
Figures and figure supplements

SIRT2 inhibition protects against cardiac hypertrophy and ischemic injury

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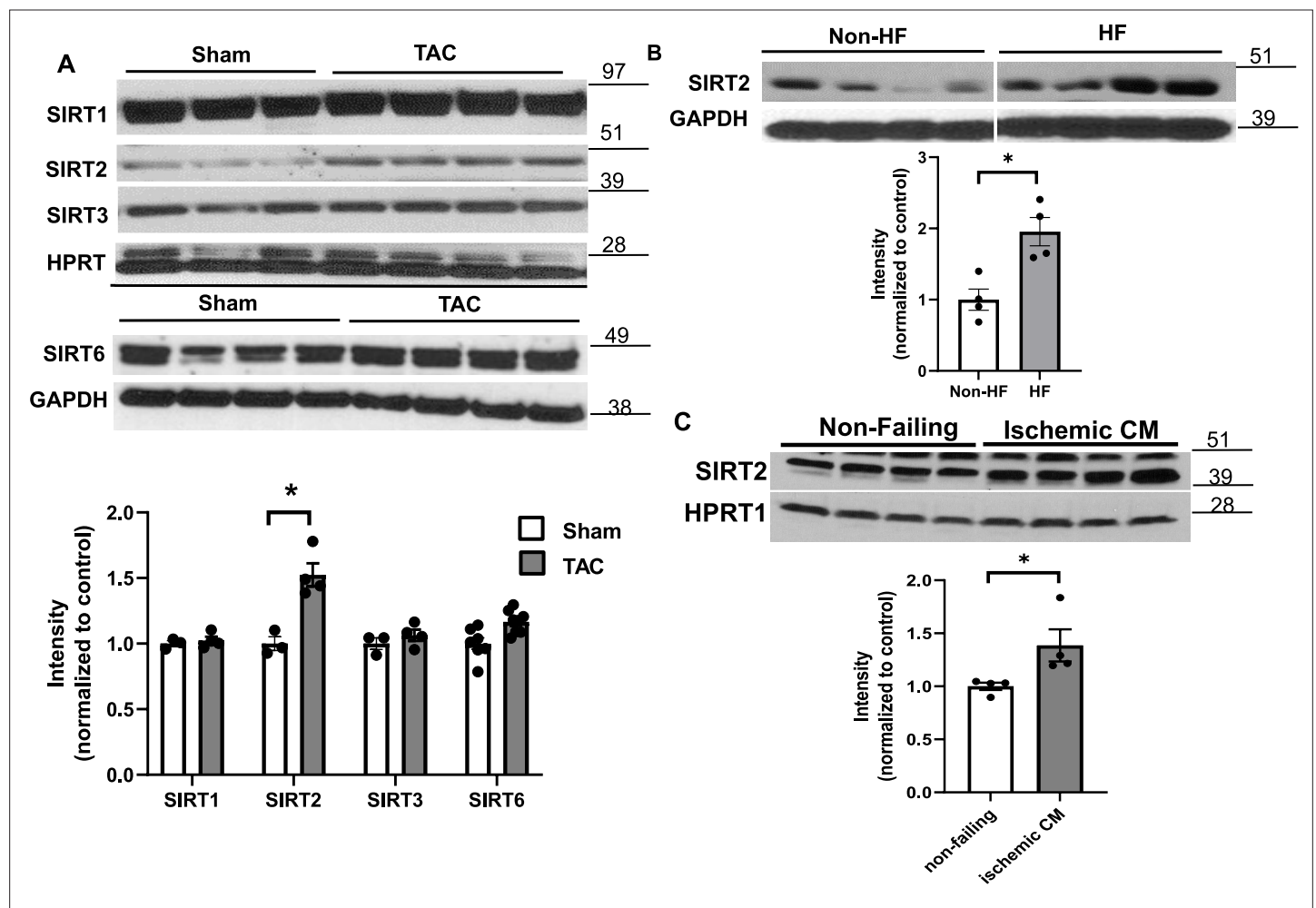


Figure 1. SIRT2 is upregulated in heart failure (HF). (A) SIRT1, SIRT2, SIRT3, and SIRT6 in mouse hearts after trans-aortic constriction (TAC). (B) SIRT2 in human hearts from healthy patients and patients with dilated cardiomyopathy. (C) SIRT2 protein levels in the hearts of control individual and patients with ischemic heart failure. * $p < 0.05$ by Student's t-test. Data presented as mean \pm SEM.



Figure 1—figure supplement 1. SIRT2 protein in different mouse tissues, including the heart (A), and in various cell lines, including H9c2 cells (B).

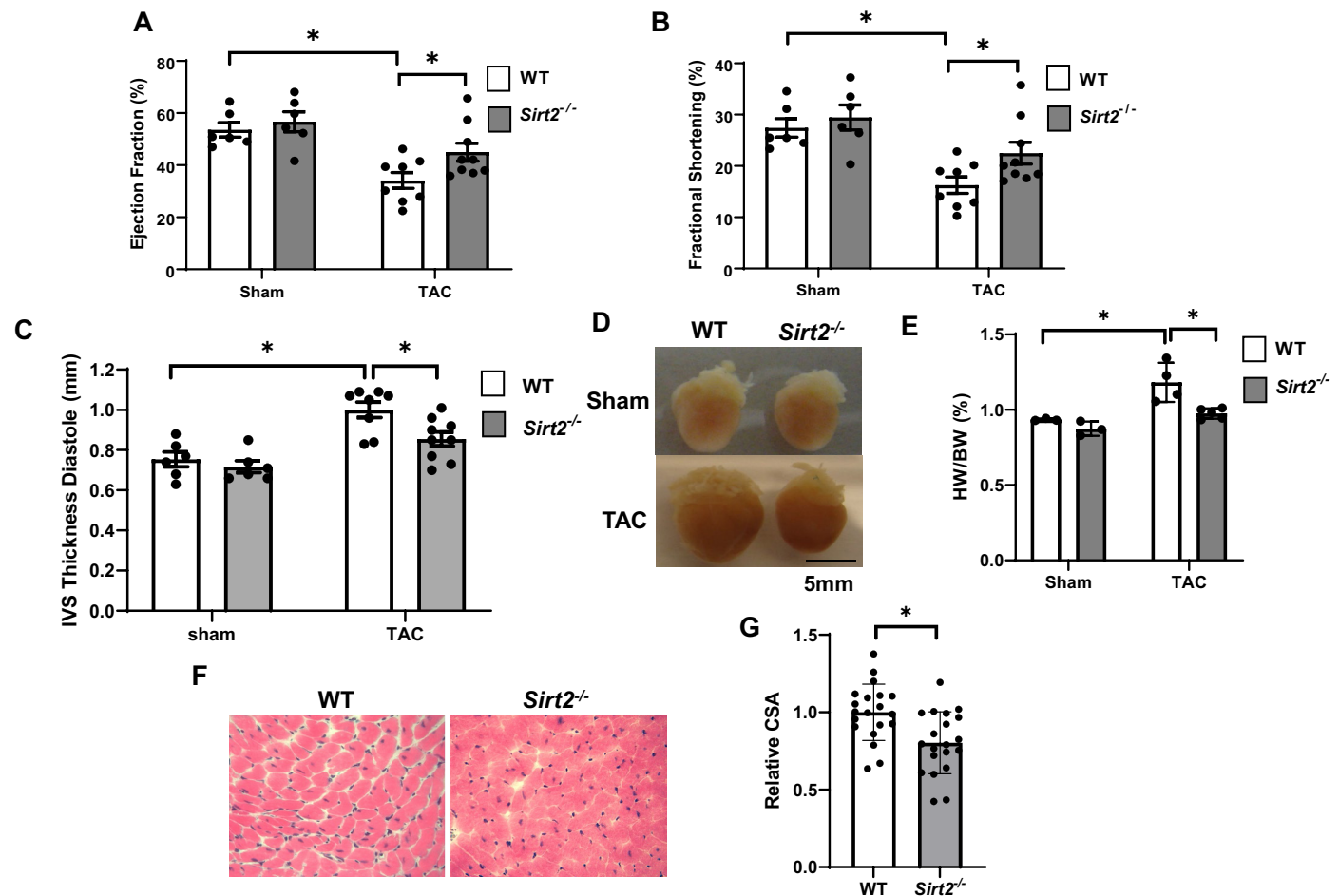


Figure 2. *Sirt2* deficiency protects the heart against cardiac dysfunction after trans-aortic constriction (TAC). *Sirt2*^{-/-} and wild-type (WT) littermates were subjected to TAC and ejection fraction (EF) (A), fractional shortening (FS) (B), and interventricular septal thickness during diastole (C) were assessed 4 weeks later (N=6–9). (D–F) Representative hearts (D), HW/BW (E) (N=3–5), H&E staining, (F) and the summary of cross-sectional area of cardiomyocytes (G) in WT and *Sirt2*^{-/-} hearts (N=20 cardiomyocytes), *p<0.05 by one-way ANOVA and post hoc Tukey analysis (A, B, C, and E) and unpaired Student's t-test (G). Bars represent group mean.

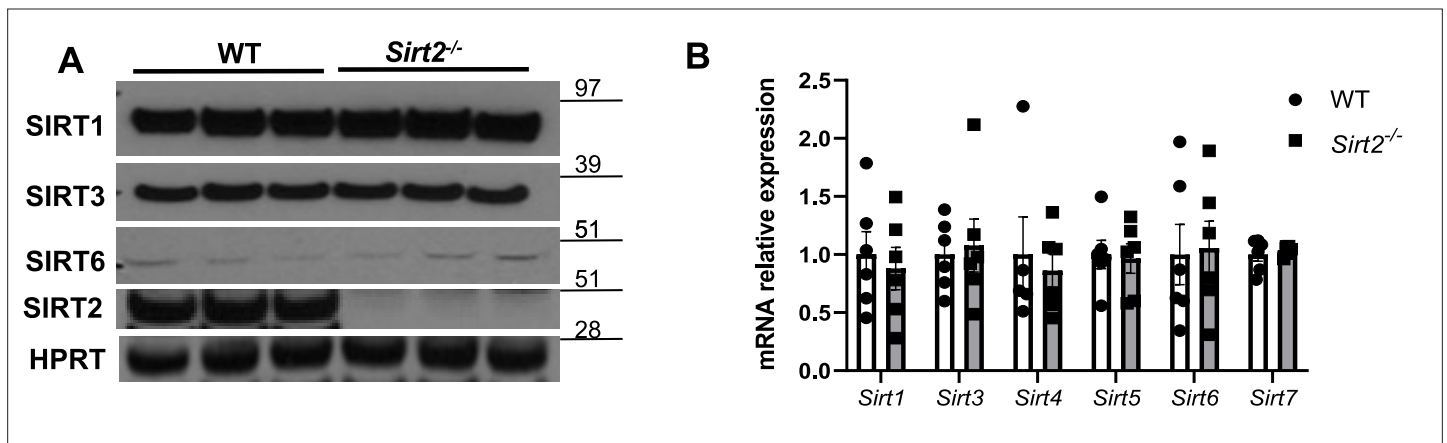


Figure 2—figure supplement 1. Expression of protein (A) and mRNA (B) of other sirtuin family members in the hearts of *Sirt2*^{-/-} mice. N=6. Data presented as mean ± SEM.

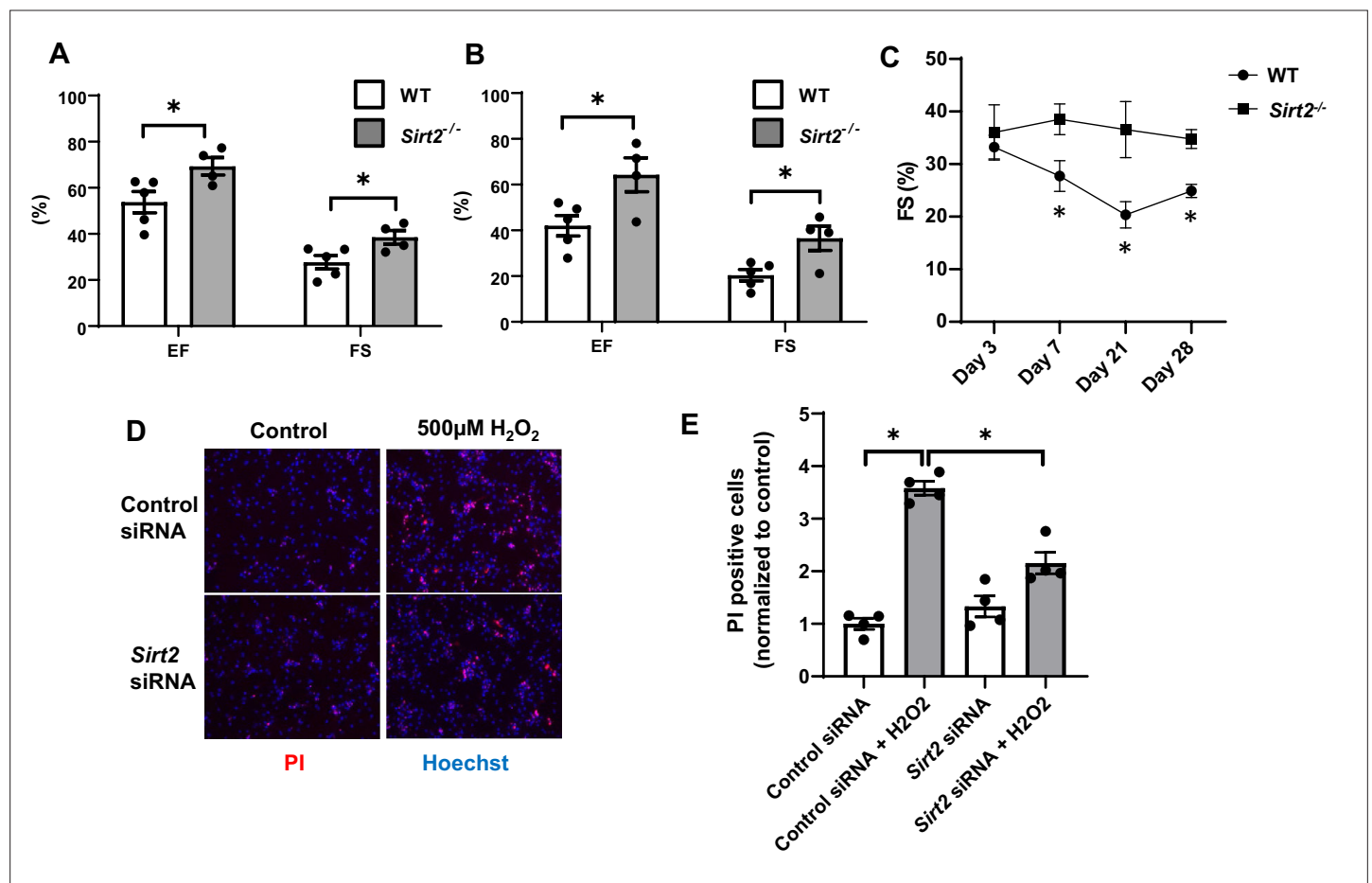


Figure 3. Hearts from *Sirt2*^{-/-} mice are protected against ischemia-reperfusion (I/R) injury. Ejection fraction (EF) and fractional shortening (FS) in wild-type (WT) and *Sirt2*^{-/-} mice 7 (A) and 21 days (B) after I/R (N=4–5). (C) Time course of FS in *Sirt2*^{-/-} mice after I/R injury (N=4–5). (D, E) Cell death assessed by propidium iodide (PI), in neonatal rat cardiomyocyte (NRCM) treated with control or *Sirt2* siRNA and with 500 μM of H₂O₂. *p<0.05 by ANOVA for all panels except for panel C, where Student's t-test was used for comparison between the two time points. Bars represent mean (A, B), and data presented as mean ± SEM.

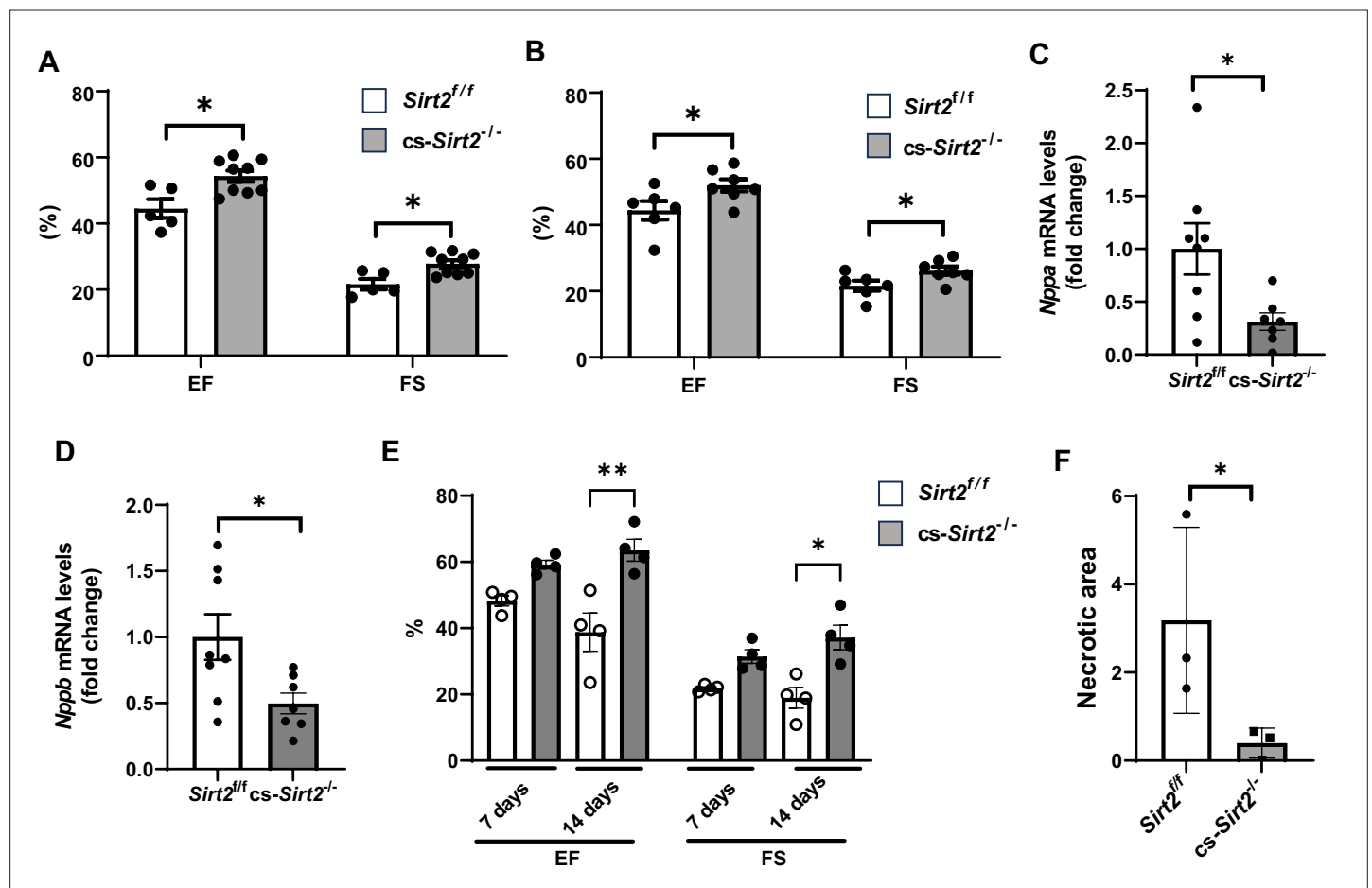


Figure 4. *cs-Sirt2*^{-/-} hearts are protected against trans-aortic constriction (TAC) and ischemia-reperfusion (I/R). Ejection fraction (EF) and fractional shortening (FS) in *Sirt2*^{fl/fl} and *cs-Sirt2*^{-/-} mice 7 (A) and 14 days (B) after TAC (N=5–9). (C,D) mRNA levels of *Anf* (C) and *Bnp* (D) in the hearts of *Sirt2*^{fl/fl} and *cs-Sirt2*^{-/-} mice 4 weeks after TAC (N=7–8). (E) EF and FS in *Sirt2*^{fl/fl} and *cs-Sirt2*^{-/-} mice 7 and 14 days after I/R (N=4). (F) Necrotic area (representing the degree of ischemic damage) in *Sirt2*^{fl/fl} and *cs-Sirt2*^{-/-} mice 14 days after MI. **p*<0.05 by ANOVA for panels A and B, and Student's *t*-test was used for panels C and D. Data are presented as mean ± SEM.

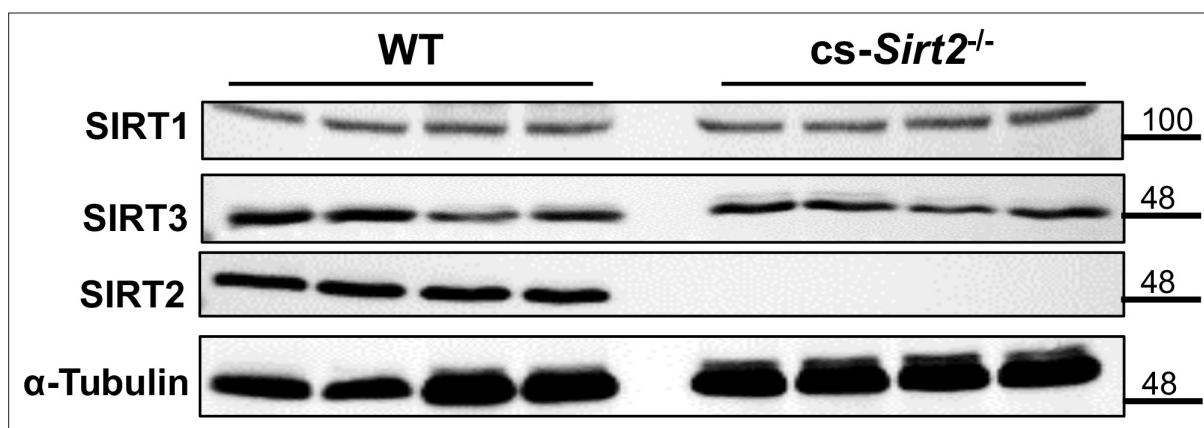


Figure 4—figure supplement 1. SIRT1, SIRT3, and SIRT2 protein in the hearts of *cs-Sirt2^{-/-}* mice.

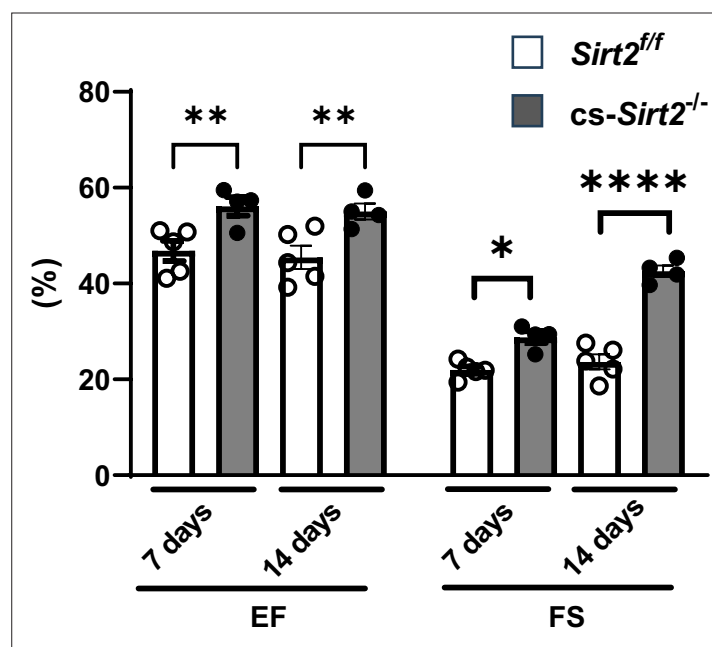


Figure 4—figure supplement 2. *cs-Sirt2*^{-/-} hearts from female mice are protected against trans-aortic constriction (TAC). Ejection fraction (EF) and fractional shortening (FS) in female wild-type (WT) and *cs-Sirt2*^{-/-} mice 7 and 14 days after TAC (N=4).

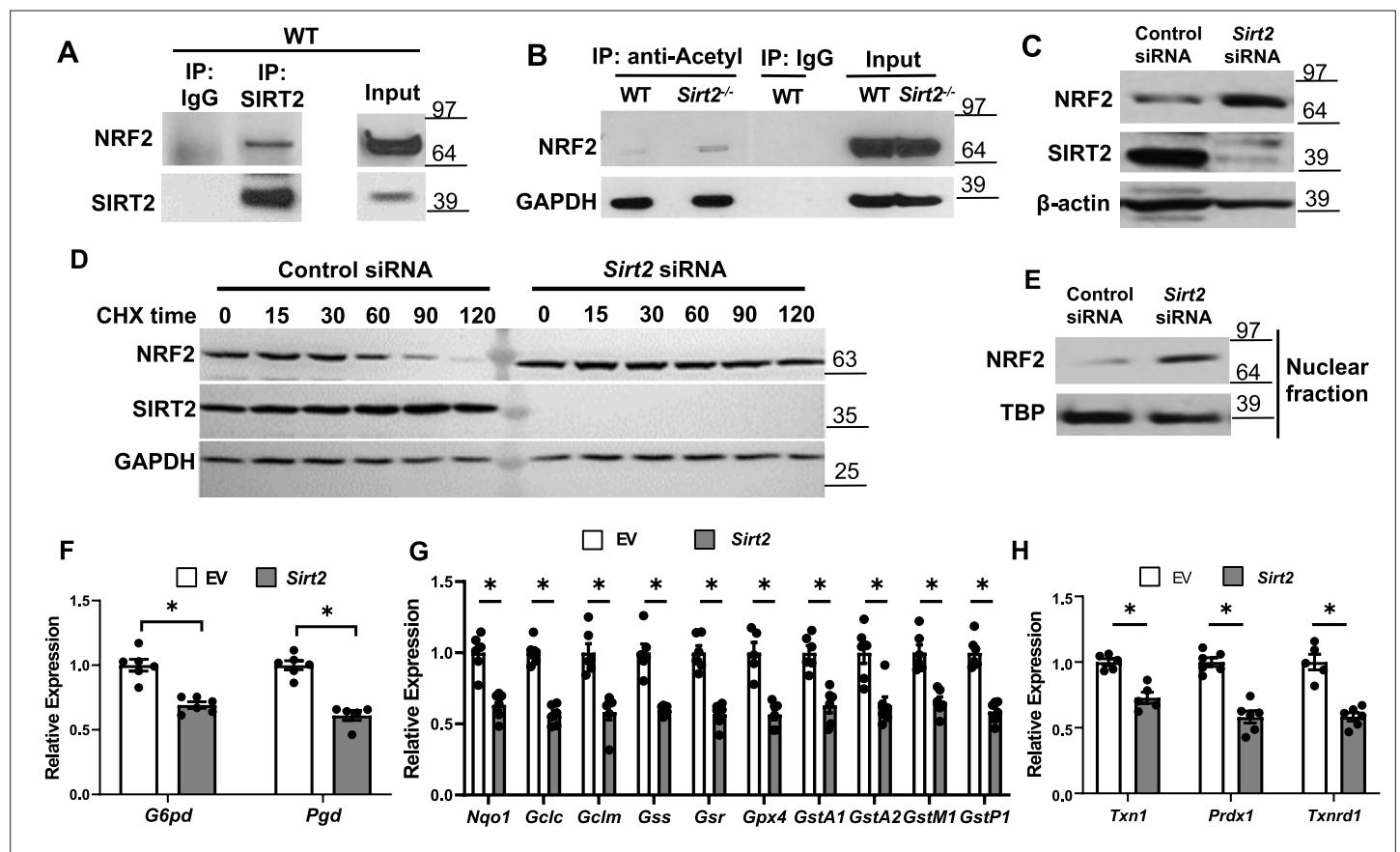


Figure 5. SIRT2 interacts with nuclear factor (erythroid-derived 2)-like 2 (NRF2) and regulates its activity in the heart. (A) Co-immunoprecipitation (IP) of SIRT2 and NRF2 in extracts of hearts from wild-type (WT) mice. (B) Endogenous NRF2 acetylation levels in the hearts of WT and *Sirt2*^{-/-} mice at the baseline. Acetylated proteins were IPed by anti-acetyl antibody followed by immunoblotting with anti-NRF2 antibody. (C) NRF2 protein levels in neonatal rat cardiomyocytes (NRCMs) treated with *Sirt2* siRNA. (D) NRF2 protein levels in H9c2 cells treated with control or *Sirt2* siRNA and harvested at different time points after treatment with 100 µg/ml of CHX. (E) NRF2 protein levels in the nucleus in NRCMs treated with control or *Sirt2* siRNA. (F–H) mRNA levels of NRF2 target genes in pentose phosphate pathway (F), quinone and glutathione-based detoxification (G), thioredoxin production (H) in H9c2 cells overexpressing empty vector (white bars) or SIRT2 (gray bars). **p*<0.05 by Student's *t*-test.

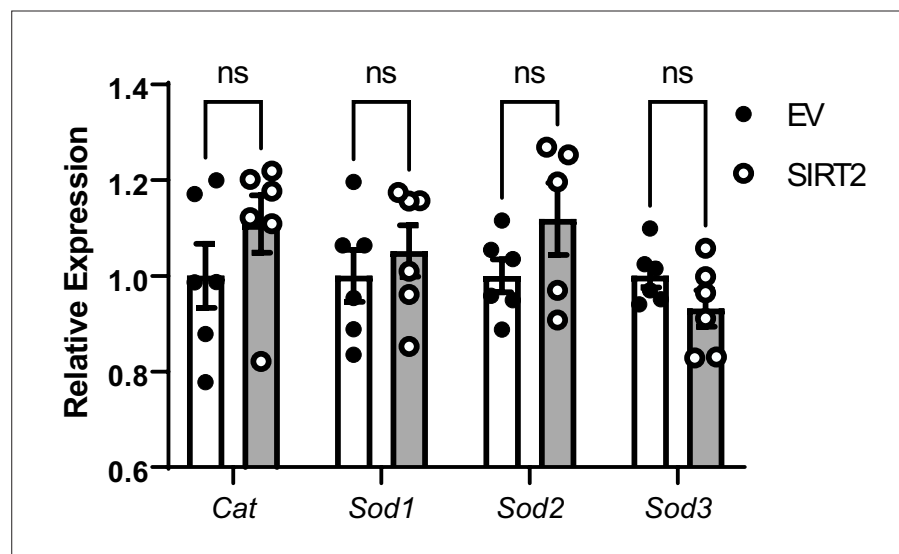


Figure 5—figure supplement 1. Effects of SIRT2 overexpression on mRNA levels of non-nuclear factor (erythroid-derived 2)-like 2 (NRF2) targeted antioxidant genes. N=5–6. Data presented as mean \pm SEM.

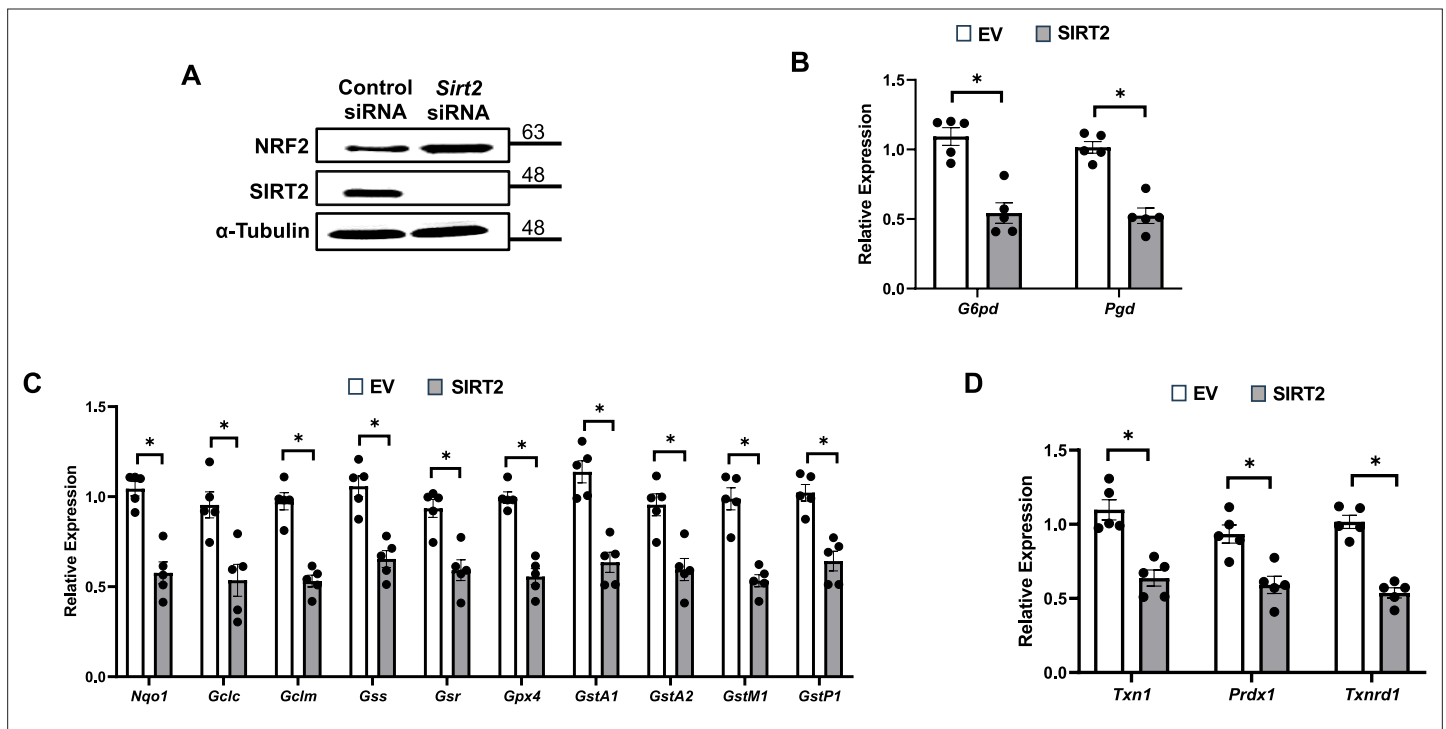


Figure 5—figure supplement 2. SIRT2 regulates nuclear factor (erythroid-derived 2)-like 2 (NRF2) and its target proteins. **(A)** NRF2 protein levels in HL-1 cells treated with *Sirt2* siRNA. **(B–D)** mRNA levels of NRF2 target genes in pentose phosphate pathway **(B)**, quinone and glutathione-based detoxification **(C)**, thioredoxin production **(D)** in HL-1 cells overexpressing empty vector (white bars) or SIRT2 (gray bars). * $p < 0.05$ by Student's t-test.

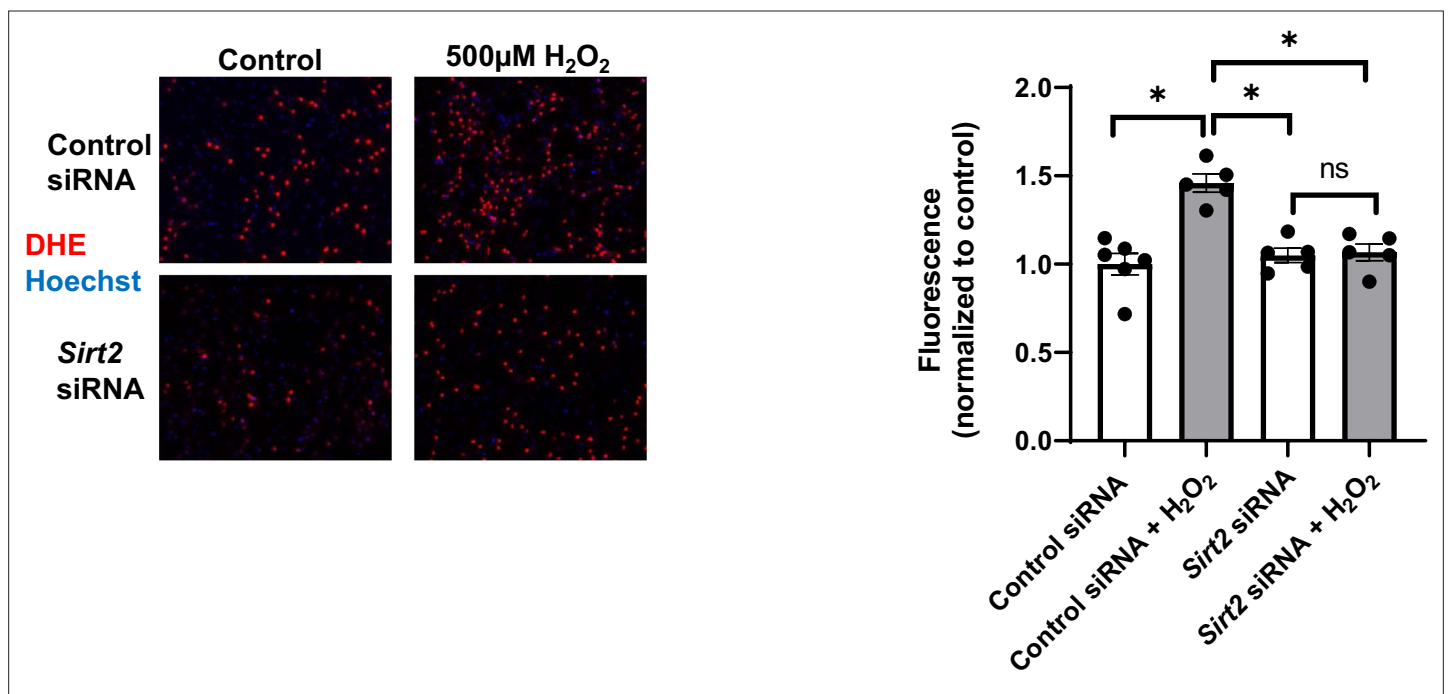


Figure 5—figure supplement 3. Reactive oxygen species (ROS) levels as assessed by dihydroethidium (DHE) staining in neonatal rat cardiomyocytes (NRCMs) treated with control or *Sirt2* siRNA after treatment with 500 μ M H₂O₂. N=5–6. Data presented as mean \pm SEM.

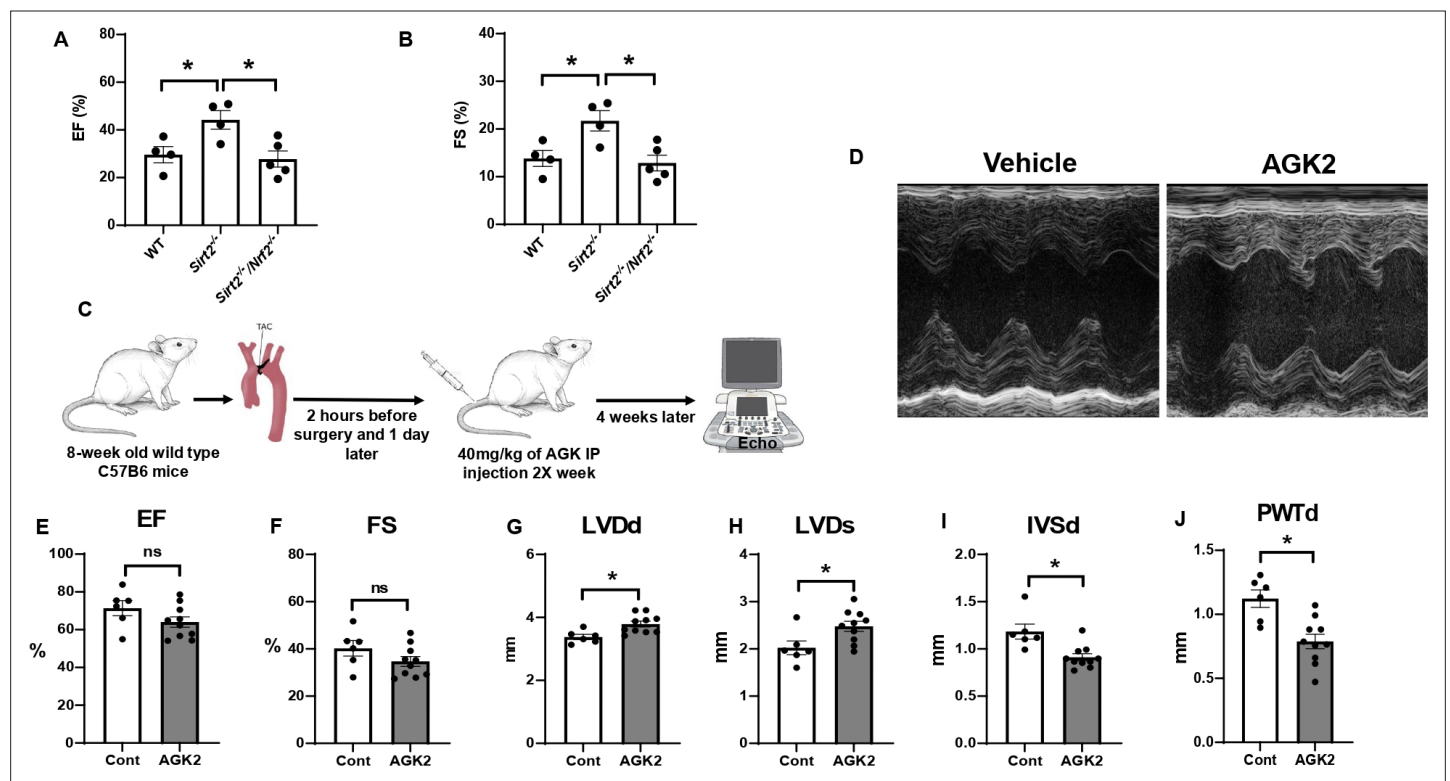


Figure 6. *Nrf2* deletion and SIRT2 inhibitors protected against cardiac damage and cardiac hypertrophy. Ejection fraction (EF) (A) and fractional shortening (FS) (B) in wild-type (WT), *Sirt2*^{-/-}, and *Sirt2*^{-/-}/*Nrf2*^{-/-} double knockout (KO) mice 28 days after ischemia-reperfusion (I/R) (N=4–5). (C) Protocol for treatment of mice with SIRT2 inhibitor, AGK2. (D) Echo images of hearts from WT mice treated with either vehicle or AGK2. (E–J) EF (E), FS (F), left ventricular diameter during diastole (LVDd) (G), left ventricular diameter during systole (LVDs) (H), IVSd (I), and posterior wall thickness during diastole (PWTd) (J) in WT mice treated with AGK after trans-aortic constriction (TAC) according to the protocol in panel C (N=6–10). *p<0.05 by ANOVA for panels A–B or Student's t-test for panels E–J.