Supporting Information

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SI Methods

Gene Targeting. A bacterial artificial chromosome (BAC) containing the mouse AKAP150 genomic region was obtained from Children's Hospital Oakland Research Institute (clone RPCI-24). An AKAP150 targeting construct was engineered using bacterial recombineering. Both 5′ and 3′ loxP recombination sequences were inserted, again by bacterial recombineering, in addition to a neomycin resistance cassette 3′ of the AKAP150 exon. Electroporation of embryonic stem (ES) cells, blastocyst injections, and generation of chimeric and agouti mice were performed at the University of Cincinnati Mouse Transgenic Core according to established protocols. Correctly targeted ES cells were identified by PCR using primers that annealed inside and outside of the targeted genomic DNA. Chimeric offspring were bred with C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) to establish germ-line transmission, and colony expansion. The FLPeR, B6.FVB-Tg(EIIa-cre) C5379Lmgd/J (Cre deleter), and C57BL/6 mouse strains were purchased from The Jackson Laboratory.

Immunocytochemistry. DIV13–15 neurons were fixed in 4% paraformaldehyde in PBS, pH 7.4, for 20 min and permeabilized in 0.1% Triton X-100 PBS for 10 min before staining. Antibodies used for immunofluorescence were: rabbit polyclonal antibody (VO88) to AKAP150, mouse monoclonal antibody to PKA_C (BD Biosciences PharMingen), Alexa Fluor 488 goat-anti-rabbit IgG and AlexaFluor 568 goat-anti-mouse IgG (Invitrogen). Stained cells were mounted in Prolong Antifade reagent (Invitrogen) and visualized on a Bio-Rad MRC1024 laser-scanning confocal microscope. Representative images are from a merged z-series.

Modifications to Electrophysiology Experiments. Signals were sampled at 2 kHz, filtered at 1 kHz, and acquired using pClamp software (version 9, Axon Instruments). Recording of AMPA currents in hippocampal neurons was performed as described using the following solutions (10). Patch pipettes (2–4 MΩ) were filled with intracellular solution containing 140 mM Cs methanesulfonate, 5 mM ATP, 3 mM BAPTA, 5 mM $MgCl_2$, 10 mM Hepes (pH 7.3). 10 μM PKI was added in the indicated experiments. The external solution consisted of 140 mM NaCl, 3 mM KCl, 0.1 mM $CaCl_2$, 11 mM glucose, 10 mM Hepes (pH7.4), 100 μM cyclothiazide, 10 μM bicuculline, 10 μM ZD7288 and 1 μM TTX.

M Current Recording in SCG Neurons: The perforated patch method was used as described in ref. 11. Amplitudes of the M-currents were measured as deactivating currents during 1-s test pulses to −50 mV from a holding potential of −30 mV.

Adult mice (>3 months) were perfused with 4% paraformaldehyde, and brains dissected. Coronal and saggital cryosections (20 μl thickness) were prepared and stained with rabbit polyclonal antibody (VO88) to AKAP150 and Alexa Fluor 568 goat-anti-rabbit IgG (Invitrogen). After antibody staining, brain slices were briefly stained with NeuroTrace 500 nm Fluorescent Nissl stain (Invitrogen). Stained slices were visualized on a Bio-Rad MRC1024 laser-scanning confocal microscope.

Recording in Hippocampal Slices. Adult male mice (8–16 weeks old) were anesthetized with pentobarbital (60 mg/kg, i.p.) and decapitated. Brains were removed within 1 min of decapitation and immediately submerged in ice-cold, sucrose-modified artificial Cerebral Spinal Fluid (ACSF) for hippocampal dissection and slicing: 110 mM sucrose, 60 mM NaCl, 2.5 mM KCl, 28 mM $NaHCO_3$, 1.25 mM NaH_3PO_4, 0.5 mM $CaCl_2$, 7 mM $MgCl_2$, 5 mM glucose, and 0.6 mM sodium ascorbate, pH 7.4 at 4°C (presaturated by bubbling with 95% O_2/5% CO_2 at 21–22°C). Hippocampal slices (400 μm, transverse) were prepared using a vibratome and an agar backing (2%), and each slice was transferred to warm ACSF: 125 mM NaCl, 2.5 mM KCl, 22.6 mM $NaHCO_3$, 1.25 mM NaH_3PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 11.1 mM glucose, pH 7.4 that was continuously saturated with 95% O_2/5% CO_2, for 30 min at 37°C. Slices from the dorsal and ventral thirds of the hippocampus were discarded. After recovery, the holding chamber was equilibrated at 21–22°C, and slices were held for 2–8 h before recording. For measurements of synaptic strength, submerged hippocampal slices were suspended in pairs on nylon mesh in a small (350–400 μl) recording chamber and perfused with ACSF at a rate of 2.5–3 ml/min. The temperature in the recording chamber was raised and held at 32°C for the duration of the experiment. Synaptic responses were evoked by Schaffer collateral stimulation using a bipolar tungsten electrode (tip spacing, 140 μm; Frederick Haer Company) and a 100-μs square wave test pulse (typically 30–40 μA) delivered at 60-s intervals. The stimulation intensity was adjusted to produce a basal response of 1.2–1.4 mV. Recordings were made with ACSF-filled glass micropipettes (2–4 MΩ) placed in the stratum radiatum area of CA1 and connected via head stages to an A-M Systems (Carlsborg, WA) model 1800 amplifier. Signals were digitized at 100 kHz using Axon Instruments Digidata 1200 series interface running Clampex 8.0, and the initial slope (linear portion of the first millisecond) of the fEPSPs was analyzed with Clampfit 8.0 software. LTD was induced with low frequency stimulation (900 pulses delivered at 1 Hz). The first data point after LTD induction was taken after an interval of 20 s.

Behavioral Analysis. For behavioral analysis (10 mice for each genotype unless otherwise stated) were littermates from heterozygous mating pairs that had been backcrossed for at least 2 generations (indicate age and sex of mice). Data are expressed as mean ± SEM. Differences among means were evaluated by ANOVA using genotype as between-subjects factors, followed by Tukey–Kramer or Dunnett's post hoc tests using Prism and SPSS software. The null hypothesis was rejected at the 0.05 level for all analyses.

Pilocarpine-Induced Seizures. Age and sex-matched (8–10 months) mice were weighed (31–43 g) and injected IP with scopolamine (1 mg/kg) in 50–70 μl of saline 30 min before pilocarpine injection (300 mg/kg in 200 μl of saline; Sigma). Behavior was monitored for 1 h by two observers (genotype blind). Progression through the five stages of status epilepticus was evaluated visually.

Histology and Spine Density Analysis. Brains from three adult wild-type or AKAP150 null mice were prepared according to the Golgi procedure as described (1: Glaser, E.M., van der Loos, H. Analysis of thick brain section by obverse-reverse computer microscopy: Application of a new, high clarity Golgi-Nissl stain. *J Neuros Meth, 5, 117.* 1981 and 2: Soderling, S. H., Guire, E. S., Kaech, S., White, J., Zhang, F., Schutz, K., Langeberg, L. K., Banker, G., Raber, J., and Scott, J. D., A WAVE-1 and WRP signaling complex regulates spine density, synaptic plasticity, and memory, *J Neurosci, 27*, 355, 2007). Briefly mouse brains were impregnated with stain for 14 d, and 100 μm sections were cut using a cryostat and mounted on cover slips with Cytoseal 60. Image collection and analysis was done using the Leica AS MDW imaging system and the Leica DEBLUR software.

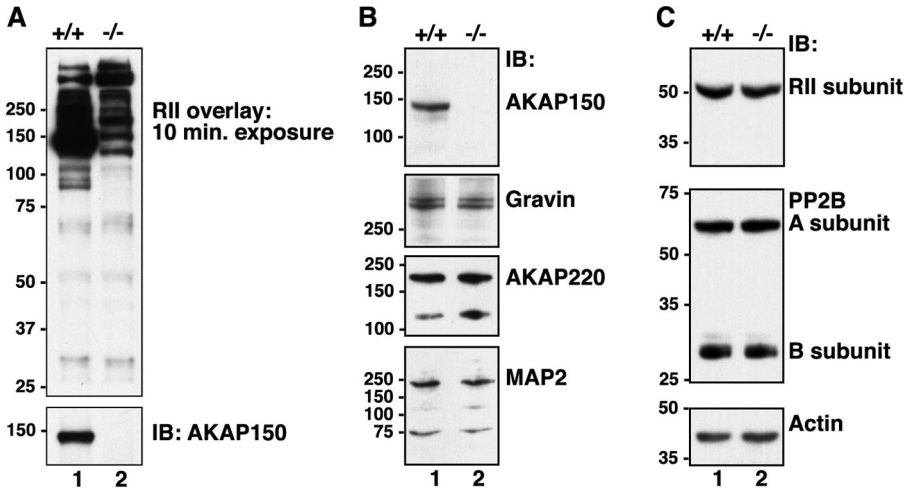

Fig. S1. Representatative RII-overlay and Western blot analyses of AKAPs and AKAP-binding proteins in AKAP150 wild-type and knockout brains. *A)* Autoradiograph showing extended exposure (10 min) of ^{32}P-RII overlay (top). Other brain AKAPs are evident at this exposure time. Western blot of AKAP150 from the same wild-type and AKAP150 null mice whole brains extracts. (*B*) Western blot analyses of AKAP150 wild-type and null whole brain extracts show no compensatory increase in other brain AKAPs such as gravin, AKAP220 and MAP2. (*C*) Western blot detection shows equivalent expression of PKA RII subunit and PP2B subunits in wild-type and AKAP150 null mice brain extracts. Western blot detection of actin serves as a loading control.

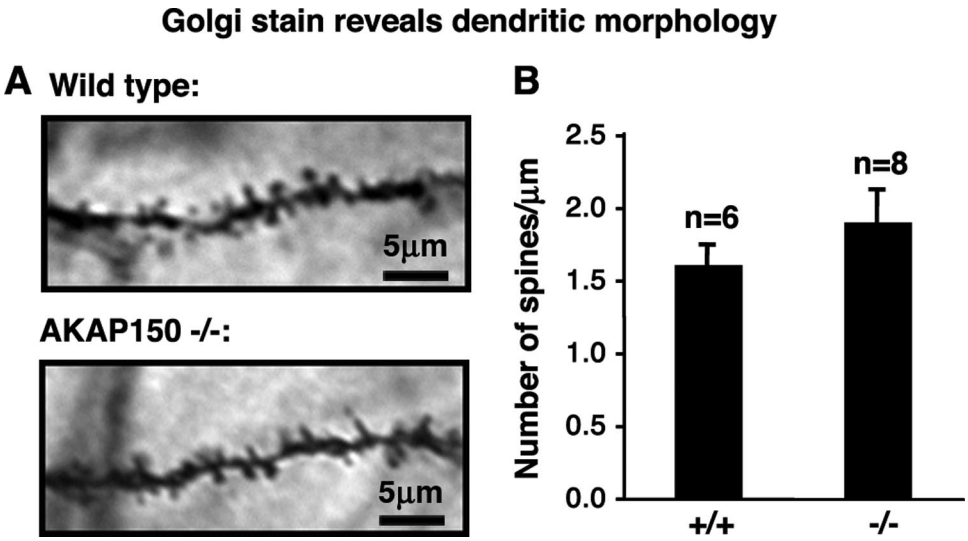

Fig. S2. Golgi procedure staining of whole brain *in situ* reveals dendritic morphology. *A)* Dendritic spine morphology is hippocampal slices remains intact in AKAP150 null mice (bottom) and indistinguishable from that of wild-type littermates (top). (*B*) Quantification of spine density (number of spines/μm) in dendrites from AKAP150 wild-type and knockout slices. N equals neurites examined and image analysis was done using Leica DEBLUR software (v2.1.5).

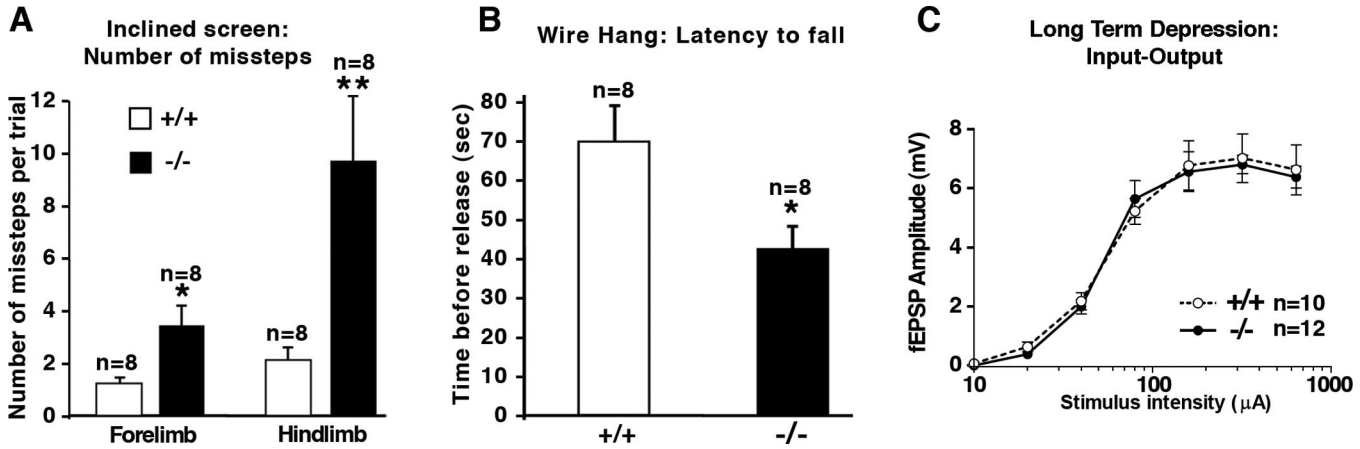

Fig. S3. Cerebellar associated behavior. *A)* Inclined screen test for coordination by measuring frequency of missed steps while climbing an inclined screen. Missteps by AKA150 −/− (black bars) and +/+ (white bars) mice are shown for forelimbs (*Left*, *, $P < 0.02$) and hindlimbs (right, **, $P < 0.008$). Error bars indicate S.E.M. (*B*) Wire hang tests strength by measuring the sec. +/+ (white bar) and AKAP150 −/− mice (black bar, *, $P < 0.03$) can hang a horizontal wire before releasing. Error bars indicate S.E.M.