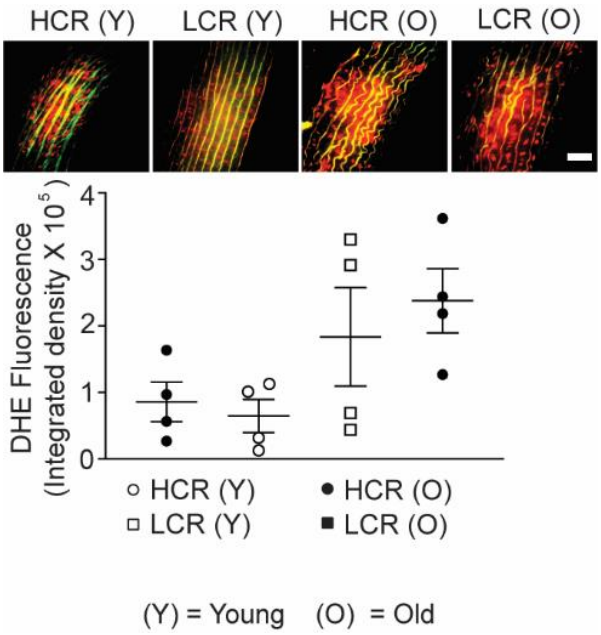
Supplementary Material

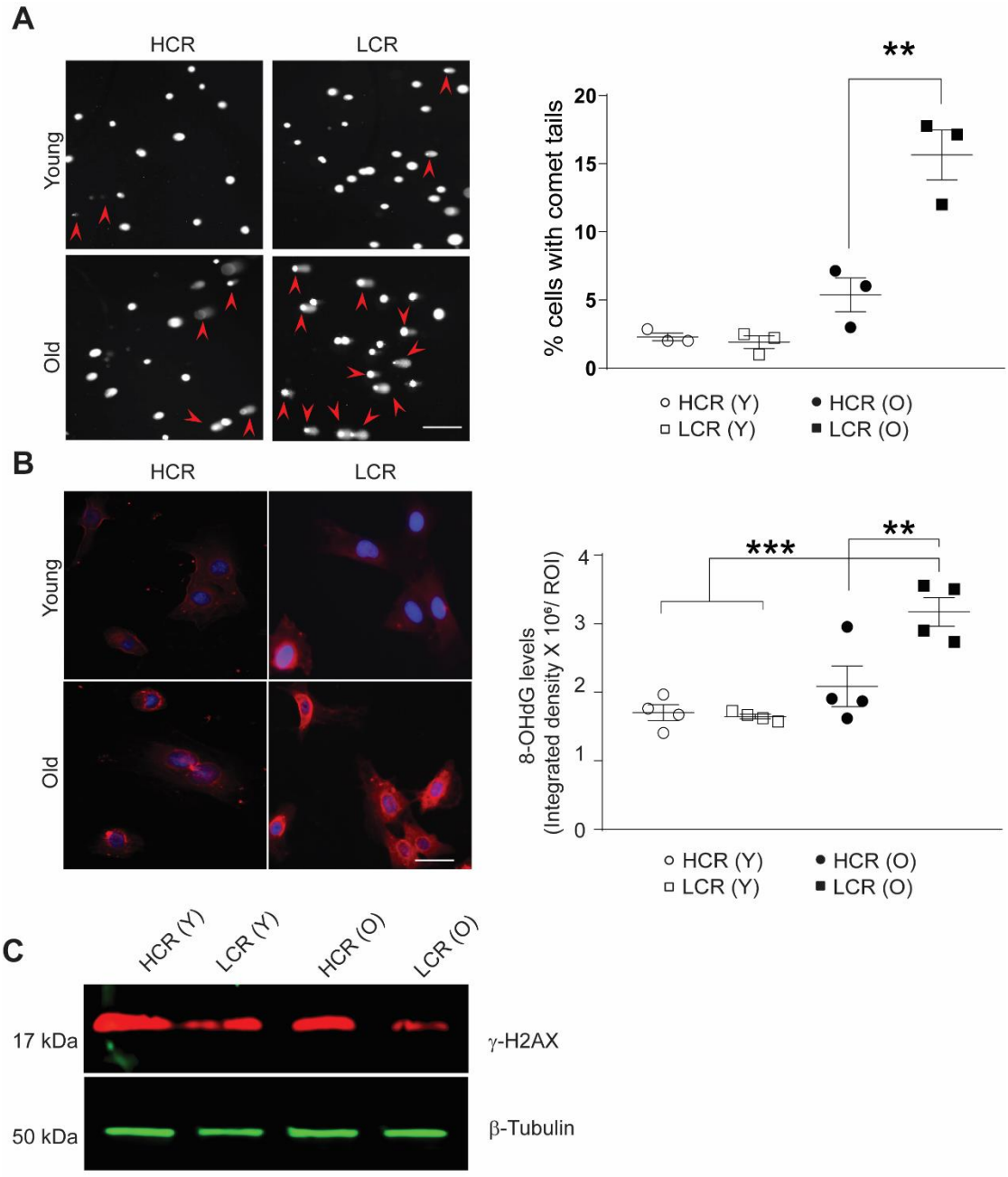
Mitochondrial DAMPs-dependent inflammasome activation during aging induces vascular smooth muscle cell dysfunction and aortic stiffness in low aerobic capacity rats

Supplemental Table 1: Antibodies used in this study

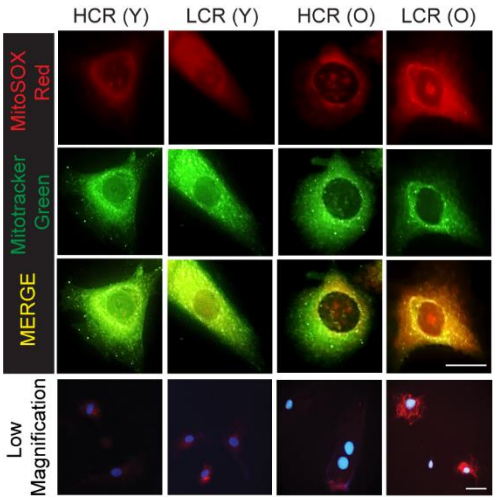
S.No.	Name	Company	Catalog number
1	eNOS (D9A5L) Rabbit mAb	Cell Signaling Technology	#32027
2	Phospho-eNOS (Ser1177) Antibody	Cell Signaling Technology	#9571S
3	AIM2 (3C4G11)	Santa Cruz Biotechnology Inc.	sc-293174
4	NLRP3 Polyclonal Antibody	Bioss Antibodies	bs-10021R
5	Caspase-1 Antibody (14F468)	Santa Cruz Biotechnology Inc.	sc-56036
6	IL-18(H-173)	Santa Cruz Biotechnology Inc.	sc-7954
7	IL-18/IL-1F4 Antibody	R & D Systems	AF521-SP
8	LC3B Antibody	Cell Signaling Technology	#12741S
9	BNIP3L/Nix (D4R4B) Rabbit mAb	Cell Signaling Technology	#12396
10	PINK1 antibody [N4/15]	ABcam	ab186303
11	TOM20	Proteintech	11802-1-AP
12	VCAM1	ThermoFisher Scientific	11802-1-AP
13	LAMP-1 (H4A3)	Santa Cruz Biotechnology Inc.	sc-20011
14	β-Tubulin antibody	Sigma-Aldrich	T8328
15	GAPDH (14C10)	Cell Signaling Technology	#2118
16	Actin, α-Smooth Muscle	Sigma-Aldrich	A2547
17	P62	Novus Biologicals	MAB8028
18	P202 (53BP1)	Novus Biologicals	NB100-304
19	TFAM	Cell Signaling Technology	#7495
20	Phospho-Histone H2A.X (Ser139)	Cell Signaling Technology	#9718S
21	TGF-β	Cell Signaling Technology	#3711
22	Alexa Fluor 488 (goat anti rat)	Invitrogen	A11006
23	Alexa Fluor 488 (goat anti rabbit)	Invitrogen	A11070
24	Alexa Fluor 594 (goat anti mouse)	Invitrogen	11020



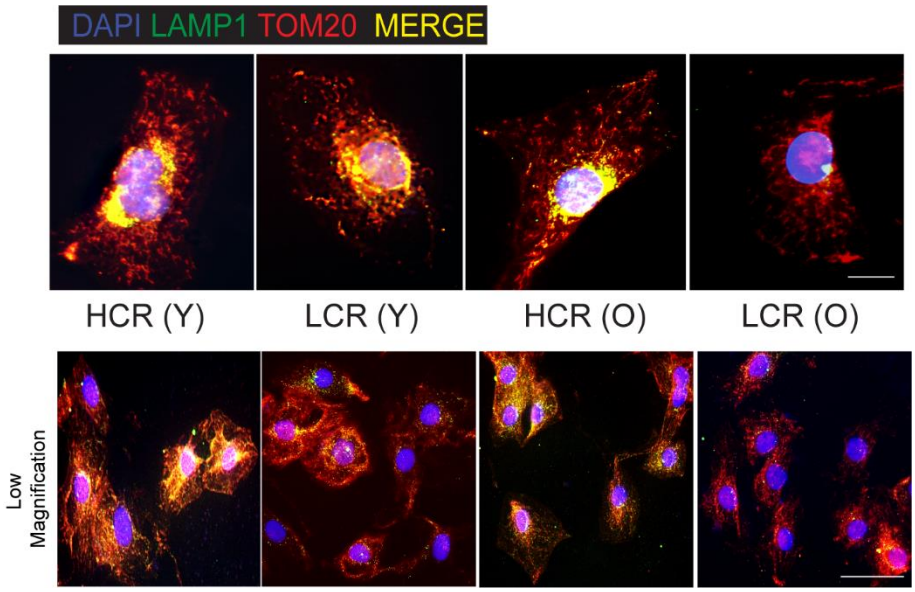
Supplemental Figure 1: Representative images and quantification of DHE stained aortic sections from young and old age HCR and LCR rats (scale =100 μm). Data are presented as average integrated density/ROI ± SEM where *n* = 4.



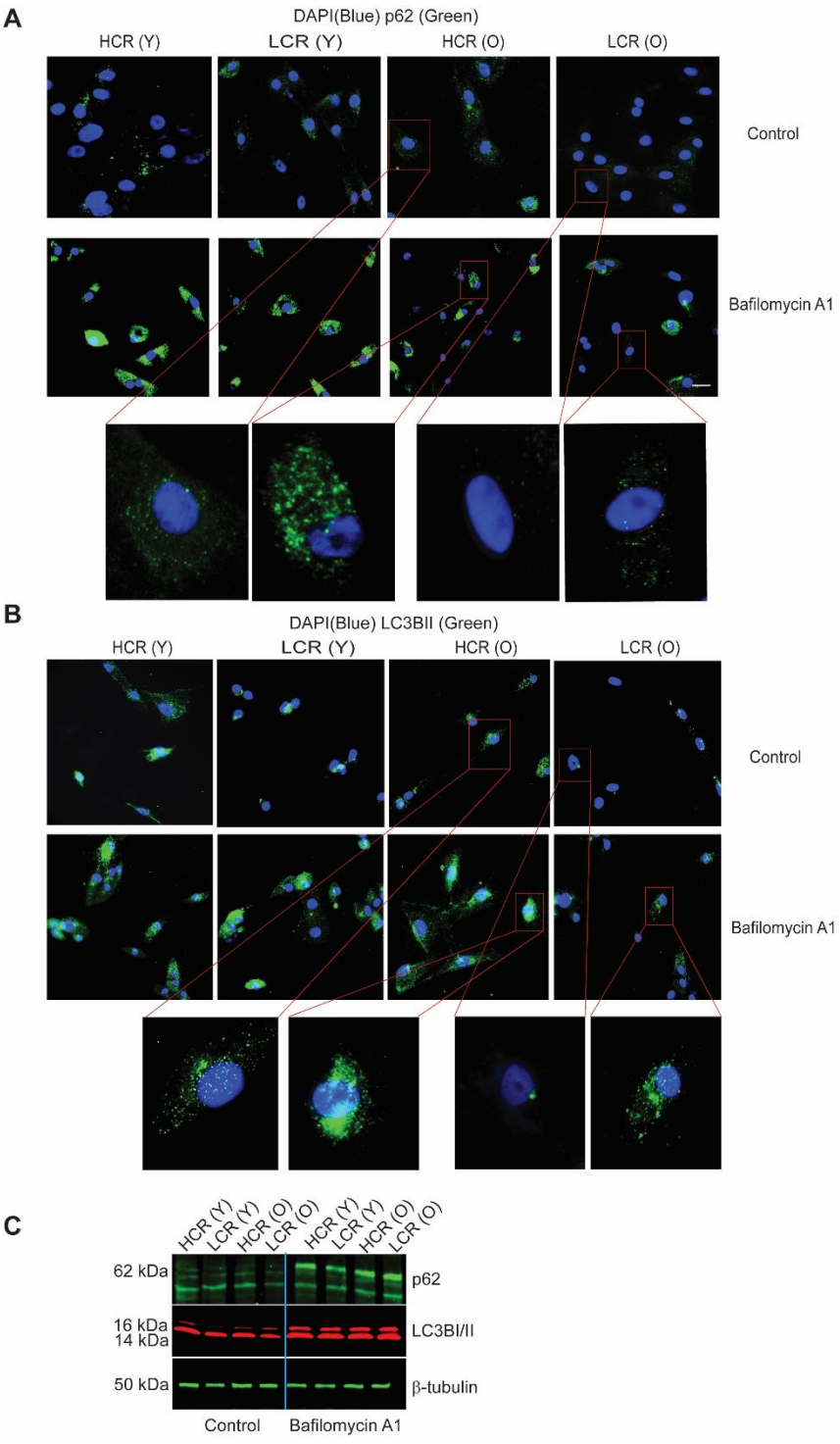
Supplemental Figure 2: (A) Comet assay analysis of VSMC from young and aged HCR and LCR rats (left panel, scale = 100 μm). Data are mean +/- SEM of percent cells with comet tails (right panel, *n* = 4). (B) Representative fluorescence images of VSMC stained with 8-OHdG antibody (left panel, scale = 50 μm). Data are mean +/- SEM of integrated density of red fluorescence (Red channel). (C) Representative image of the Western analysis for γ-H2AX levels.



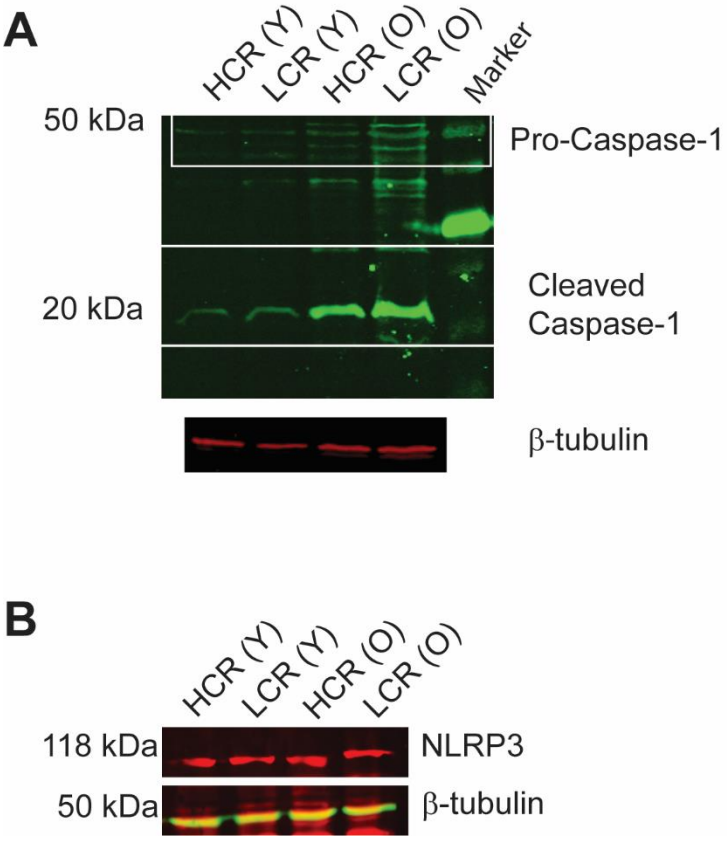
Supplementary Figure 3. Magnified representative fluorescence images of VSMC stained with MitoSOX Red (red channel) and MitoTracker Green (MTG) (green channel). The merge of red and green yields yellow color representing mitochondrial ROS levels from young and old age HCR and LCR rats. Scale represents 10 μm. Lower panel (row 4) represents low magnification images of the specified VSMC stained with MitoSOX Red (red channel) and DAPI (blue channel) (scale = 50 μm).



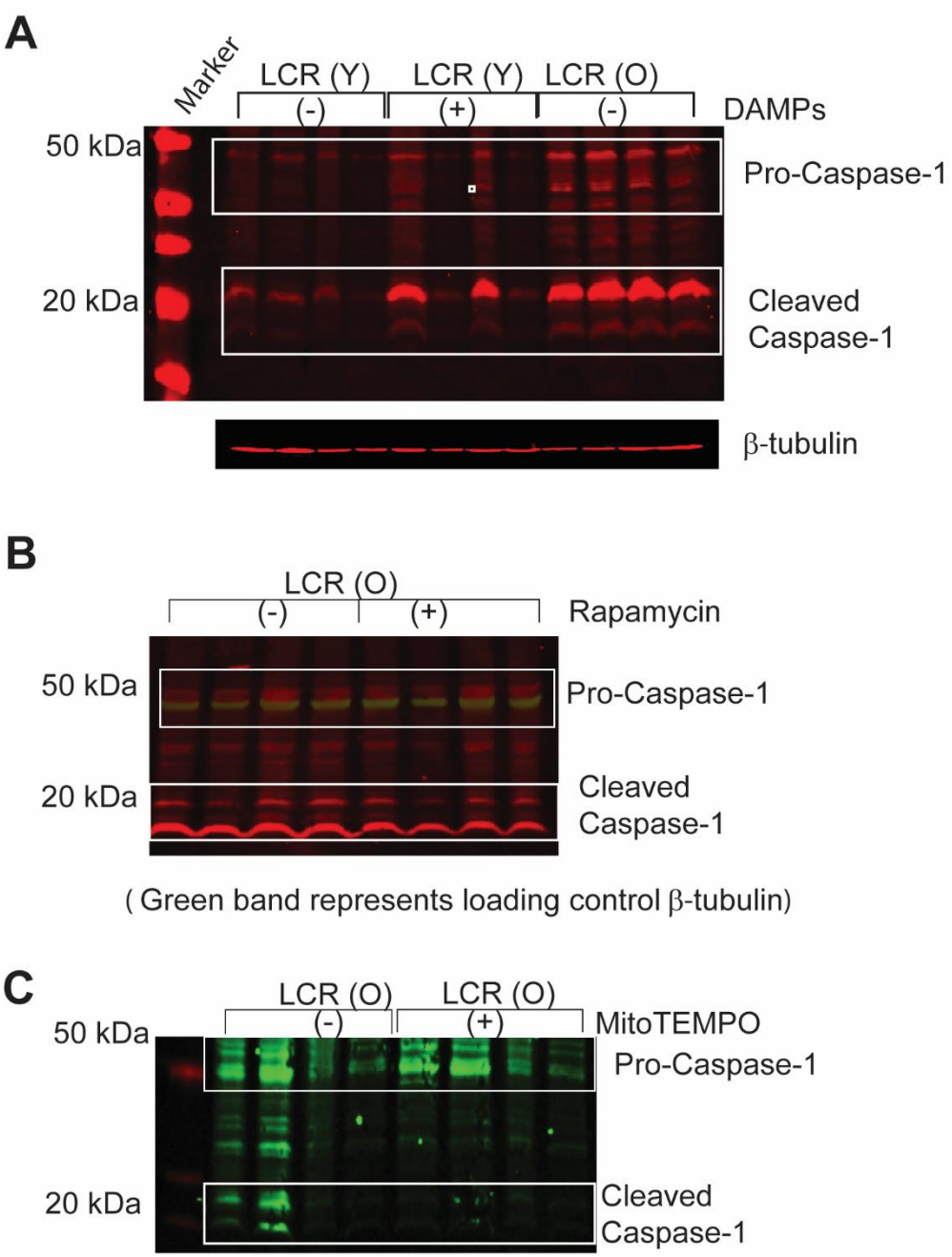
Supplemental Figure 4: High magnification (top panel) and low magnification (bottom panel) representative fluorescence images of VSMC stained with DAPI (blue channel), LAMP1 (green channel), TOM20 (red channel). The merge of red and green yields yellow color representing mitophagy levels from young and old age HCR and LCR rats. Scale represents 10 μm and 50 μm for top and bottom panels, respectively.



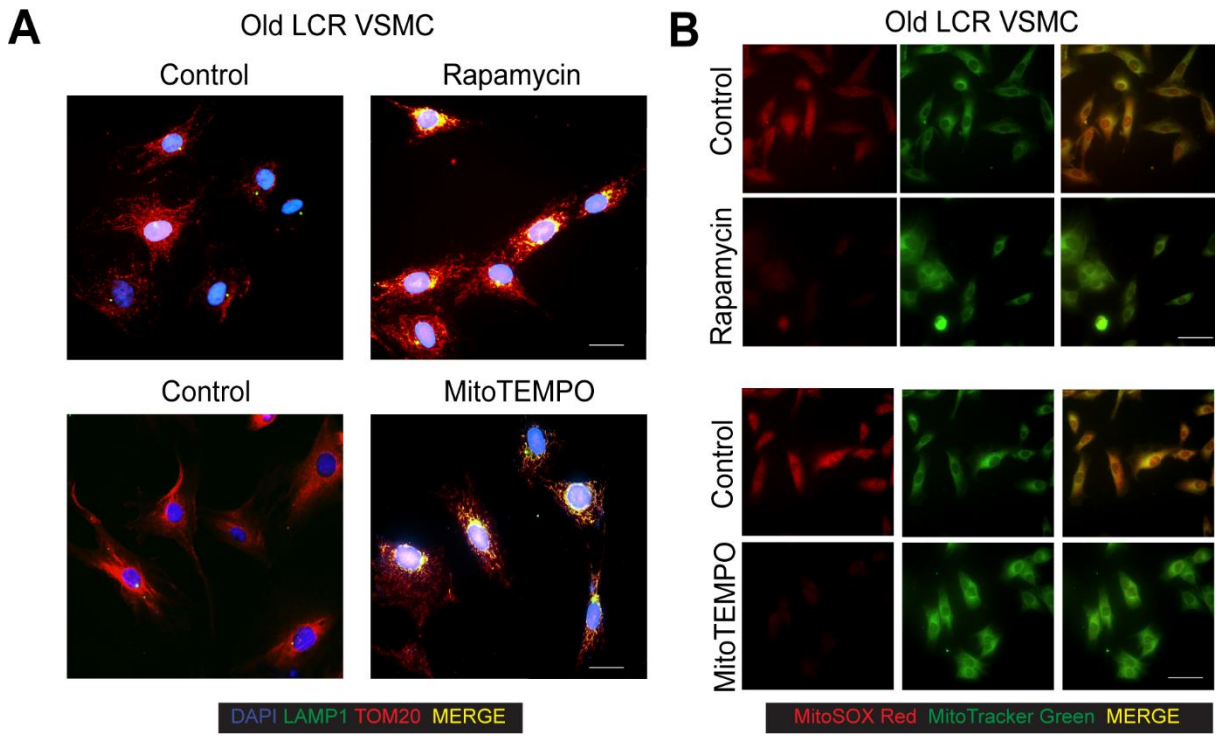
Supplemental Figure 5: Effect of Bafilomycin A1 on autophagosome accumulation in rat VSMC. (A) Representative images of autophagosomal marker p62 (green channel) (B) LC3BII (green channel) with and without autophagy inhibitor, Bafilomycin A1 (n = 4, scale = 50 μm). (C) Western analysis of autophagosomal markers p62 and LC3BI/II from young and aged VSMC. There was no difference in the house keeping marker β-tubulin between the samples.



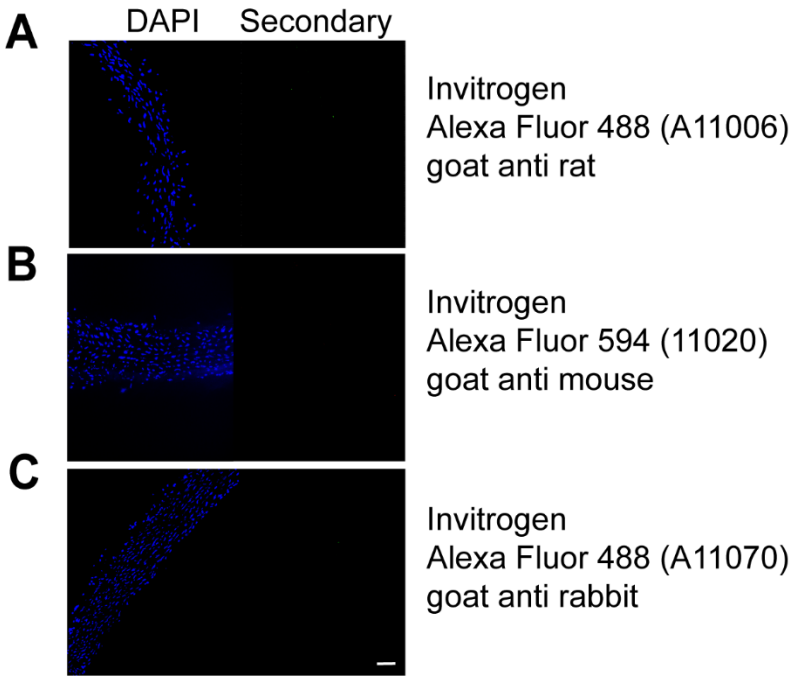
Supplemental Figure 6: (A) Full length western blots for cleaved caspase 1 (represented in main figures 5) showing pro- and cleaved- caspase1 bands. (B) Western analysis of NLRP3 in young and aged LCR and HCR rat VSMC.



Supplemental Figure 7: Full length western blots for cleaved caspase 1 (represented in main figures 6) showing pro- and cleaved- caspase1 bands.



Supplemental Figure 8: (A) Representative low magnification fluorescence images of VSMC stained with DAPI (blue channel), LAMP1 (green channel), TOM20 (red channel). The merge of red and green yields yellow color representing mitophagy levels from old age LCR rats with and without rapamycin (top panels) or MitoTEMPO (bottom panels). Scale represents 50 μm. (B) Representative Low magnification fluorescence images of VSMC stained with MitoSOX Red (red channel) and MitoTracker Green (MTG) (green channel). The merge of red and green yields yellow color representing mitochondrial ROS levels from old age LCR rats with and without rapamycin (top panels) or MitoTEMPO (bottom panels). Scale represents 100 μm.



Supplemental Figure 9: Representative images for secondary antibodies used in this study. DAPI (blue channel) and specific secondaries in green channel (A and C) and red channel (B) are shown. Scale is 50 μm.