# Transcriptomic Profiling of Human iPSC-derived Sensory Neurons as a Model for Non-Opioid Pain Therapeutic Drug Discovery

**Dezmond Taylor-Douglas,** Scott Schachtele, Lida Zeighami, Yash Raj, Rebecca Fiene, Lisa Harms, Kiranmayee Bakshy, Ali Fathi, Jing Liu, Coby Carlson

FUJIFILM Cellular Dynamics, Inc., Madison, WI USA

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## Abstract

With the recent FDA approval of Vertex's compound Suzetrigine, a highly selective pain signal inhibitor that targets the NaV1.8 sodium channel, there is renewed biopharma focus on non-opioid based pain drugs. Rodent models have historically supported pre-clinical research, but there is a clear biological and translational gap when moving from animal studies into human clinical trials. The desire to improve predictability is fueling interest in new human-relevant models, with a strong focus on induced pluripotent stem cell (iPSC)-derived peripheral sensory neurons. These cells offer an exciting opportunity for advancing pain research; however, a deeper understanding of how these cells can recapitulate relevant expression and function of non-opioid pain targets is needed. In this study, we report the large-scale, directed-differentiation of human iPSC-derived sensory neurons (a.k.a., iCell® Sensory Neurons) and establish a baseline transcriptomic characterization of these cells in comparison to both human dorsal root ganglion (hDRG) cells and other iPSC-derived sensory neurons from published protocols.

The data presented in this poster demonstrate that iPSC-derived sensory neurons have a transcriptional profile of sensory neuron and pain-related genes and offer a human relevant *in vitro* model for non-opioid drug discovery and chemotherapy-induced peripheral neuropathy. Importantly, iCell Sensory Neurons have an advanced gene expression profile with the majority of cells expressing premier non-opioid pain targets and a population that is enriched in neural maturation markers with ion channel and intracellular signaling pathways upregulation that correlates with electrophysiological and sensory function.

## Methods

iCell Sensory Neurons (FUJIFILM Cellular Dynamics) were thawed and maintained in culture using iCell Sensory Neurons Medium. Cells were evaluated at various timepoints for gene expression, immunocytochemistry and morphology. For bulk RNA sequencing, cells were lysed in RTL lysis buffer (QIAGEN) and total RNA was extracted (QIAsymphony, QIAGEN). Total RNA sequencing was performed by Novogene. Cells were prepared for single cell RNA sequencing by following a slightly modified version of the fixed tissue protocol from 10X genomics (CG000553). Briefly, cells were lifted off the plate with gentle swirling in dPBS, then fixed in PFA containing buffer overnight. Cells were then digested to a single cell suspension with papain (Worthington), washed several times in quenching buffer to remove debris and frozen in storage buffer. Samples were stored at -80°C until sequencing, which was performed by Washington University's Genome Technology Access Center. Comparative hiPSC-derived sensory neurons and hDRG RNASeq datasets were downloaded from Gene Expression Omnibus (GEO) database. For hiPSC-derived sensory neurons, the raw reads were downloaded from GEO (Deng 2024: PRJNA783035, Plumbly 2022: GSE187345, Zeidler 2021: GSE161530, Truong 2024: GSE275412) and processed through the FCEI bulk RNASeq pipeline (HiSat2/FeatureCount) to create TPM/FPKM files. For hDRG RNASeq, the Quantile-normalized TPMs file was downloaded and compared with our Quantile-normalized TPMs data. Functional assays using hiPSC-derived sensory neurons were performed at 2-4 weeks in culture. Calcium assays were performed following iCell Lab Note Calcium Influx instructions and recorded using the Hamamatsu FDSS µCell.

| iCell® Sensory Neurons | | | |
|---|---|---|---|
| Catalog # | Donor | Sex | Vial Size |
| C1259 | 01279 | Male | 6M |
| C1260 | 01279 | Male | 1M |
| C1261 | 21527 | Female | 6M |
| C1262 | 21527 | Female | 1M |
| Cells Cultured w/ iCell Sensory Neuron Medium Kit (Cat # R1259) | | | |

## Background on Human iPSC-derived Sensory Neurons

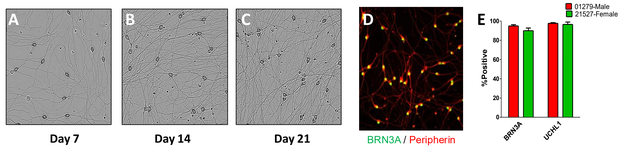



**Figure 1. Brightfield Morphology and cell purity by ICC. (A-C)** iCell Sensory Neurons were plated onto Geltrex-coated 6-well plate (1x10$^6$ cells/well). Sensory neurons mature with a bright cell body, extensive neurite networks, and pseudo-unipolar morphology. **(D)** ICC staining showed majority of the culture expressed sensory neuron markers Brn3A and peripherin. **(E)** Quantification of % positive marker expression across 4-6 lots each donor.

## Comparison of Human iPSC-derived Sensory Neurons to Human DRG

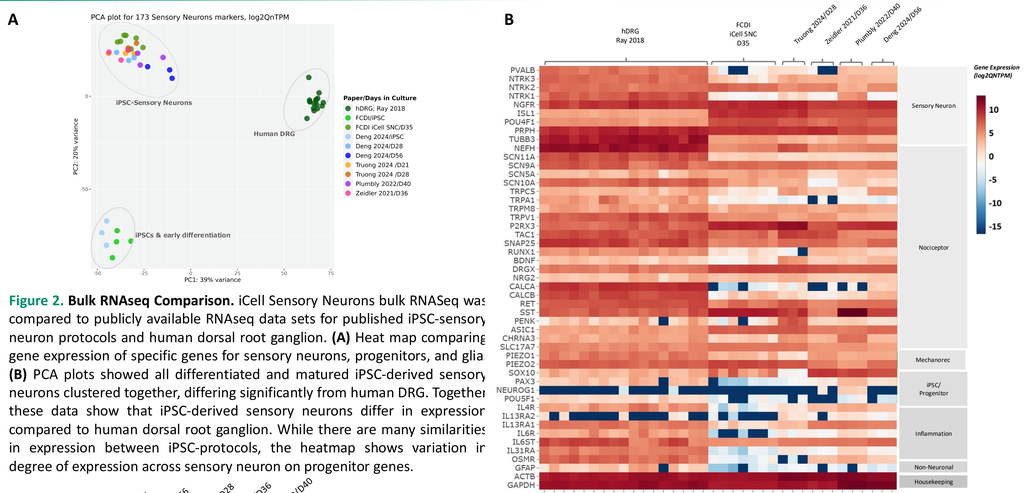



**Figure 2. Bulk RNAseq Comparison.** iCell Sensory Neurons bulk RNASeq was compared to publicly available RNAseq data sets for published iPSC-sensory neuron protocols and human dorsal root ganglion. **(A)** Heat map comparing gene expression of specific genes for sensory neurons, progenitors, and glia **(B)** PCA plots showed all differentiated and matured iPSC-derived sensory neurons clustered together, differing significantly from human DRG. Together these data show that iPSC-derived sensory neurons differ in expression compared to human dorsal root ganglion. While there are many similarities in expression between iPSC-protocols, the heatmap shows variation in degree of expression across sensory neuron on progenitor genes.

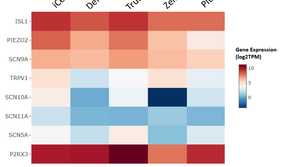



**Figure 3. iCell Sensory Neurons Show High Expression of Non-opioid Pain Targets.** Bulk RNAseq analysis was performed on key non-opioid and sensory neuron targets across iPSC-derived sensory neuron protocols. These data show variation of these marker expressions across iPSC-derived sensory neurons, with most cells expressing key pain targets to some degree. Importantly, iCell Sensory Neurons display an enhanced expression of TRPV1 and SCN10A, both genes important for nociceptor function and active targets for non-opioid pain research.

## Single Cell Analysis of Sodium Channels and Non-Opioid Pain Targets

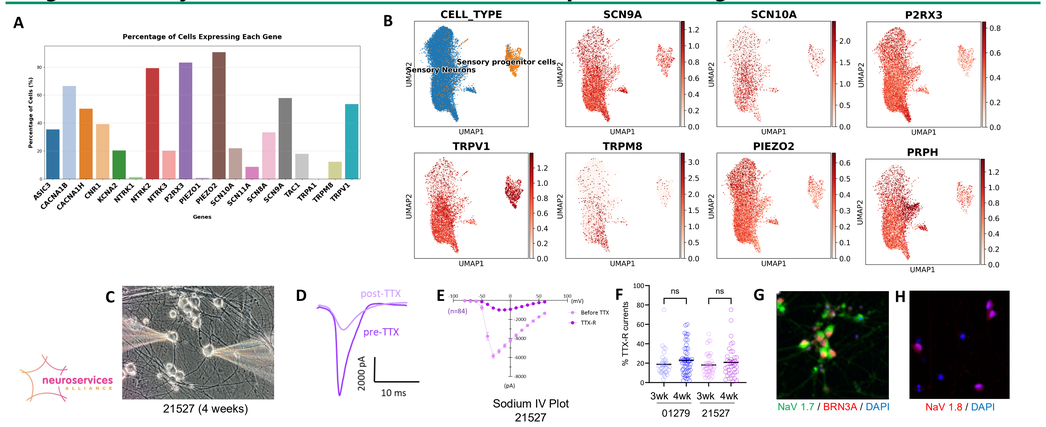
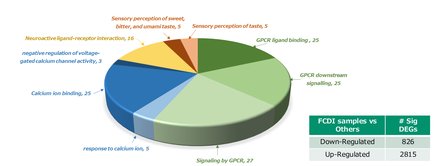



**Figure 4. Single Cell Expression and Function of Sodium Channels.** iCell Sensory Neurons from donor 21527 (female) were analyzed for single cell gene expression across two manufactured lots. **(A)** Bar graph showing % of cells expressing each gene within the entire population sample. Notedly, close to 60% of the population expressed TRPV1 and SCN9A, and 22% expressed SCN10A which is consistent with the patch clamp data collected by Neuroservices-Alliance **(C-F)**. **(B)** uMAP plots showing the majority of cells cluster as mature sensory neurons (~91%) and with the remaining cells classifying as sensory progenitor (~9%). Individual uMAP plots of non-opioid pain targets show they are expressed within the mature sensory neuron population. **(C)** Representative brightfield image iCell Sensory Neurons cultured for 28 days and patched manually at weeks to evaluate TTX-r and TTX-s sodium currents. **(D-E)** Representative current traces (Na IV plot) from iCell Sensory Neurons showing sodium currents pre- and post-TTX treatment. **(F)** Quantification of TTX-sensitive (TTX-s) and TTX-resistant (TTX-r) currents show ~20% of sodium current is TTX-r for both donors and at both three and four weeks in culture. **G-H)** Immunocytochemistry staining for NaV1.7 and NaV1.8, demonstrating single cell protein level expression of the sodium channels.

## Robust Calcium Response with Enhanced Calcium Pathway Gene Expression




| FCDI samples vs Others | # Sig DEGs |
|---|---|
| Down-Regulated | 826 |
| Up-Regulated | 2815 |

**Figure 5. Robust Calcium Responses to Sensory Receptor Agonists.** RNAseq pathway and Go analysis comparing iCell Sensory Neurons vs. all other protocols identified significantly up- or down-regulated pathways. Importantly, calcium handling pathways were significantly enriched in iCell Sensory Neuron, which is beneficial for sensory agonist calcium influx assays.

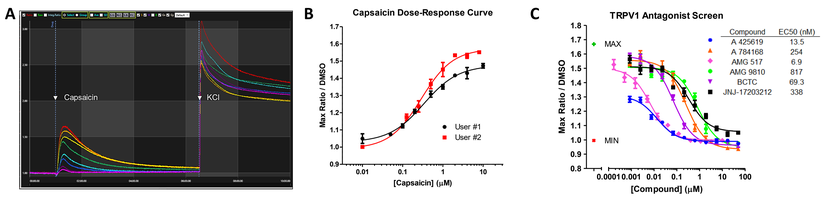



**Figure 6. Robust Calcium Responses to Sensory Receptor Agonists. (A)** Representative traces of calcium response to a titration of Capsaicin (0→8 µM, TRPV1 agonist) from the FDSS/µCell. **(B)** Assay reproducibility demonstrated by two independent operators generating similar results from a dose-response of Capsaicin with the same lot of iCell Sensory Neurons. **(C)** Compound profiling in 384-well format: six different TRPV1 antagonists (dose-response, n=3) were incubated for 1 h prior to stimulation with 1 µM Capsaicin. $EC_{50}$ values are sub-micromolar.

## Expression and Function of Migraine-Related Pain Targets

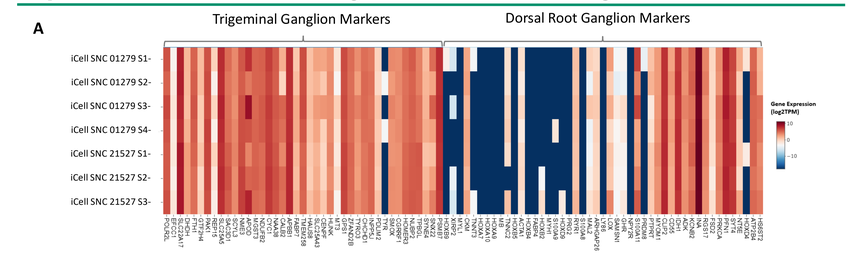



**Figure 7. Potential Human *In Vitro* Model for Migraine Research. (A)** Comparison of bulk RNAseq gene expression in iCell Sensory Neurons across identified trigeminal and dorsal root ganglion genes (*Megat et al., J. of Nsci., 2019*), suggesting a human trigeminal neuron genotype. **(B)** iCell Sensory Neurons released CGRP when stimulated by various pain modulators as measured by ELISA (KCl as positive control). **(C)** Quantitative dose-dependent SNAP25 cleavage upon exposure to a broad range of Botulinum Toxin A (BoNT/A) detected by Western blot.

## Conclusions

- iCell Sensory Neurons have a distinct bulk RNASeq profile compared to hDRG.
- Express many non-opioid pain-relevant genes, including Nav1.7, Nav1.8 and TRPV1. Some genes are enhanced compared to sensory neurons derived using alternate differentiation protocols.
- Demonstrate enhanced calcium pathway gene expression and robust calcium influx responses to sensory agonists, indicating their utility for high throughput drug screening.
- Release CGRP when stimulated, show SNAP25 cleavage in response to BoNT/A, and expression of trigeminal neuron markers, demonstrating usage as a model system for migraine study.

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