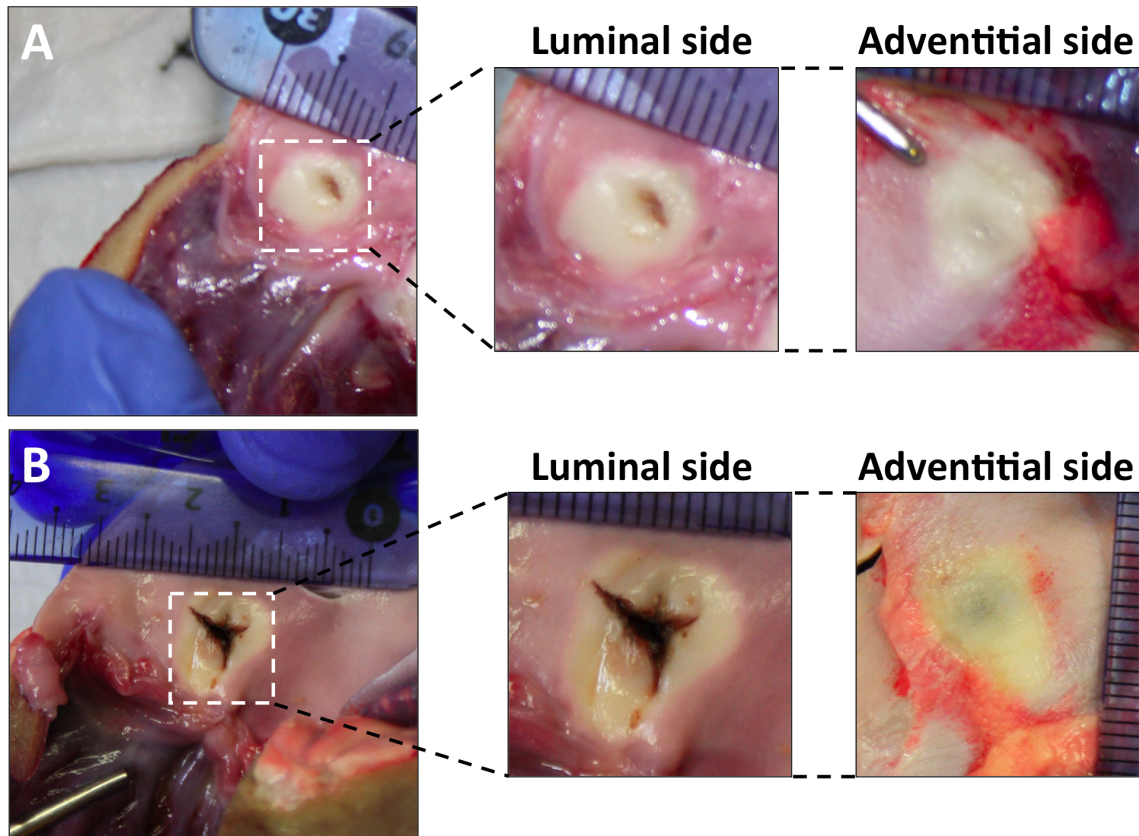


SUPPLEMENTAL MATERIAL

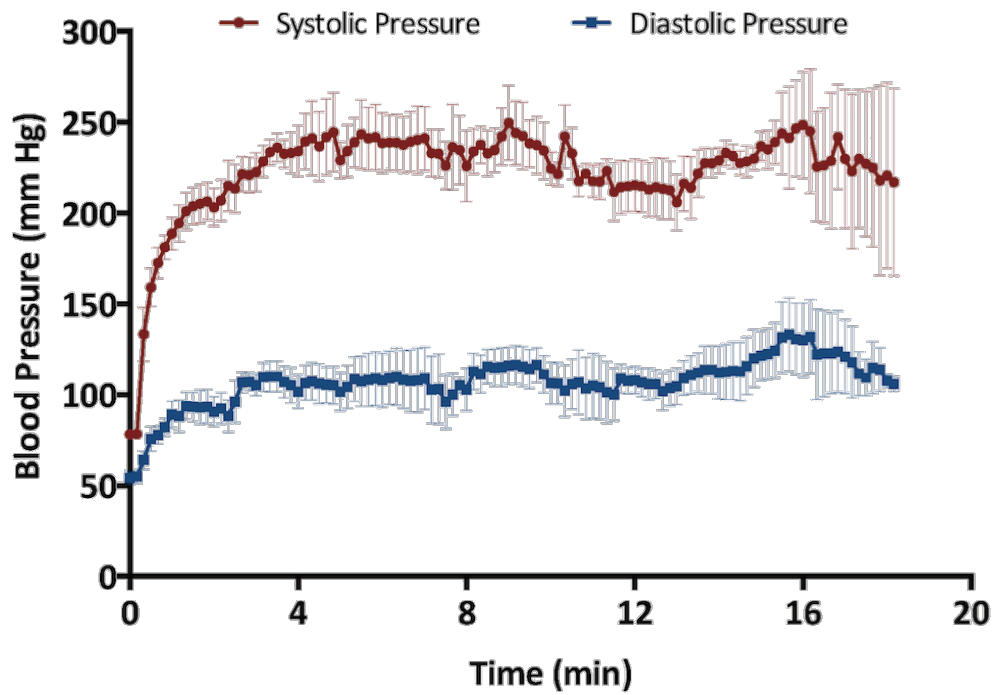
Multiphoton microscopy scanning.

Multiphoton microscopy is coupled with second harmonic generation (SHG) imaging, which enables to visualize highly ordered extracellular matrix macromolecules such as collagen, without the need of staining. Elastin fibers generate autofluorescence signals without the need for staining either. Therefore, vascular elastin and collagen fibers can be scanned simultaneously with minimal tissue manipulation. After dissection, the arteries were immersed in 70% formalin/30% sucrose solution overnight at 4°C for cryoprotection. Then, the samples were embedded in optimal cutting temperature compound (OCT) within histology cassettes and frozen with pulverized dry ice. Then, the tissue samples were cut into 30- μ m thick cross-sections and the OCT was washed out with PBS 1X. Arterial cross-sections were scanned overnight using a multiphoton and confocal system (Zeiss LSM 780) coupled with an AxioExaminer Z1 upright microscope and a pulsed infra-red laser (Spectra-Physics Mai Tai DeepSee eHP, 690-1040 nm) with a water dipping objective W Plan-Apochromat 20x, numerical aperture 1.0 (Zeiss, Jena, Germany). Z-stack and Tile Scan modes were performed to scan the entire sample, including both the lesion region and the surrounding healthy area. The samples were excited at 800 nm wavelength and second harmonic generation signals (SHG) originating from collagen fibers were obtained at 400 nm wavelength. The autofluorescence elastin-related signals were obtained at 450-650 nm wavelength. Image processing and analysis were performed with the ZEN 2011 SP7 Software (Version 14.0.0.201, Zeiss).

Supplemental Figures.



Suppl. Figure 1. Macroscopic view of acute radiofrequency-related vascular damage after steam pop at the root of the pulmonary artery. A, Relatively mild wall vascular damage after steam pop. B, Severe vascular damage with overt wall rupture at the luminal side after steam pop.



Suppl. Figure 2. Ascending aortic pressures during wall stress testing in animals with chronic lesions. Continuous recording of ascending aortic pressures after clamping the ascending aorta and acute increase in left ventricular afterload. The data depict mean \pm standard error of systolic and diastolic aortic pressures during the wall stress test.

Supplemental Movie 1 Legend: Catheter position (CF 41 g) at the left coronary sinus before radiofrequency delivery. Simultaneous coronary angiogram of the left main coronary artery, left descending coronary artery and circumflex artery.