Supporting information

Spherical α-helical polypeptide-mediated E2F1 silencing against myocardial ischemia and reperfusion injury (MIRI)

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Materials, cell lines, and animals

All reagents were purchased from Sinopharm chemical reagent Co., Ltd (Suzhou, China) and all chemicals were purchased from Energy Chemical (Anhui, China) unless otherwise specified. The amine-terminated, third-generation polyamidoamine (PAMAM, G3-NH_2) was purchased from Weihai CY Dendrimer Technology Co., Ltd (Weihai, China). Branched polyethylenimine with a molecular weight of 25 kDa (25k PEI) was purchased from Sigma-Aldrich (St Louis, MO, USA). γ-(4-Propargyloxybenzyl)-$_L$-glutamic acid-*N*-carboxyanhydride (POBLG-NCA) and 6-azide hexaguanidine were synthesized (Figure S1 and S4) as previously reported[1,2].

siRNA and primers were all supplied by GenePharma (Shanghai, China) and all the sequences were listed in Table S1 and S2. Primary antibody of E2F1 was purchased from Sangon Biotech (Shanghai, China). Primary antibodies of TNF-α and GAPDH were purchased from Abcam (Cambridge, UK). Secondary antibodies were purchased from Beyotime (Nanjing, China). Pierce BCA protein assay kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Trizol was obtained from Biosharp (Shanghai, China). PrimeScript RT Reagent Kit and SYBR Premix Ex Taq were purchased from Takara (Suzhou, China). Lysotracker deep red and Hoechst 33258 were purchased from Invitrogen (Carlsbad, CA, USA). DAPI was purchased from Beyotime (Nanjing, China).

H9C2 cells (rat cardiomyocyte) were purchased from Bioleaf (Shanghai, China) and cultured in DMEM containing 10% fetal bovine serum (FBS).

Male Sprague-Dawley (SD) rats (250-300 g, 8-10 weeks) were purchased from Shanghai Slaccas Experimental Animal Co., Ltd., and were housed in a clean room, two to a cage, with access to water ad libitum, a 12:12 h light-dark cycle (7:00 am - 7:00 pm), and a temperature of 25 ± 1 °C. All the animal experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Soochow University. The animal experimental protocols were performed in accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985).

Synthesis of linear α-helical polypeptide (LPP)

LPP was synthesized according to the route in Figure S5. POBLG-NCA (50 mg, 0.16 mmol) was dissolved in dimethylformamide (DMF, 1 mL) in a glove box, and the DMF solution of hexamethyldisilazane (HMDS, 16 μL, 0.1 mol/L) was added. The reaction progress was monitored by Fourier transform infrared spectrum (FTIR) for 72 h at RT until the monomer conversion was higher than 99%. After the reaction ended, the mixture was added dropwise into cold, anhydrous methanol (20 mL), and the precipitate was washed with anhydrous methanol (10 mL × 3). The product PPOBLG was obtained as white solid (35 mg, 82% yield). Gel permeation chromatography (GPC) was used to determine the degree of polymerization (DP) and the molecular weight distribution (*Đ*) using DMF as the eluent.

In a glove box, PPOBLG (30 mg, 0.11 mmol alkyne group) and 6-azide hexaguanidine (26 mg, 0.14 mmol) were dissolved in DMF (3 mL), into which *N*,*N*,*N'*,*N''*,*N''*-pentamethyldiethylene-triamine (PMDETA, 34 μL, 0.16 mmol) and CuBr (18 mg, 0.16 mmol) were added and reacted for 36 h at RT. Then, the mixture was exposed to air for oxidation of CuBr, and hydrochloric acid (3-4 mL, 1 mol/L) was added until the solution changed from blue to colorless. The mixture was dialyzed against DI water for 3 d (MWCO = 3500 Da) and lyophilized. The product LPP was obtained as white solid (42 mg, 84% yield). The secondary structure of LPP in deionized (DI) water at 0.2 mg/mL was characterized by circular dichroism (CD).

Synthesis of spherical α-helical polypeptide (SPP)

SPP was synthesized according to the route shown in Figure S6. In a glove box, POBLG-NCA (50 mg, 0.16 mmol) was dissolved in DCM (1 mL) and DMF solution of G3-NH_2 (10 mg/mL, 35 μL, the amount of amino substance was 1.6 μmol) was added, and the reaction progress was monitored by FTIR for 1 h at RT until the monomer conversion was greater than 99%. Then, the mixture was added dropwise into anhydrous methanol (50 mL), and the crude product was washed with anhydrous methanol (10 mL × 3). The product PAMAM-PPOBLG was obtained as white solid

(36 mg, 85% yield). ^{1}H NMR analysis was used to characterize the structure of SPP, and GPC analysis was used to characterize the DP and *Đ* of SPP.

In a glove box, PAMAM-PPOBLG (30 mg, 0.11 mmol alkyne group) and 6-azide hexaguanidine (26 mg, 0.14 mmol) were dissolved in DMF (3 mL), into which PMDETA (33 μL, 0.16 mmol) and CuBr (18 mg, 0.16 mmol) were added and the reaction lasted for 36 h at RT. Then, the mixture was exposed to air for oxidation of CuBr, and hydrochloric acid (3-4 mL, 1 mol/L) was added until the solution changed from blue to colorless. The mixture was dialyzed in DI water for 3 d (MWCO = 3500 Da) and lyophilized. The product SPP was obtained as white solid (39 mg, 78% yield) and the secondary structure of SPP at 0.2 mg/mL in DI water was characterized by CD.

Preparation and characterization of SPP/siE2F1 nanocomplexes (NCs)

To prepare SPP/siE2F1 NCs or LPP/siE2F1 NCs, polypeptide solution (5 mg/mL in DEPC water) and siRNA solution (0.1 mg/mL in DEPC water) were mixed at various polypeptide/siRNA weight ratios, vortexed for 5 s, and incubated at RT for 30 min. To evaluate the siRNA condensation, the freshly prepared NCs were subjected to electrophoresis in 2% agarose at 90 mV for 20 min. siRNA migration was visualized by using a gel imaging system.

Zeta potentials and particle sizes of NCs were determined using Malvern Zetasizer Nano ZS. The morphology of SPP/siEF1 NCs was visualized by transmission electron microscopy (TEM).

To further study the siRNA stability against rat serum, freshly prepared SPP/siE2F1 NCs (70 μL, containing 5 μg siRNA) or naked siE2F1 (5 μg) was incubated with rat serum (100 μL) at 37 °C for different time (0.5, 1, 2, and 4 h), and the mixture was then denatured at 85 °C for 5 min. Heparin (1000 U/mL) was added to dissociate siRNA from NCs, and the mixture was loaded on 2% agarose gel (400 ng siRNA/well) followed by electrophoresis at 90 mV for 20 min. The integrity of siRNA was visualized by using a gel imaging system.

Cellular uptake and intracellular kinetics

H9C2 cells were seeded on 12-well plates (5×10^4 cells/well) and cultured for 24 h. The medium was replaced with serum-free DMEM, and various FAM-siRNA-containing NCs (SPP/FAM-siRNA or LPP/FAM-siRNA, w/w = 10; 25k PEI/FAM-siRNA, w/w = 5) were added (0.5 μg FAM-siRNA/well) and incubated with cells for 4 h. Cells were then digested with trypsin, washed three times with cold heparin-containing PBS (20 U/mL), resuspended in PBS, and subjected to flow cytometric analysis. Cells without NCs treatment severed as the blank.

To further compare the cell uptake level of SPP/FAM-siRNA NCs and LPP/FAM-siRNA NCs, H9C2 cells were seeded on 96-well plates (1.5×10^4 cells/well) and cultured for 24 h. The medium was replaced with serum-free DMEM, and FAM-siRNA-containing NCs (SPP/FAM-siRNA or LPP/FAM-siRNA, w/w = 10) were added (0.1 μg FAM-siRNA/well) and incubated with cells for 4 h. Cells were then washed three times with cold heparin-containing PBS (20 U/mL) and lysed with the RIPA lysis buffer (100 μL/well). The content of FAM-siRNA in the lysate was determined by spectrofluorimetry (λ_{ex} = 488 nm, λ_{em} = 520 nm), and the total protein content was determined using the BCA kit. The uptake level was expressed as the amount of FAM-siRNA associated with per milligram of cellular protein.

The study the internalization mechanism of SPP/siRNA NCs, the cellular uptake study was performed in cells pre-treated with various endocytic inhibitors. H9C2 cells were seeded on 96-well plates (1×10^4 cells/well) and cultured for 24 h. Cells were pre-incubated with chlorpromazine (CPZ, 10 μg/mL), genistein (GNT, 100 μg/mL), methyl-β-cyclodextrin (mβCD, 50 μM), or wortmannin (WTM, 50 nM) for 1 h. Then, the medium was replaced with serum-free DMEM, and SPP/FAM-siRNA NCs (w/w = 10, 0.1 μg FAM-siRNA/well) were added and incubated with cells at 37 °C for 4 h. Cells were washed three times with cold heparin-containing PBS (20 U/mL) and lysed with the RIPA lysis buffer (100 μL/well). FAM-siRNA content in the lysate was determined by spectrofluorimetry (λ_{ex} = 488 nm, λ_{em} = 520 nm) and the protein content was determined by the BCA kit. Uptake level was calculated as the amount of

FAM-siRNA per milligram of cellular protein. Results were expressed as the percentage uptake level of NCs in control cells that were not pre-treated with endocytic inhibitors.

To study the endosomal escape of SPP/FAM-siRNA NCs, H9C2 cells were seeded on coverslips in 24-well plates (5×10^4 cells/well) and cultured for 24 h. The medium was replaced with serum-free DMEM, and SPP/FAM-siRNA NCs (0.5 μg FAM-siRNA/well) were added and incubated with cells at 37 °C for different time (2 or 4 h). Cells were washed three times with cold heparin-containing PBS (20 U/mL), and stained with Lysotracker deep red (200 nM, 1 h, for endolysosomes) and Hoechst 33258 (5 μg/mL, 20 min, for nuclei) before observation by confocal laser scanning microscopy (CLSM, Leica, TCS SP5, Germany). The colocalization ratio between green fluorescence (FAM-siRNA) and red fluorescence (Lysotracker deep red-stained endolysosomes) was calculated using the Image J software.

In vitro gene silencing in H9C2 cells

H9C2 cells were seeded on 6-well plates (1×10^6 cells/well) and cultured for 24 h. The medium was replaced with serum-free DMEM and various NCs (SPP/siE2F1, w/w = 10, LPP/siE2F1, w/w = 10, SPP/siSC, w/w = 10, 25k PEI/siE2F1, w/w = 5, 2 μg siRNA/well) were added and incubated with cells at 37 °C for 4 h. The medium was replaced with 10% FBS-containing DMEM and cells were cultured for another 20 h. Cells were further incubated in an anoxic incubator (1% O_2) for another 6 h before the mRNA levels of E2F1, Pink1, and miR421 were determined by real-time PCR. Cells challenged by anoxia but without treatment with NCs served as the control (100% expression level).

Mitochondrial depolarization

The mitochondrial dye JC-1 (Beyotime, Shanghai, China) was used to determine the mitochondrial membrane potential (Ψm)[3]. H9C2 cells were seeded on coverslips in 24-well plates (5×10^4 cells/well) and cultured for 24 h. The medium was replaced with serum-free DMEM and NCs (SPP/siE2F1 or SPP/siSC, w/w = 10, 0.5 μg

siRNA/well) were added and incubated with cells for 4 h. The medium was replaced with 10% FBS-containing DMEM and cells were cultured for another 20 h. Cells were then incubated in an anoxic incubator (1% O_2) for another 6 h, stained with JC-1 solution (0.5 mL, 20 min), washed with washing buffer (1 mL × 3), stained with Hoechst 33258 (5 μg/mL, 10 min), and observed by CLSM.

To quantitatively analyze the Ψm of cells, H9C2 cells on 24-well plates were treated with NCs and challenged by anoxia as described above. After JC-1 staining, cells were digested with trypsin and collected by centrifugation (600 g, 4 min, 4 °C). Cells were suspended in the buffer (10^5 cells/mL), transferred to a black 96-well plate, and subjected to spectrofluorimetric analysis (λ_{ex} = 525 nm, λ_{em} = 590 nm) on the microplate reader. The relative Ψm was denoted as the percentage fluorescence intensity at 590 nm of NCs-treated cells to cells without any treatment.

Intracellular reactive oxygen species (ROS) level

Intracellular ROS level in H9C2 cells was detected using a ROS probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). H9C2 cells were seeded on coverslips in 24-well plates (5×10^4 cells/well) and cultured for 24 h. The medium was replaced with serum-free DMEM and NCs (SPP/siE2F1 or SPP/siSC, w/w = 10, 0.5 μg siRNA/well) were added and incubated with cells for 4 h. The medium was replaced with 10% FBS-containing DMEM and cells were cultured for another 20 h. Cells were then incubated in an anoxic incubator (1% O_2) for another 6 h, stained with DCFH-DA (10 μM, 20 min), fixed with 4% paraformaldehyde, stained with DAPI (5 μg/mL, 10 min), washed with PBS for three times, and observed by CLSM.

Intracellular ATP level

Intracellular ATP level in H9C2 cells was detected using an ATP assay kit. H9C2 cells were seeded on 6-well plates (1×10^6 cells/well) and cultured for 24 h. The medium was replaced with serum-free DMEM, and SPP/siE2F1 NCs (w/w = 10, 1 μg siRNA/mL) were added and incubated with cells for 4 h at 37 °C. The medium was replaced with DMEM containing 10% FBS and cells were incubated for another 20 h.

Cells were then incubated in an anoxic incubator (1% O_2) for another 6 h and were lysed with the lysis buffer (200 μL/well) at 4 °C for 20 min according to the manufacturer's protocol. The supernatant (20 μL) was collected by centrifugation (12,000 g, 5 min, 4 °C), mixed with the APT working solution (100 μL), and subjected to spectrofluorimetric analysis. The ATP concentration in the supernatant was calculated according to the ATP standard curve.

Anti-apoptosis efficiency of NCs

To study the cell apoptosis, H9C2 cells were seeded on 6-well plates (1×10^6 cells/well) and cultured for 24 h. The medium was replaced with serum-free DMEM, and SPP/siE2F1 NCs (w/w = 10, 1 μg siRNA/mL) were added and incubated with cells for 4 h at 37 °C. The medium was replaced with DMEM containing 10% FBS and cells were incubated for another 20 h. Cells were then incubated in an anoxic incubator (1% O_2) for another 6 h. Cells were then collected, stained with the Annexin V-FITC/PI apoptosis kit (Beyotime, Shanghai, China) according to the manufacturer's protocol, and analyzed by flow cytometry.

***In vitro* cytotoxicity of NCs**

To study the cytotoxicity of NCs, H9C2 cells were seeded on 96-well plates (1×10^4 cells/well) and cultured for 24 h. The medium was replaced with serum-free DMEM, and NCs (SPP/siSC, LPP/siSC, or 25k PEI/siSC) at various polymer/siSC weight ratios were added to cells at the final concentration of 1 μg siRNA/mL and incubated for 4 h. Then, the NCs-containing medium was replaced by fresh 10% FBS-containing medium, and cells were cultured for another 20 h before determination of the cell viability by the MTT assay. Cells without NCs treatment served as the control (100% viability).

Establishment of rat MIRI model and *in vivo* administration

Rat MIRI model was established as reported previously[4,5]. Briefly, rats were anesthetized via intraperitoneal injection of pentobarbital sodium (80 mg/kg), and

were intubated and ventilated mechanically using a rodent ventilator (2 mL/100 g). A left intercostal thoracotomy was performed to expose the heart through a 2-cm incision and the left coronary artery was ligated with a 6-0 prolene suture for 30 min. The ischemic condition was confirmed by the evidence of immediate changes, including sudden pallor, distinct dilatation, and paralysis of the affected portion of the left ventricle. The suture was loosened to allow coronary reperfusion, and at 10 min post reperfusion, saline or different NCs (250 μg siRNA/kg) including SPP/siSC NCs (w/w = 10), LPP/siE2F1 NCs (w/w = 10), SPP/siE2F1 NCs (w/w = 10), and 25k PEI/siE2F1 NCs (w/w = 5) were injected into the myocardium through three points around the infarct site. Chest was closed after expelling of the air from the chest cavity using a rubber tube needle. Sham-operated rats receiving the same surgical treatment except coronary artery ligation served as the sham control.

***In vivo* gene silencing efficiency**

Twenty-four hours after intramyocardial injection of NCs, rats were sacrificed and the hearts were harvested. The mRNA and protein levels of E2F1 in the ischemic myocardium were determined by real-time PCR and Western blot, respectively. The dilutions of each antibody used in the Western blot analysis were as follows: anti-E2F1 (1:1000), anti-GAPDH (1:10000), and HRP-conjugated anti-rabbit IgG (1:1000).

***In vivo* anti-apoptosis efficiency**

On day 7 post NCs administration, rats were sacrificed, and the hearts were harvested, fixed with 10% formalin, embedded in paraffin, and sectioned at 10 μm in thickness. Colorimetric TUNEL apoptosis assay kit (Beyotime, China) was used to stain the myocardial sections and evaluate the cardiac cell apoptosis. Apoptotic cells (brown) in five random high power fields per slice were counted and the myocardial apoptosis ratio was denoted as the percentage of apoptotic cells in total cells.

Infarct size analysis

To measure the infarct size, the hearts were collected on day 7 post NCs administration, frozen promptly at -80 °C, sectioned into 2-mm slices, stained with 2,3,5-triphenyte-trazoliumchloride (TTC, 1%) for 15 min at 37 °C, and fixed in 10% formalin buffer at RT for 24 h. The infarcted area (white) was distinguished from the normal area (red) by visualization with a digital scanner. The infarct size (%) was calculated as the weight of infarct tissues normalized by that of the total heart.

Histological analysis

On day 7 post NCs administration, rats were sacrificed, and the hearts were harvested, fixed with 10% formalin, embedded in paraffin, and sectioned at 10 μm in thickness. The tissue sections were individually stained with hematoxylin & eosin (H&E) and Masson's trichrome (MT) to study the histological pathology and myocardium fibrosis, respectively. Fibrotic area (blue) was observed in five random high power fields per slice and calculated with the Image J software. The fibrosis ratio (%) was denoted as the fibrotic area normalized by the total area.

The protein level of TNF-α was determined by Western blot analysis. The dilutions of each antibody used in the Western blot analysis were as follows: anti-TNF-α (1:1000), anti-GAPDH (1:10000), and HRP-conjugated anti-rabbit IgG (1:1000). Immunofluorescence staining was further used to monitor the TNF-α level in the ischemic myocardium. Harvested hearts were dehydrated in 30% (w/v) sucrose solution overnight, embedded in the OTC medium, frozen at -80 °C, and then cryo-sectioned at 10-μm in thickness. The sections were fixed in iced acetone for 5 min, rinsed with PBS for three times, and then immunofluorescence staining was carried out through the following steps, including blocking with 5% bovine serum albumin for 2 h, incubation with primary antibody at 4 °C overnight, incubation with secondary antibody for 1 h at RT, and staining with DAPI (10 μg/mL) for 10 min, followed by observation by CLSM. The concentrations of each antibody were as follows, primary antibody, anti-TNF-α (1:200), secondary antibody, Cy3-goat anti-mouse IgG (1:200).

Echocardiographical analysis

The cardiac function of rats was evaluated by echocardiographic analysis with a Philips high-resolution ultrasound system on day 3 post NCs administration. Ejection fraction (EF, %) and fractional shortening (FS, %), two critical indicators for the cardiac function, were calculated based on the average over three consecutive cardiac cycles.

Biocompatibility evaluation

Saline (200 μL) or SPP/siE2F1 NCs (250 μg siE2F1/kg) were intramyocardially injected to male SD rats as described above. Blood and major organs (heart, liver, spleen, lung, and kidney) were then collected. The major organs were fixed in 10% buffered formalin, embedded in paraffin, cross-sectioned at 8 μm in thickness, stained with H&E, and observed by overall perspective optical microscopy.

Hematological assessment was performed on a Cobas501 automatic hematology analyzer (Roche, USA) including red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), coefficient of variation of red blood cell distribution width (RDW-CV), standard deviation of red blood cell distribution width (RDW-SD), % lymphocyte (LY), and mean platelet volume (MPV). Serum levels of biochemical parameters were determined using a BC-5380 automatic chemistry analyzer (Mindray, China) including cholinesterase (CHE), lactate dehydrogenase (LDH), creatine kinase (CK), alanine aminotransferase (ALT), aspartate amino transferase (AST), total cholesterol (TC), urea (UR), uric acid (UA), creatinine (CR), total protein (TP), albumin (ALB), globulin (GLO), ALB/GLO (A/G), prealbumin (PAB), α-hydroxybutyrate dehydrogenase (α-HBDH), lipase (LIP), and amylase (AMY).

Statistical analysis

All the experimental data were presented as the mean ± standard deviations, and statistical analysis was performed using Student's t-test. The differences between two experimental groups were assessed to be significant at $*p < 0.05$ and very significant at $**p < 0.01$ and $***p < 0.001$.

Supplementary Tables

Table S1. Sequences of siRNA.

siRNA	Sequence (5’-3’)
siE2F1 sense	AUCUGACCACCAAACGCUUdTdT
siE2F1 antisense	AAGCGUUUGGUGGUCAGUdTdT
siSC sense	UUCUUCGAACGUGUCACGUdTdT
siSC antisense	ACGUGACACUUCGGAGAAdTdT

Table S2. Forward (F) and reverse (R) primer sequences.

Primer	Sequence (5’-3’)
E2F1-F	GTATGTATGTATGTATGTATGTATG
E2F1-R	GAGCAAAATAGGACATAGC
Pink1-F	GTATGAAGCCACCATGCCCA
Pink1-R	ACGACATCTGGGCCTTTTCC
miR421-F	CCTGGCTGGCCTCATTAAAT
miR421-R	TATGGTTCTTCACGACTGGTTCAC
GAPDH-F	GACATGCCGCCTGGAGAAAC
GAPDH-R	AGCCCAGGATGCCCTTTAGT

Supplementary Figures

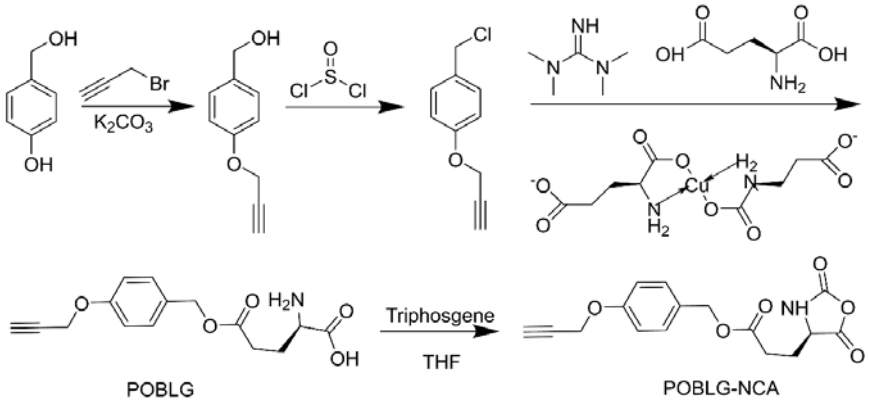

Figure S1. Synthetic route of POBLG-NCA.

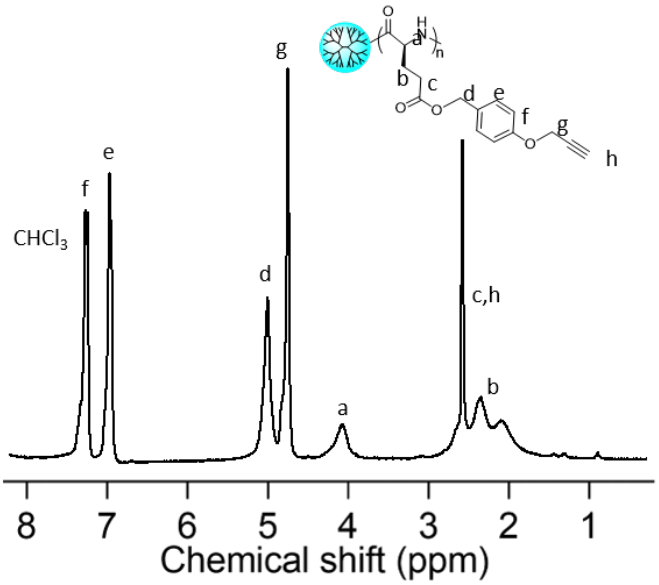

Figure S2. ^{1}H NMR spectrum of PAMAM-PPOBLG ($CDCl_3$, 400 MHz).

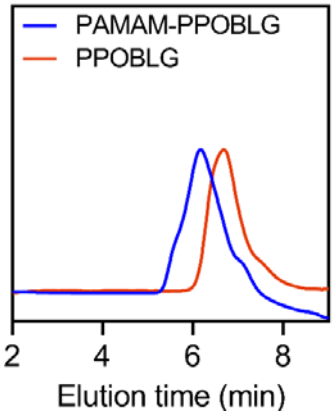

Figure S3. GPC curves of PAMAM-PPOBLG and PPOBLG.

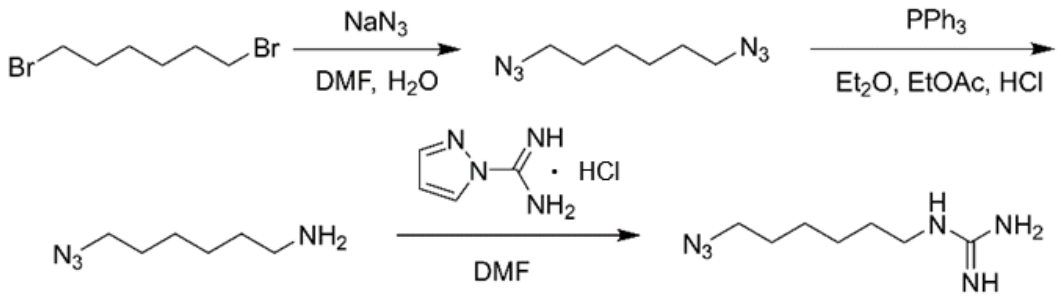

Figure S4. Synthetic route of 6-azide hexaguanidine.

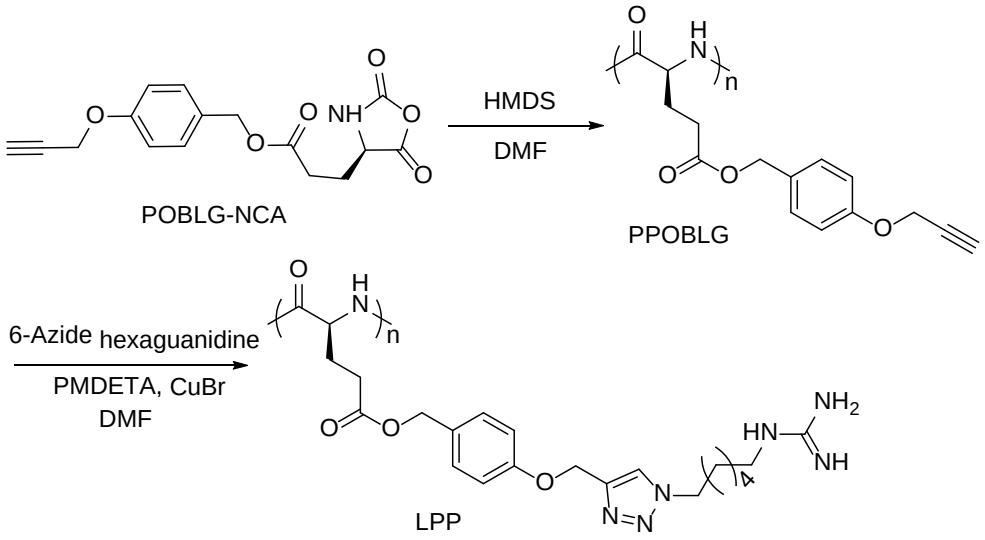

Figure S5. Synthetic route of LPP.

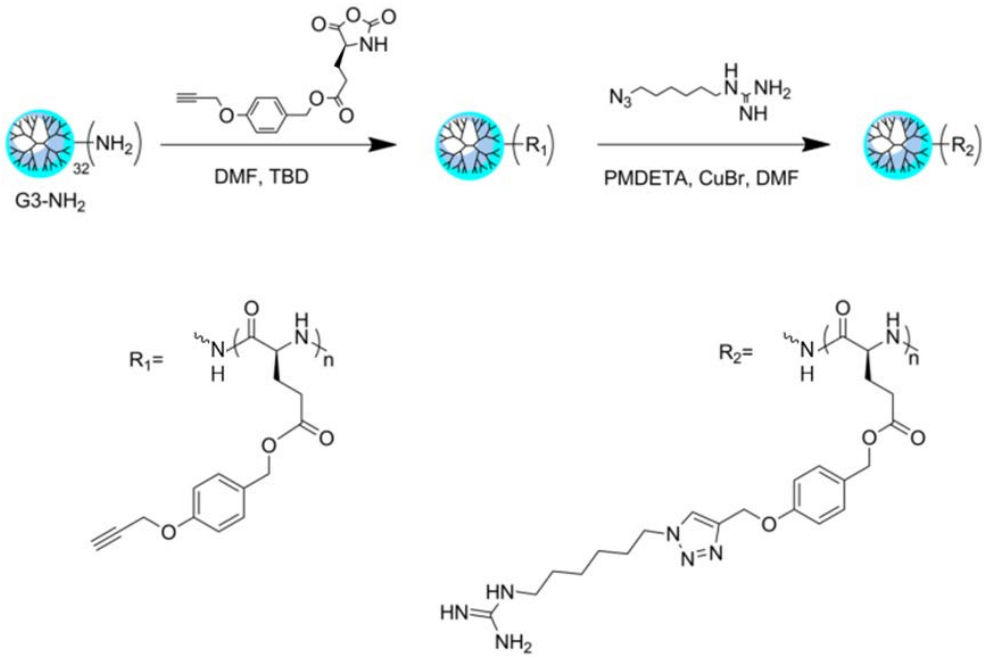

Figure S6. Synthetic route of SPP.

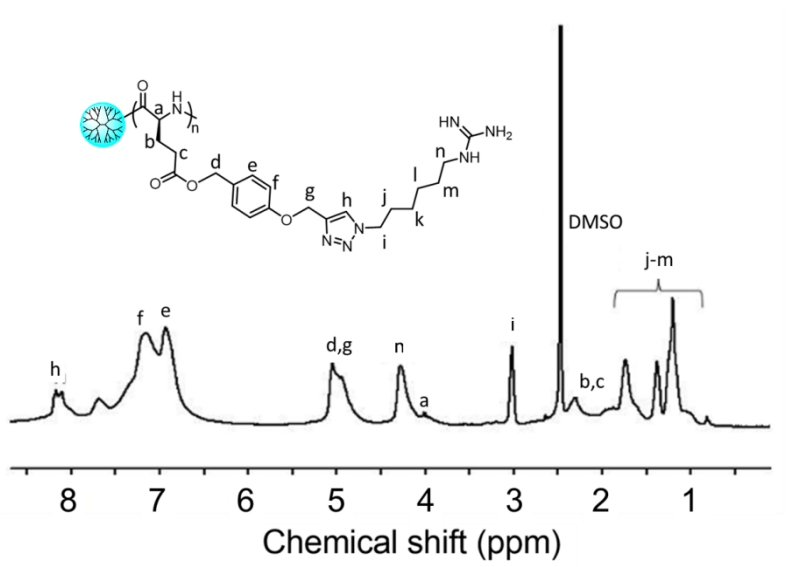

Figure S7. ^{1}H NMR spectrum of SPP (DMSO, 400 MHz).

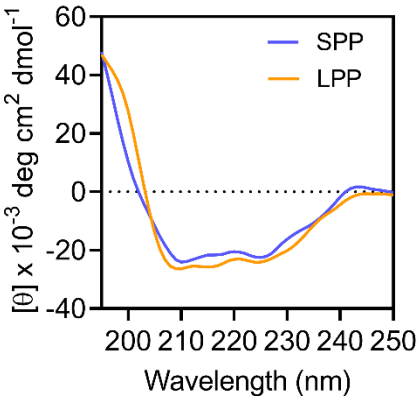

Figure S8. CD spectra of LPP and SPP in DI water (0.2 mg/mL).

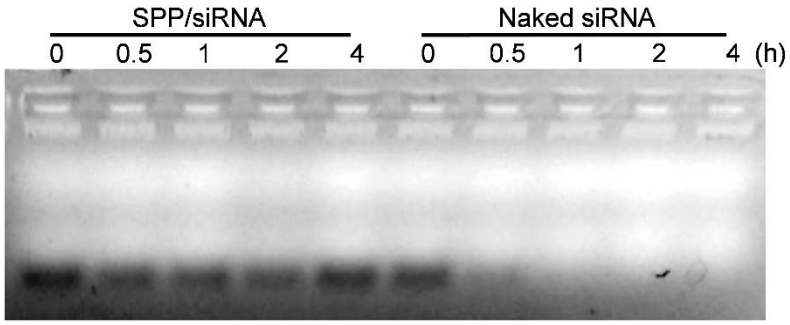

Figure S9. siRNA integrity in SPP/siRNA NCs after incubation with rat serum for different time. Naked siRNA incubated with serum for different time served as the control.

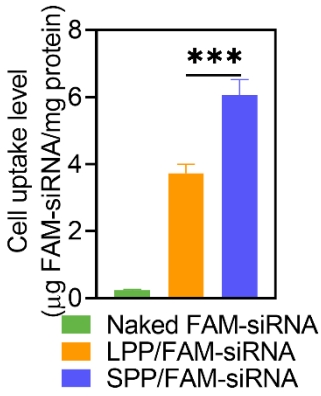

Figure S10. Cellular uptake levels of various FAM-siRNA-containing NCs in H9C2 cells after 4-h incubation as determined by spectrofluorimetry (1 μg FAM-siRNA/mL, polymer/siRNA = 10, w/w, *n* = 3).

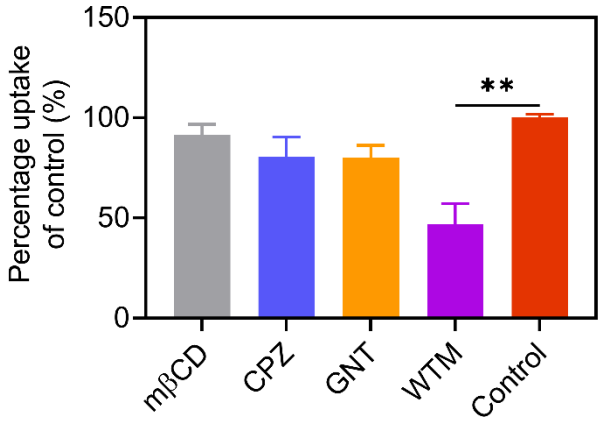

Figure S11. Relative uptake level of SPP/FAM-siRNA NCs in H9C2 cells pre-treated with various endocytic inhibitors (SPP/FAM-siRNA = 10, w/w, *n* = 3).

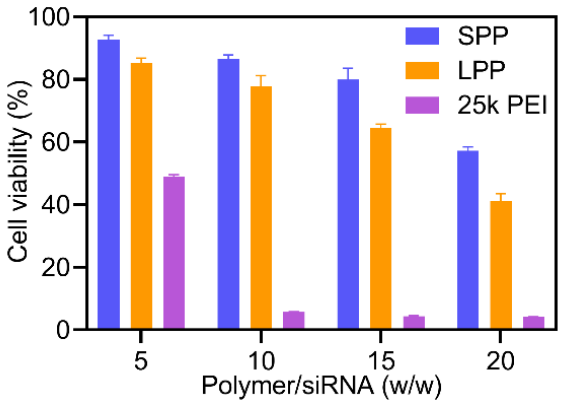

Figure S12. Viability of H9C2 cells after treatment with various NCs at different polymer/siRNA weight ratios (*n* = 3).

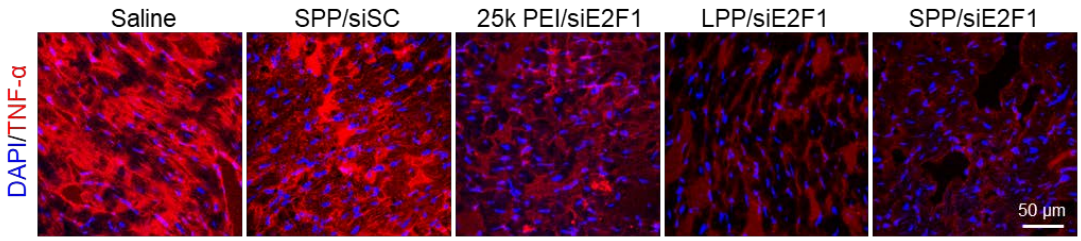

Figure S13. Representative CLSM images of rat ischemic myocardium sections immunostained for TNF-α (red).

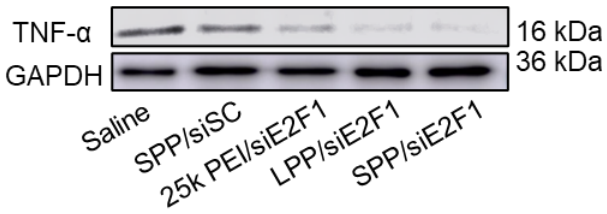

Figure S14. TNF-α protein level in the ischemic heart at 24 h post MIRI as determined by Western blot analysis.

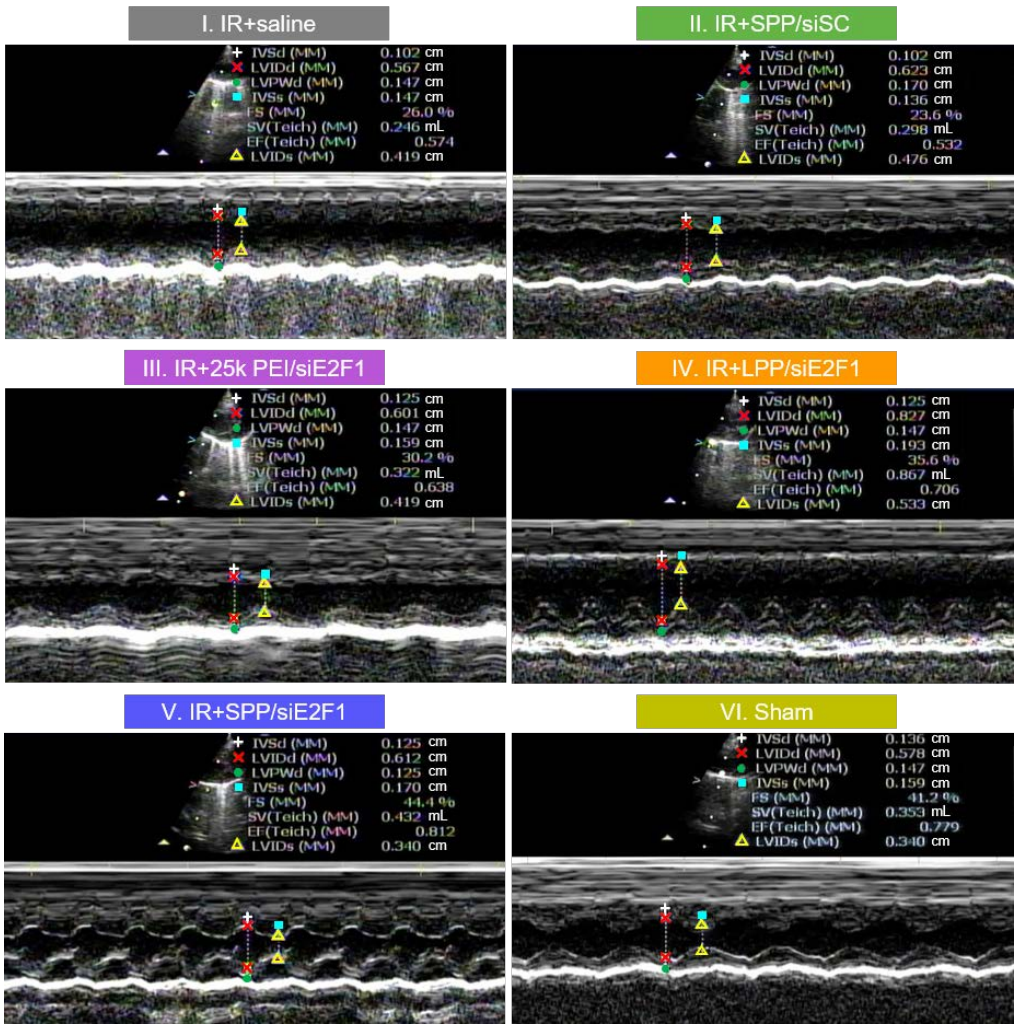

Figure S15. Echocardiographs of MIRI rats post treatment with saline or various NCs. The left-side and right-side marks indicate left ventricular end-diastolic and end-systolic dimensions, respectively.

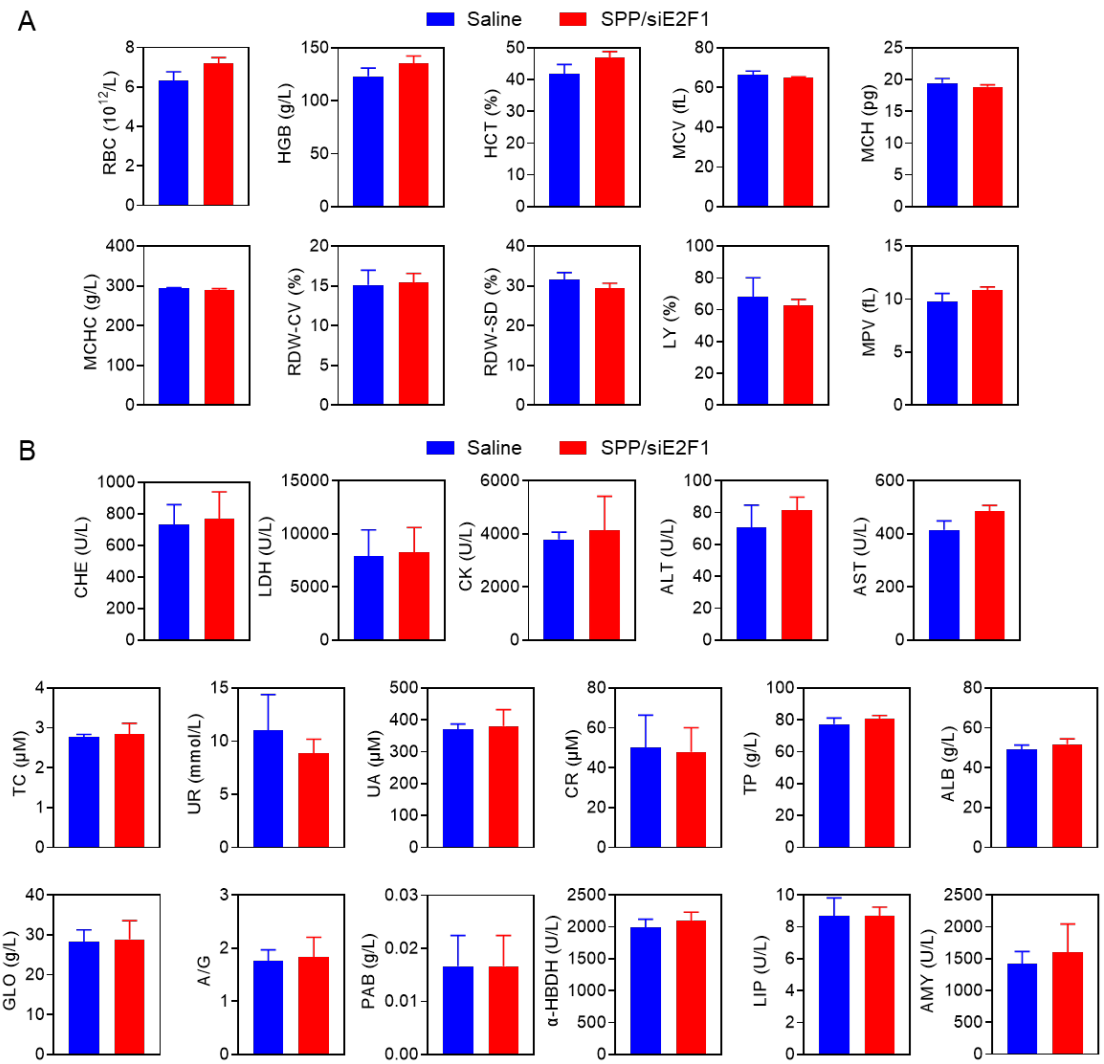

Figure S16. Hematological (A) and biochemical (B) parameters of rats after intramyocardial injection of saline or SPP/siE2F1 NCs (250 μg siE2F1/kg, n = 3).

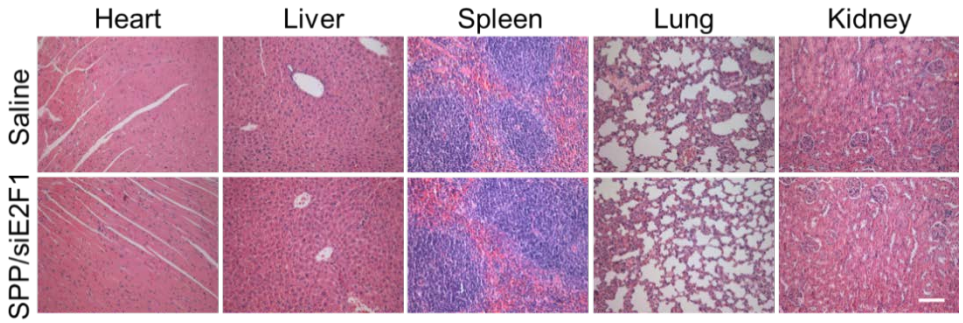

Figure S17. H&E-stained major organ sections of rats after intramyocardial injection of saline or SPP/siE2F1 NCs (250 μg siE2F1/kg, scale bar = 100 μm).

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