Supplementary material

Preparation of L-IR-775

IR-775, cholesterol, and egg yolk lecithin was weighed at room temperature and then were transferred into a 50 mL round bottom flask, and an appropriate amount of chloroform was added. The mixture was completely dissolved by sonication. The flask was placed on a rotary evaporator, and the chloroform was completely evaporated at 37°C to form a film. Afterwards, the flask was added the appropriate amount of saline and was shaken until the film completely dissolved. The reaction solution was filtered through 0.45 μm and 0.22 μm millipore membrane filters, respectively, to obtain L-IR-775.

Preparation of L-DOX

Appropriate amounts of egg yolk lecithin and cholesterol were weighed accurately, transferred into a 50 mL round bottom flask, and an appropriate amount of chloroform was added. After evaporation at reduced pressure for 2 hours at 37°C, chloroform was completely removed and a translucent or white honeycomb membrane was formed in the bottle wall. Afterwards, the appropriate amount of 0.155 mol/L ammonium sulfate solution was added and shaken vigorously until the film dissolved completely. The reaction solution in the flask was then incubated at 50°C in a water bath for 2 hours, and sonic disruption was performed over intervals up to ten minutes. The solution was then filtered through 0.45 μm and 0.22 μm millipore membrane filters in order to obtain unilamellar liposomes.

The above-mentioned blank liposome suspension was placed in a dialysis bag and dialyzed twice in 1 L PBS buffer (pH=7.4). Then the appropriate amount of doxorubicin hydrochloride aqueous

solution with exact concentration was added to the appropriate amount of blank liposome solution. After that, the liquid was incubated in a 50°C water bath for 1 hour in order to obtain L-DOX.

Tissue distribution of L-DOX

We prepared 60% ethanol containing 0.3 $mol \cdot L^{-1}$ HCl as an extraction buffer. Then tissue homogenate samples were extracted using this extraction buffer (2mL extraction buffer per 0.2 g organ samples). After centrifugation at 11000 rpm/min for 20 minutes, the supernatant was transferred to a fresh tube and stored at -80°C until further analysis. A standard curve was used to calculate the concentration of DOX based on fluorescence intensity. Concentration data were dose normalized. The results of biodistribution were expressed as the amount of DOX uptake per gram of organ (ng/g).

Detection of cardiomyocyte apoptosis of the LAA

Frozen LAA samples were cut into 5 μm sections, then fixed in 4% paraformaldehyde at room temperature for 20 minutes, rinsed with PBS for 30 minutes and permeabilized in 0.1% Triton-X 100 at 4°C for 2 minutes, and rinsed twice with PBS for 5 minutes. TUNEL reaction solution (terminal deoxynucleotidyl transferase and fluorescein-labeled dUTP) was then added, and samples were incubated in a humidity chamber protected from light for 60 minutes, and rinsed three times with PBS for 5 minutes. DAPI was then added for 4 minutes, and samples were then rinsed three times with PBS for 5 minutes; anti-fade mounting medium was then used for mounting. With a confocal laser-scanning microscope (LSM510META, Zeiss, Germany), TUNEL-positive cardiomyocytes were counted in 5 randomly selected fields per section and quantified by using the Image J software. The rate of apoptosis was expressed as the ratio of TUNEL-positive cardiomyocyte nuclei (green) to the total

number of cardiomyocyte nuclei (blue).

Western blot analysis of proteins of the LAA

Protein extraction reagents containing protease inhibitors (1 mL protein extraction reagents was added 5 μl protease inhibitors mixture, 5 μl PMSF, and 5 μl phosphatase mixture) were prepared initially. The frozen LAA samples were cut into small pieces and transferred into small tubes. The pre-cooled extraction reagents were then added to these tubes (1 mL per 250 mg tissue). Samples were homogenized with a homogenizer for 30 seconds at low speed, followed by separation in an ice bath for 1 minute, until the tissue was completely lysed. Solubilized proteins were collected following centrifugation at 14000×g for 15 minutes. The supernatant was collected into new tubes and stored at -80°C.

Whole cell proteins were assessed to test the expression of apoptotic genes, including Bax, Bcl-2, and cleaved-caspase-3. Sample protein concentrations were quantified using an enhanced BCA (bicinchoninic acid) Protein Assay kit (Beyotime Biotechnology, Haimen, China).

Semi-quantitative immunohistochemical examination

After one week, we sacrificed experimental rabbits, collected the LAA specimens and used 10% buffered neutral formalin solution to fix them. After being dehydrated with gradual ethanol (70%-100%), cleared with xylene, these specimens were embedded in wax, and cut into 5 μm sections. Then the sections were deparaffinized, rehydrated, and antigen repaired with 10 mmol/L sodium citrate (pH=6). We immersed these slides in 3% hydrogen peroxide at room temperature for 30 minutes in order to quench the endogenous peroxidase activity and used 1% (w/v) bovine serum

albumin in PBS was used as a blocking solution to block sections for 1 hour before the addition of appropriate antibodies. The slides were incubated overnight at 4°C after applying the primary antibodies (rabbit monoclonal Bax, Bcl-2, and cleaved-caspase-3 antibody). Appropriate secondary antibodies labelled with HRP were then applied to the samples. DAB chromogen was then applied for 2 minutes. After rinsing DAB, counterstaining with Mayer Hematoxylin and cover slipping was performed prior to slide examination under the light microscope.

Table S1 The rate of electrical conduction block in different phases

Group	Rate of electrical conduction block（%）			
	Phase1	**Phase2**	**Phase3**	**Phase4**
RFCA	0 (0/10)	100 (10/10)	30 (3/10)	20 (2/10)
RFCA+L-DOX	0 (0/10)	100 (10/10)	80 (8/10)	80 (8/10)
RFCA+DOX	0 (0/10)	100 (10/10)	/	/

Notes: n=10 per group.

Abbreviations: RFCA, radiofrequency catheter ablation; L-DOX, liposomal doxorubicin; DOX, doxorubicin.

Table S2 The drug targeting index (DTI) of L-DOX for each organ on the second day after intravenous drug administration

Organ	DTI ± SD
LAA	1.872 ± 0.22
Heart	0.679 ± 0.01
Liver	1.615 ± 0.13
Spleen	1.706 ± 0.36
Lung	1.074 ± 0.28
Kidney	0.353 ± 0.02

Notes: n=5 per group, data presented as means±SD.

DTI = The concentration of L-DOX of organ / The concentration of DOX of organ

Abbreviations: DOX, doxorubicin; L-DOX, liposomal doxorubicin; LAA, Left Atrial Appendage.

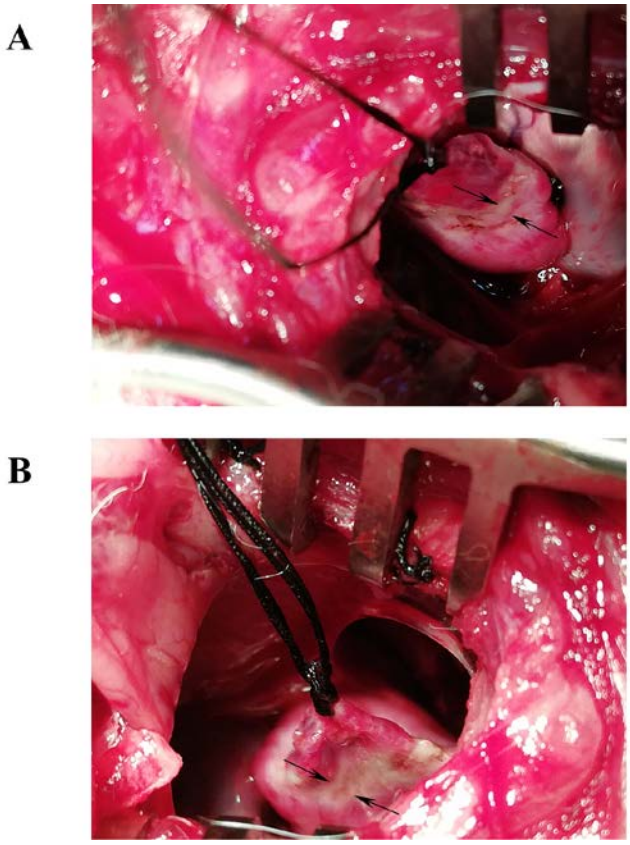

Figure S1 The circular ablation around the anterior wall (**A**) and posterior wall (**B**) of LAA. The black arrow indicates the LAA lesion after RFCA.

Abbreviations: LAA, left atrial appendage; RFCA, radiofrequency catheter ablation.

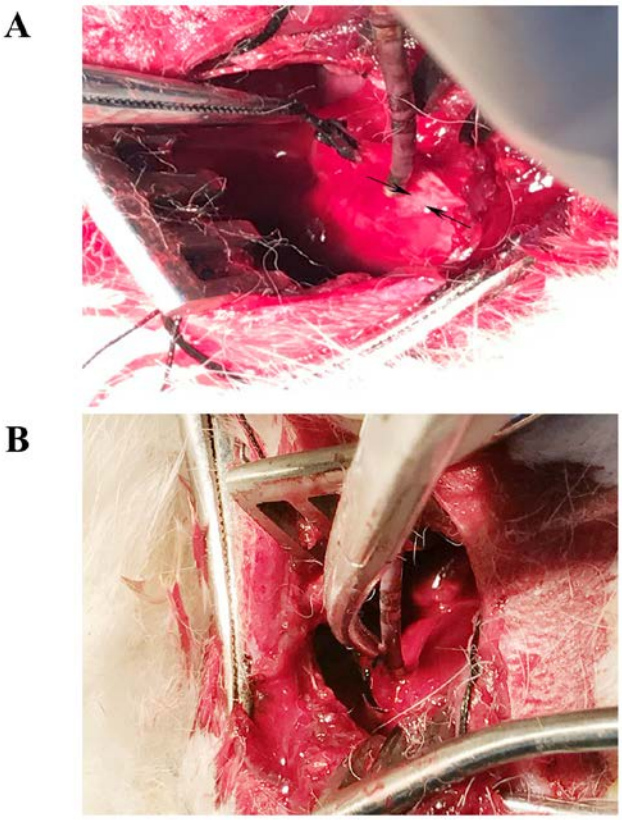

Figure S2 The tip of stimulating electrode was respectively attached to the anterior wall (**A**) or posterior wall (**B**) of the LAA inside the loop ablation lesion. The black arrow indicates the lesion of the LAA after RFCA.

Abbreviations: LAA, left atrial appendage; RFCA, radiofrequency catheter ablation.

Assessments of LAA captured

Two methods were used to ensure LAA capture following pacing:

(1) Prior to pacing, we adjusted the pacing output voltage to pace other areas of the heart, including the ventricle. Once the ventricle was effectively paced, the identified pacing output voltage (15 mV) was used to pace the LAA. Through this assessment, we could ensure that the pacing output voltage reached or was greater than the pacing threshold (Figure S3).

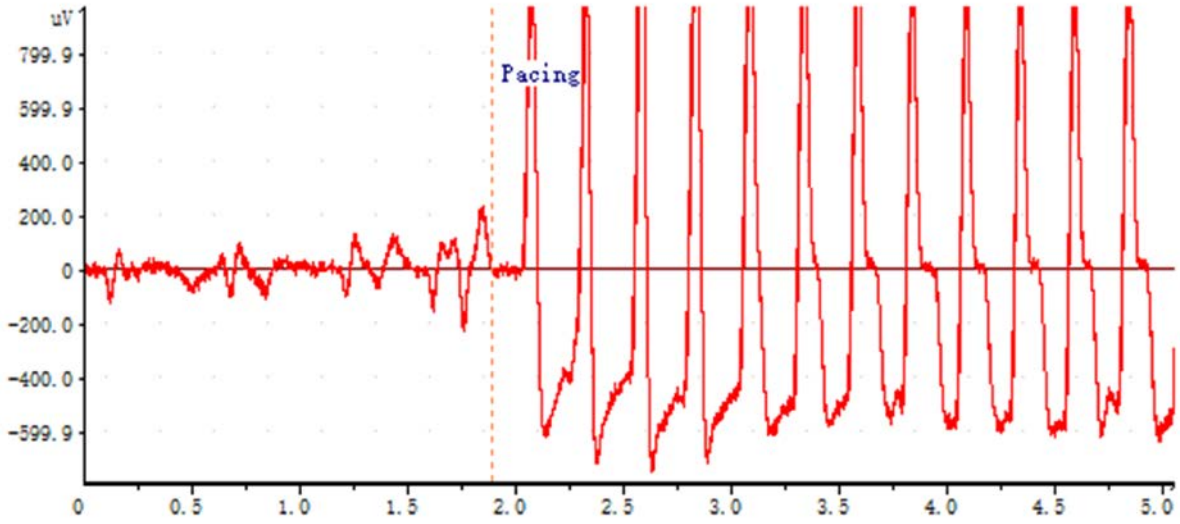

Figure S3 Successful pacing of the ventricle.

(2) An electromyogram (EMG) channel (Channel 2) was established to record the potential (units = μv) of the LAA independently. The potential could be observed when pacing the LAA with a frequency consistent with the pacing frequency. The waveform was initially unstable as the tip of the stimulating electrode was inaccurate for the LAA. This confirmed that the LAA was captured through the pacing signal (Figure S4).

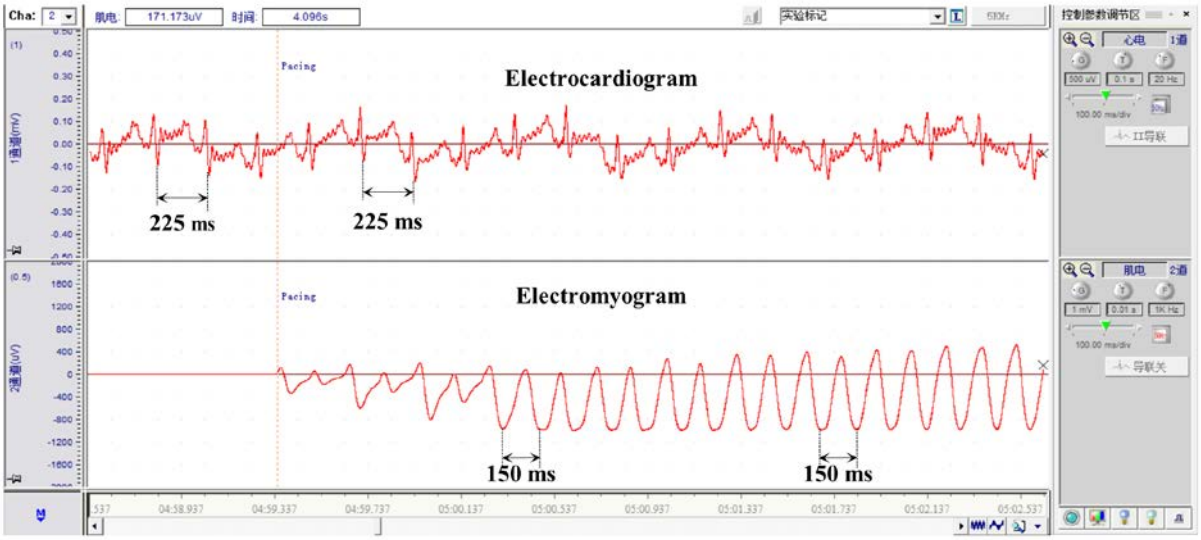

Figure 4 LAA capturing using the pacing signal.

Abbreviations: LAA, left atrial appendage.

Assessments of electrical conduction block

When assessing exit block, the output energy used to pace the LAA reached the pacing threshold, and the pacing rate exceeded that of the heart rhythm. The myocardial tissue was therefore close to the ablation point and could be greatly excited. Thus, the myocardial electrogram can reflect the excitatory activity of the LAA muscle to verify its capture by the pacing signal.

However, when detecting entrance block, the LAA potential was recorded prior to ablation (Figure S5A), and could not be recorded post-ablation (Figure S5B). This was because the LAA potential after ablation is so small, it cannot be distinguished from the interference signal. Consequently the entrance block following ablation could not be detected. In addition, verification of the exit block was sufficient to highlight the purpose of these experiments.

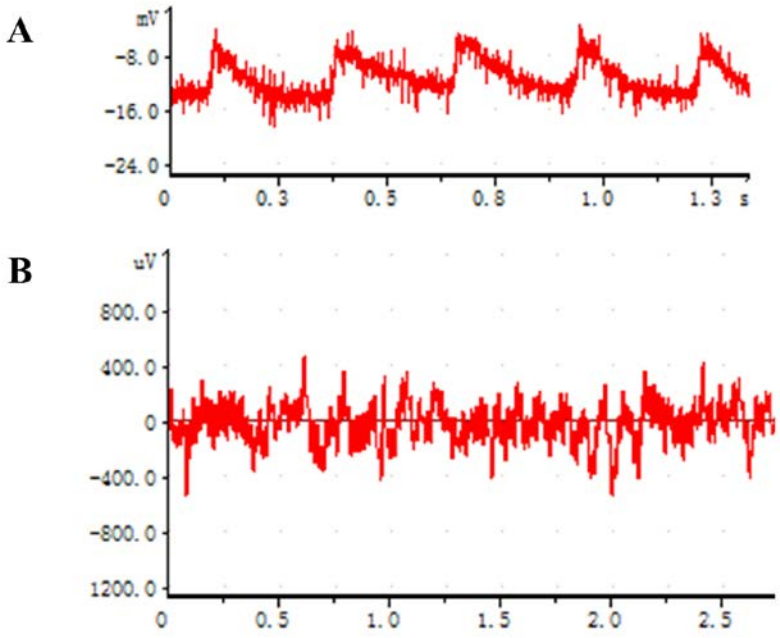

Figure S5 LAA potential before (A) and after (B) ablation.

Abbreviations: LAA, left atrial appendage.

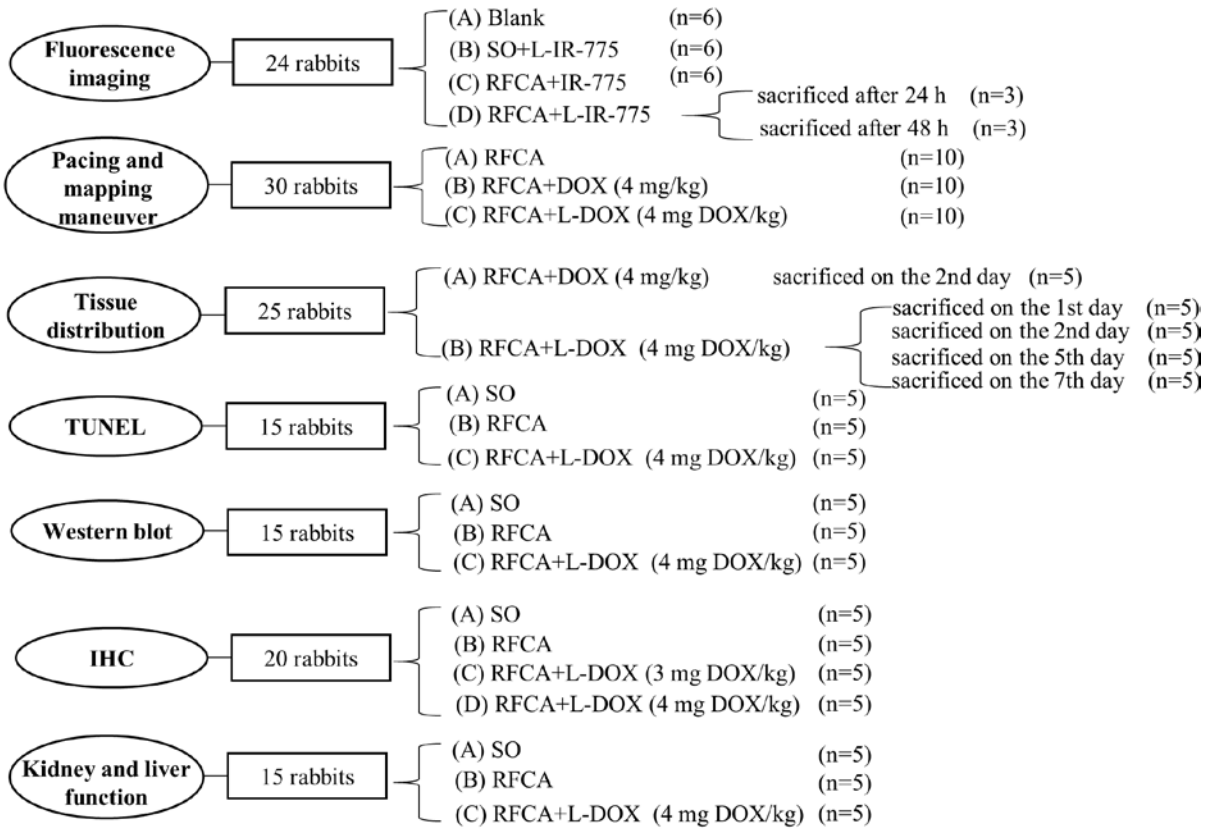

Figure S6 The number and group assignment of the animals used in the experiments.

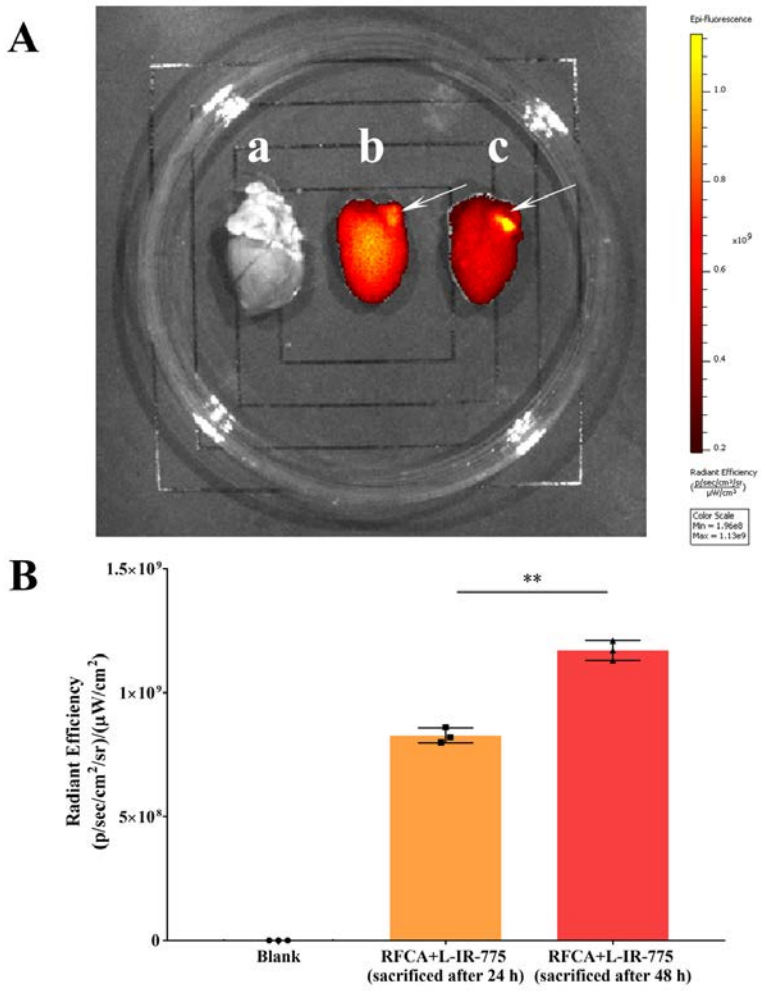

Figure S7 Fluorescence imaging (**A**) and semi-quantative data (**B**) from rabbit hearts in appropriate treatment groups. Group A, blank (**a**); Group B, RFCA+L-IR-775 and the rabbits were sacrificed 24 hours after intravenous administration (**b**); Group C, RFCA+L-IR-775 and the rabbits were sacrificed 48 hours after intravenous administration (**c**). The white arrow indicates the LAA ablation site.

Notes: **P<0.01 vs 24 hours after intravenous administration, n=3 per group, data are presented as means ± SD.

Abbreviations: RFCA, radiofrequency catheter ablation; L-IR-775, IR-775 liposome; LAA, left atrial appendage.

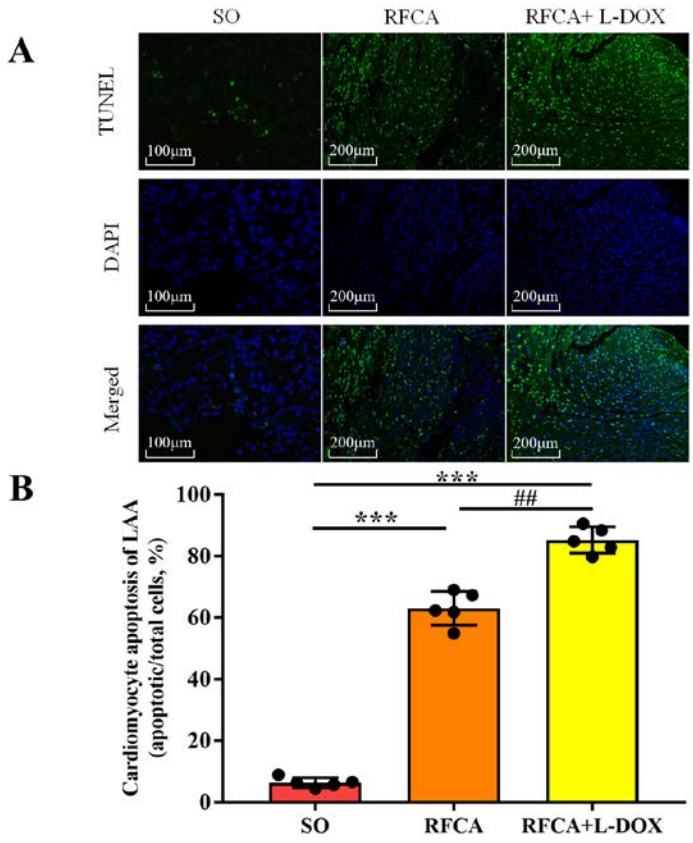

Figure 8 (**A**) Cardiomyocyte apoptosis in different groups was determined via TUNEL staining. (**B**) The apoptosis rate was expressed as the ratio of TUNEL-positive cardiomyocyte nuclei to total number of cardiomyocyte nuclei.

Notes: ***P<0.001 vs SO treatment; $^{\#\#}$P<0.01 vs RFCA treatment. n=5 per group, data are presented as means ± SD.

Abbreviations: SO, sham-operated; RFCA, radiofrequency catheter ablation; L-DOX, liposomal doxorubicin .

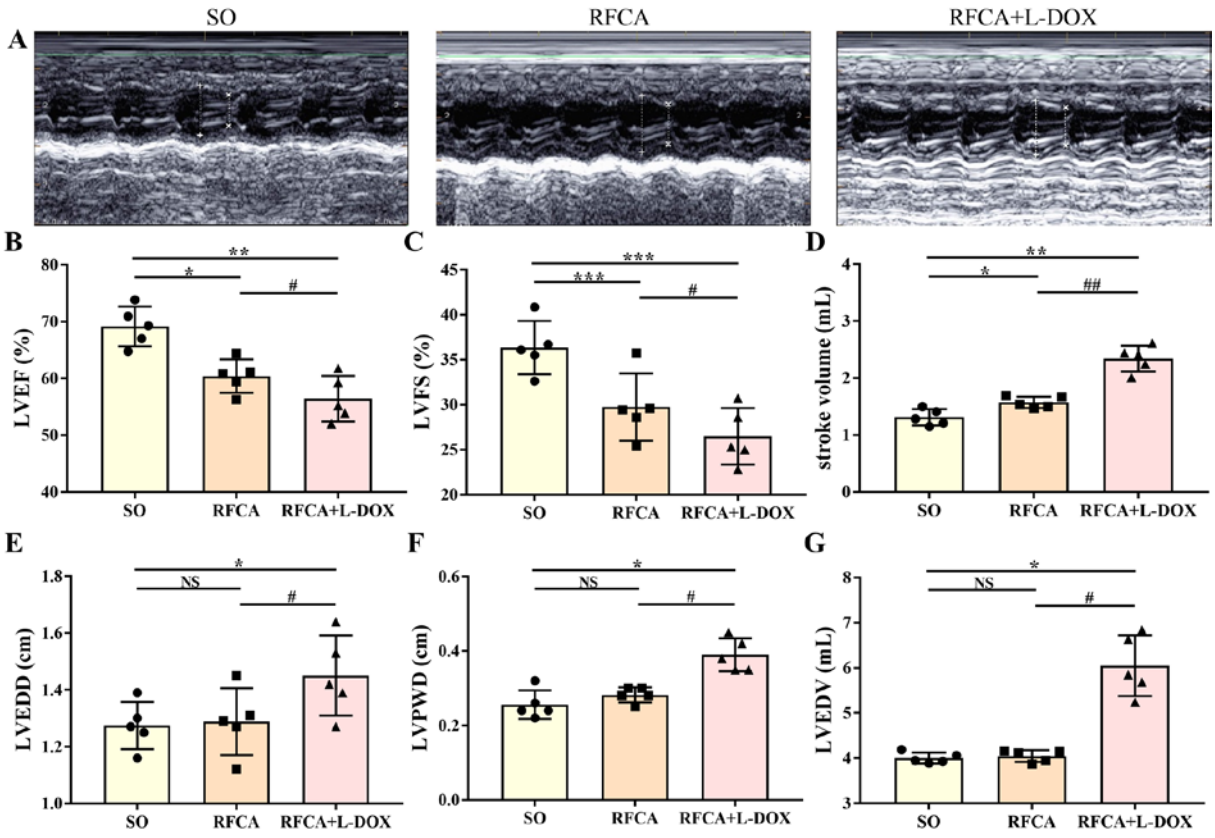

Figure 9 The echocardiography of different treatment groups. The representative M-mode echocardiographic images (**A**) and echocardiographic parameters including LVEF (**B**), LVFS (**C**), stroke volume (**D**), LVEDD (**E**), LVPWD (**F**) and LVEDV (**G**) in different groups.

Notes: *P<0.05, **P<0.01, ***P<0.001 vs SO treatment; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs RFCA treatment. n=5 per group, data are presented as means ± SD.

Abbreviations: SO, sham-operated; RFCA, radiofrequency catheter ablation; L-DOX, liposomal doxorubicin ; LVEF, left ventricular ejection fraction; LVFS, left ventricular fraction of shorting; LVEDD, left ventricular end diastolic diameter; LVPWD, left ventricular posterior wall depth; LVEDV, left ventricular end diastolic volume.