Metabolic Measurements of Disease-relevant Human iPSC-derived Cells Using Seahorse XF Assay Technology

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Abstract

OBJECTIVE/RATIONALE: Terminally differentiated cell types generated from human induced pluripotent stem cells (iPSC) offer an important source of specialized material that is not often readily available. Furthermore, iPSC technology enables "disease-in-a-dish" studies though the differentiation of such cell types from both healthy and disease-relevant iPSC lines. A revealing feature of human iPSC-derived cell types is their basal metabolism, and a key application is to detect discrete changes in their cellular bioenergetics based on disease state, cell activation, or toxicity. The metabolic profile of various iPSC-derived cell types and the corresponding disease models (mutation vs. isogenic control) was determined by measuring the Mitochondrial Stress Test Profile on the Seahorse XF Pro Analyzer (Agilent).

METHODS/RESULTS: Seahorse protocols were developed using commercially available, cryopreserved iPSC-derived neural cell types (iCell products) from FUJIFILM Cellular Dynamics. Seahorse XF Cell Mito Stress Test Kit was used to comprehensively evaluate mitochondrial respiration and bioenergetics. All cells were thawed/cultured according to manufacturer's instructions with media and supplements provided. Assay variables were cell density, extracellular matrix, assay media, day of assay, and [FCCP]. XF measurements were normalized to cell number by nuclei count from high content image analysis.

Results include Seahorse data comparing iCell Microglia (wild-type; WT) with TREM2 mutants (AD-relevant hetero- and homozygous TREM2 functional knockouts), where WT microglia consistently displayed a 1.5X higher spare capacity. iCell Induced Excitatory Neurons, WT and progranulin mutation (GRN R493X) cell lines, demonstrated higher OCR profiles when compared to iCell GlutaNeurons and iCell GABANeurons. Additionally, respiration was the highest for the GRN R493X neurons, which correlates with higher neuronal spike activity recorded on microelectrode array (MEA). Finally, iCell DopaNeurons generated from Michael J. Fox Foundation (MJFF) patient-derived iPSC lines harboring PD-relevant mutations (LRRK2 G2019S or GBA N370S) and the corrected isogenic controls were compared. A high cell density (125K cells/well) was needed to achieve robust OCR signal and the GBA N370S line had the largest spare capacity.

CONCLUSIONS: Seahorse XF cell analysis provides sensitive label-free measurements that are useful in detecting changes in mitochondrial function between different human iPSC-derived neural cell types, as well as between wild-type and disease-mutation containing cells. The combination of these two technologies is a powerful tool for disease modeling.

Keywords: Metabolism, iPSC, Oxidative Stress

Materials and Methods

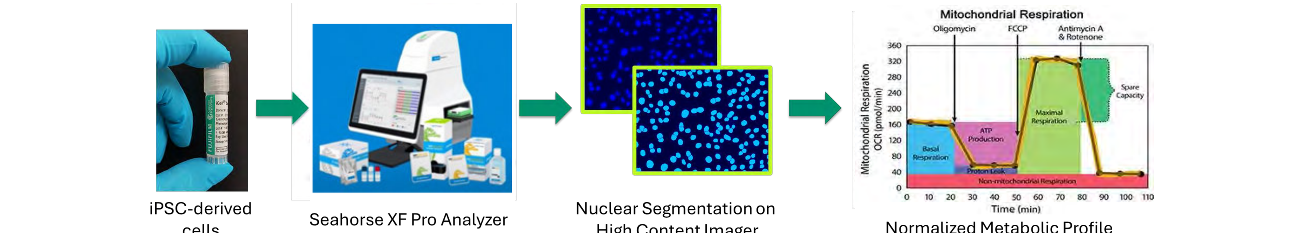
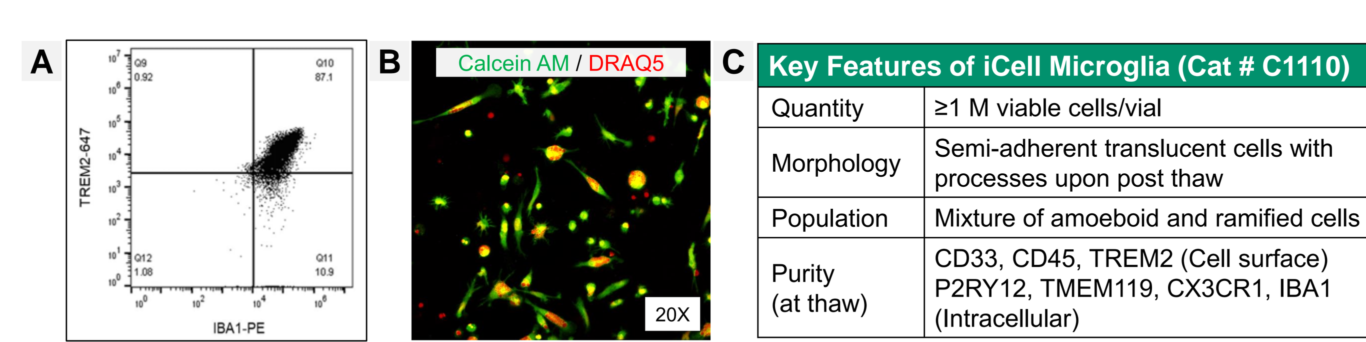


Figure 1. Seahorse XF assay workflow. Cryopreserved iPSC-derived cells are thawed according the manufacturer's instructions and cultured in maintenance media until the day of assay. Mito Stress Test assays were performed on the XF Pro Analyzer and then nuclear cell counts were used to normalize cell numbers. Data is analyzed using Excel-based software templates from Agilent.

- iCell products were thawed and plated according to the User's Guide or Quick Guide.
- Complete Maintenance Medium for each cell type was prepared and stored according to recommendations.
- Seahorse XF Pro 96-well plates were either PDL Cell Culture Microplates (Agilent 103799-100) or standard XF Pro M Cell Culture Microplates (1103774-100) coated with the appropriate ECM.
- Cell density per well was optimized for each cell type and generally used Maximal Respiration over 3 time points as the key metric. On Day 0, cells were seeded in 80 µl/well of media and not in the corners of the plate.
- On the day before assay, an XF Sensor Cartridge was hydrated overnight.
- On day of assay, culture medium was exchanged into supplemented XF DMEM Assay Medium.
- Assay medium typically includes 10 mM glucose, 1 mM Sodium Pyruvate, and 2 mM L-Glutamine.
- All data in this poster features the Seahorse XF Cell Mito Stress Test Kit (103015-100).
- Run Seahorse XF Assay as recommended by Agilent. Refer to guidelines and instructions here: https://www.agilent.com/en/product/cell-analysis/how-to-run-an-assay
- FCCP concentration was optimized for each cell type. Oligomycin = 1 µM. Rot/AntA = 0.5 µM.
- After each XF assay was completed, cells were labeled with nuclear dye in XF Assay Medium (20 µL of 1:1000 working solution to each well) for 15 minutes.
- Nuclear images were acquired on a fluorescent microscope or high content imager; cell segmentation and counting was performed, and data was exported to Excel.
- Cell number data was copy/pasted into the "Normalize" tab in results page of XF Pro Controller Software. Alternatively, data could be uploaded to cloud-based Seahorse Analytics: https://seahorseanalytics.agilent.com/Account/Login

iCell Microglia



Key Features of iCell Microglia (Cat # C1110)	
Quantity	≥1 M viable cells/vial
Morphology	Semi-adherent translucent cells with processes upon post thaw
Population	Mixture of amoeboid and ramified cells
Purity (at thaw)	CD33, CD45, TREM2 (Cell surface) P2RY12, TMEM119, CX3CR1, IBA1 (Intracellular)

Figure 1. Characterization of iCell Microglia. iCell Microglia are a biologically relevant cell type amenable to research for neuroinflammation and cytokine signaling, modeling Alzheimer's Disease, drug screening, and co-culture systems w/ neurons and astrocytes. (A) MGL are a highly pure population of microglia (TREM2 vs. IBA1) derived from human iPSC donor 01279. These cells are differentiated based on technology developed by the Blurton-Jones laboratory (Abud et al. *Neuron* 2017) for which FUJIFILM CDI holds the exclusive license. (B) Cryopreserved cells are provided as a kit with optimized media with supplements that support high cell viability and functionality. (C) Key features of iCell Microglia are summarized in the table.

D iCell Microglia: FCCP Titration

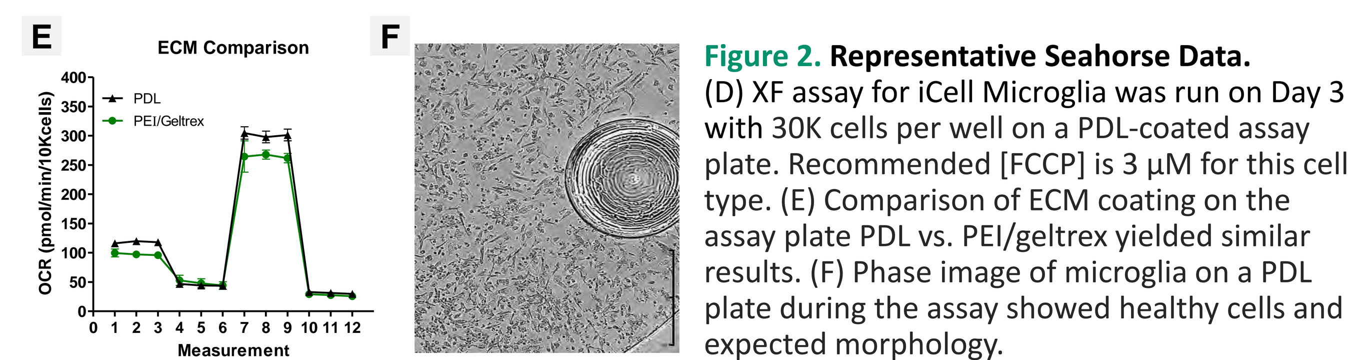


Figure 2. Representative Seahorse Data. (D) XF assay for iCell Microglia was run on Day 3 with 30K cells per well on a PDL-coated assay plate. Recommended [FCCP] is 3 µM for this cell type. (E) Comparison of ECM coating on the assay plate PDL vs. PEI/geltrex yielded similar results. (F) Phase image of microglia on a PDL plate during the assay showed healthy cells and expected morphology.

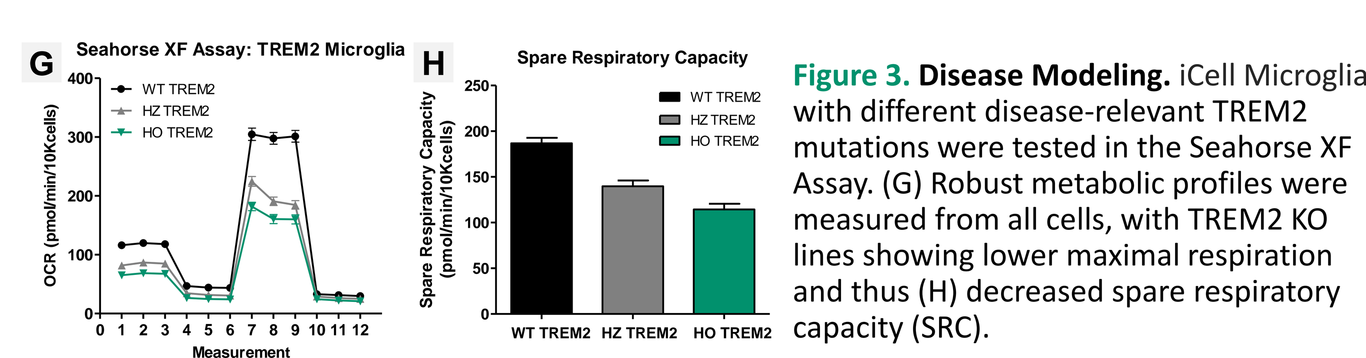
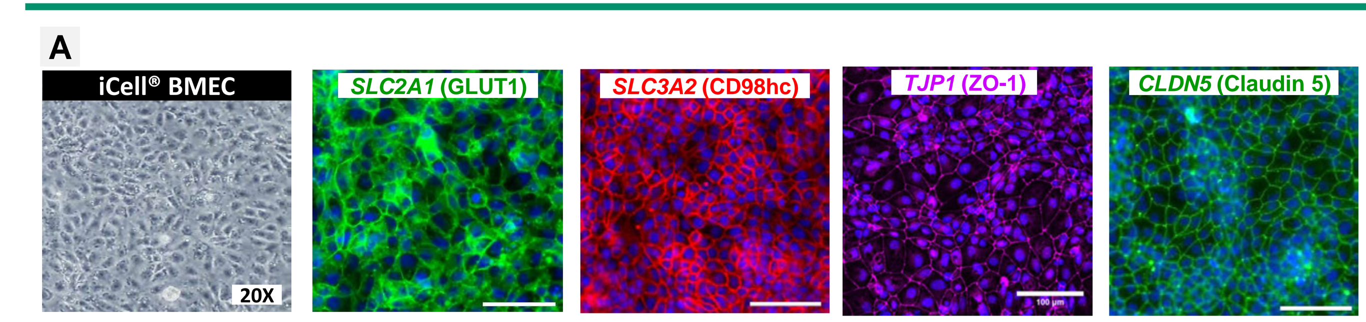
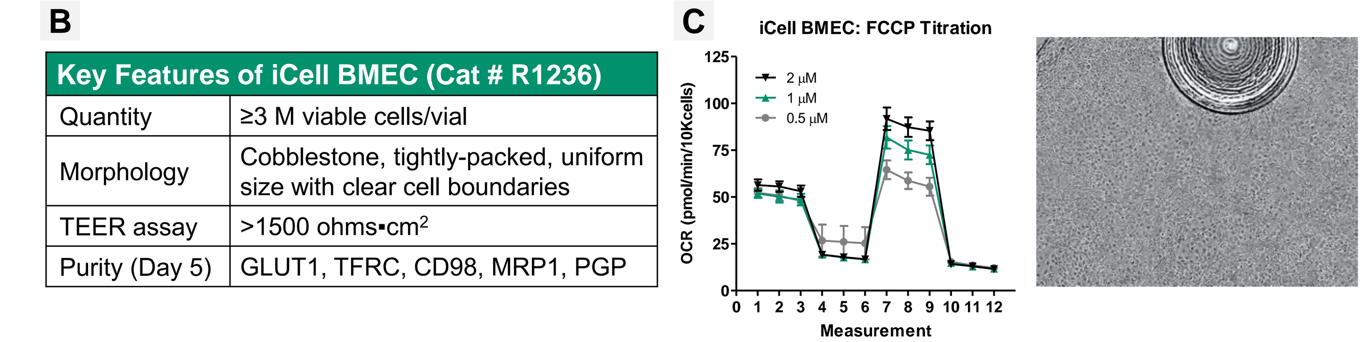


Figure 3. Disease Modeling. iCell Microglia with different disease-relevant TREM2 mutations were tested in the Seahorse XF Assay. (G) Robust metabolic profiles were measured from all cells, with TREM2 KO lines showing lower maximal respiration and thus (H) decreased spare respiratory capacity (SRC).

iCell Brain Microvascular Endothelial Cells (BMEC)

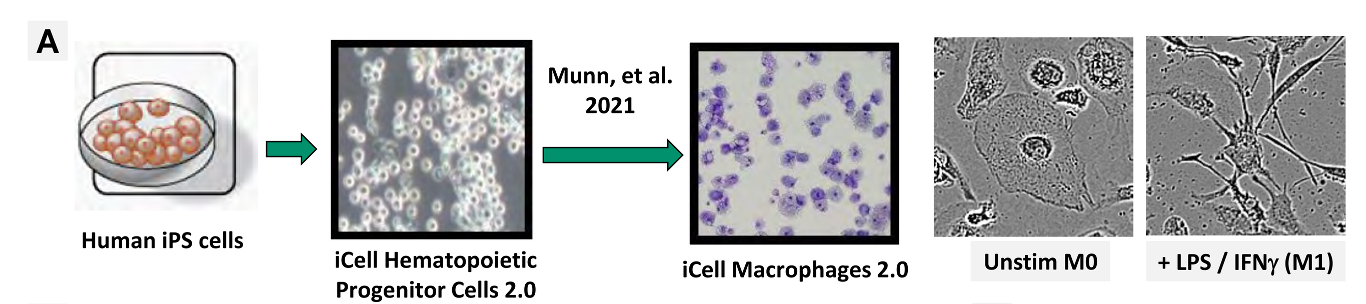


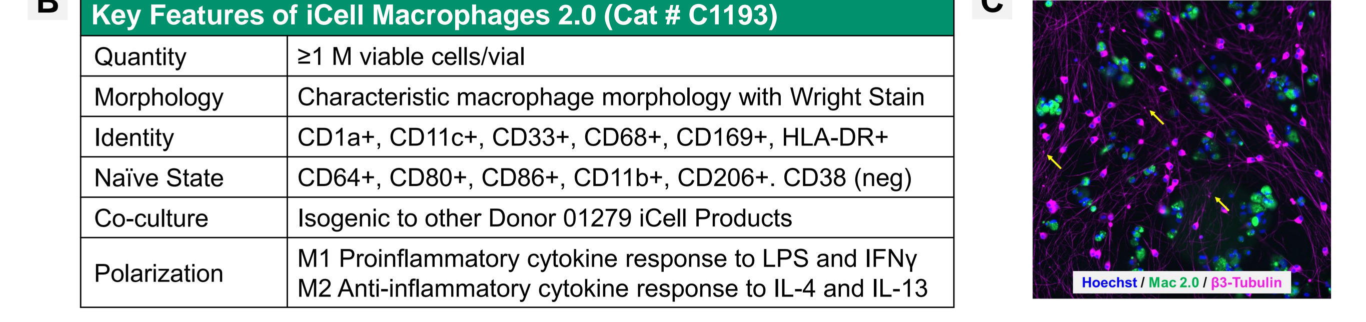


Key Features of iCell BMEC (Cat # R1236)	
Quantity	≥3 M viable cells/vial
Morphology	Cobblestone, tightly-packed, uniform size with clear cell boundaries
TEER assay	>1500 ohms•cm²
Purity (Day 5)	GLUT1, TFRC, CD98, MRP1, PGP

Figure 4. Characterization of iCell Brain Microvascular Endothelial Cells. BMEC are a central element of the blood-brain barrier (BBB), together with astrocytes and pericytes, to form an interface between nervous tissue and circulating blood. (A) BMEC differ from other endothelial cells in that they have cobblestone morphology as tightly packed cells with uniform size and clear cell boundaries. Marker expression as measured by ICC reveals the endothelial markers (Claudin 5, ZO-1), transporters (GLUT1, CD98hc), and efflux/ influx proteins (data not shown). (B) Key features of iCell BMEC include high cell purity by flow cytometry (≥90% positive) using prototypical BMEC markers. (C) Analysis of BMEC metabolism by Seahorse XF Assay was performed on Day 5 with 75K cells per well on a fibronectin-coated (10 ug/mL) assay plate. Optimized conditions for iCell BMEC utilized 2 µM FCCP.

iCell Macrophages 2.0





Key Features of iCell Macrophages 2.0 (Cat # C1193)	
Quantity	≥1 M viable cells/vial
Morphology	Characteristic macrophage morphology with Wright Stain
Identity	CD1a+, CD11c+, CD33+, CD68+, CD169+, HLA-DR+
Naïve State	CD64+, CD80+, CD86+, CD11b+, CD206+. CD38 (neg)
Co-culture	Isogenic to other Donor 01279 iCell Products
Polarization	M1 Proinflammatory cytokine response to LPS and IFNγ M2 Anti-inflammatory cytokine response to IL-4 and IL-13

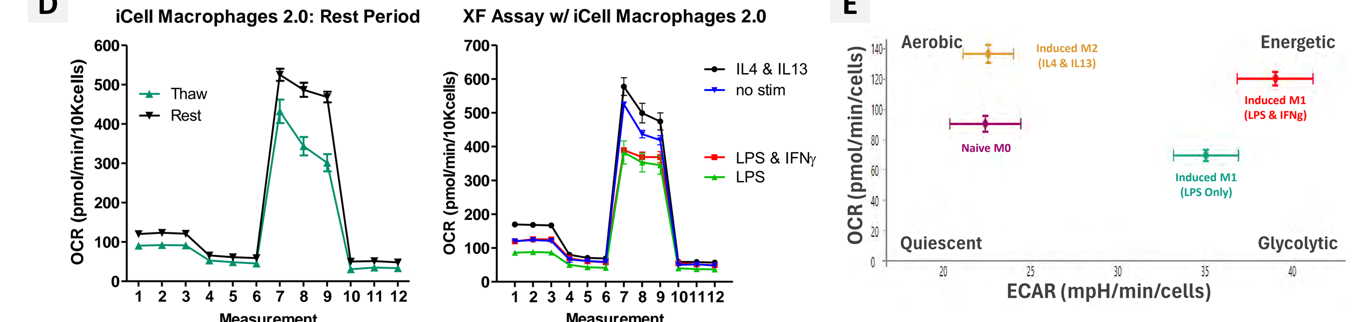
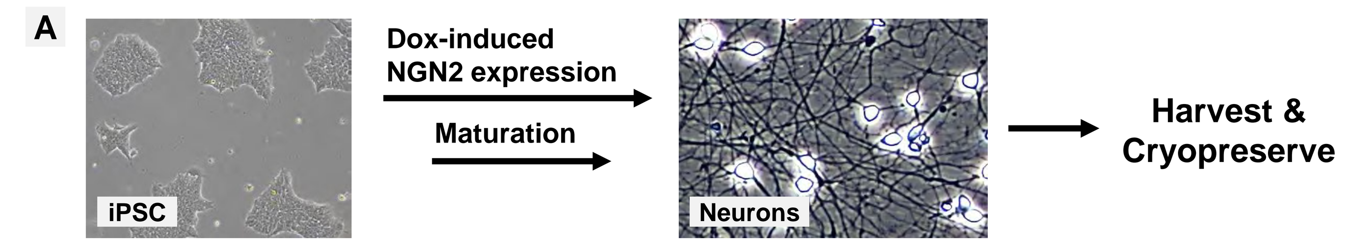


Figure 5. Characterization of iCell Macrophages 2.0. (A) iCell Mac 2.0 are differentiated from iPS cells through a hematopoietic progenitor intermediate. (B) Key features of iCell Mac 2.0 include M1/M2 polarization and that they are isogenic to other Donor 01279 iCell Products. (C) iCell Mac 2.0 can be successfully co-cultured with iCell Sensory Neurons to investigate immune-mediated effects on pain. (D) Cryopreserved cells can be assayed immediately at thaw or following a 3-day rest period. Data suggests that macrophages are more stable and consistent in their response to compounds (e.g., FCCP) in the Mito Stress Test kit when allowed to recover post-thaw. Further testing revealed that M1 polarization with LPS (and IFNγ) for 24 hours decreased OCR signal whereas anti-inflammatory M2 stimuli increased the metabolic profile. Assays were performed with 30K macrophages per well on DIV 3. (E) These data can be plotted as a bioenergetic profile of OCR vs. ECAR to describe the relative metabolic states of these iPSC-derived macrophages.

iCell Induced Excitatory Neurons: AHN vs. FTD Mutation



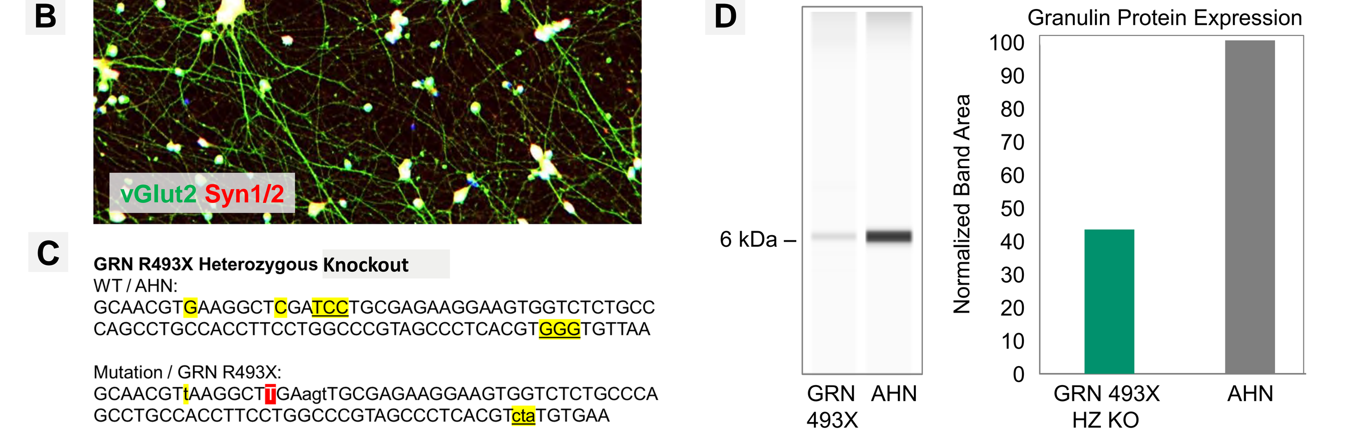
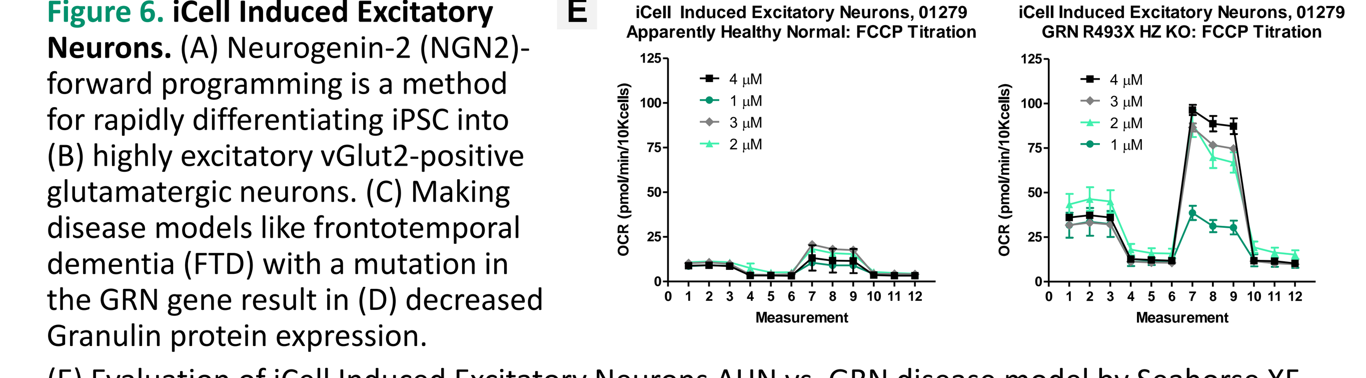
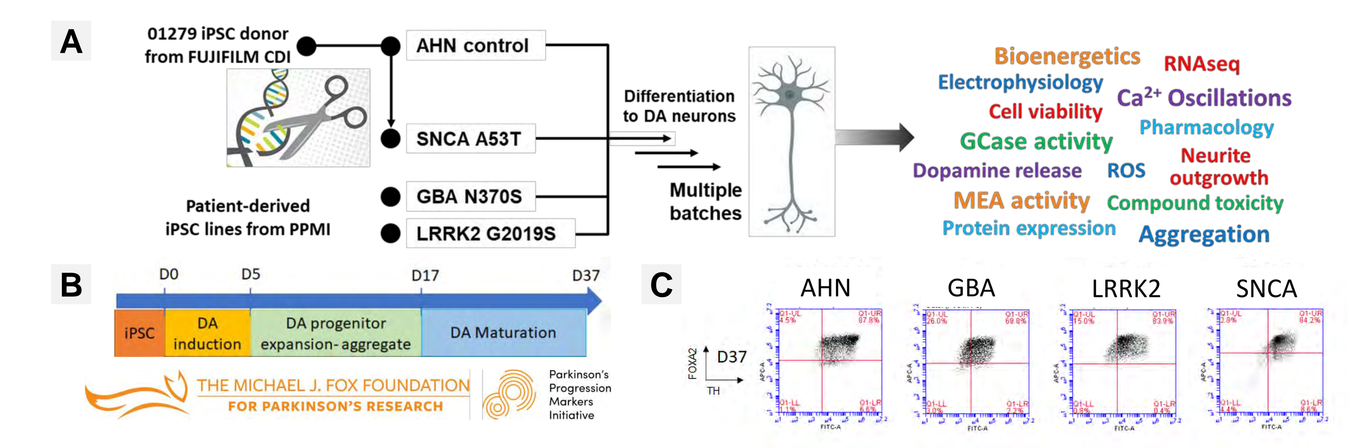


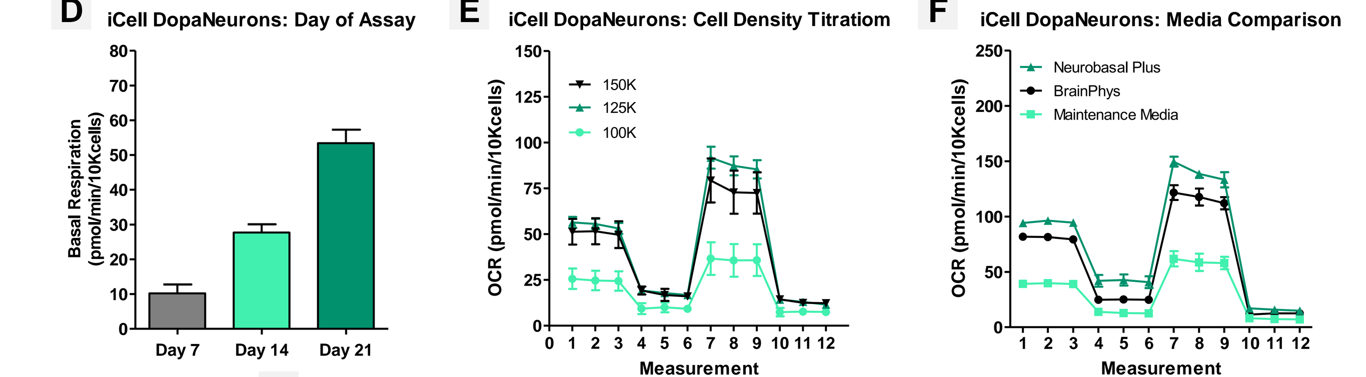
Figure 6. iCell Induced Excitatory Neurons. (A) Neurogenin-2 (NGN2)-forward programming is a method for rapidly differentiating iPSC into (B) highly excitatory vGlut2-positive glutamatergic neurons. (C) Making disease models like frontotemporal dementia (FTD) with a mutation in the GRN gene result in (D) decreased Granulin protein expression.



(E) Evaluation of iCell Induced Excitatory Neurons AHN vs. GRN disease model by Seahorse XF Assay revealed that these iPSC-derived neurons are relatively "quiet" and were measured at the lower end of sensitivity for the system. Interestingly, preliminary results with the GRN mutant showed a stronger metabolic profile under the same experimental conditions (30K cells/well, same day of assay, ECM, culture medium, etc.). Additional testing with this cell type is ongoing.

iCell DopaNeurons: Parkinson's Disease Panel





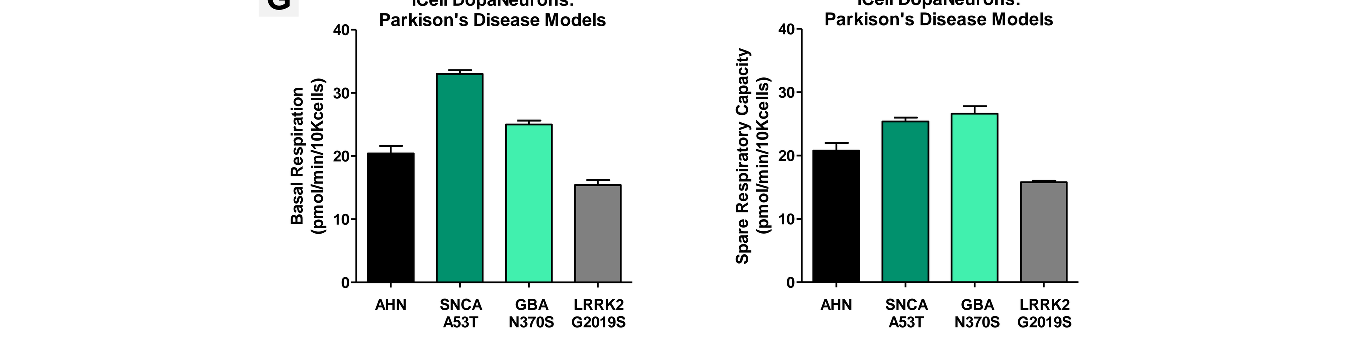


Figure 7. iCell DopaNeurons and Parkinson's Disease Models. (A) and (B) In partnership with the Parkinson's Progression Markers Initiative (PPMI) and The Michael J. Fox Foundation (MJFF), FUJIFILM CDI generated iPSC lines from clinically symptomatic PD patients carrying known risk-associated gene mutations. (C) The resulting iPSC-derived dopaminergic midbrain neurons were highly pure cell populations ready for use in numerous applications. Seahorse XF Assay with iCell DopaNeurons, AHN control cells was optimized for (D) day of assay, (E) cell density per well, and FCCP concentration (data not shown). While good results can be obtained at Day 7 and 14 in culture, the most consistent data was achieved at Day 21, which is when the optimal cell density of 125K cells/well was determined. (F) Alternate cell culture media impacted the bioenergetic profile of iCell DopaNeurons and these preliminary results are being tested further. (G) From the PD panel of cells tested on Day 14, SNCA A53T had the highest basal respiration, while GBA N370S showed the largest spare capacity. Isogenic mutation-corrected control cells have been generated for these lines and are being tested in the Seahorse XF Assay. Results are coming soon!

iPSC lines for the LRRK2 and GBA are part of the Parkinson's Progression Markers Initiative (PPMI) iPS cell bank.

Summary and Future Directions

iPSC-derived Cell Type	ECM / Matrix	Cells/Well	DIV	Oligomycin	FCCP	Rot / Ant A
iCell® Microglia	PDL	30,000	>3	1	3	0.5
iCell® BMEC	Fibronectin	75,000	>3	1	2	0.5
iCell® Macrophages 2.0	None	60,000	>3	1	2	0.5
iCell® Induced Neurons	PLO/Matrigel	>30,000	>9	1	4	0.5
iCell® DopaNeurons	PEI/Laminin	125,000	>21	1	1	0.5

Measurement of metabolic profiles for each of the human iPSC-derived cell types featured here resulted in robust response to compounds in the Mito Stress Test Kit on the Seahorse XF Pro Analyzer. This assay is very sensitive, and many factors can influence the OCR signal, such as cell density, media formulation, and compound concentrations. Therefore, assay optimization must be considered carefully for each cell type and even for different genetic variants. For example, the TREM2 diseased microglia lines consistently displayed lower spare capacity compared to WT; however, the PD DopaNeurons did not always result in a lower spare respiratory capacity.

This poster aimed to establish a starting point for customers to use these cell types and measure effects of toxicity, cellular maturation and polarization, and find unique disease phenotypes. The Seahorse XF data presented here will add power to the characterization of FUJIFILM CDI iCell products and facilitate the drug discovery and therapeutic development possibilities.

Additional information about the complete metabolic profile of these cells can be obtained combining the mitochondrial function studies with the Seahorse ATP Rate Assay, that delivers simultaneous quantitative assessment of both glycolytic and mitochondrial activity. In addition, the XF Substrate Oxidation Stress test kits can allow to deeper understand the main fuels that sustain mitochondria respiration as well as changes in the metabolic flexibility of the different genetic variants.