

Supplementary Materials for

Raman spectroscopy imaging reveals interplay between atherosclerosis and medial calcification in the human aorta

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Supplementary Materials

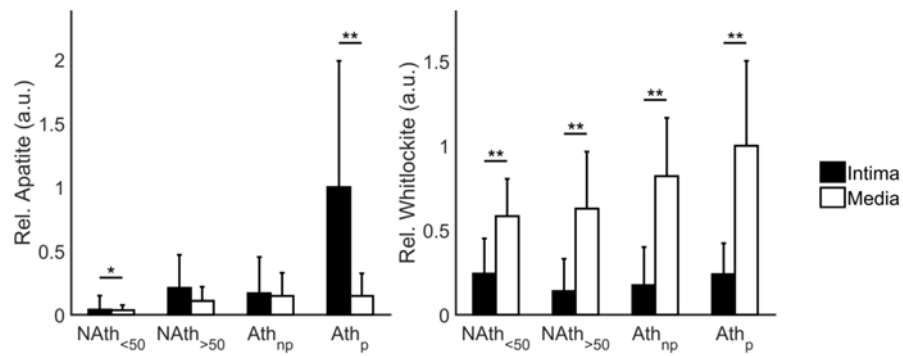


fig. S1. Comparison of the relative concentrations of apatite and whitlockite between the aortic intima and the media. Averages of the relative concentrations within the intima and the media, normalized to the maximum for each component and to area of the Raman images based on VCA results. Statistical significance was defined by Wilcoxon rank-sum test, error bars denote ± 1 SD, * $p < 0.05$ ** $p < 0.01$, $n \geq 9$ donors.

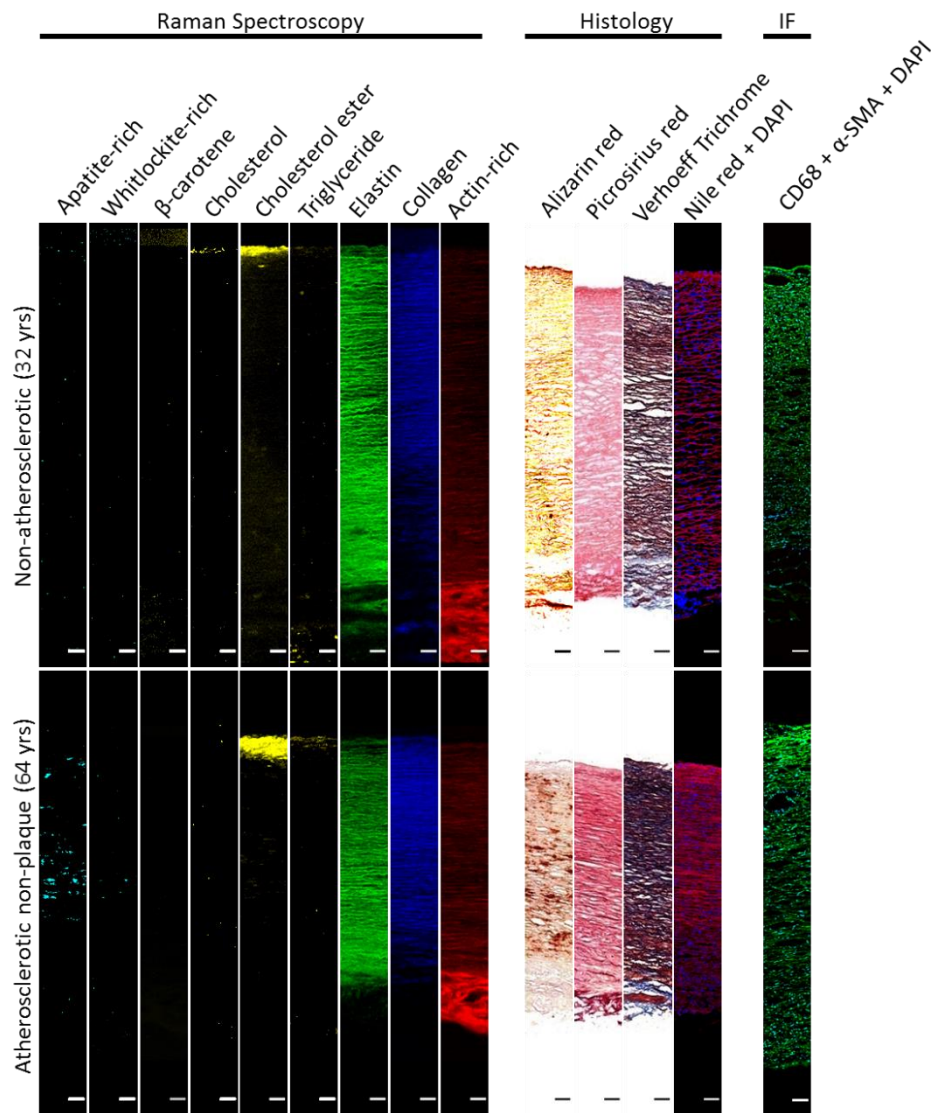


fig. S2. VCA on Raman spectroscopy images and relevant histology images.

Representative VCA images, associated histology and immunofluorescence (red: CD68, green: α -SMA, blue: DAPI) of aortic tissues from a healthy 32-year-old donor and a 64-year-old donor with atherosclerosis (tissue sample taken from non-plaque region). Scale bars 100 μ m. IF: immunofluorescence.

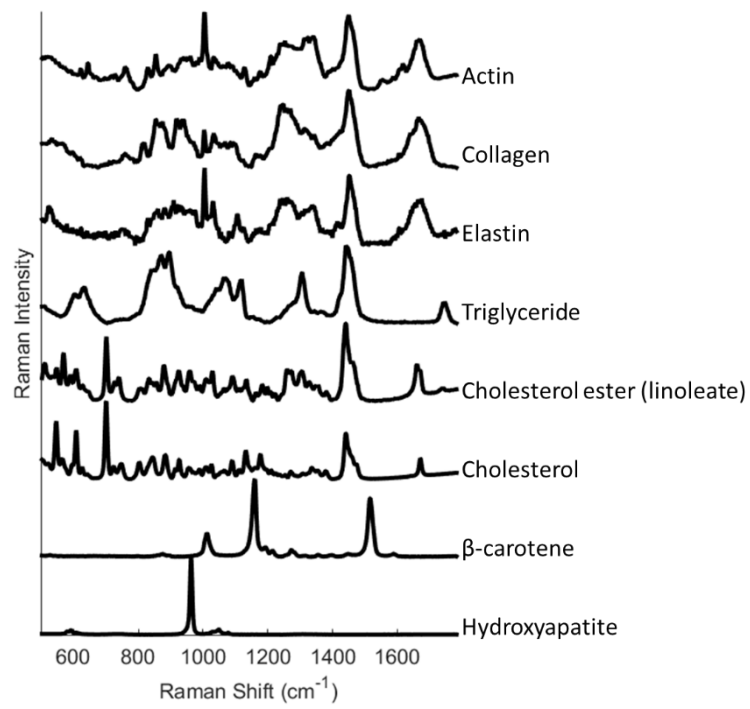


fig. S3. Raman spectra of pure commercial powders.

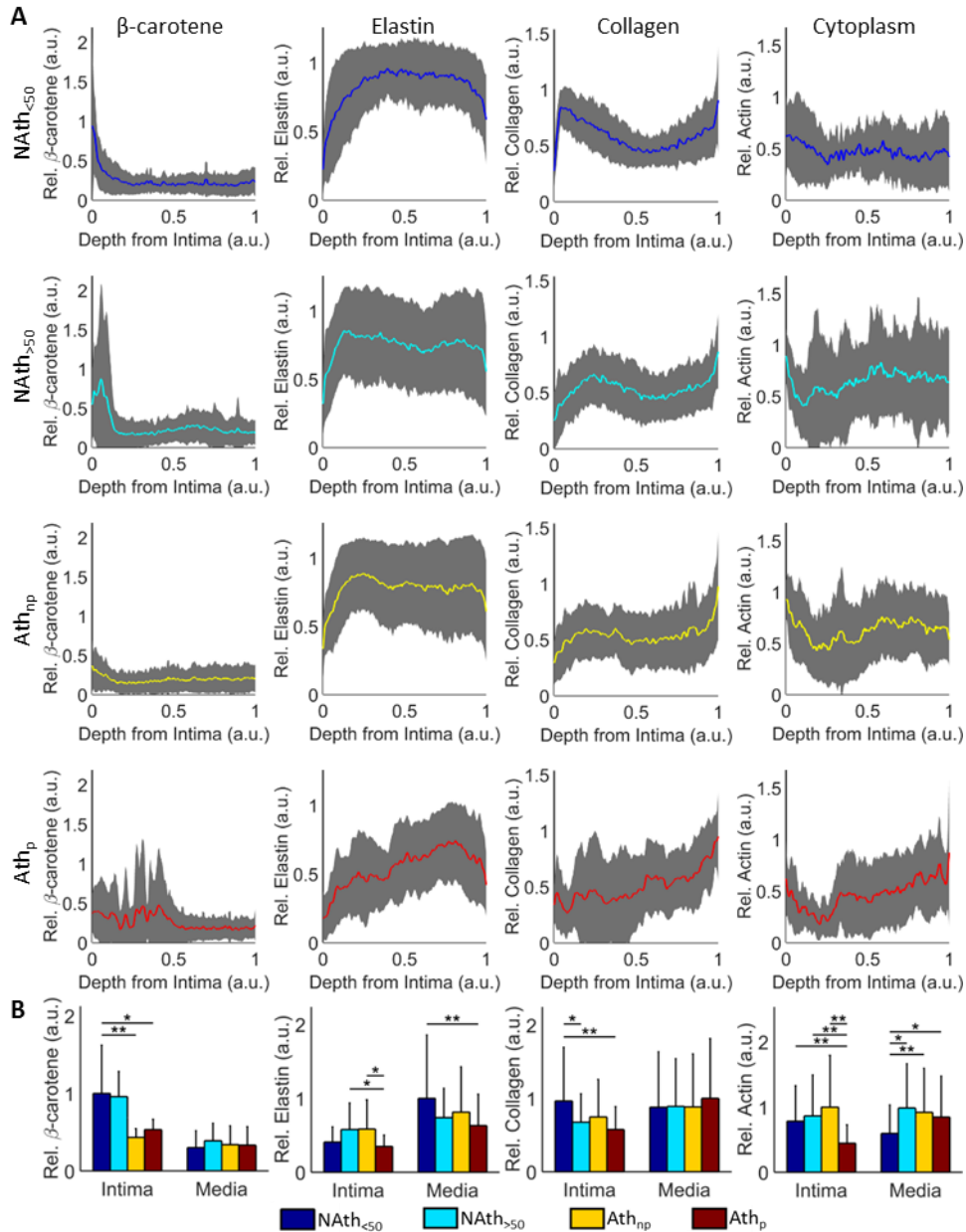


fig. S4. Depth-dependent profiles of β -carotene, elastin, collagen, and actin in the aortic intima and media based on VCA results, normalized to the maximum for each component. The relative concentration (Rel.) was summed along the normalized depth of the aorta from the surface of the intima, assigned 0 to the media-adventitia interface, assigned 1, plotted with ± 1 SD (**A**). Averages of the relative concentrations within the intima and the media, normalized to area of the Raman images, and to the maximum for each component, error bars denote 95% confidence intervals (**B**). Statistical significance was defined using generalized linear model (GLM) regression followed by ANOVA, * $p < 0.05$ ** $p < 0.01$, $n \geq 9$ donors per group.

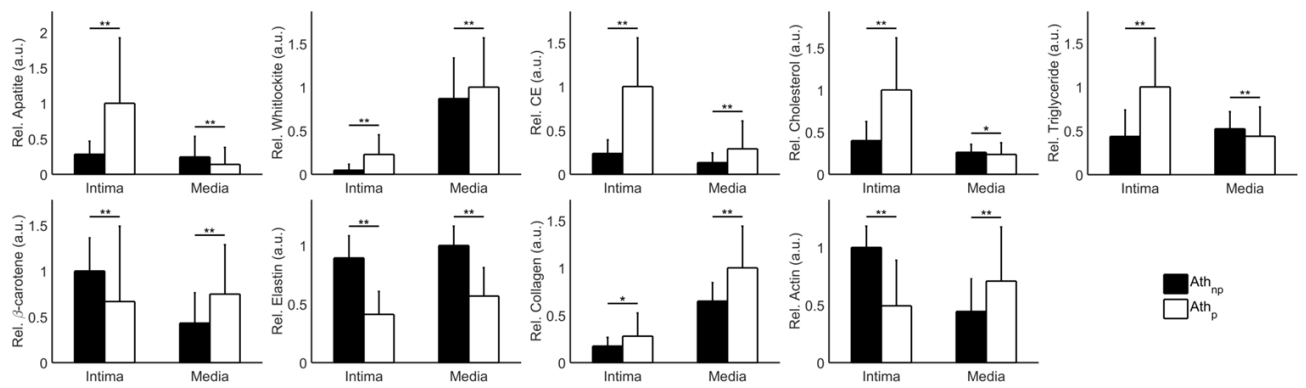


fig. S5. Representative comparison within one donor, for the relative concentrations of major components within the aortic intima and media. Averages of the relative concentrations within the intima and the media, normalized to the maximum for each component and to area of the Raman images based on VCA results. CE: Cholesterol ester. Statistical significance was defined by Wilcoxon rank-sum test, error bars denote ± 1 SD, $*p < 0.05$ $**p < 0.01$, $n \geq 2000$ data points from Raman spectroscopy map.