## Supplemental material

Schreiber et al., http://www.jcb.org/cgi/content/full/jcb.201506048/DC1



Figure S1. **GKAP and SHANK1 levels are not affected in Trim3**<sup>-/-</sup> **mice.** Hippocampal synapse-enriched fractions were prepared from wild-type and Trim3<sup>-/-</sup> mice under control conditions (home cage) and 2 h after contextual fear conditioning (shock). Samples were immunoblotted and stained for GKAP and SHANK1. Normalized protein levels were calculated by dividing immunoblot (IB) signal intensities by the total protein intensities (stain-free gel) and expressed relative to wild-type levels. No differences in normalized protein levels were found for either GKAP (wild-type home cage:  $1 \pm 0.04$ , Trim3<sup>-/-</sup> shock:  $0.80 \pm 0.15$ ) or SHANK1 (wild-type home cage:  $1 \pm 0.07$ , Trim3<sup>-/-</sup> home cage:  $0.95 \pm 0.07$ , wild-type shock:  $0.98 \pm 0.05$ , Trim3<sup>-/-</sup> shock:  $0.89 \pm 0.07$ ; means  $\pm$  SEM, n = 4 per genotype).



Figure S2. Hippocampal morphology and cytoarchitecture are not affected in *Trim3*-/- mice. (A) Nissl staining showed normal gross morphology of the hippocampus in *Trim3*-/- mice (A) compared with wild-type controls (A') at 4 mo of age. Bars, 250  $\mu$ m. (B–D) Golgi-cox staining showed normal cellular morphology of CA1 (B) and CA3 (C) pyramidal neurons and of granule neurons in the dentate gyrus (D) in *Trim3*-/- mice compared with wild-type controls (B'-D'). CA, cornu ammonis; DG, dentate gyrus. Bars, 50  $\mu$ m.

## Table S1. Behavioral analysis of Trim3-/- mice at 8 mo of age

Test/test measure	Unit	Wild type (n = 14)	Knockout (n = 12)	P-value
Open field/Ethovision				
Number of entries into the center area		37.29 ± 3.67	41.08 ± 4.06	0.493
Time spent in the center area	S	65.0 ± 6.4	85.8 ± 20.0	0.303
Total distance traveled	cm	4,729 ± 341	5,290 ± 303	0.238
Open Field/SEE analysis/velocity				
Lingering progression threshold speed	cm/s	12.52 ± 0.67	14.83 ± 0.78	0.034
Median of move segment maximum speed	cm/s	21.55 ± 1.10	24.94 ± 1.09	0.040
Quantile 95 of move segment maximum speed	cm/s	30.22 ± 1.13	34.18 ± 1.33	0.031
Lingering mean speed	cm/s	1.77 ± 0.10	1.84 ± 0.14	0.687
Median of lingering segment maximum speed	cm/s	$4.41 \pm 0.40$	4.07 ± 0.35	0.540
Open field/SEE analysis/acceleration				
Latency to maximum half speed	sec	10.71 ± 2.87	13.01 ± 4.07	0.642
Median segment acceleration to maximum speed	cm/s <sup>2</sup>	13.59 ± 0.36	14.55 ± 0.57	0.155
Open field/SEE analysis/other				
Number of progression segments	segments	112.5 ± 8.3	114.8 ± 6.0	0.828
Median length of progression segments	cm	23.83 ± 2.03	30.66 ± 1.99	0.026
Median duration of progression segments	S	1.58 ± 0.08	1.76 ± 0.06	0.075
Quantile 5 of duration of progression segments	S	0.59 ± 0.05	0.67 ± 0.03	0.238
Quantile 95 of duration of progression segments	S	3.35 ± 0.15	3.68 ± 0.13	0.119
Number of stops per distance	segments/cm	$0.03 \pm 0.00$	$0.03 \pm 0.00$	0.109
Time proportion of lingering episodes		0.67 ± 0.03	0.63 ± 0.02	0.389
Median radius of turn	cm	75.43 ± 4.17	83.35 ± 5.29	0.245
Median turn rate	degrees/s	13.23 ± 0.54	13.45 ± 0.79	0.812

Entries show mean ± SEM values unless otherwise indicated.

Table S2 is provided as an Excel table and shows a list of proteins identified as potential TRIM3 substrates.