

# Neural progenitor cells - potent models of normal and disease neurobiology

Brian Shapiro, Ph.D.

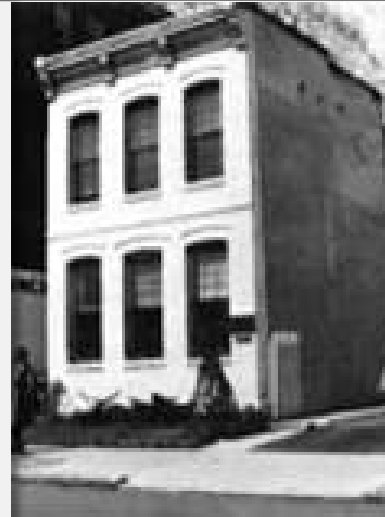
*Technical Writer, Cell Biology, ATCC*

November 19, 2015



# About ATCC

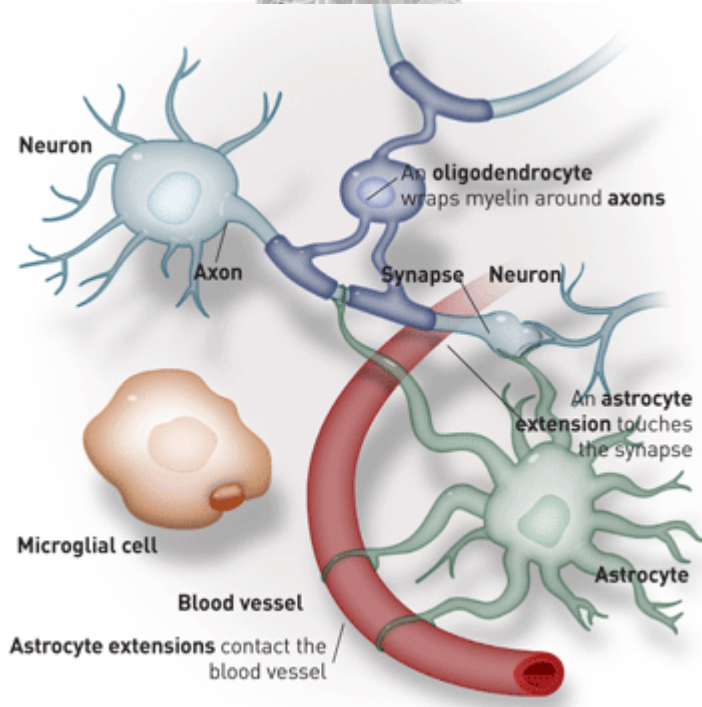
- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with, and supports, the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one-third with advanced degrees



Established partner to global researchers and scientists



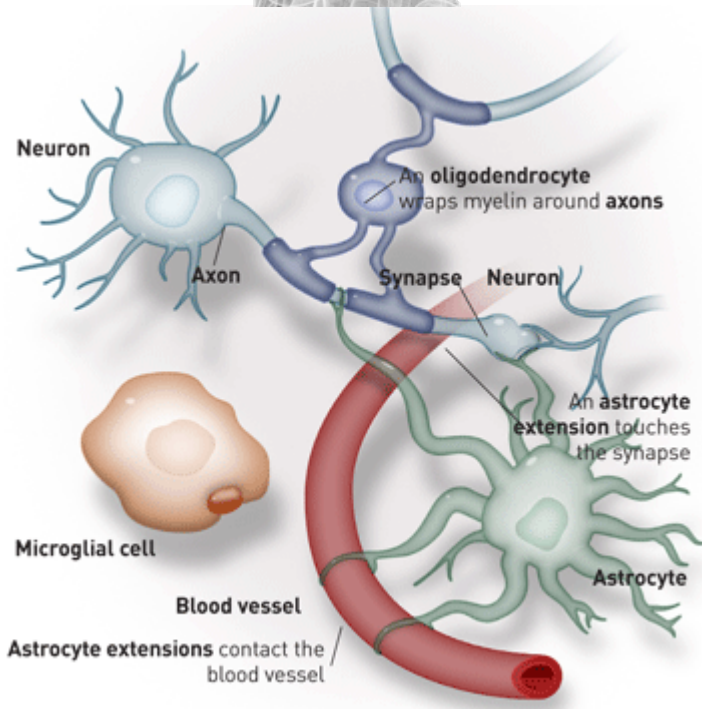
# ATCC neuroscience cell lines



Stanford Medicine, 2009

Brain cell type	ATCC cell lines
Astrocyte	✓
Oligodendrocyte	✓
Microglia	✓
Neural progenitors	✓
Region of Study	ATCC cell lines
Cortex	✓
Cerebellum	✓
Hippocampus	✓
Locus Cereulus	✓
Medullary Raphe	✓
Mesencephalon	✓
Pituitary	✓
Retina/Visual System	✓

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Stanford Medicine, 2009

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Astrocyte	✓
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Microglia	✓
Neural progenitors	✓
Neuro-Disorder	ATCC cell lines
Alzheimer's	✓
Cushing's	✓
Epilepsy	✓
Gigantism/Acromegaly	✓
Neurooncology	✓
Parkinson's	✓
Retinoblastoma	✓
Pituitary	✓



# Overview

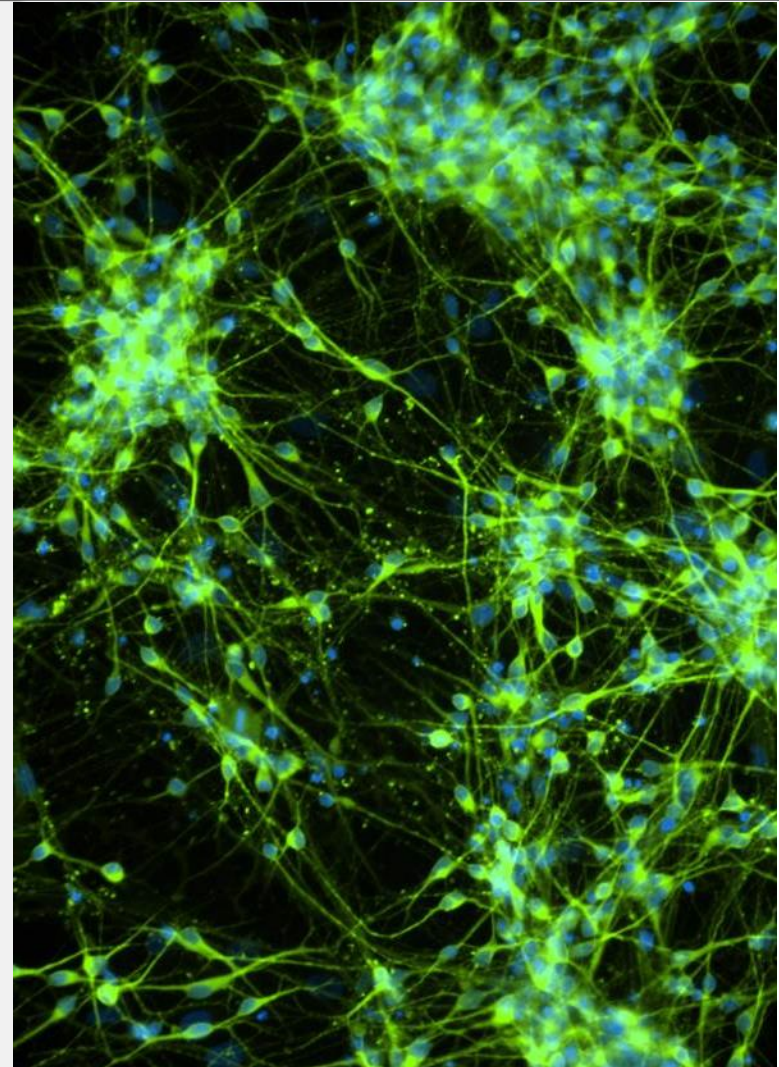
## Introduction

- Neural differentiation
- Neural progenitor cells (NPCs)

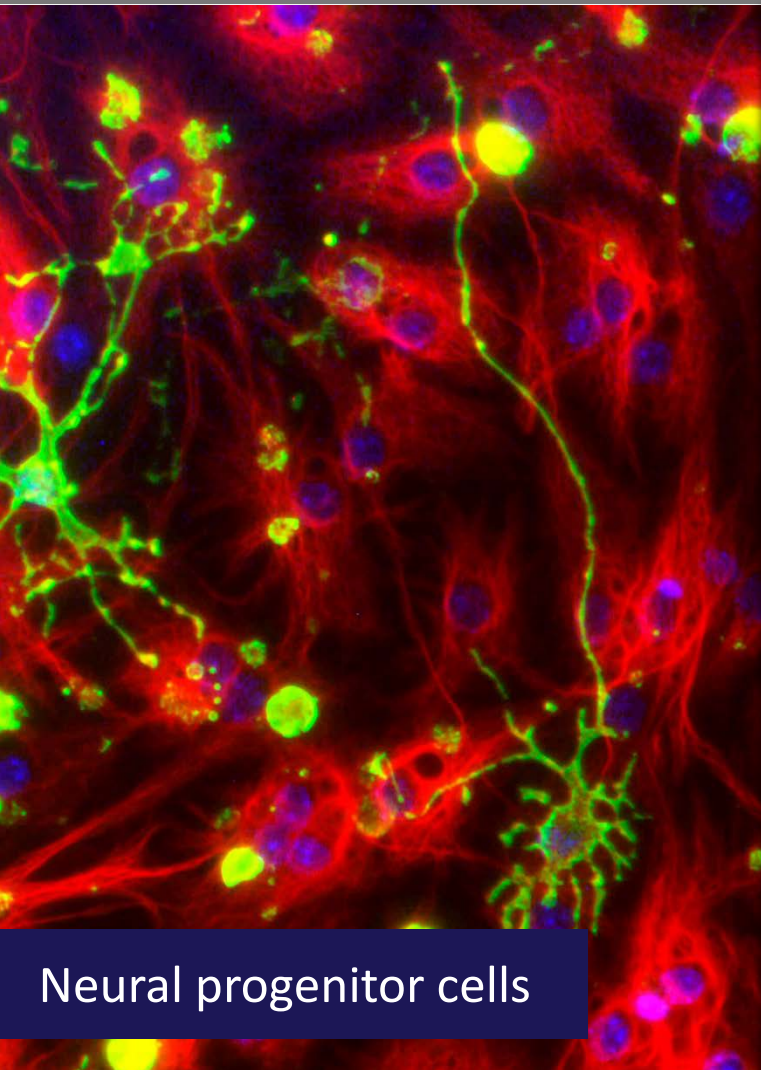
## Expanding and differentiating neural progenitors

- Fibroblast- and CD34<sup>+</sup>-derived NPCs
- Lineage-specific GFP or NanoLuc<sup>®</sup>-HaloTag<sup>®</sup> reporter NPCs
- The advantages of our complete NPC system

## ATCC NPC availability and summary



# Why use NPCs?



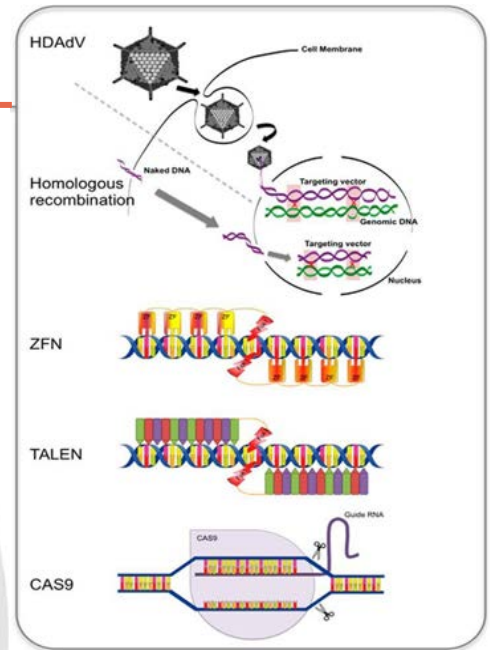
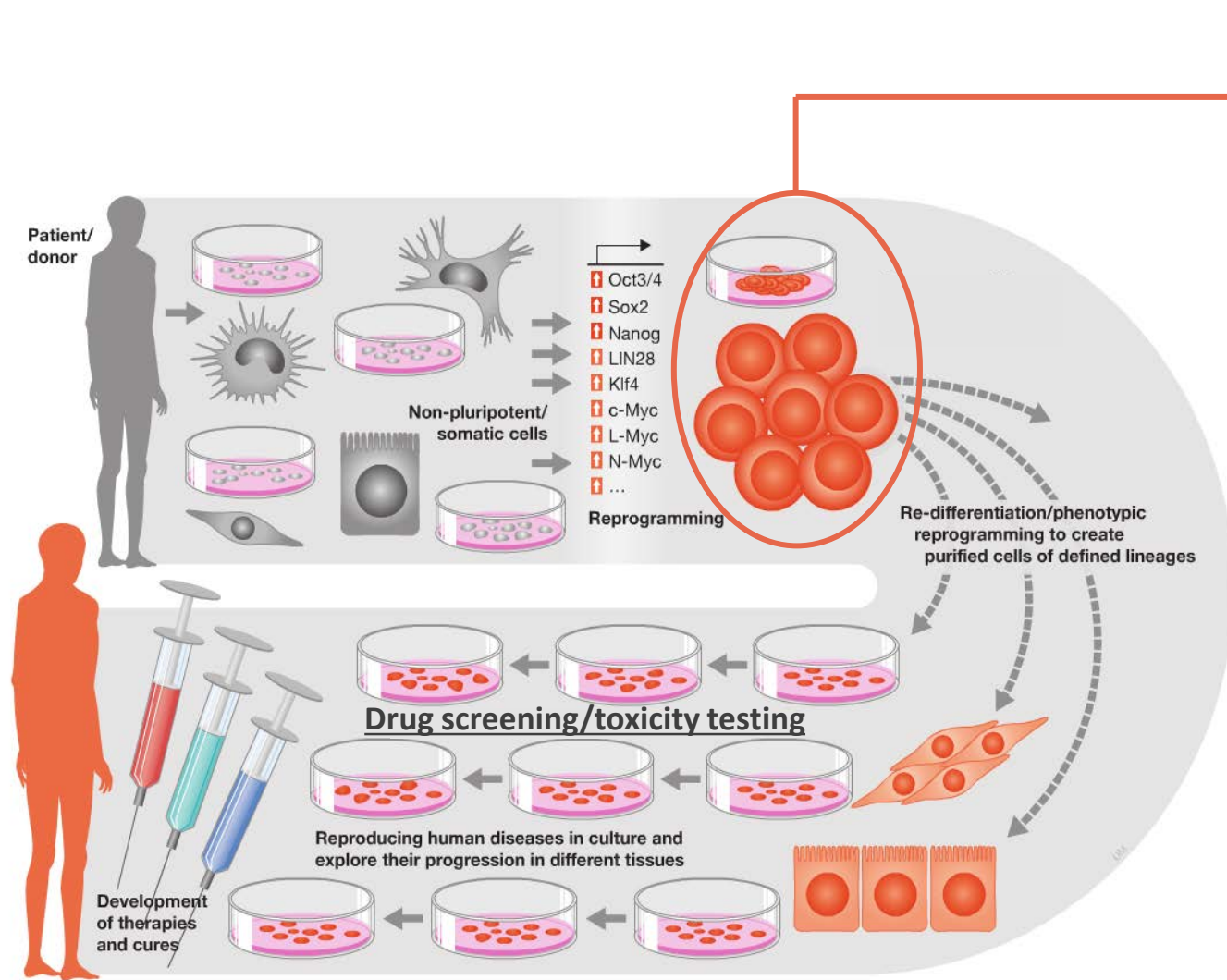
## Human NPCs are widely used:

- Drug discovery
- Toxicological assessment
- Preclinical studies

## Advantages of using NPCs:

- Human models with no donor variation
  - Biologically relevant results/predictive system
- Cells exhibit full differentiation spectrum
  - Neurons
  - Astrocytes
  - Oligodendrocytes
- Easy to use
  - Complete system of cells and media will be available
  - Live imaging is possible
  - Markers allow for easy endpoint readout
- Saves time

# iPSCs for generating NPCs

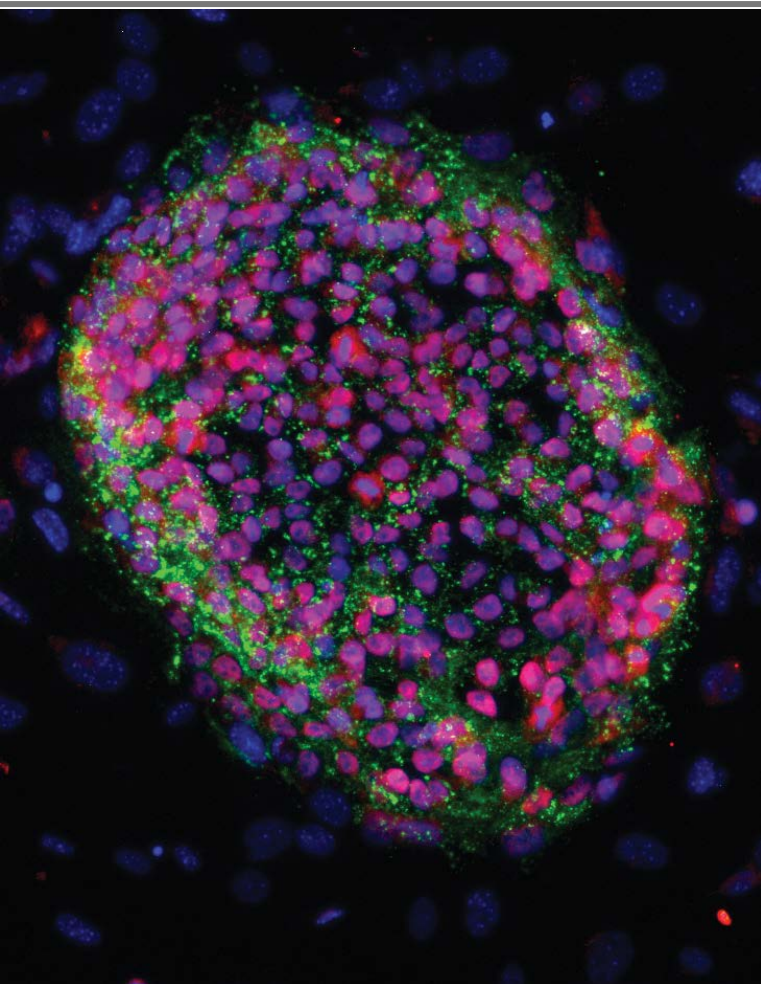


Gene editing to correct inherited diseases or to insert reporters





# iPSCs for generating NPCs

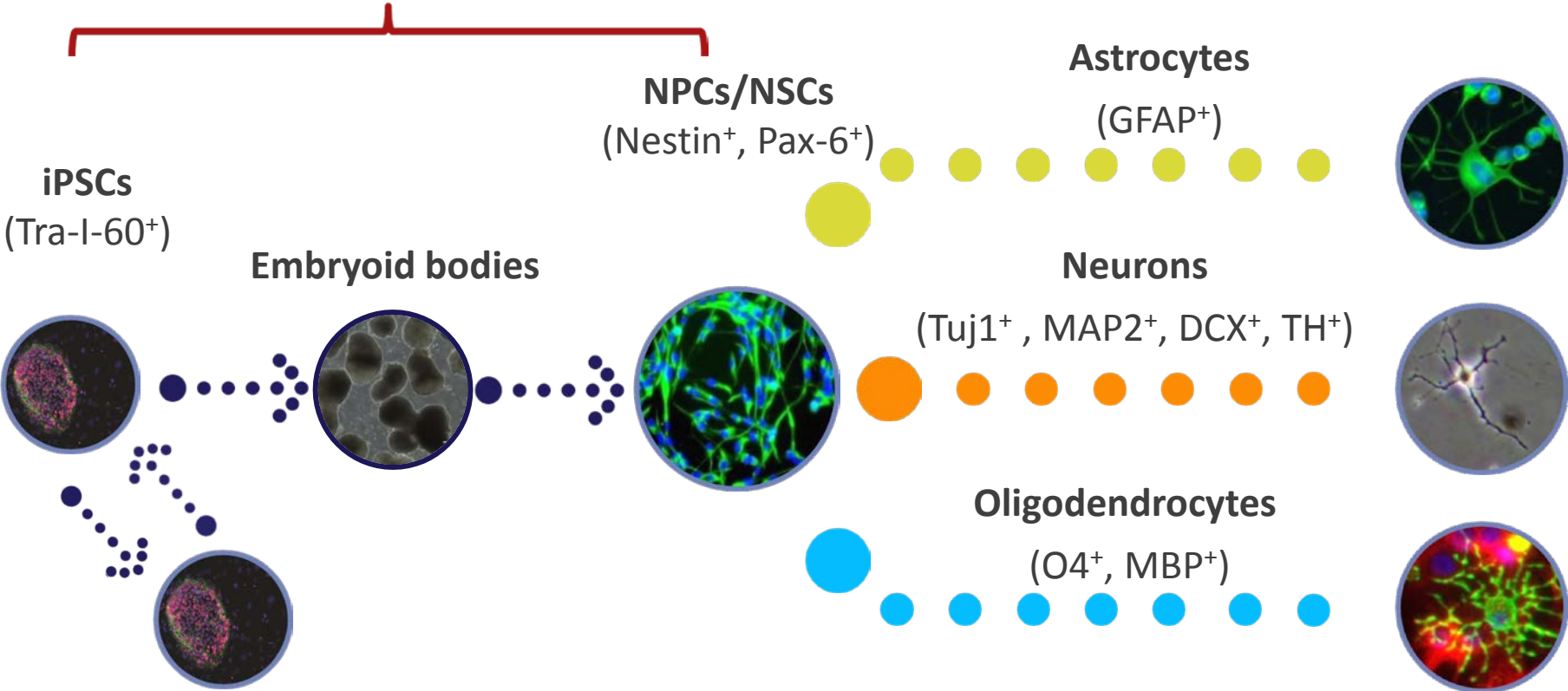


ATCC® No.	Designation	Gender	Ethnicity
ACS-1024™	ATCC-BYS0110	Male	African American
ACS-1028™	ATCC-BYS0114	Female	African American
ACS-1025™	ATCC-BYS0111	Male	Hispanic
ACS-1029™	ATCC-BXS0115	Female	Hispanic
ACS-1026™	ATCC-BYS0112	Male	Caucasian
ACS-1030™	ATCC-BXS0116	Female	Caucasian
ACS-1027™	ATCC-BYS0113	Male	Asian
ACS-1031™	ATCC-BXS0117	Female	Asian



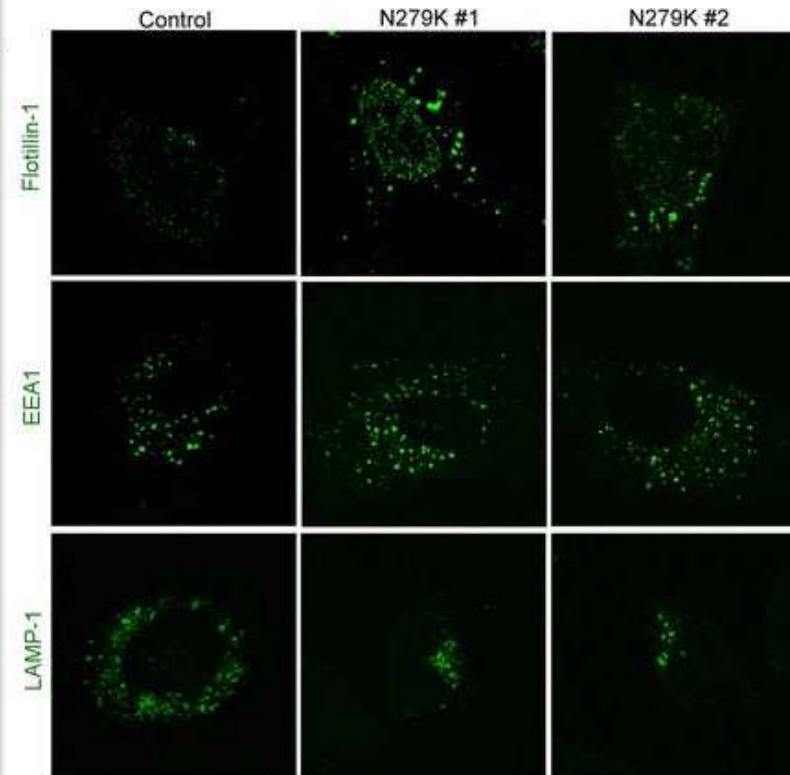
# Neuronal differentiation of iPSCs

4 to 8 weeks



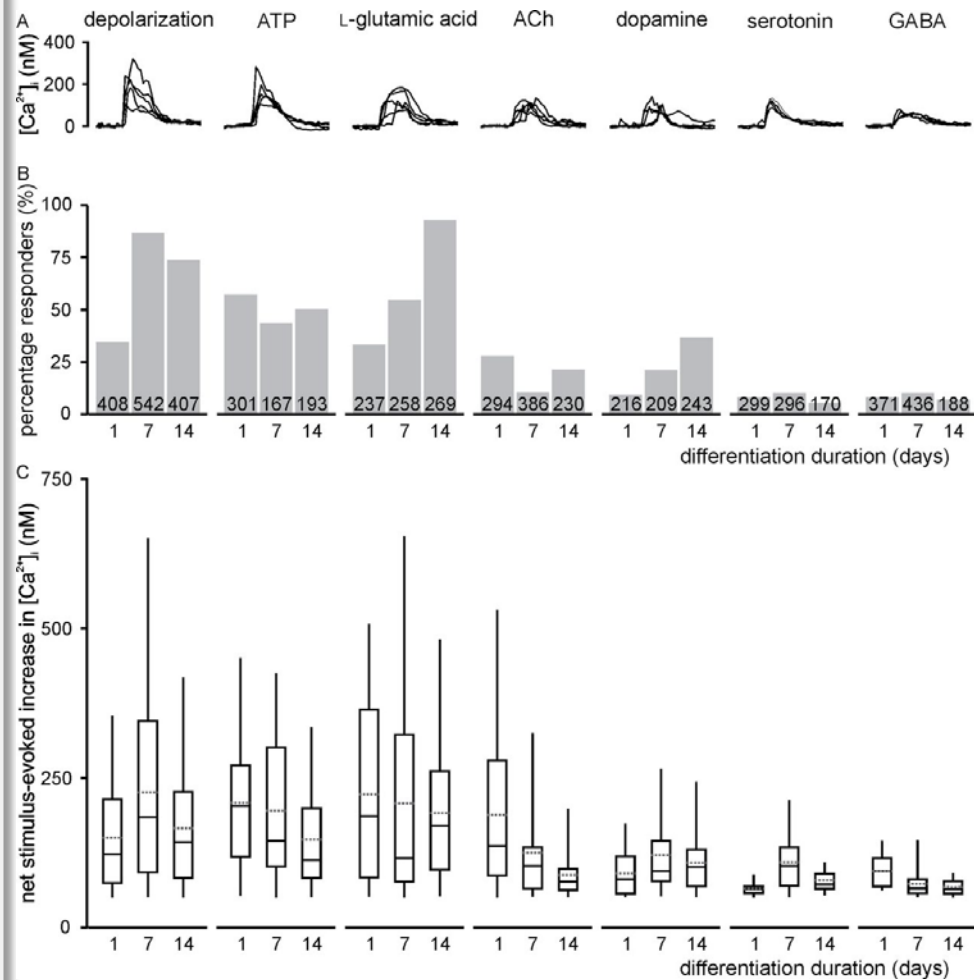
# iPSC-derived NPCs are useful for disease modeling

- Wren and colleagues created iPSCs from microtubule-associated protein tau (MAPT) N279K Parkinson's disease patients
- NPCs were created
- N279K causes increases in 4 repeat to 3 repeat tau domains in MAPT
- Since MAPT binds microtubules vesicle trafficking was investigated
- NPCs derived from patients with mutation displayed impaired endocytic trafficking



# NPCs for toxicological studies

- NPCs can be used to develop high throughput toxicological studies
- Embryonic mouse brains NPCs were isolated and differentiated
- Calcium ion flux
- Multi-electrode array experiments
- Cells were sensitive to neurotransmitters
  - Acetylcholine
  - Dopamine
  - Serotonin
  - GABA



# Overview

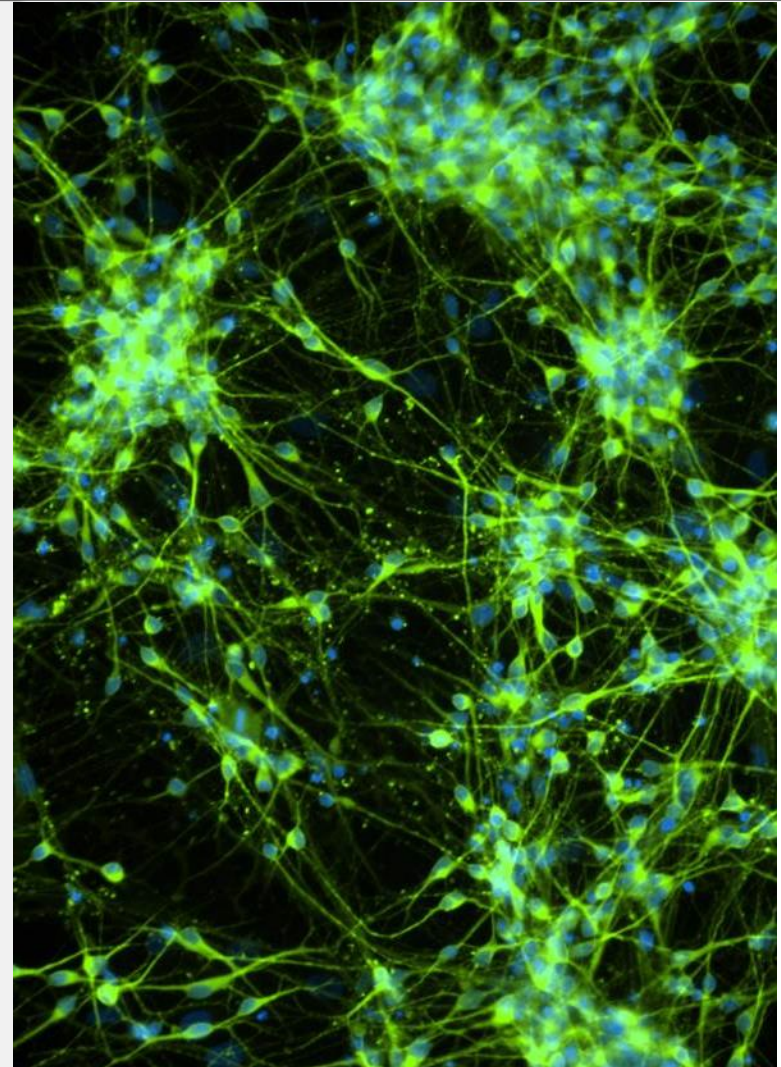
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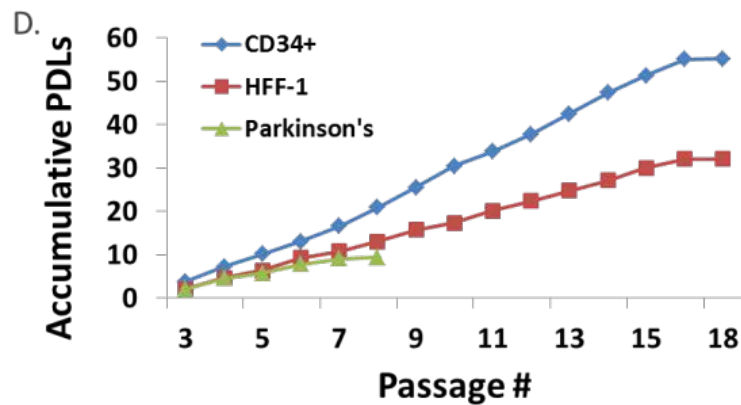
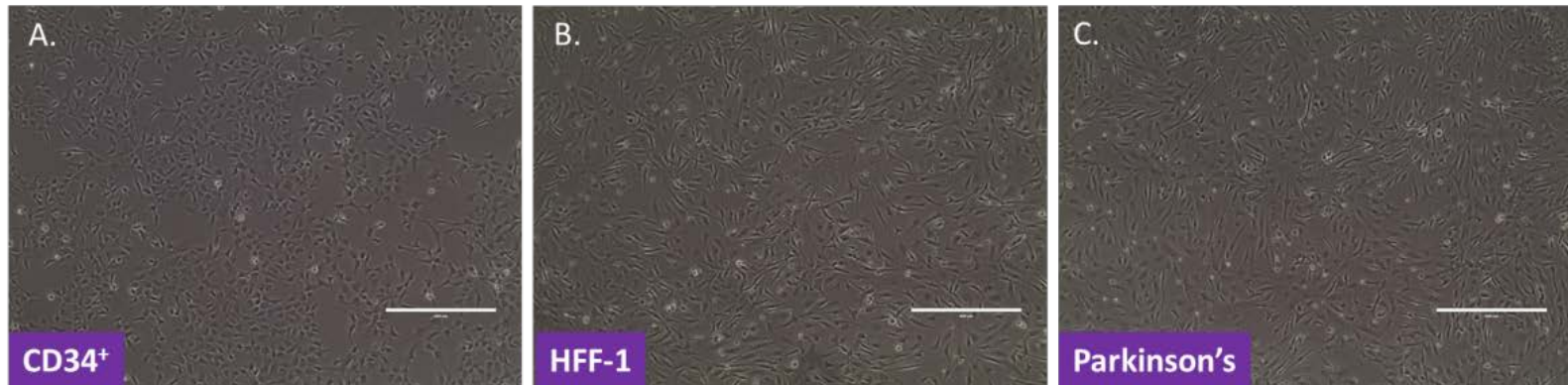




# QC testing of ATCC® NPCs

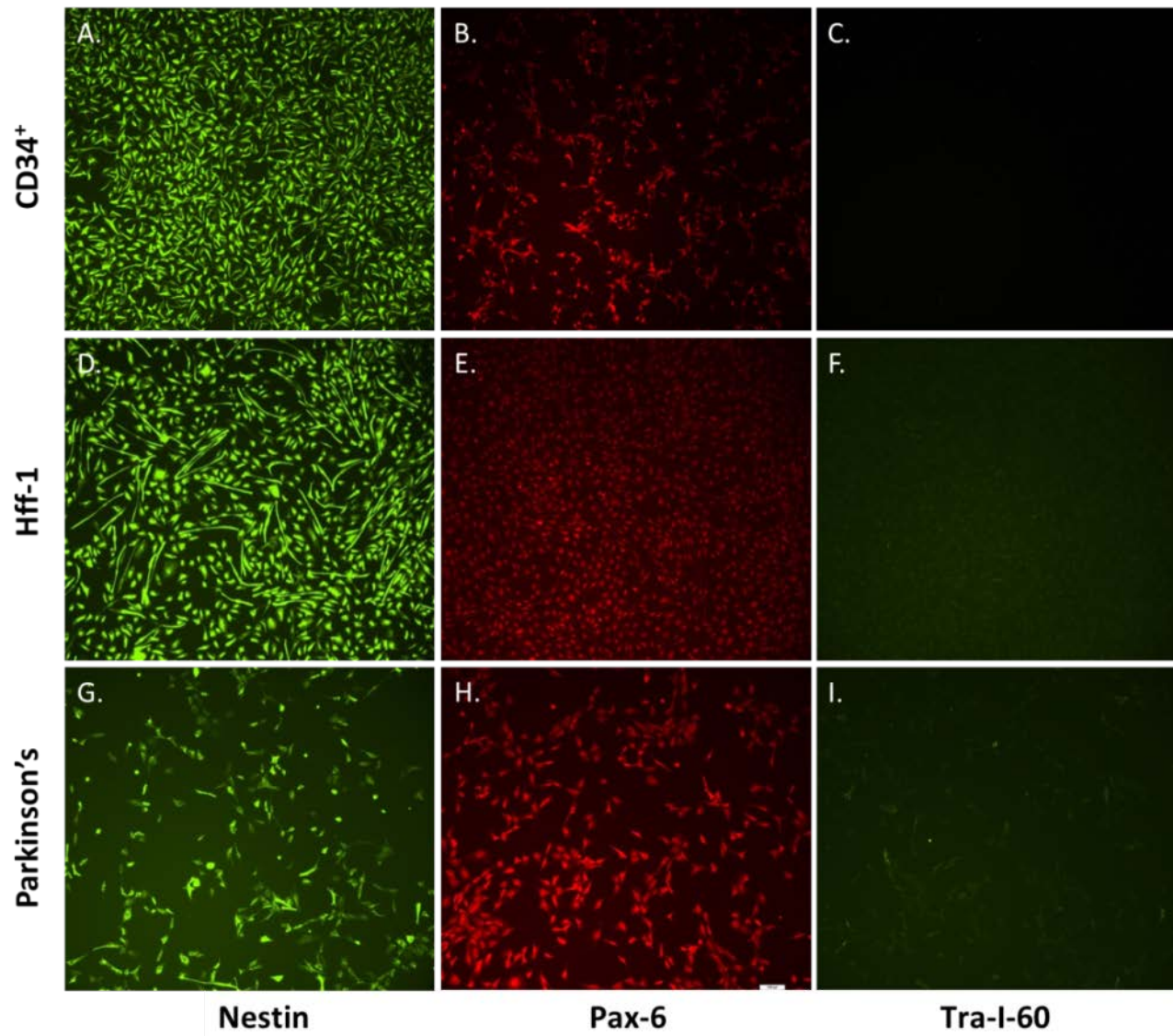
- Post-thaw cell viability: >80%
- Post-thaw viable cell number: >1x10<sup>6</sup> cells/vial
- Longevity: >15 PDLs or 5 passages
- NPC marker expression: Nestin<sup>+</sup>, Pax-6<sup>+</sup>, and Tra-I-60<sup>-</sup>
- Differentiation potential:
  - >70% Tuj1<sup>+</sup> early neurons and
  - >10% TH<sup>+</sup> dopaminergic neurons
- Identity: STR profile matching parental iPSC line
- Sterility, Mycoplasma, and viral panel testing: None detected

# Morphology and growth curves of NPCs derived from CD34<sup>+</sup>, HFF-1, and Parkinson's iPSC lines

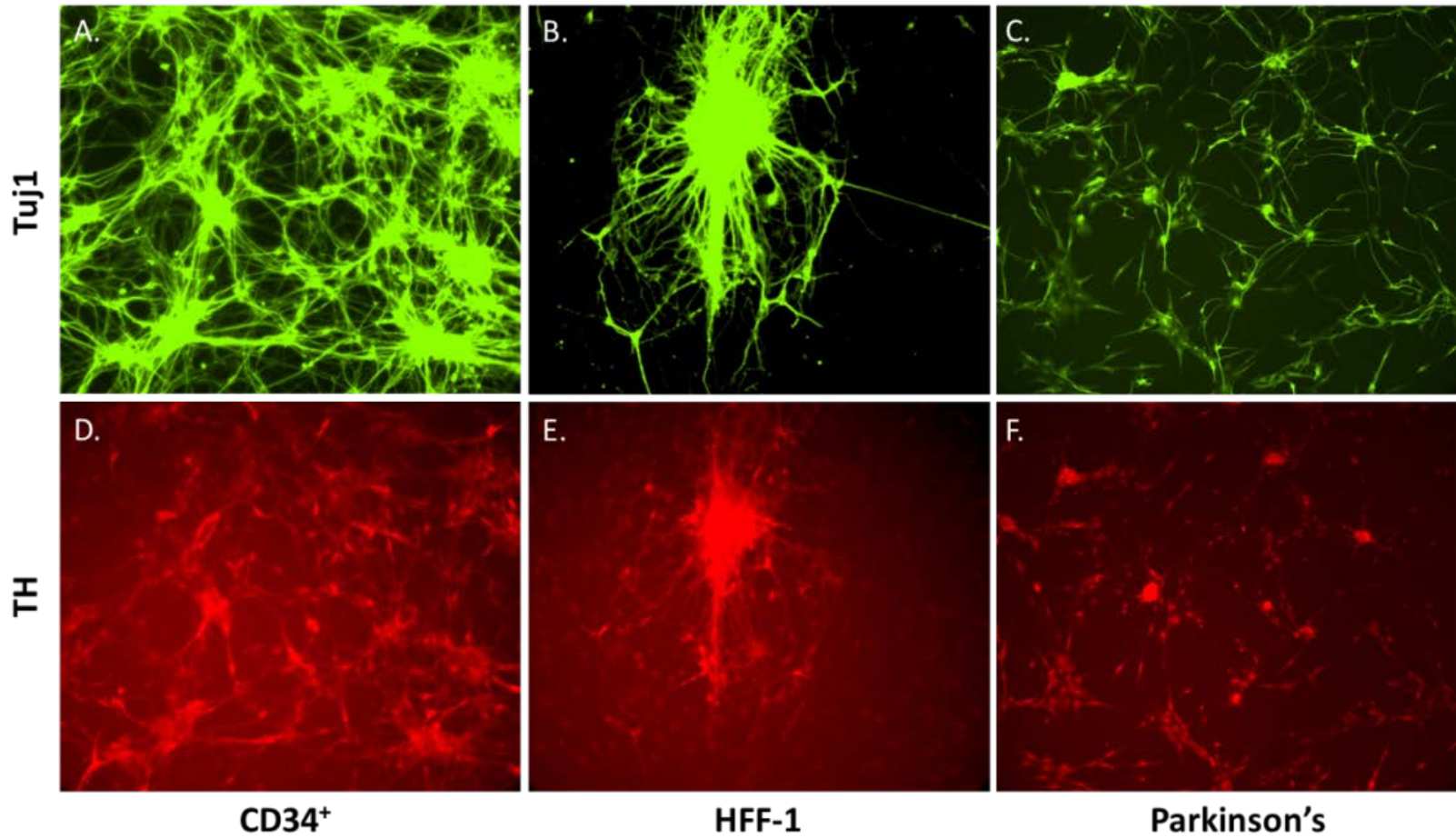


- NPCs derived from foreskin fibroblasts and CD34<sup>+</sup> demonstrated greater proliferative capacity than Parkinson's disease
- CD34<sup>+</sup>-derived NPCs exhibited better morphology than the other types

# NPCs derived from CD34+, HFF-1, and Parkinson's iPSC lines expressed Nestin and Pax-6 NPC markers



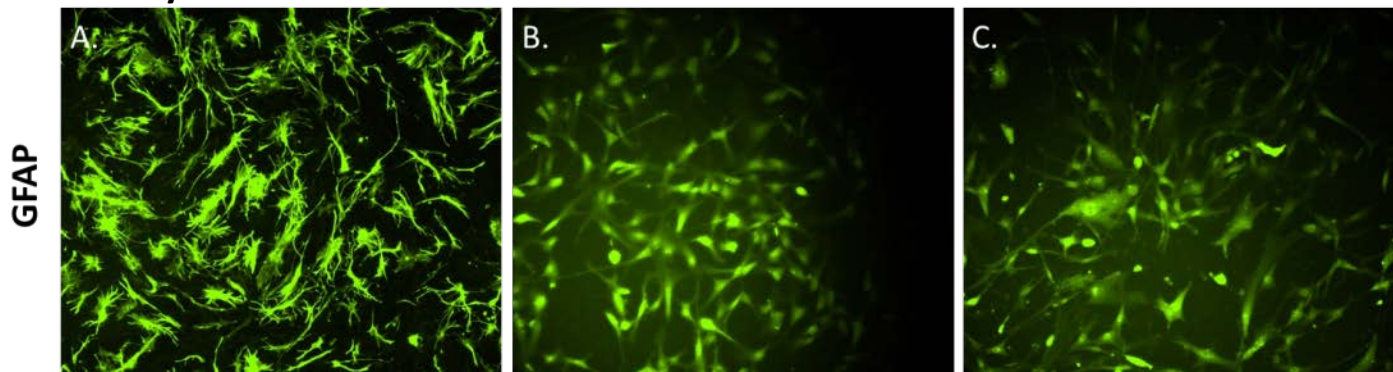
# Dopaminergic neuron differentiation of NPCs



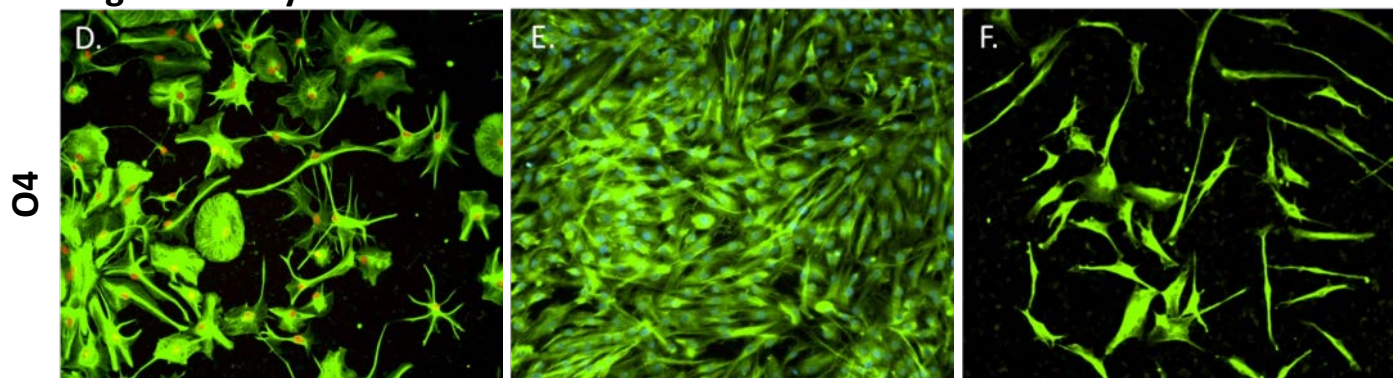


# Astrocyte and oligodendrocyte differentiation of NPCs

## Astrocyte differentiation



## Oligodendrocyte differentiation



CD34<sup>+</sup>

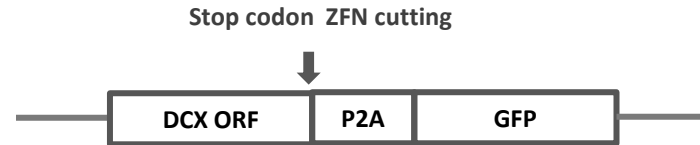
HFF-1

Parkinson's

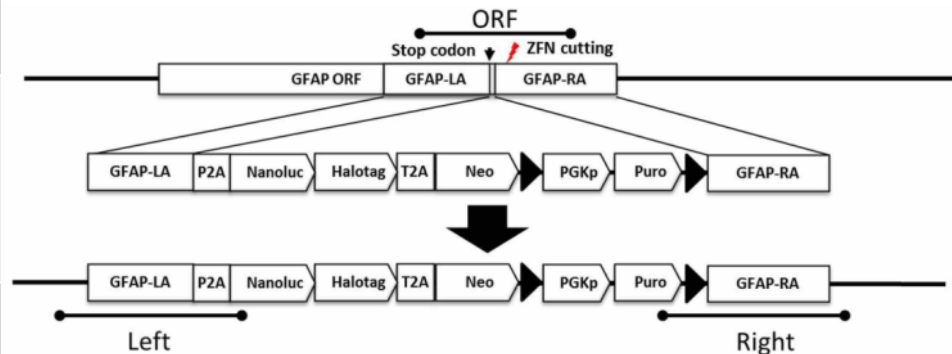
# NPC Reporter lines and constructs

ATCC® No.	Designation
ACS-5005™	DCXp-GFP Neural Progenitor Cells, Human, Normal Origin: XCL-1 hiPSCs
ACS-5006™	GFAP-Nanoluc-Halotag Neural Progenitor Cells, Human, Normal Origin: XCL-1 hiPSCs
ACS-5007™	MAP2-Nanoluc-Halotag Neural Progenitor Cells, Human, Normal Origin: XCL-1 hiPSCs

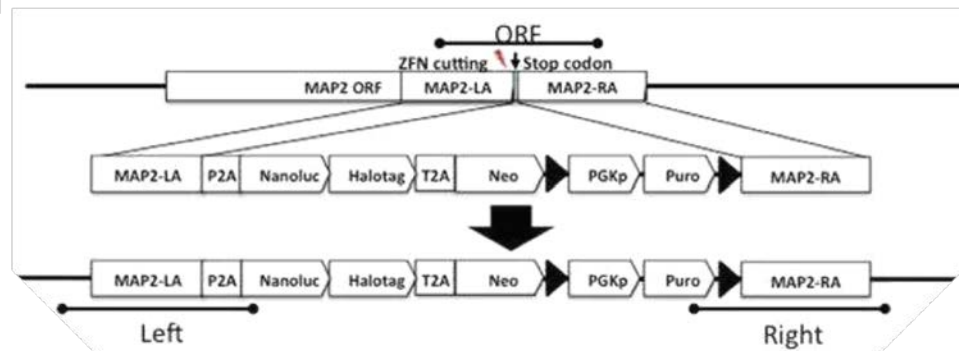
- DCXp-GFP: Mature neuron reporter line



- GFAP-Nanoluc-Halotag: Astrocyte reporter line

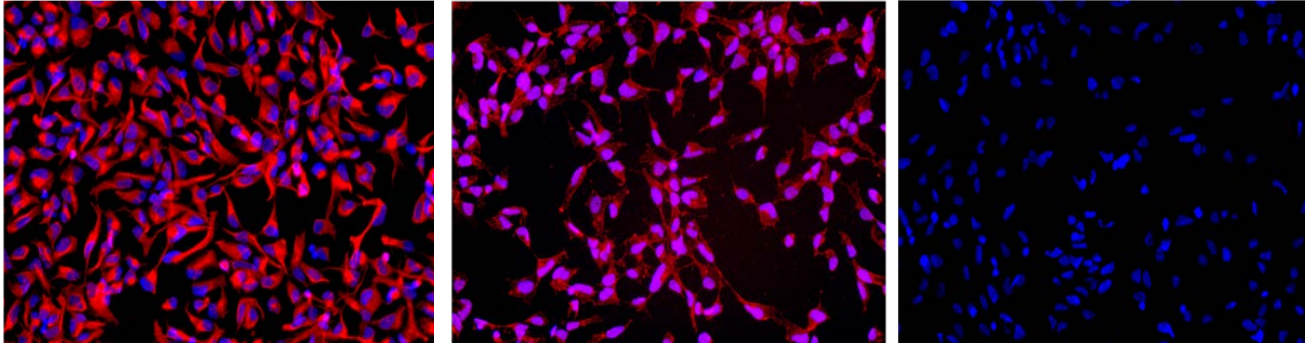


- MAP2-Nanoluc-Halotag: Early neuron reporter line

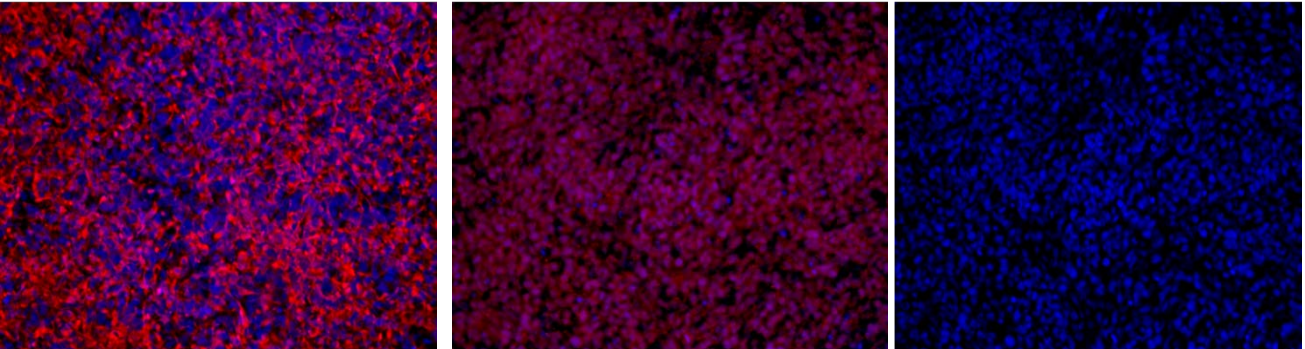


# NPC marker expression in reporter NPC lines

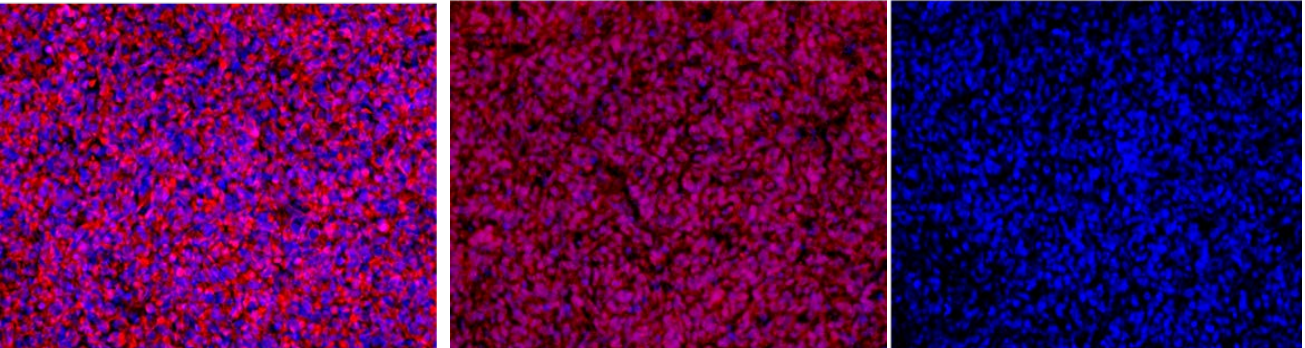
DCXp-GFP



GFAP-Nanoluc-Halotag



MAP2-Nanoluc-Halotag



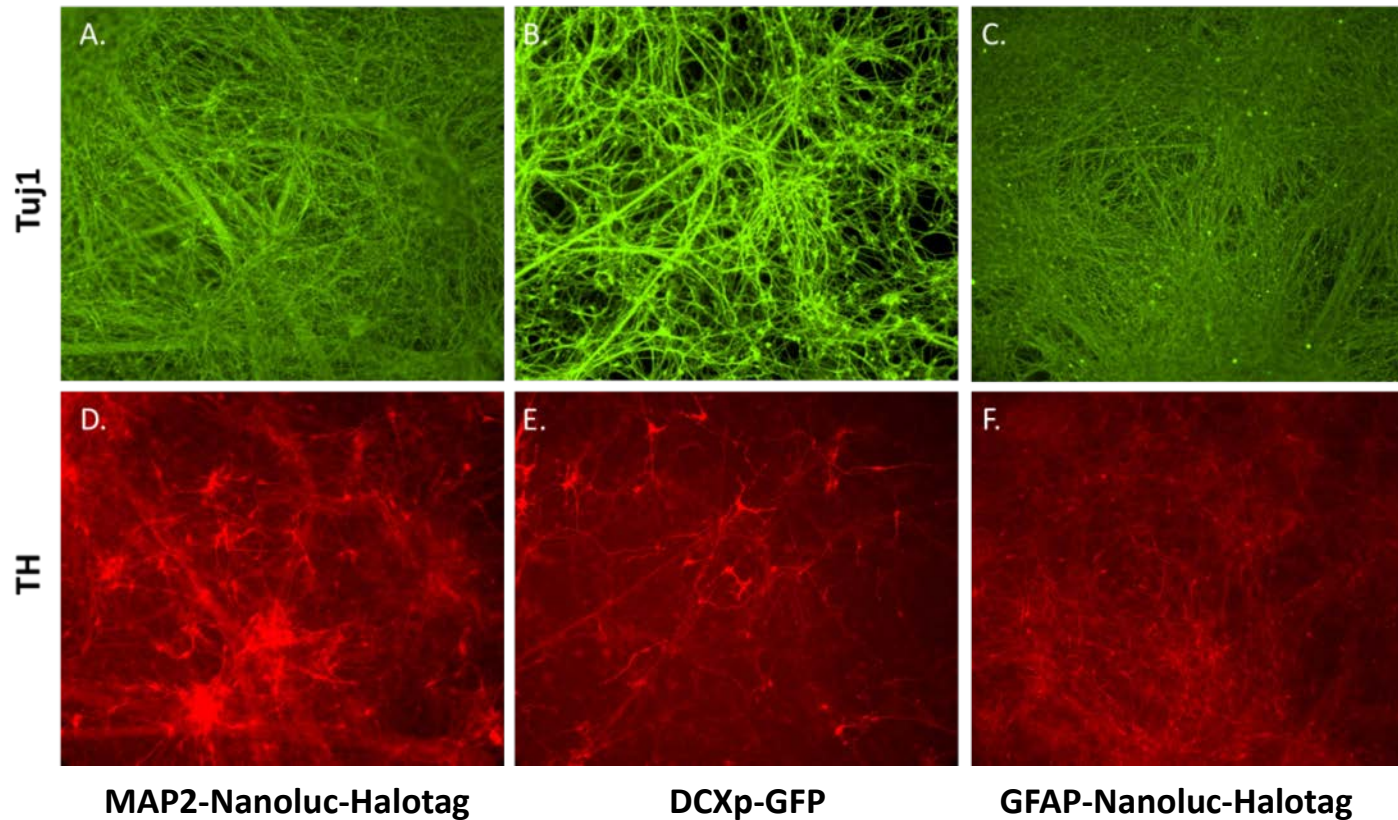
Nestin + DAPI

Pax-6 + DAPI

Tra-I-60 + DAPI



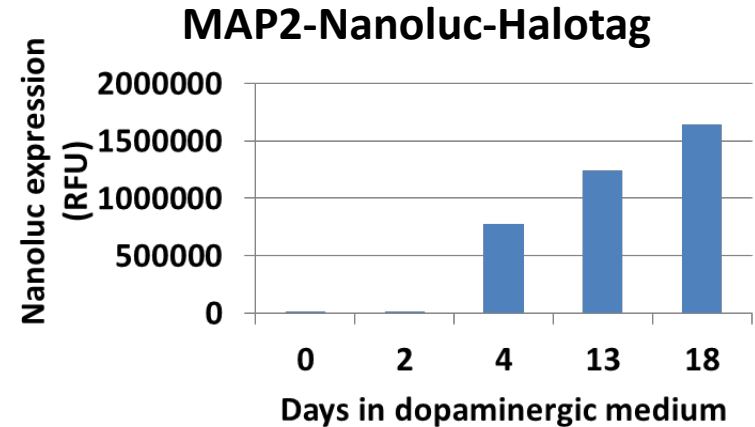
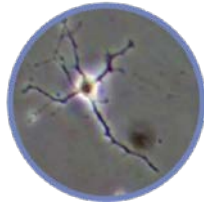
# Dopaminergic neuron differentiation of NPC reporter lines



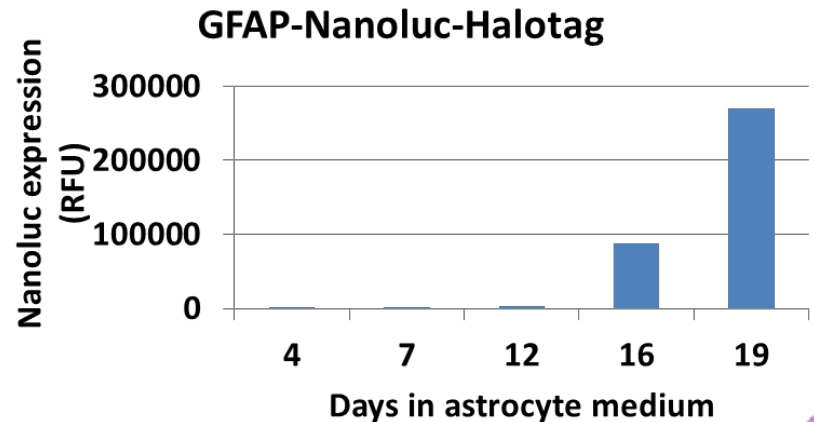
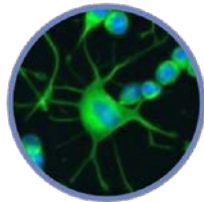


# Expression of the luciferase reporter during dopaminergic neuron or astrocyte differentiation

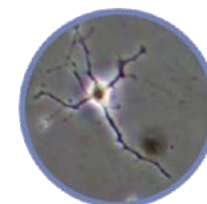
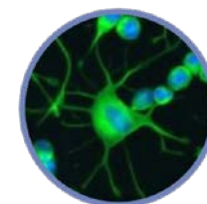
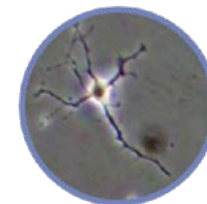
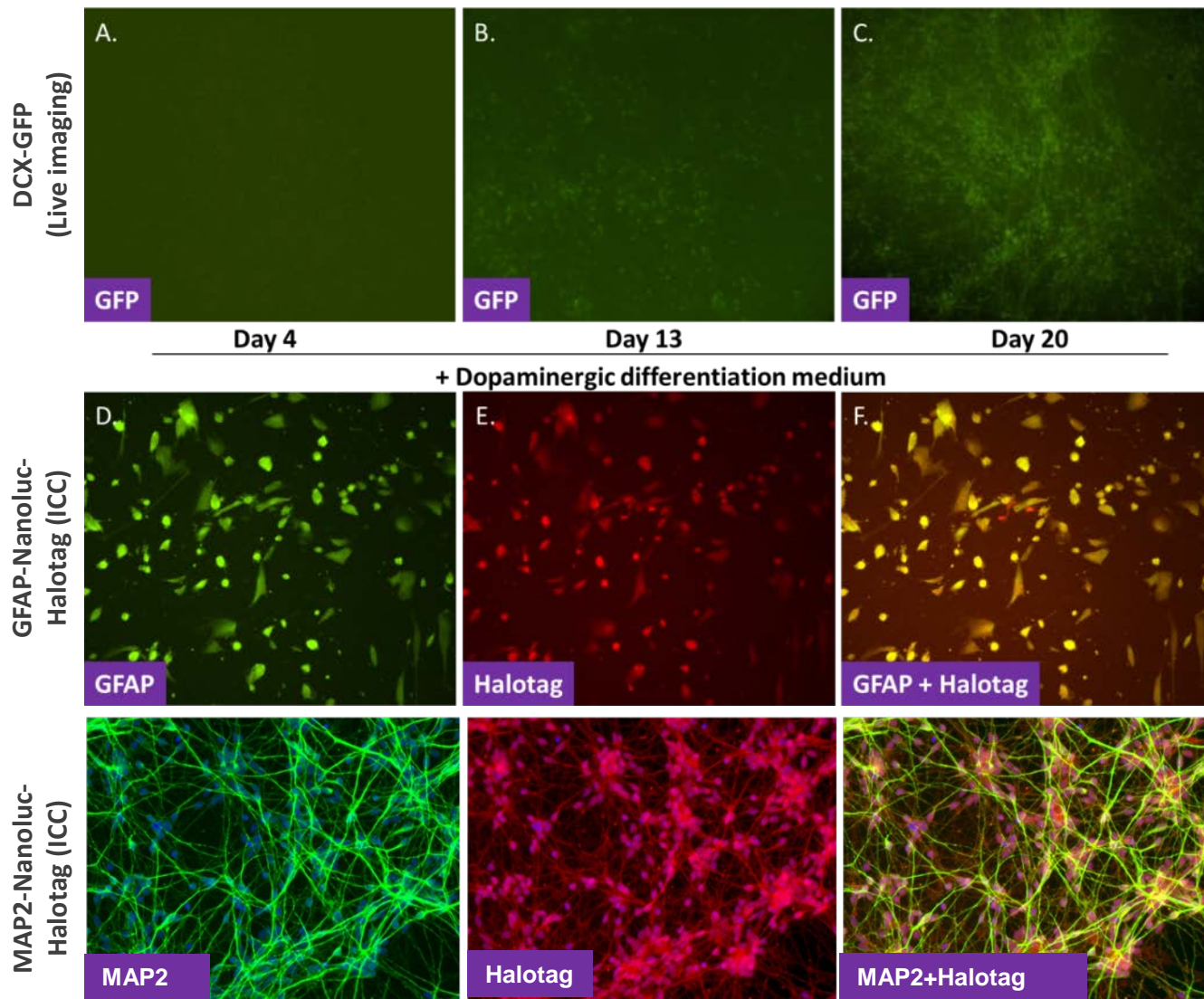
Luciferase secretion during dopaminergic neuron differentiation of MAP2-Nanoluc-Halotag NPCs



Luciferase secretion during astrocyte differentiation of GFAP-Nanoluc-Halotag NPCs

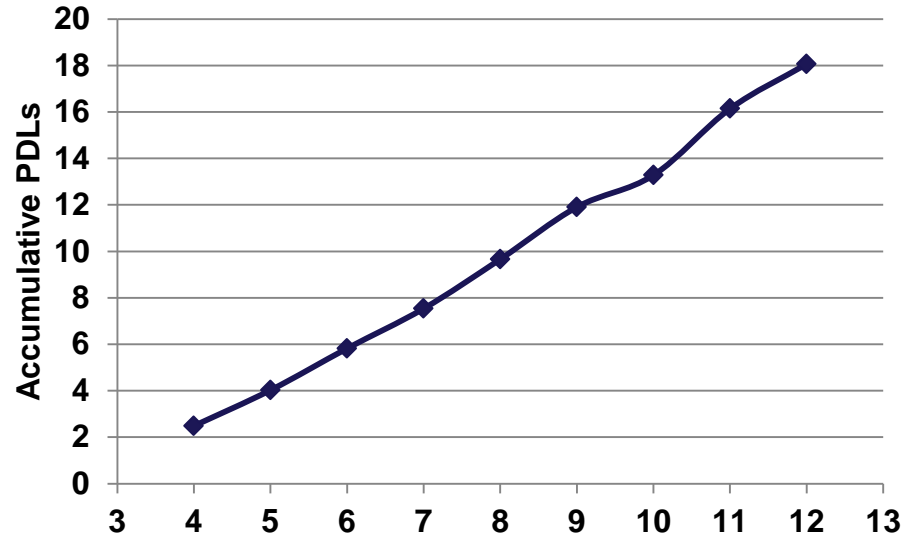
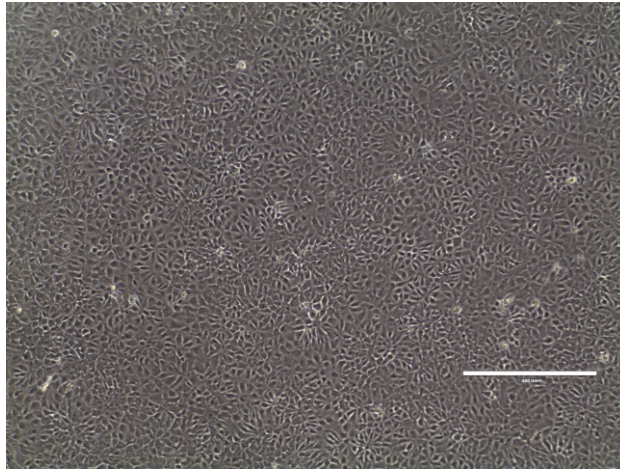


# Expression of the GFP or Halotag reporter during dopaminergic neuron or astrocyte differentiation

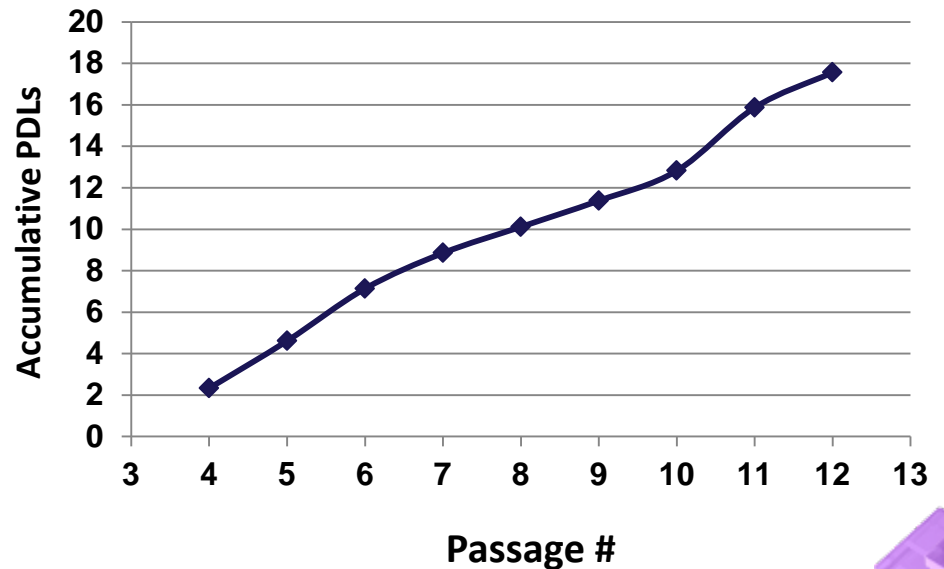
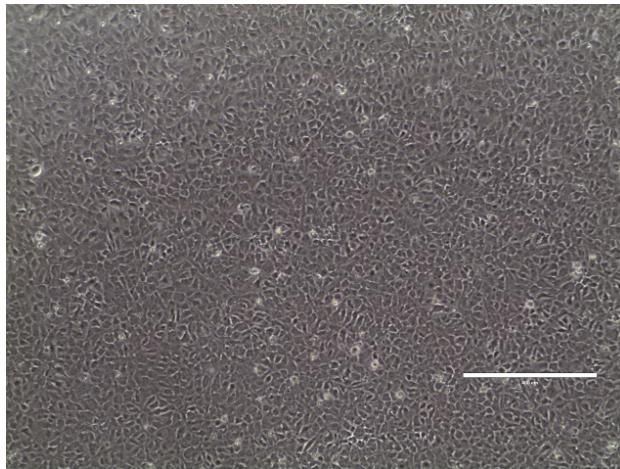


# Development of ATCC NPC growth media

ATCC NPC  
growth media



Company B NPC  
growth media



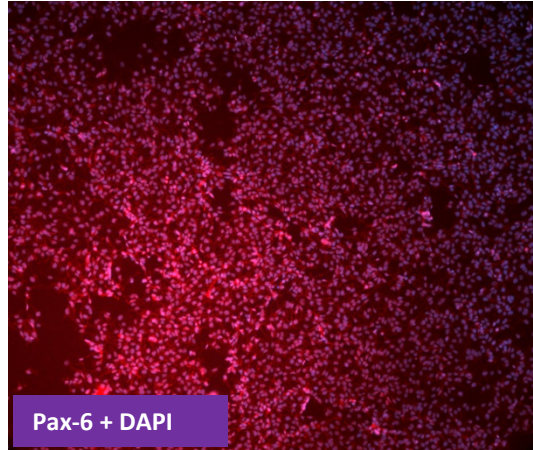
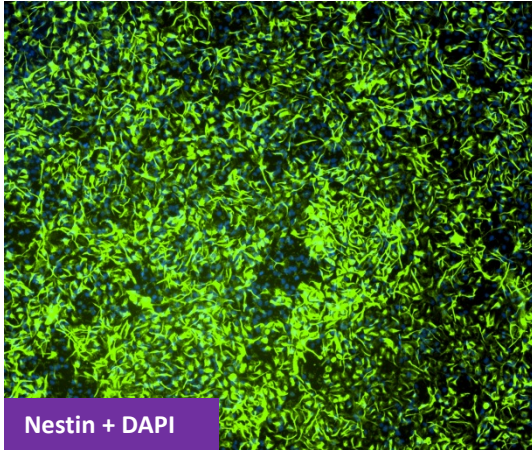
P7; Day 3

Passage #

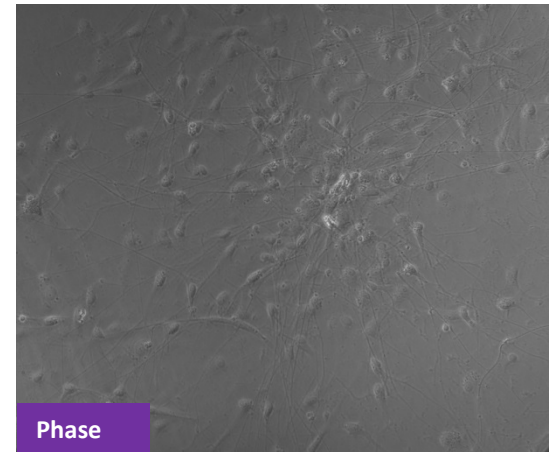
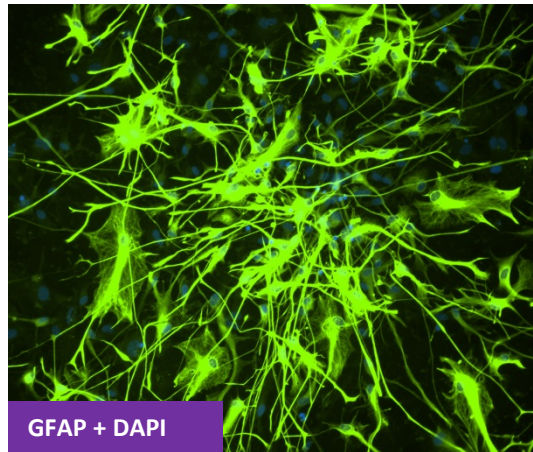
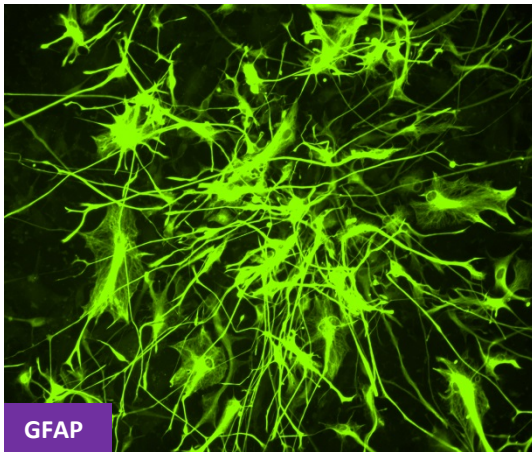


# Marker expression and astrocyte differentiation of NPCs cultured in ATCC growth media

NPCs (P3)



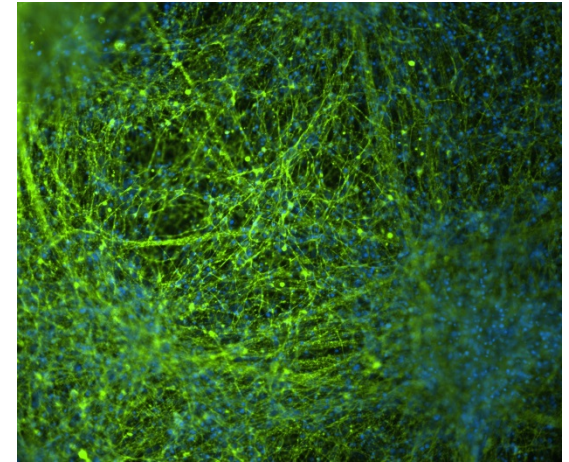
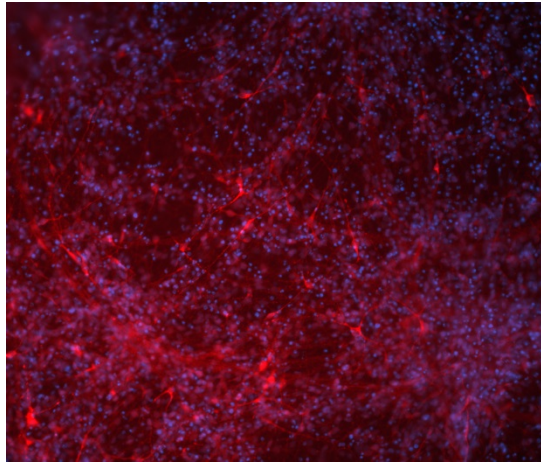
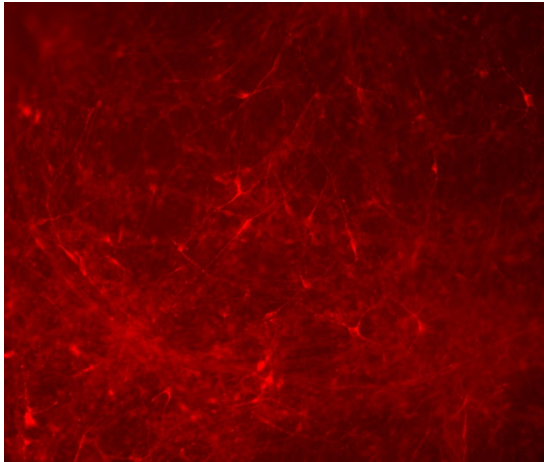
Astrocytes



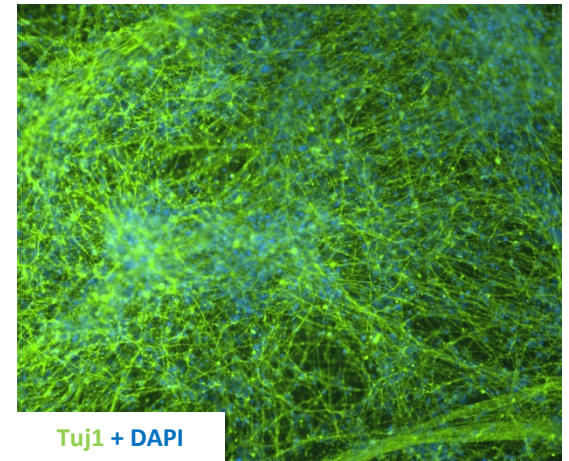
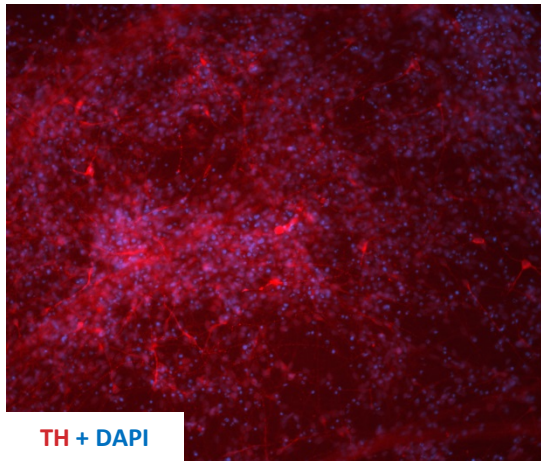
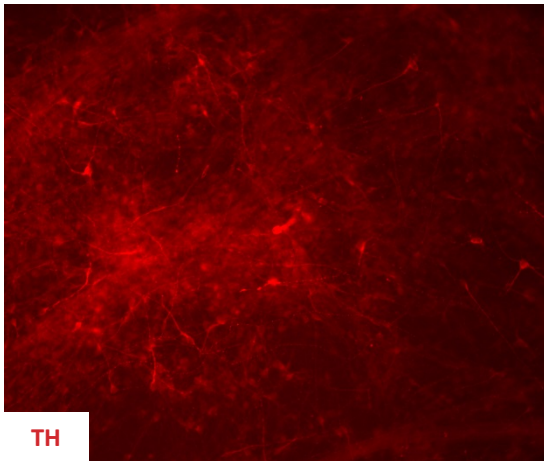


# Development of ATCC dopaminergic neuron differentiation media (ATCC® ACS-3004™)

ATCC NPC  
growth media



Company A NPC  
growth media



TH

TH + DAPI

Tuj1 + DAPI

# Overview

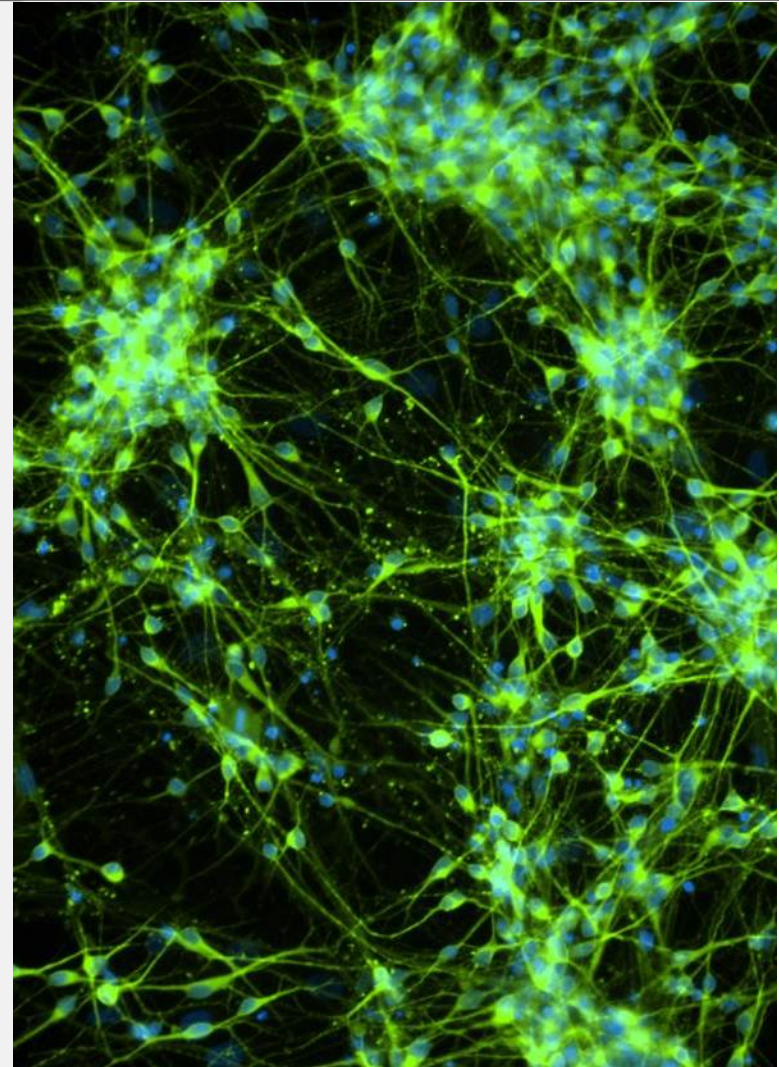
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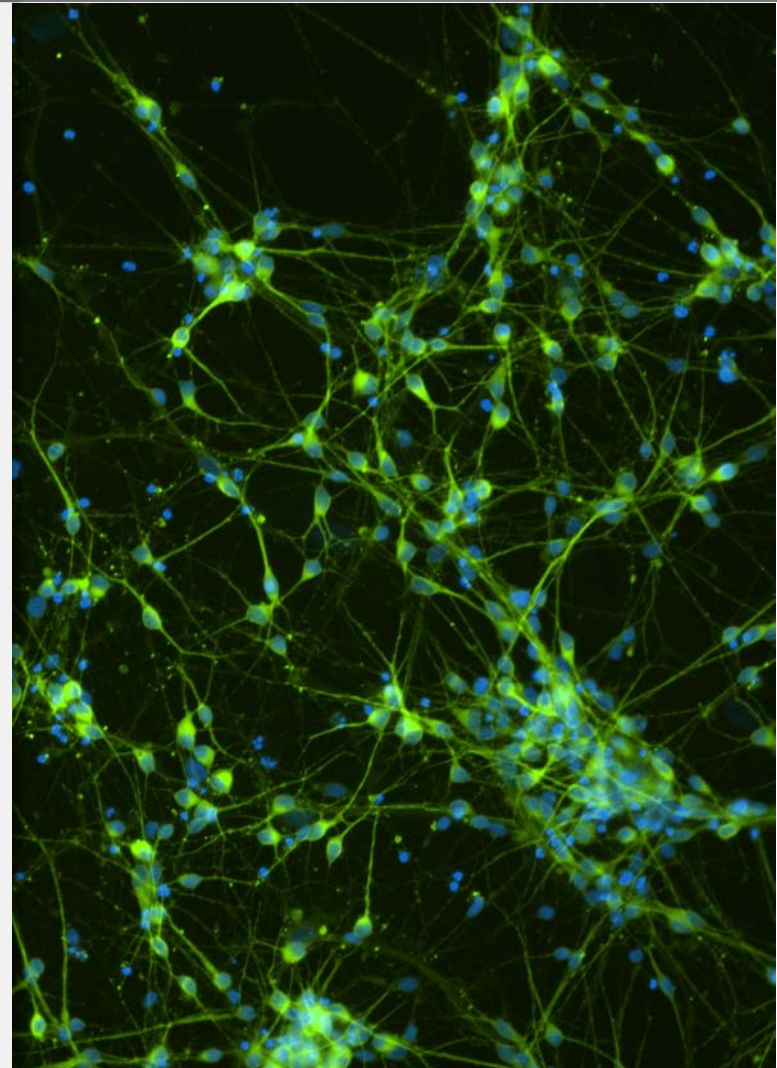


# ATCC initial NPC offerings

ATCC® No.	Designation	Availability
ACS-3003™	Neural Progenitor Cell Growth Kit	Late 2015
ACS-3004™	Dopaminergic Differentiation Kit	Late 2015
ACS-5001™	Neural Progenitor Cells, Parkinson's Origin: ATCC-DYS0530 (ACS-1013™) hiPSCs	In development
ACS-5003™	Neural Progenitor Cells, Normal Origin: ATCC-BXS0117 (ACS-1031™) hiPSCs	Late 2015
ACS-5004™	Neural Progenitor Cells, Normal Origin: ATCC-BYS0112 (ACS-1026™) hiPSCs	Late 2015
ACS-5005™	DCXp-GFP Neural Progenitor Cells, Normal Origin: XCL-1 hiPSCs	Late 2015
ACS-5006™	GFAP-Nanoluc-Halotag Neural Progenitor Cells, Normal Origin: XCL-1 hiPSCs	Late 2015
ACS-5007™	MAP2-Nanoluc-Halotag Neural Progenitor Cells, Normal Origin: XCL-1 hiPSCs	Late 2015

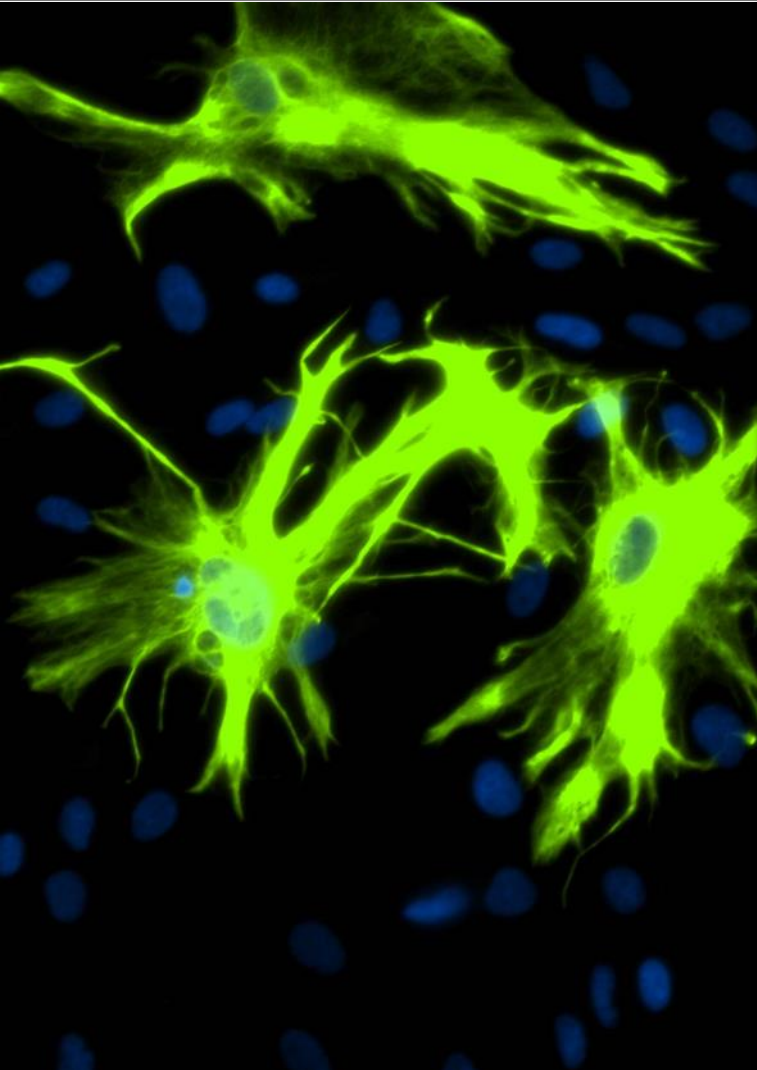
# Summary

- Developed a process enabling generation of unlimited supply of neural progenitor cells
- Optimized culture conditions for the expansion and tri-lineage differentiation of NPCs
- Starting iPSC lines played an important role in morphology, proliferative capacity, and differentiation potential of NPCs
- CD34<sup>+</sup>-derived NPCs exhibited a better proliferative capacity and greater efficiency of tri-lineage differentiation
- Three gene-edited NPC reporter lines expressed GFP, NanoLuc<sup>®</sup>, or HaloTag<sup>®</sup> during lineage specific differentiation
- ATCC complete solution of NPC products including NPCs and culture media provides a powerful tool for disease modeling and drug screening





# Acknowledgments



## **Project team:**

Leelamma Jacob, M.S., Ph.D.

Michelle Spencer, M.S.

Dezhong Yin, Ph.D.

## **Trademarks**

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