SUPPLEMENTAL MATERIAL

Vascular smooth muscle-specific progerin expression accelerates atherosclerosis and death in a mouse model of Hutchinson-Gilford progeria syndrome

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Table I. **Predominant *Lmna* gene products in different cells and organs of mouse models used in the study.**

Genotype	Vascular smooth muscle cells	Myeloid cells (Macrophages)	Other organs/tissues*
***Apoe*$^{-/-}$ *Lmna*$^{+/+}$**	Lamin A, lamin C	Lamin A, lamin C	Lamin A, lamin C
***Apoe*$^{-/-}$ *Lmna*$^{G609G/G609G}$**	**Progerin**, lamin C, lamin A (residual)	**Progerin**, lamin C, lamin A (residual)	**Progerin**, lamin C, lamin A (residual)
***Apoe*$^{-/-}$ *Lmna* $^{LCS/LCS}$**	Lamin C	Lamin C	Lamin C
Apoe*$^{-/-}$ *Lmna*$^{LCS/LCS}$ *SM22aCre	**Progerin**, lamin C, lamin A (residual)	Lamin C	Lamin C#
Apoe*$^{-/-}$ *Lmna*$^{LCS/LCS}$ *LysMCre	Lamin C	**Progerin**, lamin C, lamin A (residual)	Lamin C

* kidney, liver, spleen, heart.

appreciable progerin expression in the heart.

Table II. Plaque features in *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* mice.

	Necrotic core (% of plaque area)	Iron deposits (% of animals showing iron deposits in the plaque)	SMC content (% of SMA-positive plaque area)	SMC content in the fibrous cap (% of SMA-positive area in 6 µm below the endothelium)
***Apoe*$^{-/-}$ *Lmna*$^{+/+}$**	0.89 +/- 0.55	0%	3.25 +/- 1.13	8.29 +/- 2.69
***Apoe*$^{-/-}$ *Lmna*$^{G609G/G609G}$**	38.10 +/- 6.54	40%	1.52 +/- 0.21	3.92 +/- 1.04
***P* value**	0.0005	-	0.1723	0.1681
***Apoe*$^{-/-}$ *Lmna* $^{LCS/LCS}$**	6.48 +/- 1.80	0%	4.75 +/- 1.00	13.50 +/- 1.81
Apoe*$^{-/-}$ *Lmna*$^{LCS/LCS}$ *SM22aCre	30.25 +/- 4.29	60%	3.15 +/- 0.76	6.92 +/- 2.00
***P* value**	0.0009	-	0.2381	0.0406

Mice were fed a high-fat diet for 8 weeks starting at 8 weeks of age. The table summarizes quantification of atherosclerotic plaque features in mice of the indicated genotypes. Necrotic cores were quantified in Hoechst 33342-stained sections. Iron deposits were analyzed in Perls' Prussian blue-stained sections. Smooth muscle cell (SMC) content was quantified in anti-smooth muscle cell actin (SMA)-stained sections. Each plaque characteristic was quantified in three sections obtained from different aortic root regions, and the mean was used for further analysis; n=5. Data are mean ± SEM. Statistical differences were assessed by the two-tailed *t*-test.

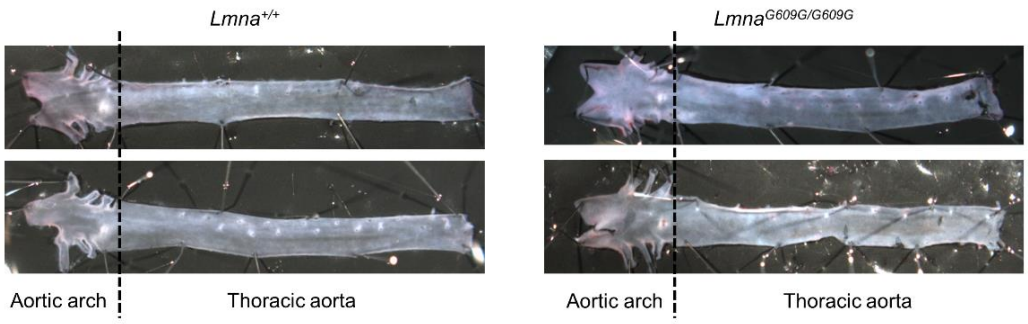

Figure I. Lack of atherosclerosis in *Lmna*$^{G609G/G609G}$ mice. *Lmna*$^{G609G/G609G}$ and *Lmna*$^{+/+}$ mice were fed a high-fat diet for 8 weeks starting at 8 weeks of age. Mice were sacrificed at 16 weeks (close to their median survival) and aortas were stained with Oil Red O to visualize lipid-rich atheroma plaques.

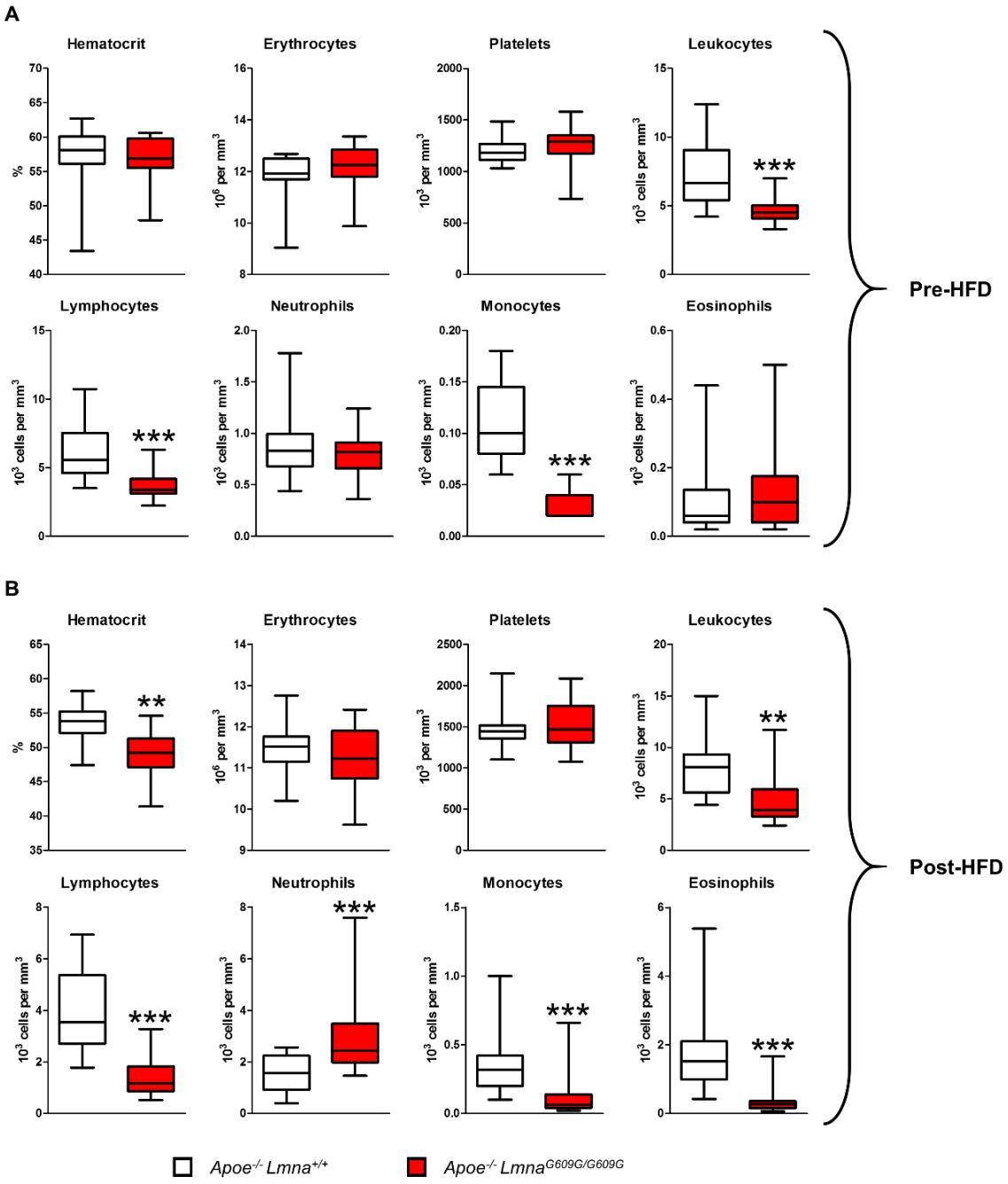

Figure II. *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice present alterations in the leukocyte subpopulations pre- and post-high-fat diet (HFD). Mice were challenged with HFD for 8 weeks starting at 8 weeks of age. **(A)** Pre-HFD hematology results for 8-week-old *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice (n=18) and *Apoe*$^{-/-}$*Lmna*$^{+/+}$ *mice* (n=18). **(B)** Post-HFD hematology results for 16-week-old *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice (n=17) and *Apoe*$^{-/-}$*Lmna*$^{+/+}$ mice (n=17). Data are shown as median with interquartile range and minima and maxima. Statistical differences were analyzed by two-tailed Mann-Whitney test. ***P*<0.01, ****P*<0.001.

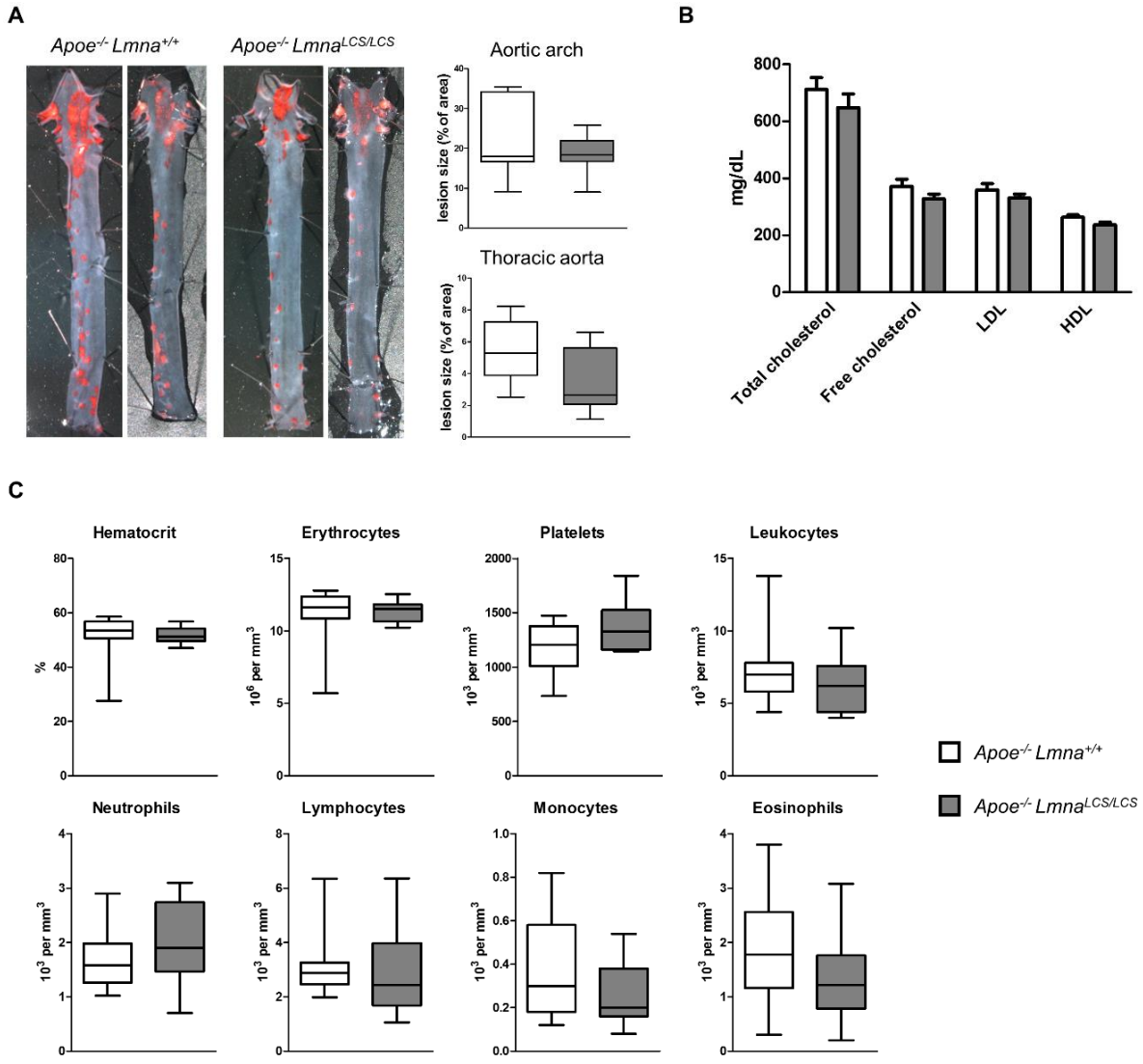

Figure III. *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ mice expressing only lamin C show no more atherosclerosis than *Apoe*$^{-/-}$*Lmna*$^{+/+}$ control mice expressing wild-type lamin A/C. Mice were fed a high-fat diet (HFD) for 8 weeks starting at 8 weeks of age and sacrificed at 16 weeks of age. **(A)** Representative examples of aortas stained with Oil Red O, and quantification of atherosclerosis burden in *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ mice (n=8 aortic arches, n=11 thoracic aortas) and *Apoe*$^{-/-}$*Lmna*$^{+/+}$ mice (n=7 aortic arches, n=11 thoracic aortas). **(B)** Post-HFD serum levels of total cholesterol, free cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) in *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ (n=6) and *Apoe*$^{-/-}$*Lmna*$^{+/+}$ (n=7) mice. **(C)** Post-HFD hematology results for *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ mice (n=11) and *Apoe*$^{-/-}$*Lmna*$^{+/+}$ mice (n=11). Data are shown as median with interquartile range and minima and maxima in **A** and **C** and as mean ± SEM in **B**. Statistical differences were analyzed by two-tailed Mann-Whitney test in **A** and **C** and by two-tailed *t*-test in **B**.

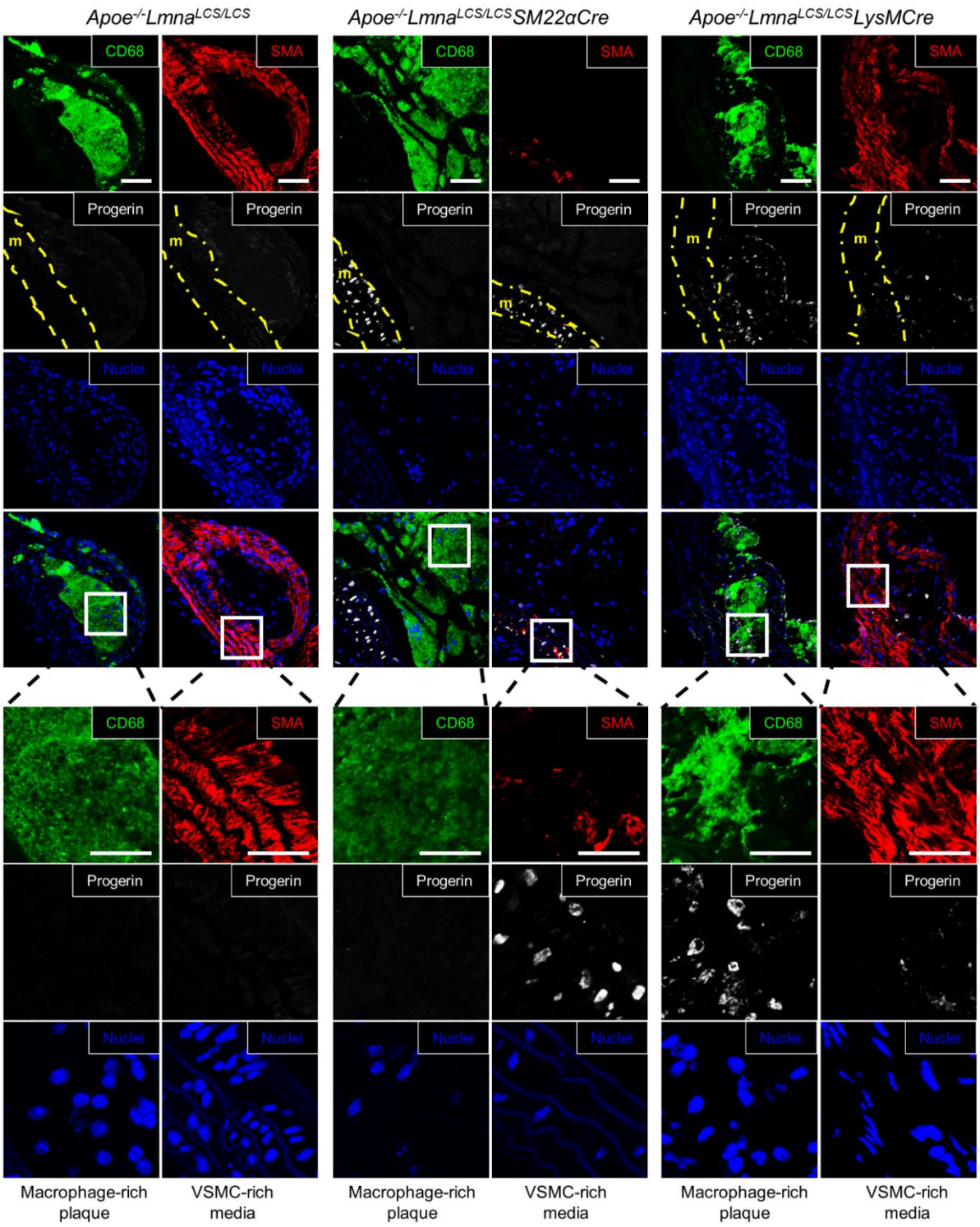

Figure IV. Progerin is expressed specifically in vascular smooth muscle cells (VSMCs) of *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* aorta and in macrophages of *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*LysMCre* aorta. Representative images of atheroma-containing aortas from 16-week-old mice fed a high-fat diet for 8 weeks starting at 8 weeks of age. Progerin was visualized in white, macrophages were detected with anti-CD68 antibody (green), and VSMCs with anti-smooth muscle actin (SMA) antibody (red). Nuclei were stained with Hoechst 33342 (blue). Amplified images show VSMC-rich media and a macrophage-rich atherosclerotic plaque. Scale bar: 50 µm (entire image) and 25 µm (magnified view). m: media.

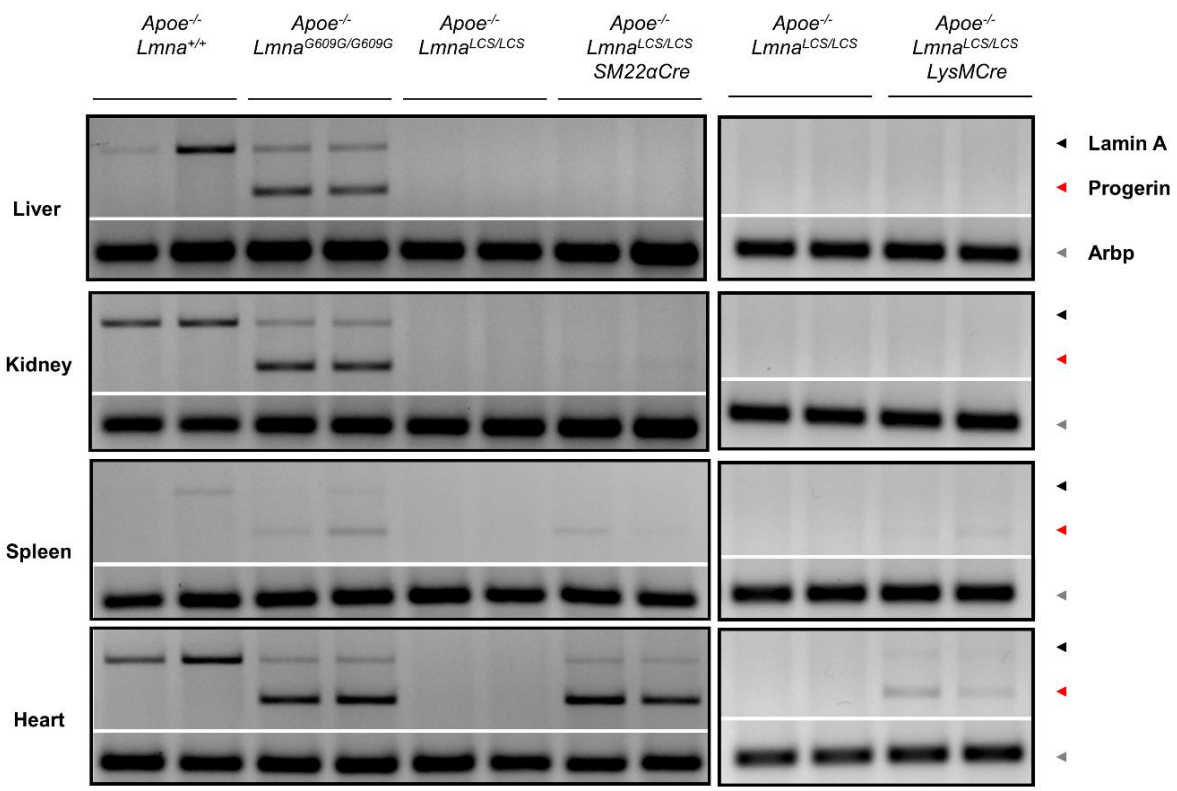

Figure V. Progerin and lamin A mRNA expression in different organs of progeroid and control mice. Representative images of PCR products showing lamin A and progerin mRNA expression in liver, kidney, spleen, and heart of 8-week-old mice of the indicated genotypes. *Arbp* was used as an endogenous control.

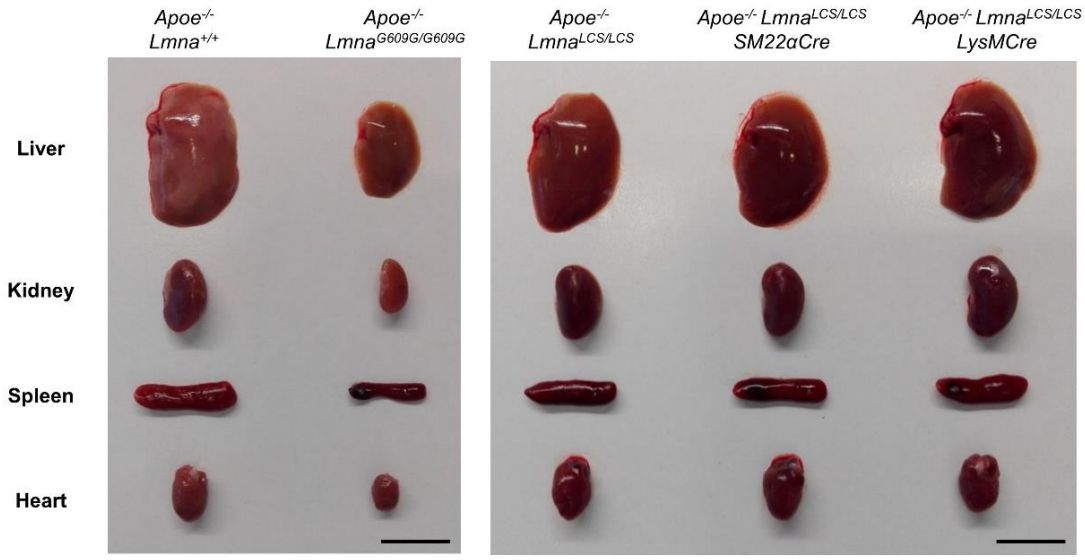

Figure VI. *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*LysMCre* mice, unlike *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice, show normal organ size. Representative images of liver, kidney, spleen, and heart of 16-week-old mice of the indicated genotypes. Scale bar: 1 cm.

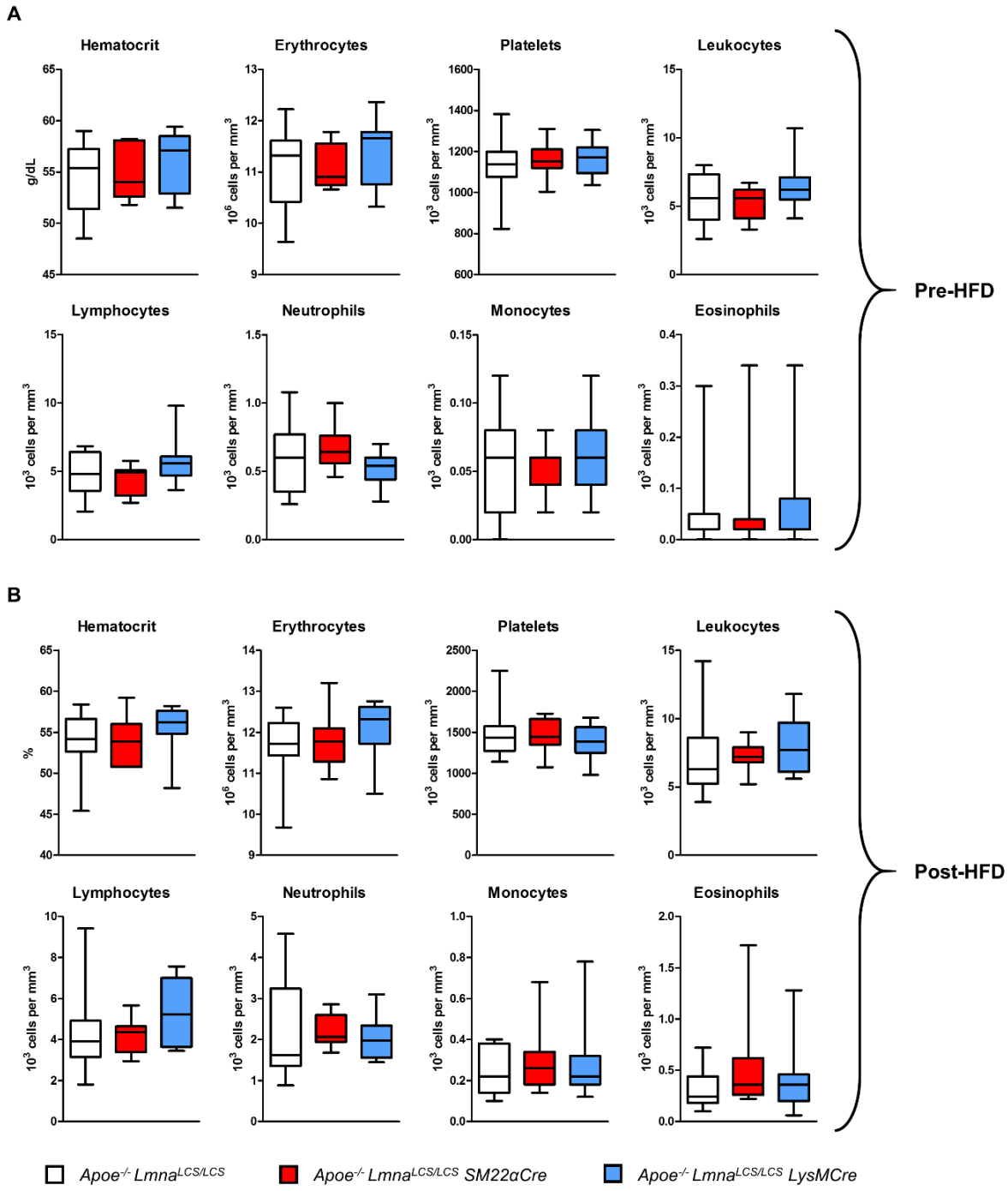

Figure VII. Normal hematological parameters in *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*LysMCre* mice. Mice were fed a high-fat diet (HFD) for 8 weeks starting at 8 weeks of age. **(A)** Pre-HFD hematology results for 8-week-old *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ mice (n=17), *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* mice (n=11), and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*LysMCre* mice (n=11). **(B)** Post-HFD hematology results for 16-week-old *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ mice (n=17), *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* mice (n=11), and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*LysMCre* mice (n=11). Data are shown as median with interquartile range and minima and maxima. Statistical differences were analyzed by Kruskal-Wallis test with Dunn´s *post hoc* test.

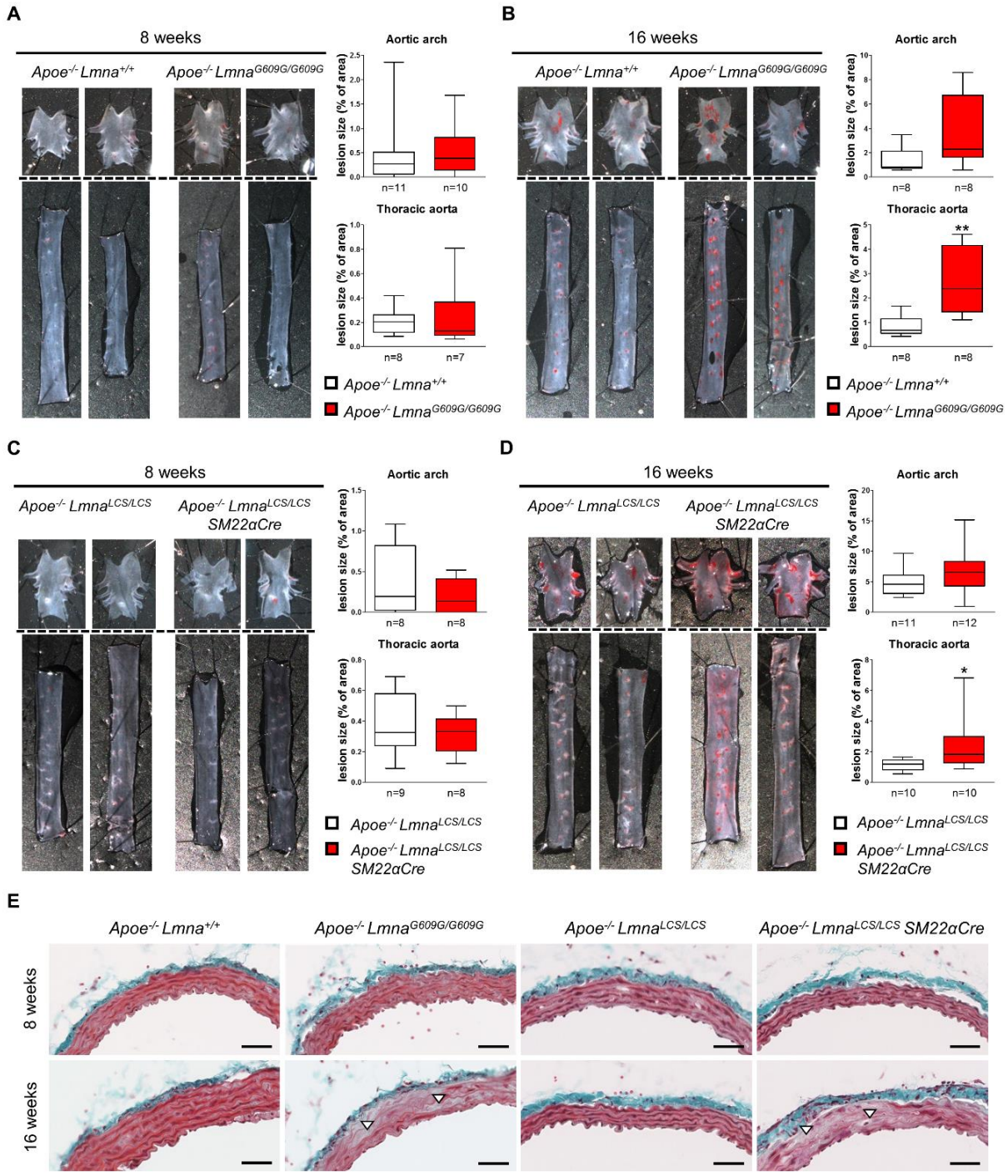

Figure VIII. Progression of vascular disease in *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* mice fed normal chow. Mice were fed normal chow and sacrificed at 8 or 16 weeks of age. Controls for *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* mice were *Apoe*$^{-/-}$*Lmna*$^{+/+}$ and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$, respectively. **(A-D)** Representative images of aortas stained with Oil Red O and quantification of atherosclerosis burden in mice of the indicated ages and genotypes. The number of mice is indicated below each graph. **(E)** Representative images of Masson's Trichrome-stained aortic arches from mice of the indicated ages and genotypes. White arrowheads indicate regions with vascular smooth muscle cell loss. Scale bar: 50 µm. Data in **A-D** are shown as median with interquartile range and minima and maxima. Statistical differences were analyzed by two-tailed Mann-Whitney test. **P*<0.05, ***P*<0.01.

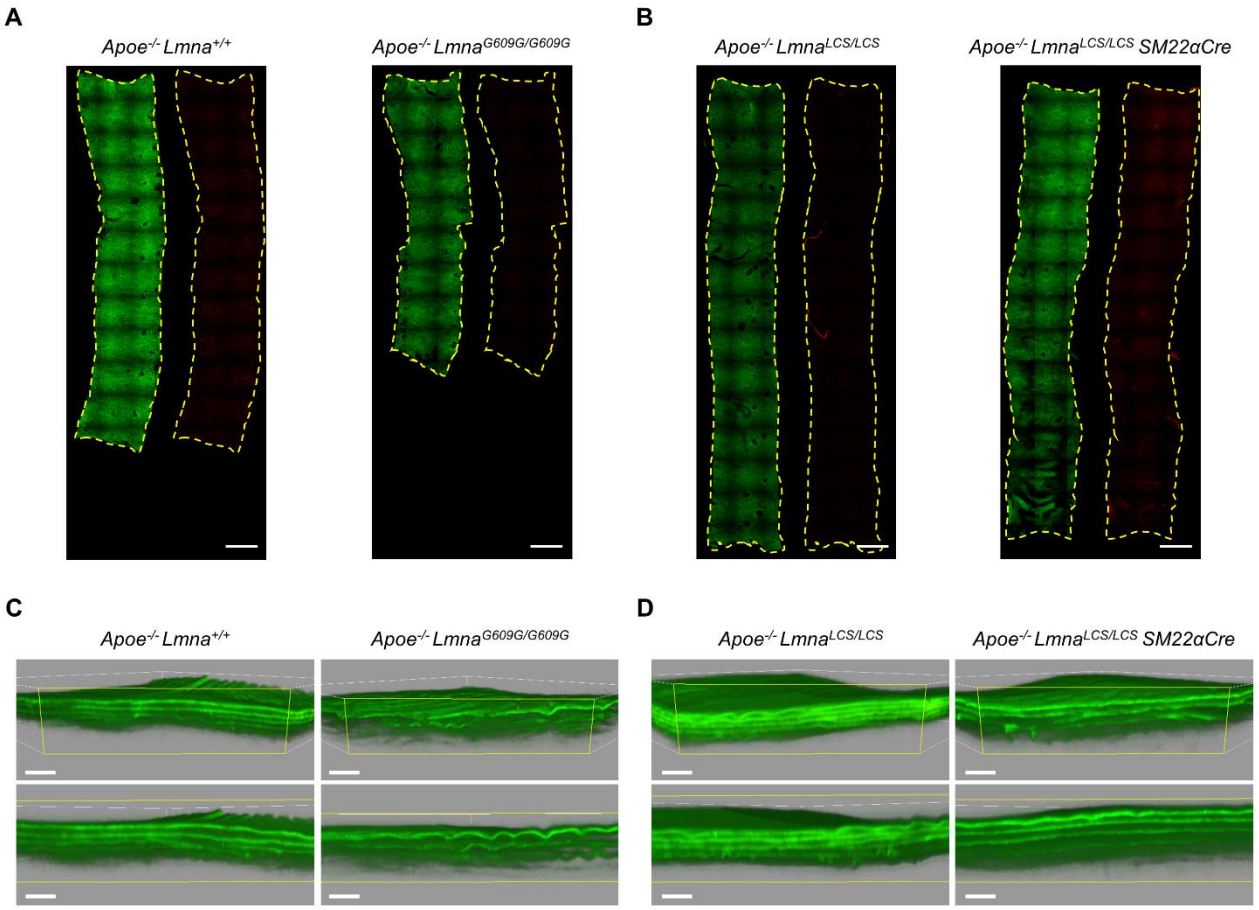

Figure IX. Autofluorescence levels in the aortas of *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$, *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre, Apoe*$^{-/-}$*Lmna*$^{+/+}$ and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ mice. Mice of the indicated genotypes fed normal chow were sacrificed at 16 weeks of age and used as controls for the LDL retention experiments (littermates not injected with Atto565-LDL). Tile scan fluorescence images were acquired with a confocal microscope. **(A, B)** Representative green (*left*) and red (*right*) fluorescence images of thoracic aortas. Scale bar: 1 mm. **(C, D)** Still frame images of 3D reconstruction videos, showing autofluorescence of aortas in the green and red channels (see Supplemental Movies XIII-XVI). Scale bar: 50 µm (*top*), 30 µm (*bottom*).

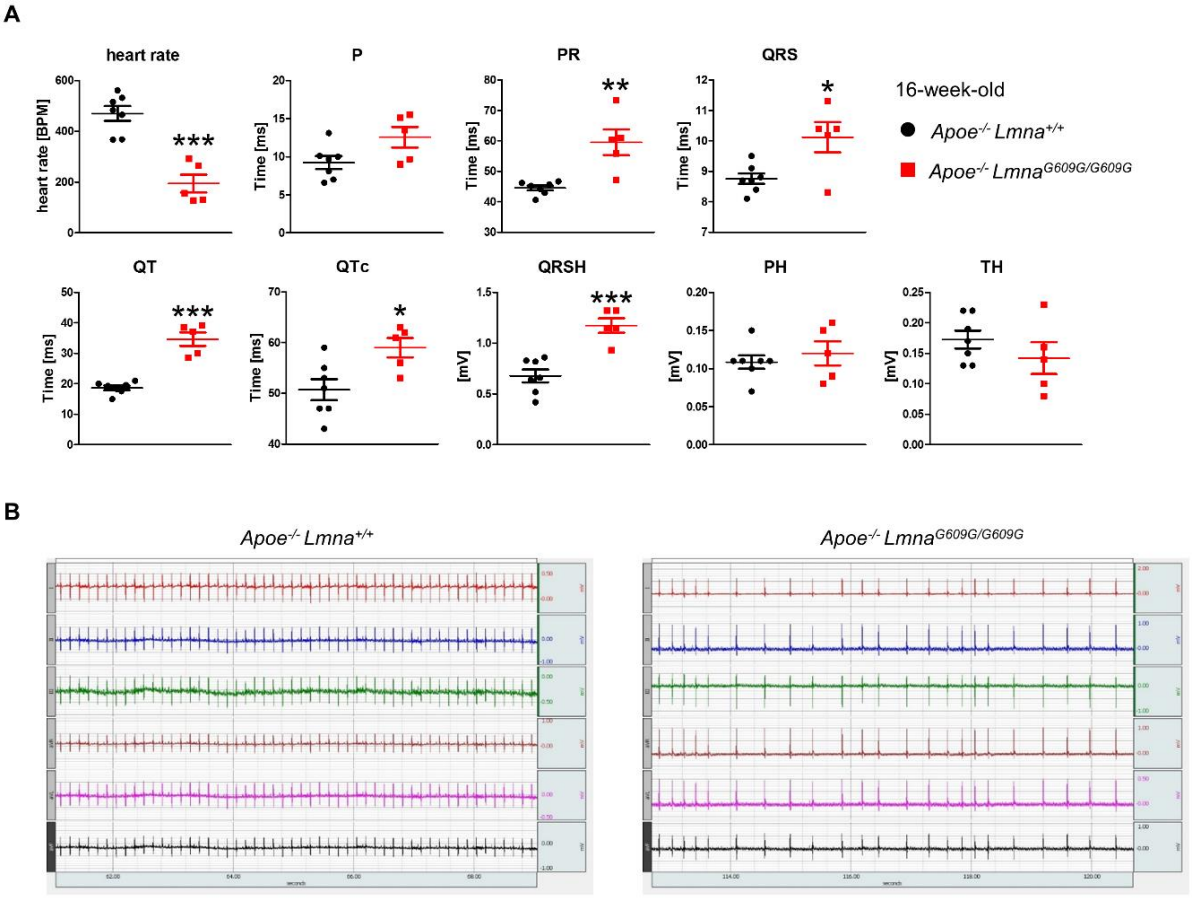

Figure X. *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice develop cardiac electrical defects and arrhythmias. Electrocardiography (ECG) was performed in isoflurane-anesthetized 16-week-old mice fed normal chow. **(A)** ECG parameters for *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ (n=5) and *Apoe*$^{-/-}$*Lmna*$^{+/+}$ (n=7) mice. **(B)** Representative ECG recordings. *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice have bradycardia and arrhythmia. QRSH, PH, and TH indicate amplitudes of QRS complex, P, and T waves, respectively. Data are presented as mean ± SEM. Statistical differences were analyzed by two-tailed *t*-test. **P*<0.05, ***P*<0.01, ****P*<0.001.

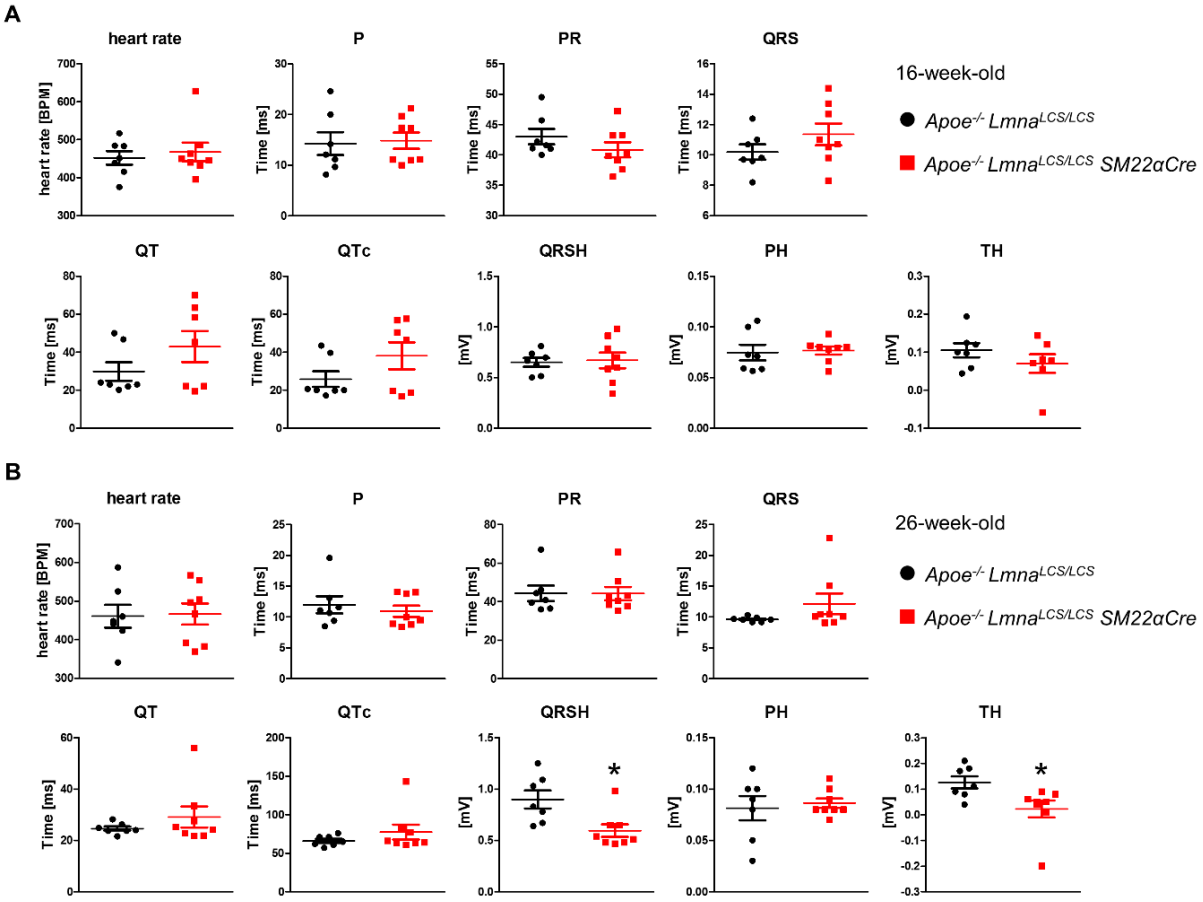

Figure XI. *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* mice show normal cardiac electrical function at 16 weeks of age and lower voltage of QRS complex and T wave at 26 weeks of age. Electrocardiography (ECG) was performed in isoflurane-anesthetized 16- and 26-week-old mice fed normal chow. **(A)** ECG parameters for 16-week-old *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* (n=7) and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ (n=7) mice. **(B)** ECG parameters for 26-week-old *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* (n=8) and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ (n=7) mice. QRSH, PH, and TH indicate amplitudes of QRS complex, P, and T waves, respectively. Data are presented as mean ± SEM. Statistical differences were analyzed by two-tailed *t*-test. **P*<0.05.

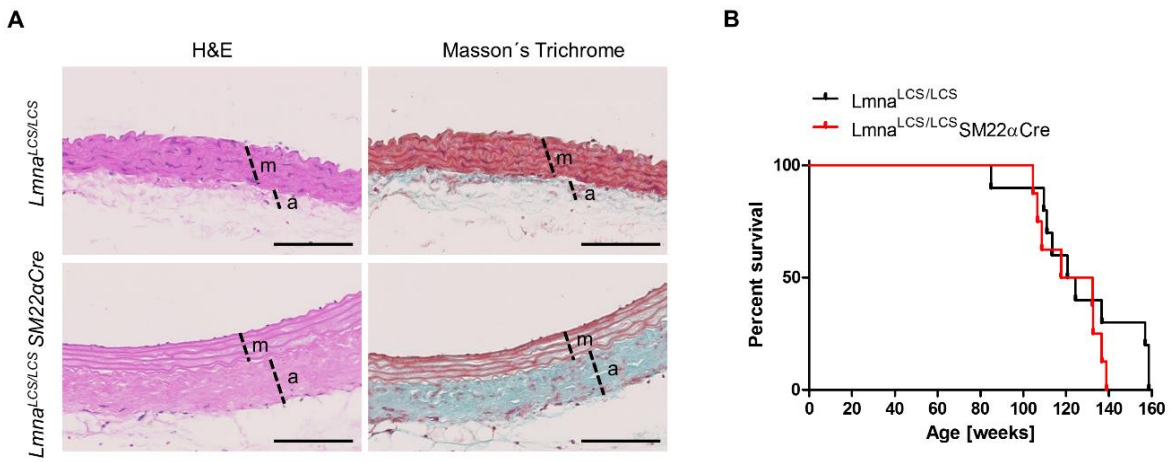

Figure XII. *Lmna*$^{LCS/LCS}$*SM22αCre* mice with an intact *Apoe* gene show aortic vascular smooth muscle cell loss and adventitial thickening but do not develop atherosclerosis and have a normal lifespan. *Lmna*$^{LCS/LCS}$ mice were used as controls. **(A)** Representative histological sections of aortic arches stained with hematoxylin & eosin (H&E) and Masson's Trichrome. Mice were fed normal chow and sacrificed at 38 weeks of age. Scale bar: 100 µm. m: media, a: adventitia. **(B)** Kaplan-Meier survival curves for *Lmna*$^{LCS/LCS}$*SM22αCre* mice (n=8) and *Lmna*$^{LCS/LCS}$ mice (n=10). A long-rank test disclosed no statistically significant between-genotype differences.

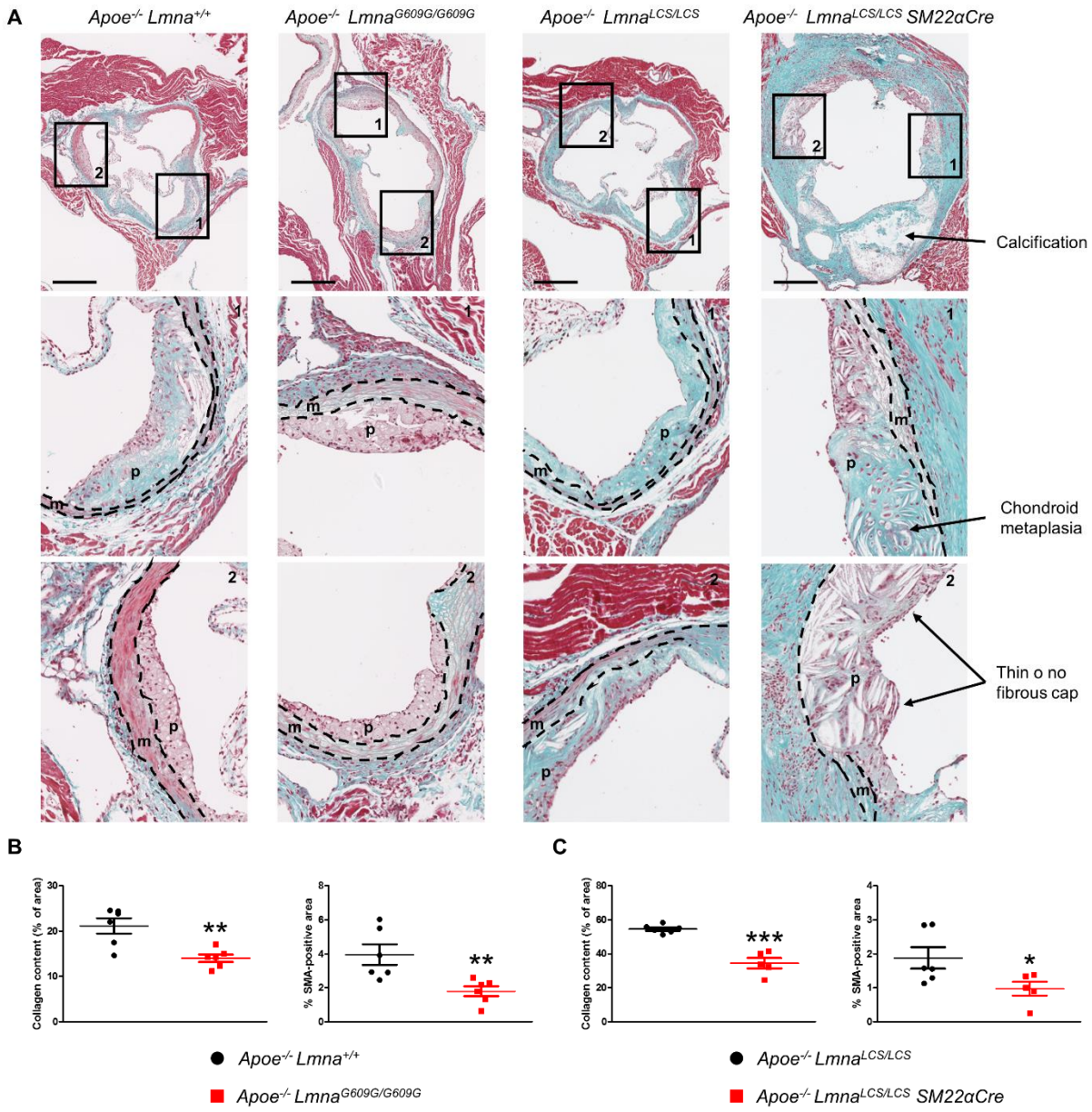

Figure XIII. More severe atherosclerosis in the aortic root in *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* mice than *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice at ages close to their maximum survival. Mice fed normal chow were sacrificed at either 21-23 weeks of age (*Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ and control *Apoe*$^{-/-}$*Lmna*$^{+/+}$ mice) or 51 weeks of age (*Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* and control *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ mice). **(A)** Representative aortic root sections from *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$, *Apoe*$^{-/-}$*Lmna*$^{+/+}$, *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ mice stained with Masson's Trichrome. *Middle* and *bottom* panels show higher magnification of atherosclerotic plaques. Scale bar: 400 μm. m: media, p: plaque. **(B, C)** Quantification of collagen and smooth muscle cell (smooth muscle actin, SMA) content in the plaques of mice of the indicated genotypes; n=5-6. Data are presented as mean ± SEM. Statistical differences were analyzed by two-tailed *t*-test. **P*<0.05, ***P*<0.01, ****P*<0.001.

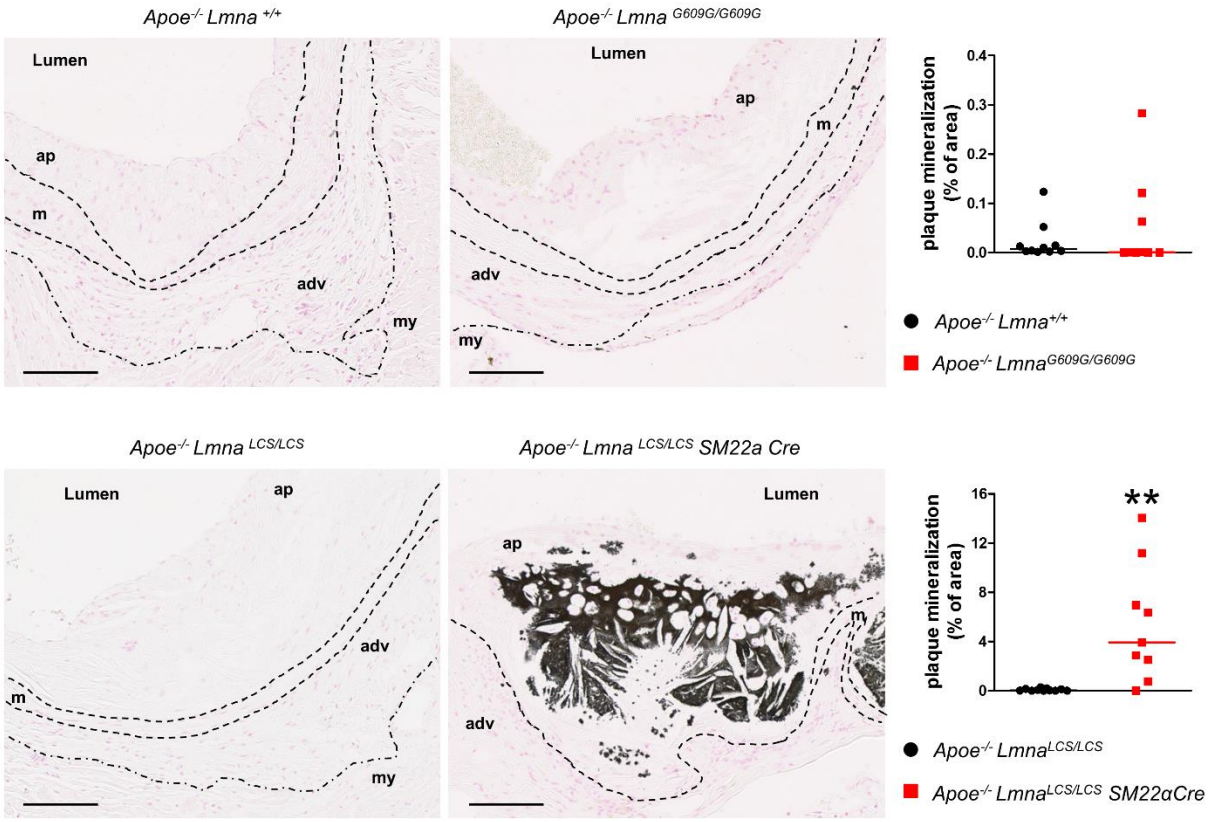

Figure XIV. Severe plaque calcification in *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* mice close to their maximum lifespan. Mice fed normal chow were sacrificed at 21-23 weeks of age (*Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ and control *Apoe*$^{-/-}$*Lmna*$^{+/+}$ mice) or at 51 weeks of age (*Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* and control *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ mice). Images show representative aortic root sections of the indicated genotypes stained with von Kossa to visualize tissue mineralization (black staining). Scale bar: 100 µm. Graphs show quantification of plaque mineralization as % of the black-stained area; n=9-11. Data are presented as median. Statistical differences were analyzed by two-tailed Mann-Whitney test. ***P*<0.01. m: media, ap: atherosclerotic plaque, adv: adventitia, my: myocardium.

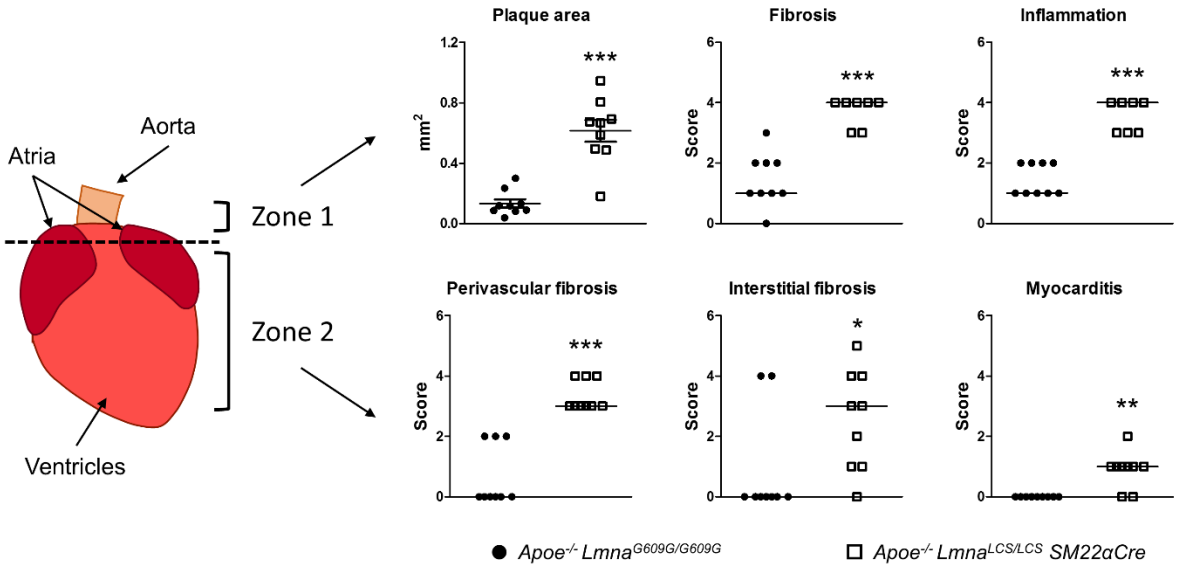

Figure XV. *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* mice have more severe aortic root pathologies than *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice at ages close to their maximum lifespan. Mice fed normal chow were sacrificed at 21-23 weeks of age (*Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$) or at 51 weeks of age (*Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre*). Atherosclerotic plaque area was quantified in the aortic root (at 3 levels of the aortic valve). Cardiac pathologies were analyzed in the upper part of the heart near the aortic valves (Zone 1) and in at least 6 levels within the ventricles (Zone 2). A score (from 0 to 5) was assigned to each animal for each of 5 pathological parameters. Graphs show plaque area and scores for fibrosis and inflammation in Zone 1 (*top*) and for perivascular fibrosis, interstitial fibrosis, and myocarditis in Zone 2 (*bottom*); n=9. Data are presented as mean ± SEM for plaque area and as median for other parameters. Statistical differences were analyzed by two-tailed *t*-test for plaque area and by two-tailed Mann-Whitney test for other parameters. **P*<0.05, ***P*<0.01, ****P*<0.001.

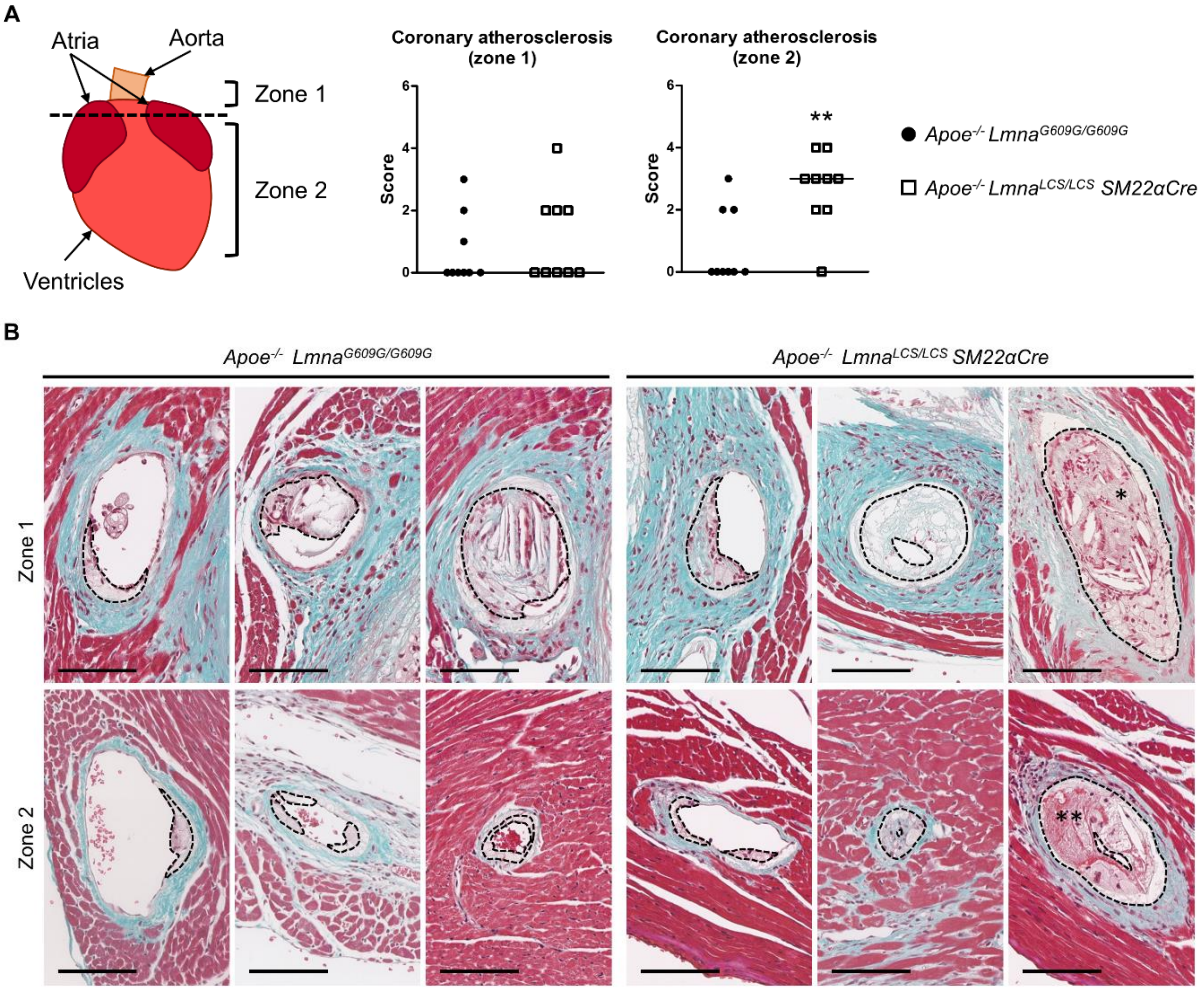

Figure XVI. Coronary atherosclerosis in *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* mice at ages close to their maximum lifespan. Mice fed normal chow were sacrificed at 21-23 weeks of age (*Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$) or at 51 weeks of age (*Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre*). **(A)** Coronary atherosclerosis was analyzed in the upper part of the heart near the aortic valve (Zone 1) and in at least 6 levels within ventricles (Zone 2). A coronary atherosclerosis score (from 0 to 5) was assigned to each animal. Graphs show the coronary atherosclerosis scores in both zones; n=9. Data are presented as median. Statistical differences were analyzed by two-tailed Mann-Whitney test. ***P*<0.01. **(B)** Masson's Trichrome-stained sections showing examples of coronary atherosclerosis in each zone. Dashed lines indicate atherosclerotic lesions, ranging from very early (intimal hyperplasia and intimal xanthoma) to advanced (*: healed thrombus; **: intraplaque hemorrhage). Scale bar: 100 µm.

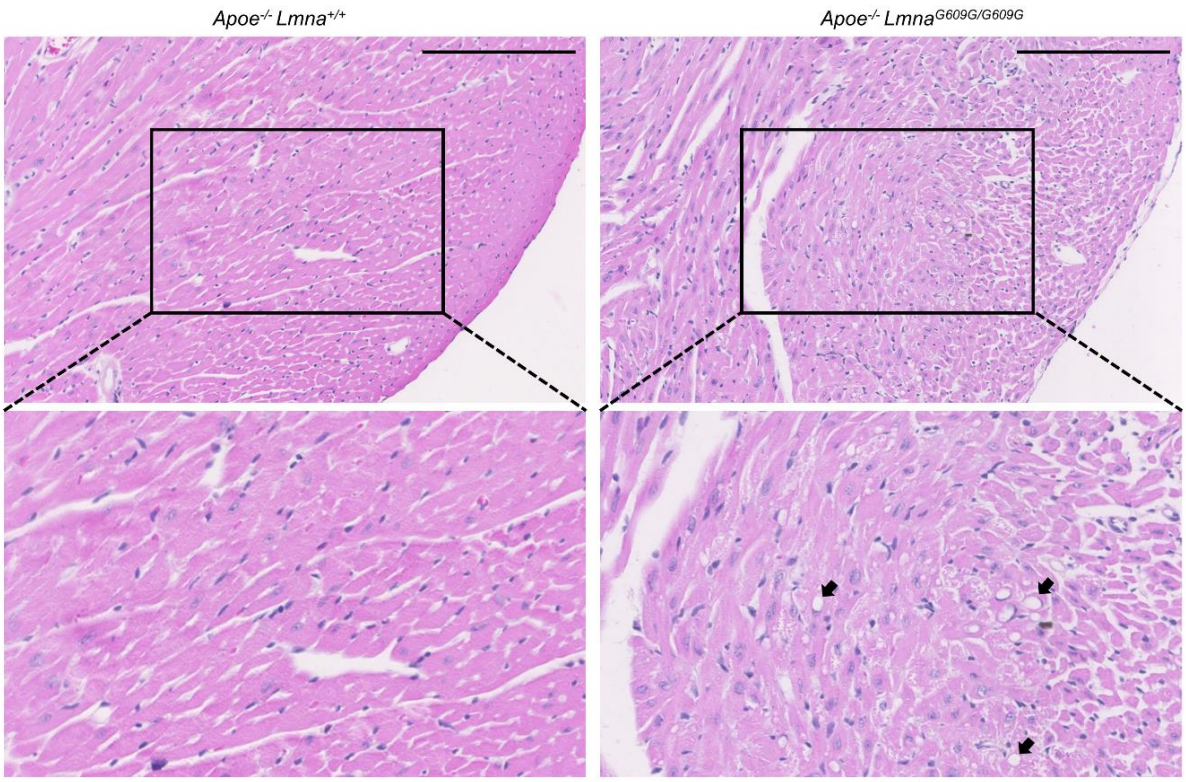

Figure XVII. *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ hearts exhibit vacuolization of cardiomyocytes. Mice fed normal chow were sacrificed at 21-23 weeks of age (close to *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice maximum survival). Photographs show hematoxylin & eosin-stained cardiac tissue from *Apoe*$^{-/-}$*Lmna*$^{+/+}$ mice (*left*) and *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice (*right*). Scale bar: 200 µm. Black arrows indicate examples of cardiomyocyte vacuolization.

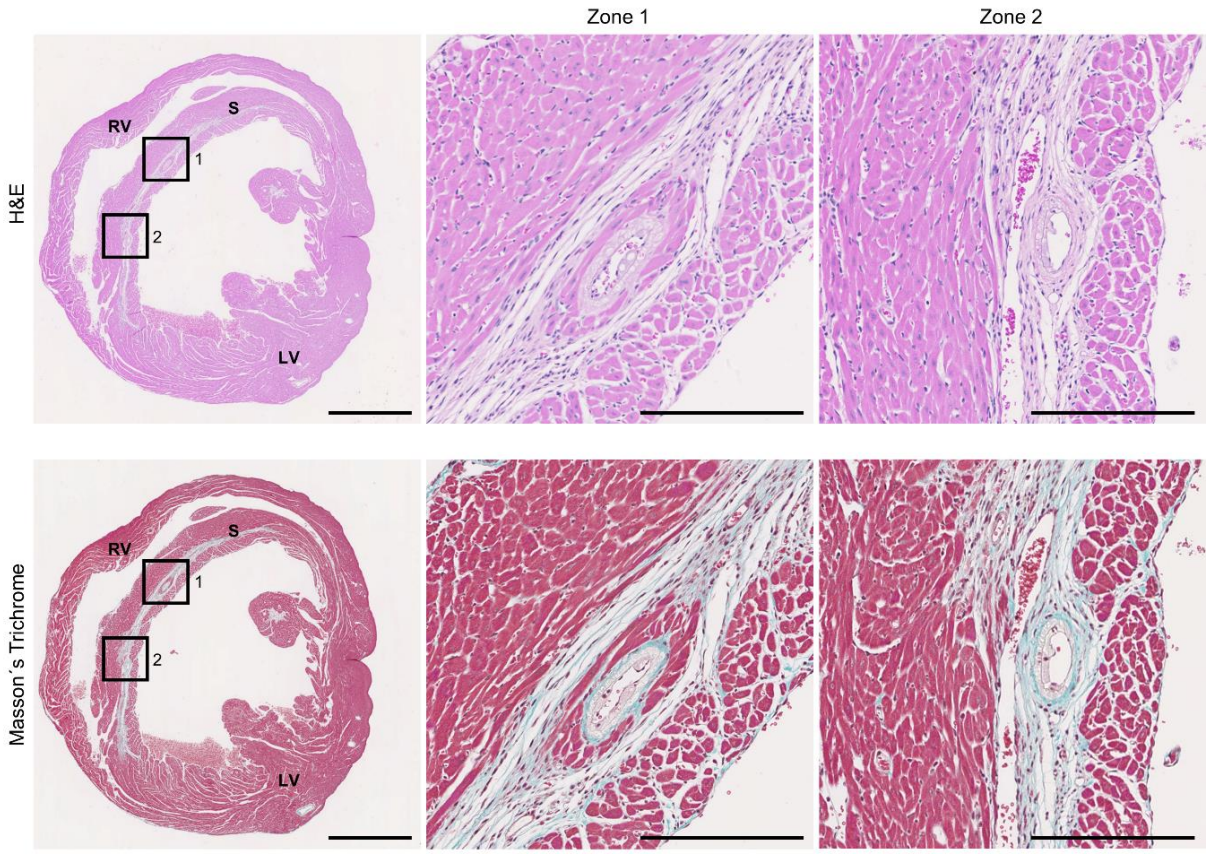

Figure XVIII. Evidence of coronary atherosclerosis and myocardial infarction in *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice. Mice fed normal chow were sacrificed at 21-23 weeks of age (close to their maximum survival). Photographs show consecutive sections of an *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ heart stained with hematoxylin & eosin (H&E) and Masson´s Trichrome, showing coronary atherosclerosis and infarct in the septum. Scale bar: 1 mm (whole cross section) and 200 µm (magnified view in insert). RV: right ventricle, S: septum, LV: left ventricle.

LEGENDS OF SUPPLEMENTAL MOVIES

Movies I-XII show 3D reconstruction of the aortic wall from mice of the indicated genotypes injected with Atto565-low-density lipoprotein (LDL), green channel – autofluorescence (elastin), red channel – Atto565 fluorescence (LDL).

Movies XIII-XVI show 3D reconstruction of the aortic wall from mice of the indicated genotypes not injected with Atto565-LDL, green channel – autofluorescence (elastin), red channel – autofluorescence.

Movie I. Wild type lamin AC 1: *Apoe*$^{-/-}$*Lmna*$^{+/+}$ - zone without lesion

Movie II. Wild type lamin AC 2: *Apoe*$^{-/-}$*Lmna*$^{+/+}$ - zone with intimal lesion

Movie III. Wild type lamin AC 3: *Apoe*$^{-/-}$*Lmna*$^{+/+}$ - zone with intimal lesion in the bifurcation

Movie IV. Ubiquitous progerin 1: *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ - zone without lesion

Movie V. Ubiquitous progerin 2: *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ - zone with intimal lesion

Movie VI. Ubiquitous progerin 3: *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ - zone with intimal lesion in the bifurcation

Movie VII. Lamin C only 1: *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ - zone without lesion

Movie VIII. Lamin C only 2: *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ - zone with intimal lesion

Movie IX. Lamin C only 3: *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ - zone with intimal lesion in the bifurcation

Movie X. VSMC specific progerin 1: *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* - zone without lesion

Movie XI. VSMC specific progerin 2: *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* - zone with intimal lesion

Movie XII. VSMC specific progerin 3: *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* - zone with intimal lesion in the bifurcation

Movie XIII. Wild type lamin AC without LDL: *Apoe*$^{-/-}$*Lmna*$^{+/+}$ not injected with Atto565-LDL

Movie XIV. Ubiquitous progerin without LDL: *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ not injected with Atto565-LDL

Movie XV. Lamin C only without LDL: *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ not injected with Atto565-LDL

Movie XVI. VSMC specific progerin without LDL: *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22αCre* not injected with Atto565-LDL