Supplemental Data

Methods

Cell Attachment Assay – HCAECs were pre-treated as in the migration assay and seeded in triplicate into a 96-well plate coated with 250 μg/mL Matrigel. The cells were allowed to adhere for 4 hours at 37ºC and the plate was then washed twice with PBS. HCAECs were then stained with 0.5% Crystal Violet in 10% formalin for 15 minutes and washed again with PBS. Attachment was quantified by measuring absorbance at 600 nm on a Benchmark Plus microplate spectrophotometer (Bio-Rad, Inc., Hercules, CA).

$p27^{Kip1}$ mRNA Measurements – HCAECs were stimulated with EGM-2 MV supplemented with 20% FBS for 1, 16, and 24 hours in presence of vehicle or 100 nmol/L rapamycin. 250 ng total RNA from each sample was analyzed to determine the relative amounts of mRNA encoding $p27^{Kip1}$ and β-actin using QuantiTect SYBR Green one-step RT-PCR (Qiagen, Inc., Valencia, CA) and QuantiTect primer assays for human p27 (QT00998445) and human actin (QT01680476) (Qiagen). The expression of $p27^{Kip1}$ was normalized to the expression of β-actin for each sample and the fold difference between the rapamycin treated and untreated samples was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blots – HCAECs were grown under identical conditions as in the migration assays. Western blots were prepared as previously described (21) and probed with an antibodies for pRb (Cell Signaling Technology, Danvers, MA).

Fig. S1. Rapamycin pretreatment does not alter attachment of HCAECs. HCAECs were grown in growth medium supplemented with either rapamycin (100 nmol/L) or vehicle and the cells were incubated for 24 hours. HCAECs were then seeded into 96-well plates coated with Matrigel and cell attachment was measured using Crystal Violet staining and spectroscopy after 4 hours. Columns represent mean ±SEM (n=3).

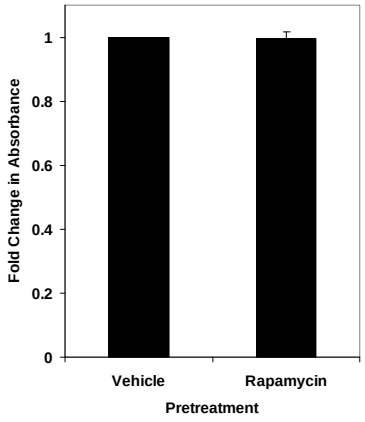

Fig. S2. Comparison of the levels of mRNA encoding $p27^{Kip1}$ in HCAECs treated with 100 nmol/L rapamycin for the indicated times to those treated with vehicle. Columns represent mean ± SE (n=4).

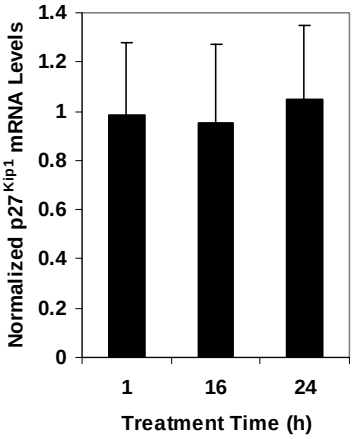

Fig. S3. Rapamycin treatment inhibits pRb phosphorylation in HCAECs. HCAECs were grown in growth medium supplemented with either rapamycin (100 nmol/L) or vehicle and the cells were incubated for 24 hours. A representative western blot illustrates the decrease in intensity of the phosphorylated (β) bands compared to the faster migrating non-phosphorylated protein bands (α). Columns represent mean ± SE (n=3) for the ratio of the intensity of the α-band to the total pRb intensity. The bracket indicates $p < 0.01$