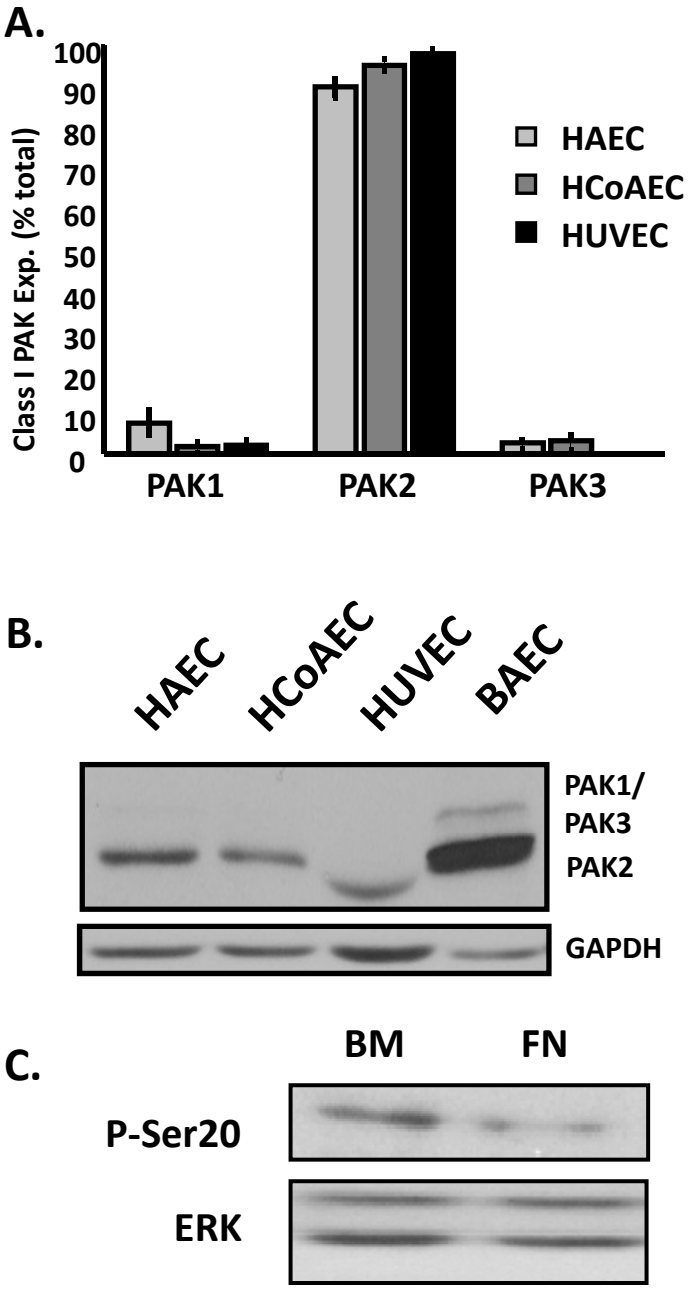
Supplemental Table I. qRT-PCR primers.

Gene	Forward Primer	Reverse Primer
PAK1	5' - GCTGTTCTGGATGTGTTGGA - 3'	5' - TTCTGAAACTGGTGGCACTG - 3'
PAK2	5' - ACAGAAGCACCCGCAGTAGT - 3'	5' - AAAGACTTGGCAGCACCATC - 3'
PAK3	5' -AGTCAGAAGTTCAGTTCGCC - 3'	5' - CCACATGAATCGTATGCTCA - 3'
ICAM-1	5' - TGTCCCCCTCAAAAGTCATC - 3'	5' - TAGGCAACGGGGTCTCTATG - 3'
GAPDH	5' -GAAGGTGAAGGTCGGAGTC - 3'	5' - GAAGATGGTGATGGGATTTC - 3'
B2M	5' - AGCATTCGGGCCGAGATGTCT - 3'	5' - CTGCTGGATGACGTGAGTAAACCT - 3'

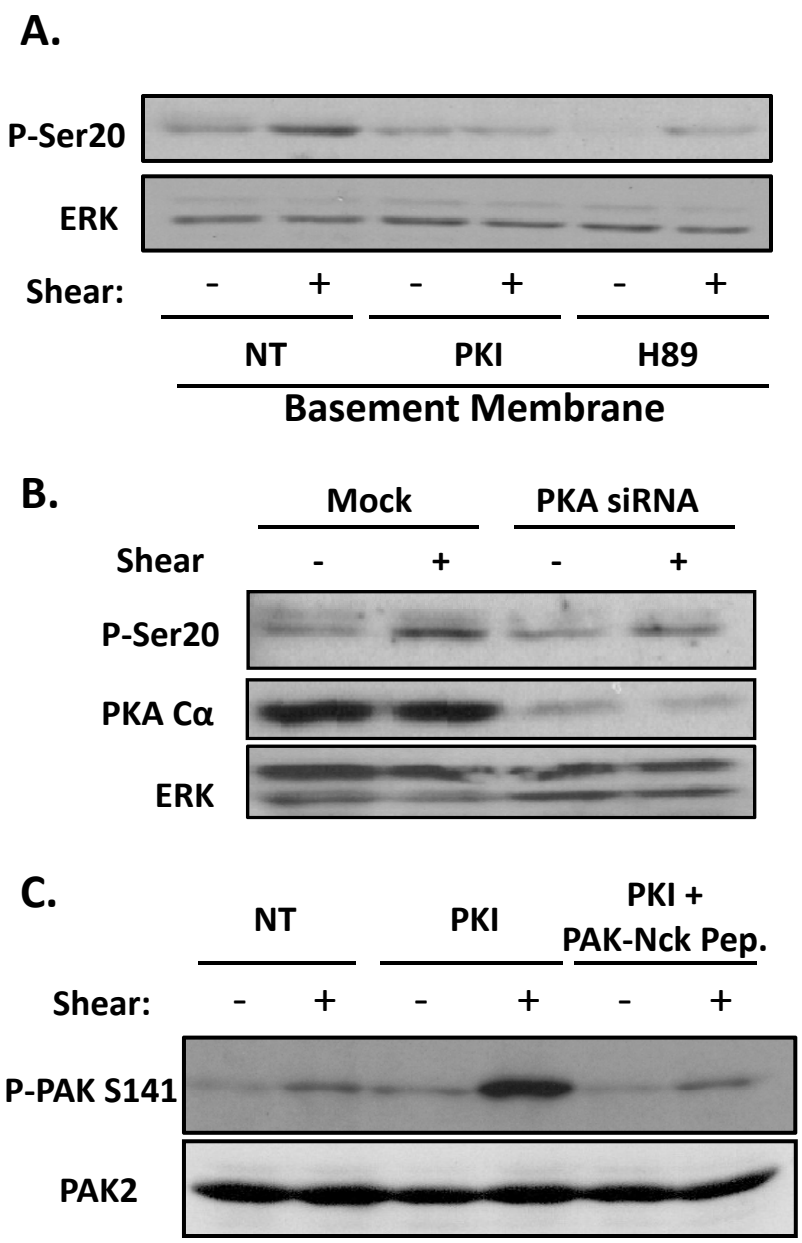
PAK1, p21 activated kinase-1; PAK2, p21-activated kinase 2; PAK3, p21-activated kinase 3; ICAM-1, intercellular adhesion molecule-1; GAPDH, glyceraldehydes 3-phosphate dehydrogenase ; B2M, β2-microglobulin.

Supplemental Figure 1.



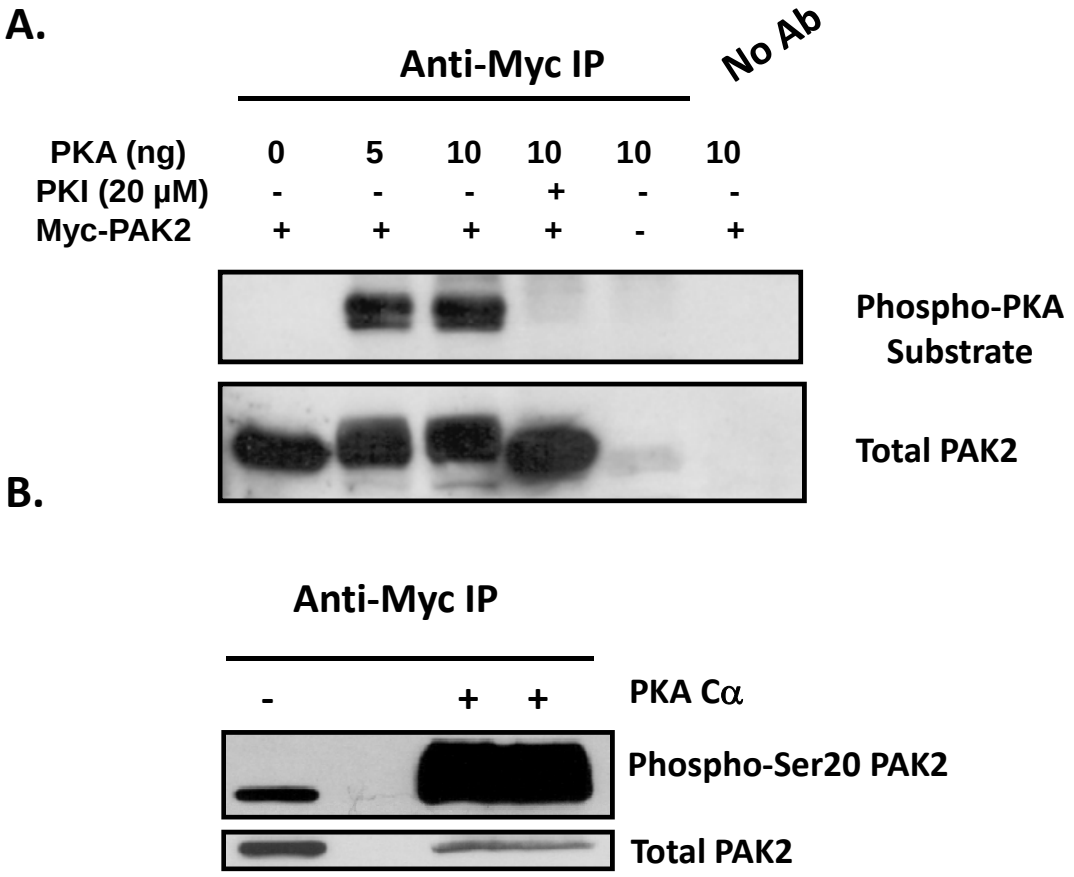
(A) mRNA isolated from endothelial cells derived from human aorta (HAEC), human coronary artery (HCoAEC), and human umbilical vein (HUVEC) were analyzed for class I PAK expression by qRT-PCR. Results were normalized to GAPDH and expressed as a percent of the total group I PAK expression. n = 3-4. (B) Class I PAK expression was determined by Western blotting cell lysates from HAEC, HCoAEC, HUVEC, and bovine aortic endothelial cells (BAEC) with an antibody that recognizes all three group I PAK isoforms. n = 3. (C) Endothelial cells plated on BM or FN were exposed to oscillatory shear stress for 18 hours and PAK2 Ser20 phosphorylation was determined by Western blotting. Representative images are shown. n = 4.

Supplemental Figure 2.



(A) BAECs were treated with the PKA inhibitors PKI (20 μM) or H89 (5 μM) for 30 minutes prior to application of fluid shear stress (15 min.), and Ser20 phosphorylation was determined by immunoblotting. Representative images are shown (B) Endothelial cells transfected with siRNA against PKA Cα were sheared for 15 minutes, and Ser20 phosphorylation was determined. Representative blots are shown. (C) Endothelial cells were treated with PKI (20 μM) in the absence or presence of the PAK-Nck blocking peptide (20 μg/ml), and shear stress-induced PAK2 activation was determined by Western blotting as previously described. Representative images are shown.

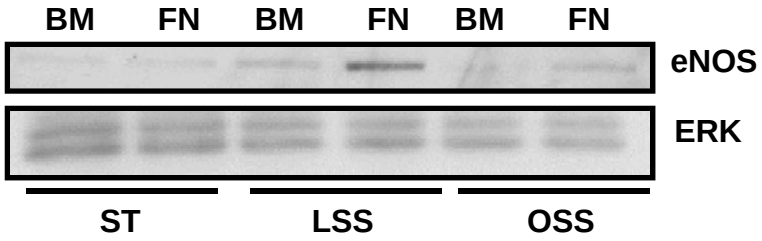
Supplemental Figure 3.



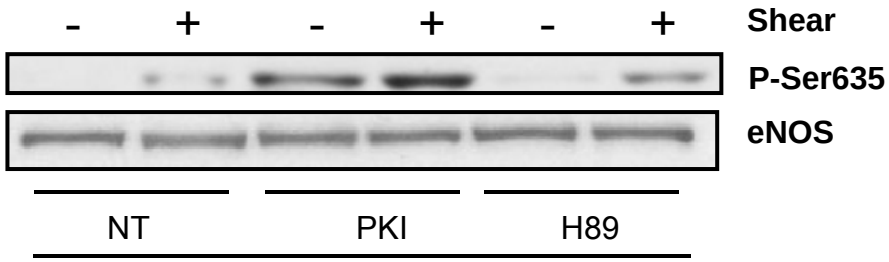
(A) Myc-PAK2 was immunoprecipitated from BAECs and subjected to PKA in vitro kinase assay with recombinant active PKA Cα. PKA phosphorylation was assessed using an antibody that recognizes phospho-PKA substrate epitopes. Addition of the PKA inhibitor PKI was utilized to verify the phosphorylation was attributable to PKA. (B) Multi-epitope PAK2 was treated as in (A) and phosphorylation on Ser20 was determined using phospho-Ser20 antibodies.

Supplemental Figure 4

A.

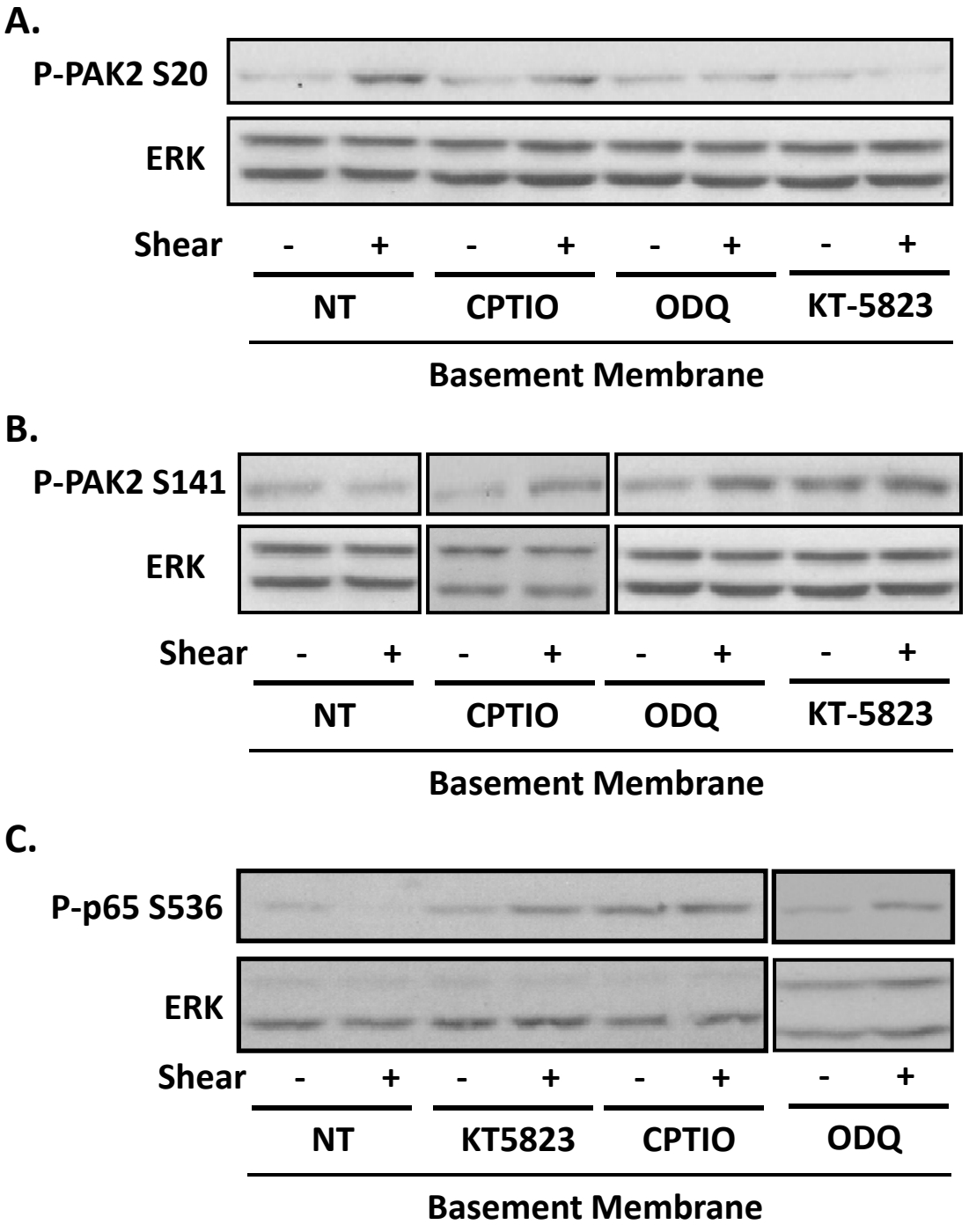


B.



(A) BAECs plated on basement membrane proteins (BM) or fibronectin (FN) were stimulated for 18 hrs with either laminar flow (LSS) or oscillatory flow (OSS). eNOS expression was determined by Western blotting. Representative images are shown. n = 3. (B) BAECs were treated with the PKA inhibitors PKI (20 μM, 15 min), or H89 (5 μM, 15 min) and exposed to shear stress for 30 minutes. Phosphorylation of eNOS on Ser635 was determined by Western blotting and normalized to eNOS. Representative images are shown. n = 3.

Supplemental Figure 5



BAECs were left untreated (NT) or treated CPTIO (200 μM, 30 min.), ODQ (10 μM, 30 min.) or KT5823 (2 μM, 1 hr.). Cells were sheared for 15 min and (A) Ser20 and (B) Ser141 phosphorylation was determined. Representative images are shown. (C) Endothelial cells treated as in (A) were sheared for 30 minutes and p65 Ser536 phosphorylation was analyzed by Western blotting. Representative images are shown.