SUPPLEMENTAL MATERIAL

Reactivation of the *Nkx2.5* cardiac enhancer after myocardial infarction does not presage myogenesis

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Supplemental Methods

Transgenic Mouse Strains

Mice were housed in an accredited facility in compliance with the European Community Directive related to laboratory animal protection (2010/63/EU). All procedures implemented in mice were approved by the German Heart Center and the local legislation on protection of animals (Commission on Animal Protection, Regierung von Oberbayern, Munich, Germany, 55.2-1-55-2532-141-14, 55.2-1-54-2532.3-17-13) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

All transgenic mouse lines have been previously described in detail. The *Nkx2.5* cardiac enhancer GFP (NkxCE-GFP) mouse was described by Wu et al.[1] as a reporter mouse for embryonic bi- or tri-potent cardiac progenitor cells. The pNTK-mouse was also developed in the laboratory of Sean Wu[2]. It carries the same *Nkx2.5* cardiac enhancer element as the NkxCE-GFP mouse. However, this element is doxycycline-inducible and is followed by a GFP-Cre fusion protein. Mice from the C57Bl/6J strain (Jackson Labs, Stock No 000664) were used as wild type controls. All animal organ or embryo extractions were performed in accordance with the European regulations for animal care and handling (2010/63/EU). For extraction of embryos or organs mice were first anesthetized with isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) and then euthanized by cervical dislocation. All procedures implemented in mice were approved by the German Heart Center and the local legislation on protection of animals (Commission on Animal Protection, Regierung von Oberbayern, Munich, Germany).

Embryos of the transgenic pNTK mice were collected on E8 from timed matings (a positive mating plug indicated E 0.5). Half of the dams were substituted with doxycycline in the drinking water from the beginning of the mating to repress the doxycycline-inducible GFP-Cre expression.

Embryos of double-transgenic *pNTK-ROSA26*^{YFP} mice were collected on E9.5 from timed matings (a positive mating plug indicated E 0.5). Half of the dams were substituted with doxycycline in the drinking water from the beginning of the mating to repress the doxycycline-inducible GFP-Cre and subsequent YFP expression.

Induction of myocardial infarction (MI) by LAD ligation

Adult mice (NkxCE-GFP, pNTK-ROSA^{YFP}) older than eight weeks were anesthetized using midazolam (5.0 mg/kg), medetomidine (0.5 mg/kg) and fentanyl (0.05 mg/kg) via intraperitoneal injection (i.p.), then intubated and ventilated with oxygen enriched air using a small animal respirator (Mouse Ventilator MiniVent Type 845, Harvard apparatus, Holliston, MA, USA). Buprenorphine (0.05 mg/kg) was administered subcutaneously prior to surgery for analgesia. For surgery, mice were placed on a heating pad to maintain body temperature. The chest wall was shaved and a thoracotomy

was performed in the second or third left intercostal space. After placement of traction sutures at the upper and lower rib edges using braided 5/0 suture (Ethibond, Johnson & Johnson Medical GmbH, Ethicon, Norderstedt, Germany), the left ventricle was visualized, the pericardium was then opened and the left anterior descending artery (LAD) was permanently ligated using a 8/0 monofilament suture (Prolene, Johnson & Johnson Medical GmbH, Ethicon, Norderstedt, Germany) at the site of its emergence from under the left atrium. Significant blanching at the ischemic area was considered indicative of successful coronary occlusion. The thoracotomy and the skin were closed with a 5/0 braided suture in a continuous fashion. Narcotics were antagonized by subcutaneous injection of flumazenil (0.5 mg/kg) and atipamezol (2.5 mg/kg). The endotracheal tube was removed once spontaneous respiration resumed. Animals remained on the heating pad until the mice were completely awake. Sham-operated control mice underwent the same surgical procedure except that the suture around the coronary artery was not tied. Preemptive analgesia was provided by buprenorphine (0.05mg/kg BW s.c.) three times per day. After different periods infarcted hearts were extracted and subsequent analyses were performed (described under the respective sections).

For the Thymosin beta 4 (TB4) *in vivo* stimulation experiments NkxCE-GFP mice were primed with TB4 (12mg/kg BW i.p.) daily for seven days before inducing MI. As a control group NkxCE-GFP mice were injected with PBS daily for seven days before inducing MI. Then, in both groups MI was induced by LAD ligation as described above. Surgery was performed in a blinded fashion. The surgeon did not know which mouse belonged to which group. After MI mice achieved additional doses (for 4 days) of TB4 and PBS. Mice were imaged by *in vivo* magnetic resonance imaging (MRI) using a 7T GE/Agilent MR901 at one week after induction of MI to evaluate left ventricular function (LVEF) and infarct size (%LV) (see MRI). Subsequently, hearts were harvested, digested and prepared for flow cytometry analysis (see Flow Cytometry).

Heterotopic cervical heart transplantation

Hetereotpoic heart transplantation was performed as described previously [3]. Briefly, the animals were anesthetized by intraperitoneal injection of midazolam (5.0 mg/kg), medetomidine (0.5 mg/kg) and fentanyl (0.05 mg/kg). Buprenorphine (0.05 mg/kg) was administered subcutaneously prior to surgery. To remove the donor heart, a midline abdominal incision was made and 50 IU heparin was injected into the inferior vena cava. A bilateral thoracotomy was performed and the anterior chest wall was reflected superiorly. The thymus was removed and the superior vena cava was incised. The ascending aorta was punctured with a 30 G needle and the heart was retrogradually perfused with 3 ml of 4 °C Custodiol HTK solution. The venae cavae and the pulmonary vein were ligated and transected. The aorta and pulmonary artery were transected distally and the donor heart was removed. Until implantation the graft was stored on ice in cardioplegic solution (6 h).

To prepare the recipient animal, a transverse skin incision was made from the jugular notch to the right mandibular angle. The right lobe of the submandibular gland was removed. The external jugular vein was mobilized and divided distally between ligatures. The vessel was pulled through a 23 G cuff and occluded proximally with a vascular clamp. The ligature was removed and the vessel lumen was irrigated with 1:10 heparinized saline. The vessel wall was everted over the cuff and fixed with a 8-0 silk ligature. The common carotid artery was mobilized and divided between ligatures just below the carotid bifurcation. The artery was pulled through a 20 G cuff and occluded proximally with a Yasargil clamp. The ligature was removed, the vessel wall was gently dilated, everted over the cuff and fixed with a 8-0 silk ligature.

For graft implantation, the aorta was pulled over the cuff with the carotid artery and the pulmonary artery was pulled over the cuff with the external jugular vein. Anastomoses were secured with circumferential 8-0 silk ligatures. The clamps were removed and 37 °C saline was dropped onto the graft. Normally, the heart developed sinus rhythm within one minute. The skin was closed with a single 8-0 continuous resorbable suture.

Narcotics were antagonized by subcutaneous injection of flumazenil (0.5 mg/kg) and atipamezol (2.5 mg/kg). Preemptive analgesia was provided by 0.05 mg/kg buprenorphine three times per day.

After 1 week of reperfusion donor hearts and recipient original hearts were extracted and subsequent flow cytometry analysis was performed.

Induction of pressure overload-induced cardiac hypertrophy by Transverse Aortic Constriction (TAC)

Transverse aortic constriction (TAC) was performed to create pressure overload-induced cardiac hypertrophy and heart failure. When compared to a complete occlusion of the left anterior descending (LAD) coronary artery, TAC provides a more gradual time course in the development of heart failure[4]. Adult mice (> 8 weeks) were anaesthetized by intraperitoneal injection of midazolam (5.0 mg/kg), medetomidine (0.5 mg/kg) and fentanyl (0.05 mg/kg), intubated, ventilated with a rodent ventilator (Harvard Apparatus, 0.2 mL tidal volume, 150 breaths per minute), and placed in a supine position on a heating pad. Buprenorphine (0.05 mg/kg) was administered subcutaneously prior to surgery. A partial sternotomy was performed to the second intercostal space, the thymus was separated and the aortic arch was identified. Aortic banding was performed by ligating the aorta between the innominate artery and left common carotid artery with a 27-gauge needle using a 5-0 silk suture[5]. The needle was quickly removed, the sternum was readapted, skin was sutured. Narcotics were antagonized by subcutaneous injection of flumazenil (0.5 mg/kg) and atipamezol (2.5 mg/kg) and mice were left to recover until fully conscious. Preemptive analgesia was provided by buprenorphine (0.05mg/kg BW s.c.) three times per day. Hearts were extracted after 1 week or 6 weeks and flow cytometry analysis was performed.

Flow Cytometry

Mouse hearts (MI, TAC, HTX, sham, ctr without operation) were extracted after animals were euthanized by cervical dislocation after anesthetizing them with 2 % isoflurane. As described previously[6], the hearts were immediately isolated and placed in cold 1x PBS. Atria were discarded for the subsequent flow cytometry analysis. Each heart was mechanically dissociated into small pieces, followed by enzymatic digestion with a collagenase II (10000 U/ml, Worthington Biochemical Corporation, Lakewood, NJ)/DNase I (10000 U/µl, Roche) mixture rotating or shaking for 1 h at 37°C including erythrocyte lysis (Red blood cell lysis solution, Miltenyi Biotec, Bergisch-Gladbach, Germany) and a 70 µm-filtration step to obtain single cell suspension. By this filtration step the cardiac cell suspension gets depleted from cardiomyocytes (size discrimination). Dissociated heart cells were washed with PBS and resuspended in PBS/0.5 % BSA/2 mM EDTA for flow cytometry. Dead cells were stained with propidium iodide solution (2 µg/ml, Sigma-Aldrich) or DAPI (4,6-Diamidino-2-phenylindole; 1 µg/ml, BioLegend, SanDiego, CA).

Whole blood from infarcted NkxCE-GFP mice was obtained before harvesting the hearts one week post-MI and erythrocyte lysis (Red blood cell lysis solution, Miltenyi Biotec) was performed before resuspending blood cells in FACS buffer (PBS/0.5 % BSA/2 mM EDTA).

Bone marrow cells from infarcted NkxCE-GFP mice were obtained by flushing tibiae and femora of donor mice with PBS one week post-MI. Rinsed bone marrow was then filtrated by a 30µm filter and flow through was supplied to erythrocyte lysis (Red blood cell lysis solution, Miltenyi Biotec). Bone marrow cells were finally resuspended in FACS buffer (PBS/0.5 % BSA/2 mM EDTA).

Antibody staining of single cell suspensions from infarcted NkxCE-GFP hearts was performed one week post-MI. The cardiomyocyte-depleted cell fractions were further filtrated by a 30µm filter and cells were then incubated for 20min at room temperature with 5% goat serum/PBS to block unspecific bindings. First antibodies (CD45, CD31, CD90, Sca1; all primary antibodies are specified in Suppl Table 2) were incubated over night at 4°C. Alexa 555-conjugated secondary antibodies (1:2000) matching to the species and subclasses of the primary antibodies were subsequently used to label the target cells (e.g. anti-rabbit Alexa Fluor 555 and anti-rat Alexa Fluor 555; both Abcam). CD45 was already conjugated with PE-Cy7 and Sca1 with APC. Stained cells were then resuspended in FACS buffer (PBS/0.5 % BSA/2 mM EDTA). Dead cells were stained with propidium iodide solution (2 µg/ml, Sigma-Aldrich) or DAPI (4,6-Diamidino-2-phenylindole; 1 µg/ml, BioLegend, SanDiego, CA).

Flow cytometry data (cell counting) were acquired using a BD LSRFortessa™ cell analyzer equipped with three lasers (blue, red, violet) (BD Biosciences, San Jose, CA) and the BD FACSDiva software version 8.0.1 (BD Biosciences). Doublet discrimination and exclusion was performed by gating cells according to their FSC-H versus FSC-W and SSC-H versus SSC-W distributions. The results were analyzed with the FlowJo 7.6.5 software (Tree Star, Ashland, OR).

Cell sorting for RNA extraction was performed using a FACS ARIA^{TM} Illu flow cytometer (BD Biosciences, San Jose, CA) and the BD FACSDiva software version 6.1.2 (BD Biosciences). Doublet discrimination and exclusion was performed by gating cells according to their FSC-H versus FSC-W and SSC-H versus SSC-W distributions. NkxCE-GFP-positive cells and their negative counterparts were directly sorted in RNA Lysis Buffer (PeqGold, PeqLab, Wilmington, DE). Flow cytometry data were additionally acquired. The results were analyzed with the FlowJo 7.6.5 software (Tree Star, Ashland, OR).
Cell sorting for *in vitro* cultivation of MICs was performed using a MoFlo^{TM} Legacy flow cytometer (Beckman Coulter, Brea, CA) and the Summit software version 4.3 (Beckman Coulter). Flow cytometry data were additionally acquired. The results were analyzed with the FlowJo 7.6.5 software (Tree Star, Ashland, OR).

Histological analysis of cardiac sections (immunofluorescent, Movat's penatchrome stain)

One week after MI or heterotopic heart transplantation hearts were perfused with phosphate buffered saline (PBS) and then harvested. Hearts were fixed with 4% paraformaldehyde for 12 h at 4°C. Then, hearts were washed 3x with PBS and incubated over night in two parts 30% Sucrose in PBS mixed with one part optimal cutting temperature (OCT, Tissue-Tek, Hartenstein) compound at 4°C until sedimentation. Finally, hearts were embedded in OCT for frozen sections and stored at -80°C for further use (immunohistochemical staining or Movat's stain). OCT-embedded hearts were sectioned with a cryotome (Cryostat, LEICA CM1850) (8µm per section).

Immunohistochemical staining with fluorescent antibodies

Immunohistochemical staining procedure was performed similar to a previously published method[2]. In brief: The frozen sections were first permeabilized in 0.4 % Triton X-100 (Carl Roth, Karlsruhe, Germany) for 10 min and then blocked with 10% normal goat serum (Abcam, Cambridge, UK) for 1 h at room temperature. Cells expressing GFP (MICs) were identified by a primary antibody against GFP (chicken polyclonal, Abcam) 2 h at 37°C (all primary antibodies are specified in Suppl Table 2) which was subsequently labelled with an Alexa Fluor 488-conjugated secondary antibody for 1 h at room temperature (1:1000, Abcam). GFP+ cells were co-stained by primary antibodies against Sca1, Fap, Thy1, Myh10, Wt1, Col1, smooth muscle actin-a (aSMA), Vimentin, Phospho-Histon H3 (PH3) or CD31 (all primary antibodies are specified in Suppl Table 2) to clarify whether they were smooth muscle cells, endothelial cells or fibroblasts/myofibroblasts, respectively. Alexa 555-conjugated secondary antibodies matching to the species and subclasses of the primary antibodies were subsequently used to label the target cells (e.g. anti-rabbit Alexa Fluor 555 1:500 and anti-rat Alexa Fluor 555 1:500; both Abcam). All primary and secondary antibodies were diluted in 1.5 % normal goat serum. Negative controls were performed with secondary antibodies only. After washing the sections two times with 1x PBS and one time with Aq. bidest they were embedded in fluoroshield

mounting medium with 4′,6-diamino-2-phenylindole (DAPI) to counterstain the nuclei (Abcam). Pictures were taken with an AxioCam MRm on an Axiovert 200M fluorescent microscope supported by the AxioVison Rel 4.8 software.

Movat's Pentachrome staining

Movat's Pentachrome staining was performed to identify fibrotic areas (collagen fibers are stained in yellow) and to distinguish them from intact muscle tissue (muscle is stained in red). Adjacent sections from immunofluorescent stainings were used to identify fibrotic and remote areas. Movat's pentachromic staining was performed with the Verhöff staining kit (Morphisto, Frankfurt, Germany) according to the manufacturer's protocols.

In situ 2-photon-microscopy

Infarcted hearts of NkxCE-GFP mice were harvested one week after MI. Hearts were cross-sectioned with a scalpel to expose the infarct area. No fixation or staining procedure was performed. Hearts were analyzed *in situ*. Images were acquired with a TrimScope (LaVision Biotech) connected to an upright Olympus microscope, equipped with a MaiTai Laser (Spectra-Physics) and a 20x water immersion objective (numerical aperture 0.95, Olympus). Scans were performed using a wavelength of 800 nm in a frame of 558 µm × 558 µm with 1030 × 1030 pixels, and a *z*-step of 3 µm in a range of approximately 30 µm.

Cultivation of cells for global MicroArray analysis (ES-derived CPCs, CFs and TTFs)

Murine ES cell line, ES cell culture and in vitro differentiation to produce GFP+ CPCs

The NkxCE-GFP embryonic stem (ES) cell line is a reporter cell line for cardiac progenitor cells/early cardiomyocytes[1], hereafter called CPCs. Upon activation of the NkxCE CPCs begin to express GFP usually on day 5 or 6 of *in vitro* differentiation. As described previously[6, 7] NkxCE-GFP ES cells were grown on a monolayer of mitomycin-inactivated mouse embryonic fibroblasts (MEFs) with murine ES cell medium (Dulbecco's MEM (DMEM with 4.5 g/l glucose; Biochrom, Cambridge, UK), 15 % FCS (Thermo Fisher), 200 U/ml : 200 µg/ml penicillin/streptomycin, 2 mM L-glutamine, 0.1 mM non-essential amino acid solution (Life Technologies), 0.1 mM β-mercaptoethanol (Sigma-Aldrich), and 10^3 U/ml leukemia inhibitory factor (LIF) (Millipore, Billerica, MA, USA). Cells were refreshed with new medium daily and passaged every second day at a 1:6 ratio. Sub-passaging of cells was done by trypsinization. Cells were examined by an Axiovert 200M microscope (Carl Zeiss AG, Jena, Germany).

In vitro differentiation assays of NkxCE-ES cells were performed according to Huang & Wu[8]. In brief, NkxCE-ES cells were prepared for differentiation by growing them on feeder-free gelatin-coated dishes with Iscove's Modified Dulbecco's Media (IMDM) as a medium base (IMDM (PAA), 15 %

FCS, 200 U/ml : 200 µg/ml penicillin/streptomycin, 2 mM L-glutamine, 0.1 mM monothioglycerol (Sigma Aldrich), and 10^3 U/ml LIF) for two days. On the day of differentiation cells were trypsinized and hanging droplets (11 µl) were prepared with a cell concentration of $2*10^5$/ml (about 250 drops per 15 cm plate). Plates are stored upside down till day two when differentiation medium (IMDM, 15 % FCS, 200 U/ml : 200 µg/ml penicillin/streptomycin, 2 mM L-glutamine, 50 µg/ml ascorbic acid (Sigma-Aldrich), 0.1 mM monothioglycerol) was added to the plates after so called embryoid bodies have formed. Cells were differentiated till day 7 and after achieving single cell suspension by collagenase type 2/DNaseI digestion for 60 min at 37°C GFP-positive CPCs were isolated by flow cytometry. Before digestion, pictures were taken with an AxioCam MRm on an Axiovert 200M microscope supported by the AxioVison Rel 4.8 software. CPCs were directly sorted by FACS into RNA-lysis buffer to stabilize total RNA for subsequent RNA purification. Schematic descriptions of *in vitro* differentiation assays and further procedures are provided in the respective Figs (Suppl Fig S6B). CPCs from three different differentiation assays were used for Micro Array analysis.

Cultivation of CFs and TTFs

Murine cardiac fibroblasts (CFs) and murine tail tip fibroblasts (TTFs) from NkxCE-GFP mice were prepared by digesting either hearts without the atria or tailtips with collagenase type 2/DNaseI for 1 h at 37°C. After centrifugation cells and remaining tissue were resuspended in fibroblast medium (Dulbecco's MEM (DMEM; Biochrom, Cambridge, UK) supplemented with 10% FCS (Thermo Fisher), 4.5 g/l glucose, 2 mM L-glutamine and 100 U/ml penicillin/100 µg/ml streptomycin) and plated on gelatin-coated 6 well plates. On the next day cells were extensively washed with PBS to remove tissue residues and cells that have not settled down. Remaining cells were cultivated in fibroblast medium. Passaging was performed several times by trypsinization at a 1:3 ratio. Cells were directly lysed in the cell culture dishes with RNA lysis buffer (PeqLab) for further RNA isolation (Suppl Fig S6C-D). CFs and TTFs from three different mice were used for Micro Array analysis.

Total RNA isolation

Total RNA was extracted from flow cytometry isolated GFP+ MICs (one week post MI), the corresponding GFP negative cell populations, from *in vitro* differentiated CPCs (7 days) and from cardiac and tailtip fibroblasts as described previously[6]. In brief, total RNA (including mRNA, microRNA and other small RNAs) was isolated using the peqGold total RNA Kit according to the manufacturer's instructions (Peqlab, Erlangen, Germany).

Assessment of gene expression by qRT PCR

Total RNA was reverse transcribed into first strand cDNA with random hexamer primers (Invitrogen, CA, USA) by M-MLV reverse transcriptase (Invitrogen) for subsequent gene expression analysis as described previously[6, 7]. Amplification was performed on a LightCycler 1.5 (Roche Diagnostics, Basel, Switzerland) using gene-specific primer sets (ELLA Biotech, Munich, Germany) and QuantiTect SybrGreen (Qiagen, Hilden, Germany) for 40 cycles. The sequences of the used primers are indicated in Supplemental Table S1. PCR conditions: 95°C for 15 min to activate *Taq* polymerase, followed by 40 cycles of 95°C for 10 sec, annealing at 60°C for 20 sec and synthesis at 72°C for 20 sec. ΔCT calculations were performed by a Light Cycler 1.5 (Roche). β-actin was used to normalize gene expression.

micro Arrays (mRNA)

For MicroArray analysis total RNA samples from NkxCE-GFP+ MICs (one week post MI), murine ES-derived CPCs (in-vitro differentiated for 7 days), murine CFs and TTFs (cultivated for several passages < 4) were used (each n = 3). First, the integrity of the total RNA was assessed through analysis on an Agilent Bioanalyzer 2100 (Agilent Technologies, Böblingen, Germany). Samples with a RIN less than seven were excluded from further analyses. *A two-round amplification and labeling was done with the TargetAmp-Pico Labeling Kit (Epicentre, Illumina, CA, USA) starting with 500 pg total RNA and finally Biotin-aRNA was analyzed with the Mouse WG-6 v2.0 Expression BeadChip (Illumina).* BeadChips were scanned using the Illumina Bead Array Reader.

Bioinformatic Analysis of MicroArray data

Mouse WG-6 v2.0 chip (Illumina) raw intensity data were imported in Genome Studio v2.0.2 for background subtraction, quantile normalization and $\log_2$ transformation signal values. Moderated *t*-test analysis with Benjamini and Hochberg (BH) multiple testing correction was utilized in GeneSpring GX v13.0 to obtain genes whose fold change between comparisons was ≥ 1.5 (with a p value cut-off of ≤ 0.05). Average linkage hierarchical clustering was performed, and the Euclidean complete distance metric was used as a measure of similarity between the gene expression profile samples based on $\log_2$-transformed signal values across the differentially-expressed genes. The differential expression gene list derived from moderated *t*-tests was plotted on Heat-Maps and divided in clusters generated by pathway analysis tool IPA (Ingenuity Systems, www.ingenuity.com) used for gene set enrichment analysis and gene network analysis. GO-terms for gene clusters were analyzed by DAVID 6.8 Functional Annotation Tool[9, 10]. The Venn diagram was performed by R statistical environment with the package "venneuler"[11] (R package version 1.1-0. https://CRAN.R-project.org/package=venneuler). Comparisons of groups was submitted to principal component analysis (PCA) performed using R

statistical environment [R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/].

Assessment of microRNA expression by qRT PCR

For microRNA expression analysis total RNA samples from NkxCE-GFP+ MICs (one week post MI), murine ES-derived CPCs (in-vitro differentiated for 7 days), murine CFs and TTFs (cultivated for several passages < 4) were used (each at least n = 3). Total RNA was reverse transcribed using a miRCURY LNA Universal RT microRNA PCR Kit, Universal cDNA Synthesis Kit II (Exiqon, Vedbaek, Denmark) in a total volume of 10 µl (6 µl sample RNA, 2 µl 5x buffer, 1 µl RNA spike (UniSp6) and 1 µl enzyme mix). The mixture was incubated for 1 h at 42 °C, followed by 5 min at 95 °C before storage at −20 °C. Polymerase chain reactions were conducted with a ViiA™ 7 Real-Time PCR system (Applied Biosystems, CA, USA) with cycle settings recommended by Exiqon. The qRT-PCR reaction mixture (10 µl) contained 4 µl cDNA (1:80 dilution), 5 µl ExiLent SYBR Green Mix (Exiqon) and 1 µl LNA PCR primer sets (Exiqon). LNA PCR Primer sets (Exiqon) used for microRNA analysis were specific for: U6snRNA (hsa,mmu,rno, #203907), hsa-miR-1 (#304344), mmu-miR-133a-5p (#205320), hsa-miR-21-5p (#204230), mmu-miR-29-1-5p (#205411). Initial activation/denaturation took place at 95 C for 10 min, followed by 40 cycles each consisting of 10 s denaturation at 95 °C and 60 s annealing and extension at 60 °C (ramp rate of 1.6 °C/s). Melting curves were plotted at the end of each cycle series to verify the purity of the products. With the use of synthetic RNA templates (UniSp6) we controlled for isolation yield, cDNA synthesis and PCR efficiency. Expression levels of the measured miRNAs were normalized against U6 snRNA. The delta delta Ct method was performed to obtain the relative miRNA expression levels

In vitro cultivation of MICs including stimulation by various substances

Infarcted NkxCE-GFP mice were euthanized after 1 week, hearts were extracted, single cell suspension was prepared, and 20 GFP+ MICs were sorted by FACS moFloII directly into collagen-coated 96 well plates filled with 100µl standard differentiation medium with ascorbic acid (Iscove's medium (IMDM; Biochrom), 15 % FCS, 200 U/ml : 200 µg/ml penicillin/streptomycin, 2 mM L-glutamine, 50 µg/ml ascorbic acid (Sigma-Aldrich), 0.1 mM monothioglycerol). One 96 well plate was cultivated for one week, a second plate for two weeks. After the respective period cells were fixed with 4% paraformaldehyde and nuclei were counterstained with DAPI to easily count the cells. By this, 96 wells were counted after1 week and 96 wells were counted after 2 weeks.

Calculation of doubling time

Doubling times were calculated with the following formula: Doubling Time = {duration * \log(2) \over \log(final_concentration) - log(initial_concentration)}, where "log" is the logarithm to base 10 or 2 or any other base (Roth V. 2006 Doubling Time Computing, available from: http://www.doubling-time.com/compute.php).

Stimulation

Infarcted NkxCE-GFP mice were euthanized after 1 week, hearts were extracted, single cell suspension was prepared, and 20 GFP+ MICs were sorted by FACS moFloII directly into collagen-coated 96 well plates filled with 100µl standard differentiation medium with ascorbic acid (IMDM, 15 % FCS, 200 U/ml : 200 µg/ml penicillin/streptomycin, 2 mM L-glutamine, 50 µg/ml ascorbic acid (Sigma-Aldrich), 0.1 mM monothioglycerol). Two days after plating MICs were subjected to stimulation by several substances: ActivinA (recombinant hu/mu/rat ActivinA, 100ng/ml and 500ng/ml; R&D Systems, MN, USA, according to Chen et al. (2012)[12]), Bone morphogenic protein 4 (BMP4) (recombinant mu BMP4, 100ng/ml and 500ng/ml; R&D Systems, according to Chen et al. (2012)[12]), Thymosin β4 (TB4) (100ng/ml and 500ng/ml; RegenRX, Maryland, USA; we are greatful to Paul Riley from the Oxford University for the TB4 gift), A83-01, a small molecule ALK4,5,7 inhibitor (1 µM and 10µM; Tocris-Bioscience, Bristol, UK; according to Chen et al. (2012)[12]). Every second day MICs were refreshed with new medium substituted with the above mentioned substances. For each condition at least 6 wells were treated on the same 96 well plate. To exclude well-specific effects that are well known to affect proliferation of cells a control plate without stimulation was cultured. For control-calculations only the same wells as on the stimulation plate were considered. After 1 week of stimulation cells were fixed with 4% paraformaldehyde, permeabilized with 0.25 % Triton X-100 (Carl Roth, Karlsruhe, Germany) in PBS for 15 min and nuclei were stained with DAPI to easily count the cells. Two 96 well plates were subjected to stimulation, one 96 well plate served as untreated control.

Immunocytochemistry of in vitro cultivated MICs

Infarcted NkxCE-GFP mice were euthanized after 1 week, hearts were extracted, single cell suspension was prepared, and 20 GFP+ MICs were sorted by FACS moFloII directly into collagen-coated 96 well plates filled with 100µl standard differentiation medium with ascorbic acid (IMDM, 15 % FCS, 200 U/ml : 200 µg/ml penicillin/streptomycin, 2 mM L-glutamine, 50 µg/ml ascorbic acid (Sigma-Aldrich), 0.1 mM monothioglycerol). After different cultivation times (1 - 3 weeks) on collagenized 96 well plates MICs were fixed with 4% paraformaldehyde for 15 min at room-temperature (RT) and washed with 1x PBS once. MICs were then permeabilized with 0.25 % Triton X-100 (Carl Roth) in PBS for 15 min. After blocking with 5 % normal goat serum (Abcam) in PBST for 1 h at RT cells were incubated with primary antibodies which were diluted in 1.5 % normal goat serum for 1 h at 37°C. Primary antibodies that were used are specified in Supplemental Table 2. After

two washes with PBS, fluorochrome-conjugated secondary antibodies (e.g. anti-chicken Alexa Fluor 488 1:1000, anti-rabbit Alexa Fluor 555 1:500, anti-rat Alexa Fluor 555 1:500; Abcam) matching to the species and subclasses of the primary antibodies were used for detection (1 h at RT, in the dark). Negative controls were performed with secondary antibodies only. After washing MICs twice with 1x PBS and 1 time with Aq. bidest cells were embedded in fluoroshield mounting medium (Abcam) with DAPI to counterstain the nuclei. At least 24 wells were stained with each antibody.
All immunostainings were examined by an Axiovert 200M microscope (Zeiss, Oberkochen, Germany) and images were taken with an AxioCam MRm (Zeiss) and the AxioVison Rel 4.8 software version 2.9 (Zeiss).

Epigenetic analysis of the Nkx2.5 genomic locus

Previously published Hi-C[13, 14], DNA methylation[14-16], RNA-seq[14, 16] and ChIP-seq[16, 17] data were reanalyzed. Principal component analysis was performed on Hi-C data using the HiC-Explorer[18]. RNA-seq and ChIP-seq data were mapped and genome wide read coverage was calculated. DNA methylation data was mapped using Bismark[19]. Coverage tracks were calculated using deepTools[20] and the HiC-Explorer[18] was used for visualization.

Magnetic Resonance Imaging (MRI)

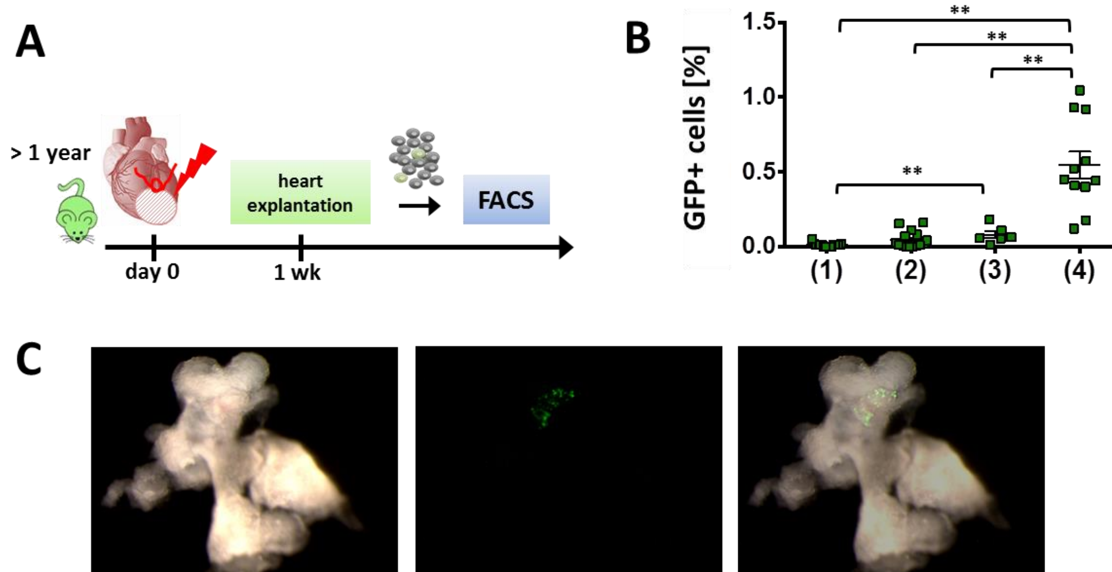
Mice were imaged by *in vivo* magnetic resonance imaging (MRI) using a 7T GE/Agilent MR901 at one week after induction of LAD ligation to evaluate left ventricular function (LVEF) and infarct size (%LV). Control mice without infarction were additionally imaged. All mice were pre-anaesthetized in a closed chamber with 3% isofluorane in oxygen. Anaesthesia was then maintained at 1.5% isofluorane in oxygen.
Cardiac function parameters were assessed by short axis based views in gradient echo pulse sequences and infarcted myocardium was delineated in late gadolinium enhancement (LGE) images, with a flexible coil array, ECG and respiratory gating (Rapid Biomedical GmbH). Imaging was performed 20-25 minutes after intraveneous injection of 0.6 mmol/kg Gd-DTPA, with the following imaging parameters: echo time, 2.6 ms; 16 frames per RR interval (TR 4.3-6.9 ms); resolution 256 x 256 x 1000 μm^3; NEX 2; flip angle 60°(3). Quantitative analysis of the gradient echo pulse sequence MRI was calculated using a semi-automated approach employing a software package MunichHeart/MR. The epi- and endocardial contours of the LV slices were manually traced at end-diastolic (EDV) and end-systolic (ESV) phases for calculation of the corresponding LV volumes, as well as the left ventricle ejection fraction (LVEF).

Data Analysis and Statistics

Data are presented as mean values ± standard error of the mean (S.E.M.). Statistical differences were evaluated using the unpaired Student's t-test or the Mann-Whitney-U test. Comparison of several groups was performed by one way ANOVA or Kruskal-Wallis test on ranks including appropriate post-hoc tests. A value of $p < 0.05$ was considered to be statistically significant. In all figures statistical significance is indicated as follows: * means $p < 0.05$ and ** means $p < 0.01$.

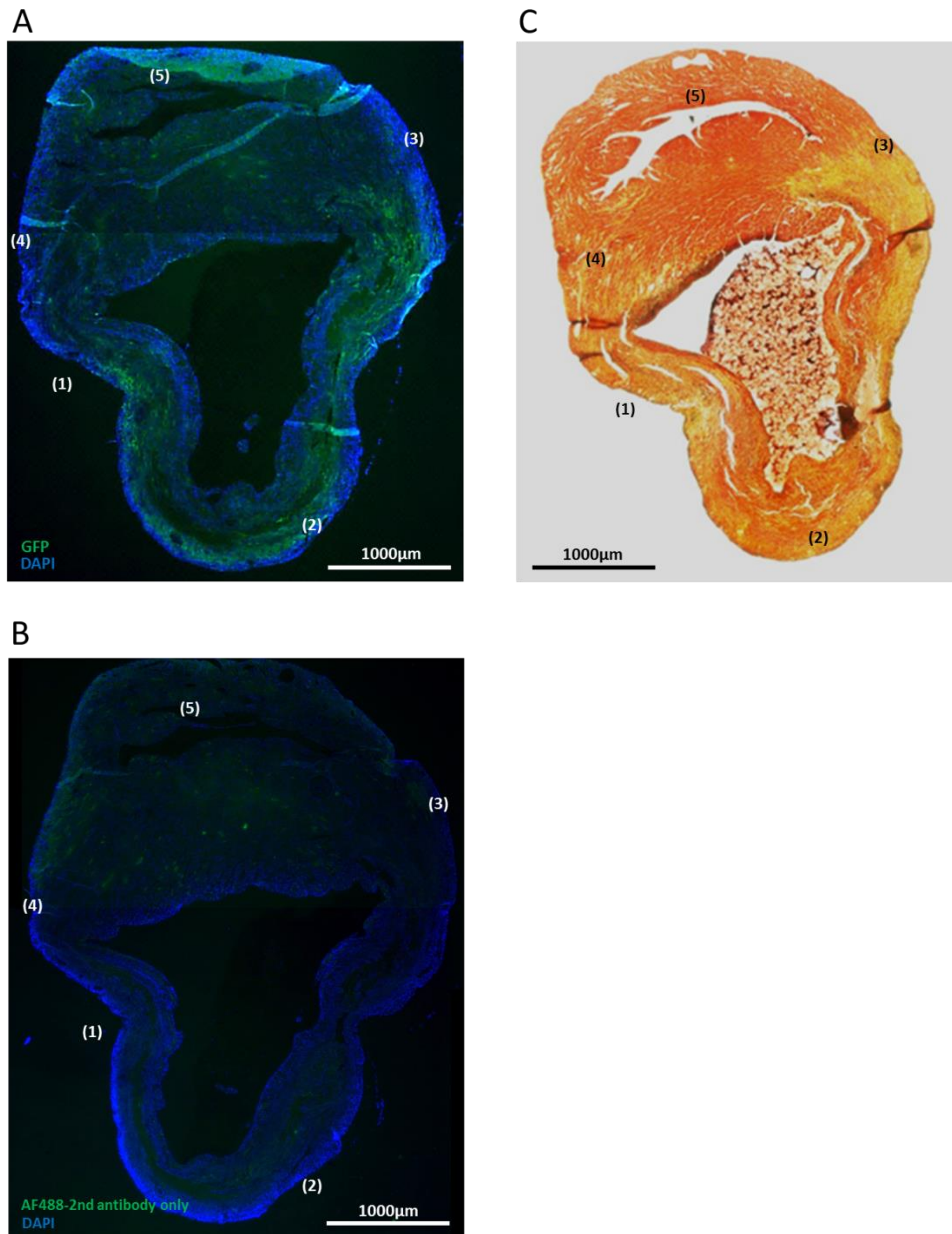
Data Availability

The datasets generated during and/or analysed during the current study will be available in the GEO repository from NCBI (https://www.ncbi.nlm.nih.gov/gds).



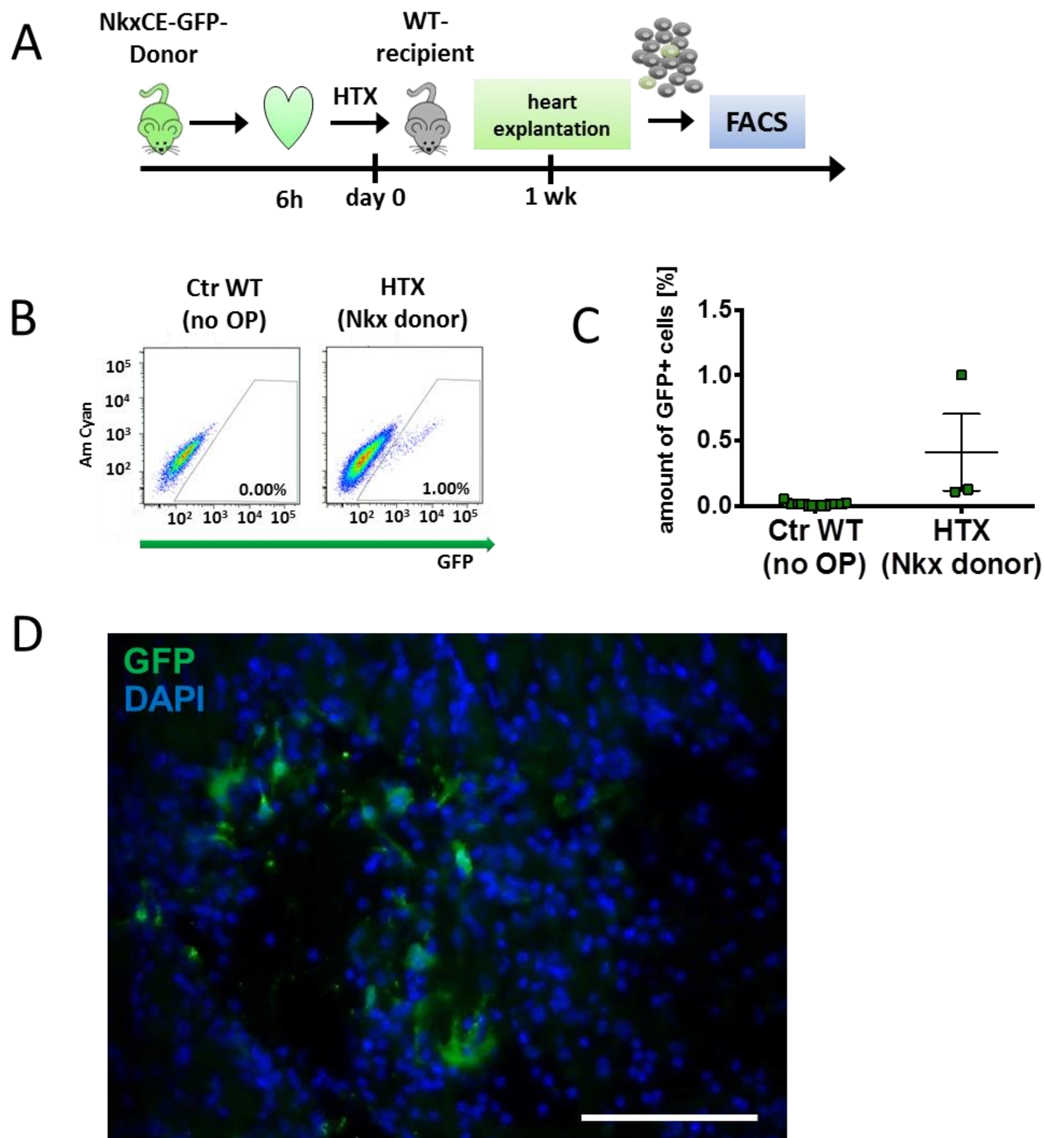
Supplemental Figure S1. Identification of NkxCE-GFP-positive cells in adult mouse hearts after myocardial infarction (MICs).

(A) Myocardial infarction (MI) was induced by ligation of the left anterior descending artery (LAD) in NkxCE-GFP mice older than 1 year; heart explantation 1 week post MI. Heart tissue was digested to achieve single-cell suspension which was cardiomyocyte-depleted due to several filter-steps. GFP-positive cells were quantified by flow cytometry analysis (FACS). (B) About 0.5% of GFP+ cells were detected in aged (> 12 months) infarcted NkxCE-GFP mice. Groups: (1) Ctr WT (no OP): Wild type mouse (C57Bl/6) without surgery. (2) Ctr Nkx (no OP): NkxCE-GFP mouse without surgery. (3) Ctr Nkx (sham): NkxCE-GFP mouse with sham operation. (4) MI Nkx (> 12 mths): NkxCE-GFP mouse with LAD ligation (> 12 months). ** $p < 0.01$ (Kruskal-Wallis, postHoc: Dunn's Method). (C) Cardiac-specific weak GFP-fluorescence in an NkTGC-embryo (E8) whole-mount in-situ under a fluorescent microscope without doxycycline substitution of the drinking water of the mother during embryonic development.



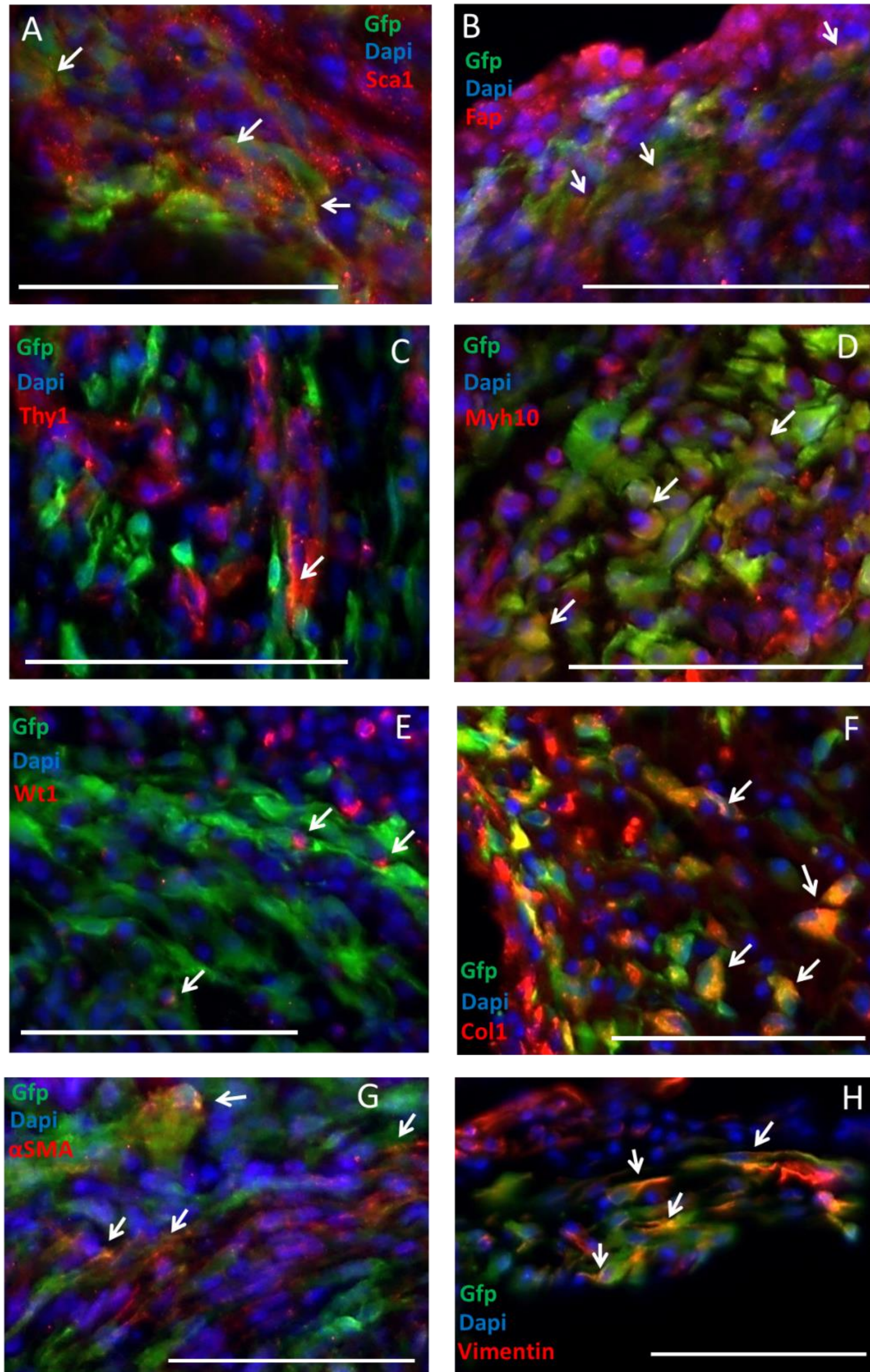
Supplemental Figure S2. Localization of NkxCE-GFP+ MICs after MI.

(A) NkxCE-GFP heart, 1 week after MI. Section was stained for GFP (green), (DAPI = nuclei, blue). GFP+ MICs are visible in the infarction area and border zone (1, 2, 3, 4) but not in the remote myocardium (5). (B) NkxCE-GFP heart, 1 week after MI. This section served as a negative control for (A) since only the second antibody (conjugated with AF488, green) was used to show the autofluorescence level), (DAPI = nuclei, blue). No GFP+ MICs are visible in the whole section indicating that the GFP-fluorescence in (A) is specific. (C) Movat's Pentachrome staining of an adjacent section to A. to depict the infarction area (yellow, collagen fibers) and the healthy remote area (red, muscle). Yellow areas (collagen fibres) overlap with areas where GFP+ MICs are visible in the infarction area and border zone (1, 2, 3, 4) but not in the remote myocardium (5).



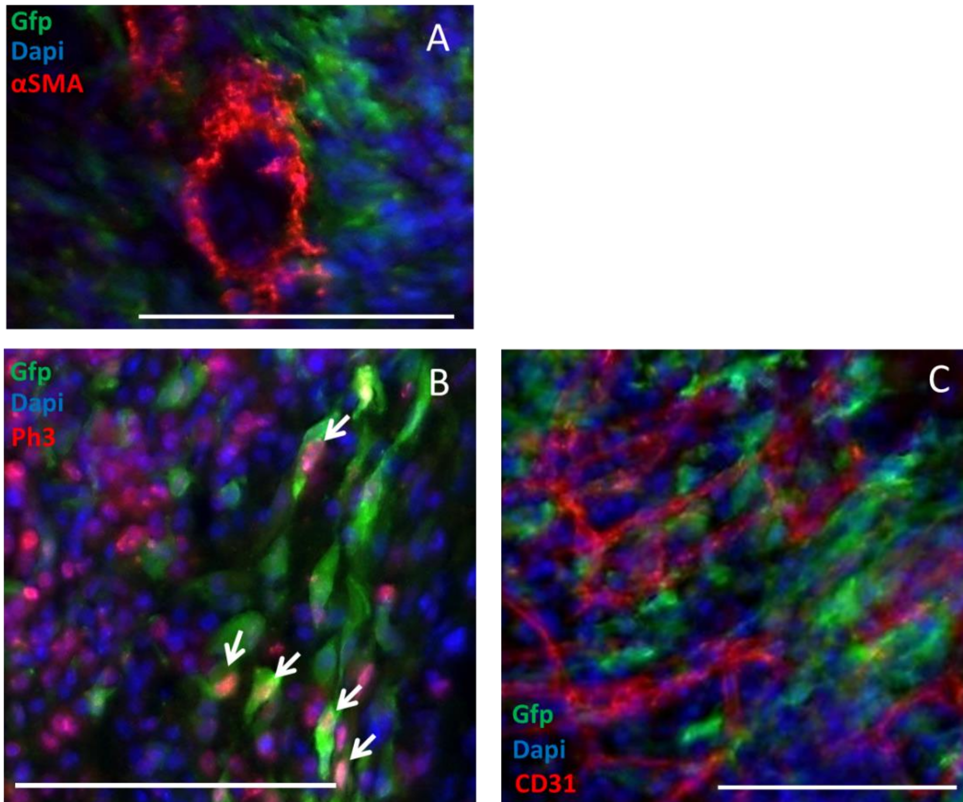
Supplemental Figure S3. Origin of NkxCE-GFP+ MICs in the adult mouse heart after MI.

(A) Hearts of transgenic NkxCE-GFP mice were harvested and transplanted into C57Bl/6 wild type recipients after 6 h of ischemia. After 1 week hearts were explanted and GFP+ cells were quantified by FACS. (B) Exemplary FACS plots show an induction of GFP+ cells in transplanted transgenic hearts. Ctr WT (no OP): Wild type mouse (C57Bl/6) without surgery. HTX (Nkx donor): Cervically transplanted heart of transgenic NkxCE-GFP mouse. (C) In ischemia/reperfusion (I/R) donor hearts from NkxCE-GFP mice a similar amount of GFP+ MICs as in MI-NkxCE-GFP hearts emerged. (groups see B.). (D) Staining of emerging GFP+ MICs (anti-GFP-antibody, green) after I/R injury in a transplanted NkxCE-GFP heart (DAPI = nuclei, blue). Scale bar: 100µm.



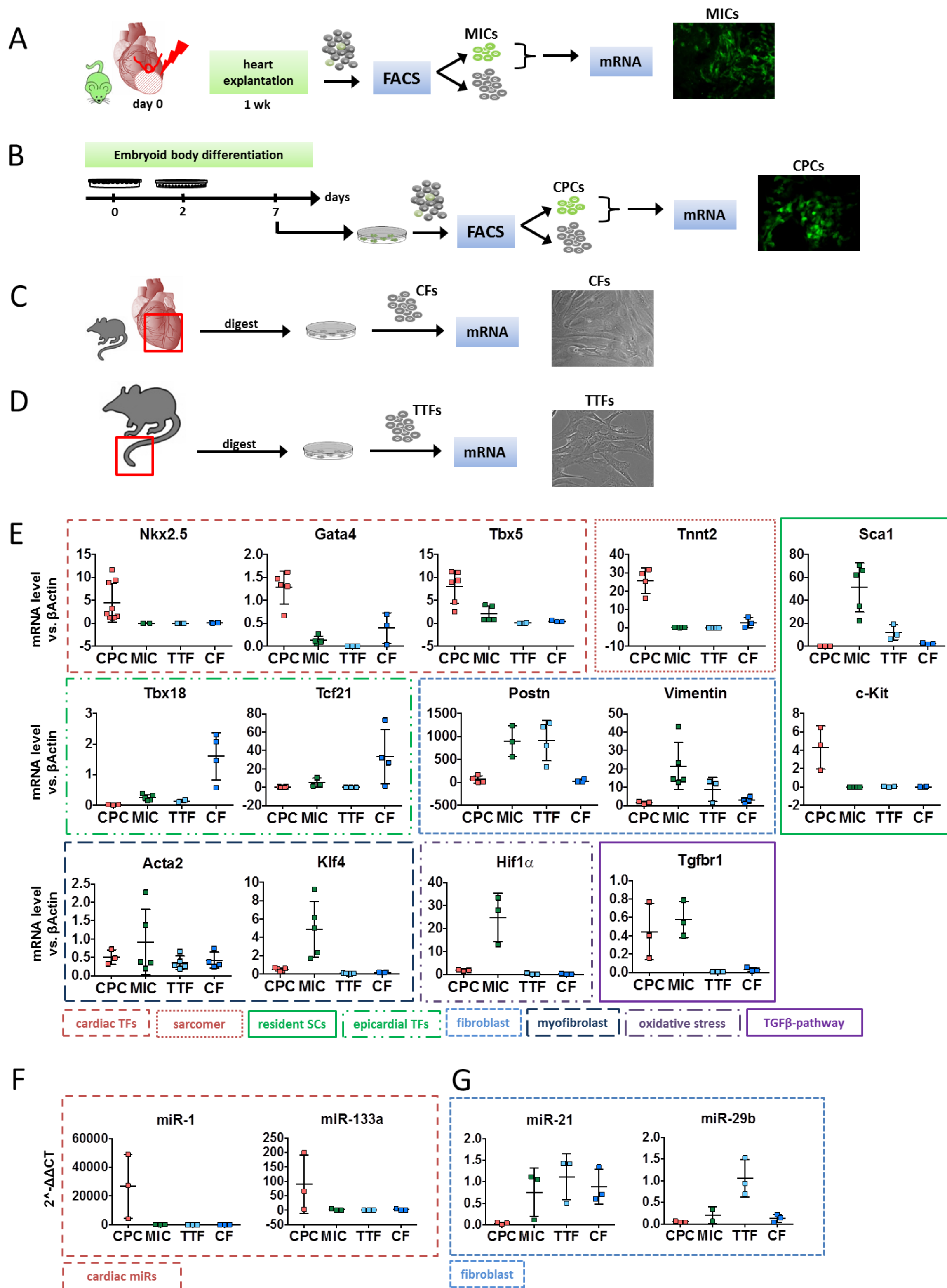
Supplemental Figure S4. Immunohistochemical staining of NkxCE-GFP+ MICs after MI.

Immunofluorescent staining of NkxCE-GFP infarcted heart sections one week after MI. Double staining for GFP (MICs, green) and select additional antibodies (red) were performed as indicated. (A) Gfp/Sca1. (B) Gfp/Fap. (C) Gfp/Thy1. (D) Gfp/Myh10. (E) Gfp/Wt1 (F) Gfp/Col1. (G) Gfp/αSMA. (H) Gfp/Vimentin. Double positive cells were detected for all different antibodies indicating that MICs express respective proteins. Nuclei were counterstained with DAPI. Scale bars: 100 µm. White arrows indicate select double positive MICs.



Supplemental Figure S5. Immunohistochemical staining of NkxCE-GFP+ MICs after MI.

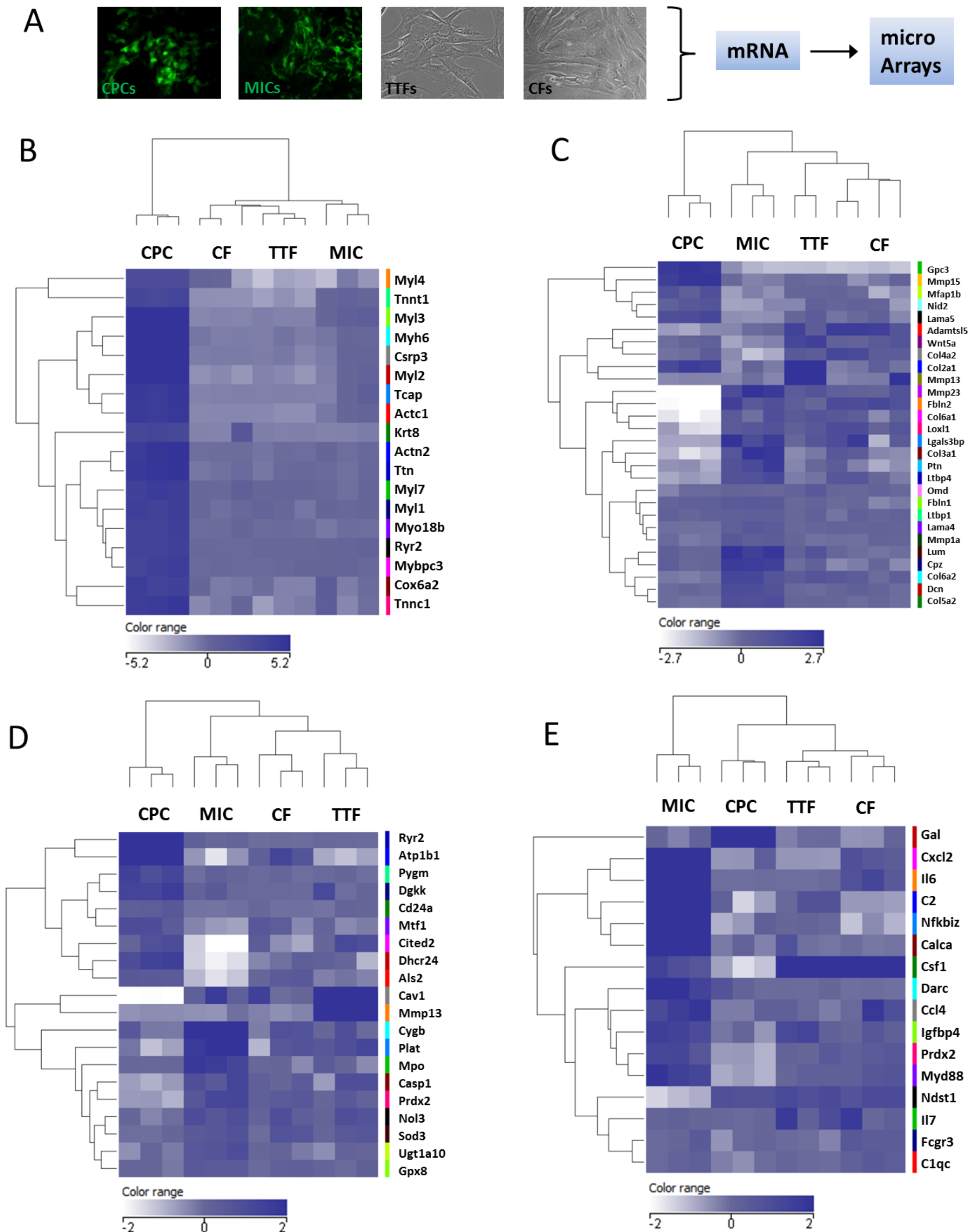
Immunofluorescent staining of NkxCE-GFP infarcted heart sections one week after MI. Double staining for GFP (MICs, green) and selected additional antibodies (red) were performed. Nuclei were counterstained with Dapi. Scale bars: 100µm. (A) αSMA antibody stains vascular smooth muscle cells. (B) A marked amount of MICs was positive for the proliferation marker PH3 (phospho histon H3). (C) No MICs were positive for CD31.



Supplemental Figure S6. Characterization of NkxCE-GFP+ MICs after MI.

(A) MI was induced in NkxCE-GFP mice. After 1 week hearts were explanted, GFP-positive MICs were sorted and total RNA was extracted for Microarray Analysis. (B) Transgenic NkxCE-GFP embryonic stem cells were *in vitro* differentiated by the hanging drop method. Embryoid bodies formed after 2 days when plates were flooded with differentiation medium. GFP-positive cardiac

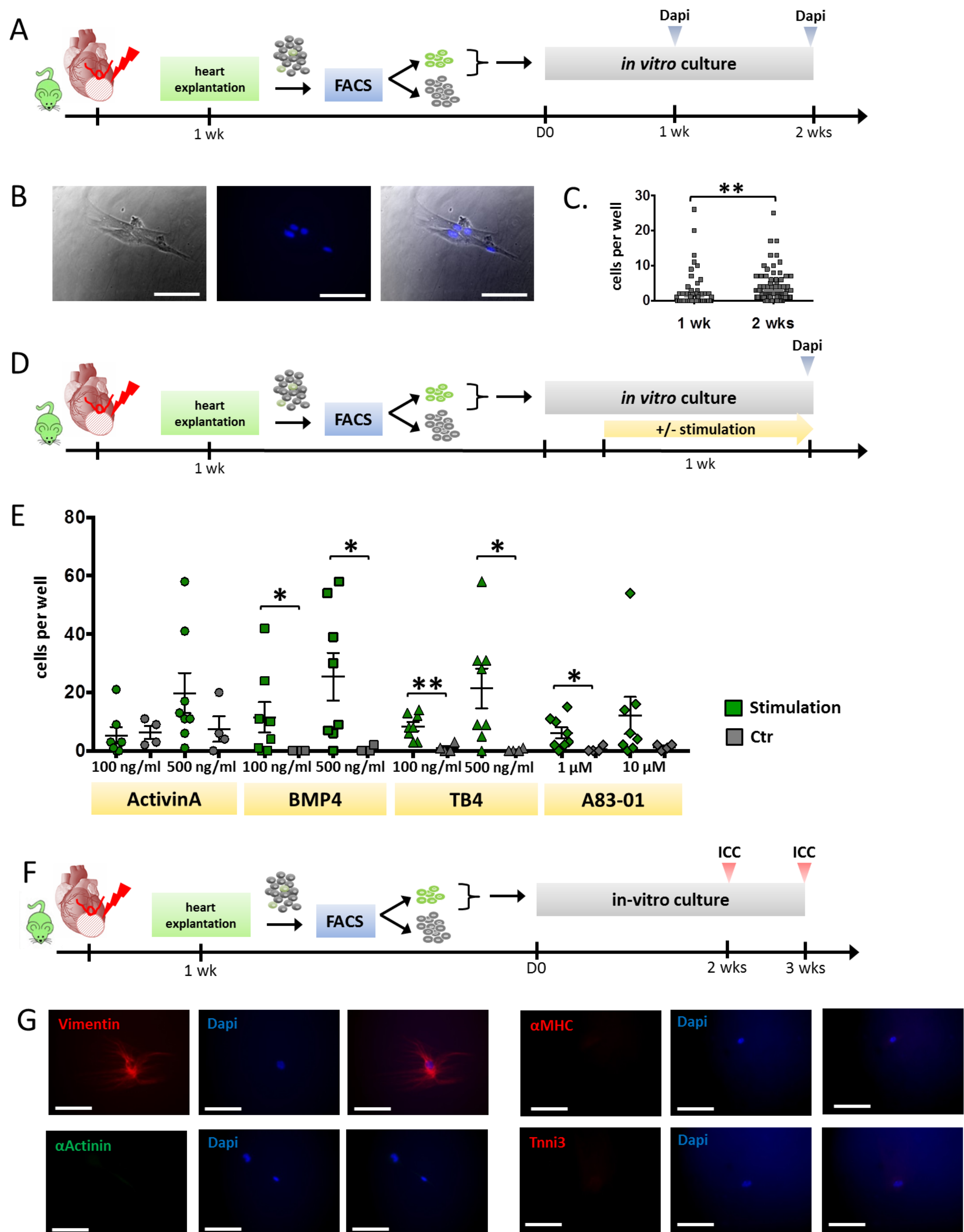
progenitor cells (CPCs corresponding to early cardiomyocytes) were sorted after 1 week of differentiation and total RNA was extracted for Microarray Analysis. (C) Cardiac fibroblasts (CFs) were generated from murine hearts by performing collagenase type II-digestion and plating the cell suspension on cell culture ware with standard fibroblast medium. Cells were harvested after passaging the cells a few times and total RNA was extracted for Microarray Analysis. (D) Murine tailtip fibroblasts (TTFs) were generated from murine tailtips by performing collagenase type II-digestion and plating the cell suspension on cell culture ware with standard fibroblast medium. Cells were harvested after passaging the cells a few times and total RNA was extracted for Microarray Analysis. (E) Gene expression analysis by qRT-PCR of a panel of cardiac developmental transcription factors (*Nkx2.5, Gata4, Tbx5*), sarcomeric proteins (*Tnnt2*), adult cardiac resident stem cell marker (*Sca1*), epicardial developmental marker (*Tbx18, Tcf21*), fibroblast marker (*Postn*), mesenchymal and myofibroblast marker (*Vimentin*), smooth muscle cell and myofibroblast marker (Acta2), myofibroblast marker (*Klf4*), marker for oxidative stress (*Hif1α*) and *Tgfbr1* (*Alk1*, target of A83-01[12]). (F) MicroRNA analysis by qRT-PCR for cardiac or cardiomyocyte-specific microRNAs miR-1 and miR-133a. MICs show similar levels like CFs or TTFs while CPCs express high amounts of miR-1 and miR-133a. (G) Typical fibroblast microRNAs miR-21 and miR-29b are highly expressed in fibroblasts and MICs, while expression is low in CPCs.



Supplemental Figure S7. Characterization of NkxCE-GFP+ MICs after MI. Focus Heat Maps

(A) Total RNA was isolated from GFP+ MICs, embryonic-stem-cell derived day 7 *in vitro* differentiated cardiac progenitor cells (CPCs; transgenic NkxCE-GFP), murine tailtip fibroblasts (TTFs) and murine cardiac fibroblasts (CFs) and directed to microarray analysis. (B) A focus heat map on differentially expressed genes annotated to GO terms corresponding to cardiac muscle shows that MICs cluster with TTFs and CFs whereas CPCs are separated by high expression of sarcomeric and cardiac muscle genes. (C) A focus heat map on differentially expressed genes annotated to GO terms corresponding to extracellular matrix presents that MICs again cluster with TTFs and CFs. One cluster

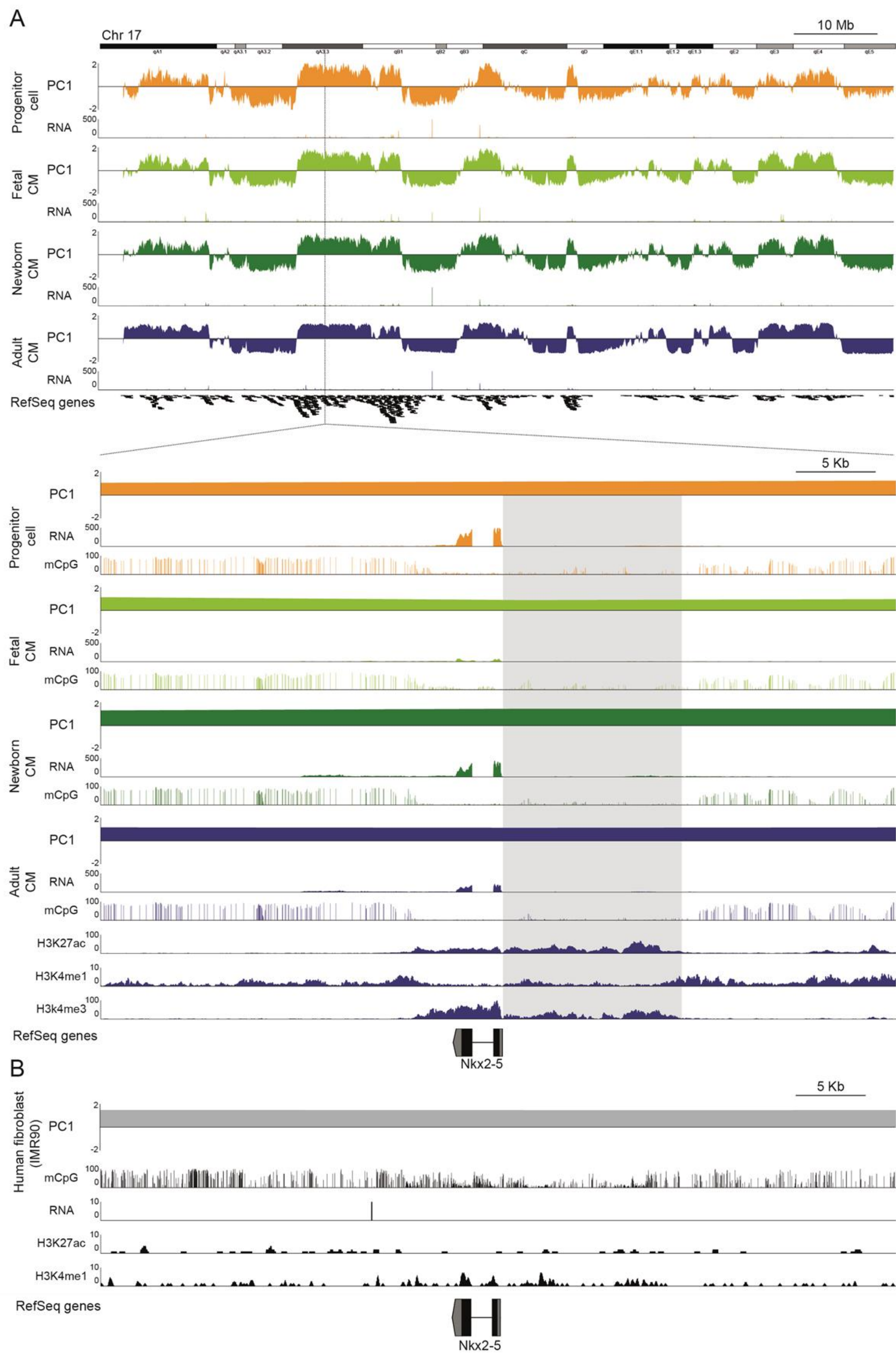
of ECM genes is highly expressed in CPCs whereas two other clusters are up-regulated in MICs. (D) A focus heat map on differentially expressed genes annotated to GO terms corresponding to oxidative stress once more exhibits that MICs cluster with TTFs and CFs. One cluster of genes is highly expressed in CPCs whereas a second cluster is up-regulated in MICs. (E) In the focus heat map on differentially expressed genes annotated to GO terms corresponding to inflammation MICs are separated from the other cells up-regulating several inflammatory genes.



Supplemental Figure S8. ***In-vitro*** **culture of NkxCE-GFP+ MICs isolated after MI: proliferation and stimulation.**

(A) Hearts from infarcted NkxCE-GFP mice were explanted 1 week after MI and 20 GFP-positive MICs were directly sorted to collagen-coated 96-well-plates. MICs were cultured up to two weeks. One 96 well plate was stained with DAPI after 1 week and a second plate after two weeks to count the

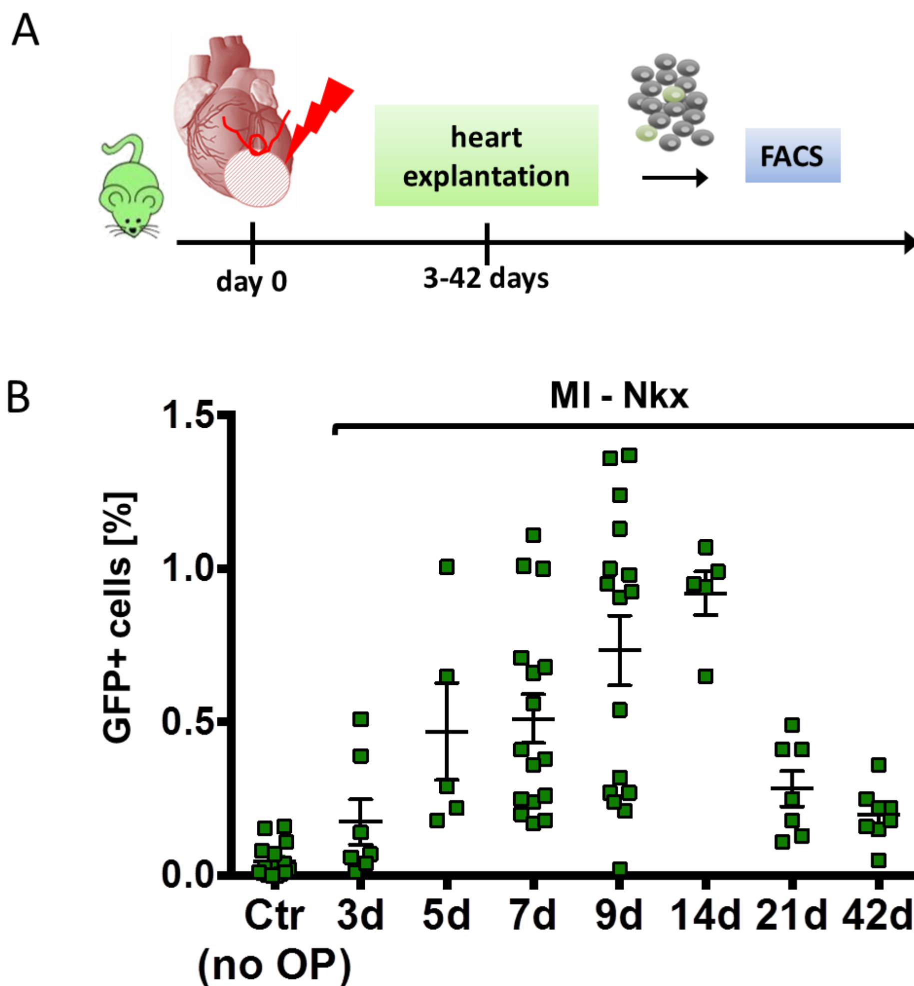
amount of cells per well. (B) Fibroblast-like MIC-morphology after 1 week (DAPI stains nuclei, blue). Scale bars: 100µm (C) The double amount of cells was detected per well after 2 weeks of culture compared to 1 week of culture indicating slow proliferation. ** $p < 0.01$ (D) Hearts from infarcted NkxCE-GFP mice were explanted 1 week after MI and 20 GFP-positive MICs were directly sorted to collagen-coated 96-well-plates. In standard differentiation medium they were stimulated with different substances. After 1 week of stimulation 96 well plates were stained with DAPI to count the amount of cells per well. (E) Amount of cells per well after 1 week of stimulation with different substances. Significant proliferation was induced by Bmp4, TB4 and A83 (1µM) compared to untreated controls (same wells on a second untreated 96 well plate). * $p<0.05$; ** $p<0.01$ (Mann-Whitney test). (F) Hearts from infarcted NkxCE-GFP mice were explanted 1 week after MI and 20 GFP-positive MICs were directly sorted to collagen-coated 96-well-plates. They were cultured for up to three weeks. Cells were fixed after two or three weeks and stained with different antibodies (Immunocytochemistry - ICC). (G) Vimentin (mesenchymal marker) stained MICs well but no positive staining for any cardiomyocyte marker (αActinin, αMHC or Tnni3) could be detected (exemplary pictures). Scale bars: 100µm



Supplemental Figure S9. Chromatin state analysis of CPCs, cardiomyocytes and fibroblasts.

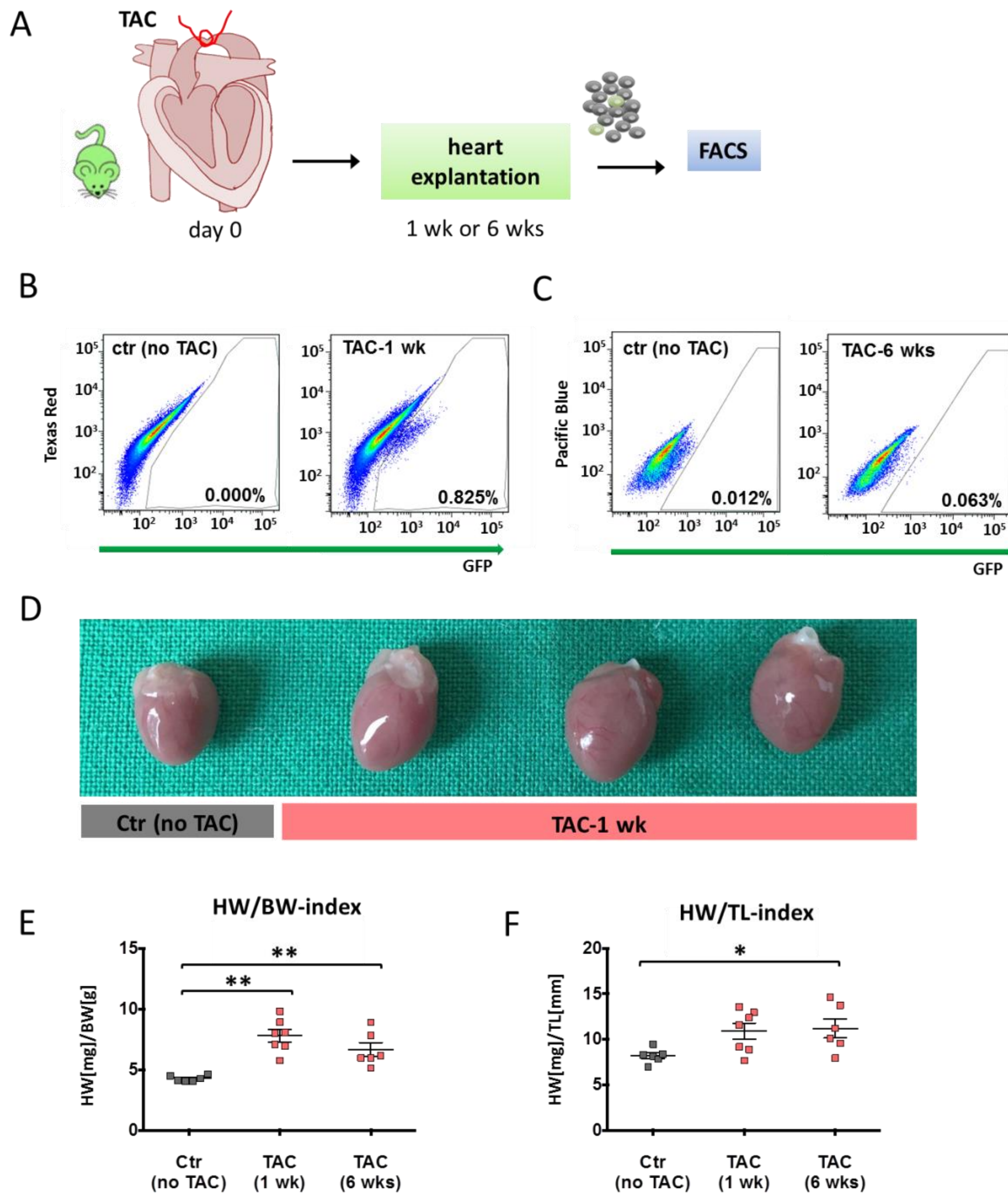
(A-B) Hi-C data was used to annotate active (A, PC1 >0) and inactive (B, PC1 <0) compartments in genomic regions flanking the *Nkx2.5* gene. Regulatory regions (enhancer and promoter; shaded in grey) enriched for H3K4me1/3 and H3K27ac and depleted for CpG methylation were annotated. Expressed genes were determined by RNA-seq. Shown are CpG methylation (%); ChIP-seq (RPKM;

reads per kilobase per million reads); RNA-seq (FPKM; fragments per kilobase of mapped reads) and the eigenvector (PC1) of chromatin interactions measured by Hi-C. (A) Depicted are data from cardiac progenitor cells (CPC) as well as fetal (E14), postnatal (P1) and adult cardiomyocytes (CM). (B) Fibroblast data was obtained from human fibroblasts (IMR90).



Supplemental Figure S10. Characterization of NkxCE-GFP+ MICs after MI. Timecourse of MIC-appearance.

(A) Infarcted NkxCE-GFP hearts were explanted 3, 5, 7, 9, 14, 21 and 42 days (n=5-16) after MI and GFP-positive MICs were quantified by FACS. (B) MICs appeared from day 3 after MI, achieved their maximum after about two weeks and then vanished.



Supplemental Figure S11. Functional analysis of NkxCE-GFP+ MICs after transverse aortic constriction (TAC).

(A) One (n=5) or six weeks (n=3) after transverse aortic constriction (TAC) was applied to NkxCE-GFP mice hearts were explanted and GFP+ cells were quantified by FACS. (B-C) Exemplary FACS-plots 1 week (1 wk) and 6 weeks (6 wks) after TAC. Left panels: Ctr-mouse without TAC operation (ctr no TAC). Right panels: Mouse with TAC. After 1 week TAC-treated mice exhibit 0.825% of GFP+ cells in their hearts while no GFP+ cells were detectable in TAC-hearts 6 weeks post operation. (D) Hearts of TAC-operated mice morphologically appear bigger one week post injury compared to a healthy control heart. (E-F) Heart weight (HW) versus body weight (BW) index and heart weight versus tibia length (TL) index both indicating hypertrophic hearts after TAC operations. * $p<0.05$; ** $p<0.01$ (Mann-Whitney test).

Supplemental Tables

Supplemental Table 1: Primer-sequences for qPCR (alphabetical order).

Gene name	Forward (5‘ → 3‘)	Reverse (5‘ → 3‘)
β-Actin (housekeeping gene)	ccaaccgtgaaaagatgacc	accagaggcatacagggaca
Acta2 (αSMA)	ccaaccgggagaaaatgac	cagttgtacgtccagaggcata
Actc1 (cardiac αActinin)	ggtcatcaccattggcaac	atgccagcagattccatacc
CD31 (Pecam1)	gttgcagccaaatgctactt	gaaatcttctcgctgttgga
Col3a1	aggacccactggcagttct	ggaggaccaggaagaccac
Fap (Fapα)	agacgggggactgactttct	taacactctggctgcaagga
Flk1 (Kdr, Vegfr2)	cagtggtactggcagctagaag	acaagcatacgggcttgttt
Gata4	ggaagacaccccaatctcg	catggccccacaattgac
Hif1α	gcactagacaaagttcacctgaga	cgctatccacatcaaagcaa
Il6	tctaattcatatcttcaaccaagagg	tggtccttagccactccttc
Klf4	cgggaagggagaagacact	gagttcctcacgccaacg
Mef2c	atgggcggagatctgaca	ttcttgttcaggttaccaggtg
Myh10 (NMMHC-IIB)	aagatgcaggcgcacatt	tgattttagcctctgctgtcac
Myh6 (αMHC)	cctatgcttctgctgataccg	tcatcagcttgttcagattttcc
Myl2 (Mlc2v)	gactgagccctgaaccacag	acatcatcaacttgcggtca
Nkx2.5	gagcctacggtgaccctga	gtggtctctcggcgccat
Pdgfrα	acctcccaccaggtctttct	tcattctcgtttgggaggat
Periostin (Postn)	tgtcattgaccgtgtcctg	tccaagaggtcagaggtgatg
Sca1 (Ly6a)	tcagtcctcctgcagacctt	actcccaccttggagcttct
Smarcd3 (Baf60c)	gcgacaagtattccagcaga	gacgtggttgatcacaatgg
Tbx18	tcgacaagctgaaacttaccaa	ttggtatttgtgcatagaatgaaga
Tbx5	ggatgtctcggatgcaaagt	ggttggaggtgactttgtgc
Tcf21	cattcacccagtcaacctga	ccagactcgcacctccaa
Tgfbr1	agaagagcgttcatggttcc	cgtccatgtcccattgtct
Tgfbr2	tgtggagagcatgaaagacag	tcacacacgatctggatgc
Thy1 (CD90)	aactcttggcaccatgaacc	gttcaccaggcaggctgt
Tnnt2 (TropT)	ttcgacctgcaggaaaagtt	cttcccacgagttttggaga
Vimentin	ccaaccttttcttccctgaa	tgagtgggtgtcaaccagag
Wt1	gaaatggacagaagggcaga	accccgtgggtgtgtattc

Supplemental Table 2: Primary antibodies applied for flow cytometry, immunohistochemistry and immunocytochemistry (alphabetical order).

abbreviation	Product name	Company, order-nr	Description	Application
αActinin (Actc1)	Anti-Sarcomeric Alpha Actinin antibody	Abcam, ab9465	Mouse monoclonal IgG1 to Sarcomercic Alpha Actinin	ICC (1:200)
αSMA (Acta2)	Anti-alpha smooth muscle Actin antibody	Abcam, ab5694	Rabbit polyclonal to alpha smooth muscle Actin	ICC (1:20) IHC (1:20)
CD31 (Pecam1)	Anti-CD31 antibody	Abcam, ab 28364	Rabbit polyclonal to CD31	ICC (1:50) IHC (1:50) FACS (1:200)
CD45	Anti-Mouse CD45 PE-Cyanine7	Affymetrix, eBioscience, 25-0451	Rat monoclonal IgG2b [30-F11) to CD45 conjugated with PE-Cy7	FACS (1:25)
Col1	Anti-Collagen I antibody	Abcam, Ab21286	Rabbit polyclonal to Col I	ICC (1:200) IHC (1:200)
Fap (Fapα)	Anti-Fibroblast activation protein, alpha antibody	Abcam, ab 53066	Rabbit polyclonal to FAP	ICC (1:20) IHC (1:20)
GFP	Anti-GFP antibody	Abcam, ab13070	Chicken polyclonal to GFP	ICC (1:2000) IHC (1:1000)
Myh10 (NMMHC-IIB)	Anti-non-muscle Myosin IIA antibody	Abcam, ab24762	Rabbit polyclonal to non-muscle Myosin IIA	ICC (1:100) IHC (1:100)
Myh6 (αMHC)	Myosin Heavy Chain Antibody	R&D Systems, MAB4470	Monoclonal Mouse IgG2B [MF20]	ICC (1:100)
Ph3	Anti-Histone H3 (phospho S10) antibody	Abcam, ab5176	Rabbit polyclonal to Histone H3 (phospho S10)	IHC (1:250)
Sca1 (Ly6A/E)	Anti-Sca1/Ly6A/E antibody	Abcam, ab51317	Rat monoclonal [E13 161-7] to Sca1/Ly6A/E	IHC (1:200)
Sca1 (Ly6A/E)	Rat Anti-Mouse Ly-6A/E (Sca-1)	Beckman Coulter	Rat monoclonal [D7) to Sca1/Ly6A/E conjugated with Allophycocyanin	FACS (1:400)

			(APC)	
Thy1 (CD90)	Anti-CD90/Thy1 antibody	Abcam, ab3105	Rat monoclonal [IBL-6/23] to CD90/Thy1	IHC (1:100) FACS (1:400)
Tnni3	Anti-Cardiac Troponin I antibody	Abcam, ab200080	Mouse monoclonal [B1561M] to Cardiac Troponin I	ICC (1:100)
Vimentin	Anti-Vimentin antibody	Abcam, ab45939	Rabbit polyclonal to Vimentin	ICC (1:500) IHC (1:200)
Wt1	Anti-Wilms Tumor Protein antibody	Abcam, ab15249	Rabbit polyclonal to Wilms Tumor Protein	IHC (1:300)

Abbreviations: ICC (immunocytochemistry), IHC (immunohistochemistry), FACS (Flow cytometry)

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