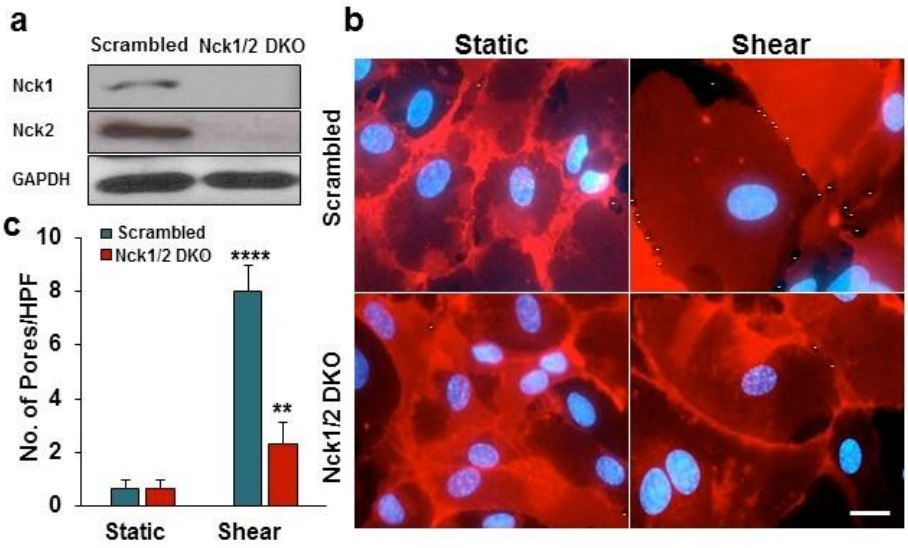
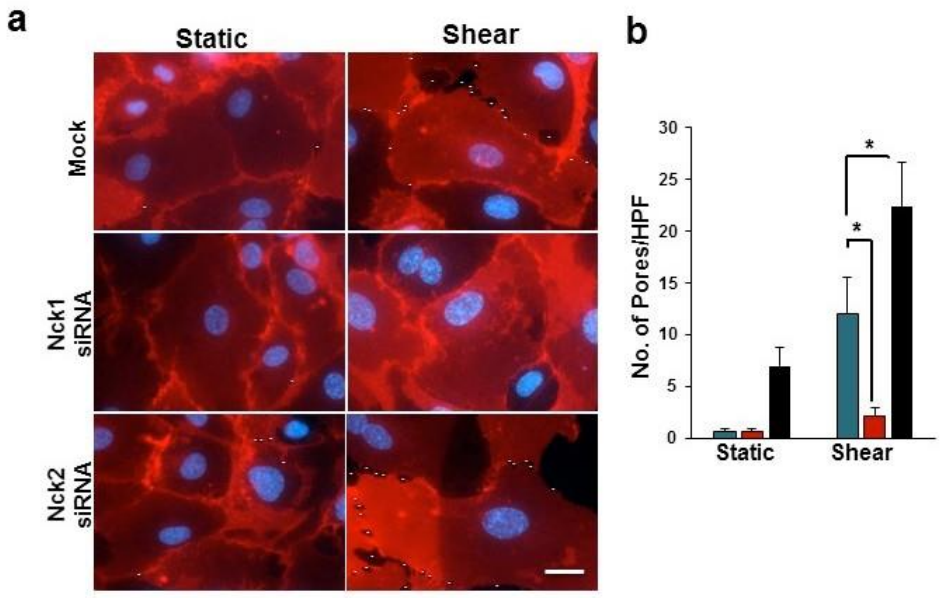
SUPPLEMENTAL MATERIAL

Figure S1. Deletion of Nck1 and Nck2 adaptor proteins blunt shear stress-induced endothelial permeability.



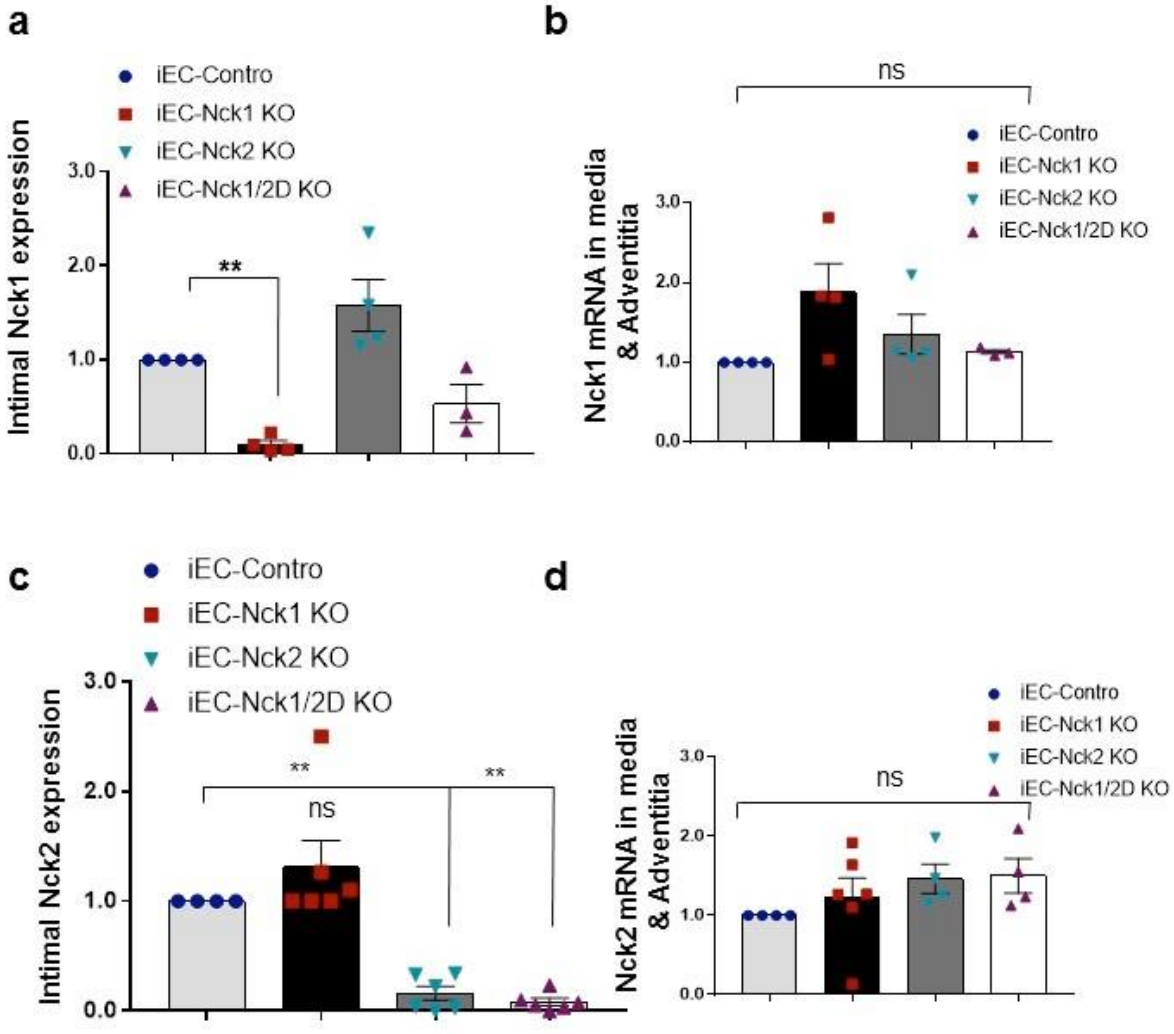
a) A representative blot showing deletion of Nck1 and Nck2 in Nck1/2 DKO cells. **b-c)** Endothelial monolayer integrity was assessed in Nck1/2 DKO or Scramble cells after shear stress (30 minutes) and stained with PECAM-1 (Red) for paracellular pore formation (stars). All data are from n=4, mean ± SEM, analyzed by 2-way ANOVA, and Bonferroni's post-test, **p<0.01. ****p<0.0001. Scale bars=100µm.

Figure S2. Ablation of Nck1 but not Nck2 adaptor protein blunts shear stress-induced endothelial permeability.



(**a &b)** PECAM-1 stained cells showing paracellualr formation (arrows) in Nck1 and Nck2 siRNA treated cells. Images analyzed using Nis Elements software from n=4. Scale bars=200µm. Data are analyzed by 2-Way ANOVA and Bonferroni's post-test,
*p<0.05.

Figure S3. Conditional Nck1 and Nck2 KO is endothelial specific after tamoxifen injection.



Intimal and medial / adventitial Carotid mRNA analysis from iEC-Control, iEC-Nck1 KO, iEC-Nck2 KO, iEC-Nck1/2 DKO mice after tamoxifen injection and two weeks recovery. a-b) Nck1 mRNA and (c-
d) Nck2 mRNA levels are normalized to the house keeping gene RPL13a and are from n=3-6/ group. Data are mean ± SEM, analyzed by 1-Way ANOVA and Tukey's post-test, **p<0.01, ns, indicates non-significance.

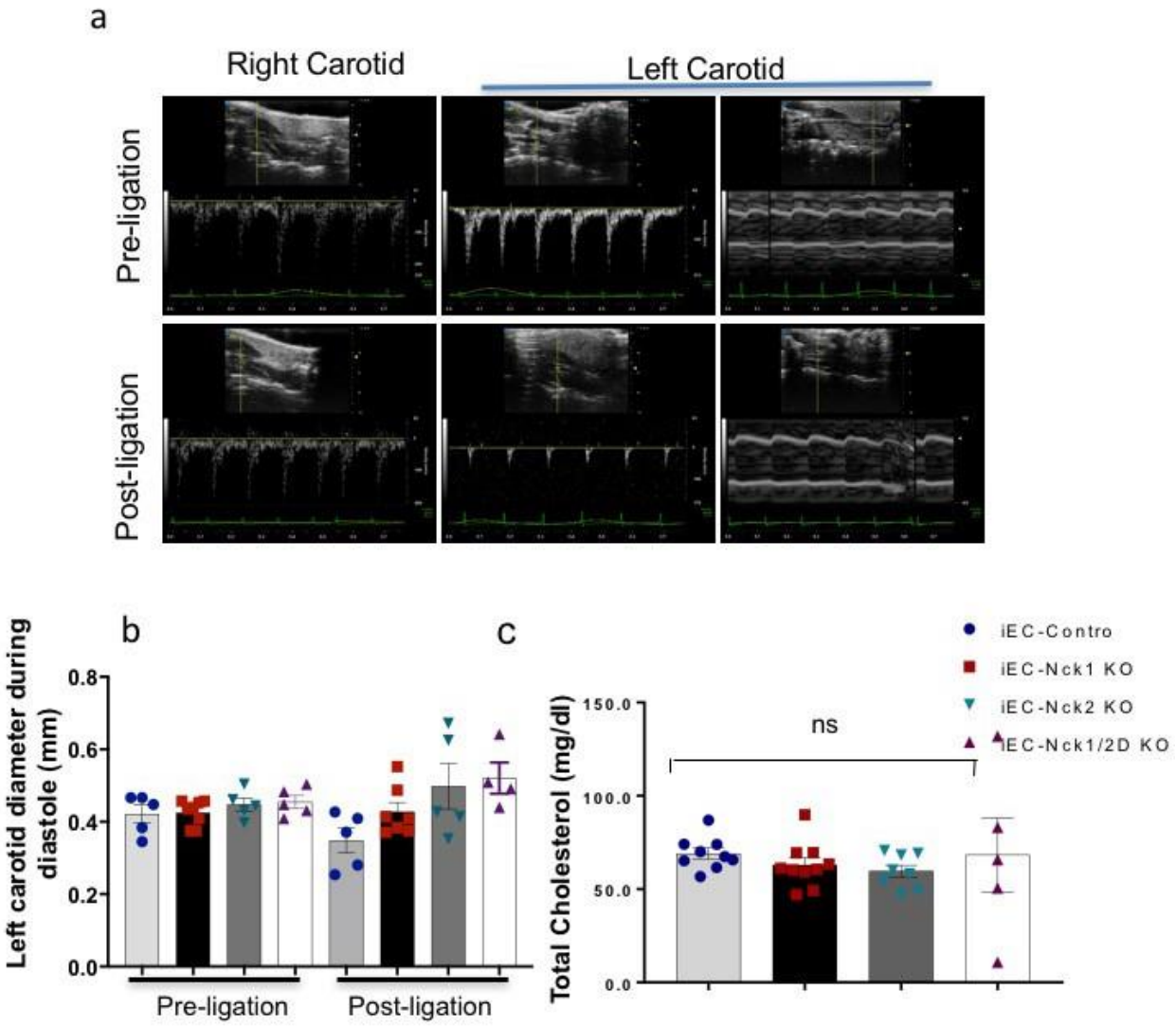


Figure S4. a) A representative Doppler carotid echocardiographic images from mice subjected to partial carotid ligation surgery showed a low and oscillatory flow. b) wall dimension changes of left carotid artery before and after ligation among experimental groups. c) Plasma total cholesterol levels showing non-significant (ns) changes among experimental groups. Data are mean ± SEM, analyzed by 1-Way ANOVA.

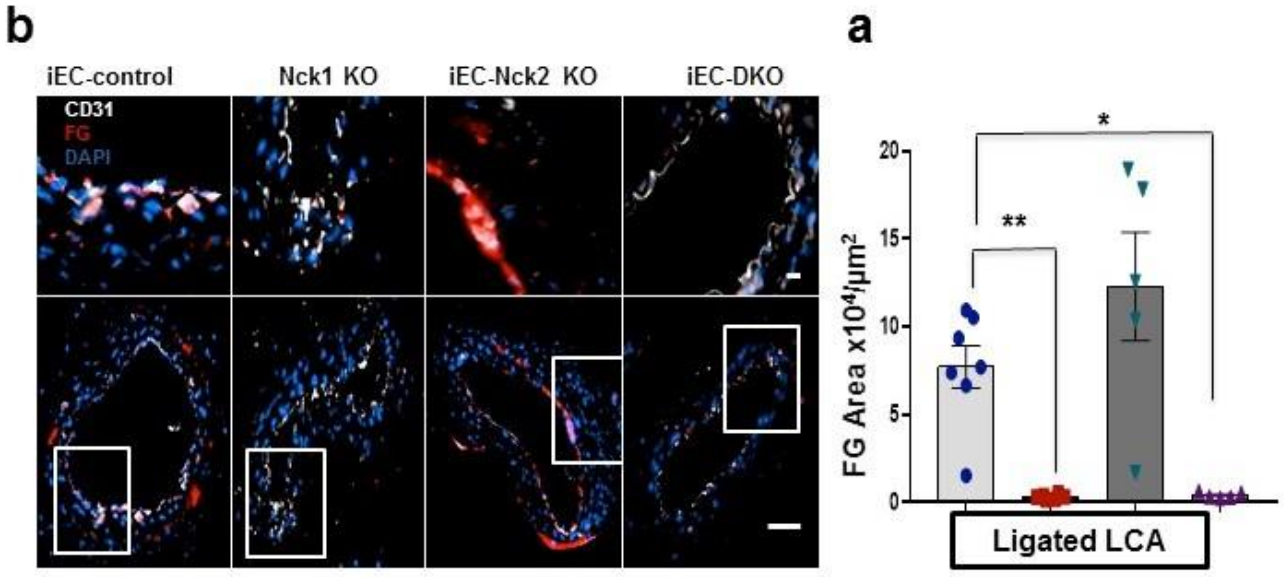


Figure S5. a-b) Nck1 KO mice showed significantly less Fibrinogen (FG, red) staining in ligated carotid arteries. Images analyzed using NIS-Elements software, from n=5-7 mice per group. Data are mean ± SEM, analyzed by 1-Way ANOVA and Tukey's post-test, *p<0.05, **p<0.01. Sale bars= 100-200µm.

Figure S6. A representative blot from endothelial cell lysates from either Nck1siRNA treated or untreated cells after oscillatory shear stress (± 5 dynes/cm^2 with 1 dyne/cm^2 forward) showing phospho PAK s141, total PAK and Nck1 levels. β-tubulin was used a loading control, from n=3.

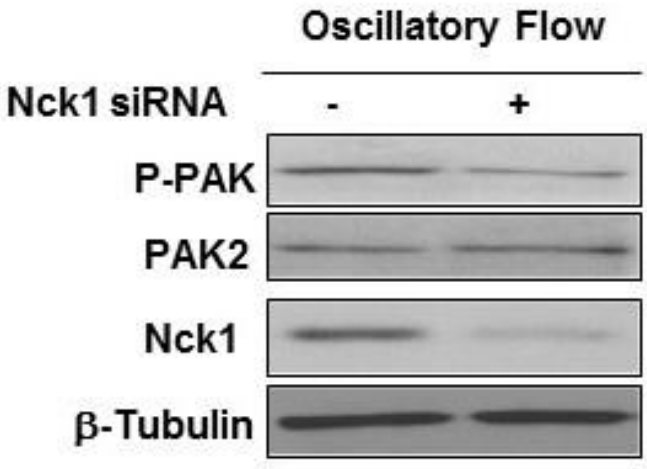


Figure S7. A representative blot from Nck1 co-immunoprecipitations after being treated with or without AX-024 (50nM) and the cells were subjected to shear stress for 30minutes, showing phospho PAK s141, and Nck1 levels, from n=3.