

**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 μ M and 5.0 μ M. **(a)** Apoptosis induced by CCCP at 2.5 μ M was measured using flow cytometry. **(b)** Apoptosis induced by CCCP at 5.0 μ M 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 \pm 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 \pm 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 \pm 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 \pm 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

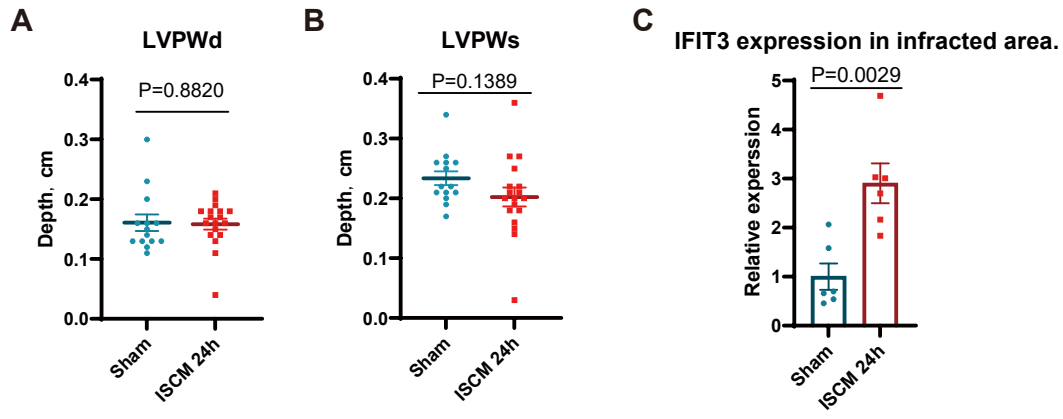
interferons, specifically IFN- α -1b, IFN- α -2a, and IFN- α -2b (10,000 IU/L) for 24 hours, with n=3. **b.** The detection and quantification of IFIT3 expression induced by IFN- α -1b at concentrations of 100, 1,000, and 10,000 IU/L were performed in HL-1 cells, with n=3. **c.** Western blot analysis 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 \pm 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 μ g/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 μ g/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 μ g/mL, for 2 to 24 hours). All results were analyzed using the t-test and are presented as mean \pm 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.

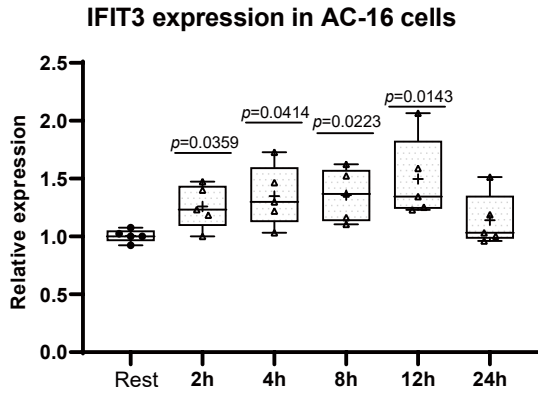
Supplement Figure 7. S100A1 and S100A9 stimulated IFIT3 overexpression and induced AC-16 cells apoptosis. **a-d.** Western blot analysis was conducted to quantitatively assess the expression levels of CASP3 (**a**) and Cleaved-CASP3 (**b**) in AC-16 cells treated with S100A1 (2.0 µg /mL, panels **a, 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).

S.Figure 1

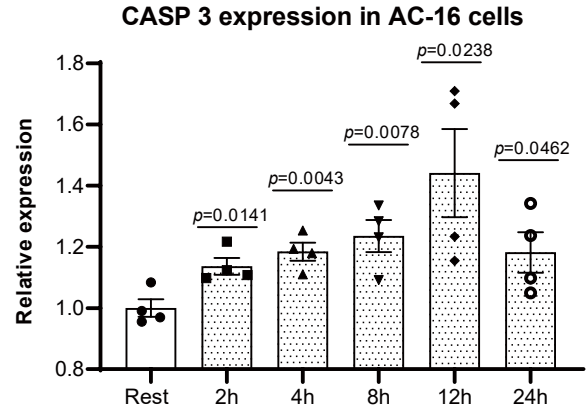


S.Figure 2

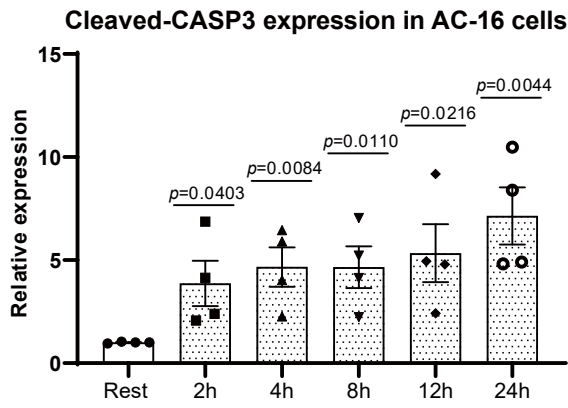
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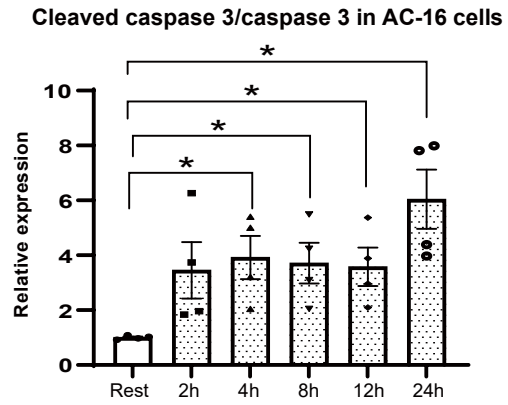
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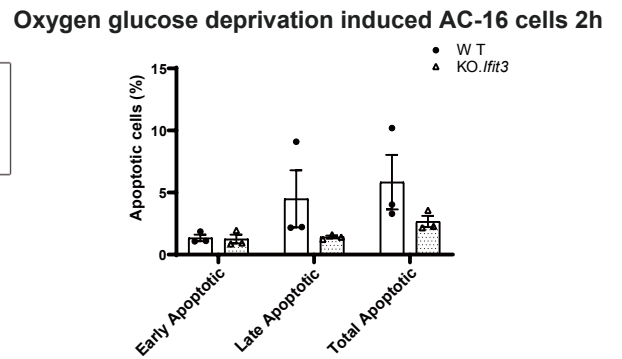
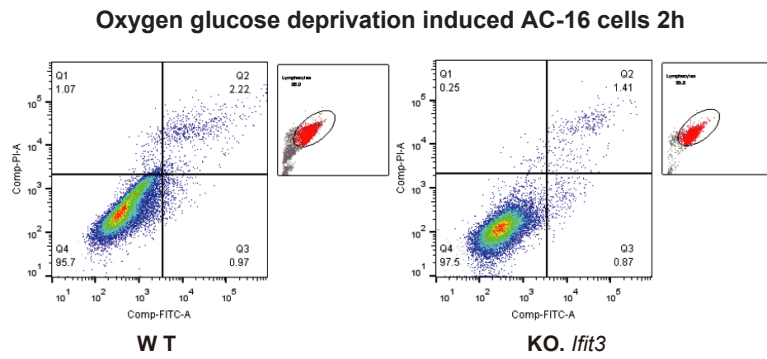
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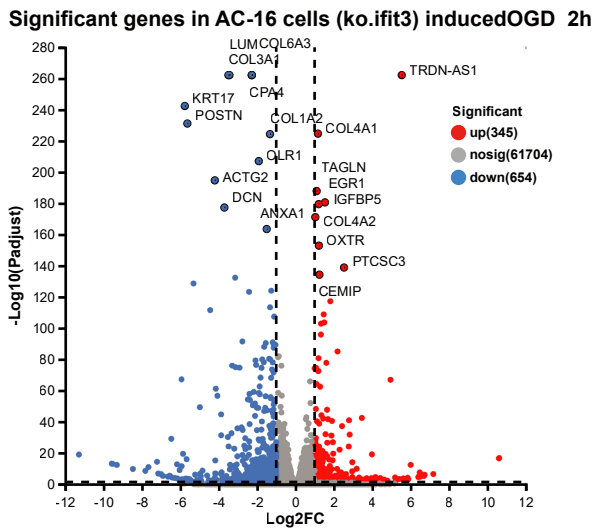
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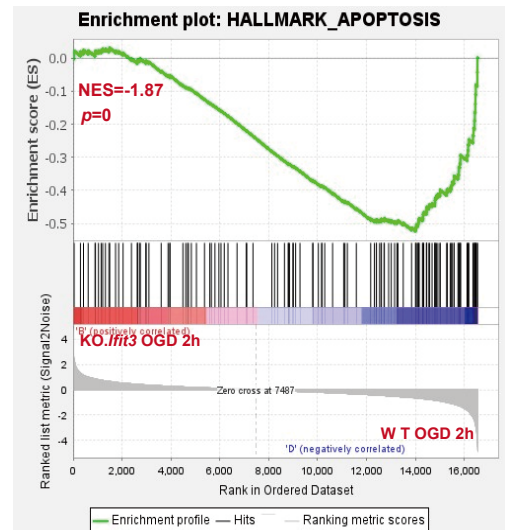
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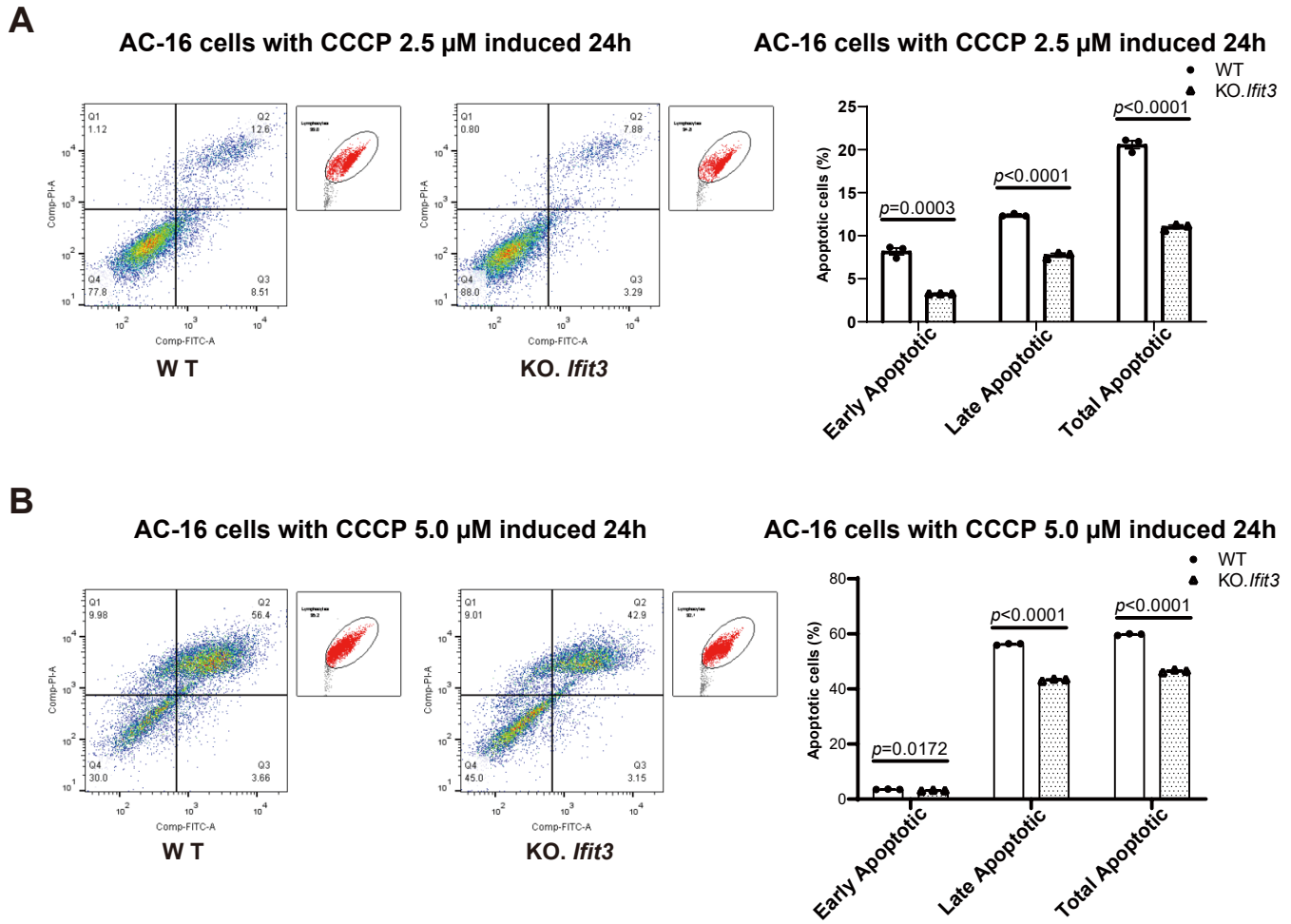
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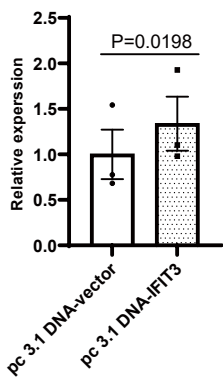
S Figure 3



S. Figure 4

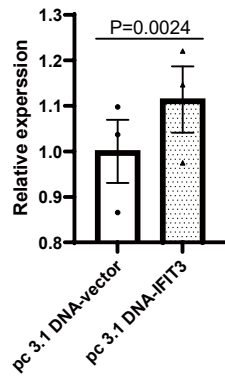
A

Cleaved caspase3/caspase 3

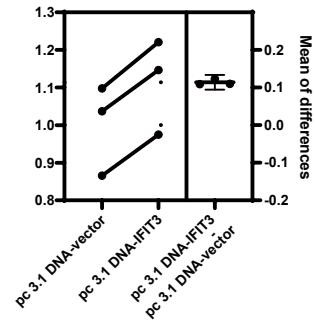


B

Cleaved caspase8/caspase 8

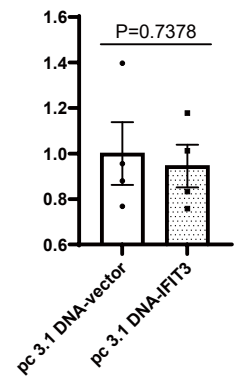


Cleaved caspase8/caspase 8

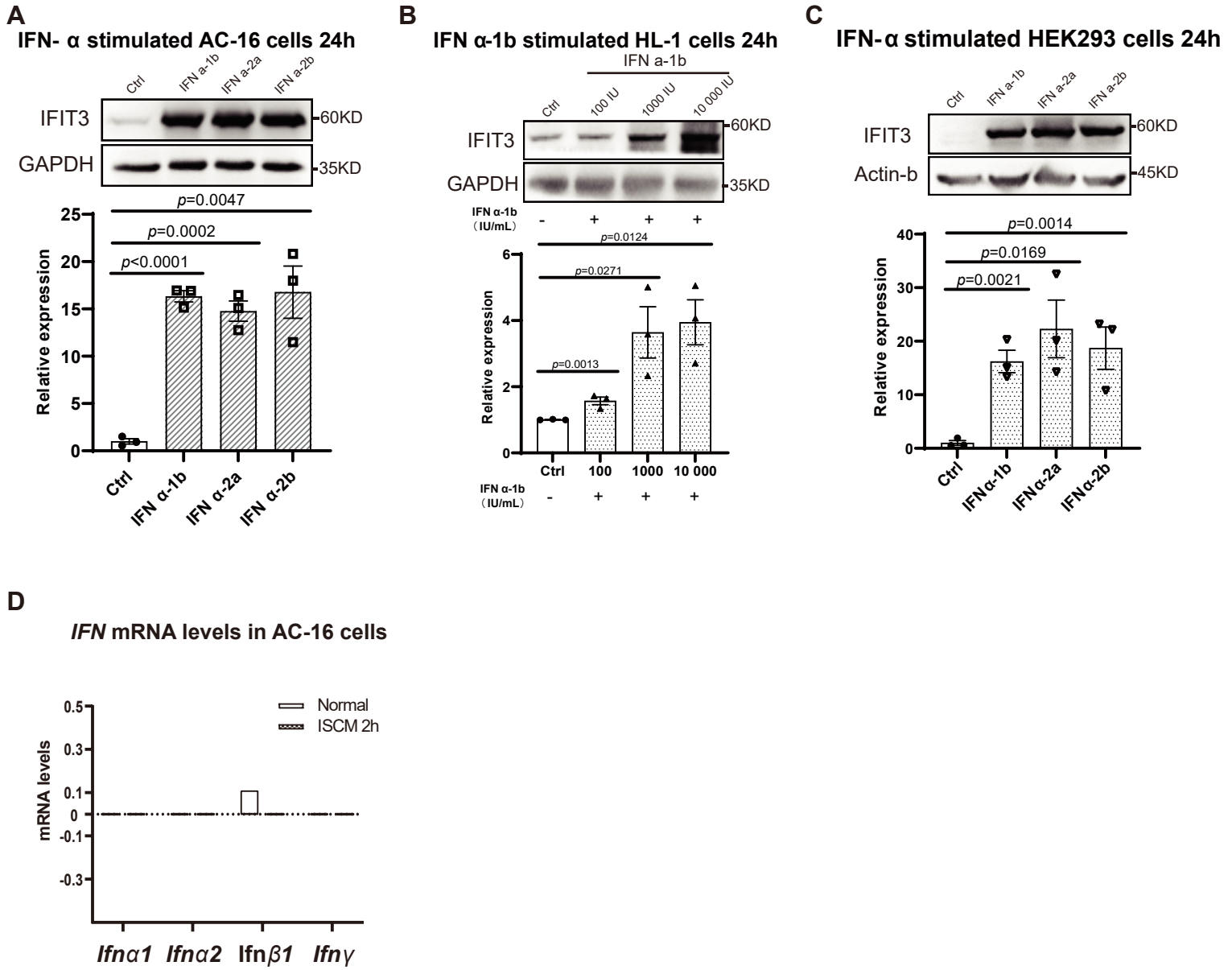


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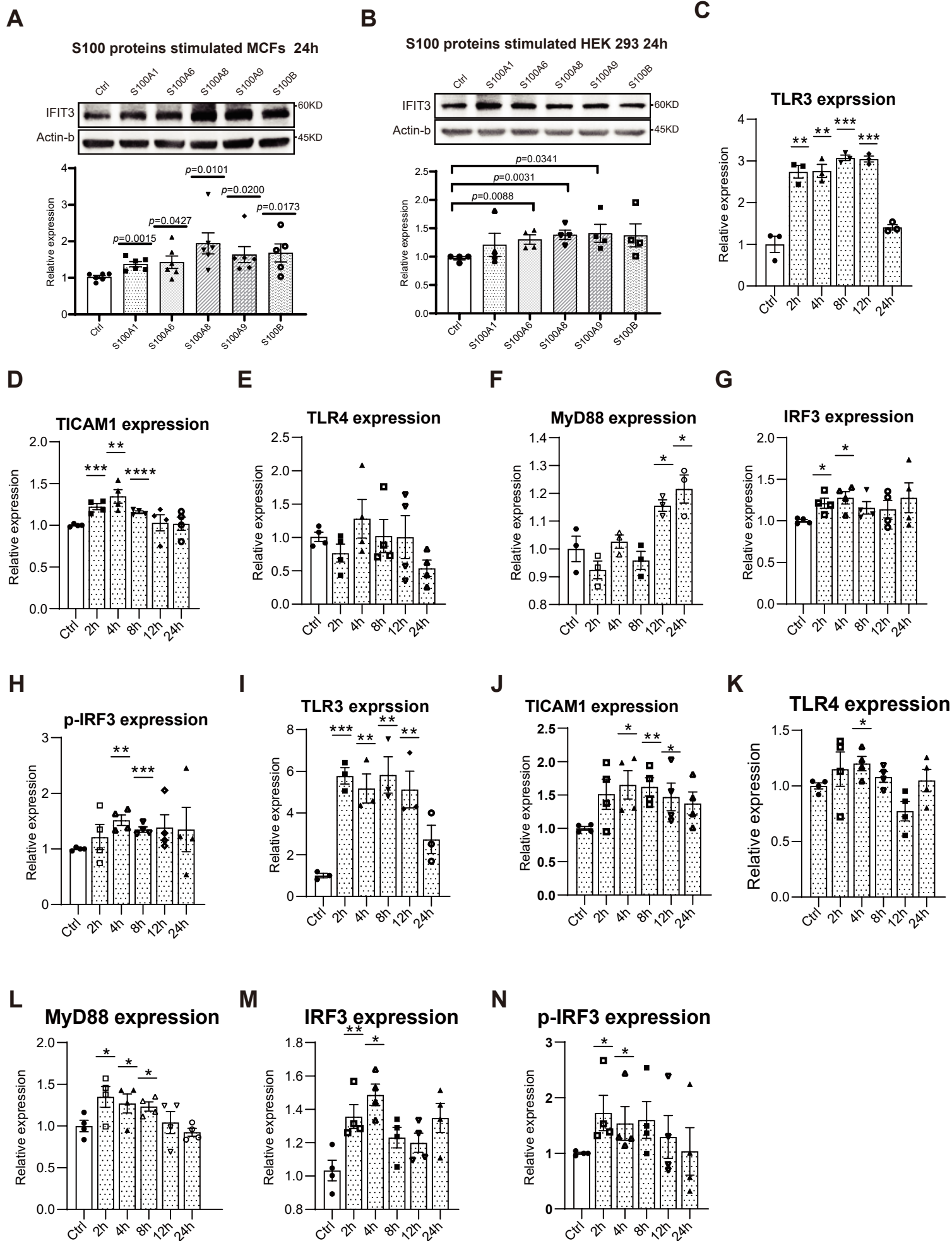
Cleaved caspase9/caspase 9



S Figure 5



S Figure 6



S Figure 7

