Supplemental Figure I

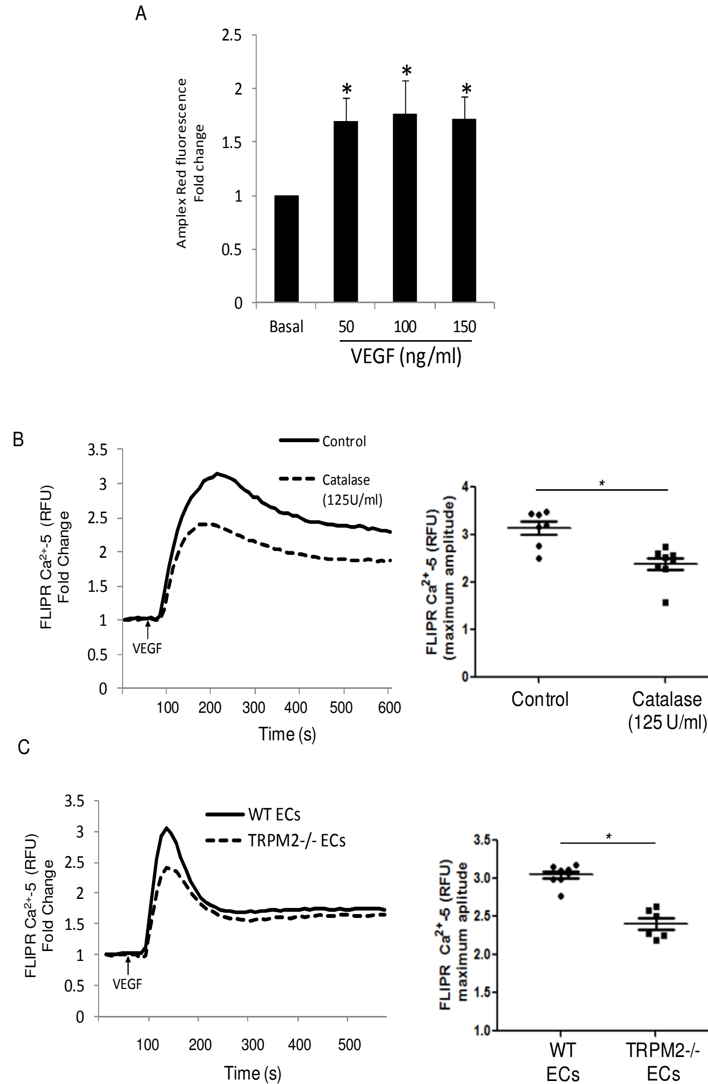


Fig. I: A) Amplex Red assay to measure ROS generation in ECs. Confluent human lung EC monolayers were seeded on 96-well plate and were stimulated with VEGF at the indicated concentration. An increase in fluorescence reflects the formation of red-fluorescent oxidation product, resorufin by H_2O_2 released from ECs. B) Ca^{2+} transients were measured using FLIPR calcium-5 dye following VEGF stimulation in control and catalase (125 U/ml) treated cells. The cells were treated with PEG-catalase for 24 h. The tracing is an average response from 8 replicates of 96 well plate. Mean of maximum responses is expressed in a scattered dot plot (with SEM). C) Ca^{2+} transients measured using FLIPR calcium-5 dye following VEGF stimulation of WT and *TRPM2*$^{-/-}$ ECs. The tracing is an average response from 8 replicates of 96 well plate. Mean of maximum responses is expressed in a scattered dot plot (with SEM).

Supplemental Figure II

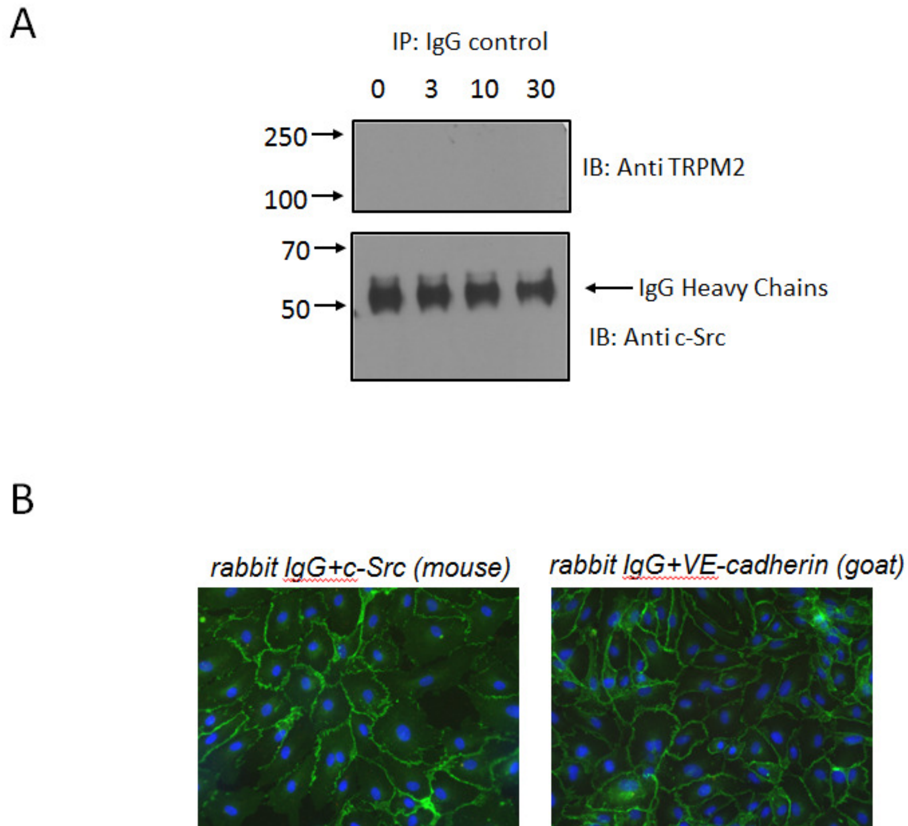


Fig II: A) IgG control for co-immunoprecipitation experiment. ECs lysate at different time points was immunoprecipitated with rabbit IgG and immublotted with TRPM2 and cSrc antibody. Except IgG heavy chain bands, no band was observed for TRPM2 or cSrc. Similar results were obtained when goat IgG was used and blotted for VE-cadherin (data not shown). B) Negative control for PLA assay. No in situ PLA signal was observed when either rabbit IgG and VE-cadherin (goat) or rabbit IgG and c-Src (mouse) were used.

Supplemental Figure III

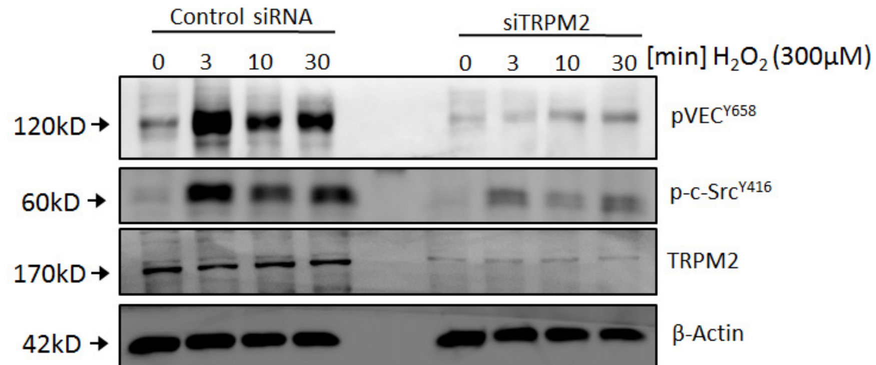


Fig. III. Effects of H_2O_2 on VE-cadherin phosphorylation in TRPM2 depleted ECs. Confluent monolayer of human ECs were stimulated with H_2O_2 at the indicated concentrations and blotted for phospho anti-VE cadherin Tyr658. Knockodown of TRPM2 suppressed phosphorylation of VE-cadherin and c-Src after stimulation with H_2O_2.

Supplemental Figure IV

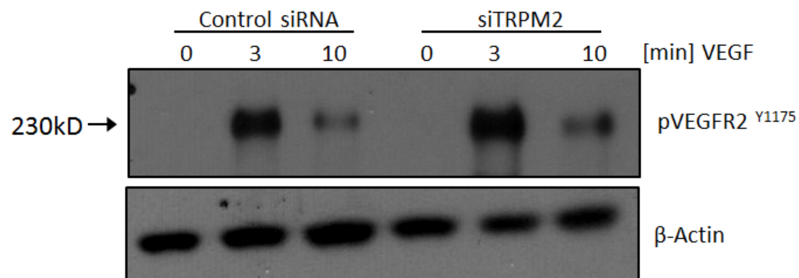
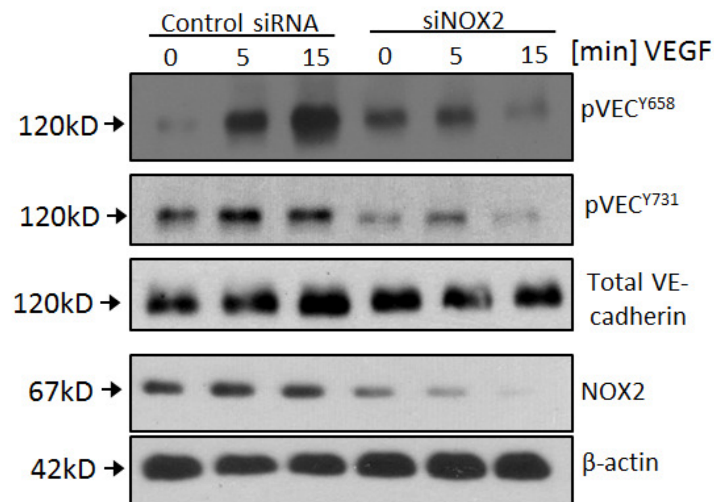


Fig IV: Immunoblot showing phosphorylation of VEGFR2 at active site Y1175 after VEGF stimulation in control and TRPM2 siRNA treated cells. Knockdownof TRPM2 did not affect the phosphorylation of VEGFR2 in ECs.

Supplemental Figure V



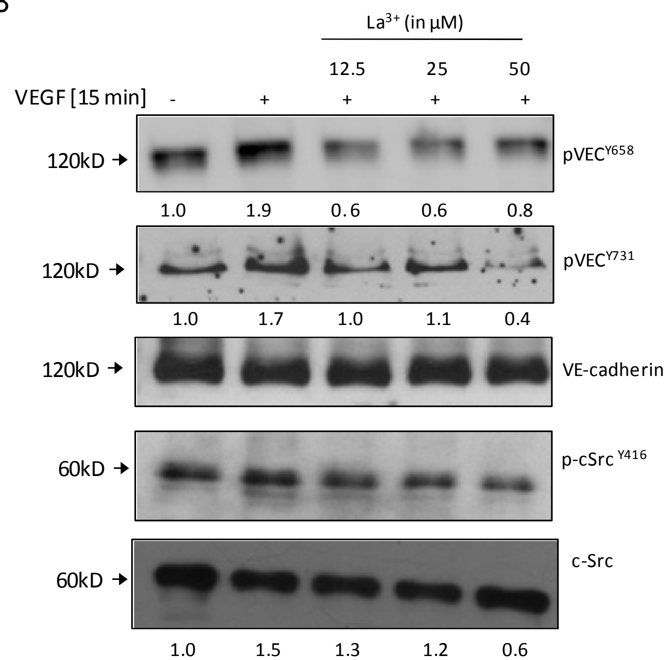


Fig. V: A) Immunoblots showing that knockdown of NOX2 in ECs suppressed VE-cadherin phosphorylation at Tyr658 and Tyr731 on VEGF stimulation indicating that ROS generated by NOX2 plays a crucial role in VEGF-activated VE-cadherin phosphorylation. B) Human lung ECs were pre-treated with $LaCl_3$ at the indicated concentration for 30 min then stimulated with VEGF for 15 min. The total cell lysate was blotted for anti- phospho VE-cadherin and anti-phospho c-Src antibodies. Representative blot is shown and experiment was repeated twice.

Supplemental Figure VI

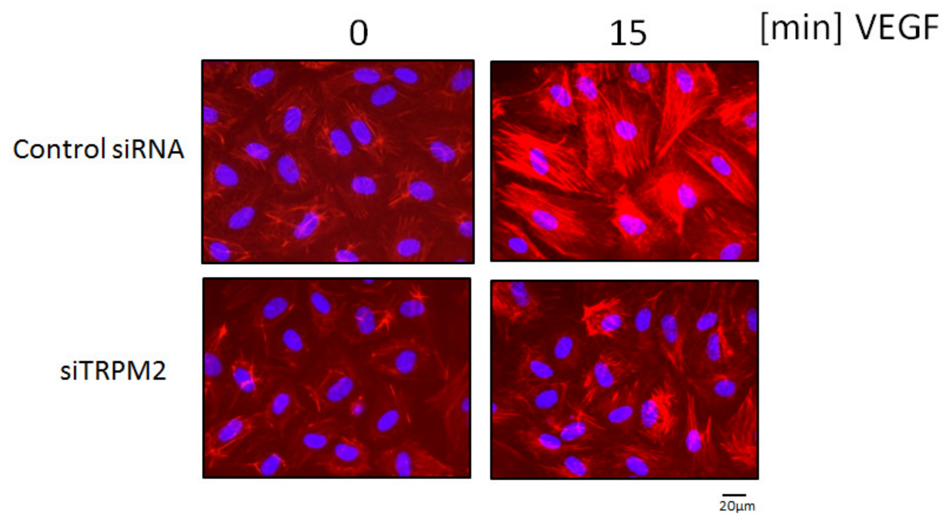


Fig VI: Human ECs were transfected with control and TRPM2 siRNA and were stimulated with VEGF for 15 min. The cells were stained with phalloidin for actin fiber staining. Formation of actin fibers on VEGF stimulation was suppressed after TRPM2 knockdown.

Supplemental Figure VII

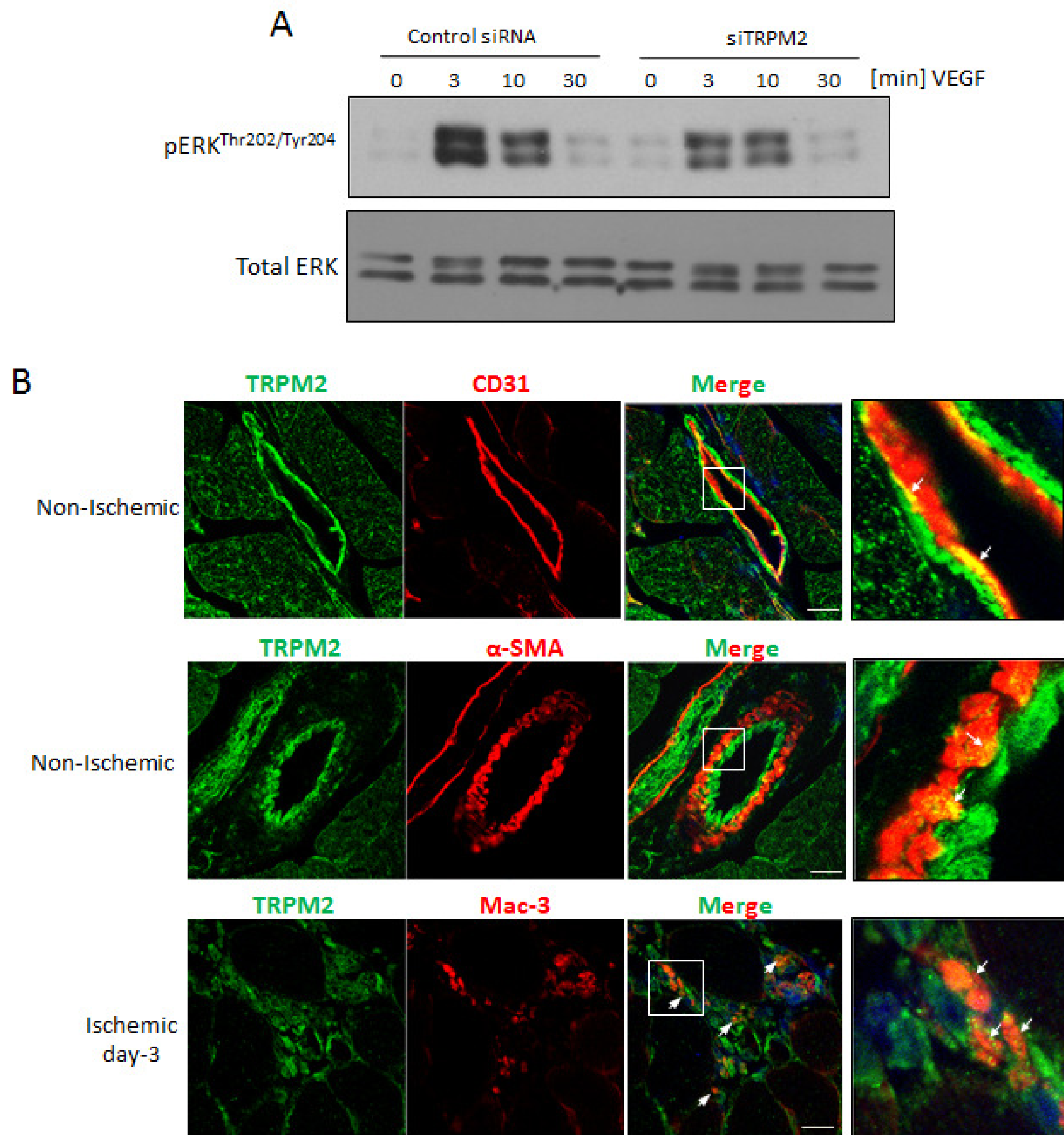


Figure VII: A) Immunnoblot showing VEGF-induced phosphorylation of ERK in ECs treated with control siRNA and TRPM2 siRNA. Activation of ERK was impaired in endothelial cells treated with TRPM2 siRNA compared to control siRNA treated cells. B) Co-localisation of TRPM2(green) with CD31 (red), α-SMA (red) and Mac-3 (red) on GC muscle of wild type mice. Expression of TRPM2 was localised in both endothelium and smooth muscle layer of the vessels. Expression of TRPM2 was also localised with Mac-3 (red) in mononuclear phagocytic cells recruited in response to ischemic injury.