Supplementary information, Figure S3

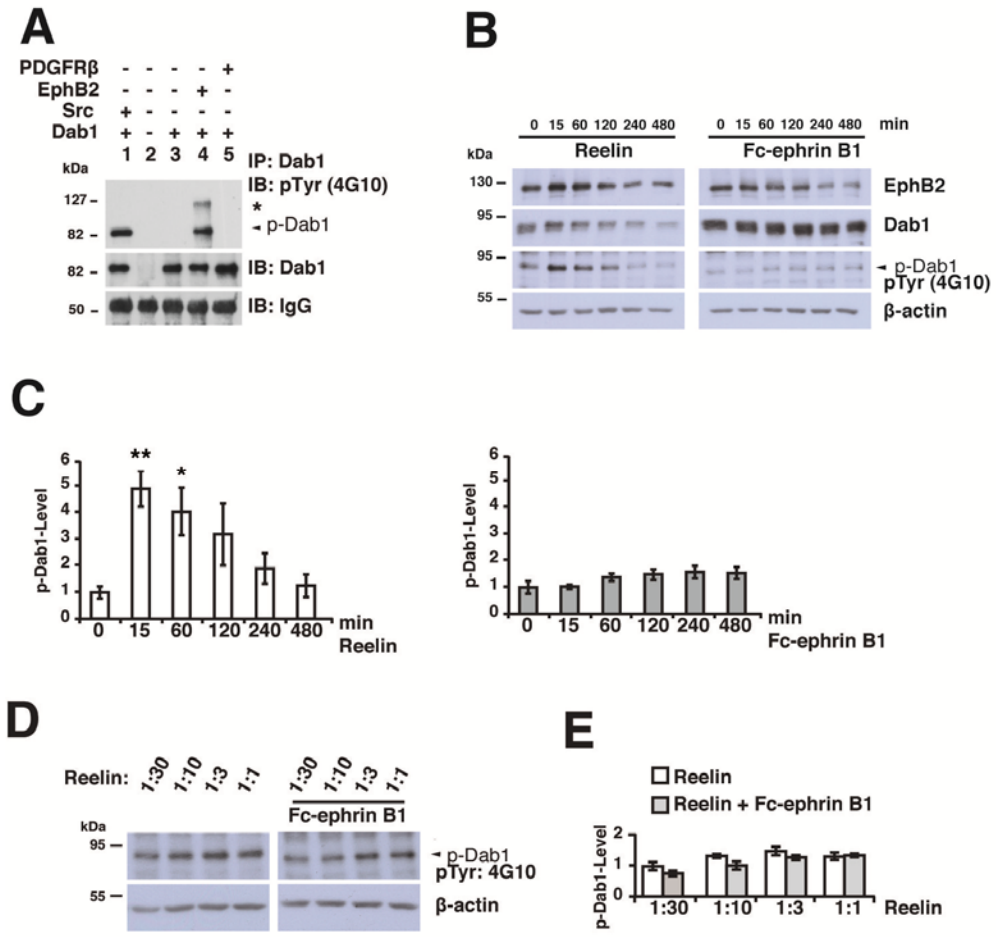

Figure S3 Activation of EphB2 forward signaling by ephrin B1 does not induce Dab1 phosphorylation. **(A)** Dab1 phosphorylation in HEK-293 cells co-expressing Dab1 and EphB2. HEK-293 cells were transfected with the indicated plasmids, lysed and analyzed by western blotting (IB) using the indicated antibodies after immunoprecipitation (IP) of Dab1. Co-transfection of Dab1 and Src (lane 1) or Dab1 and EphB2 (lane 4) but not of Dab1 with the receptor tyrosine kinase PDGFRβ (lane 5) leads to tyrosine phosphorylation of Dab1 (arrowhead), as assessed by phosphotyrosine blotting with the 4G10 monoclonal antibody. The asterisk indicates the position of autophosphorylated EphB2 kinase co-immunoprecipitating with Dab1.

(B) Effect of ephrin B1-induced EphB2 forward signaling on Dab1 tyrosine phosphorylation in primary neurons. Cortical neurons (E15.5, DIV5) were treated with recombinant Reelin (left) or preclustered ephrin B1 ectodomain (right) for the indicated times. Note the lack of Dab1 tyrosine phosphorylation and Dab1 degradation in the ephrin B1-treated neurons. **(C)** The relative p-Dab1 signal intensities as determined by western blotting with the 4G10 antibody were quantified (s.d., $*P < 0.05$, $**P < 0.01$ for treatment with Reelin, $n = 3$, white bars). The effect of preclustered Fc-ephrin B1 on p-Dab1 levels (grey bars) did not reach statistical significance. **(D)** Fc-ephrin neither augments nor inhibits the effect of Reelin on Dab1 phosphorylation. Cortical neurons (E15.5, DIV5) were treated with different dilutions of recombinant Reelin alone (left), or Reelin plus preclustered ephrin B1 ectodomain (right) for 1 h. The lysates were separated by SDS-PAGE and blotted with the 4G10 phosphotyrosine antibody. Actin served as a loading control. **(E)** Quantification of the effect of cotreatment with Reelin and Fc-ephrin B1 on Dab1 tyrosine phosphorylation (s.d., $n = 3$).