Organ-on-a-chip and organoids to model the cardiac pathophysiology

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What are stem cells?

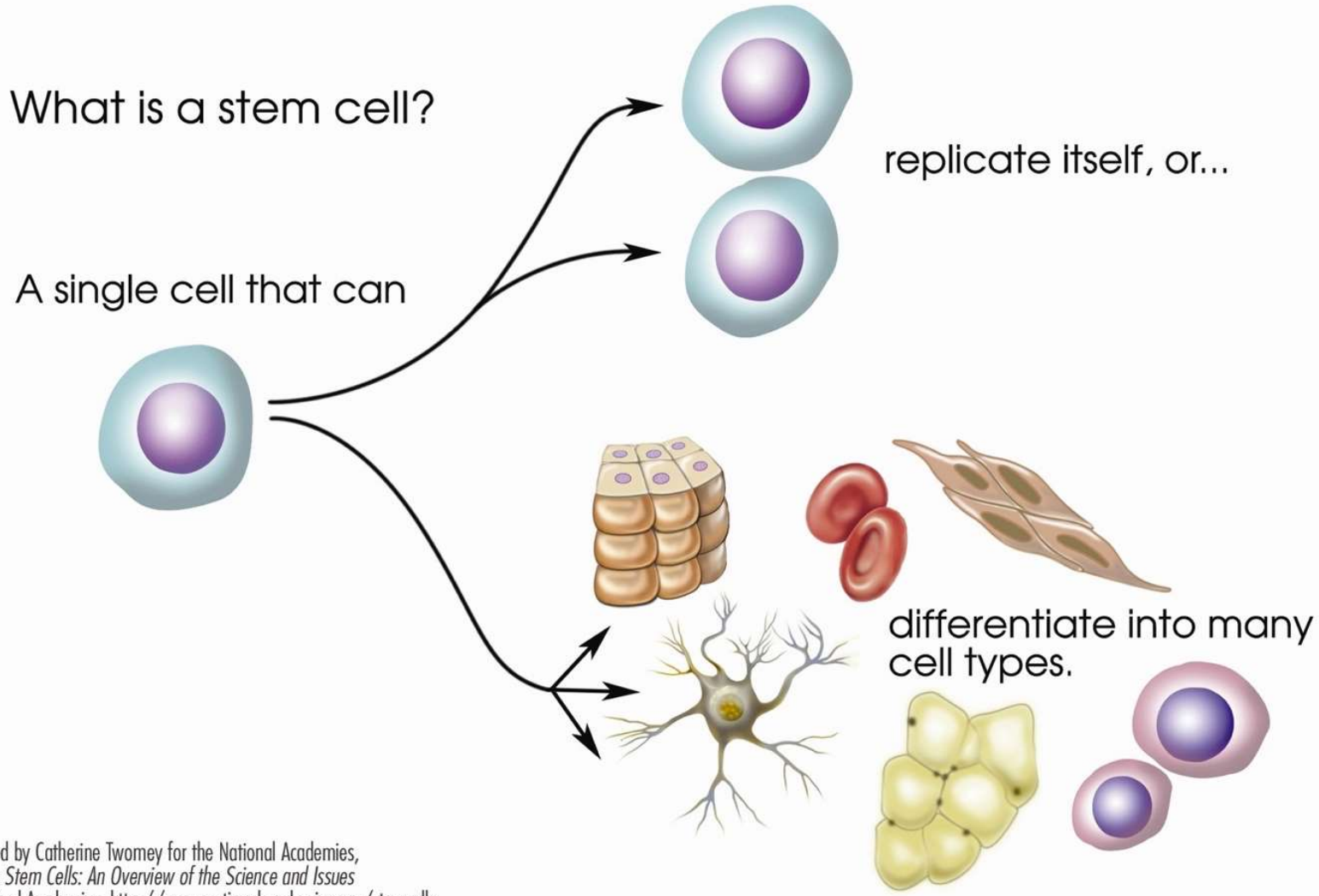


Image prepared by Catherine Twomey for the National Academies,
Understanding Stem Cells: An Overview of the Science and Issues
from the National Academies, http://www.nationalacademies.org/stemcells.
Academic noncommercial use is permitted.

Cell

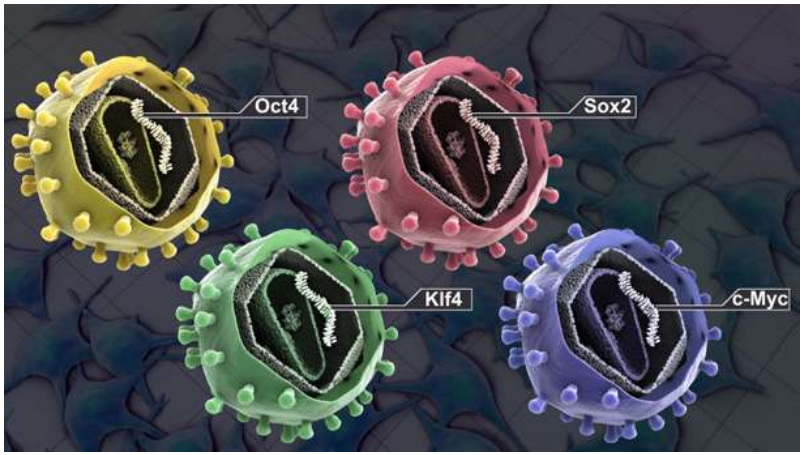
Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanal
DOI 10.1016/j.cell.

Shinya Yamanaka
Kyoto University

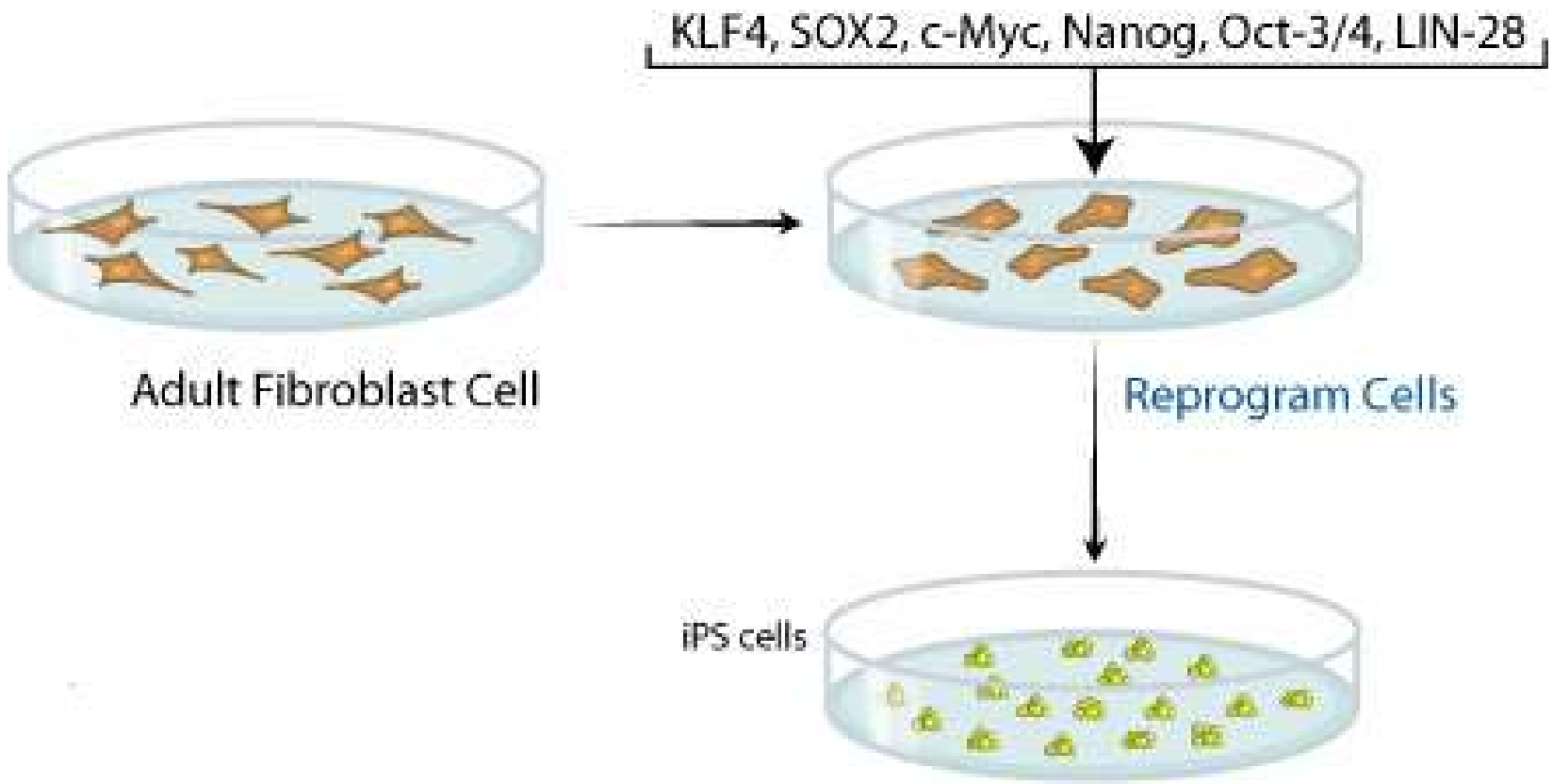
Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

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,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
[3]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
[4]Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019



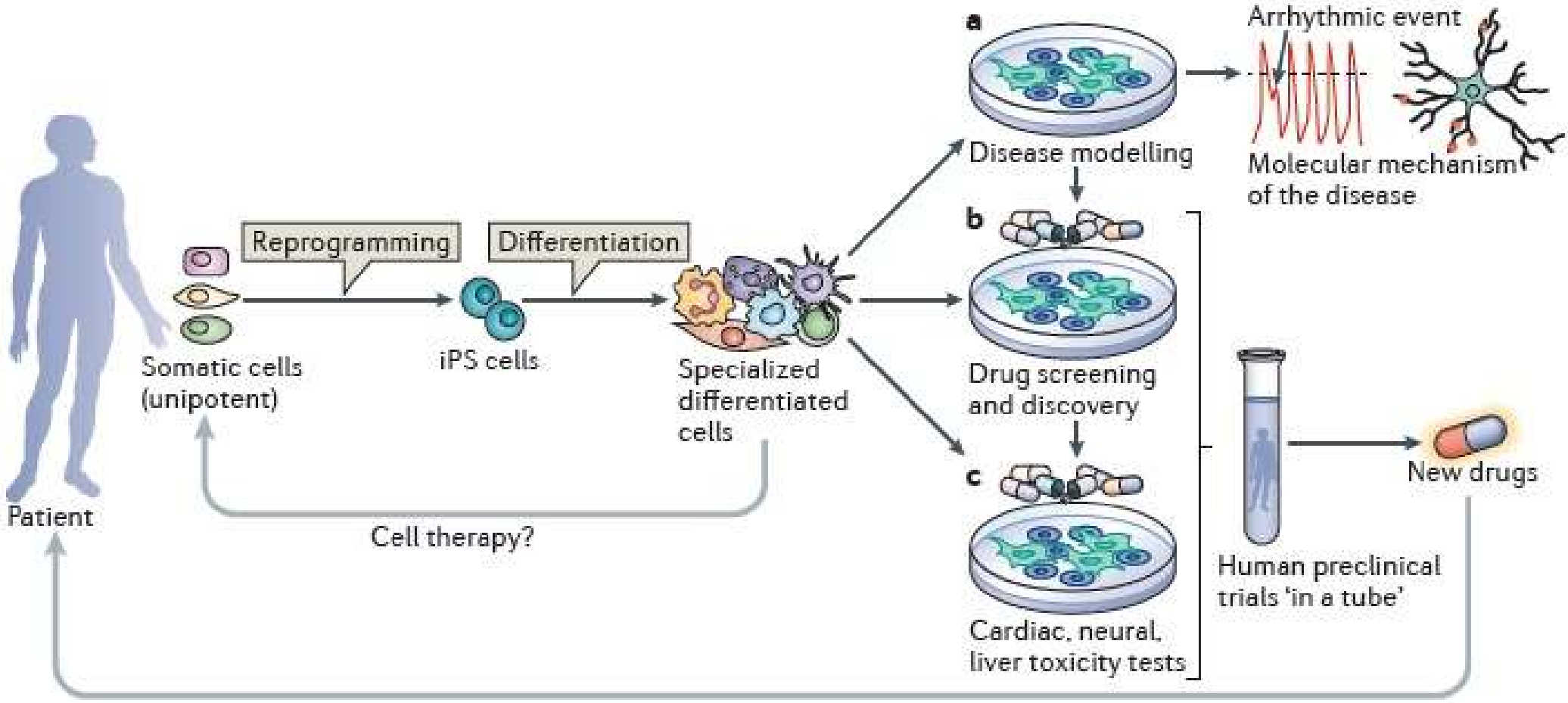
Reprogramming factors

iPS reprogramming has…



… no ethical issue compared to hES cells!

Human iPS cell derivation, differentiation and applications



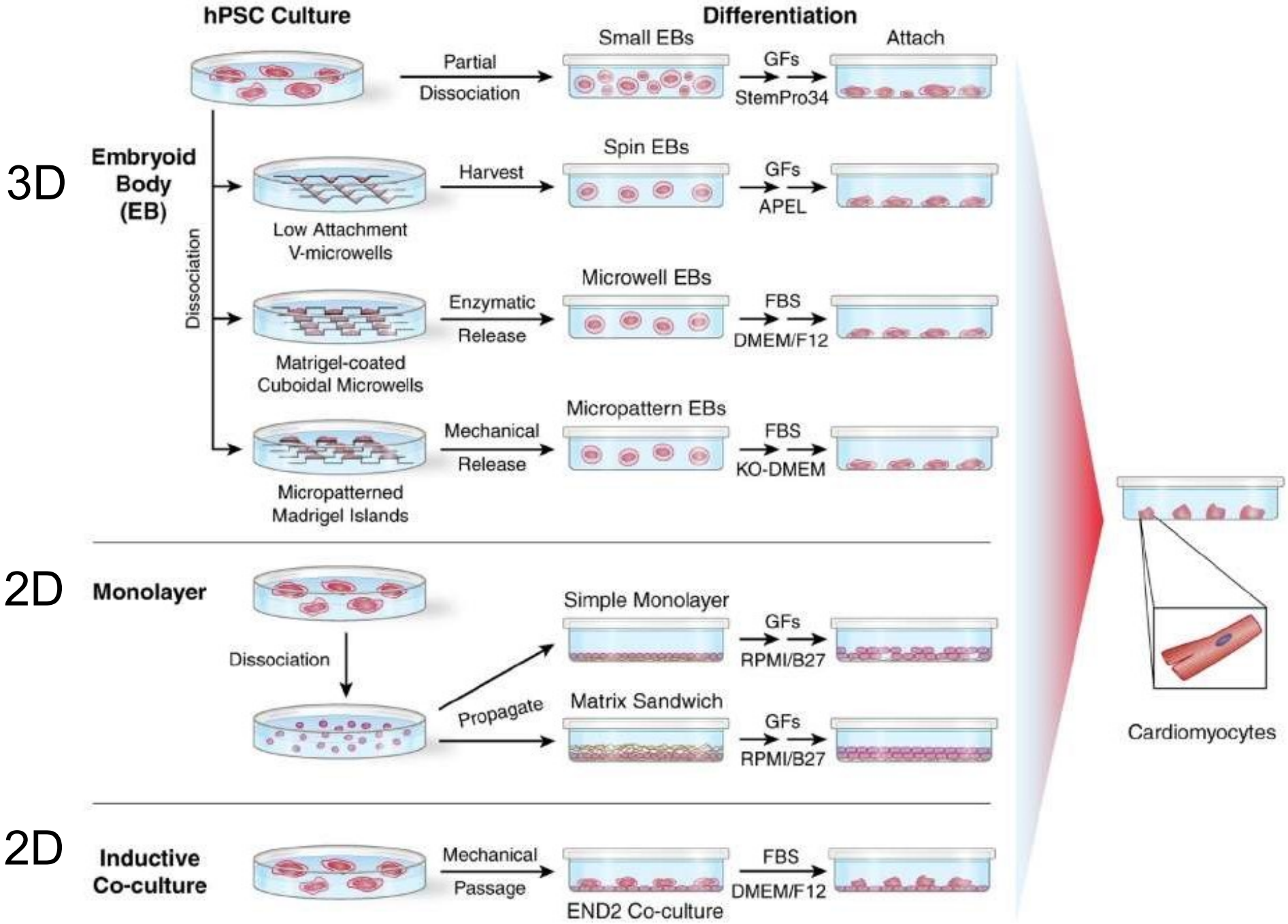
Adapted from Bellin *et al.*, 2012

Differentiation of hiPSC in cardiomyocytes

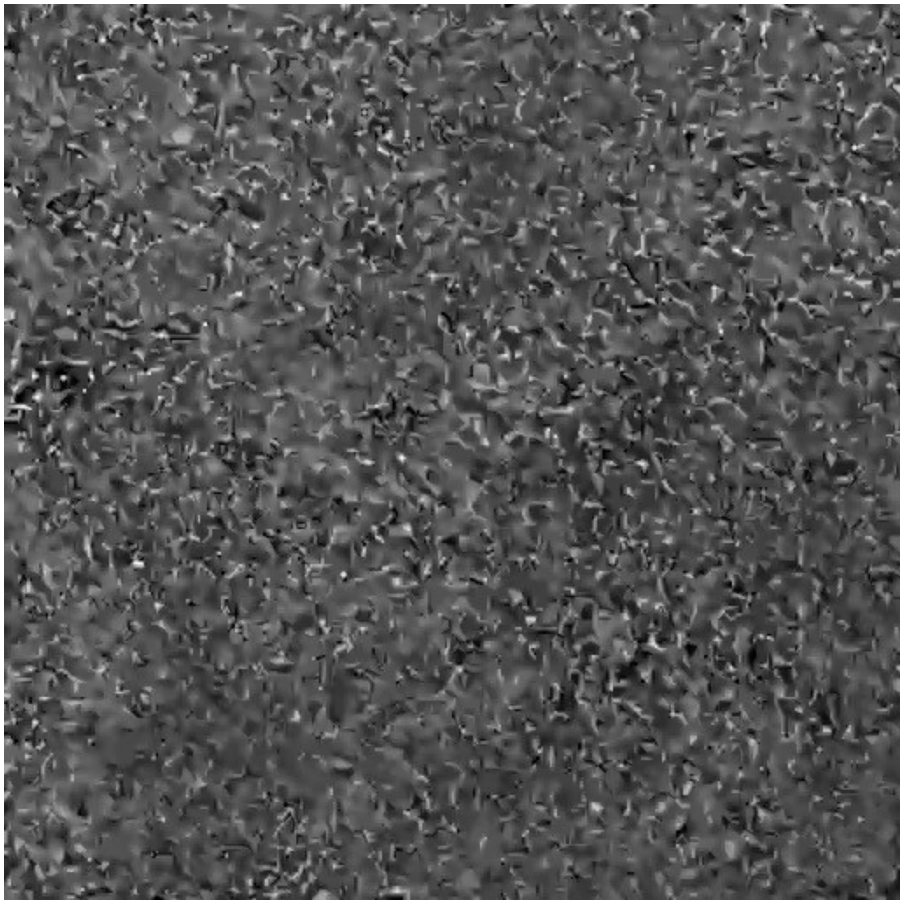
conditions and assumptions:

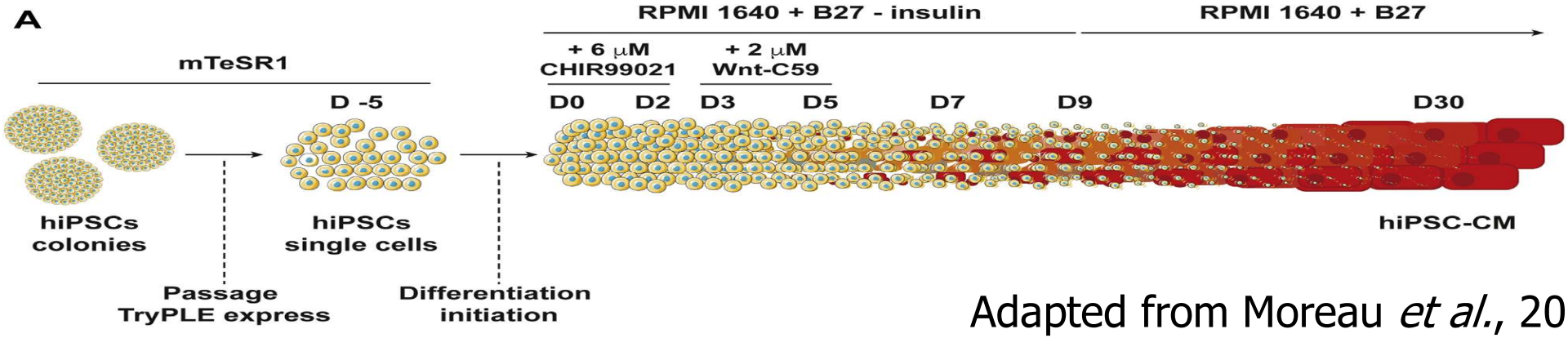
- ✓ Differentiation of specific populations of CMs (ventricular vs atrial vs nodal)
- ✓ Purified cardiac population
- ✓ Obtain mature (adult) hiPSC-CMs

Current methods for cardiac differentiation of hPSC



Monolayer-based cardiac differentiation protocol





Adapted from Moreau *et al.*, 2017
Sleiman *et al.*, 2020

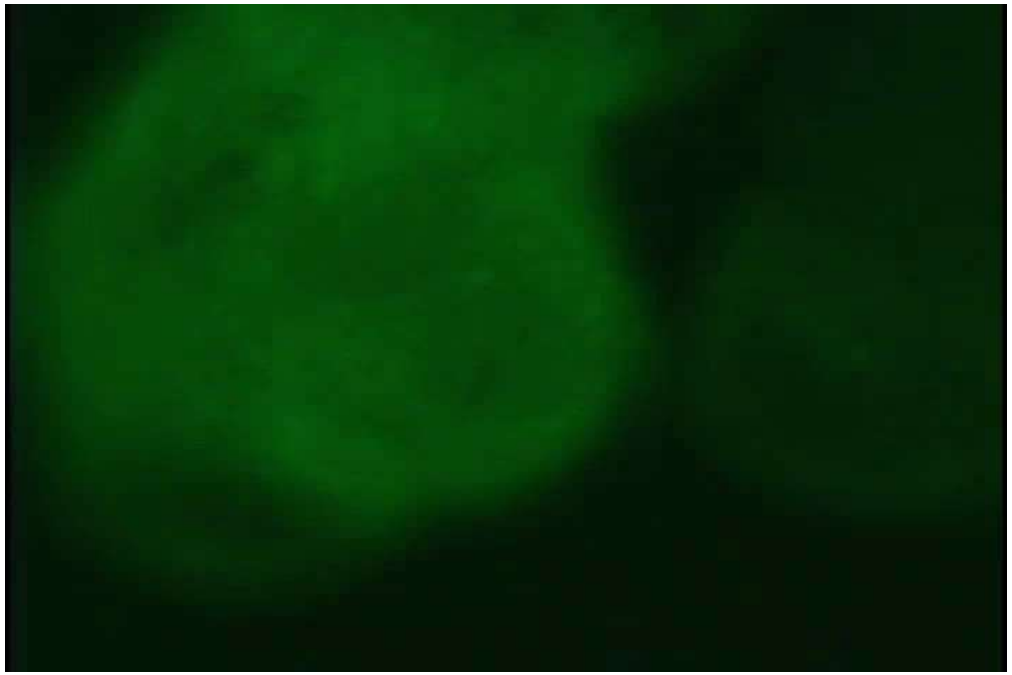
nature biotechnology

SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells

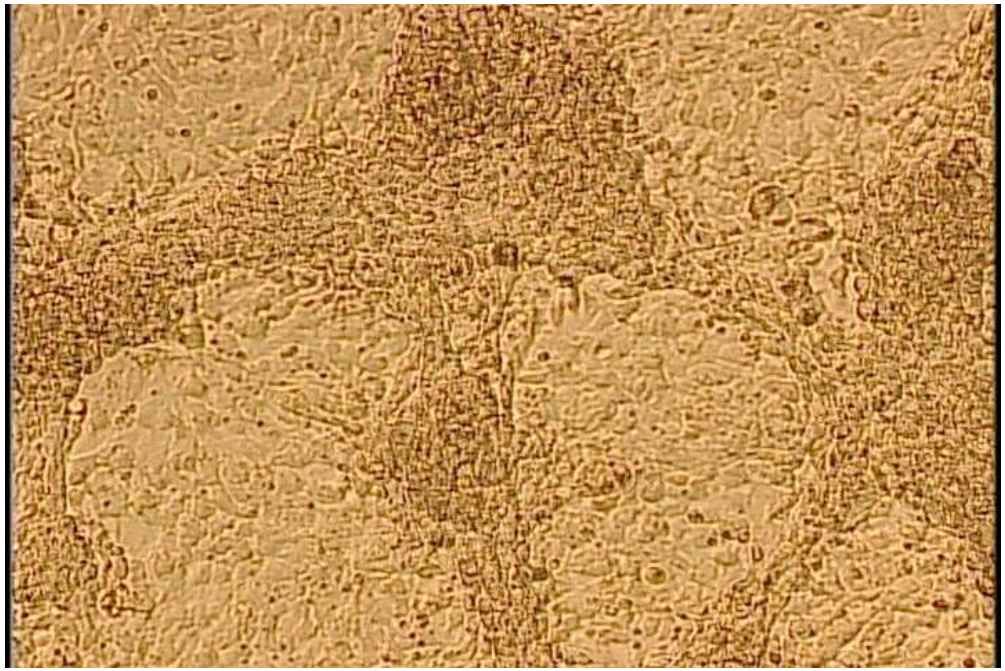
Nicole C Dubois[1], April M Craft[1], Parveen Sharma[2], David A Elliott[3], Edouard G Stanley[3], Andrew G Elefanty[3], Anthony Gramolini[2] & Gordon Keller[1]

To identify cell-surface markers specific to human cardiomyocytes, we screened cardiovascular cell populations derived from human embryonic stem cells (hESCs) against a panel of 370 known CD antibodies. This screen identified the signal-regulatory protein alpha (SIRPA) as a marker expressed specifically on cardiomyocytes derived from hESCs and human induced pluripotent stem cells (hiPSCs), and PECAM, THY1, PDGFRB and ITGA1 as markers of the nonmyocyte population. Cell sorting with an antibody against SIRPA allowed for the enrichment of cardiac precursors and cardiomyocytes from hESC/hiPSC differentiation cultures, yielding populations of up to 98% cardiac troponin T-positive cells. When plated in culture, SIRPA-positive cells were contracting and could be maintained over extended periods of time. These findings provide a simple method for isolating populations of cardiomyocytes from human pluripotent stem cell cultures, and thereby establish a readily adaptable technology for generating large numbers of enriched cardiomyocytes for therapeutic applications.

Purified beating syncytium from hPSC-CMs



NKX2-5-GFP embryoid bodies



SIRPA^{+}- cell derived population

Metabolic sorting of hPSC-CMs

Cell Stem Cell
Resource

Distinct Metabolic Flow Enables Large-Scale Purification of Mouse and Human Pluripotent Stem Cell-Derived Cardiomyocytes

Shugo Tohyama,[1,3] Fumiyuki Hattori,[1,4,*] Motoaki Sano,[1] Takako Hishiki,[2] Yoshiko Nagahata,[2,5] Tomomi Matsuura,[2,5] Hisayuki Hashimoto,[1] Tomoyuki Suzuki,[6] Hiromi Yamashita,[1,4] Yusuke Satoh,[1] Toru Egashira,[1] Tomohisa Seki,[1] Naoto Muraoka,[1] Hiroyuki Yamakawa,[1] Yasuyuki Ohgino,[1] Tomofumi Tanaka,[4] Masatoshi Yoichi,[4] Shinsuke Yuasa,[1] Mitsushige Murata,[1] Makoto Suematsu,[2,5] and Keiichi Fukuda[1,*]

[1]Department of Cardiology
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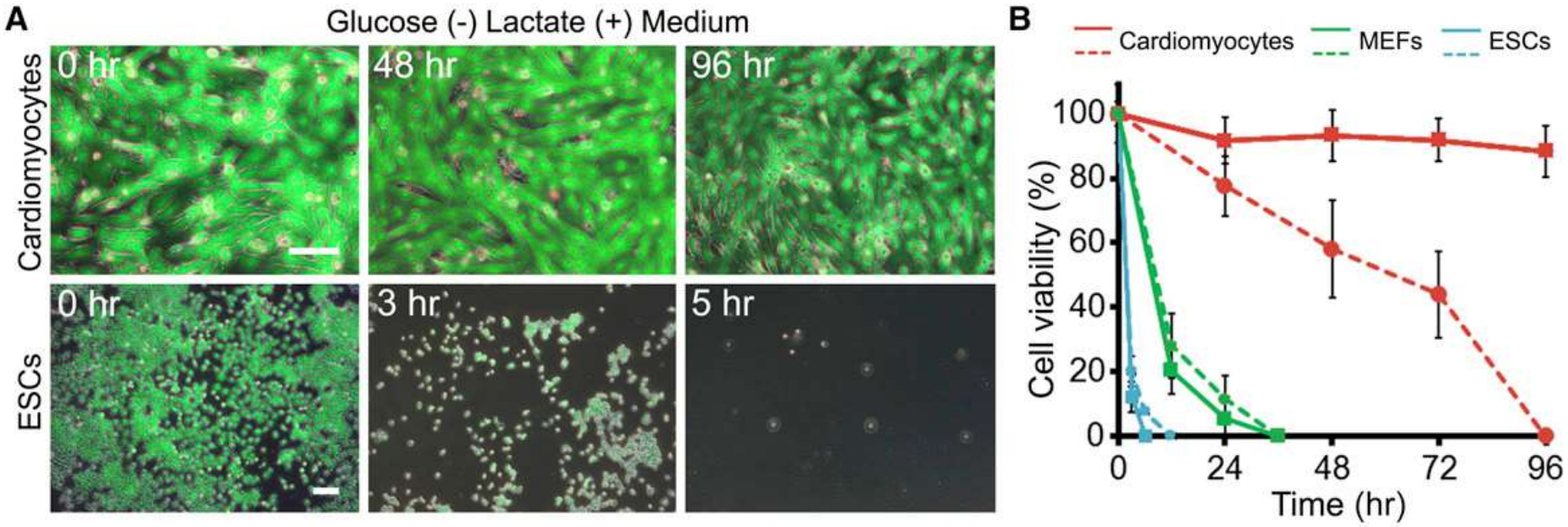
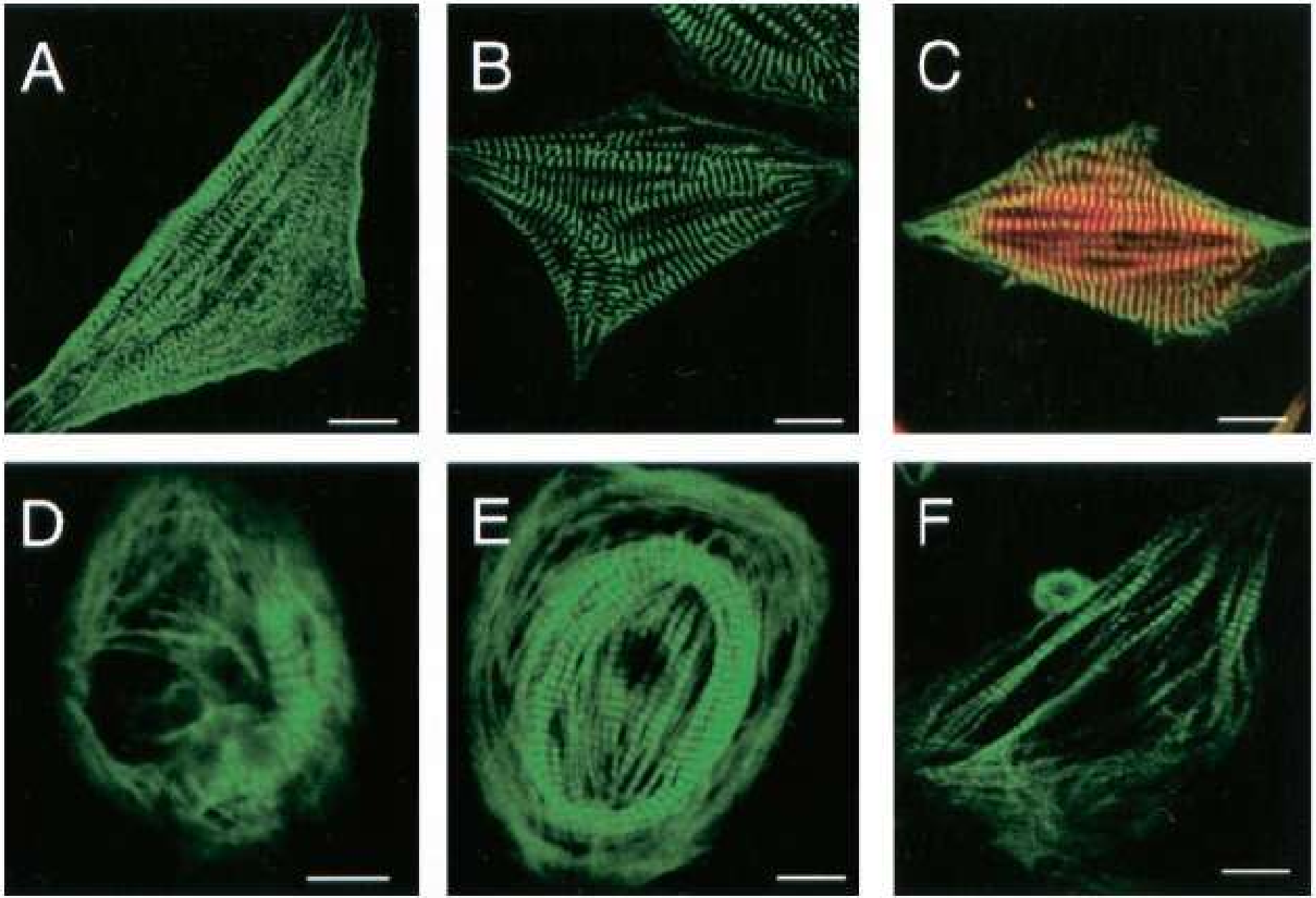
Keio University School of Medicine, Tokyo 160-8582, Japan
[3]Japan Society for the Promotion of Science, Tokyo 102-8472, Japan
[4]Asubio Pharma, Kobe 650-0047, Japan
[5]Japan Science and Technology Agency (JST), Exploratory Research for Advanced Technology (ERATO) Suematsu Gas Biology Project, Tokyo 160-8582, Japan
[6]Department of Cardiovascular Research, Research Institute of Environmental Medicine, Nagoya University, Nagoya 464-8601, Japan
*Correspondence: hattori.fumiyuki.ef@asubio.co.jp (F.H.), kfukuda@a2.keio.jp (K.F.)
http://dx.doi.org/10.1016/j.stem.2012.09.013

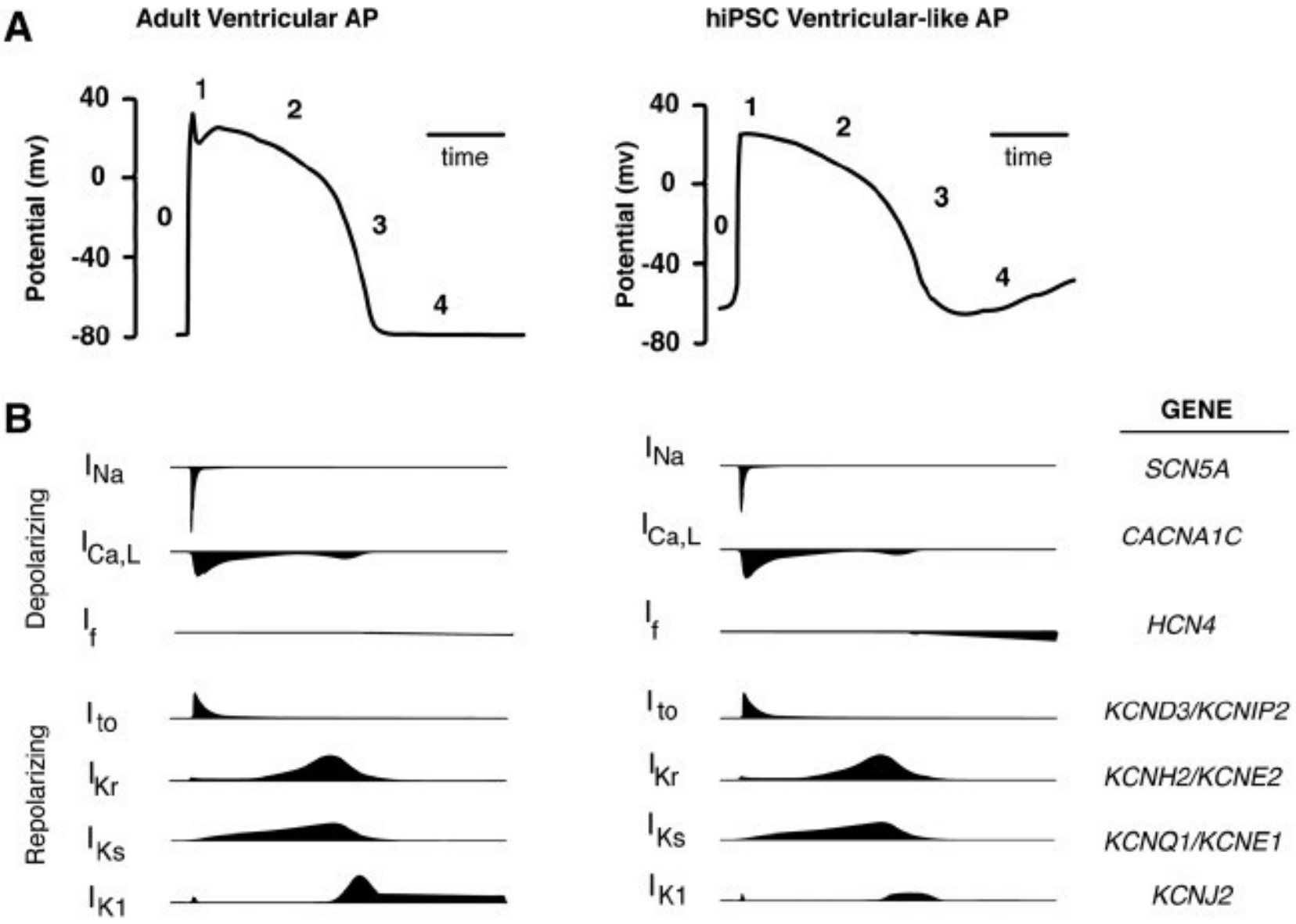
Metabolic sorting of hPSC-CMs



How mature are hPSC-derived CMs in the dish?



Comparison of action potentials



hiPSC-CMs exhibit spontaneous automaticity!

European Heart Journal
doi:10.1093/eurheartj/ehs096

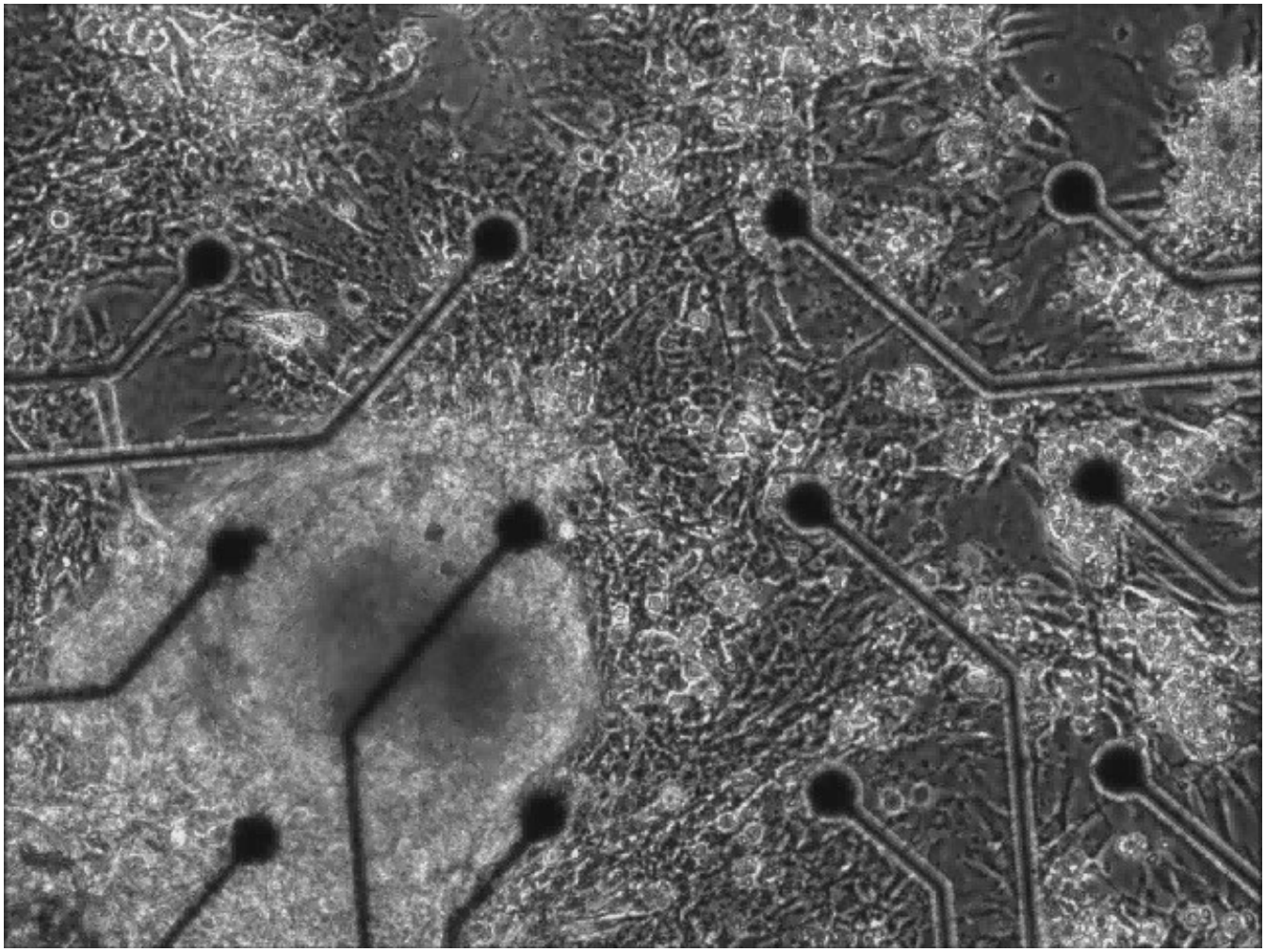
BASIC SCIENCE

Derivation and cardiomyocyte differentiation of induced pluripotent stem cells from heart failure patients

Limor Zwi-Dantsis[1,2], Irit Huber[1], Manhal Habib[1], Aaron Winterstern[1], Amira Gepstein[1], Gil Arbel[1], and Lior Gepstein[1,3]*

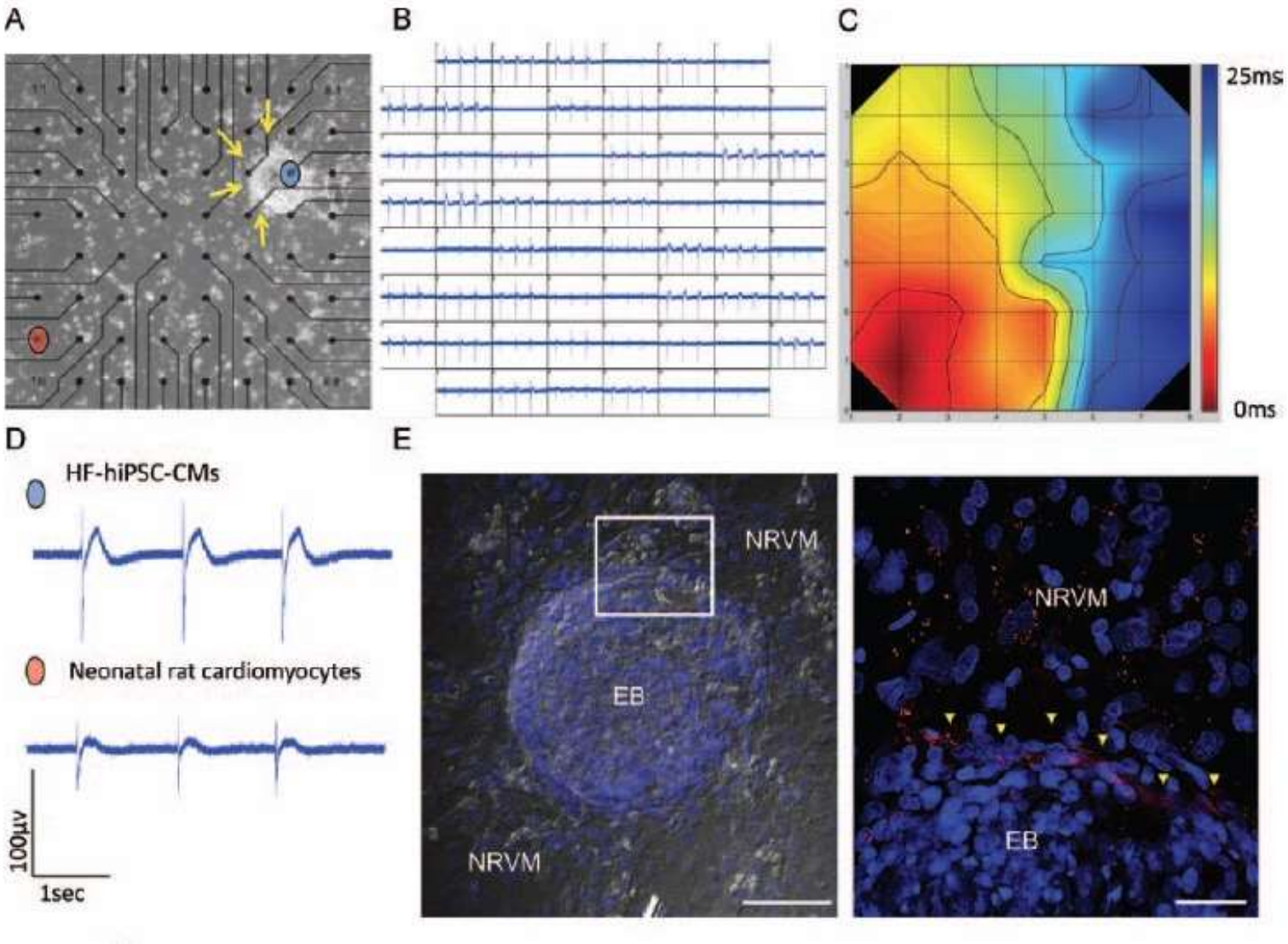
[1]Sohnis Research Laboratory for Cardiac Electrophysiology and Regenerative Medicine, The Bruce Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, POB 9649, Haifa 31096, Israel; [2]Biotechnology Interdisciplinary Unit, Technion–Israel Institute of Technology, Haifa, Israel; and [3]Cardiology Department, Rambam Medical Center, Haifa, Israel

Integration of HF hiPSC-CM in host cardiac tissue



Integrated hiPSC-CMs with rat CMs

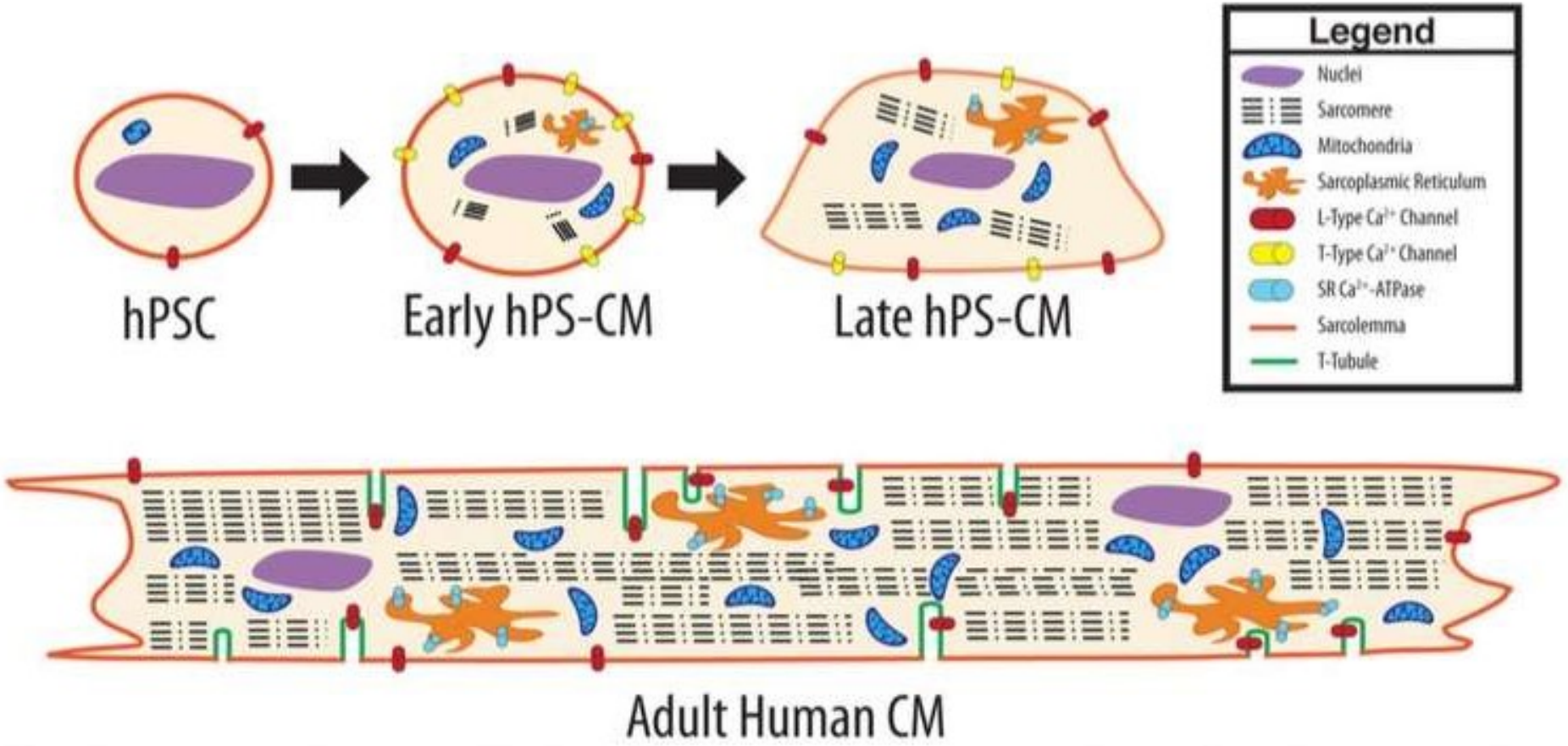
Integration of HF hiPSC-CM in host cardiac tissue



Integrated hiPSC-CMs with rat CMs

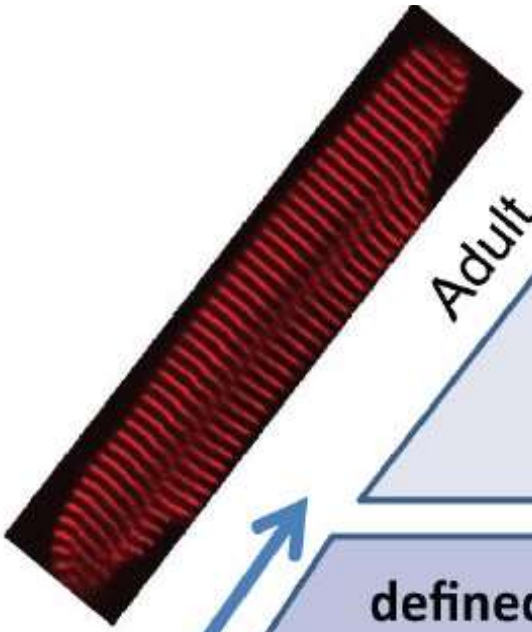
How to improve the maturity of hiPSC-derived cardiomyocytes?

Stem cell maturation



Robertson et *al.*, Stem cells, 2013

Developmental progression of cardiac myocytes

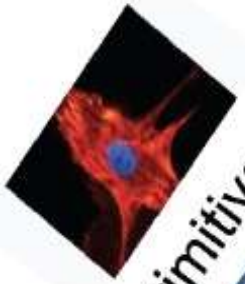


Adult

Adult
cTnI isoform
Rod-shaped

defined sarcomere formation
Developmental and chamber-specific sarcomere isoforms
Refined Ca2+ cycling and beating

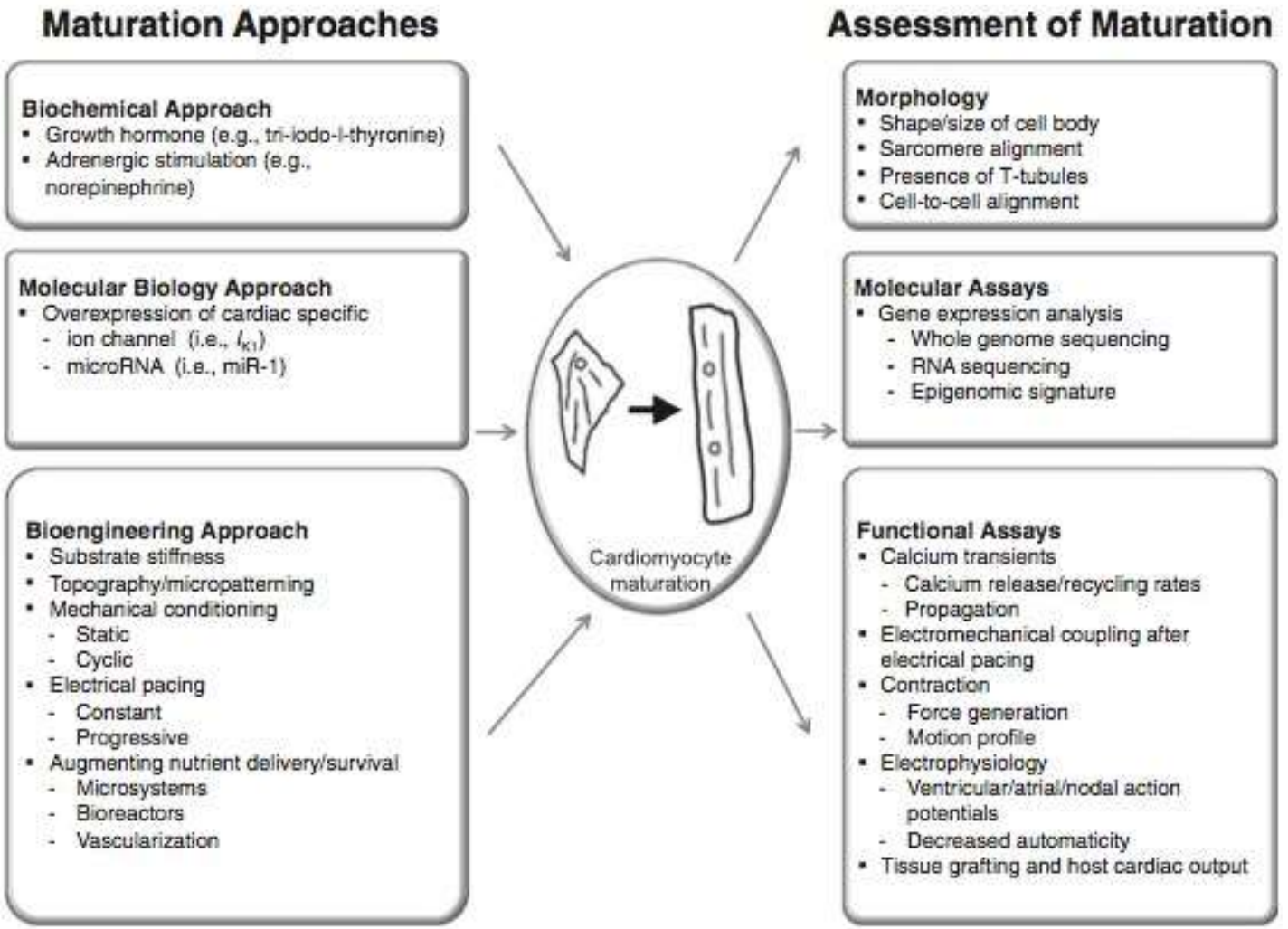
Disease



Primitive

Nascent sarcomere formation
Embryonic/fetal isoforms
Amorphic cell structure
Rudimentary Ca2+ cycling and beating
ssTnI

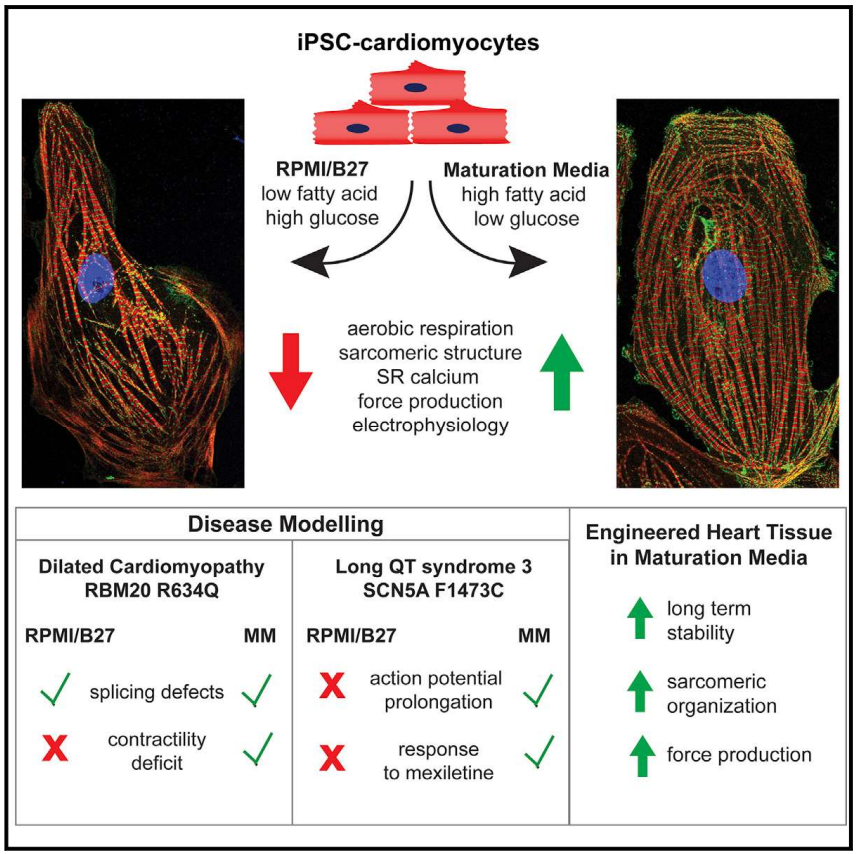
Stem cell maturation



Cell Reports

Metabolic Maturation Media Improve Physiological Function of Human iPSC-Derived Cardiomyocytes

Graphical Abstract



Highlights

- We developed a defined maturation medium for hiPSC-CMs
- The media improve electrophysiological and mechanical characteristics of hiPSC-CMs
- The media improve the fidelity of disease modeling

Authors

Dries A.M. Feyen, Wesley L. McKeithan, Arne A.N. Bruyneel, ..., Thomas Eschenhagen, Christian M. Metallo, Mark Mercola

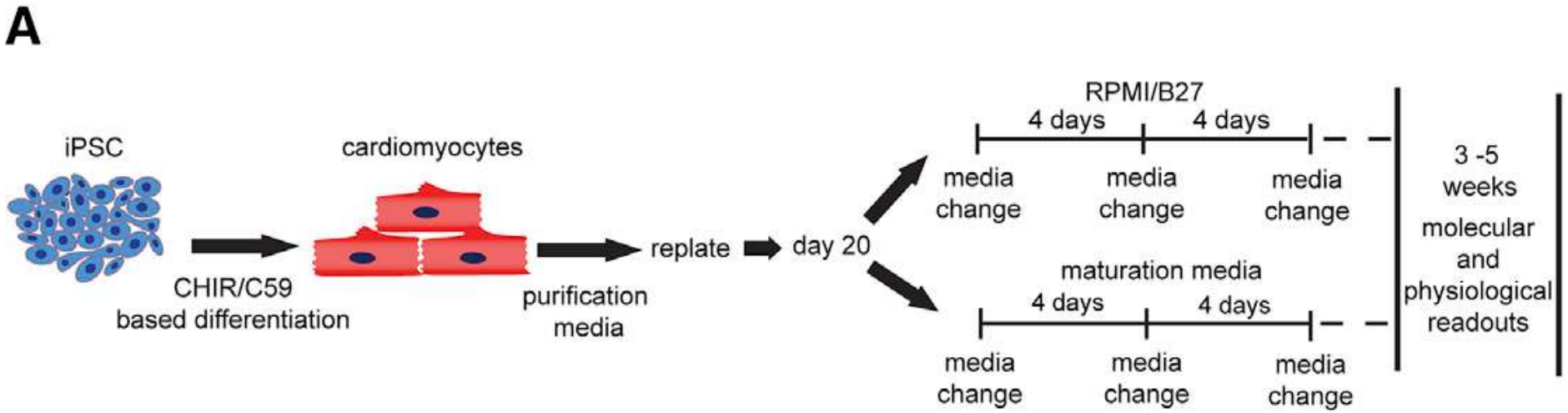
Correspondence

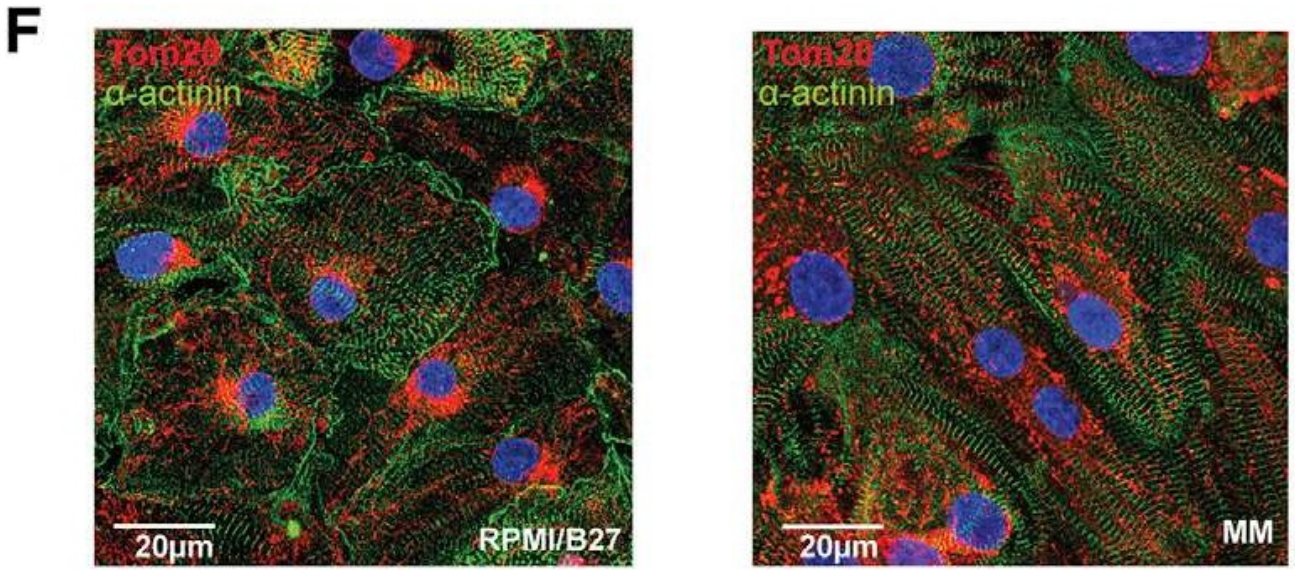
mmercola@stanford.edu

In Brief

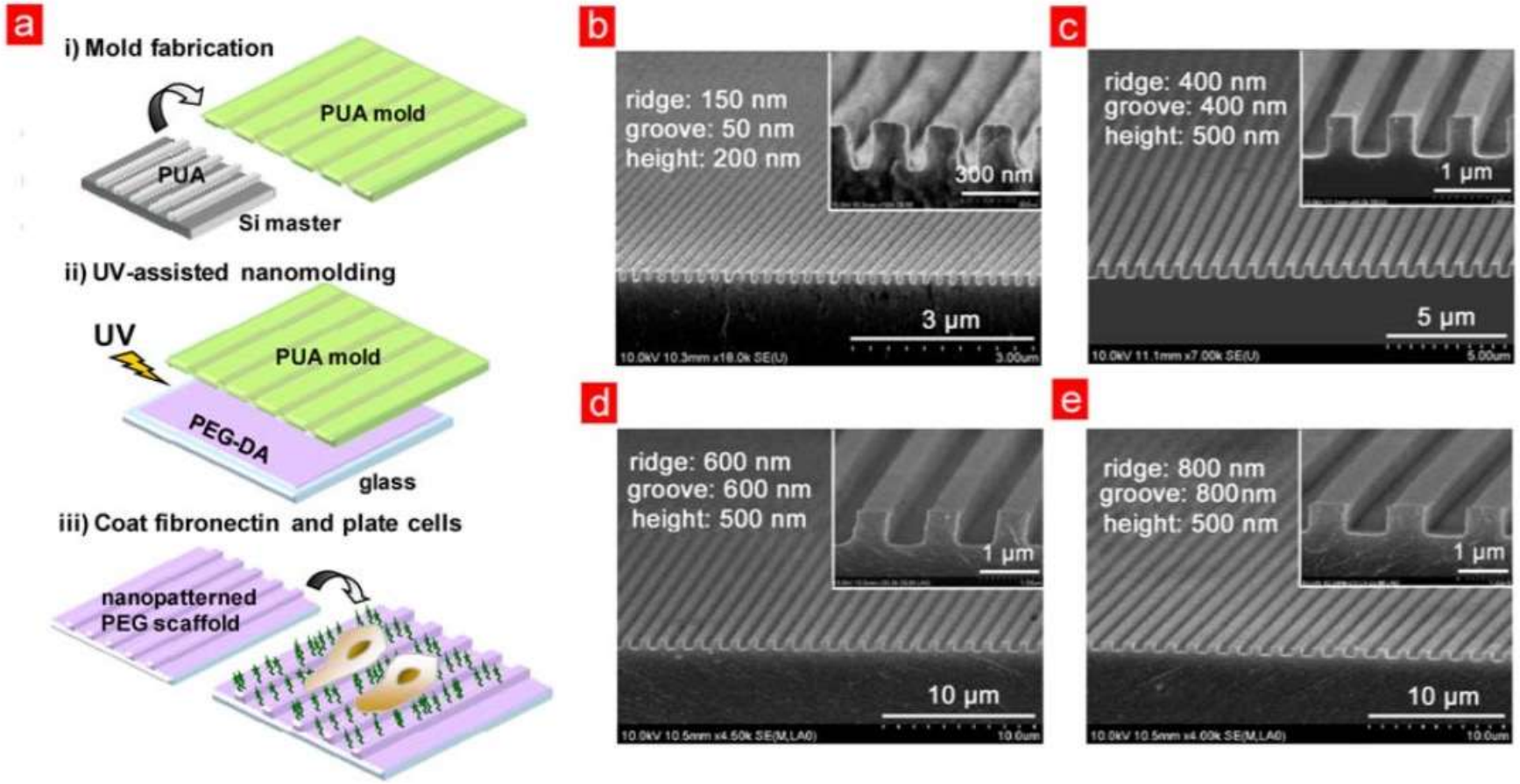
Physiological immaturity of iPSC-derived cardiomyocytes limits their fidelity as disease models. Feyen et al. developed a low glucose, high oxidative substrate media that increase maturation of ventricular-like hiPSC-CMs in 2D and 3D cultures relative to standard protocols. Improved characteristics include a low resting V_m, rapid depolarization, and increased Ca^{2+} dependence and force generation.

Fatty acids improve the hPSC-CMs





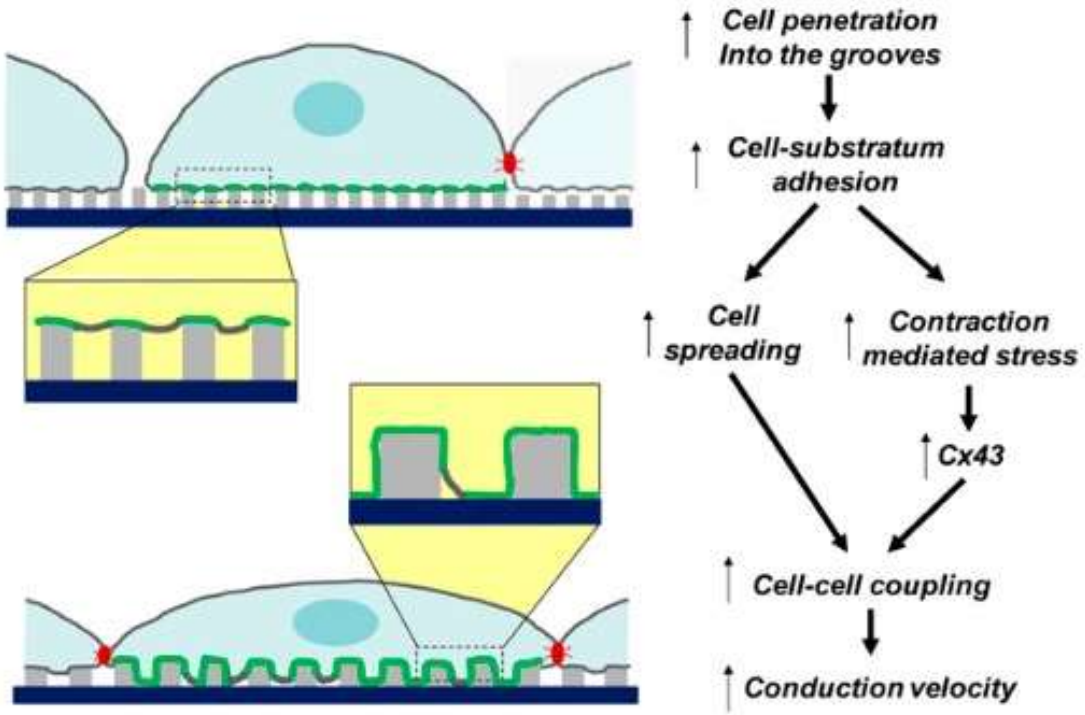
Monolayer

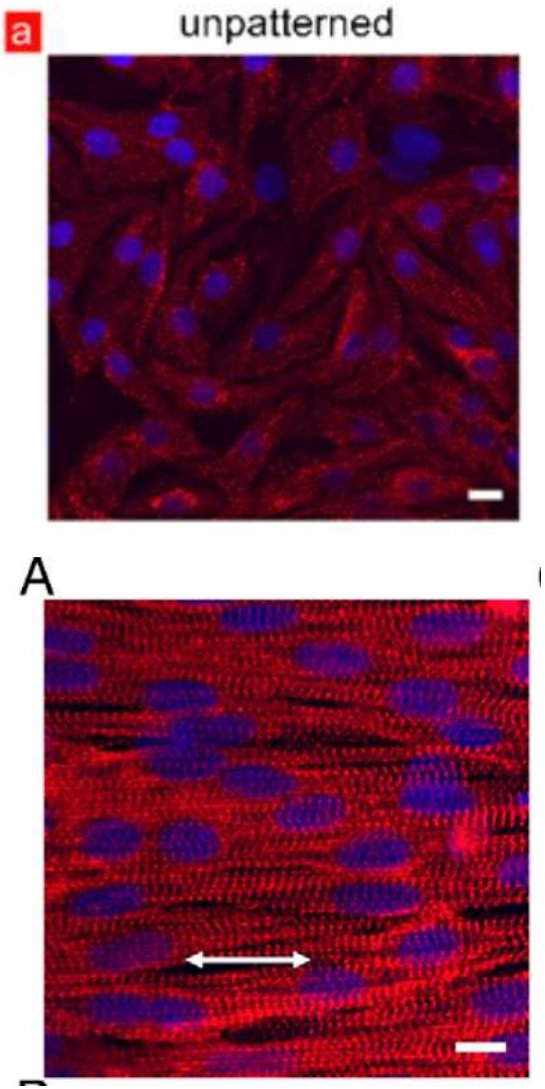


Biocompatible polyethylene glycol (PEG) hydrogel arrays imitating the myocardial ECM and allowing formation of engineered cardiac tissue constructs.

Kim *et al.*, PNAS 2010

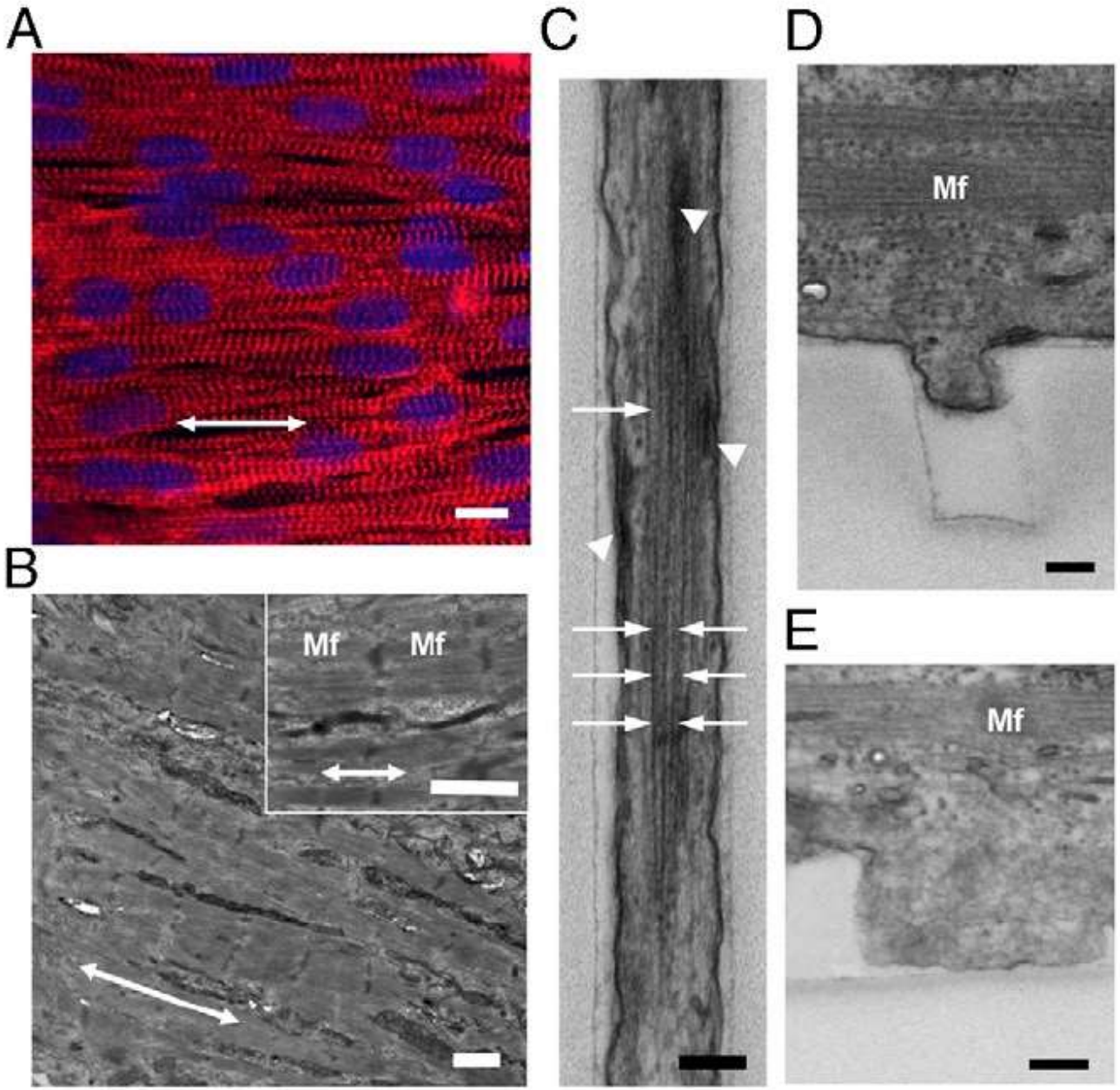
Monolayer





Kim *et al.*, PNAS 2010

Monolayer



Kim *et al.*, PNAS 2010

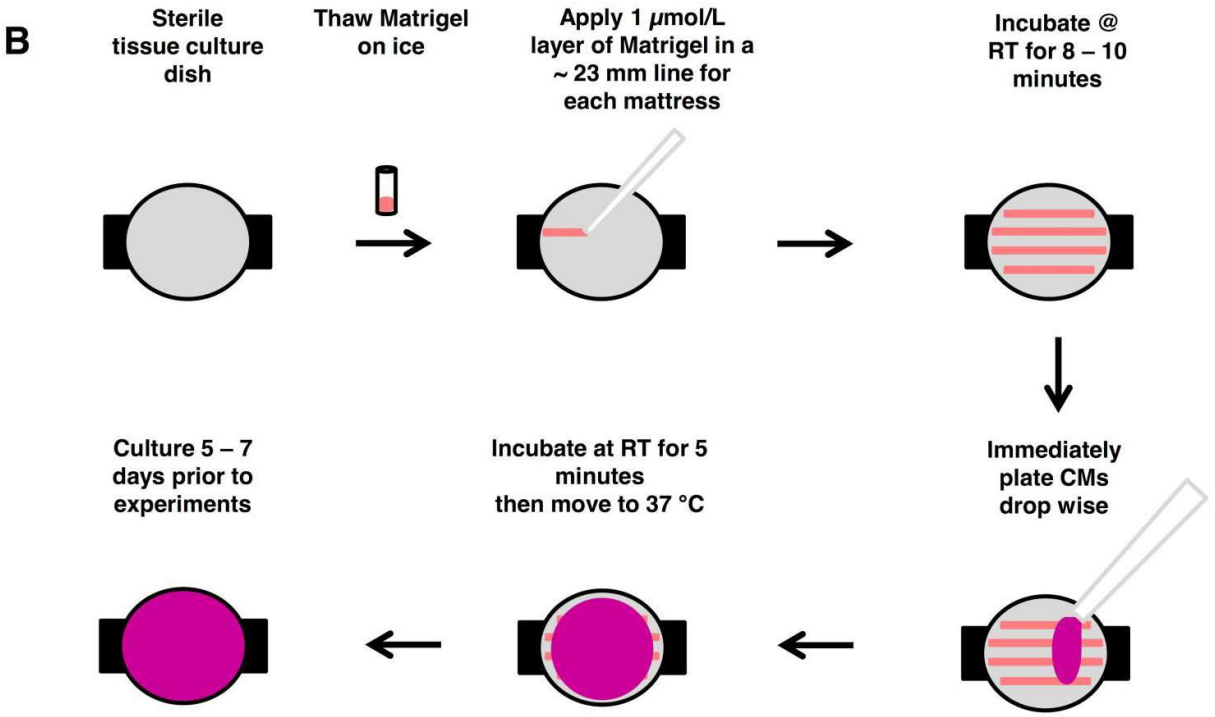
Brief UltraRapid Communication

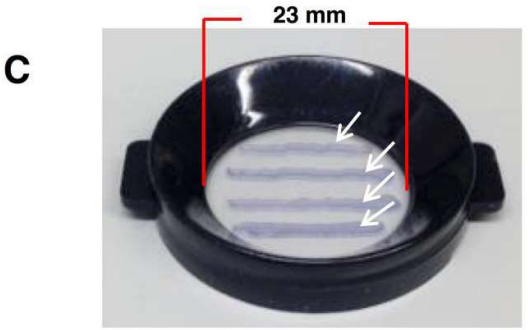
Matrigel Mattress

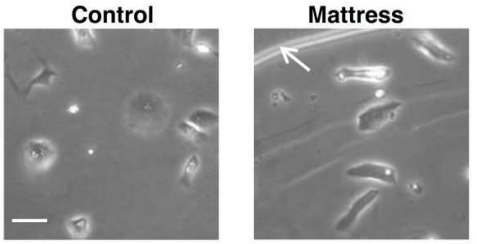
A Method for the Generation of Single Contracting Human-Induced Pluripotent Stem Cell–Derived Cardiomyocytes

Tromondae K. Feaster, Adrian G. Cadar, Lili Wang, Charles H. Williams, Young Wook Chun, Jonathan E. Hempel, Nathaniel Bloodworth, W. David Merryman, Chee Chew Lim, Joseph C. Wu, Björn C. Knollmann, Charles C. Hong

Matrigel mattress

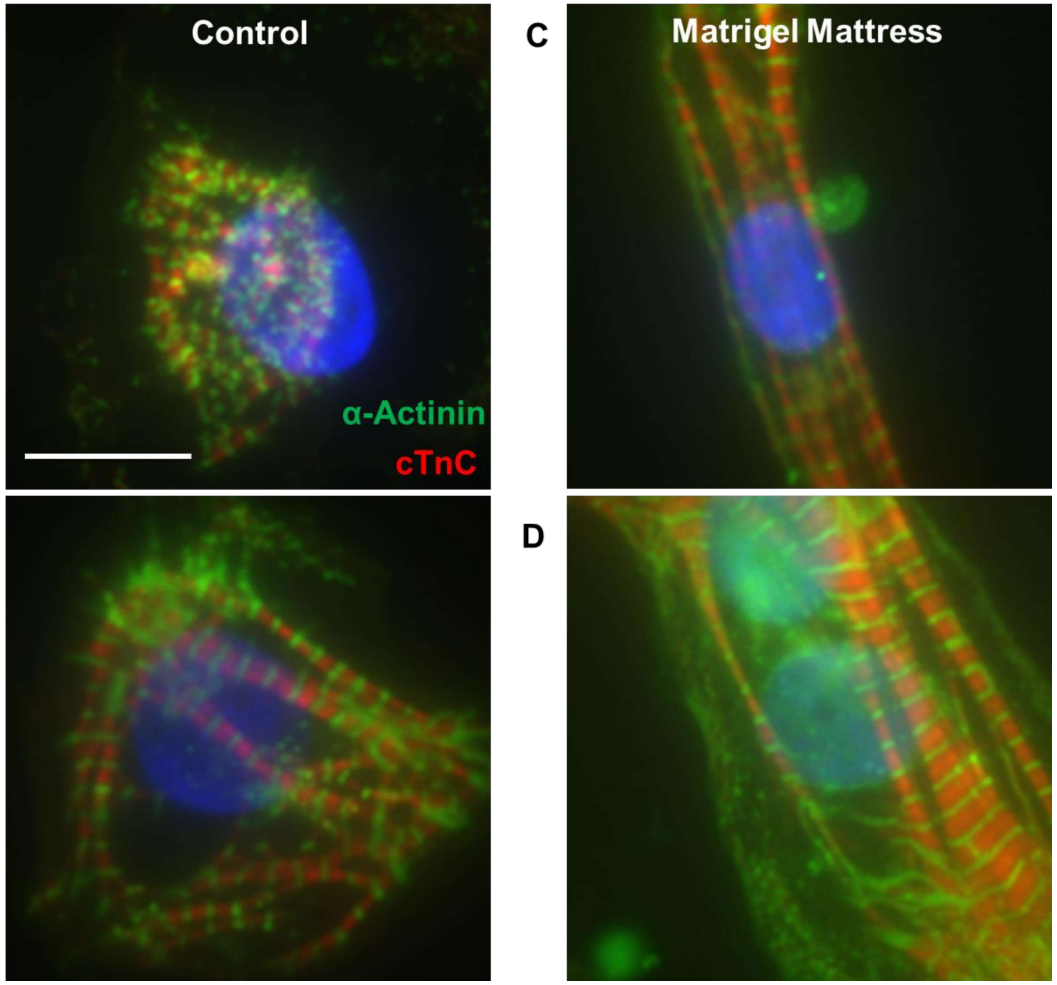


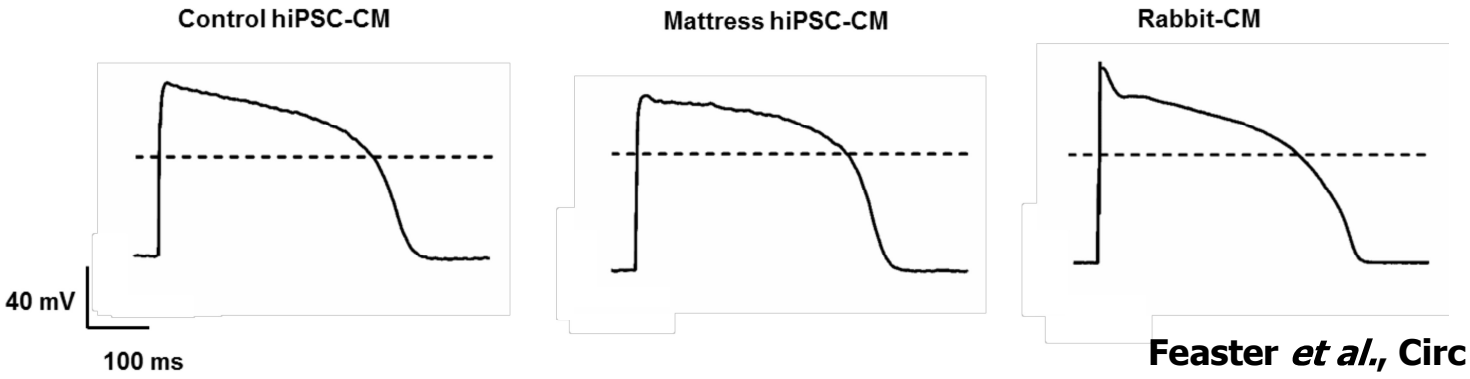




Feaster *et al.*, Circ Res 2016

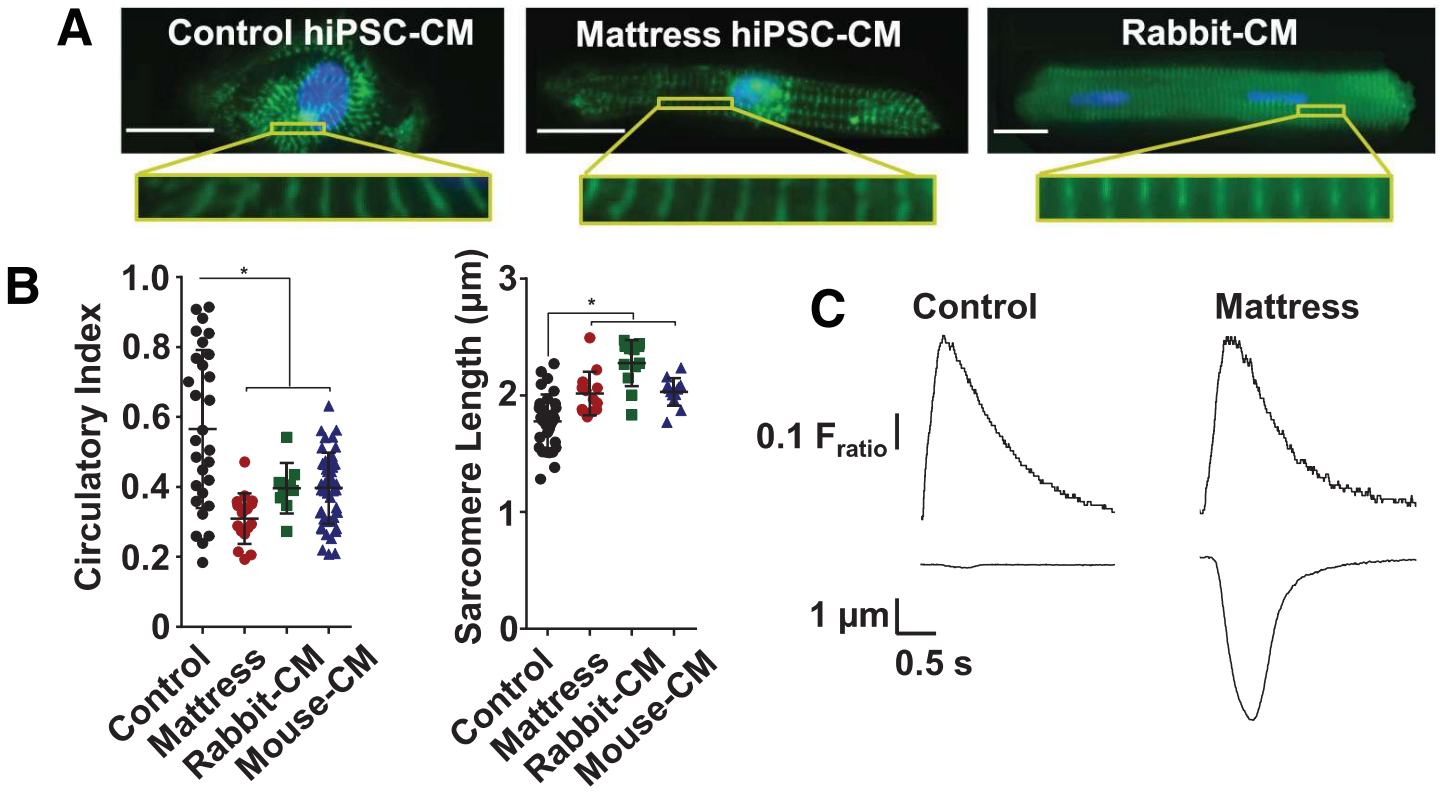
Matrigel mattress





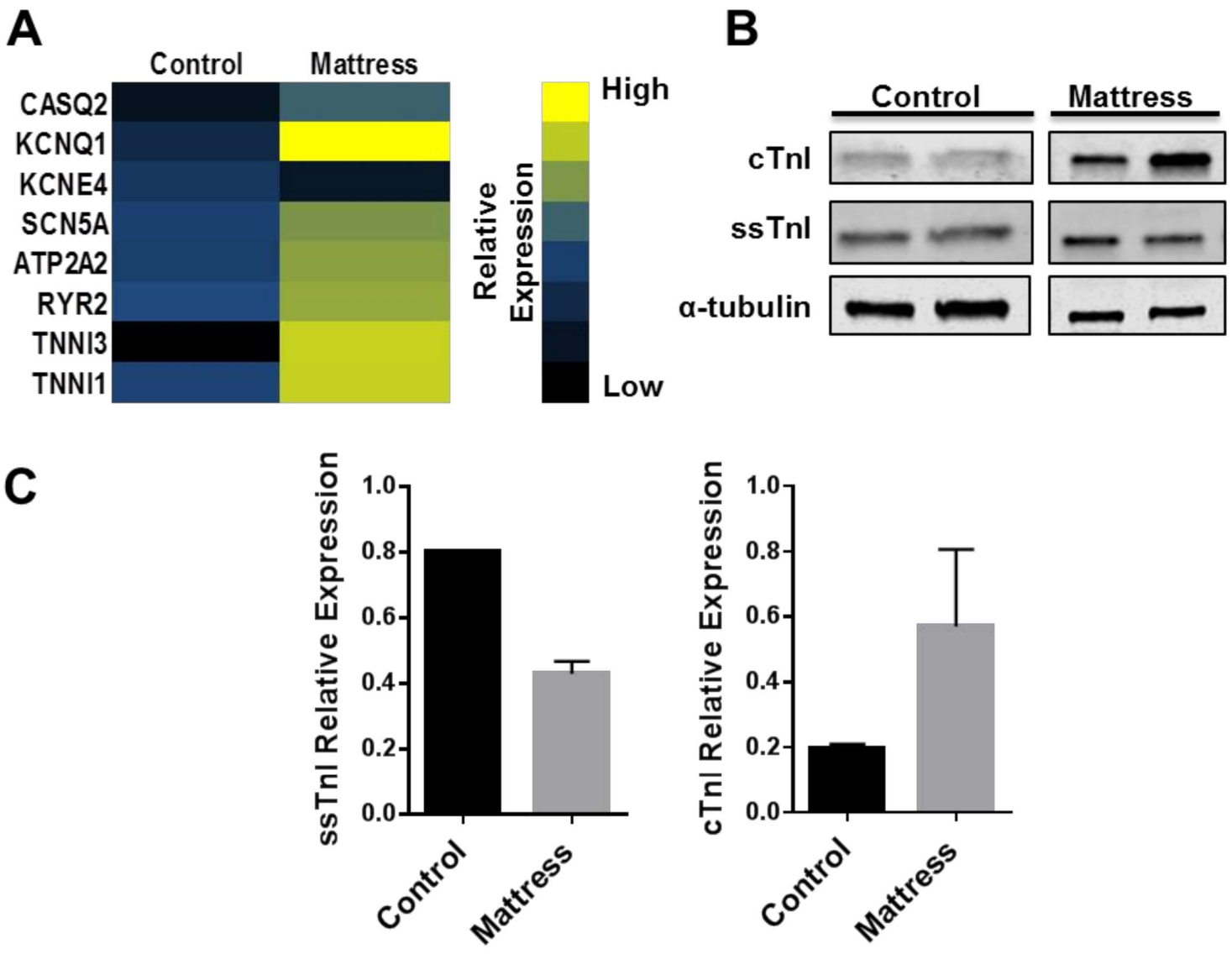
Feaster *et al.*, Circ Res 2016

Matrigel mattress



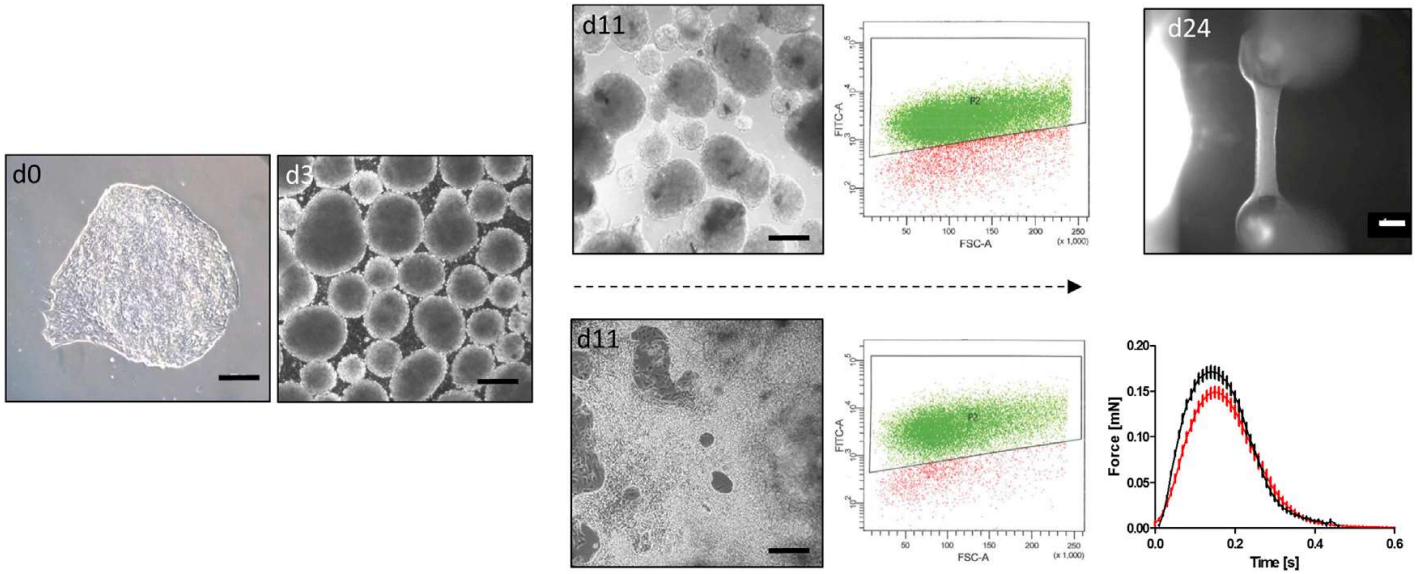
Feaster *et al.*, Circ Res 2016

Matrigel mattress



Feaster *et al.*, Circ Res 2016

Engineered heart tissues



EB-Formation
Spinner flasks

Mesodermal differentiation
Suspension culture
BMP4
Activin A
bFGF
Minus Insulin

Cardiac differentiation
Adhesion culture or suspension culture
Small molecule WNT inhibitor DS-07
Minus insulin: day 4-6
Plus insulin: day 7-13

Dissociation of CM
Generation of EHTs

Contraction analysis

Mannhardt *et al.*, Stem Cell Report 2016

Engineered heart tissues

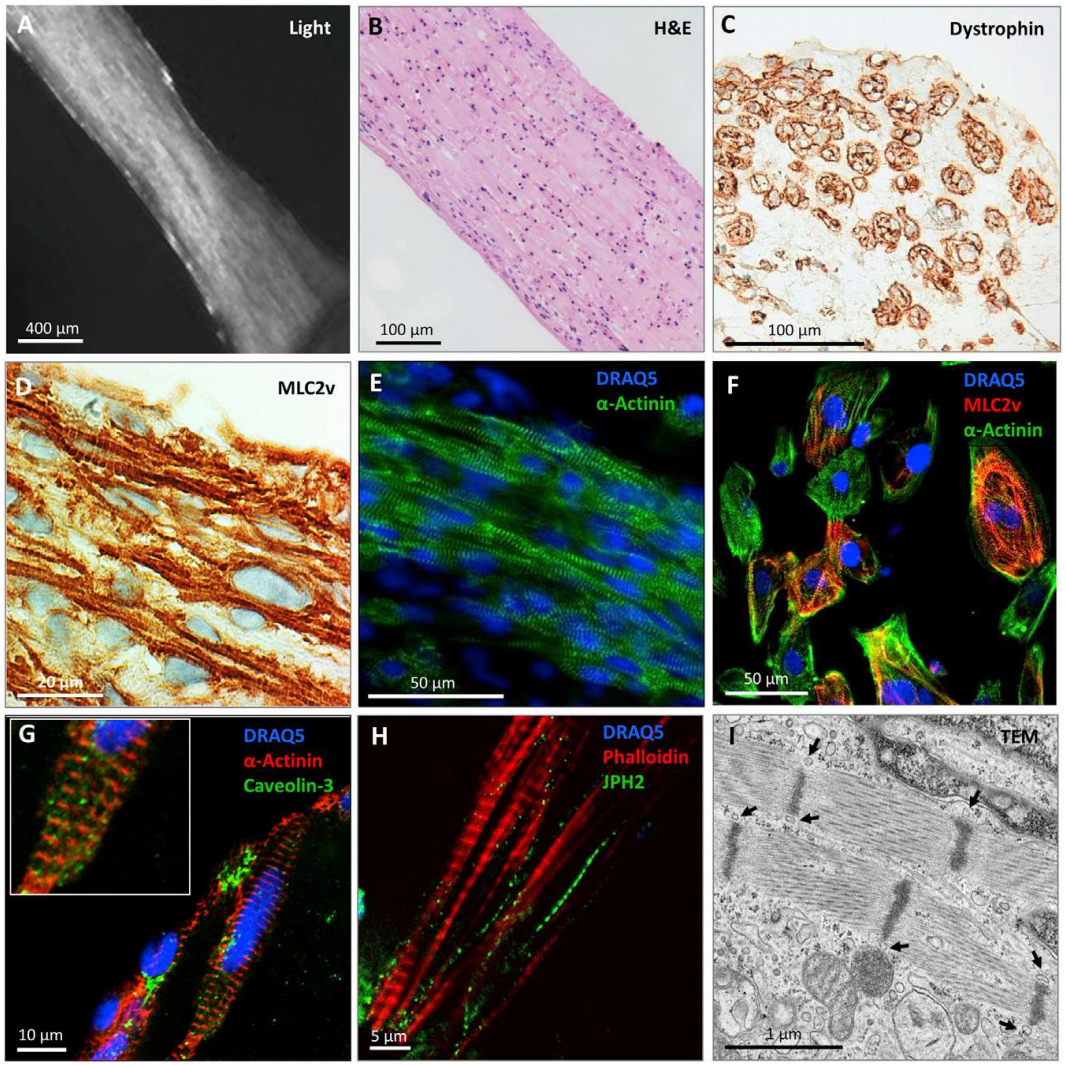
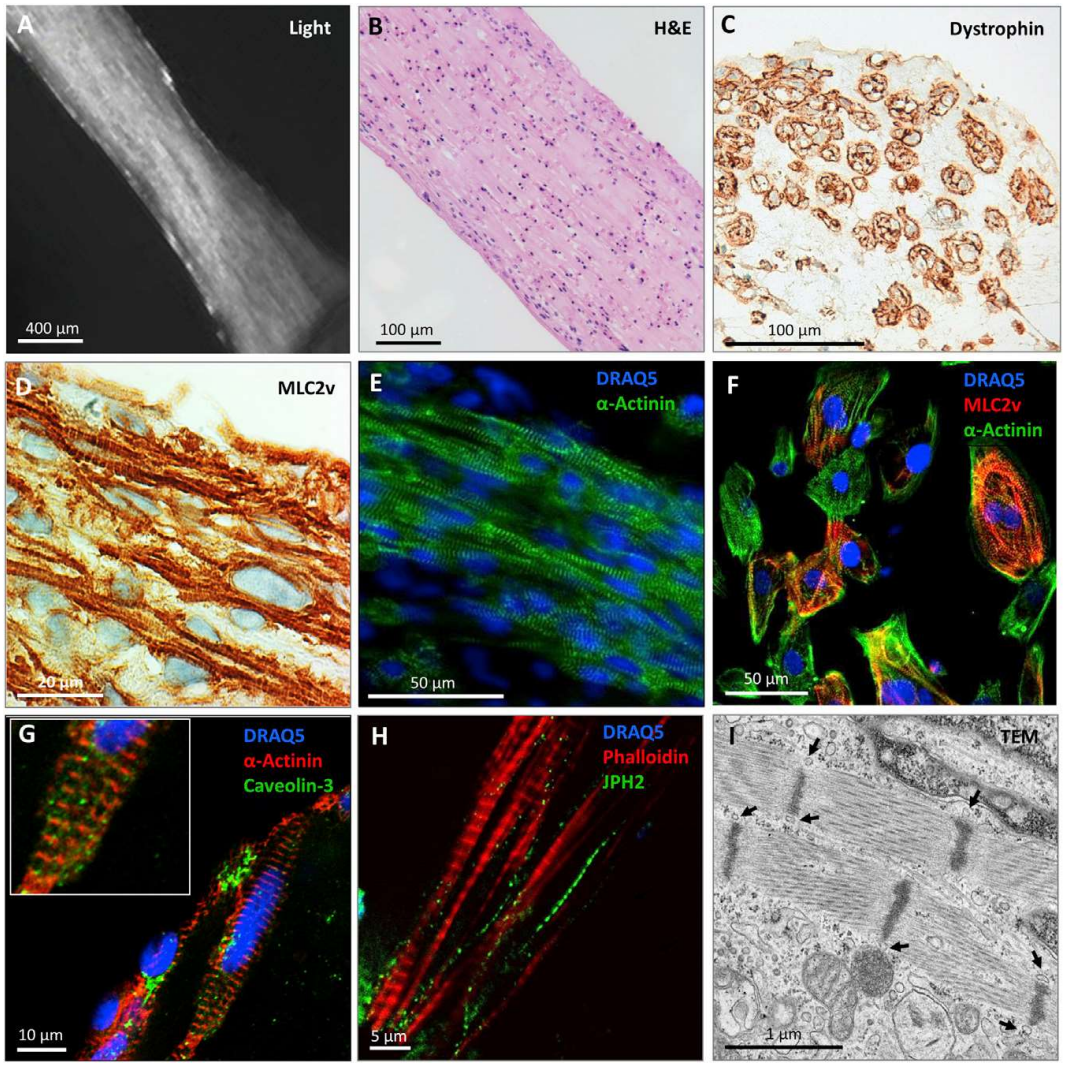


Figure 2. Histological Evaluation of hiPSC-EHTs

(A) Representative still view of a living EHT.
(B) Longitudinal section stained with H&E.
(C) Cross section stained for dystrophin.
(D) Longitudinal section stained for MLC2v.
(E) Whole-mount immunofluorescence confocal microscopic section of EHT stained with DRAQ5 (nuclei, blue) and α-actinin (green).
(F) Confocal analysis of hiPSC-CM cultured in 2D for 30 days stained with DRAQ5 (blue) and antibodies against cardiac MLC2v (red) and α-actinin (green).
(G and H) Whole-mount immunofluorescence confocal microscopic section of 30- to 35-day-old EHTs stained with DRAQ5 (blue) and antibodies against α-actinin (red; G) and caveolin-3 (green; G) or phalloidin (red; H) and an antibody against junctophilin-2 (green; H).
(I) Transmission electron microscopy of 35-day-old EHT. Arrows indicate structures resembling t tubules.
See also Figure S2.

Mannhardt *et al.*, Stem Cell Report 2016

Engineered heart tissues



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Mannhardt *et al.*, Stem Cell Report 2016

Engineered heart tissues

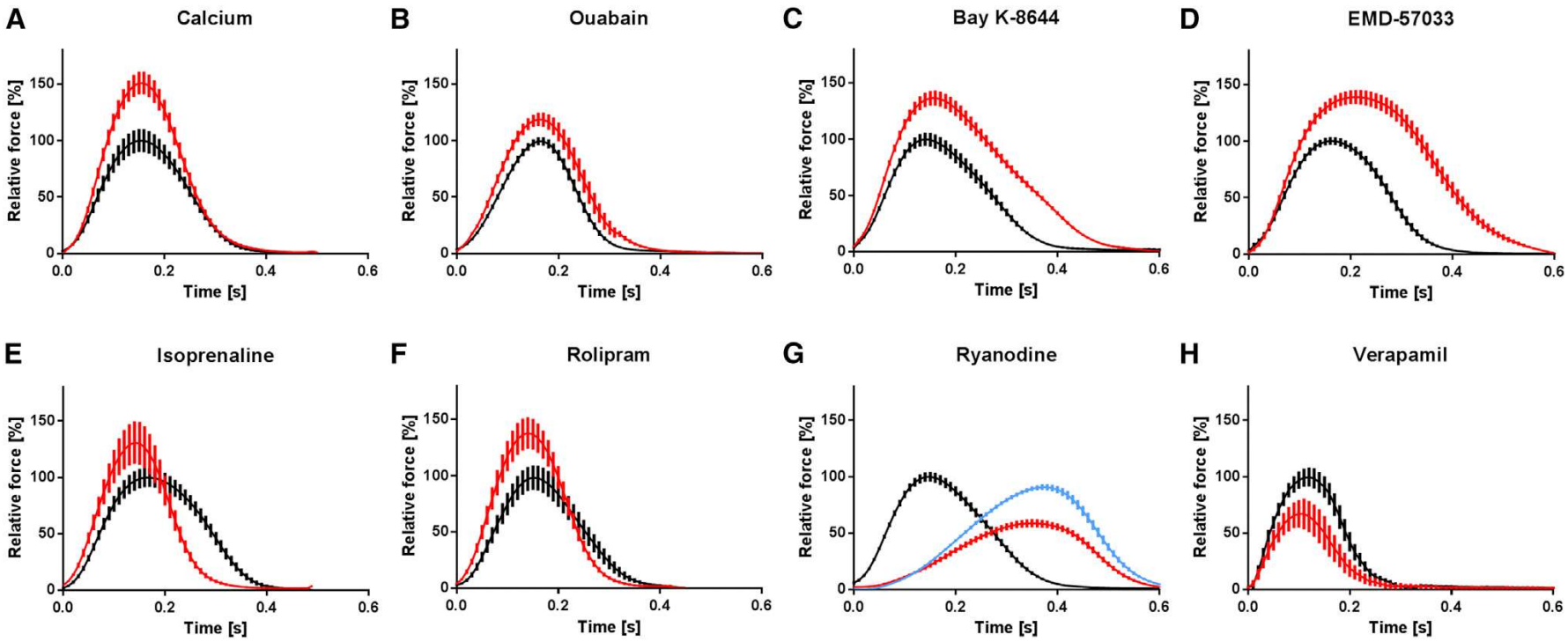

Figure 5. Regulation of Contractile Function of hiPSC-EHTs by Inotropic Drugs

Average contraction peaks before (black) and after (red) the respective inotropic drug intervention.

(A–H) Positive inotropic drugs (A–F) were tested at submaximal (0.5–0.6 mM), and negative inotropic drugs (G and H) at high (1.8 mM; H) and submaximal (0.5 mM; G) calcium concentrations. Depicted is the electrically stimulated (1.5–2 Hz) mean relative force in percentage of baseline maximum ± SEM in modified Tyrode's solution; replicates are indicated as EHTs/number of independent experiments. (A) calcium (5 mM; n = 8/2). (B) ouabain (100 nM; n = 6/2). (C) Bay K-8644 (300 nM; n = 4/1). (D) EMD-57033 (10 μM; n = 4/1). (E) isoprenaline (100 nM; n = 4/1). (F) rolipram (10 μM) + isoprenaline (100 nM, red) versus isoprenaline (100 nM, black; n = 11/2). (G) ryanodine (0.3 μM, red; 10 μM, blue; n = 6/2). (H) verapamil (1 μM; n = 18/2).

See also Figure S5.

Mannhardt *et al.*, Stem Cell Report 2016

LETTER

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Advanced maturation of human cardiac tissue grown from pluripotent stem cells

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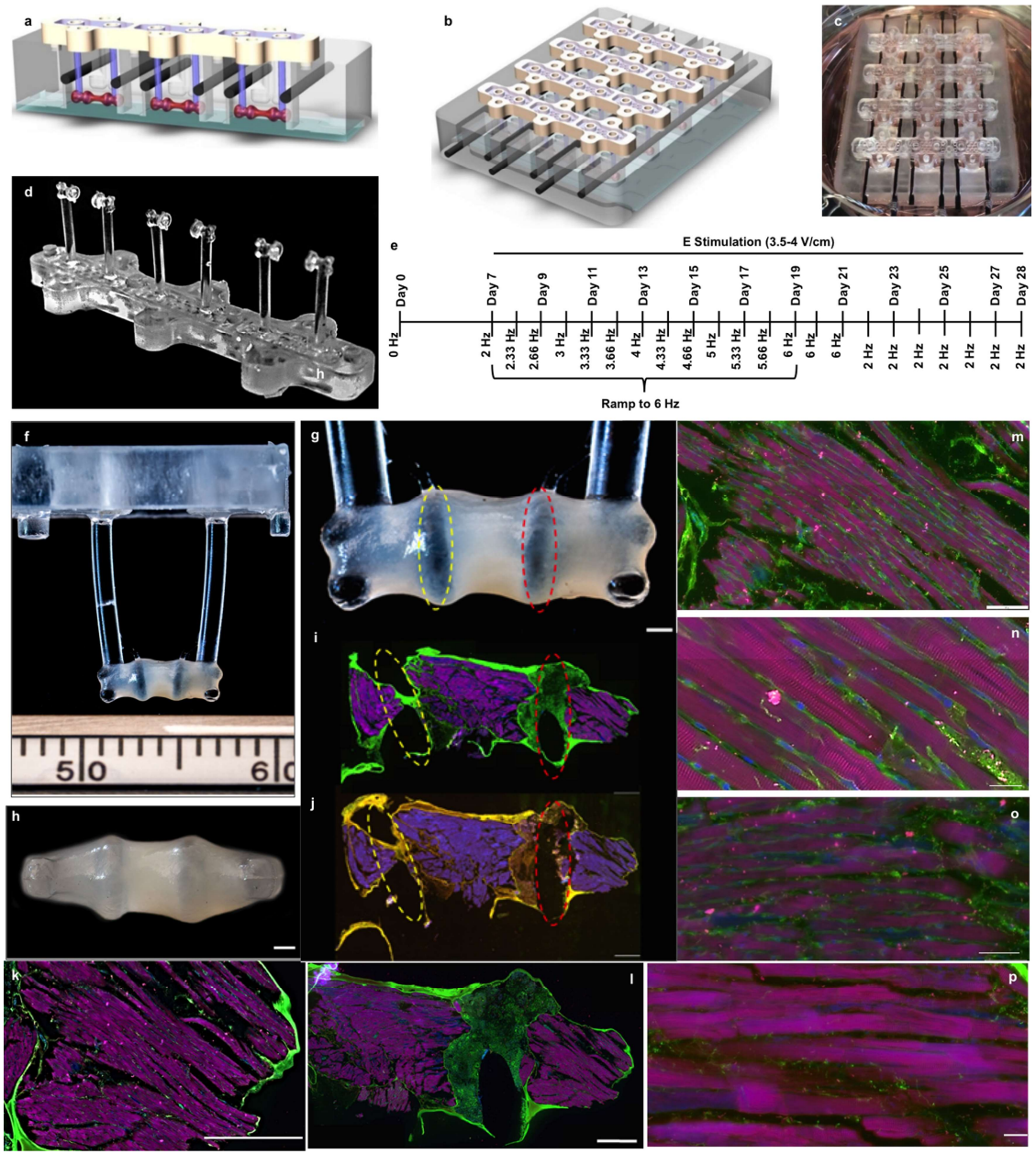
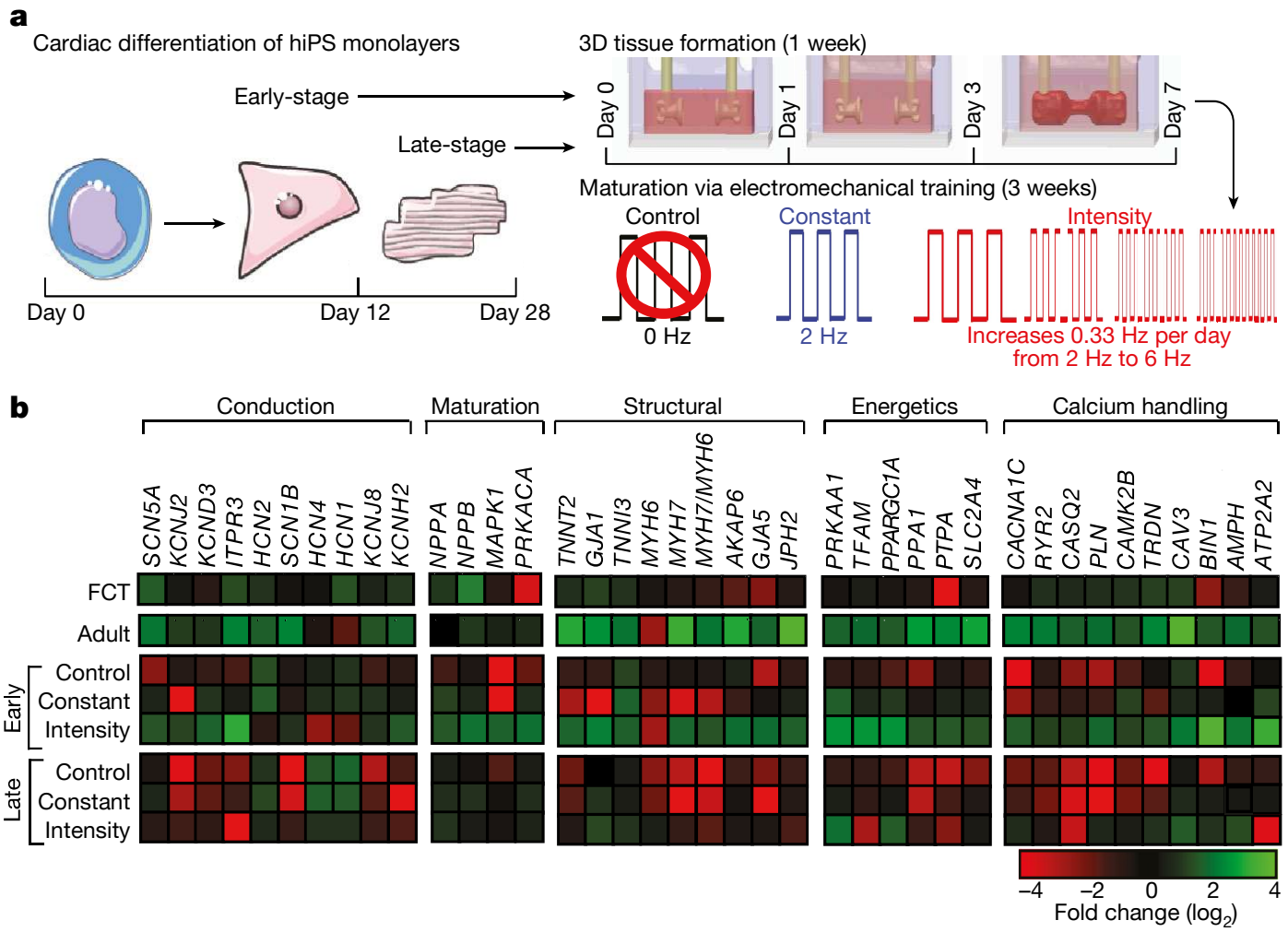
Early-stage intensity-trained tissues



Ronaldson-Bouchard *et al.*, Nature 2018

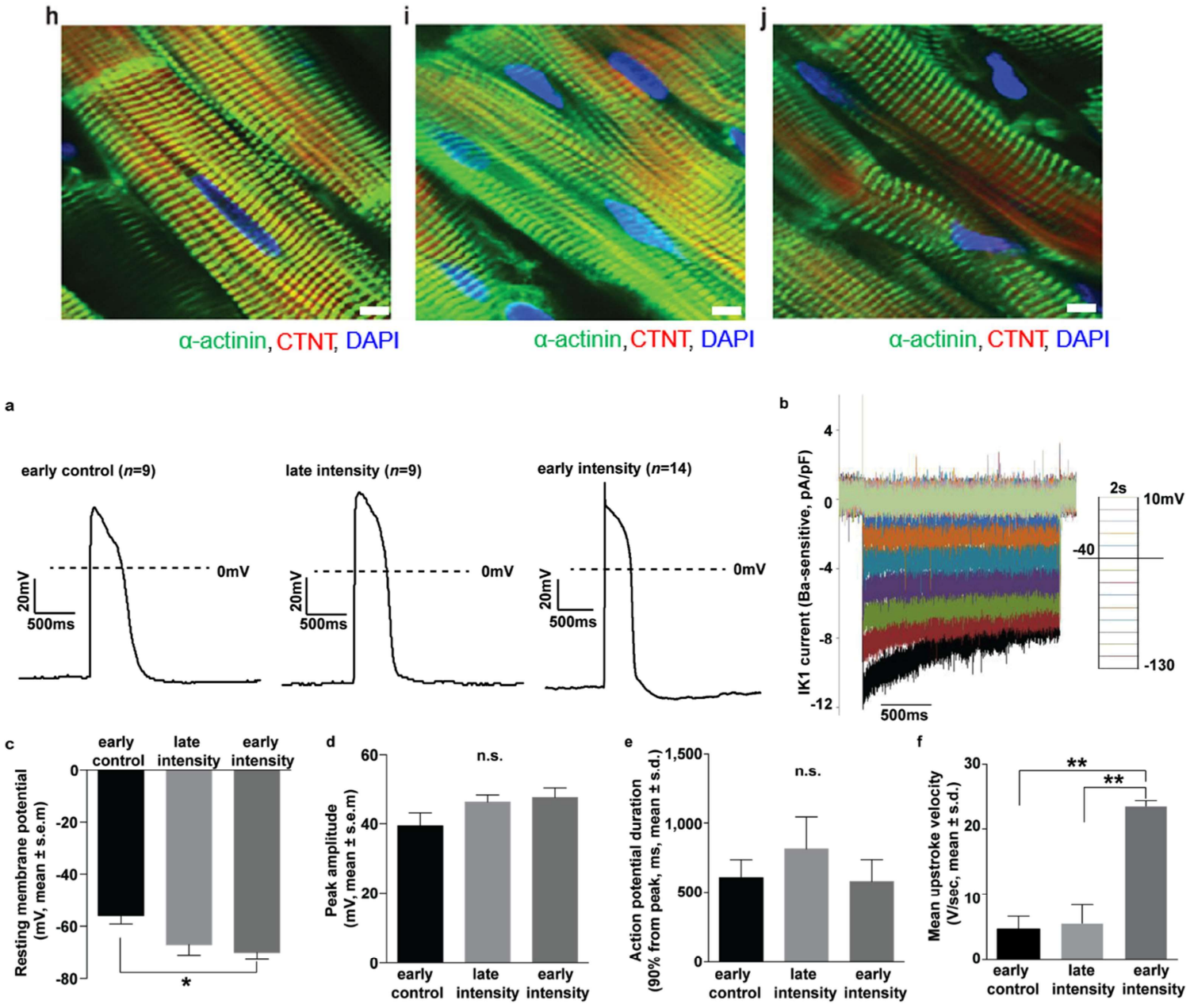
Early-stage intensity-trained tissues

RESEARCH LETTER



Ronaldson-Bouchard *et al.*, Nature 2018

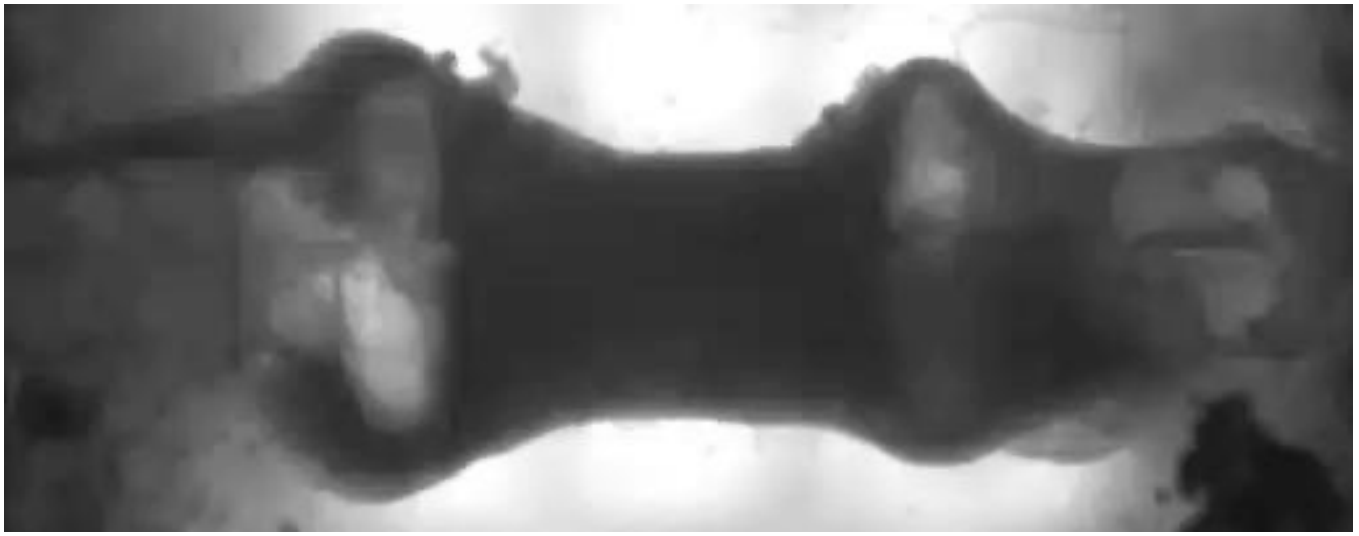
Early-stage intensity-trained tissues



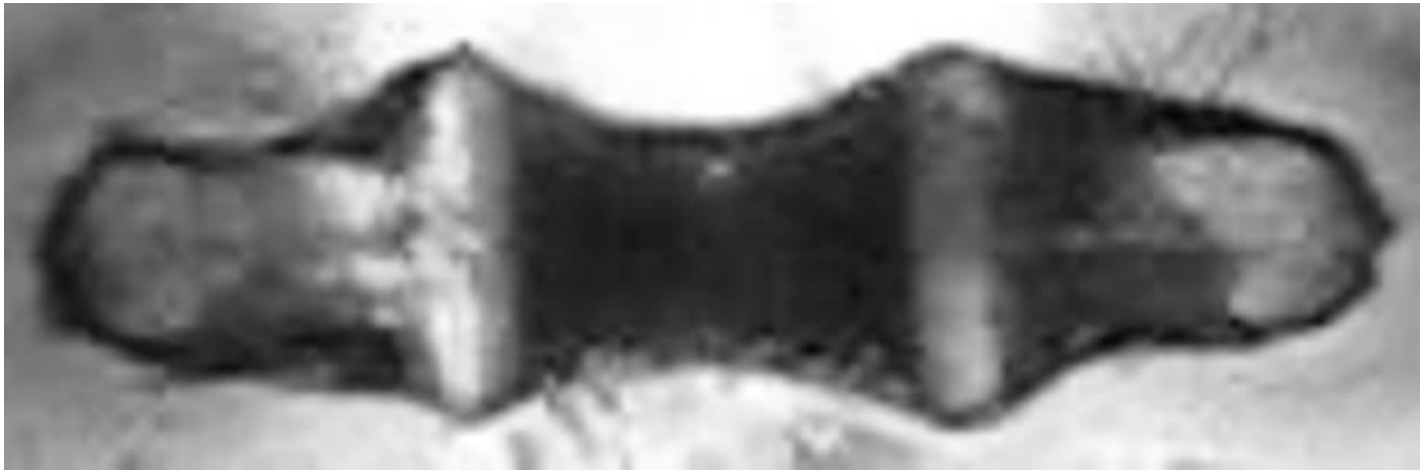
Ronaldson-Bouchard *et al.*, Nature 2018

Early- vs late-stage intensity-trained tissues

Early-stage intensity-trained tissues

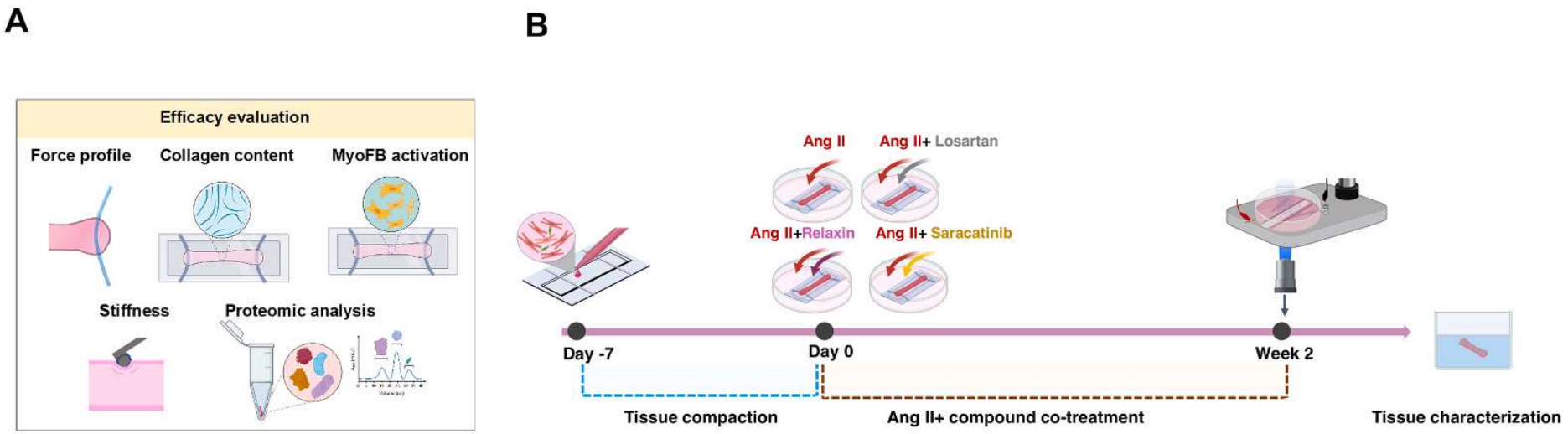


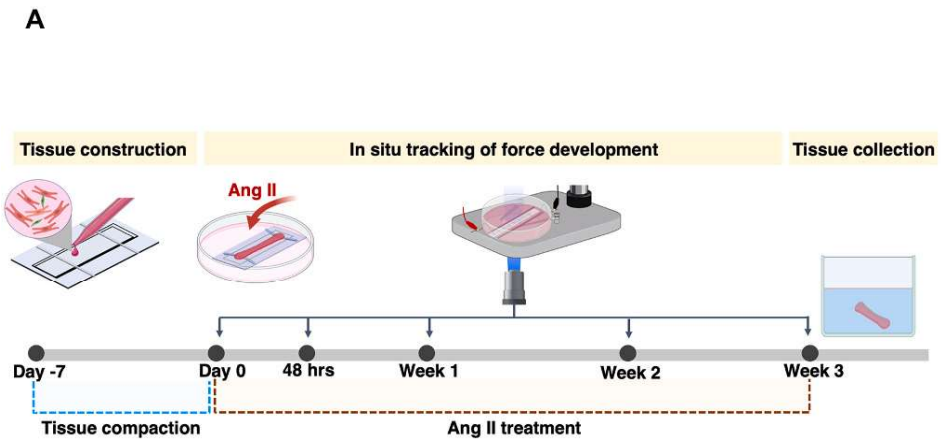
Late-stage intensity-trained tissues

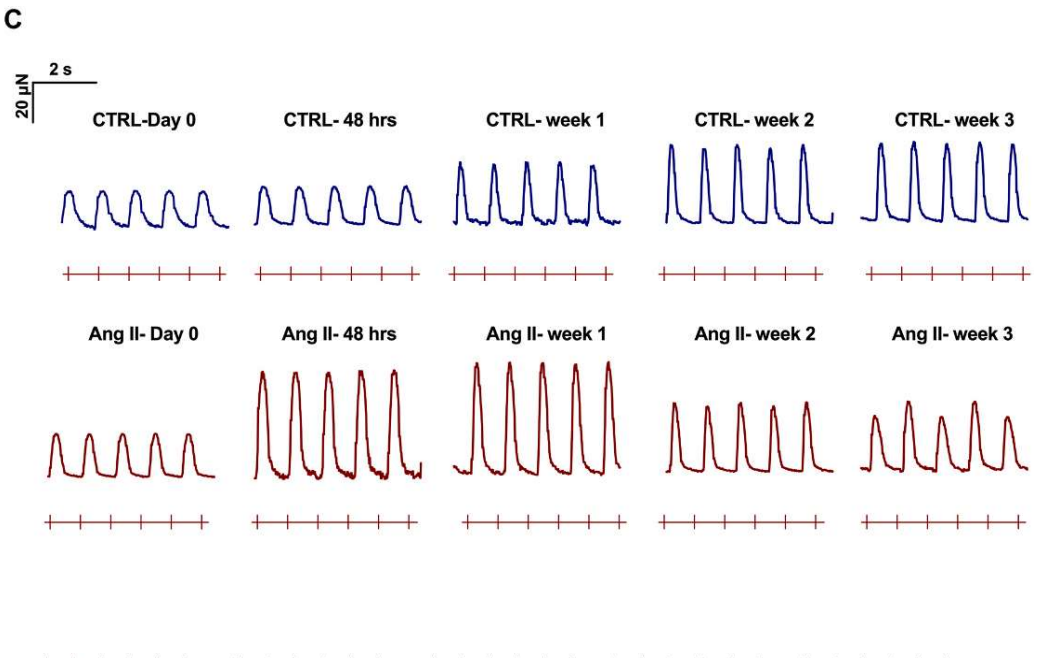


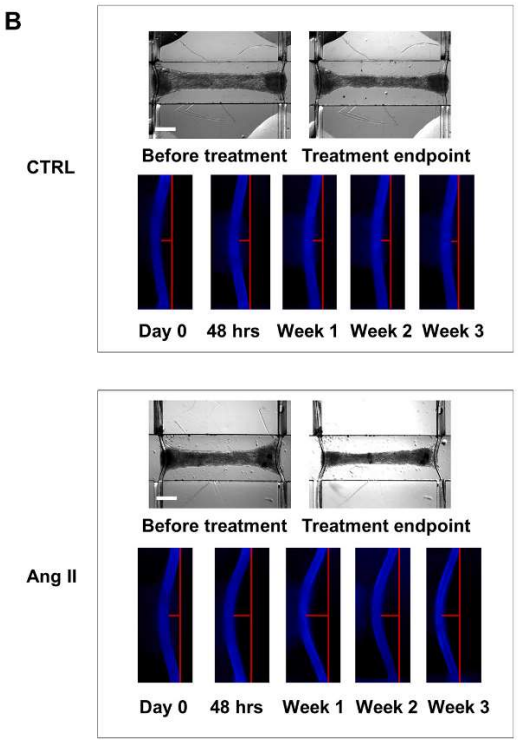
An organ-on-a-chip model for pre-clinical drug evaluation in progressive non-genetic cardiomyopathy

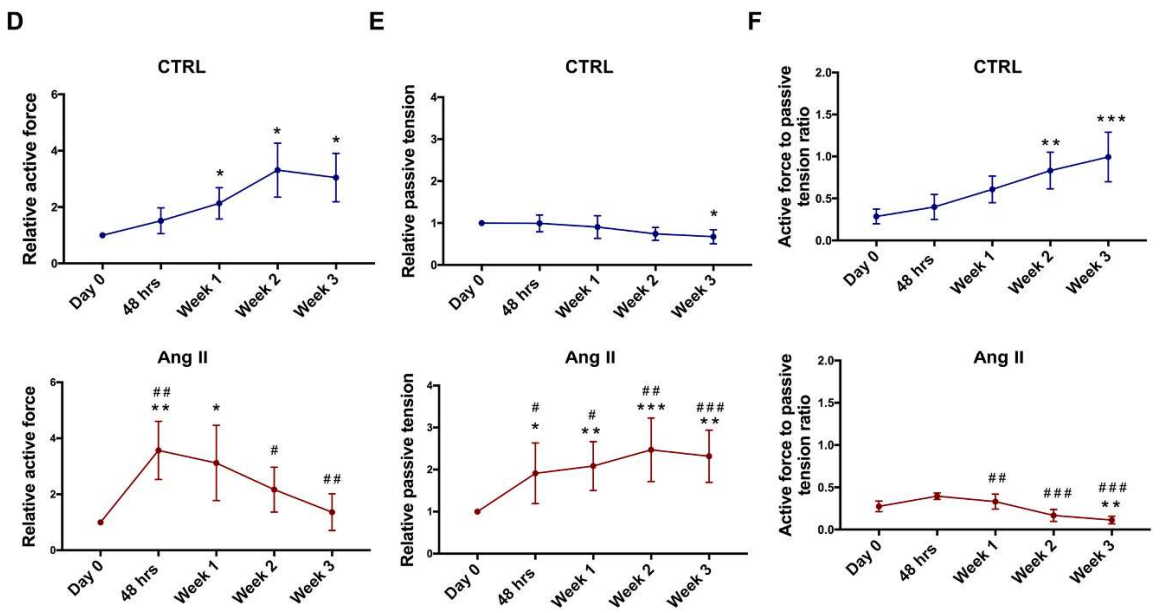
Erika Yan Wang [a], Uros Kuzmanov [b,c], Jacob B. Smith [d], Wenkun Dou [e], Naimeh Rafatian [f], Benjamin Fook Lun Lai [a], Rick Xing Ze Lu [a], Qinghua Wu [a], Joshua Yazbeck [a], Xiao-Ou Zhang [g], Yu Sun [e], Anthony Gramolini [b,c], Milica Radisic [a,d,f,*]











One-way repeated measures ANOVA over time (p-value)				
Comparison	48 hrs-day 0	week 1-day 0	week 2-day 0	week 3-day 0
CTRL	NS	0.0461	0.0261	0.0271
Ang II	0.0027	0.013	NS	NS

One-way repeated measures ANOVA over time (p-value)				
Comparison	48 hrs-day 0	week 1-day 0	week 2-day 0	week 3-day 0
CTRL	NS	NS	NS	0.0429
Ang II	0.0366	0.0096	0.0004	0.0014

One-way repeated measures ANOVA over time (p-value)				
Comparison	48 hrs-day 0	week 1-day 0	week 2-day 0	week 3-day 0
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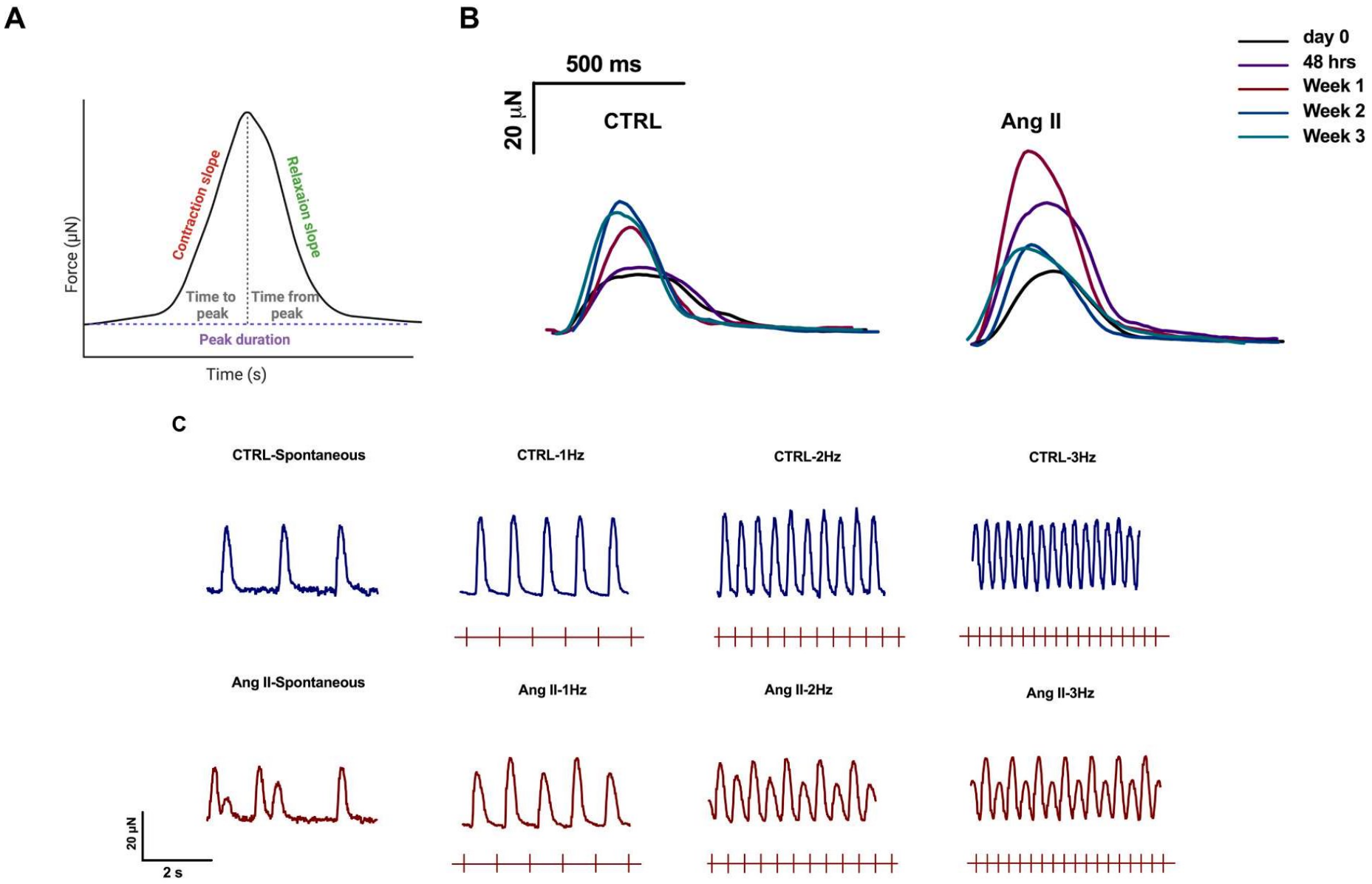
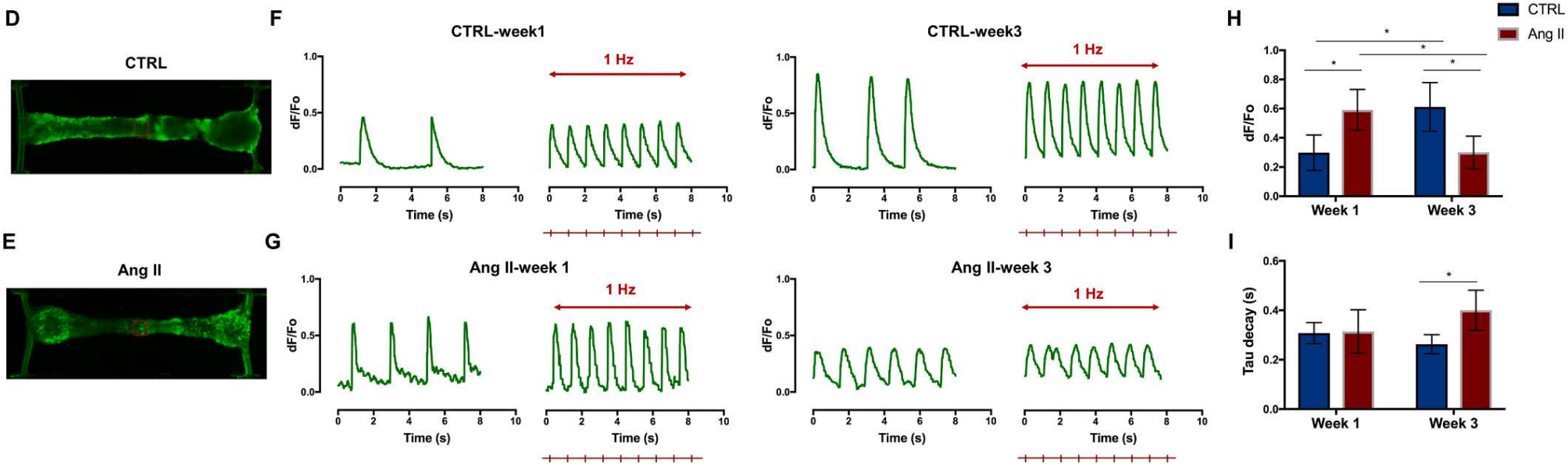
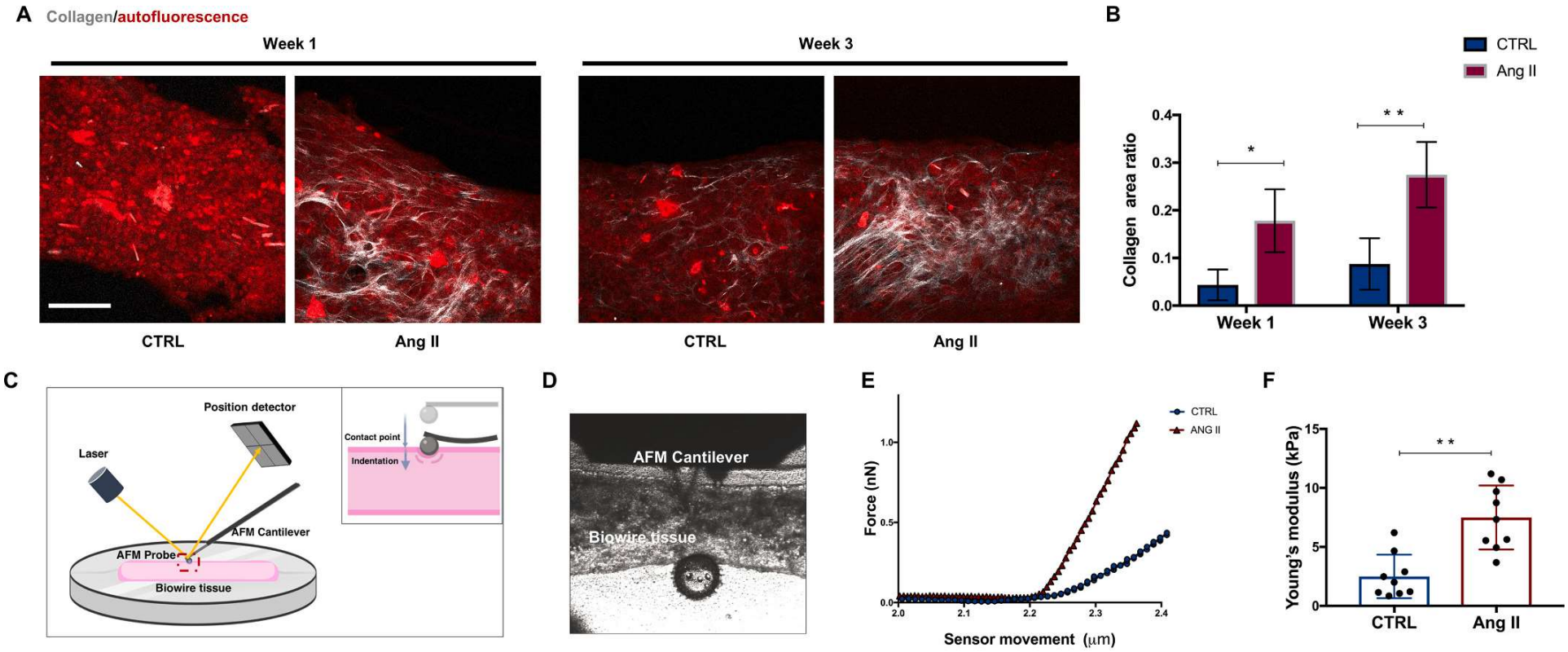
Ang II	NS	NS	NS	0.0065


A
Force (μN)
Contraction slope
Relaxaion slope
Time to peak
Time from peak
Peak duration
Time (s)
B
500 ms
20 μN
CTRL
Ang II
day 0
48 hrs
Week 1
Week 2
Week 3
C
CTRL-Spontaneous
CTRL-1Hz
CTRL-2Hz
CTRL-3Hz
Ang II-Spontaneous
Ang II-1Hz
Ang II-2Hz
Ang II-3Hz
20 μN
2 s


D
CTRL
E
Ang II
F
CTRL-week1
1 Hz
dF/Fo
Time (s)
CTRL-week3
1 Hz
G
Ang II-week 1
1 Hz
Ang II-week 3
1 Hz
H
CTRL
Ang II
dF/Fo
Week 1
Week 3
I
Tau decay (s)
Week 1
Week 3


A
Collagen/autofluorescence
Week 1
Week 3
CTRL
Ang II
CTRL
Ang II
B
CTRL
Ang II
Collagen area ratio
0.4
0.3
0.2
0.1
0.0
*
* *
Week 1
Week 3
C
Position detector
Laser
AFM Cantilever
AFM Probe
Biowire tissue
Contact point
Indentation
D
AFM Cantilever
Biowire tissue
E
CTRL
ANG II
Force (nN)
1.0
0.5
0.0
2.0
2.1
2.2
2.3
2.4
Sensor movement (μm)
F
Young's modulus (kPa)
15
10
5
0
* *
CTRL
Ang II