

Supplement Material

Novel Mechanism of Aortic Aneurysm Development in Mice
Associated with Smoking and Leukocytes (ATVB/2012/300208D)

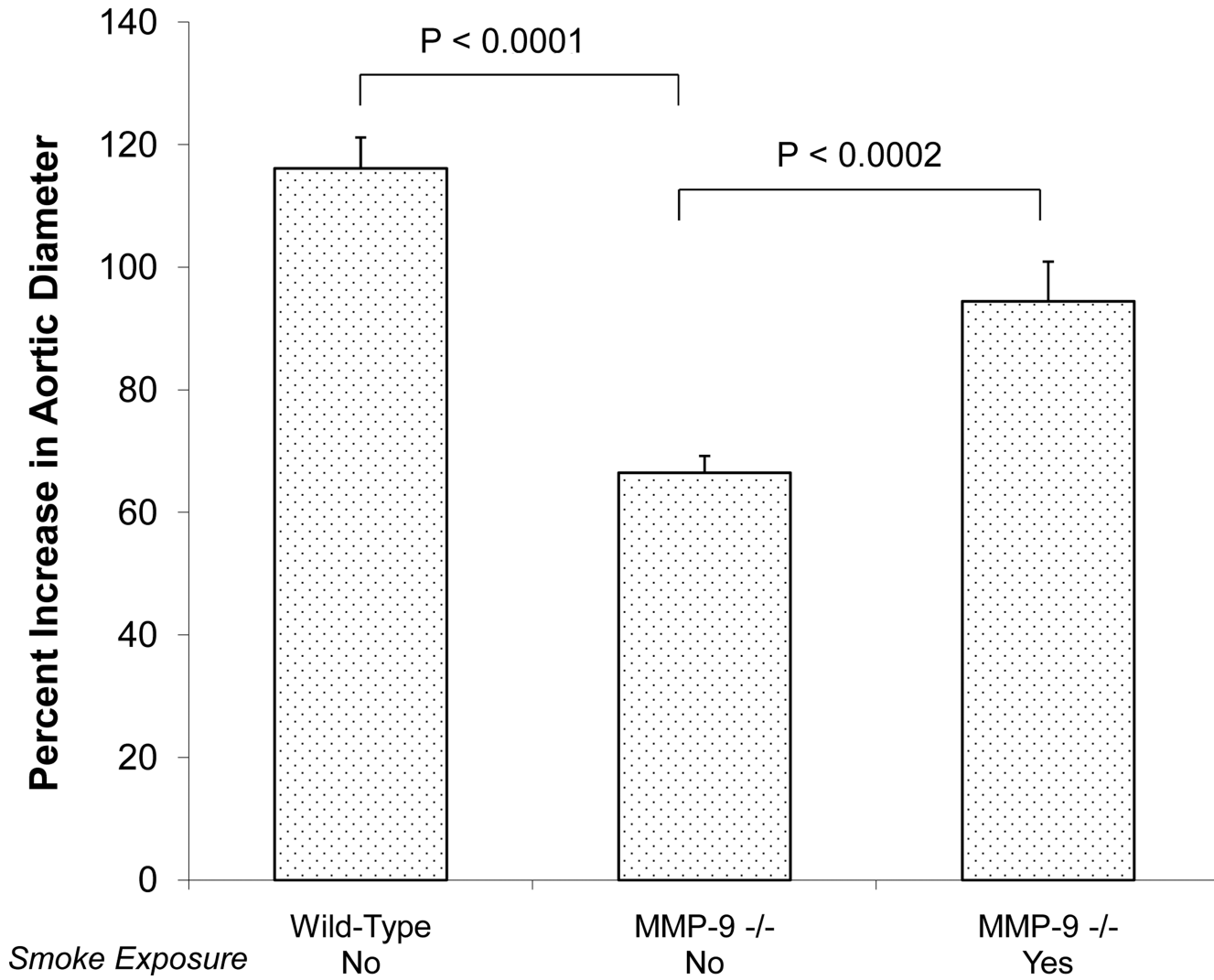
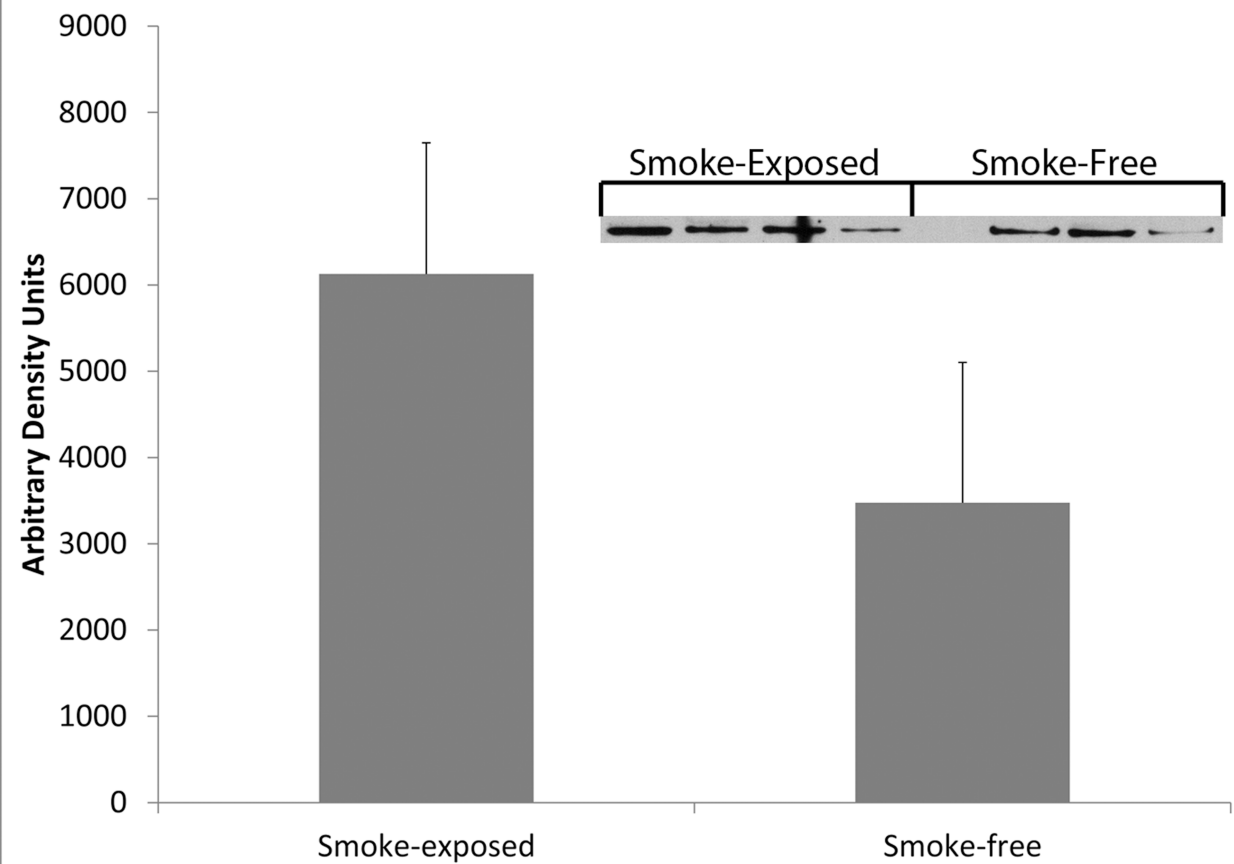


Figure I: Effect of tobacco smoke exposure and MMP-9 deficiency in 129/SvEv mice

Consistent with previous studies, the absence of MMP-9 in smoke-free 129/SvEv mice significantly reduced the overall increase in $\% \Delta AD$, from $116 \pm 5\%$ to $66 \pm 3\%$ ($n=18$ and $n=8$ respectively, $P < 0.0001$), as well as the incidence of AAAs (maximal $\% \Delta AD \geq 100\%$), from 78% to 0% ($P < 0.0002$ by Chi-Squared). However, when MMP-9 $-/-$ 129/SvEv mice ($n=6$) were exposed to TS there was a significant increase in the extent of aortic dilatation ($94 \pm 6\%$) compared to smoke-free MMP-9 $-/-$ mice ($P < 0.0002$), with a substantially higher incidence of AAAs (22%)

A



B

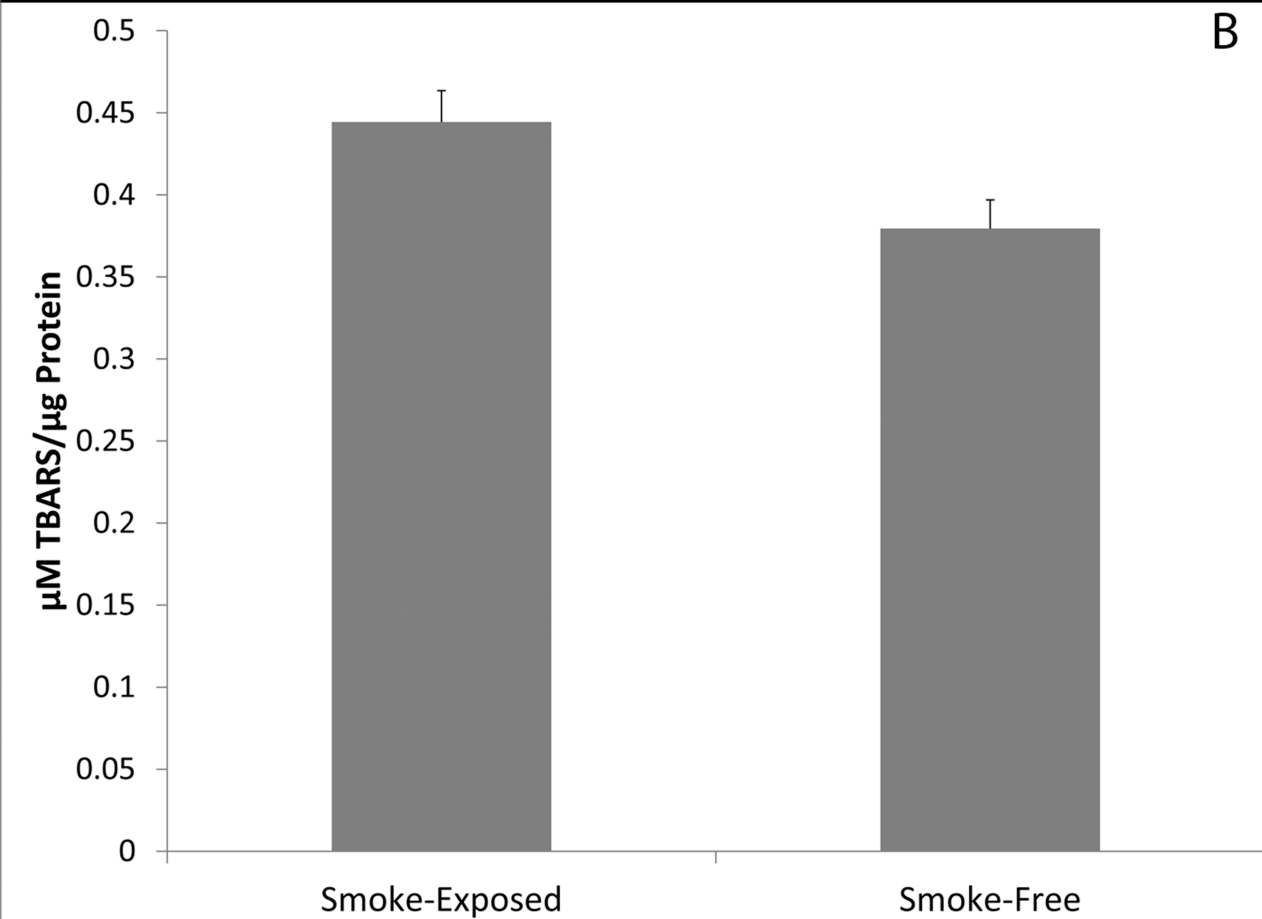


Figure II: No change in Oxidative Stress or Tissue Oxidation in the aortas of animals exposed to tobacco smoke.

Aortic tissue was removed from C57/Bl6 mice after 6 weeks of exposure to tobacco smoke or from littermate mice maintained for an identical period of time in smoke-free conditions. Protein was extracted from the tissues and analyzed for markers of oxidative stress and tissue oxidation. **(A)** Oxidative stress was evaluated with Heme oxygenase-1 production by Western blot (n=4 smoke-exposed and n=4 smoke-free), and tissue oxidation was evaluated by assaying for **(B)** thiobarbituric acid reactive substances (TBARS) (n=9 smoke-exposed and n=10 smoke-free). We did not find any significant difference in the results of these assays between smoke-exposed and smoke-free animals.