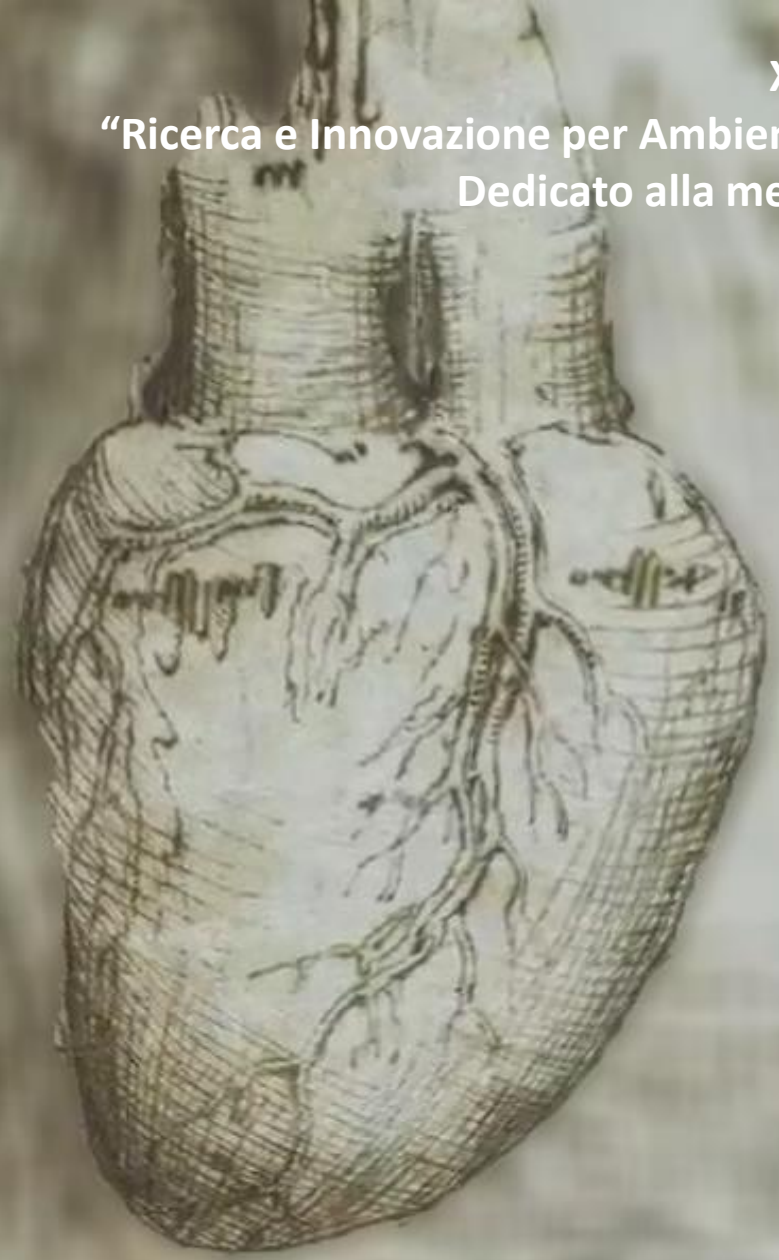
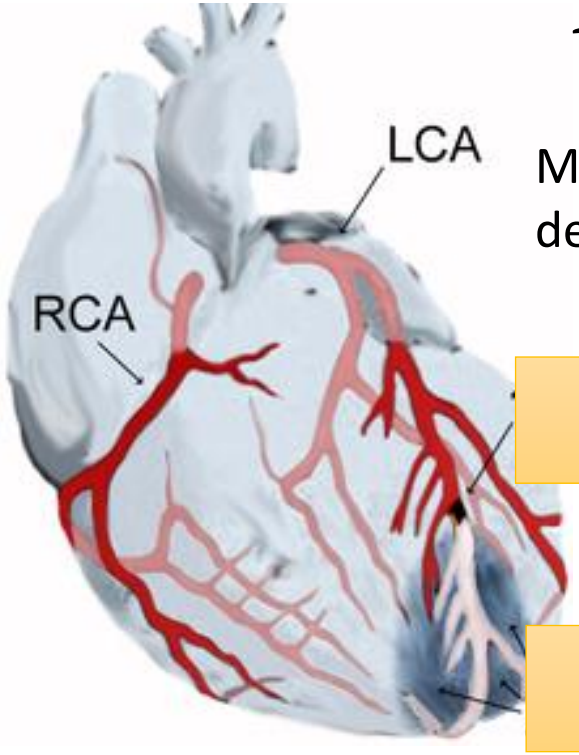
XIV Convegno Nazionale INBB
“Ricerca e Innovazione per Ambiente, Salute ed Alimentazione”
Dedicato alla memoria del Prof. Gustavo Mita
Roma
18.11.2022

Cardiogenesi diretta: nuove strategie molecolari per la rigenerazione del cuore



Gabriele D’Uva
University of Bologna
Bologna, Italy

The need for cardiac regenerative strategies



Myocardial infarction along with the consequent heart failure is a leading cause of death worldwide.

Myocardial infarction

↓

- **Death and loss of cardiomyocytes**
- **Formation of scar tissue**

↓

Inability of the heart to function properly, heart failure and death

THE ADULT MAMMALIAN HEART IS UNABLE TO REGENERATE FOR TWO MAJOR REASONS:

- **the absence of biologically important endogenous cardiac stem cell populations**
- **the very low proliferative rate of adult mammalian cardiomyocytes**

The development of cardiac regenerative strategies is an urgent clinical need, which could help million of heart failure patients worldwide

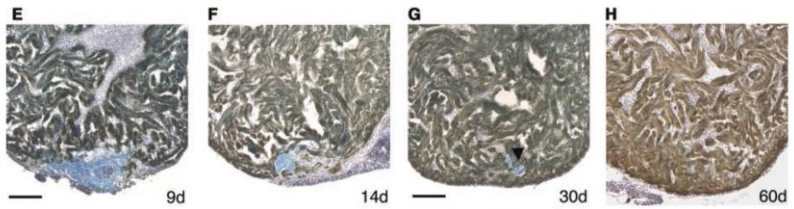
Spontaneous heart regeneration in zebrafish via proliferation of endogenous cardiomyocytes

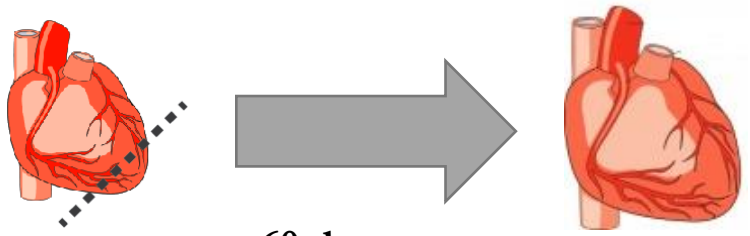
Science AAAS

Heart Regeneration in Zebrafish

Kenneth D. Poss,* Lindsay G. Wilson, Mark T. Keating*

2002





60 days

zebrafish heart injury

SPONTANEOUS CARDIAC REGENERATION

nature LETTERS

2010

Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation

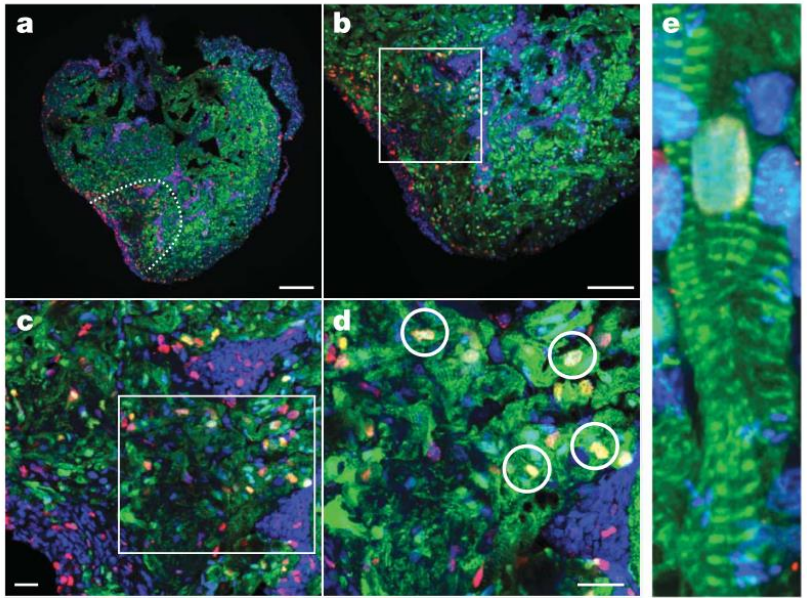
Chris Jopling[1], Eduard Sleep[1,2]†, Marina Raya[1]†, Mercè Martí[1], Angel Raya[1,2,3]† & Juan Carlos Izpisúa Belmonte[1,2,4]

nature LETTERS

2010

Primary contribution to zebrafish heart regeneration by *gata4*$^{+}$ cardiomyocytes

Kazu Kikuchi, Jennifer E. Holdway, Andreas A. Werdich, Ryan M. Anderson, Yi Fang, Gregory F. Egnaczyk, Todd Evans, Calum A. MacRae, Didier Y. R. Stainier & Kenneth D. Poss

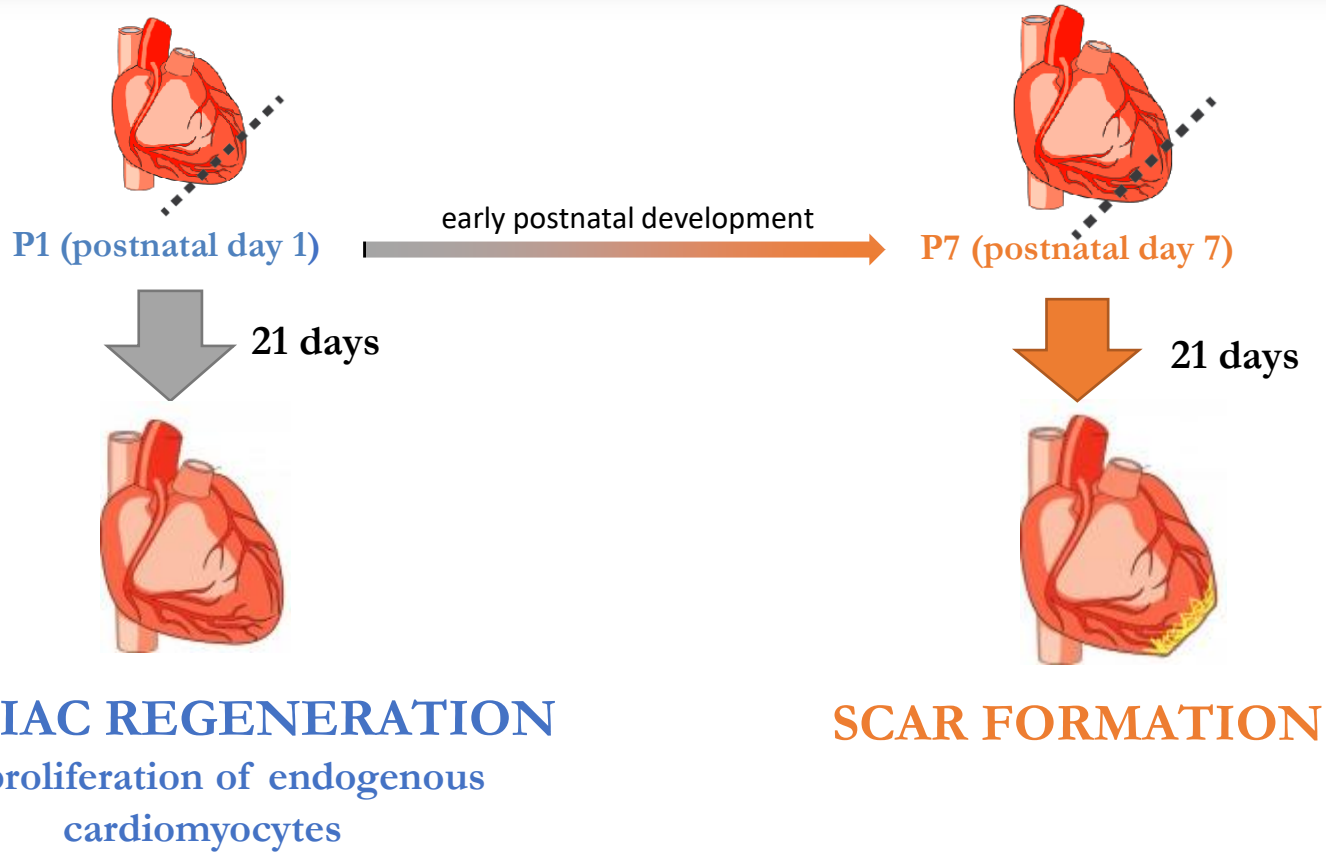


Transient Regenerative Potential of the Neonatal Mouse Heart

Enzo R. Porrello[1], Ahmed I. Mahmoud[2], Emma Simpson[3], Joseph A. Hill[1,2], James A. Richardson[1,3], Eric N. Olson[1,*], Hesham A. Sadek[2,*]

Science, 2011

Science
AAAS



Cardiac regeneration is possible in mammals,

however it seems restricted in the early post-natal period

A low rate of cardiomyocyte renewal exists even in adult mammals

LETTER

nature

LETTERS

Mammalian heart renewal by pre-existing cardiomyocytes

Samuel E. Senyo[1], Matthew L. Steinhauser[1], Christie L. Pizzimenti[1], Vicky K. Yang[1], Lei Cai[1], Mei Wang[4,5], Ting-Di Wu[2,3], Jean-Luc Guerquin-Kern[2,3], Claude P. Lechene[4,5] & Richard T. Lee[1,6]

Science, 2013

Science

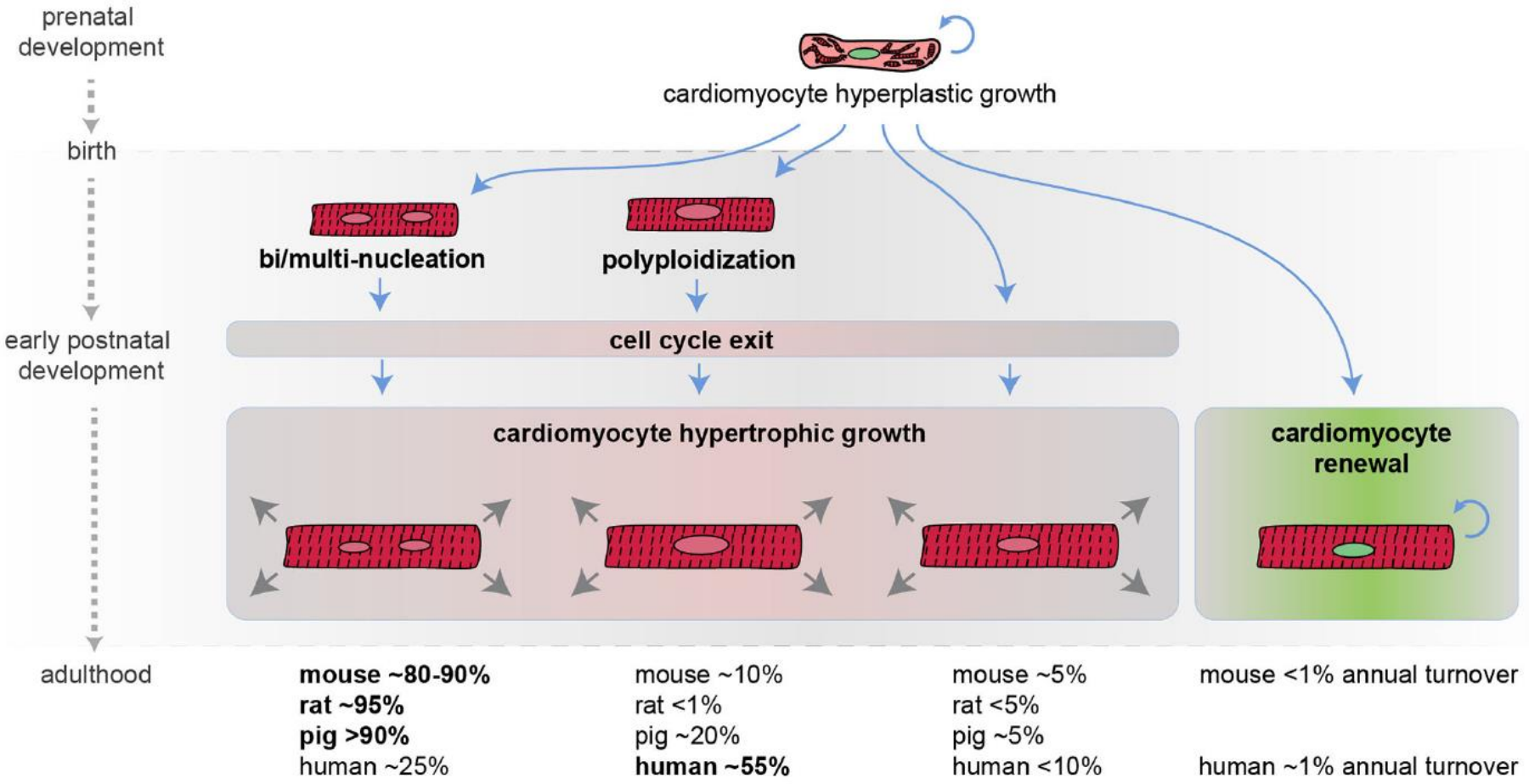
AAAS

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]†

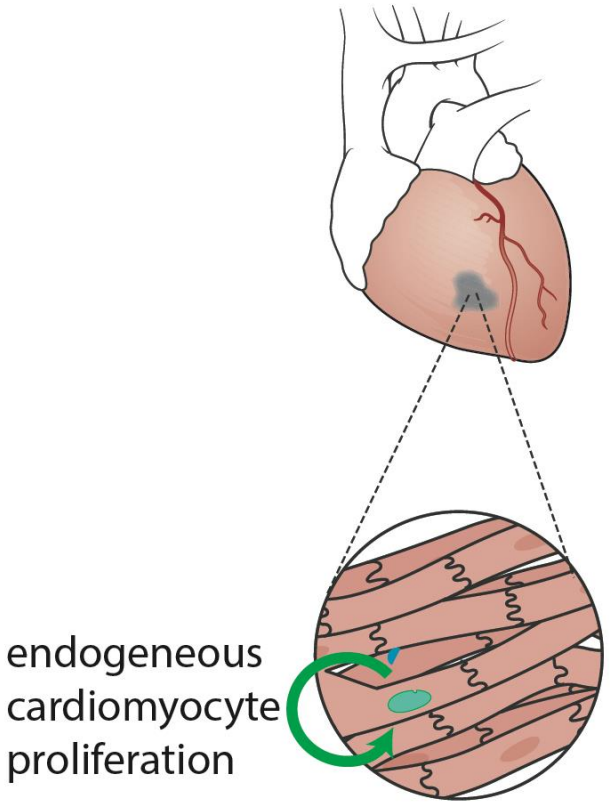
Science, 2009

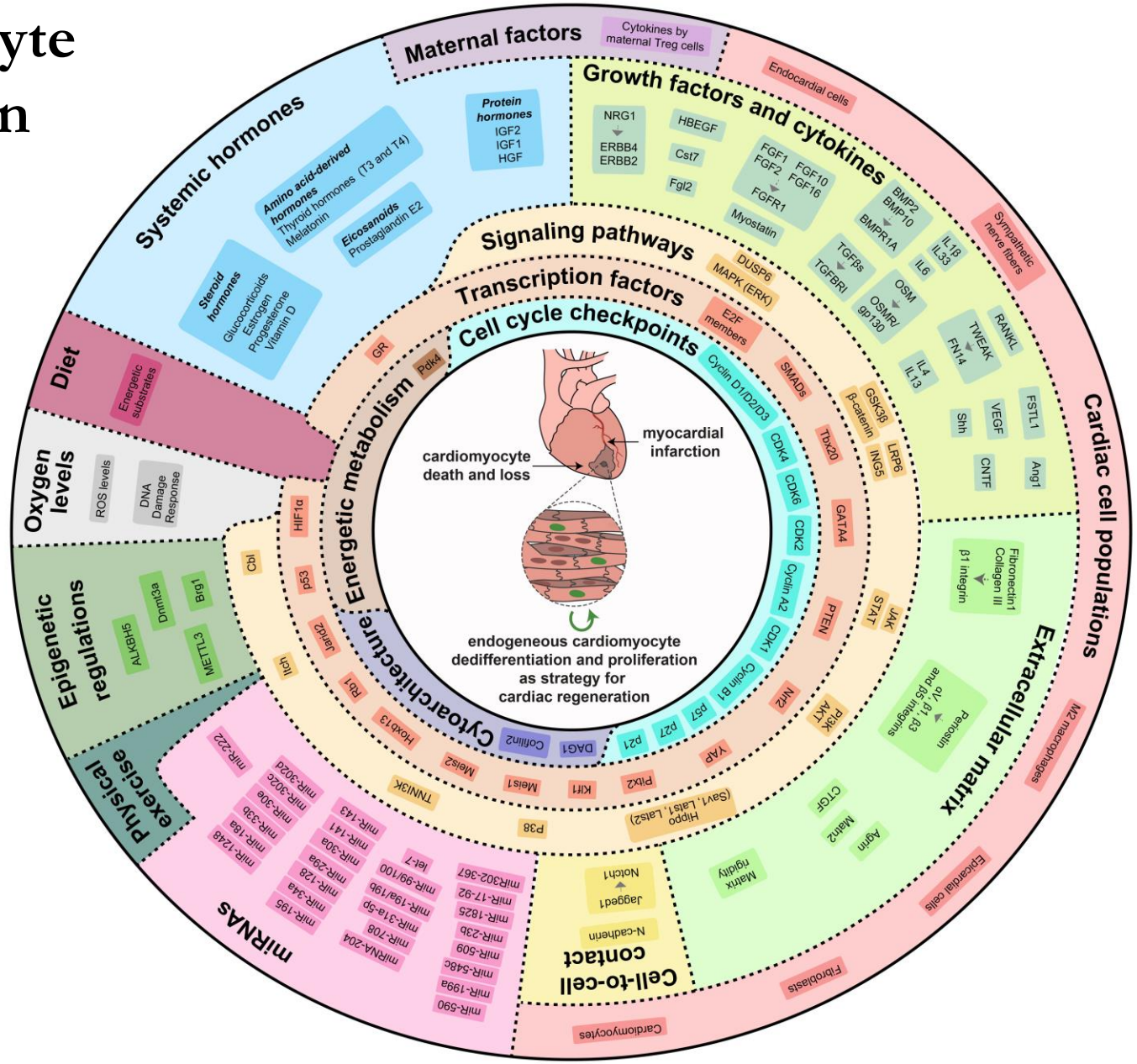
Most mammalian cardiomyocytes exit from the cell cycle during the early postnatal development



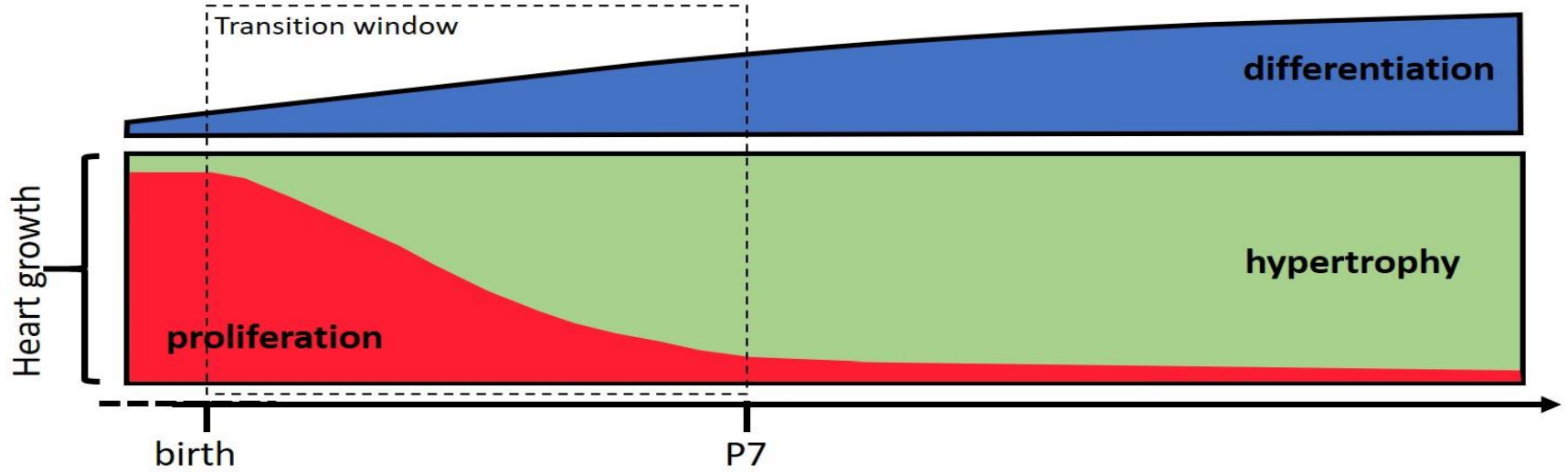
reviewed in Bongiovanni … & D'Uva, Frontiers in Cardiovascular Medicine, 2021

Direct stimulation of cardiomyocyte dedifferentiation and proliferation as a novel approach for cardiac regeneration





reviewed in Bongiovanni … & D'Uva, Frontiers in Cardiovascular Medicine, 2021

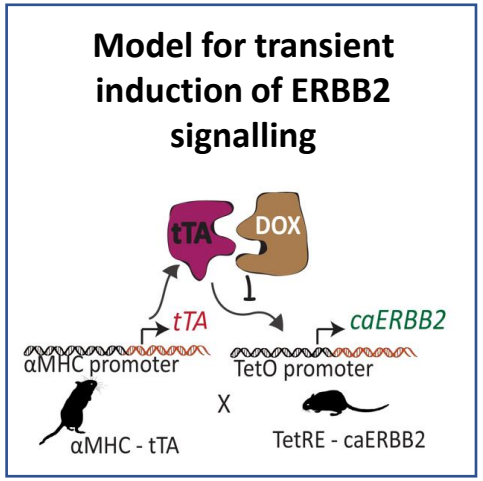


Several adaptations from intrauterine to extrauterine life starting at birth and continuing in the immediate neonatal period are known to induce cardiomyocyte maturation, cell cycle exit and hypertrophic growth, concurring to the loss of the mammalian cardiac regenerative ability.

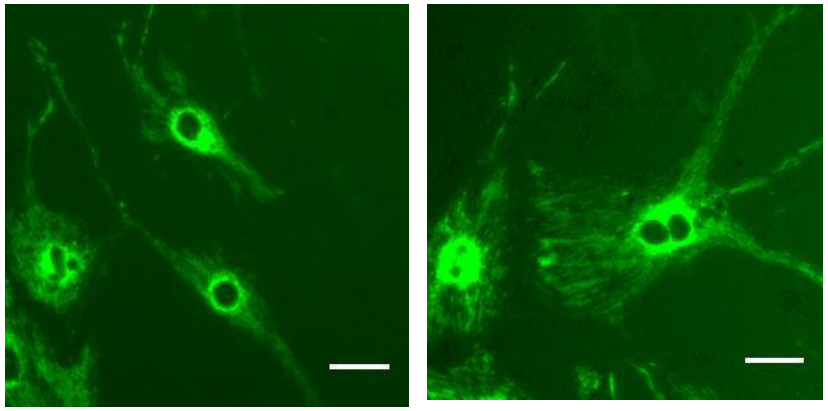
Do hormones and growth factors play a role in this transition?

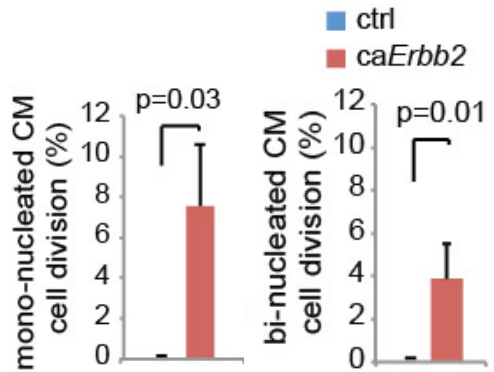
reviewed in Bongiovanni … & D'Uva, Frontiers in Cardiovascular Medicine, 2021

Reactivation of the co-receptor Erbb2 promotes cardiomyocyte proliferation and heart regeneration

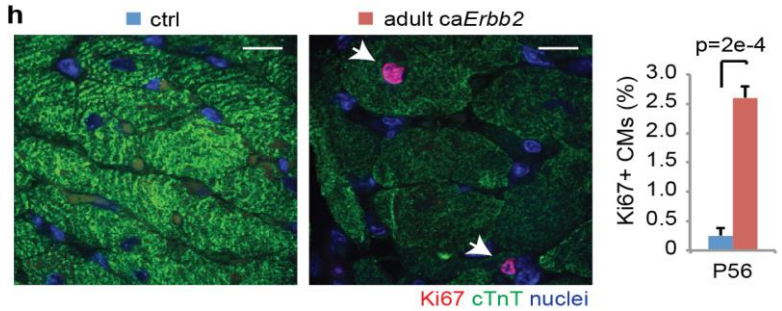


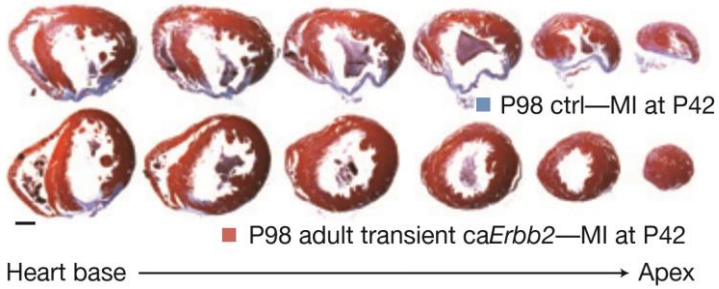
1. Induction of ERBB2 signaling induces **cell division of mono-nucleated and bi-nucleated post-mitotic cardiomyocytes**

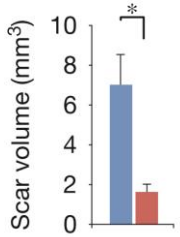




2. Transient induction of ERBB2 signaling in cardiomyocytes **shortly after myocardial infarction** in adult mice is sufficient to promote **cardiomyocyte proliferation and cardiac regeneration**

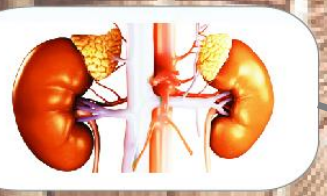






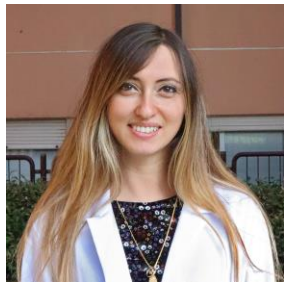
D'Uva, […] and Tzahor, Nature Cell Biology, 2015

Novel regulators of cardiomyocyte regenerative plasticity



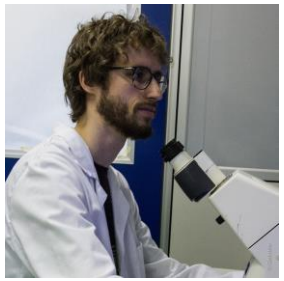
Cardiac growth factors (in particular BMP7)

Bongiovanni … & D'Uva (under consideration)

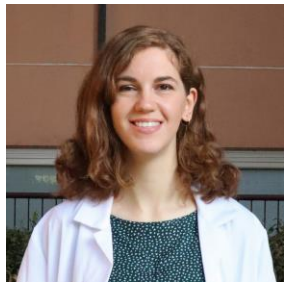


Chiara Bongiovanni

Hormones (glucocorticoids)



Nicola Pianca



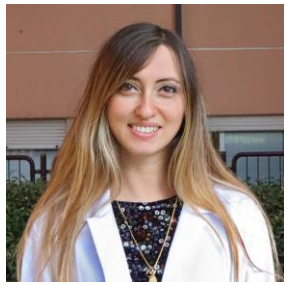
Francesca Sacchi

Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

Novel regulators of cardiomyocyte regenerative plasticity

Cardiac growth factors (in particular BMP7)

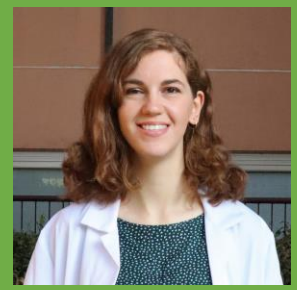
Bongiovanni … & D'Uva (under consideration)



Chiara Bongiovanni

Hormones (glucocorticoids)

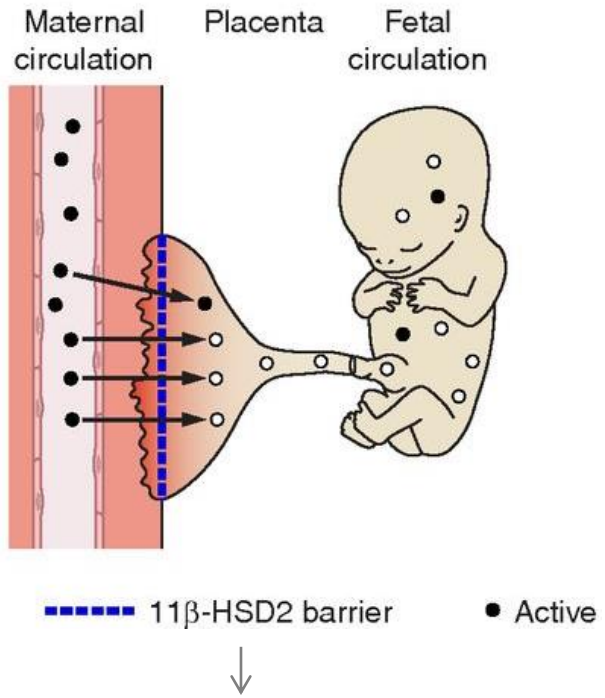
Nicola Pianca



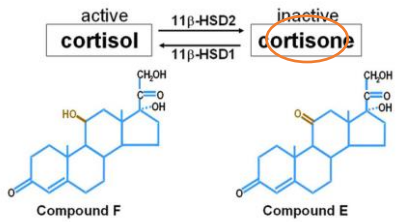
Francesca Sacchi

Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

Active glucocorticoids (GCs) rise dramatically shortly before birth to promote the maturation of the lungs and other organs



Corticosteroid 11-beta-dehydrogenase isozyme 2



GC activity in the fetus is quite low

Shortly before birth

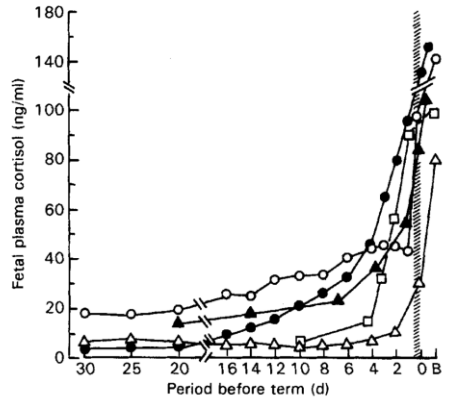


Fig. 1. Mean fetal concentrations of plasma cortisol with respect to time period (d) from delivery in the sheep (●), pig (○), man (▲), guinea-pig (□), and horse (△). (▩), Labour; B, birth. (Data from Silver & Fowden, 1988.)

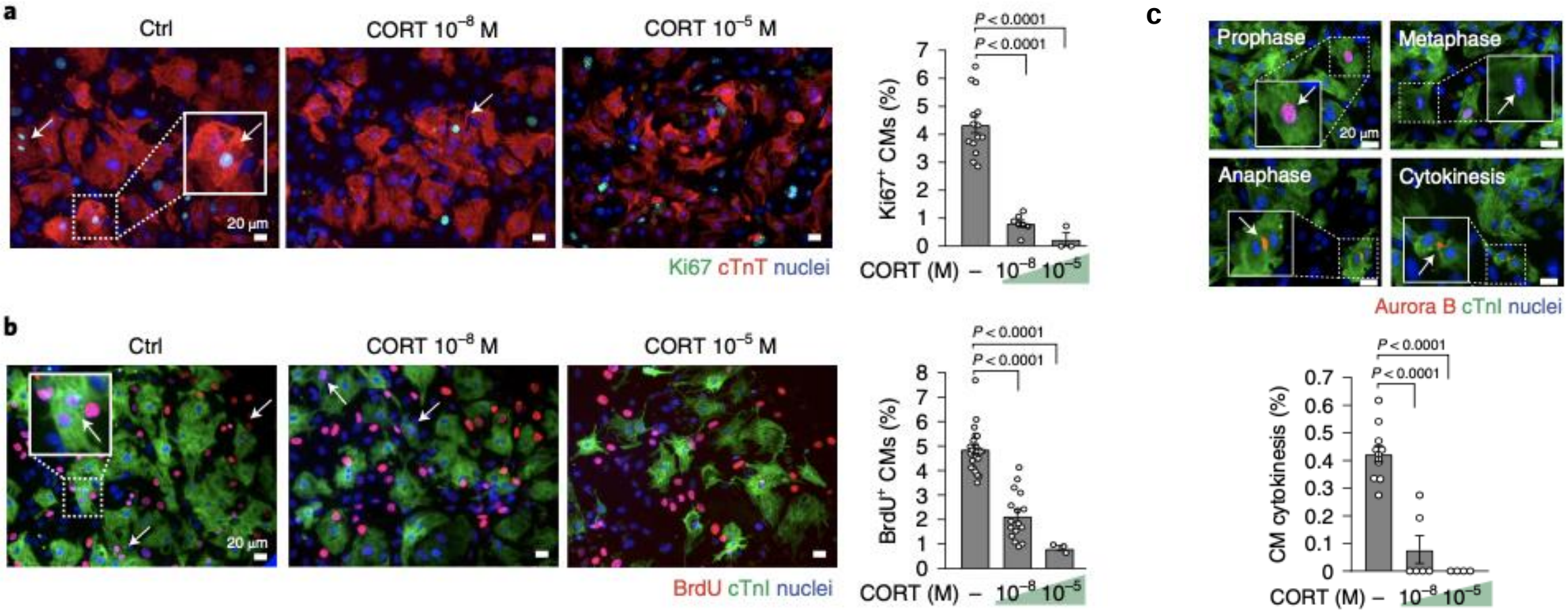
circulating active GCs
(corticosterone in mice and cortisol in humans)

maturation of the lungs

cardiomyocyte maturation in late gestation

Do glucocorticoids regulate postnatal cardiomyocyte differentiation thus impacting on their proliferative ability?

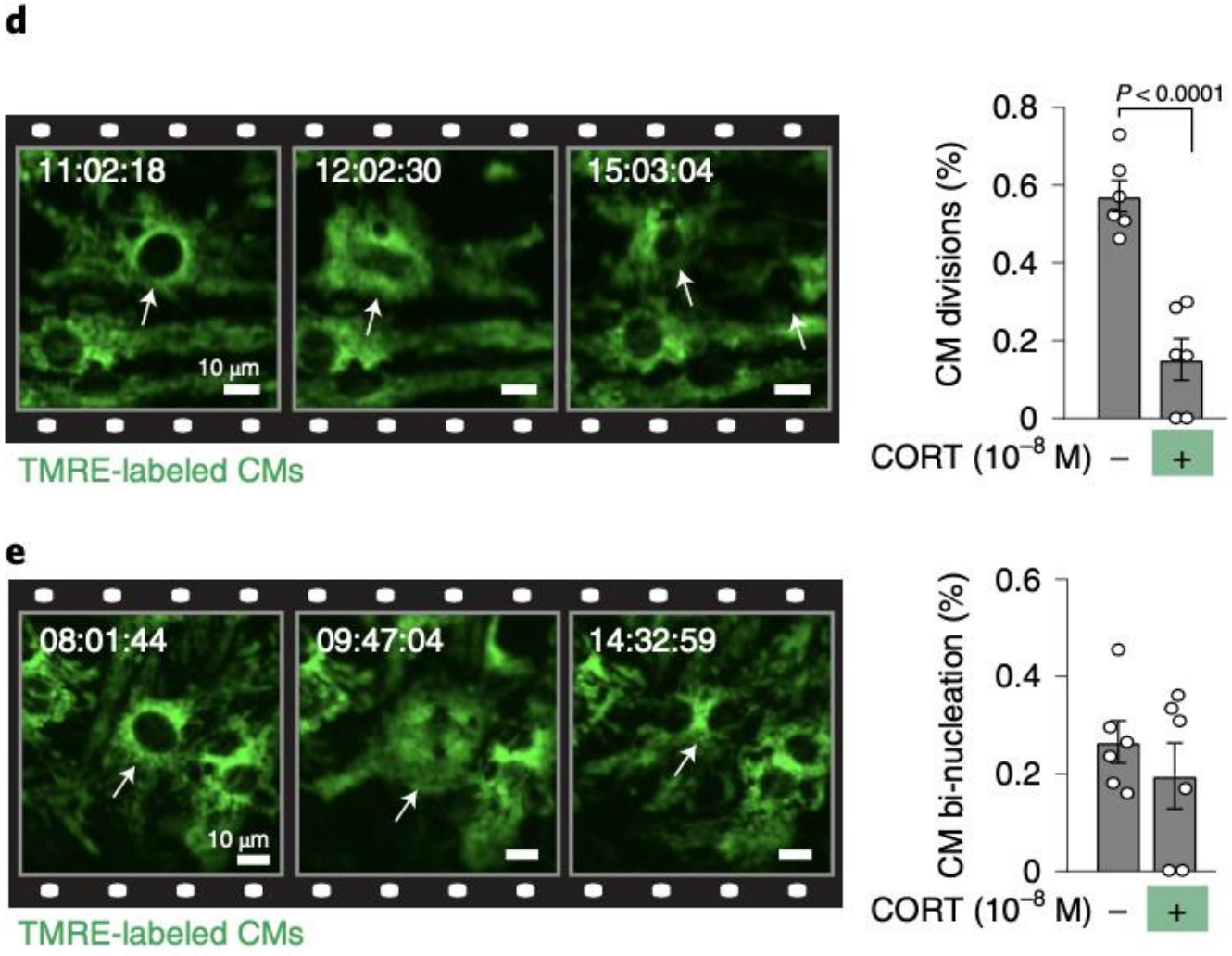
Endogenous glucocorticoids restrain the proliferative ability of neonatal cardiomyocytes



CORT = major endogenous glucocorticoid in the mouse model

Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

Endogenous glucocorticoids selectively reduces cardiomyocyte cell division rather than cell binucleation

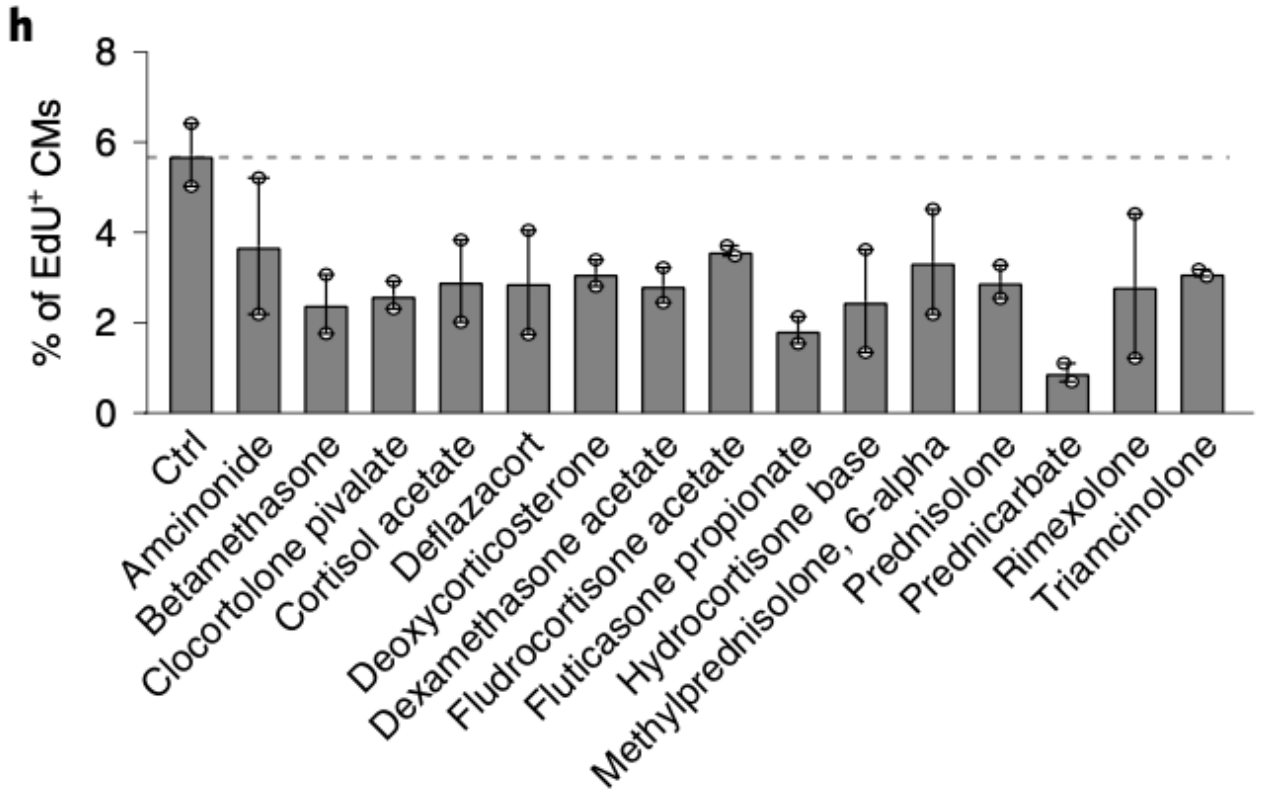


CORT = major endogenous glucocorticoid in the mouse model

Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

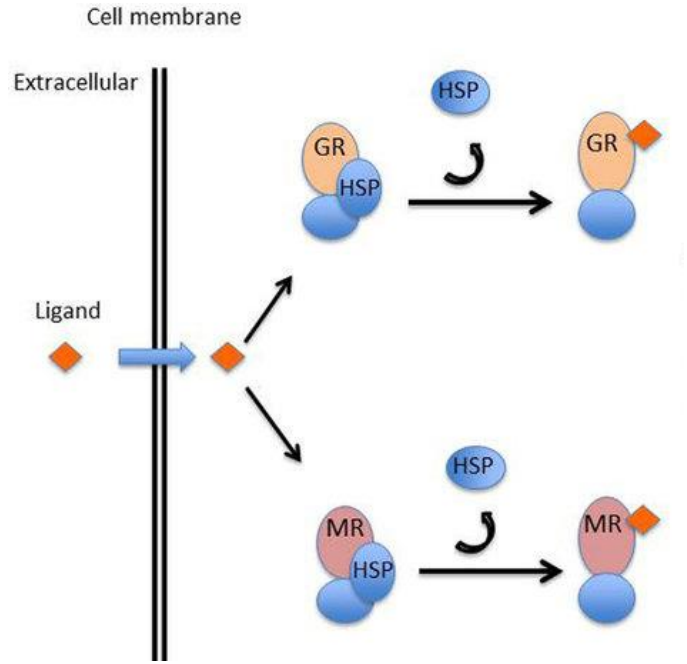
Synthetic glucocorticoids reduces the proliferation of neonatal cardiomyocytes

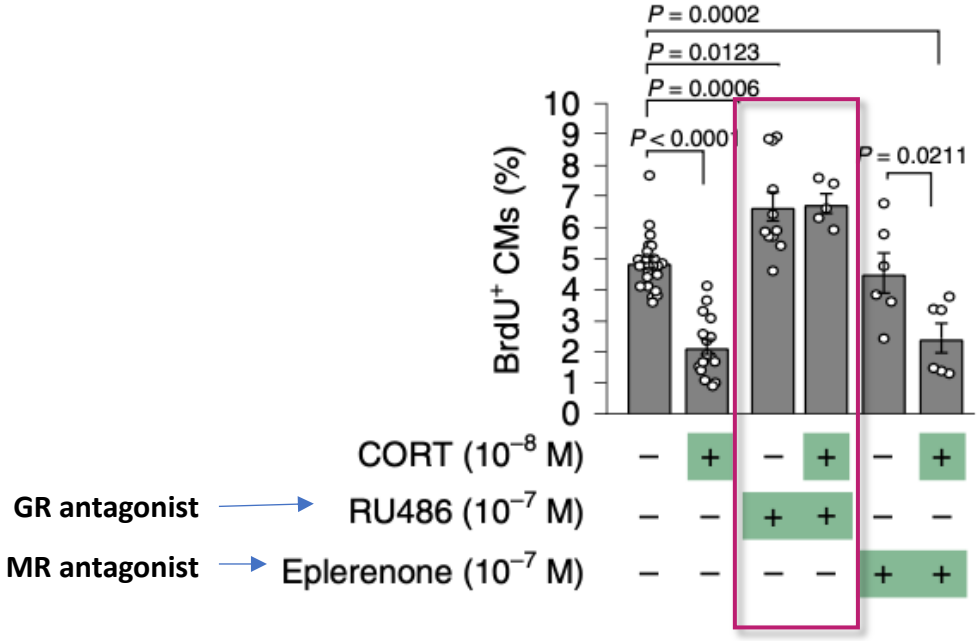
in collaboration with Prof. Giacca lab (King's College, UK)



Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

Endogenous glucocorticoids reduces the proliferation of neonatal cardiomyocytes via activation of the glucocorticoid receptor (GR)

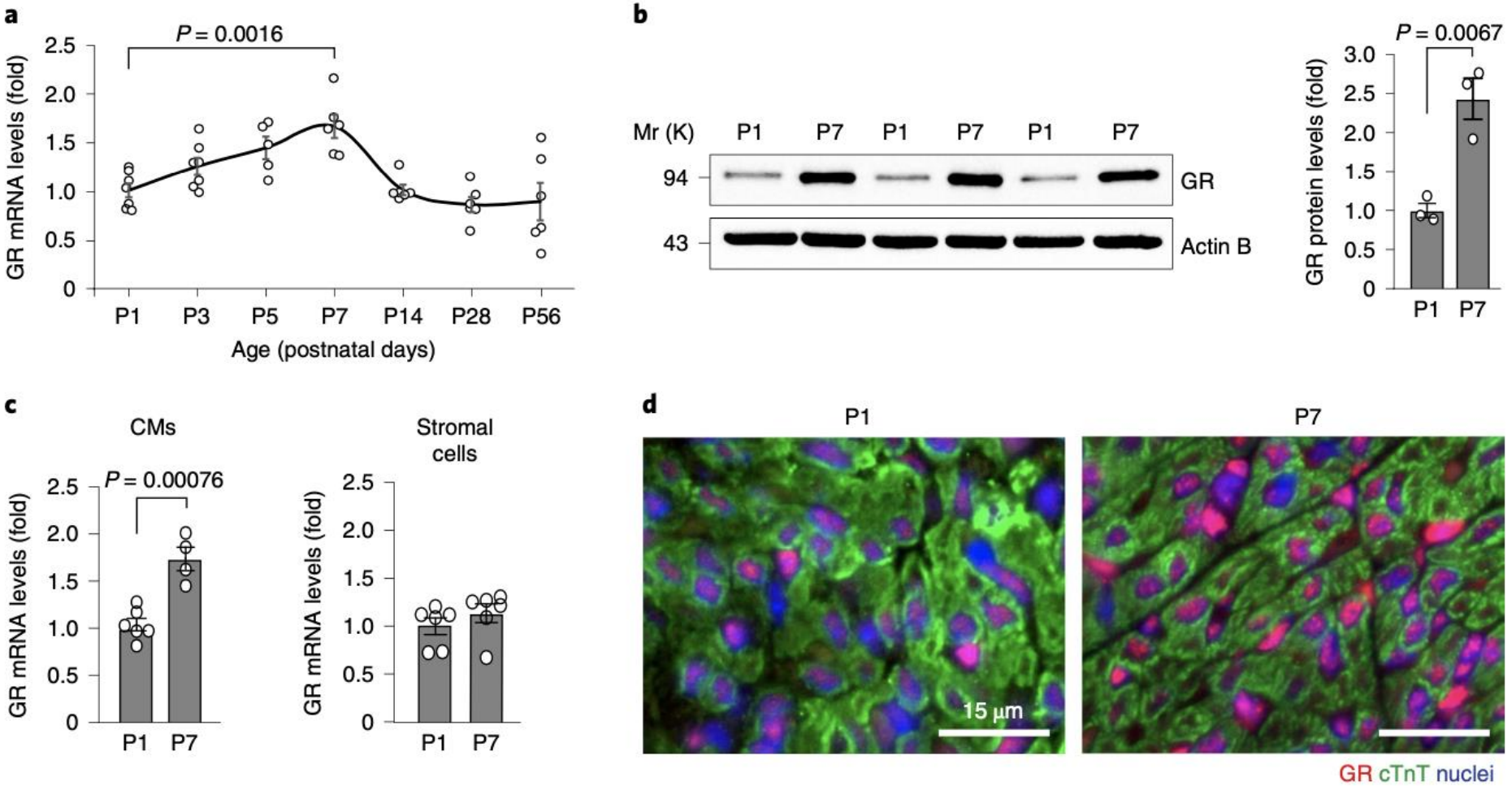




CORT = major endogenous glucocorticoid in the mouse model

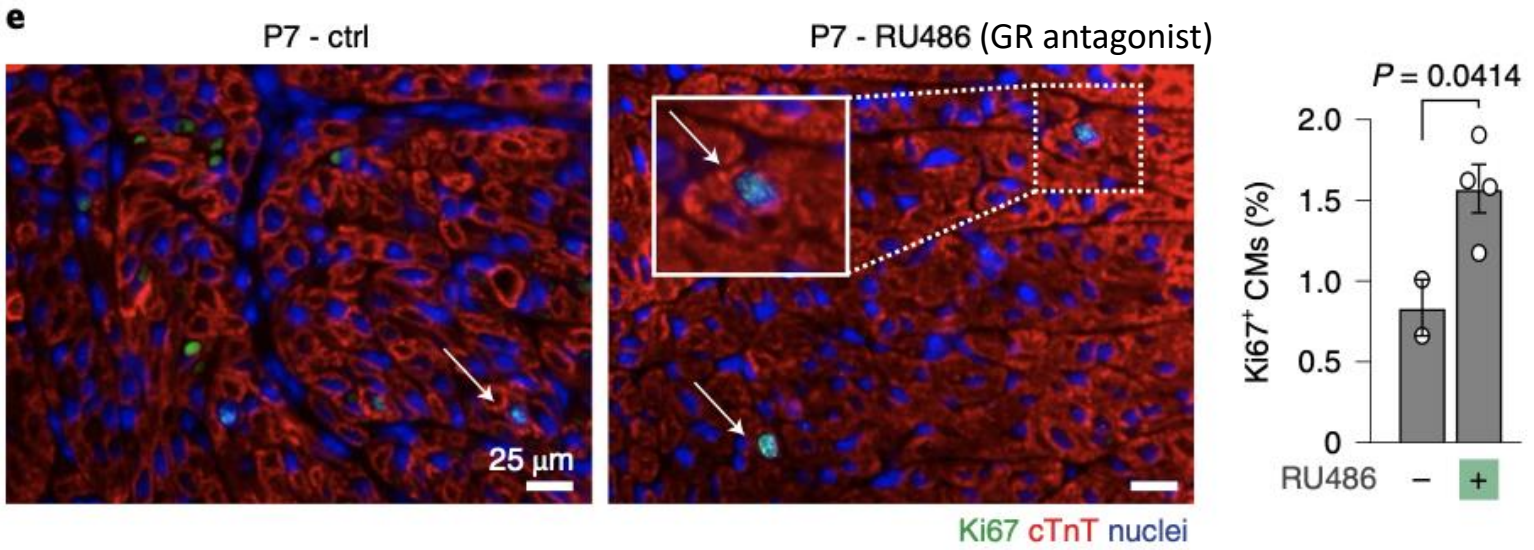
Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

GR abundance transiently in cardiomyocytes increase during the early postnatal period



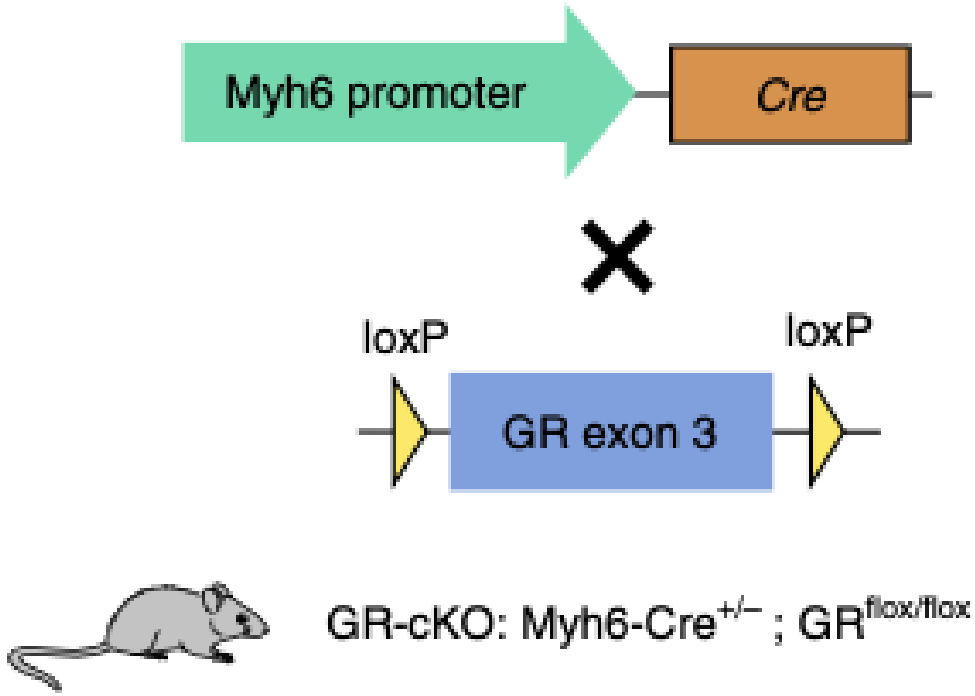
Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

Glucocorticoids/GR axis concurs to postnatal cardiomyocyte cell cycle exit



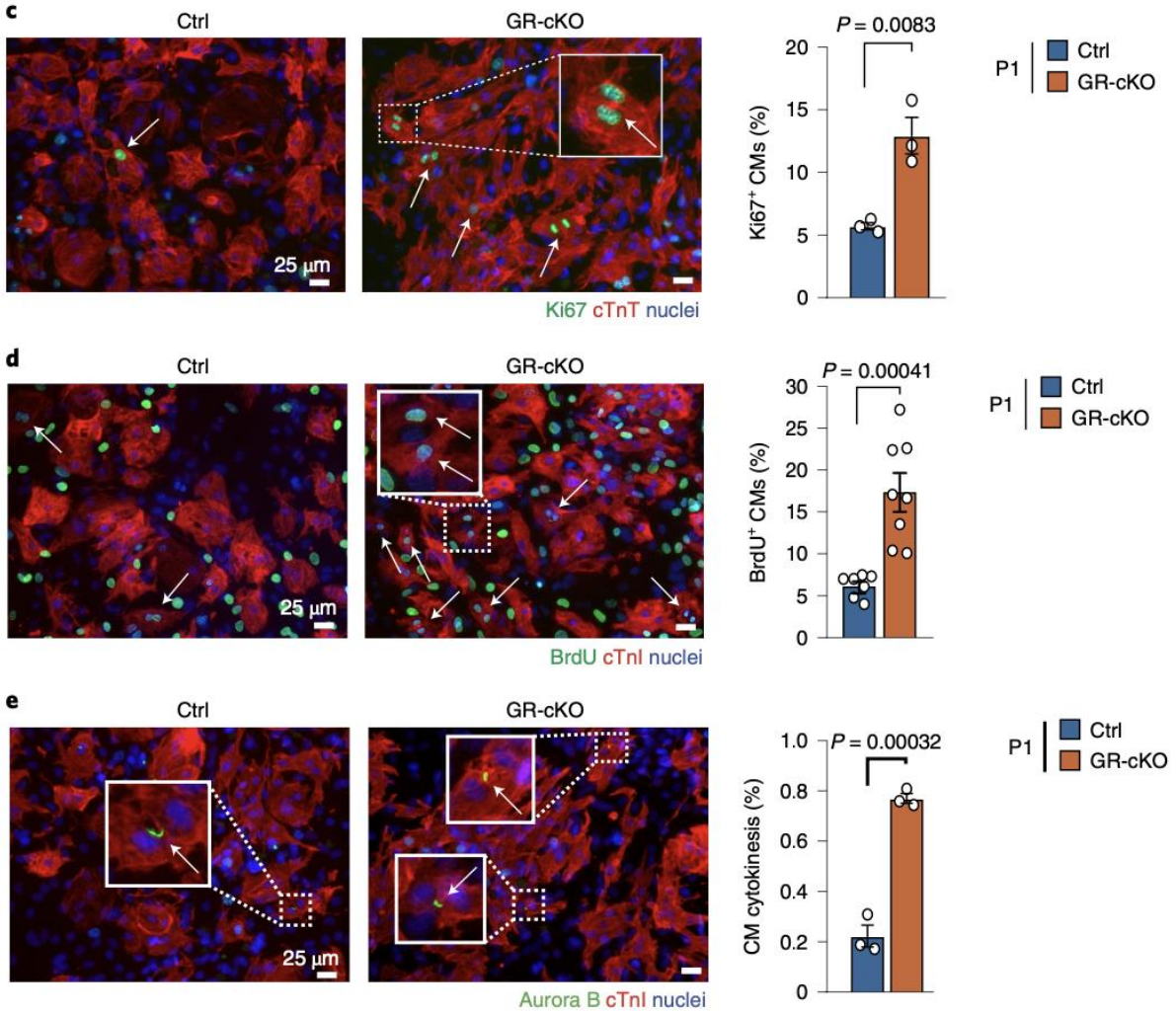
Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

In vivo cardiomyocyte specific Knock-out model for Glucocorticoid Receptor (GR-cKO)



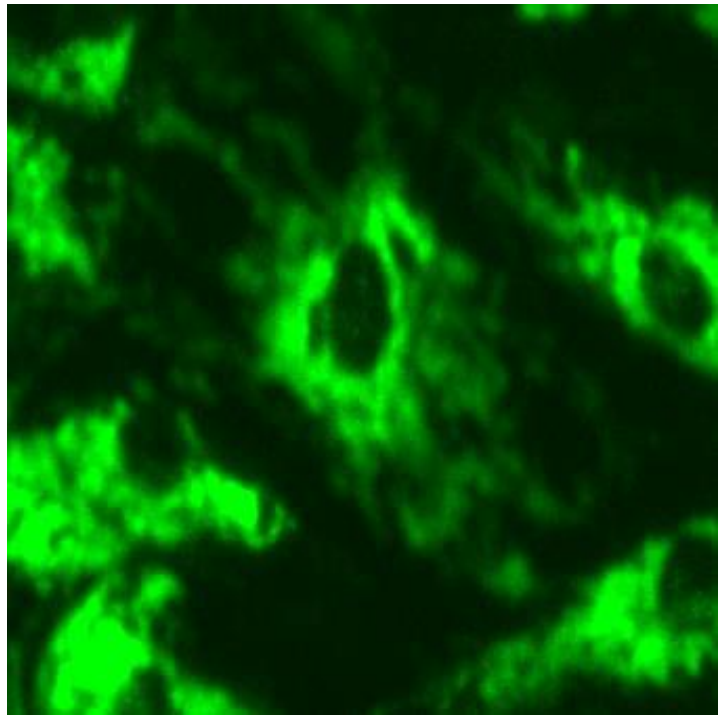
Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

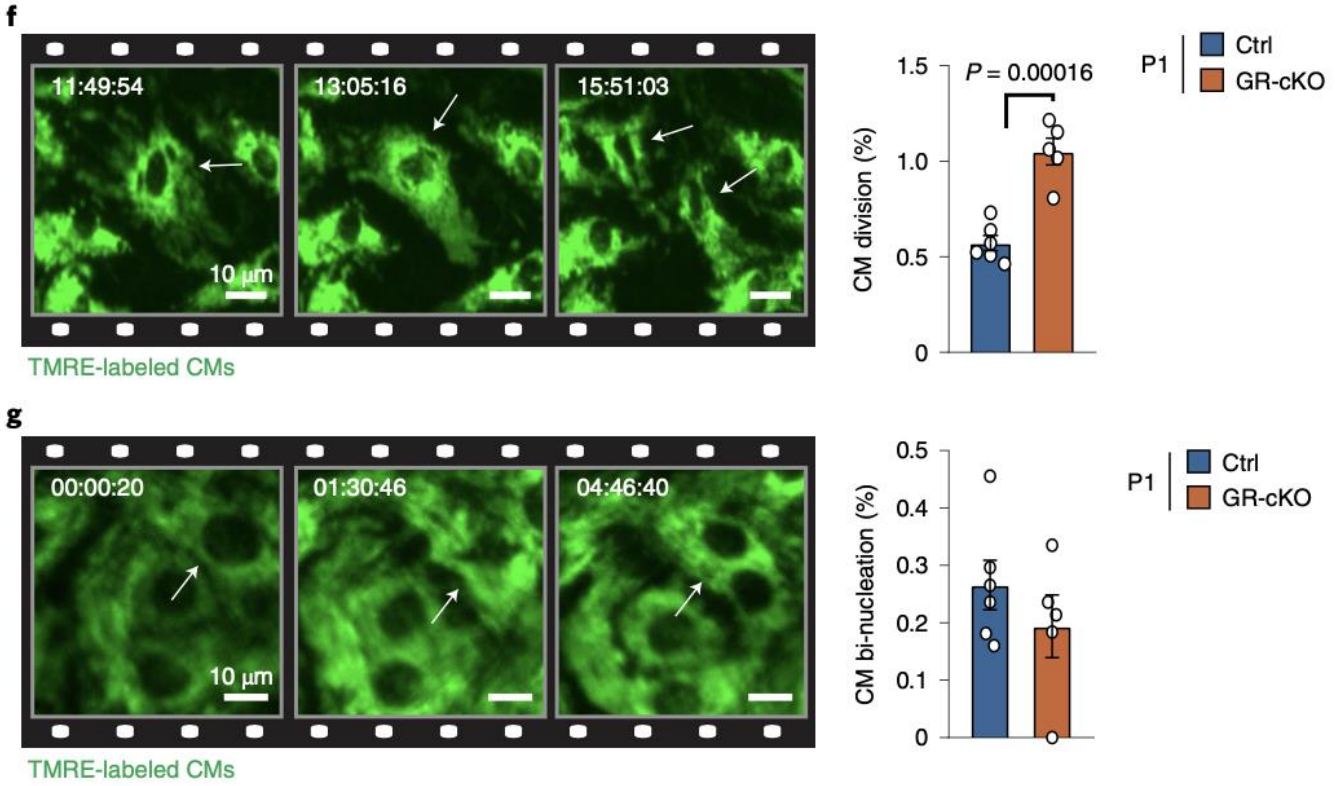
Cardiomyocyte-specific GR deletion increases neonatal cardiomyocyte proliferation



Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

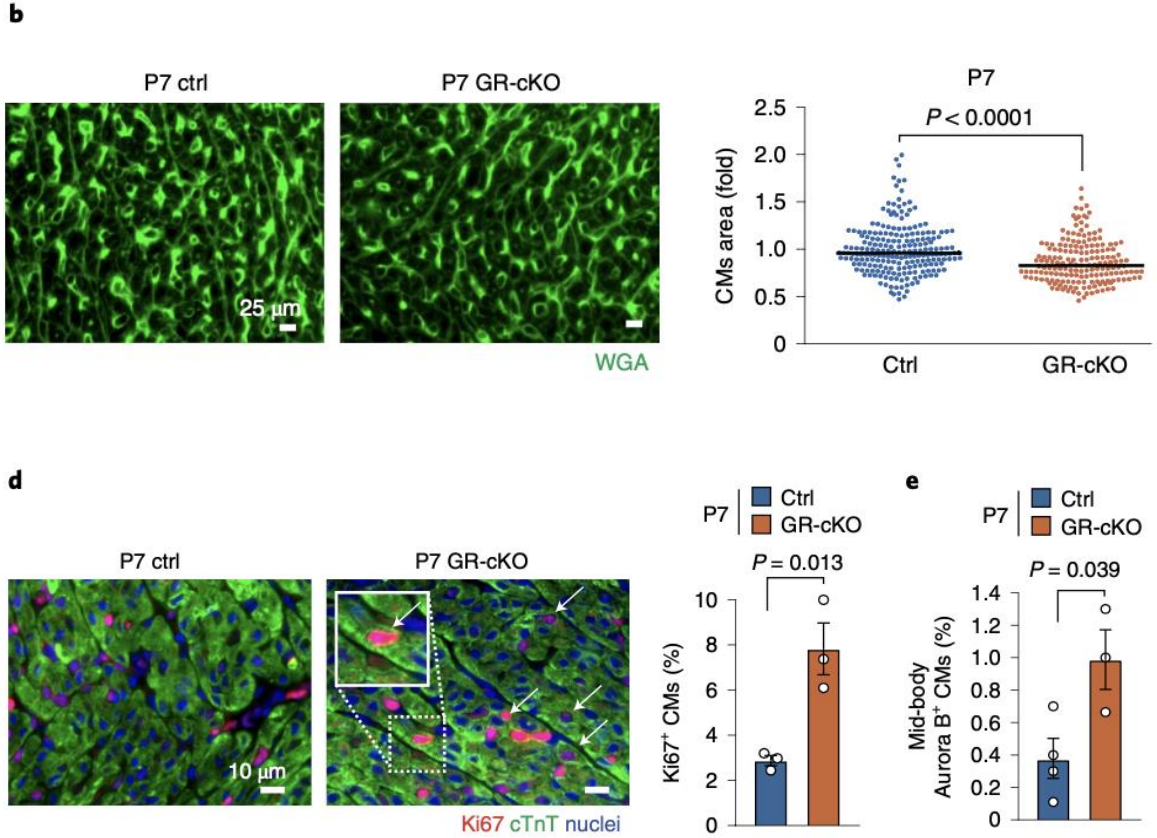
Cardiomyocyte-specific GR deletion increases neonatal cardiomyocyte division

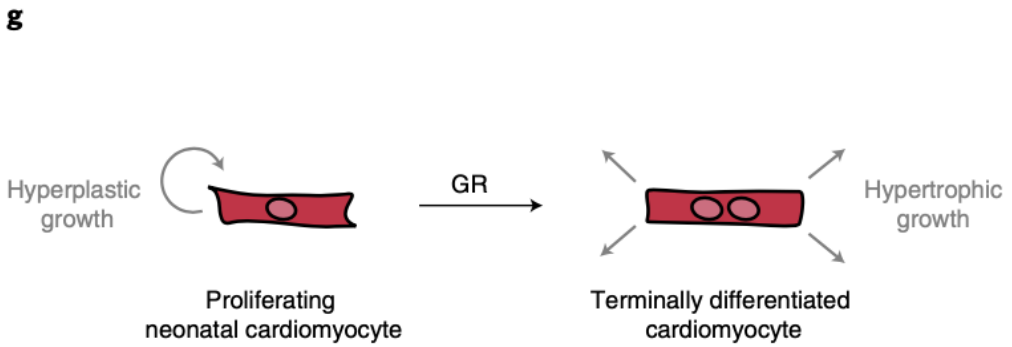




Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

Cardiomyocyte-specific GR ablation reduces the hyperplastic-to-hypertrophic transition occurring during early postnatal cardiac development

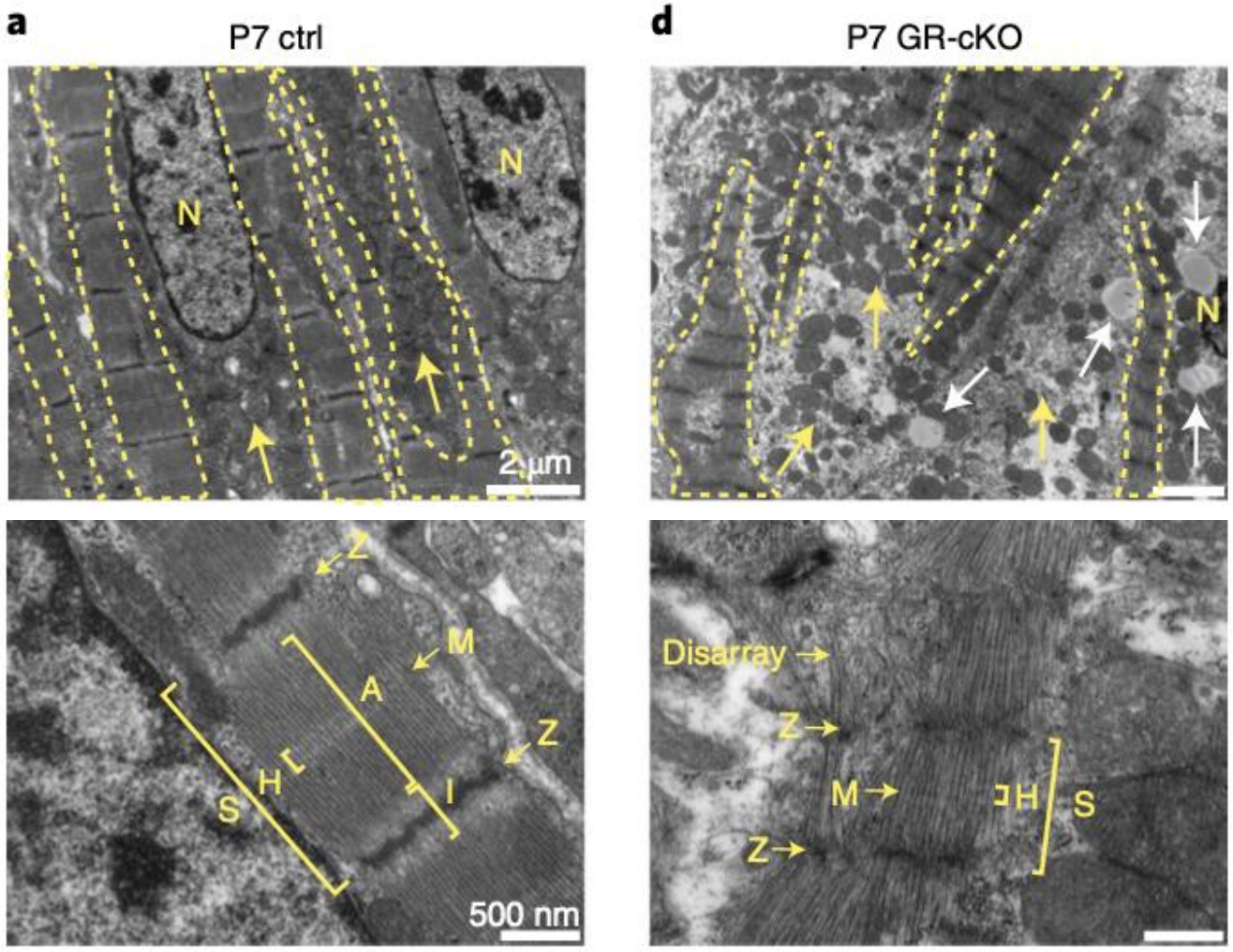




Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

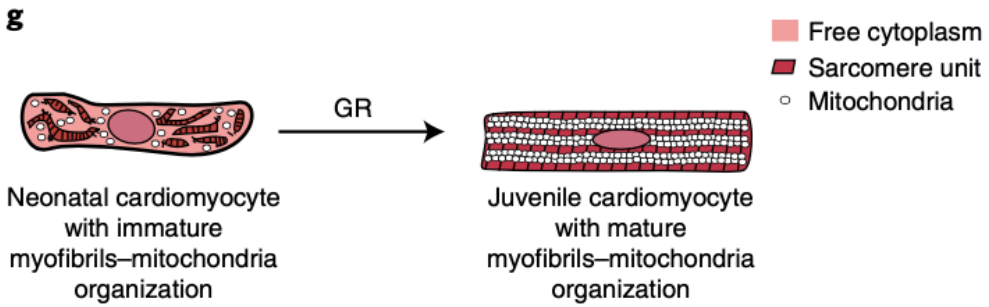
GR promotes the maturation of myofibrils–mitochondria organization during the early postnatal development

in collaboration with Prof. Cenacchi lab (Bologna University, Italy)



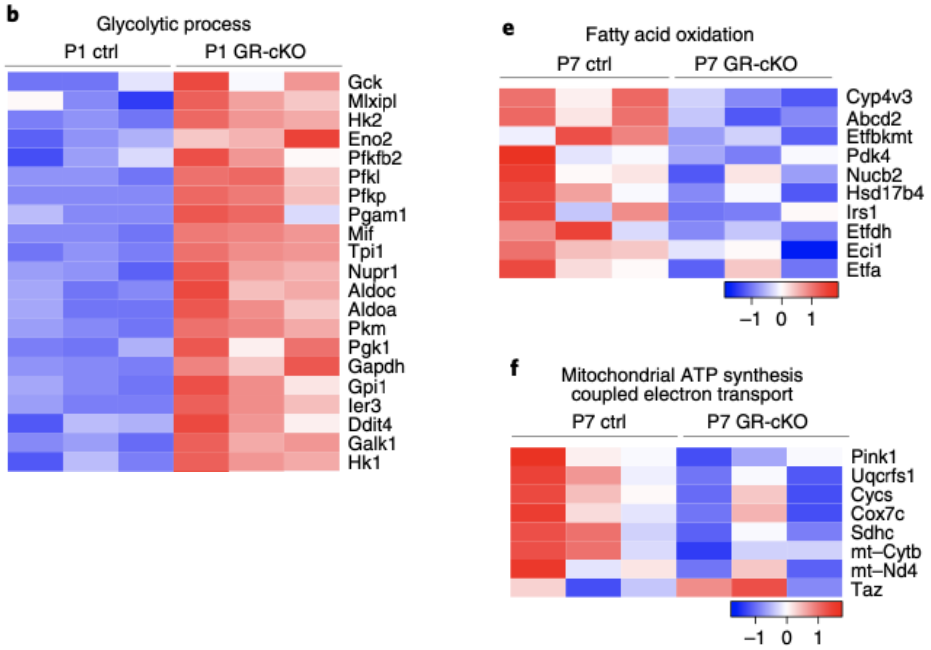
P7 GR-cKO vs ctrl:

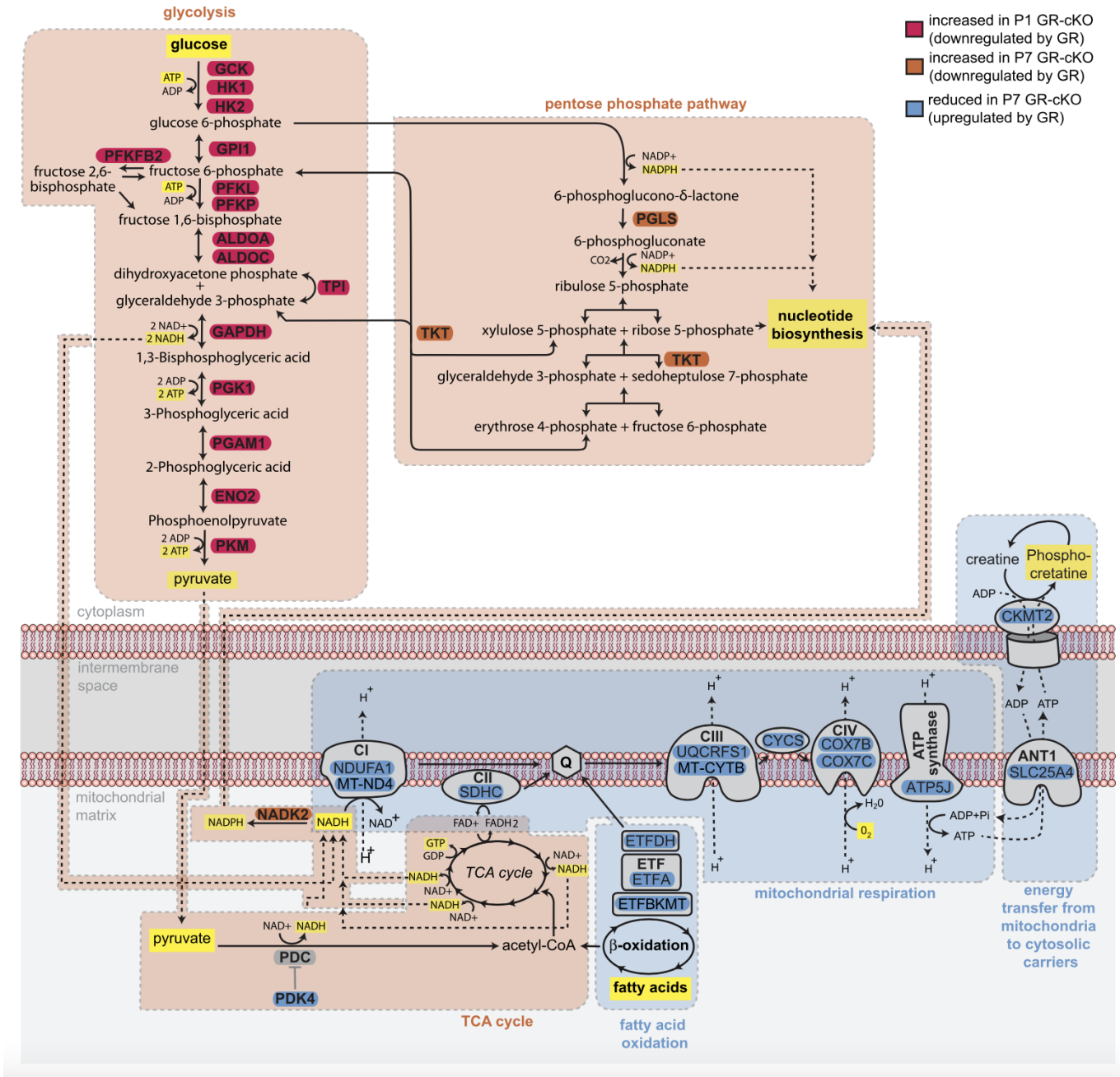
- high content of free cytoplasm
- irregular and misaligned pattern of myofibrils with loss of the major axis orientation and arrangement around isolated mitochondria



Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

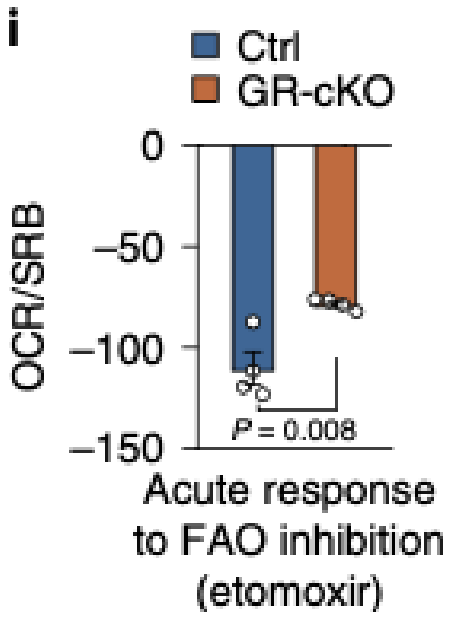
Ablation of GR increases the expression of genes involved in glycolysis while reducing the expression of those involved in fatty acid oxidation and mitochondrial respiration



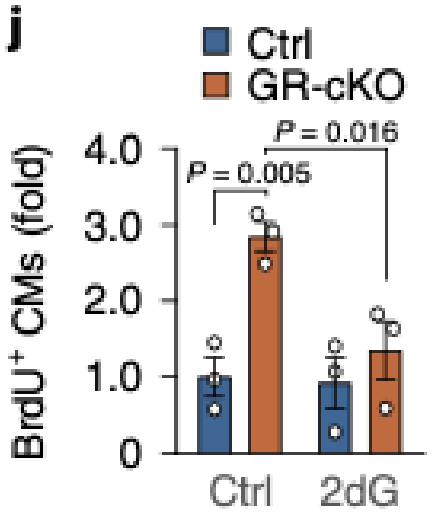


Pianca, Sacchi …]& D'Uva, Nature Cardiovascular Research 2022

Ablation of GR promotes cardiomyocyte proliferation by favoring glucose catabolism over fatty acid oxidation



GR-cKO CMs are less dependent on fatty acid oxidation (FAO) for their mitochondrial respiration.



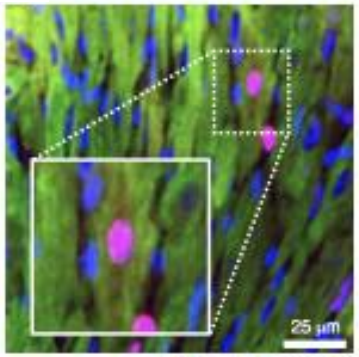
Inhibition of glucose catabolism reduces the proliferation of GR-cKO CMs while having no significant effect on the proliferation rate of control cardiomyocytes.

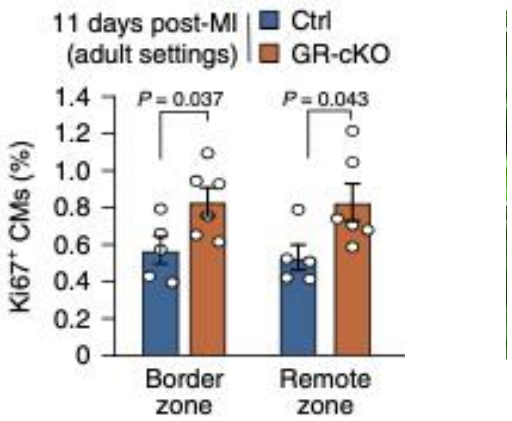
Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

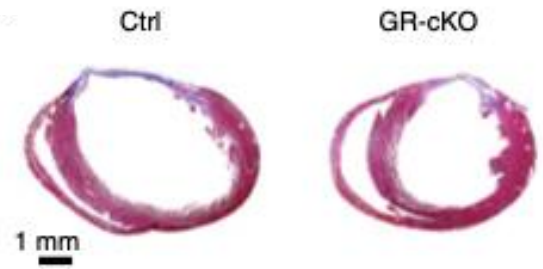
Ablation or pharmacological inhibition of GR enhances cardiac regenerative ability after myocardial infarction

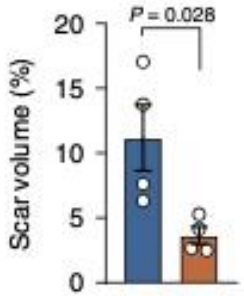
1. MI in GR ablation model

in collaboration with Prof. Rizzi (La Sapienza University of Rome, Italy)



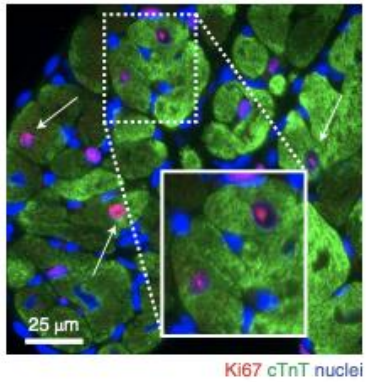


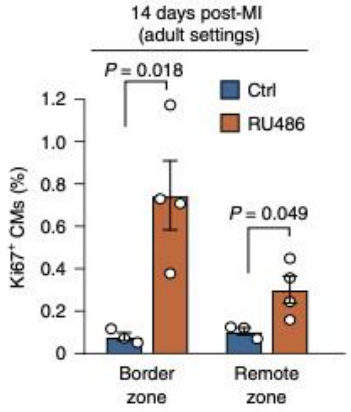


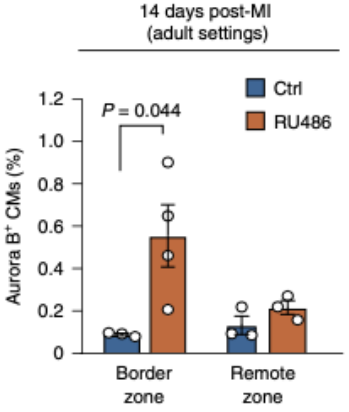


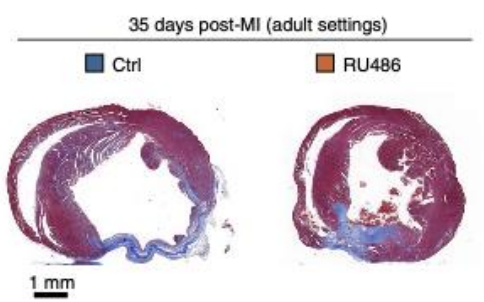
2. transient GR inhibition (RU486) post-MI

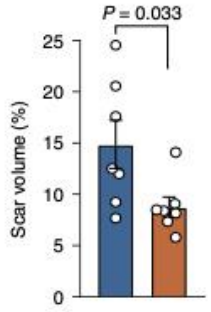
in collaboration with Prof. Tzahor lab (Weizmann Institute of Science, Israel)





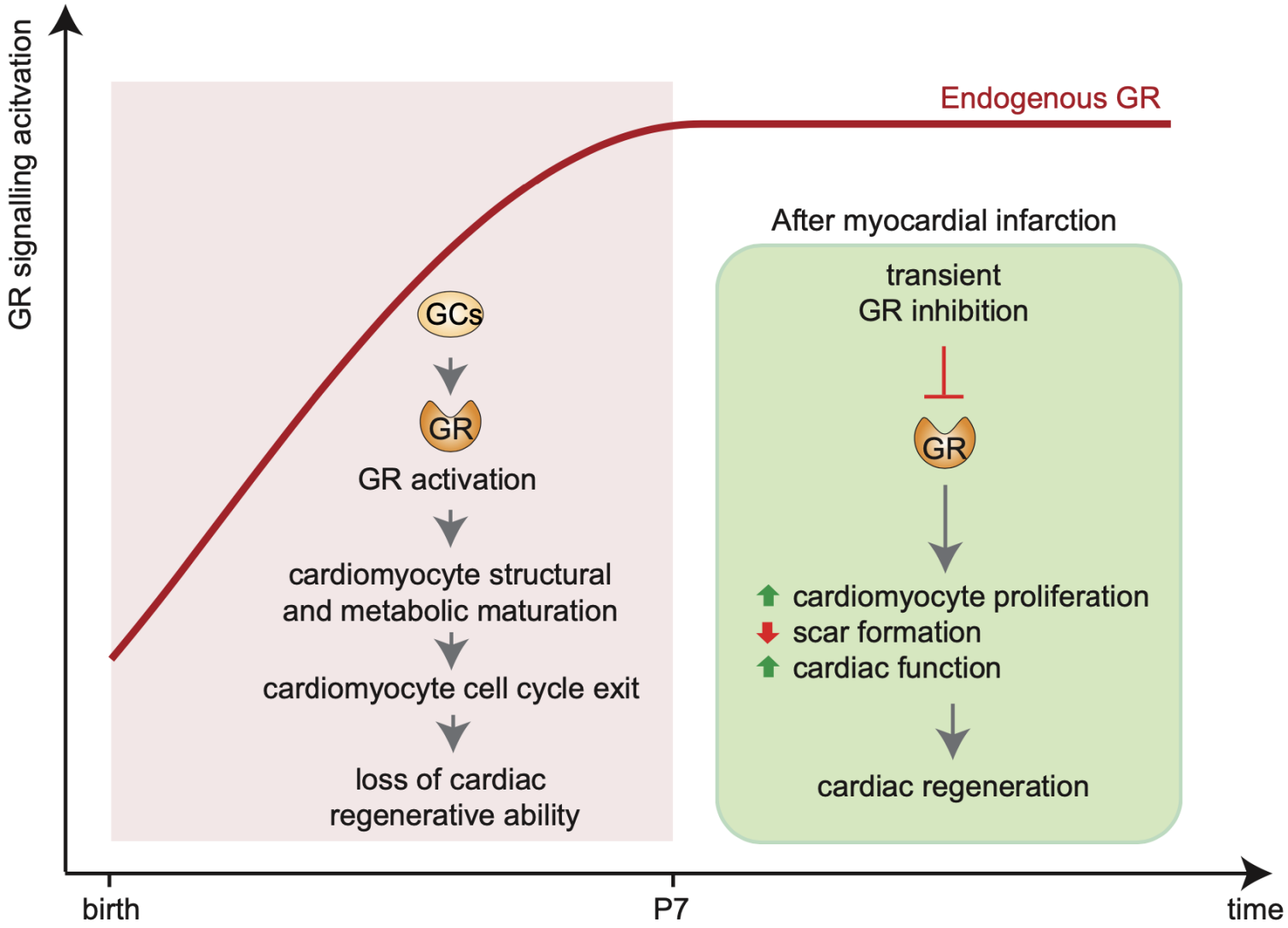






Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

Take home messages



During the early postnatal development:

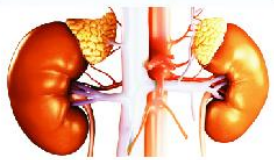
- GR expression levels increase in cardiomyocytes
- Glucocorticoids (GCs)/GR axis promotes cardiomyocytes cytoarchitectural and metabolic maturation, which results in cell cycle exit and loss of cardiac regenerative ability

In juvenile and adult stages:

- GR-cKO or transient GR antagonization facilitates cardiomyocyte proliferation and promotes heart regeneration upon myocardial infarction in adult mice

Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

Novel regulators of cardiomyocyte regenerative plasticity

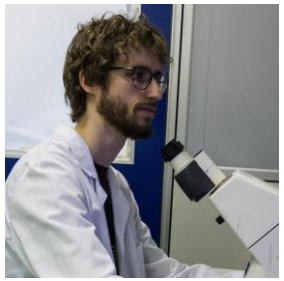


Cardiac growth factors (in particular BMP7)

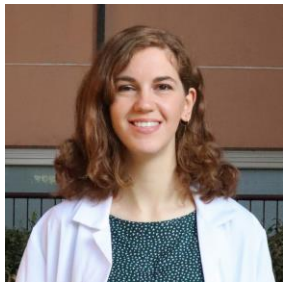
Bongiovanni … & D'Uva (under consideration)

Chiara Bongiovanni

Hormones (glucocorticoids)



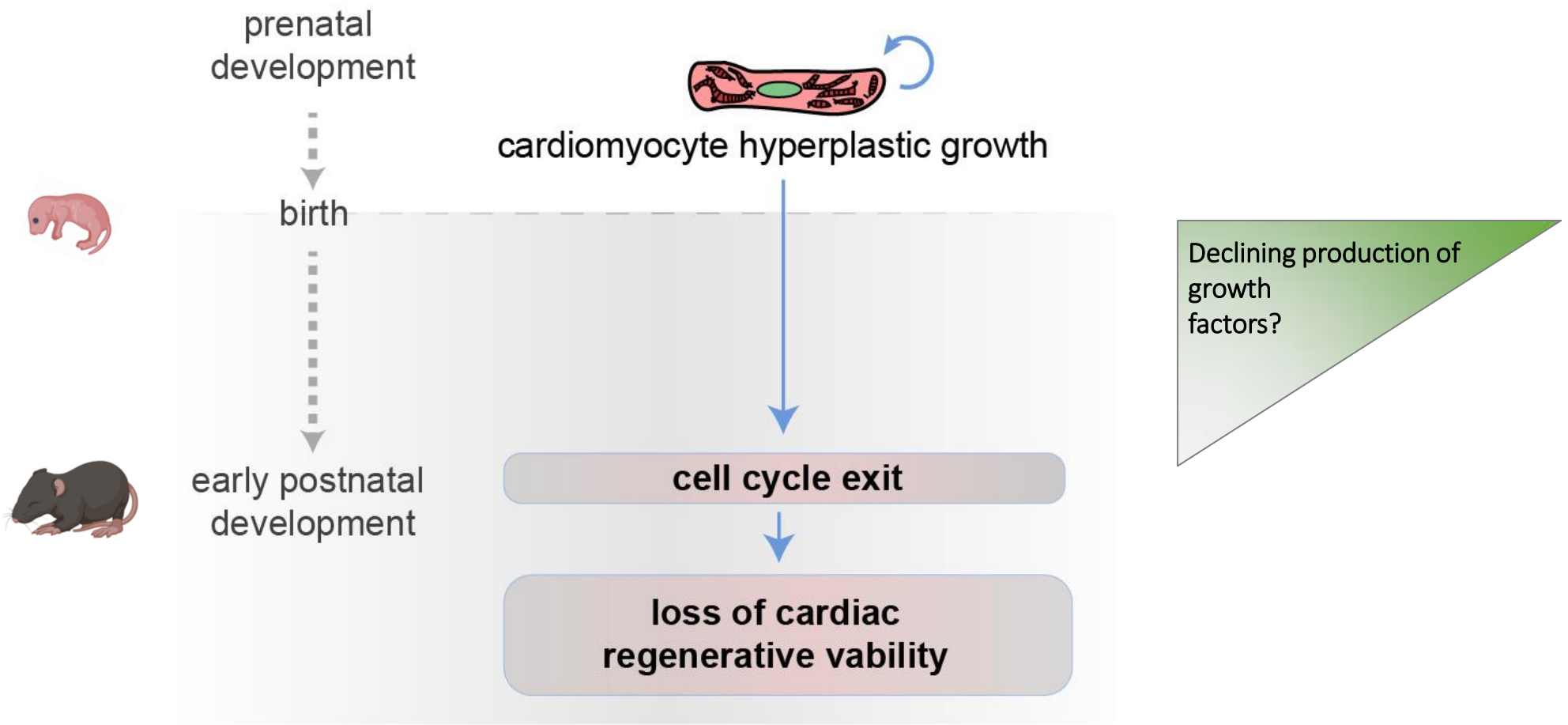
Nicola Pianca



Francesca Sacchi

Pianca, Sacchi … & D'Uva, Nature Cardiovascular Research 2022

Project hypothesis

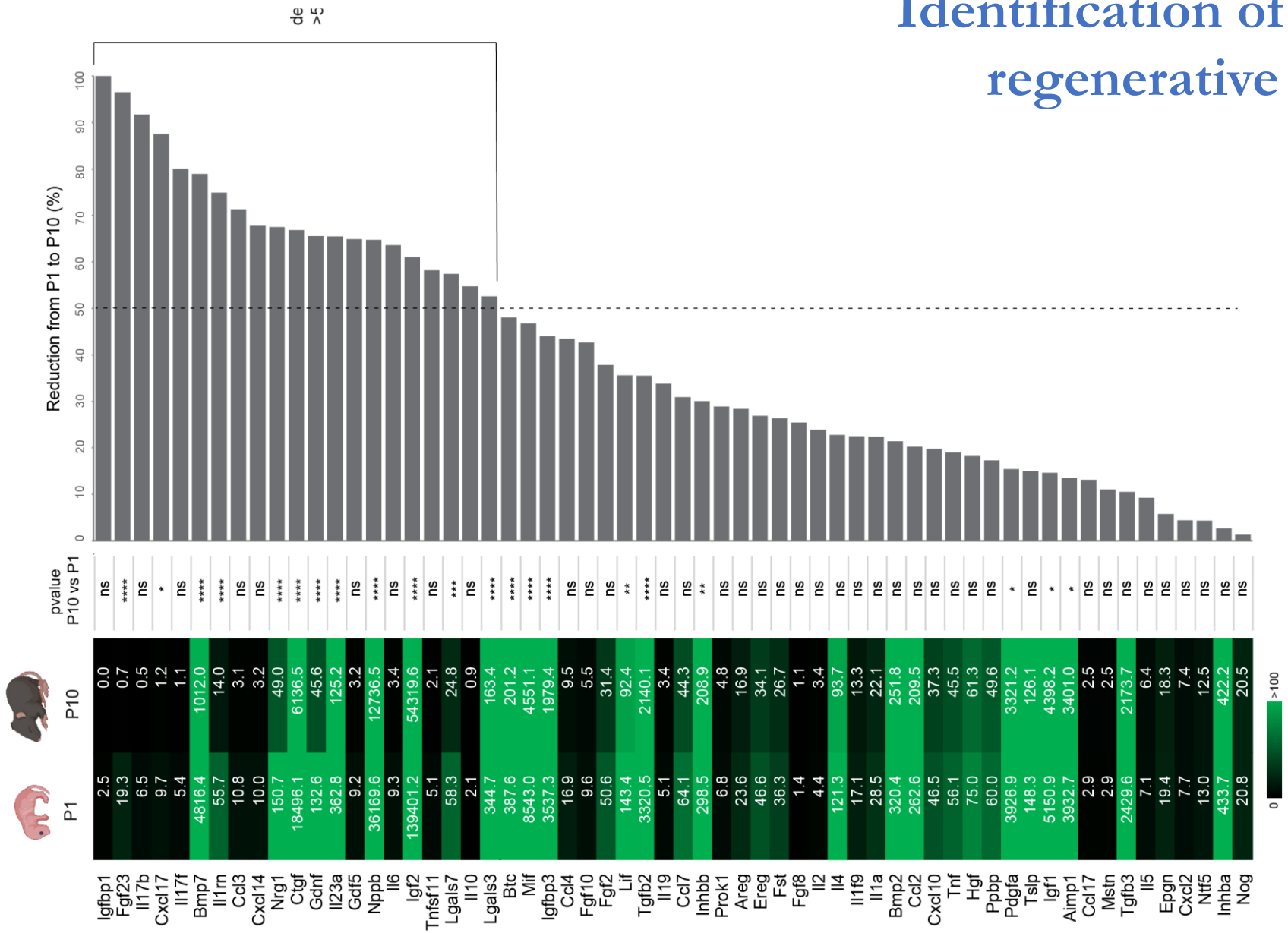


A declining production of specific growth factors contributes to cardiomyocyte cell cycle exit and consequent reduction of cardiac regenerative ability during the early postnatal mammalian development.

Bongiovanni … & D'Uva (under consideration)

21 growth factors with a decrease over 50%

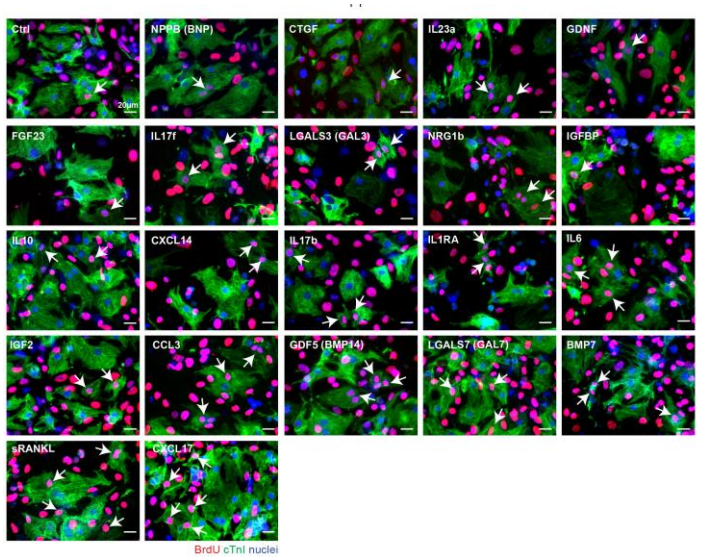
Identification of candidate cardiac regenerative growth factors

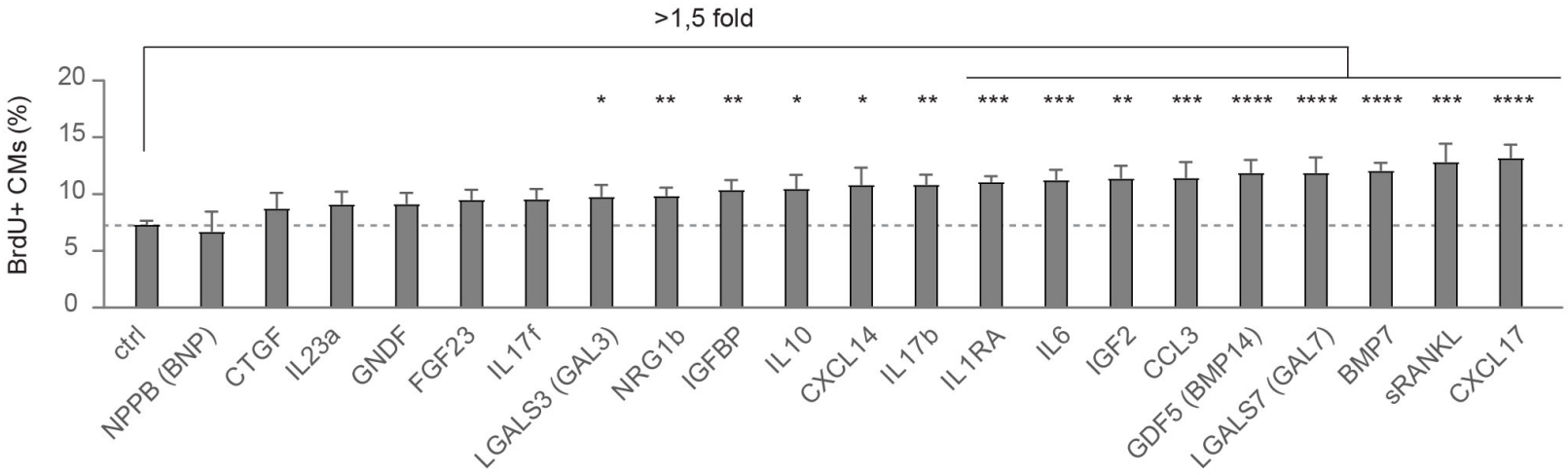


metanalyses of Haubner et al., 2012

Bongiovanni … & D'Uva (under consideration)

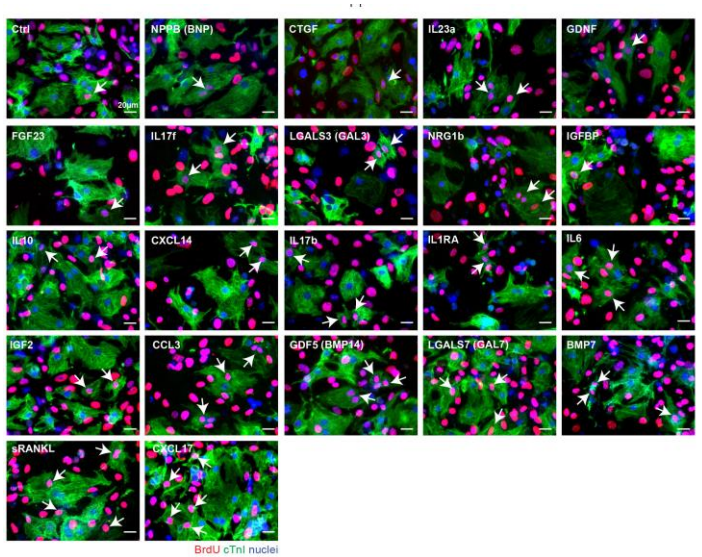
Several growth factors declining in expression during the early postnatal period promote cell cycle progression of neonatal cardiomyocytes

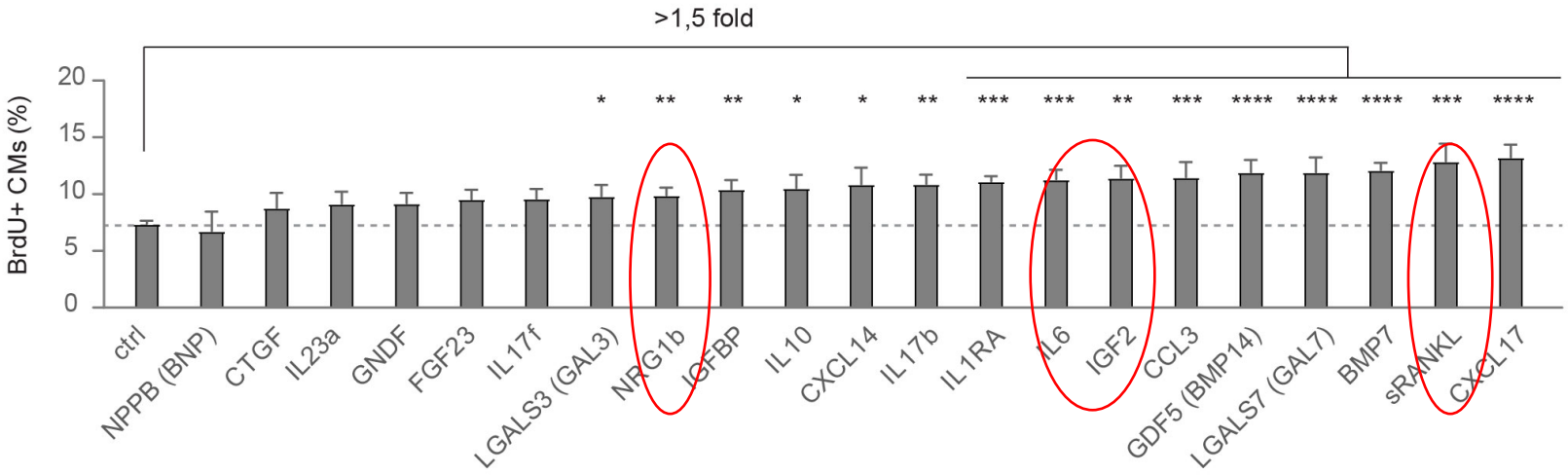




Bongiovanni … & D'Uva (under consideration)

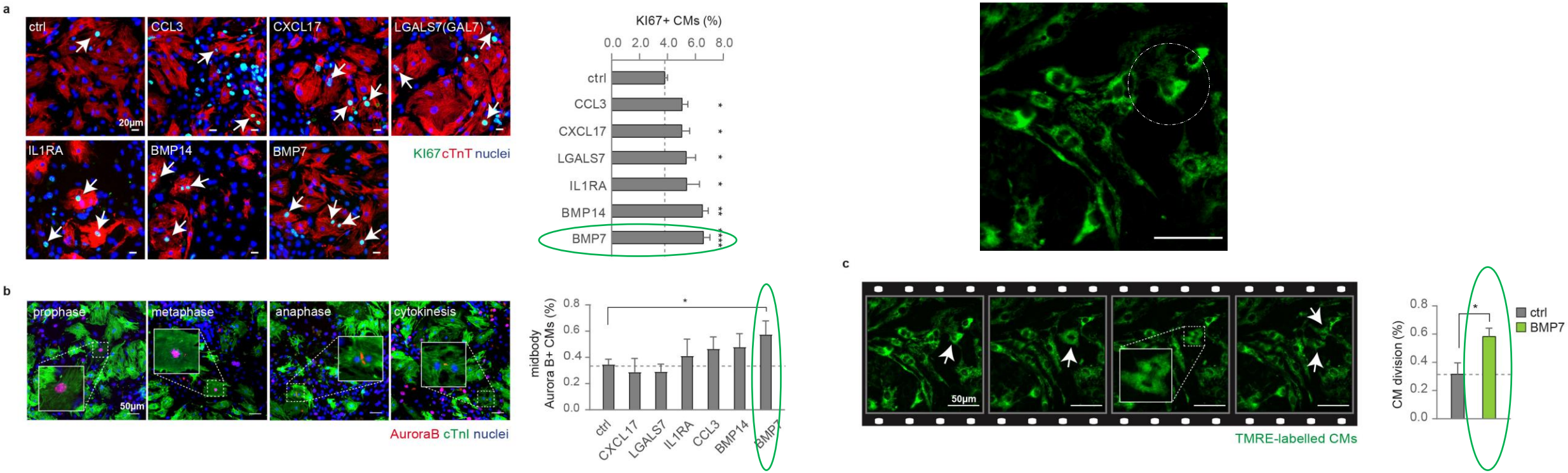
Several growth factors declining in expression during the early postnatal period promote cell cycle progression of neonatal cardiomyocytes





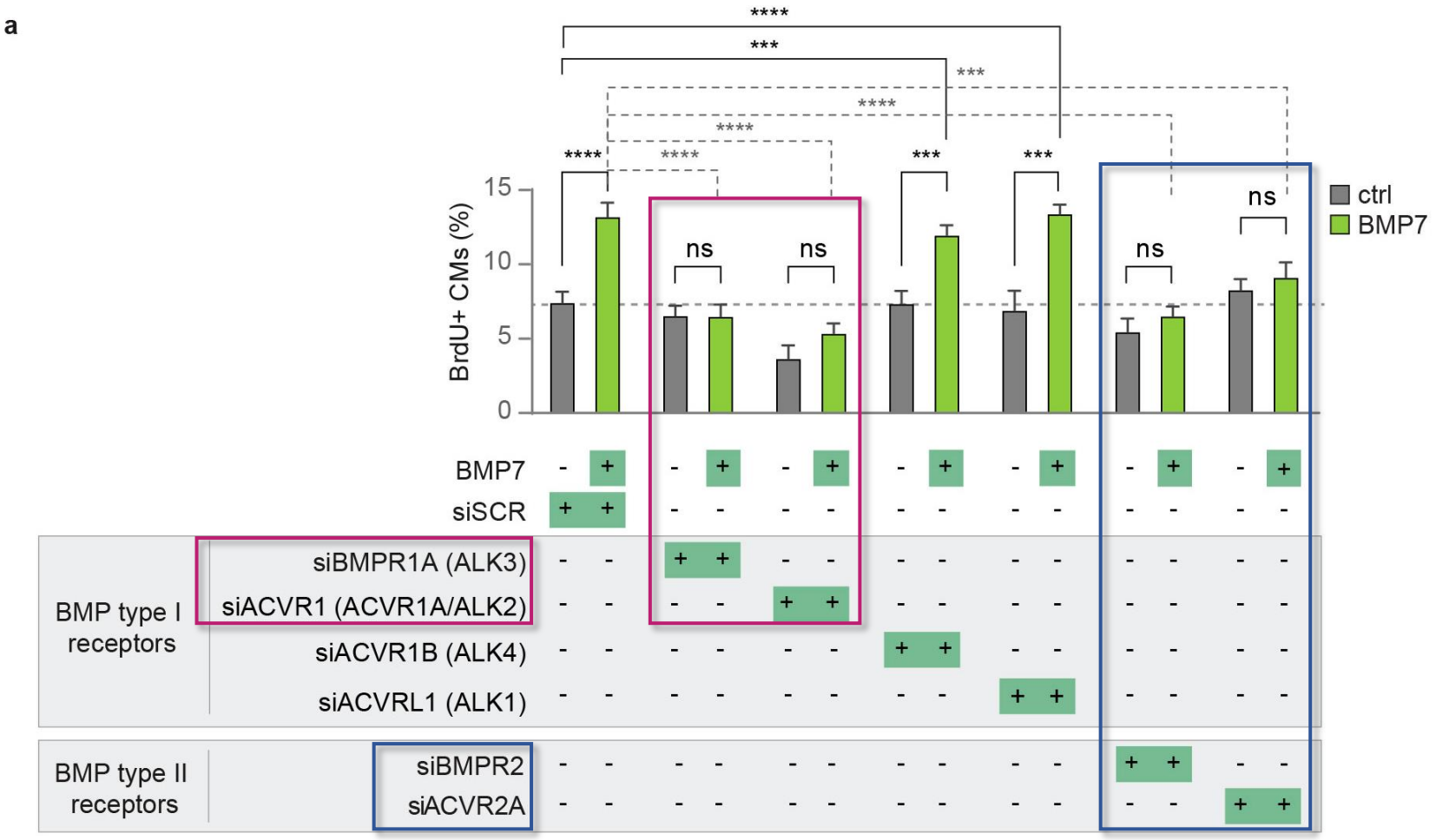
Bongiovanni … & D'Uva (under consideration)

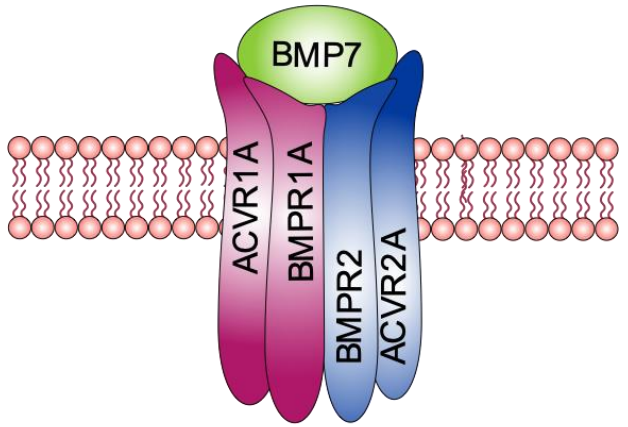
BMP7 robustly induces cardiomyocyte cell cycle re-entry and cell division



Bongiovanni … D'Uva (under consideration)

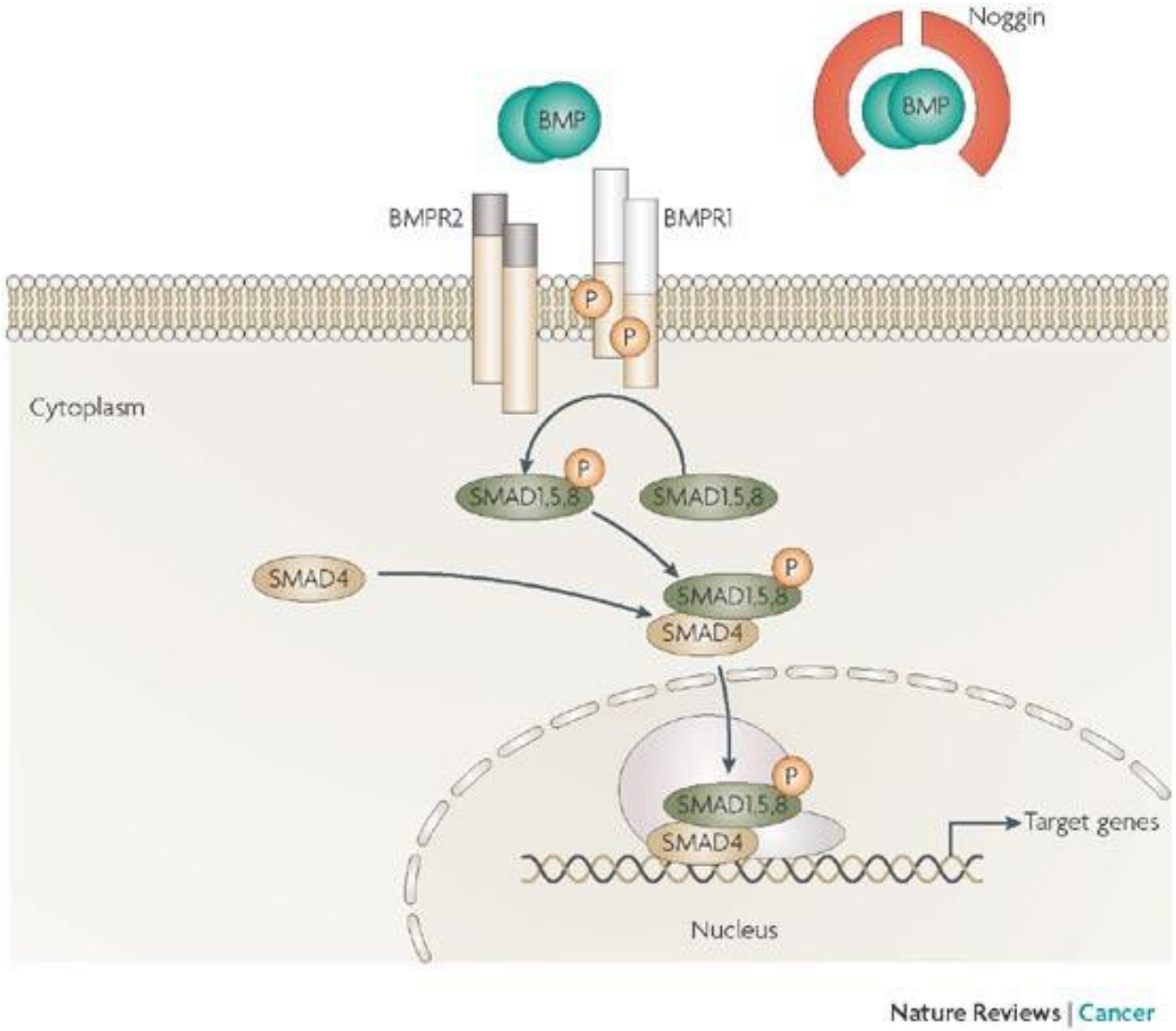
BMPR1A/ACVR1 and ACVR2/BMPR2 receptors mediates BMP7-induced cardiomyocyte proliferation





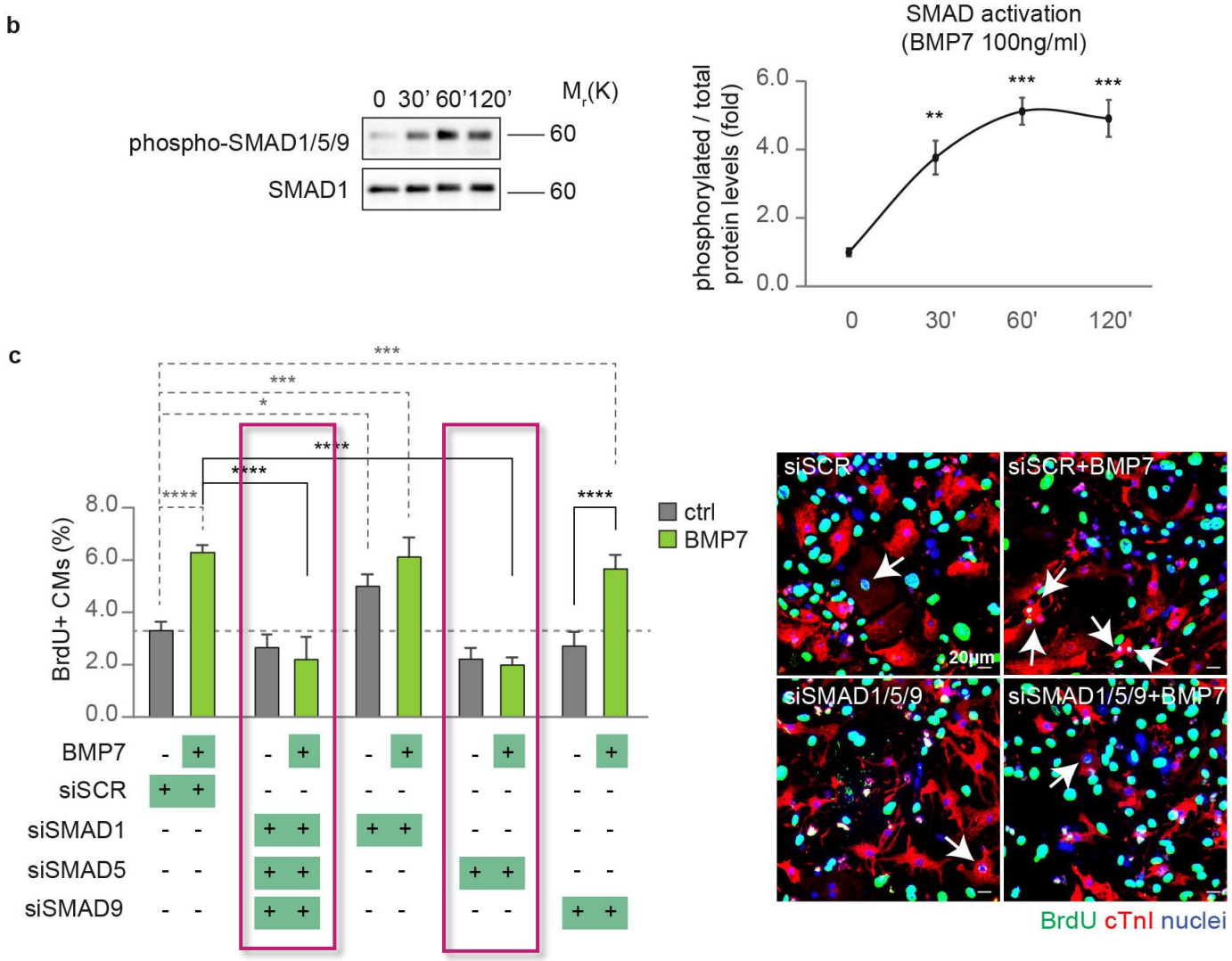
Bongiovanni […] & D'Uva (under consideration)

BMP7 downstream canonical pathway



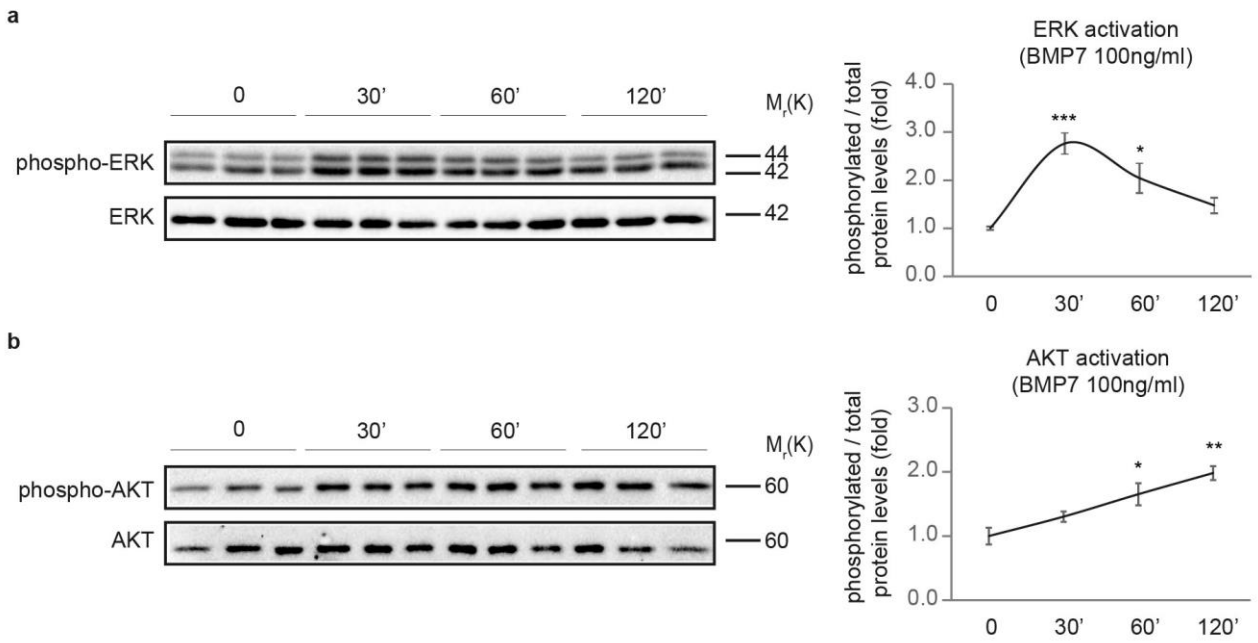
adapted from Hardwick … & van den Brink, Nature reviews Cancer 2008

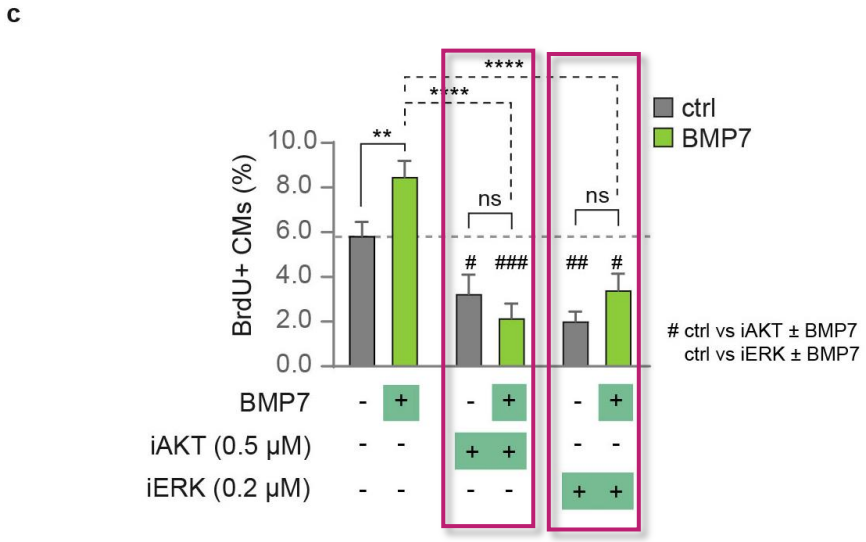
BMP7 induces cardiomyocyte proliferation by activation of canonical SMAD5 signalling

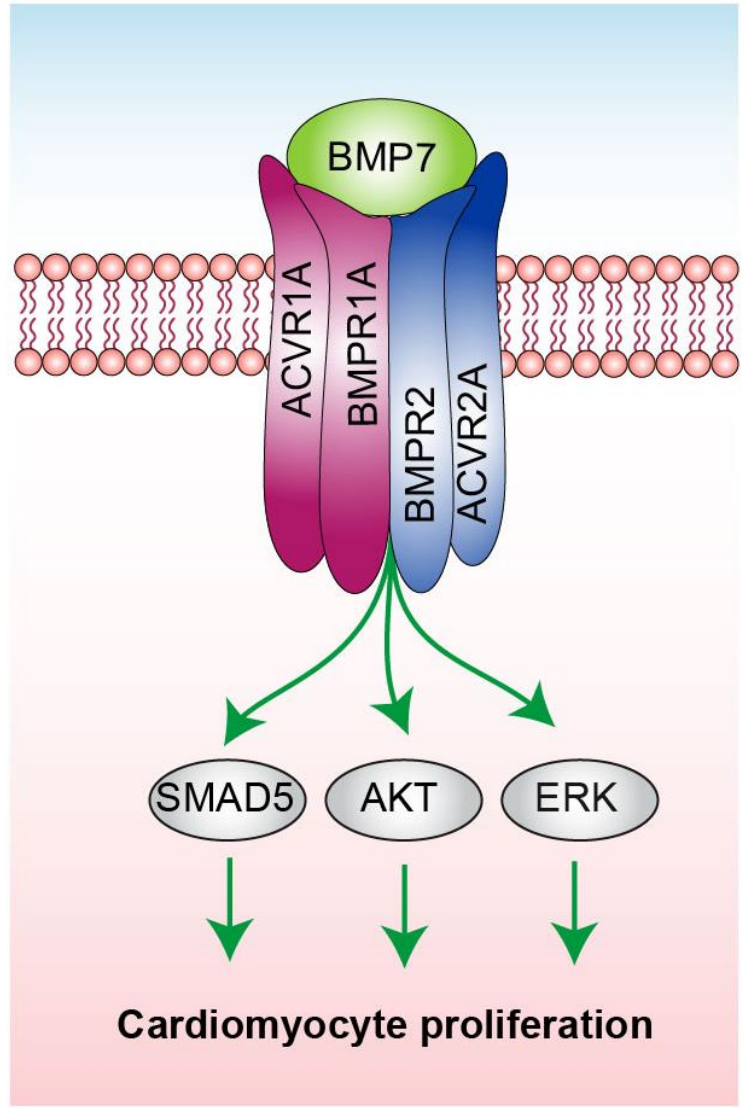


Bongiovanni … & D'Uva (under consideration)

Non-canonical pathways take part in BMP7 mitogenic signal transduction

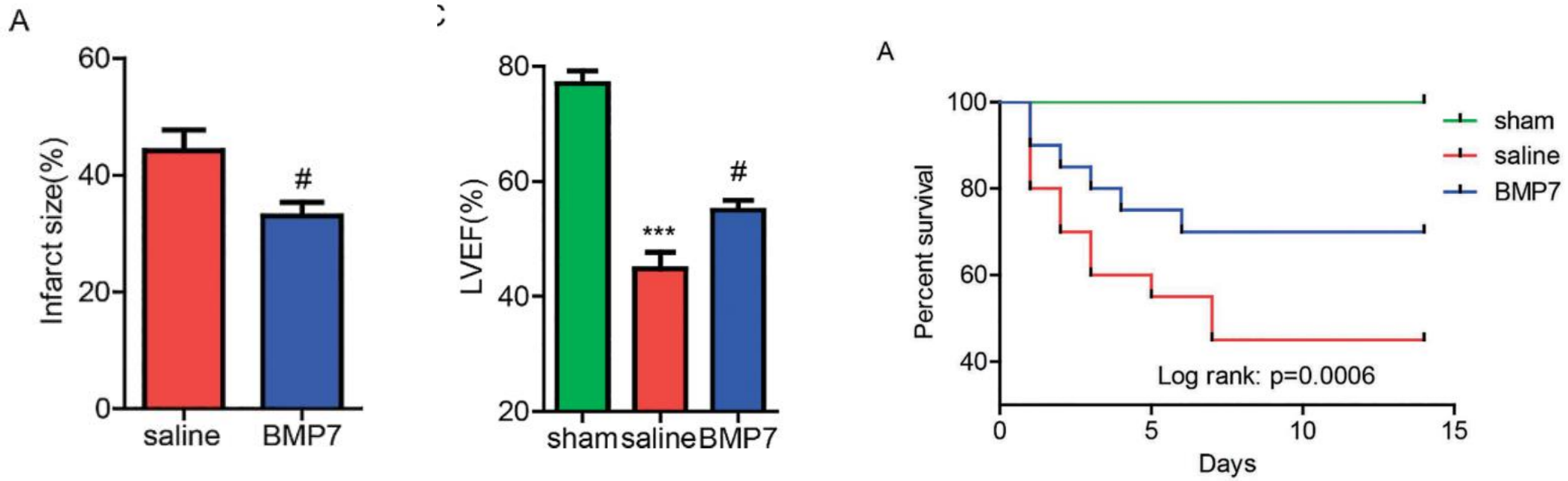






Bongiovanni … & D'Uva (under consideration)

BMP7 counteracts TGF-β1 signaling pathway and exerts antifibrotic effects



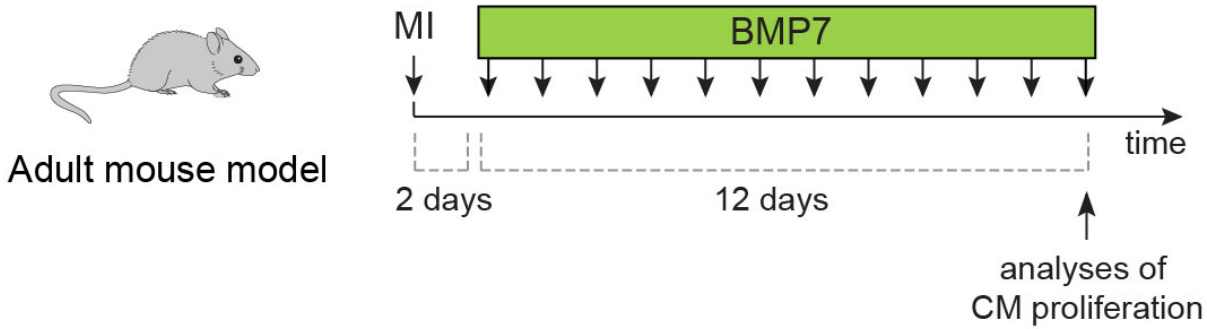
Administration of BMP7 for 2 weeks after myocardial infarction in rats has been shown to attenuate myocardial fibrosis by counteracting the TGF-β1 signalling pathway, leading to improved cardiac function and mice survival.

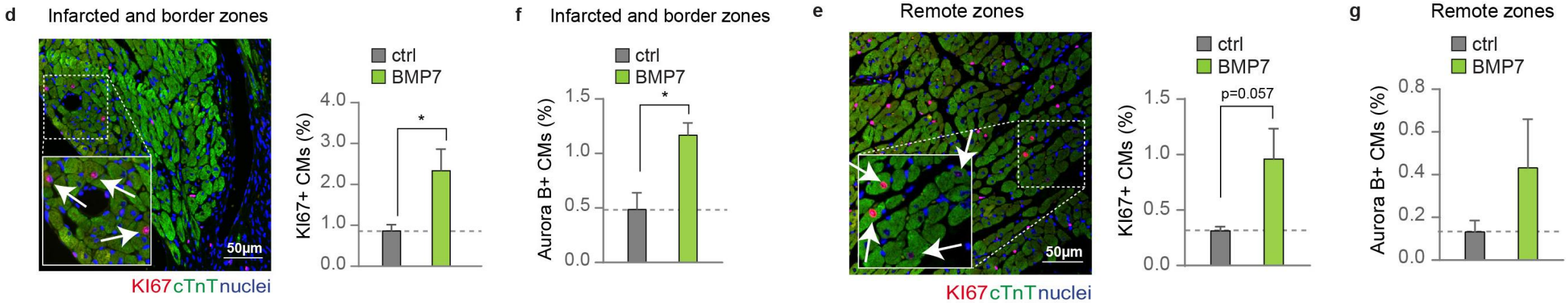
However, the potential effect of BMP7 administration in promoting the regeneration of adult mammalian cardiomyocytes after a cardiac injury was not evaluated

adapted from Jin … & Li, Tohoku, J. Exp. Med. 2018

The administration of BMP7 triggers mammalian cardiomyocyte proliferation in adult mice following myocardial infarction

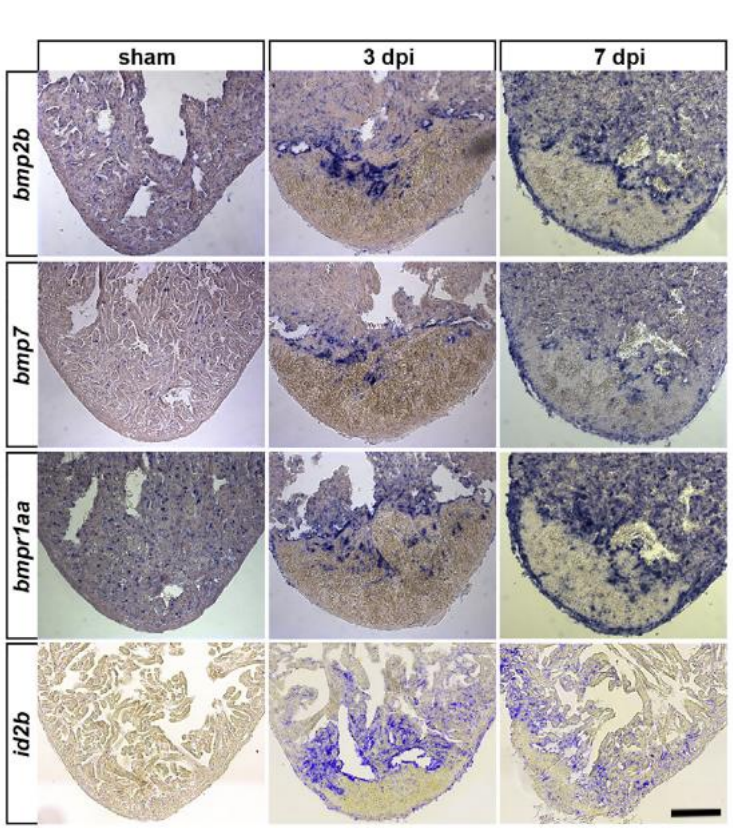
in collaboration with Prof. Tzahor lab (Weizmann Institute of Science, Israel)

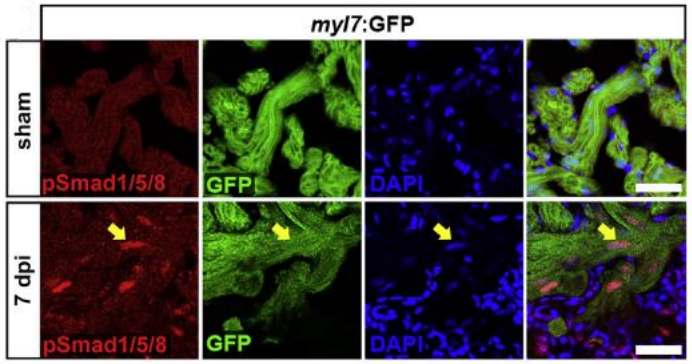


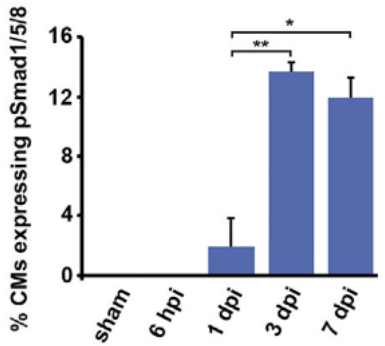


Bongiovanni … & D'Uva (under consideration)

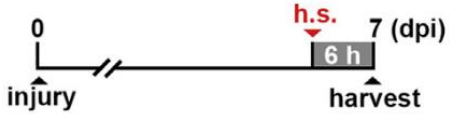
In zebrafish, BMP signaling is activated during the spontaneous regeneration process

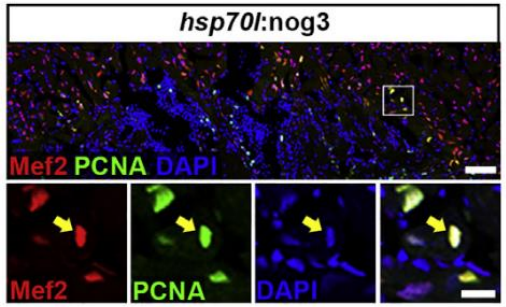


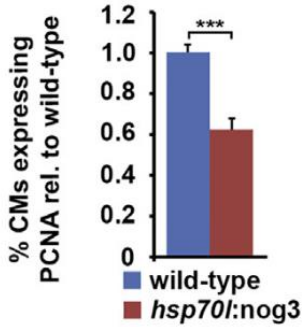




BMP signaling increases after cardiac injury in zebrafish







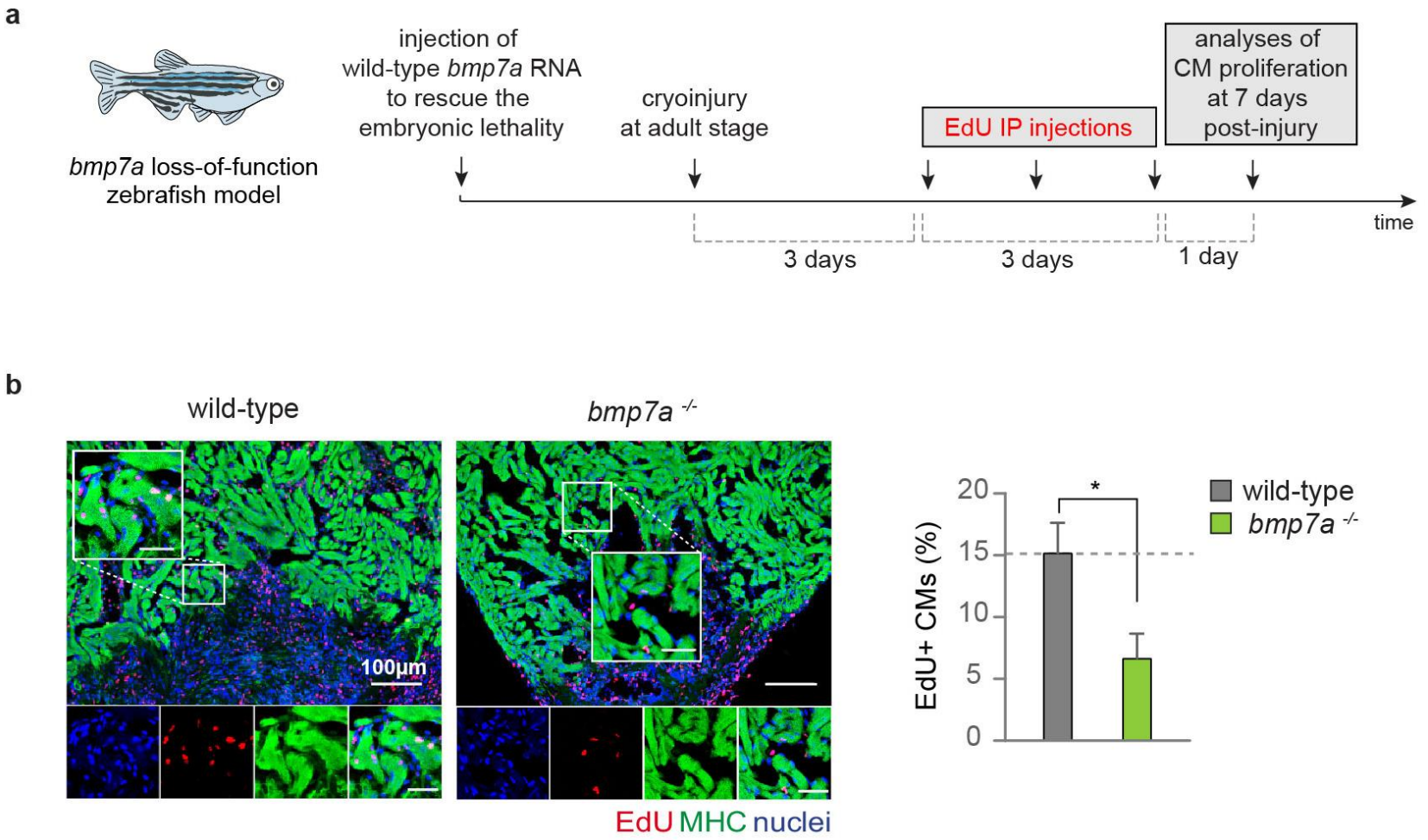
Inhibition of BMP signaling post cardiac injury reduces cardiomyocyte proliferation

Nevertheless, the role of specific BMP ligands in injury-induced cardiomyocyte proliferation was not previously explored

adapted from Wu … Weidinger & Bakkers, Developmental Cell, 2016

Bmp7 loss-of-function reduces cardiomyocyte proliferation during zebrafish heart regeneration

Collaboration with Prof. Weidinger lab (University of Ulm)

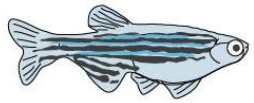


Bongiovanni … & D'Uva (under consideration)

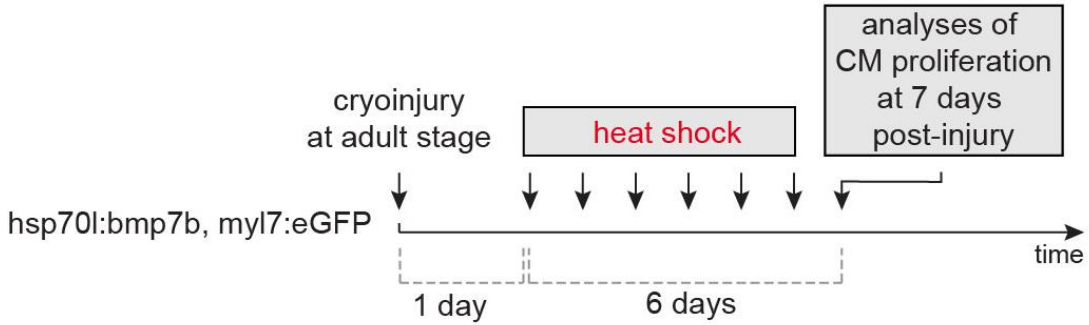
Bmp7 gain-of-function increases cardiomyocyte proliferation during zebrafish heart regeneration

in collaboration with Prof. Weidinger lab (University of Ulm, Germany) and Prof. Heermann lab (University of Freiburg, Germany)

c



bmp7b gain-of-function zebrafish model



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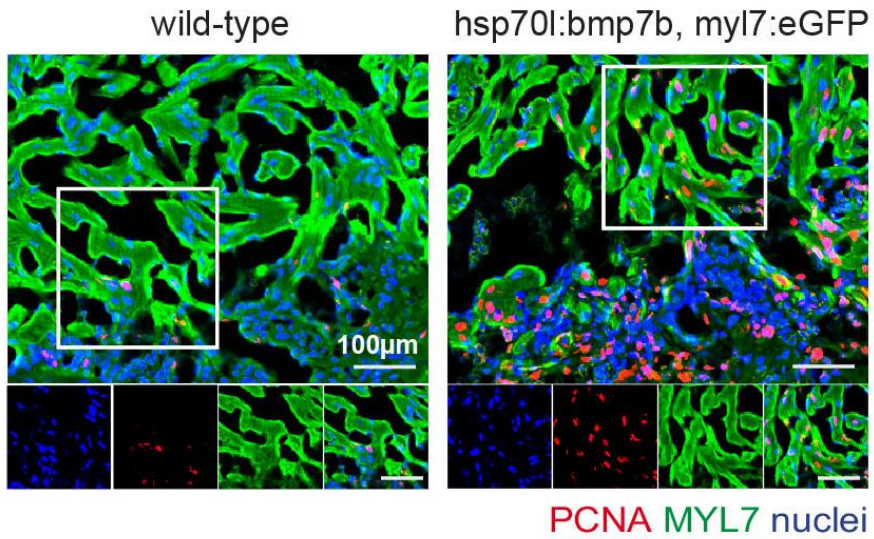
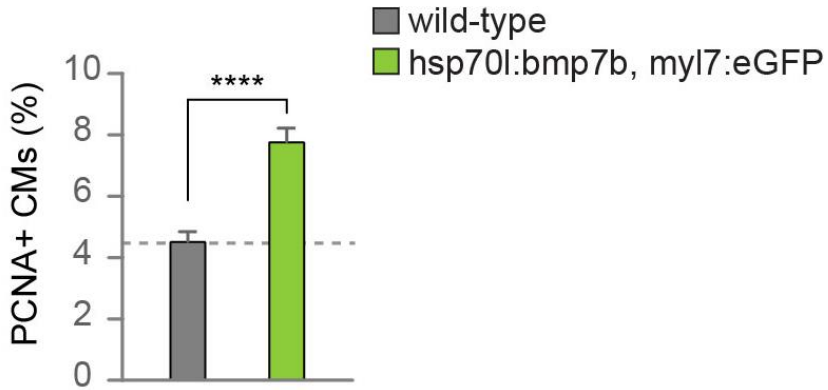
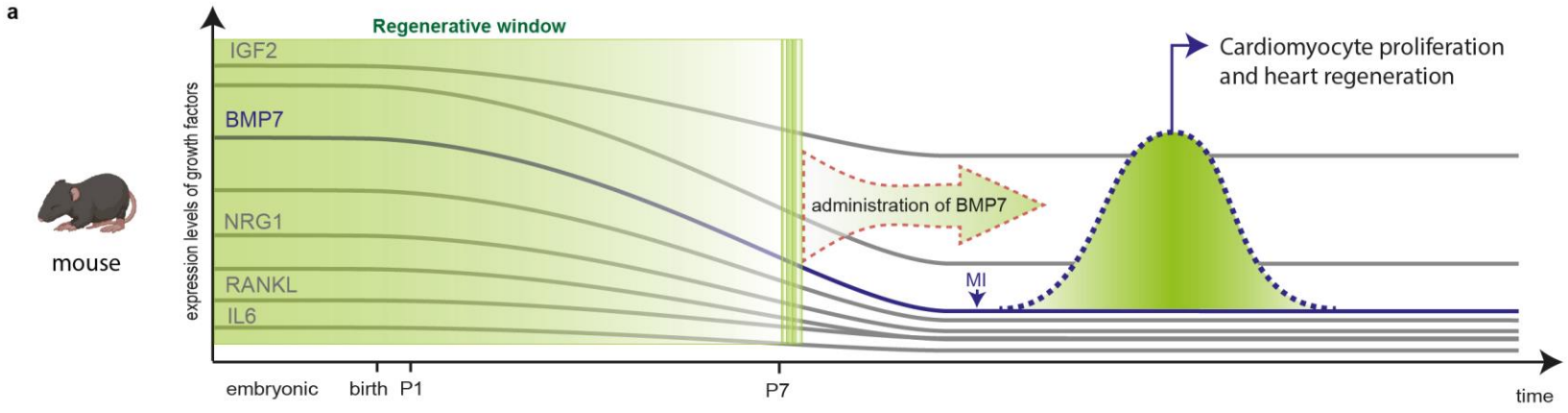




Bongiovanni … & D'Uva (under consideration)

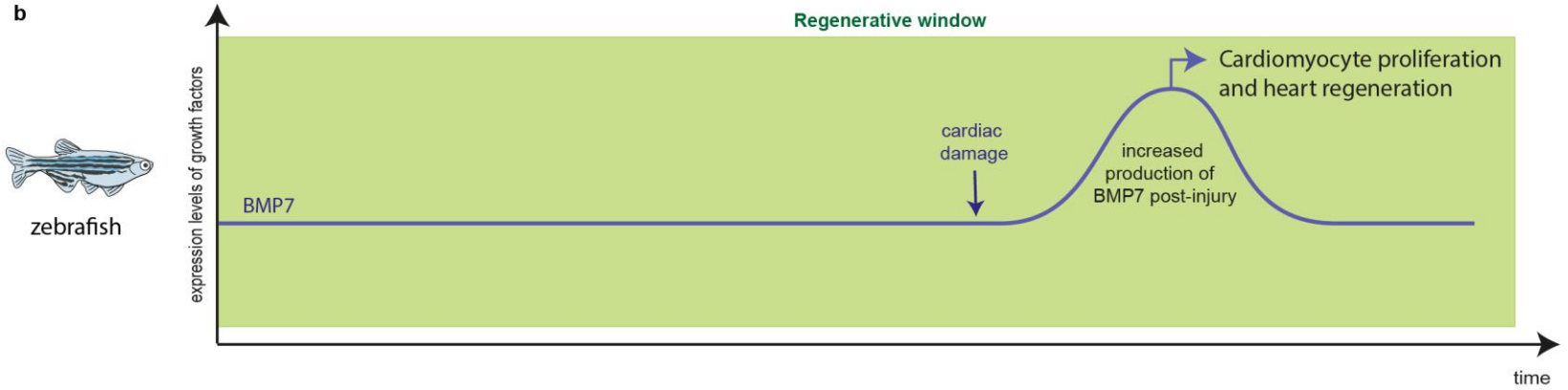
Take home messages



- Several candidate growth factor declining in expression levels in the early postnatal development, including the pro-regenerative NRG1, IGF2, RANKL, IL6 as well as BMP7, can promote the proliferation of neonatal cardiomyocytes

- BMP7, a member of the bone morphogenic proteins, is a potent inducer of cardiomyocyte cell cycle re-entry and division
- Mechanistically, BMP7-induced pro-proliferative effects are mediated by type I BMP receptors BMPR1A and ACVR1 and type II BMP receptors ACVR2A and BMPR2. Analyses of the downstream signaling cascade unveiled the involvement of the canonical SMAD5 and non-canonical AKT and ERK signalling.
- Administration of BMP7 triggers cardiomyocyte regeneration after cardiac injury in adult mammals.

A decline in the production of specific growth factors, including NRG1, IGF2, RANKL, IL6 as well as the newly identified BMP7, contributes to cardiomyocyte cell cycle exit during postnatal mammalian development resulting in the reduction of the cardiac regenerative ability



- Endogenous Bmp7 promotes cardiomyocyte cell cycle activity during the naturally occurring cardiac regeneration process in the zebrafish model.

Bongiovanni … & D'Uva (under consideration)

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