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Supplemental information

Tau reduction affects excitatory and inhibitory neurons differently, reduces excitation/inhibition ratios, and counteracts network hypersynchrony

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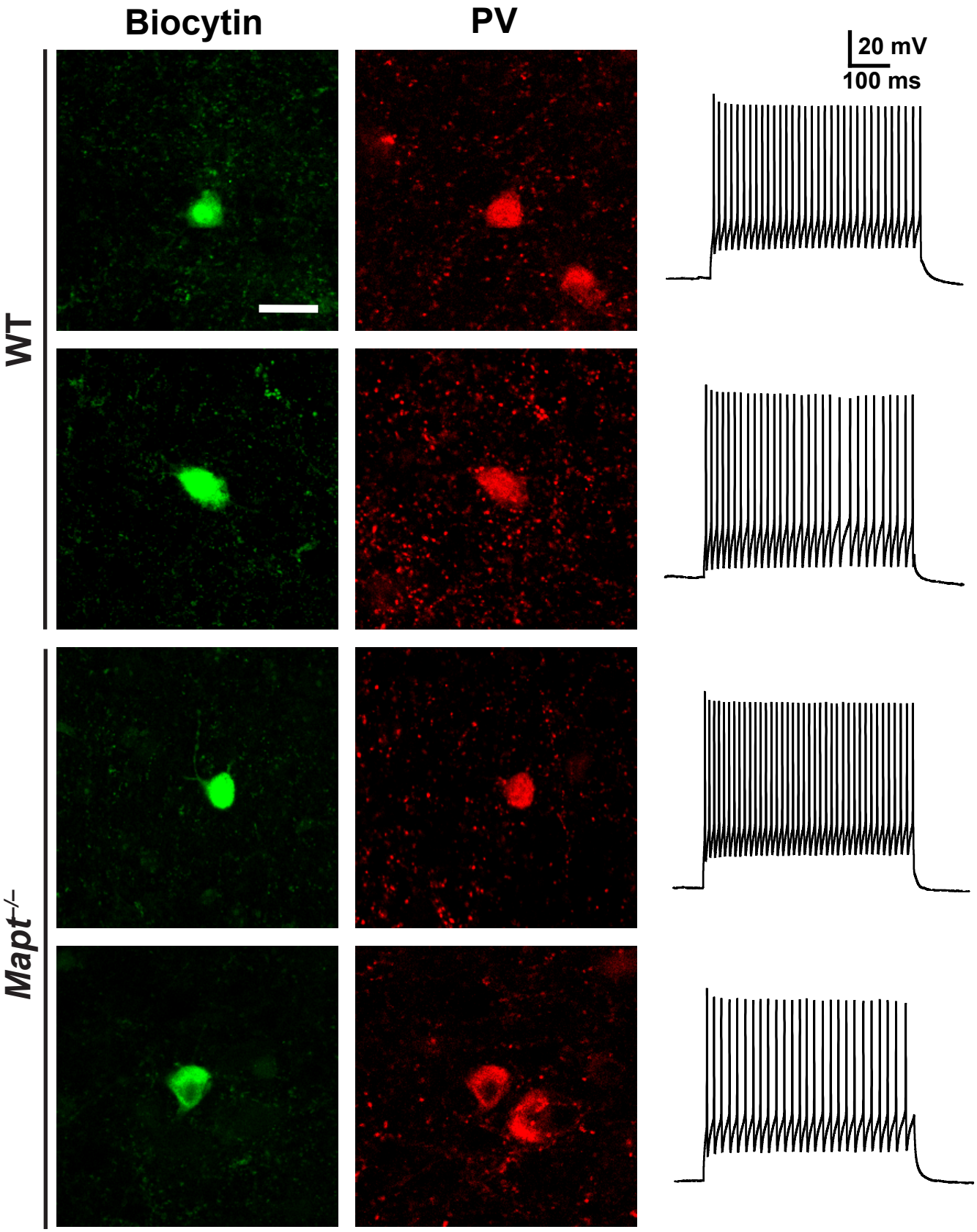

Figure S1 (Related to Figure 2). Neurons in layer 5 of the somatosensory cortex exhibit fast-spiking firing patterns and express PV

Neurons in layer 5 of the somatosensory cortex in acute cortical slices from 24–28-day-old WT and *Mapt*$^{-/-}$ mice were patched to record their AP firing patterns, filled with biocytin, and stained for biocytin and PV . Recordings and labelings of four representative PV cells are shown. Scale bar: 15 µm.

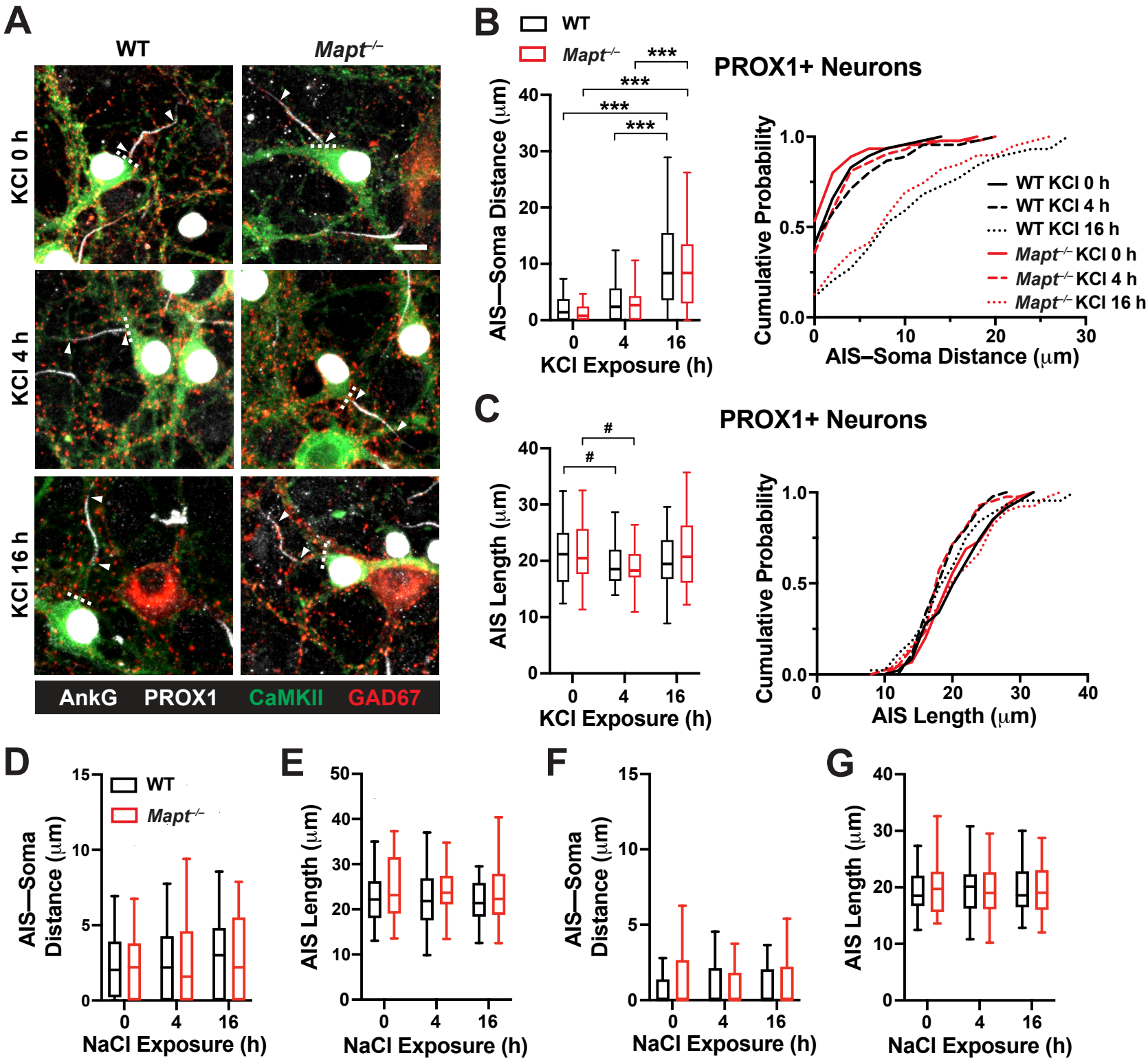
A
WT
Mapt−/−
KCl 0 h
KCl 4 h
KCl 16 h
AnkG
PROX1
CaMKII
GAD67
B
WT
Mapt−/−
***
***
***
***
PROX1+ Neurons
AIS—Soma Distance (μm)
KCl Exposure (h)
Cumulative Probability
AIS–Soma Distance (μm)
WT KCl 0 h
WT KCl 4 h
WT KCl 16 h
Mapt−/− KCl 0 h
Mapt−/− KCl 4 h
Mapt−/− KCl 16 h
C
#
#
PROX1+ Neurons
AIS Length (μm)
KCl Exposure (h)
Cumulative Probability
AIS Length (μm)
D
WT
Mapt−/−
AIS—Soma Distance (μm)
NaCl Exposure (h)
E
AIS Length (μm)
NaCl Exposure (h)
F
AIS—Soma Distance (μm)
NaCl Exposure (h)
G
AIS Length (μm)
NaCl Exposure (h)

Figure S2 (Related to Figure 3). Tau ablation does not alter AIS responses of excitatory neurons to depolarization of primary cultures with KCl, and NaCl treatment does not alter AISs of excitatory or inhibitory neurons

(A–C) At DIV13–14, primary hippocampal neuronal cultures from WT and *Mapt*$^{-/-}$ mice were or were not exposed to 15 mM KCl (10 mM added plus 5 mM in medium) for 4 or 16 h, fixed, immunostained for markers of the AIS (AnkG, white), excitatory cells (CaMKII, cytoplasmic, green), including granule cells (PROX1, nuclear, white), and interneurons (GAD67, cytoplasmic, red), and analyzed by confocal microscopy.

(A) Representative images depicting AISs of granule cells. Arrowheads mark the start and end of AISs, and dashed lines the soma boundary. Scale bar: 20 μm.

(B and C) Quantitations of the AIS–soma distance (B) and AIS length (C) in granule cells. Cumulative probability curves binned at 2 μm are shown on the right.

(D–G) At DIV13–14, primary hippocampal neuronal cultures from WT and *Mapt*$^{-/-}$ mice were or were not treated with NaCl (10 mM) for 4 or 16 h, fixed, immunostained for markers of the AIS (AnkG), excitatory cells (CaMKII), granule cells (PROX1), and interneurons (GAD67), and analyzed by confocal microscopy.

(D and E) Quantitations of the AIS–soma distance (D) and AIS length (E) in interneurons.

(F and G) Quantitations of the AIS–soma distance (F) and AIS length (G) in granule cells.

n = 39–47 AISs of PROX1-positive neurons (from 2–3 mice) per genotype and treatment; 6–27 AISs were analyzed per well and 1–2 wells per mouse (B, C). *n* = 23–33 AISs of GAD67-positive neurons (D, E) and 28–42 AISs of PROX1-positive neurons (F, G) (from 3–4 mice) per genotype and treatment; 4–13 AISs were analyzed per well and 1 well per mouse.

$^{\#}p < 0.2$, ***$p < 0.001$ by two-way ANOVA with Holm-Sidak test (B, C). No significant differences were identified between genotypes and treatments by permutation test with Holm-Sidak correction (D, F) or two-way ANOVA with Holm-Sidak correction (E, G).

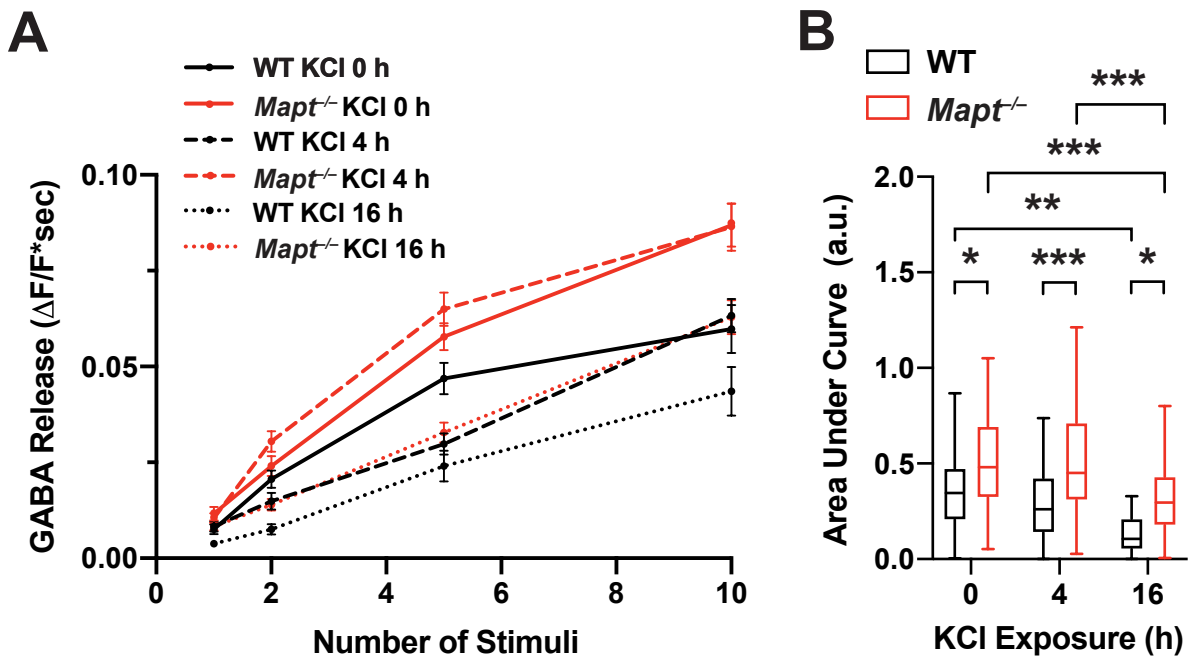

Figure S3 (Related to Figure 4). Tau ablation maintains input-output responses of inhibitory neurons after stimulation of neuronal cultures

(A and B) Primary hippocampal neuronal cultures from WT and *Mapt*$^{-/-}$ mice were transduced on DIV4 to express iGABASnFR. At DIV13–14, they were or were not exposed to 15 mM KCl (10 mM added plus 5 mM in medium) for 4 or 16 h, followed by incubation in conditioned medium (without KCl addition) for 1 h. Fluorescence changes indicating GABA release were then triggered by increasing numbers of electrical field stimuli (1 ms, 90 mA, 100 Hz) and monitored by fluorescence microscopy.

(A) GABA release (area of fluorescence change) plotted as a function of the number of stimuli (input-output curves).

(B) Quantitations of areas under input-output curves.

n = 64–84 cells (from 5 mice) per genotype and treatment; 7–29 cells were analyzed per coverslip and 1–2 coverslips per mouse.

*p < 0.05, **p < 0.01, ***p < 0.001 by permutation test with Holm-Sidak correction (B). Table S4 provides p values for all comparisons. Values in (A) are means ± SEM.

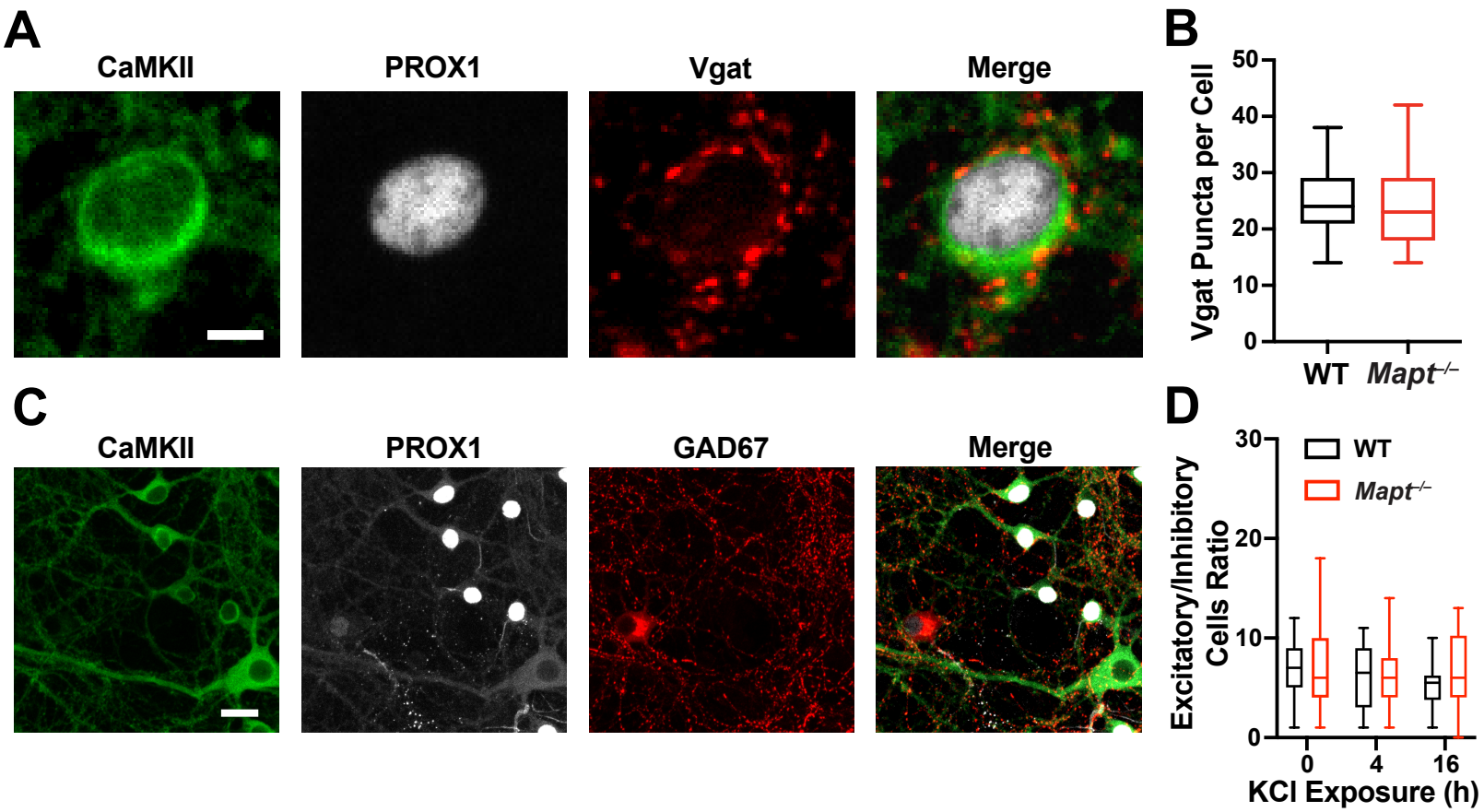

Figure S4 (Related to Figure 4). Similar numbers of inhibitory synapses on excitatory neurons and comparable ratios of excitatory to inhibitory neurons in neuronal cultures from WT and *Mapt*$^{-/-}$ mice

(A) Primary hippocampal neuronal cultures from P0–1 WT and *Mapt*$^{-/-}$ mice were immunostained for markers of excitatory cells (CaMKII, cytoplasmic, green), including granule cells (PROX1, nuclear, white), and inhibitory synapses (Vgat, puncta, red), and analyzed by confocal microscopy. Scale bar: 5 μm.

(B) Number of Vgat puncta on PROX1-positive cells.

(C) Primary hippocampal neuronal cultures from WT and Mapt–/– mice were or were not treated at DIV13–14 with KCl (15 mM) for 4 or 16 h, fixed, immunostained for markers of excitatory cells (CaMKII, cytoplasmic, green), including granule cells (PROX1, nuclear, white), and interneurons (GAD67, cytoplasmic, red), and analyzed by confocal microscopy. Scale bar: 20 μm.

(D) Quantitations of ratios of CaMKII-positive to GAD67-positive cells. For this analysis, fields were chosen that contained both excitatory and inhibitory neurons. Many other fields contained only excitatory neurons (data not shown).

n = 47 cells (from 4 mice) per genotype; 10–13 cells were analyzed per well and 1 well per mouse (B); n = 28–42 fields (from 3 mice) per genotype and treatment; 9–15 fields (0.02 mm^2 each) were analyzed per well and 1 well per mouse (D). No significant differences were identified between genotypes by paired, two-tailed Student's t test in (B) or between genotypes and treatments by two-way ANOVA and Holm-Sidak test in (D).

Table S1 (Related to Figure 1). Additional properties of APs and PSCs in PCs

	Characteristics of single AP	
	Amplitude (pA)	**FWHM (ms)**
WT	93.1 ± 0.97	1.615 ± 0.03
***Mapt*$^{-/-}$**	93.31 ± 1.00	1.554 ± 0.05
	sEPSC	
	Amplitude (pA)	**FWHM (ms)**
WT	12.11 ± 0.80	2.30 ± 0.23
***Mapt*$^{-/-}$**	14.05 ± 0.61**	2.47 ± 0.21
	mEPSC	
	Amplitude (pA)	**FWHM (ms)**
WT	8.96 ± 0.24	1.21 ± 0.07
***Mapt*$^{-/-}$**	9.61 ± 0.26	1.38 ± 0.07
	sIPSC	
	Amplitude (pA)	**FWHM (ms)**
WT	28.74 ± 1.57	7.04 ± 0.25
***Mapt*$^{-/-}$**	32.92 ± 1.91	6.31 ± 0.26
	mIPSC	
	Amplitude (pA)	**FWHM (ms)**
WT	16.24 ± 0.68	5.78 ± 0.34
***Mapt*$^{-/-}$**	17.47 ± 0.61	5.54 ± 0.32

FWHM: Full width at half maximum

**p < 0.01 by Mann-Whitney test

Table S2 (Related to Figure 2). Additional properties of APs and EPSCs in FS cells

	Characteristics of single AP	
	Amplitude (pA)	**FWHM (ms)**
WT	75.5 ± 1.8	0.68 ± 0.03
***Mapt*$^{-/-}$**	76.9 ± 1.3	0.67 ± 0.02
	sEPSC	
	Amplitude (pA)	**FWHM (ms)**
WT	17.84 ± 0.85	1.30 ± 0.04
***Mapt*$^{-/-}$**	18.19 ± 0.80	1.32 ± 0.06
	mEPSC	
	Amplitude (pA)	**FWHM (ms)**
WT	16.16 ± 0.65	1.28 ± 0.05
***Mapt*$^{-/-}$**	15.76 ± 0.60	1.28 ± 0.07

FWHM: Full width at half maximum

Table S3 (Related to Figure 4). Properties of single channel bursts and network bursts

	Single channel bursts			
	Frequency		**Spikes per burst**	
KCl Exposure (h)	**0**	**4**	**0**	**4**
WT	0.32 ± 0.04	0.34 ± 0.04	38.87 ± 5.66	34.89 ± 4.80
***Mapt*$^{-/-}$**	0.27 ± 0.03	0.27 ± 0.04	25.87 ± 3.60$^{\#}$	27.23 ± 3.57
	Network bursts			
	Frequency		**Spikes per burst**	
KCl Exposure (h)	**0**	**4**	**0**	**4**
WT	1.17 ± 0.18	0.92 ± 0.1	147.4 ± 24.40	184.2 ± 23.81
***Mapt*$^{-/-}$**	0.63 ± 0.09**	0.63 ± 0.07	115.1 ± 19.38	145.9 ± 23.31

$^{\#}p = 0.06$, **$p < 0.01$ by two-way ANOVA with Holm-Sidak test