Figure S1

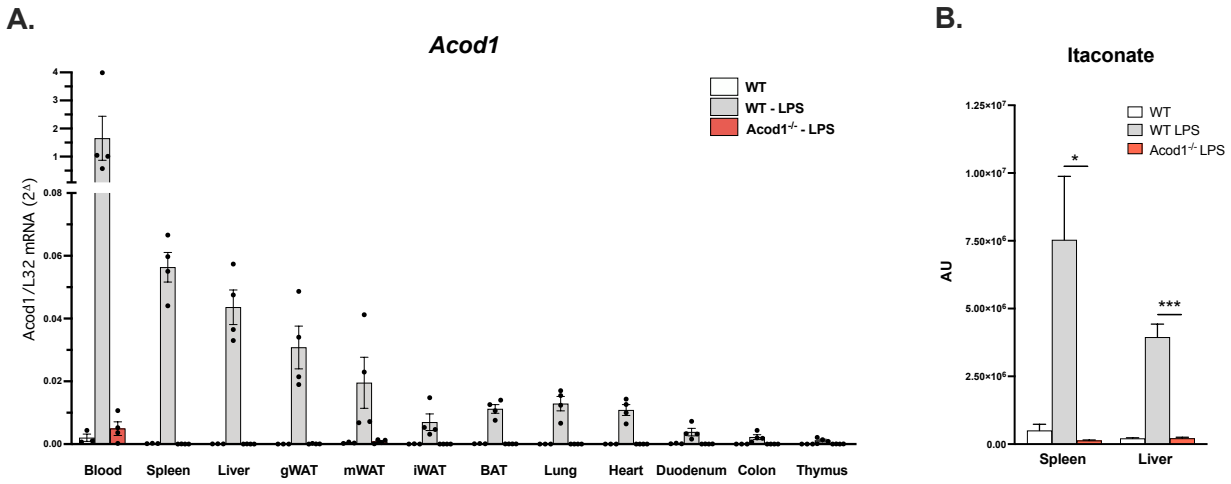

Figure S1. Induction of Acod1 mRNA expression and itaconate production during systemic inflammation. (A) mRNA gene expression of Acod1 6 h after LPS treatment in WT and Acod1$^{-/-}$ mice (B) Itaconate levels in spleen and liver after LPS induction of Acod1 expression. *P < 0.05, ***P < 0.001.

Figure S2

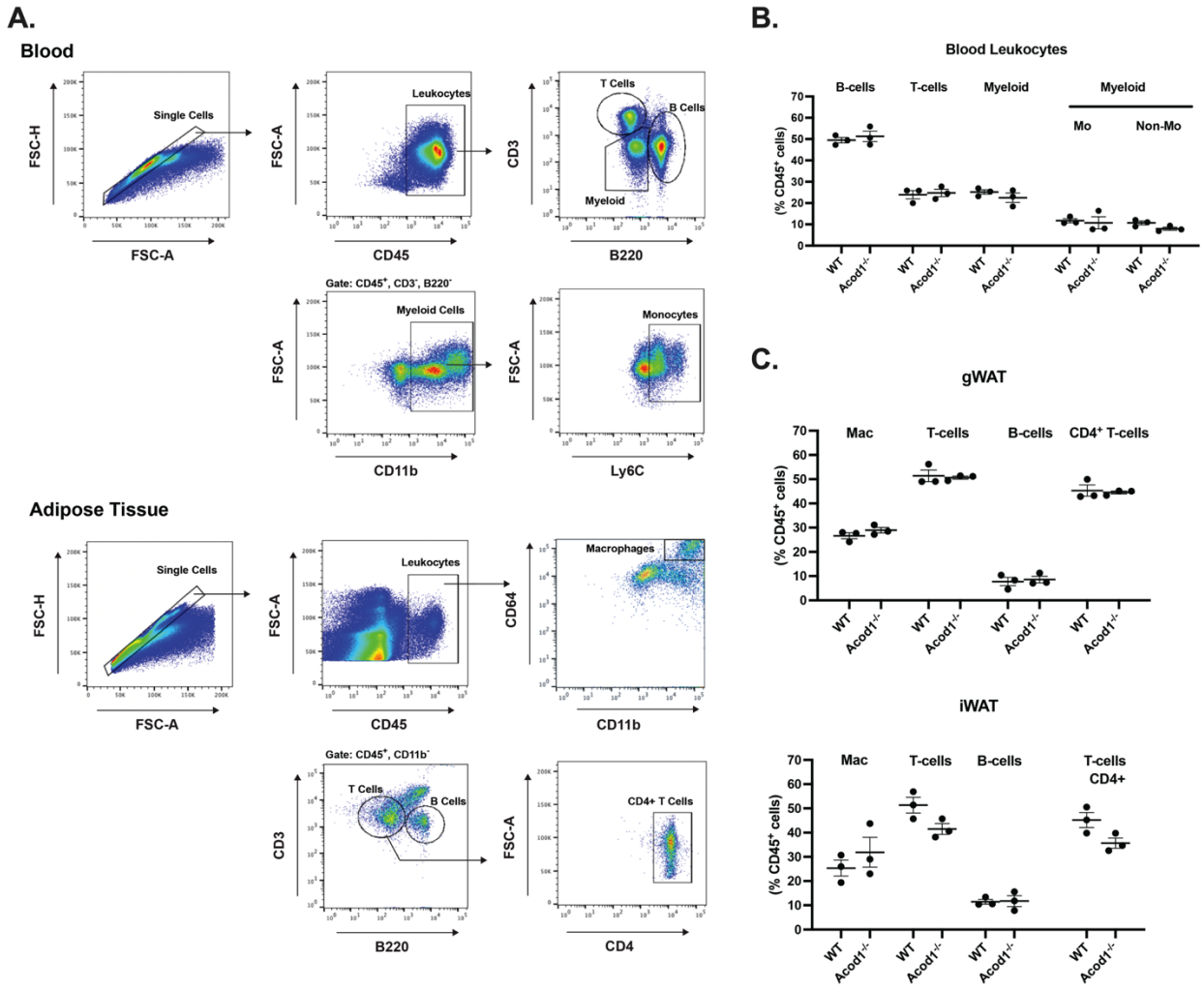

Figure S2. Flow cytometry analysis of blood and tissue leukocytes during DIO. (A) Gating strategy for analysis of leukocytes in blood and adipose tissue. Leukocyte percentage of $CD45^{+}$ cells in (B) blood, and (C) gWAT and iWAT.

Figure S3

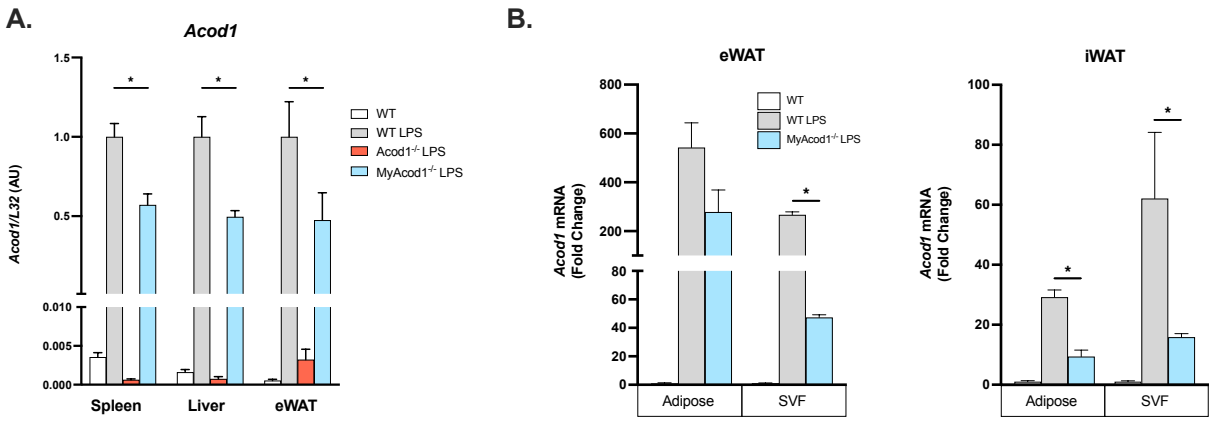

Figure S3. Non-myeloid cell type-specific expression of Acod1. (A) mRNA expression of Acod1 in spleen, liver and eWAT of WT, Acod1$^{-/-}$, and myeloid Acod1$^{-/-}$ (MyAcod1$^{-/-}$) mice after 6 h LPS treatment (B) Acod1 mRNA expression from whole adipose tissue and isolated SVF fraction of adipose tissue after 6 h LPS treatment. n = 3-4 per group. *P < 0.05, **P < 0.01.

Figure S4

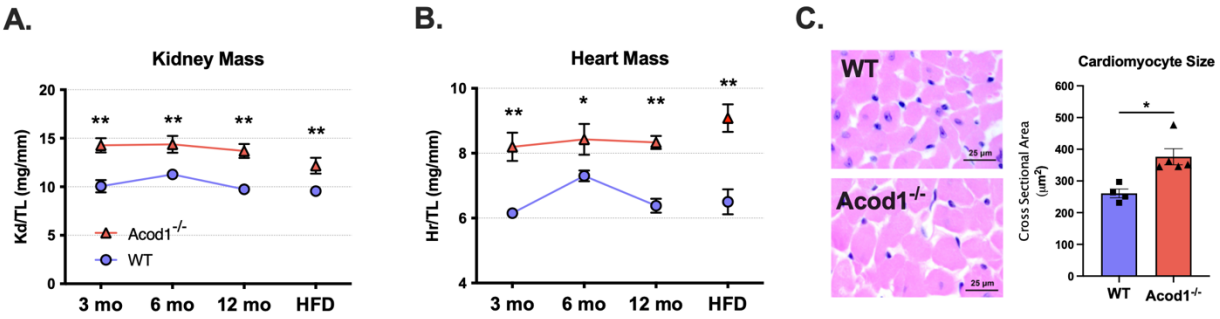

Figure S4. Organomegaly in Acod1 knockout mice. (A) kidney mass and (B) heart mass in WT and Acod$^{-/-}$ mice. (C) Photomicrograph of cardiomyocytes and quantification of cardiomyocyte size in 12 mo old WT and Acod$^{-/-}$ mice. n = 4-7 per group. *P < 0.05, **P < 0.01.

SUPPLEMENTAL METHODS

Itaconate analysis

Tissues were lysed in 80% cold methanol, centrifuged and supernatants were dried in a SpeedVac. Metabolite pellets were resuspended in a 50:50 methanol water mixture and metabolites were run on an Agilent 1260 UHPLC combined with a 6520 Accurate-Mass Q-TOF LC/MS. For data analysis, Agilent MassHunter Workstation Software Profinder B.08.00 with Batch Targeted Feature Extraction and Batch Isotopologue Extraction and Qualitative Analysis B.07.00 was used.

Isolation of Stromal Vascular Fraction from adipose tissue

Adipose tissue depots were dissected, mechanically dissociated by mincing, and then digested in 1 mg/mL collagenase II (Sigma-Aldrich) at 37°C for 30 minutes. Cells were then rinsed with RPMI + BSA media and filtered through 100 μm nylon cell strainer. Cell suspension was centrifuged at 500 x g for 10 min at 4 °C to separate adipocytes and SVF.

Indirect calorimetry

Whole body oxygen consumption (VO2), carbon dioxide production (VCO2), spontaneous motor activity and food intake were measured using an integrated open-circuit calorimeter (CLAMS, Columbus Instruments). Mice were weighed before measurements and were placed in individual sealed chambers with free access to food and water and measured continuously for 72 hours.

Flow cytometry

Adipose tissue depots were dissected, mechanically dissociated by mincing, and then digested in 1 mg/mL collagenase II (Sigma-Aldrich) at 37°C for 30 minutes. Samples were passed through a 50 μM cell strainer, centrifuged, and red blood cell lysis was performed.

Single cell suspensions were stained with anti-mouse CD45 FITC, CD3 PerCP Cy5.5, CD64 PE, CD11b BV421, Ly6G APC, CD4 PE Cy7, B220 BV605 antibodies. Flow cytometry data were acquired using a BD LSRFortessa cell analyzer and were analyzed using FlowJo software.

Body composition

Body composition analysis of body fat, lean mass, and free fluid was determined using an NMR-based analyzer (EchoMRI 1100). Conscious mice were placed individually in a measuring tube with measurements lasting less than 2 minutes. The EchoMRI analyzer was checked daily using a reference sample (canola oil) as recommended by the manufacturer.

LPS-induced systemic inflammation

Mice were injected intraperitoneally with LPS (5 mg/kg, *Escherichia coli* serotype O127:B8, Sigma-Aldrich, L3129) dissolved in PBS. Mice were euthanized after 6 h and tissues were harvested for gene expression and metabolite analysis.