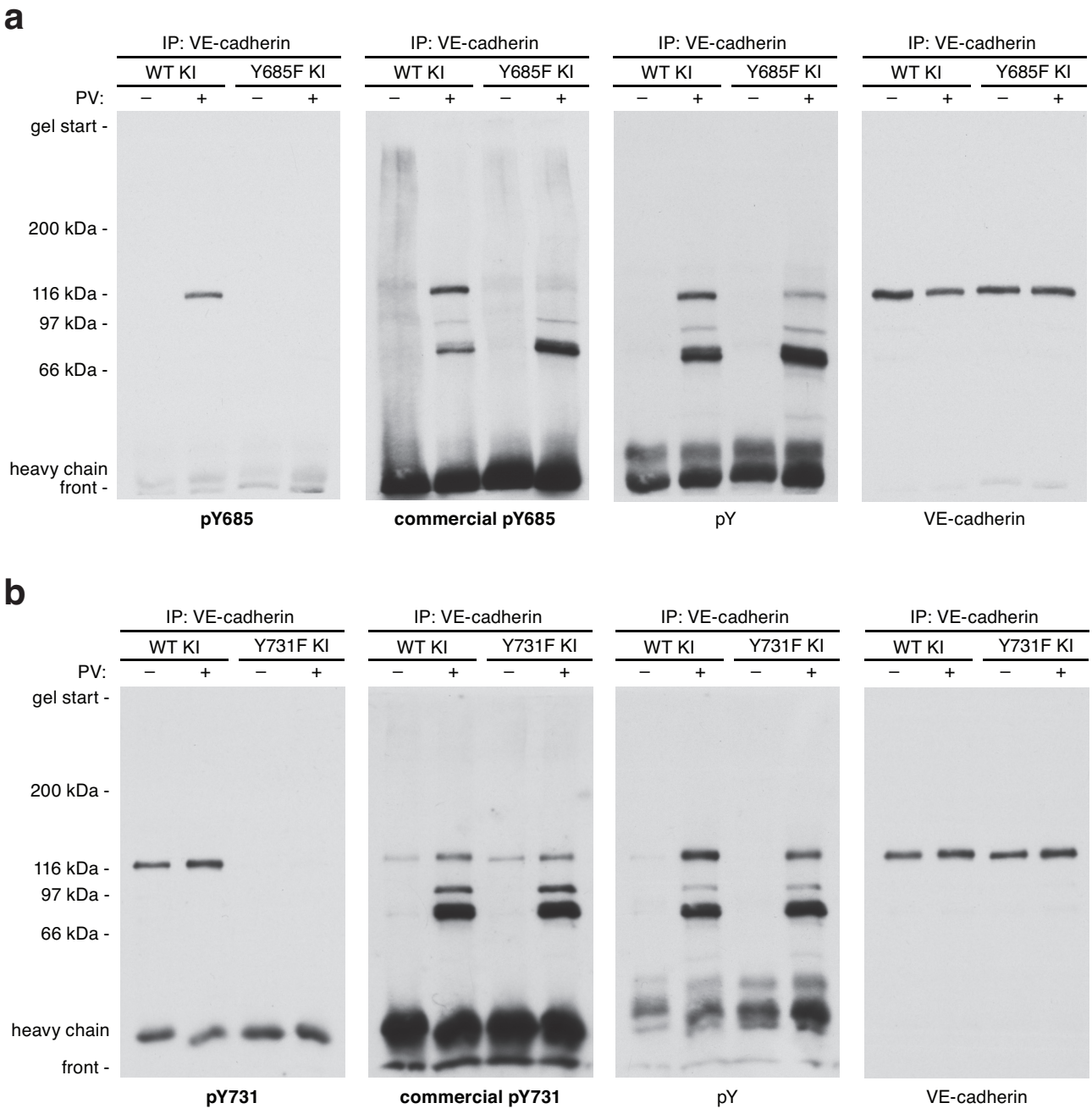
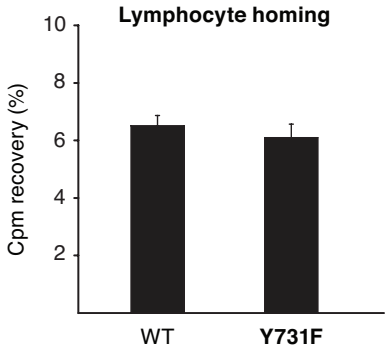
Supplementary Figure 1



Specificity of newly generated mAbs for VE-cadherin pY685 and pY731 in comparison with commercially available pY-antibodies. (**a**+**b**) Immunoblots of VE-cadherin immunoprecipitated from lung lysates of VEC-WT and VEC-Y685F mice (**a**) and VEC-Y731F mice (**b**) that had been anesthetized and intravenously injected with PBS containing (+) or not containing (-) peroxyvanadate (PV). The same membranes were sequentially immunoblotted with the following antibodies: mp685 (**a**) or mp731 (**b**); commercial pY685 (**a**) or commercial pY731 (**b**); general anti pY antibody 4G10 (**a**+**b**) and VE-cadherin antibodies (**a**+**b**). Transfer of the complete gel with start of the separating-gel and electrophoresis front are displayed. Data are representative for 2 independent experiments.

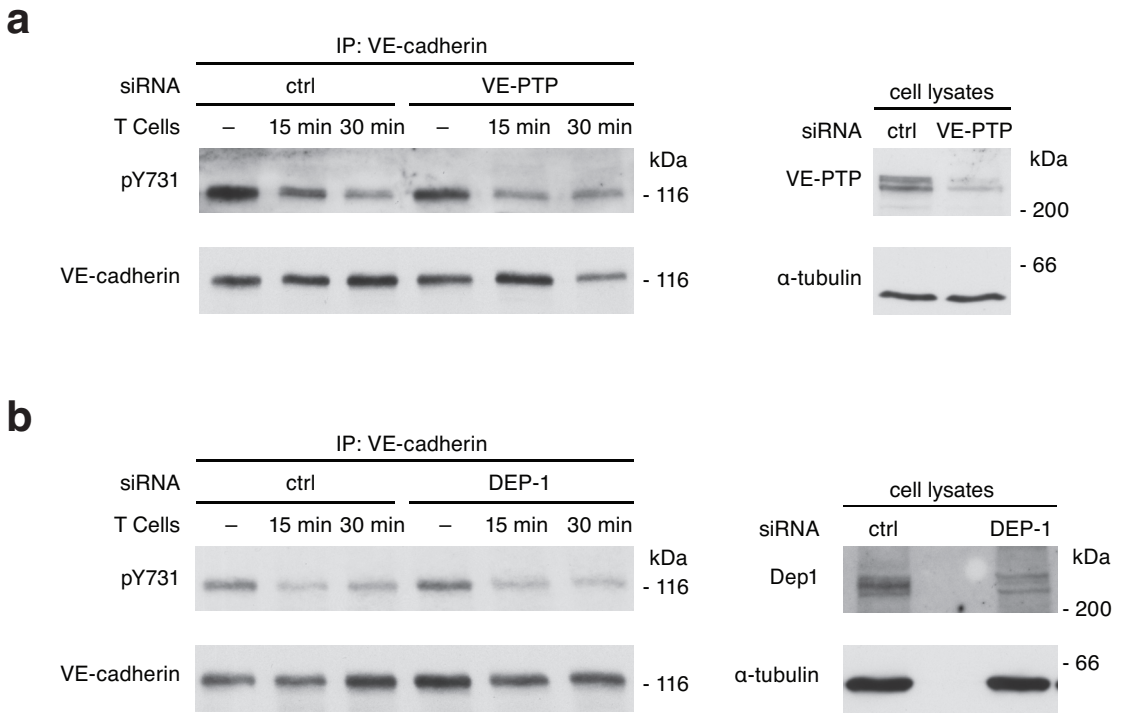
Nature Immunology: doi:10.1038/ni.2824

Supplementary Figure 2



Homing of naive lymphocytes to lymph nodes is not affected in Y731F-KI mice. Radiolabelled naive lymphocytes from C57Bl6 mice were injected intravenously into WT and Y731F KI mice and the percentage of radioactivity in lymph nodes compared to residual body radioactivity was determined 3 h later. Results are from three independent experiments with at least four mice per group and experiment (n=14 mice).

Supplementary Figure 3



Knock down of the expression of VE-PTP or DEP-1 by siRNA does not interfere with leukocyte-induced dephosphorylation of VE-cadherin-Y731. Immunoblots of VE-cadherin immunoprecipitated from TNF-activated bEnd.5 cells that had been either treated with VE-PTP siRNA (**a**) or with DEP-1 siRNA (**b**) and control siRNA (as indicated above). Blots were incubeated with mp731 or anti VE-cadherin antibodies (left panels). Knock-down efficiency of VE-PTP and DEP-1 was confirmed in total cell lysates by immunoblotting with anti VE-PTP or anti DEP-1 antibodies and equal loading was controlled with anti α-tubulin antibodies (right panels). Data are representative for 2 (**a**) and 3 (**b**) independent experiments.

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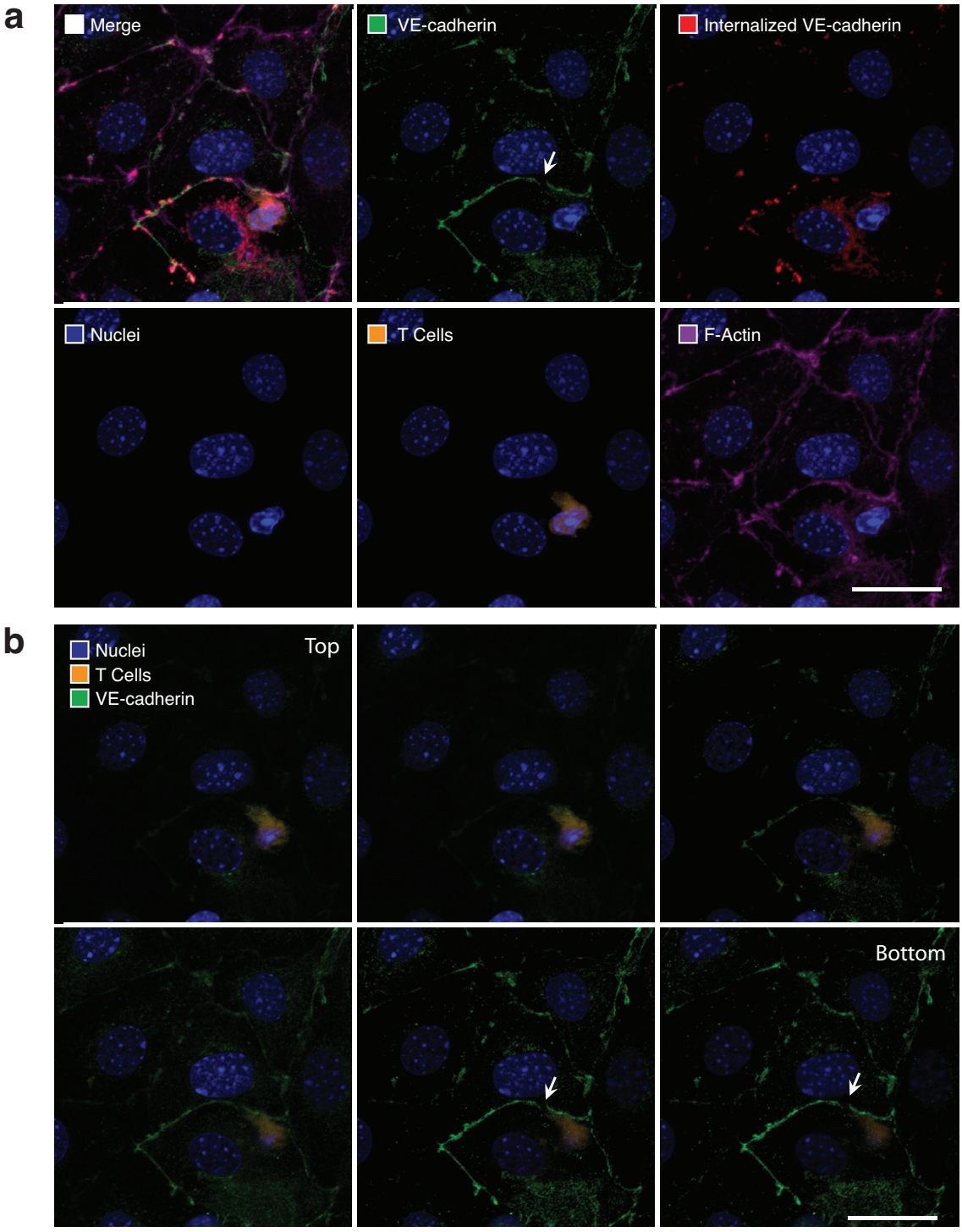
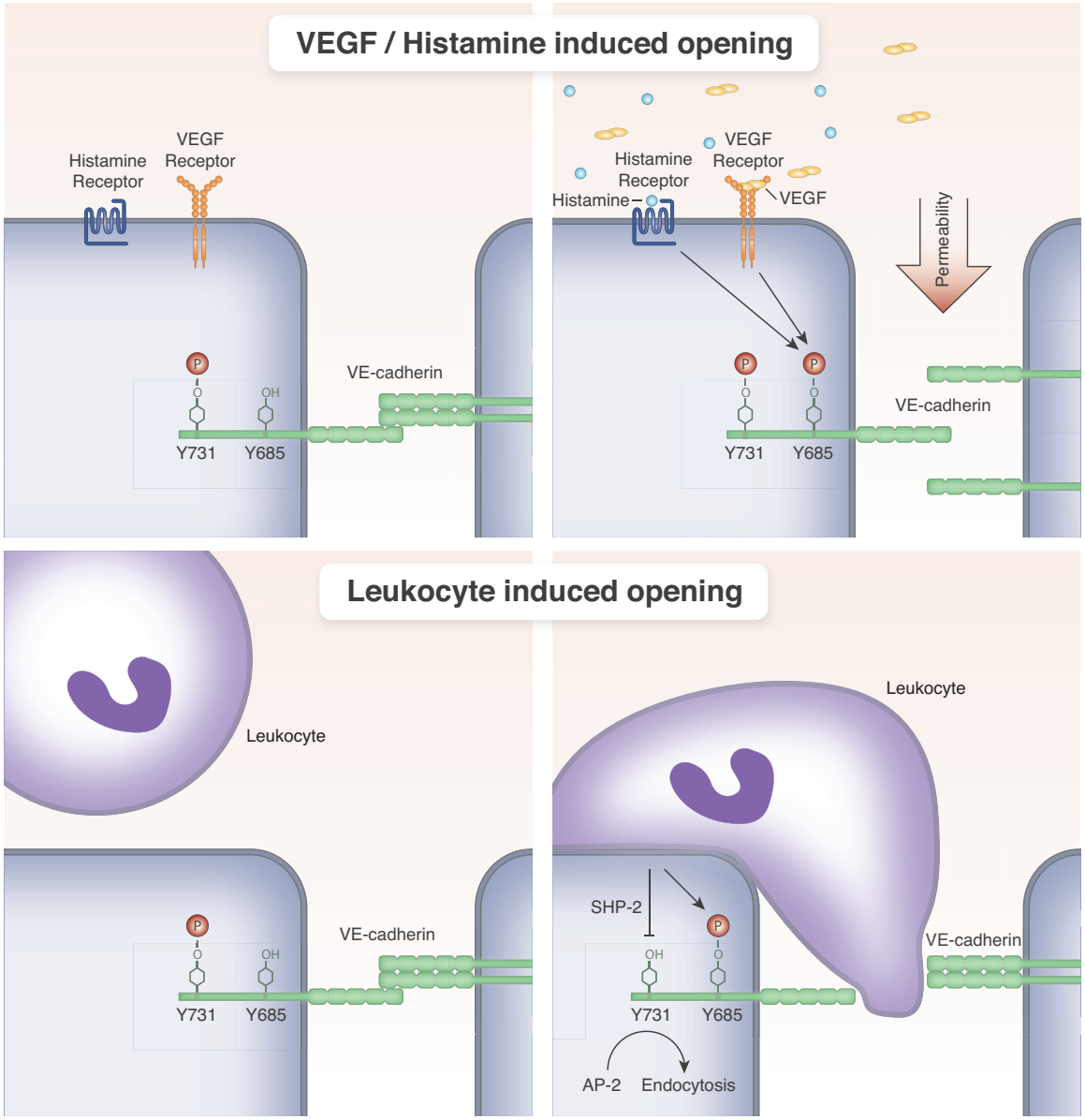
Supplementary Figure 4



Endocytosis of VE-cadherin induced by single T cells. (**a**) T cells (labeled with cell tracker orange) were added for 15 min to TNF-α-stimulated primary isolated mouse endothelial cells at a ratio of 1:5 (one T cell per five endothelial cells). Uptake of VE-cadherin was monitored with mAb BV13 as described under Material & Methods (red). After 15 min endothelial cells were carefully washed to avoid removal of T cells. VE-cadherin at cell contacts was stained with a polyclonal antibody (green). F-actin was stained with phalloidin (magenta) and nuclei were stained with Hoechst 33342 (blue). Note that VE-cadherin-containing endocytic vesicles have accumulated close to the single associated lymphocyte. VE-cadherin junctional staining reveals a gap at the site where the lymphocyte is probing the cell contact (white arrow). (**b**) Serial optical sections of the same cells displaying only the staining for VE-cadherin at cell contacts, the lymphocyte and nuclei to highlight the gap (white arrow) in VE-cadherin staining at junctions. The leukocyte is polarized towards the site of junctional loss of VE-cadherin. Scale bar 20 μm. Data are representative of 2 independent experiments with more than 100 lymphocytes analyzed.

Supplementary Figure 5



Induction of endothelial permeability relies exclusively on phosphorylation of VE-cadherin-Y685, whereas efficient extravasation of leukocytes depends exclusively on the dephosphorylation of Y731.

Upper panel: Under resting conditions VE-cadherin is not significantly phosphorylated at Y685, but strongly phosphorylated at Y731. Vascular permeability-inducing factors such as VEGF or histamine strongly enhance Y685 phosphorylation whereas high baseline phosphorylation of Y731 is unaltered. Only Y685, but not Y731 is required for proper induction of vascular permeability in vivo.

Lower panel: In contrast to permeability-inducing stimuli, leukocytes rapidly dephosphorylate Y731. This is mediated by the phosphatase SHP2 and followed by binding to AP-2 and subsequent rapid endocytosis. Only Y731, but not Y685 supports extravasation of leukocytes in vivo. Collectively, the induction of permeability and the extravasation of leukocytes regulate the phosphorylation of different tyrosine residues on VE-cadherin in different ways resulting in different signatures of phosphorylation sites. Each of the two sites is exclusively involved in only one of the two processes.

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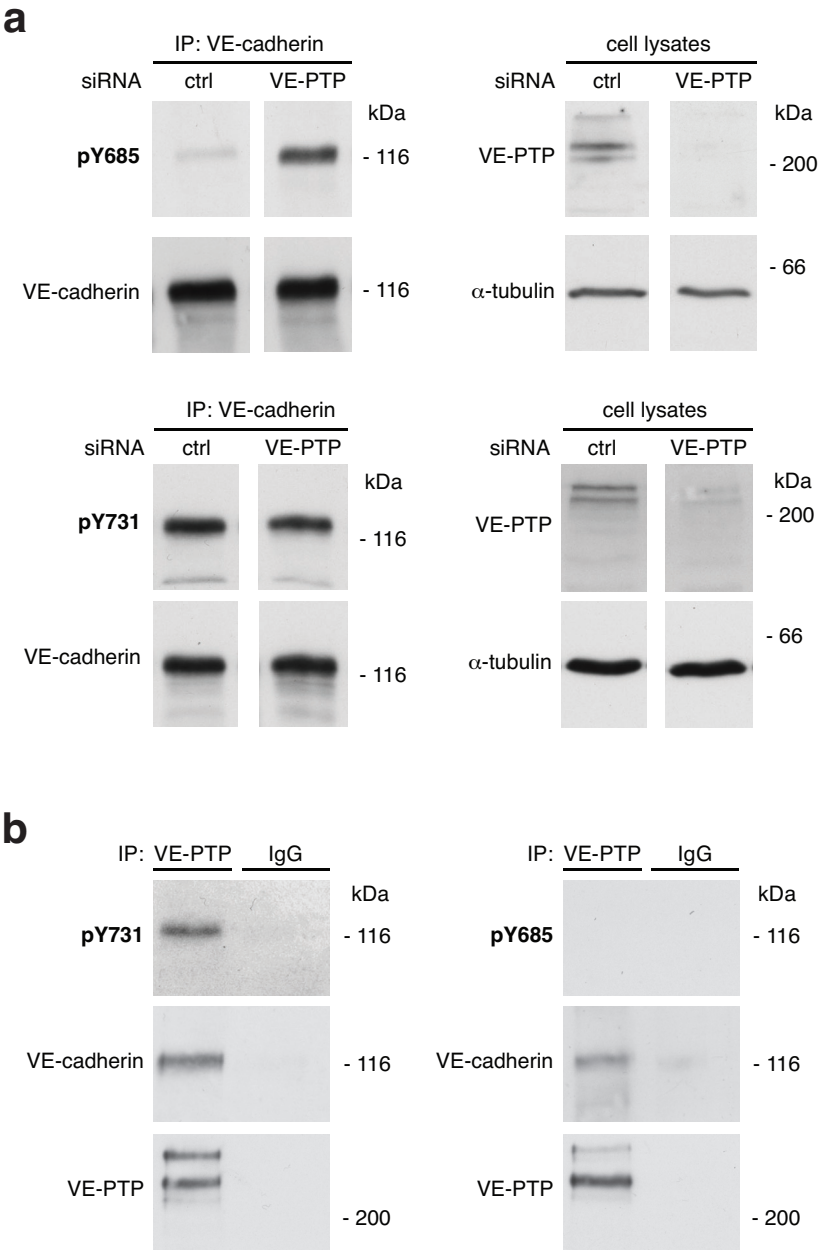
Supplementary Figure 6



VE-PTP counteracts phosphorylation of Y685 but not of Y731. (**a**) 24 h after downregulation of VE-PTP expression by siRNA in bEnd.5 cells, VE-cadherin was immunoprecipitated and precipitates were immunoblotted with the mAb mp685, mp731 or VE-cadherin antibodies (left panels). Knock-down of VE-PTP was confirmed in total cell lysates by immunoblotting with anti VE-PTP and gel loading was controlled with anti α-tubulin antibodies (right panels). (**b**) VE-PTP-associated VE-cadherin is phosphorylated at Y731, but not at Y685. VE-PTP was immunoprecipitated from highly confluent bEnd.5 cells. Phosphorylation of co-precipitated VE-cadherin was checked in immunoblots using the mAb mp731 (left panels) and mp685 (right panels). Co-precipitated VE-cadherin-levels and precipitated VE-PTP were analyzed in immunoblots with the respective antibodies Data in (**a**) and (**b**) are representative for 3 independent experiments.