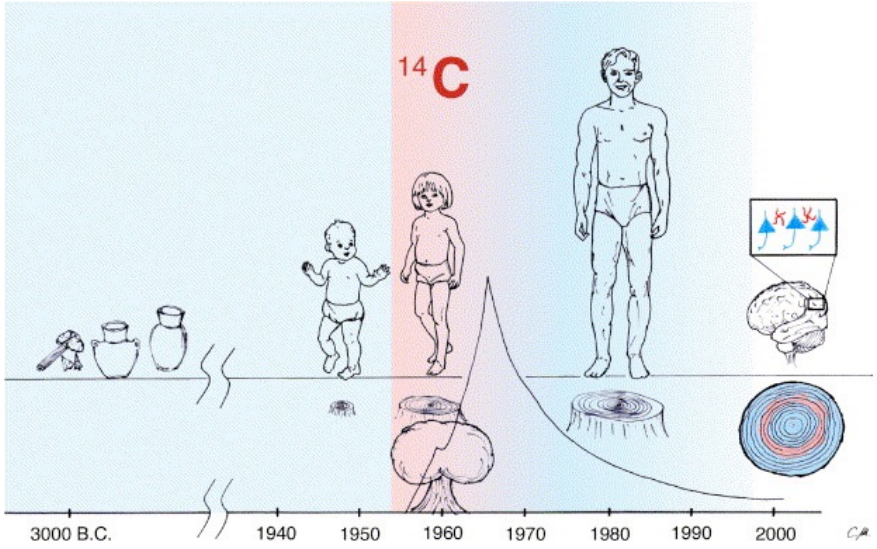
How does adult cardiomyocyte renewal occur?

Carbon dating of human tissues

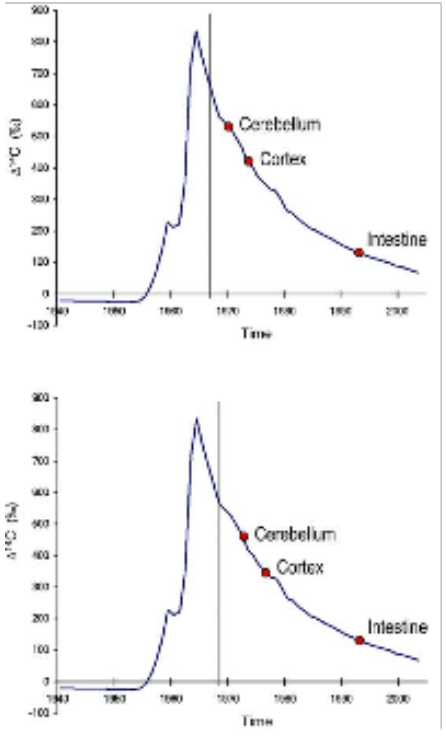
After the Second World War, tests of nuclear bombs spewed carbon-14 pollution into the atmosphere. This isotope was incorporated into plants and the people who consumed them. After above-ground tests were stopped in 1963, levels of the isotope started to fall. The ^{14}C in a cell's DNA corresponds to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

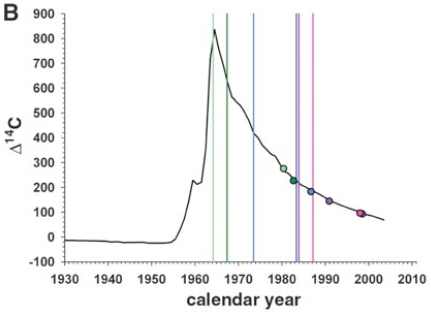
People born before 1955 had levels of ^{14}C in their cardiomyocytes that were higher than was present in the atmosphere at the time of their birth, so some of these cells must have arisen later on in their lives. Further work and mathematical modelling allowed to calculate that a 50-year-old heart still 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

Olaf Bergmann,[1]* Ratan D. Bhardwaj,[1]* Samuel Bernard,[2] Sofia Zdunek,[1] Fanie Barnabé-Heider,[1] Stuart Walsh,[3] Joel Zupicich,[1] Kanar Alkass,[4] Bruce A. Buchholz,[5] Henrik Druid,[4] Stefan Jovinge,[3,6] Jonas Frisén[1]†

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.

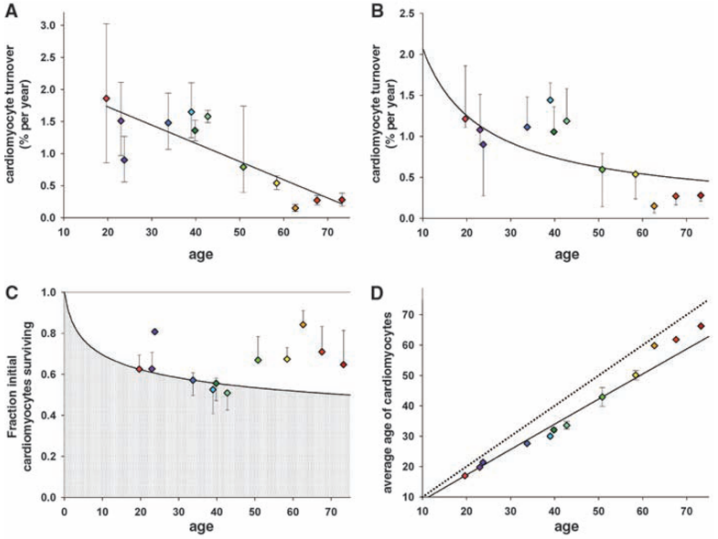


Fig. 4. Dynamics of cardiomyocyte turnover. (**A**) Individual data fitting assuming a constant turnover (see supporting online text) reveals an almost linear decline of cardiomyocyte turnover with age (R = −0.84; P = 0.001). A constant-turnover hypothesis might therefore not represent the turnover dynamics 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.

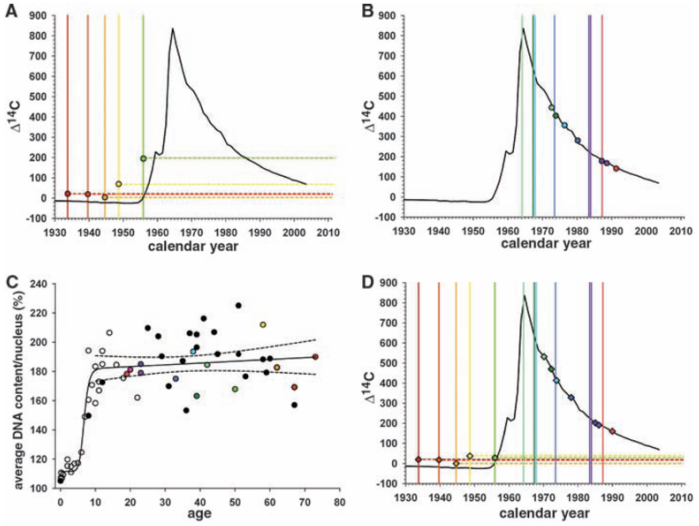


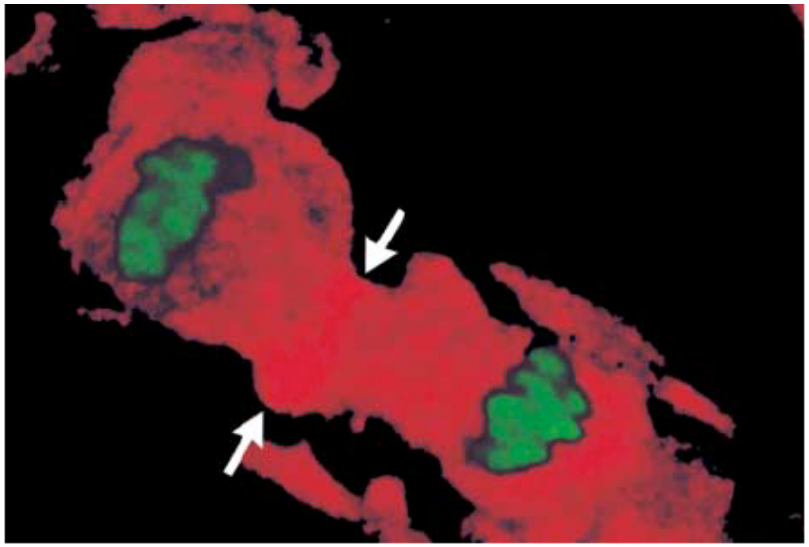
Fig. 3. Cardiomyocyte turnover in adulthood. (**A**) The ^{14}C concentrations in cardiomyocyte DNA from individuals born before the time of the atmospheric radiocarbon increase correspond to time points after the birth of all individuals. The vertical bar indicates year of birth, with the correspondingly 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.

A considerable amount of cardiomyocyte division was shown in the failing and infarcted human myocardium (mitotic index of 0.015% and 0.08%, respectively)

The New England Journal of Medicine

EVIDENCE THAT HUMAN CARDIAC MYOCYTES DIVIDE AFTER MYOCARDIAL INFARCTION

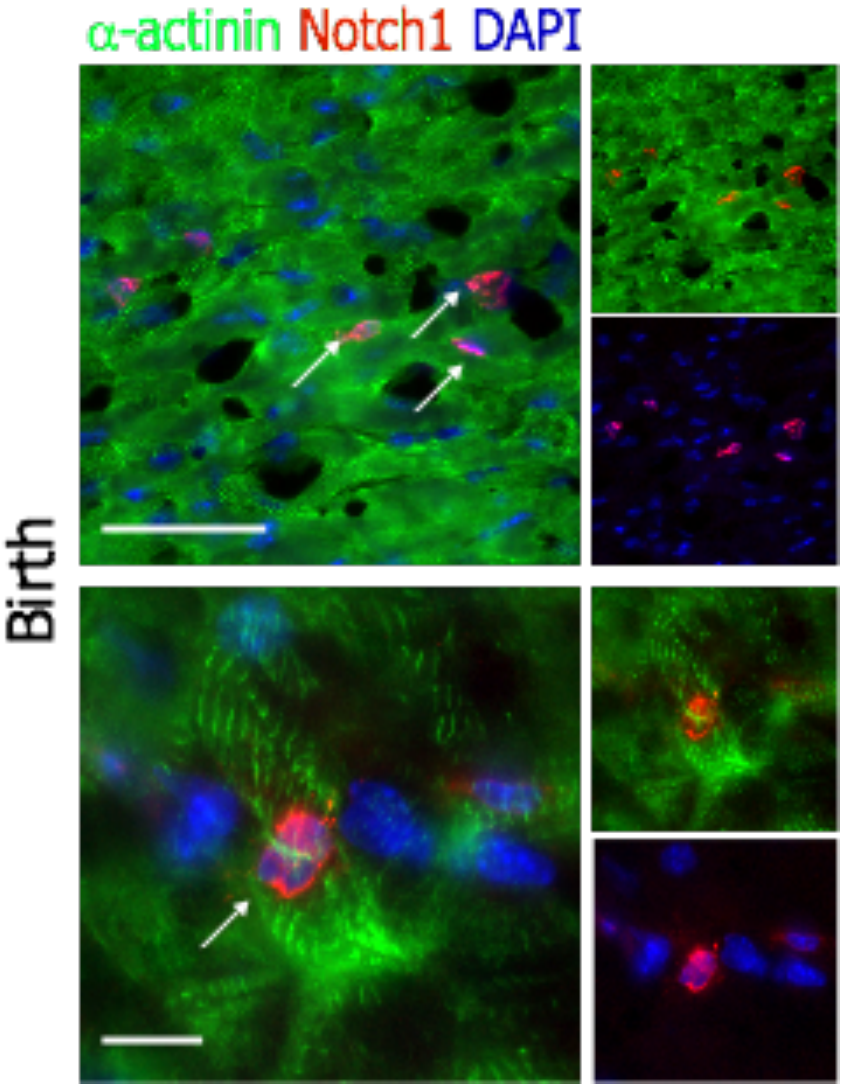
ANTONIO P. BELTRAMI, M.D., KONRAD URBANEK, M.D., JAN KAJSTURA, PH.D., SHAO-MIN YAN, M.D., NICOLETTA FINATO, M.D., ROSSANA BUSSANI, M.D., BERNARDO NADAL-GINARD, M.D., PH.D., FURIO SILVESTRI, M.D., ANNAROSA LERI, M.D., C. ALBERTO BELTRAMI, M.D., AND PIERO ANVERSA, M.D.

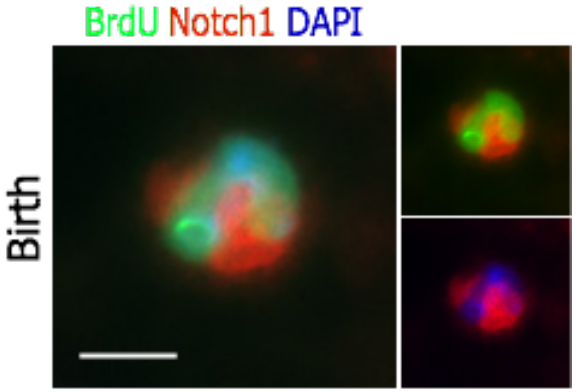


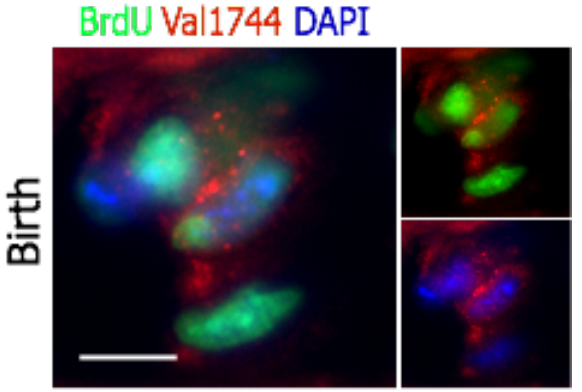
NEJM, 2001

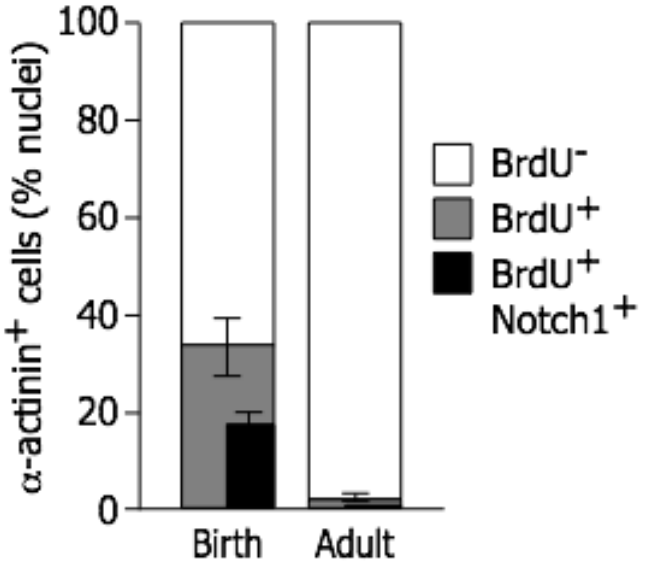
When does cardiomyocyte proliferation stop?

Proliferating neonatal cardiomyocytes express Notch1

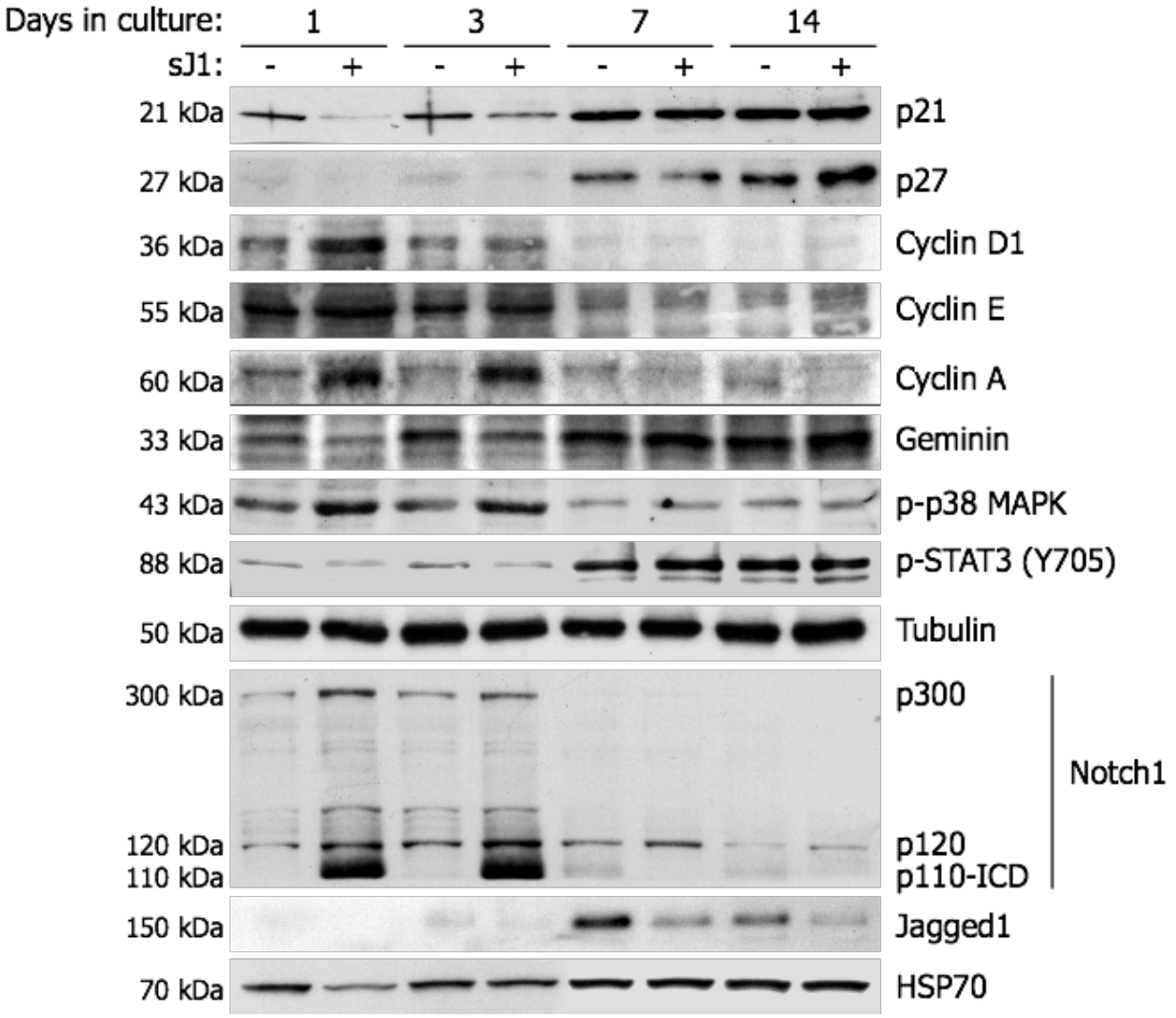






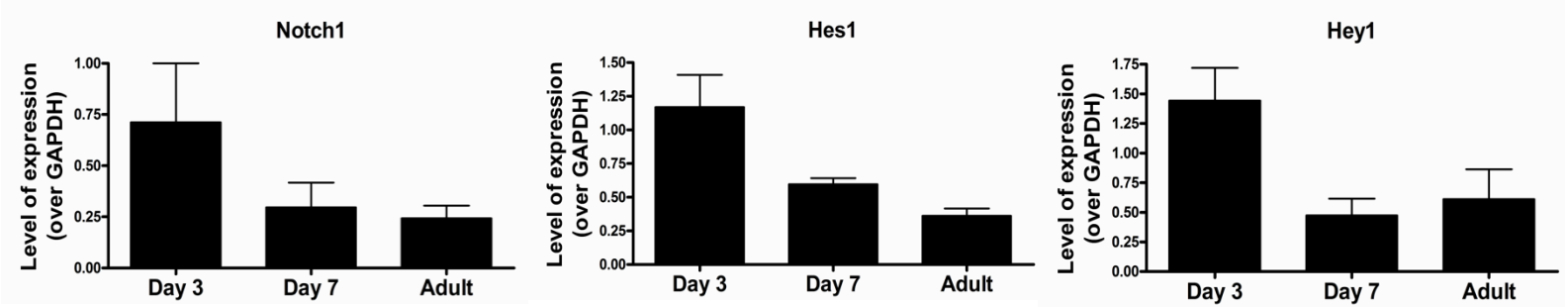


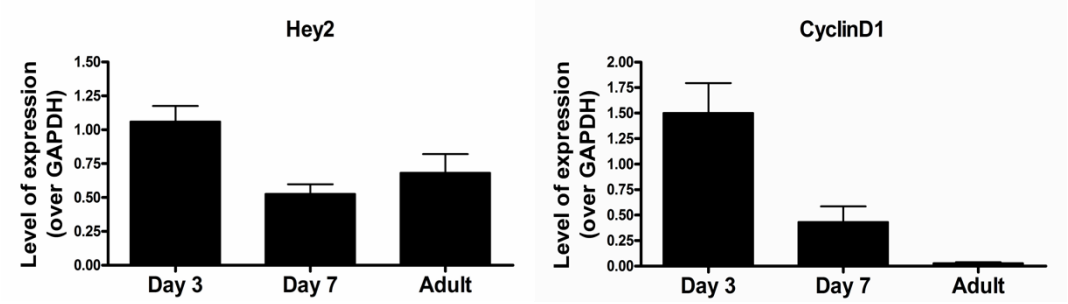
sJagged1-mediated Notch1 activation parallels cardiomyocyte proliferation

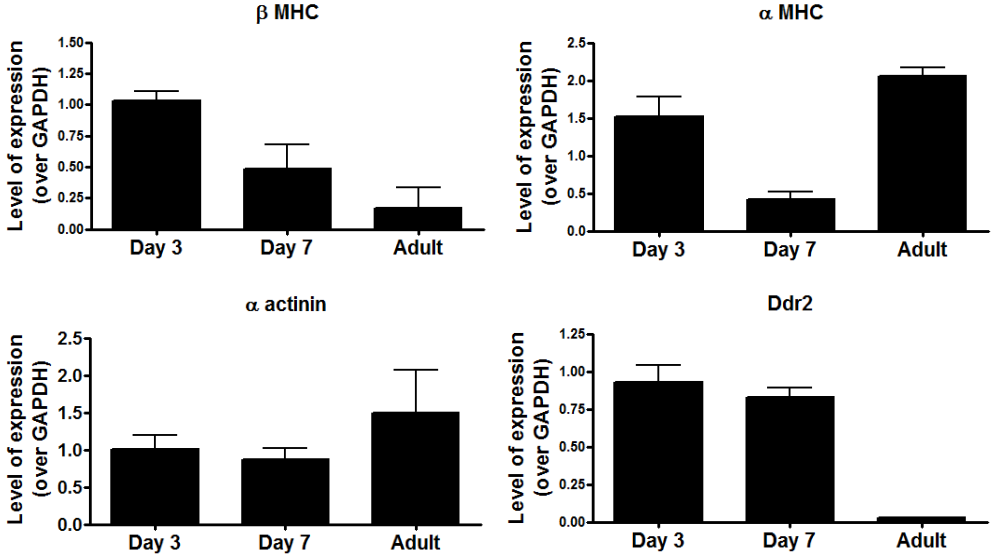


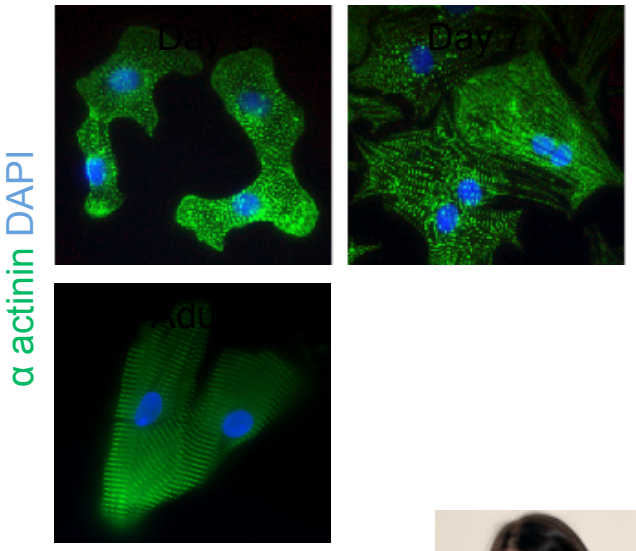
What about adult cardiomyocytes?

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



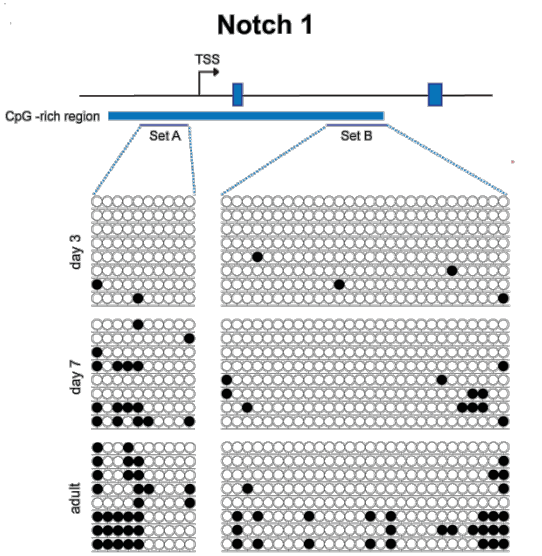




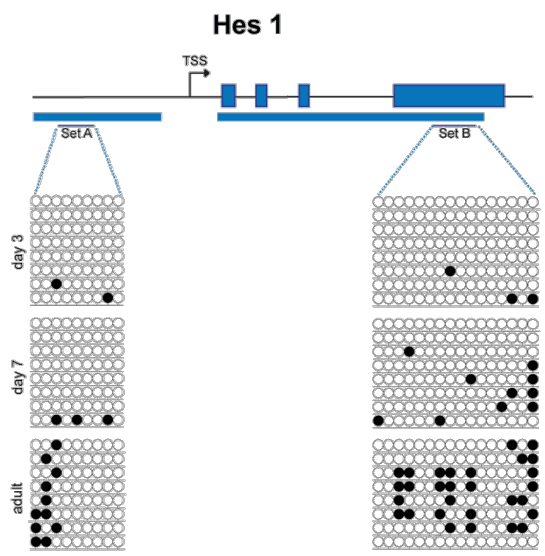


Giulia Felician

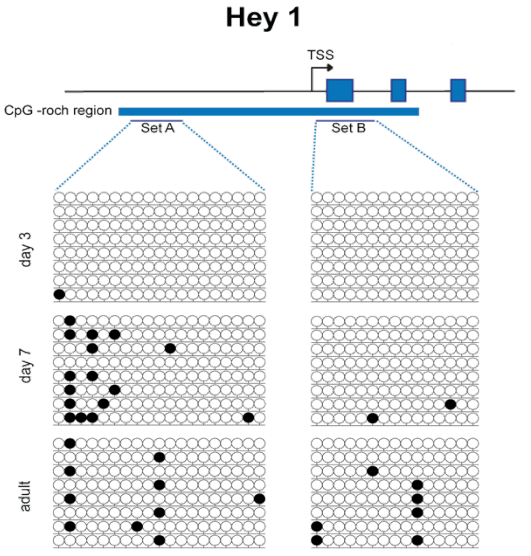
Methylation of promoters of Notch target genes impairs AAV9-sJagged1 and AAV9-N1ICD effect



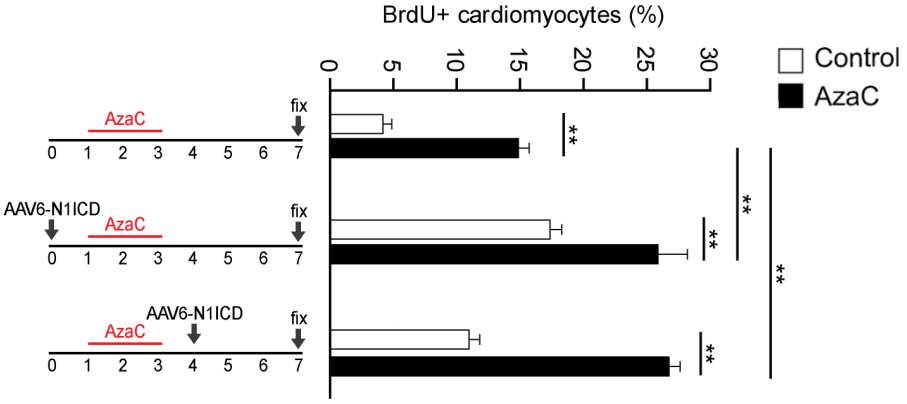
	day 3	day 7	adult
Set A	2/80 (2.5%)	16/80 (20.0%)	29/80 (36.2%)
Set B	4/224 (1.8%)	11/224 (4.9%)	29/224 (12.9%)

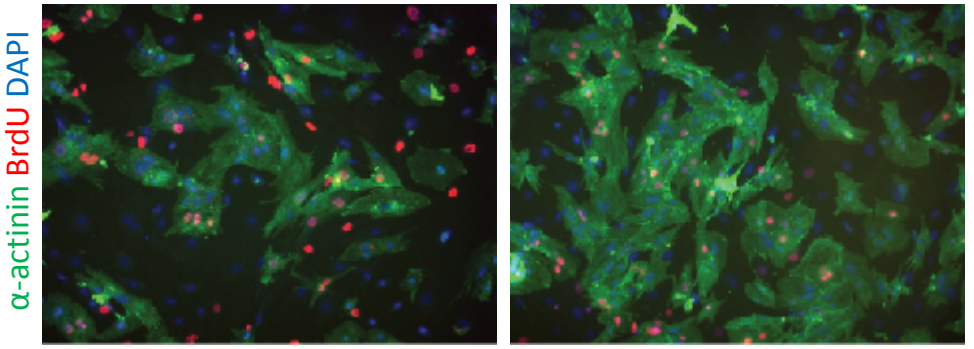


	day 3	day 7	adult
Set A	2/72 (2.7%)	3/72 (4.2%)	11/72 (15.3%)
Set B	3/128 (2.3%)	11/128 (8.6%)	29/128 (22.7%)



	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

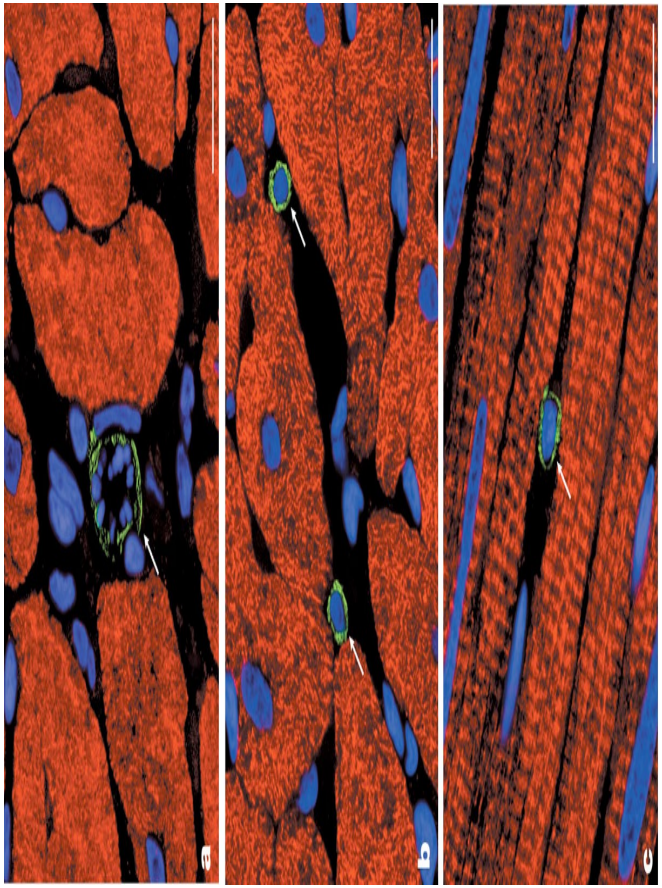
Notch1 pathway stimulation extends the proliferative capacity of neonatal cardiomyocytes ex vivo

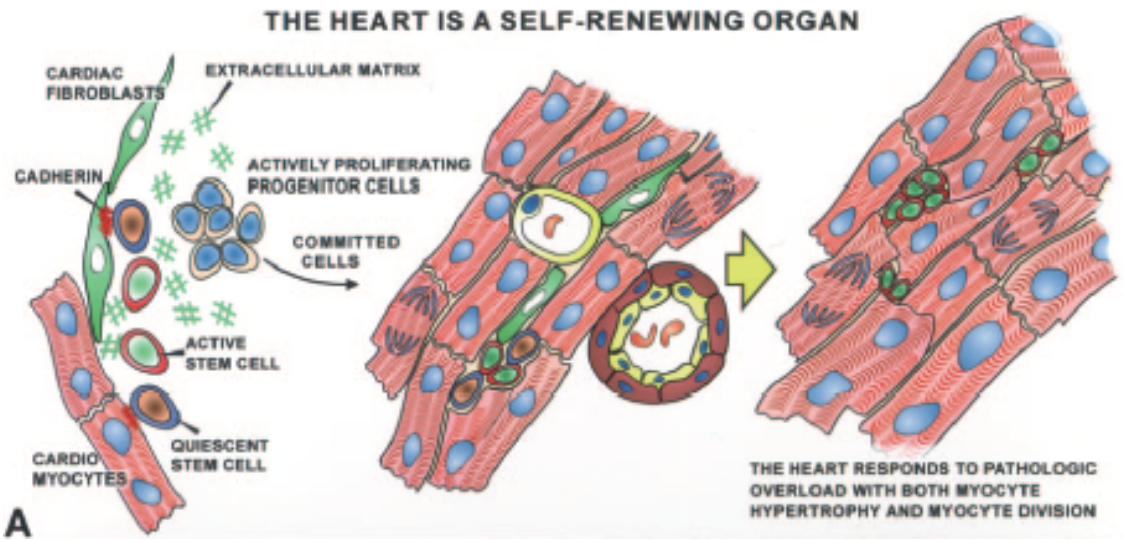
Cardiomyocyte proliferative renewal is exquisitely sensitive to stimulation by Notch signalling and Notch post-translational modification by acetylation is a powerful amplifier of this effect both in vitro and in vivo.

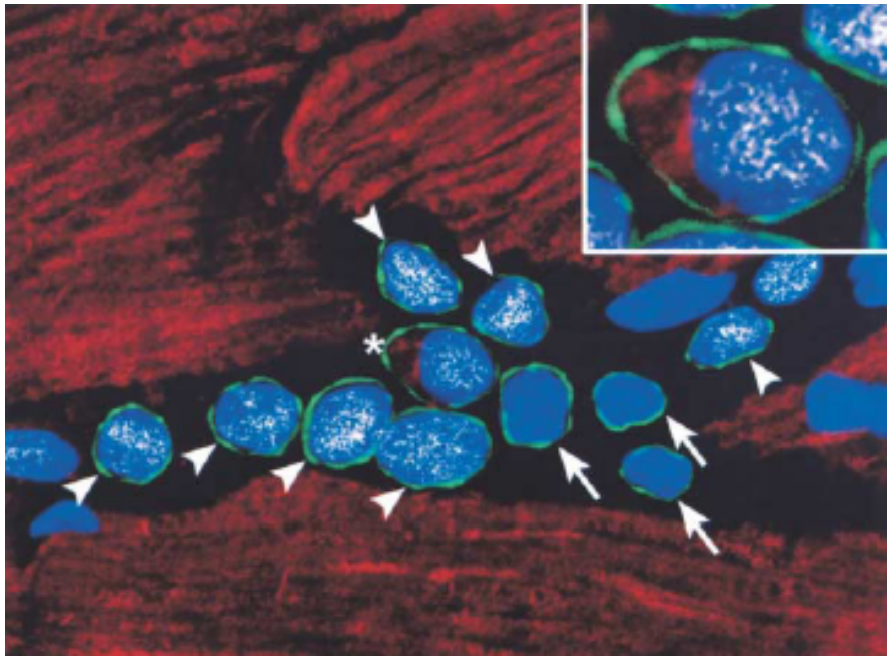
Stimulation of Notch1 pathway does not induce cardiac regeneration in adult mice after myocardial infarction due to target gene promoter methylation

Cardiac resident stem cells?

Cardiac stem cells (CSCs): do they exist?



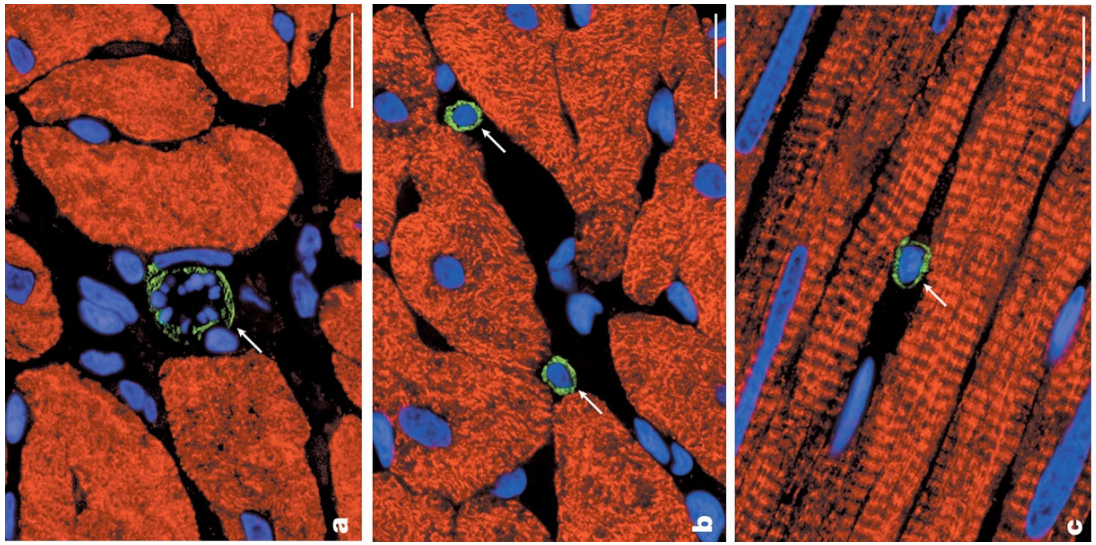


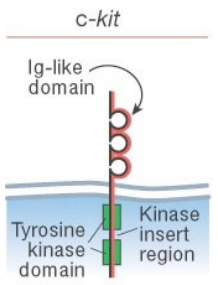


Adult cardiac stem cells are multipotent and support myocardial regeneration.
Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P.

Cardiac stem cells (CSCs): do they exist?

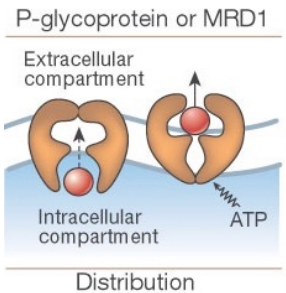
Nature, 2002





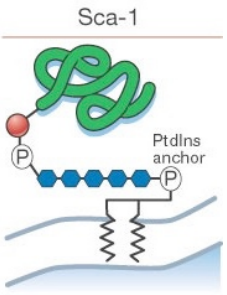
Distribution
- Melanocytes
- Mast cells
- Germ cells
- Stem cells

Functions
- Proliferation
- Migration
- Differentiation
- Secretion



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

Functions
- Transmembrane efflux pump
- Inhibition of apoptosis



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

Functions
- Cell adhesion
- Cell signalling
- T-cell activation

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

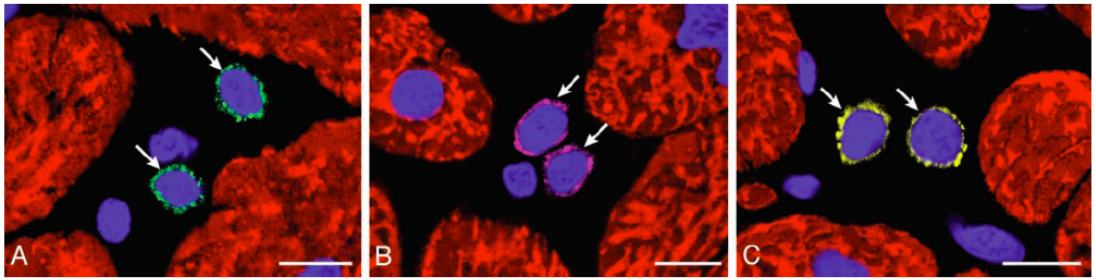
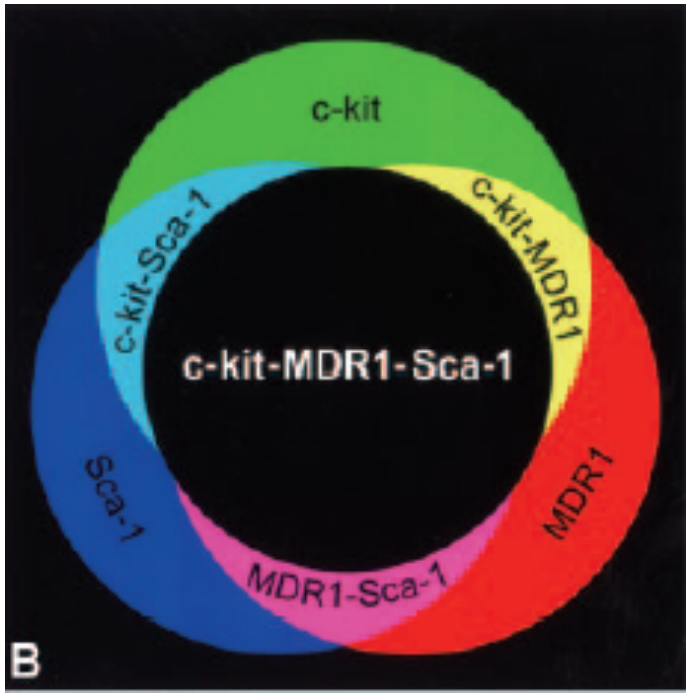
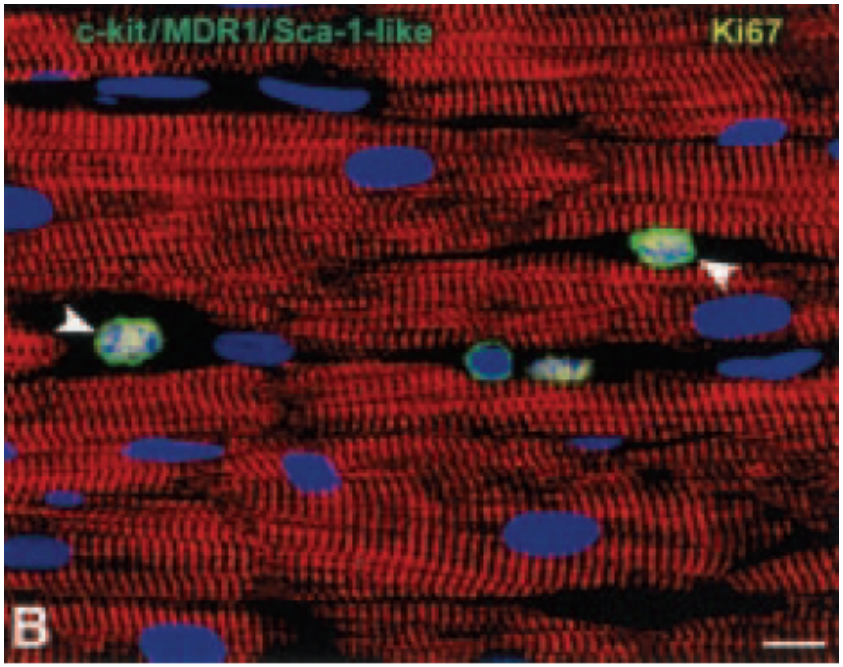
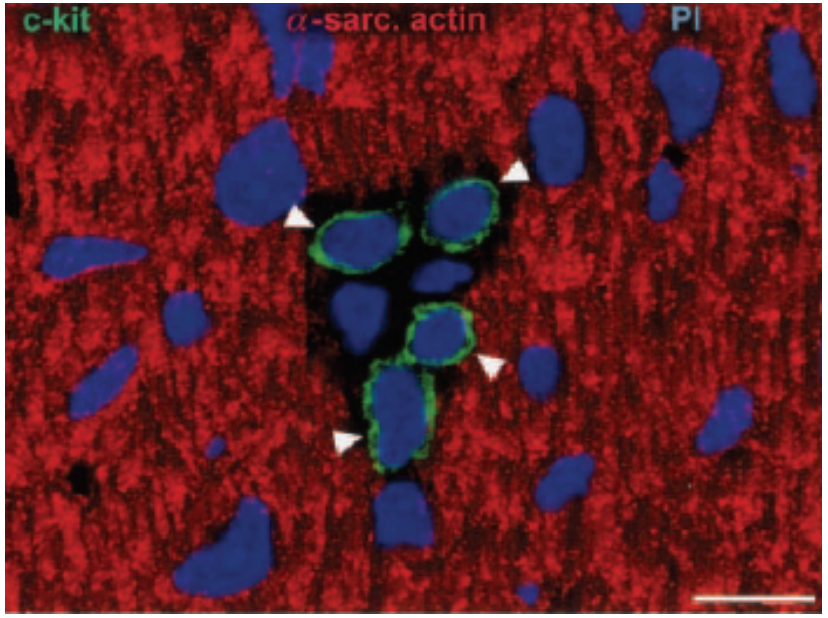


Fig. 1. Putative cardiac stem cells. Shown are detection of c-kit (*A*, green), MDR1 (*B*, purple), and Sca-1-reactive protein (*C*, yellow) in primitive cells (arrows) of hypertrophied hearts. Nuclei are stained by propidium iodide (PI; blue) and myocytes by cardiac myosin (red). (Bars = 10 μm.)







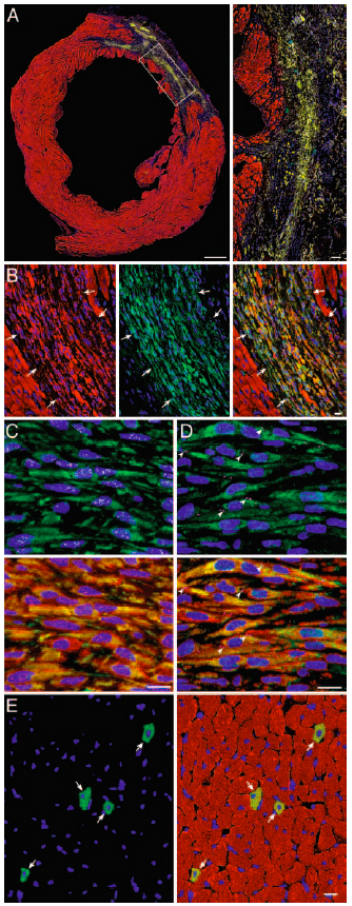
Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function

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

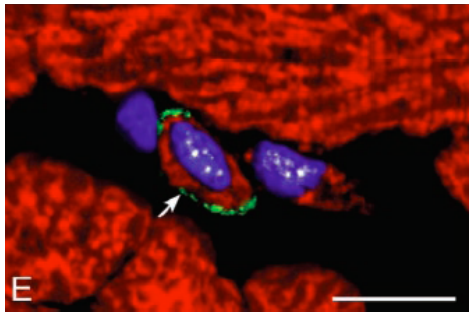
Adult cardiac stem cells are multipotent and support myocardial regeneration.

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.

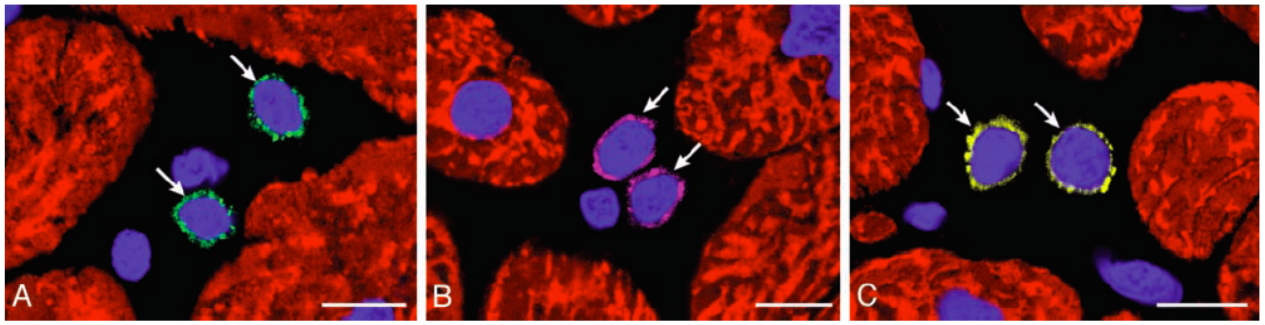
Cell, 2003 Sep 19



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*‡



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

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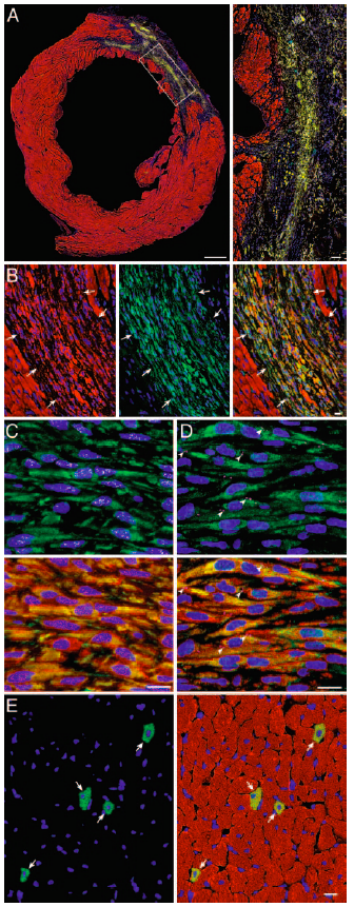
Ventricular function monitored by echocardiography

Myocardial regeneration by histology



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

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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.

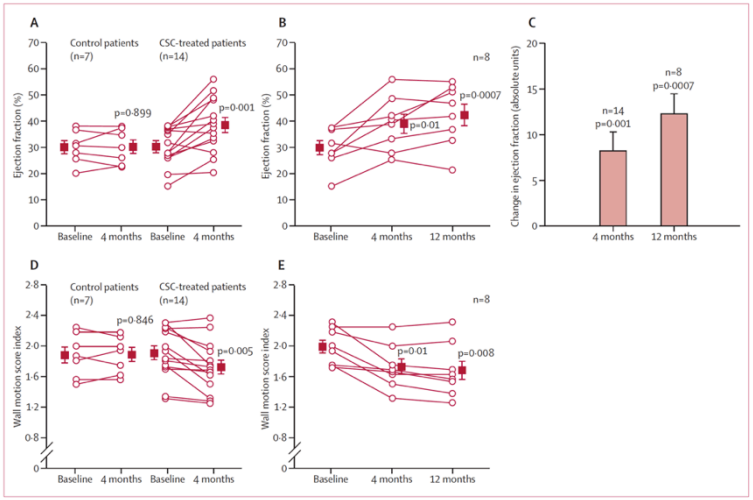


Figure 4: **Echocardiographic analysis of CSC-treated patients and controls**
(A) Left ventricular ejection fraction (measured by use of three-dimensional echocardiography) at 4 months after baseline in control and CSC-treated patients. (B) Ejection fraction at 4 months and 12 months after baseline in 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.

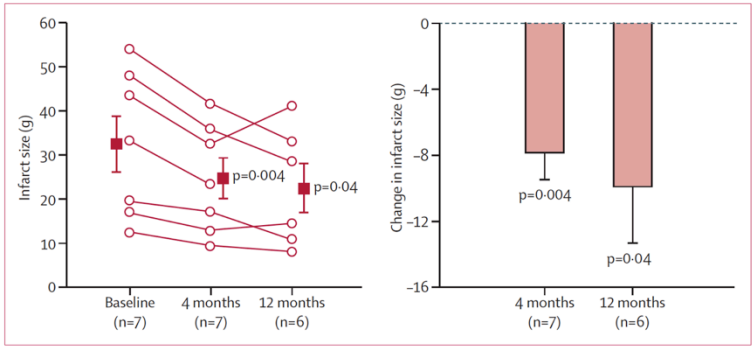
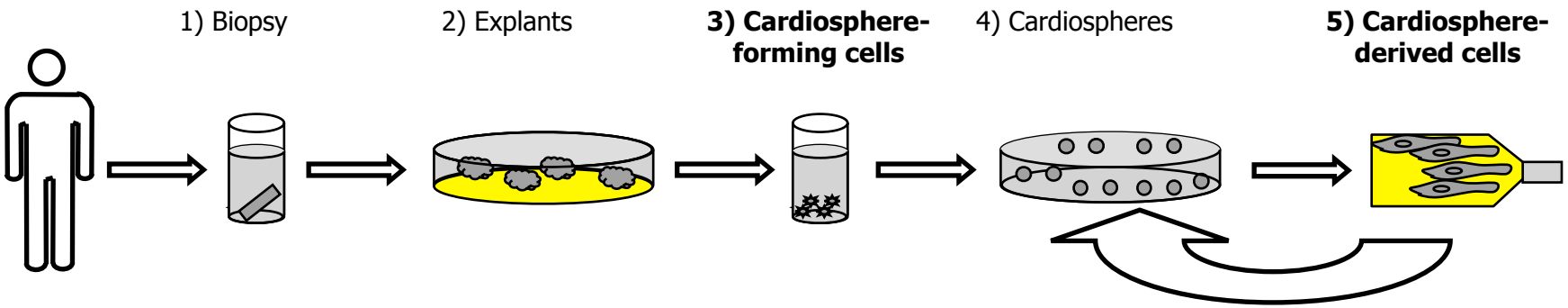
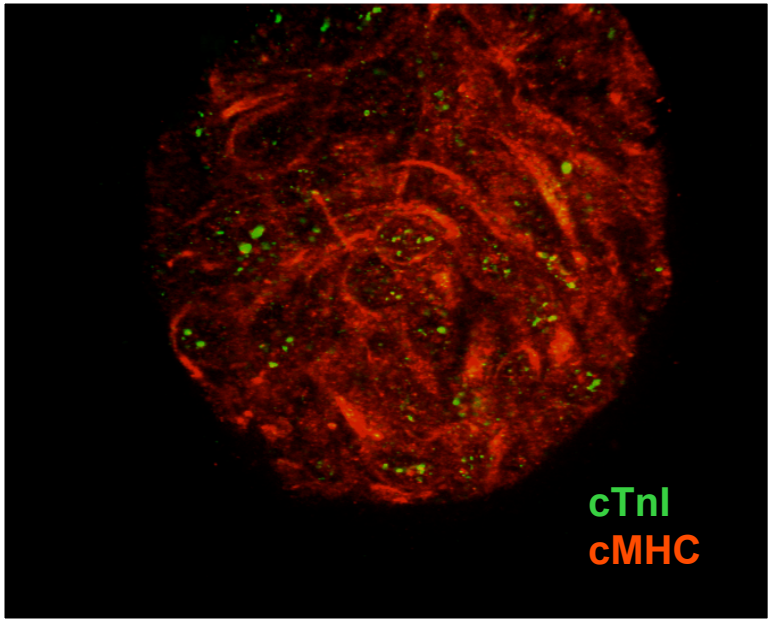


Figure 5: **Infarct size and change in infarct size at 4 months and 12 months after baseline in patients administered cardiac stem cells**
p values are reported for difference between baseline and 4 months and between baseline and 12 months. Boxes and bars represent the mean values and error bars represent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

Cardiospheres





Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial

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Summary

Background Cardiosphere-derived cells (CDCs) reduce scarring after myocardial infarction, increase viable myocardium, and boost cardiac function in preclinical models. We aimed to assess safety of such an approach in patients with left ventricular dysfunction after myocardial infarction.

Methods In the prospective, randomised CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction (CADUCEUS) trial, we enrolled patients 2–4 weeks after myocardial infarction (with left ventricular ejection fraction of 25–45%) at two medical centres in the USA. An independent data coordinating centre randomly allocated patients in a 2:1 ratio to receive CDCs or standard care. For patients assigned to receive CDCs, autologous cells grown from endomyocardial biopsy specimens were infused into the infarct-related artery 1·5–3 months after myocardial infarction. The primary endpoint was proportion of patients at 6 months who died due to ventricular tachycardia, ventricular fibrillation, or sudden unexpected death, or had myocardial infarction after cell infusion, new cardiac tumour formation on MRI, or a major adverse cardiac event (MACE; composite of death and hospital admission for heart failure or non-fatal recurrent myocardial infarction). We also assessed preliminary efficacy endpoints on MRI by 6 months. Data analysers were masked to group assignment. This study is registered with ClinicalTrials.gov, NCT00893360.

Findings Between May 5, 2009, and Dec 16, 2010, we randomly allocated 31 eligible participants of whom 25 were included in a per-protocol analysis (17 to CDC group and eight to standard of care). Mean baseline left ventricular ejection fraction (LVEF) was 39% (SD 12) and scar occupied 24% (10) of left ventricular mass. Biopsy samples yielded prescribed cell doses within 36 days (SD 6). No complications were reported within 24 h of CDC infusion. By 6 months, no patients had died, developed cardiac tumours, or MACE in either group. Four patients (24%) in the CDC group had serious adverse events compared with one control (13%; p=1·00). Compared with controls at 6 months, MRI analysis of patients treated with CDCs showed reductions in scar mass (p=0·001), increases in viable heart mass (p=0·01) and regional contractility (p=0·02), and regional systolic wall thickening (p=0·015). However, changes in end-diastolic volume, end-systolic volume, and LVEF did not differ between groups by 6 months.

Interpretation We show intracoronary infusion of autologous CDCs after myocardial infarction is safe, warranting the expansion of such therapy to phase 2 study. The unprecedented increases we noted in viable myocardium, which are consistent with therapeutic regeneration, merit further assessment of clinical outcomes.

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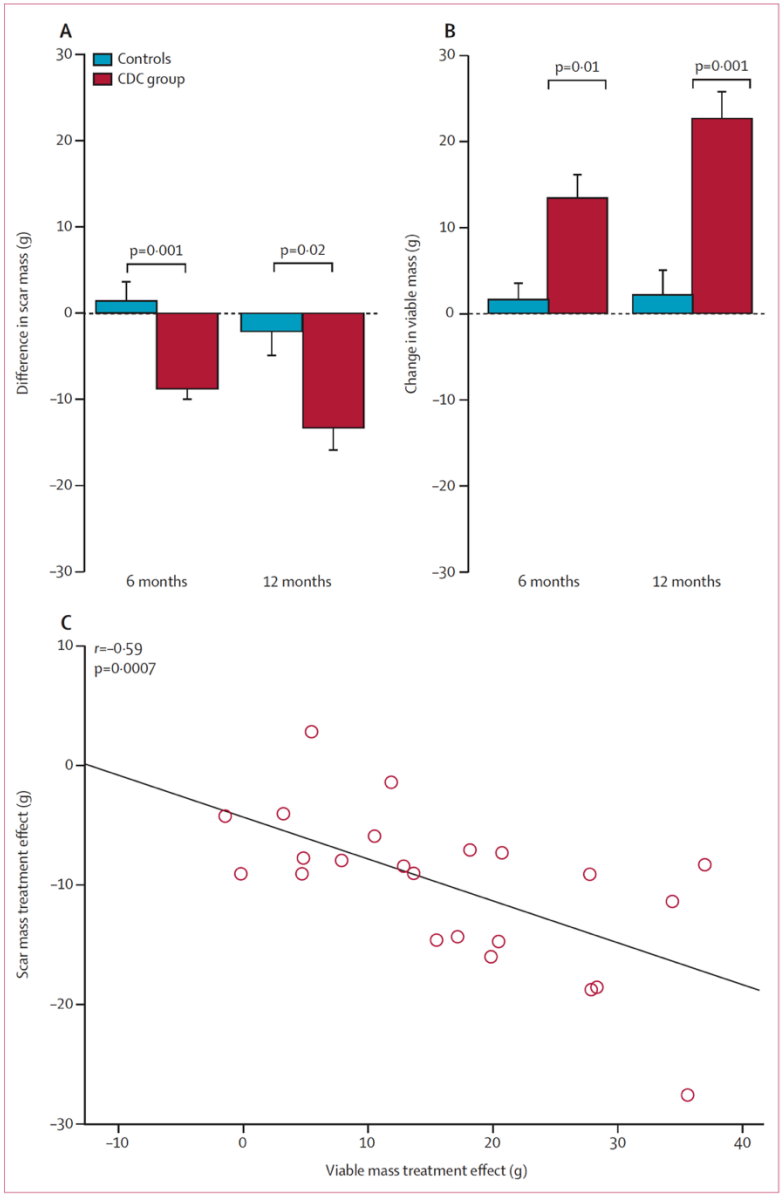

Figure 4: **Scar mass and viable left ventricular mass on MRI**
We noted decreases in scar mass and increases in viable mass on MRI in patients treated with CDCs but not controls. (A) Differences in scar mass between groups from baseline to 6 months or 12 months. (B) Differences in viable left ventricular mass from baseline to 6 months or 12 months. (C) Correlation between the change in scar mass and the change in viable mass in individual patients at 6 and 12 months compared with baseline. CDC=cardiosphere-derived cell.

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Notch1 regulates the fate of cardiac progenitor cells

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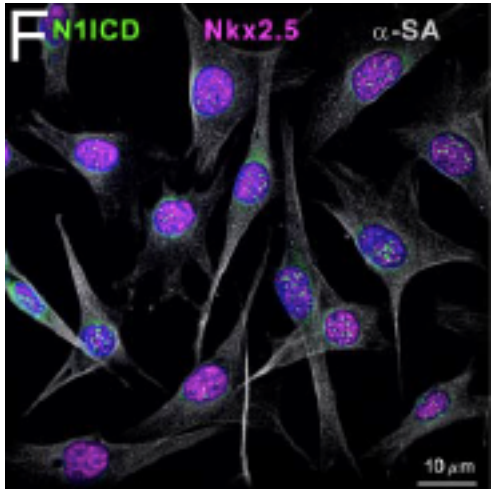

- Cardiac progenitor cells (CPCs) in the niches express Notch1 receptor, and the supporting cells exhibit the Notch ligand Jagged1.
- N1ICD and RBP-Jk form a protein complex, which in turn binds to the Nkx2.5 promoter initiating transcription and myocyte differentiation.

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