## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

*Invasion assays*- $5.0x10^4$ cells in 0.05% FBS/DMEM were seeded in the top chamber of a Biocoat Matrigel invasion chamber (BD Biosciences) with 1.5% FBS/DMEM in the bottom chamber. At 8hrs cells were fixed, permeabilized and stained with rhodamine-phalloidin. For each membrane, five fields of cells were counted. For assays with Y-27632, cells were treated with 10μM Y-27632 (Calbiochem) for 5hr prior to and during invasion assays.

*Image analysis*- Densiometry for Western blots, and image analysis for RhoA biosensor, invasion and tube formation were performed with ImageJ.

*Western blotting*- 100 μg of protein were separated by Western blot and blotted with anti-RhoA (Santa Cruz, 26C4) or anti-phospho-MLC2 (Cell Signaling, Ser19) antibody or mouse monoclonal anti-CCM2 antibody (Johnson lab, UNC 48.8.5).

## SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1. Quantitation of shRNA knockdown of CCM expression. (**A**) Expression of CCM1, 2, and 3 mRNA in stable lentiviral shRNA infected cells. Expression of CCM1, 2, and 3 mRNA in shCCM1, 2, and 3 cells are knocked down by 70, 96 and 95% relative to pLKO.1 cells, respectively. Real-Time PCR was used to measure expression levels and data are the means ± SEM of three independent experiments performed in triplicate. (**B**) Western blot of WT and shCCM2 cells showing that CCM2 protein expression is lost in shCCM2 cells.

Supplemental Fig. 2. Invasion is decreased in shCCM1,2 or 3 endothelial cells and rescued by ROCK inhibitor Y-27632 or shRNA for ROCK2. (**A**) shCCM1, 2 or 3 cells were seeded in the top of a Boyden chamber invasion chamber. Cells invaded to the bottom of the membrane were stained with Rhodamine phalloidin and imaged. Five images per membrane were taken and one image per condition is shown. Invasion of shCCM1, 2 or 3 cells is decreased relative to WT, but rescued upon treatment with 10μM Y-27632 or upon shRNA knockdown of ROCK2. (**B**) Bar graph showing the fold change in the number of invaded shCCM cells relative to pLKO.1. Invasion in shCCM1, 2 or 3 cells is decreased respectively 70%, 70% and 60% relative to pLKO.1 cells. Data represents mean ± SEM for minimum of three independent experiments. Error bars ***$p<0.001$, **$p<0.02$, *$p<0.05$.

Supplemental Fig. 3. Tube formation in shCCM1, 2, and 3 endothelial cells is rescued by shROCK2 or Y-27632 treatment. (**A**) Tube formation in shCCM1, 2, and 3 cells is not rescued with extended incubation. At 48 hours, cells congregate but still fail to form tubes. Cells die and detach after 48 hours. Image is a 9 panel montage of 10x fields, and bar represents 300μM. (**B**) Y-27632 and shROCK2 rescue tube formation in CCM1, 2, and 3 knockdown cells. Image is a 9 panel montage of 10x fields, and bar represents 300μM.

Supplemental Fig. 4. Expression levels of CCM1, 2, 3, and ROCK2 in single and double knockdown stable cell lines. Knockdown was achieved through lentiviral shRNA and was measured by Real-Time PCR. Data are the means ± SEM of three independent experiments performed in triplicate and normalized to empty vector (pLKO.1) cells. (**A**) Expression of ROCK2 in shCCM1, 2, and 3 cell lines. (**B**) Expression of CCM1, 2, and 32 in shCCM/shROCK2 cells. CCM1, 2, and 3 knockdown is 50, 92, and 95 %, respectively. ROCK2 knockdown is 79, 68, and 81% in shCCM1/shROCK2, shCCM2/shROCK2, and shCCM3/shROCK2 cells, respectively.

Supplemental Fig. 5. CCM1, 2, and 3 knockdown endothelial cells generate filopodia but are unable to form lamellipodia and undergo cobblestone to cuboidal transition. Migration is also impaired. Data shows images of cells taken at approximately 1 and 4 hours. shROCK2 and Y-27632 rescue tube

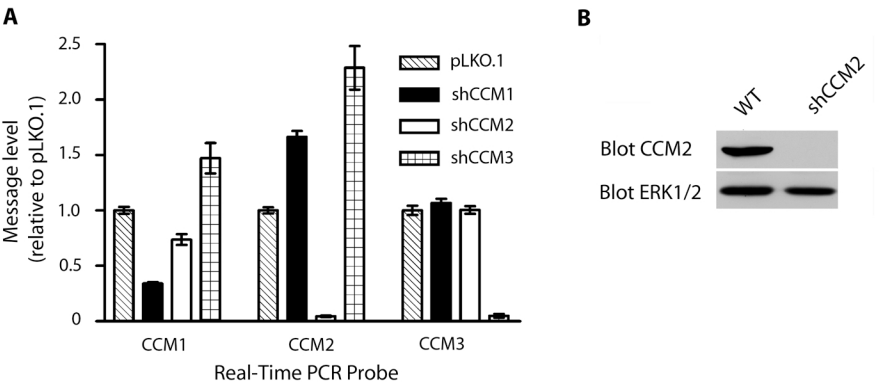
formation. Arrow denotes filopodia, arrowhead denotes cells with pronounced membrane protrusions that form nucleation centers for tube formation. Bar represents 50μM

Supplemental Movie 1. pLKO.1 and shCCM2 MEECs were incubated at 37° C/7% $CO_2$ on Matrigel-coated 24-well plates and imaged at 10x on a Cellomics Arrayscan (Thermo Scientific) via transmitted light every 10 minutes for 18 hours.

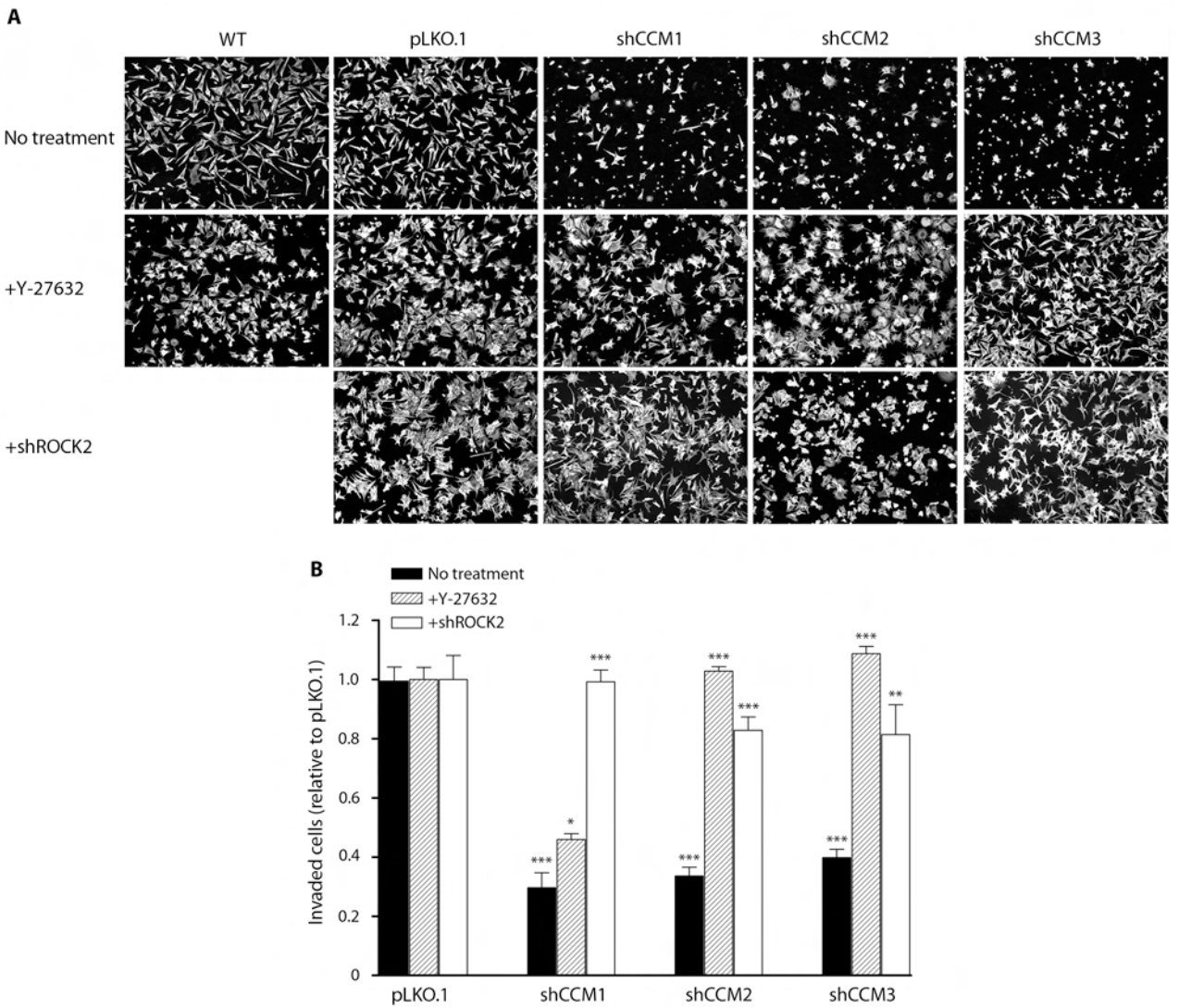
Supplemental Movie 2. pLKO.1 and shCCM2 MEECs were incubated and imaged as described in figure S1 in the presence of 10μM Y-27632.

Supplemental Movie 3. pLKO.1/shROCK2 and shCCM2/shROCK2 MEECs were incubated and imaged as described in figure S1.

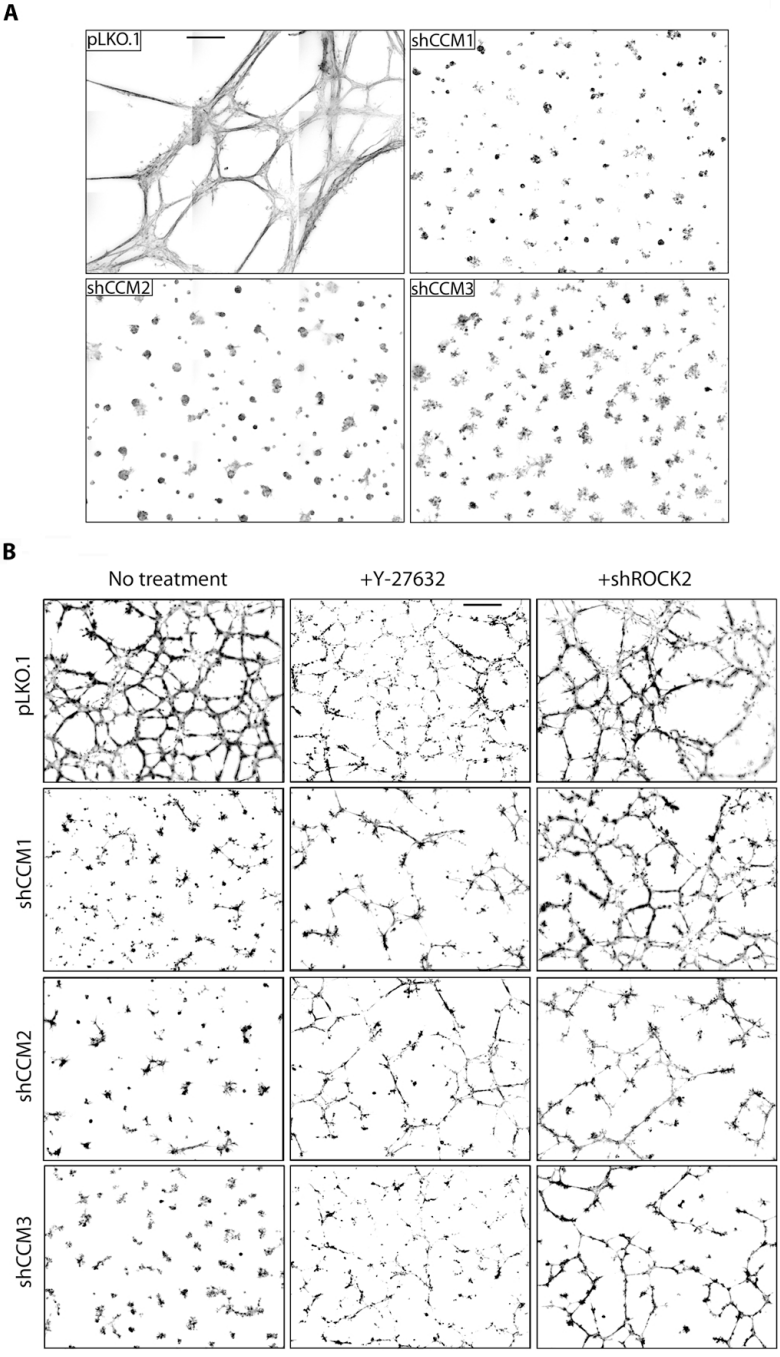
**Supplemental Figure 1**



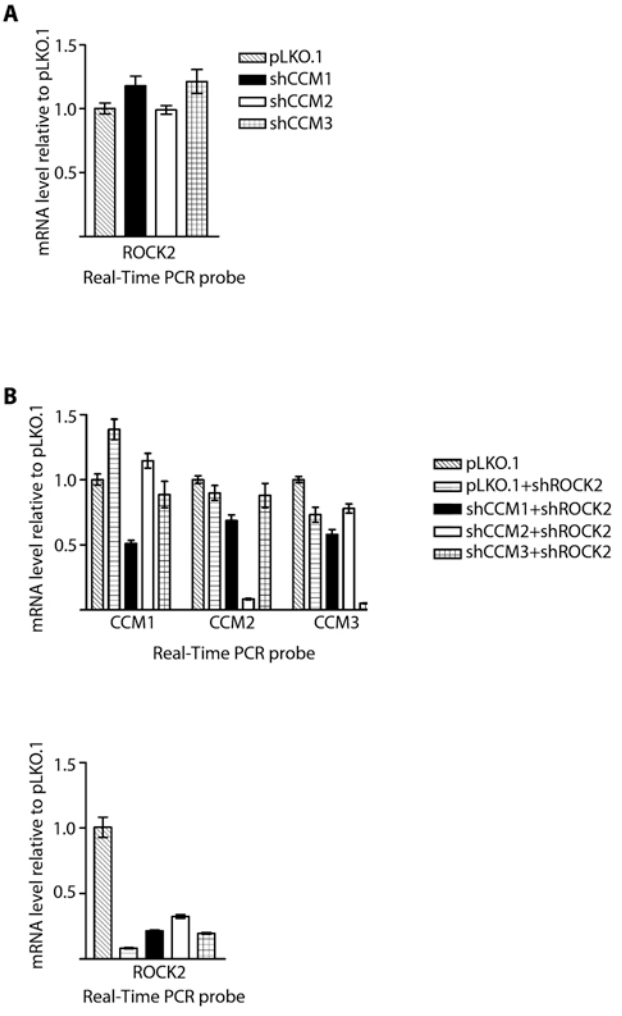
**Supplemental Figure 2**



**Supplemental Figure 3**



**Supplemental Figure 4**



**Supplemental Figure 5**