SUPPLEMENTARY MATERIAL

Supplementary Methods

Donor MI with parasternotomy or left thoracotomy

For parasternotomy MI, donor mice randomly underwent the parasternotomy MI procedure but with the ligation at different positions of the LAD artery to induce different infarct sizes. The LAD artery was ligated ~2 mm and ~5 mm below the tip of the left atrial appendage, which induces roughly > 40% (large) and < 10% (small) ischemia of the left ventricle, respectively. The *donor* EF measured on day 2 post-MI was ~31% in large MI donor mice and ~45% in small MI donor mice. For thoracotomy MI, donor mice randomly underwent left thoracotomy (no bone injury) MI procedure with the LAD ligation ~2 mm below the tip of the left atrial appendage. Sham surgical procedure involved exposing the mouse heart via the same parasternotomy with a suture passed the beneath of the LAD artery to damage myocardium, but without actual ligation of the artery.

BMC harvest and injection

Mouse femurs and tibias were harvested, the ends were cut, and bone marrow was flushed with cold Hank's buffered saline solution (HBSS) with 0.5% BSA. The suspension was strained through a 70 μm filter and washed twice with HBSS. The cell viability was checked using Trypan Blue with an average of 98% viability before cell injection. The cell concentration was adjusted to 10^8 viable cells/ml. 10^6 cells were injected into myocardium at the infarct border zone as two 5 μl injections with a glass

Hamilton syringe and 30 g. needle under ultrasound visualization using a Vevo660 micro-ultrasound system (VisualSonics Inc., Toronto), equipped with a 30-MHz linear transducer, under 2% isoflurane anesthesia.

Immunofluorescent histology

For detection of implanted BMCs in recipient hearts, BMCs harvested from GFP^+ transgenic mice were injected into wild-type mouse hearts. Hearts were harvested, and subjected to retro-perfusion from aorta with perfusion solution (10 mM Tris-HCl buffer containing 140 mM Na^+, 2 mM Ca^{2+}, 2 mM Mg^{2+} and 0.1% adenosine, pH 7.4; Sigma-Aldrich) for 2 minutes and fixation solution (1.5% paraformaldehyde/PBS; Fisher Scientific) for 2 minutes. Then hearts were immersed in 0.5% paraformaldehyde/PBS for 1-2 hours and switched in 20% sucrose/PBS overnight at room temperature. Hearts were embedded in O.C.T. compound (Sakura Finetek USA, Torrance, CA), frozen in a bath of 2-methylbutane (Fisher Scientific) with dry ice and stored at -80°C. The hearts were sliced transversely from apex to base of ventricle at 10-μm thickness with an interval of 300 μm between each collected section. GFP^+ cells were doubly detected by (1) direct intrinsic fluorescence (green) and (2) use of a polyclonal rabbit anti-GFP IgG at 1:1000 dilution (Invitrogen) followed by a secondary goat anti-rabbit IgG conjugated to Alexa Fluor 647 at 1:200 dilution (far red; Invitrogen).

For detection of GFP^+ BMC proliferation, frozen heart sections as described above for GFP^+ BMC tracking were fixed with 1.5% paraformaldehyde/PBS for 15 minutes, washed with PBS and then blocked with 2% normal goat serum (NGS), 0.3% Triton X-100, and sodium azide blocking buffer for 30 minutes and subsequently with rodent block

M (Biocare, Concord, CA) for 20 minutes. They were then washed with blocking buffer 3 times. Primary antibodies rabbit anti-mouse Ki-67 (Abcam, Cambridge, MA; 1:300 dilution) and rat anti-mouse CD45 (BD Biosciences, San Diego, CA; 1:500 dilution) were applied to the sections overnight at 4˚C. After washing with blocking buffer, sections were stained with Hoechst 33258 (Invitrogen) and secondary Alexa Fluor 647 goat anti-rabbit/rat antibodies (Invitrogen; 1:200 dilution) for one hour at room temperature. The Ki-67^+ GFP^+ BMCs were counted with a fluorescence microscope.

Flow cytometry

The following fluorescent antibodies for cell-surface markers were used: anti-panNK-FITC, anti-Gr-1-PE, anti-MHCII-PE, anti-CD3-PerCPCy5.5, anti-CD25-PerCPCy5.5, anti-CD45R-PerCPCy5.5, anti-CD11b-PerCPCy5.5, anti-CD11c-PECy7, anti-CD69-PECy7, anti-CD11b-Pacific Blue, anti-CD44-Pacific Blue, anti-CD80-APC, anti-CD19-Alexa647, anti-CD4-APC750, anti-Gr-1-APC750 (eBiosciences); anti-Ly6C-FITC, anti-CD19-APCCy7(BD Biosciences); anti-CD86-Pacific Blue (Biolegend); anti-CD62L-Alexa647 (UCSF Hybridoma Core); anti-CD8-Pacific Orange (Invitrogen). Anti-CD11b-Pacific Blue, anti-CD11b-PerCPCy5.5, anti-Gr-1-PE, anti-Gr-1-APC750, anti-Ly6C-FITC and anti-CD11c-PE were used for identifying monocytes, macrophages, and neutrophils. Data were acquired on an LSRII (Becton Dickson) and analyzed with FlowJo v.8.8.6.

Cytospin histology

For cytospin preparation, BMCs were harvested as described for *BMC harvest and injection* and prepared as a cell suspension of 0.5×10^6 cells/ml of HBSS, and given to the UCSF Mouse Pathology Core facility to conduct the cytospin procedure. The morphological recognition of bone marrow hematopoietic cells at various developmental stages was blindly analyzed.

Cytokine array and enzyme-linked immunosorbent assay (ELISA)

Cytokine levels were assayed using a mouse cytokine array kit (R&D Systems, MN). Briefly, mouse blood samples were collected to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000×g, and store at -20°C. Sera were diluted and used to detect a panel of cytokines, chemokines and other related proteins with a mouse cytokine array kit. Arrays were exposed to film and results were quantified by densitometry. Data in Figure 5C are shown as % changes normalized to the no-MI sample, and the raw pixel density data is supplied in Supplemental Figure 2 (n = 2 per group, tested in duplicate).

Serum levels of cytokine IL-6 were assayed using a mouse IL-6 immunoassay kit (R&D Systems, MN) and following the assay procedure. Each sample was tested in duplicate (n = 2-4 per group).

Supplementary Figures

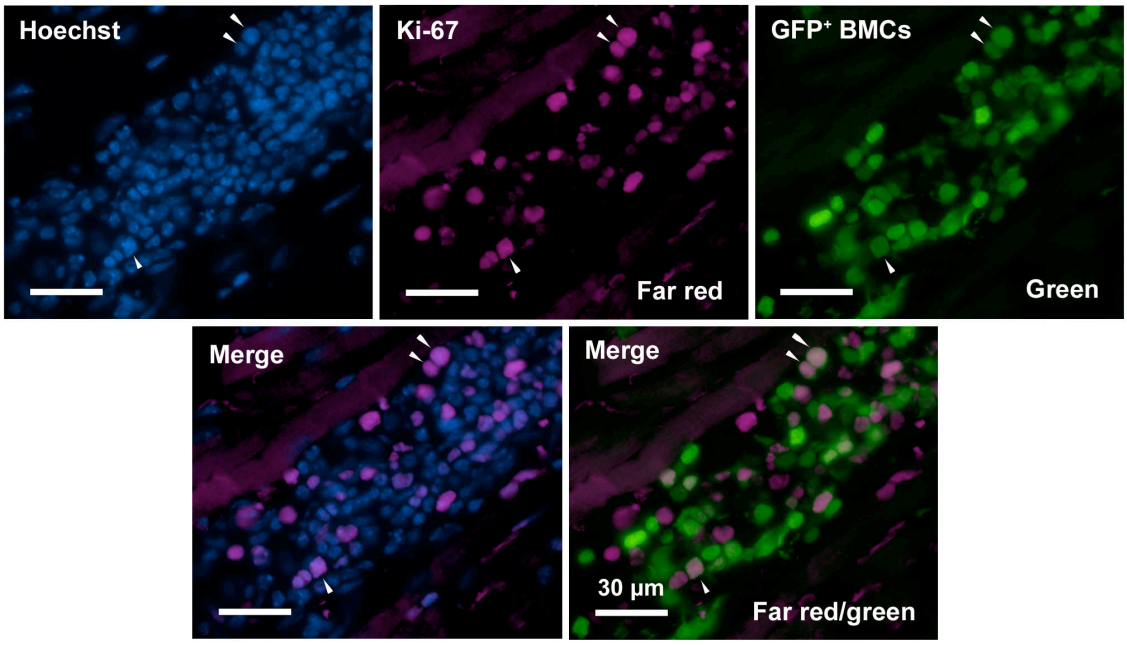

Fig. S1. Proliferation of implanted GFP^+ BMCs. BMCs harvested from GFP^+ transgenic mice under healthy and 3 day post-MI conditions were injected into the myocardium of 3 day post-MI wild-type mice. For the detection of the implanted GFP^+ BMC proliferation, frozen heart sections were stained with Hoechst 33258 for detection of nuclei (blue fluorescence), and stained with Ki-67 antibody for cell proliferation using a far red secondary antibody (pseudocolored magenta). Proliferating GFP^+ BMCs were identified as those visible by both green and far red fluorescence (shown as arrows at the lower right panel).

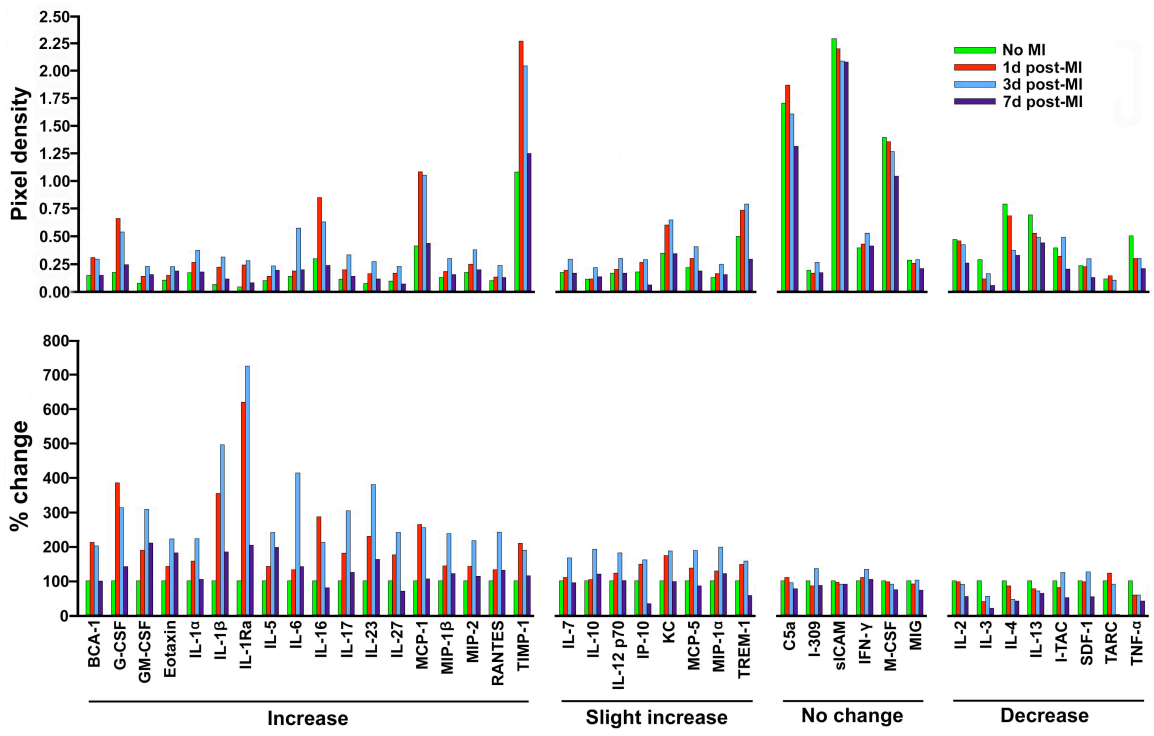

Fig. S2. Changes in cytokines at varying times post-donor-MI. Results are shown first as raw data from densitometry analysis of exposed films, and then normalized to the no-MI sample as in Figure 5C. Cytokine array analysis showed differences in serum levels of pro-inflammatory cytokines and other inflammation-related proteins at 1 to 7 days post-donor-MI. Abbreviations: BCA, B-cell attracting chemokine; C5a, complement component 5a; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; IFN, interferon; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; IP-10, interferon-inducible protein-10; I-TAC, interferon-inducible T cell alpha chemoattractant; KC, keratinocyte chemoattractant; MCP, monocyte chemoattractant protein; M-CSF, macrophage colony stimulating factor; MIG, monokine induced by γ-interferon; MIP, macrophage inflammatory protein; RANTES, regulated upon activation normal T-cell expressed and secreted; SDF, stromal cell-derived factor; sICAM, soluble intercellular adhesion molecule; TARC, thymus and

activation regulated chemokine; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; TREM, triggering receptor expressed on myeloid cells.

Supplementary Tables

Table S1. Recipient echocardiographic parameters and general data before (day 2 post-MI) and after (day 28 post-MI) the implantation of BMCs from healthy and infarcted donors

Donor condition (recipient number)	HR bpm	BW g	EF %	ESV µl	EDV µl	AWTd mm	PWTd mm	Border zone mm	Infarct mm
Healthy (n = 15)									
Baseline	463±39	24.9±1.2	50.1±2.2	33.2±3.4	66.4±5.1				
Day 2 post-MI	563±33*	23.8±1.2*	32.1±3.4$^{\triangle}$	42.1±5.6$^{\triangle}$	61.9±7.3	0.73±0.04	0.69±0.05	0.63±0.04	0.51±0.06
Day 28 post-MI	520±55$^{\#\Delta}$	26.9±1.2$^{\#}$	39.0±5.9$^{\triangle\ddagger}$	41.2±6.7†	67.6±9.6$^{\Diamond}$	0.72±0.04	0.67±0.05	0.59±0.04‡	0.49±0.06
Infarcted (n = 15)									
Baseline	482±44	24.1±1.6	50.3±4.8	32.1±5.7	64.3±8.1				
Day 2 post-MI	560±32*	23.1±1.9*	31.6±4.4$^{\triangle}$	43.7±6.8$^{\triangle}$	63.7±8.5	0.74±0.05	0.72±0.06	0.64±0.04	0.52±0.06
Day 28 post-MI	524±72$^{\#\Delta}$	25.6±1.8$^{\#}$	31.1±6.2$^{\triangle\square}$	50.6±12.4$^{\triangle\ddagger}$	72.6±12.0$^{\#\Diamond}$	0.73±0.04	0.68±0.03$^{\Diamond}$	0.54±0.07‡	0.45±0.11$^{\Diamond}$
HBSS (n = 14)									
Baseline	473±41	24.8±1.5	50.1±4.4	33.3±4.7	66.7±6.3				
Day 2 post-MI	580±38$^{\#}$	23.1±2.6*	31.8±3.5$^{\triangle}$	42.9±6.3$^{\triangle}$	62.9±7.9	0.73±0.02	0.71±0.02	0.63±0.03	0.54±0.06
Day 28 post-MI	597±34$^{\#}$	27.0±1.2$^{\#}$	21.4±6.4$^{\triangle\S\Omega}$	82.5±26.0$^{\triangle\ddagger\Omega}$	103.4±25.5$^{\triangle\S\Omega}$	0.67±0.04$^{\S\Omega}$	0.70±0.01	0.46±0.09$^{\S\square\infty}$	0.33±0.09$^{\S\square\infty}$

AWTd, anterior wall thickness in diastole; BMCs, bone marrow cells; BW, body weight; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; HBSS, Hank's buffered saline solution; HR, heart rate; MI, myocardial infarction; PWTd, posterior wall thickness in diastole. * P<0.05 vs baseline and day 28 post-MI; ⌂ P<0.0001 vs baseline; † P<0.01 vs baseline; # P<0.05 vs baseline; § P<0.0001 vs day 2 post-MI; ‡ P<0.01 vs day 2 post-MI; ◊ P<0.05 vs day 2 post-MI; Δ P<0.01 vs HBSS control group; ¤ P<0.001 vs healthy donor group; Ω P<0.001 vs healthy and infarcted donor groups; ∞ P<0.05 vs infarcted donor group.

Table S2. Recipient echocardiographic parameters at 28 days post-MI after the implantation of BMCs from healthy and infarcted donor groups

Donor condition (recipient number)	EF %	ESV µl	EDV µl
Healthy (*n* = 15)	39.0±5.9	41.2±6.7	67.6±9.6
MI-small (*n* = 13)	32.3±3.2$^{*\#}$	48.9±9.0$^{\#}$	72.0±10.8$^{\#}$
MI-large‡ (*n* = 15)	27.5±6.7$^{*\#\Delta}$	59.7±19.0$^{*\#}$	81.1±18.4$^{\Omega\#}$
HBSS (*n* = 14)	21.4±6.4*	82.5±26.0*	103.4±25.5*
MI-parasternotomy‡ (*n* = 15)	27.5±6.7$^{*\#\S}$	59.7±19.0$^{*\#\S}$	81.1±18.4$^{\Omega\#}$
MI-thoracotomy (*n* = 14)	26.9±6.5$^{*\triangle\S}$	63.5±14.6$^{*\#\S}$	86.2±13.7$^{*\#\S}$
Sham-whole (*n* = 14)	33.2±6.4$^{\Omega\#}$	48.2±12.2$^{\#}$	71.5±12.2$^{\#}$
1h post-MI (*n* = 15)	35.5±4.9	46.6±9.0	71.9±10.6
1d post-MI (*n* = 12)	32.2±5.8$^{*\wedge}$	54.0±17.6*	78.4±20.1
3d post-MI‡ (*n* = 15)	27.5±6.7*†	59.7±19.0*†	81.1±18.4*
5d post-MI (*n* = 14)	29.2±5.1*†	56.6±8.2*†	79.8±8.0$^{\Omega}$
7d post-MI (*n* = 14)	31.2±5.3*	53.3±17.1*	76.5±19.9
21d post-MI (*n* = 14)	33.4±6.4$^{*\wedge}$	53.6±14.9*	79.4±14.9$^{\Omega}$

BMCs, bone marrow cells; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; HBSS, Hank's buffered saline solution; MI, myocardial infarction. ‡ MI-large, MI-parasternotomy and 3d post-MI are same donor group; * $P<0.01$ vs healthy donor group; # $P<0.01$ vs HBSS control group; Δ $P<0.05$ vs MI-small donor group; § $P<0.05$ vs sham-whole donor group; ⌂ $P<0.05$ vs HBSS control group; Ω $P<0.05$ vs healthy donor group; † $P<0.01$ vs 1h post-MI donor group; ^ $P<0.05$ vs 3d post-MI donor group.