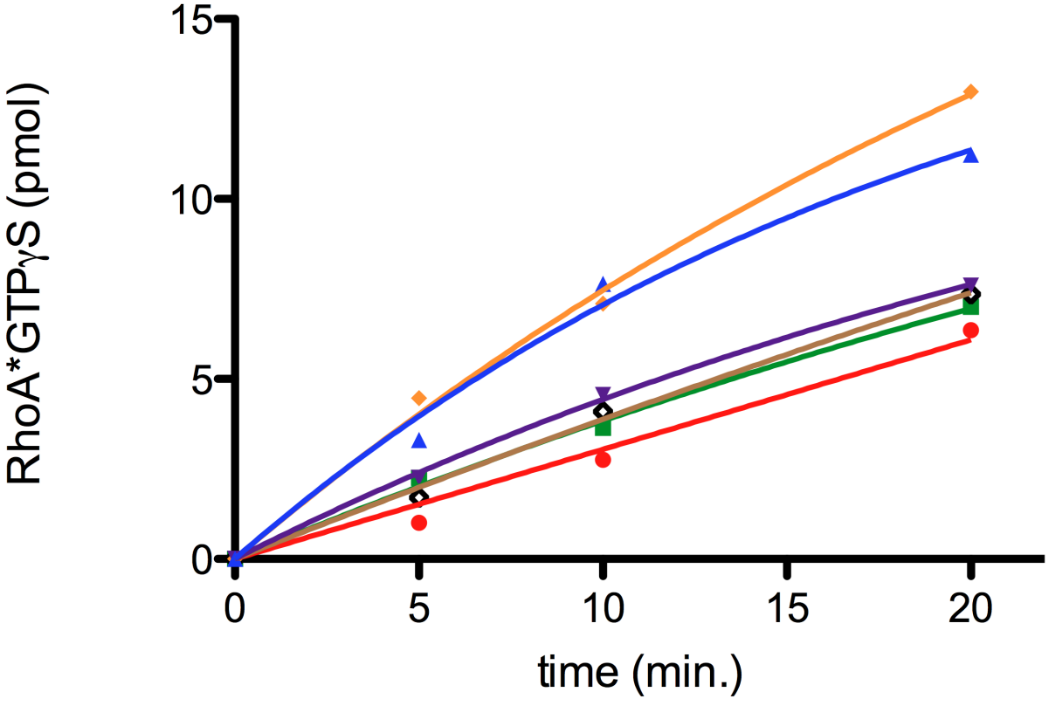
Supplemental Figure Legends

Supplemental Figure 1: The T274E mutation in Gα13 impairs RhoA activation *in vitro*. GTPγS binding to RhoA (final concentration 500 nM) was measured in the presence of buffer, 5 nM p115RhoGEF, or 5 nM p115RhoGEF and 100 nM AlF_4^--activated Gα13 (wild-type or mutant). Samples were incubated at 30 °C and quenched in ice-cold buffer containing 10 mM $MgSO_4$ at the indicated times. Samples are: RhoA only (●); p115RhoGEF (■); p115RhoGEF + Gα13 T274E/N278A (□); p115RhoGEF + Gα13 T274E (◊); p115RhoGEF + Gα13 N278A (□); p115RhoGEF + Gα13 (▲). Data presented are from a single experiment.

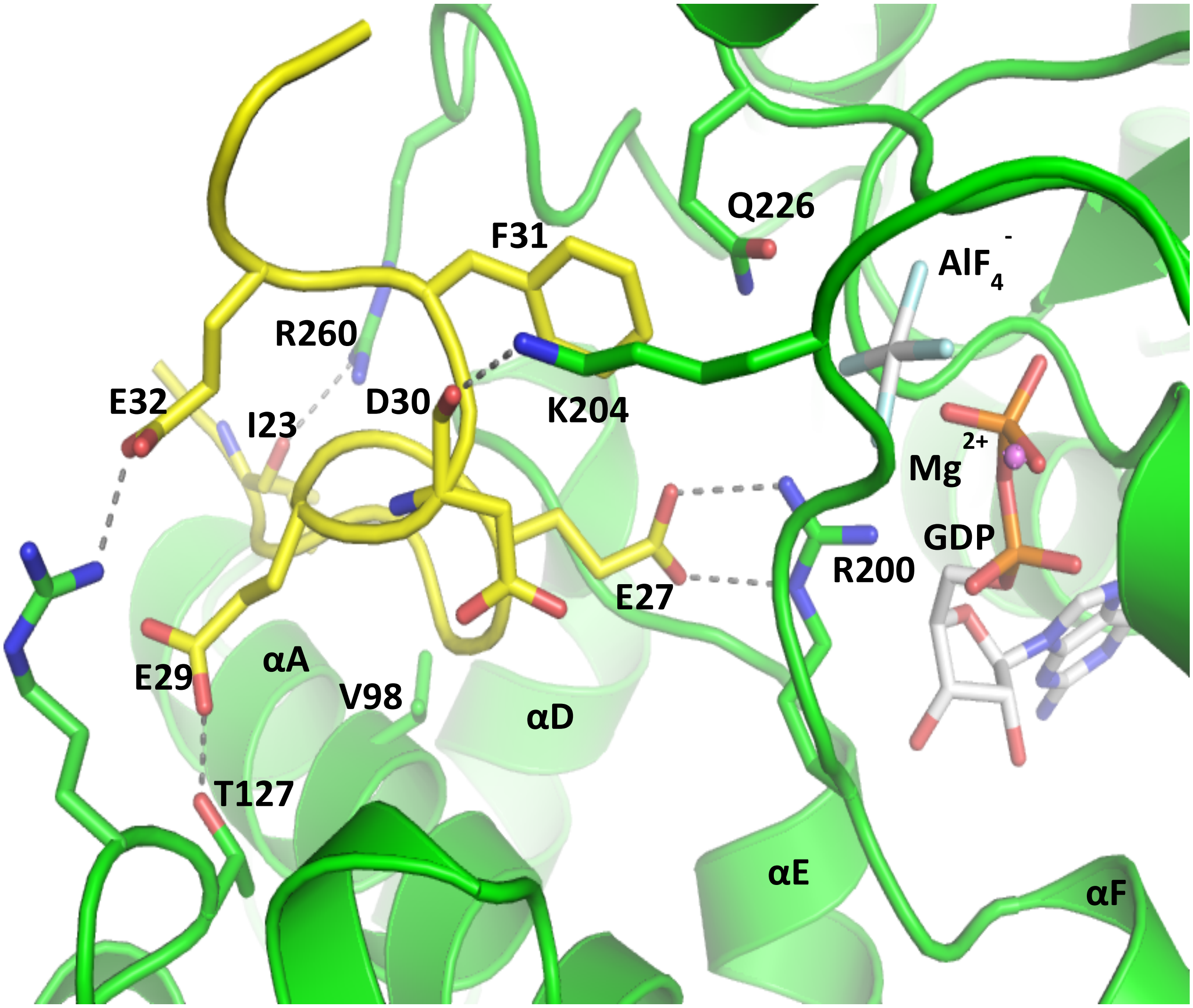
Supplemental Figure 2: A detailed view of the Gα13-p115 RH GAP interface. The complex is shown as a ribbon diagram with Gα13 colored in green and the RH domain of p115RhoGEF colored in yellow. Oxygen and nitrogen atoms are colored red and blue, respectively. Phosphate atoms are colored orange, while aluminum and fluoride atoms are light grey and light blue, respectively. GDP and AlF_4^- are depicted as stick models, while the Mg^{2+} co-factor is a purple sphere. Hydrogen bonds are depicted as dashed lines.

Supplemental Figure 3: Mutational analysis of the Gα12-RH-RhoGEF effector interface. **Upper panel**: The T277E and N281A mutations in Gα12 impair Rho activation in cells. HeLa cells were transiently transfected with empty vector or the indicated Gα12 QL construct. The luciferase activity of cell lysates was determined as described in Materials and Methods. Data are presented as the mean (± S.E.M.) of triplicate determinations from a single experiment, representative of two independent experiments with similar results. Total cell lysate was immunoblotted for either Gα12 or GAPDH. Data were analyzed by one-way ANOVA, followed by Dunnett's post-test. Statistically significant difference from Gα12 QL: ns, not significant; **, $p<0.01$. **Lower panel**: The T277E mutation in Gα12 impairs binding to the RH domain of p115RhoGEF. HEK293 cells were transiently transfected with wild-type Gα12 or the indicated mutant. Detergent-soluble cell extracts were incubated with 250 pmoles of GST-p115 RH in the presence or absence of AlF_4^- and GST-p115 RH was pulled down with glutathione Sepharose beads. Bound proteins were released by the addition of SDS-PAGE sample buffer and boiling, and separated by SDS-PAGE. Gα12 was detected by immunoblotting, and GST-p115 RH was stained with Coomassie Brilliant blue. Data presented are from one experiment, representative of two independent experiments with similar results.

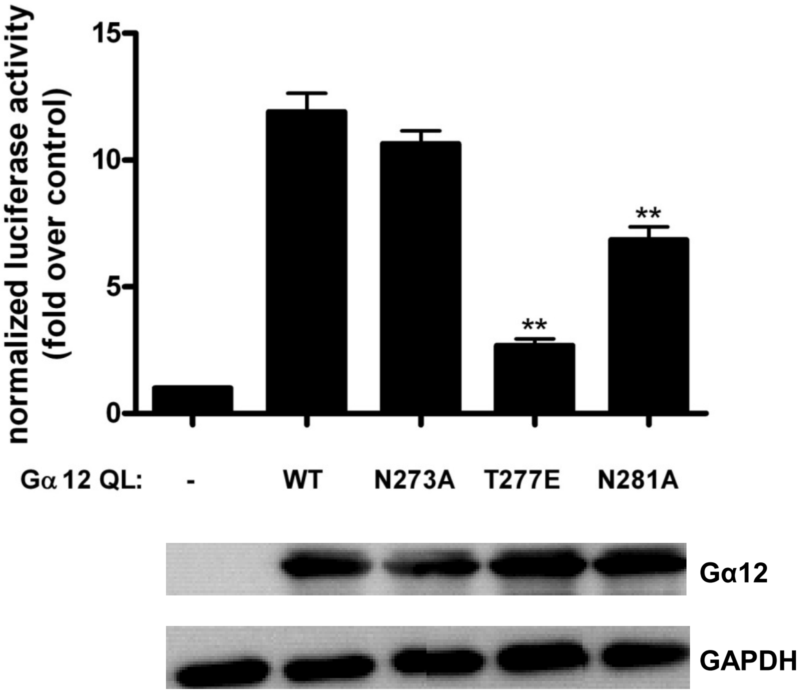
Supplemental Figure 1

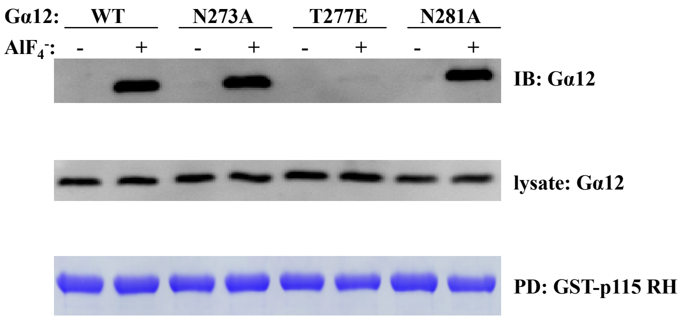


Supplemental Figure 2



Supplemental Figure 3





Supplemental Table I

p115RhoGEF residues (Chain B)

G$\alpha_{i/13}$ residues (Chain A)

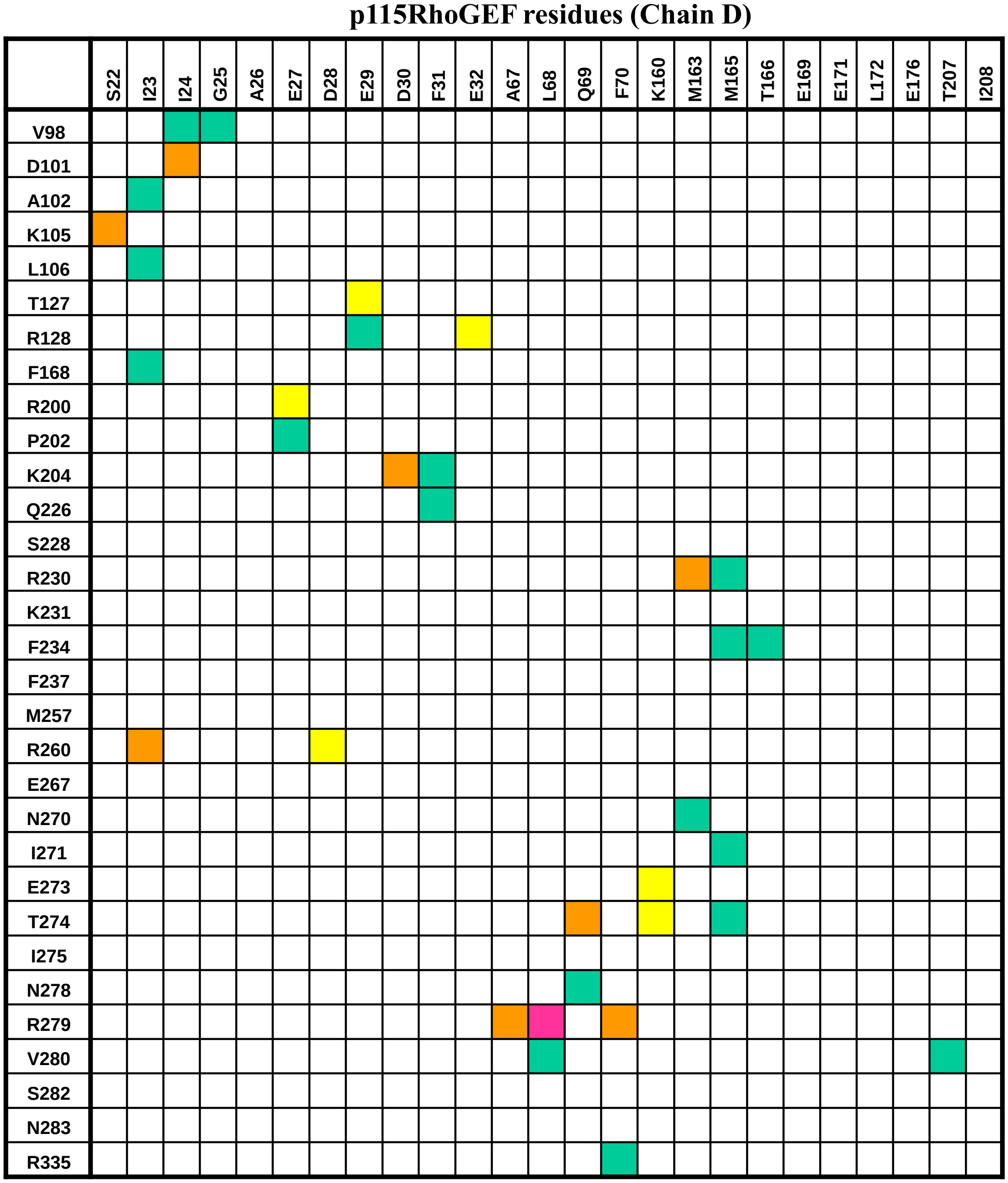
	S22	I23	I24	G25	A26	E27	D28	E29	D30	F31	E32	A67	L68	Q69	F70	K160	M163	M165	T166	E169	E171	L172	E176	T207	I208
V98					🟩																				
D101			🟧																						
A102																									
K105	🟧	🟩																							
L106																									
T127								🟨																	
R128								🟩			🟨														
F168																									
R200						🟨																			
P202						🟩																			
K204									🟧	🟩															
Q226										🟩															
S228																									
R230																	🟧	🟩							
K231																				🟩					
F234																		🟩	🟩						
F237																									
M257										🟩															
R260		🟧																							
E267																									
N270																🟧	🟩								
I271																		🟩							
E273																🟨									
T274														🟧											
I275																		🟩							
N278													🟩												
R279												🟧	🟥		🟧										
V280													🟩											🟩	
S282																									
N283																									
R335															🟩										

Residues involved in

🟩 **hydrophobic contact(s)** 🟧 **M-S hydrogen bond(s)** 🟨 **S-S hydrogen bond(s)** 🟥 **M-M hydrogen bond(s)**

M- main chain
S- side chain

Supplemental Table II



p115RhoGEF residues (Chain D)

$G\alpha_{i/13}$ residues (Chain C)

	S22	I23	I24	G25	A26	E27	D28	E29	D30	F31	E32	A67	L68	Q69	F70	K160	M163	M165	T166	E169	E171	L172	E176	T207	I208
V98			🟩	🟩																					
D101			🟧																						
A102		🟩																							
K105	🟧																								
L106		🟩																							
T127								🟨																	
R128								🟩			🟨														
F168		🟩																							
R200						🟨																			
P202						🟩																			
K204									🟧	🟩															
Q226										🟩															
S228																									
R230																	🟧	🟩							
K231																									
F234																		🟩	🟩						
F237																									
M257																									
R260		🟧					🟨																		
E267																									
N270																	🟩								
I271																		🟩							
E273																🟨									
T274														🟧		🟨		🟩							
I275																									
N278														🟩											
R279												🟧	🟥		🟧										
V280													🟩											🟩	
S282																									
N283																									
R335															🟩										

Residues involved in

🟩 hydrophobic contact(s) 🟧 M-S hydrogen bond(s) 🟨 S-S hydrogen bond(s) 🟥 M-M hydrogen bond(s)

M- main chain
S- side chain

Supplemental Table III

PDZ-RhoGEF residues

G$\alpha_{i/13}$ residues

	L307	I308	I309	G310	P311	E312	-	E313	D314	Y315	D316	L349	S350	Q351	A352	K439	L442	L444	G445	-	S446	L447	N451	K480	IY481
V98				🟩																					
D101			🟧																						
A102																									
K105																									
L106		🟩																							
T127								🟨																	
R128																									
F168		🟩																							
R200						🟨																			
P202						🟩																			
K204																									
Q226										🟩															
S228										🟧															
R230																	🟧								
K231																					🟧				
F234																					🟩		🟩		
F237																						🟩			
M257										🟩															
R260		🟧																							
E267																	🟩								
N270																									
I271																									
E273																🟨									
T274														🟧		🟩		🟩							
I275																									
N278														🟩											
R279													🟪												🟩
V280																									
S282																								🟨	
N283																								🟨	
R335														🟧											

Residues involved in

🟩 **hydrophobic contact(s)** 🟧 **M-S hydrogen bond(s)** 🟨 **S-S hydrogen bond(s)** 🟪 **M-M hydrogen bond(s)**

M- main chain
S- side chain