JHC Express
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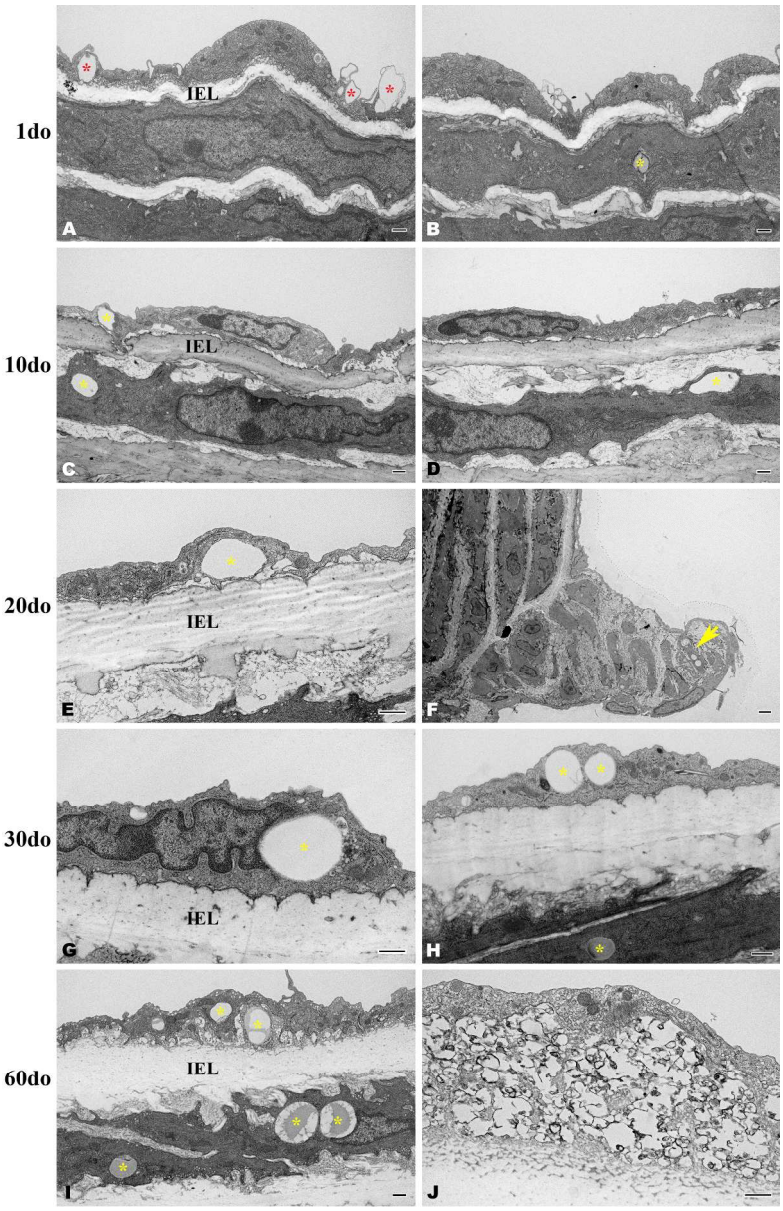

Figure 7. Transverse, osmicated electron micrographs showing the deposition of lipid within the distal thoracic aortic wall of SR-BI-/-/ApoeR61h/h mice aged 1-60 days old (do). Variably sized, intracellular lipid can be observed within endothelial cells (C, E, G-I) and within smooth muscle cells (B, C, H-I) directly beneath the internal elastic lamina (IEL) across all age groups, (yellow asterisks). At 1do, lipid is being engulfed by endothelial cells (A, red asterisks). Within an intercostal ostial ridge, lipid droplets are pictured accumulating within a smooth muscle cell (F, yellow arrow). At 60do, an accumulation of intraendothelial, clustered and varaibly sized lipid droplets admixed with calcified/mineralized debris (black amorphous material) is indicative of early atherosclerotic lesion formation (J). Scale bars = 500 nm (A-E and G-J) and 2 µm (F).
222x343mm (300 x 300 DPI)

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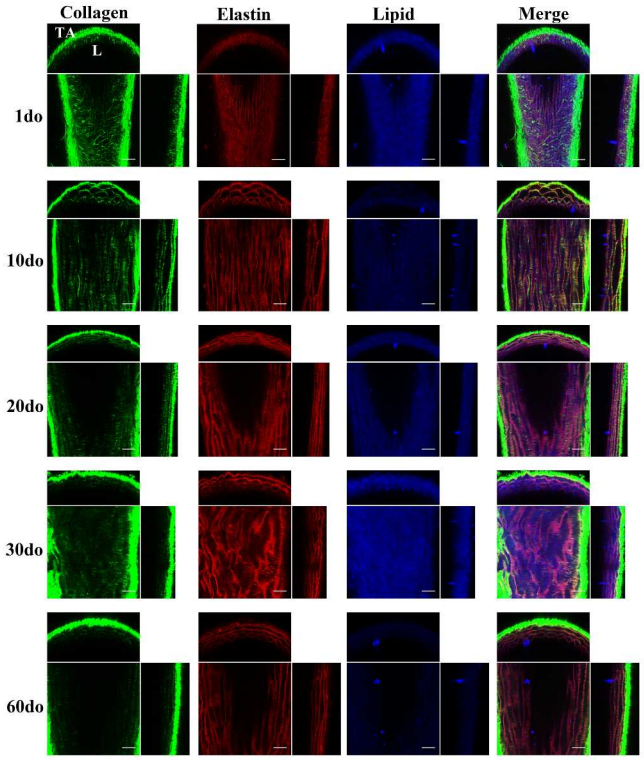

Supplemental image 1. Depicted is the same image panel represented in Figure 3 prior to post image analysis by ICA used for comparison. Note the background signal present in the lipid channel is greater before ICA processing resulting in less signal to noise in unprocessed images. L = lumen; TA = tunica adventitia. Scale bar, 50μm.
279x361mm (300 x 300 DPI)

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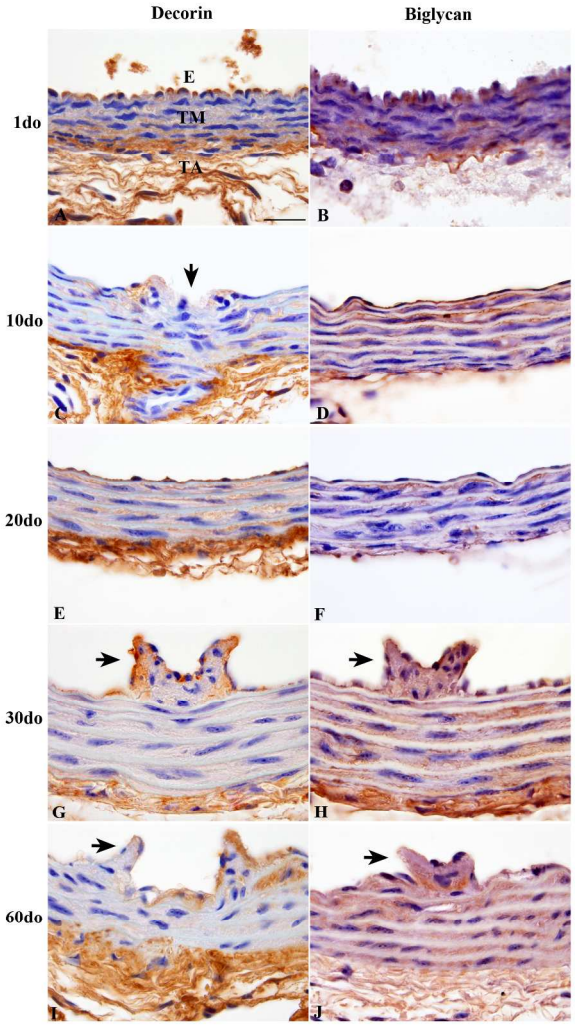

Supplemental Image 2. Distribution of proteoglycans decorin (A, C, E, G, and I) and biglycan (B, D, F, H, and J) within the extracelluar matrix of proximal thoracic aortas (PTAs) from SR-BI-/-/ApoeR61h/h mice aged 1-60do. Notice the prominent positive immunoreactivity (brown chromogen) in the tunica adventitia (TA) and subendothelium (E) for both decorin and biglycan across all age groups. Decorin staining within the tunica media (TM) appears to decrease with age while remaining similar for biglycan. Intercostal anterior ostia (black arrows, C and G-J) exhibit strong immunoreactivity for both decorin and biglycan within the subendothelium. All images are oriented with luminal surfaces at the top and external adventitial surfaces at the bottom of the photomicrographs. Scale bar = 20 µm, A-J.
215x279mm (300 x 300 DPI)

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Supplemental Material Legends

Supplemental Image 1. Depicted is the same image panel represented in Figure 3 prior to post image analysis by ICA used for comparison. Note the background signal present in the lipid channel is greater before ICA processing resulting in less signal to noise in unprocessed images. L = lumen; TA = tunica adventitia. Scale bar, 50 μm.

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Supplemental avi movies I-X. Avi video clips were produced from a full-thickness *z*-stack (ranging from ~150-200μm-thick tissue image acquisitions) obtained within the ventral (anterior) surface of the proximal thoracic aorta of representative SR-BI$^{-/-}$/*ApoeR61*$^{h/h}$ mice aged 1 (movies I-II), 10 (movies III-IV), 20 (movies V-VI), 30 (movies VII-VIII) and 60 (movies IX-X) days old. En-face projections coupled with cross sections followed by a maximum projection, three-dimensional rendering for each age group are provided. Collagen second harmonic generation (SHG) is green, elastin auto-fluorescence is red and nile red (NR) lipid droplet fluorescence is blue. Image acquisition was optimized during all experiments to fill the digitizer. ICA was

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applied during post image processing and the elastin fluorescence signal was optimized across all age groups for comparison purposes.

Supplemental avi movies XI-XII. Avi video clips produced from a full-thickness *z*-stack obtained within the ventral (anterior) surface of the proximal thoracic aorta of a 3-month old SR-BI$^{-/-}$/*ApoeR61*$^{h/h}$ mouse. Collagen second harmonic generation (SHG) is green, elastin auto-fluorescence and Syto® 24 fluorescent nucleic acid stain are both red, and nile red (NR) lipid droplet fluorescence is blue. Note that the wide emission spectra of Syto® 24(~475-600 nm) when excited with an 820 nm wavelenghth essentially masks any lipid labelled with NR. En-face projections (movie XI) and a merged cross section (movie XII) are supplied. ICA was applied during post image processing. These movies provide confirmation of complete penetration of the vessel wall by a fluorescent nuclear dye in this in-situ mouse thoracic aorta preparation.

Supplemental avi movies XIII-XIV. Avi video clips produced from a full-thickness *z*-stack obtained within the ventral (anterior) surface of the proximal thoracic aorta of a 3-month old SR-BI$^{-/-}$/*ApoeR61*$^{h/h}$ mouse. Collagen second harmonic generation (SHG) is green, elastin auto-fluorescence is red, and the vascular endothelium (di-8-ANEPPS) and phospholipid bilayers (di-8-ANEPPS) are blue. En-face projections (movie XIII) and a merged cross section (movie XIV) are supplied. ICA was applied during post image processing. Unlike lipid stained as solid spheres as observed with NR, lipid stained with di-8-ANEPPS appears as black hollow spheres with phospholipid bilayers outlined in blue. Lipid is appreciated resting on the stained vascular endothelium (blue) indicating that the lipid droplets within this *z*-stack are likely captured on the vascular luminal surface directly on the endothelium and within the endothelial cell cytoplasm.