SUPPLEMENTARY MATERIAL

MATERIALS AND METHODS

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Eighth Edition, Revised 2010) and with the guidelines of the Animal Care and Use Committee of the University of Louisville, School of Medicine (Louisville, KY, USA).

Isolation, culture, and transfection of c-kit^{POS} CPCs. CPCs were isolated from the entire heart (atria and ventricles) of adult male Fischer 344 rats as described. [1] The tissues were cut into small pieces (<1 mm^3) and suspended in 15 ml of Ham's F12 medium (Gibco) containing 1,600 units/ml of collagenase II (Worthington Labs). After 45 min of digestion, cells were plated into T75 flasks containing Ham's F12 medium supplemented with 10% fetal bovine serum (Seradigm), 5% horse serum (Gibco), 10 ng/ml recombinant human basic fibroblast growth factor (PeproTech), 10 ng/ml leukemia inhibitor factor (Millipore), 5 mU human erythropoietin (Sigma), 0.2 mM L-glutathione (Sigma), and 50 µg/ml porcine gelatin (Sigma). Cells were expanded for 5 days and subjected to immunomagnetic sorting for c-kit. Cells were then trypsinized, resuspended in 1 ml/10^7 cells of MACS buffer (Miltenyi), and then incubated with 50 µl/10^7 cells of anti-c-kit antibody (H-300, Santa Cruz) for 30 min on ice. After washing, cells were resuspended in 240 µl/10^7 cells of MACS buffer and incubated with 60 µl/10^7 cells of microbeads (anti-rabbit microbeads, Miltenyi) for 15 min at 4°C. After washing, cells were applied onto an LS column (Miltenyi) and the positive fraction was plated into a T25 flask. The c-kit^{POS} CPCs were transfected at passage 2 or 3 with a lentiviral vector expressing GFP or mCherry protein. After selection with blasticidin (Invitrogen) and fluorescence-activated cell sorting (FACS), the GFP or mCherry positive cells were expanded and cryopreserved at passage 5 to 6. Then, cells were thawed, cultured, and injected into the rat heart at passages 6 to 8. The rationale for using male donor CPCs was that the fate of transplanted CPCs can be tracked down by detecting Y-chromosome^{pos} cells, although previous studies have suggested that bone marrow-derived mesenchymal stromal cells differ significantly between male and female sources, with female cells usually performing more robustly.[2]

Characterization of c-kit^{POS} CPCs. Small samples of CPCs were fixed in 4% paraformaldehyde for 20 min at room temperature at the same passage that was used for injection. Fixed cells were incubated for 1 h at room temperature with an antibody against c-kit (C19), a hematopoietic lineage marker (CD45), an endothelial marker (CD31), or mesenchymal markers (CD90/105/73/29). Then, cells were incubated for 1 h at room temperature with a secondary antibody if the primary antibodies were not directly conjugated. FACS analysis was done with Accuri C6 (Accuri Cytometers) or BD LSR II (BD Biosciences) instruments.[1]

Surgical procedures. The rat model of old MI has been described previously.[3] Briefly, adult female Fischer 344 rats (age, 4 months; weight 175 ± 2 g) were anesthetized with ketamine (37 mg/kg) and xylazine (5 mg/kg), intubated, and ventilated with a rodent respirator. Anesthesia was maintained with 1% isoflurane inhalation and body temperature was kept at 37°C with a

heating pad. After administration of antibiotics, the chest was opened and the heart was exposed. All rats underwent a 2-h occlusion of the left anterior descending coronary artery followed by reperfusion. Ketoprofen was given for three days after surgery (5 mg/kg).

Treatment protocol. Thirty days after surgery, the animals were randomly allocated to three treatment groups: vehicle (control), single dose, or multiple doses (Fig. 1). Randomization was performed using the MS Excel random group generator. All rats received an echo-guided intraventricular injection, which was performed using the Vevo 2100 Imaging System (VisualSonics) equipped with a 20-MHz transducer, a Vevo Image Station with Injection Mount and micro-manipulation controls, and a Chemyx NanoJet Stereotaxic syringe pump (Chemyx Inc. TX). Before the procedure, rats were anesthetized with 3% isoflurane. The anterior chest was shaved and the animals were placed on the imaging table in the right lateral decubitus position with the left lateral side facing the injection mount. Light anesthesia was maintained with 1% isoflurane. With the imaging transducer aligned perpendicularly to the injection mount, the left ventricle was initially imaged in the parasternal plane and a good 2D long-axis view was procured by adjusting the angle and position of the imaging table. The transducer was then turned 90 degrees clockwise and the left ventricle was scanned in the 2D short-axis and color Doppler views from apex to base to determine the optimal site for needle insertion, a site that did not include the infarct scar or a coronary artery. Under the guidance of a real-time B-mode view, a 30 G injection needle (1" length) was then carefully inserted from the left lateral side using the micro-manipulation controls and advanced into the center of the LV cavity.

Rats received an infusion of CPCs (12 x 10^6 cells in 5 ml of DPBS (Dulbecco's phosphate-buffered saline, Gibco) or vehicle (5 ml of DPBS) into the LV cavity with the injection pump at a rate of 1.25 ml/min for 4 min. The single-dose and multiple-dose groups received CPCs whereas the vehicle group received DPBS. Thirty-five days later, this procedure was repeated; rats received a second infusion of CPCs (multiple-dose group) or an infusion of DPBS (single-dose and vehicle groups). Thirty-five days later, rats received a third infusion of CPCs (multiple-dose group) or another infusion of DPBS (single-dose and vehicle groups). mCherry-labeled CPCs were used for the 1st treatment, non-labeled CPCs for the 2nd treatment, and GFP-labeled CPCs for the 3rd treatment. To monitor formation of new cells, in all groups 5-bromo-2'-deoxyuridine (BrdU, Sigma) was given for 35 days after the 1st treatment and 5-iodo-2'-deoxyuridine (IdU, Sigma) for 35 days after the 3rd treatment (both were given in the drinking water at a final concentration of 0.1%). At 35 days after the 3rd treatment, rats were subjected to hemodynamic studies and euthanized for histologic studies or PCR analysis (Fig. 1).

Before initiating the protocol, pilot studies were conducted to identify a dose of CPCs that would results in LV myocardial retention similar to that observed after intracoronary infusion of 1 million CPCs, the dose used in our previous studies in rats.[1, 3, 4] Rats underwent a 90-min coronary occlusion and reperfusion. Four hours after reperfusion, they received 1.0 $\times 10^6$ CPCs (in 1 ml of DPBS) via intracoronary infusion as in our previous studies[1, 3-5], 3.0, 9.0, or 12.0 $\times 10^6$ CPCs (in 5 ml of DPBS over 4 min) using echo-guided injection into the LV cavity, or 3.0x10^6 CPCs i.v.. Rats were euthanized 24 h after CPC delivery and the heart was harvested to measure the number of CPCs in the tissue by real-time PCR.

Echocardiographic studies. All echocardiographic analyses were performed by investigators who were blinded to treatment allocation. Serial echocardiograms were obtained at five time-

points: baseline (three days before coronary artery occlusion), 30 days after MI (before the 1st treatment), 35 days after the 1st treatment (before the 2nd treatment), 35 days after the 2nd treatment (before the 3rd treatment), and 35 days after the 3rd treatment (before euthanasia)(Fig. 1 of the main manuscript). The echocardiographic studies were performed as described[1, 3-5] using a Vevo 2100 Imaging System equipped with a 20-MHz transducer. Briefly, rats were lightly anesthetized with 3% isoflurane. The anterior chest was shaved and the animals were placed in the left lateral decubitus position. A rectal temperature probe was inserted, and the body temperature was carefully maintained between 37.0-37.5°C with a heating pad throughout the study. Anesthesia was maintained with 1% isoflurane. The parasternal long-axis and parasternal short-axis views were used to obtain 2D and M-mode images for the measurement of LV mass, end-diastolic and end-systolic thickness of the infarcted wall (IWTd and IWTs, respectively) and posterior (noninfarcted) wall (PWTd and PWTs), infarcted and posterior LV wall systolic thickening fraction (ThF) (IWThF and PWThF), end-diastolic and end-systolic LV volume (LVEDV and LVESV), stroke volume (SV), cardiac output (CO), ejection fraction (EF), and fractional area change (FAC).[1, 3-5] The akinetic endocardial length (AL), expressed as a percentage of the entire LV endocardial length, was also calculated.[6] Measurements were performed according to the American Society for Echocardiography, and were averaged over three consecutive cardiac cycles.

Hemodynamic studies. All hemodynamic analyses were performed by investigators who were blinded to treatment allocation. The hemodynamic studies were conducted 35 days after the 3rd treatment, just before euthanasia (Fig. 1). The protocol has been described. [1, 3, 5] Briefly, rats were anesthetized with 2-3% isoflurane, intubated, and mechanically ventilated. Anesthesia was maintained with 1% isoflurane and the core temperature carefully kept at 37.0°C with a heating pad throughout the study. A 2F microtip pressure-volume (PV) catheter (SPR-869, Millar Instruments) was inserted into the right carotid artery and advanced into the LV cavity. The right jugular vein was cannulated for fluid administration. After 30 min of stabilization, the PV signals were recorded continuously with an ARIA PV conductance system (Miller Instruments) coupled with a Powerlab/4SP A/D converter (AD Instruments), stored, and displayed on a personal computer. PV relations were assessed by transiently compressing the inferior vena cava with a cotton swab. Parallel conductance from surrounding structures was calculated by injecting a small bolus of 15% NaCl through the jugular vein. Hemodynamic indexes were calculated using the PVAN3.2software (Millar Instruments).[1, 3, 5]

Histologic studies. The protocol for histologic analyses has been described.[1, 3, 5] Briefly, after the hemodynamic measurements, a polyethylene catheter filled with phosphate buffer (0.2 M, pH 7.4) and heparin (100 IU/ml) was advanced to the ascending aorta via the right carotid artery. In rapid succession, the heart was arrested in diastole by injecting 1 ml of a mixture of cadmium chloride (100 mM)/potassium chloride (3 M) through the aortic catheter. The heart was then excised and perfused retrogradely with phosphate buffer for ~3 min to flush out residual blood in the coronary circulation, followed by perfusion with 10% neutral buffered formalin solution for 10 min. Perfusion pressure was maintained between 60 and 80 mmHg while end-diastolic pressure was kept at 8 mmHg. After perfusion-fixation, the atria and right ventricle were dissected from the left ventricle. The LV weight was measured; LV volume was

calculated as the difference between the weight of the water-filled and empty left ventricle by converting weight to volume (1 g H_2O = 1 ml H_2O).[1, 3, 5, 7]

The heart was cut into four transverse slices (~2 mm thick), which were processed, embedded in paraffin, sectioned at 4-µm intervals, and stained with Masson's trichrome, picrosirius red, or antibodies against GFP, mCherry and cell-type specific markers. Images were acquired digitally and analyzed using NIH ImageJ (1.48v). From the Masson's trichrome–stained images, morphometric parameters including total LV area, risk region area, scar area, and LV wall thickness in the risk and noninfarcted regions were measured in each section.[1, 3, 5] Myocardial collagen content was quantitated on picrosirius red-stained heart images acquired under polarized light microscopy by determining collagen density (arbitrary unit) per mm^2 of risk region or noninfarcted region with NIH ImageJ software.[1, 3]

Immunohistochemistry. Immunohistochemistry was performed in formalin-fixed, paraffin-embedded, 4-µm-thick heart sections. CPC proliferation was assessed by immunofluorescent staining of nuclei with specific antibodies for BrdU and IdU (Santa Cruz). Myocytes were stained with anti-cardiac troponin I (cTnI) antibody (Santa Cruz) or with anti-α-sarcomeric actin (α-SA) antibody (Sigma). Myocyte membranes were stained with FITC-conjugated wheat germ agglutinin (WGA) (Sigma) to facilitate the identification of individual myocytes for analysis of myocyte cross-sectional area and myocyte density. To determine vessel density, heart sections were stained with specific FITC-conjugated Isolectin B4 (IB4) antibody (Sigma).[8] Triplicate immunofluorescent staining was conducted with specific anti-BrdU, anti-IdU, and anti-cTnI antibodies for evaluation of proliferating myocytes, and with specific anti-BrdU, anti-IdU, and anti-Isolectin B4 antibodies for evaluation of proliferating vessels. Nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole).

Fluorescent *in situ* hybridization (FISH) analysis. To assess the fate of the transplanted male CPCs, single-labeled Y chromosome was detected by fluorescence *in situ* hybridization (FISH) according to the modified manufacturer's protocol (ID Labs, London, ON).[1, 9] 4-µm-Thick serial tissue sections were cut from paraffin-embedded heart blocks. Heart tissue sections on coated slides were deparaffinized in xylene and rehydrated gradually, followed by an antigen retrieval procedure. After treatment with pepsin and air-drying, a denatured rat FITC-conjugated Y chromosome probe was added to each section and covered by coverslip. After overnight hybridization at RT°C, the coverslip was removed and serial washings were performed. Male and female rat heart tissue sections were used as positive and negative controls, respectively, in the FISH staining procedure. Proliferation of transplanted male CPCs in the heart was assessed by triplicate fluorescent staining with a FITC-conjugated Y-chromosome probe and specific anti-BrdU and anti-IdU antibodies (Santa Cruz). Myocardial morphology was examined with the confocal transmitted light channel's detector (ChD) in which the pseudocolor selected for myocardial background in the ChD channel was gray white. Nuclei were counterstained with DAPI. To minimize auto fluorescence, slides were incubated with 0.1% Sudan Black B (Sigma), rinsed in PBS, and then mounted with ProLong Gold antifade reagent (Invitrogen).[10] Immunohistochemical signals were imaged by confocal microscopy and quantitatively analyzed by Image J (1.46, NIH). In each heart, Y-chromosome^{POS} nuclei, Y-chromosome^{POS} myocytes, Y-chromosome^{POS}/BrdU^{POS} myocytes, Y-chromosome^{POS}/IdU^{POS} myocytes, Y-chromosome^{POS}/BrdU^{POS}/IdU^{POS} myocytes , total

myocytes, and total nuclei were counted in 25 confocal images (5 from each border zone, 10 from the scar region, and 5 from the noninfarcted region). For Y-chromosome^{POS} nuclear analysis, an average of 1,274 nuclei was counted in a 0.354 mm^2 area per rat heart (n=8/group).

Measurement of transplanted cells by real-time PCR. To determine the absolute number of transplanted CPCs, a quantitative real-time PCR-based method was used as previously described.[11, 12] A subset of hearts from the single-dose group (n=8) and the multiple-dose group (n=11) were frozen just after excision. The left ventricle was divided into two regions: the risk region (anterior wall and septum), which included the border zone, and the noninfarcted region (posterior LV wall). The tissue samples were placed in screw-cap tubes containing an excess volume of Buffer ATL (tissue lysis buffer) plus Proteinase K. The internal standard (250,000 female human peripheral mononuclear cells [hPBMCs]) was resuspended using 50 µl of the above tissue digestion solution and added to each tube. After overnight incubation, the samples were treated with RNase A (Qiagen) to prevent RNA contamination of genomic DNA. The DNA concentrations of the samples were measured using standard UV spectrophotometry. For each real-time PCR, 200 ng of genomic DNA was amplified in a 20-µl reaction using SYBR Green PCR Master Mix and a StepOne Plus real-time PCR machine. Each sample was run in triplicate and analyzed using the following sets of primers: rat *Sry* (for male rat DNA) 5'-TGCAGCGTGAAGTTGCCTCAACA-3' and 5'-ACTGGTGTGCAGCTCTAGCCCA-3'; *HLA-DMA* (for human DNA) 5'-TACAAACCTCAGCTACCTTCGTGGC-3' and 5'-AACCCAGCTGACTCTGGGTGG-3'; rat *Gapdh* (for total rat DNA) 5'-GCGCATGCCGGTCTGGCTAA-3' and 5'-CGAGCCTTCCCCAGGTCCGA-3'. The amount of target DNA (human and male rat DNA) present in the PCR sample was calculated using the corresponding standard curve. The total number of cells present in each tissue was calculated using the following equation: $F = (f*H)/h$, where F, total number of (female) rat cells in the tissue; f, number of rat cells represented in the DNA sample; H, total number of human cells added to the tissue sample (i.e., 250,000); h, number of human cells represented in the DNA sample. The total number of male rat CPCs present in each tissue sample was calculated using the following equation: $M = (m*F)/f$, where M, total number of male rat cells in the tissue and m, number of rat cells presented in the DNA sample. The above two equations can be merged into a simplified equation: $M = (m*H)/h$.

Statistical analysis. All data are expressed as means ± SEM. Echocardiographic data were analyzed with two-way repeated-measures ANOVA followed by Student's t-tests with Bonferroni correction for intra- and inter-group comparisons, as appropriate. All parametric data including morphometric, histologic, immunohistochemical, and hemodynamic data were analyzed by one-way ANOVA followed by Student's t-tests with Bonferroni correction for inter-group comparisons.[13-15] Mortality was analyzed by the chi-square test. All analyses were conducted with SigmaStat 3.5. P values <0.05 were considered significant.

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contractile function. A pathophysiological basis for chronic myocardial "stunning". *J Clin Invest*. 1995;96:1066-84.

SUPPLEMENTARY TABLES

Supplementary Table I: Enrollment and exclusions

	Vehicle	Single dose	Multiple doses
Initial enrollment of rats for MI studies	85		
Death after MI (all deaths were within seven days)	13 (15.3%)		
Rats assigned to group	18	24	30
Death after either the 1st, 2nd, or 3rd infusion due to intrathoracic bleeding from the LV injection site	2 (11.1%)	3 (12.5%)	4 (13.3%)
Completed the protocol	16	21	26
Excluded	0	1 (EF drop <15 units after MI by echocardiography)	1 (very small scar size [<10% of risk region] by trichrome stain analysis)
Total rats included in final analyses	16	20	25
Hearts used for assessment of LV function	16	16	18
Hearts used for pathology (all of these hearts were used for assessment of LV function)	16	12	14
Hearts used for real-time PCR after assessment of LV function	0	4	4
Hearts used for real-time PCR only	0	4	7
Hearts excluded from final analysis of real-time PCR (due to extremely high CPC number in myocardium)	0	0	2
Total hearts included for final real-time PCR data report	0	8	9

Supplementary Table II. Body Weight and Gross Measurements

	Initial Body Weight, g	Final Body Wight, g	LV weight, mg	LV Chamber Volume, mm^3
Vehicle (n=16)	173.9±3.3	215.7±1.8	707.3±23.8	195.5±24.6
Single dose (n=12)	179.7±4.8	224.3±3.7	704.6±38.2	164.5±30.3
Multiple doses (n=14)	178.0±3.1	220.7±3.8	706.3±34.3	153.5±24.0

Data are means ± SEM

Supplementary Table III. Echocardiographic data

	Vehicle Control (n=16)	Single dose (n=16)	Multiple doses (n=18)
Baseline (before MI)			
Heart rate (bpm)	326±6	327±6	325±4
IW Thd (mm)	1.52±0.03	1.50±0.02	1.52±0.03
IW Ths (mm)	2.44±0.04	2.37±0.05	2.42±0.05
LVEDD (mm)	5.69±0.06	5.66±0.09	5.84±0.07
LVESD (mm)	2.88±0.08	2.87±0.08	2.98±0.07
PWd (mm)	1.57±0.03	1.55±0.03	1.53±0.02
PWs (mm)	2.50±0.05	2.51±0.04	2.53±0.05
EF (%)	79.5±0.7	78.7±0.7	79.3±0.6
FS (%)	21.9±0.6	21.4±0.9	21.0±0.8
FAC (%)	69.7±1.2	70.4±0.8	70.0±0.8
LVEDV (μl)	193.6±6.4	190.2±5.9	189.5±5.2
LVESV (μl)	39.8±2.2	40.6±2.2	39.1±1.4
CO (ml/min)	49.9±1.3	49.0±1.3	49.4±1.4
SV (μl)	153.9±4.9	149.5±4.2	150.4±4.3
IW ThF (%)	65.6±2.7	68.7±2.1	69.8±2.0
PW ThF (%)	59.1±2.4	62.9±3.1	65.2±2.5
LV mass (mg)	523.5±16.8	509.0±18.5	536.3±12.9
AL (%)	0	0	0
30 days after MI (before treatment)			
Heart rate (bpm)	322±5	328±4	326±5
IW Thd (mm)	0.75±0.04	0.73±0.04	0.69±0.04
IW Ths (mm)	1.01±0.05	0.89±0.05	0.82±0.05
LVEDD (mm)	6.73±0.15	6.78±0.14	7.13±0.15
LVESD (mm)	4.11±0.21	4.23±0.21	4.70±0.22
PWd (mm)	1.61±0.05	1.63±0.04	1.48±0.03
PWs (mm)	2.45±0.04	2.49±0.07	2.30±0.03
EF (%)	52.1±1.4	50.0±1.3	49.6±1.4
FS (%)	8.6±0.8	7.6±0.6	9.4±0.5
FAC (%)	53.7±1.9	53.1±2.7	48.5±2.0
LVEDV (μl)	290.0±12.1	280.7±11.6	303.2±11.2
LVESV (μl)	139.5±8.0	141.2±7.8	154.7±9.2
CO (ml/min)	48.3±2.0	45.7±1.8	48.5±1.6
SV (μl)	150.5±6.4	139.5±5.5	148.5±3.8
IW ThF (%)	35.5±2.7	21.0±2.7[b]	18.2±2.0[b]
PW ThF (%)	54.0±4.4	53.3±2.3	56.1±3.4
LV mass (mg)	646.7±16.0	640.1±24.7	586.7±20.1
AL (%)	28.8±2.8	28.5±1.9	28.7±2.6

	Vehicle Control (n=16)	Single dose (n=16)	Multiple doses (n=18)
35 days after 1st treatment			
Heart rate (bpm)	326±6	322±4	322±4
IW Thd (mm)	0.64±0.04[f]	0.82±0.03[bf]	0.79±0.03[bf]
IW Ths (mm)	0.79±0.05[f]	1.08±0.04[bef]	1.00±0.05[bef]
LVEDD (mm)	6.67±0.15	6.38±0.12[f]	6.88±0.19[e]
LVESD (mm)	4.37±0.20	3.90±0.18[ae]	4.34±0.26[f]
PWd (mm)	1.55±0.08	1.63±0.04	1.56±0.04
PWs (mm)	2.33±0.12	2.51±0.05	2.37±0.06
EF (%)	46.7±1.7[f]	54.0.8±1.2[bf]	53.0±1.6[af]
FS (%)	8.2±0.7	10.3±1.0[e]	9.4±0.8
FAC (%)	48.0±1.8[f]	56.1±1.9[a]	50.9±2.3
LVEDV (µl)	306.1±12.6	292.5±10.6	300.2±15.4
LVESV (µl)	164.1±9.7[f]	135.1±7.1[a]	144.5±11.8[e]
CO (ml/min)	46.2±2.1	50.8±2.1[f]	50.1±1.7
SV (µl)	141.9±6.4	157.4±5.8[f]	155.7±5.4
IW ThF (%)	21.6±2.4[f]	32.3±2.5[bf]	26.7±2.8[f]
PW ThF (%)	50.3±3.4	54.9±3.2	52.4±2.8
LV mass (mg)	609.6±34.4	610.1±18.5	637.3±20.3
AL (%)	33.2±2.8[f]	22.6±2.4[bf]	23.1±3.2[af]
35 days after 2nd treatment			
Heart rate (bpm)	329±8	323±8	322±5
IW Thd (mm)	0.61±0.03[f]	0.85±0.03[bef]	0.90±0.05[bf]
IW Ths (mm)	0.75±0.05[f]	1.11±0.05[bf]	1.21±0.06[bf]
LVEDD (mm)	6.93±0.19	6.49±0.12	6.92±0.18[e]
LVESD (mm)	4.70±0.21[f]	4.00±0.14[b]	4.22±0.24[f]
PWd (mm)	1.59±0.07	1.56±0.04	1.55±0.03
PWs (mm)	2.31±0.10	2.39±0.05	2.49±0.03[f]
EF (%)	44.4±2.0[f]	54.4±1.0[bf]	57.0±1.7[bf]
FS (%)	8.7±0.9	9.9±1.0	12.2±0.8[bf]
FAC (%)	45.3±1.9[f]	53.8±2.0[b]	53.8±2.0[bf]
LVEDV (µl)	313.3±16.2	312.7±12.4[e]	311.4±13.0
LVESV (µl)	175.7±12.5[f]	143.0±7.5[a]	136.5±10.6[af]
CO (ml/min)	45.0±2.4	54.9±2.5[bf]	56.2±1.8[bf]
SV (µl)	137.6±7.8	169.6±6.5[bf]	174.9±5.3[bf]
IW ThF (%)	22.4±2.8[f]	30.2±2.1[af]	34.7±2.6[bf]
PW ThF (%)	45.0±4.3	53.9±3.8	61.4±2.0[b]
LV mass (mg)	639.3±32.7	627.4±26.7	662.3±19.0
AL (%)	35.9±1.7[f]	22.6±1.7[bf]	18.2±2.5[bf]

	Vehicle Control (n=16)	Single dose (n=16)	Multiple doses (n=18)
Final echo (35 days after 3rd treatment)			
Heart rate (bpm)	330±8	323±8	322±5
IW Thd (mm)	0.54±0.03[f]	0.88±0.03[bef]	1.01±0.05[bcf]
IW Ths (mm)	0.65±0.04[f]	1.13±0.05[bf]	1.42±0.08[bdf]
LVEDD (mm)	6.96±0.16	6.74±0.13	6.84±0.18[f]
LVESD (mm)	4.96±0.18[f]	4.09±0.16[b]	4.02±0.20[be f]
PWd (mm)	1.58±0.09	1.64±0.04	1.56±0.03[e]
PWs (mm)	2.19±0.12[e]	2.51±0.05[a]	2.52±0.04[bf]
EF (%)	40.1±1.9[f]	54.7±0.8[bf]	62.5±1.5[bdf]
FS (%)	7.9±0.9	10.2±0.9[f]	12.8±0.9[bcf]
FAC (%)	41.7±2.5[f]	54.8±1.6[b]	59.8±1.5[bcf]
LVEDV (μl)	329.7±18.5[e]	328.0±11.3[f]	343.0±18.2[f]
LVESV (μl)	196.9±12.0[f]	149.1±6.9[b]	130.4±10.6[bf]
CO (ml/min)	43.6±3.2	58.1±2.2[bf]	69.9±3.8[bcf]
SV (μl)	132.7±10.2	178.8±5.3[bf]	212.5±10.0[bdf]
IW ThF (%)	20.1±2.4[f]	29.1±2.2[bef]	40.5±3.2[bcf]
PW ThF (%)	38.9±3.4[f]	54.1±2.2[b]	61.4±2.3[bc]
LV mass (mg)	634.8±43.6	695.6±27.2	692.0±21.4
AL (%)	37.0±2.6[f]	22.7±2.0[b f]	15.1±2.5[b cf]

Data are means ± SEM.

IW Thd, infarcted wall thickness at end-diastole; **IW Ths**, infarcted wall thickness at end-systole; **LVEDD**, left ventricular end-diastolic diameter; **LVESD**, left ventricular end-systolic diameter; **PWd**, posterior wall thickness at end-diastole; **PWs**, posterior wall thickness at end-systole; **EF**, ejection fraction; **FS**, fractional shortening; **FAC**, fractional area change; **LVEDV**, left ventricular end-diastolic volume; **LVESV**, left ventricular end-systolic volume; **CO**, cardiac output; **SV**, stroke volume; **IW ThF**, infarcted wall systolic thickening fraction; **PW ThF**, posterior wall systolic thickening fraction; **AL**, akinetic endocardial length (expressed as a percentage of the LV endocardial length). [a], $P<0.05$, and [b], $P<0.01$ vs. vehicle; [c], $P<0.05$, and [d], $P<0.01$ vs. single dose; [e], $P<0.05$, and [f], $P<0.01$ vs after MI;

Supplementary Table IV. Hemodynamic data

	Vehicle (Control) (n=16)	Single dose (n=16)	Multiple doses (n=18)
Heart rate (bpm)	324±6	330±6	335±4
LV end-diastolic volume (μL)	295.1±17.7	225.4±18.6 [a]	193.2±14.2[b]
LV end-systolic volume (μL)	170.9±8.1	101.7±6.4[b]	73.7±5.4[bd]
LV end-systolic pressure	100.5±3.2	111.2±2.6 [a]	112.4±2.3 [b]
LV end-diastolic pressure	10.9±0.7	8.3±0.2[b]	7.1±0.4[bc]
Ejection fraction (%)	46.8±1.1	60.3±1.0[b]	65.7±1.1[bd]
dP/dt_{max} (mmHg/sec)	6365±294	7614±203[b]	7983±135[b]
dP/dt_{min} (mmHg/sec)	-6334±249	-7688±275[b]	-8175±169[b]
$P@dP/dt_{max}$ (mmHg)	70.0±3.6	78.9±1.5 [a]	78.8±2.1 [a]
$V@dP/dt_{max}$ (μL)	286.1±16.6	219.2±16.7 [b]	175.4±12.3 [bc]
$V@dP/dt_{min}$ (μL)	167.1±8.3	102.9±6.2 [b]	74.2±5.9 [bd]
Tau_w (msec)	12.7±0.4	10.9±0.4 [b]	10.4±0.3[b]
PAMP (mWatts/μL^2)	7.6±0.6	16.1±2.0[b]	19.4±1.7[b]
E*es*	0.62±0.05	0.90±0.06[b]	1.19±0.08[bd]
PRSW	62.4±5.3	72.6±5.5	98.1±6.9[bd]
E_{max}	1.47±0.27	1.86±0.18	2.63±0.37[a]
dP/dt-EDV	25.5±2.2	42.0±6.1[a]	53.2±4.3[b]
dP/dt_{max}-EDV	22.6±1.5	36.8±3.2[b]	45.1±3.2[b]

Data are means ± SEM.

Tau_w, Tau-Weiss method; **PAMP**, preload-adjusted maximal power; **E*es***, end-systolic elastance; **PRSW**, preload-recruitable stroke work; **E*max***, maximal elastance during a cardiac circle; **EDV**, end-diastolic volume. [a], $P<0.05$, and [b], $P<0.01$ vs. vehicle; [c], $P<0.05$, and [d], $P<0.01$ vs. single dose.

Supplementary Table V. Morphometric data

	Vehicle Control (n=16)	Single dose (n=12)	Multiple doses (n=14)
LV weight (mg, postmortem)	707.3±23.8	704.6±38.2	706.3±34.3
Total LV wall area (mm^2)	30.7±1.5	31.9±2.1	32.7±1.3
Risk region area (mm^2)	14.6±1.1	15.6±1.5	16.1±1.8
Non-risk region area (mm^2)	16.1±1.2	16.3±1.7	16.5±1.4
Scar area (mm^2)	7.1±0.7	6.2±0.7	5.2±0.6[a]
Risk region weight (mg)	336.3±24.9	341.9±27.0	345.6±34.8
Scar weight (mg)	166.6±18.8	136.2±15.0	115.7±16.4
Viable tissue weight in non-risk region (mg)	371.0±21.9	362.7±36.9	360.7±30.6
Viable tissue weight in risk region (mg)	169.6±10.8	205.7±18.0	229.9±25.4[a]
Total viable tissue weight (mg)	540.6±19.8	568.3±26.3	590.6±20.2
Risk (% of total LV)	47.5±2.9	49.0±3.5	48.8±4.2
Scar (% of risk region)	48.5±2.3	35.1±3.9[a]	32.0±3.0[b]
Scar (% of LV)	23.3±2.1	18.9±1.4	15.6±1.5[b]
Viable tissue (% of risk region)	51.5±2.3	60.6±2.9[a]	66.7±2.9[b]
Average IW thickness (mm)	0.92±0.07	1.18±0.06[a]	1.41±0.07[b]
IW thickness (mm)	0.67±0.06	0.82±0.12	0.96±0.09[a]
NIW thickness (mm)	2.06±0.12	2.12±0.12	1.99±0.13

Data are means ± SEM.
IW thickness, infarcted wall thickness; **NIW thickness**, non-infarcted wall thickness. [a], $P<0.05$, and [b], $P<0.01$ vs. vehicle; [c], $P<0.05$, and [d], $P<0.01$ vs. single dose.

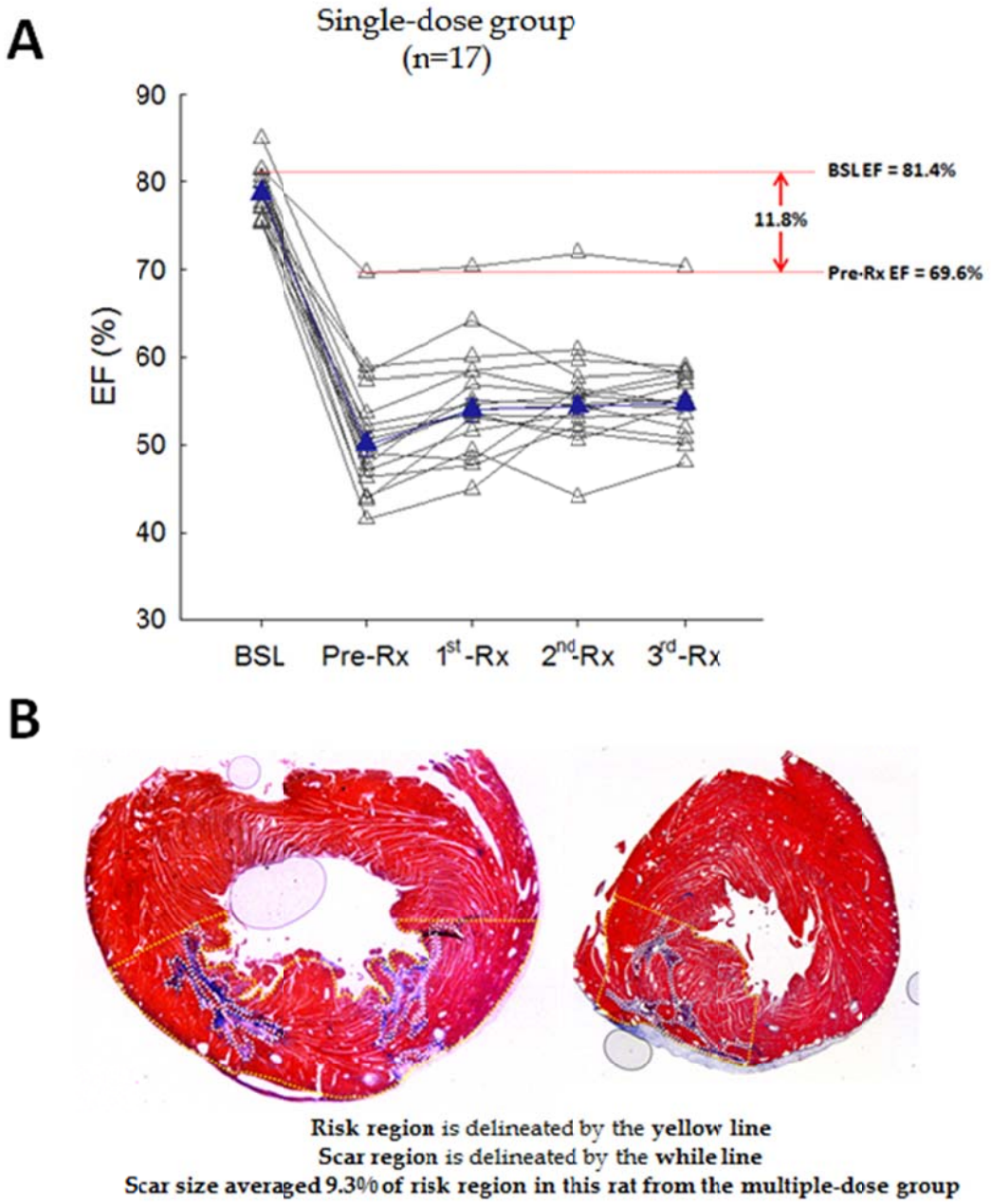
Supplementary Table VI. Number of male CPCs at 105 days after the 1st treatment

Group	CPCs/heart
Rats with detectable CPCs	
Single dose (n=5)	3,330 ± 931
Multiple dose (n=7)	5,078 ± 1,409
All rats (included rats no detectable CPCs)	
Single dose (n=8)	2,081 ± 1,060
Multiple dose (n=9)	3,949 ± 1,310

Data are means ± SEM.

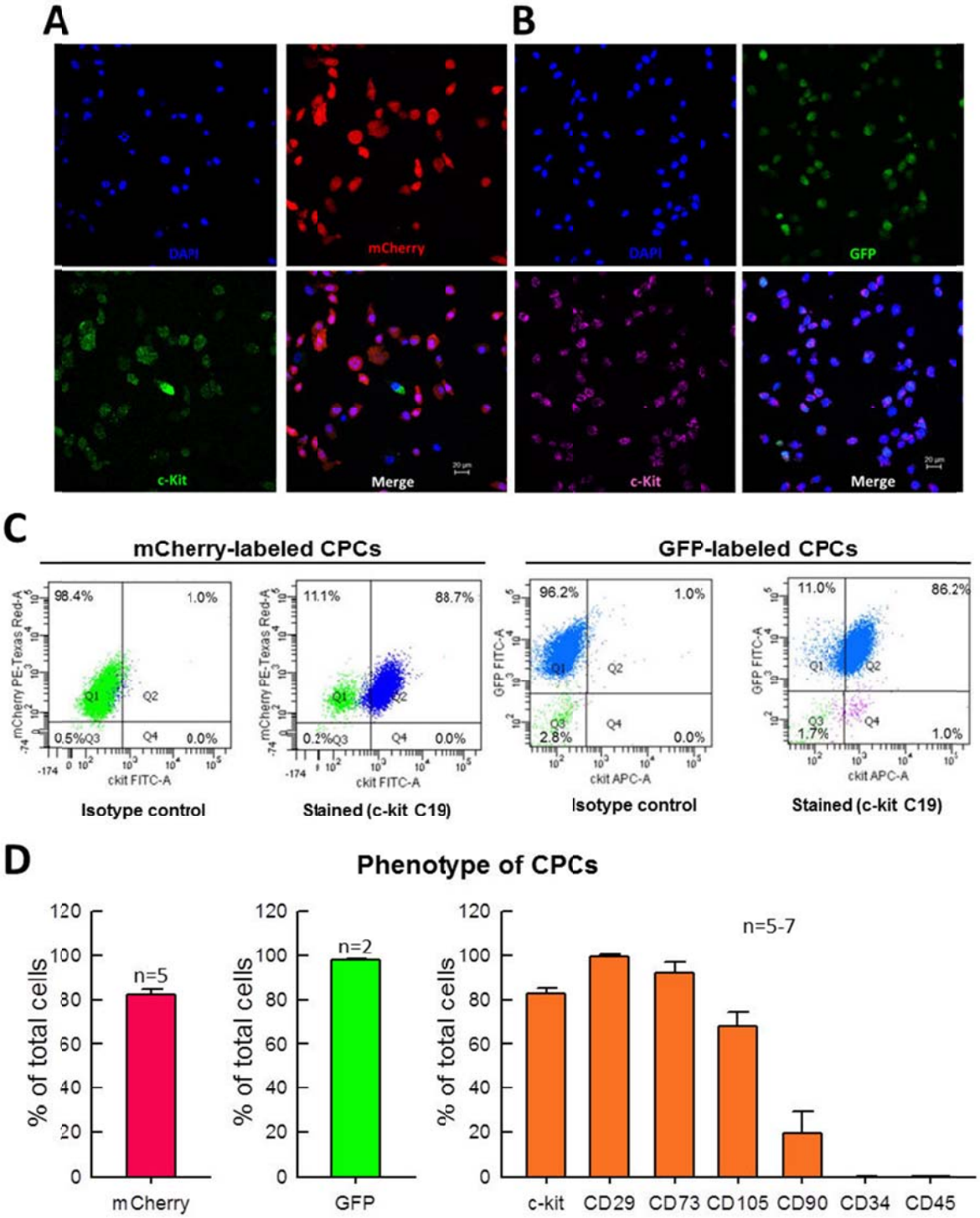
SUPPLEMENTARY FIGURES

Supplementary Figure I



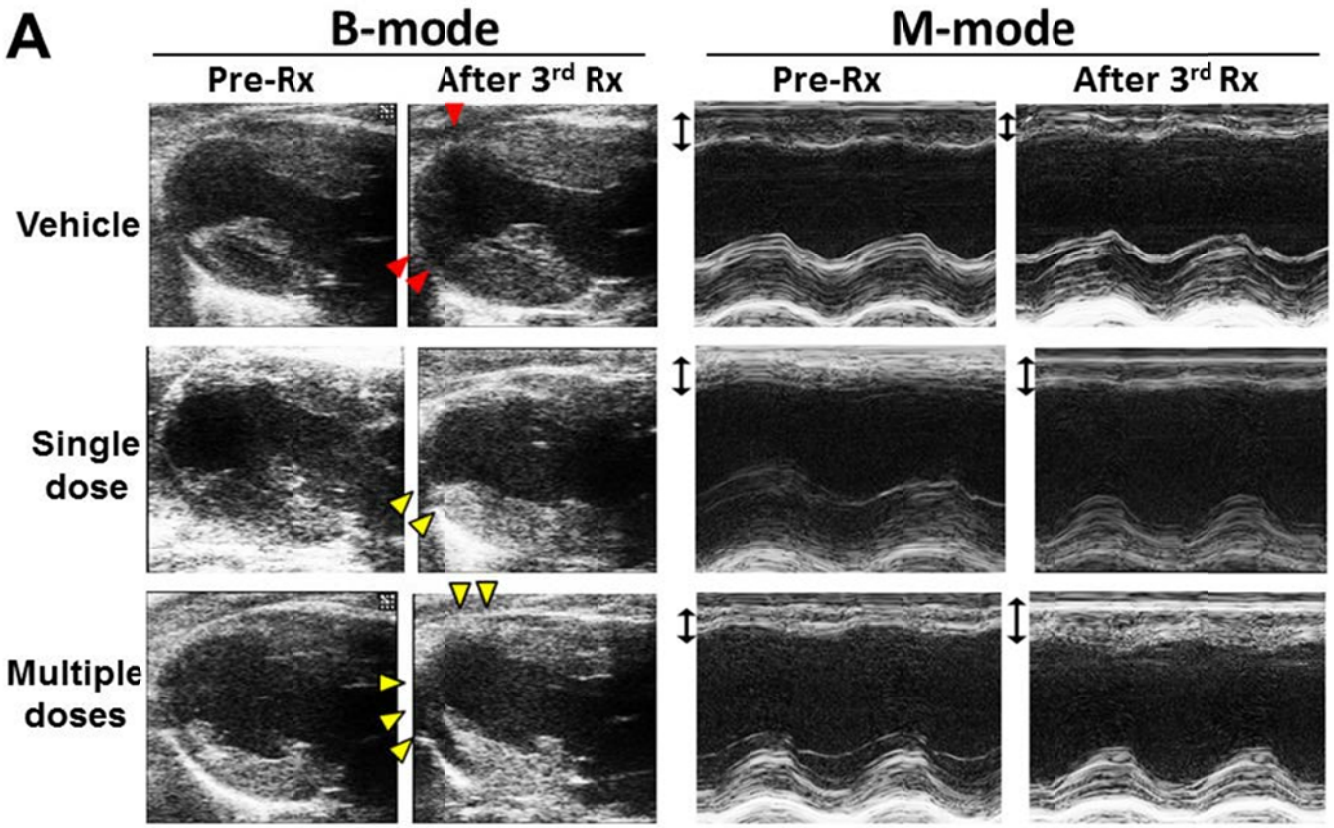
Supplementary Figure I. Exclusion of two rats because of prespecified criteria: LVEF drop <15 units after MI and scar size <10% of risk region. A. Echocardiographic assessment of LV EF in the single-dose group showing that the excluded rat was an outlier since LVEF decreased only by 11.8 units after MI (before treatment, Pre-Rx), suggesting a small infarction (the prespecified criterion for exclusion was a drop in LVEF <15 units). **B.** Trichrome images of two LV sections in a heart from the multiple-dose group showing that the average scar size was 9.3% of the risk region (the prespecified criterion for exclusion was scar size <10% of the risk region). These two rats were excluded from the final report.

Supplementary Figure II



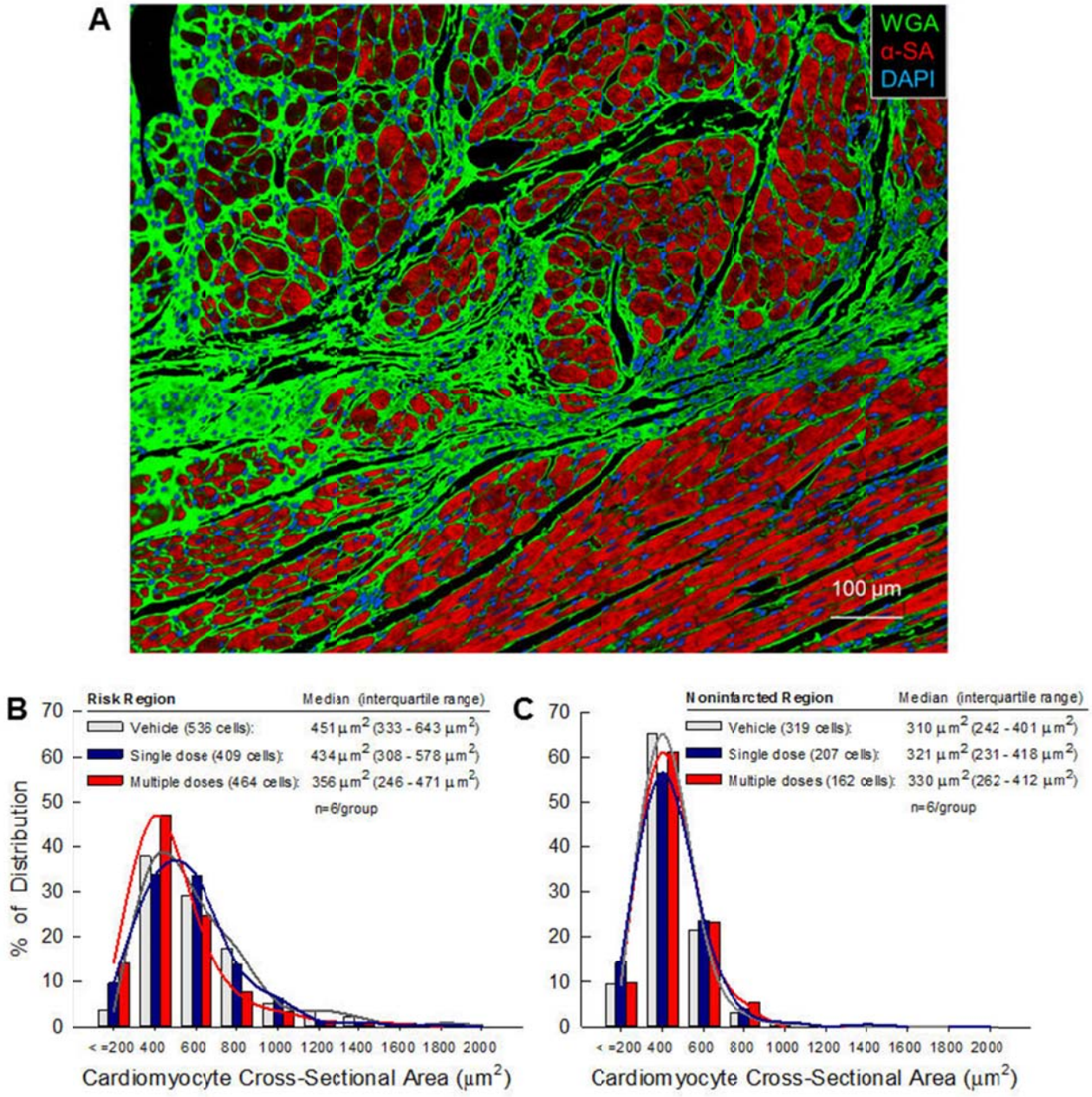
Supplementary Figure II. Phenotype of CPCs. Immunofluorescent staining of mCherry-labeled CPCs (**A**) and GFP-labeled CPCs (**B**). **C.** Representative results of FACS analysis of mCherry-labeled CPCs (left panels) and GFP-labeled CPCs (right panels) stained with isotype control or anti-c-kit C19 antibody. **D**. Quantitative analyses of CPC phenotype. Data are means ± SEM. Bar is 20 μm.

Supplementary Figure III



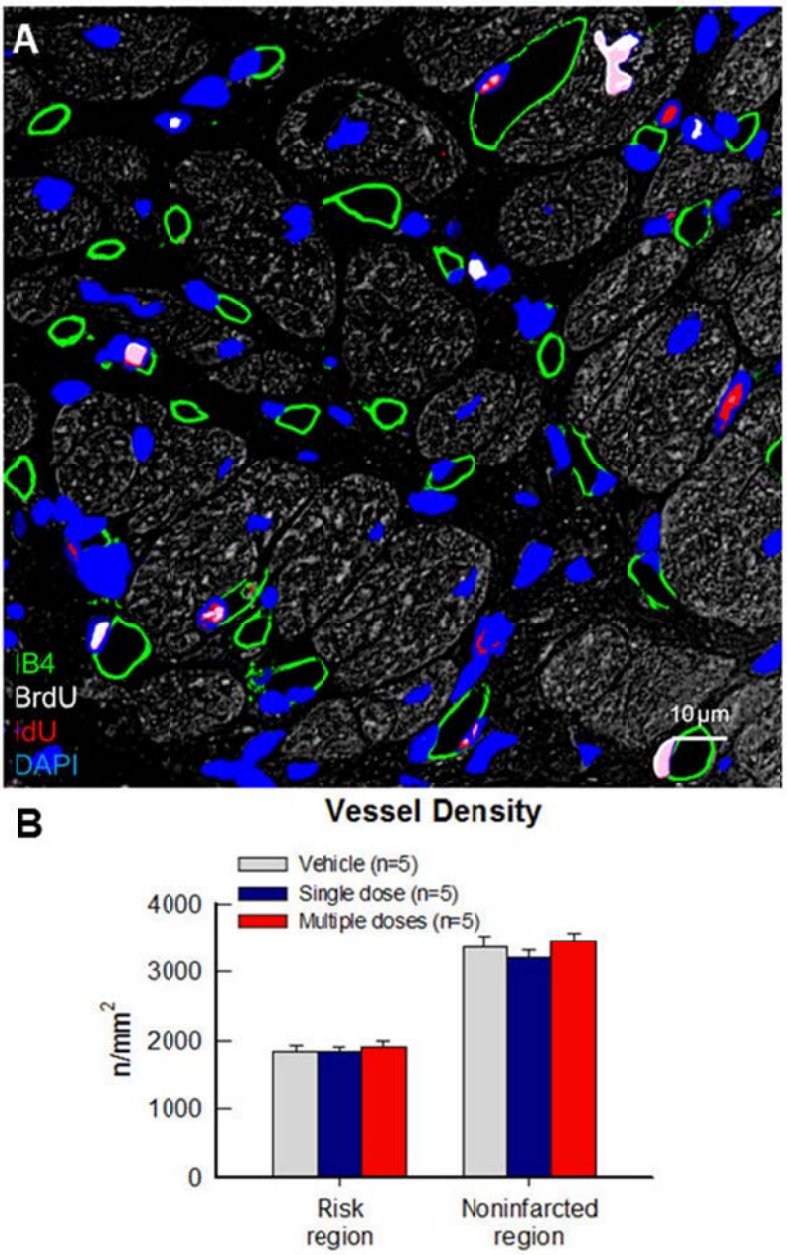
Supplementary Figure III. Echocardiographic assessment of LV volume and function. Representative B-mode long axis images in systole **(left)** and M-mode images **(right)** obtained before the 1st treatment (Pre-Rx) (30 days after MI) and at 35 days after the 3rd treatment (3rd Rx). **Red arrows**, LV segments that deteriorated; **yellow arrows**, LV segments that improved; **two-headed arrows**, thickness of the infarcted wall.

Supplementary Figure IV



Supplementary Figure IV. Evaluation of myocyte cross-sectional area. **A.** Representative epifluorescent image sequentially acquired from 9 fields of the border zone of a vehicle-treated rat at a microscopic magnification of x300. WGA (to identify the sarcolemma of myocytes) is shown in green, the cardiac protein marker α-SA in red, and nuclei were stained with DAPI in blue. Myocytes with round nuclei and clearly defined sarcolemmal borders were selected for analysis of cross-section area. **B-C**. Distribution frequency of myocyte cross-sectional areas in the risk region (**B**) and noninfarcted region (**C**) region. The risk region comprises both the border zones and the infarcted region. Bar is 100 µm.

Supplementary Figure V



Supplementary Figure V. **Analysis of vascular density**. **A.** Representative confocal microscopic image acquired from the border zone. Immunofluorescent staining was performed with a specific anti-Isolectin B4 antibody to identify vascular endothelial cells in green, anti-BrdU antibody to identify newly-formed cells after the 1st infusion (in white), and anti-IdU antibody to identify newly-formed cells after the 3rd infusion (in red). Nuclei were stained with DAPI in blue. Myocardial morphology was examined with the confocal transmitted light channel's detector (ChD) in which the pseudocolor selected for myocardial background in the ChD channel was gray white. **B.** Quantitative analysis of vessel density. The risk region comprises both the border zones and the infarcted region. Data are means ± SEM. Bar is 10 µm.