Trimetazidineprevents macrophage mediated septic myocardial dysfunction via Sirt1

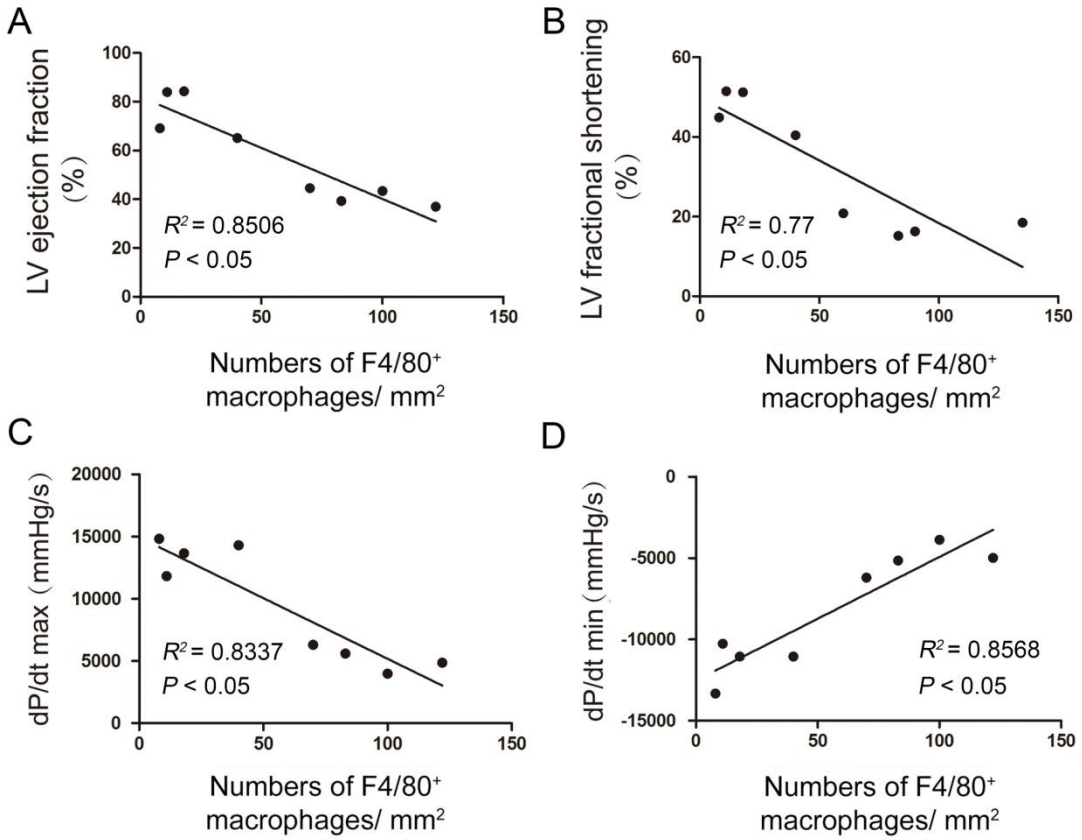

Figure S1. Cardiac function in recipient mice was association with the numbers of F4/80$^+$ macrophages in heart tissues. Scatter plot of cardiac function parameters (y-axis) and the numbers of F4/80$^+$ macrophages (x-axis) determined by immunohistochemical staining in heart tissues. (A) Correlation between LVEF values and the number of F4/80$^+$ macrophages. (B) Correlation between LVFS values and the number of F4/80$^+$ macrophages. (C) Correlation between dp/dt max values and the number of F4/80$^+$ macrophages. (D) Correlation between dp/dt min values and the number of F4/80$^+$ macrophages. Pearson's correlation coefficient was used to analyze the linear relationship between numbers of F4/80$^+$ macrophages and the performance of cardiac function (LVEF values, LVFS values, dp/dt max values and dp/dt min value).

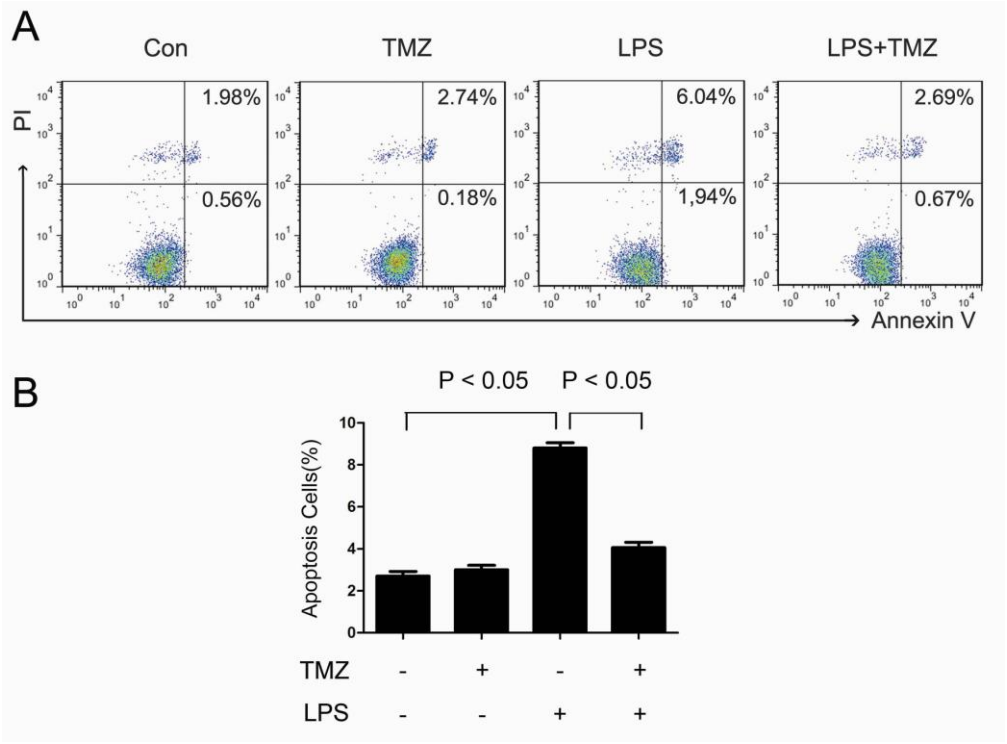

Figure S2. TMZ attenuated LPS-induced cardiomyocyte apoptosis. Neotanal cardiomyocyte were pretreated with TMZ (20 μM) for 1 hour and then stimulated with LPS (5 $\mu g \cdot ml^{-1}$) for 6 hours. (A) Flow cytometry detection of cardiomyocytes apoptosis was analyzed with annexin V and propidium iodide (PI). (B) The apoptosis rate of different groups and P values. Data were presented as mean ± SEM of three separate experiments. Data were analyzed by one-way ANOVA analysis using SPSS software.

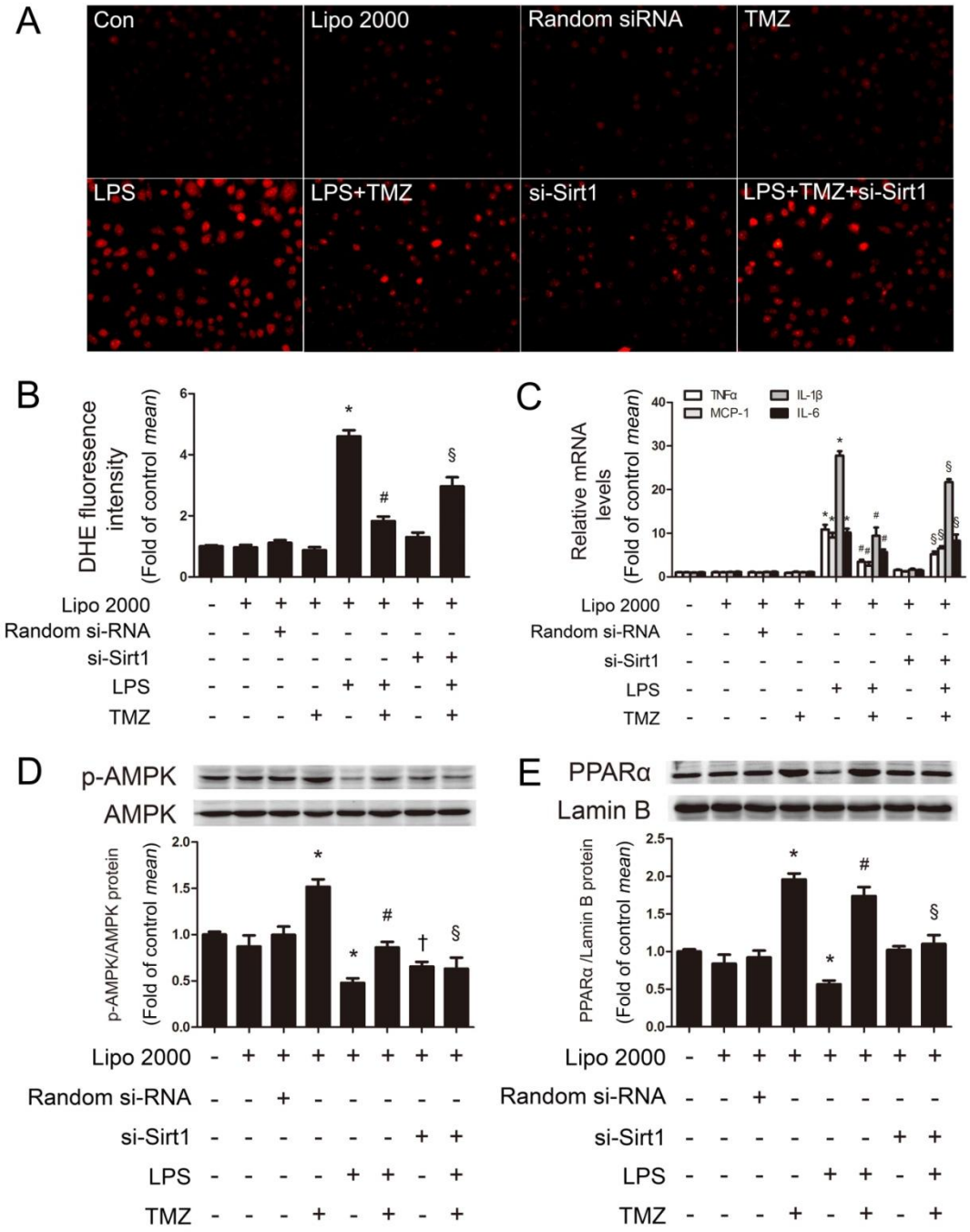

Figure S3. TMZ attenuated ROS-mediated macrophage proinflammatory response via Sirt1. Peritoneal macrophages were first transfected with si-Sirt1 or random siRNA using lipo 2000 for 24 hours. After transfection, cells were pretreated with TMZ (20 μM) for 1h and then stimulated with LPS (5 μg ml^{-1}) for 6h. (A) The ROS levels in macrophages were measured by DHE staining. (B) ROS productions were evaluated by qutification of mean fluorescence intensity in DHE staining. (C) RT-PCR revealed the expression of proinflammatory cytokines. Cell lysates were prepared and analyzed for p-AMPK (D) and nuclear PPARα (E) expression by western blotting. Values

below the western blots represent the densitometry analysis of p-AMPK/AMPK and nuclear PPARα/Lamin B. Data were presented as mean ± SEM of three separate experiments. Data were analyzed by one-way ANOVA analysis using SPSS software (*$p < 0.05$ vs. control; $^{\#}p < 0.05$ vs. LPS; $^{\S}p < 0.05$ vs. LPS + TMZ treated group; $^{\dagger}p < 0.05$ vs. Lipo 2000 control).

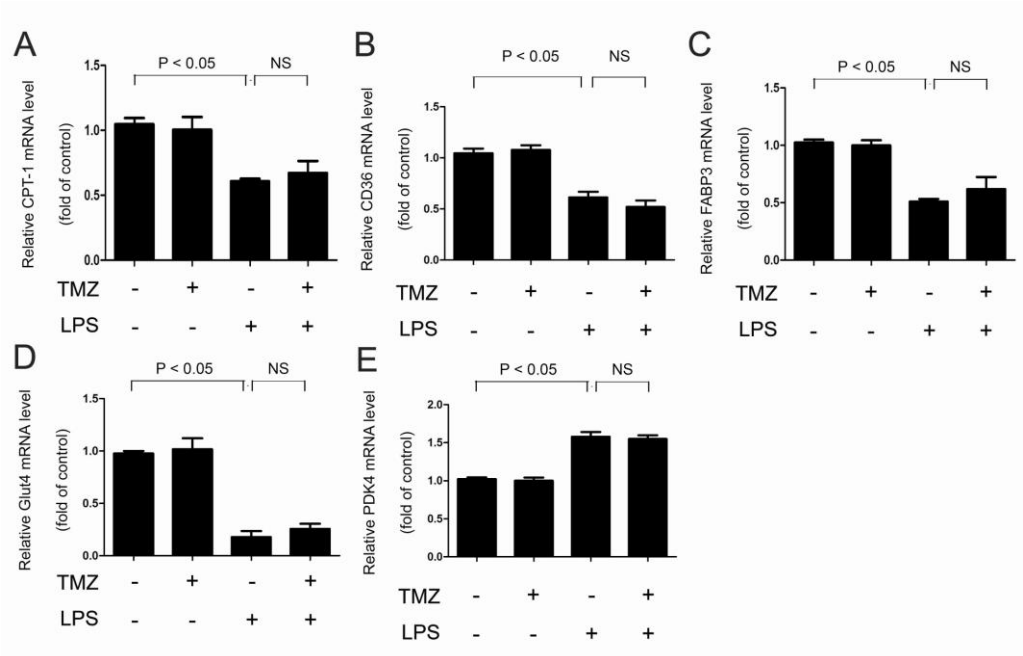

Figure S4. TMZ did not affect the shift from fatty acids to glucose metabolism in LPS-stimulated heart. C57BL/6 mice were pretreated with TMZ (20 mg • kg^{-1}, i.g, Tid) or saline three times a day for 3 days, followed by i.p injection of LPS (15mg kg^{-1}) (n=8 in each group). The mice were sacrificed 6 hours after LPS stimulation and the heart tissue were collected to prepare RNA samples. (A-F) RT-PCR analysis showed the relative mRNA levels of CPT-1, CD36, FABP3, GLUT4 and PDK4. All values were normalized internally to 18S RNA expression and to the normal control sets. Data were presented as mean ± SEM of three independent experiments. Data were analyzed by one-way ANOVA analysis using SPSS software.