Supplementary Information

Activation of Rac1 and RhoA preserve corneal endothelial barrier function

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Supplementary Figure 1. RhoA-ROCK and Rac1 inhibition do not prevent endothelial barrier dysfunction in response to hyperosmotic stress. Absolute resistance values. (A) HCECs were grown to confluency in plastic dishes and the effect of C3 transferase and Ehop-016 on Rho and Rac1 activation, respectively, were measured by pulldown assays. Ct, control cells in HBSS. Note the shift in RhoA molecular weight upon C3 transferase treatment. (B-D) Absolute resistance values of experiments shown Figure 4. HCECs were grown to confluency in ECIS arrays containing gold electrodes and absolute levels of TEER (resistance) were analyzed by ECIS. (B) Effect of C3-transferase (0.5 µg/ml) on barrier contraction induced with 50 mM excess NaCl and the subsequent recovery upon return to iso-osmolarity. (C) Effect of Y-27632 (5 µM) on barrier contraction and the subsequent recovery upon return to iso-osmolarity. (D) Effect of Ehop-016 (5 µM) on barrier contraction and the subsequent recovery upon return to iso-osmolarity.
Supplementary Figure 2. Murine cornea dissection for *ex vivo* experiments. Adult female mice were sacrificed according to the institutional guidelines from the CBMSO animal house facility and murine eyes were enucleated. Lens and iris were removed and the cornea maintained in Tissue C medium at room temperature before hyperosmotic assays and barrier recovery assays were carried out.
Supplementary Figure 3: Effect of RhoA-ROCK inhibition during barrier contraction in HUVECs. ZO-1 immunostaining and F-actin labeling of confluent HUVEC treated with C3-transferase (0.5 µg/ml) for 2 h and Y-27632 (5 µM) for 30 min when indicated. Acute contraction was induced with 1 U/ml thrombin for 30 min when indicated. Note that C3-transferase and Y-27632 treatment induced a clear decrease of F-actin in unstimulated and thrombin-stimulated cells. Scale bar, 10 µm.
Supplementary Figure 4: Effect of Act-I and Act-II on RhoA, Rac1 and Cdc42. Active Rho GTPases were analyzed by pulldown assays in untreated HCECs and in HCECs treated with Act-I or Act-II for 2 h. Act-I (I) mainly induced Rac1 activation. Act-II (II) specifically induced RhoA activation. Ct, control cells in HBSS.
Supplementary Figure 5. RhoA and Rac1 activation preserve endothelial barrier function. Absolute resistance values of experiments shown Figure 7. HCECs were grown to confluence in ECIS arrays containing gold electrodes and TER (resistance) was analyzed by ECIS. (A) Effect of pretreatment with 1 mg/ml Act-I (RhoA and Rac1), Act-I + 5 mM Y-27632 (Rac1), and 5 mg/ml Act-II (RhoA) on barrier integrity. Contraction upon osmotic stress was induced with an excess of 50 mM NaCl. (B) Effect of treatment with Act-I (RhoA and Rac1 activation), Act-I + Y-27632 (Rac1 activation), and Act-II (RhoA activation) during the return to iso-osmolarity (recovery). To better analyze recovery upon stress withdrawal, cells were exposed to osmotic stress with an excess of 150 mM NaCl, which induced greater barrier disruption.