Supplementary Figure Legends

Suppl. Figure 1. Expression patterns of Rap1, Rap2, SPAR and EphA4 in hippocampal neurons. ***A,*** Specificity of the anti-SPAR antibodies. Affinity-purified SPAR antibodies were used for immunoblotting of hippocampal lysates (HC) and lysates of HEK 293T cells transfected with SPAR or control pcDNA3. In the right lanes, the SPAR antibody used for immunoblotting was preincubated with the peptide antigen. * indicates that the film was exposed to the blot for 15 sec while for the other lanes, which were from the same blot, the fild was exposed for 5 min. Immunoblotting for actin is shown as a control for protein loading. ***B,*** Time course of expression of Rap1, Rap2, SPAR and EphA4 in freshly plated hippocampal neurons. Immunoblotting for GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and actin was used as control for protein loading. ***C,*** Primary hippocampal neurons transfected with a vector encoding actin fused to enhanced yellow fluorescent protein (YFP-actin, green) were double labeled for EphA4 (blue) and Rap1, Rap2 or SPAR (red). Arrows point to growth cones. Bar, 10 μm.

Suppl. Figure 2. Dominant negative Rap1, dominant negative Rap2 and Rap1GAP cause inactivation of both Rap1 and Rap2 and the constitutively active Rap1 and Rap2 show higher GTP binding than the corresponding wild-type proteins. HEK 293T cells were transfected in (***A***) with HA-tagged dominant negative Rap1 S17N, Myc-tagged dominant negative Rap2 S17N or pcDNA3 as a control and in (***B***) with SPAR, Rap1GAP or pcDNA3 as a control. HT22 cells were transfected in (***C***) with HA-tagged Rap1 or constitutively-active Rap1 G12V or Myc-tagged Rap2 or constitutively-active Rap2 Q63E. Active endogenous Rap1 and Rap2 (***A,B***) or transfected Rap1 (***C***) were isolated by pull-down with GST-RalGDS RBD and detected with anti-Rap1, anti-Rap2 or anti-HA antibodies. The total levels of endogenous Rap1 and Rap2 in

the lysates were also verified by immunoblotting. Transfected Rap1 and Rap2 (which have a substantially larger apparent molecular weight compared to the endogenous proteins) were detected in the lysates using antibodies to their respective tags.

Suppl. Figure 3. Expression patterns of Rap1, Rap2, SPAR and EphA4 in synaptosomal fractions. ***A,*** Synaptosomal fractions were prepared from 1 month old rat brains and probed by immunoblotting with the indicated antibodies. TX sol, TritonX-100 soluble fraction; TX insol, TritonX-100 insoluble fraction; PSD, postsynaptic density fraction. ***B,*** Primary hippocampal neurons cultured for 10 days were cotransfected with EGFP and pcDNA3-Myc (ctrl) or Myc-tagged wild type Rap2 (wtRap2) or constitutively active HA-tagged Rap2 Q63E. Neurons were fixed at 21 days in culture and stained for F-actin (red). Bars, 50 μm (top) and 2 μm (bottom). ***C,*** Graphs show cumulative percentages of spine lengths and widths shorter or more narrow, respectively, than the size indicated. Histograms show mean dendritic spine lengths, widths and densities ± SE. Primary hippocampal neurons transfected with Rap2 constructs have significantly shorter spines and slightly decreased spine width (** $p<0.01$ by KS-test and 1-way ANOVA and Dunnett's post-test). Spine density was decreased after transfection of Rap2 Q63E but not wild-type Rap2 (** $p<0.01$, 1-way ANOVA and Dunnett's post-test).