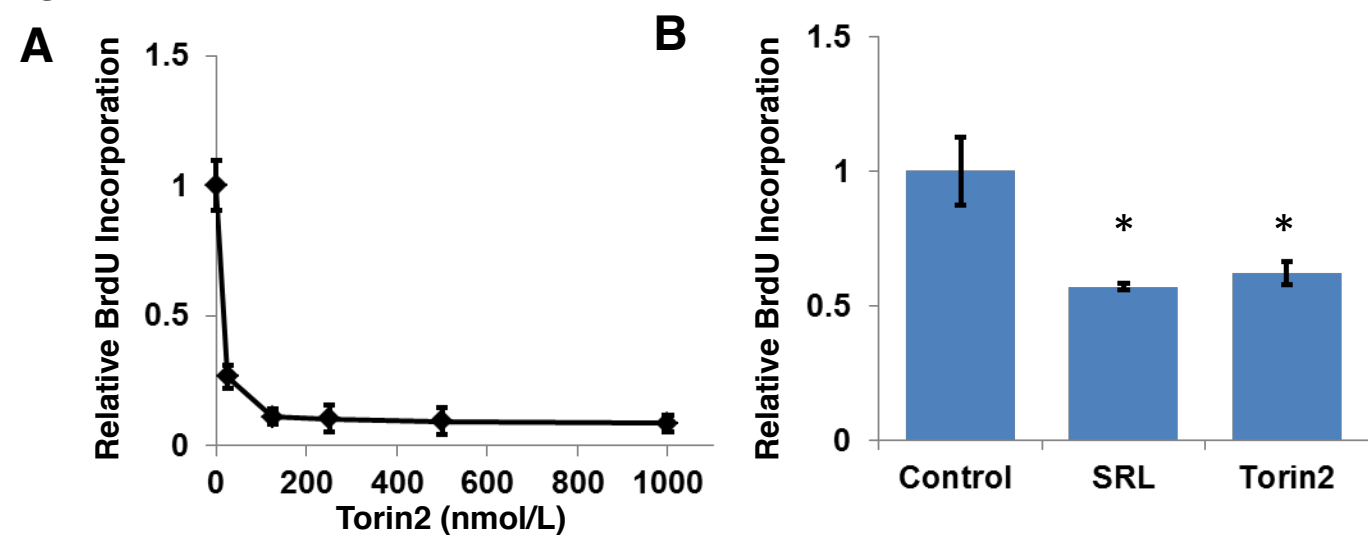


Figure II



Supplemental Figure Legends

Figure I. (A) HAECs treated with siRNA for FKBP12.6 and PKC α and immunoblotted (IB) for the respective antibodies with representative examples shown (B) p120 was immunoprecipitated (IP) from HAEC lysates after siRNA transfection for FKBP12.6 and non-targeting siRNA (Scr) and precipitates were immunoblotted for pPKC α and VE-cadherin showing increased p120-pPKC α interaction and decreased p120-VE cadherin interaction after transfection. Total cell lysates were also immunoblotted (IB) for the respective antibodies with representative examples shown. Experiments was repeated four times (n = 4). (C) Densitometry was performed for the association of p120 with pPKC α and VE-cadherin (mean \pm SD, n = 4, * and # p < 0.01 compared to Scr). (D) Immunofluorescent imaging of HAECs with VE cadherin (green) and p120 (red) was performed after siRNA transfection for FKBP12.6 and non-targeting siRNA (Scr) shown in 60x. Negative control (NC) shown with secondary antibody only and DAPI nuclear counterstain. White bar is 10 μ m. (E) Representative 2-D florescent intensity plots for immunofluorescent images for each treatment group (shown in D) with pearson correlation coefficients (r) for the co-localization of p120 and VE cadherin pixels shown in inset (mean \pm SD, p < 0.01 for Scr v. siFKBP12.6, n > 4 fields of > 10 cells).

Figure II. Torin2 inhibits Human Aortic Endothelial Cells (HAEC) Proliferation. (A) Torin2, an ATP-competitive mammalian of rapamycin (mTOR) inhibitor, inhibits HAEC in a dose dependent manner (0 – 1000 nmol/L, n = 4). (B) IC₅₀ for proliferation for Torin2 and Sirolimus is 25 nmol/L and 500 nmol/L, respectively, and both significantly inhibited proliferation compared with control (n = 4, * p < 0.05).

Figure S3

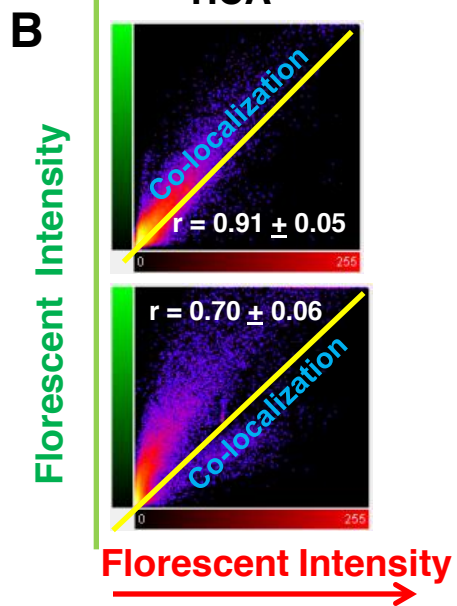
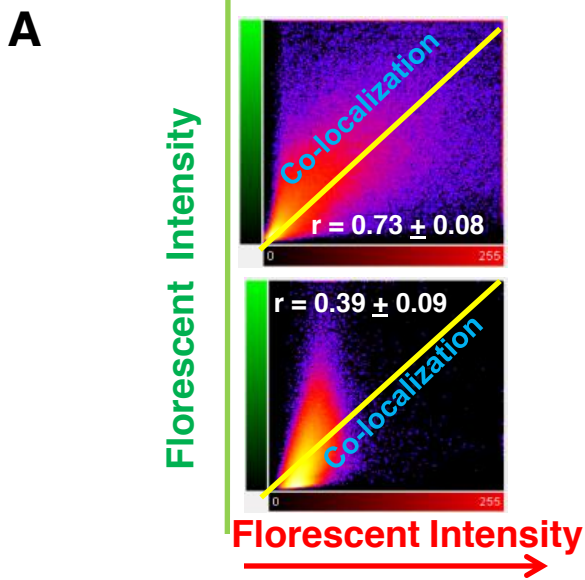


Figure S4

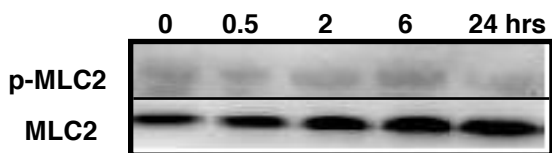
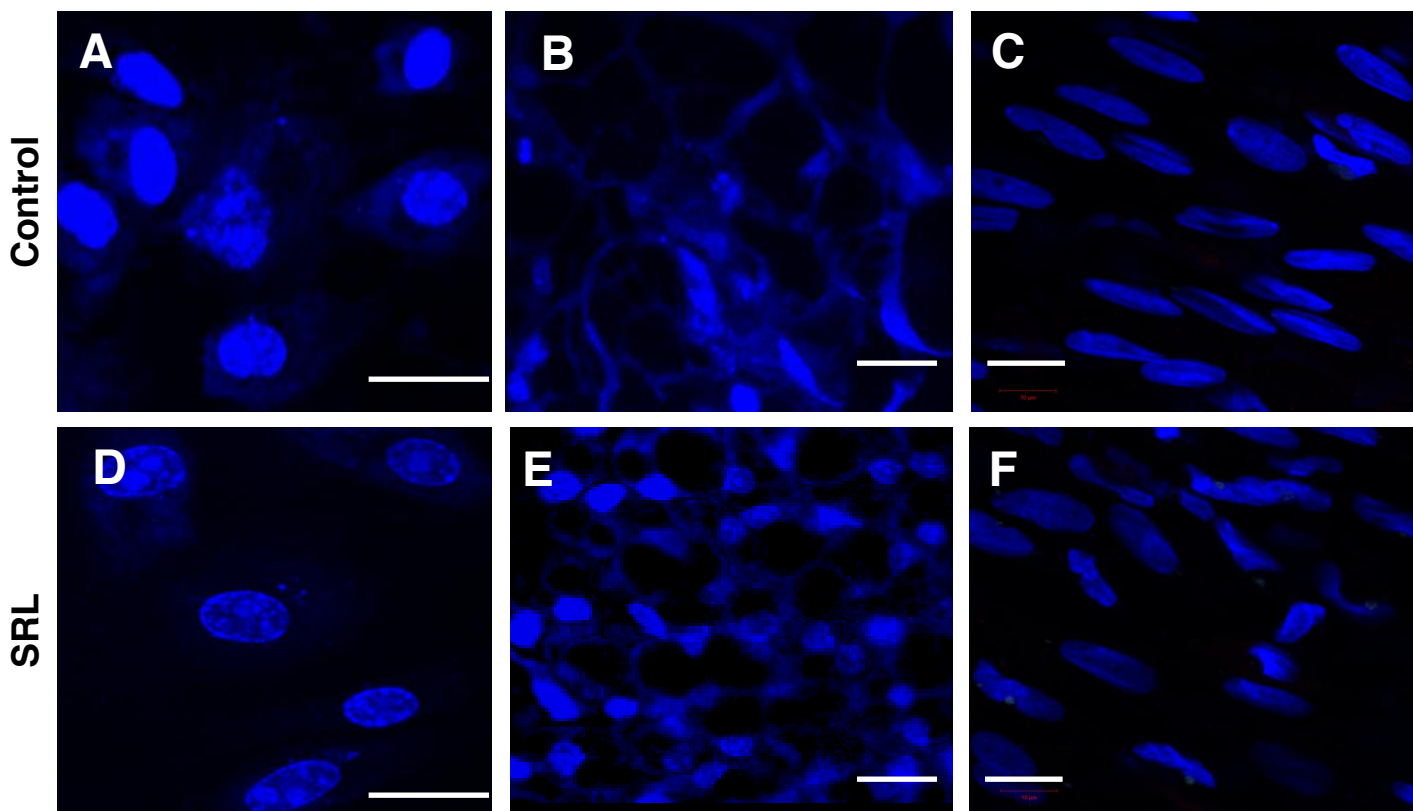


Figure S5

HAEC

MAE

HCA



Supplemental Figure Legends

Figure III. Sirolimus Decreases p120-VE cadherin Colocalization *in vivo* and *ex vivo*. (A) Representative 2-D florescent intensity plots for immunofluorescent images of mouse aortic endothelium (MAE) for vehicle (top) and SRL-treated (bottom) groups (images shown in figure 3C) with pearson correlation coefficients (r) for the co-localization of p120 and VE cadherin pixels shown in inset (mean \pm SD, $p < 0.01$ for cont v. SRL, $n > 4$ fields of > 10 cells). (B) Representative 2-D florescent intensity plots for immunofluorescent images of human coronary endothelium (HCA) for vehicle (top) and SRL-treated (bottom) groups (images shown in figure 3C) with pearson correlation coefficients (r) for the co-localization of p120 and VE cadherin pixels shown in inset (mean \pm SD, $p < 0.01$ for cont v. SRL, $n > 4$ fields of > 5 cells).

Figure IV. Representative western blots of phosphorylated Myosin Light Chain 2 (pMLC-2, Ser18/Thr19) and total MLC-2 treated with SRL (500 nm) for the indicated time intervals. Repeated 4 times ($n = 4$)

Figure V. Negative control images for p120 and VE cadherin for HAEC, MAE and HCA, respectively. (A-C) Human aortic endothelial cells (HAEC), mouse aortic endothelium (MAE) and human coronary artery (HCA) dual immunofluorescence for p120 (red) and VE cadherin (green) using secondary antibodies only in control treated groups. Magnification at 60x with white bar indicating 10 μm for (A), 20x with the white bar indicating 20 μm for (B) and Images shown in 40x with the white bar indicating 10 μm for (C). (D-F) Human aortic endothelial cells (HAEC), mouse aortic endothelium (MAE) and human coronary artery (HCA) dual immunofluorescence for p120 (red) and VE cadherin (green) using secondary antibodies only in SRL-treated groups. Magnification at 60x with white bar indicating 10 μm for (D), 20x with the white bar indicating 20 μm for (E) and Images shown in 40x with the white bar indicating 10 μm for (F) DAPI (blue) is the nuclear counterstain.

Supplemental Table I. Baseline Transendothelial Electrical Resistance (TEER) and Average TEER over Measured Period

Group (n = 4)	TEER at t = 0	Average TEER ($\Omega \text{ cm}^2$) \pm SD
Control	908	858 \pm 33
Sirolimus (SRL)	795	686 \pm 34
Ryanodine (Ryan)	997	1061 \pm 29
SRL + Ryan	992	1026 \pm 64
Scr	941	930 \pm 30
Scr + SRL	914	803 \pm 71
siPKC α	1087	1000 \pm 46
siPKC α + SRL	928	949 \pm 30
siFKBP12.6	811	739 \pm 22
siFKBP12.6 + Ryan	920	856 \pm 63
Torin 2	1157	1203 \pm 20

Supplemental Table II. Average FITC-Dextran Permeability (florescent intensity [AU])

Group (n = 4)	Average FITC-Dextran Permeability
No cells	880 \pm 26
Control	133 \pm 13
Sirolimus (SRL)	168 \pm 11
SRL + Ryanodine	122 \pm 8