Supplemental Information

Supplemental Figure Legends

Figure S1. HCN1 is not targeted to distal dendritic regions in dissociated hippocampal cultures. Related to Figure 1.

(A) Example of a dissociated hippocampal PN at DIV28 stained for HCN1 (A_1), MAP2 (A_2), and the overlay (A_3) of HCN1 (green) and MAP2 (magenta). Scale bar = 50 µm.

(B) Mean HCN1 intensity decreases as a function of scaled dendritic distance from the soma (n = 12).

(C) Mean MAP2 shows a similar decrease in staining intensity as a function of scaled dendritic distance from the soma.

(D) The ratio of HCN1 to MAP2 staining does not change as a function of scaled dendritic distance from the soma.

(E and F) Examples of CA1 region of hippocampal organotypic cultures stained for HCN1 and MAP2 grown without (E) or with EC attached (F). Scale bar = 100 µm.

(G) Mean HCN1 intensity (in AU +/- SEM) of CA1 region in somatic (S), proximal (P), or distal (D) dendritic regions in cultures grown without and with EC (n = 7,9, unpaired t-test, $p > 0.05$).

(H). Mean MAP2 intensity in cultures grown with EC or without EC ($p > 0.05$).

Figure S2. Deletion of NGL-1 does not alter HCN1 distal enrichment. Related to Figure 1.

(A) HCN1 staining in coronal hippocampal slices from a wild-type littermate mouse (A_1) and an NGL-1 knockout mouse (A_2). Scale bar = 200 µm.

(B) Mean HCN1 staining intensity (AUs) in CA1 region of wild-type and NGL-1 knockout mice in *SP, SR*, and *SLM* (n = 3,3, $p > 0.05$).

Figure S3. Reeler mice show a loss of distal dendritic enrichment of HCN1 and GIRK1. Related to Figure 1.

(A) HCN1 staining in hippocampi from wild type (WT)(A_1) and reeler mice (A_2). Scale bar = 200 µm for all images.

(B) GIRK1 staining intensity in WT (B_1) and reeler mice (B_2).

(C) MAP2 staining intensity in WT (C_1) and reeler mice (C_2).

(D) Mean HCN1 intensity in CA1 region of WT and reeler mice (n = 4,4, in *SLM* p = 0.021).

(E) Mean GIRK1 intensity in CA1 region of in WT and reeler mice (in *SP* p = 0.025; in *SLM* p = 0.045).

(F) Mean MAP2 intensity in CA1 region of in WT and reeler mice (p > 0.05).

Figure S4 rAAV Cre-GFP expression in Dab$^{f/f}$ mice leads to a loss of Dab1 protein. Related to Figure 1.

(A) Hippocampal slices from hemisphere contralateral (A_1) and ipsilateral (A_2) to site of injection of rAAV Cre-GFP. Cre-GFP+ neurons are observed only in the injected hemisphere, not contralaterally. Scale bar = 200 µm for all images.

(B) Dab1 intensity was greatest in *SLM* in the contralateral control hippocampus (B_1). Dab1 staining intensity was reduced in the injected hippocampus (shown above) only (B_2).

(C) Mean Dab1 intensity in *SP, SR*, and *SLM*. Dab1 intensity in injected hippocampi was significantly reduced in all regions compared with control (in *SP* p = 0.013; in *SR* p = 0.019; in *SLM* p = 0.005).

(D) Western blot of four hippocampi expressing increasing amounts of Cre-GFP. Lane 1 shows the western blot of an un-injected hippocampus. Increasing amounts of GFP were detected from hippocampi shown in lanes 2, 3, 4 were associated with decreasing levels of active SFK signal.

Bands of the predicted molecular weights were detected by antibodies and are shown for Dab1, total SFK, and kinase-active SFK.

Figure S5. Dab1 knockdown reduces distal enrichment of TRIP8b. Related to Figure 2.

A) GFP signal (A_1) and TRIP8b staining (A_2) in the hippocampus contralateral to site of injection. Scale bar = 50 µm for all images.

(B) GFP signal (B_1) and TRIP8b staining (B_2) in the Dab1 knockdown hippocampus.

(C) Mean TRIP8b intensity was significantly reduced in Dab1 knockdown hippocampi compared with contralateral hippocampi (in *SP* p = 0.031; in *SR* p = 0.108; in *SLM* p = 0.023).

Figure S6. Dab1 knockdown does not alter structure of CA1 PN dendrites. Related to Figure 3 and Extended Experimental Procedures.

(A) Biocytin fills of Cre-GFP- (A_1) and Cre-GFP+ (A_2) CA1 PNs. Scale bar = 50 µm.

(B) Sholl analysis showing number of intersections of raw traces of the apical dendrites as a function of scaled distance from the soma of Cre-GFP- and Cre-GFP+ neurons (n = 6,4).

(C) Mean total dendritc length (C_1), distal dendritic (terminal 100 µm) length (C_2), total dendritic volume (C_3), distal dendritic volume (C_4), total surface area (C_5), and distal surface area (C_6) of Cre-GFP- and Cre-GFP+ neurons.

(D) Viral overexpression mCherry (D_1) did not alter HCN1 intensity (D_2).

(E) HCN1 staining as a function of distance from the soma along the apical dendrites of a wild-type mouse (E_1) and a mCherry overexpression mouse (E_2).

Figure S7. Dab1 knockdown increases input resistance by reduction of GIRK1-mediated currents in distal dendrites of CA1 PNs. Related to Figure 3.

(A) Whole cell current-clamp voltage recordings from distal dendrites of Cre-GFP- (A_1) and Cre-GFP+ (A_2) CA1 PNs before (black traces) and after application of 200 μM barium (red traces). Voltage responses are shown to a series of 500 ms hyperpolarizing and depolarizing current pulses. Scale bars = 50 mV, 0.1 sec.

(B) Mean Rin (MOhms) measured in distal dendrites of Cre-GFP- and Cre-GFP+ neurons before and after application of barium. Barium significantly increased Rin in Cre-GFP- neurons (paired t-test, $p < 0.007$, $n = 6$), but not in Cre-GFP+ neurons ($n = 4$). In the absence of barium, Rin was significantly greater in Cre-GFP+ neurons than in Cre-GFP- neurons (unpaired t-test, $p = 0.0172$).

(C) Mean series resistance ($R_{s,}$ MOhm) of Cre-GFP- and Cre-GFP+ neurons was measured at the beginning of whole cell somatic and dendritic recordings, and monitored throughout the experiment.

(D) Mean AP threshold (mV) of Cre-GFP- and Cre-GFP+ neurons for the soma and the distal dendrites.

(E) Mean amplitude (mV) of the depolarization of the first AP peak of Cre-GFP- and Cre-GFP+ neurons for the soma and the distal dendrites from -70mV resting membrane potential.

Extended Experimental Procedures

Antibodies

The following antibodies were used in this study. When two antibodies for the same protein are listed the second antibody was used for verification:

Antibody	Company	Catalog #	Dilution
HCN1	SantaCruz	Sc-19706	1:200
HCN1	NeuroMab	73-110	1:50
TRIP8b (17.3)	NeuroMab	73-244	1:200

MAP2	AbCam	Ab5392	1:2000 – 1:5,000
Dab1	Rockland	400-555	1:1000
GIRK1	AbCam	Ab80970	1:200
GIRK1	Alomone	APC-005	1:200
GluR1	Millipore	Ab1504	1:200
Netrin G ligand1	AbCam	Ab31983	1:200
Total SRC	Invitrogen	AHO1152	1:200
Total SFK	Abcam	Ab47405	1:200
"Active" phospho SRC family	Cell Signaling	2101	1:200
GFP	Invitrogen	A11122	1:1000

Viruses

Adeno-associated viruses were purchased from the Penn Vector Core in the School of Medicine Gene Therapy Program at the University of Pennsylvania. rAAV2/1 CMV.GFP.CRE virus was used to drive expression of Cre-GFP under control of the CMV promoter. rAAV2/1 CAG.mCHERRY virus was used to drive expression of mCherry under control of the CAG promoter.

Western blots

Western blots were performed as previously described (Santoro et al., 2009). Brain tissue was homogenized in ice-cold homogenization buffer (10 mM Tris-HCl (pH7.4), 320 mM sucrose, 5 mM EDTA, with protease inhibitor cocktail (CompleteMini, Roche)) and centrifuged for 5 min at 3000 x g. The supernatant was collected and centrifuged for 30 min at 12,000 x g. The pellet was resuspended in homogenization buffer with 150 mM NaCl and 1% Triton added. Protein concentration was determined using Micro BCA reagent (Pierce).

Animals

All mouse lines were maintained in standard conditions in accordance with guidelines established by the National Institute of Health and by the Institutional Animal Care and Use Committee. C57B/6 wild-type mice were obtained from the Jackson Laboratory. *Reeler* knockout mice and wild-type littermates were purchased from the Jackson Laboratory (stock number 000235). $Dab1^{f/f}$ mice were generously provided by Gabriella D'Arcangelo at Rutgers University and Ulrich Mueller at the Scripps Research Institute. NGL1 knockout mice were a gift from Shigeyoshi Itohara at the Riken Institute.

Organotypic culture preparation

Organotypic cultures were prepared from neonatal Sprague Dawley rats (Charles River) at postnatal day 5 to 7 as previously described (Gogolla et al., 2006)**.** In brief, rat pups were decapitated, and the brain was rapidly removed and placed in ice-cold Hank's Buffered Salt Solution (HBSS) for 4 minutes. The hippocampi were dissected out and chopped along the transverse axis into 400 µM sections with a McIlwain tissue chopper (Stoelting). Sections were gently transferred with a paintbrush to organotypic culture inserts (Millipore) in High Serum Media (50% DMEM with Glutamax, 25% Heat-inactivated Donor Equine Serum, 25% HBSS, 10mM HEPES). Media was exchanged every 2 to 3 days with fresh High Serum Media.

All experimental treatments in organotypic cultures began on day *in vitro* (DIV) 8 and were terminated after 48 hours on DIV 10. The following drugs were used: PP2 (5 µM, Tocris), PP3 (5 µM, Tocris), GST-RAP (50 µg/mL, Enzo Scientific), and GST-GABARAP (50 µg/mL, Enzo Scientific).

Upon completion of the experiment, cultures were washed briefly in PBS and fixed for 1hr in 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS), and immunohistochemistry was performed.

Imaging

Because HCN1 staining intensity varies in different regions of the hippocampus and the neocortex along the rostral-caudal axis, we also compared staining intensity within individual coronal slices that span both hemispheres, thereby minimizing location-dependent variability in HCN1 expression. Square ROIs were placed in *SP*, the middle of *SR*, and *SLM* and average pixel intensity was measured. For organotypic cultures, the border of *SR*/*SLM* was not clearly defined. We therefore do not use the labels, *SP, SR*, and *SLM*, but instead refer to somatic (S), proximal (P), and distal (D). Distal is defined as with 50 µm of the fissure. Proximal is defined as the midpoint between S and D.

Adult Viral Injections

Adult mice were anesthetized with inhaled isoflourane and virus was delivered to the hippocampus by stereotactic injection. A small, ~7-mm incision was made in the scalp of the animal with medical scissors to allow access to the skull. The exposed region was swabbed with betadine and a small <1-mm hole in the skull was drilled with a sterile dentist's drill. Borosilicate glass pipettes were used to deliver 0.1-1 µl of high titer virus. The pipettes were inserted through the hole in the skull using the stereotactic manipulator to a position corresponding to the mouse's hippocampal CA1 region. After injection, the pipette was removed and the scalp wound was sutured and disinfected with Betadine. The mouse was given an IP injection of buprenorphine 0.05-0.1 mg/kg as an analgesic.

Electrophysiology Solutions

Recordings were performed using standard artificial cerebrospinal fluid (ACSF) for the extracellular solution. ACSF consisted of (in mM): NaCl (125), $NaHCO_3$ (25), KCl (2.5), NaH_2PO_4 (1.25), $MgCl_2$ (1), $CaCl_2$ (2), glucose (22.5), Na-pyruvate (3), ascorbate (1). Hippocampal slices were prepared and incubated in sucrose-enriched modified ACSF containing (in mM): NaCl (10), NaH_2PO_4 (1.2), KCl (2.5), $NaHCO_3$ (25), glucose (25), $CaCl_2$ (0.5), $MgCl_2$ (7), sucrose (190), pyruvate (2). The ACSF had a pH of 7.3, osmolarity of 305-320

mOsm and was saturated with 95% O_2 and 5% CO_2. The intracellular patch pipette solution contained (in mM): $KMeSO_4$ (135), KCl (5), NaCl (2), EGTA (0.2), HEPES (10), phosphocreatineNa_2 (10), MgATP (5), Na_2GTP (0.4), Alexa Fluor 594 cadaverine (0.1) and Biocytin (0.2%).

All experiments were performed in the presence of the $GABA_A$ receptor antagonist SR95531 (2 μM) and the $GABA_B$ receptor antagonist CGP55845 (1 μM) (obtained from Tocris). In a subset of experiments, the HCN channel blocker ZD7288 (10 μM) was applied to the bath 10-15 mins after the start of the recordings. In other experiments 200 μM $BaCl_2$ (Sigma-Aldrich) was applied to the bath to block GIRK channels.

Somatic and Dendritic Electrophysiology

Horizontal hippocampal slices were prepared on a Leica VT1200S and incubated at 34°C for approximately 30 mins in sucrose-enriched ACSF. Slices were then transferred to a recording chamber beneath an upright microscope (Olympus, BX51) and perfused with fresh ACSF at 34°C. Hippocampal CA1 pyramidal neurons were visualized with Dodt gradient contrast optics (Luigs & Neumann) or differential interference contrast optics, using an IR filter (Schott Glass), and 40x/60x 0.8 NA water immersion objectives, coupled to a PCO Sensicam or Hamamatsu ORCA-R2 CCD camera using ImageJ *μ*-Manager (Edelstein *et al.* 2010). Fluorescence-guided, targeted patch clamp recordings were performed for the soma. As GFP labeling was restricted to the soma, dendrites were intracelullarly filled with Alexa 594 and biocytin and identified as being GFP+ or GFP- at the end of the experiment. Fluoresence was visualized using a metal-halide lamp epifluorescence illumination system (X-cite 120Q, EXFO/Lumen Dynamics) and ET-GFP and mCherry filter sets (Chroma).

Patch clamp recordings were performed using borosilicate glass pipettes (Sutter) with tip resistances of 2-5 MΩ for somatic and 9-16 MΩ for dendritic recordings. Pipette capacitance (Cp), Series resistance (Rs) and whole cell capacitance (Cm) were compensated under voltage clamp initially with maximal allowable prediction and correction (95% for soma and 75%. for

dendrites). These values were used as a guide to estimate the pipette capacitance compensation and bridge balance under current clamp. For somatic recordings, the mean series resistance was 7.66 ± 0.33 MΩ (SEM) for GFP- neurons and 8.41 ± 0.52 MΩ for GFP+ neurons (n=29,17); the series resistance voltage drop was compensated 100% through the bridge circuit. For dendritic recordings the average series resistance was 31.30 ± 2.511 MΩ for GFP- neurons and 34.67 ± 2.4 MΩ for GFP+ neurons (n=7, 10); the majority of the series resistance was compensated with the bridge circuit. The maximal voltage error during the largest current injection step (-200 to -250 pA) in individual experiments used to hyperpolarize the membrane to voltages between -140 mV and -160 mV was less than 5 mV (under the conservative assumption of only 50% bridge compensation). Series resistance compensation was monitored throughout the course of the experiment using a small step current. Cells for which series resistance increased by ≥ 15% during the course of experiment were excluded from the analysis.

Voltage sag resulting from activation of Ih was elicited by injecting hyperpolarizing current into the cells. The amplitude of the current step was adjusted so that each cell reached a chosen value of peak hyperpolarization (Vmin = –110 to –120 mV) to assure uniform activation of Ih during the hyperpolarization in different cells and under different conditions. Synaptic responses were evoked by electrical stimulation of the perforant path (PP) or Schaffer collaterals (SC), using focal glass pipette stimulating electrodes coupled to constant current stimulators (WPI). Stimulus strengths were adjusted so that PP and SC PSPs were less than 50% of their maximal amplitude, and basal transmission was monitored every 15 s with PP and SC stimuli spaced 2 s apart**.** Individual and paired stimuli (5 pulses at 20 Hz and 50 Hz) were administered at subthreshold (~40 μA) and suprathreshold (~80 μA) current strengths for measuring PSP summation and for evoking dendritic spikes, respectively. Sag and summation measurements were performed by holding the soma or dendrite at a RMP of -68 mV via current injection through the patch pipette.

Data Analysis

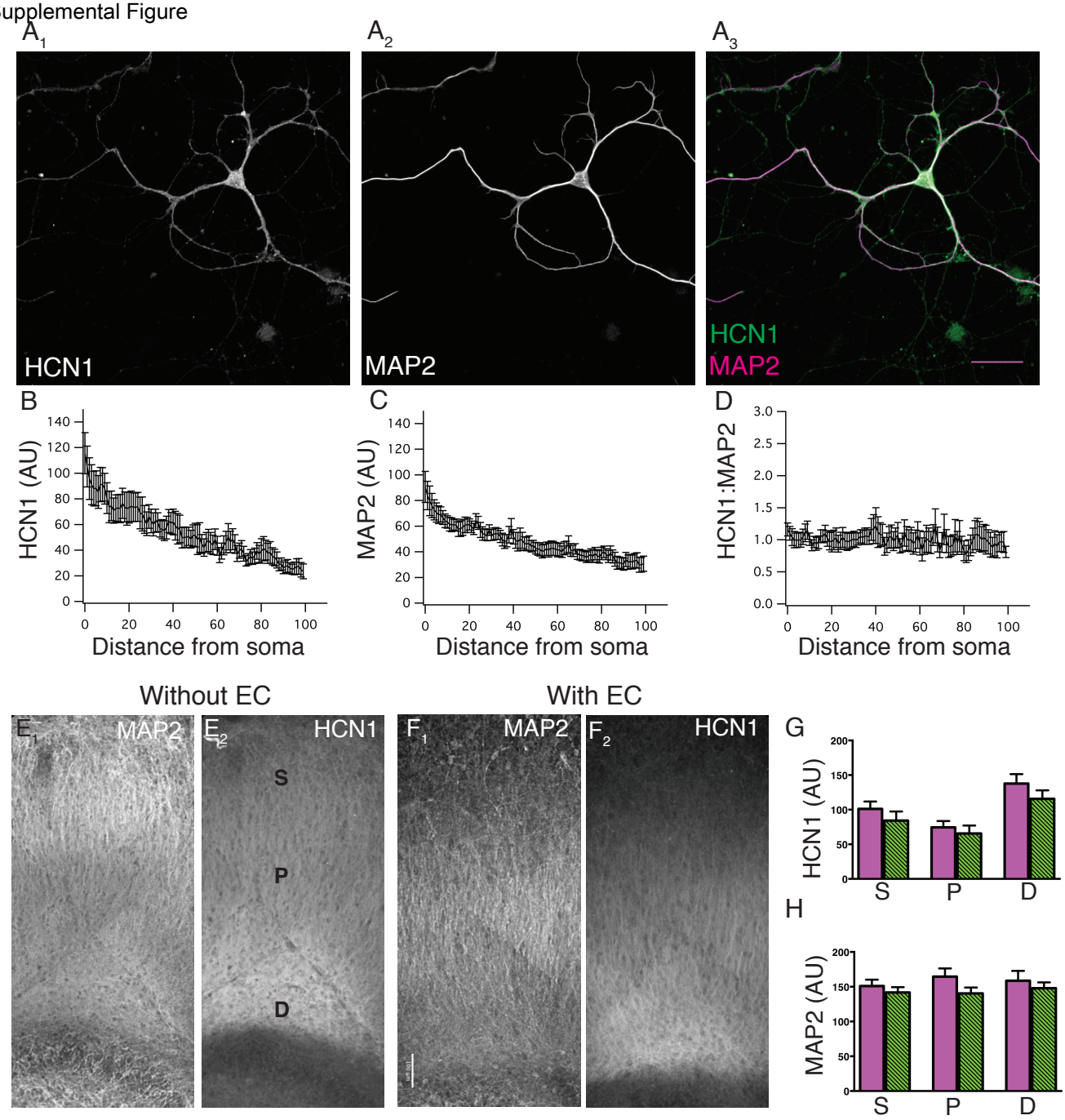
Axograph X and Matlab (Mathworks) were used for electrophysiology data analysis. Kaleidagraph (Synergy) and Prism (Graphpad) were used for plotting data and statistical analysis. The sag ratio was measured as (1 – ΔVss/ΔVmin) x 100%, where ΔVss is the steady-state hyperpolarization (relative to resting/membrane holding potential) at the end of the hyperpolarizing pulse and ΔVmin is the peak hyperpolarization near the beginning of the current step. To measure summation, for each recording the peak EPSP amplitude of the fifth EPSP was normalized by that of the first EPSP after baseline subtraction. Individual and mean average summation ratios are plotted.

***Post-hoc* Identification and Reconstruction of Recorded Neurons**

In addition to online visualization of recorded cells with Alexa Fluor 594 (Invitrogen), all cells were filled with 0.2% biocytin to allow for enhanced visualization with a streptavidin-bound fluorophore (Streptavidin Alexa Fluor 555, Invitrogen) during somatic and dendritic patch clamp recordings. Immediately after recording from either an acute brain slice or an organotypic culture, the tissue was placed into a 4% formaldehyde solution, overnight at 4°C. The tissue was then thoroughly rinsed (3×15 min) with phosphate-buffered saline and processed for immunohistochemistry.

Slices were processed using strepavidin-555 (Invitrogen) and imaged on the Zeiss LSM700 microscope before reconstructions were done with the NeuroLucida system (MicroBrightField). Apical dendrite length, volume, and surface and Sholl analyses were measured for both total apical dendrite and the most distal 100 uM of the apical dendrite were measured.

Supplemental Figure



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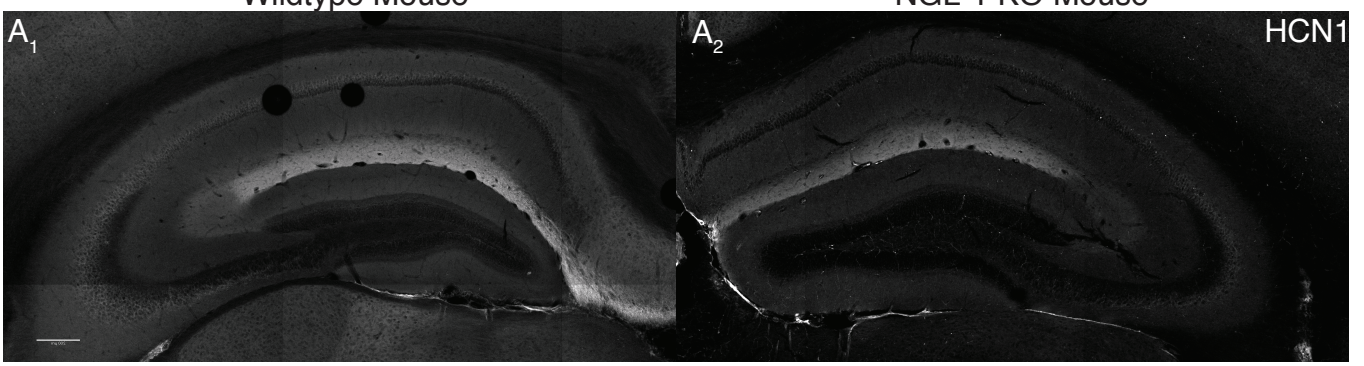

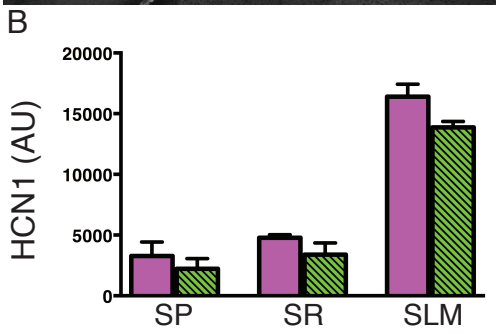

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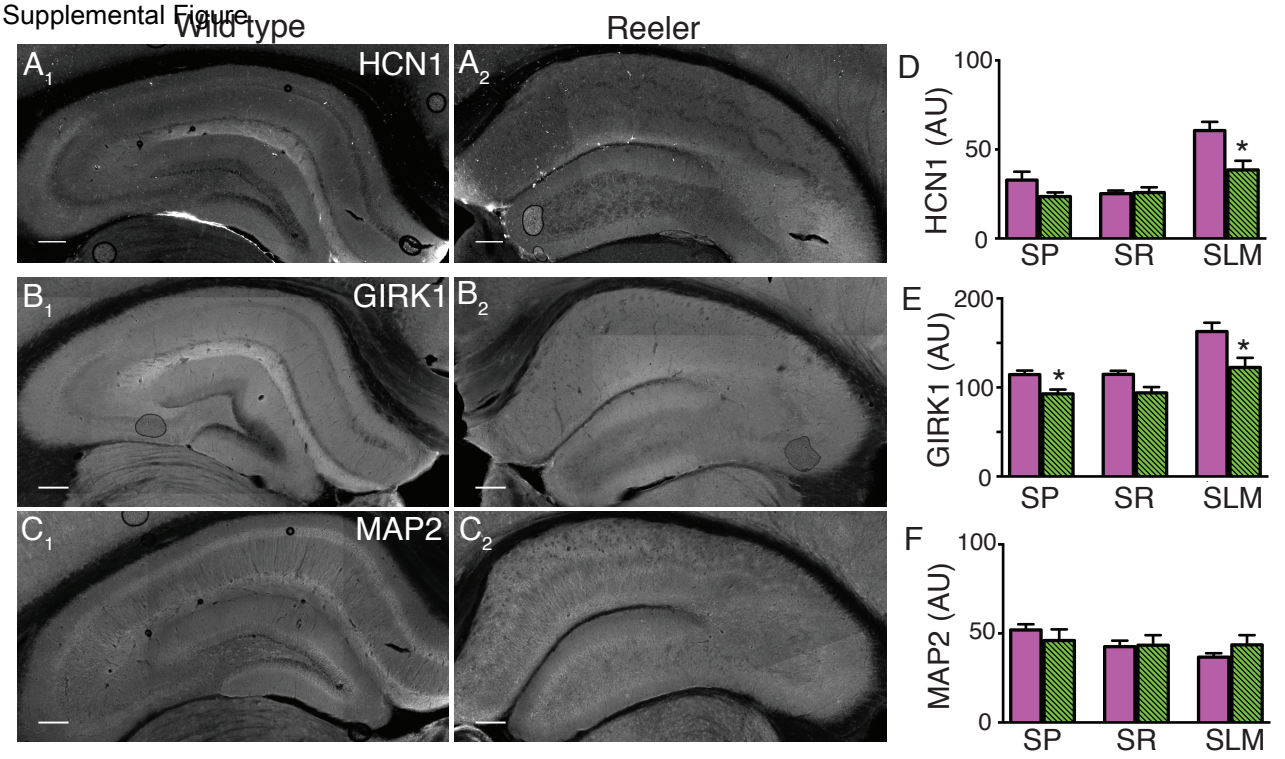

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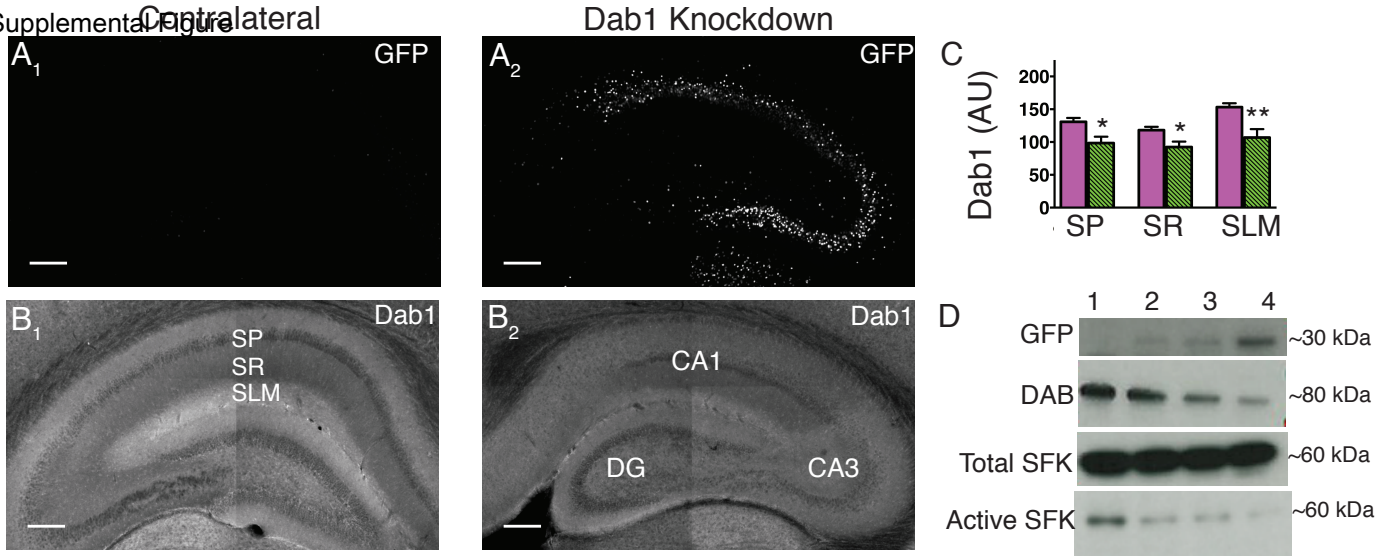

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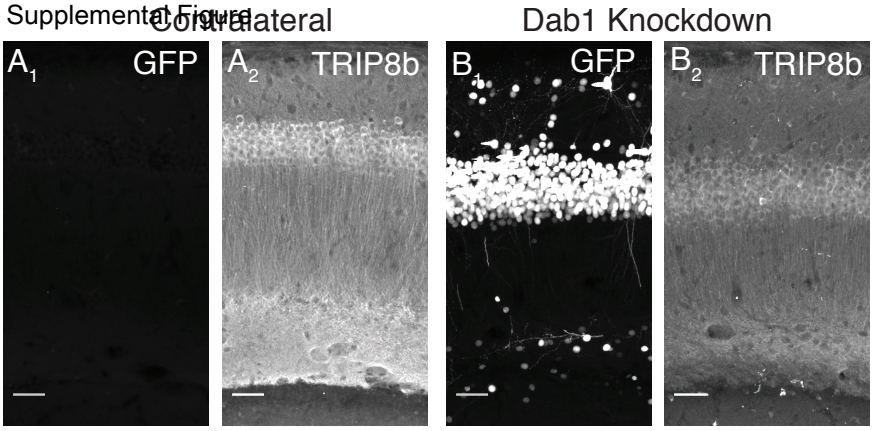

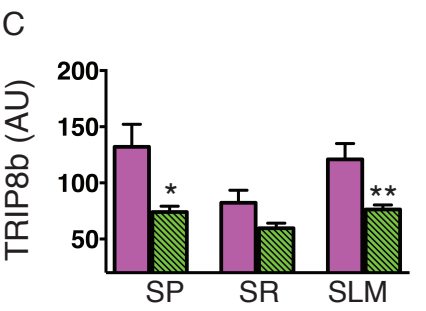

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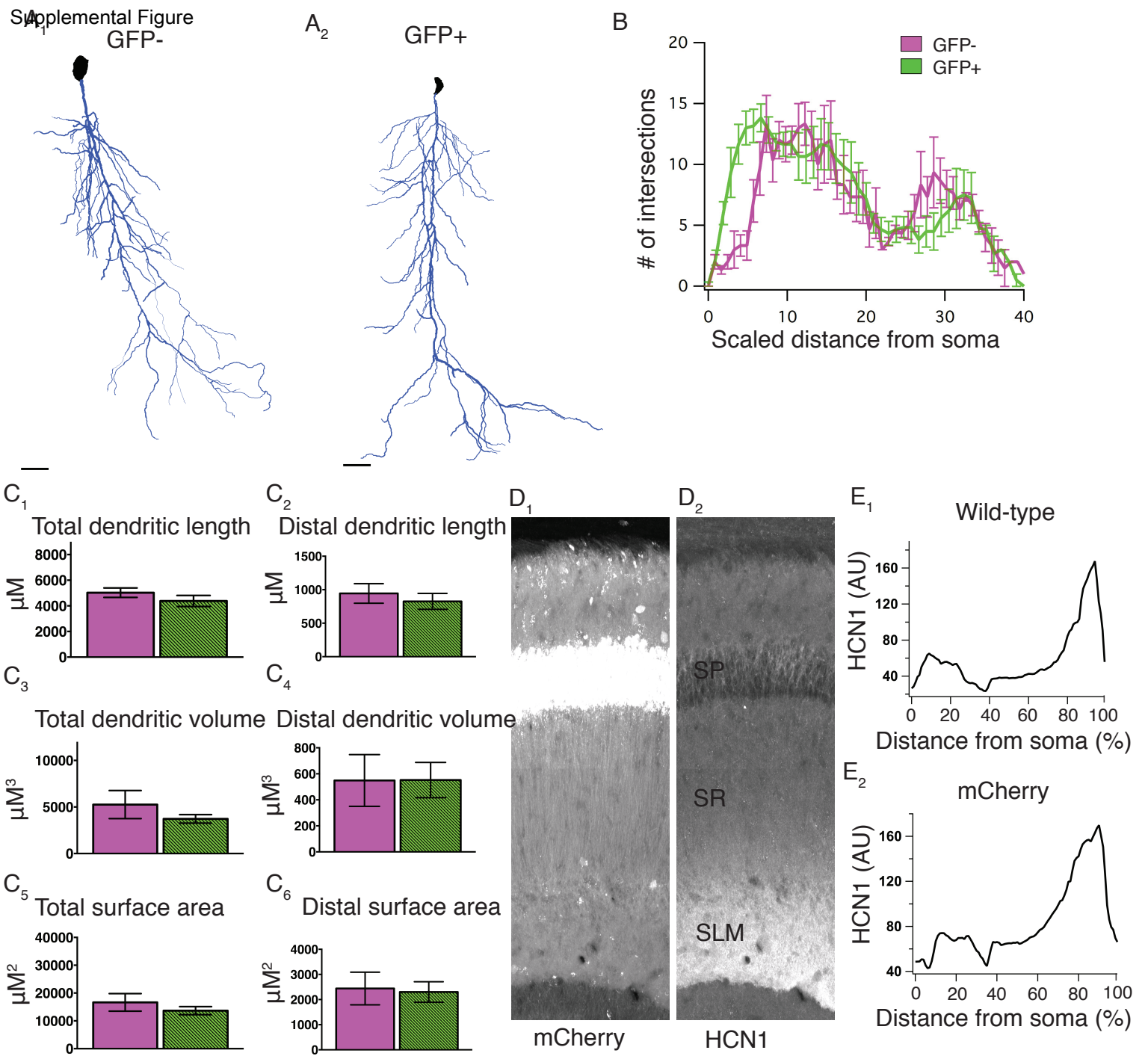

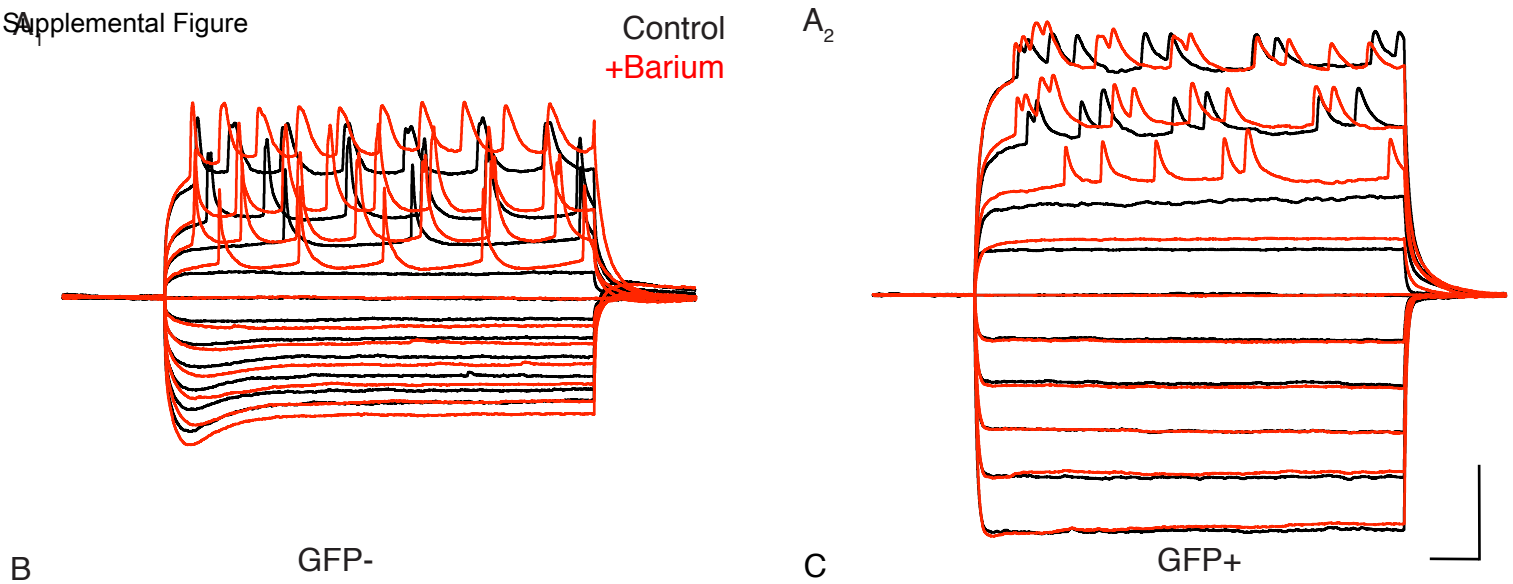
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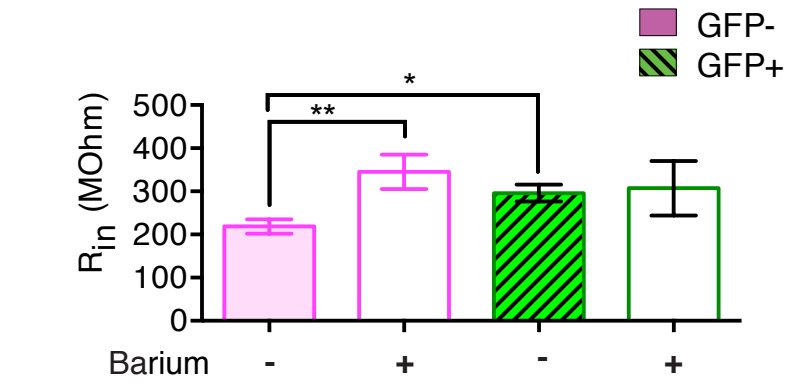
Control
+Barium
A2
GFP-
GFP+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GFP+
Rin (MOhm)
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300
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100
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Barium
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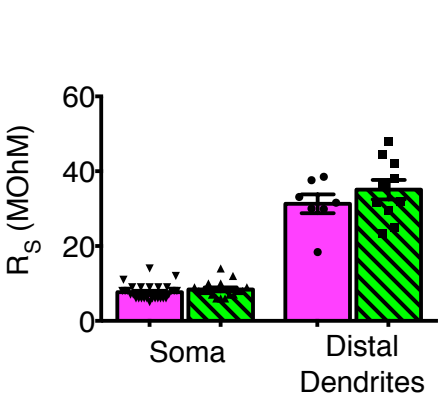
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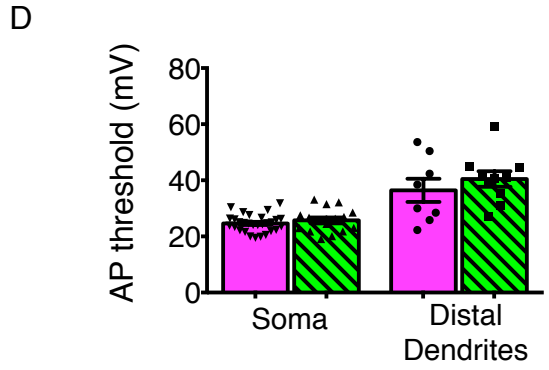
Soma
Distal Dendrites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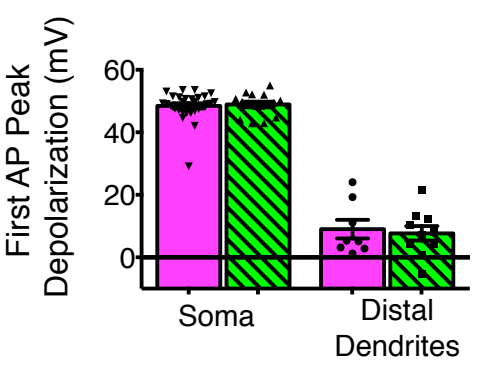
Soma
Distal Dendrites


E
First AP Peak Depolarization (mV)
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40
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Distal Dendrites