice. Brain sections of hippocampus and cerebral cortex were subjected to Nissl staining at P35. Scale bar: 300 μ m.

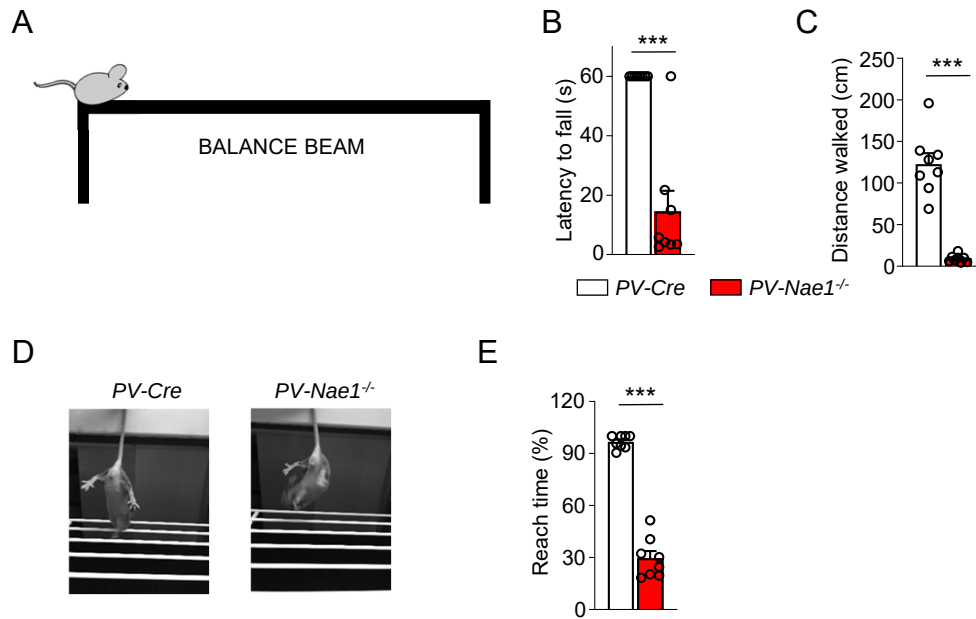
(C) Labeling of layer-specific markers, Cux1 (green) and Foxp2 (red), in brain coronal sections from *PV-Cre* and *PV-Nae1^{-/-}* mice at P35. Nuclei were labeled with DAPI (blue). Scale bar: 250 μ m.

(D) Quantification of the thickness of cerebral layers, expressed as relative value to the total thickness of the somatosensory cortex. Data were normalized to values of the *PV-Cre* group. n = 6 slices of three mice per genotype.

(E) NeuN staining showing comparable numbers of neurons in the somatosensory cortex of *PV-Cre* and *PV-Nae1^{-/-}* mice at P35. Scale bar: 100 μ m.

(F) Quantification of NeuN+ cells in different cortical layers. n = 6 slices of three mice per genotype; $P > 0.05$ in all comparisons.

Data represent mean $\pm$ SEM. ns, no significant difference. unpaired two-tailed Student's *t*-test.



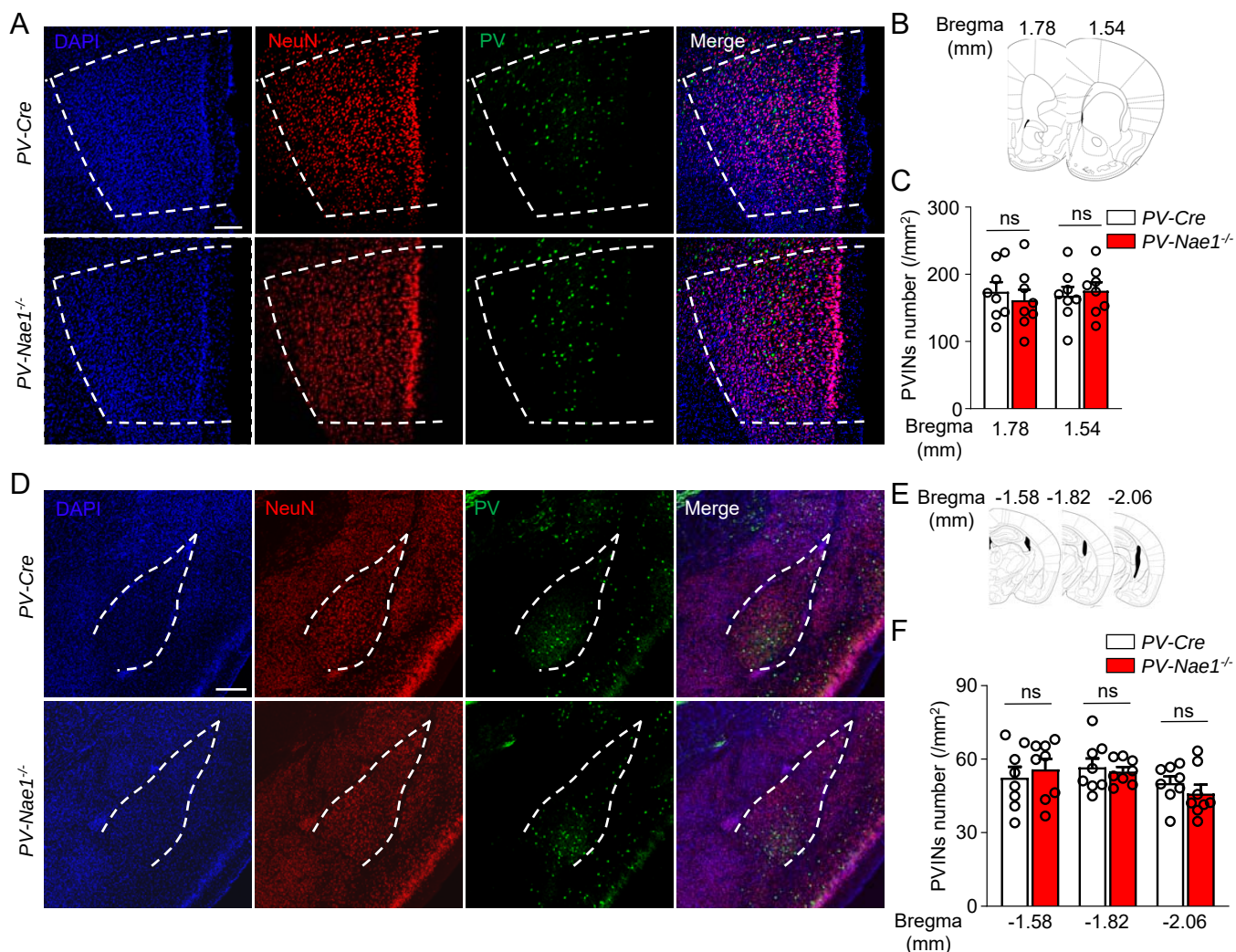
Supplemental Figure 3 Ataxia phenotypes in *PV-Nae1* deficient mice.

(A-C) Reduced latency to fall and distanced walked in balance beam test in *PV-Nae1*^{-/-} mice. (A) Schematic illustration of balance beam test. Latency (B) and distance walked (C) before falling from the beam. n = 8 mice per genotype.

(D) Curved body posture and failure to reach cage bars of *PV-Cre* mice in tail suspension test.

(E) Quantification showing decreased time spent in reaching for the cage bars in *PV-Nae1*^{-/-} mice compared with *PV-Cre* mice. n = 8 mice per genotype.

Data represent mean $\pm$ SEM. ***P < 0.001. unpaired two-tailed Student's *t*-test.



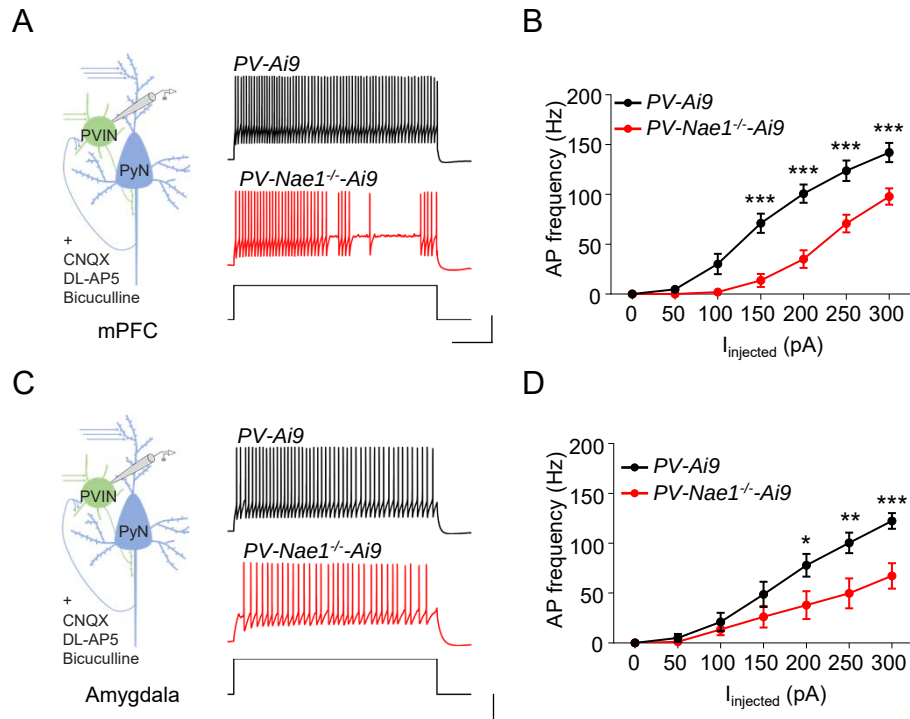
Supplemental Figure 4 No alteration in PVIN number under Nae1 deficiency.

(A-C) No change in PVIN number in mPFC of PV-Nae1^{-/-} mice. (A) Fluorescence images showing PVINs in mPFC; (B) Diagram of coronal sections of mPFC. Numbers indicate the distance to Bregma. (C) Quantification of PVINs in mPFC at the two anterior-posterior coordinates; n = 8 slices respectively from three mice per genotype. Scale bar: 150 μ m.

(D-F) No change in PVIN number in amygdala of PV-Nae1^{-/-} mice. (D) Fluorescence images showing the PVINs in amygdala; (E) Diagram of coronal sections of amygdala. Numbers indicate the distance to Bregma. (F) Quantification of PVINs in amygdala at the three anterior-posterior coordinates; n = 8 slices respectively from three mice per genotype. Scale bar: 300 μ m.

Slices were stained with anti-NeuN (red) and PV (green) antibodies, nuclei were labeled by DAPI (blue).

Data represent mean $\pm$ SEM. ns, no significant difference. unpaired two-tailed Student's *t*-test.

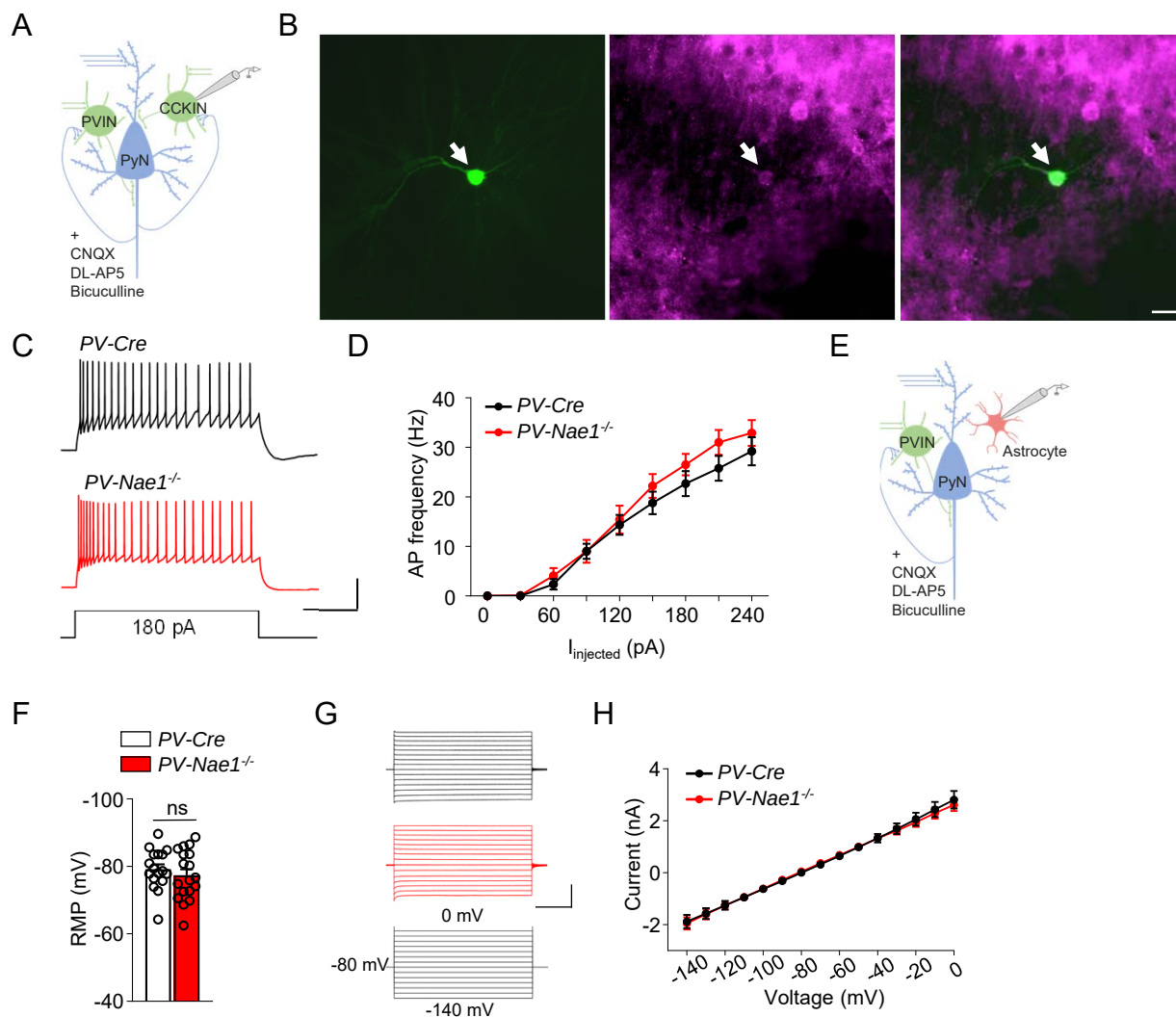


Supplemental Figure 5 Reduced PVIN excitability in mPFC and amygdala of *PV-Nae1* deficient mice.

(A and B) Reduced PVIN intrinsic excitability in mPFC. Schematic diagram of recording (Left) and representative AP traces (Right) in PVINs (A), quantification of current injection-induced APs (B). $n = 10$ neurons, 3 mice for each genotype. Scale bar: 0.2 s, 30 mV.

(C and D) Reduced PVIN intrinsic excitability in amygdala. Schematic diagram of recording (Left) and representative AP traces (Right) in PVINs (C), quantification of current injection-induced APs (D). Scale bar: 0.2 s, 30 mV.

Data represent mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, no significant difference. Two-way ANOVA.



Supplemental Figure 6 No change in CCKIN excitability and astrocyte electrophysiological properties in CA1 of PV-Nae1 deficient mice.

(A) Schematics diagram of recording in CCKINs of hippocampal slices.

(B) Confocal images showing a biocytin-filled interneuron (arrow) stained by anti-CCK antibody. Scale bar, 20 μ m.

(C and D) Representative AP traces (C) and quantification (D) of current injection-induced APs in CCKINs. Scale bar: 0.3 s, 30 mV.

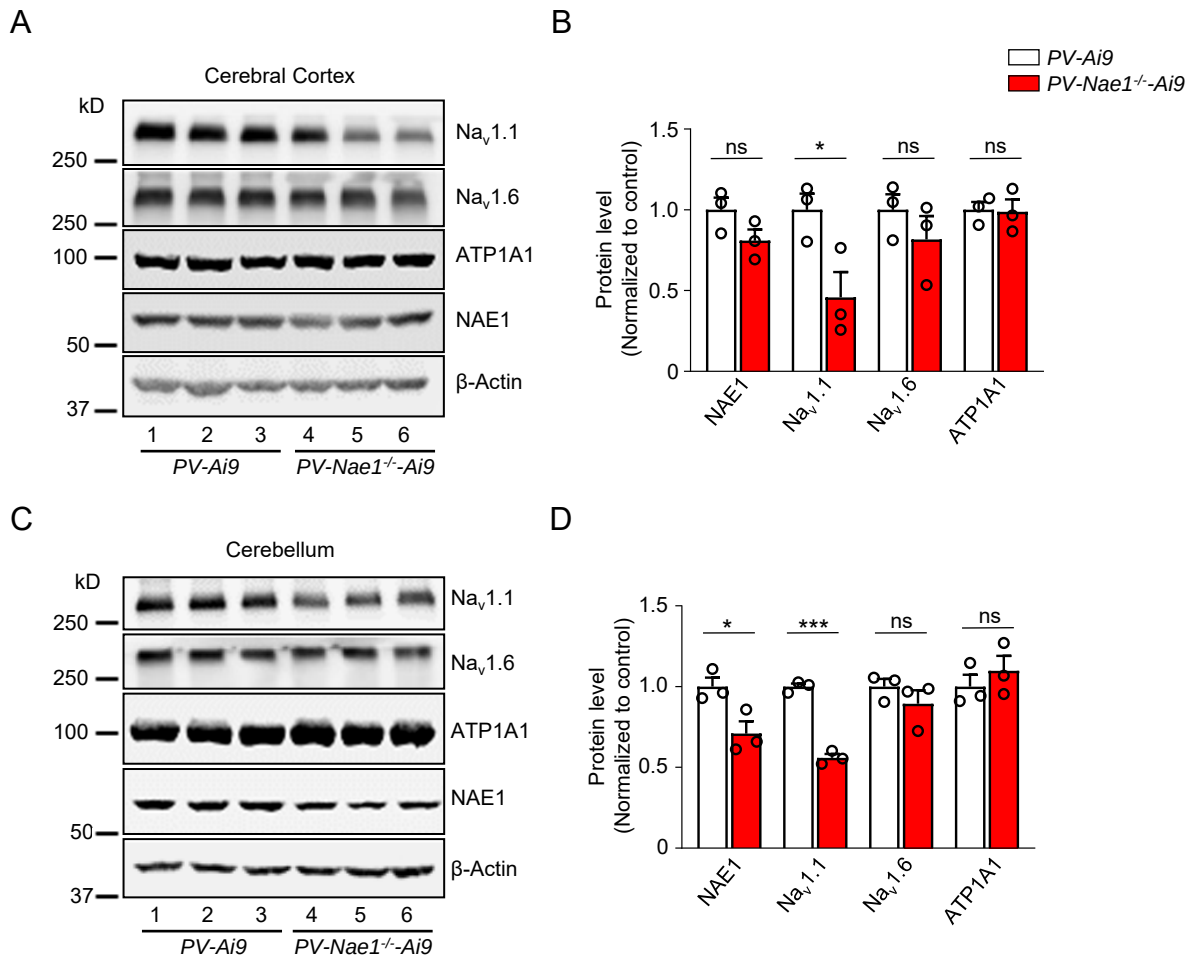
(E) Schematics diagram of recording astrocyte labeled by SR101 in hippocampal slices.

(F) No difference in astrocyte resting membrane potential between PV-Cre and PV-Nae1^{-/-} mice. n = 17-18 cells, 4 mice for both genotypes.

(G) Representative traces of membrane current of astrocyte in hippocampus. Scale bar: 200 ms, 1 nA.

(H) Quantification of current-voltage relationship in astrocytes.

Data were expressed as mean $\pm$ SEM. ns, no significant difference. unpaired two-tailed Student's *t*-test (F). Two-way ANOVA (D and H).



Supplemental Figure 8 Reduced Na_v1.1 protein level in cerebral cortex and cerebellum.

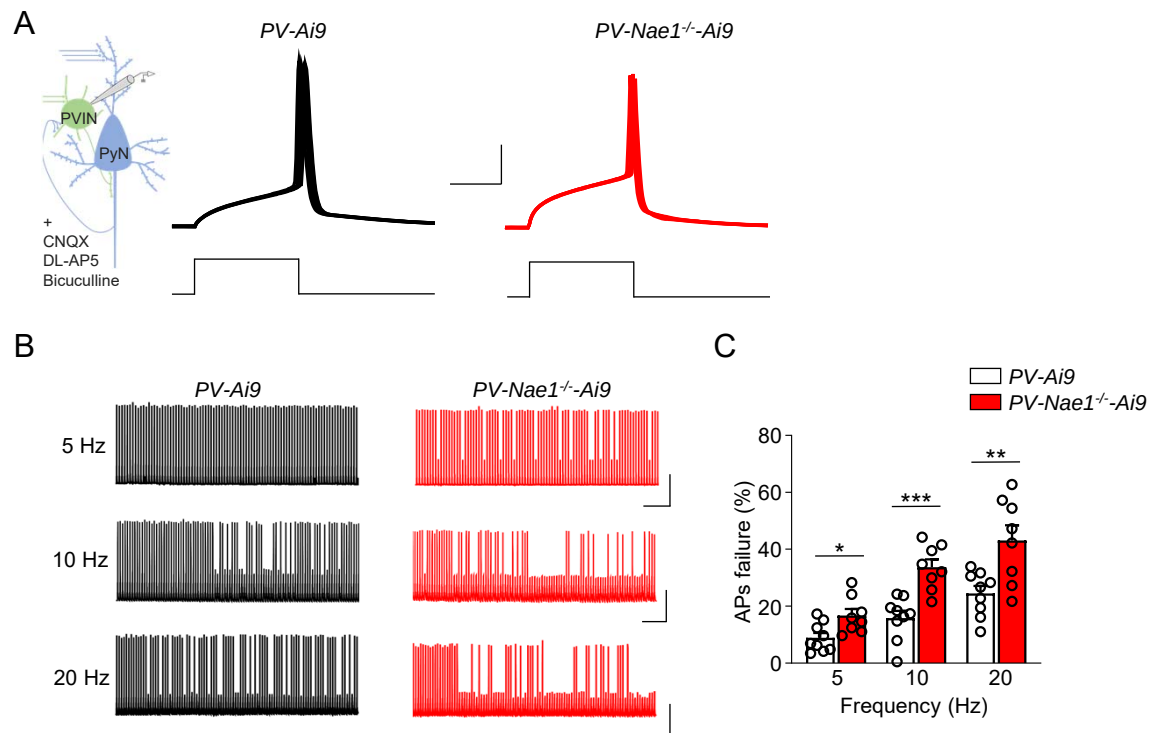
(A) Immunoblot analysis of NAE1, Na_v1.1, Na_v1.6 and ATP1A1 in cerebral cortex. Protein was extracted from cerebral cortex of indicated genotypes after homogenization and subjected to Immunoblot. Data were collected from three mice per genotype.

(B) Quantification of data in A showing selective decrease in Na_v1.1 protein level in PV-Nae1^{-/-}-Ai9 mice.

(C) Immunoblot analysis of NAE1, Na_v1.1, Na_v1.6 and ATP1A1 in cerebellum. Equal amount of proteins were analyzed in three mice per genotype.

(D) Quantification of data in C showing decrease in both NAE1 and Na_v1.1 protein in PV-Nae1^{-/-}-Ai9 mice.

Data were expressed as mean $\pm$ SEM. * $P < 0.05$; *** $P < 0.001$; ns, no significant difference. unpaired two-tailed Student's t -test.



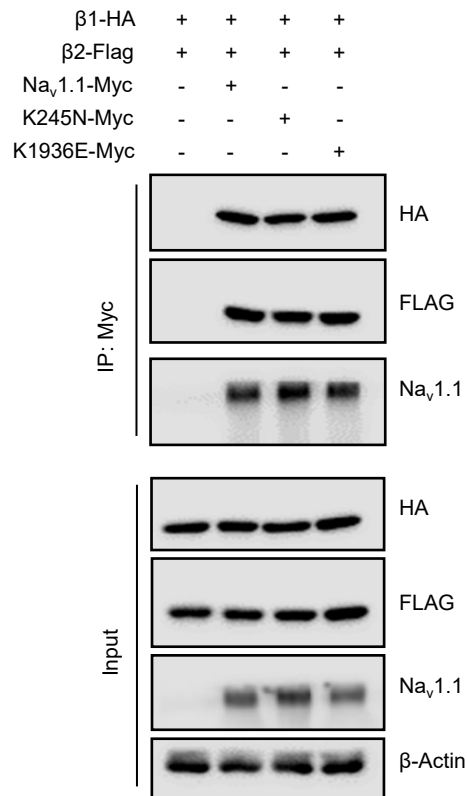
Supplemental Figure 9 Impaired AP induction in PV-Nae1 deficient mice.

(A) Representative AP traces of PVINs in response to 1 Hz train. Left, schematic diagram of recording. Scale bar: 5 ms, 20 mV.

(B) Representative AP traces of PVINs in response to 5 Hz, 10 Hz and 20 Hz trains.

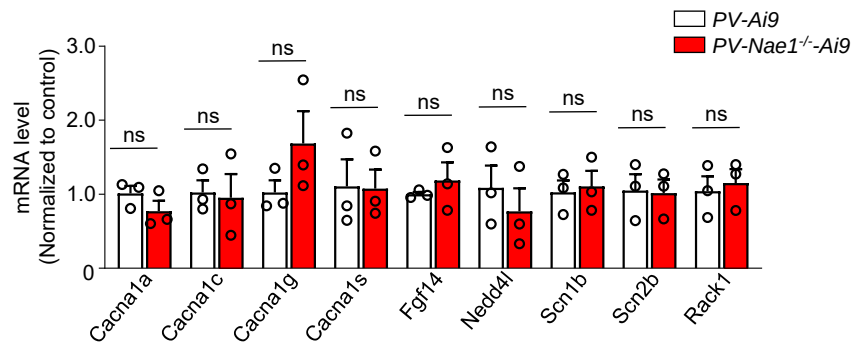
(C) Quantification of the AP failure ratio in response to trains of different frequencies. n = 8 - 9 neurons, 3 mice for each group. Scale bar: 2 s, 30 mV (5 Hz); 1 s, 30 mV (10 Hz); 0.5 s, 30 mV (20 Hz).

Data were expressed as mean $\pm$ SEM. * P < 0.05; ** P < 0.01; *** P < 0.001. unpaired two-tailed Student's t -test.



Supplemental Figure 10 No alteration in interaction between Na_v1.1 and β subunits by K245N or K1936E mutation.

Na_v1.1 interacts with β 1 or β 2 subunits in tsA-201 cells, which was not affected by K245N or K1936E mutation. Cells were transfected with hemagglutinin-tagged β 1 subunit (β 1-HA) and β 2-Flag with Myc-tagged WT-Na_v1.1 (Na_v1.1-Myc) or Myc-tagged mutated Na_v1.1. Lysates were incubated with anti-Myc antibody-conjugated beads, and precipitates were blotted with indicated antibodies.

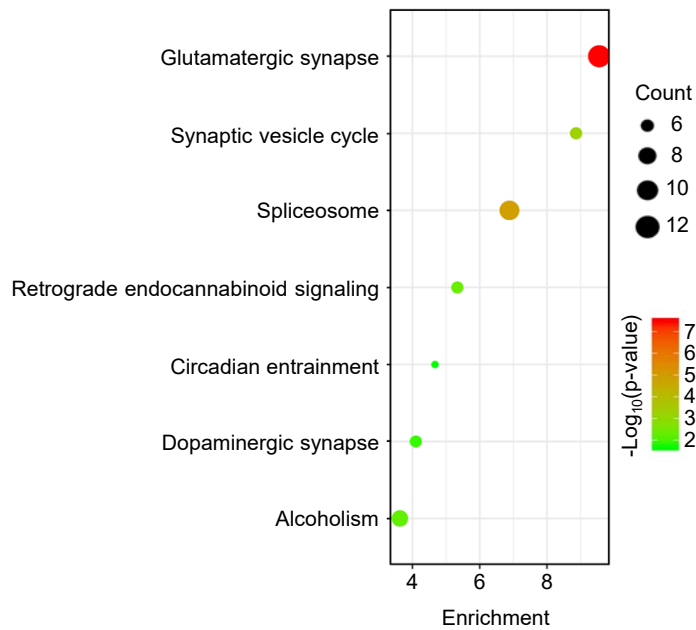


Supplemental Figure 11 No change in the genes implicated in regulating expression and functions of sodium channels in PV-Nae1 deficient mice.

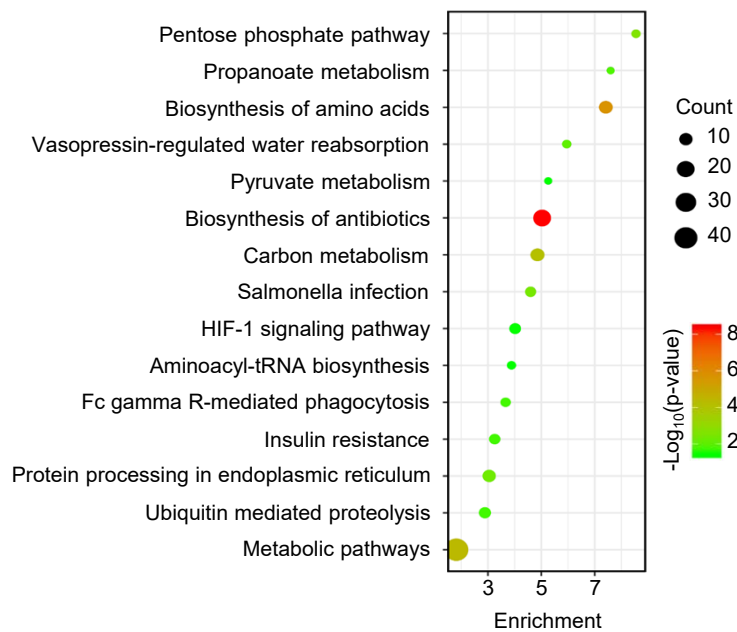
Hippocampal tissues of indicated genotypes were subjected to RT-PCR analyses of indicated genes.

Data were expressed as mean $\pm$ SEM. ns, no significant difference. unpaired two-tailed Student's *t*-test.

A



B



Supplemental Figure 12 Bubble plot of significantly enriched KEGG pathways of differentially expressed proteins.

(A) Bubble plot of significantly enriched KEGG pathways of down-regulated proteins.

(B) Bubble plot of significantly enriched KEGG pathways of up-regulated proteins.

Supplemental Table 1 Proteomics analysis of brain samples between *PV-Nae1^{-/-}-Ai9* and *PV-Ai9* mice, Related to Figure 7.

Supplemental Table 2 GO analysis of down-regulated proteins in *PV-Nae1^{-/-}-Ai9* samples, Related to Figure 7.

Supplemental Table 3 KEGG analysis of down-regulated proteins in *PV-Nae1^{-/-}-Ai9* samples, Related to Supplemental Figure 12.

Supplemental Table 4 GO analysis of up-regulated proteins in *PV-Nae1^{-/-}-Ai9* samples, Related to Figure 7.

Supplemental Table 5 KEGG analysis of up-regulated proteins in *PV-Nae1^{-/-}-Ai9* samples, Related to Supplemental Figure 12.

Supplemental Video 1 An example *PV-Nae1^{-/-}* mouse showing imbalanced or uncoordinated walking.

Supplemental Video 2 An example *PV-Nae1^{-/-}* mouse showing spontaneous epileptic seizures.