What about mammals?

LETTER

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Mammalian heart renewal by pre-existing cardiomyocytes

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Although recent studies have revealed that heart cells are generated in adult mammals, the frequency of generation and the source of new heart cells are not yet known. Some studies suggest a high rate of stem cell activity with differentiation of progenitors to cardiomyocytes[1]. Other studies suggest that new cardiomyocytes are born at a very low rate[2–4], and that they may be derived from the division of pre-existing cardiomyocytes. Here we show, by combining two different pulse–chase approaches—genetic fate-mapping with stable isotope labelling, and multi-isotope imaging mass spectrometry—that the genesis of cardiomyocytes occurs at a low rate by the division of pre-existing cardiomyocytes during normal ageing, a process that increases adjacent to areas of myocardial injury. We found that cell cycle activity during normal ageing and after injury led to polyploidy and multinucleation, but also to new diploid, mononucleate cardiomyocytes. These data reveal pre-existing cardiomyocytes as the dominant source of cardiomyocyte replacement in normal mammalian myocardial homeostasis as well as after myocardial injury.

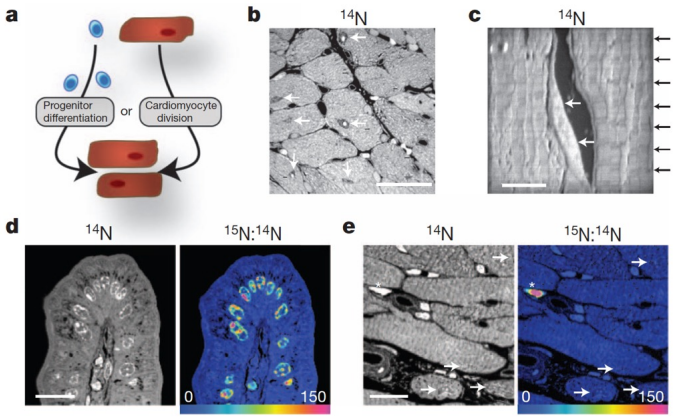

Figure 1 | Use of MIMS to study cardiomyocyte turnover. **a**, Primary question: are new cardiomyocytes derived from progenitors or from pre-existing cardiomyocytes? **b**, ^{14}N mass image. Subcellular details are evident, including cardiomyocyte nuclei (white arrows). Scale bar, 20 μm. **c**, MIMS resolves periodic sarcomeres (black arrows) in cardiomyocytes. Non-cardiomyocytes (white arrows) are seen outside cardiomyocyte borders. Scale bar, 5 μm. **d**, Right, ^{15}N:^{14}N hue–saturation–intensity image of small-intestinal epithelium after labelling with [^{15}N]thymidine. The scale ranges from blue, where the ratio is equivalent to natural ratio (0.37%, expressed as 0% above natural ratio (enrichment over natural ratio)), to red, where the ratio is 150% above natural ratio. ^{15}N labelling is concentrated in nuclei in a pattern resembling chromatin. Scale bar, 15 μm. **e**, Right, ^{15}N:^{14}N hue–saturation–intensity image of heart section (left ventricle). [^{15}N]Thymidine was administered for 1 week. Asterisk, rare $^{15}N^{+}$ interstitial cells. Cardiomyocyte nuclei (white arrows) are unlabelled. Scale bar, 15 μm.

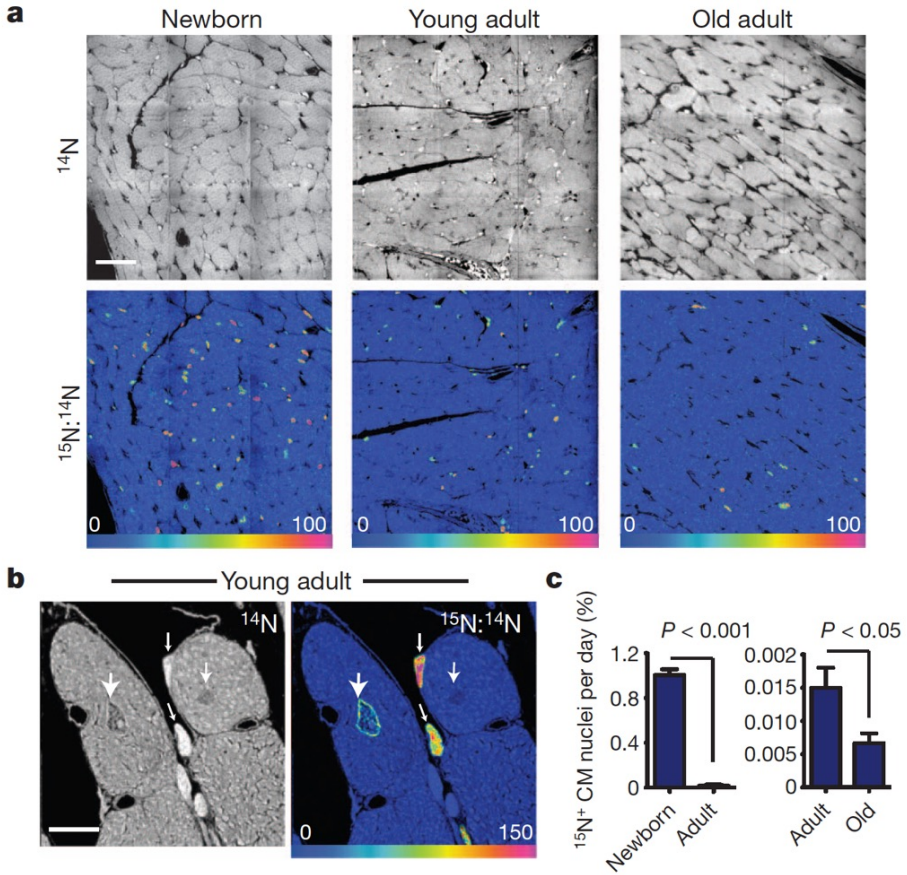

Figure 2 | Cardiomyocyte DNA synthesis decreases with age. **a**, [^{15}N]Thymidine was administered for 8 weeks to mice of different ages: newborn, starting at postnatal day 4; young adult, starting at 2 months; old adult, starting at 22 months. Top, ^{14}N mass images show histological details. Bottom, ^{15}N:^{14}N hue–saturation–intensity images show ^{15}N^{+} nuclei. Mosaics are constructed from nine tiles, 60 μm each. Scale bar, 30 μm. **b**, High-magnification analysis shows a cardiomyocyte from the young adult with nuclear ^{15}N labelling (large arrow), two labelled non-cardiomyocytes (small arrows) and an adjacent unlabelled cardiomyocyte nucleus (medium arrow). Scale bar, 10 μm. **c**, Age-related decline in cardiomyocyte DNA synthesis. Left, comparison of newborn with young adult. Right, scale reduced to compare young adult with old adult ($n = 3$ mice per group). Error bars indicate s.e.m.

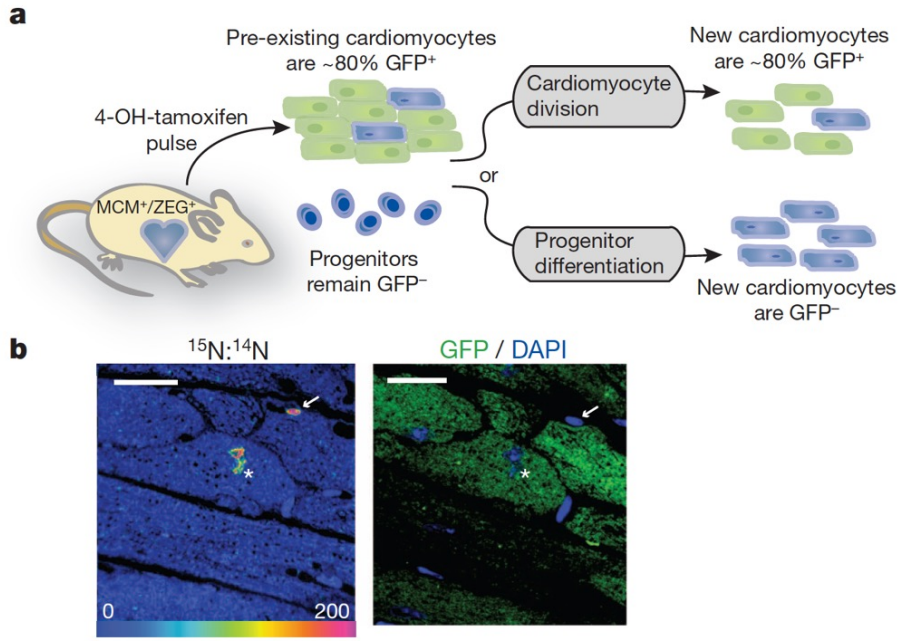

Figure 3 | New cardiomyocytes are derived from pre-existing cardiomyocytes during ageing. **a**, Experimental strategy. MerCreMer^{+}/ZEG^{+} (MCM^{+}ZEG^{+}) mice ($n = 4$) were treated for 2 weeks with 4-OH-tamoxifen to induce cardiomyocyte-specific GFP expression. [^{15}N]Thymidine was administered continuously during a 10-week chase, then cycling cells were identified by ^{15}N labelling. New cardiomyocytes (^{15}N^{+}) derived from pre-existing cardiomyocytes should express GFP at a rate similar to that of the surrounding quiescent (^{15}N^{-}) cardiomyocytes. New cardiomyocytes (^{15}N^{+}) derived from progenitors should be GFP^{-}. **b**, Left, ^{15}N:^{14}N hue–saturation–intensity image showing a [^{15}N]thymidine-labelled cardiomyocyte nucleus (white asterisk) and a ^{15}N^{+} non-cardiomyocyte (white arrow). Right, immunofluorescent image showing that the ^{15}N^{+} cardiomyocyte is GFP^{+}. Scale bars, 15 μm.

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Transient Regenerative Potential of the Neonatal Mouse Heart

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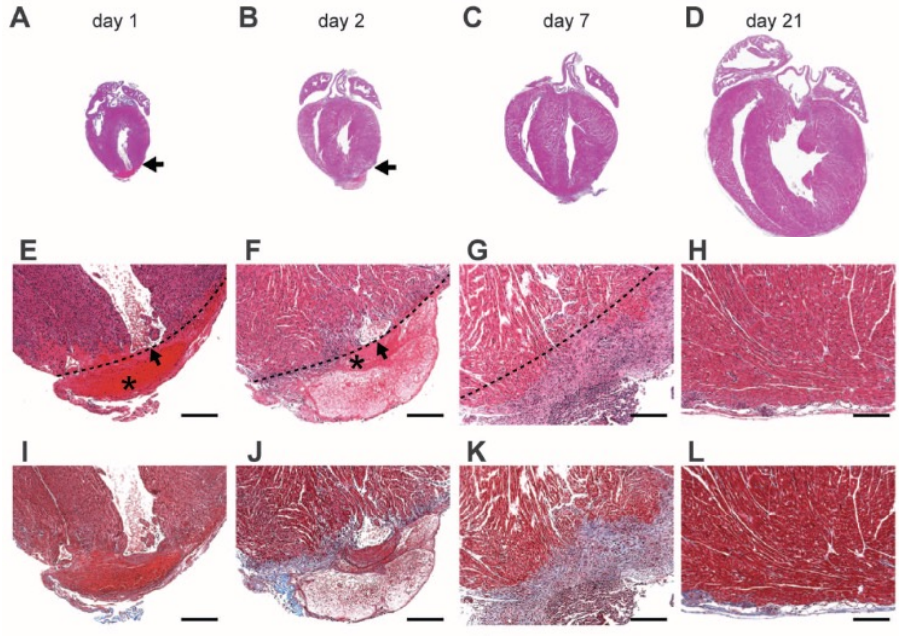
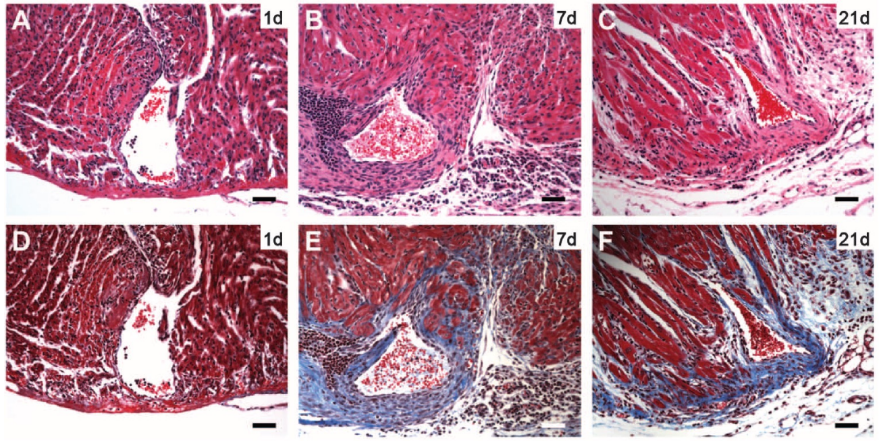
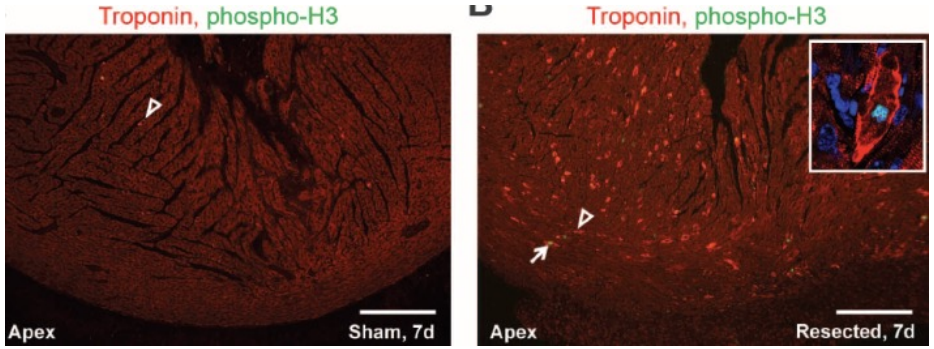
Eric N. Olson,[1]* Hesham A. Sadek[2]*





- Hearts of 1-day-old neonatal mice can regenerate after partial surgical resection, but this capacity is lost by 7 days of age.
- The regenerative response was characterized by cardiomyocyte proliferation with minimal hypertrophy or fibrosis.
- The majority of cardiomyocytes within the regenerated tissue originated from preexisting cardiomyocytes.



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Surgical models for cardiac regeneration in neonatal mice

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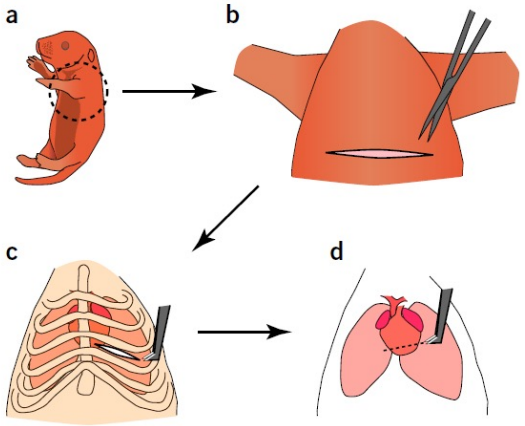
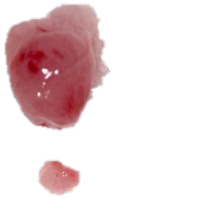
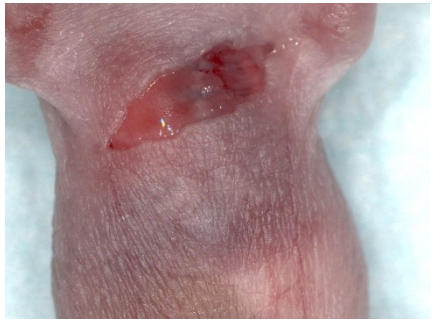
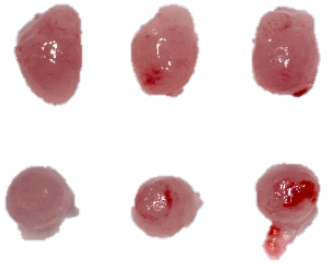


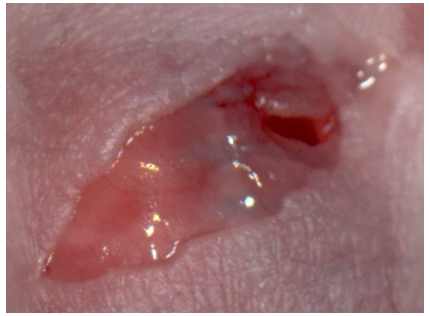
(Mahmoud et al., 2014)

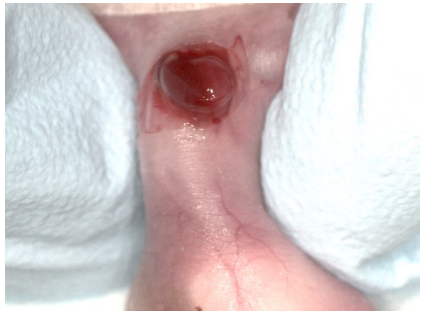
Apical resection surgical model

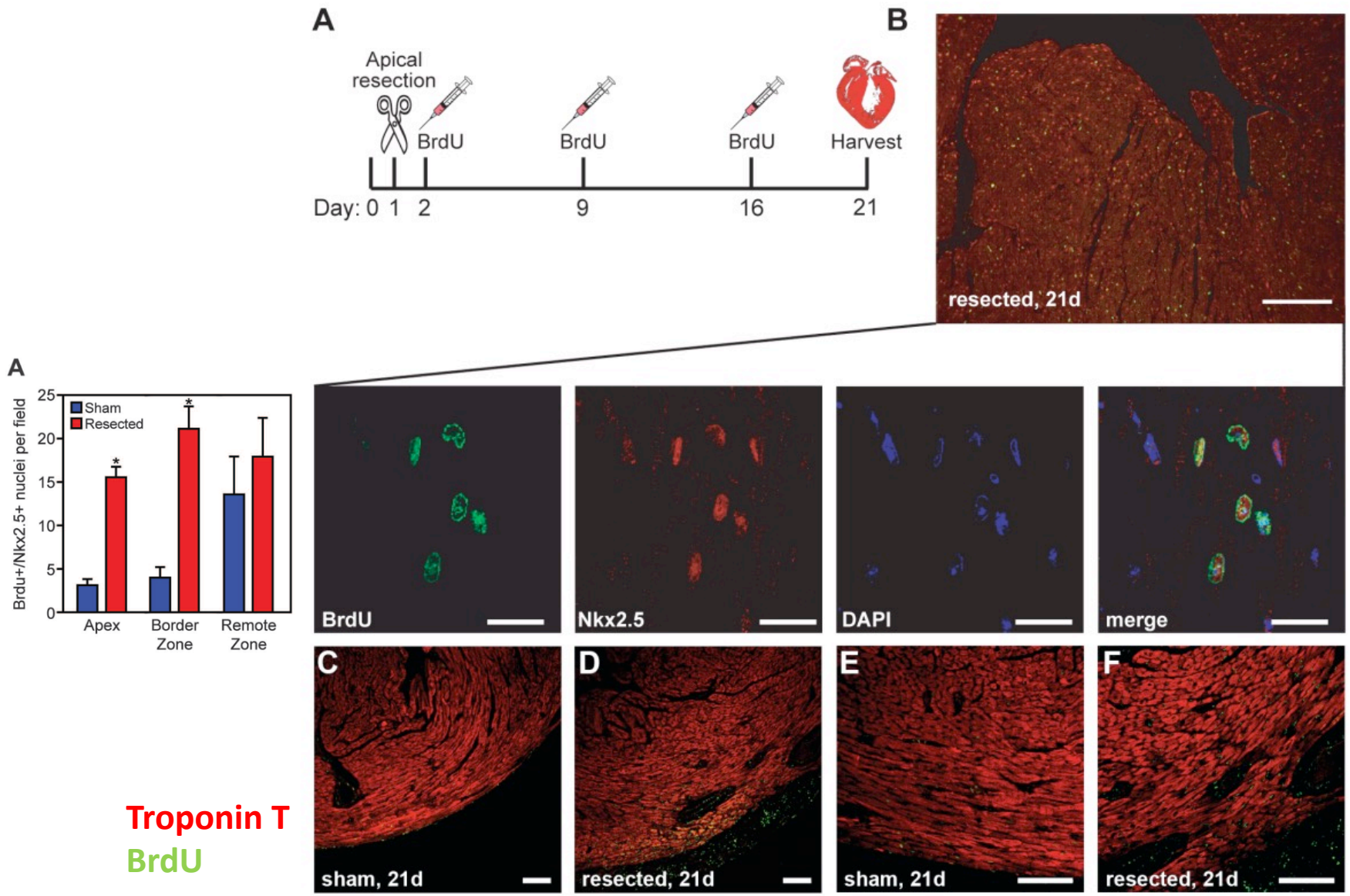








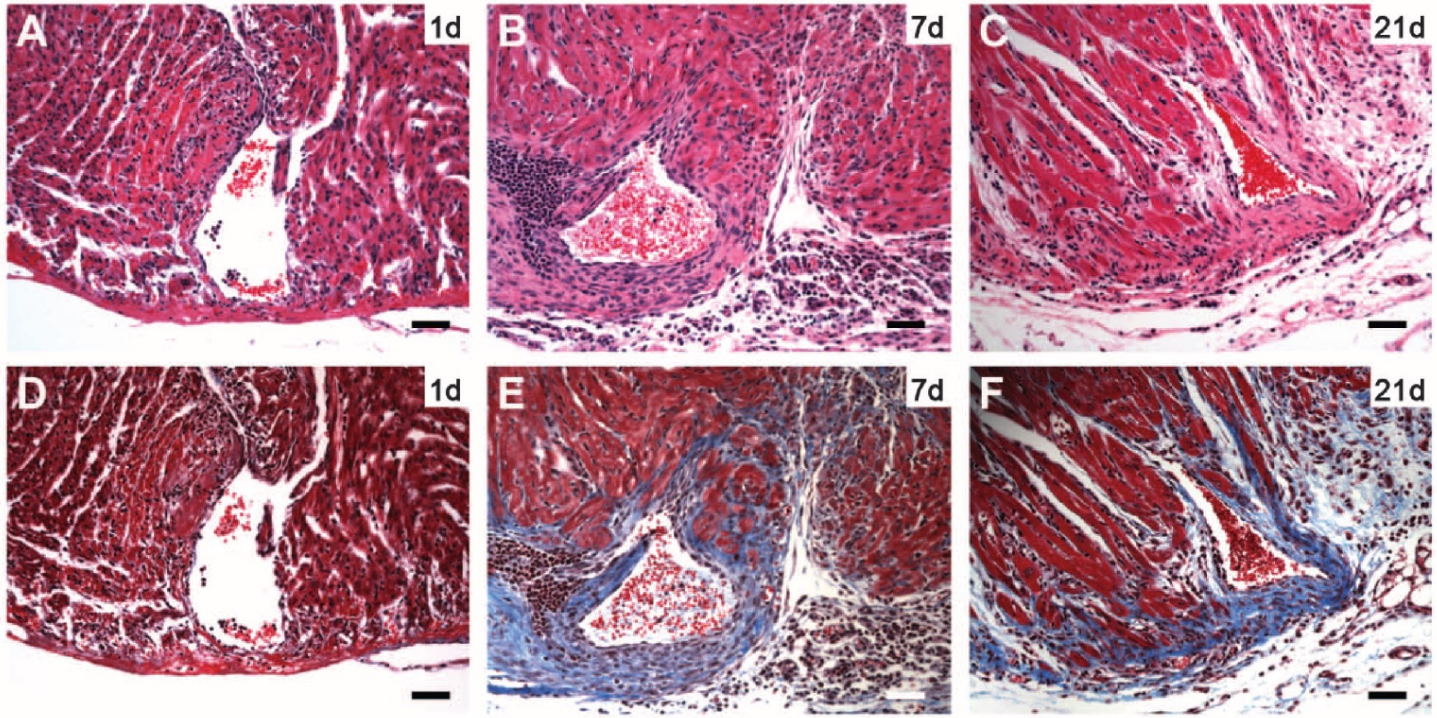




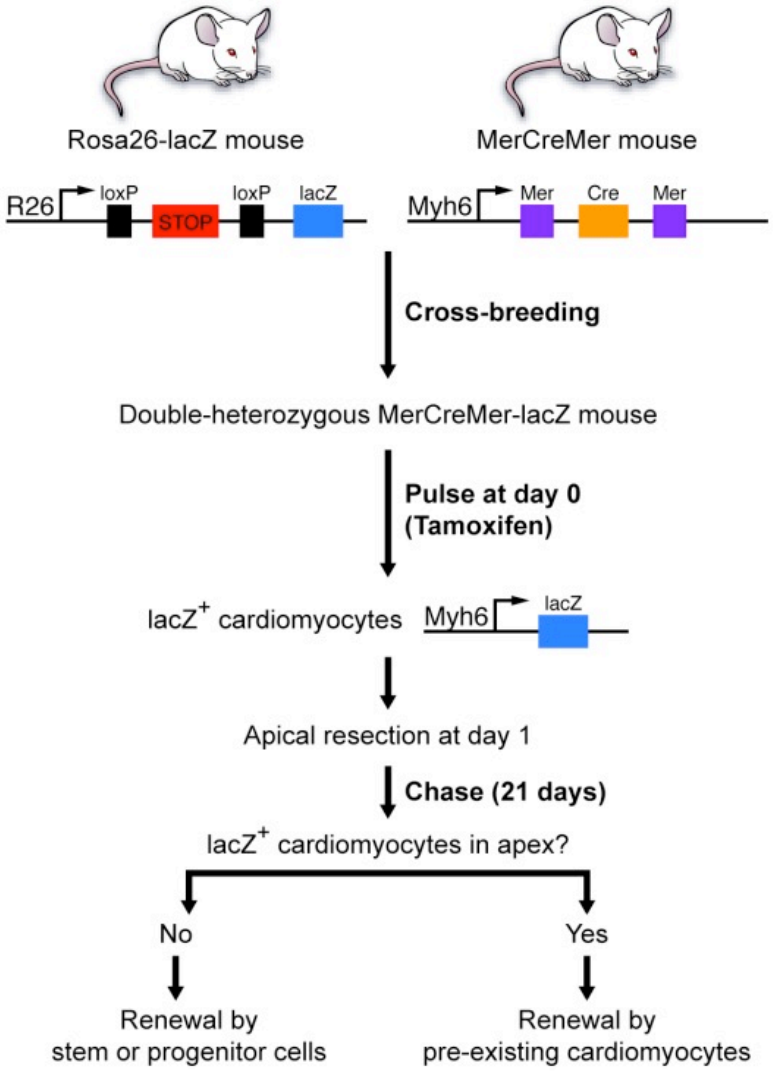
Newly formed cardiomyocytes

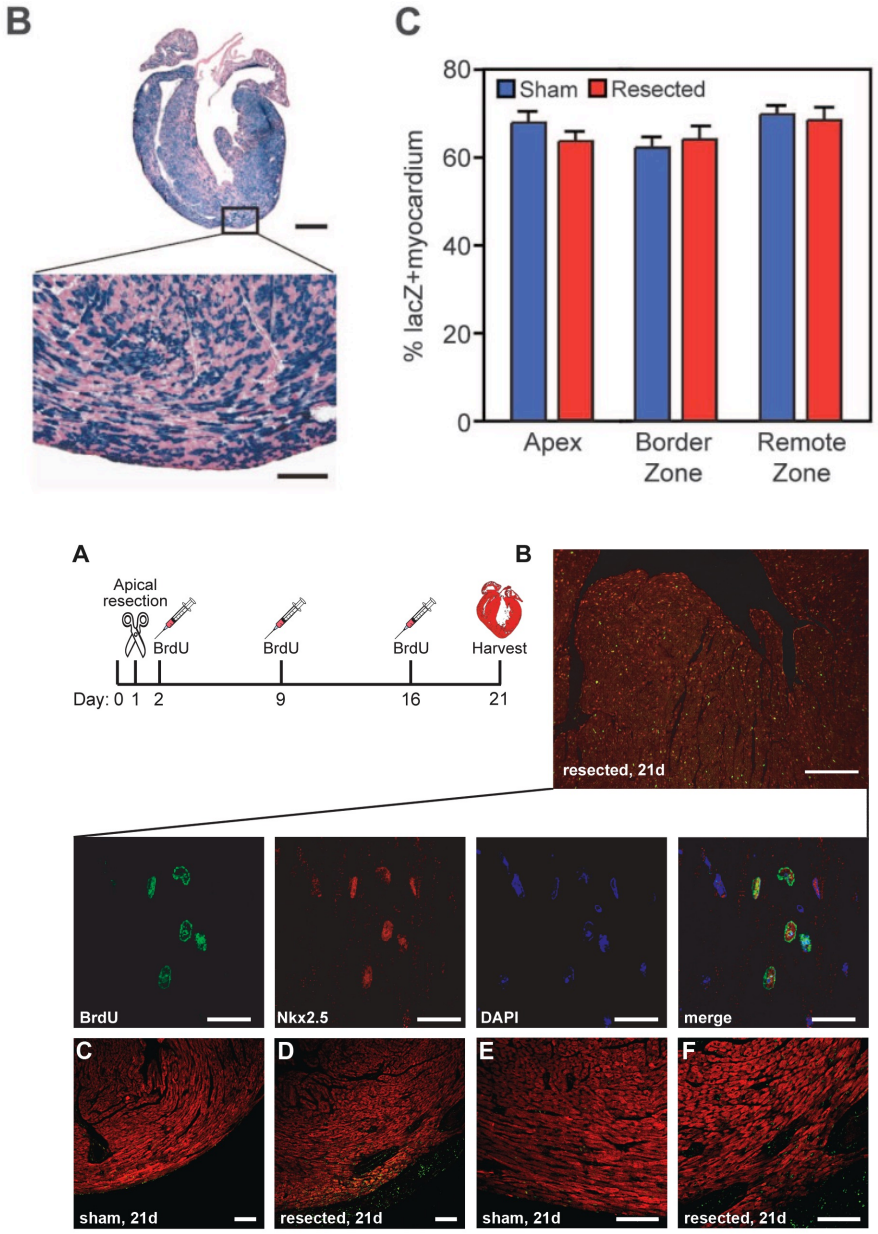
(Porrello et al., 2011)

Fig. 4. Lack of regeneration after apical resection of 7-day-old mice. (**A** to **C**) H&E staining at 1, 7, and 21 dpr, respectively. (**D** to **F**) Trichrome staining at 1, 7, and 21 dpr. Note fibrotic scar (blue staining) surrounding resected ventricular chamber at 7 and 21 dpr [(E) and (F)]. Scale bars, 200 μm.



Which cells do they derive from ?





(Porrello et al., 2011)

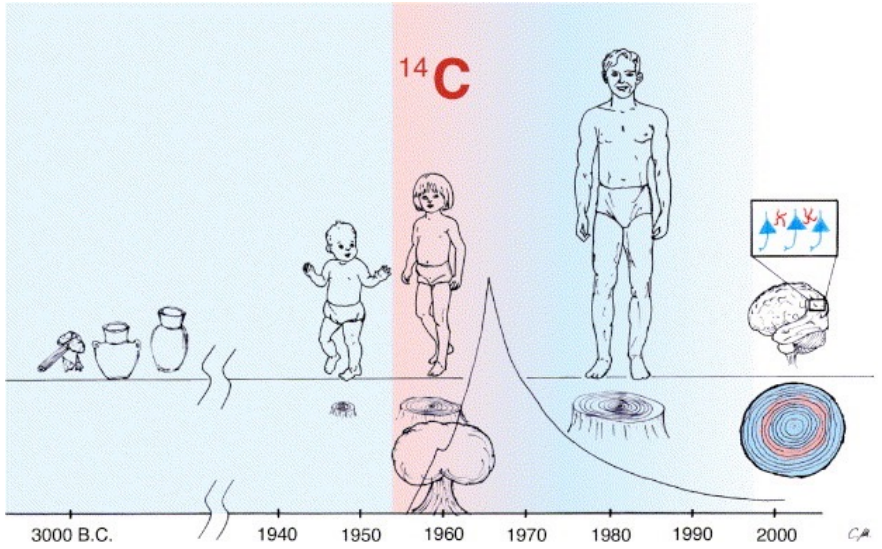
How does adult cardiomyocyte renewal occur?

Is the heart really a post-mitotic organ?

Carbon dating of human tissues

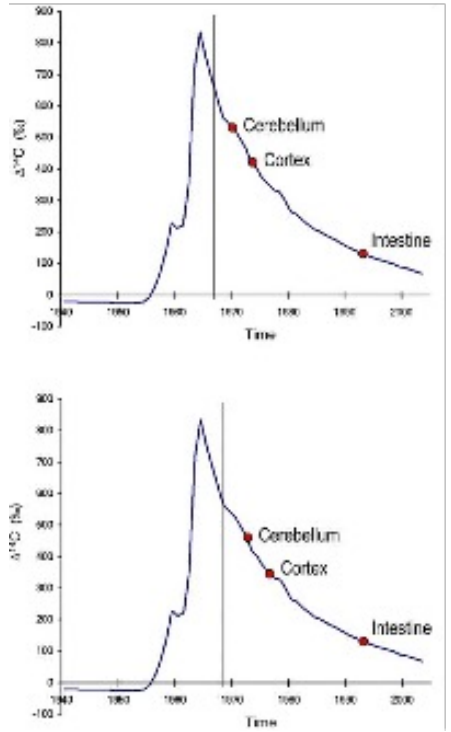
After the Second World War, tests of nuclear bombs spewed carbon-14 pollution into the atmosphere. This isotope was incorporated into plants and the people who consumed them. After above-ground tests were stopped in 1963, levels of the isotope started to fall. The ^{14}C in a cell's DNA corresponds to the amount of the isotope in the atmosphere at the time it was dividing, providing a way to date a cell's birth.

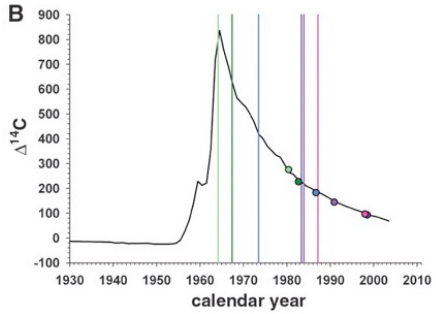
People born before 1955 had levels of ^{14}C in their cardiomyocytes that were higher than was present in the atmosphere at the time of their birth, so some of these cells must have arisen later on in their lives. Further work and mathematical modelling allowed to calculate that a 50-year-old heart still contains more than half the cells it had at birth and that the turnover slows down with time. A 25-year-old heart replaces about 1% of all cardiomyocytes over a year; a 75-year-old about half that.



Although extensive regeneration is unlikely to occur in most of mammalian tissues, evidence has accumulated in recent years suggesting that mammalian cardiac myocytes do retain the capacity to divide. Carbon dating of cardiomyocytes in human hearts has been suggested to indicate a lifetime turnover rate of 50%.

Nevertheless, the ability of adult mammalian myocytes to regenerate injured tissue is limited. Perhaps during the course of evolution, mammalian hearts have simply lost the capacity for regeneration because it wasn't needed. After all, heart disease occurs later in life after we have reproduced. In addition, repair became more important. The mammalian heart works at high pressure, whereas the fish heart doesn't.





The vertical bar indicates the date of birth of each individual, and the similarly colored dots represent the ^{14}C data for the same individual.

3 APRIL 2009 VOL 324 SCIENCE

Evidence for Cardiomyocyte Renewal in Humans

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It has been difficult to establish whether we are limited to the heart muscle cells we are born with or if cardiomyocytes are generated also later in life. We have taken advantage of the integration of carbon-14, generated by nuclear bomb tests during the Cold War, into DNA to establish the age of cardiomyocytes in humans. We report that cardiomyocytes renew, with a gradual decrease from 1% turning over annually at the age of 25 to 0.45% at the age of 75. Fewer than 50% of cardiomyocytes are exchanged during a normal life span. The capacity to generate cardiomyocytes in the adult human heart suggests that it may be rational to work toward the development of therapeutic strategies aimed at stimulating this process in cardiac pathologies.

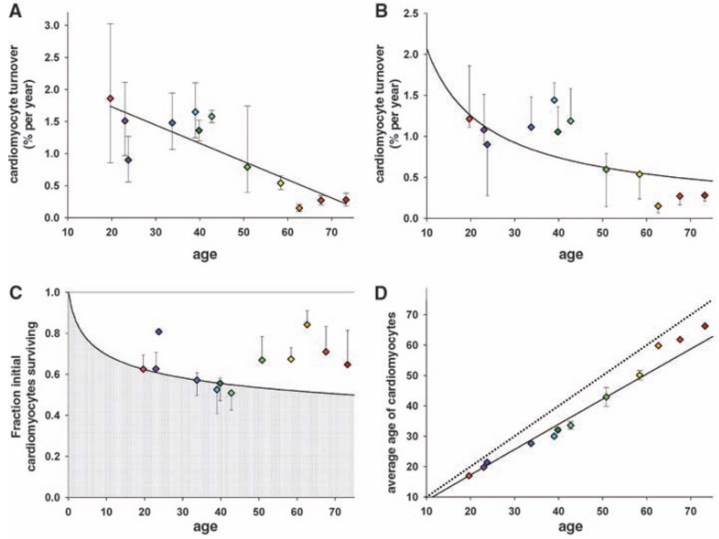


Fig. 4. Dynamics of cardiomyocyte turnover. (**A**) Individual data fitting assuming a constant turnover (see supporting online text) reveals an almost linear decline of cardiomyocyte turnover with age (R = −0.84; P = 0.001). A constant-turnover hypothesis might therefore not represent the turnover dynamics accurately. (**B**) Global fitting of all data points (see supporting online text, error sum of squares = 1.2×10^4) shows an age-dependent decline of cardiomyocyte turnover. (**C**) The gray area depicts the fraction of cardiomyocytes remaining from birth, and the white area is the contribution of new cells. Estimate is from the best global fitting. (**D**) Cardiomyocyte age estimates from the best global fitting. The dotted line represents the no-cell-turnover scenario, where the average age of cardiomyocytes equals the age of the individual. The black line shows the best global fitting. Colored diamonds indicate computed data points from ^{14}C-dated subjects. Error bars in (A) are calculated from the errors on ^{14}C measurements. Error bars in all other graphs are calculated for each subject individually and show the interval of possible values fitted with the respective mathematical scenario.

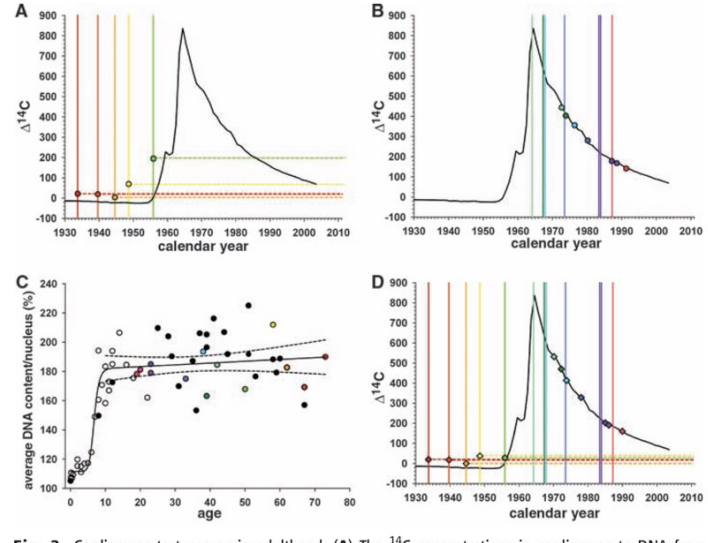
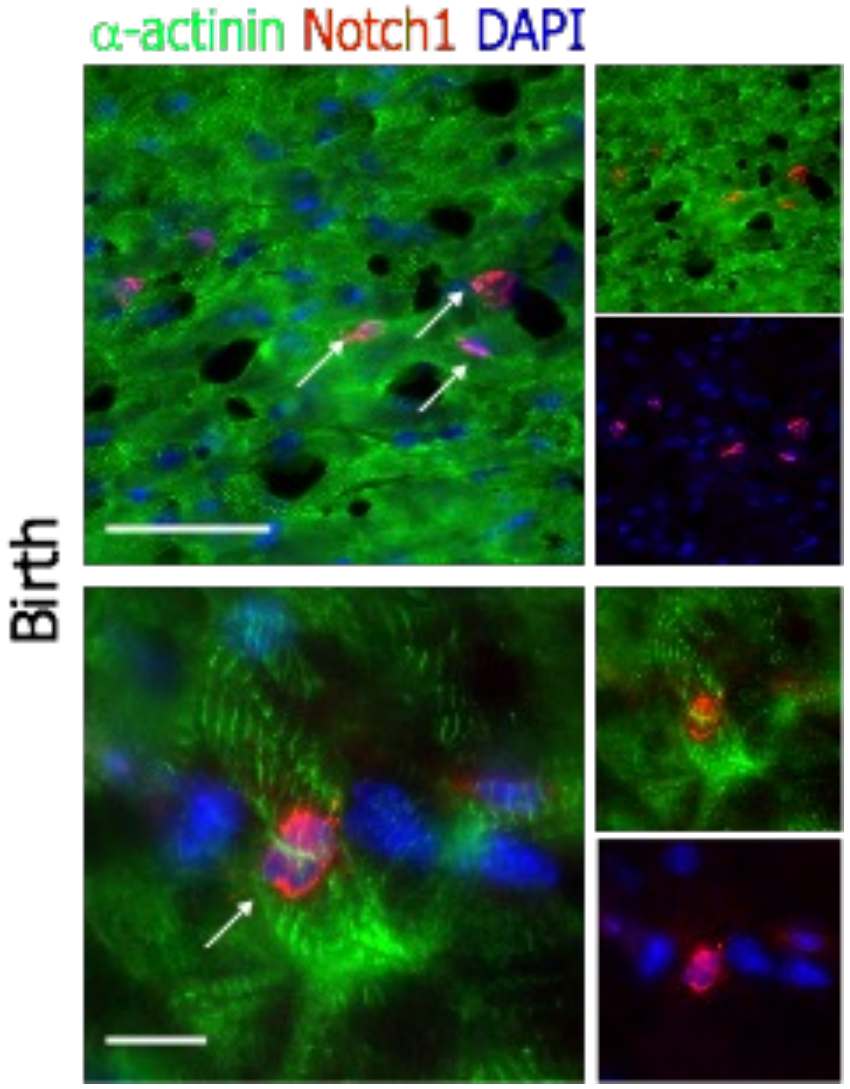
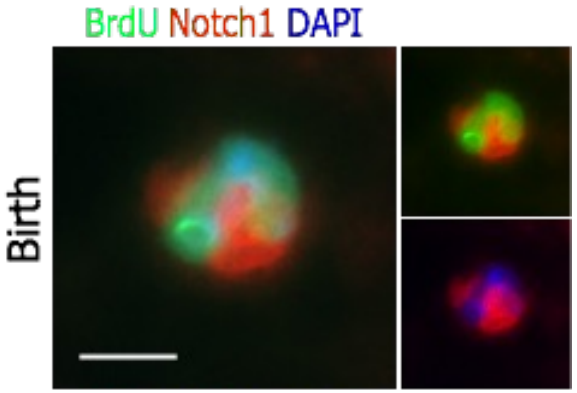
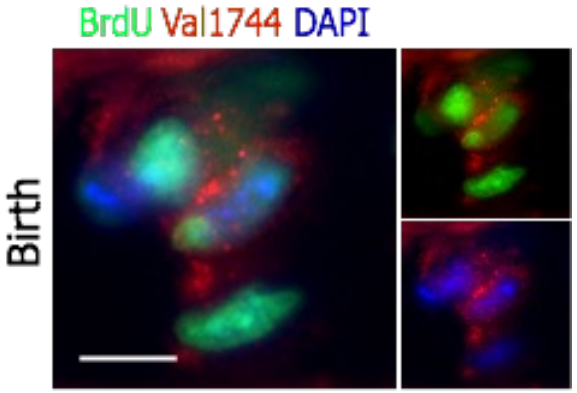


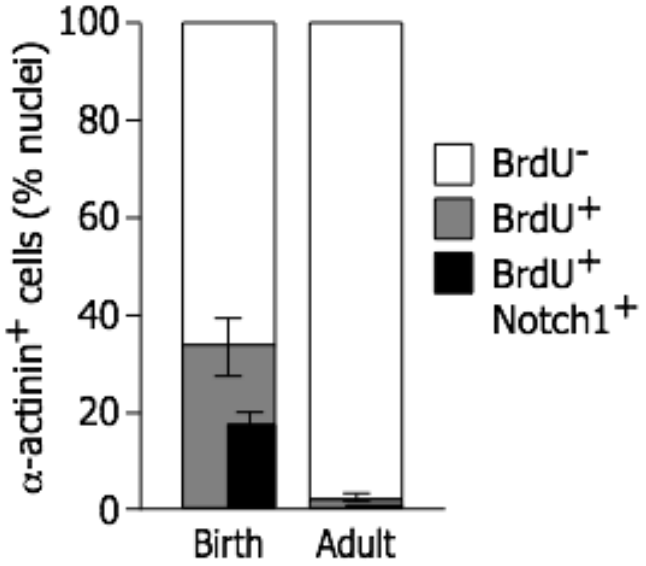
Fig. 3. Cardiomyocyte turnover in adulthood. (**A**) The ^{14}C concentrations in cardiomyocyte DNA from individuals born before the time of the atmospheric radiocarbon increase correspond to time points after the birth of all individuals. The vertical bar indicates year of birth, with the correspondingly colored data point indicating the Δ^{14}C value. (**B**) ^{14}C concentrations in cardiomyocyte DNA from individuals born after the time of the nuclear bomb test. (**C**) Average DNA content ($2n$ = 100%) per cardiomyocyte nucleus from individuals (without severe heart enlargement; see fig. S5) of different ages. Ploidy was measured by flow cytometry. Colored data points identify individuals analyzed for ^{14}C (n = 13). Black data points are from individuals analyzed only with regard to ploidy level (n = 23), and white data points are taken from Adler *et al.* (n = 26) (*24*, *26*). The dashed lines indicate the 95% confidence interval for the regression curve. (**D**) ^{14}C values corrected for the physiologically occurring polyploidization of cardiomyocytes during childhood for individuals born before and after the bomb-induced spike in ^{14}C concentrations, calculated on the basis of the individual average DNA content per cardiomyocyte nucleus. The ^{14}C content is not affected in individuals where the polyploidization occurred before the increase in atmospheric ^{14}C concentrations.

Proliferating neonatal cardiomyocytes express Notch1









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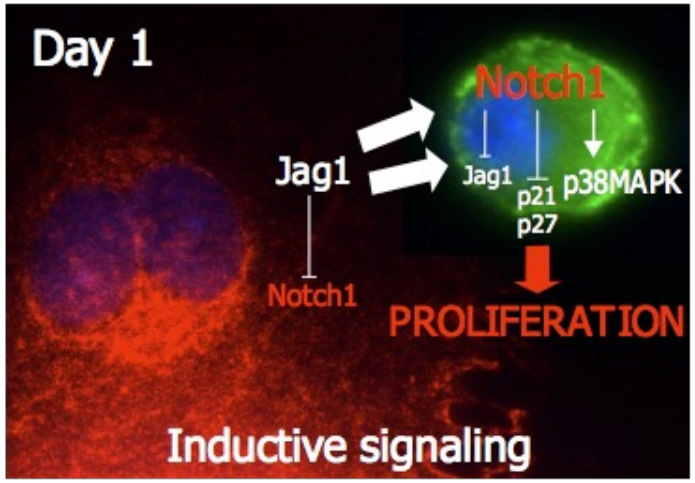
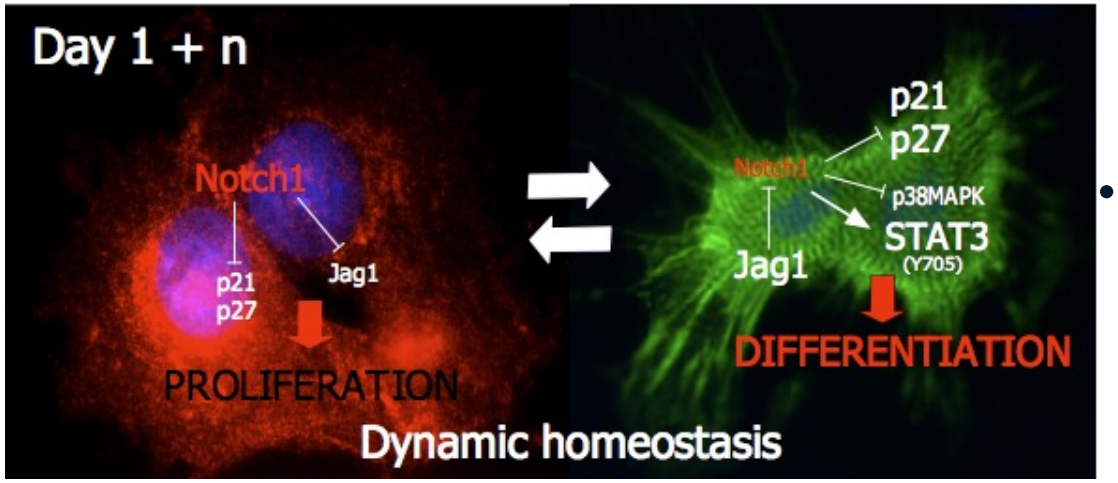
Notch1 signaling stimulates proliferation of immature cardiomyocytes





- Loss of cardiomyocyte proliferation after birth in vivo parallels loss of Notch signaling
- Neonatal cardiomyocyte proliferation in vitro requires activated Notch ICD
- Cardiomyocyte proliferation in vitro can be stimulated by Notch pathway stimulation
- In vivo, AAV9-N1ICD transduction induces the infiltration of the myocardium with BrdU+, proliferating cells.

Published October 6, 2008

Notch activates cell cycle reentry and progression in quiescent cardiomyocytes

Víctor M. Campa, Raquel Gutiérrez-Lanza, Fabio Cerignoli, Ramón Díaz-Trelles, Brandon Nelson, Toshiya Tsuji, Maria Barcova, Wei Jiang, and Mark Mercola

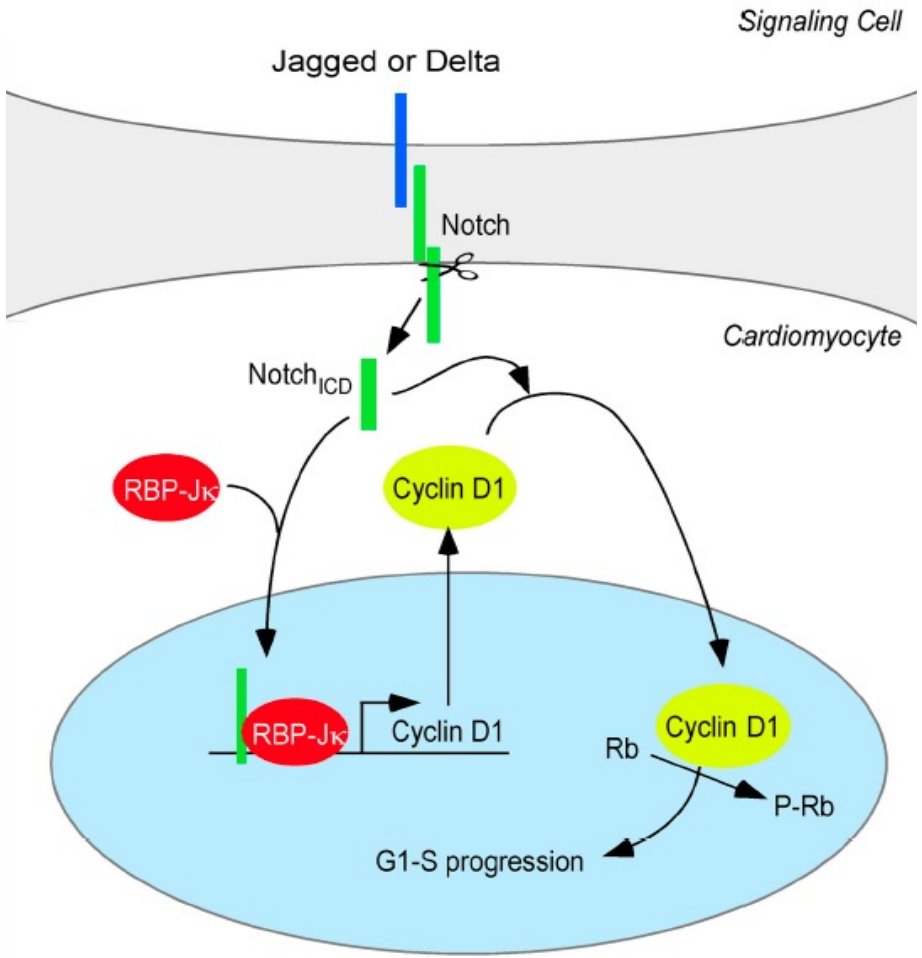
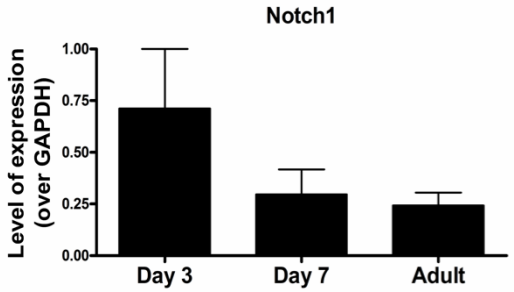
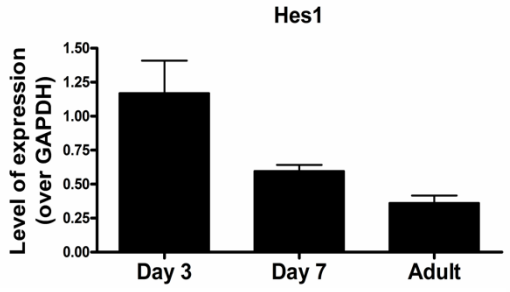


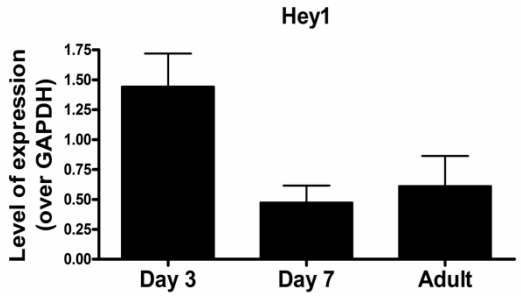
Figure 9. **Summary of Notch2-induced cell cycle entry.** RBP-Jκ–dependent transcription leads to accumulation of cyclin D1 in the cytosol. Notch ICD regulates entry into the cell cycle by controlling nuclear localization of cyclin D1 independently of RBP-Jκ.

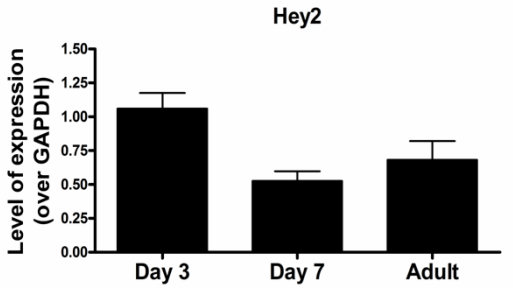
What about adult cardiomyocytes?

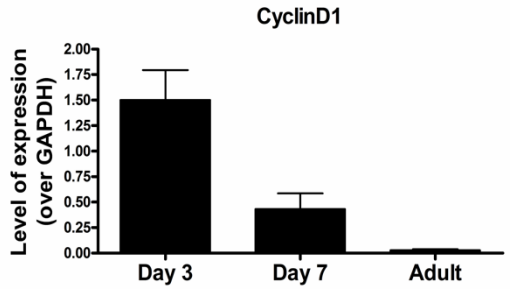
Loss of cardiomyocyte proliferative potential correlates with downregulation of Notch1 and its target genes

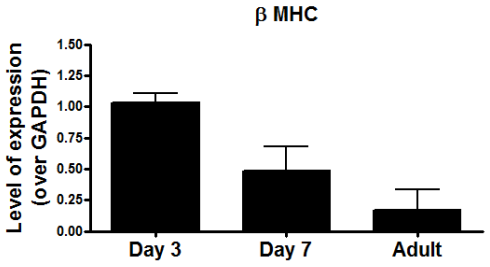


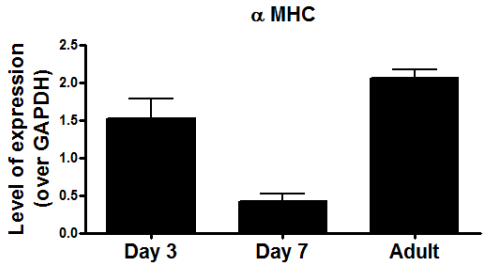


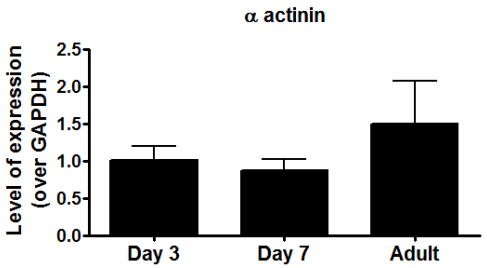


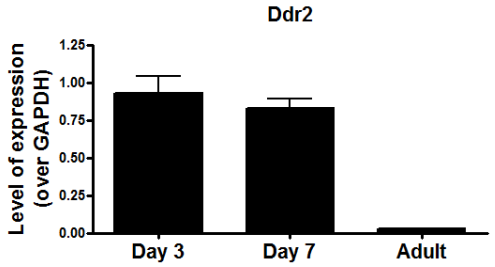


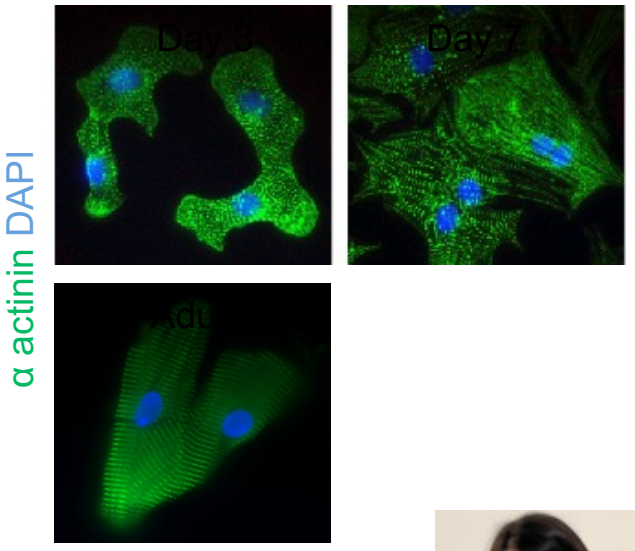






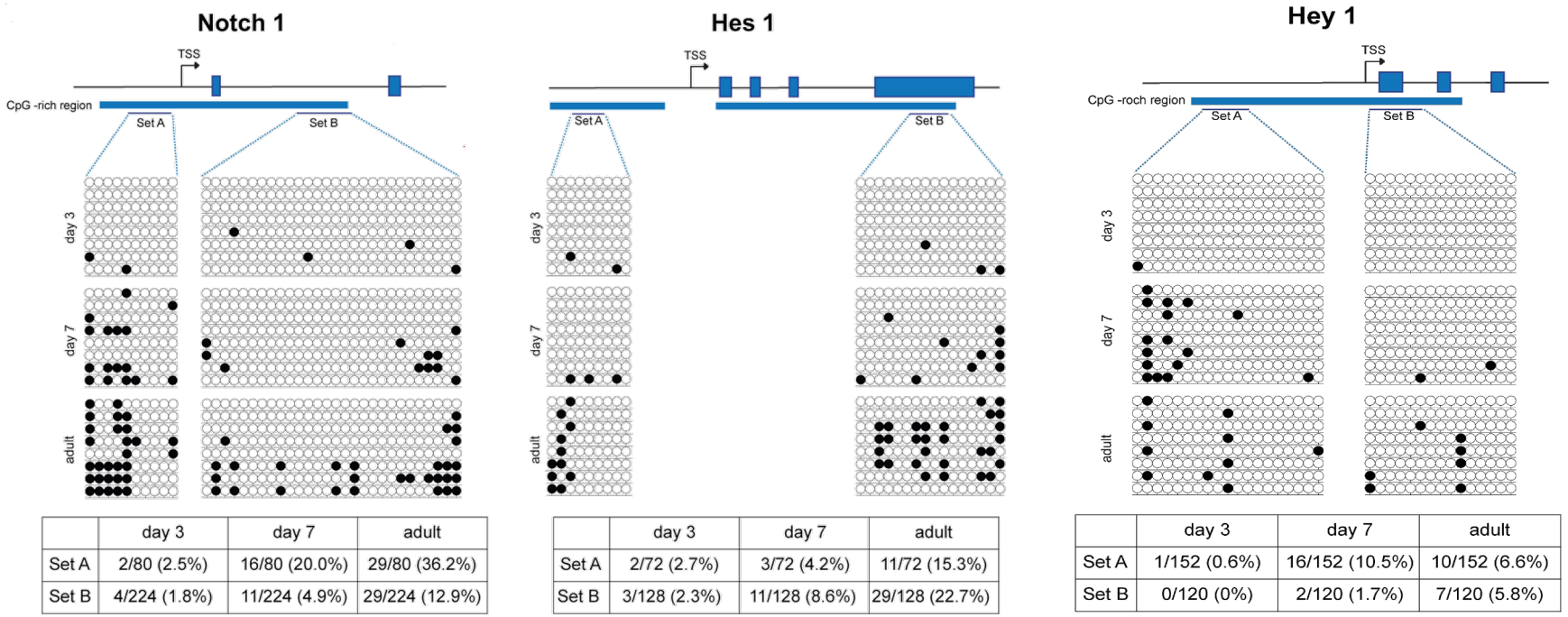






Giulia Felician

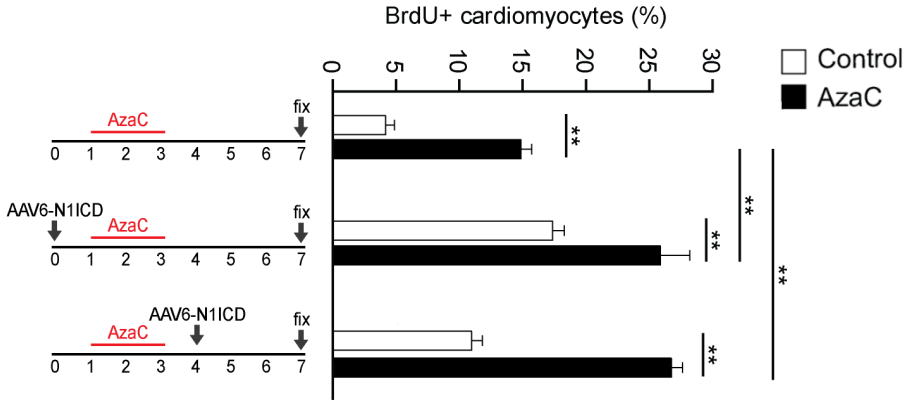
Methylation of promoters of Notch target genes impairs AAV9-sJagged1 and AAV9-N1ICD effect

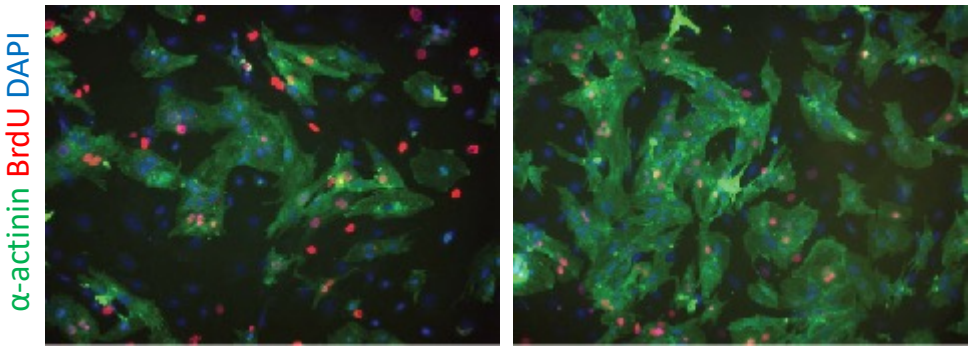


	day 3	day 7	adult
Set A	2/80 (2.5%)	16/80 (20.0%)	29/80 (36.2%)
Set B	4/224 (1.8%)	11/224 (4.9%)	29/224 (12.9%)

	day 3	day 7	adult
Set A	2/72 (2.7%)	3/72 (4.2%)	11/72 (15.3%)
Set B	3/128 (2.3%)	11/128 (8.6%)	29/128 (22.7%)

	day 3	day 7	adult
Set A	1/152 (0.6%)	16/152 (10.5%)	10/152 (6.6%)
Set B	0/120 (0%)	2/120 (1.7%)	7/120 (5.8%)

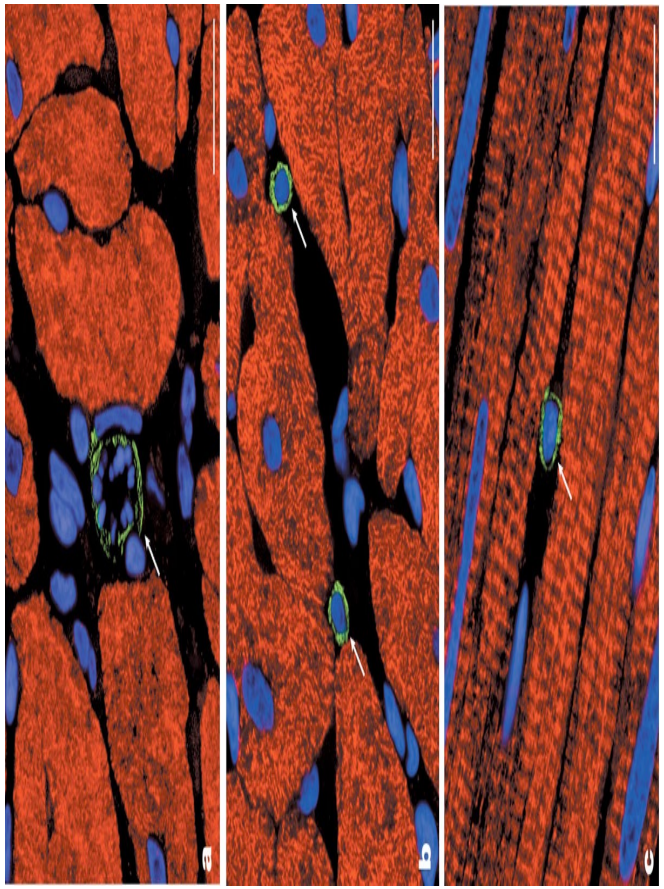


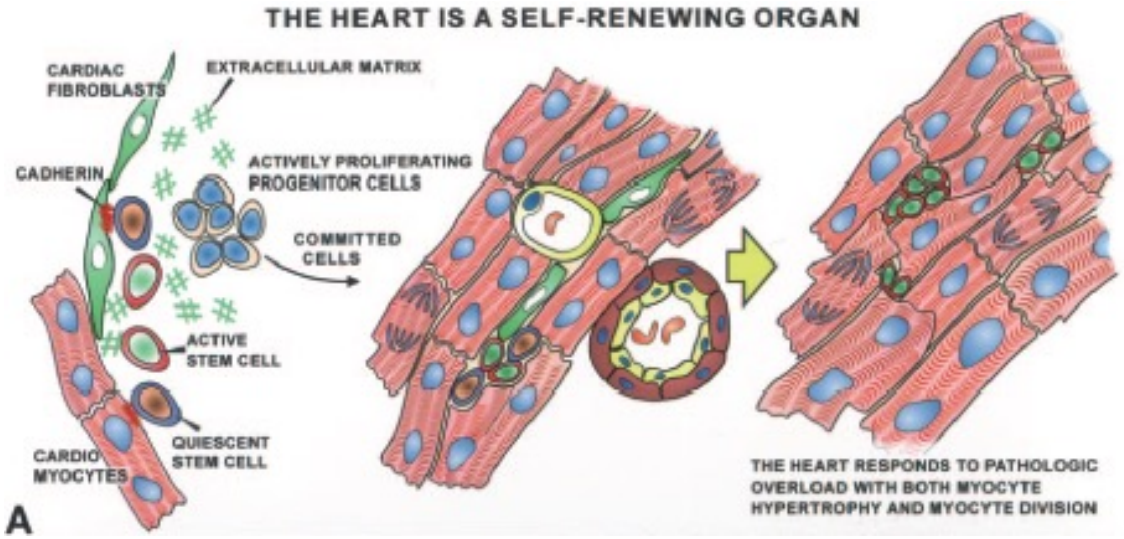


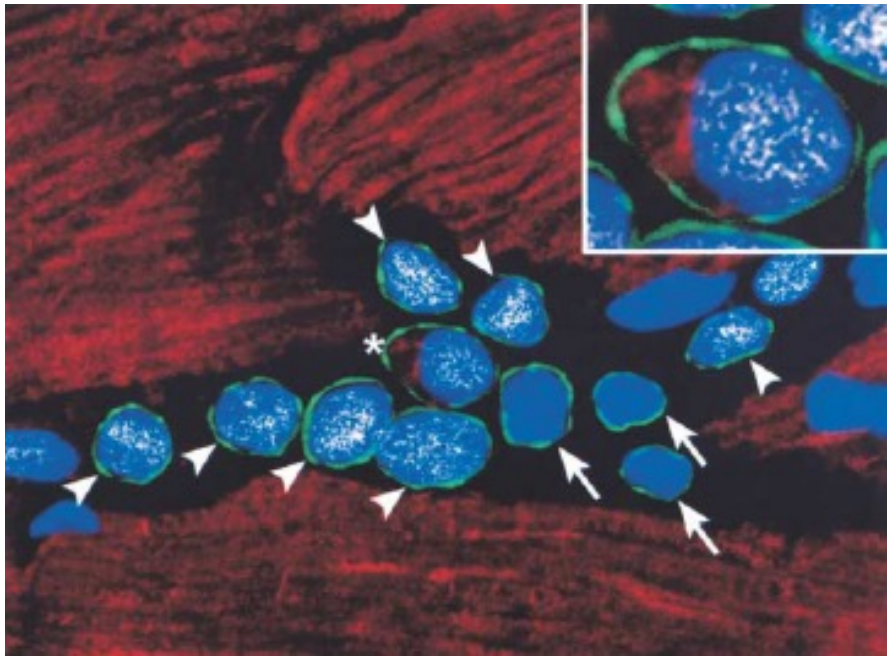
Felician G. et al., Circ. Res., 2014

Cardiac resident stem cells?

Cardiac stem cells (CSCs): do they exist?







Adult cardiac stem cells are multipotent and support myocardial regeneration.
Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P.

Life and Death of Cardiac Stem Cells

A Paradigm Shift in Cardiac Biology

Piero Anversa, MD; Jan Kajstura, PhD; Annarosa Leri, MD; Roberto Bolli, MD

Circulation March 21, 2006

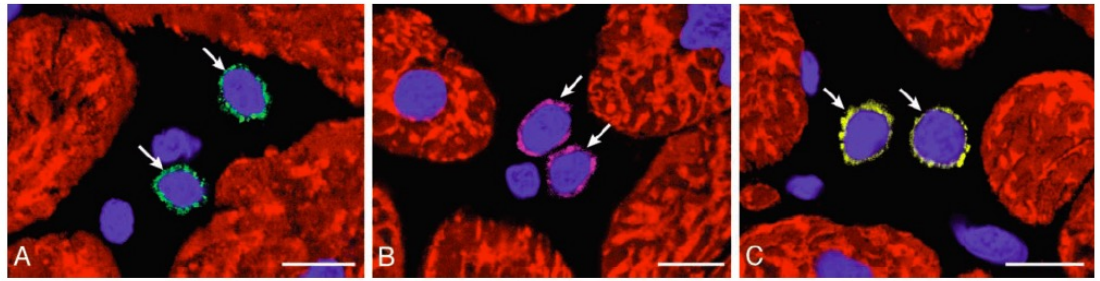
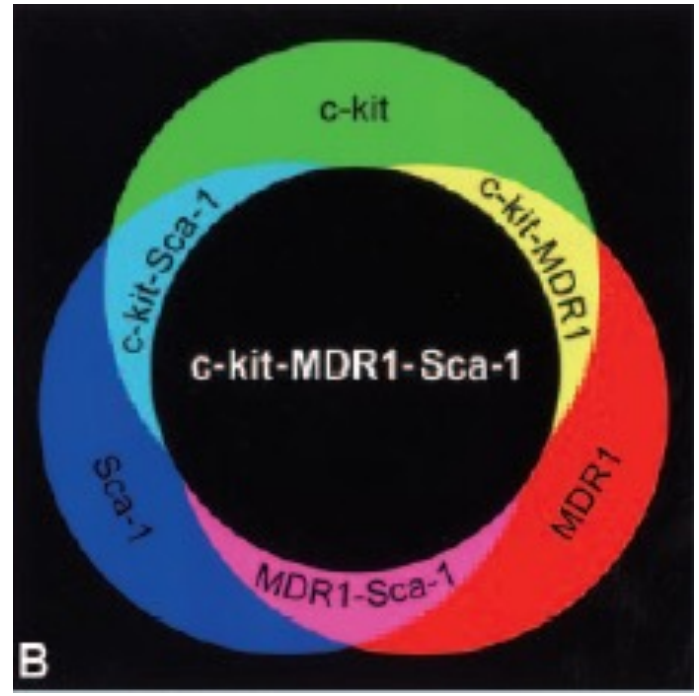
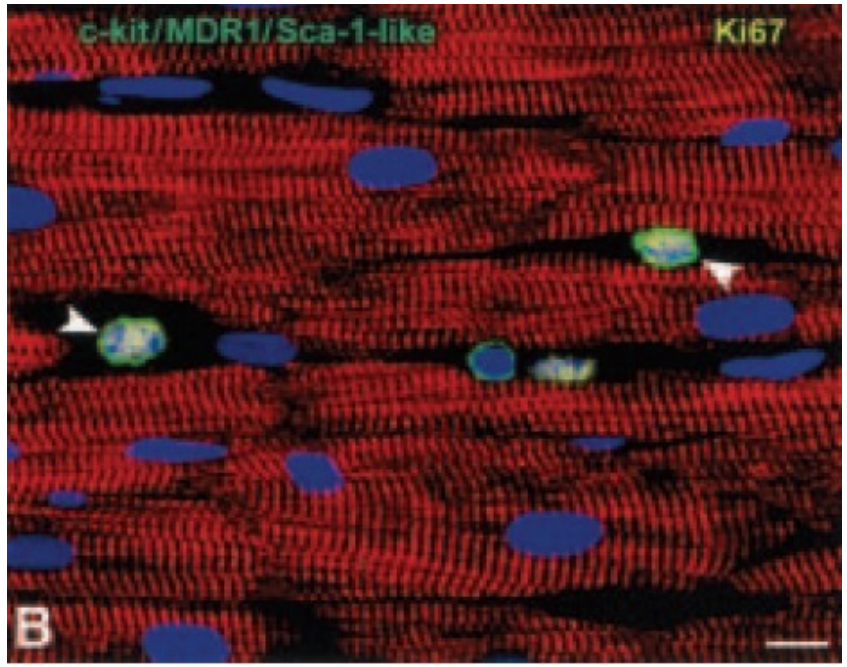
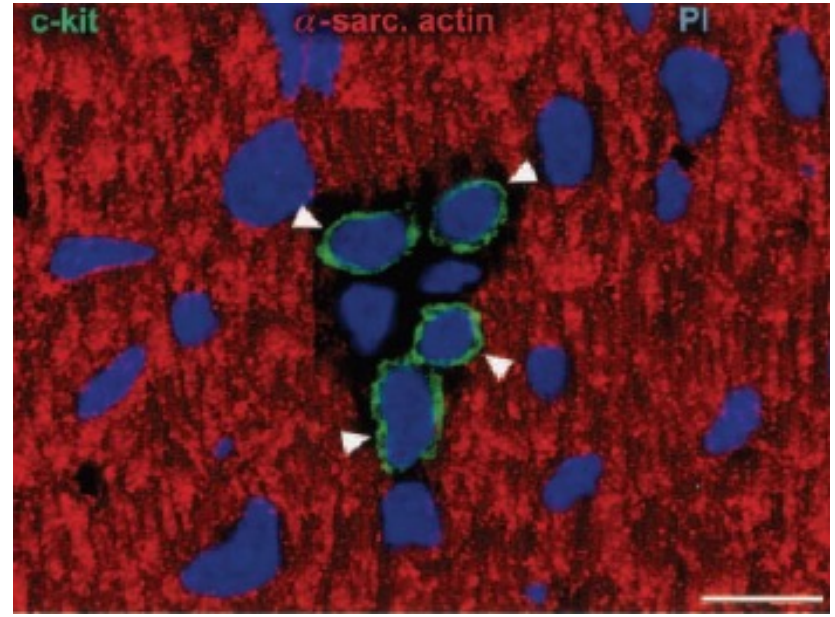
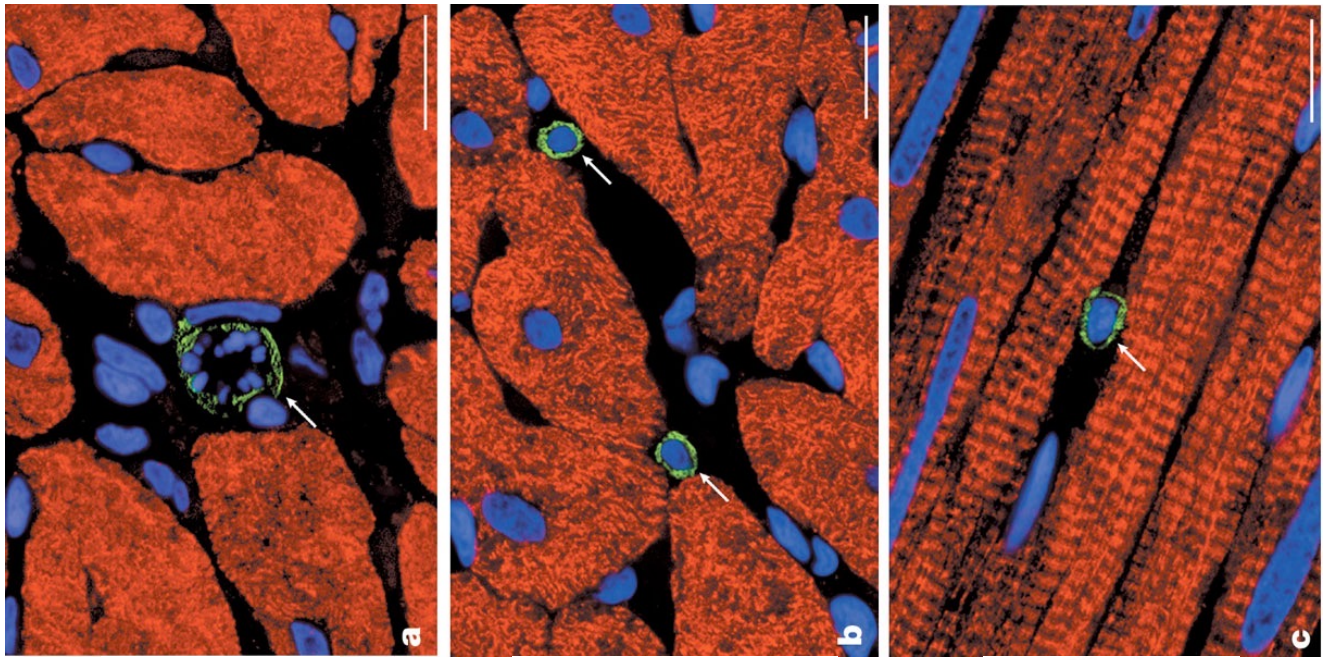


Fig. 1. Putative cardiac stem cells. Shown are detection of c-kit (*A*, green), MDR1 (*B*, purple), and Sca-1-reactive protein (*C*, yellow) in primitive cells (arrows) of hypertrophied hearts. Nuclei are stained by propidium iodide (PI; blue) and myocytes by cardiac myosin (red). (Bars = 10 μm.)

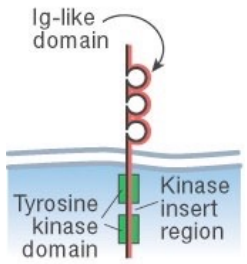








c-*kit*



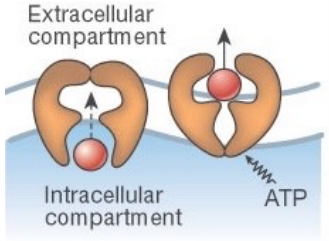
Distribution

- Melanocytes
- Mast cells
- Germ cells
- Stem cells

Functions

- Proliferation
- Migration
- Differentiation
- Secretion

P-glycoprotein or MRD1



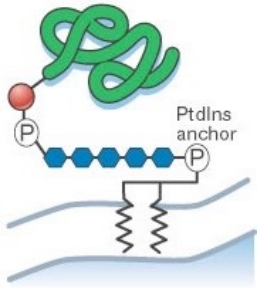
Distribution

- Hepatocytes–cholangiocytes
- Brush border cells
- Renal tubular cells
- Endothelial cells (brain)
- Cancer cells
- Stem cells

Functions

- Transmembrane efflux pump
- Inhibition of apoptosis

Sca-1



Distribution

- Vessel wall
- Kidney cortical tubules
- Thymus, spleen
- T lymphocytes
- Stem cells

Functions

- Cell adhesion
- Cell signalling
- T-cell activation

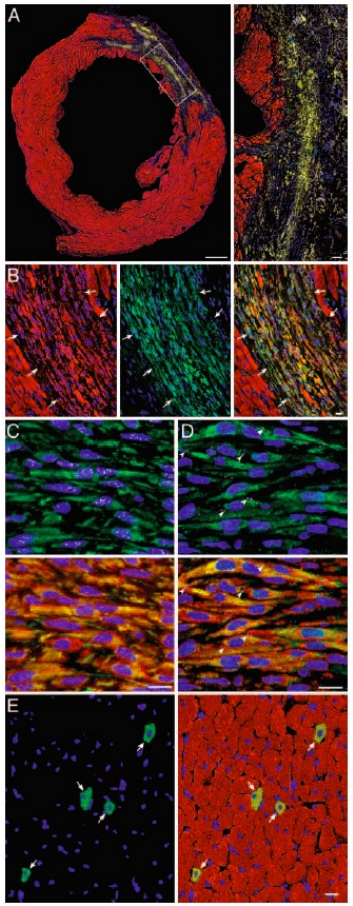
Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function

Buddhadeb Dawn*, Adam B. Stein*, Konrad Urbanek†, Marcello Rota†, Brian Whang†, Raffaella Rastaldo†, Daniele Torella†, Xian-Liang Tang*, Arash Rezazadeh*, Jan Kajstura†, Annarosa Leri†, Greg Hunt*, Jai Varma*, Sumanth D. Prabhu*, Piero Anversa†, and Roberto Bolli*‡

GFP-labeled CSCs delivered to the coronary arteries 4 hr after ischemia-reperfusion

Ventricular function monitored by echocardiography

Myocardial regeneration by histology



3766–3771 | PNAS | March 8, 2005 | vol. 102 | no. 10

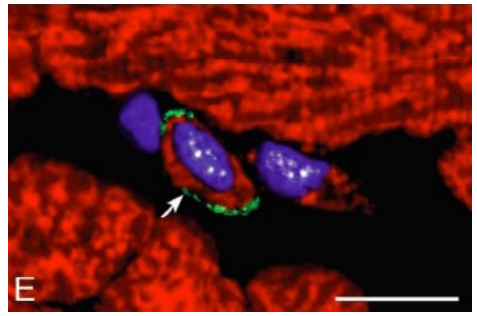
Adult cardiac stem cells are multipotent and support myocardial regeneration.

Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P.

Cardiovascular Research Institute, Department of Medicine, New York Medical College, Valhalla, NY 10595, USA

The notion of the adult heart as terminally differentiated organ without self-renewal potential has been undermined by the existence of a subpopulation of replicating myocytes in normal and pathological states. The origin and significance of these cells has remained obscure for lack of a proper biological context. We report the existence of Lin(-) c-kit(POS) cells with the properties of cardiac stem cells. They are self-renewing, clonogenic, and multipotent, giving rise to myocytes, smooth muscle, and endothelial cells. When injected into an ischemic heart, these cells or their clonal progeny reconstitute well-differentiated myocardium, formed by blood-carrying new vessels and myocytes with the characteristics of young cells, encompassing approximately 70% of the ventricle. Thus, the adult heart, like the brain, is mainly composed of terminally differentiated cells, but is not a terminally differentiated organ because it contains stem cells supporting its regeneration. The existence of these cells opens new opportunities for myocardial repair.

Cell, 2003 Sep 19



Red: MHC
Green: c-kit
White: MEF2
Blue: DAPI

Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy

Konrad Urbanek*, Federico Quaini*, Giordano Tasca†, Daniele Torella*, Clotilde Castaldo*, Bernardo Nadal-Ginard*, Annarosa Leri*, Jan Kajstura*, Eugenio Quaini†, and Piero Anversa*‡

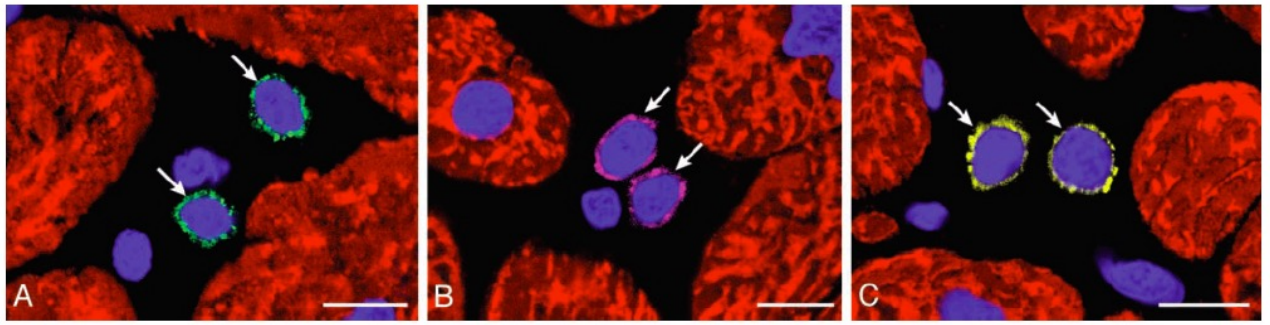


Fig. 1. Putative cardiac stem cells. Shown are detection of c-kit (*A*, green), MDR1 (*B*, purple), and Sca-1-reactive protein (*C*, yellow) in primitive cells (arrows) of hypertrophied hearts. Nuclei are stained by propidium iodide (PI; blue) and myocytes by cardiac myosin (red). (Bars = 10 μm.)

Resident cardiac stem cells

c-Kit+ cells (Anversa)
Sca-1 cells (Schneider)
Side population cells (Liao)
Islet-1 cells (Chien)
Cardiosphere-forming cells (Messina/Marban)
SSea-4+ cells (Taylor)

One of the least regenerative organ in the body has multiple non-overlapping populations of cardiomyocyte progenitors??

Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial

Roberto Bolli, Atul R Chugh, Domenico D'Amario, John H Loughran, Marcus F Stoddard, Sohail Ikram, Garth M Beache, Stephen G Wagner, Annarosa Leri, Toru Hosoda, Fumihiro Sanada, Julius B Elmore, Polina Goichberg, Donato Cappetta, Naresh K Solankhi, Ibrahim Fahsah, D Gregg Rokosh, Mark S Slaughter, Jan Kajstura, Piero Anversa

Summary

Background c-kit-positive, lineage-negative cardiac stem cells (CSCs) improve post-infarction left ventricular (LV) dysfunction when administered to animals. We undertook a phase 1 trial (Stem Cell Infusion in Patients with Ischemic cardiOmyopathy [SCIPIO]) of autologous CSCs for the treatment of heart failure resulting from ischaemic heart disease.

Methods In stage A of the SCIPIO trial, patients with post-infarction LV dysfunction (ejection fraction [EF] ≤40%) before coronary artery bypass grafting were consecutively enrolled in the treatment and control groups. In stage B, patients were randomly assigned to the treatment or control group in a 2:3 ratio by use of a computer-generated block randomisation scheme. 1 million autologous CSCs were administered by intracoronary infusion at a mean of 113 days (SE 4) after surgery; controls were not given any treatment. Although the study was open label, the echocardiographic analyses were masked to group assignment. The primary endpoint was short-term safety of CSCs and the secondary endpoint was efficacy. A per-protocol analysis was used. This study is registered with ClinicalTrials.gov, number NCT00474461.

Findings This study is still in progress. 16 patients were assigned to the treatment group and seven to the control group; no CSC-related adverse effects were reported. In 14 CSC-treated patients who were analysed, LVEF increased from 30·3% (SE 1·9) before CSC infusion to 38·5% (2·8) at 4 months after infusion (p=0·001). By contrast, in seven control patients, during the corresponding time interval, LVEF did not change (30·1% [2·4] at 4 months after CABG *vs* 30·2% [2·5] at 8 months after CABG). Importantly, the salubrious effects of CSCs were even more pronounced at 1 year in eight patients (eg, LVEF increased by 12·3 ejection fraction units [2·1] *vs* baseline, p=0·0007). In the seven treated patients in whom cardiac MRI could be done, infarct size decreased from 32·6 g (6·3) by 7·8 g (1·7; 24%) at 4 months (p=0·004) and 9·8 g (3·5; 30%) at 1 year (p=0·04).

Interpretation These initial results in patients are very encouraging. They suggest that intracoronary infusion of autologous CSCs is effective in improving LV systolic function and reducing infarct size in patients with heart failure after myocardial infarction, and warrant further, larger, phase 2 studies.

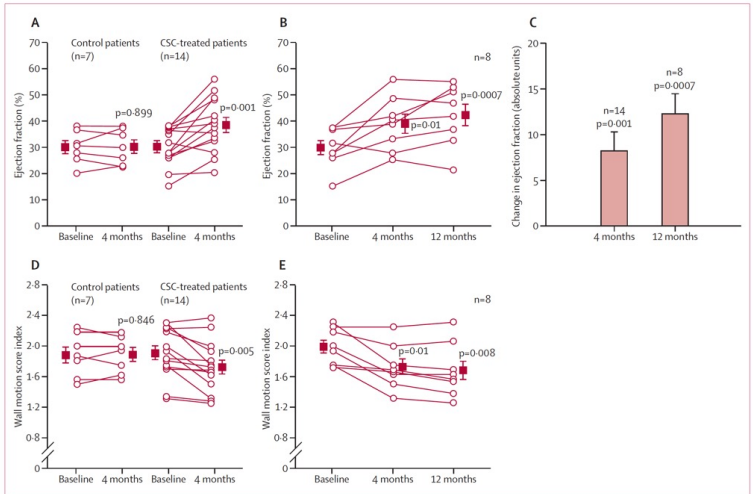


Figure 4: **Echocardiographic analysis of CSC-treated patients and controls**
(A) Left ventricular ejection fraction (measured by use of three-dimensional echocardiography) at 4 months after baseline in control and CSC-treated patients. (B) Ejection fraction at 4 months and 12 months after baseline in the CSC-treated patients who had 1 year of follow-up. (C) Change in ejection fraction from baseline at 4 months and 12 months in CSC-treated patients. (D) Wall motion score index at 4 months after baseline in control and CSC-treated patients. (E) Wall motion score index at 4 months and 12 months after baseline in the CSC-treated patients who had 1 year of follow-up. Boxes represent the mean values and error bars represent SE. p values are reported for difference between baseline and 4 months and between baseline and 12 months. CSC=cardiac stem cell.

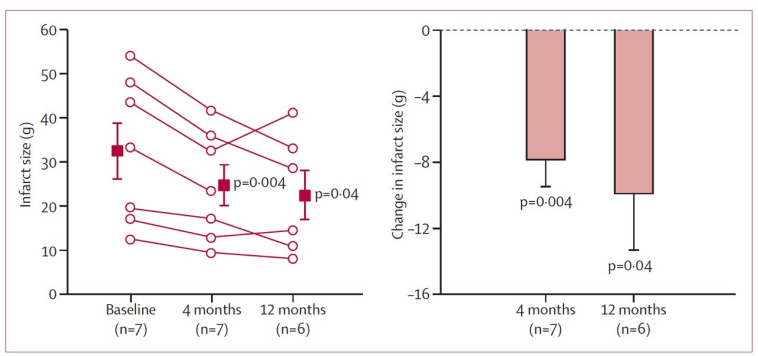


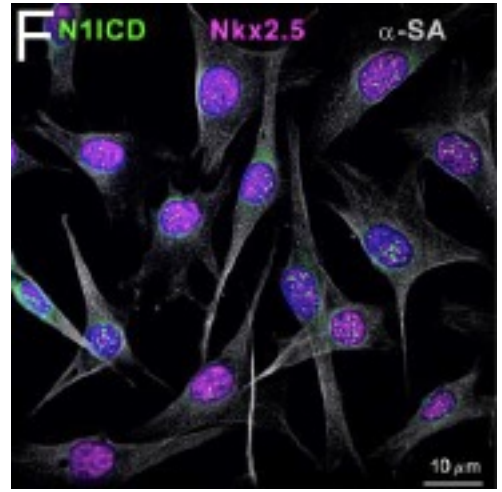
Figure 5: **Infarct size and change in infarct size at 4 months and 12 months after baseline in patients administered cardiac stem cells**
p values are reported for difference between baseline and 4 months and between baseline and 12 months. Boxes and bars represent the mean values and error bars represent the SE.

PNAS | October 7, 2008 | vol. 105 | no. 40 | 15529–15534

Notch1 regulates the fate of cardiac progenitor cells

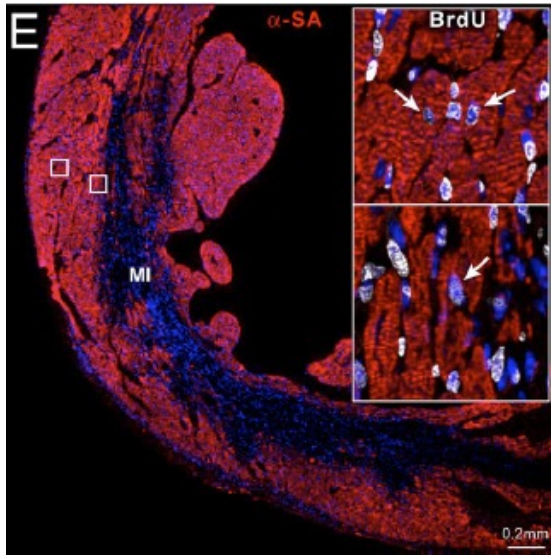
Alessandro Boni*†, Konrad Urbanek*†, Angelo Nascimbene*†, Toru Hosoda†, Hanqiao Zheng†, Francesca Delucchi†, Katsuya Amano†, Arantxa Gonzalez†, Serena Vitale†, Caroline Ojaimi‡, Roberto Rizzi†, Roberto Bolli§, Katherine E. Yutzey¶, Marcello Rota†, Jan Kajstura†, Piero Anversa†, and Annarosa Leri†‖

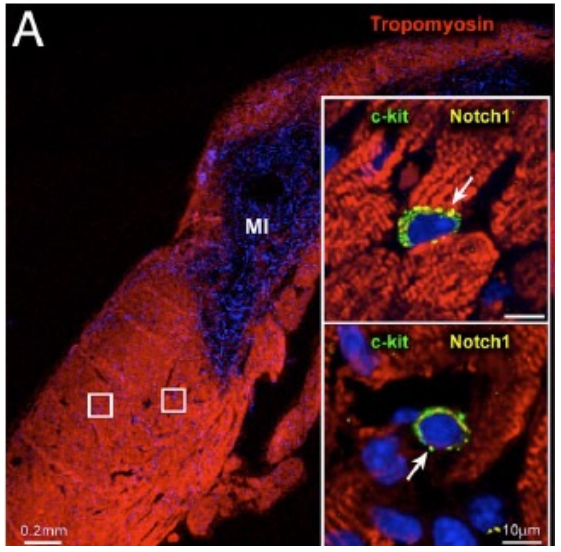
†Departments of Anesthesia and Medicine and Division of Cardiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115; ‡Department of Physiology, New York Medical College, Valhalla, NY 10595; §Institute of Molecular Cardiology, University of Louisville, Louisville, KY 40292; and ¶Divison of Molecular Cardiovascular Biology, Children's Medical Center, Cincinnati, OH 45229



- Cardiac progenitor cells (CPCs) in the niches express Notch1 receptor, and the supporting cells exhibit the Notch ligand Jagged1.
- N1ICD and RBP-Jk form a protein complex, which in turn binds to the Nkx2.5 promoter initiating transcription and myocyte differentiation.

- Notch1 favors the early specification of CPCs to the myocyte phenotype but maintains the newly formed cells in a highly proliferative state.

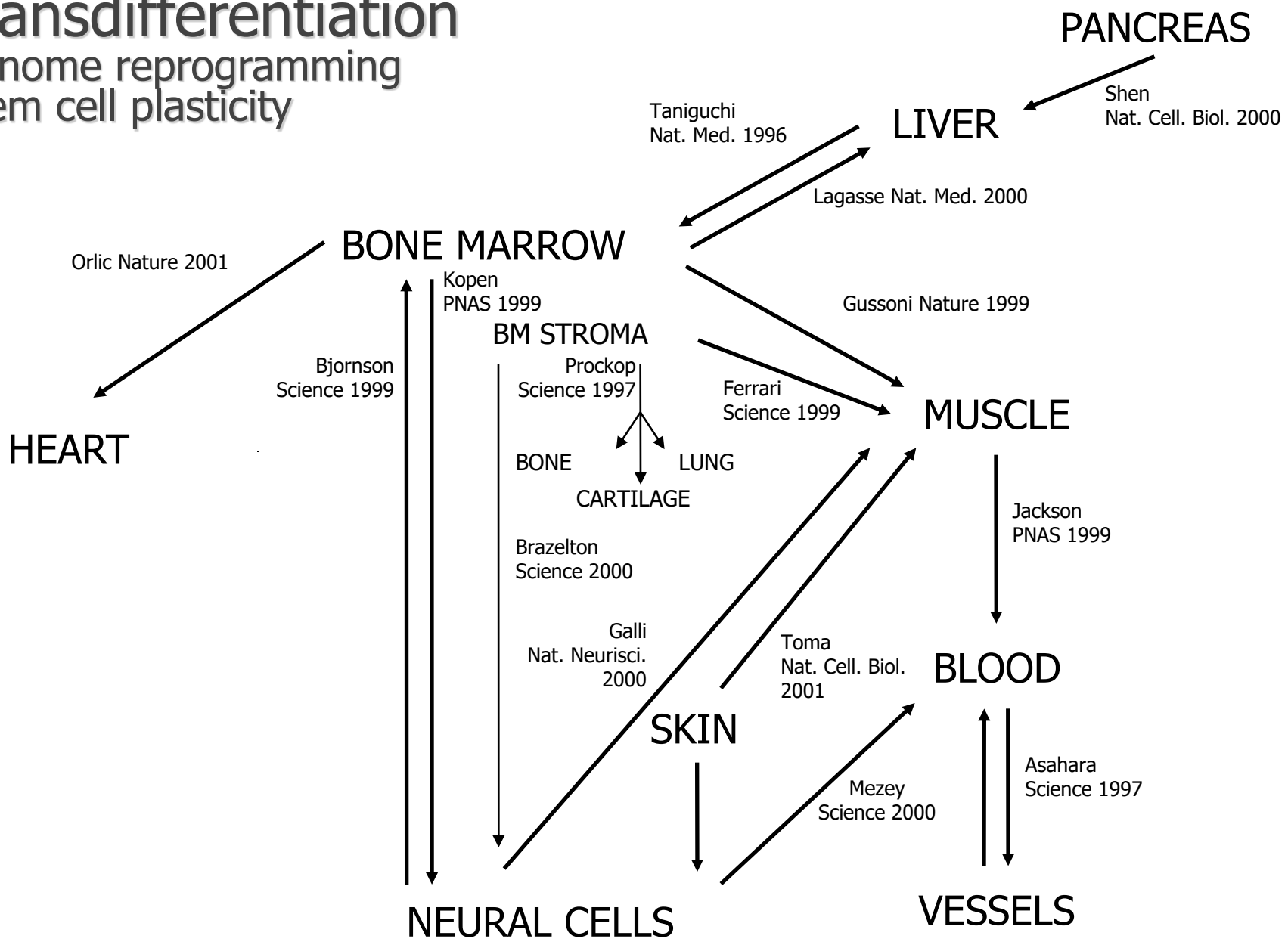




"Stem cells" from bone marrow

Transdifferentiation

Genome reprogramming
Stem cell plasticity



Neovascularization of ischemic myocardium by human bone-marrow–derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function

A.A. KOCHER[1], M.D. SCHUSTER[1], M.J. SZABOLCS[3], S. TAKUMA[2], D. BURKHOFF[2], J. WANG[1], S. HOMMA[2], N.M. EDWARDS[1] & S. ITESCU[1,2]

Kocher AA., Nature Medicine, Apr. 2001

Bone marrow cells regenerate infarcted myocardium

Donald Orlic†, Jan Kajstura*, Stefano Chimenti*, Igor Jakoniuk*, Stacie M. Anderson†, Baosheng Li*, James Pickel‡, Ronald McKay‡, Bernardo Nadal-Ginard*, David M. Bodine†, Annarosa Leri* & Piero Anversa*

NATURE | VOL 410 | 5 APRIL 2001

Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells

Kathyjo A. Jackson,[1] Susan M. Majka,[1,2] Hongyan Wang,[1] Jennifer Pocius,[1] Craig J. Hartley,[1] Mark W. Majesky,[2,3] Mark L. Entman,[1] Lloyd H. Michael,[1] Karen K. Hirschi,[1,4] and Margaret A. Goodell[1]

The Journal of Clinical Investigation | June 2001 | Volume 107 | Number 11

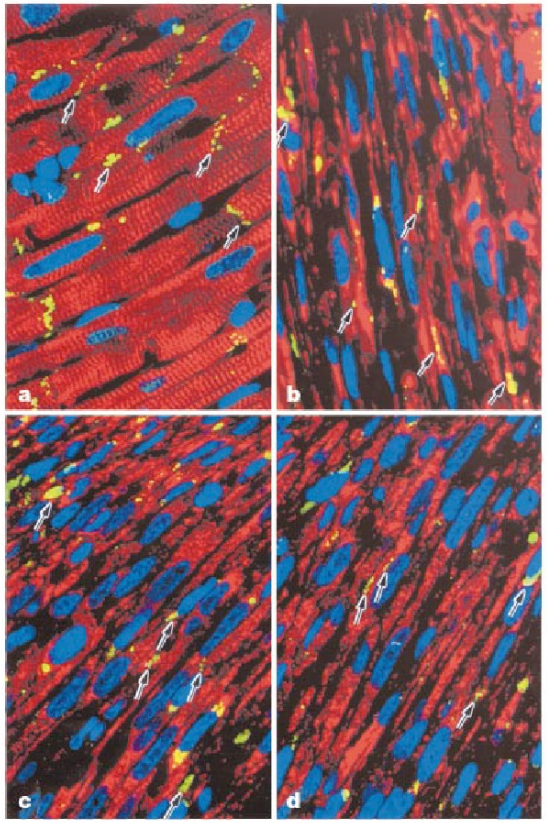


Figure 4 Myocardial repair and connexin 43. **a**, Border zone; **b**–**d**, regenerating myocardium. Shown are connexin 43 (yellow–green; arrows indicate contacts between myocytes) and α-sarcomeric actin (red), and PI-stained nuclei (blue). Original magnification, ×500 (**a**), ×800 (**b**–**d**).

Bone marrow cells regenerate infarcted myocardium

Donald Orlic†, Jan Kajstura*, Stefano Chimenti*, Igor Jakoniuk*, Stacie M. Anderson†, Baosheng Li*, James Pickel‡, Ronald McKay‡, Bernardo Nadal-Ginard*, David M. Bodine†, Annarosa Leri* & Piero Anversa*

** Department of Medicine, New York Medical College, Valhalla, New York 10595, USA*
† Hematopoiesis Section, Genetics and Molecular Biology Branch, NHGRI, and ‡ Laboratory of Molecular Biology, NINDS, NIH, Bethesda, Maryland 20892, USA

Myocardial infarction leads to loss of tissue and impairment of cardiac performance. The remaining myocytes are unable to reconstitute the necrotic tissue, and the post-infarcted heart deteriorates with time[1]. Injury to a target organ is sensed by distant stem cells, which migrate to the site of damage and undergo alternate stem cell differentiation[2–5]; these events promote structural and functional repair[6–8]. This high degree of stem cell plasticity prompted us to test whether dead myocardium could be restored by transplanting bone marrow cells in infarcted mice. We sorted lineage-negative (Lin^-) bone marrow cells from transgenic mice expressing enhanced green fluorescent protein[9] by fluorescence-activated cell sorting on the basis of c-*kit* expression[10]. Shortly after coronary ligation, Lin^- c-kit^{POS} cells were injected in the contracting wall bordering the infarct. Here we report that newly formed myocardium occupied 68% of the infarcted portion of the ventricle 9 days after transplanting the bone marrow cells. The developing tissue comprised proliferating myocytes and vascular structures. Our studies indicate that locally delivered bone marrow cells can generate *de novo* myocardium, ameliorating the outcome of coronary artery disease.

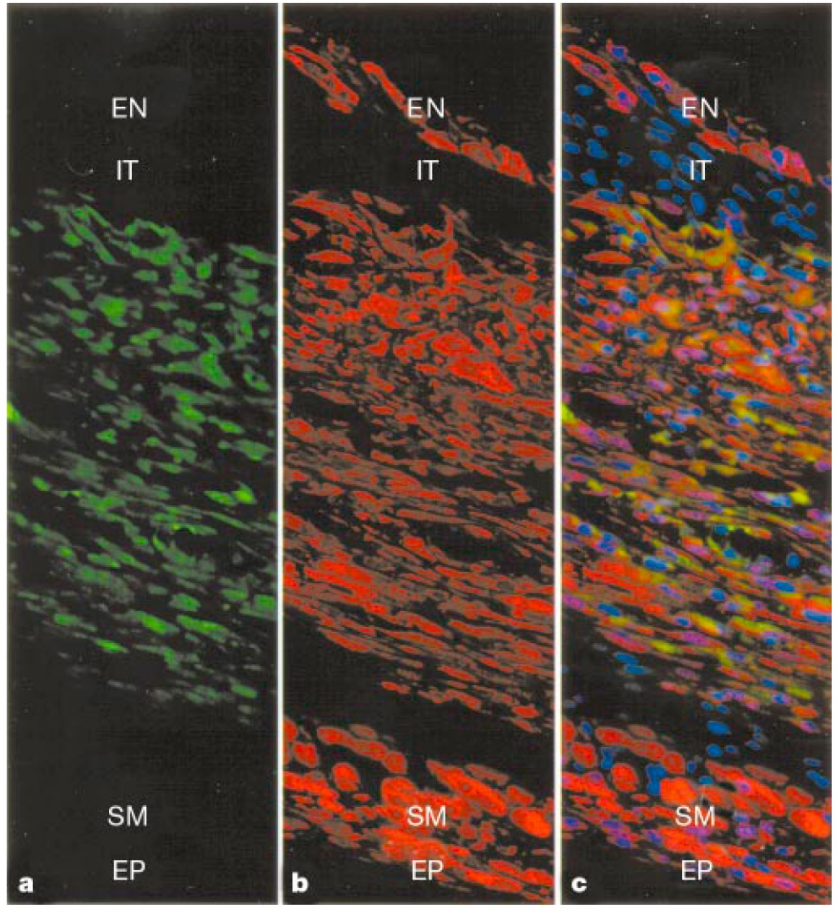
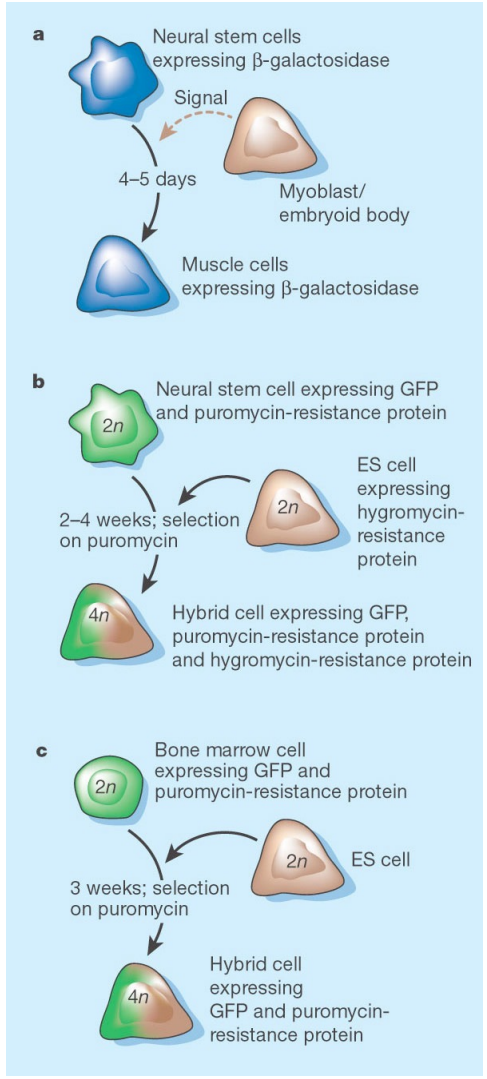


Figure 2 Myocardial infarct injected with Lin^-c-kit^{POS} cells; myocardium is regenerating from endocardium (EN) to epicardium (EP). **a**, EGFP (green); **b**, cardiac myosin (red); **c**, combination of EGFP and myosin (red–green), and propidium-iodide-stained nuclei (blue). Infarcted tissue (IT) can be seen in the subendocardium, spared myocytes (SM) can be seen in the subepicardium. Original magnification, ×250 (**a**–**c**).

Is it true plasticity? It might be, but there are other possibilities…



Stem cells

Cell fusion causes confusion

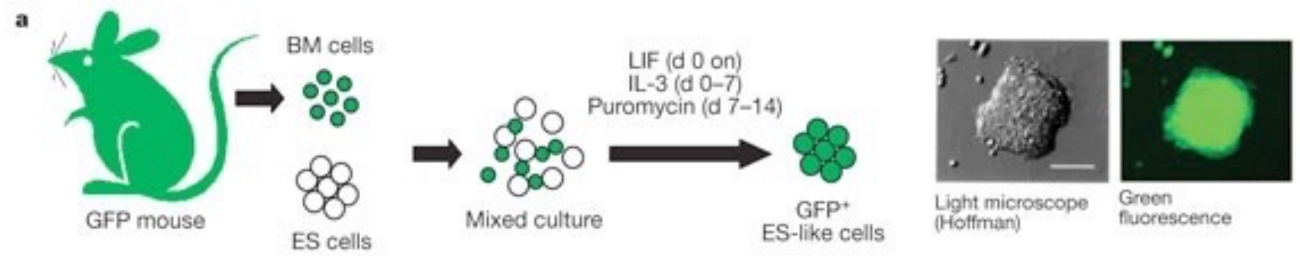
Andrew E. Wurmser and Fred H. Gage

'Transdifferentiation' is a poorly understood process invoked to explain how tissue-specific adult stem cells can generate cells of other tissues. New results challenge its existence.

Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion

Naohiro Terada*†, Takashi Hamazaki*, Masahiro Oka*, Masanori Hoki*, Diana M. Mastalerz*, Yuka Nakano‡, Edwin M. Meyer‡, Laurence Morel*, Bryon E. Petersen*† & Edward W. Scott†§

** Department of Pathology, † Program in Stem Cell Biology, Shands Cancer Center, ‡ Department of Pharmacology, § Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, Florida 32610, USA*



Lost in translation

Kenneth R. Chien

The potential use of stem cells as agents of repair in human disease makes them the subject of high-profile studies. But we should be wary of prematurely pushing laboratory research into clinical practice.

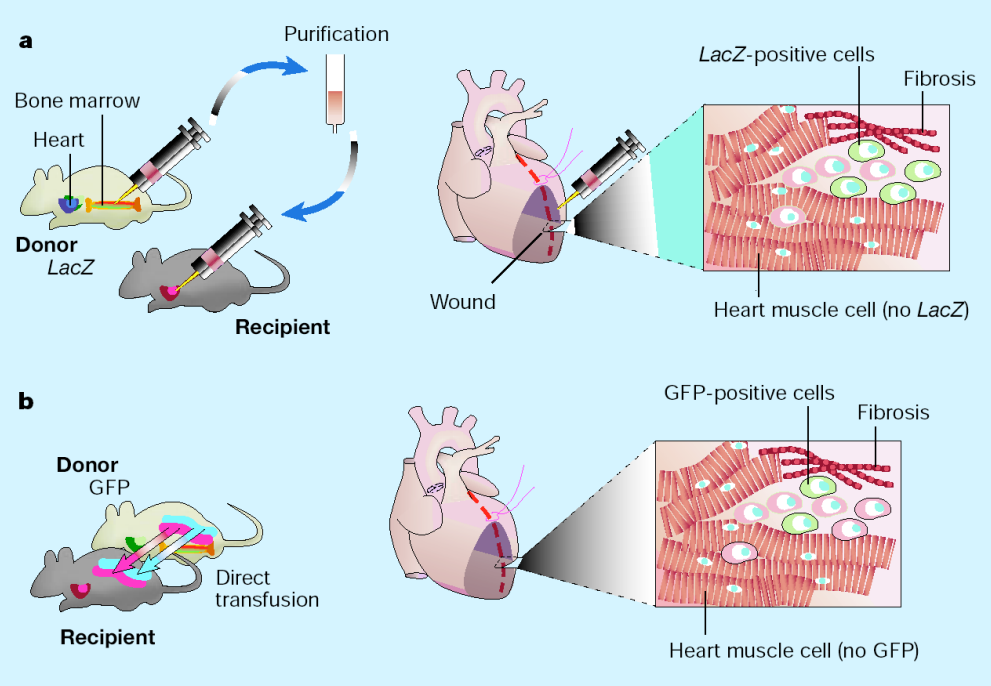


Figure 1 **Two strategies used to show that bone-marrow stem cells do not take on the role of damaged heart cells.** a, **Murry *et al.*[2] isolated and purified genetically modified bone-marrow stem cells from mice. The modification 'tagged' the cells (with *LacZ*), enabling them to be detected in the recipient mouse heart, into which the cells were directly injected. Closer inspection of the recipient heart showed that the label could not be detected in heart muscle cells.** b, **Similar results were shown by Balsam *et al.*[3], although the approach was slightly different. Donor bone-marrow stem cells were transfused directly into the circulation of recipients. Again, the tag (GFP; green fluorescent protein) could not be detected in heart muscle cells of the donor; indeed, the bone-marrow cells continued to differentiate into blood cells while in the heart.**

Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium

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Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts

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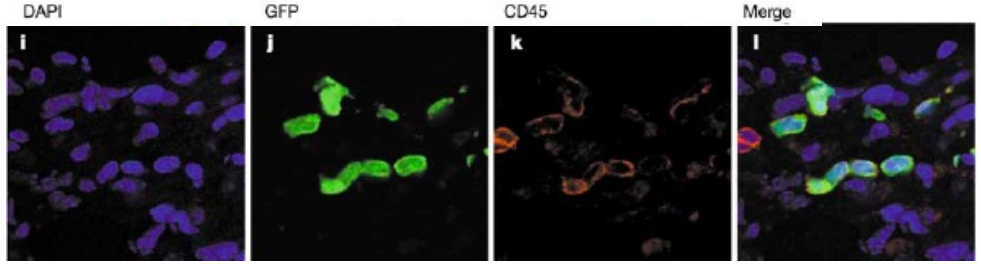
Table 1 **Estimated number of GFP$^+$ cells present in injured myocardium**

Animal number	Cell type	Original no. of GFP$^+$cells implanted	Time of death (days)	Estimated number of GFP$^+$ cells at death	Original GFP$^+$ cells present at death (%)
1	c-kit^{Err}	1×10^6	10	190,430	19.0
2	c-kit^{Err}	1×10^6	10	471,930	47.2
3	c-kit^{Err}	1×10^6	30	3,600	0.4
4	c-kit^{Err}	1×10^6	30	3,000	0.3
5	c-kit^{Err}	1×10^6	30	0	0
6	c-kit^{Err}	1×10^6	30	0	0
7	KTLS LT-HSC	4×10^3	10	25,980	649.5
8	KTLS LT-HSC	4×10^3	10	11,820	295.5
9	KTLS LT-HSC	4×10^3	10	16,440	411.0
10	KTLS LT-HSC	4×10^3	30	0	0
11	KTLS LT-HSC	4×10^3	30	0	0
12	KTLS LT-HSC	4×10^3	30	720	18.0
13	Lin$^-$ c-kit$^+$	6×10^5	10	61,800	10.3
14	Lin$^-$ c-kit$^+$	6×10^5	10	64,890	10.8



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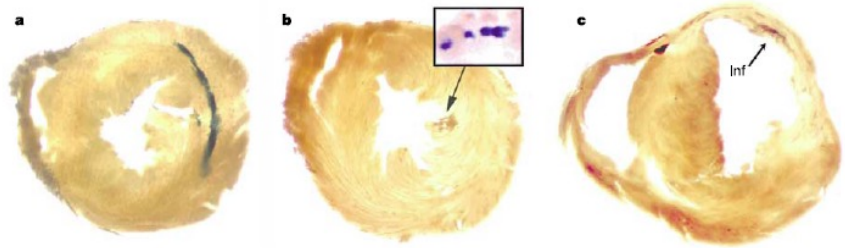
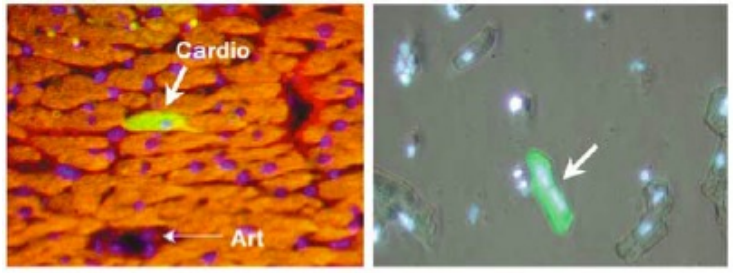


Transgenic mice in which the cardiac-specific MHC promoter drives the expression of a nuclear beta-gal



A rare GFP cardiomyocyte in the peri-infarct region, after BMT (MHC staining) and a single rod-shaped enzymatically dispersed cardiomyocyte

The biological toolbox for cardiac regeneration

Stem cells

- Embryonic stem cells (from embryos, through cloning)
- iPs cells
- Bone marrow stem cells (?)
- Mesenchymal stem cells (?)
- Adult cardiac stem cells
- Cardiospheres

Direct transdifferentiation of fibroblasts to cardiomyocytes

- Transcription factors

Stimulation of adult cardiomyocyte proliferation

- microRNAs