Dedifferentiation of Adult Cardiac Myocytes

Joanne Stocks

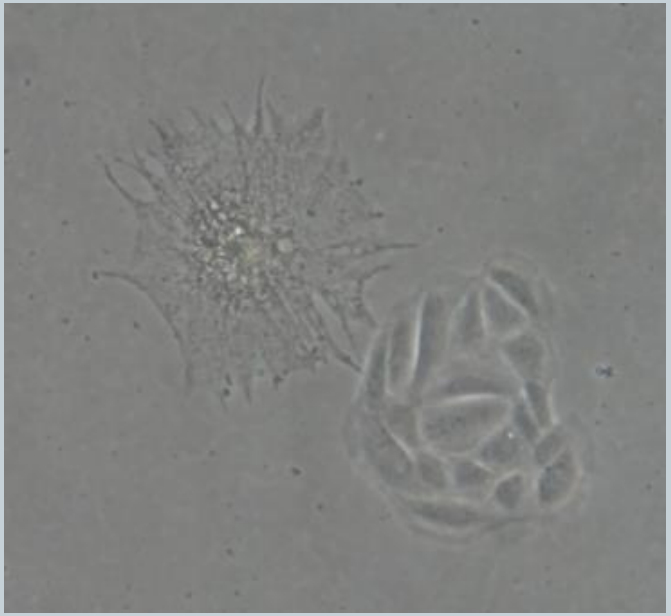
Icahn School of Medicine at Mount Sinai

Background

- Adult mammalian myocardium has no regenerative capacity.
- Cardiomyocyte loss as a result of injury or disease is irreversible.
- Regeneration of the myocardium is a new area being investigated for future therapy against heart failure

Current Therapeutic Ideas

- Cell replacement strategies -potential to restore cardiac function?
- Promising results from both basic animal studies and clinical trials using:
  - embryonic stem cells
  - skeletal myoblasts
  - haematopoietic stem cells
  - endothelial progenitor cells
  - mesenchymal stem cells



Current Therapeutic Ideas

- Reverse terminal differentiation
  - Activating committed progenitor cells
  - Encourage differentiated cells to re enter cell cycle
- Could cardiac derived stem/progenitor cells differentiate into cardiac myocytes?
- Could grafted healthy myocytes couple with host myocytes in a diseased heart?

- Myocyte proliferation reported in vivo but not in vitro.
- Stem cell differentiation?
- Cardiac Environment?
- Paracrine factors released by stem cells?

"Paracrine factors released by modified MSCs potentially can exert effects on various aspects of cardiac pathophysiology such as myocardial cell survival, angiogenesis, remodelling, contractility, and even myocyte regeneration. "

Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. M Gnecchi,VJ Dzau et al. Nature Medicine 11, 367 - 368 (2005)

Hypothesis

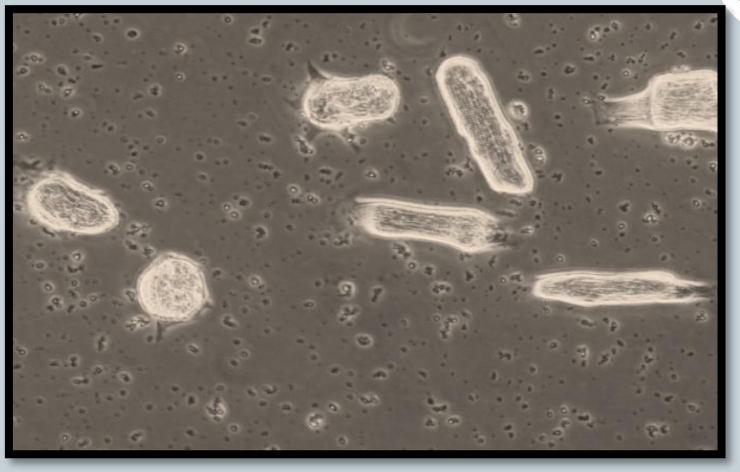
Paracrine factors released by C kit positive cardiac derived progenitor cells can have a protective effect adult cardiac myocytes.

Paracrine factors released by C kit positive cardiac derived progenitor cells induce dedefferentiation of adult cardiac myocytes.

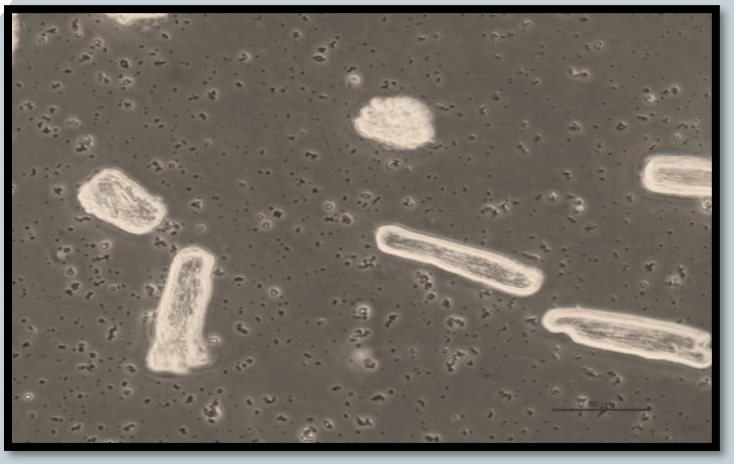
Method

- C kit^{+ve} cardiac derived progenitor cells (CDPC) and cardiomyocytes were isolated from 6-8 week old adult rats (200-250g).
- C kit^{+ve} CDPC's grown in culture for 4 days.
- Supernatant is collected and diluted 50:50 with αMEM, 10% FCS and AraC.
- Myocytes allowed to adhere for 2-12hrs on laminin coated plates before culturing with conditioned media.

Changes in Myocyte Morphology in Culture



Day 0 40x



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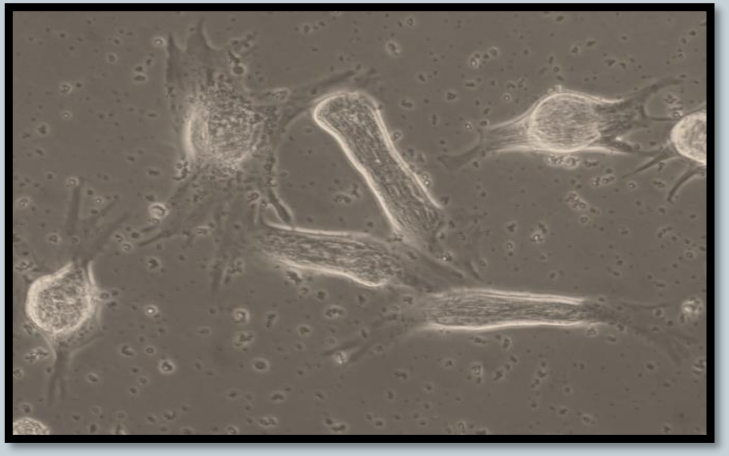

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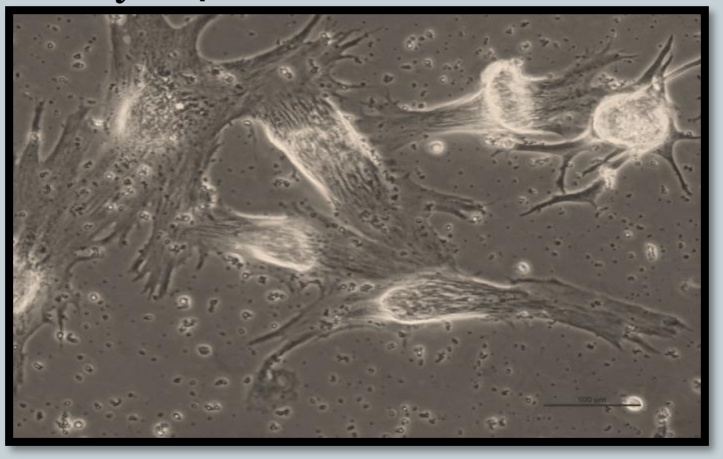
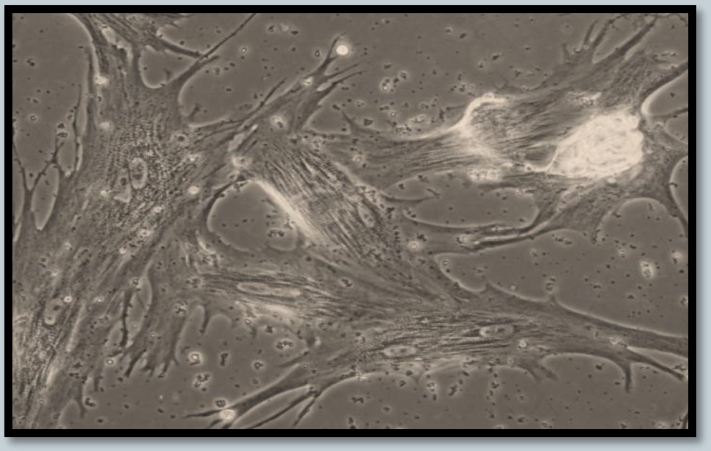


Day 3 40x

Changes in Myocyte Morphology in Culture



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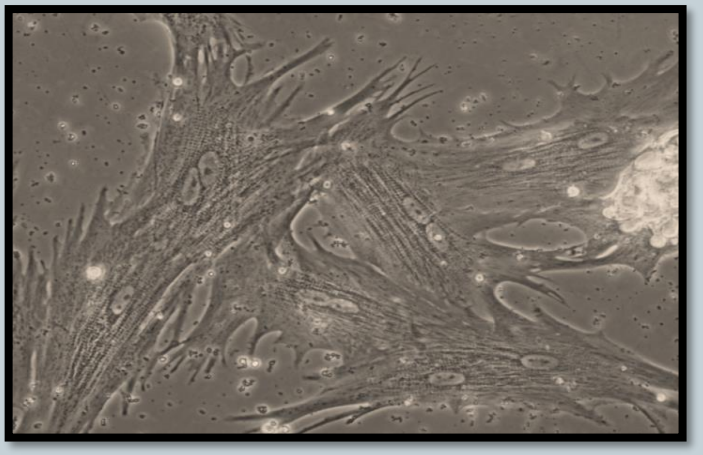

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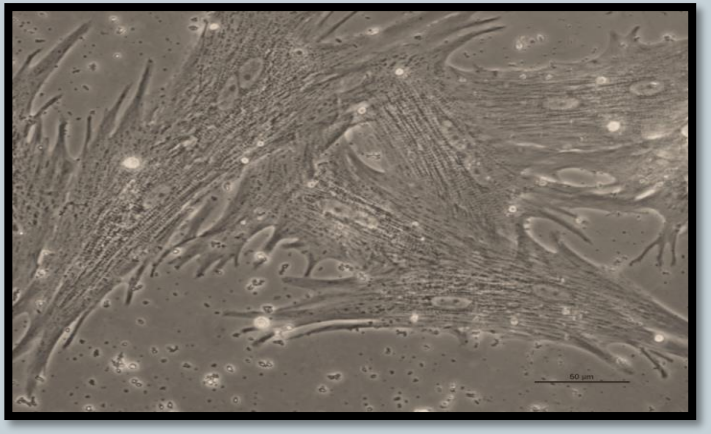

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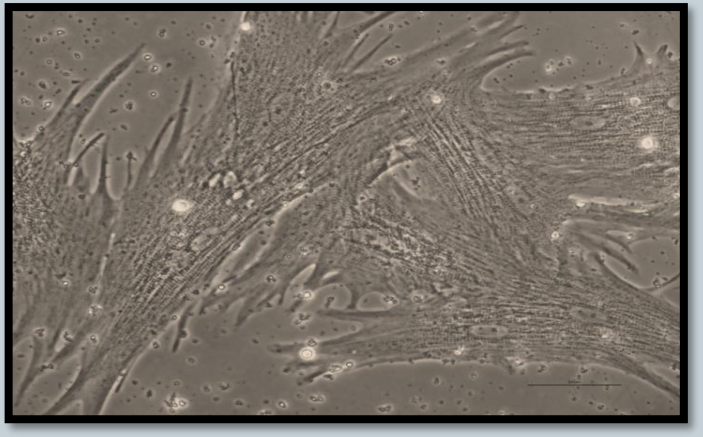
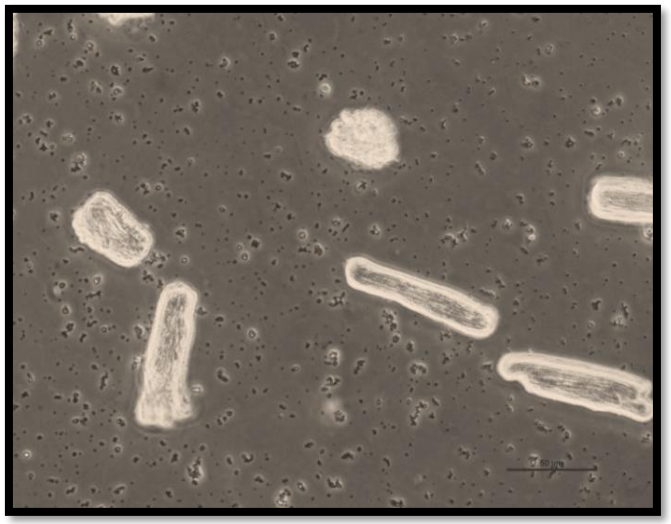
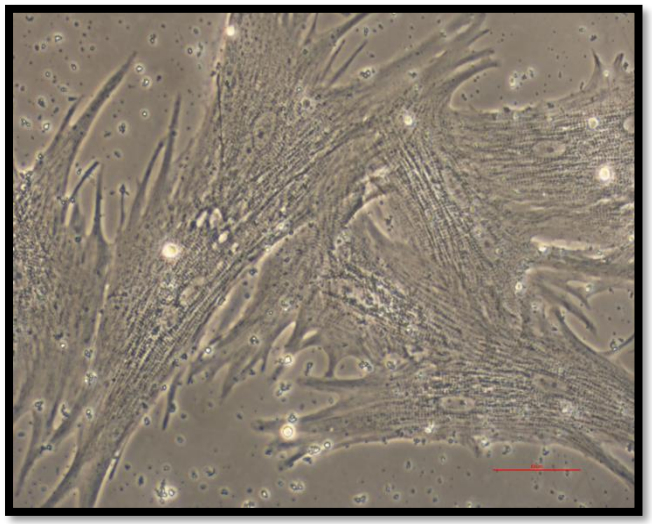


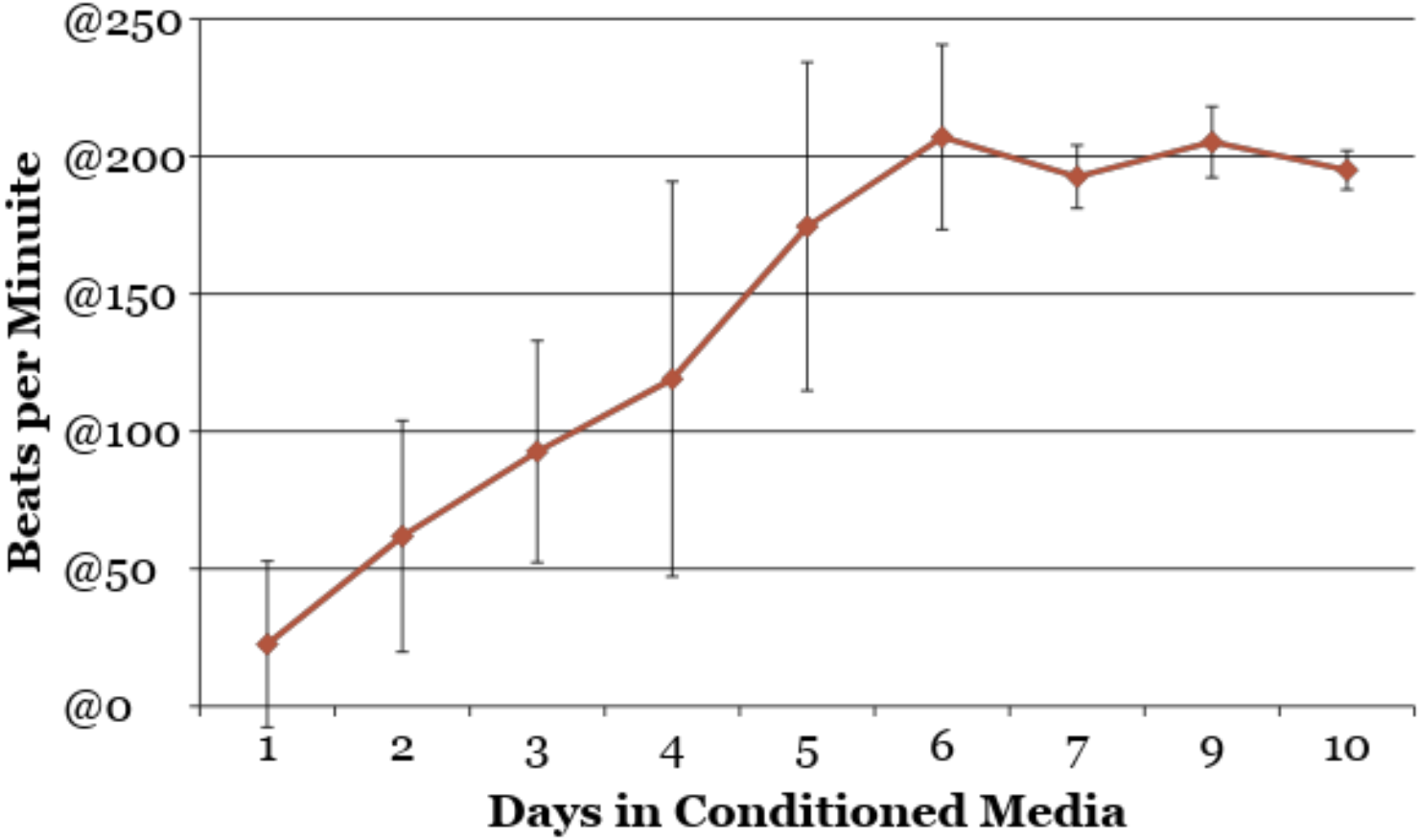
Day 7 40x





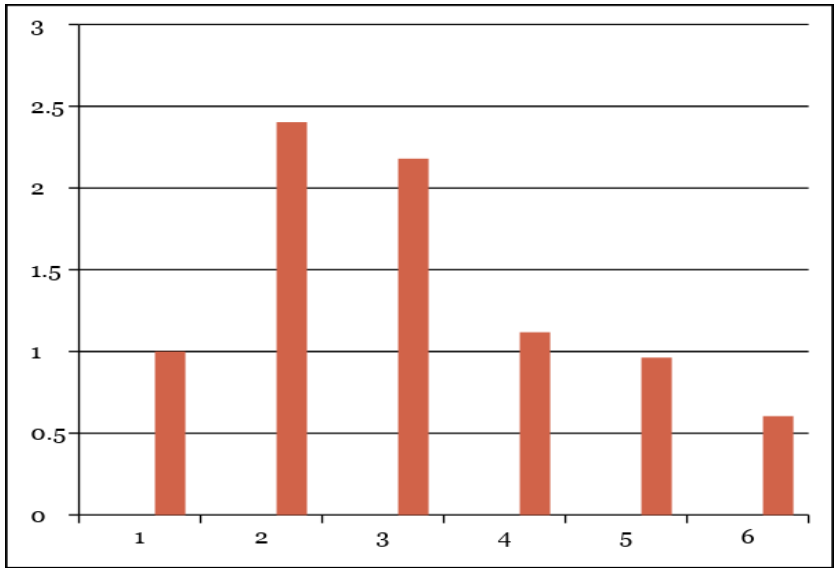
Day 0

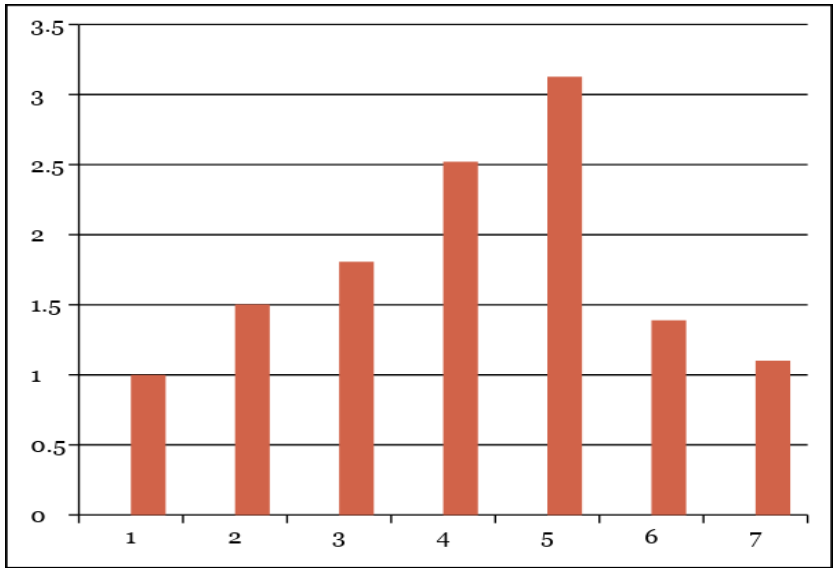
Day 7



Single cell beating rate per minute

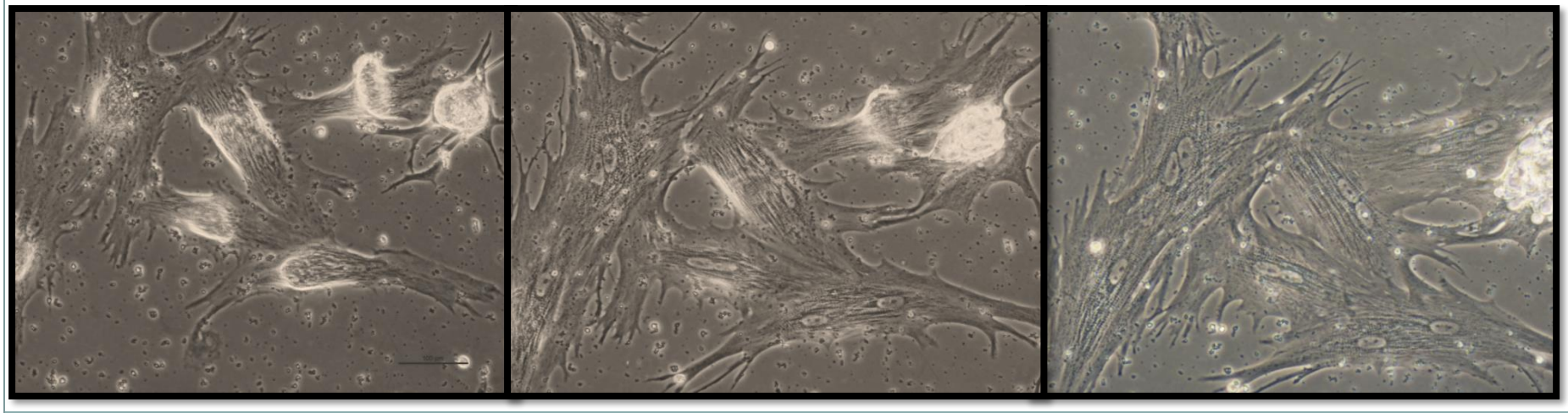
Expression of Connexin 43 mRNA





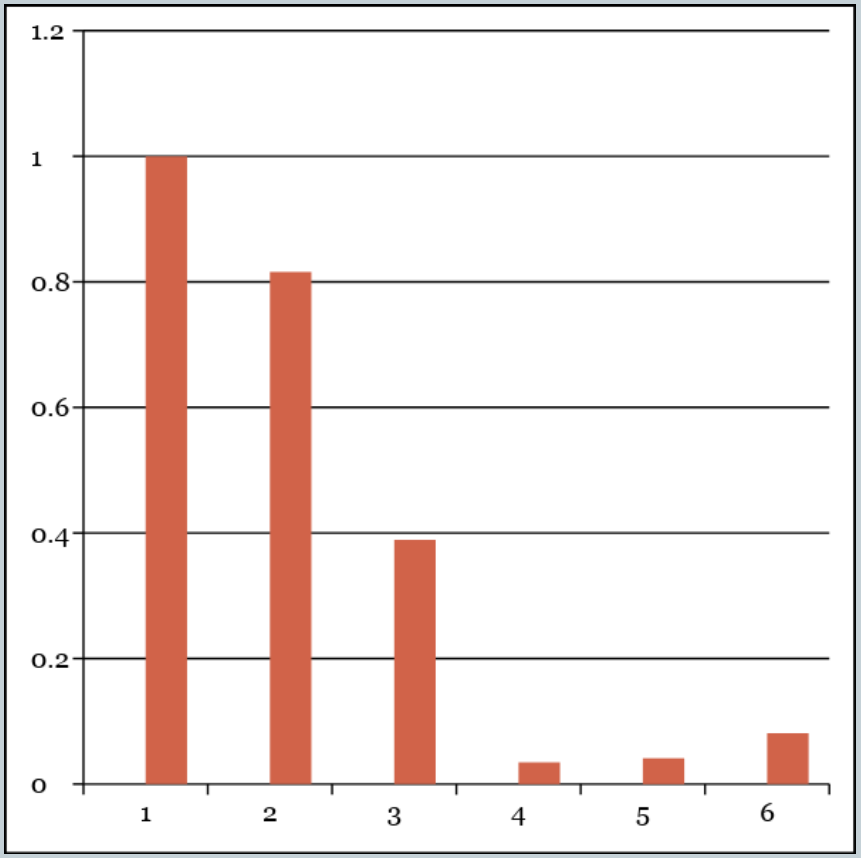
Normal Adult Myocyte Media

Conditioned Stem Cell Media

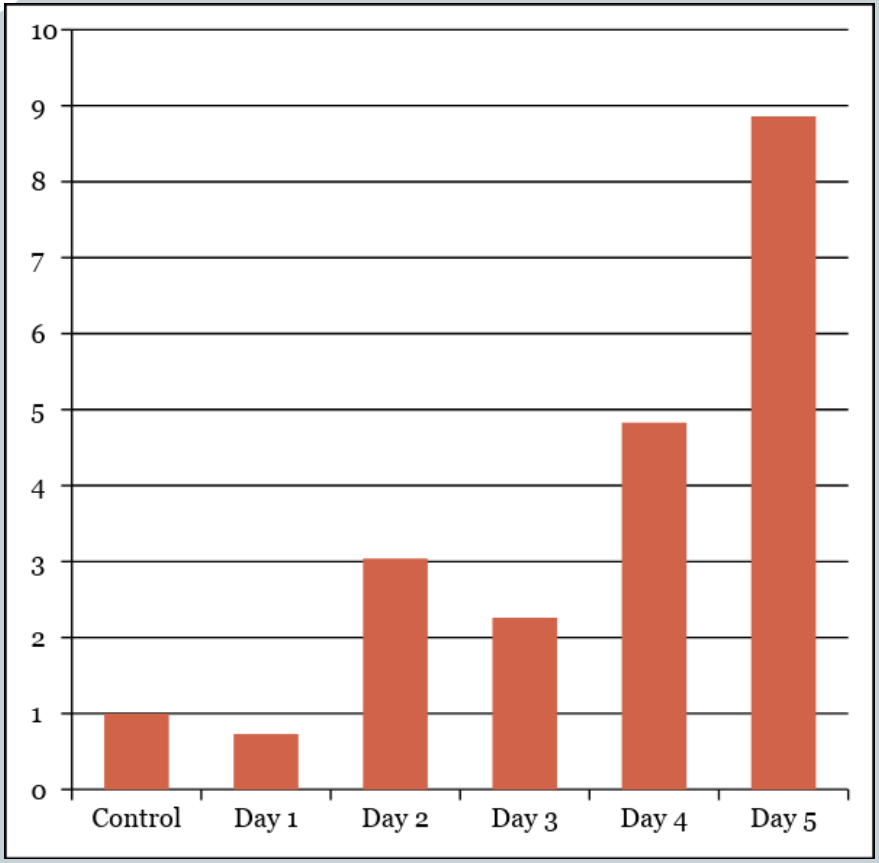


Day 3 Day 4 Day 5

Expression of bMHC mRNA

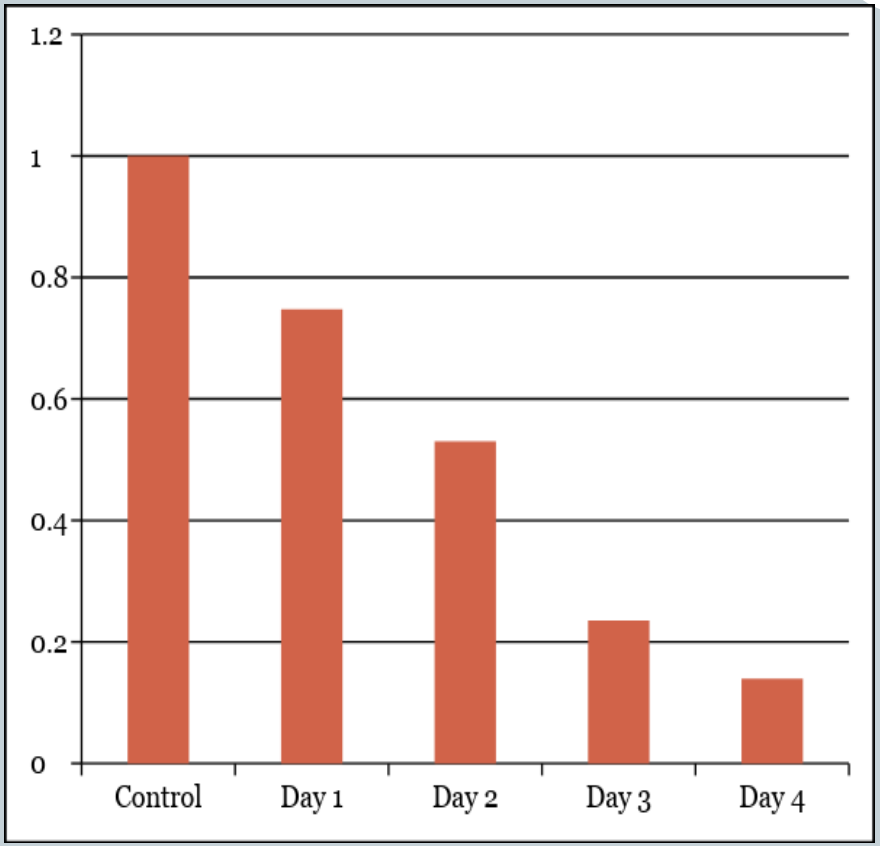


Normal Adult Myocyte Media

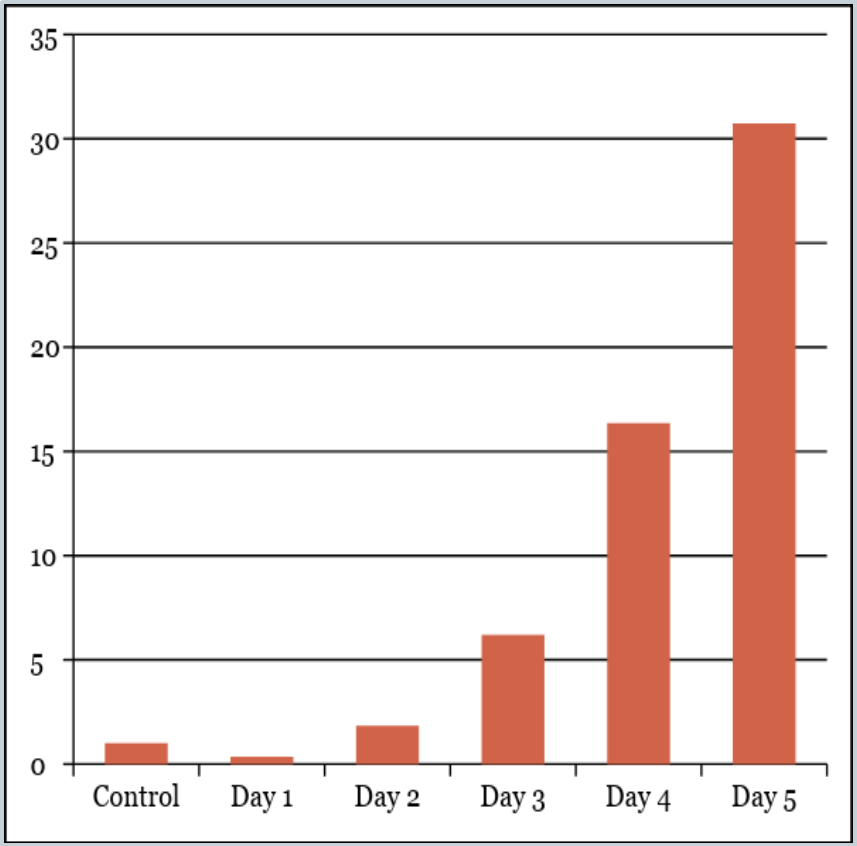


Conditioned Stem Cell Media

Expression of ANF mRNA



Normal Adult Myocyte Media



Conditioned Stem Cell Media

Our cells express neonatal phenotype

Exciting considering previous work in

- Cardiomyocytes derived from mouse embryonic stem cells.
- Primary cultures of foetal cardiomyocytes transplanted into the connective tissue of mice or dogs.
- Cardiomyocyte transplantation from rat neonatal myocytes.

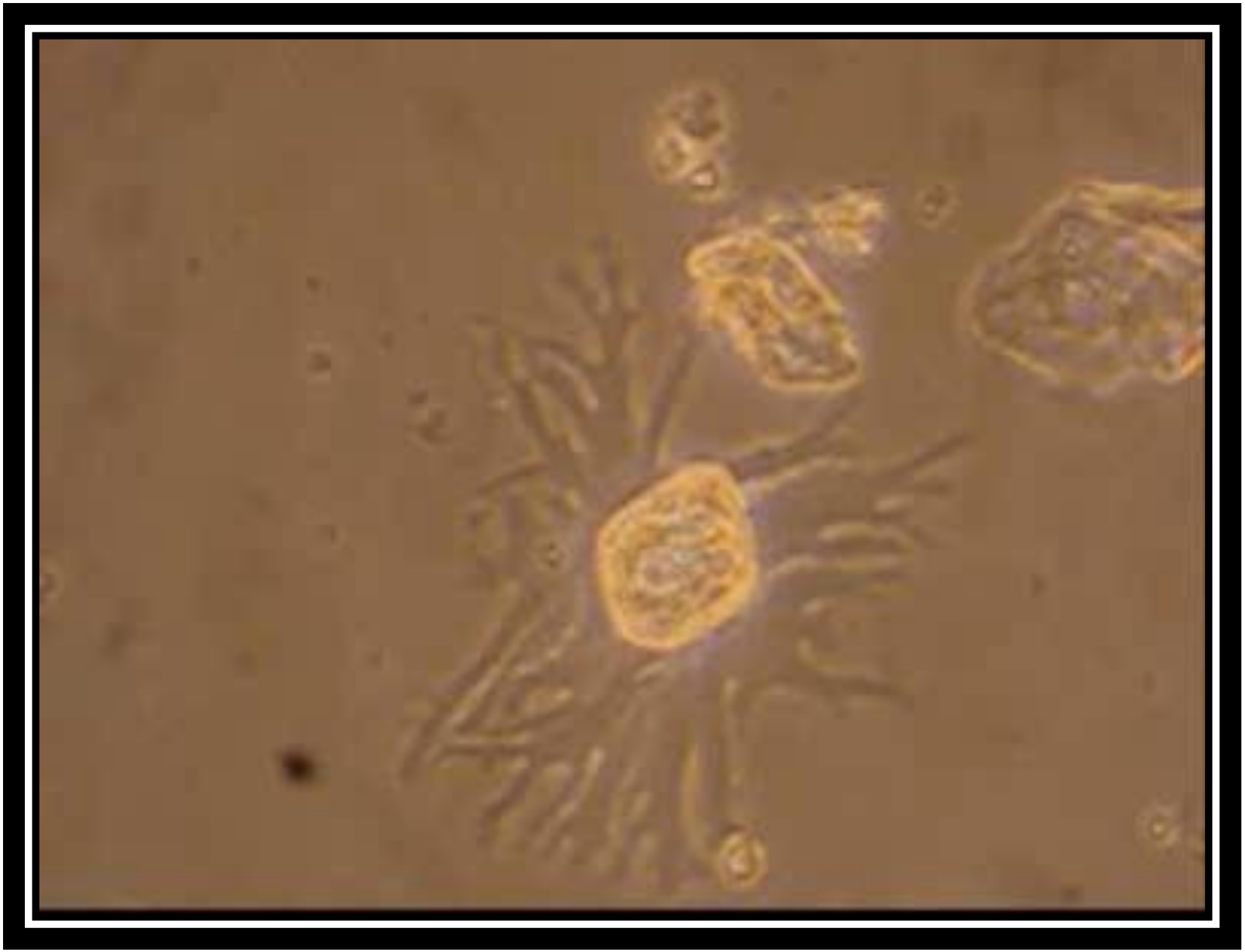
Improved clinical outcomes observed using these myocytes

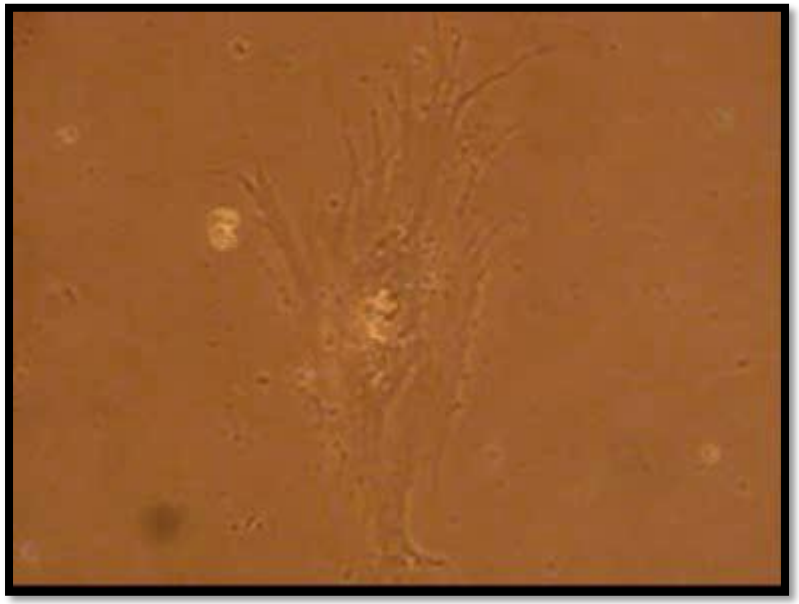
- Associated with smaller infarcts,
- Prevented cardiac dilatation
- Prevented remodelling
- Improved myocardial performance.

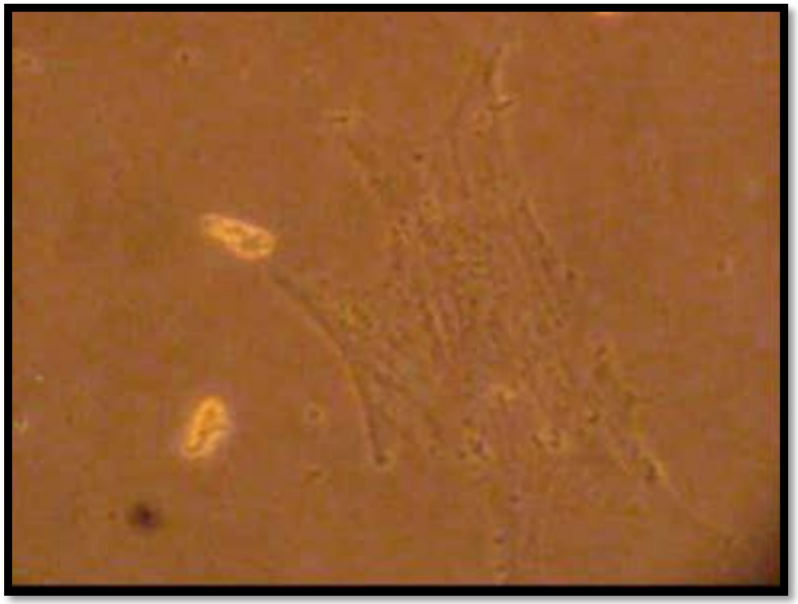
Cryopreservation

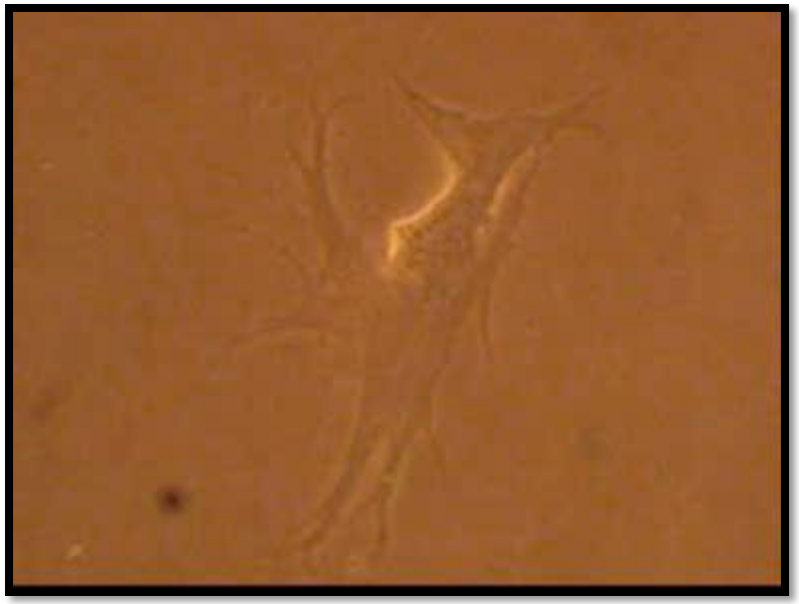
If adult myocytes with neonatal phenotype are to be used to repair the heart they need to be collected from culture once dedifferentiated and to be remain viable once they have been cryopreserved

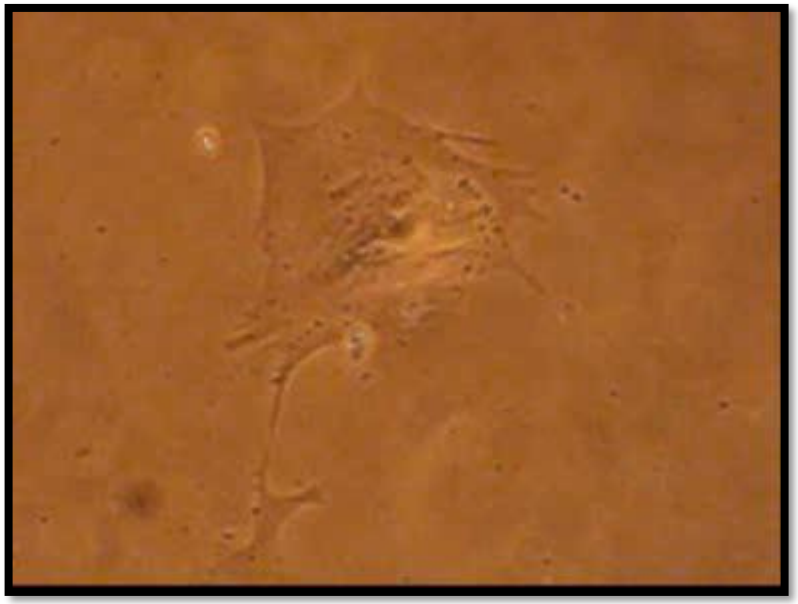
- **Cells cultured for 7 days in conditioned media**
- **Frozen for 72hrs (90% FCS 10% DMSO)**
- **Re-plated and filmed 6hrs later**







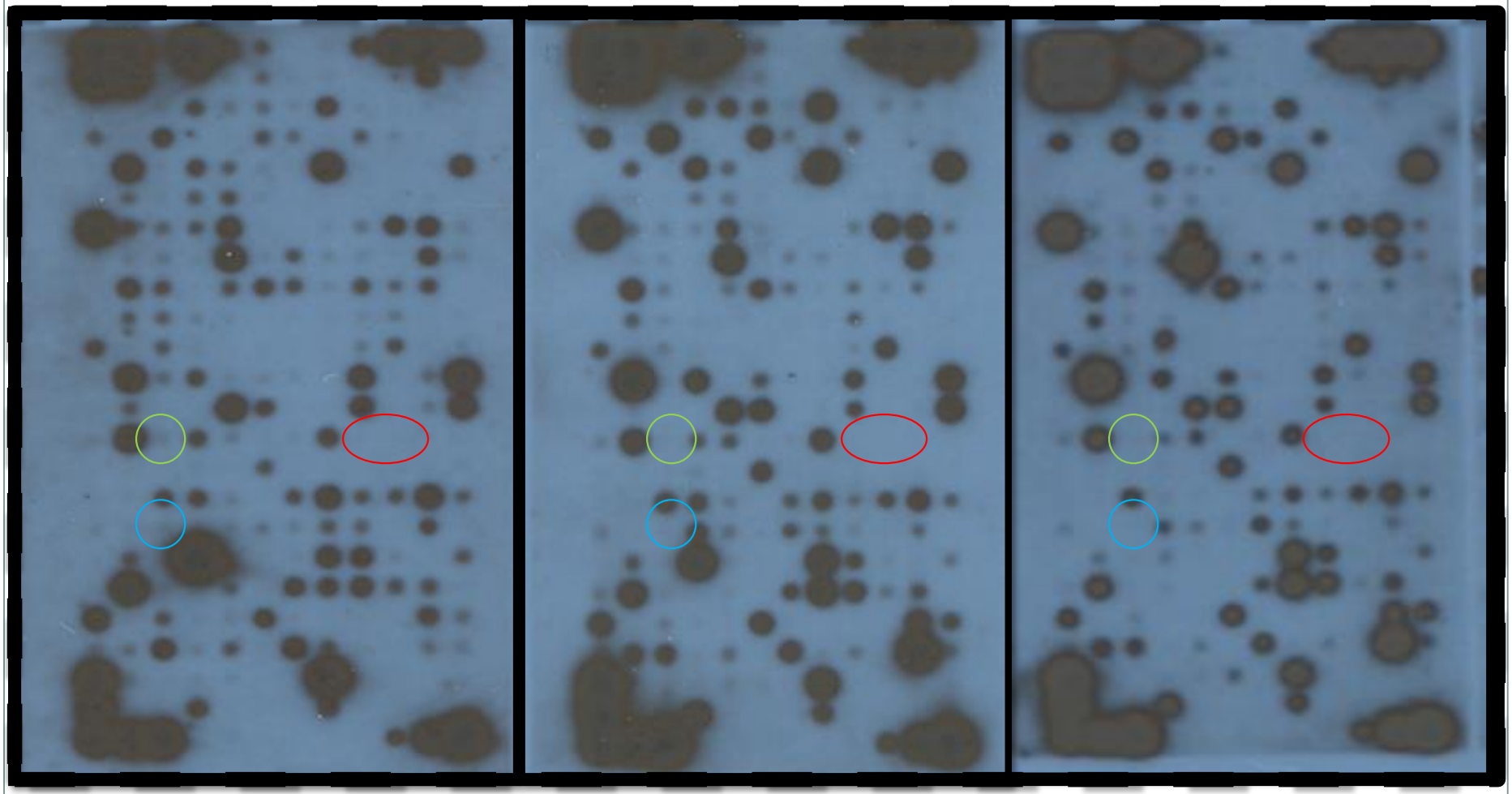




"From a therapeutic standpoint, intrinsic cardiomyocyte proliferation rates as low as 0.05% to 0.1% could, over the course of many months, result in a significant increase in cardiomyocyte number and, consequently, partial restoration of myocardial function in a diseased heart."

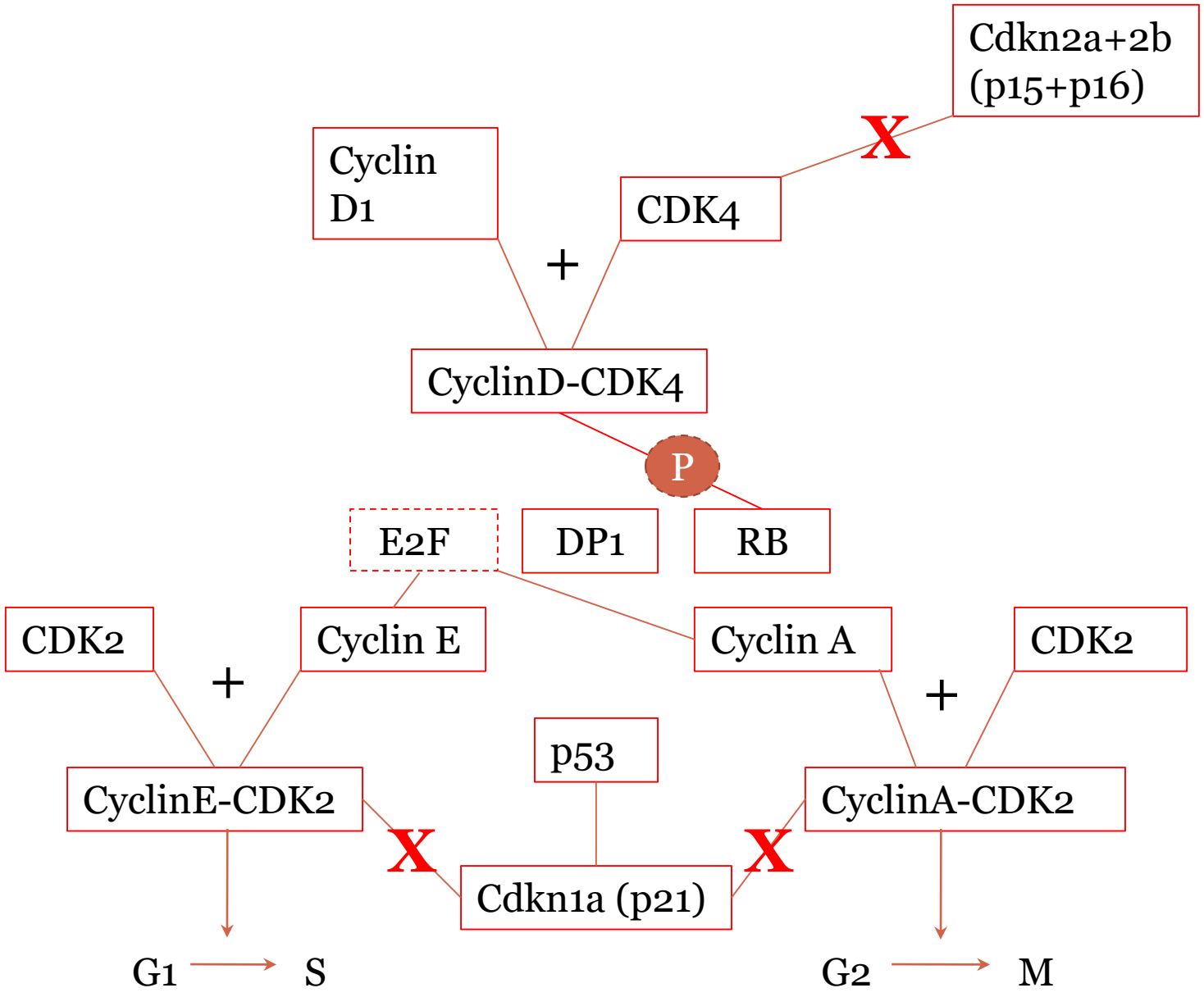
Soonpaa, M.H. and L.J. Field, Survey of studies examining mammalian cardiomyocyte DNA synthesis. Circ Res, 1998. ***83****(1): p. 15-26.*

Day 0 Day 4 Day 14

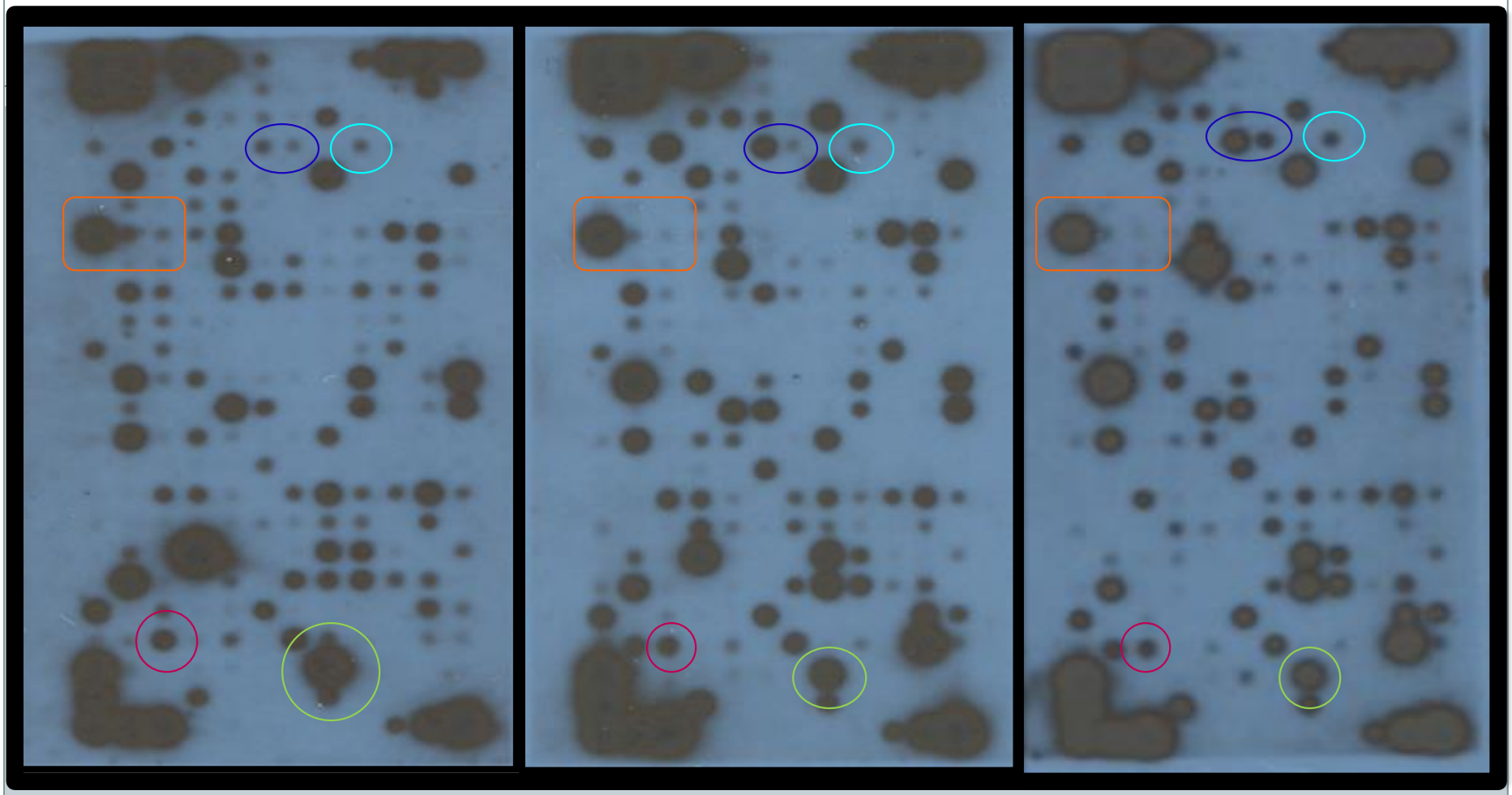


C Kit and receptor
Nanog
Isl-1

Stem cell associated markers

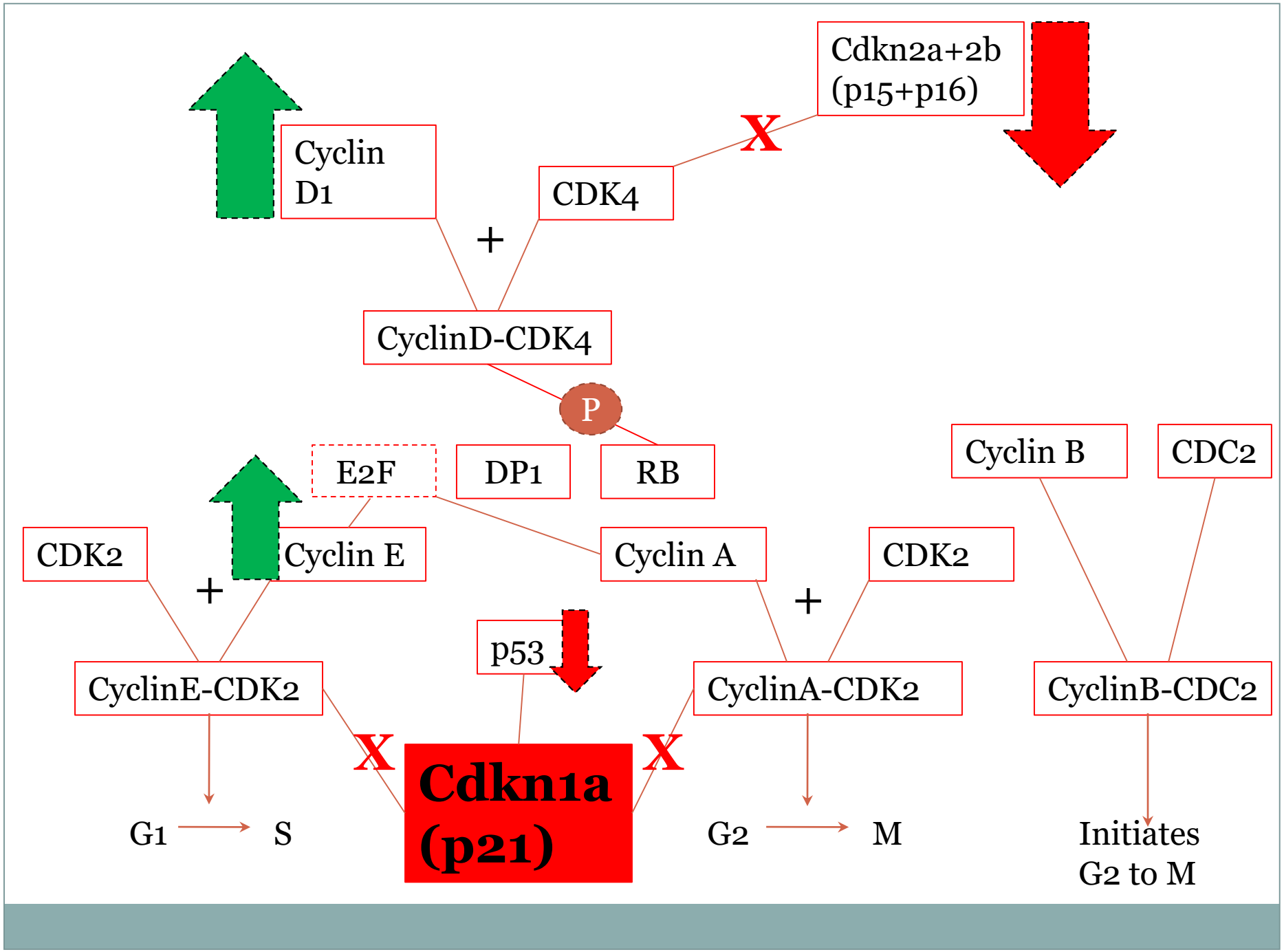

Cdkn2a+2b
(p15+p16)
X
Cyclin
D1
CDK4
+
CyclinD-CDK4
P
E2F
DP1
RB
CDK2
+
Cyclin E
Cyclin A
CDK2
+
p53
CyclinE-CDK2
X
X
CyclinA-CDK2
Cdkn1a (p21)
G1
S
G2
M

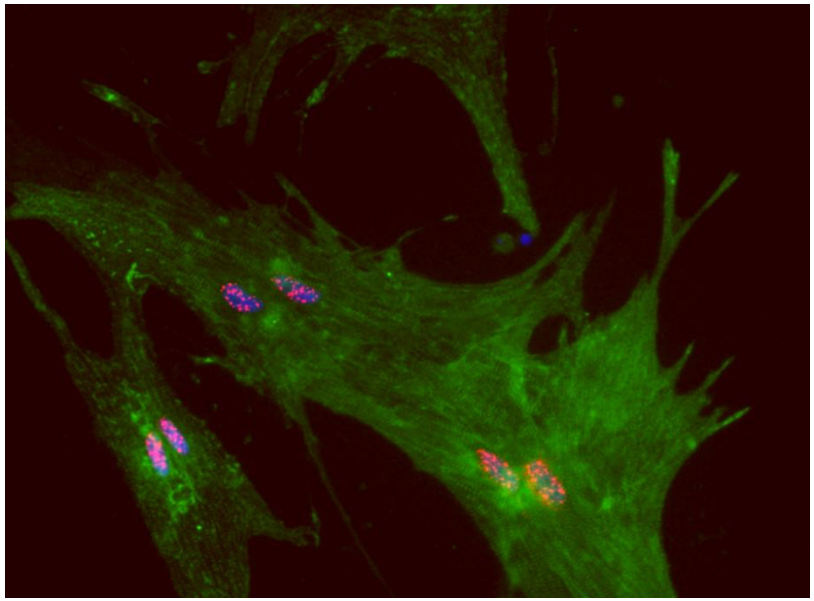
Day 0 Day 4 Day 14

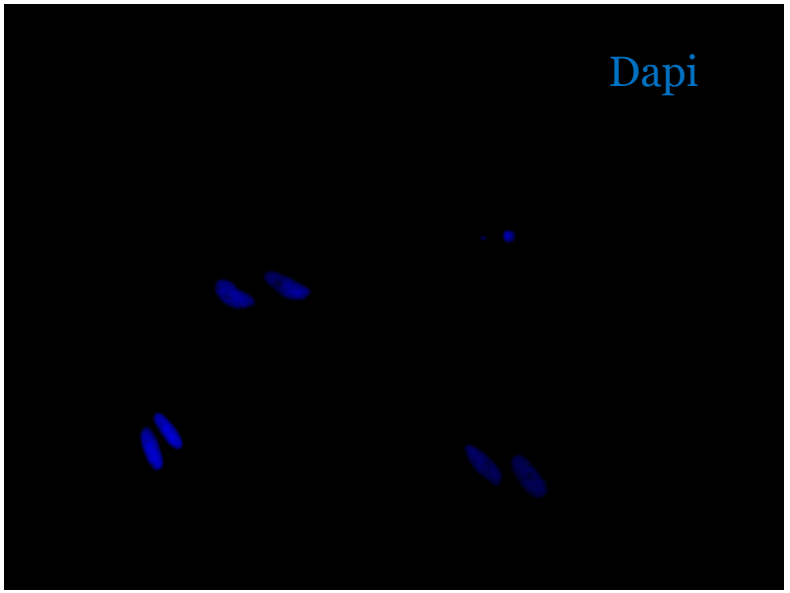


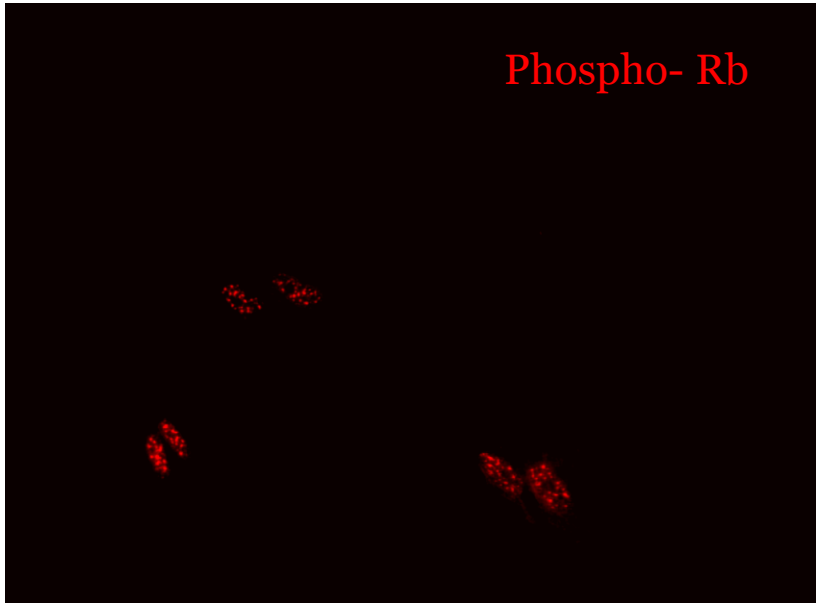
Cyclin D1, D2
Cyclin E

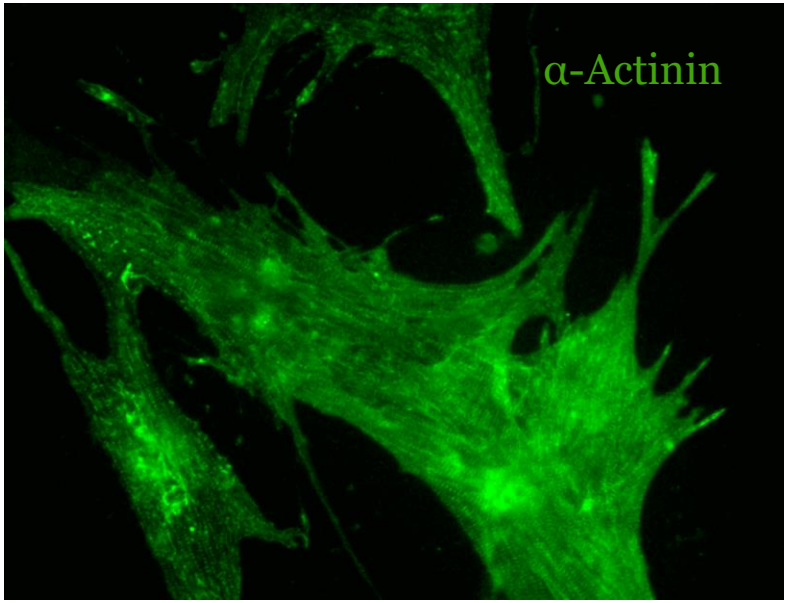
p21, p16,p15
Rb **p53**


Cdkn2a+2b
(p15+p16)
X
Cyclin
D1
CDK4
+
CyclinD-CDK4
P
E2F
DP1
RB
Cyclin B
CDC2
CDK2
Cyclin E
Cyclin A
CDK2
+
+
p53
CyclinE-CDK2
CyclinA-CDK2
CyclinB-CDC2
X
X
Cdkn1a
(p21)
G1
S
G2
M
Initiates
G2 to M

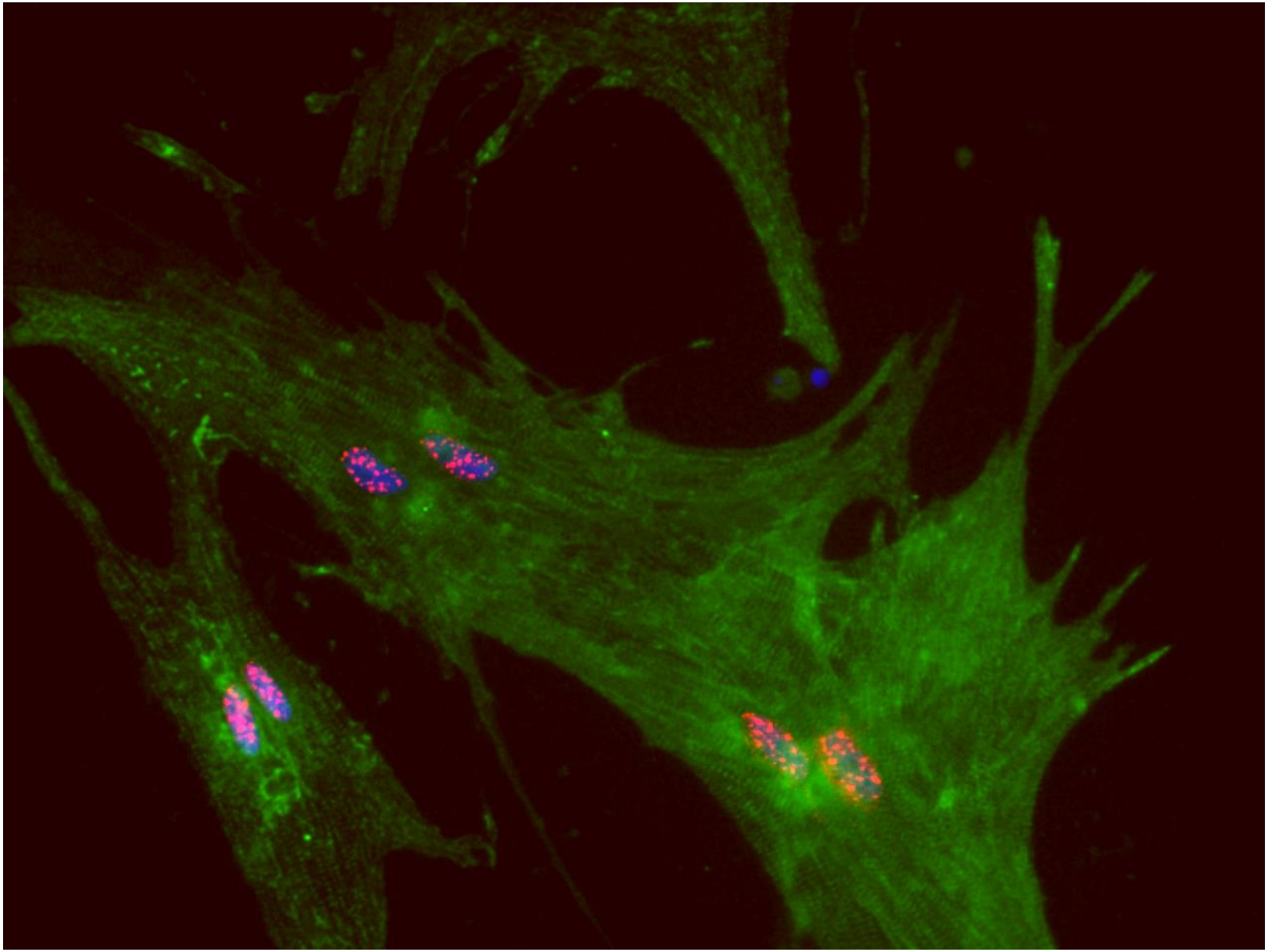


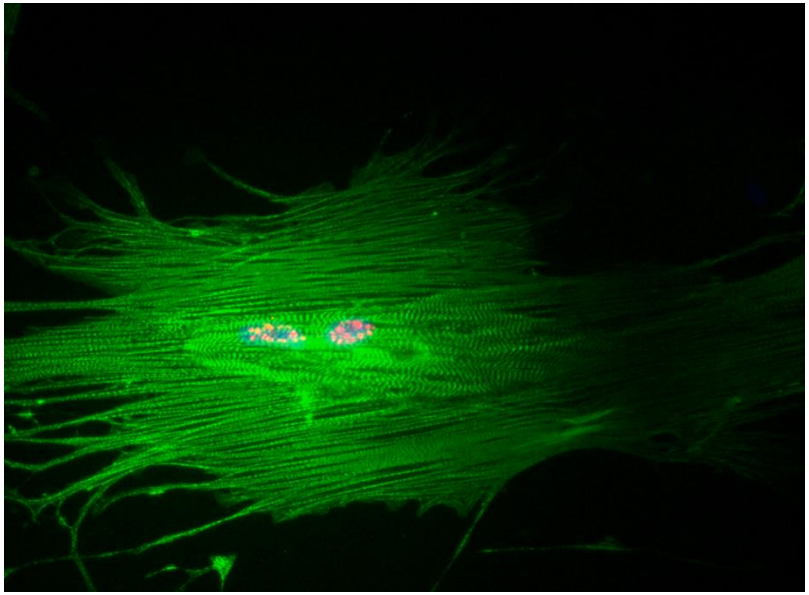


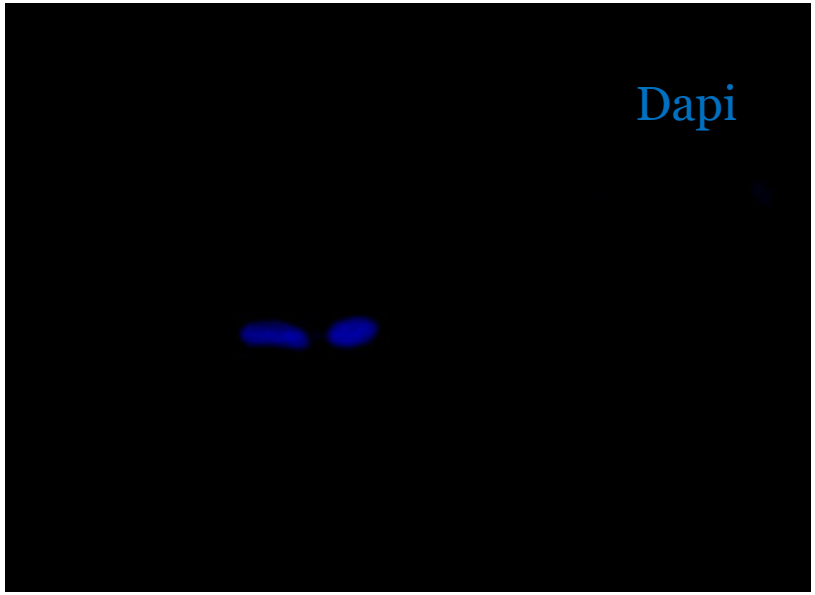


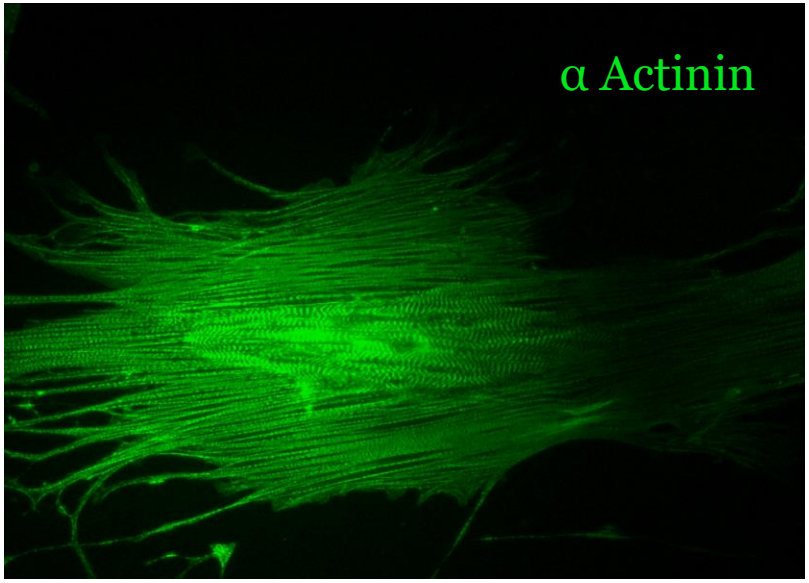


4day untreated cells

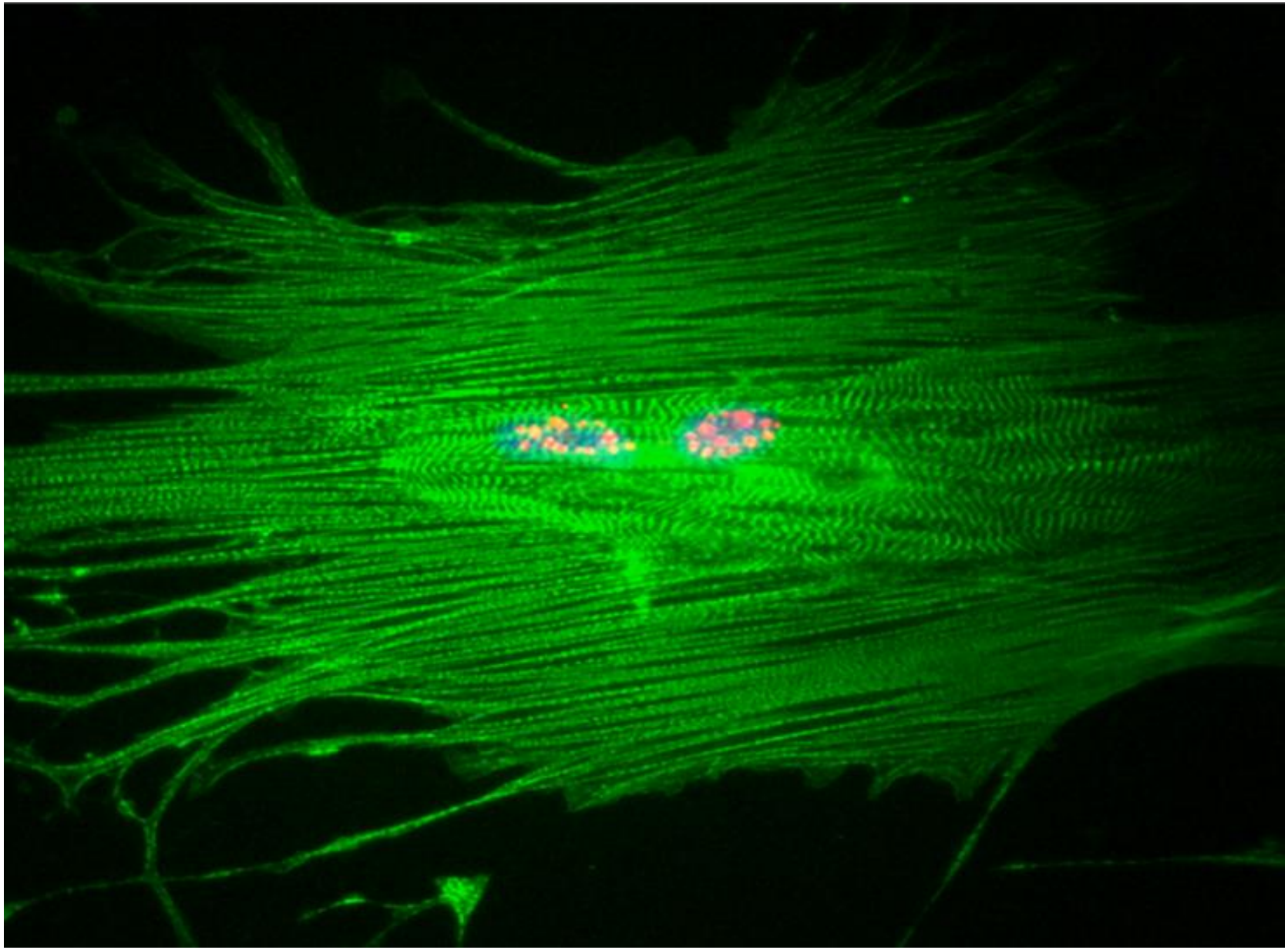








Untreated 4 dys



Conclusion

- Secreted factors from cardiac progenitor cells appear to rapidly dedifferentiate adult myocytes to a neonatal phenotype and morphology.
- Secreted factors from cardiac progenitor cells may have a protective paracrine effect on adult myocytes.
- Dededifferentiated adult myocytes can be cryopreserved and regain contractile properties.
- **Dededifferentiated adult myocytes have a greater potential to proliferate.**

Future

- Can adult dedifferentiated cells integrate into host myocardium?
- Can this conditioned media cause dedifferentiation in vivo?
- Can injecting conditioned media encourage proliferation to repair the injured myocardium?
- Can paracrine factors from cardiac progenitor cells have a protective effect on injured myocardium?