Supporting Information

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SI Materials and Methods

Reagents, Plasmids, and Antibodies. B27 supplement, GlutaMAX, penicillin-streptomycin, Neurobasal A, Hanks' balanced salt solution, trypsin, Hepes, Lipofectamine 2000, and Prolong Gold Antifade Reagent were from Life Technologies. Pipes, gelatin from cold water fish skin, poly-D-lysine, laminin, paraformaldehyde, DNase, phalloidin, taxol, and polyethylene glycol (molecular mass 35 kDa) were from Sigma-Aldrich. Triton X-100 was from MP Biomedicals. BSA was from Gemini Bioproducts. Histochoice clearing agent was from VWR. Tween 20 was from Calbiochem. Electron microscopy-grade glutaraldehyde was from Polysciences.

Rat 190-kDa (1) and 270-kDa (2) ankyrin-G-GFP were previously described. The sequence encoding the giant exon of rat ankyrin-G was identified in the rat genome based on exon 37 of the predicted human transcript of ANK3 (ENST00000280772). This sequence was synthesized by Biomatik including the endogenous ClaI and EcoRV sites found in the 270-kDa ankyrin-G-GFP plasmid and subcloned using ClaI and EcoRV, and the sequence was validated by full-length sequencing across the entire coding region. Mutants for 480-kDa ankyrin-G-GFP were generated using the Quikchange II XL kit (Agilent), subcloned back into 480-kDa ankyrin-G-GFP, and verified by full-length sequencing. For long-term neuronal expression of 190- and 480-kDa ankyrin-G-GFP, the CMV promoter from eGFP-N1 was replaced with a CAG promoter. The numbering of amino acid residues corresponds to the location in human ankyrin-G peptides. Transferrin receptor-YFP and TGN38-YFP were gifts from Antonius M. J. VanDongen, Duke University, Durham, NC. LAMP-1-YFP was purchased from Addgene. Cre-2A-BFP plasmid was generated by cloning Cre recombinase, a viral 2A peptide, and TagBFP under control of a CAG promoter into pLenti6-V5-DEST. The 2A peptide mediates cotranslational cleavage and allows efficient expression of both Cre and BFP. TagBFP-N1 was a generous gift from James Bear, University of North Carolina, Chapel Hill, NC. The 480-kDa ankyrin-G–specific shRNA was generated using the sequence 5′-GAGGTAGAGAGAAGCTGATGT-3′ cloned into pLentilox 3.7 BFP.

Rabbit anti–480-kDa ankyrin-G (2735–2935 of rat 480-kDa ankyrin-G) and rabbit anti-β4 spectrin (800–1094 of mouse β4 spectrin sigma 6, NP_001186163.1) antibodies were generated by cloning the indicated sequences into a pMAL-6xHis-DEST vector. Proteins were expressed in *Escherichia coli* and purified first by nickel chromatography and then by amylose resin. Proteins were cleaved from the amylose resin using Precission Protease (GE Healthcare), covalently coupled to ovalbumin, and injected into rabbits with Freund's complete adjuvant (first injection) and Freund's incomplete adjuvant for all subsequent injections. All rabbit manipulations were performed by Duke Laboratory Animal Resources. Serum was collected, cleared on MBP and ovalbumin columns, and affinity-purified using beads covalently coupled to the original antigen. Finally, antibodies were eluted with 4 M $MgCl_2$ and dialyzed into storage buffer [150 mM NaCl, 10 mM phosphate buffer, 1 mM EDTA, 1 mM NaN_3, and 50% (vol/vol) glycerol]. Goat anti–C-terminal (total) ankyrin-G (3), rabbit anti–C-terminal (total) ankyrin-G (4), and anti-neurofascin FNIII (5) antibodies were previously described. Chicken anti-MAP2 (ab5392) and anti-GFP (ab13970) antibodies were from Abcam. Rabbit anti-GFP antibodies were laboratory-generated. Mouse (S8809) and rabbit (S6936) anti-pan VGSC and mouse anti-MAP2 (M4403) and anti-calbindin (C9848) antibodies were from Sigma-Aldrich. Rabbit anti-KCNQ2 (PA1-929) antibodies were from Thermo Fisher. Guinea pig anti-VGAT (131 004) antibodies were from Synaptic Systems. Mouse anti-Caspr (75-001) antibodies were from Neuromab. All AlexaFluor-conjugated secondary antibodies were from Life Technologies. Pan ankyrin-G antibodies that recognize ankyrin-G, -B, and -R were previously described (6).

Neuronal Culture and Transfection. For hippocampal rescue into an AnkG-null background, exon 22–23 total AnkG floxed mice (7) were maintained as homozygous for the mutant floxed allele. Dissociated hippocampal neurons from flox/flox pups were transfected with Cre-2A-BFP (blue fluorescent protein) plasmid to excise Ank3 in vitro. All experiments were performed in accordance with the guidelines for animal care of the Animal Care and Use Program at Duke University.

Neurons and medium were prepared as described (8). Briefly, hippocampi of postnatal day 0 mouse pups were isolated, treated for 18 min at 37 °C with 0.25% trypsin in HBSS with 10 mM Hepes and 100 μg/mL DNase, and then gently triturated through a glass pipette with a fire-polished tip. The dissociated cells were plated onto poly-D-lysine and laminin-coated MatTek dishes in Neurobasal-A medium containing 10% (vol/vol) FBS, B27 supplement, 2 mM glutamine, and penicillin/streptomycin. On the following day, the medium was replaced with fresh Neurobasal-A medium containing B27, glutamine, penicillin/streptomycin, and 1% FBS.

For rescue of ankyrin-G into an ankyrin-null background, neurons from exon 22–23 flox/flox pups were transfected at DIV3 with Cre-2A-BFP (to excise endogenous ankyrin-G) with or without the rescue plasmid. For Cre-2A-BFP alone, 1 μg of cDNA was used. For 190-kDa and 270-kDa ankyrin-G-GFP rescue, 1 μg of total cDNA was used (0.5 μg of Cre, 0.5 μg of rescue plasmid). For 480-kDa ankyrin-G-GFP and mutant rescue experiments, 1.5 μg of cDNA were used (0.5 μg of Cre, 1.0 μg of rescue plasmid). Briefly, cDNA was added to 100 μL of Neurobasal A, and Lipofectamine 2000 was resuspended in a second tube in 100 μL of Neurobasal A in a 3:1 ratio to cDNA (3-4.5 μL). Tubes were mixed and incubated at room temperature for 15 min. Neuronal growth medium was removed, cells were washed once with Neurobasal A, and transfection mixture was added for 40 min at 37 °C. Transfection mixture was then aspirated, and original growth medium plus 2.5 μg/mL Ara-C was added. Cells were maintained in culture until DIV7 to -9 and imaged live or processed for immunofluorescence as described below.

For DIV21 hippocampal cultures, cells were transfected as described previously (9). Briefly, calcium phosphate transfection was used to introduce DNA/Ca^{2+} phosphate complexes directly onto the cell layer at days 3 in vitro (DIV3). Generally, 1 μg of cDNA in a 25-μL $CaCl_2$/water solution was mixed with 2× Hanks' balanced salt solution followed by gentle vortexing (Clontech). The DNA–Ca^{2+}–phosphate complex was formed after incubation for 15 min at room temperature and then added dropwise to DIV3 neurons prewashed with Neurobasal media on Mat-tek plates. Cells were incubated in a humidified 5% CO_2 chamber at 37 °C for 1 h. Precipitate was dissolved by incubating cells with Neurobasal media preequilibrated in 10% CO_2 in a humidified 5% CO_2 chamber at 37 °C for 20 min. Finally, cells were fed with original growth media containing 2.5 μg/mL Ara-C and maintained until DIV21 for immunofluorescence staining as described below.

Spinal Cord Preparation. For spinal cord immunohistochemistry, P18–P20 were killed by cardiac perfusion, and the spinal cord

was immediately removed and fixed overnight in 4% (wt/vol) paraformaldehyde followed by a standard single-day paraffin preparation protocol (PBS wash followed by dehydrations through 70%, 95%, and 100% ethanol with final incubations in xylene and hot paraffin under vacuum). Paraffin sections were cut at 7 μm using a Leica RM2155 microtome. Sections were deparaffinized and rehydrated using a standard protocol of washes: 3 × 3-min Histochoice Clearing Reagent washes, 3 × 2-min 100% ethanol washes, and 1 × 2-min 95%, 80%, and 70% ethanol (each) followed by at least 5 min in PBS. Sections were then processed for antigen retrieval using 10 mM sodium citrate in the microwave for 20 min. Sections were allowed to cool, washed in PBS, and blocked using blocking buffer. Slides were incubated overnight at 4 °C with primary antibodies diluted in blocking buffer. After extensive PBS washing, slides were incubated in blocking buffer with the appropriate secondary antibodies for 1 h at room temperature, washed, and mounted with Prolong Gold Antifade reagent.

Immunofluorescence. For all antibodies except pan VGSC, dissociated hippocampal neurons were fixed for 15 min at room temperature with 4% (wt/vol) paraformaldehyde, permeabilized for 10 min with 0.2% Triton X-100 in PBS, and blocked with blocking buffer [5% (wt/vol) BSA, 0.2% Tween 20] in PBS. Primary antibodies were diluted in blocking buffer and incubated overnight at 4 °C. For mouse pan VGSC, cells were fixed for 15 min at room temperature with 4% (wt/vol) paraformaldehyde and permeabilized and blocked simultaneously with gelatin buffer [5% (wt/vol) fish gelatin, 0.25% Tween in PBS] for 30 min at room temperature. Primary antibodies were diluted in gelatin buffer and incubated for 2 h at room temperature. All cells were washed with PBS, incubated with secondary antibodies diluted in blocking buffer for 1–2 h at room temperature, washed, and mounted with Prolong Gold.

For immunohistochemistry, P18–P20 were killed by cardiac perfusion, and brain was immediately removed and fixed overnight in 4% (wt/vol) paraformaldehyde followed by a standard single day paraffin preparation protocol (PBS wash followed by dehydrations through 70%, 95%, and 100% ethanol with final incubations in xylene and hot paraffin under vacuum). Paraffin sections were cut at 7 μm using a Leica RM2155 microtome. Sections were deparaffinized and rehydrated using a standard protocol of washes: 3 × 3-min Histochoice Clearing Reagent washes, 3 × 2-min 100% ethanol washes, and 1 × 2-min 95%, 80%, and 70% ethanol (each) followed by at least 5 min in PBS. Sections were then processed for antigen retrieval using 10 mM sodium citrate in the microwave for 20 min. Sections were allowed to cool, washed in PBS, and blocked using blocking buffer. Slides were incubated overnight at 4 °C with primary antibodies diluted in blocking buffer. After extensive PBS washing, slides were incubated in blocking buffer with the appropriate secondary antibodies for 1 h at room temperature, washed, and mounted with Prolong Gold Antifade reagent.

The following antibody dilutions were used: anti–480-kDa ankyrin-G (1:500), anti–total ankyrin-G (1:500), chicken or mouse anti-MAP2 (1:1,000), mouse anti-pan VGSC (1:100, for dissociated hippocampal cultures), rabbit anti-pan VGSC (1:100, for brain slices), rabbit anti-β4 spectrin (1:500), rabbit anti-GFP (1:1,000), rabbit anti-neurofascin (1:500), mouse anti-Caspr (1:1,000), guinea pig anti-VGAT (1:1,000), mouse anti-calbindin (1:1,000), rabbit anti-KCNQ2 (1:250), and all secondary antibodies (1:250).

Confocal Microscopy and Live Imaging. Samples were imaged on a Zeiss LSM 780 with a 40 × 1.3 Plan-Apochromat objective, and excitation was accomplished using 405-, 488-, 561-, and 633-nm lasers. For live imaging experiments, a humidified, temperature-controller chamber was used to maintain the neuronal cultures at 37 °C and 5% CO_2 in physiological salt solution (141 mM CaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5.6 mM glucose, 15 mM Hepes, pH 7.4).

Electron Microscopy. Hippocampal cultures grown on MatTek dishes were prepared essentially as described previously (10). Cells were extracted for 3 min with 1% Triton X-100, 2% (vol/vol) PEG-35,000, 2 μM phalloidin, 2 μM taxol in PEM buffer (100 mM Pipes, pH 6.9, 5 mM EGTA, 5 mM $MgCl_2$) at room temperature. Cells were washed three times with PEM buffer containing 2 μM phalloidin and 2 μM taxol and then fixed in 2% (vol/vol) glutaraldehyde in 0.1 M sodium cacodylate, pH 7.3 for 20 min at room temperature. Coverslips were then removed from the dish and prepared for electron microscopy and imaged as described previously (10). In brief, detergent-extracted samples were fixed with 2% (vol/vol) glutaraldehyde, tannic acid, and uranyl acetate; critical point dried; coated with platinum and carbon; and transferred onto electron microscopic grids for observation. Samples were examined using a transmission electron microscope (JEM 1011; JEOL) operated at 100 kV. Images were acquired by a charge-coupled device camera (ORIUS 832.10W; Gatan) and presented in inverted contrast. Gold particles in replica electron microscopy images were identified at high magnification after contrast enhancement to distinguish them from other bright objects in the samples.

Western Blot. Mouse brains were dissected and hand-dounced on ice in 10 volumes/weight homogenization buffer (10 mM phosphate buffer, pH 7.4, 0.32 M sucrose, 1 mM EDTA, 1 mM NaN_3, plus protease inhibitors), mixed 1:1 with 5× PAGE buffer [5% (wt/vol) SDS, 25% (wt/vol) sucrose, 50 mM Tris, pH 8, 5 mM EDTA, bromophenol blue], sonicated 10 pulses, and heated to 65 °C for 10 min. Samples (10-μL volume) were run on a 3.5–17.5% gradient gel in 1× Tris buffer, pH 7.4 (40 mM Tris, 20 mM NaOAc, and 2 mM NaEDTA) with 0.2% SDS (11). Transfer to nitrocellulose was performed overnight at 300 mA at 4 °C in 0.5× Tris buffer with 0.01% SDS. Membranes were blocked with Blot buffer I (150 mM NaCl, 1 mM NaN_3, 1 mM EDTA, 0.2% Triton X-100, and 10 mM phosphate buffer, pH 7.4) with 2% (wt/vol) BSA and incubated overnight at 4 °C with primary antibodies (rabbit total ankyrin-G, 1:5,000) diluted in blocking buffer. Membranes were then incubated with I125-labeled protein A/G (1:1,000). Membranes were placed on a storage phosphor screen, and signal was detected using a Typhoon imager (GE Healthcare).

In Vitro Electrophysiology Recording and Analysis. For whole-cell patch-clamp recordings, brains from animals (WT, 3; KO, 3) were removed quickly into ice-cold solution bubbled with 95% O2–5% CO_2 containing the following: sucrose (194 mM), NaCl (30 mM), KCl (2.5 mM), $MgCl_2$ (1 mM), $NaHCO_3$ (26 mM), NaH_2PO_4 (1.2 mM), and D-glucose (10 mM). After 4–5 min, the brains were blocked, and coronal slices were taken at 250 μm. During the recovery period (30 min), the slices were placed and stabilized at 35.5 °C with oxygenated artificial cerebrospinal fluid (aCSF) solution containing the following: NaCl (124 mM), KCl (2.5 mM), $CaCl_2$ (2 mM), $MgCl_2$ (1 mM), $NaHCO_3$ (26 mM), NaH_2PO_4 (1.2 mM), and D-glucose (10 mM) with pH adjusted to 7.4 with HCl and osmolarity set to 319 mosM. Internal solution for the pipette (3–5 MΩ) contained: potassium gluconate (150 mM), $MgCl_2$ (2 mM), EGTA (1.1 mM), Hepes (10 mM), sodium ATP (3 mM), and sodium GTP (0.2 mM) with pH adjusted to 7.2 with KOH and osmolarity set to 315 mosM. All recordings were performed under continuous perfusion of aCSF at 28–29 °C with 2–3 mL/min flow rate, and with a MultiClamp 700B amplifier (Molecular Device). To measure neuronal excitability, current-clamp recording was performed from coronal mouse brain slices. Recorded signals were filtered at 10 kHz and digitized at

20 kHz with a Digidata 1440A digitizer (Molecular Devices). Data were analyzed using pCLAMP10 (Molecular Devices).

In Vivo Electrophysiology Recording and Analysis. Surgery (WT, 2; Exon 37-null, 3) was performed under anesthesia with isoflurane (2%). A craniotomy was performed over the motor cortex locations according to known stereotaxic coordinates [from bregma in μm, the coordinates were anteroposterior (AP) 1.1 and mediolateral (ML) 1.2]. The electrode arrays used in this study consisted of custom-made 2 × 8 platinum-coated tungsten microwire electrodes (35 μm diameter) with 150 μm between microwires, and 200 μm between rows. The arrays were lowered to the appropriate stereotaxic depth (~1 μm) to target layer 5 in the primary motor cortex and fixed in place with dental acrylic. After 6 h, we started recording local field potentials (LFPs, five sessions from KO and four sessions from WT, each session lasting at least 10 min). LFP signals were filtered with a third order high-pass filter and seventh order low-pass filter (0.1-Hz to 5-Hz cutoffs). The analysis of LFP power was performed using Neuroexplorer. The power spectra were calculated using Welch's method (512 frequencies between 1 and 55 Hz). Alpha and gamma were defined as 8–15 Hz and 30–55 Hz in frequency, respectively. In addition, electrode placement was verified postmortem after fixation with 10% (vol/vol) formalin, followed by thionin staining in 100-μm coronal sections.

Phylogenetic Analysis. Alignment of the ankyrin family was done using ankyrin sequences with the giant exon removed to accurately compare homology of ankyrin-R (which does not contain a giant insert) with ankyrin-B and ankyrin-G. Alignments were performed using the ClustalW2 website (www.ebi.ac.uk/Tools/msa/clustalw2/), and the resulting alignments were used to generate a rooted phylogenetic tree (iubio.bio.indiana.edu/treeapp/treeprint-form.html).

qRT-PCR. For gene-expression studies, animals were killed by decapitation, and tissues were quickly dissected and frozen in liquid nitrogen. Total RNA was isolated using RNeasy mini kit (Qiagen) according to the manufacturer's instructions. First strand synthesis was carried out using SuperScript III reverse transcriptase (Life Technologies). Quantitative RT-PCR (qRT-PCR), duplex primer-probe PrimeTime assays (IDT) were designed to span exon–exon boundaries to specifically amplify 190-kDa or 480-kDa ankyrin-G. For 190-kDa ankyrin-G, the primer sequences were 5′-GCTTTGCCTCCCTAGCTTTA-3′ and 5′- GATATCCGTCCG-CTCACAAG-3′ and the probe was 5′- TTGACTGAACCCAGC-ATGAGTCCG-3′. For 480-kDa ankyrin-G, the primer sequences were 5′-GAGGCACCGCCCTTAAA-3′ and 5′-GCCAGCTCTG-TCCAACTAA-3′ and the probe was 5′-AGAGTCCTTGTG-AGCGGACGGATA-3′. Ankyrin-G probes were Zen-modified and FAM-labeled, and standard GAPDH probes were Zen-modified and HEX labeled. RT-PCR was performed in duplex using TaqMan Gene Expression Master Mix (Applied Biosystems) and a StepOnePlus PCR system (Life Technologies) according to the manufacturer's instructions.

Statistical and Data Analysis. Measurements were taken using Zeiss Zen or NIH ImageJ software and repeated for at least three independent experiments. Statistical analysis was performed using Graphpad Prism software. For quantification of axon initial segment (AIS) density, a background subtracted value for the region of interest corresponding to the AIS of the transfected neuron was expressed as a ratio to a corresponding region of interest from a nearby nontransfected (WT) neuron. Data shown are mean ± SEM. Student's *t* test was used for comparisons between two groups whereas a one-way ANOVA with Tukey post hoc test was used to compare three or more groups.

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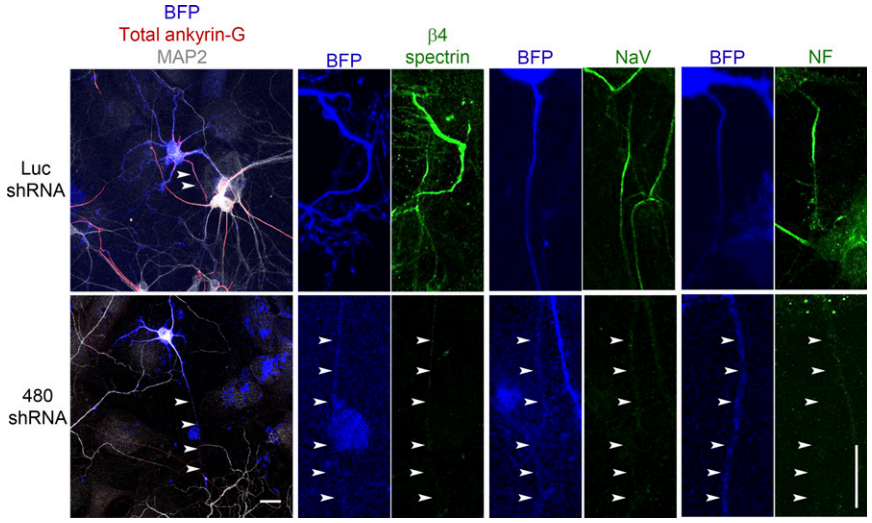

Fig. S1. The 480-kDa ankyrin-G–specific shRNA completely abolishes clustering of known AIS proteins in the proximal axon. Representative images from cultured hippocampal neurons expressing a control shRNA to luciferase (*Top*) or shRNA to 480-kDa ankyrin-G. BFP shown in blue, total ankyrin-G staining shown in red. MAP2 shown in white. (Scale bar: 20 μm.) Higher magnification images of axon shown on *Right* for BFP (blue) and either β4 spectrin, VGSC, or NF (green).

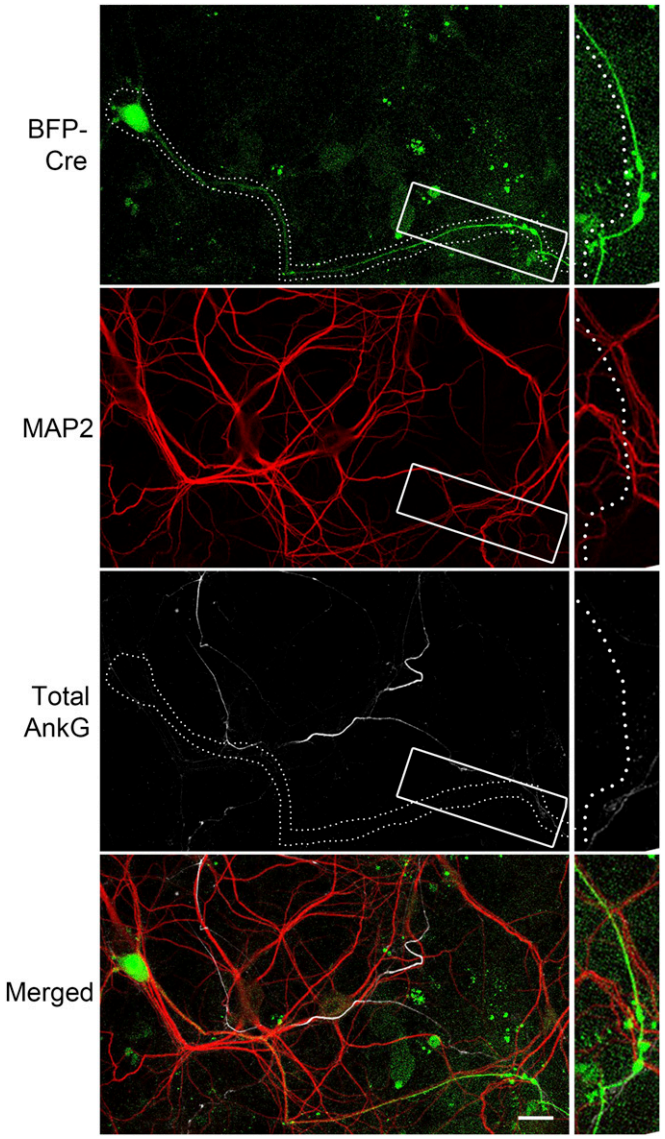

Fig. S2. Cre transfection at DIV3 completely abolishes ankyrin-G clustering at AIS by DIV7. Representative images of a cultured exon 22–23 flox/flox hippocampal neurons transfected with Cre-2A-BFP (pseudocolored green). MAP2 is shown in red, and total ankyrin-G staining is shown in white. Higher magnification of distal axon region of interest shown on *Right*. (Scale bar: 20 μm.)

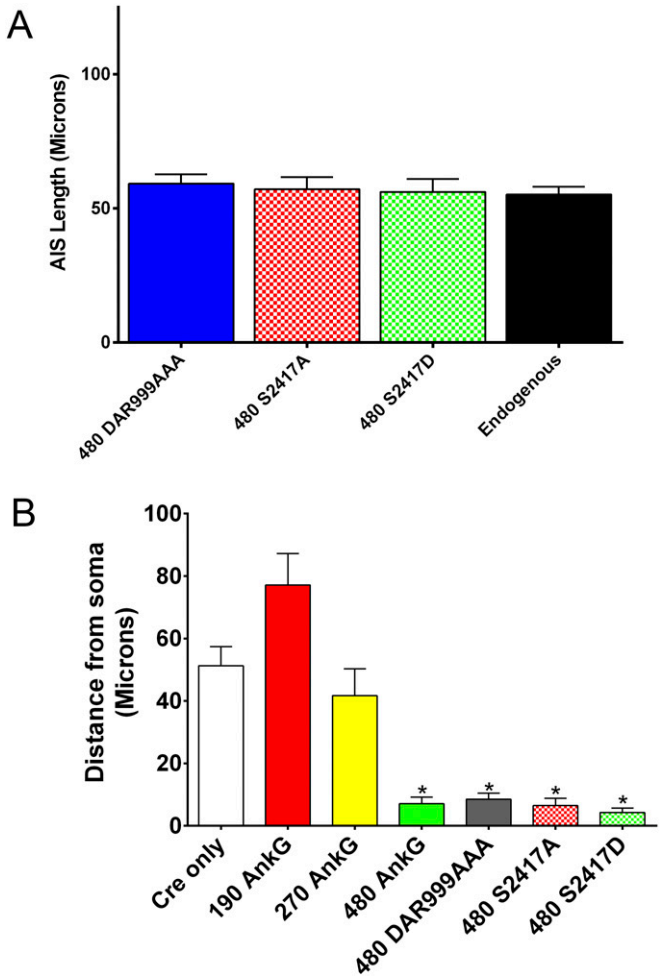

Fig. S3. Measurements of cluster length and AIS position relative to endogenous AIS. (*A*) Average measurements of AIS clustering of 480-kDa ankyrin-G-GFP with the indicated mutations relative to endogenous AIS ($P = 0.9099$, one-way ANOVA, $n = 18–21$ per group). (*B*) Average distance from soma for transition between dendritic character (MAP2-positive) and axonal character (MAP2-negative) in total ankyrin-G–null neurons rescued with the indicated constructs. ($P < 0.0001$, one-way ANOVA followed by Tukey post hoc test; $^*P < 0.05$ relative to total ankyrin-G–null, Cre only).

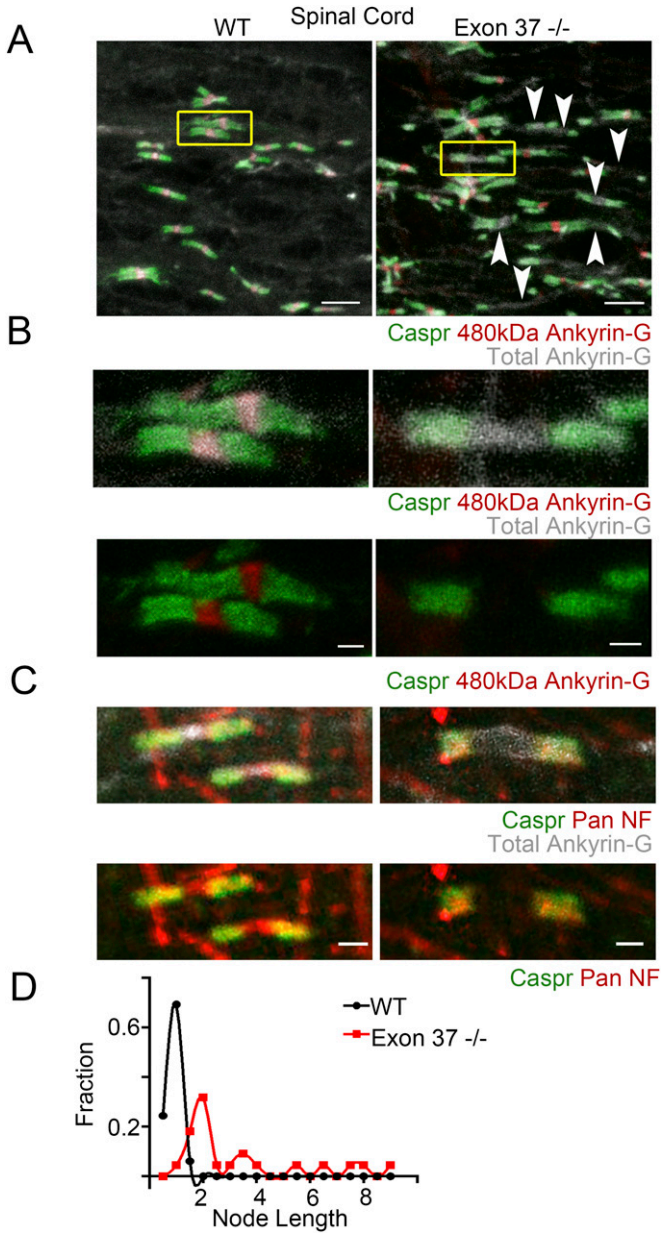

Fig. S4. A subset of nodes of Ranvier in the spinal cord are affected by nestin-Cre–mediated deletion of exon 37. (*A*) Representative images of sections through spinal cord from p20 WT or exon 37-null mice. Sections were stained with antibodies to the paranode marker Caspr (green), 480-kDa ankyrin-G (red), and total ankyrin-G (white). (Scale bars: 5 μm.) (*B*) Higher magnification of nodes of Ranvier from yellow regions of interest in *A*. (Scale bars: 1 μm.) (*C*) Higher magnification images of nodes from WT or exon 37-null mice. Caspr (green), NF (red), and total ankyrin-G (white). (Scale bars: 1 μm.) (*D*) Histogram of node of Ranvier length from WT (black) or exon 37-null (red) nodes of Ranvier from p20 spinal cord. Nodes were determined to be exon 37-null by loss of 480-kDa AnkG staining.

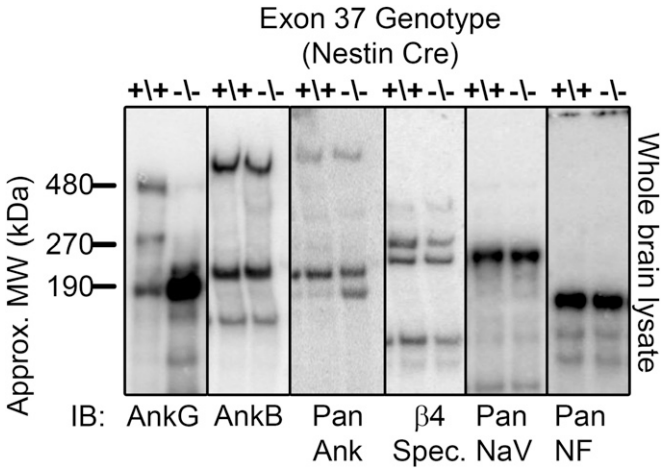

Fig. S5. No changes detected in protein expression of other ankyrin isoforms or known ankyrin-G partners. Western blot analysis from whole-brain lysate of P20 WT (+/+) or exon 37-null (–/–) animals. Blots were probed with antibodies to total ankyrin-G (AnkG), total ankyrin-B (AnkB), pan ankyrin (Pan Ank), β4 spectrin, pan voltage-gated sodium channel (Pan NaV), and Pan neurofascin (Pan NF).

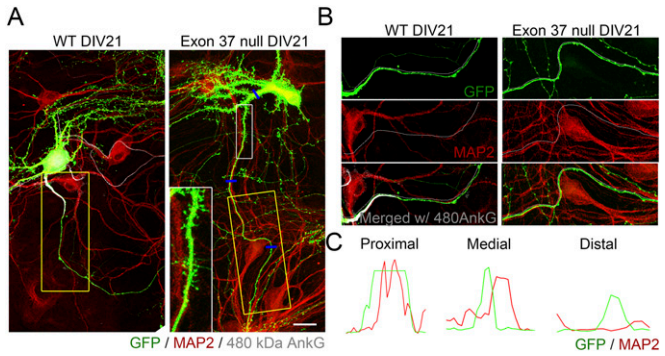

Fig. S6. DIV21 Exon 37-null hippocampal neurons exhibit profound proximal axon polarity defects but maintain distal polarity. (*A*) Representative images of WT (*Left*) or exon 37-null (*Right*) DIV21 cultured hippocampal neurons. Soluble GFP shown in green. MAP2 shown in red. Staining for 480-kDa ankyrin-G staining shown in white. (Scale bar: 20 mμ.) Blue bars represent position of line scan analysis shown in *C*. Exon 37-null *Inset* demonstrates acquisition of dendrites in proximal axon. (*B*) Higher magnification images of yellow regions of interest shown in *A* demonstrating loss of MAP2 in distal axon of exon 37-null neuron. (*C*) Line fluorescent intensity analysis of blue bars shown in *A* in proximal, medial, or distal axon. GFP shown in green. MAP2 shown in red.

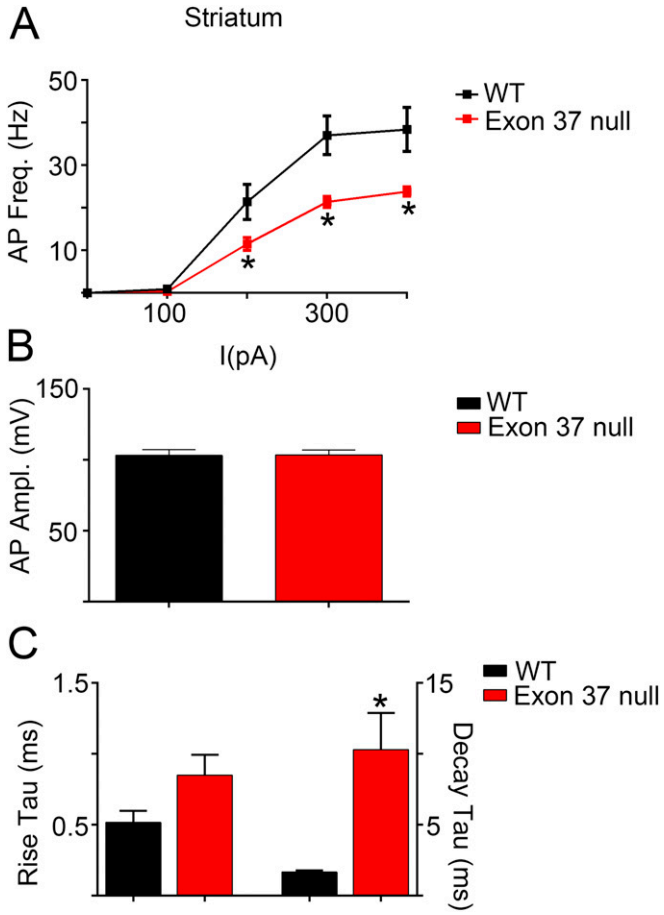

Fig. S7. Action potential frequency and dynamics are affected in the striatum of the exon 37-null mouse. (*A*) Elicited action potential frequency from striatal neurons from WT (black) or exon 37-null (red) acute brain slices. Data shown are mean ± SEM, **P* < 0.05 compared with WT. (*B*) Action potential amplitude from WT (black) or exon 37-null (red) striatal neurons at +400 pA current injection (WT, 103.2 ± 4.050 mV, *n* = 10; exon 37-null, 103.5 ± 3.532 mV, *n* = 10). (*C*) Time constants (τ) for action potential rise (*Left*) or decay (*Right*) from WT (black) or exon 37-null (red) striatal neurons at +400 pA current injection (rise τ, *P* = 0.0586; WT, 0.5 ± 0.1 ms, *n* = 10; exon 37-null, 0.9 ± 0.1, *n* = 10; decay τ, **P* = 0.0036; WT, 1.7 ± 0.1 ms, *n* = 10; exon 37-null, 10.3 ± 2.6 ms, *n* = 10).

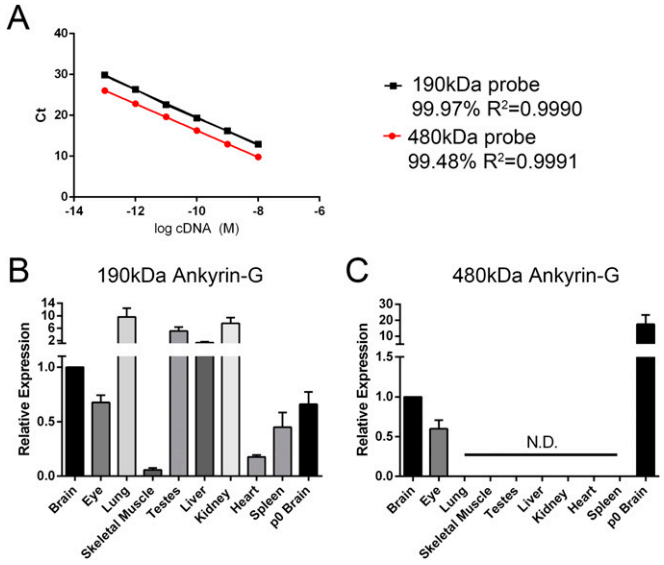

Fig. S8. The 480-kDa ankyrin-G expression is restricted to neuronal tissues. (*A*) Probe validation for qRT-PCR probes specific for 190-kDa (black) or 480-kDa (red) ankyrin-G using cDNA encoding each isoform as a template. (*B*) Relative190-kDa ankyrin-G gene expression from P30 WT mouse tissues or P0 brain. Data normalized to GAPDH as endogenous control and to P30 brain. (C) Relative 480-kDa ankyrin-G gene expression from P30 WT mouse tissues or P0 brain. Data normalized to GAPDH as endogenous control and to P30 brain.