ONLINE SUPPLEMENTARY MATERIAL

METHODS

All procedures and animal care were approved by our institutional research committee and conformed the animal care guideline in Mount Sinai School of Medicine

Generation of Recombinant Adenovirus Ad.SCF.

The Ad.SCF recombinant adenovirus was generated using the AdEasy XL Adenoviral Vector System (Stratagene). The full length of the SCF membrane isoform cDNA was cloned into the pShuttle-IRES-hrGFP2 vector. The linearized shuttle vector was recombined in *Escherichia coli* strain BJ5183 with AdEasy, a serotype 5 first-generation adenoviral backbone. The recombinant viral backbones were transformed into AD-293 cells and grown at large scale. Adenoviruses were purified by standard CsCl ultracentrifugation and desalting. Ad-EGFP-β-gal (Ad.β-gal) adenovirus was used as a control. Cardiac myocytes were infected with recombinant adenoviruses for 8 hours at a multiplicity of infection 50 for Ad.β-gal and 100 for Ad.SCF for an additional 24 to 48 hours to ensure transgene expression.

Gene transfer.

Male Sprague-Dawley rats were anesthetized with pentobarbital (60 mg/kg) by intraperitoneal injection and placed on a ventilator. The chest was accessed from the left side through the fourth intercostal space and the pericardium was incised. The left anterior descending coronary artery (LAD) was identified and encircled with a 7-0 silk suture at the apex of the left ventricle. The suture was briefly snared to confirm the ligation by blanching the arterial region. Ad.β-gal and Ad.SCF adenoviruses (200 μl at a concentration of ~$5x10^{11}$ pfu/ml) were injected into the peri-infarct zone after permanent LAD ligation..

Quantitative RT-PCR.

Total RNA (2 μg) was isolated with Trizol (Sigma-Aldrich) and transcribed to cDNA (Applied Biosystems). Gene expression was analyzed by quantitative PCR performed in an ABI PRISM Sequence Detector System 7500 (Applied Biosystems) with SYBR Green (Bio-Rad Laboratories) as fluorescent and ROX (Bio-Rad Laboratories) as reference dyes. Fragments were amplified for 40 cycles with the following specific primers:

Notch1 forward: GTTTGTGCAAGGATGGTGTG

Notch1 reverse: CCTTGAGGCATAAGCAGAGG

Hox B4 forward: GCACGGTAAACCCCAATTA

Hox B4 reverse: GGCAACTTGTGGTCTTTTTT

Cyclin D1 forward: TGAGTCTGGCACATTCTTGC

Cyclin D1 reverse: TCCTGGGAGTCATTGGTAGC

Gene transfer evaluation by X-gal staining.

One week after LAD ligation and gene transfer, hearts were harvested, embedded in OCT and cryosectioned into 8 μm sections. β-gal/LacZ staining was determined to determine gene transfer expression.

Histological analysis.

Frozen heart blocks were cut into 8 μm sections and prepared for hematoxylin and eosin staining and immunohistochemistry. Paraformaldehide-fixed cryostat heart sections were subjected to confocal microscopy for detection (and merged) images of c-kit and phosphohistone H3 (all from Cell Signaling), Ki67 and Aurora B kinase (all from Abcam) expression in cardiac tissue, using specific antibodies and Zeiss LSM510 META confocal microscope. Masson Trichrome (Sigma) and Picrosirius Red (Sigma) staining, and Sircol Collagen Assay (Biocolor) were conducted according to the manufacturer guidelines.

Cardiomyocyte isolation.

Rats were anesthetized with intraperitoneal Ketamine and Xylazine 50/5 mg/Kg IP. The chest was opened and the pericardium removed. The transverse aorta between the carotid arteries was cut and the isolated heart immediately cannulated via the aorta and perfused ex vivo to obtain cardiomyocytes.

Cell culture.

Primary cultures of adult cardiomyocytes from 2 month-old Sprague-Dawley rats (Charles River Laboratories) were prepared. After perfusing the heart, ventricular tissue was enzymatically digested with collagenase IV and dissociated. Cell suspensions were plated onto laminin-coated dishes and cultured in cardiac myocyte culture medium.

Flow cytometry.

The following antibodies were used for hematopoietic c-kit$^+$ population: anti-c-Kit (2B8), -CD19 (1D3), -CD11b, -CD49b (DX5), -Ter-119, -CD3$_\varepsilon$ (145-2C11), and -Gr-1(RB6-8C5) (all from BD Pharmingen). Antibodies were directly coupled to phycoerythrin or allophycocyanin. For cardiac c-kit$^+$ population characterization we used the following antibodies: anti-c-kit (2B8), -MEF2. -MEF2C, -Nkx2.5, -GATA4 and -α- sarcomeric actin (all from Abcam), and -CD20, -CD45, -P-GP (BD). c-kit$^+$ population was gated and analyzed in a FACSCalibur cytometer (BD). For isolated cardiomyocytes population characterization we used Troponin T (TnT from abcam).

Western blot.

Cardiac tissue was minced and subsequently homogenized in RIPA buffer containing a protease inhibitor cocktail (Sigma-Aldrich). Protein extracts (10 μg) were separated on 10% SDS-PAGE and transferred onto PVDF membrane (Millipore). Antibody binding was visualized by ECL (Immobilon Western, Chemiluminescent HRP Substrate, Millipore). Monoclonal antibodies used in western blotting included anti-c-kit, -GSK3β, -phospho-GSK3β (P-GSK3β), -β-catenin, -phosphohistone H3 (P-H3) and -CyclinD1 (all from Cell Signaling) and –PCNA, -collagen I, -collagen III and -TGBβ (all from Abcam). Protein loading was controlled by GAPDH (Sigma-Aldrich)

expression. Peroxidase-conjugated secondary antibodies (Sigma-Aldrich) were employed.

Echocardiography measurements.

Echocardiography was performed while the rats were under ketamine sedation (60 mg/kg, i.p.). Heart rate was maintained at ≈400 bpm by adjusting ketamine sedation. Short-axis views were obtained using a 14 MHz GE-i1 3L probe as recommended by the American Society of Echocardiography. M-mode and bidimensional images were taken at the midpapillary level of the left ventricle from the parasternal short-axis views. The transducer position was carefully adjusted until the short-axis image of the LV cavity appeared to be circular, indicating perpendicular intersection beam with the long axis of the LV. LV wall thickness and cavity dimensions were measured in systole and diastole with M-mode echocardiography. Additional measurements were made to determine the ejection fraction, LV end-diastolic and LV end-systolic volumes using the area-length method.

Survival.

Survival analysis was performed in sham, controls (Ad.b-gal) and SCF-treated groups. During the study period (3 months), rats were inspected daily for deceased animals, examined for MI presence.

Myocardial apoptosis.

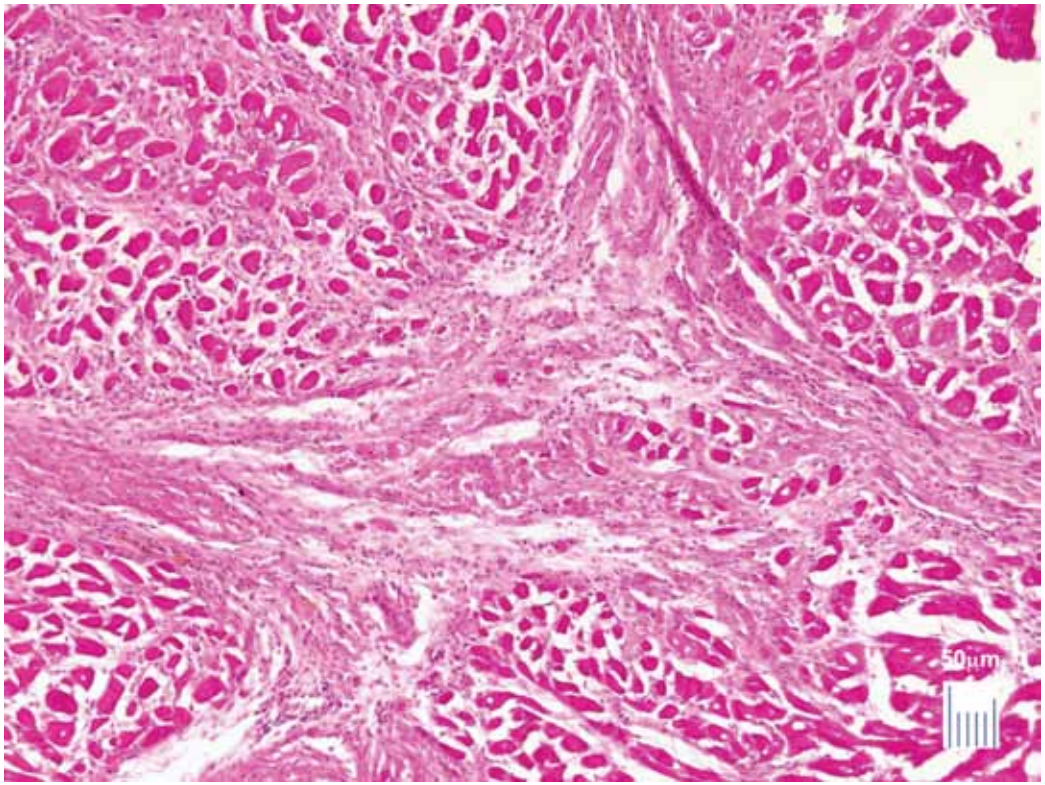
Frozen heart blocks were cut into 8 μm sections, paraformaldehide-fixed and prepared for tunel assay (Millipore). Tunel positive-cardiac cell nuclei were counted and normalized per total nuclei. The proportion of apoptotic cells was counted in the peri-border area.

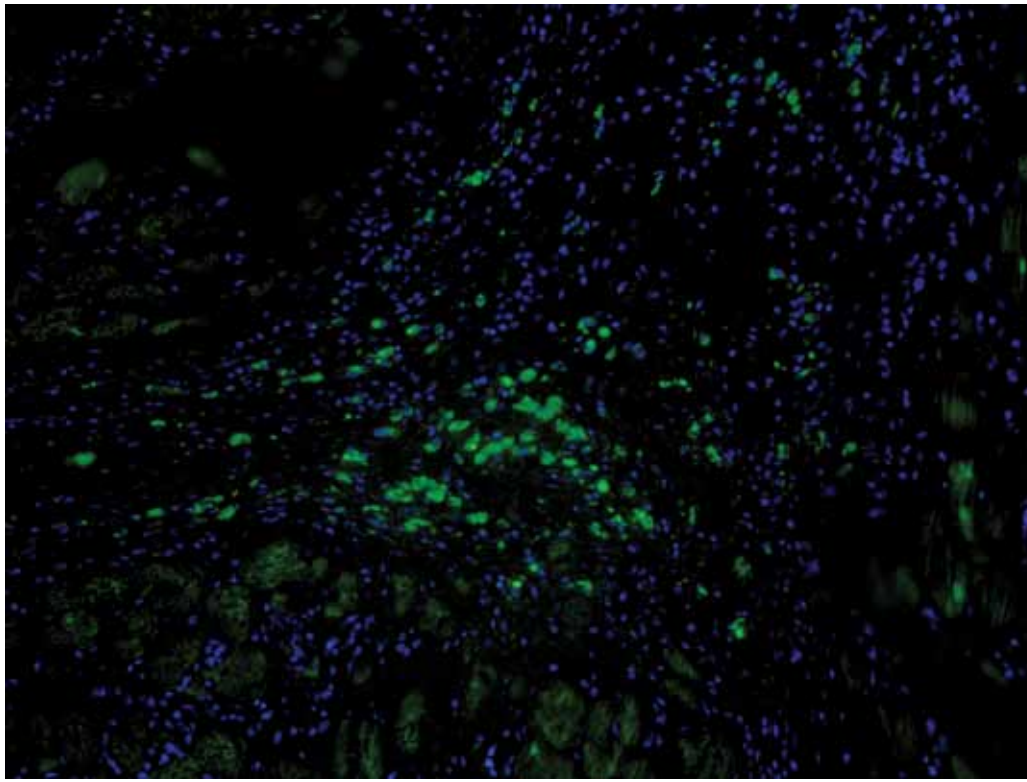
Cardiac MRI.

Cardiac MRI scans were performed in the same animals 2 days, 2 weeks and 1 month post-MI. Myocardium segmentation was performed among 7 LV short-axis slices outlining endocardial and epicardial borders in all cardiac frames. The end-diastolic volumes (EDV) and end-systolic volumes (ESV) were considered the largest and smallest areas, respectively, of the LV cavity in each slice. Apical and basal slices without semicircular muscular ring at either end-systole or end-diastole were disregarded and, without including papillary muscle and LV outflow tract. LV function and infarct volume was calculated by Segment software. Infarct area was defined as the brightest area on short-axis images with the largest area of hyperenhancement in both, end-systole and end-diastole frames.

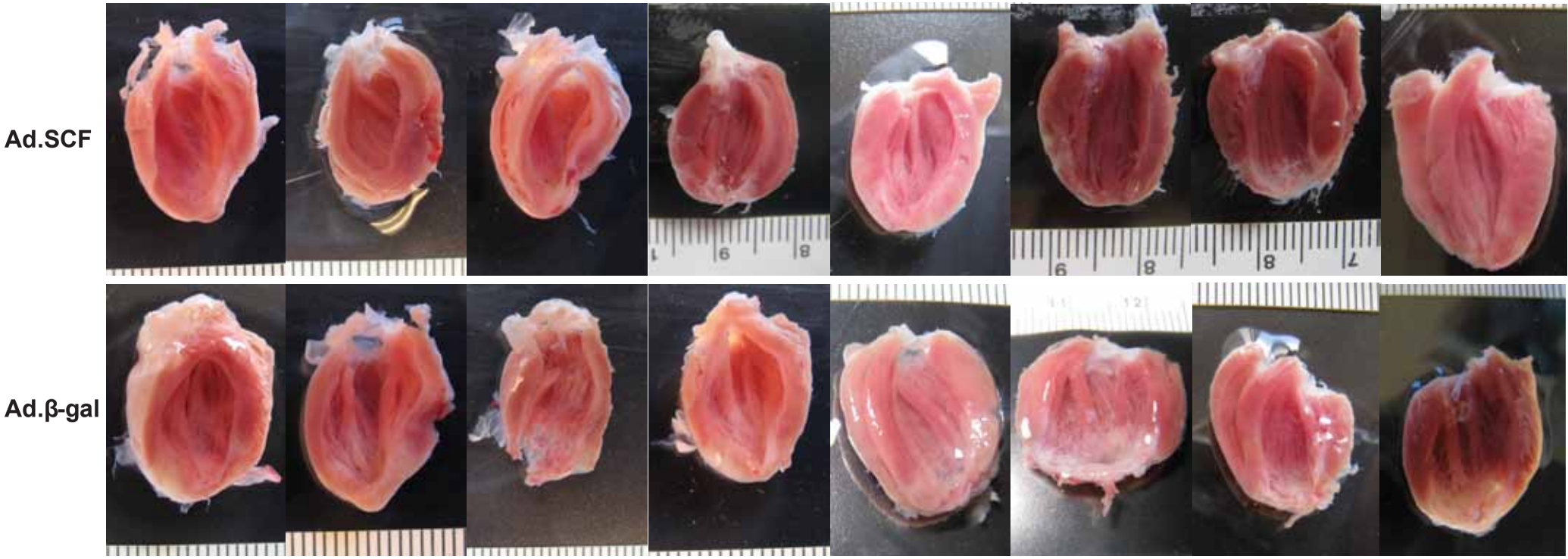
Statistical analysis.

The data of this manuscript are expressed as mean ± SEM. Survival analysis was performed by Kaplan-Meier method. Statistical differences were analyzed using one-way ANOVA and pairwise comparisons were determined using Tukey's honest significance test with $P<0.01$.

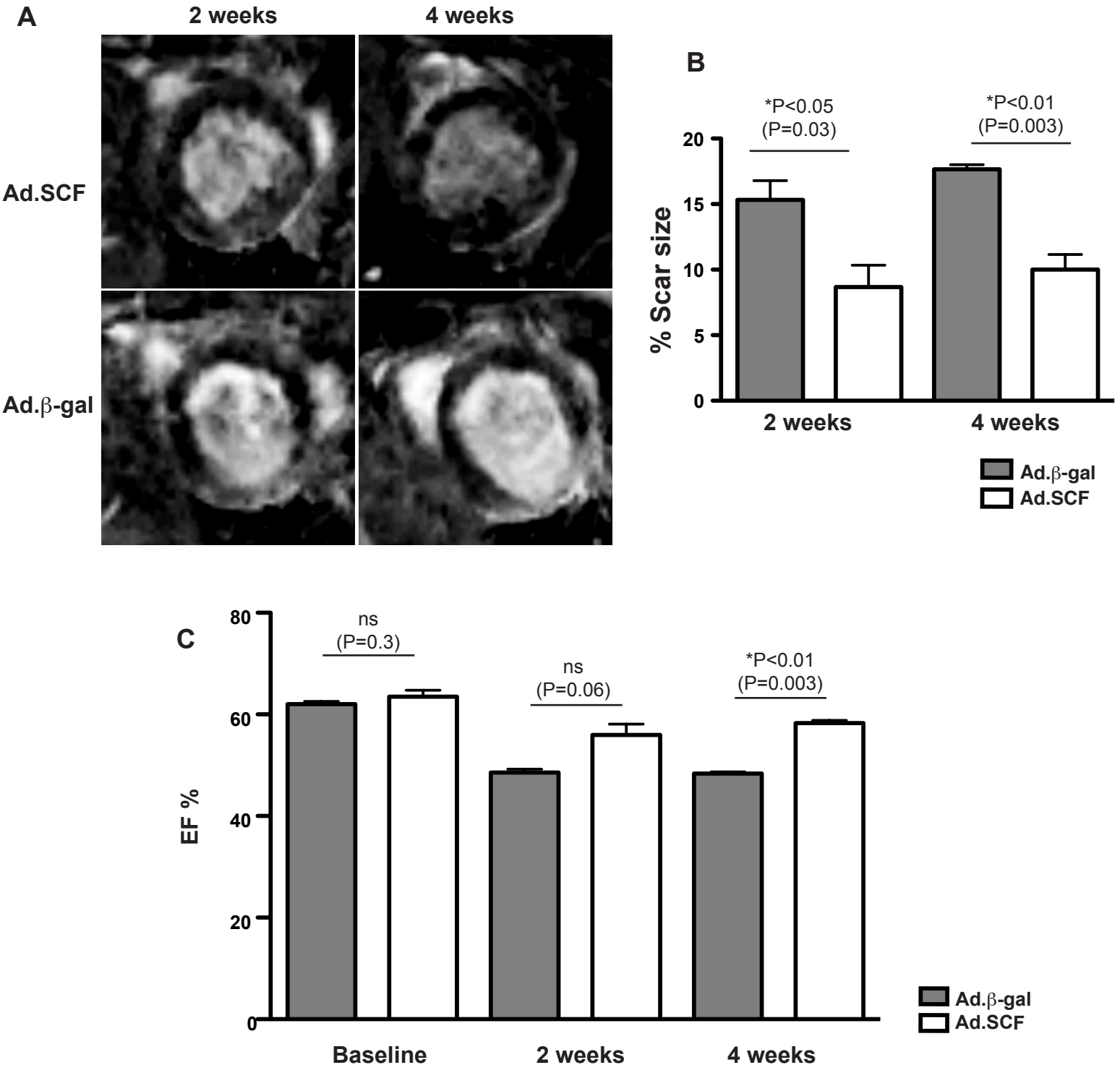




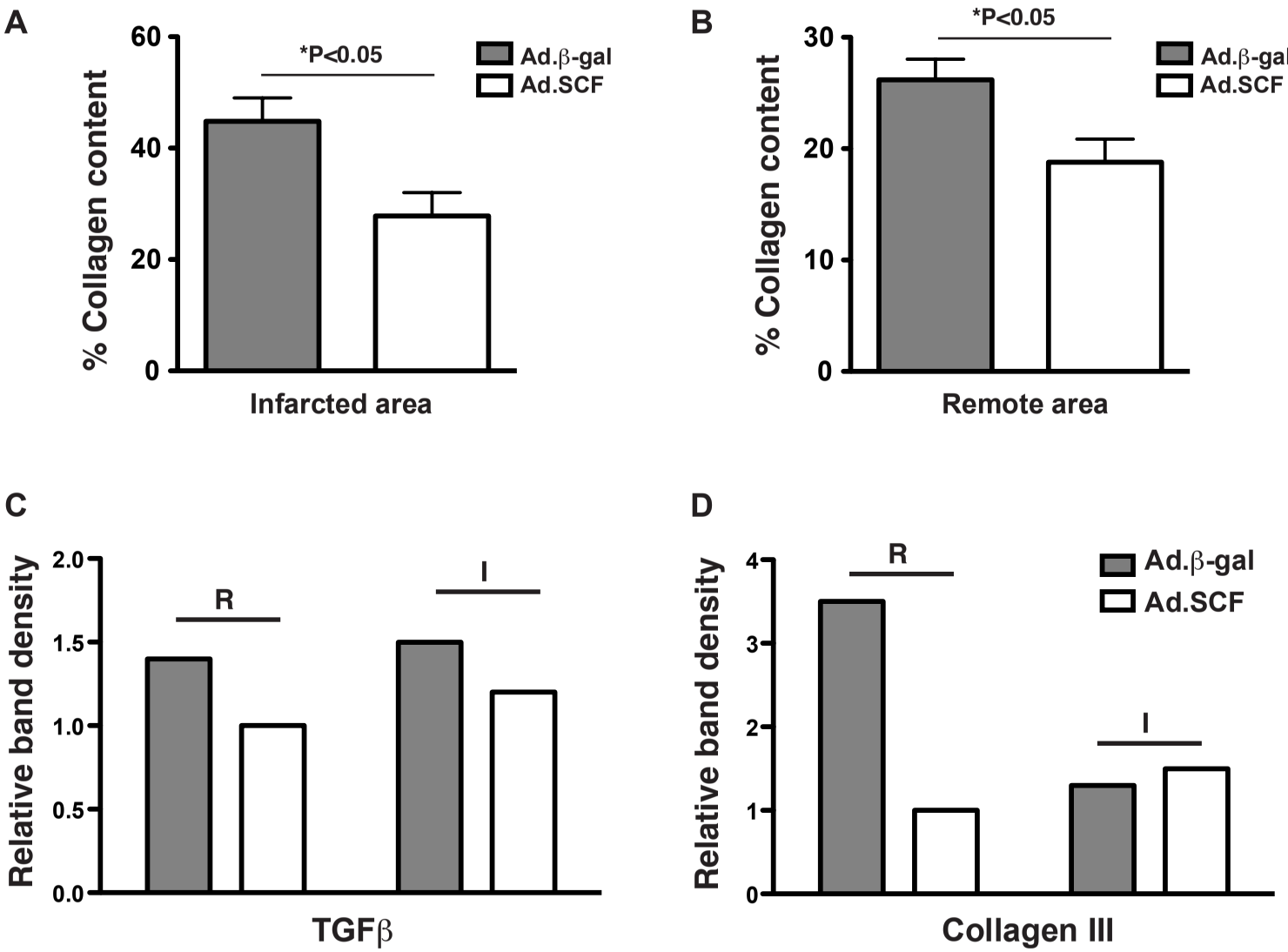
Online Figure I. SCF expression in the myocardium one week post-MI. A) Hematoxylin-eosin staining in the border area from SCF-treated section heart. **B)** Confocal image of the border area from Ad.SCF-treated section heart confirming SCF gene transfer. Blue, DAPI; green, SCF.



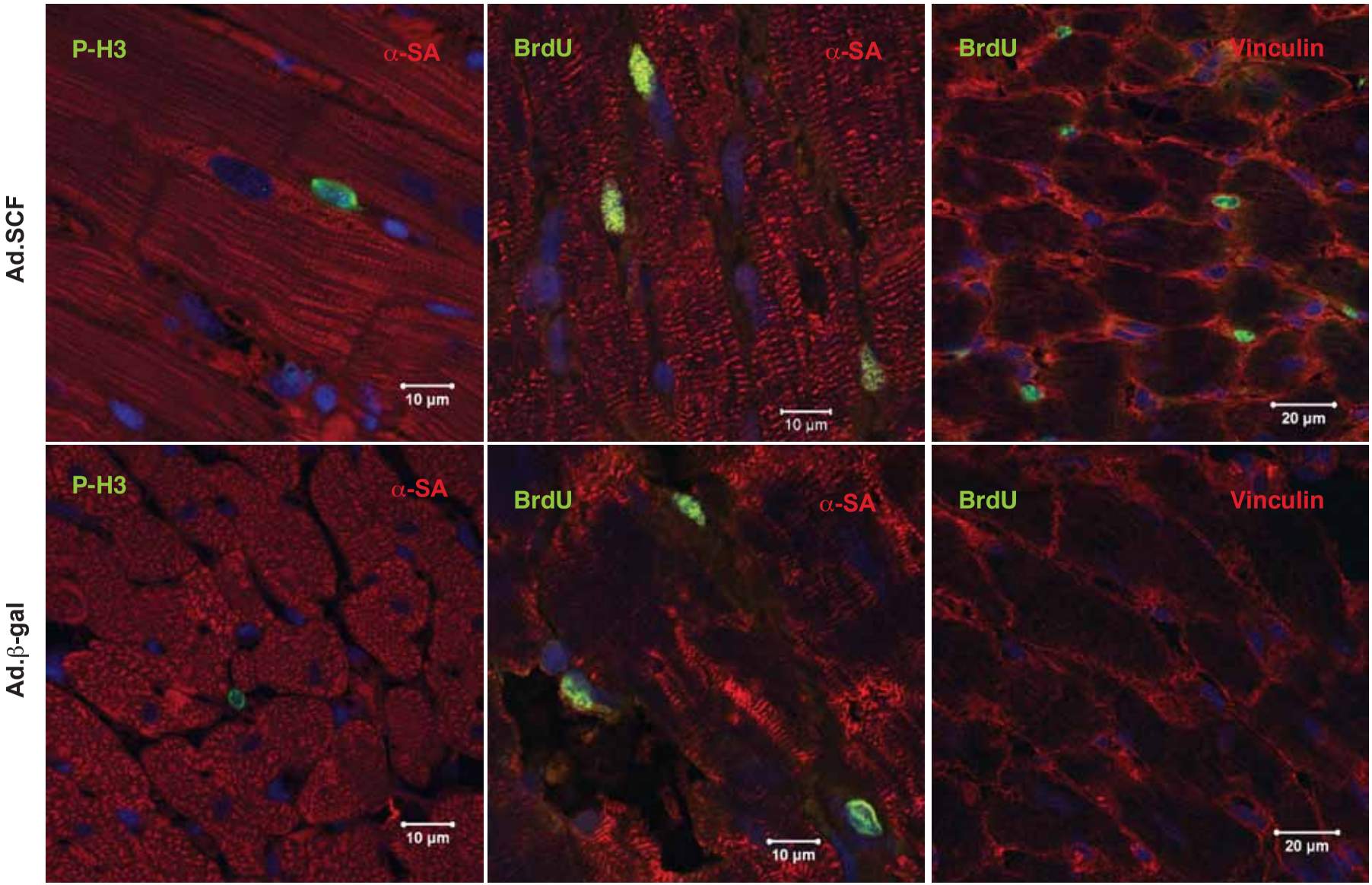
Online Figure II. SCF therapy reduces scar size after myocardium infarction. Representative images of SCF-treated and control hearts. The infarcted area is characterized by LV bleaching.



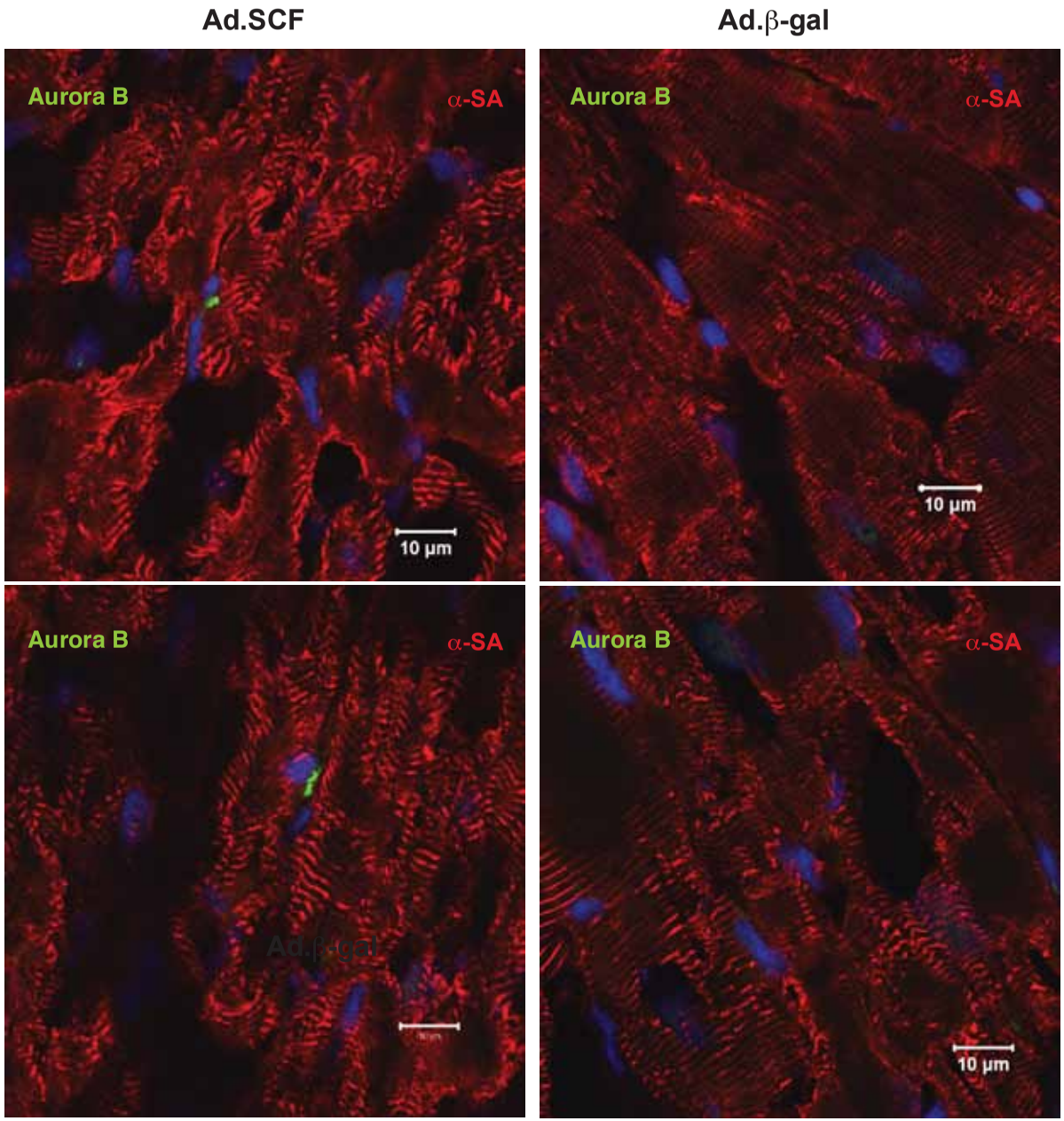
Online Figure III: SCF over-expression decreases infarct size. A) Short-axis cMRI images at 2 and 4 weeks post-MI. Infarct size was determined by hyperintensity bleached area of the scar tissue. **B)** Analysis of scar size at 2 weeks post-MI (15.3%±1.4% Ad.β-gal vs 8.6%±1.6% Ad.SCF; P<0.05) and 4 weeks post-MI (17.7%±0.3% Ad.β-gal vs 7.6%±1.2% Ad.SCF; P<0.01). **C)** Ejection fraction analysis by cMRI at baseline (2 days post-MI) (62.0%±0.5% Ad.β-gal vs 63.5%±1.2% Ad.SCF; P=0.31), at 2 weeks post-MI (48.7%±0.8% Ad.β-gal vs 56.5%±2.9% in Ad.SCF; P=0.06) and at 4 weeks post-MI (48.3%±0.4% in Ad.β-gal vs 58.4%±0.6% in Ad.SCF; P<0.01) post-MI.



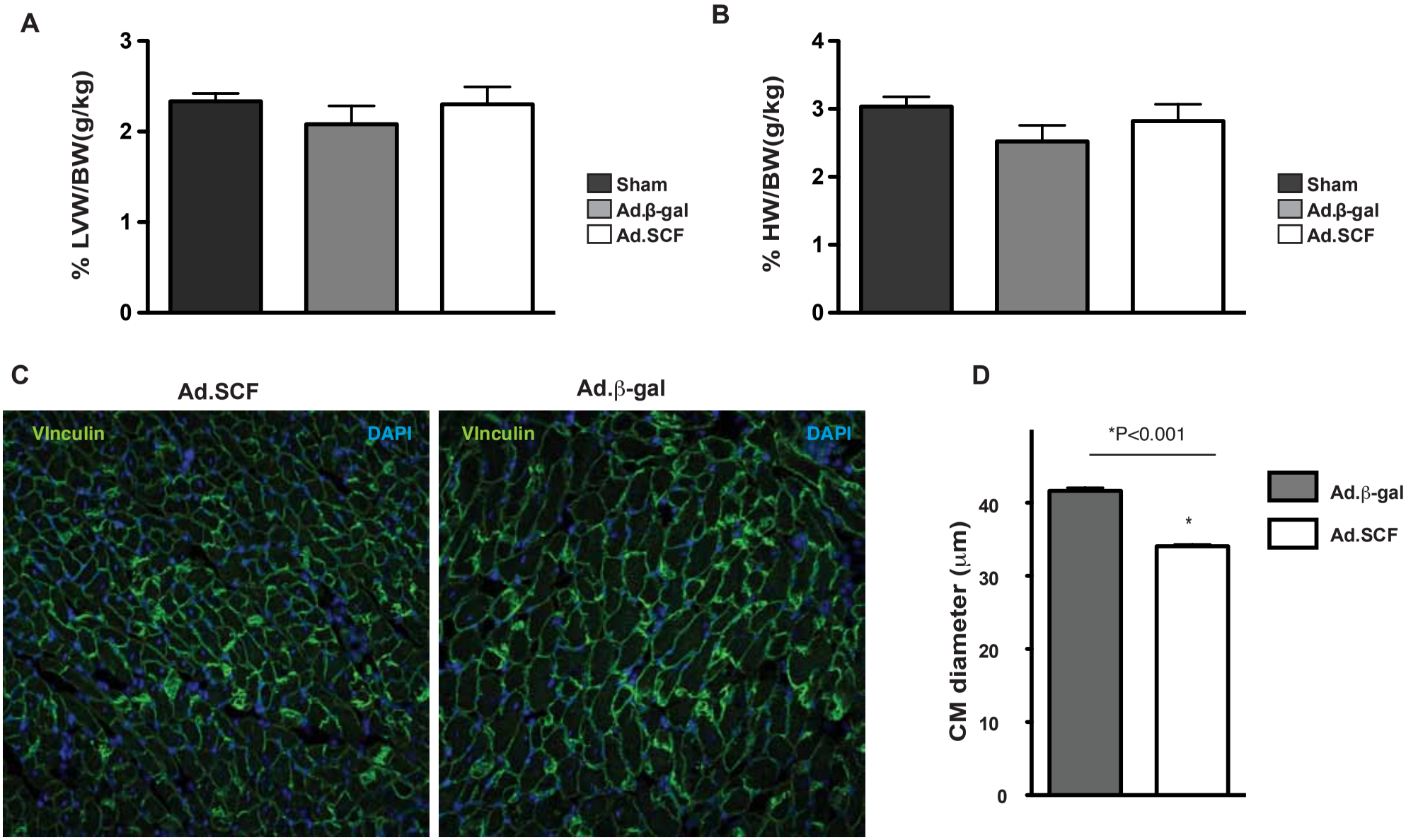
Online Figure IV. Cardiac fibrosis is decreased 3 months post-MI after SCF therapy. **A, B)** Collagen content quantification through Sircol Collagen Assay, in the infarcted (**A**) (44.8%±4.1% in Ad.β-gal vs 27.8%±4.1% in Ad.SCF; $p<0.05$) and remote area (**B**) (26.2%±1.8% in Ad.β-gal vs 18.7%±2.0% in Ad.SCF; $p<0.05$). **C, D)** Quantification of TGFβ (**C**) and collagen III protein levels (**D**), from representative immunoblots in controls and SCF-treated rats at and 3 months post-MI, in the remote (R) and infarcted (I) area.



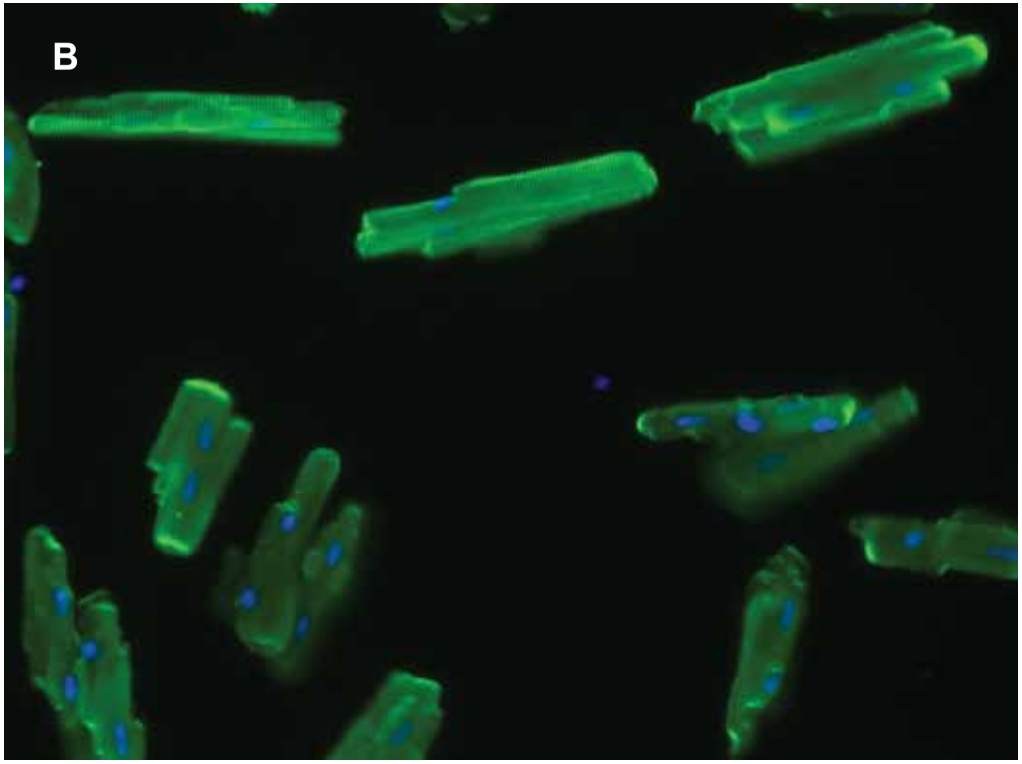
Online Figure V. Induction of cardiomyocytes cell cycle activation by SCF therapy. A) Cardiomyocytes co-expressing α-SA and P-H3 and BrdU or BrdU⁺ cells co-stained with vinculin were detected in the border area 1 week post-MI after SCF gene transfer.

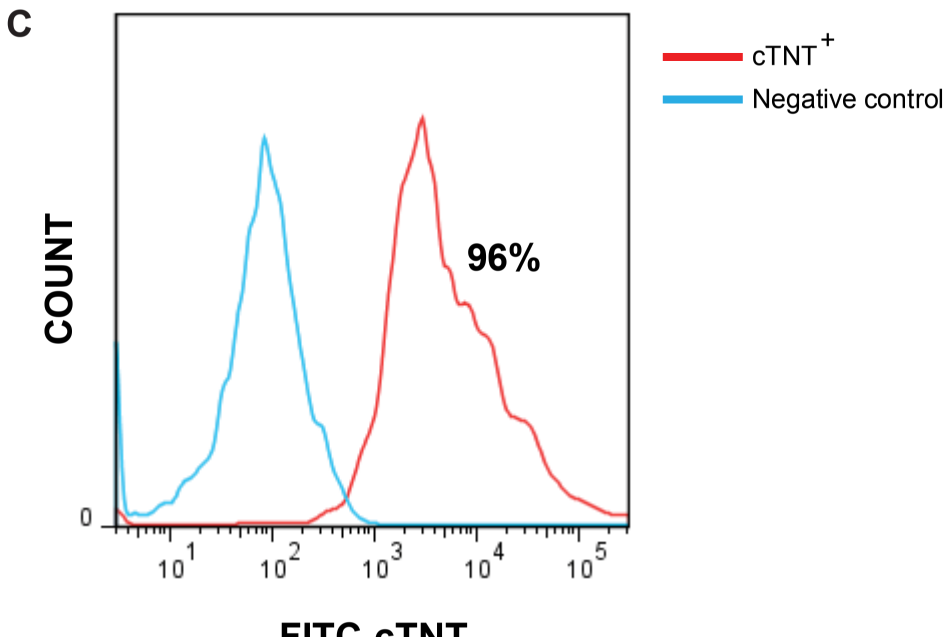


Online Figure VI. SCF over-expression stimulates Aurora B expression 1 week post-MI. Confocal images of representative sections from infarcted hearts at 1 week post-MI in control (Ad.β-gal) and Ad.SCF-treated rats. Blue, DAPI-stained nuclei; Green, Aurora B kinase; Red, α-SA-stained myocardium.



Online Figure VII. SCF over-expression reduces hypertrophy after MI. A) Analysis of left ventricular mass one week post-MI after SCF over-expression. Ratio of LVW/BW (**A**) and HW/BW (**B**). LVW, left ventricular weight; BW, body weight; HW, heart weight. **C**) Confocal images showing vinculin staining of cardiomyocytes cross-sections. Blue, DAPI; green, vinculin. **D**). Quantitative analysis of cardiomyocytes (CM) diameter in controls and SCF-treated hearts (*P<0.0001 vs Ad.β-gal).





Online Figure VIII. Cardiomyocytes isolation from the myocardium at 1 week post-MI. A) Bright field images from isolated cardiomyocytes at 1 week post-MI. **B)** Fluorescence images from isolated cardiomyocytes. Blue, DAPI-stained nuclei; green, α-sarcomeric actin-stained myocardium;. **C,** Analysis by FACS of isolated cardiomyocytes stained with troponin T (cTNT).

	Ad.SCF R vs Ad.β-gal R *P<0.0001	Ad. β-gal I vs Ad.β-gal R *P<0.05	Ad.SCF I vs Ad.β-gal R *P<0.0001
Notch1	2.1±0.3	1.3±0.08	1.6±0.1
HoxB4	5.8±0.5	4.1±1.7	8.2±0.4
Cyclin D1	7.1±0.3	3.7±1.1	7.0±0.3

Online Table I. qPCR analysys of Notch 1, HoxB4 and cyclin D1 expression at 2 weeks post-MI.