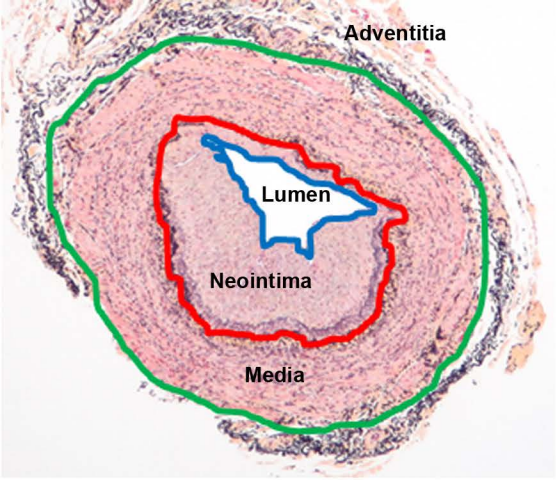
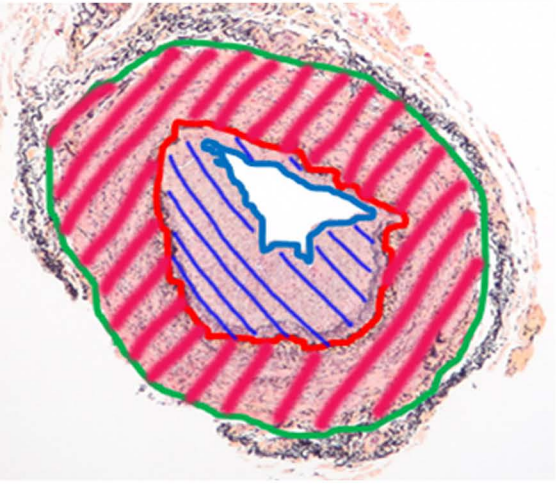




External Elastic Lamina Length (EEL)

Internal Elastic Lamina Length (IEL)

Lumen Area

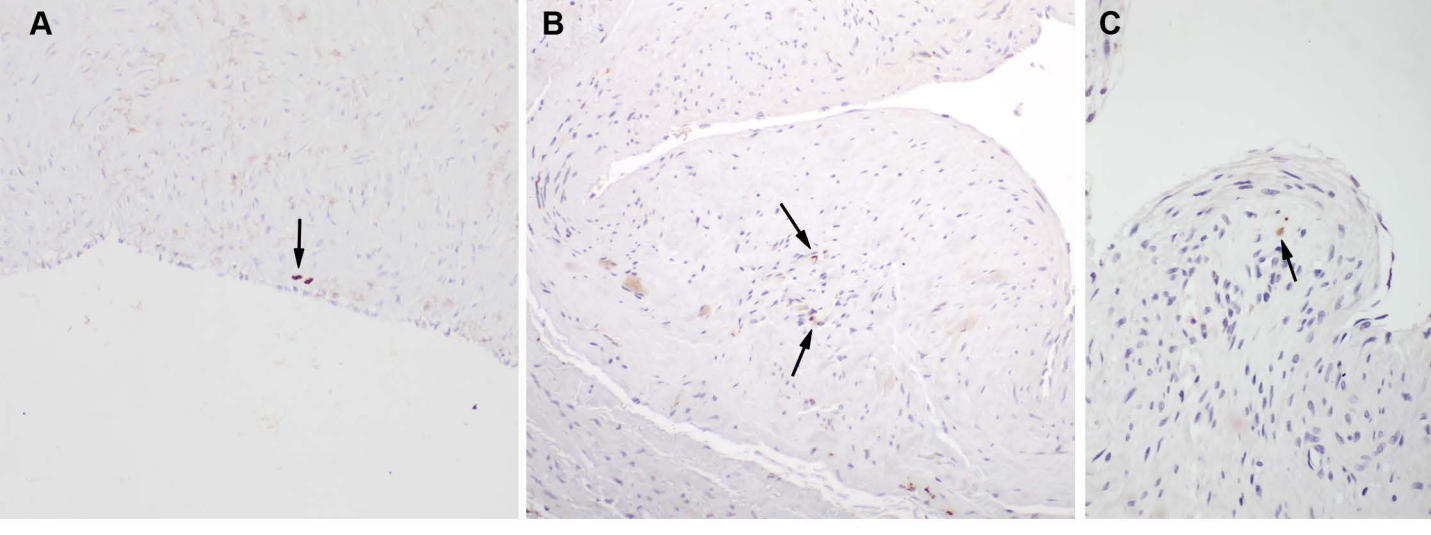
Medial Area

Neointimal/Lesion Area

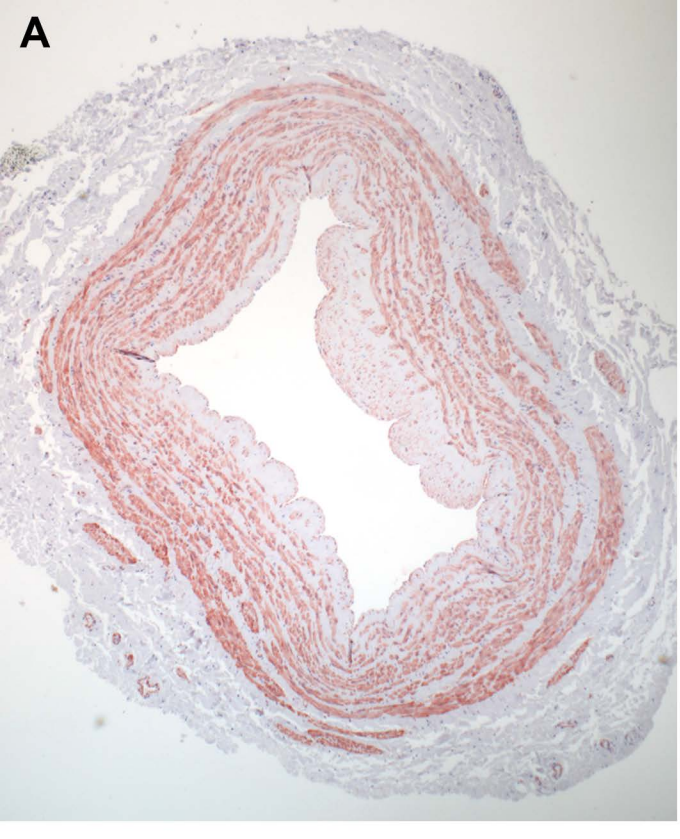
Total Area = + + Lumen Area

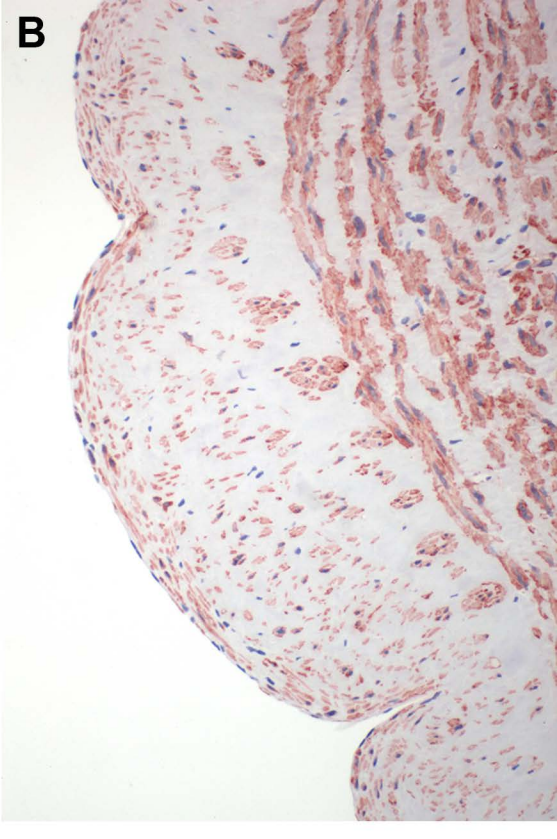
Maximal Lumen Area = + Lumen Area

Supplemental Figure 1

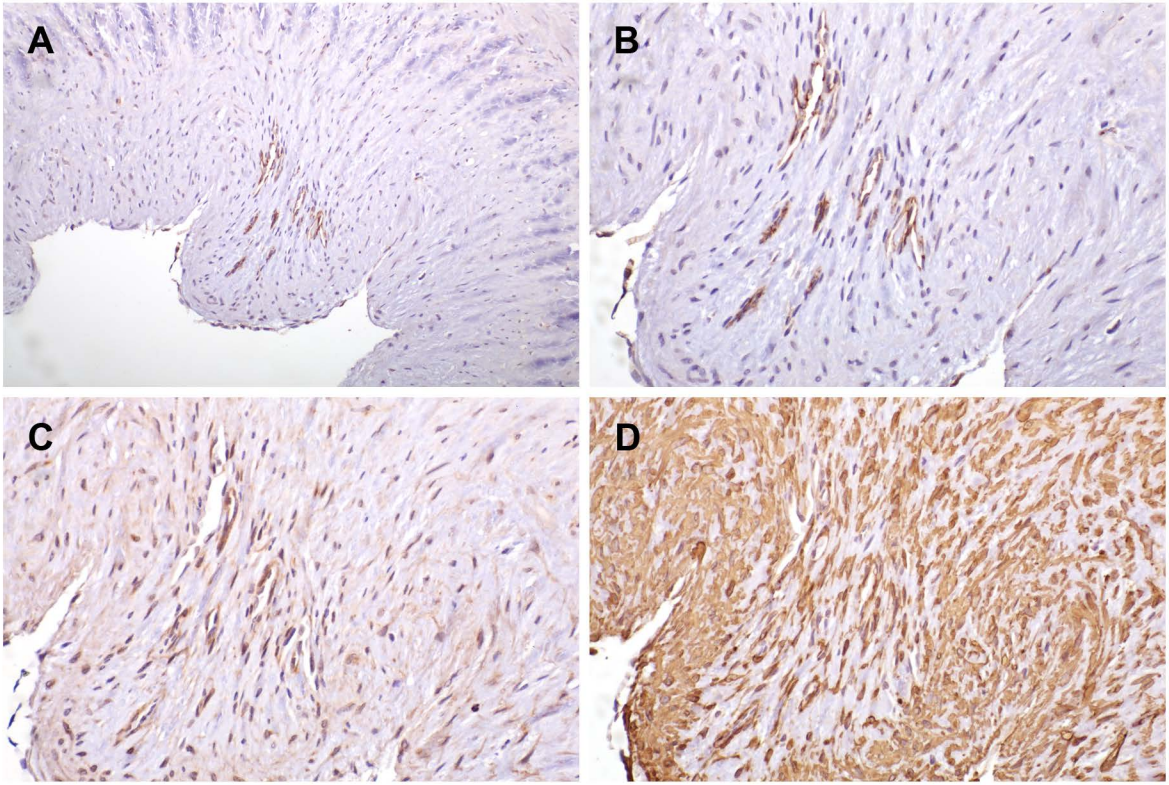


Supplemental Figure 2





Supplemental Figure 3



Supplemental Figure 4

Supplemental Figure 1: Pictorial depiction of vein composition and definition of regions measured in this study. The media is that portion of the vessel wall located between the internal and external elastic lamina (EEL). The internal elastic lamina (IEL), which stains black with a Movat's stain, serves as a landmark that separates the intima from the media. Normally the intima occupies such a limited portion of the vessel wall (just a lining of endothelial cells) as to be histologically miniscule. Expanded intimal tissue, or neointima is defined as occupying the space between the IEL and the vessel lumen. The maximal lumen area (neointima plus patent lumen) was calculated, with the ratio of patent lumen to maximal lumen area termed “luminal patency” and its complement, the ratio of neointimal area to maximal lumen area, used as a measure of extent of luminal narrowing due to neointimal fibroplasia. The adventitia is composed of connective tissue external to the EEL, but has no clear outer boundary and thus was not measured.

Supplemental Fig 2. Immunohistochemical characterization of proliferation, inflammation, and cell death in veins with intimal hyperplasia. A. Immunostaining of vein sample with Ki-67, a marker of proliferation. Positive immunostaining is detected by rust color. In this atypical example, two cells (arrows) located beneath the endothelial lining in a vein with little neointimal hyperplasia were stained positively, as indicated by the rust color, indicative of engagement in the cell cycle. B. Immunostaining with CD68, a marker of monocyte/macrophages, showed only a few positive cells as illustrated in this vein sample (arrows). C. Immunostaining with anti-caspase 3 antibody indicated

that caspase 3 expressing cells are also very rare within veins from this cohort (arrow). Proliferating cells, monocyte/macrophages and caspase 3 expressing cells were infrequent in veins from this cohort.

Supplemental Fig 3. Immunostaining of vein with alpha-smooth muscle actin antibody. Positive staining is indicated by rust color. Low (A) and high (B) power views of a typical vein with a neointima demonstrating that smooth muscle cells that express alpha-smooth muscle actin populate the medial portions of the vein. Myofibroblasts, fibroblasts that have become more proliferative, migratory and secrete more extracellular matrix, also express alpha-smooth muscle actin. The great majority of cells in the neointima also express alpha-smooth muscle actin, indicative of a smooth muscle cell or myofibroblast or "myofibroblast-like" phenotype.

Supplemental Fig 4. Immunostaining of vein with antibody markers of endothelial cells, pericytes , and smooth muscle cells and/or myofibroblasts. Serial tissue sections were stained with CD31 (A. low power and B. higher power), PDGFRβ (C), and smooth muscle actin (D) showing that neointimal microvessels lined by CD31 expressing endothelial cells are covered by PDGFRβ positive pericytes. The small number of microvessels relative to the large number of smooth muscle actin expressing cells indicates that the majority of the actin expressing cells in the neointima are either smooth muscle cells and/or myofibroblasts. Staining of replicate tissue sections for markers of endothelial cells (CD31), pericytes (platelet-derived growth factor receptor beta), show pericytes co-localizing with penetrating capillaries within neointimas, but

that the great preponderance of smooth muscle actin expressing cells were not pericytes.