Supplemental Material.

DETAILED METHODS.

Mice. The *cKit*$^{CreERT2/+}$ and *IRG*, mice that were employed in this study, have been previously described [1].

Tamoxifen-induced recombination. To induce *CreER*^{T2}-mediated recombination in *cKit*^{CreERT2}*;IRG* neonates, tamoxifen (Sigma) was dissolved in peanut oil (Sigma) at a final concentration of 20mg/ml by shaking overnight at 37°C, and was administered via single subcutaneous injections (50µl/injection) at selected time points. For inducing *CreER*^{T2}-mediated recombination *in-vitro*, the active metabolite of tamoxifen, (Z)-4-hydroxytamoxifen (4-OH tamoxifen; Sigma), was dissolved in 100% ethanol (Sigma) and used at a final concentration of 1µM, supplemented in the culture medium.

Porcine MSC isolation, expansion and inactivation. Swine MSCs were isolated and expanded from a single, healthy Yorkshire donor as previously described (*7, 44*). Briefly, bone marrow was obtained from the iliac crest, and aspirates were passed through a density gradient to eliminate undesired cell types and were plated with 25ml MEM Alpha media (Mediatech, Manassas, VA) containing 20% fetal Bovine Serum (Hyclone) in 162cm^2 culture flasks (Fisher Scientific, Pittsburgh, PA). At 5-7 days after plating, non-adherent cells were washed away during medium changes and the remaining, plastic adherent, purified MSC population was expanded in culture. All used cells were harvested when they reached 80–90% confluence at passage 3-4, and inactivated for 3h at 37°C with 10µg/ml of mitomycin-C (Sigma). Inactivated porcine MSCs were collected by trypsinization and cryopreserved in liquid nitrogen until use. For all experiments, MSCs were seeded at a concentration of 2x10^5 cells/ml a day before use.

E*x-vivo* analysis of mouse CSCs. Three-day old neonatal mouse hearts (PN3) were harvested, washed in ice-cold HBSS and cleaned from unwanted tissues under a stereomicroscope (VistaVision). They were then transferred in a tissue-cultured hood and, after additional washing

steps with DMEM (GIBCO) under sterile conditions, were minced into ~2-3mm^3 fragments and digested in a solution of DMEM/F12 (GIBCO), 20% FBS, 1% penicillin/streptomycin and 200 units/ml Collagenase-Type II solution (Worthington) at 37°C. Digested tissue explants were then collected and washed twice with DMEM to remove residual enzyme. Single tissue fragments were then hand-picked under sterile conditions with a stereomicroscope and a micropipette, and cultured individually in each well of gelatin-coated 24-well plates, with or without 1x10^5 MSCs. Samples were fed every other day. The basic myocardial explant feeding medium consisted of DMEM/F12, 15% FBS (Atlas), 1% penicillin/streptomycin (GIBCO), 1% β-mercaptoethanol (GIBCO), 1000units/ml recombinant mouse LIF (Millipore), 1ng/ml recombinant mouse bFGF (Peprotech), and 0.1mM nonessential aminoacids (GIBCO). The impact of SCF was tested by supplementing the medium with 100ng/ml recombinant murine SCF (Peprotech). For cKit neutralization, 100ng/ml of rat anti-murine cKit antibody (eBioscience, clone 2B8) were supplemented to the medium. For assessing the role of SDF1/CXCR4, AMD3100 (Sigma) was supplemented to the medium, at a final concentration of 1μM. Samples were then monitored daily, and growth of EGFP$^+$ cells was quantified every other day for 5-8 consecutive days based on EGFP epifluorescence, under a fluorescent microscope (Olympus IX81). Analysis of *Cre*-recombination efficiency was performed using flow cytometric analysis of EGFP epifluorescence and cKit immunofluorescence co-localization (anti-cKit APC, eBiosciences) in a BD LSR-II analyzer. To explore whether DSRED$^+$ cardiac explant derived cells correspond to CSCs or could activate expression of cKit in culture, DSRED$^+$ cells were manually picked from 1-week old, 4-OH treated cardiac explant cultures under a fluorescence microscope (EVOS), and propagated in Geltrex-coated dishes (Invitrogen) for 50 days, in the presence of 4-OH tamoxifen.

Generation of CSCs from iPSC^{kit}. The generation of iPSC^{kit} lines has been previously described [1]. Differentiation into CSCs was performed as previously described [1]. Briefly, iPSC^{kit} were differentiated into embryoid bodies (EBs), via transient antagonism of the bone morphogenetic protein pathway (BMP) with Noggin (R&D systems) or the small molecule dorsomorphin (Tocris).

4-OH tamoxifen was supplemented in the medium from EB-Day 10 onwards, in order to monitor the induction of $EGFP^+$ CSCs. Successful differentiation of $iPSC^{kit}$ into CSCs was monitored with gene-expression analysis as well as the development of spontaneously contracting EGFP+ embryoid bodies after EB-Day 10.

Human CSCs and MSCs. All human cells were manufactured by the Foundation for Accreditation of Cellular Therapy (FACT)-accredited Good Manufacturing Practice (GMP) Cell Production Facility at the Interdisciplinary Stem Cell Institute, University of Miami Miller School of Medicine, as previously described[2]. MSCs were derived from the bone marrow (iliac crest aspiration) of three healthy donors. CSCs were isolated from single,1-2 mm endomyocardial biopsies, obtained from the septal wall of the right ventricle, as previously described [3, 4]. Briefly, for CSC extraction, biopsies were grown in Ham's F12 medium (Life Technologies, Grand Island, NY) containing 10% fetal bovine serum (FBS), 0.2mM L-glutathione, 5 mU/mL erythropoietin, 10 ng/mL human fibroblast growth factor (Recombinant Human FGF-basic PeproTech), and 1% glutamine/penicillin/streptomycin (GPS; Life Technologies). CSCs that migrated out of biopsies were isolated using the Human CD117 Microbeads kit (Miltenyi cat#130-091-332) and processed through the MACS separation system (Miltenyi). MSCs were released for further studies after meeting the following criteria: negative for mycoplasma via polymerase chain reaction, ≥70% cell viability, growth assay via colony forming unit-fibroblasts assay, positive for CD105 (>80%) and negative for CD45 by flow cytometry, and no growth of bacteria. CSCs were released for further studies after meeting the following criteria: negative for mycoplasma via polymerase chain reaction, ≥70% cell viability, growth assay via colony forming unit-fibroblasts assay, positive for CD117 (>80%) and negative for CD45 by flow cytometry, and no growth of bacteria. The following antibodies were used for flow cytometry: IgG2bk-APC Isotype control (e-Biosciences 174031-82); cKit APC (e-Biosciences 17-1179-42); IgG1 FITC Isotype control (Beckman C, IM0639U); CD45 FITC (Beckman, IM0782U); CD105 PE (Fitzgerald, 61R-cd105cHUPE).

Migration Assays. For the trans-well migration assays, $1x10^5$ cryopreserved hCSCs were suspended in cell migration medium (DMEM (GIBCO), 1% glutamine/penicillin/streptomycin (GPS; Life Technologies), and 0.1% bovine serum albumin fraction V (ThermoFisher Scientific) and plated in triplicates on the upper well of a modified Boyden chamber with 8μm pores (Corning), in 24 well plates (Corning). For testing the MSCs chemotaxis, $1x10^5$ cryopreserved hMSCs were re-suspended in cell migration medium and plated in triplicates in the 24-well plates, one day before thawing the CSCs. Treatment with AMD 3100was performed as described above. All samples were cultured under 5% CO^2 atmosphere at 37°C, either in 20% O_2 or in a hypoxia chamber at 0.5% O_2 (Coy Labs). For quantification of cell migration, cells at the upper chamber of the Boyden chamber were wiped with cotton swab 24h after plating. The lower chamber of the transwell insert was fixed for 10min with 70% ethanol and the migrated cells were visualized with crystal violet (Sigma) and manually quantified in an inverted light microscope (Zeiss) using a hemocytometer.

Immunohistochemistry and confocal microscopy. Samples were fixed for 1-1.5 h in 4% PFA (EMS) at room temperature followed by overnight incubation in 30% sucrose (Calbiochem) at 4°C. Next day, samples were embedded in OCT (EMS) and flash-frozen in liquid nitrogen. Cryosectioning was performed as previously described (*46*). For immunohistochemistry, 10μm-thick samples were post-fixed for 10min with 4% PFA and blocked for 1h at RT with 10% normal donkey serum (Chemicon), followed by overnight incubation at 4°C with the primary antibody. Subsequently, samples were visualized by incubating the sections for 1h at 37°C with FITC, Cy3 and Cy5- conjugated $F(ab')_2$ fragments of affinity-purified secondary antibodies (Jackson Immunoresearch) or Alexa 488 and Alexa 546 dyes (Molecular probes). The primary antibodies used were EGFP (1:500 dilution, Aves GFP-1020), Nkx2.5 (1:50 dilution, Santa Cruz Biotechnologies, SC-14033 and SC-8697), CD68 (1:100 dilution, Santa Cruz Biotechnologies, SC-9139), CD45 (1:100 dilution, Abcam, ab10558), Cardiac Troponin I (1:100, Abcam, ab10231) and Tropomyosin (1:100 dilution, Abcam ab7786). Samples were counterstained with DAPI,

mounted with ProLong Antifade Gold reagent (Molecular Probes,) and stored at 4°C until further examination. Microscopic evaluations and image acquisitions were performed with a Zeiss LSM-710 Confocal Microscope (Carl Zeiss MicroImaging, Inc. Thornwood, NY), using the Zeiss ZEN software (version 2009, Carl Zeiss Imaging Solutions, GmbH); or with an Olympus IX81 fluorescent microscope using the Image Pro Premier software (Version 9.04; 64-bit; Media Cybernetics).

Gene-expression analysis. Total RNA was extracted from *iPSC^{kit}* at selected time points before and during the course of their differentiation into cardiomyocytes, using the RNeasy mini plus kit, according to manufacturers' instructions (Qiagen). Complementary DNA synthesis was performed using the high capacity cDNA reverse transcription kit, according to manufacturers' instructions (Applied Biosystems). Quantitative PCR was performed using Taqman Universal Master mix in a iQ5 real time PCR detection system (BioRad). All samples were run in triplicates and normalized to a GAPDH endogenous control. Relative fold change was calculated using the $2^{-\Delta\Delta Ct}$ method. The IDs for the Taqman Gene-expression assays are the following: SDF1α: Hs03676656_mH; CXCR4: Hs00607978_s1; GAPDH: Hs02758991_g1.

CXCR4 activity assays.

We employed a Chem-1 cell line, transduced to overexpress human CXCR4 and the G protein Gα15 (Millipore, HTS004RTA), in which the effects of CXCR4 modulators can be genetically tracked via fluorescence-based assays of intracellular Ca2+ mobilization. The CXCR4-Chem1 cell line was loaded with a Fluo-8 Ca2+ dye (Abcam, ab112129), and dose-dependent responses of intracellular Ca^{2+} mobilization to recombinant human SDF1α (Thermo Scientific), AMD3100 (Abcam), or human MSC were assessed in a 96-well plate format, with a microplate reader (Molecular Devices, Spectramax, M5), according to manufacturer's instructions. For the condition medium, $1x10^6$ hMSCs per well were plated in 6-well plates with regular MSC medium. The next day, the medium was replaced with 2ml of serum-free CXCR4-Chem1 cell medium (Millipore),

and returned to the incubator for 24h. For the 96-well assay, 20µl of CXCR4-Chem1 hMSC-conditioned medium were used per well.

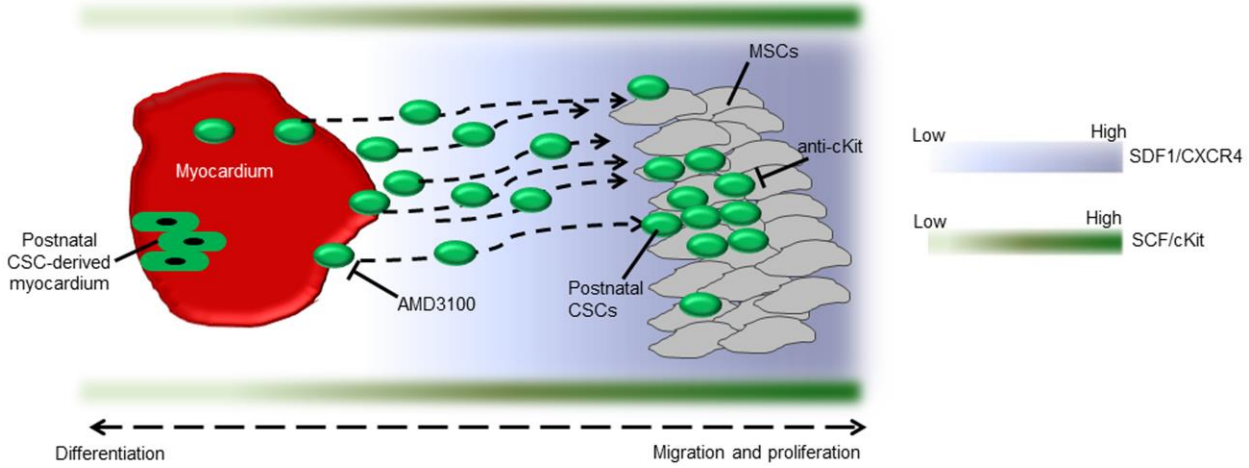
Statistics.

Statistical analyses were performed using GraphPad Prism version 5.00 for Windows. A two-way repeated measures ANOVA, followed by Bonferroni post-hoc tests, was employed for comparing *in-vitro* quantification data of $EGFP^+$ cells. A one-way ANOVA, followed by Tukey's post-hoc tests, was performed to compare migration of human cells and gene-expression analysis. All data met the assumptions of the tests. To analyze the effect of AMD3100 in cardiomyocyte differentiation of CSCs, a Mann-Whitney test was employed to compare differences between groups. This test was chosen due to a non-normal distribution of the values and significant differences in the variation within groups (Bartlett's test). A $p<0.05$ was considered statistically significant. All values are reported as mean±SEM.

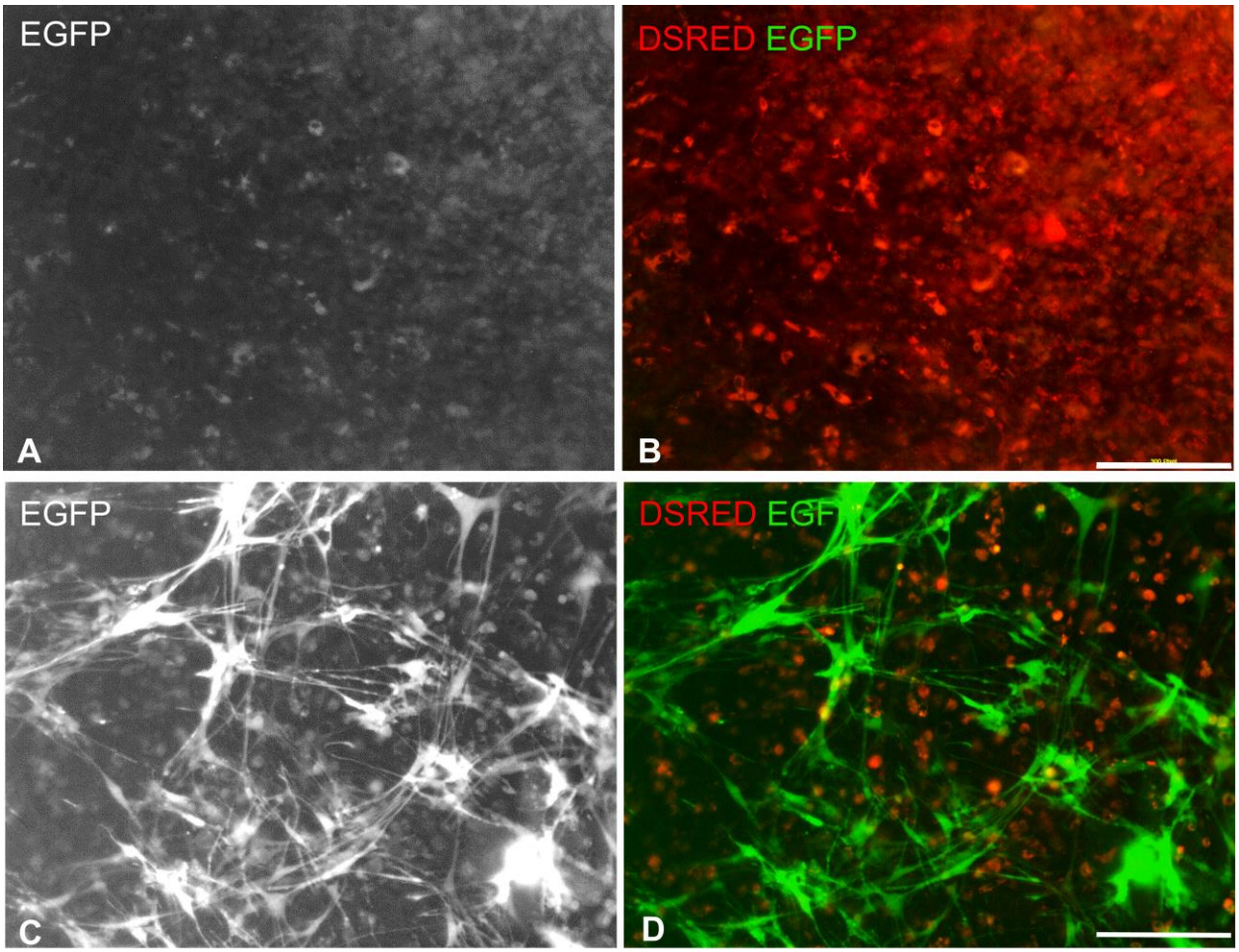
Study Approval.

The heart failure patients and healthy MSC subjects recruited for this study are enrolled in POSEIDON-DCM (NCT01392625), "A Phase I/II, Randomized Pilot Study of the Comparative Safety and Efficacy of Transendocardial Injection of Autologous Mesenchymal Stem Cells Versus Allogeneic Mesenchymal Stem Cells in Patients with Nonischemic Dilated Cardiomyopathy". This study had institutional review board approval from the University of Miami Miller School of Medicine, and all patients gave written informed consent prior to inclusion in the study [2]. The animal studies were reviewed and approved by the University of Miami Institutional Animal Care and Use Committee and complies with all Federal and State guidelines concerning the use of animals in research and teaching as defined by *The Guide for the Care and use of Laboratory Animals* (National Institutes of Health, revised 2011).

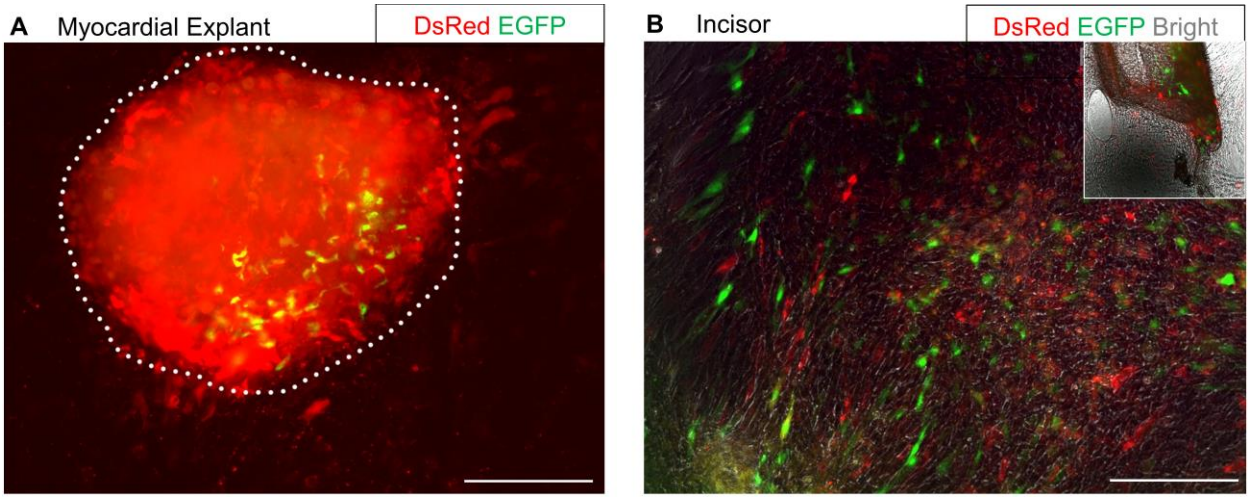
SUPPLEMENTAL FIGURES AND FIGURE LEGENDS:



Online Figure I. **Summary Figure**. Lineage fate-mapping analysis illustrates that postnatal CSCs (green, oval), are a proliferative and migratory cell population that generate de-novo cardiomyocytes (green, rounded rectangle) in the neonatal mouse heart (red). Cell-cell interactions with MSCs (grey) enhance proliferation and migration of CSCs, via the SCF/cKit (green gradient) and SDF1/CXCR4 (blue gradient) signaling pathways. Blocking the SDF1/CXCR4 pathway by AMD3100, inhibits proliferation and migration of CSCs, while inducing their differentiation into cardiomyocytes.



Online Figure II. Explant-derived cells are not CSCs. **A-B,** Live-cell epifluorescence imaging of DSRED and EGFP expression in DSRED+ explant-derived cells, manually-picked from *cKit*$^{CreERT2/+}$*;IRG* heart explant cultures, and propagated for 50 days in the presence of 4-OH tamoxifen. DSRED+ cells do not acquire expression of EGFP with prolonged culture indicating that they are not CSCs. **C-D**, Live-cell epifluorescence imaging of DSRED and EGFP expression in EGFP^{+} explant-derived cells, manually-picked from the same *cKit*$^{CreERT2/+}$*;IRG* heart explant culture that cells in panels A-F were derived, and propagated for 50 days in the presence of 4-OH tamoxifen. Scale bars, 200μm.

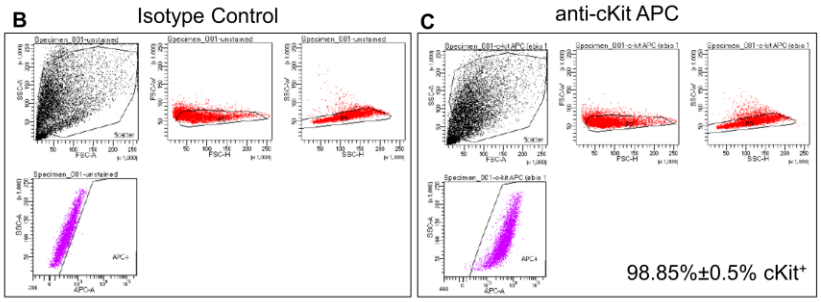


Online Figure III. Migratory capacity of cardiac- and dental- explant-derived cells. **A**, Live-tissue epifluorescence of an explanted myocardial sample, 4 days after plating on a gelatin-coated vessel. Cell outgrowth from myocardial explants is robust. However, expression of EGFP is confined in cells within the explant tissue borders. **B,** Live-tissue epifluorescence of an explanted developing incisor. In contrast to the myocardial explant, dental explant-derived $EGFP^{+}$ cells hold full capacity to migrate along with $DSRED^{+}$ cells, suggesting that the lack of migratory activity of cardiac $EGFP^{+}$ cells is unlikely to be due to the hypomorphic *Kit-CreERT2/+* knocked-in allele. Inset depicts a low-power, magnification, live-tissue epifluorescent image of the incisor. Scale bars, 200μm.

A

Flow cytometric analysis of human MSCs

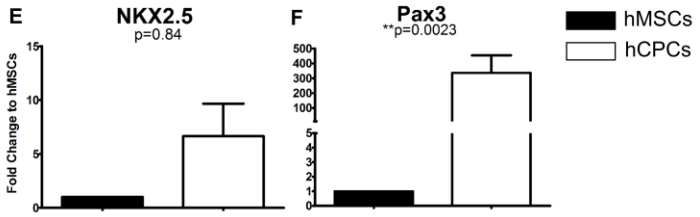
Sample ID	Passage #	% CD105⁺	% CD45⁺
MSC009	P2	100.00%	1.00%
MSC010	P2	99.30%	0.80%
MSC012	P2	99.70%	0.37%



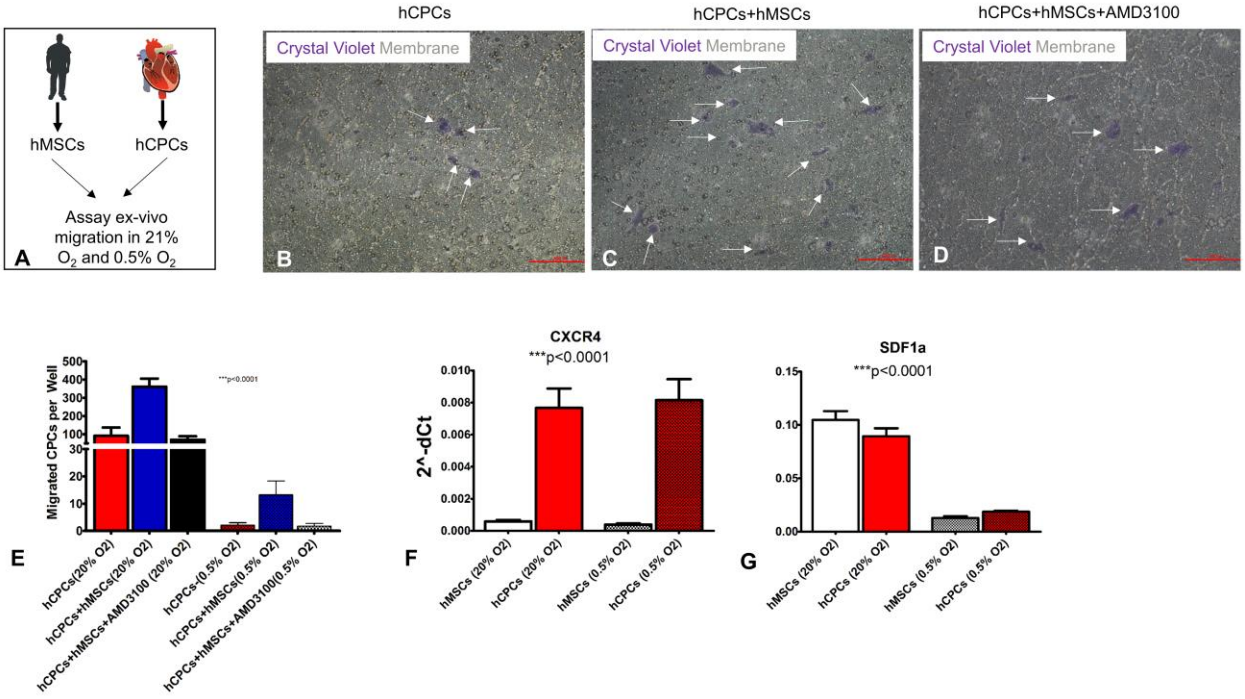
D

Flow cytometric analysis of human CPCs

Sample ID	Passage #	% cKit⁺	% CD45⁺
DCM-033	P3	99.70%	1.08%
DCM-036	P3	99.50%	0.09%
DCM-037	P3	98.90%	0.19%



Online Figure IV. Characterization of hMSCs and hCSCs. A, Flow cytometric analysis of CD105 and CD45 expression in the 3 hMSCs lines. B**-C,** Representative **examples** of cKit expression in culture-expanded CSCs. **D**, Flow cytometric analysis of cKit and CD45 expression in the 3 hCSCs lines **E,** Gene-expression analysis of Nkx2.5 in hCSCs and hMSCs. **F,** Gene-expression analysis of Pax3 in hCSCs and hMSCs.



Online Figure 5. Human MSCs stimulate CSCs from patients with dilated cardiomyopathy. A-C, Fluorescent immunocolocalization of EGFP (A) and Nkx2.5 (B) in cardiac explant derived cells. Panels D-F are blown-up images of the areas delineated with insets in panels A-C, respectively. Scale bars, 200μm.

Supplemental References.

1. Hatzistergos KE, Takeuchi LM, Saur D, Seidler B, Dymecki SM, Mai JJ, White IA, Balkan W, Kanashiro-Takeuchi RM, Schally AV and Hare JM. cKit+ cardiac progenitors of neural crest origin. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;112:13051-6.

2. Premer C, Blum A, Bellio MA, Schulman IH, Hurwitz BE, Parker M, Dermarkarian CR, DiFede DL, Balkan W, Khan A and Hare JM. Allogeneic Mesenchymal Stem Cells Restore Endothelial Function in Heart Failure by Stimulating Endothelial Progenitor Cells. *EBioMedicine*. 2015;2:467-75.

3. Williams AR, Hatzistergos KE, Addicott B, McCall F, Carvalho D, Suncion V, Morales AR, Da Silva J, Sussman MA and Heldman AW. Enhanced Effect of Combining Human Cardiac Stem Cells and Bone Marrow Mesenchymal Stem Cells to Reduce Infarct Size and to Restore Cardiac Function After Myocardial InfarctionClinical Perspective. *Circulation*. 2013;127:213-223.

4. Karantalis V, Suncion-Loescher VY, Bagno L, Golpanian S, Wolf A, Sanina C, Premer C, Kanelidis AJ, McCall F, Wang B, Balkan W, Rodriguez J, Rosado M, Morales A, Hatzistergos K, Natsumeda M, Margitich I, Schulman IH, Gomes SA, Mushtaq M, DiFede DL, Fishman JE, Pattany P, Zambrano JP, Heldman AW and Hare JM. Synergistic Effects of Combined Cell Therapy for Chronic Ischemic Cardiomyopathy. *J Am Coll Cardiol*. 2015;66:1990-9.

Supplemental Legends for Video Files.

Online Video I. Explant culture of *cKit^{CreERT2};IRG* neonatal mouse heart. EGFP epifluorescence in a myocardial explant from a tamoxifen-pulsed PN3 *cKit^{CreERT2};IRG* mouse heart. EGFP becomes detectable within 48h after culture. EGFP^{+} CSCs are primarily located within the spontaneously beating myocardial explant, whereas cells outgrowing from the tissue are exclusively non-contractile DSRED^{+} cells.

Online Video II. Modeling CSCs migration and differentiation in iPSCs. Live epifluorescent imaging illustrating that the development of spontaneously contracting EGFP^{+} clusters of iPSC^{kit}-derived CSCs involves their outgrowth from the embryoid bodies. Note that the EGFP^{+} cells retained within the embryoid body (bottom right corner) are not contractile.

Online Video III. AMD3100 induces differentiation of CSCs. Spontaneously contracting EGFP^{+} cells in a PN3 *cKit^{CreERT2};IRG* myocardial explant, four days after culturing in the presence of AMD3100. The EGFP^{+} cell in green inset depicted at higher magnification in the right panel.

Online Video IV. De-novo postnatal cardiomyogenesis from CSCs in the right ventricular myocardium. Live-tissue epifluorescent imaging in the right ventricle of a PN7 spontaneously beating *cKit^{CreERT2};IRG* mouse heart illustrates contribution of postnatal CSCs in spontaneously beating, right-ventricular EGFP^{+} cardiomyocytes.

Online Video V. De-novo postnatal cardiomyogenesis from CSCs in the left ventricular myocardium. Live-tissue epifluorescent imaging in the left ventricle of a PN7 spontaneously beating *cKit^{CreERT2};IRG* mouse heart illustrates contribution of postnatal CSCs in spontaneously beating, left-ventricular EGFP^{+} cardiomyocytes.

Online Video VI. De-novo postnatal cardiomyogenesis from CSCs in the cardiac apex. Live-tissue epifluorescent imaging in the apex of a PN7 spontaneously beating *cKit^{CreERT2};IRG* mouse heart illustrates contribution of postnatal CSCs in spontaneously beating, EGFP^{+} cardiomyocytes.

Online Video VII. In-vivo cardiomyocyte differentiation of neonatal CSCs. Confocal z-stack imaging illustrates cardiomyocyte differentiation of postnatal CSCs within the neonatal mouse heart, as indicated by the co-localization of EGFP (green) with Tropomyosin (red). DAPI is depicted in white.