SUPPLEMENTARY MATERIAL

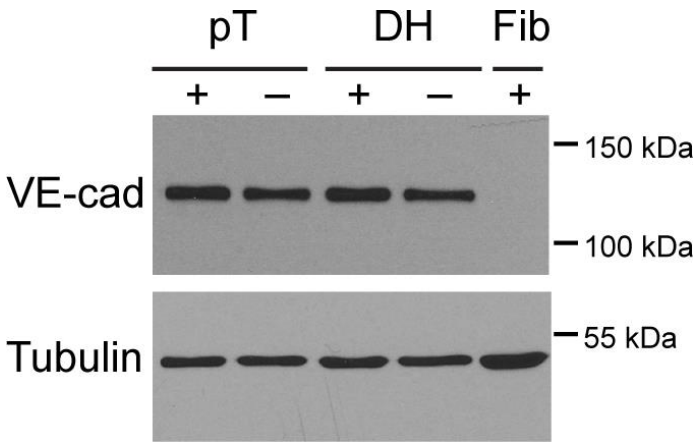

Fig. S1. Characterization of immortalized endothelial wild-type (+) and plectin deficient (-) cell lines derived from kidney (pT) and lung (DH). Protein extracts from pT and DH cell lines were analysed by immunoblotting using antibodies to VE-cadherin and tubulin (loading control). Cell extracts of cultured wild-type mouse fibroblasts were included as controls for VE-cadherin-negative cells (Fib). Note similar levels of VE-cadherin in all endothelial cell lines tested.

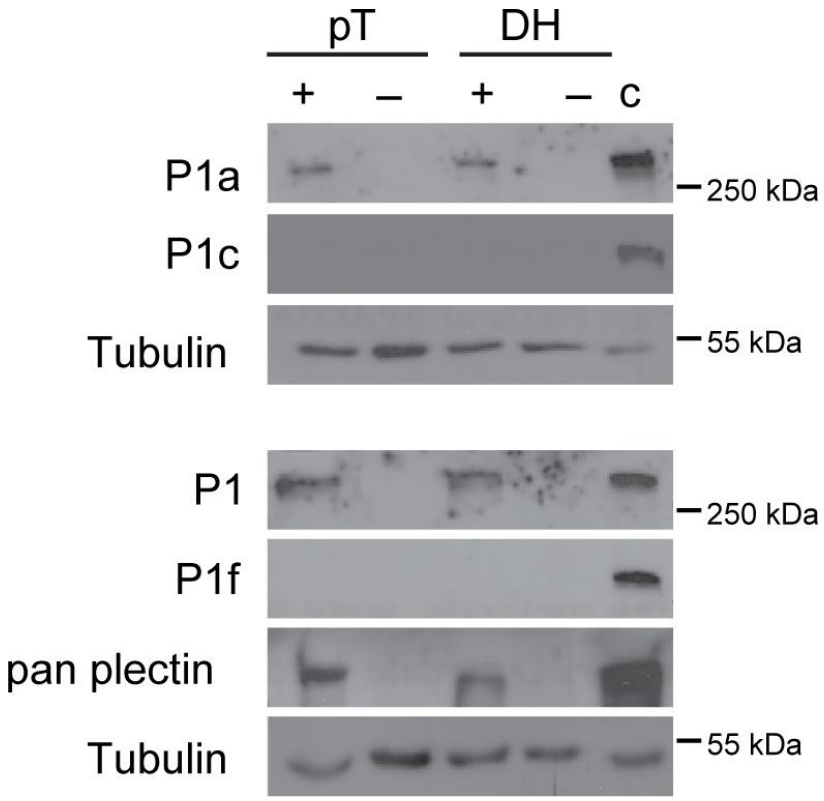

Fig. S2. Expression pattern of plectin isoforms in wild-type (+) and plectin-deficient (-) endothelial cell lines. Protein extracts of pT and DH cell lines were subjected to immunoblotting using affinity-purified, isoform-specific antibodies to P1, P1a, P1c, and P1f, and anti-pan plectin antiserum #9. Lysates of immortalized wild-type keratinocytes/fibroblasts served as positive controls (c) for plectin isoforms P1a and P1c/P1 and P1f, respectively. Tubulin, loading control.

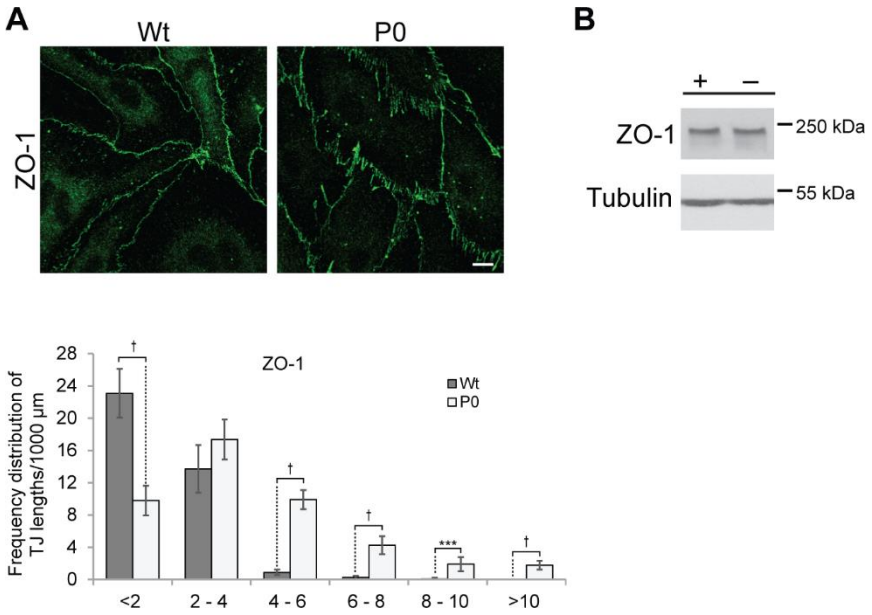

Fig. S3 . Immunolocalization and expression levels of tight junction protein ZO-1 in Wt and P0 endothelial cells. (A) Upper panels, confluent Wt and P0 endothelial cell layers (~24 h adhesion) were immunolabeled using antibodies to ZO-1. Scale bar, 10 µm. Lower panel, bar graph showing spike lengths categorized and statistically evaluated as described in Fig. 2F. >1000 junctions were evaluated for each genotype, 3 independent experiments. ***P<0.001, $^{\dagger}P$<0.0001. (B) Immunoblotting of Wt and P0 cell lysates using antibodies to ZO-1. Tubulin, loading control. Note equivalent protein levels of ZO-1 in both cell types.

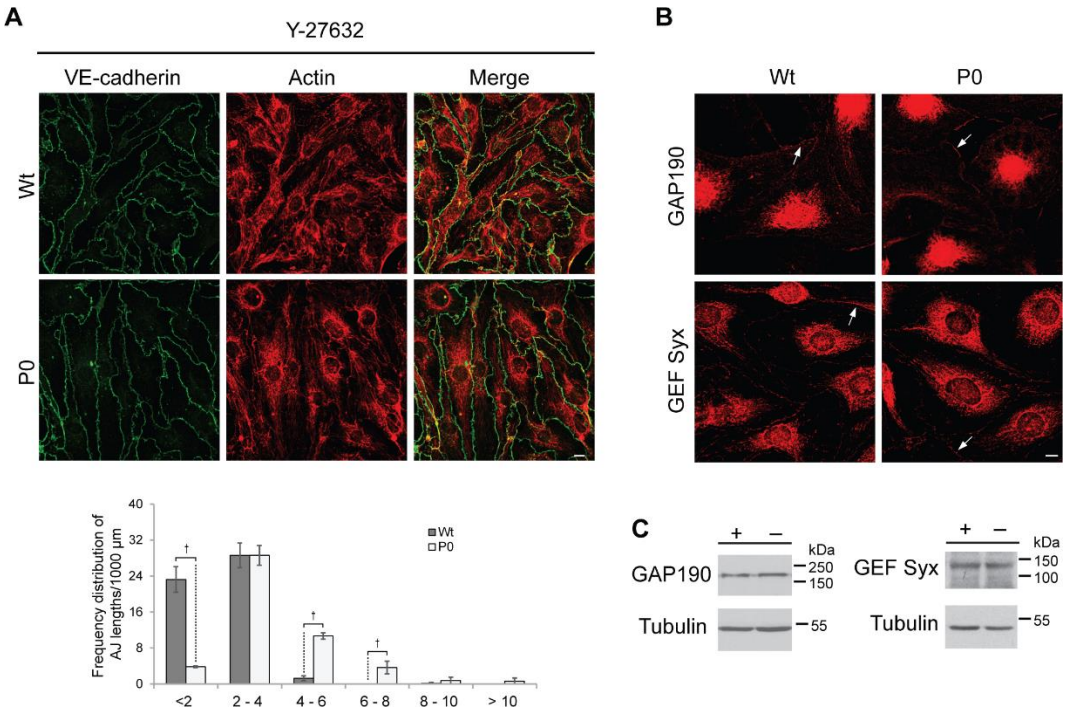

Fig. S4. Unaltered levels and distribution of RhoA regulators GAP190 and GEF Syx in P0 cells and partial restoration of AJ discontinuity by Rho kinase inhibition. (A) Double immunolabeling (VE cadherin and actin) of confluent Wt and P0 endothelial cell layers (~24 h adhesion) after treatment with 20 µM ROCK-inhibitor Y-27632 for 1 h. Scale bar, 10 µm. Bar graph shows AJ lengths categorized and statistically evaluated as described in Fig. 2F. >600 junctions were evaluated per genotype, 3 independent experiments. †P<0.0001. (B) Immunofluorescence microscopy of Wt and P0 cells using antibodies to GAP190 and GEF Syx. Arrows, cell-cell junctional areas. (C) Immunoblotting of Wt and P0 endothelial cell lysates using antibodies to GAP190 and GEF Syx. Tubulin, loading control.