

*Supplemental Figure 1.* Representative traces of a single iNOS<sup>-/-</sup> popliteal lymphatic vessel contractile activity during the pressure step protocol (**A & B**) and the acetylcholine dose-response protocol (**C & D**). **A & B:** This vessel was exposed to the same pressure steps (top trace, labeled) as before in the absence and presence of L-NAME, for the same length of time. **C & D:** The acetylcholine dose-response protocol was then performed on the same iNOS<sup>-/-</sup> vessel, while pressure was held at 3 cmH<sub>2</sub>O. Vertical lines on the diameter tracing, below, mark where each successive dose of acetylcholine was added to the bath.

*Supplemental Figure 2.* Summary of lymphatic contractile parameters in iNOS<sup>-/-</sup> vessels exposed to pressure steps in the absence and presence of L-NAME. **A-F:** End diastolic diameter (EDD), tone, contraction amplitude (AMP), contraction frequency (FREQ), ejection fraction (EF), and fractional pump flow (FPF) are all plotted as a function of pressure. Means ±SEM are reported. Data in each graph are fit to a curve or line, as appropriate, except for AMP, which is splined. \*, indicates significant differences between closed vs. open data points (p<0.05); ‡, closed and open data points both differ from their respective first data point at 0.5 cmH<sub>2</sub>O; †, only closed data points differ significantly from the first data point at 0.5 cmH<sub>2</sub>O; ^, only open data points differ significantly from the first data point at 0.5 cmH<sub>2</sub>O.

*Supplemental Figure 3.* Summary of lymphatic contractile parameters of iNOS<sup>-/-</sup> vessels exposed to increasing acetylcholine doses before and during L-NAME treatment. **A-F:** The same contraction parameters as appear in Figure 5 are graphed as a function of acetylcholine concentration. Closed data points indicate data from untreated iNOS<sup>-/-</sup>

vessels, while open points indicate data from L-NAME-treated iNOS<sup>-/-</sup> vessels. Means  $\pm$ SEM are reported. Data in each graph are fit to a sigmoidal dose-response curve (3 parameter fit). \*, indicates significant differences between closed vs. open data points ( $p < 0.05$ ); †, only closed data points differ significantly from the control data point at 0 M acetylcholine.

*Supplemental Figure 4.* Summary of lymphatic contractile parameters in two-valve WT vessels exposed to pressure steps in the absence and presence of L-NAME. **A-F:** End diastolic diameter (EDD), tone, contraction amplitude (AMP), contraction frequency (FREQ), ejection fraction (EF), and fractional pump flow (FPF) are all plotted as a function of pressure. Means  $\pm$ SEM are reported. Data in each graph are fit to a curve or line, as appropriate, except for tone, which is splined. \*, indicates significant differences between closed vs. open data points ( $p < 0.05$ ).

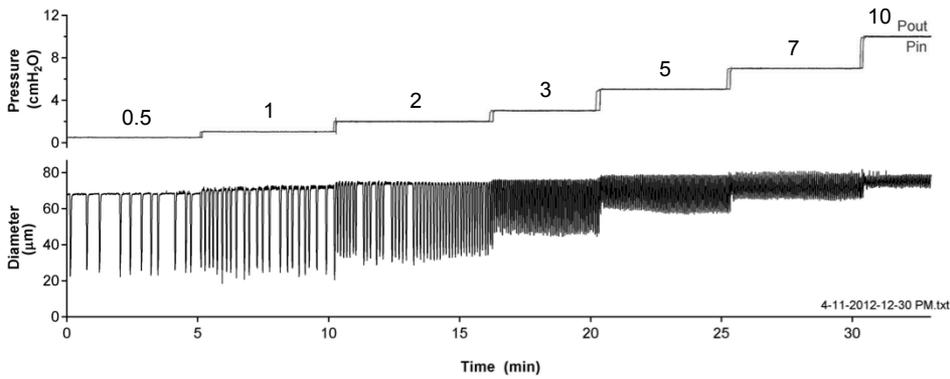
*Supplemental Figure 5.* Summary of lymphatic contractile parameters in two-valve eNOS<sup>-/-</sup> vessels exposed to pressure steps in the absence and presence of L-NAME. **A-F:** End diastolic diameter (EDD), tone, contraction amplitude (AMP), contraction frequency (FREQ), ejection fraction (EF), and fractional pump flow (FPF) are all plotted as a function of pressure. Means  $\pm$ SEM are reported. Data in each graph are fit to a curve or line, as appropriate, except for tone and FPF, which are splined. \*, indicates significant differences between closed vs. open data points ( $p < 0.05$ ).

*Supplemental Figure 6.* Direct comparison of lymphatic contractile parameters between two-valve WT and eNOS<sup>-/-</sup> vessels exposed to pressure steps. **A-F:** End diastolic diameter

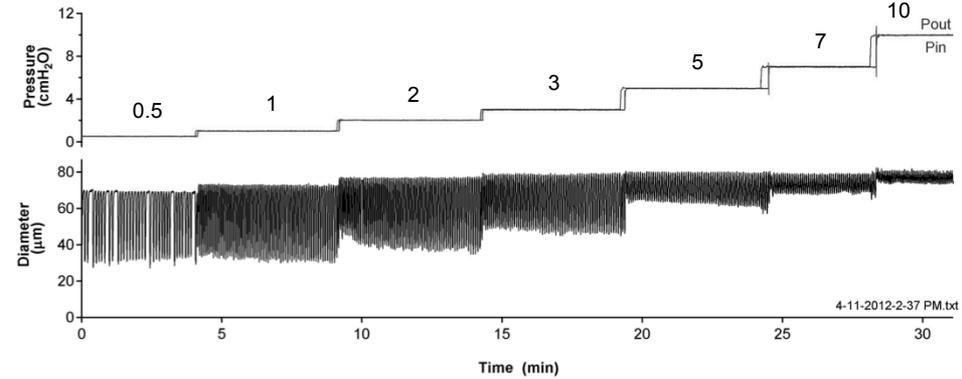
(EDD), tone, contraction amplitude (AMP), contraction frequency (FREQ), ejection fraction (EF), and fractional pump flow (FPF) are all plotted as a function of pressure. Means  $\pm$ SEM are reported. Data in each graph are fit to a curve or line, as appropriate, except for tone and FPF, which are splined. \*, indicates significant differences between closed vs. open data points ( $p < 0.05$ ).

# Supplemental Figure 1. Raw Responses of iNOS<sup>-/-</sup> Vessels to Pressure and Acetylcholine

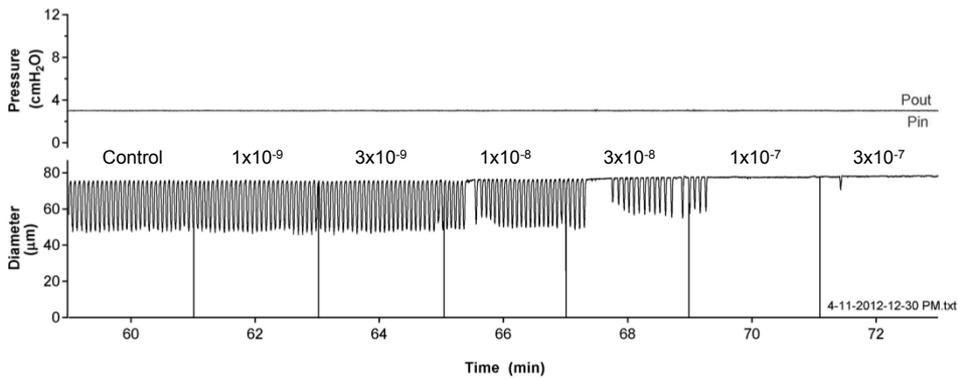
**A** iNOS<sup>-/-</sup>



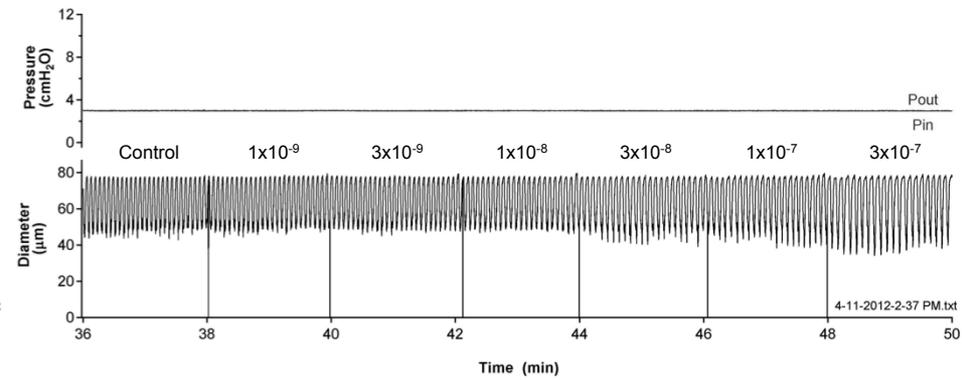
**B** iNOS<sup>-/-</sup> with L-NAME



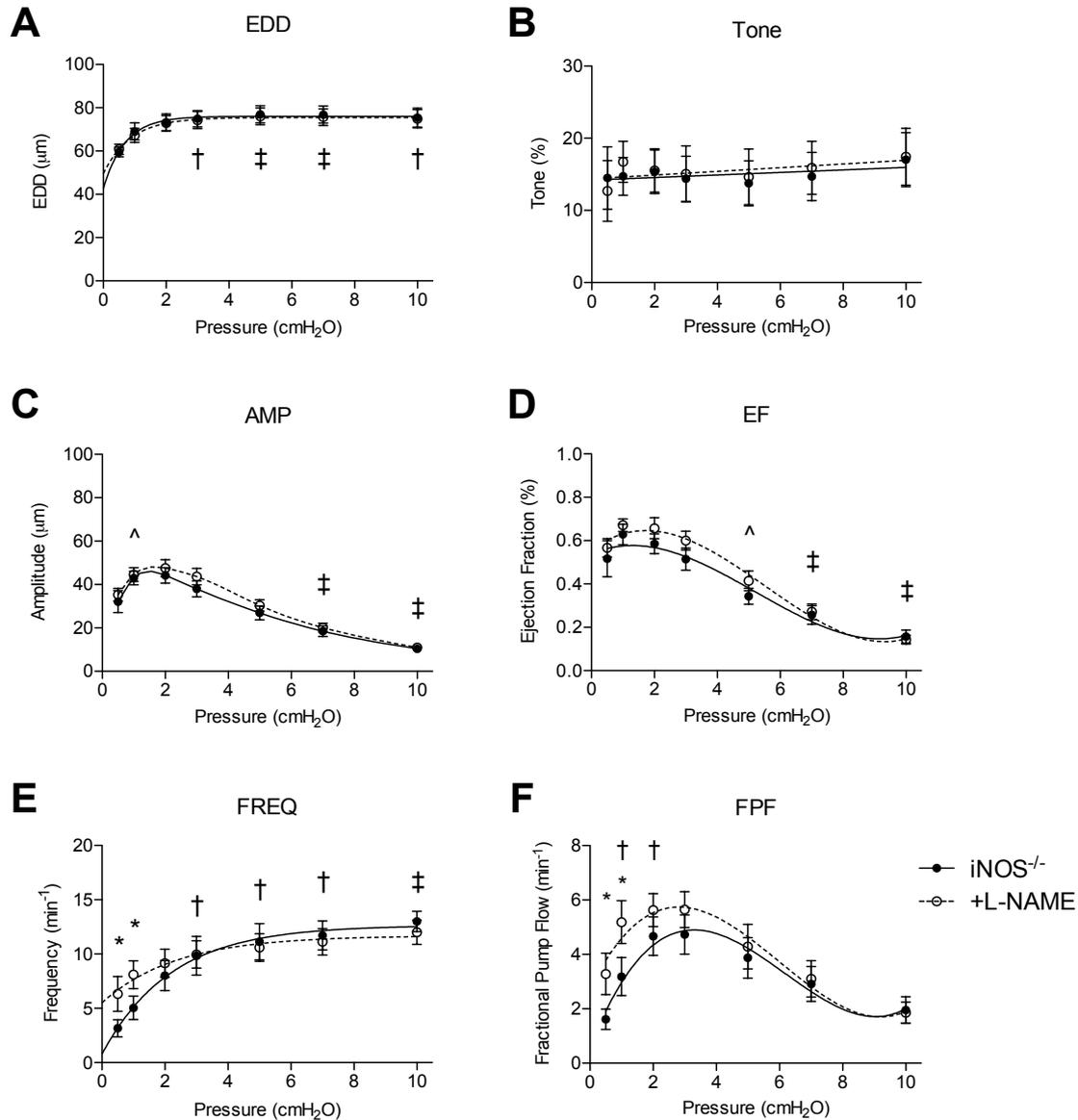
**C** iNOS<sup>-/-</sup>



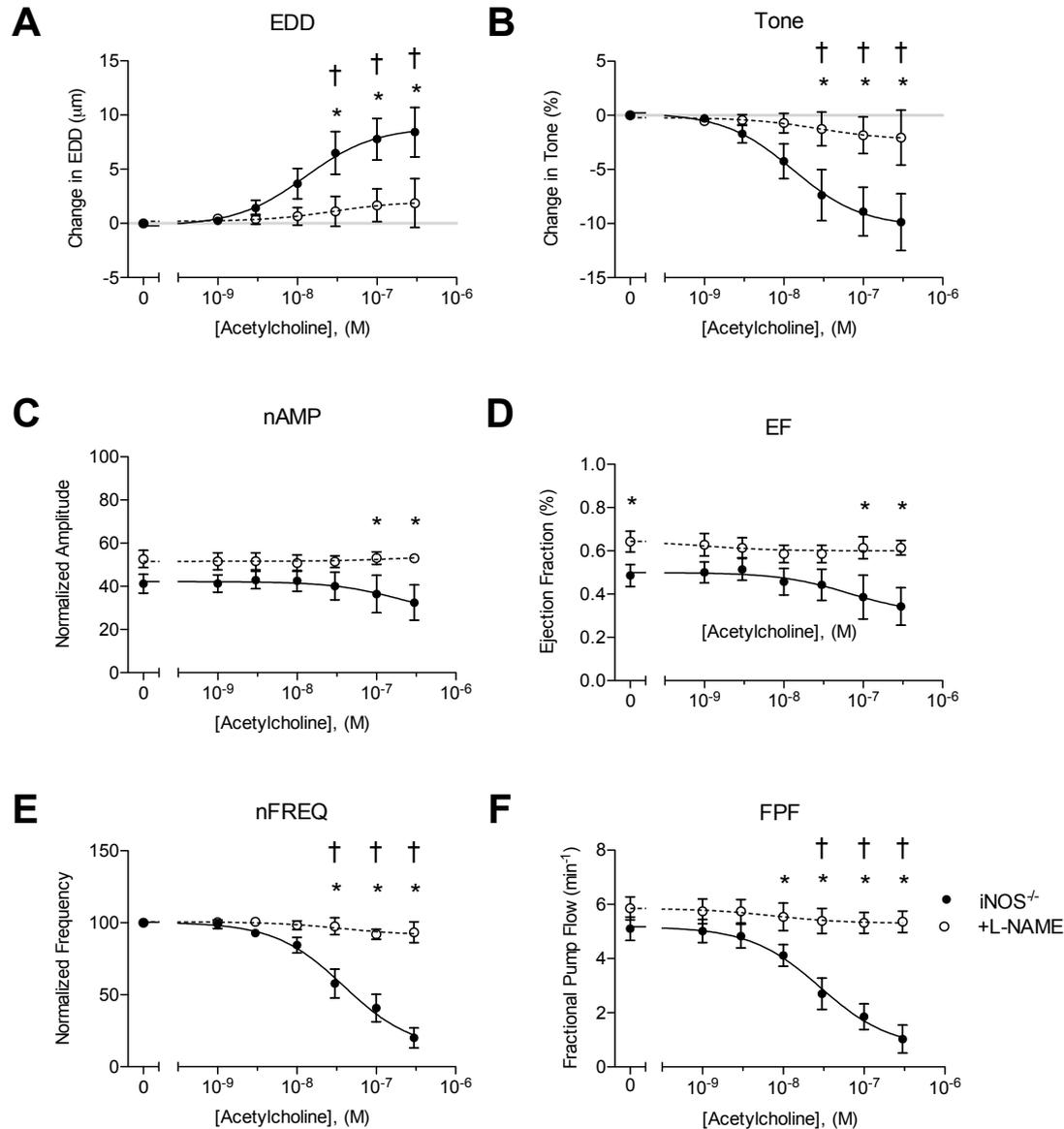
**D** iNOS<sup>-/-</sup> with L-NAME



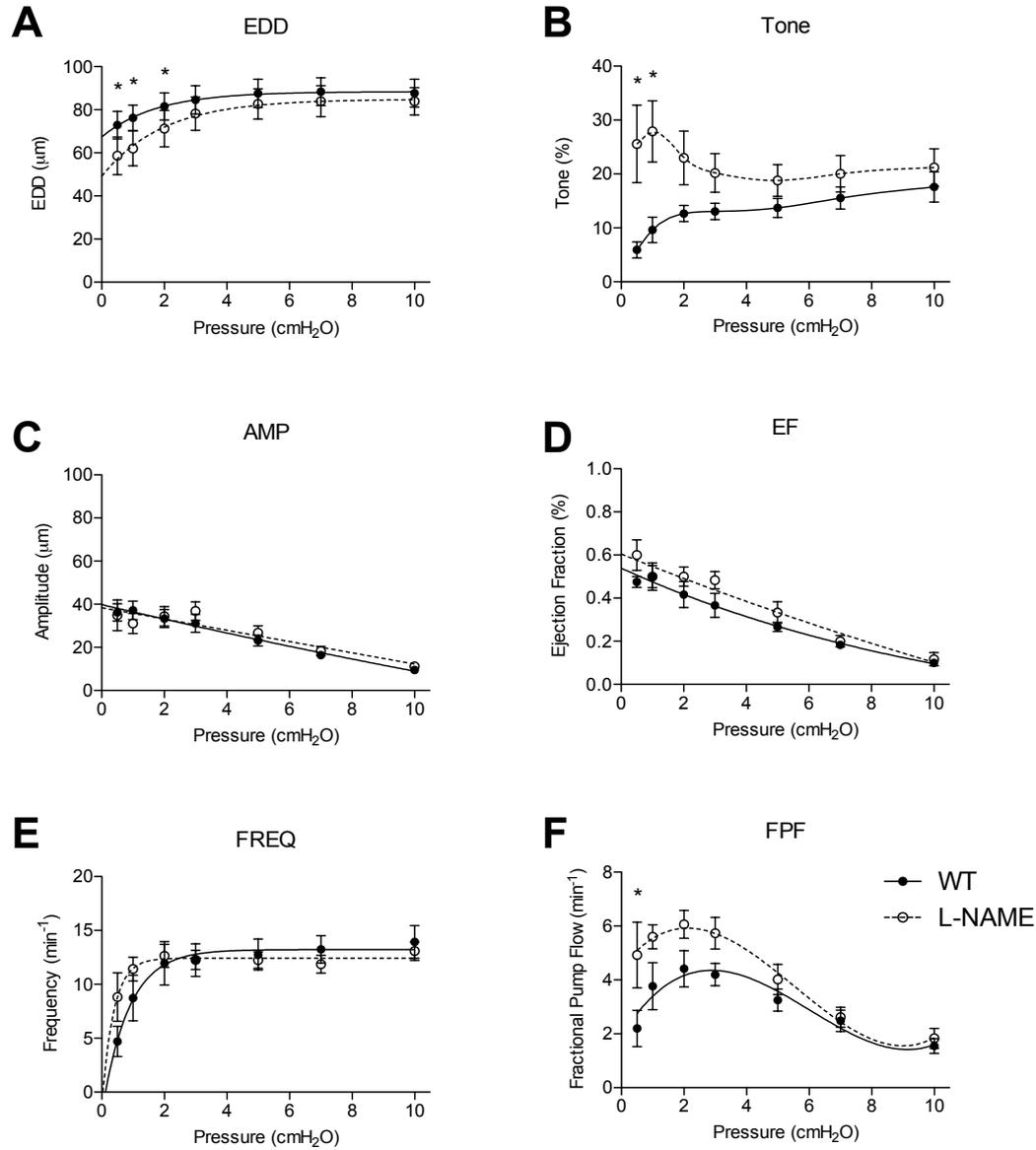
# Supplemental Figure 2. Effects of Basal NO After Genetic Removal of iNOS



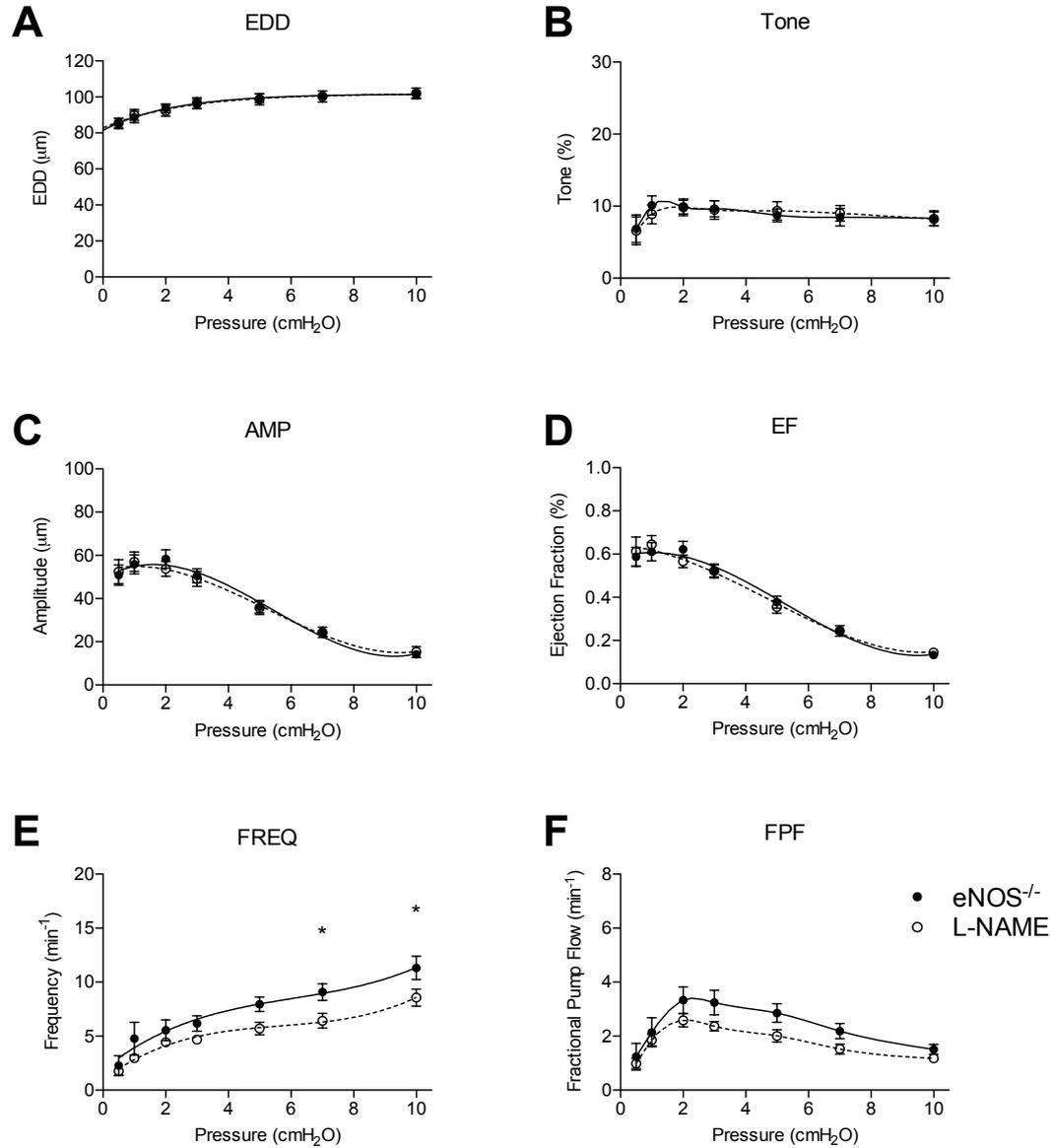
# Supplemental Figure 3. Acetylcholine-Stimulated NO Release in iNOS<sup>-/-</sup> Vessels



# Supplemental Figure 4. Two Valve WT Vessels Treated with L-NAME



# Supplemental Figure 5. Two Valve eNOS<sup>-/-</sup> Vessels Treated with L-NAME



# Supplemental Figure 6. Two Valve WT versus Two Valve eNOS<sup>-/-</sup> Vessels

