Antibodies. For FACS analysis biotin-conjugated rat anti-mouse Flk-1 (clone Avas12a1) and for isotype-specific control rat IgG2a, κ, were used followed by streptavidinphycoerythrin. PECAM-1 was detected with FITC-conjugated anti-mouse CD31 (PECAM-1) rat monoclonal antibody (clone 390, all from eBioscience, San Diego, CA). For immunofluorescence rat anti-mouse CD144 (VE-cadherin) monoclonal antibody (clone 11D4.1, BD Biotechnologies) was used, followed by Cy™2-conjugated donkey anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA). Western blotting was performed with an affinity-purified rabbit polyclonal antibody against Rap1 (121), followed by an anti-rabbit IgG conjugated to HRP (both from Santa Cruz Biotechnologies, Santa Cruz, CA).

FACS analysis of surface receptor expression. Wild-type and Rap1b-deficient endothelial cells were labeled with biotin-conjugated anti-mouse VEGFR2 rat antibody or unrelated, biotin-conjugated isotype-matched control, followed by streptavidinphycoerythrin (eBioscience; San Diego, CA) and analyzed using a FACS LSR2 flow cytometer (BD Biosciences).

Immunofluorescence. Wild-type and Rap1b-deficient endothelial cells were cultured on fibronectin-coated coverslips as described until they reached confluence. Cells were rinsed in PBS, fixed in 3.7% formaldehyde/PBS, permeabilized in 0.2% Triton X100/PBS, blocked in 5% horse serum/PBS and stained with an anti-CD144 antibody. Stained cells were imaged using Leica TCS SP2® Laser Scanning Confocal microscope.

Analysis of Rap1 protein expression. Total Rap1 protein expression was analyzed using western blotting of endothelial cell lysates from wild-type and *rap1b*-/- mice with a rabbit polyclonal Rap1-specific antibody.