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### The balance between $G\alpha_{12/13}$ -Cdc42/Rac and $G\alpha_{12/13}$ -RhoA pathways determines endothelial barrier regulation by sphingosine-1-phosphate

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**DOI**

[10.1091/mbc.E17-03-0136](https://doi.org/10.1091/mbc.E17-03-0136)

**Publication date**

2017

**Document Version**

Other version

**Published in**

Molecular Biology of the Cell

[Link to publication](#)

**Citation for published version (APA):**

Reinhard, N. R., Mastop, M., Yin, T., Wu, Y., Bosma, E. K., Gadella, T. W. J., Goedhart, J., & Hordijk, P. L. (2017). The balance between  $G\alpha_{12/13}$ -Cdc42/Rac and  $G\alpha_{12/13}$ -RhoA pathways determines endothelial barrier regulation by sphingosine-1-phosphate. *Molecular Biology of the Cell*, 28(23), 3371-3382. <https://doi.org/10.1091/mbc.E17-03-0136>

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# 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  $n=14$ .

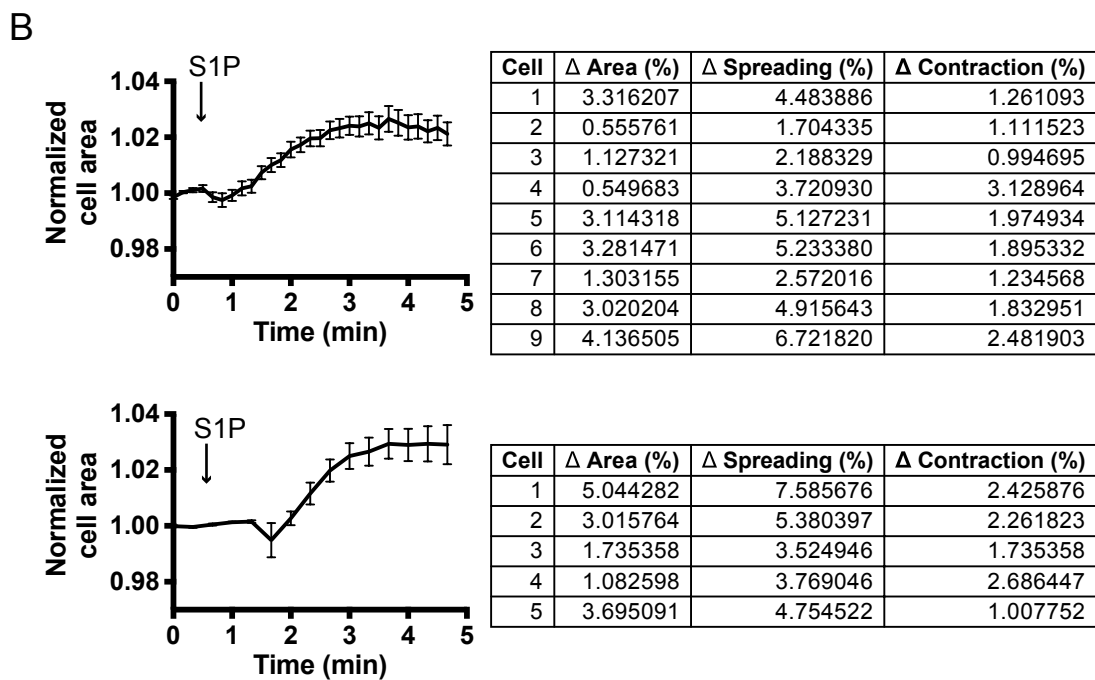
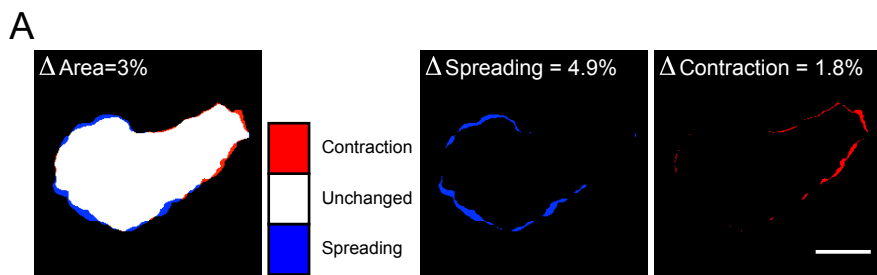
**Figure S2.** Rac1 activation via recruitment of TIAM induces an increase in cell area, while p63RhoGEF-mediated activation of Rho induces a decrease in cell area. (A and B) Normalized mean EC area changes, before and after rapamycin addition at  $t = 1:10$ . Plots on the right represent total cell 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 ( $500\text{ nM}$ ) 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.

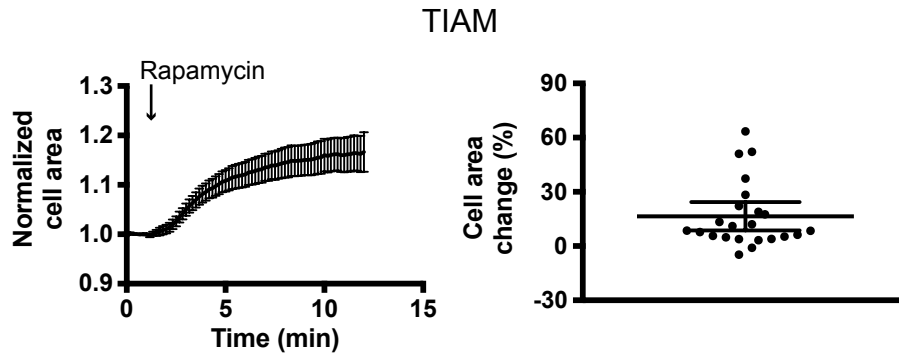
**Figure S4.** S1P stimulation of Rac1 FRET sensor expressing cells, shows two populations of responders. (A and B) Normalized mean YFP/CFP ratio traces ( $\pm\text{SEM}$ ) for (A) Rac1 FRET sensor expressing EC reaching an  $\text{YFP/CFP}_{\text{max}}$  above baseline ( $n=18$ ) and (B) Rac1 FRET sensor 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 ( $100\text{ng/ml}$ ) at  $t = 1:51$  before stimulation with S1P (Figure 6D). Endothelial resistance ( $4000\text{ Hz}$ ) was measured using the ECIS.

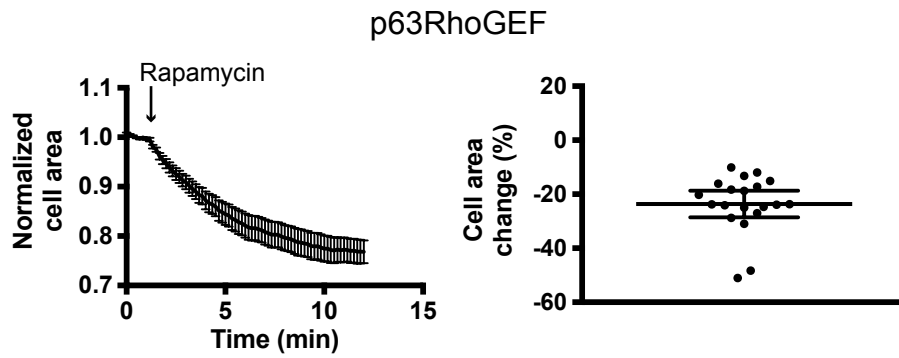
**Figure S6.** Membrane-linked p115-RGS most efficiently inhibits  $G\alpha_{13}$ -QL-mediated RhoA activation. YFP/CFP ratios (error bars depict 95% CI) of HeLa cells transfected with RhoA FRET sensor + mCherry (n=55), RhoA FRET sensor + mCherry +  $G\alpha_{13}$ -QL (n=74), RhoA FRET sensor + RGS-mCherry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

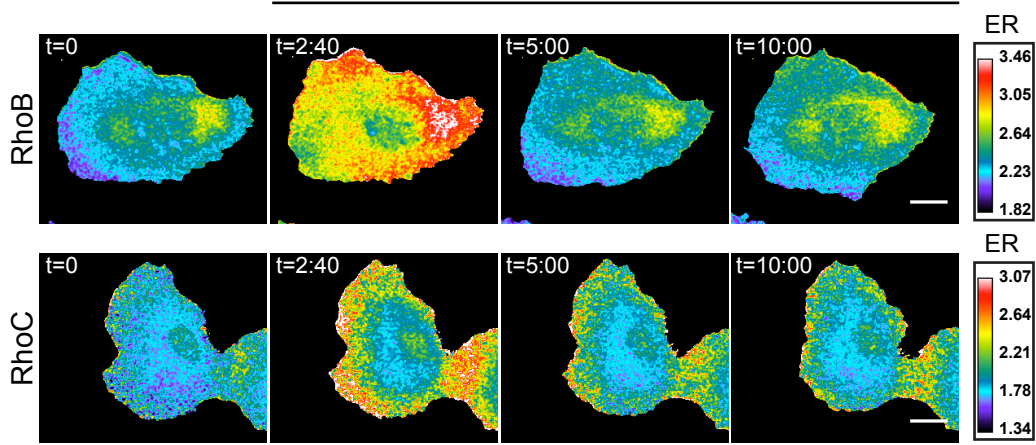


B

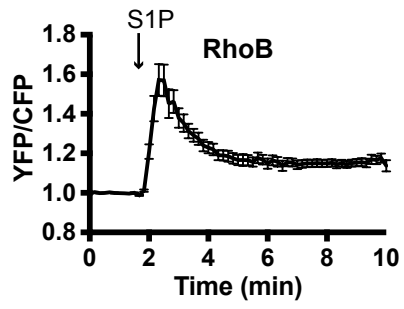


A

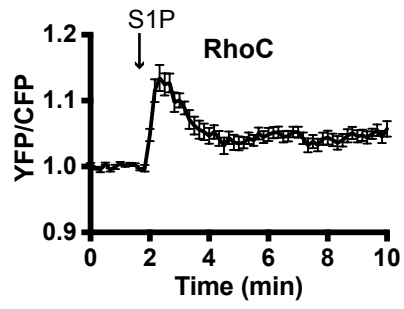
S1P



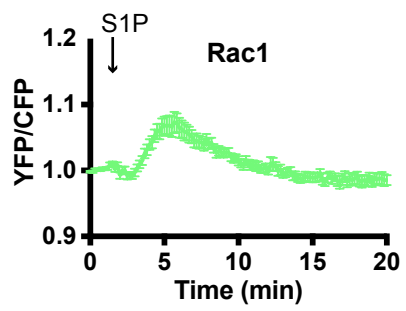
B



C



A



B

