SUPPLEMENTAL METHODS

Immunofluorescence: Cells were grown on Matrigel-coated glass coverslips (1:50, BD Biosciences) and immunofluorescence was performed as previously described. To determine phospho-MLC2 association with stress fibers, five representative images for each experimental condition were masked for rhodamine-phalloidin intensity and the mean intensity of phospho-MLC2 was measured on this mask.

In vitro tube formation assay: Tube formation was performed as previously described.

HUVEC Permeability Assay: Permeability assays were performed as previously described. Every 15 minutes for 1.5 hours, 50 μL aliquots were removed from the lower chamber, and FITC intensity was measured using a PHERAstar microplate reader (BMG Labtech). Relative fluorescence units (RFU) were plotted against time and reported as the average change in RFU per minute between duplicate Transwells.

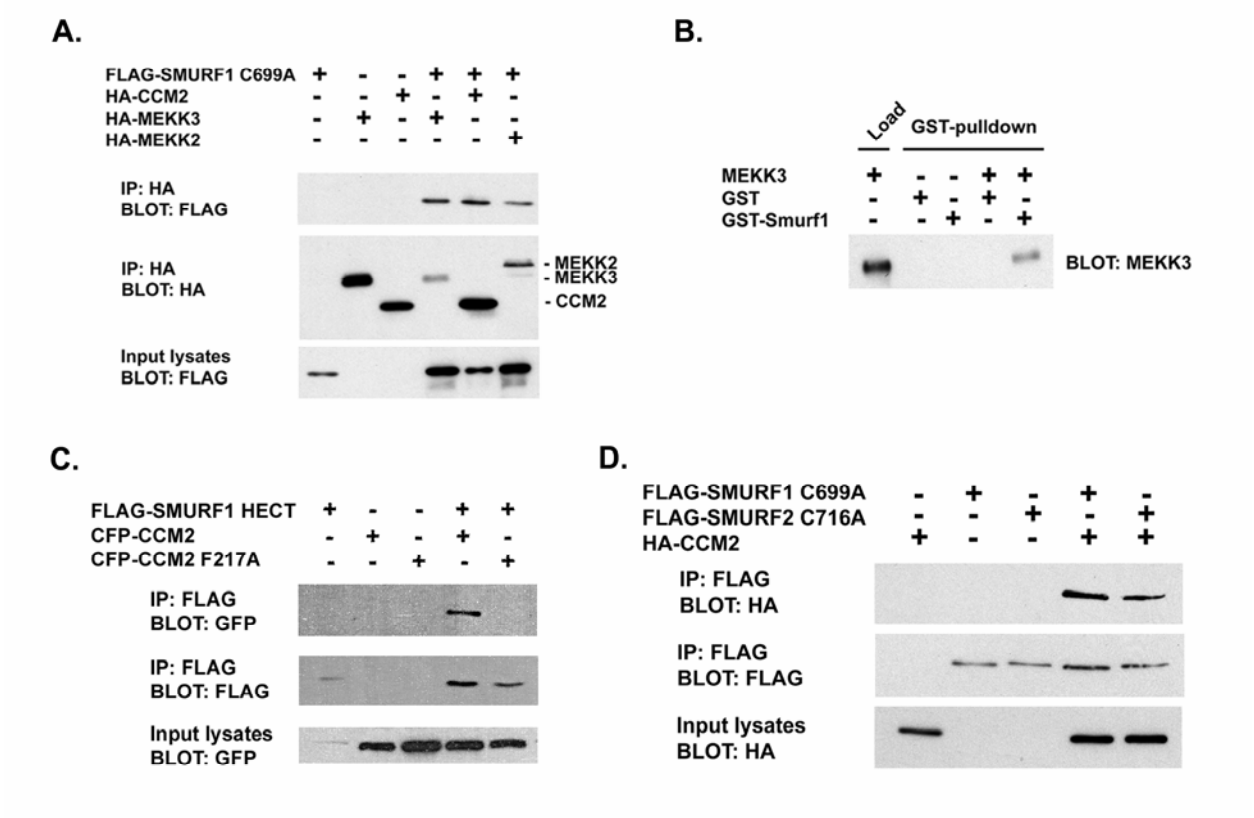
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1. Smurf1 binds MEKK3 and CCM2. A) Cells expressing HA-tagged CCM2, MEKK2, or MEKK3 with Flag-Smurf1 C699A were immunoprecipitated with anti-HA antibody and associated Flag-Smurf1 C699A was determined by western blot. B) The Smurf1-MEKK3 interaction is direct. Purified GST or GST-Smurf1 was incubated with purified His-MEKK3 and associating MEKK3 was determined by western blot using anti-MEKK3 antibody. C) CCM2 interacts with the Smurf1 HECT domain in a PTB domain dependant manner. Flag-Smurf1 HECT was expressed with either CFP-CCM2 or CFP-CCM2 F217A in cells. Cell lysates were incubated with anti-Flag and associating CFP-CCM2 was determined by western blot. D) CCM2 can associate with either Smurf1 or Smurf2. Cells expressing HA-CCM2 and either Flag-Smurf1 C699A or Flag-Smurf2 C716A were lysed and immunoprecipitated with anti-Flag antibody. Associating HA-CCM2 was determined western blot with anti-HA antibody.

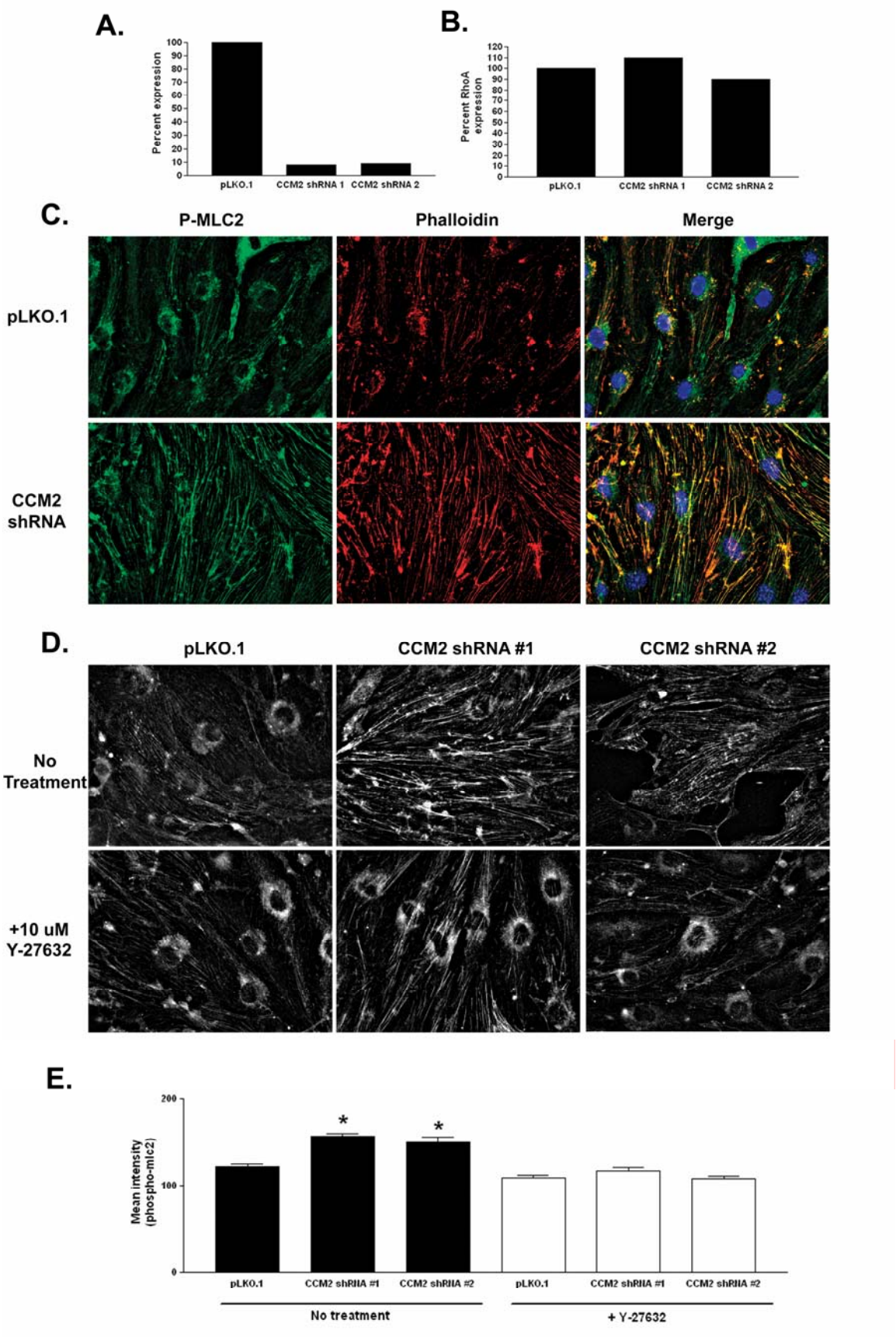
Supplemental Fig. 2. CCM2 knockdown leads to cytoskeletal changes in brain endothelial cells. A) Relative quantitation of CCM2 RNA expression was determined by real time RT-PCR quantitation. Representative data shown. CCM2 RNA levels were standardized to β-actin RNA levels. B) Relative quantitation of RhoA RNA expression was determined by real time RT-PCR quantitation. Representative data shown. RhoA RNA levels were standardized to β-actin RNA levels. C) bEND.3 cells stably expressing pLKO.1 or CCM2 shRNA were plated on Matrigel-coated coverslips and stained for phalloidin (red), phospho(Ser19)-MLC2 (green), and DAPI (blue). D) Association of phospho-MLC2 with stress fibers is abrogated with treatment with the ROCK inhibitor Y-27632. Top: bEND.3 cells grown on coverslips were treated with or without Y-27632 for 24 hours and then stained for phospho(Ser19)-MLC2. E) Intensity of phosphorylated MLC-2 associated with rhodamine-phalloidin was determined as described in *Materials and Methods.*

Supplemental Fig. 3. CCM2 is necessary for endothelial barrier function and endothelial tubule formation. A) HUVECs electroporated with either CCM2 SMARTpool siRNA or scrambled siRNA were plated on duplicate Matrigel-coated Transwells and were allowed form monolayers for 48 hours. The medium was then removed and replaced with EBM-2 containing 0.625 mg/mL FITC-dextran, with or without 4 mM EGTA. Aliquots (50 μL) were removed from the bottom well at 15 minute increments for 1.5 hours and fluorescence was read using a PHERAstar microplate reader. Data is expressed as the change in relative fluorescence units (RFU) per minute ± the standard deviation of the duplicates. Results are representative of three experiments. The average percent knockdown of CCM2 RNA levels compared to scrambled controls was ~60%. B) Mouse embryonic endothelial cells stably expressing either pLKO.1 or CCM2 shRNA were subjected to an in vitro tube formation assay as described in *Materials and Methods.*

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3