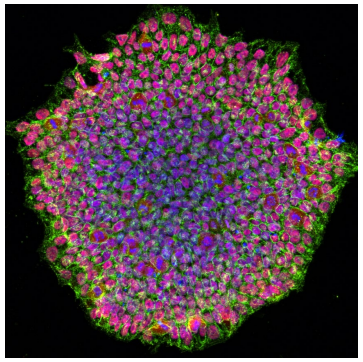
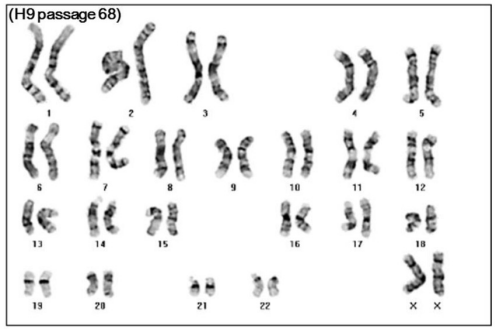
Developmental Biology of iPSC-derived Cardiomyocytes

Human Pluripotent Stem Cells

- Indefinite self-renewal

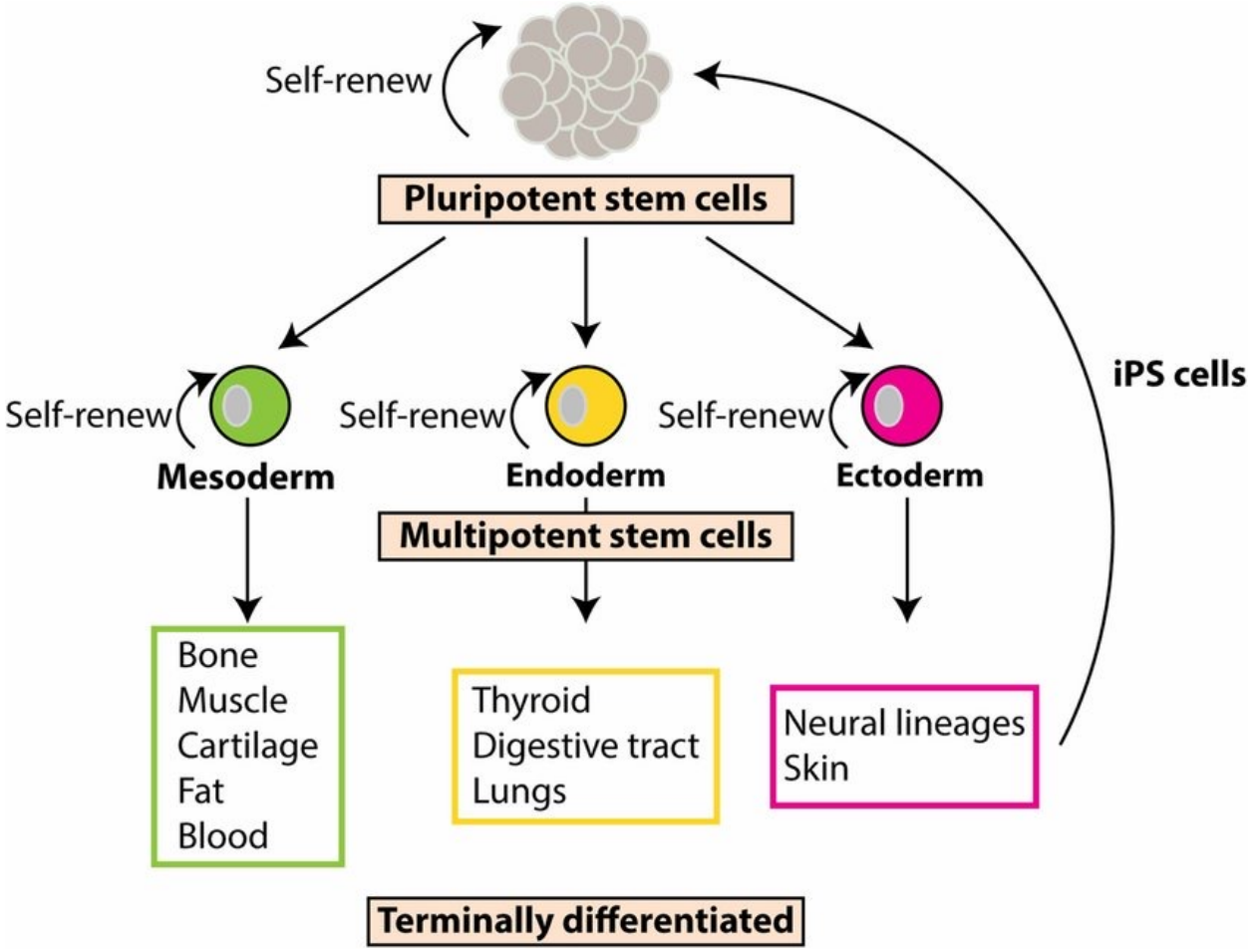


OCT4, SSEA4



Karyotype analysis

- Capability to derive tissues of all three germ lineages



Human Pluripotent Stem Cells

Embryonic Stem Cell Lines Derived from Human Blastocysts

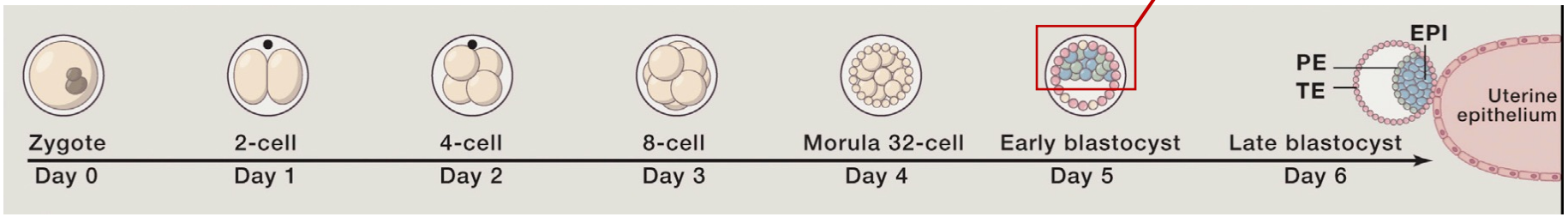
JAMES A. THOMSON, JOSEPH ITSKOVITZ-ELDOR, SANDER S. SHAPIRO, MICHELLE A. WAKNITZ, JENNIFER J. SWIERGIEL, VIVIENNE S. MARSHALL, AND JEFFREY M. JONES

Authors Info & Affiliations

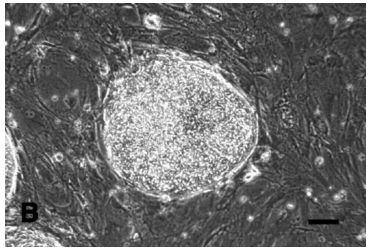
SCIENCE • 6 Nov 1998 • Vol 282, Issue 5391 • pp. 1145-1147 • DOI: 10.1126/science.282.5391.1145

1998

Inner Cell Mass



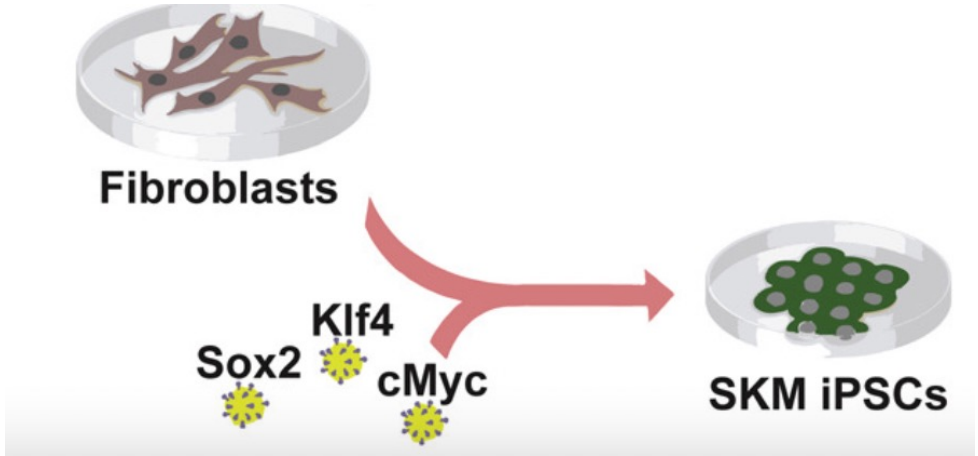
First derivation of hESCs

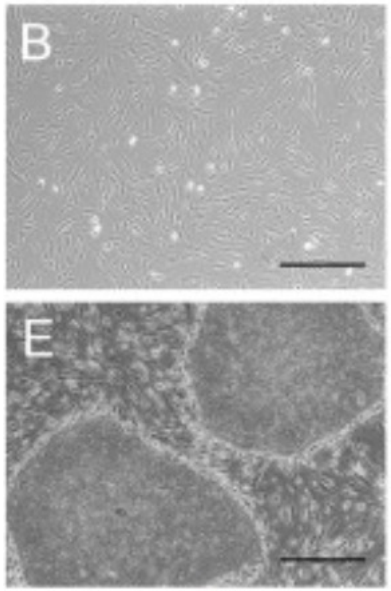


Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

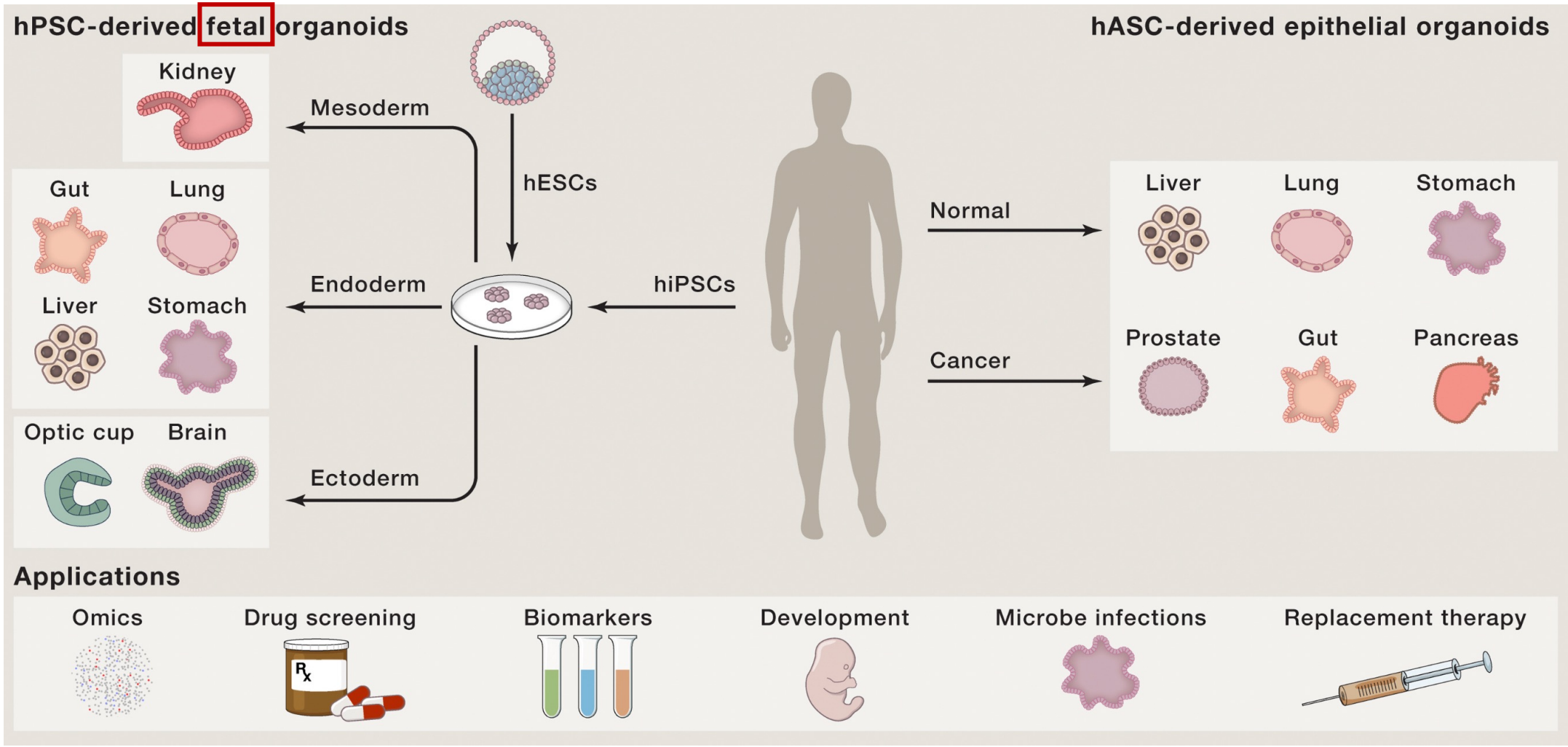
2007

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]



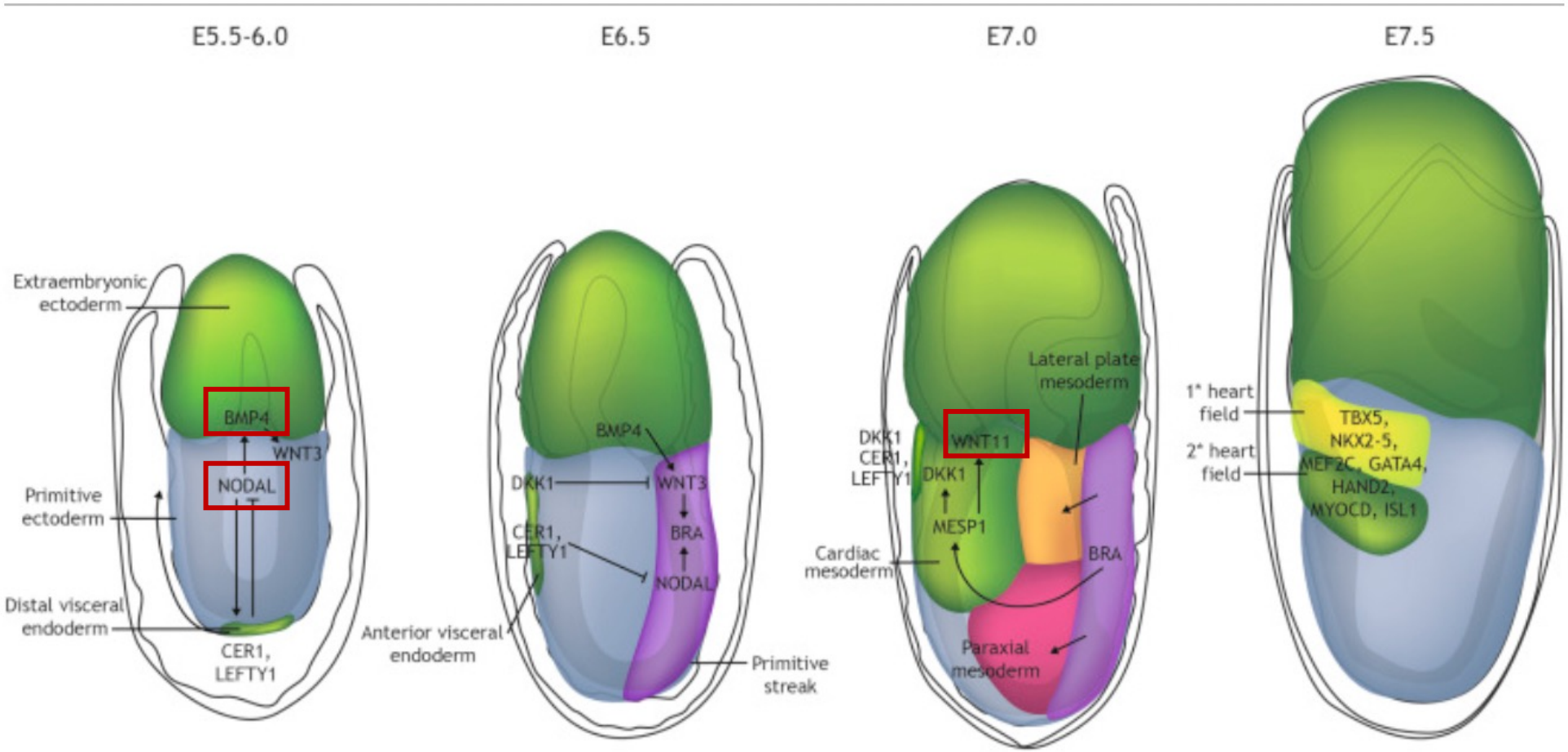


Modeling human development and disease



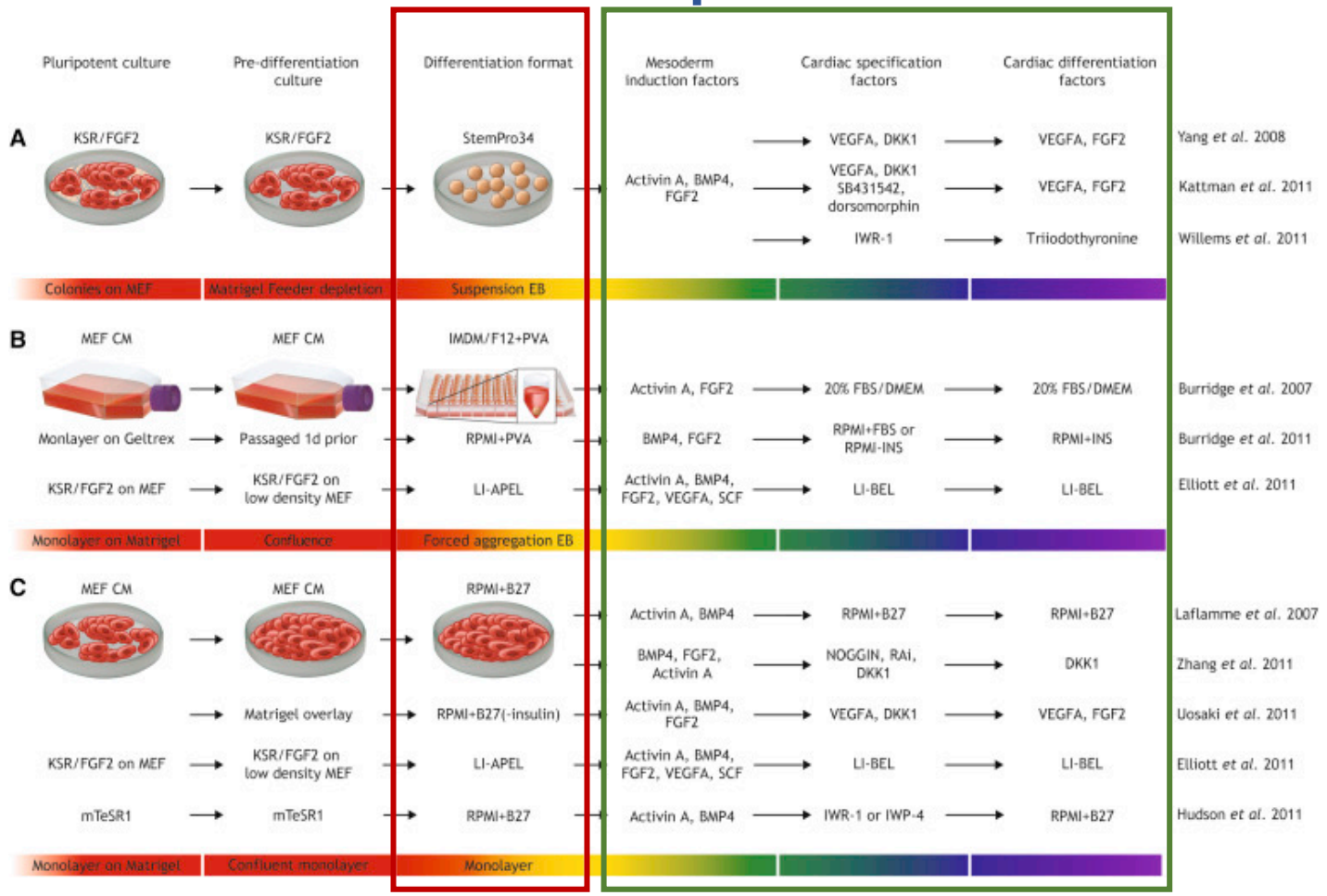
Wu et al. 2016

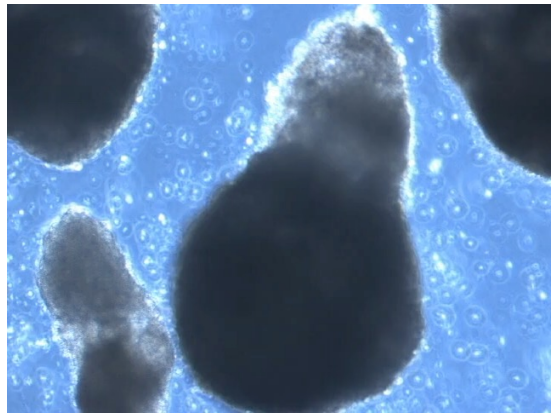
Cardiac Embryonic Development

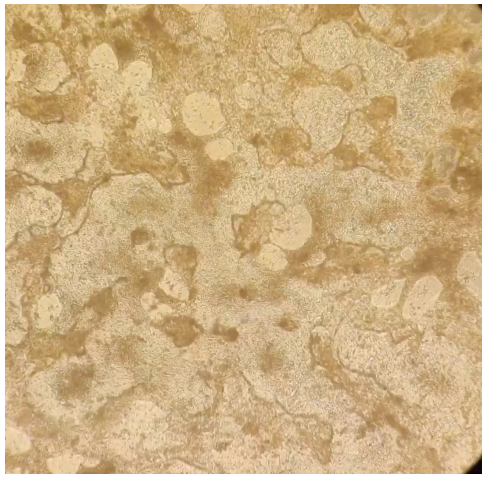


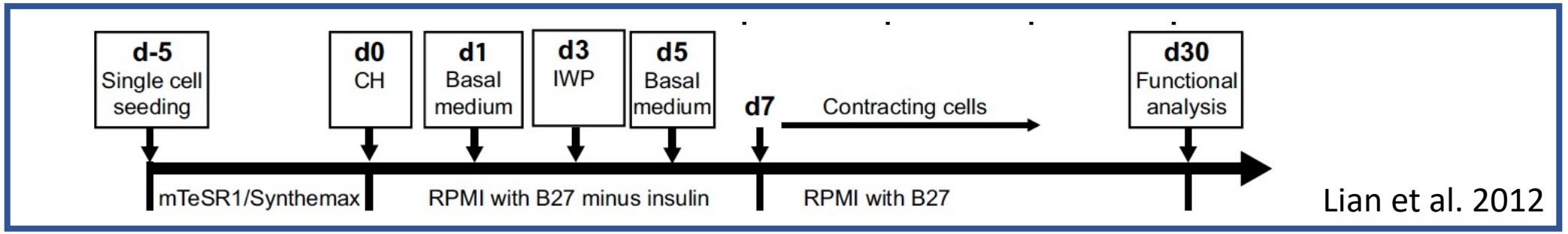
Burridge et al. 2012

Methods for the Differentiation of Human Pluripotent Stem Cells





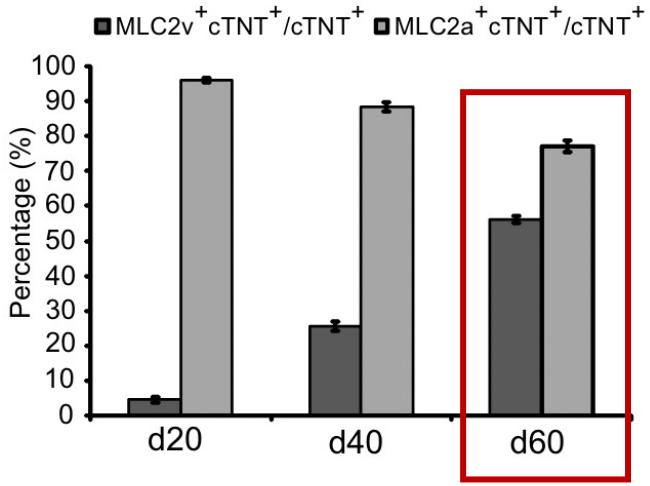




Lian et al. 2012

Structural and functional characterization of cardiomyocytes

- Molecular Level

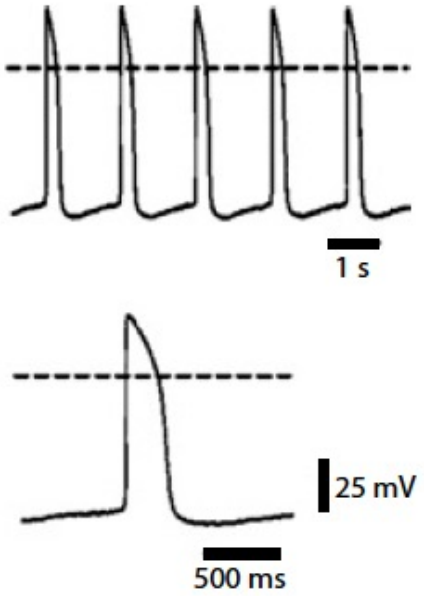


- MLC2V: mature ventricular cardiomyocyte marker.
- MLC2A: Atrial and immature ventricular cardiomyocyte marker.

- Physiological Level

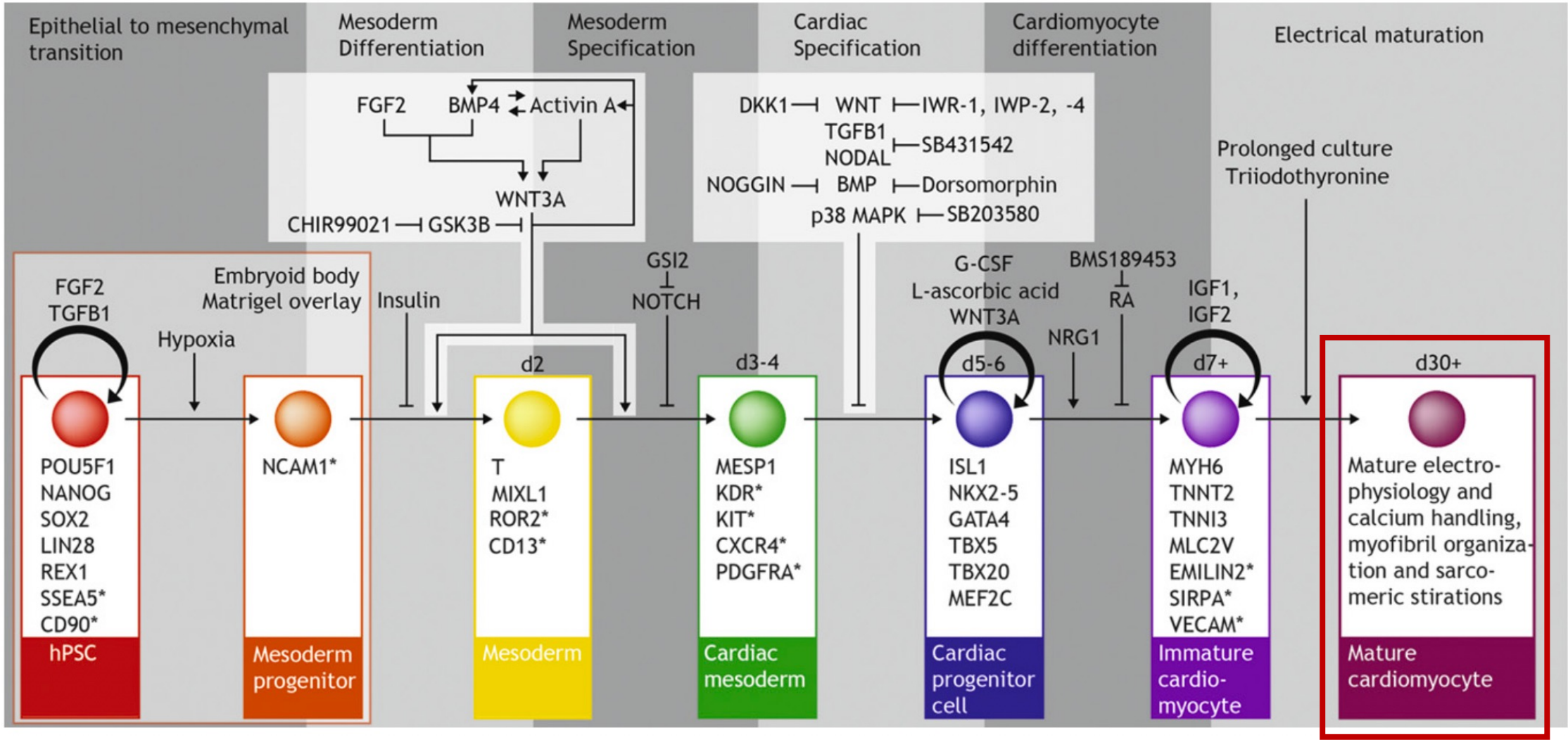
Spontaneously contracting cardiomyocytes:

- Ventricular-like action potential morphology (32/35, 91.5%).
- Atrial-like action potentials were observed less commonly (3/35, 8.5%).
- nodal-like action potentials were not observed (0/35, 0%).



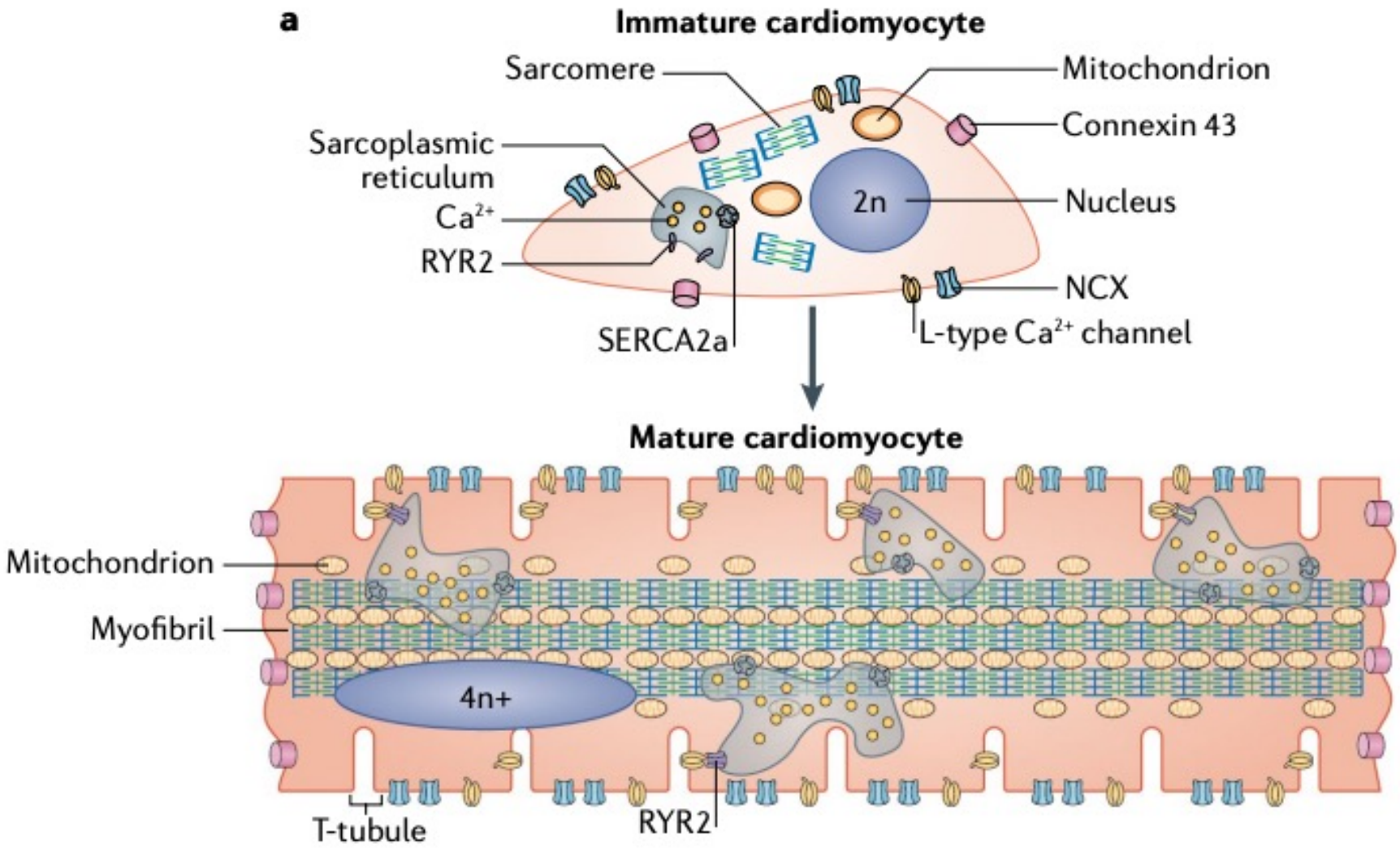
Lian et al. 2012

Six major steps of hPSC cardiac differentiation



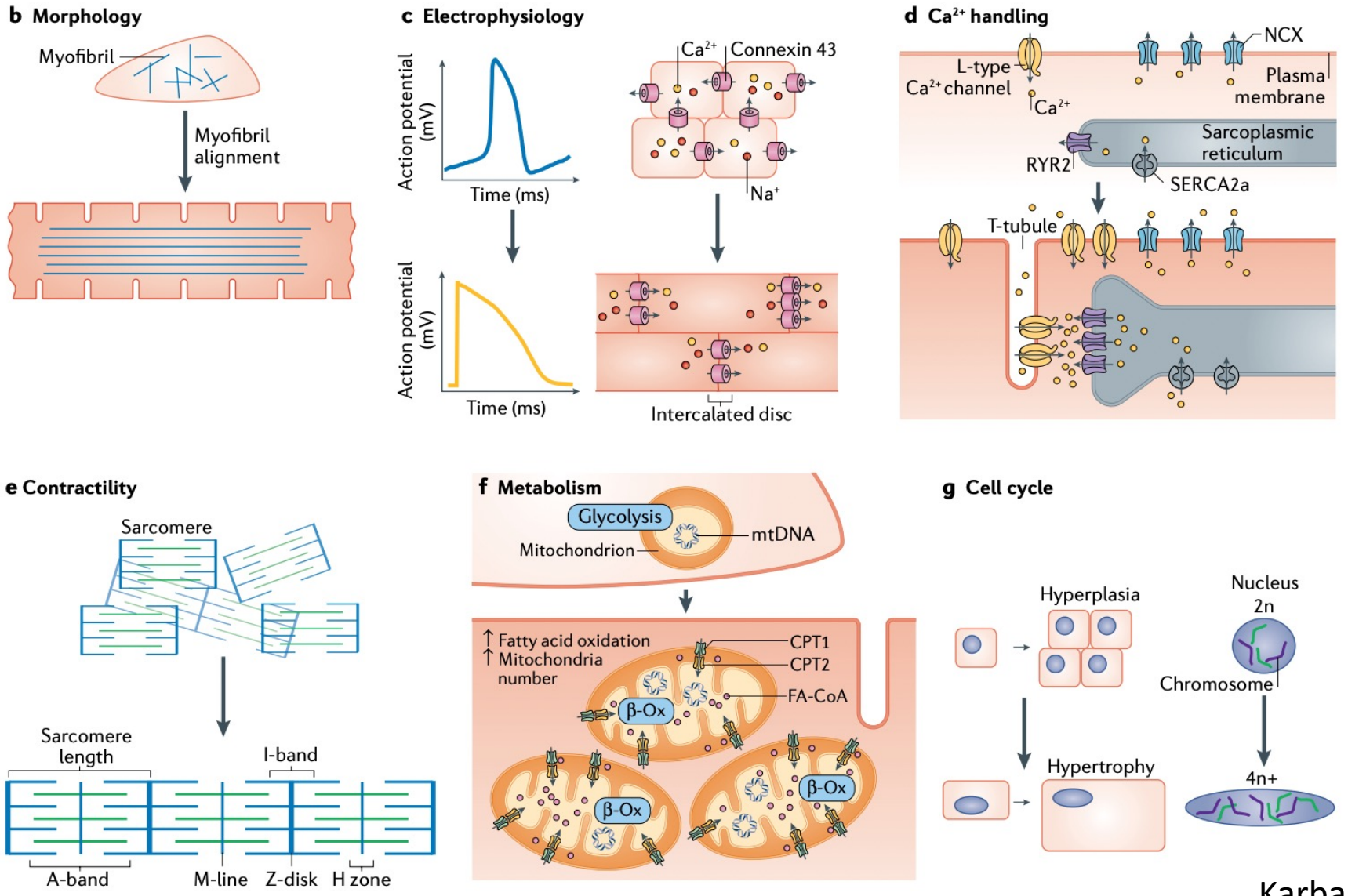
Burridge et al. 2012

Cardiomyocyte maturation



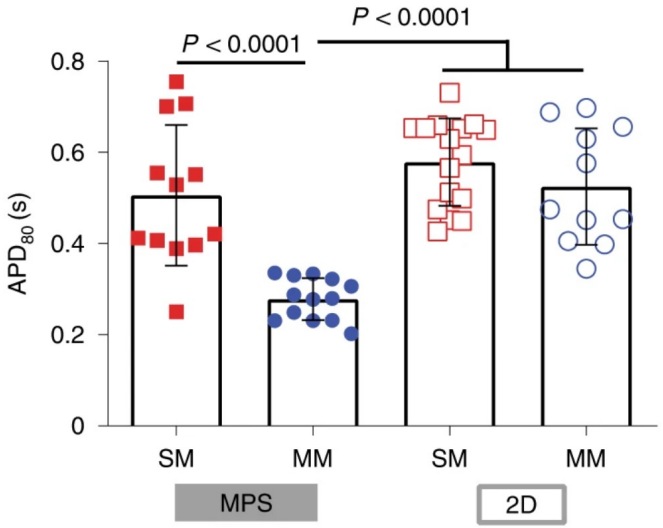
Karbassi et al. 2020

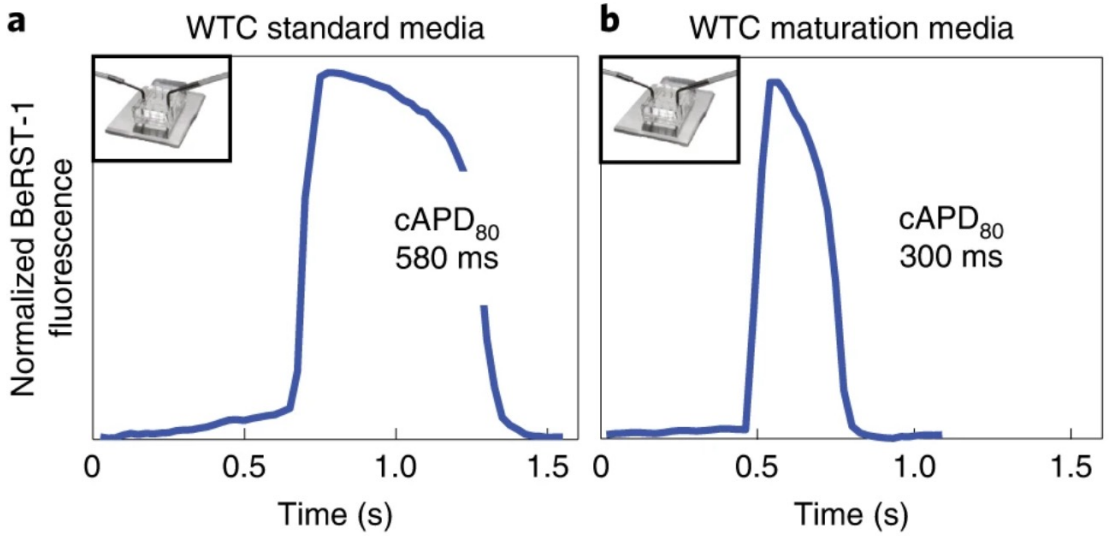
Cardiomyocyte maturation features



Karbassi et al. 2020

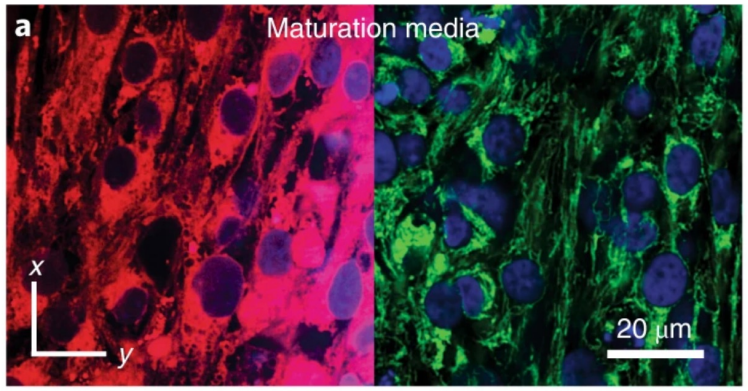
Metabolically driven maturation of human-induced-pluripotent-stem-cell-derived cardiac microtissues on microfluidic chips

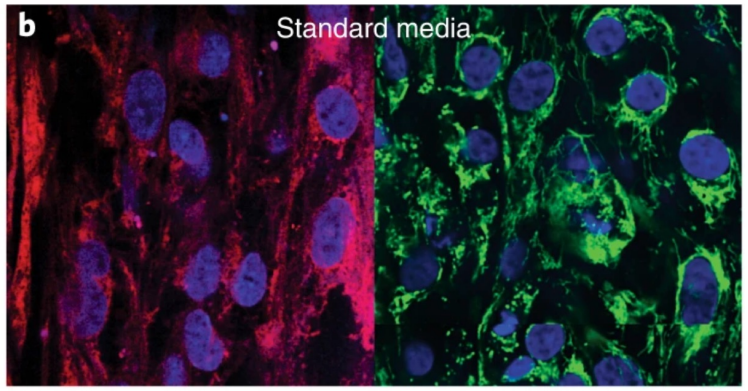




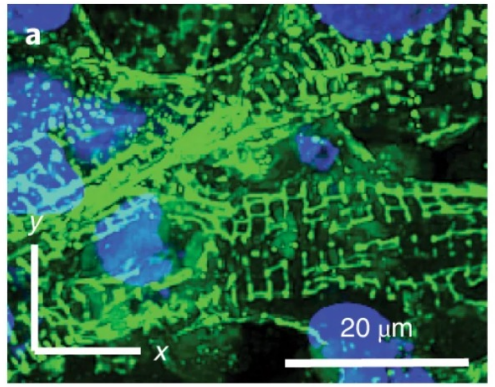
Metabolic switch

Sarcomeric α-actinin

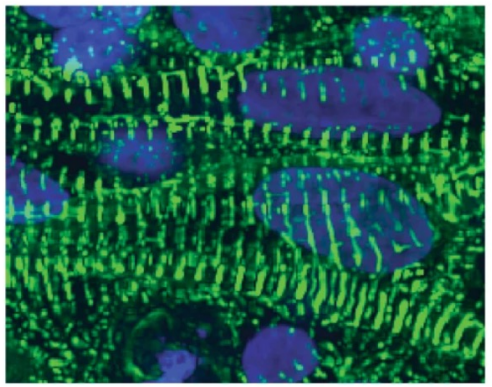




Mitotracker, Mitochondrial antibody, DRAQ5



Standard media



Maturation media

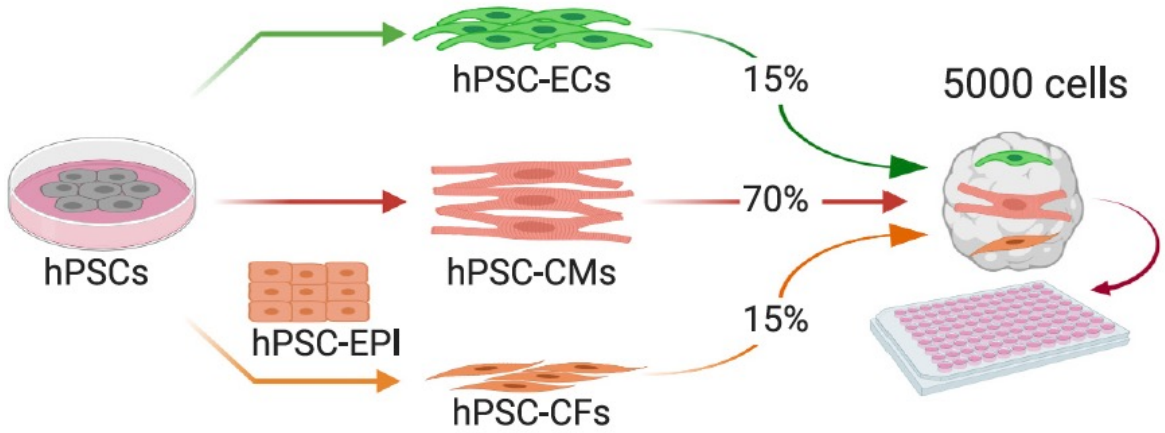
Huebsch et al. 2022

Other strategies to mature hPSC-CM

Article

Human-iPSC-Derived Cardiac Stromal Cells Enhance Maturation in 3D Cardiac Microtissues and Reveal Non-cardiomyocyte Contributions to Heart Disease

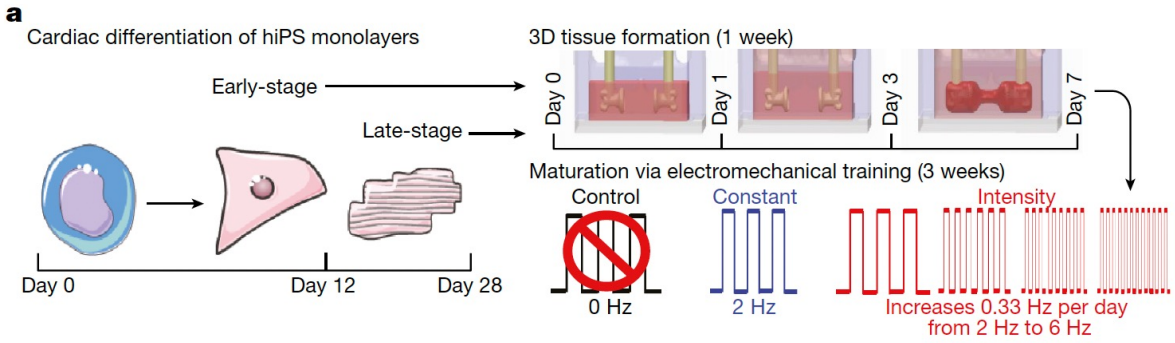
Enhanced Maturation of Human PSC-derived Cardiomyocytes in 3D Cardiac Microtissues



Giacomelli et al. 2020

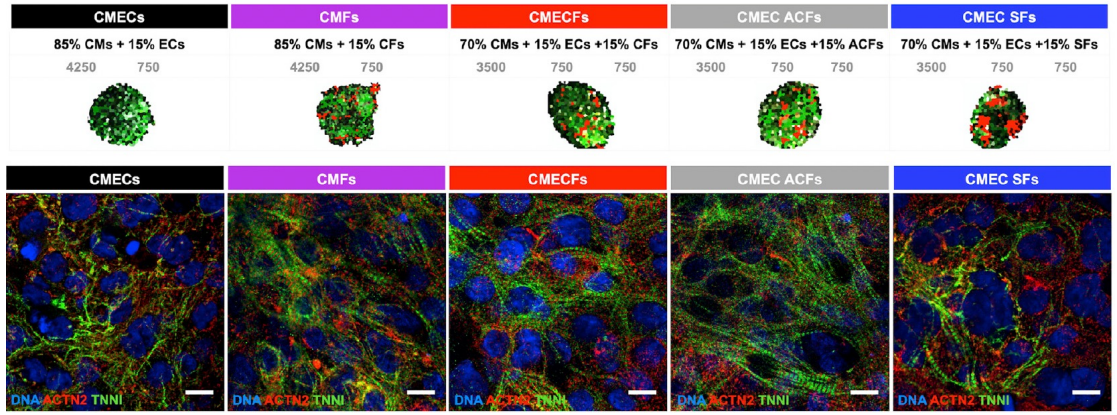
Advanced maturation of human cardiac tissue grown from pluripotent stem cells

Kacey Ronaldson-Bouchard[1], Stephen P. Ma[1], Keith Yeager[1], Timothy Chen[1], LouJin Song[2], Dario Sirabella[1], Kumi Morikawa[2], Diogo Teles[1,3,4], Masayuki Yazawa[2] & Gordana Vunjak-Novakovic[1,5]*

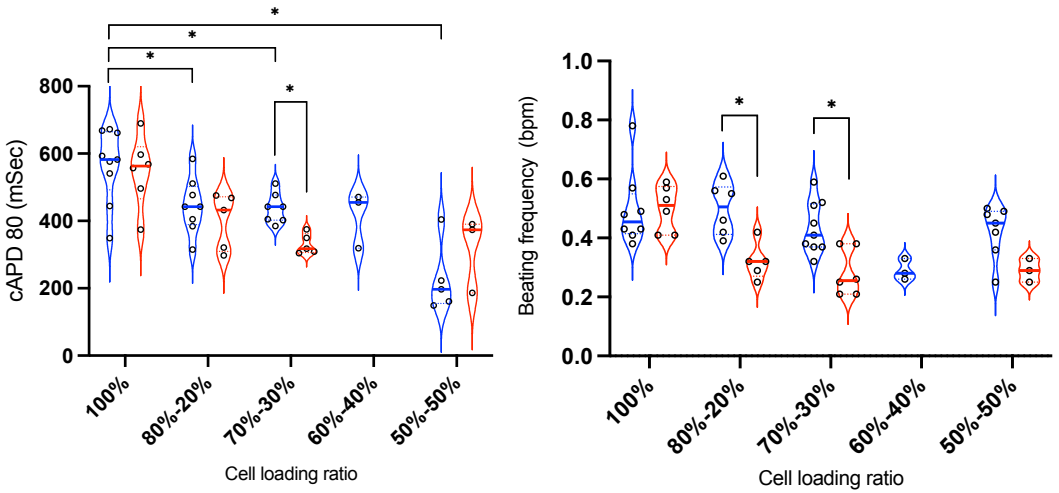


Ronaldson-Bouchard et al. 2018

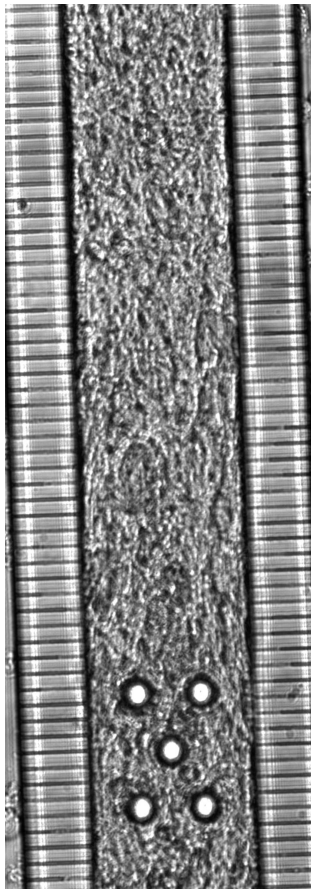
Enhancing cardiac maturation by adding human cardiac fibroblasts into the cardiac MPS



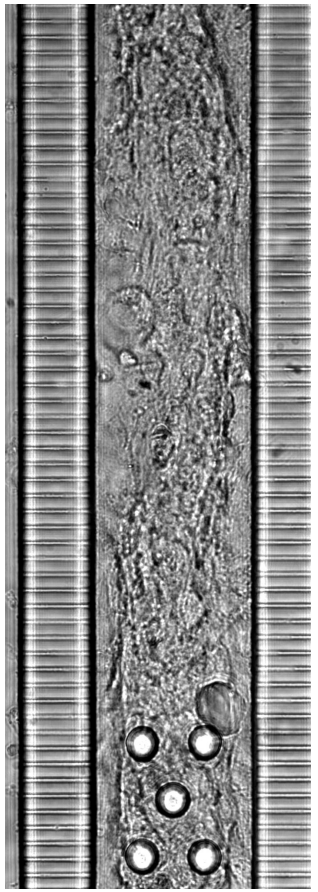
Giacomelli et al. 2020



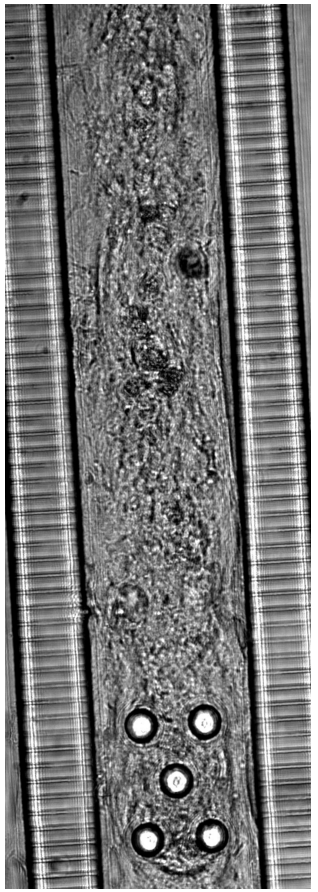
100% iCM



70% iCM – 30% HCF



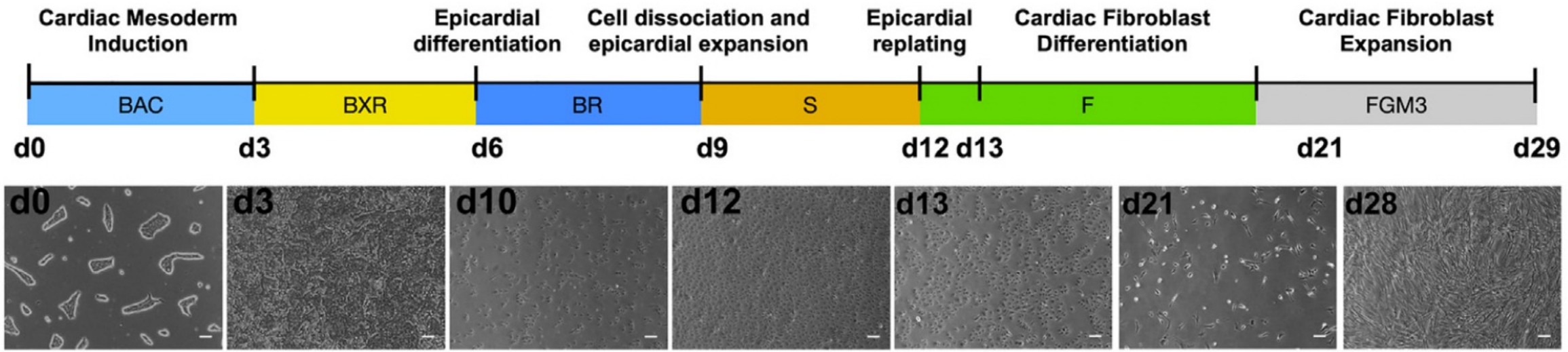
50 iCM – 50% HCF



Challenges of iso-genic multiculture microtissues

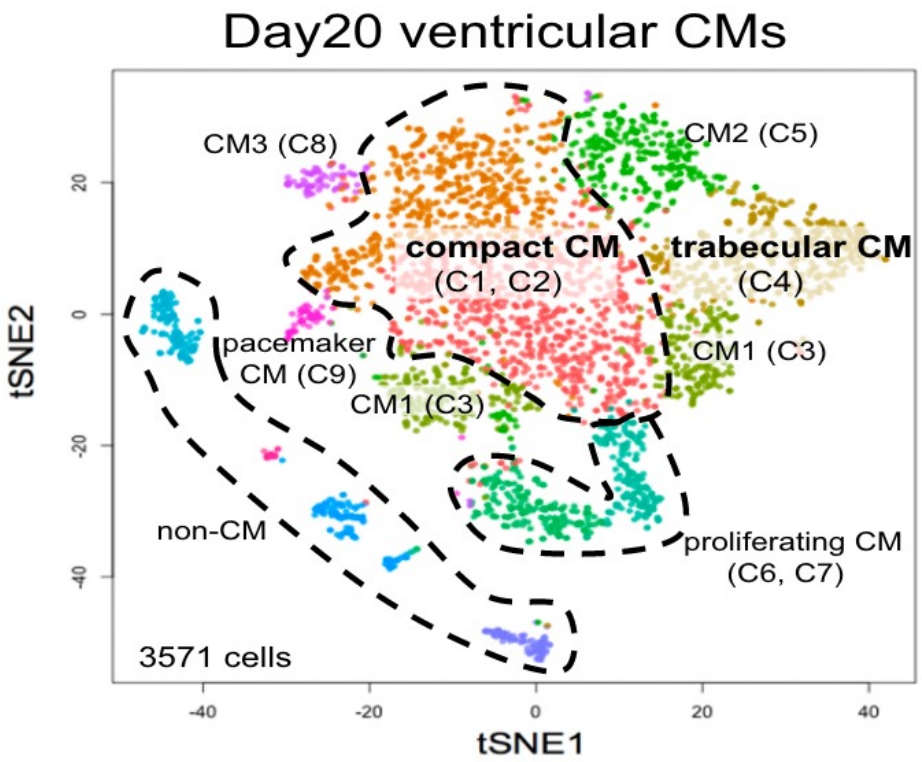
- Cardiac Endothelial Differentiation

- Cardiac Fibroblast Differentiation



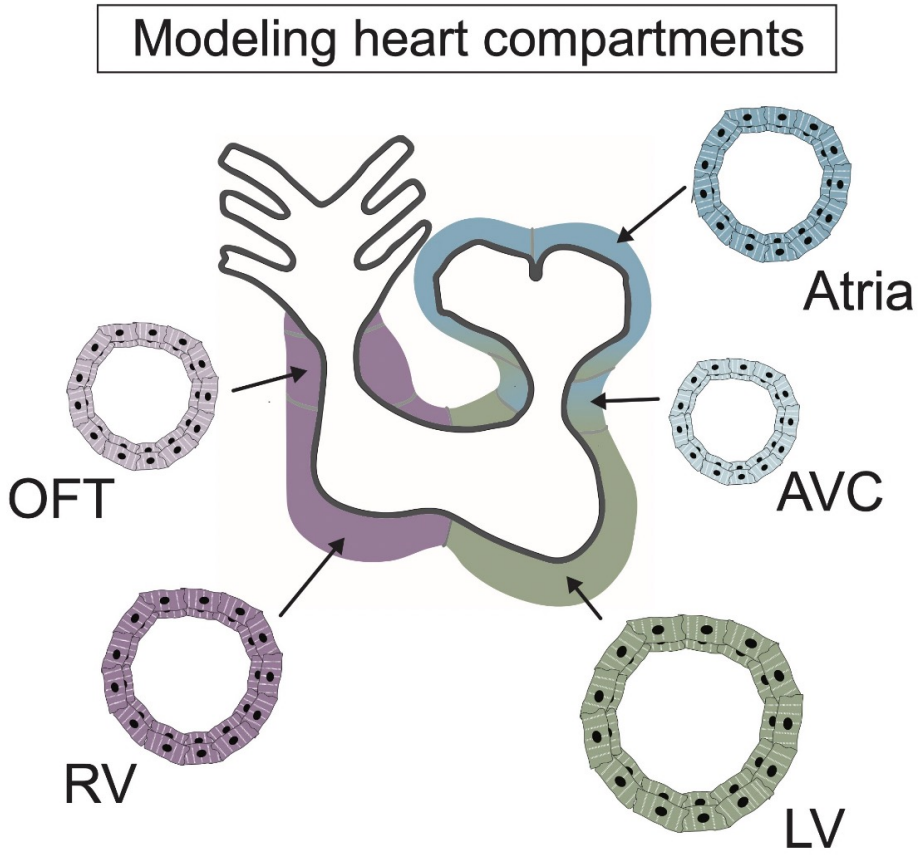
Differentiation to Ventricular and Atrial Cardiomyocytes

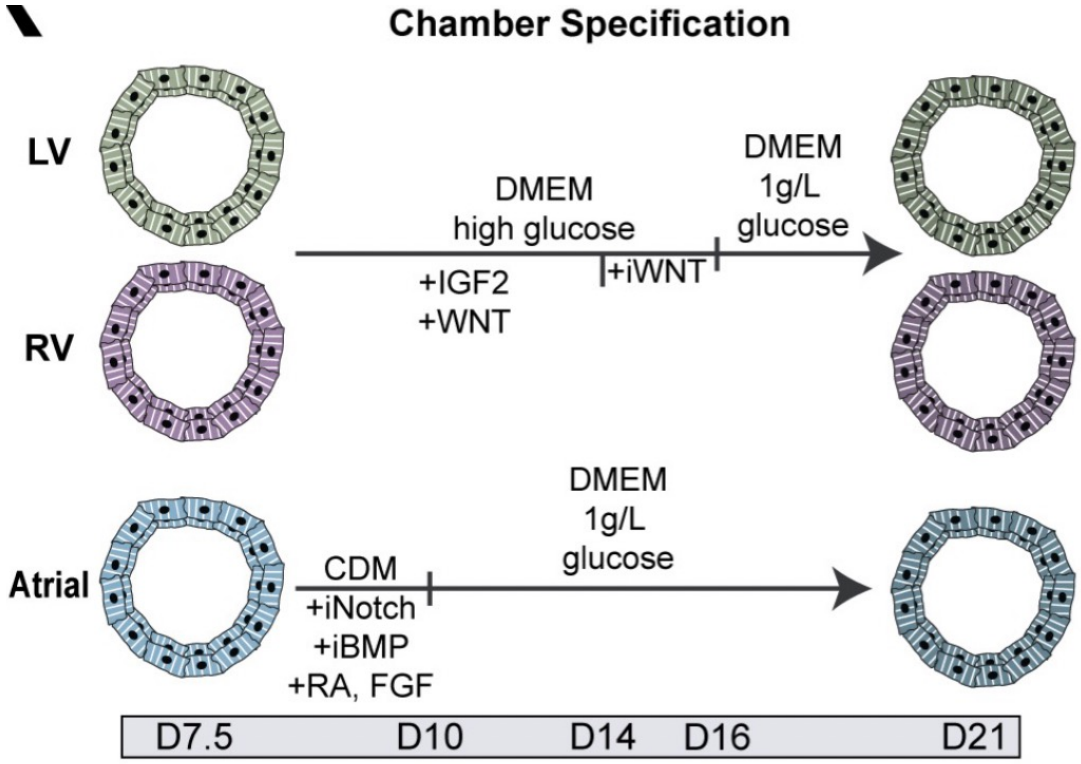
Cell Type	Differentiation	Characterization
Cardiomyocyte		
Ventricular CM *Yang et al., Nature (2008)* *Kattman et al., Cell Stem Cell (2011)* *Lee & Protze et al., Cell Stem Cell (2017)* *Lian et al., PNAS (2012)* *Burridge et al., Nat Methods (2014)* *Zhang et al., Cell Stem Cell (2019)*	hPSCs d1 → high Nodal BMP FGF → CD235a⁺PDGFRα⁺ d3 → WNT → d5 → VEGF → d7 → cTNT⁺SIRPα⁺ MLC2v⁺CD90⁻ d20 d1 → WNT → d3 → WNT → d5 → d7 → cTNT⁺MLC2v⁺ d20 d5 → TBX5⁺ → d7 → LV	**Marker Genes** *TNNT2⁺ MYL2⁺ IRX4⁺* **Electrophysiology** Vmax > 10 V/s APD30/90 ≥ 0.3 I_f low I_{KACH} low I_{Na} high I_{Kur} low Carbachol: no effect on APD Vernakalant: no effect on APD Conduction velocity: fast
Atrial CM *Zhang et al., Cell Res (2011)* *Devalla et al., EMBO Mol (2015)* *Lee & Protze et al., Cell Stem Cell (2017)*	hPSCs d1 → low Nodal BMP FGF → RALDH2⁺PDGFRα⁺ d3 → RA WNT → d5 → d12 → cTNT⁺SIRPα⁺ MLC2v⁺CD90⁻ d20	**Marker Genes** *TNNT2⁺ NPPA⁺ KCNJ3⁺* *NR2F2⁺ SLN⁺ CACNA1D⁺* *TBX5⁺ MYL7⁺ KCNA5⁺* **Electrophysiology** Vmax > 10 V/s APD30/90 < 0.3 I_f low I_{KACH} high I_{Na} high I_{Kur} high Carbachol: shortens APD Vernakalant: prolongs APD Conduction velocity: fast
Sinoatrial CM *Birket et al., Nat Biotechnol (2015)* *Protze et al., Nat Biotechnol (2017)*	hPSCs PDGFRα⁺ d1 → Nodal BMP WNT VEGF SCF → d3 → d4.75 → MYC → d6 → Nodal → MYC IGF Hh → d12 → FGF Nodal BMP → PDGFRα⁺ Podoplanin⁺ → Hh FGF BMP TGFβ WNT IGF T3 DEX → d20 → cTNT⁺NKX2-5⁻ Podoplanin⁺ hPSCs d1 → low Nodal → RALDH2⁺ PDGFRα⁺ d3 → RA BMP FGF Nodal → d5 → VEGF → d12 → cTNT⁺SIRPα⁺ MLC2v⁻CD90⁻NKX2-5⁻ d20	**Marker Genes** *NKX2-5⁻ SHOX2⁺ HCN4⁺* *TBX3⁺ ISL1⁺ KCNJ3⁺* *TBX18⁺ COUPTFII⁺* **Electrophysiology** Vmax ≤10 V/s I_f high I_{KACH} high I_{Na} low I_{Kur} high Conduction velocity: slow



Protze et al. 2019

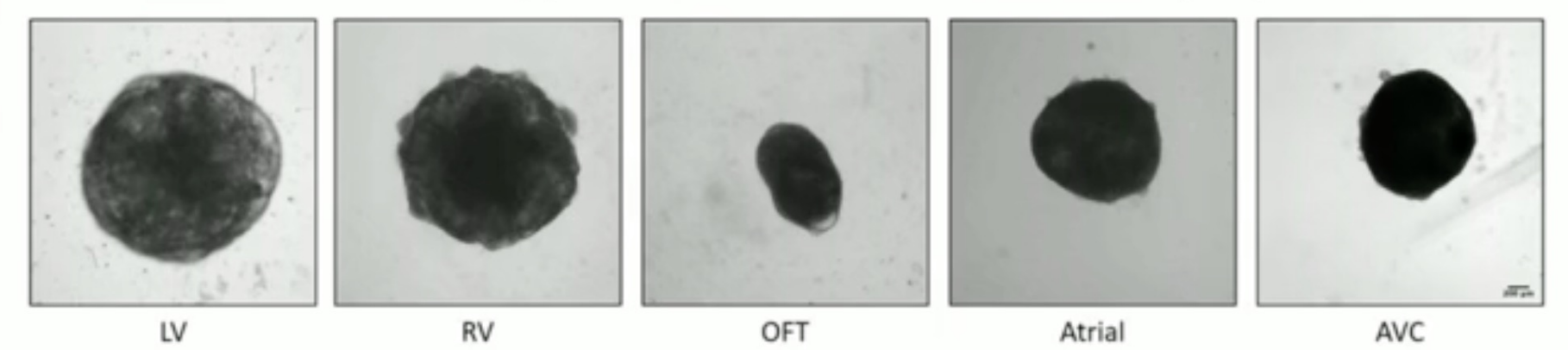
Modeling heart compartments

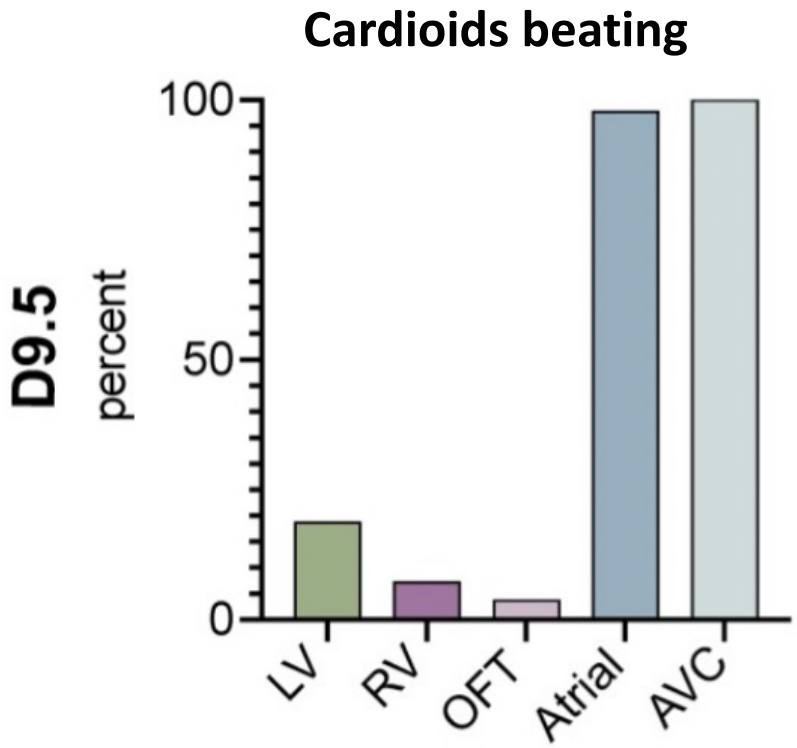


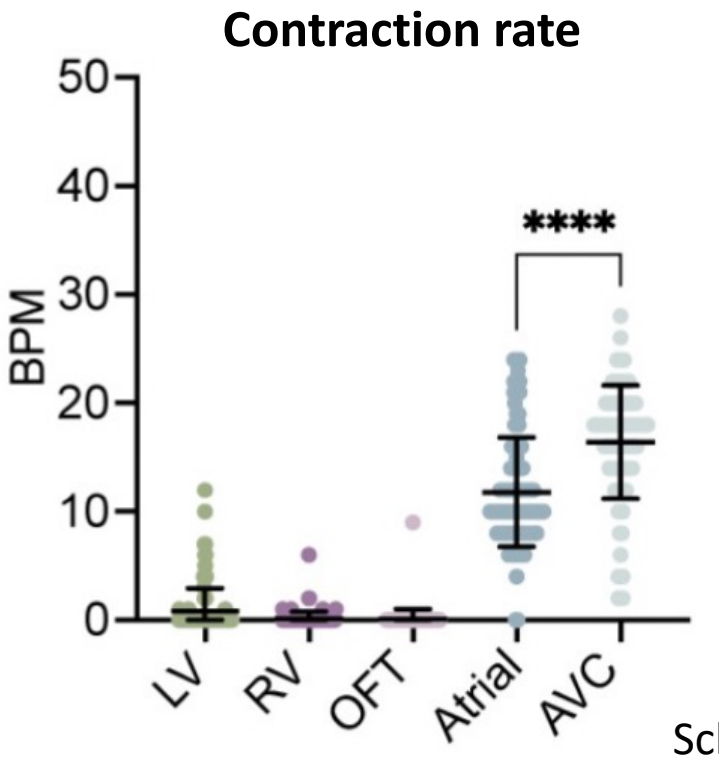


Schimdt, et al. 2022. Preprint.

Cardiac subtypes have different contraction properties

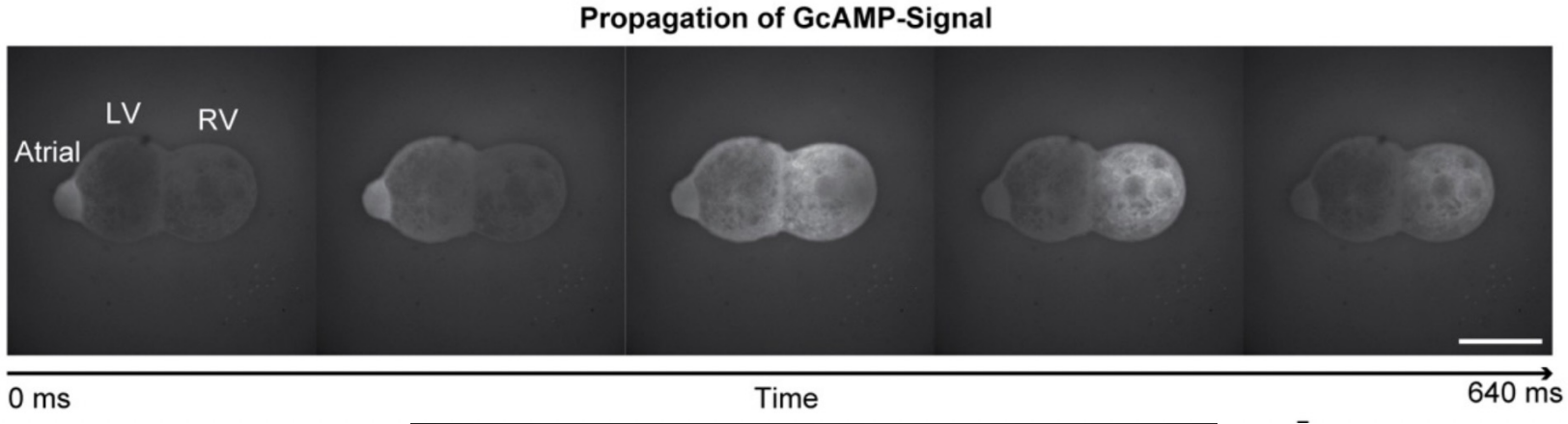


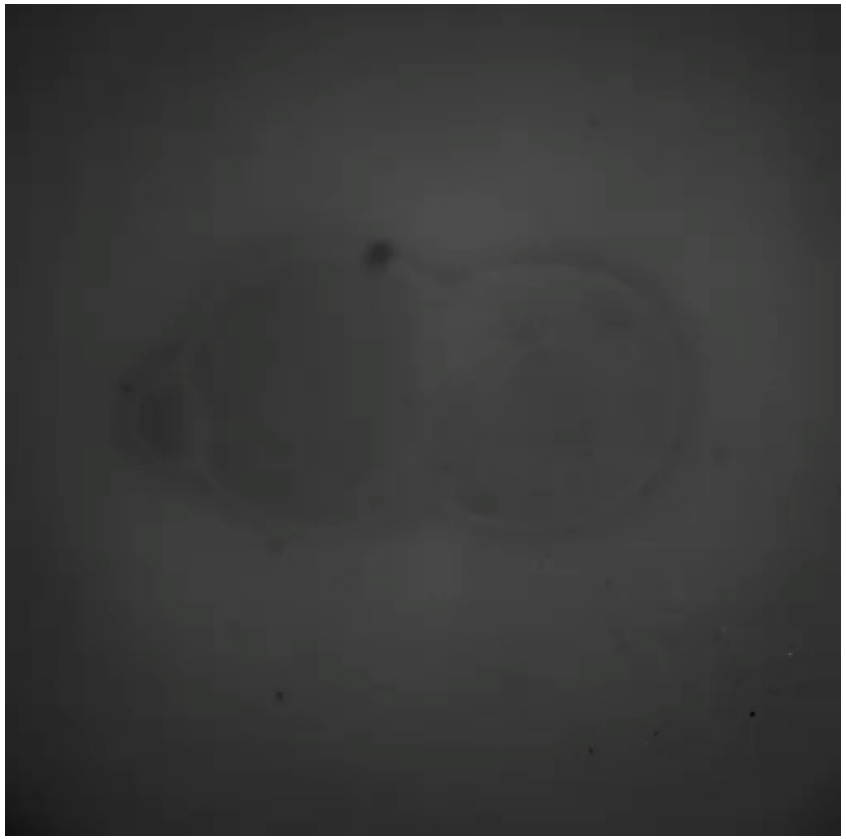




Schimdt, et al. 2022. Preprint.

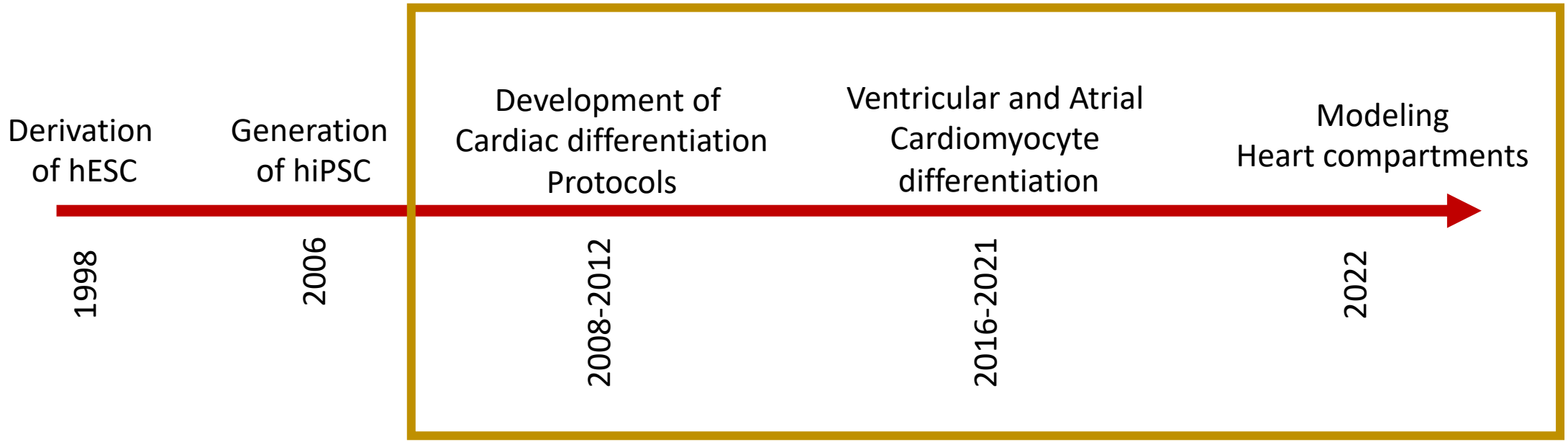
3-chambered cardioid showing the direction of signal propagation





Schimdt, et al. 2022. Preprint.

Optimization of the cardiomyocyte maturation



Overcome the issues of modeling human diseases in mice resulting from species differences in heart physiology

Organ-on-a-chip systems for biomedical research