干细胞心肌分化与应用转化

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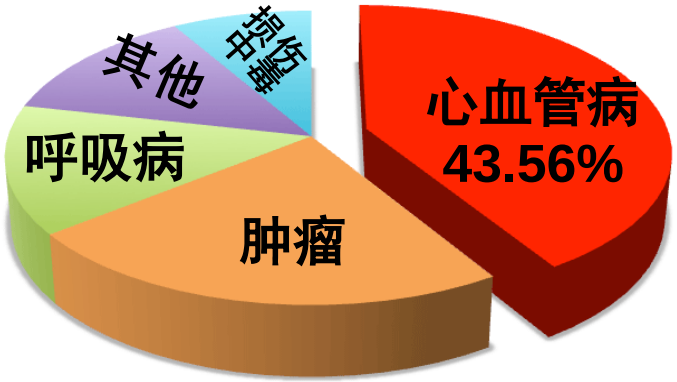
中国科学院上海营养与健康研究所

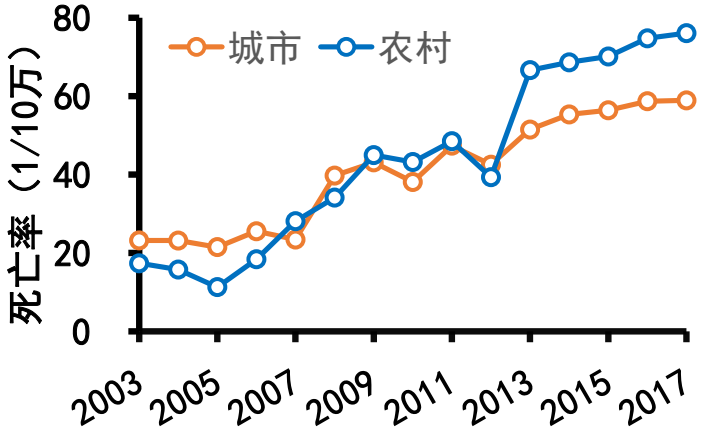
心血管病是我国疾病致死的主要原因

心血管病为国人第一杀手



2017年中国城市居民主要疾病死因构成比

心梗死亡率持续上升

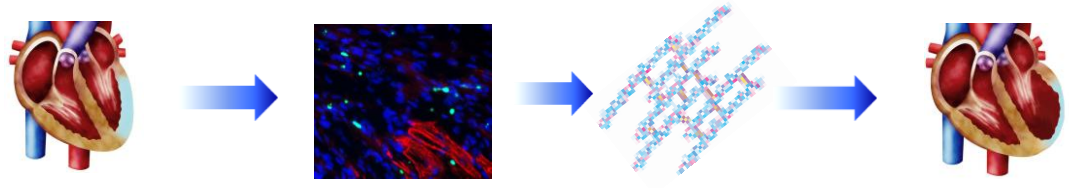


《中国心血管健康与疾病报告2019》

- >40%心血管病患者死于心梗及心力衰竭（心衰）
- 心衰5年死亡率近50%

亟需发展新的手段降低心梗后心衰死亡率

Cell therapy: a promising option for repair infarcted hearts



- Recover function
- Reduce CM death
- Limit fibrosis

- Replace the lost cells?
- Stimulate endogenous repair mechanisms?

Cell therapy

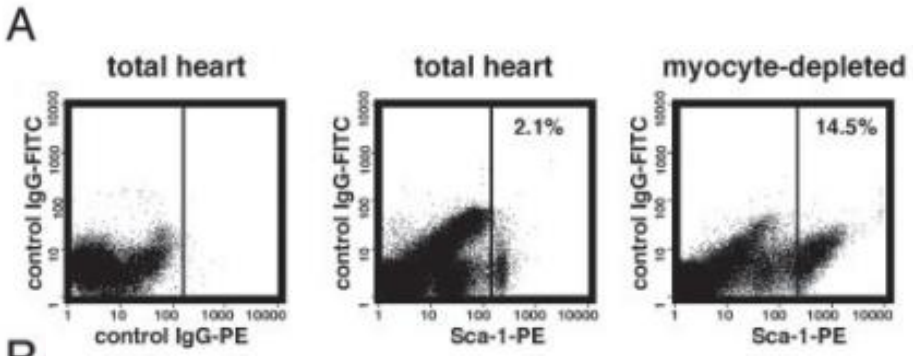
What we are going to discuss…

- Adult heart-derived cardiac progenitor cells/cardiac stem cells
- Differentiation of pluripotent stem cells (PSCs) into cardiomyocytes
- PSC-derived cardiovascular progenitor cells
- Perspectives

What we are going to discuss…

- **Adult heart-derived cardiac progenitor cells/cardiac stem cells**
- **Differentiation of PSCs into cardiomyocytes**
- **PSC-derived cardiovascular progenitor cells**
- **Perspectives**

Sca-1^{+} Cardiac progenitor cells from adult myocardium



Sca1^{+} ratio:
- **2.1% of total cardiac cells**
- **14–17% of "myocyte-depleted" fraction**

Sca1^{+} cells location:
- **Adjacent to the basal lamina;**
- **Typically coexpressing CD31 and in proximity with endothelial Sca-1^{-} CD31^{+}cells**

Prof Michael D. Schneider
Faculty of Medicine, National Heart & Lung Institute
Chair in Cardiology

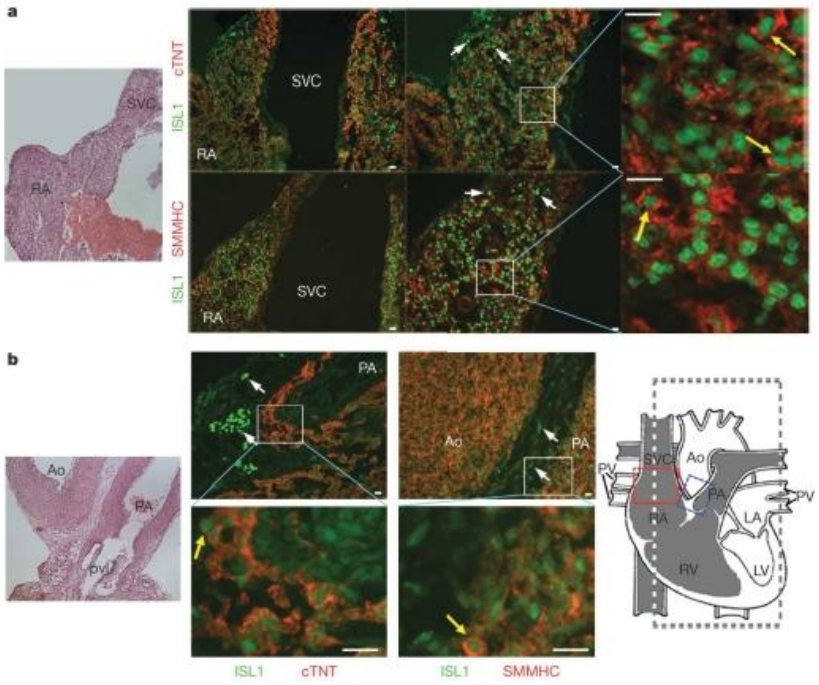
Characteristics of Sca1+ cells:

- **Lack blood cell lineage markers**
- **Express most cardiogenic TFs but not cardiac structural genes**
- **Differentiate into cardiomyocytes *in vitro***
- **Homing to injured myocardium, offering auspicious properties for cardiac repair**

Oh H, Bradfute SB, Gallardo TD, et al., PNAS 2003

New cardiomyocytes generated in human life span

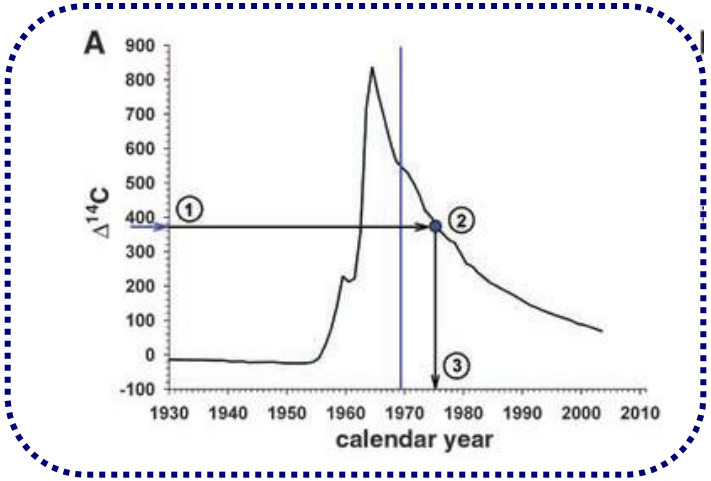
A **ISL1⁺ progenitor cells in the human fetal heart**



Lei Bu;...Kenneth R.Chien. Nature, 460(2009)113-118

B **New cardiomyocytes generated in adult human**

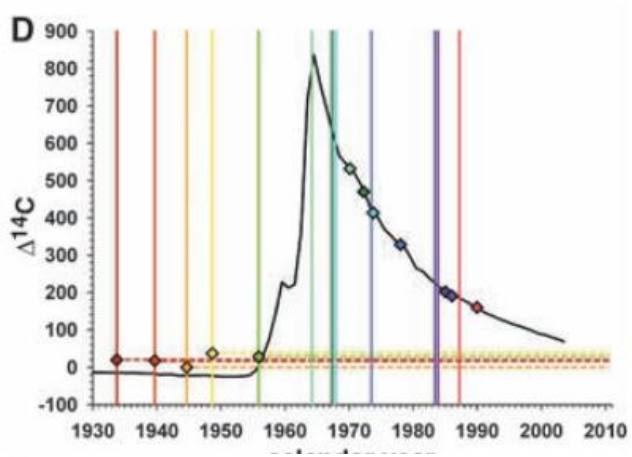
- Turnover rate 1.5% per year <25 yr
- Turnover rate <1% per year >25 yr
- 50% of CMs are exchanged during a normal life span



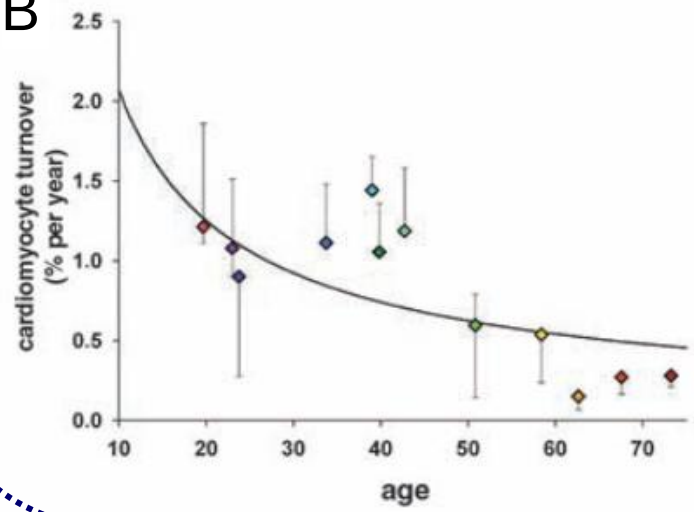
***Bergmann* et al., Science 2009**

人类出生后心肌是否具有再生能力?

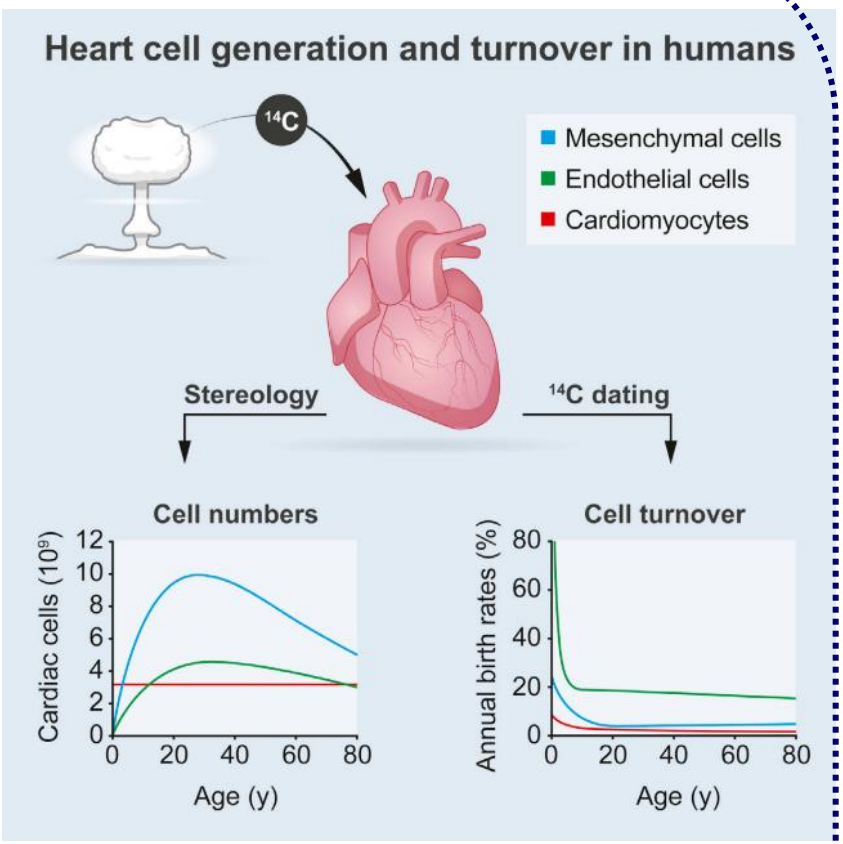
A



B



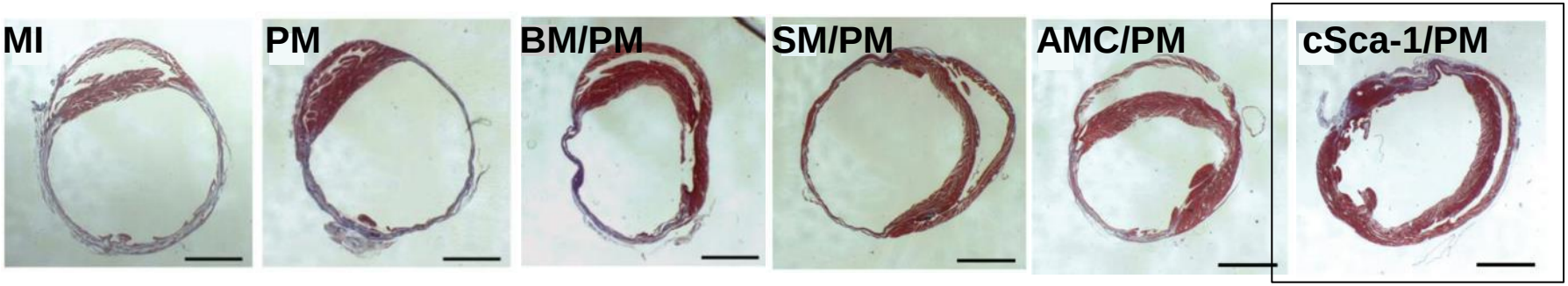
C



出生后-75岁，约50. 4%心肌细胞被更新

Bergmann et al.,2009, Science, 324, 98-102;
Bergmann et al., 2015, Cell, 161, 1566–1575.

c-Kit、cSca-1阳性细胞移植后可改善心肌梗死



MI：non-treated myocardial infarction
PM：Puramatrix™
BM：bone marrow mononuclear cells
SM：skeletal myoblasts
AMC：adipose tissue-derived mesenchymalcells
cSca-1：clonal stem cell antigen-1 positive cardiac progenitors

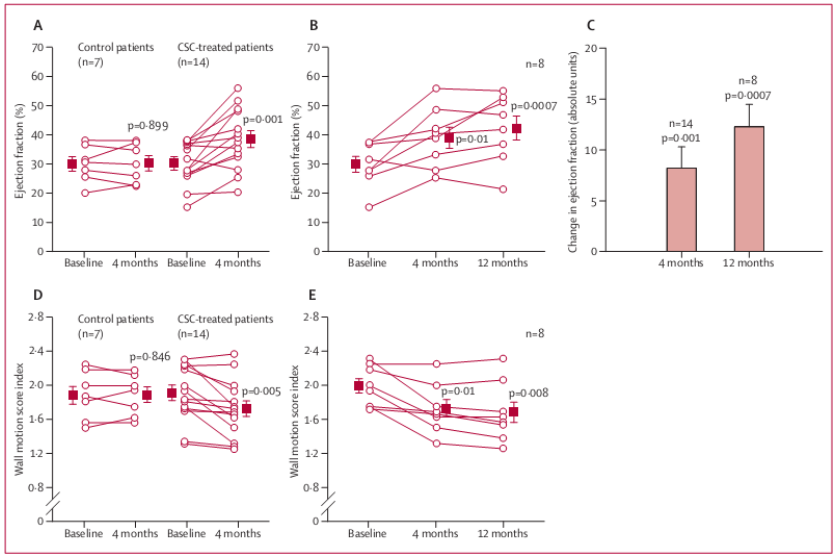
Table 1
Echocardiographic measurement of hearts 2 weeks after cell transplantation.

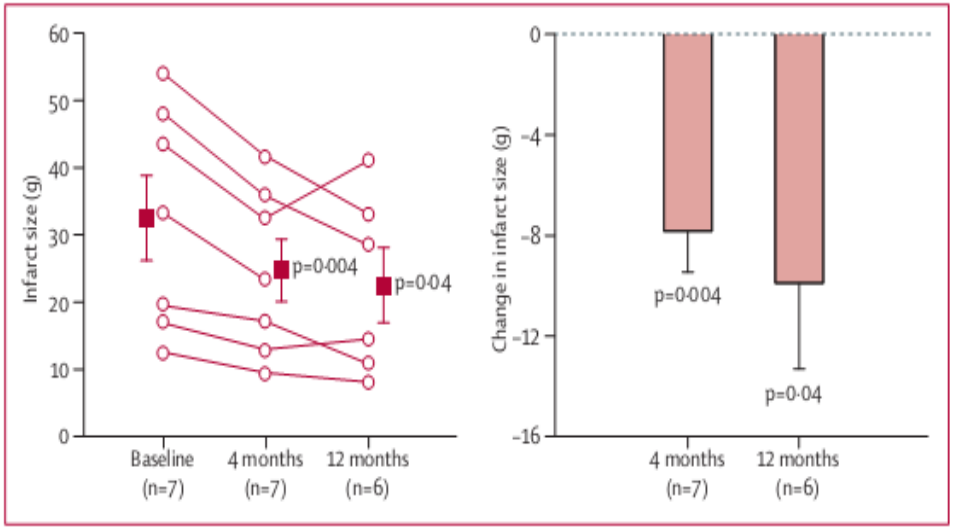
Parameter	MI	PM	BM/PM	SM/PM	AMC/PM	cSca-1/PM	cSca-1
n	13	20	8	11	16	24	14
Age (week)	13.5±2.1	13.0±1.8	12.8±1.5	14.1±1.1	13.8±0.7	13.6±1.0	14.0±2.3
BW (g)	24.8±2.2	25.1±2.0	24.7±1.3	24.9±2.0	25.0±1.6	25.4±1.4	26.5±2.4
HW (mg)	172±46	172±39	175±25	181±37	155±26	168±38	177±32
HW/BW (mg/g)	7.0±1.9	6.8±1.4	7.1±1.2	7.3±1.5	6.2±1.0	6.6±1.4	6.7±1.0
HR (min^{-1})	697±55	711±40	729±50	712±48	700±60	689±42	689±61
LVIDD (mm)	5.9±0.6	5.5±0.7	5.8±0.6	5.3±1.0	5.0±1.0	4.8±0.9[a]	5.0±1.1
LVISD (mm)	5.5±0.6	4.9±0.9	5.2±0.8	4.7±1.2	4.3±1.3[a]	4.1±1.1[a]	4.4±1.3
%FS	6.5±2.3	11±5.5	9.6±4.6	12.2±8.2	16.5±11[a]	16.2±11[a]	14.8±9.4

[a] $p<0.05$ in comparison with MI.

M Tokunaga, Meilan Liu, et al. ***J Mol Cell Cardiol.*** *, 2010*

Retraction--- Human c-kit^{+} Cardiac Stem Cells in Patients with Ischemic Cardiomyopathy (SCIPIO): Initial Results of a Randomised Phase 1 Trial





Echocardiographic analysis of CSC-treated patients and controls

- **16 patients, 7 control**

Infarct size and change in infarct size at 4 mon and 12 mon after baseline in patients administered cardiac stem cells

- **LVEF increased from 30·3 % (SE1.9) to 38·5% (2.8),4 mon, n=14, p=0·001)**
- **Infarct size decreased from 32·6 g by 7·8 g (24%) at 4 mon (p=0·004) 9·8 g (30%) at 1 year (p=0·04) by MRI.**

Roberto Bolli, et al. Lancet, 2011; 378: 1847-1857

(Retracted article)

ARTICLE

Nature. 2014 May 15; 509(7500): 337–341.

doi:10.1038/nature13309

c-kit^{+} cells minimally contribute cardiomyocytes to the heart

Jop H. van Berlo[1,2*], Onur Kanisicak[1*], Marjorie Maillet[1], Ronald J. Vagnozzi[1], Jason Karch[1], Suh-Chin J. Lin[1], Ryan C. Middleton[3], Eduardo Marbán[3] & Jeffery D. Molkentin[1,4]

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c-Kit-Cre 谱系追踪

Molkentin Lab

- Regulation of Cell Death Through the Mitochondria
- Regulation of Cardiac Hypertrophy
- Regulation of Skeletal Muscle Development and Hypertrophy
- Regulation of Fibroblast Activity
- Stem Cells in Cardiac Regeneration
- Regulation of Transcription
- Mechanisms of Duchenne and Miyoshi Myopathy

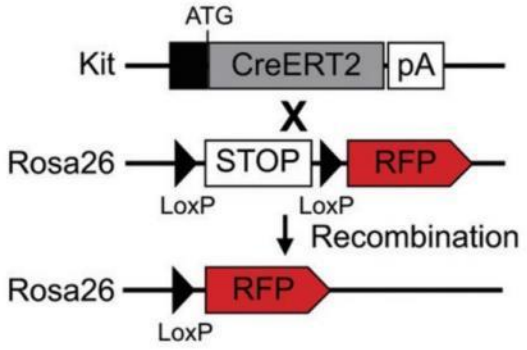
fate-mapping studies

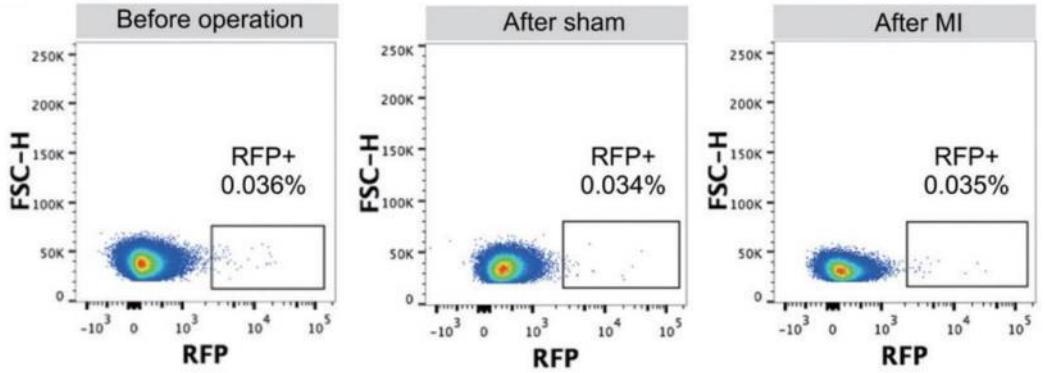
Open
ORIGINAL ARTICLE

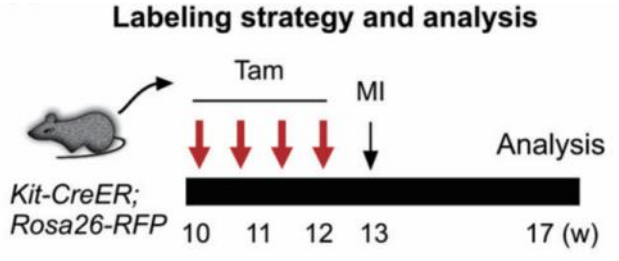
Cell Research (2015) :1-12.
www.nature.com/cr

Genetic lineage tracing identifies *in situ* Kit-expressing cardiomyocytes

Qiaozhen Liu[1, *], Rui Yang[1, *], Xiuzhen Huang[1], Hui Zhang[1], Lingjuan He[1], Libo Zhang[1], Xueying Tian[1], Yu Nie[2], Shengshou Hu[2], Yan Yan[3], Li Zhang[4], Zengyong Qiao[5], Qing-Dong Wang[6], Kathy O Lui[7], Bin Zhou[1, 8, 9]







Kit⁺ cells contribute minimally to cardiomyocytes during homeostasis or after injury

nature COMMUNICATIONS

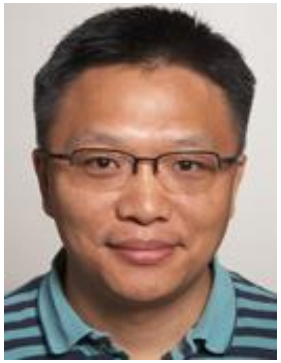
ARTICLE

Received 31 Jul 2015 | Accepted 22 Sep 2015 | Published 30 Oct 2015

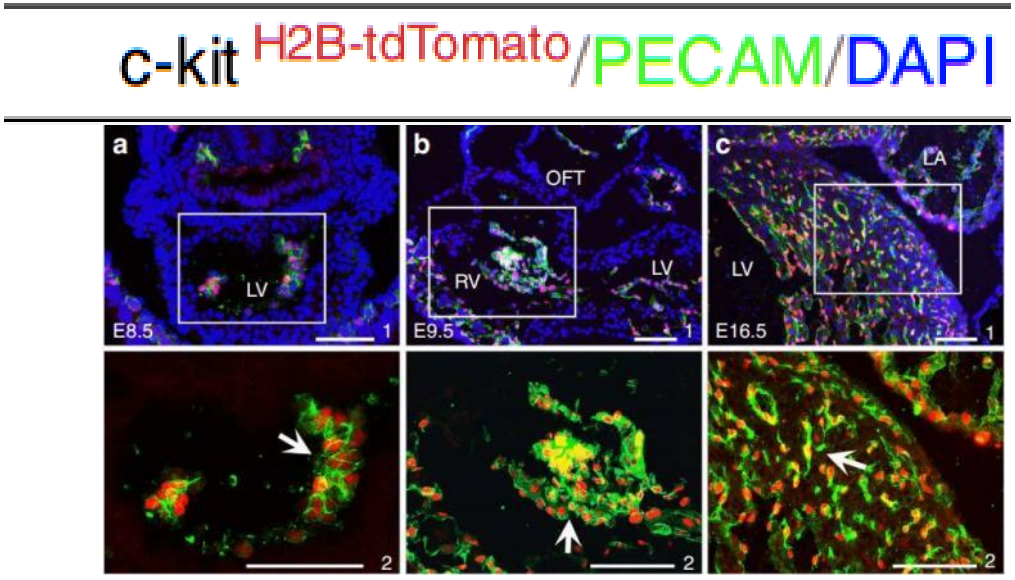
DOI: 10.1038/ncomms9701 OPEN

Resident c-kit^{+} cells in the heart are not cardiac stem cells

Nishat Sultana[1,*], Lu Zhang[1,*], Jianyun Yan[1,*], Jiqiu Chen[2], Weibin Cai[1], Shegufta Razzaque[1], Dongtak Jeong[2], Wei Sheng[3], Lei Bu[4], Mingjiang Xu[5], Guo-Ying Huang[3], Roger J. Hajjar[2], Bin Zhou[6], Anne Moon[7] & Chen-Leng Cai[1]



Assoc Prof. Chenleng Cai
Dept of Developmental and Regenerative Biology
美国西奈山医学院



Endothelial nature of cardiac resident c-kit cells

Major findings

- **Endothelial nature of cardiac resident c-kit cells;**
- **c-kit is not an appropriate marker of resident CSCs, including CSCs destined for an endothelial fate;**
- **c-kit+ endothelial cells rarely de-differentiate into CSCs to contribute to myocardial repair.**

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

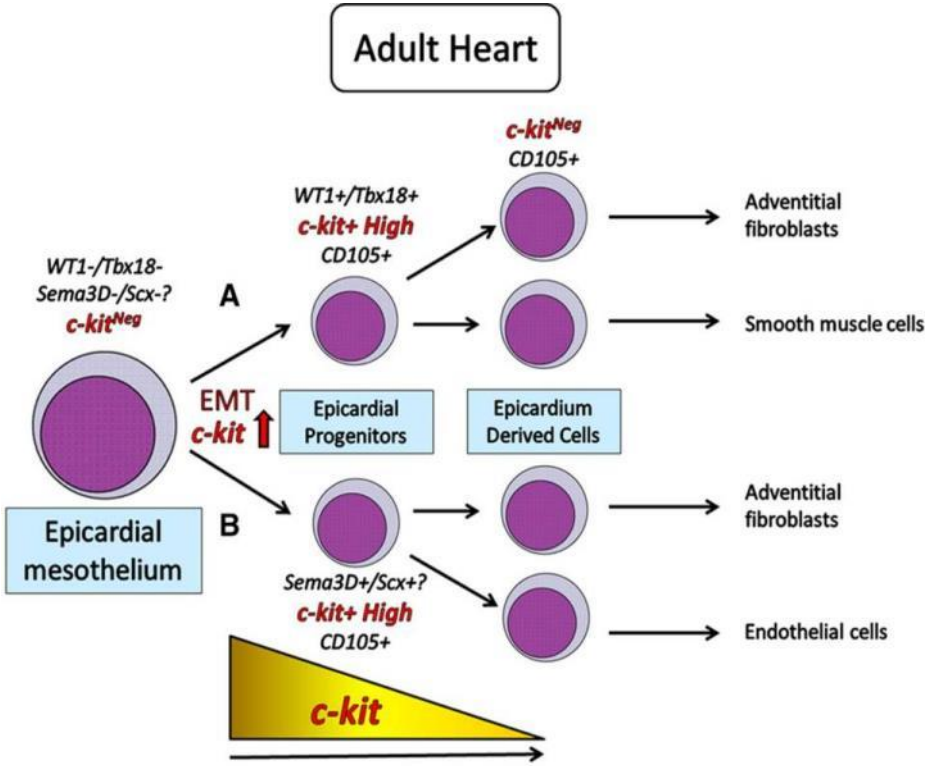
American Heart Association

Circ Res. 2015;116:1216-1230

"String Theory" of c-kit^{pos} Cardiac Cells: A New Paradigm Regarding the Nature of These Cells That May Reconcile Apparently Discrepant Results

Matthew C.L. Keith and Roberto Bolli

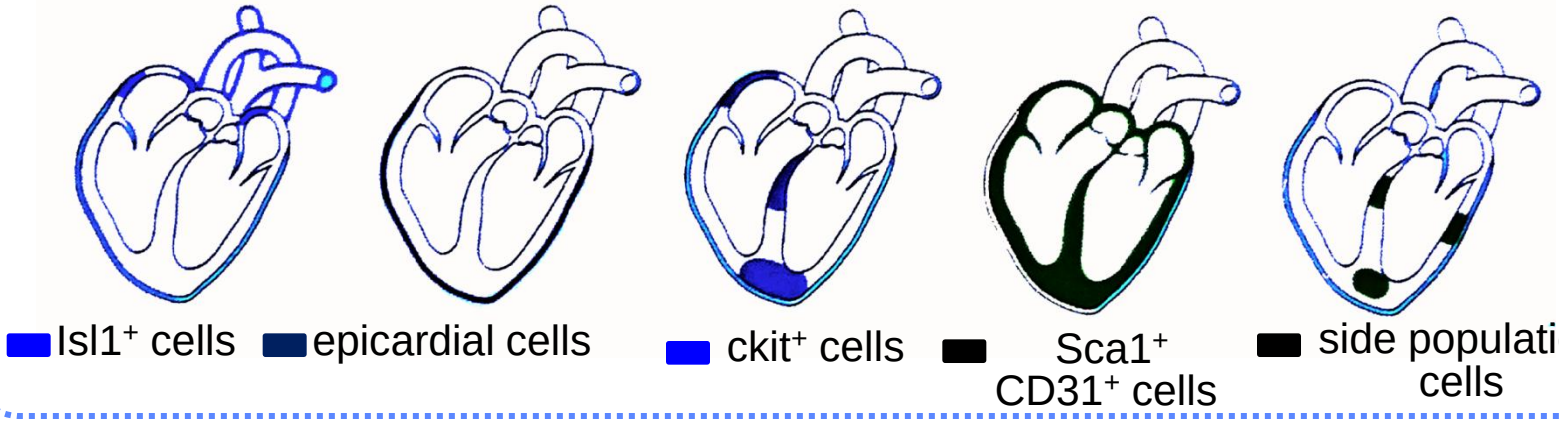
Proposed origin of c-kit^{+} cells in the adult heart



EPDCs generate Adventitial Cells/Fibroblasts/cMSCs >> Smooth Muscle > Endothelium but not Cardiomyocytes

- In the adult heart, c-kit^{+} cells are intermediates derived from c-kit^{neg} epicardial progenitors that undergo epithelial-to-mesenchymal transition (EMT). (EMT is known to be associated with expression of c-kit)
- These c-kit^{+} intermediates have a mesenchymal phenotype and give rise to c-kit^{-} epicardium-derived cells (EPDCs) with differentiation potential limited to noncardiomyocytic lineages.

心肌再生新生心肌细胞来源?
---终末分化的心肌细胞重新进入增殖周期



- 心脏干细胞(CSCs) /前体细胞(CPCs)难以分化为心肌细胞
- 移植以后有一定心肌修复作用、安全

- 丰富了对多种成体细胞的认识；
- 揭示了心肌内源性修复机制；
- 建立了临床前和临床评价移植细胞有效性和安全性的标准方案；

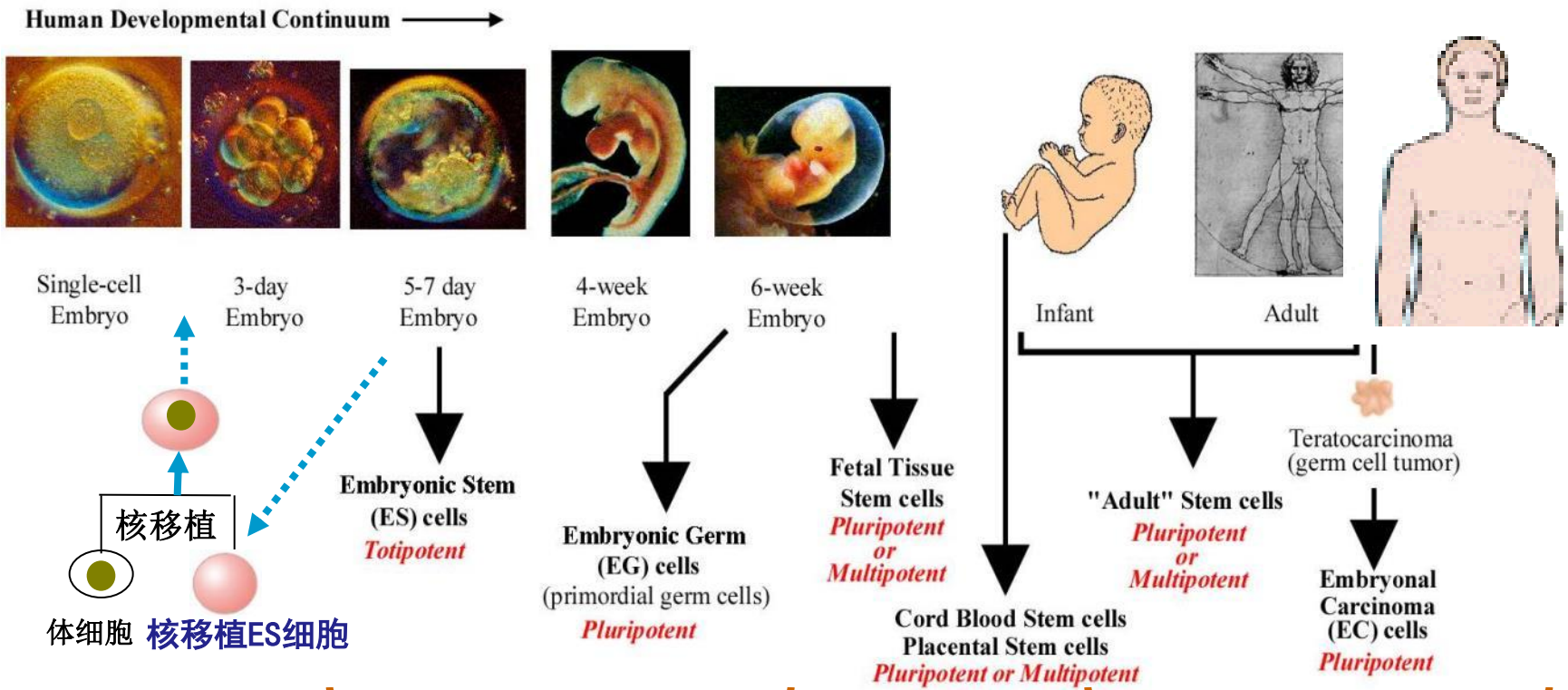
Questions need to be answered

- 成体心脏有干细胞或祖细胞吗？
- 心脏干细胞或祖细胞有哪些表面标志物？
- 它们的全貌是什么？生物学功能是什么？
- 是否还有目前没有发现的心脏干细胞或祖细胞？
- 这些干细胞或祖细胞的微环境是什么？如何激活这些细胞？
- 如何激活成年心肌细胞进入增殖周期。。。。。。

What we are going to discuss…

- Adult heart-derived cardiac progenitor cells/cardiac stem cells
- **Differentiation of PSCs into cardiomyocytes**
- PSC-derived cardiovascular progenitor cells
- Perspectives

Stem Cells



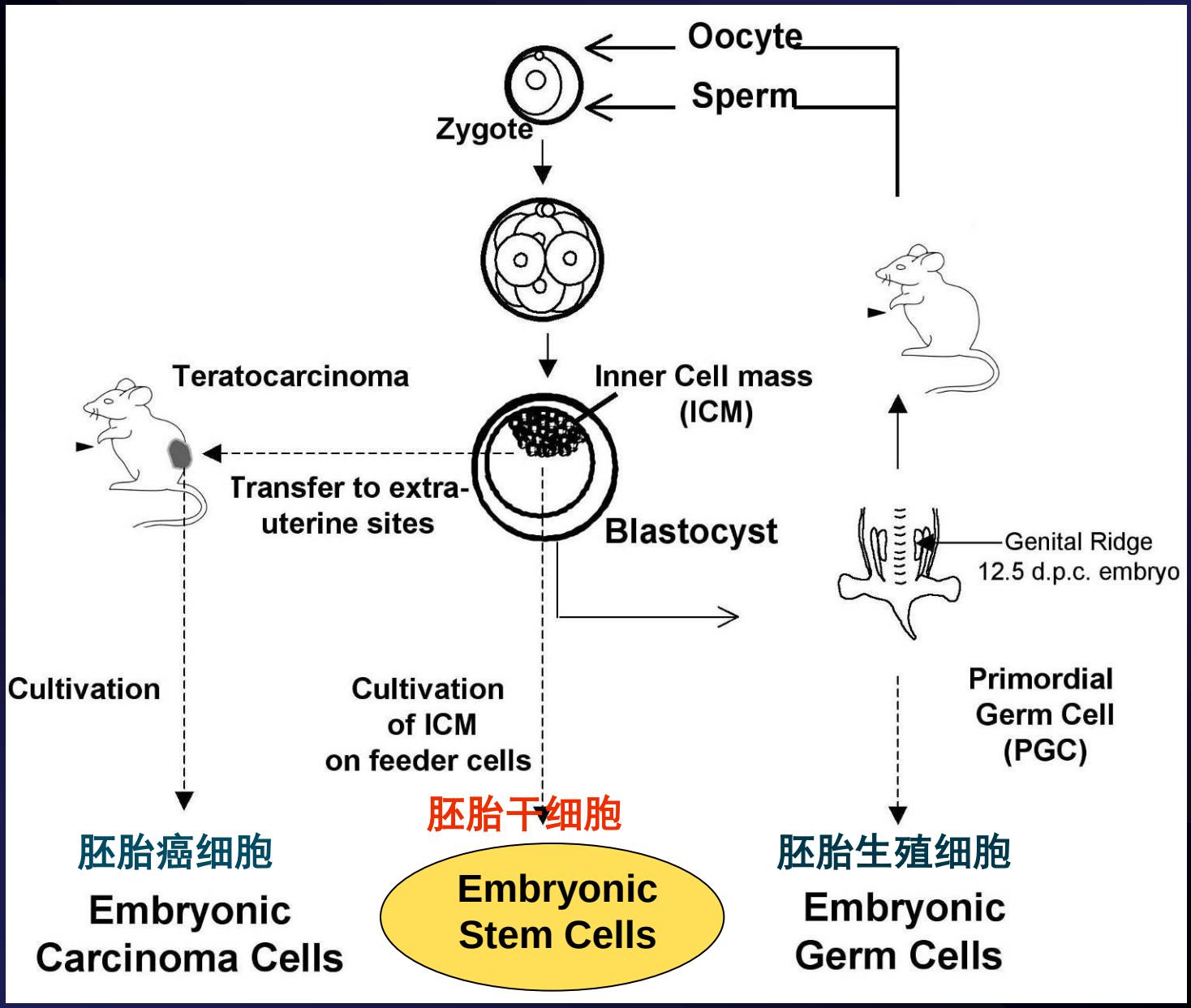
PSCs

ES cells **iPS cells** **tissue stem cells**

胚胎干细胞 **诱导性多能干细胞** **组织干细胞**

Self-renewal

Differentiation potential


Oocyte
Sperm
Zygote
Teratocarcinoma
Inner Cell mass
(ICM)
Transfer to extra-
uterine sites
Blastocyst
Genital Ridge
12.5 d.p.c. embryo
Cultivation
Cultivation
of ICM
on feeder cells
Primordial
Germ Cell
(PGC)
胚胎干细胞
胚胎癌细胞
Embryonic
Carcinoma Cells
Embryonic
Stem Cells
胚胎生殖细胞
Embryonic
Germ Cells

History: pluripotent stem cells

1954	mEG cells	Chiquoine AD
1981	mES cells	Evans MJ & Kaufmann MH
1981	mEC cells	Martin G
1980	hEC cells	Andrews PW et al.
1989	hEC cells	Pera MF et al. (multipotent)
1998	hES cells	Thomson JA
1998	hEG cells	Shamblott MJ, Gearheart JD
2006	miPS cells	Takahashi K & Yamanaka S
2007	hiPS cells	Yamanaka S & George Q. Daley
2008	rES cells	Smith A & Ying QL

ESC lines of other species

Sheep ES cells. Handyside A, Hooper ML, Kaufman MH, Wilmut I. Roux's Arch Dev Biol 196:185–90, 1987

Rabbit ES cells. Graves KH, Moreadith RW. Mol Reprod Dev 36:424–33, 1993

Swine ES cells. MB Wheeler. Reprod Fertil Dev 6:563–8, 1994

Bovine ES cells. Cherny RA, Stokes TM, Merei J, Lom L, Brandon MR, Williams L. Reprod Fertil Dev 6:569–75, 1994

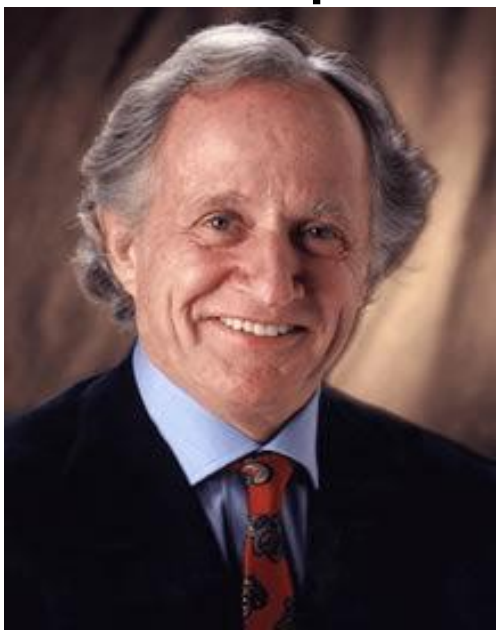
ES-like cells from early **zebrafish** embryos. Sun L, Bradford CS, Ghosh C, Collidi P, Barnes DW. Mol Mar Biol Biotechnol 4:193–9, 1995

Primate ES cells. Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Becker RA, et al. Proc Natl Acad Sci USA 92:7844–8, 1995

Avian ES cells. Pain B, Clark ME, Shen M, Nakazawa H, Sakurai M, Samarut J, et al. Development 122:2339–48, 1996

… …

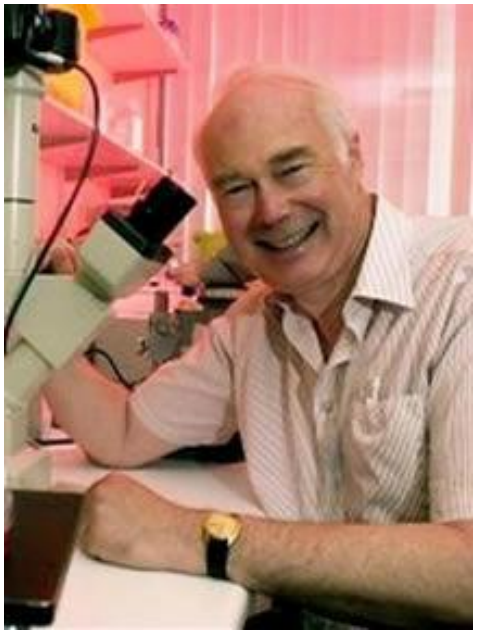
Mario R. Capecchi



1937, Verona, Italy
University of Utah,
Howard HughesMedical Institute

Gene Targeting

Sir Martin J. Evans



1941, United Kingdom
Cardiff University,
Cardiff, United Kingdom

Using Mouse ESCs for Gene Targeting & Gene Trap

Oliver Smithies

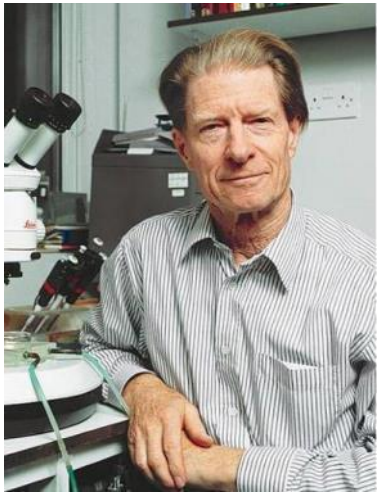
1925, Halifax,United Kingdom
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Gene KO in mice

Prize motivation: "for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells."

The Nobel Prize in Physiology or Medicine 2007

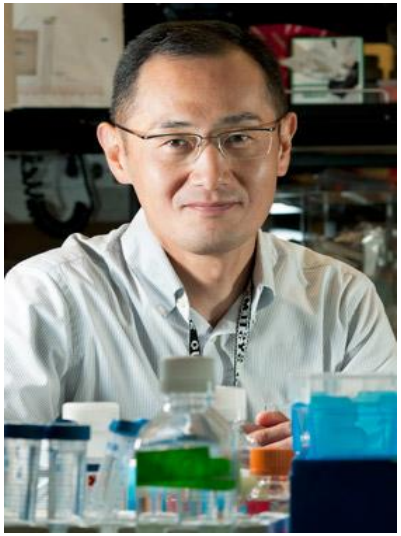
John B. Gurdon



1933, United Kingdom
Gurdon Institute, Cambridge, United Kingdom

细胞核移植与克隆

Shinya Yamanaka

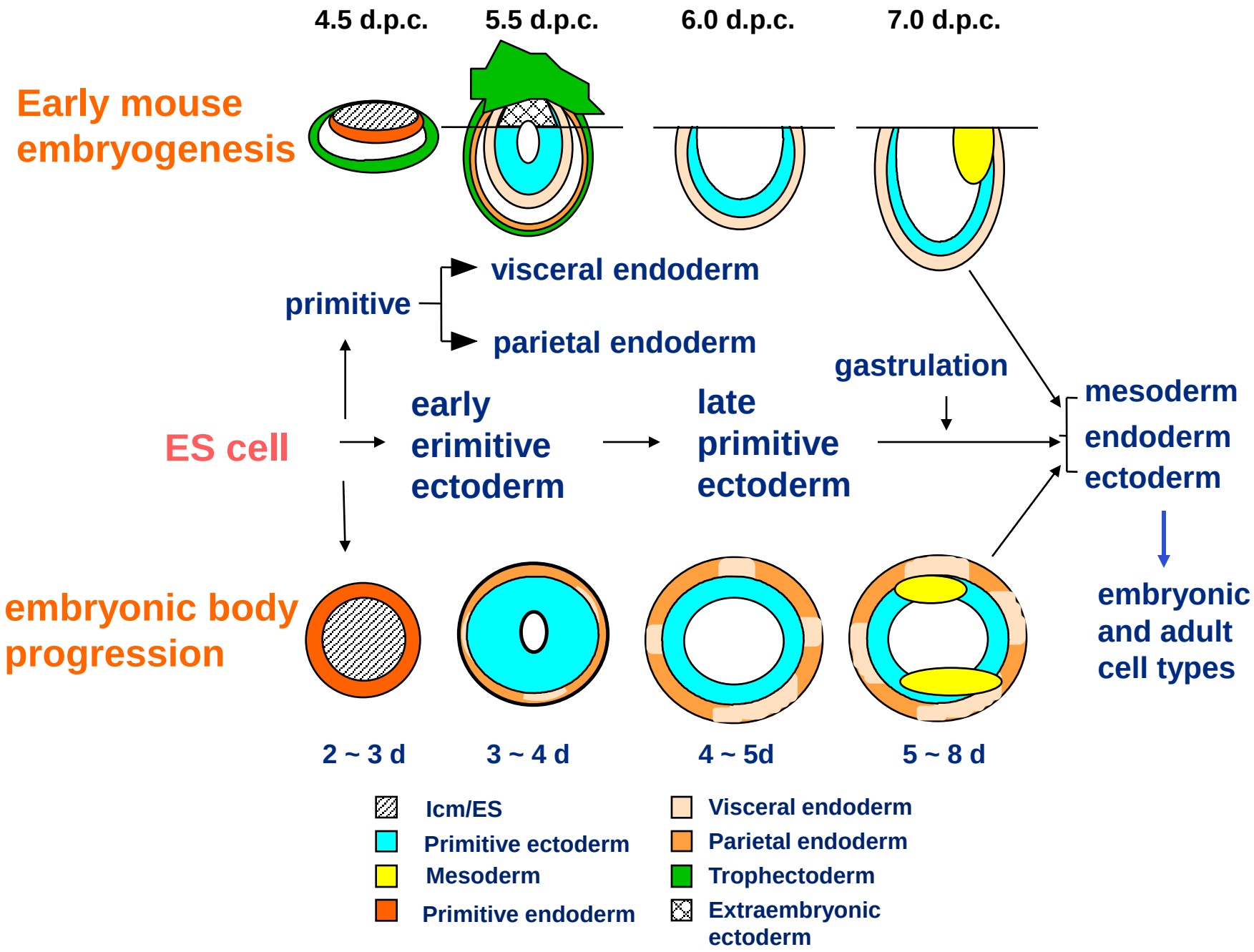


1962, Osaka, JapanKyoto University, Kyoto, Japan & Gladstone Institutes, San Francisco, CA,USA

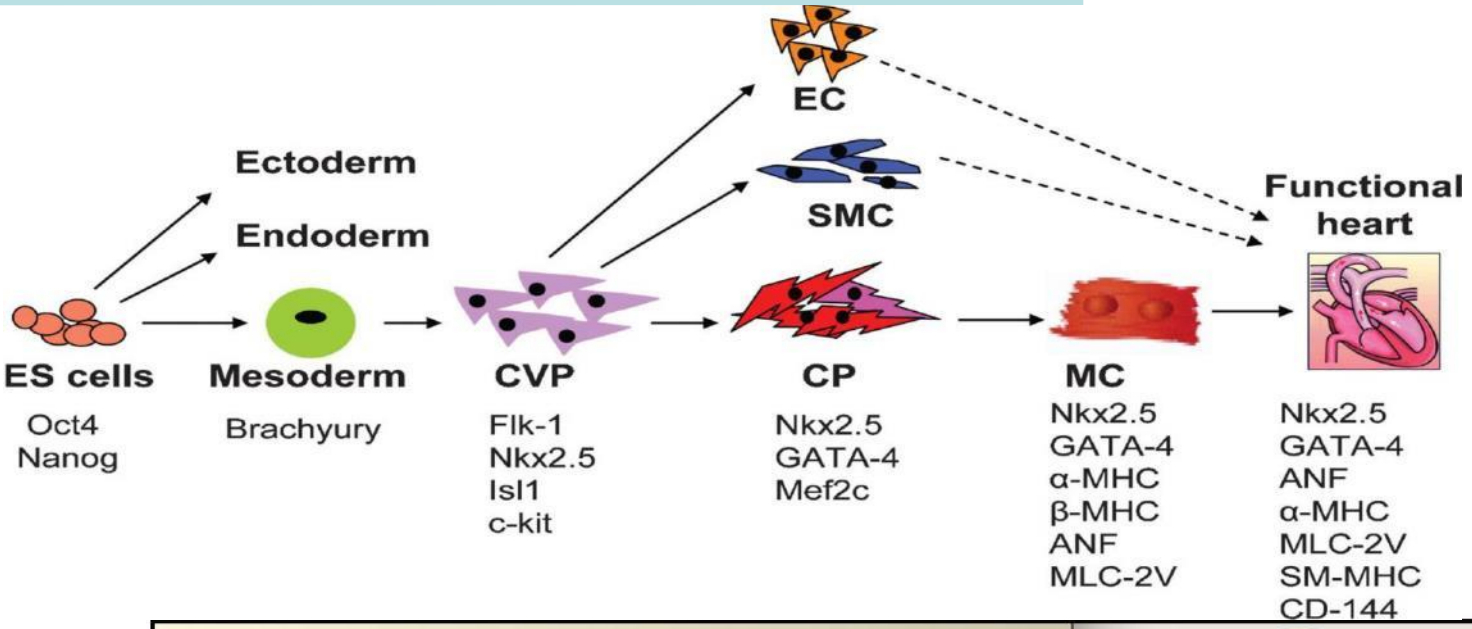
iPSCs

Prize motivation: "for the discovery that mature cells can be reprogrammed to become pluripotent

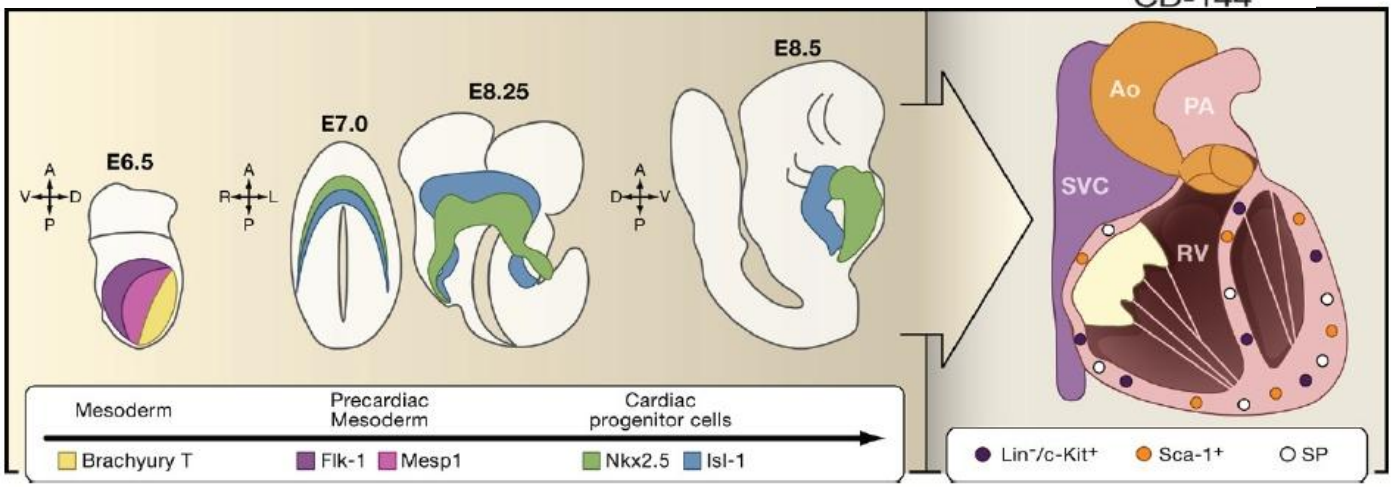
The Nobel Prize in Physiology or Medicine 2012


4.5 d.p.c.
5.5 d.p.c.
6.0 d.p.c.
7.0 d.p.c.
Early mouse embryogenesis
primitive
visceral endoderm
parietal endoderm
gastrulation
ES cell
early erimitive ectoderm
late primitive ectoderm
mesoderm
endoderm
ectoderm
embryonic body progression
embryonic and adult cell types
2 ~ 3 d
3 ~ 4 d
4 ~ 5d
5 ~ 8 d
Icm/ES
Primitive ectoderm
Mesoderm
Primitive endoderm
Visceral endoderm
Parietal endoderm
Trophectoderm
Extraembryonic ectoderm

再现了人类胚胎早期发育过程



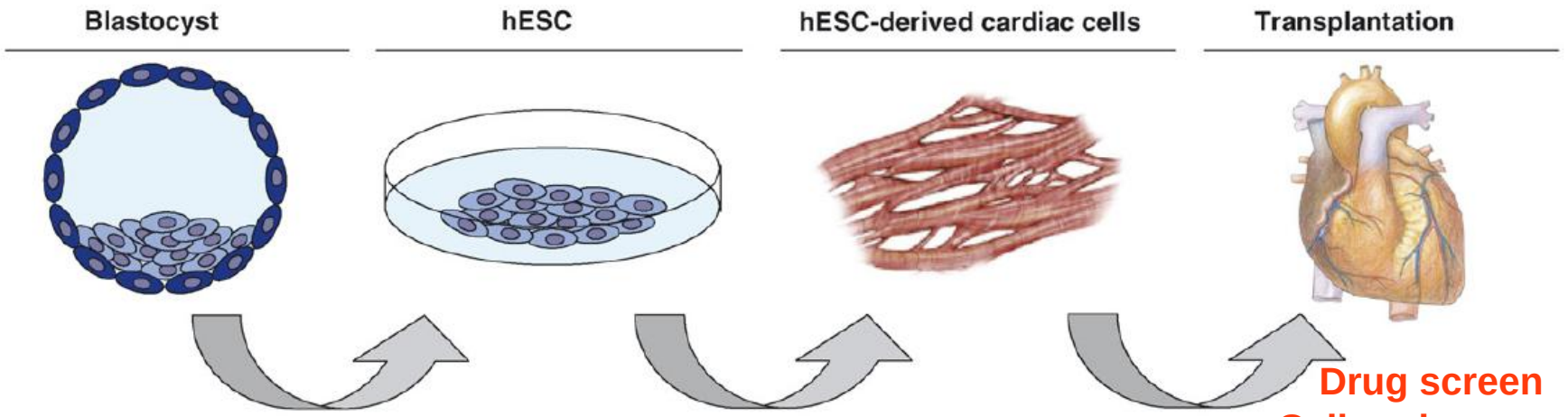
CVP,
cardiovascular progenitors
EC,
endothelial cells
SMC: smooth muscle cells
CP,
cardiomyocyte progenitors
MC,
mature cardiomyocytes



Origins of Cardiac Progenitor Cells in the Developing Heart

Wu & Mummery et al. *Cell* 2008,132,537

Differentiation to tissue cells is a prerequisite for the application of PSCs in clinic



Derivation
- Reproducible isolation techniques
- Ethical considerations

Culture
- (Epi)genetic stability
- Animal-free culture environment

Differentiation
- Reproducible cardiac differentiation system

Purification
- Teratoma formation

Delivery
- Intramuscular/ intracoronary/intravenous?
- Engineered scaffolds?

Integration
- Electromechanical coupling
- Paracrine function

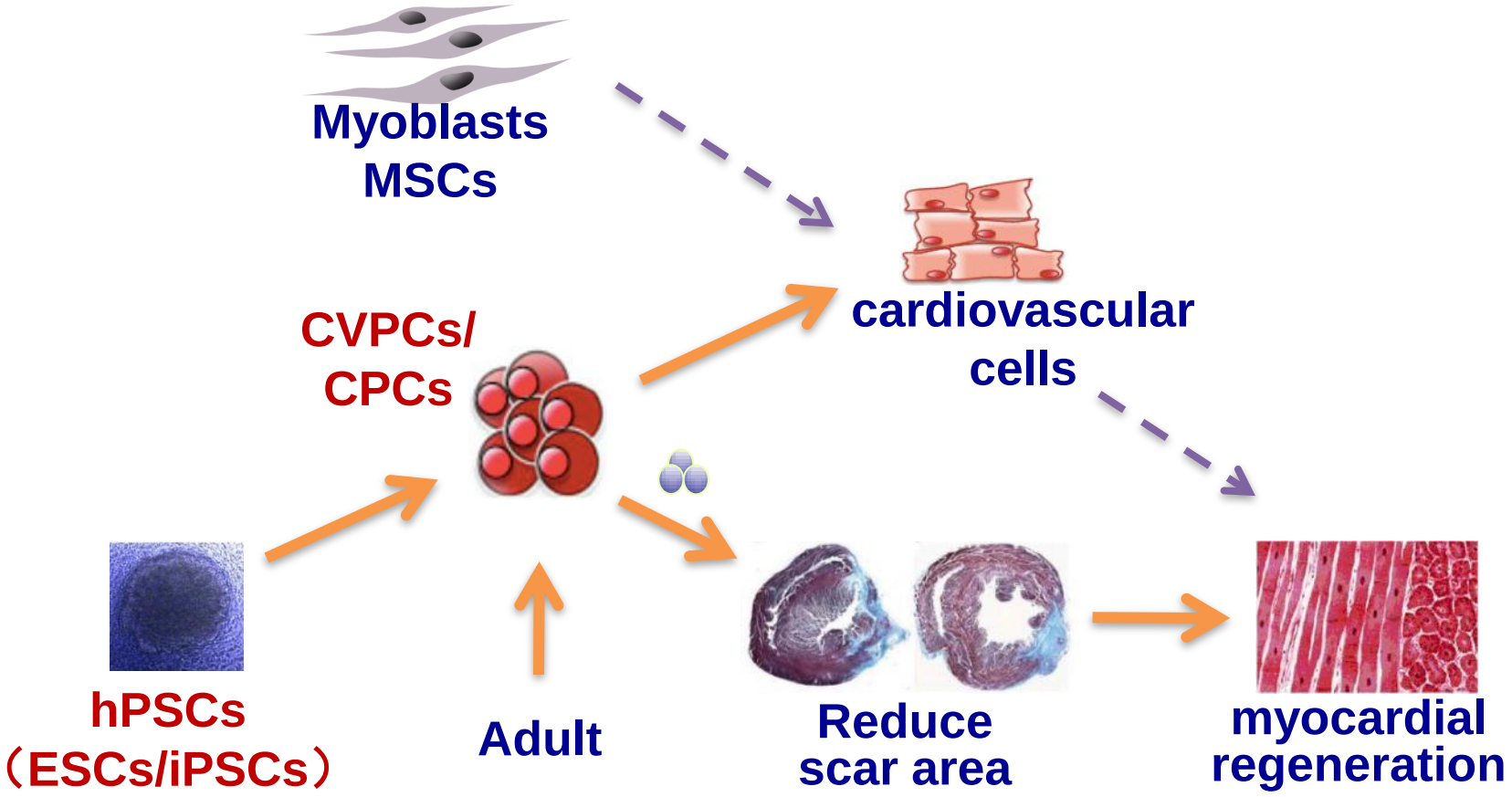
Survival
- Immune rejection
- Non-specific cell death

Current Opinion in Biotechnology

From laboratory to bedside:
myocardial differentiation!

Swijnenburg RJ, … Wu JC. Curr Opin Biotechnol. 2007, 18:38–45

What are ideal cells for myocardial regeneration?



Cao N. … Yang HT Cell Res 2012*
Cao N. … Yang HT Cell Res 2013*
Cao N. … Yang HT Methods in Mol Bio 2015*

Menasche et al., European Heart J 2015
Birket MJ, Mummery CL. Circ Res 2015
Ye L. … Zhang J. Cell Stem Cell 2014

Q1: how to induce?

Strategies for *in vitro* differentiation of murine ES cells to cardiomyocytes

- 培养方式： 3D aggregates (EBs, embryoid bodies; monolayer culture)
- 诱导物:
  - Treatment with compounds/growth factors;
  - Different inducers added at different differentiation stages

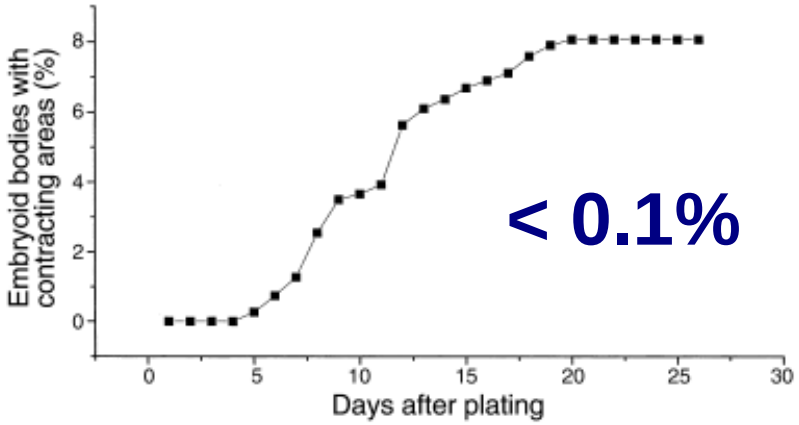
Differentiation of human PSCs into cardiomyocytes

Strategies for *in vitro* differentiation of hESCs to cardiomyocytes

心肌细胞定向诱导分化策略的发展路程：

1. **Human EBs (I Kehat et al. J. Clin. Invest. 2001; 108:407–414)**
2. **Supplementation with 5-aza-dC (DNA甲基转移酶抑制剂，70% EBs with contracting areas, C hunhui Xu et al. Circ Res. 2002;91:501-508)**
3. **Co-culture with visceral endoderm-like cells (END-2, 35±10% beating areas, Christine Mummery et al. Circ. 2002;107:2733-2740)**
4. **Serum free + co-culture with END-2 (Robert P. et al. Stem Cells 2005;23:772–780)**
5. **Stage specific optimization (Gordon M. Keller, Sean M. Wu et al.)**
6. **Induction with small molecules and growth factors**

1. hESC-derived cardiomyocytes in vitro



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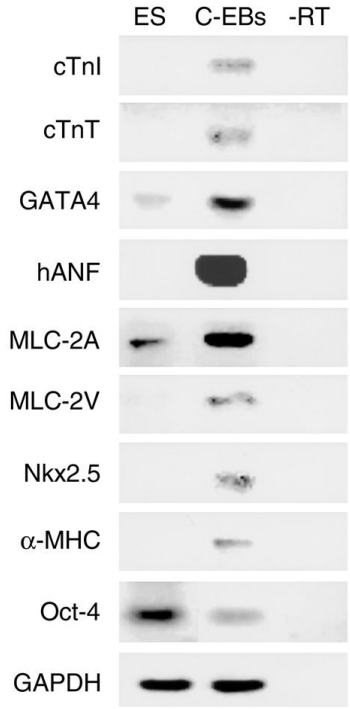
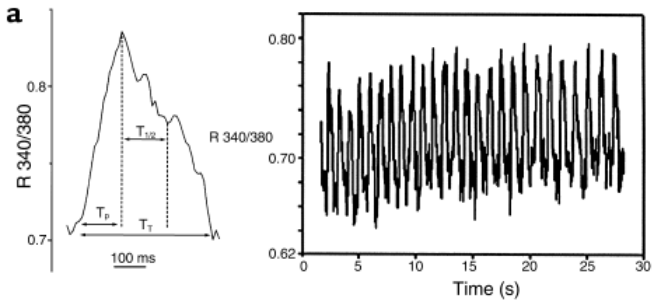
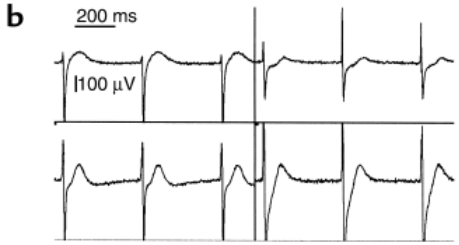
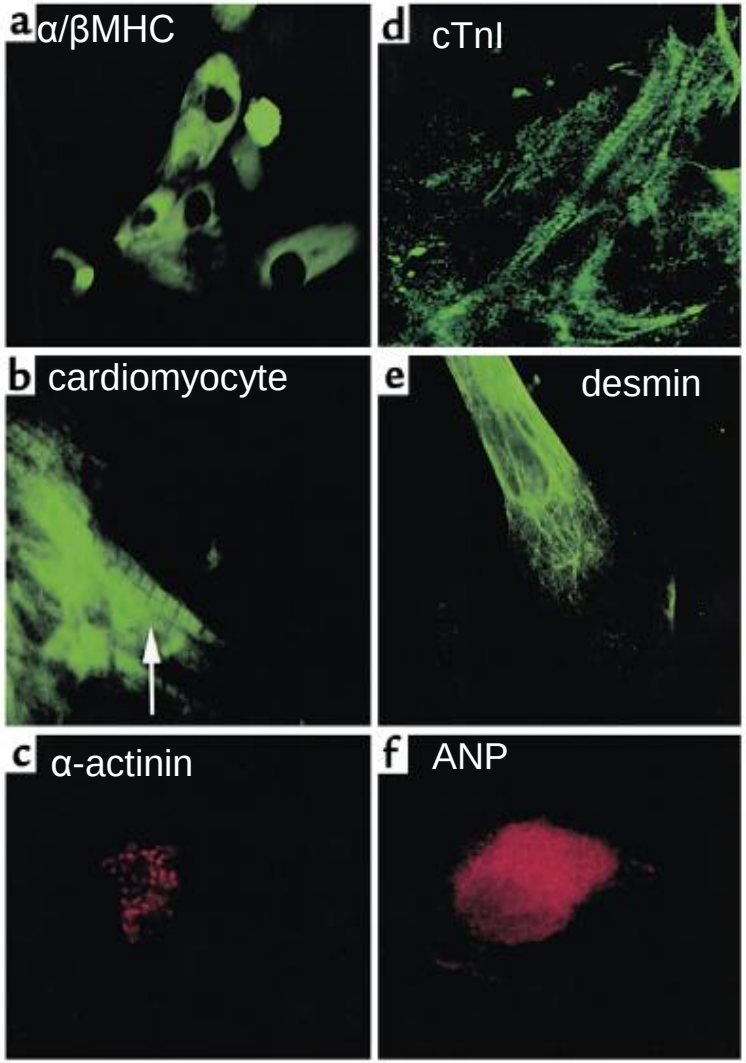






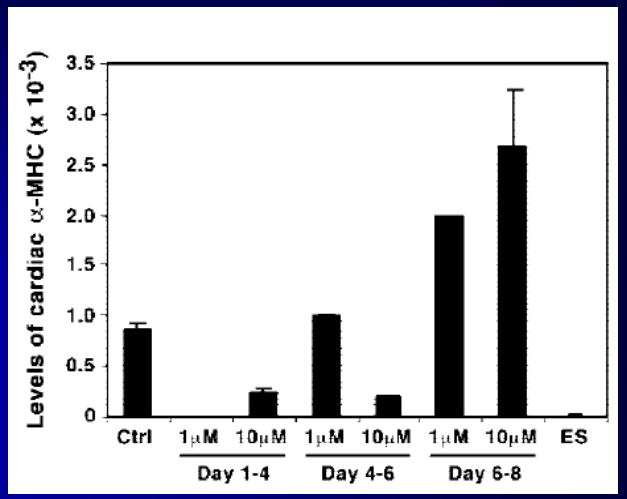


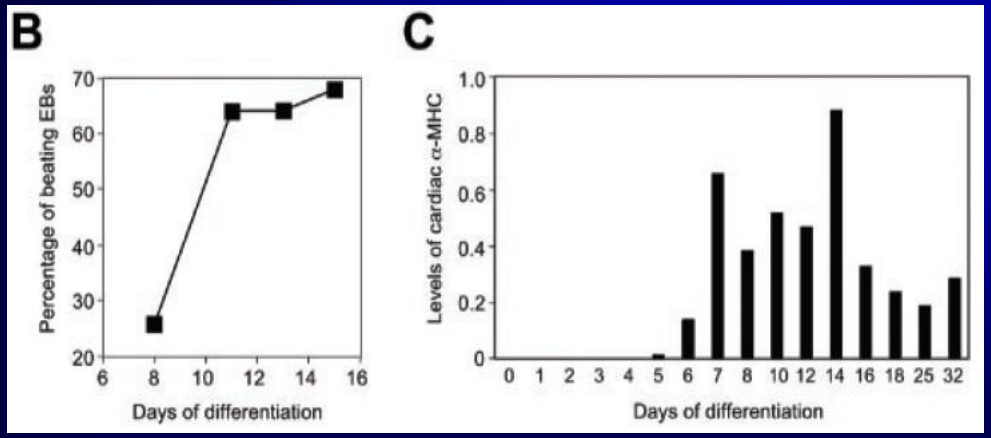
Kehat, I. …Gepstein L. J Clin Invest. 2001;108, 407-414

2. Supplementation with 5-aza-2-deoxycytidine

Undiff hES cells **form EBs** **cardiomyocytes**

5-aza-dC treatment

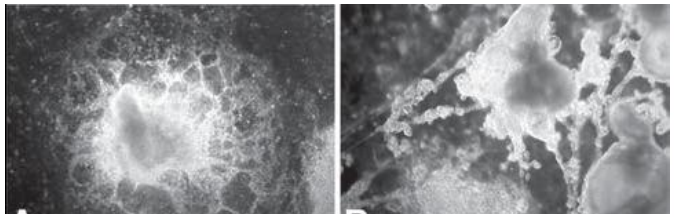




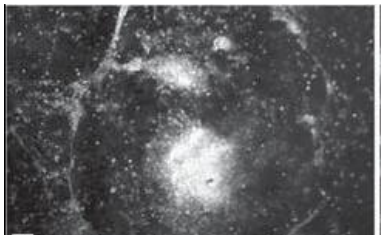
(H1, H7, H9, H9.1, and H9.2)

Xu, C. et al. Circ Res 2002; 91,501-508

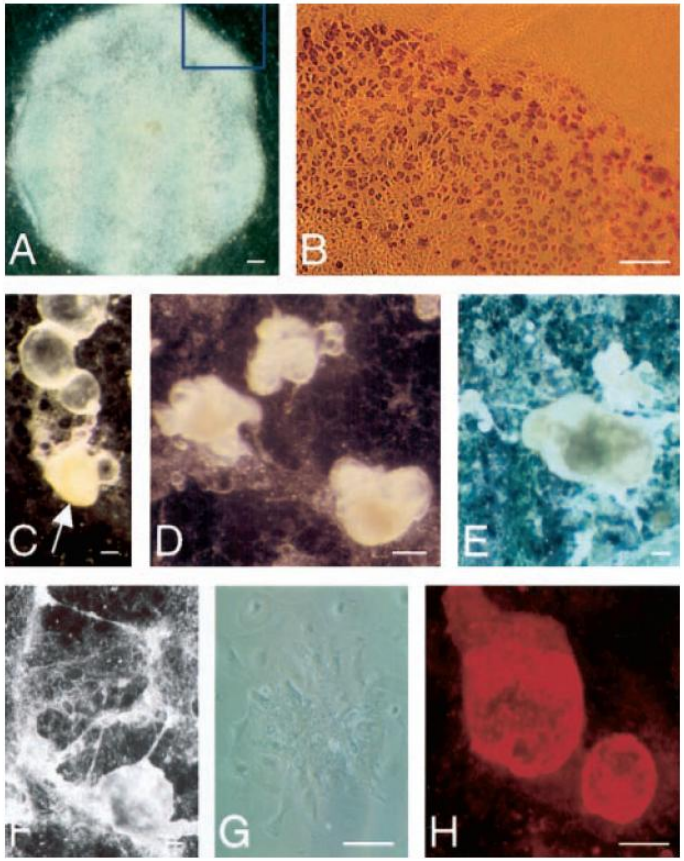
3. Co-culture with a visceral endoderm-like cells line (END-2)



On END-2



On MEF

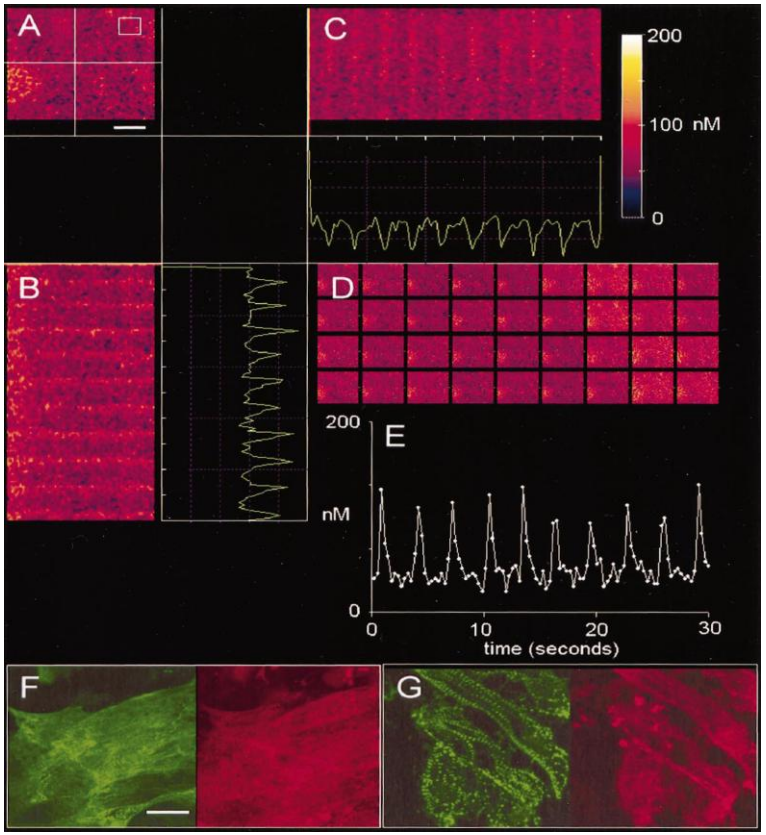


A. Undifferentiated hES cell colony
B. Nuclear oct-4 staining
C. hES cells after 11 days of co-culture with beating aggregate(arrow).
D. Morphologies of beating muscle aggregates
E. Phase-contrast image of beating area in hES/END-2 coculture.
F. Phase-contrast image of a nonbeating area with cardiomyocyte morphology.
G. Dissociated hES aggregate
H. Cystic structures stained with -fetoprotein

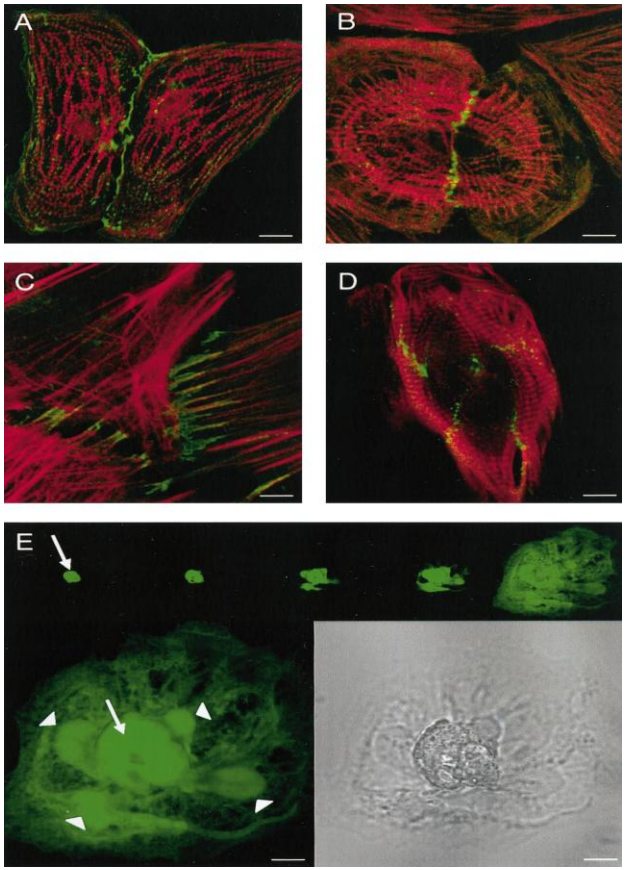
Christine Mummery et al.
Circulation. 2002 107:2733-2740

Mummery, C. et al. Circulation. 2003 Jun 3;107(21):2733-40
Passier R., … Mummery, C. Stem Cells 2005; 23:772-780

Differentiation of hESCs to cardiomyocytes: role of coculture with visceral endoderm-like cells



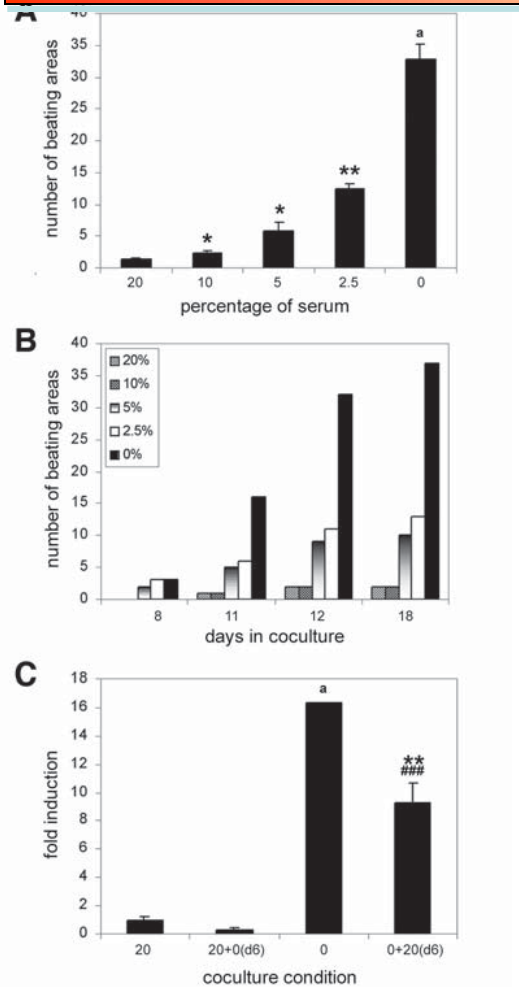
Calcium transients and L-type calcium channels

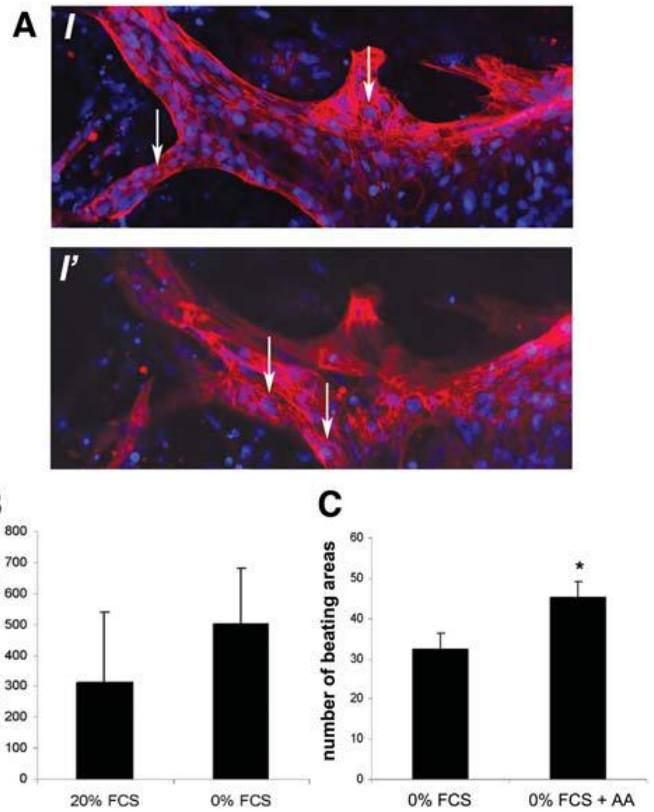


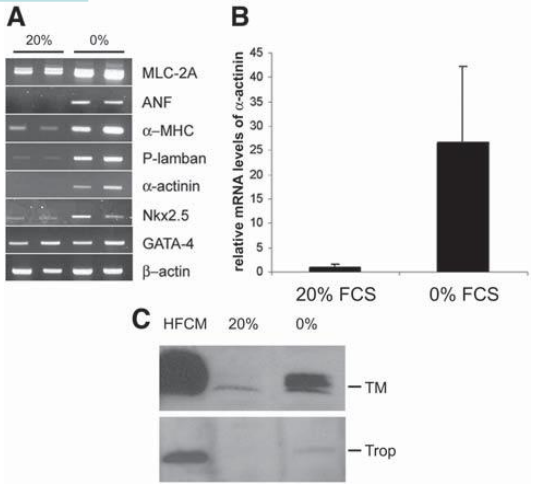
Junctional communication in hES-derived and human fetal cardiomyocytes

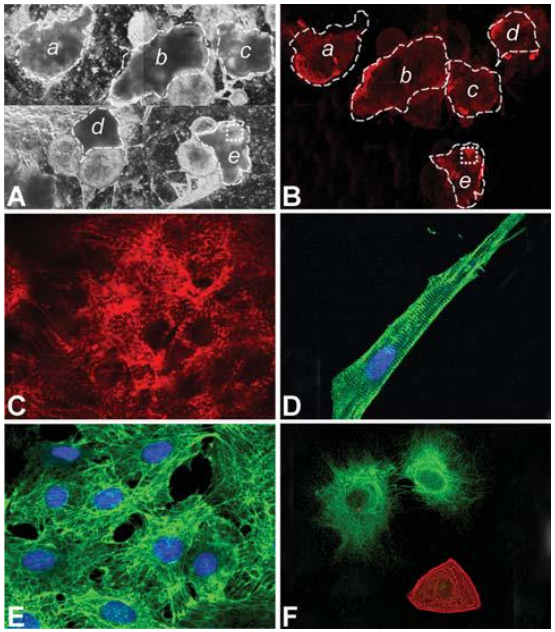
Christine Mummery et al. *Circulation*. 2002 107:2733-2740

4. Increased cardiomyocyte differentiation from hESCs in serum-free cultures







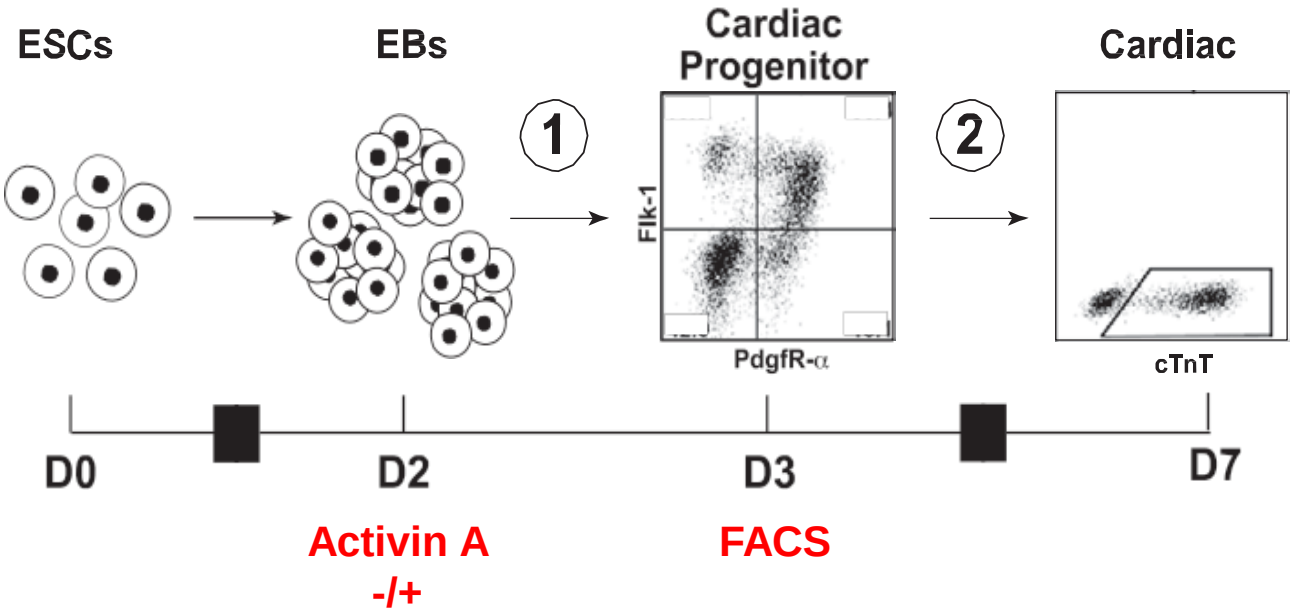


Effects of serum on the number of beating areas in hESC-END-2 cocultures

Robert P. et al. *Stem Cells* 2005 23:772–780

5. Stage-Specific Optimization of Activin/Nodal and BMP Signaling Promotes Cardiac Differentiation of Mouse and Human Pluripotent Stem Cell Lines

Steven J. Kattman,[1] Alec D. Witty,[1,2] Mark Gagliardi,[1] Nicole C. Dubois,[1] Maryam Niapour,[1] Akitsu Hotta,[3] James Ellis,[3,4] and Gordon Keller[1,*]



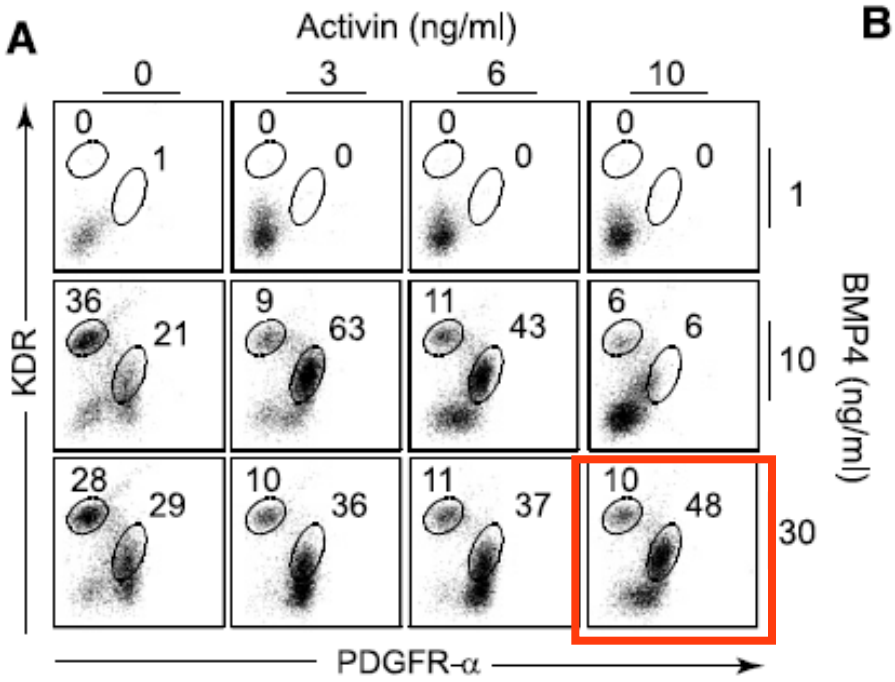
Gordon Keller, PhD
Senior Scientist, Canada
Ontario Cancer Institute

Stage 1, with various concentrations of Activin A and BMP4;
Stage 2, whole unsorted or FACS-sorted cells are cultured in "cardiac conditions" (+VEGF, DKK1, bFGF, FGF10), analyzed with FACS at d7.

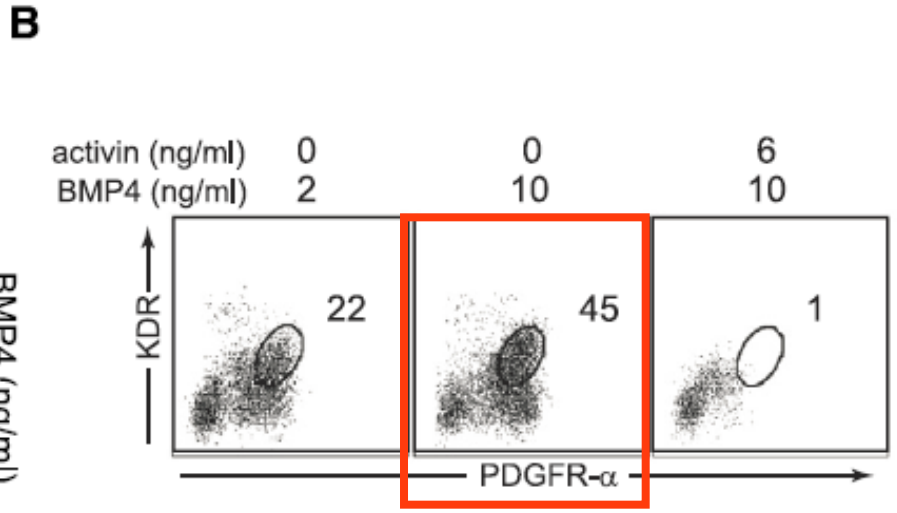
Steven J. Kattman, et al; Cell Stem Cell. Feb., 8:228-240; 2011

6. Activin and BMP4 Induce KDR^{+}PDGFR-α^{+} Cardiogenic Mesoderm in hESC and hiPSC Cultures

human ESCs



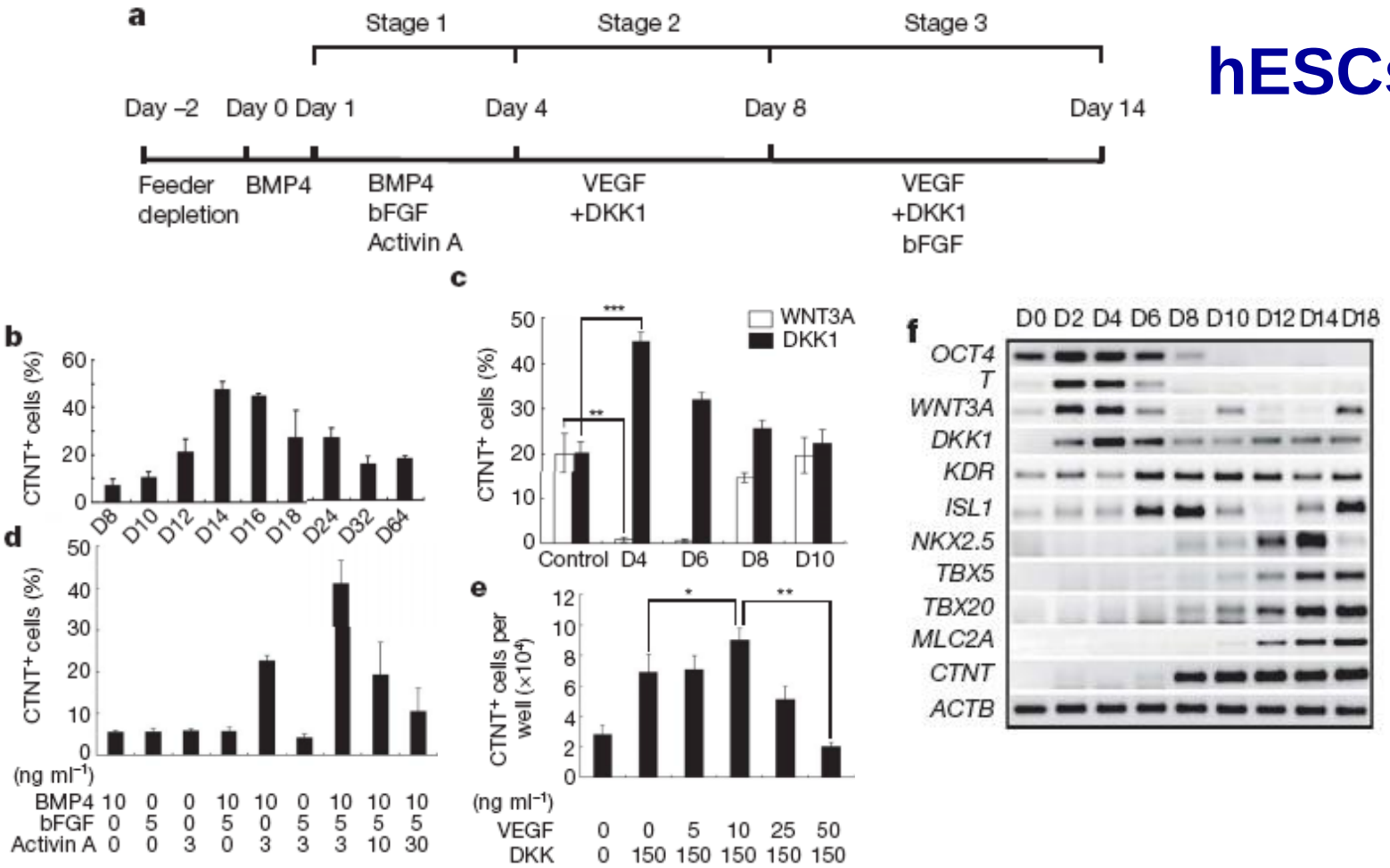
human iPSCs



Steven J. Kattman et al; Cell Stem Cell. 8: 228-240; 2011

6. Induction with small molecules and growth factors

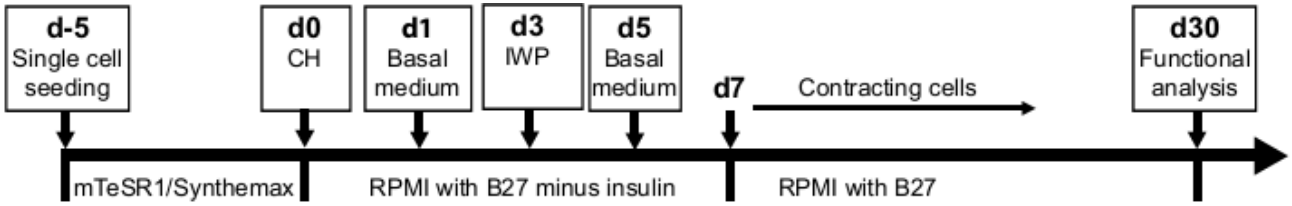
hESCs



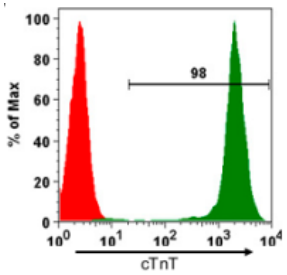
CTNT：cardiac troponin T
T：brachyury

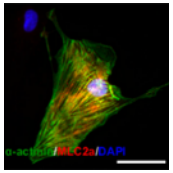
Lei Yang，… Gordon M. Keller. Nature. 2008 May 22; 453(7194):524-8.

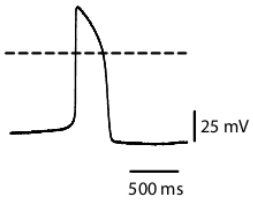
Robust cardiomyocyte differentiation from hPSCs via temporal modulation of canonical Wnt signaling



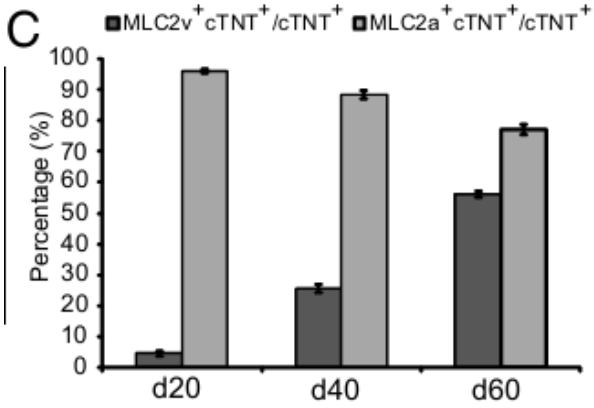
Sean P. Palecek
Associate Professor
WiCell Research Institute,
Madison, WI







15 CMs /input hPSC
>50% cTnT⁺/MLC2v⁺ cells



- ***Simple, highly efficient and reproducible,***
- ***Fully defined, growth factor-free conditions***
- ***Low variability between cell lines***

CH, IR99021, GSK3β inhibitor

IWP: Wnt signaling inhibitor

Lian Xiaojun, …, Palecek Sean P. PNAS, 2012; E1848-E1857

Robust cardiomyocyte differentiation from human pluripotent stem cells

Paul Burridge, PhD
Instructor

Joseph C Wu, MD, PhD
Professor

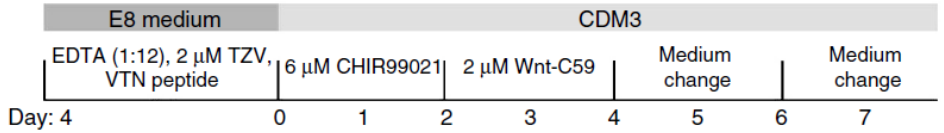
Stanford Cardiovascular Institute

Chemically defined generation of human cardiomyocytes

Paul W Burridge[1–3], Elena Matsa[1–3], Praveen Shukla[1–3], Ziliang C Lin[4], Jared M Churko[1–3], Antje D Ebert[1–3], Feng Lan[1–3], Sebastian Diecke[1–3], Bruno Huber[1–3], Nicholas M Mordwinkin[1–3], Jordan R Plews[1–3], Oscar J Abilez[1–3], Bianxiao Cui[5], Joseph D Gold[1] & Joseph C Wu[1–3]

NATURE METHODS, Jun 15th, 2014, Epub ahead of print

a



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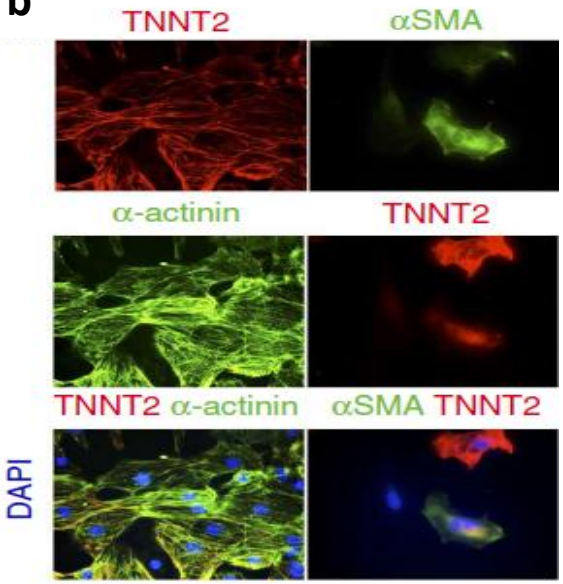

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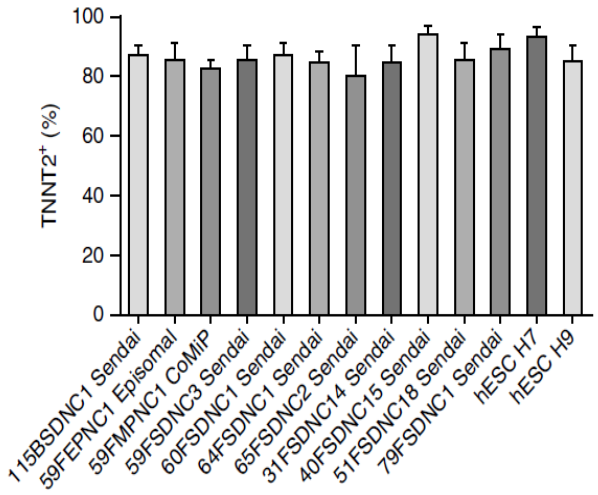
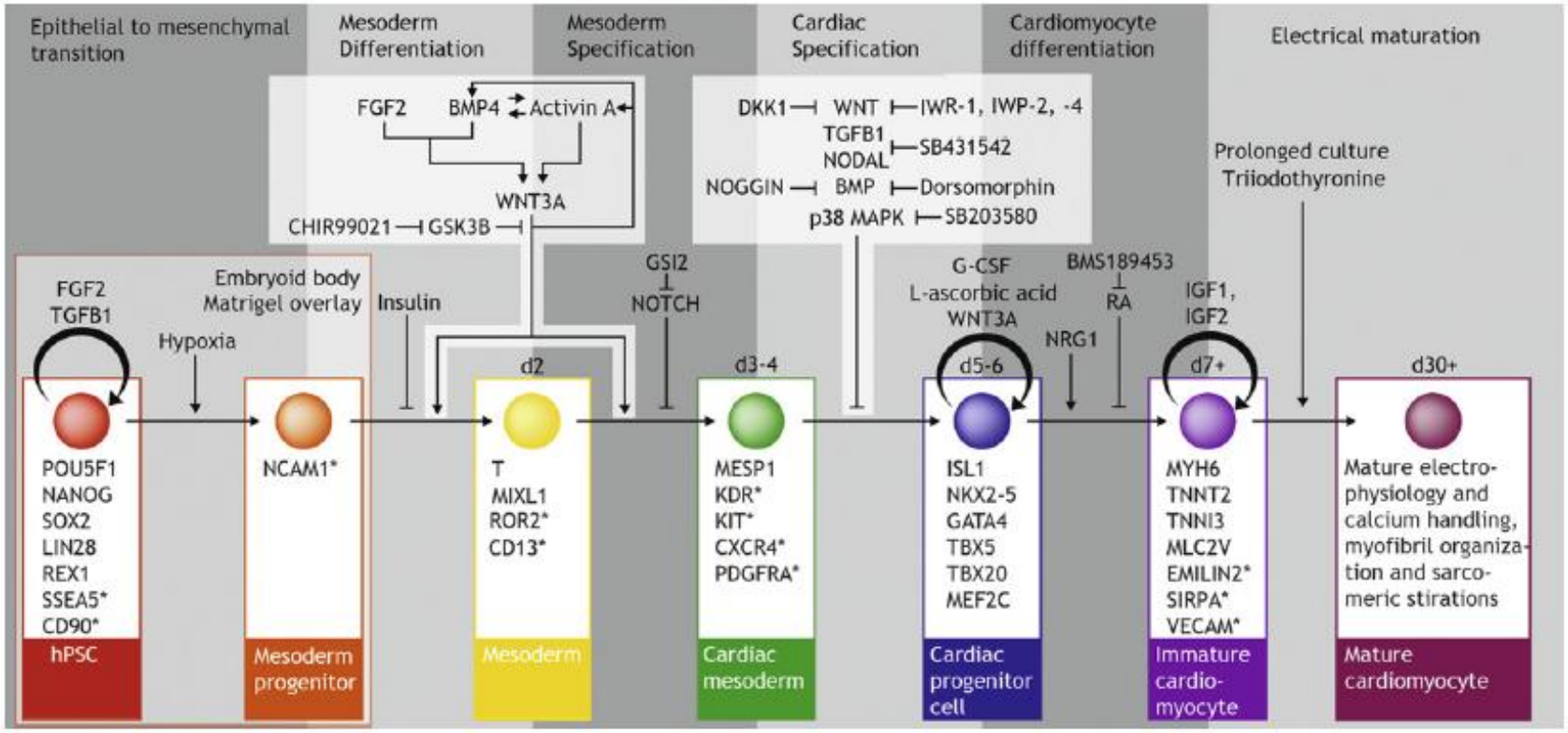


TZV: Thiazovivin, ROCK inhibitor
CHIR99021: GSK3β inhibitor
Wnt-C59: Wnt inhibitor

- **Universal and high efficiency, up to 95% cTnT⁺ cells**
- **Chemically defined**
- **Low cost**

Schematic of current knowledge on the factors involved in hPSC cardiac differentiation



epithelial to mesenchymal transition | **mesoderm differentiation** | **mesoderm speciation** | **cardiac specification** | **CM differentiation** | **electrical maturation**

Paul W. Burridge, Gordon Keller, Joseph D. Gold, and Joseph C Wu Cell Stem Cell 2012 Jan

Cardiac differentiation of iPSC

iPSCs

Induction of pluripotent stem cells from mouse and human embryonic and adult fibroblast cultures by defined factors

通过向胚胎和成体成纤维细胞中转入特定基因使之逆分化为多潜能干细胞 (*OCT4, SOX2, KLF4, c-MYC*)

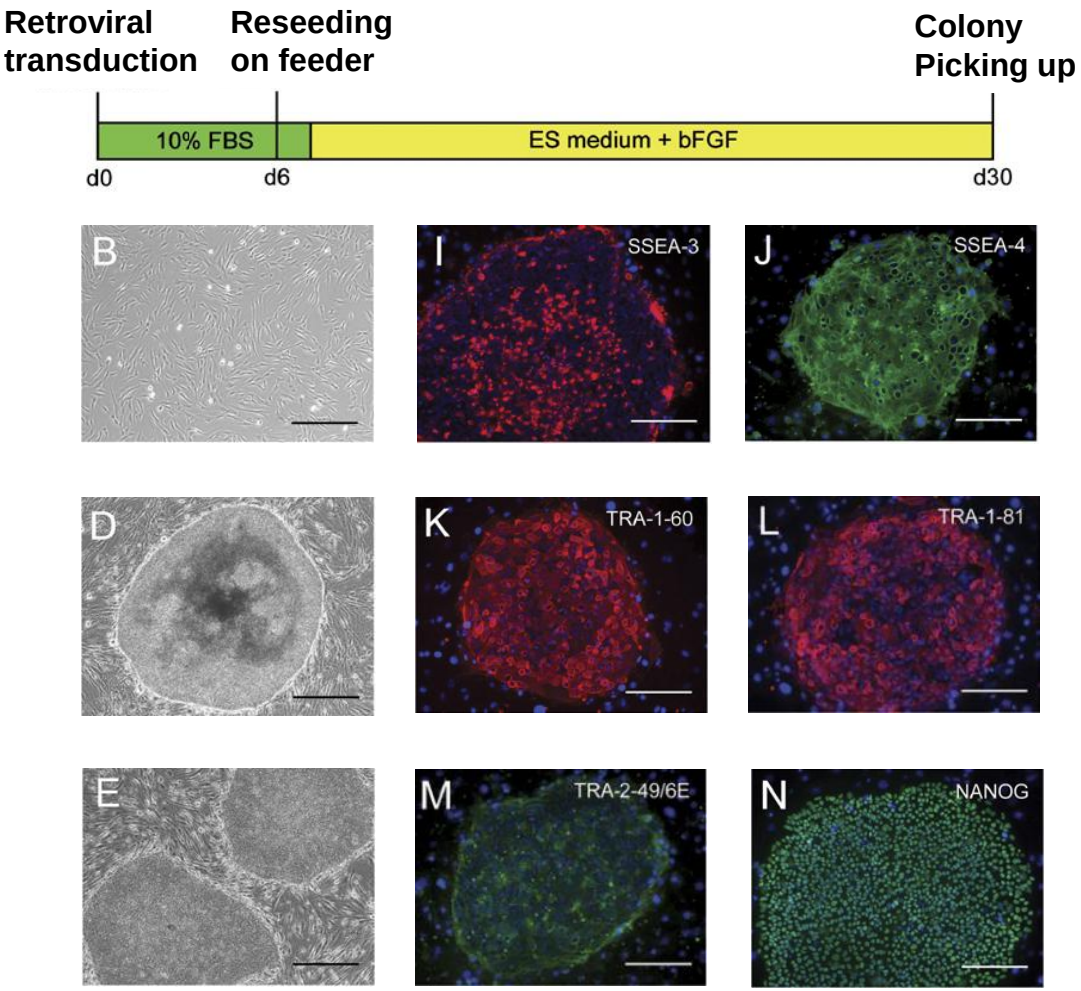
Kazutoshi Takahashi, Shinya Yamanaka

Cell. 2006 Aug 25; 126(4):663-76

Cell 131, 1–12, November 30, 2007

Dept of Stem Cell Biology
Institute for Frontier Medical Sciences
Kyoto University

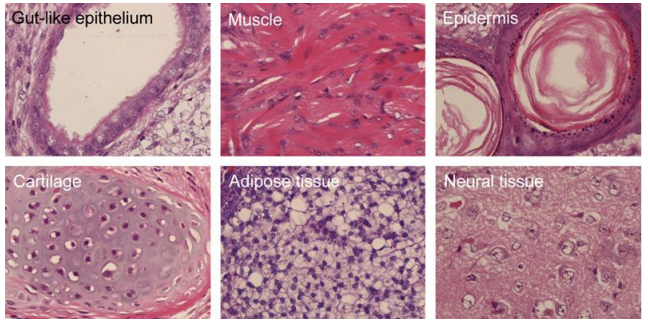
Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors



OCT4, SOX2, KLF4, c-MYC

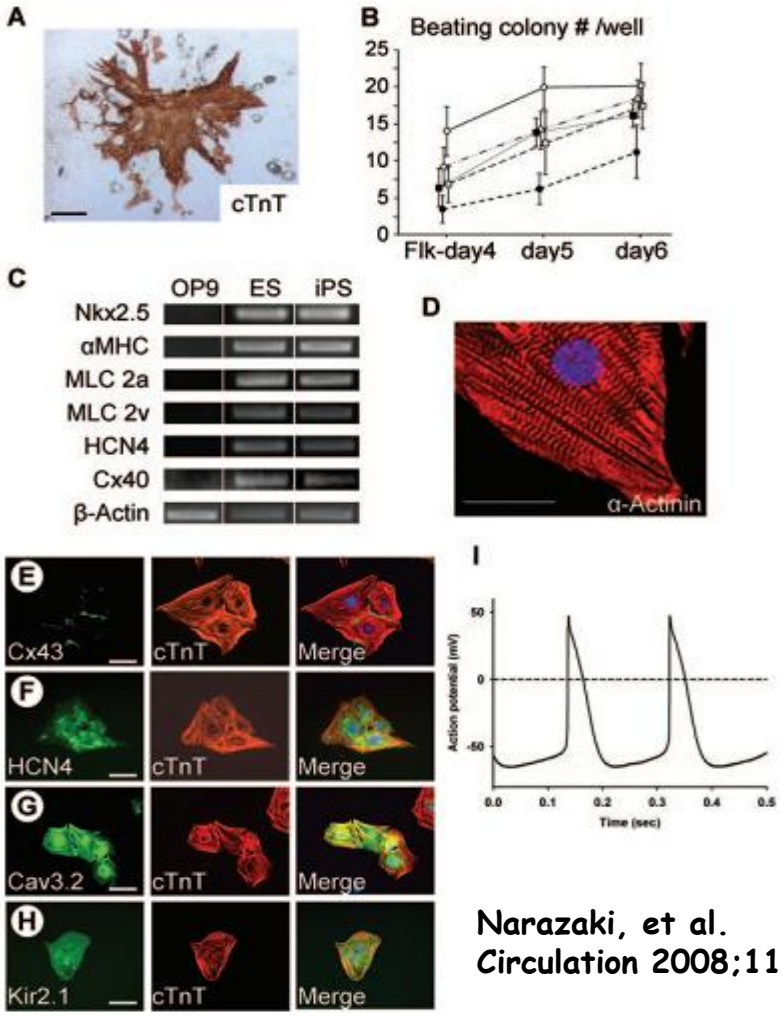
(A) Time schedule of iPS cell generation.
(B) Morphology of HDF.
(D) Typical image of hES cell-like colony.
(E) Morphology of established iPS cell line at passage number 6 (clone 201B7).
(I–N) Immunocytochemistry for SSEA-3 (I), SSEA-4 (J), TRA-1-60 (K), TRA-1-81 (L), TRA-2-49/6E (M), and Nanog (N). Nuclei were stained with Hoechst 33342 (blue). Bars = 200 mm (B–E), and 100 mm (I–N).

Teratoma Formation from Human iPS Cells

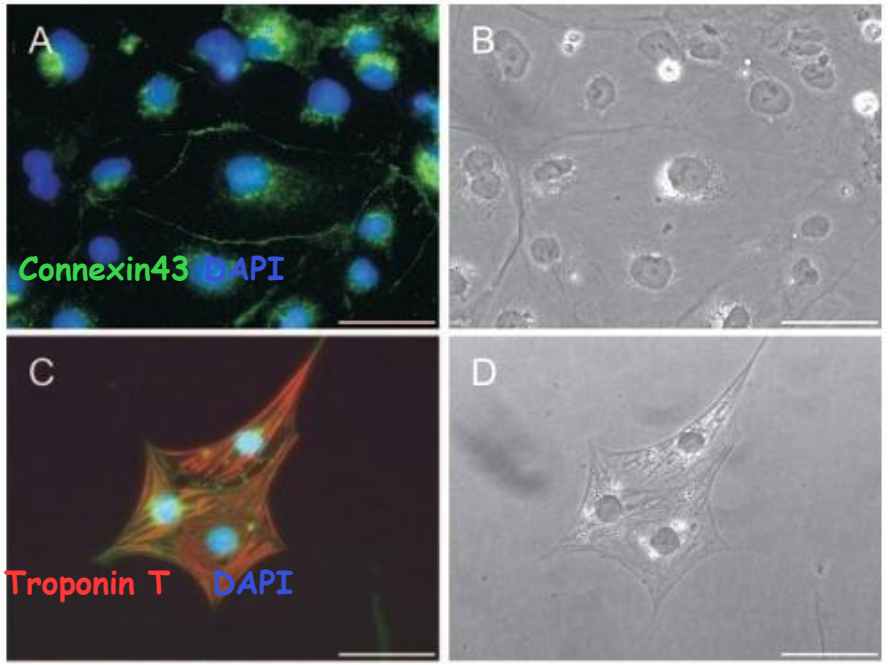


Kazutoshi Takahashi, Shinya Yamanaka
Cell 131, 1–12, November 30, 2007

iPSCs can differentiate into cardiomyocytes



Narazaki, et al.
Circulation 2008;118;498-506



Mauritz C &Martin U et al. *Circulation*. 2008 118(5):507-517

Big variation in differentiation!

Reprogramming of mouse fibroblasts into cardiomyocytes using a direct strategy

Copy number variation and selection during reprogramming to pluripotency

Samer M. Hussein[1,2]*, Nizar N. Batada[3]*, Sanna Vuoristo[2], Reagan W. Ching[4], Reija Autio[5,6], Elisa Närvä[5], Siemon Ng[3], Michel Sourour[1], Riikka Hämäläinen[1,2], Cia Olsson[2], Karolina Lundin[2], Milla Mikkola[2], Ras Trokovic[2], Michael Peitz[7], Oliver Brüstle[7], David P. Bazett-Jones[4], Kari Alitalo[8], Riitta Lahesmaa[5], Andras Nagy[1,9] & Timo Otonkoski[2,10]

Hussein SM, et. al. Nature. 2011 Mar 3;471(7336):58-62

A high-resolution single nucleotide polymorphism array showed that a significantly more CNVs are present in early-passage human iPS cells than intermediate passage human iPS cells, fibroblasts or human ES cells.

Somatic coding mutations in human induced pluripotent stem cells

Athurva Gore[1]*, Zhe Li[1]*, Ho-Lim Fung[1], Jessica E. Young[2], Suneet Agarwal[3], Jessica Antosiewicz-Bourget[4], Isabel Canto[2], Alessandra Giorgetti[5], Mason A. Israel[2], Evangelos Kiskinis[6], Je-Hyuk Lee[7], Yuin-Han Loh[3], Philip D. Manos[3], Nuria Montserrat[5], Athanasia D. Panopoulos[8], Sergio Ruiz[8], Melissa L. Wilbert[2], Junying Yu[4], Ewen F. Kirkness[9], Juan Carlos Izpisua Belmonte[5,8], Derrick J. Rossi[10], James A. Thomson[4], Kevin Eggan[6], George Q. Daley[3], Lawrence S. B. Goldstein[2] & Kun Zhang[1]

Gore A, et. al. Nature. 2011 Mar 3;471(7336):63-7

Found that each hiPSC line contained an average of five protein-coding point mutations enriched in genes mutated or having causative effects in cancers by exomes sequencing.

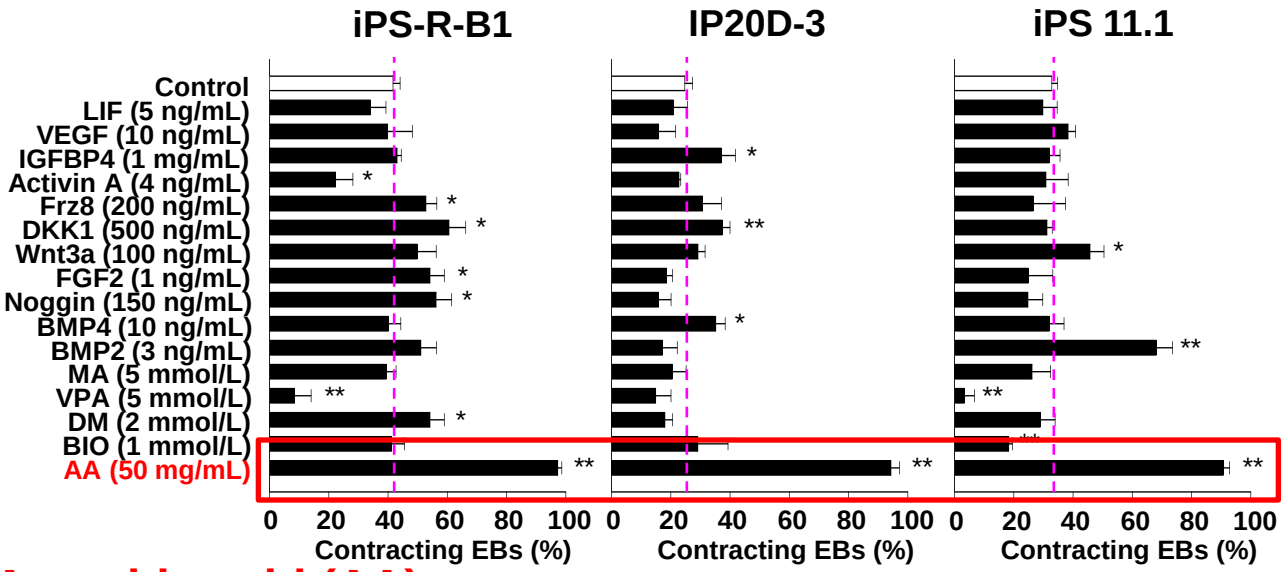
Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells

Ryan Lister[1]*, Mattia Pelizzola[1]*, Yasuyuki S. Kida[2], R. David Hawkins[3], Joseph R. Nery[1], Gary Hon[3], Jessica Antosiewicz-Bourget[4,5], Ronan O'Malley[1], Rosa Castanon[1], Sarit Klugman[3], Michael Downes[2], Ruth Yu[2], Ron Stewart[4,5], Bing Ren[3,6], James A. Thomson[4,5,7,8], Ronald M. Evans[2] & Joseph R. Ecker[1]

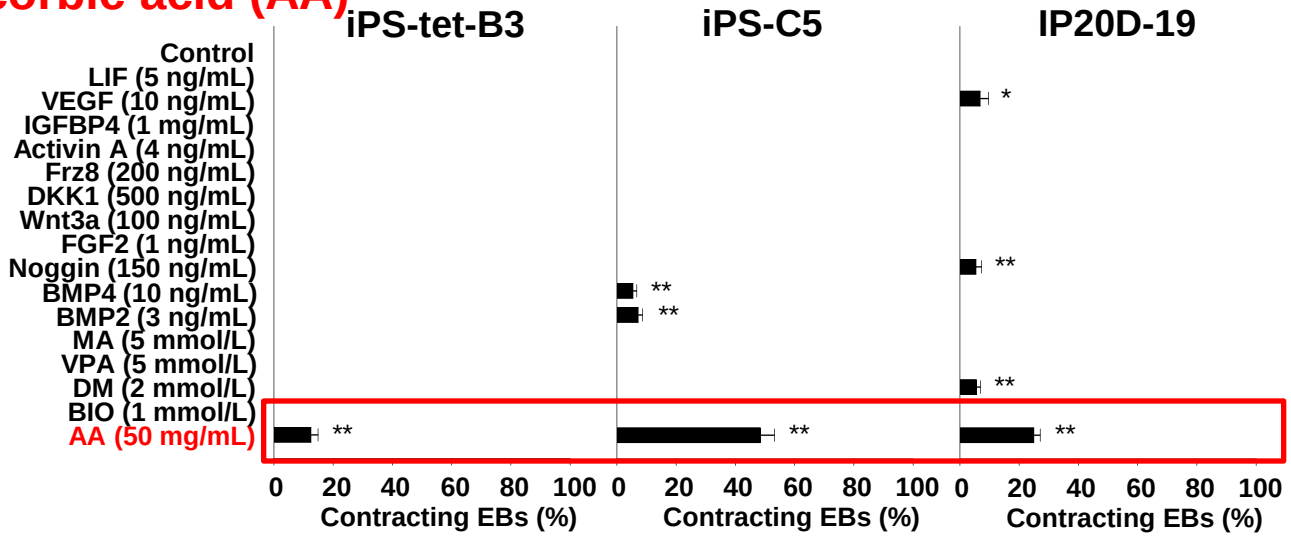
Lister R, et. al. Nature. 2011 Mar 3;471(7336):68-73

Reported the first whole-genome profiles of DNA methylation at single-base resolution and discovered a significant reprogramming variability, including somatic memory and aberrant reprogramming of DNA methylation in human iPS cells.

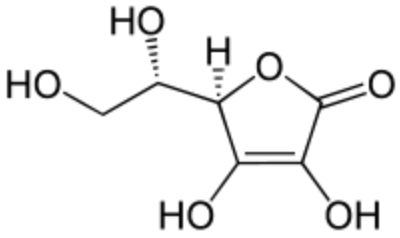
Screening of suitable cardiomyocyte inducers

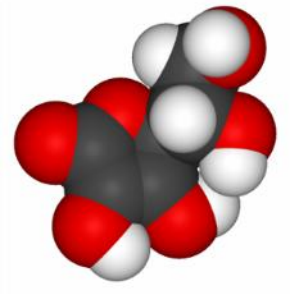


Ascorbic acid (AA)



Ascorbic acid (AA)



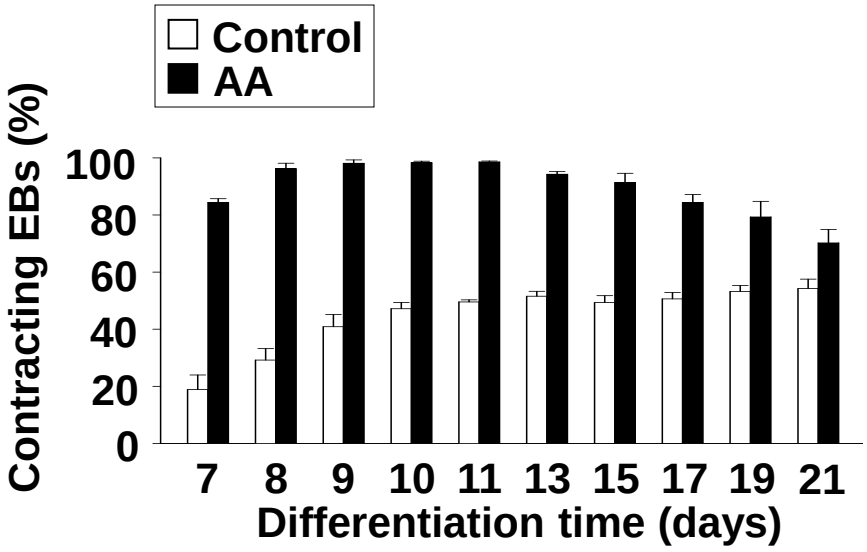


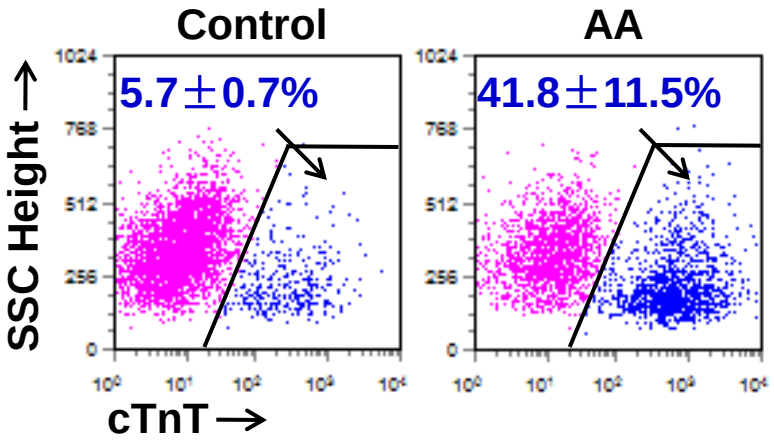
Szent-Györgyi

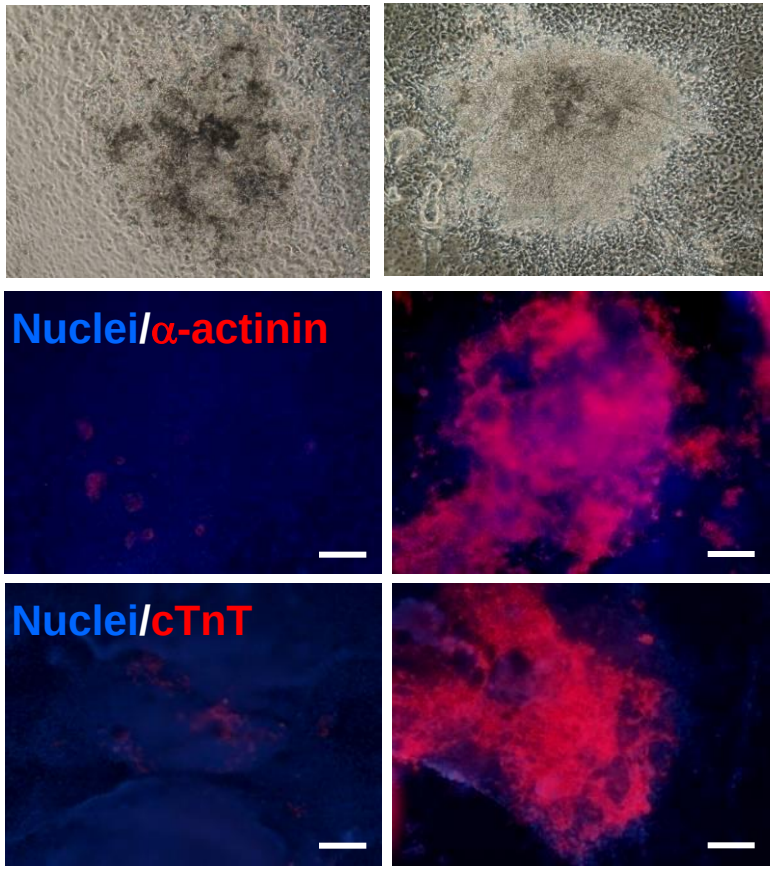
1932 Nobel Prize for purification of AA

- A highly effective antioxidant
- An important enzyme cofactor
- Promote the synthesis of collagen
- Alleviate cell senescence

AA Robustly Enhances the Cardiac Differentiation of iPSCs

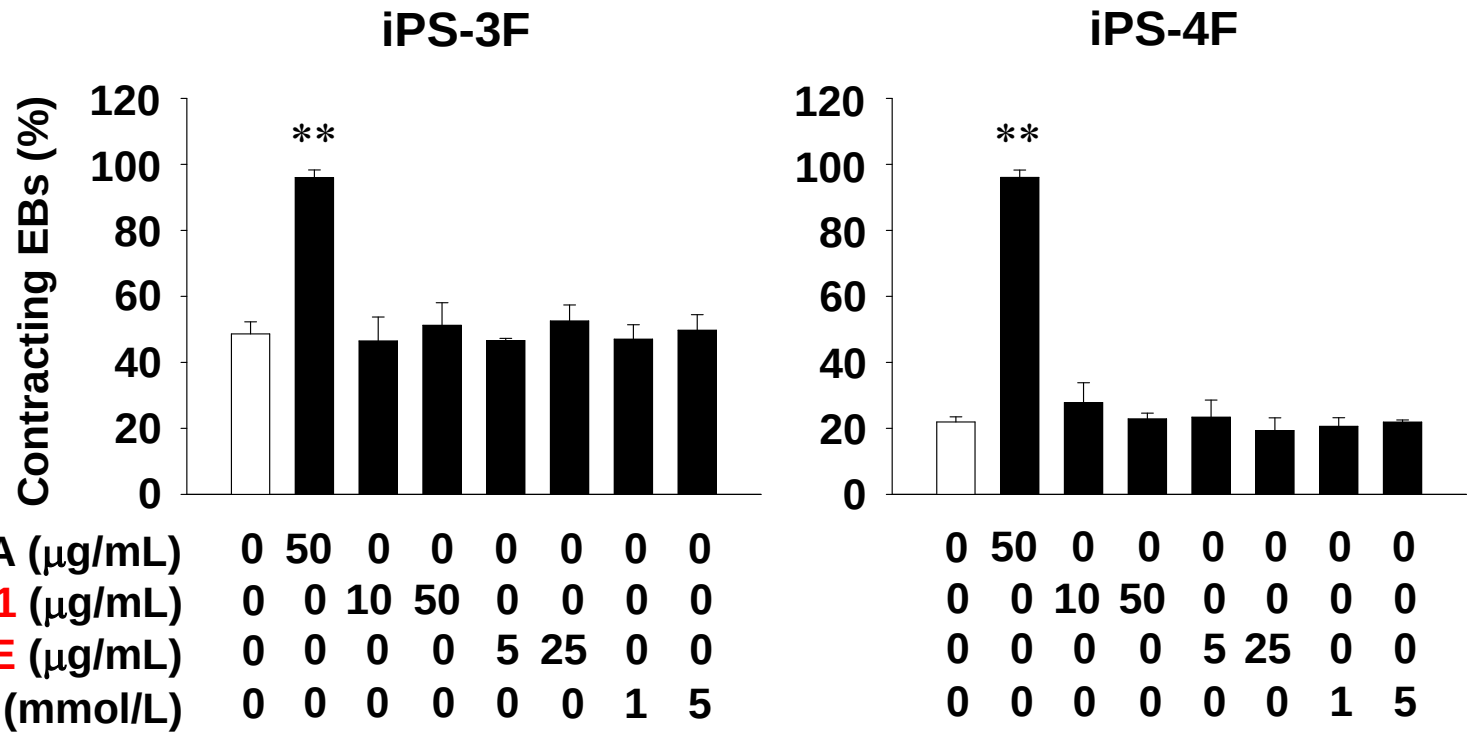






Cardiomyocyte-Promoting Effect of AA is Independent of Its Antioxidative Property

Antioxidative Property

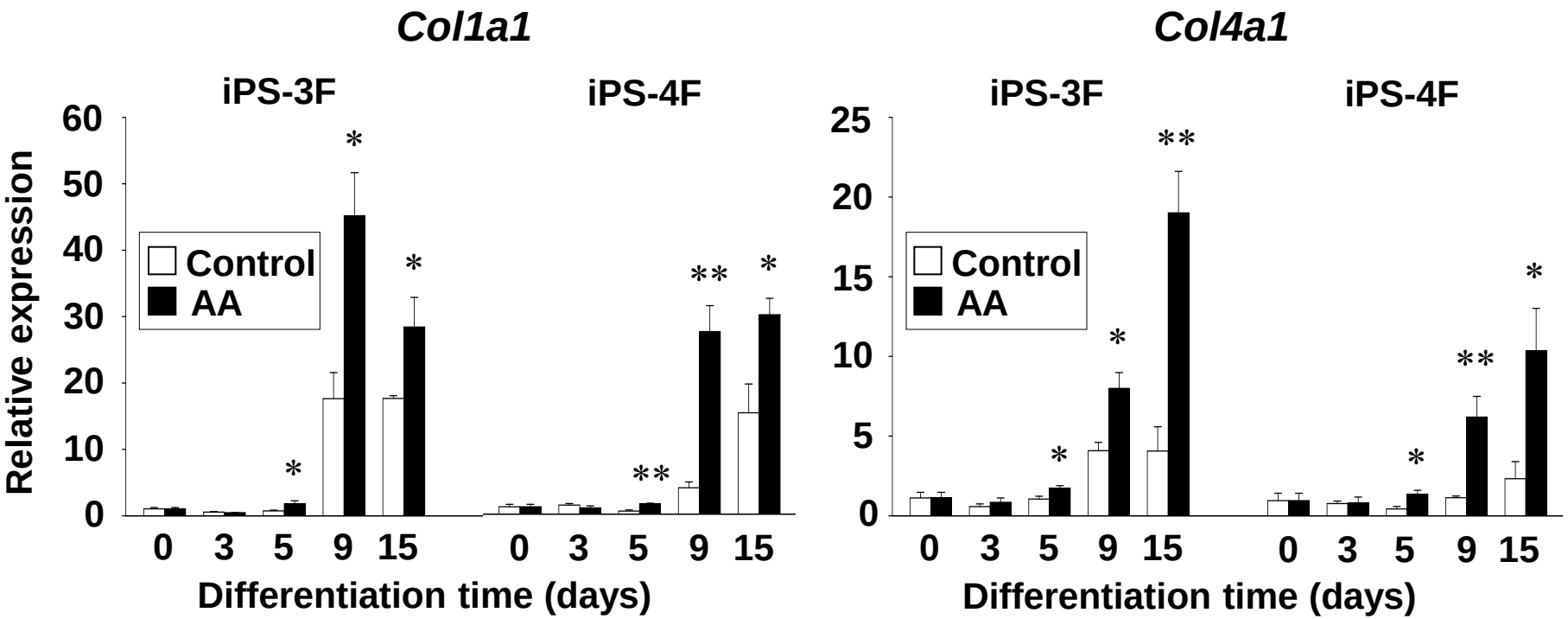


Alternative antioxidants:
Vb1, vitamin B1; GMEE, reduced gluthatione; NAC, N-acetyl-L-cysteine

*P<0.05, **P<0.01 vs. control.

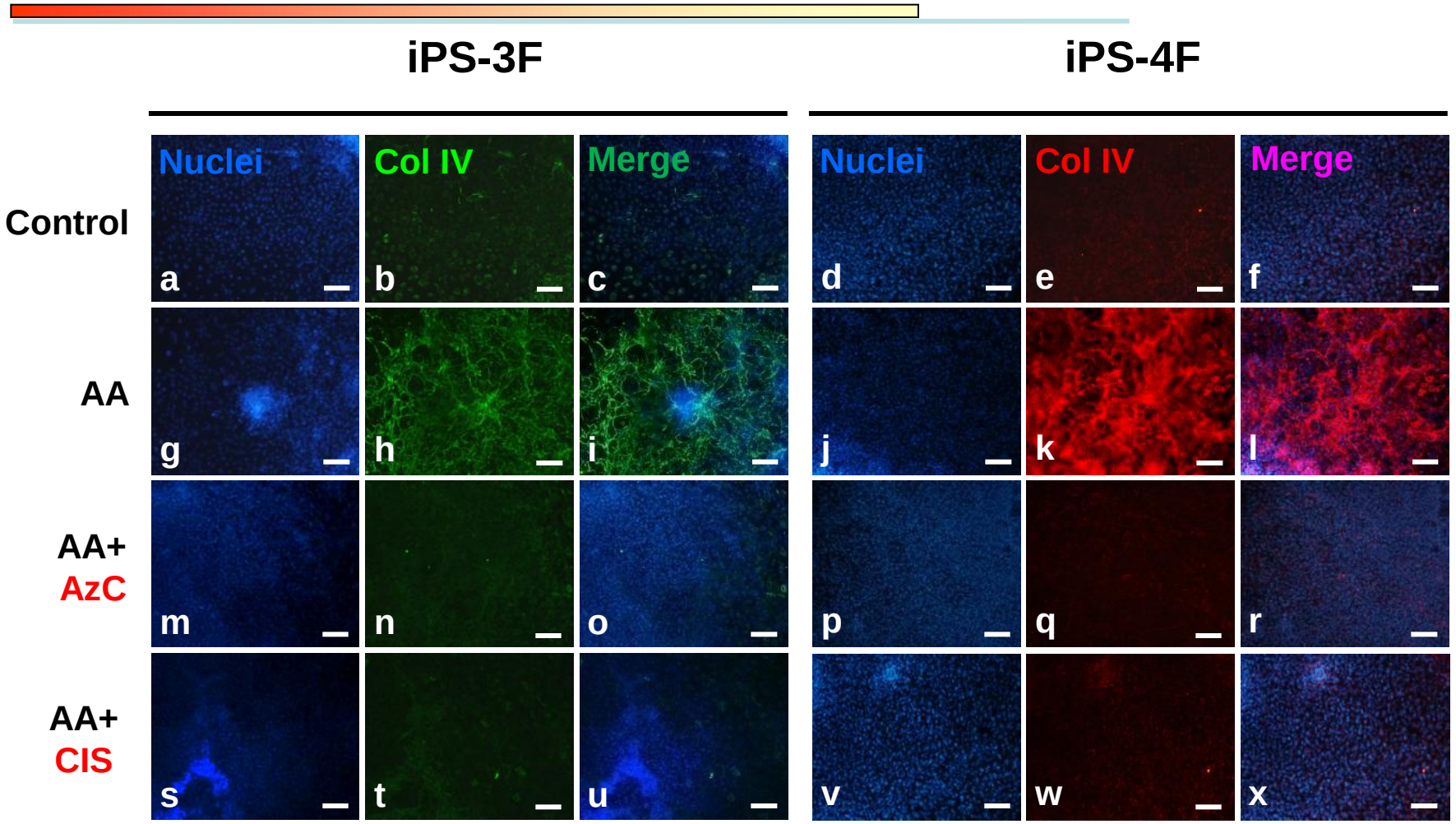
AA Promotes the Expression of Collagen during Cardiac Differentiation

Is collagen involved in the AA-enhanced cardiac differentiation?



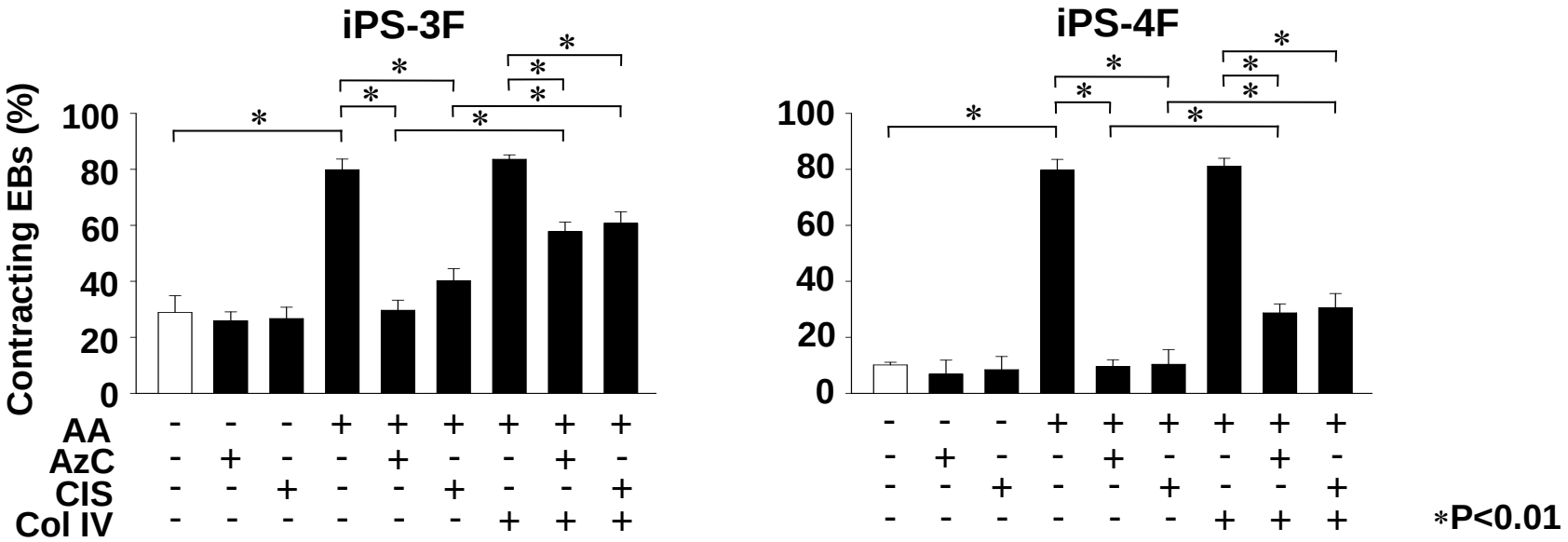
Col1a1 and *Col4a1*, transcripts of type I and IV collagen. ∗P<0.05, ∗∗P<0.01 vs. control.

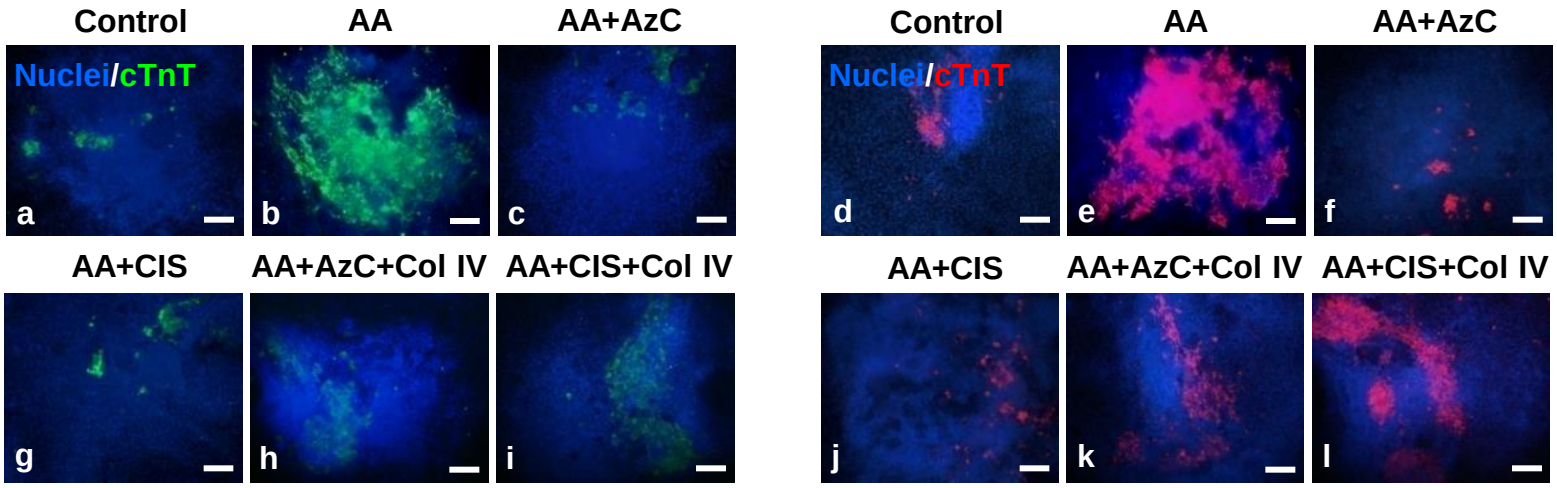
AA Promotes the Expression of Collagen during Cardiac Differentiation



AzC and CIS, two collagen synthesis inhibitor. Col IV, type IV collagen

AA-promoted cardiogenesis is abolished by the inhibition of collagen synthesis





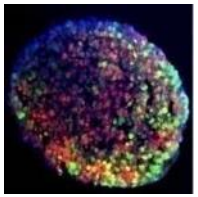
AzC and CIS, collagen synthesis inhibitors. Col IV, type IV collagen; cTnT, cardiomyocyte marker

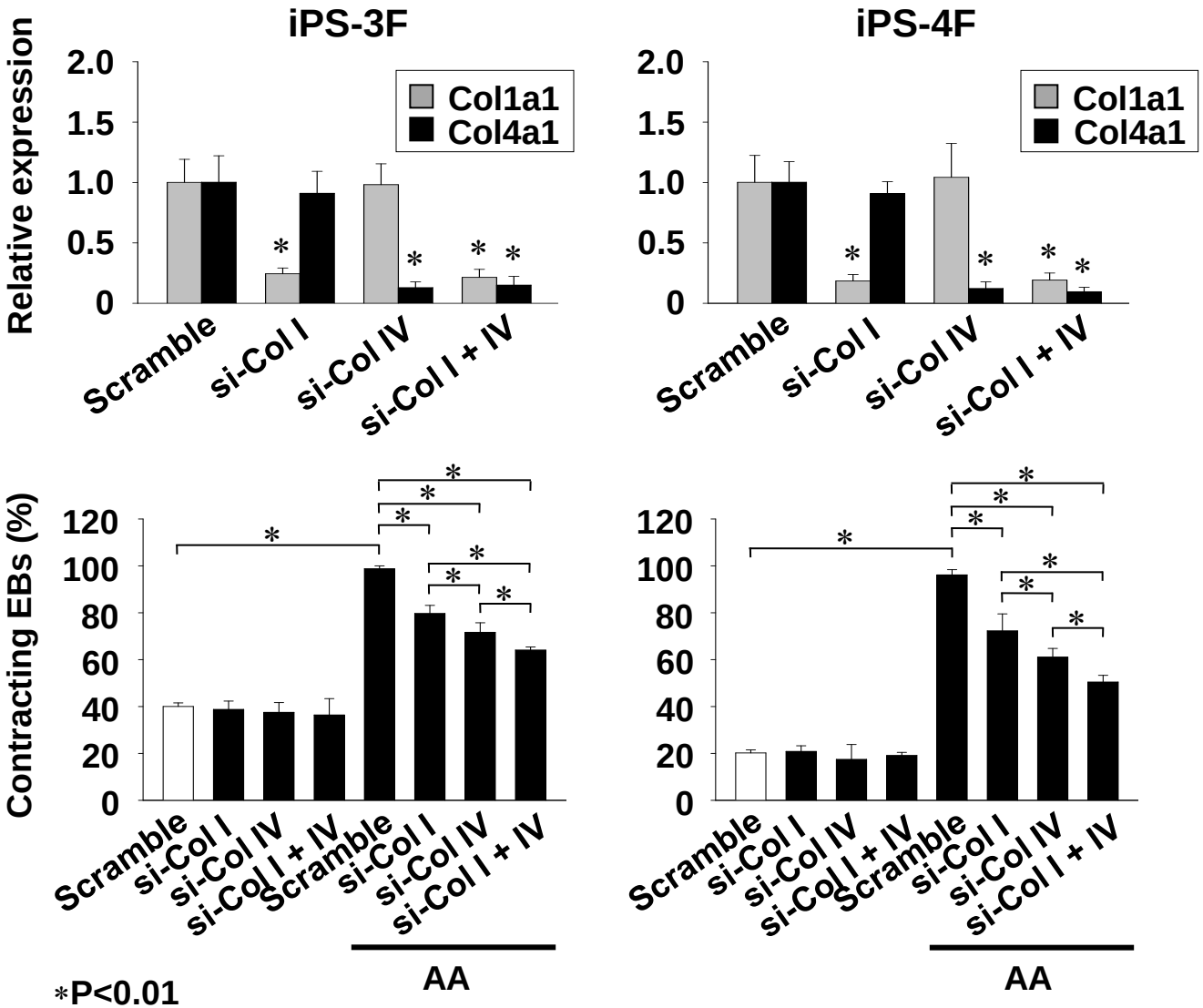
Col I and Col IV are Required for the AA-Promoted Cardiogenesis

shRNA

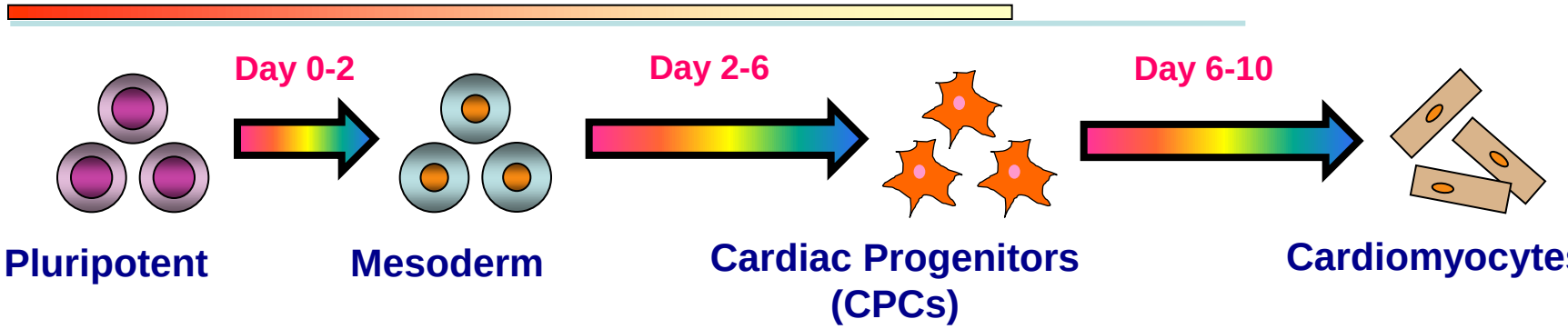
Lentivirus

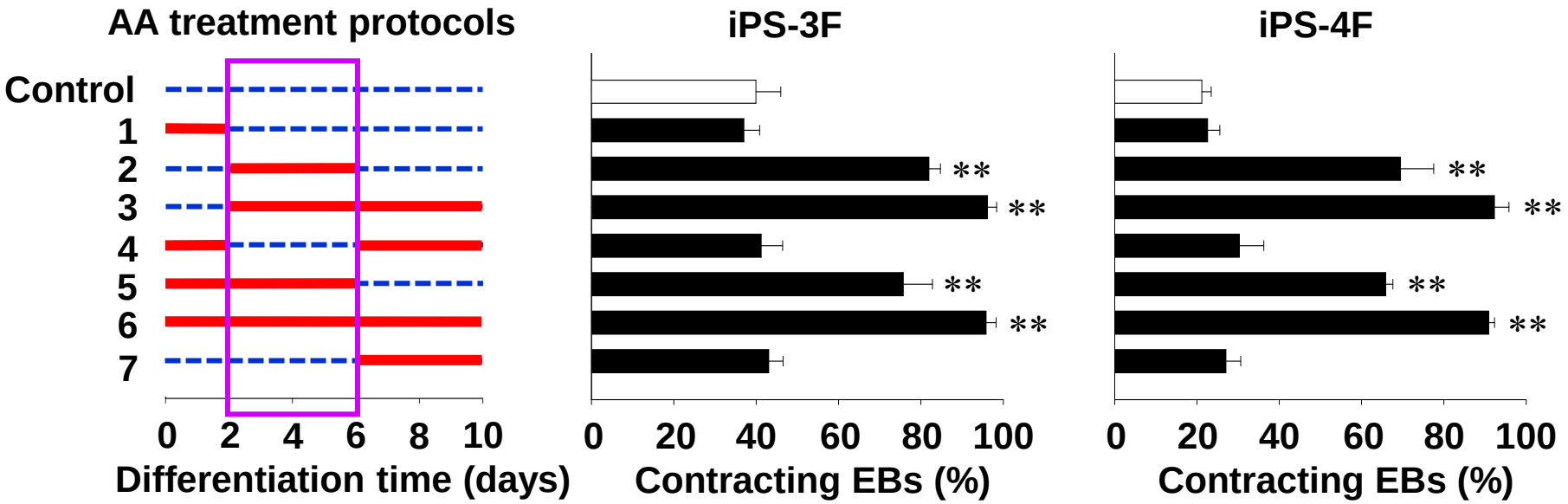
Stable iPSC line





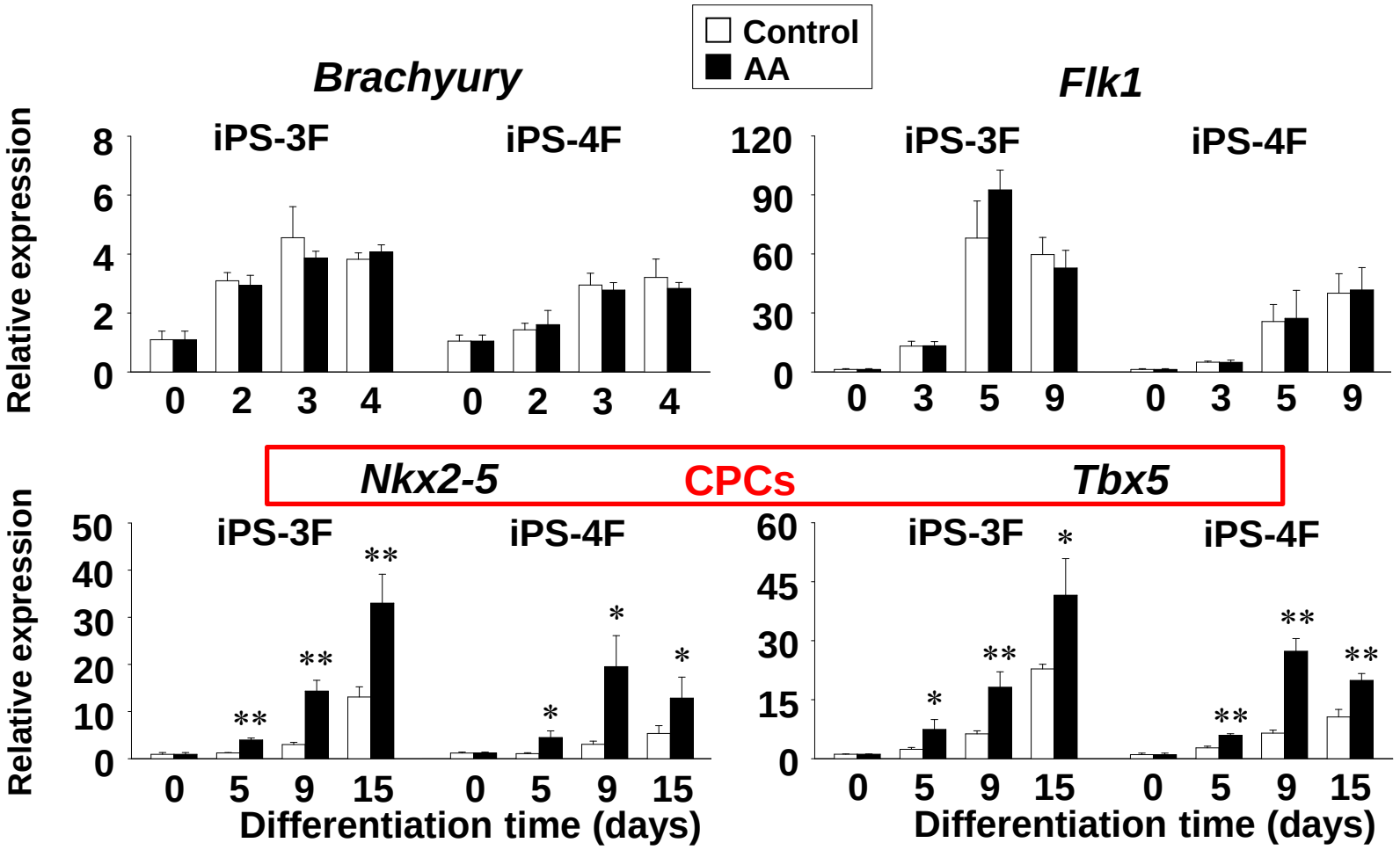
Stage for Cardiac Progenitor Cell Specification Is Critical for AA to Take Effect





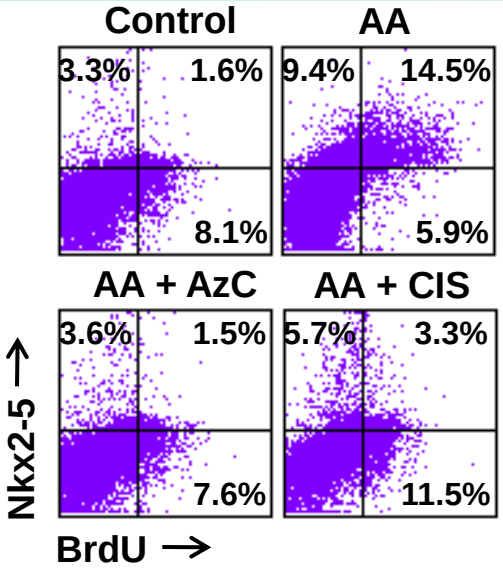
**P<0.01 vs. control.

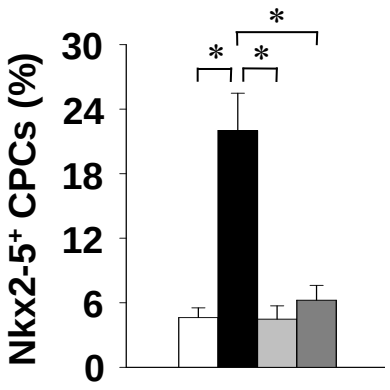
AA Increases the Expression of CPC-Specific but not Mesodermal Genes

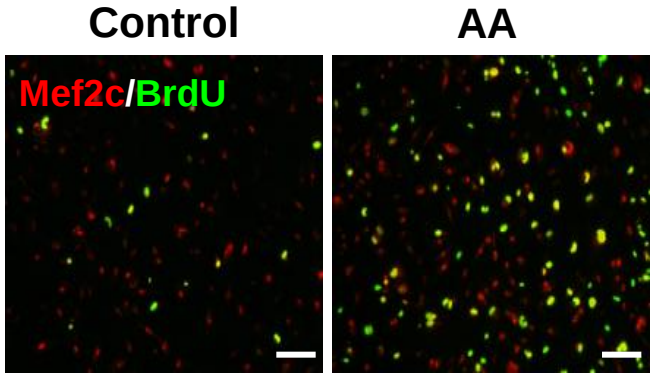


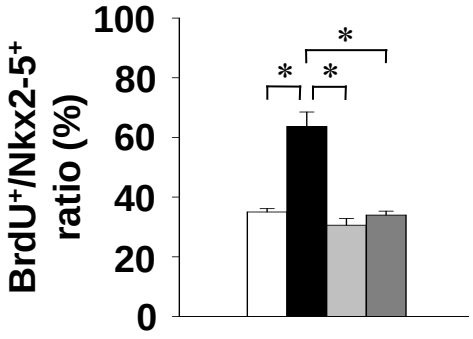
*P<0.05, **P<0.01 vs. control.

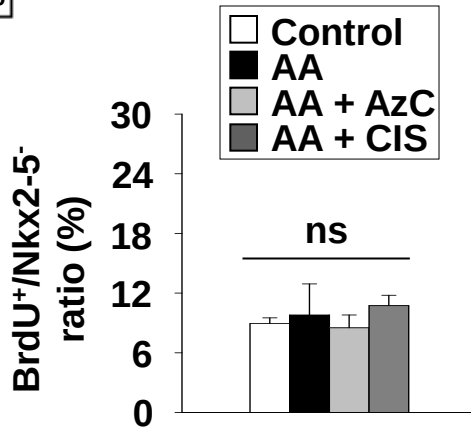
AA Enhances Cardiac Differentiation through Promoting the Proliferation of CPCs in a collagen synthesis-dependent mechanism

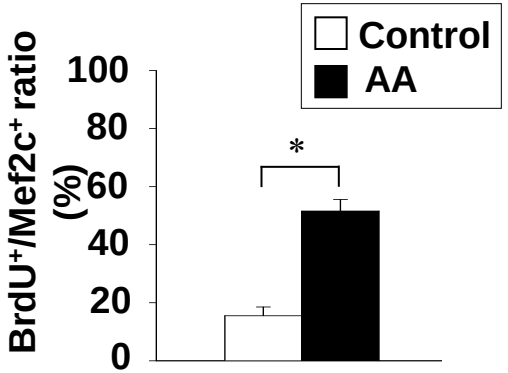




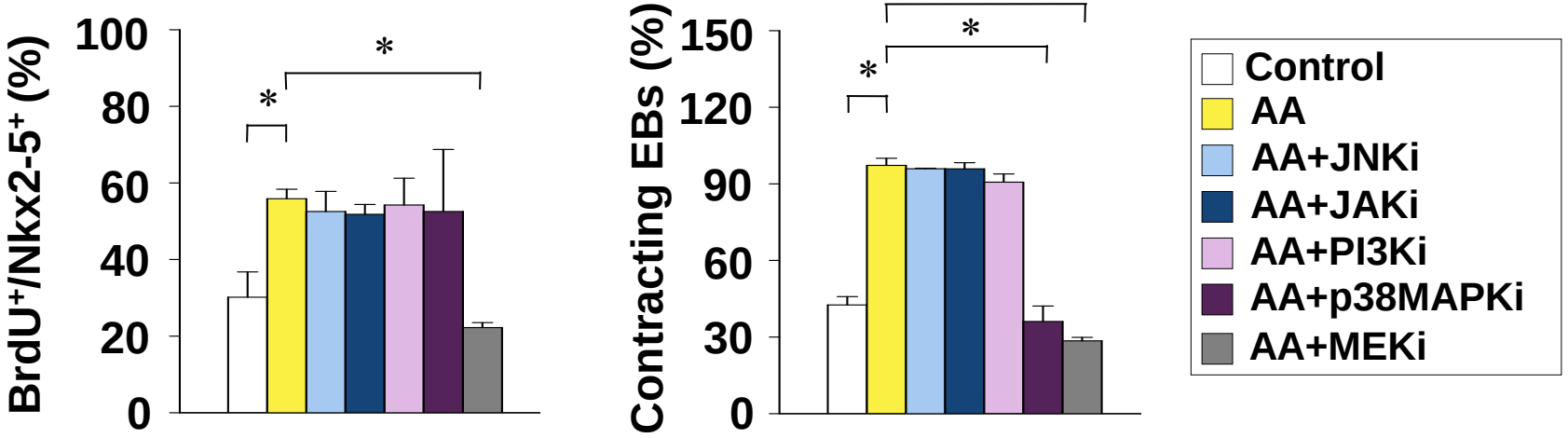


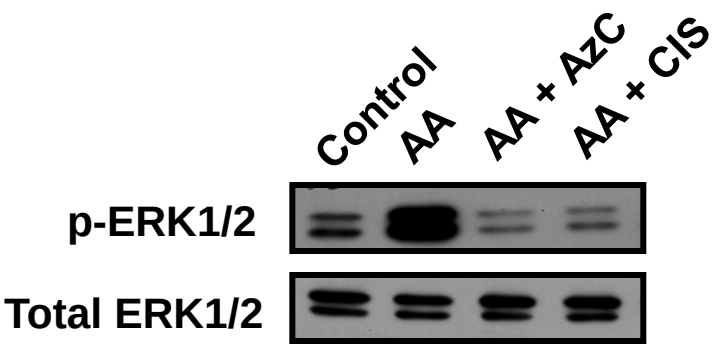


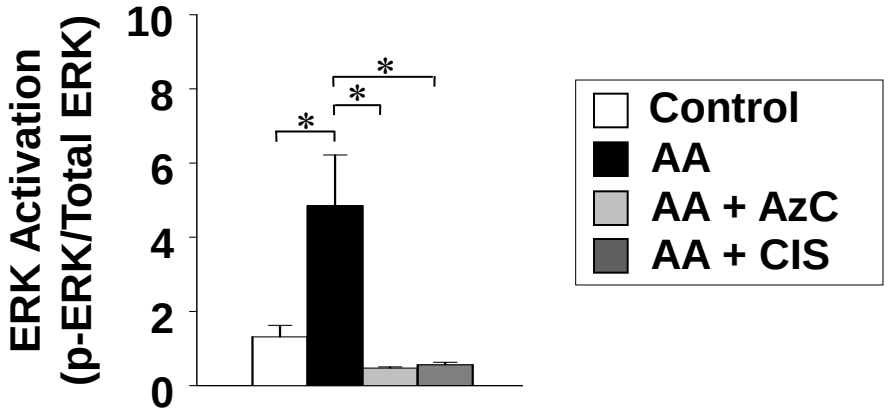




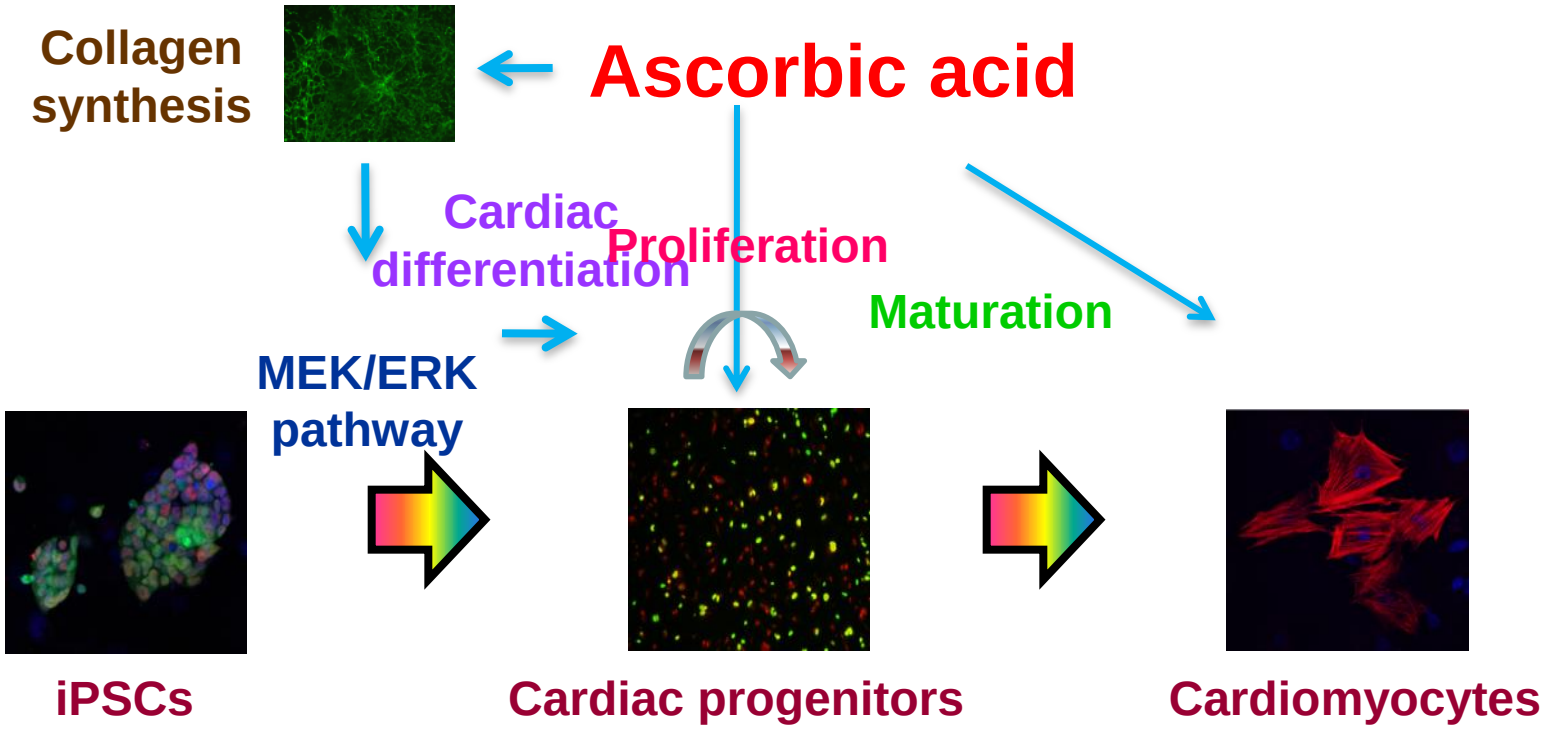
AA promotes the proliferation of iPSC-derived cardiac progenitors via the MEK-ERK1/2 pathway







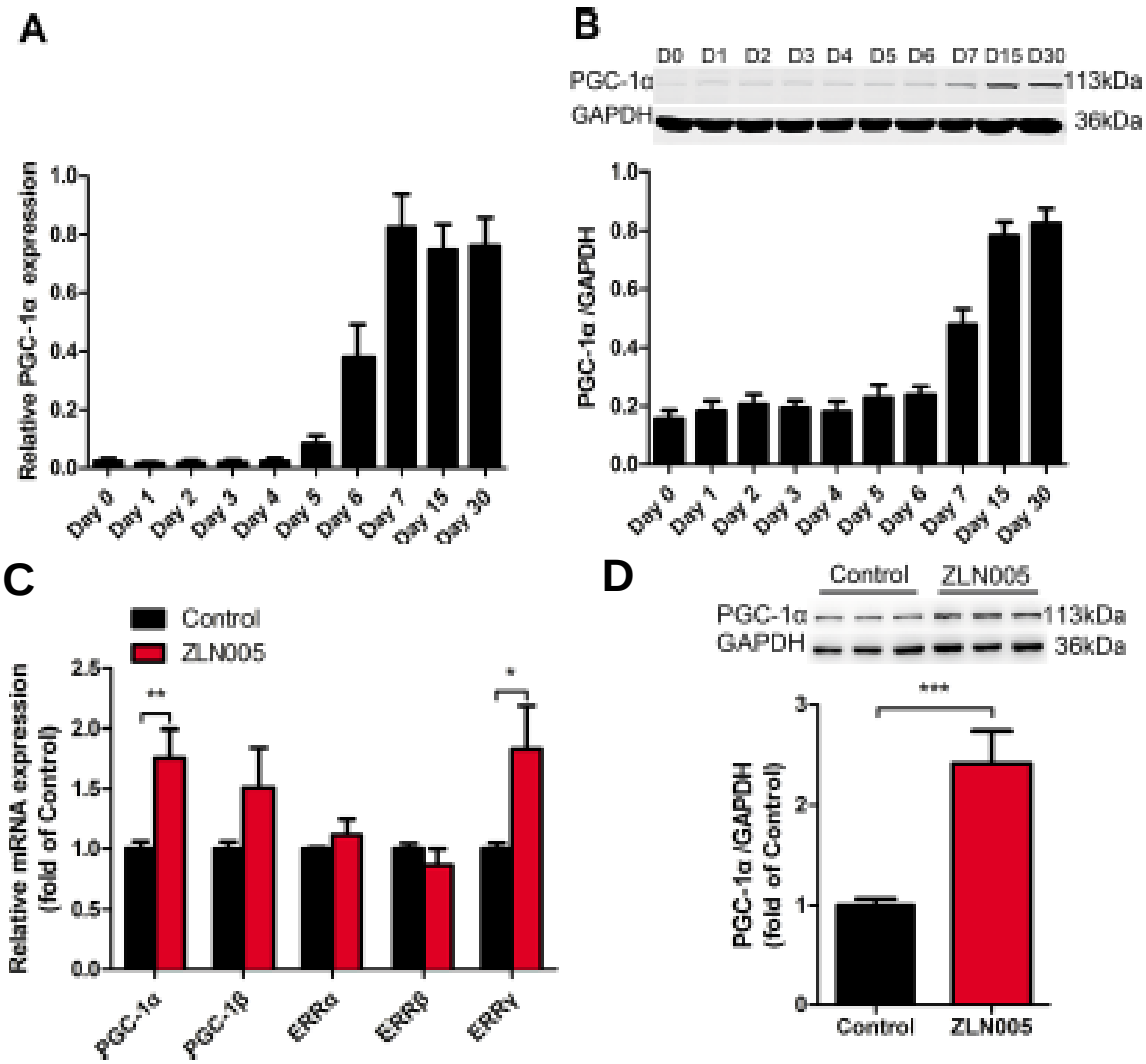
Regulation of microenvironment for cardiac progenitor cells critically promotes cardiac differentiation



Cao et al., Cell Res 2012 Jan

Q2. 如何促进hPSC源心肌细胞成熟？

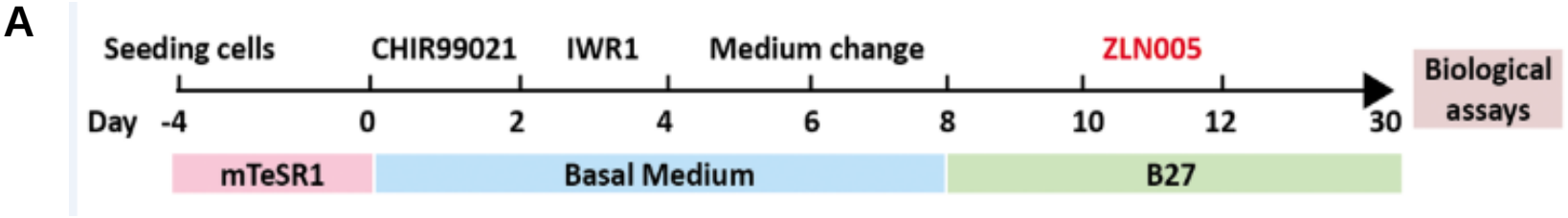
The expression of PGC-1α is upregulated during cardiomyocyte differentiation of hESCs

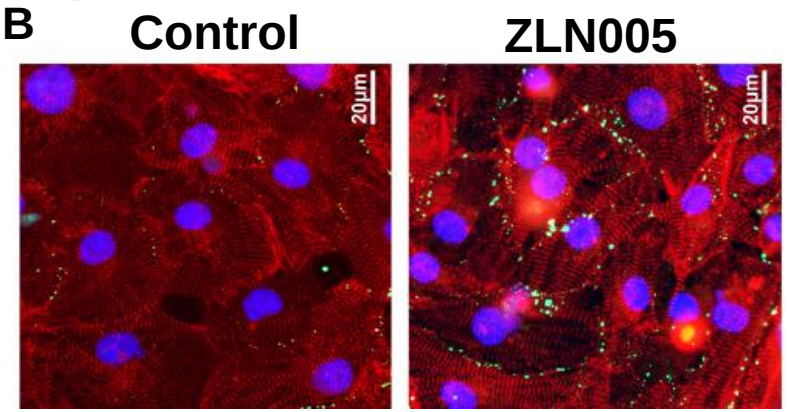


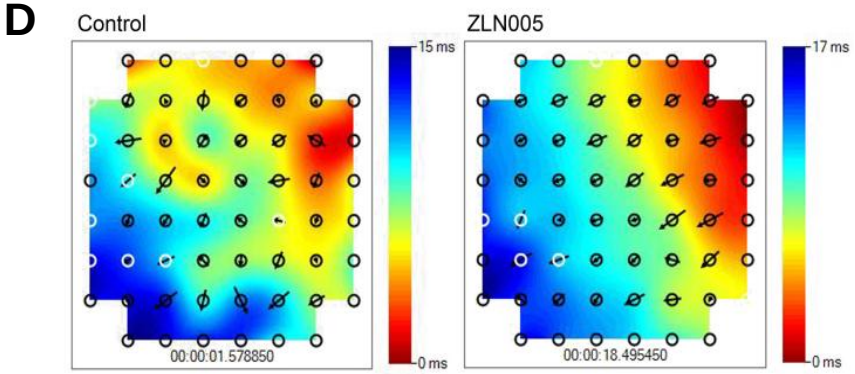
- **PGC-1 family: PGC-1α, PGC-1β, PGC-1-related coactivator (PRC), function as transcriptional co-activators**
- **PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1α): a key regulator of mitochondrial biogenesis and metabolism**
- **ZLN005: PGC-1 activator**

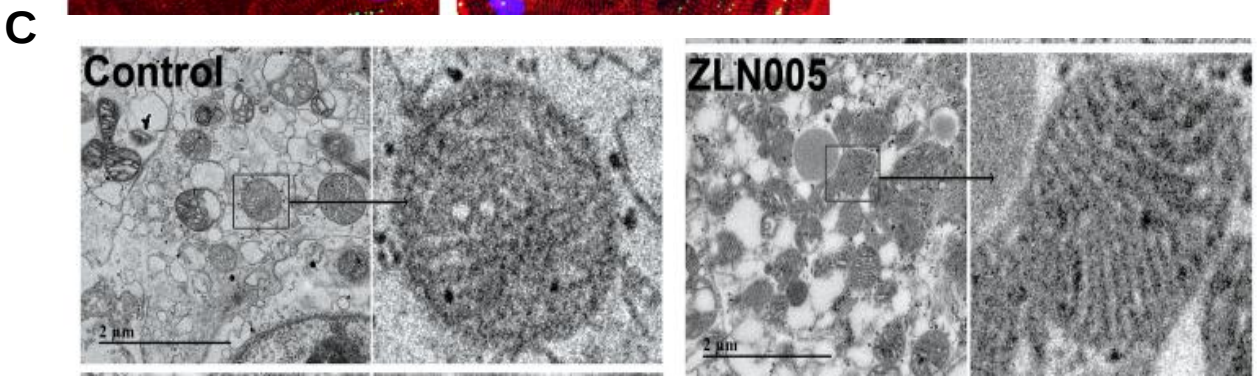
ERRs: estrogen-related receptors

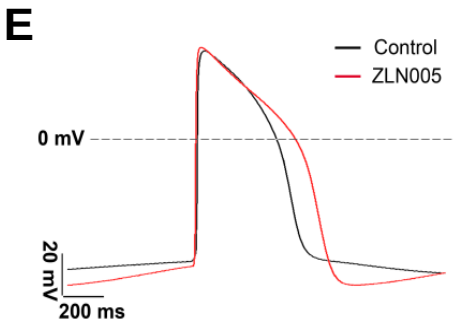
ZLN005 improved cardiac structural and functional maturation











Mitochondrial biogenesis enhanced by ZLN005

Liu et al. ***Aging-USA***. 20202020 Apr 28;12(8):7411-7430.doi: 3088

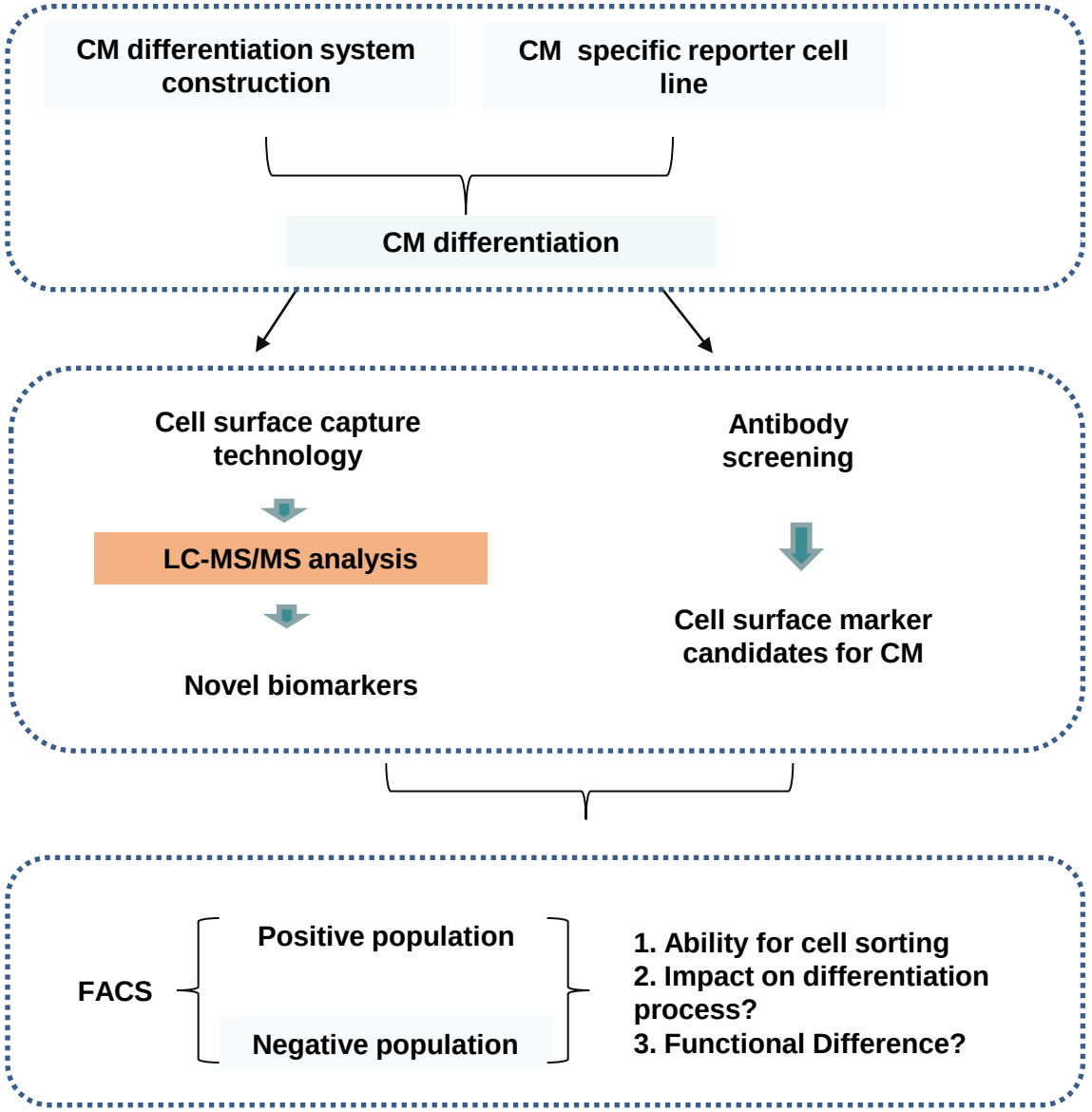
Q3. 如何区分/分离到更为成熟的hPSC源活心肌细胞？

Strategies

Systems construction

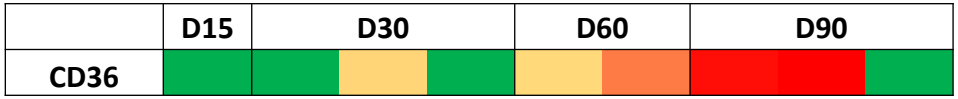
Candidates screening

Functional confirmation



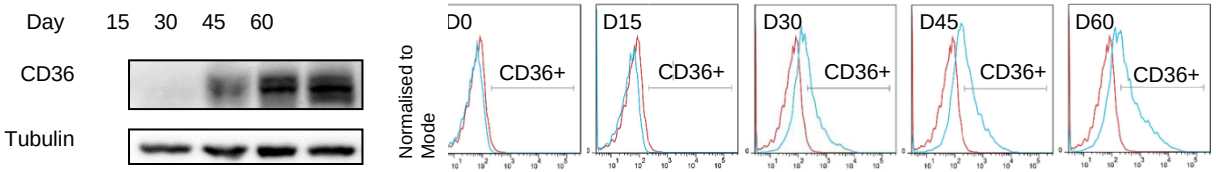
CD36 transcripts significantly increase during both mouse and human cardiac development of PSC

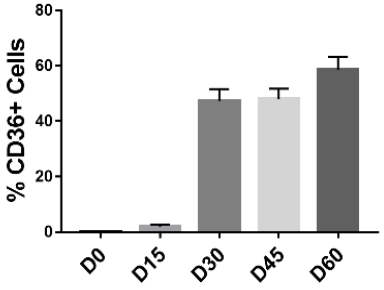
(A)



Low High

(B)

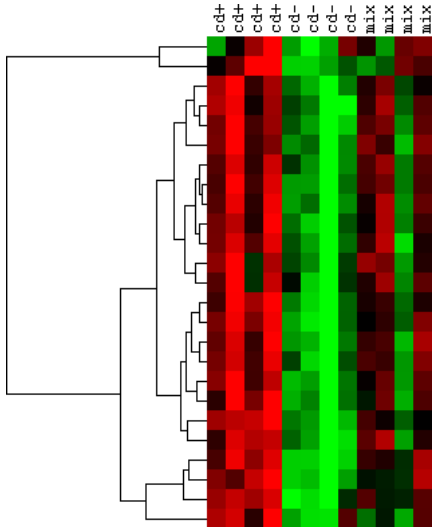




(C)

Term	PValue
GO:0005743~mitochondrial inner membrane	2.46E-17
GO:0005739~mitochondrion	3.20E-15
GO:0006123~mitochondrial electron transport, cytochrome c to oxygen	8.11E-11
GO:1902600~hydrogen ion transmembrane transport	1.20E-06
GO:0007062~sister chromatid cohesion	8.04E-06
GO:0042776~mitochondrial ATP synthesis coupled proton transport	1.42E-04
GO:0019915~lipid storage	2.44E-04
GO:0007059~chromosome segregation	2.56E-04
GO:0000070~mitotic sister chromatid segregation	2.87E-04
GO:0006120~mitochondrial electron transport, NADH to ubiquinone	4.31E-04

APC Intensity

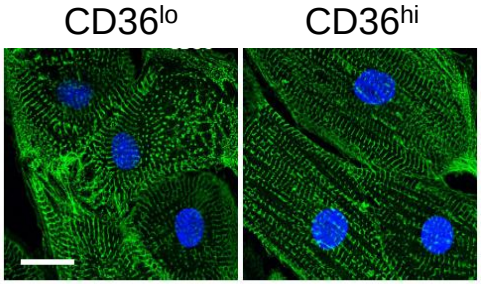


Gene Symbol	P-value
PPIF	9.13E-06
COX5A	1.51E-05
UQCRFS1	1.80E-05
UQCRC1	1.12E-04
CYCS	1.86E-04
NDUFA8	5.64E-04
SDHA	6.14E-04
NDUFB9	6.75E-04
NDUFV1	6.91E-04

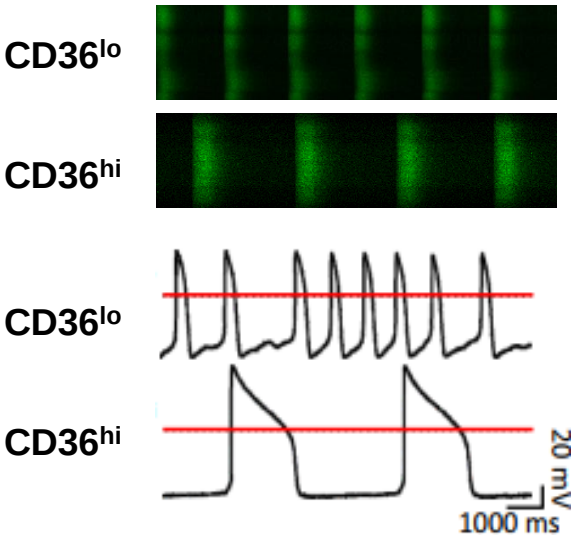
CD36, a fatty acid translocase

CD36 identifies a sub-population of mitochondria-rich cardiomyocytes

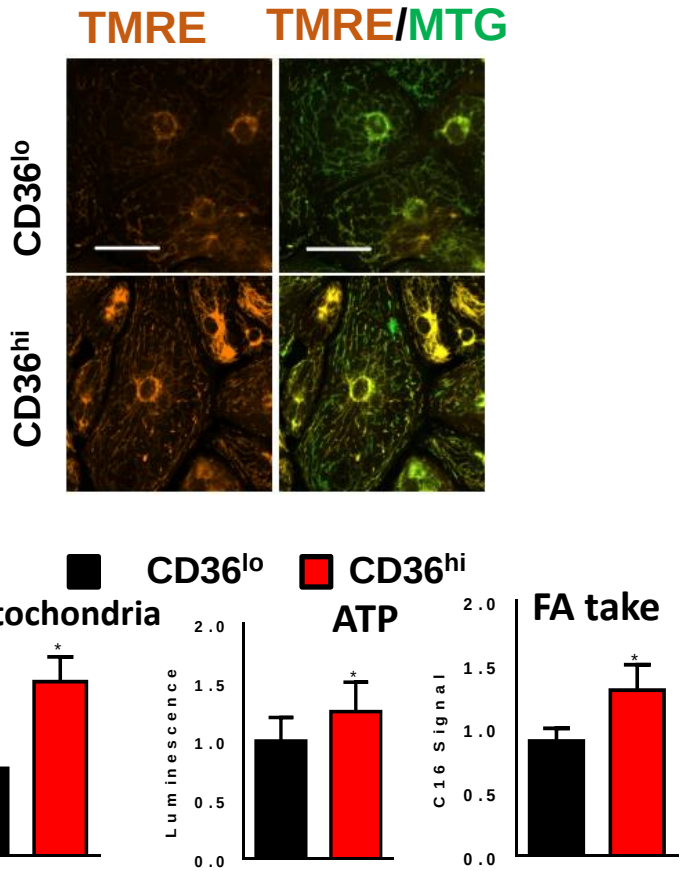
A CD36^{hi}具有更规则的肌丝结构



B CD36^{hi}具有更成熟的功能特征



C CD36^{hi}具有更成熟的线粒体特征

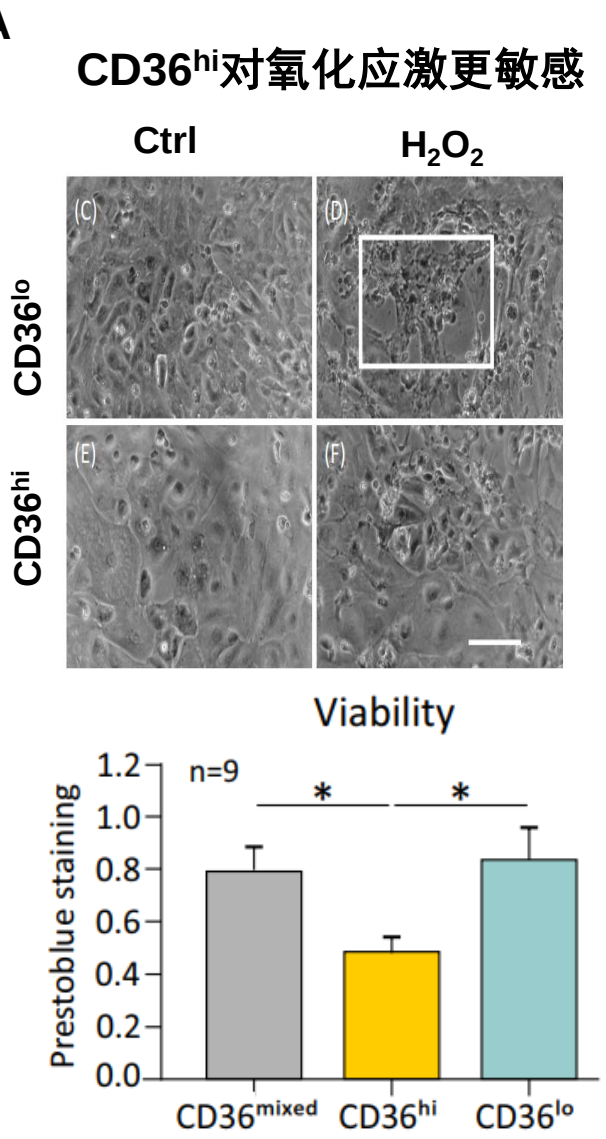


细胞表面捕捉技术

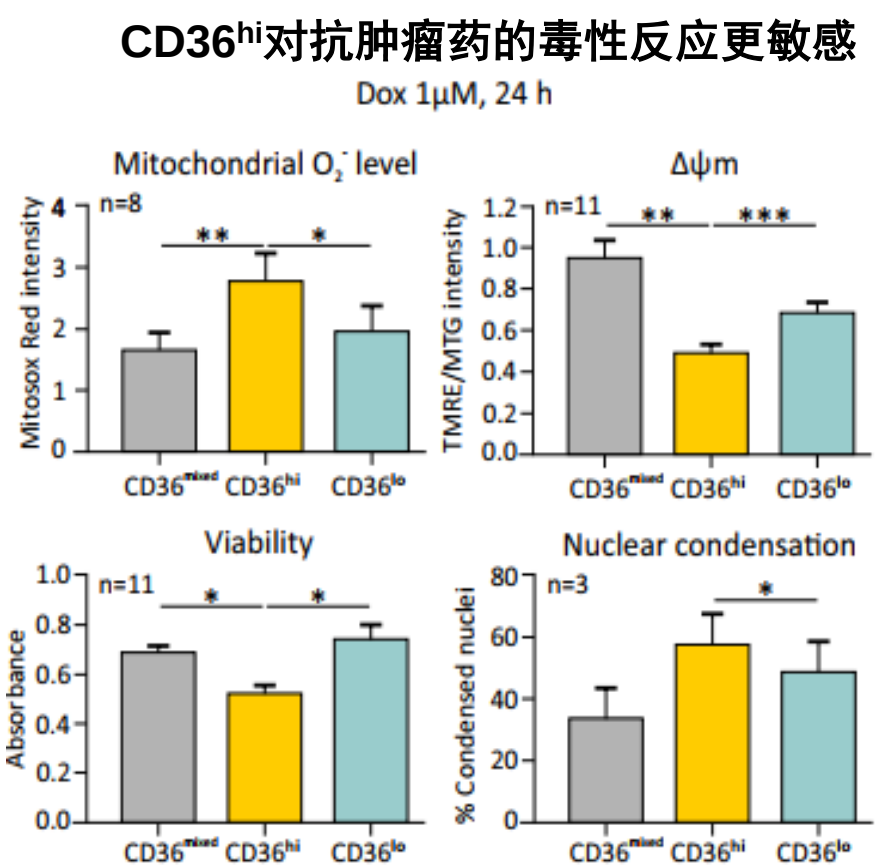
Poon, et al., Cell Res. 2020 Jul;30(7):626-629. doi: 10.1038/s41422-020-0292-y.

CD36^{hi} hPSC-CMs are much more sensitive to oxidative stress

A



B



Poon, et al., Cell Res. 2020 Jul;30(7):626-629. doi: 10.1038/s41422-020-0292-y.

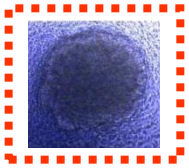
What we are going to discuss…

- Adult heart-derived cardiac progenitor cells/cardiac stem cells
- Differentiation of PSCs into cardiomyocytes
- **PSC-derived cardiovascular progenitor cells**
- Perspectives

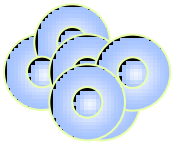
CVPCs-Derived from hPSCs Holds Great Promise for the Basic Research and Application

- Multiple growth factors with stage switch (BMP, FGF, Activin/Nodal, VEGF, Wnts)
- Efficiency at 10%-60%
- Co-culture
- Difficulty in expansion

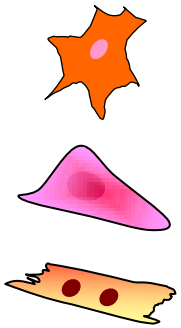
Yang L et al., Nature. 2008; 453(7194):524-8
Wu SM et al., Cell 2008; 132:537-43
Bu L et al., Nature 2009; 460:113-118.
Mauritz C et al., Eur Heart J 2011; 32:2634-41



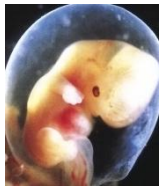
hPSCs
(ESCs & iPSCs)



CVPCs/CPCs
(ISL1^{+}/KDR^{low}/C-KIT^{neg})



Cardiomyocytes
Smooth muscle cells
Endothelial cells



Early development

Disease model

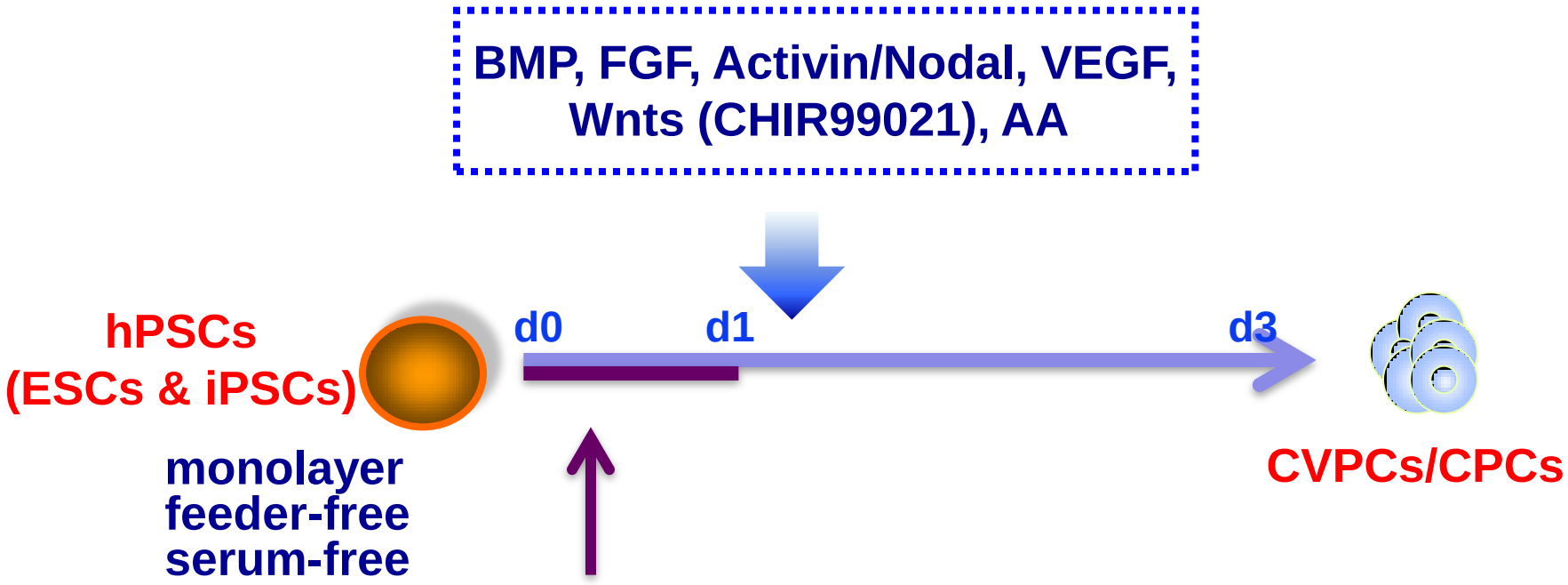
Cell transplantation

Drug screen

Highly desirable

- Renewable source
- Scaled up
- Reproducible
- Carry gene mutation

Capturing critical signaling pathways involved in the generation of cardiovascular progenitor cells (CVPCs) from hPSCs



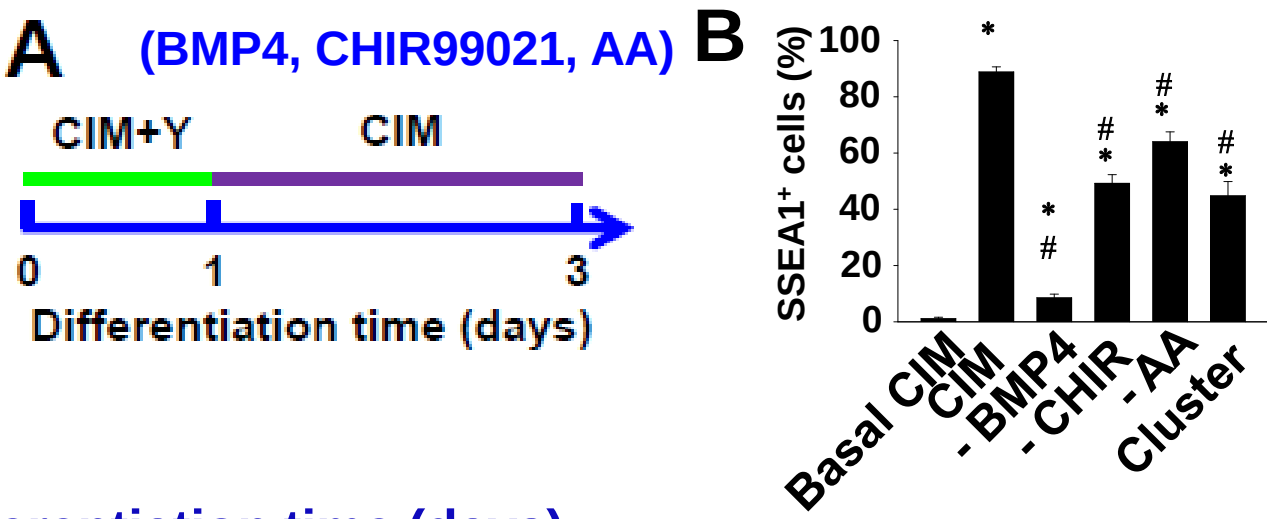
(Watanabe K et al., Nat Biotechnol 2007; 25:681-6)

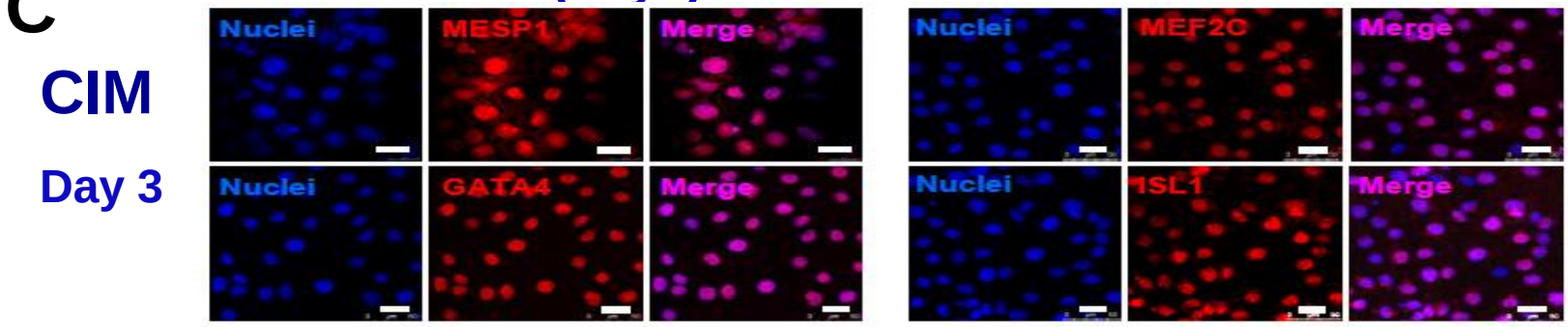
AA, ascorbic acid

CHIR99021, GSK3 inhibitor;

CIM, CVPC induction medium

BMP signaling combining with GSK inhibition and AA is sufficient for the efficient conversion of hPSCs into SSEA1$^+$ cells





Scale Bars = 25 μm

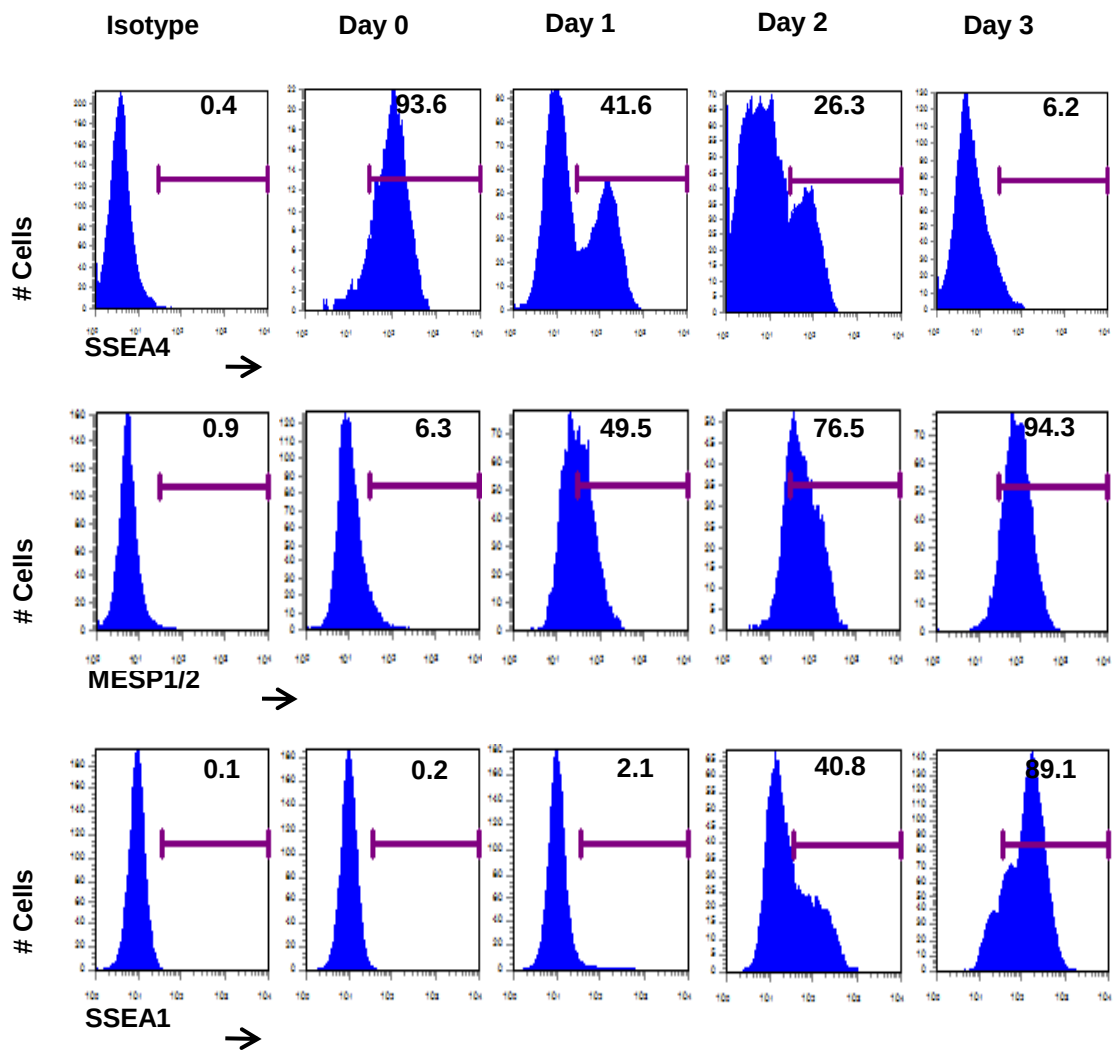
Basal CIM, without BMP4, CHIR99021, AA

CIM: CVPC induction media: BMP4, bone morphogenetic protein

CHIR: CHIR99021, GSK3β inhibitor; AA: ascorbic acid

****P*<0.01 *vs.* Basal CIM; #*P*<0.01 *vs.* CIM**

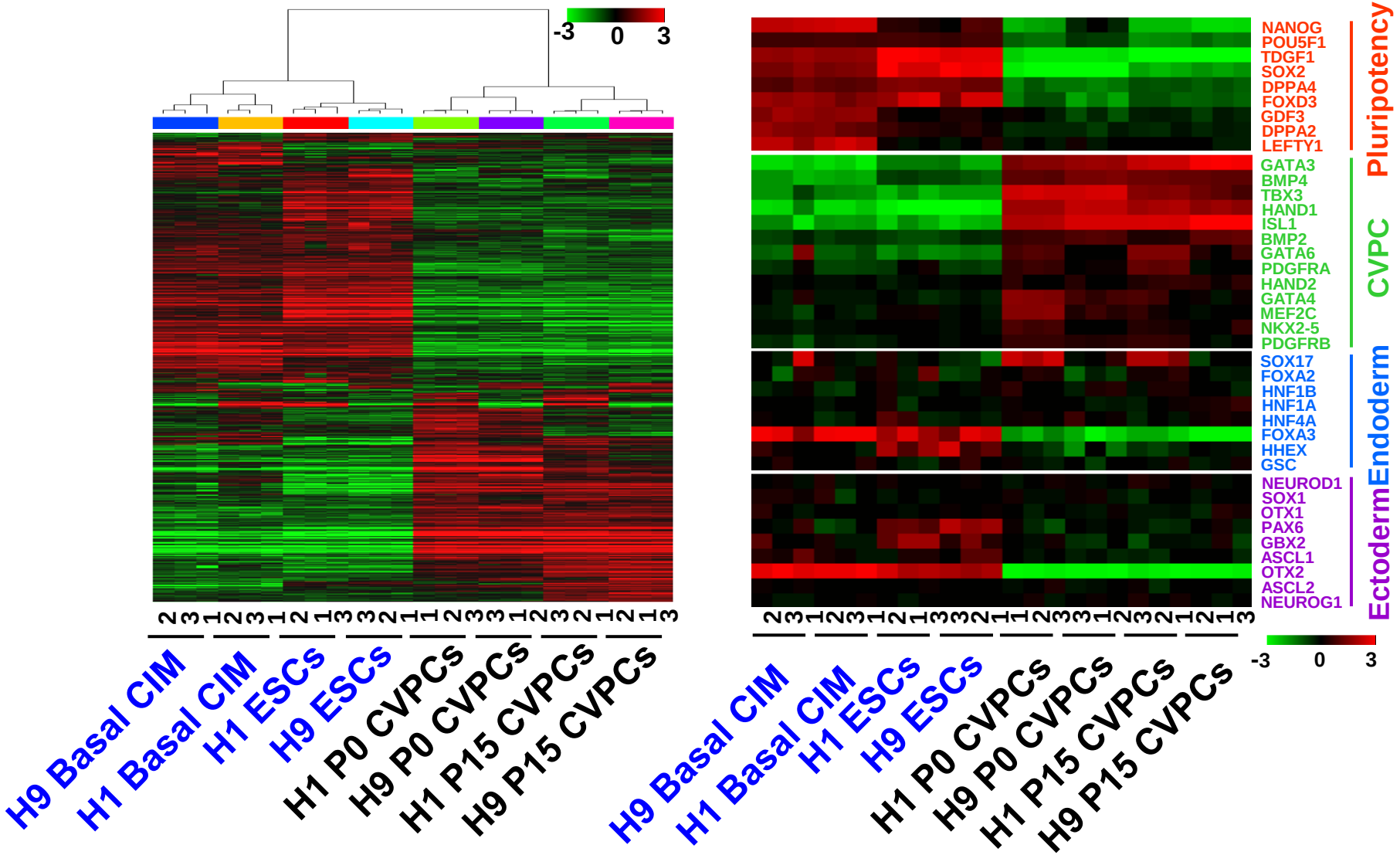
BMP signaling combines with GSK inhibition and AA allows a rapid conversion hPSCs into enriched CVPCs in defined medium



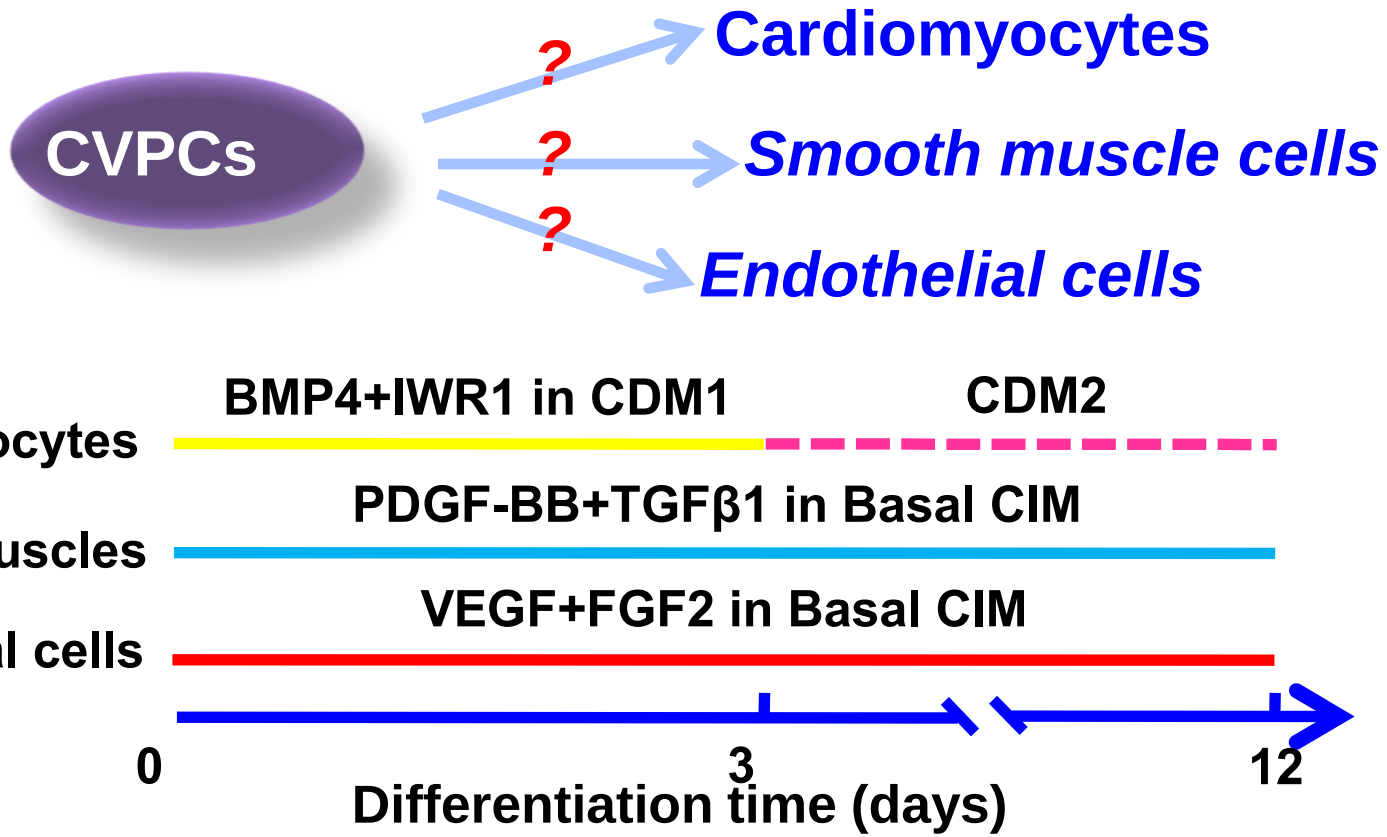
Cells	N	SSEA1^{+} (%)
H1	3	89.7±1.9
H9	15	86.8±2.2
hiPSC	3	86.5±2.2

Cells	N	MESP1/2^{+} (%)
H1	3	94.0±2.3
H9	7	94.4±2.4
hiPSC	3	92.4±4.2

Global changes in gene expression occurring after CVPC induction



Do hPSC-derived CVPCs have multi-cardiovascular lineage differentiation potential in vitro?



CDM1: cardiac differ media 1 (RPMI1640, 1x B27 supplement, 1% L-glutamine, and 1% penicillin/streptomycin, BMP4 and Wnt antagonist IWR-1);

CDM2, CDM without BMP4 + IWR-1

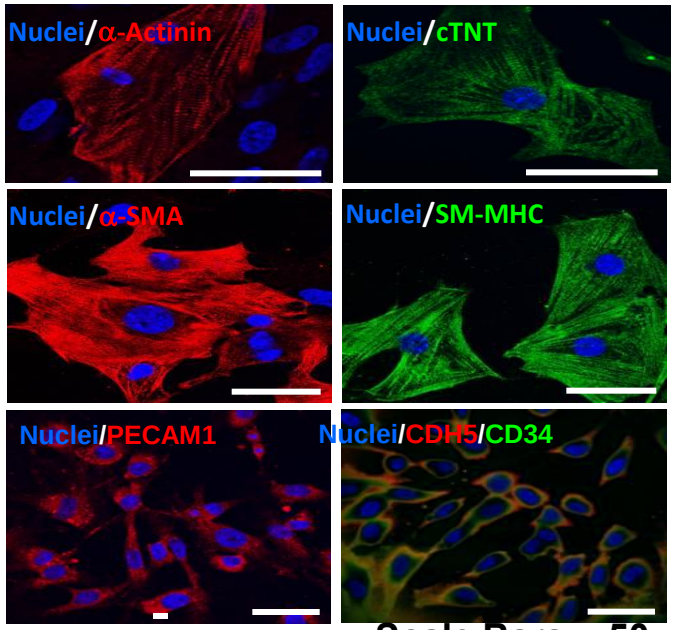
IWR-1: Wnt antagonist; TGFβ1: transforming growth factor β1;

PDGF-BB, platelet-derived growth factor-BB

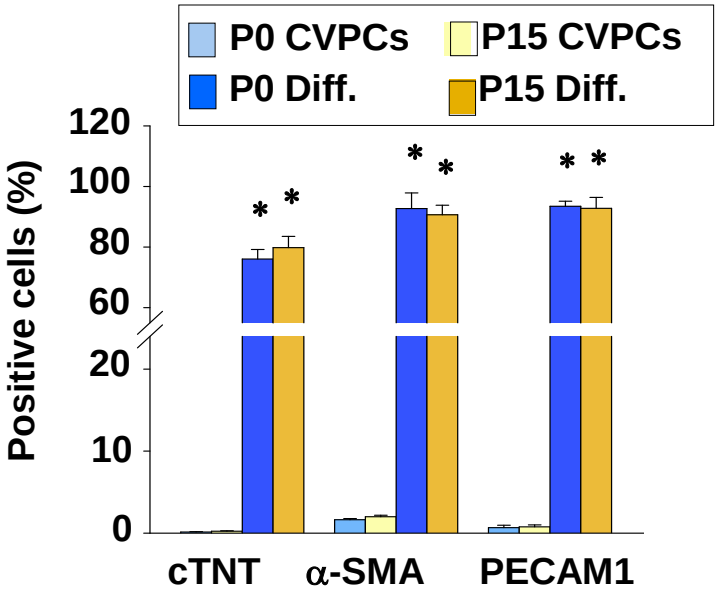
hPSC-derived CVPCs have multi-cardiovascular lineage differentiation potential in vitro

A

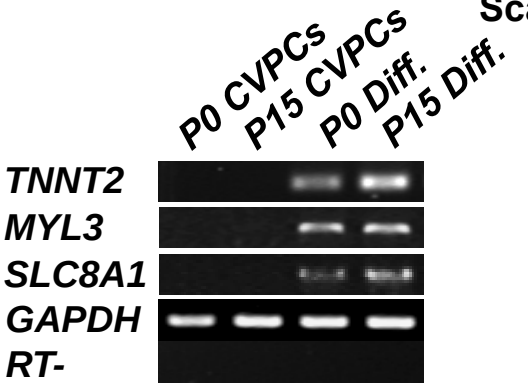
Differentiation of CVPCs



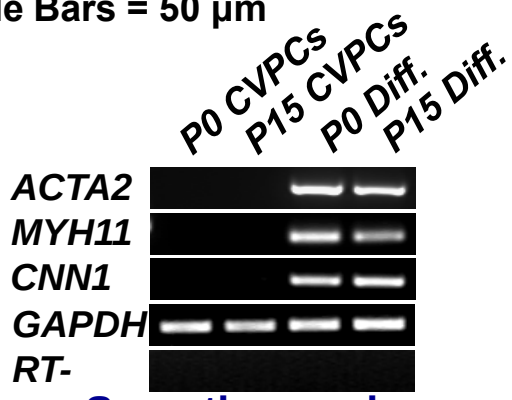
Scale Bars = 50 μm



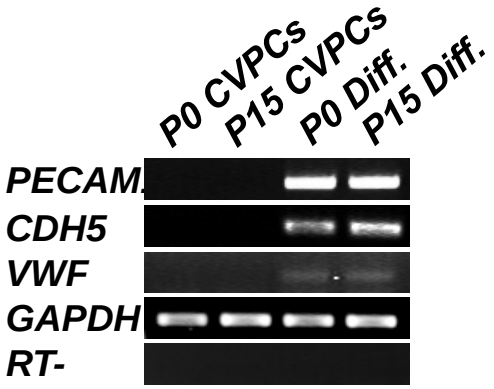
B



Cardiomyocytes

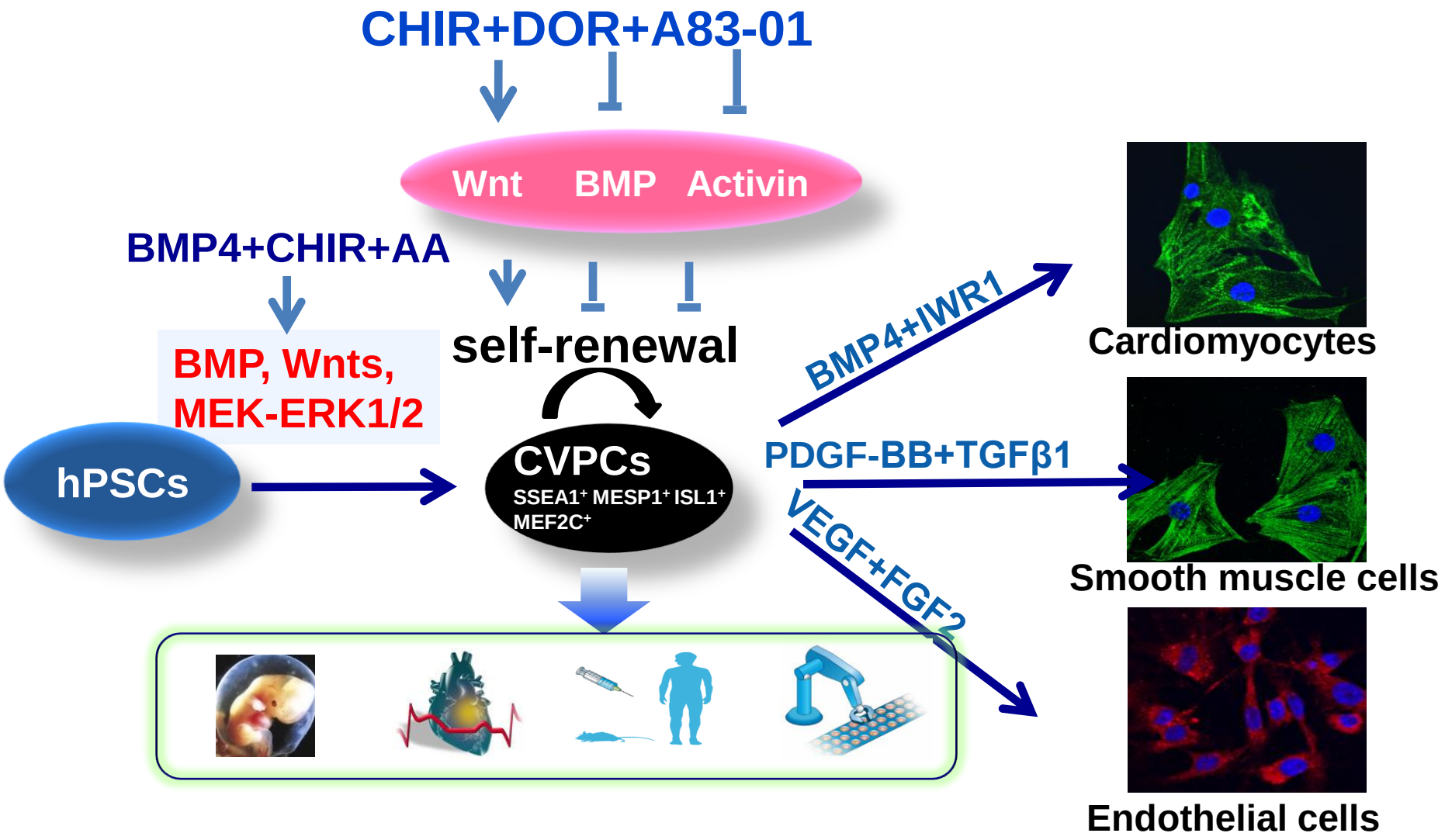


Smooth muscles



Endothelial cells

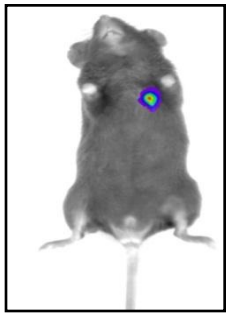
Efficient generation of hCVPCs from hPSCs



Cao et al., *Cell Res* (2013) 23:1119–1132; doi:10.1038/cr. 2013.102

Can PSC-derived cardiovascular progenitors repair infarcted hearts?

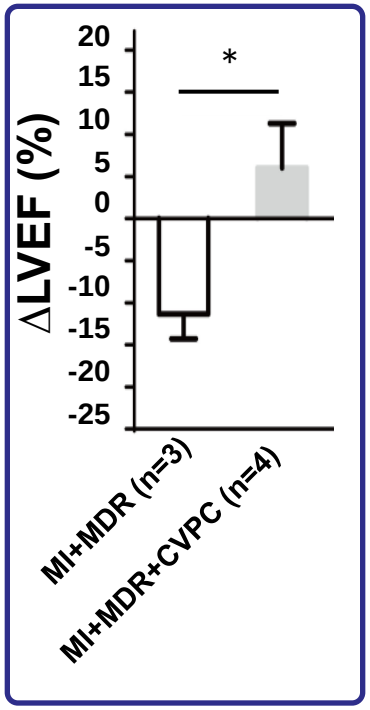
hCVPCs improve heart function after transplanted into acute infarcted mice hearts



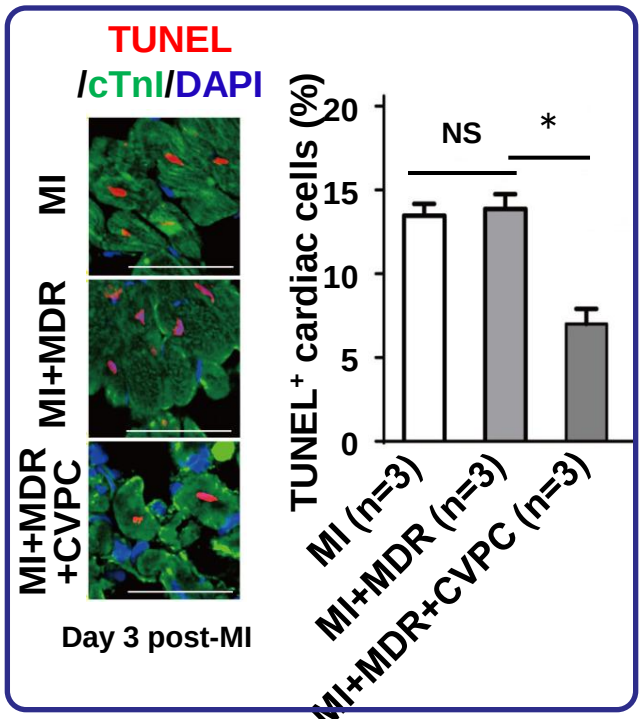
hCVPCs: hESC-CVPCs;
hcFbs: human cardiac fibroblasts;
PM: puramatrix.

hCVPCs benefit infarcted hearts of non-humane primates

心功能改善

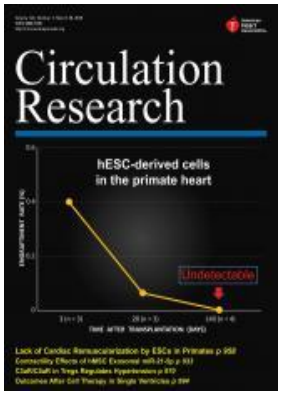


促进心肌细胞存活



ΔLVEF=(LVEF at D28)-(LVEF at D3)
MDR: Multiple-drug regimen

Zhu et al. ***Circ Res*** 2018;122:958-969
封面论文

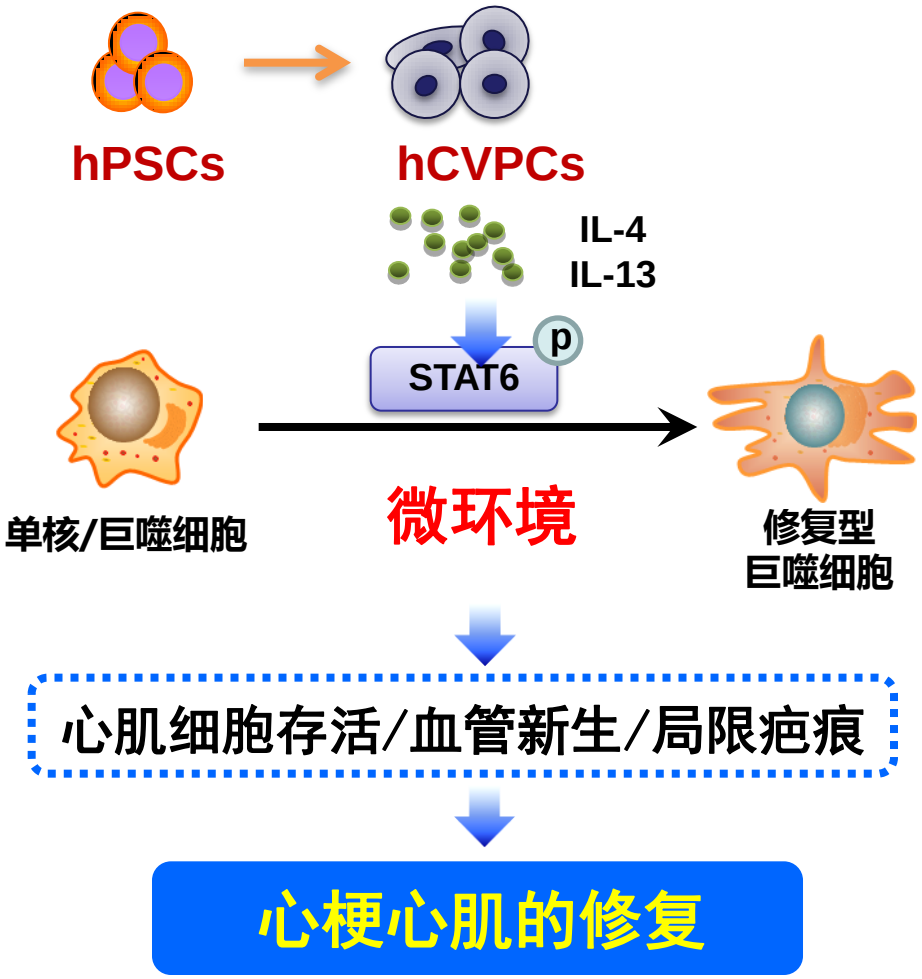


- 心血管领域全球最大规模非人灵长类大动物的研究 （32只）
- 发现三种免疫抑制剂联用显著提高心血管前体细胞移植后驻留率
- 揭示心血管前体细胞移植改善心梗后心功能和心肌细胞存活

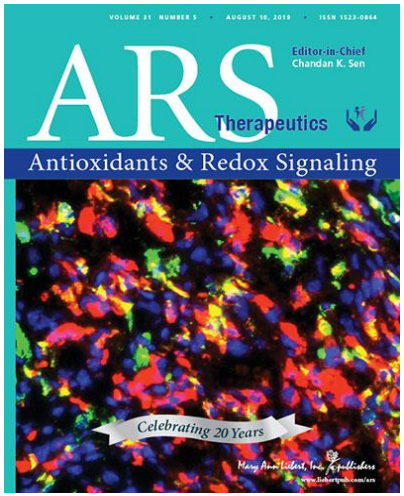
- 心梗急性期是个重要的细胞治疗窗口

这些细胞是如何修复心肌的？

Working model I



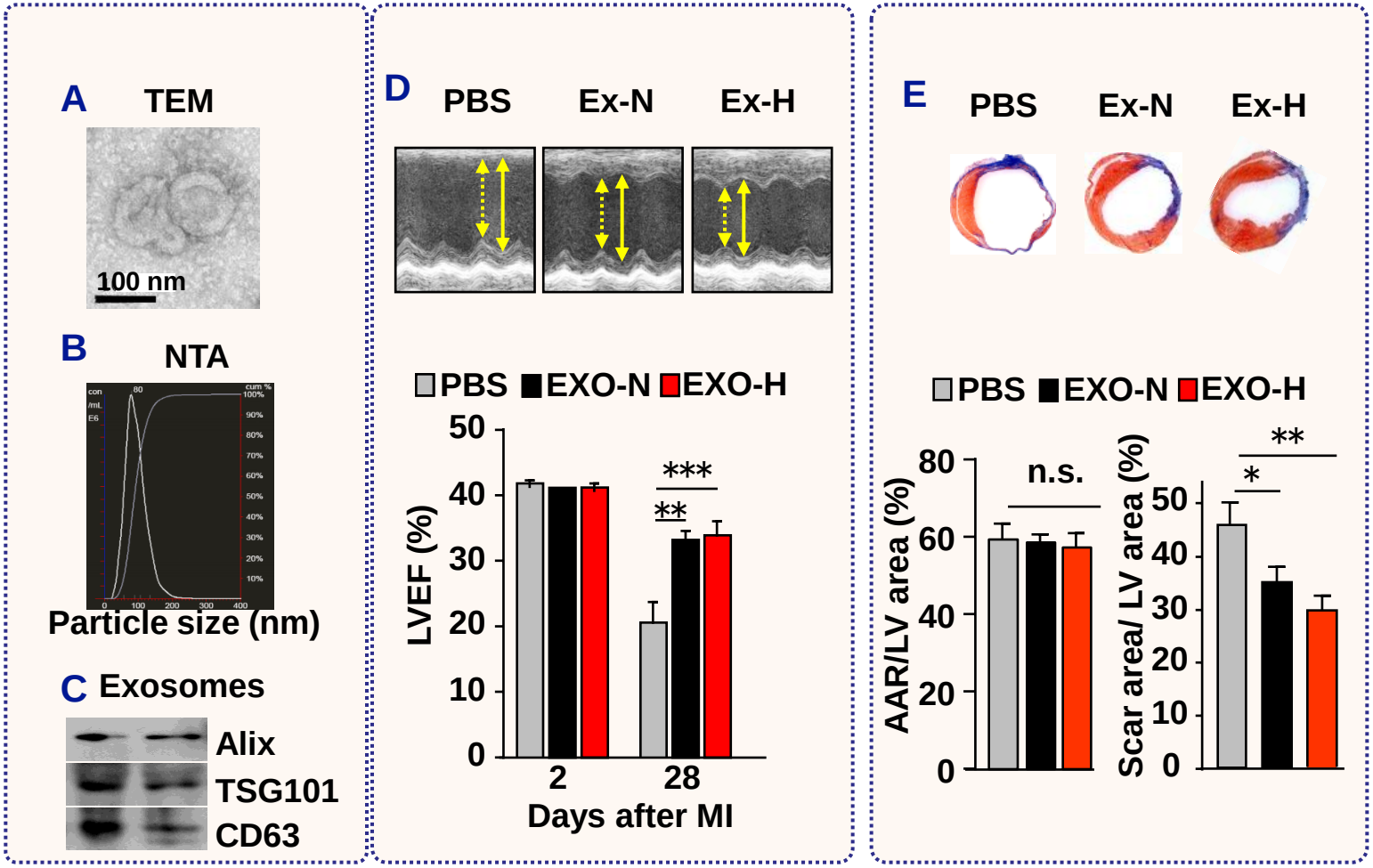
- 心梗急性期是损伤修复的重要窗口
- hCVPC通过调节炎症反应促进心梗心肌损伤修复



Wang et al., ***Antioxid Redox Signal.*** *2019*;31:369-386 (Cover)

Rusinkevich V. ***APS.*** 2019; 40:1168-1183

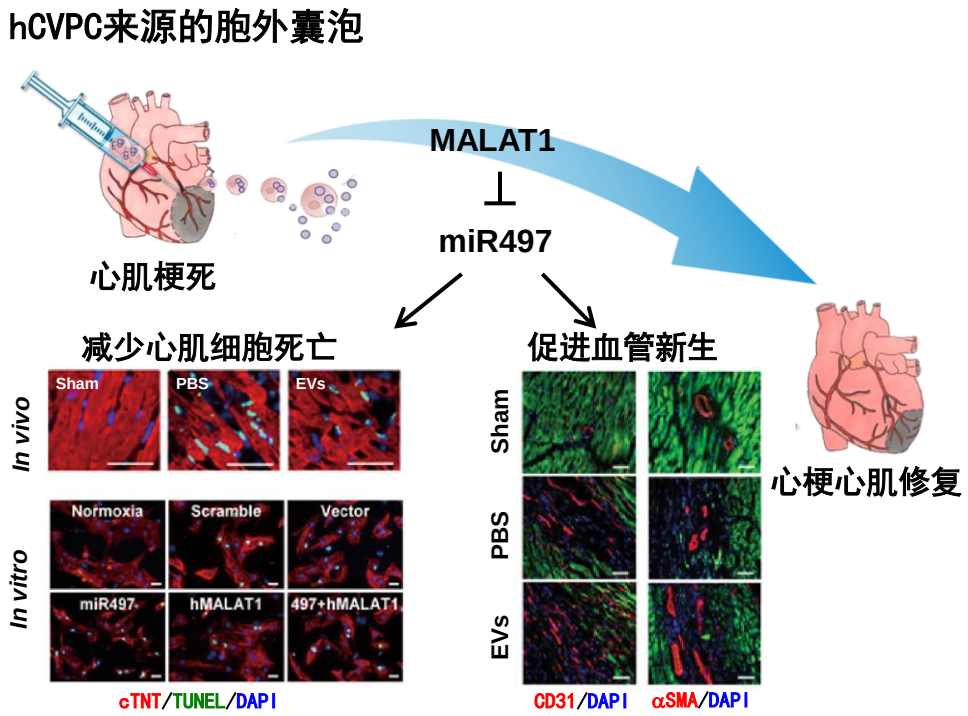
hCVPC-extracellular vesicles (EVs) promote healing of the infarcted hearts



EV-H or EV-N: EVs from hCVPCs with or without the hypoxic treatment in the culture

移植hCVPCs分泌的胞外囊泡（EVs)通过lncRNA促进心梗心肌修复

Working model II



- 首次证明心梗急性期心肌注射hCVPC-EVs改善心功能、抑制纤维疤痕；
- hCVPC-EVs 促进内皮细胞迁移和成管，改善心肌细胞容量；
- 低氧预处理hCVPCs可显著提升hCVPC-EVs中长链非编码RNA MALAT1含量；
- MALAT1通过靶向抑制miR-497表达，促进血管生成和抑制心肌细胞凋亡。

Wu et al. ***Cell Death & Disease***. 2020;11:354.
https://www.nature.com/articles/s41419-020-2508-y

人胚胎干细胞源心脏前体细胞首例临床试验

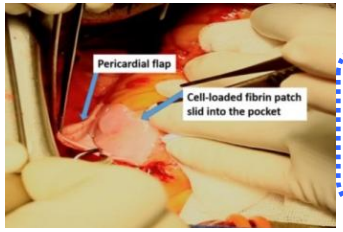
European Heart Journal Advance Access published May 19, 2015

European Heart Journal
doi:10.1093/eurheartj/ehv189

EHJ BRIEF COMMUNICATION
Heart failure/cardiomyopathy

Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report

Philippe Menasché[1,2,3*], Valérie Vanneaux[4,5], Albert Hagège[2,3,6], Alain Bel[1], Bernard Cholley[2,7], Isabelle Cacciapuoti[4,5], Alexandre Parouchev[4,5], Nadine Benhamouda[8], Gérard Tachdjian[9], Lucie Tosca[9], Jean-Hugues Trouvin[10,11], Jean-Roch Fabreguettes[12], Valérie Bellamy[3], Romain Guillemain[8], Caroline Suberbielle Boissel[13], Eric Tartour[2,3,8], Michel Desnos[2,3,6], and Jérôme Larghero[4,5,14]



- No arrhythmia
- No tumor formation

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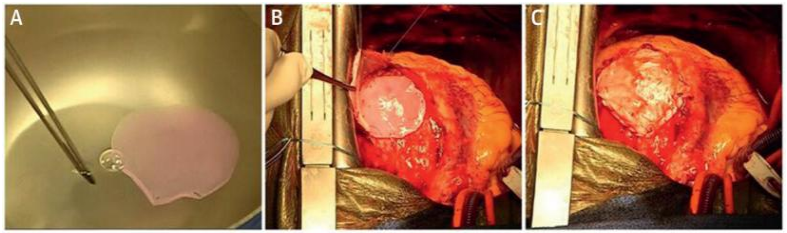
Transplantation of Human Embryonic Stem Cell–Derived Cardiovascular Progenitors for Severe Ischemic Left Ventricular Dysfunction

Philippe Menasché, MD,[a,b,c] Valérie Vanneaux, PharmD,[d,e] Albert Hagège, MD,[b,c,f] Alain Bel, MD,[a] Bernard Cholley, MD,[b,g] Alexandre Parouchev, PhD,[d,e] Isabelle Cacciapuoti, MSc,[d,e] Reem Al-Daccak, PhD,[h] Nadine Benhamouda, MSc,[i] Hélène Blons, PhD,[j] Onnik Agbulut, PhD,[k] Lucie Tosca, PhD,[l] Jean-Hugues Trouvin, PharmD,[m,n] Jean-Roch Fabreguettes, PharmD,[o] Valérie Bellamy, BASc,[c] Dominique Charron, MD,[p,q] Eric Tartour, MD,[b,c,i] Gérard Tachdjian, MD,[l] Michel Desnos, MD,[b,c,f] Jérôme Larghero, PhD[d,e,q]



Eur Heart J 2015;36:2011-2017
JACC 2018; 71: 429-438

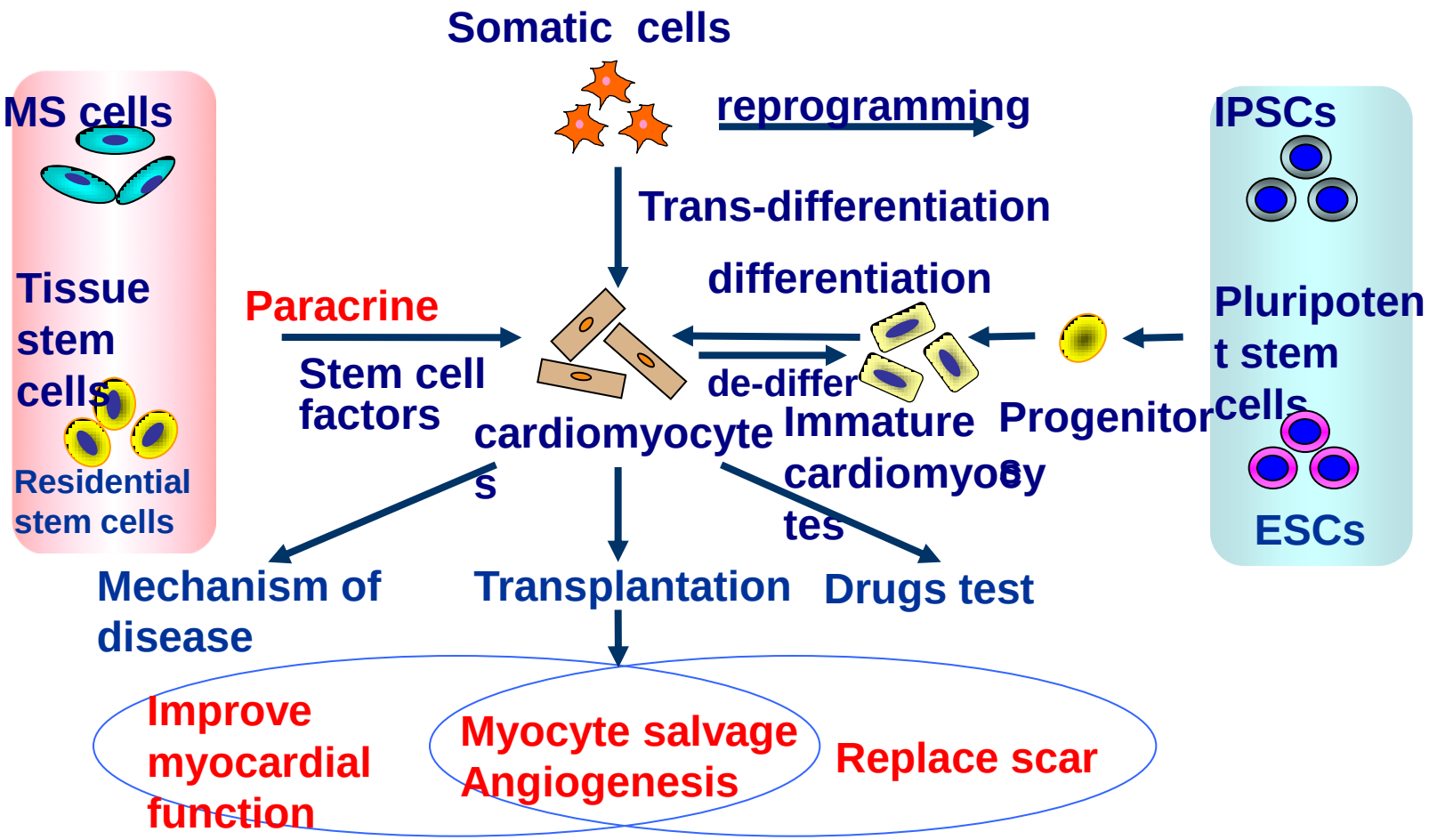
From bench to bed for hPSCs

- Understanding precise regulatory mechanisms;
- Improving stability and scalability;
- Reducing cost (eliminating growth factors);
- Better strategies for full development of maturation (E-C coupling machinery);
- Classifying the subtype of cardiomyocytes;
- Produce homogenous populations of the CVPCs and CPCs;
- Which cell type (s) is better for cardiac repair?
- Assessing safety and efficiency in human-size hearts (pigs);
- … …

What we are going to discuss…

- Adult heart-derived cardiac progenitor cells/cardiac stem cells
- Characteristics of PSCs
- Differentiation of PSCs into cardiomyocytes
- **Perspectives**

Multiple approaches for regenerative therapies



Improvement of contractile performance

Prevention of myocardial remodeling