

# Neural progenitor cells – Toxicological models for the 21<sup>st</sup> century

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# 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



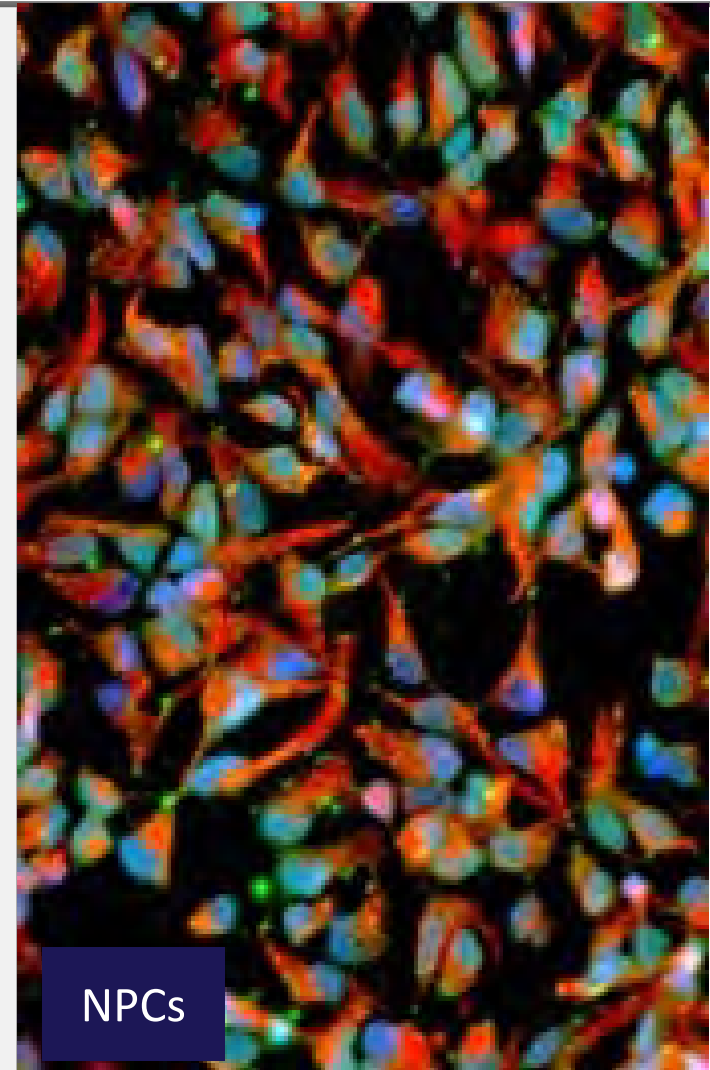
Established partner to global researchers and scientists



# Agenda

## Neural Progenitor Cells (NPCs) and Media

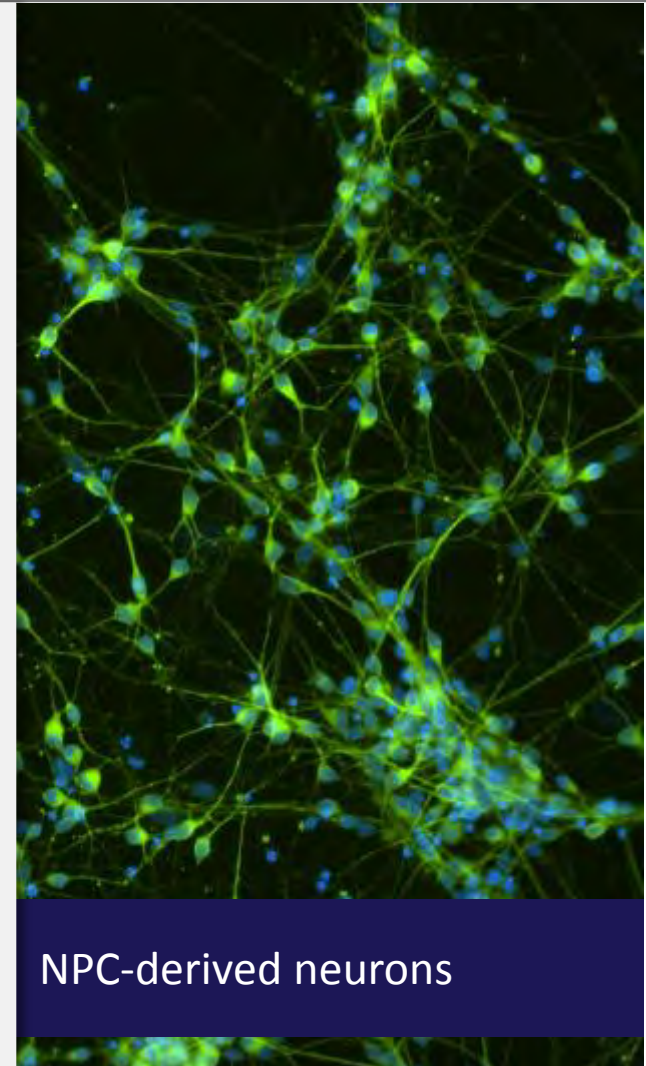
- **Background information**
- Differentiation potential of ATCC NPCs
- Toxicological studies
- Summary



NPCs

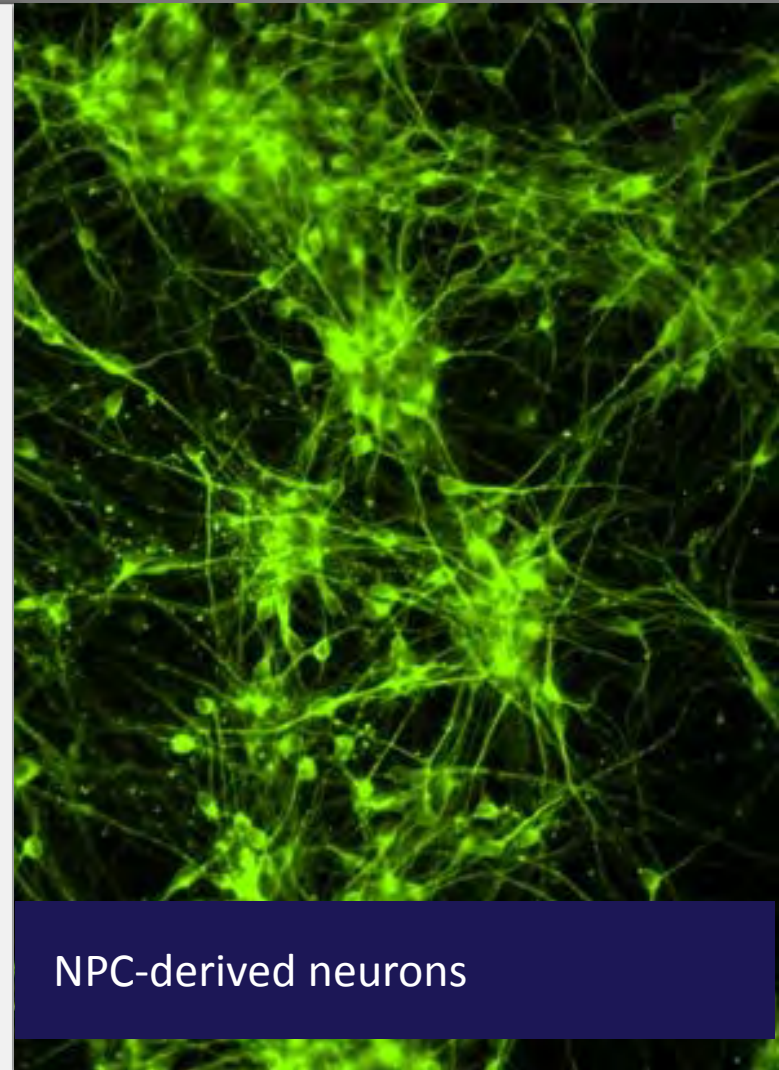
# The current status of neurobiology research

- Primary cells from animals (mouse and rat neurons)
  - Not predictive
  - Donor variation
- Continuous cell lines (originally isolated from tumors)
  - Not normal
  - Not predictive
- Induced pluripotent stem cells (iPSCs; commercial or self-made)
  - Time and labor intensive
  - Often not validated for neural development



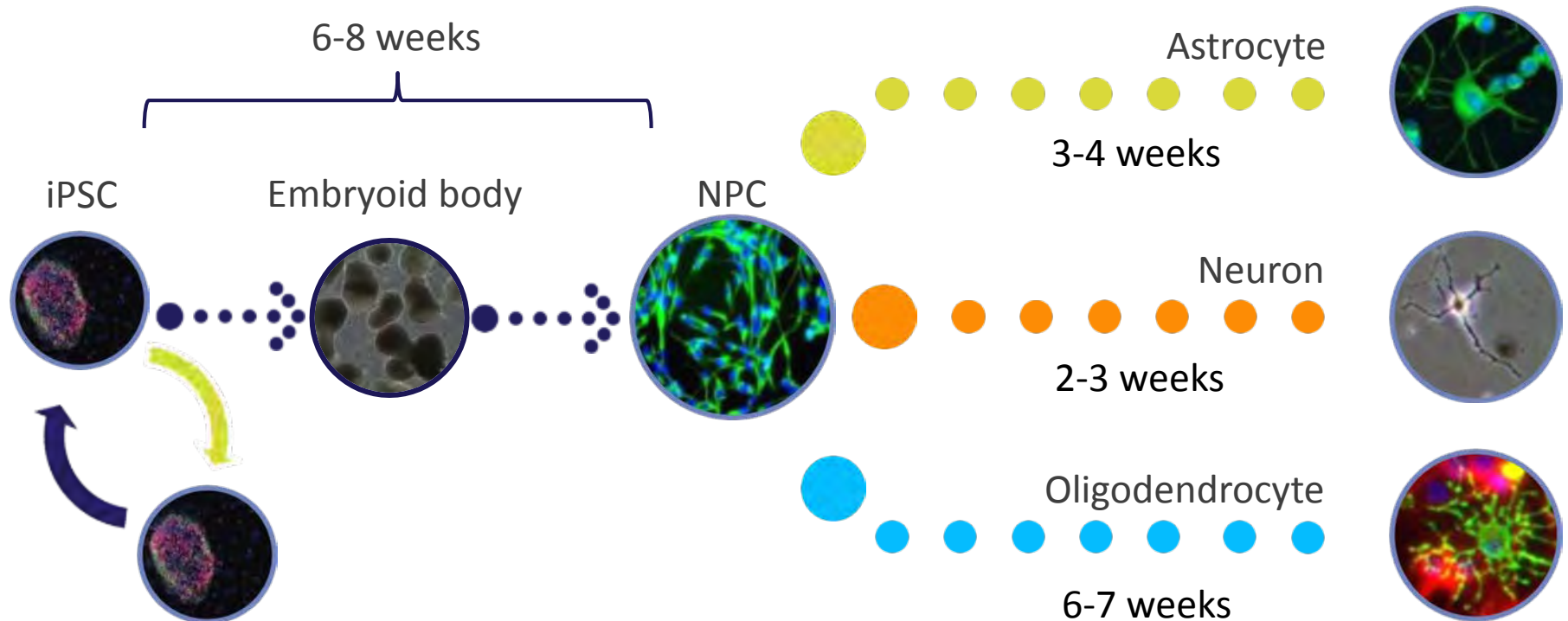
# What is needed?

- Biologically relevant models
- Validated neural functioning
- Predictive for screening applications



NPC-derived neurons

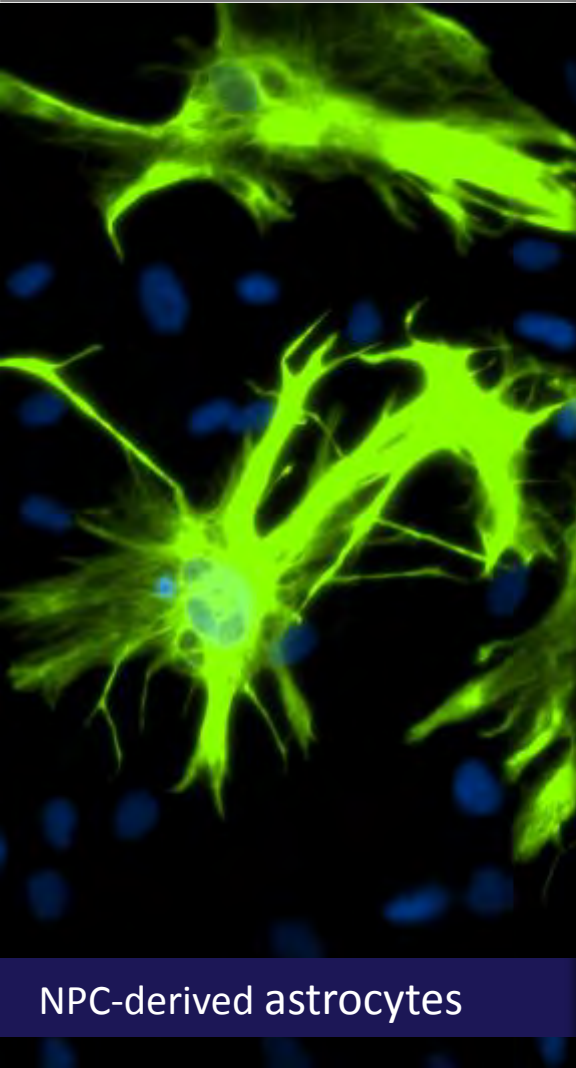
# Neural progenitor cells (NPCs) - Neuronal differentiation



NPCs:

- Shorten research time
- Human model
- Predictive screening

# Neural Progenitor Cells (NPCs) from ATCC



NPC-derived astrocytes

## Value:

- Human models with no donor variation
- Live imaging is possible
- Cells exhibit full differentiation spectrum
- Complete system of cells and media will be available

## Key benefits:

- More biologically relevant results/more predictive system
- Markers allow for easy endpoint readout
- Can differentiate to neuronal and glial cells
- Easy to use and saves time

# ATCC® NPC offerings

ATCC® No.	Designation
ACS-3003	NPC Growth Kit – <i>add to DMEM/F12</i>
ACS-3004	NPC Dopaminergic Differentiation Kit – <i>add to DMEM/F12</i>
ACS-5003	NPCs derived from ATCC-BXS0117 (ACS-1031)
ACS-5004	NPCs derived from ATCC-BYS0112 (ACS-1026)
ACS-5005	Neural Progenitor Cells derived from XCL-1 DCX-GFP <i>(for late neuron differentiation)</i>
ACS-5006	Neural Progenitor Cells derived from XCL-1 GFAP-Nanoluc®-Halotag® <i>(for astrocyte differentiation)</i>
ACS-5007	Neural Progenitor Cells derived from XCL-1 MAP2-Nanoluc®-Halotag® <i>(for early neuron differentiation)</i>
ACS-2103	Screening Fee – For Profit

ATCC® ACS-1026 – iPSC derived from bone marrow CD34+ cell from Caucasian male

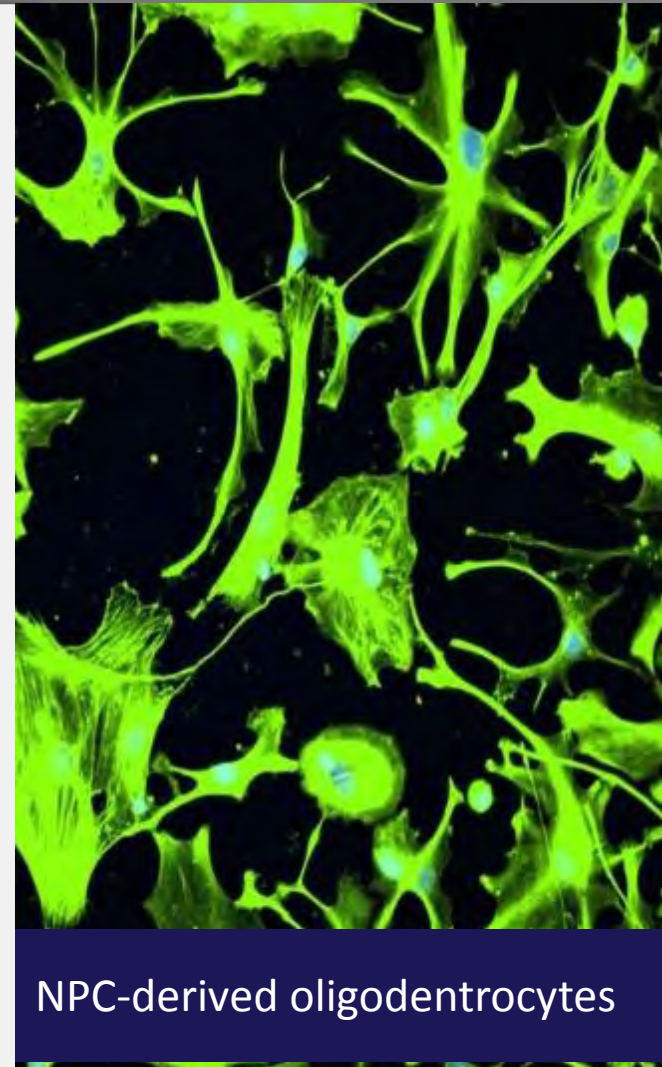
ATCC® ACS-1031 – iPSC derived from bone marrow CD34+ cell from Asian female

Reporter lines from iPSC derived from cord blood CD34+ from a Caucasian male  
(XL-1 iPSCs from NIH)



# QC testing of ATCC<sup>®</sup> NPCs

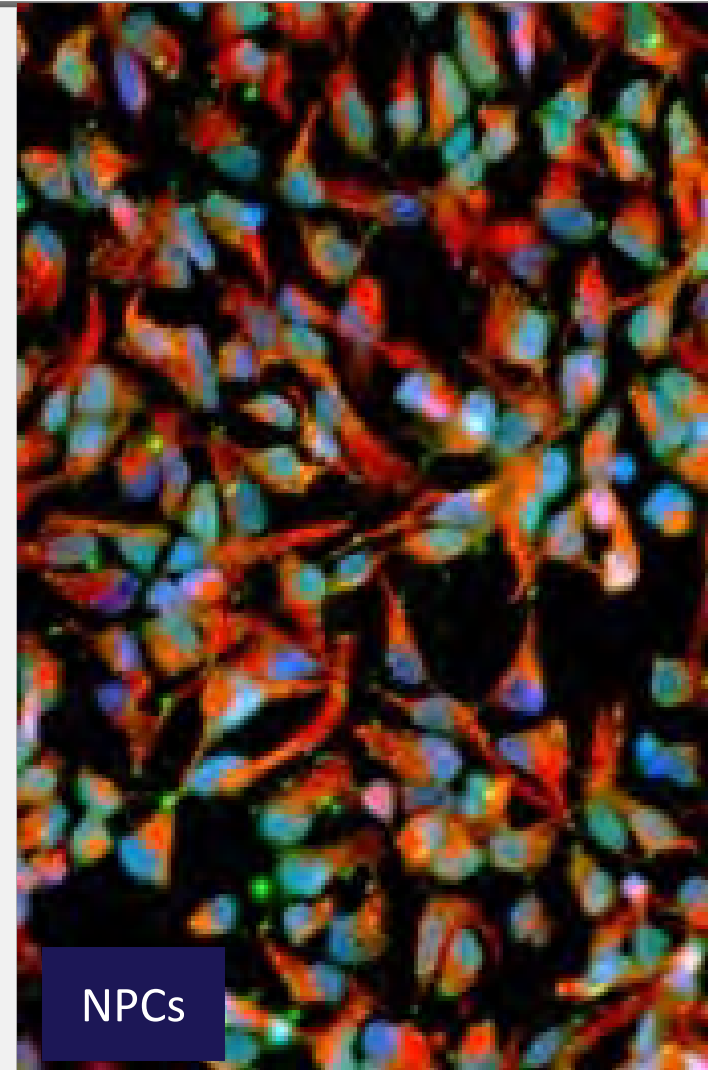
- Post-thaw cell viability: >80%
- Post-thaw viable cell number: >1x10<sup>6</sup> cells/vial
- Longevity: >15 PDLs or 5 passages
- NPC marker expression: Nestin<sup>+</sup>, Pax-6<sup>+</sup>, and Tra-I-60<sup>-</sup>
- Differentiation potential:
  - >70% Tuj1<sup>+</sup> early neurons and
  - >10%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



# Agenda

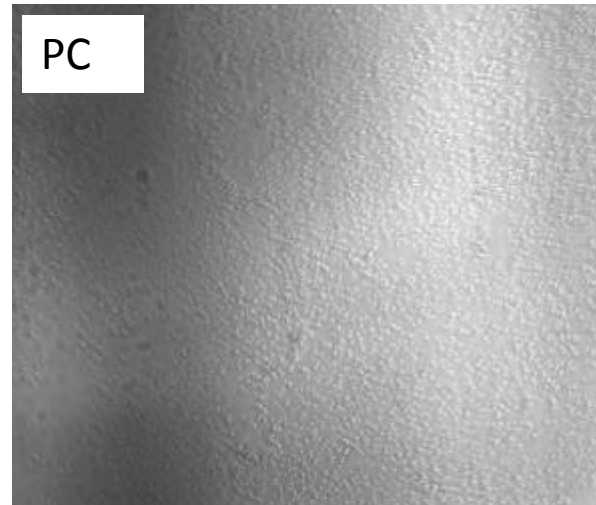
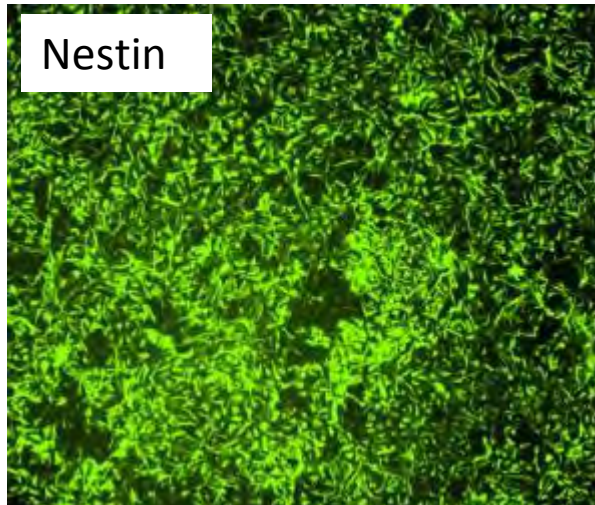
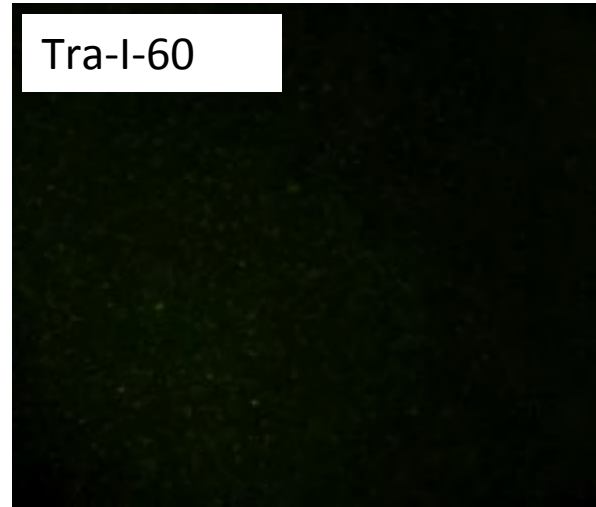
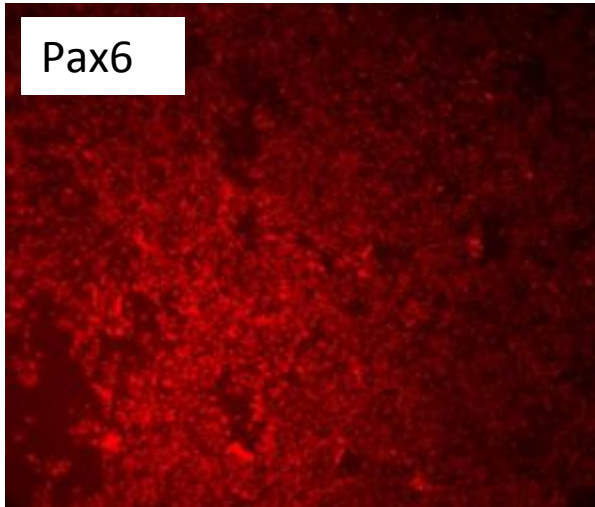
## NPCs and Media

- Background information
- **Differentiation potential of ATCC NPCs**
- Toxicological studies
- Summary



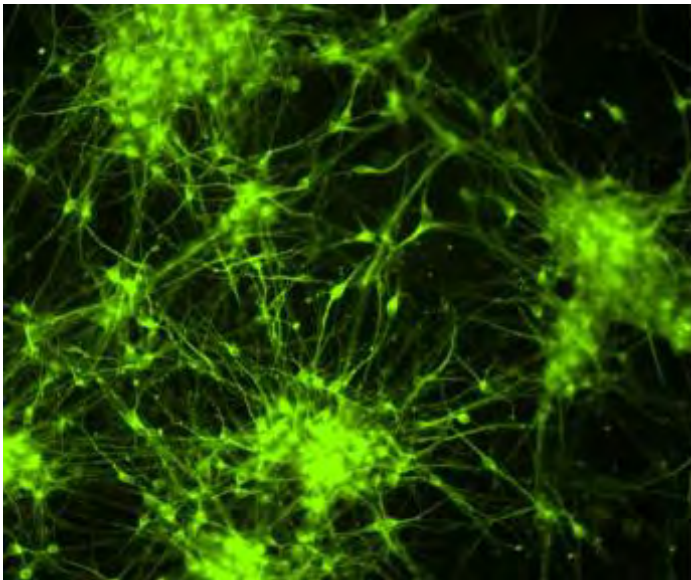
NPCs

# ATCC NPCs express NPC markers but **not** iPSC markers

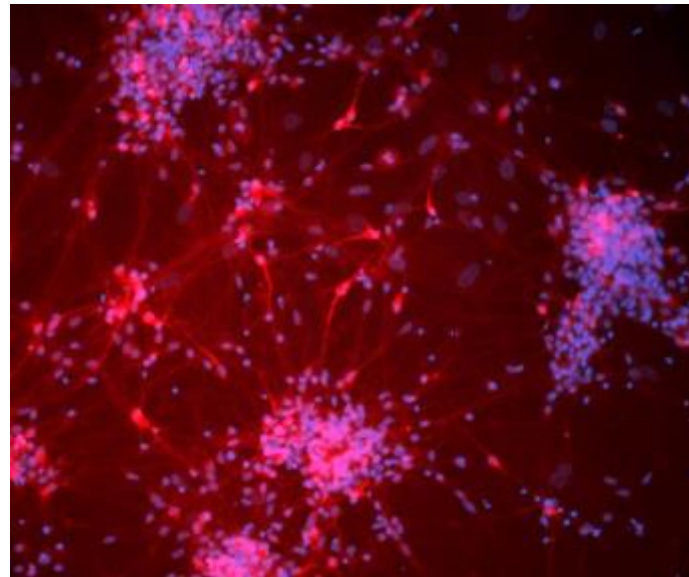


- NPC Marker
- Nestin
  - Pax-6
- iPSC Marker
- Tra-I-60

# Dopaminergic neuron differentiation of NPCs



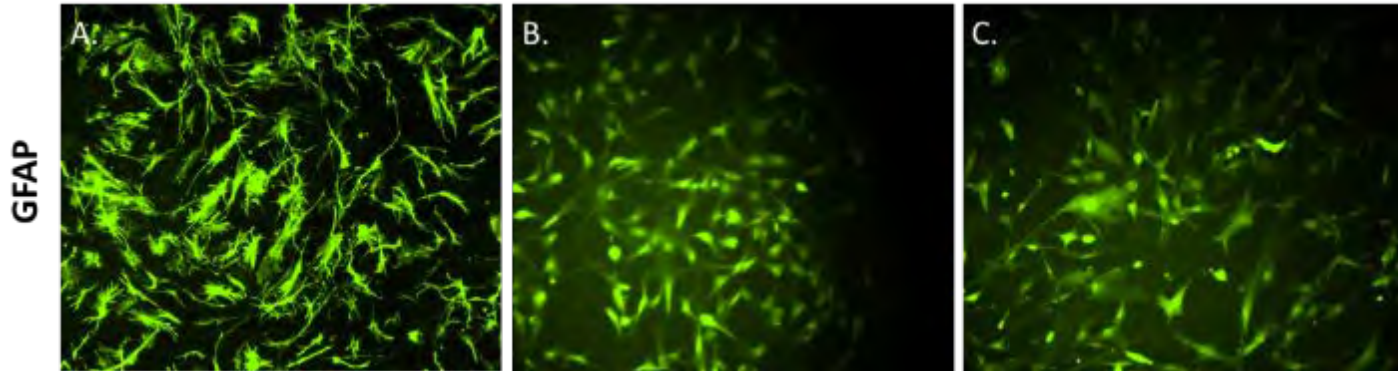
Tuj1



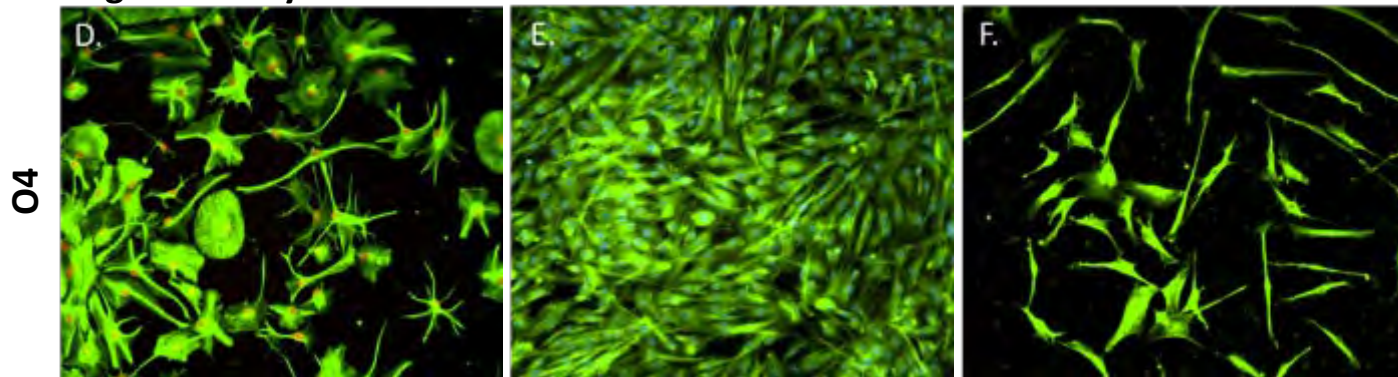
TH/DAPI

# Astrocyte and oligodendrocyte differentiation

## Astrocyte differentiation



## Oligodendrocyte differentiation

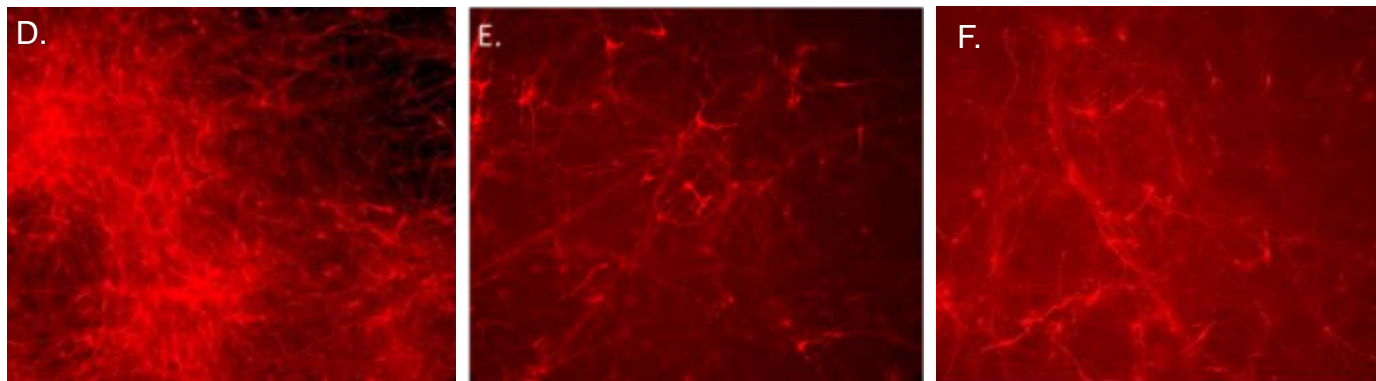
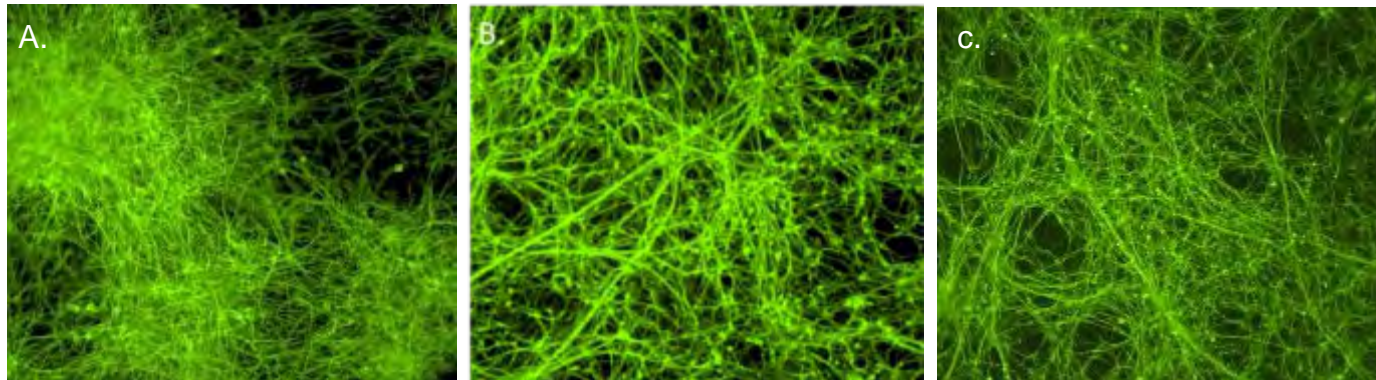


CD34<sup>+</sup>

HFF-1

Parkinson's

# Dopaminergic neuron differentiation of NPC reporter lines



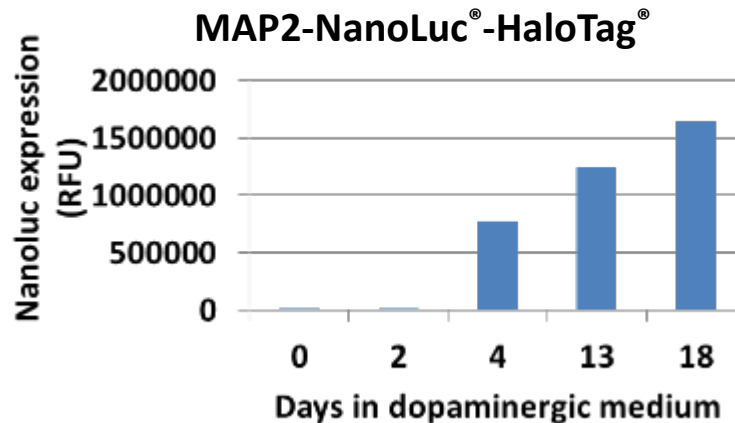
**MAP2- NanoLuc<sup>®</sup>-HaloTag<sup>®</sup>  
(ACS-5007)**

**DCX-GFP  
(ACS-5005)**

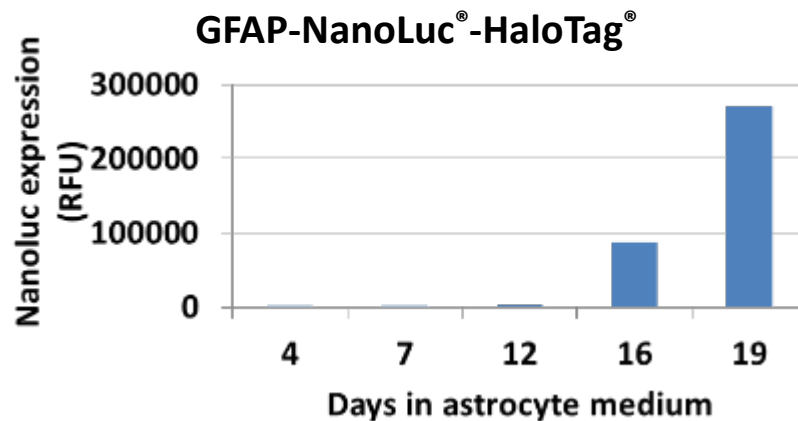
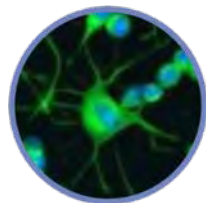
**GFAP-NanoLuc<sup>®</sup>-HaloTag<sup>®</sup>  
(ACS-5006)**

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

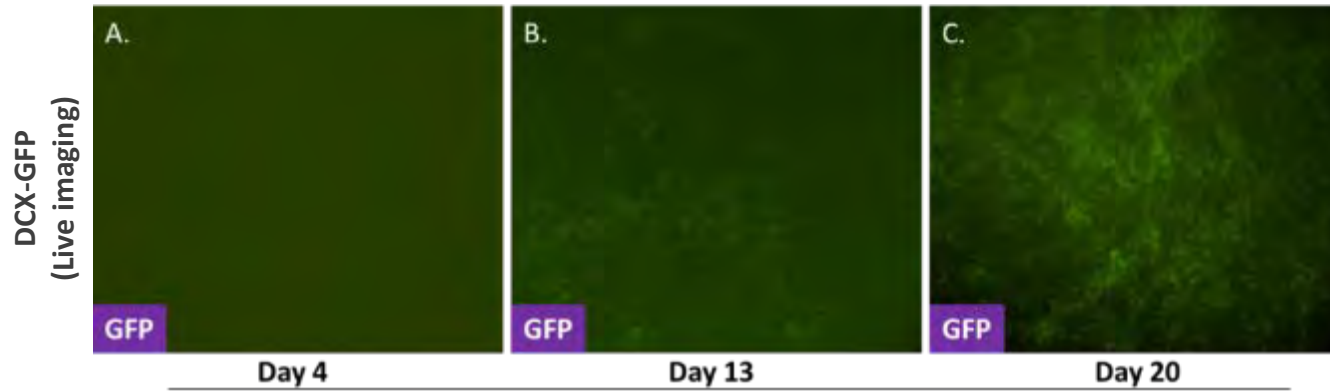
Luciferase secretion during dopaminergic neuron differentiation of NanoLuc<sup>®</sup>-HaloTag<sup>®</sup> NPCs



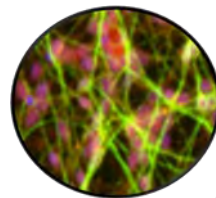
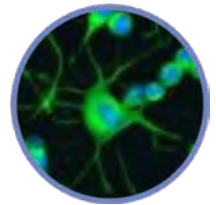
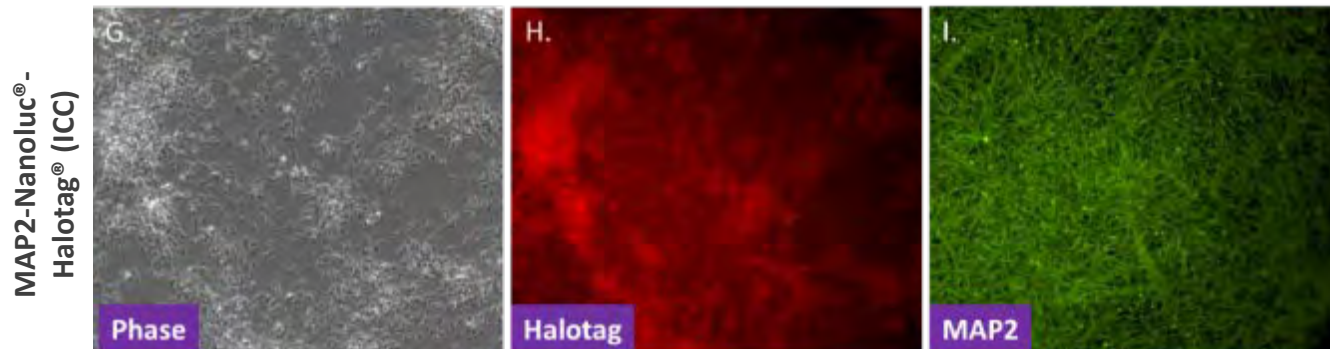
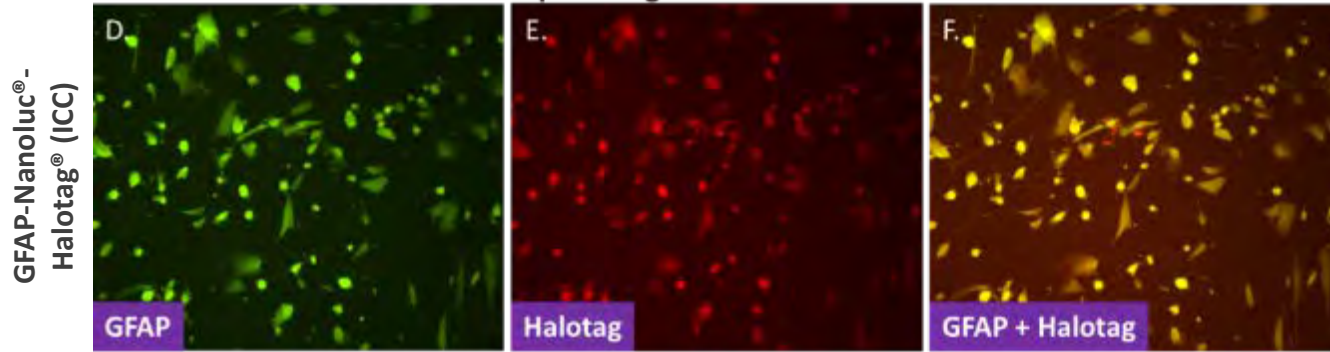
Luciferase secretion during astrocyte differentiation of GFAP-NanoLuc<sup>®</sup>-HaloTag<sup>®</sup> NPCs



# Expression of the GFP or HaloTag<sup>®</sup> reporter during dopaminergic neuron or astrocyte differentiation



+ Dopaminergic differentiation medium

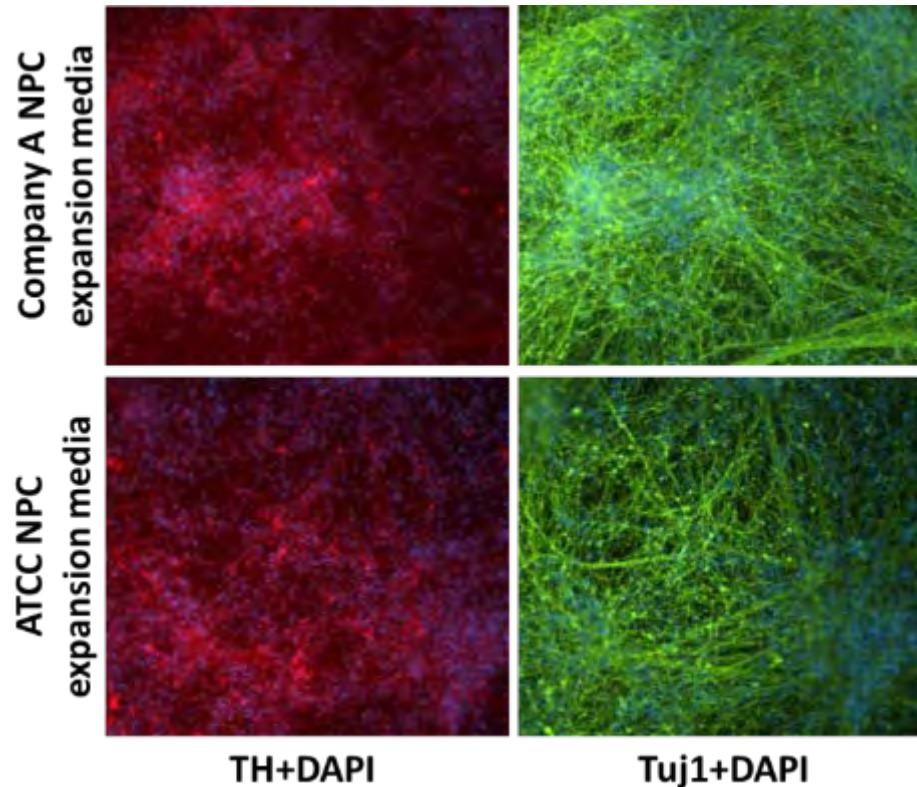




# Development of ATCC's NPC expansion and dopaminergic differentiation media

NPCs cultured in company A NPC expansion media (top row) or ATCC NPC Growth Kit (bottom row) for 3 passages prior to differentiation using ATCC's NPC Dopaminergic Differentiation Kit

ATCC® No.	Designation
ACS-3003	NPC Growth Kit
ACS-3004	NPC Dopaminergic Differentiation Kit



# Expression of genes associated with the differentiation of NPCs

**TaqMan<sup>®</sup> primers were used to identify the presence of other types of neurons during dopaminergic neuron differentiation using ATCC<sup>®</sup> ACS-3004<sup>™</sup> media**

Dopaminergic neurons: TH, Nurr1, VMAT2, AADC

Glutamatergic neurons: GLS2, vGLUT1, vGLUT2

Gabaergic neurons: GABA (GABRB3)

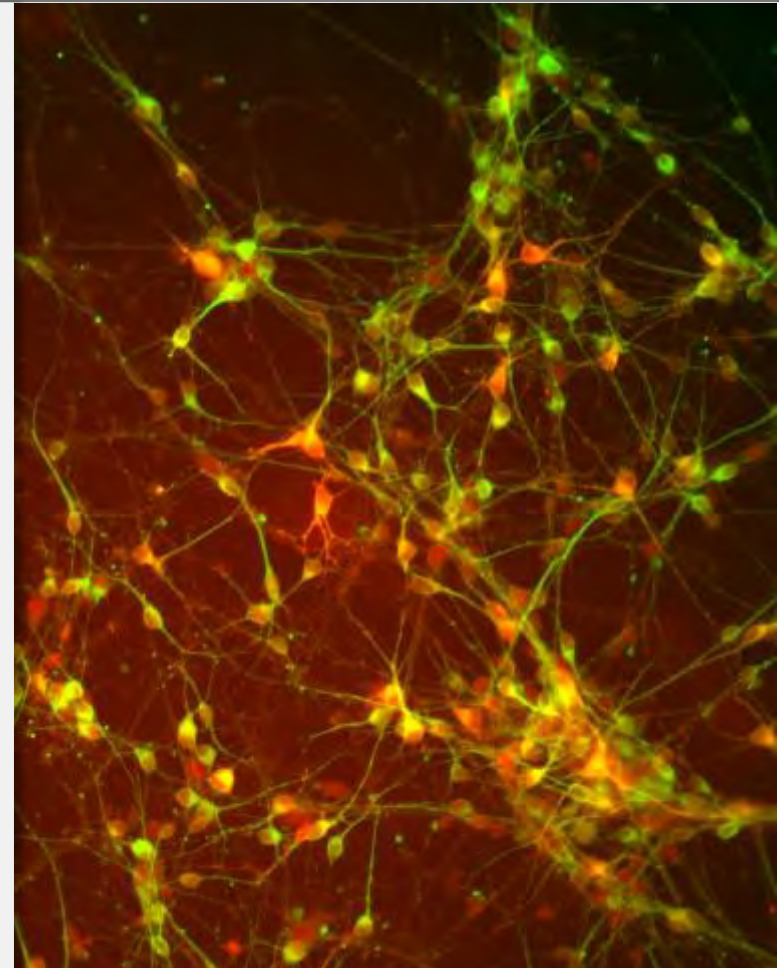
Motor neurons: EN1, LIM3, and Hb9

Cholinergic neurons: ChAT



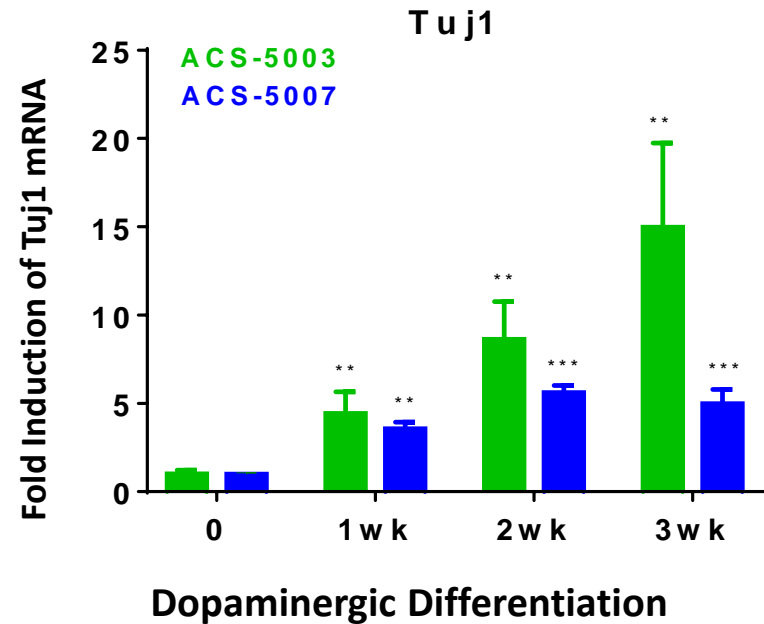
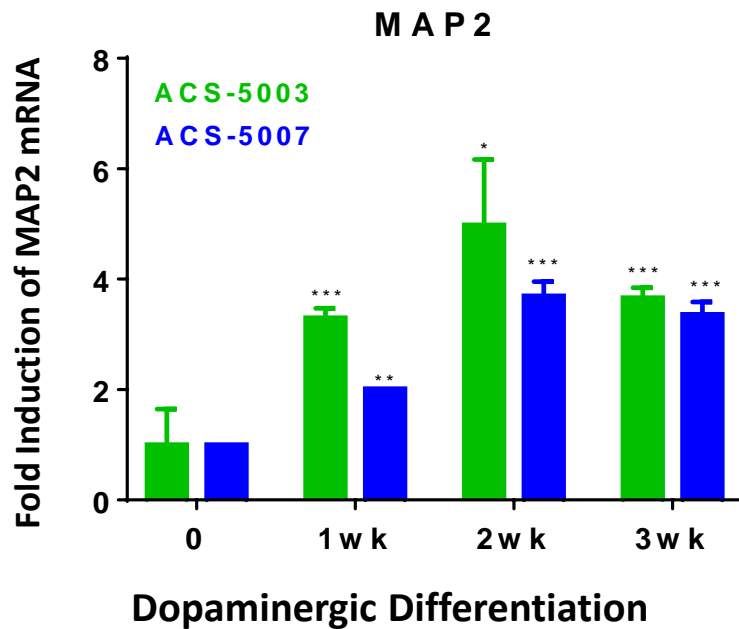
# Early and dopaminergic neuron gene expression

**Upregulation of early and dopaminergic neuron genes in ACS-5003 and ACS-5007 NPCs during dopaminergic neuron differentiation**

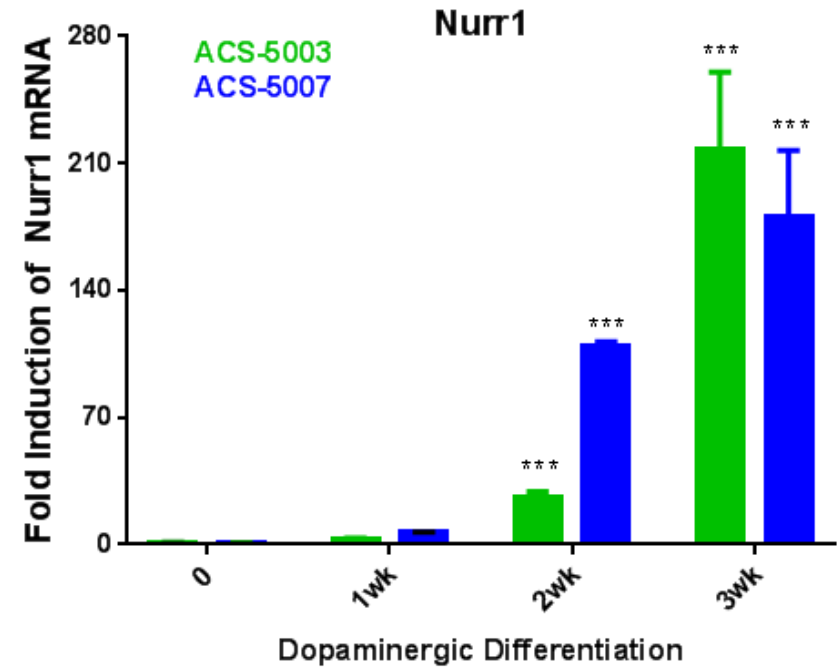
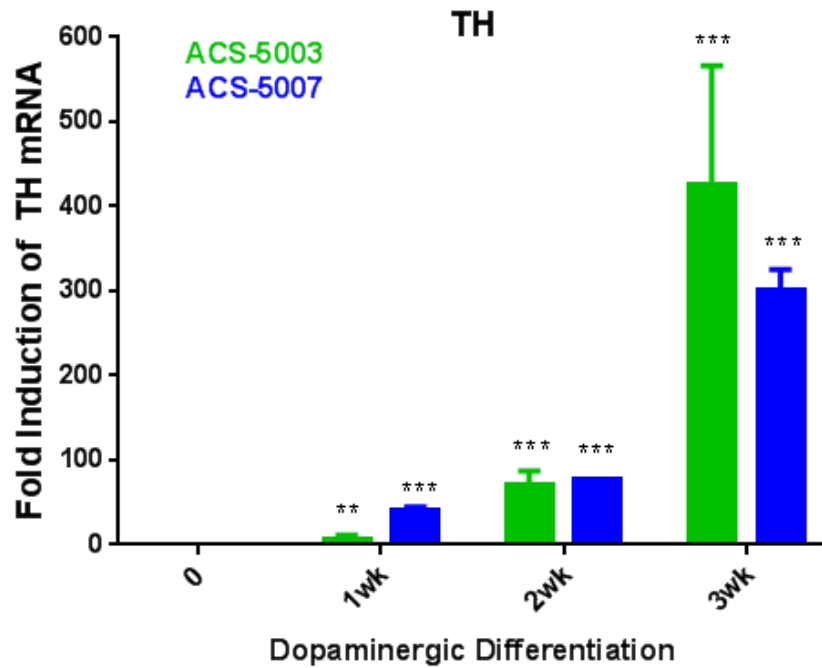


NPC-derived dopaminergic neurons

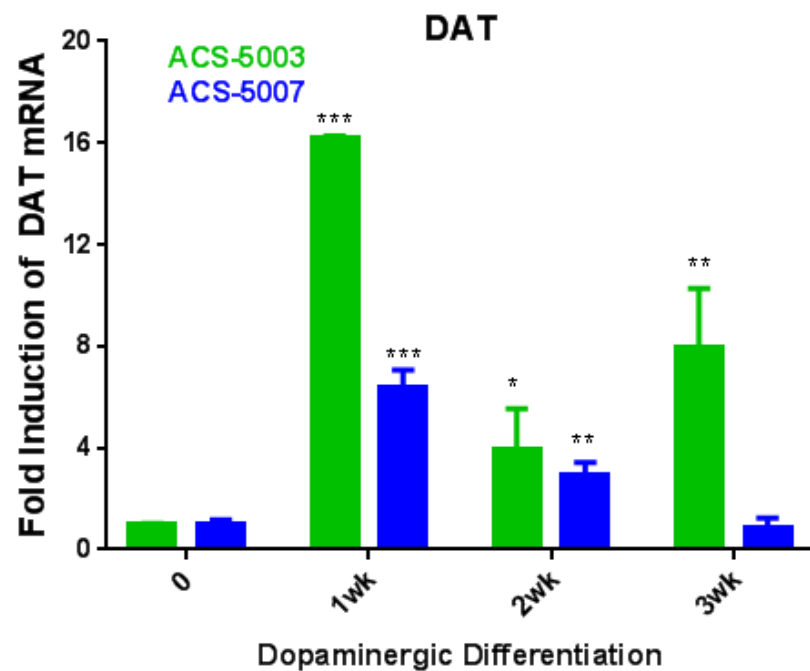
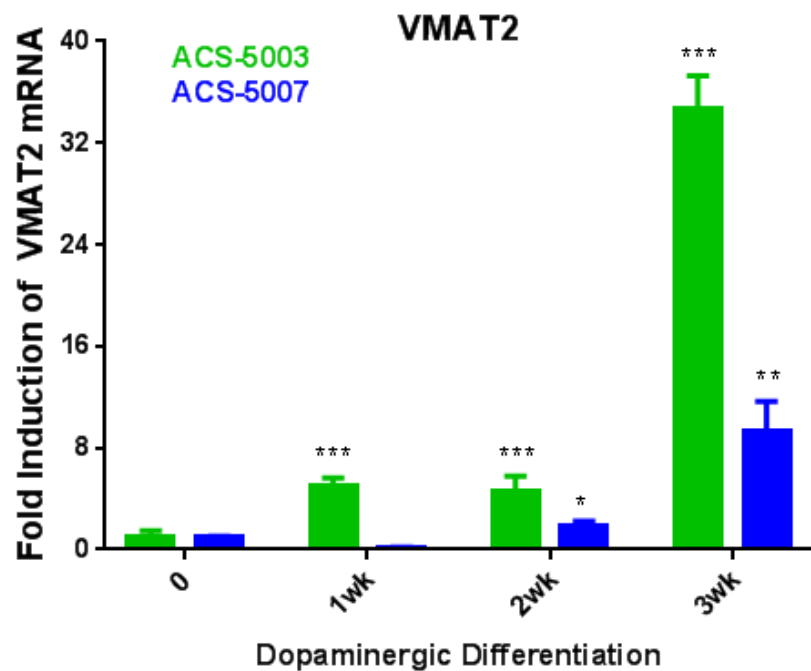
# Expression of early neuron genes (MAP2 and Tuj1) in ACS-5003 and ACS-5007 NPCs



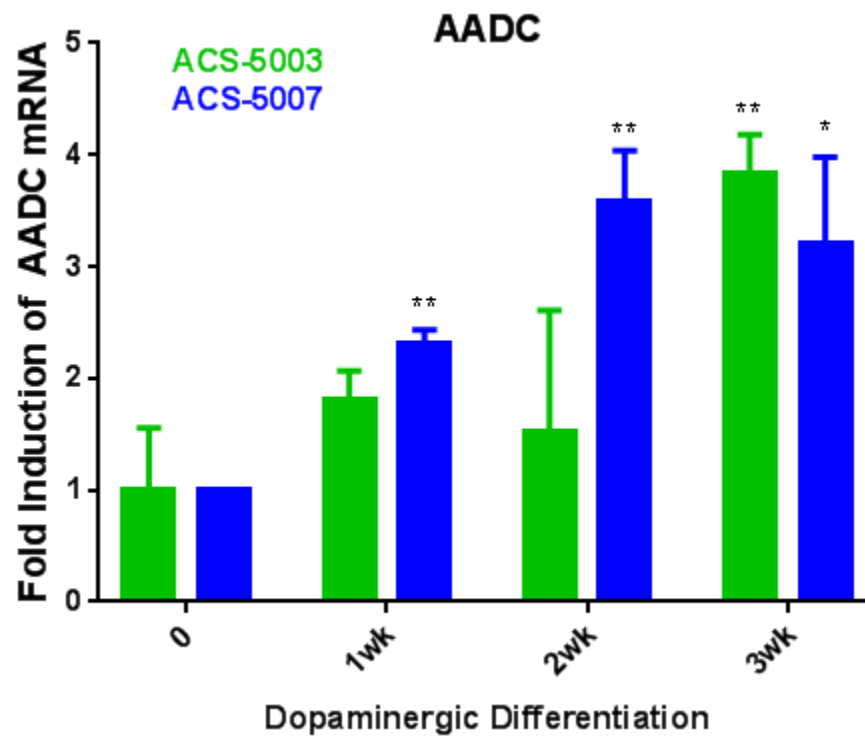
# Expression of dopaminergic neuron genes, TH and Nurr1



# Expression of VMAT2 and DAT

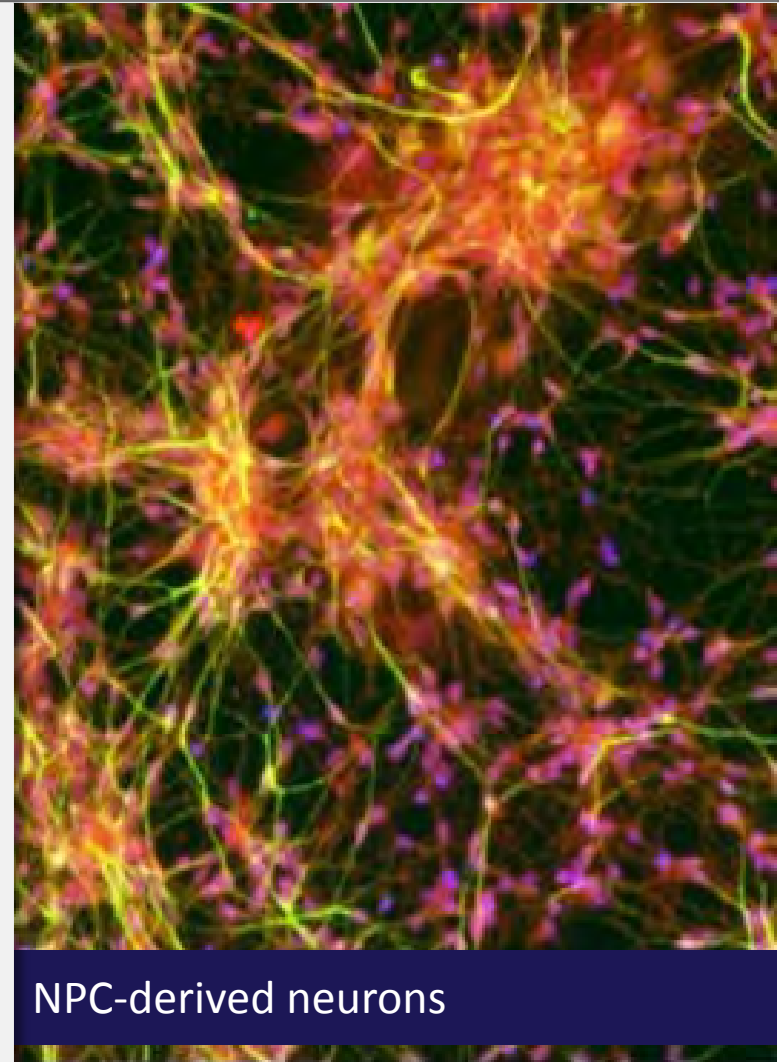


# Expression of AADC



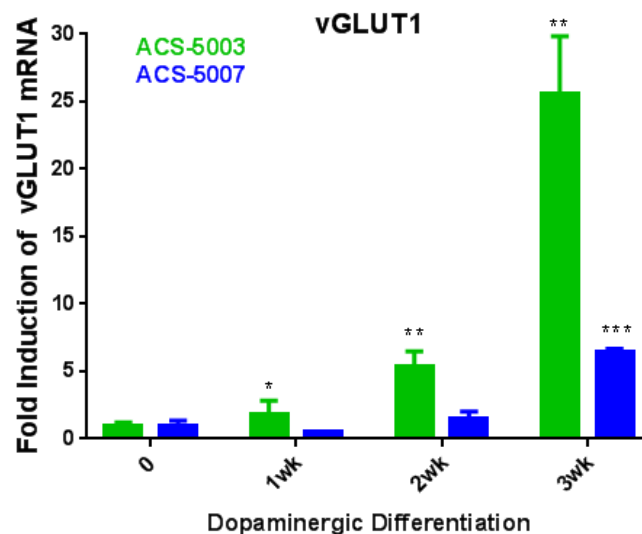
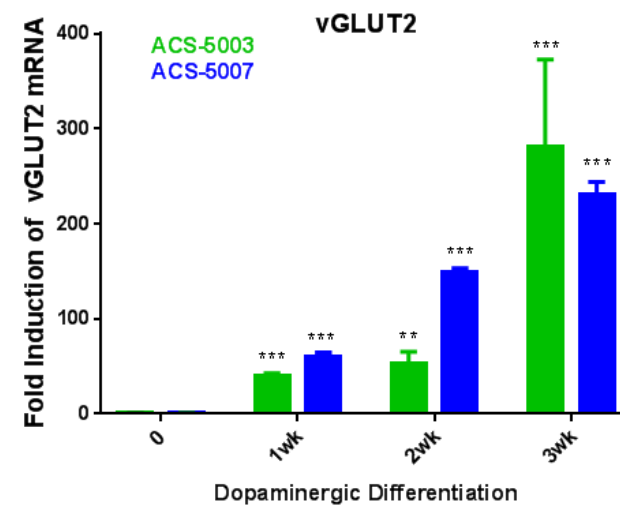
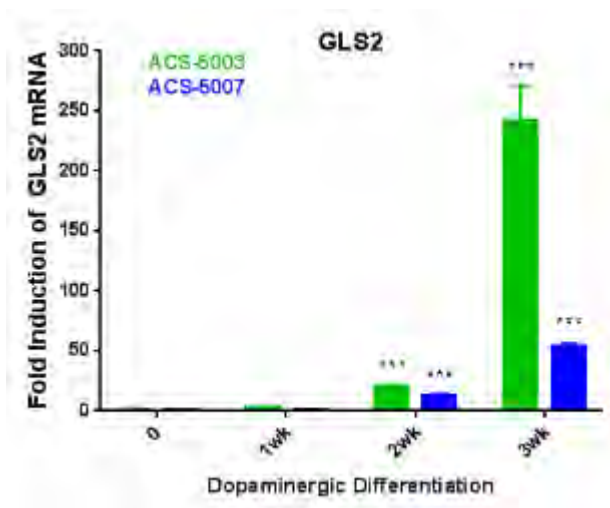
# Glutamatergic and GABAergic gene expression

**Upregulation of glutamatergic and GABAergic neuron genes in ACS-5003 and ACS-5007 NPCs during dopaminergic neuron differentiation**

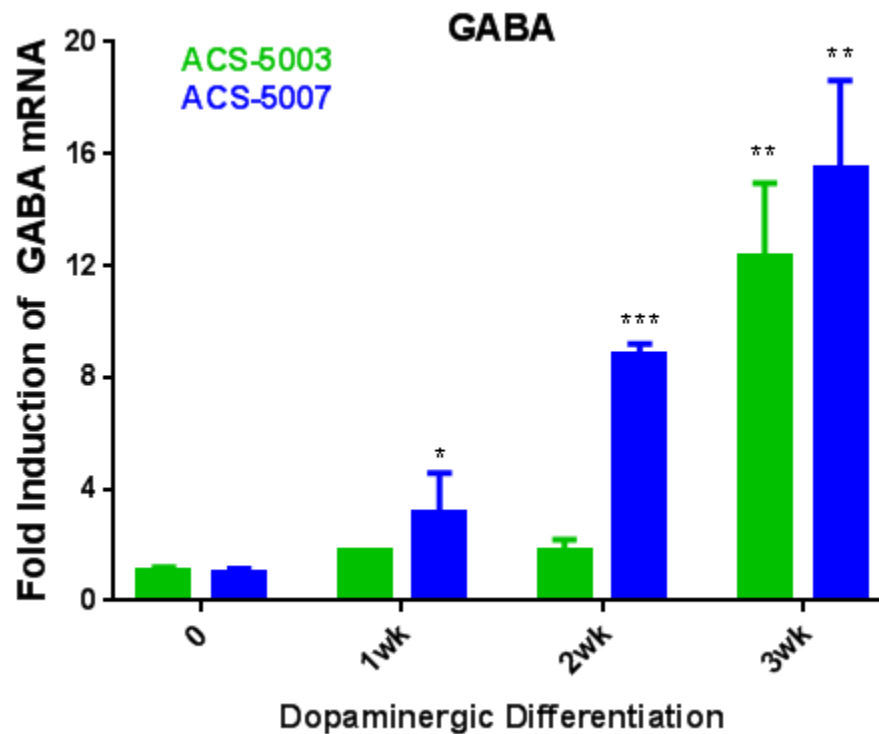




# Expression of GLS2, vGLUT2 and vGLUT1



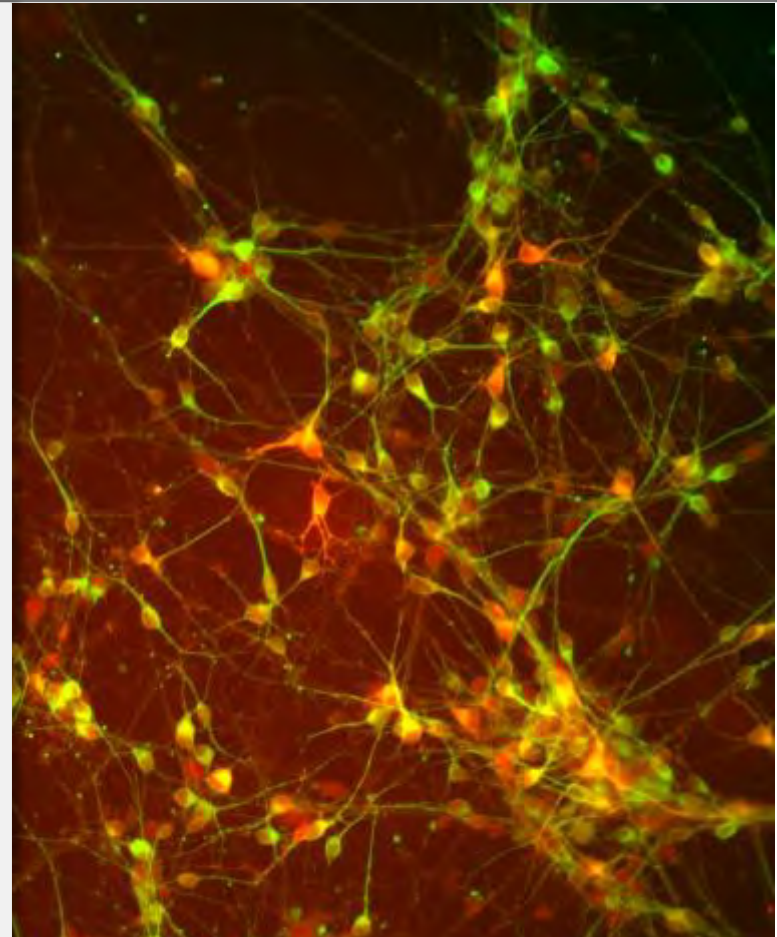
# Expression of GABA receptor B3 (GABRB3)



# Motor and cholinergic gene expression

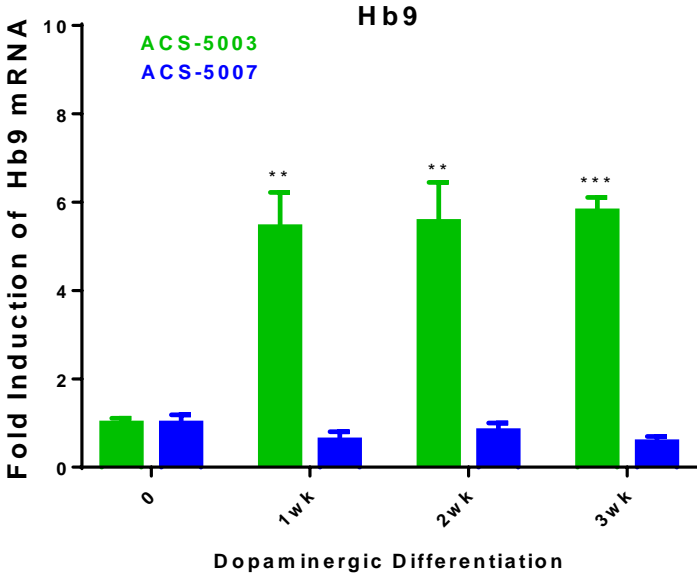
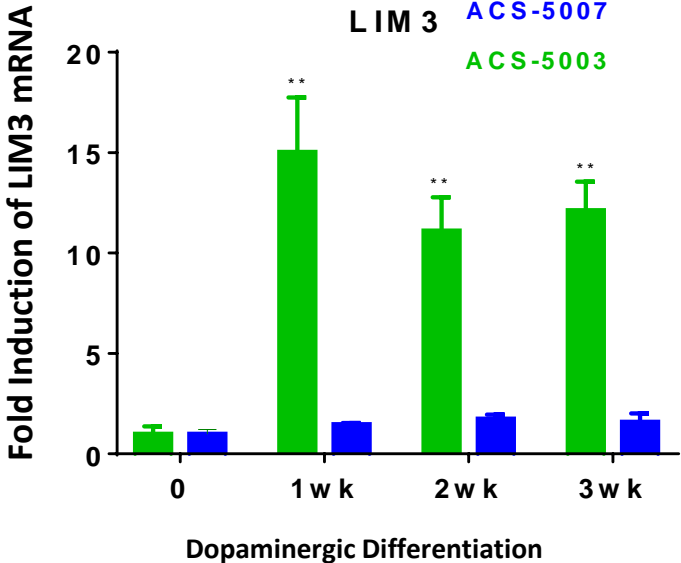
Upregulation of neuron genes in ACS-5003 and ACS-5007 NPCs during dopaminergic neuron differentiation:

- Motor
  - LIM3
  - Hb9
  - EN1
- Cholinergic
  - ChAT

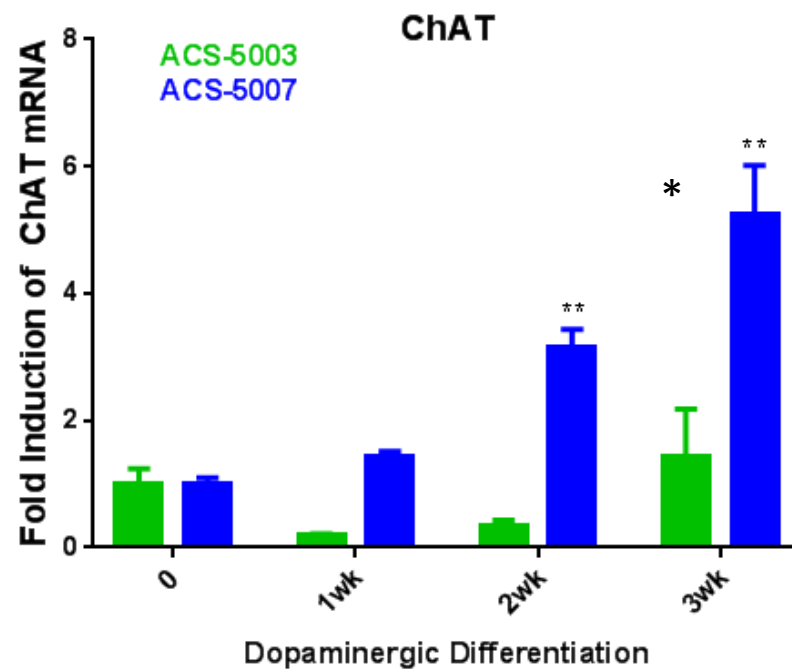
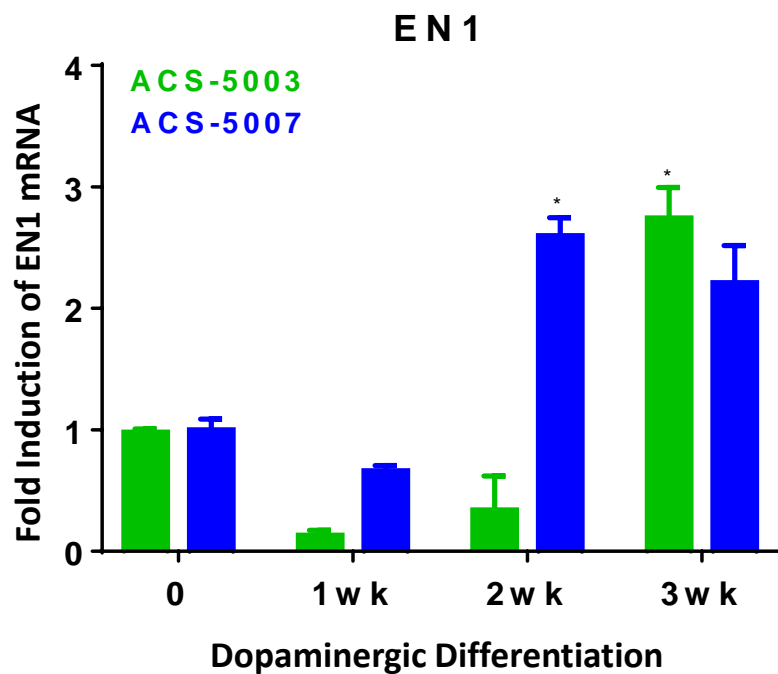


NPC-derived dopaminergic neurons

# Expression of LIM3 and Hb9

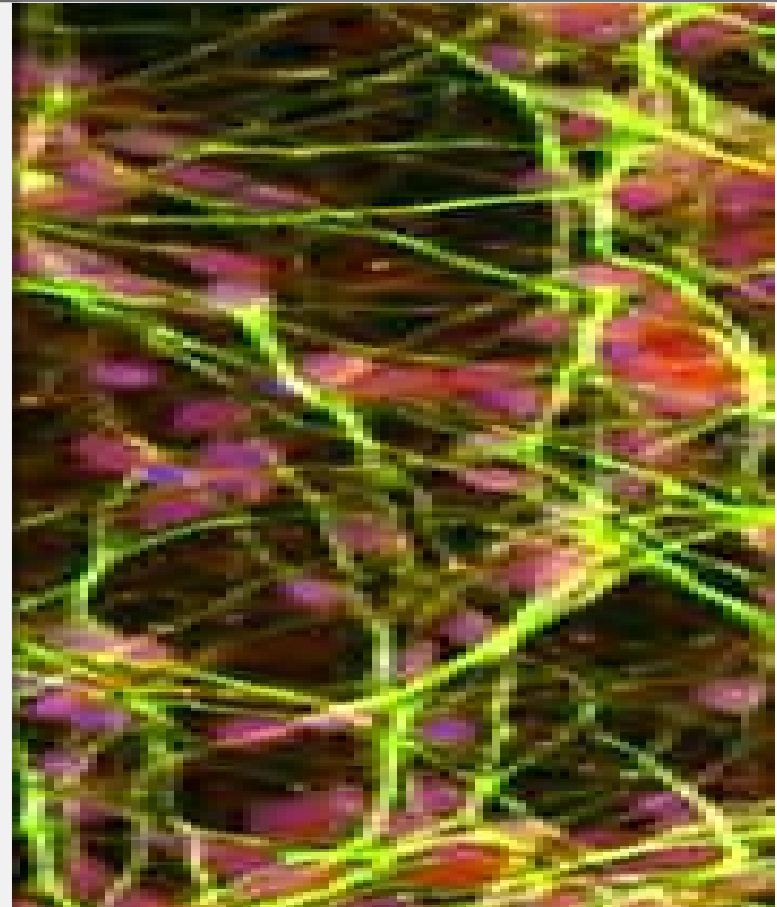


# Expression of EN1 and ChAT



# Protein expression

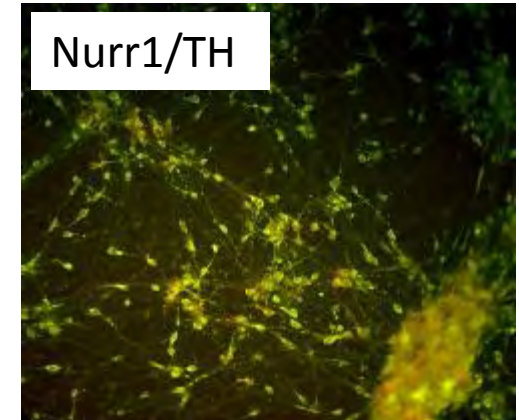
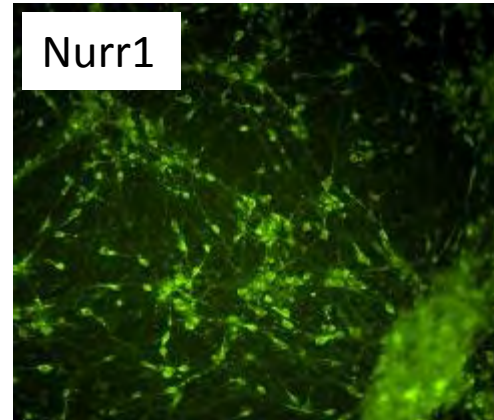
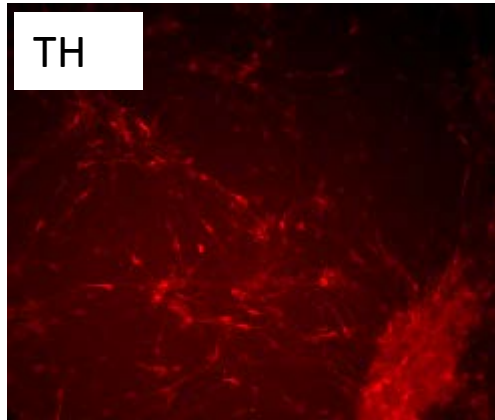
Confirmation of protein expression in **ACS-5003** and **ACS-5007** NPCs during dopaminergic differentiation by ICC



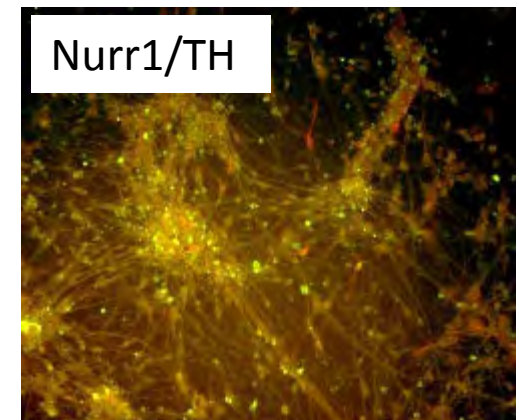
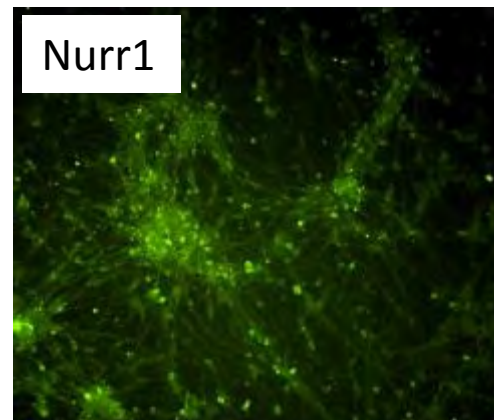
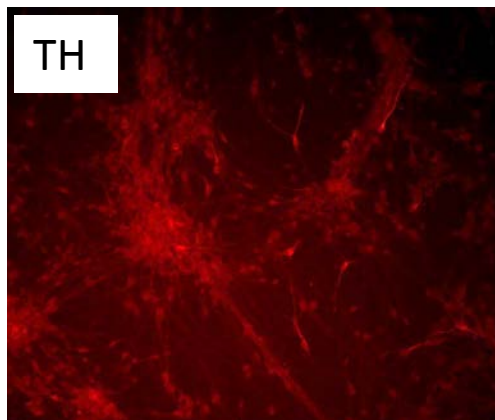
NPC-derived neurons

# Confirmation of dopaminergic neuronal-specific protein expression during differentiation by ICC

ACS-5007

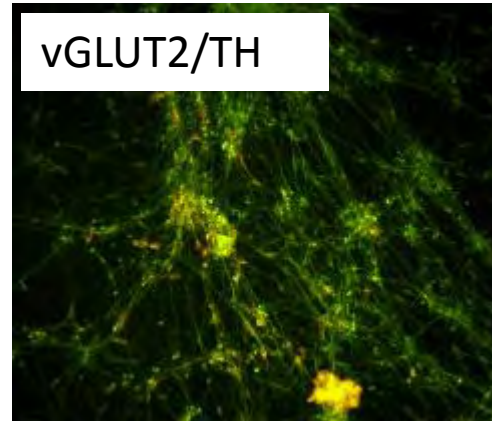
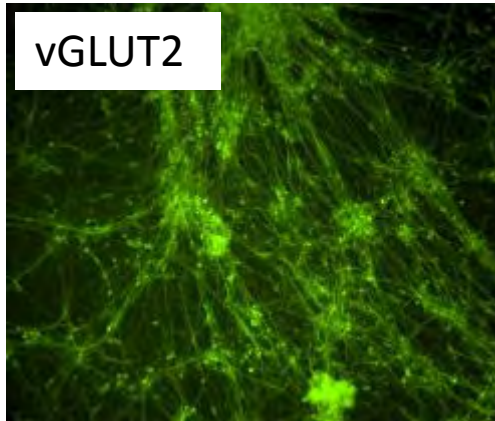


ACS-5003

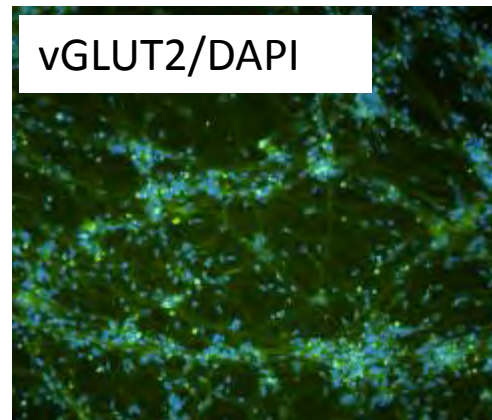
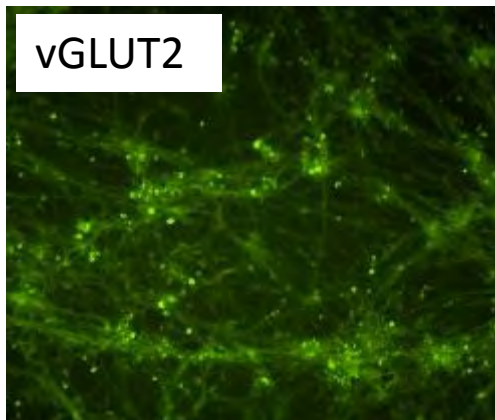


# Confirmation of glutamatergic neuron-specific protein expression during differentiation by ICC

ACS-5007



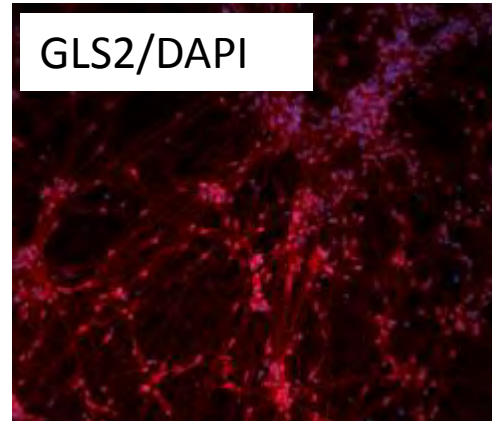
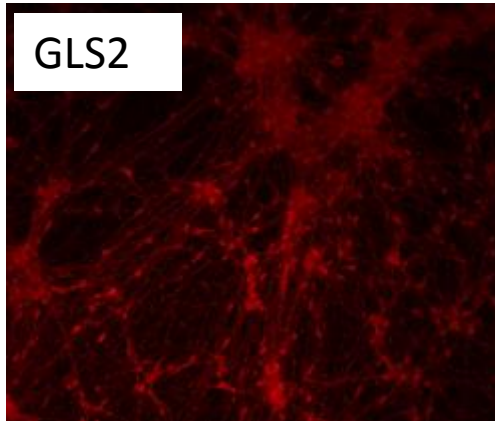
ACS-5003



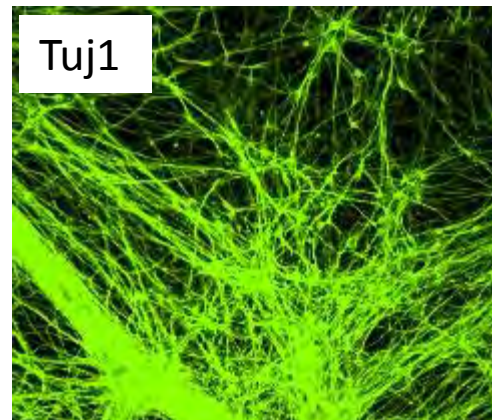
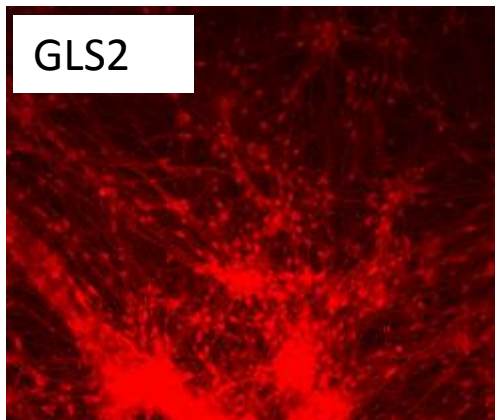


# Confirmation of glutamatergic neuron-specific protein expression during differentiation by ICC

ACS-5007

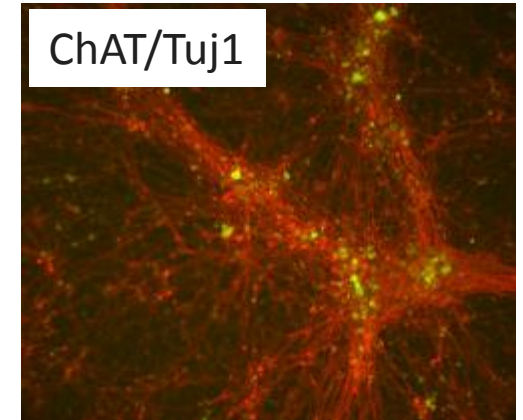
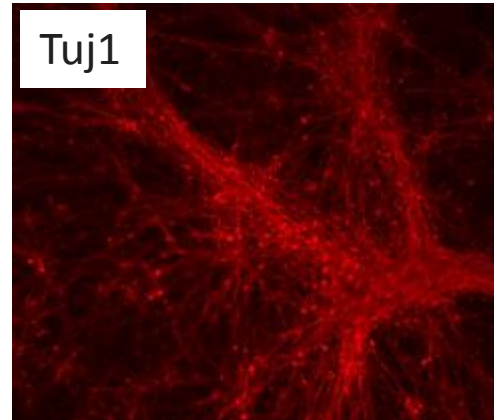
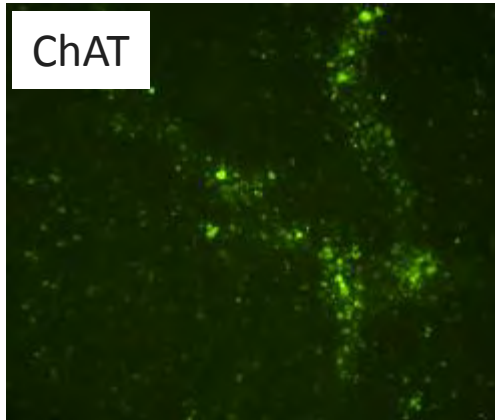


ACS-5003

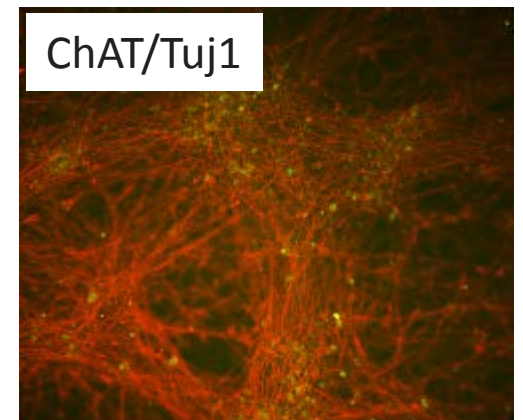
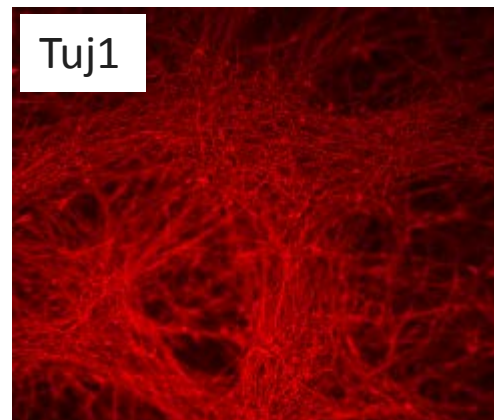
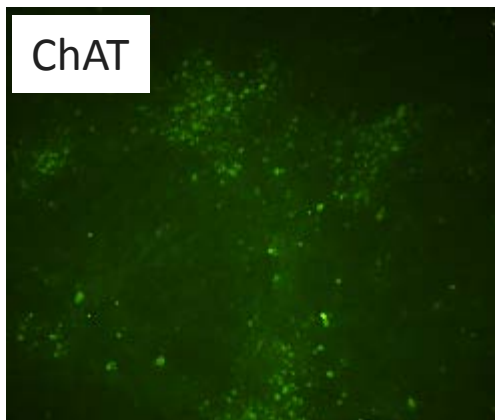


# Confirmation of cholinergic neuron-specific protein expression during differentiation by ICC

ACS-5007



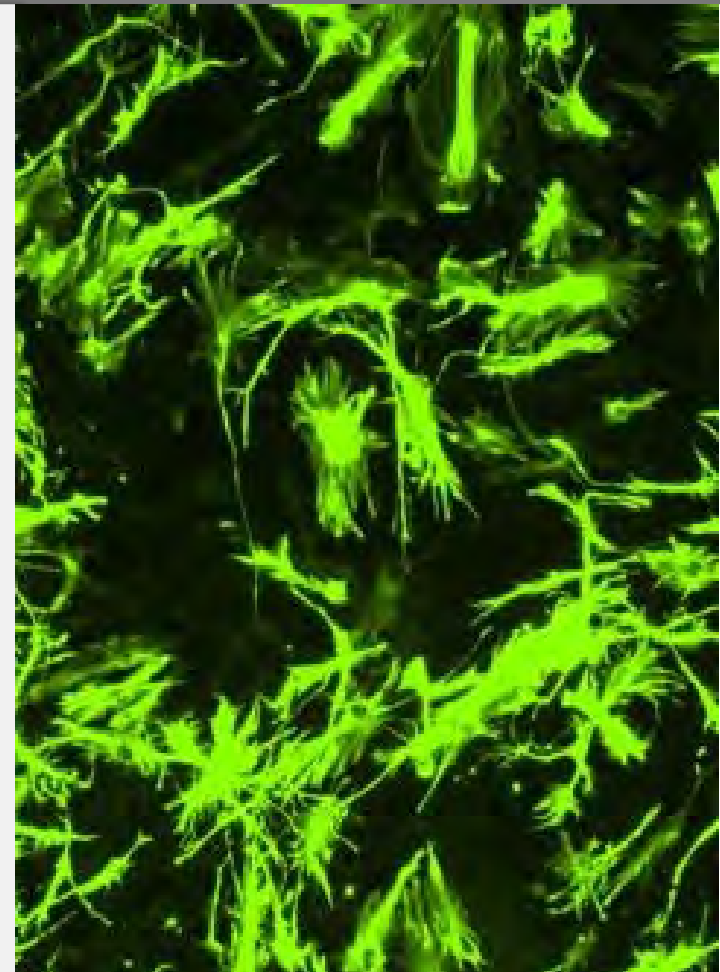
ACS-5003



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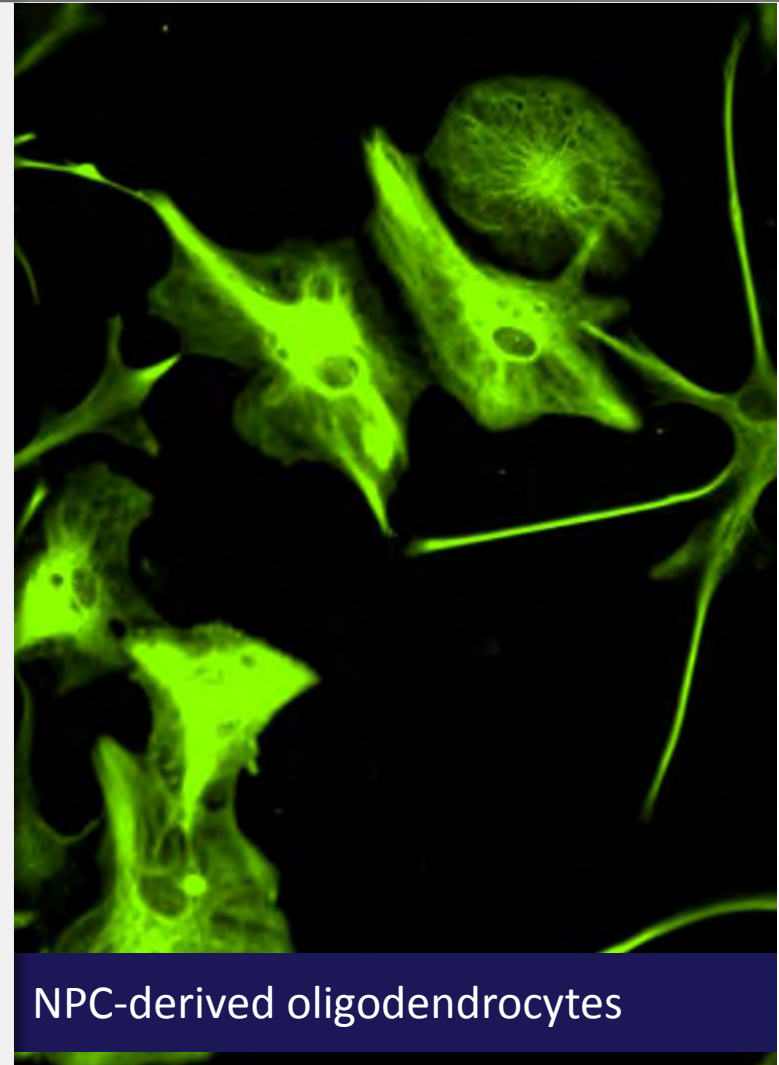


NPC-derived astrocytes

# Neurotoxicity studies

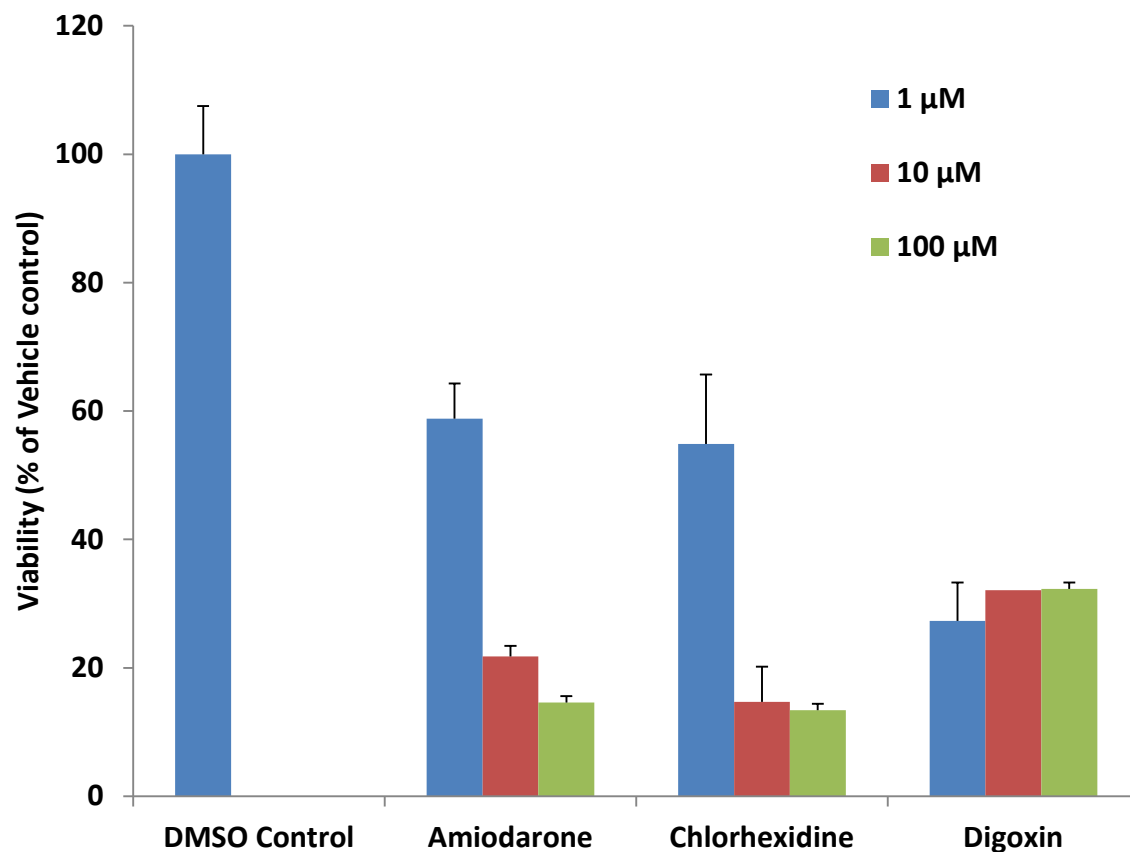
## Neurotoxicity studies with ACS-5003 and ACS-5007 NPCs and NPCs-derived Neurons

- Resazurin viability
- High-content imaging assays



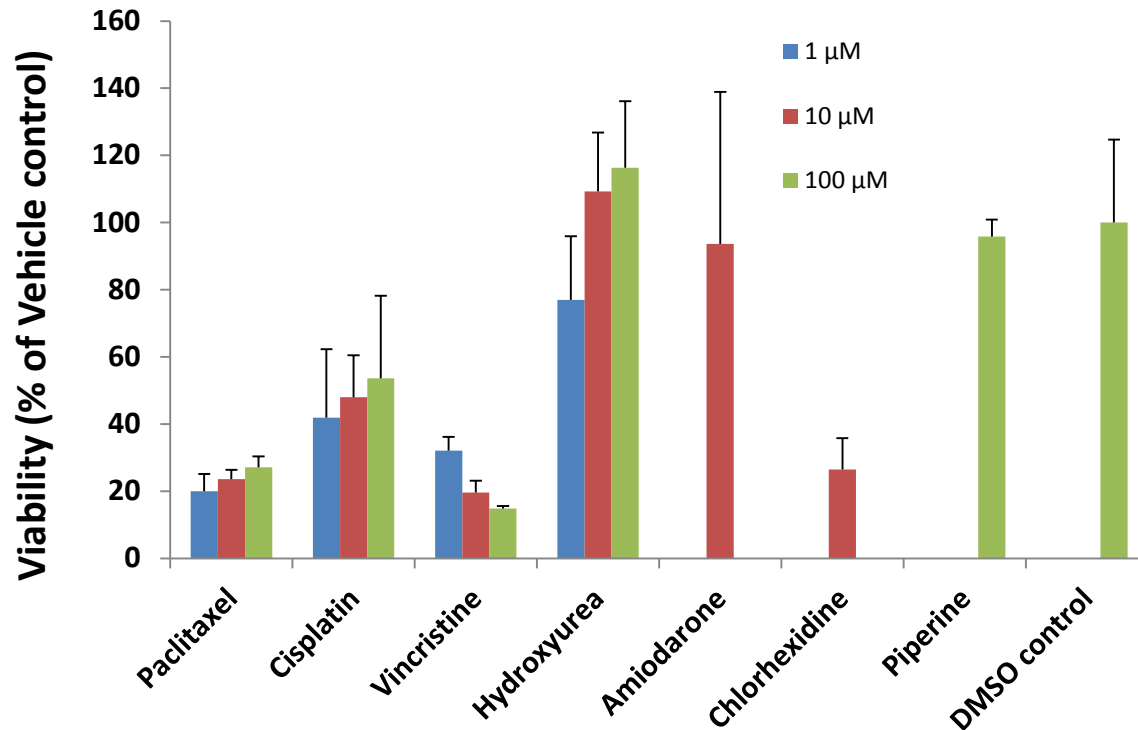
# Neurotoxicity studies – undifferentiated NPCs

Effect of amiodarone, chlorhexidine, and digoxin on cytotoxicity of ACS-5003 NPCs



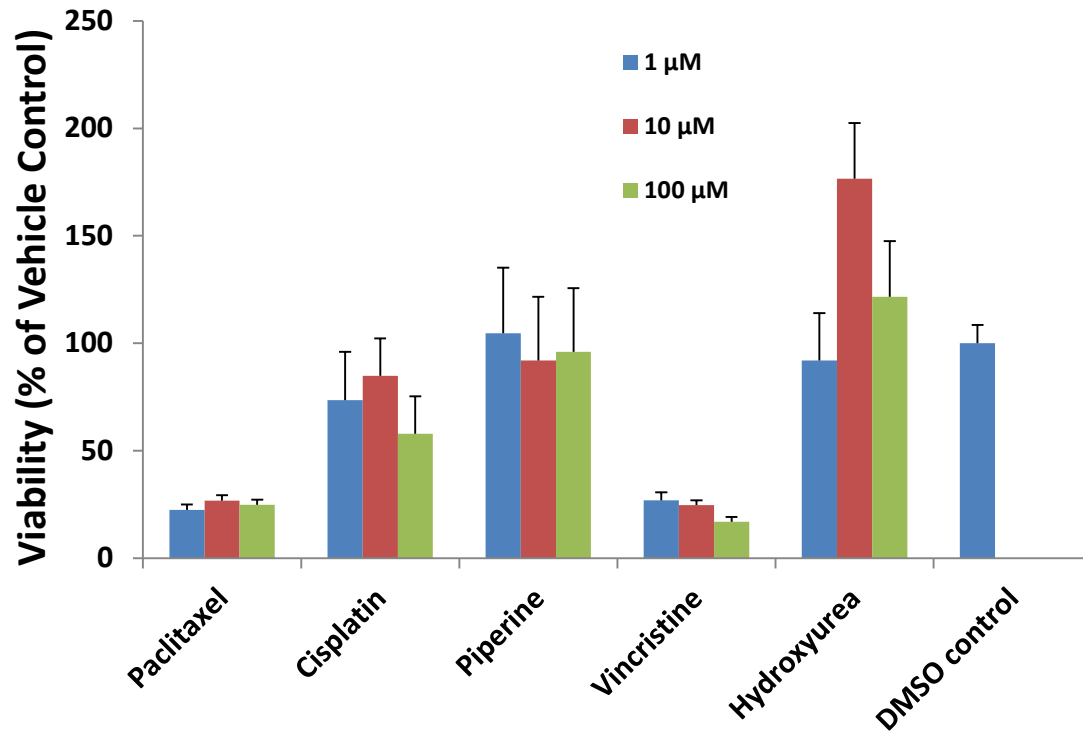
# Neurotoxicity studies – undifferentiated NPCs

Effect of paclitaxel, cisplatin, piperine, vincristine, and hydroxyurea on cytotoxicity of ACS-5003 NPCs, P8



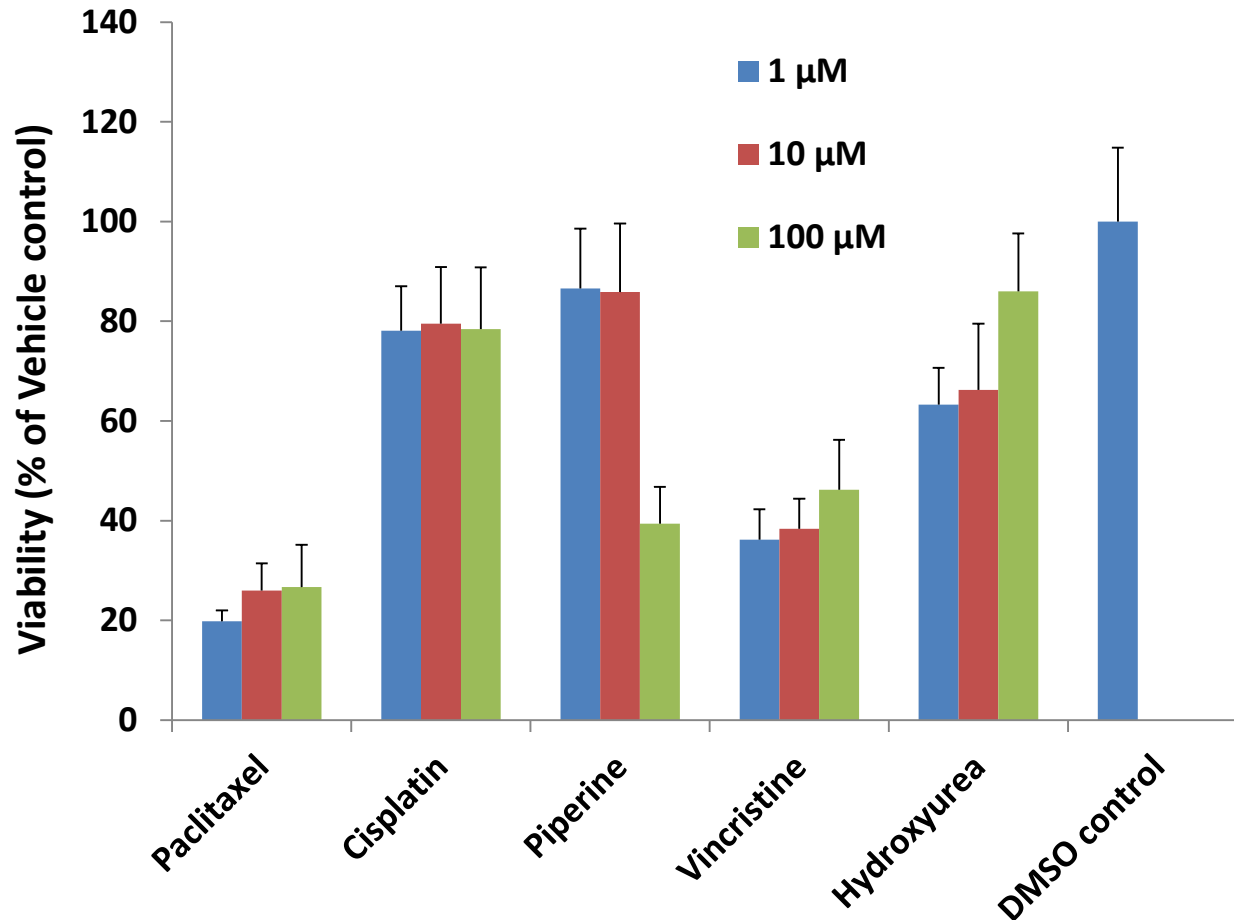
# Neurotoxicity studies – undifferentiated NPCs

Effect of paclitaxel, cisplatin, piperine, vincristine, and hydroxyurea on cytotoxicity of ACS-5003 NPCs, P7



# Neurotoxicity studies – undifferentiated NPCs

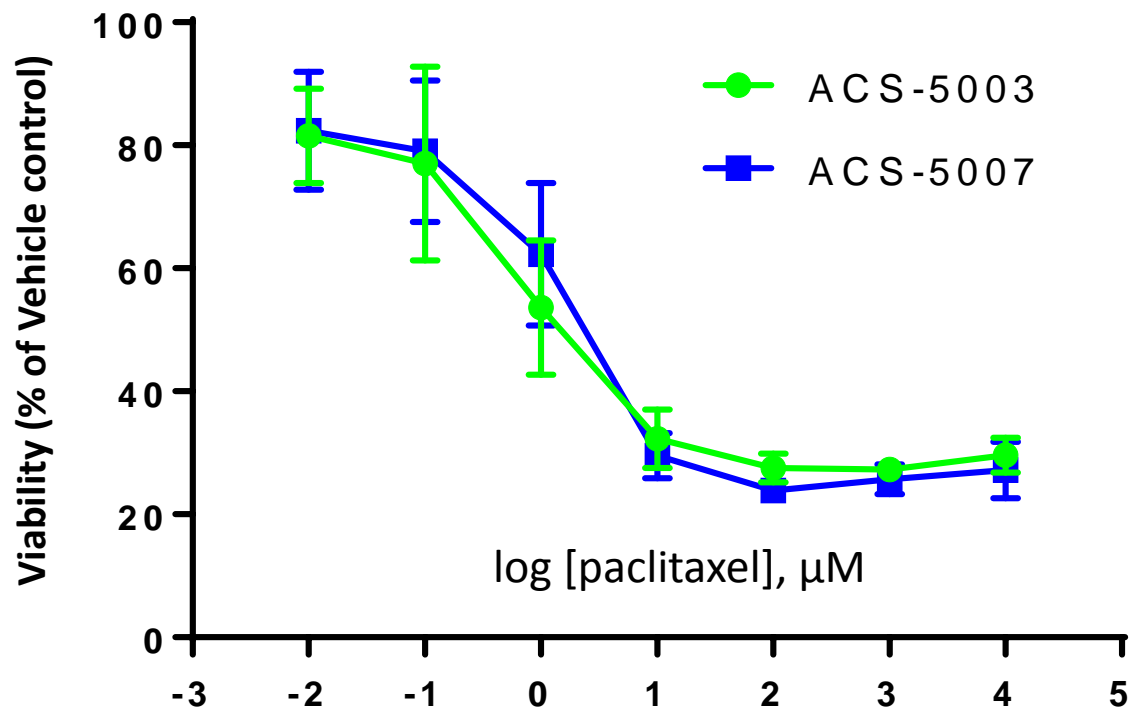
Effect of paclitaxel, cisplatin, piperine, vincristine, and hydroxyurea on cytotoxicity of ACS-5007 NPCs, P10





# Neurotoxicity studies – undifferentiated NPCs

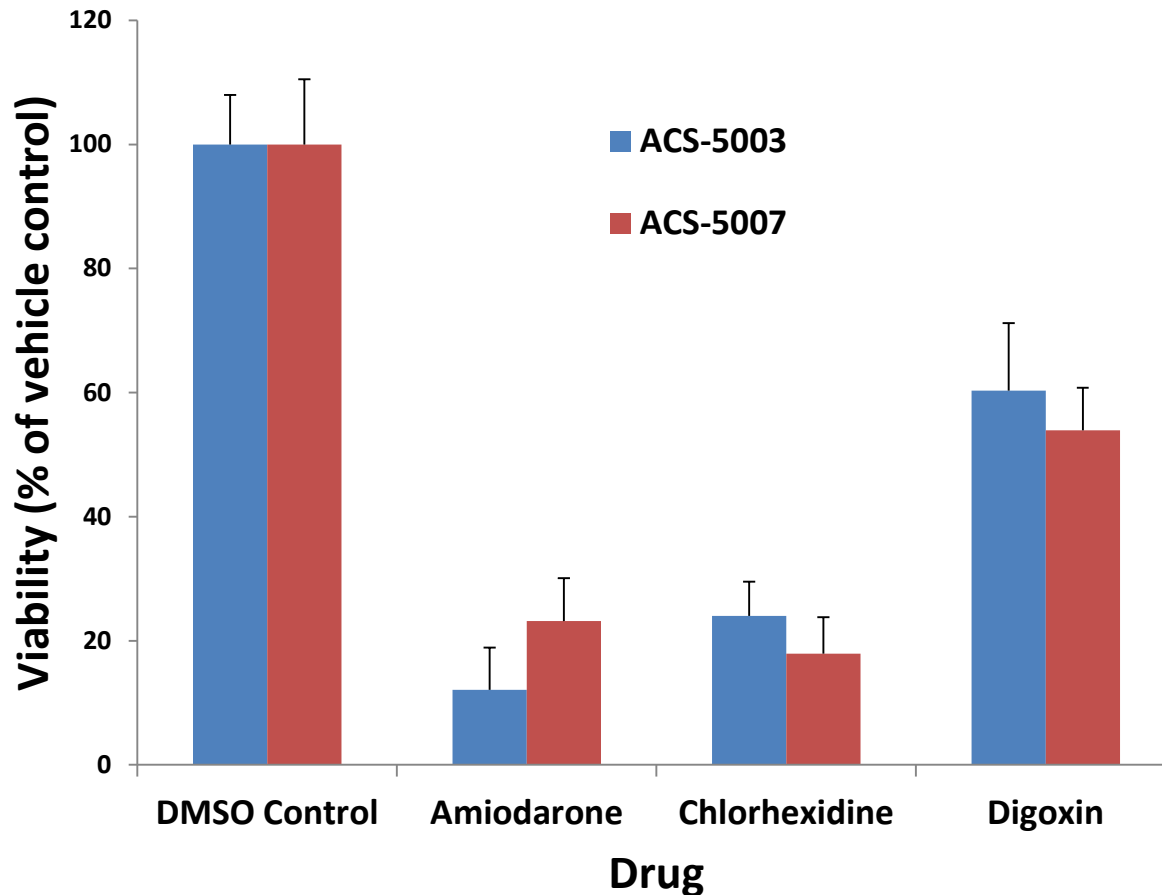
Effect of paclitaxel on cytotoxicity of ACS-5003 (P9) and ACS-5007 (P8) NPCs (n=12)



IC50: ACS-5003=0.9 μM; ACS-5007=1.6 μM

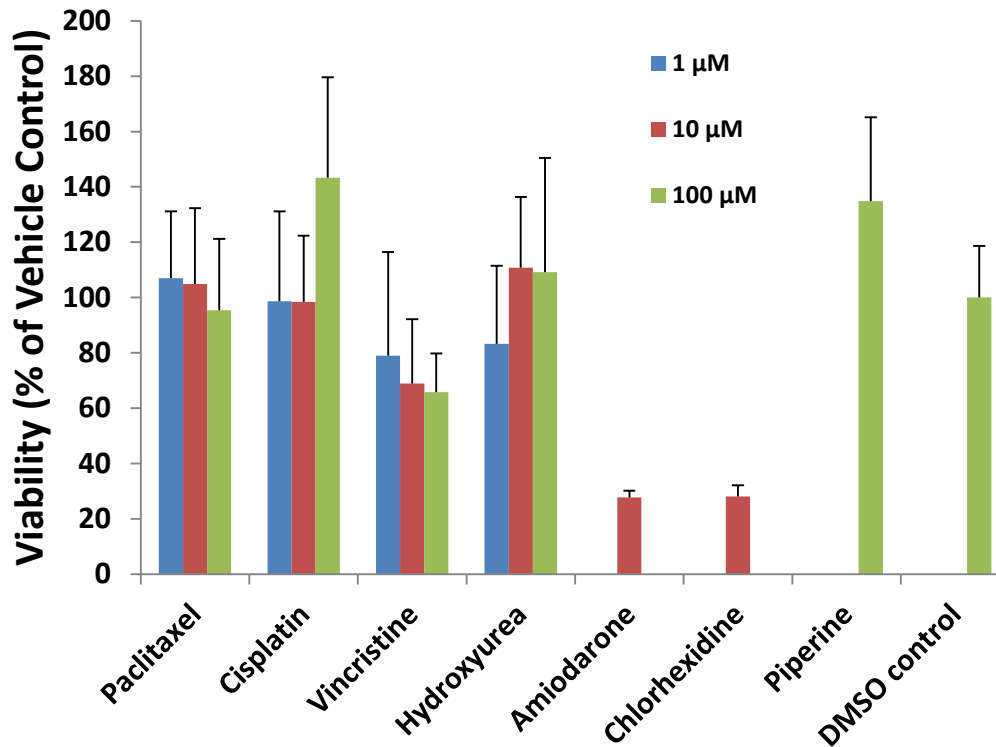
# Neurotoxicity studies – differentiated NPCs

Effect of amiodarone, chlorhexidine, and digoxin on cytotoxicity of ACS-5003 and ACS-5007 NPC-derived neurons



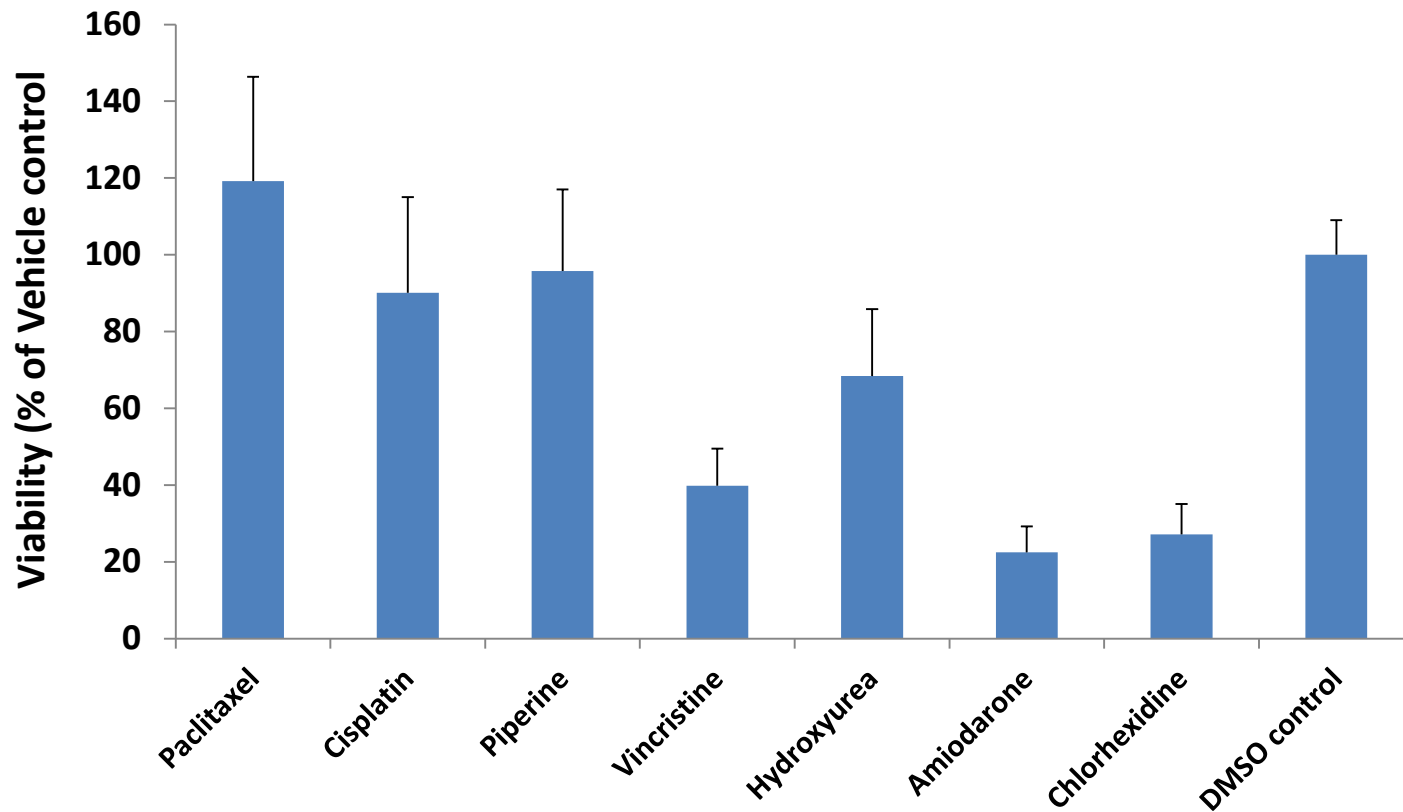
# Neurotoxicity studies – differentiated NPCs

Effect of paclitaxel, cisplatin, piperine, vincristine, and hydroxyurea on cytotoxicity of ACS-5007-derived neurons



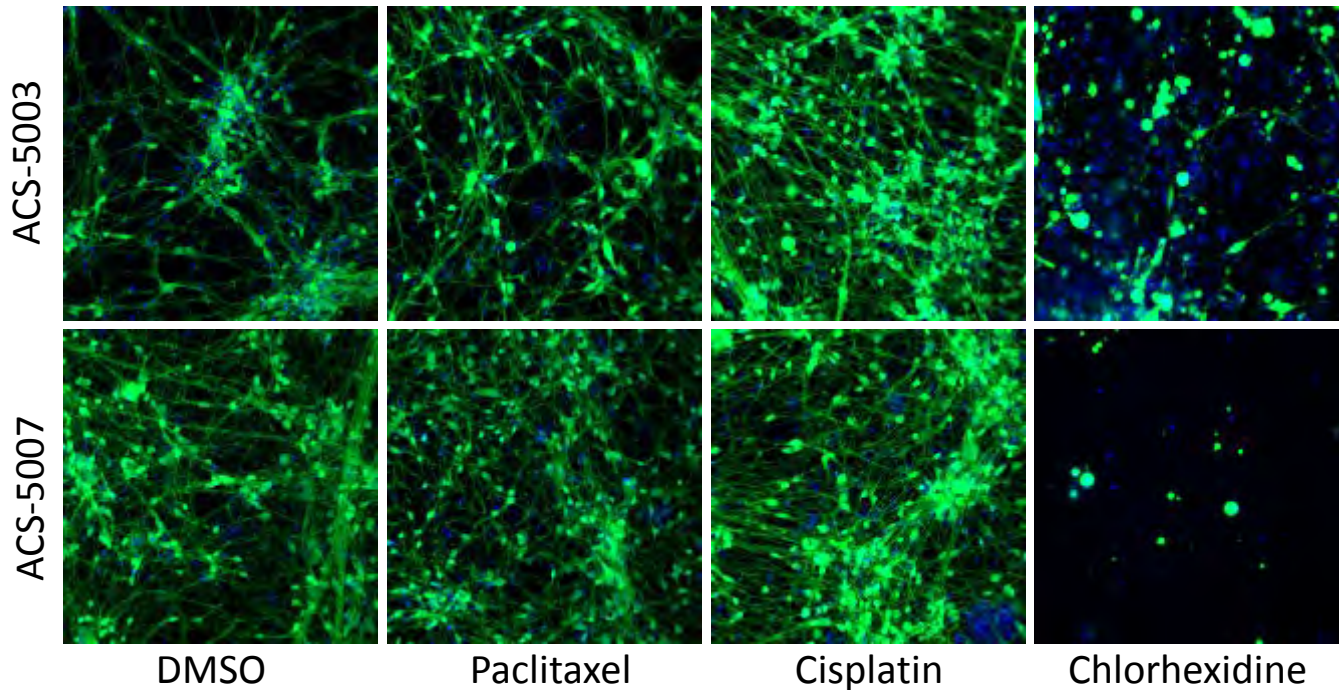
# Neurotoxicity studies – differentiated NPCs

Effect of amiodarone (10  $\mu$ M), chlorhexidine (10  $\mu$ M), paclitaxel (100  $\mu$ M), cisplatin (100  $\mu$ M), piperine (100  $\mu$ M), vincristine (100  $\mu$ M), and hydroxyurea (100  $\mu$ M) on ACS-5007 NPC-derived neurons



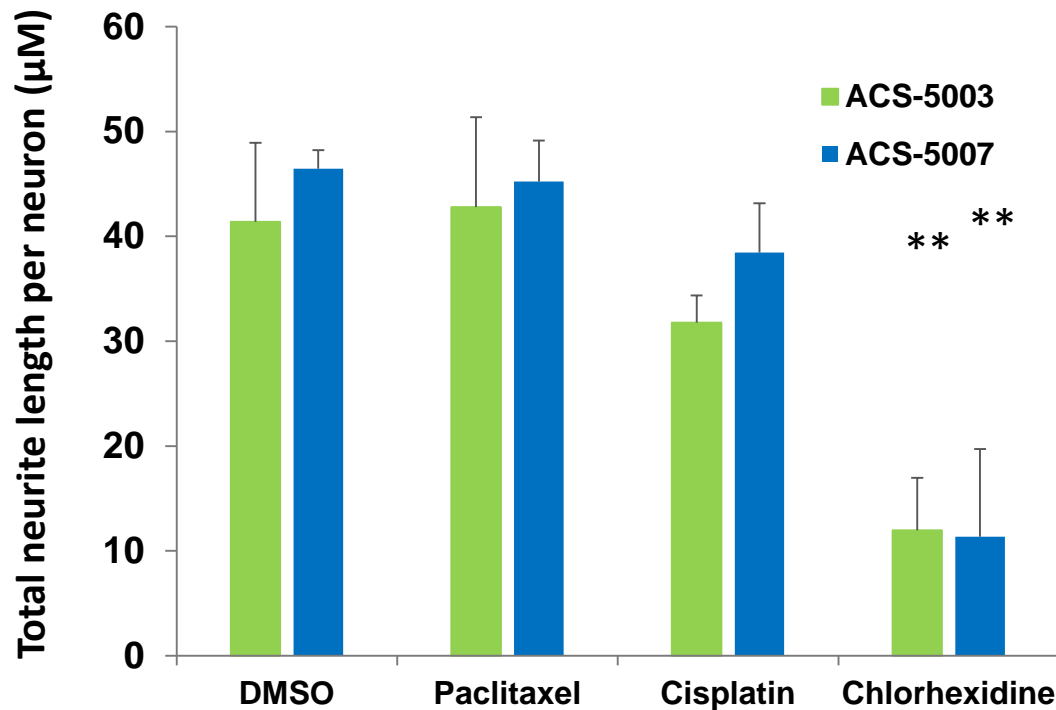
# Neurotoxicity studies – differentiated NPCs

High content imaging analysis of in NPC-derived neurons stained with Calcein Green AM, and Hoechst 33342



# Neurotoxicity studies – differentiated NPCs

High content imaging analysis of 10  $\mu\text{M}$  paclitaxel, cisplatin, and chlorhexidine in ACS-5003 and ACS-5007 NPC-derived neurons by using a CX7 imager



# Neurotoxicity studies – ACS-5003

Toxin	ACS-5003	Neuron derived from ACS-5003
Amiodarone	Toxic	Toxic
Chlorhexidine	Toxic	Toxic
Digoxin	Toxic	Toxic
Cisplatin	Toxic	Resistant
Piperine	Resistant	Not tested
Vincristine	Toxic	Not tested
Hydroxyurea	Toxic	Not tested
Paclitaxel	Toxic	Resistant

# Neurotoxicity studies – ACS-5007

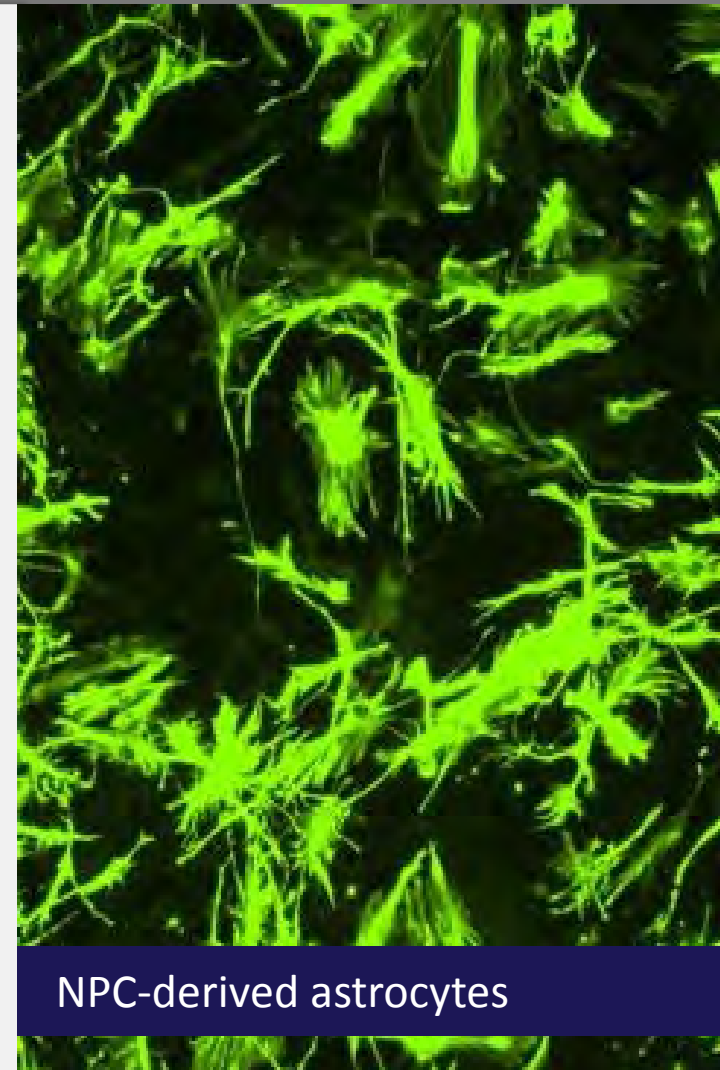
Toxin	ACS-5007	Neuron derived from ACS-5007
Amiodarone	Not tested	Toxic
Chlorhexidine	Not tested	Toxic
Digoxin	Not tested	Toxic
Cisplatin	Toxic	Resistant
Piperine	Resistant	Resistant
Vincristine	Toxic	Toxic
Hydroxyurea	Toxic	Toxic
Paclitaxel	Toxic	Resistant



# Agenda

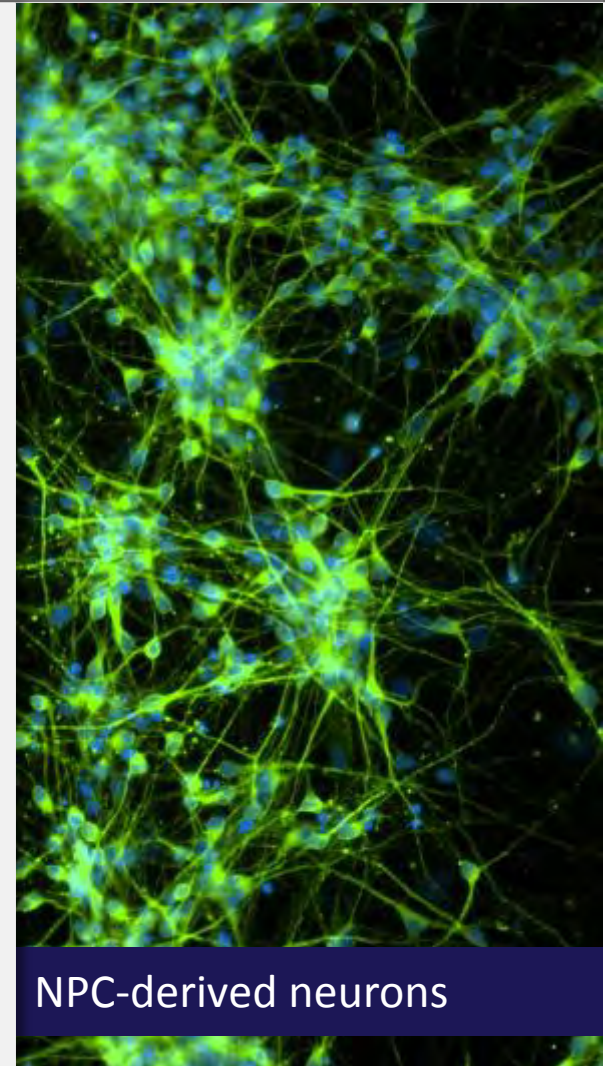
## Neural Progenitor Cells and Media

- Background information
- Differentiation potential of ATCC NPCs
- Toxicological studies
- **Summary**



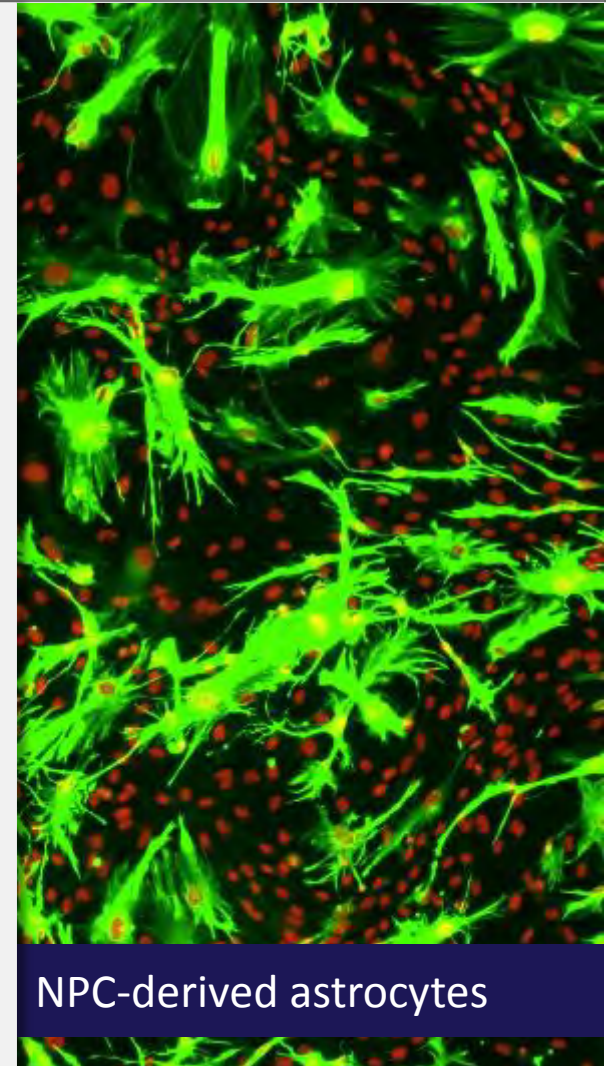
# NPCs – Summary

- Cells and media with easy to use protocols
  - Expansion and Differentiation Medium
- Human model with no donor variation
  - Ability to expand and bank
- Differentiation across a wide spectrum of neural and glial lineages
  - Neurons
  - Astrocytes
  - Oligodendrocytes
- Live imaging of differentiation
  - GFP expression upon neural differentiation



# NPCs – Summary

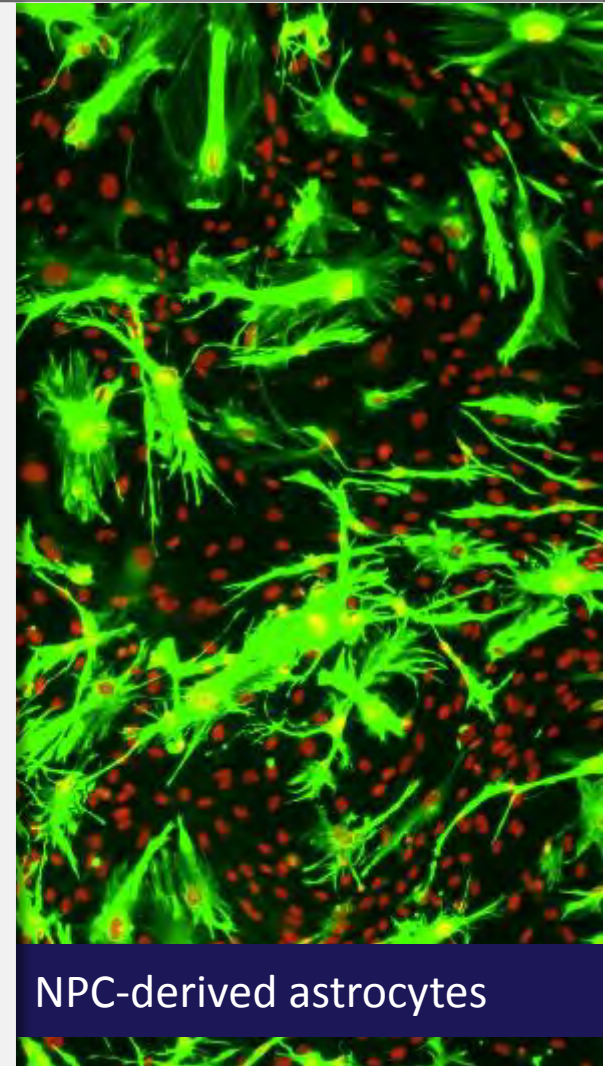
- Our studies demonstrated that ATCC NPCs have the potential to be differentiated into:
  - Dopaminergic neurons
  - GABAergic neurons
  - Glutamatergic neurons
  - Motor neurons
  - Cholinergic neuronsafter treatment of NPCs with ATCC dopaminergic differentiation media
- ATCC NPCs and NPCs-derived neurons are suitable for drug screening applications



NPC-derived astrocytes

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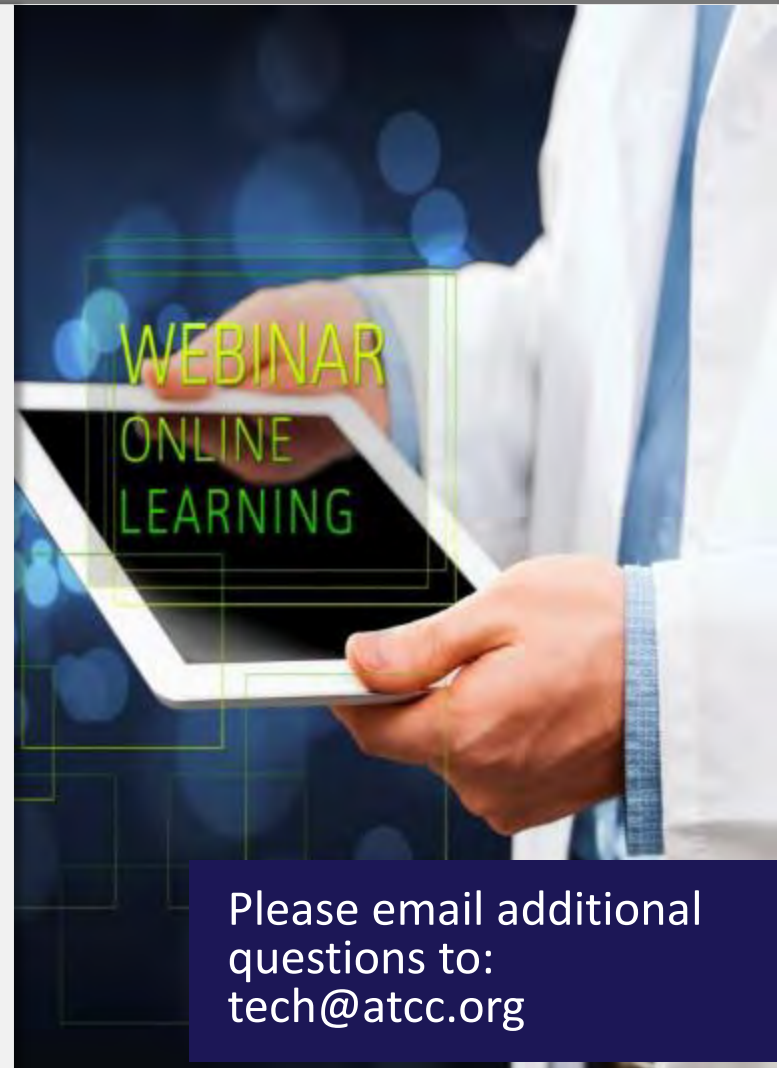


NPC-derived astrocytes

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- **August 4, 2016**  
**12:00 PM EST**  
Kevin Grady, *Product Line Business Manager*, ATCC  
*Get Ready for a Better Angiogenesis Model*
- **August 18, 2016**  
**12:00 PM EST**  
Cara Wilder, Ph.D., *Technical Writer*, ATCC  
*Improving the Detection of Shiga Toxin-producing Escherichia coli*



Please email additional questions to:  
[tech@atcc.org](mailto:tech@atcc.org)