# Efficient, Large-Scale Production and Functional Characterization of Nociceptors Derived from Human Pluripotent Stem Cells

**Tao Deng, Pinar Ormanoglu, Pei-Hsuan Chu, Carlos A. Tristan, Claire Malley, Christopher P. Austin, Anton Simeonov, Ilyas Singeç**

National Center for Advancing Translational Sciences (NCATS), Stem Cell Translation Laboratory (SCTL), NIH, Rockville, MD, 20850

## Abstract

Development of new non-addictive analgesics would greatly benefit from directed differentiation of hPSCs into relevant cell types. Here, we devised a highly efficient step-wise protocol that differentiates human pluripotent stem cells (hPSCs) exclusively into nociceptors under fully defined conditions. By manipulating critical cell signaling pathways using small molecule inhibitors, hPSCs were first converted into SOX10+ neural crest stem cells followed by differentiation into *bona fide* nociceptors. Time-course RNA-Seq analysis (Day 0-28) and immunocytochemistry experiments confirmed that nociceptors expressed typical neuronal markers, transcription factors, neuropeptides, and ion channels. Focusing on pain-relevant receptors expressed by hPSC-derived nociceptors (e.g. P2RX3, opioid receptors), we could demonstrate robust functional activities in multi-electrode array experiments and differential responses to nociceptive stimuli and specific drugs including natural and synthetic opioids. The nociceptor differentiation protocol was then automated by using a robotic cell culture system (CompacT SelecT™) enabling multiple high-throughput projects that require large numbers of cells. In summary, the scalable human nociceptor platform developed here will aid in the discovery of new pain medications as part of the effort to tackle the opioid crisis.

## Materials and Methods

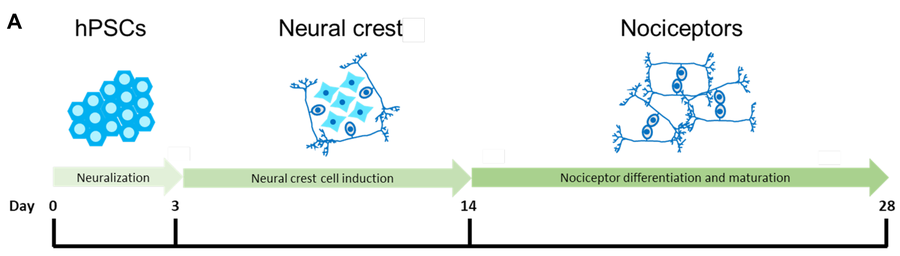


**Fig. 1: Automated nociceptor differentiation protocol. A.** Controlled stepwise differentiation of hPSCs into functional nociceptors using small molecules and chemically defined conditions. **B.** Differentiation protocol was adapted to an automated cell culture system enabling standardized and scalable production of nociceptors from numerous iPSC lines.

## Results

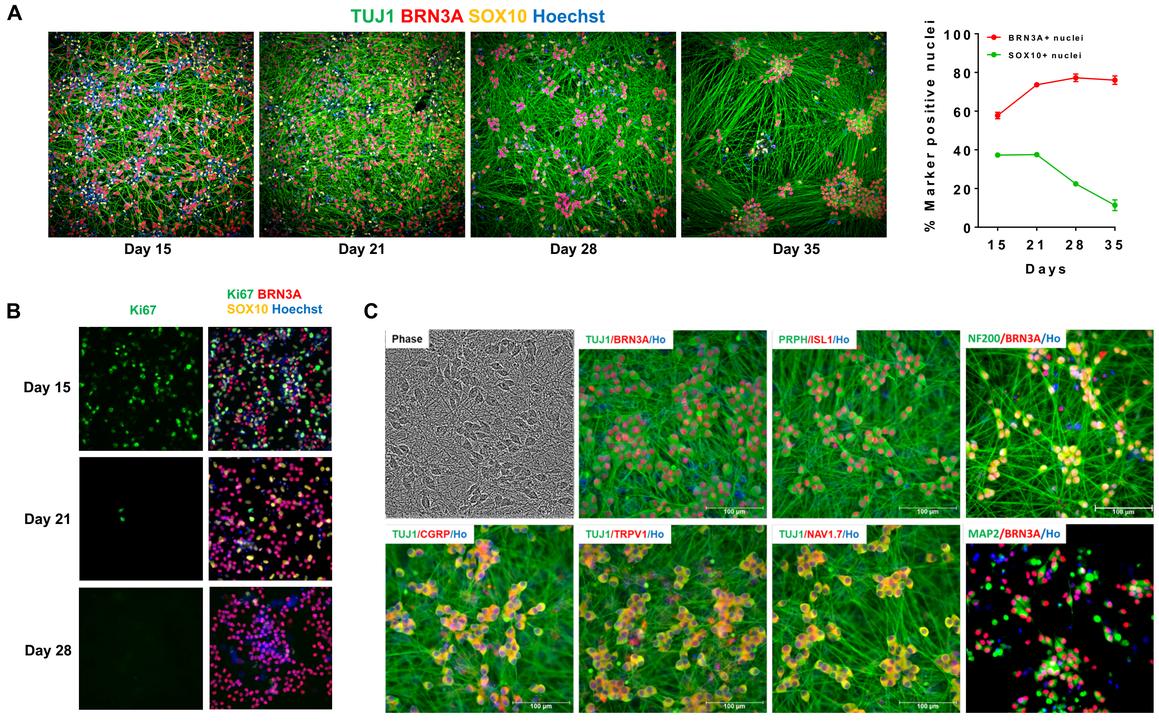


**Fig. 2: Characterization of hPSCs-derived nociceptors. A.** Quantification of immunolabeled cells at different stages demonstrate the efficiency of the nociceptor differentiation protocol. **B.** Ki67 immunostaining showing the elimination/maturation of proliferating cells during the differentiation process. **C.** Phase-contrast and various immunostainings showing that nociceptors express typical markers after 4 weeks of differentiation. Note the absence of MAP2+ dendrites consistent with the expected pseudo-unipolar anatomy of nociceptors.

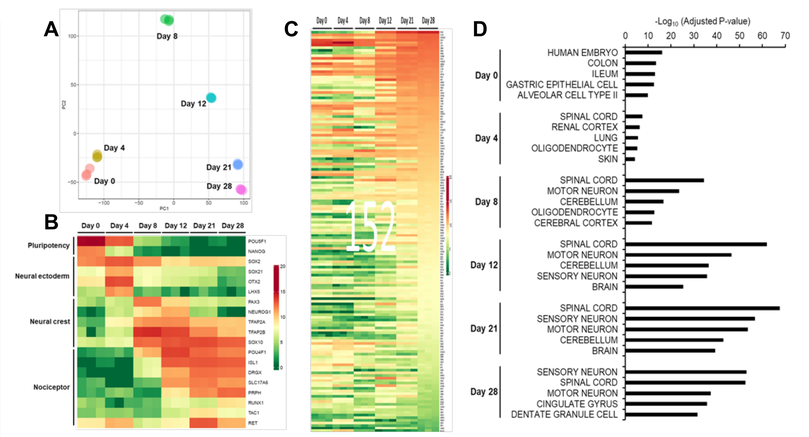


**Fig. 3: Gene expression profiling of differentiating nociceptors (RNA-seq). A.** PCA plot showing distinct signatures at different time points during nociceptor differentiation. **B.** Heat map illustrating the time-course of pluripotent and neuronal marker expression. **C.** Heat map of ion channels expressed by hPSC-derived nociceptors. **D.** EnrichR analysis comparing the transcriptome of nociceptors at different differentiation stages to that of human tissue samples in the ARCH4 database.

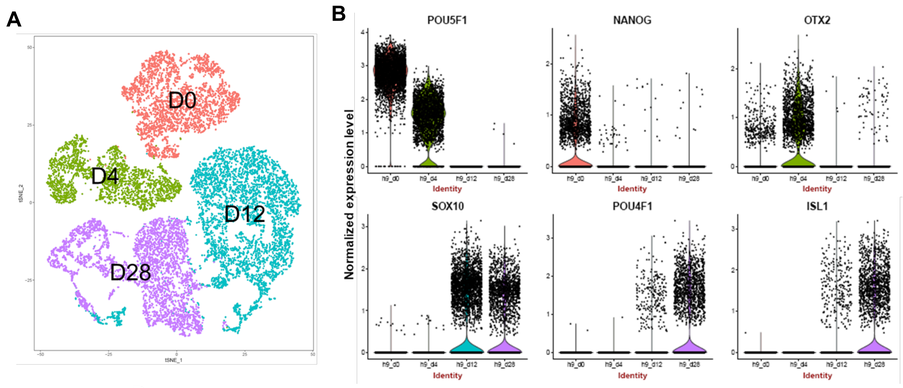


**Fig. 4: Single-cell analysis (scRNA-seq) of differentiating nociceptors. A.** tSNE plot shows the distribution of the cell populations at different stages during nociceptor differentiation. **B.** Violin plots illustrate the proportion of cells expressing pluripotent or neuronal markers across different stages of differentiation.

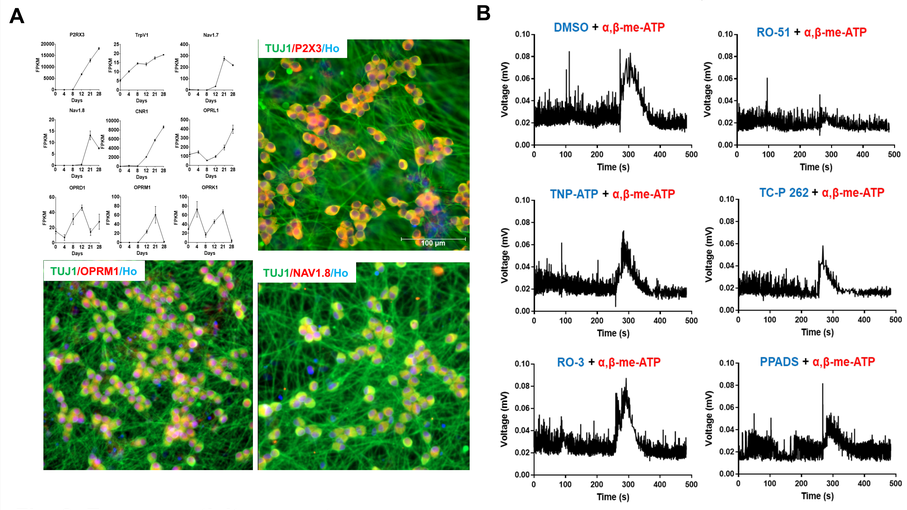


**Fig. 5: Functional analysis of nociceptors. A.** RNA-seq and immunostainings document expression of important ion channels and receptors in hPSC-derived nociceptors. **B.** Multi-electrode array (MEA) analysis demonstrate that P2RX3 ion channels can be activated by ATP and modulated by various selective P2RX3 antagonists.

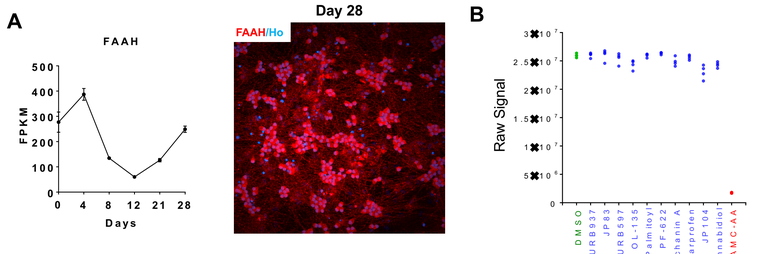


**Fig. 6: Enzyme activity assay to demonstrate the utility of hPSCs-derived nociceptors for screening and validating FAAH (fatty acid amide hydrolase) inhibitors. A.** RNA-seq and immunostaining show the expression of FAAH in hPSC-derived nociceptors. **B.** Cell-based assay showing FAAH activities in hPSC-derived nociceptors. Note the lack of efficiency of several presumable FAAH inhibitors reported previously.

## Summary

1. We successfully established a highly efficient, scalable protocol to differentiate hPSCs into functional nociceptors under chemically defined conditions.
2. We adapted the protocol to an automatic cell culture system to standardize and scale-up the production of nociceptors.
3. Human PSCs-derived nociceptors express typical markers, ion channels, neuropeptides, and display electrical activity that can be modulated by various stimuli.
4. Well-characterized human nociceptors will play key roles in disease modeling, pain research, and drug development.

**Funding Source: NIH Common Fund; NCATS Intramural Research**