

Supplementary Materials

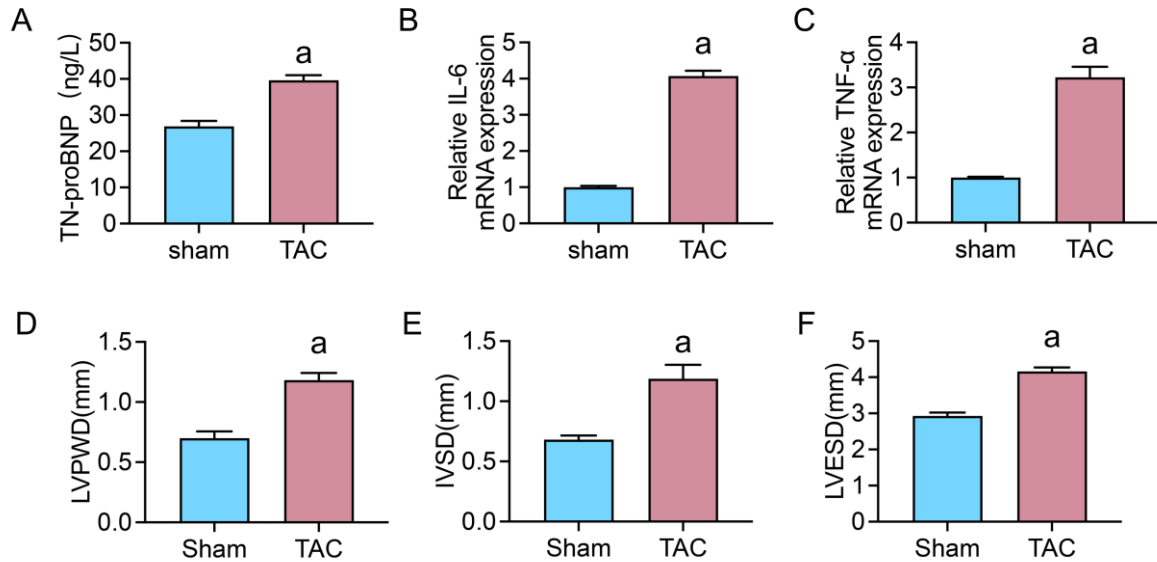


Figure S1. Phenotypic validation of the TAC-induced heart failure model.

(A) Plasma NT-proBNP levels in Sham and TAC mice ($n = 3$). (B) Relative mRNA expression of IL-6 in myocardial tissue from Sham and TAC mice ($n = 3$). (C) Relative mRNA expression of TNF- α in myocardial tissue from Sham and TAC mice ($n = 3$). (D) Echocardiographic measurement of left ventricular posterior wall thickness at end-diastole (LVPWd) ($n = 3$). (E) Echocardiographic measurement of interventricular septal thickness at end-diastole (IVSd) ($n = 3$). (F) Echocardiographic measurement of left ventricular end-systolic diameter (LVESD) ($n = 3$). *a* indicates a statistically significant difference compared with the Sham group ($P < 0.05$).

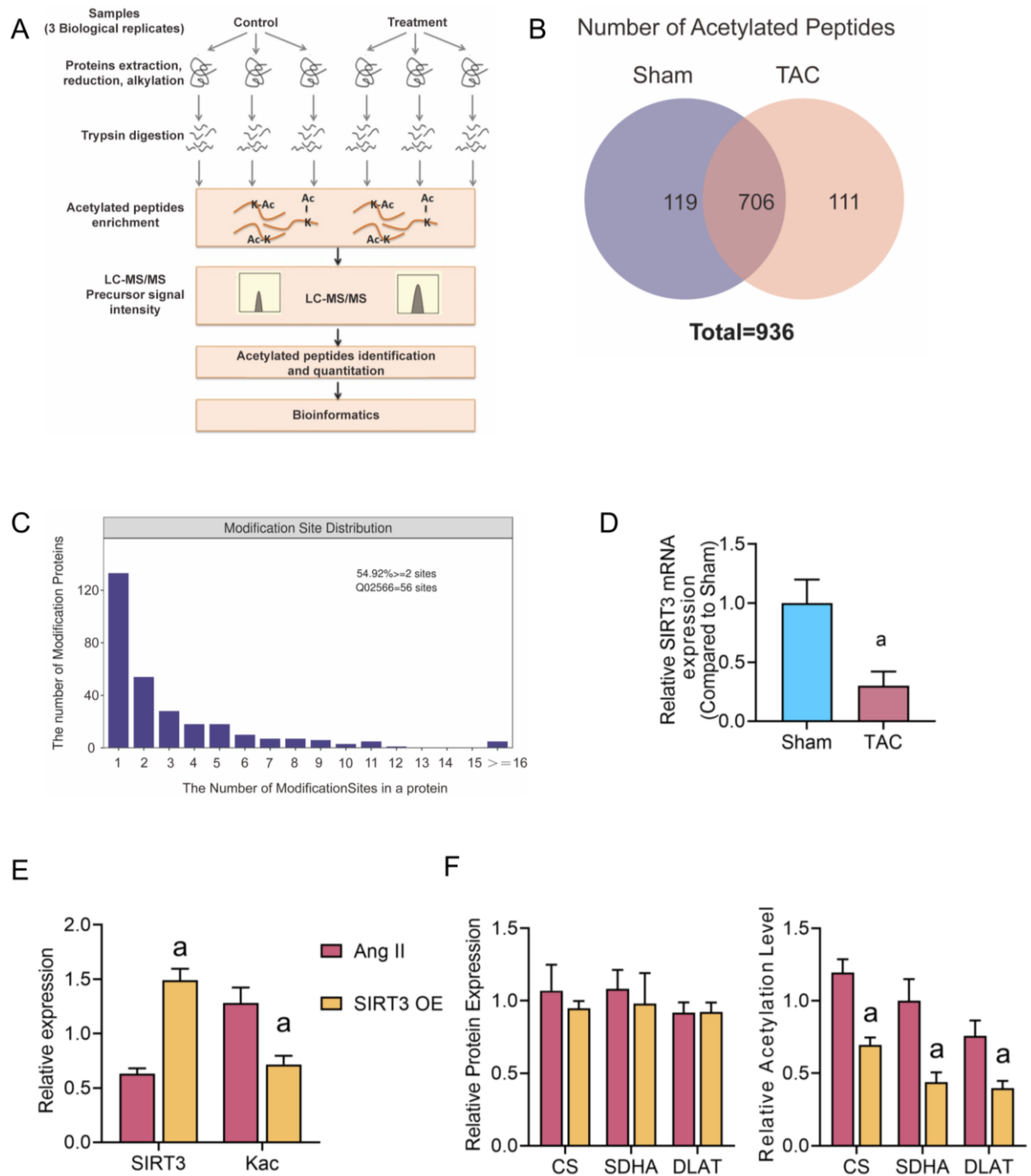


Figure S2. Global acetyl-proteomics profiling and SIRT3-dependent regulation of TCA cycle enzyme acetylation.

(A) Schematic workflow of acetyl-proteomics analysis, including protein extraction, tryptic digestion, acetylated peptide enrichment, LC-MS/MS identification, and bioinformatics analysis. **(B)** Venn diagram showing the number and overlap of acetylated peptides identified in Sham and TAC myocardium. **(C)** Distribution of acetylation site numbers per protein in Sham and TAC groups. **(D)** Relative SIRT3 mRNA expression in myocardial tissue from Sham

and TAC mice ($n = 3$). **(E)** Statistical analysis of SIRT3 expression and global lysine acetylation (Kac) levels in HL-1 cells treated with Ang II or SIRT3 overexpression ($n = 3$). **(F)** Quantification of acetylation levels of CS, SDHA, and DLAT in HL-1 cells under Ang II and SIRT3 overexpression conditions ($n = 3$). *a* indicates $P < 0.05$ vs. Sham group (D) or Ang II group (E–F).

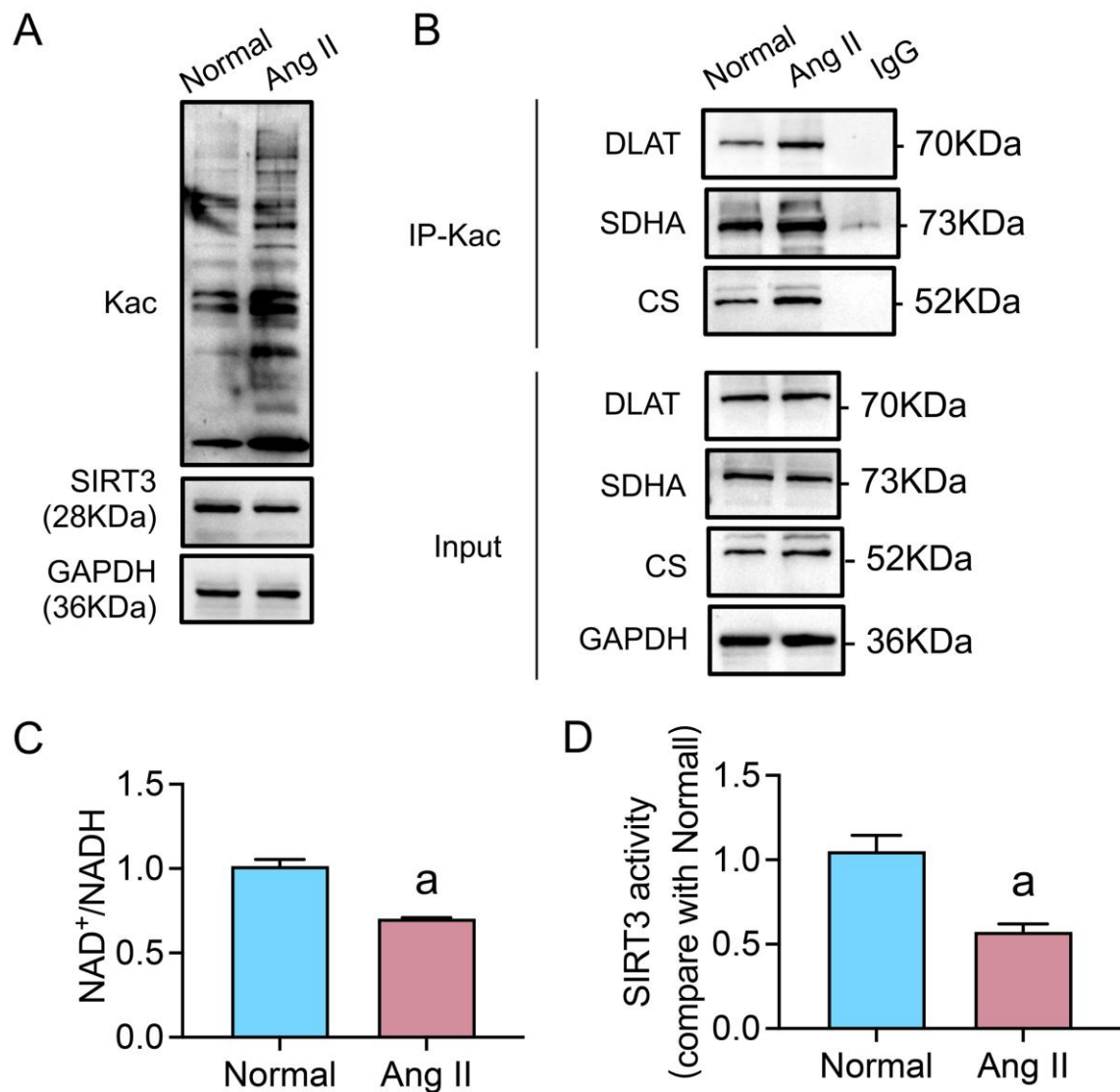


Figure S3. SIRT3 overexpression improves mitochondrial quality and respiration in heart failure via deacetylation

(A) Representatives immunoblot of total lysine acetylation (Kac) and SIRT3 protein expression in Normal and Ang II-treated HL-1 cardiomyocytes. **(B)** Co-immunoprecipitation (IP-Kac)

analysis of DLAT (70 kDa), SDHA (73 kDa), and citrate synthase (CS, 52 kDa) acetylation levels in Normal and Ang II–treated cells. IgG was used as a negative control, and input blots indicate comparable protein loading (n = 3/group). **(C)** Intracellular NAD⁺/NADH ratio in HL-1 cells under Normal and Ang II conditions (n = 3/group). **(D)** SIRT3 enzymatic activity in Normal and Ang II–treated HL-1 cells, normalized to the Normal group (n = 3/group). *a* indicates $p < 0.05$ vs. Normal group.

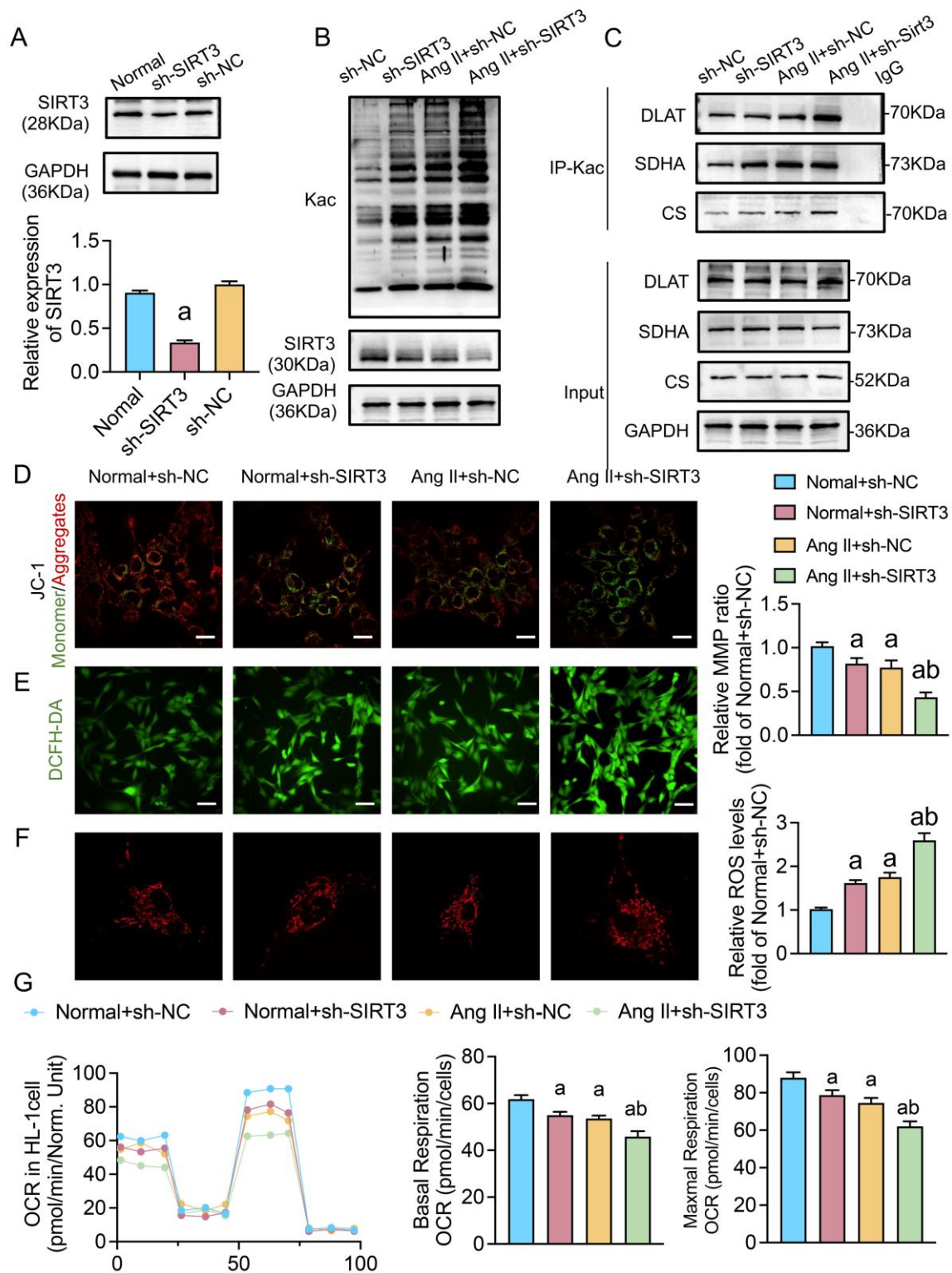


Figure S4. SIRT3 knockdown aggravates mitochondrial hyperacetylation, oxidative stress, and respiratory dysfunction in cardiomyocytes.

(A) Validation of SIRT3 knockdown efficiency in HL-1 cardiomyocytes. Representative Western blot images and quantitative analysis of SIRT3 protein levels in Normal, sh-SIRT3,

and sh-NC groups (n = 3 per group). **(B)** Western blot analysis of total lysine acetylation (Kac) and SIRT3 expression in Normal and Ang II-treated HL-1 cells under sh-NC or sh-SIRT3 conditions. GAPDH was used as a loading control. **(C)** Co-immunoprecipitation using anti-acetyl-lysine antibody (IP-Kac), followed by immunoblotting for DLAT (70 kDa), SDHA (73 kDa), and citrate synthase (CS, 52 kDa), showing that Ang II treatment and SIRT3 knockdown markedly increase acetylation of key TCA cycle enzymes (n = 3 per group). **(D)** JC-1 staining showing mitochondrial membrane potential (MMP) in HL-1 cells from the Normal+sh-NC, Normal+sh-SIRT3, Ang II+sh-NC, and Ang II+sh-SIRT3 groups, with quantitative analysis of the red/green fluorescence ratio. Scale bar = 10 μ m (n = 3 per group). **(E)** Representative fluorescence images and quantitative analysis of intracellular reactive oxygen species (ROS) levels in the four groups. Scale bar = 40 μ m (n = 3 per group). **(F)** Seahorse extracellular flux analysis showing oxygen consumption rate (OCR) curves, basal respiration, and maximal respiration in HL-1 cells from the four groups (n = 3 per group). *a indicates $p < 0.05$ vs. Normal+sh-NC; b indicates $p < 0.05$ vs. Ang II+sh-NC.*