

**Supplementary Figure 4. Immunohistochemistry of Mouse Aortas for p-eNOS (S1177). Determination of antibody specificity for the phospho-protein.**

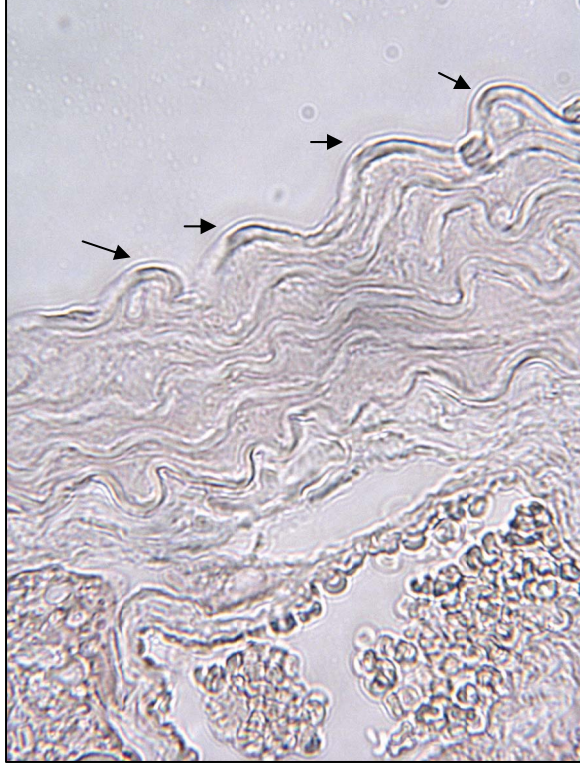
**A** pS1177 p-eNOS (Upstate) 1:100 (Exercised)



**PP2A Treated**

**B**

pS1177 p-eNOS (Upstate) 1:100 (Upstate) 1:100 (Exercised)



**No PP2A Treatment,  
only PP2A buffer.**

Both A & B are slices of aortas from exercised mice. The aorta in panel A was treated with 0.1U PP2A for 30 minutes at 37C in PP2A buffer (50mM imidazole pH 7.2, 0.2 mM EGTA, 0.02%  $\beta$ -mercaptoethanol, 0.1mg/ml BSA, and 1mM NiCl<sub>2</sub>), washed three times with PBS then incubated overnight with the Ab indicated above the panel. B) Was treated similarly to A, except that it was only incubated with PP2A's reaction buffer for the same 30 minutes. Post secondary antibody reactions, both aortas were exposed to the DAB reagent for 20 min (a long exposure) to bring out any staining that could occur. Arrows point at positively staining ECs. (63x magnification)