SUPPLEMENTARY DATA

SIRT2 Delays Vulnerable Plaque Progression by Modulating Vascular Smooth Muscle Cell Senescence

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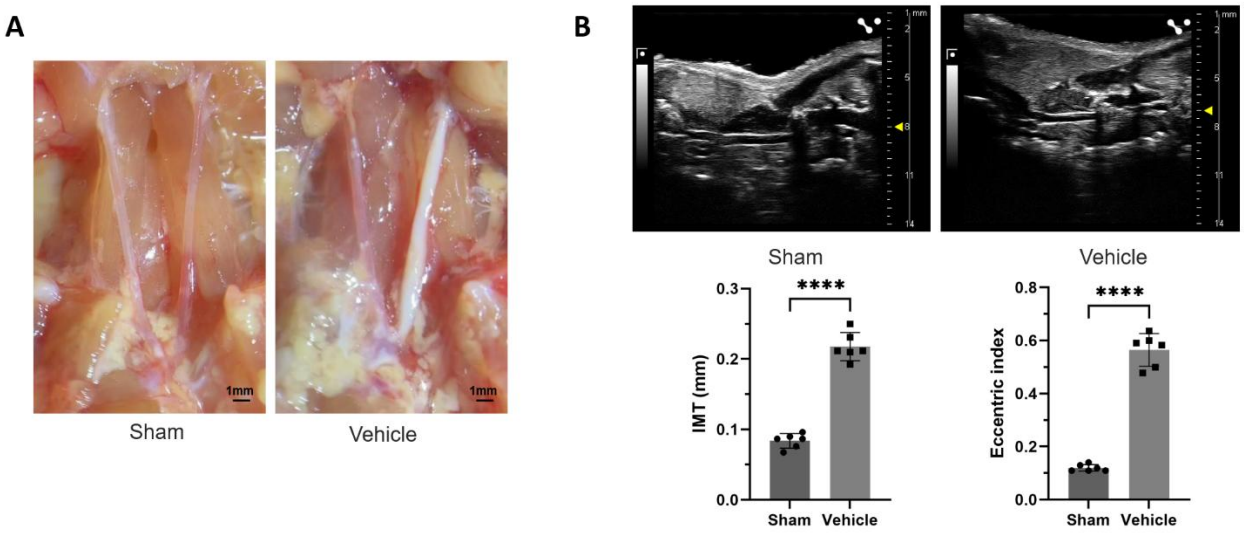
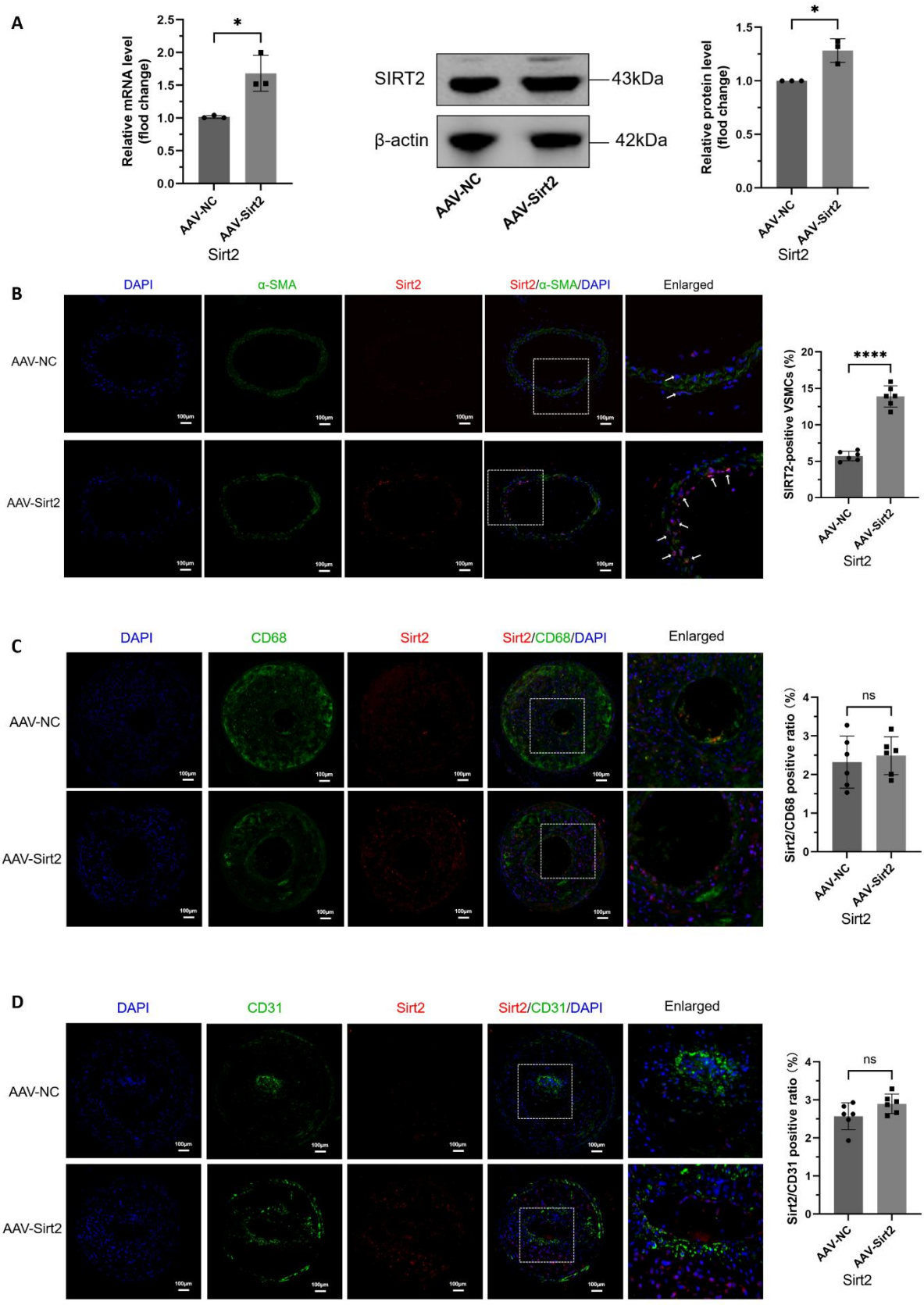
SUPPLEMENTARY DATA



Supplementary Figure 1 Mouse left carotid vulnerable plaque model. A: Representative microscopic images of the left common carotid artery harvested from 16-week-old mice in the sham group and vehicle group. Scale bar = 1 mm. B: Micro-ultrasound longitudinal views of the left common carotid artery in 16-week-old ApoE$^{-/-}$ mice, with quantification of intima-media thickness (IMT) and eccentricity index (EI). n=6 per group. All data were analyzed by unpaired two-tailed t-test. Ns: not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

SUPPLEMENTARY DATA



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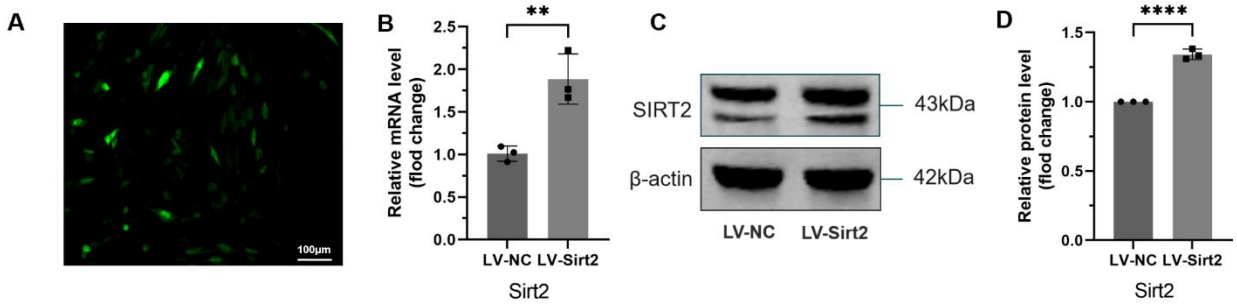
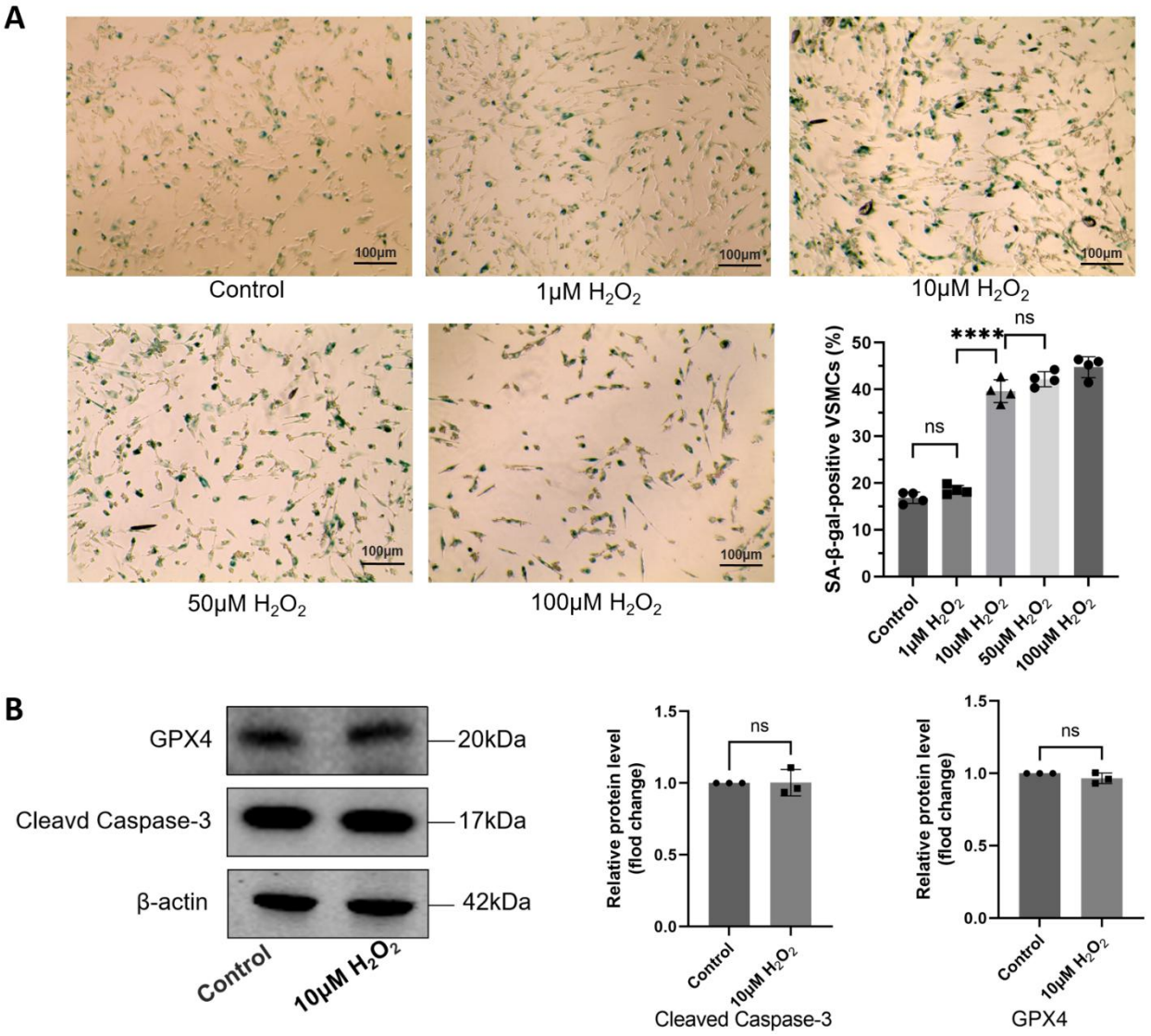
Supplementary Figure 2. Validation of adeno-associated virus transduction efficiency. A: Sirt2 mRNA and protein expression levels in mouse aortas after AAV9-NC or AAV9-Sirt2 infection for 4 weeks (n=3 per group). Data are normalized to the AAV-NC group. B: Immunofluorescence images showing Sirt2 (red) co-localization with α-SMA (green) in VSMCs, with quantification of Sirt2 expression in VSMCs. Arrows indicate Sirt2-positive VSMCs. After tissue collection from 16-week-old mice, Sirt2 expression in macrophages and endothelial cells was further examined: C: Immunofluorescence images showing Sirt2 (red) co-localization with CD68 (green) in macrophages, with quantification. D: Immunofluorescence images showing Sirt2 (red) co-localization with CD31 (green) in endothelial cells, with quantification. All data were analyzed by unpaired two-tailed t-test. Note: Scale bar = 100 μm; Each group n=6; ns: no statistical difference; **P*< 0.05; ***P*< 0.01; ****P*< 0.001; *****P*< 0.0001.



Supplementary Figure 3. Validation of lentiviral transfection efficiency. A: Fluorescence image 72 hours after infection with pLVX-mSirt2-3xFLAG-ZsGreenPuro. After puromycin selection, mRNA and protein were extracted from VSMCs for validation. B: Sirt2 mRNA expression levels in VSMCs infected with empty lentivirus (LV-NC) or LV-Sirt2 (n=3 per group). **C, D:** Western blot analysis of Sirt2 protein expression normalized to β-actin (n=3 per group). Data are normalized to the LV-NC group (set to 1). All data were analyzed by unpaired two-tailed t-test. Note: ns: no statistical difference; **P*< 0.05; ***P*< 0.01; ****P*< 0.001; *****P*< 0.0001.

SUPPLEMENTARY DATA



Supplementary Figure 4. Establishment of H_2O_2-induced VSMC senescence model. A: Representative images and quantification of SA-β-gal senescence staining in primary VSMCs treated with different concentrations of H_2O_2 for 72 hours (n = 4 per group) (one-way ANOVA with Holm-Šídák's post hoc test). Scale bar=100μm. B: Western blot analysis and quantification of Cleaved Caspase-3 and GPX4 expression normalized to β-actin in VSMCs treated with 10 μM H_2O_2 for 72 hours (n = 3 per group) (unpaired two-tailed t-test). Data are normalized to the Control group (set to 1). Note: ns: no statistical difference; **P*< 0.05; ***P*< 0.01; ****P*< 0.001; *****P*< 0.0001.