Supplementary Materials

Supplemental Figure Legends

Figure S1. Plasma lipids levels in young and aged Apoe$^{-/-}$ compared with Apoe$^{-/-}$/Sod2$^{+/-}$ mice. A-B, Plasma levels of total cholesterol (A) and triglycerides (B). Data are mean ± SEM, n=12.

Figure S2. Transfection of VSMC from middle-age wild-type mice with *Capn2* siRNA:p5RHH nanocomplexes effectively reduces calpan-2 but not calpain-1 expression levels. Western blot analysis of calpain-2 and calpain-1 expression in VSMC transfected with scrambled siRNA or *Capn2* siRNA:p5RHH nanocomplexes.

Figure S3. Features of plaque VSMC transdifferentiation to macrophage-like cells in *Apoe*$^{-/-}$ and *Apoe*$^{-/-}$/*Sod2*$^{+/-}$ mice. (A) Representative fluorescent microscopy images of fresh frozen transverse aortic sections stained for immunoreacative CD68 (red), smooth muscle SM-MHC (green), and DAPI (blue) and quantification of proportion of CD68^{+} SM-MHC^{+} cells relative to total core CD68^{+} cells (mean ± SEM, n=6). (B) Representative fluorescent microscopy images of fresh frozen transverse aortic sections stained for immunoreacative Mac3 (green), smooth muscle α-actin (red), and DAPI (blue) and quantification of proportion of Mac3^{+} α-actin^{+} cells relative to total plaque Mac3^{+} cells (mean ± SEM, n=6). *P<0.05. Scale is 100 μm.

Figure S4. Systemic effects of MitoTEMPO treatment in middle-aged *Apoe*$^{-/-}$ and *Apoe*$^{-/-}$/*Sod2*$^{+/-}$ mice. A-E, Plasma levels of total cholesterol (A), triglycerides (B), blood urea nitrogen (BUN) (C), creatinine (D), and alanine aminotransferase (E). Data are mean ± SEM, n=8. F-G, Representative images of mouse kidney (F) and liver (G) sections stained with H&E. N=4. Scale is 100 μm.

Figure S5. MMP2 expression in CD68^{+} macrophages in atherosclerotic lesions of middle-aged mice treated with MitoTEMPO. Representative fluorescent microscopy images of fresh frozen

transverse aortic sections stained for immunoreacative MMP2 (green), CD68 (red) and DAPI (blue) and quantification of MMP2 expression in $CD68^+$ cells represented as fluorescence integrated density (IntDen) per number of cells (mean ± SEM, n=6). Scale is 100 μm. *P<0.05; **P<0.01.

Figure S1.

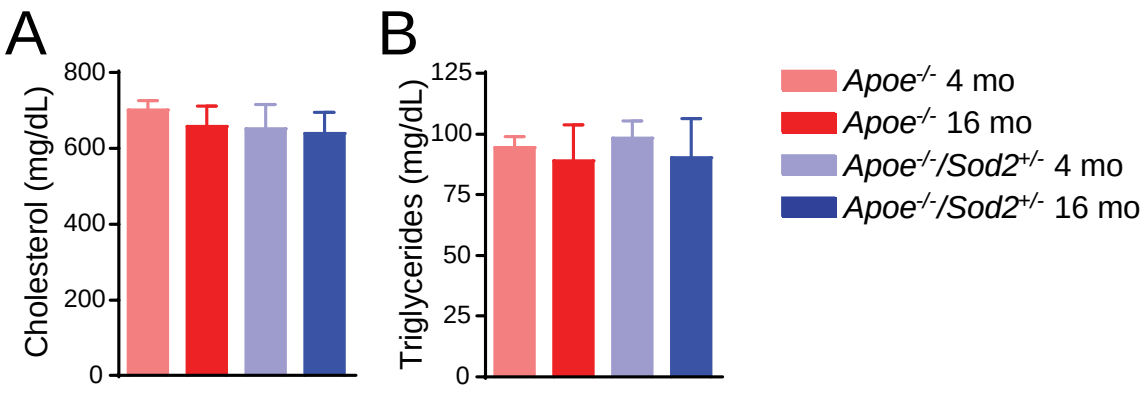

A
Cholesterol (mg/dL)
800
600
400
200
0
B
Triglycerides (mg/dL)
125
100
75
50
25
0
Apoe-/- 4 mo
Apoe-/- 16 mo
Apoe-/-/Sod2+/- 4 mo
Apoe-/-/Sod2+/- 16 mo

Figure S2.

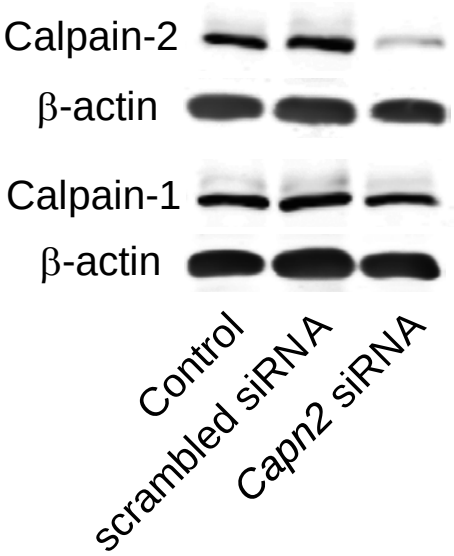

Calpain-2
β-actin
Calpain-1
β-actin
Control
scrambled siRNA
Capn2 siRNA

Figure S3.

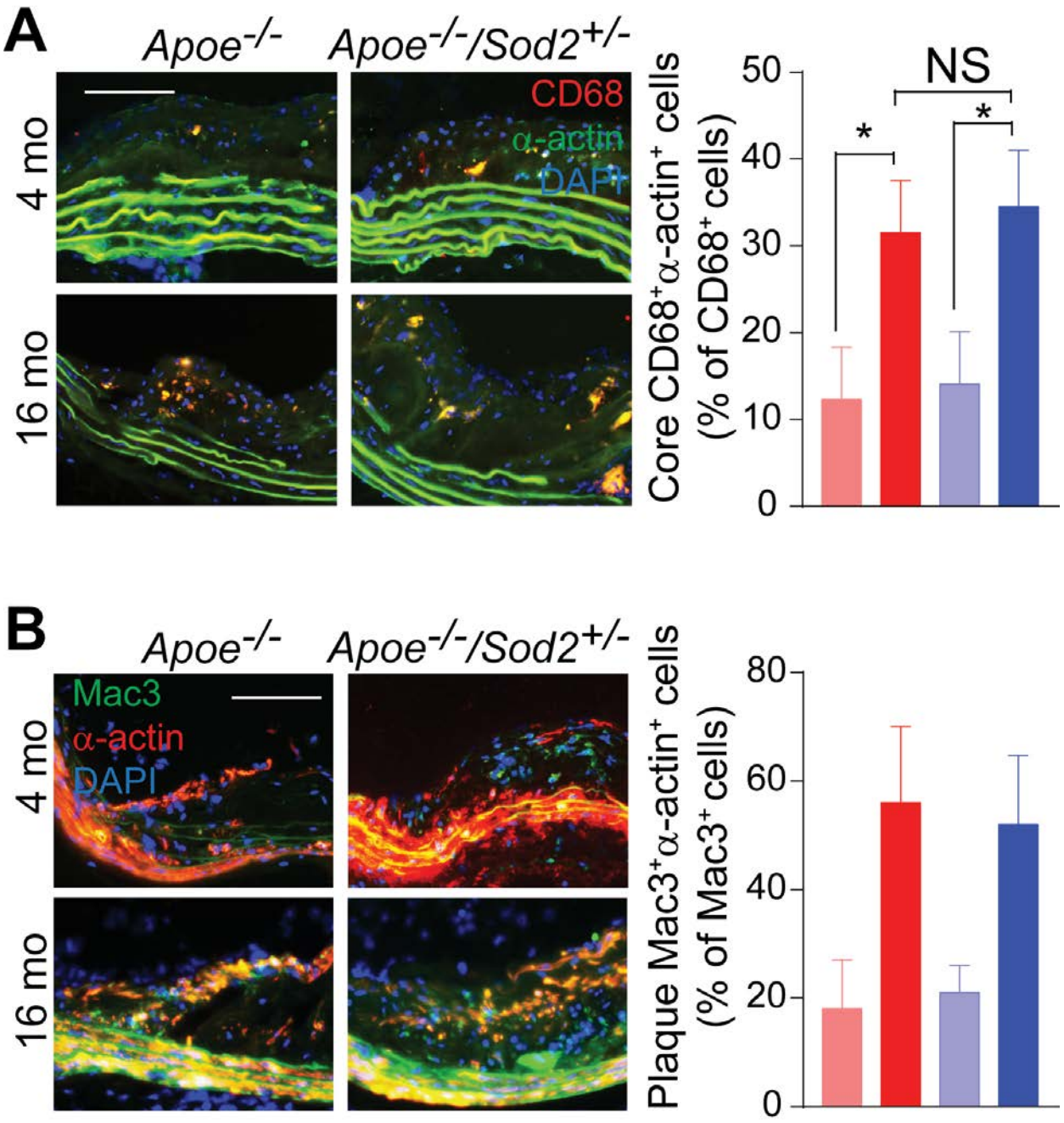

A
Apoe-/-
Apoe-/-/Sod2+/-
4 mo
16 mo
CD68
α-actin
DAPI
Core CD68+α-actin+ cells
(% of CD68+ cells)
NS
*
*
50
40
30
20
10
0
B
Apoe-/-
Apoe-/-/Sod2+/-
4 mo
16 mo
Mac3
α-actin
DAPI
Plaque Mac3+α-actin+ cells
(% of Mac3+ cells)
80
60
40
20
0

Figure S4.

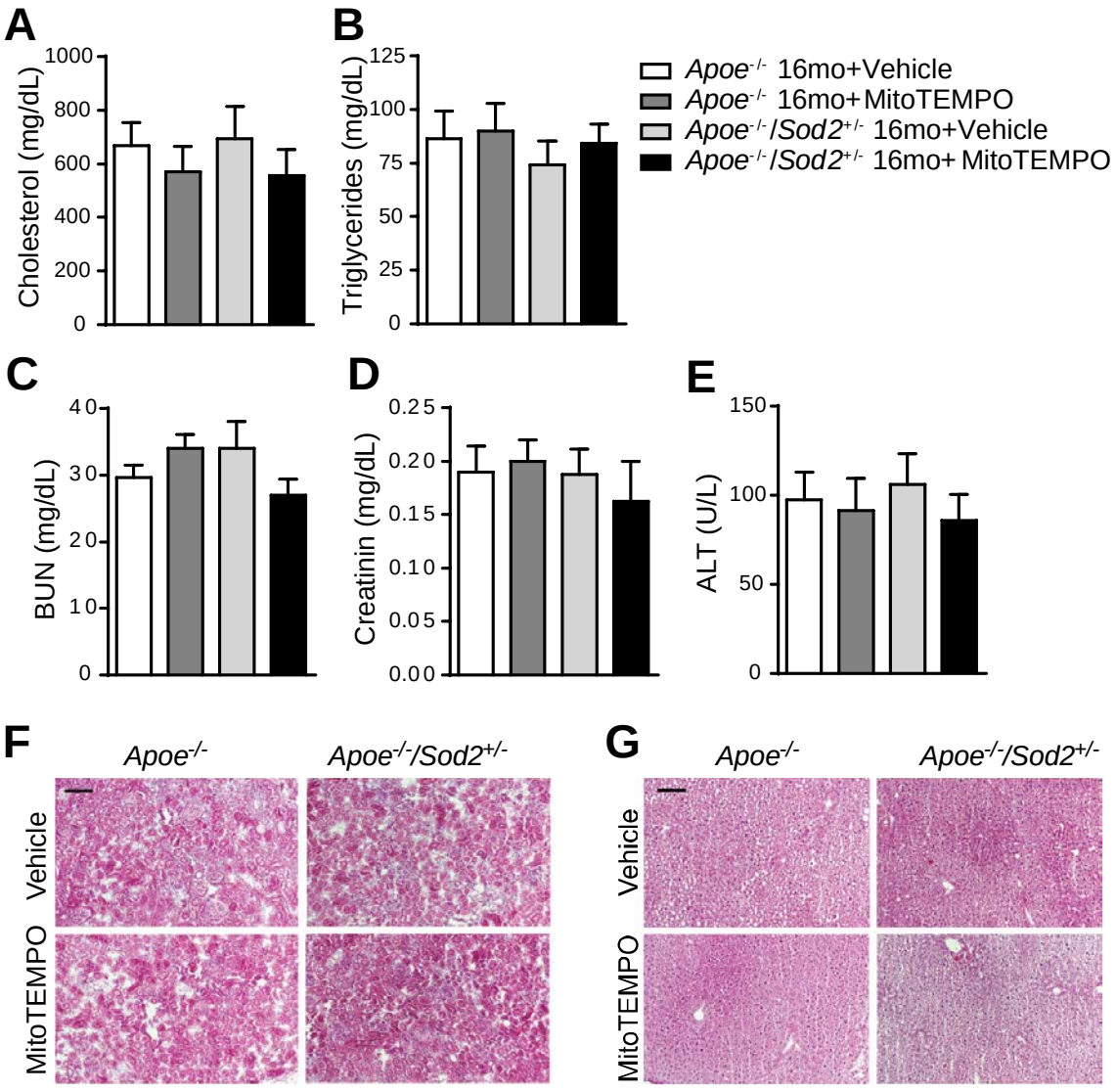
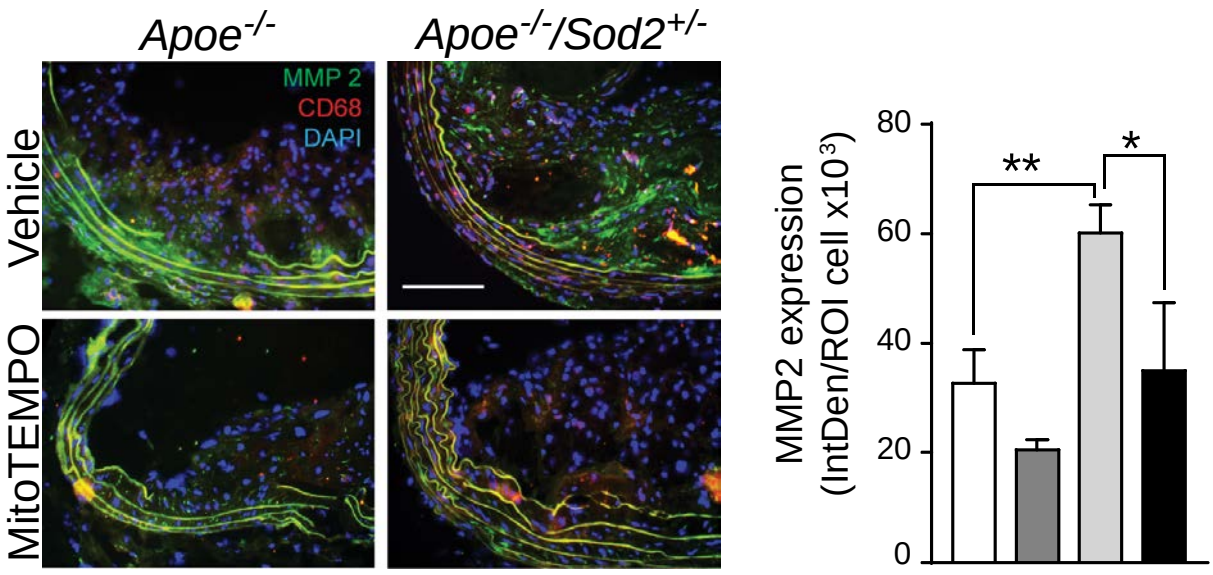

A
Cholesterol (mg/dL)
1000
800
600
400
200
0
B
Triglycerides (mg/dL)
125
100
75
50
25
0
Apoe-/- 16mo+Vehicle
Apoe-/- 16mo+MitoTEMPO
Apoe-/-/Sod2+/- 16mo+Vehicle
Apoe-/-/Sod2+/- 16mo+ MitoTEMPO
C
BUN (mg/dL)
40
30
20
10
0
D
Creatinin (mg/dL)
0.25
0.20
0.15
0.10
0.05
0.00
E
ALT (U/L)
150
100
50
0
F
Apoe-/-
Apoe-/-/Sod2+/-
Vehicle
MitoTEMPO
G
Apoe-/-
Apoe-/-/Sod2+/-
Vehicle
MitoTEMPO

Figure S5.


Apoe-/-
Apoe-/-/Sod2+/-
MMP 2
CD68
DAPI
Vehicle
MitoTEMPO
MMP2 expression
(IntDen/ROI cell x10³)
80
60
40
20
0
**
*