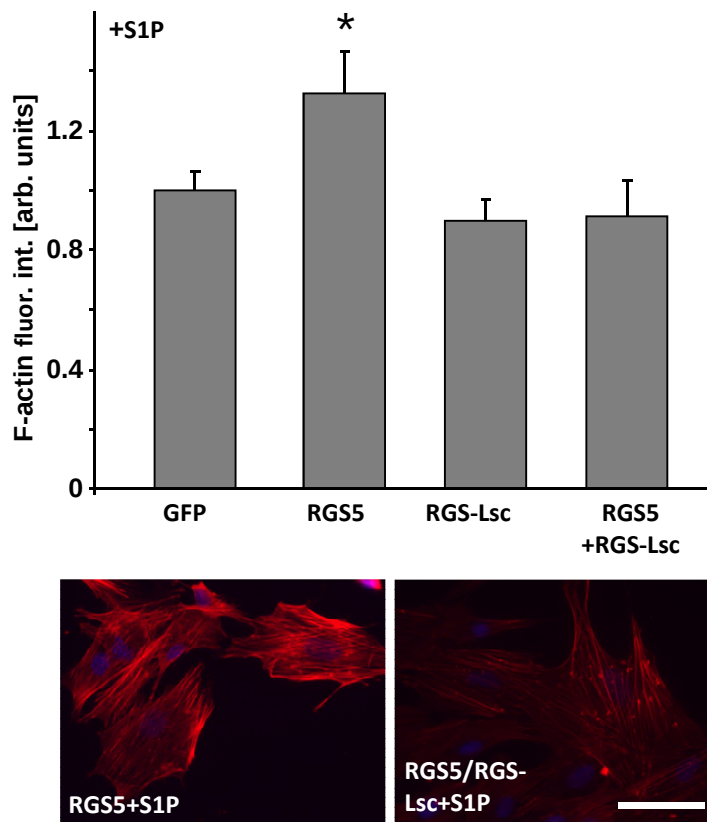


Supplemental Figure 1



Analysis of the impact of RGS-Lsc on S1P-induced stress fiber formation in RGS5-overexpressing SMCs

Umbilical artery SMCs were transduced with an adenoviral control vector (GFP), RGS5 expression vector (RGS5) or RGS-Lsc expression vector (RGS-Lsc) alone or both RGS5 and RGS-Lsc expression vectors. Stress fibers (F-actin) were visualized after stimulating the cells with S1P (15 min, 10 μ mol) and exposing them to TRITC-labelled phalloidin (1:200) for 20 minutes.

Stress fiber formation in S1P-stimulated, RGS5-over-expressing cells was enhanced while simultaneous overexpression of RGS5 and RGS-Lsc abrogated this effect (* p <0.05 vs. control GFP-overexpressing cells; shown are the means $\pm$ SD of one experiment summarizing three randomly selected fields of view per condition determining the cumulative phalloidin fluorescence intensity of at least four (transduced) cells, scale bar 20 μ m). This result suggests that S1P-stimulated Rho-dependent stress fiber formation in RGS5-overexpressing cells is due to the activation of $G_{\alpha 12/13}$ and subsequent RGS-Lsc activation.