

Supplementary Materials for
Plexins Are GTPase-Activating Proteins for Rap and Are Activated by
Induced Dimerization

Yuxiao Wang, Huawei He, Nishi Srivastava, Sheikh Vikarunnessa, Yong-bin Chen,
Jin Jiang, Christopher W. Cowan, Xuewu Zhang*

*To whom correspondence should be addressed. E-mail: xuewu.zhang@utsouthwestern.edu

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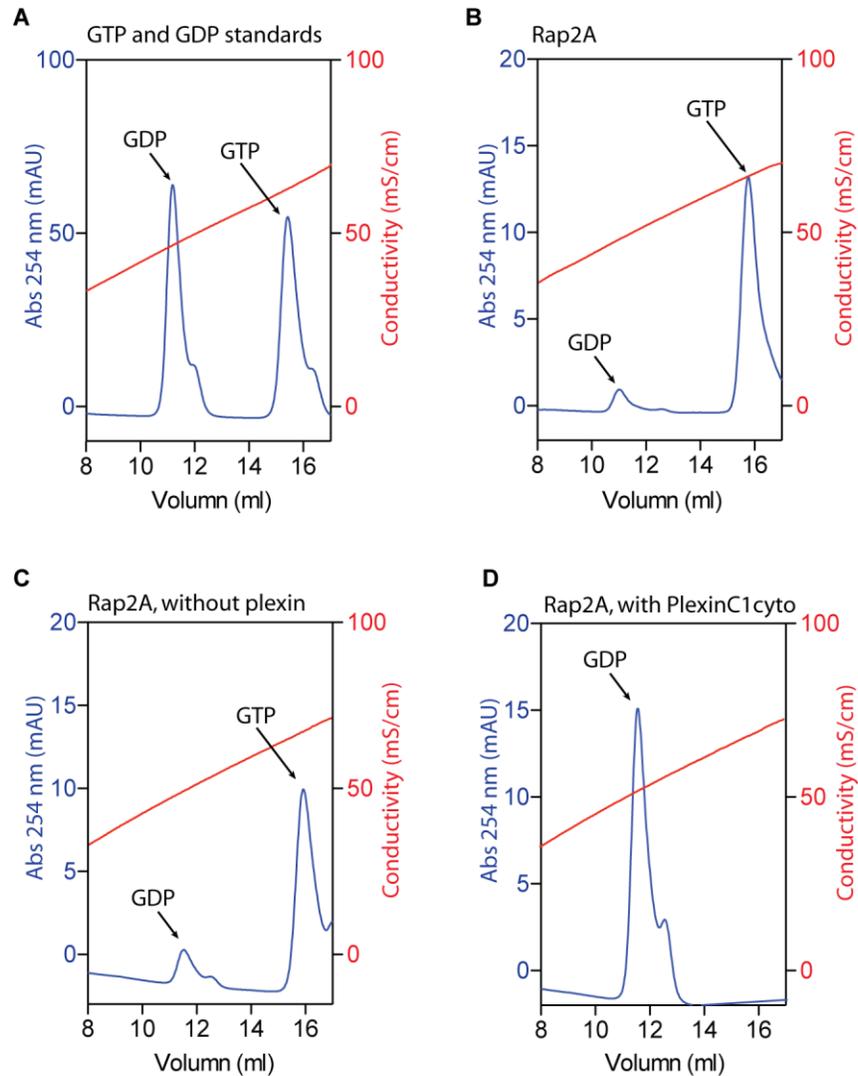


Figure S1. HPLC-based assay for plexin RapGAP activity.

(A) Anion exchange chromatogram of GDP and GTP standards. GDP and GTP are eluted at conductivities of ~40 mS/cm and ~60 mS/cm, respectively.

(B) Rap2A purified from *E. Coli* is ~90% GTP bound, perhaps due to its low intrinsic GTPase activity.

(C) Incubation of Rap2A at room temperature for 30 min does not change the GTP/GDP ratio.

(D) Incubation of Rap2A in the presence of PlexinC1_{cyto} at room temperature for 30 min leads to complete hydrolysis of bound GTP to GDP.

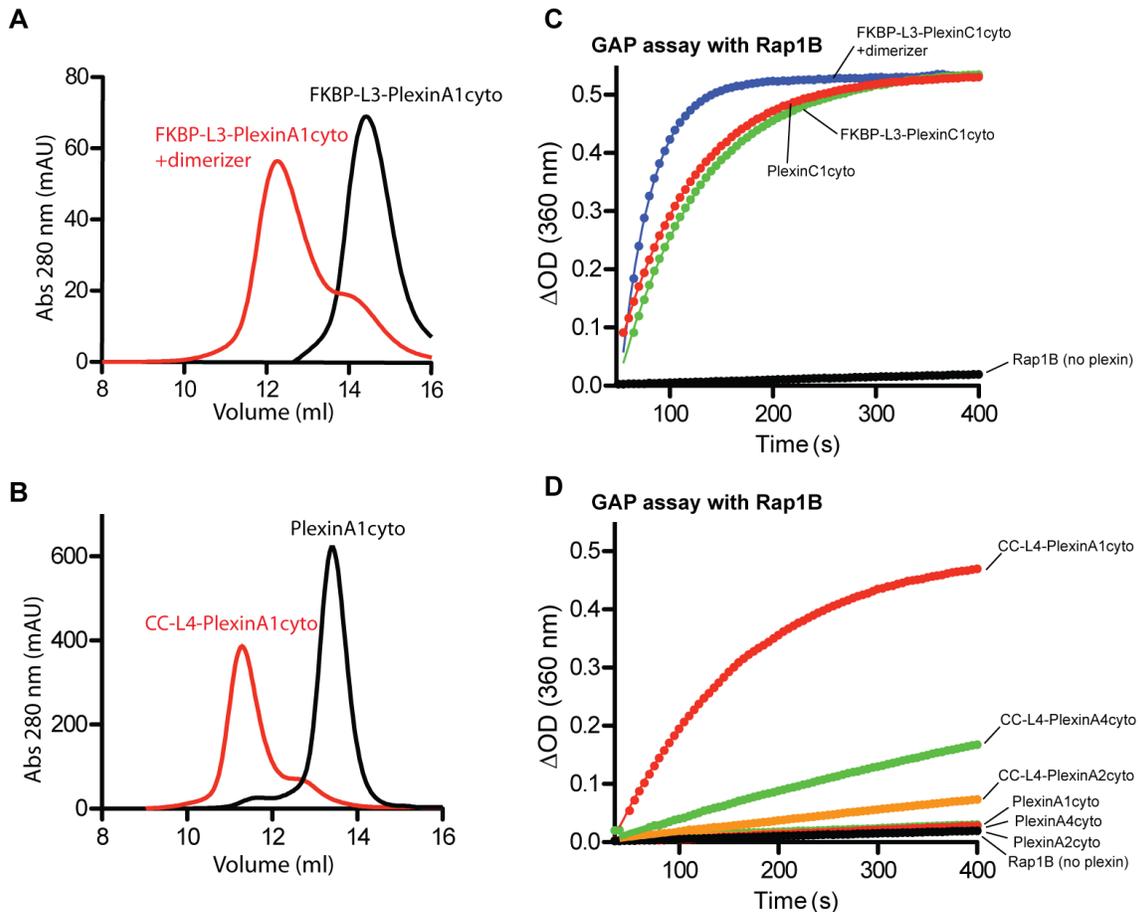


Figure S2. The RapGAP activity of plexins_{cyto} is stimulated by induced dimerization.

(A) Gel filtration profiles of FKBP-L3-PlexinA1_{cyto} with or without the dimerizer AP20187. (B) Gel filtration profiles of native PlexinA1_{cyto} and CC-L4-PlexinA1_{cyto}. The results in (A) and (B) show that PlexinA1_{cyto} is predominantly monomeric in solution, but can be induced to dimerize through the FKBP/AP20187 system or the coiled-coil dimerizer. The peak positions in (A) and (B) are not comparable because different columns were used. (C) Stimulation of the RapGAP activity of FKBP-L3-PlexinC1_{cyto} by dimerization. (D) Coiled-coil enforced dimerization increases the RapGAP activity for PlexinA1_{cyto}, PlexinA2_{cyto}, and PlexinA4_{cyto}. CC-L4-PlexinA1_{cyto} displays the highest degree of activation (28-fold). CC-L4-PlexinA2_{cyto} and CC-L4-PlexinA4_{cyto} are 3-fold and 8-fold more active than their respective native proteins. The GAP assay conditions are the same as in Fig. 1. Data shown are representative of at least 3 independent experiments.

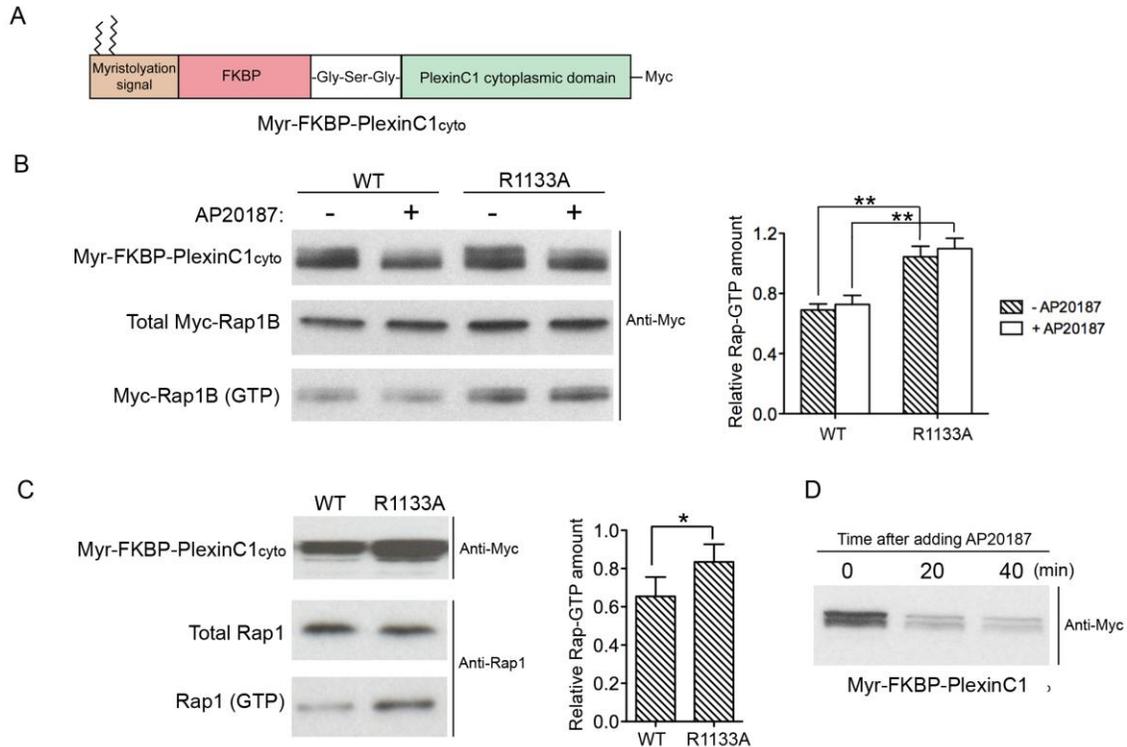


Figure S3. GST-RalGDS pull-down assays for plexin RapGAP activity in cells.

(A) Diagram of the construct of the membrane-localized FKBP-L3-PlexinC1_{cyto} (named Myr-FKBP-PlexinC1_{cyto}). The myristoylation signal from the kinase Yes (residues 1-11) was fused to the N-terminus of FKBP-L3-PlexinC1_{cyto} to target the protein to both plasma membrane and intracellular membranes.

(B) GST-RalGDS pull-down assays for transfected Myc-tagged Rap. Myc-tagged Rap1B was cotransfected with either the wild-type (WT) or the catalytically dead mutant (R1133A) of Myr-FKBP-PlexinC1_{cyto} into HEK293T cells. The GST-RalGDS pull-down assay was employed to determine the amount of GTP-bound Myc-Rap1B, which was normalized to the amount of total Myc-Rap1B to calculate the relative Rap-GTP amount. Cells expressing wild-type PlexinC1 show significantly lower amounts of GTP-bound Rap1B compared with those expressing the R1133A mutant ($p < 0.01$ by two-way ANOVA followed by Bonferroni post-hoc test, $n = 5$ experiments). Stimulation with AP20187 (the dimerizer) did not cause significant change in the amounts of GTP-bound Rap1B ($F_{1,16} = 0.54$, $p = 0.47$ by two-way ANOVA, $n = 5$ experiments). This lack of change could be because AP20187 treatment caused rapid loss of the plexin protein [shown in both (B) and (D)], and the ~3-fold increase in the RapGAP activity of FKBP-PlexinC1_{cyto} as shown by our in solution assays (fig. S2C) is offset by the loss of the plexin protein.

Western blots were quantified using ImageJ. Error bar: standard error of the mean. Asterisks (**) indicate statistical significance ($p < 0.01$).

(C) GST-RalGDS pull-down assays for endogenous Rap1 in HEK293T cells. The pull-down experiments and data analysis were performed as in (B), and an anti-Rap1 antibody (BD Transduction) was used for probing endogenous Rap1. Cells expressing the wild-type PlexinC1 have decreased amounts of GTP-bound endogenous Rap1 compared to those expressing the R1133A mutant. Error bar: standard error of the mean. Western blots were quantified using ImageJ. Asterisk (*) indicates statistical significance ($p < 0.05$ by Student's t-test, $n = 5$ experiments).

(D) Loss of the expressed Myr-FKBP-PlexinC1_{cyto} protein after AP20187 stimulation. The lower band likely corresponds to the N-terminally partially degraded protein because the Myc-tag is at the C-terminus. The rapid loss of the protein after AP20187 treatment is likely due to degradation as well, the mechanism of which is not understood at present.

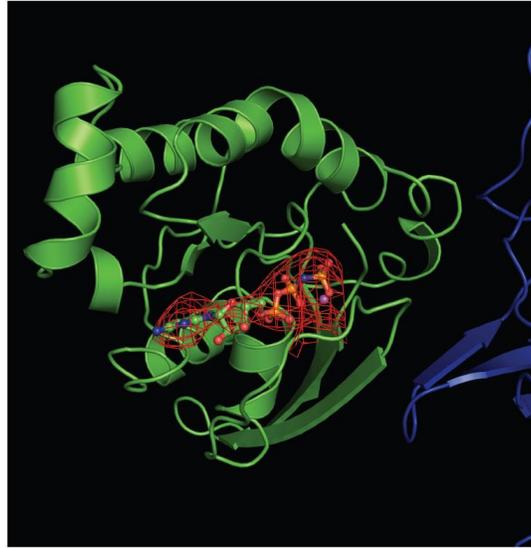
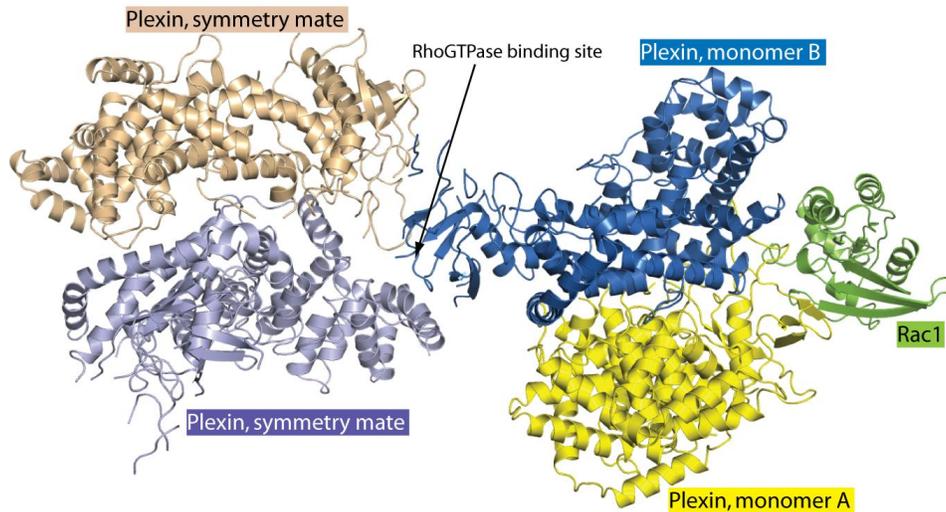
A**B**

Figure S4. Crystal structure of the PlexinA1_{cyto}-Rac1 complex.

(A) Electron density showing the presence of GMP-PNP/Mg²⁺ at the nucleotide-binding site in Rac1. The GMP-PNP/Mg²⁺ molecules in the final refined model are shown as a stick-and-ball model. Rac1 and plexin (partially shown) are colored in green and blue, respectively. The ($F_o - F_c$) electron density contoured at 3σ (red) was calculated prior to including GMP-PNP/Mg²⁺ in the model.

(B) Molecular packing in the crystal. The asymmetric unit contains two plexin monomers. Monomer A (yellow) is bound to Rac1 (green), whereas the binding site in monomer B (blue) is empty. The space between the RhoGTPase binding site of monomer B and two symmetry related plexin molecules is too small to accommodate Rac1.