# Supplemental Materials

# Detailed Methods

## Rat CDC culture

Rat hearts were excised and biopsies of the left ventricle were cut into 1 to 3 $mm^3$ pieces with a sterile scalpel. The minced tissues were digested with 0.25% trypsin (Invitrogen, Carlsbad, CA, USA) for 5-10 min and then cultured on fibronectin-coated tissue culture dishes using media consisting of Iscove's Modified Dulbecco's Medium (IMDM, Invitrogen) supplemented with 20% fetal bovine serum (FBS), 100 U/mL penicillin G, 100 µg/mL streptomycin, and 0.1 mmol/L 2-mercaptoethanol. This media was referred to as cardiosphere media (CEM). After 10-20 days, a layer of fibroblast-like adherent cells and a smaller number of phase-bright cells migrated from the tissue explants. The cells were washed with PBS and detached with TrypLE$^{TM}$ Select (Invitrogen) at room temperature. The harvested cells were then seeded into poly-D-lysine coated 6-well plates ($1x10^5$ cells/well), in CEM containing 10% FBS. Under this suspension culture condition, the cells self-aggregate into cardiospheres in 3-10 days. These cardiospheres were harvested, seeded in fibronectin-coated tissue culture flasks for expansion as monolayers in CEM containing 20% FBS. These cells express several stem cell markers and are referred to as cardiosphere-derived cells or CDCs.

## SPM labelling and *in vitro* toxicity experiment

After 2 passages, rat CDCs were labelled with Dragon-green fluorescence-conjugated SPM particles by co-incubation of the cells with SPMs for 24 hr. For assessment of cell proliferation, 200,000 SPM-labelled and non-labelled cells were seeded into T25 tissue culture flasks. After 2 and 6 days of culture, cells were harvested from the flasks and

viable cells were manually counted by Trypan Blue exclusion to determine the proliferative activity of CDCs. For assessment of cell adhesion activity, both SPM-labelled and control cells were seeded at the same initial density onto fibronectin-coated dishes. At 30 min, 2 hours and 4 hours after cell seeding, the media was removed and the flask washed by PBS 3 times to remove floating cells. Attached cells were then harvested, counted and quantified as a percentage of the initial seeding number. Cell apoptosis and necrosis were assessed by TUNEL staining (In Situ Cell Death Detection Kit, TMR red, Roche, Germany). Reactive oxygen species (ROS) fluorescence was detected by confocal imaging using the Image-iT™ LIVE Green Reactive Oxygen Species Detection Kit (Invitrogen). Quantitative ROS measurement was performed by staining cells with 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate di(acetoxymethyl ester) (Invitrogen) and then measuring fluorescence intensity with a SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA). Plain CDCs and $H_2O_2$-treated CDCs were included as negative and positive controls, respectively.

**Flow cytometry**

Flow cytometry experiments were performed using a LSRII equipment (BD Biosciences, San Jose, CA). Monoclonal antibodies were conjugated with fluorophores using commercially available kits (Molecular Probes, Eugene, OR). Flow cytometry analysis of cell apoptosis/necrosis was performed with the Annexin V-PE Apoptosis Detection Kit I (BD Pharmingen 559763). For phenotypic characterization, the following monoclonal antibodies and conjugated fluorochromes were used with corresponding isotype controls: CD31 (BD Pharmingen 555445), CD34 (Chemicon CBL555F); CD90-FITC (Dianova

DIA120); c-Kit (BD Pharmingen 550412). Fluorescent compensation was included using single-labeled controls. The percentage of positive cells was defined as the percent of the population falling above the 99th percentile of an isotype control cell population. Data analysis was performed using flow cytometry software (Flow-Jo 7.2.2 Treestar Inc., Ashland, OR).

**Animal model**

Animal care was in accordance to Institutional Animal Care and Use Committee guidelines. Female WKY rats (Charles River Laboratories, Wilmington, MA) (n=88 total) underwent left thoracotomy in the 4$^{th}$ intercostal space under general anesthesia. The heart was exposed and myocardial infarction was produced by permanent ligation of the left anterior descending coronary artery, using a 9-0 silk suture, immediately before cell injection. CDCs (total of 1 million; SPM-labeled or non-labeled; suspended in 100 μl of PBS) were injected directly into the myocardium, at 4 sites into the border zone of infarction (i.e., 250,000 cells in 25 μl PBS per site), using a 29G needle. For magnetic targeting, a 1.3 Tesla circular NdFeB magnet (Edmund Scientifics, Tonawanda, NY) was placed above the heart on the retractor (Online Movie II) during and 10 min after the cell injection. The chest was closed and animal was allowed to recover after all procedures.

**Fluorescence imaging**

Representative animals from each cell-injected group were euthanized at 24 hours and 3 weeks after cell injection for fluorescence imaging purposes. The heart, lung and spleen were harvested. Organs were placed in an IVIS 200 imaging system (previously

Xenogen Corporation; now Caliper Life Sciences, Mountain View, CA) to detect flash-red fluorescence. Extensive PBS wash was performed to remove any cells adherent to the epicardium. Excitation was set at 640 nm and emission was set at 680 nm. Exposure time was set at 5 seconds and maintained the same during each imaging experiment. Fluorescence signals (photon/s) from a fixed region of interest (ROI) were measured and quantified with the Xenogen software. Organs from the CDC group (animals receiving non-labeled CDCs) were used as controls for background noise.

**Quantification of engraftment by real time PCR**

Quantitative PCR was performed 24 hr and 3 weeks after cell injection in 6 animals from each cell-injected group to quantify cell retention/engraftment. We injected CDCs from male donor WK rats into the myocardium of female recipients to utilize the detection of SRY gene located on the Y chromosome as target. The whole heart was harvested, weighed, and homogenized. Genomic DNA was isolated from aliquots of the homogenate corresponding to 12.5mg of myocardial tissue, using commercial kits (DNA Easy minikit, Qiagen). The TaqMan® assay (Applied Biosystems, CA) was used to quantify the number of transplanted cells with the rat SRY gene as template (forward primer: 5'-GGA GAG AGG CAC AAG TTG GC-3', reverse primer: 5'-TCC CAG CTG CTT GCT GAT C-3', TaqMan probe: 6FAM CAA CAG AAT CCC AGC ATG CAG AAT TCA G TAMRA, Applied Biosystems). A standard curve was generated with multiple dilutions of genomic DNA isolated from male hearts to quantify the absolute gene copy numbers. All samples were spiked with equal amounts of female genomic DNA as control. The copy number of the SRY gene at each point of the standard curve is

calculated with the amount of DNA in each sample and the mass of the rat genome per cell. For each reaction, 50 ng of template DNA was used. Real time PCR was performed with an Applied Biosystems 7900 HT Fast real-time PCR System. All experiments were performed in triplicate. The number of engrafted cells per heart was quantified by calculating the copy number of SRY gene in the total amount of DNA corresponding to 12.5 mg of myocardium and then extrapolating to the total weight of each heart.

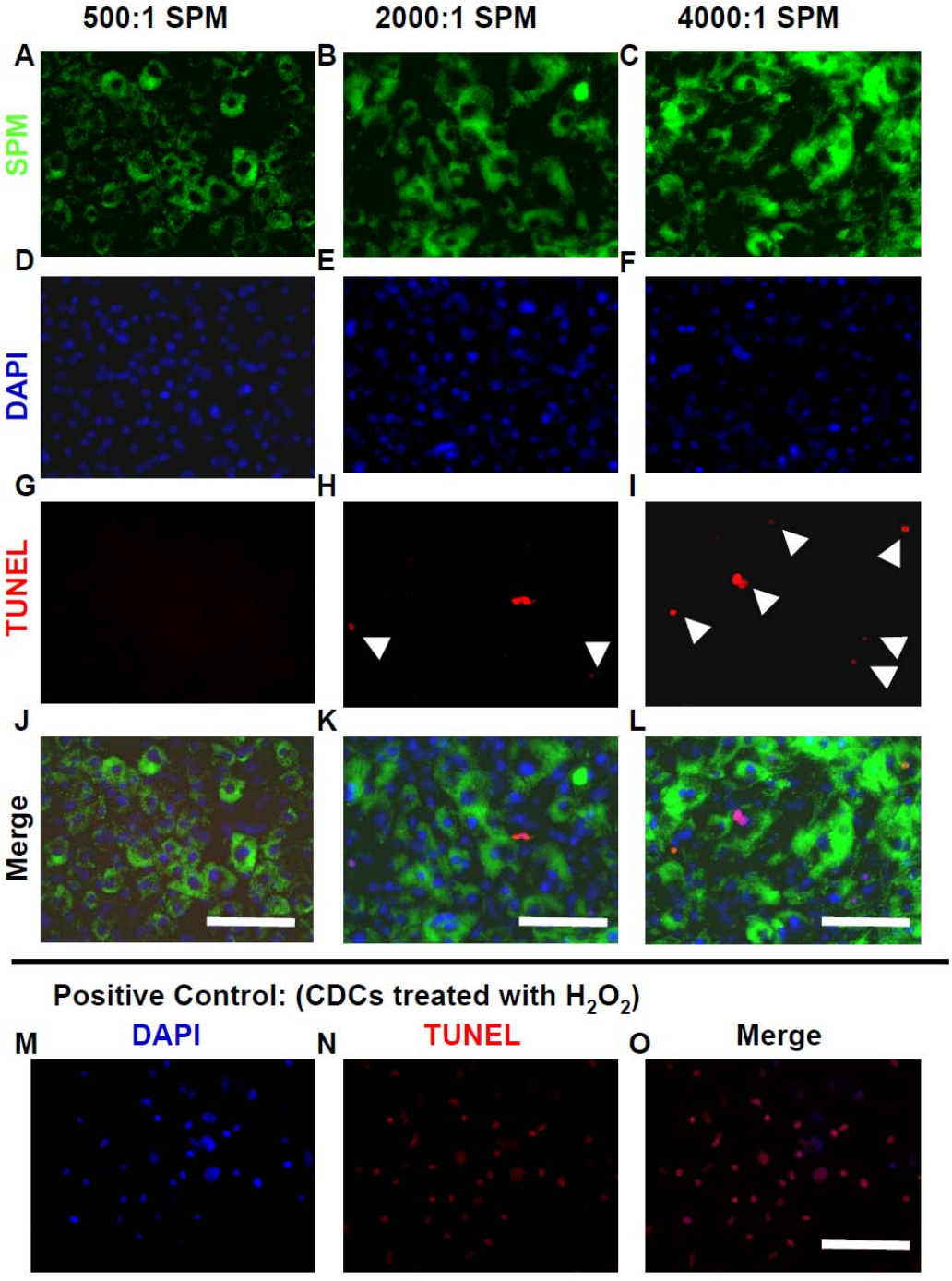
**Morphometric heart analysis**

For morphometric analysis, 5-6 animals in each group were euthanized at 3 weeks and the hearts were harvested and frozen in OCT compound. Sections every 100 μm (10 μm thickness) were prepared. Masson's trichrome staining was performed as previously described[1]. Images were acquired with a PathScan Enabler IV slide scanner (Advanced Imaging Concepts, Princeton, NJ). From the Masson's trichrome-stained images, morphometric parameters including LV cavity circumference, total LV circumference, risk region area, scar area, non-infarcted region wall thickness and infarct wall thickness were measured in each section with NIH ImageJ software. To quantify both the degree of LV dilation and the degree of infarct wall thinning, the LV expansion index was calculated as previously described[2, 3]: LV Expansion index = (LV cavity circumference /total LV circumference) x (non-infarcted region wall thickness/risk region wall thickness). The percentage of viable myocardium as a fraction of the risk region was quantified as described[3].

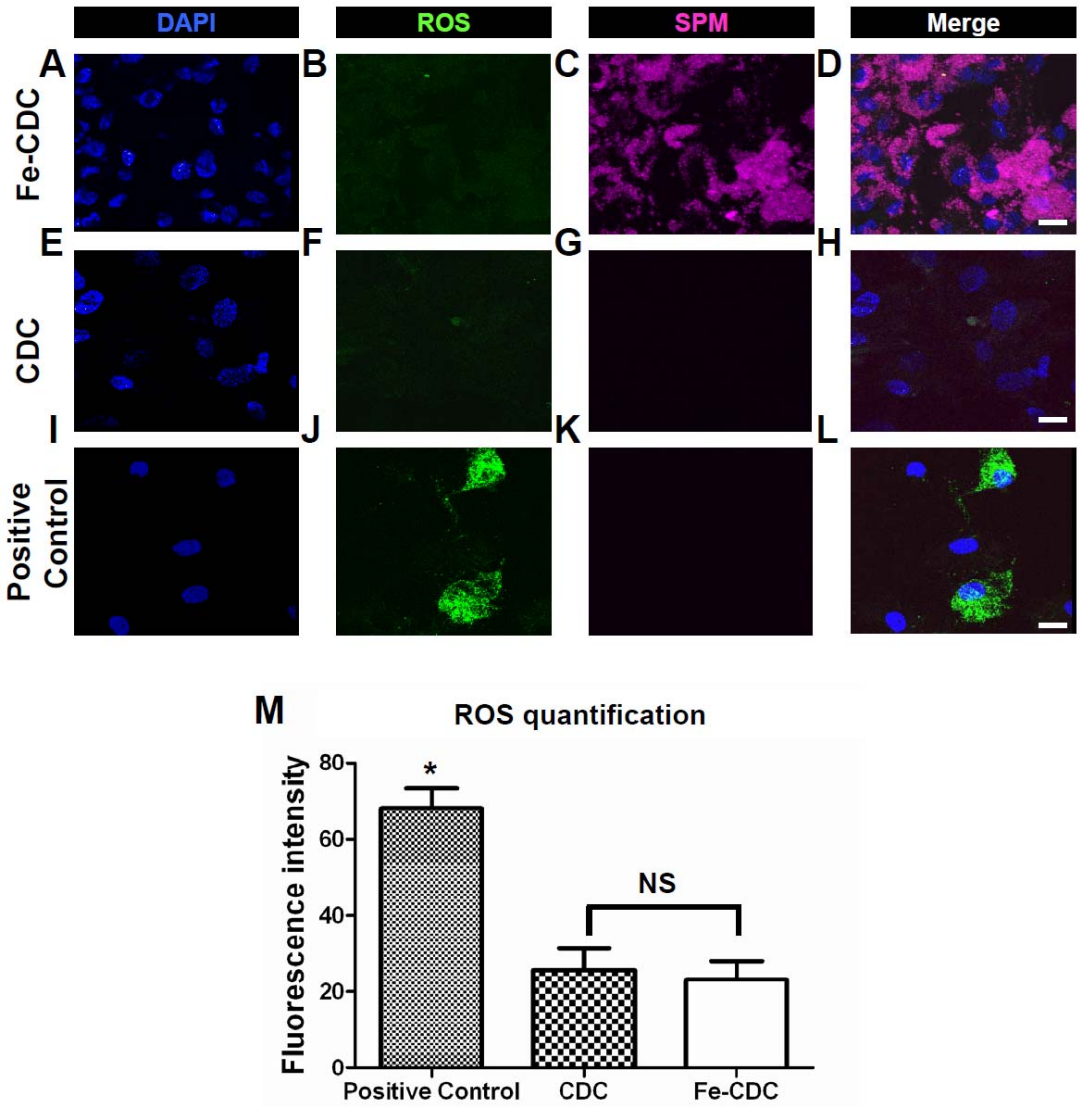
**Histology**

For histology analysis, a subpopulation of animals in each group received Fe-CDCs or CDCs over-expressing GFP. At 24 hours or 3 weeks, the animals were euthanized and the hearts were harvested and frozen in OCT compound. Sections every 100 μm of the infarct and infarct border zone area (10 μm thickness) were prepared and immunocytochemistry for GFP and CD-68 (macrophages) was performed, using a rabbit anti-GFP (Abcam, Cambridge, MA, USA) and a Mouse anti rat CD68 (Abcam, Cambridge, MA, USA) primary antibody respectively. At the 3 week time point, immunocytochemistry for cardiomyocytes and endothelial cells were performed using a mouse anti-alpha-sarcomeric actin (Sigma) and rabbit anti-von Willebrand factor (Abcam) primary antibody respectively. Images were taken by a Leica TCS SP5 X confocal microscopy system.

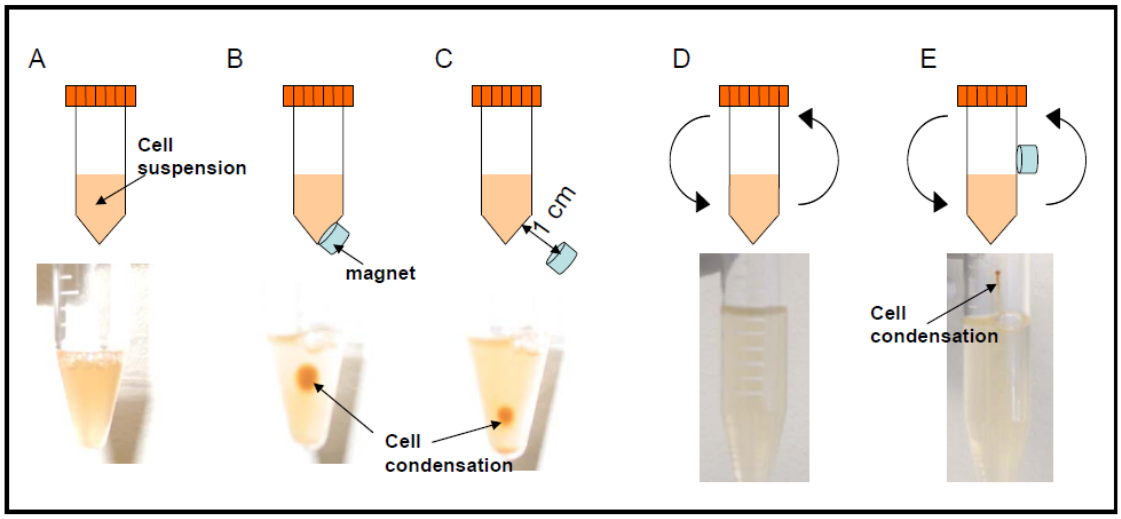
# Online Figures and Figure Legends



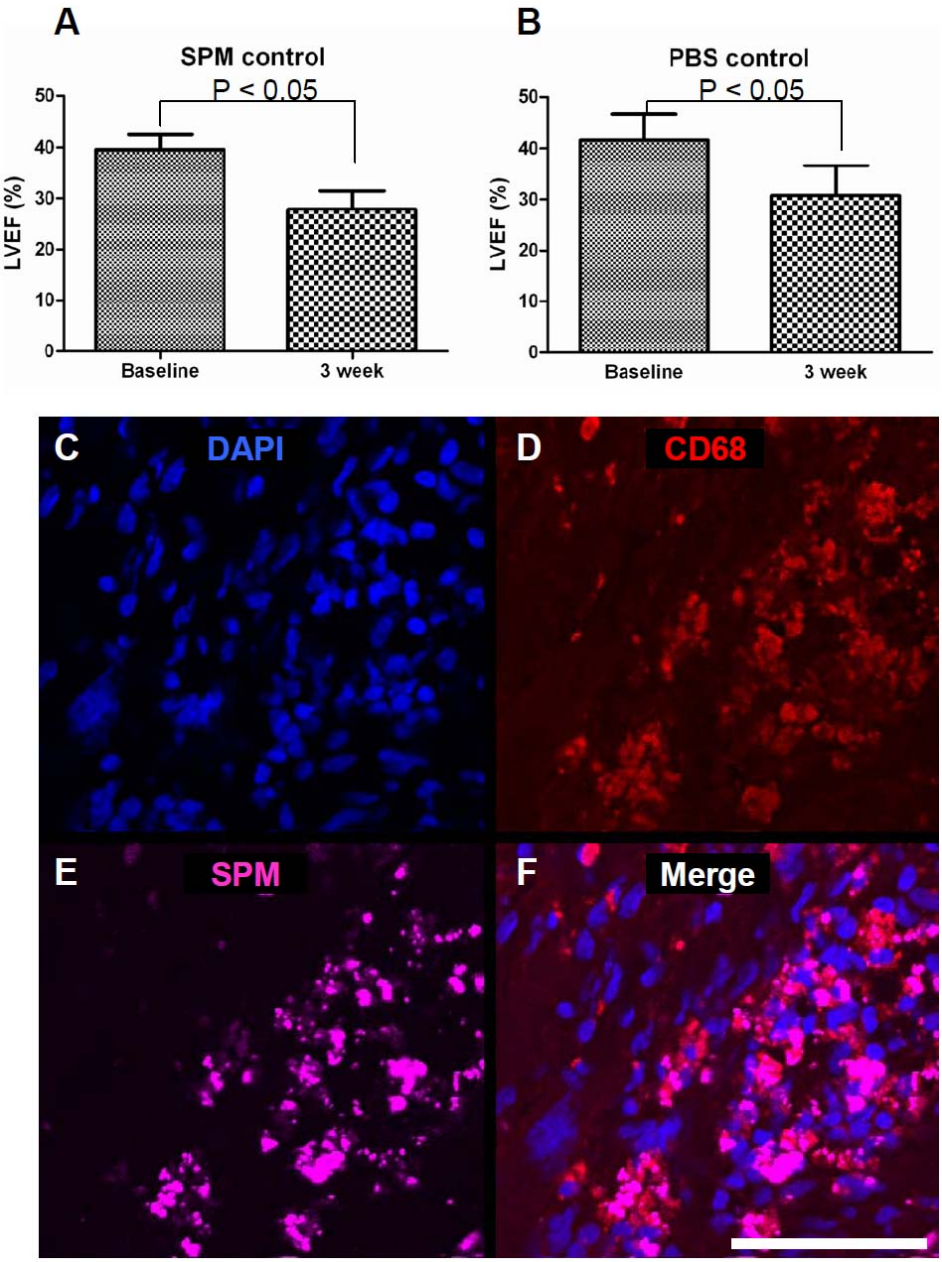

**Online Figure I. Effects of SPM labeling on cell death.** CDCs were co-incubated with SPMs for 24 hours at varying SPM:cell ratios: 500:1 (A, D, G, J); 2000:1 (B, E, H, K); 4000:1 (C, F, I, L). Apoptotic cells (red color) are highlighted by white arrowheads. M-O, images from positive controls (CDCs treated with $H_2O_2$). Bars = 50 μm.



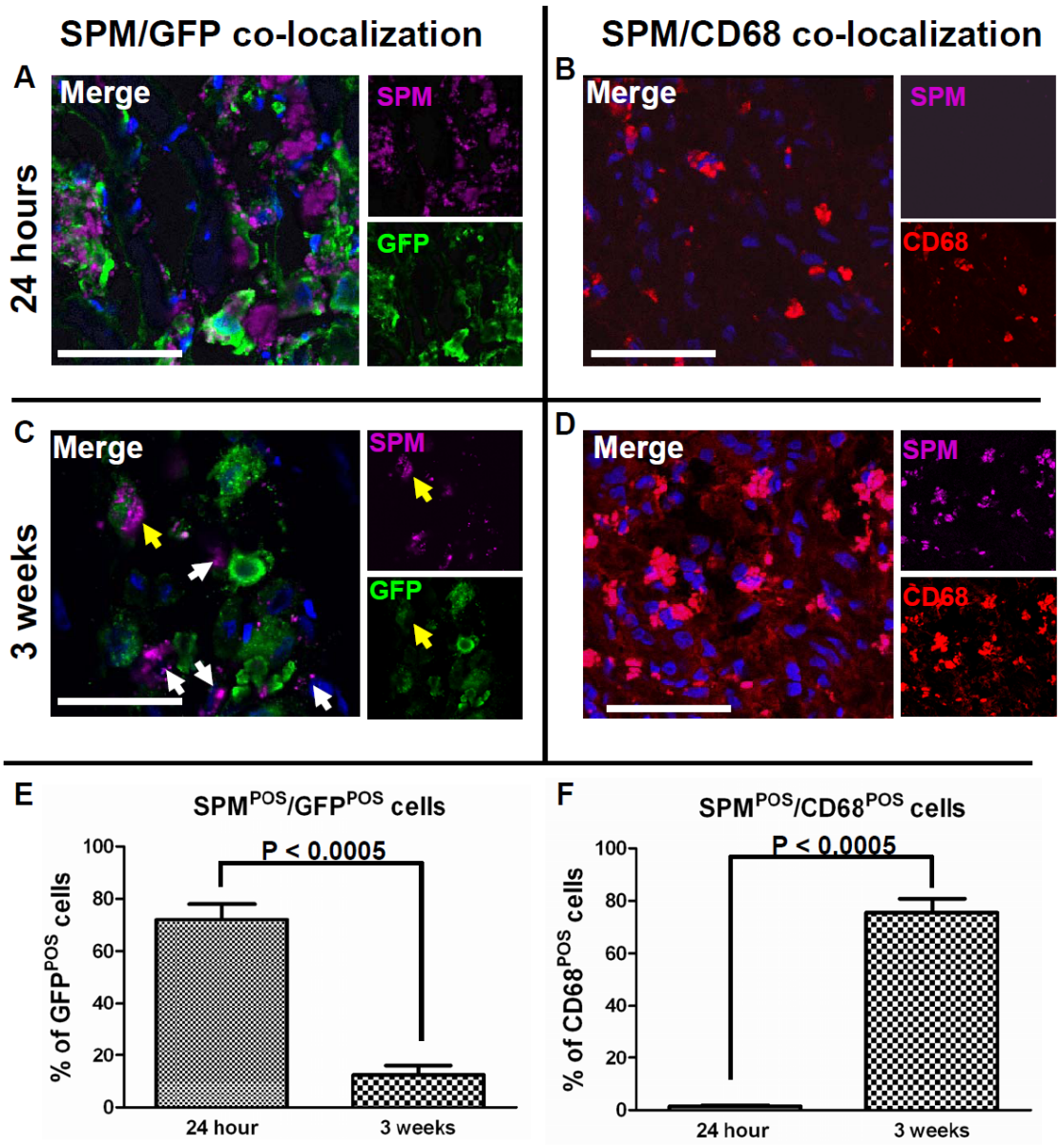

**Online Figure II. Effects of SPM labeling on Reactive oxygen species (ROS) generation.** A-D, Fe-CDCs: CDCs were co-incubated with SPMs for 24 hours at 500:1 SPM particle:cell ratio. E-H, plain CDCs. I-L, positive control: CDCs were treated with 100 μM $H_2O_2$ for 24 hours. ROS staining was detected by confocal microscopy imaging using the Image-iT™ LIVE Green Reactive Oxygen Species Detection Kit. M, Quantitative ROS measurement was performed by incubating cells with 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate di(acetoxymethyl ester) (Invitrogen) for 60 min at a concentration of 10 μM. After that the fluorescence intensity was measured by a SpectraMax M5 plate reader. The ROS fluorescence intensities from the CDC and Fe-CDC group are indistinguishable, suggesting SPM labeling were not likely to elevate ROS generation. Bars = 10 μm. * indicates $P < 0.05$ when compared to the CDC or Fe-CDC group.



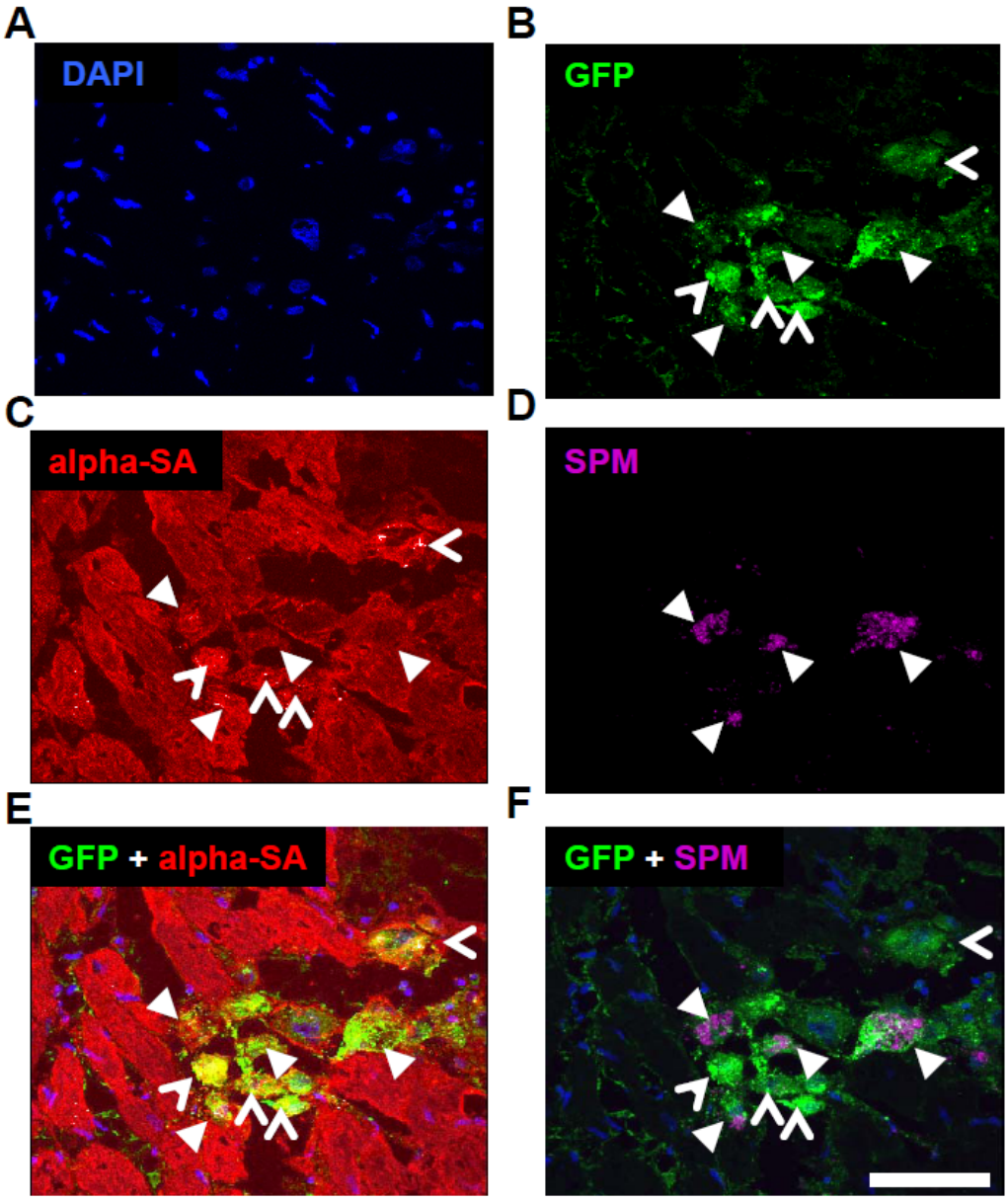

**Online Figure III. Capturing of Fe-CDCs by magnet *in vitro*.** A, Fe-CDCs were suspended in PBS (1 million cells/mL) in a 15 mL conical tube. Uniform cell suspension was obtained. B, cell condensate rapidly formed on the inner tube wall when a 1.3 Tesla magnet was placed on the outer tube wall for 20 seconds. C, a smaller cell condensate was formed when the same magnet was moved 1 centimeter away from the tube. D, without a magnet mounted on the tube, uniform cell suspension was obtained when the conical tube was rotated for 24 hours at 60 RPM. E, cell condensate was formed when the magnet was mounted on the tube during the rotation.



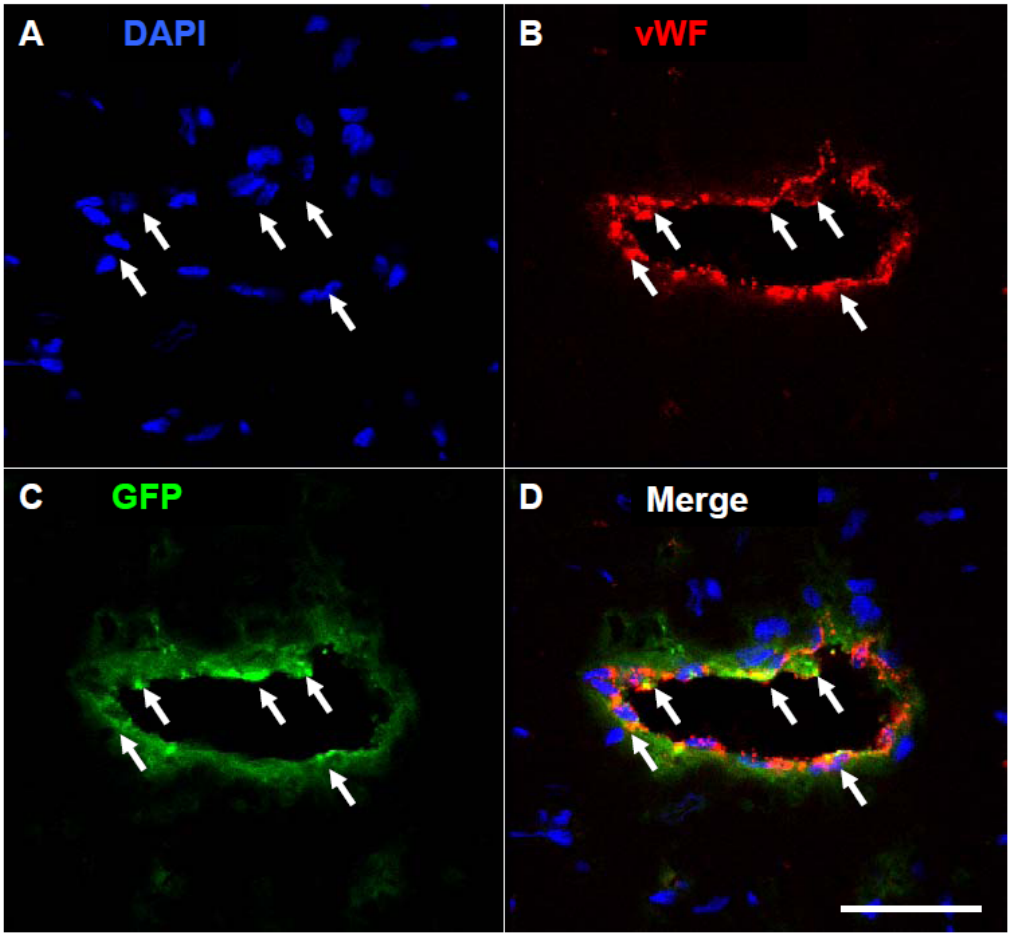

**Online Figure IV. Injection of SPMs alone does not improve heart function.** A, left ventricular ejection fraction (LVEF) measured by echocardiography at baseline and 3 weeks after injection of 500 million SPM beads in 100 µL PBS (n=9). B, LVEFs of PBS-injected animals (n=9; data reproduced from Figure 6). The effect of the SPM injection resembled that of PBS injection, as the LVEF decreased over time. This indicates that injection of SPMs alone did not have therapeutic benefits. C-F, immunohistochemistry staining of sections from SPM-injected hearts at 3 weeks. SPMs were present in the myocardium, with a large amount taken up by macrophages. Bar = 100 µm.




**Online Figure V. Colocalization of SPM, GFP and CD-68.** Subpopulations of animals from the Fe-CDC+Magnet group were euthanized at 24 hours and 3 weeks after injection. At the 24 hour time point, the majority of transplanted CDCs (GFP; green) were SPM positive (Flash red; magenta) (A). In contrast, the SPMs present in CD-68-positive macrophages (Texas red; red) were negligible (B). At the 3 week time point, only very few CDCs (C; yellow arrow) still contained SPMs. SPMs were found outside of the GFP-positive cells (C; white arrows). CD-68 staining confirmed the presence of macrophages and the majority of them were SPM positive (D). E & F, quantification of SPM/GFP and SPM/CD-68 colocalization at 24 hours and 3 weeks after cell injection. Bars = 50 µm.




**Online Figure VI. Expression of cardiac marker in $SPM^{POS}/GFP^{POS}$ cells.** Representative confocal images showing colocalization of GFP with alpha-sarcomeric actin (alpha-SA) in the peri-infarct region of a Fe-CDC+Magnet animal. A, DAPI; B, GFP; C, alpha-SA; D, SPM; E, GFP + alpha-SA; F, GFP + SPM. $SPM^{POS}/GFP^{POS}/alpha\text{-}SA^{POS}$ cells (solid white arrowheads "►") were detected in the region, indicating that remnant SPMs in the cytoplasm did not prevent CDCs from differentiating into a cardiomyocyte phenotype. $SPM^{NEG}/GFP^{POS}/alpha\text{-}SA^{POS}$ cells were also detected (empty white arrowheads ">"), indicative of cells that exocytosed the SPMs before or after acquiring a cardiomyocyte phenotype. Bars = 50 µm.




**Online Figure VII. Expression of endothelial proteins in GFP$^{POS}$ cells.** Representative confocal images showing colocalization of GFP with von Willebrand factor (vWF) in an arteriole in the peri-infarct region of a Fe-CDC+Magnet animal. A, DAPI; B, vWF; C, GFP; D, merge. GFP$^{POS}$/vWF$^{POS}$ cells are indicated by white arrows. The colocalization of GFP with vWF suggests that transplanted CDCs participated in regeneration of vascular structures, differentiating into an endothelial phenotype. Bars = 50 μm.

# Online Table

| Cell retention rate (%) | Time point | Cell type | Delivery method | Study model | References |
|---|---|---|---|---|---|
| **11.1** | 10 min | Microspheres | IM | pig | [4] |
| **17.6** | 1 hr | CDCs | IM | rat | [5] |
| **5.5** | 1 hr | CD34+ cells | IC | human | [6] |
| **11** | 1 hr | PMNCs | IM | pig | [7] |
| **2.6** | 1 hr | PMNCs | IC | pig | [7] |
| **3.2** | 1 hr | PMNCs | RCV | pig | [7] |
| **1.3-2.6** | 75 min | BM stem cells | IC | human | [8] |
| **2.03** | 24 hr | EPCs | IV | rat | [9] |
| **4.70** | 24 hr | EPCs | ILV | rat | [9] |
| **< 1** | 24 hr | CDCs | IC | pig | [10] |
| **8** | 24 hr | CDCs | IM | pig | [11] |

**Online Table I. Survey of short-term cell retention.** Short-term cell retention rates from several recent studies are summarized here. IM=intramyocardial, IC=intracoronary, RCV=retrograde coronary venous, IV=intravenous, ILV=intra LV cavity.

## Supplemental References:


**1.** Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, Giacomello A, Abraham MR, Marban E. Regenerative Potential of Cardiosphere-Derived Cells Expanded From Percutaneous Endomyocardial Biopsy Specimens. *Circulation.* 2007;115:896-908.

**2.** Hochman JS, Choo H. Limitation of myocardial infarct expansion by reperfusion independent of myocardial salvage. *Circulation.* 1987;75:299-306.

**3.** Tang X-L, Rokosh G, Sanganalmath SK, Yuan F, Sato H, Mu J, Dai S, Li C, Chen N, Peng Y, Dawn B, Hunt G, Leri A, Kajstura J, Tiwari S, Shirk G, Anversa P, Bolli R. Intracoronary Administration of Cardiac Progenitor Cells Alleviates Left Ventricular Dysfunction in Rats With a 30-Day-Old Infarction. *Circulation.*121:293-305.

**4.** Teng CJ, Luo J, Chiu RCJ, Shum-Tim D. Massive mechanical loss of microspheres with direct intramyocardial injection in the beating heart: Implications for cellular cardiomyoplasty. *The Journal of Thoracic and Cardiovascular Surgery.* 2006;132:628-632.

**5.** Terrovitis J, Bonios M, Fox J, Engles JM, Yu J, Leppo MK, Pomper MG, Wahl RL, Seidel J, Tsui BM, Bengel FM, Abraham R, Marban E. Noninvasive Quantification and Optimization of Acute Cell Retention by In Vivo Positron Emission Tomography After Intramyocardial Cardiac-Derived Stem Cell Delivery. *Journal of the American College of Cardiology.* 2009;54:1619-1626.

**6.** Didier B, Michel T, Guy B, Micheline L, Philippe U, Nicolas P, Eric S, Dominique E, Jean-Paul D, Michel G, Serge G. Myocardial Homing of Nonmobilized Peripheral-Blood CD34+ Cells After Intracoronary Injection. *Stem Cells.* 2006;24:333-336.

**7.** Hou D, Youssef EA-S, Brinton TJ, Zhang P, Rogers P, Price ET, Yeung AC, Johnstone BH, Yock PG, March KL. Radiolabeled Cell Distribution After Intramyocardial, Intracoronary, and Interstitial Retrograde Coronary Venous Delivery: Implications for Current Clinical Trials. *Circulation.* 2005;112:I-150-156.

**8.** Hofmann M, Wollert KC, Meyer GP, Menke A, Arseniev L, Hertenstein B, Ganser A, Knapp WH, Drexler H. Monitoring of Bone Marrow Cell Homing Into the Infarcted Human Myocardium. *Circulation.* 2005;111:2198-2202.

**9.** Aicher A, Brenner W, Zuhayra M, Badorff C, Massoudi S, Assmus B, Eckey T, Henze E, Zeiher AM, Dimmeler S. Assessment of the Tissue Distribution of Transplanted Human Endothelial Progenitor Cells by Radioactive Labeling. *Circulation.* 2003;107:2134-2139.

**10.** Johnston PV, Sasano T, Mills K, Evers R, Lee S-T, Smith RR, Lardo AC, Lai S, Steenbergen C, Gerstenblith G, Lange R, Marban E. Engraftment, Differentiation, and Functional Benefits of Autologous Cardiosphere-Derived Cells in Porcine Ischemic Cardiomyopathy. *Circulation.* 2009;120:1075-1083.

**11.** Lee S-T, White AJ, Matsushita S, Li T-S, Malliaras K, Chowdhury S, Glenn A, Zeng H, Zhang Y, Terrovitis I, Simsir S, Makkar R, Marban E. Abstract 3976: Direct Intramyocardial Injection of Autologous Cardiosphere-Derived Cells

Yields Consistent Engraftment and Improves Cardiac Function in a Porcine Model of Ischemic Cardiomyopathy. *Circulation.* 2009;120:S898-b-.

## Legends for the Supplemental Video Files

**Online Movie I.** Representative video capturing cell injection procedure without magnetic targeting (Fe-CDC). The yellow-brown cells wash out quickly after injection.

**Online Movie II.** Representative video capturing cell injection procedure with magnetic targeting (Fe-CDC + Magnet). The yellow-brown cells are drawn towards the apex and persist in the heart after injection.