Cell Reports, Volume *21*

Supplemental Information

GRIP1 Binds to ApoER2 and EphrinB2 to Induce Activity-Dependent AMPA Receptor Insertion at the Synapse

Sylvia Pfennig, Franziska Foss, Diane Bissen, Eva Harde, Julia C. Treeck, Marta Segarra, and Amparo Acker-Palmer

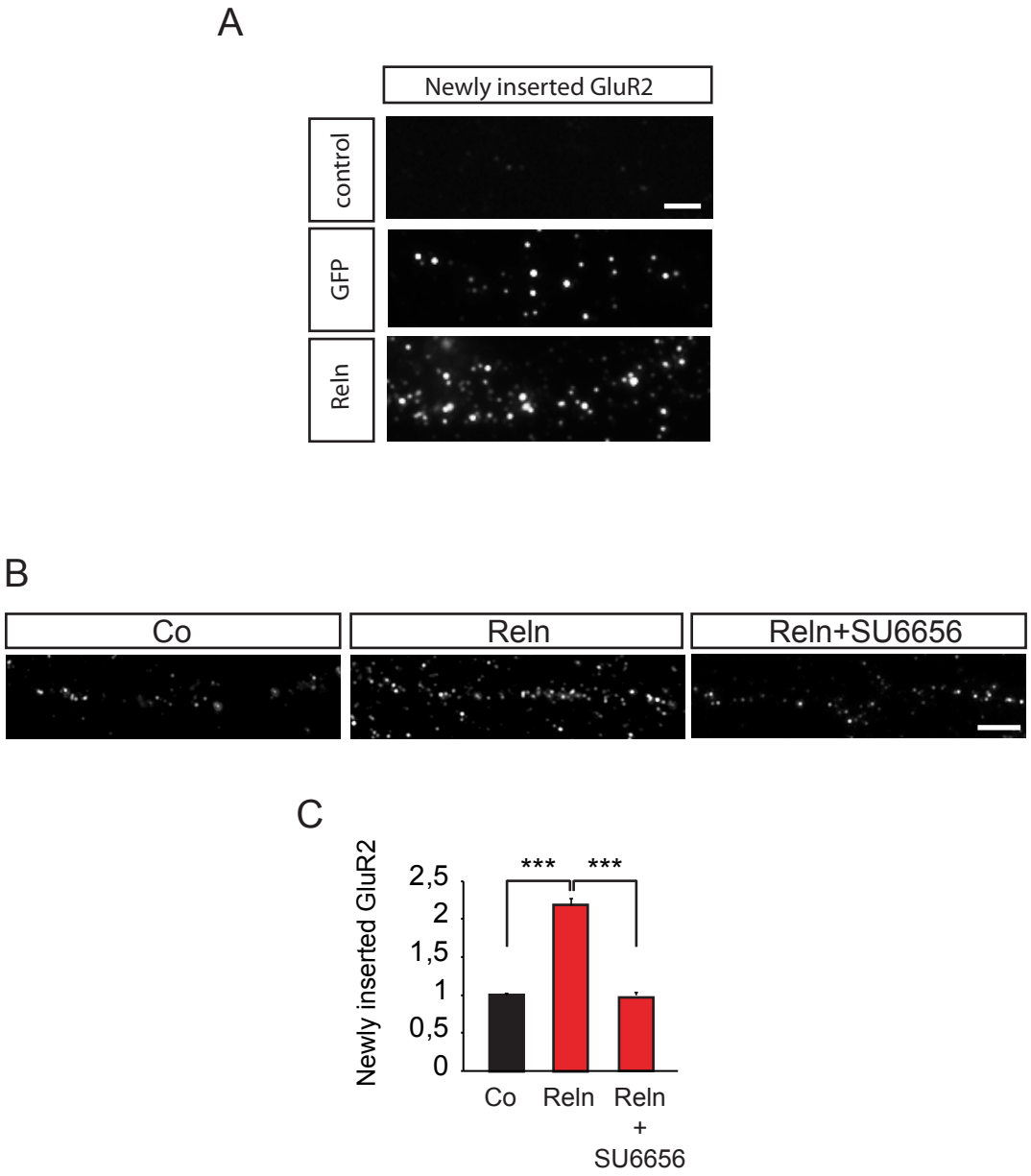
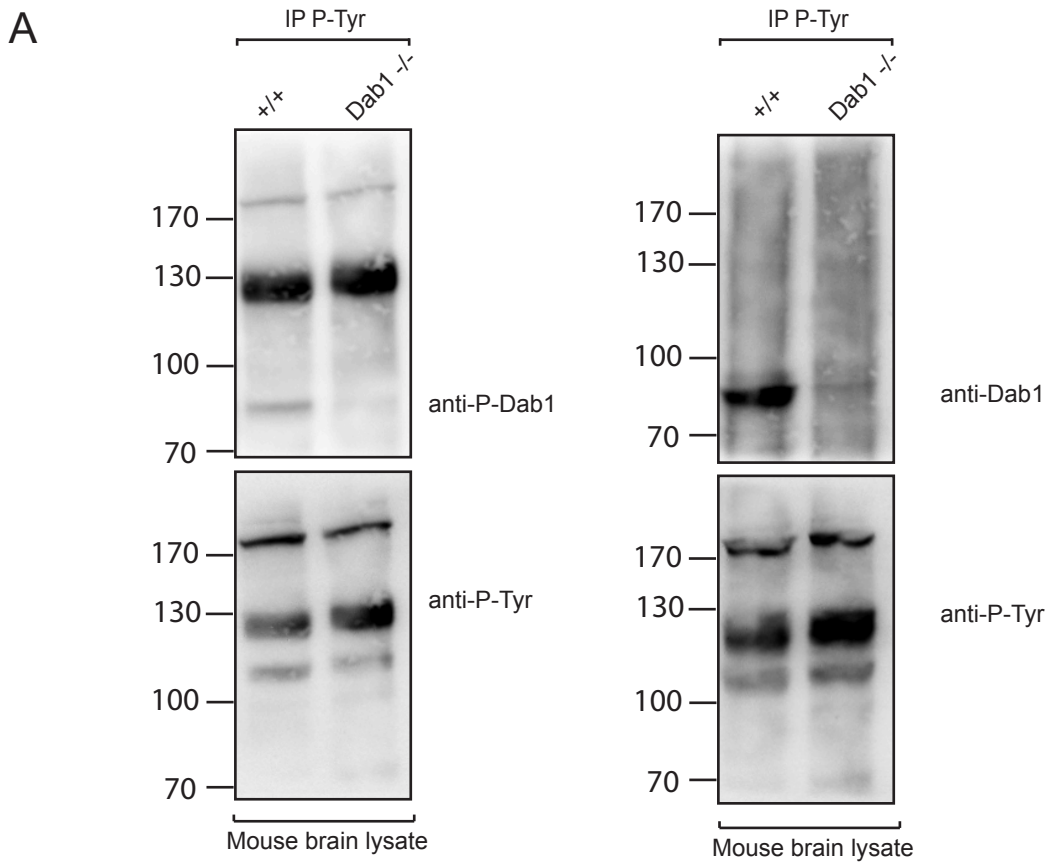


Figure S1, related to Figure 1. ApoER2- mediated insertion of AMPA receptor requires SFK activation.
(A) The unconjugated secondary antibody efficiently blocks surface AMPA receptors present before stimulation. Immunofluorescence images of dendrites obtained from primary hippocampal neurons 14 DIV that have been subjected to the newly inserted AMPA receptor assay. The unconjugated secondary antibody masks efficiently surface AMPARs (control, top panel) so that indeed only newly inserted GluR2 positive AMPA receptors are detectable upon GFP (middle panel) or Reln (bottom panel) stimulation. Scale bar 2µm.
(B, C) Src family kinases are necessary for AMPA receptor membrane insertion induced by Reelin. Wild type hippocampal neurons, examined for newly inserted AMPA receptors, were stimulated with Reelin for 3 hours or Reelin after pre-incubation with the SFK-inhibitor SU6656. SFK-inhibition was performed for 1 hour prior to the stimulation step. Fluorescent images of GluR2 inserted into the dendritic membrane (B). Relative fluorescence intensities of newly inserted GluR2 in dendrites of neurons stimulated with Reelin (Reln) or Reelin in combination with SU6656 (Reln+SU6656). n=4 (C).
Scale bar: 5µm; Bar graphs show mean ± SEM ***P < 0.001.



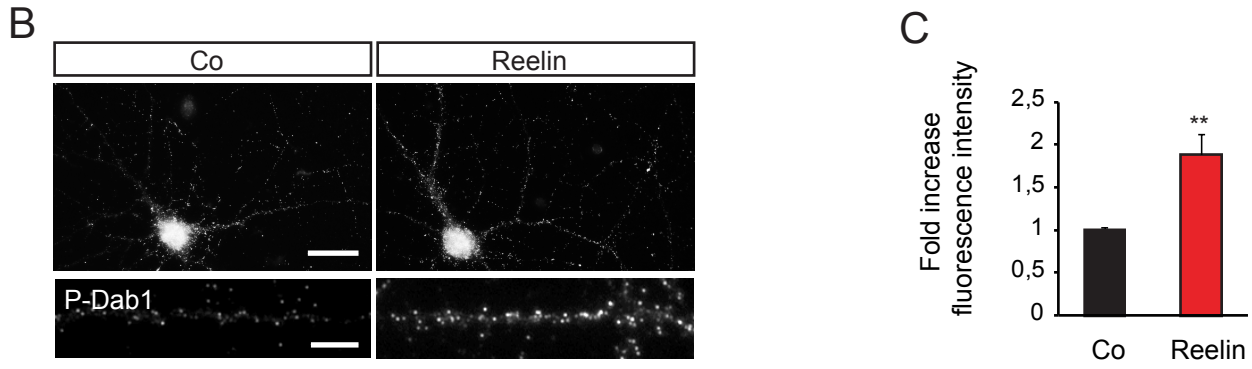


Figure S2, related to Figure 2, A-F. Levels of Dab1-phosphorylation in dendrites increase after Reelin stimulation. (A) The P-Dab1 (Y232) antibody is specific for Dab1. Total brain lysates from wild type (+/+) and Dab1 knockout (Dab1$^{-/-}$) mice were subjected to immunoprecipitation assays using an anti-phospho-Tyrosine antibody. Western blots were probed with antibodies for pDab1 (Y232), Dab1, and phospho-Tyrosine. (B-C) Primary hippocampal neurons were isolated from wild type mice at E 17.5 and stimulated at 14 DIV with Reelin for 60 min. Fluorescent images showing dendritic pDab1 staining are shown in (B). Quantification of relative pDab1 fluorescence intensity upon stimulation with Reelin. n=3 (C). Scale bar in (B) 20µm; scale bar in (B, higher magnification) 5µm. Bar graphs show mean ± SEM **P < 0.01.

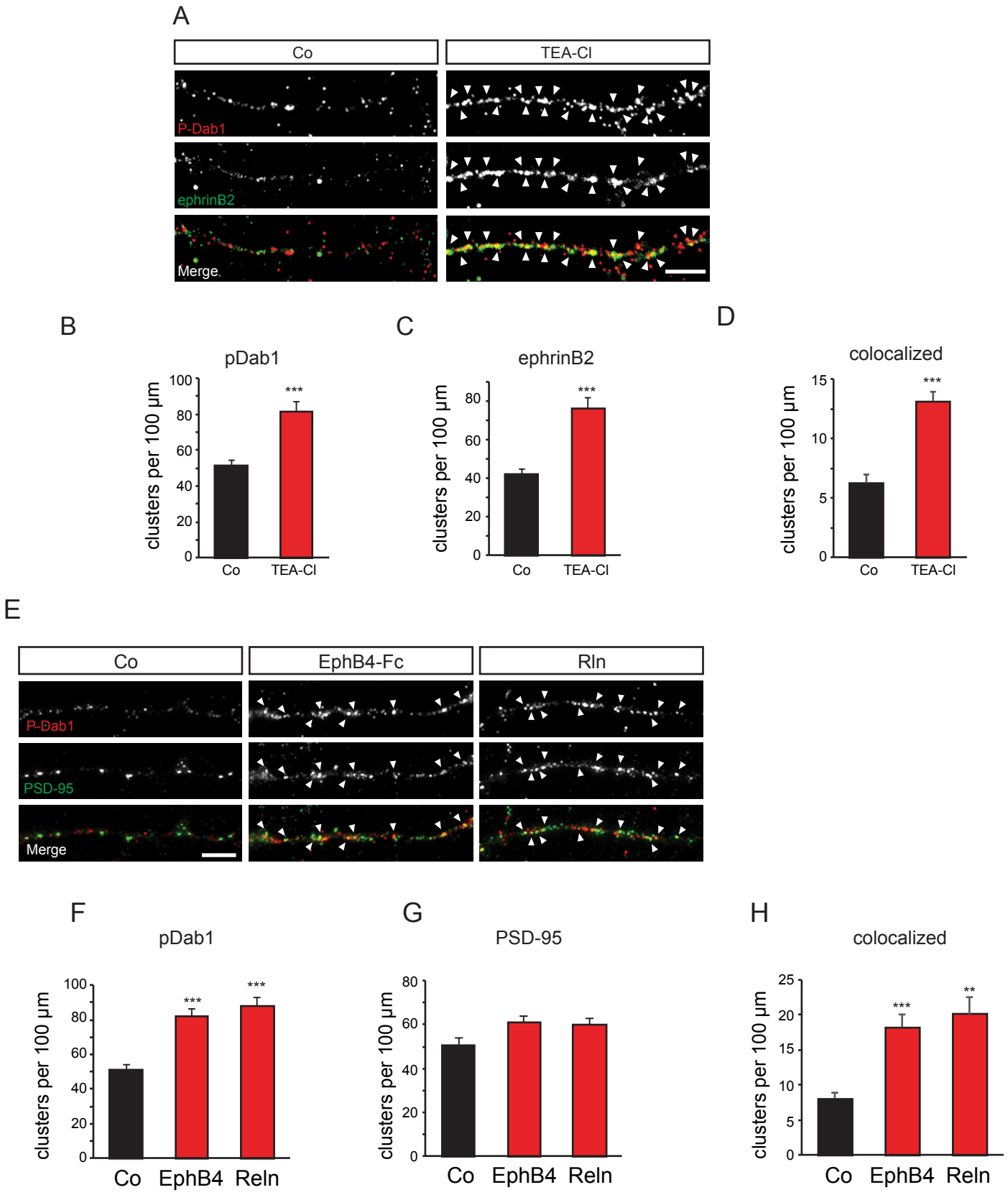


Figure S3, related to Figure 2, G-J. Clustering of P-Dab1 at ephrinB2 and PSD95 positive sites.
(A-D) Induction of chemical LTP with TEA-Cl increases Dab1 phosphorylation and ephrinB2 co- clustering. Wild type primary hippocampal neurons were stimulated with 25mM TEA-Cl for 10 min and labeled for pDab1 and ephrinB2. Fluorescent images of dendrites showing pDab1 (red) and ephrinB2 (green) staining. Arrowheads indicate co-localization of pDab1 and ephrinB2 (A). Quantification of pDab1 clusters (B), ephrinB2 clusters (C) and pDab1-ephrinB2 co-clustering upon stimulation with TEA-Cl (D). n=5 neurons. (E-H) Activation of ephrinB2 and ApoER2 leads to co-clustering of pDab1 and PSD-95. Primary hippocampal neurons isolated from wild type mice at E17.5 were stimulated with pre-clustered EphB4-Fc or concentrated Reelin supernatants after 14 DIV. Immunocytochemistry was performed using anti-pDab1 and anti-PSD-95 antibodies. Visualization of pDab1 and PSD-95 in dendritic branches. Arrowheads indicate co-clustering of pDab1 (red) and PSD-95 (green) (E). Quantification of pDab1 clusters (F), PSD-95 clusters (G), and pDab1-PSD-95 coclusters (H) upon EphB4-Fc (EphB4) and Reelin stimulation. n=5 neurons.
Scale bars 5µm. Bar graphs show mean ± SEM; **P < 0.01; ***P < 0.001.

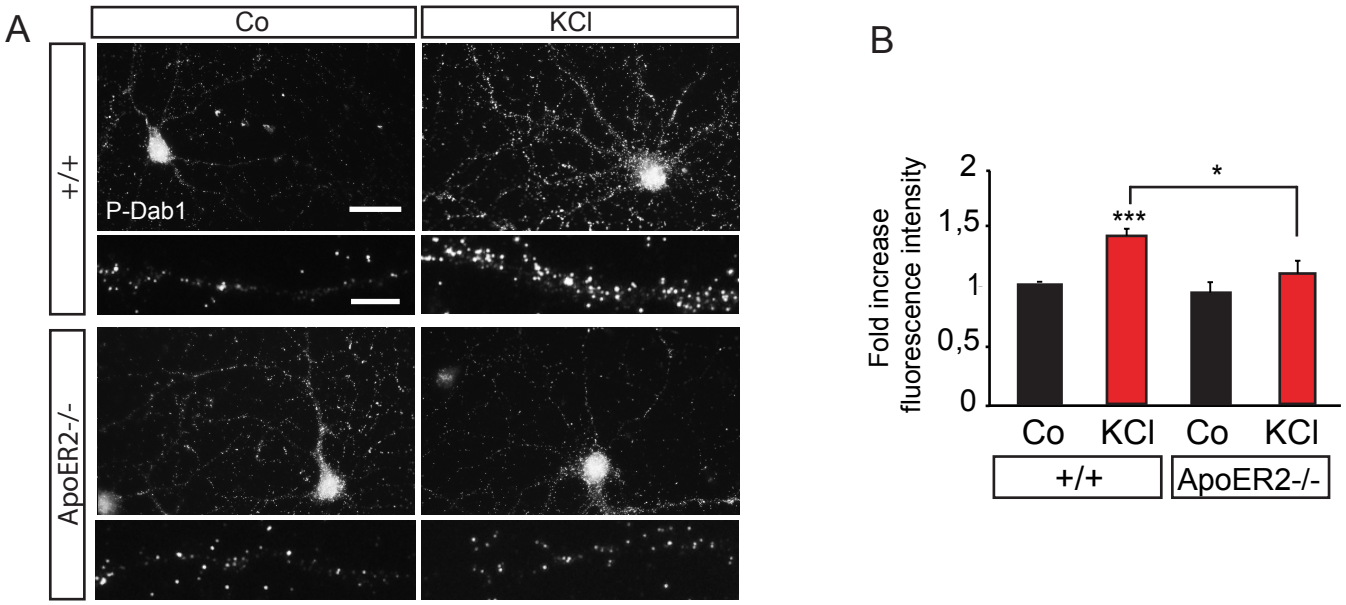


Figure S4, related to Figure 2, K-L. Dab1 phosphorylation upon KCl stimulation depends on ApoER2 receptors.
(A, B) Dab1 phosphorylation upon KCl stimulation depends on ApoER2 receptors. Primary hippocampal neurons were isolated from ApoER2 knockout embryos at E 17.5. At 14 DIV neurons were stimulated with 10 mM KCl for 10 min and immunocytochemistry for pDab1 was performed. Fluorescence images of dendrites showing pDab1 in wild type (+/+) and ApoER2 knockout (ApoER2$^{-/-}$) neurons stimulated with KCl (A). Quantification of relative pDab1 fluorescence intensity in ApoER2 knockout neurons. n=6 (B).
Scale bar in (A) 20µm; scale bar in (A, higher magnification) 5µm. Bar graphs show mean ± SEM, *P < 0.05 ***P < 0.001.

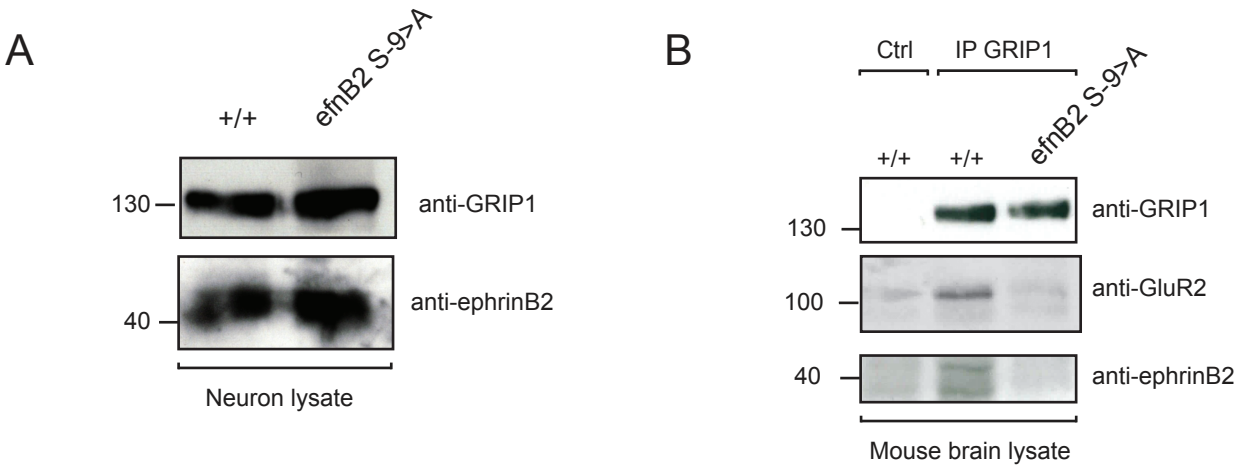


Figure S5, related to Figure 6A. Serine-9 of ephrinB2 is required for the formation of the GRIP1, GluR2, and ephrinB2 complex.

(A) EphrinB2 expression levels are unaltered in ephrinB2 S-9>A knock-in mutant mice. Hippocampal neurons were cultured from wild type (+/+) and ephrinB2 S-9>A (efnB2 S-9>A) embryos. At 14DIV neurons were lysed and analyzed for ephrinB2 and GRIP1 expression by Western blot. (B) The mutation of serine-9 of ephrinB2 to alanine abolishes the GRIP1-GluR2-ephrinB2 complex formation. Total brain lysates from wild type (+/+) and ephrinB2 S-9>A (efnB2 S-9>A) mice were immunoprecipitated by anti-GRIP1 antibody and examined for ephrinB2 and GluR2 binding.

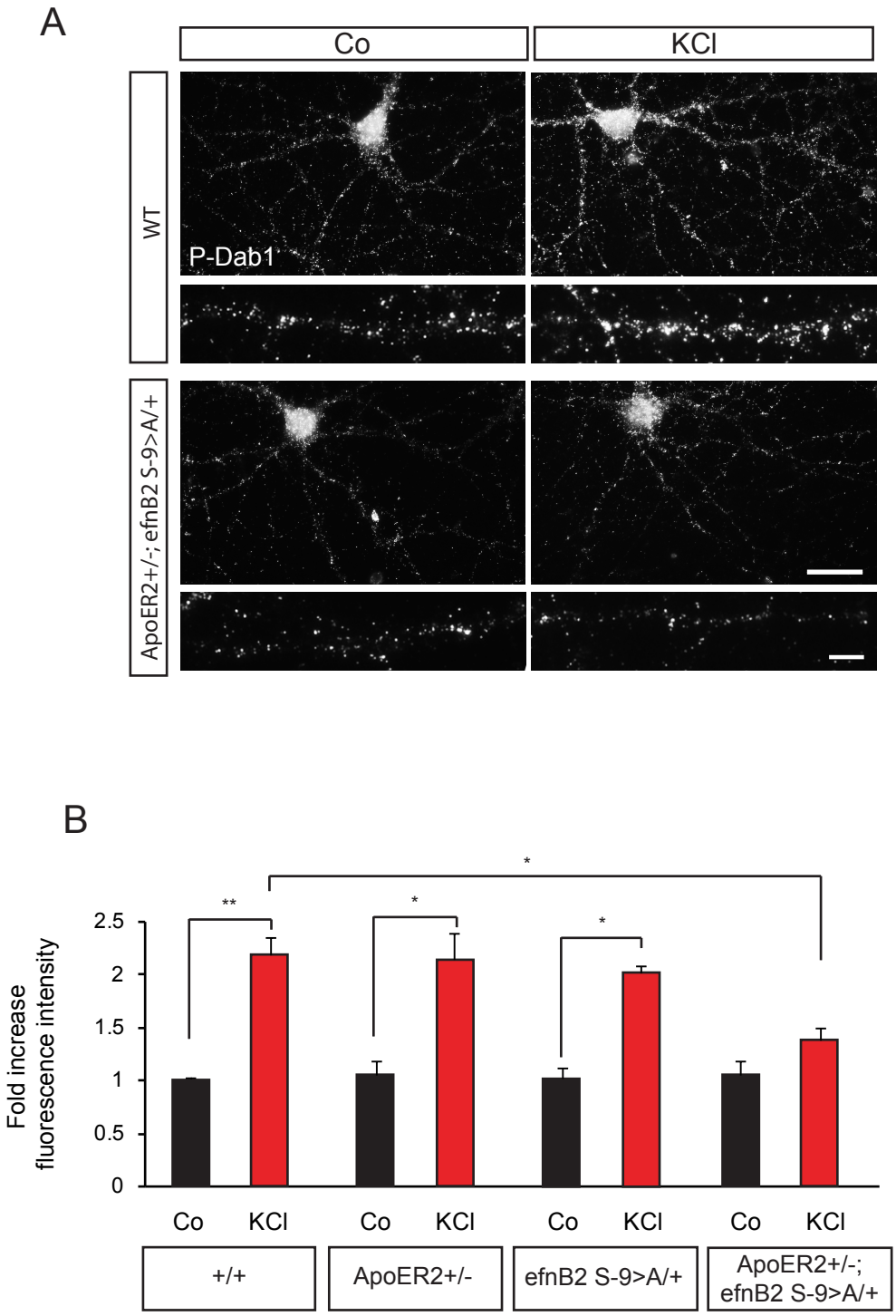


Figure S6, related to Figure 6, D-G. Functional interaction of ApoER2 and serine-9 of ephrinB2 is required for KCl-induced Dab1 phosphorylation.

(A, B) ApoER2$^{+/-}$; ephrinB2$^{S\text{-}9>A/+}$ compound neurons show reduced levels of pDab1 after KCl stimulation compared to wild type neurons. ApoER2$^{+/-}$; ephrinB2$^{S\text{-}9>A/+}$ compound neurons, wild type neurons and single heterozygous neurons were stimulated with 10 mM KCl at 14DIV. Fluorescent images of pDab1 staining in wild type (+/+) and ApoER2$^{+/-}$; ephrinB2$^{S\text{-}9>A/+}$ compound neurons (A). Quantification of pDab1 fluorescence intensities in wild type, ApoER2$^{+/-}$, ephrinB2$^{S\text{-}9>A/+}$ and compound neurons. Single heterozygous neurons show normal pDab1 levels. n=3-4 (B).

Scale bar in (A) 20µm; scale bar in (A higher magnifications) 5µm. Bar graphs show mean ± SEM *P < 0.05; **P < 0.01.

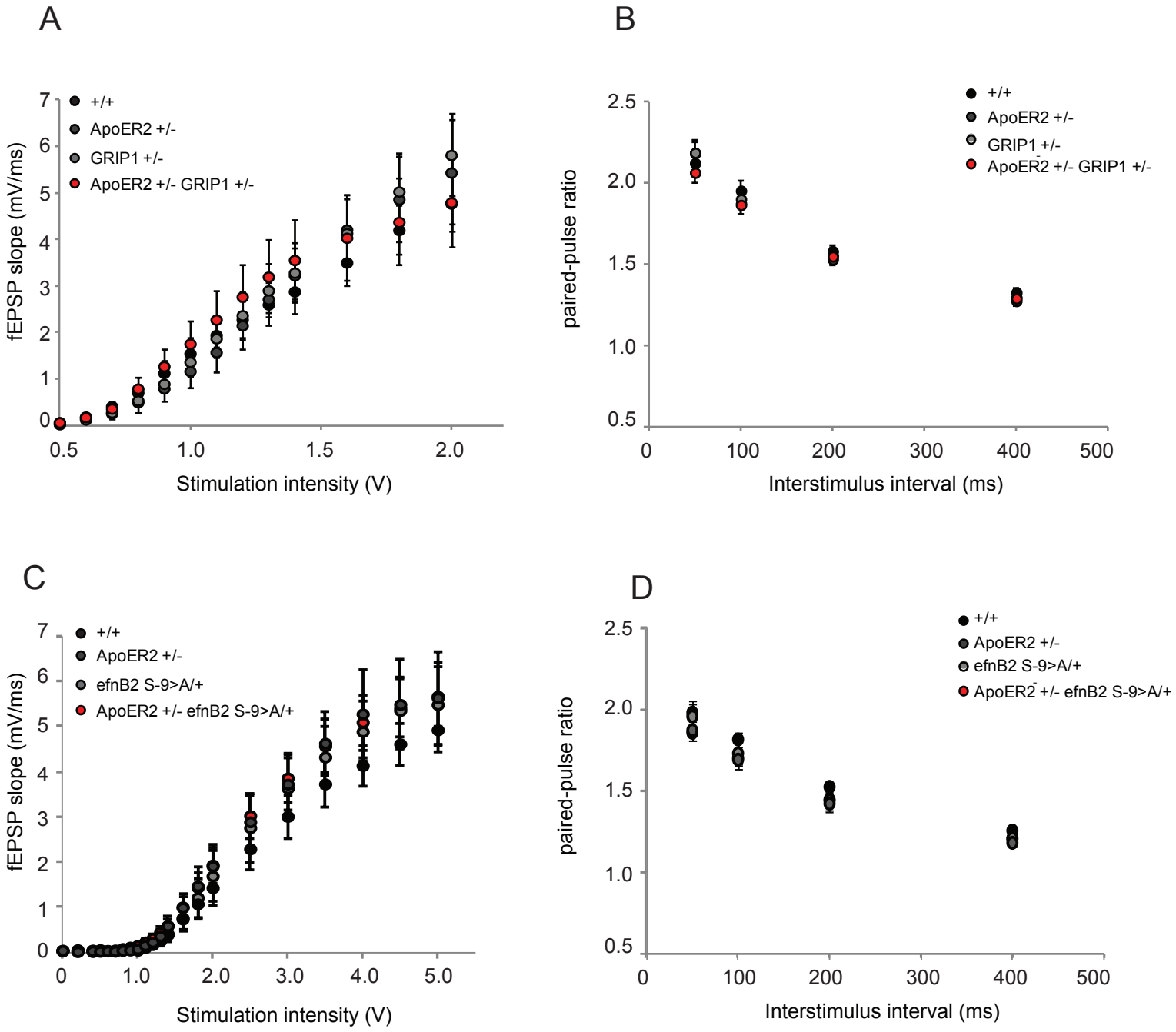


Figure S7, related to Figure 7. ApoER2$^{+/-}$; GRIP1$^{+/-}$ and ApoER2$^{+/-}$; efnB2$^{S\text{-}9>A/+}$ compound mice show normal synaptic hallmarks.

Basal synaptic transmission and presynaptic properties are unchanged in ApoER2$^{+/-}$; GRIP1$^{+/-}$ (A, B) and in ApoER2$^{+/-}$; efnB2$^{S\text{-}9>A/+}$ (C, D) compound mice. The input-output relation of fEPSP slope at various stimulation intensities does not show significant differences among the respective groups (A, C). Paired-pulse facilitation was measured at different interstimulus intervals. No significant differences were obtained between the corresponding different genotypes (B, D).

Data are represented as mean ± SEM as calculated across slices. ApoER2 - GRIP1: n = 7 wild type mice (16 slices), 6 ApoER2$^{+/-}$ mice (13 slices), 9 GRIP1$^{+/-}$ mice (16 slices), 8 ApoER2$^{+/-}$; GRIP1$^{+/-}$ compound mice (21 slices). ApoER2 - ephrinB2: n = 6 wild type mice (13 slices), 4 ApoER2$^{+/-}$ mice (9 slices), 4 efnB2$^{S\text{-}9>A/+}$ mice (8 slices), 6 ApoER2$^{+/-}$; efnB2$^{S\text{-}9>A/+}$ compound mice (15 slices).