Evidence for Minimal Cardiogenic Potential of Sca-1 Positive Cells in the Adult Mouse Heart

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Abstract

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Despite modern pharmacotherapy, heart failure remains a major medical burden. The heart has a limited regenerative capacity, and bolstering regeneration might represent new therapeutic approaches for heart failure patients. Various progenitor cells have been proposed to have cardiomyogenic properties, but this evidence is based mostly on cell culture and transplantation studies. One population of interest is characterized by the expression of Stem Cell Antigen-1 (Sca-1). Here we tested the hypothesis that Sca-1+ cells are endogenous progenitors for cardiomyocytes. We evaluated the innate cardiogenic potential of Sca-1 positive cells in vivo by generating a novel mouse model to genetic lineage-trace the fate of Sca-1+ cells. This was accomplished by introducing a tamoxifen-inducible Cre-recombinase into the Sca-1 locus (Sca-1mCm/+). Crossing this mouse line to a Cre dependent tdTomato reporter line allowed for genetic lineage-tracing of endogenous Sca-1+ cells (Sca-1mCmtdTomato). We validated the genetic lineage tracing mouse model in bone marrow and heart. Unlike previous publications suggesting significant cardiogenic potential, we found that less than 0.1% of cardiomyocytes were derived from Sca-1+ cells in the adult heart under homeostatic conditions and there was no significant

change in contribution to cardiomyoctyes six months after myocardial infarction. Our results show that Sca-1+ cells in the adult heart have minimal cardiogenic potential under homeostatic conditions or in response to myocardial infarction.

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INTRODUCTION

Cardiac regeneration has been investigated for more than 100 years and the ability of the adult heart to generate new myocytes has been under debate ever since[1,2]. The current consensus is that *de-novo* cardiomyogenesis is limited to ~1% per year in the healthy adult mammalian heart[3,4]. To what extent this limited ability to generate cardiomyocytes is increased after injury remains to be determined. However, even minimal *de novo* cardiomyogenesis might represent a target for regenerative therapeutic interventions. Most evidence points towards proliferation of pre-existing myocytes as the main source of newly formed cardiomyocytes[5,6]. However, several resident cardiac progenitor cell (CPC) types have been described and these CPCs may minimally contribute to the generation of *de novo* cardiomyocytes in the adult heart[7,8]. The cardiogenic potential of these different CPC populations has been evaluated in transplantation studies[9–11], however, it is increasingly recognized that transplantation studies do not always reveal the endogenous functions of stem cells[12]. More recent fate-mapping studies demonstrated that a putative CPC population, characterized by the expression of c-kit, arguably one of the most studied CPC population, does not significantly contribute to cardiomyocytes in vivo[7,13,14].

Another candidate CPC population is marked by the expression of the cell surface marker Stem Cell Antigen-1 (Sca-1). Sca-1 was initially described as a surface marker of murine hematopoietic stem cells[15] but it is also expressed on mature B- and T-lymphocytes and in multitude of cells in the thymus, brain, kidney, lung, and the heart[16,17]. Oh et al. were the first to describe this perivascular cell type that is characterized by the expression of Sca-1 but does not express CD45, CD34 or CD31[18], suggesting it is neither a leukocyte nor an endothelial cell. These Sca-1 positive cells were reported to differentiate into endothelial cells, smooth muscle cells and also cardiomyocytes after transplantation[18]. Further characterization revealed that the expression of PDGFRα marks the clonogenic Sca-1 positive (Sca-1^{+}) cells that displayed cardiogenic potential[19].

To further assess the ability of endogenous Sca-1 expressing cells to generate cardiomyocytes, a previous attempt was made to genetically lineage-trace Sca-1 expressing cells in the adult heart[20]. These studies appeared to show contribution of Sca-1 expressing cells to *de novo* cardiomyocytes, although precise quantifications were not provided. More importantly, the data were derived from a transgenic construct that showed widespread expression than in just Sca-1 expressing cells, complicating interpretation of the results[21]. Adequate fidelity is a major concern for all fate-mapping studies and might explain contrary results. To reliably trace the descendants of Sca-1^{+} cells *in vivo*, we generated a tamoxifen-inducible Sca-1 mer-Cre-mer knock-in mouse model using a strategy that has been successfully applied in the hematopoietic system[22] and sought to test the hypothesis that endogenous Sca-1^{+} cells are progenitors for cardiomyocytes *in vivo* under physiological and pathophysiological conditions. Using this model, we report that Sca-1^{+} cells do not form new cardiomyocytes in healthy myocardium or after myocardial infarction, but they appear to contribute robustly to the endothelial cell population.

MATERIALS AND METHODS

Animals

All animal procedures were conducted in accordance with the US NIH Public Health Service Policy on Humane Care and Use of Laboratory Animals, and all procedures complied with and were approved by either the University of Washington or the University of Minnesota Institutional Animal Care and Use Committee. Both male and female mice between 8-12 weeks of age and weighing an average of 25g were used and randomly assigned to each experimental group. Mice were group housed by sex throughout the experiments in ventilated cages and were of normal immune status.

Generation of the Sca-1 merCremer and Sca-1^{mCm}R26^{tdTomato} mouse models:

We generated the knock-in mouse model adapting a strategy from Hanson et al.[22] The second exon of the Ly6a gene was targeted in W4 mouse embryonic stem cells (strain 129S6/SvEvTac). Correct

integration was confirmed with Southern blot and PCR. A correctly targeted clone was aggregated with morula stage embryos, transferred into pseudopregnant mice, and chimeric offspring was back-bred onto C57Bl/6J mice (strain 000664 The Jackson Laboratory). To create inducible tdTomato labeling of Sca-1^{+} cells, we cross-bred Sca-1^{MCM} mice with Cre dependent reporter mice. *Gt(ROSA)26Sor*$^{tm4(ACTB\text{-}tdTomato,\text{-}EGFP)Luo}$/J, (R26^{mTmG}; strain 007676 The Jackson Laboratory) and *Gt(ROSA)26Sor*$^{tm1(CAG\text{-}lacZ,\text{-}EGFP)Glh}$/J, previously cross-bred with Rosa26-Flpe mice and back-crossed to C57Bl/6 (R26^{GFP}; strain 012429 The Jackson Laboratory) were initially used. As we did not see recombination in the bone marrow, we eventually switched to (*Gt(ROSA)26Sor*$^{tm4(ACTB\text{-}tdTomato,\text{-}EGFP)Luo}$/J (tdTomato; strain 007914 The Jackson Laboratory) to generate Sca-1^{MCMtdtomato} mice. Genotypes were confirmed via PCR screening.

Genotyping

1mm punch biopsies were obtained from ear pinna samples of mice and DNA was prepared using the REDExtract-N-Amp™ Tissue PCR Kit Protocol (Sigma-Aldrich, XNAT). PCR was performed to detect the presence of Cre recombinase using primers 5' – ACGACCAAGTGACAGCAATG (forward) and ACCAGAGACGGAAATCCATCGCT (reverse). PCR conditions were 94 C for 5 minutes to separate strands, followed by 30 cycles of amplification (94 °C for 30 s, 57 °C for 30 sec, 72 °C for 30 s).

Animal procedures

Tamoxifen was suspended in corn oil and administered by daily intraperitoneal injections (2 mg) for 7 days to activate the inducible Cre-recombinase. Myocardial infarction surgery was performed after a 7 day chase period. For myocardial infarction surgery, 8-12 week-old mice were anesthetized by intraperitoneal injection of ketamine and xylazine. Mice were intubated, and mechanically ventilated while lateral thoracotomy was performed and the left anterior descending coronary artery was ligated as previous described[23]. Mice received buprenorphine analgesia and were monitored regularly until endpoint analysis.

Cell isolation

Bone marrow was harvested by flushing femurs and tibia with cold Hanks Balanced Salt Solution (HBSS). Cells were centrifuged at 400 g for 10 minutes at 4°C and pellets were re-suspended in HBSS. Red blood cell lysis was performed with a lysis buffer containing NH_4Cl (155 mM), $NaHCO_3$ (12 mM) and EDTA (0.1 mM). Non-myocytes were isolated from minced adult hearts by enzymatic digestion with Collagenase Type II (Worthington 240U/mg) for 30 minutes at 37 °C. Cells were then filtered through a 70 µm mesh. Bone marrow and non-myocytes were stained with antibodies against Sca-1 (Milteny Biotec; clone: D7), CD31 (BioLegend, clone MEC13.3), Lineage marker (BioLegend, clone, anti-mouse CD3e, clone 145-2C11; anti-mouse Ly-6G/Ly-6C, clone RB6-8C5; anti-mouse CD11b, clone M1/70; anti-mouse CD45R/B220, clone RA3-6B2; anti-mouse TER-119/Erythroid cells, clone Ter-119), cardiac troponin T (Thermo Scientific, clone 13-11) and tdTomato (Abcam, ab62341). Flow cytometry was performed using a BD FACSCanto™ and a FACSAria II flow cytometry system. Cells were gated by forward scatter versus side scatter to eliminate debris. A minimum of 50,000 events was counted for each analysis. Analysis of flow cytometry data was performed in FlowJo.

Isolation of adult mouse cardiomyocytes

Mouse cardiomyocytes were isolated using an enzymatic digestion and mechanical dispersion method previously described[24]. In brief, after retrograde perfusion with Tyrode buffer (126mM NaCl, 5.4mM KCl, 0.33mM NaH_2PO_4, 1mM $MgCl_2$, 10mM HEPES, 10mM Glu, 20mM Taurine, 25uM Blebbistatin) and digestion with collagenase solution (Collagenase Type II, Worthington 240U/mg), the ventricular myocytes were separated using a fine scalpel and scissors. Afterwards cells were fixed in 4% paraformaldehyde and processed for immunohistochemical analysis.

Immunohistochemical analysis

Histological stains and subsequent analysis were conducted as described previously by our group [25–27]. In brief, hearts were perfused with PBS and 150 mM KCl solution after harvesting, fixed in 4% paraformaldehyde and cryo-preserved in 30% sucrose solution. Sections of the heart were sliced along the transverse axis into 5-mm-thick sections, cryosectioned, and stained with appropriate primary and secondary antibodies. Immunofluorescent images were collected by a Zeiss Axio Imager M1 upright microscope or a Nikon A1 Confocal System attached to a Nikon Ti-E inverted microscope platform. For figure preparation, images were processed to convert colors by Nikon NIS Elements software.

RESULTS

Generation of tamoxifen-inducible Sca-1-dependent genetic lineage-tracing mouse model and selection of reporter lines

To track the lineage of Sca-1^{+} cells in the heart, we performed traditional gene-targeting and 'knocked in' a cDNA cassette encoding for tamoxifen-inducible Cre recombinase (mER-Cre-mER) into the Ly6a locus, which codes for the Sca-1 protein, of W4 mouse embryonic stem cells (Fig. 1A). Proper heterozygous integration was determined by Southern blotting and PCR (Suppl. Fig. 1A). A correctly targeted clone was aggregated with morula stage embryos to generate chimeric mice. These were backcrossed onto C57Bl/6 mice to generate the Cre-driver strain, which we refer to as Sca-$1^{mCm/+}$ hereafter. Previously there has been debate and confusion regarding expression of the allelic genes Ly6A and Ly6E [15,28,29]. To conclusively verify gene targeting of the correct Ly6 gene in the Sca-$1^{mCm/+}$, backcrossed to C57Bl/6J, we generated homozygous Sca-$1^{mCm/mCm}$ mice, which showed no Sca-1 expression by flow cytometry on either bone marrow cells or the non-cardiomyocyte population in the heart (Fig.1B and 1C).

Since it is known that different responder lines have different sensitivity for Cre recombinase [30], we next screened different Cre-reporter mice to identify an optimal system for Sca-1 lineage analysis. Initially, we

crossbred Sca-1$^{mCm/+}$ mice with Rosa26^{mTmG} mice and Rosa26^{GFP}. When adult mice were pulsed daily with tamoxifen for one week and studied 7 days later, there was extensive recombination in the heart (Suppl. Fig. 2). Flow cytometry demonstrated that on average 35% of the cells expressing Sca-1 protein expressed GFP. However, there was no significant recombination in bone marrow hematopoietic compartment with both reporter lines, where the non-endothelial Sca-1^{+} cells were essentially negative for GFP. These results could be due to insufficient tamoxifen to nuclearize the fusion Cre protein, or due to overall low abundant Cre fusion protein, or potentially due to inefficiency of Cre mediated recombination of the reporter allele. In an attempt to maximize Cre fusion protein abundance, we generated homozygous Sca-1$^{mCm/mCm}$ mice crossbred with Rosa26^{GFP} reporter mice. On histology we noticed extensive labeling of endothelial-like cells in the heart (although no labeled cardiomyocytes were observed), but with flow cytometry of bone marrow cells, we still did not observe labeling in that compartment (Suppl. Fig. 2E). To ensure we would not underestimate the cardiogenic potential of Sca-1^{+} cells, both hematopoietically derived cells as well as those residing in the heart, we tested a third reporter strain. In studies of gastric stem cells[31] the Rosa26-tdTomato mouse line was identified as a sensitive Cre reporter strain. Thus, we crossbred this responder line with Sca-1$^{mCm/+}$ mice and now observed recombination rates in the bone marrow compartment of 0.25-2% in response to tamoxifen treatment (Fig. 2A and B). Additionally, we were able to detect a small population of tdTomato^{+} cells in the bone marrow that was also Lin-positive (i.e. expressed markers for the lymphoid, erythroid or granulocytic lineages; Fig. 2C), indicating that we had induced recombination in hematopoietic stem cells which then differentiated into mature blood cells. Importantly, there was recombination in the heart averaging 33% in Sca-1^{+}/CD31^{+} populations and 13% in the Sca-1^{+}/CD31^{-} population (Fig. 2D and 2E). All subsequent studies were performed in mice obtained by crossing Sca-1$^{mCm/+}$ driver line to the tdTomato mouse, which we refer to as Sca-1^{mCm}R26^{tdTomato} mice (Fig. 2A).

Sca-1$^+$ lineage marks microvascular endothelium and perivascular cells in the normal heart

Adult Sca-1^{mCm}R26^{dTomato} animals were treated with tamoxifen daily for 7 days and hearts were harvested one week after the last tamoxifen injection to assess recombination by flow cytometry and histology. No tdTomato$^+$ cells were identified by flow cytometry in Sca-1^{mCm}R26^{dTomato} mice that did not receive tamoxifen (data not shown). Histology demonstrated an endothelial tdTomato expression pattern in Sca-1^{mCm}R26^{dTomato} hearts that co-localized with Sca-1 expression (Fig. 3A). TdTomato was not expressed in cardiomyocytes identified using phalloidin staining, but did illustrate an endothelial pattern, often co-localized with CD31 (Fig. 3A). Additionally, tdTomato expression in tissues known to express Sca-1 (kidney, liver, ileum, lung, heart) confirmed the presence of tdTomato$^+$ cells (Fig. 3B).

Lineage tracing in injured hearts reveals low numbers of Sca-1 derived cardiomyocytes

Next, we set out to test whether new cardiomyocytes were derived from descendants of Sca-1$^+$ cells after myocardial infarction. Adult Sca-1^{mCm}R26^{dTomato} mice were treated with tamoxifen, followed by a 7 day chase period, while control mice were not exposed. The mice then underwent myocardial infarction, induced by permanent ligation of the left coronary artery. Mice were euthanized approximately 6 months after infarction for examination of the heart (Fig. 4A). Initially, we performed double immunostaining for cTnT and tdTomato to identify cardiomyocytes derived from Sca-1 descendants. Although no significant numbers of tdTomato$^+$ cardiomyocytes were identified, the high number of tdTomato$^+$ cells visualized in niches between cardiomyocytes provided too much background to count rare events. We then adapted a method of enzymatically dissociating cardiomyocytes, followed by immunostaining for cTnT and tdTomato. This allowed unambiguous distinction of cardiomyocytes and non-myocytes (Fig. 4B). Using this method, a minimum of 40,000 cardiomyocytes was counted from each mouse (n=7), including tamoxifen-negative controls (n=4). We detected abundant numbers of tdTomato$^+$ non-myocytes.

However, using this rigorous screening method allowed the detection of only 19 tdTomato^{+} cardiomyocytes. This accounts for an average of 0.008% tdTomato^{+} cardiomyocytes (Fig. 4C and 4D and Suppl. Fig. 3). Importantly, not a single cardiomyocyte expressed tdTomato in animals that were not exposed to tamoxifen.

DISCUSSION

The results of this study do not support the hypothesis that Sca-1 expressing cells have the ability to significantly contribute new cardiomyocytes to the heart after myocardial infarction. Despite using the best available techniques for lineage tracing, including knocking an inducible Cre recombinase into the Sca-1 gene, using a sensitive Cre-reporter strain, and enzymatically dispersing cardiomyocytes from the non-myocytes to allow for single cell screening, we were unable to detect significant contributions of the Sca-1 lineage to cardiomyocytes. We conclude that Sca-1^{+} cells are not endogenous cardiac progenitor cells in the adult heart.

The results of our study contrast data from Uchida et al., who showed a significant contribution of Sca-1^{+} cells to cardiomyocytes[20]. The main difference between these two studies concerns the way lineage-tracing was initiated, i.e. the Cre-driver lines. We generated a knock-in mouse line where a tamoxifen-inducible Cre-recombinase was inserted into the endogenous Ly6a locus. Uchida et al. used a transgenic approach with a 14 kb fragment of the Sca-1 gene, a system that has been reported to generate *false-positives*, meaning expression of the promoter fragment in cells that normally do not express Sca-1[21]. Therefore, we believe the transgenic promoter fragment may have overestimated the 'true' cardiogenic potential of endogenous Sca-1 expressing cells.

Our study is in line with recent work on c-kit lineage-tracing in the heart[7]. C-kit^{+} cells were reported to contribute to cardiomyocytes in the adult heart[32], yet, eventually a knock-in approach that allowed to lineage trace the fate of c-kit^{+} cells *in vivo,* demonstrated that they do not contribute to cardiomyocytes in relevant numbers[7,8]. Similar to the differences between Uchida and our study, the first c-kit study used a short promoter fragment that was known have more widespread expression, and therefore likely overestimated the 'true' cardiogenic potential of c-kit expressing cells[32].

A different problem is the possibility of activation of Sca-1 within cardiomyocytes. Recently, it was shown that the low level of cardiomyocyte labeling in c-kit genetic lineage tracing experiments is likely not derived from differentiation of c-kit expressing cells, but rather the result of a fusion event, or due to expression of c-kit within cardiomyocytes[33]. Inducible lineage tracing studies of cardiac progenitors have this inherent risk of producing *erroneous positives* due to transient Cre recombinase activity in pre-existing cardiomyocytes. Although we cannot rule out this possibility, it is likely of no relevance given the extremely low overall rate of cardiomyocyte labeling.

A third problem that might limit interpretation of genetic lineage tracing strategies is incomplete activity of Cre recombinase giving rise to *false negative* cells. We acknowledge that our recombination rates in the bone marrow were lower than expected, considering that the majority of murine hematopoietic stem cells in the bone marrow should express Sca-1. However, the recombination rates in the heart were reasonably abundant, but still only resulted in 0.008% of cardiomyocytes to become labeled, which might be a slight underestimation. These limitations are especially important to consider as all biological assays have some background noise and we probably reach the sensitivity threshold of even the most rigorous assay when detecting only 0.008% positive cells. However, our assay was able to readily detect differentiated non-cardiomyocyte cells in the heart, further validating our assay. Moreover, using our rigorous approach of isolating adult cardiomyocytes from the heart to unambiguously assess tdTomato

status, we did not detect a single tdTomato$^+$ cardiomyocyte in animals that were not subjected to tamoxifen, indicating a lack of leakiness of the inducible Cre recombinase.

In conclusion, we did not find evidence in the adult mouse heart that cardiomyocytes are derived in significant numbers from Sca-1 progenitor cells. Although there is no orthologue for Sca-1 in the human genome, these findings demonstrate that the two most discussed putative CPCs do not significantly contribute to cardiomyocytes. These findings raise the possibility that there is no CPC population in the hearts of adult mice and potentially all mammalian species.

FIGURES

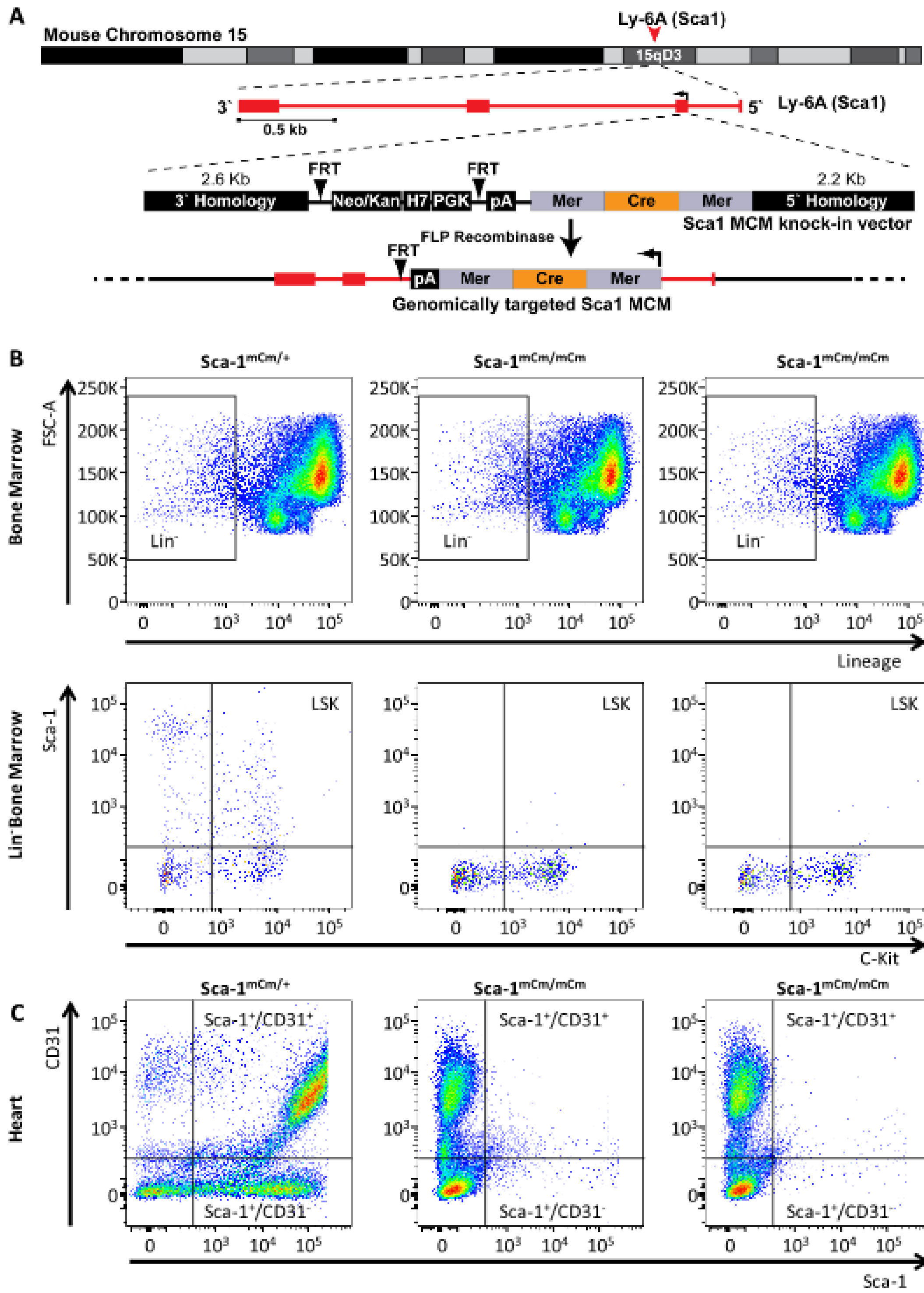


Figure 1: Generation of a tamoxifen-inducible Sca-1 Cre mouse line. A) Exon 2 of the Ly6a locus was targeted to generate the Sca-$1^{mCm/+}$ line. **B**) Heterozygous animals showed regular Sca-1 staining in the

bone marrow by flow cytometry, whereas homozygous Sca-1$^{mCm/mCm}$ did not express Sca-1 protein. **C**) Heterozygous animals showed regular Sca-1 staining in the heart by flow cytometry, whereas homozygous Sca-1$^{mCm/mCm}$ hearts did not express Sca-1 protein.

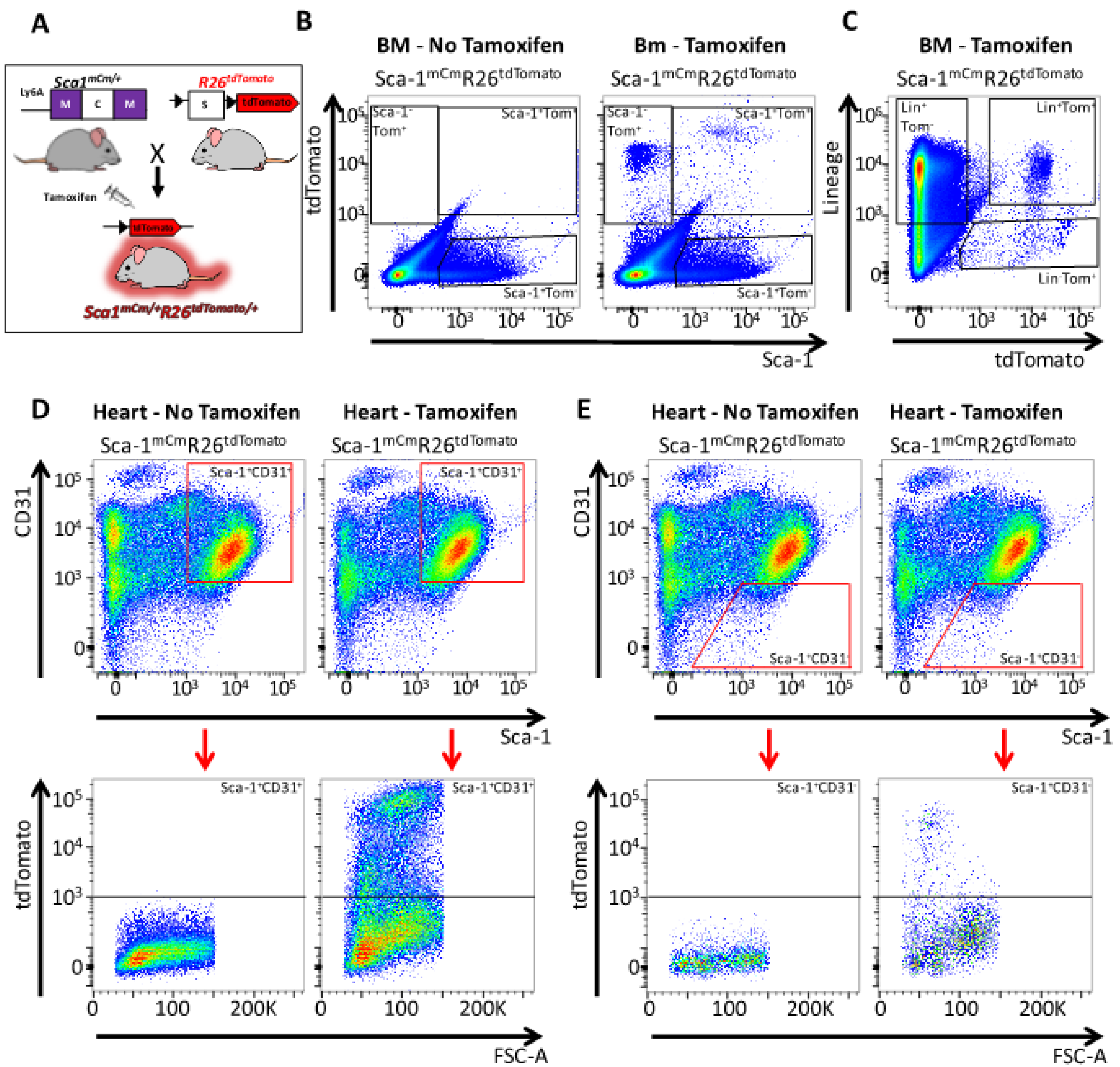


Figure 2: Characterization of Sca-1^{mCm}R26^{tdTomato} mice. A) Heterozygous Sca-1$^{mCm/+}$ mice were crossbred to a cre-dependent tdTomato reporter line and treated with tamoxifen. **B)** Tamoxifen administration resulted in labeling of bone marrow stem cells (Sca-1^{+}Tom^{+}) that over the pulse period differentiated into Sca-1 negative hematopoetic cells (Sca-1^{-}Tom^{+}). **C)** Lineage staining revealed a population of Lin^{+}tdTomato^{+} cells (differentiated bone marrow lineages), as well as Lin^{-}tdTomato^{+} cells

D) Sca-1^{+}CD31^{+} cells isolated from Sca-1^{mCm}R26^{tdTomato} hearts demonstrated successful recombination after tamoxifen induction. **E)** Sca-1^{+}CD31^{-} cells isolated from Sca-1^{mCm}R26^{tdTomato} hearts demonstrated successful recombination after tamoxifen induction, albeit at lower efficiency.

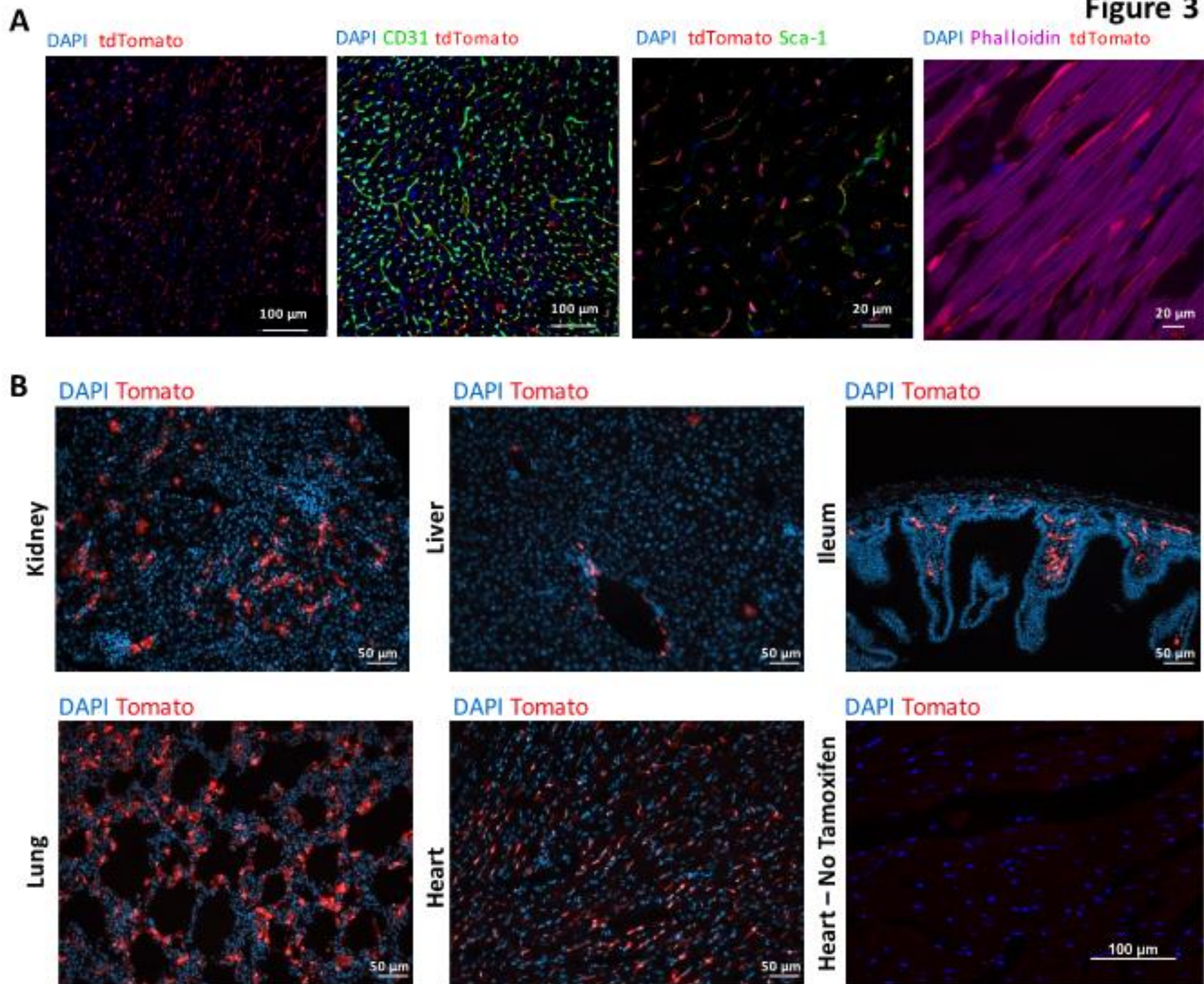


Figure 3: Sca-1⁺ lineage marks microvascular endothelium and perivascular cells in the normal heart. A) TdTomato expression demonstrated an endothelial like staining pattern and mainly co-localized with Sca-1 and CD31 staining. TdTomato was not expressed in cardiomyocytes but co-localized with Sca-1 staining. **B**) TdTomato was also expressed in organs known to express Sca-1 (Kidney, Liver, Lung, Ileum, and Heart). Tissue from animals that had not received tamoxifen did not express tdTomato.

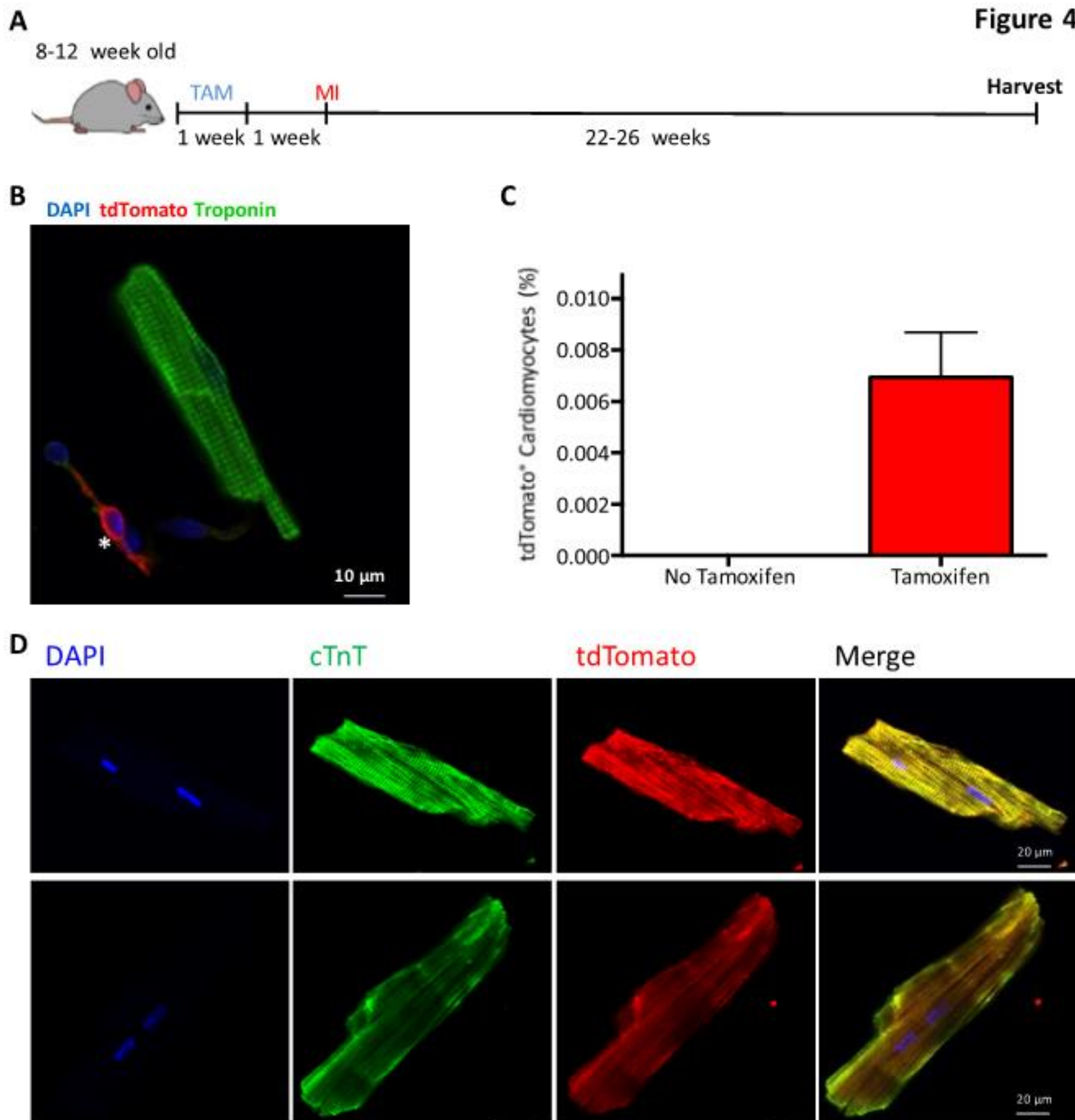
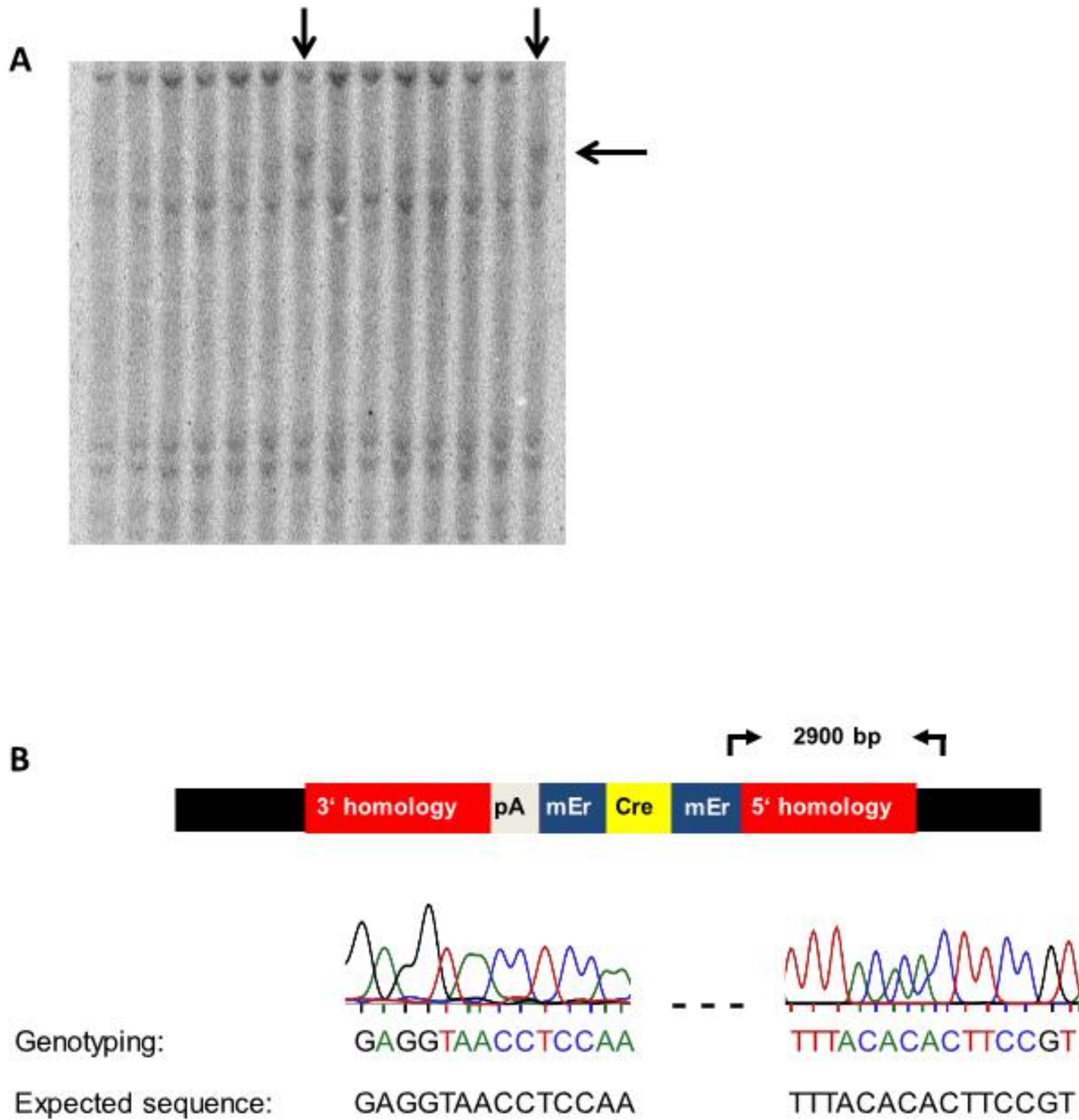


Figure 4: Lineage tracing in injured hearts reveals low numbers of Sca-1 derived cardiomyocytes

A) Sca-1^{mCm}R26^{tdTomato} animals were pulsed with tamoxifen for 1 week. LAD-ligation was performed

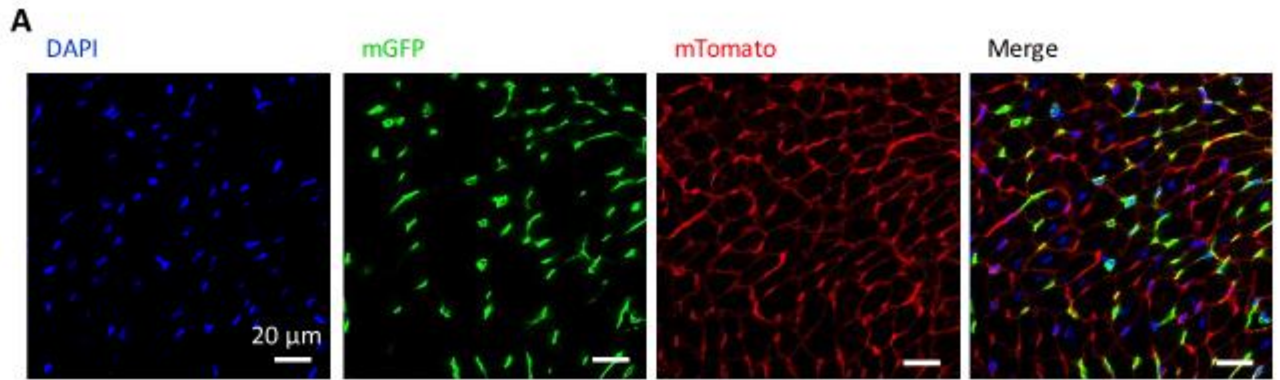
after a chase period of another 7 days and hearts were harvested after 6 months. **B)** Enzymatic digestion allowed for an unambiguous differentiation between cardiomyocytes and non-myocytes. Asterisk indicates non-cardiomyocyte. **C)** In total 19 tdTomato^{+} cardiomyocytes were detected in 7 animals accounting for a derived percentage of 0.008% Sca-1^{+}-derived cardiomyocytes. No tdTomato^{+} cardiomyocytes were observed in the absence of tamoxifen treatment. **D)** Examples of tdTomato^{+} cardiomyocytes.

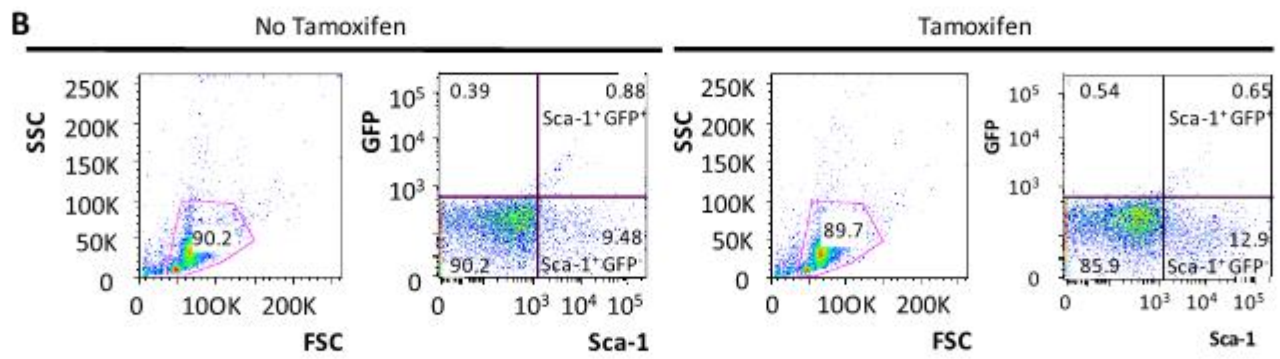
SUPPLEMENTAL FIGURES

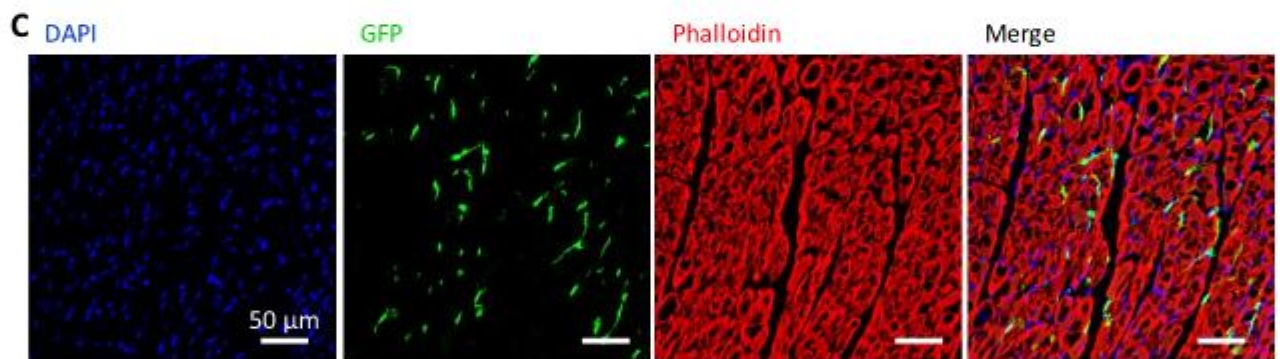


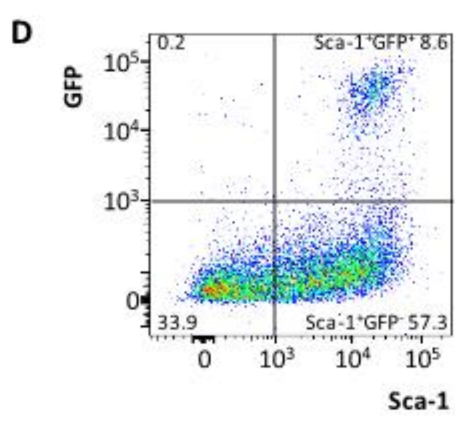
Supplemental Figure 1: Genotyping of Sca-1$^{mCm/+}$ animals was performed by Southern blot and PCR. A) Southern blot shows positive band indicated by arrow. Two positives clones were identified on

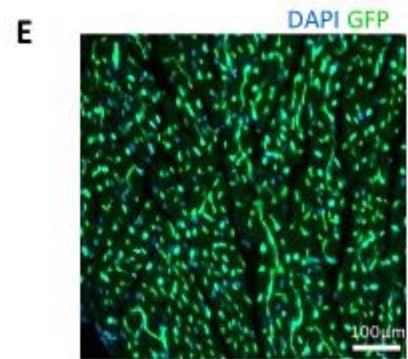
this Southern blot. **B)** Long-range PCR with sequencing result shows correct targeting of mCm into Ly6A allele.


A
DAPI
mGFP
mTomato
Merge
20 µm

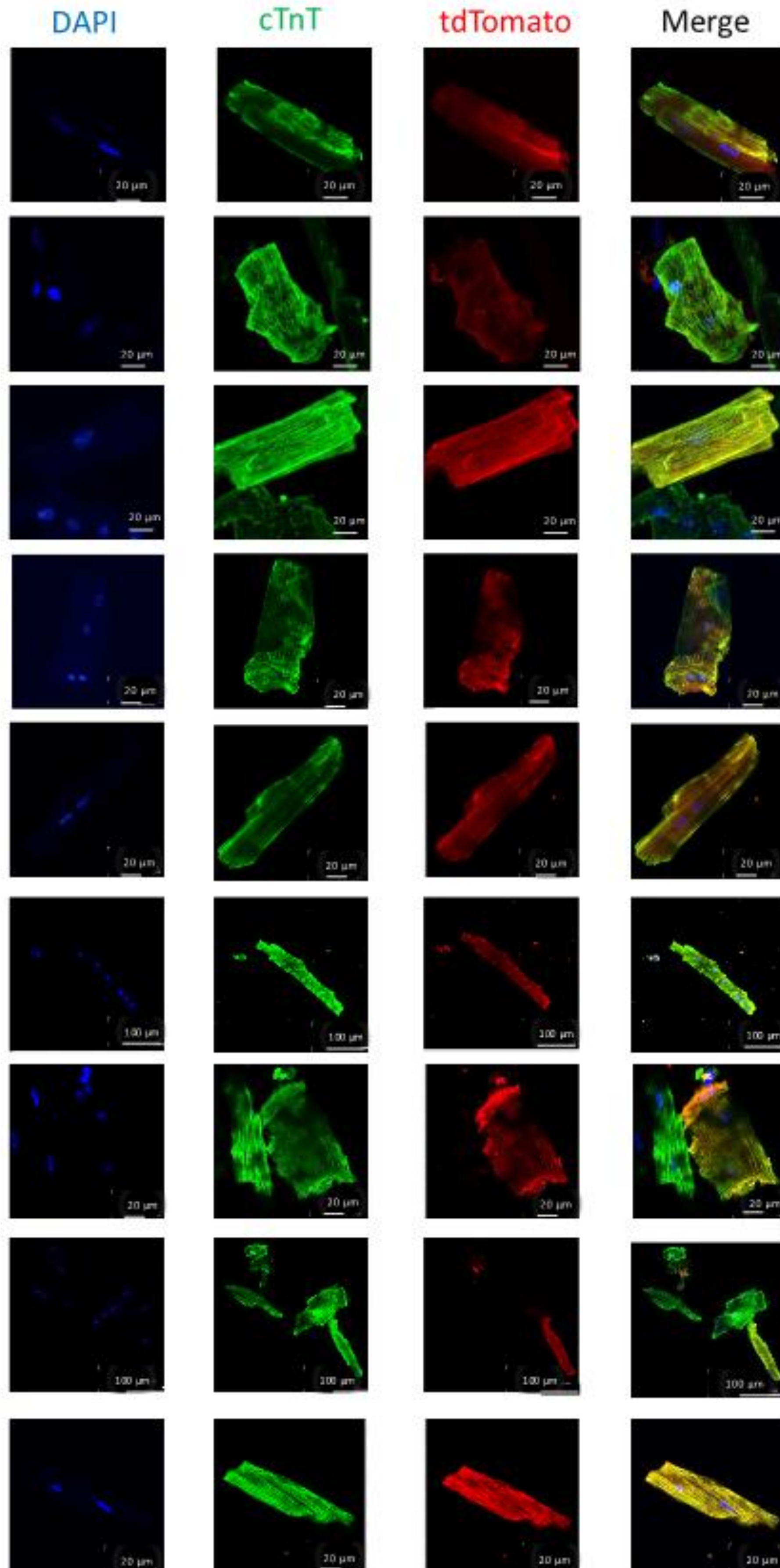

B
No Tamoxifen
Tamoxifen
SSC
FSC
90.2
GFP
Sca-1
0.39
0.88
Sca-1⁺GFP⁺
9.48
Sca-1⁺GFP⁻
89.7
0.54
0.65
12.9
85.9


C
DAPI
GFP
Phalloidin
Merge
50 µm


D
GFP
Sca-1
0.2
Sca-1⁺GFP⁺ 8.6
33.9
Sca-1⁺GFP⁻ 57.3


E
DAPI GFP
100µm

Supplemental Figure 2: Characterization of Sca-1^{mCm}R26^{mTmG} and Sca-1^{mCm}R26^{GFP} animals. Breeding of Sca-1$^{mCm/+}$ mice to **A+B**) R26^{mTmG} The Jackson Laboratory 007676; and **C-E**) R26^{GFP} The Jackson Laboratory 012429, resulted in recombination in the endothelium of the heart but lacked recombination in the bone marrow. **A)** Immunohistochemistry of heart tissue from Sca-1^{mCm}R26^{mTmG} treated with tamoxifen shows endothelial recombination pattern. **B)** Flow cytometry of bone marrow from Sca-1^{mCm}R26^{mTmG} mice shows lack of recombination. **C)** Immunohistochemistry of heart tissue from Sca-1^{mCm}R26^{GFP} treated with tamoxifen shows endothelial recombination pattern. **D)** Flow cytometry of cardiac non-myocytes from Sca-1^{mCm}R26^{GFP} mice shows low-level recombination. **E)** Immunohistochemistry of homozygous Sca-1$^{mCm/mCm}$R26$^{GFP\ shows}$ extensive endothelial recombination pattern. No cardiomyocytes were observed.


DAPI
cTnT
tdTomato
Merge
20 μm
100 μm

Supplemental Figure 3: Overview of tdTomato^{+} cardiomyocytes. Cells were stained with antibodies against cardiac Troponin T (cTnT) and tdTomato.

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