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Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We thank G. Bucher, M. Weber and M. Klingler for the *pu11* enhancer-trap line, R. White for the FP6.86 antibody, DSHB for the 4D9 antibody and G. Pflugfelder for sharing information about *omb* degenerate primers. We thank K. Leonard for maintaining beetle stocks, T. Shippy for discussion and reading and S. Brown, R. Beeman, S. Haas and all the Manhattan beetle/insect laboratory members for discussion and comments. Y.T. thanks A. Sato and T. Yamaguchi for discussion. This work was supported by the international Human Frontier Science Program Organization (Long-term Fellow) and the National Science Foundation.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to Y.T. (tomoyasu@ksu.edu). The sequences are available in GenBank under the following accession numbers. *Tc-sal*: AY600513; *Tc-iro*: AY600514; *Tc-omb*: AY600516.

Postnatal *Isl1*⁺ cardioblasts enter fully differentiated cardiomyocyte lineages

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The purification, renewal and differentiation of native cardiac progenitors would form a mechanistic underpinning for unravelling steps for cardiac cell lineage formation, and their links to forms of congenital and adult cardiac diseases^{1–3}. Until now there has been little evidence for native cardiac precursor

cells in the postnatal heart⁴. Herein, we report the identification of *Isl1*⁺ cardiac progenitors in postnatal rat, mouse and human myocardium. A cardiac mesenchymal feeder layer allows renewal of the isolated progenitor cells with maintenance of their capability to adopt a fully differentiated cardiomyocyte phenotype. Tamoxifen-inducible Cre/lox technology enables selective marking of this progenitor cell population including its progeny, at a defined time, and purification to relative homogeneity. Co-culture studies with neonatal myocytes indicate that *Isl1*⁺ cells represent authentic, endogenous cardiac progenitors (cardioblasts) that display highly efficient conversion to a mature cardiac phenotype with stable expression of myocytic markers (25%) in the absence of cell fusion, intact Ca²⁺-cycling, and the generation of action potentials. The discovery of native cardioblasts represents a genetically based system to identify steps in cardiac cell lineage formation and maturation in development and disease.

In skeletal muscle, satellite cells are considered specialized endogenous muscle precursors (that is, myoblasts) that are pre-programmed to enter the skeletal muscle lineage^{5,6}. Despite intensive inquiry, there has been no clear evidence for a native cardiac progenitor population with similar characteristics in the myocardium. Various cell surface markers have been used to purify precursor cells from heart muscle including *c-kit* and *sca-1* (refs 7, 8), but the precise role of these cells in *in vivo* cardiogenesis is unclear and the efficiency of conversion to fully differentiated myocytes, in the absence of fusion, is relatively low^{9–11}. Taking advantage of a developmental lineage marker for undifferentiated cardiogenic precursor cells as a requirement for a heart-specific origin, we have identified in the postnatal heart a novel cardiac cell type. The LIM-homeodomain transcription factor *islet-1* (*Isl1*) marks a cell population that makes a substantial contribution to the embryonic heart, comprising most cells in the right ventricle, both atria, the outflow tract and also specific regions of the left ventricle¹².

A subset of *Isl1*⁺ undifferentiated precursors remains embedded in the embryonic mouse heart after its formation and their number decreases progressively from embryonic day 12.5 (ED12.5) to ED18.5 (Fig. 1a–d). After birth, relatively few *Isl1*⁺ cardioblasts were still detectable, averaging 500 to 600 in the myocardium of a 1–5-day-old rat (Fig. 1e, f). Their organ distribution matched the defined contributions of *Isl1*⁺ embryonic precursors (Fig. 1i), suggesting these cells are developmental remnants of the fetal progenitor population. Clusters of *Isl1*⁺ cardioblasts were observed in both atria, whereas in the ventricles they occurred mostly as single cells (Fig. 1e, f). The localization of *Isl1*⁺ progenitors in specific cardiac segments (atrial muscle wall, intra-atrial septum, conus muscle, right ventricle) was conserved among diverse species: mouse, rat and human (Fig. 1g, h; Supplementary Table 1).

Using conditional genetic marking techniques in the mouse, we performed Cre-recombinase-triggered cell lineage tracing experiments to irreversibly mark *Isl1*-expressing cells as well as their differentiated progeny during embryonic development (Fig. 2). *Isl1*-IRES-Cre mice¹³ were crossed into the conditional Cre reporter strain R26R¹⁴, in which Cre-mediated removal of a stop sequence results in the ubiquitous expression of the lacZ gene under the control of the endogenous *Rosa26* promoter (Fig. 2a). In neonatal mice bearing both alleles a high proportion of right ventricular myocardium expressed β -galactosidase (β -gal) detected by 5-bromo-4-chloro-3-indolyl- β -D-galactoside (X-gal) staining (Fig. 2a). Around 30–40% of cardiac myocytes isolated from double heterozygous hearts stained positive for X-gal and displayed co-expression of β -gal and sarcomeric α -actinin (Fig. 2b), demonstrating that a significant proportion of myocytes originate from *Isl1*⁺ cardiac progenitors.

To achieve temporal and spatial control of Cre expression

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required for tracing the fate of *isl1*⁺ progenitors resident in the postnatal heart, we generated mice that harbour a knockin of the tamoxifen-dependent Cre recombinase (*isl1*-mER-Cre-mER) into the *isl1* locus (Fig. 3). The presence of two mutated oestrogen-receptors (mERs) results in the sequestration of Cre in the cytoplasm¹⁵. Tamoxifen induces a rapid nuclear translocation of the mER-Cre-mER protein, which permits Cre-mediated recombination^{16,17}. As a readout for Cre activity, we again used the reporter strain R26R¹⁴. Thus, application of tamoxifen to double heterozygous *isl1*-mER-Cre-mER/R26R mice leads to β -gal expression in cells expressing *isl1* at the time of exposure (Fig. 3a). Experiments with *isl1*-mER-Cre-mER/R26R mice were performed to assess the specificity of cell labelling. β -gal⁺ cells are found only within the looping heart in double heterozygous animals after tamoxifen

injection at ED7.5 and X-gal staining at ED9.5 (Fig. 3b). Double immunofluorescent analysis confirmed the co-expression of *isl1* and Cre in undifferentiated cardiac progenitors and demonstrated that nuclear or cytosolic Cre localization was dependent on tamoxifen exposure (Fig. 3c). At ED12, sections of right ventricle and atrial tissue, heart regions that have been shown to originate from *isl1*⁺ precursors¹², exhibited expression of α -myosin heavy chain but were negative for *isl1* and Cre (Fig. 3d). Thus, tamoxifen injection leads to a highly specific marking of *isl1*-expressing progenitors and Cre protein is not detectable \sim 54 h after the promoter has been inactivated. After birth, β -gal⁺ cells can be detected with highest frequency in the conus muscle, the right atrium and the right ventricle by X-gal stain in *isl1*-mER-Cre-mER/R26R double heterozygous neonatal hearts after tamoxifen injection at ED17

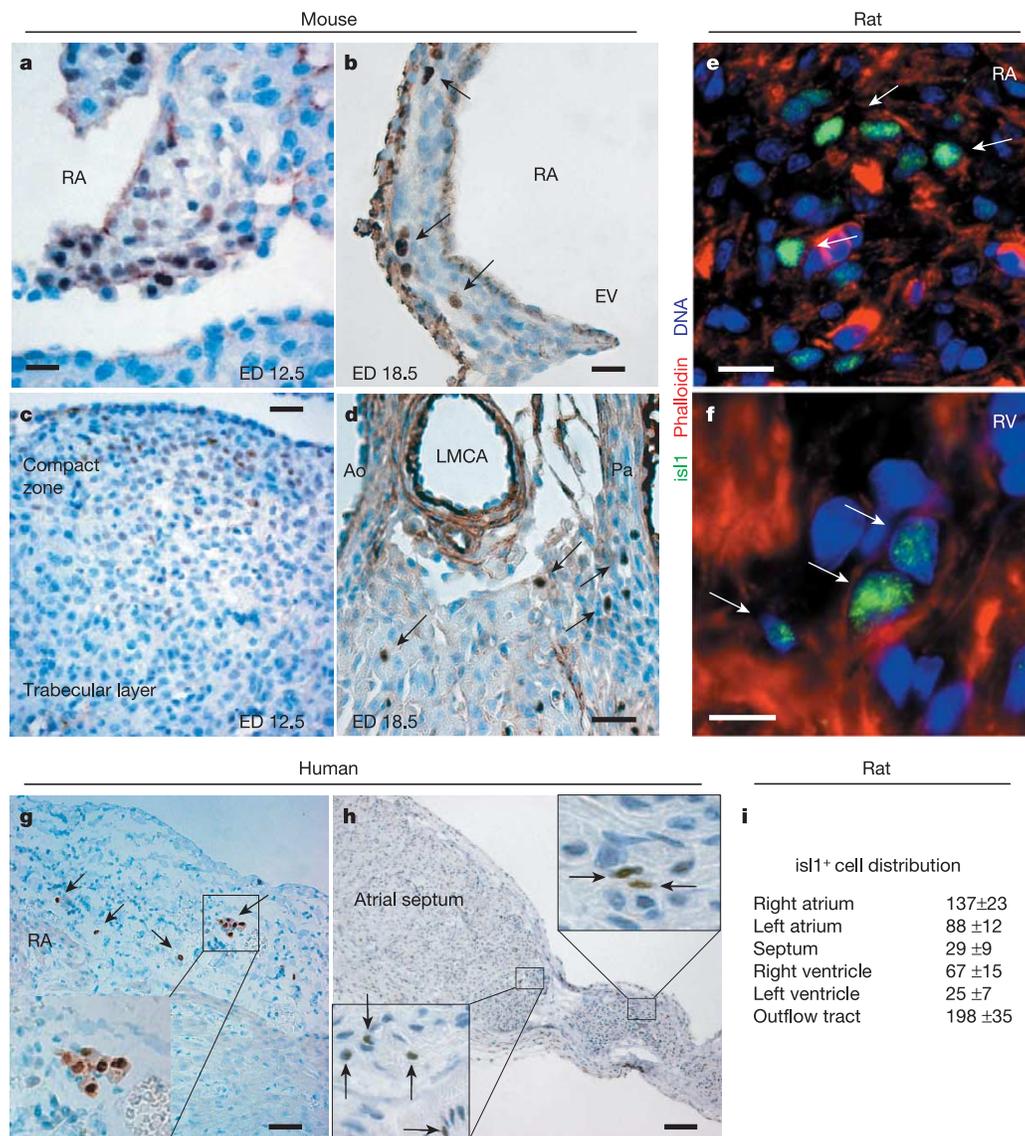


Figure 1 *Isl1*⁺ progenitors in the late embryonic and postnatal heart. **a–d**, *Isl1*⁺ progenitors in mouse sections at ED12.5 (**a**, atrial septum; **c**, ventricular tissue) and 18.5 (**b**, free atrial wall; **d**, ventricular arterial region). Scale bar, 20 μ m (**a, b, d**), 50 μ m (**c**). EV, eustachian valve; RA, right atrium; Ao, aorta; LMCA, left main coronary artery; Pa, pulmonary artery. **e, f**, Cluster of *Isl1*⁺ cardiac precursors embedded in right atrial tissue and in the right ventricle of a postnatal day 1 rat heart. Scale bars, 10 μ m (**e**), 15 μ m (**f**).

g, h, *Isl1*⁺ progenitors in human right atrial tissue from an 8-day-old patient and intra-atrial septum from a 2-day-old patient. Scale bars, 75 μ m (**g**), 150 μ m (**h**). **i**, Quantification and localization of *Isl1*⁺ cells in postnatal day 1 rat hearts. Mean values \pm s.e.m. from three hearts. Arrows designate *Isl1*⁺ cells in different species and insets represent a magnification of the areas of interest.

(Fig. 3e), and represent a rare subset of cells in the adult right atrium and outflow tract (Supplementary Fig. 1).

Following cell isolation from postnatal *isl1*-mER-Cre-mER/R26R mouse hearts and reporter gene induction *in vitro*, most β -gal⁺ cells were found in the cardiac mesenchymal cell (CMC) fraction (Fig. 4a). Mesenchymal cells isolated from postnatal rat hearts also contain *isl1*⁺ progenitors (Supplementary Fig. 2). The CMC culture environment maintains *isl1* expression in the progenitor population and promotes their expansion in culture without differentiation (Fig. 4b and Supplementary Fig. 2).

β -gal⁺ cells can be purified from the mesenchymal cells using fluorescence-activated cell sorting (FACS) after labelling with a fluorogenic, lipophilic β -gal substrate (C₁₂FDG) (Fig. 4c, d). *Isl1*⁺ progenitors were isolated as a distinct population presenting seven to ten times higher C₁₂FDG fluorescence than background at a purity of 90–95%. They corresponded to 0.5% of the mesenchymal cell fraction when FACS-sorted during an early stage in culture (4–6 days) and reached ~8% after 15–18 days culture (Fig. 4c, d). As phenotypic characteristics of an undifferentiated state, β -gal⁺ precursors expressed *isl1* and early specification markers for cardiac mesoderm, *Nkx2.5* and *GATA-4*, while lacking transcripts of mature myocytes (Fig. 4e).

FACS analysis of the FDG⁺ cell fraction, expanded *in vitro* over 10 days, revealed negative staining for *sca-1* and no correlation between β -gal expression and the efflux of the DNA-binding dye Hoechst 33342, demonstrating that *isl1*⁺ cardioblasts are distinct from cardiac side population cells (Fig. 4f–h and Supplementary Table 2)^{8,18}. In addition, *isl1*⁺ cells did not express *c-kit*.

After exposure to 4-hydroxytamoxifen (4-OH-TM) *in vitro*, a rare subset of β -gal⁺ cells coexpressing cardiac troponin T was observed at day 4 ($n = 51 \pm 12$), day 7 ($n = 81 \pm 29$) and day 10 ($n = 157 \pm 10$) in the myocyte fraction (Fig. 4i, j), resulting in ~75% of the total β -gal⁺ cells. The fact that *isl1* is repressed as

soon as the cells acquire a differentiated phenotype¹² and the lack of β -gal expression in the absence of 4-OH-TM (Fig. 3b) strongly suggest that *isl1*⁺ cardioblasts can spontaneously acquire myocytic characteristics when dispersed and cultured in the presence of differentiated myocytes.

To assess the potential of β -gal⁺ progenitors to adopt a myocytic phenotype, we performed co-culture experiments of FACS-sorted precursors with neonatal cardiac myocytes. After 3 days in co-culture, β -gal⁺ cells were found within the myocytic syncytium and expressed cardiac specific proteins, that is, α -sarcomeric actinin or troponin T in a partially or completely organized pattern (Fig. 4k, l). Moreover, a subset of β -gal⁺ cardioblasts showed contractile activity and intact electromechanical coupling to neighbouring cells, as documented by expression of the gap-junctional protein connexin 43 (data not shown). The differentiation capacity of β -gal⁺ precursors did not significantly differ between cells maintained in culture for only a few days (early stage) and cells expanded for 15–18 days (late stage) (Fig. 4l).

We addressed the role of cell fusion in the progenitor-to-cardiac myocyte transition, by performing co-culture experiments in which neonatal myocytes were pre-fixed with paraformaldehyde to stabilize cellular components and render the cells refractory to cell fusion¹⁹. Paraformaldehyde treatment disrupted the exclusion of propidium iodide by the myocytes, indicating that these cells were non-viable (Fig. 4m). FACS-sorted β -gal⁺ and β -gal⁻ cells were labelled with semiconductor red-fluorescent nanocrystals (QD₆₅₅)²⁰ and co-cultured with pre-fixed myocytes in the presence of conditioned medium from viable myocytes. Under these conditions, within 5 days around 30% of the QD₆₅₅-marked β -gal⁺ progenitors started to express α -actinin (Fig. 4m), indicating that cardioblasts enter the myocytic program independently from cell fusion. In contrast, all β -gal⁻ cells stained negative for

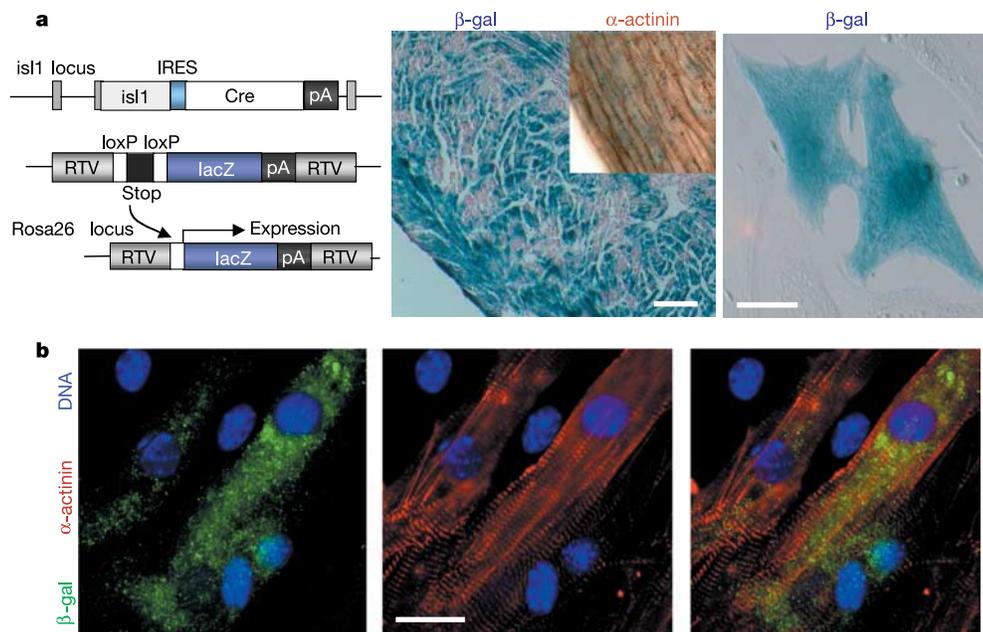


Figure 2 Genetic marking of *isl1*⁺ progenitors and myocytic cell fate. **a**, Mice carry one *isl1*-IRES-Cre allele and one R26R reporter gene. Cre expression catalyses excision of the stop cassette, resulting in selective lacZ expression and genetic marking of *isl1*-expressing cells and their differentiated progeny. RTV, retroviral integration sequence. Shown are β -gal⁺ cardiomyocytes in the right ventricle (middle panel; inset demonstrates

α -actinin expression; scale bar, 180 μ m) and after cell isolation (right panel; scale bar, 20 μ m) from double heterozygous hearts of 4-month- or 1-day-old animals, respectively. **b**, Immunocytochemistry for β -gal and sarcomeric α -actinin in isolated cardiac myocytes from animals carrying both alleles. Scale bar, 15 μ m.

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α -actinin, as well as β -gal⁺ precursors when cultured in the absence of pre-fixed myocytes, demonstrating that differentiation is cell-specific and requires secreted and membrane-bound factors (Fig. 4m).

To characterize whether the β -gal⁺ cardioblasts could adopt a

fully differentiated cardiac phenotype, C₁₂FDG-labelled cells in co-culture were analysed by real-time intracellular calcium [Ca²⁺]_i imaging. Differentiated C₁₂FDG⁺ progenitors showed periodic [Ca²⁺]_i oscillations similar to and synchronized with those in adjacent myocytes (Fig. 5a, b; Supplementary Video). Isoproterenol

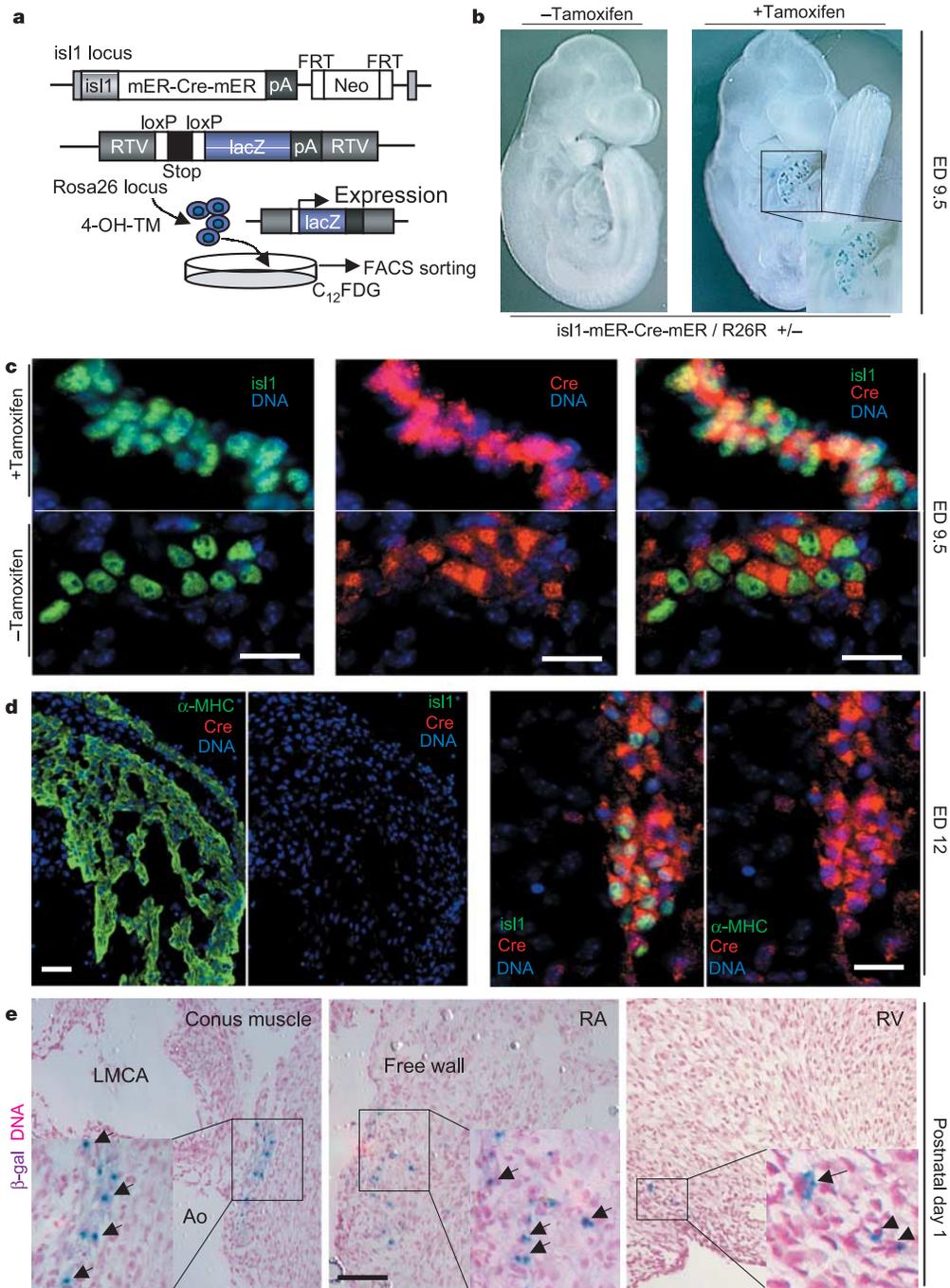


Figure 3 Specificity of recombination and distribution of β -gal⁺ cells in *isl1*-mER-Cre-mER/R26R hearts. **a**, Tamoxifen injection of *isl1*-mER-Cre-mER/R26R double heterozygous mice or administration of 4-OH-TM in culture results in heritable expression of *lacZ*. Labelling with C₁₂FDG allows FACS purification of *isl1*⁺ cells. **b**, X-gal stain in whole-mount double heterozygous embryos with or without tamoxifen injection into pregnant mothers at ED7.5. **c**, Cre localization in *isl1*⁺ cells from *isl1*-mER-Cre-mER/R26R embryos is cytosolic without tamoxifen and nuclear with tamoxifen injection. Scale

bar, 25 μ m. **d**, Downregulation of *isl1* and Cre in sequential sections of right ventricular and atrial tissue (left panels; scale bar, 80 μ m) but not of dorsal mesocardium (right panels; scale bar, 30 μ m). **e**, Sections from hearts of 1-day-old double heterozygous mice injected with tamoxifen at ED17, after X-gal stain and haematoxylin counterstain. LMCA, left main coronary artery; Ao, aorta; RA, right atrium; RV, right ventricle. Scale bar, 150 μ m. Arrows designate β -gal⁺ cells and insets represent a magnification of the areas of interest.

stimulation induced a 30% increase of the Ca^{2+} transient amplitude in differentiated β -gal⁺ cells, suggesting the existence of a functional β -adrenergic signalling pathway²¹ (Fig. 5c). The $[Ca^{2+}]_i$ oscillations responded to increasing frequencies of external field stimulation (Fig. 5d). Of the C₁₂FDG-labelled cardioblasts in the co-culture 2.3% showed active intracellular Ca^{2+} transients. Functional maturity was confirmed by analysing action potential characteristics in QD₆₅₅-marked progenitors and neighbouring myocytes. Beating differentiated cardioblasts displayed continuous electrical activity or action potentials characterized by upstroke velocity (dV/dt_{max}), amplitude (APA), duration (APD) and maximum diastolic

potential (MDP) comparable to those of neonatal cardiac myocytes (Fig. 5e, f). Repeated impalements of individual QD₆₅₅-marked differentiated progenitors revealed reproducible action potentials with distinct morphologies, nodal-like and atrial-like, supporting the concept that these cells are able to differentiate into a distinct subset of cardiac lineages, including conduction system myocytes²².

We demonstrate that the mammalian heart harbours an organ-specific progenitor cell that can be localized, purified, expanded and differentiated into mature cardiac myocytes (Fig. 5g). It will be interesting to investigate whether $isl1^+$

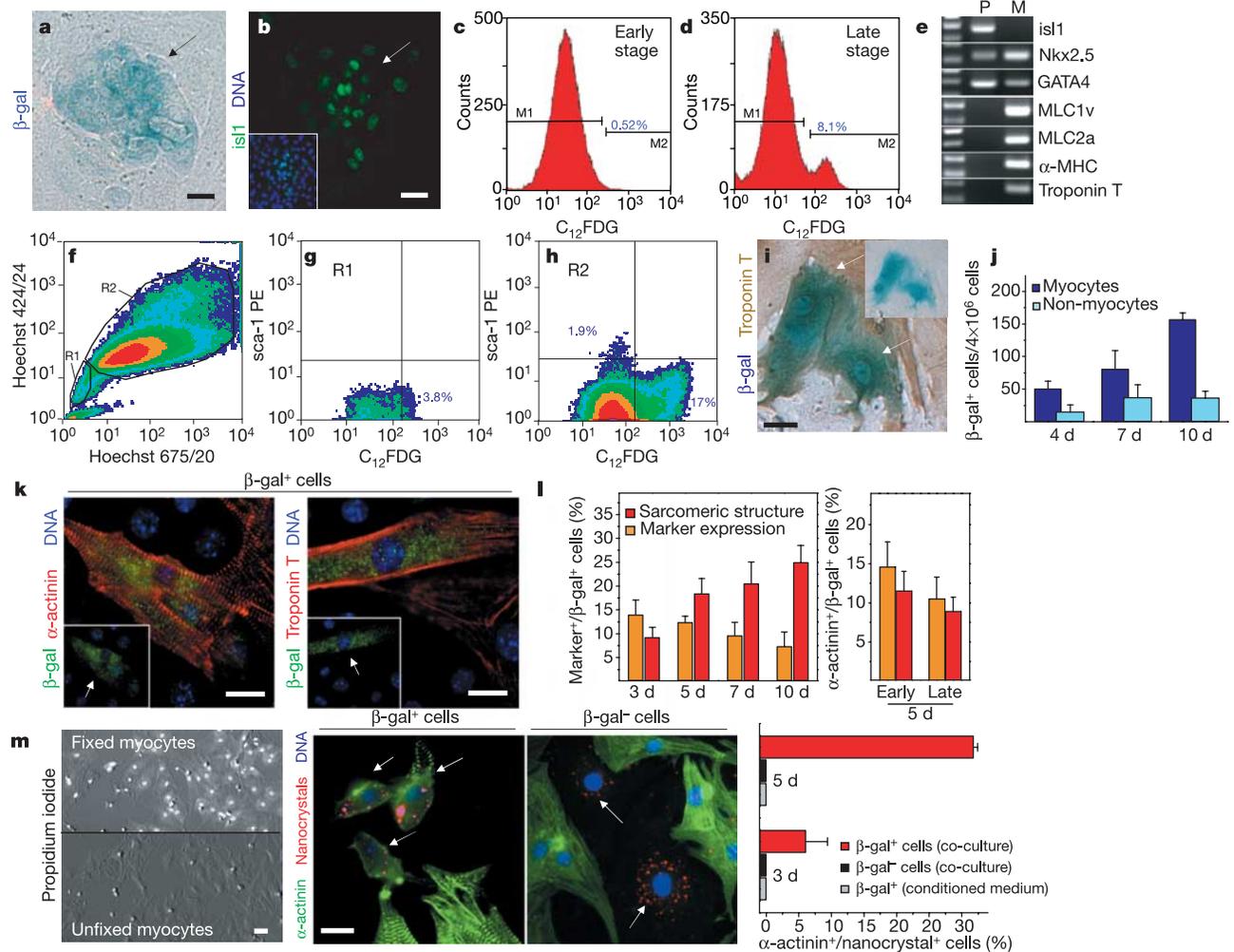


Figure 4 Amplification, characterization and myocytic differentiation of $isl1^+$ cardioblasts *in vitro*. **a, b**, Mesenchymal cell fractions from $isl1$ -mER-Cre-mER/R26R hearts at 10 days in culture after 4-OH-TM treatment. Arrows point to β -gal⁺ cardioblasts detected by X-gal stain (**a**) and $isl1$ expression (**b**). Scale bars, 15 μ m. **c, d**, Histograms of FACS-sorted β -gal⁺-C₁₂FDG-labelled cells in cardiac mesenchymal fractions after 5 days (**c**) and 14 days (**d**) in culture. **e**, RT-PCR analysis for myocytic and progenitor markers in FACS-sorted progenitors (P) and neonatal myocytes (M). **f-h**, Flow cytometry profile of Hoechst 33342 dye efflux in cardiac mesenchymal cell fractions from double heterozygous animals after C₁₂FDG and sca-1 labelling. **i, j**, Neonatal myocytes isolated from $isl1$ -mER-Cre-mER/R26R mice at 4 days in culture. Arrows designate β -gal⁺ cells coexpressing troponin T and inset shows the same cells before immunocytochemistry. Scale bar, 15 μ m (**i**). Frequency of β -gal⁺ myocytes and non-myocytes over time (**j**, mean value \pm s.e.m., $n = 3$). Exposure to 1 μ M 4-OH-TM was performed at the first day in culture.

k, Images of differentiated β -gal⁺ progenitors in co-culture with wild-type neonatal myocytes. Arrows mark progenitor-derived β -gal⁺ myocytes. Scale bar, 15 μ m. **l**, Quantification of differentiation events over time in co-culture of β -gal⁺ progenitors FACS-sorted at day 10–14 (left panel) and comparison at 5 days co-culture between β -gal⁺ progenitors expanded for 5 days (early) or 14 days (late) in culture (right panel). Mean values \pm s.e.m. from six experiments ($n = 1,200$ cells per group). **m**, Cell fusion independent cardioblast-myocyte conversion. Propidium-iodide stain in pre-fixed myocytes (scale bar, 25 μ m). Arrows point to QD₆₅₅-labelled precursors expressing α -actinin in β -gal⁺, but not in β -gal⁻ cells during co-culture with pre-fixed myocytes. Scale bar, 15 μ m. The diagram represents a quantification of fusion-independent myocytic transition. Grey columns indicate the percentage of β -gal⁺ cells expressing α -actinin in absence of pre-fixed myocytes. Mean values \pm s.e.m. ($n = 3$).

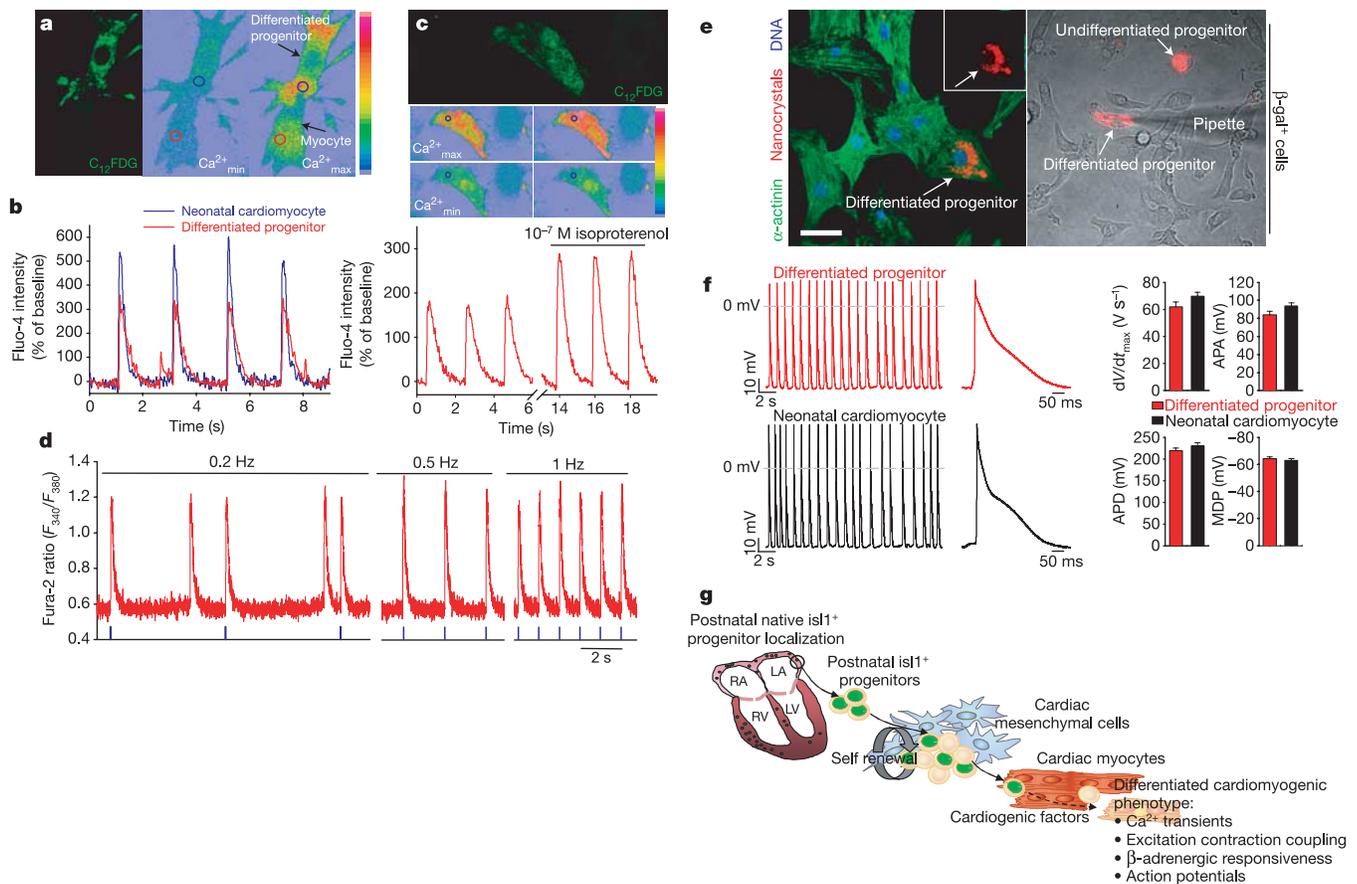


Figure 5 Real time $[Ca^{2+}]_i$ transients and action potentials in FACS-sorted β -gal⁺ precursors after myocytic differentiation in co-culture. **a, b**, Identification of β -gal⁺ cells by C_{12} FDG labelling, before cell loading with the Ca^{2+} indicator fluo-4. Pseudo-colour images show minimal (Ca^{2+}_{min}) and maximal (Ca^{2+}_{max}) fluo-4 fluorescence intensity and circles indicate the Ca^{2+} measuring areas as outlined in the fluo-4 intensity traces in **b**. Eighteen measured cells: $n = 11$ differentiated progenitors, $n = 7$ neonatal myocytes. **c**, Increase in $[Ca^{2+}]_i$ transient amplitude of a β -gal⁺ cell under isoproterenol (10^{-7} M) stimulation. **d**, Calcium transients of differentiated C_{12} FDG⁺ precursor in response to electrical pacing at different frequencies after cell loading with the Ca^{2+} indicator fura-2.

Six measured C_{12} FDG⁺ cells. **e**, QD₆₅₅-marked differentiated progenitors stained for α -actinin (left panel; scale bar, 20 μ m) and impaled by an intracellular electrode to measure electrical activity (right panel). Inset represents the QD₆₅₅ cell. **f**, Action potentials in a differentiated progenitor and a neonatal cardiomyocyte. Summarized data (means \pm s.e.m.) showing dV/dt_{max} (the maximum rate of rise of the action potential stroke), APA, APD₉₀ (action potential duration at 90% of repolarization) and MDP (the maximum diastolic potential). We analysed 160 action potentials from four different cells for each cell type. **g**, Working model of $isl1^+$ cardiac progenitors for self-renewal and cardiomyocytic differentiation.

precursors can contribute to the cardiac lineage in the absence of fusion after cellular transplantation *in vivo*, and to analyse functionally on a single-cell level the differentiated progeny²³. Cardioblasts may represent an *in vitro* system to study signalling pathways for cardiac cell lineage differentiation, which will ultimately give new insights into key steps that govern entry into specific contractile and conduction system lineages; recent work has now documented defects in later stages of ventricular lineage specification and forms of human congenital heart disease²⁴. Furthermore, this study suggests the feasibility of isolating $isl1^+$ cardiac progenitors from mammalian embryonic stem cell systems during cardiogenesis. □

Methods

Mice

Isl1-IRES-Cre were provided by T. M. Jessel and have been previously described¹³. An IRES-Cre SV40 pA and a pgk-neomycin cassette were inserted into the exon encoding the second LIM homeodomain of *isl1*. The conditional Cre reporter mouse line R26R was generated by P. Soriano¹⁴. The *isl1*-mER-Cre-mER targeting construct was generated by an in-frame insertion of a mER-Cre-mER SV40 pA cassette¹⁵ along with a neo-selectable

marker flanked by FRT sites into exon 1 of the genomic *isl1* locus. The generation of *isl1*-mER-Cre-mER knockin mice will be described in detail elsewhere. *Isl1*-IRES-Cre/R26R and *isl1*-mER-Cre-mER/R26R double heterozygous mice were generated by crossing single heterozygous mice. Mice are in a mixed 129 \times C57Bl/6 background. The *isl1*-mER-Cre-mER line showed exclusively a tamoxifen-dependent expression of Cre. Tamoxifen (Sigma) was dissolved in corn oil and injected twice intraperitoneally in the pregnant mothers at a dose of 75 μ g g⁻¹ body weight at ED17 and ED18, unless specified otherwise²⁵. Animal care and all procedures were approved by the Institutional Animal Care Committee at UCSD.

Isolation of mouse cardiac progenitors and cell culture conditions

For cell isolation, we used 40–60 1–5-day-old pups that were double heterozygous for *isl1*-mER-Cre-mER and R26R alleles. Hearts were cut into four pieces, washed repetitively in ice-cold Hank's balanced salt solution without Ca^{2+} (HBSS) and predigested overnight in 0.5 mg ml⁻¹ trypsin-HBSS solution at 4 °C under constant shaking, to remove blood and dead cells. Cardiac cells were obtained by four rounds of 10 min digestions with 240 U ml⁻¹ collagenase type II (Worthington) in HBSS at 37 °C. The mesenchymal cell fraction containing most β -gal⁺ progenitor cells was separated from myocytes and endothelial cells by two rounds of 1 h differential plating on plastic in DMEM/M199 (4:1 ratio) medium containing 10% horse serum and 5% fetal bovine serum at 37 °C in the presence of 5% CO₂. Under these conditions preferentially mesenchymal and β -gal⁺ precursor cells attach on plastic. After rigorous washing to remove non-adherent cells, CMC and cardioblasts were cultured for 48 h in DMEM containing penicillin (100 U ml⁻¹), streptomycin (100 μ g ml⁻¹), HEPES (25 mM),

glutamine (2 mM), 10% newborn calf serum and 5% fetal bovine serum (FBS). Culture medium was exchanged to DMEM/F12 containing B27 supplement, 2% FBS and 10 ng ml⁻¹ EGF on the second day in culture when the CMC reached confluency, to stimulate Isl1 progenitor growth. 4-OH-TM (stock solution 1 mM in ethanol; Sigma) was applied in culture one day after cell plating at a concentration of 1 μM and maintained for 48 h.

Methods regarding flow cytometry analysis, co-culture experiments, immunocytochemistry and lacZ staining, polymerase chain reaction with reverse transcription (RT-PCR), detection of [Ca²⁺]_i transients and electrophysiological action potential recordings are described in the Supplementary Methods section.

Received 12 October; accepted 22 November 2004; doi:10.1038/nature03215.

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Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We thank W. Giles for discussions and comments on the real-time Ca²⁺ measurements and electrophysiology, the National Center for Microscopy and Imaging Research at UCSD for the use of the high-speed multi-photon laser-scanning microscope (Bio-Rad RTS2000) and custom plug-ins for the ImageJ software (H. Hakoziaki, S. Chow and M. Ellisman), the UCSD Cancer Center Digital Imaging Shared Resource for deconvolution microscopy (S. McMullen and J. Feramisco). We would like especially to acknowledge D. Young for his expertise and help in the FACS sorting analysis (UCSD Cancer Center Flow Cytometry Laboratories). This work was supported by the NIH and the Jean Le Ducq Foundation. K.-L.L. is a Heisenberg Scholar of the German Research Foundation.

Competing interests statement The authors declare that they have no competing financial interests.

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The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA–ssDNA junction

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The BRCA2 tumour suppressor¹ is essential for the error-free repair of double-strand breaks (DSBs) in DNA by homologous recombination^{2,3}. This is mediated by RAD51, which forms a nucleoprotein filament with the 3′ overhanging single-stranded DNA (ssDNA) of the resected DSB, searches for a homologous donor sequence, and catalyses strand exchange with the donor DNA⁴. The 3,418-amino-acid BRCA2 contains eight ~30-amino-acid BRC repeats that bind RAD51 (refs 5, 6) and a ~700-amino-acid DBD domain that binds ssDNA⁷. The isolated BRC and DBD domains have the opposing effects of inhibiting^{8,9} and stimulating recombination⁷, respectively, and the role of BRCA2 in repair has been unclear. Here we show that a full-length BRCA2 homologue (Brh2) stimulates Rad51-mediated recombination at substoichiometric concentrations relative to Rad51. Brh2 recruits Rad51 to DNA and facilitates the nucleation of the filament, which is then elongated by the pool of free Rad51. Brh2 acts preferentially at a junction between double-stranded DNA (dsDNA) and ssDNA, with strict specificity for the 3′ overhang polarity of a resected DSB. These results establish a BRCA2 function in RAD51-mediated DSB repair and explain the loss of this repair capacity in BRCA2-associated cancers.

Isolated BRC repeats can block the assembly of the RAD51 nucleoprotein filament *in vitro* when present in excess over RAD51 (refs 9, 10), and can also interfere with DSB repair in a dominant-negative fashion *in vivo*⁸. These effects have been suggested to reflect a negative control mechanism of BRCA2 over RAD51 (ref. 11). In contrast, the findings that the DBD domain (BRCA2^{DBD}) binds ssDNA and has a putative helix–turn–helix (HTH) dsDNA-binding motif⁷, coupled with the demonstration that a stoichiometric amount of the BRCA2^{DBD} can stimulate RAD51-mediated strand exchange *in vitro*⁷, indicated that BRCA2 might facilitate RAD51-mediated recombination and might have a role at the dsDNA–ssDNA (ds–ss) junction of the resected DSB⁷.

One model for reconciling these opposing biochemical activities is that BRCA2 helps to nucleate the filament by recruiting RAD51 to the DNA, and possibly to the ds–ss junction. Once there, RAD51 could dissociate from BRCA2 and partition onto ssDNA, nucleating filament formation by the pool of free RAD51. Alternatively, RAD51 could remain bound to the BRC repeat and still nucleate the filament. This is a possibility because in the RAD51–BRC crystal structure¹⁰ the BRC repeat blocks only one of the two polymerization ends of RAD51, and the second end is in principle available to bind an additional RAD51 molecule and elongate the filament in a unidirectional manner (Supplementary Fig. 1).

To test this model, we purified the full-length BRCA2 homologue Brh2 from the fungus *Ustilago maydis*. Like BRCA2, Brh2 is required for recombination, DSB repair and genomic stability¹². The 1,075-amino-acid Brh2 has homology to the DBD of human BRCA2 (ref. 13) and contains one consensus BRC repeat¹². The number of

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doi:10.1038/nature05790

Postnatal *Isl1*⁺ cardioblasts enter fully differentiated cardiomyocyte lineages

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Nature 433, 647–653 (2005)

It has been drawn to *Nature's* attention that K.R.C., S.E., C.-L.C., A.M. and K.-L.L. filed a patent application relevant to this work (patent number WO 2004/070013) in 2004, which should therefore have been declared as a competing financial interest.

CORRIGENDUM

doi:10.1038/nature05791

Global warming and climate forcing by recent albedo changes on Mars

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Nature 446, 646–649 (2007)

It has been brought to our attention that there was some ambiguity in the wording of the first boldface paragraph of this Letter regarding our prediction of the change in temperature on Mars that would have resulted from changes in albedo. As is clear from Table 1, we predict a total change of 0.65 degrees Celsius over the period from the 1970s to the 1990s, rather than 0.65 degrees Celsius per year.

Published online 5 April 2007.