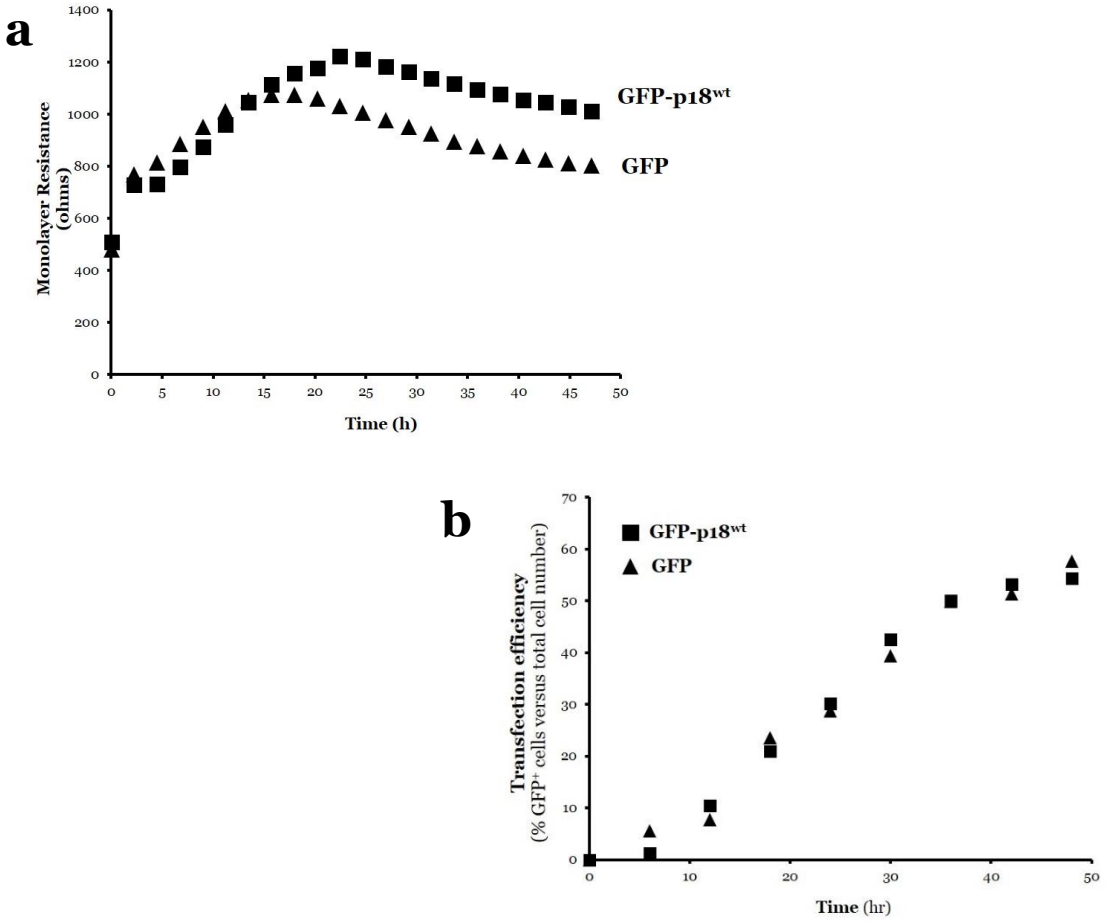
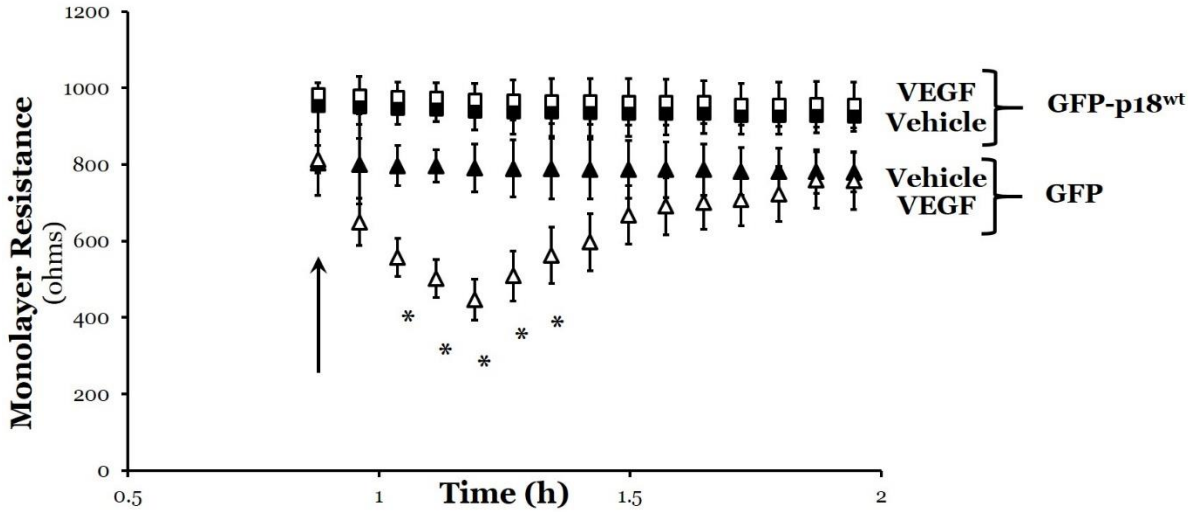
Supplementary Figure S1



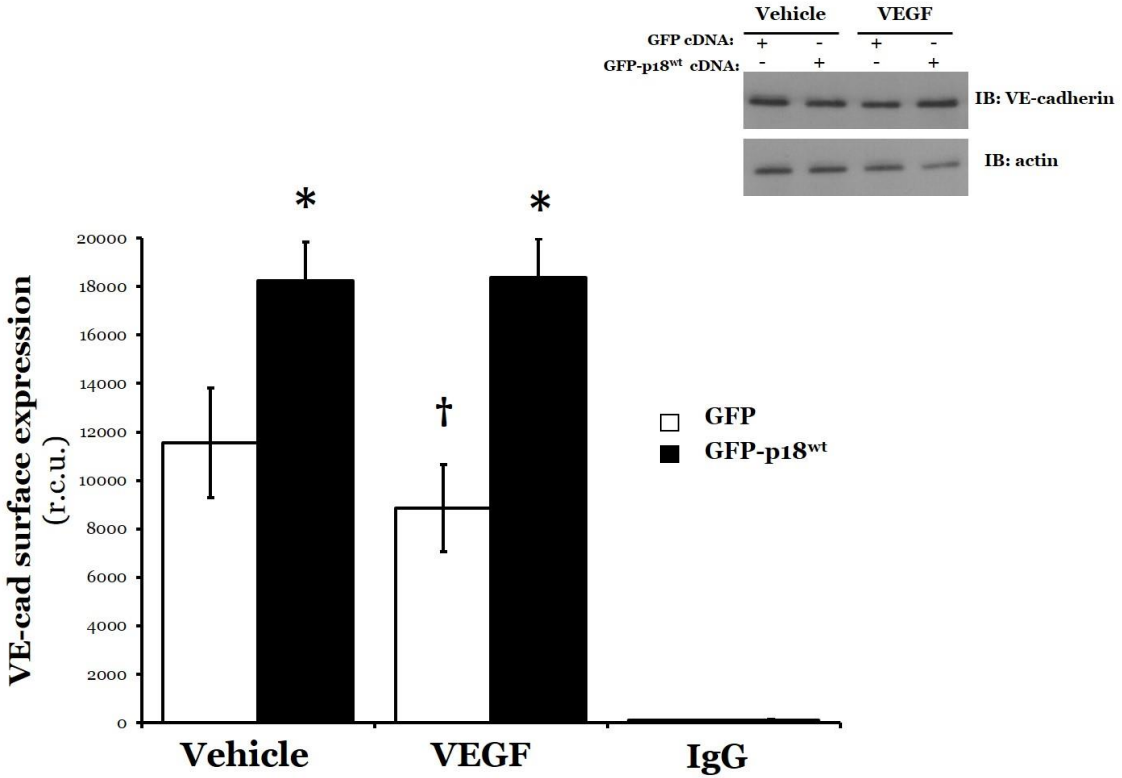
Supplementary Figure S1: p18^{wt} overexpression enhances endothelial barrier dysfunction. Equivalent numbers of LMVEC were transiently transfected with GFP (triangle symbol) or GFP-p18^{wt} (square symbol) cDNA. *Panel a:* Changes in endothelial monolayer resistance were measured using ECIS immediately following transfection and seeding. *Panel b:* Overexpression was assessed by images captured at 6 hour intervals immediately following transfection and seeding. Transfection efficiency was measured as the percentage of GFP-positive cells versus total cells. Representative plots are shown (n=3).

Supplementary Figure S2



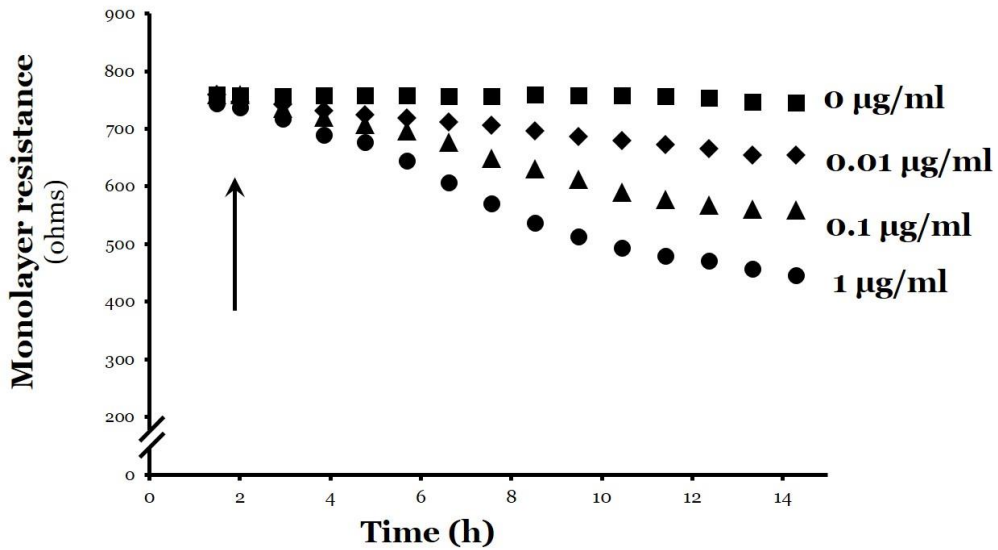
Supplementary Figure S2: p18^{wt} overexpression attenuates VEGF-induced endothelial barrier dysfunction. Equivalent numbers of LMVEC were transiently transfected with GFP (triangle symbol) or GFP-p18^{wt} (square symbol) cDNA. Following 48 h, changes in endothelial monolayer resistance were measured using ECIS, in the presence (open shape) and absence (solid shape) of VEGF (50 ng/ml). Arrow indicates addition of VEGF. Data are presented as mean ± SD. n=4-5. *p<0.05 vs vehicle.

Supplementary Figure S3



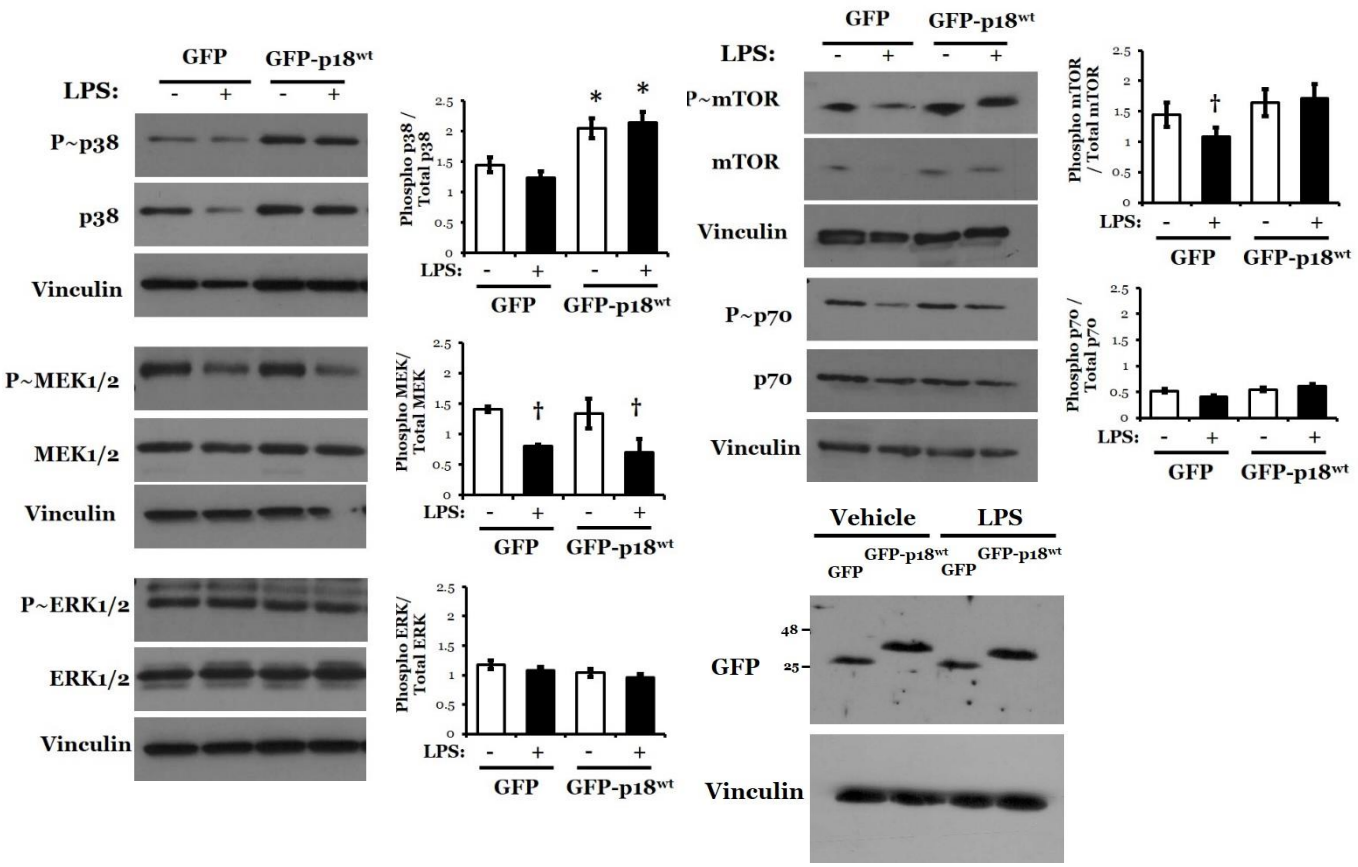
Supplementary Figure S3: p18^{wt} overexpression attenuates VEGF-induced internalization of VE-cadherin. Equivalent numbers of LMVEC were transiently transfected with GFP (open bar) or GFP-p18^{wt} cDNA (closed bar). Following 48 h, LMVEC were exposed to VEGF (50 ng/ml, 30 min) and cell surface expression was determined with whole-cell indirect ELISA using chemiluminescence. Expression of VE-cadherin in whole cell lysates was assessed by immunoblot analysis of lysates from transiently transfected cells with an antibody specific to VE-cadherin (*inset*). Blots were stripped and reprobed for actin as a loading control. r.c.u: relative chemiluminescence units. Data are presented as mean ± SD. n=7-8. *p<0.05 vs GFP, †p<0.05 vs vehicle.

Supplementary Figure S4



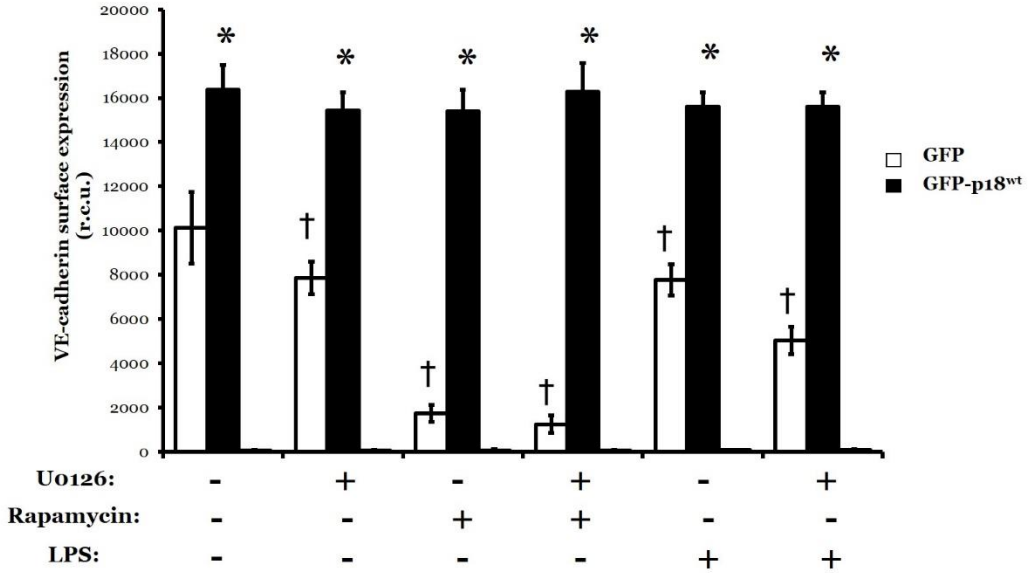
Supplementary Figure S4: Dose-dependent increase in LPS-induced endothelial barrier dysfunction. Endothelial monolayer resistance was assessed in LMVEC using ECIS, in the presence and absence of varying LPS concentrations (0.1-1 µg/ml). Arrow indicates addition of LPS. A representative graph is shown.

Supplementary Figure S5



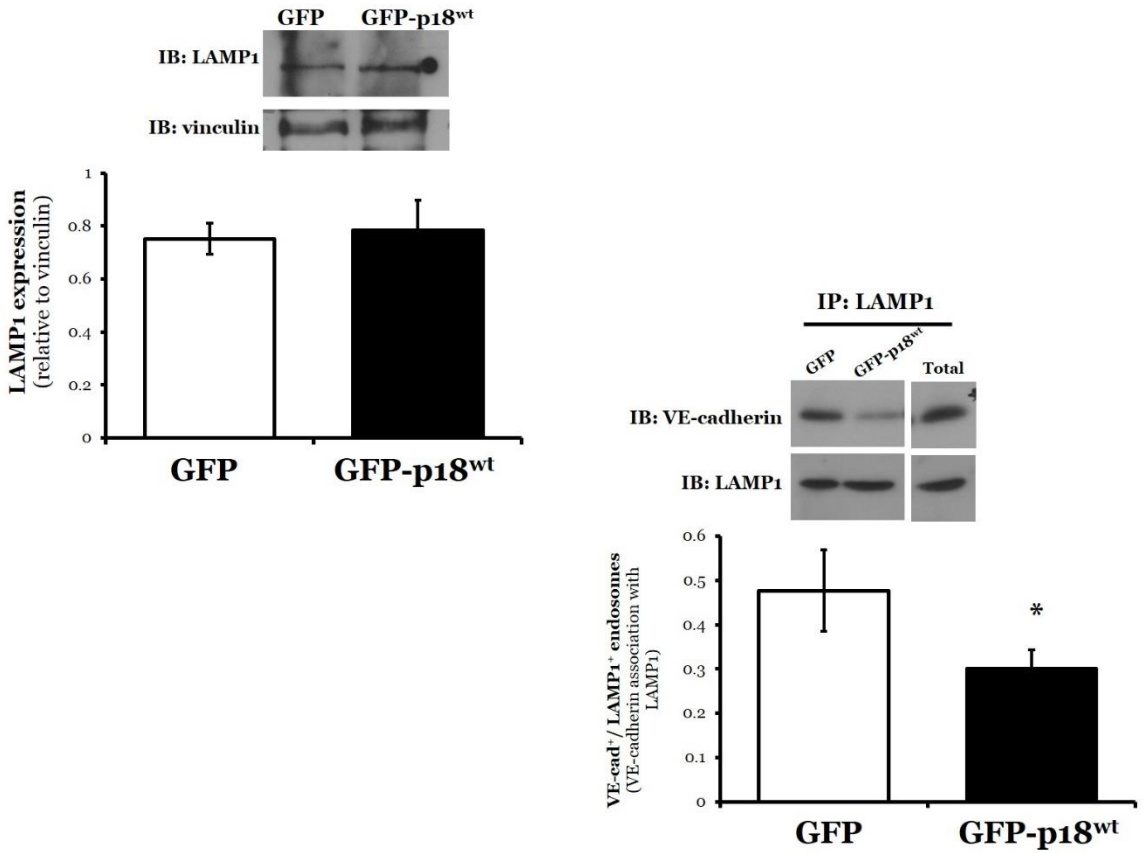
Supplementary Figure S5: p18^{wt} overexpression enhances p38, but not MEK or ERK, activation. Equivalent numbers of LMVEC were transiently transfected with GFP or GFP-p18^{wt} cDNA. Following 48 h, LMVEC were exposed to LPS (1 µg/ml, 6 h). Phosphorylation of p38, MEK1/2, ERK1/2, mTOR and p70 was assessed in whole cell lysates by immunoblot analysis of lysates from transiently transfected cells with an antibody specific to each phosphorylated protein. Blots were stripped and reprobed for total protein expression and vinculin as a loading control. Representative blots are shown. Data are presented as mean ± SD. n=3. *p<0.05 vs GFP, †p<0.05 vs vehicle.

Supplementary Figure S6



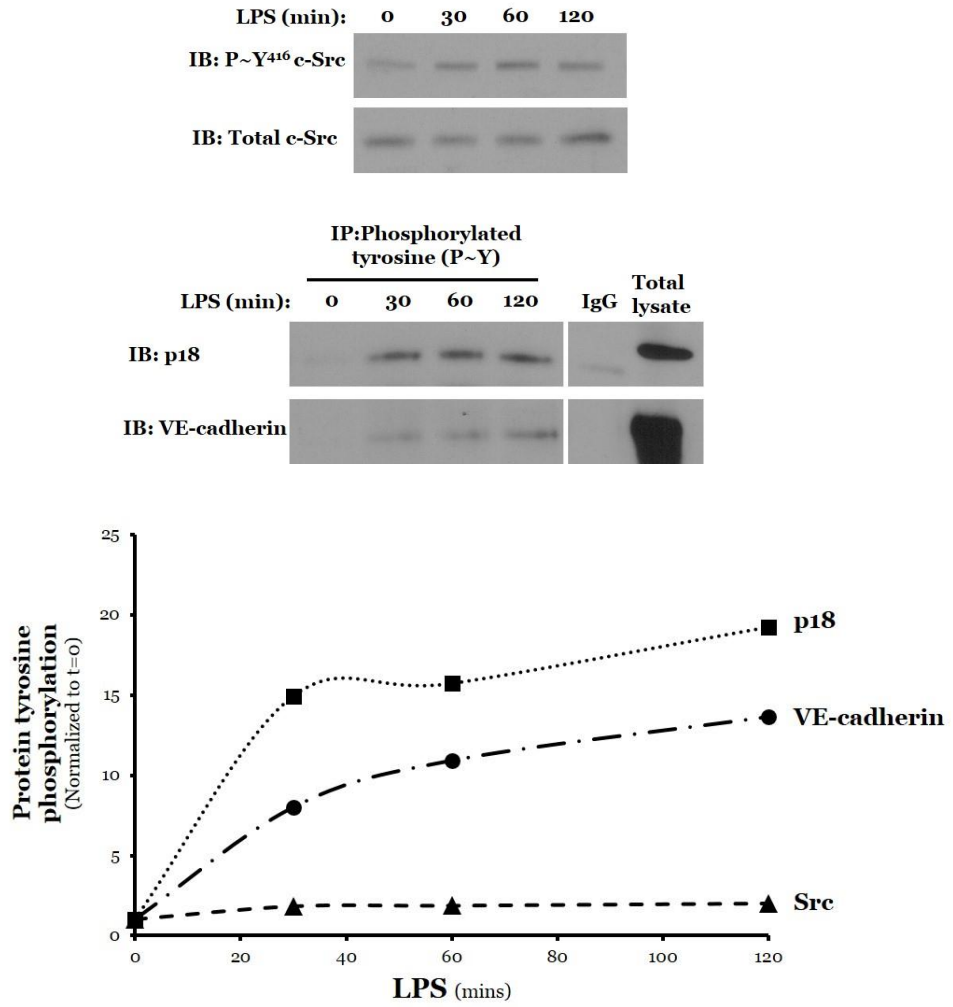
Supplementary Figure S6: p18^{wt} overexpression attenuates internalization of VE-cadherin induced by MEK or mTOR inhibition. Equivalent numbers of LMVEC were transiently transfected with GFP (open bar) or GFP-p18^{wt} (closed bar) cDNA. Following 48 h, LMVEC were pre-incubated with MEK inhibitor U0126 (10 µM, 30 min) followed by exposure to LPS (1 µg/ml, 6 h) or rapamycin (10 nM, 24 h) and cell surface expression was determined with whole-cell indirect ELISA using chemiluminescence. r.c.u: relative chemiluminescence units. Data are presented as mean ± SD. n=4-5. *p<0.05 vs GFP, †p<0.05 vs vehicle.

Supplementary Figure S7



Supplementary Figure S7: Overexpression of p18 decreases the formation of VE-cadherin-positive late endosomes. Equivalent numbers of LMVEC were transiently transfected with GFP (open bar) or GFP-p18^{wt} (closed bar) cDNA. Following 48 h, LMVEC were assessed for LAMP1 expression (*left panels*) or subjected to endosome preparation *(right panels*). Late endosomes were immunoprecipitated with an antibody specific to the lysosomal-associated membrane protein 1 (LAMP1) and subjected to immunoblot analysis with an antibody specific to VE-cadherin. Blots were stripped and reprobed for LAMP1. A representative blot (*top panel*) and densitometry analysis (*bottom panel)* are shown. Data are presented as mean ± SD. n=3. *p<0.05 vs GFP.

Supplementary Figure S8



Supplementary Figure S8: LPS induces tyrosine phosphorylation of p18, Src and VE-cadherin. LMVEC were exposed to LPS (1 µg/ml) for 30-120 mins. LMVEC were lysed and subjected to immunoblot analysis for phosphorylated Src. Blots were stripped and re-probed for total Src levels. Lysates were also immunoprecipitated for tyrosine phosphorylation using the 4G10 clone antibody. Immunocomplexes and whole cell lysates were subjected to immunoblot analysis with an antibody specific to p18 or VE-cadherin. Representative blots (*left panel)* and densitometry analysis (*right panel*) are shown (n=3).