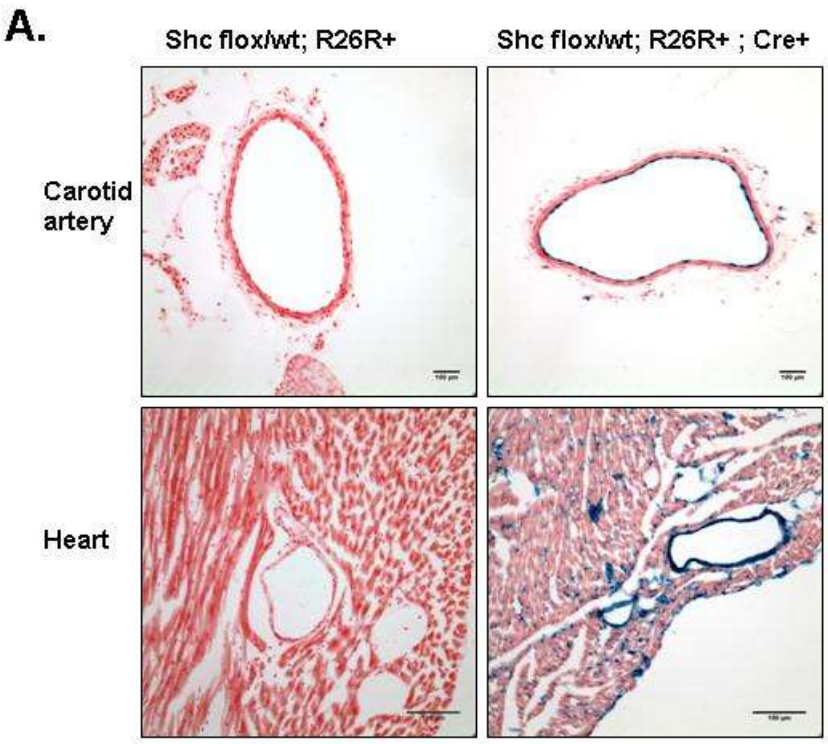

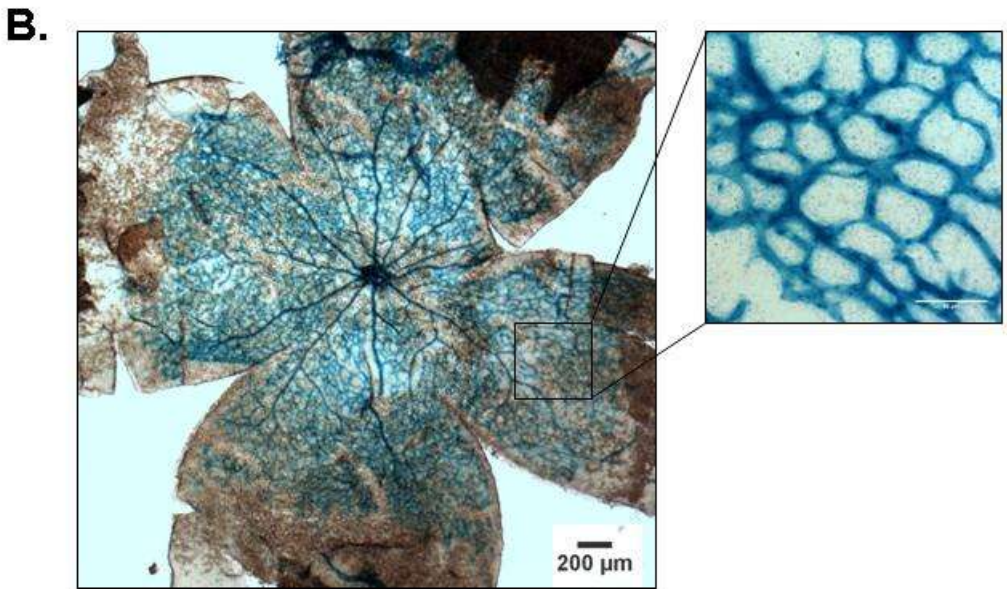

Figure S1. Shc knockout is restricted to ECs in *Shcflox/flox; Tie2-Cre+* mice (A) To confirm that Shc gene excision was restricted to endothelial cells, *Shcflox/flox; Tie2-Cre+* males were crossed to Rosa26Reporter mice to yield *Shcflox/WT; Tie2-Cre+; R26R* animals. Heart and arteries were isolated and sections were stained with X-Gal and counterstained with Nuclear Fast Red. (B) Retinas were isolated from P5 pups were stained with X-gal to visualize Tie2-Cre expression in the retina.

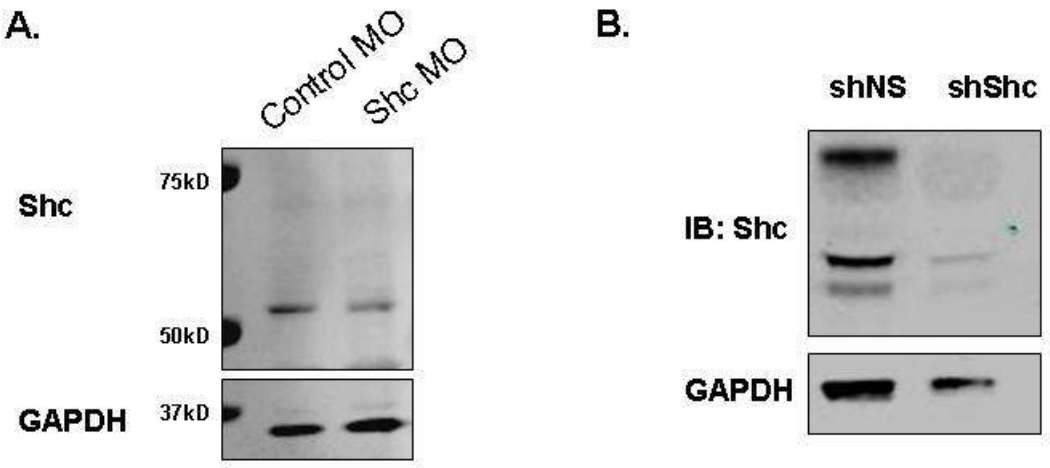

Figure S2. Shc knockdown in zebrafish and HUVECs

(A) Shc is expressed as a single isoforms of approximately 52 kDa in zebrafish. Total cell lysate from 48 hpf zebrafish embryos were analyzed by western blot to confirm Shc depletion by Shc-MO injection. (B) Lentivirus-mediated Shc knockdown in HUVEC. HUVEC infected with lentivirus expressing short hairpin RNA against Shc (shShc) or a non-specific target (shNS) were lysed and immunoblotted to confirm efficient Shc knockdown.

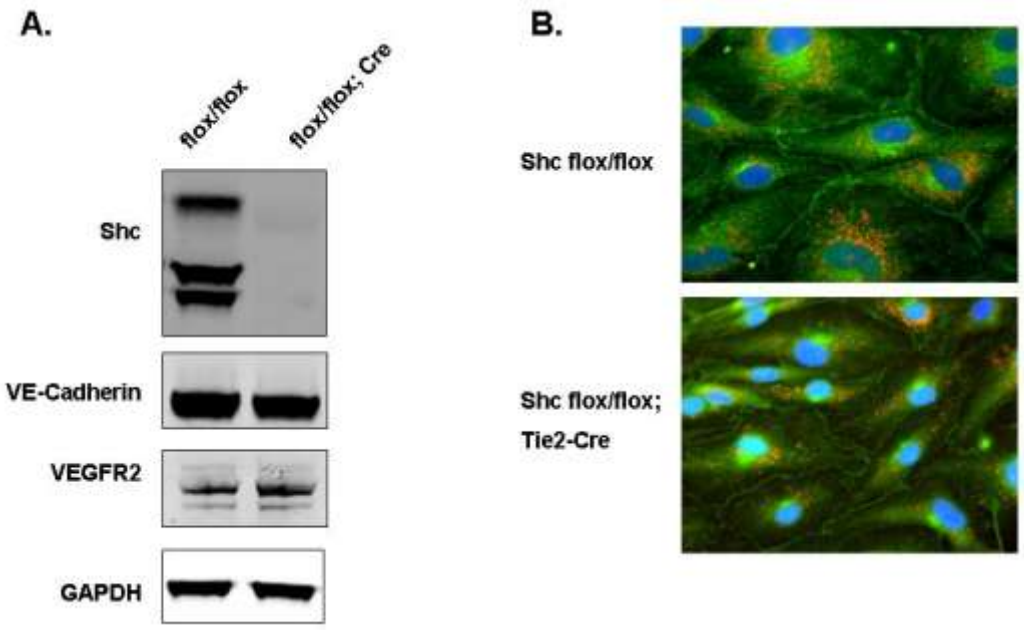

Figure S3. Validation of mouse lung endothelial cells (MLECs)
(A) Endothelial cells were isolated from P6-P9 pups using CD31-coated Dynabeads to select for PECAM-positive cells. Cell lysates were separated using SDS-PAGE and immunoblotted for Shc, GAPDH, VE-Cadherin and VEGFR-2 to confirm Shc knockout in ECs from *Shcflox/flox; Tie2-Cre*+ mice. Similar results were obtained from 4 different clones per genotype. (B) Immunofluorescence staining shows expression and proper localization of PECAM-1 (green) and Ac-LDL (red) uptake by MLECs.

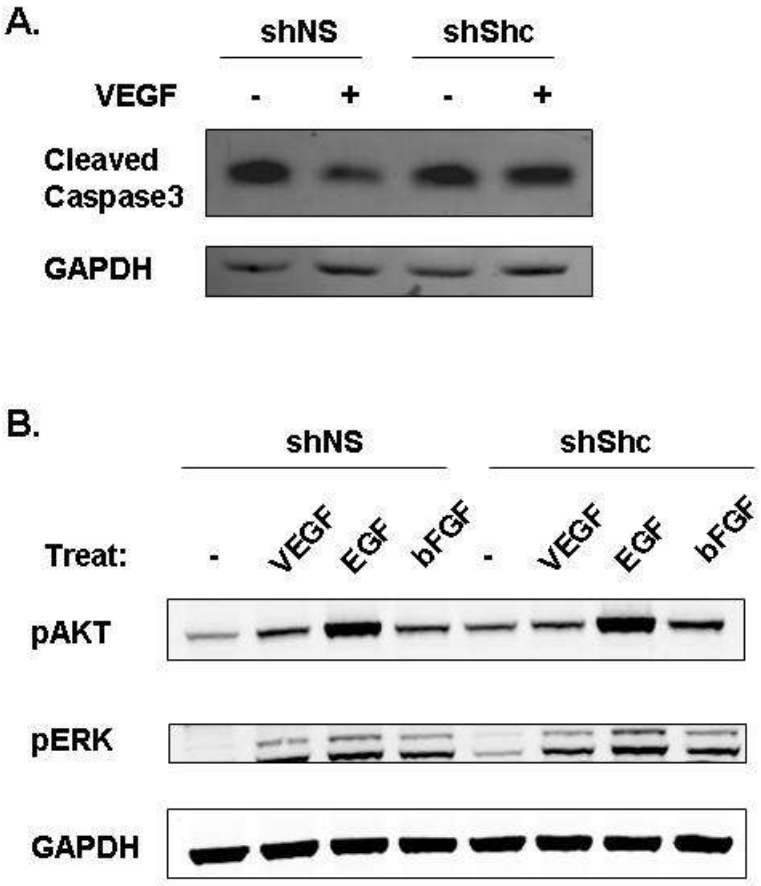

Figure S4. Shc is required for VEGF-induced survival and requirement for Shc in Akt activation is specific to VEGF signaling

(A) Lentivirus infected HUVECs were grown to confluence on FN coated plates and then serum starved for 24 hrs with or without 100ng/ml VEGF to induce apoptosis. Cells were lysed and 50ug total protein was loaded onto an SDS-PAGE gel. Lysates were immunoblotted for cleaved caspase 3, a well characterized marker and executor of apoptosis, and GAPDH as a loading control. (B) To assay the specificity of Shc in signaling activated by VEGF and other growth factors in ECs, HUVECs were serum starved 4 hrs to reduce basal signaling and then treated for 5 min with 100ng/ml VEGF, 100ng/ml EGF, 100ng/ml bFGF or vehicle. Cell lysates were immunoblotted for phos-Akt, phos-ERK,and GAPDH.