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Supplementary Information for

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Supplementary Information

Material and Methods

Genetic testing

Exome sequencing was performed using genomic DNA isolated from whole blood of proband and parents (MagnaPure, Roche). Libraries were prepared using the Ion AmpliSeq™ Exome Kit (Life Technologies) and quantified by qPCR. The enriched libraries were prepared using Ion Chef™ and sequenced on PI™ Chip in the Ion Proton™ System (Life Technologies) to provide >90% of amplicons covered with at least 20x. Signal processing, base calling, alignment and variant calling were performed on a Proton™ Torrent Server using the Torrent Suite™ Software. Variants were aligned with the reference genome (build 37 of the hg19 genome) and annotated using Ion Reporter™ Software, and pedigree analysis was performed using the Genetic Disease Screen (GDS) trio workflow. Variant filtering and prioritization were performed with an in-house software program and a local database. Candidate variants were visualized using IGV (Integrative Genomics Viewer). Candidate variants were evaluated based on stringent assessments at both the gene and variant levels taking into consideration both the patient's phenotype and the inheritance pattern. Variants were classified following the guidelines of the American College of Medical Genetics and Genomics (ACMG).

Animals

All animal experiments were conducted in accordance with the Duke University Institutional Animal Care and Use Committee guidelines (IACUC Protocol Number: A083-18-04). $AG^{E22\text{-}23}$fl/fl mice, where *ANK3* exons 22 and 23 were flanked by LoxP sites, were previously generated by our lab(1). Introducing Cre recombinase in neurons from these mice knocks out 190kDa, 270kDa and 480kDa isoforms of AnkG.

Plasmids and antibodies

The gAnkG expression vectors were modified from pEGFP-C vector driven by chicken beta-actin promoter (CAG) with the GFP tag fused to the C-end. A linker (QSTVPRARDPPVAT) encoded with (5'CAGTCGACGGTACCGCGGGCCCGGGATCCACCGGTCGCCACC3') was used to replace the stop codon of gAnkG coding sequence and was in-frame with GFP. Cre-

2A-BFP plasmid was driven by CAG promoter in pLenti6-V5-DEST vector (1,2) . Briefly, the sequence encoding the giant exon of rat ankyrin-G was synthesized and subcloned to 270 kDa ankyrin G-GFP plasmid using ClaI and EcoRV sites. Due to the relatively large (~14K) coding sequence of giant AnkG, we therefore generated mutants for 480-kDa ankyrin-G-GFP using cloning based strategy. In detail, wild type giant AnkG-GFP plasmid was digested with restriction enzymes to remove a fragment that contains the target point mutation site. The removed sequence was replaced by 2 fragments generated by PCR using the primers that contains the point mutations sites. Fragment 1 was created by a forward primer that contains a 15bp overlap with the cut vector and a reverse primer that contains the mutated site and 15bp overlap with fragment 2. Fragment 2 was created by a forward primer that contains the mutated site and 15bp overlap with fragment 1 and a reverse primer that contains 15bp overlap with the cut vector. The size of these fragments was around 1K-3K, which is much smaller than the entire coding sequence of gAnkG and could be amplified efficiently. The vector, fragment 1 and fragment 2 were assembled using In-Fusion® clone kit (Takara Bio USA, Inc; Cat # 638920). Primers used to generate giant AnkG mutations are listed in Table S3.

β4-Spectrin-Σ1-Halo and β4-Spectrin-Σ6-Halo plasmids were generated by flipping the mouse B4-Spectrin coding sequencing from B4-Spectrin-S1-HA and B4-Spectrin-S6-HA plasmids (gift from Dr.Matthew Neil Rasband, Baylor College of Medicine) into a vector modified from pEGFP-C vector (gift from Dr. Gray Banker, OHSU), which was driven by CAG promotor and the C-end GFP coding sequence was replaced by halo coding sequence. A linker (ASLEPTTEDLYFQSDNDGS) encoded by (5'GCTAGCCTCGAGCCAACCACTGAGGATCTGTACTTTCAGAGCGATAACGATGGATCC3') was used to replace the stop codon of inserted protein and was in-frame with halo. The vector was cut using AscI/NheI. Forward primers overlapping with AscI cutting site for β4-Spectrin-Σ1-Halo is 5'-CCCAAGCTTGGCGCGCCATGGCACAAGTACCAGGGG-3' and for β4-Spectrin-Σ6-Halo is 5'-CCCAAGCTTGGCGCGCCATGGATCCATGAGAAGATGCTGATGG-3'. The same reverse primer was used for both Σ1 and Σ6: 5'-TTGGCTCGAGGCTAGCCTTCCGGCGCCCG-3'.

β4-Spectrin-∑6-Y1901A-Halo mutation was generated using the same strategy employed with giant AnkG mutations. Primers used for generating 2 fragments are listed in table S3. Target gRNA sequence used to knock out b4-spectrin by crispr-Cas9 is in gene *SPTBN4*: 5'-3' GCAAGACGTAGACCCCGGTG. The gRNA sequence was cloned into a vector that contains the coding sequence of Crispr-Cas9-GFP. The transfected cell will be GFP positive. 3 other gRNA sequence in *SPTBN4* were also used to confirmed the results (5'-3': ACCTAATTTCTTGCGCACAG, GCAAGAAATTAGGTGAGATC, GCTTCGCGGAGCTGGACAAG). The knock out efficiency of β4-spectrin-gRNA was confirmed by immunostaining of transfected neurons with anti- β4-spectrin antibody (2).

AnkG rabbit polyclonal antibody against the C-terminal domain expressed in bacteria was generated by our lab (1:1000 for neuronal immunostaining and PLA) (2). AnkG mouse monoclonal antibody against the spectrin binding domain is from Life Technologies, Inc (Cat#: 33-8800) (1:50 for neuronal immunostaining and PLA). B4-Spectrin rabbit polyclonal antibody was previously generated by our lab (1:1000 for neuronal immunostaining) (2). 186 kDa neurofascin rabbit polyclonal antibody was previously generated by our lab (1:250 for neuronal immunostaining) (4). Pan Voltage gated Sodium channel mouse monoclonal antibody was from Sigma-Aldrich (Cat#: S8809) (1:100 for neuronal immunostaining). Alexa Fluor® 488, 594, 647 secondary antibody anti-mouse or rabbit were from Life Technologies, Inc (1:500 for neuronal immunostaining).

Hippocampal neuron culture, transfection and immunostaining

Primary hippocampal neurons were co-cultured with glia cell feeder layers without antibiotics as described previously (5). Briefly, hippocampi were dissected from PND 0-1 mice, trypsinized, dissociated, and plated onto poly-L-lysine coated 18-mm glass coverslips in pre-conditioned glia feeder dishes with plating media (MEM supplemented with glucose 0.6% wt/vol, hourse serum 10% vol/vol) as ~200,000 neurons/60mm culture dish. 2-3 hours after plating, coverslips containing adhered neurons were flipped into pre-conditioned neuron growth media (Neurobasal-A medium plus 2% vol/vol B27 supplement, 1XGlutMAX®) and maintained at 37°C incubator with a 5% CO_2.

For transfection, at day3, coverslips with neurons growing on them were flipped to glia feeder dishes with pre-conditioned neuronal growth media. Plasmids for Cre-2A-BFP (0.5μg/coverslip) and giant AnkG (0.5μg/coverslip) were mixed with 100μl opti-MEM (Thermo Fisher, Cat# 31985062) at room temperature for 5 minutes. Then the DNA mixture was combined with 3μl Lipofectamine 2000® (Thermo Fisher, Cat# 11668019) and 100μl opti-MEM (Thermo Fisher, Cat# 31985062) for 10 minutes at room temperature. 50μl of the DNA/lipofectamine mix was added gently on each coverslip and incubated for 30 minutes in the incubator. Then coverslips were flipped back to the home glia feeder dishes. Transfected hippocampal neurons were maintained in the incubator until fixation.

On the day of fixation, 1ml of 37°C pre-warmed fixation buffer (4% wt/vol PFA with 4% wt/vol sucrose in PBS) was added on each coverslip and incubated for 10 min at 37 °C before washing with PBS for all conditions except VGSC. For VGSC staining, neurons were fixed with 2% PFA in PBS for 10 mins at 37 °C. Cells expressing constructs with Halo-tag were treated with 50 nM JF-549 ® dye (6) for 10 minutes and washed with PBS for 10 min prior to fixation or imaging.

For immunostaining, fixed neurons were permeabilized with 0.25% TritonX-100 for 5 min, washed with PBS and then blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibodies were diluted in blocking buffer and incubated at 4 °C overnight. On the next day, neurons were washed with PBS and incubated with the appropriate fluorescent secondary antibodies in blocking buffer for 1 hour at room temperature. Finally, neurons were mounted with Prolong® Diamond (Thermo Fisher) onto glass slides and allowed to cure for 24 hours before imaging.

HEK293T cell culture and transfection

HEK293T cells are maintained in DMEM High Glucose medium containing L-glutamine (200 mM), Fetal Bovine Serum (5% v/v), Penicillin (10,000 U/mL) /Streptomycin (10,000 μg/mL) in 37 °C incubator. 1 day before the transfection, HEK293T cells were trypsinized and seeded on 18mm poly-L-lysine pre-coated coverslips in 12 well plates. Cell density should reach 50–80% confluence on the day of transfection. On the day of transfection, 1μg of total constructs was mixed with 50μl opti-MEM (Thermo Fisher,

Cat# 31985062) at room temperature for 5 minutes. Then DNA mixture was combined with 3μl Lipofectamine 2000® (Thermo Fisher, Cat# 11668019) and 50μl opti-MEM (Thermo Fisher, Cat# 31985062) for 10 minutes at room temperature. The 100μl of DNA/lipofectamine mix was added gently in 1 ml medium and incubated in the incubator until further experiment.

HEK293T cell lysis and in-gel western

After 2 days of transfection, HEK293T cells were washed 3 times with cold PBS and collected with pre-warmed lysis buffer [8M urea, 5% SDS (wt/vol), 50mM Tris pH 7.4, 5mM EDTA, 5mM N-ethylmelanamide, protease and phosphatase inhibitors] and heated for 15min at 65 degrees. Cell lysate was mixed with 5x PAGE buffer [5% SDS (wt/vol), 25% sucrose (wt/vol), 50mM Tris pH 8, 5mM EDTA, bromophenol blue] and heated for 15min at 65 degrees. Lysate was centrifuged at 132,000 rpm for 5 mins and the supernatant was loaded on a 3.5-17.5% 0.75mm gradient gel in Fairbanks Running Buffer [40mM Tris pH 7.4, 20mM NaAc, 2mM EDTA, 0.2%SDS (wt/vol)]. Gels for in-gel westerns were immediately fixed in 50% (vol/vol) isopropanol plus 7% (vol/vol) glacial acetic acid for 15min and washed 2x for 10min with deionized water. Gels were incubated with primary antibodies (rabbit anti giant ankyrin-G, 1:2000) in 5% BSA (wt/vol) in TBST overnight at 4 degrees. Gel was washed 3 times with TBST then incubated with secondary antibodies (goat anti-rabbit 800CW, 1:10,000; Licor 926-32211; Lincoln, NE) in 5% BSA (wt/vol) in TBST for 2 hours at room temperature. Gels were extensively washed with TBST for 2 hours at room temperature with 2 final washes in TBS. Gels were imaged on the Licor Odyssey at 0.37mm custom offset.

Imaging and data analysis

Immunofluorescent stained neurons were imaged with confocal microscopy at the duke light microscopy core facility (Zeiss LSM780 or Zeiss LSM880 inverted confocal microscopes; Oberkochen, Germany). Images for AIS intensity quantification used either a 63x NA/1.4 oil objective or a 40x NA/1.4 Zeiss oil objective. For the whole neuron morphology images, a 40x NA/1.3 oil objective was used. All experiments were repeated in 3 independent experiments. The final quantification was generated from 10-15 neurons from one coverslip to minimize the experimental variations. Neurons from the same

experiment were imaged under same microscope settings to ensure reproducibility of intensity measurements. A standard AIS image is a 3 step Z-series image with an interval of 0.25μm in a Z distance. Z-series AIS images were processed with maximum intensity projection function from the Zen black software (Zeiss). Maximum intensity images were used for the AIS intensity quantification.

AIS intensity quantification was adapted from Berger et al. (7). Briefly, maximum intensity projected images were opened by Fiji (https://fiji.sc). To trace the intensity of fluorescence at the AIS, a 6-pixel wide line was manually drawn on the center of the axon from the soma for 100μm down toward the distal axon. Average intensity for the 6-pixel wide line was plotted along the distance of axon. For all of the experiments, AnkG signal was used to identify the AIS except for neurons lacking AIS. For AnkG null neurons, the proximal 100μm of axon, were traced. AIS intensity profiles were exported to spread sheets and processed using a MATLAB script adapted from Berger. Background intensity from an empty region of each image was subtracted from all pixels of that image prior to further analysis. Data are presented as average intensities ± SEM of all traces in each condition.

Giant AnkG purification and phosphorylation detection

Wild type PND60 days mouse brains were dissected and homogenized in 65 °C prewarmed lysis buffer (8M Urea, 5% SDS, 50mM Tris pH 7.4, 5mM sodium EDTA, 50mM sodium Fluoride, 10mM sodium pyrophosphate, 5mM N-ethylmaleimide, 100ug/ml AEBSF, 10ug/ml pepstatin, 10ug/ml leupeptin, 100ug/ml benzamidine, Halt Protease/Phosphatase inhibitor) for about 30 secs on 75% power output of a homogenizer (OMNI-TH01) until tissue are complete homogenized. Brain lysate was incubated at 65 °C for another 15 minutes or until the solution cleared. Urea was removed from the lysate by dialysis using 10-14K tubing in 1L dialysis buffer (50mM Tris pH 7.4, 5mM sodium EDTA, 1% SDS, 1mM sodium azide) for 4 times at least 6 hours each. SDS was reduced by adding KCl to a final concentration 0.1M, and carbonyl-free TritonX-100 was added to 0.1% (v/v). The dialyzed lysate was placed on ice for 1 hour to promote precipitation of potassium dodecylsulfate (KDS). Then, the mixture was centrifuged at 100,000g for 30 min to remove the precipitated KDS. AnkG polypeptides were isolated from the brain

lysate supernatant using anti-AnkG C-terminal domain antibody-coated protein-A/G magnetic beads (Pierce™, Cat# 88802) at 4 °C, rotated. On the next day, beads were washed with binding buffer (2M Urea, 0.1M glycine, 1% Triton X-100) for 3 times. After washing 3 times with 0.1M ammonium bicarbonate, beads were digested on ice for 15 minutes by 1μg/ml heat activated trypsin (Promega, Cat# V5111). Supernatants were collected for Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) to unbiasedly detect the post-translational modifications.

The sample in 50mM ammonium bicarbonate was supplemented with 0.2% Rapigest SF acid-labile surfactant prior to reduction with 10 mM DTT for 30 min at 70°C and alkylation with 25mM iodoacetamide for 45 minutes at RT. 500ng of Trypsin was added and the sample was digested for 18hr at 37°C. Prior to LC-MS, samples were acidified to pH2.5 with formic acid and spun to remove hydrolyzed Rapigest surfactant. The sample was subjected to chromatographic separation on a Waters Acquity UPLC equipped with a Waters HSS T3 1.7μm 75μm I.D. X 15cm reversed-phase column. The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. Following a 3μL injection, peptides were trapped for 3 minutes on a 5 μm Symmetry C_{18} 180μm I.D. X 20 mm column at 5μl/min in 99.9% A. The analytical column was then switched in-line and a linear elution gradient of 5% B to 40% B was performed over 90 min at 400nL/min. The analytical column was connected to a fused silica PicoTip emitter (New Objective, Cambridge, MA) with a 10μm tip orifice and coupled to a Fusion Lumos Orbitrap mass spectrometer (Thermo Fisher) through an electrospray interface operating in a data-dependent mode of acquisition. The instrument was set to acquire a precursor MS scan from *m/z* 375-1675 in the OT (120k res, target AGC 2e5 ions) with MS/MS spectra acquired for the ten most abundant precursor ions in the IT (rapid, target AGC 5e3). For all experiments, HCD energy settings were 30v and a 20s dynamic exclusion was employed for previously fragmented precursor ions.

Raw LC-MS/MS data files were processed in Proteome Discoverer (Thermo Fisher) and then submitted to independent Mascot searches (Matrix Science) against a SwissProt database (*Mouse* taxonomy) with a custom AnkG sequence and containing both forward and reverse entries of each protein. Search tolerances were 5ppm for precursor ions and

0.8Da for product ions using trypsin specificity with up to two missed cleavages. Carbamidomethylation (+57.0214 Da on C) was set as a fixed modification, whereas oxidation (+15.9949 Da on M), and phosphorylation (+79.97 Da on STY) were considered dynamic mass modifications. All searched spectra were imported into Scaffold (v4.3, Proteome Software) and scoring thresholds were set to achieve a peptide false discovery rate of 1% using the PeptideProphet algorithm.

Proximity ligation assay

Protein proximity ligation assay was assessed using the Duolink PLA kit following the manufactory protocol (Sigma-Aldrich, Cat# DUO92101). Briefly, neurons were fixed and permeabilized as descried above. Neurons were blocked with the blocking solution from the Duolink PLA kit for 1 hour at room temperature. Mouse anti-AnkG-N-terminal antibody (1:50) and Rabbit anti-AnkG-C-terminal antibody (1:1000) were mixed and diluted with the antibody dilution solution from the kit. Neurons were incubated with primary antibody mixture overnight at 4°C. The following secondary antibody incubation, ligation and amplification steps were processed exactly as outlined in the commercial manual. Coverslips were mounted in the Duolink® *In Situ* Mounting Medium with DAPI (Sigma-Aldrich, Cat# DUO82040) containing DAPI. The images were collected using a 40x/NA1.3 oil objective on a LSM780 inverted confocal microscope (Zeiss) for both fluorescent PLA images and bright contrast image to visualize the cell morphology. The PLA signal was quantified as number of PLA dots/100μm. All experiments were repeated in 3 independent experiments.

Statistical Analysis and Graphing of Data

Statistical analyses and graph generation were performed using GraphPad Prism 7 (La Jolla, CA) unless otherwise noted. The data are presented as means ± SEMs. Student's t-test was used for comparisons between two groups and a one-way ANOVA with Tukey's post-hoc or Bonferroni pair-wise comparisons test or Dunnett's multiple comparison test were used for comparisons of more than two groups.

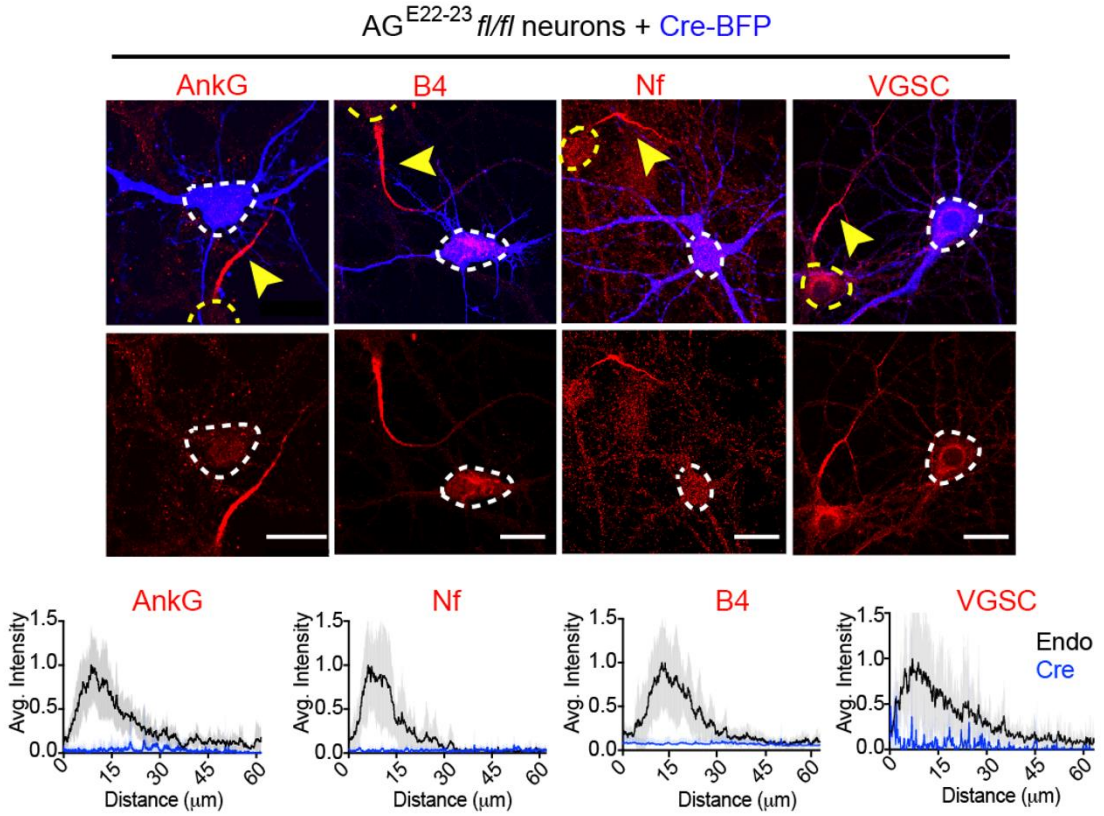

Fig. S1. Impaired accumulation of AnkG AIS binding partners in AG^{E22-23}-fl/fl/Cre-BFP AnkG null neurons. Loss of AnkG and its AIS binding partners accumulation in AnkG null neurons. DIV3 neurons of AG^{E22-23}-fl/fl mice were transfected with a plasmid encoding Cre-BFP and stained for AnkG, β4-spectrin (β4), neurofascin (Nf) or voltage gated sodium channels (VSVG). Representative images of transfected neurons for Cre and indicated antibody staining are displayed. White dash line indicates the cell body of a transfected neuron. Yellow arrow head indicates the AIS of a neighboring non-transfected neuron. Scale bar is 20μm. Average intensity of indicated antibody staining at the AIS was plotted and aligned for transfected neurons (Cre, blue) and non-transfected neurons (endo, black). n=10 and results were repeated in 3 independent experiments.

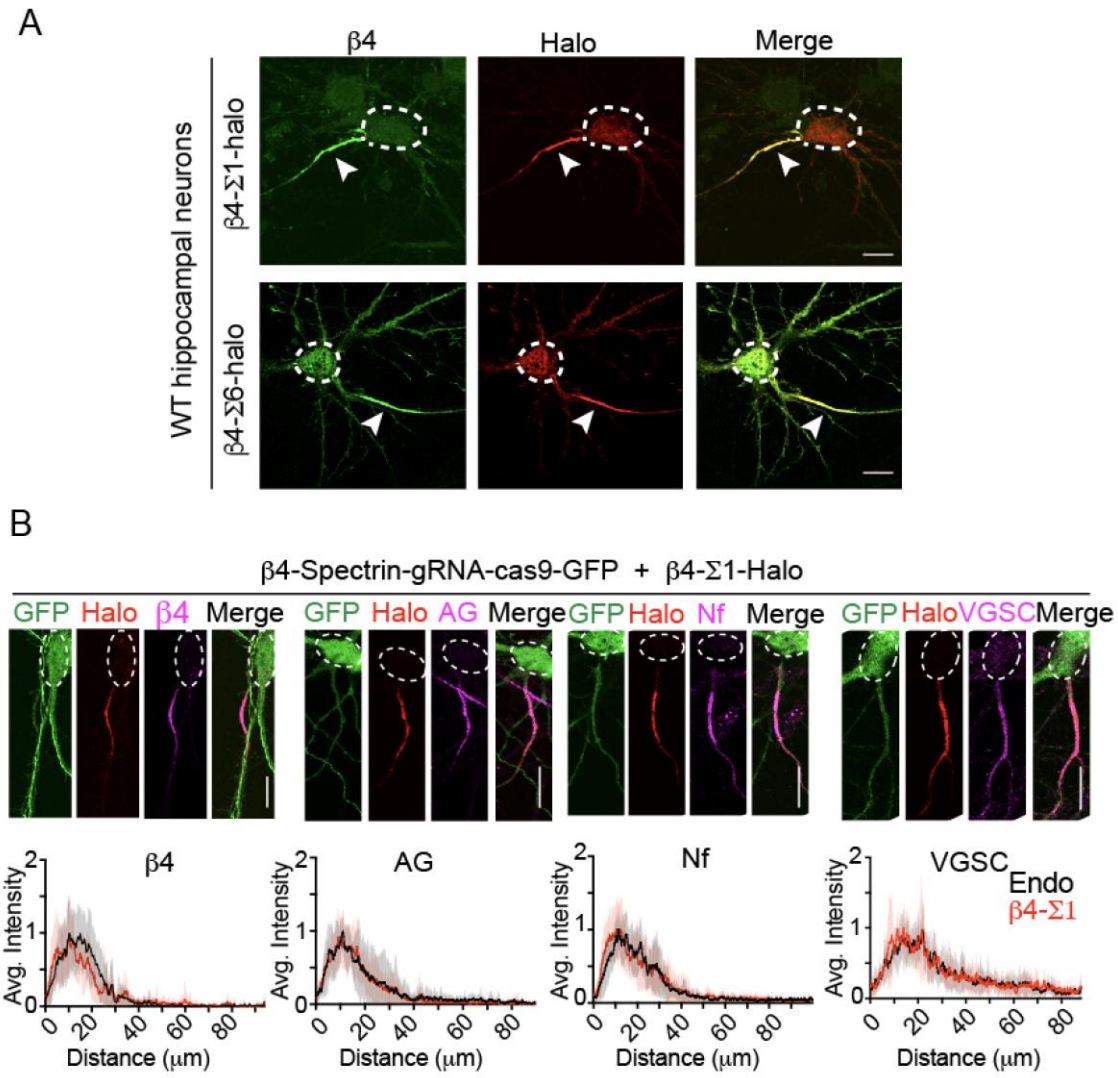

Fig. S2. Loss of β4-spectrin in neurons rescued by β4-spectrin-Σ1 or Σ6. *(A)* Wild type hippocampal neurons were transfected at 3 DIV with plasmids encoding β4-spectrin-Σ1 or Σ6 fused with a halo tag. Neurons were labeled with JF594® halo dye, then stained with β4-spectrin antibody at 7 DIV. Scale bar is 20μm. White circles indicate the soma of transfected neurons. White arrow heads point to the associated AIS of the same cell. *(B)* Hippocampal neurons from AG$^{E22\text{-}23}$-fl/fl mice were co-transfected with crispr-cas9- β4-spectrin gRNA and β4-spectrin-Σ1 cDNA at 3 DIV. Neurons were stained with indicated antibody at 7 DIV. Scale bar is 25μm. Average intensity of indicated antibody at the AIS were plotted and aligned

for transfected neurons (β4-spectrin-Σ1, red line) and non-transfected neurons (endo, black line). n=10 and results were repeated in 3 independent experiments.

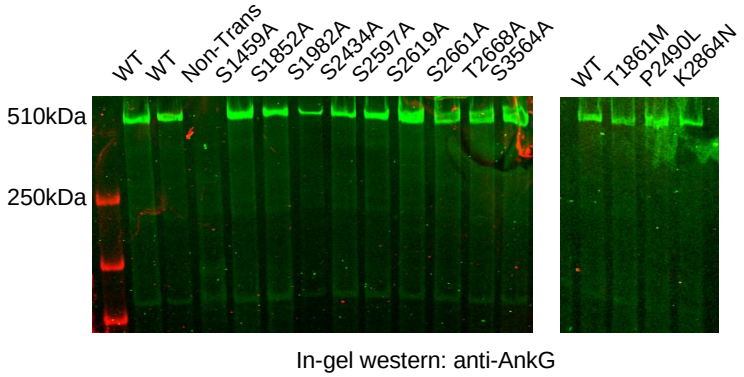

Fig. S3. Phospho-mutants of gAnkG express as full-length polypeptides in HEK cells. HEK 293T cells were transfected with plasmids encoding wild type gAnkG or gAnkG bearing individual serine/threonine to alanine mutations. 2 days post-transfection, cell lysates were collected and immunoblotted with anti-AnkG antibody by in-gel western. Results were repeated in 3 independent experiments.

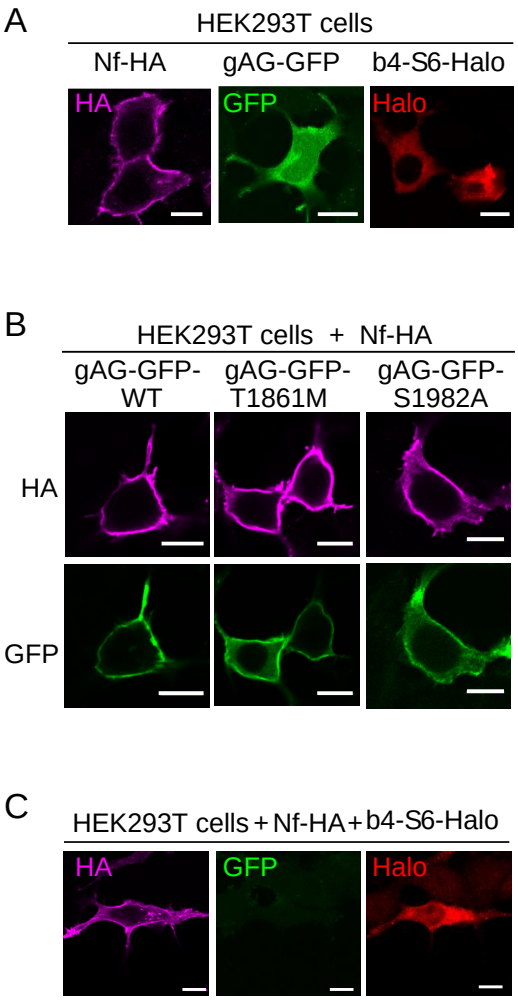

Fig. S4. HEK193T cell membrane recruitment of T1861M and S1982A gAnkG by neurofascin. *(A)* HEK293T cells were transfected with plasmids encoding neurofascin-HA, gAnkG-GFP and β4-spectrin-Σ6-Halo respectively. *(B)* HEK293T cells were co-transfected with neurofascin-HA and wild type gAnkG or gAnkG mutants. *(C)* HEK293T cells were co-transfected with cDNA of neurofascin-HA and β4-spectrin-Σ6-Halo. 1-2 days post transfection, cells were labeled with

JF594® halo dye, then stained with antibody against neurofascin or AnkG. Scale bar is 10μm. Results were repeated in 3 independent experiments.

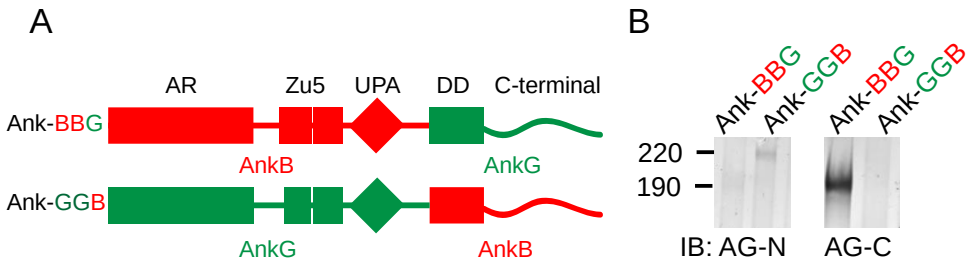

Fig.S5. Evaluating the specificity of antibodies against N- or C-terminal domains of AnkG. *(A)* Schematic representation of the AnkB/G chimeric proteins. Red, AnkB counterparts; green, AnkG counterparts. *(B)* HEK 293T cells were transfected with AnkB/G chimeric cDNAs. 2 days post transfection, cell lysates were collected and immunoblotted with anti-AnkG-N-or C- terminal antibody by in-gel western.

Table. S1. Clinical Features of Individuals with giant ANK3 mutations

Compound heterozygous Individual	c.5582C>T (p. Thr1861Met)	Het	c.5582C>T (p.Thr1861Met)	Het
	c.7469C>T (p.Pro2490Leu)	Het	c.8592G>T (p.Lys2864Asn)	Het
Age/Sex	5years / Male		6 years / Female	
Full scale IQ	73		59	
Brain MRI	normal		White matter abnormality*	
Speech delay	+		+	
Autism like feature	+		+	
Developmental delay	NA		+	
Seizure	NA		+	
Cognitive impairment	NA		+	
Other symptoms			Ataxia, Cerebellar atrophy, Cerebral atrophy, a rod-cone retinal dystrophy, small stature	

* Symmetric, mild to moderate generalized cerebellar atrophy with developmental hypoplasia of the cerebellar vermis; thinning of the corpus callosum; delayed myelination.

Table. S2. Phosphorylated peptides sequence of gAnkG in the mouse brain.

Phosphorylated Residue (aa Human)	Amino Acid Sequence (*= phosphorylated)	% Abundance**
Serine 1459	(R)RQ**S***FASLALR(K)	28
Serine 1852	(K)SAAALL**S***PIKTLTTETRPQPHFNR(T)	12
Serine 1982	(K)SDKGH**S***PEDDWTEFSSEEIR(E)	30
Serine 2406***	(R)VNTPG**S***QEEDSRPSSAQLLSDDSYK(T)	8
Serine 2434	(K)LLSQH**S***VEYHDDELSDLRGESYR(F)	25
Serine 2597	(K)LNDELQ**S***PEK(K)	20
Serine 2619	(K)DYSSQSSTSS**S***PEKVLTELLASNDEWVK(A)	50
Serine 2661	(R)SN**S***PENRVPTQQSEDDQPPEEAKR(T)	67
Threonine 2668	(R)SNSPENRVP**T***QQSEDDQPPEEAKR(T)	17
Serine 3564	(R)TPTDESTPT**S***EPNPFPFHEGK(M)	12

** Abundance= (number of phosphorylated peptides/number of total peptides) ×100

***This site is 2417 in the mouse sequence (2). S2417A mutation results in loss of beta-4 spectrin recruitment (2).

Table. S3. Primers used for generating point mutation constructs.

Giant AnkG Mutagenesis		
	Forward Primer (5’-3’)	**Reverse Primer (5’-3’)**
EcoRI cut site	TTTTGGCAAAGAATTCATGGC	
EcoRV cut site		TTTTTGACTGGAATTCGAGAC
BstZI cut site	CATCGATAATCACAGTGCCA	
SpeI cut site		ATGGGAAGGGGTTAGGCTCA
S1459A	ACGCCAGGCCTTCACATCCCTAGCC	GTGAAGGCCTGGCGTCTGTCTGCCTTC
S1852A	TTTGCTGGCGCCCATTAAAAC	ATGGGCGCCAGCAAAGCCGC
T1861M-Hu	ATTGACTATGGAGACACGTCCTCAG	GTCTCCATAGTCAATGTTTTAATGGGC
S1982A	GGACACGCTCCAGAAGATGACTG	TTCTGGAGCGTGTCCTTTGTCACTC
S2434A	AGTCAGCACGCCGTAGAGTAC	ACTCTACGGCGTGCTGACTC
P2490L-Hu	AGGACCCCTTAGCTCAGAGTTA	GAGCTAAGGGGTCCTGCATG
S2597A	CTGCAGGCCCCGGAGAAAAAG	CTCCGGGGCCTGCAGTTCGTC
S2619A	AGCAGTGCCCCCGAGAAAGTG	CTCGGGGGCACTGCTGGTG
S2661A	TCCAGCGCCCCCGAGAAGAGGG	CTCGGGGGCGCTGGACCCACTG
T2668A	GGTCCCTGCCCAACAGATTGAG	TGTTGGGCAGGGACCCTCTTC
K2864N-Hu	ACAATAACGCTCAGAAAGAACAACTC	TCTGAGCGTTATTGTTATTGGCCC
S3564A	CATCGATAATCACAGTGCCA	GGTTAGGCTCACTAGTTGGGGTAGCTTCG
B4-Spectrin-S6-YA Mutagenesis		
AscI cut site	CCCAAGCTTGGCGCGCCATGGATCCATGAGAAGATGCTGATGG	
NheI cut site		TTGGCTCGAGGCTAGCCTTCCGGCGCCCG
Y1901A	CGGACGGTGGCCGCTGGCGAG	GGCCACCGTCCGCAGCTG

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