Supplementary information

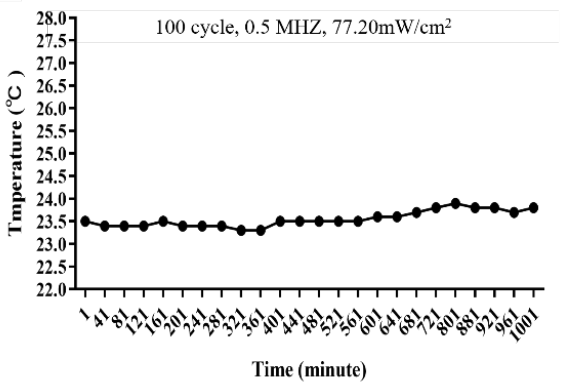

Fig. S1 Results of the temperature test. We used a temperature test paper (TMCHallcrest, USA) to test the temperature of the culture media in the cell dishes placed at room temperature for 6 h, and the results showed that there were hardly any changes in temperature under our ultrasound conditions during LIPUS procedures.

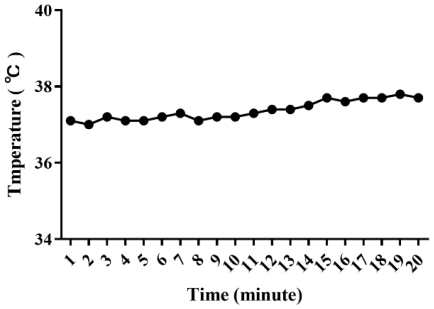

Fig. S2 Results of the temperature test. We used a temperature test paper (TMCHallcrest, USA) to test the temperature of the culture media in the cell dishes placed in the 37 ℃ incubator, and the results showed that there were hardly any changes in temperature under our ultrasound conditions during LIPUS procedures.

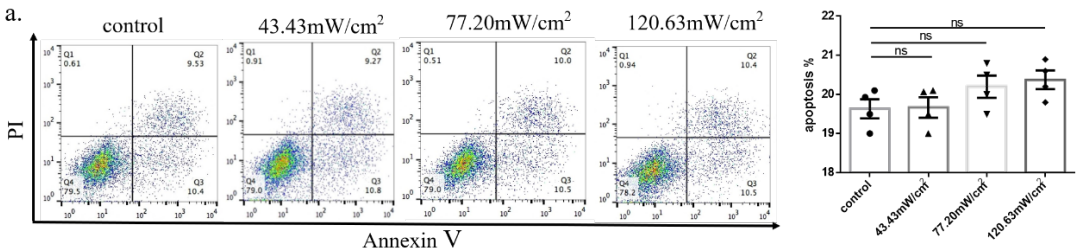

Fig. S3 Apoptotic effects of LIPUS on cardiomyocytes. The results of flow cytometry on apoptosis in cardiomyocytes stimulated by different doses of ultrasound intensities (left) and the corresponding quantitative analysis (right); *n*>3 per group. The results are expressed as the mean±SEM (ns indicates not significant, **P*<0.05, ***P*<0.01, ****P*<0.001, compared with the control or model group).

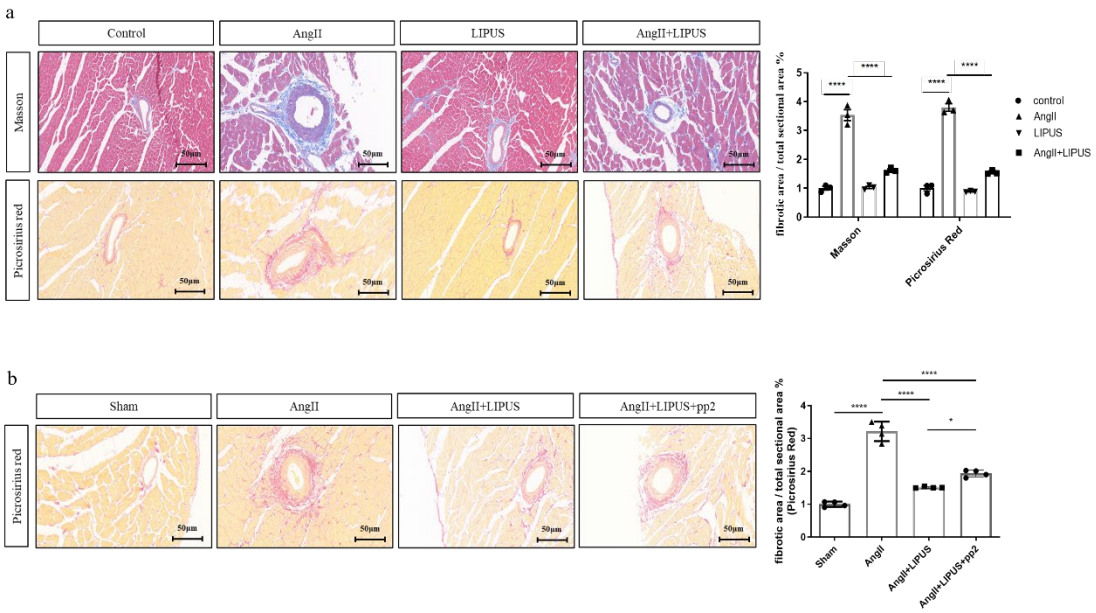

Fig. S4 Role of LIPUS irradiation in AngII-induced coronary artery fibrosis. (a) Representative images of sections of coronary arteries stained with Masson's method (scale bar, 50 μm) (top) and picrosirius red (scale bar, 50 μm) (bottom) from the different groups (*n*=3 per group) (left), and the corresponding quantitative analysis. (b) Representative images of sections of coronary arteries stained with picrosirius red (scale bar, 50 μm) from the different groups (*n*=3 per group), and the corresponding quantitative analysis. The results are expressed as the mean±SEM (NS indicates not significant, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001).

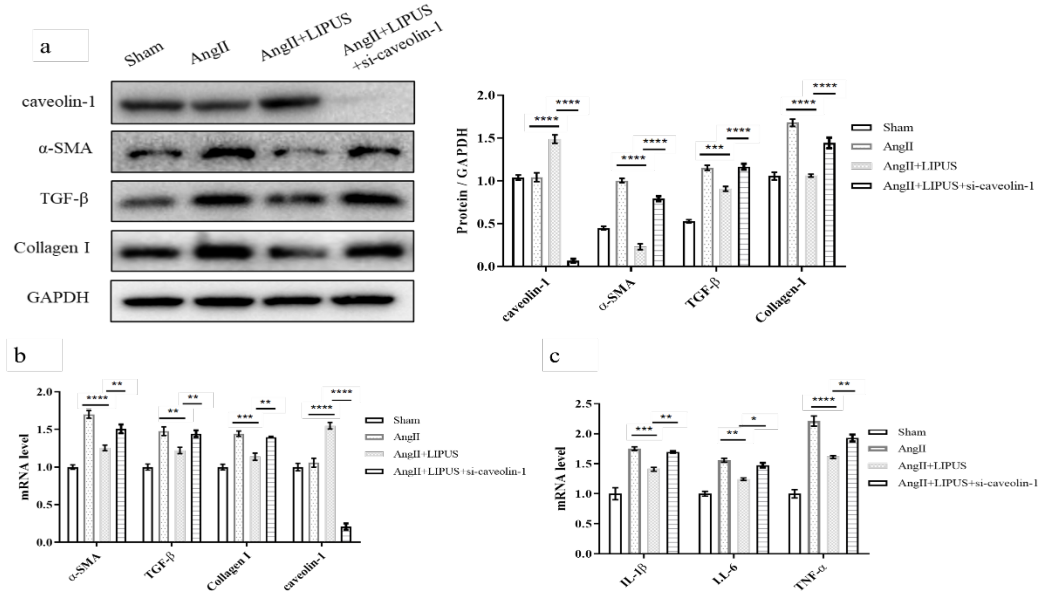

Fig. S5 Role of si-caveolin-1 in the antifibrotic effects of LIPUS irradiation on AngII-induced cardiac fibrosis. (a) Protein expression levels of caveolin-1, α-SMA, TGF-β, and Collagen I in cardiac fibroblasts from different groups (left) and the corresponding densitometric analysis. (b, c) The mRNA expression of α-SMA, TGF-β, Collagen I, and caveolin-1 (b) as well as IL-1β, IL-6, and TNF-α (c) in cardiac fibroblasts from different groups. *n*=3 per group, all mRNA and protein expression levels were normalized to GAPDH. The results are expressed as the mean±SEM (NS indicates not significant, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001).

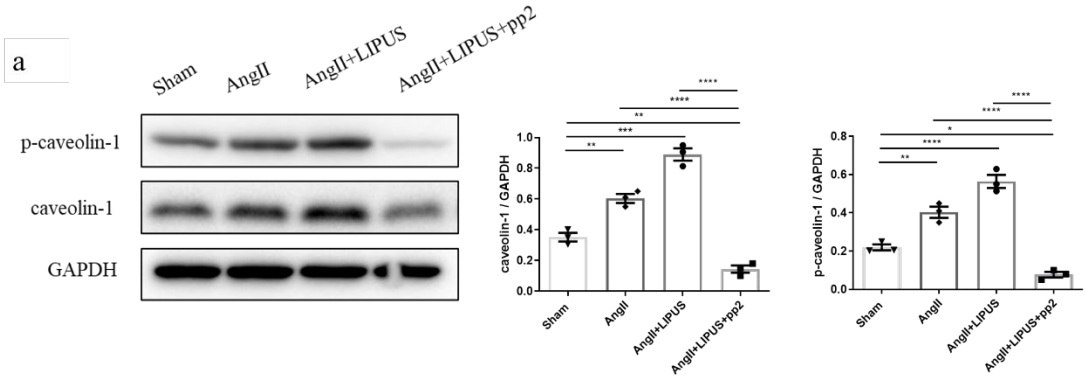

Fig. S6 LIPUS irradiation promoted caveolin-1 activation in AngII-induced mice models. Protein expression levels of p-caveolin-1, and caveolin-1 in heart tissues from different groups (left) and the corresponding densitometric analysis (right). *n*=3 per group; protein expression levels were normalized to GAPDH. The results are expressed as the mean±SEM (NS indicates not significant, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001).

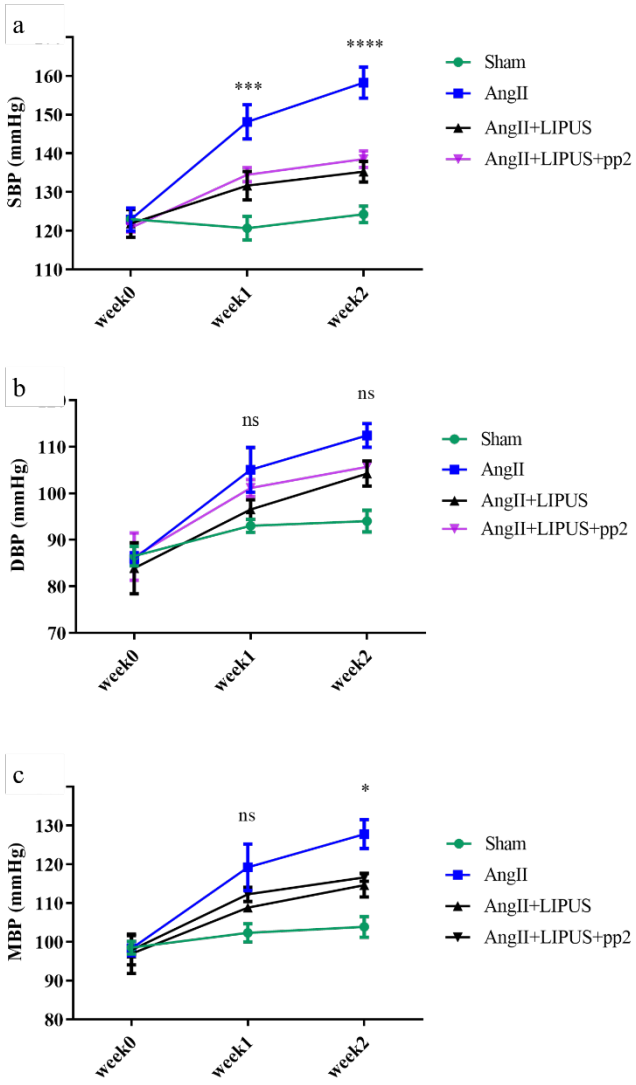

Fig. S7 Measurements of blood pressure of mice during the first two weeks after AngII infusion. (a-c) LIPUS irradiation downregulated the AngII-induced increase of systolic blood pressure (SBP) (a) and mean blood pressure (MBP) (c) at two weeks after AngII infusion. However, there was almost no significant difference in diastolic blood pressure (DBP) between the AngII group and the AngII+LIPUS group (b). *n*=5 per group. The results are expressed as the mean±SEM (NS indicates not significant, **P*<0.05, ***P*<0.01, ****P*<0.001, compared with the AngII group). Blood pressure was measured weekly in the tails using a noninvasive computerized tail-cuff system (Kent Scientific Corporation, CT, USA). The mice were warmed at 28 ℃ for 10–20 min before the measurement, allowing tail arterial and steady pulsation. The SBP, DBP, and MAP of the tail artery were set as the average of at least 10 measurements.

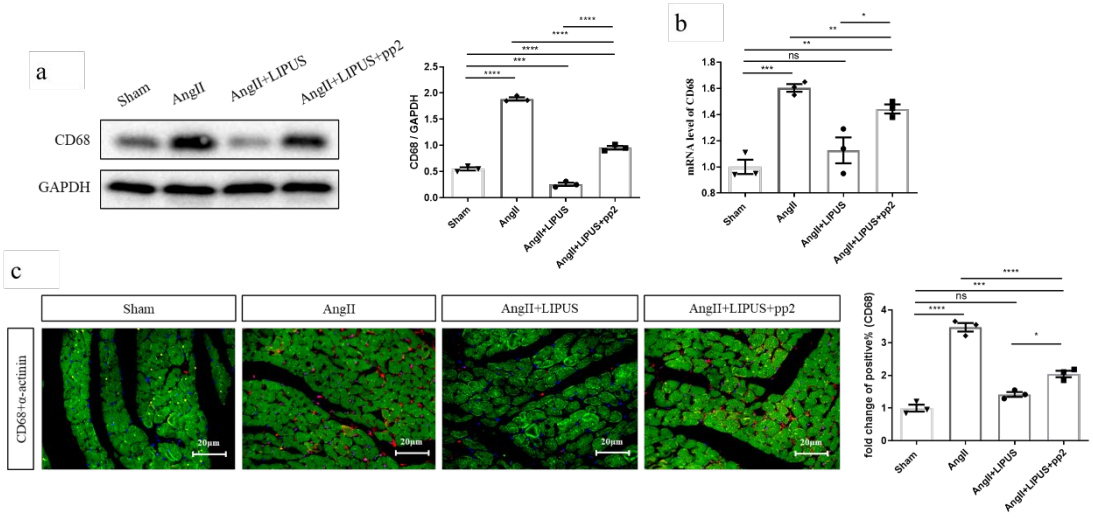

Fig. S8 pp2 pretreatment blocked the inhibitory effects of LIPUS irradiation on AngII-induced CD68 infiltration in vivo. (a, b) Protein (a) and mRNA (b) expression levels of CD68 in heart tissues from different groups. The mRNA and protein expression levels were normalized to GAPDH. (c) Representative immunofluorescence images of heart tissues labeled with CD68, α-actinin, and DAPI (CD68, red; α-actinin, green; DAPI, blue. Scale bars, 20 μm) (left), and the corresponding quantitative analysis (right). N=3 per group; the results are expressed as the mean±SEM (NS indicates not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

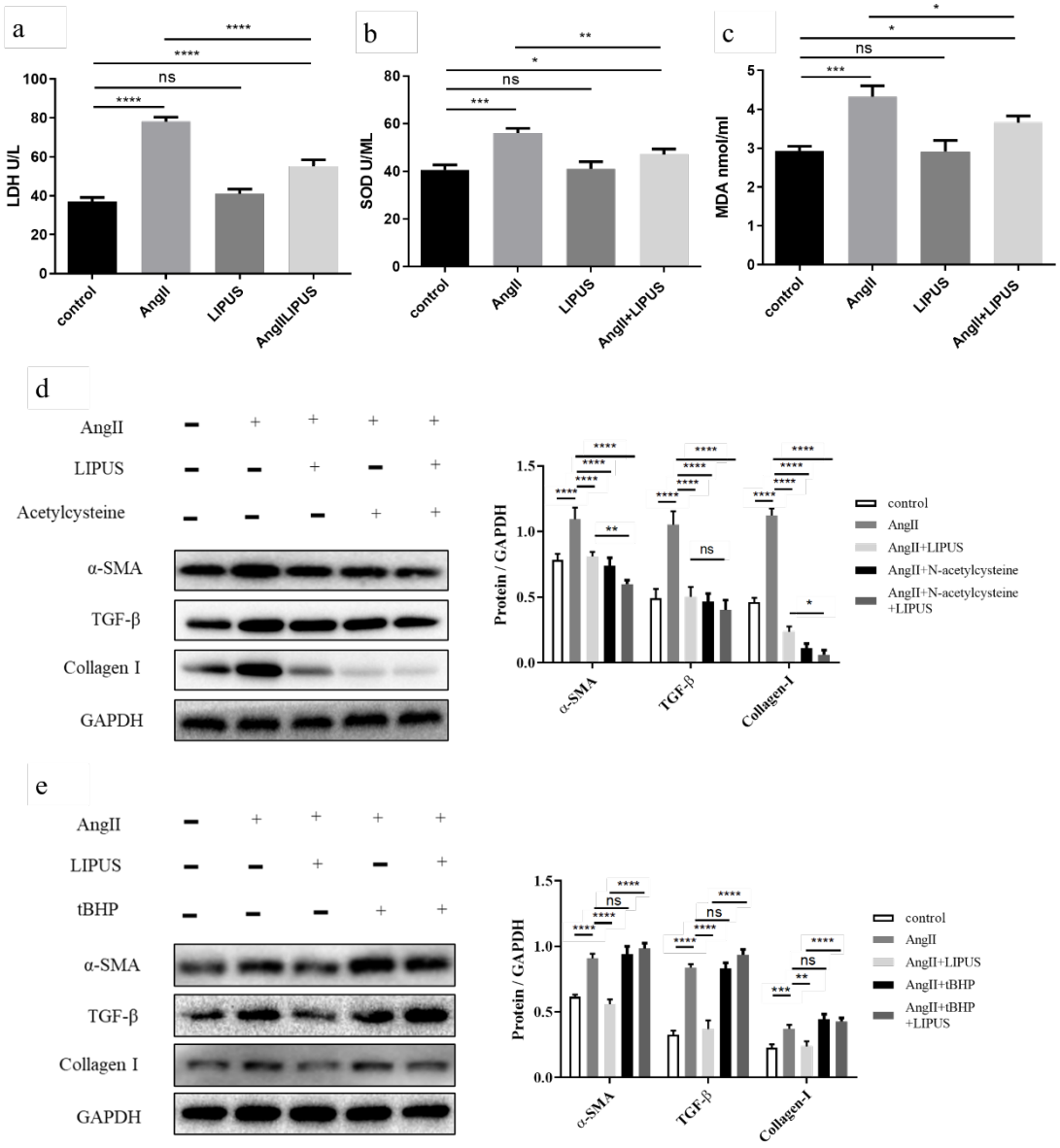

Fig. S9 Role of oxidative stress in the effects of LIPUS irradiation on ameliorating AngII-induced cardiac fibrosis. (a-c) Effects of LIPUS irradiation on LDH (b), SOD (c), and MDA (d) concentrations in the supernatant liquid of cardiac fibroblasts from indicated groups. (d, e) The protein expression levels of α-SMA, TGF-β, and Collagen I in cardiac fibroblasts from the control group indicated that N-acetylcysteine mimicked the antifibrotic effects of LIPUS irradiation on AngII-induced cardiac fibrosis (d), and tBHP reversed this effect (e). *n*=3 per group, GAPDH was detected as the loading control. All the results are expressed as mean±SEM (NS indicates not significant, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001).

Table S1 List of primers used for q-PCR

Gene	Species	Forward primer	Reverse primer
TGF-β	Rat	TCTGCATTGCACTTATGCTGA	AAAGGGCGATCTAGTGATGGA
α-SMA	Rat	GCATCCACGAAACCACCTA	CACGAGTAACAAATCAAAGC
Collagen I	Rat	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
GAPDH	Rat	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA
TGF-β	Mouse	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
α-SMA	Mouse	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
Collagen I	Mouse	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
Collagen III	Mouse	TGGTCCTCAGGGTGTAAAGG	GTCCAGCATCACCTTTTGGT
GAPDH	Mouse	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

Table S2 Different ultrasound intensities and their corresponding voltages used in our study

Burst period	Number of cycles	Voltage	Intensity
10 ms	100	100 mvpp	19.30 mw/cm^2
		150 mvpp	43.43 mw/cm^2
		200 mvpp	77.20 mw/cm^2
		250 mvpp	120.63 mw/cm^2

Table S3 Echocardiographic data of left ventricular function 2 weeks after AngII infusion

Variables	The control group	The model group	The LIPUS control group	The treatment group
LVEF (%)	76.81±2.08	58.11±2.84*	72.39±1.60	61.56.±2.91
LVFS (%)	41.96±0.92	31.51±2.56	39.05±1.04	32.67±1.63

The mice were randomly assigned to the control group, model group, LIPUS control group, and treatment group. The mice were implanted with an ALZET 2004 osmotic mini-pump filled with either AngII (2.5 mg/(kg·d)) or PBS subcutaneously under anesthesia for 4 successive weeks. The treatment group and the LIPUS control group underwent LIPUS irradiation for 20 min under isoflurane anesthesia every 2 days from 1 week before surgery to 4 weeks after surgery. Transthoracic echocardiographic images of mice hearts were acquired at the end of 2 weeks after surgery using an ultrasound system. The results are expressed as the mean±SEM (LVEF: left ventricular ejection fraction; LVFS: left ventricular fractional shortening; *P<0.05 versus the control group; #P<0.05 versus the model group).