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# Innate Regeneration in the Aging Heart: Healing From Within

**Piero Anversa, MD** and **Annarosa Leri, MD**
Department of Anesthesia, Department of Medicine, and Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

## Abstract

The concept of the heart as a terminally differentiated organ incapable of replacing damaged myocytes has been at the center of cardiovascular research and therapeutic development for the past 50 years. The progressive decline in myocyte number as a function of age and the formation of scarred tissue after myocardial infarction have been interpreted as irrefutable proofs of the postmitotic characteristic of the heart. However, emerging evidence supports a more dynamic view of the heart in which cell death and renewal are vital components of the remodeling process that governs cardiac homeostasis, aging, and disease. The identification of dividing myocytes in the adult and senescent heart raises the important question concerning the origin of these newly formed cells. In vitro and in vivo findings strongly suggest that replicating myocytes derive from lineage determination of resident primitive cells, supporting the notion that cardiomyogenesis is controlled by activation and differentiation of a stem cell compartment. It is the current view that the myocardium is an organ permissive of tissue regeneration mediated by exogenous and endogenous progenitor cells.

A fundamental issue pertaining to the ability of the heart to sustain cardiac diseases of ischemic and nonischemic origin is whether myocardial regeneration occurs in the adult organ or whether this growth adaptation is restricted to prenatal life, severely limiting the response of the myocardium to pathologic stresses.[1] The concept of the heart as a terminally differentiated organ incapable of replacing damaged myocytes has been at the center of cardiovascular research for the past 50 years.[2–6] The accepted view has been that the postnatal, adult, and aging heart reacts to an increase in workload by hypertrophy of existing myocytes only. Hypertrophied cardiomyocytes express the senescence-associated proteins $p16^{INK4a}$ and $p53^{7–11}$ and are prone to undergo apoptosis and necrosis, possibly because of the high intracellular content of reactive oxygen species. Cell death is restricted to $p16^{INK4a}$-positive myocytes, but the process is inefficient, resulting in the progressive accumulation of poorly functional myocytes[9]; they are characterized by changes in the expression of contractile protein isoforms and by profound alterations in intracellular calcium cycling and electrical properties.[12–14] These defects, together with myocyte loss and the development of foci of myocardial scarring, inevitably contribute in the long term to the onset of ventricular dysfunction and its progression to overt failure.



**Correspondence:** Address to Piero Anversa, MD, Department of Anesthesia, Department of Medicine, and Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St, Boston, MA 02115 (panversa@partners.org).

## REGENERATIVE CAPACITY OF ADULT ORGANS

The ability of stem cells to continuously replenish the compartment of undifferentiated and lineage-committed cells is a typical property of higher organisms with mitotic soma. In these cases, tissues are capable of renewal and repair, and the extent of regeneration positively correlates with animal lifespan.[15,16] The reduced longevity of lower organisms, including *Caenorhabditis elegans* and D*rosophila*, is linked to the lack of regenerative potential of their postmitotic tissues in adulthood.[15] Dying cells cannot be replaced, leading to a rapid decline in organ function. Conversely, cell turnover by proliferation and commitment of resident progenitor cells is active in mammals, and old injured cells lost as a result of normal wear and tear may be restored by new, better-functioning cells. However, regeneration is hampered in the presence of damage, which creates a barrier to restitutio ad integrum and promotes a repair process leading to the formation of a scar.[1,17] The scar does not possess the biochemical, physical, and functional properties of the intact tissue, negatively affecting the remodeling of the diseased heart, a critical determinant of cardiac performance.[17,18]

Despite the presence of resident adult stem cells, the repair mechanism triggered by ischemic injury results in collagen accumulation and scarring. This phenomenon is not restricted to the heart but occurs in all the organs, whether their parenchymal cells are highly proliferating, slowly cycling, or terminally differentiated. This intrinsic limitation was interpreted as the inability of the adult myocardium to generate de novo cardiomyocytes.[16,17] However, the outcome of infarction is essentially identical throughout the organism. Occlusion of a major conductive artery or a large branch results in loss of tissue in the skin, kidney, intestine, brain, liver, and reproductive organs in a manner identical to the heart.[17,19–23] This common outcome is conditioned by 2 crucial factors: (1) stem cells in the infarct die as do all other cells deprived of oxygen supply and (2) resident stem cells activate a local regenerative response but do not migrate from the viable tissue to the damaged area to replace the necrotic tissue (Figure 1).

The main factors that dictate the evolution of a lesion into scarring rather than regeneration have not been identified with certainty. Some insights come from embryonic development. Repair in embryos is rapid, efficient, and scar free.[17] Skin wounds in the early mammalian embryo heal with restitutio ad integrum, whereas wounds in late gestational fetuses and adult mammals result in scarring. This difference may be related to the intrinsic characteristics of embryonic fibroblasts or to external stimuli. Fibroblasts in embryos are less prone to synthesize and release collagen, attenuating the amount of fibrosis after injury.[17,24] The presence of hyaluronic acid and fibronectin in the amniotic fluid may also interfere with scar formation during wound healing. Moreover, the inflammatory response is attenuated; a low number of poorly differentiated inflammatory cells accumulate in the region of damage, and the growth factors present at the site of healing are different from those in the adult organism.[17,25–27] Scar-free regeneration of damaged organs is well known in amphibians and zebra fish.[17,28] Epimorphic regeneration corresponds to the complete restoration of organs after the development of body plans and cellular differentiation.[16,17,29] However, this occurs only in invertebrates and lower vertebrates, including urodeles and fish. Conversely, mammals retain limited capacity for spontaneous regeneration in the adult liver and infant fingertips,[16,17,29,30] suggesting that during evolution animals gradually lose their ability to reconstitute damaged organs. Characterization of the processes that drive regeneration in lower vertebrates may provide some information for the implementation of regenerative strategies in humans. Regeneration of the adult heart occurs in newts and zebra fish and has been claimed to involve the reentry of cardiomyocytes into the cell cycle[31,32] or dedifferentiation.[11]

Dedifferentiation refers to a regression of a mature cell in its own lineage; this process is typically coupled with attainment of the proliferative capacity. Dedifferentiation is considered the natural regenerative response to cardiac injury in zebra fish, newts, and planaria. After disassembly of the sarcomeric contractile apparatus, which impedes cytokinesis, differentiated cardiomyocytes replicate to reconstitute up to 20% of the zebra fish ventricle after resection. 11 By cre/lox cell lineage analysis, the regenerating myocardium has been shown to derive primarily from a subpopulation of GATA4-positive cardiomyocytes rather than from nonmyocyte sources, such as stem cells. The poorly organized sarcomeric structure of replicating GATA4-positive myocytes has led to the hypothesis that dedifferentiation constitutes the primary regenerative step.[32] However, the failure in the induction of the early myocyte commitment genes Nkx2.5 and Hand2 in the replicating myocytes indicate that the cells of origin underwent limited dedifferentiation or, most likely, that replicating GATA4-positive cardiomyocytes correspond to cells at the late stage of commitment of stem cell differentiation. In the mouse heart, Nkx2.5 expression precedes GATA4 in transit-amplifying cardiomyocytes originated by lineage specification of cardiac stem cells (CSCs) (Figure 2).[33]

A comparable regenerative response has been observed after surgical resection of the apex of the left ventricle in the neonatal mouse heart.[34] Again, cardiomyocyte proliferation was considered as the cellular adaptation supporting the repair process. Genetic fate mapping strategies have been used to distinguish the contribution of preexisting myocytes and stem/progenitor cells to regeneration of the zebra fish and mouse heart. This approach, however, has several intrinsic limitations. The specificity of the promoter and its transactivated protein for the population of cells to be studied is an essential prerequisite of lineage tracing. Cardiac stem cells engineered with fluorescent constructs placed under the control of promoters encoding myocyte-specific transcription factors and sarcomeric proteins show transgene expression.[35] Although *GATA4* and α-*myosin heavy chain* promoters are generally considered to be active primarily in cardiomyocytes, the presence of the reporter gene in c-kit—positive CSCs reflects a very early stage of cardiogenic lineage commitment of these mother cells, which, after the loss of the stem cell antigen, give rise to a large compartment of transit-amplifying myocytes (Figure 3). Thus, the lack of specificity severely hampers the validity of the findings obtained with fate mapping strategies when promoters encode alleged myocyte-restricted proteins.[10,11]

The essential foundation of meticulously performed lineage tracing protocols consists of expression of the fluorescent label in the pool of cells hierarchically located upstream of the progeny to be tracked; the use of promoters regulating contractile proteins introduces a serious bias into the method. The availability of cre/lox mice carrying fluorescent tags under the control of stem cell antigens will clarify this controversial issue. Data collected in mice with spontaneous inactive mutations of c-kit and mice carrying a deletion of Sca-1 strongly suggest that stem cells contribute significantly to cardiomyogenesis during homeostasis and regulate the magnitude of myocyte regeneration after injury.[36–38] Thus, current observations in zebra fish and neonatal, adult, and old mice do not exclude that CSCs participate in myocardial growth.

Evidence of dedifferentiation, ie, reentry of terminally differentiated cardiomyocytes into the cell cycle, is based exclusively on cellular morphology. Cells characterized by an intermediate phenotype between forming and mature myocytes have arbitrarily been considered the product of dedifferentiation.[10,11,39] However, the lack of structural markers typical of a given cell type is not by itself indicative of the developmental stage of the cell[10]; it is impossible to define in vivo whether the cell of interest is undergoing differentiation or whether it is in the process of reverting to an earlier immature state. As discussed previously herein, the possibility of dedifferentiation was raised by observations conducted in

vertebrates with exceptional regenerative abilities. This conclusion, however, has recently been challenged. During salamander limb reconstitution, cells from muscle, bone, cartilage, nerve sheath, and connective tissues are believed to dedifferentiate into a pool of proliferating cells known as the regeneration blastema.[40–42] But the possibility that preexisting unipotent or multipotent stem cells constitute the pool of proliferating blastema cells[43] questions the notion of dedifferentiation and subsequent redifferentiation of a common pool of cells responsible for the repair of the amputated limb.[43] Similarly, the hypothesis has been raised that clonally dominant cardiomyocytes with properties similar to those of stem cells direct heart morphogenesis in zebra fish from the early stages of development to adulthood.[44]

## MYOCARDIAL REGENERATION: THE CONTROVERSY

A critical issue of cardiac pathobiology concerns whether myocardial regeneration occurs in the adult organ or whether this growth adaptation is restricted to prenatal life and ceases shortly after birth.[1–6,10,11] The concept that the heart is a postmitotic, terminally differentiated organ unable to replace its myocyte compartment has been at the center of cardiovascular research for several decades.[1–6,10,11,45] The accepted view is that the myocardium reacts to an increased workload by hypertrophy of the existing myocytes, and when this cellular response is exhausted, ventricular dysfunction supervenes. Based on this assumption, molecular cardiology has focused in the past 30 years on identification of the multiple signaling pathways regulating the activation and depression of genes implicated in the hypertrophic growth of cardiomyocytes during postnatal maturation or after abnormal pathologic states or chronological aging.[1,10,11,17,45–50] The possibility that the heart can renew its parenchymal cells has been largely dismissed, and, even today, myocardial repair is viewed with suspicion and trepidation.[40,41]

The engrained paradigm that promotes a rather uninteresting biological perspective of the developing, old, and diseased heart has been shaken by a variety of studies indicating that myocyte regeneration occurs in humans and animals after infarction,[8,51] after prolonged pressure overload,[7] and in the decompensated senescent heart.[52,53] Although some of these studies were published almost 25 years ago[54] and systematically continued to appear in the literature (for reviews, see references 10, 11, 40, and 41), the traditional establishment tends to reject this alternative notion of cardiac biology, defending a territory that was considered unwavering and immovable.

The recognition that myocyte death by apoptosis and necrosis occurs continuously in the adult heart of humans and animals[9,17,55,56] emphasizes the necessity for an equivalent degree of myocyte formation to preserve cardiac mass and function. The discovery of resident CSCs and their ability to differentiate into cardiomyocytes, vascular endothelial cells, and smooth muscle cells has provoked a rather negative response in part of the scientific community; CSCs were either ignored or claimed to lack biological significance.[5,57,58] Similarly, the documentation that hematopoietic stem cells transdifferentiate, generating cardiomyocytes and coronary vessels after infarction,[1,59,60] was immediately rejected, prompting a series of negative studies that challenged the validity of the early observations.[61,62] Some comments about the history of the heart as a postmitotic organ may be relevant for understanding the shift in paradigm required for the implementation of the novel field of regenerative cardiology.

## THE HEART AS A POSTMITOTIC ORGAN

Numerous studies of the human heart from 1850 to 1911 held the view that myocardial hypertrophy was the consequence of hyperplasia and hypertrophy of existing myocytes.[10,46,63–65] However, subsequent reports from 1921 to 1925 questioned the ability

of myocytes to proliferate, suggesting that the increase in cardiac muscle mass in the pathologic heart was the result of pure cellular hypertrophy.[10,46,66–68] The concept that myocytes cannot divide originated from difficulty in identifying mitotic figures in these cells in the absence of quantitative evaluations of myocyte size and number.[68] This conviction gained support from autoradiographic analysis of thymidine incorporation in the hearts of animals during postnatal growth[69] and after pressure overload.[70–72] DNA synthesis in myocyte nuclei was not detected, prompting the conclusion that the heart survives and exerts its function until the death of the organism with the same cells that are present at birth.[73]

Accordingly, ventricular myocytes in humans are terminally differentiated cells, and their life-span corresponds to the lifespan of the organism. The number of myocytes attains an adult value a few months after birth,[74] and the same myocytes are believed to contract 70 times per minute throughout life. Because a certain fraction of the population reaches 100 years of age or more, an inevitable consequence of this paradigm is that cardiac myocytes are immortal, functionally and structurally.[1,10,17,46]

In view of this conviction, we discuss myocardial aging and the potential mechanisms involved in acquisition of the cardiac senescent phenotype. In addition, we address the differences in the rate of myocyte turnover claimed by various laboratories and the potential role that resident human CSCs have in organ homeostasis and regeneration as a function of age. This information is critical to define whether the aging myopathy is a stem cell disease, and strategies can be developed to modify the progression of ventricular dysfunction in the old heart.[75]

## MYOCARDIAL AGING IN HUMANS

The aging myopathy typically manifests itself with diastolic dysfunction and preserved ejection fraction (EF).[76] More than 50% of patients with heart failure have normal or near-normal EF, and the incidence and prevalence of this condition increases with age.[76–80] Although the claim is commonly made that age-associated physiologic changes predispose older adults to development of heart failure with a normal EF, the etiology of diastolic heart failure is unknown. The difficulty in defining myocardial aging and the mechanisms involved further complicates recognition of the cellular processes underlying impaired diastolic relaxation. Morbidity and mortality for chronic heart failure continue to increase and parallel the extension in median life-span of the population,[81] pointing to aging as the major risk factor of the human disease. At present, there are 5.1 million patients with chronic heart failure in the United States alone, with an incidence of 670,000 new cases per year,[82] and most individuals with chronic heart failure are 65 years or older.

Currently, we have little understanding of the etiology of myocardial aging. Rarely, studies in animals and humans have considered aging as an independent process and time as the major cause of the manifestations of the aging myopathy. Aging has been interpreted as a variable, which cooperates with a variety of diseases, to define the old heart.[83–85] Only occasional reports have characterized cardiac aging in humans independent of concomitant pathologic states.[9,53,86] Myocardial aging differs in women and men, emphasizing a sex difference in the adaptive response of the myocardium to physiologic aging alone or together with cardiovascular diseases. Diastolic heart failure predominates in old women,[82] and, although it is frequently observed in men, systolic and diastolic function is impaired more commonly in old men. The senescent male heart has an attenuated ability to sustain increases in pressure load, possibly mediated by the decrease in the number of myocytes early in life, which increases with age.[8,87,88] Conversely, myocyte number remains constant in women up to nearly 90 years of age,[88] and this difference may explain the higher incidence of systolic-diastolic heart failure in men.[89]

## Myocardial Aging and CSCs

The concept of myocardial aging is complex, and this difficulty is dictated by the identification of parameters that define the consequences of time alone on the heart independent of cardiac and noncardiac pathologic abnormalities. Cardiac aging has been confounded by the notion that the heart is a postmitotic organ characterized by a predetermined number of myocytes, which is established at birth and preserved until the death of the organ and organism.[2–5] According to this old paradigm, the generation of myocytes is restricted to the fetal neonatal heart, and organ hypertrophy in the adult occurs only by myocyte enlargement. Based on this premise, cellular, organ, and organism age coincide; at any given time, the heart is composed of a homogeneous population of myocytes of identical age. Because of this static view, aging has been construed as a time-dependent process that interacts with ischemic injury, hypertension, diabetes, and other disorders, which together define the clinical phenotype.[75,82–84]

The discovery that stem cells live in the heart and differentiate into the various cardiac cell lineages has dramatically changed our understanding of myocardial biology.[90–99] The CSCs are multipotent cells capable of generating cardiomyocytes and coronary vessels, providing the missing link between the identification of small dividing myocytes and the uncertainty concerning the origin of these repopulating cells (see references 10, 17, 100, and 101). Most importantly, this information has imposed a reconsideration of our view of cardiac homeostasis and myocardial aging. A new paradigm has emerged: the heart is a self-renewing organ characterized by resident CSCs stored in niches (Figure 4). The niches control the physiologic turnover of cardiac cells and the growth, migration, and commitment of CSCs that leave the niches, replacing dying cells in the myocardium.[33,102–104] This information has imposed a reconsideration of myocardial aging.

In the past, myocardial aging has been attributed to genetic modifications, accumulation of oxidative DNA damage, mutations in mitochondrial DNA, and telomeric dysfunction, triggering growth arrest, cellular senescence, and death.[105–109] Although these determinants of organ aging are all valid, the cellular target in the heart may have changed. Aging effects on myocytes, smooth muscle cells, endothelial cells, and fibroblasts may be only an epiphenomenon dictated by CSC aging. Organ aging may reflect a process in which temporal alterations in CSC behavior determine the phenotypic properties of the myocardium. Myocardial aging may be regulated by time-dependent changes in the biology of CSCs. Growth defects at the level of the controlling cell, ie, the CSC, may perturb the structural integrity and function of the old heart. Old myocytes nested in proximity to CSCs may secrete a variety of inflammatory molecules and factors favoring cellular senescence and apoptosis, altering the physiologic behavior of stem cell niches in the myocardium.[110] A typical example is provided by the enhanced synthesis and release of angiotensin II from hypertrophied cardiomyocytes,[111–114] resulting in the activation of angiotensin II type 1 receptors present in CSCs.[56,115] This process is commonly coupled with initiation of the apoptotic pathway. The effects of time on CSCs are critical for understanding the mechanisms conditioning CSC senescence, myocyte and vascular aging, and, ultimately, cardiac aging and organism lifespan.

The telomere-telomerase axis is a determinant of cellular aging. Human CSCs (hCSCs) have telomeres of approximately 10 kilobase pairs (kbp), and with each cell division there is a loss of approximately 130 base pairs of telomeric DNA.[92] Telomere length reflects the past replicative history and cumulative oxidative DNA damage occurring during the life cycle of the cell.[105,107,109] Telomerase activity delays but cannot prevent telomere erosion, which is mediated by down-regulation of telomerase, formation of reactive oxygen species, and loss of telomere-related proteins.[106] When telomere length reaches 1.5 to 2.0 kbp, replicative senescence and irreversible growth arrest occur.[116] The presence of telomere-induced

dysfunction foci in hCSCs is coupled with expression of the senescence-associated proteins $p16^{INK4a}$ and p53[117] and, eventually, initiation of the apoptotic pathway (Figure 5).

Growth activation of hCSCs with a wide range of telomere lengths leads to a progeny with distinct phenotypes. Chronological age results in telomere attrition of hCSCs, and these cells form myocytes that rapidly express $p16^{INK4a}$.[9] Daughter cells inherit the shortened telomeres of the maternal cells, and after a few rounds of division and terminal differentiation, they typically show an old cell phenotype.[9,56] Telomere length in hCSCs and human myocytes shows a consistent pattern; if hCSCs exhibit long telomeres, the myocytes formed possess comparable telomere length. Conversely, hCSCs with short telomeres generate a myocyte progeny with severe telomere attrition. This phenomenon affects the aging female and male human heart; however, the proportion of hCSCs/myocytes with short telomeres is higher in men than in women, and the fraction of hCSCs/myocytes with long telomeres is larger in women than in men.[9] Telomere length in hCSCs defines cellular aging independent of chronological age and organism lifespan. Oncogenic stress[118]may be viewed as an additional stimulus that accelerates senescence in amplifying myocytes. Oncogenic proliferative signals are coupled with several growth inhibitory responses generally seen with the induction of cellular aging and activation of the endogenous cell death pathway.[119] Insulinlike growth factor 1 delays cardiac aging and rescues the infarcted myocardium experimentally,[55,56,119,120] although it fails to initiate the division of terminally differentiated postmitotic myocytes. This parenchymal cell compartment may react with abortive mitosis[121] or the formation of anaphase bridges.

## Function of hCSCs

Human beings up to 104 years of age possess a significant number of hematopoietic stem cells with long telomeres and remarkable growth reserve that, however, are in a quiescent state.[9] Conversely, most activated hCSCs in the senescent myocardium have short telomeres that are inherited by the specialized progeny. In the young healthy heart, the asymmetrical kinetics of stem cell growth efficiently preserves organ homeostasis. The structural and functional decline of the old and diseased heart may be coupled with defects in the hierarchical growth of the organ, suggesting that quantitative and qualitative alterations occur in resident hCSCs or in the pool of transit-amplifying cardiomyocytes. Both possibilities have been documented in stem cell–regulated organs.

The number of epithelial stem cells in the small intestine is gradually reduced with age,[122] and the function of hematopoietic stem cells changes with time.[123] Whether aging of the skin is conditioned by a decreased frequency of epidermal stem cells or by alterations in the kinetics of the transit-amplifying cell pool remains controversial.[122,124] Similarly, the environmental and cell-autonomous mechanisms that maintain “young” hCSCs in a state of long-term quiescence remain to be identified. However, the existence of a pool of hCSCs with intact telomeres, 8 to 12 kbp, in senescent female and male hearts and in explanted hearts[9,117] is of great clinical relevance. This category of hCSCs with high growth reserve is expected to generate a young myocyte progeny in the failing and senescent heart. Because each division of hCSCs results in the loss of approximately 130 base pairs of telomeric DNA,[93] an extremely large number of cardiomyocytes can be formed by these cells before critical telomeric shortening and growth arrest occur. From a clinical perspective, the recognition that a subset of telomerase-competent hCSCs with long telomeres persists at all ages and with chronic heart failure has raised the possibility that autologous cell-based therapy may be feasible in patients with severe ventricular dysfunction. Recently, a method has been developed to isolate this compartment of functionally competent hCSCs from endomyocardial biopsies of patients undergoing cardiac transplant or left ventricular assist device implantation.[125] After in vitro amplification, a clinically relevant number of hCSCs with high myogenic and vasculogenic potential was obtained. Expanded hCSCs possess a

significant growth reserve as documented by the short population doubling time, high telomerase activity, and relatively long telomeres.

The phase 1 trial SCIPIO (Stem Cell Infusion in Patients with Ischemic cardiOmyopathy) involves the delivery of autologous c-kit–positive lineage-negative hCSCs for the treatment of severe chronic heart failure of ischemic origin.[126,127] Patients with EF lower than 40% at 4 months after coronary artery bypass grafting were enrolled in the treatment and control groups. Treated patients received a single intracoronary infusion of 1 million autologous hCSCs. The primary end point was short-term treatment safety, and the secondary end point was efficacy. No hCSC-related adverse effects were reported. In 14 CSC-treated patients who were analyzed, EF increased from 30% to 38% at 4 months after infusion. In contrast, 7 control patients, during the corresponding interval, did not show any change in this functional parameter. The beneficial effects of CSCs were even more pronounced at 1 year. In 7 treated patients, infarct size decreased 24% and 30% at 4 and 12 months, respectively.[126,127] These initial results are encouraging and warrant further studies.

## CONCLUSION

The human heart is a highly dynamic organ regulated by a pool of resident hCSCs that modulate cardiac homeostasis and condition organ aging. Hopefully, recent findings will resolve the debate that has divided the scientific community into strong opponents and passionate supporters of the regenerative potential of the human heart. A common ground can now be found to translate this different perspective of cardiac biology into the development of novel strategies for the management of the human disease.

## Abbreviations and Acronyms

| | |
|---|---|
| **CSC** | cardiac stem cell |
| **EF** | ejection fraction |
| **hCSC** | human cardiac stem cell |

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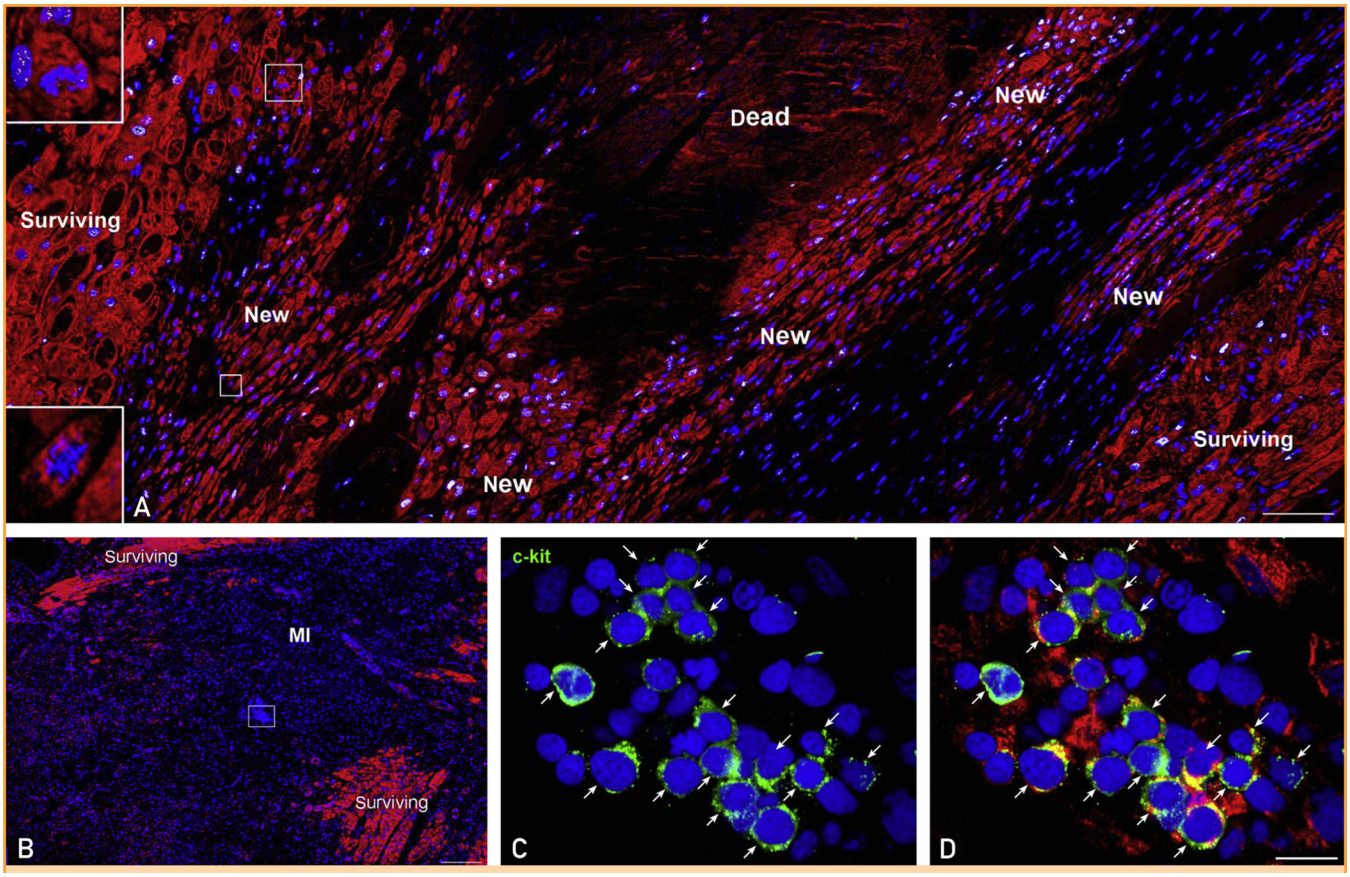
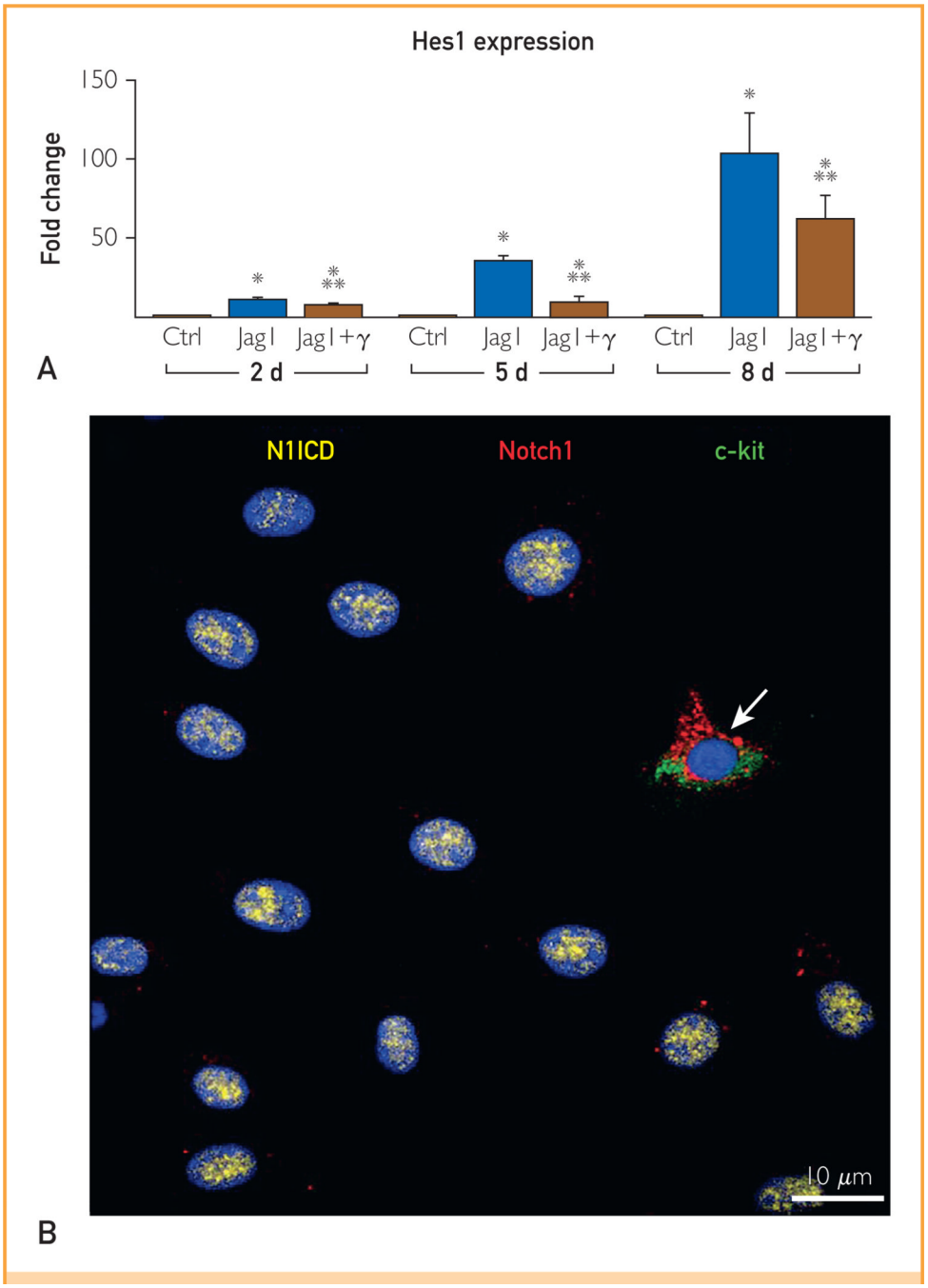



**FIGURE 1.**

Heart failure and myocardial regeneration. A, Area of regenerating myocardium in the infarct. Proliferating, small, developing myocytes are positive for the cell-cycle marker MCM5 (white). Myocytes are labeled by cardiac myosin (red) and nuclei by DAPI (blue). Two small myocytes in mitosis are shown in the insets (magnification 300×). B, The cluster of cells included in a rectangle in the middle of an acute infarct is shown at higher magnification in C and D. These cells express c-kit (green, arrows) and at times cardiac myosin (red; D), reflecting undifferentiated cardiac stem cells and early committed progenitors, respectively. MI = myocardial infarction. Scale bars: A and B, 100 μm; C and D, 10 μm.



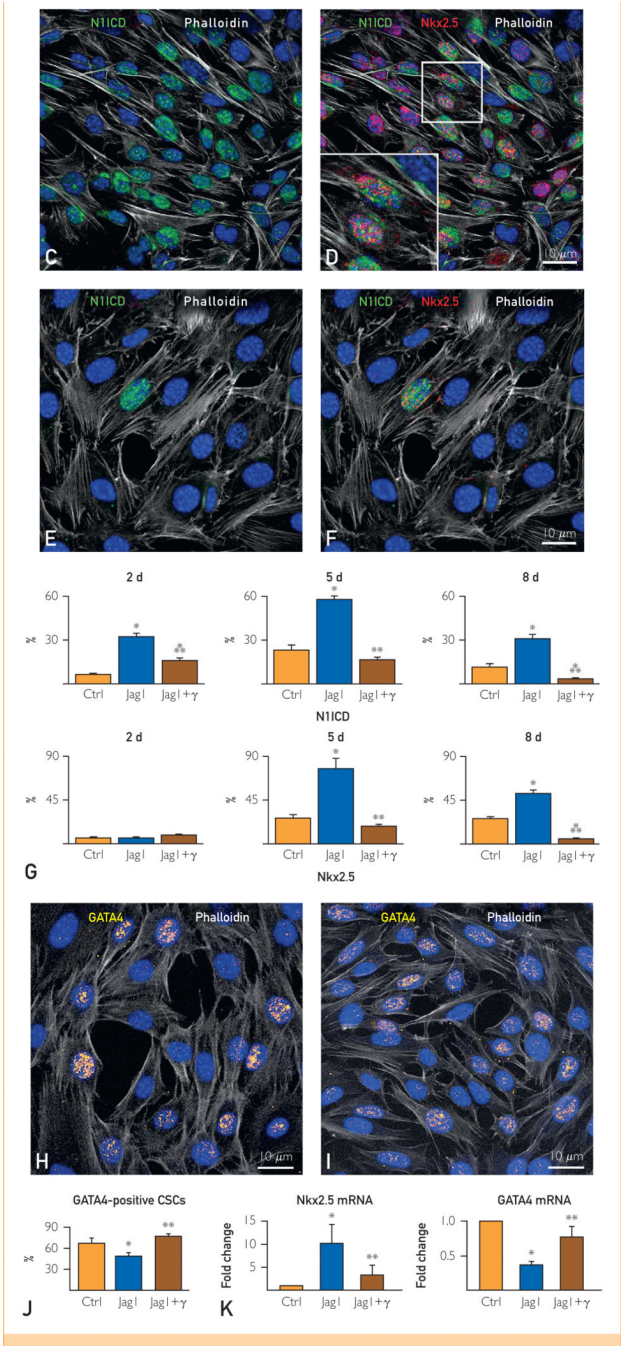



**FIGURE 2.**

Notch1 up-regulates Nkx2.5 but not GATA4 during early commitment of cardiac stem cells (CSCs). A, *Hes1* transcript was measured in CSCs at 2, 5, and 8 days under control conditions (Ctrl) and in the presence of Jagged1 (Jag1) and Jag1 and γ-secretase inhibitor (Jag1 + γ). *Hes1* quantity is shown as fold changes vs the corresponding Ctrl. B, After Jag1 stimulation, CSCs are c-kit negative and display nuclear localization of the active fragment of Notch1 (Notch1 intracellular domain [N1ICD], yellow). One CSC continues to express c-kit (green) together with the extracellular domain of the Notch1 receptor (red, arrow). C and D, Jag1-stimulated CSCs express N1ICD (green) together with Nkx2.5 (red). The area included in the square is shown at higher magnification in the inset (magnification: 1000×).

E and F, After γ-secretase inhibition, Jag1-stimulated CSCs are negative for N1ICD and Nkx2.5. G, Percentage of CSCs positive for N1ICD and Nkx2.5 at 2, 5, and 8 days. H and I, The expression of GATA4 (yellow) is shown in CSCs stimulated by Jag1 in the (H) absence and (I) presence of γ-secretase inhibitor. J, Percentage of CSCs positive for GATA4 at 5 days. K, *Nkx2.5* and *GATA4* transcripts were analyzed in CSCs at 5 days. mRNA = messenger RNA. **P*<.05 vs Ctrl. ***P*<.05 vs Jag1. ***Statistically different vs both Ctrl and Jag1. Data are shown as mean ± SD.

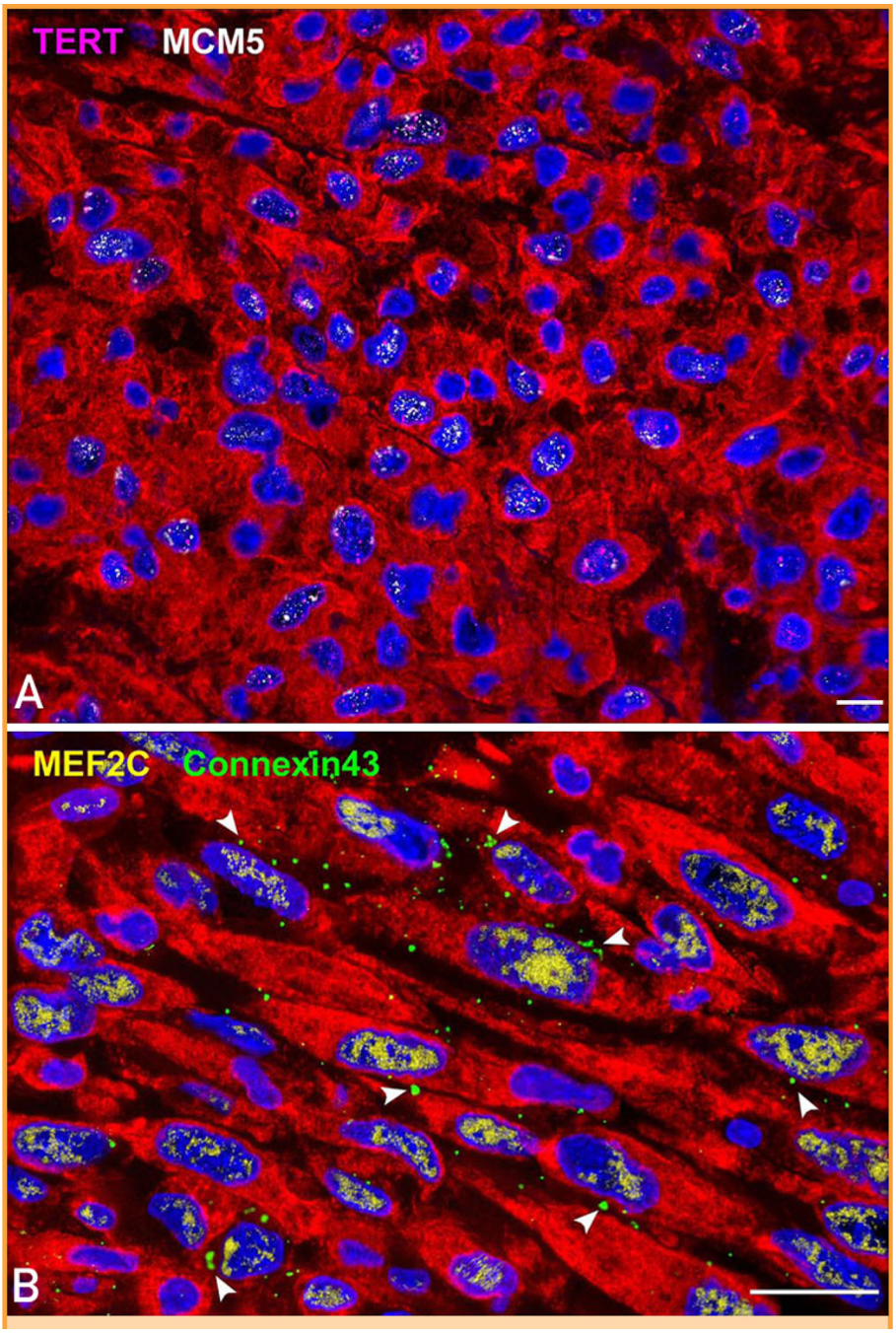



**FIGURE 3.**

Transit-amplifying myocytes. Small developing myocytes in the infarct are positive for (A) telomerase (magenta) and MCM5 (white) and for (B) MEF2C (yellow) and connexin 43 (green, arrowheads). TERT = telomerase reverse transcriptase. Scale bars, 10 mm.

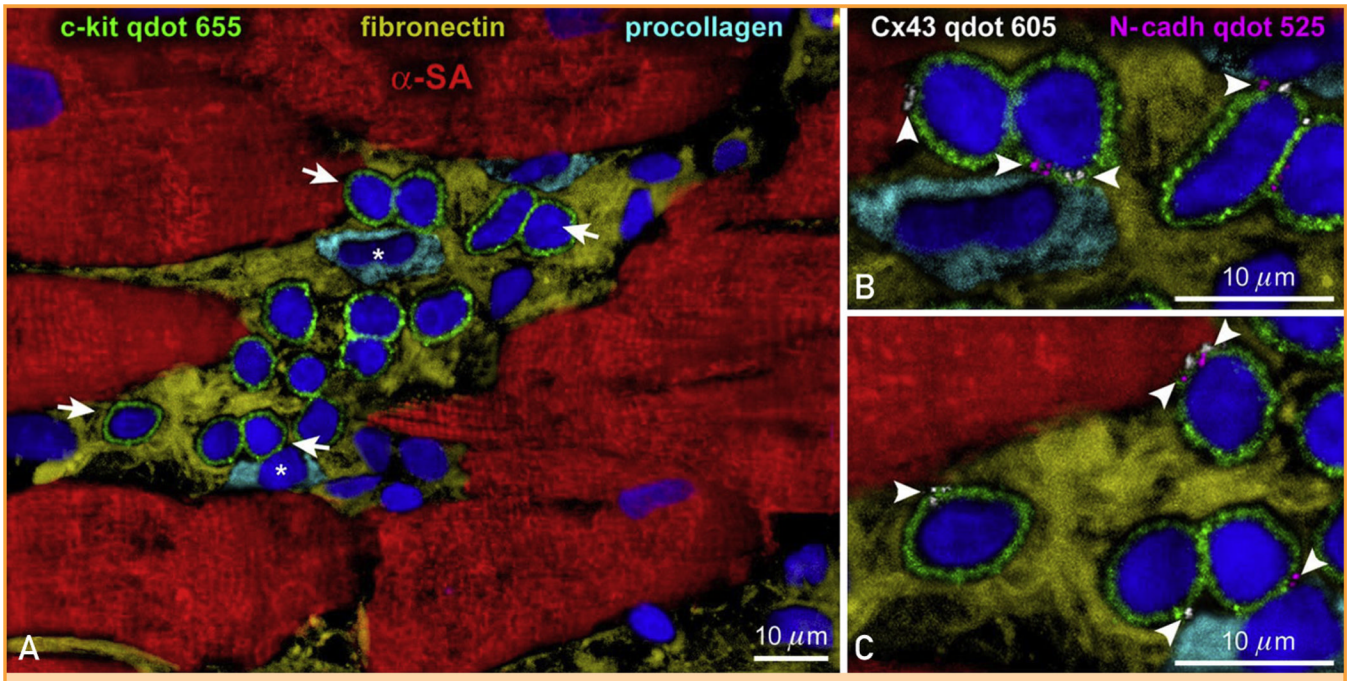



**FIGURE 4.**

Cardiac niches in the human heart. A–C, Cluster of c-kit–positive cardiac stem cells (CSCs) (green). Arrows in A define the areas shown at higher magnification in B and C (magnification 1600×). Gap junctions (connexin 43 [C×43], white; arrowheads) and adherens junctions (N-cadherin [N-cadh], magenta; arrowheads) are illustrated. Connexin 43 and N-cadh are present between CSCs and myocytes (α-sarcomeric actin [SA], red) and fibroblasts (procollagen, light blue; asterisks). Fibronectin, yellow. qdot = quantum dots.

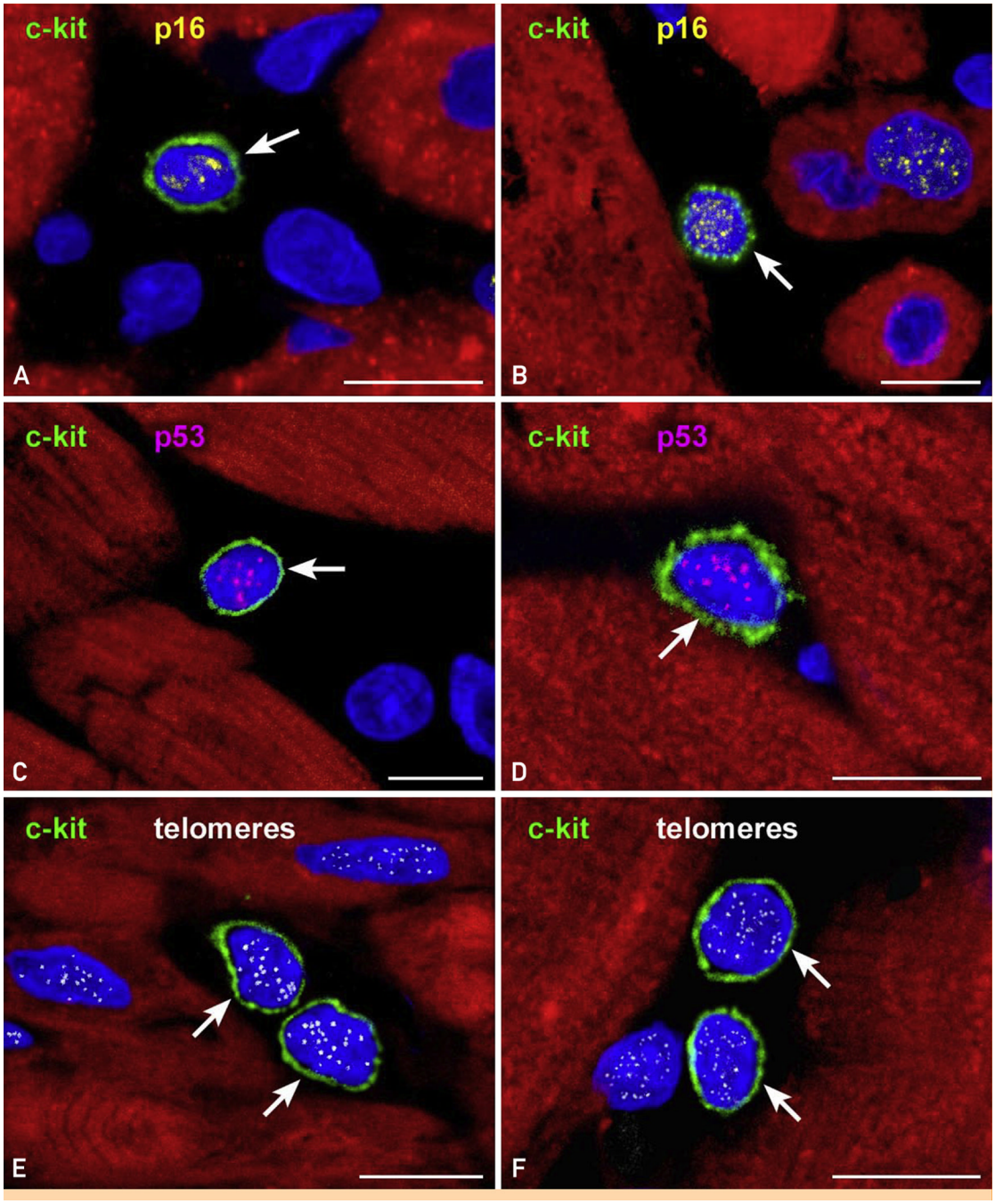



**FIGURE 5.**

Cardiac stem cell (CSC) senescence. A–F, Two examples each of c-kit–positive CSCs (green) expressing (A and B) $p16^{INK4a}$ (yellow) and (C and D) p53 (magenta) are shown. E and F, Similarly, telomeres in c-kit–positive CSCs are illustrated in 2 examples by small white fluorescence dots. Arrows indicate c-kit–positive CSCs. Scale bars, 10 μm.