

SUPPLEMENTAL MATERIAL

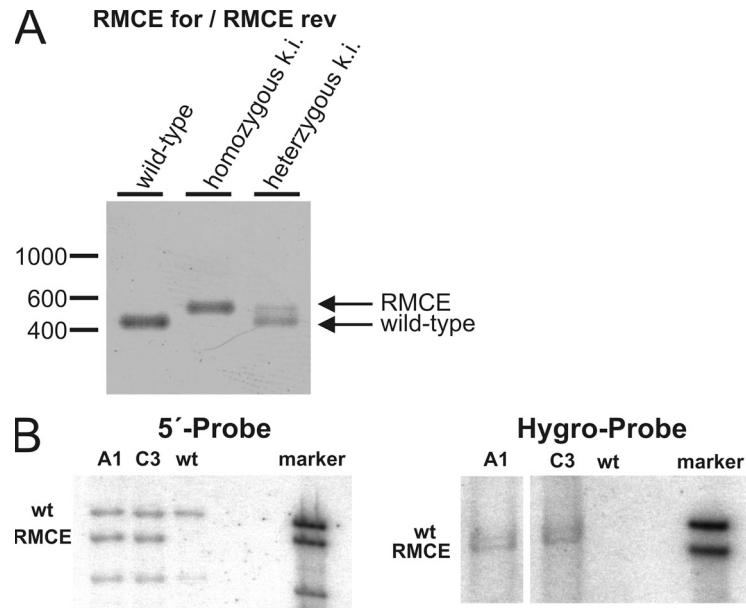
Broermann et al., <http://www.jem.org/cgi/content/full/jem.20110525/DC1>

Figure S1. Analysis of KI mice and ES cells. (A) Genotyping of KI mice was performed by PCR with primers RMCEfor/RMCErev resulting in 520- and 565-bp products for the WT and the KI allele, respectively. (B) ES cells were analyzed by Southern blot confirming correct homologous recombination. The two clones (A1 and C3) were analyzed using an external probe (5'-Probe) and an internal Probe (Hygro-Probe); WT indicates the wild-type control. Data are representative of two independent experiments (A-C).

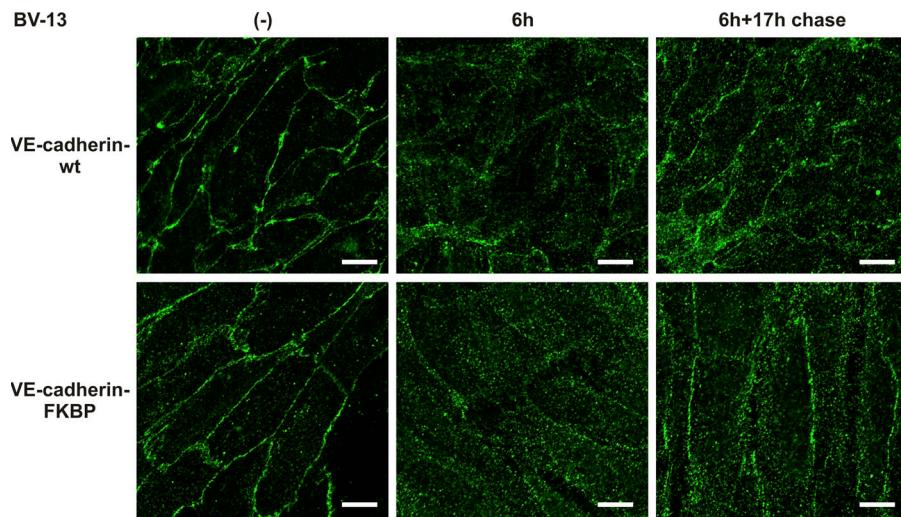


Figure S2. Antibodies mobilize VE-cadherin-FKBP away from junctions as efficiently as VE-cadherin. Primary murine endothelial cells isolated from lungs of WT mice (top row) or from VE-cadherin-FKBP KI mice (bottom row) were grown to confluence on transwell filters. Cells were either left untreated (-), incubated with anti-VE-cadherin mAb BV-13 (50 µg/ml) for 6 h, or incubated with BV13 for 6 h, followed by removal of the antibody and additional culturing for 17 h. Cells were fixed and VE-cadherin was visualized by indirect immunofluorescence staining with a rabbit antibody against VE-cadherin and analysis by laser scanning microscopy using an LSM 780 inverted microscope. Bars, 10 µm. Data are representative of two independent experiments.

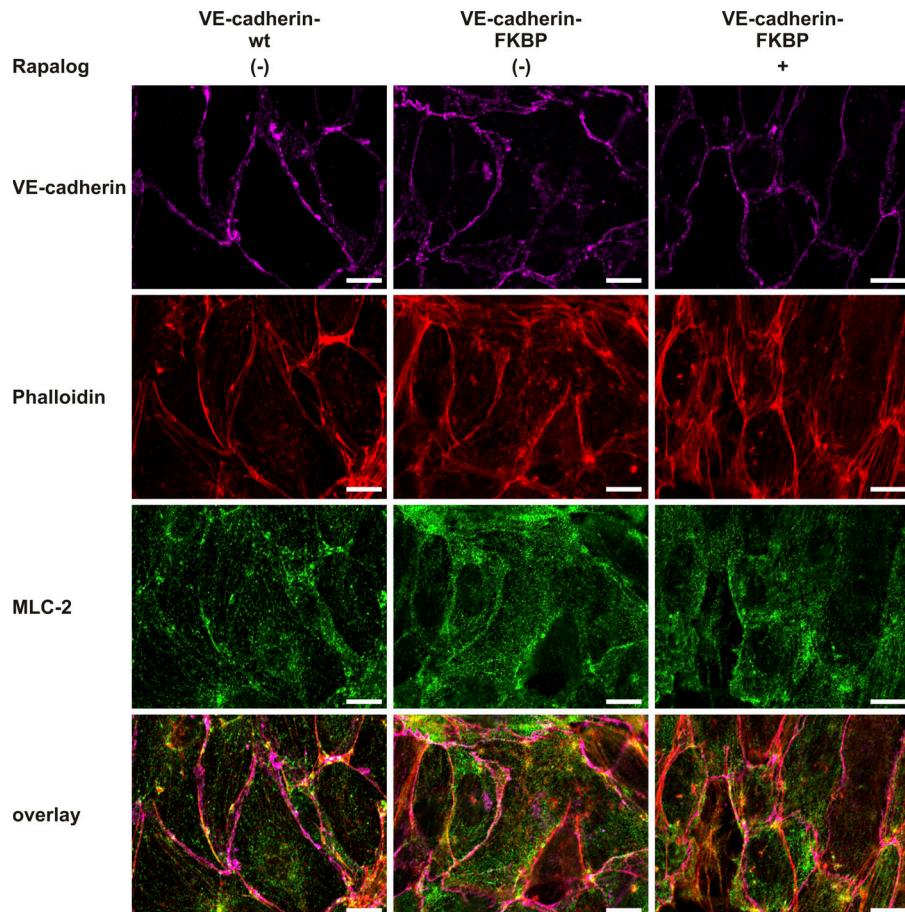


Figure S3. Expression of VE-cadherin-FKBP and application of rapalog does not alter actin organization. Primary murine endothelial cells isolated from lungs of WT mice (left row) or from VE-cadherin-FKBP KI mice (middle and right row) were grown to confluence on transwell filters. Before fixation and immunostaining, cells were either treated with 250 nM rapalog (+) or vehicle (−) alone for 45 min. Expression and localization of VE-cadherin, actin, and MLC-2 (as indicated) was analyzed using indirect immunofluorescence. Visualization was done by confocal laser scanning microscopy using a LSM 780 inverted microscope. Bars, 10 μ m. Data are representative of two independent experiments.

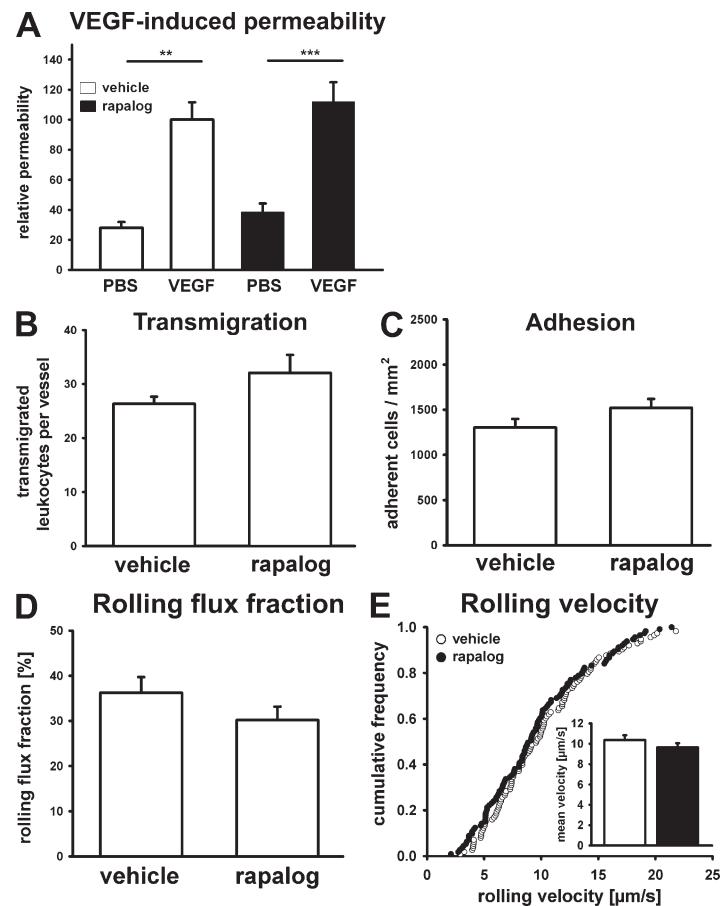


Figure S4. Rapalog does not inhibit permeability induction or leukocyte recruitment in WT mice.(A) WT mice were injected i.v. with rapalog (filled bars) or vehicle alone (open bars) 8 and 4 h before the assay. At the start of the assay, Evan's blue dye was i.v. injected, followed by intradermal injection of VEGF or PBS 10 min later. After 30 min, mice were sacrificed and the dye was extracted from skin samples and quantified. Results were confirmed in two independent experiments with three to five mice per group. (B-E) WT mice were i.v. injected with either rapalog or vehicle alone (as indicated) 8 and 4 h before the assay, followed by an intrascrotal injection of 50 ng IL-1 β . The cremaster muscle was prepared for intravital imaging 4 h later. Numbers of extravasated (B) and adherent leukocytes (C), rolling flux fraction (D), and rolling velocity (E) were determined. The results are displayed as mean \pm SEM of at least 20 vessels from 3 independent animals in each group. **, P < 0.01; ***, P < 0.001.