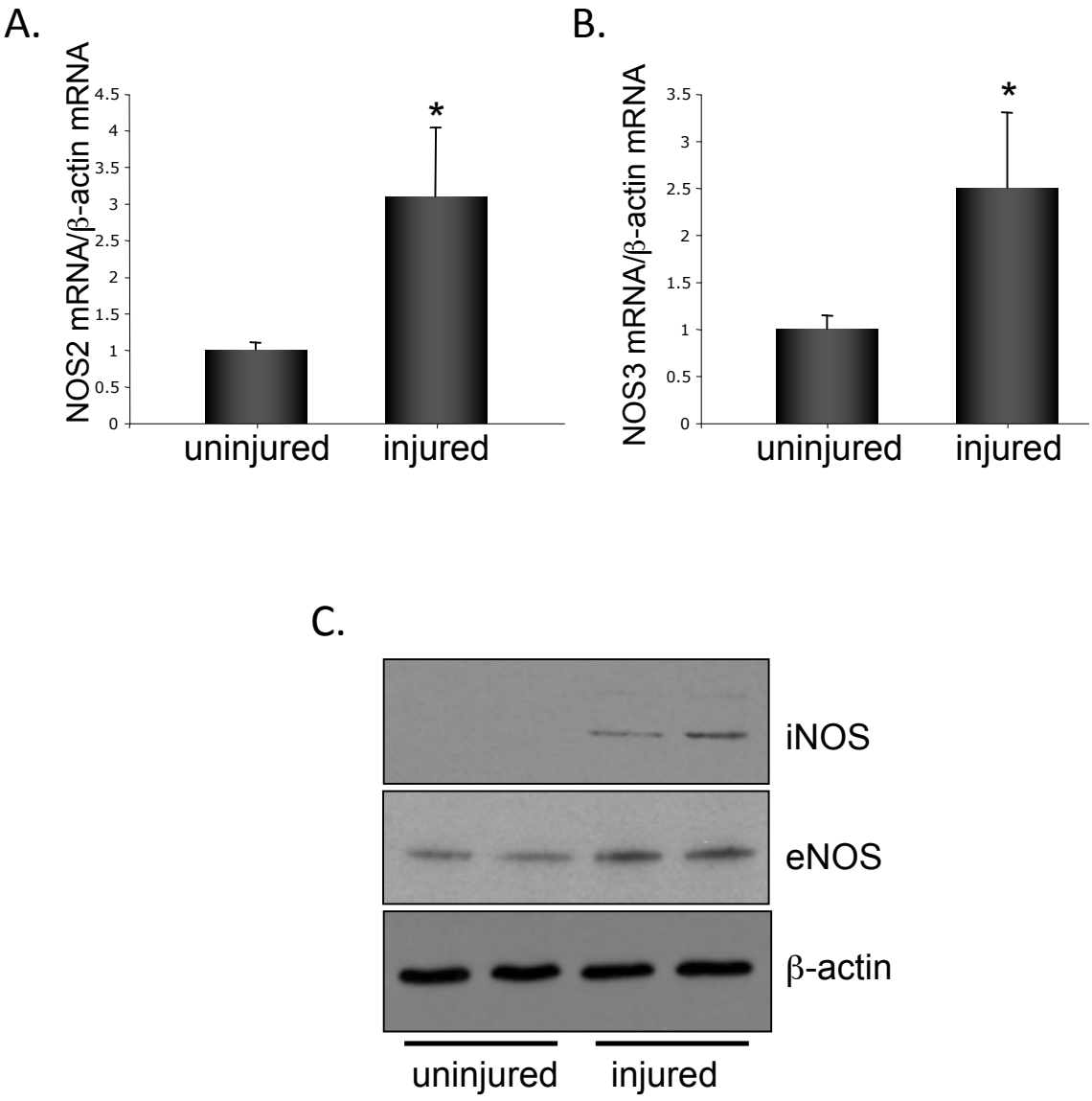
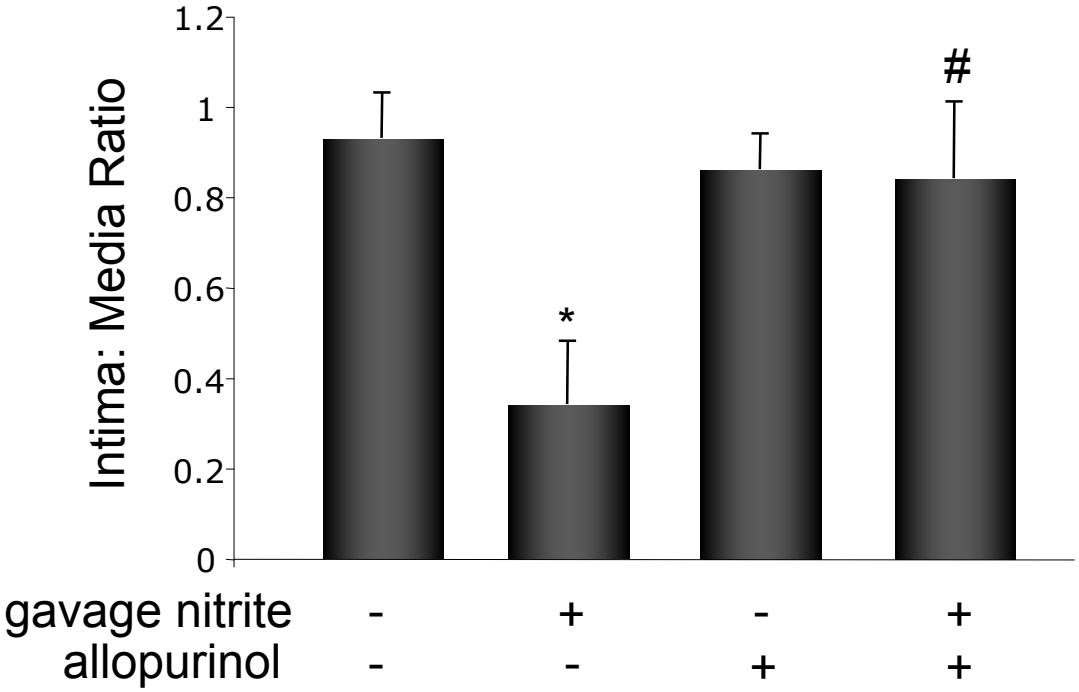
Supplemental Figure 1

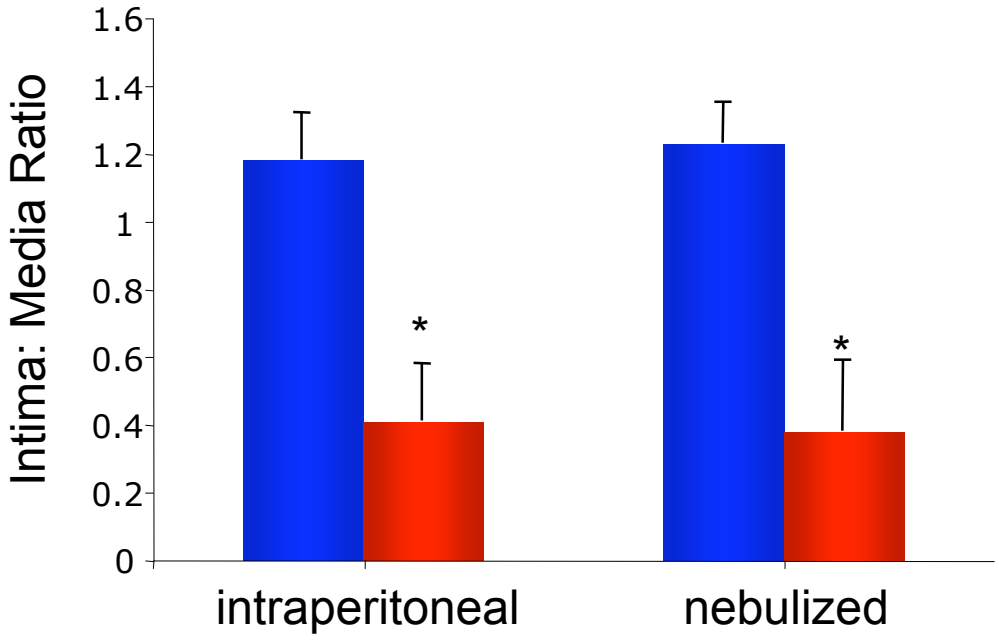


Supplemental Figure 2

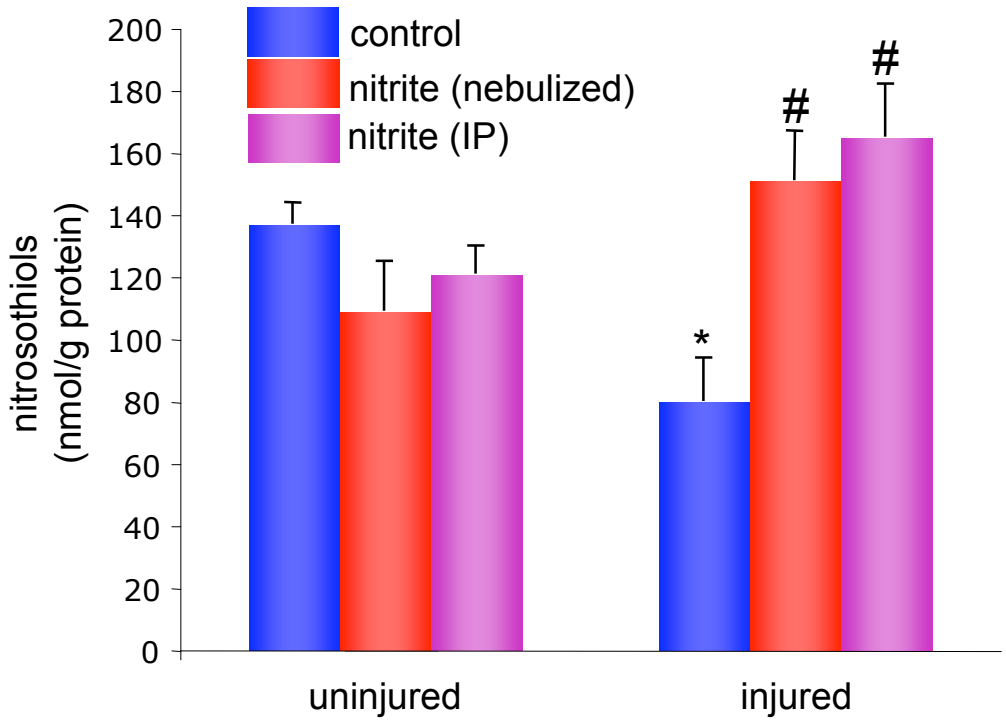
A.



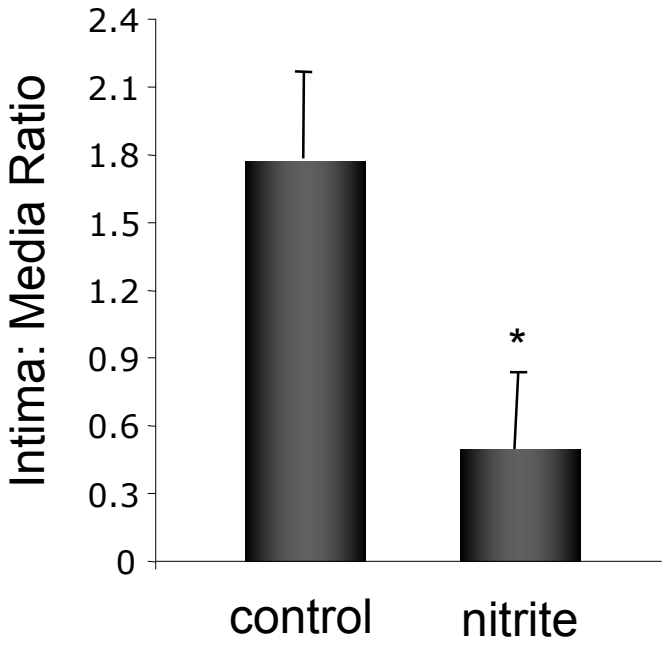
B.



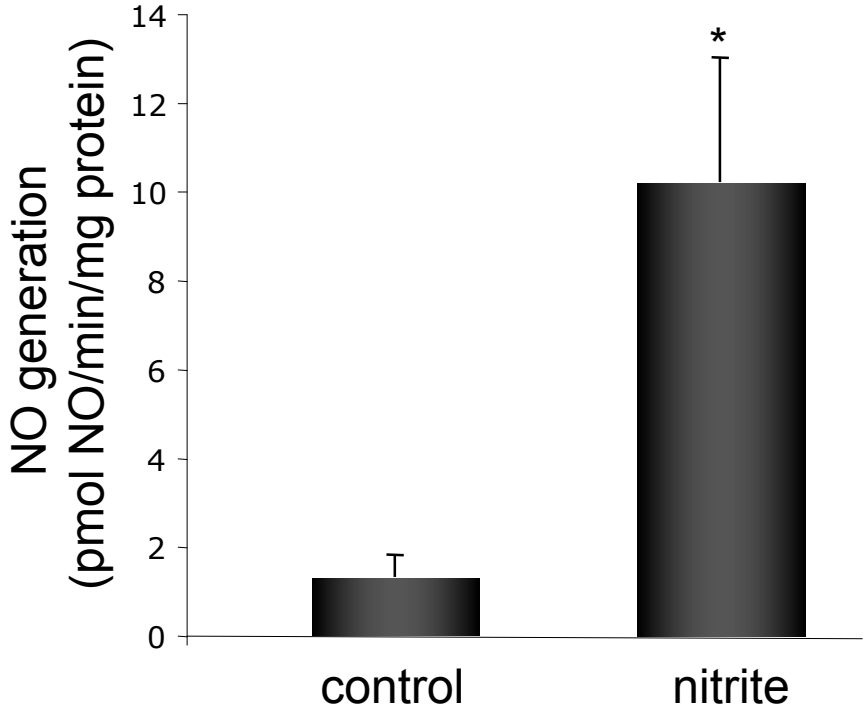
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplementary Figure Legends

Supplementary Figure 1. Vascular injury results in increased nitric oxide synthase (NOS) mRNA expression (**A, B**) and protein levels (**C**). Seven days after balloon injury, rat carotid arteries were harvested and RNA or protein lysates were collected. rtPCR for inducible NOS (iNOS; NOS2), endothelial NOS (eNOS; NOS3), and β-actin or Western blotting for iNOS, eNOS, and β-actin were performed. rtPCR data is from 4 rats per group and Western blotting is performed on 2 rats per group.

Supplementary Figure 2. Sodium nitrite protects against the development of or reverses established intimal hyperplasia. **A**. Intima:Media ratios were determined in rats 14 days following balloon injury. Rats had been randomized to receive water supplemented with allopurinol (100 μM/kg/day) for 48 hours before and 24 hours after vascular injury. Additionally, rats in each group were further randomized to receive a single gavage fed dose of sodium nitrite (500 nM) 90 minutes prior to vascular injury. Nitrite induced protection against intimal hyperplasia was reversed by allopurinol. **B**. Sodium nitrite delivered via nebulization (30 mg over 20 minutes through nebulizing chamber) or intraperitoneal dosing (500 nM) once daily from days 15-28 reversed intimal hyperplasia determined four weeks after vascular injury. All experiments were performed with n=5 rats per group.

Supplementary Figure 3. Reduction in S-nitrosothiol levels in vascular injury is reversed by nitrite treatment. Rats underwent carotid artery balloon injury and uninjured and injured carotid arteries were harvested 4 weeks after injury. Rats were randomized to no further treatment (control) or to receive sodium nitrite delivered via nebulization (30

mg over 20 minutes through nebulizing chamber) or intraperitoneal dosing (500 nM) once daily from days 15-28. N=4 carotid arteries per group.

Supplementary Figure 4. Sodium nitrite reverses intimal hyperplasia following mouse carotid artery injury. C57BL/6 mice underwent left carotid artery injury with a 0.018-inch guide wire. Mice were randomized to no further treatment (control) or to receive sodium nitrite supplemented drinking water (9.6 μg/kg/day). Intima:media ratios were decreased by 71± 20% compared to controls. N= 4 mice per group.

Supplementary Figure 5. Nitrite treatment increases NO generation in human arterial tissues. Segments of human aorta explanted as part of organ harvests for transplantation (generously provided by Dr. Michael DeVera) were homogenized and NO generation was analyzed with or without the addition of sodium nitrite (250 μM). N= 4 arteries per group and 4 independent measurements per artery.

Supplementary Methods:

Mouse carotid injury model, Mouse carotid injury model was performed following administration of Nembutal. Dissection of the left carotid artery and control of the vasculature was performed in a manner identical to that of the rat model. Injury was performed by insertion of a 0.018-inch guide wire (Cook, Bloomington, IN) through an external carotid arteriotomy into the common carotid artery, rotated 360 degrees three times. The wire was removed and the process was repeated two additional times. The external carotid artery was ligated above and below the arteriotomy and flow was restored to the common and internal carotid arteries. Intima:media ratios were determined 4 weeks after injury.

SYBR Green real-time RT-PCR. RNA was prepared by utilizing a silica-gel based membrane method using the RNeasy Midi Kit (Qiagen) according to the manufacturer's instructions. An on-column DNase digestion using RNase-free DNase (Qiagen) was performed to rid the samples of genomic DNA. One μg of RNA was used to generate cDNA using oligo dT primers (Qiagen) and Omniscript (Qiagen) reverse transcriptase. PCR reaction mixtures were prepared using SYBR Green PCR master mix (PE Applied Biosystems). SYBR Green two-step real-time RT-PCR for iNOS, eNOS and β-actin was performed as described. All samples were run in duplicate. The level of gene expression for each sample was normalized to β-actin mRNA expression using the comparative C_t method.