Supplement information

Materials and Reagents.

Tetraethyl orthosilicate (TEOS), Tris(2',2'-bipyridyl) dichlororuthenium-(II) hexahydrate (Ru(bpy)32+), Triton X-100, branched polyethylenimine (PEI, 800 Da), Dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich Trading Company, Ltd. (Shanghai); Cyclohexane and 1-hexanol were obtained from Sinopharm Chemical Reagent Company, Ltd. (Shanghai, China). The oligonucleotides used in the study were purchased from GenePharma Co.Ltd. (Suzhou, China). Their sequences were as follows- miR-24 mimic (5'-UGGCUCAGUUCAGCAGGAACAG-3'), miR-24 mimic NC (5'-UUCUCCGAACGUGUCACGUTT-3'), miR-24 inhibitor (5'-CUGUUCCUGCUGAACUGAGCCA-3'), miR-24 inhibitor NC (5'-CAGUACUUUUGUGUAGUACAA-3'). FAM-labeled FAM-miR-24-mimic (5'-UGGCUCAGUUCAGCAGGAACAG-3'-FAM).

Preparation of Silica nanoparticles and DSP-PEI

Silica nanoparticles were prepared with modification, according to the microemulsion method. Briefly, 22.5 mL of cyclohexane, 5.3 mL of Triton X-100, 5.4 mL of 1-hexanol, 1.5 mL of 6.7 mg/mL $Ru(bpy)^{32+}$ aqueous solution, and 350μL of $NH_3{\cdot}H_2O$ were mixed with continuous stirring for 30 mins flowed by the addition of 300 μL of TEOS. The reaction was allowed to continue for 24 h at room temperature. After centrifugation of the solution mixture, the precipitate (Silica nanoparticles) was collected, washed thoroughly with ethanol and DMF, and dried under a vacuum.

For the construction of the crosslinker DSP-PEI, 2.5 mL of 10 mg/mL DMSO solution was added dropwise to 5 mL of 50 mg/mL PEI, stirring for 2 hours at room temperature after all the additions were completed. The above chemical reaction finally allows the NHS-ester in the DSP to undergo transesterification with primary ammonia in the PEI, thereby interconnecting the PEI through disulfide bonds.

Cardiomyocytes Isolation and Cell culture

Cardiomyocytes were extracted from 1-day-old Sprague-Dawley (SD) rats according to our previously described method. Briefly rats were sacrificed, and the heart was quickly immersed in ice-cold PBS. Atria was removed, and the remaining ventricular tissues were treated with Trypsin-EDTA for 30 minutes. Cell suspensions were then collected by centrifuging and re-suspension in DMEM medium supplemented with 15% fetal bovine serum and 1% streptomycin/penicillin. The cells were then cultured at 37℃ and 5% CO_2. All experimental protocols were approved by the National Center for Nanoscience and Technology Animal Experiment Ethics Committee.

Measurements of cardiomyocyte viability in H_2O_2-induced hypoxia

To induce hypoxia-like apoptosis, 100 μM H_2O_2 was added into cardiomyocyte culture for 0, 6, 12, 24, or 48 h. Cardiomyocytes were plated in 96-well plates. Cell viability was assessed using a CCK-8 assay. After H_2O_2 treatment, 10 μL CCK-8 reagents were added to the culture medium of DMEM + 2% FBS for 4 h, and the absorption at 450 nm was evaluated with a Synergy HT multi-detection microplate reader (Bio-Rad, USA). The percentages of viable cardiomyocytes with H_2O_2 treatment were estimated against the percentage under control (0 h H_2O_2 treatment).

Cell viability assays

The cell viability of cardiomyocytes were determined by using a Live/Dead Viability/Cytotoxicity Kit. (KeyGen Biotech. Company Ltd. Nanjing, China). In brief, Neonatal rat ventricular myocytes (NRVMs) were plated on 24-well plates and incubated for 24 h. Then the growth medium was replaced with a fresh growth medium containing F-Silica with various concentrations (0/0.1/0.2/0.4/0.6 mg/mL). The cells were grown for another 1 or 3 d at 37 ℃ in the cell incubator. Then, the growth medium was removed, and cells were incubated for 30 min with 2 μM calcein-AM and 8 μM PI in phosphate-buffered saline (PBS). Finally, the dye was aspirated, washed twice with PBS, and observed under a fluorescence microscope (Nikon AZ-100 multipurpose microscope, Japan).

Agarose gel electrophoresis

To study the stability and strength of miR-24 binding to the F-Silica, we incubated different final concentrations of F-Silica (0.05/0.1/0.15/0.20/0.25 mg/mL) with an equal amount of miR-24 (final concentration of 200 nM) for 30 min. Then those samples were loaded on a 1% agarose gel containing 0.01% ethidium bromide and run at 90 V for 30 min.

Cellular uptake study

Confocal fluorescence microscopy was used to assess the intracellular trafficking of miRNA. NRVMs were seeded and grown on a confocal dish ($1*10^5$ cells/well). 100 μL RNase-free water was added to the miR-24 group; 100 μL PEI-FAM-miR-24(final concentration of miR-24 was 200 nM) was added to PEI-miR-24 group; F-Silica-miR-24 group was transfected with FAM-miR-24(final concentration of miR-24 was 200 nM) attached with F-Silica. The redundant nanoparticles were removed by thoroughly washing twice with PBS after 24 h incubation. Then 200 μL of 4% paraformaldehyde solution was added at room

temperature for 30 min to fix the cells. After additionally washing three times with PBS and incubated with 0.3% Triton X-100 for 5 minutes, the cells were stained with DAPI for 15 min, washed three times with PBS for 3 min, added anti-fluorescence quencher, and analyzed under a confocal microscope with Volocity Demo 6.1.1 (Zeiss).

Quantitative real-time PCR

NRVMs were inoculated into 6-well plates and cultured in DMEM with 15% fetal bovine serum until 80% confluence was reached. The control group did not receive any treatment; the H_2O_2 group was treated with 100 μM H_2O_2 for 24 h; the other gene transfection groups were pre-transfected with F-Silica-loaded 200 nM oligonucleotides for 24 h before H_2O_2 treatment, the gene transfection groups were: miR-24-mimic group; miR-24-mimic NC group; miR-24-inhibitor group; miR-24-inhibitor NC group. Total RNA was extracted using RNAiso plus reagent (Invitrogen) according to the manufacturer's protocol. The relative expression levels of miR-24 in each group were tested according to the instructions of Bulge-LoopTM miRNA qRT-PCR, a miRNA detection kit independently developed by Guangzhou Ruibo Biotech Co., Ltd., U6snRNA provided by the company's primer kit was used as an internal reference.

Western blot analysis

NRVMs were prepared and transfected as described above. Total proteins were extracted using protein lysis buffer and measured using a BCA Protein Assay Kit (Beyotime).50 μg of total protein was resolved on a 12% SDS-PAGE gel and transferred to a PVDF membrane. Incubation of primary antibody against Bim (1:1000, Abcam, UK) was applied overnight at 4°C. On the second day, the respective horseradish peroxidase-coupled secondary antibody (Cell Signaling, USA) was added for 1 hour at room temperature. The blot was visualized with ECL chemiluminescence reagents, and GAPDH was used as the control protein.

TUNEL Staining for Apoptosis Detection

Mouse hearts were removed 24 h after coronary artery ligation to quantify apoptosis in cardiomyocytes, fixed with 0.5% PFA, and routinely frozen embedded in OCT and processed for sectioning and staining with TUNEL and α-Actinin (rat; 1:800; Sigma-Aldrich) as described above. DAPI was used for nuclear counterstaining. The number of apoptotic cells was counted under laser scanning confocal microscopy. Five slices were randomly selected from the same specimen, and five visual fields were randomly selected from the same section for counting.

Rat AMI model

Rats were anesthetized with 2.4% isoflurane/97.6% oxygen and placed in a supine position on a heating pad (37 ℃). All surgeries and subsequent analyses were performed in a blinded fashion for intervention. AMI was induced by permanent ligation of the left anterior descending artery with a 6-0 Prolene suture. Immediately after coronary artery ligation, 100 μL PBS, or 100 μL miR-24 mimic (200 nM) connected with F-Silica, or 100 μL miR-24 inhibitor (200 nM) connected with F-Silica were administrated by intramyocardial injection in 3 regions of the border zone of the infarcted area. 2 weeks after occlusion, echocardiography was performed to evaluate cardiac function and left ventricular shortening fraction (LVFS) and left ventricular ejection fraction (LVEF) were measured. After echocardiography evaluation, the hearts were removed and cut into two transverse slices from the middle of the infarcted area. The sections were stained with Masson staining. Afterward, infarct sizes were determined under a microscope.

Live subject statement

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Chinese Academy of Sciences University and studies were approved by the Institutional Animal Care and Use Committee (IACUC) of the National Center for Nanoscience and Technology (Beijing, China).