

Triggering Calcium Responses in Various Human iPSC-derived Neural Cell Types

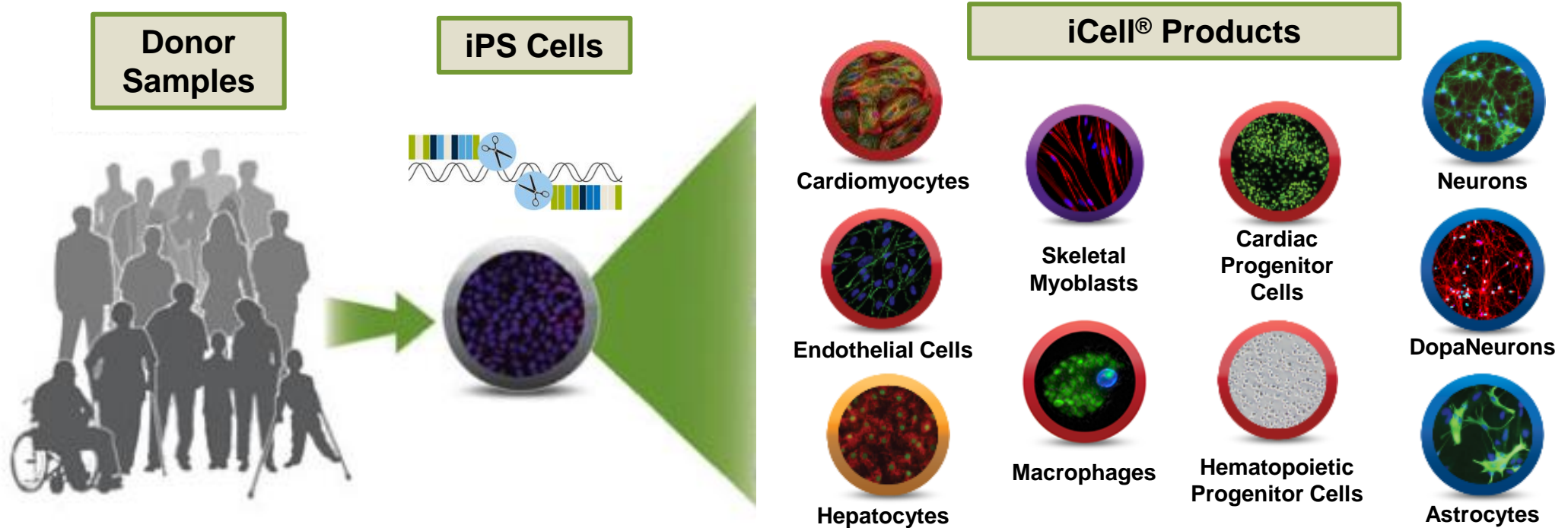
Giorgia Salvagiotto, PhD

June 2016



Transformative Potential of iPSC Technology:

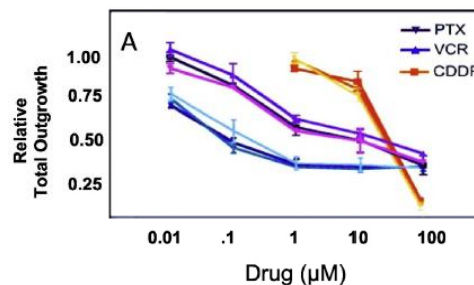
Enabling for Drug Discovery, Toxicology, and Regenerative Medicine



CDI Manufacturing Benchmarks (cells per month, >95% purity)

- 70 billion iPS cells
- 30 billion cardiomyocytes
- 30 billion neurons
- 20 billion endothelial cells
- 20 billion hepatocytes

Lot-to-Lot Consistency



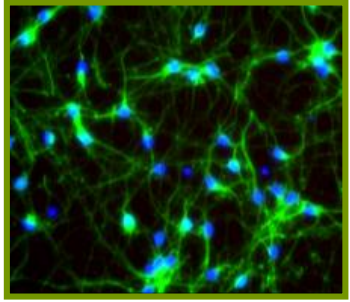
Diversity and Disease Portfolio

- Diversity
Gender, Ethnicity, etc
- Disease Cohorts
Cardiac, hepatic, neuro, etc
- Cell Banks
CIRM, NHLBI, etc.

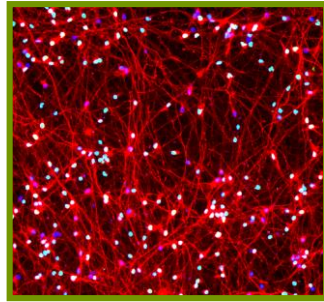
MyCell® Products

- Custom Reprogramming, Engineering, and Differentiation From Any Donor

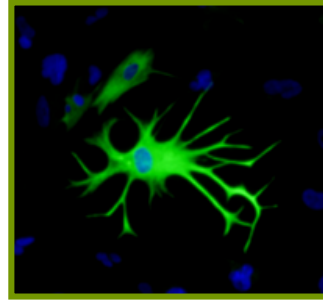
Several Different Human Neural Cell Types Available...



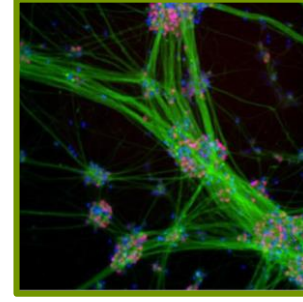
iCell Neurons



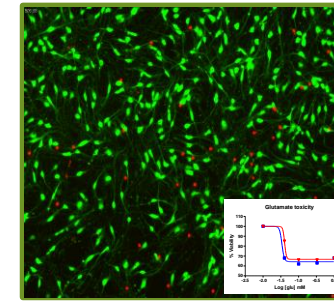
iCell DopaNeurons



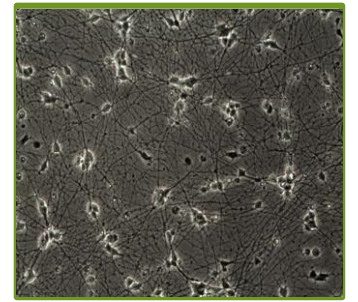
iCell Astrocytes



iCell Motor
Neurons

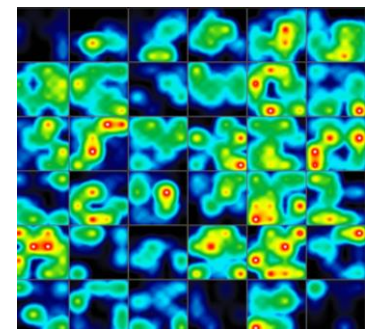
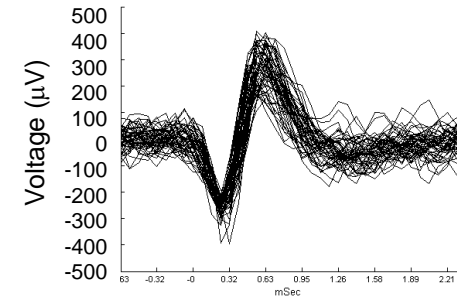
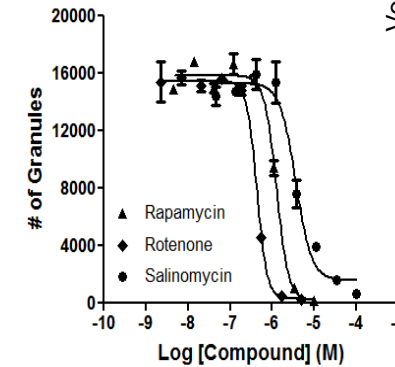
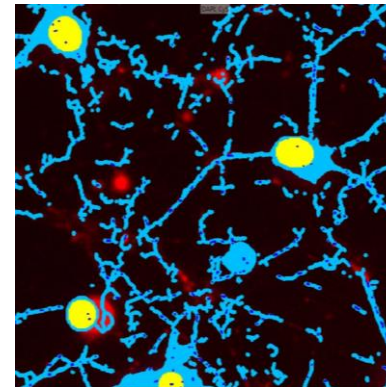
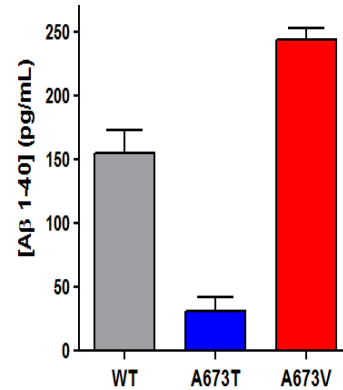
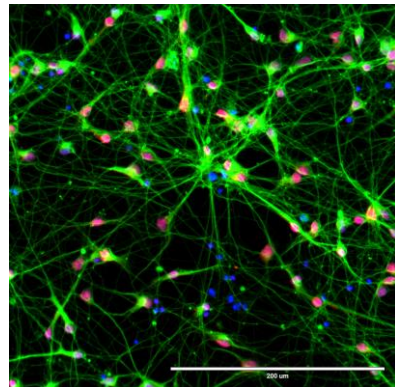
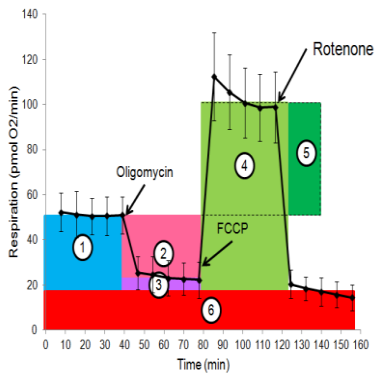


iCell GlutaNeurons

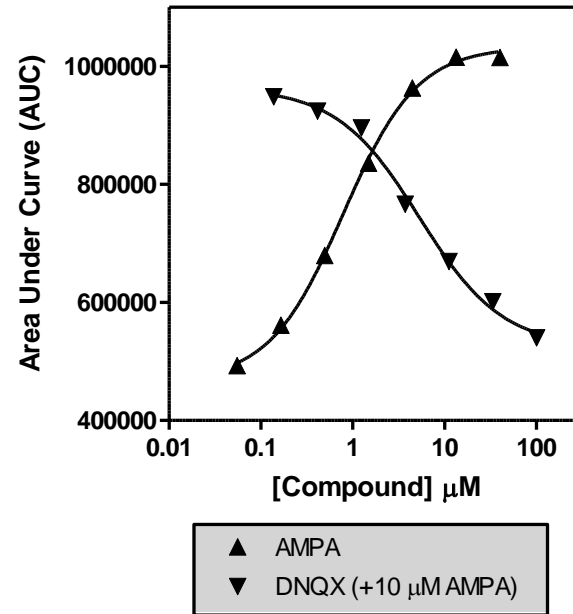
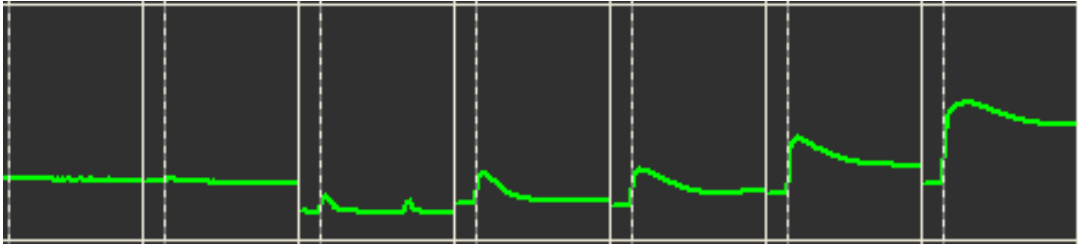


iCell Induced
Neurons

... with Numerous Different Applications



Area of Interest – Calcium Signaling Assays in Neurons



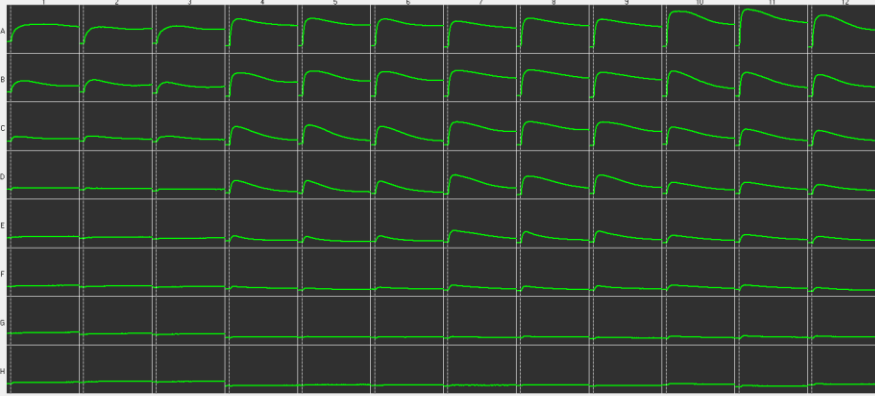
- Calcium plays an important role in cell signaling
 - Direct signal transduction or as 2nd messenger

- Calcium is involved in:
 - Membrane excitability and depolarization
 - Synaptic plasticity and neuronal transmission

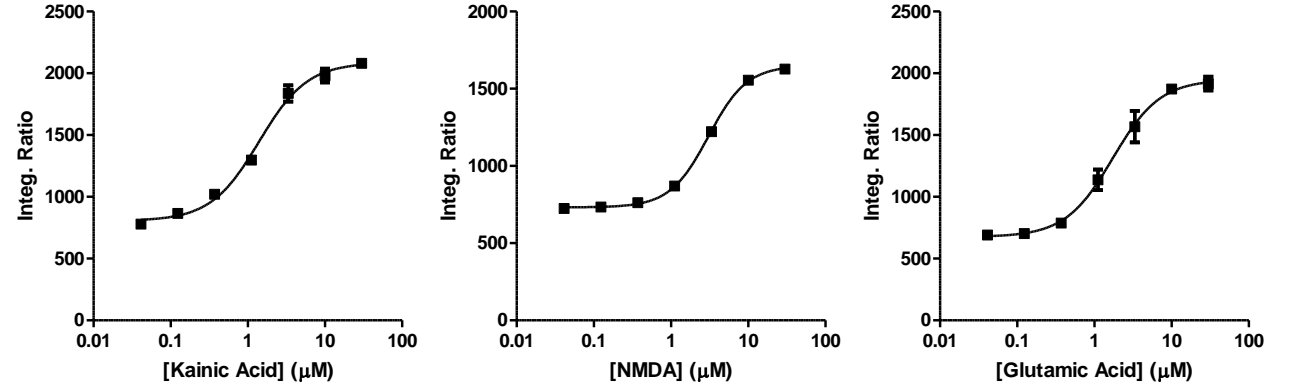
- There is interest in human iPSC-neurons because they possess relevant markers/channels that are present at endogenous expression levels:
 - Voltage-gated calcium channels (VGCCs)
 - Internal Ca^{2+} stores (IP3 and ryanodine receptors)
 - Ionotropic glutamate receptors (NMDA and AMPA)
 - Metabotropic Glu receptors (mGluRs)

1st Tier Approach: Ligand-induced Ca²⁺ Flux

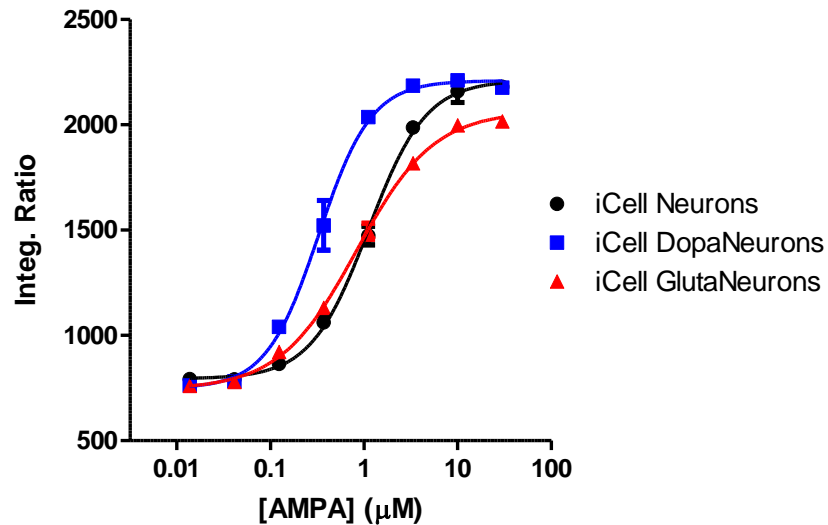
96-well plates; HTS-compatible



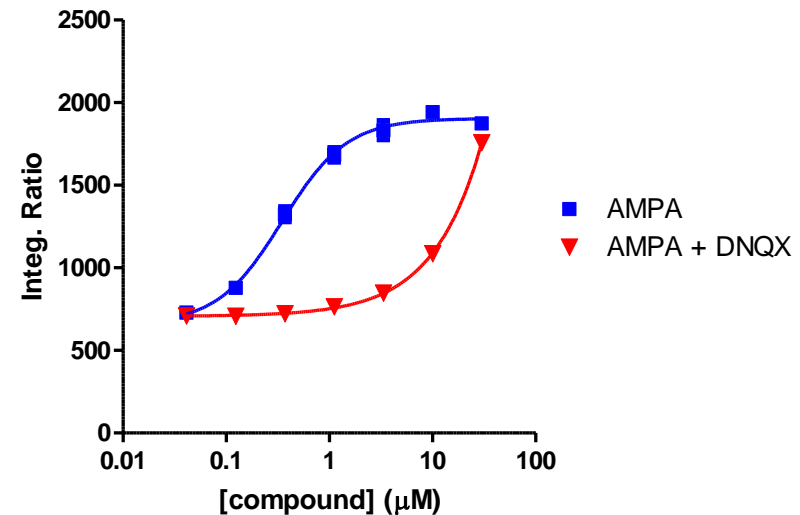
Profiling of multiple receptor agonists



Measurable across multiple neuronal subtypes

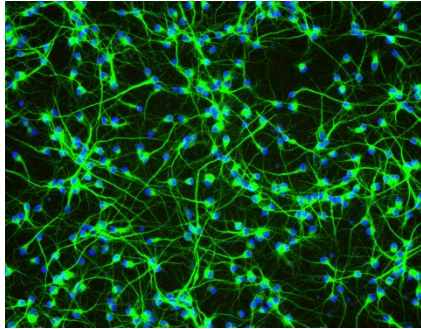


Ca²⁺ signal can be inhibited / competitively antagonized

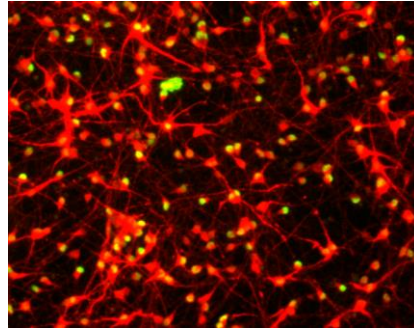


2nd Tier Approach: Measuring Network Connectivity

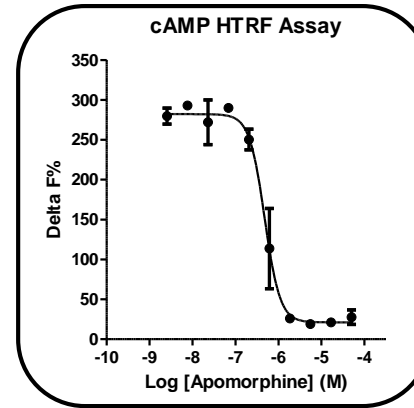
iPSC-derived Dopaminergic Neurons



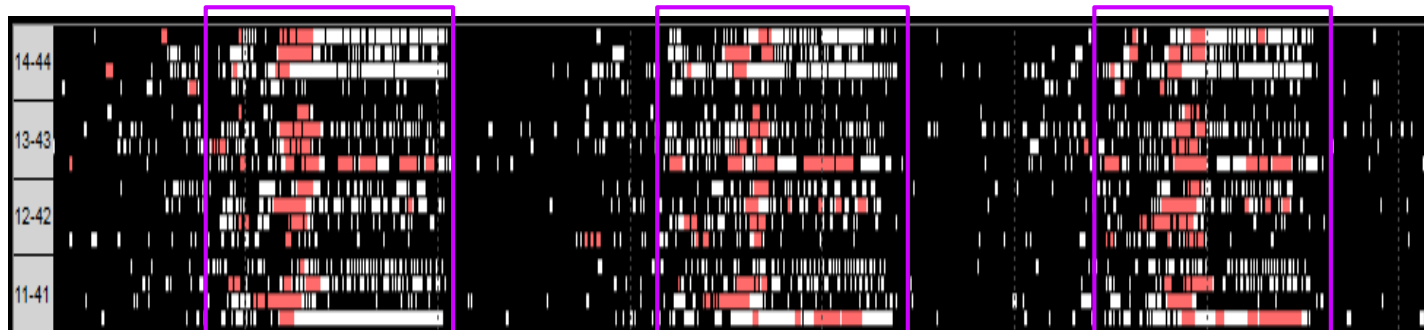
Map2 / Nestin / Hoechst



TH / FoxA2 / Hoechst



- ✓ High neuron purity (>95% MAP2+)
- ✓ High TH expression (>80% DIV 14)
- ✓ Appropriate gene expression (cells are more *excitatory* than *inhibitory*; VGLUT2 > VGAT)
- ✓ Responsive to various DA-specific pharmacology (cAMP HTRF assay)
- ✓ Develops organized network-level bursting patterns on MEA (DIV >14)

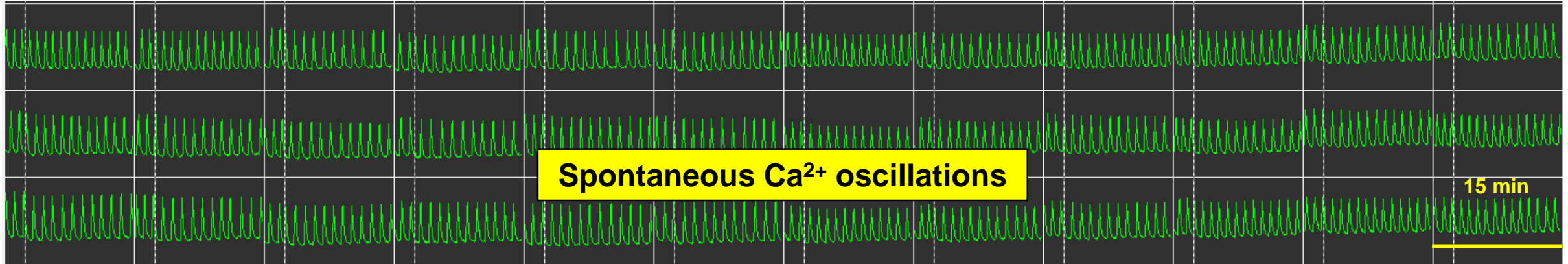


Network Bursts

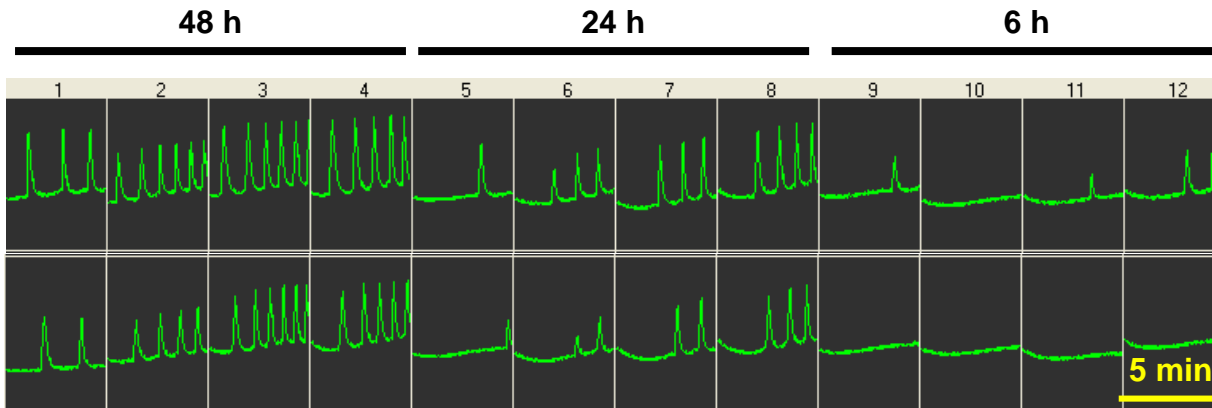
2nd Tier Approach: Measuring Network Connectivity

Assay Development Highlights

Consistent phenotype / assay signal from well-to-well



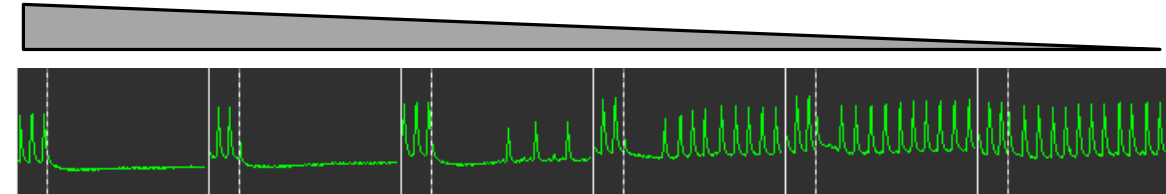
Timing and media considerations



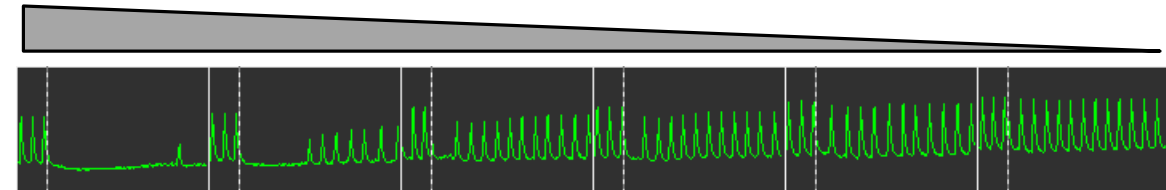
DIV >14

Pharmacological modulation of signal

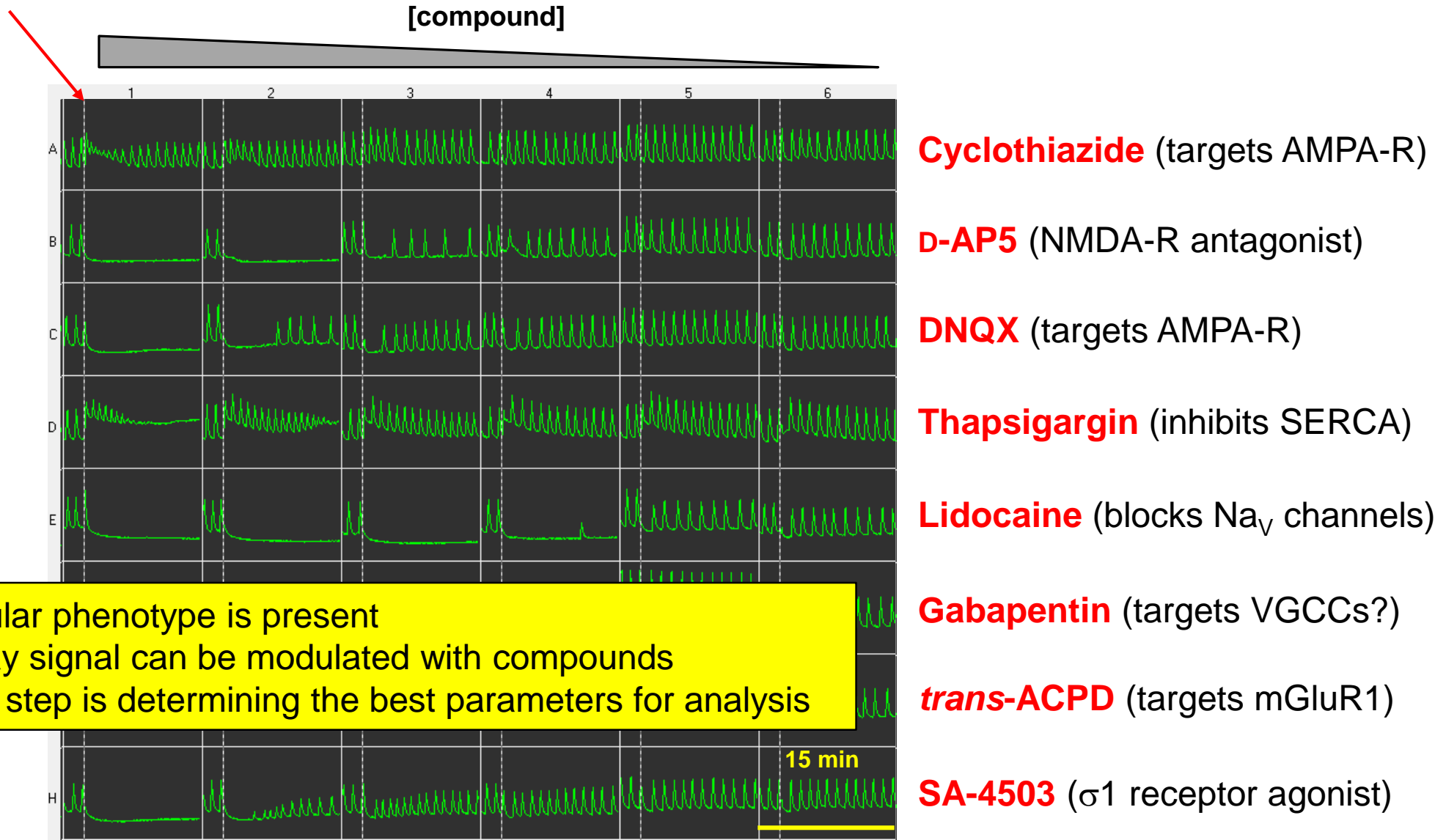
20 μ M D-AP5



5 μ M DNQX



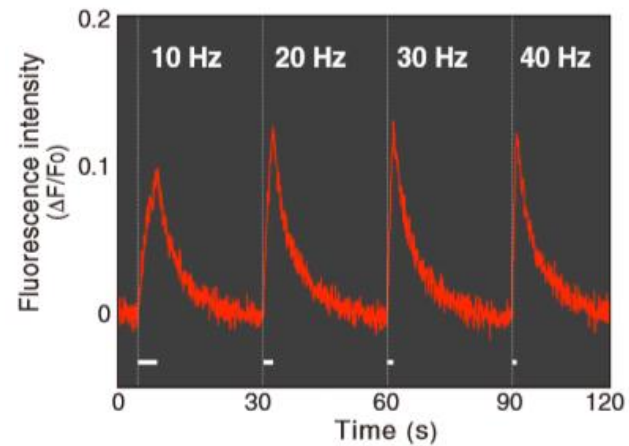
Example Pharmacology with iCell DopaNeurons



1. Cellular phenotype is present
2. Assay signal can be modulated with compounds
3. Next step is determining the best parameters for analysis

3rd Tier Approach: 'Next Gen' Assays with FDSS/ μ Cell

Ca²⁺ response evoked by EFS

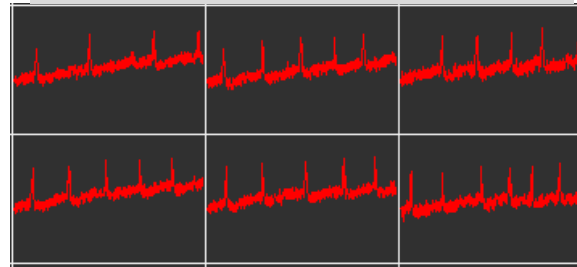


Electric field stimulation (EFS) parameters

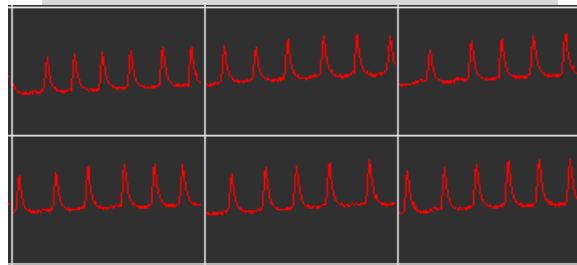
Voltage	20 V
Pulse Width	2.0 ms
Number of pulse	50 times at each stimulation
Frequency	10, 20, 30, 40 Hz

Membrane Potential

Voltage (FluoVolt dye)

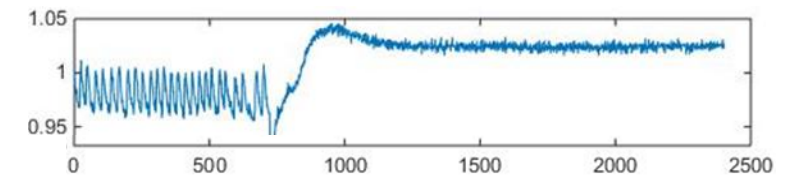
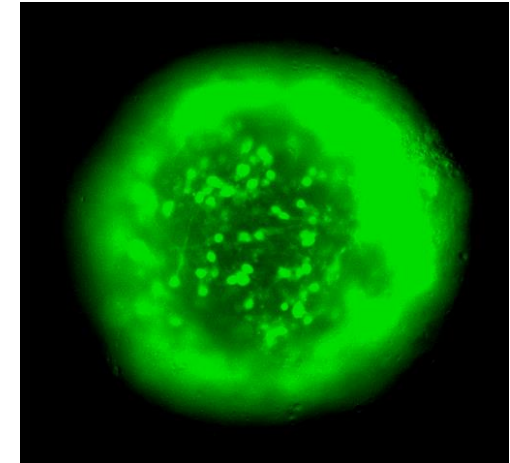


Calcium (Fluo-4 dye)

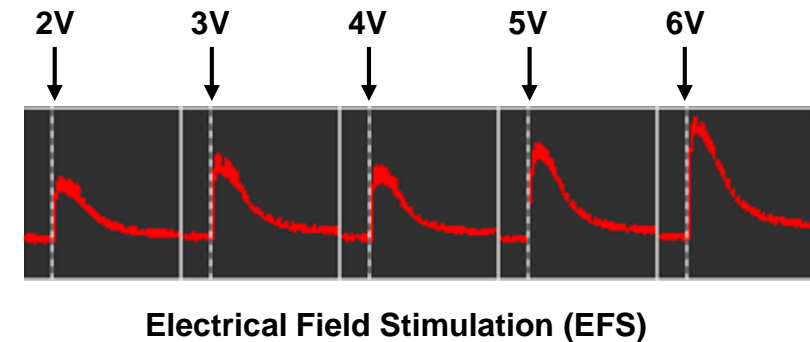
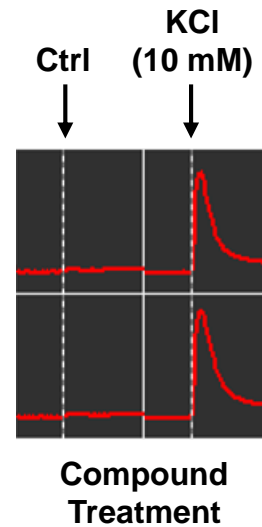
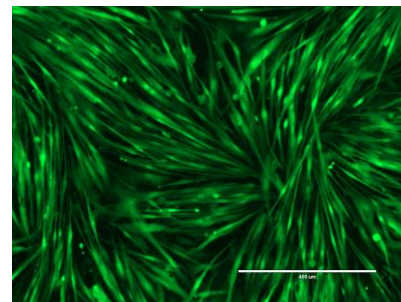
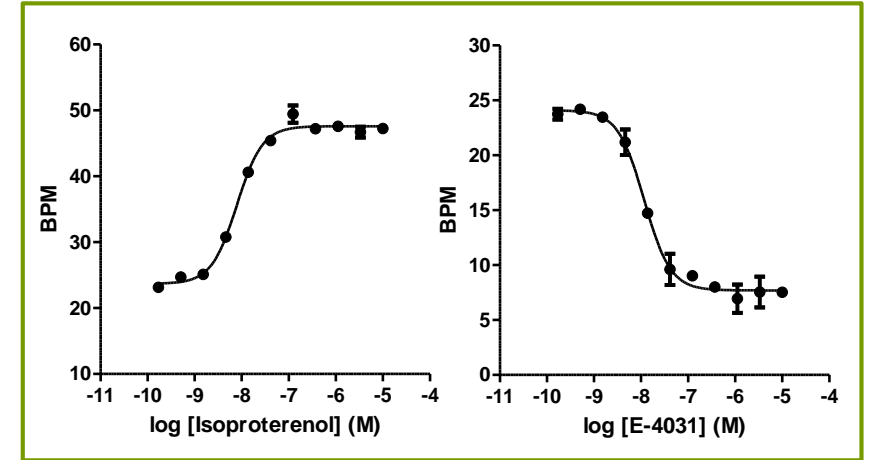
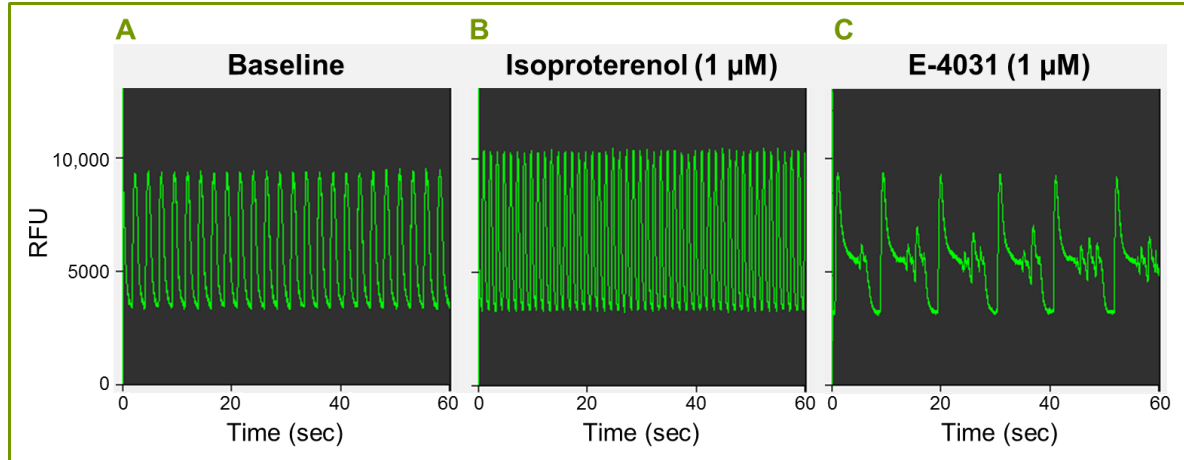
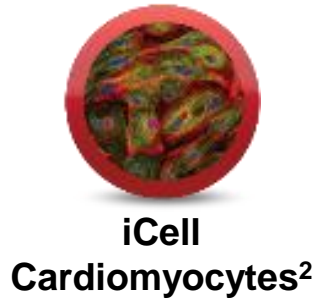


(Data from cells on same plate)

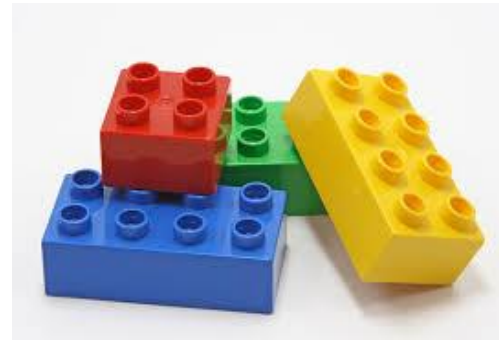
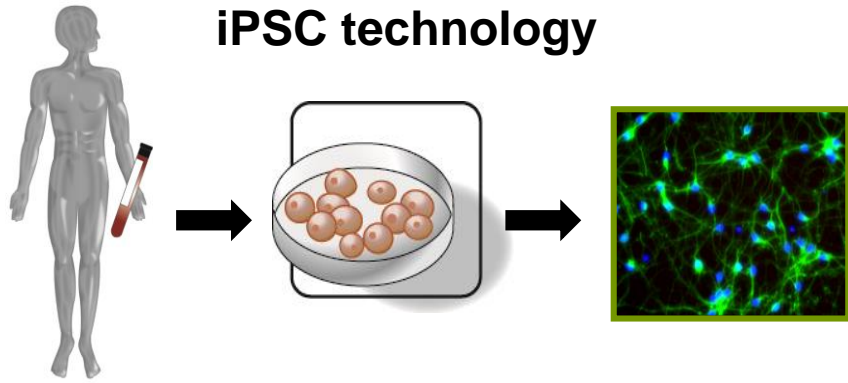
3D Cell Culture / Co-Culture



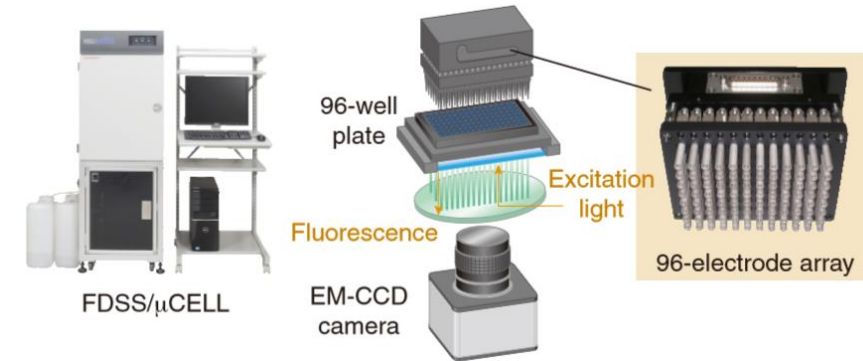
Other *Functionally Relevant* Human Cell Types from iPSC



Final Thoughts



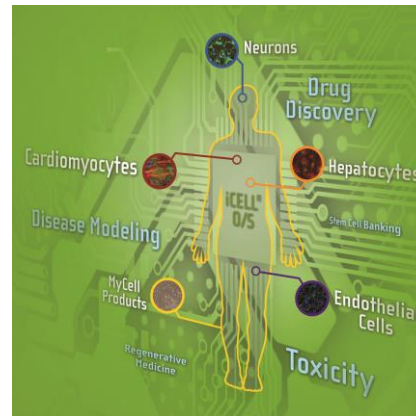
Lego building blocks



**Platform provider;
Data analysis**



**CDI provides
highly pure
cryopreserved
cells**



iCell Operating System



Study neurological diseases