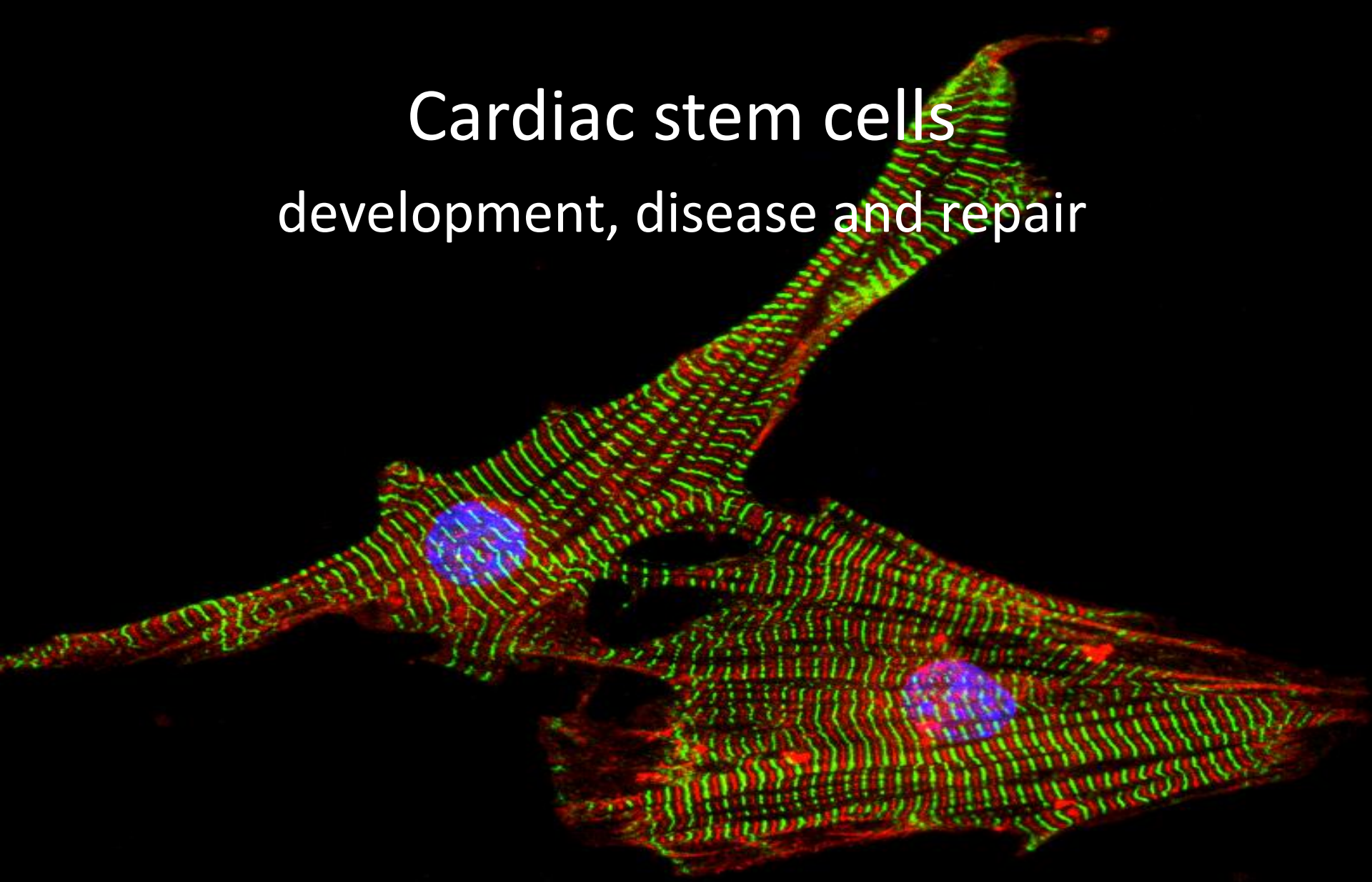
Cardiac stem cells

development, disease and repair



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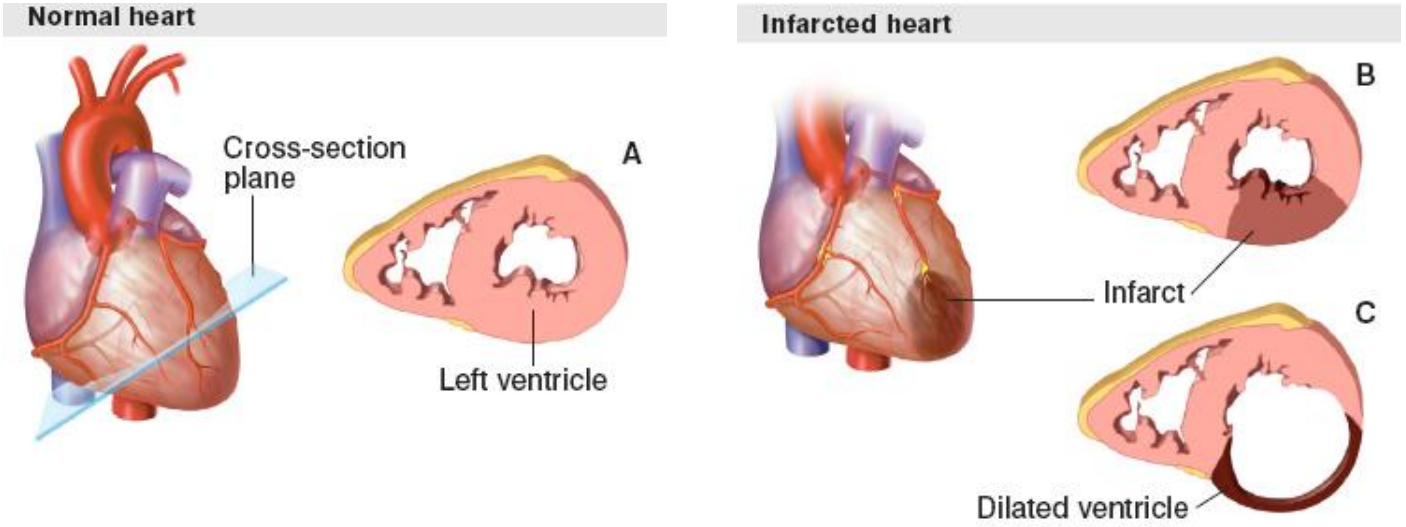
What kind of stem cells can be identified?

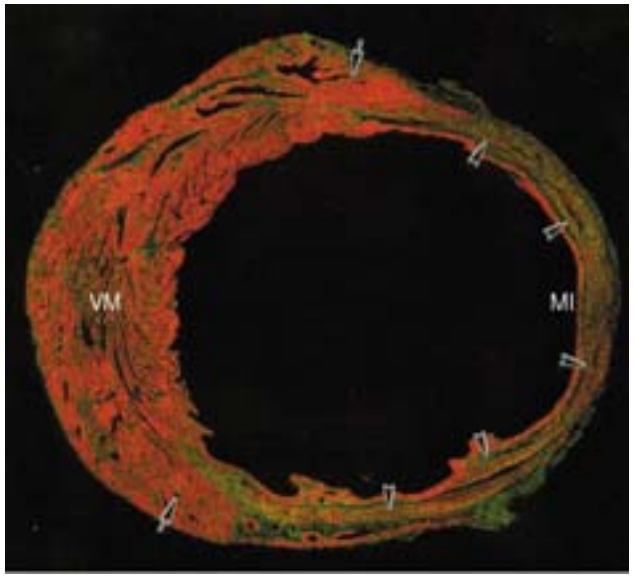
- *adult or somatic stem cells*
  - Present in all tissues and organs (adult or fetal) with the capacity to repair after injury
  - Differentiation capacity (uni- or multipotent) and number of cells are limited
  - Not ethically sensitive since autologous cells (from the patient) can be transplanted.
  - Endogenous activation/differentiation may be possible
- *embryonic stem cells*
  - Derived from blastocyst-stage embryo's (in human:1998)
  - Pluripotent
  - Ethically sensitive, but many cell(s) lines available and can differentiate to all cells of the human body
- *Induced pluripotent stem cells*
  - Derived from reprogrammed somatic cells (in human:2007)
  - Pluripotent (similar to ESCs)
  - Not ethically sensitive, since cells can be derived from adults (patients). Could be used for autologous transplantation

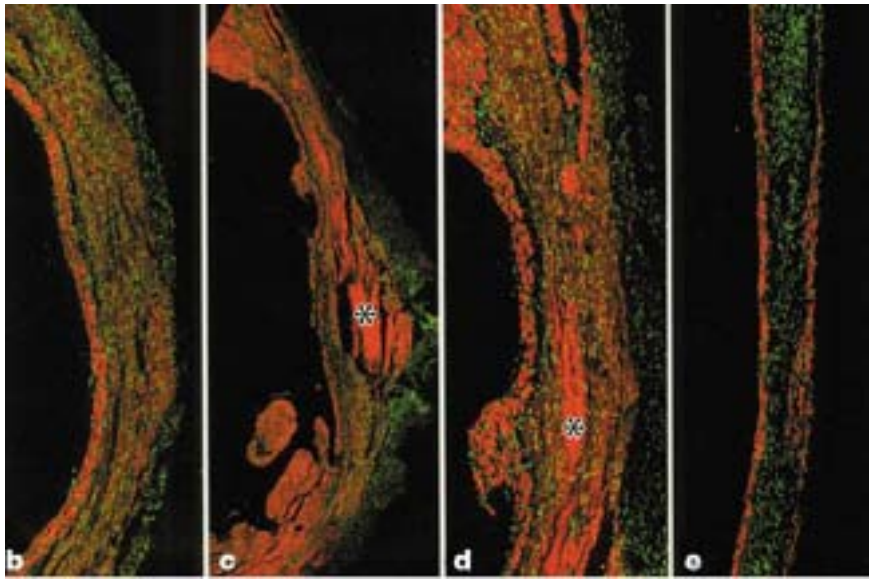
Stem cells for cardiac repair

- Loss of cardiomyocytes in cardiovascular diseases (myocardial infarction)
- Intrinsic myocardial regeneration is limited



Bone marrow cells (c-kit^{+}, Lin^{-}) for cardiac repair





Bone marrow cells and myocardial regeneration. a, Myocardial infarct (MI) injected with Lin- c-*kit*POS cells from bone marrow (arrows). Arrowheads indicate regenerating myocardium; VM, viable myocardium

Red, cardiac myosin; green, propidium iodide labelling of nuclei.

Orlic et al Nature. 2001;410:701.

Adult stem cells for cardiac repair

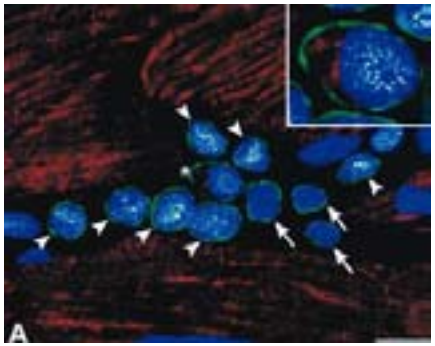
- Clinical trials with bone marrow cells with limited success
  - Slight improvement of heart function
  - No cardiac regeneration

- Mesenchymal stem cells (MSCs)
  - Stromal cells obtained from the bone marrow, but also from many other tissues
    - Adipose tissue, umbilical cord blood, placenta, pericvascular tissues, etc.
  - Self-renewal capacity
  - Multipotent differentiation capacity
    - chondrocytes, osteoblasts, adipocytes, cardiomyocytes)

- Role of MSCs in cardiac repair
  - Improved heart function
    - Migration to injury site
    - Immunosuppressive properties
    - Increased vascularization
    - Release of growth factors (VEGF, IGF-1)
    - Cardiac differentiation from MSCs is limited

cardiac stem cells

Clusters of primitive and early committed cells could be found in the heart



Beltrami et al. Cell. 2003;114:763

Small cluster of c-kit+ cells (green) positive for Nkx2.5 (white)

Lin^- $c\text{-}kit^+$ CSCs injected into an ischemic heart resulted in the formation of blood-carrying new vessels and myocytes

Markers identifying cardiac stem cells:

- C-kit+
- Sca-1+
- Isl-1+
- Flk-1+
- SP+

Cardiospheres of culture explants of human heart biopsies represent a potential source of endogenous cardiac stem cells

- Clnical trial in MI patients (CADUCEUS)

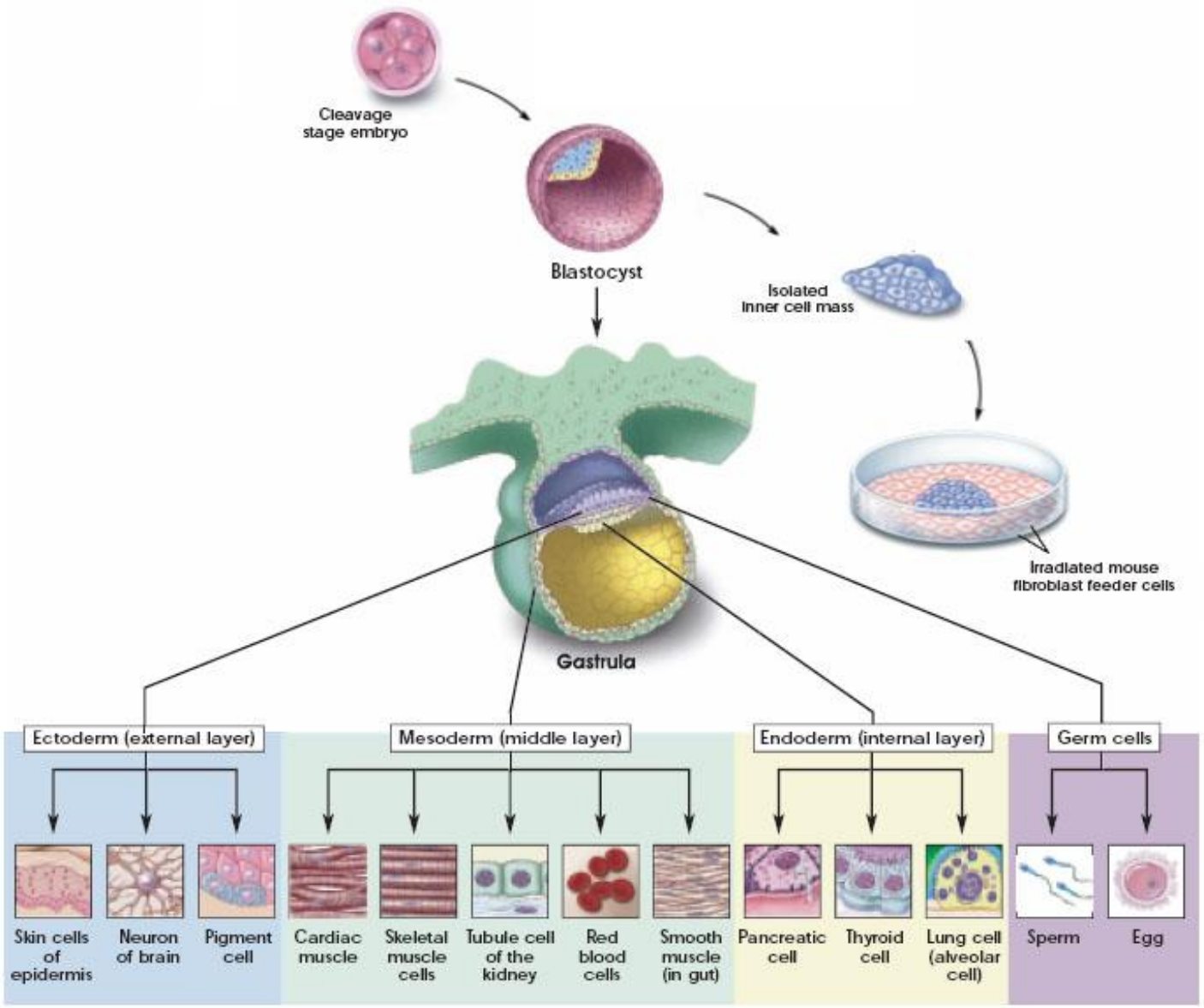
Epicardial cells (WT1+/TBX18+) represent another cell population that may contribute to endogenous repair under the right conditions (Smart et al Nature 2011)

- Enhanced regeneration in the presence of Thymosin Beta 4

Human Pluripotent Stem Cells

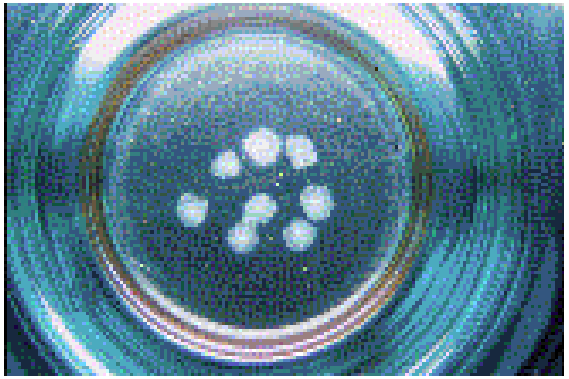
generation of cardiac progenitor cells and cardiomyocytes

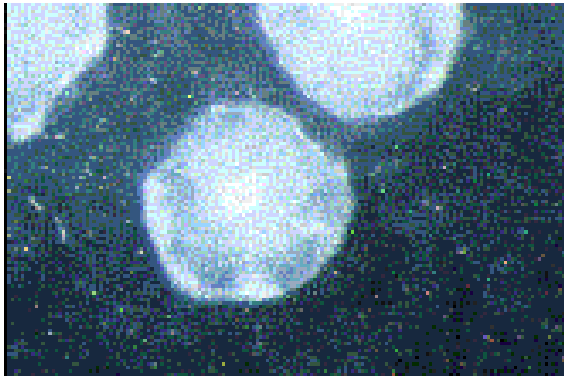
embryonic stem cells



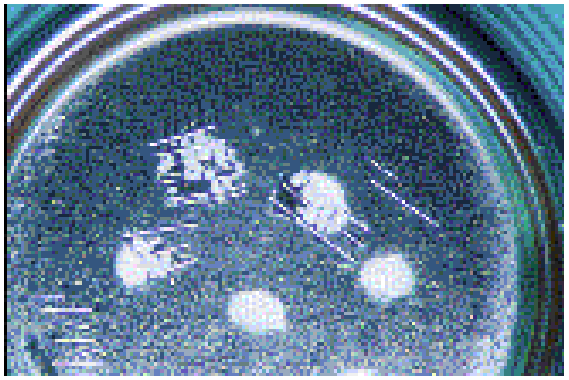
Culturing human embryonic stem cells

hESC on mouse feeders, ready to passage

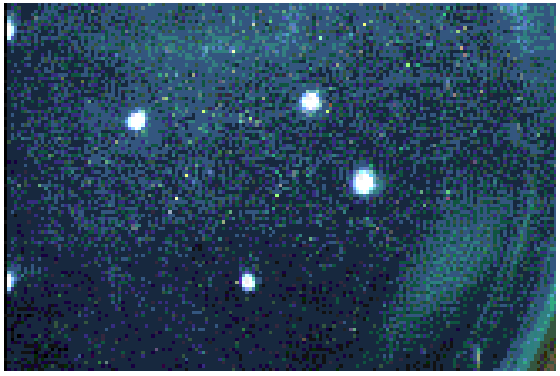




cutting colonies

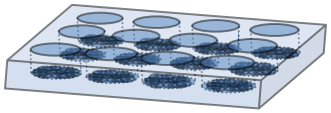


hESC, 1 day after transfer



hESC-END-2 co-culture
improving cardiomyocyte differentiation

undifferentiated hESC



END-2

12 days

beating area

cardiomyocyte differentiation efficiency

Mummery et al. 2003

2003

20% FCS

Passier et al. 2005

2005

no FCS

24-fold ↑

Freund et al. 2008

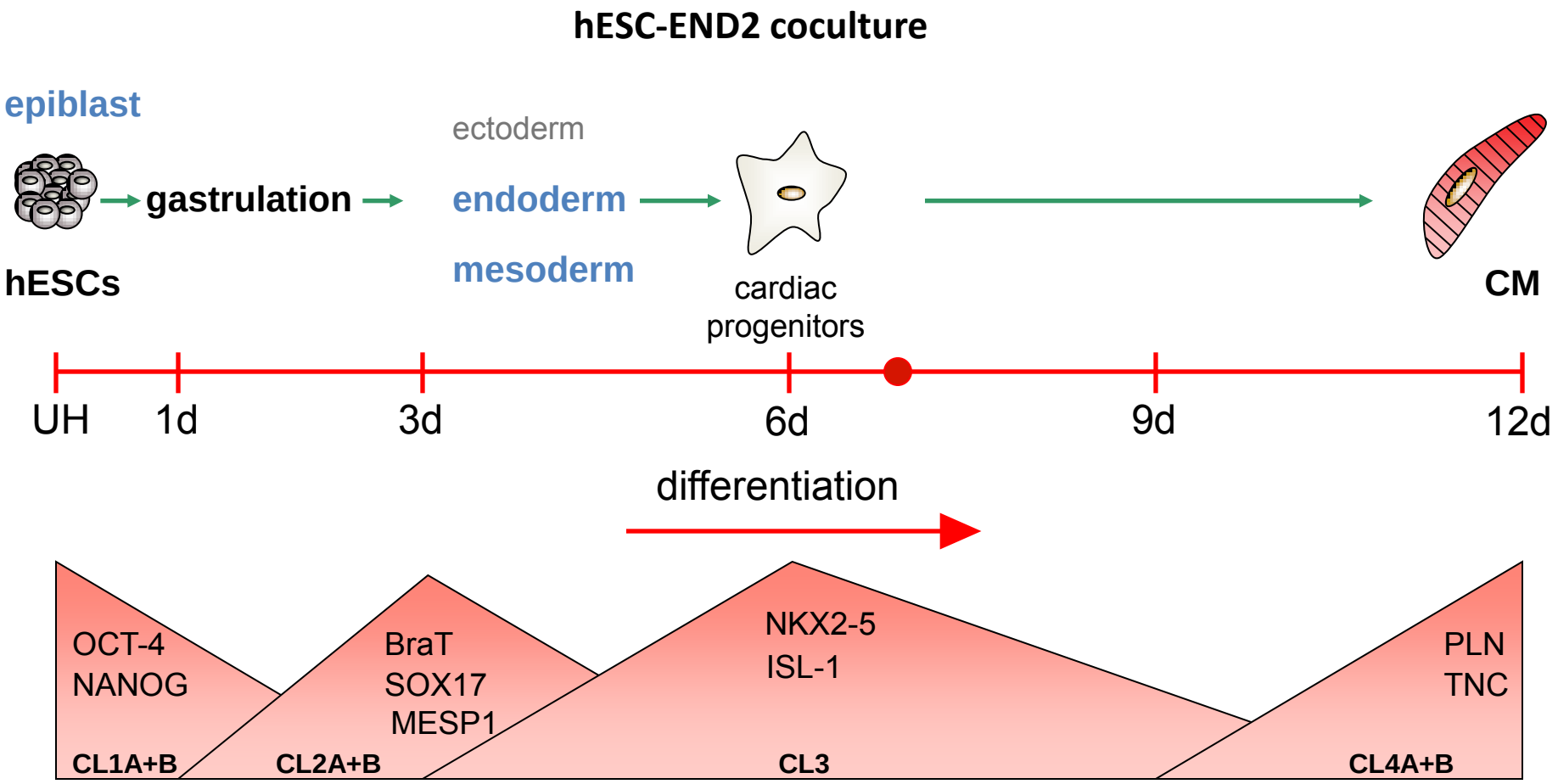
2008

no FCS
no insulin

3-fold ↑

72-fold ↑

hESC differentiating to cardiomyocytes

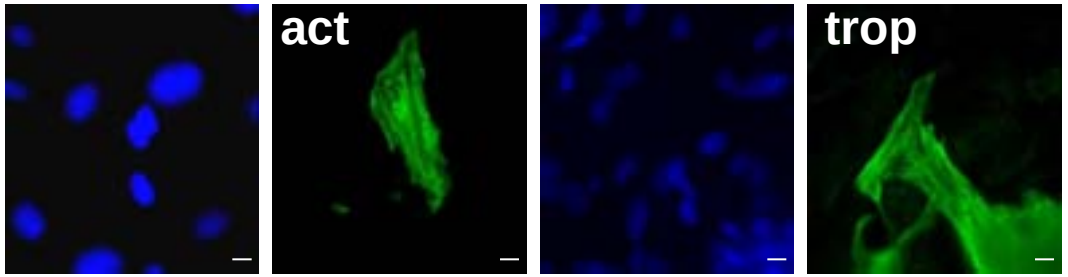


Differentiating hESC-CM follow "waves" of expression comparable to in vivo cardiac development!

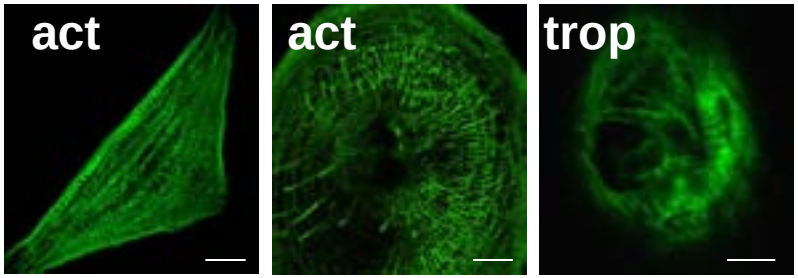
Beqqali et Stem Cells 2006

Cardiac proteins in hESC, human fetal and adult cardiomyocytes (CM)

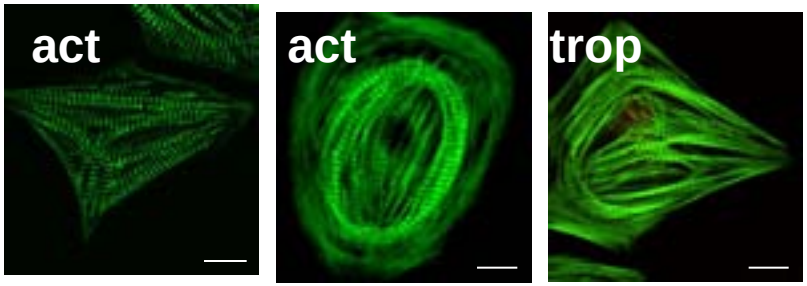
hESC-CM



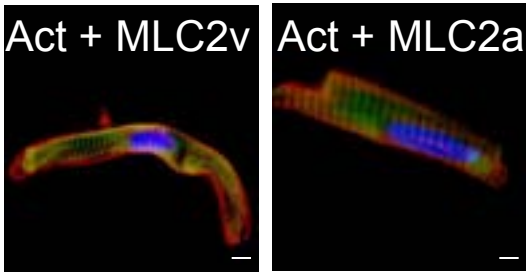
hESC-CM



Fetal hCM

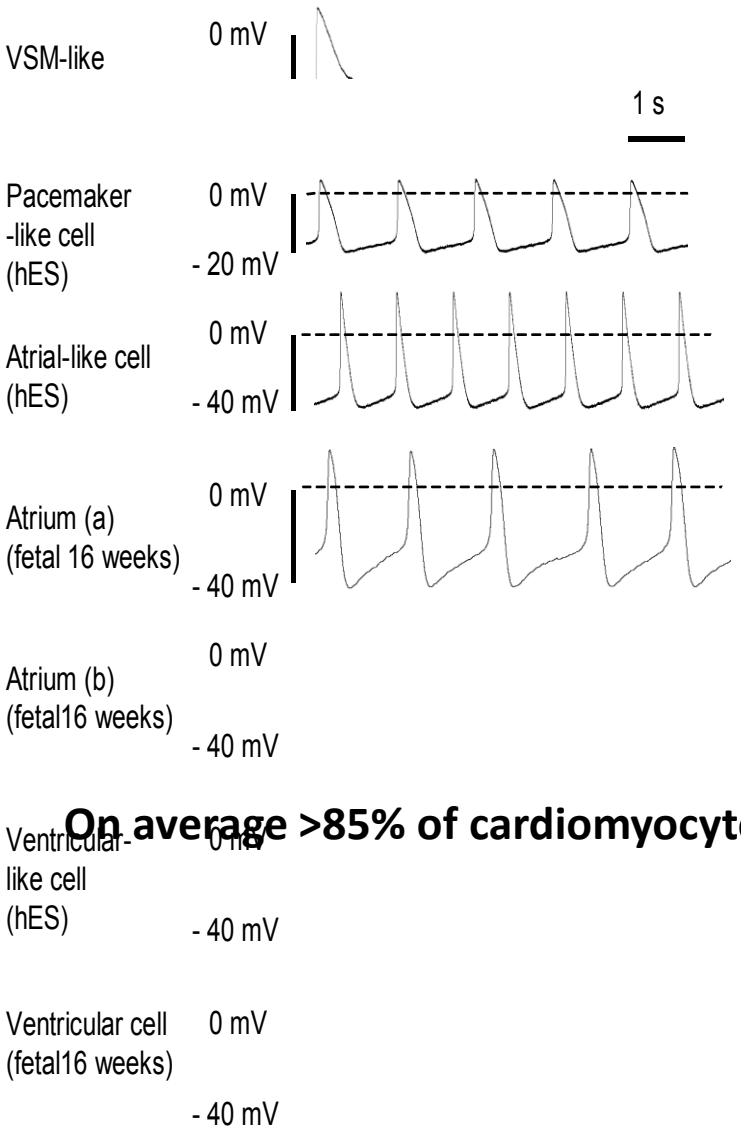


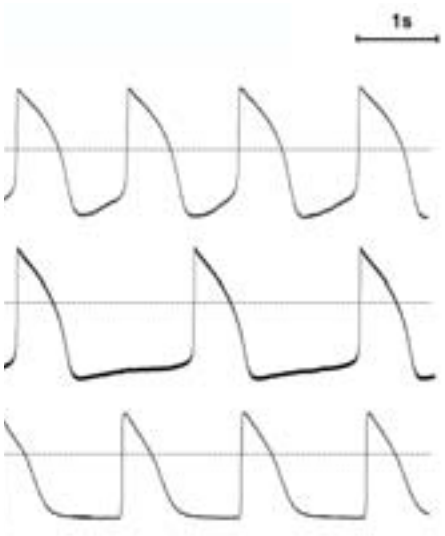
Adult hCM



Electrophysiological characterization of hESC-CM

A





On average >85% of cardiomyocytes show a ventricular-like action potential

Human pluripotent stem cell-derived cardiomyocytes

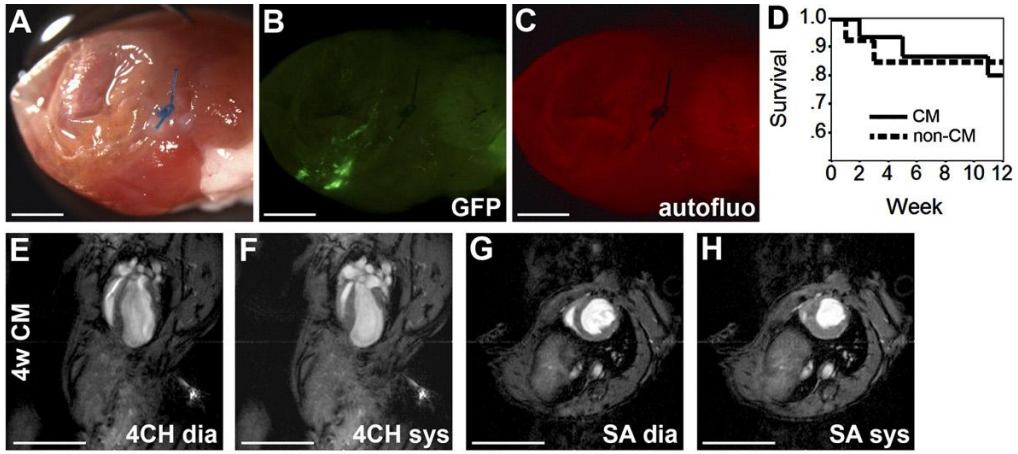
cell transplantation for cardiac repair?

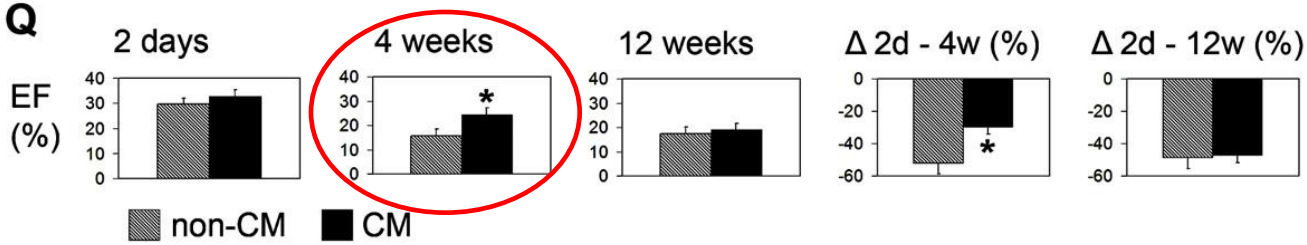
Effect on cardiac function?

Model of acute myocardial infarction

Male SCID mice (n=13-15 per group)

- MI (LAD ligation) +
  - 1 million GFP-HES3 from beating areas END-2 co-culture (20% CM)
  - 1 million non-CM differentiated from GFP-HES3
- MRI (9.4 T) after 2 days, 4 weeks, 12 weeks





Cardiac function improvement at 4 weeks not sustained!

Why do we need human stem cell-derived cardiomyocytes?

hPS d as:

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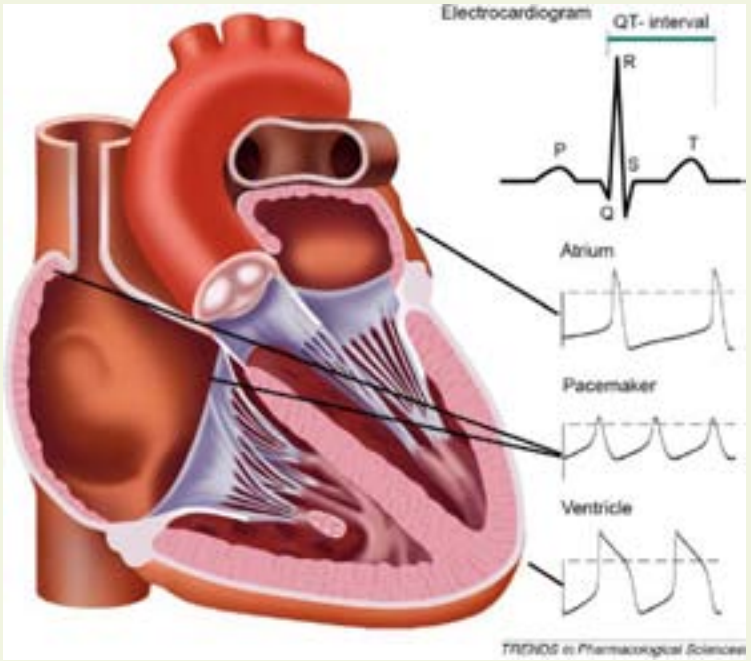
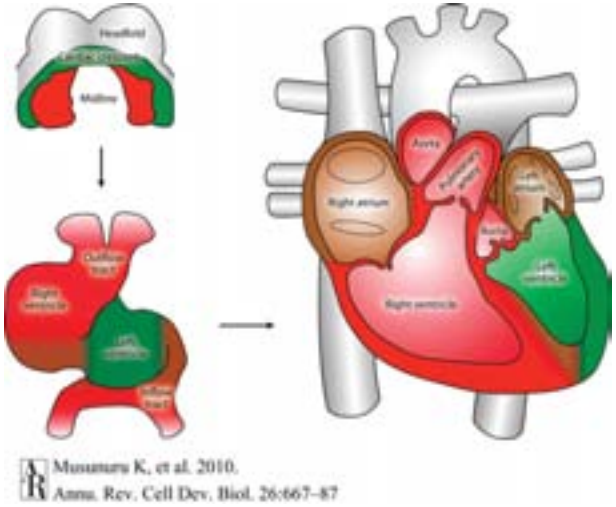


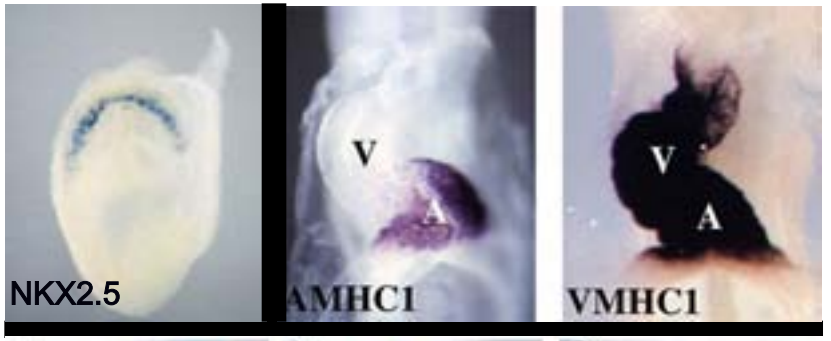
end-stage

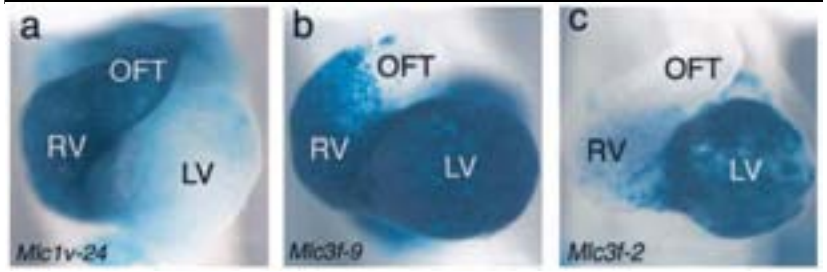
(*Beqqali et al.*

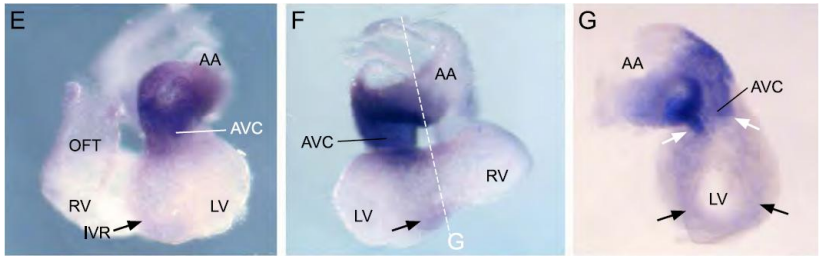
Higher predictabillity and succes can be achieved with more homogeneous cardiac subtype populations or controlled mixtures of cells

Identification of cardiac subtype populations



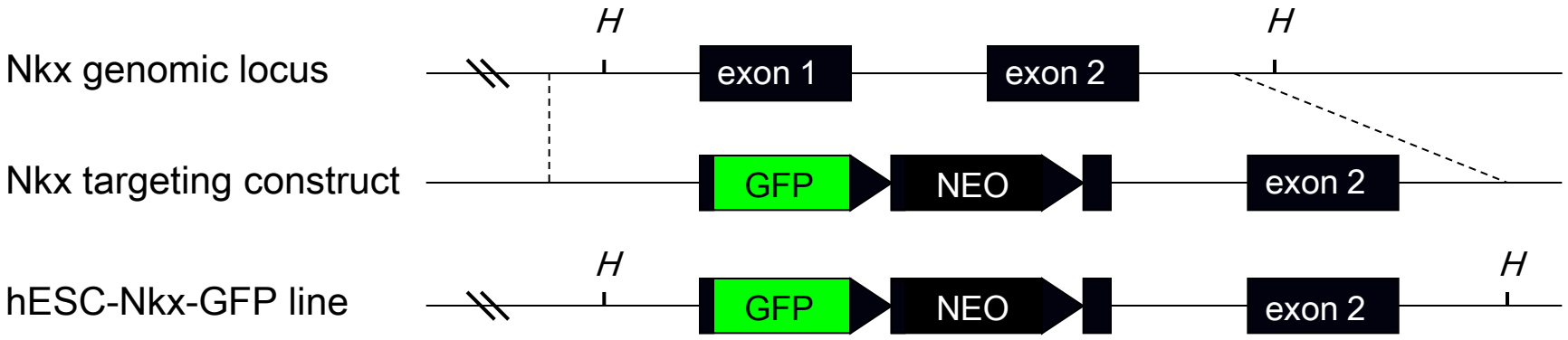


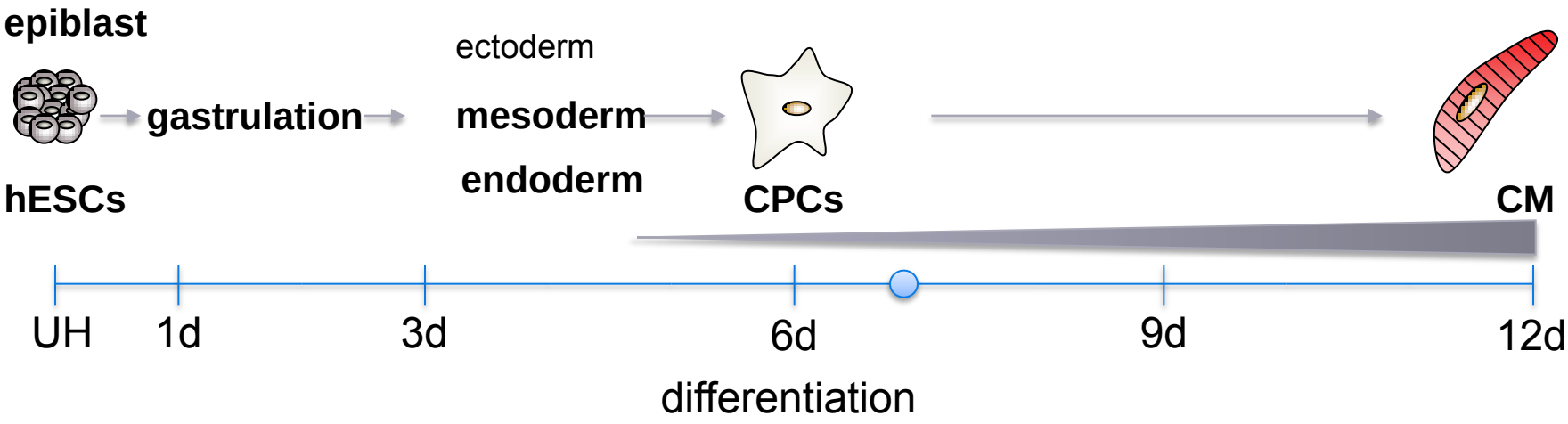




Reporter lines for cardiac conduction system

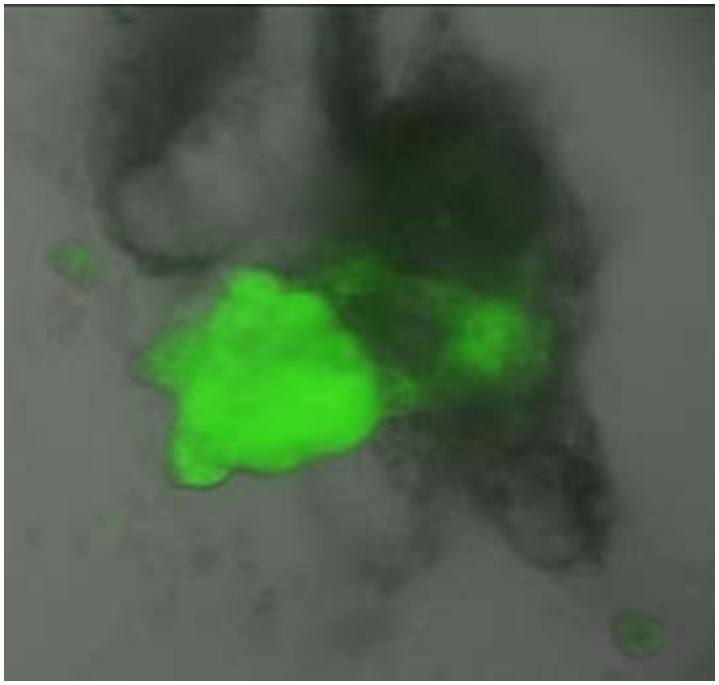
Building a cardiac reporter line

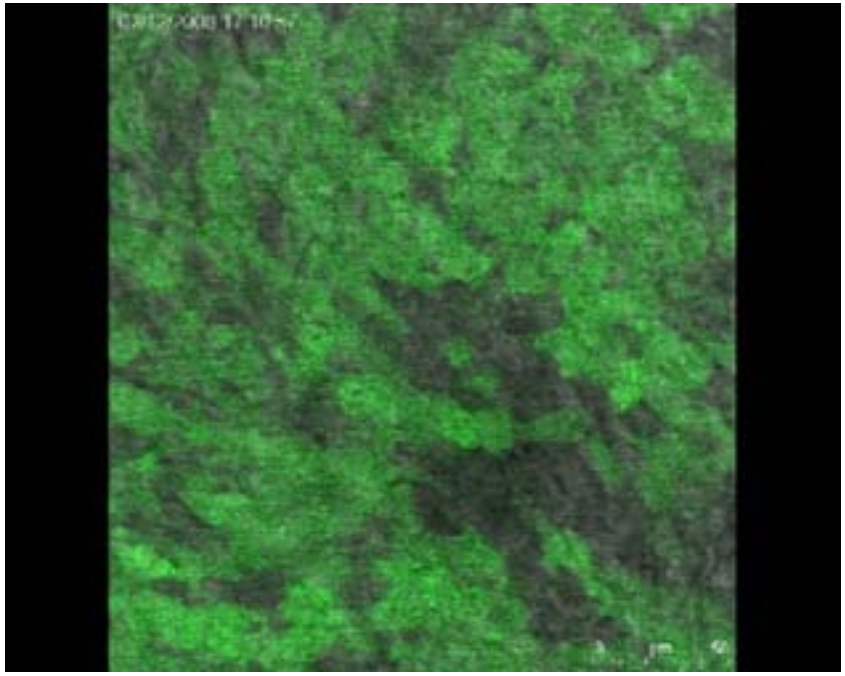




EGFP expression by the endogenous NKX2-5 promoter

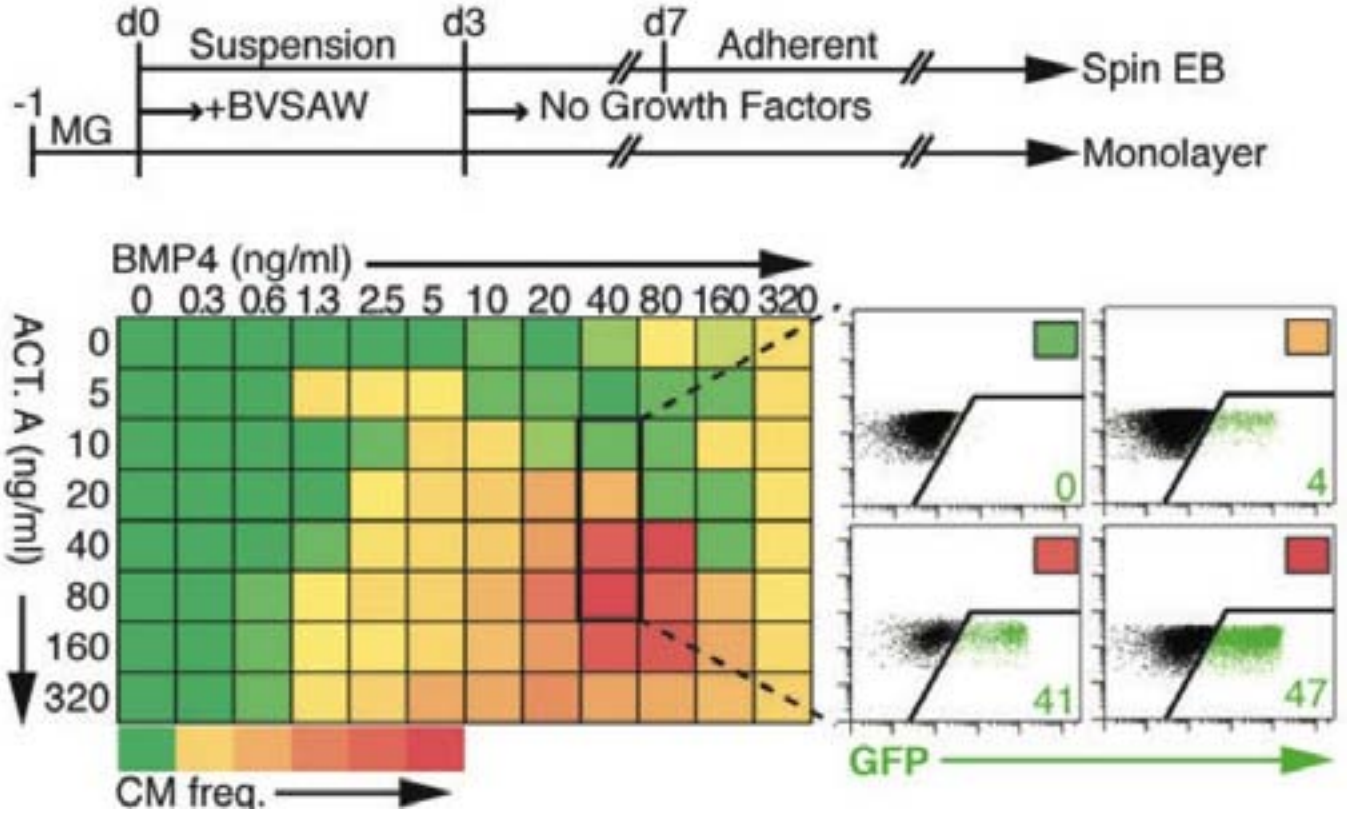
Homologous recombination following electroporation in hESC





Beating differentiating hESC

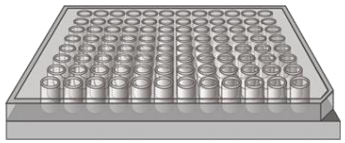
Controlled differentiation



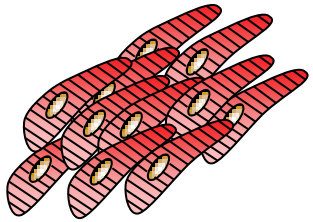
Elliott et al. Nat Methods 2011

cardiac cell differentiation and purification

Defined, HT differentiation

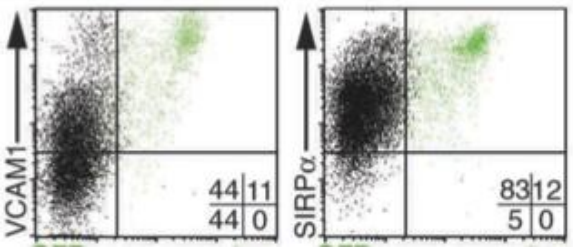


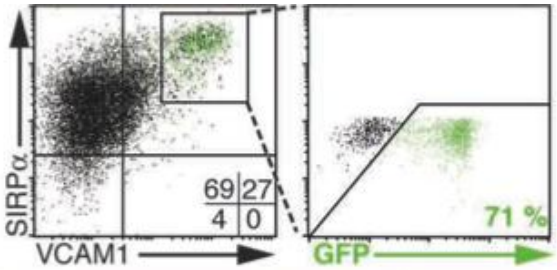
Differentiation in 96-well plates
(scalable technology)



> 50% Cardiomyocytes

Purify cardiomyocytes: sorting (SIRP +VCAM1)



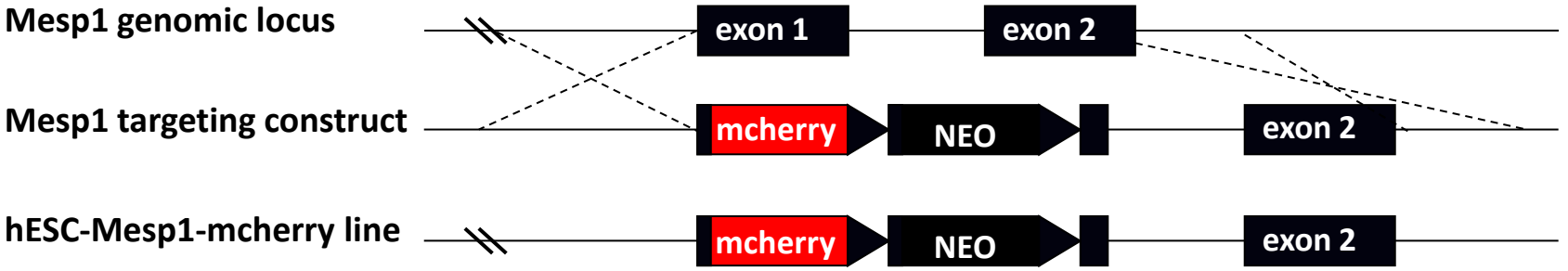


Elliot et al. Nat. Meth. 2011, 8:1037

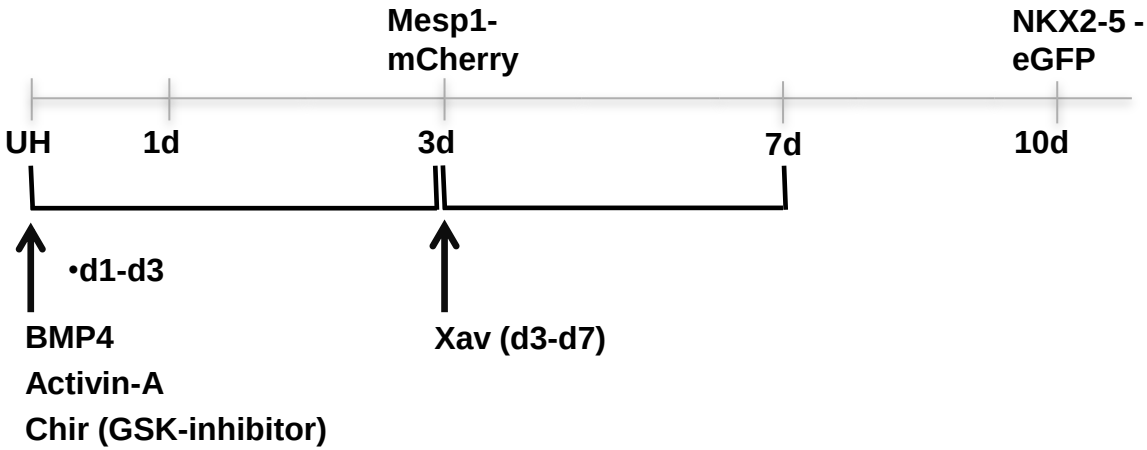
Generation of a cardiac mesodermal hESC line

Generated in Nkx2.5-GFP background: double transgenic hESC line (Mesp1-mCherry/Nkx2.5-GFP)

MESP1 reporter hESC line

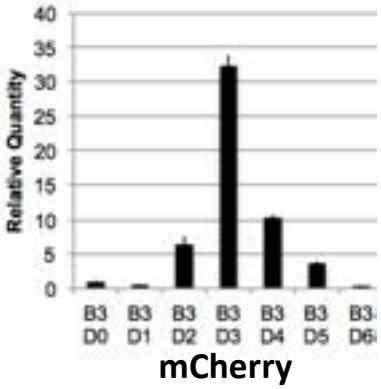


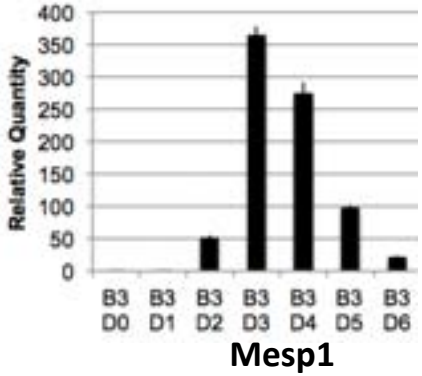
cardiac differentiation



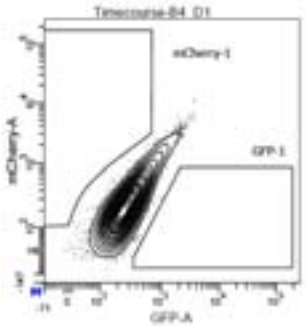
Mesp1-mCherry/Nkx2.5-GFP reporter hESC-line

RT-PCR

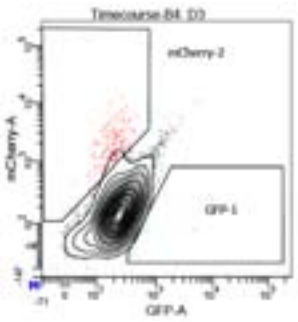




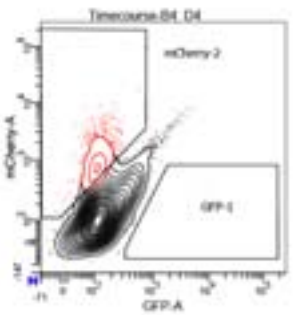
FACS



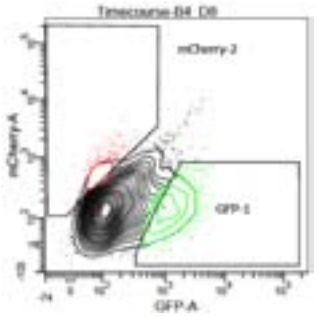
D1



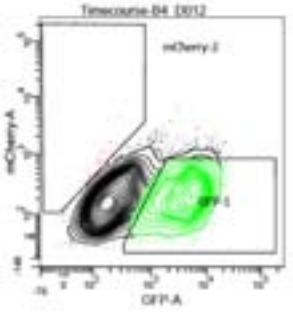
D3



D4

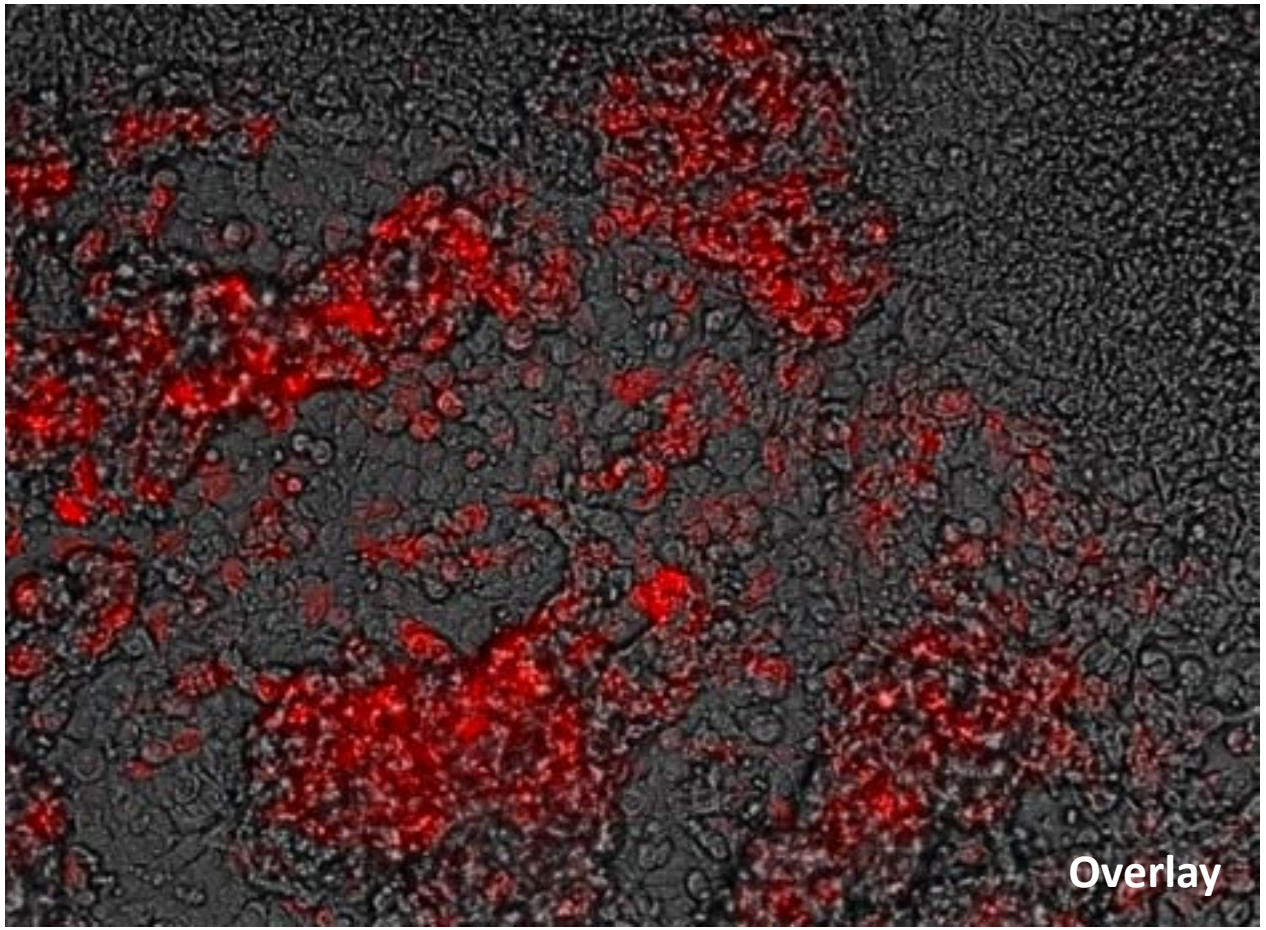


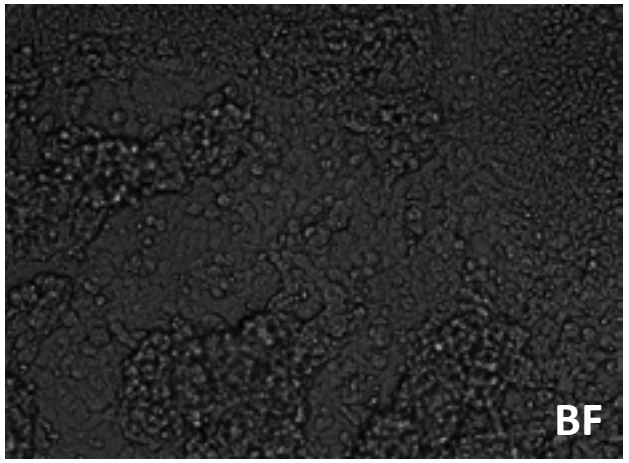
D8

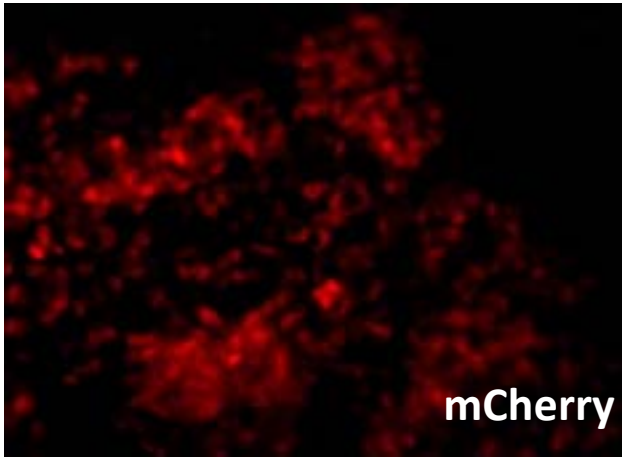


D12

Mesp1-mCherry expressing Cells at Day 3 of Differentiation

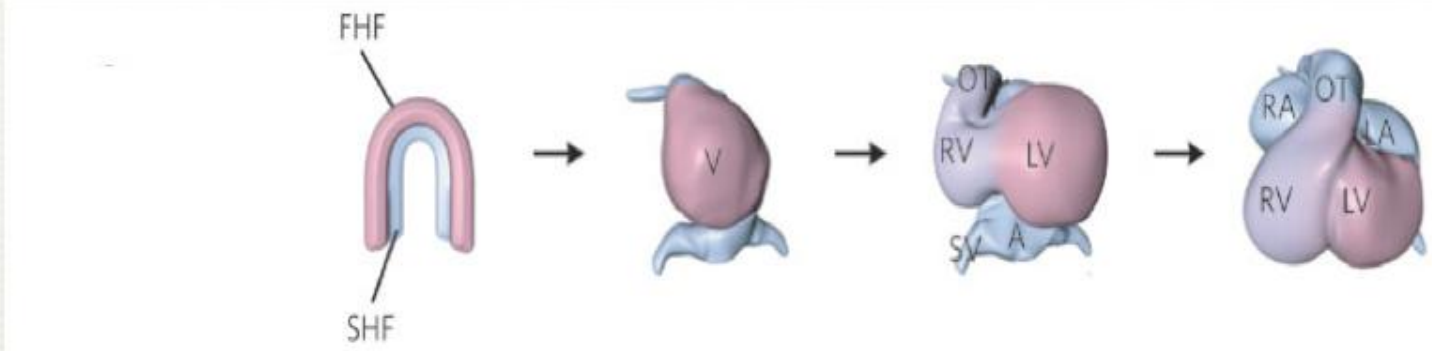






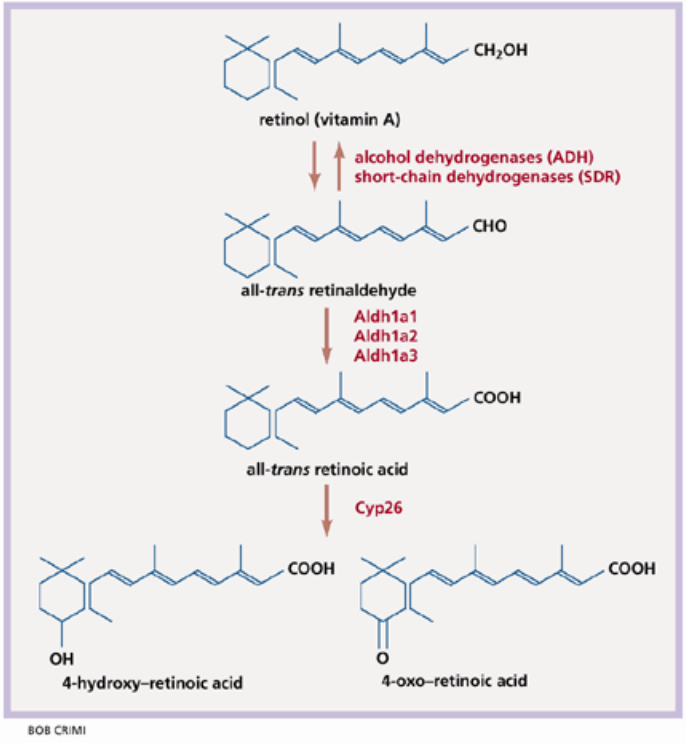
How do we get cardiac subtype populations?
(atrial, ventricular, pacemaker cells)

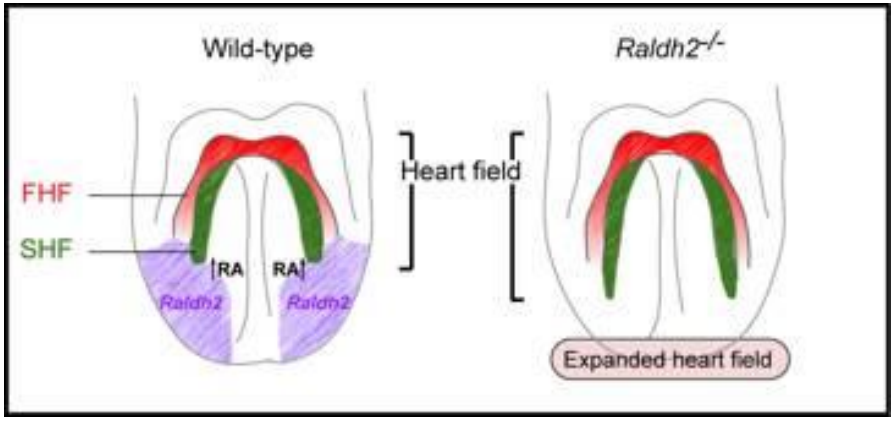
Heart development at a glance



Stage:	Cardiac crescent	Linear heart tube	Looping heart	Chamber formation
Mouse embryo day:	E7.5	E8	E9	E10
Human embryo day:	Day 15	Day 20	Day 28	Day 32
	WNT BMP ACTIVIN/NODAL	SHH WNT NOTCH	RA NODAL LEFTY	NRG RA NOTCH
	MESP1/2 NKX2.5 GATA4	TBX5 MEF2 SRF	PITX2 HAND1 HAND2	HEY1 HEY2 IRX4

Retinoic acid in heart development

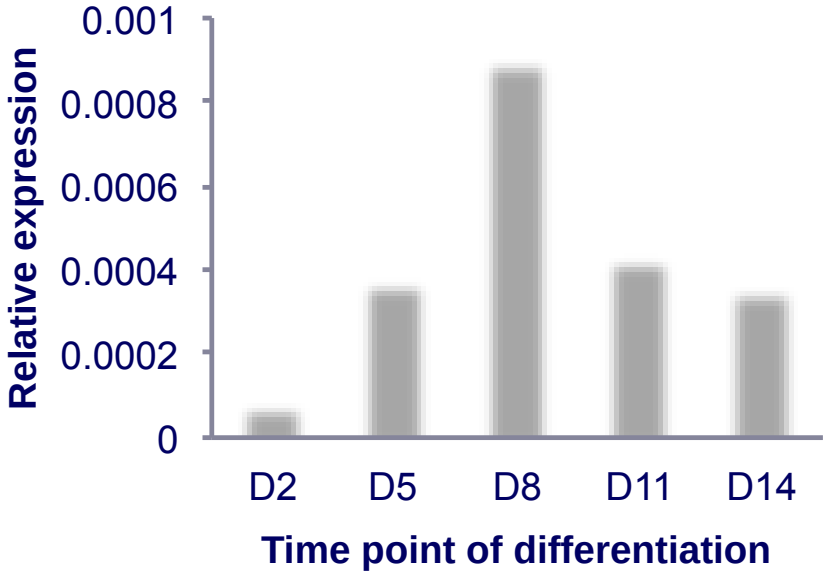


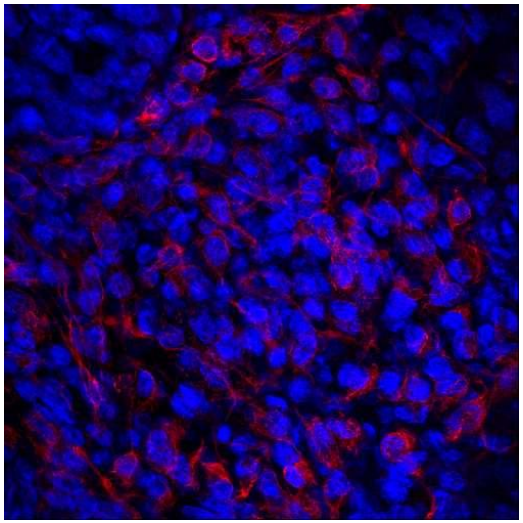


Keegan et al. Science 2005

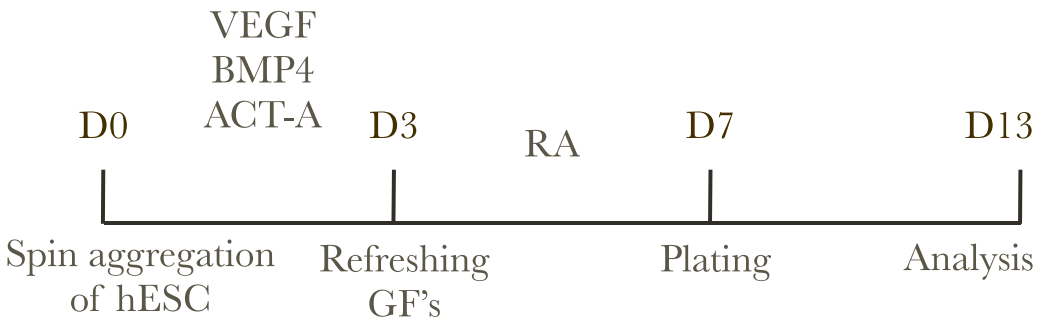
Gassanov et al. Differentiation 2008: RA induces atrial differentiation in mES

Expression of RALDH2 in differentiating hESC-CM

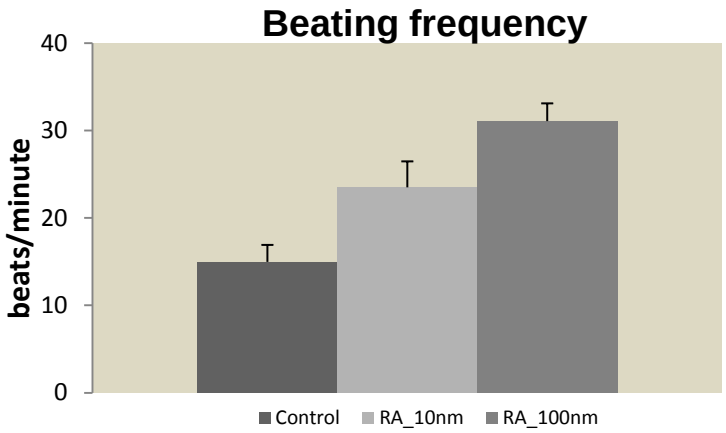




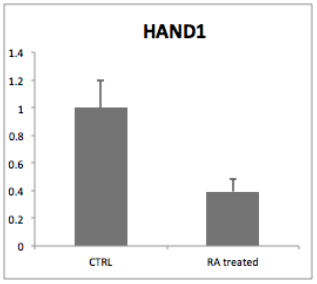
RALDH2
DAPI

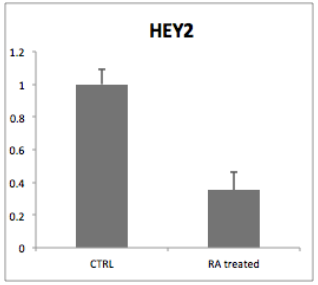


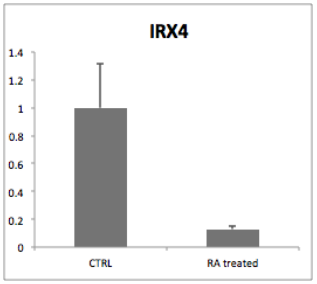
Retinoic acid treatment: shift from ventricular to atrial cells

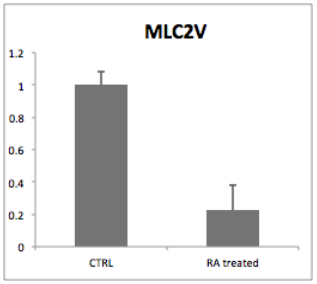


ventricular genes

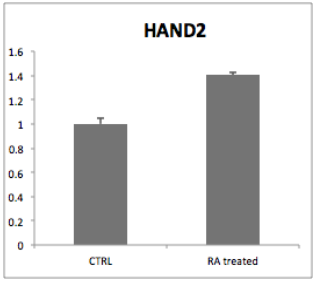


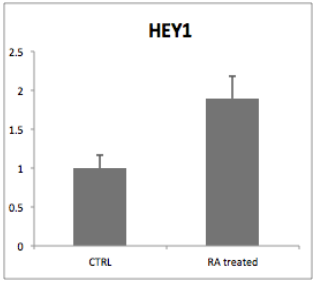


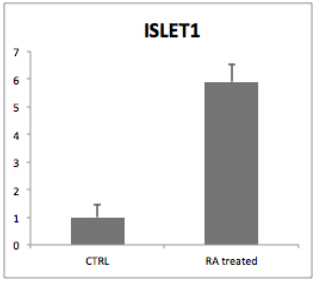


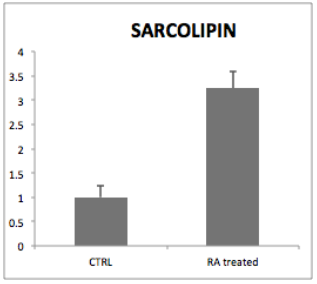


atrial genes

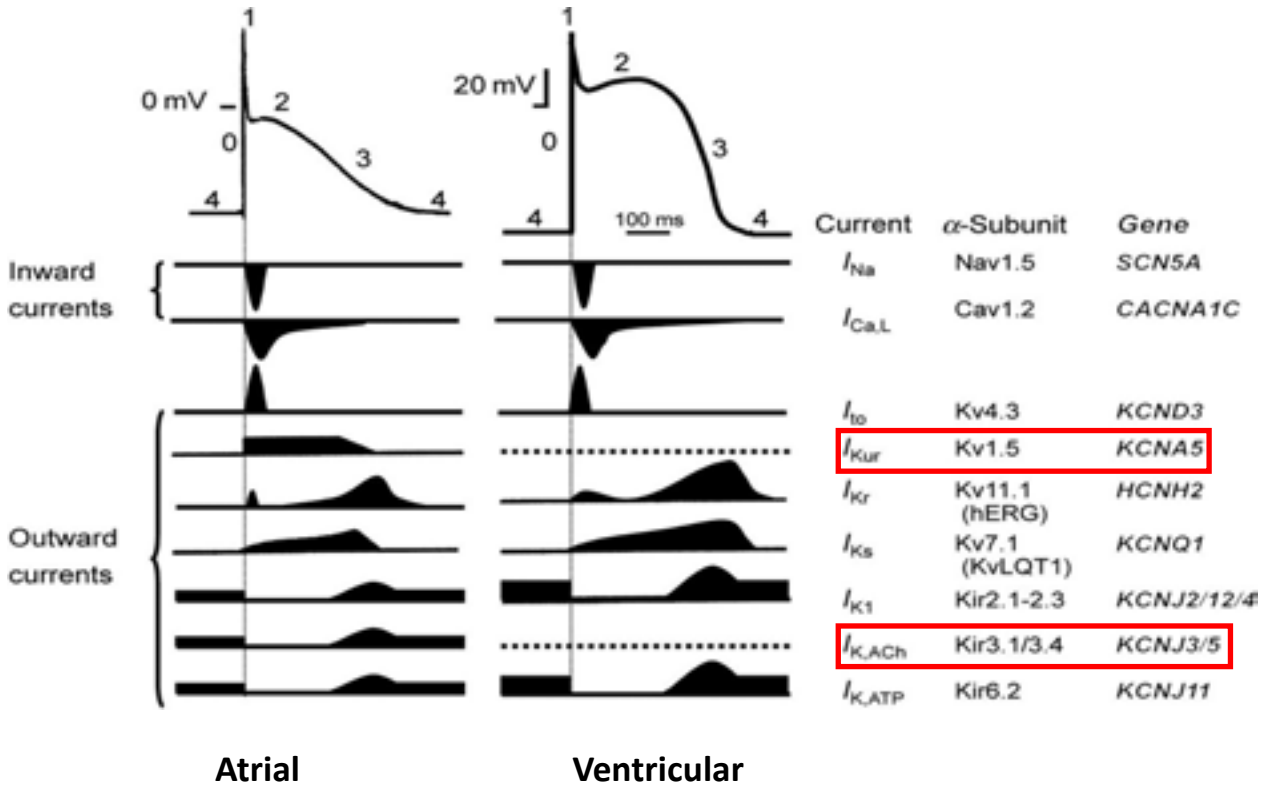




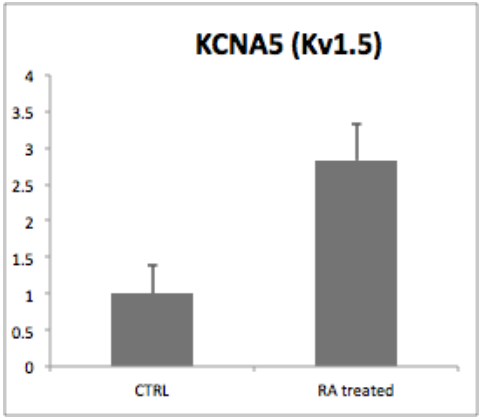


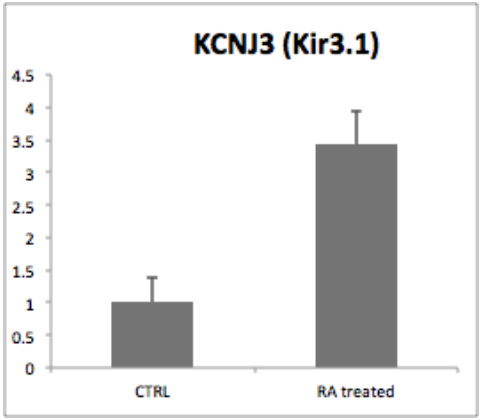


ION CURRENTS CONTRIBUTING TO ATRIAL & VENTRICULAR ACTION POTENTIALS



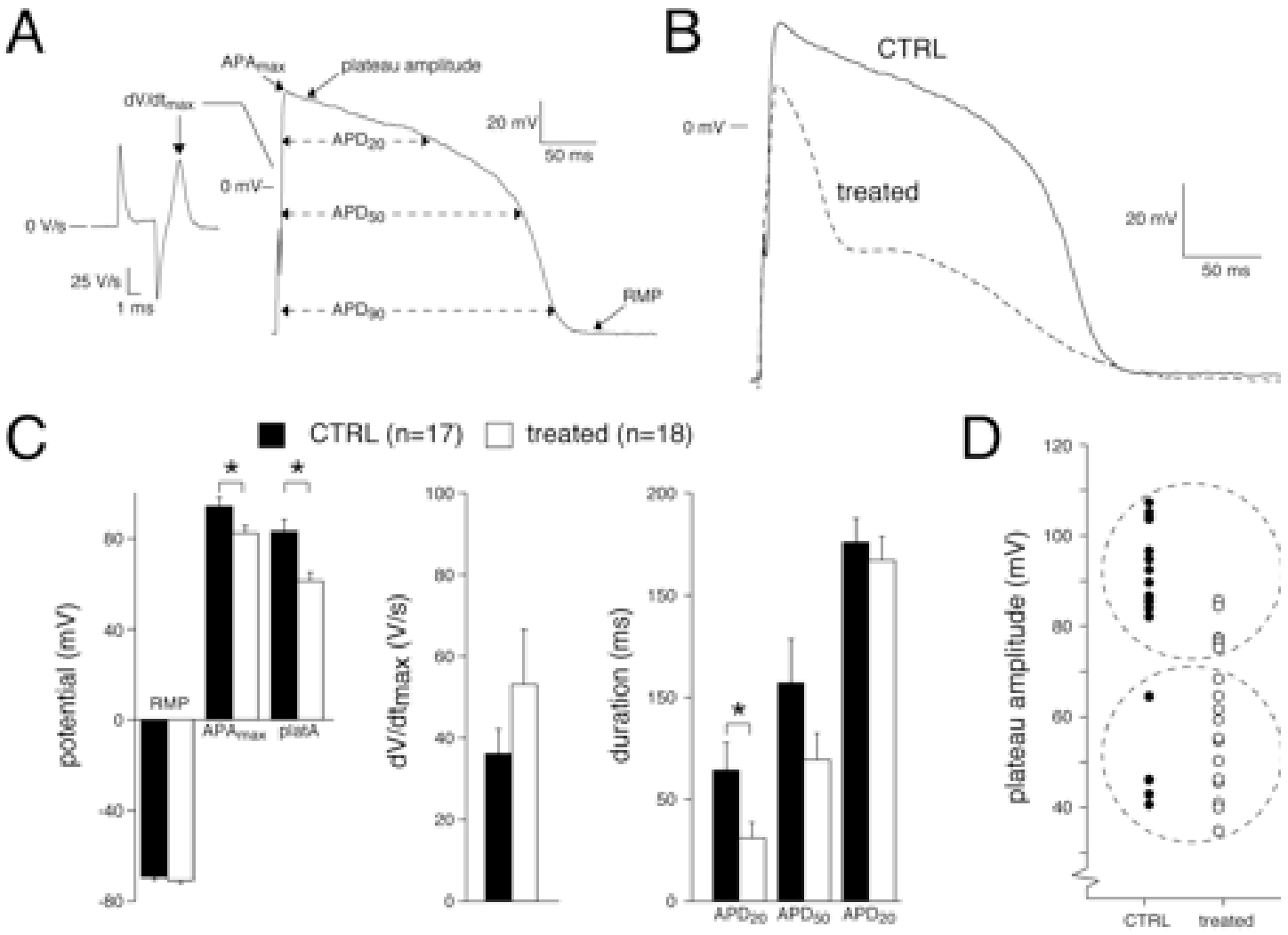
Current	α-Subunit	Gene
I_{Na}	Nav1.5	*SCN5A*
$I_{Ca,L}$	Cav1.2	*CACNA1C*
I_{to}	Kv4.3	*KCND3*
I_{Kur}	Kv1.5	*KCNA5*
I_{Kr}	Kv11.1 (hERG)	*HCNH2*
I_{Ks}	Kv7.1 (KvLQT1)	*KCNQ1*
I_{K1}	Kir2.1-2.3	*KCNJ2/12/4*
$I_{K,ACh}$	Kir3.1/3.4	*KCNJ3/5*
$I_{K,ATP}$	Kir6.2	*KCNJ11*





Supported by Zhang et al. Cell Research 2011: RA signalling affects differentiation of atrial and ventricular cells

Action potential properties of RA treated cardiomyocytes



Conclusions

- Mesenchymal stem cells and cardiac stem cells are promising cell sources for the treatment of cardiac disease.
  - Transplantation, tissue engineering, endogenous activation
- Human pluripotent stem cells for transplantation: tissue engineering using mixtures of cardiac cells from defined differentiation cultures will be the next step
- Genetic cardiac reporter lines faithfully recapitulate the “in vivo” lineage
  - Molecular mechanisms for expansion and differentiation can be studied
- Refined protocols enable cardiac subtype specification (retionic acid → atrial CMs)
  - Advantageous for tissue engineering, drug screening, disease modeling

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