Supplementary information, Figure S3 Similar effects of endophilin A1 and p140Cap knockdown on spine morphology.

(A) HEK293 cells were cotransfected with constructs expressing Flag-tagged p140Cap and shRNA targeting p140Cap for 48 h and lysed for immunoblotting analysis. **(B)** Quantification of p140Cap expression. Statistical test: *** $P < 0.001$; one-way ANOVA followed by Dunnett's multiple-comparison post hoc tests. $n = 3$ independent experiments. **(C)** Hippocampal neurons were transfected with shRNA constructs coexpressing DsRed and shRNA at DIV16-17 followed by immunostaining with antibodies to DsRed and p140Cap at DIV21. Shown are representative confocal images. Filled arrowheads, cell bodies of transfected neurons. Scale bar, 10 µm. **(D)** Quantitative analysis of p140Cap mean intensity in transfected neurons. Statistical test: *** $P < 0.001$; one-way ANOVA followed by Dunnett's multiple-comparison post hoc tests. All values are shown as mean ± s.e.m. 20-25 neurons. **(E)** Representative images of cultured hippocampal neurons transfected with shRNA constructs

coexpressing shRNA and DsRed at DIV16-17 followed by immunostaining with antibodies to DsRed at DIV21. Arrows, spines; Open arrows, filopodia; open arrowheads, filopodia growing from existing spine heads. Scale bar, 5 μm. **(F)** Quantification of dendritic protrusion density of transfected neurons in **E**. (number of cells analyzed, Ctrl-shRNA: 31, EENA1-shRNA #1: 20, EENA1-shRNA #2: 15, p140Cap-shRNA #1: 33, p140Cap-shRNA #2: 26). In all, more than 600 protrusions were measured for each group. All values are shown as mean ± s.e.m. Statistical test: ## $P < 0.01$ (total protrusions), ** $P < 0.01$ (spines), $$ $P < 0.01$, $ $P < 0.05$ (filopodia); one-way ANOVA followed by Dunnett's multiple-comparison post hoc tests.