



Supplementary information, Figure S4 Regulation of spine morphogenesis by endophilin A1 requires not only binding to p140Cap but also its membrane association.

(A) HEK293 cells cotransfected with constructs encoding Myc-tagged p140Cap and Flag-tagged mutants of endophilin A1 for 48 h were lysed for immunoprecipitation with immobilized Flag antibody. Input and bound proteins were analyzed by

immunoblotting with antibodies against Myc and Flag. **(B)** Cultured hippocampal neurons were cotransfected with constructs expressing shRNA and RNAi-resistant Flag-tagged mutants of endophilin A1 (indicated by asterisk) at DIV16-17 followed by immunostaining with antibodies to Flag and DsRed at DIV21. Shown are representative confocal images. Filled Arrows, spines; Open arrows, filopodia. **(C)** Quantitative analysis of dendritic protrusion density of transfected neurons in **B** (number of cells analyzed, Ctrl-shRNA: 14, EENA1-shRNA: 15, + Y299*: 18, + F301*: 15, + E308*: 15, + W327*: 13, + Y343*: 18). More than 550 protrusions were analyzed for each group. All values are shown as mean \pm s.e.m. Statistical test: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$; one-way ANOVA followed by Dunnett's multiple-comparison post hoc tests. **(D)** Cultured hippocampal neurons were cotransfected with constructs expressing shRNA and RNAi-resistant Flag-tagged mutants of endophilin A1 (indicated by asterisk) at DIV16-17 followed by immunostaining with antibodies to Flag and DsRed at DIV21. Shown are representative confocal images. Filled arrows, spines; Open arrows, filopodia. **(E)** Quantitative analysis of dendritic spine density of transfected neurons in **D** (number of cells analyzed, Ctrl-shRNA: 13, EENA1-shRNA: 12, EENA1-shRNA + WT*: 12, EENA1-shRNA + KKK-EEE*: 21, EENA1-shRNA + BAR- Δ H11*: 19). More than 450 protrusions were measured for each group. All values are shown as mean \pm s.e.m. Statistical test: *** $P < 0.001$, * $P < 0.05$; one-way ANOVA followed by Newman-Keuls multiple comparison post hoc tests. Scale bars, 5 μ m.