Supplementary data

Targeted miR-21 loaded liposomes for acute ischemic myocardial disease

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MATERIALS AND METHODS

Immunohistochemistry Examination

The pathological changes of cTnT was evaluated by immunohistochemistry. Normal rats and MI rats with LAD ligation for 1-7 days were prepared. The rats were killed and perfused with 4% paraformaldehyde for 10 minutes. The isolated hearts were soaked in 4% paraformaldehyde and 30% sucrose solution successively. Then the heart tissues were incised with Frozen Section Media with the thickness of 5 μ m. The frozen tissue sections were incubated with 3% H₂O₂ for 5 minutes at room temperature. 5% BSA was added for 15 minutes at 37 °C. The primary antibody anti-cTnT (1:200) was incubated

overnight at 4 $^{\circ}$ C, before adding the secondary antibody at 37 $^{\circ}$ C. The sections were visualized with a diaminobenzidine (DAB) and restained with hematoxylin for 5 minutes. The images were taken by Leica DMI3000 fluorescence microscope.



Figure S1. The particle size (A) and zeta potential (B) of cT-21-LIPs detected by Malvem etasizer Nano ZS 90. (C) The release kinetics curve of 21-LIPs and cT-21-LIPs (n=3, error bar = standard deviation). (D) The interaction of rhodamine-labeled LIPs and rhodamine-labeled cT-LIPs with T proteins by micro thermography (MST). The normalized fraction of the combined LIPs or cT-LIPs is plotted as a correlation curve against protein concentration.



Figure S2. The cell viability of 21-LIPs and cT-21-LIPs on hypoxia cardiomyocyte treated by CCK-8 (n=6. $\Box \Box \Box p < 0.005$, # # # p < 0.005 vs. 0 nM).



Figure S3. ECG recordings from healthy rats (A) and myocardial infarction rats (B).



Figure S4. Bio-distribution of various DIR-liposomes in major organs. After 12 h injection the rats were sacrificed and the organs were examined ex-vivo by optical in vivo imaging to evaluate accumulation of NPs (in vivo images of the brain, heart, liver, spleen, lung and kidneys). healthy

rats were administrated NaCl (Ctl), AMI rats were respectively administrated DiR-labeled liposomes (LIPs), DiR-labeled anti-cTnT Ab modified liposomes (cT-LIPs) and free dye (Free), and Sham-operated rats were administrated equal volume of cT-DiR-LIPs (sham).



Figure S5. Time-dependent expressions of cTnT in ischemic myocardium after AMI.