Figure EV1. Generation of adipose- and myeloid cell-specific SIRT1 knockout mice.

A Strategy for tissue-specific knockout of SIRT1. WT: wild type; SIRT1fl/fl: exon-4 of Sirt1 is flanked by loxP sites; locations of the forward and reverse primers for PCR analysis of genomic deletion are shown. The primer set spans floxed region of mouse Sirt1 gene and amplifies a band of 900 and 450 bp in WT and KO mice, respectively, as a result of Cre recombinase-mediated deletion.

B PCR analysis for genomic deletion of Sirt1 in fractionated adipocytes (adi), stromal vascular fraction (SVF) of epididymal adipose tissue (epi), and peritoneal macrophage (mφ).

C Western blotting of SIRT1 protein in fractionated mature adipocytes from epididymal fat depots and several other tissues. BAT, brown adipose tissue.

D, E Western blotting for acetylated (Ac) and total (T) p53 in mature adipocytes and SVF of epididymal fat from WT and SIRT1 knockout mice. (E) Quantification of (D). Data are expressed as means ± SD (n = 6) from three independent repeats with two-way repeated-measures ANOVA and Tukey test. *P < 0.05.

Source data are available online for this figure.
Figure EV2. SIRT1 deficiency in adipocytes accelerates glucose intolerance and hyperinsulinemia independent of adiposity.

A  Body weight of AKO, MKO, and WT mice on standard chow diet.
B  Body composition of 45-week-old mice measured by in vivo NMR.
C, D GTT performed in 8-, 18-, 30-, and 45-week-old mice. GTT of AKO, MKO, and WT mice at 30 weeks (C) and AUC of GTT performed at different time points (D).
E  Plasma levels of fasting (left panel) and fed (right panel) insulin measured at different time points.

Data information: Data are expressed as means ± SEM (n = 8) from three independent repeats with two-way repeated-measures ANOVA and Tukey test. *P < 0.05, **P < 0.01.
Figure EV3. WT, AKO, and MKO mice have similar body weight, composition, and energy expenditure.

A–D Body weight (A) of WT, AKO, and MKO mice on HFD. Representative picture (B), body composition measured by in vivo NMR (C), and food intake (D) of mice after 16 weeks of HFD feeding. Data are expressed as means ± SEM (n = 6) from three independent repeats with two-way ANOVA and Tukey test.

E–I CLAMS analysis of mice at 16 weeks after HFD feeding. O₂ consumption (VO₂) (E, F), CO₂ production (VCO₂) (G), and RER (H, I). Data are expressed as means ± SEM (n = 6) from three independent repeats with two-way ANOVA and Tukey test.
Figure EV4. Deletion of SIRT1 does not affect insulin sensitivity in adipocytes.

A SVF cells isolated from epididymal adipose tissues of AKO mice or WT littermates were differentiated into mature adipocytes. Insulin-stimulated glucose uptake was measured using $^3$H-labeled 2-DG. Data are expressed as means ± SEM (n = 8) from three independent repeats with two-way ANOVA.

B Insulin-stimulated phosphorylation of Akt was examined by Western blotting with rabbit anti-phospho-Akt (S473) and mouse anti-total Akt antibodies. Data are representative of three independent repeats.

Source data are available online for this figure.

Figure EV5. Eosinophils and neutrophils are unaltered in obese WT and AKO mice.

A, B WT and AKO mice were fed with HFD for 4 weeks, and eosinophils (A) and neutrophils (B) in epididymal adipose tissue were quantified by flow cytometry. Data are expressed as means ± SEM (n = 8) from three independent repeats with two-sided Student's t-test.