Figure S1

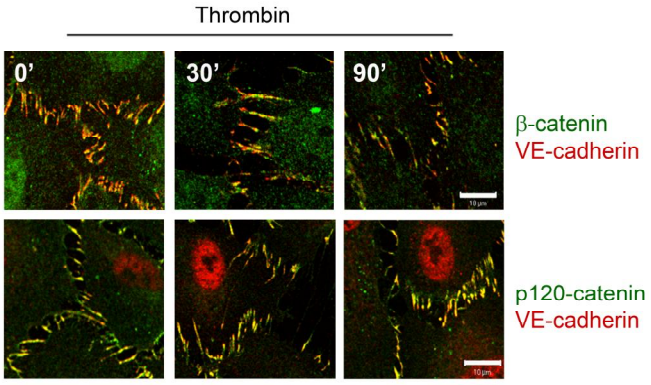

Figure S1. Cadherin-catenin complex localization after thrombin stimulation. HUVECs were cultured on FN-coated glass covers, grown to confluency and stimulated with thrombin. Immunostaining for VE-cadherin (red), β-catenin (upper panel, green) and p120-catenin (lower panel, green) shows that β-catenin and p120-catenin co-localize with VE-cadherin at cell-cell junctions during thrombin stimulation. Bar, 10 μm.

Figure S2

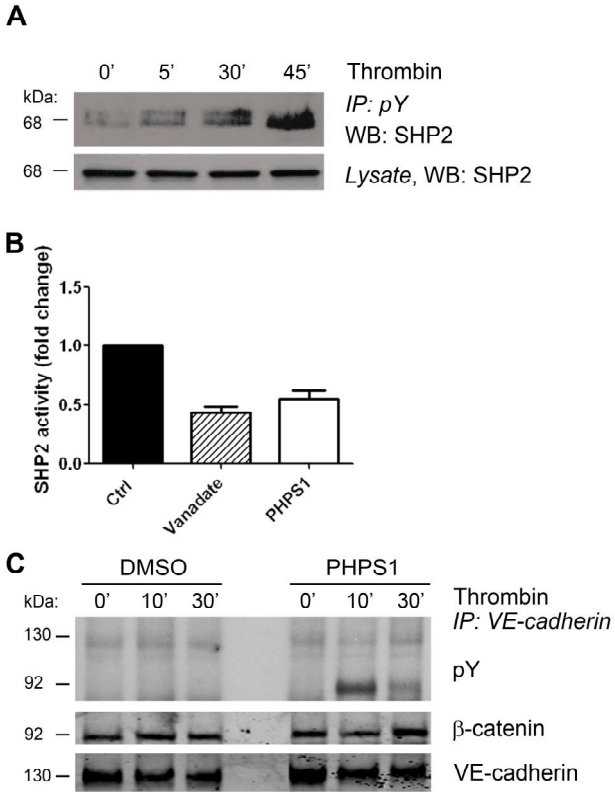

Figure S2. Thrombin induces SHP2 phosphorylation and SHP2-mediated de-phosphorylation of VE-cadherin-associated β-catenin. (A) HUVECs were stimulated with thrombin as indicated, lysed and tyrosine phosphorylated proteins (pY) were immunoprecipitated (IP) and subsequently analyzed by Western blotting. Blot shows increased tyrosine phosphorylation of SHP2 after thrombin stimulation. Lower panel shows SHP2 protein expression in the cell lysate. (B) SHP2 was immunoprecipitated from HUVECs treated with 15 μM SHP2 inhibitor PHPS1 for 20 hours or with 1 mM vanadate for 30 minutes and subjected to a tyrosine phosphatase activity assay, as described in Materials and Methods. (C) HUVECs were treated with SHP2 inhibitor PHPS1 for 20 hours, stimulated with thrombin as indicated and lysed. An IP for VE-cadherin was performed. Phosphorylation levels of the VE-cadherin complex were determined by Western Blotting with an anti-phosphotyrosine (pY) antibody. Blot shows prolonged and elevated tyrosine phosphorylation of a ~92 kDa VE-cadherin-associating protein (likely β-catenin) when SHP2 activity is inhibited.

Figure S3

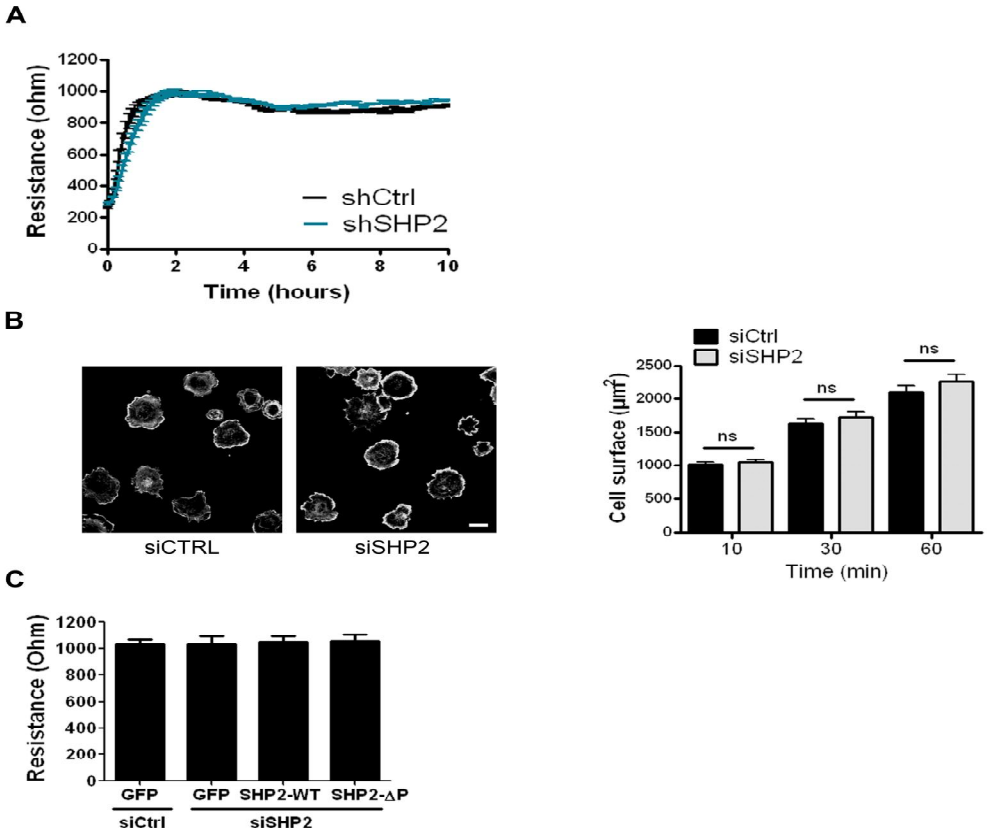

Figure S3. Silencing of SHP2 does not affect endothelial cell spreading and basal endothelial monolayer integrity. (A) Cell spreading of control and SHP2 shRNA-treated endothelial monolayers, analyzed by ECIS technique. Experiment was performed as described in Figure 4C. Similar to siRNA-based knockdown of SHP2, no significant difference in the electrical resistance during cell spreading and monolayer formation was measured between shRNA control and shRNA SHP2 treated endothelial cells. Representative image is shown from one out of two separate experiments, which were performed in quadruplicate. (B) Left panel: Immunostaining for F-actin in control and SHP2-depleted HUVEC spread on fibronectin for 10 minutes showed no effect on actin organization in spreading cells. Bar, 20 μm. Right panel: Measurement of cell surface, 10, 30 and 60 minutes after cell seeding on fibronectin, showed no significant difference in spreading of control and SHP2-depleted endothelial cells. Data are mean values ± SD of two independent experiments. In total, cell surface of 60 control and 60 knockdown cells was measured for each time-point. ns, not significant. (C) Experiment was performed as described in 5F, except that the bar graph shows basal electrical resistance monitored before thrombin addition. Transfection of HUVECs with SHP2 siRNA and infection with adenovirus containing pIRES-GFP-SHP2-WT or a phosphatase domain-deleted mutant (ΔP) did not affect basal endothelial monolayer resistance. Data are mean ± SD of three independent experiments, which were performed in duplicate.

Figure S4

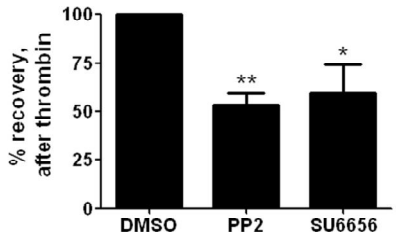

Figure S4. Src family kinase inhibition delays endothelial barrier recovery upon thrombin stimulation. HUVECs were pre-treated with DMSO or the Src family kinase inhibitors PP2 or SU6656, cultured on FN-coated ECIS electrode-arrays and stimulated with thrombin. Bar graph represents the percentage recovery of the endothelial monolayer resistance after thrombin at time-points when control monolayers were completely restored. Both PP2 and SU6656 reduced recovery of the endothelial monolayer resistance. Data are mean ± SD of two independent experiments, which were performed in duplicate. * $P<0.05$; ** $P<0.01$.

Figure S5

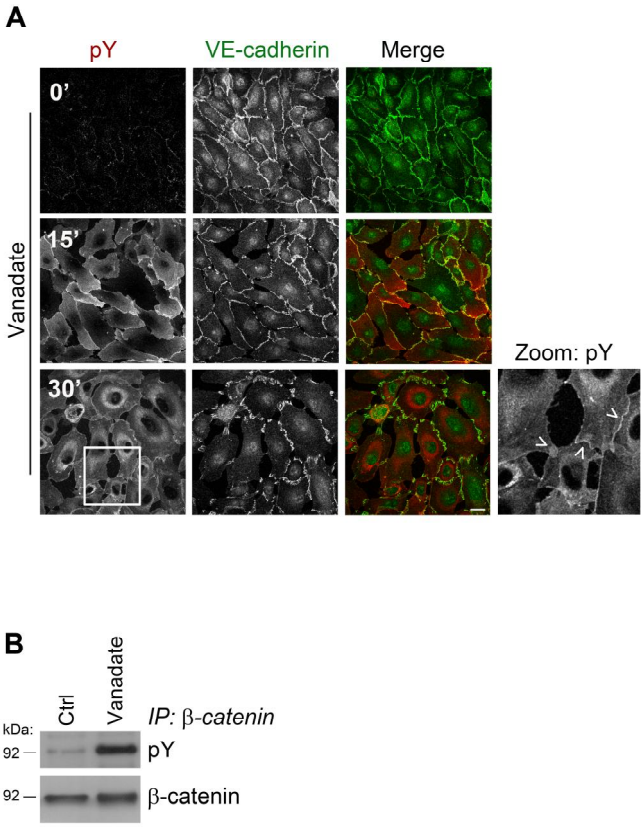

Figure S5. Vanadate induces tyrosine phosphorylation of junctional proteins. (A) HUVEC were grown on FN-coated glass covers, treated with 25µM vanadate for 15 and 30 minutes, processed, and stained for phosphotyrosine (pY, red) and VE-cadherin (*green*). Confocal images show that vanadate induces an increase in tyrosine phosphorylation levels in general, also at cell-cell junctions, eventually resulting in cell-cell junction disruption. Bar, 20 µm. (B) Vanadate-treated HUVECs were lysed and an IP for β-catenin was performed and analyzed for tyrosine phosphorylation levels by Western Blotting. Immunoblot shows increased tyrosine phosphorylation of β-catenin after vanadate treatment.

Figure S6

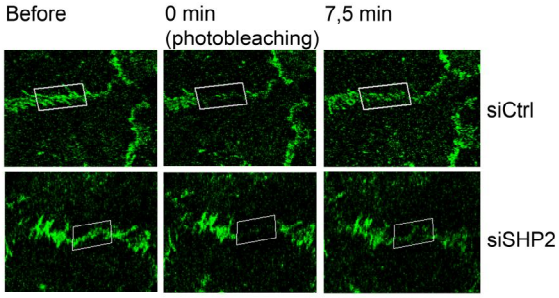

Figure S6. FRAP of VE-cadherin-GFP. HUVECs were transfected with control or SHP2 siRNA, transduced with adenovirus encoding VE-cadherin-GFP and stimulated with thrombin. Representative images of FRAP experiments are shown, in which the boxes indicate the photobleached area. Recovery of the fluorescent intensity of VE-cadherin-GFP in these regions was measured in time and was reduced in thrombin-treated SHP2-depleted cells.