A novel S100A-TLR3-IFIT3 signaling axis promotes cardiomyocyte apoptosis during myocardial infarction

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Figure Legend

Supplement Figure 1. a. LVPWd: LV posterior wall thickness in diastole. **b.** LVPWs: LV posterior wall thickness in systole. **c.** The statistical results of IFIT3 expression in the infarcted area of the left ventricle of rats. Data were represented as mean±SEM, unpaired t-test, compared to the sham group, n=6.

Supplement Figure 2. Western blot analysis the expression levels of IFIT3 **(a)**, CASP3 **(b)**, Cleaved CASP3 **(c)** and Cleaved CASP3/CASP3 **(d)**. These data were represented as mean±SEM, unpaired t-test, n=4, compared to the rest group. **e.** AC-16 apoptosis induced by persistent oxygen-glucose deprivation for 2h. The apoptosis was detected by flow cytometry and about 20,000 cells were detected **(e). (f)** The analysis revealed significant changes in gene expression in the IFIT3 knockout AC-16 cells following 2 hours of persistent OGD. Genes upregulated are indicated in red, downregulated genes in blue, and genes without statistically significant differences in gray (n=3). Significant genes are fold change over 2. All results were used t-test and expressed mean±SEM, compared with the control group (WT), n=3. **(g)** GSEA indicated that the Hallmark apoptosis pathway was significantly enriched.

Supplement Figure 3. Apoptosis induced by carbonyl cyanide m-chlorophenyl hydrazone (CCCP) was investigated in IFIT3 knockout cardiomyocytes. Compared to

the wild-type (WT) control group, the knockout of IFIT3 significantly reduced cardiomyocyte apoptosis induced by CCCP at concentrations of 2.5 μM and 5.0 μM. **(a)** Apoptosis induced by CCCP at 2.5 μM was measured using flow cytometry. **(b)** Apoptosis induced by CCCP at 5.0 μM was also assessed via flow cytometry. Approximately 10,000 cells were analyzed in each experiment. All results were statistically analyzed using a t-test and are expressed as the mean ± standard error of the mean (SEM), with comparisons made to the wild-type group (n=3).

Supplement Figure 4. a. The ratio of Cleaved caspase3/caspase 3 at overexpression IFIT3 48h. All results were statistically analyzed using a t-test and are expressed as the mean ± standard error of the mean (SEM), with comparisons made to the pc 3.1 DNA-vector (n=3). **b.** The ratio of Cleaved caspase8/caspase 8 at overexpression IFIT3 48h. the result was statistically analyzed using a t-test and are expressed as the mean ± standard error of the mean (SEM), with comparisons made to the pc 3.1 DNA-vector group (n=3). **c.** The ratio of Cleaved caspase9/caspase 9 at overexpression IFIT3 48h. The result was statistically analyzed using a t-test and are expressed as the mean ± standard error of the mean (SEM), with comparisons made to the pc 3.1 DNA-vector group (n=3).

Supplement Figure 5. Interferons induce the IFIT3 expression. a. Western blot analysis detected IFIT3 expression in AC-16 cells following induction with type I

interferons, specifically IFN-α-1b, IFN-α-2a, and IFN-α-2b (10,000 IU/L) for 24 hours, with n=3. **b.** The detection and quantification of IFIT3 expression induced by IFN-α-1b at concentrations of 100, 1,000, and 10,000 IU/L were performed in HL-1 cells, with n=3. **c.** Western blot analysis detected IFIT3 expression in HL-1 cells following induction with IFN-α-2b at concentrations of 100, 1,000, and 10,000 IU/L for 24 hours, with n=3. **d.** The expression level of IFIT3 in HEK293 cells was detected and quantified after induction with IFN-α-1b, IFN-α-2a, and IFN-α-2b (10,000 IU/L) for 24 hours, with n=3. **e.** Whole transcriptome analysis of IFN mRNA expression was conducted following 2 hours of glucose and oxygen deprivation. At this time point, no significant differences were observed in the mRNA expression of all interferons (n=3). All results were analyzed using the t-test and are presented as mean ± SEM, compared to the control group (Ctrl).

Supplement Figure 6. S100 family proteins induced IFIT3 overexpression in mouse cardiac fibroblasts and HEK293. a. The expression levels of IFIT3 induced by S100 proteins (2.0 μg/mL: S100A1, S100A6, S100A8, S100A9, and S100B) were detected and quantified in MCFs after 24 hours (n=6). **b.** The expression levels of IFIT3 induced by S100 proteins (2.0 μg/mL: S100A1, S100A6, S100A8, S100A9, and S100B) were also detected and quantified in HEK293 cells after 24 hours (n=4). **c-n.** The quantitative analysis of TLR3/4 signaling pathway activation in AC-16 cells following the induction of S100A1 (c-n) and S100A9 (i-n) (2.0 μg/mL, for 2 to 24 hours). All results were analyzed using the t-test and are presented as mean ± SEM, compared to

the control group (Ctrl), n=3 or n=4. Statistical significance is indicated as follows: * $p < 0.05$; ** $p < 0.01$, **** $p < 0.0001$.

Supplement Figure 7. S100A1 and S100A9 stimulated IFIT3 overexpression and induced AC-16 cells apoptosis. **a-d.** Western blot analysis was conducted to quantitatively assess the expression levels of CASP3 **(a)** and Cleaved-CASP3 **(b)** in AC-16 cells treated with S100A1 (2.0 μg /mL, panels **a, b**) or S100A9 (2.0 μg/mL, panels **c, d**). **e-j.** The effect of IFIT3 knockout on the expression of CASP3 and Cleaved-CASP3 induced by S100A1 (panels **e-g**) and S100A9 (panels **h-j**) was evaluated. Statistical analyses were performed using a t-test or ANOVA test, and results were expressed as mean ± SEM (n>3). Significance was determined at * $p < 0.05$; ** $p < 0.01$, **** $p < 0.0001$ compared with the control group (Ctrl).