Stemonix

Accelerating the Discovery of New Medicines

Morphologies, Motions and Markers of In Vitro Cardiovascular Screening Assessments

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The speaker is a paid employee of StemoniX Inc., a human-based stem cell company that provides screening services and next-generation iPSC-derived cardiomyocytes and neurons in patterned and 3D ready-to-use formats.

Morphologies, Motions and Markers of In Vitro Cardiovascular Screening Assessments

Goal: Provide an overview of iPSC cardiomyocyte biology and use in functional toxicity testing

Outline:

iPSC Primer

iPSC Cardiomyocyte Functional Overview

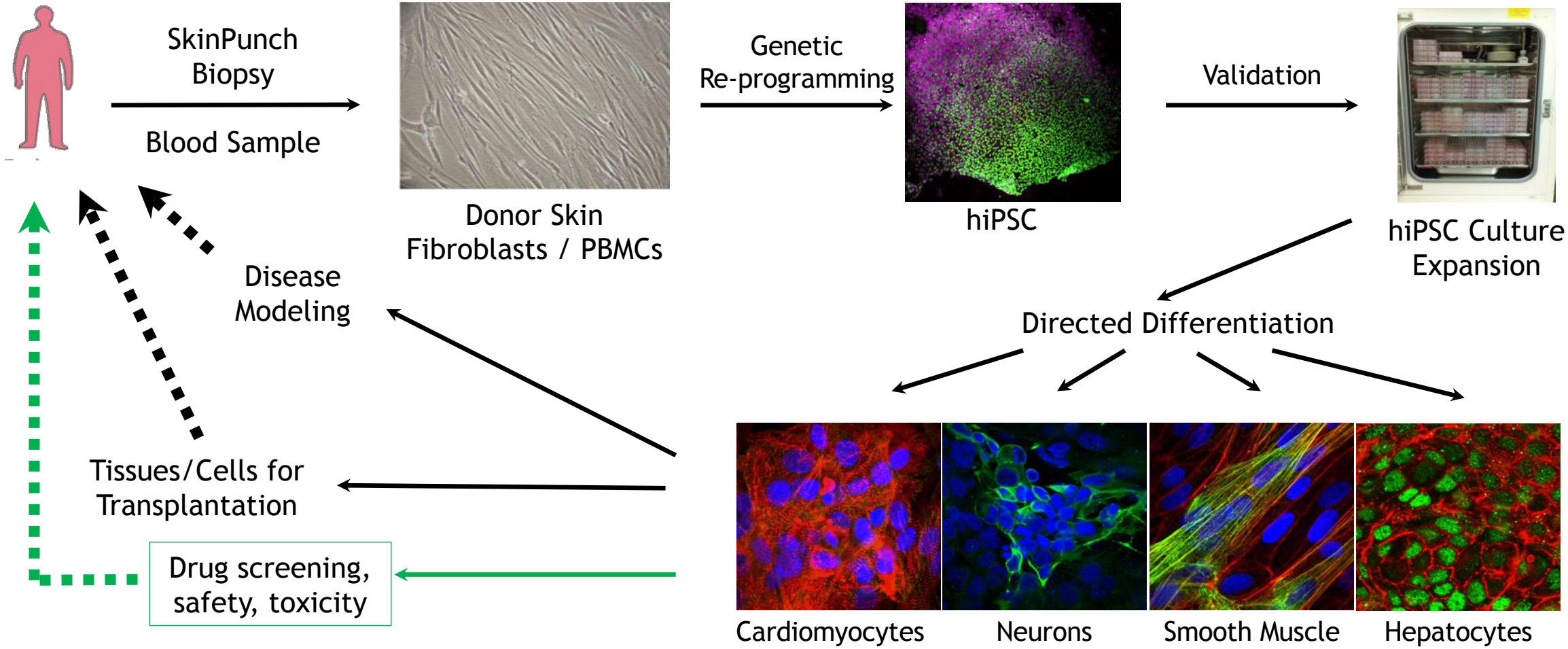
Screening endpoints (markers)

- Electrophysiology
- Ca^{2+} Signaling
- Contraction
- Energetics

Morphology

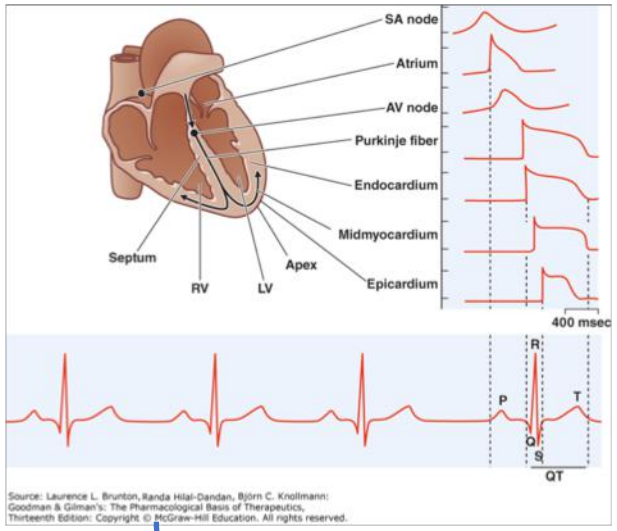
Summary

Human induced Pluripotent Stem Cells (hiPSCs) Provide Human Material for Basic and Applied Research

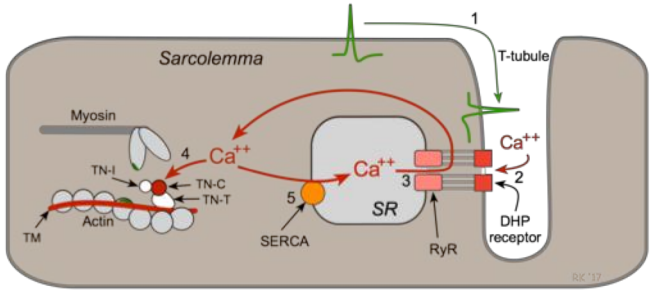


IPSC Cardiomyocytes Provide Markers for Functional Assessment

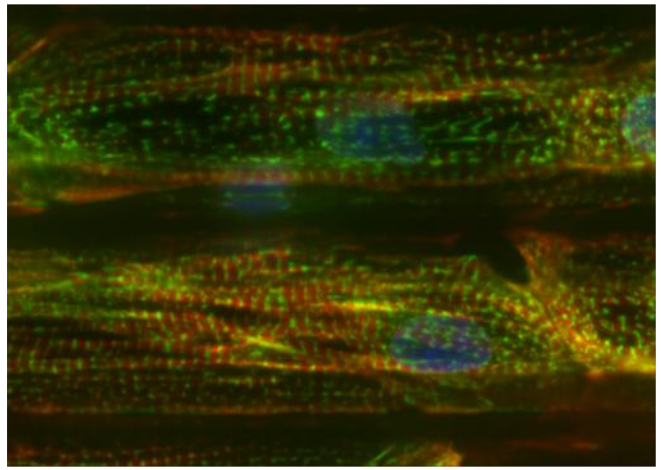
Membrane Electrical Activity (Ion channels, receptors, etc)



Ca^{2+} signaling (EC Coupling)



Contraction



DNA Sarc. αActinin cTnT

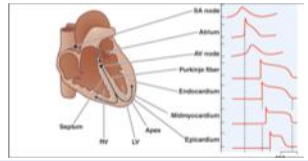
Electrical activity at the membrane causes calcium release from the SR which in turn enables contraction

Energetics

Functional activity is highly energy dependent

Each of these nodes is critical for proper cardiac function and thus offer markers for CV toxicity assessment

IPSC Cardiomyocytes Show Typical Electrophysiology

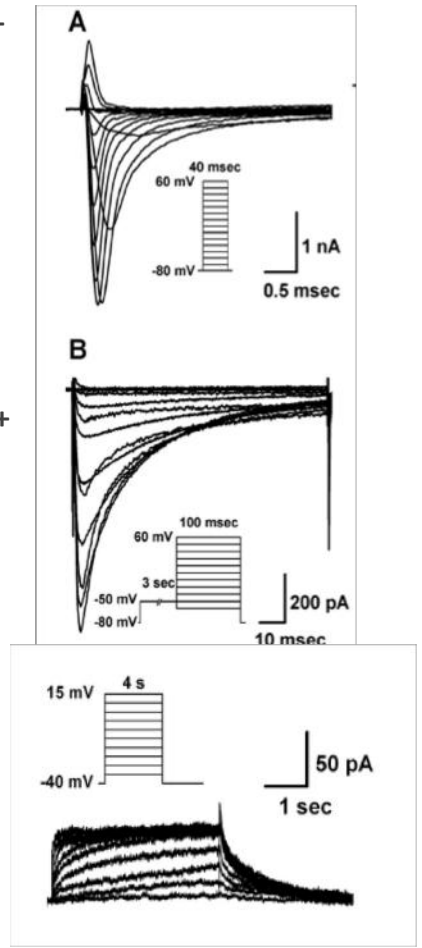


Ionic Currents

Na^+

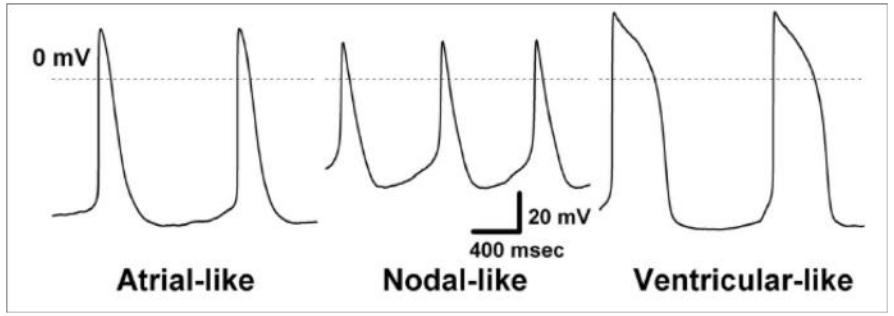
Ca^{2+}

K^+

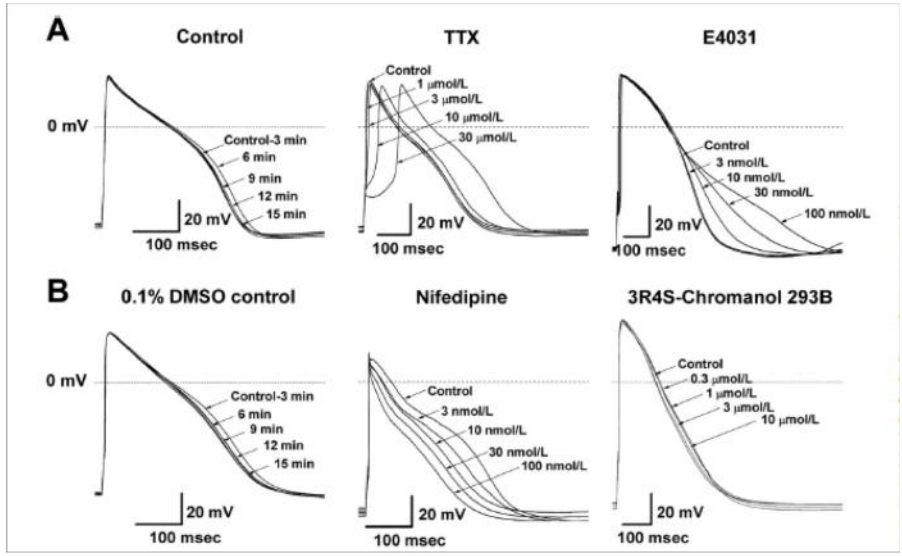


Ma et al., 2011

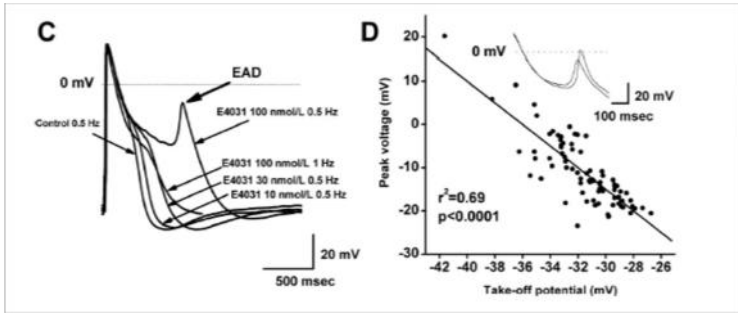
Action Potentials



Drug Block



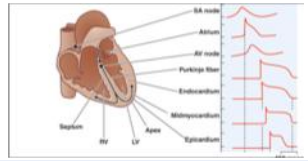
Arrhythmogenic Triggers



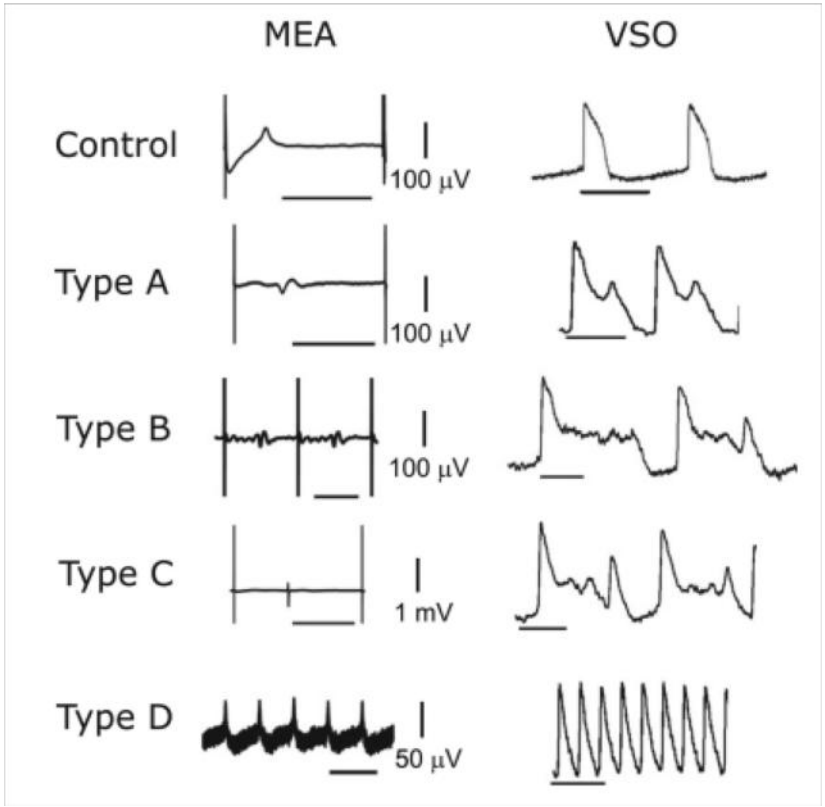
iPSC cardiomyocytes show 'typical' electrophysiology and drug induced effects

A basic biomarker is altered electrical activity

IPSC Cardiomyocytes Electrophysiology and Clinical Translation

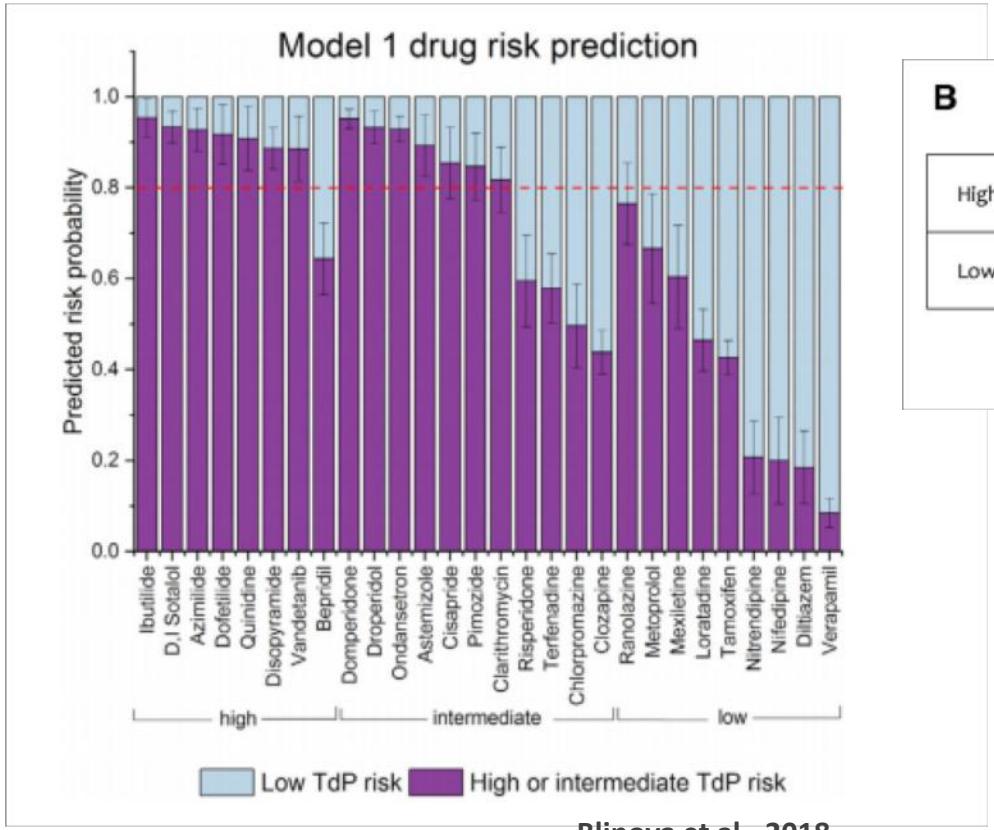


MEA and VSO Measurements



Blinova et al., 2018

Translation to the clinic



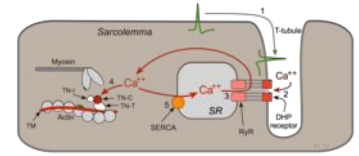
Blinova et al., 2018

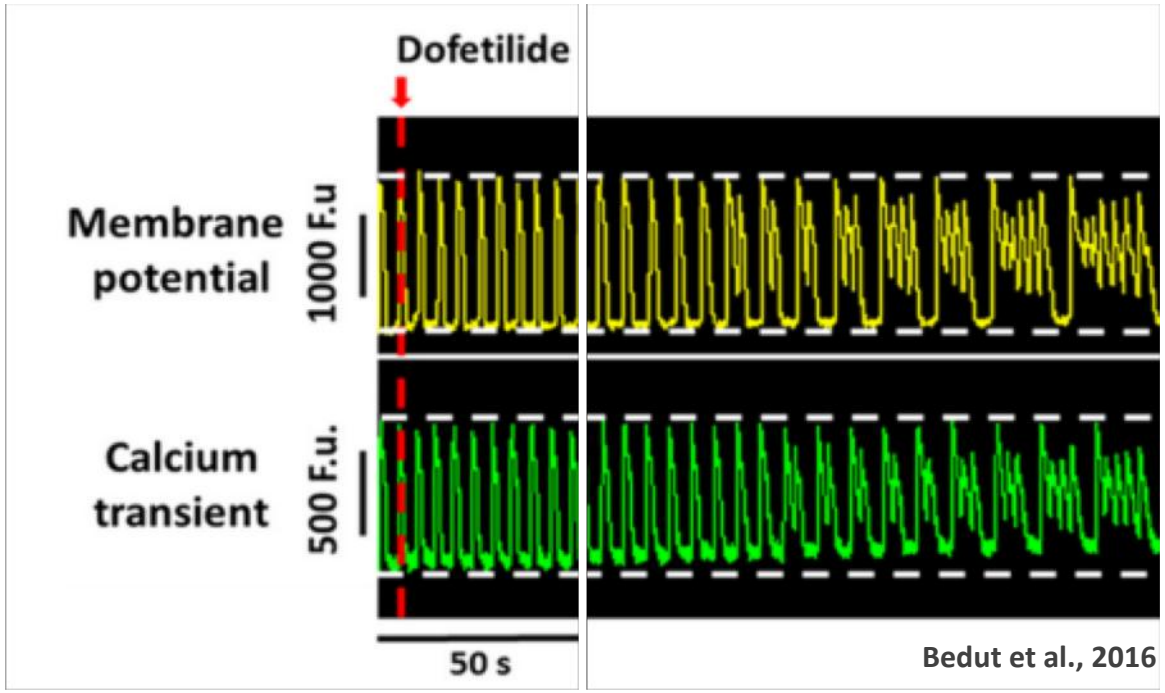
B	Known risk of TdP	Other categories	
High risk	17	2	
Low risk	4	13	
	81	87	83
	Sensitivity	Specificity	Accuracy

Ando et al., 2017

Basic electrophysiology markers translate to clinical effects

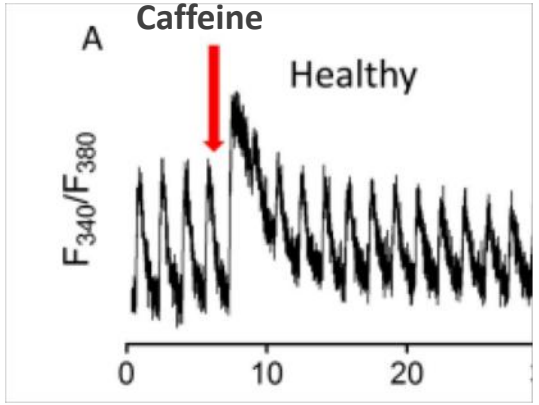
IPSC Cardiomyocytes and Ca^{2+} handling





Bedut et al., 2016

Typically, Ca^{2+} cycling follows membrane voltage, making it difficult to separate the two processes

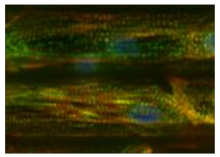


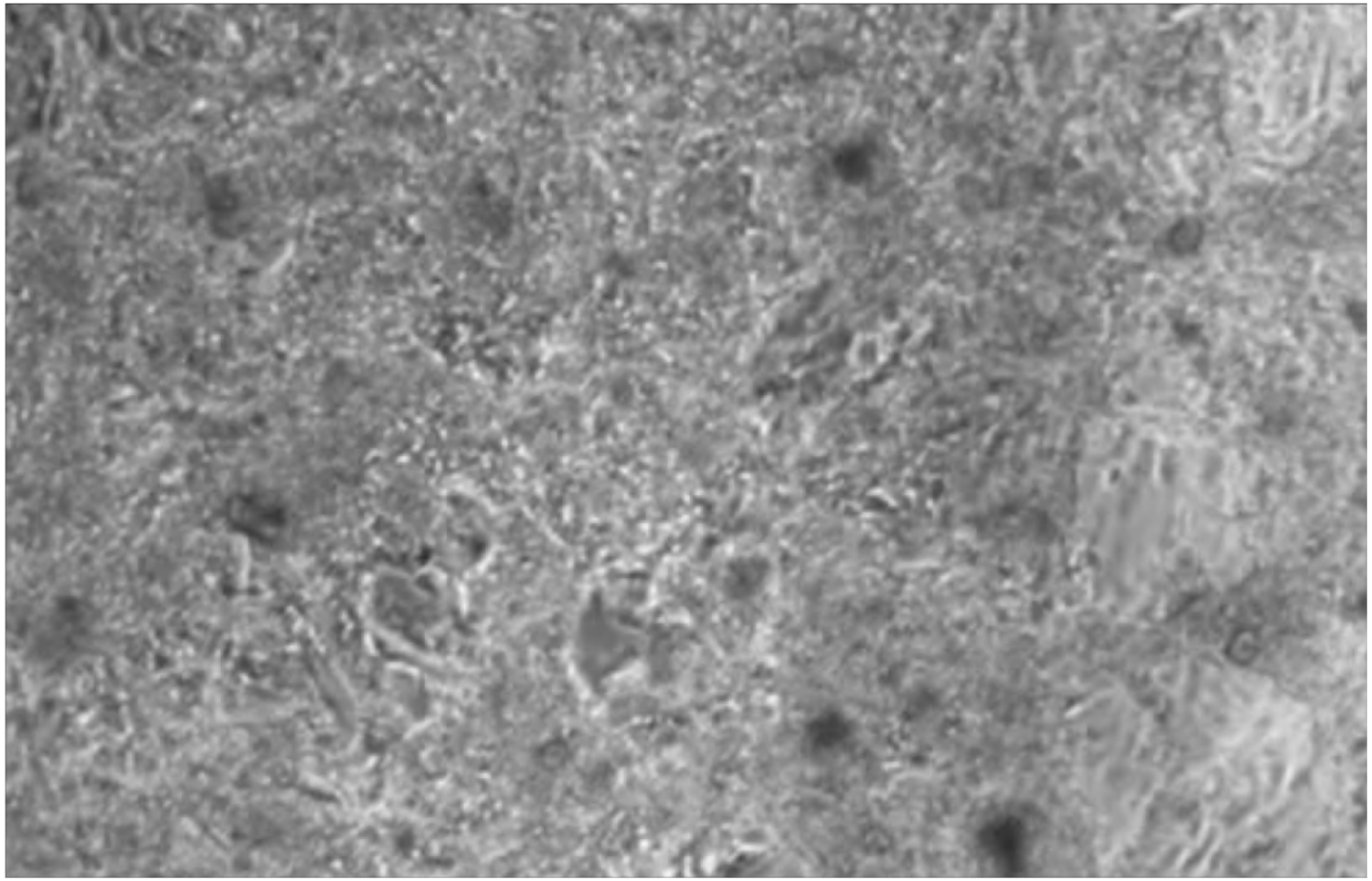
Schick et al., 2018

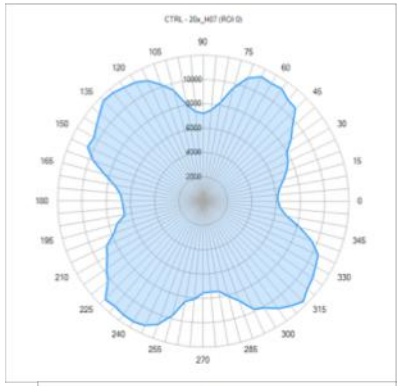
Compounds, such as caffeine, target Ca^{2+} cycling

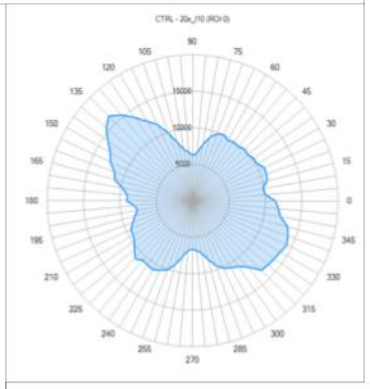
Additional testing with compounds targeting RYR and/or SERCA under normal and stressed conditions may be necessary to isolate Ca^{2+} cycling specific effects.

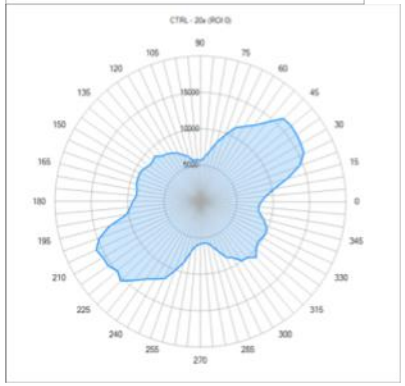
IPSC Cardiomyocytes and Contractility







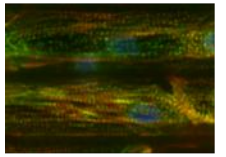




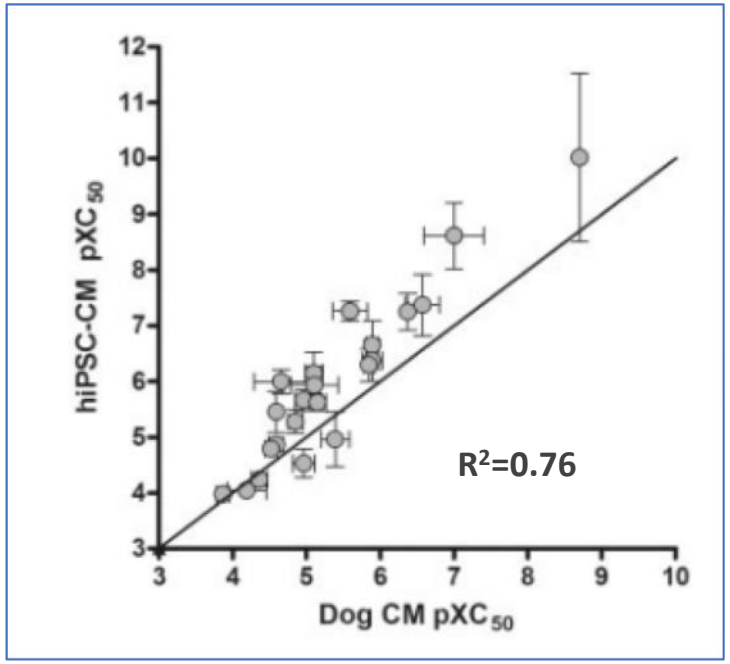
Contraction is multidimensional in non-patterned iPSC cardiomyocytes

SI8000 Motion Vector Software

IPSC Cardiomyocytes and Contractility – Comparison with current models



iPSC cardiomyocytes show comparable potency to gold standard canine sarcomeric shortening



Scott et al., 2014

iPSC cardiomyocytes show comparable assay parameters to other preclinical models

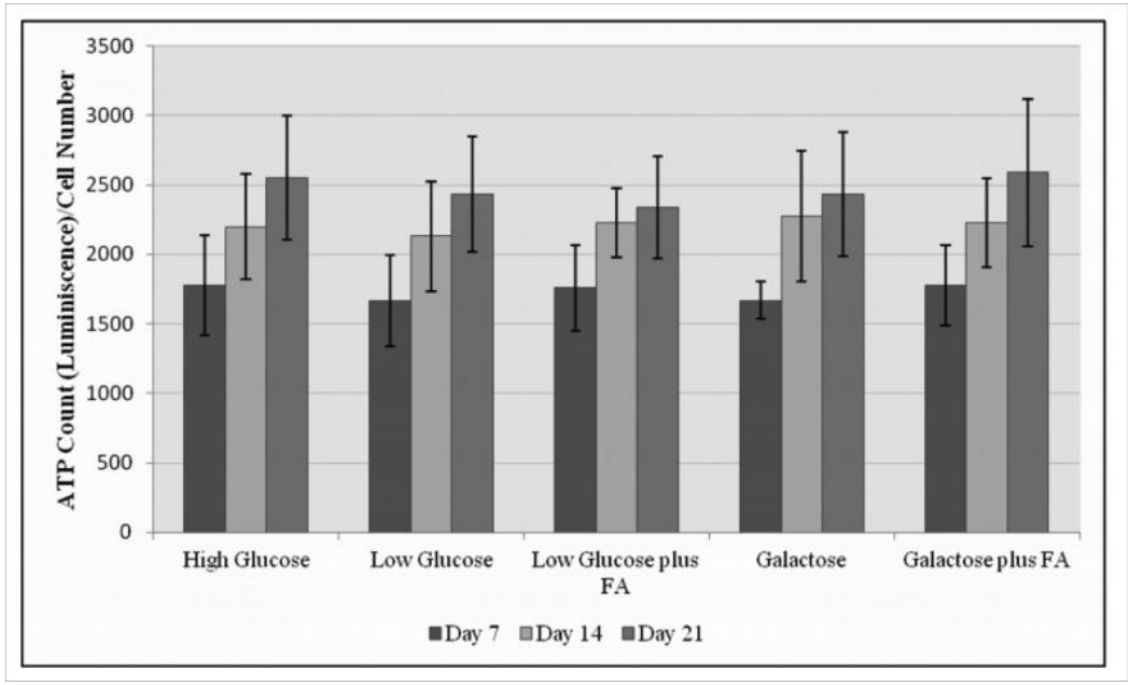
TABLE 2. Translational Predictivity of CM Assays

Assay parameter	hiPSC-CM impedance	Rat CM impedance	Dog CM sarcomere shortening
Sensitivity	90%	77%	83%
Specificity	74%	74%	84%
Accuracy	82%	74%	82%
Neg predictivity	82%	67%	76%
Pos predictivity	84%	82%	89%

iPSC-cardiomyocyte impedance measurements show good correlation across preclinical species

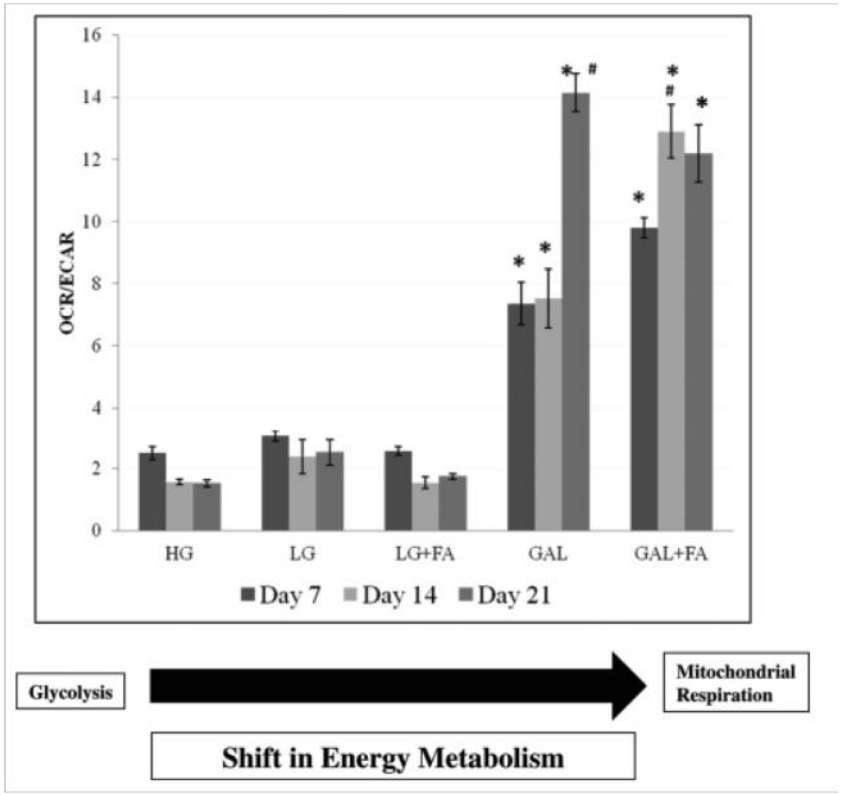
IPSC Cardiomyocytes and Energetics

ATP levels are relatively constant under various environmental conditions



Rana et al., 2012

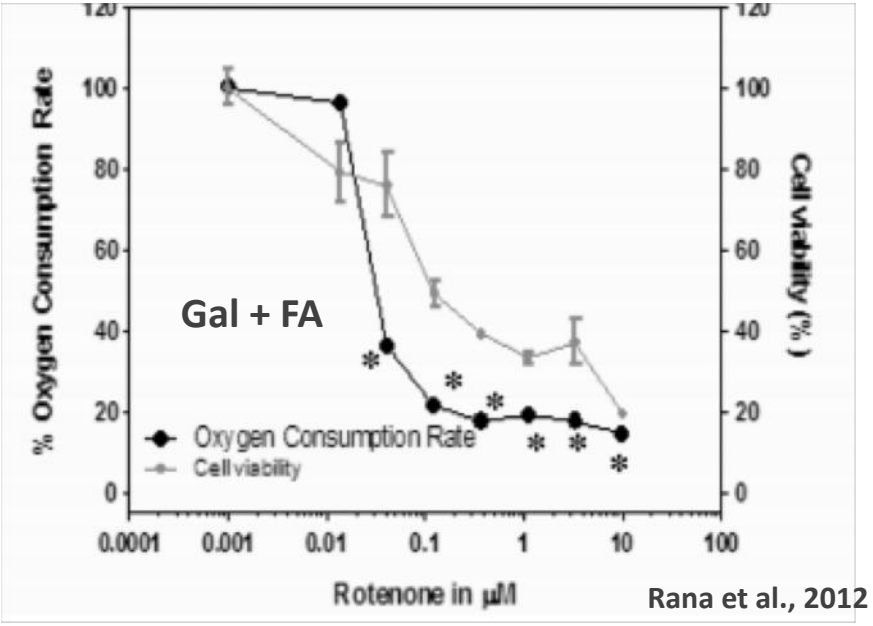
Metabolic processes shifted depending on environmental conditions



Similar to adult cardiomyocytes, iPSC-cardiomyocytes utilize available energy resources

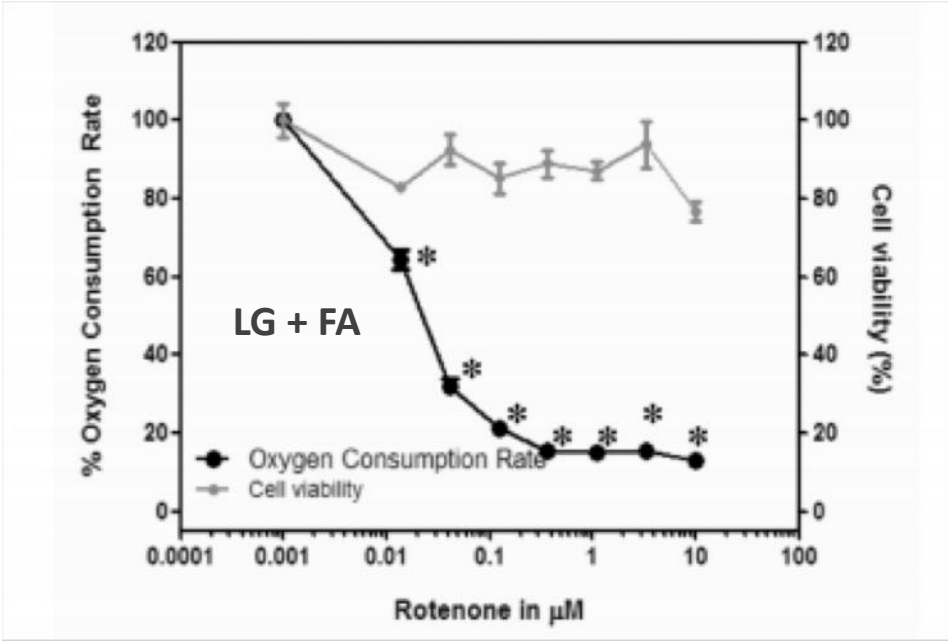
IPSC Cardiomyocytes Measuring Mitochondrial Toxicity

Oxygen consumption is a biomarker for mitochondrial toxicity



Rana et al., 2012

Overt toxicity can be masked when glucose is available



Biomarkers for mitochondrial toxicity need to directly measure mitochondrial function

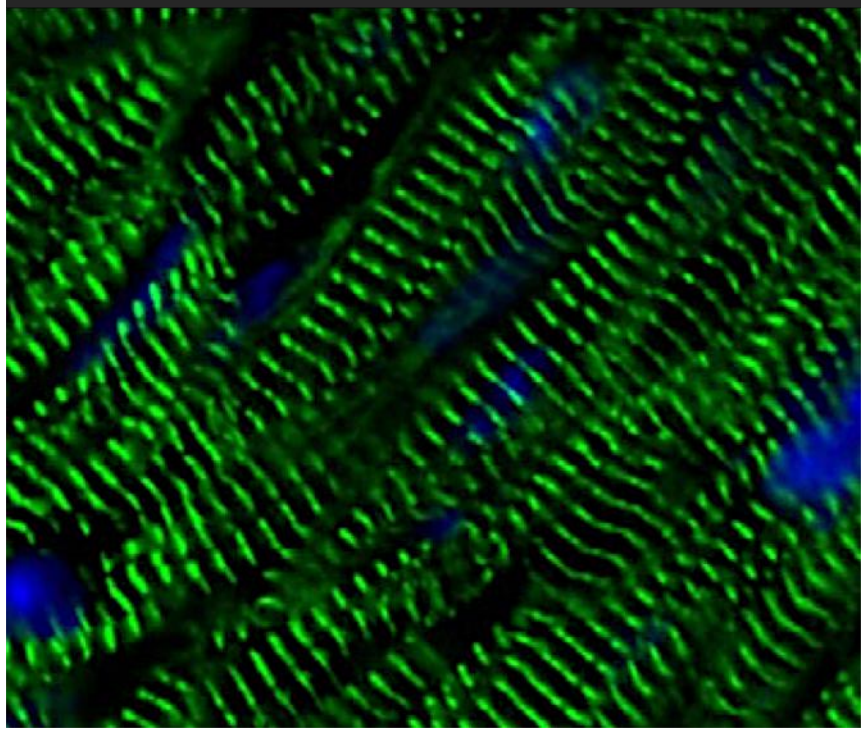
IPSC Cardiomyocytes Provide Markers for Functional Assessment

Biological process /	Biomarker	Measurement Techniques
Electrophysiology	Trans-membrane current Action potential	Manual and automated patch clamp MEA Voltage sensitive dyes Ca^{2+} sensitive dyes (surrogate) Impedance (surrogate)
Intracellular Ca^{2+}	Calcium sensitive dyes	Ca^{2+} sensitive dyes
Contractility	Shape / attachment relative to substrate	Impedance
	Movement	Vector Analysis Video microscopy
Mitochondrial Toxicity	Mitochondrial respiration	Oxygen consumption
	Mitochondrial membrane potential	Various dyes and kits

A variety of robust biomarkers and measurement techniques exist for functional assessment of cardiotoxicity

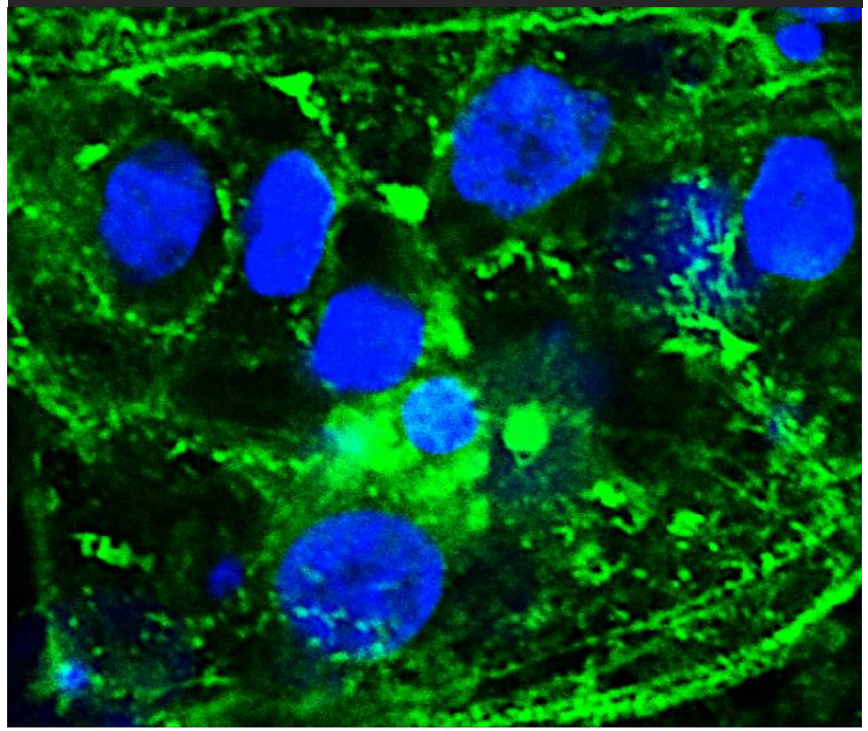
IPSC Cardiomyocytes and Morphology

Heart Tissue



Boyer et al., 2010

iPSC Cardiomyocytes



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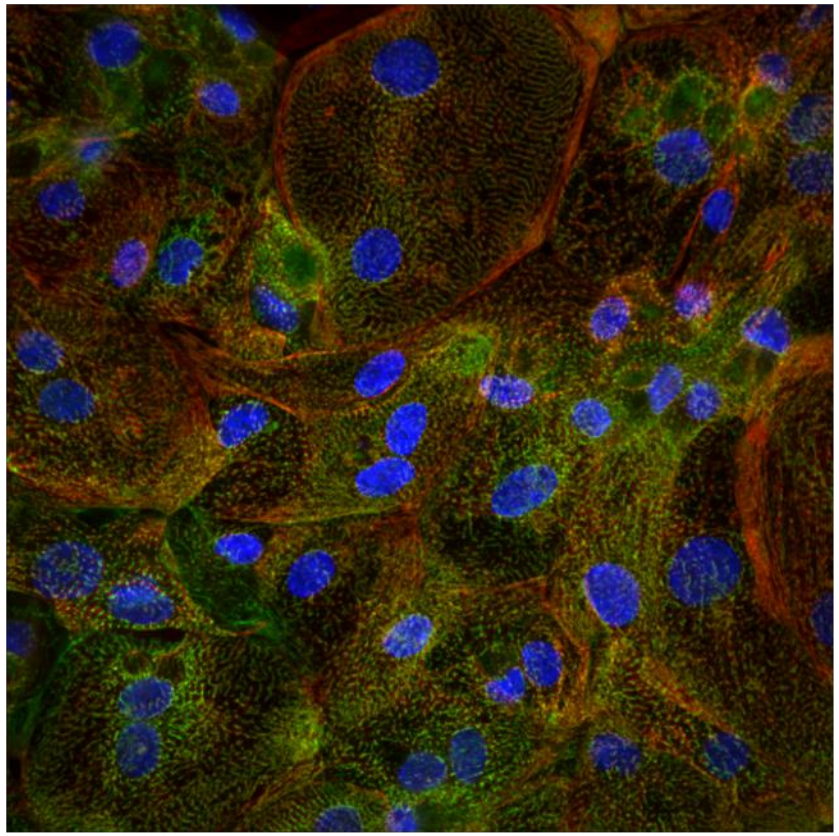
iPSC Cardiomyocytes on unstructured plates have an unstructured morphology

IPSC Cardiomyocytes Provide Markers for Functional Assessment

Typical 2D plating



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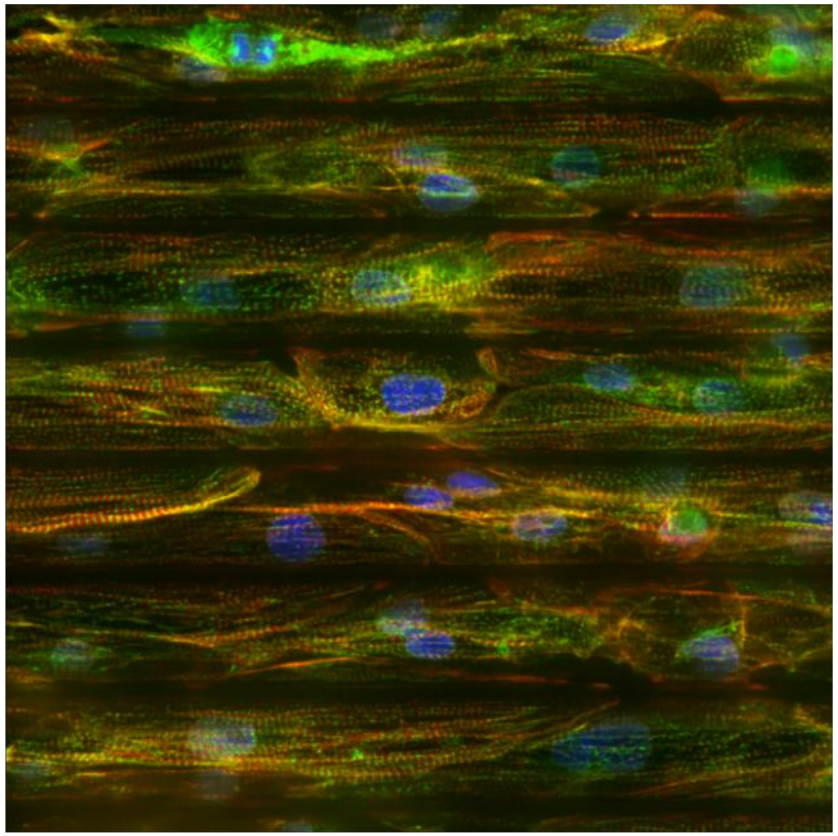
Plating on grooved surface

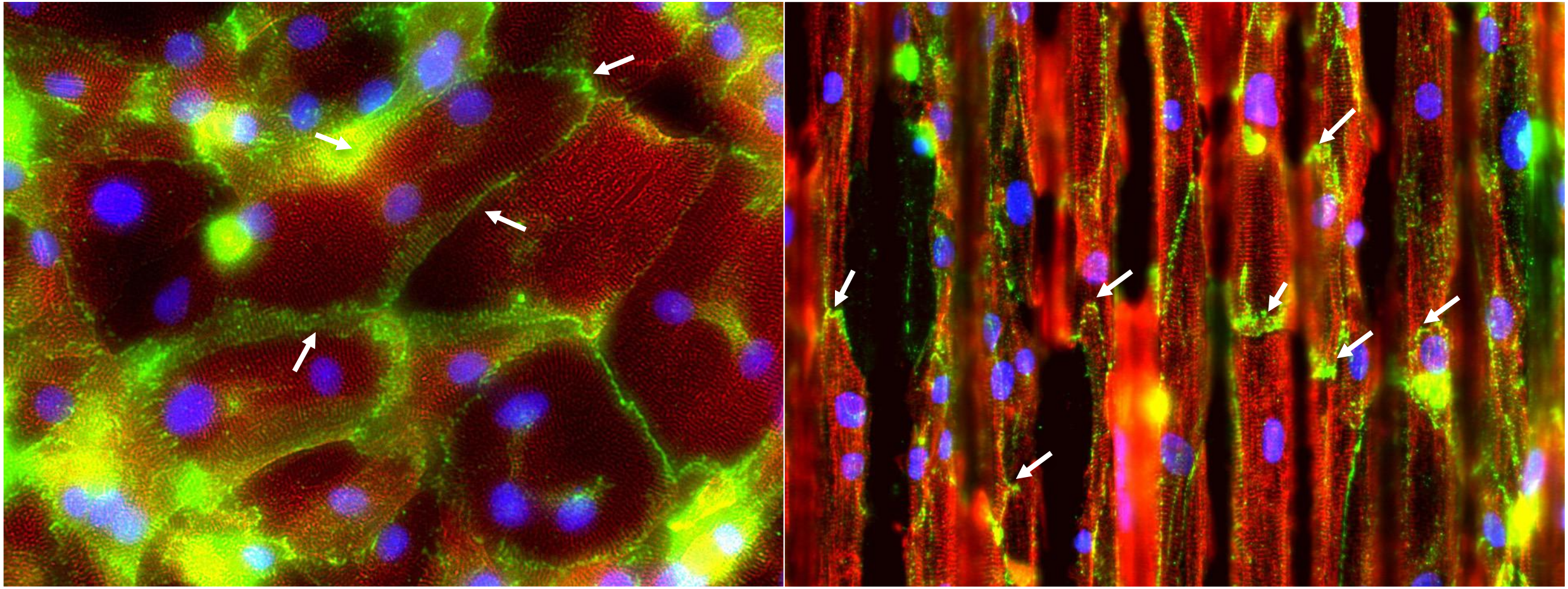


Stemonix microHeart

iPSC Cardiomyocytes can be structured

Alignment/Morphology has Consequences – Protein localization

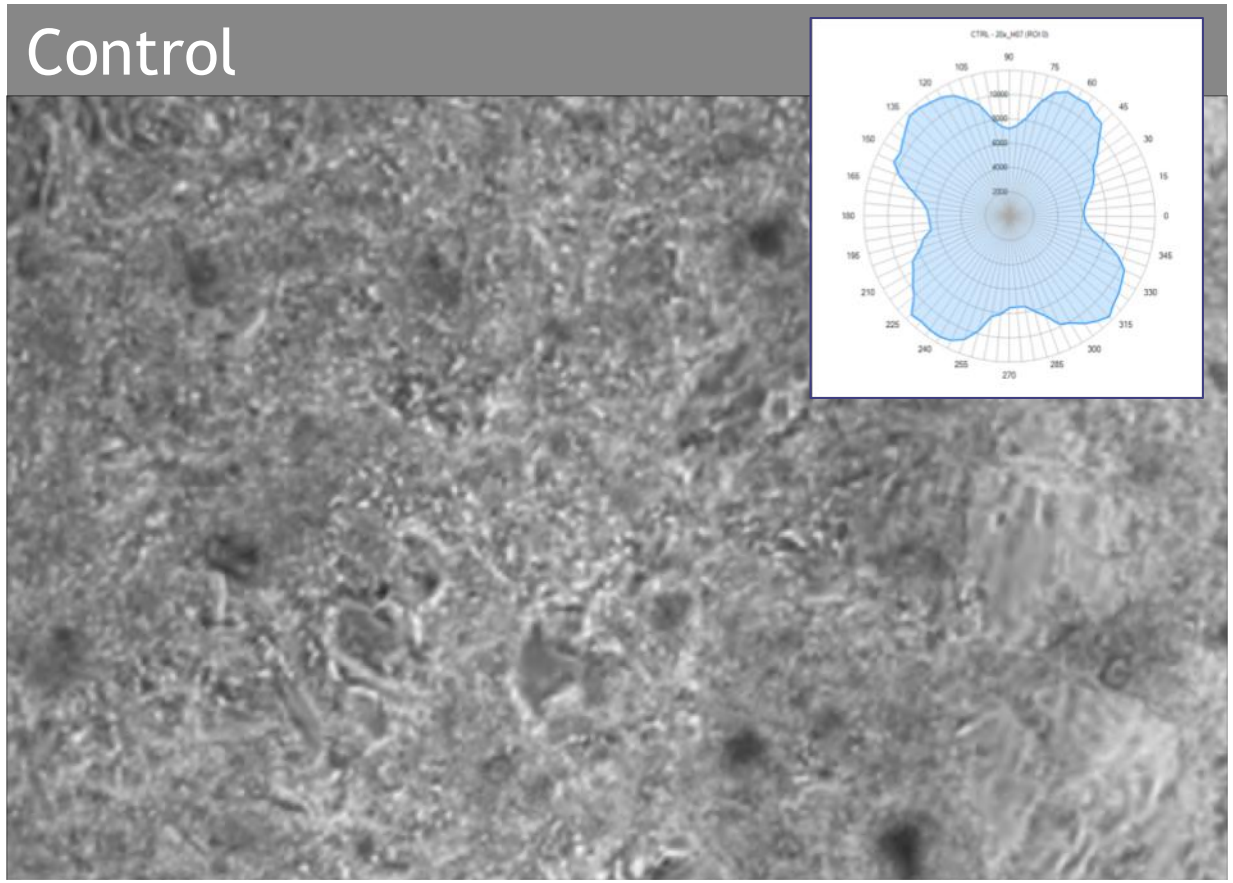
Control	microHeart

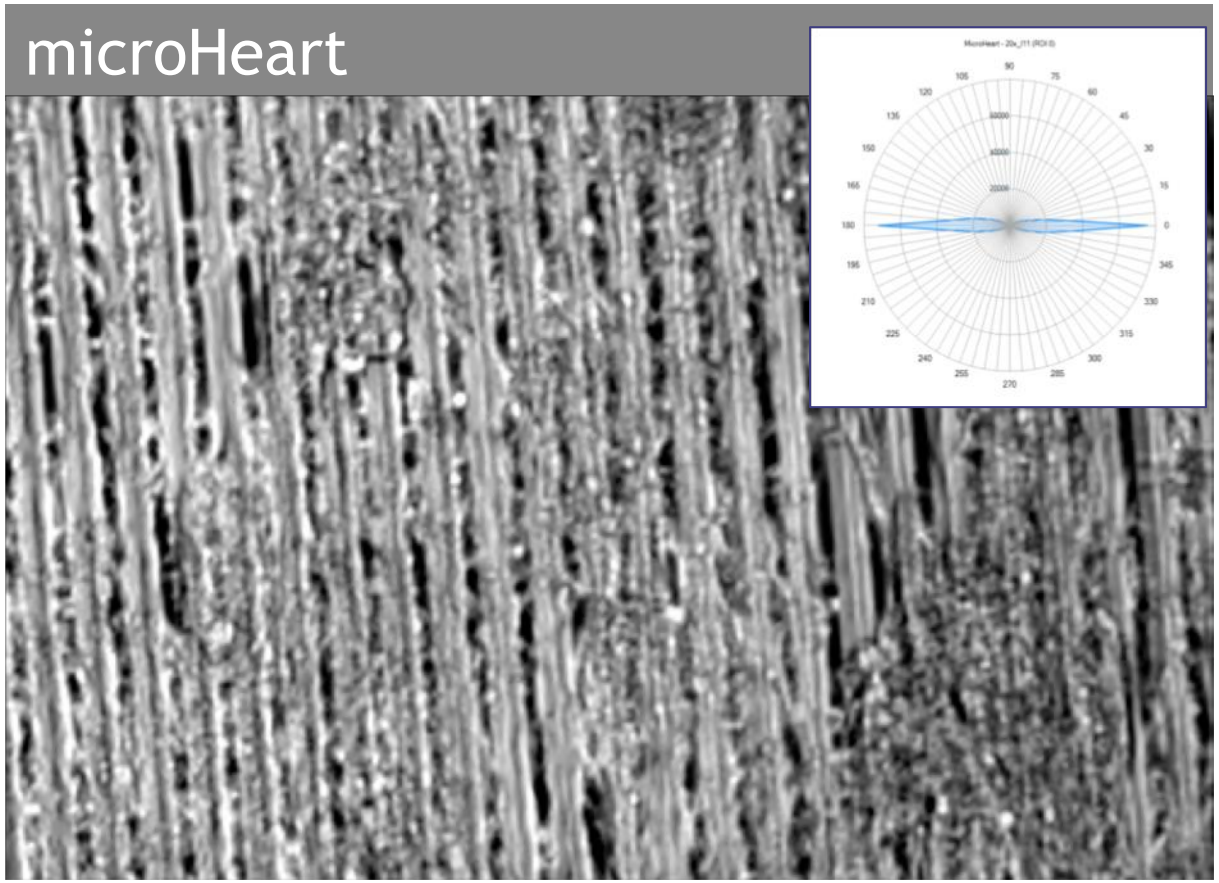


DNA Sarcomeric αActinin Plakoglobin

Gap junctions are located more distally on the fiber

Alignment/Morphology has Consequences - Unidirectional Contraction of hiPSC-CMs



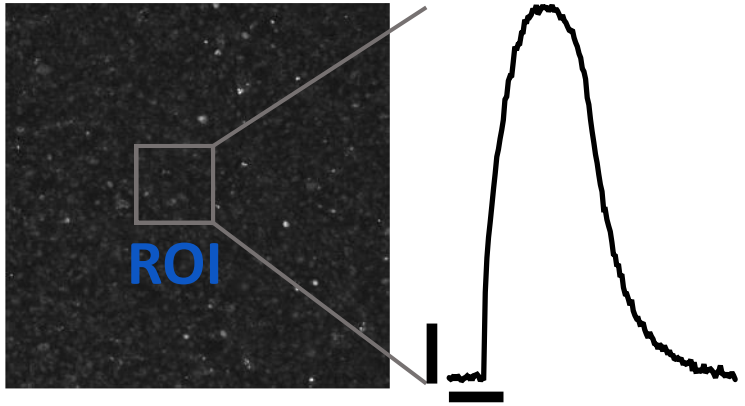


SI8000 Motion Vector Software

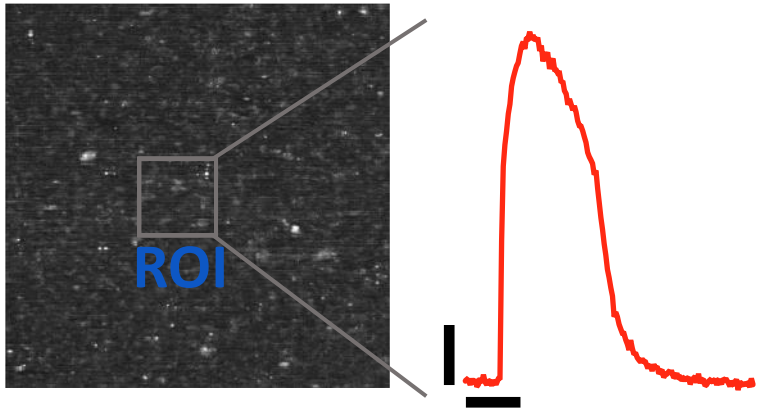
Alignment/Morphology has Consequences – Ephys / Ca^{2+} handling

High Speed Calcium Imaging
Acquisition Rate: 100Hz

Control

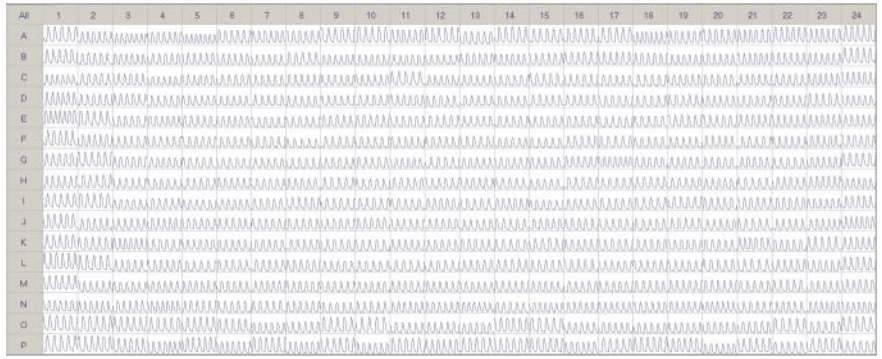


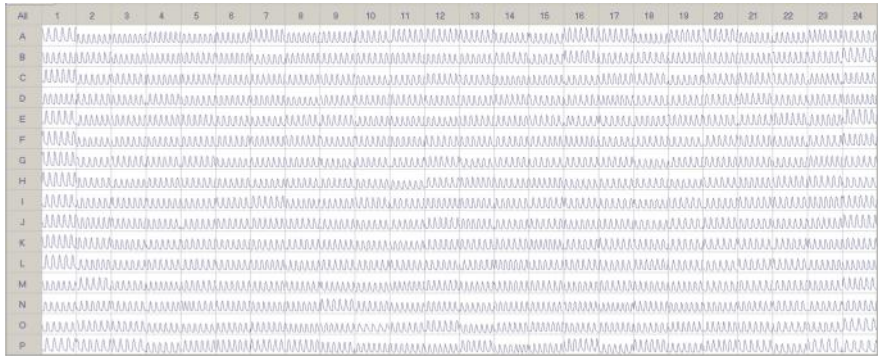
microHeart



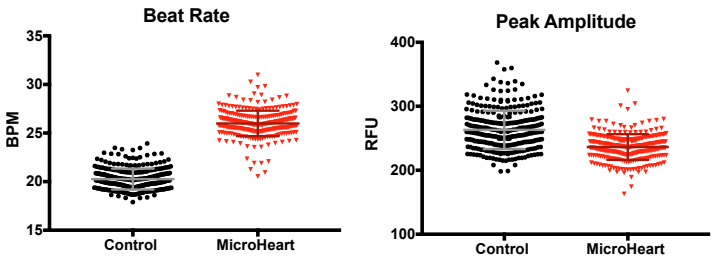
ImageXpress micro Confocal

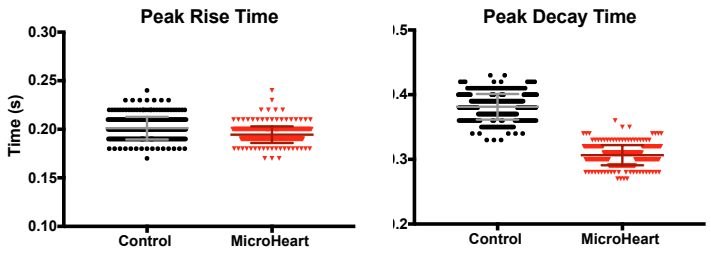
High Throughput Calcium Flux





FLIPR^{TETRA}





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* p<0.001

Thank-you

Goal: Provide an overview of iPSC cardiomyocyte biology and use in functional toxicity testing

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iPSC Cardiomyocyte Functional Overview

Screening endpoints (markers)

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Critical functions have multiple biomarkers with good translation

Morphology → Can have a structural and functional impact

Summary