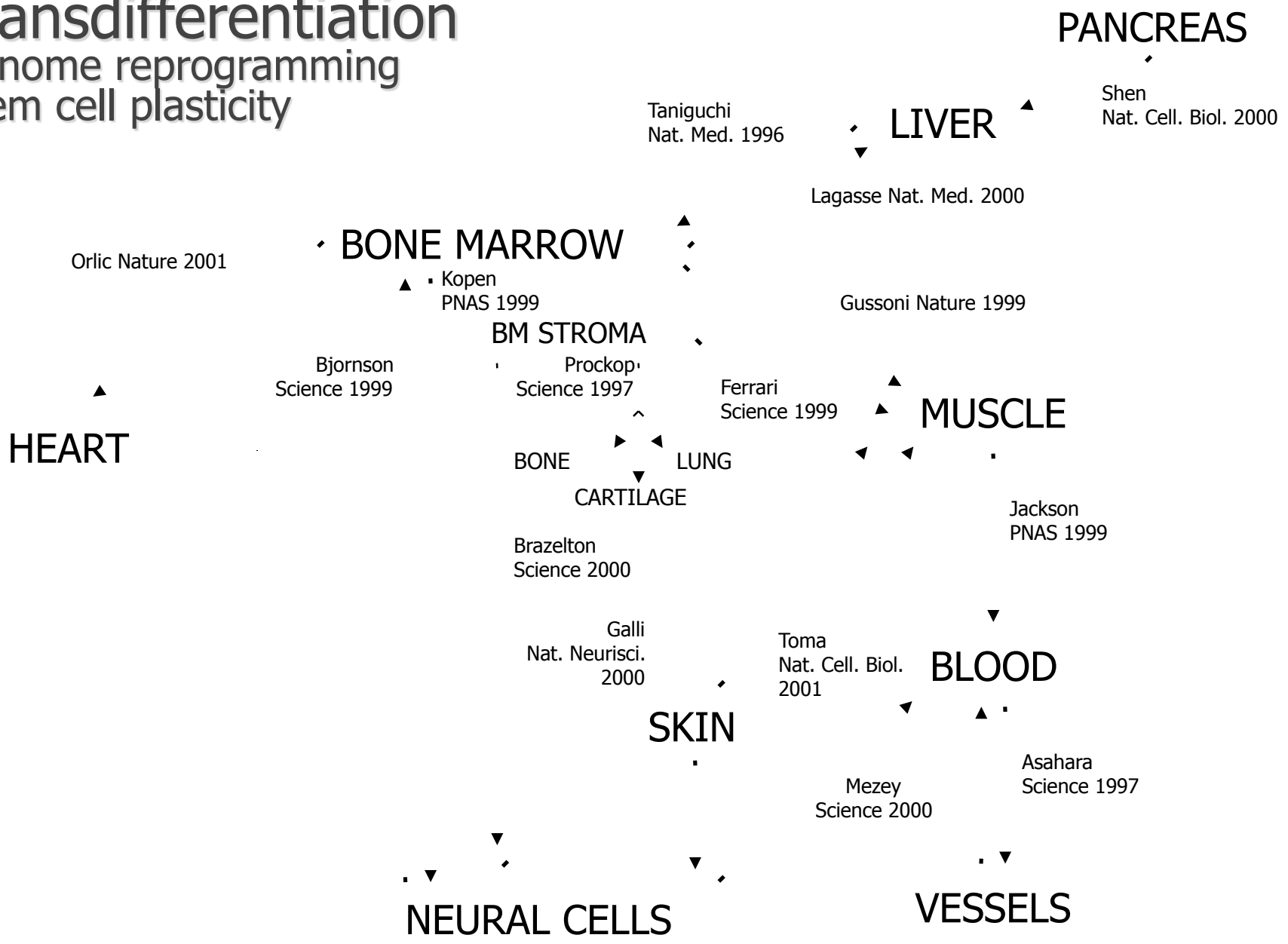
"Stem cells" from bone marrow

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

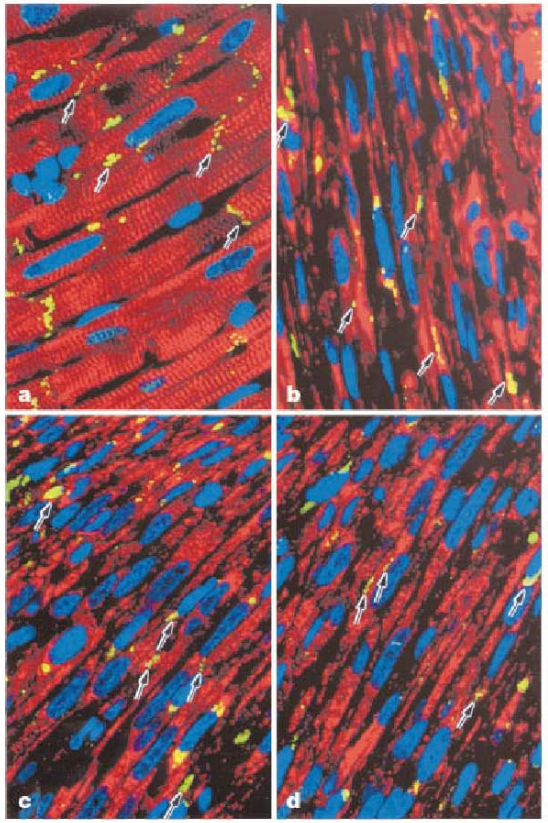


Figure 4 Myocardial repair and connexin 43. **a**, Border zone; **b**–**d**, regenerating myocardium. Shown are connexin 43 (yellow–green; arrows indicate contacts between myocytes) and α-sarcomeric actin (red), and PI-stained nuclei (blue). Original magnification, ×500 (**a**), ×800 (**b**–**d**).

Bone marrow cells regenerate infarcted myocardium

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** Department of Medicine, New York Medical College, Valhalla, New York 10595, USA*

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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.

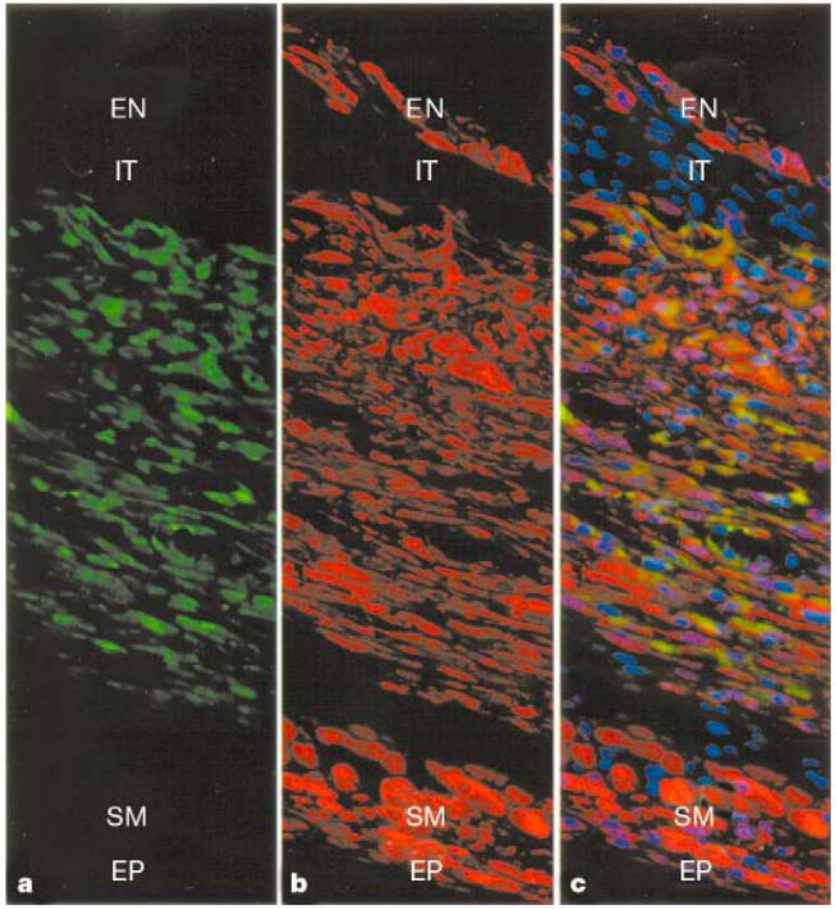
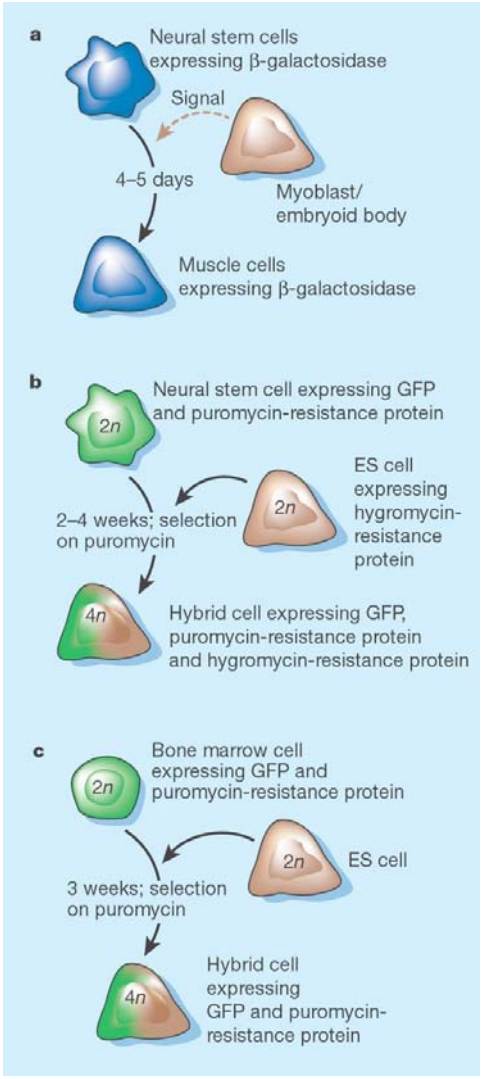


Figure 2 Myocardial infarct injected with Lin^-c-kit^{POS} cells; myocardium is regenerating from endocardium (EN) to epicardium (EP). **a**, EGFP (green); **b**, cardiac myosin (red); **c**, combination of EGFP and myosin (red–green), and propidium-iodide-stained nuclei (blue). Infarcted tissue (IT) 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

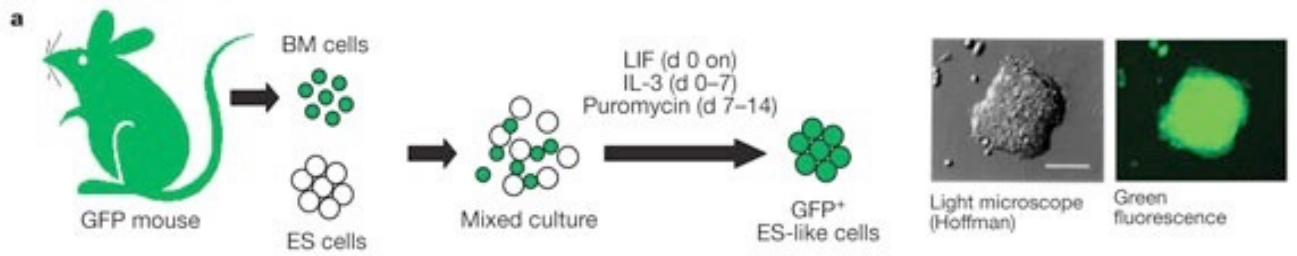
Andrew E. Wurmser and Fred H. Gage

'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.

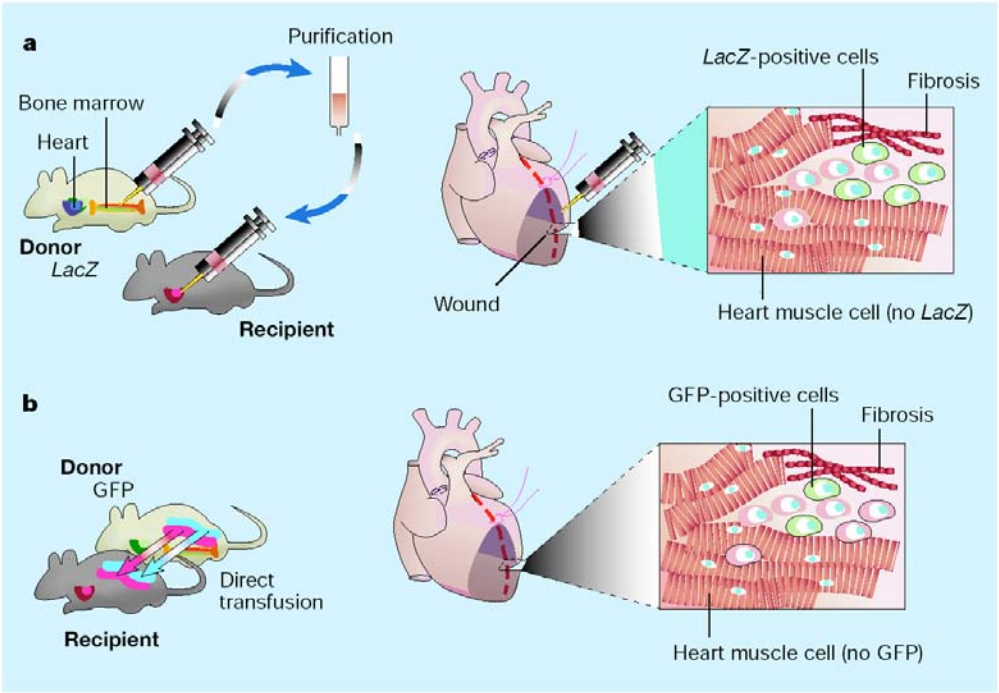


Figure 1 **Two strategies used to show that bone-marrow stem cells do not take on the role of damaged heart cells.** a, Murry *et al.*[2] isolated and purified genetically modified bone-marrow stem cells from mice. The modification 'tagged' the cells (with *LacZ*), enabling them to be detected in the 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

Leora B. Balsam[1], Amy J. Wagers[2,3], Julie L. Christensen[2,3], Theo Kofidis[1], Irving L. Weissman[2,3] & Robert C. Robbins[1]

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

Charles E. Murry[1], Mark H. Soonpaa[2], Hans Reinecke[1], Hidehiro Nakajima[2], Hisako O. Nakajima[2], Michael Rubart[2], Kishore B. S. Pasumarthi[2]*, Jitka Ismail Virag[1], Stephen H. Bartelmez[3], Veronica Poppa[1], Gillian Bradford[2], Joshua D. Dowell[2], David A. Williams[2]* & Loren J. Field[2]

[1]*Department of Pathology, Box 357470, Room D-514 HSB, University of Washington, Seattle, Washington 98195, USA*
[2]*Wells Center for Pediatric Research, Indiana University, 1044 West Walnut Street, R4 Bldg, Room W376, Indianapolis 46202-5225, USA*
[3]*Department of Pathobiology, University of Washington, Seattle, Washington 98195, USA*

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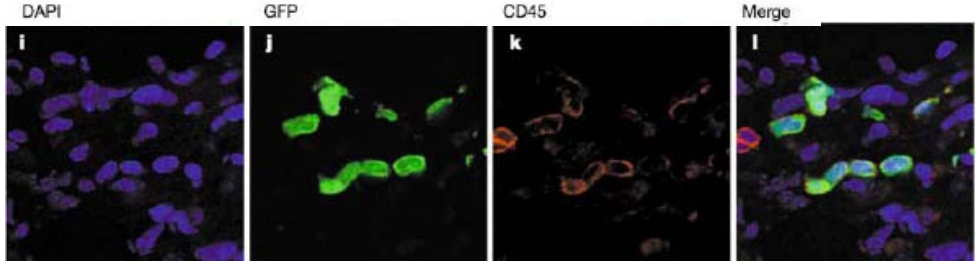
NATURE | VOL 428 | 8 APRIL 2004 | www.nature.com/nature

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^{Bry}	1×10^6	10	190,430	19.0
2	c-kit^{Bry}	1×10^6	10	471,930	47.2
3	c-kit^{Bry}	1×10^6	30	3,600	0.4
4	c-kit^{Bry}	1×10^6	30	3,000	0.3
5	c-kit^{Bry}	1×10^6	30	0	0
6	c-kit^{Bry}	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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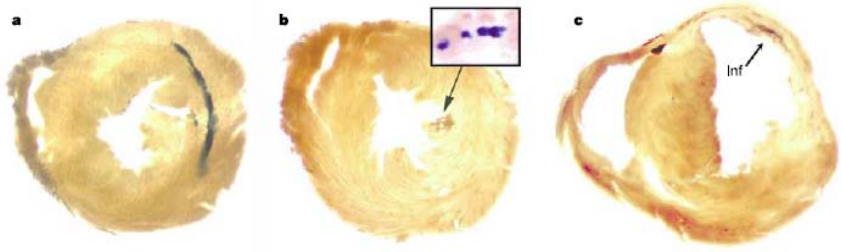
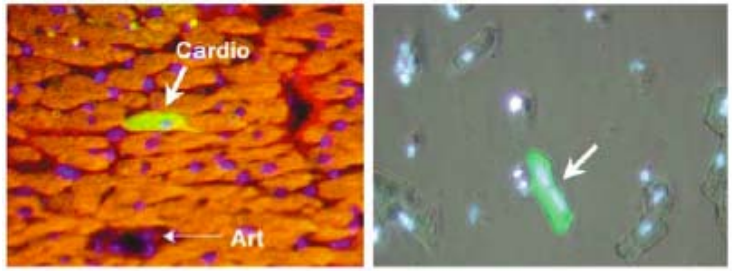


Transgenic mice in which the cardiac-specific MHC promoter drives the expression of a nuclear beta-gal



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

Therapeutic nucleic acids

Protein-coding cDNAs

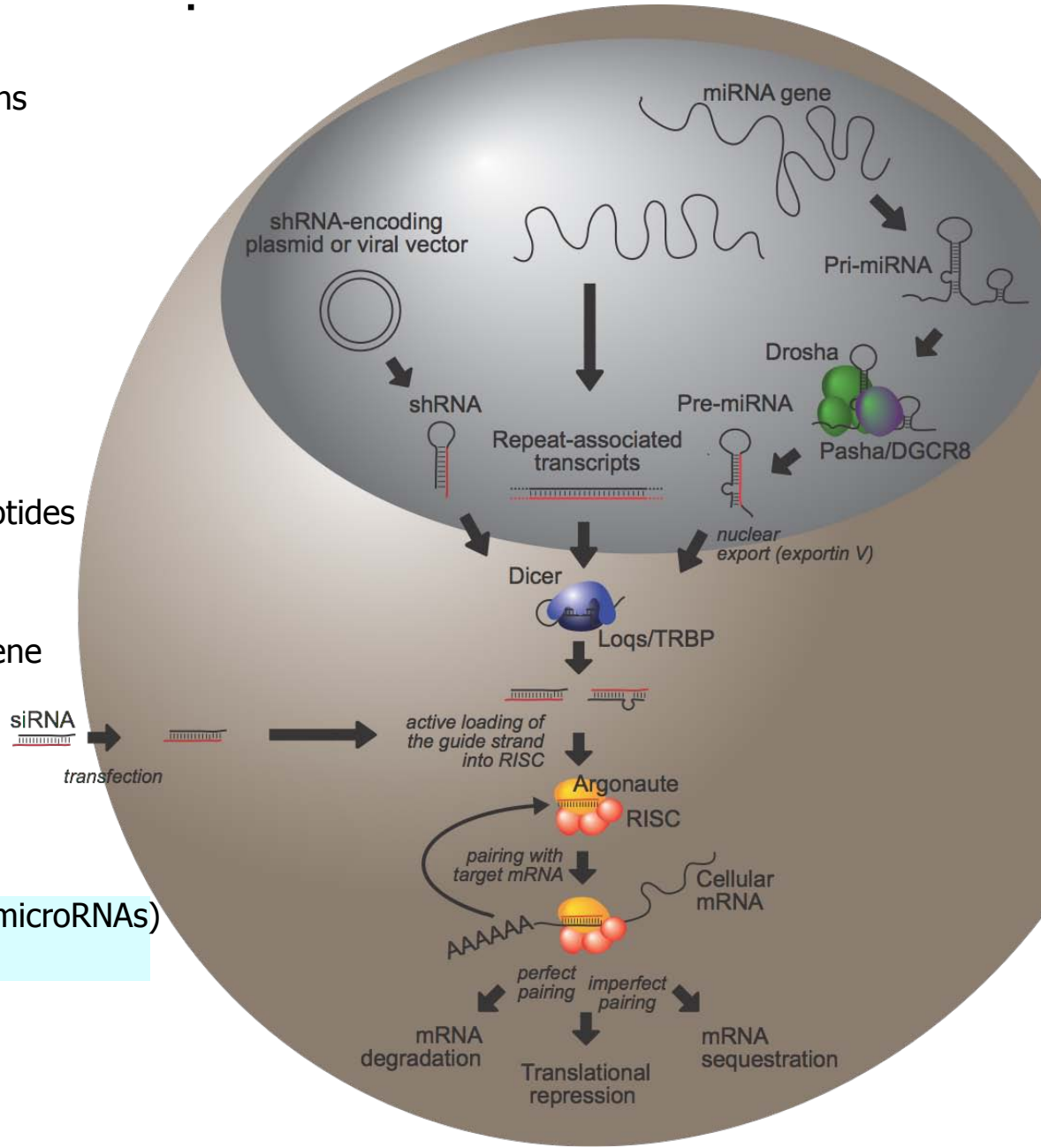
Proteins replacing missing cellular functions
Proteins modulating cellular functions
Proteins regulating cell survival
Proteins activating the immune system
Antibodies and intracellular antibodies

Small, non-coding DNAs and RNAs

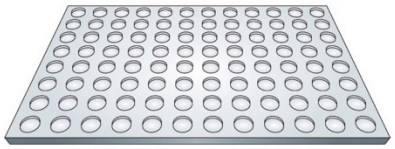
Oligonucleotides and modified oligonucleotides
- Phosphorothioate oligonucleotides
- 2' ribose-modified oligonucleotides
- Locked Nucleic Acids (LNA) and Ethylene Bridged Nucleic Acids (ENA)
- Morpholino (PMO)
- Peptide Nucleic Acids (PNA)

Catalytic RNAs and DNAs (ribozymes and DNAzymes)

Small regulatory RNAs (siRNAs, shRNAs, microRNAs)



Screening for cardiomyocyte proliferation using a library of microRNA mimics

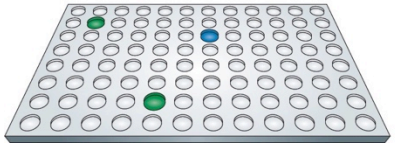


microRNA mimics
arrayed on 96-well plates
(988 mature sequences)

0h — add transfection reagent
add cardiomyocytes isolated from newborn rats

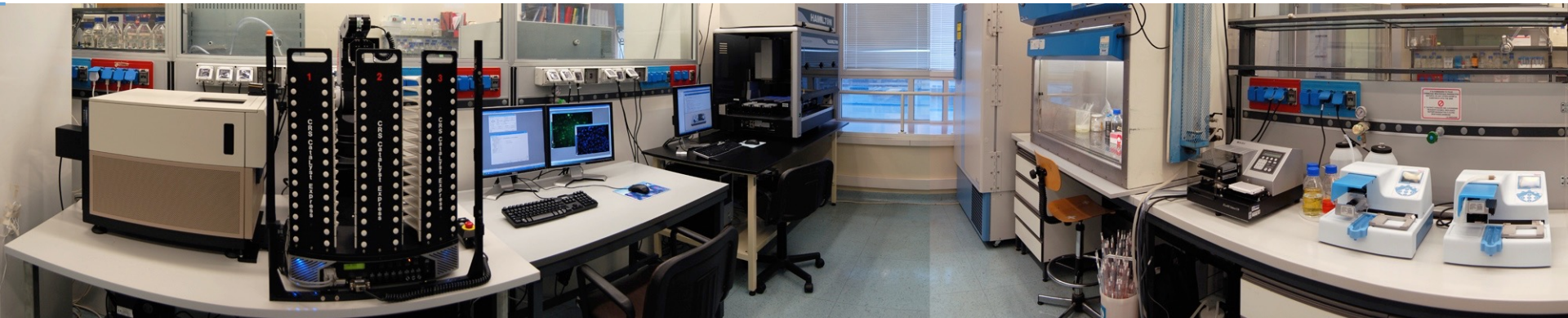
52h — add EdU

72h

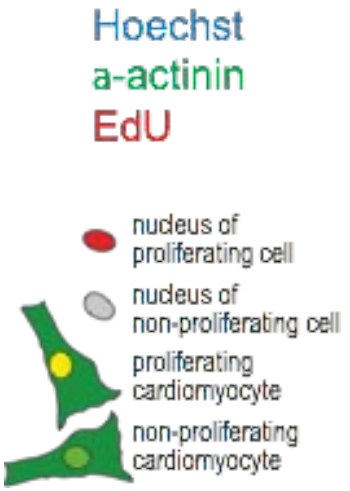


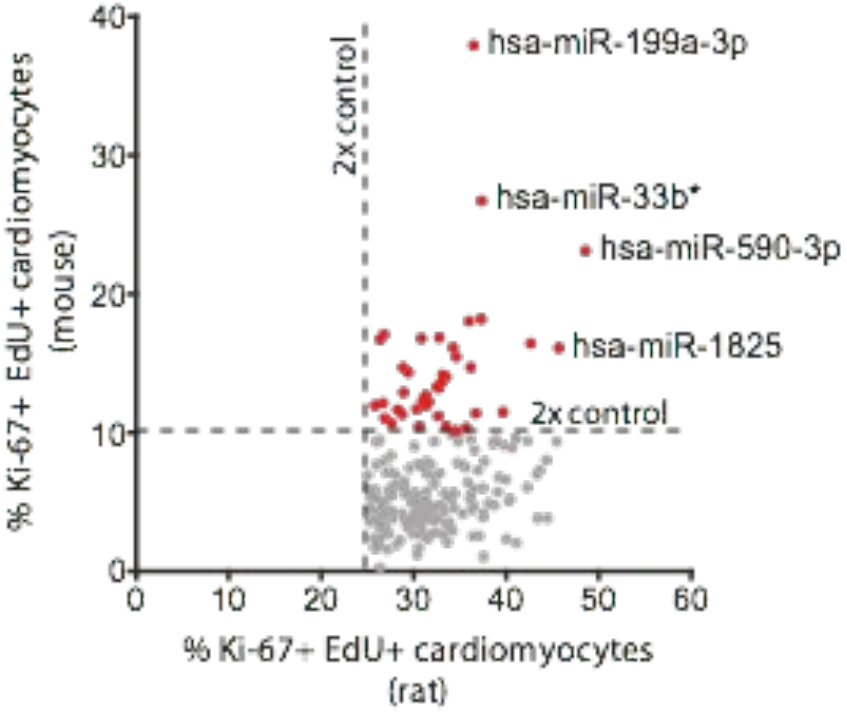
cell fixation and fluorescence staining
(Hoechst, alpha-actinin, Ki-67 and EdU)

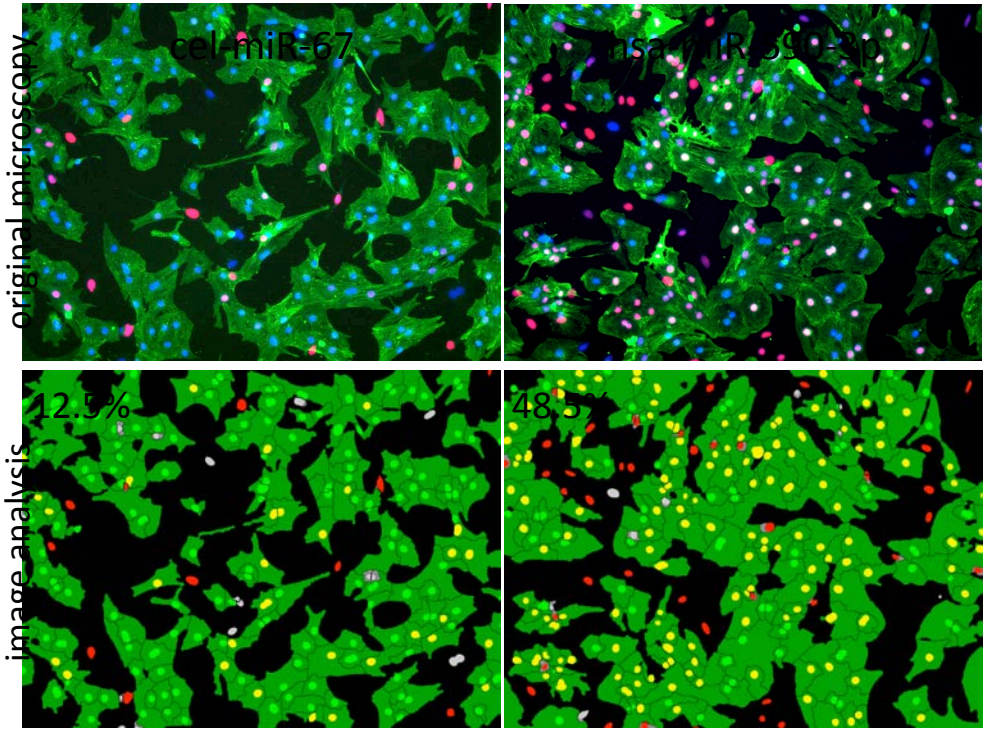
Ana
Eulalio



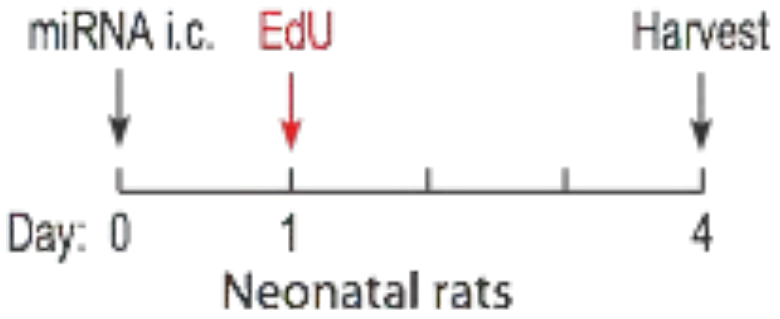
40 human miRNAs increase both rat and mouse cardiomyocyte proliferation





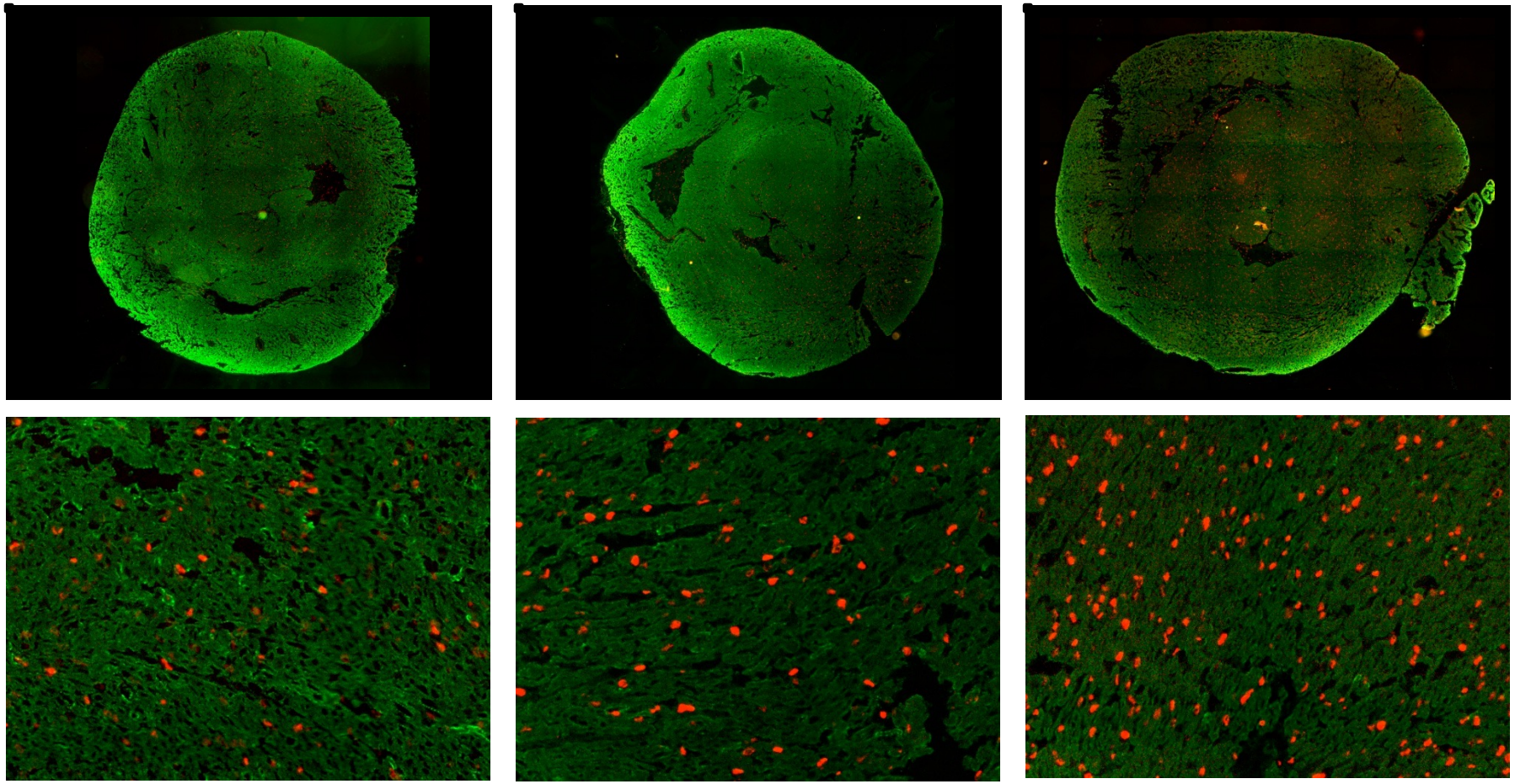


Intracardiac injection of the miRNAs increasing cardiomyocyte proliferation in the newborn rat heart

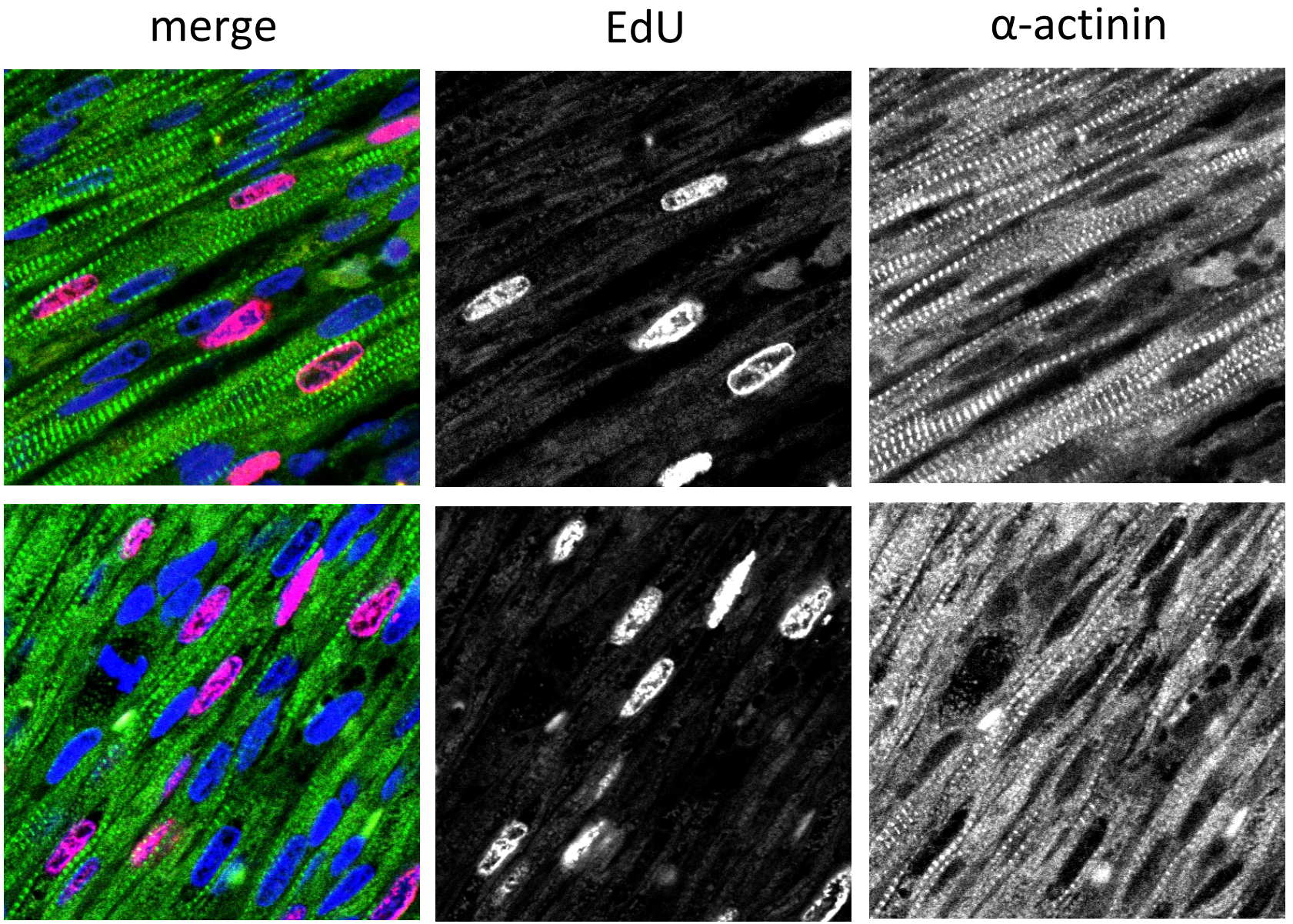


cel-miR-67 | hsa-miR-199a | hsa-miR-590

α-actinin / EdU

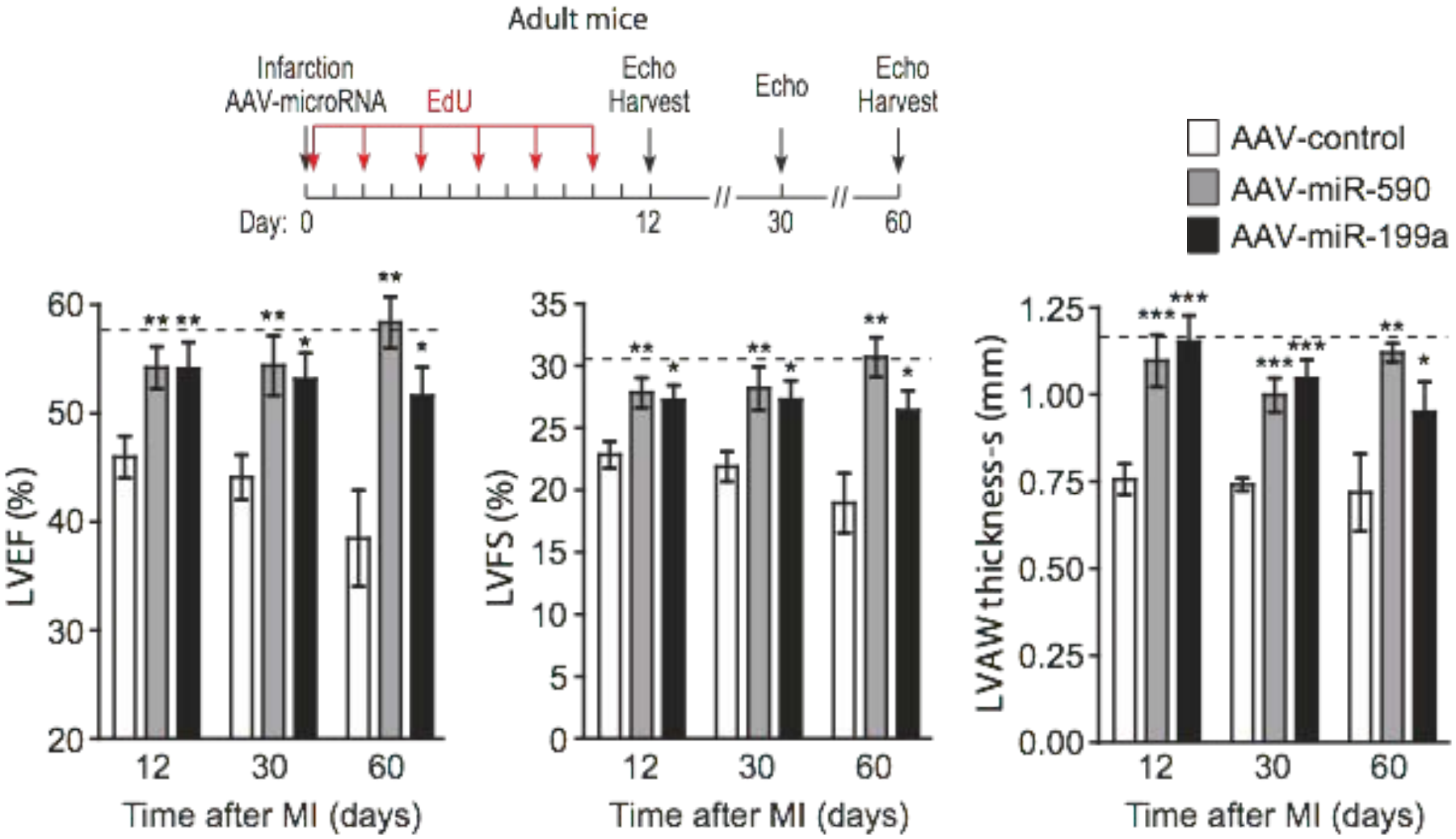


miRNAs increasing CM proliferation *in vivo*

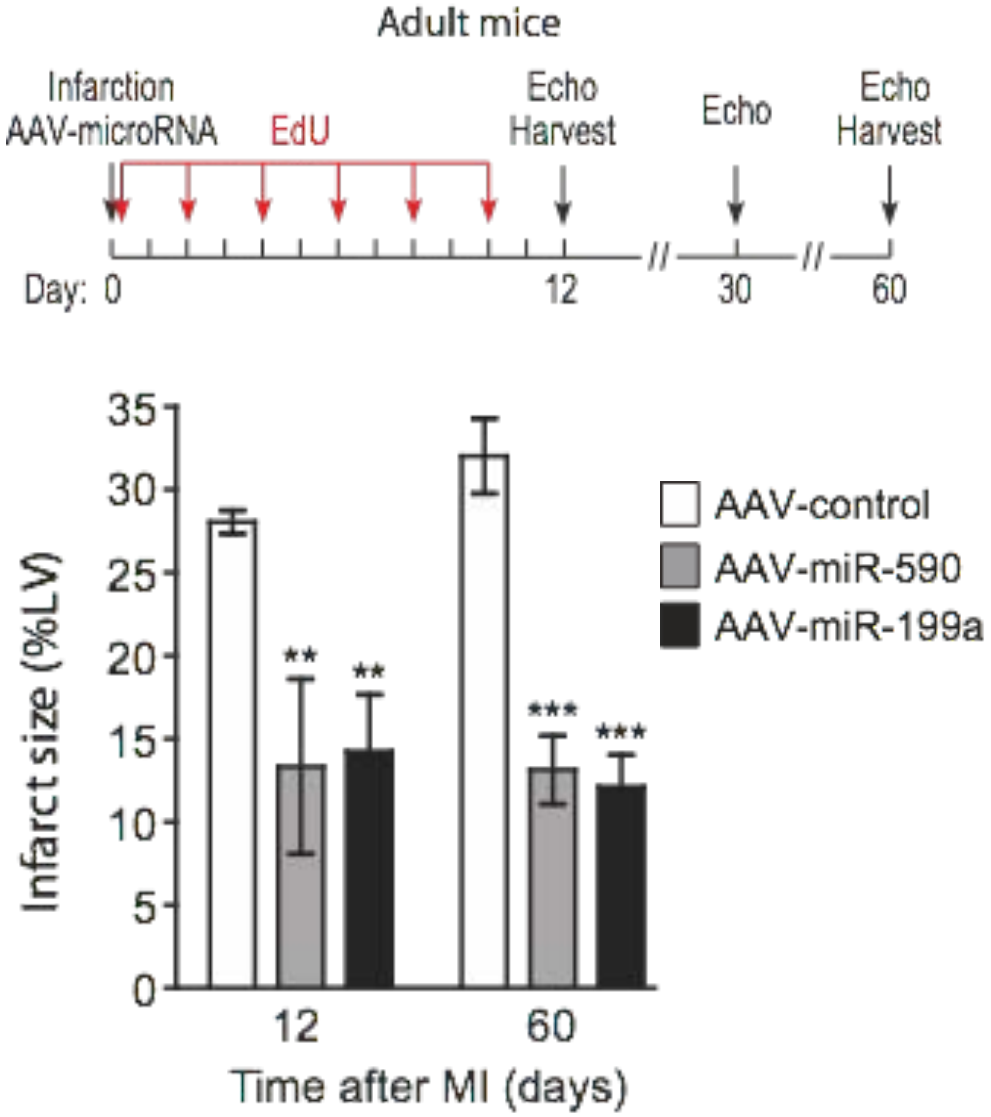


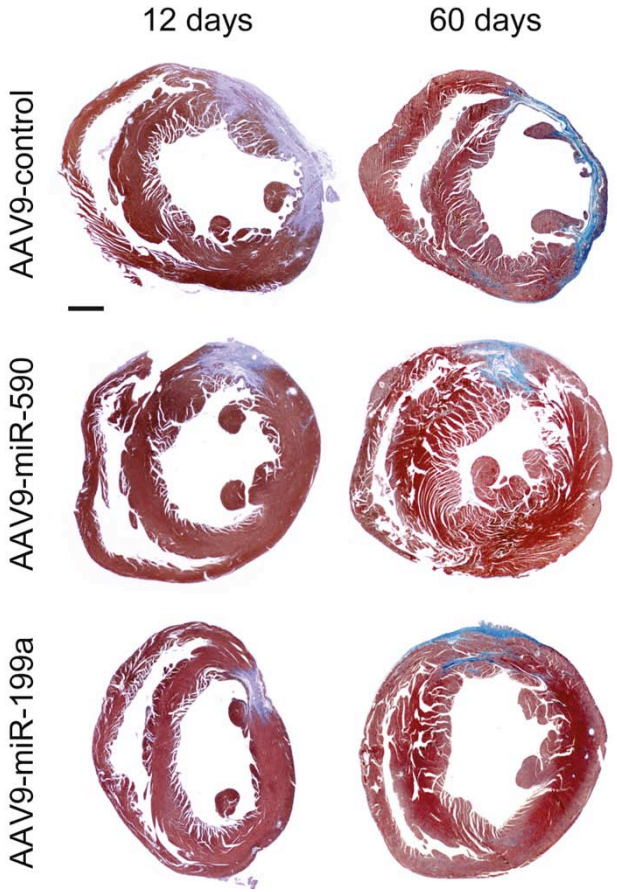
Effect of miRNA prolonged expression in vivo?

miR-590 and miR-199a preserve myocardial function after MI



miR-590 and miR-199a markedly reduce infarct size

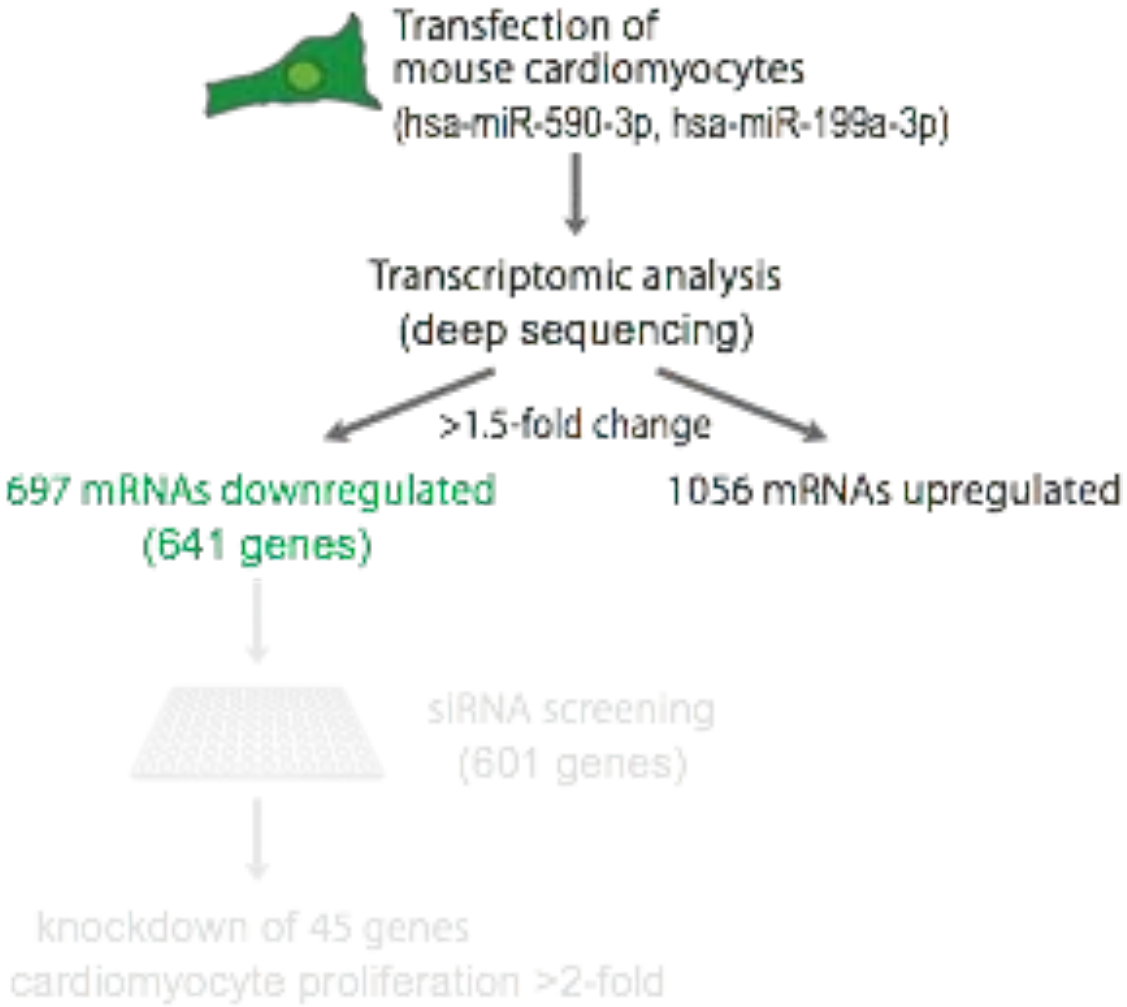




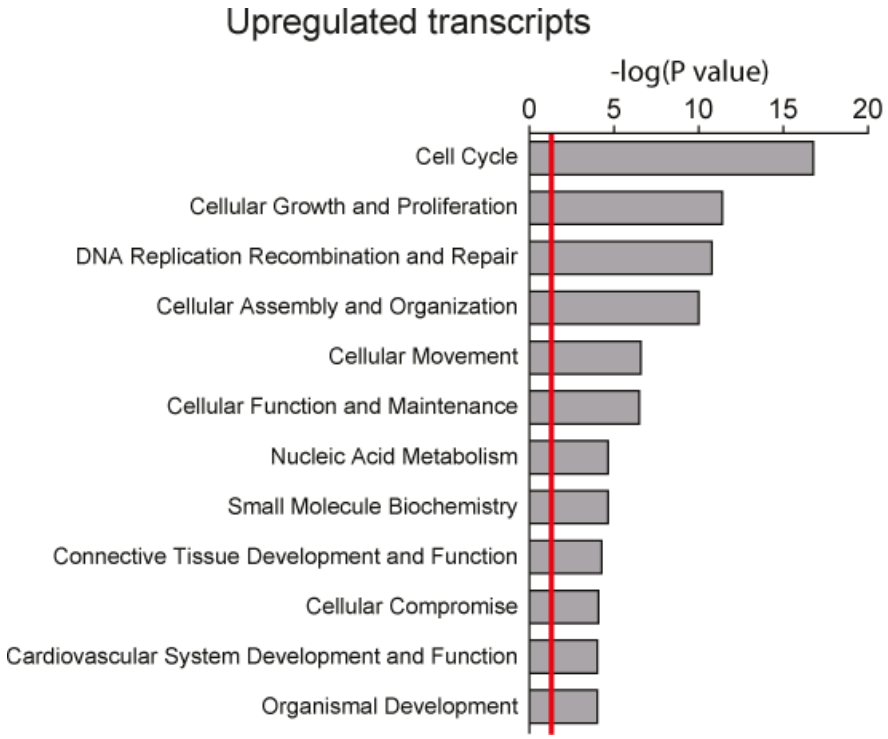
Masson Trichrome staining

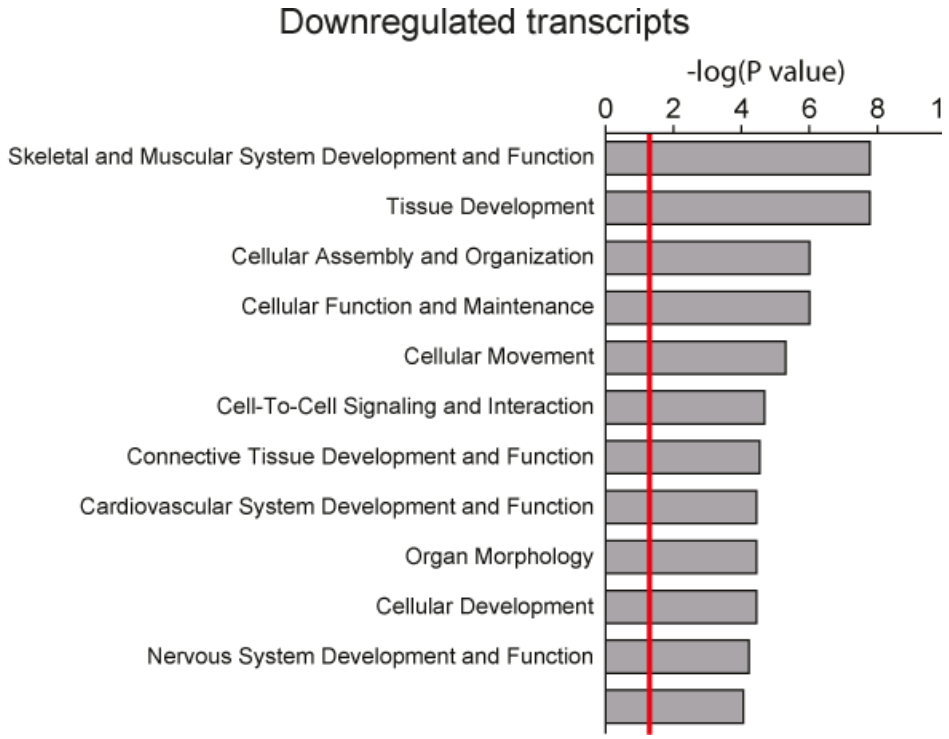
Mechanism?

Identification of miR-590 and miR-199a target genes



Functional analysis (IPA) of transcripts up- and down-regulated by hsa-miR-590-3p and hsa-miR-199a-3p





Among the 641 genes downregulated by miR-590-3 and miR-199a-3p are:

Myomesin 1 (Myom1)
Myomesin 2 (Myom2)
Myosin light polypeptide 4 (Myl4)
Nebulin-related anchoring protein (Nrap)
Myosin IB (Myo1b)
Titin (Ttn)
Troponin T1, skeletal slow (Tnnt1)
Troponin T2 cardiac (Tnnt2)
Cofilin2 (Cofilin2)
Dynamin1-like (Dnm1l)
Ankyrin repeat domain 52 (Ankrd52)
Nebulette (Nbl)

What about large animal models?

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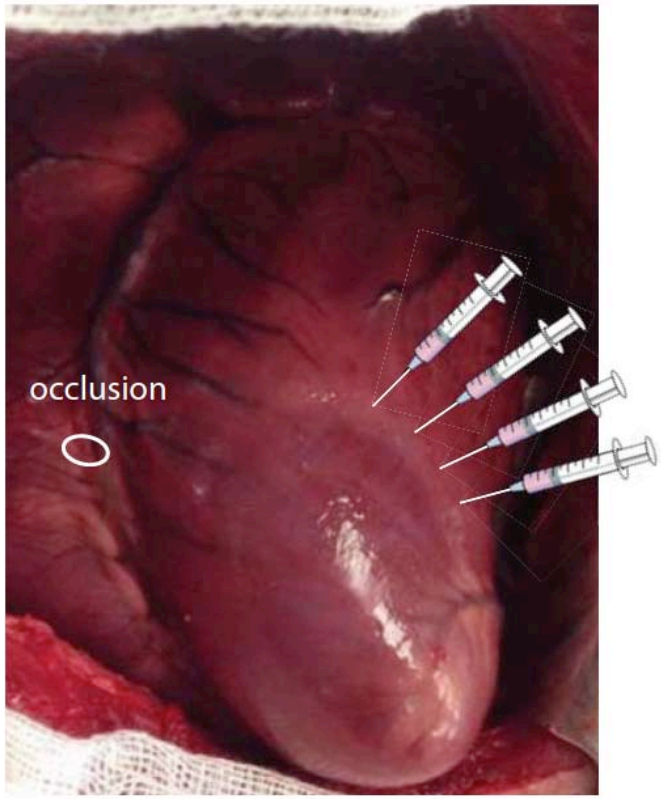
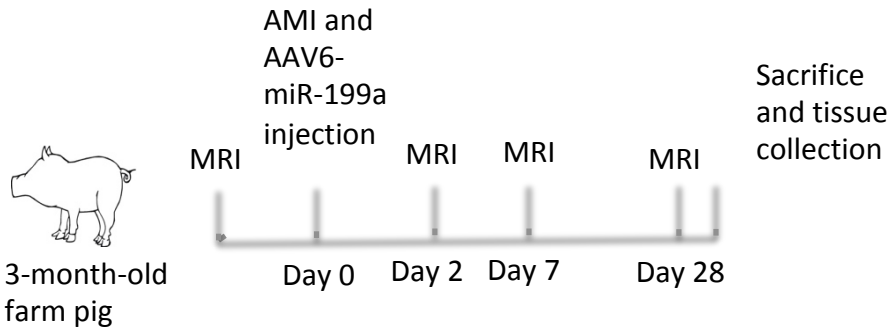
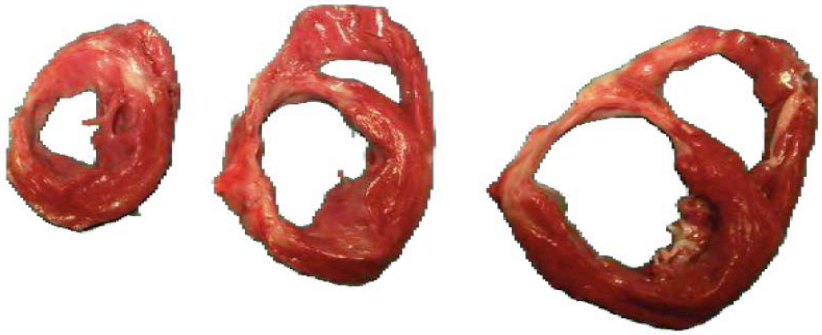
Open chest MI model in farm pig



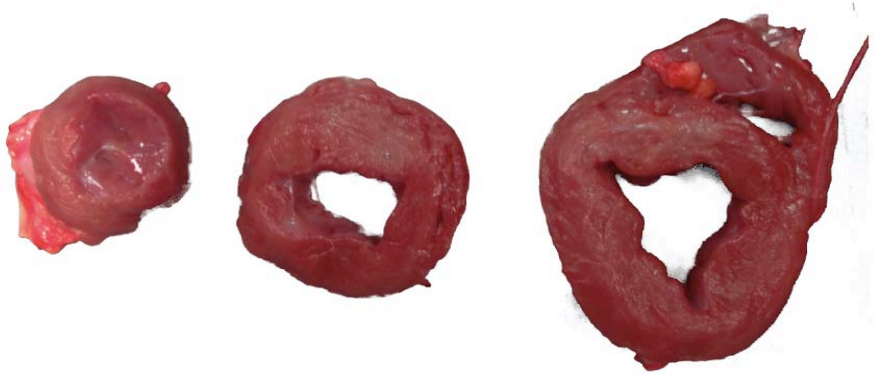
AAV6-miR-199a reduces infarct size after MI

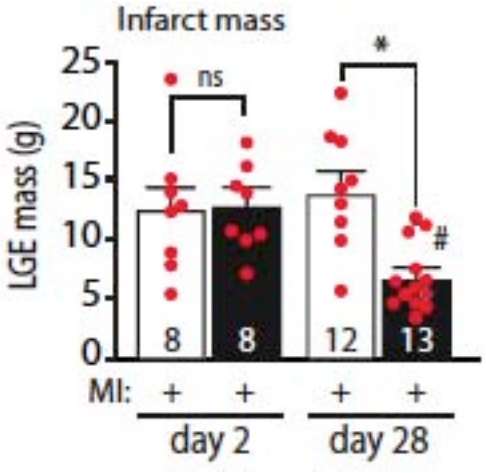


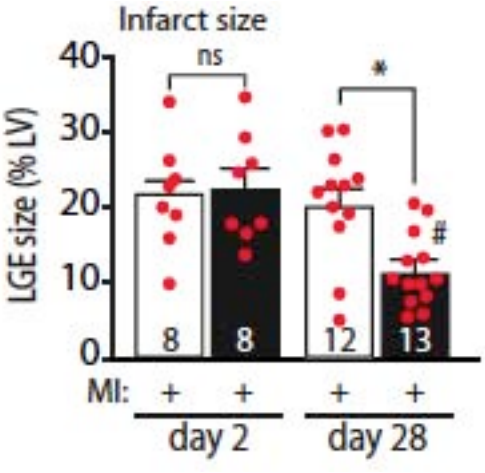
AAV6 empty

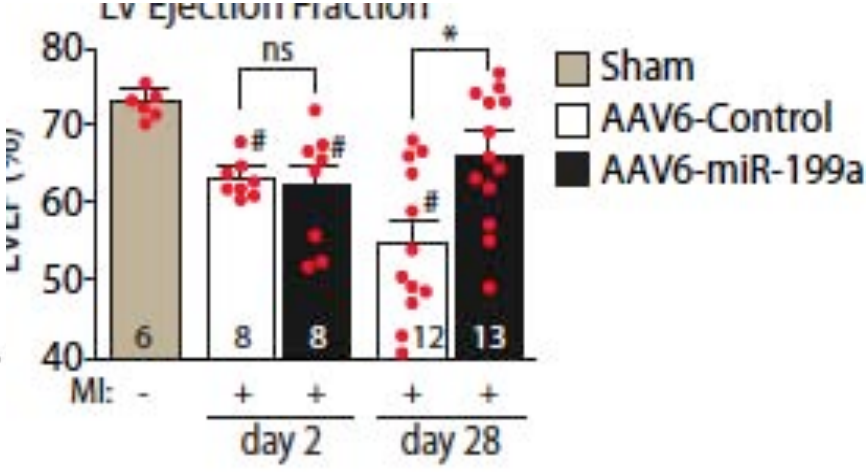


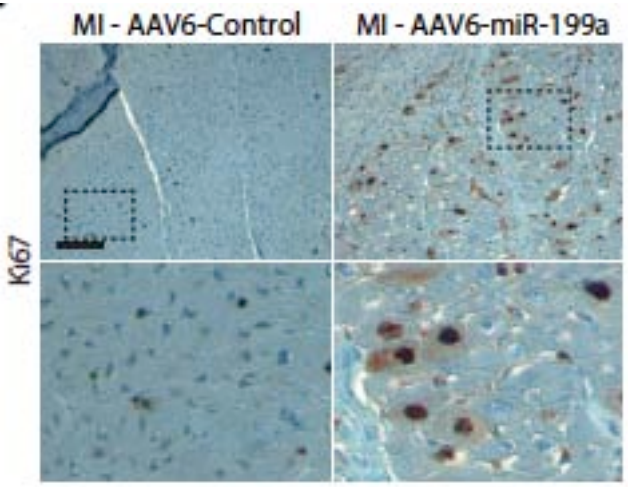
AAV6 miR-199a

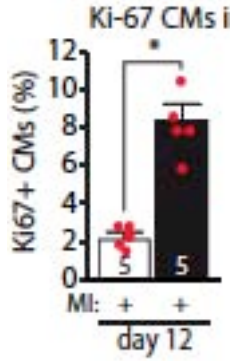




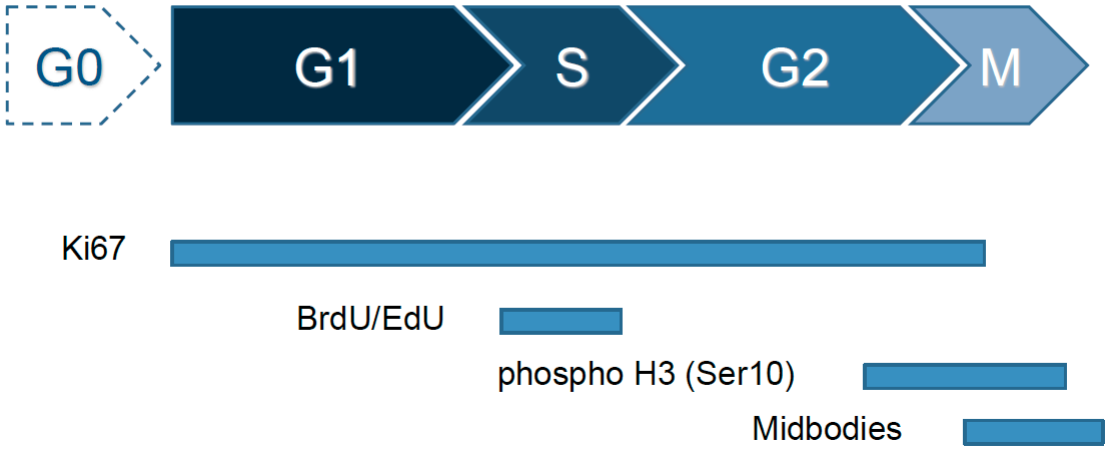


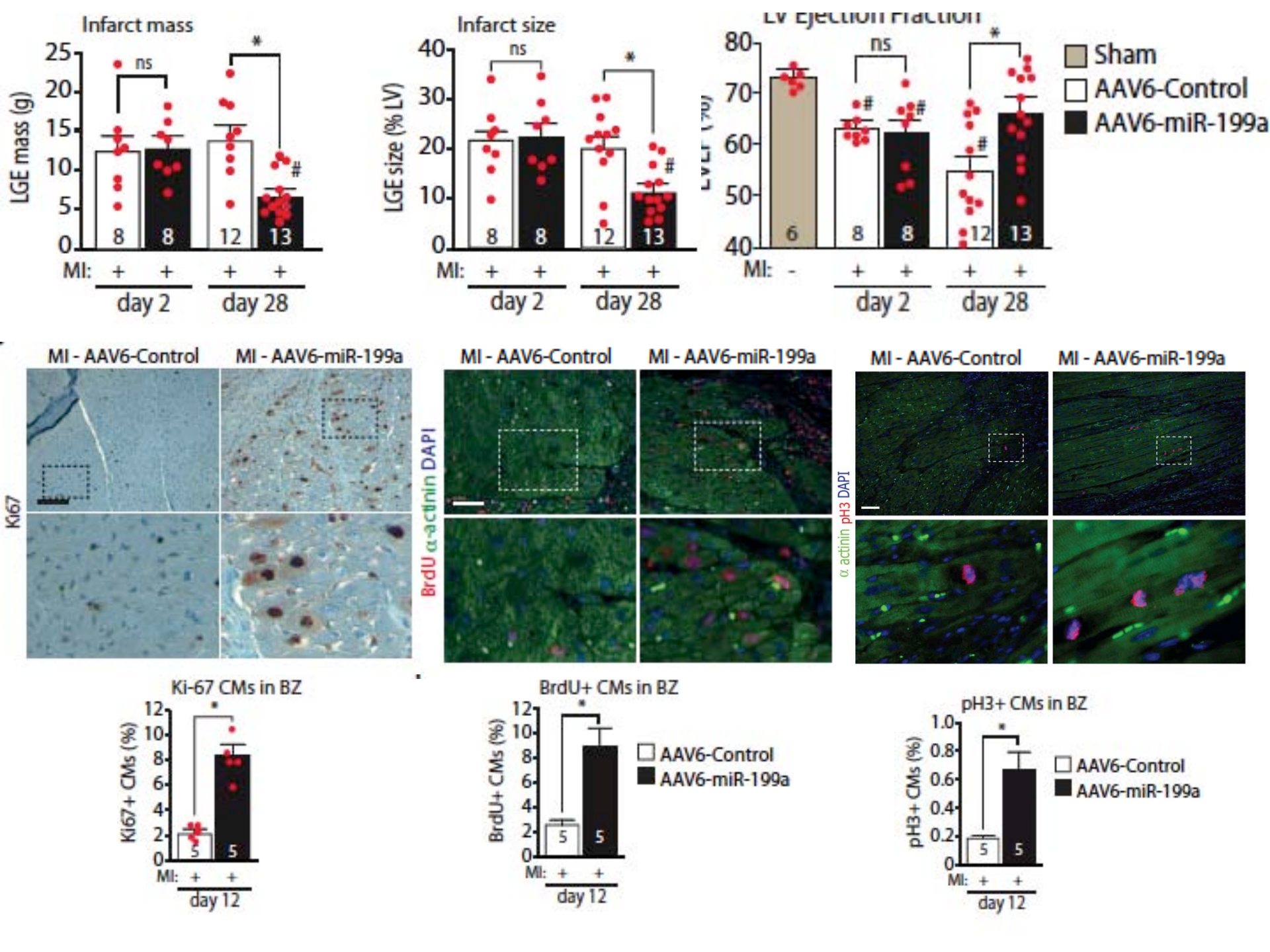


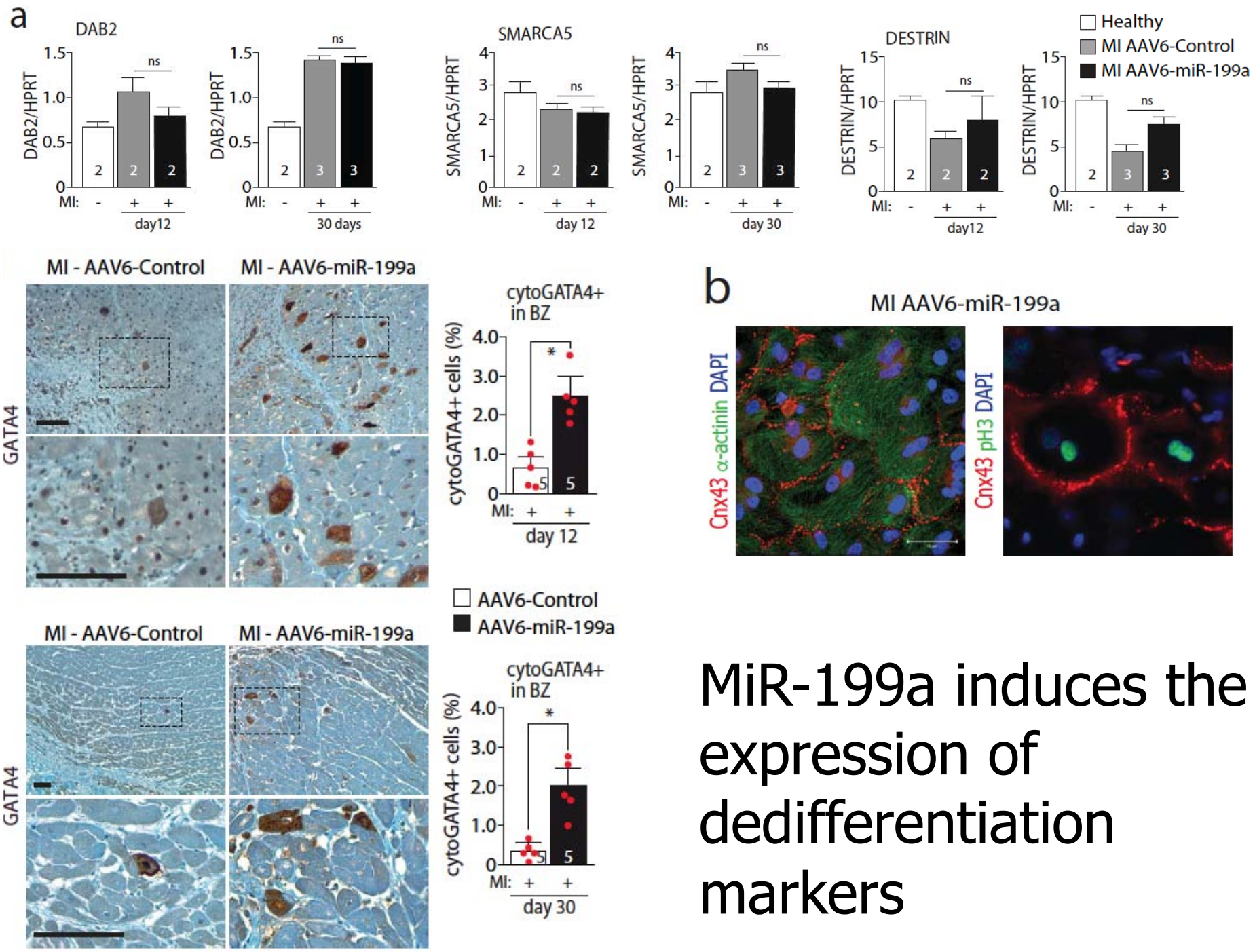




Common markers of cell cycle progression




Infarct mass
LGE mass (g)
ns
*
#
8
8
12
13
MI: + + + +
day 2
day 28
Infarct size
LGE size (% LV)
LV Ejection Fraction
6
Sham
AAV6-Control
AAV6-miR-199a
MI: - + + + +
MI - AAV6-Control
MI - AAV6-miR-199a
Ki67
BrdU α-actinin DAPI
α actinin pH3 DAPI
Ki-67 CMs in BZ
Ki67+ CMs (%)
BrdU+ CMs in BZ
BrdU+ CMs (%)
pH3+ CMs in BZ
pH3+ CMs (%)
day 12



MiR-199a induces the expression of dedifferentiation markers

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