Supplemental Data for

SIRT1 decreases Lox-1-mediated foam cell formation in atherogenesis

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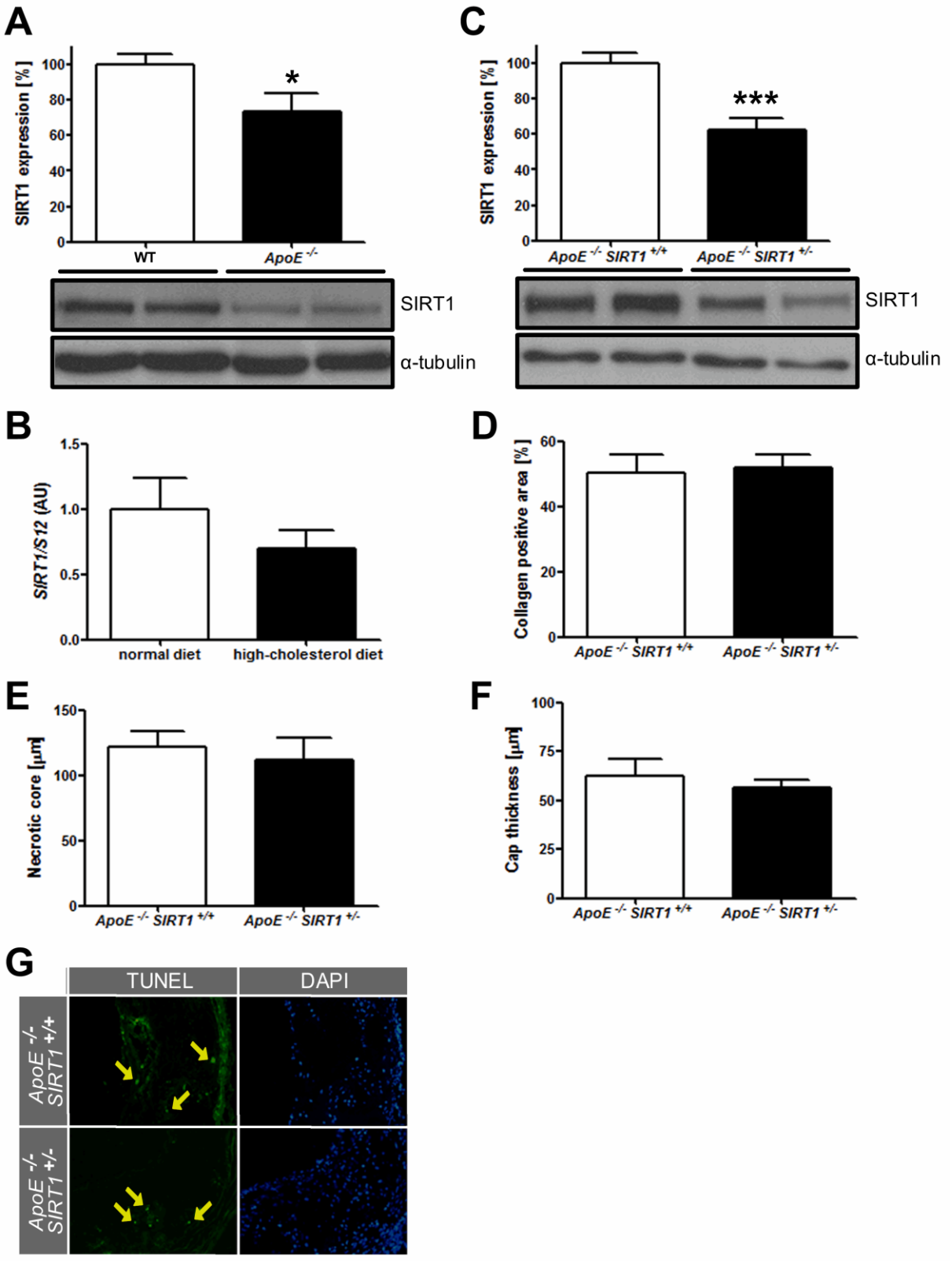
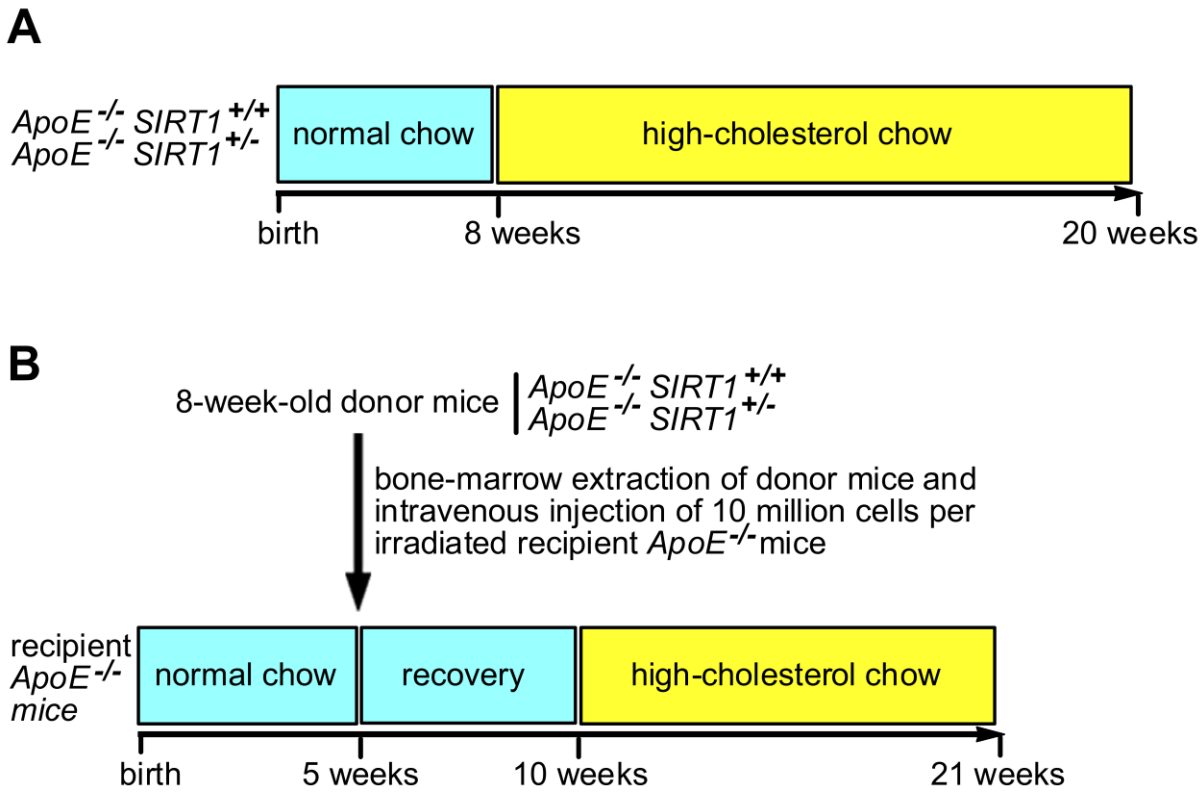
SUPPLEMENTARY FIGURES

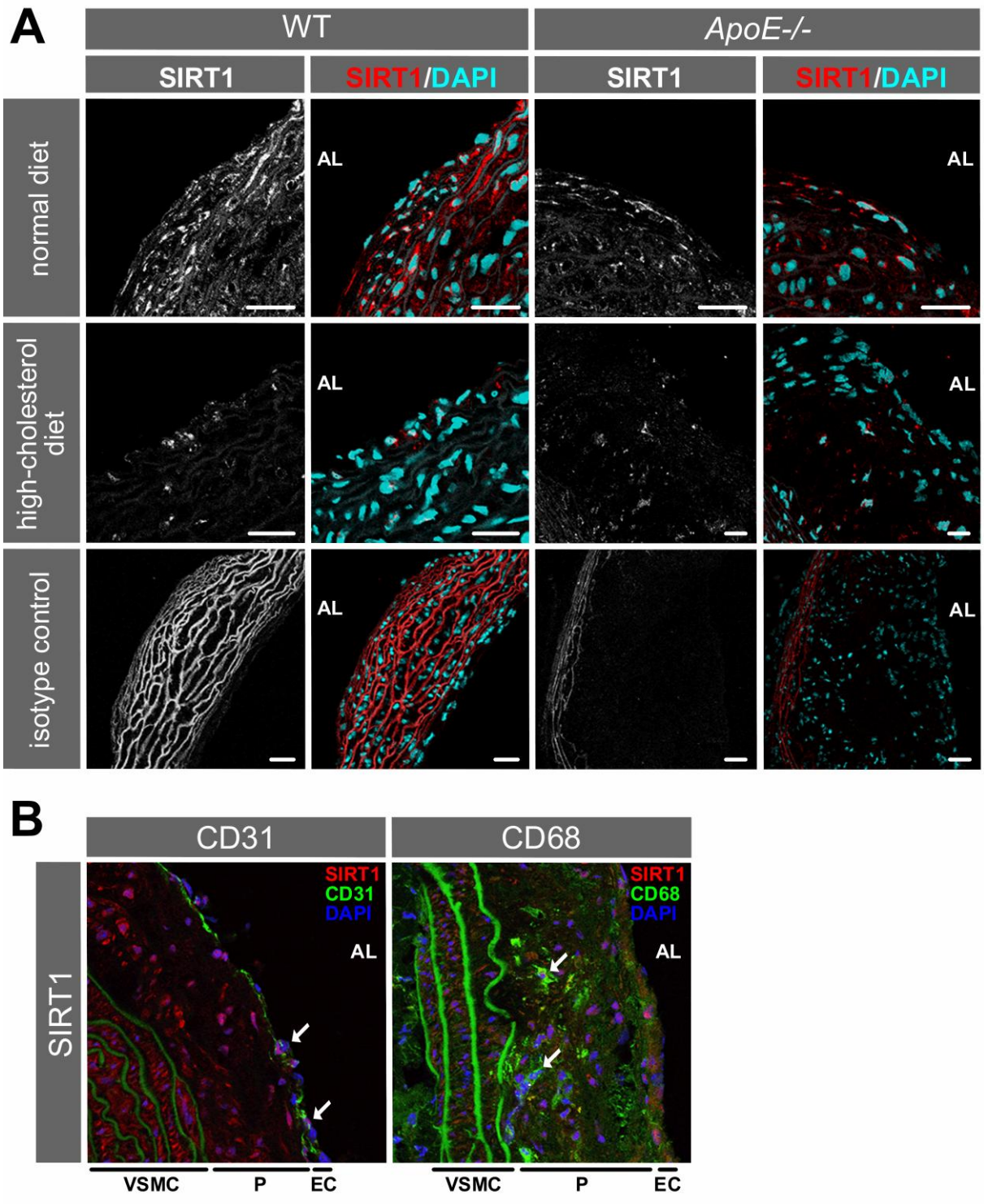


Supplemental Figure 1. Correlation between SIRT1 and atherogenesis. (A) Protein expression of SIRT1 and α-tubulin in *ApoE-/-* and WT aortic lysates. n=6 per genotype. (B)

SIRT1 mRNA levels in aortic tissue of wild-type mice treated a normal or high-cholesterol diet. n=4 per treatment group. (C) *ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ mice express approximately 60% of *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ protein levels in aortic lysates. n=9 per genotype. (D-F) Quantification of collagen content (D), necrotic core size (E), and cap thickness in plaques from aortic sinus. n=8 per genotype. (F) TUNEL staining revealed no difference in amount of apoptotic cells. n=6 per genotype.

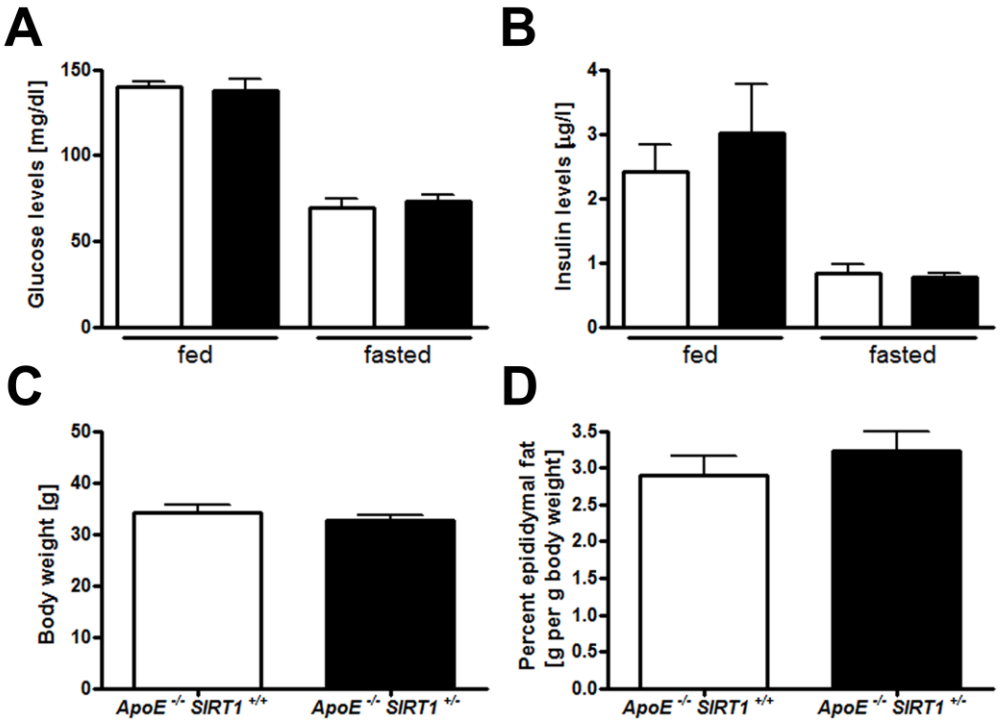


Supplemental Figure 2. Diet schemes. **(A)** 8 weeks after birth, *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ and *ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ mice were kept on a high-cholesterol diet for 12 weeks. 20-week-old male animals were euthanized for tissue harvesting. **(B)** Bone-marrow from 8-week-old donor mice (*ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ and *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$) was extracted, and 10^6 bone-marrow cells were injected intravenous into irradiated recipient *ApoE*$^{-/-}$ mice. Transplanted mice were allowed to recover for 5 weeks, and were then fed a high-cholesterol diet for 11 weeks prior to tissue harvesting.

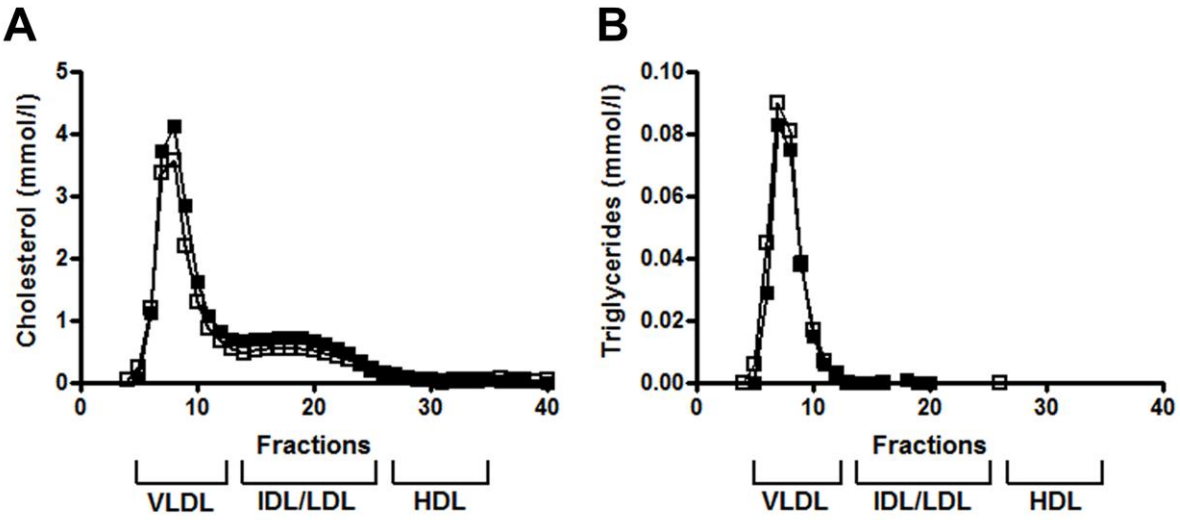


Supplemental Figure 3. SIRT1 staining in aortic plaque of wild-type (healthy) and ApoE-/- (diseased) mice. (**A**) Aortic expression of SIRT1 in wild-type and *ApoE*$^{-/-}$ mice fed a normal or high-cholesterol (HC) diet. Large plaques are only observed in *ApoE*$^{-/-}$ mice fed a HC diet. Isotype controls were exposed longer and show only unspecific staining of the connective tissue. Bar = 25 µm. AL: Arterial lumen. (**B**) SIRT1 colocalizes with endothelial cells (CD31;

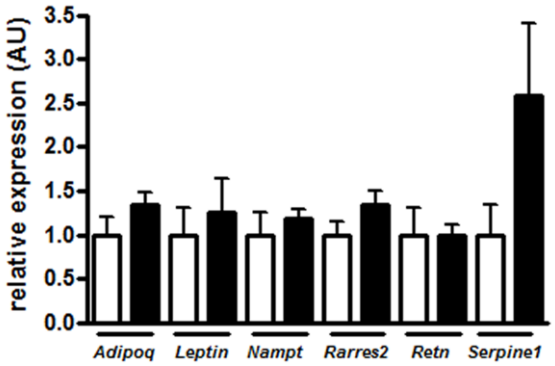
arrows), and with macrophages (CD68; arrows) in atherosclerotic plaques. EC: endothelial cell layer; P: Plaque; VSMC: Vascular smooth muscle cells; AL: Arterial lumen.



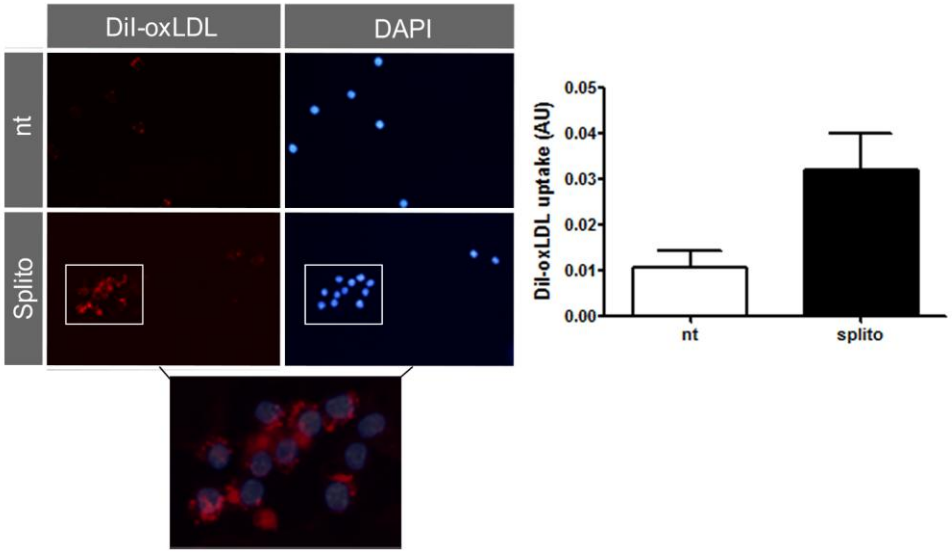
Supplemental Figure 4. No difference between *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ (white columns) and *ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ (black columns) mice regarding glucose plasma levels, body weight, and weight of epididymal fat pad. (**A**) Plasma glucose levels in fed and fasted animals. (**B**) Plasma insulin levels in fed and fasted animals. (**C**) Total body weight of mice prior to harvesting. (**D**) Percent epididymal fat, given as the percentage of the epididymal fat per total body weight. n≥10 per genotype.



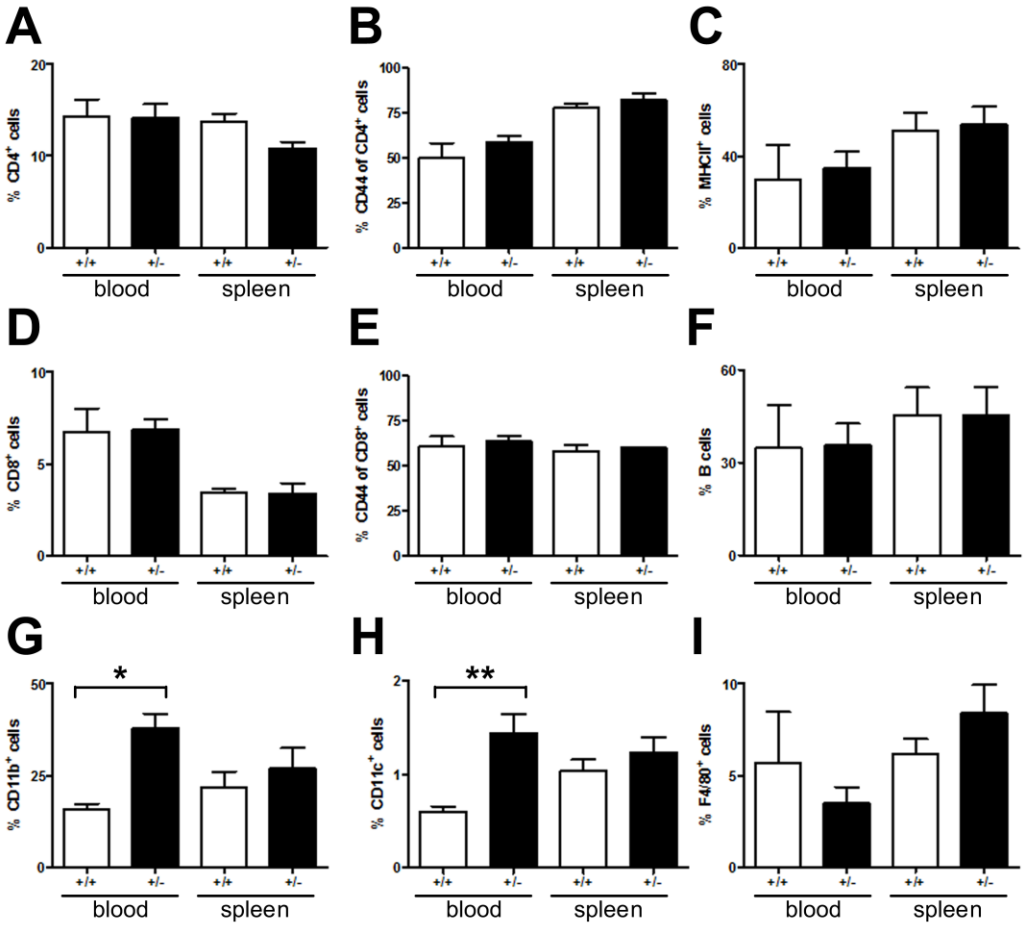
Supplemental Figure 5. No alterations of plasma lipid distribution in *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ & *ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ mice. (**A, B**) Distribution of cholesterol and triglycerides in the plasma of *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ mice (■, n = 10 (pooled)) or *ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ mice (□, n = 15 (pooled)). No differences in the distribution of cholesterol or triglycerides are observed between *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ & *ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ mice. VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.



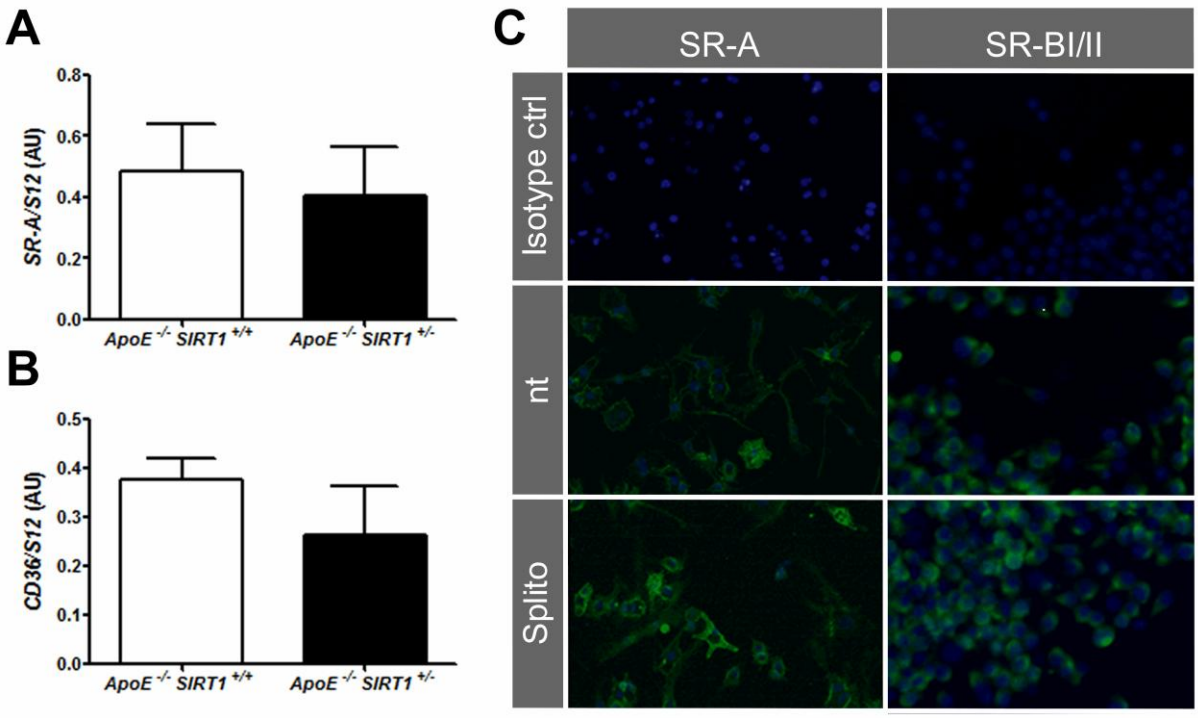
Supplemental Figure 6. Epididymal white adipose tissue expression of Adiponectin (Adipoq), Leptin, Visfatin (Nampt), Chemerin (Rarres2), Resistin (Retn) and Plasminogen activator inhibitor 1 (PAI-1 or Serpine1) in ApoE$^{-/-}$ SIRT1$^{+/+}$ (white columns) and ApoE$^{-/-}$ SIRT1$^{+/-}$ (black columns) mice. n=6 per genotype.



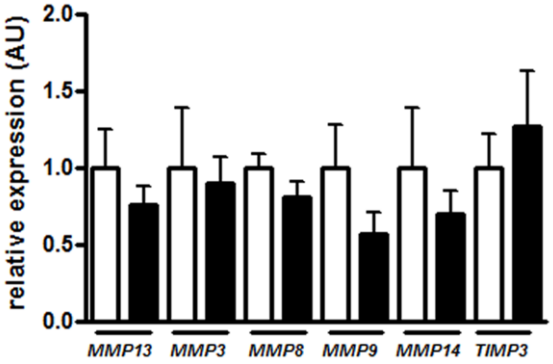
Supplemental Figure 7. SIRT1 inhibition with splitomicin (splito) shows a trend towards increased accumulation of oxLDL in RAW 264.7 cells compared to non-treated cells.



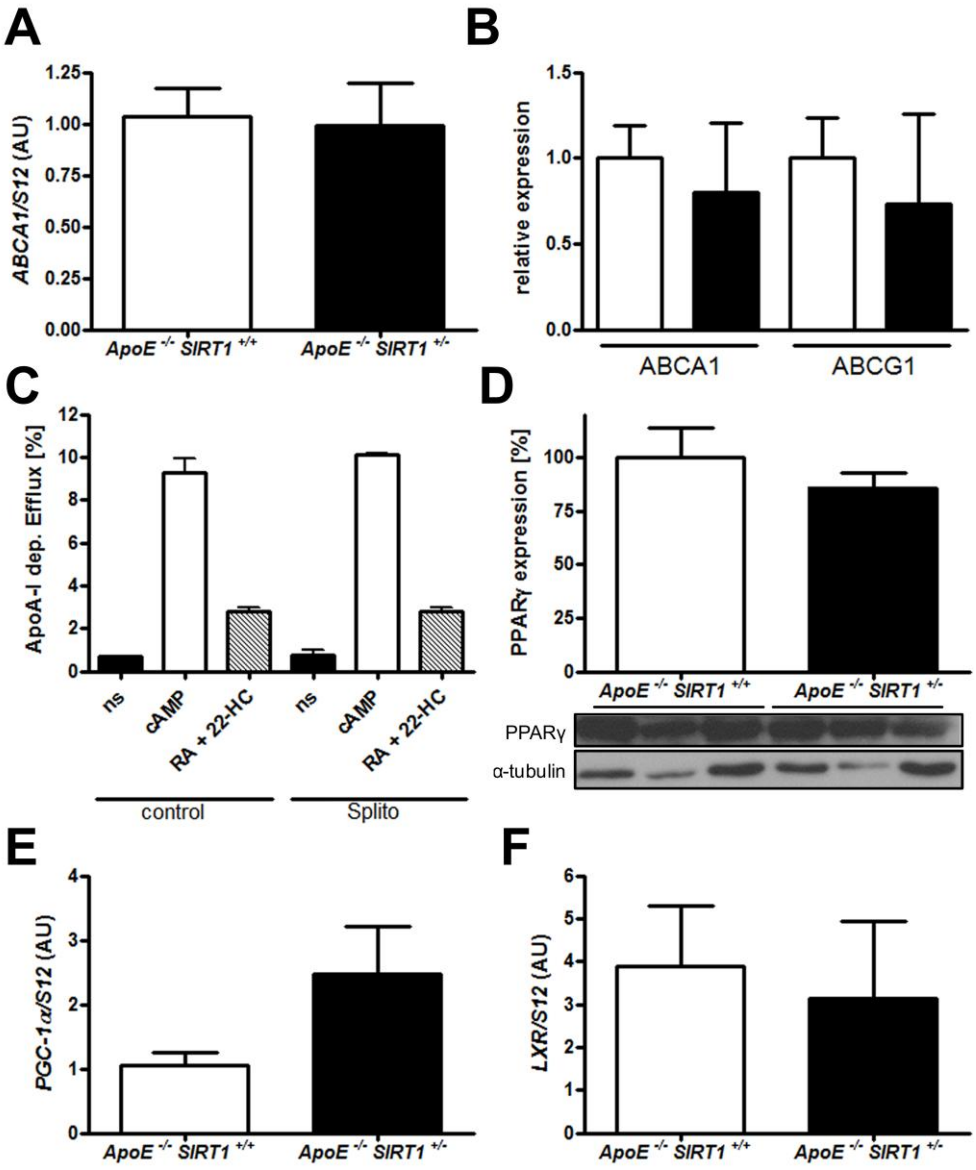
Supplemental Figure 8. Blood and spleen analysis of bone-marrow transplanted mice. Percental FACS analyses of **(A)** $CD4^+$ cells, **(B)** $CD44^+$ population of $CD4^+$ cells, **(C)** $MHCII^+$ cells, **(D)** $CD8^+$ cells, **(E)** $CD44^+$ population of $CD8^+$ cells, **(F)** B cells, **(G)** $CD11b^+$ cells, **(H)** $CD11c^+$ cells, **(I)** $F4/80^+$ cells. n=3/transplanted genotype. *p<0.05; **p< 0.01**.**



Supplemental Figure 9. SIRT1 does not affect the expression of SR-A, CD36, or SR-B. Aortic RNA levels of *SR-A* (**A**) and *CD36* (**B**). **(C)** SR-A and SR-B immunofluorescence in RAW 264.7 macrophages reveals no difference upon splitomicin (Splito) treatment compared with non-treated cells (nt).



Supplemental Figure 10. Aortic expression of *MMP13*, *MMP3*, *MMP8*, *MMP9*, *MMP14*, and *TIMP3* in *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ (white columns) and *ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ (black columns) mice. n=10 per genotype.



Supplemental Figure 11. SIRT1 does not affect cholesterol efflux in macrophages. (**A**) Aortic RNA levels of *ABCA1*. n=6 per genotype. **(B)** Expression of ABCA1 and ABCG1 in *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ (white columns) and *ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ (black columns) peritoneal macrophages. n=4 per genotype. (**C**) ApoA-1-dependent cholesterol efflux in RAW 264.7 macrophages is not affected by 100 μM splitomicin (Splito) compared with untreated control groups (nt). 9 *cis*-retinoic acid (RA) + 22-hydroxycholesterol (22-HC)-stimulation is done to analyze LXR/RXR-dependent efflux, cAMP-stimulation to study LXR/RXR-independent efflux. n=3 per treatment group. **(D)** Aortic expression of PPARγ. n=6 per genotype. **(E, F)** Aortic RNA levels of PGC-1α (E, n=10 per genotype) and LXRα (F, n=6 per genotype).

SUPPLEMENTARY TABLES

Supplemental Table 1: Plasma lipid profiles of *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ and *ApoE*$^{-/-}$ *SIRT1*$^{+/-}$.

	***ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ (n=10)**	***ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ (n=14)**
Total cholesterol (mmol/l)	45.54 ± 6.15	39.72 ± 2.56
Triglycerides (mmol/l)	2.12 ± 0.24	2.03 ± 0.18
Free fatty acids (mmol/l)	0.99 ± 0.13	0.93 ± 0.09

mean ± SEM.

Supplemental Table 2: Plasma cytokine values of *ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ and *ApoE*$^{-/-}$ *SIRT1*$^{+/-}$.

	***ApoE*$^{-/-}$ *SIRT1*$^{+/+}$ (n=10)**	***ApoE*$^{-/-}$ *SIRT1*$^{+/-}$ (n=15)**
VCAM-1 (ng/ml)	1146 ± 59.7	1266 ± 62.8
ICAM-1 (ng/ml)	843 ± 51.4	813 ± 31.1
TGF-β	6431 ± 644.5	6167 ± 332.7
IFN-γ (pg/ml)	7.27 ± 3.67	16.15 ± 8.26
IL-6 (pg/ml)	65.36 ± 21.17	127.54 ± 57.67
IL-10 (pg/ml)	86.40 ± 40.27	189.30 ± 85.90
mKC	110.44 ± 22.01	138.81 ± 15.64

mean ± SEM.