

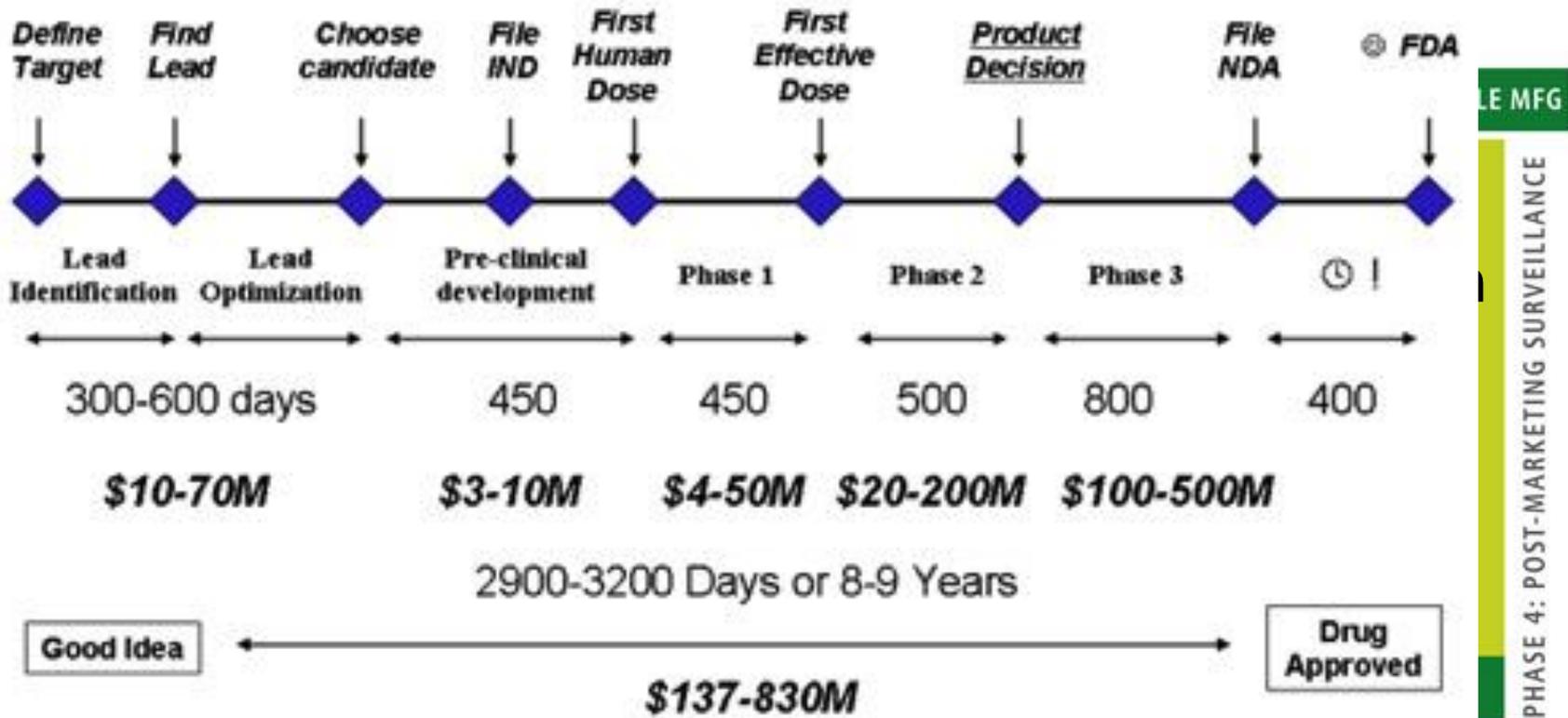
The use of iPSC-derived cells (& primary cells) as *in vitro* models for toxicity screening

*15th March 2016
SOT
New Orleans
Booth #419*

Drug Discovery & Development

“A long, risky road”

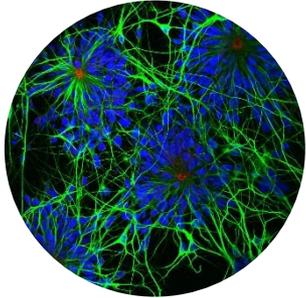
Need for early toxicity testing and improved prediction



Slide taken from the Pivotal Point Group, LLC

Source: Pharmaceutical Research and Manufacturers of America

Overview

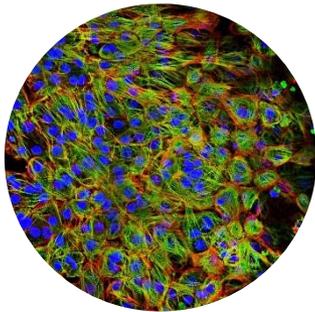


iPSC-Derived Neural Stem Cells

Neurotoxicity in drug safety testing

Functional Integrity

*Gene Expression, Electrophysiology,
Multi-Electrode Array,
Effects of developmental neurotoxin*



iPSC-Derived Cardiomyocytes

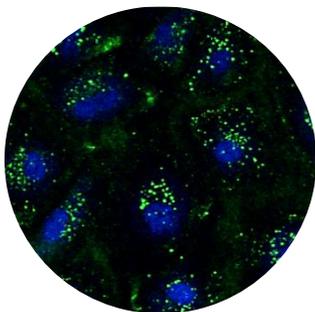
Cardiotoxicity in drug safety testing

Functional Integrity

*Express major cardiac-selective markers
Beat spontaneously in culture, Ca²⁺ imaging*

Electrophysiology

Pharmacology

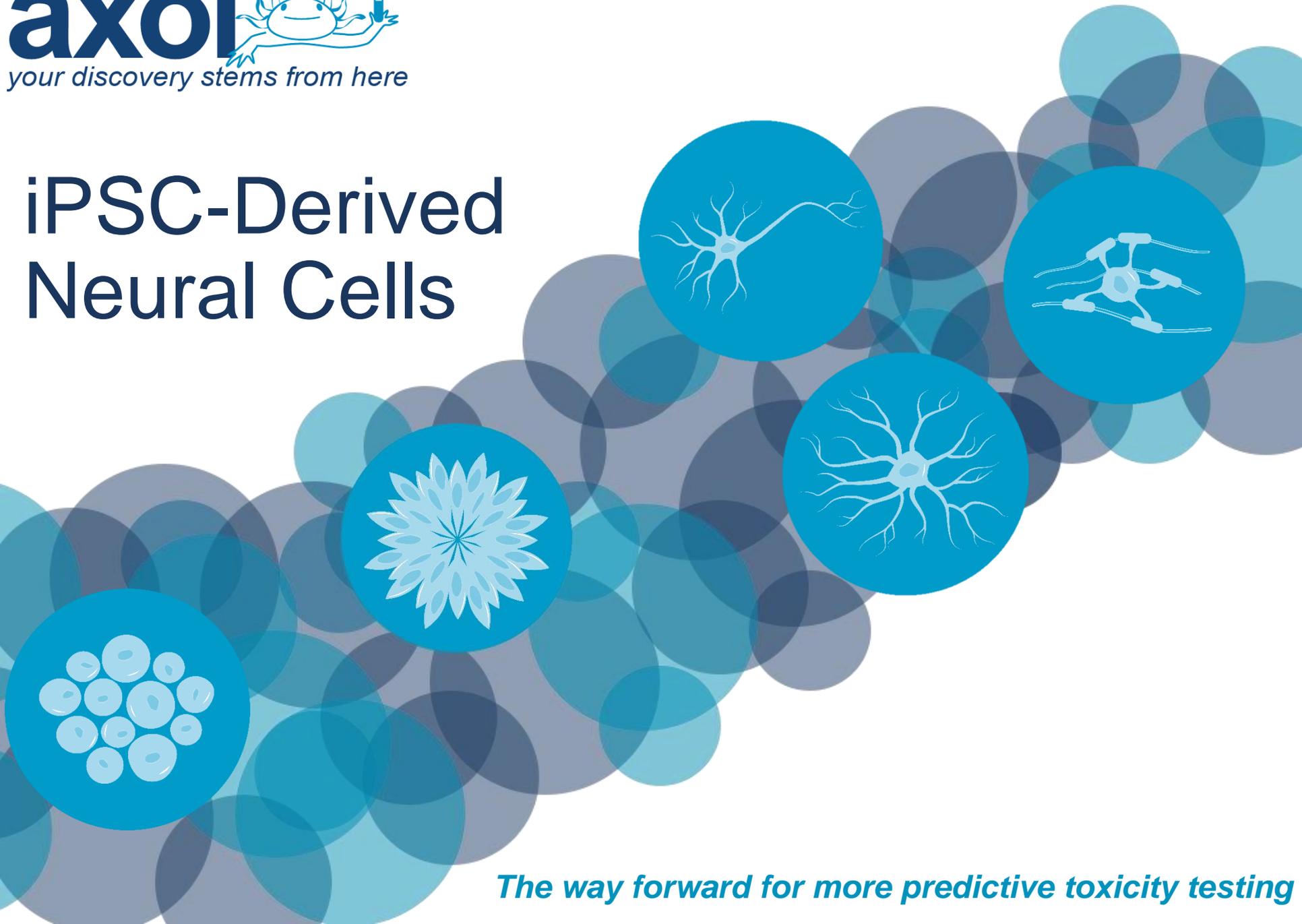


Hepatocytes

Hepatotoxicity in drug safety testing

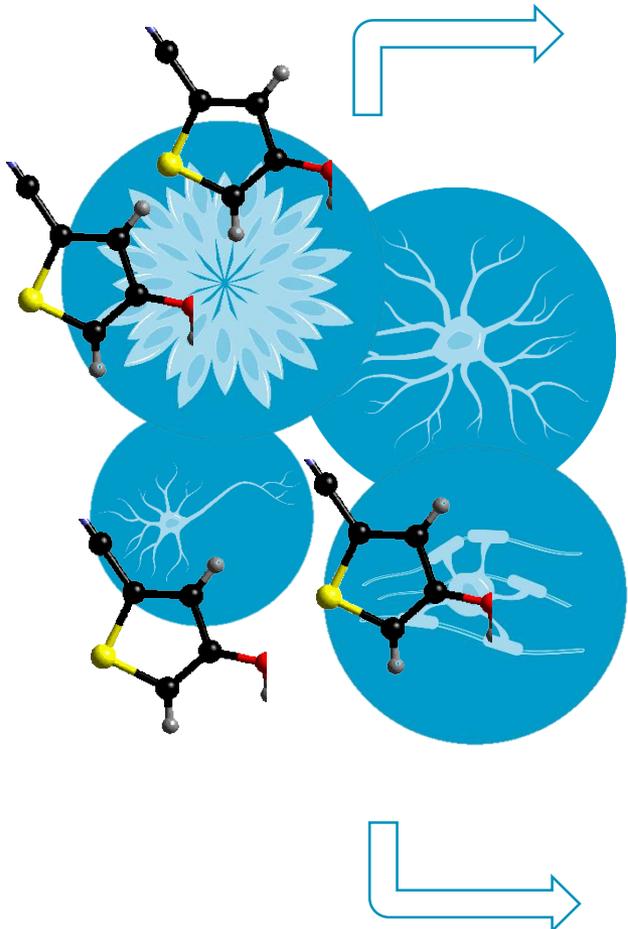
*Metabolism studies, Hepatotoxicity studies,
Genotoxicity micronucleus studies*

iPSC-Derived Neural Cells



The way forward for more predictive toxicity testing

Neurotoxicity in Drug Safety Testing



Functional Integrity

Gene Expression
Protein Expression
Electrophysiology
Multi-Electrode Array
Whole Cell Patch

Disease Modeling

Responsive to drug treatments
Expression disease-relevant phenotypes

Patch clamp

Gene expression

Biochemical analysis

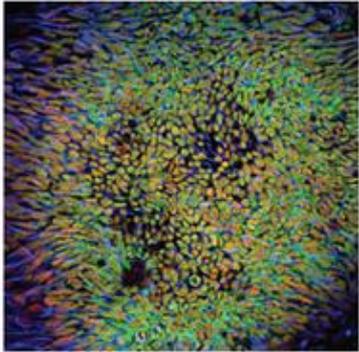
Multi-Electrode Array

Neurite outgrowth

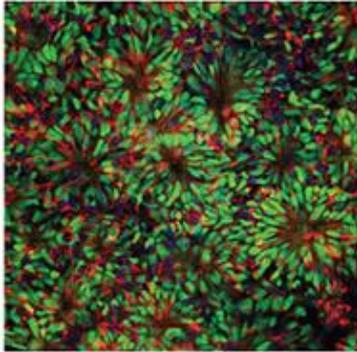
General Characterization of NSCs

We confirmed expression of neural stem cell markers like SOX2, PAX6, Ki67 and ZO1

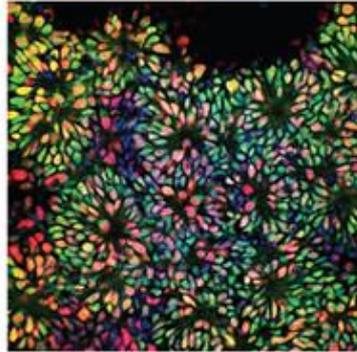
Foxg1/Sox2/Nestin



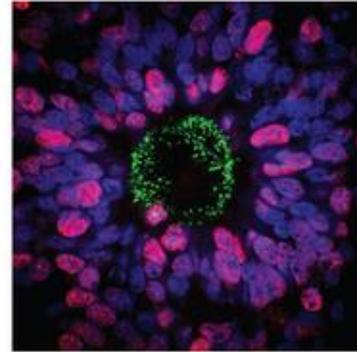
Pax6/Vimentin/DAPI



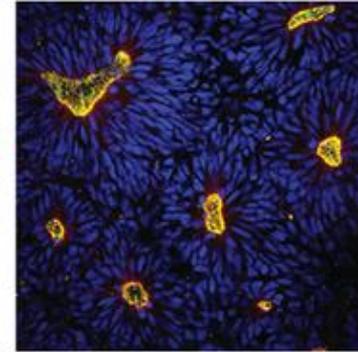
Otx/Ki67/DAPI



ASPM/Ki67/DAPI

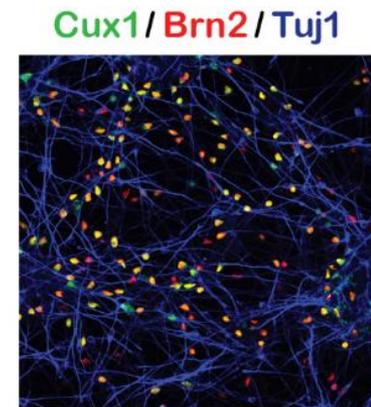
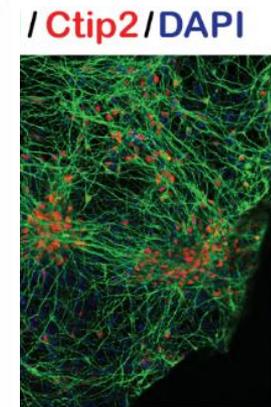
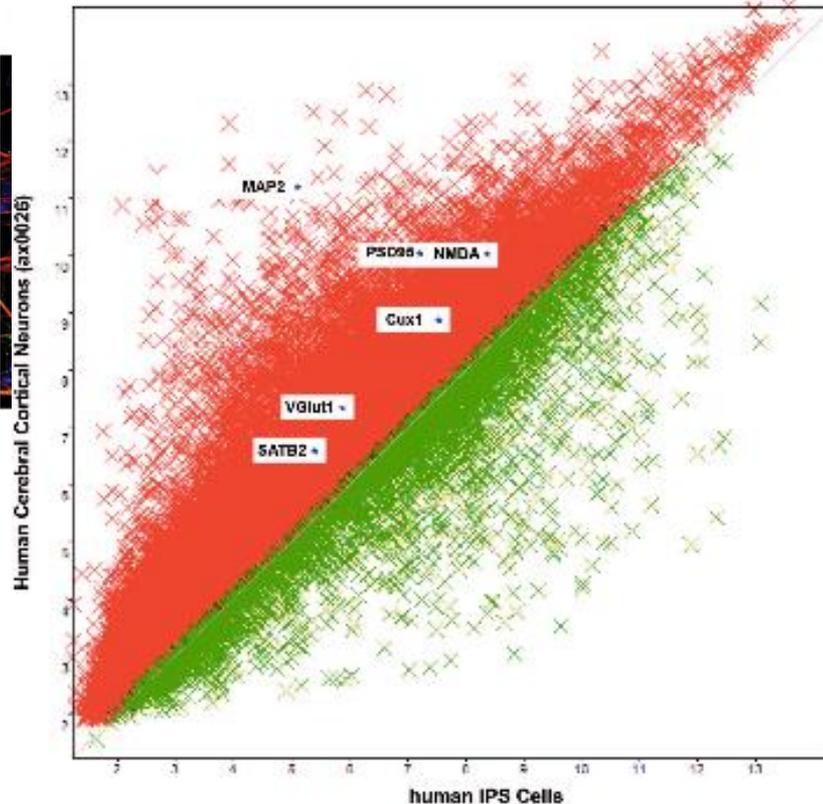
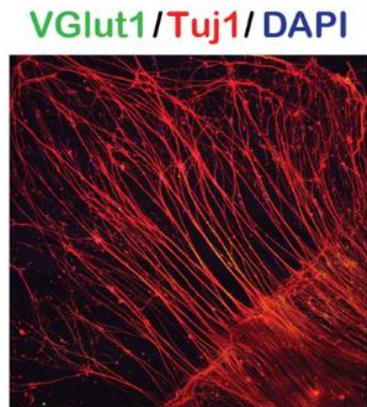


ZO1/NCad/DAPI



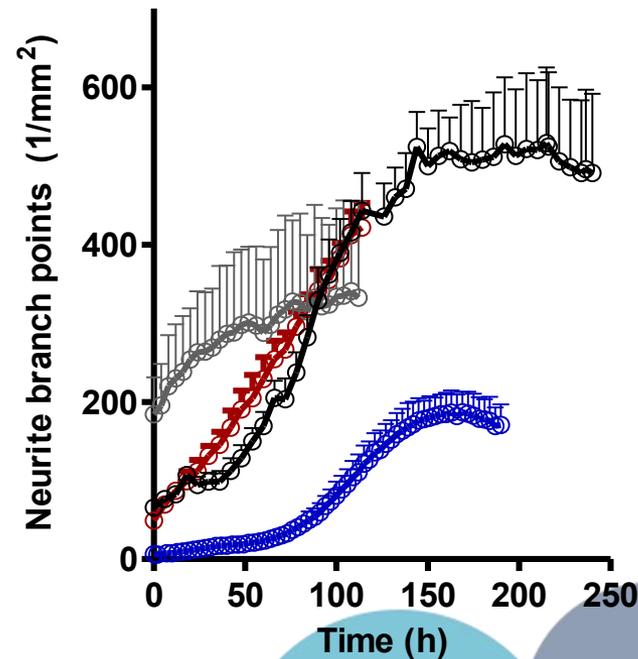
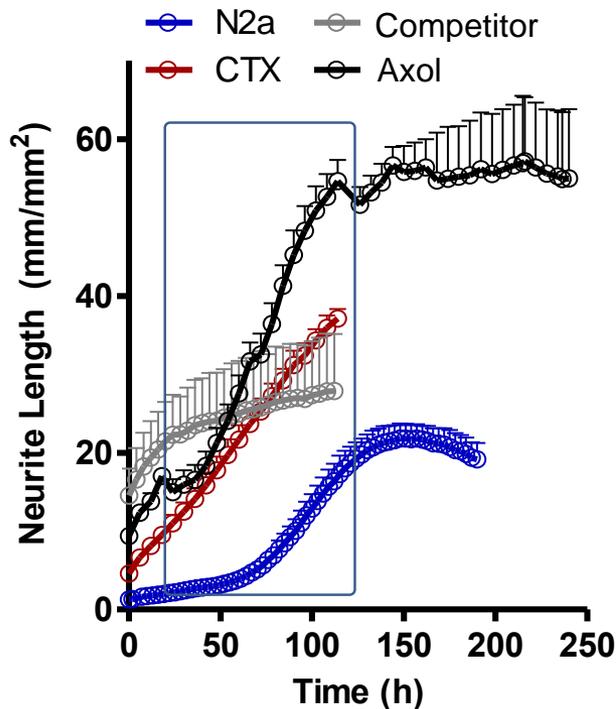
Characterization of Cortical Neurons

Our transcriptomic data confirmed down regulation of iPSC markers and up regulation of cortical neuronal markers like MAP2, NMDA, VGlut1, Cux1 etc

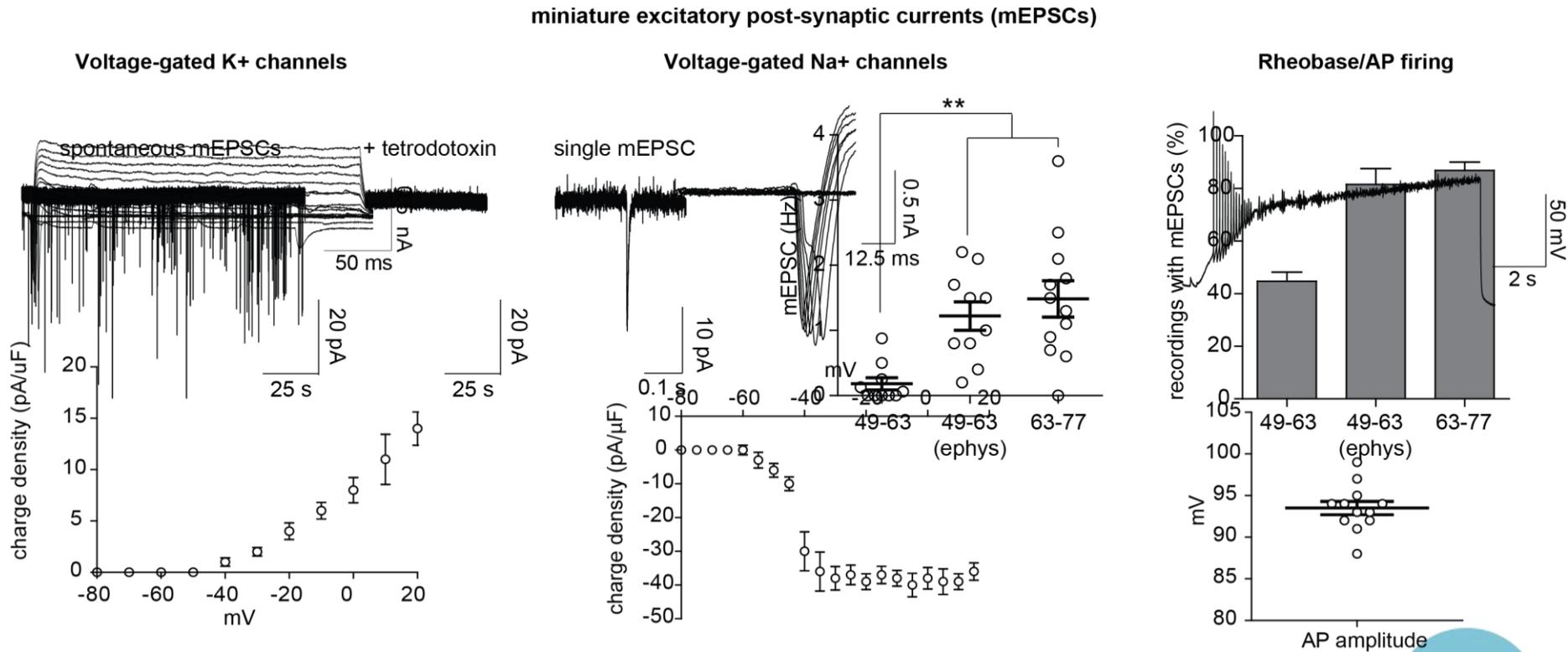


Functional Characterization

We confirmed the functional integrity by looking into neural networks with increased neurite length and branching in cortical neurons

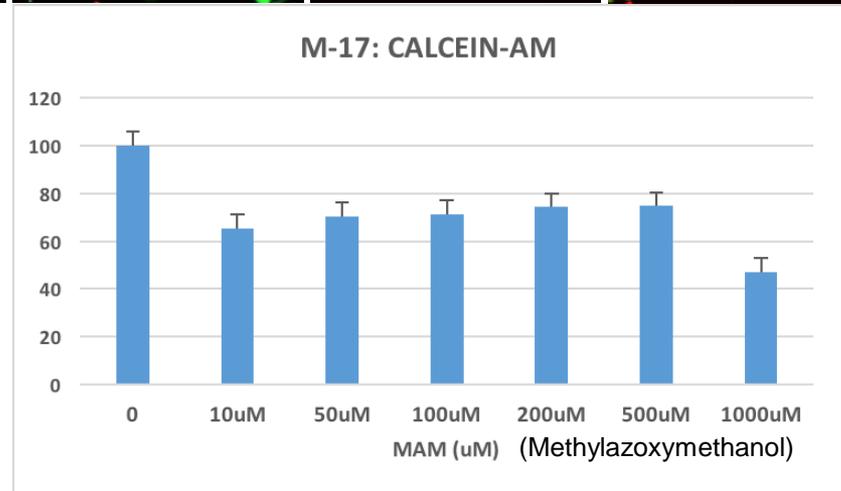
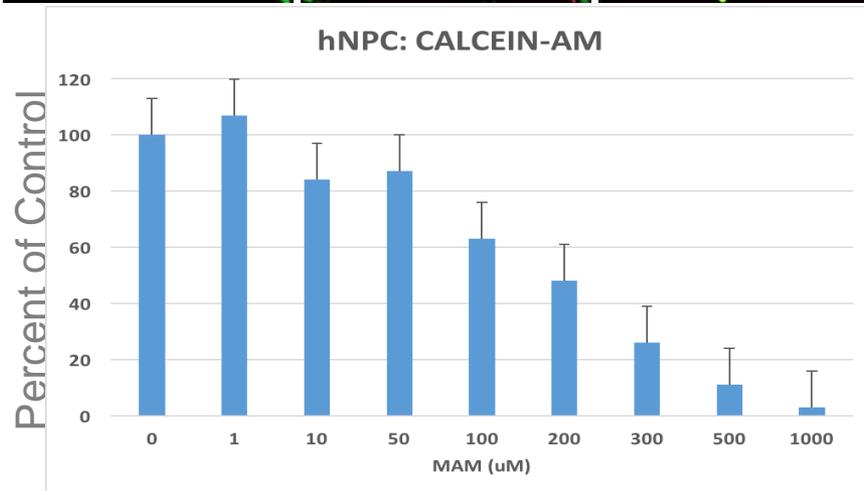
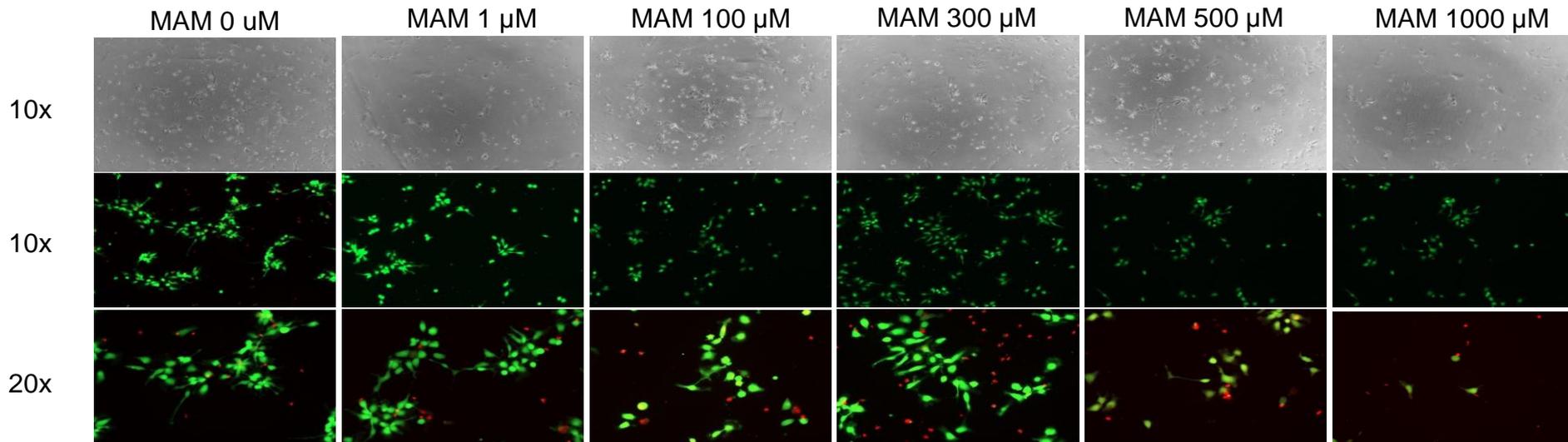


Electrophysiological Characterization



Spontaneous activity

Neurotoxin Effects on iPSC-Derived Neural Stem Cells

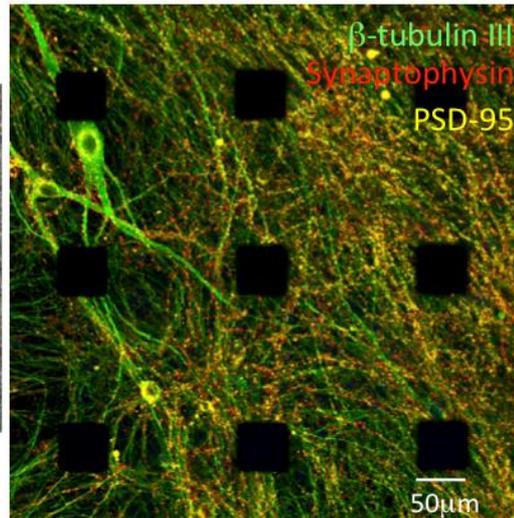


Data provided by Dr Kisby's lab by Michael Czulinski and Morgan Florek

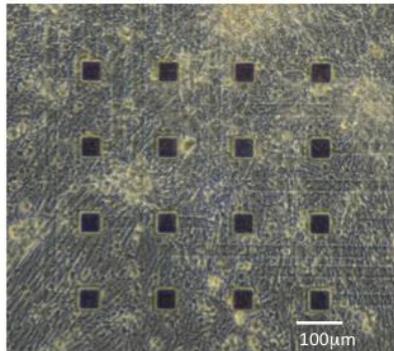
iPSC-Derived Cortical Neurons as *in-Vitro* Models for Drug Screening



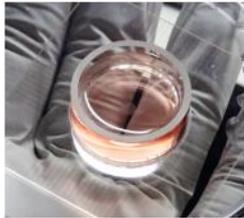
300 days culture on the MEA dish



294 days culture on the MEA dish



Multi-electrode array chip



Alpha Med Scientific Inc



iPSC-derived neural cells used to demonstrate LTP & LTD on an MEA platform

Biochemical and Biophysical Research Communications 469 (2016) 856–862

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journal homepage: www.elsevier.com/locate/ybbrc



ELSEVIER

Induction of long-term potentiation and depression phenomena in human induced pluripotent stem cell-derived cortical neurons

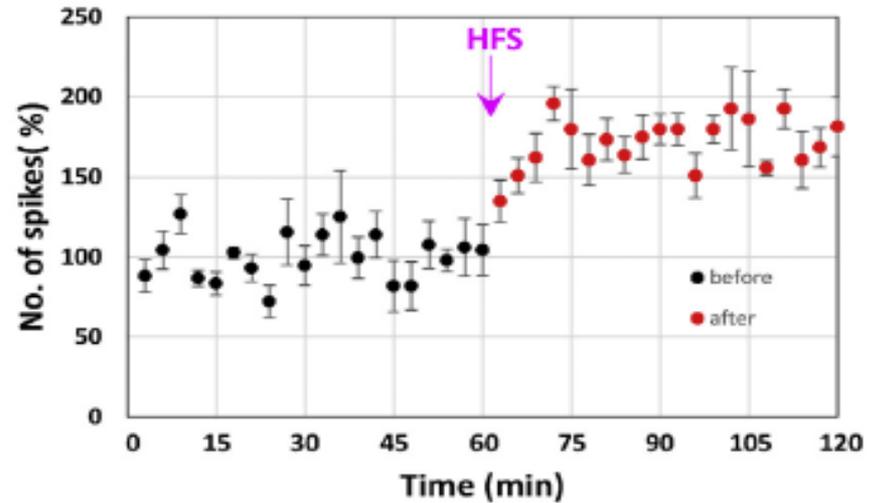
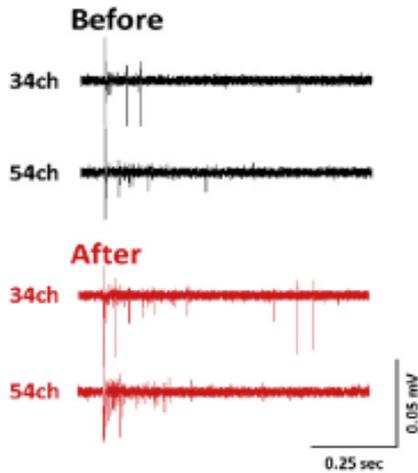
A. Odawara ^{a,c,1}, H. Katoh ^{a,1}, N. Matsuda ^b, I. Suzuki ^{a,b,*}



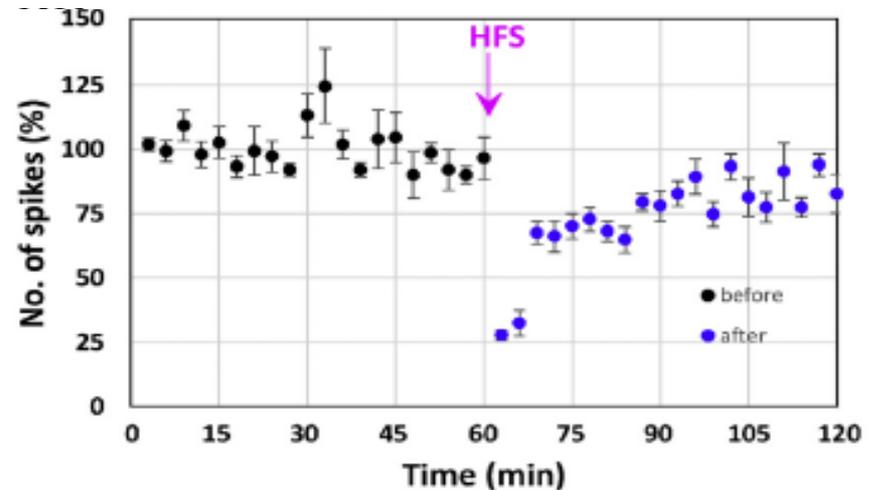
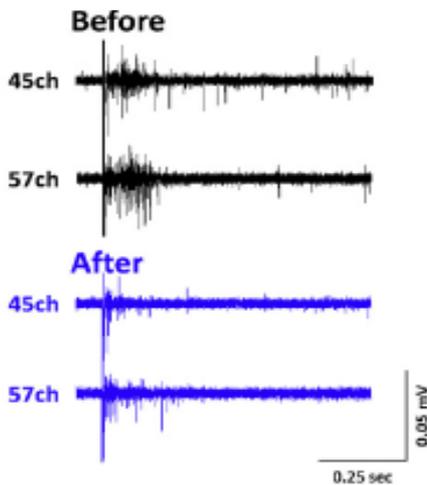
iPSC-Derived Neurons Show Potential for Synaptic Plasticity

Induction of long-term potentiation (LTP) and long-term depression (LTD) by high-frequency stimulation (HFS) (112 DIV)

Long-Term Potentiation



Long-Term Depression

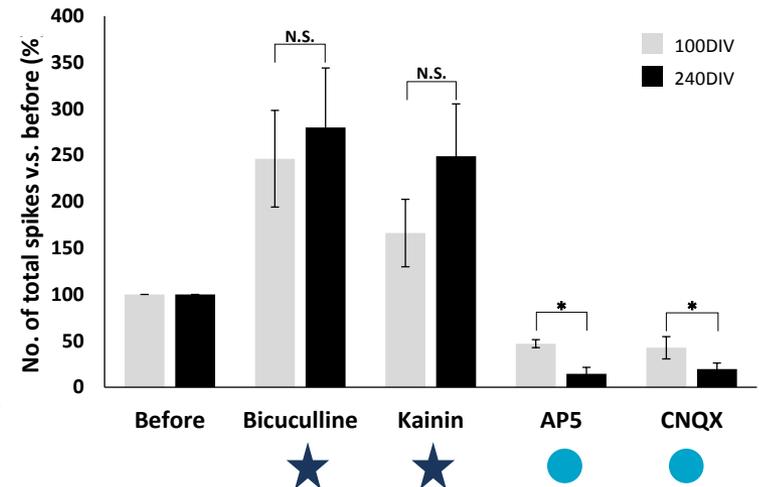
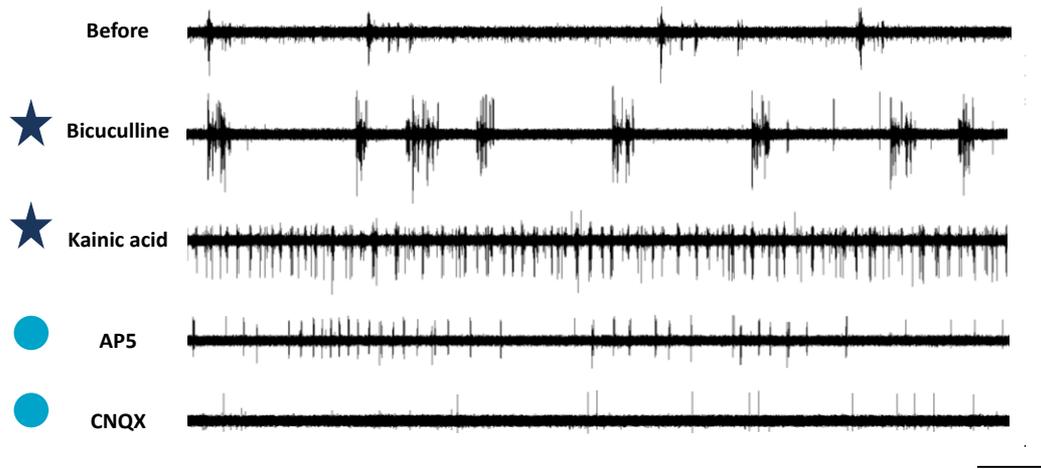


iPSC-Derived Neurons Respond to Drug Application



iPSC-derived neurons in response to drug application:

- ★ Synapse agonists (Bicuculline & Kainin acid)
 - Increase in firing
 - No change over days in culture
- Synaptic antagonists (CNQX & AP5)
 - Inhibit firing
 - Decrease with days in culture (100 v 240)



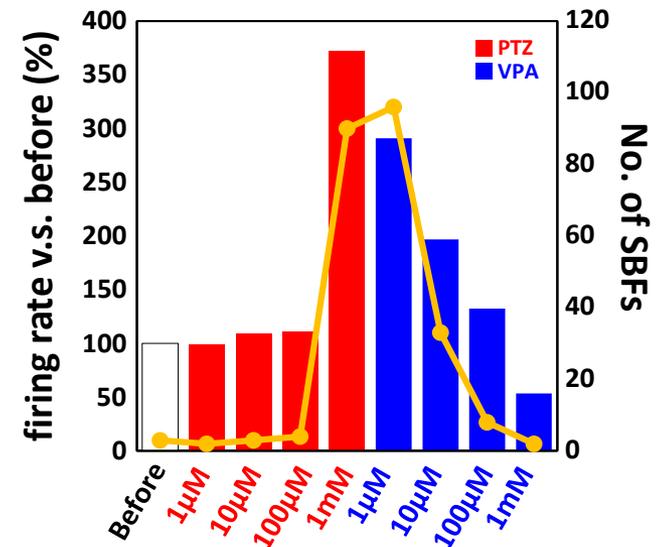
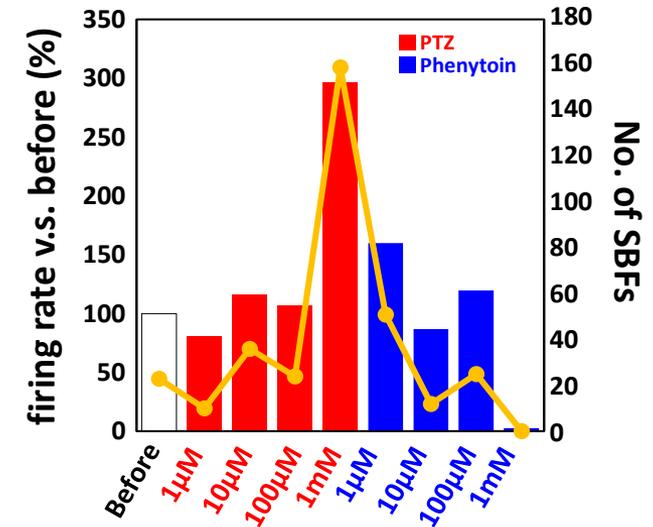
SOT poster number: 1826/P132
Copies available at booth #419

Induction of Epileptiform Activity & axol



- Induced epilepsy by adding PTZ (pentylentetrazole) (>1mM)
- Anti-epilepsy drugs, **phenytoin** & **sodium valproate (VPA)** were able to reverse the high frequency synchronized bursts evoked with PTZ

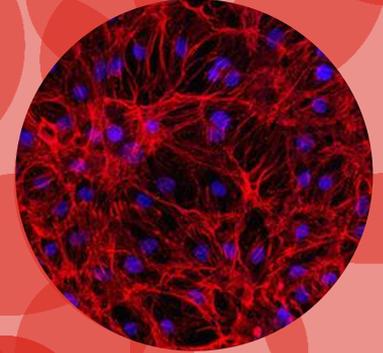
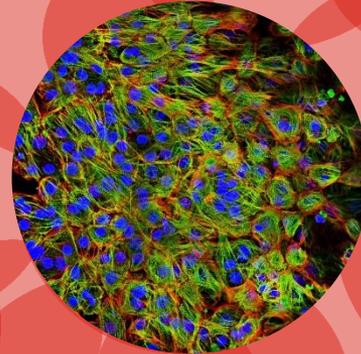
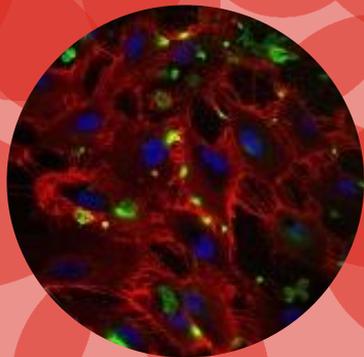
*These results suggested that long-term electrophysiological measurements in iPSC-derived neurons using a MEA system may be beneficial for **drug screening applications***



Neurotoxicity Summary

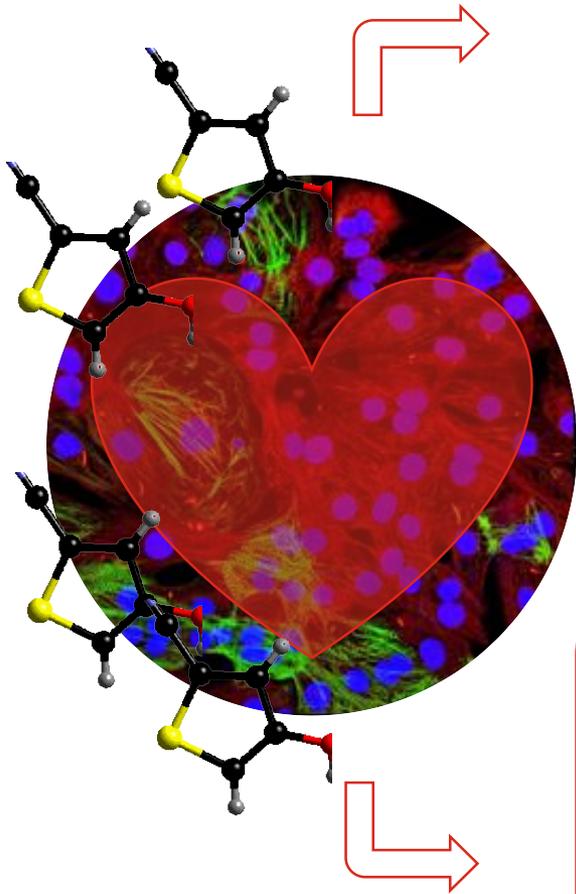
- iPSC-derived NSC
 - Express neural markers at gene & protein level
 - Excellent neurite outgrowth
 - Electrophysiologically functional
 - Capable of synaptic plasticity
- iPSC-derived NSCs are more sensitive to the developmental neurotoxin MAM & can replace routinely cell lines use for screening for neurotoxins
- Responsive to drug treatment
- Can be cultured long-term
- Physiologically relevant tool for drug discovery & toxicity studies

iPSC-Derived Cardiomyocytes



A way forward for more predictive toxicity testing

Cardiotoxicity in Drug Safety Testing



Electrophysiology

Contractility
QT prolongation
Na⁺ & Ca²⁺ channels
Pharmacology

Patch clamp

Impedance

Biochemical
analysis

Immuno-
cytochemistry

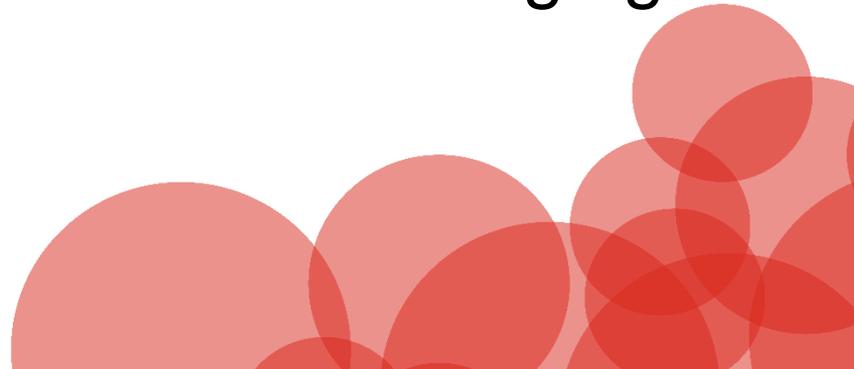
Multi-electrode
Array

Functional Integrity

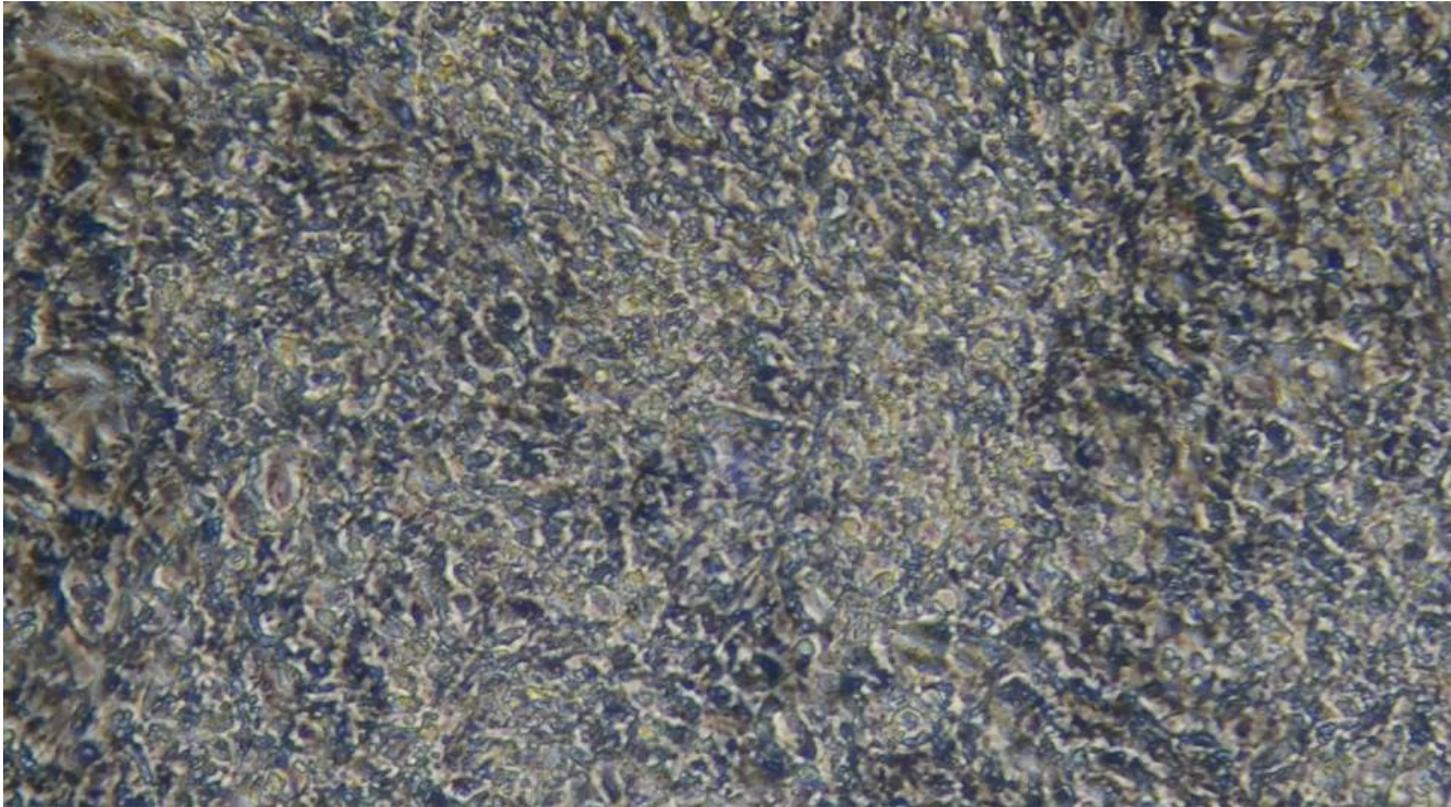
Ca²⁺ signaling
Morphology
Stress & toxic response markers

Why iPSC-Derived Cardiomyocytes?

- Benefits of a synchronously beating monolayer
 - React as a unit syncytium of cells, electrically coupled
- Robust & reproducible
- Large quantities available
- High purity
- Functional on xCelligence, for calcium imaging & for electrophysiology



iPSC-Derived Cardiomyocytes Showing Synchronized Beating

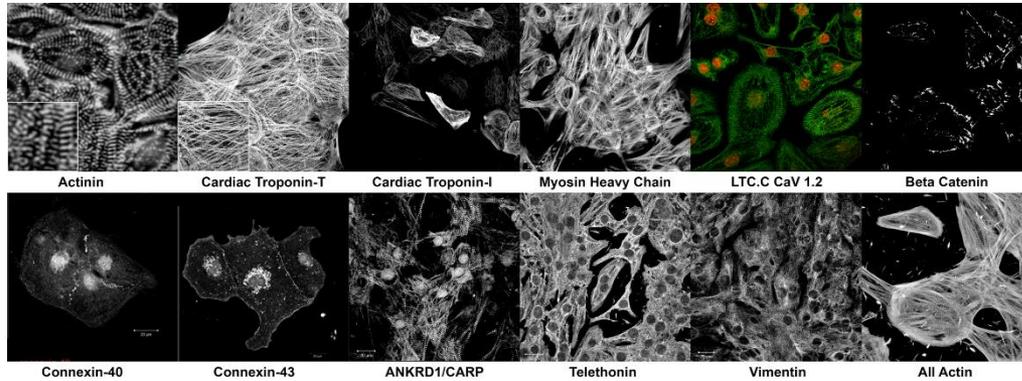


Benefits of a synchronously beating monolayer

- Electrically coupled
- Physiologically relevant to human heart

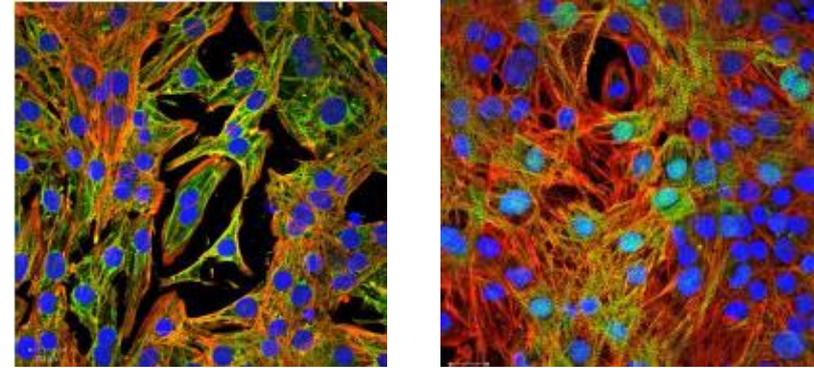
Functional iPSC-Derived Cardiomyocytes

Protein Expression

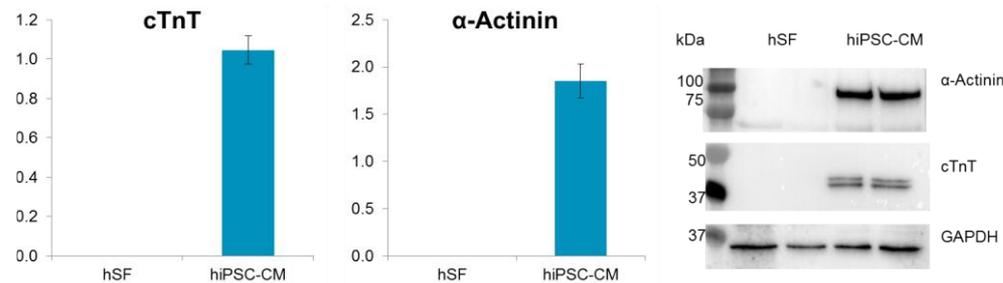


Data from Dr Christian Zuppinger

Signaling & Stress-Response



Data from Dr Christian Zuppinger

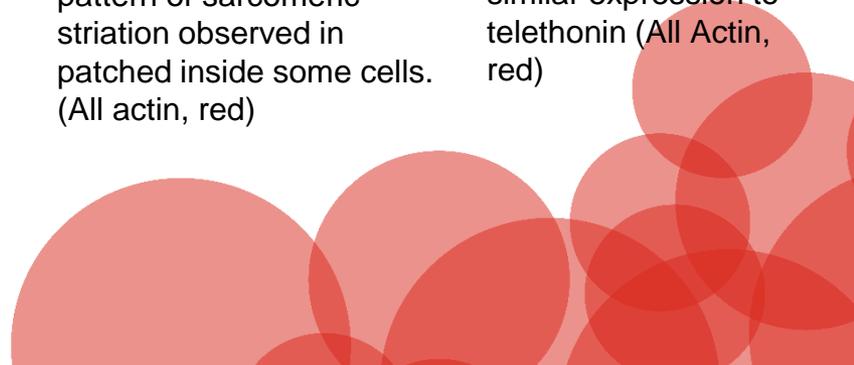


Human iPSC-CMs (hiPSC-CMs) express more cardiac troponin-T (cTnT) & α-Actinin than human skin fibroblasts (hSFs)

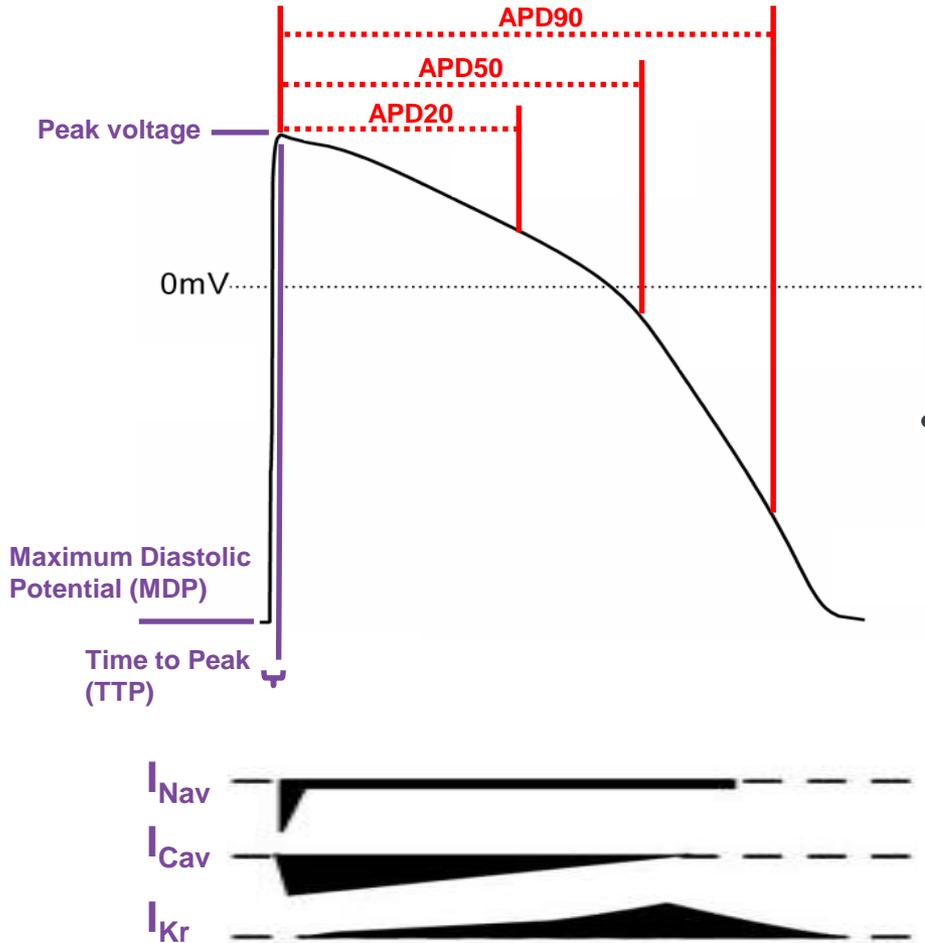
Data from Abigail Robertson from University of Manchester

Telethonin (green) suggested signalling & stress-response functions is present iPSC-CMs with a pattern of sarcomeric striation observed in patched inside some cells. (All actin, red)

Ankyrin repeat domain 1 (ANKRD1) (green) could be used a marker of toxic stress, showed similar expression to telethonin (All Actin, red)



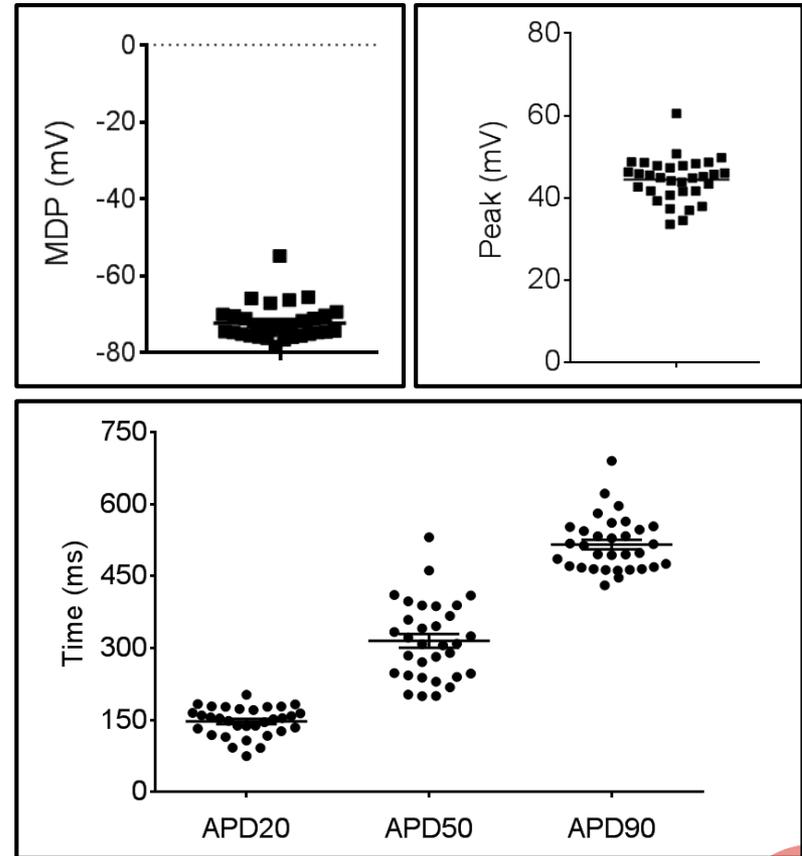
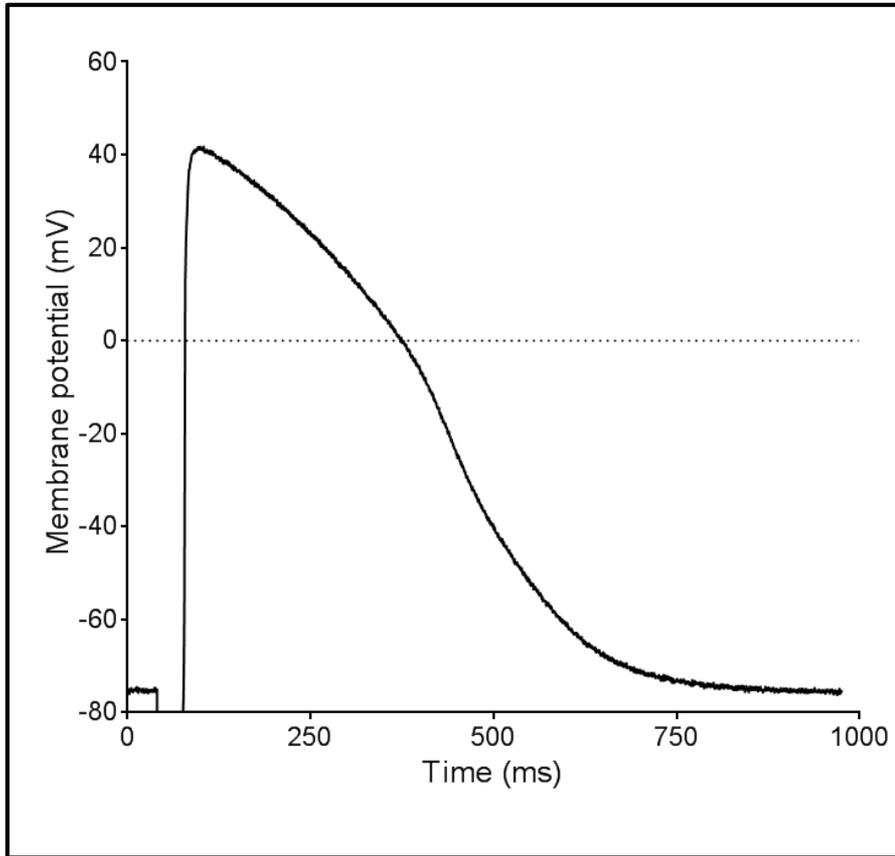
Methods, Tools & Recording Parameters



- Patched 7-14 days post seeding
 - Action potentials (AP) recorded from syncytial cells (field stimulation)
 - Perforated patch clamp (100 $\mu\text{g/ml}$ gramicidin)
- Pharmacological tools:

Compound	Ion channel
Carbachol	I_{KACH}
TTX	I_{Nav}
Mexiletine	I_{Nav}
Nifedipine	I_{Cav}
Verapamil	I_{Cav} & I_{Kr}
Dofetilide	I_{Kr}

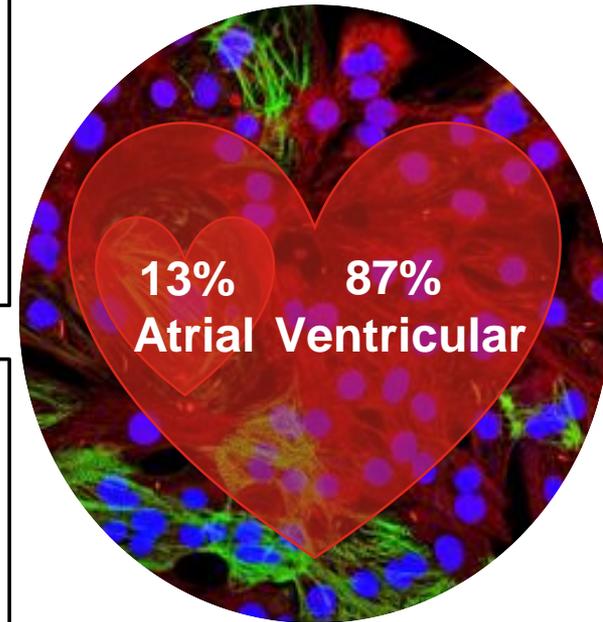
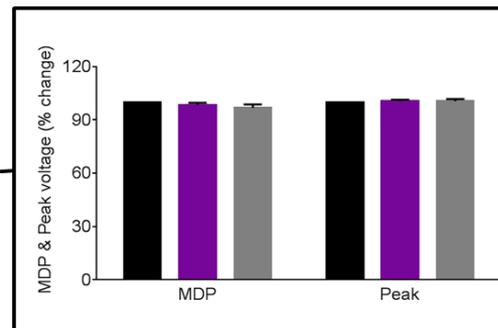
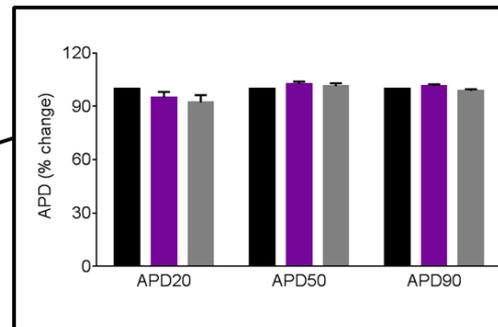
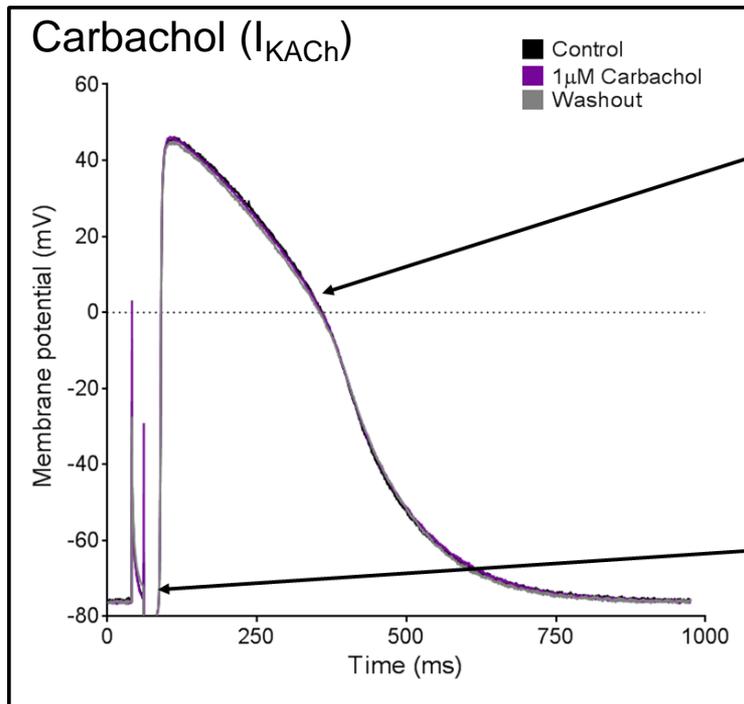
AP Parameters



n = 32 control recordings

Cells paced at either 0.5 or 1Hz

Pure Population Ventricular Cardiomyocytes

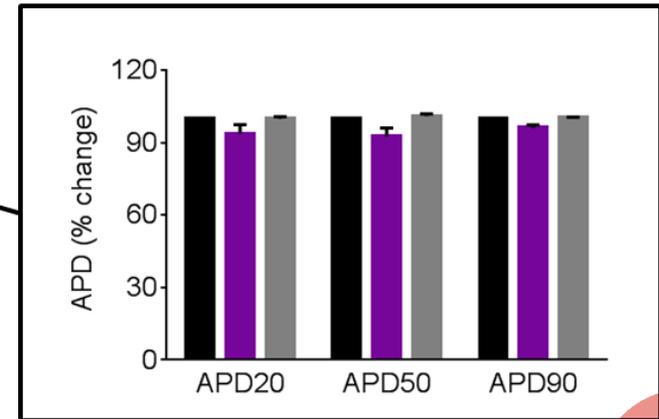
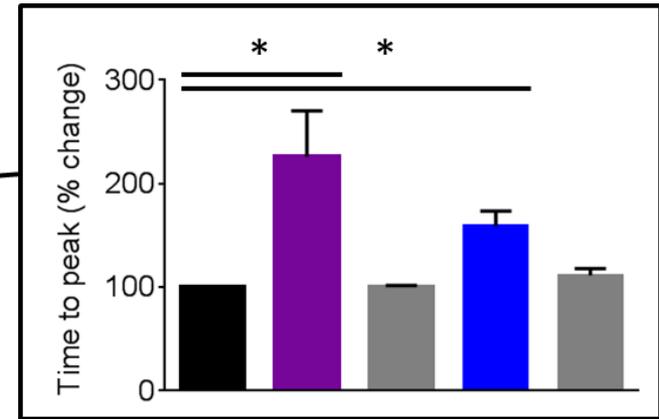
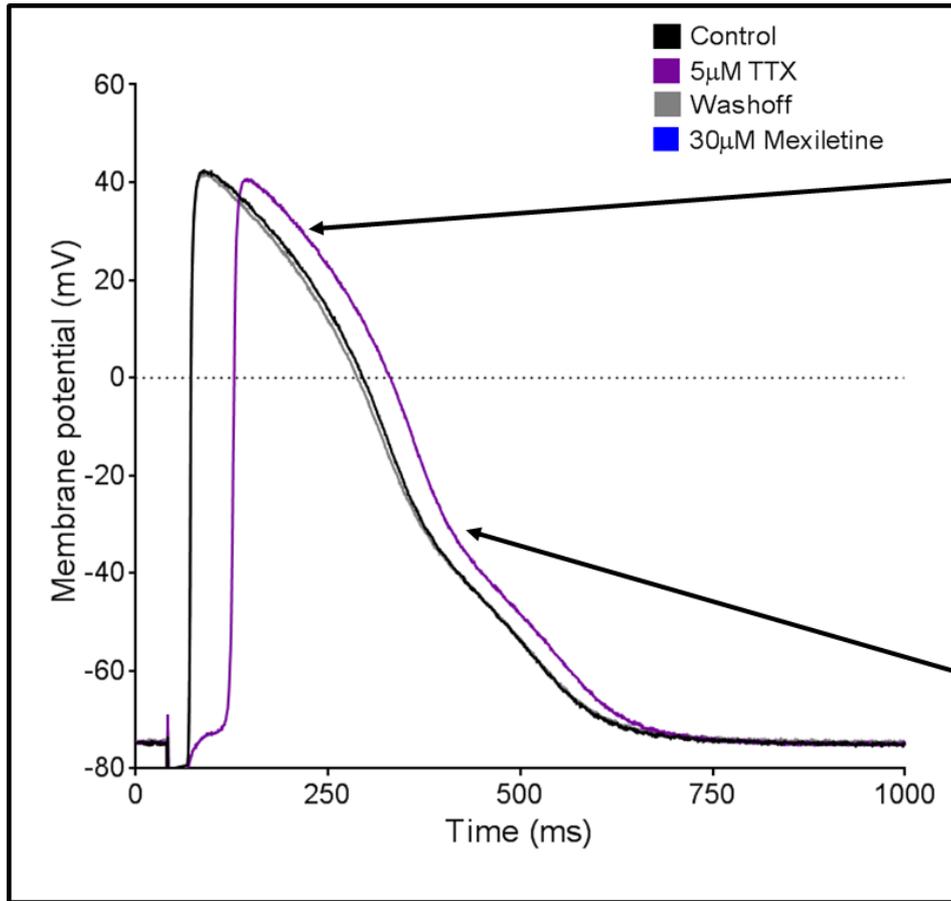


- Negligible effect on AP parameters (n=8)
 - Positive effect of carbachol observed with atrial-derived HL-1 cells
 - Suggests majority of cells do not display an atrial phenotype

Ventricular myosin light chain (87%)
and atrial myosin light chain (13%)

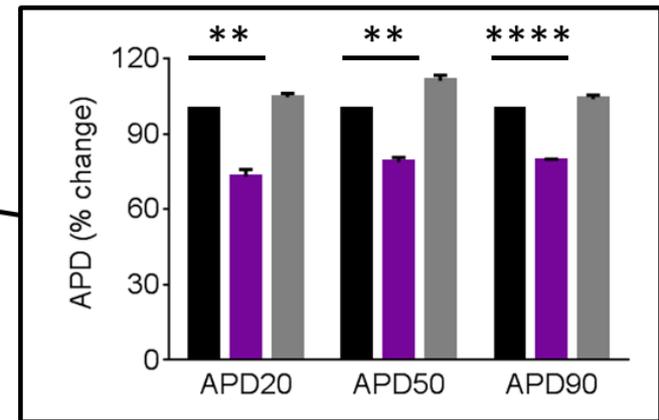
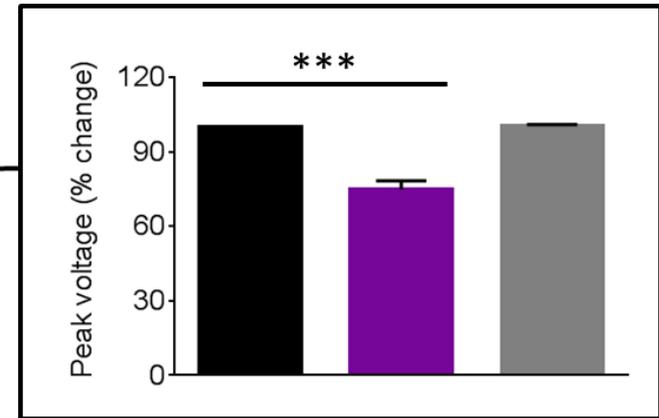
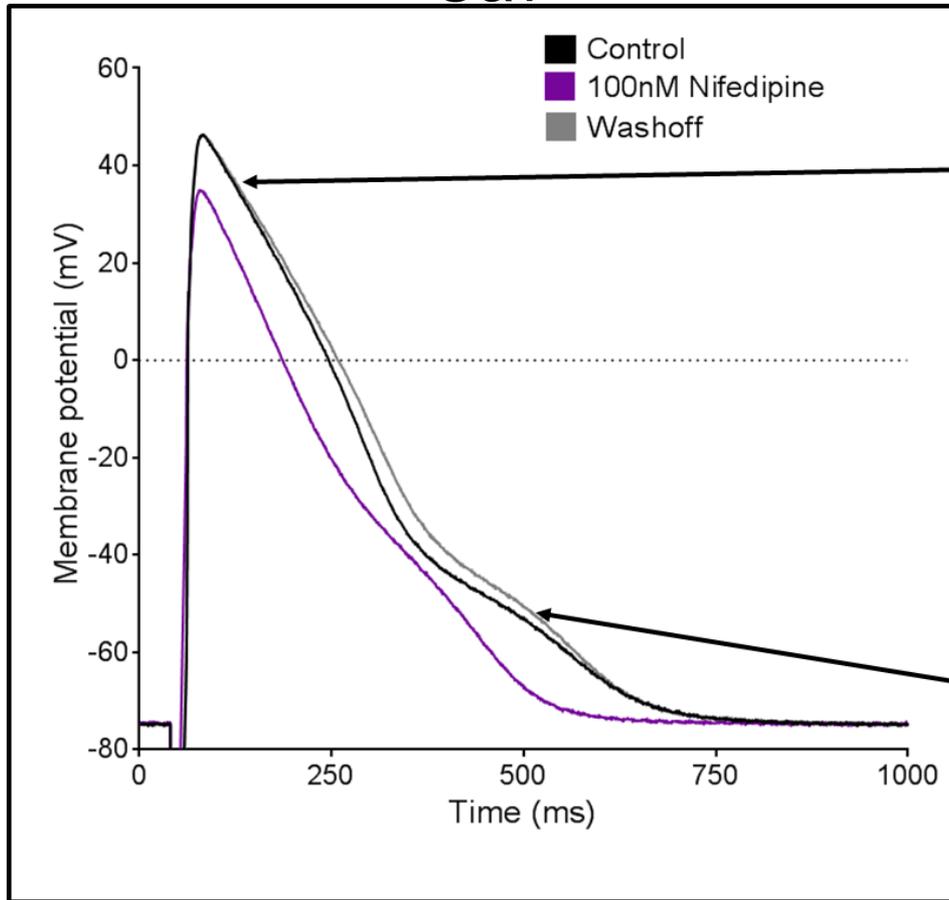
(Does not include nodal population)

TTX & Mexiletine (I_{Nav})



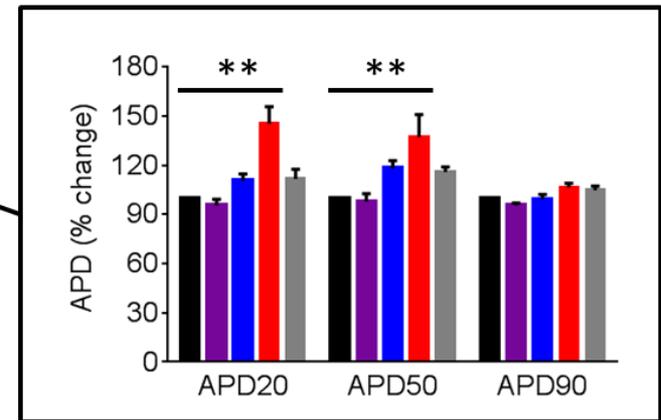
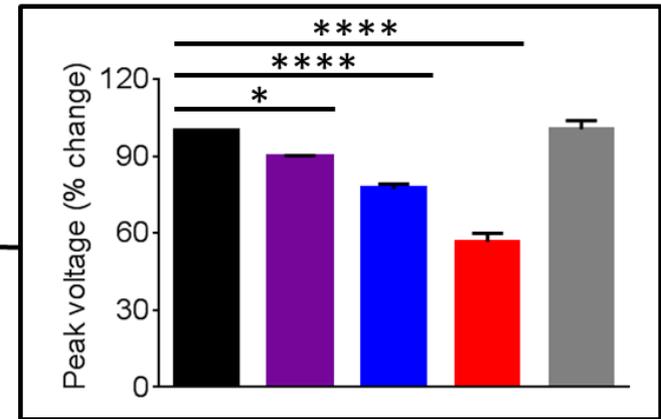
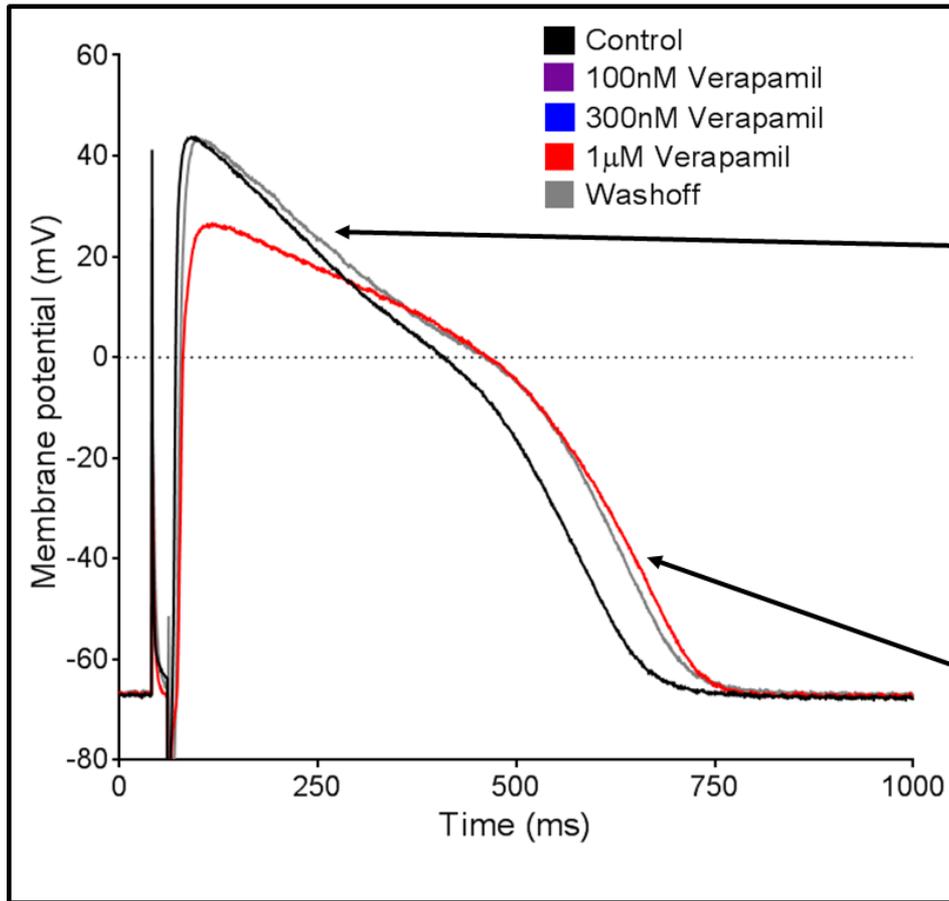
- Significantly prolonged the TTP
- Negligible effect on other AP parameters
- Similar effect observed with Mexiletine

Nifedipine (I_{CaV})



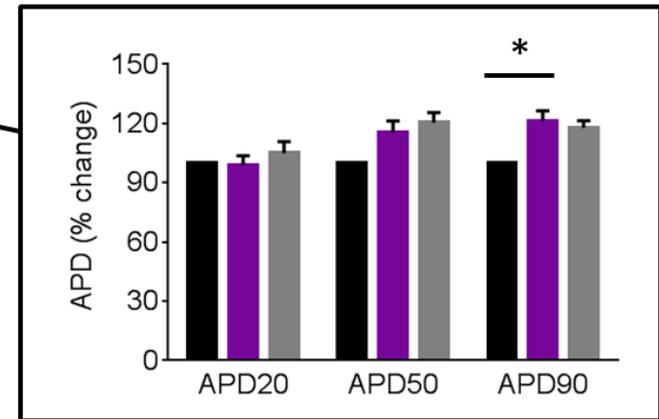
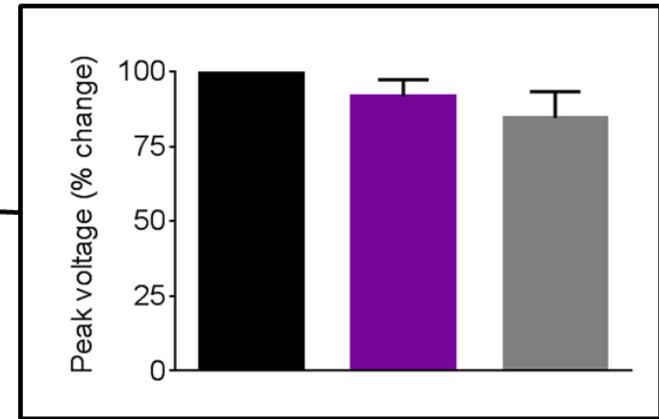
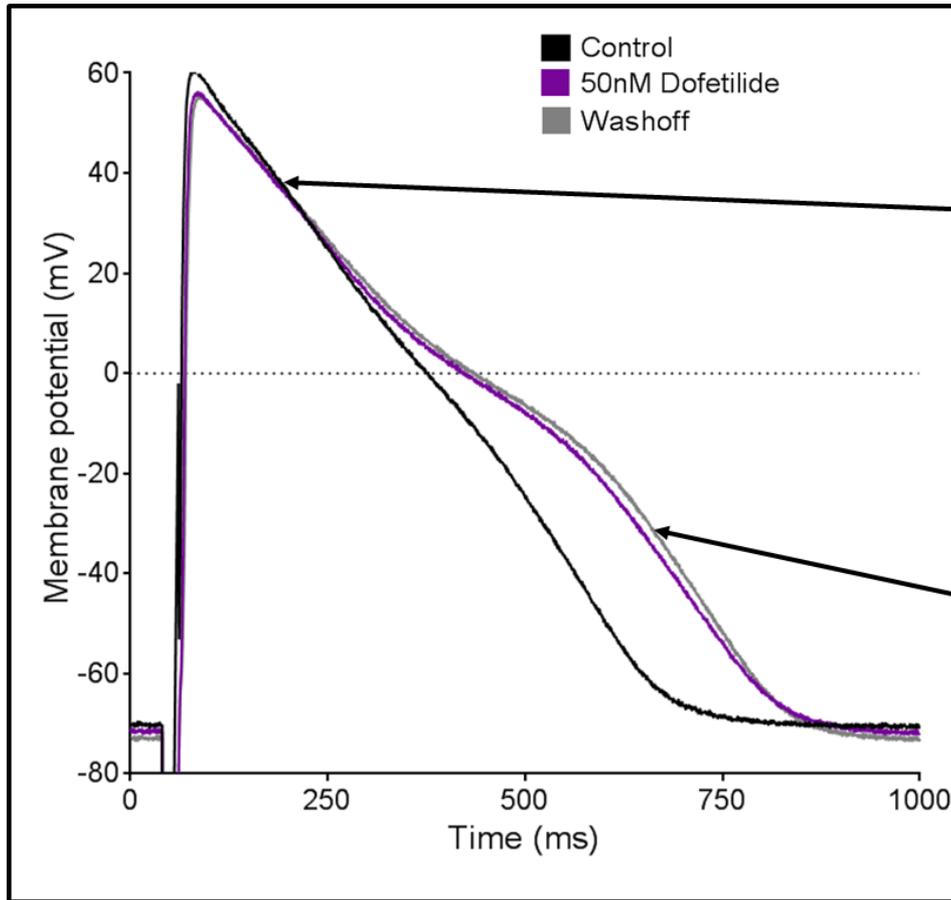
- Significantly reduced the peak voltage
- Significant shortening of APD20, APD50 & APD90

Verapamil (I_{Cav} & I_{Kr})

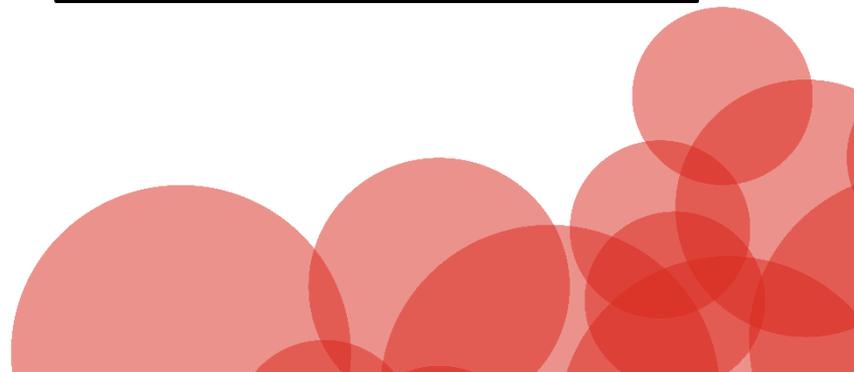


- Significant reduction to the peak voltage (all concentrations)
- Significant reduction in TTP(1µM)
- Significant prolongation of APD20 & 50 (1µM) but not APD90

Dofetilide (I_{Kr})



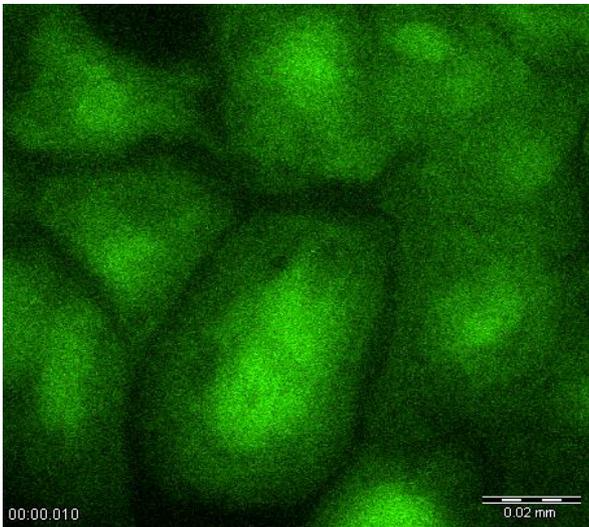
- Significant prolongation to APD90
- Negligible effect on other AP parameters



Effect of Dofetilide on Calcium Imaging

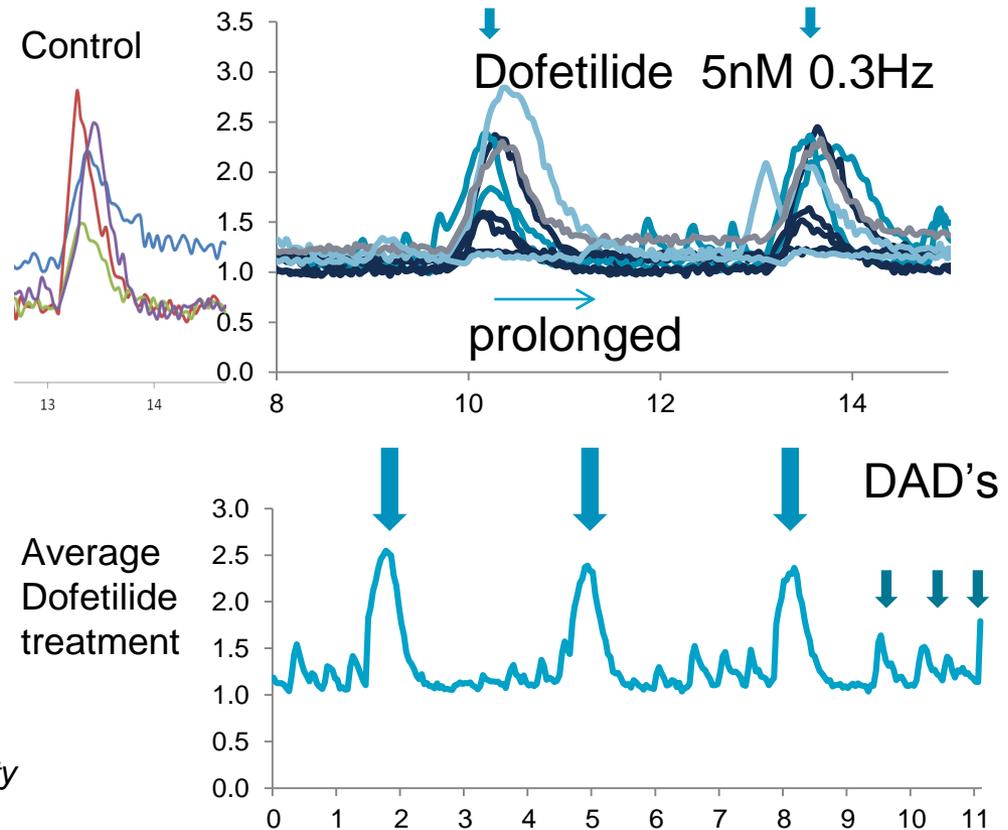
Without treatment

Using Fluo-4 calcium dye to measure calcium transients



Data provided by Dr Frances Brook at Oxford University

Dofetilide treatment prolongs the calcium transient

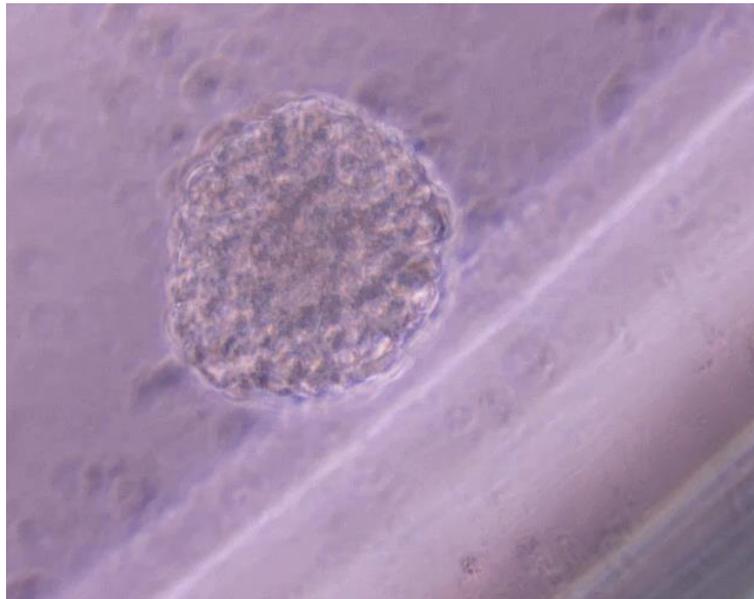


Delayed after depolarization (DAD)
apparent in some cells

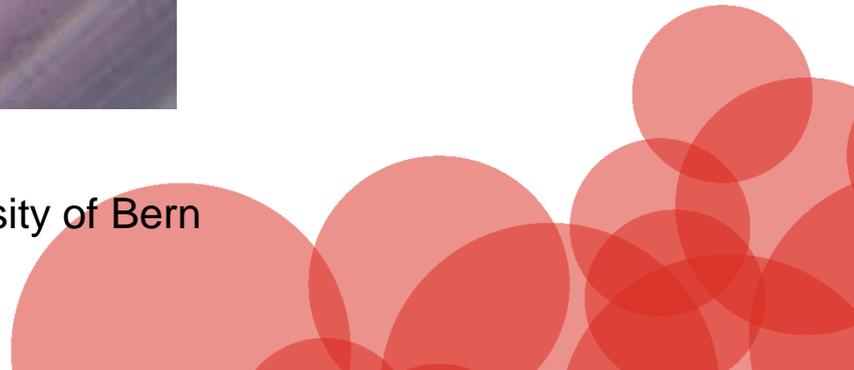
iPSC-Derived Cardiomyocytes in 3D culture



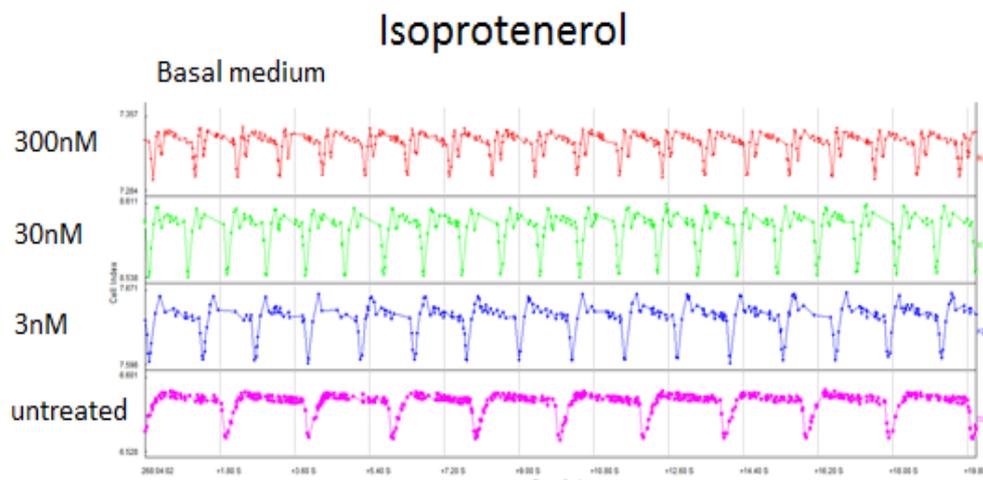
Cardiosperoids are essential for successful co-culturing of iPSC-derived cardiomyocytes & endothelial cells



Dr Christian Zuppinger, University of Bern

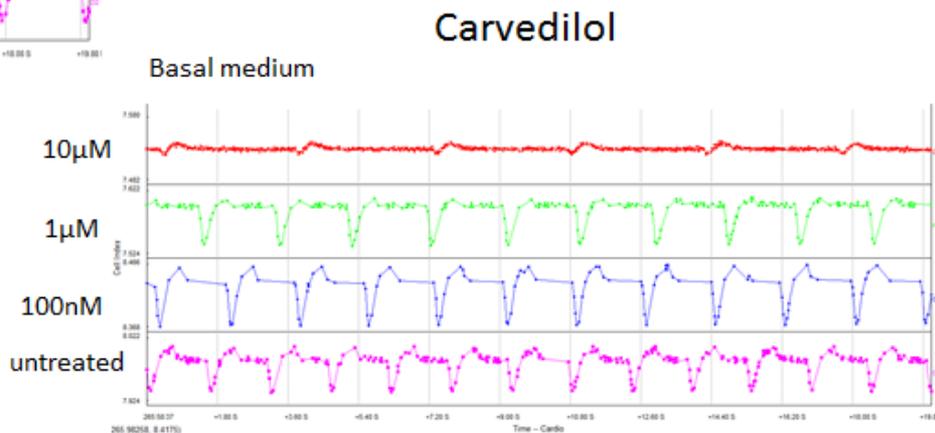


In-Vitro Models for Cardiotoxicity Studies



Isoproterenol (β -adrenoceptor antagonist) & carvedilol (β -adrenoceptor agonist) were added at 265h

Data shared by Dr Jason Gill, Durham University



iPSC-derived cardiomyocytes responded to both compounds in a dose-dependent fashion & strongly indicates the clinical relevance of these cells & their utility for drug screening applications

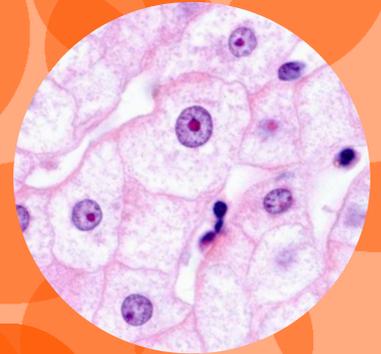
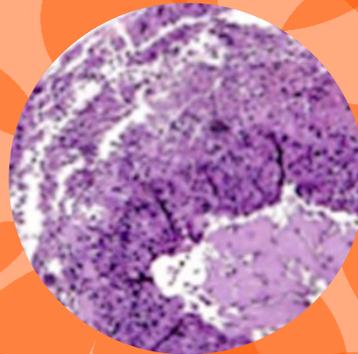
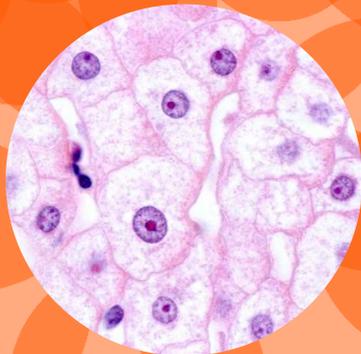
Cardiotoxicity Summary

iPSC-derived cardiomyocytes (CMs) could be used in cardiotoxicity & cardiomyocyte pharmacology studies

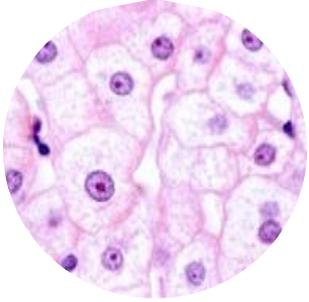
- iPSC-derived CMs express definitive cardiac markers & form organized sarcomeres
- iPSC-derived CMs show synchronized beating as a monolayer culture at high confluency
- Electrophysiological measurement of APs, pharmacology consistent with expression of INav, ICav & IKr
- Functional on xCelligence & for calcium imaging



Hepatocytes



A way forward for more predictive toxicity testing



Hepatotoxicity in Drug Safety Testing

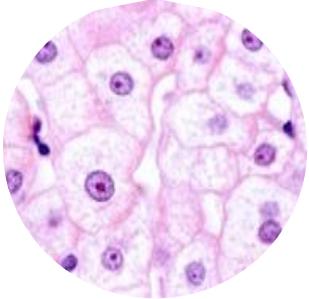


We need:

- Reliable genotoxicity testing, predictive hepatotoxicity screens
- Cells expressing adult hepatocyte markers & no fetal phenotype
- Large batch sizes from the same donor for consistency for toxicity and high-throughput screening

Human primary hepatocytes have much greater functionality than iPSC-derived hepatocytes

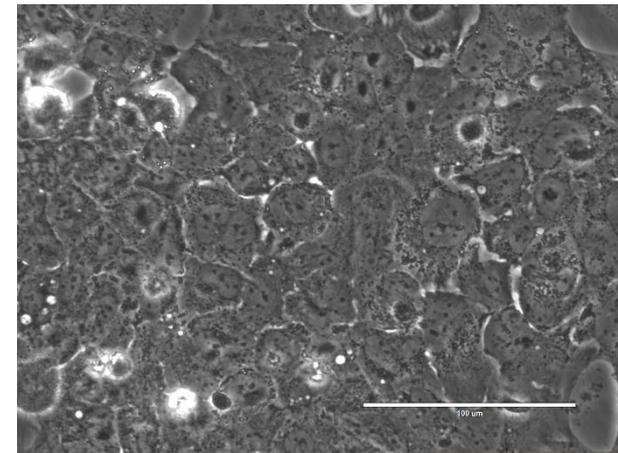
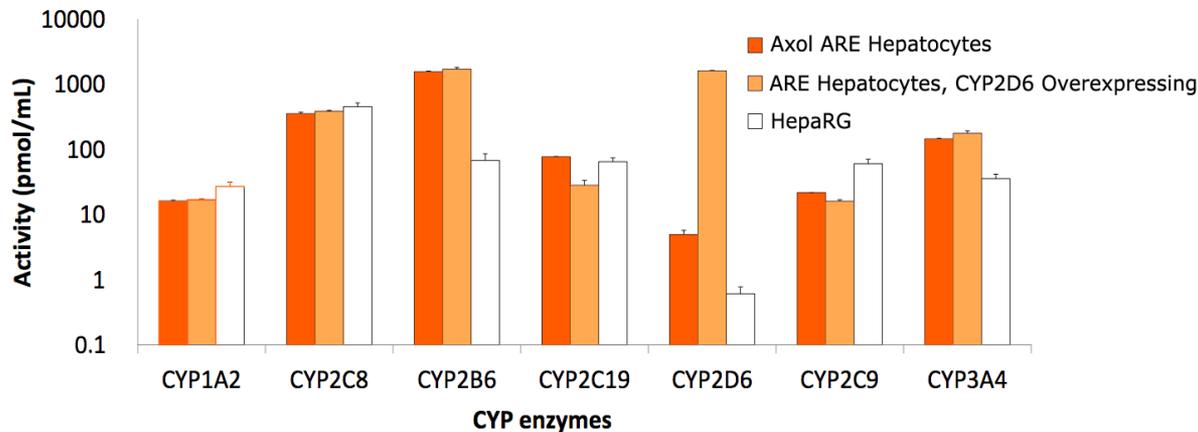




Assay-Ready Expanded (ARE) Hepatocytes

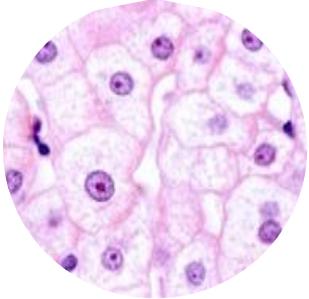


- Expanded hepatocytes that retain many characteristics of primary human hepatocytes
- Metabolically functional & express cytochrome P450 (CYP) enzymes



Cobblestone morphology

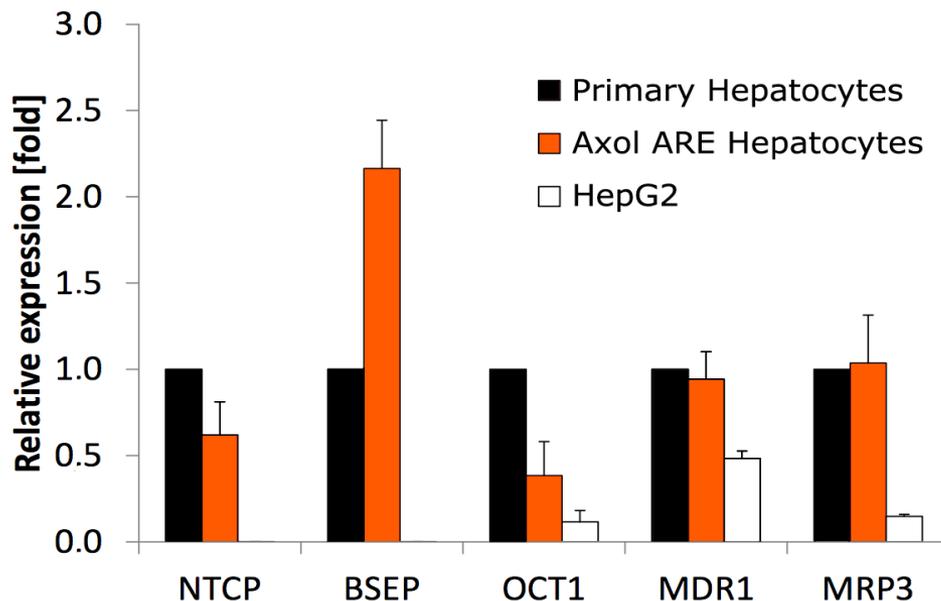
Comparison of the Phase I CYP enzyme activity between ARE Hepatocytes, ARE Hepatocytes (CYP2D6 Overexpressing) & HepaRG cells



Assay-Ready Expanded (ARE) Hepatocytes

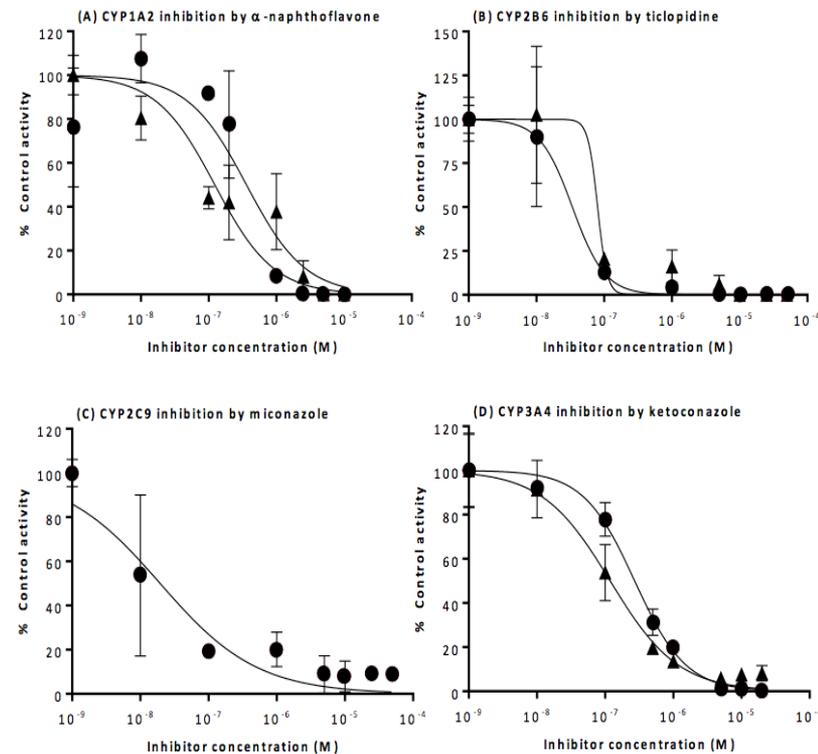


Compound uptake studies

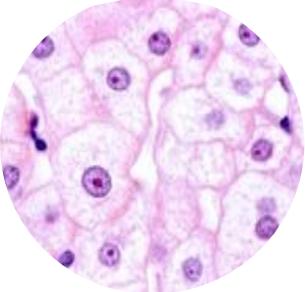


Expression of hepatic transporter genes in primary hepatocytes, ARE hepatocytes & HepG2 cells

Inhibition Studies



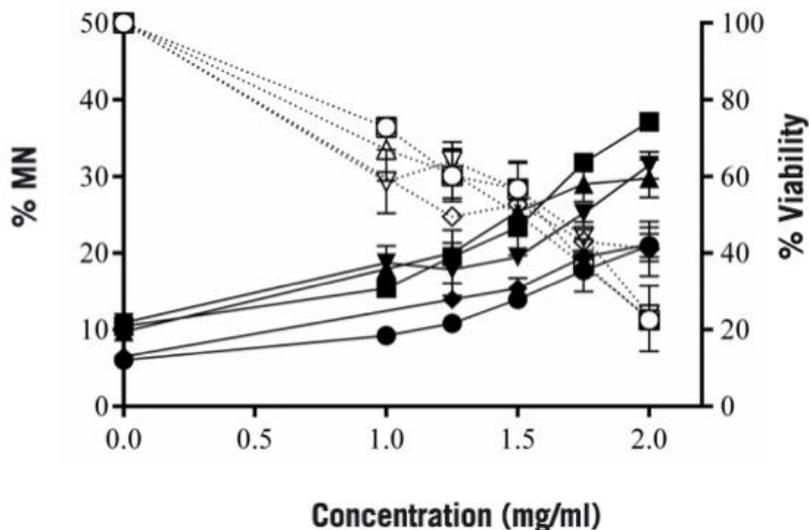
Reproducible CYP induction & inhibition in a donor-specific manner by prototypical inducers/inhibitors



Assay-Ready Expanded (ARE) Hepatocytes

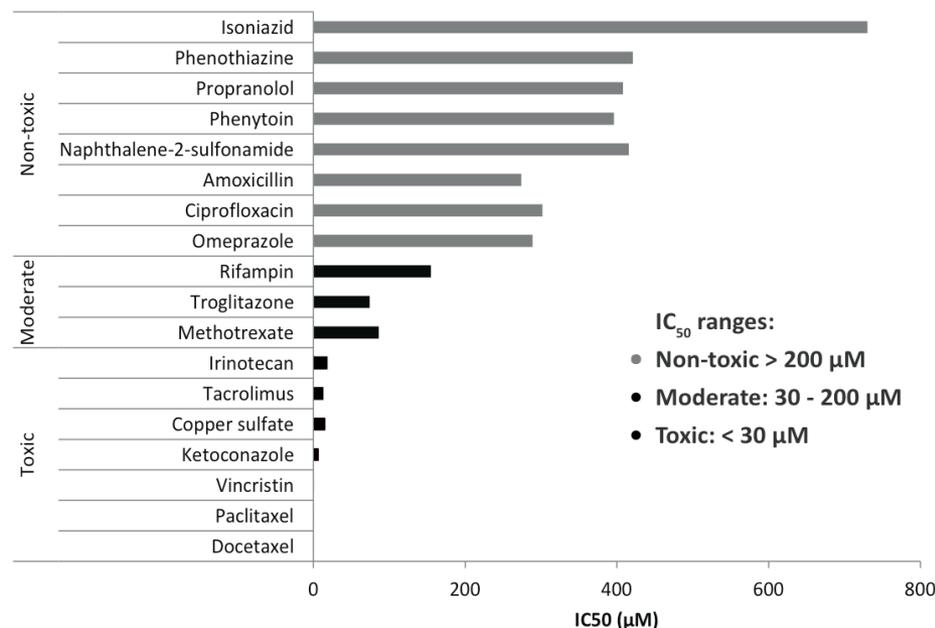


Genotoxicity studies



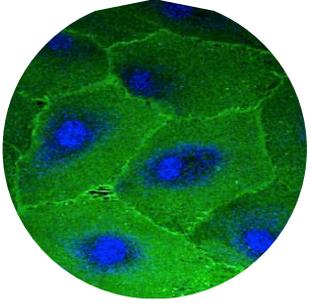
Increasing cyclophosphamide concentration affects the percentage of cells with micronuclei (% MN) & cell viability

Hepatotoxicity studies



Sensitivity to hepatotoxic compounds



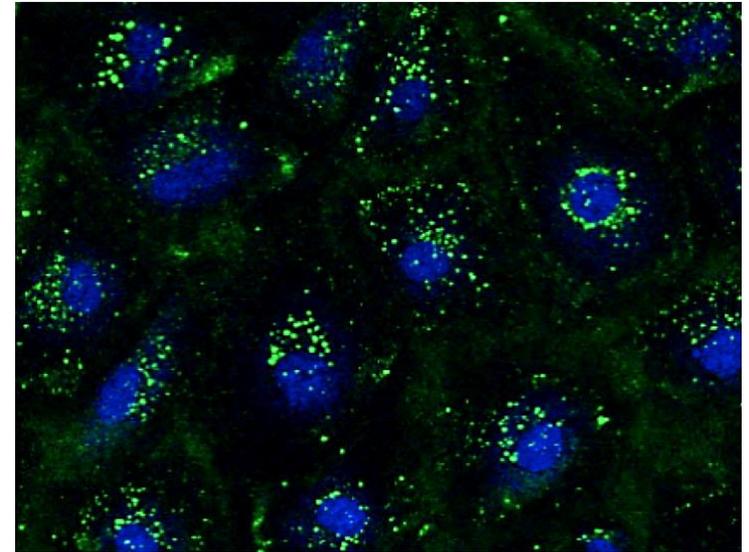


Assay-Ready Expanded (ARE) Liver Sinusoidal Endothelial Cells

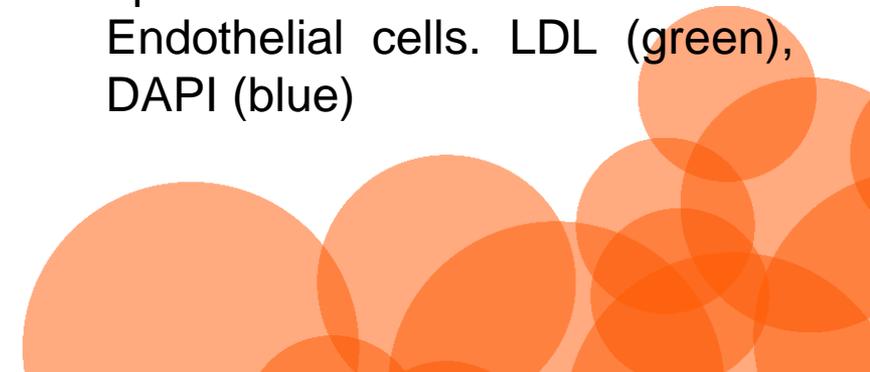


ARE Liver Sinusoidal Endothelial Cells are primary liver endothelial cells that have been expanded *in-vitro*

3D cultures can be generated by co-culturing with ARE Hepatocytes



Low Density Lipoprotein (LDL) uptake in ARE Liver Sinusoidal Endothelial cells. LDL (green), DAPI (blue)



Hepatotoxicity Summary

- ARE Hepatocytes display a primary liver cell phenotype
- ARE Hepatocytes are metabolic competent cells expressing liver specific transporters and metabolizing enzymes
- Large batch sizes from the same donor for consistency for toxicity & high-throughput screening
- Sensitivity to hepatotoxic compounds & reliable genotoxicity testing
- ARE Hepatocytes can be co-cultured with liver sinusoidal endothelial cells



Conclusion

Our aim is to provide physiologically relevant *in-vitro* disease models for drug discovery & toxicity studies

Axol iPSC-derived NSC

- Express neural markers at gene and protein level
- Excellent neurite outgrowth
- Electrophysiologically functional
- Capable of synaptic plasticity

Axol iPSC-derived Cardiomyocytes

- Expressing definitive cardiac markers and form organized sarcomeres
- Synchronous beating monolayers, electrophysiologically functional
- Functional on xCelligence & for calcium imaging

ARE Hepatocytes

- Display a primary liver cell phenotype
- Metabolic competent cells expressing liver specific transporters and metabolizing enzymes
- Sensitivity to hepatotoxic compounds & reliable genotoxicity testing



Thank you!

SOT Booth #419

your discovery stems from here

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