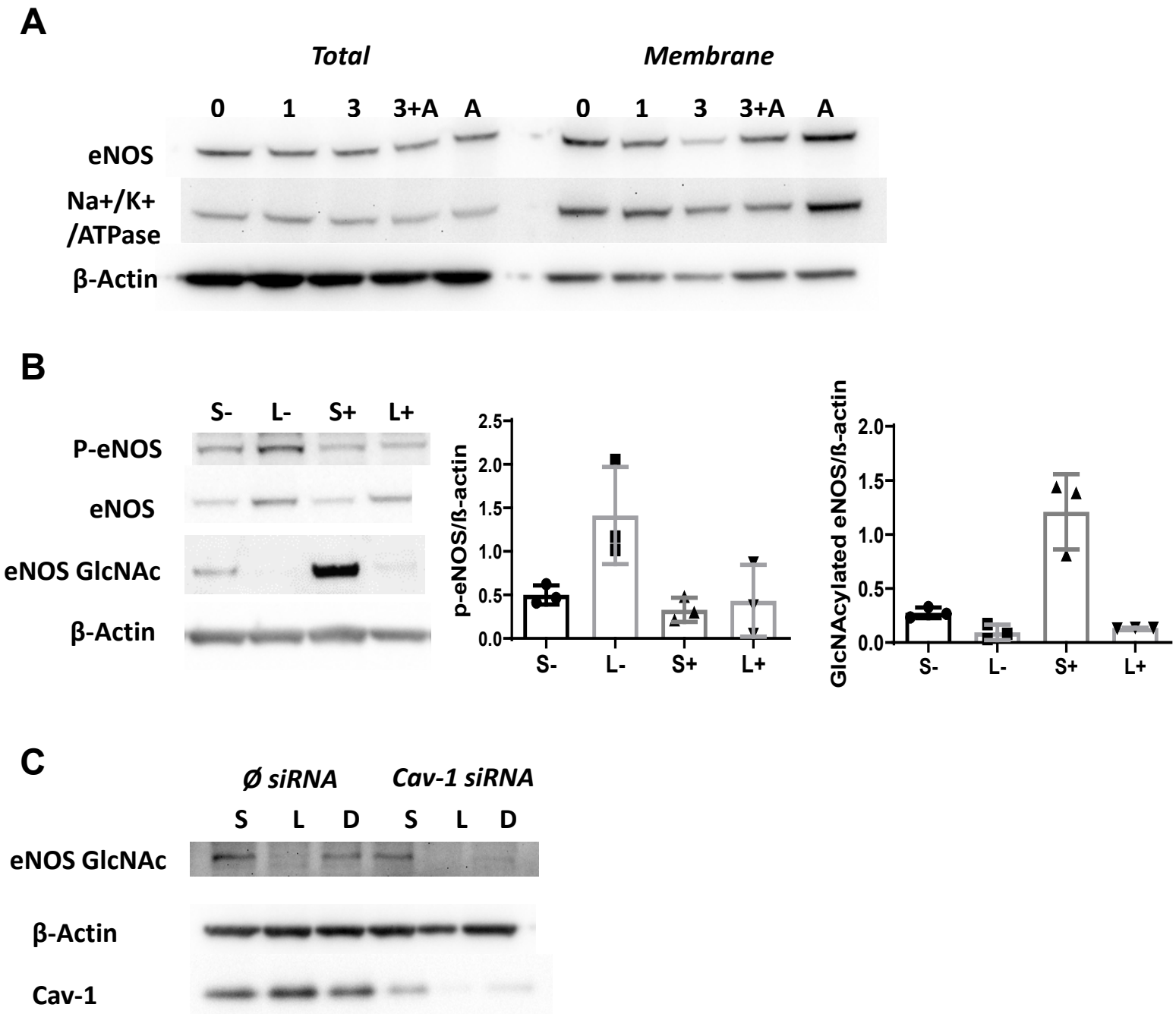
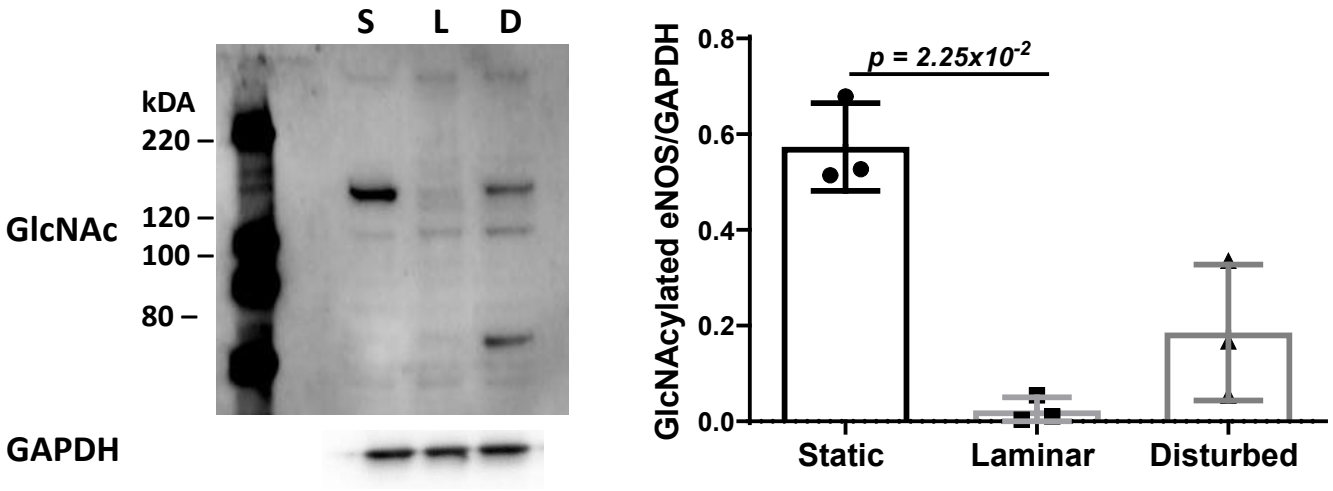
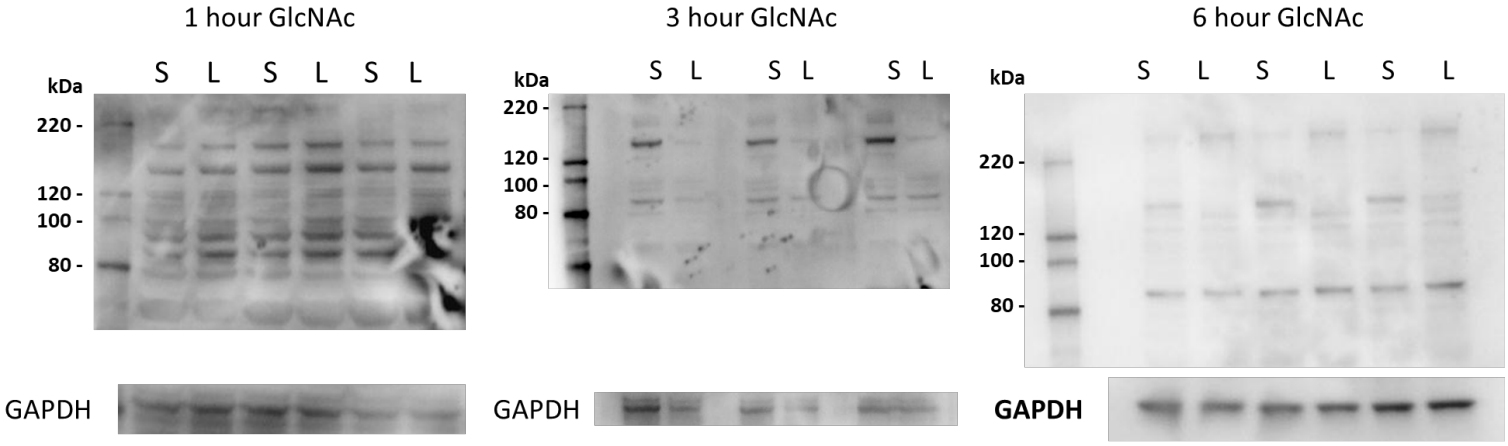
Supplemental Material

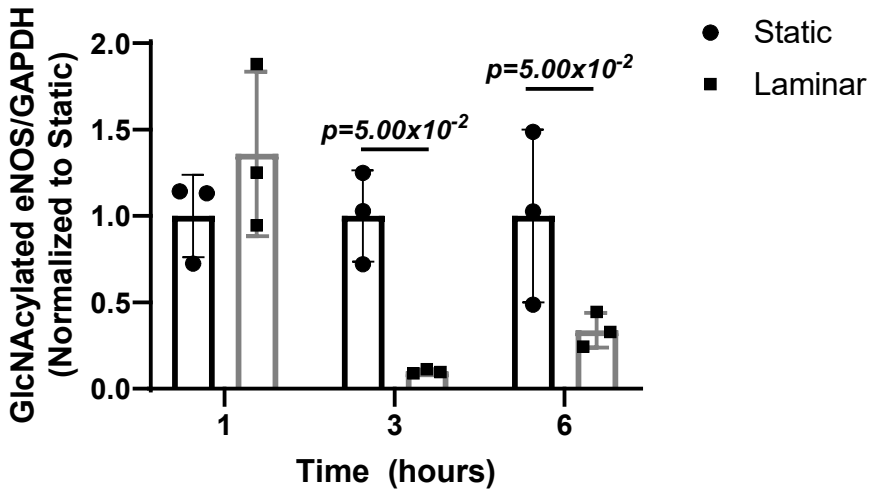


Online Figure I: Caveolae disruption via methyl-β-cyclodextrin (MβCD) decreased p-eNOS in steady laminar flow but did not decrease eNOS O-GlcNAcylation. Knocking down Caveolin-1 (Cav-1) via siRNA did not significantly change eNOS O-GlcNAcylation in steady laminar or oscillating disturbed flow. (A) Western blot and quantification of eNOS subcellular fractionation in HUVEC incubated in 0 mM MβCD, 1 mM MβCD, 3 mM MβCD, 3 mM MβCD + 100 μM ATP, or 100 μM ATP for 24 hours in static culture. (B) Western blot and quantification of p-eNOS and eNOS O-GlcNAcylation in HUVEC exposed to 24 hours static ('S') or steady laminar flow ('L') with and without 3 mM MβCD (+/- MβCD, caveolae disrupter). n = 3 samples. $p = 5.02 \times 10^{-2}$ (p-eNOS) and $p = 1.17 \times 10^{-3}$ (GlcNAc eNOS) by Kruskal-Wallis test. (C) Western blot and quantification of eNOS O-GlcNAcylation in HUVEC exposed to 24 hours static ('S'), steady laminar flow ('L'), and oscillating disturbed flow ('D') with and without Cav-1 siRNA knockdown (+/- Cav-1 siRNA).

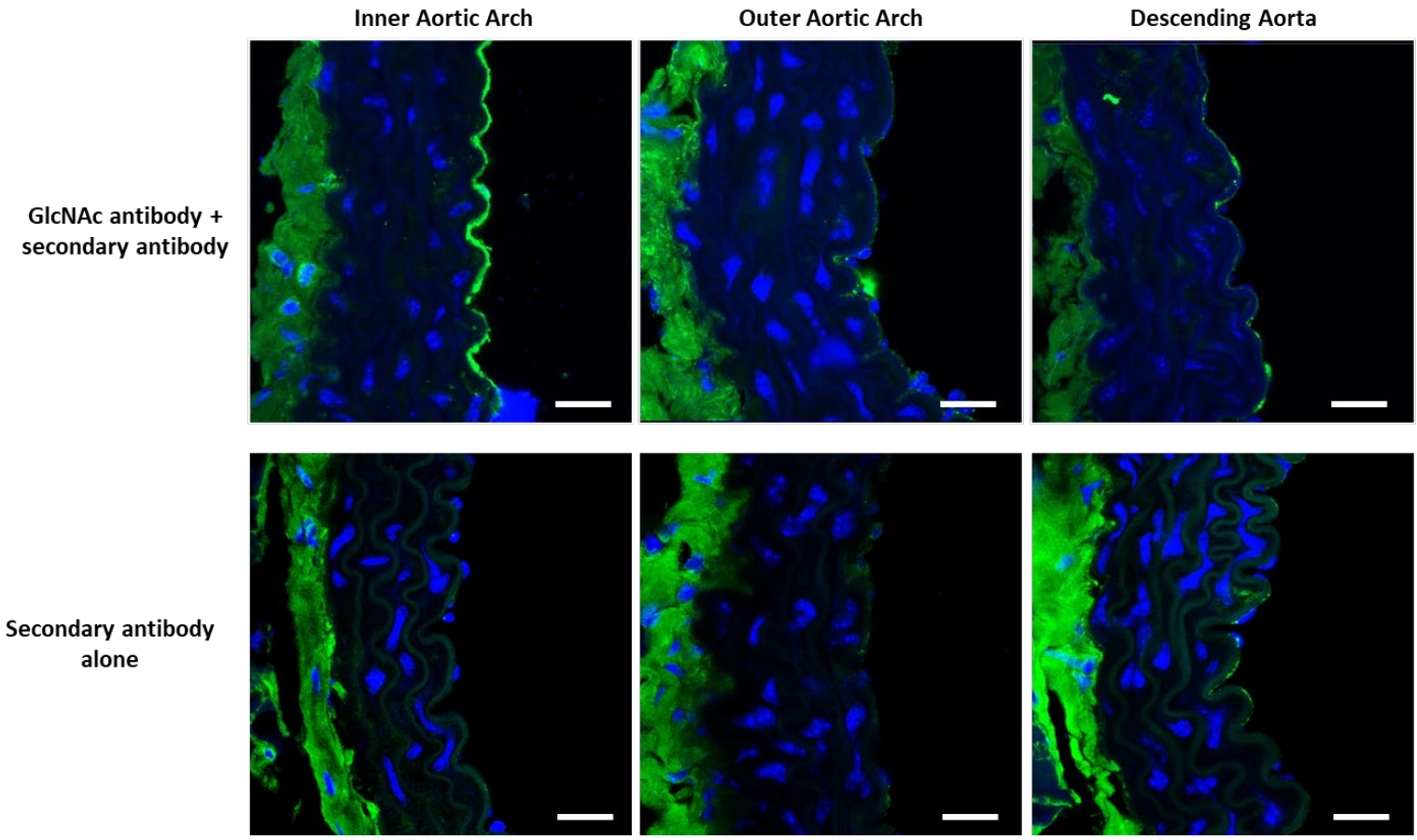


Online Figure II: Steady laminar flow decreased eNOS O-GlcNAcylation in pulmonary artery endothelial cells. Western blot for all O-GlcNAcylated proteins with quantification of GlcNAcylation of the ~140 kDa protein in human pulmonary artery endothelial cells (hPAEC) adapted to static culture ('S'), steady laminar ('L', 20 dynes/cm2 for 24 hours), oscillating disturbed flow ('D', 4±6 dynes/cm2) for 24 hours. n = 3 samples per experiment. 1 representative experiment shown out of 3 total experiments. $p = 1.07x10^{-2}$ by Kruskal-Wallis test, with p value on the figure determined by Dunn's multiple comparisons test (all samples compared to steady laminar flow).





Online Figure III: eNOS O-GlcNacylation decreased over time for HUVEC exposed to steady laminar flow. By 3 hours, the decrease in O-GlcNAcylated eNOS was statistically significant by Western blot. n = 3 samples per experiment. 1 representative experiment shown of 3 total experiments. Statistical significance determined by Mann-Whitney test.



Online Figure IV: Reaction control for aortic labeling of O-GlcNAc shows minimal endothelial labeling with secondary antibody alone. Confocal microscopy images of aortic cross sections from 2-month-old male C57BL/6J mice labeled with either the O-GlcNAc antibody and the secondary antibody (top, green) or the secondary antibody alone (bottom). Blue = nuclei. Scale bar = 20 μm.