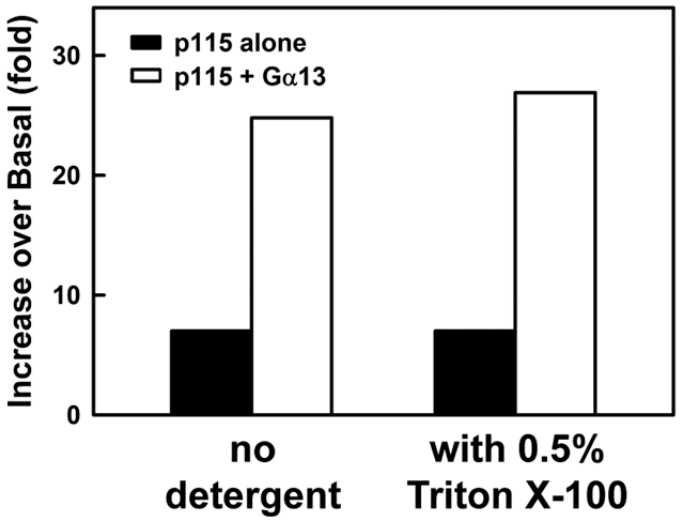
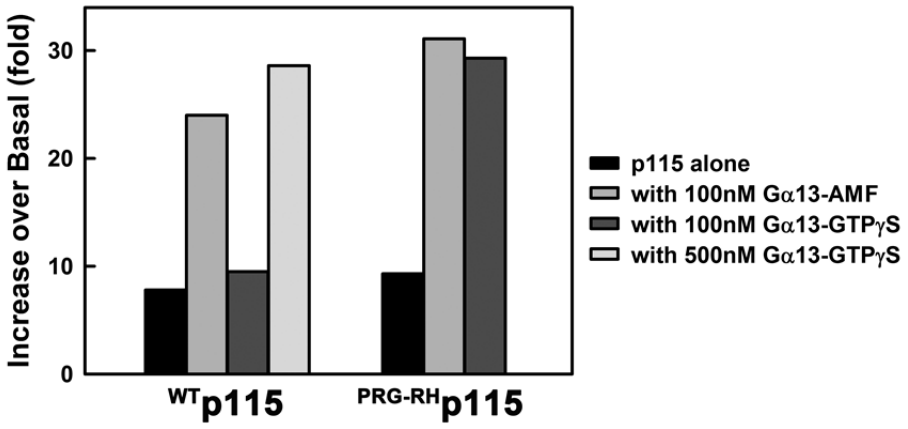
Supplementary Figure S1



Supplementary Figure S1. The presence of Triton X-100 had no effect on stimulation of GEF activity by Gα13. Nucleotide exchange assays with p115 RH-L-DH/PH and RhoA were carried out as described in Figure 1. Stimulation of the GEF activity of RH-L-DH/PH was measured in the presence of activated Gα13 bound to GDP●AlF_4^-●Mg^{2+} (GDP●AMF).

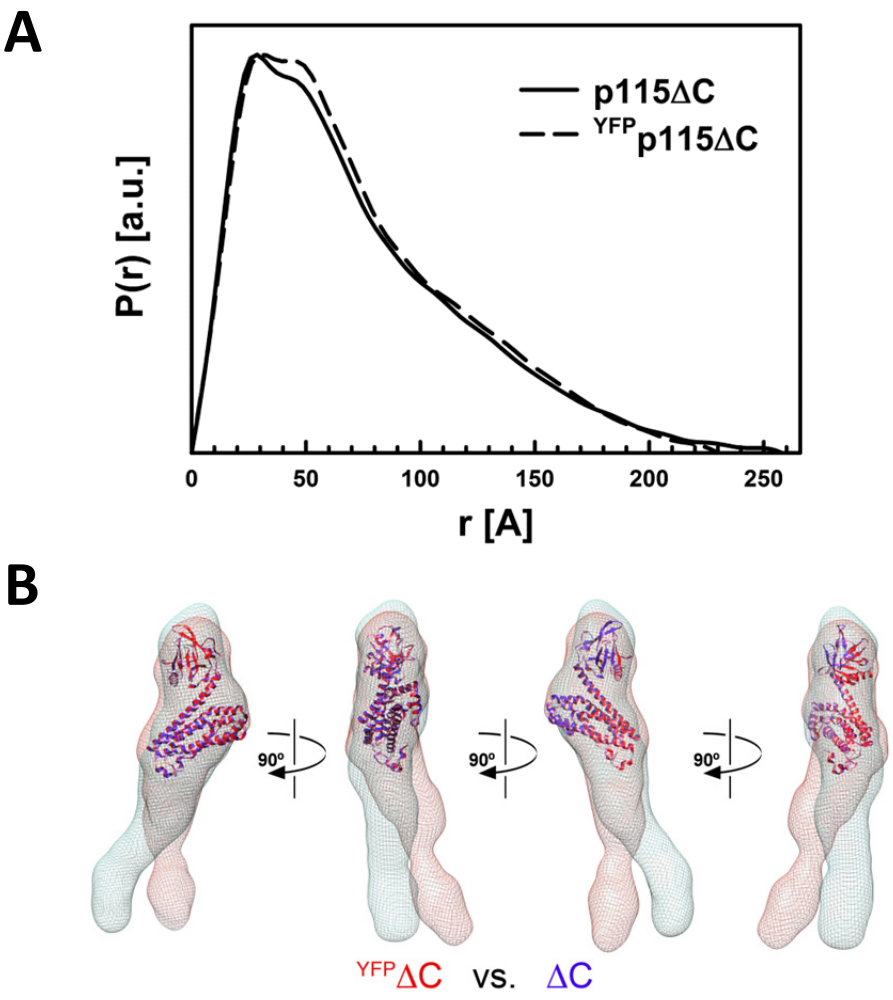
Supplementary Figure S2



Supplementary Figure S2. GAP activity of RH is not required for stimulation of GEF activity by Gα13. Nucleotide exchange assays with p115 RH-L-DH/PH and RhoA were carried out as described in Figure 1. Stimulation of GEF activity was measured in the presence of activated Gα13 bound to GDP●AMF or GTPγS as indicated. Replacement of the RH domain of p115 with RH from PRG had no effect on the stimulation of GEF activity by activated Gα13 bound to GDP●AMF. Gα13 bound to GTPγS was more efficient at stimulating the GEF activity of the chimeric ^{PRG}*RH*-L-DH/PH due to the higher binding affinity between the PRG-RH domain and Gα13●GTPγS (*1*).

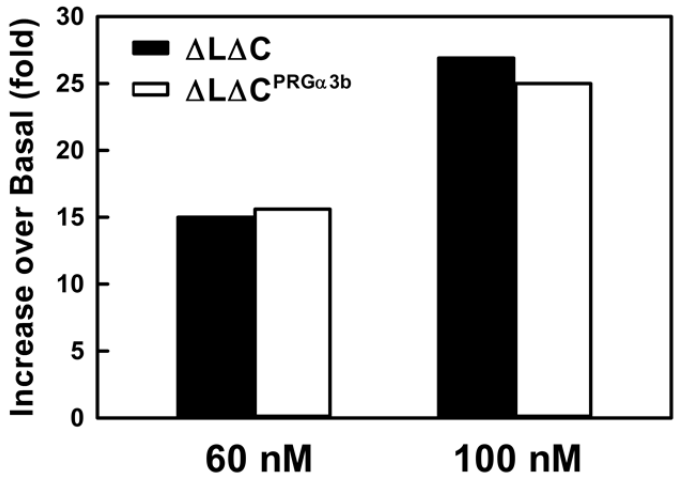
1. Chen, Z., Singer, W. D., Danesh, S. M., Sternweis, P. C., and Sprang, S. R. (2008) Recognition of the activated states of Galpha13 by the rgRGS domain of PDZRhoGEF, *Structure 16*, 1532-1543.

Supplementary Figure S3



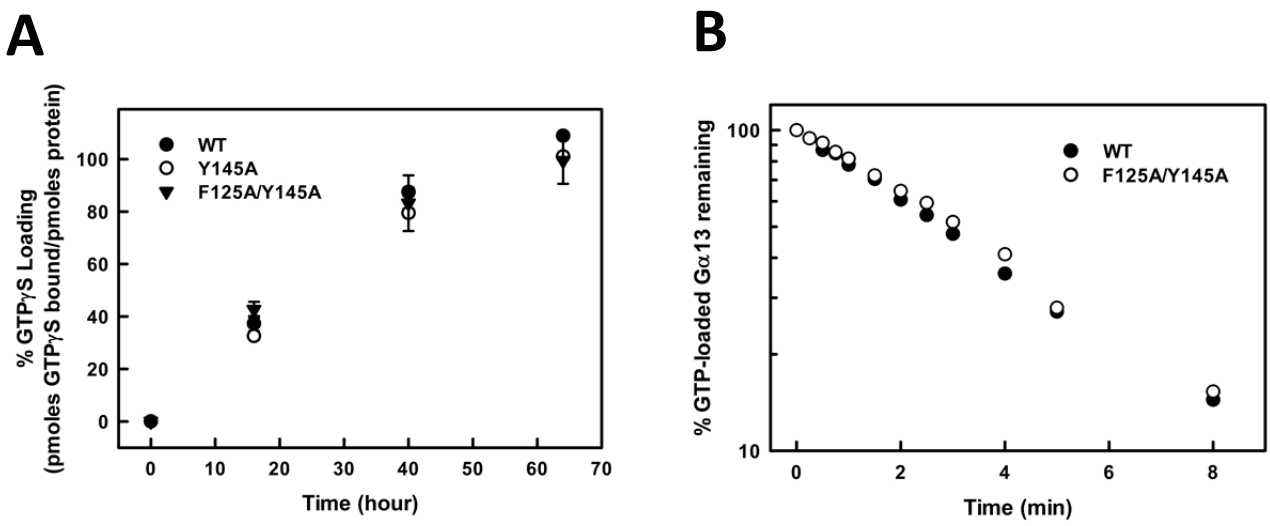
Supplementary Figure S3. Comparison of the solution structures of wild-type RH-L-DH/PH and ^{YFP}RH-L-DH/PH. **(A)** Comparison of the distance distribution functions of wild-type p115 and the YFP-fusion protein. **(B)** Molecular envelopes of the wild-type p115 and the YFP-fusion proteins were superimposed based on the modeled DH/PH structure. Insertion of YFP within the RH domain caused significant changes in conformation beneath the DH domain.

Supplementary Figure S4



Supplementary Figure S4. The replacement of the α3b region in p115 with that from PRG had no effect on the GEF activity of the RH-DH/PH (ΔLΔC) fragment of p115. p115 has significantly higher basal exchange activity towards RhoA in the absence of the auto-inhibitory linker region. In the $\Delta L\Delta C^{PRG\alpha 3b}$ construct, residues 503-507 from p115 were substituted with corresponding residues from PRG. As in the cases shown in Figure 5 and Figure 7, such substitution had no effect on the GEF activity of RH-DH/PH. Taken together, these data demonstrated that mutations to the α3b region of p115 do not compromise intrinsic capacity of the protein to activate RhoA. Nucleotide exchange assays with RH-DH/PH and RhoA were carried out as described in Figure 1. Two concentrations (60 nM and 100 nM) of RH-DH/PH were tested.

Supplementary Figure S5



Supplementary Figure S5. (A) Mutations at the αB-αC region did not affect binding of Gα13 to guanine nucleotide. GTPγS-binding assays of Gα13 were carried out at 20 °C: wild-type (WT, closed circles), Y145A (open circles), F125A/Y145A (closed triangles). Reactions contained 10 μM Gα13 (wild-type or mutant) and 100 μM [^{35}S]GTPγS, in a solution containing 20 mM Na·HEPES, pH 8.0, 1 mM EDTA, 1 mM DTT, 100 mM NaCl and 1 mM $MgCl_2$. At the indicated time points, 10 uL aliquots were removed from the reaction mixture and assessed for protein-bound [^{35}S]GTPγS by filter binding. **(B)** Mutations at the αB-αC region did not affect the intrinsic GTPase activity of Gα13. Single-turnover GTPase assays (4 °C) of wild-type Gα13 (WT, closed circles) or the F125A/Y145A mutant (open circles) were carried out as described (*2*).

2. Chen, Z., Singer, W. D., Sternweis, P. C., and Sprang, S. R. (2005) Structure of the p115RhoGEF rgRGS domain-Galpha13/i1 chimera complex suggests convergent evolution of a GTPase activator, *Nat Struct Mol Biol 12*, 191-197.