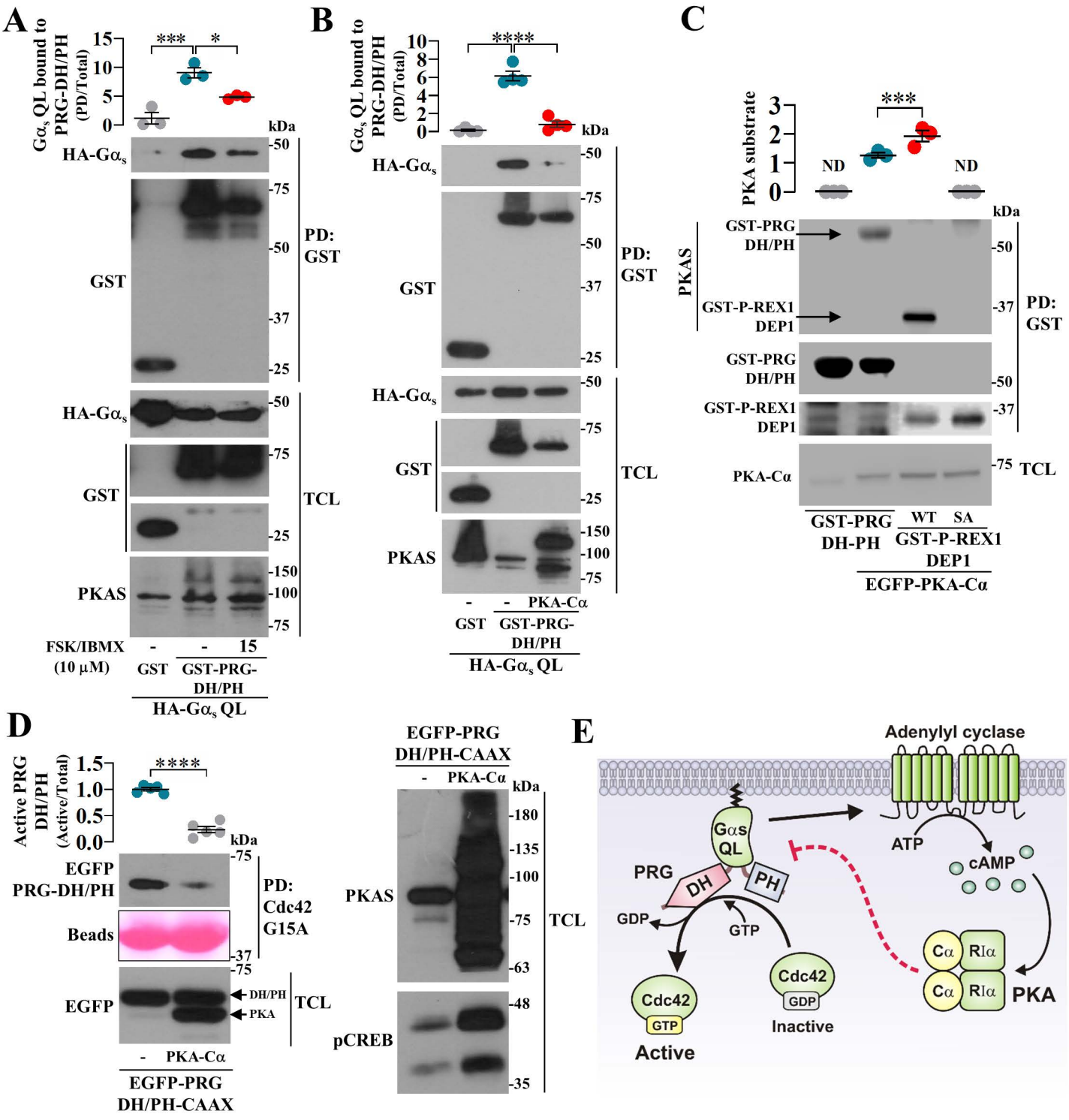

Supplementary Figure 1. cAMP/PKA signaling attenuates $G\alpha_s$:PRG interaction and reduces PRG-DH/PH:Cdc42-G15A binding. A-B) Effect of cAMP/PKA signaling on $G\alpha_s$:PRG interaction was studied by pulldown of GST-PRG-DH/PH and HA-$G\alpha_s$-QL using lysates of HEK293T cells that were stimulated or not with forskolin/IBMX to activate adenylyl cyclase (**A)** or transfected with PKA catalytic subunits **(B)**. Graphs represent the mean ± S.E. (n=3); *p=0.0196, ***p=0.009, ****p<0.0001, one-way ANOVA followed Tukey. **C)** Potential phosphorylation of PRG-DH/PH by PKA was analyzed in HEK293T cells transfected with GST-PRG-DH/PH without or with EGFP-PKA-Cα. Cell lysates were subjected to GST-pulldown followed by western blot with PKAS antibodies to detect PKA substrates. GST-P-Rex1-DEP1 WT and SA (non-phosphorylatable mutant) were used as controls. Graphs represents the mean ± S.E.; n=3, ***p=0.0006, t-test. **D)** Effect of catalytic PKA subunit on PRG-DH/PH:Cdc42-G15A binding was evaluated using lysates of HEK293T cells transfected with EGFP-PRG-DH/PH-CAAX and EGFP-PKA-C. Graphs represents the mean ± S.E.; n=5, ****p<0.0001, t-test. **E)** Model postulates that the Gαs:PRG:Cdc42 signaling pathway is fine-tuned by the PKA.