Fate Tracing of Isl1$^+$Cells in Adult Mouse Hearts under Physiological and Exercise Conditions

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ABSTRACT

Myocardial damage due to dysfunctional myocardium has been increasing, and the prognosis of pharmacological and device-based therapies remain poor. Isl1-expressing cells were thought to be progenitor cells for cardiomyocyte proliferation after specific stimuli. However, the true origin of the proliferating myocardiac cells and the role of Isl1 in adult mammals remain unresolved. In this study, Isl1-CreERT2 knock-in mouse model was constructed using CRISPR/Cas9 technology. Using tamoxifen-inducible Isl1-CreERT/Rosa26R-LacZ system, Isl1$^+$cells and their progeny were permanently marked by lacZ-expression. X-gal staining, immunostaining, and quantitative PCR were then used to reveal the fate of Isl1$^+$cells under physiological and exercise conditions in mouse hearts from embryonic stage to adulthood. Isl1$^+$cells were found to localize to the sinoatrial node, atrioventricular node, cardiac ganglia, aortic arch, and pulmonary roots in adult mice heart. However, they did not act as cardiac progenitor cells under physiological and exercise conditions. Although Isl1$^+$cells showed progenitor cell properties in early mouse embryos (E7.5), this ability was lost by E9.5. Furthermore, although the proliferation and regeneration of heart cell was observed in response to exercise, the cells associated were not Isl1 positive.

ABBREVIATIONS

Isl1	islet-1
CRISPR	clustered regularly interspaced short palindromic repeat
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction

Introduction

Cardiovascular diseases are among the most prevalent diseases worldwide. Current treatment strategies do not address the problem of myocardial tissue loss and repair. Recent studies indicated that the adult mammalian heart retains regeneration ability in normal conditions or in response to specific stimuli, such as exercise [1–3]. However, the optimal cell types for this approach remain elusive. Although several populations of cardiac stem cells have been described in the mammalian heart [4], the origins and in vivo functions of each of these remain largely unexplored.

The LIM-homeodomain transcription factor Islet-1 (Isl1), a marker of cardiovascular progenitors during embryogenesis [5, 6], plays essential roles in cell proliferation, differentiation, and survival [7, 8]. Isl1 marks a cell population that makes substantial contribution to the embryonic heart, accounting for most cells in the atria, right ventricle, specific regions of the left ventricle, and the outflow tract during cardiac development [9, 10]. Isl1$^+$cells are thought to be endogenous cardiac progenitor cells that can differentiate into

about 2/3 of the cells needed for heart development, myocardial cell lines, endothelial cell lines, smooth muscle cell lines, as well as all major cell types of the cardiovascular compartment and conduction system [11, 12]. Isl1$^+$cells, present from birth to adulthood [13], are the only progenitor cell type found in the heart that can be identified by a single marker, which is useful for the study of related lineages. Therefore, Isl1$^+$cells are favorable potential endogenous progenitor cells for heart regeneration. However, it is not clear whether Isl1$^+$cells act as cardiac progenitor cells in adult mouse hearts.

Appropriate exercise is known to enhance the function of the heart and promote the regeneration of cardiomyocytes [14–17]. Based on their presence from embryonic development to adulthood and their roles in the regulation of myocardial hypertrophy and proliferation, Isl1$^+$cells are a potential source of endogenous cardiomyocyte progenitor cells induced by exercise. However, the link between exercise and the self-renewal and differentiation of Isl1$^+$cells has not been established.

In this study, we used the genome editing system CRISPR/Cas9 to insert a cre-ERT2 cassette with a polyA sequence into the mouse *Isl1* gene by homologous recombination to determine the origin of proliferating cells. Genetic fate-mapping of Isl1$^+$cells in the heart was performed using Isl1-CreERT2/Rosa26-loxP-neo-loxP-LacZ reporter mice. The spatial and temporal distributions of Isl1$^+$cells and its progeny cells during cardiac development were studied by directional induction at various developmental periods. Histopathological analysis, real-time quantitative PCR, and immunofluorescence were used to examine the characteristics of progenitor cells. Echocardiography was used to test the effects of heart function before and after physical training interference. Thus, the regeneration of cardiac cells was studied at the molecular, cellular, and morphological levels.

Materials and Methods

Ethics statement and animals

All animal experiments in this study were approved by the Animal Care and Use Committee of the Shanghai Research Center for Model Organisms (approval number SRCMO-IACUC No. 20140002). Specific pathogen-free (SPF) C57/B6 mice were purchased from the SLAC Laboratory Animal Corporation of the Chinese Academy of Sciences (Shanghai, China). Rosa26-loxP-neo-loxP-LacZ transgenic reporter mice and Isl1-CreERT2 were supplied by the Shanghai Model Organisms Center, Inc (Shanghai, China). Mice were maintained in an SPF facility under a 12-h light/dark cycle and received free access to sterilized food and autoclaved water. Animal welfare and experimental operations strictly abided by the regulations for the control of laboratory animals. We have read and understood IJSM's ethical standards document [18] and confirm that our study meets the ethical standards.

Mouse preparation and genotyping

The Isl1-CreERT2 knock-in mouse model was constructed using CRISPR/Cas9 technology [19, 20]. The CreERT2-BGHpolyA expression cassette was site-specifically inserted into the translation start codon ATG in exon 1 of the *Isl1* gene (Ensembl: ENSMUSG00000042258). The information regarding the Isl1-CreERT2 knock-in mouse model is described in our previous article [21]. The Isl1-CreERT2 mice were mated with Rosa26-loxP-neo-loxP-LacZ transgenic reporter mice (Rosa26-lacZ) to obtain Isl1-CreERT/Rosa26-LacZ mice ▶ **Fig. 1S**. The genotypes were verified by PCR and confirmed by sequencing of the PCR products. The primers used for genotyping are listed in (▶ **Table 1S**).

Genetic labeling of Isl1$^+$cells

Tamoxifen (Sigma-Aldrich, St. Louis, MO, USA) was used to induce CreERT2 translocation to the nucleus and loxP site recombination in Isl1-CreERT/Rosa26-LacZ mice. Tamoxifen was diluted in corn oil (Sigma-Aldrich, USA). Isl1-CreERT/Rosa26-LacZ mice (8-week old) received intraperitoneal (i.p.) injection of tamoxifen (120 mg/kg) every other day for three times [6]. To examine the temporal pattern of Cre activity, the hearts were subjected to X-gal staining after tamoxifen injection at different time points (1 week, 8 weeks, and 24 weeks). Pregnant mice were treated with i.p. injections of tamoxifen (75 mg/kg body weight) at E7.5 or E9.5 [6, 10], and embryos were harvested at E14.5 and subjected to X-gal staining.

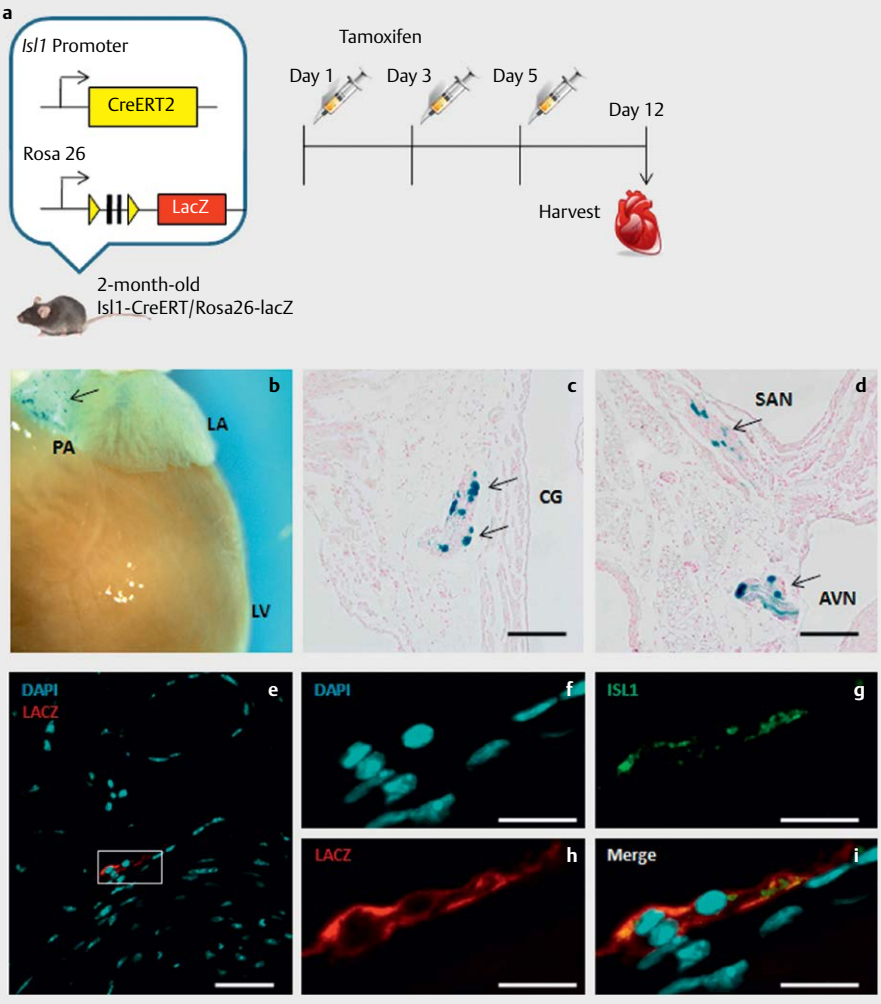
X-gal staining

All mice were electively sacrificed at various time points from week 1 to week 24, as shown in ▶ **Fig. 1a**, ▶ **2a**, and ▶ **3a**. The hearts were harvested and rinsed with cold 4 % PFA and perfusion-fixed in 2 % PFA for 4 h. The fixed samples were stained overnight in X-gal (Beyotime Biotechnology, Shanghai, China) solution (5 mM $K_4Fe(CN)_6$, 5 mM $K_3Fe(CN)_6$, 2 mM $MgCl_2$, 0.01 % NP-40, 0.1 % deoxycholate, 0.1 % X-gal in PBS) at 37 °C [6]. The samples were paraffin-embedded in Optimal Cutting Temperature compound (OCT; Thermo, Waltham, MA, USA) for sectioning (5 µm). The nuclei were stained using Nuclear Fast Red (Sigma-Aldrich). Images were acquired and analyzed using Image-pro Plus 6.0 (Media Cybernetics, Rockville, MD, USA).

Embryos were harvested in cold PBS and fixed for 1–2 h in 4 % PFA. To optimize tissue fixation and improve the penetration of the X-gal substrate (Roche Molecular, Indianapolis, IN, USA), the heart was harvested. Whole hearts were incubated in X-gal solution. For high-resolution analysis of β-Gal activity, stained hearts were paraffin-embedded, sectioned, and counter-stained with Nuclear Fast Red. After X-gal staining, the hearts were cut longitudinally and embedded in paraffin. For histology and immunohistochemistry, the heart sections (5 µm in thickness) were processed either with hematoxylin or for immunostaining.

Immunostaining

The paraffin sections were blocked with Immunol Staining Blocking Buffer (Beyotime Biotechnology) for 1 h and then incubated with primary antibodies (▶ **Table 2S**) overnight at 4 °C. The sections were then incubated with fluorescent-labeled secondary antibodies (▶ **Table 3S**) for 1 h at room temperature (21–22 °C) after washing with 0.25 % TritonX-100 in PBS. Nuclear DNA was stained using 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen, Carlsbad, CA, USA). Images were acquired from three sections per sample (n = 3 per group). Positive staining signals were counted from five visual fields (magnification, ×400) per section.



▶ **Fig. 1** Tamoxifen-induced lacZ staining of heart tissues and co-staining experiments confirmed that lacZ-positive cells are Isl1-positive. **a**: Experimental scheme: Isl1-CreERT/Rosa26-lacZ adult mice were injected with tamoxifen every other day for 3 times. Mice hearts were dissected after a 1-week and stained with X-gal. **b**-**d**: Observation of X-gal staining of the hearts of Isl1-CreERT/Rosa26-lacZ adult mice under a stereoscope and histological analysis of X-gal staining of heart tissues. Scale bar represents 100 µm. Arrows in **b**, **c** and **d** indicate X-gal-positive cells within the sinoatrial node, atrioventricular node, cardiac ganglion, outflow tract, and aortic arch. **e**: Immunofluorescence staining in the Isl1-CreERT/Rosa26-lacZ adult mouse heart. Nuclei were stained using DAPI (blue). ISL1 (green) cells and lacZ (red) cells were colocalized. Scale bar represents 100 µm. **f**-**i**: enlarged portions of E. Abbreviations: LA: left atria; LV: left ventricle; CG: cardiac ganglion; SAN: sinoatrial node; PA: pulmonary artery. N = 3.

Exercise intervention

We divided 2-month old adult male Isl1-CreERT/Rosa26-LacZ heterozygote mice into 2 groups (9 mice/group): control (HC), and aerobic exercise group (HA). Mice in HA underwent 8-week's training program using small motorized animal treadmill. Aerobic exercise was carried out as follows [22]: mice from the exercise group were subjected to 10 min of running on a motorized treadmill two times daily for the first 3 days to adapt to experimental conditions. Then, the running time was increased to 90 min once a day, 6 times a week for the next 8 weeks (refer to supplementary ▶ **Table 4S**). Mice in the control group (HC) were just in a home cage with no intervention. The mice were closely observed during training to avoid injury.

RNA extraction and quantitative PCR

Total RNA was isolated from the heart tissues of mice using TRIzol Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. The cDNA was generated from 1000 ng of RNA with ReverTra Ace qPCR RT Master Mix (FSQ-301; Toyobo, Osaka, Japan) in a 20 µl reaction mixture, according to the manufacturer's protocol. The cDNA was diluted 5-fold and 2.5 µl of the diluted template was used in a 10 µl real-time PCR mixture with SYBR Green (DBI-2224; DBI Bioscience, Shanghai, China). Primers used for qPCR are shown in ▶ **Table 1S**.

Statistical analysis

Results are expressed as means ± standard error. Student's t-test was utilized to compare two means and one-way ANOVA followed by Bon-

ferroni tests was used to compare multiple means. Statistical analyses were performed using Prism v.6.0 (GraphPad Software, San Diego, CA, USA). A value of $p < 0.05$ was considered statistically significant.

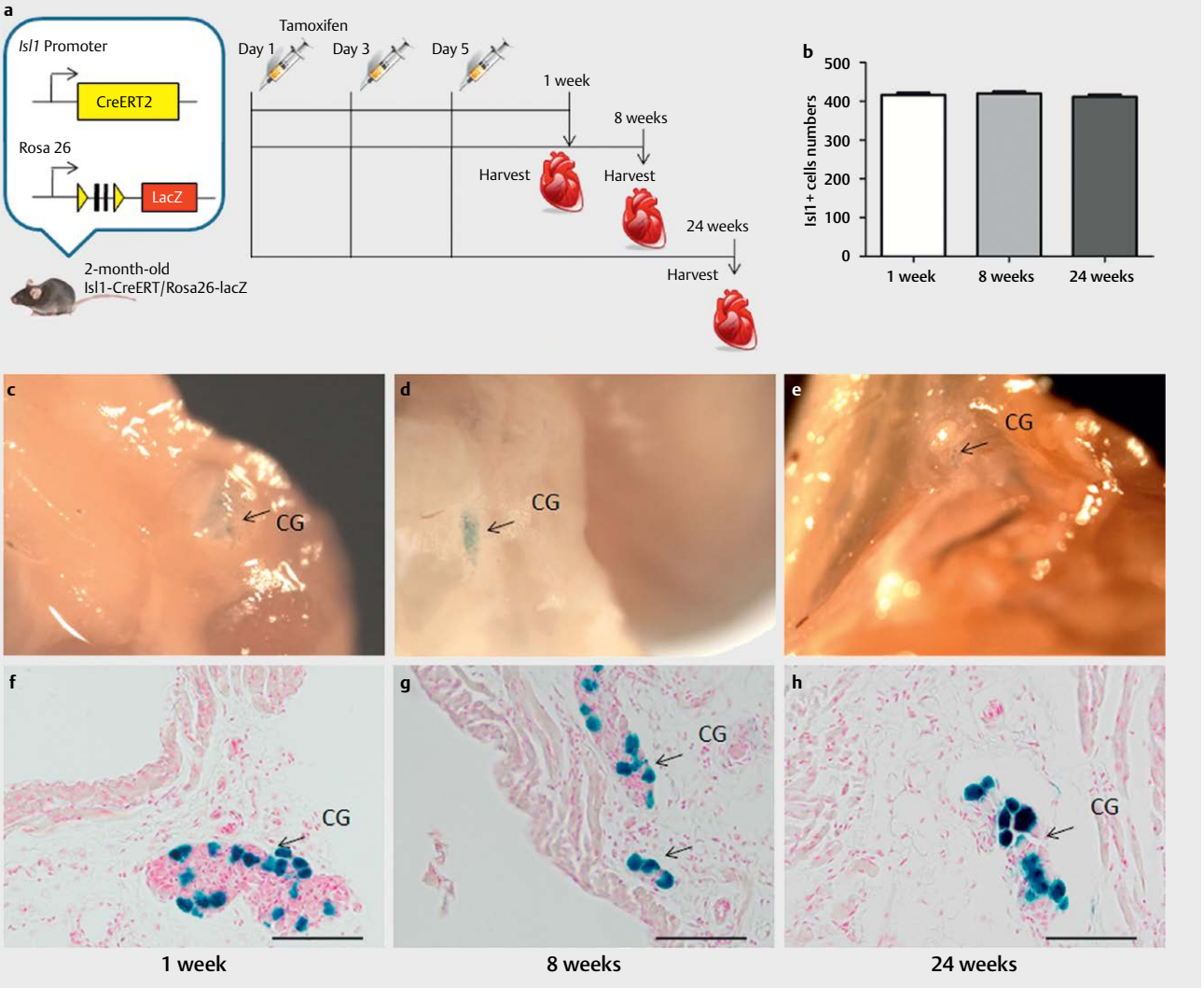
Results

Genetic labeling of Isl1$^+$cells in the adult mouse heart

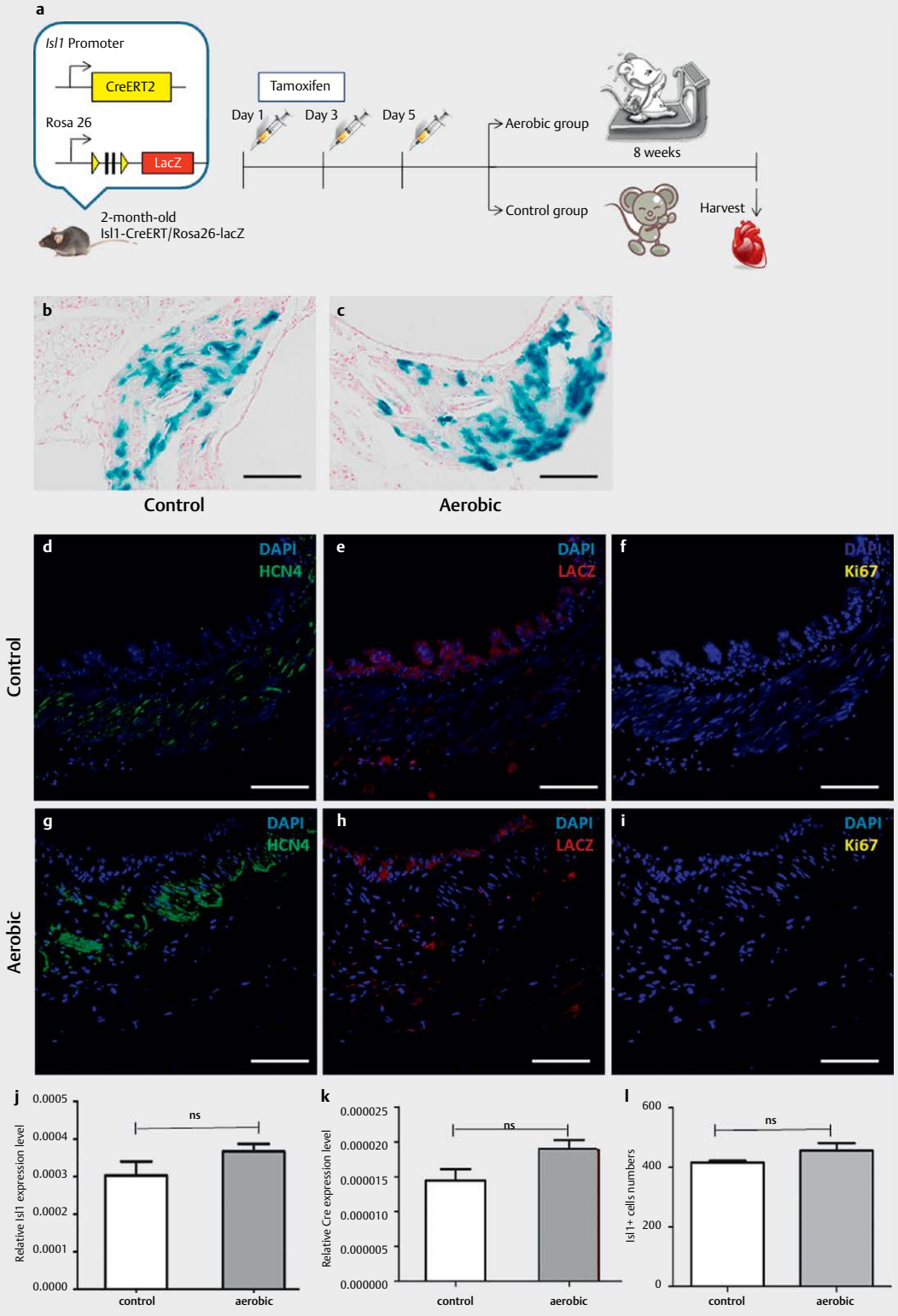
To investigate whether Isl1$^+$cells exist in the adult heart, tamoxifen was injected into 2-month-old Isl1-CreERT/Rosa26-lacZ mice every other day for 3 times (▶ **Fig. 1a**). The heart tissues were gathered 1 week later and stained with X-gal and sliced. Tamoxifen-induced Cre/loxP recombinant cells were detected in the heart sinoatrial node, cardiac ganglia, aortic arch, and pulmonary roots in Isl1-CreERT/Rosa26-lacZ adult mice (▶ **Fig. 1b**), consistent with previous results [5, 23, 24]. The expression of CreERT2 cells in these tissues was further confirmed by using X-gal staining in frozen and paraffin-embedded sections of heart tissues from heterozygote (▶ **Fig. 1c, d**). Furthermore, double immunostaining for lacZ and Isl1 showed that the lacZ$^+$cells co-localized with Isl1$^+$cells (▶ **Fig. 1e-i**). The data showing CreERT was co-expressed with Isl1 in Isl1-CreERT/Rosa26-lacZ adult mouse hearts, and that the expression of CreERT2 was consistent with endogenous Isl1 in these mice. Thus, we established a system for tracing Isl1-expressing cells and their progeny cells.

Isl1$^+$cells are not cardiac progenitor cells in adult mice under physiological conditions

To investigate the fate of Isl1$^+$cells in the adult mouse heart under normal physiological conditions, tamoxifen was injected into



▶ **Fig. 2** Temporal and spatial distribution of Isl1$^+$cells and its progeny cells in the hearts of Isl1-CreERT/Rosa26-lacZ adult mice. **a**: Experimental scheme: Isl1-CreERT/Rosa26-lacZ adult mice were injected with tamoxifen 3 times. Then, mice were sacrificed and hearts were dissected for analysis after 1, 8, and 24 weeks. **b**: Quantification of isl1t cells in adult mice hearts after 1, 8, and 24 weeks with tamoxifen 3 times. Mean values ± SEM; from three hearts. **c**–**h**: Observation of X-gal staining of hearts of Isl1-CreERT/Rosa26-lacZ adult mice at three stages under a stereoscope and histological analysis of X-gal staining of heart tissues of three stages. **c** and **f**: After a 1-week washout period; **d** and **g**: After an 8-week washout period; **e** and **h**: After a 24-week washout period. CG: cardiac ganglion. Scale bar represents 100 µm.



▶ **Fig. 3** Lineage tracing of Isl1 + cells in an anaerobic exercise model. **a**: Experimental scheme: Isl1-CreERT/Rosa26-lacZ adult mice were injected with tamoxifen 3 times. Then, mice were sacrificed and hearts were dissected for analysis after 8 weeks of aerobic exercise (n = 9). **b**-**c**: Histological analysis of X-gal staining of heart tissues from the exercise group and control group (normal diet and no intervention). **d**-**i**: Double immunostaining of heart tissues for lacZ (red), HCN4 (green), and Ki67 (yellow). Nuclei were stained using DAPI (blue). Scale bars represent 100 µm. **j**-**k**: Isl1 and Cre expression levels in each group, determined by qPCR. **l**: Quantification of Isl1 + cells number. Results are expressed as means ± SEM.

2-month-old Isl1-CreERT/Rosa26-lacZ male mice. Hearts were harvested at 1, 8, and 24 weeks after injection and stained with X-gal (▶ **Fig. 2a**). Isl1$^+$cells and their progeny cells were detected in the sinoatrial node, cardiac ganglion, and pulmonary artery, and this localization remained unchanged after 1, 8, and 24 weeks of injection (▶ **Fig. 2c-h**), indicating that localization of Isl1$^+$cells is not affected with age within the time points analyzed. Furthermore, there were no significant differences in the lacZ$^+$cell number and distribution between these groups and the number of Isl1$^+$cells did not change significantly over time (▶ **Fig. 2b**). These results suggested that Isl1$^+$cells do not serve as cardiac progenitors in adult heart regeneration under normal physiological conditions.

Isl1$^+$cells in adult mouse hearts do not have the potential for proliferation in response to exercise

To test whether Isl1$^+$cells are progenitor cells in heart regeneration stimulated by exercise, 2-month-old Isl1-CreERT/Rosa26R-lacZ mice were subjected to training on a treadmill for 8 weeks of aerobic training; littermates served as controls for each group (▶ **Fig. 3a**). Hearts were obtained 24 h after the last day of exercise and X-gal and immunofluorescence staining were performed. Echocardiography, histopathological analysis, real-time quantitative PCR, and immunofluorescence were used to determine patterns of cell movement and morphology.

The localization and number of lacZ$^+$cell was similar in the exercise and control groups (▶ **Fig. 3b, c, l**). Based on immunostaining of heart tissues for lacZ, the sinus node marker hyperpolarization activated cyclic nucleotide-gated cation channel 4 (HCN4), and LacZ-positive cells were located within or near the sinus node (▶ **Fig. 3d-i**). There was no significant difference between the exercise and control groups in Isl1 and Cre expression, as indicated by qPCR (▶ **Fig. 3j, k**). In addition, exercise did not cause significant change in the number and distribution of lacZ$^+$cell in the heart tissues. Thus, our results indicated that Isl1$^+$cells in adult mouse hearts do not have the potential for proliferation in response to exercise.

Cardiomyocyte proliferation in response to exercise in adult mouse hearts

The Isl1-CreERT/Rosa26R-lacZ mice were subjected to aerobic training for 8 weeks and hearts were removed 24 h after the last exercise period and paraffin sections were prepared for immunofluorescence staining. Confocal microscopy was used to analyze TNNI3 (marker for heart cardiac muscle) and Ki67 (cell proliferation marker). In response to regular intensity-controlled exercise training, Ki67 staining was detected in the cardiomyocytes in the exercise group (▶ **Fig. 4e**, ▶ **Fig. S2**)), but not in the control group (▶ **Fig. 4b**). Cyclin-D1 expression measured by qPCR was also higher in hearts from the exercise group than in that from the control group (▶ **Fig. 4i**). Thus, exercise promoted the proliferation of cardiomyocytes in adult mice, suggesting that cell proliferation and regeneration occur in the heart in response to exercise stimulation. However, Isl1 + or LacZ staining was not carried out, and hence whether the proliferative cells were from Isl1$^+$could not been determined.

Isl1$^+$cells lose cardiac progenitor properties at E9.5

To determine when Isl1$^+$cells lose their progenitor property, tamoxifen was injected into pregnant dams at E7.5 or E9.5. Embryos were harvested at E14.5 and stained with X-gal to reveal the lineages derived from cells expressing Isl1 at the time of injection (▶ **Fig. 5a**). We found that Isl1$^+$cells gave rise to the atria, the right ventricle, and the outflow tract in the embryonic mouse heart (▶ **Fig. 5c, d**). Isl1$^+$cells had the characteristics of cardiac progenitor cells in early mouse embryos (E7.5). However, when tamoxifen induction was carried out at E9.5, only a few lacZ$^+$cell were present within the outflow tract (▶ **Fig. 5f, g**). Isl1$^+$cells and their progeny cells in the embryonic heart at E14.5 were significantly reduced compared with those in the embryonic heart induced at E7.5. Moreover, expression of Isl1 was decreased in the late stage of embryonic development. These results indicate that Isl1 + cell may lose the role of cardiac progenitor cells by E9.5.

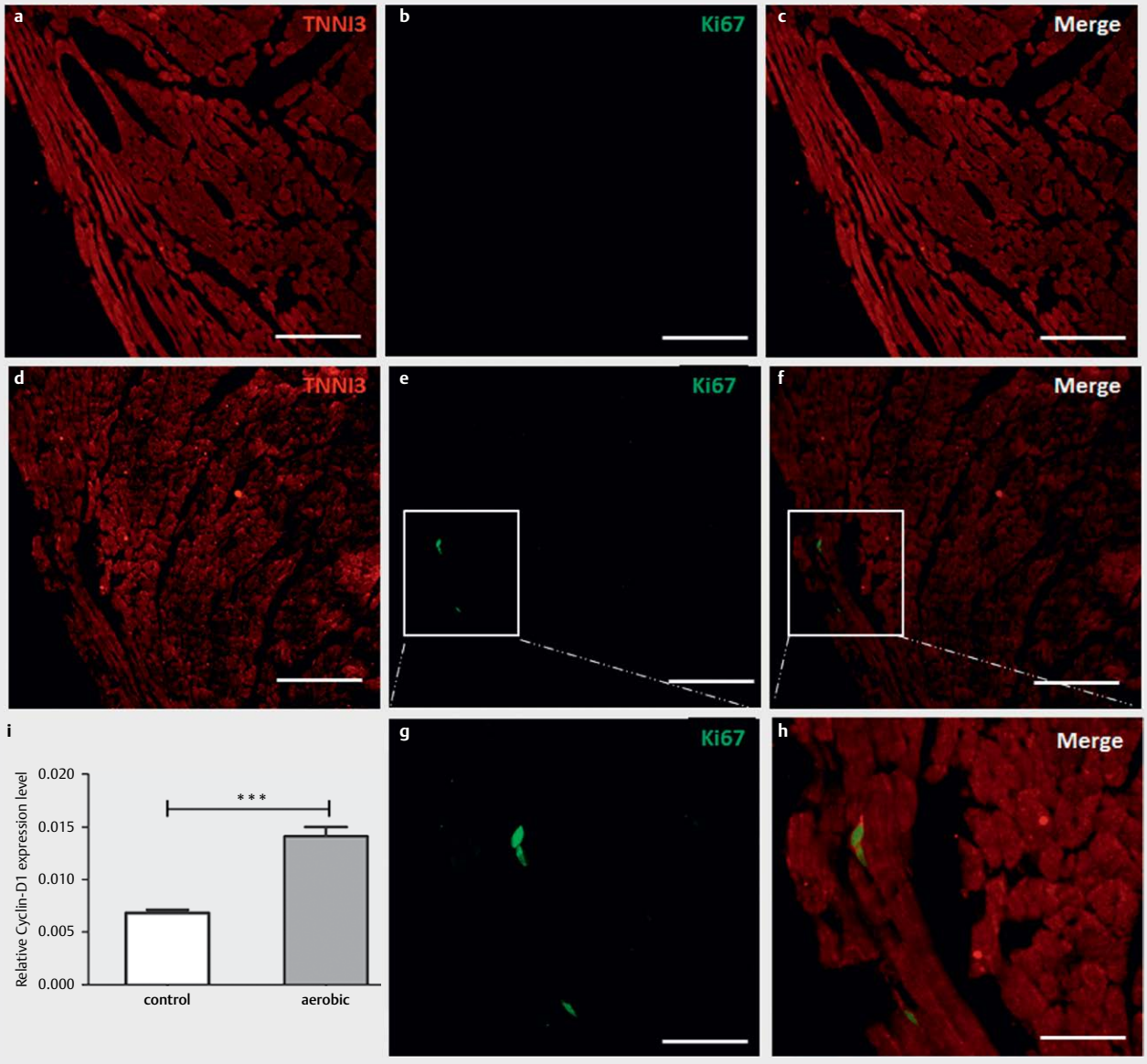
Discussion

Cardiomyocytes in the adult human heart can renew, with a gradual decrease from 1 % turnover annually at the age of 25 to 0.45 % at the age of 75 [25]. Isl1 as a pleiotropic transcription factor regulates many downstream target genes at various time points and in different tissue types, and plays an important role in cardiac development. Isl1 gene knockout mice suffer from cardiovascular insufficiency and die at E10.5 [7]. The normal expression of Isl1 during early development is necessary for the proliferation, migration, and survival of cardiac progenitor cells. Some studies have found that it interacts with multiple genes, such as Gata4, Mef2c, and Nkx2.5 [26–29].

In this study, we found that Isl1$^+$progeny cells were widely distributed in the atria, the right ventricle, and the outflow tract in early mouse embryos (E7.5), highlighting their potential significance in cardiac development. Previous studies have shown that different cardiac cell types arise from common multipotent cardiac progenitor cells during heart development. Although Isl1$^+$cells exist in adult heart tissues in several species, including mice, rats, and humans [30–32], it is not clear whether these cells function as progenitor cells in adult hearts.

Isl1 nuclear LacZ (nLacZ) knock-in mice have been generated in previous studies [5, 6, 8, 10]. Several studies have revealed that Isl1$^+$cells are strictly confined to certain heart structures, but there is no evidence suggesting that Isl1$^+$cells are cardiac progenitor cells in adult mouse hearts. Although the Isl1-nlacZ mouse model can be used to detect the existence of Isl1$^+$cells, they cannot be used to trace progeny cells produced by Isl1$^+$cells that do not express Isl1. Therefore, it is not an effective model for lineage tracing experiments focused on cardiac stem cell differentiation.

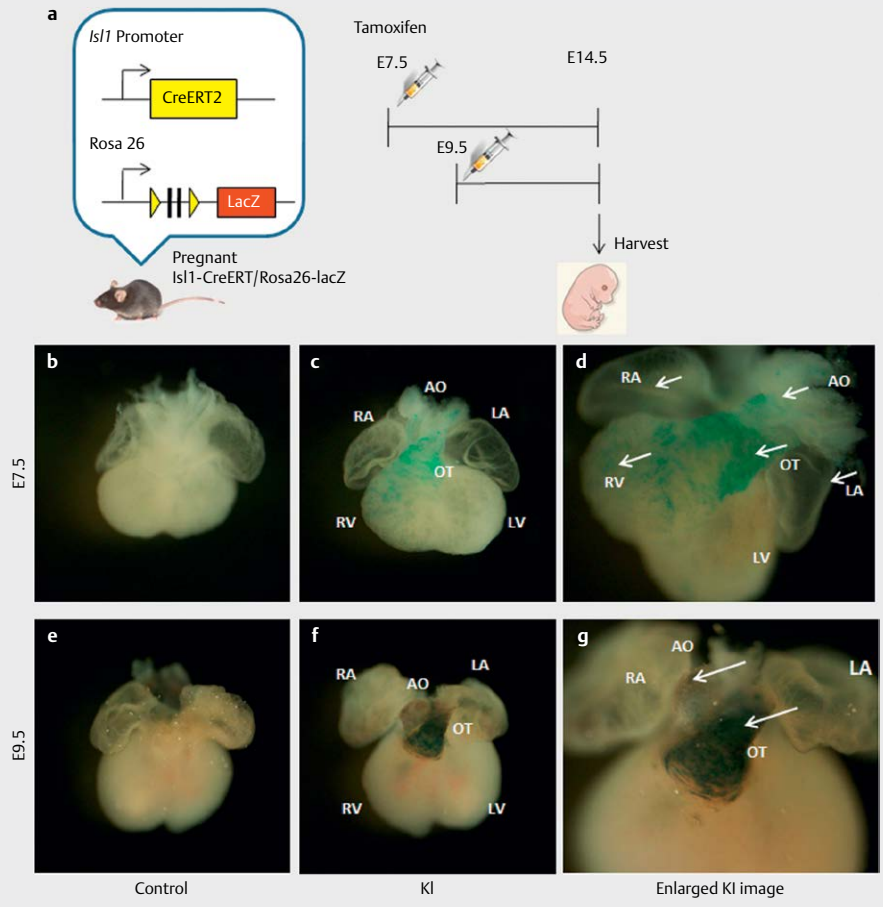
In this study, we used the CRISPR/Cas9 genome editing system to insert CreERT2 into the mouse Isl1 endogenous gene promoter for cell lineage tracing. The Isl1-CreERT/Rosa-lacZ mouse model that we developed could be used to detect both Isl1$^+$cells and their progeny cells. Using this model, we found that Isl1$^+$cells in adult mouse hearts were mostly located in the sinoatrial node, cardiac ganglia, aortic arch, and pulmonary roots. We also found that Isl1$^+$cells do not have progenitor cell properties in adult mouse



▶ **Fig. 4** Exercise–induced cardiomyocyte proliferation in Isl1-CreERT/Rosa26-lacZ adult mice. Immunofluorescence staining of the Isl1-CreERT/Rosa26-lacZ adult mouse heart after 8 weeks of intervention. **a**-**c**: control group; **d**-**f**: aerobic exercise group. Scale bar represents 100 µm. **g** and **h** show enlarged portions of **e** and **f**. Scale bar represents 50 µm. Proliferation marker Ki67 (green) and cardiomyocyte-selective protein TNNI3 (red). **i**: Cyclin-D1 expression differences between groups (determined by qPCR). Results are expressed as means ± SEM; * $p < 0.05$. N = 3.

heart under physiological conditions. A previous study using myocardial infarction induction model indicated that Isl1$^+$cells were absent in the myocardial infarction area [9]. Therefore, under normal physiological and pathological conditions, in the adult heart, there is no evidence that Isl1$^+$cells are progenitor cells for myocardial regeneration.

Other studies have shown that exercise promotes cardiomyocyte cell division in response to specific stimuli [2, 3, 15, 16, 22]. In preliminary analyses, we found that regular moderate aerobic exercise could induce improvement in cardiac structure and physiological function in wild-type mice and has substantial effects on morphological indices, such as heart size, weight, ventricular wall thickness, and angiogenesis [17]. It could improve the heart coefficient, shorten the left ventricular short axis, and improve heart function. Isl1 expression was elevated in the whole heart in both the aerobic exercise group and overload exercise group [33]. Therefore, we used exercise as a non-traumatic endogenous stimulus to determine whether Isl1$^+$cells act as cardiac progenitor cells in the hearts of adult mice stimulated with exercise. Our results suggested that myocardial cell proliferation occurred after exercise. However, the proliferating cells were not from Isl1$^+$cells. Instead, our results suggest that Isl1$^+$cells may lose their cardiac progenitor properties as early as E9.5.



▶ **Fig. 5** Lineage analysis of Isl1-cre-expressing cells during heart development. **a**: Experimental scheme: Pregnant Isl1-CreERT/Rosa26-lacZ females were divided into two groups and were injected with tamoxifen at E7.5 or E9.5. Then, the embryos were harvested at E14.5 and stained with X-gal. **b**-**d**: Whole-mount X-gal staining view of E14.5 embryonic hearts induced at E7.5. **e**-**g**: Whole-mount X-gal staining view of E14.5 embryonic hearts induced at E9.5. Hearts from Isl1-CreERT/Rosa26-lacZ embryos (KI) were X-gal-positive (**c** and **f**), while those in the control group were X-gal-negative (**b** and **e**, control). Arrows in D indicate X-gal-positive cells within the atria, right ventricle, outflow tract, and aortic arch. Arrows in G indicate X-gal-positive cells within the aortic arch and outflow tract. Abbreviations: LA, Left atria; RA, Right atria; OT, outflow tract; AO, Aortic arch; LV, left ventricle; RV, right ventricle. N = 3.

Recent studies have shown that stem cells do not exist in adult hearts of mice under normal physiological conditions which is inconsistent with previous studies. Our results demonstrate that Isl1$^+$cells do not contribute to the effects of myocardial cell proliferation associated with exercise, likely because the stress caused by exercise is not sufficiently severe. Therefore, it remains unknown whether certain Isl1$^+$cells have the potential to contribute to proliferation under more extreme conditions (such as in acute myocardial infarction). In future studies, we will establish a model of coronary artery ligation to study the role of Isl1$^+$cells in response to heart damage. In addition, we found that Isl1-CreERT/Rosa26-LacZ heterozygotes have a different phenotype compared with wild-type mice under exercise condition (data not shown). The specific mechanism will be the focus of follow-up studies.

In conclusion, our study shows that Isl1$^+$cells hold progenitor cell properties in early mouse embryos (E7.5), but this function is lost at E9.5. Importantly, Isl1$^+$cells do not act as cardiac progenitor cells under physiological conditions. Furthermore, although myocardial cell proliferation occurred after exercise, the proliferating cells were not from Isl1$^+$cells.

Authors' Contributions

Y. Zhou and J. Fei were responsible for conceptualization and supervision; Y. Zhou and J. Fei acquired funding; M. Zhang and Z. Wang performed investigation; J. Fei and Z. Wang conceived of the methodology; J. Shi and R. Sun designed the sgRNA and CRISPR/Cas9; Y. Zhou, H. Yang, S.Yang and N. Wang analyzed the role of isl1 in embryonic period; Y. Zhou, H. Yang , J. Shi and S. Yang performed the exercise assay. Y. Zhou, H. Yang and J. Fei performed the data analyses; Y. Zhou wrote the original draft; and H. Yang and J. Fei reviewed and edited the draft.

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Conflict of Interest

Authors declare that they have no conflict of interest.

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