Supplemental Material for

Cardiac stem cell hybrids enhance myocardial repair

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Supplemental Methods

Study design

In vitro studies were designed to predict the reparative potential of CPCs, MSCs, CPC + MSC and CardioChimeras (CCs) in a mouse model of injury. Cell-to-cell fusion yielded 18 CC clones from a total of 192 wells. For ease in naming clones, we identified CCs based on well number followed by A (1st 96-well plate) or B (2nd 96-well plate). Selected CCs were analyzed for proliferation, survival and paracrine-mediated effects on cardiomyocytes and/or expression of cardiomyogenic commitment markers (Online Figure III). The 18 CCs exhibited variable proliferative capabilities relative to individual CPCs and MSCs and were classified as slow, slow-medium, medium-fast and fast growing clones based on a 1-2 fold, 2-4 fold, 4-6 fold or >6 fold change in fluorescence relative to day of plating respectively (Online Figure IA). A number of slow-growing CCs were excluded due to a low expansion rate (Online Figure IA and Online Table I). We further characterized two-three clones per proliferative status for analysis of cell death after oxidative stress and morphological features such as cell size. With the addition of 40μM hydrogen peroxide, 4 CCs were excluded D.6 (A), B.10 (A), D.10 (A), E.10 (A) due to poor cell survival (Online Figure IB and Online Table I). Although cell size was not a defining reason for exclusion, cell morphological assessments correlated CC phenotype to individual parent cells. CC1 (F.7 (A)) and A.3 (A) exhibited a similar cell surface area to MSCs, whereas the majority of CCs including CC2 (E.2(A)) had a similar surface area to CPCs (Online Figure IC). Furthermore, co-culturing CCs with NRCMs mediated the identification of clones that could facilitate survival and growth of cardiomyocytes as compared to parent MSCs. Neonatal rat cardiomyocytes (NRCMs) were maintained in low serum (0.5% Fetal Bovine Serum) for 24 hours followed by the addition of CCs, CPCs, MSCs or CPCs and MSCs combined for an additional 24 hours and cardiomyocyte size and cell death was measured (Online Figure I, D and E). CCs were considered based on the ability to promote cardiomyocyte growth and prevent cardiomyocyte cell death following co-culture (Online Table I). Of the four candidates for adoptive transfer studies, F.9(B) and G.7(B) were eliminated due to a lower proliferative status (Online Table I). These initial analyses facilitated the identification of CCs that are suitable for cardiac therapy during acute damage by identification of clones with optimal proliferation and survival properties in addition to displaying the potential to secrete protective factors to preserve cardiomyocyte health.

For the adoptive transfer analysis, we required approximately 16 animals per cell group to allow for analysis of at 3-5 mice per time point (4, 12 and 18 WPI) without impacting on statistical significance obtained during longitudinal assessment by echocardiography. This number was chosen based on an average of 65-75% survival rate immediately after injury. Mice with a measured EF between 35-50% one-week post infarction were included in the experiment. EF >50% or <35% were excluded from the experiment. Throughout the time course, mice were not subjected to echocardiography if mice were perceived to be in distress. The study was concluded after determining statistical significance in wall thickness recovery and EF at 18 WPI. The study was not randomized, but was blinded to the operator during echocardiographic acquisition and analysis.

CPC and MSC isolation

CPCs were isolated and maintained as previously described[1]. CPCs were used during passages 10-20. Mesenchymal stem cells were isolated from 12 week old female FVB mice by flushing the femur and tibiae with 5% Fetal Bovine Serum in PBS through a 40-μm filter and centrifuged (10 minutes, 600g,

4°C). Cells were resuspended in media consisting of α modified minimum essential media and 15% FBS. Cells were plated in 150mm dish and media was changed every two days to remove non-adherent cells. Adherent cells created colonies in approximately two weeks. MSCs were passaged using 0.25% Trypsin and used during passages 2-4 for experiments.

Lentiviral constructs and stem cell transduction

A third generation enhanced green fluorescent protein (eGFP) lentivirus with a phosphoglycerate kinase (PGK) and puromycin (puro) selection marker was purchased from Addgene. pLenti PGK GFP Puro was used as a backbone to sub clone mcherry in the place of eGFP and bleomycin (bleo) to replace the puro gene in order to create pLenti PGK mcherry Bleo. MSCs at passage 1 were lentivirally transduced with pLenti PGK GFP Puro at a multiplicity of infection (MOI) of 50 and maintained in puromycin supplemented MSC media for one week starting at 48 hours post-infection. CPCs at passage 10 were lentivirally transduced with pLenti PGK mcherry Bleo at a MOI of 10 and subjected to fluorescent activated cell sorting (FACS) to purify mcherry positive CPCs. Fluorescent protein expression in MSCs (MSC-GFP) and CPCs (CPC-mcherry) was confirmed by fluorescent light microscopy and flow cytometric analysis.

Light microscopy and measurement of cell morphology

Images of stem cells were obtained on a Leica DMIL microscope and cell outlines were traced using ImageJ software. Relative surface area was determined as previously described[2].

Centromere labeling (fluorescence *in situ* hybridization)

Cells were fixed on glass two chamber slides in 3:1 ethanol:acetic acid for 30 minutes and then passed through graded alcohol series 70, 90, 100% (2 minutes each step). Slides were baked at 65°C for 15 minutes and then transferred to acetone for 10 minutes. Slides were then incubated for 1h at 37°C in 2X SSC (NACl/NA Citrate) + RNase (100µg/ml). Cell were treated with pepsin, 10mM HCl mixed with 0.5µl of stock pepsin solution (1mg/ml) at room temperature for 2-3 minutes and then dehydrated through ethanol series. Denaturing cellular DNA was done by immersing slides in 70% formamide in 2X SSC at 70°C for 2 minutes and then placed in ice cold 70% ethanol for 2 minutes followed by passing through an ethanol series. Prior to hybridization the centromere probe, CENPB-Cy3 (PNA Bio; ATTCGTTGGAAACGGGA), was warmed to 37°C for 5 minutes. The probe was denatured for 10 minutes at 85°C then immediately chilled on ice before applying probe to the slides. The hybridization protocol required 16 hours at 37°C. Post hybridization washes for 5 minutes at 37°C in 2X SSC were followed by two washes in 50% formamide/2X SSC 37°C, for 5 min each time and final wash in 2X SSC, twice for 5 min each time. DAPI (Sigma-Aldrich) was added to the final wash. Cell nuclei were visualized using a Leica TCS SP8 confocal microscope and the Z-stacking feature. Measurements of nuclear size and centromere intensity were determined after outlining the nucleus and getting the area (µm2) and mean gray values (fluorescent intensity/µm2) after creating a projection of Z-Stack scans.

Proliferation assay and cell doubling time

Cell proliferation was determined using the CyQuant Direct Cell Proliferation Assay (Life Technologies) according the manufacturer's instruction and as previously described[2]. Population doubling times were calculated using the readings from CyQuant Direct Proliferation Assay and use of a population doubling time online calculator (http://www.doubling-time.com/compute.php).

Cell death assay

Stem cells were plated in a 6-well dish (80,000 cells per well) and incubated in starvation media (growth factor and FBS depleted media) with 1% PSG for 18 hours. The cells were then treated with either 40µM or 80µM hydrogen peroxide for 4 hours. Cells were resuspended with Sytox Blue (Life Technologies) to label necrotic cells. Data was acquired on a FACS Aria (BD Biosciences) and analyzed with FACS Diva

software (BD Biosciences). Cell death was quantitated by measurement of Sytox Blue positive cells and represented as a fold change relative to cells in starvation media alone.

In co-culture conditions of stem cells with NRCMs, whole populations were analyzed and stained with Annexin V (BD Biosciences) and Sytox Blue and only the negative (non-fluorescent NRCM) population was analyzed for cell death. Cell death of NRCMs was represented as a fold change relative to cells in growth media (10% M199). NRCMs in 0.5% M199 and 0.5% plus add back of 10% M199 at the time of stem cell addition were maintained as positive and negative controls for cell death.

Neonatal rat cardiomyocyte (NRCM) co-culture with stem cells

NRCMs were isolated and plated as previously described[3]. After enzymatic digestion, cells were plated in M199 media (Life Technologies) with 15% FBS (Omega Scientific Inc.) at a density of 260,000 cells per well of a 6-well culture dish pretreated with 1% gelatin (Sigma-Aldrich). Within 18 hours, myocyte cultures were washed with PBS and incubated with M199 with 10% fetal bovine serum for 24 hours. The next morning, the cells were subjected to serum starvation (0.5% FBS in M199) for 24 hours. After low serum conditions, stem cells were added to the plate at a ratio of 1:10 (CPCs, MSCs, CPC + MSC combined, CC1 and CC2) and allowed to incubate with NRCMs for an additional 24 hours in low serum conditions. Controls for NRCMs included leaving cells in 0.5% alone, adding back 10% M199 or maintaining NRCMS in 10% M199 for the duration of the experiment. NRCM size was measured after staining cardiomyocytes with sarcomeric actinin (1:100, Sigma-Aldrich) and nuclei with TO-PRO-3 iodide and as previously described[4]. Separation of NRCM and stem cells was accomplished with fluorescent activated cell sorting (FACS) of negative cells (NRCMs) versus GFP^+, $mcherry^+$ or GFP^+/ $mcherry^+$ stem cells. After sorting, cells were centrifuged and suspended in RNAse buffer for isolation and quantitation of mRNA from NRCMs or stem cells.

Immunocytochemistry

Stem cells were placed at a density of 15,000 per well of a two-chamber permanox slide and stained according to previous studies[5]. Before scanning, cells were washed in PBS containing TO-PRO-3 iodide (Life Technologies) to stain for nuclei. Slides were visualized using a Leica TCS SP2 confocal microscope. Primary and secondary antibodies used are listed in Table S2.

Flow cytometric analysis

Cells in suspension were counted (200,000 cells per sample) and stained with primary and secondary antibodies as indicated in the Online Table III. Samples were analyzed using a FACS Canto (BD Biosciences).

mRNA isolation, cDNA synthesis and quantitative RT-PCR

RNA was enriched using the Quick RNA Mini Prep kit from ZymoResearch according to the manufacturers instructions. Reverse transcriptase was performed using protocol for the iScript cDNA Synthesis Kit (BIORAD). qRT-PCR was read after incubation of cDNA, primers (100nM) and IQ SYBR Green Supermix (BIORAD). Data was analyzed using the $\Delta\Delta C(t)$. Primer sequences are listed in the Online Table IV.

Enzyme-Linked Immuno Assay (ELISA)

The ELISA assay was performed in NRCMs alone (0.5%, 0.5% + 10% rescue, and 10% M199 treated cells), NRCMs incubated with stem cell groups and stem cells alone in normal growth media. Briefly, after 24 hour incubation with serum or stem cells, the 96-well microplate was centrifuged for 5 minutes at 1200rpm and 100µL of media supernatant was removed and used for IL-6 Mouse ELISA Kit (Life Technologies) performed according the company's instructions.

Myocardial infarction and intramyocardial cell injections

Myocardial infarctions were carried out in eleven-week old female FVB mice under 2-3% isoflurane anesthesia and by tying off the left anterior descending artery (LAD) using a modified protocol[20]. After ligation, injections with either PBS (5μL per injection, 5 injections total per mouse), parents (CPCs or MSCs), parents combined (CPC + MSC) or CCs CC1 (20,000 cells per 5μL injection, 5 injections making a total of 100,000 cells injected per mouse) were introduced to the pre-ischemic border. Placing the heart out of the chest and placing it back in the chest without ligation of the LAD was considered a sham surgery. The review board of the Institutional Animal Care and Use Committee at San Diego State University approved all animal protocols and studies.

Retroperfusion
Mice were sacrificed under chloral hydrate sedation before removing hearts from mice and as previously described[1]. After retroperfusion, hearts were processed for paraffin embedding.

Immunohistochemistry
Heart sections were deparaffinized, and incubated with primary and secondary antibodies as previously described[5]. Subsequent tyramide amplification was performed as necessary. Slides were incubated in DAPI (Sigma-Aldrich) for 10 minutes to stain for nuclei. Primary and secondary antibodies used are listed in the Online Table III.

Echocardiography and hemodynamics
Echocardiography was used to evaluate cardiac function after MI and injections using the Vevo 2100 (Visual Sonics) and as previously described[5]. Closed-chest hemodynamic assessment was performed after insertion of a microtip pressure transducer (FT111B, Scisense) and as previously described[5]. Cardiac function assessed by echocardiography 2 days post-infarction was not statistically different between infarcted/injected groups. The review board of the Institutional Animal Care and Use Committee at San Diego State University approved all animal protocols and studies.

Quantitation of c-kit cells, infarct size and cellular engraftment
Paraffin sections were probed with primary antibodies for proteins cardiac troponin T, c-kit, GFP and mcherry and visualized on a Leica TCS SP8 Confocal Microscope. Nuclei were visualized after DAPI staining. For infarct size, cTNT was probed to visualize live myocardium and DAPI to determine nuclei distribution and area of infarction. Area of live versus dead myocardium was measured using the drawing tool in the Leica Software and normalized to the total area of the left ventricular free wall and converted to percentage. In this area, c-kit$^+$ cells were counted. For engraftment, area of mcherry$^+$ (CPCs, CPCs in CPC + MSC group, CC1 and CC2) or GFP$^+$ (MSCs alone and MSCs in CPC + MSC group) was measured and normalized to total area. 4 and 12 week sections had an N=3-4 hearts per group.

Isolectin staining and measurement of capillary dimensions
Paraffin sections were probed with Isolectin B4-488 (Life Technologies) in combination with cTNT and DAPI. Scans consisted of border zone and infarct regions for each heart analyzed. The analysis software on the Leica SP8, quantitated the number of positive cells in each field of view. The area of the field of view was measured and used to normalize capillary numbers per mm^2. An N=3-4 hearts per group was measured.

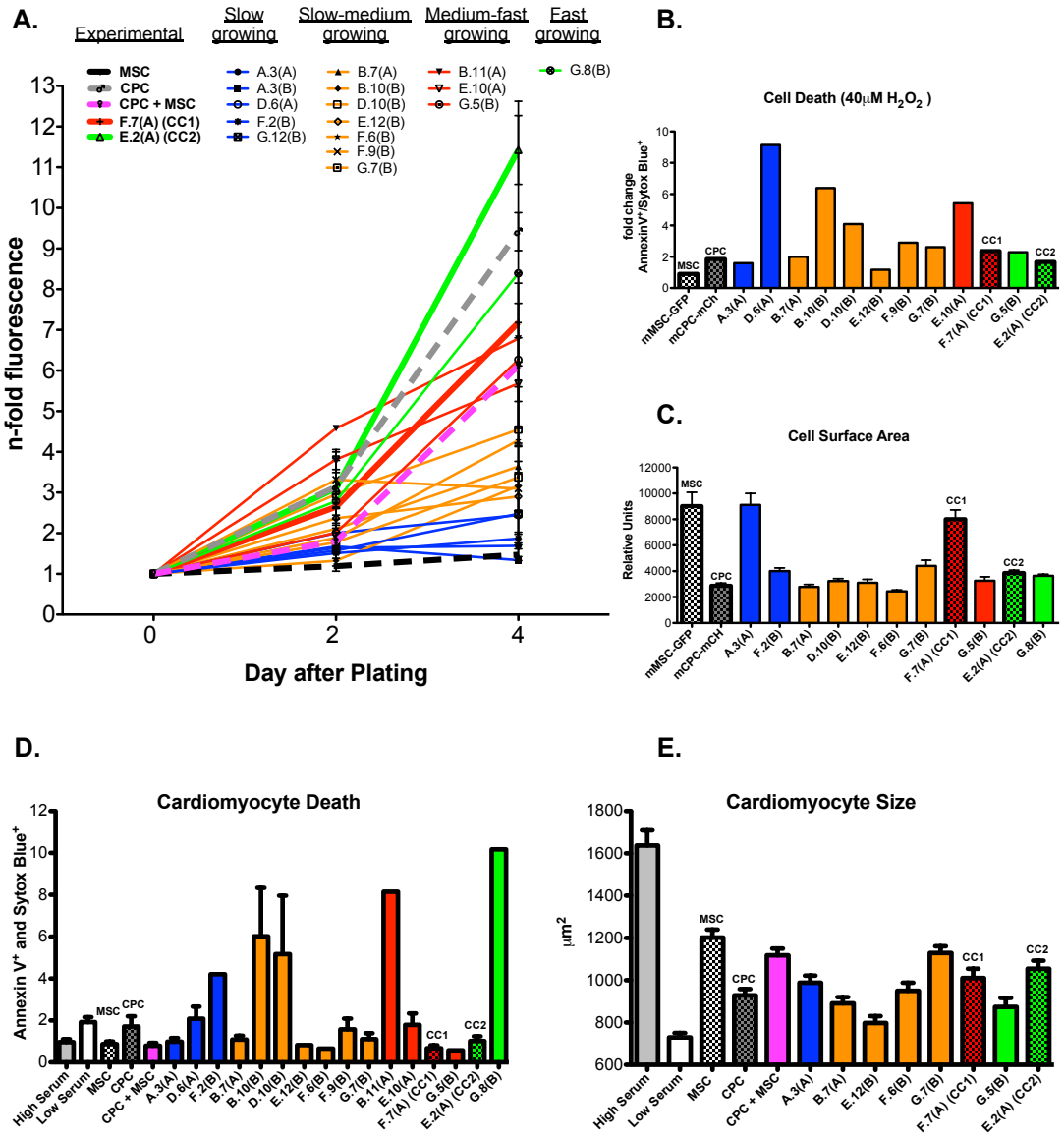
Measurement of cardiomyocyte hypertrophy
Paraffin sections were stained for cTNT to visualize live myocardium, wheat germ agglutinin-555 (Life Technologies) to outline cardiomyocyte membrane and DAPI to visualize nuclei and area of infarction. Myocytes were measured in the border zone of the infarct or in this infarct. Cross-sectional views of cardiomyocytes were considered and measured using the drawing tool to determine area using the SP8 TCS Leica Software. An N=3-4 hearts per group was measured.

Masson's Trichrome

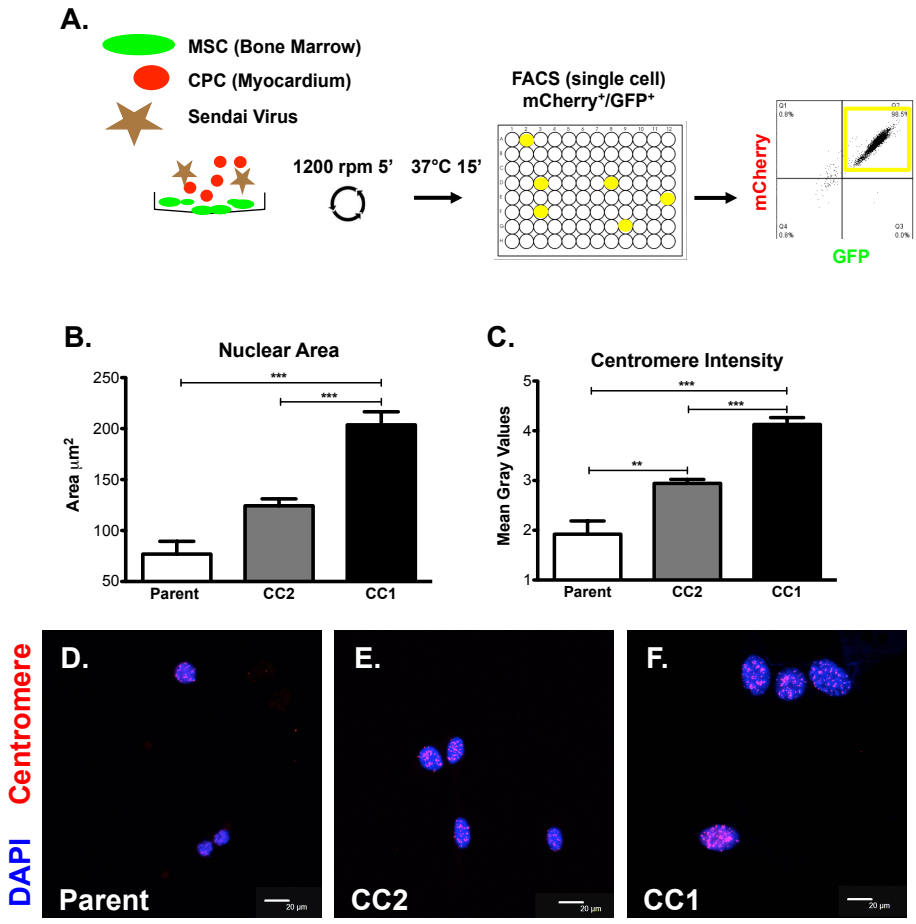
Trichrome (Masson) kit was used to stain for collagen deposition in infarcted hearts according to manufacturer's protocol and based on previous reports[1]. Staining was visualized using a Leica DMIL microscope.

Statistical analyses

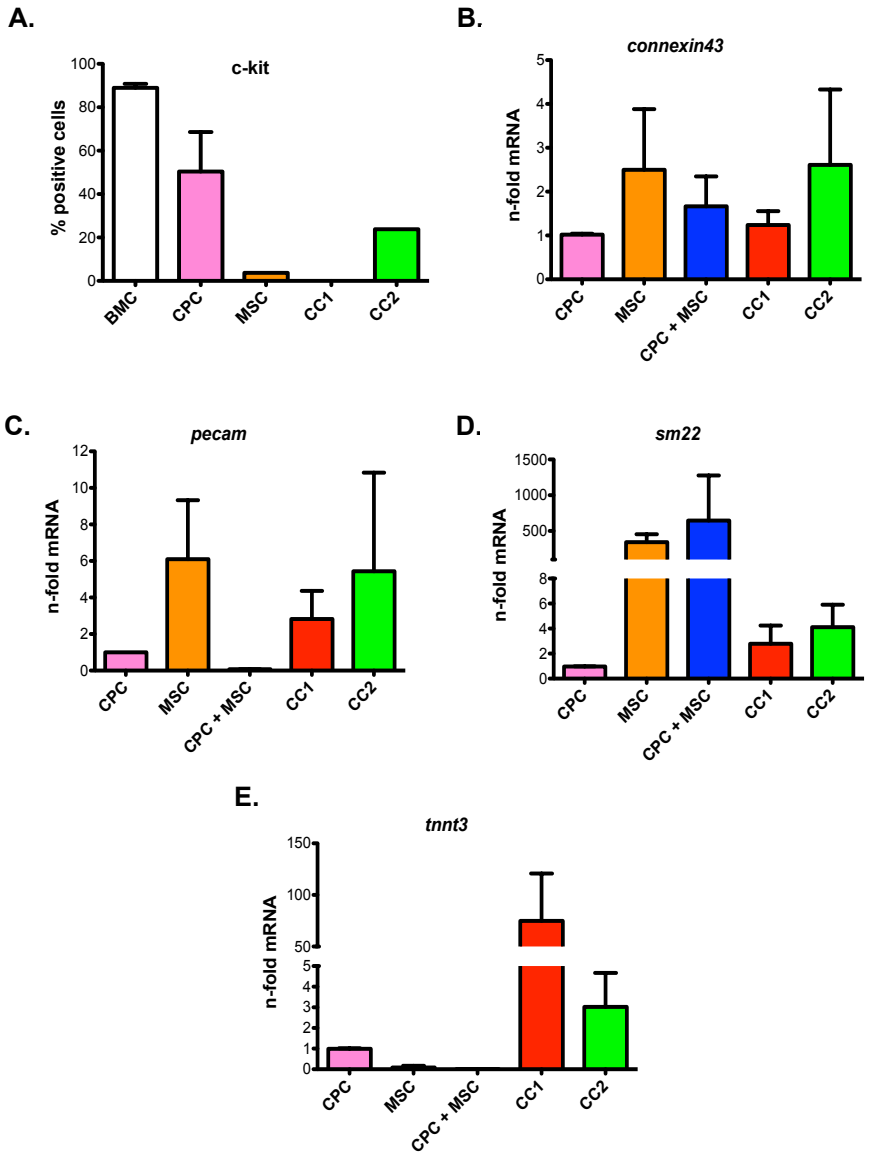
All data are expressed as mean +/- SEM. Statistical analyses was done using paired or unpaired Student's t-test, one-way ANOVA or two-way ANOVA with a Dunnett post-test to compare groups to a control group using Graph Pad Prism v5.0. A value of $p<0.05$ was considered statistically significant**.**



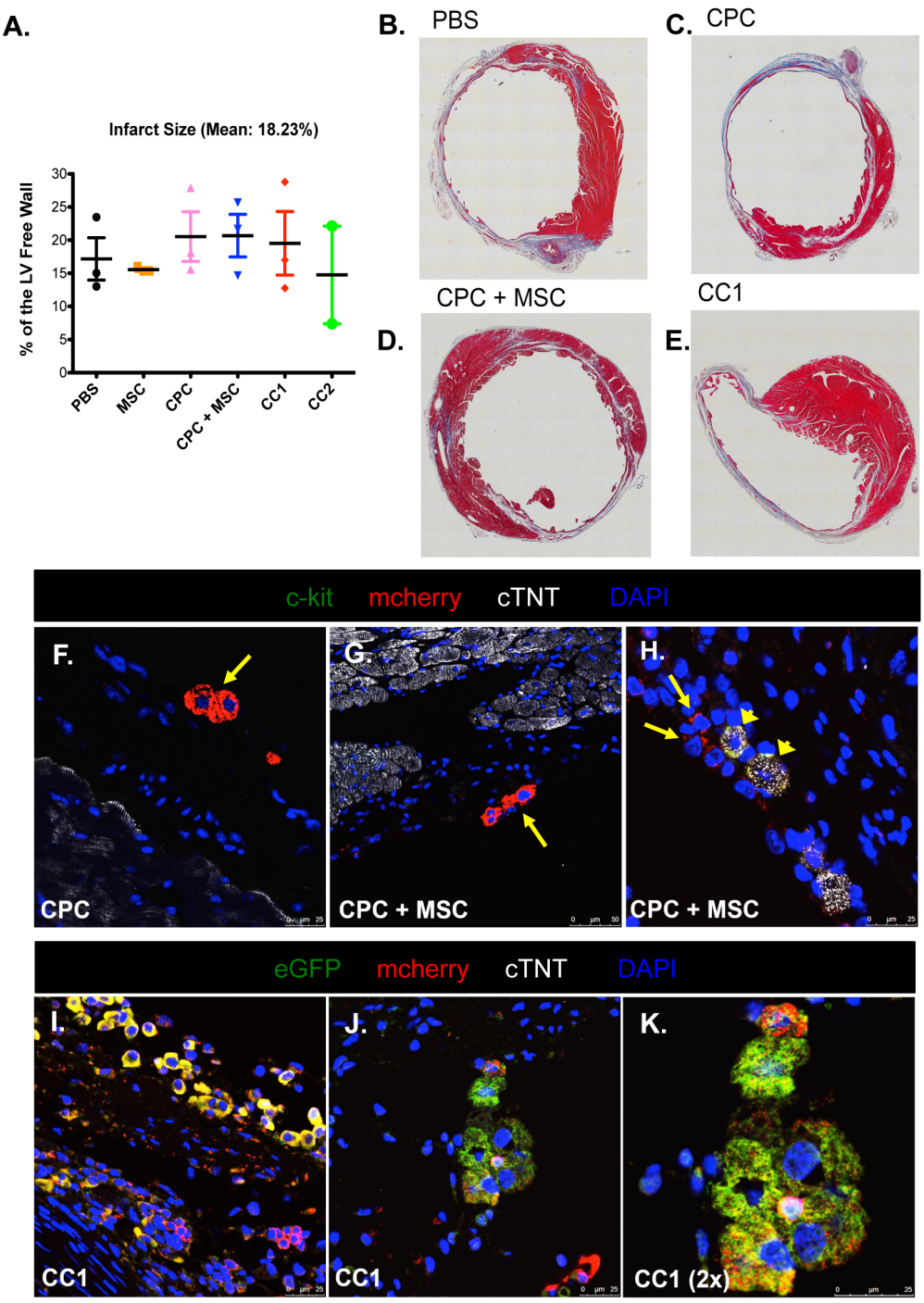
Online Figure I. Phenotypic Characterization of CardioChimera clones. (A) Proliferation data for the 18 CardioChimera clones relative to day of plating using a direct-fluorescent based assay (CyQuant Assay). CardioChimeras are categorized as slow (blue), slow-medium (orange), medium-fast (red) and fast (green) growing. Experimental control groups (CPC, MSC and CPC + MSC) cell lines are represented as dashed bold lines. CardioChimera 1 and 2 are represented as solid bold lines. (B) CardioChimera death after treatment with hydrogen peroxide stimulus. Values are represented as a fold change of Annexin V^+ and Sytox $Blue^+$ compared to cells in growth media alone. (C) Neonatal rat cardiomyocytes incubated in high or low serum or with the addition of parent cells, parent cells combined or CardioChimeras. (D) Cell death was quantitated by measuring a fold change of Annexin V^+ and Sytox $Blue^+$ cardiomyocytes relative to cardiomyocytes in high serum. (E) Cardiomyocyte size was quantitated in high serum or with the addition parent cells, parent cells combined or CardioChimeras.



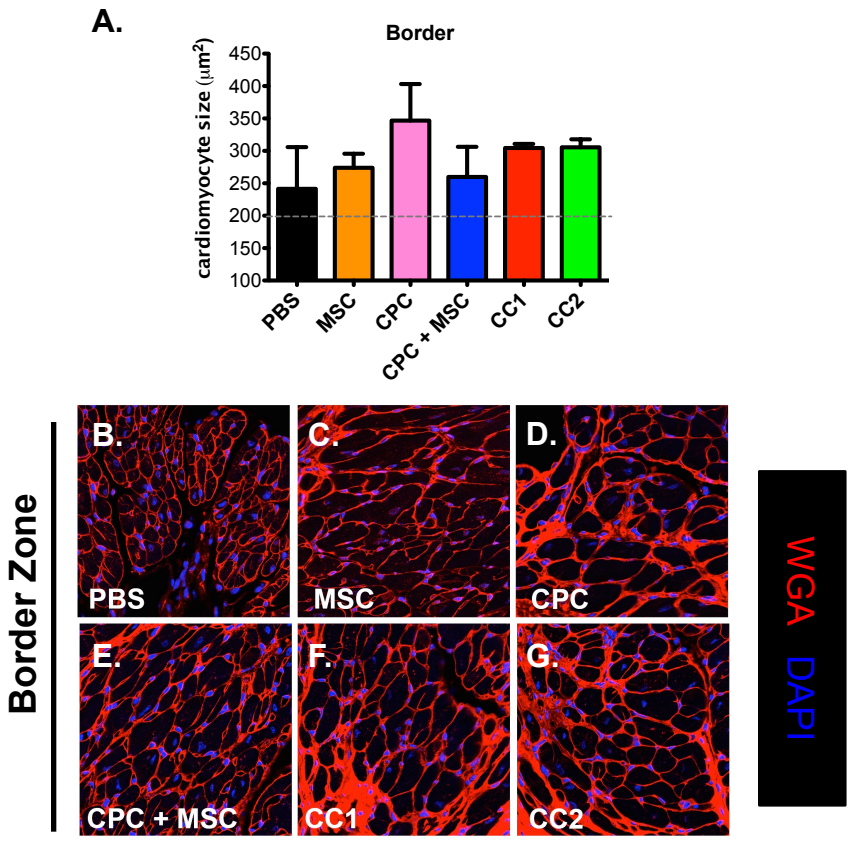
Online Figure II. CardioChimeras have increased nuclear size and DNA content. (A) Detailed protocol for the fusion and clonal expansion of CardioChimeras. Briefly, mouse CPCs were co-incubated with mouse MSCs at a 1:1 ratio with addition of Sendai virus. Cells were centrifuged to force cell contact and single cell sorted based on fluorescent expression of mcherry and GFP. Clones were confirmed by flow cytometric analysis. (B) Measurement of nuclear size and (C) Centromere intensity in parent MSCs, CC2 and CC1. (D) Representative images of nuclei in parent (E) CC2 and (F) CC1. Blue represents DAPI staining of DNA content and red represents centromere probe binding. Scale bar is 20µm. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Online Figure III. CardioChimeras have increased expression of cardiomyogenic commitment markers at basal levels. (A) C-kit protein expression as analyzed by flow cytometry. C-kit purified bone marrow cells were utilized as a positive control. (B) *connexin 43*, (C) *pecam* (*cd31*), (D) *sm22* and (E) *cTNT* (*tnnt3*) gene expression was analyzed by qRT-PCR in CPC, MSC, CPC + MSC, CC1 and CC2 after normalization to ribosomal 18s. Values are represented as a fold change relative to CPCs.


A.
Infarct Size (Mean: 18.23%)
% of the LV Free Wall
30
25
20
15
10
5
0
PBS
MSC
CPC
CPC + MSC
CC1
CC2
B. PBS
C. CPC
CPC + MSC
D.
CC1
E.
c-kit mcherry cTNT DAPI
F.
CPC
G.
CPC + MSC
H.
CPC + MSC
eGFP mcherry cTNT DAPI
I.
CC1
J.
CC1
K.
CC1 (2x)

Online Figure IV. Cellular Engraftment of CardioChimeras 4 weeks after damage. (A) Infarct size was not significantly different between infarcted groups (mean=18.23%). N=2-4 per group. (B-E) Masson's Trichrome staining and representative images of (B) PBS, (C) CPC, (D) CPC + MSC and (E) CC1 hearts to visualize scar size and fibrosis. Scale bar is 250μm.(F) $Mcherry^+$ CPCs detected in the infarct area in CPC treated hearts. (G) $Mcherry^+$ CPCs detected in the infarct area in CPC + MSC treated hearts. (H) $Mcherry^+$ CPCs adjacent to $c\text{-}kit^+$ /$cTNT^+$ cardiomyocytes in CPC + MSC treated hearts. (I) and (J) CC1 expressing eGFP and mcherry in the infarcted area. (K) 2x zoom of CC1. Scale bar is 25μm for confocal images. Scale bar is 50μm in (G).



Online Figure V. Cardiomyocyte size is unaffected in the border zone region after treatment. (A) (A) Mean cardiomyocyte size in the border zone regions. Sample size is 3-4 mice per group. (B-G) Representative images of border zone area cardiomyocytes. Red=Wheat germ agglutinin, White=cardiac troponin T and Blue=DAPI to stain for nuclei. Scale bar is 25μm.* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Growth Rate	Proliferation	Cell Death	Cell Surface Area/Morphology	Cardiomyocyte Growth	Cardiomyocyte Death	Exclusion Reason
	MSC	MSC	MSC	MSC	MSC	
	CPC	CPC	CPC	CPC	CPC	
	CPC + MSC			CPC + MSC	CPC + MSC	
slow growing	A.3 (A)	A.3 (A)	A.3 (A)	A.3 (A)	A.3 (A)	very slow growing
	A.3 (B)					did not grow/expand
	D.6 (A)	D.6 (A)			D.6 (A)	poor cell survival
	F.2 (B)		F.2 (B)			promoted cardiomyocyte death
	G.12 (B)					very slow growing
slow-medium growing	B.7 (A)	B.7 (A)	B.7 (A)	B.7 (A)	B.7 (A)	did not induce CM growth
	B.10 (B)	B.10 (B)				poor cell survival and promoted cardiomyocyte death
	D.10 (B)	D.10 (B)	D.10 (B)			poor cell survival and promoted cardiomyocyte death
	E.12 (B)	E.12 (B)	E.12 (B)	E.12 (B)	E.12 (B)	did not induce CM growth
	F.6 (B)		F.6 (B)	F.6 (B)	F.6 (B)	did not induce CM growth
	F.9 (B)	**F.9 (B)**			**F.9 (B)**	**Potential CardioChimera Candidate**
	G.7 (B)	**G.7 (B)**	**G.7 (B)**	**G.7 (B)**	**G.7 (B)**	**Potential CardioChimera Candidate**
medium-fast growing	B.11 (A)					promoted cardiomyocyte death
	E.10 (A)	E.10 (A)			E.10 (A)	poor cell survival
	F.7 (A) (CC1)	**F.7 (A) (CC1)**	**F.7 (A) (CC1)**	**F.7 (A) (CC1)**	**F.7 (A) (CC1)**	**CardioChimera Candidate 1**
	G.5 (B)	G.5 (B)	G.5 (B)	G.5 (B)	G.5 (B)	did not induce cardiomyocyte growth
fast growing	**E.2 (A) (CC2)**	**E.2 (A) (CC2)**	**E.2 (A) (CC2)**	**E.2 (A) (CC2)**	**E.2 (A) (CC2)**	**CardioChimera Candidate 2**
	G.8 (B)		G.8 (B)			promoted cardiomyocyte death

Online Table I. Phenotypic characterization of the 18 CardioChimeras. Individual clones were analyzed for phenotypic properties such as proliferation, cell death and and cell surface area and potential for paracrine mediated effects on cardiomyocytes (Cardiomyocyte growth and Cardiomyocyte Death). The last panel specifies exclusion reason(s).

HEART RATE (bpm)

	SHAM			PBS			CPC			MSC			CPC + MSC			CC1			CC2		
WEEK	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N	MEAN	SEM	N
1	442.01	9.59	14.00	437.76	31.15	16.00	470.12	14.68	10.00	430.31	22.14	9.00	482.20	17.02	13.00	447.60	14.93	12.00	471.75	13.97	16.00
2	481.89	21.14	15.00	388.82	35.11	15.00	432.29	21.09	10.00	456.65	16.74	13.00	451.13	13.77	16.00	435.68	12.17	12.00	455.07	12.08	15.00
3	463.60	20.09	15.00	418.29	30.32	15.00	458.29	13.70	10.00	481.08	8.04	13.00	474.35	17.10	15.00	428.87	18.10	11.00	437.59	10.37	14.00
4	410.89	15.05	15.00	415.45	31.30	15.00	468.65	14.55	12.00	448.78	11.57	13.00	448.64	10.43	16.00	454.86	10.32	12.00	453.41	14.50	16.00
6	450.04	14.43	13.00	408.62	37.45	12.00	452.84	30.45	9.00	460.82	21.66	10.00	432.77	16.38	11.00	456.33	12.18	10.00	442.09	8.87	12.00
8	462.46	16.23	13.00	418.66	33.42	13.00	448.29	10.66	8.00	437.37	16.02	10.00	431.76	15.49	11.00	443.27	16.91	9.00	446.78	12.22	13.00
12	447.88	10.27	10.00	402.83	62.11	7.00	418.22	18.53	9.00	398.17	18.97	8.00	399.69	21.15	9.00	450.84	13.17	10.00	444.61	16.72	13.00
15	415.57	6.42	7.00	390.88	65.85	6.00	431.98	40.89	6.00	412.93	33.16	6.00	451.78	25.30	6.00	465.01	14.56	6.00	448.01	14.28	8.00
18	459.99	20.90	6.00	375.44	75.95	5.00	411.75	23.07	6.00	427.62	23.90	6.00	492.13	9.90	7.00	436.17	18.29	6.00	454.12	21.22	8.00

ANTERIOR WALL THICKNESS; SYSTOLE (mm)

	SHAM		PBS		CPC		MSC		CPC + MSC		CC1		CC2	
WEEK	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
1	1.52	0.07	0.61	0.04	0.82	0.10	0.77	0.11	0.85	0.09	1.01	0.10	1.01	0.10
2	1.52	0.06	0.69	0.09	0.87	0.14	0.70	0.08	0.98	0.10	0.96	0.10	0.91	0.12
3	1.54	0.06	0.67	0.10	0.75	0.15	0.60	0.06	0.72	0.09	0.78	0.15	0.77	0.13
4	1.54	0.05	0.43	0.05	0.80	0.10	0.56	0.10	0.75	0.10	0.84	0.12	0.82	0.12
6	1.52	0.10	0.60	0.10	0.87	0.16	0.71	0.16	0.81	0.15	1.19	0.14	0.73	0.14
8	1.53	0.06	0.44	0.06	0.71	0.18	0.56	0.14	0.71	0.15	1.10	0.14	0.85	0.13
12	1.69	0.12	0.52	0.16	1.04	0.18	0.49	0.09	0.97	0.13	1.33	0.11	0.98	0.14
15	1.56	0.08	0.57	0.11	0.98	0.21	0.88	0.21	1.23	0.21	1.07	0.17	0.86	0.21
18	1.42	0.03	0.47	0.04	0.65	0.20	0.57	0.17	0.84	0.17	1.09	0.21	0.78	0.18

ANTERIOR WALL THICKNESS; DIASTOLE (mm)

	SHAM		PBS		CPC		MSC		CPC + MSC		CC1		CC2	
WEEK	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
1	0.99	0.05	0.46	0.03	0.60	0.07	0.58	0.07	0.65	0.07	0.73	0.08	0.72	0.08
2	1.02	0.05	0.54	0.06	0.62	0.10	0.54	0.07	0.67	0.06	0.74	0.10	0.72	0.10
3	0.97	0.05	0.55	0.08	0.49	0.09	0.45	0.04	0.54	0.07	0.63	0.10	0.59	0.09
4	0.96	0.05	0.30	0.04	0.54	0.08	0.41	0.07	0.56	0.09	0.61	0.08	0.56	0.08
6	0.98	0.05	0.45	0.09	0.61	0.13	0.54	0.13	0.58	0.11	0.83	0.11	0.56	0.10
8	1.01	0.05	0.33	0.04	0.55	0.14	0.40	0.12	0.58	0.11	0.82	0.12	0.66	0.09
12	1.06	0.05	0.40	0.14	0.73	0.11	0.38	0.09	0.70	0.10	1.02	0.11	0.84	0.11
15	1.02	0.08	0.46	0.10	0.66	0.15	0.57	0.14	0.92	0.15	0.79	0.13	0.85	0.16
18	0.99	0.03	0.31	0.05	0.42	0.13	0.46	0.13	0.57	0.11	0.78	0.17	0.60	0.10

POSTERIOR WALL THICKNESS; SYSTOLE (uL)

	SHAM		PBS		CPC		MSC		CPC + MSC		CC1		CC2	
WEEK	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
1	1.40	0.08	0.70	0.08	0.64	0.07	0.73	0.07	0.71	0.07	0.65	0.04	0.75	0.07
2	1.44	0.06	0.62	0.06	0.79	0.13	0.71	0.07	0.72	0.05	0.58	0.08	0.79	0.10
3	1.55	0.07	0.63	0.06	0.64	0.10	0.61	0.05	0.72	0.06	0.57	0.07	0.62	0.09
4	1.34	0.05	0.61	0.09	0.52	0.08	0.72	0.10	0.53	0.08	0.64	0.06	0.74	0.10
6	1.59	0.09	0.49	0.06	0.66	0.11	0.50	0.07	0.54	0.09	0.56	0.07	0.68	0.11
8	1.43	0.05	0.50	0.06	0.61	0.07	0.62	0.07	0.65	0.12	0.58	0.09	0.70	0.11
12	1.52	0.07	0.49	0.06	0.45	0.06	0.40	0.04	0.54	0.08	0.61	0.08	0.75	0.11
15	1.45	0.08	0.45	0.08	0.46	0.05	0.68	0.14	0.56	0.08	0.66	0.14	0.79	0.19
18	1.38	0.12	0.50	0.09	0.51	0.07	0.50	0.13	0.62	0.08	0.59	0.10	0.87	0.13

POSTERIOR WALL THICKNESS; DIASTOLE (mm)

	SHAM		PBS		CPC		MSC		CPC + MSC		CC1		CC2	
WEEK	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
1	0.99	0.05	0.68	0.06	0.57	0.06	0.65	0.08	0.67	0.05	0.62	0.05	0.70	0.06
2	1.00	0.04	0.56	0.06	0.67	0.10	0.69	0.05	0.70	0.04	0.58	0.06	0.66	0.08
3	1.09	0.06	0.56	0.05	0.62	0.08	0.61	0.06	0.68	0.05	0.53	0.07	0.56	0.07
4	0.93	0.06	0.53	0.07	0.48	0.05	0.70	0.08	0.54	0.06	0.59	0.05	0.64	0.08
6	1.23	0.08	0.41	0.05	0.58	0.08	0.46	0.07	0.51	0.08	0.48	0.06	0.57	0.10
8	1.08	0.07	0.46	0.04	0.57	0.07	0.66	0.06	0.59	0.09	0.56	0.06	0.69	0.10
12	1.16	0.05	0.35	0.04	0.46	0.06	0.37	0.03	0.52	0.07	0.56	0.07	0.65	0.10
15	1.18	0.08	0.43	0.03	0.44	0.04	0.59	0.13	0.54	0.10	0.50	0.06	0.74	0.14
18	1.06	0.07	0.55	0.07	0.52	0.11	0.50	0.12	0.57	0.07	0.57	0.06	0.83	0.11

LEFT VENTRICULAR VOLUME; SYSTOLE (uL)

	SHAM		PBS		CPC		MSC		CPC + MSC		CC1		CC2	
WEEK	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
1	8.23	1.31	32.47	2.71	21.03	1.80	24.51	2.00	25.37	2.80	30.37	3.60	26.51	1.92
2	5.71	0.74	42.31	4.03	27.89	3.09	31.23	3.13	27.85	4.27	36.91	3.65	31.34	2.55
3	6.53	0.74	45.85	4.12	35.43	4.58	38.66	4.70	37.90	4.67	33.21	3.28	37.42	2.90
4	6.65	0.64	49.14	6.23	34.82	4.42	37.97	4.64	30.97	3.27	34.27	3.91	38.98	3.51
6	4.34	0.74	54.55	7.42	32.75	6.76	37.03	4.55	33.67	4.71	33.84	5.29	35.89	4.63
8	6.90	1.08	59.25	6.65	33.71	6.31	39.67	4.93	31.64	4.58	35.42	4.08	38.15	4.12
12	6.23	0.63	72.93	6.54	37.02	7.69	45.63	6.28	33.84	4.06	35.66	6.08	43.39	5.92
15	7.06	1.30	77.61	8.10	38.78	9.59	53.58	9.01	33.34	8.37	34.60	7.44	38.17	7.43
18	6.41	0.65	85.10	6.17	42.06	8.13	61.12	13.11	38.13	9.37	36.42	6.82	34.07	5.75

LEFT VENRICULAR VOLUME; DIASTOLE (uL)

	SHAM		PBS		CPC		MSC		CPC + MSC		CC1		CC2	
WEEK	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
1	31.91	2.09	49.27	2.55	34.91	1.94	40.96	2.62	42.59	2.54	50.25	3.95	43.68	1.83
2	28.32	1.96	65.28	3.08	49.28	4.10	51.34	4.23	49.19	4.97	60.17	3.95	52.86	2.77
3	30.11	1.13	65.36	4.57	57.79	5.91	62.22	5.49	64.60	4.82	58.81	4.31	60.18	3.55
4	31.41	1.75	70.07	6.64	55.35	5.91	55.36	4.85	50.18	3.14	57.21	4.38	60.50	3.57
6	25.73	1.62	76.12	7.76	51.35	6.48	54.04	4.28	54.65	4.94	56.34	5.98	61.18	5.75
8	26.87	2.04	80.00	6.85	55.63	6.67	60.41	5.19	53.88	5.76	63.32	5.31	64.31	4.37
12	26.59	1.40	93.79	7.14	57.87	8.50	63.91	5.37	58.94	4.86	64.08	7.96	68.67	6.68
15	33.36	2.32	101.45	7.32	58.48	10.74	72.01	8.69	55.19	11.01	61.14	9.31	60.26	8.81
18	29.43	2.67	105.34	6.58	67.10	8.31	83.06	14.89	61.47	11.20	63.69	8.03	59.45	5.41

EJECTION FRACTION (%)

	SHAM		PBS		CPC		MSC		CPC + MSC		CC1		CC2	
WEEK	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
1	76.28	3.05	35.00	3.18	40.56	2.31	40.45	3.29	41.91	3.37	40.84	2.98	40.10	2.59
2	80.65	1.92	36.74	3.47	43.81	3.45	39.75	1.84	46.12	3.14	39.90	2.46	41.80	2.61
3	78.46	2.40	31.04	2.97	40.02	3.02	39.77	3.12	43.66	3.43	44.24	2.03	38.18	2.57
4	79.27	1.39	32.57	2.39	39.12	2.46	33.76	3.12	40.19	3.57	41.50	3.95	36.64	2.71
6	83.83	2.20	30.89	3.27	40.23	5.32	32.86	4.39	41.10	3.62	42.82	4.03	43.54	3.28
8	75.09	3.33	27.78	3.36	41.97	4.85	35.76	3.24	42.44	2.98	45.39	2.47	42.24	3.58
12	76.06	2.66	22.51	3.04	39.22	5.06	31.09	4.69	43.53	3.57	47.20	4.13	39.45	3.16
15	78.93	3.73	24.03	3.60	37.19	4.32	27.93	4.55	41.69	4.12	45.15	4.43	40.31	4.39
18	77.99	1.77	19.22	2.64	37.79	6.04	29.91	4.21	40.33	3.46	44.89	4.21	44.23	5.29

Fractional Shortening (%)

	SHAM		PBS		CPC		MSC		CPC + MSC		CC1		CC2	
WEEK	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
1	28.15	2.75	13.18	1.37	17.96	2.01	17.04	0.87	18.02	2.12	14.90	1.51	15.80	1.70
2	28.66	2.58	13.58	1.51	16.88	1.97	18.18	2.03	19.14	1.93	14.74	1.38	11.91	1.43
3	25.71	1.95	11.61	0.94	14.82	1.45	14.58	1.31	15.15	1.71	11.68	1.06	15.75	1.45
4	28.48	1.47	11.88	1.51	15.38	1.10	13.77	1.24	16.33	1.47	14.02	1.63	12.70	1.55
6	31.55	2.61	11.48	1.37	13.96	1.31	11.65	1.23	10.30	1.85	13.20	2.15	18.01	1.02
8	23.11	2.11	9.26	0.64	16.07	3.45	15.21	1.70	16.48	1.71	19.97	2.55	15.12	1.18
12	26.82	3.39	9.60	1.75	10.78	1.91	10.05	1.74	11.34	1.10	13.31	1.50	16.01	1.25
15	27.00	2.24	8.27	0.95	11.74	2.36	9.54	0.85	11.81	2.27	14.82	1.86	14.05	2.07
18	25.99	2.03	8.75	1.87	17.02	1.32	10.66	2.13	12.96	2.38	14.66	2.64	12.84	2.35

Online Table II. Heart rate and echocardiographic data. Echocardiographic data represented as mean ± SEM. Heart rate, anterior wall thickness, posterior wall thickness, left ventricular volume, ejection fraction and fractional shortening were measured at specified times after MI. (N) indicates the number of mice used in each group at the given time point.

Use: Flow Cytometry	**Company**	**Primary Antibody Dilution**	**Secondary Antibody Dilution**	**Tyramide Amplification**
Goat anti-CD117 (c-kit)	R&D Systems	1:40	Donkey anti-goat 647 (1:400)	N/A
Goat anti-IgG	Santa Cruz Biotechnology Inc.	1:40	Donkey anti-goat 647 (1:400)	N/A
Annexin V-APC	BD Biosciences	1:40	N/A	N/A
Sytox Blue	Life Technologies	1:2000	N/A	N/A
Use: Immuno-cytochemistry	**Company**	**Primary Antibody Dilution**	**Secondary Antibody Dilution**	**Tyramide Amplification**
Rat anti-mcherry	Life Technologies	1:100	Donkey anti-goat 555 (1:100)	N/A
Rabbit anti-GFP	Life Technologies	1:100	Donkay anti-goat 488 (1:100)	N/A
TO-PRO-3 iodide	Life Technologies	1:10000	N/A	N/A
Use: Immuno-histochemistry	**Company**	**Primary Antibody Dilution**	**Secondary Antibody Dilution**	**Tyramide Amplification**
Goat anti-CD117 (c-kit)	R&D Systems	1:200	Bovin anti-goat HRP (1:200)	Yes
Rat anti-mcherry	Life Technologies	1:200	Donkey anti-rat biotin (1:4500); Streptavidin HRP (1:100)	Yes
Rabbit anti-GFP	Life Technologies	1:200	Donkey anti-rabbit FITC (1:200); Sheep anti-FITC-HRP (1:400)	Yes
Mouse anti-cardiac troponin T	Thermo Scientific	1:100	Donkey anti-mouse 647 (1:100)	No
Isolectin-B_4-488	Life Technologies	1:100	N/A	N/A
Wheat germ	Life Technolgies	1:100	N/A	N/A

Online Table III. Antibody list.

mRNA Primer	Forward	Reverse
Cardiac troponin T	5'-ACCCTCAGGCTCAGGTTCA-3'	5'-GTGTGCAGTCCCTGTTCAGA-3'
Connexin-43	5'-GGACCTTGTCCAGCAGCTT-3'	5'-TCCAAGGAGTTCCACCACTT-3'
Smooth muscle 22	5'-GACTGCACTTCTCGGCTCAT-3'	5'-CCGAAGCTACTCTCCTTCCA-3'
Platelet endothelial cell adhesion molecule	5'-TGCTCTCGAAGCCCAGTATT-3'	5'-TGTGAATGTTGCTGGGTCAT-3'
p53	5'-GCAGGGCTCACTCCAGCTACCT-3'	5'-GTCAGTCTGAGTCAGGCCCCACT-3'
p16	5'-CGTACCCCGATTCAGGTGATG-3'	5'-CGGGCGGGAGAAGGTAGT-3';
Interleukin-6	5'-ATCCAGTTGCCTTCTTGGGACTGA-3'	5'-TAAGCCTCCGACTTGTGAAGTGGT-3'
18s	5'-CGAGCCGCCTGGATACC-3'	5'-CATGGCCTCAGTTCCGAAAA-3'

Online Table IV. qRT-PCR primer list.

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