Supporting Information for

Original article

Cardiac fibroblast heat shock protein 47 aggravates cardiac fibrosis post myocardial ischemia–reperfusion injury by encouraging ubiquitin specific peptidase 10 dependent Smad4 deubiquitination

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Requests by researchers to access the data, analytic methods, and study materials for the purposes of reproducing the results or replicating procedures can be made to the corresponding author who manages the information.

1. **Supporting Figures S1–S7**
2. **Supporting Tables S1–S2**

1. Supporting figures

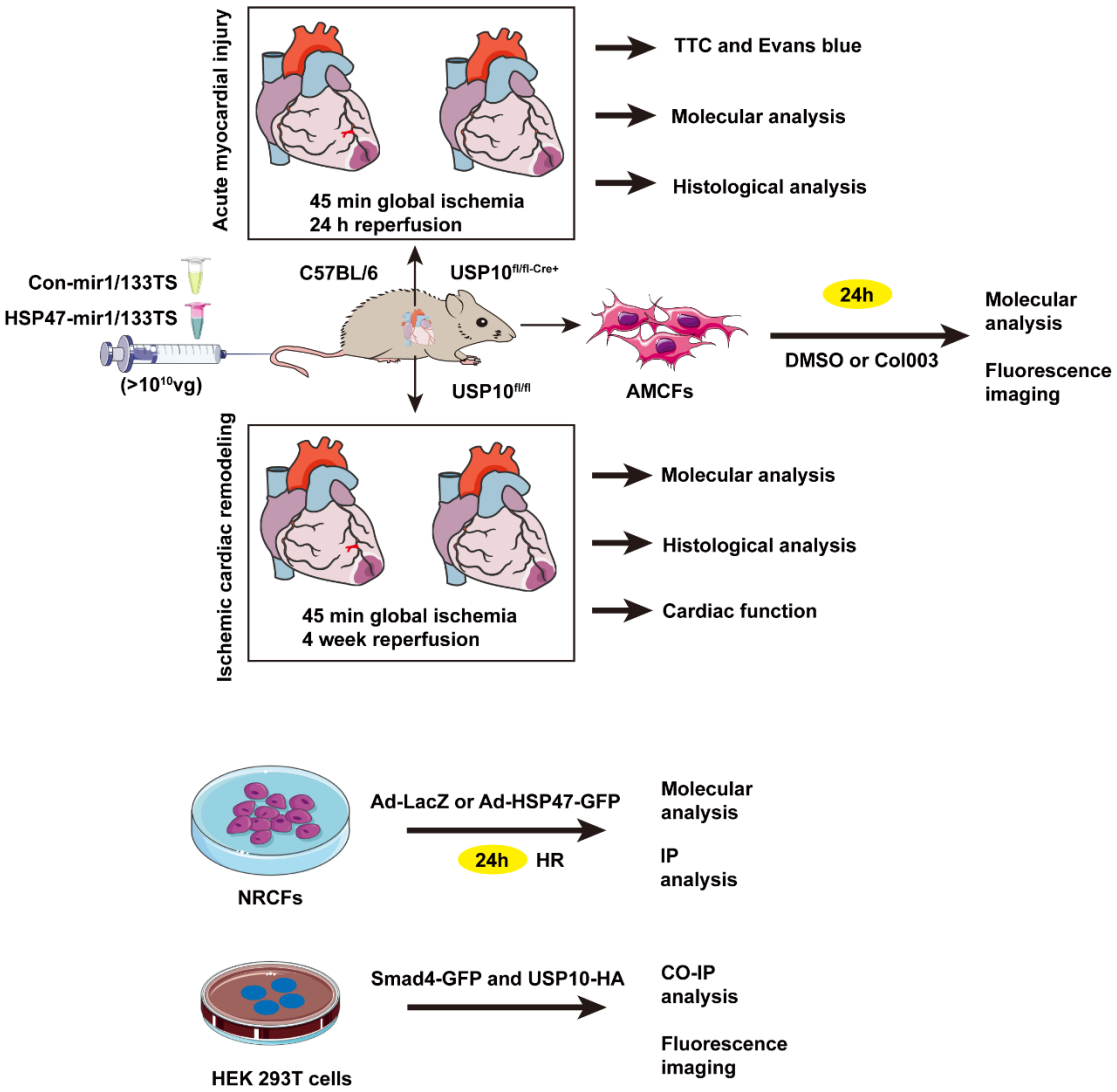

Figure S1 Protocol *in vivo* and *in vitro*.

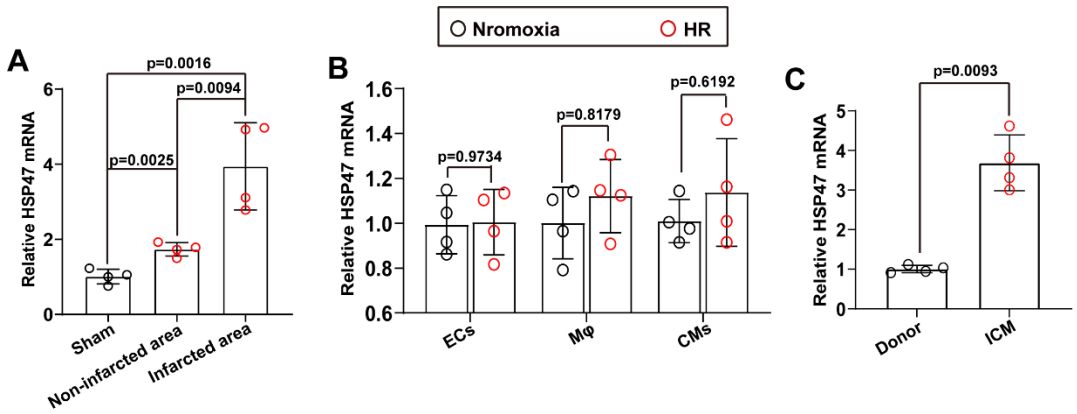

Figure 2 HSP47 expression. Related to Fig. 1 in main manuscript. (A) Total RNA isolated from indicated area was utilized for qPCR analyses. Normalized to *β*-tubulin. *n*=4 per group. Differences assessed by Student's *t* test. (B) Total RNA isolated from human umbilical vein endothelial cells (HUVECs), mouse peritoneal macrophages (*Mφ*) and cardiomyocytes were utilized for qPCR analyses. Normalized to *β*-tubulin. *n*=4 per group. Differences assessed by Student's *t* test. (C) Total RNA isolated from heart tissues of donor or ischemic cardiomyopathy (ICM) was used for qPCR analyses. Normalized to *β*-tubulin. *n*=4 per group. Differences assessed by Student's *t* test. Data are presented as the mean ± SEM, with each point represented a heart sample or a cell sample.

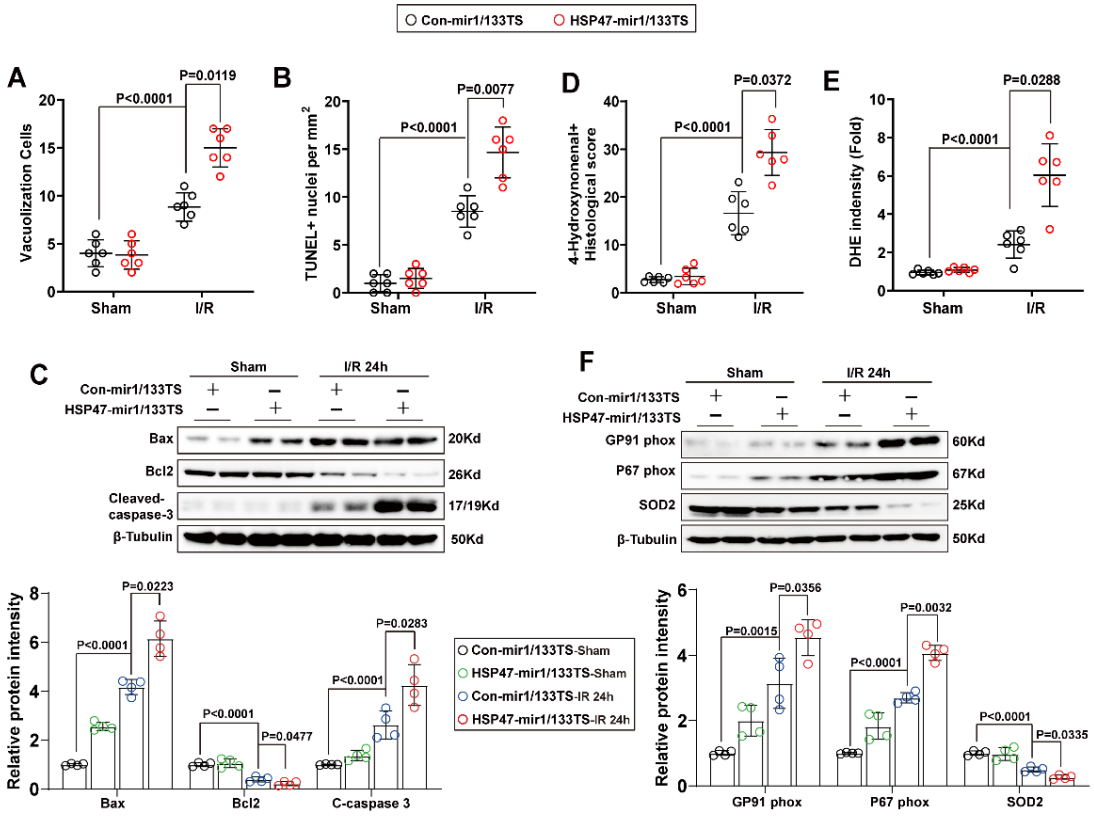

Figure S3 HSP47 overexpression in CFs accelerates apoptosis and oxidative stress in acute myocardial injury post ischemia reperfusion. Related to Fig. 2 in main manuscript. (A) Calculation of vacuolated cardiomyocytes in heart slices from indicated group. *n*=6 per group. Differences assessed by two-way ANOVA and Tukey's multiple comparison test. (B) TUNEL positive cells were counted. *n*=6 per group. Differences assessed by two-way ANOVA and Tukey's multiple comparison test. (C) Heart tissue homogenate from indicated mice was harvested for protein isolation and immunoblotting was used for Bax, cleaved caspse-3 and Bcl2 analyses, and subsequently offered quantitative analysis by image lab software. Normalized to *β*-tubulin. *n*=4 per group. Differences assessed by tow-way ANOVA and Tukey's multiple comparison test. (D) Histological score was evaluated by image pro plus 6.0. *n*=6 per group. Differences assessed by two-way ANOVA and Tukey's multiple comparison test. (E) DHE density evaluated by image pro plus 6.0. *n*=6 per group. Differences assessed by two-way ANOVA and Tukey's multiple comparison test. (F) Heart tissue homogenate from indicated mice was harvested for protein isolation and immunoblotting was used for Gp91 phox, P67 phox and Sod2 analyses, and subsequently offered quantitative analysis by image lab software. Normalized to *β*-tubulin. *n*=4 per group. Differences assessed by tow-way ANOVA and Tukey's multiple comparison test. Data are presented as the mean ± SEM, with each point represented a mouse.

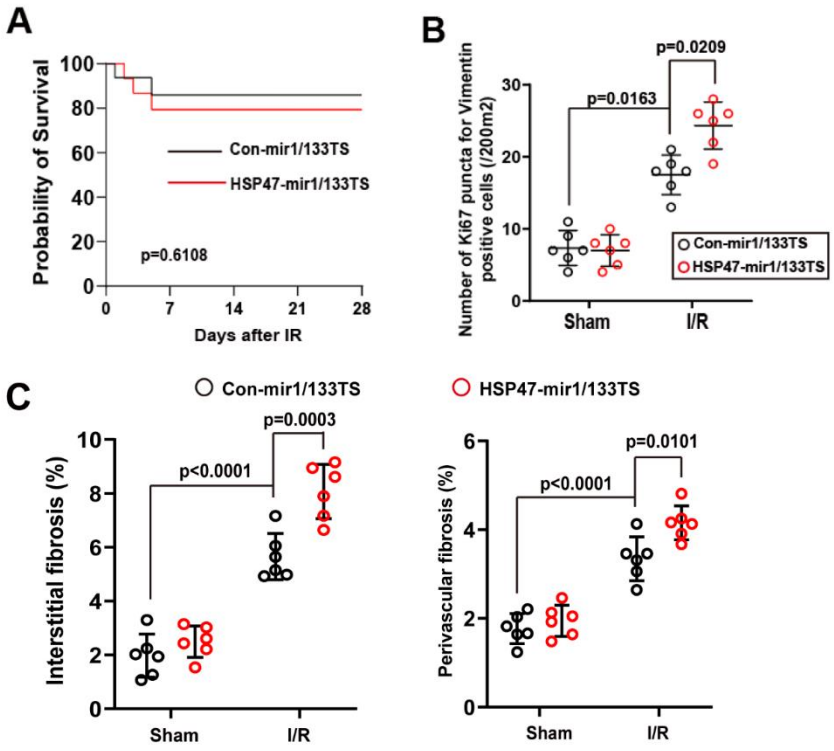

Figure S4 HSP47 promotes cell proliferation in myocardium following chronic myocardial IRI. Related to Fig. 4 in main manuscript. (A) Kaplan–Meier survival analysis of mice treated with Con-miR1/133TS or HSP47-miR1/133TS in 4 weeks after myocardial IRI. Differences assessed by Log-rank (Mantel-Cox) test. (B) Number of ki67 puncta for vimentin positive cells in heart sections, related to figure 4H (*n*=6). (C) Quantitative analyses of perivascular and Interstitial collagen volume. *n*=6 per group. Differences assessed by two-way ANOVA and Tukey's multiple comparison test. Data are presented as the mean ± SEM, with each point representing a mouse.

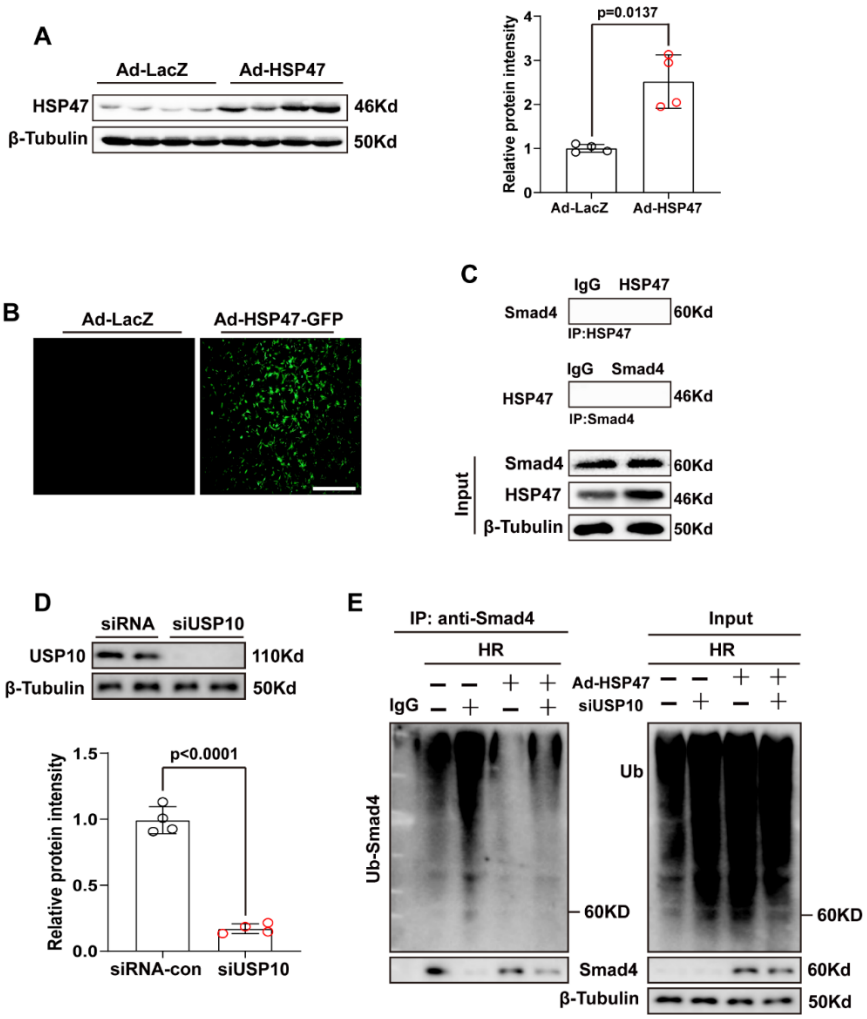

Figure S5 Verification of HSP47 overexpression in NRCFs. Related to Fig. 6 in main manuscript. (A) Proteins obtained from NRCFs transfected with Ad-HSP47-GFP or Ad-LacZ for 24 h and immunoblotting were used for HSP47 analyses, and subsequently offered quantitative analysis by image lab software. Normalized to *β*-tubulin. *n*=4 per group. Differences assessed by Student's *t* test. (B) After transfected with Ad-HSP47-GFP or Ad-LacZ for 24h, NRCFs were detected by fluorescence microscope. Scar bar=200μm. (C) NRCF lysates were immunoprecipitated with anti-Smad4 or HSP47 antibody and probed with anti-HSP47 or anti-smad4 antibody. *n*=3 independent experiments. (D) Proteins obtained from NRCFs transfected with Con-siRNA or siUSP10 for 24 h and immunoblotting were used for USP10 analyses, and subsequently offered quantitative analysis by image lab software. Normalized to *β*-tubulin. *n*=4 per group. Differences assessed by Student's *t* test. (E) Ubiquitination of Smad4 was detected by immunoprecipitation in NRCFs transfected with Ad-HSP47 or siUSP10. Data are presented as the mean ± SEM.

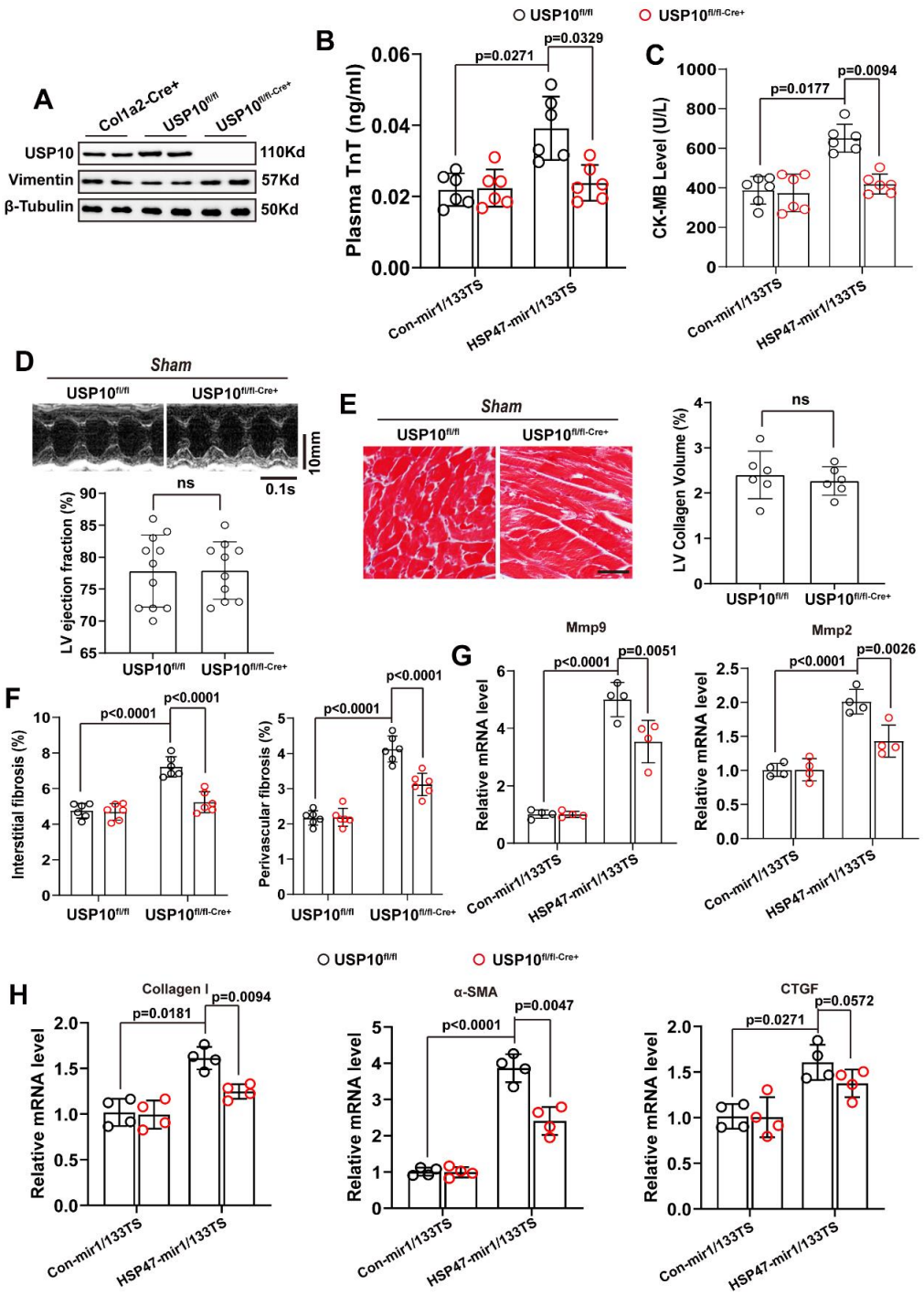

Figure S6 HSP47 overexpression promoted myocardial injury and fibrosis in myocardial IRI in USP10 dependent manner. Related to Fig. 7 in main manuscript. (A) USP10 expression was measured in cardiac fibroblasts from *Col1a2*$^{Cre+}$, *USP10*$^{fl/fl}$ and *USP10*$^{fl/fl\text{-}Cre+}$ mice by Western blot. *n*=4 per group. (B, C) The enzyme activity of CK-MB and TnT in serum were accessed in *USP10*$^{fl/fl}$ and *USP10*$^{fl/fl\text{-}Cre+}$ mice underwent sham or IR operation for 24 h by Elisa assay. *n*=6 per group. Differences assessed by two-way ANOVA and Tukey's multiple comparison test. (D) Representative M-mode echocardiography was recorded and LV ejection fraction (LVEF) was analyzed in

USP10$^{fl/fl}$ and *USP10*$^{fl/fl\text{-}Cre+}$ mice underwent sham. n=11–12 per group. (E) Representative Masson staining of heart slices from indicated mice and quantitative analyses of LV collagen volume. n=6 per group. Scar bar=80 μm. (F) Quantitative analyses of perivascular and interstitial collagen volume. n=6 per group. (G) Total RNA from *USP10*$^{fl/fl}$ and *USP10*$^{fl/fl\text{-}Cre+}$ mice underwent sham or IR operation for 4 weeks were performed to measure *Mmp9* and *Mmp2* by qPCR. Normalized to β-tubulin. n=4 per group. (H) Total RNA from *USP10*$^{fl/fl}$ and *USP10*$^{fl/fl\text{-}Cre+}$ mice underwent sham or IR operation for 4 weeks were performed to measure *collagen I*, *CTGF* and *α-SMA* by qPCR. Normalized to β-tubulin. n=4 per group. Differences assessed by two-way ANOVA and Tukey's multiple comparison test. Data are presented as the mean ± SEM, with each point representing a mouse.

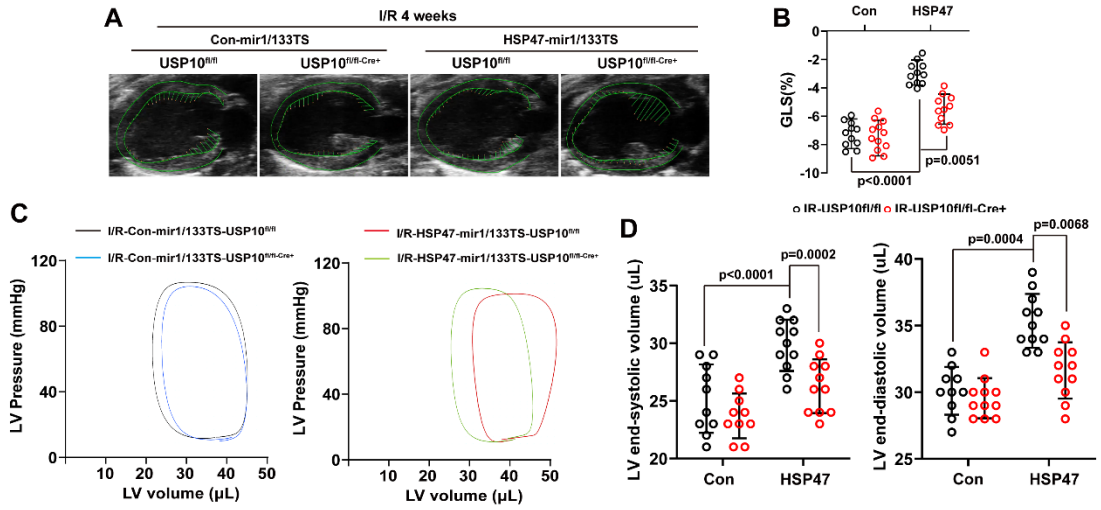

Figure S7 HSP47 overexpression promoted myocardial injury and fibrosis in myocardial IRI in USP10 dependent manner. Related to Figure 7 in main manuscript. (A) Representative ventricular wall motion echocardiography was recorded. (B) Global longitudinal strain (GLS) was measured by spot tracking technology. n=11–12 per group. Differences were assessed by two-way ANOVA and Tukey's multiple comparison test. (C) Representative PV loops of Con-miR1/133TS or HSP47-miR1/133TS pretreated *USP10*$^{fl/fl}$ or *USP10*$^{fl/fl\text{-}Cre+}$ mice following I/R for four weeks. (D) Measurement of end-systolic volume (ESV) and end-diastolic volume (EDV). n=10–11 per group. Differences assessed by two-way ANOVA and Tukey's multiple comparison test.

1. Supporting tables

Table S1 Primary antibodies used in current study.

Antibody	Customer	Product number	Dilution	Application
Collagen 1	Abcam	Ab90395	1:200	IF
			1:1000	WB
			1:100	IHC
β-Tubulin	Abcam	Ab6046	1:1000	WB
HSP47	Abcam	Ab109117	1:1000	WB
			1:200	IF
			1:100	IHC
GFP	Invitrogen	MA5-15256	1:1000	WB
4-HNE	Abcam	Ab46545	1:100	IHC
*α*SMA	Abcam	Ab5659	1:1000	WB
			1:250	IF
Fibronectin	Proteintech	15613-1-AP	1:500	WB
USP10	Invitrogen	MA5-25766	1:1000	WB
VE-cadherin	Abcam	Ab33168	1:1000	WB
			1:200	IF
Vimentin	Abcam	Ab8978	1:1000	WB
TGF*β*1	Abcam	Ab64715	1:1000	WB
p-Smad2/3	Santacruze	sc-11769	1:1000	WB
t-Smad2/3	Abcam	Ab217553	1:1000	WB
p-Smad4	Invitrogen	PA5-106038	1:1000	WB
t-Smad4	Abcam	Ab40759	1:1000	WB
Ubiquitin	CST	3936	1:1000	WB
Flag	CST	14793	1:2000	WB
			1:250	IF
HA	CST	3724	1:2000	WB
			1:250	IF

Table S2 Primers used in qPCR.

Gene	Species	Forward primer (5′→3′)	Reverse primer (5′→3′)
Serpinh1	Mouse	AGTTTCTTGGGACAGGCAGG	CCAATGCGCAACCCCAAATC
Anp	Mouse	CAACACAGATCTGATGGATTTCA	CCTCATCTTCTACCGGCATC
Bnp	Mouse	GTCAGTCGTTTGGGCTGTAAC	AGACCCAGGCAGAGTCAGAA
Myh6	Mouse	CGCATCAAGGAGCTCACC	CTGCAGCCGCAGTAGGTT
TGFβ1	Mouse	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
TGFβ3	Mouse	CCTGGCCCTGCTGAACTTG	TTGATGTGGCCGAAGTCCAAC
Fibulin2	Mouse	CTGTGAAGACCAAGACGAGTG	CGTTGAGGATATAGCCCTCTGC
Postn	Mouse	CGGGAAGAACGAATCATTACA	ACCTTGGAGACCTCTTTTTGC
Ctgf	Mouse	AGCGGTGAGTCCTTCCAAAG	TTCATGATCTCGCCATCGGG
Loxl1	Mouse	GAGTGCTATTGCGCTTCCC	GGTTGCCGAAGTCACAGGT
Loxl2	Mouse	ATTAACCCCAACTATGAAGTGCC	CTGTCTCCTCACTGAAGGCTC
Sparc	Mouse	GTGGAAATGGGAGAATTTGAGGA	CTCACACACCTTGCCATGTTT
Nupr1	Mouse	CCCTTCCCAGCAACCTCTAAA	TCTTGGTCCGACCTTTCCGA
Cola1a1	Mouse	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
β-Tubulin	Mouse	TTCCTGCCCTTGTGCCTTAG	GGTGGATTTTAGGGAGGGGC
Serpinh1	Rat	CCCAGCCCTCACAGGTCC	GAAGCCACGGTTGTCTACCA
β-Tubulin	Rat	TCGATGACGTGATGTGATGCT	GGACACAAAGGTTCAGGCGA
Serpinh1	Human	CCAGCCCGACCCAGAATGAA	TCTCATCCCAGTGTGGCTTG
β-Tubulin	Human	CCACACCCACCTATGGTGAC	CATACTCCTTCTCCCTCGGC