Supplemental Material

Detailed Methods

Antibodies and Reagents

Antibodies and reagents were purchased as follows: mouse anti Actin, mouse anti VE-cadherin and goat anti VE-cadherin antibodies (Santa Cruz); Src pY416, Src pY527, SHP2 (Cell Signaling); TXNIP (Invitrogen); Rat anti VE-cadherin (BD Biosciences Pharmingen) ; Phalloidin (Sigma-Aldrich); CSK pSer 364 (Abcam); Green fluorescent protein (Clontech)

Cell Culture and siRNA Transfection

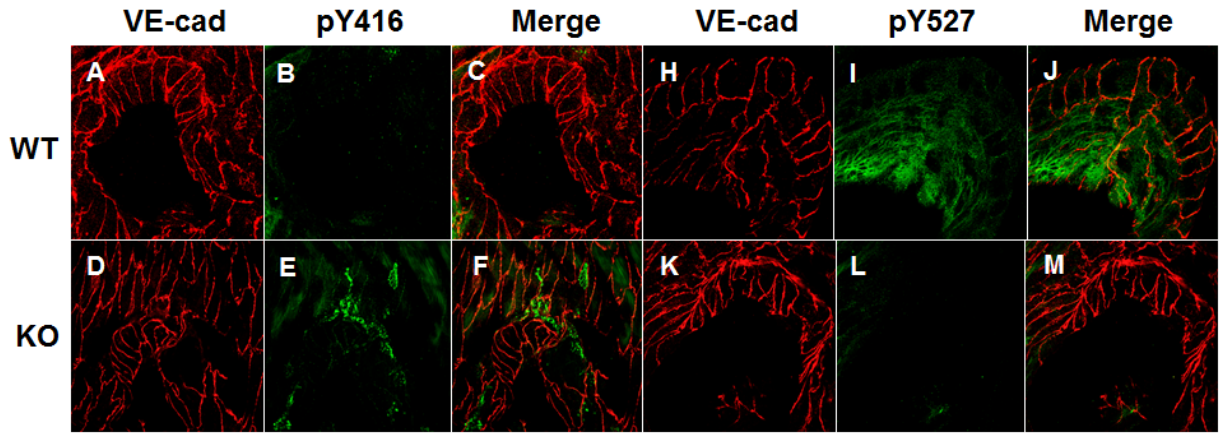
Human umbilical vein endothelial cell (HUVEC) or bovine aortic endothelial cell (BAEC) were seeded onto gelatin-coated dishes maintained in Medium 200 or Medium 199 respectively (Cascade Biologics, Portland Or) with low serum growth supplement and 5% FBS as previously described[1]. Cells were used at passages 2 to 6. Cells were transiently transfected with siRNA (TXNIP targeted or control) or plasmids as described in the text. Transfection was performed using Opti-MEM I Reduced Serum Media (Invitrogen) and Lipofectamine 2000. Experiments were performed 24 hours post-transfection.

Western Blot and Immunoprecipitation

Cells were washed twice in ice-cold PBS and harvested in lysis buffer (20 mmol/L Tris pH7.5, 150mmol/L NaCl, 1 mmol/L EDTA, 1 μmol/L EGTA, 1% Triton X-100, 2.5 μmol/L sodium pyrophosphate, 1 μmol/L β-glycerolphosphate, 100 mmol/L NaVO4, 1 mol/L NaF and protease inhibitor cocktail). Immune complex samples were separated by SDS-PAGE, transferred to nitrocellulose, and incubated with appropriate primary antibodies (overnight; 4°c). After washing and incubation with secondary antibodies (LiCor Biosciences, Lincoln NE), immunoreactive proteins were visualized with the Odyssey LiCor Infrared Imaging System. Densitometry of blots was performed using Image J software (version 1.36b, National Institutes of Health).

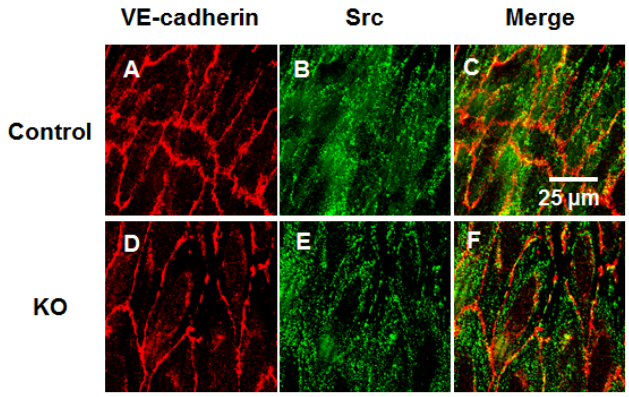
Reference

1. Yamawaki H, Haendeler J, Berk BC. Thioredoxin: a key regulator of cardiovascular homeostasis. *Circ Res.* Nov 28 2003;93:1029-1033.



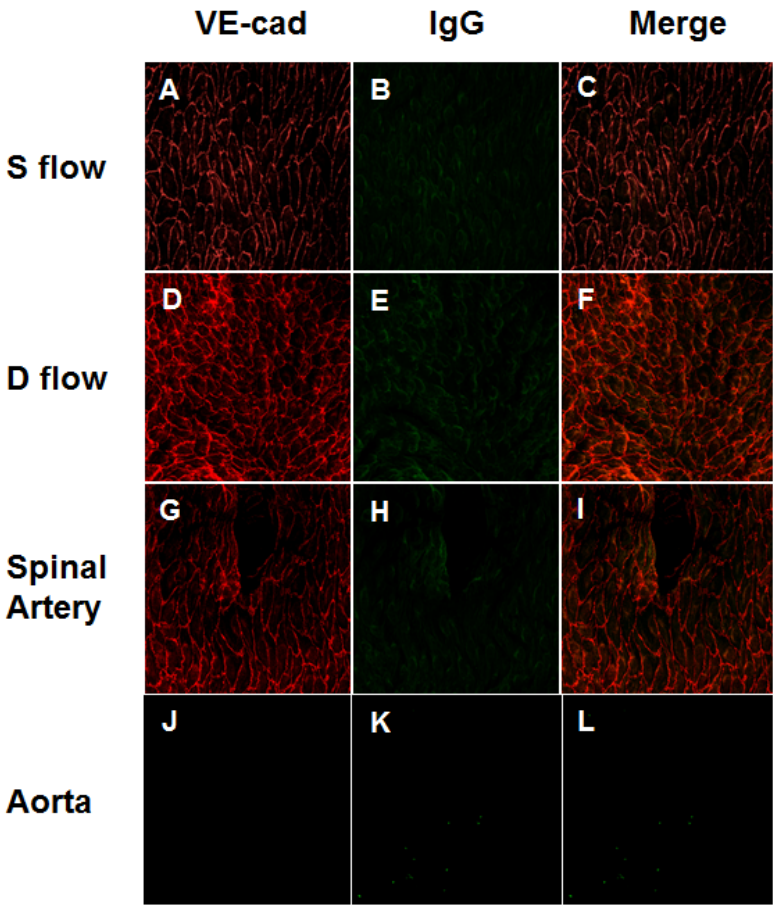
Supplemental Figure I

En face staining demonstrates differential regulation of Src pY416 and pY527 in KO animals compared to control. Aortas of control **(A-C; H-J)** or KO **(D-F; K-M)** were immuno-stained for VE-cadherin **(A, D, H, K),** Src pY416 **(B, E)**, Src pY527 **(I, L)** or presented as merged images **(C, F, J, M)**.



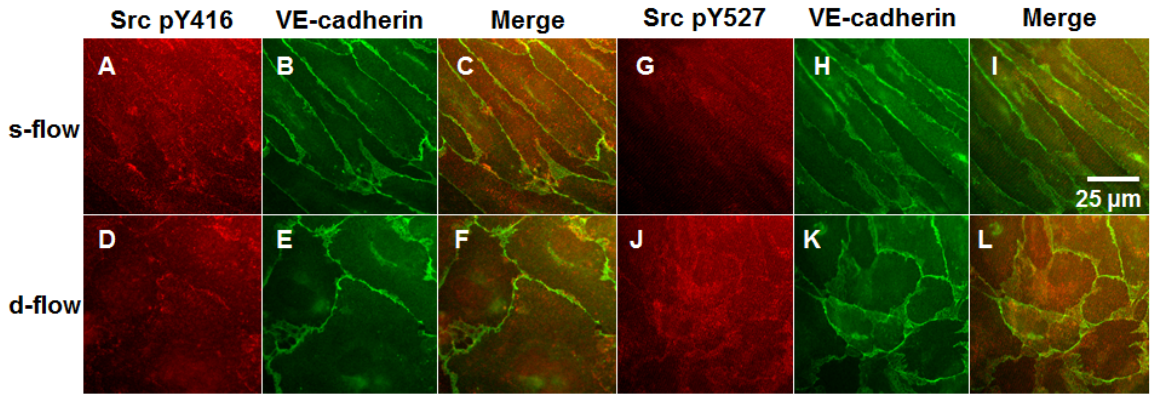
Supplemental Figure II

En face staining demonstrates equal amounts of Src in control and KO animals.



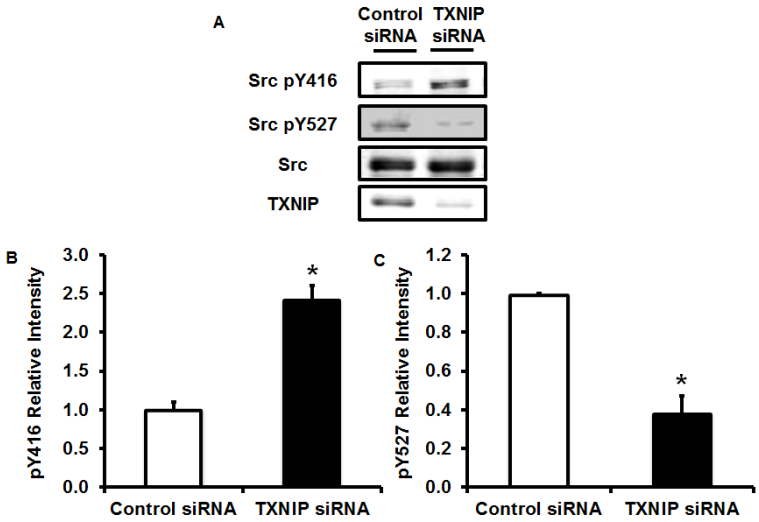
Supplemental Figure III

Negative controls for *En face* staining demonstrate low green channel background in various regions of the aorta: s-flow, d-flow and spinal artery exit. Aortas were stained for VE-cadherin **(A, D, G),** IgG control **(B, E, H)**, or no antibody and no IgG **(J, K)** or presented as merged images **(C, F, I, L)**. No significant background signals were observed.



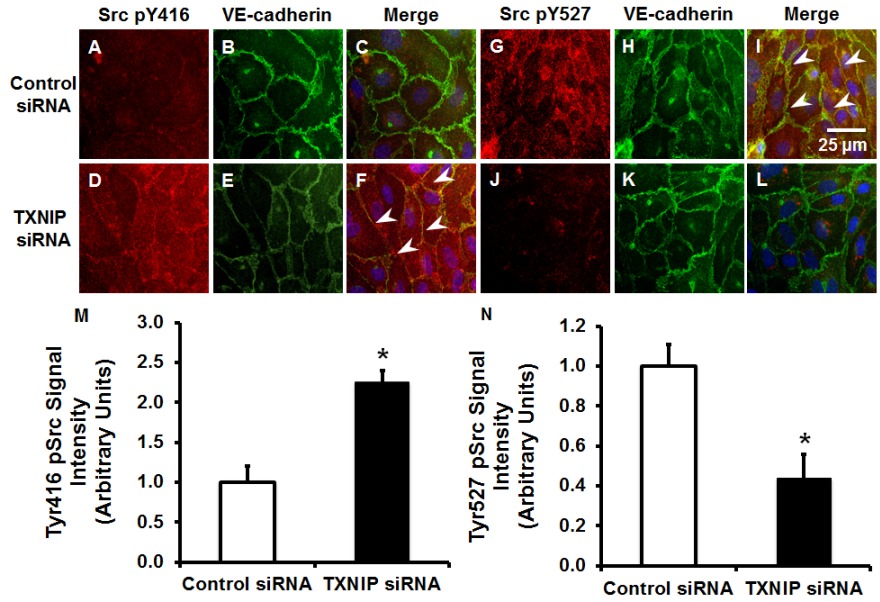
Supplemental Figure IV

Immunofluorescence assay demonstrate different cellular distribution and phosphorylation state of Src in response to flow. HUVEC exposed to s-flow **(A-C & G-I)** or d-flow (**D-F & J-L)** were immuno-stained for pY416 **(A, D)**, pY527 **(G, J)**, VE-cadherin **(B, E, H, K)** or merged **(C, F, I, L).** (N=4).



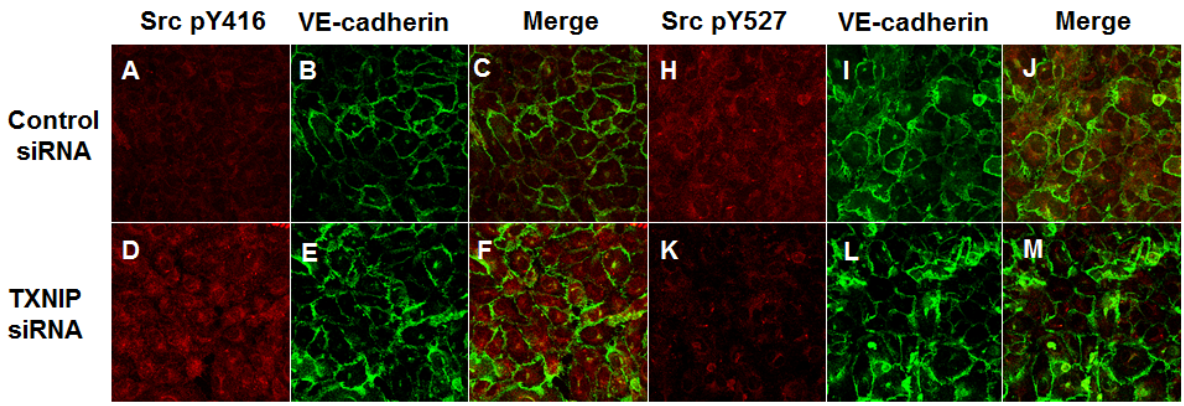
Supplemental Figure V

Western blot analysis demonstrates Src pY416 is regulated by TXNIP expression. HUVEC transfected with control siRNA or TXNIP siRNA **(A)** were left untreated and immuno-blotted for pY416, pY527, Src and TXNIP. **(B, C)** Quantification of the data using Image J (* $P<0.05$ vs. control siRNA; n=3).



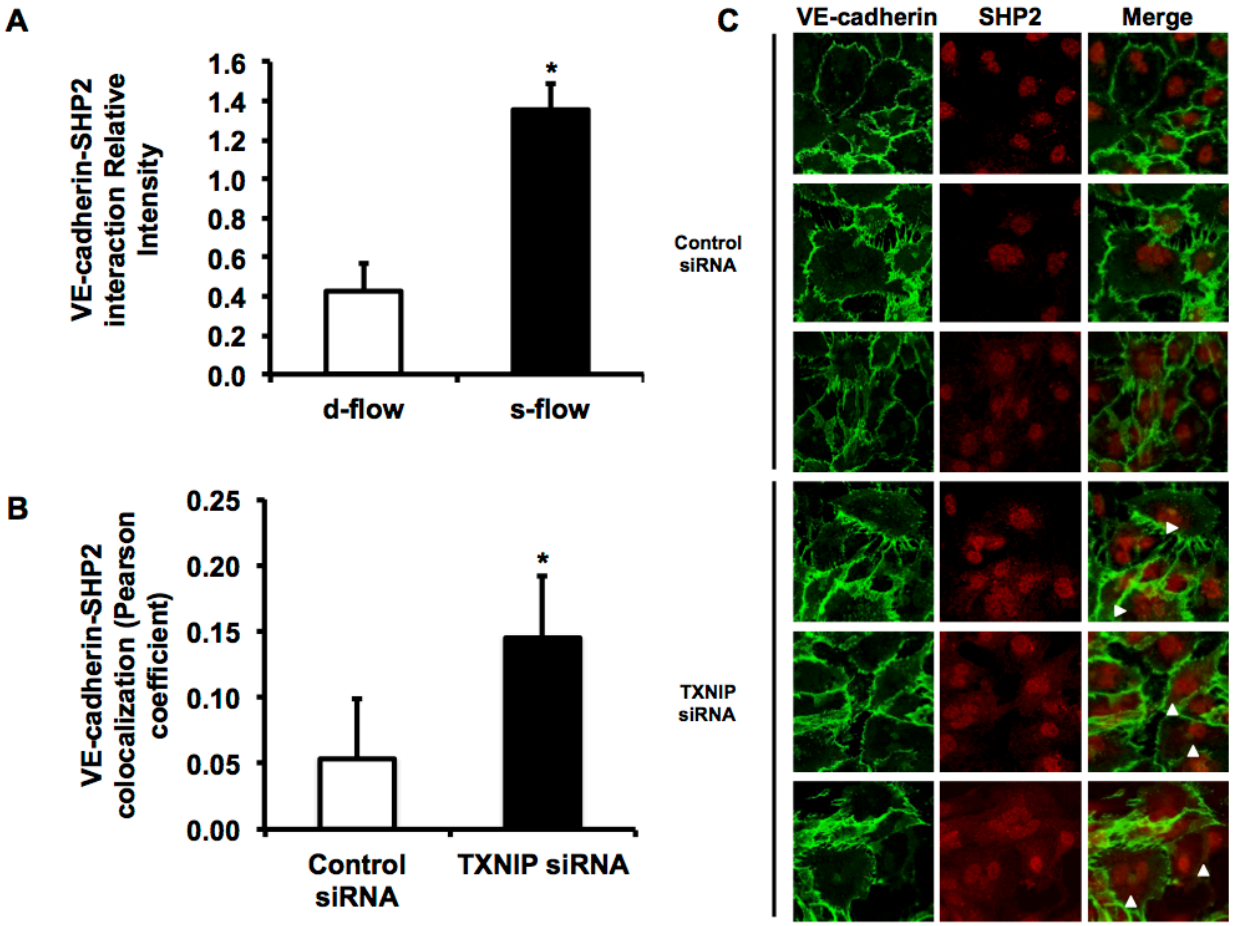
Supplemental Figure VI

Immunofluorescence assay demonstrate different cellular distribution and phosphorylation state of Src after TXNIP depletion. HUVEC transfected with control siRNA **(A-C & G-I)** or TXNIP siRNA (**D-F & J-L)** were left untreated and immuno-stained for pY416 **(A, D)**, pY527 **(G, J)**, VE-cadherin **(B, E, H, K)** or merged **(C, F, I, L)**. **(M, N)** Quantification of the data using Image J show pSrc intensity (* P<0.05 vs. control siRNA; n=4).



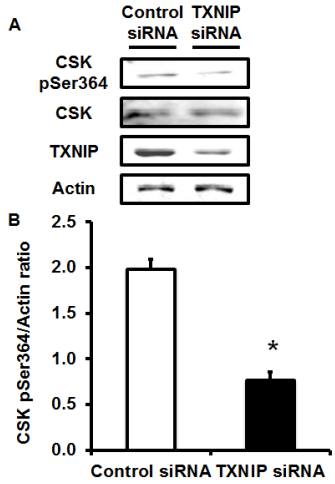
Supplemental Figure VII

Confocal microscopy demonstrates different cellular distribution and phosphorylation state of Src after TXNIP depletion. HUVEC transfected with control siRNA **(A-C & H-J)** or TXNIP siRNA (**D-F & K-M)** were left untreated and immuno-stained for pY416 **(A, D)**, pY527 **(H, K)**, VE-cadherin **(B, E, I, L)** or merged **(C, F, J, M)**.



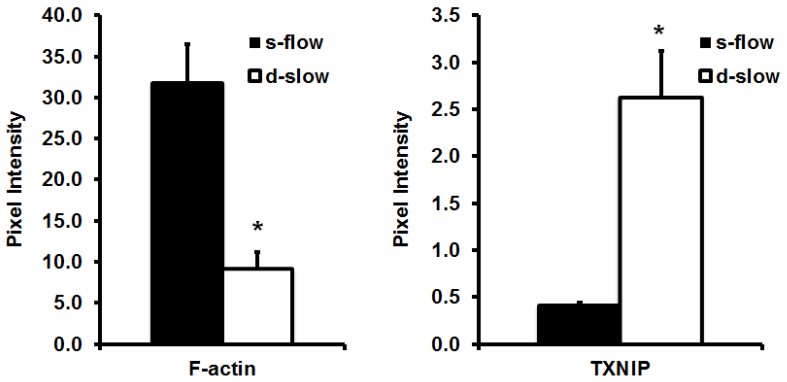
Supplemental Figure VIII

(A) Quantification of VE-cadherin-SHP2 interaction under s- or d-flow conditions (* P<0.05 vs. control siRNA; n=3). **(B)** Colocalization quantification using ImageJ of VE-cadherin-SHP2 shown in panels C (* P<0.05 vs. control siRNA; n=3). **(C)** SHP2 subcellular localization after transfection of Control or TXNIP siRNA. White arrowheads demonstrate areas of SHP2 redistribution and colocalization.



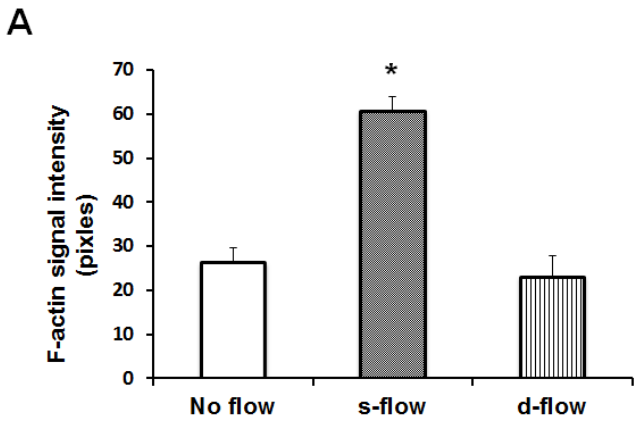
Supplemental Figure IX

Western blot analysis demonstrates CSK pSer364 is regulated by TXNIP expression. HUVEC transfected with control siRNA or TXNIP siRNA **(A)** were left untreated and immuno-blotted for CSK pSer364. **(B)** Quantification of the data using Image J (* $P<0.05$ vs. control siRNA; n=3).



Supplemental Figure X

Quantification of *in vivo* stress fiber formation and TXNIP expression experiments demonstrate differential regulation of TXNIP and stress fibers in s-flow and d-flow regions (* $P<0.05$ vs. s-flow, n=5).



Supplemental Figure XI

Quantification of *in vitro* stress fiber formation experiments demonstrate differential regulation of TXNIP and stress fibers in response to no flow, s-flow and d-flow conditions (* $P<0.05$ vs. control siRNA, n=5).