



**Supplementary information, Figure S2** Endophilin A1 knockdown impairs synapse formation and synaptic function.

(A-D) HEK293 cells were cotransfected with constructs expressing shRNA and Myc-tagged or Flag-tagged endophilins for 48 h and lysed for immunoblotting analysis. Asterisk indicates RNAi-resistant Myc-tagged endophilin A1. Low panels are quantification of endophilin expression. Statistical test: \*\*\*  $P < 0.001$ ; one-way ANOVA followed by Dunnett's multiple-comparison post hoc tests or t test. All values are shown as mean  $\pm$  s.e.m.  $n = 3$  independent experiments. (E) Hippocampal neurons

were transfected with shRNA constructs coexpressing DsRed and shRNA at DIV16-17 followed by immunostaining with antibodies to DsRed and indicated proteins at DIV21. Shown are representative confocal images. Open arrowheads, cell bodies of non-transfected neurons; filled arrowheads, cell bodies of transfected neurons. Scale bar, 10  $\mu$ m. **(F and G)** Quantitative analysis of endophilin mean intensity in transfected neurons. All values are shown as mean  $\pm$  s.e.m. 19-22 neurons. Statistical test: \*\*\*  $P < 0.001$ ; one-way ANOVA followed by Dunnett's multiple-comparison post hoc tests or t test. **(H-J)** Quantitative analysis of the fluorescence intensity **(H)**, number **(I)** and area **(J)** of PSD95 puncta in dendrites of neurons transfected with construct encoding Ctrl-shRNA or cotransfected with constructs encoding EENA1-shRNA and RNAi-resistant Flag-tagged EENA1 (indicated by asterisk), Flag-tagged EENA2, or Flag-tagged EENA3 at DIV16-17 followed by immunostaining with antibodies against PSD95 (green), Flag (blue) and DsRed at DIV21 (number of cells analyzed, Ctrl-shRNA: 16, EENA1-shRNA: 16, EENA1-shRNA + EENA1\*: 17, EENA1-shRNA + EENA2: 25, EENA1-shRNA + EENA3: 23). All values are shown as mean  $\pm$  s.e.m. Statistical test: \*\*\*  $P < 0.001$ ; one-way ANOVA followed by Dunnett's multiple-comparison post hoc tests. **(K)** Cumulative distribution of PSD95 puncta area (number of puncta analyzed, Ctrl-shRNA: 804, EENA1-shRNA: 260, + EENA1\*: 901, + EENA2: 384, + EENA3: 351). **(L)** Standardized values of mEPSC frequency and amplitude in **Fig. 3E-H**. Statistical test: \*  $P < 0.05$ ; one-way ANOVA followed by Dunnett's multiple-comparison post hoc tests. ns, not significant.