**SDC, Materials and Methods**

**Preparation of specific nanobubbles**

Dipalmitoyl phosphatidylcholine, Distearoyl phosphatidylcholine (Both from Genzyme Pharmaceuticals, Sweden), diphenylphosphoryl azide (DPPA), distearoylphosphatidylethanolamine- polyethyleneglycol (2000) biotin (DSPE-PEG) (Hercules, US) (2:1:2:1 W/W) were mixed evenly with deionized water. PEG 4000 was added into the mixture (99: 1 W/W), which was freeze-dried according to the presetted procedure. Dissolvant liquid composed of glycerine, propylene glycol and deionized water were added into the freeze-dried half-finished product (3ml:1g), the air in the vial was substituted by perfluoropropane, and the vial was sealed and oscillated subsequently. After dilution with 0.01M PBS at the ratio of 1:10, the primary product of microbubbles was centrifugated at 1000 rpm for 10 min and the underlayer liquid was abandened, which was repeated for 4 times, and then the biotinylated lipid nanobubbles were acquired. Avidin of 0.025 mg/ml (Ref: Kheirolomoom A, Dayton PA, Lum A F, Little E, Paoli EE, Zheng H, Ferrara KW. Acoustically-active microbubbles conjugated to liposomes: characterization of a proposed drug delivery vehicle. J Control Release , 2007; 118(3):275-284) was added, and the mixture was incubated for 30 min at room temperature and then was rinsed and centrifugated in the conditions same as before. Then the acquired biotinylated nanobubbles connecting avidin were mixed with the biotinylated FITC-labeled anti-CD25 antibody or isotype control antibody (20µg/ml, Biolegend, US) (1 ml: 1 ml), incubated at room temperature, rinsed and centrifugated as before, and finally the
supernatant containing nanobubbles was collected, which were the end products of the specific and the non-specific nanobubbles ($\text{NB}_{\text{specific}}$ and $\text{NB}_{\text{non-specific}}$), respectively.

**Establishment of rat heart transplantation**

Rats were anesthetized with Ketamine (100 mg/kg IP) (Sigma, USA), fixed and sterilized routinely. Donor rats were heparinized (1000 U/kg IV), and the anterior rib cage was opened to expose the heart. The inferior vena cava (IVC) and right superior vena cava (SVC) were ligated, the ascending aorta and main pulmonary artery were separated and transected separately, the left and right pulmonary veins, left SVC, left auricle and part of left atrial wall were ligated together, the left tissues were cut off, and the explanted heart was immersed in $0^\circ\text{C}$ saline.

In recipient rats, a midline abdominal incision was made, the intestinal loops were push aside, the posterior peritoneum was cut open, and the abdominal aorta and IVC were separated bluntly. The donor aorta was anastomosed to the recipient abdominal aorta, and the donor pulmonary artery was anastomosed to the recipient abdominal IVC. And then the heart was reperfused, the abdomen was closed as the cardiac rhythm restored, and the rats were allowed to recover.

**Analysis of Time-intensity Curve of MCE**

The dynamic images were transferred into a computer as DICOM file, and then were converted to JPG files by 1 frame/s with the Showcase software provided by SIEMENZ company for further analysis. All the files were analyzed with Adobe Photoshop software. The septum was selected as the region of interest in myocardium with fixed sample size, and the mean grey scale that indicated the intensity of
enhancement was detected with histogram analysis, according to which, the
time-intensity curve (TIC) was plotted by Excel, and then indices of TIC such as peak
intensity and time to peak were measured.
SDC, Figure S1  Process of myocardial enhancement during MCE with NB_{specific/non-specific} in allograft at day 6 post cardiac transplantation. A-E, MCE with NB_{specific}: A. no enhancement in myocardium at 0 s post nanobubbles injection; B. rapid enhancing of myocardium (arrows) and cardiac chamber at 4 s; C. declined enhancement of myocardium (arrows) with hyper-enhancement of cardiac chamber at 120 s; D. second enhancement of myocardium (delayed enhancement) at 150 s (arrows); E. weak enhancement (arrows) with no enhancement in cardiac chamber at 22 min, much longer than that of NB_{non-specific} (about 4 min), while the contrast in cardiac chamber was cleared. F: continuously declined enhancement at 150 s in MCE with NB_{non-specific}, which was lower than that in D. The process showed the delayed enhancement in MCE with NB_{specific} in allograft, indicating the specific binding of
NBspecific and T cells in myocardium.

SDC, Figure S2

SDC, Figure S2 Correlation of second peak parameters (MCE with NBspecific in allograft) with transplant time and pathological grade: A. positive correlation between second peak intensity and transplant time ($r=0.911$, and $P<0.001$); B. positive correlation between time to second peak and transplant time ($r=0.926$, and $P<0.001$); C. positive correlation between second peak intensity and pathological grade ($r=0.934$, and $P<0.001$); D. positive correlation between time to second peak and pathological grade ($r=0.929$, and $P<0.001$).
Pathological (HE 200×) and immunohistochemical (200×) presentations of AR myocardium: A. grade 0, no lymphocytes infiltration in myocardium; B. grade 1, local infiltration of lymphocytes in myocardium; C. grade 2, local lymphocytes infiltration with myocardial necrosis; D. grade 3, severe lymphocytes infiltration with myocardial necrosis; E. grade 4, wild infiltration of lymphocytes, acidophil and neutrophil granulocytes and multifocal myocardial necrosis with interstitial hemorrhage and edema; F-J. expression of CD25 increased with pathological grade, corresponding to pathological presentations of A-E, respectively.

Transmission electron microscopic image of nanobubble loading
anti-CD25 antibody, diameter of which is about 420 nm.