Supplemental Material

Materials and Methods

Animals and Anesthesia

All animal procedures were approved by the Temple University School of Medicine Institutional Animal Care and Use Committee. For all procedures (bone isolation, myocardial infarction, echocardiography and cardiectomy) anesthesia was induced using 3% isoflurane and maintained using 1% isoflurane (Butler Shein Animal Health; Dublin, Ohio). Adequate induction of anesthesia was confirmed prior to any intervention by observation of a negative paw- or tail-pinch reflex. For procedures involving a thoracotomy (i.e. myocardial infarction surgeries) animals were intubated after induction of anesthesia and ventilated at a rate of 180-190 breaths per minute and a tidal volume of 250-300 μL (ventilation parameters were adjusted accordingly depending on the size of each animal). For all other procedures, maintenance anesthesia was delivered via nose cone. All animals used for *in vivo* studies were 12-week-old male C57BL/6 mice (The Jackson Laboratory; Bar Harbor, ME).

Isolation and culture of cortical bone-derived stem cells

Cortical bone stem cells (CBSCs) were isolated using previously published techniques.[1] Femurs and tibias were isolated from transgenic 12-week-old male C57BL/6-Tg(CAG-EGFP)1Osb/J mice (The Jackson Laboratory; Bar Harbor, ME), which constitutively express enhanced green fluorescent protein (EGFP) off of the β-actin promoter in most cells of the body. The epiphyses of the bones were removed, and the marrow cavity was flushed three times with phosphate-buffered saline (PBS) and the marrow was discarded. The remaining cortical bone was crushed using a sterilized mortar and pestle, and bone fragments were further digested using collagenase II. Bone chunks were then plated in CBSC culture media: DMEM/F12 Media (Lonza/Biowhittaker; Basel, Switzerland) + 10% fetal bovine serum (Gibco Life Technologies; Grand Island, NY), 1% Penicillin/Streptomycin/L-glutamine (Gibco Life Technologies; Grand Island, NY), 0.2% insulin-transferrin-selenium (Lonza; Basel, Switzerland), 0.02% basic-fibroblast growth factor (Peprotech; Rock Hill, NJ), 0.02% epidermal growth factor (Sigma; St. Louis, MO), and 0.01% leukemia inhibitory factor (Millipore; Billerica, MA). Over the first week in culture, fibroblast-like stem cells began to grow out from the bone chunks, and after 1 week in culture, the remaining chunks of bone were washed away and the adherent population of CBSCs could be passaged for expansion. Expanded cells could be resuspended in CBSC culture media + 10% DMSO to be frozen and stored long-term in liquid nitrogen.

Isolation of cardiac-derived stem cells

Cardiectomy was performed on transgenic 12 week-old male C57BL/6-Tg(CAG-EGFP)1Osb/J mice (The Jackson Laboratory; Bar Harbor, ME) under general anesthesia. Hearts were cannulated and perfusion digested to dissociate stem cells from the left ventricle, and cardiac-derived stem cells (CDCs) underwent sorting for c-kit using magnetic beads (Miltenyi Biotec; Cologne, Germany) following a previously described protocol.[2, 3] Cells were cultured and stored long term under identical conditions to CBSCs as previously described.

Isolation and Culture of Mouse Left Ventricular Myocytes

Left ventricular myocytes were isolated from mice receiving MI and CBSC therapy 6 weeks after injection for myocyte staining and cell physiology experiments. Cardiectomy was performed under general anesthesia then hearts were cannulated and perfusion digested with collagenase-containing Tyrodes solution on a constant-flow Langendorff apparatus, and left ventricular myocytes were isolated

and cultured as previously described.[4, 5] All myocytes isolated from the left ventricle of each MI+CBSC mouse were plated on laminin-coated 18mm round glass coverslips. Some coverslips were used to measure fractional shortening and calcium transients, others were fixed and immunostained for cell counts, surface area analysis, and nuclei counts.

To estimate the percentage of all LV myocytes that were EGFP+ (and thus derived from injected CBSCs), an average number of 15.67±3.67 EGFP+ myocytes were counted on each coverslip. The total number of myocytes on each coverslip was estimated by counting the myocytes in ten random 10X visual fields, and an average of 12.4±1.51 total myocytes/field were counted. A 10X visual field has a surface area of 1.30^2 mm^2, and an 18mm coverslip has a surface area of 81π mm^2, so there are $81\pi/1.30^2 = 150.18$ 10X visual fields/coverslip. Thus there were an average of 12.4 * 150.18 = 1862.26 total myocytes/coverslip. So by 6 weeks post-MI, an estimated 15.67/1862.26 = 0.84% of myocytes isolated from CBSC-injected animals were EGFP+.

Fractional Shortening and Calcium Transients

Mouse left ventricular myocytes isolated from CBSC-injected animals 6 weeks post-MI were simultaneously measured for fractional shortening and calcium transients as has been previously described.[4, 5]

Flow Cytometry

For flow cytometry, $5x10^6$ CBSCs or CDCs were incubated for 15 minutes at 4°C under gentle agitation in the appropriate antibody diluted 1:11 in wash buffer (PBS+ 0.5% bovine serum albumin + 2 mM EDTA, pH = 7.3). After incubation, cells were washed with 2 mL wash buffer, centrifuged at 300 Xg for 5 min, and supernatants were aspirated and discarded. Stained cells were resuspended in PBS for flow cytometry. A second sample of CBSCs or CDCs was stained in each condition with APC-conjugated Rat IgG2b, which was used as a negative isotype control. The following conjugated antibodies or pairs of primary and secondary antibodies along were their corresponding nonspecific negative isotype controls were used:

Marker	**Primary Antibody**	**Negative Isotype Control**
Sca-1	Rat IgG2a anti-Sca-1-APC (Miltenyi Biotec; Cologne, Germany)	Rat IgG2a-APC Invitrogen; Cambridge, MA
CD29	Rat IgG anti-CD29-APC (eBiosciences; San Diego, CA)	Rat IgG-APC (eBiosciences; San Diego, CA)
CD34	Rat IgG2a anti-CD34-Alexa Fluor 647 (AbD Serotec; Kidlington, UK)	Rat IgG2a-APC (AbD Serotec; Kidlington, UK)
CD45	Rat IgG2b anti-CD45-Alexa Fluor 647 (AbD Serotec; Kidlington, UK)	Rat IgG2b-APC (AbD Serotec; Kidlington, UK)
C-kit	1° goat IgG anti-SCF-R (R&D Systems; Minneapolis, MN) 2° anti-Goat IgG Rhodamine Red-X (Jackson ImmunoResearch Labs; West Grove, PA)	1° goat IgG (R&D Systems; Minneapolis, MN) 2° anti-Goat IgG Rhodamine Red-X (Jackson ImmunoResearch Labs; West Grove, PA)
Lineage Cocktail	1° Biotin-Conjugated Lineage Cocktail (Miltenyi Biotec; Cologne, Germany) 2° anti-Biotin-APC (Miltenyi Biotec; Cologne, Germany)	2° anti-Biotin-APC only (Miltenyi Biotec; Cologne, Germany)

RNA Isolation and PCR Analysis

CDCs or CBSCs were resuspended in QIAzol Lysis Reagent, and mRNA was isolated using an RNeasy Mini Kit. DNA was eliminated from the samples using RNase-free DNase I, and then cDNA was generated using RT^2 First Strand Kit. RT^2 qPCR Primer Assays for mouse Kit (c-kit), mouse Ly-6A (Sca-1), and mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used with RT^2 SYBR Green qPCR mastermix to detect c-kit, Sca-1, or GAPDH mRNA expression, respectively. The amount of GAPDH mRNA in each cell type was determined using a 6-point standard curve with a 1:10 serial dilution of each transcript run on each primer set. The amount of transcript detected for c-kit or Sca-1 was normalized to detected levels of GAPDH and these data are presented as normalized arbitrary units. All qPCR reagents were purchased from Qiagen (Valencia, CA).

Protein Isolation and Western Analysis

CBSC or CDC lysates were prepared and analyzed using Western analysis as previously described.[5,6] The following primary antibodies purchased from Abcam (Cambridge, MA), Cell Signaling (Danvers, MA) or AbD Serotec (Kidlington, UK) were used to detect target antigens: insulin-like growth factor-1 (Abcam ab106836), angiopoietin-1 (Abcam ab95230), basic-fibroblast growth factor (Abcam, ab8880), hepatocyte growth factor (Abcam ab83760), platelet-derived growth factor (Abcam ab125268), stem cell factor (Abcam ab9753), stromal-derived factor-1 (Cell Signaling #3740S), vascular endothelial growth factor (Abcam ab46154) and Glyceraldehyde 3-phosphate dehydrogenase (AbD Serotec) . The following secondary antibodies were used: rabbit-HRP (GE#NA934V) and mouse-HRP (GE#NA931V) purchased from GE Healthcare (Little Chalfont, UK) and goat-HRP (sc-2020) purchased from Santa Cruz Biotechnology (Dallas, TX).

Enzyme-Linked Immunosorbent Assays

CBSCs or CDCs were plated at a low density of 25,000 cells/well in complete CBSC media or serum free media in a 6 well plate and allowed to proliferate to 90% confluency over 72 hours. Serum samples were collected every 24 hours and frozen at -20°C. Samples were analyzed using mouse DuoSet ELISA Kits for hepatocyte growth factor, insulin-like growth factor, stem cell factor, stromal-derived factor-1 and vascular endothelial growth factor. The presence of serum in the cell cultures did not affect cytokine production. All ELISA kits were purchased from R&D Systems (Minneapolis, MN). The data in each case is presented as a mean of 3 samples, and for each ELISA, background signal was subtracted using the mean of 3 samples containing unconditioned media only. A student's T test was used to detect any significant difference in production of each paracrine factor between CBSCs and CDCs.

In vitro Differentiation Co-cultures

Neonatal rat ventricular myocytes were isolated following the Simpson and Savion protocol[7] with minor modifications that we have previously described.[8,9] Cells were plated overnight on gelatin-coated 18mm glass coverslips in a 12-well dish. Stem cells were added the following day at low densities (1000-5000 cells/well) on top of the neonatal rat myocytes. Cells were allowed to differentiate in co-culture for 72 hours. During this time some EGFP+ cells were observed to beat. After 72 hours, coverslips were fixed and stained for α-sarcomeric actin or connexin43, and the percentage of cells expressing either marker was determined after counting cells in 10 visual fields on 5 individual coverslips

Mouse Myocardial Infarction and Intramyocardial Stem Cell Transplantation

Permanent occlusion myocardial infarction (MI) surgery was performed by ligating the left anterior descending coronary artery following a widely cited protocol.[10] Immediately after MI, 40,000

CBSCs (n=67) or CDCs (n=36) suspended in normal saline were injected intramyocardially into the infarct border zone in four x 5 uL injections. MI control animals (n=60) received saline injection only, and sham control animals (n=21) received all surgical procedures except for ligation of the coronary artery and intramyocardial injection.

Each group of animals that underwent surgery was assigned a sacrifice date prior to surgery. This prevented selection bias at the time of sacrifice, so our Kaplan-Meier analysis was designed to measure 6-week survival from the time of myocardial infarction surgery. We designed our Kaplan-Meier analysis to detect "death" as event occurrence (if the animal died prior to its assigned sacrifice date). Animals that survived until their assigned sacrifice date (either at 1, 2, or 6 weeks post-MI, depending on the group) were censored in the Kaplan-Meier analysis.

Animals selected for 24-hour sacrifice were not included in Kaplan-Meier analysis. For the MI+Saline group, 5 were sacrificed at 24 hours post-MI for infarct size analysis. For the MI+CDC or MI+CBSC groups, a total of 10 animals were sacrificed at 24 hours post-MI (5 animals for infarct size analysis and 5 animals for histology). Animals assigned to 6-week sacrifice groups underwent serial echocardiography and strain analysis using the Vevo2100 ultrasound system and these data are presented in Figures 3 and 4.

Sham (n=21): 5 animals were sham operated for 1 week sacrifice, 5 animals were operated for 2 week sacrifice, and 11 animals were operated for 6 week sacrifice for a total of 21 animals. No animals in the sham-operated group died prior to their assigned sacrifice date (100% 6 week survival). All hearts were fixed for histology at each time point.

MI+Saline (n=60): 5 animals were sacrificed 24 hours post-MI for infarct size analysis and were not included in the Kaplan-Meier analysis. The remaining 55 animals were assigned to sacrifice groups at 1, 2, or 6 weeks post-MI and were included in Kaplan-Meier analysis. Of these 55 animals, 8 animals were operated for 1-week sacrifice and 2 died prior to sacrifice (the remaining 6 were fixed for histology). 23 animals were operated for 2-week sacrifice and 10 died prior to sacrifice (the remaining 13 hearts were fixed for histology). 24 animals were operated for 6-week sacrifice and 11 died prior to sacrifice (the remaining 13 hearts were fixed for histology). Thus, for Kaplan-Meier analysis, 55 animals were included and a total of 23 died prior to sacrifice and were counted as deaths.

MI+CBSC (n=67): 10 animals were sacrificed 24 hours post-MI for infarct size analysis (n=5) or histology (n=5) and were not included in the Kaplan-Meier analysis. The remaining 57 animals were assigned to sacrifice groups at 1, 2, or 6 weeks post-MI and were included in Kaplan-Meier analysis. Of these animals, 20 were operated for 1-week sacrifice and 6 died prior to sacrifice (the remaining 14 hearts were fixed for histology). 21 animals were operated for 2-week sacrifice and 3 died prior to sacrifice (the remaining 18 hearts were fixed for histology). 16 animals were operated for 6-week sacrifice and 3 died prior to sacrifice. Of the 13 animals sacrificed at 6 weeks post-MI, 5 were digested for myocyte isolation and 8 were perfusion fixed for histology. Thus, for Kaplan-Meier analysis, 57 animals were included and a total of 12 died prior to sacrifice and were counted as deaths.

For histological analysis, 14 MI+CBSC hearts were fixed at 1-week post-MI, 18 were fixed at 2 weeks post-MI, and 8 were fixed at 6 weeks post-MI. When we analyze stem cell-injected hearts, we section each heart completely into 5 um-thick sections and stain every single slide. Because such comprehensive histological analysis is necessary, we randomly selected 6 hearts fixed at 1 week post-MI and 6 hearts fixed at 2 weeks post-MI for analysis. All hearts fixed at 6 weeks post-MI were completely sectioned, stained, and analyzed.

MI+CDC (n=36): 10 animals were sacrificed 24 hours post-MI for infarct size analysis (n=5) or

histology (n=5) and were not included in the Kaplan-Meier analysis. The remaining 26 animals were assigned to sacrifice groups at 1, 2, or 6 weeks post-MI and were included in Kaplan-Meier analysis. Of these animals, 8 were operated for 1-week sacrifice and all animals survived (all 8 hearts were fixed for histology). 8 animals were operated for 2-week sacrifice and 4 died prior to sacrifice (the remaining 4 hearts were fixed for histology). 10 animals were operated for 6-week sacrifice and 4 died prior to sacrifice (the remaining 6 hearts were fixed for histology). Thus, for Kaplan-Meier analysis, 26 animals were included and a total of 8 died prior to sacrifice and were counted as deaths.

For histological analysis, all hearts were completely sectioned and analyzed on histology (8 at 1 week post-MI, 4 at 2 weeks post-MI and 6 at 6 weeks post-MI).

Infarct Size Analysis

After 24 hours post-MI, five animals from each study group (MI+CBSC, MI+CDC, or MI+Saline) were randomly selected to undergo acute infarct size analysis. Cardiectomy was performed under general anesthesia, and the hearts were perfused with 2% Evan's Blue dye in PBS to stain the area at risk (AAR), which accounts for all areas of the myocardium except for those perfused by the ligated coronary artery. The heart was then flash-frozen in liquid nitrogen so it could be cut into 6-8 short-axis cross sections. The sections were washed in PBS to remove excess Evan's Blue dye then incubated in 2% triphenyltetrazolium chloride in PBS for 10 minutes at 37°C to stain ischemic tissues white (ischemic area, IA) and viable tissues red. Samples were washed again in PBS then photographed under a top-lit dissecting scope. AAR and IA were measured on each photograph using NIH image J software, and a mean AAR and IA for each heart was calculated as a percentage of total ventricular area.

For chronic infarct size analysis, paraffin-embedded short-axis heart sections from MI+CBSC, MI+CDC, or MI+Saline sacrificed 6 weeks post-MI were stained with hematoxylin and eosin (H&E). Brightfield photographs were acquired on a dissecting microscope using a DS-Fi1 color camera and NIS Elements software (all from Nikon Inc.; Melville, NY). Pathologically infarcted regions of the myocardium were identified and their surface area was quantified using NIH Image J software. Infarct area was calculated as a percentage of total ventricular surface area for 3-4 cross-sections

Two-Dimensional Echocardiography and Strain Analysis

Anesthetized mice underwent transthoracic echocardiography using a Vevo2100 ultrasound system (VisualSonics; Toronto, Canada). Repeated measurements were performed as previously described[4, 11, 12] at baseline and at 1, 2, 4, and 6 weeks post-MI. Images were acquired by JMD in the short-axis B-mode and M-mode for analysis of cardiac function and dimensions. Long-axis B-mode images were recorded for longitudinal and radial strain analysis using the VevoStrain software following a recently published protocol.[13] After echocardiograms were recorded, image series were randomly ordered and renumbered by CAM. All images were analyzed under their coded numbers in a blinded fashion by JMD, then the code was broken by CAM and animal data was sorted by treatment group then analyzed.

Perfusion Fixation

Cardiectomy was performed under general anesthesia and the heart was rinsed and weighed. The aorta was then cannulated and the coronary arteries were cleared by perfusion with 1 mL cold Krebs-Henseleit Buffer. The heart was then arrested in diastole by perfusion with 1 mL of 100 mM cadmium chloride/1 M potassium chloride solution. The hearts were then gravity perfused with 30 mL 10% formalin at mean arterial pressure (100 mmHg). Fixed hearts were immersed overnight in 10% formalin and then stored in 70% ethanol for up to 1 week before being processed and embedded in paraffin wax blocks.

Immunohistochemistry

For *in vitro* staining, cells were plated on gelatin-coated coverslips overnight and then fixed with 4% paraformaldehyde. Cells were permeablized with 0.1% Triton X-100 in PBS (Fluka/Sigma-Aldrich; St. Louis, MO) and stained for the following proteins: c-kit and goat IgG isotype control for c-kit (AF1356 and AB-108-C, R&D Systems; Minneapolis, MN), Rat anti-Sca-1-biotin (130-093-421, Miltenyi Biotec; Auburn, CA) and rat IgG2a-biotin isotype control for Sca-1 (IC006B, R&D Systems; Minneapolis, MN), insulin-like growth factor-1 (SC-9013, Santa Cruz Biotechnology; Dallas, TX), angiopoietin-1 (ab95230), basic-fibroblast growth factor (ab8880), hepatocyte growth factor (ab83760), platelet-derived growth factor (ab61219), stem cell factor (ab64677), stromal-derived factor-1 (ab64677), and vascular endothelial growth factor (ab46154) all purchased from Abcam (Cambridge, MA). For neonatal rat ventricular myocyte co-cultures, cells were stained for α-sarcomeric actin (A2172, Sigma; St. Louis, MO) or connexin43 (AB1728, Millipore; Billerica, MA). The following secondary antibodies were purchased from Jackson Immunoresearch Laboratories (West Grove, PA) and used for detection of primary antibodies as follows: Rhodamine Red-X donkey anti-goat IgG (705-295-147) was used to detect c-kit; Rhodamine Red-X donkey anti-biotin (200-292-211) was used to detect Sca-1; rhodamine red-X donkey anti-rabbit IgG (711-295-152) was used to detect Ang-1, bFGF, HGF, PDGF, SCF, SDF-1, VEGF, and connexin43; and rhodamine red-X donkey anti-Mouse IgM (715-295-020) was used to detect α-sarcomeric actin.

For fixed tissues, wax blocks were cut into 5 μm thick sections that were mounted on glass slides for staining. Slides were deparaffinized and underwent antigen retrieval in hot citric acid buffer. Stains were conducted against the following proteins: α-sarcomeric actin and α-smooth muscle actin (A2172 and A2547, Sigma; St. Louis, MO), EGFP and von Willebrand factor (ab111258 and ab6994, Abcam; Cambridge, MA), and connexin43 (AB1728 Millipore; Billerica, MA). The following secondary antibodies were purchased from Jackson Immunoresearch Laboratories (West Grove, PA) and used for detection of primary antibodies as follows: rhodamine red-X donkey anti-Mouse IgM (715-295-020) or Cy5 donkey anti-Mouse IgM (715-175-140) were used to detect α-sarcomeric actin; rhodamine red-X donkey anti-Mouse IgG (715-295-151) or Cy5 donkey anti-Mouse IgG (715-175-151) were used to detect α-smooth muscle actin; FITC donkey anti-goat IgG (705-095-147) was used to detect EGFP, and rhodamine red-X donkey anti-rabbit IgG (711-295-152) or Cy5 donkey anti-rabbit IgG (711-175-152) was used to detect von Willebrand factor and connexin43. Nuclei in both cells and embedded tissues were stained with 4',6-diamidino-2-phenylindole (DAPI, Millipore; Billerica, MA).

Confocal micrographs of all immunostains were acquired using a Nikon Eclipse T1 confocal microscope (Nikon Inc.; Mellvile, NY).

Statistical Analysis

All statistical analyses were overseen by the Fox Chase Cancer Center Biostatistics and Bioinformatics Facility. Survival analysis is presented using a Kaplan-Meier regression and statistical significance was determined using the log-rank test. For ELISA, infarct size analysis, blood vessel counts and isolated myocyte measurements (where discrete measurements were compared at a single time point), a two-way T test was used. For follow-up parameters with repeated measures (echocardiography and strain analysis), growth curve models with cubic splines were used. All growth curve coefficients were fitted as random effects to allow deviation of individual growth from the mean of the treatment group. Interaction term with the treatment was also included to compare the mean growth rates by treatment. For all statistical tests, a p-value < 0.05 was considered statistically significant.

References for Supplemental Materials and Methods

1. Zhu H, Guo ZK, Jiang XX, Li H, Wang XY, Yao HY, Zhang Y, Mao N. A protocol for isolation and culture of mesenchymal stem cells from mouse compact bone. *Nat Protoc*. 2010;5:550-560
2. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*. 2003;114:763-776
3. Urbanek K, Rota M, Cascapera S, Bearzi C, Nascimbene A, De Angelis A, Hosoda T, Chimenti S, Baker M, Limana F, Nurzynska D, Torella D, Rotatori F, Rastaldo R, Musso E, Quaini F, Leri A, Kajstura J, Anversa P. Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. *Circ Res*. 2005;97:663-673
4. Zhang H, Makarewich CA, Kubo H, Wang W, Duran JM, Li Y, Berretta RM, Koch WJ, Chen X, Gao E, Valdivia HH, Houser SR. Hyperphosphorylation of the cardiac ryanodine receptor at serine 2808 is not involved in cardiac dysfunction after myocardial infarction. *Circ Res*. 2012;110:831-840
5. Makarewich CA, Correll RN, Gao H, Zhang H, Yang B, Berretta RM, Rizzo V, Molkentin JD, Houser SR. A caveolae-targeted l-type ca(2)+ channel antagonist inhibits hypertrophic signaling without reducing cardiac contractility. *Circ Res*. 2012;110:669-674
6. Kubo H, Margulies KB, Piacentino V, 3rd, Gaughan JP, Houser SR. Patients with end-stage congestive heart failure treated with beta-adrenergic receptor antagonists have improved ventricular myocyte calcium regulatory protein abundance. *Circulation*. 2001;104:1012-1018
7. Simpson P, Savion S. Differentiation of rat myocytes in single cell cultures with and without proliferating nonmyocardial cells. Cross-striations, ultrastructure, and chronotropic response to isoproterenol. *Circ Res*. 1982;50:101-116
8. Gaughan JP, Hefner CA, Houser SR. Electrophysiological properties of neonatal rat ventricular myocytes with alpha1-adrenergic-induced hypertrophy. *Am J Physiol*. 1998;275:H577-590
9. Kubo H, Berretta RM, Jaleel N, Angert D, Houser SR. C-kit+ bone marrow stem cells differentiate into functional cardiac myocytes. *Clin Transl Sci*. 2009;2:26-32
10. Tarnavski O, McMullen JR, Schinke M, Nie Q, Kong S, Izumo S. Mouse cardiac surgery: Comprehensive techniques for the generation of mouse models of human diseases and their application for genomic studies. *Physiol Genomics*. 2004;16:349-360
11. Duran JM, Taghavi S, Berretta RM, Makarewich CA, Sharp Iii T, Starosta T, Udeshi F, George JC, Kubo H, Houser SR. A characterization and targeting of the infarct border zone in a swine model of myocardial infarction. *Clin Transl Sci*. 2012;5:416-421
12. Taghavi S, Duran JM, Berretta RM, Makarewich CA, Udeshi F, Sharp TE, Kubo H, Houser SR, George JC. Validation of transcatheter left ventricular electromechanical mapping for assessment of cardiac function and targeted transendocardial injection in a porcine ischemia-reperfusion model. *Am J Transl Res*. 2012;4:240-246
13. Bauer M, Cheng S, Jain M, Ngoy S, Theodoropoulos C, Trujillo A, Lin FC, Liao R. Echocardiographic speckle-tracking based strain imaging for rapid cardiovascular phenotyping in mice. *Circ Res*. 2011;108:908-916

Online Figure Legends

Online Figure I: Characterization of stem cells using quantitative real-time PCR (qPCR). Detected levels of c-kit and Sca-1 mRNA from cortical bone stem cells (CBSCs) or cardiac-derived stem cells (CDCs) were normalized to levels of GAPDH mRNA transcripts expressed by each cell type. Results are presented as normalized arbitrary units.

Online Figure II: Characterization of stem cells by immunostaining. CBSCs or CDCs were stained for **A)** c-kit or **B)** Sca-1 (red) and nuclei were labeled with DAPI (blue). Isotype controls are also shown (goat IgG for c-kit or rat IgG for Sca-1). Scale bars = 50 μm.

Online Figure III: Characterization of stem cells using flow cytometry. Flow cytometry against c-kit, Sca-1, CD29, CD34, CD45 and lineage markers (Lin). Results are shown along with negative isotype controls in which cells were labeled only with APC-conjugated Rat IgG2A.

Online Figure IV: Bone-derived stem cells differentiate *in vitro*. CBSCs were cocultured with neonatal rat ventricular myocytes for 3 days. Cells were fixed and stained red for α-sarcomeric actin (**A** and **B**) or connexin43 (**C** and **D**). Nuclei are labeled with DAPI (blue). EGFP+ CBSCs are green.

Online Figure V: Infarct size analysis. **A)** Acute infarct size analysis was performed on animals receiving MI+Saline (n=5), MI+CDC (n=5), or MI+CBSC (n=5) that were sacrificed 24 hours post-MI. Their area at risk (AAR) or infarct area (IA) was determined using Evan's Blue or triphenyltetrazolium chloride staining, respectively, and results are reported as a percentage of total ventricular area. **B)** Chronic infarct size was determined by staining short-axis cross-sections from hearts fixed at 6 weeks post-MI+Saline (n=6), MI+CDC (n=6), or MI+CBSC (n=5) with hematoxylin and eosin (H&E) and measuring the infarct area as a percent of total myocardial surface area.

Online Figure VI: Diagram of strain analysis measurements. A) Schematic showing the measurement parameters used for strain analysis on B-mode images taken in the parasternal long axis. A sample B-mode tracing of a mouse heart at baseline is included on top. B) Diagram explaining the three axes used to generate the 3D wall velocity diagrams. The X-axis represents the location along the LV endocardial surface from the base of the anterior wall (1) to the apex (2) to the base of the posterior wall (3). The Z-axis represents time (in seconds), which is measured off of the electrocardiogram. The Y-axis represents contraction (positive values/orange color) or relaxation (negative values/blue color). An example of a positive wall velocity tracing (orange) and a negative wall velocity tracing (blue) are shown next to the positive and negative Y-axes. C) Diagram demonstrating how the 3D wall velocity diagram is constructed. A single cardiac cycle is shown (one blue and one orange tracing), and below a cartoon illustrating 3 consecutive cardiac cycles is shown. This is equivalent to what is seen in the lateral view of the wall velocity diagram (D). E) Shows the rotated view of the 3D wall velocity diagram from the animal at baseline that was shown in Figure 4.

Online Figure VII: Characterization of paracrine factors secreted by cortical bone stem cells *in vivo* 2 weeks after MI. Animals receiving MI+CBSCs were sacrificed 2 weeks post-MI and EGFP+ CBSC injection sites were identified and immunostained for VEGF (red). Nuclei are labeled with DAPI (blue). **A)** Immunostain showing an EGFP+/VEGF+ cell that was selected for fluorophore colocalization analysis by confocal line scan. **B)** Intensity of red, green, and blue phlorophores across the line scan of the cell selected in Figure A. From these data, scatterplots were constructed depicting colocalization of **C)** red vs. green channel or **D)** blue vs. green channel (control). **E)** Magnified image depicting the cell in Figure A along with single color channel images.

Online Figure VIII: CBSC-treated animals have increased von Willebrand Factor+ blood vessels near the infarct border zone by 6 weeks post-MI. Slides from animals receiving MI+Saline, MI+CDC or MI+CBSC injection were stained for von Willebrand factor (purple) and α-sarcomeric actin (red), and the number of von Willebrand Factor+ blood vessels observed per visual field were quantified. Nuclei are labeled with DAPI (blue). Both a merged image and a single color image showing only the von Willebrand factor channel are shown for each group. ** = $p < 0.001$

Online Figure IX: Individual channel images and staining controls from Figure 7A and B. Scale bars = 50 μm.

Online Figure X: Low magnification images showing EGFP+ and EGFP- regions of myocardium. Scale bars = 20 μm.

Online Figure XI: Low magnification images showing EGFP+ and EGFP- regions around vasculature. Scale bars = 10 μm.

Online Figure XII: Individual color channel images and staining controls for Figure 7C. Scale bars = 20 μm.

Online Figure XIII: Individual color channel images and staining controls for Figure 7D. Scale bars = 10 μm.

Online Figure XIV: Cardiac stem cells grow and expand but do not adopt a myocyte or vascular phenotype within 6 weeks. Injection sites from MI animals receiving CDC treatment were sacrificed at 1, 2, or 6 weeks and were stained for **A)** α-sarcomeric actin (red) and EGFP (green), or **B)** connexin43 (red), EGFP (green) and α-sarcomeric actin (white). Nuclei are labeled with DAPI (blue).

Online Figure XV: Proportion of CBSCs expressing α-sarcomeric actin after 6 weeks post-MI. EGFP+ injection sites were identified in 3 animals sacrificed 6 weeks after MI+CBSC injection, and these tissues were stained for α-sarcomeric actin (α-SA). Cells expressing EGFP (n=265) were analyzed at high magnification for expression of unorganized or striated α-SA, and their numbers are expressed as percentages in the pie chart on the left.

Online Video I: Vector diagram video loop showing the direction and magnitude of endocardial strain in a mouse heart at baseline.

Online Video II: Vector diagram video loop showing the direction and magnitude of endocardial strain in a mouse heart at 6 weeks after MI+CBSC injection.

Online Video III: Vector diagram video loops showing the direction and magnitude of endocardial strain in a mouse heart at 6 weeks after MI+CDC injection.

Online Video IV: Vector diagram video loops showing the direction and magnitude of endocardial strain in a mouse heart at 6 weeks after MI+Saline injection.

Online Table I: Summary of paracrine factors produced by cortical bone stem cells as measured by FACS, Western, ELISA, and immunostaining *in vitro* and *in vivo*.

Online Table II: Summary of paracrine factors produced by cardiac-derived stem cells as measured by FACS, Western, ELISA, and immunostaining *in vitro* and *in vivo*.

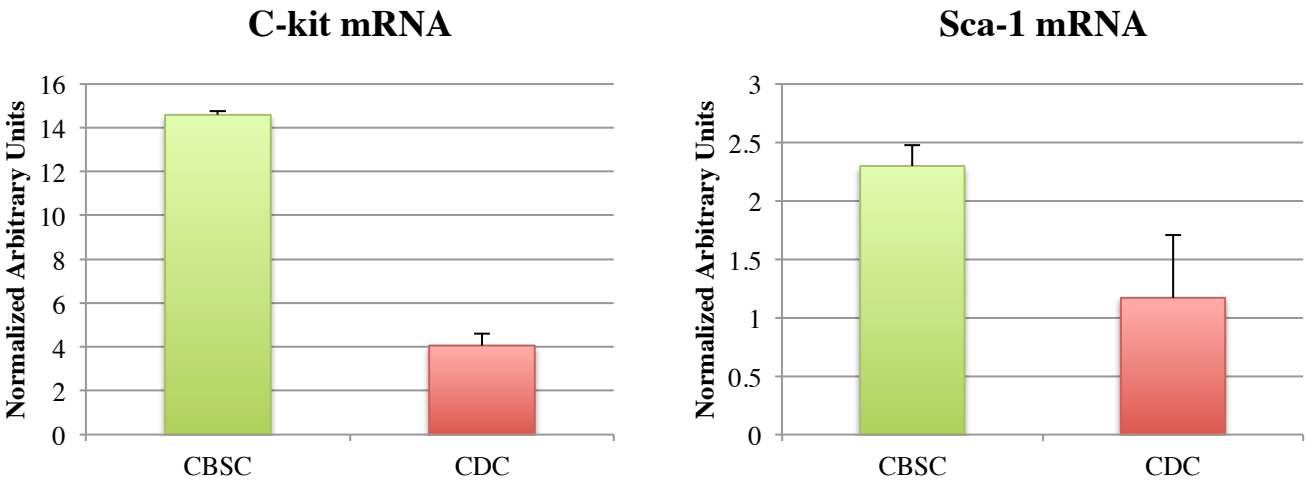
Online Table I: Summary of paracrine factors produced by cortical bone stem cells as measured by FACS, Western, ELISA, and immunostaining *in vitro* and *in vivo*.

	Western	ELISA	Staining:			
			In vitro	24 hr	1 week	2 week
AP-1	+	N/A	+	-	-	-
bFGF	+	N/A	+	+	-	-
HGF	+	-	+	-	-	-
IGF-1	+	+	+	-	-	-
PDGF	+	N/A	+	-	-	-
SCF	+	-	+	-	-	-
SDF-1	+	+++	+	-	-	-
VEGF	+	++	+	+	+	+

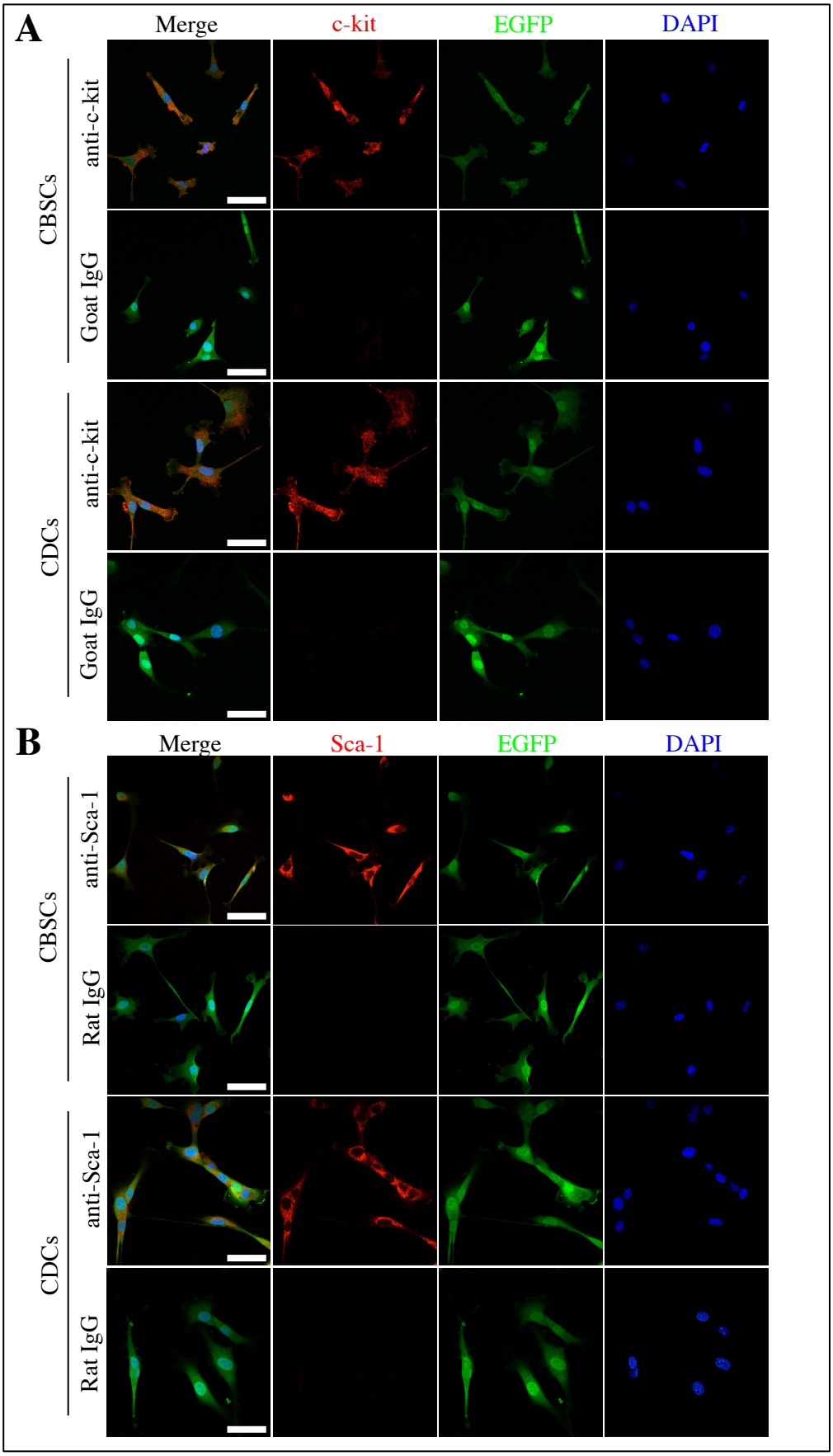
Online Table II: Summary of paracrine factors produced by cardiac-derived stem cells as measured by FACS, Western, ELISA, and immunostaining *in vitro* and *in vivo*.

	Western	ELISA	Staining:			
			In vitro	24 hr	1 week	2 week
AP-1	+	N/A	+	+	-	-
bFGF	+	N/A	+	+	-	-
HGF	+	-	+	-	-	-
IGF-1	+	+	+	-	-	-
PDGF	+	N/A	+	-	-	-
SCF	+	-	+	-	-	-
SDF-1	+	+++	+	-	-	-
VEGF	+	++	+	+	-	-

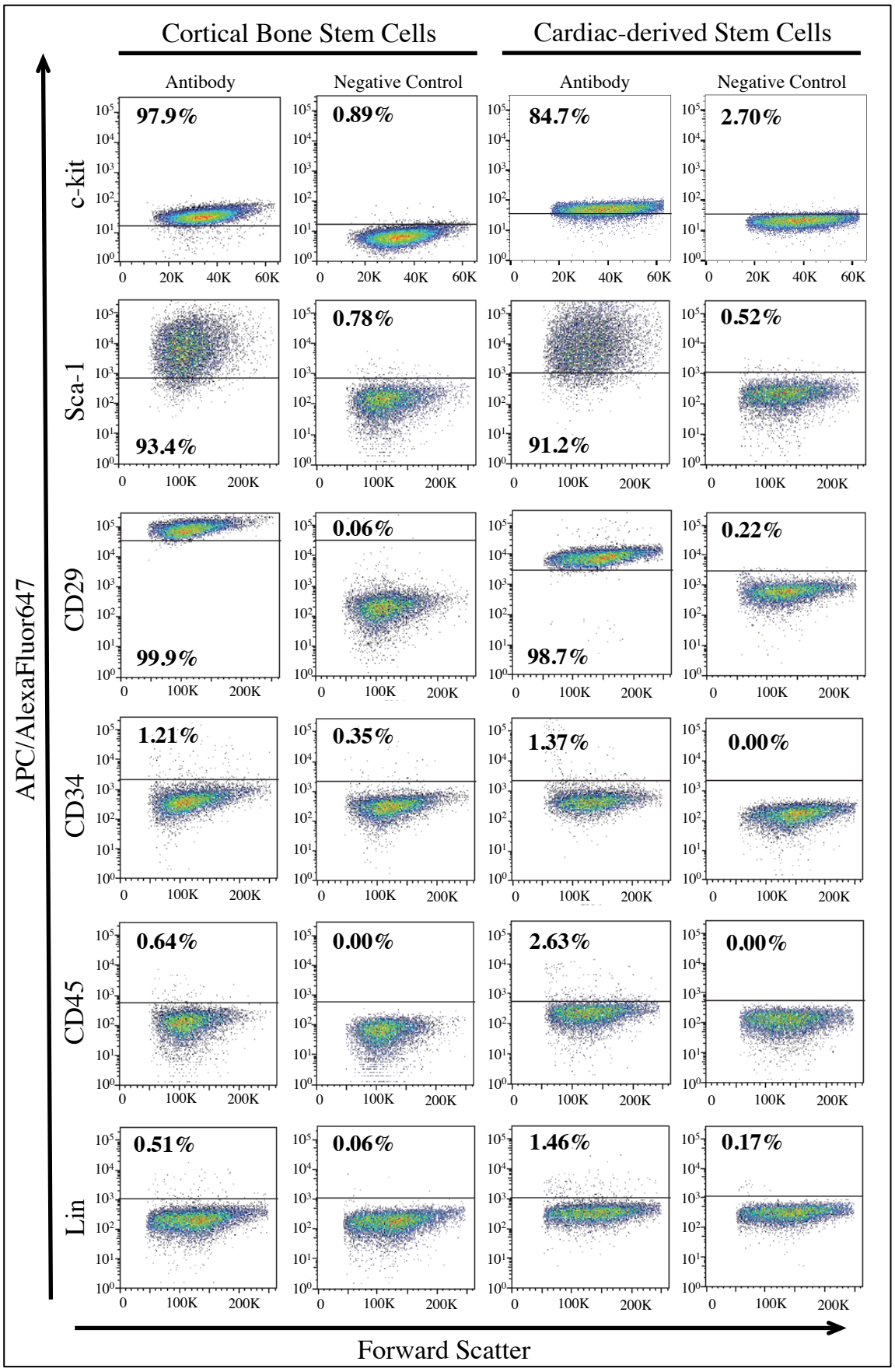
Online Figure I: Characterization of stem cells using quantitative real-time PCR (qPCR). Detected levels of c-kit and Sca-1 mRNA from cortical bone stem cells (CBSCs) or cardiac-derived stem cells (CDCs) were normalized to levels of GAPDH mRNA transcripts expressed by each cell type. Results are presented as normalized arbitrary units.



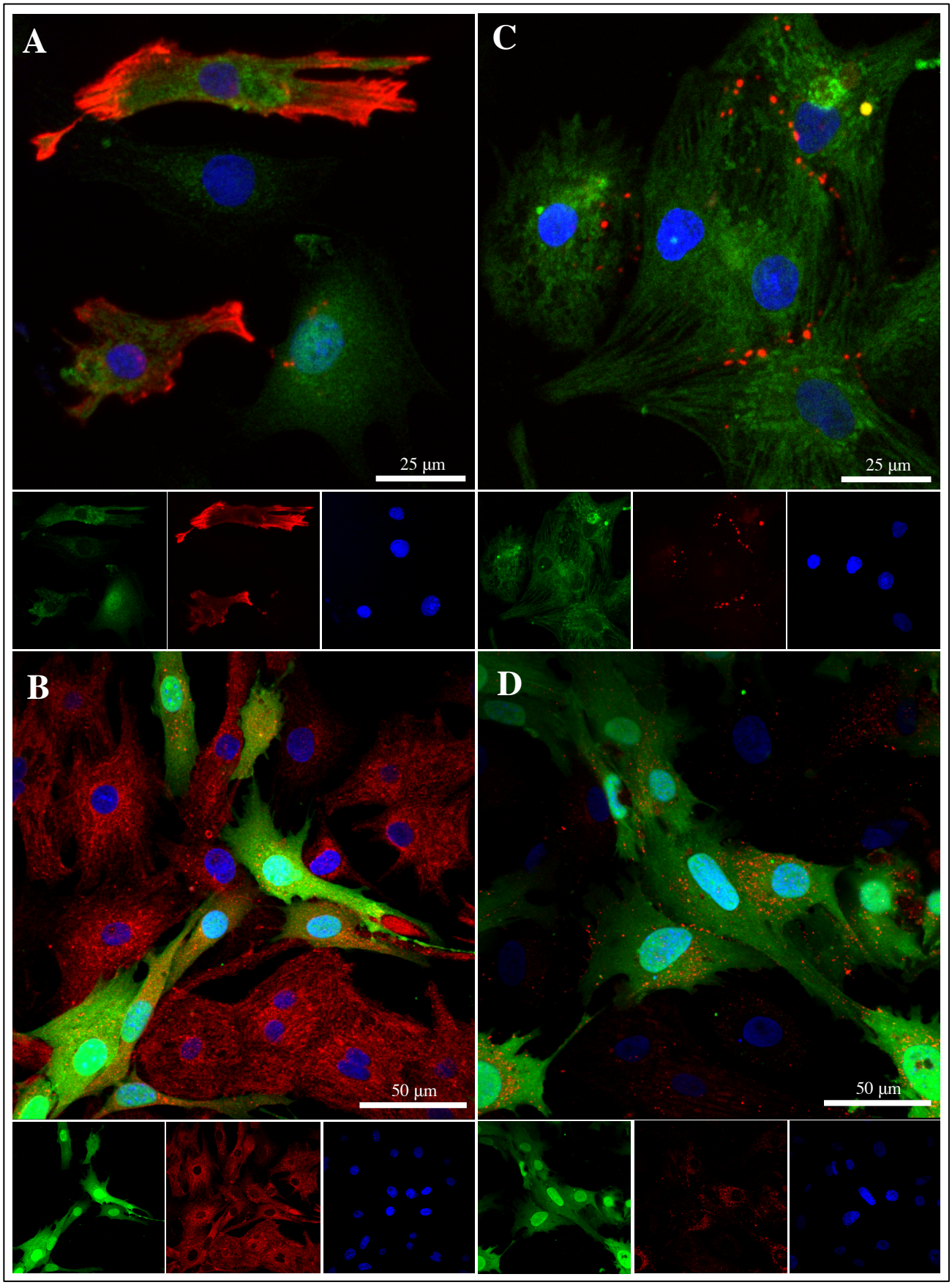
Online Figure II: Characterization of stem cells by immunostaining. CBSCs or CDCs were stained for **A)** c-kit or **B)** Sca-1 (red) and nuclei were labeled with DAPI (blue). Isotype controls are also shown (goat IgG for c-kit or rat IgG for Sca-1). Scale bars = 50 μm.



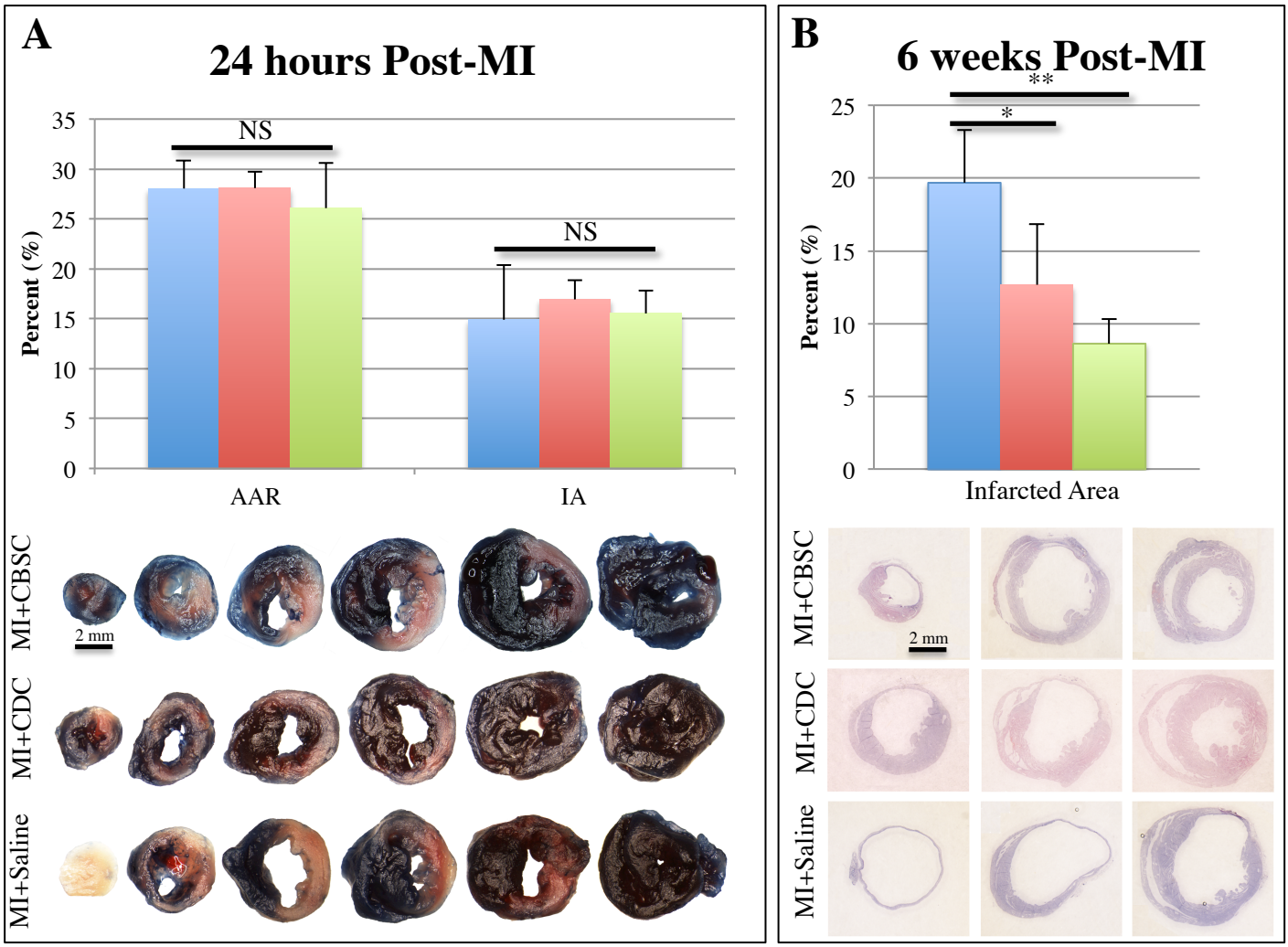
Online Figure III: Characterization of stem cells using flow cytometry. Flow cytometry against c-kit, Sca-1, CD29, CD34, CD45 and lineage markers (Lin). Results are shown along with negative isotype controls in which cells were labeled only with APC-conjugated Rat IgG2A.



Online Figure IV: Bone-derived stem cells differentiate *in vitro*. CBSCs were cocultured with neonatal rat ventricular myocytes for 3 days. Cells were fixed and stained red for α-sarcomeric actin (**A** and **B**) or connexin43 (**C** and **D**). Nuclei are labeled with DAPI (blue). EGFP+ CBSCs are green.

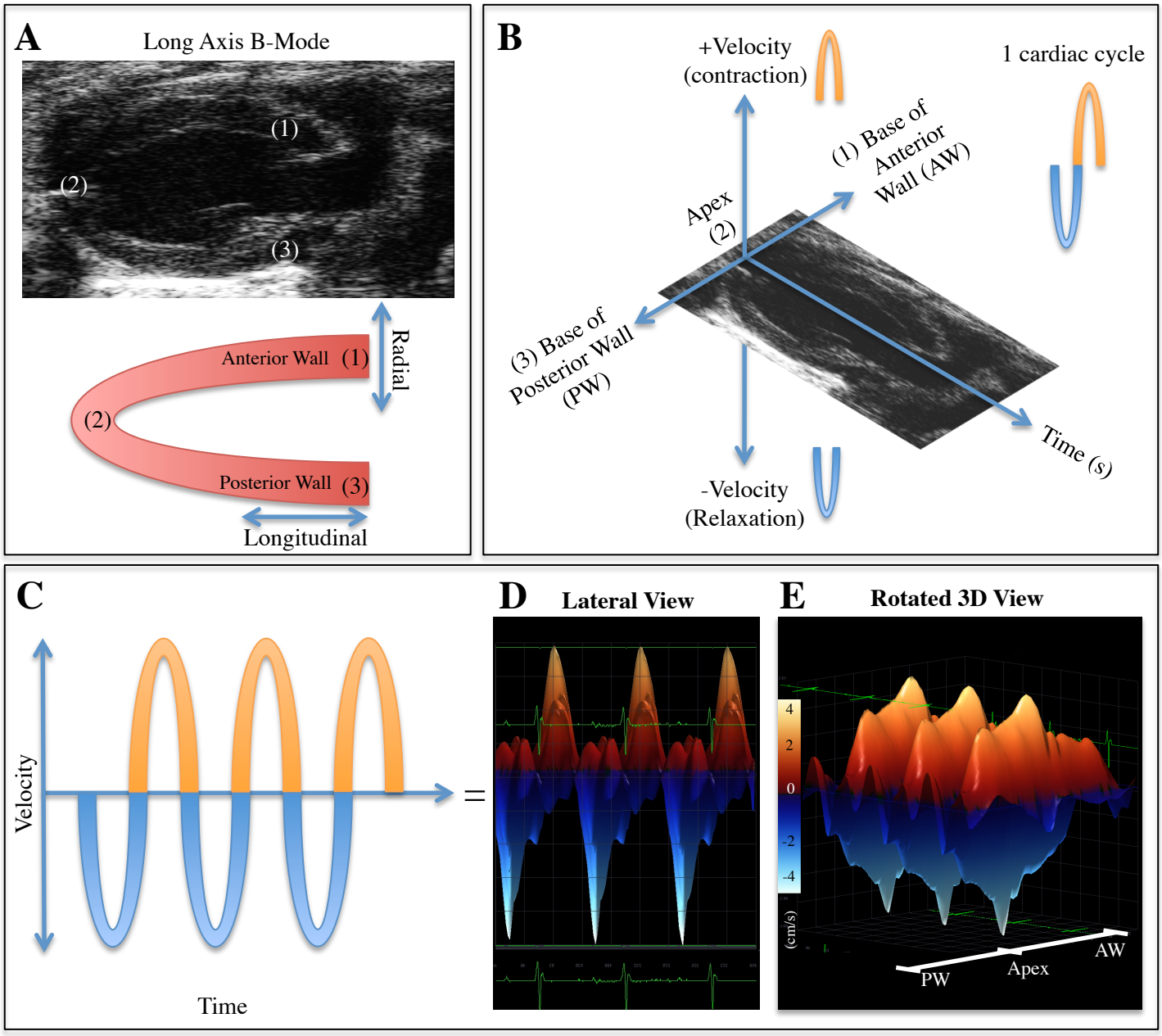


Online Figure V: Infarct size analysis. **A)** Acute infarct size analysis was performed on animals receiving MI+Saline (n=5), MI+CDC (n=5), or MI+CBSC (n=5) that were sacrificed 24 hours post-MI. Their area at risk (AAR) or infarct area (IA) was determined using Evan's Blue or triphenyltetrazolium chloride staining, respectively, and results are reported as a percentage of total ventricular area. **B)** Chronic infarct size was determined by staining short-axis cross-sections from hearts fixed at 6 weeks post-MI+Saline (n=6), MI+CDC (n=6), or MI+CBSC (n=5) with hematoxylin and eosin (H&E) and measuring the infarct area as a percent of total myocardial surface area.

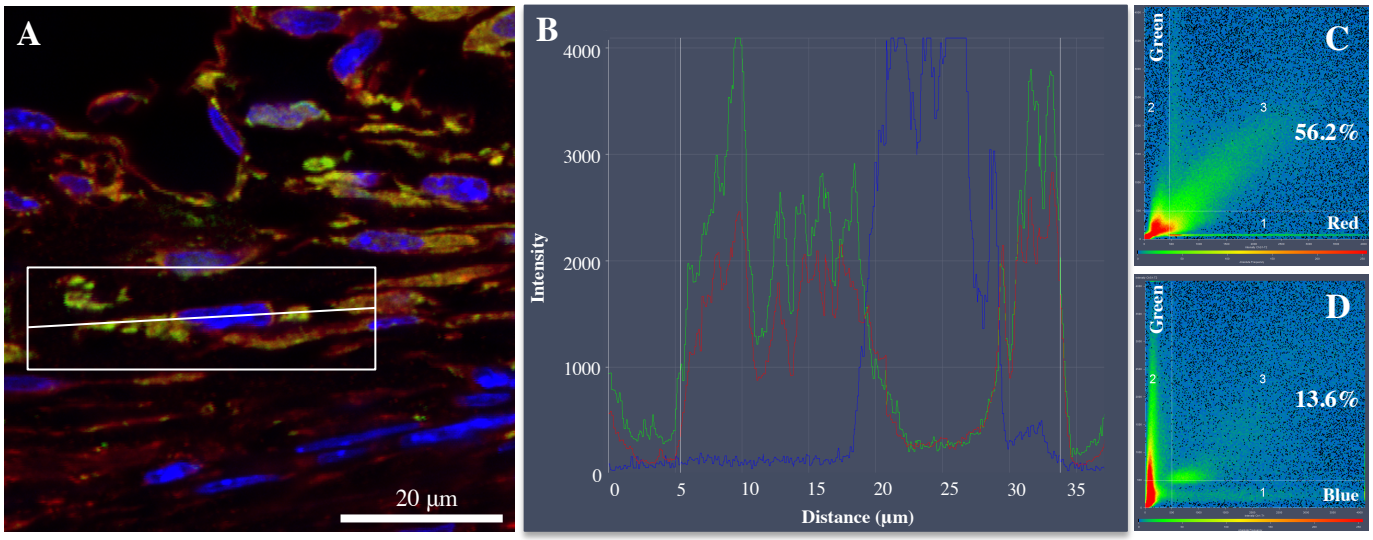


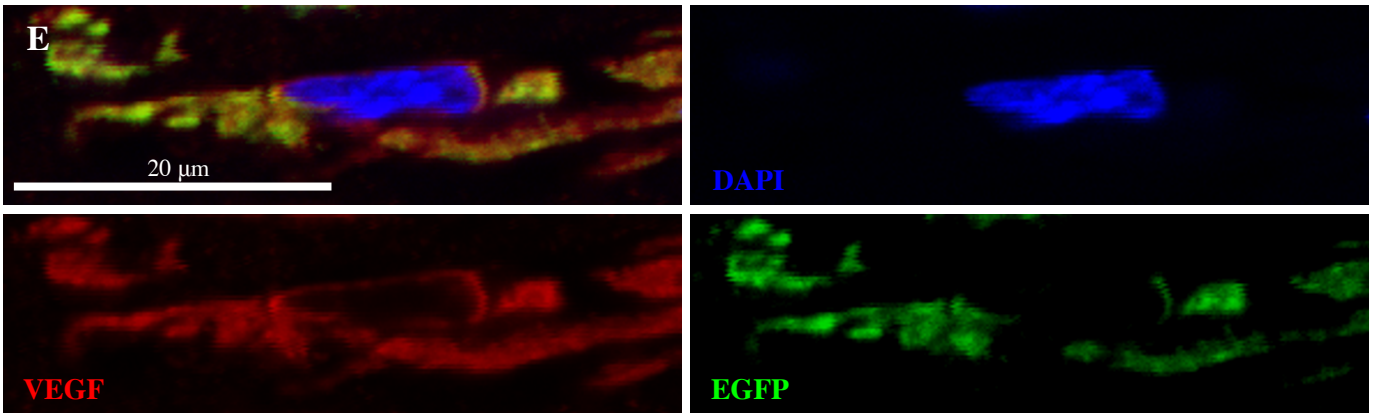
MI+Saline MI+CDC MI+CBSC * = $p < 0.05$, ** = $p < 0.01$, NS = Not Significant ($p > 0.05$)

Online Figure VI: Diagram of strain analysis measurements. **A)** Schematic showing the measurement parameters used for strain analysis on B-mode images taken in the parasternal long axis. A sample B-mode tracing of a mouse heart at baseline is included on top. **B)** Diagram explaining the three axes used to generate the 3D wall velocity diagrams. The X-axis represents the location along the LV endocardial surface from the base of the anterior wall (1) to the apex (2) to the base of the posterior wall (3). The Z-axis represents time (in seconds), which is measured off of the electrocardiogram. The Y-axis represents contraction (positive values/orange color) or relaxation (negative values/blue color). An example of a positive wall velocity tracing (orange) and a negative wall velocity tracing (blue) are shown next to the positive and negative Y-axes. **C)** Diagram demonstrating how the 3D wall velocity diagram is constructed. A single cardiac cycle is shown (one blue and one orange tracing), and below a cartoon illustrating 3 consecutive cardiac cycles is shown. This is equivalent to what is seen in the lateral view of the wall velocity diagram (**D**). **E)** Shows the rotated view of the 3D wall velocity diagram from the animal at baseline that was shown in Figure 4.

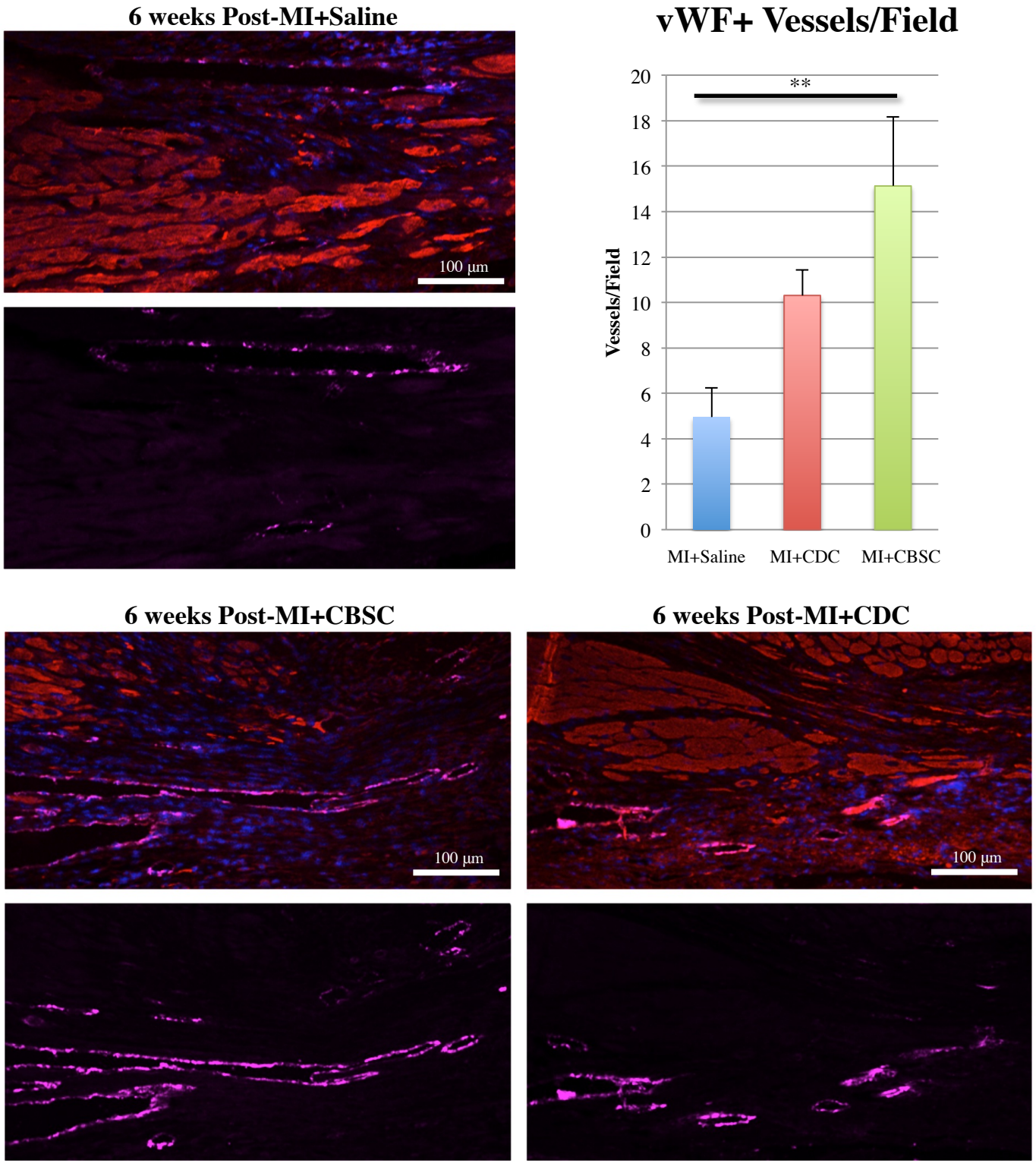


Online Figure VII: Characterization of paracrine factors secreted by cortical bone stem cells *in vivo* 2 weeks after MI. Animals receiving MI+CBSCs were sacrificed 2 weeks post-MI and EGFP+ CBSC injection sites were identified and immunostained for VEGF (red). Nuclei are labeled with DAPI (blue). **A)** Immunostain showing an EGFP+/VEGF+ cell that was selected for fluorophore colocalization analysis by confocal line scan. **B)** Intensity of red, green, and blue phlorophores across the line scan of the cell selected in Figure A. From these data, scatterplots were constructed depicting colocalization of **C**) red vs. green channel or **D)** blue vs. green channel (control). **E)** Magnified image depicting the cell in Figure A along with single color channel images.

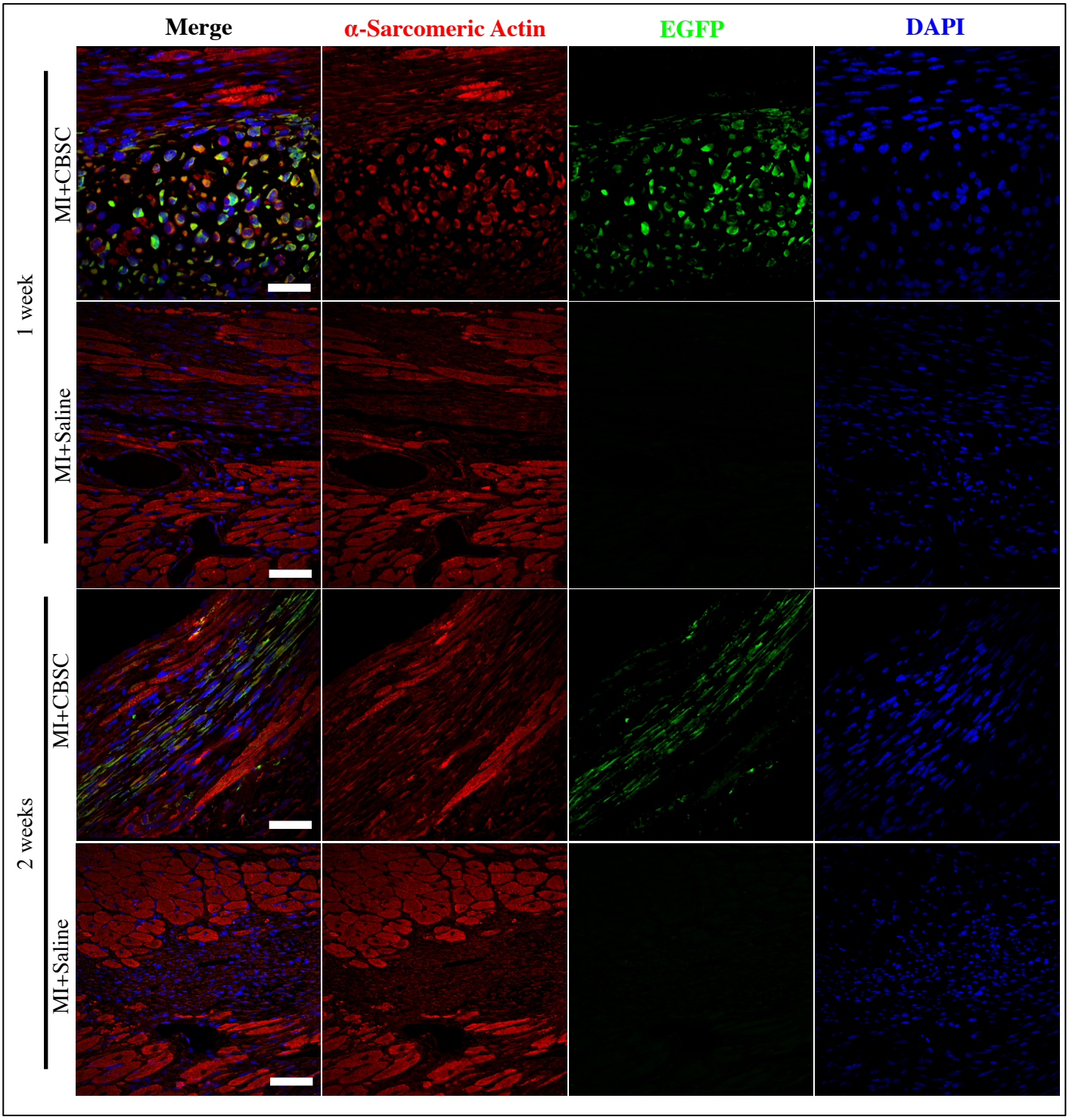




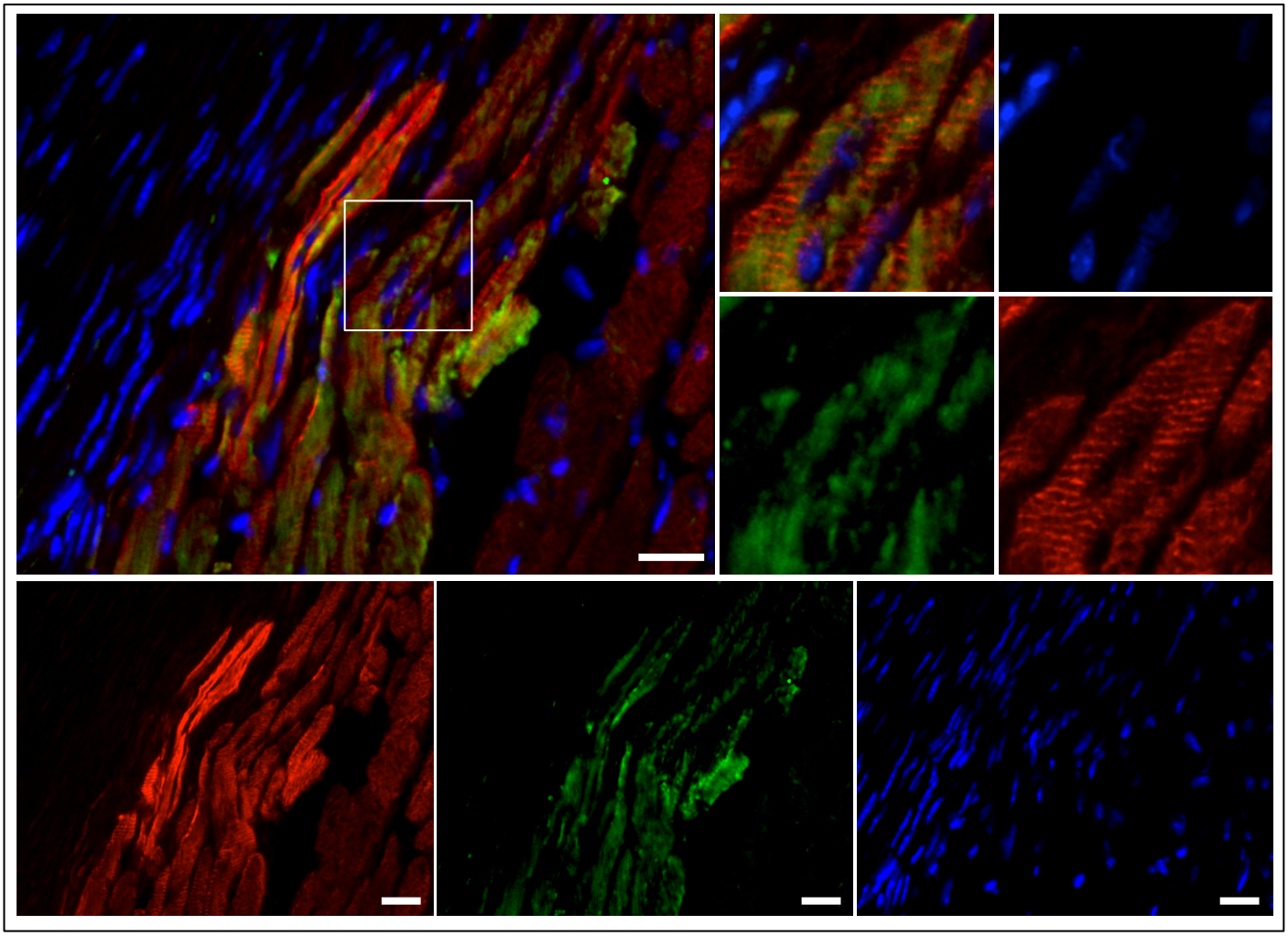
Online Figure VIII: CBSC-treated animals have increased von Willebrand Factor+ blood vessels near the infarct border zone by 6 weeks post-MI. Slides from animals receiving MI+Saline, MI+CDC or MI+CBSC injection were stained for von Willebrand factor (purple) and α-sarcomeric actin (red), and the number of von Willebrand Factor+ blood vessels observed per visual field were quantified. Nuclei are labeled with DAPI (blue). Both a merged image and a single color image showing only the von Willebrand factor channel are shown for each group. ** = $p < 0.001$



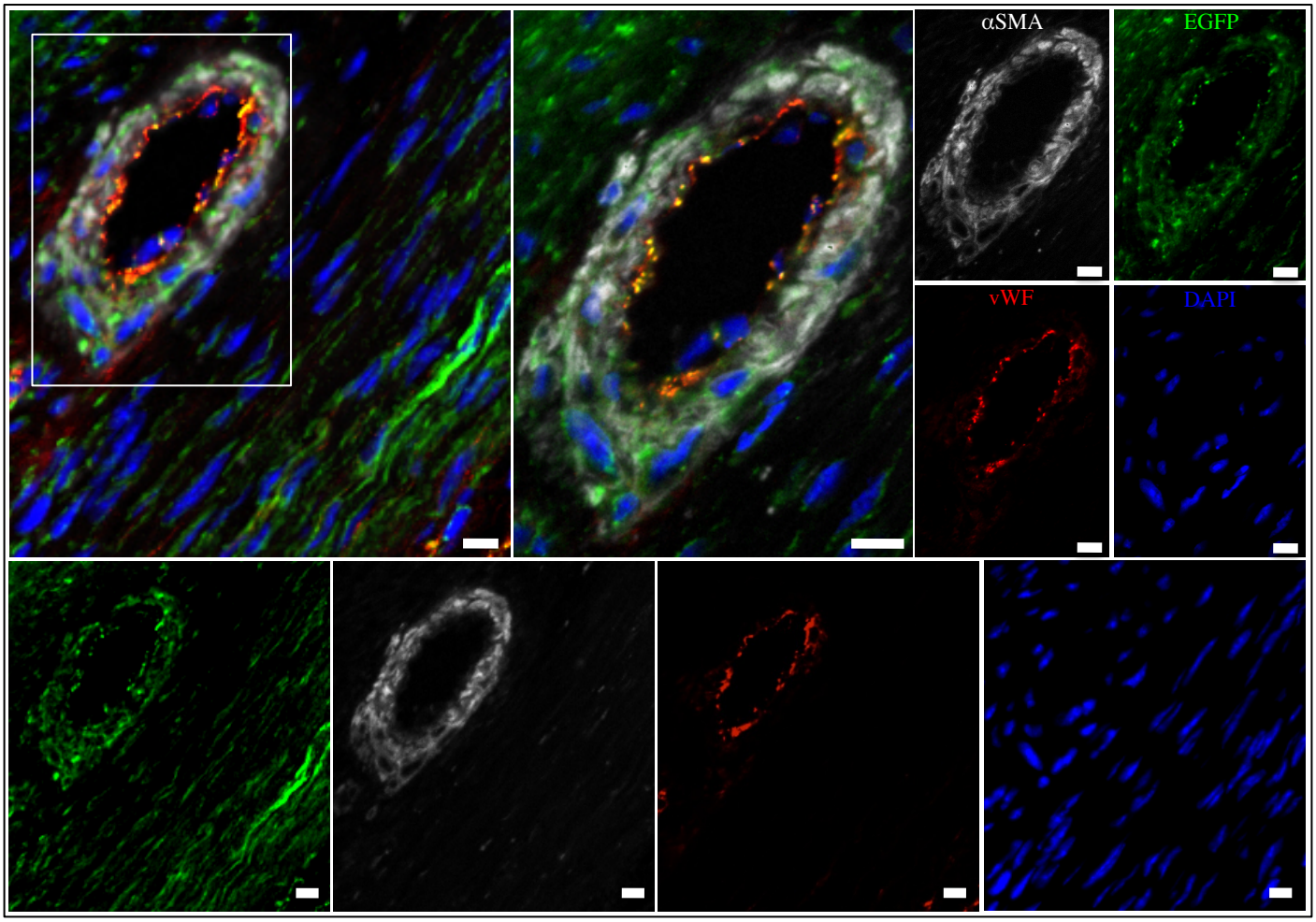
Online Figure IX: Individual channel images and staining controls from Figure 7A and B. Scale bars = 50 μm.



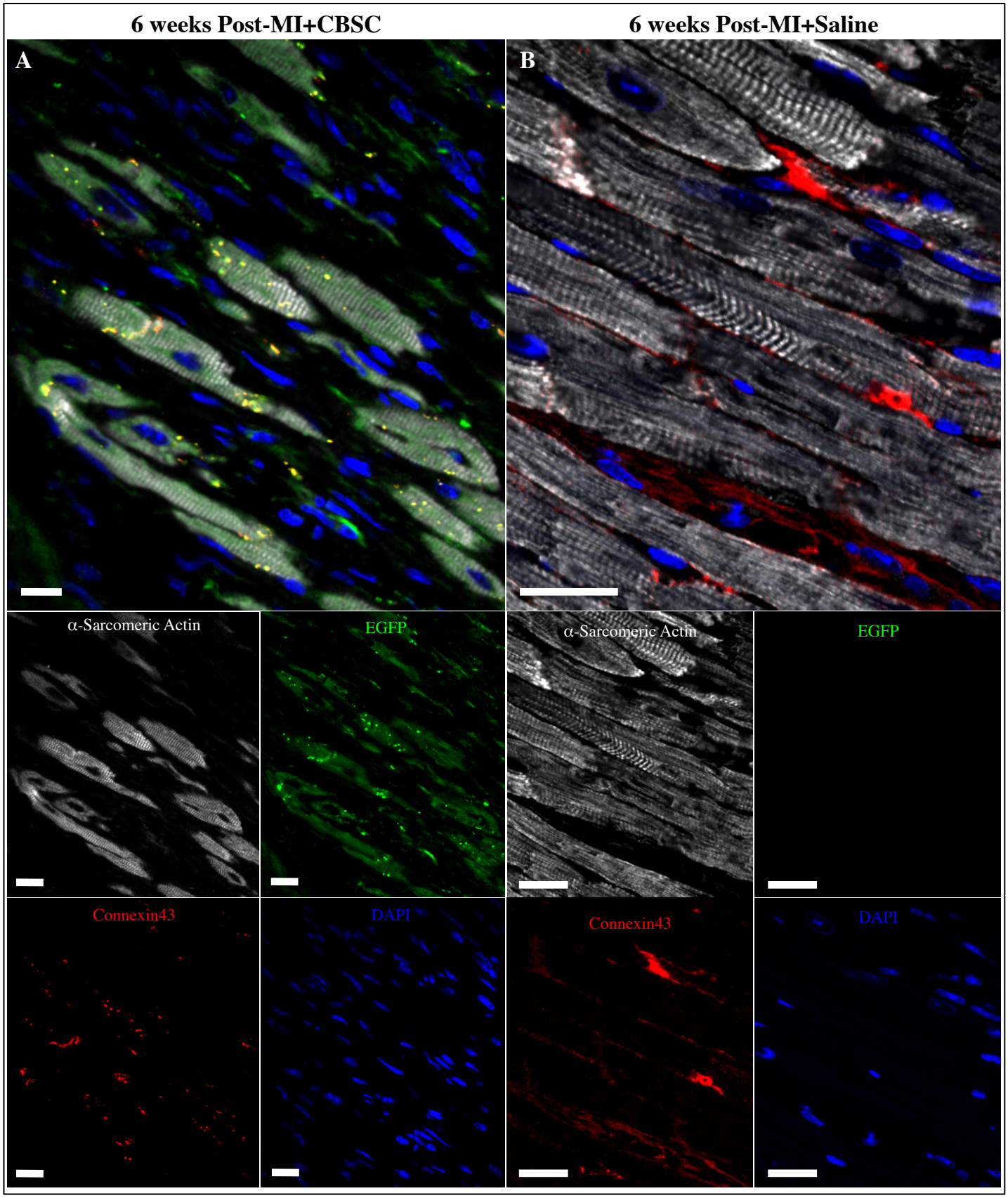
Online Figure X: Low magnification images showing EGFP+ and EGFP- regions of myocardium. Scale bars = 20 μm.



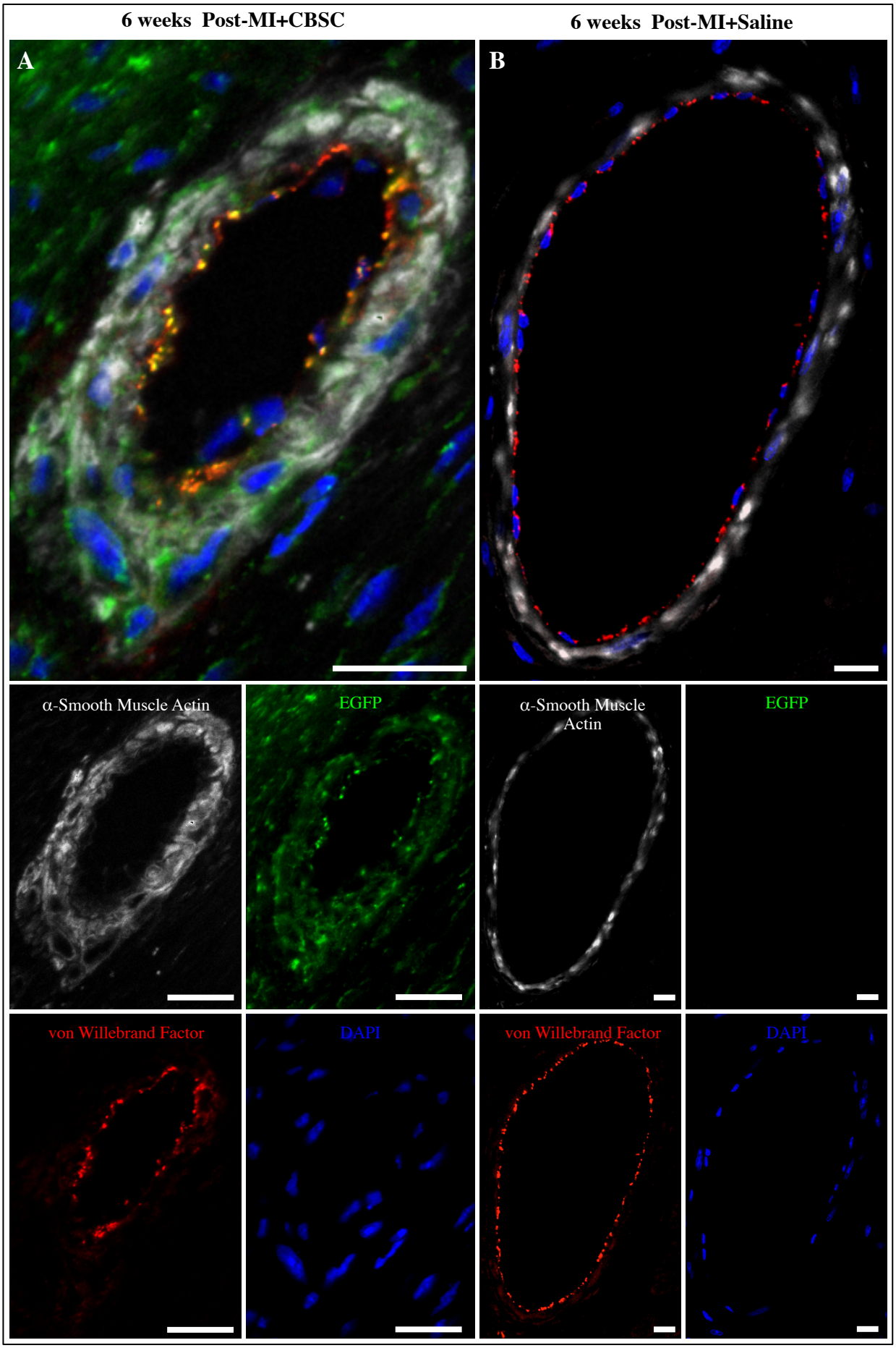
Online Figure XI: Low magnification images showing EGFP+ and EGFP- regions around vasculature. Scale bars = 10 μm.



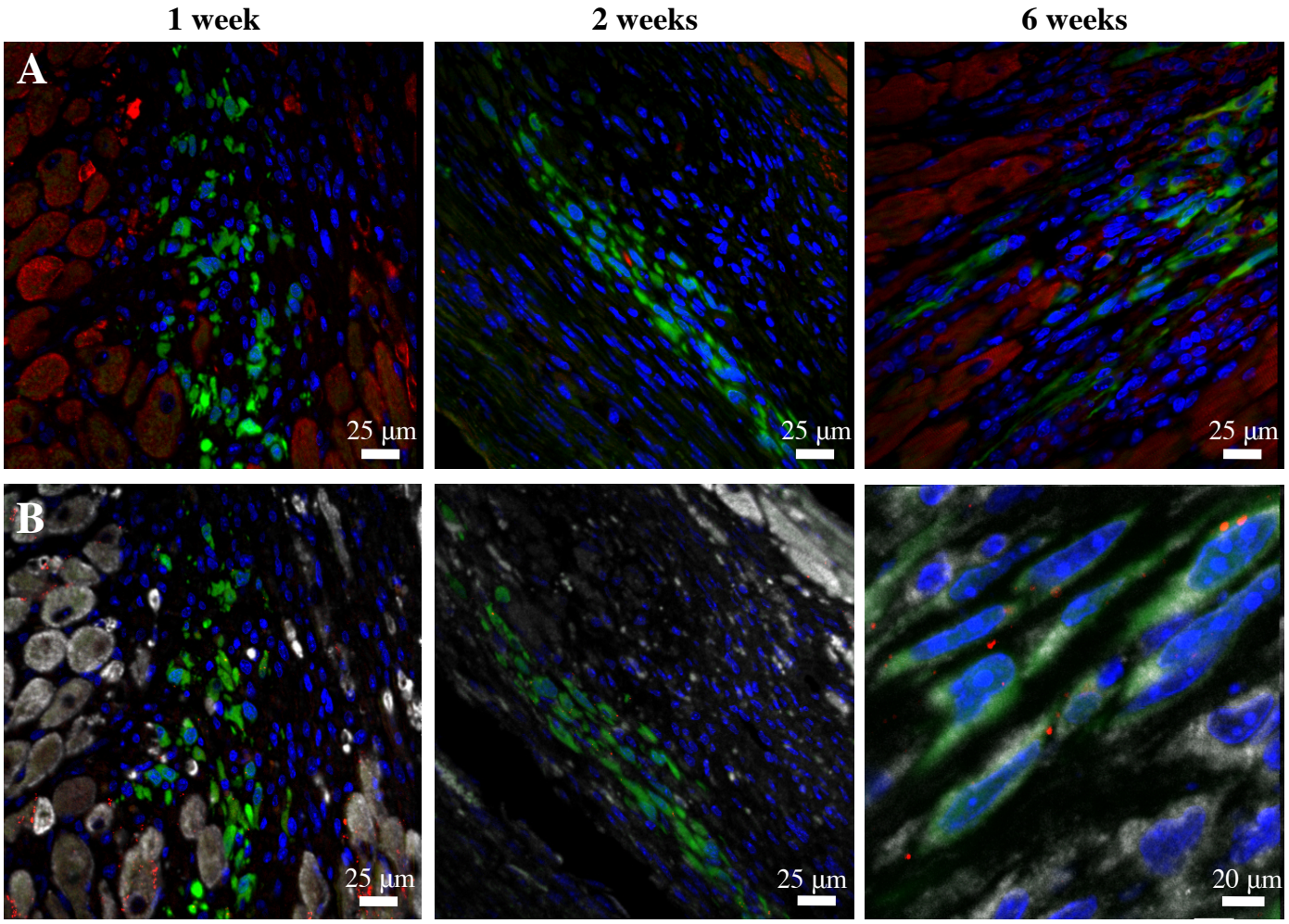
Online Figure XII: Individual color channel images and staining controls for Figure 7C. Scale bars = 20 μm.



Online Figure XIII: Individual color channel images and staining controls for Figure 7D. Scale bars = 10 um.



Online Figure XIV: Cardiac stem cells grow and expand but do not adopt a myocyte or vascular phenotype within 6 weeks. Injection sites from MI animals receiving CDC treatment were sacrificed at 1, 2, or 6 weeks and were stained for **A)** α-sarcomeric actin (red) and EGFP (green), or **B)** connexin43 (red), EGFP (green) and α-sarcomeric actin (white). Nuclei are labeled with DAPI (blue).



Online Figure XV: Proportion of CBSCs expressing α-sarcomeric actin after 6 weeks post-MI. EGFP+ injection sites were identified in 3 animals sacrificed 6 weeks after MI +CBSC injection, and these tissues were stained for α-sarcomeric actin (α-SA). Cells expressing EGFP (n=265) were analyzed at high magnification for expression of unorganized or striated α-SA, and their numbers are expressed as percentages in the pie chart on the left.