

Supplemental Materials

Molecular Biology of the Cell

Reinhard et al.

Reinhard et al., Supplementary data

Legends to supplementary figures.

Figure S1. S1P induces EC morphology changes, resulting in net EC spreading. (A) ECs, transiently transfected with mTq2, were grown to a monolayer. Local changes in the area of a single cell were measured before and after stimulation with S1P at $t = 0:30$ min. Colors represent area change following S1P stimulation (LUT on the right). Scale bar = $25\mu\text{m}$. (B) Normalized mean EC area changes before and after S1P stimulation. Graphs and tables represent two technical replicates of a total of $n=14$.

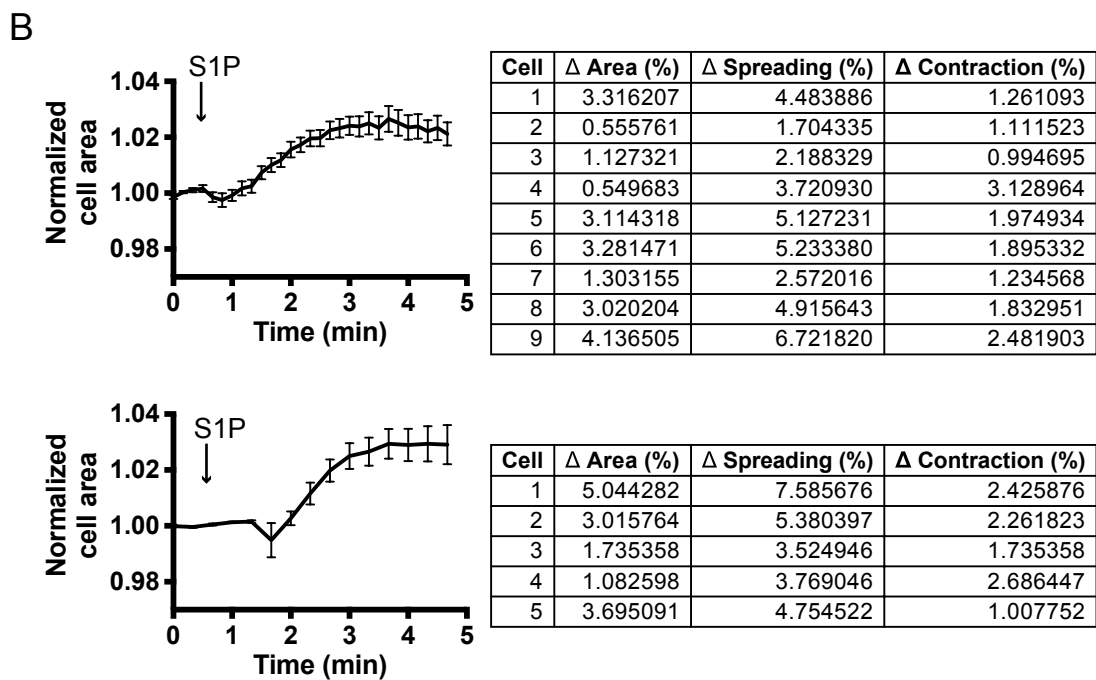
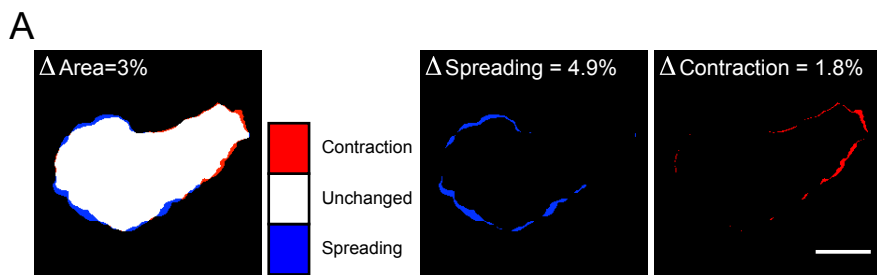
Figure S2. Rac1 activation via recruitment of TIAM induces an increase in cell area, while p63RhoGEF-mediated activation of Rho induces a decrease in cell area. (A and B) Normalized mean EC area changes, before and after rapamycin addition at $t = 1:10$. Plots on the right represent total cell area changes after 12 min. for TIAM ($n=23$) and p63RhoGEF ($n=20$).

Figure S3. S1P induces RhoB and RhoC activation. (A) Ratiometric images of ECs that were transfected with the RhoB or RhoC FRET sensor and stimulated with S1P (500 nM) at $t = 1$ min, 50 sec. Warm colors represent high activation (high YFP/CFP ratios), corresponding to emission ratio's (ER) on the right. Scale bar = $15\mu\text{m}$. (B) Normalized mean YFP/CFP ratio traces ($\pm\text{SEM}$) for RhoB ($n=23$) and RhoC ($n=17$) FRET sensor-expressing EC before and after stimulation with S1P.

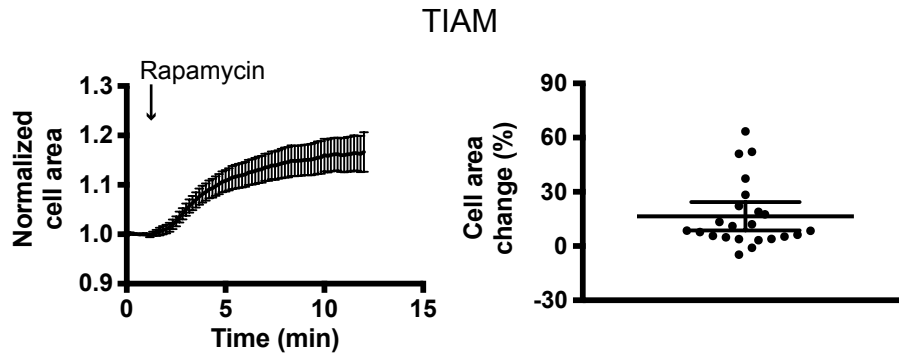
Figure S4. S1P stimulation of Rac1 FRET sensor expressing cells, shows two populations of responders. (A and B) Normalized mean YFP/CFP ratio traces ($\pm\text{SEM}$) for (A) Rac1 FRET sensor expressing EC reaching an $\text{YFP/CFP}_{\text{max}}$ above baseline ($n=18$) and (B) Rac1 FRET sensor expressing EC lacking an $\text{YFP/CFP}_{\text{max}}$ above baseline ($n=14$), before and after stimulation with S1P.

Figure S5. PTX induces a transient decrease in endothelial resistance. ECs, plated on ECIS electrodes, were stimulated with PTX (100ng/ml) at $t = 1:51$ before stimulation with S1P (Figure 6D). Endothelial resistance (4000 Hz) was measured using the ECIS.

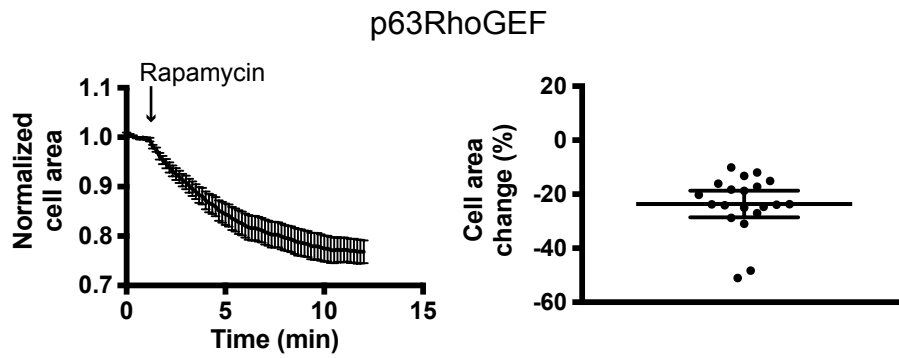
Figure S6. Membrane-linked p115-RGS most efficiently inhibits $G\alpha_{13}$ -QL-mediated RhoA activation. YFP/CFP ratios (error bars depict 95% CI) of HeLa cells transfected with RhoA FRET sensor + mCherry (n=55), RhoA FRET sensor + mCherry + $G\alpha_{13}$ -QL (n=74), RhoA FRET sensor + RGS-mCherry + $G\alpha_{13}$ -QL (n=81), RhoA FRET sensor + mCherry-RGS + $G\alpha_{13}$ -QL (n=85) or RhoA FRET sensor + Lck-mCherry-RGS + $G\alpha_{13}$ -QL (n=96).



A

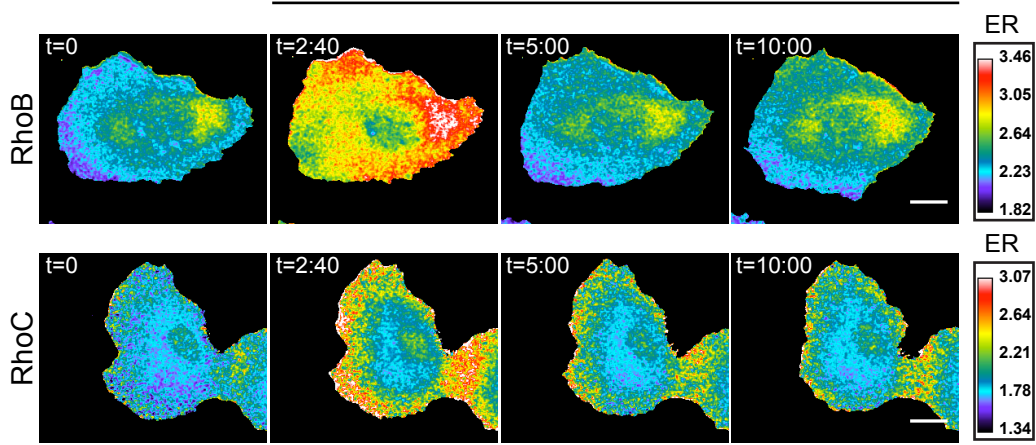


B

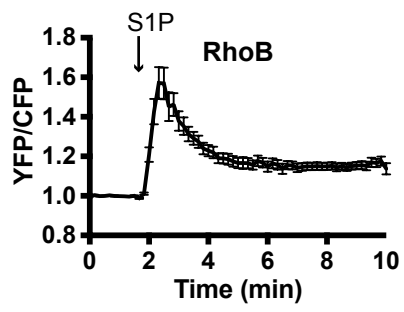


A

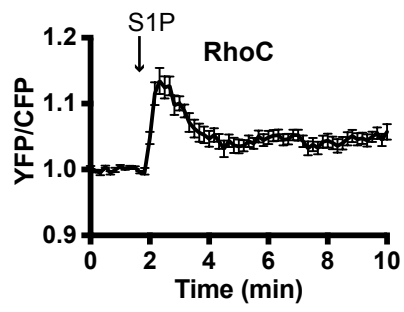
S1P



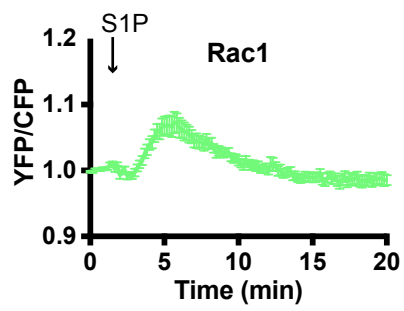
B



C



A



B

