Supplemental figures

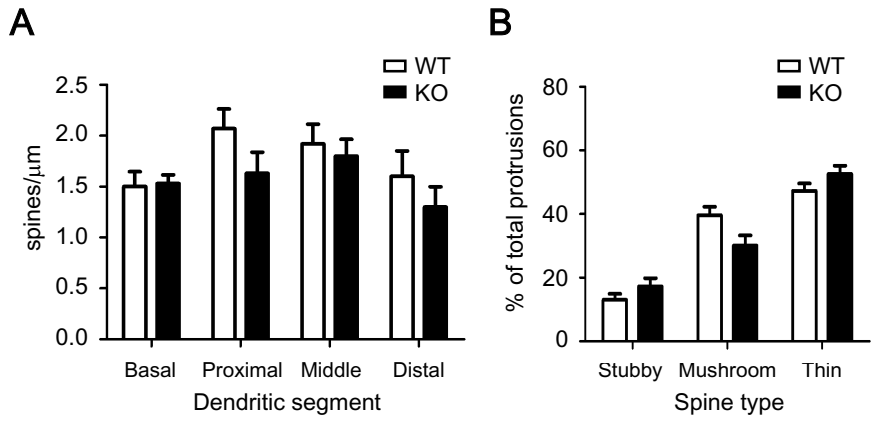
Sup. Figure 1

Mendelian ratio

Heterozygous Intercross	Expected ratio	Observed ratio
Bai1+/+	25%	22% (44/200)
Bai1+/-	50%	55% (110/200)
Bai1-/-	25%	23% (46/200)

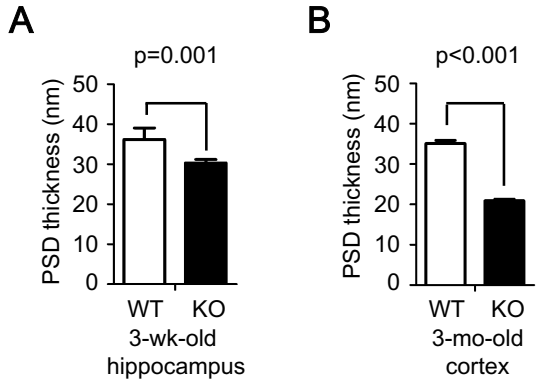
Sup. Figure 1
KO mice are obtained at the expected Mendelian ratio.

Sup. Figure 2



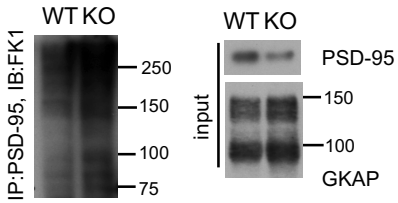
Sup. Figure 2
Quantification of 3-week-old mouse hippocampal dendritic spine density (**A**) and morphology (**B**). No significant difference was found between WT and KO CA1 neurons. P=0.11 and P=1.0, respectively, 5 mice/group, 2-way ANOVA test.

Sup. Figure 3



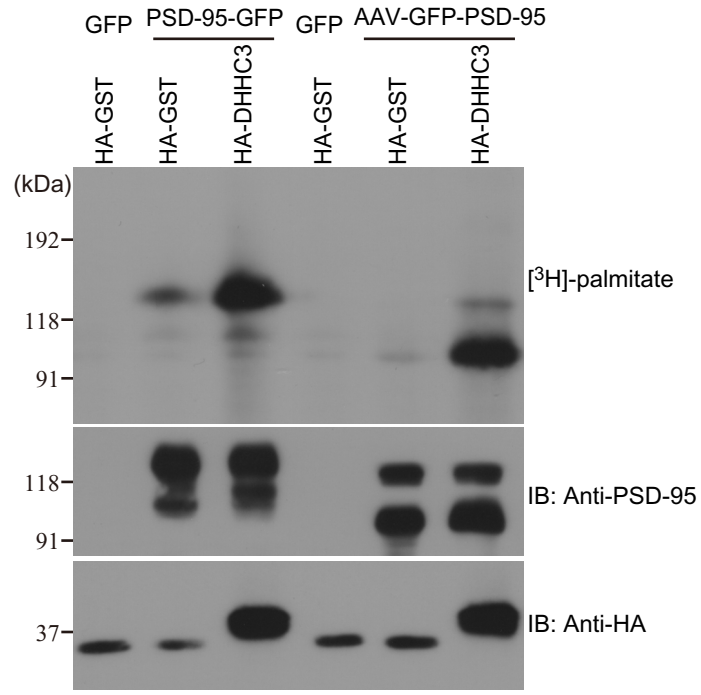
Sup. Figure 3
Decrease in PSD thickness in 3-week-old *Bai1*-/- hippocampal neurons (**A**) and in 3-month-old *Bai1*-/- cortex somatosensory neurons (**B**) by electron microscopy analysis. The histogram shows the significant difference in the mean ± SEM of PSD thickness between WT and KO mice (**A,** 5 mice/group, p=0.001; **B**, 4 mice/group, p<0.001. All tests are two-tailed t-tests).

Sup. Figure 4



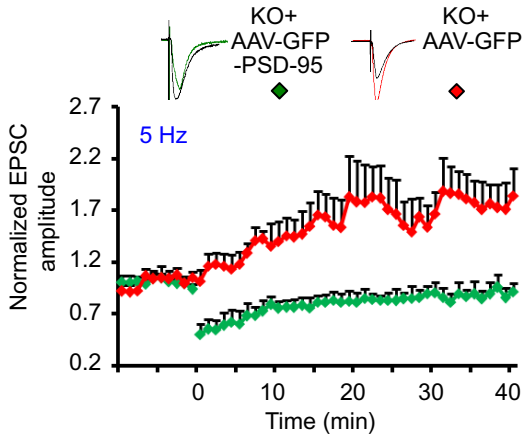
Sup. Figure 4
Western blot shows enhanced poly-ubiquitination of PSD-95 by a poly-ubiquitination specific antibody (clone FK1). Input shows reduced PSD-95 protein levels in KO brain tissue but no change in guanylate kinase-associated protein (GKAP) levels. GKAP is a PSD protein that interacts directly with PSD-95 and is regulated by the TRIM3 E3 ubiquitin ligase.

Sup. Figure 5



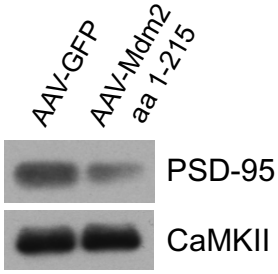
Sup. Figure 5
The PSD-95 protein expressed from the pAAV-GFP-PSD-95 expression vector is palmitoylated. HEK 293 cells were co-transfected with HA-tagged DHHC3 (Asp-His-His-Cys motif palmitoyl acyltransferase) and the pAAV-GFP-PSD-95 expression vector. Transfected cells were then labeled with 0.5 mCi/ml [^{3}H]palmitic acid for 4 hrs in the serum-free DMEM with fatty acid-free bovine serum albumin. After metabolic labeling, proteins were separated by SDS-PAGE, followed by fluorography. The main PSD-95 protein produced from the F2A containing vector migrated at ~95 kDa as expected (see lanes 5,6). PSD-95 palmitoylation was greatly enhanced by the co-expression of palmitoylating enzyme DHHC3 (top panel, last lane). A plasmid which has GFP fused to the C-terminus of PSD-95 was included as a positive control (note that the PSD-95-GFP fusion protein migrates at ~ 130 kDa; see lanes 2,3). Immunoblots were used to verify the level of transfected PSD-95 (middle panel) and HA-tagged DHHC3 (bottom panel).

Sup. Figure 6



Sup. Figure 6
Reversal of LTP following AAV-mediated restoration of PSD-95 expression in KO hippocampal neurons with 5 Hz stimulation protocols.

Sup. Figure 7



Sup. Figure 7
AAV-mediated overexpression of dominant negative Mdm2 fragment (amino acids 1-215) in neuronal cells reduces the stability of endogenous PSD-95 protein. Primary cultures of granule neuron precursors (GNPs) were obtained from WT mice cerebella on postnatal day 5 (P5) (see Material and Methods). GNPs were incubated with control (AAV-GFP) or AAV-Mdm2 (aa 1-215) viruses for 1 week, then the proteins were extracted and western blotting was performed with anti-PSD-95 and anti-CaMKII antibodies.

Author contributions:

D.Z. and E.G.V.M. initiated the project and D.Z. performed most of the experiments. C.L., J.G., S.D. and D.G.R. performed the patch clamp recording. A.M.S. and D.G.R. performed the morphological analysis of neurons and spine quantification. Y.S. and R.M.V. performed EM analysis. Z.Z. and J.J.O. performed the stereotactic neurosurgery. S.M. and E.G.V.M. designed the gene-targeting vector. T.M. and M.F. performed the in vitro palmitoylation assay. J.R.S. and R.A.H. analyzed the PSD fraction. G.N.N. performed the brain vessel quantification and marble-burying behavioral test. D.Z. and E.G.V.M. wrote the manuscript. All authors provided advice and comments on the manuscript.

Supplemental Table 1

	Frequency (Hz)	Amplitude (mV)	Half Width (ms)	Area	Rise time (ms)	Decay time (ms)	10-90 Rise time (ms)	10-90 slope
WT (n=16)	2.76 ± 0.79	0.70 ± 0.05	20.8 ± 1.7	16.7 ± 1.7	8.3 ± 0.4	24.6 ± 1.96	6.7 ± 0.4	0.12 ± 0.02
BAI1 KO (n=15)	2.69 ± 0.50	0.63 ± 0.04	22.7 ± 1.8	15.4 ± 0.8	8.3 ± 0.3	27.1 ± 1.70	6.7 ± 0.3	0.14 ± 0.05

Sup. Table 1
Properties of spontaneous EPSPs from adult WT and *Bai1*-KO mice hippocampal CA1 neurons. No significant difference was detected between WT and KO in each of the properties.

Supplemental Table 2.

Experiments	Statistical tests	Sample numbers	p value
Hidden-platform water maze, latency	2-way ANOVA, post hoc Dunnett's test	10 mice/group	P<0.001
Hidden-platform water maze, quadrant time	2-way ANOVA, post hoc Dunnett's test	10 mice/group	P<0.01
Visible-platform water maze, latency	2-way ANOVA, post hoc Dunnett's test	8 mice/group	P=0.64
Visible-platform water maze, distance traveled	2-way ANOVA, post hoc Dunnett's test	8 mice/group	P=0.32
Elevated plus maze	t-test	8 mice/group	P=0.92
Marble-burying test	t-test	7 mice/group	P=0.85
Light/dark test	t-test	8 mice/group	P=0.16
Electrophysiology, 100 Hz LTP	t-test	WT n=8 neurons/6 animals, KO n=6 neurons/4 animals	P < 0.001
Electrophysiology, 1 Hz LTD	t-test	WT n=6 neurons/3 animals, KO n=5 neurons/3 animals	P < 0.001
Electrophysiology, 5 Hz LTD	t-test	WT n=7 neurons/4 animals, KO n=5 neurons/3 animals	P<0.05
Electrophysiology, 10 Hz LTD	t-test	WT n=5 neurons/4 animals, KO n=5 neurons/3 animals	P<0.01
Electrophysiology, PPF	t-test	WT n=6 neurons/3 animals, KO n=10 neurons/5 animals	P=0.7414
Electrophysiology, PSD-95 rescue, 1 Hz LTD	t-test	Control n=5 neurons/3 animals, PSD95 rescue, n=5/3 animals	P < 0.001
Electrophysiology, PSD-95 rescue, 5 Hz LTD	t-test	Control n=5 neurons/3 animals, PSD95 rescue, n=6/3 animals	P<0.01
Electrophysiology, AAV-Mdm2 fragment, 1 Hz LTD	t-test	Control n=6 neurons/6 animals, AAV-Mdm2 (aa 1-215), n=7/6 animals	P<0.001
Quantification of spines, spine density, adult	2-way ANOVA, post hoc Dunnett's test	Neurons, n=40 (WT), n=53 (KO), 5 mice/group	P=0.55
Quantification of spines, spine type, adult	2-way ANOVA, post hoc Dunnett's test	Spines, n=1520 (WT), n=2112 (KO), 5 mice/group	P=0.99
Quantification of spines, spine density, 3-week-old	2-way ANOVA, post hoc Dunnett's test	Neurons, n=21 (WT), n=22 (KO), 5 mice/group	P=0.11
Quantification of spines, spine type, 3-week-old	2-way ANOVA, post hoc Dunnett's test	Spines, n=835 (WT), n=783 (KO), 5 mice/group	P=1.0
Quantification of PSD	t-test	n=122 (WT), n=105 (KO),	p<0.0001

density, hippocampus, adult		5 mice/group	
Quantification of PSD density, hippocampus, 3-week-old	t-test	n=174 (WT), n=170 (KO), 4 mice/group	P=0.001
Quantification of PSD density, cortex, adult	t-test	n=114 (WT), n=108 (KO), 4 mice/group	p<0.001

Power calculation:

experiments	WT mean value	KO mean value	SD of WT	Power
Hidden-platform water maze, latency	15.675	46.550	10.349	1.0
Hidden-platform water maze, quadrant time	50.610	30.170	13.631	1.0
Electrophysiology, 100 Hz LTP	1.75	2.85	0.67	1.0
Electrophysiology, 1 Hz LTD	0.67	1.41	0.24	1.0
Electrophysiology, 5 Hz LTD	1.25	1.71	0.45	0.77
Electrophysiology, 10 Hz LTD	1.32	1.85	0.27	0.99
Quantification of PSD density, adult	30.5	18.6	7.1	1.0
	Control mean	PSD-95-R mean	SD of control	Power
Electrophysiology, PSD-95 rescue, 1 Hz LTD	1.5	0.55	.22	1.0
Electrophysiology, PSD-95 rescue, 5 Hz LTD	1.66	0.75	.46	1.0
	Control mean	AAV-Mdm2	SD of control	Power
Electrophysiology, AAV-Mdm2 fragment, 1 Hz	0.56	1.03	0.24	1.0

Sup. Table 2

Details of the statistical analysis in the manuscript. The Emory Rodent Behavioral Core Facility performed the mouse behavior tests, and the mice were transferred to the facility with only the ID of each mouse shown, so the performer was blinded to the genotypes. The sample size for each experiment was determined by power calculations based on previous experience with similar experiments.