Advanced models of Parkinson's disease

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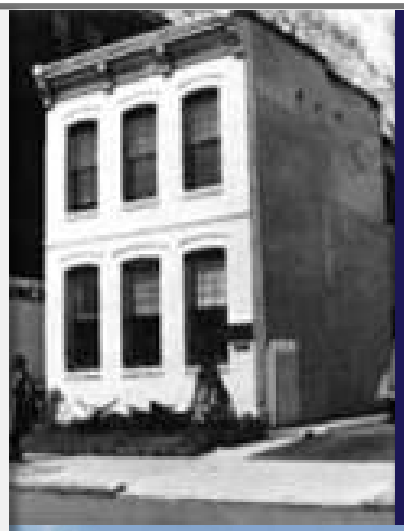
ATCC

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

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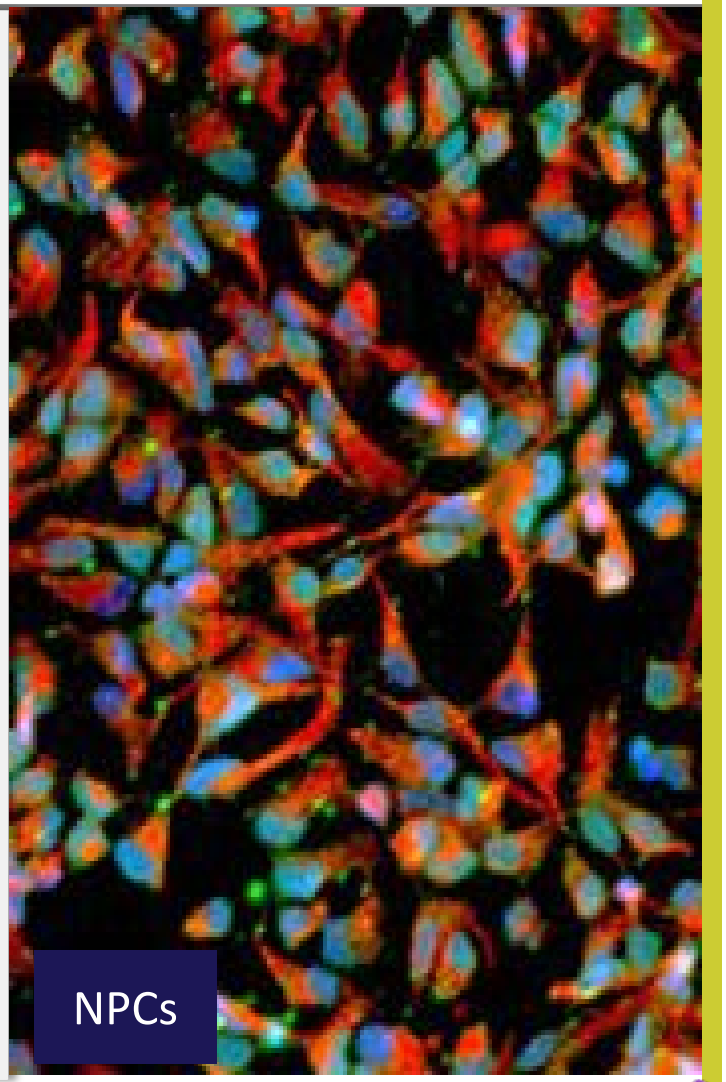
Agenda

Neural Progenitor Cells (NPCs) and Media

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



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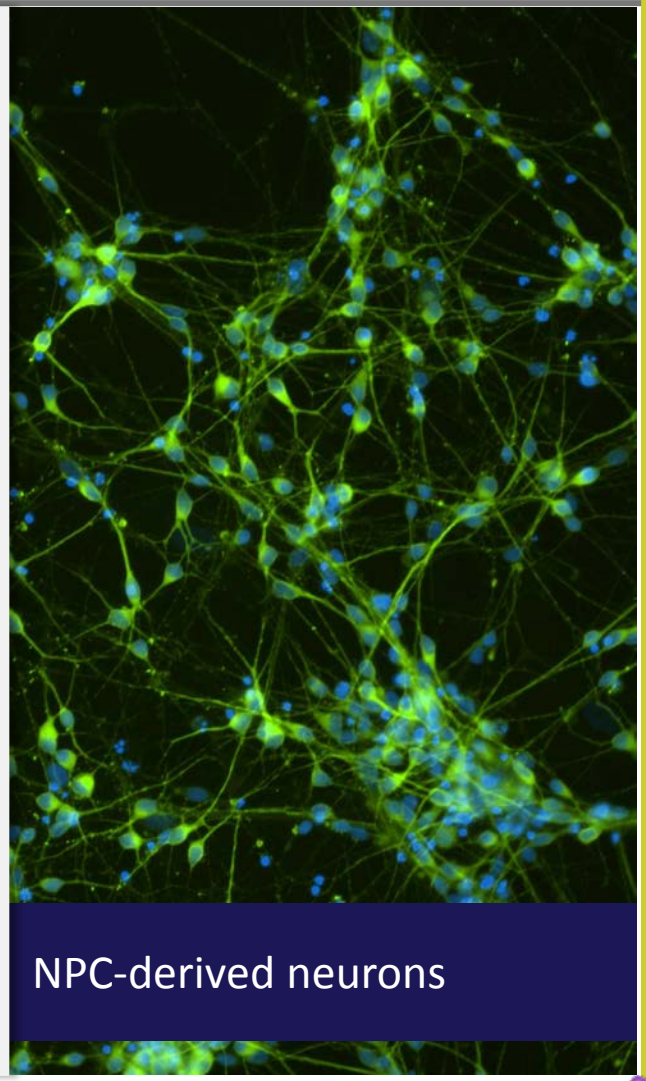
The current status of neurobiology research

- 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



NPC-derived neurons

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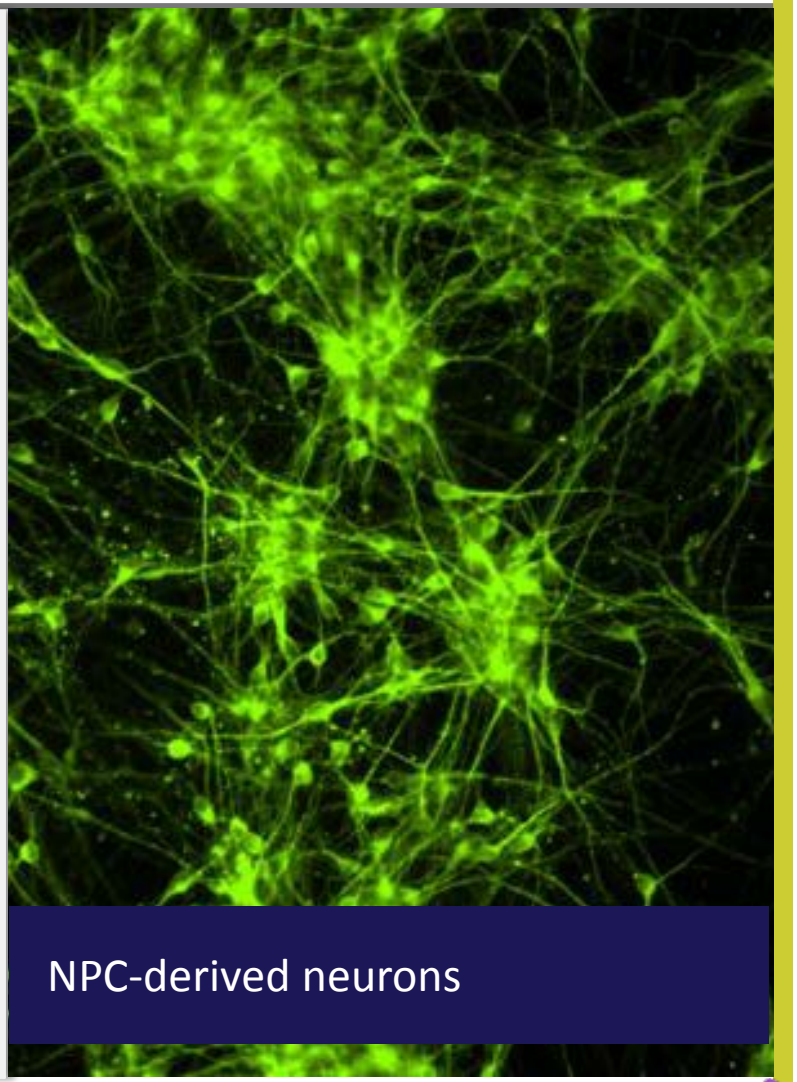
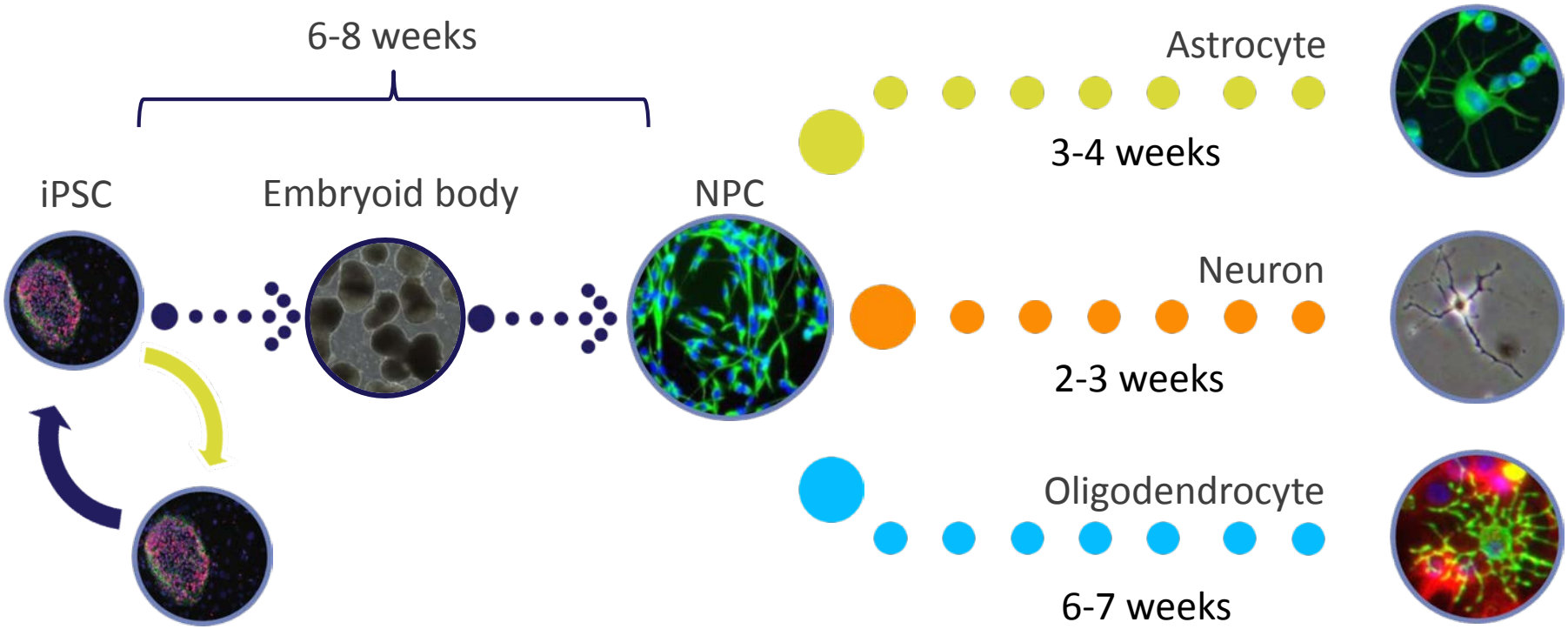
What is needed?

- Biologically relevant models
- A true disease model
- Validated neural functioning
- Predictive for screening applications



NPC-derived neurons

Neural progenitor cells (NPCs) - Neuronal differentiation



NPCs:
- Shorten research time
- Human model
- Predictive screening

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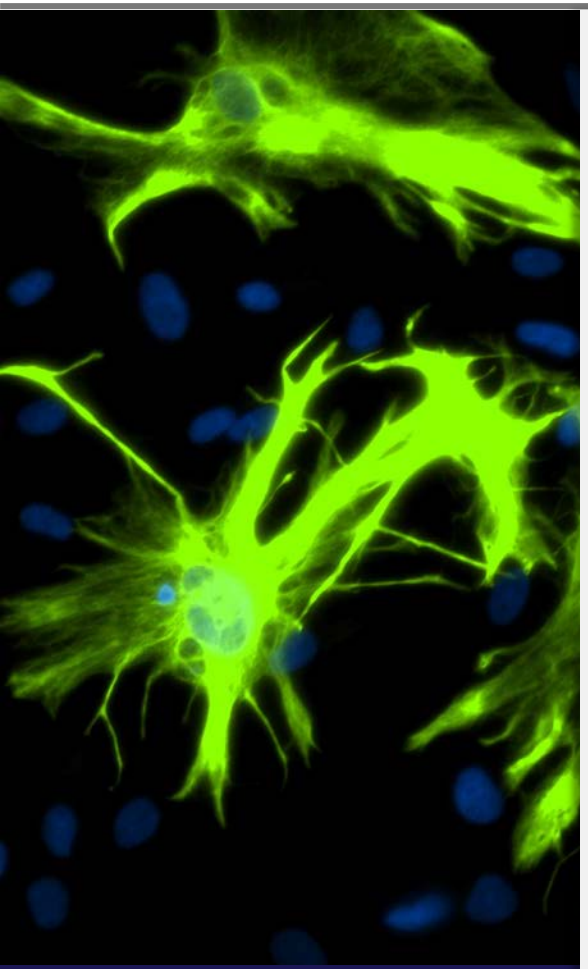
Neural Progenitor Cells (NPCs) from ATCC



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

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ATCC® NPC offerings

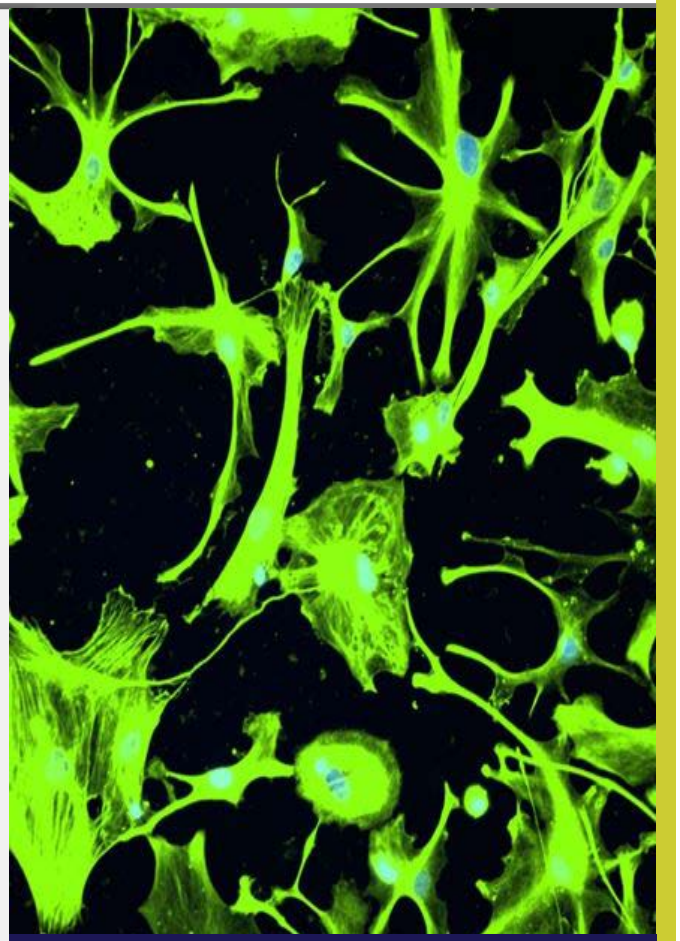
ATCC® No.	Designation
ACS-3003	NPC Growth Kit – ***add to DMEM/F12***
ACS-3004	NPC Dopaminergic Differentiation Kit – ***add to DMEM/F12***
ACS-5001	NPCs derived from ATCC-DYS0530 Parkinson's Disease (ACS-1013) ***New!***
ACS-5003	NPCs derived from ATCC-BXS0117 (ACS-1031)
ACS-5004	NPCs derived from ATCC-BYS0112 (ACS-1026)
ACS-5005	Neural Progenitor Cells derived from XCL-1 DCX-GFP ***(for late neuron differentiation)***
ACS-5006	Neural Progenitor Cells derived from XCL-1 GFAP-Nanoluc®-Halotag® ***(for astrocyte differentiation)***
ACS-5007	Neural Progenitor Cells derived from XCL-1 MAP2-Nanoluc®-Halotag® ***(for early neuron differentiation)***
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® NPCs

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

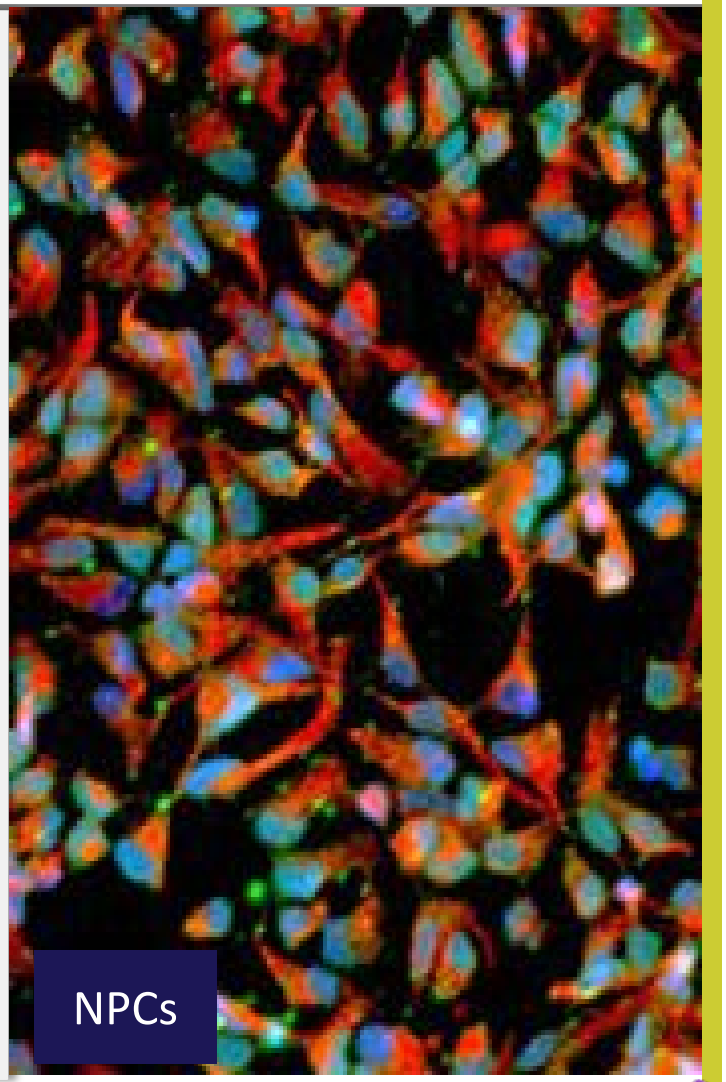


NPC-derived oligodentrocytes

Agenda

NPCs and Media

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



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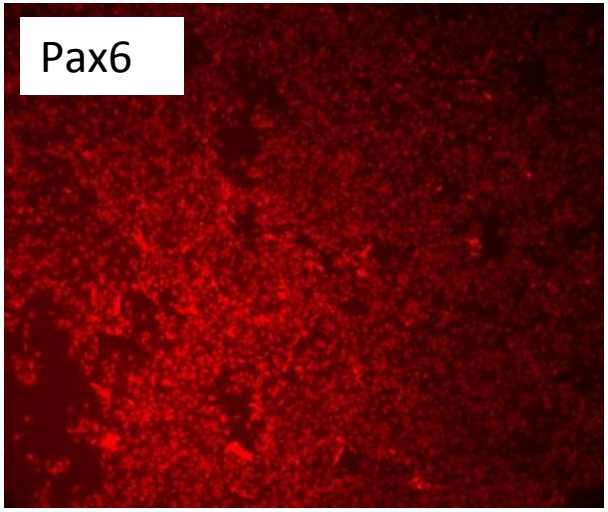
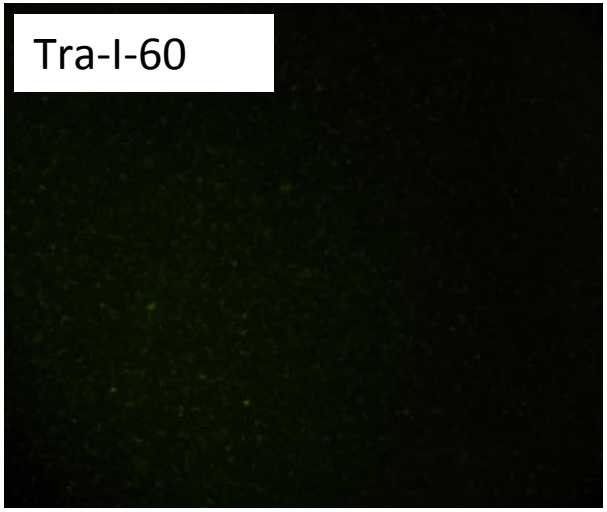
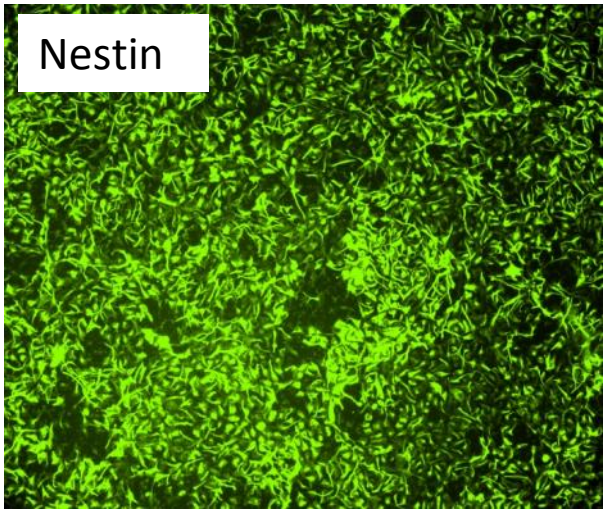
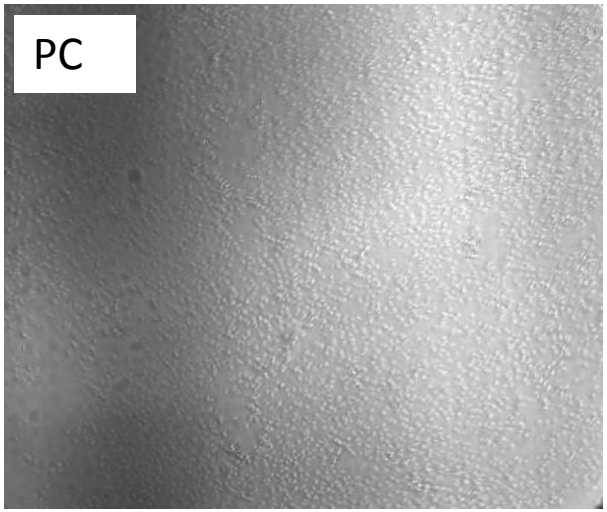
ATCC normal NPCs express NPC markers but **not** iPSC markers







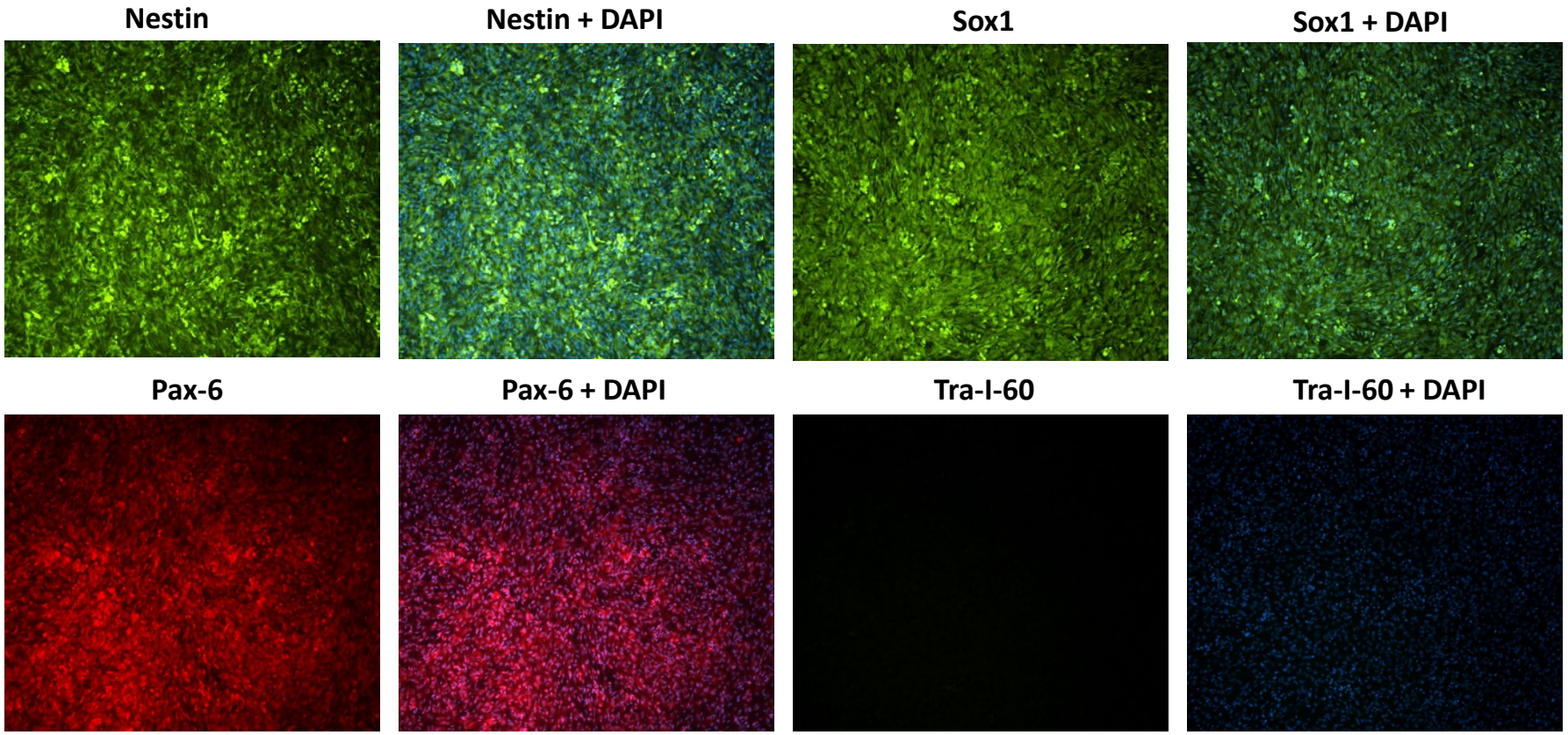


NPC Marker
- Nestin
- Pax-6

iPSC Marker
- Tra-I-60

ATCC

ATCC Parkinson's disease NPCs express NPC markers but **not** iPSC markers



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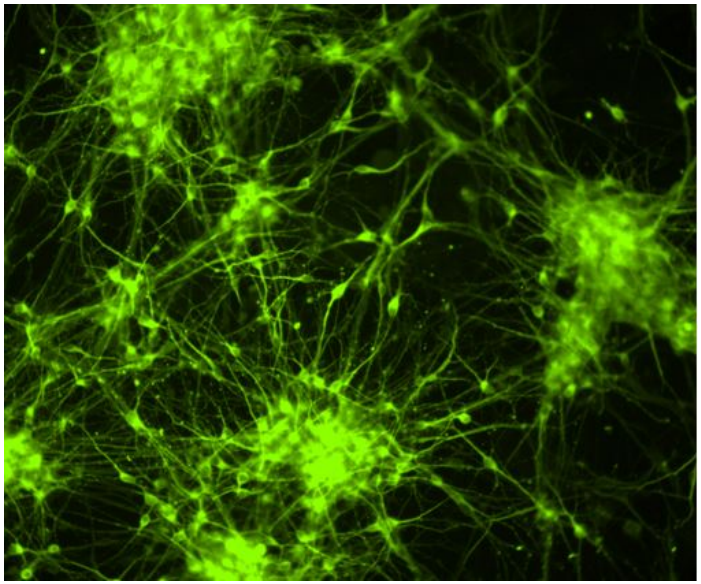
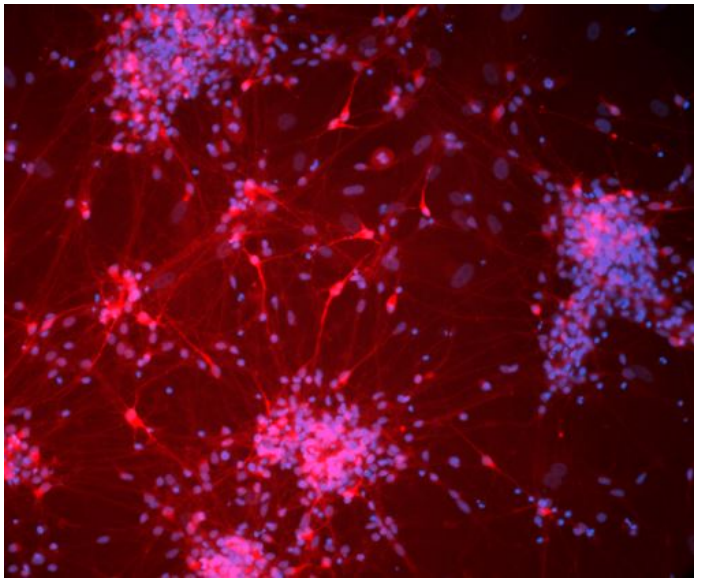
Dopaminergic neuron differentiation of NPCs



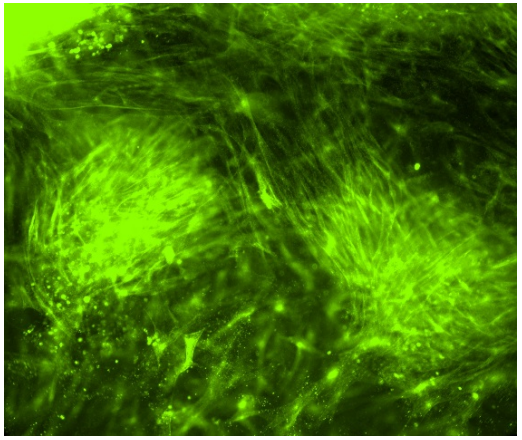
Tuj1



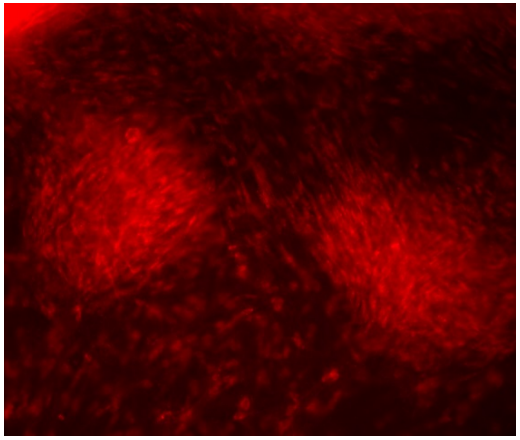
TH/DAPI

Dopaminergic neuron differentiation of Parkinson's disease NPCs

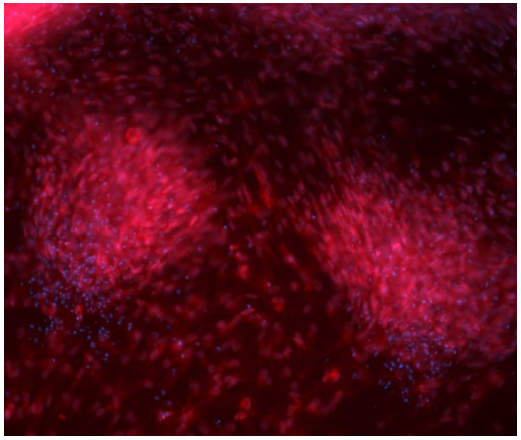
Tuj 1



TH



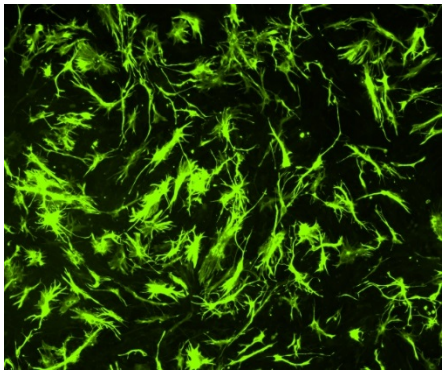
TH + DAPI

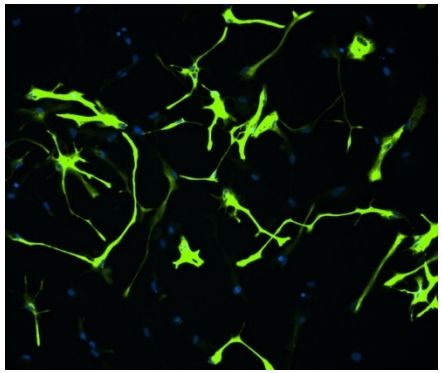


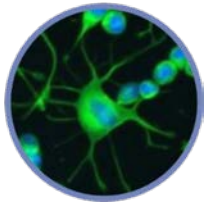
Astrocyte and oligodendrocyte differentiation

Astrocyte differentiation

GFAP

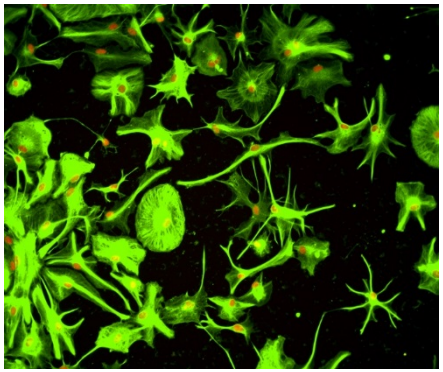


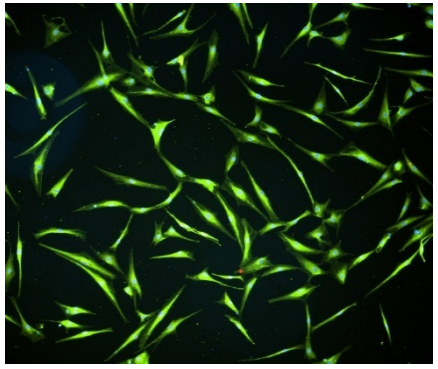


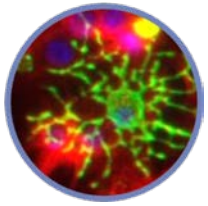


Oligodendrocyte differentiation

O4







ACS-5003

ACS-5001

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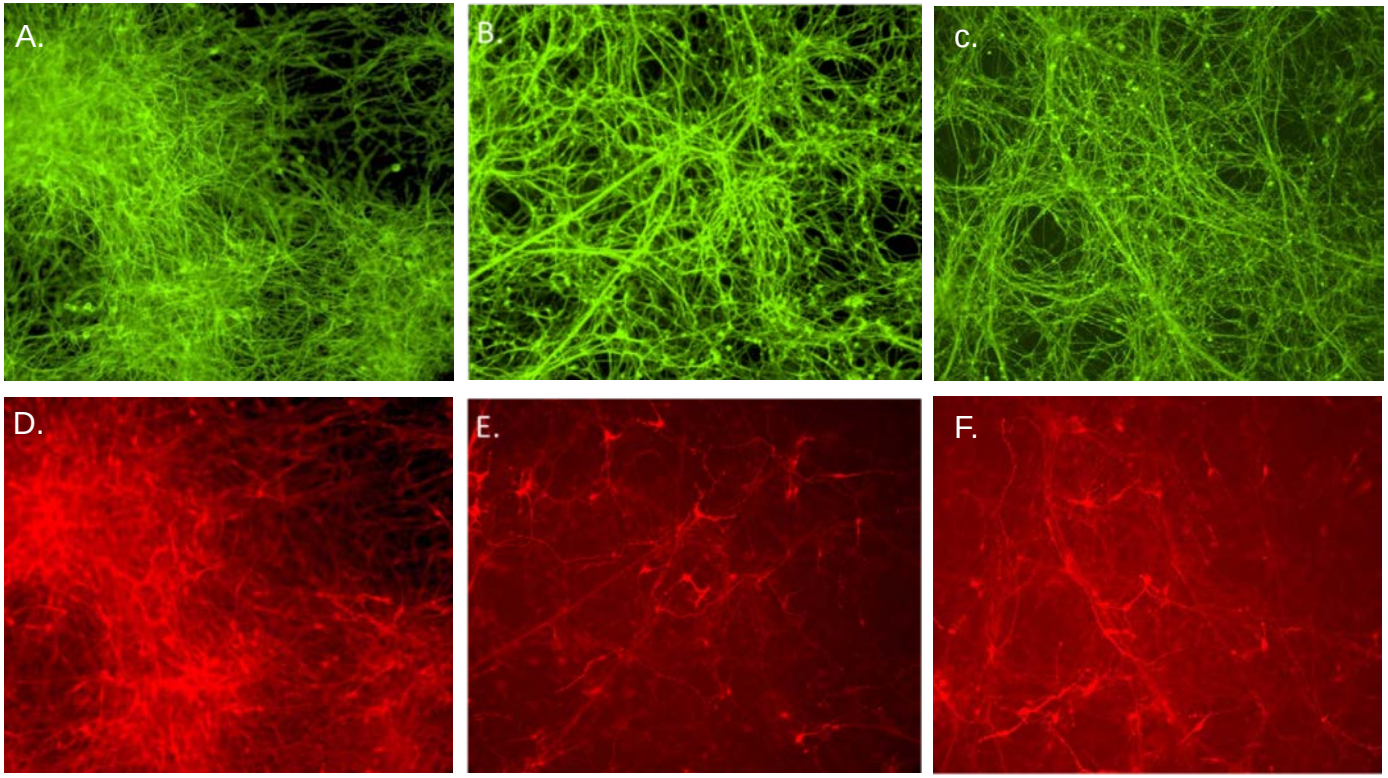
Dopaminergic neuron differentiation of NPC reporter lines



MAP2- NanoLuc®-HaloTag® (ACS-5007)

DCX-GFP (ACS-5005)

GFAP-NanoLuc®-HaloTag® (ACS-5006)

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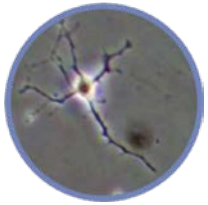
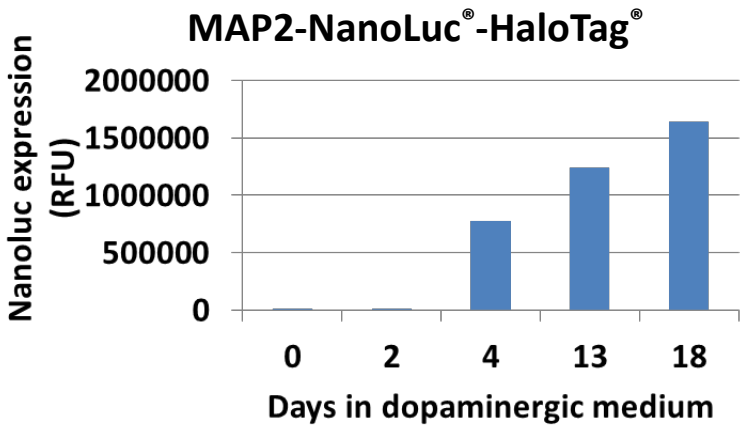
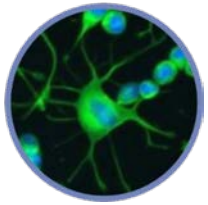
Expression of the luciferase reporter during dopaminergic neuron or astrocyte differentiation

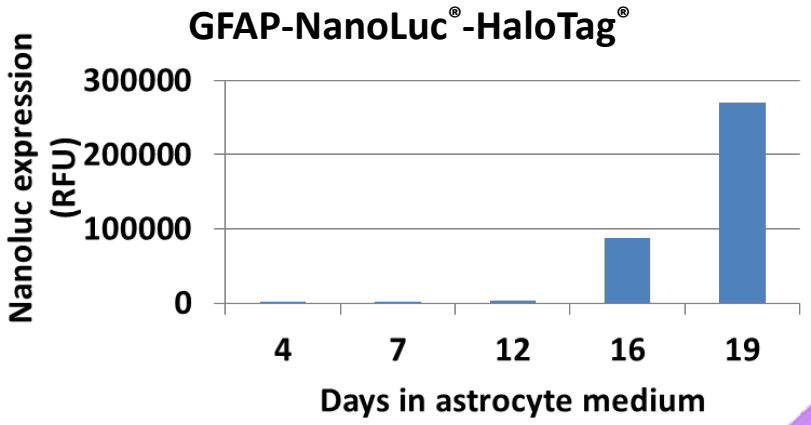
Luciferase secretion during dopaminergic neuron differentiation of NanoLuc®-HaloTag® NPCs





Luciferase secretion during astrocyte differentiation of GFAP-NanoLuc®-HaloTag® NPCs





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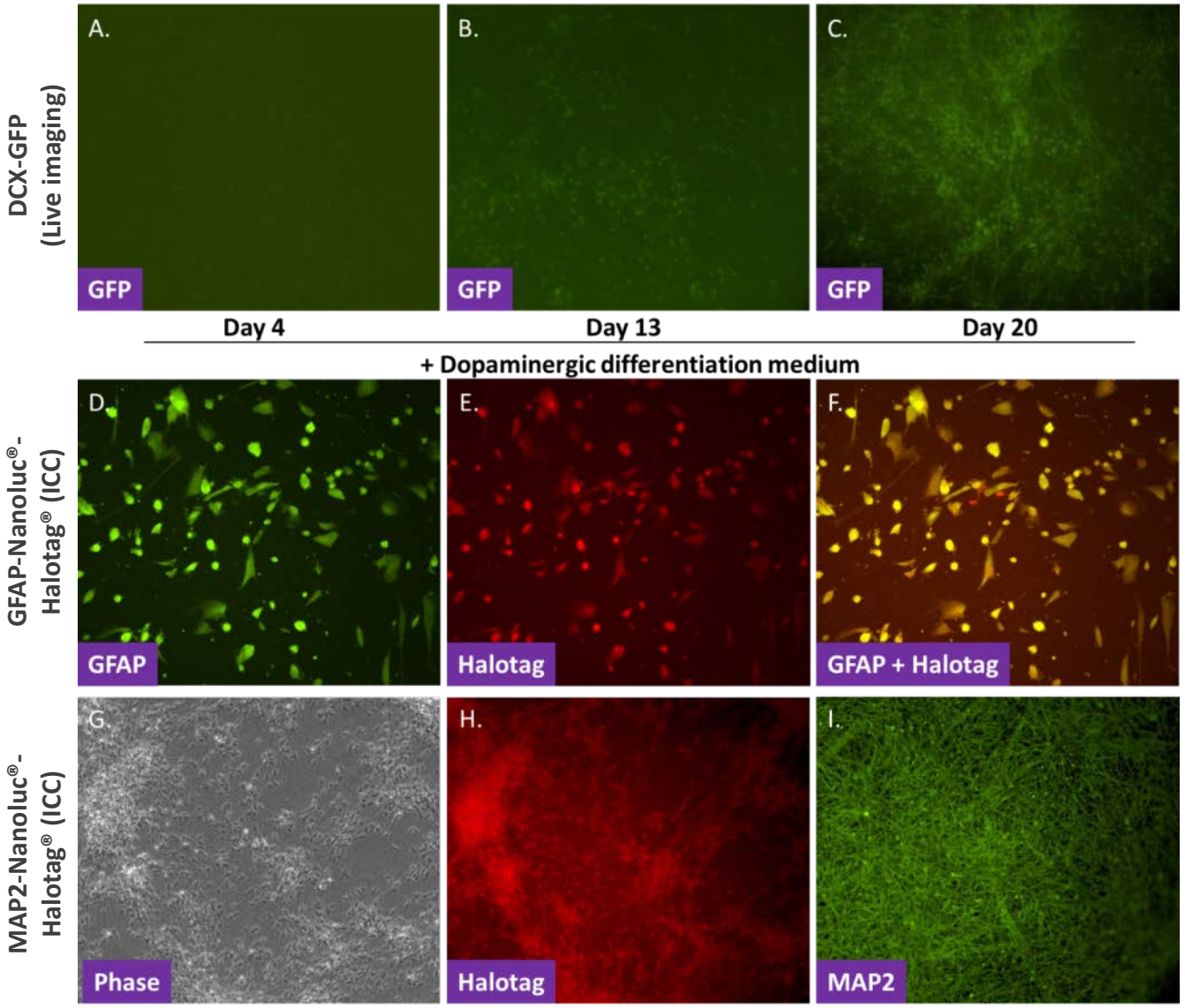
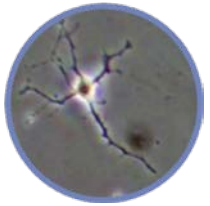
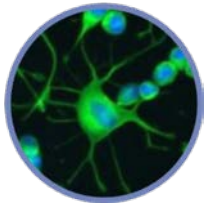
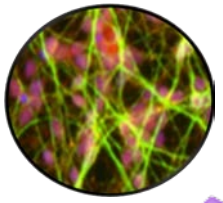
Expression of the GFP or HaloTag® reporter during dopaminergic neuron or astrocyte differentiation









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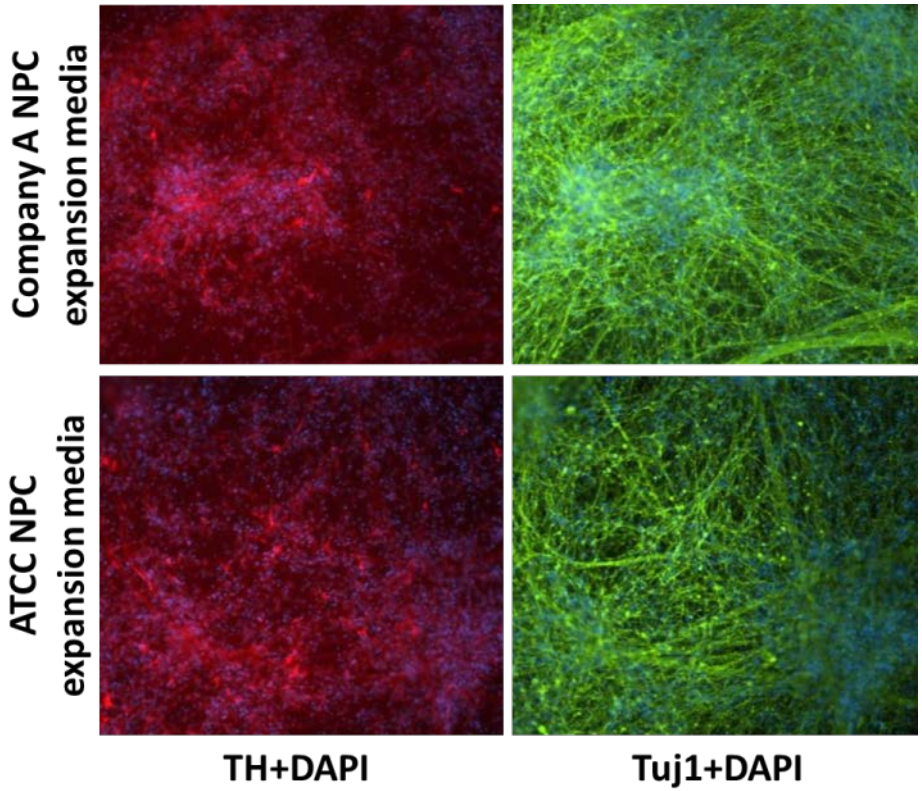
Development of ATCC's NPC expansion and dopaminergic differentiation media

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® primers were used to identify the presence of other types of neurons during dopaminergic neuron differentiation using ATCC® ACS-3004™ 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

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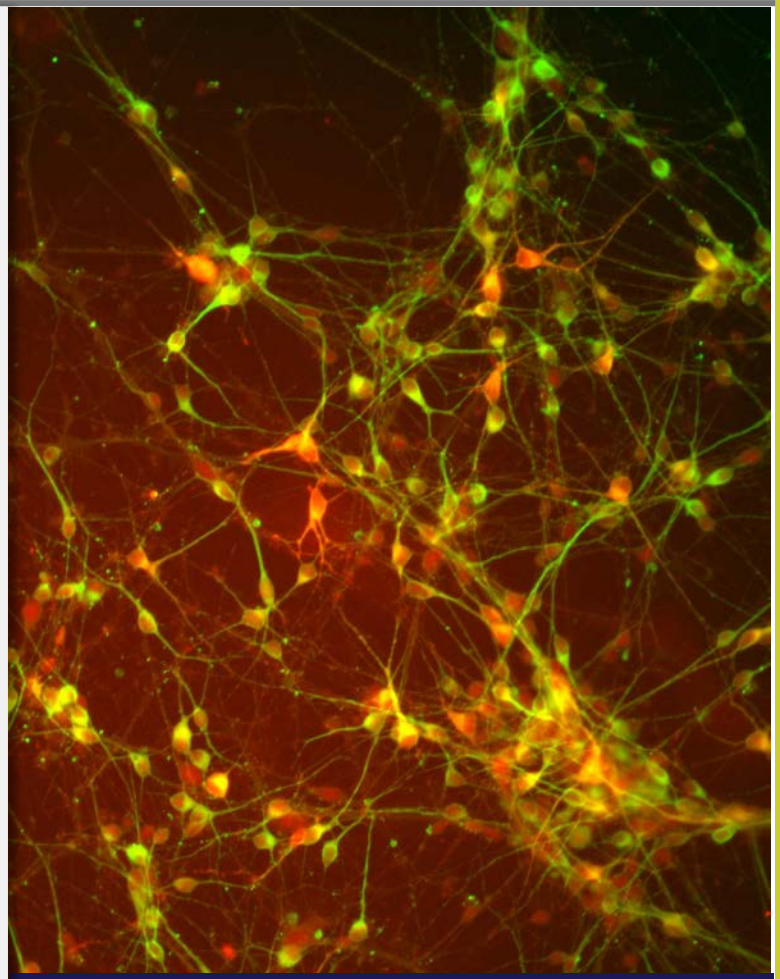
Early and dopaminergic neuron gene expression

Upregulation of early and dopaminergic neuron genes in ACS-5001, ACS-5003, and ACS-5007 NPCs during dopaminergic neuron differentiation



NPC-derived dopaminergic neurons

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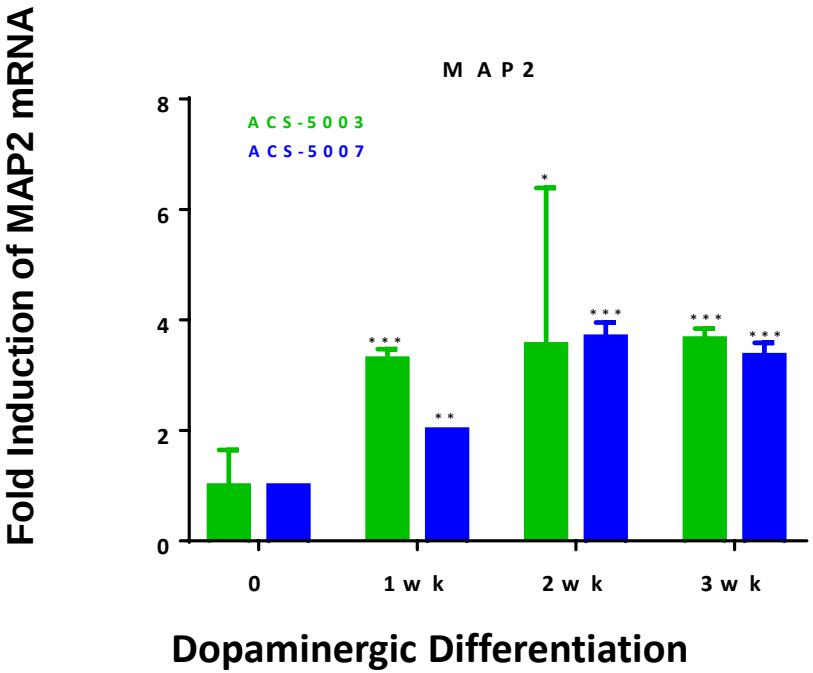
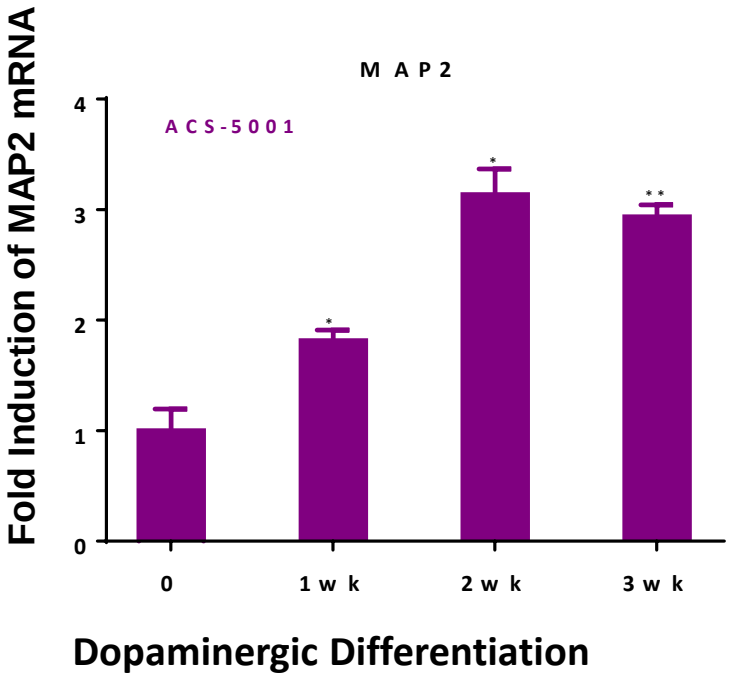
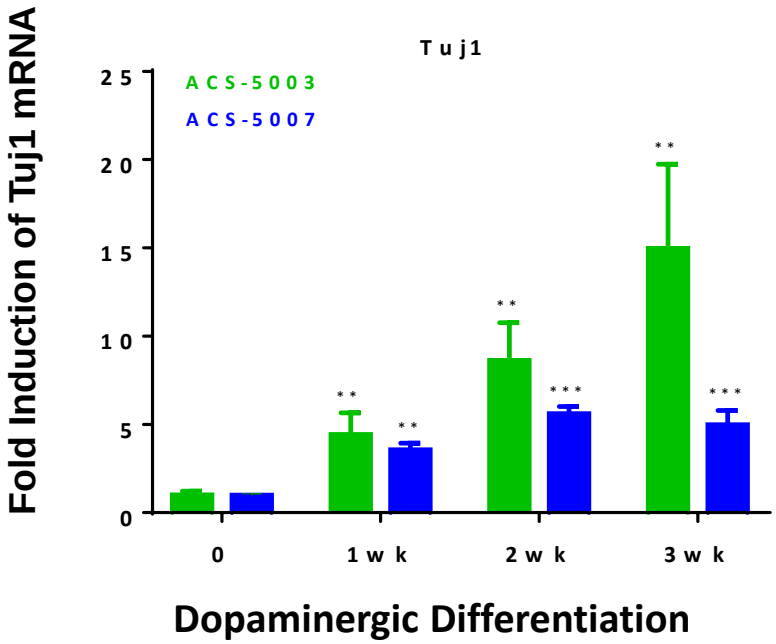
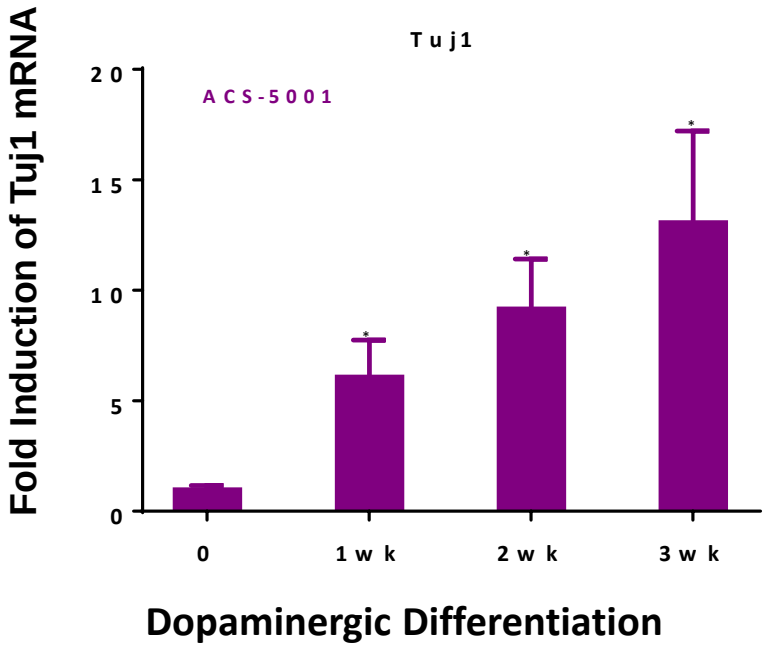
Expression of early neuron gene MAP2





Expression of early neuron gene Tuj1





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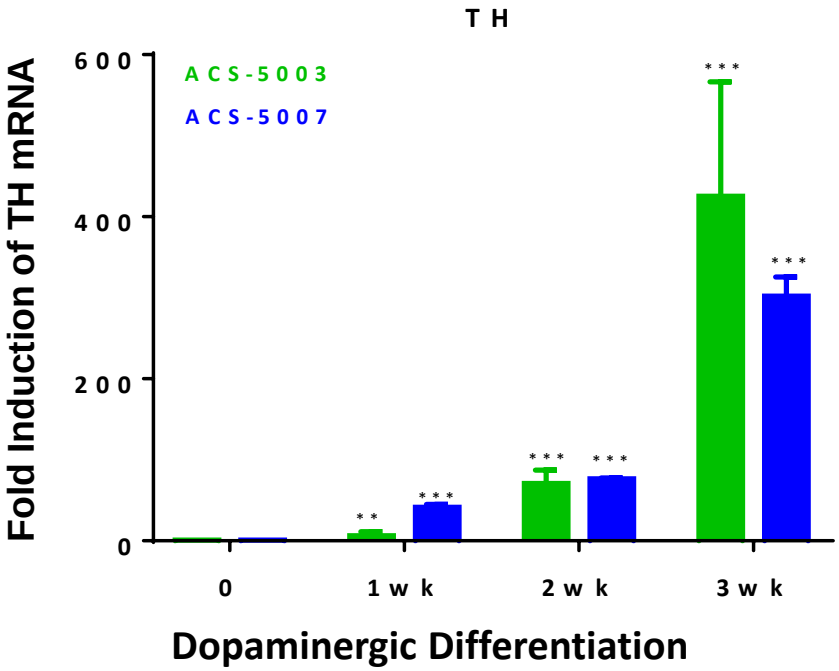
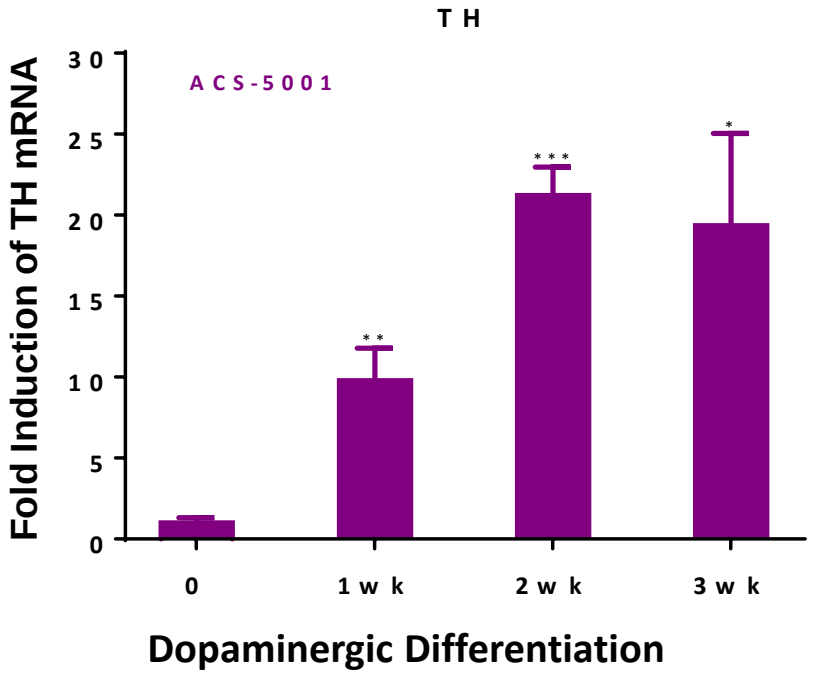
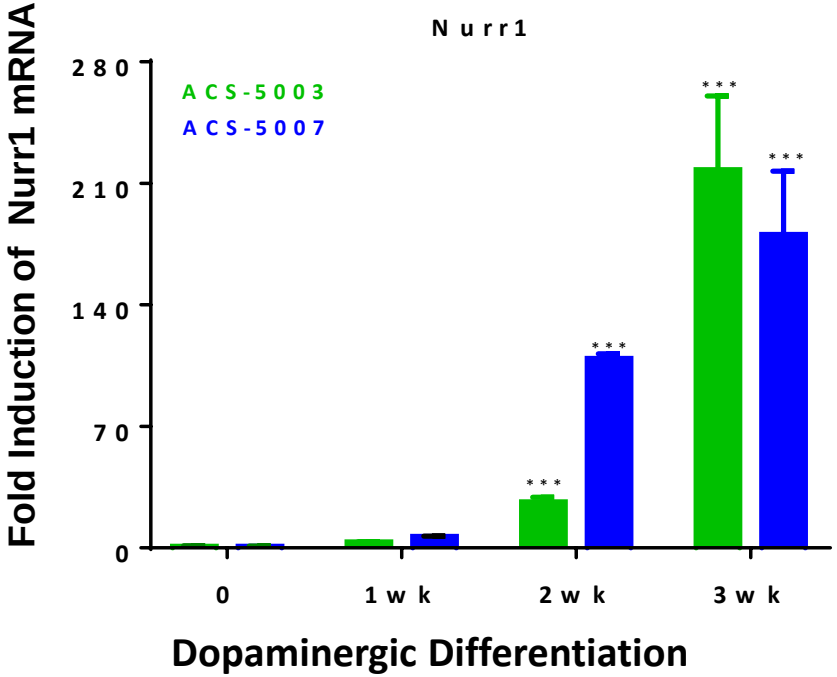
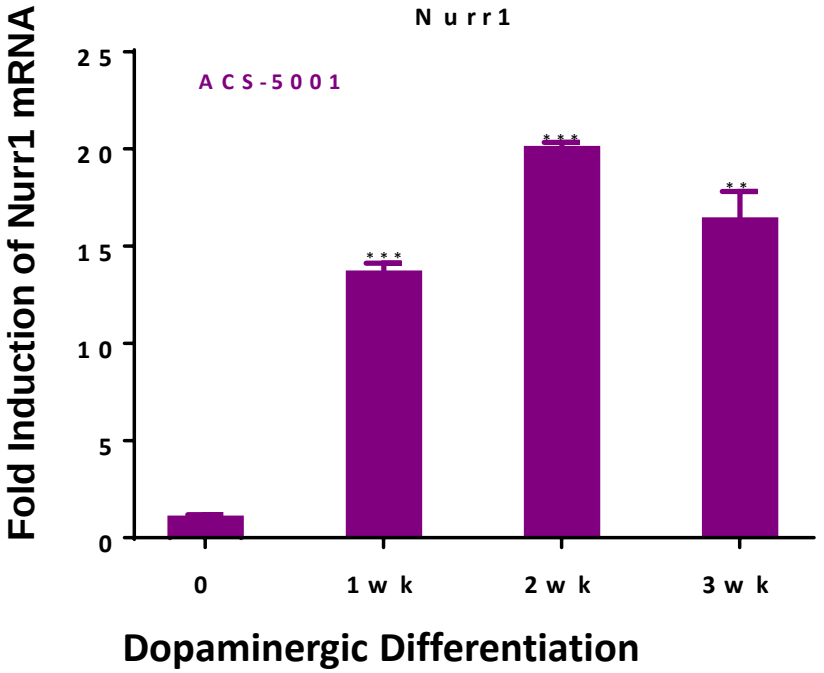
Expression of dopaminergic neuron gene TH





Expression of dopaminergic neuron gene Nurr1





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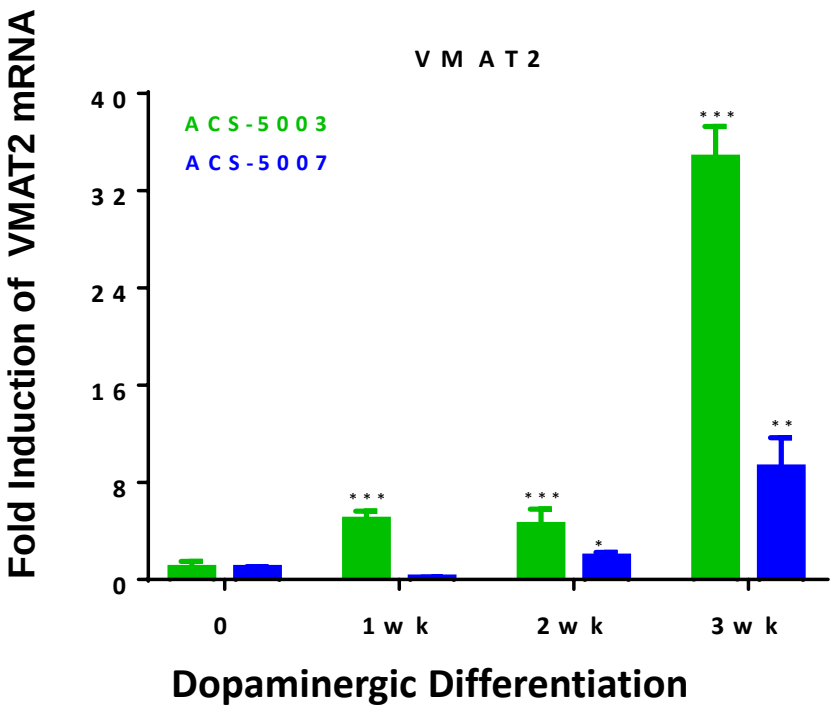
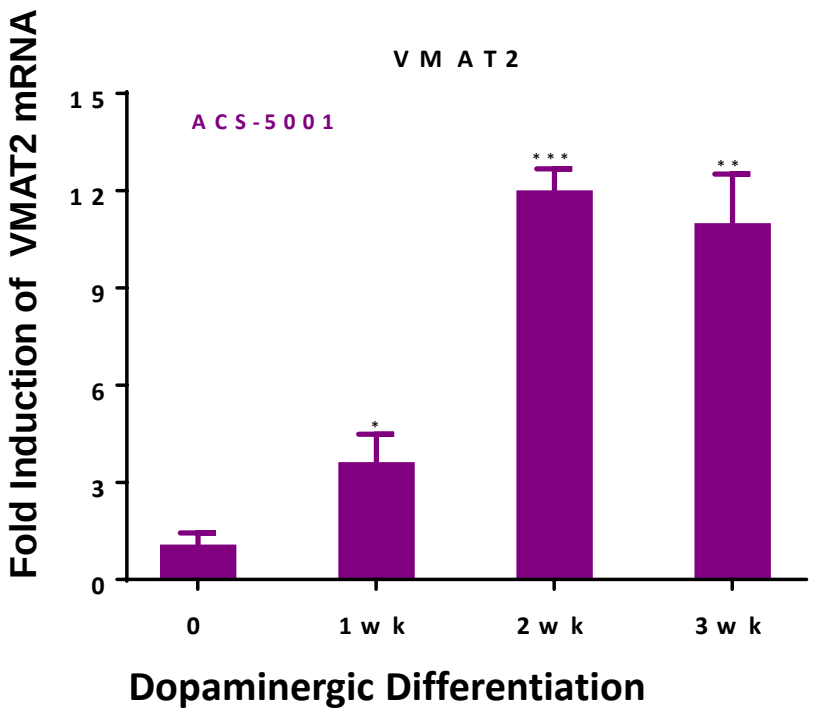
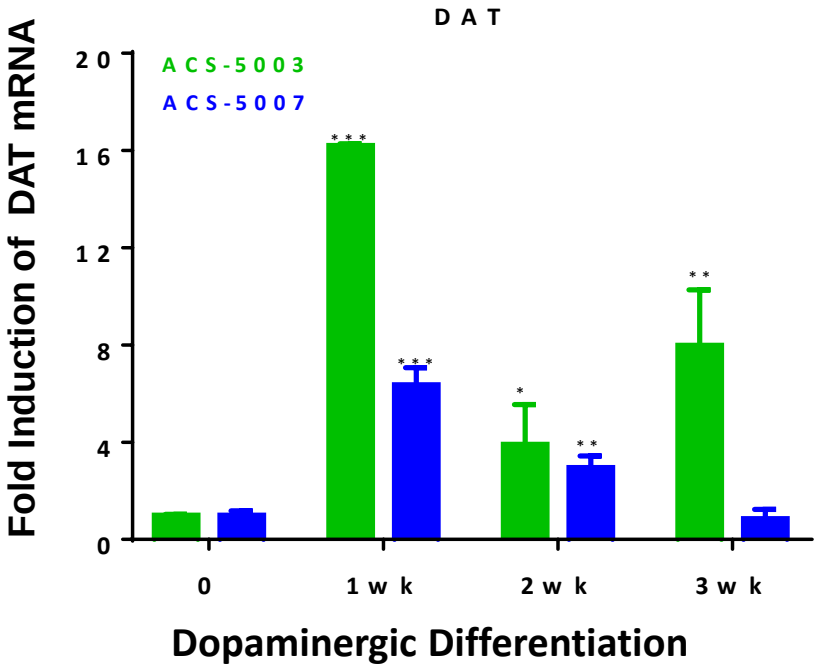
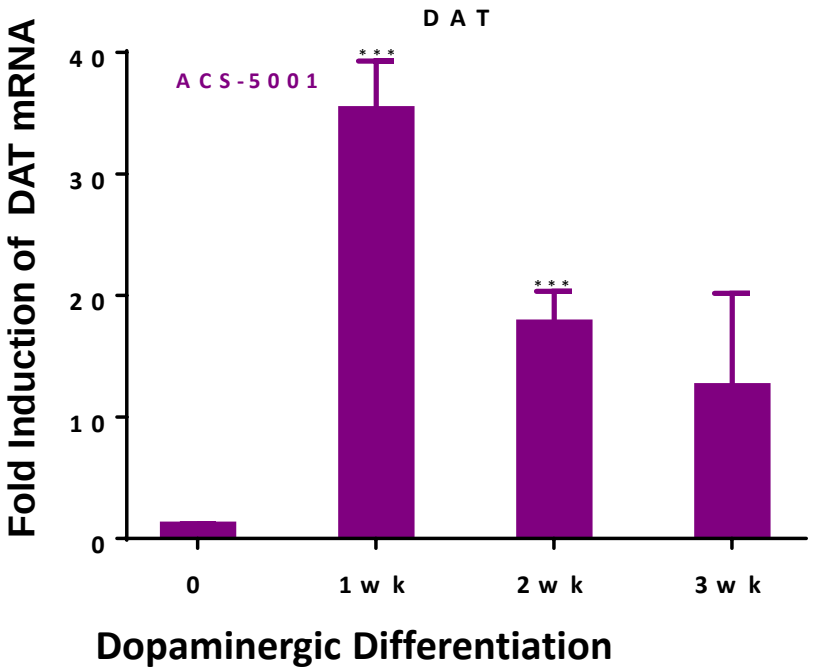
Expression of VMAT2





Expression of DAT





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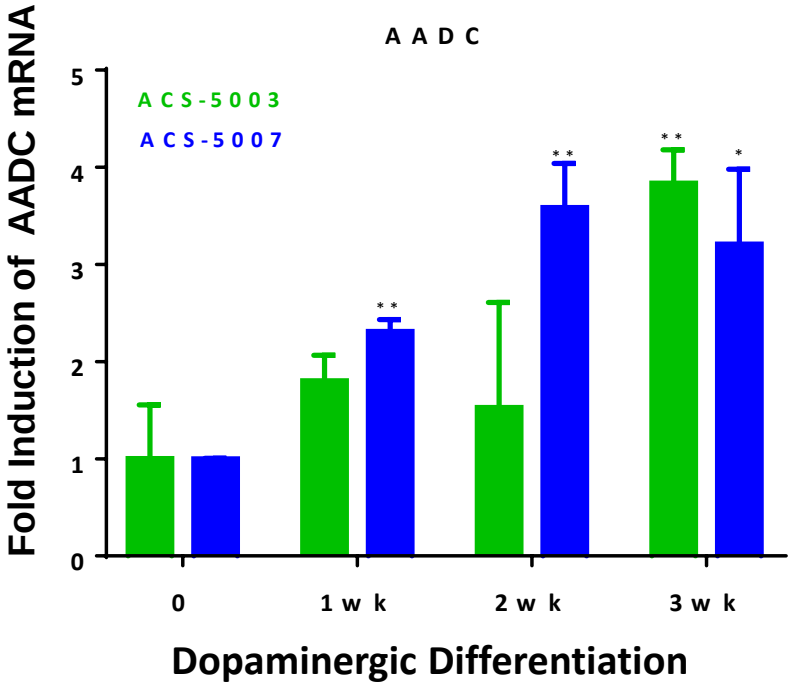
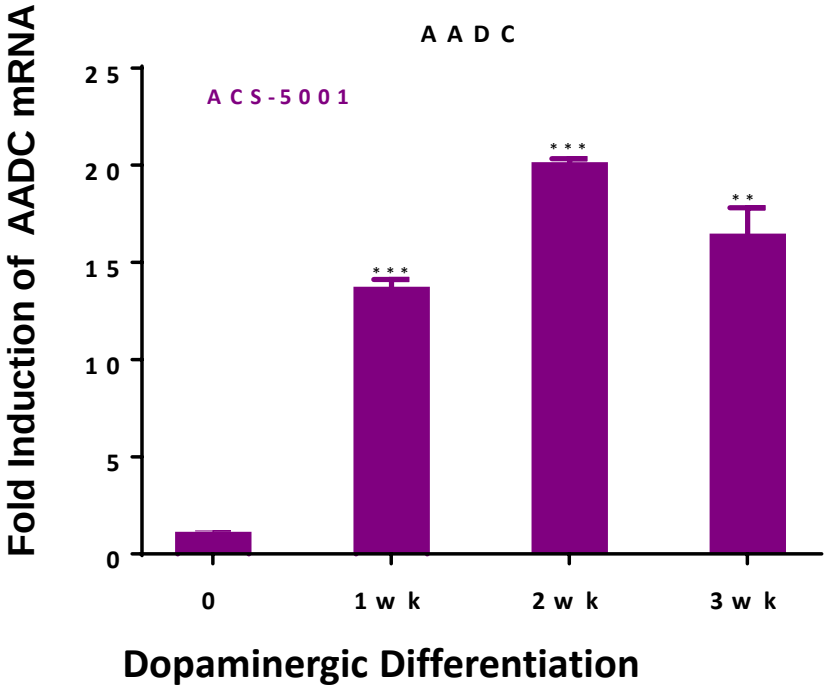
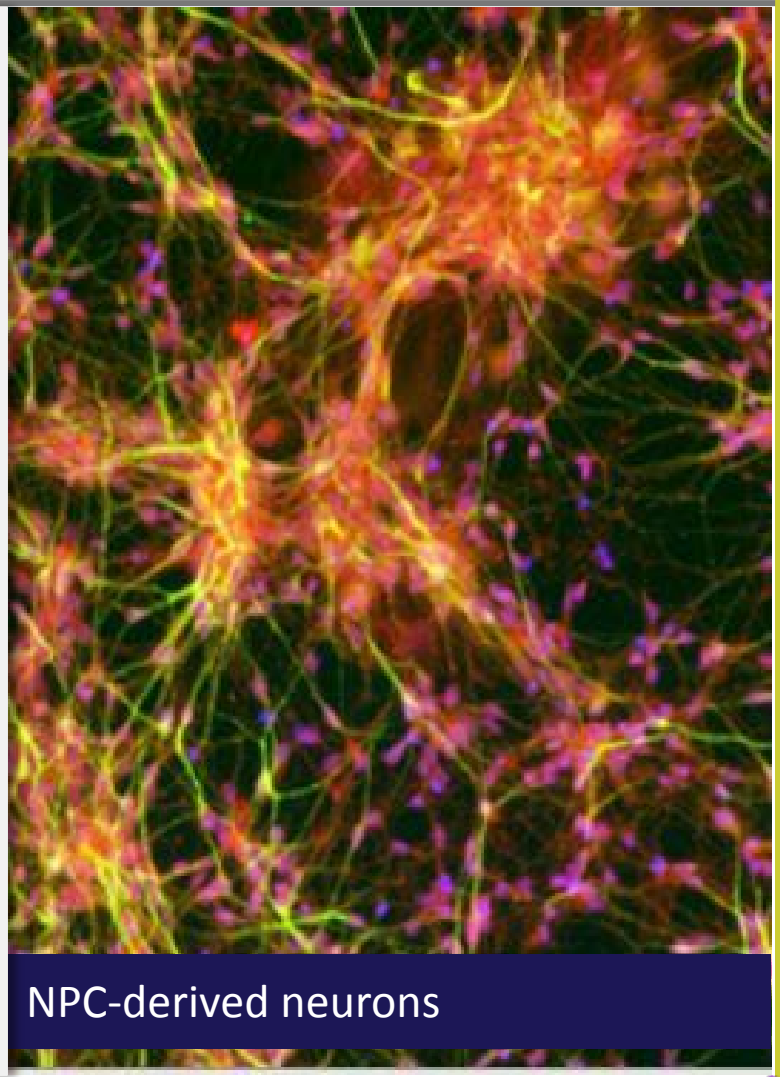
Expression of AADC





Glutamatergic and GABAergic gene expression

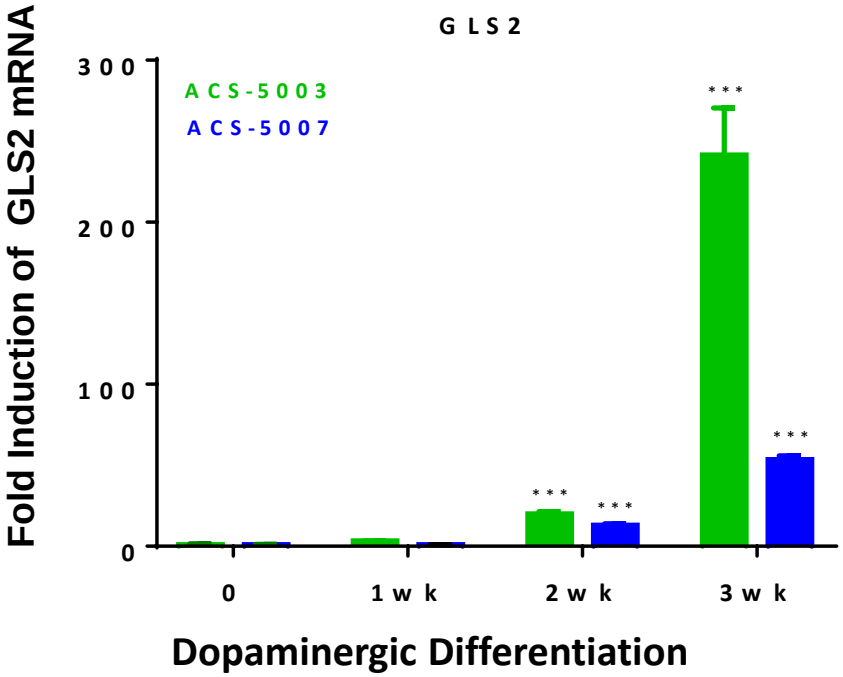
Upregulation of glutamatergic and GABAergic neuron genes in ACS-5001, ACS-5003, and ACS-5007 NPCs during dopaminergic neuron differentiation

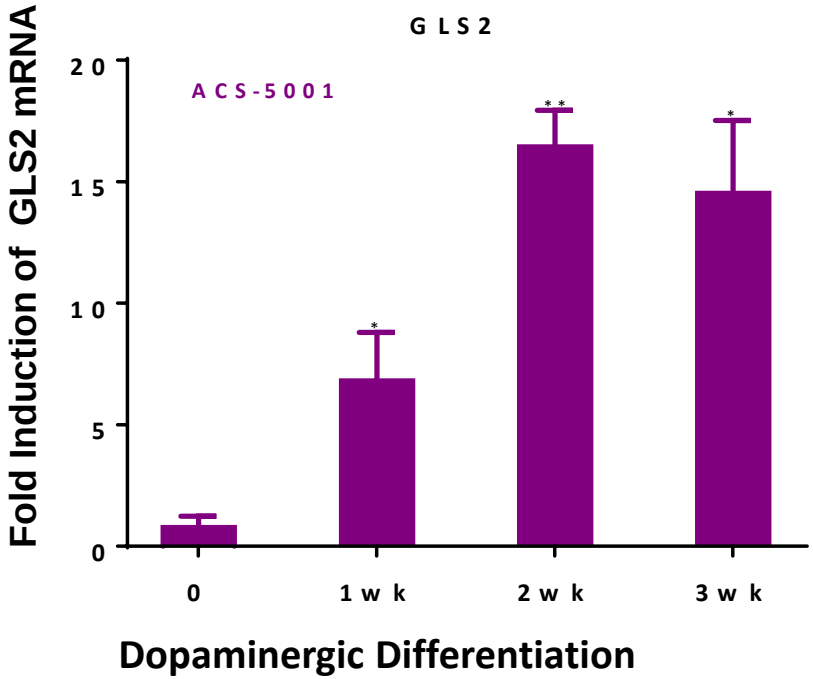


NPC-derived neurons

ATCC

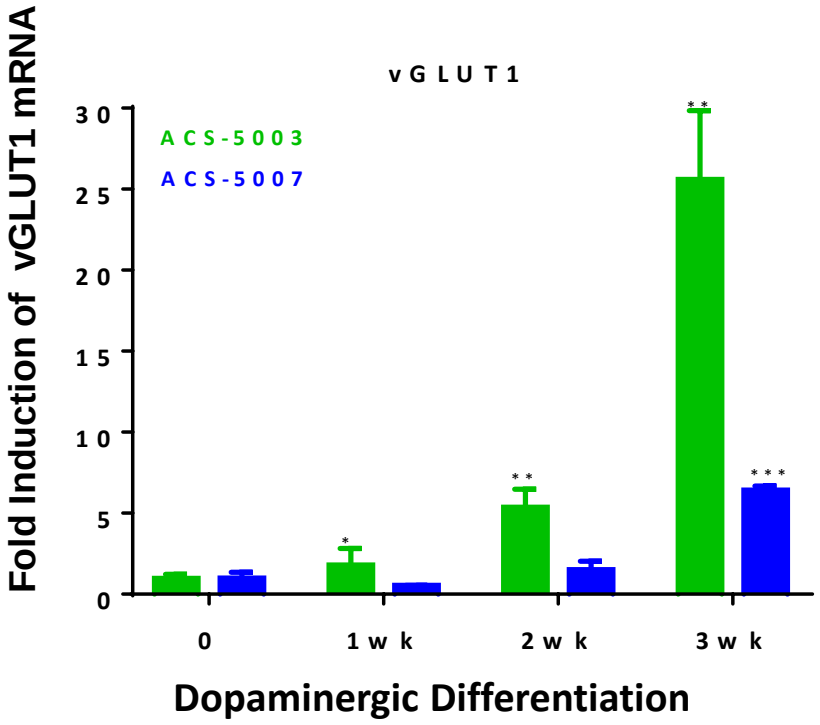
Expression of GLS2

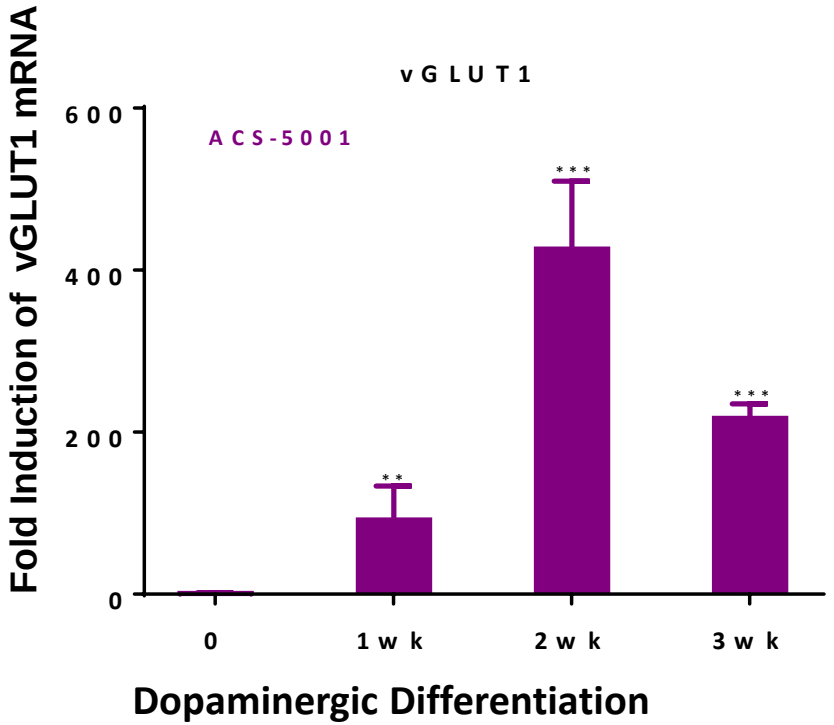




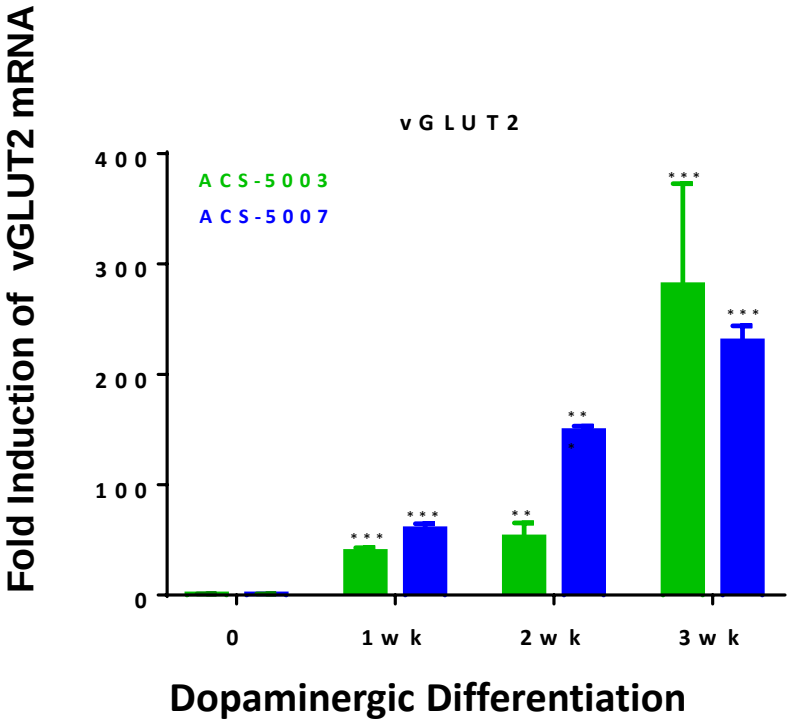
ATCC

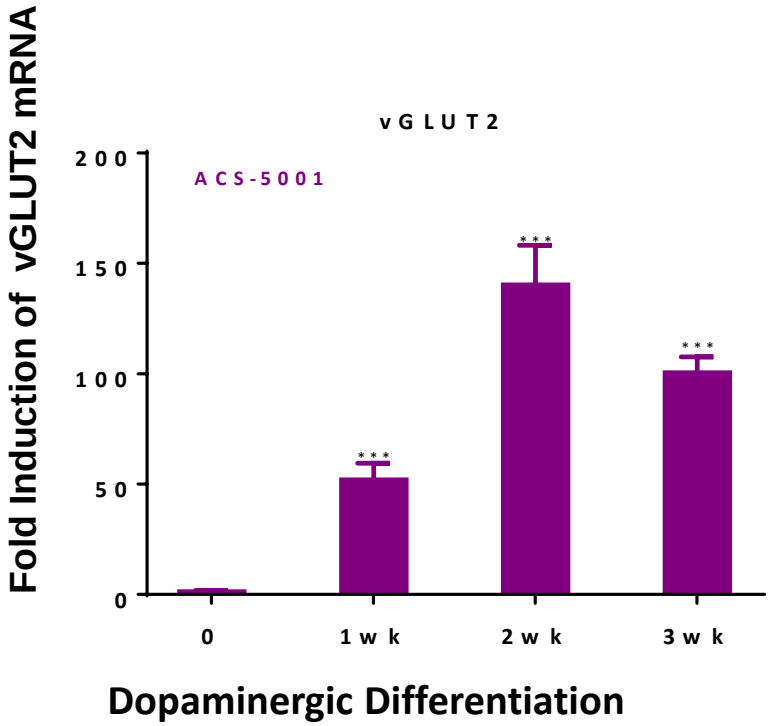
Expression of vGLUT1



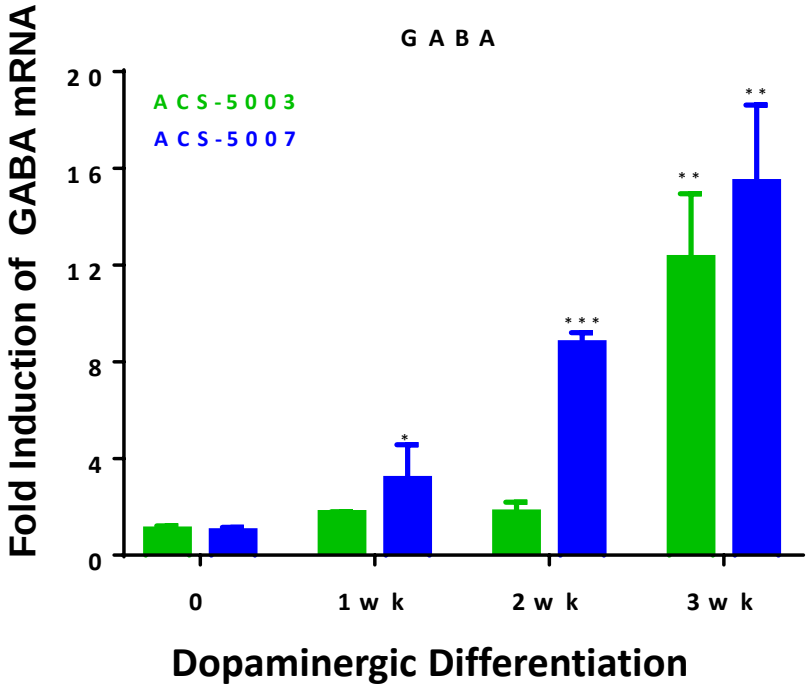


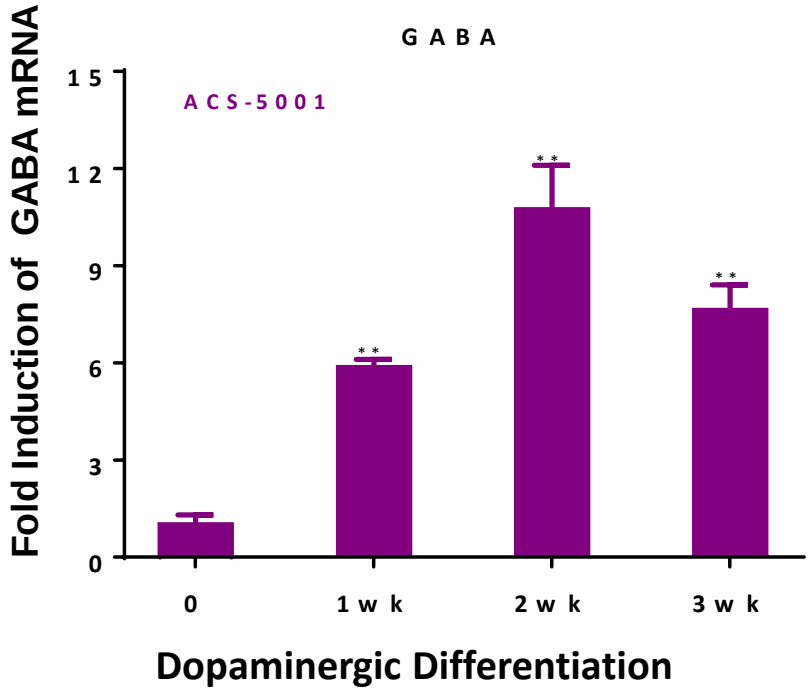
Expression of vGLUT2





Expression of GABA

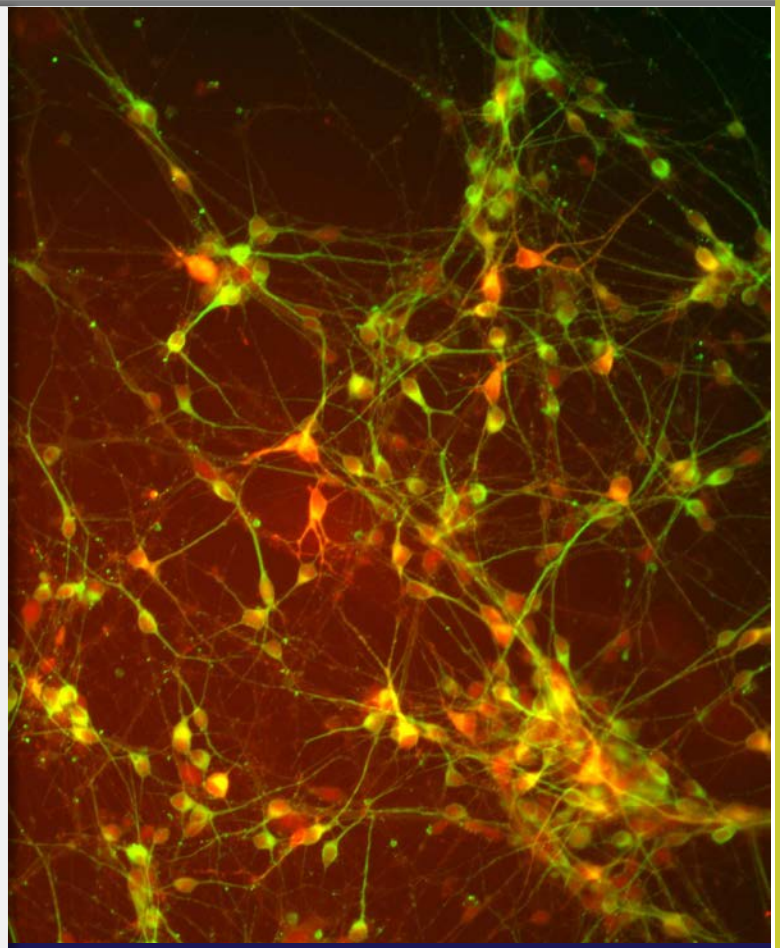




Motor and cholinergic gene expression

Upregulation of neuron genes in ACS-5001, ACS-5003, and ACS-5007 NPCs during dopaminergic neuron differentiation:

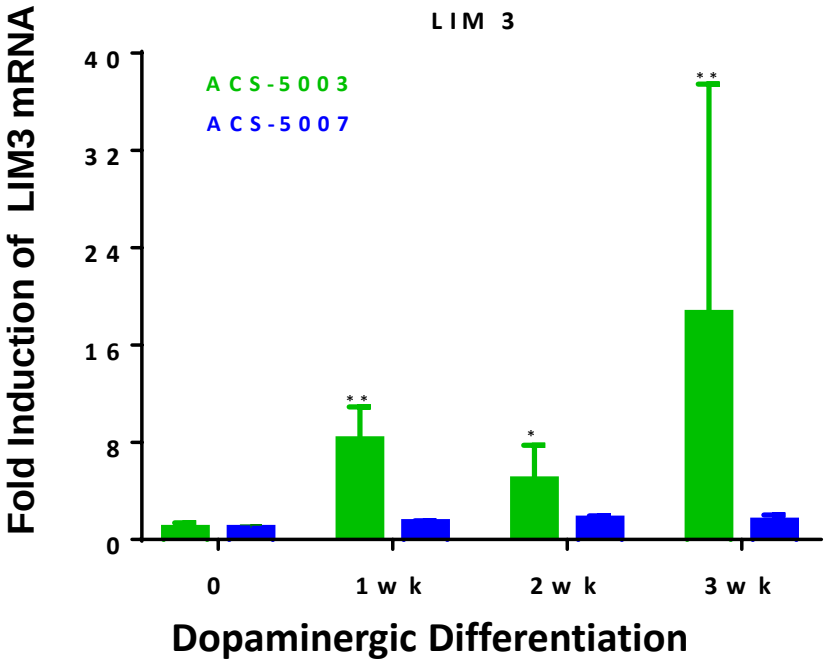
- Motor
  - LIM3
  - Hb9
  - EN1
- Cholinergic
  - ChAT

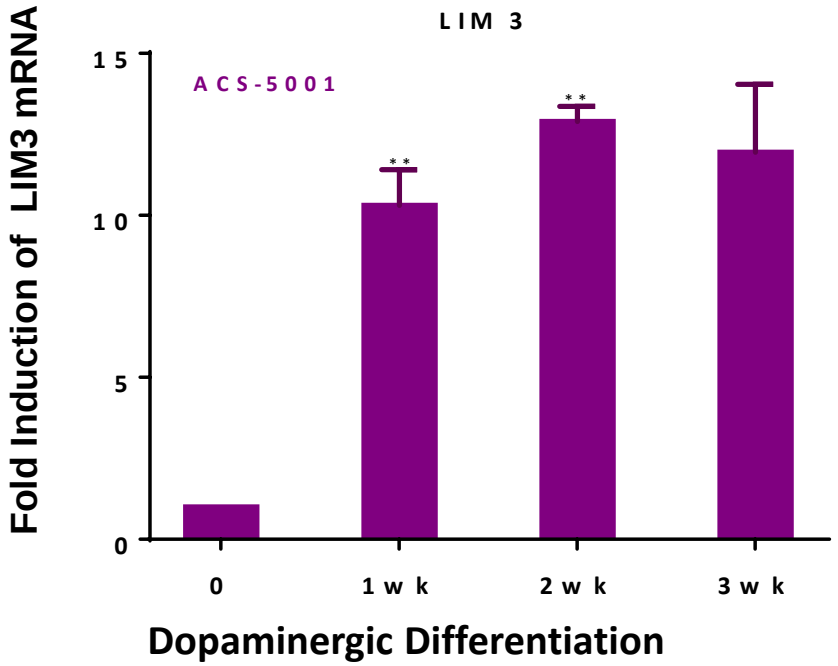


NPC-derived dopaminergic neurons

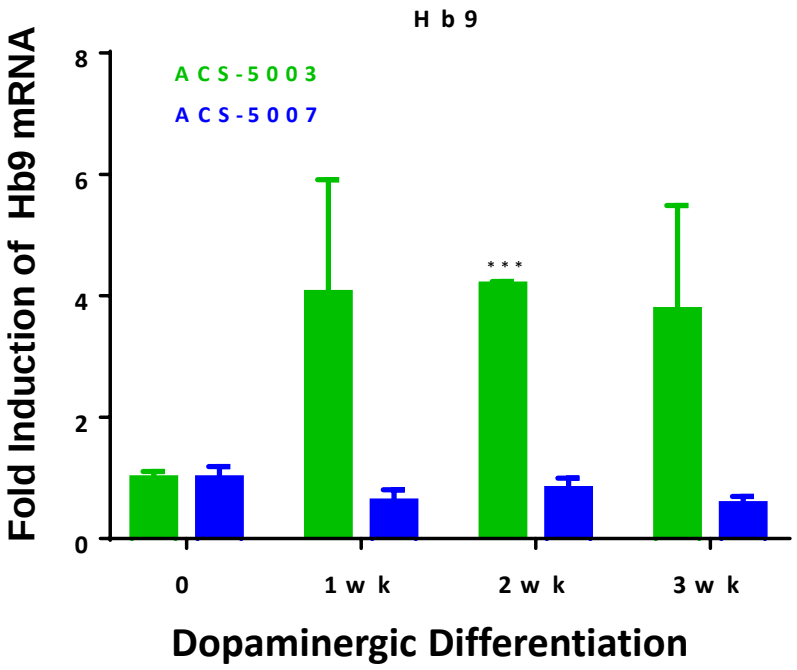
ATCC

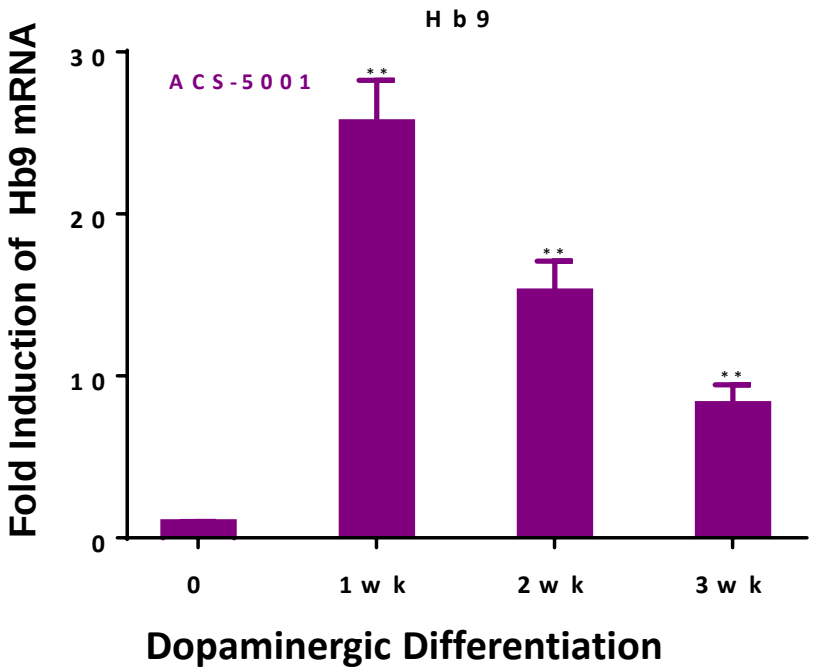
Expression of LIM3



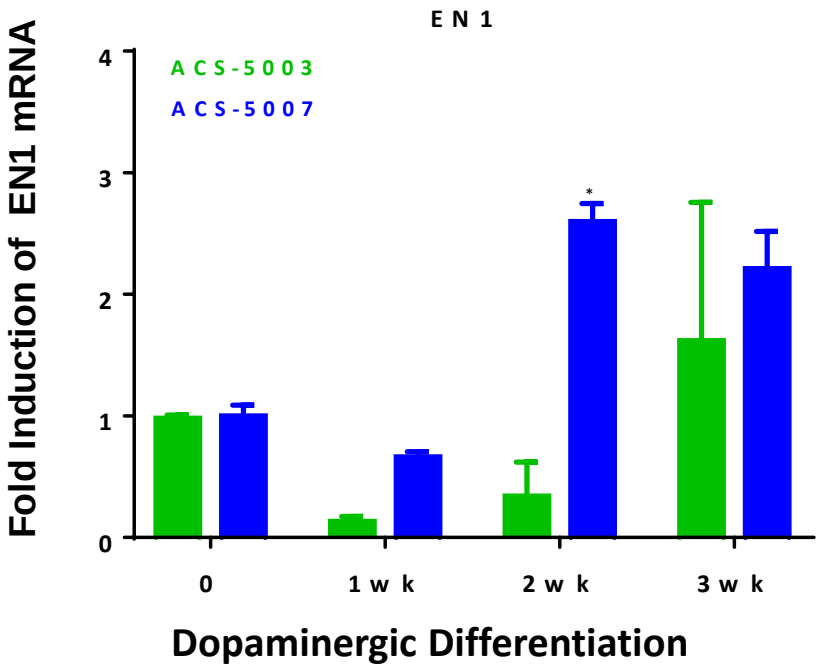


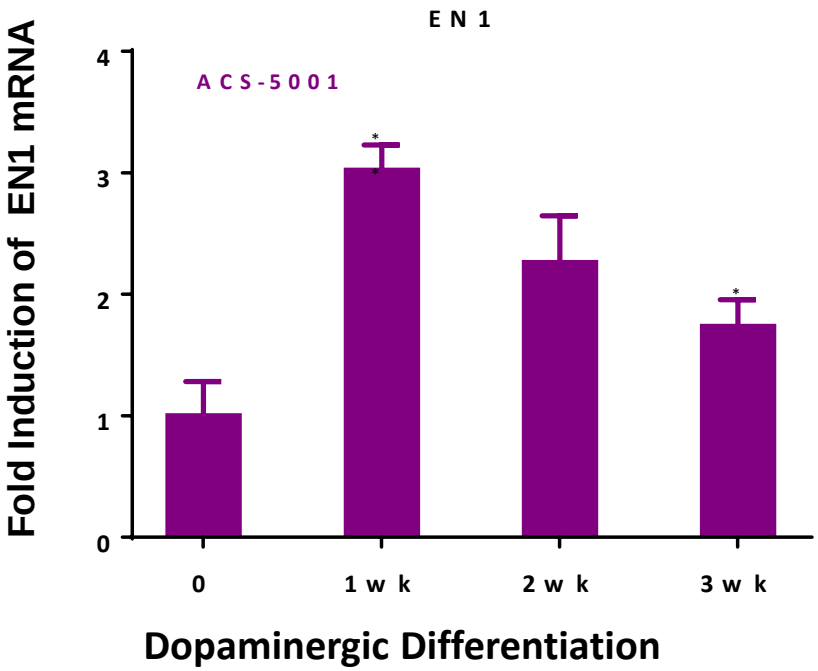
Expression of Hb9



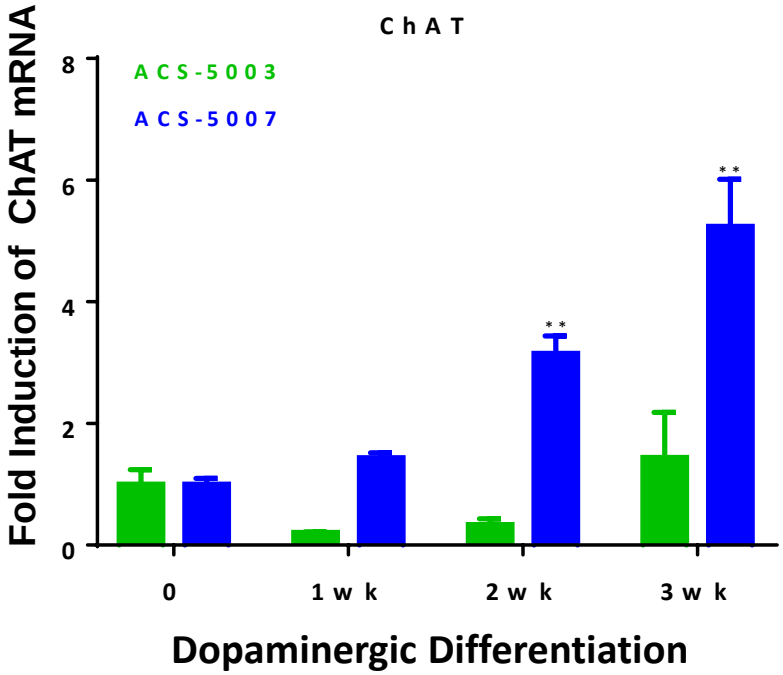


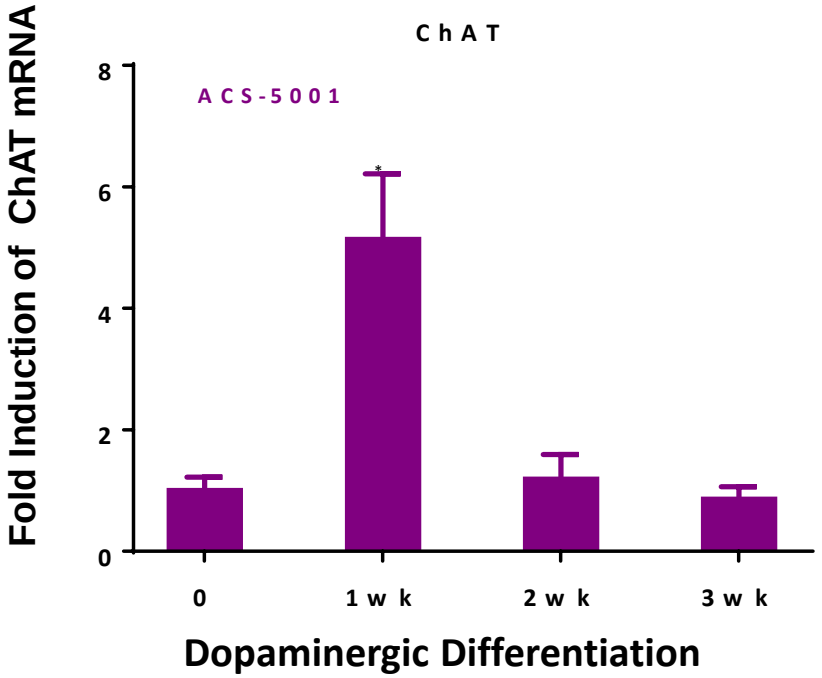
Expression of EN1





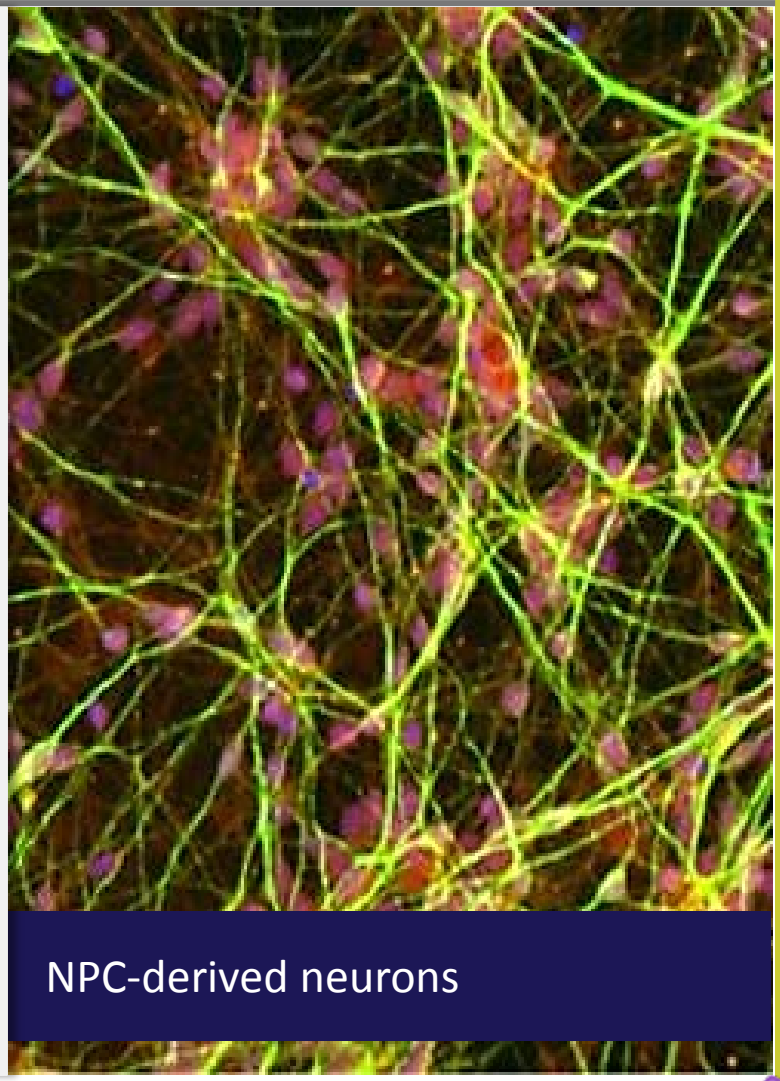
Expression of ChAT





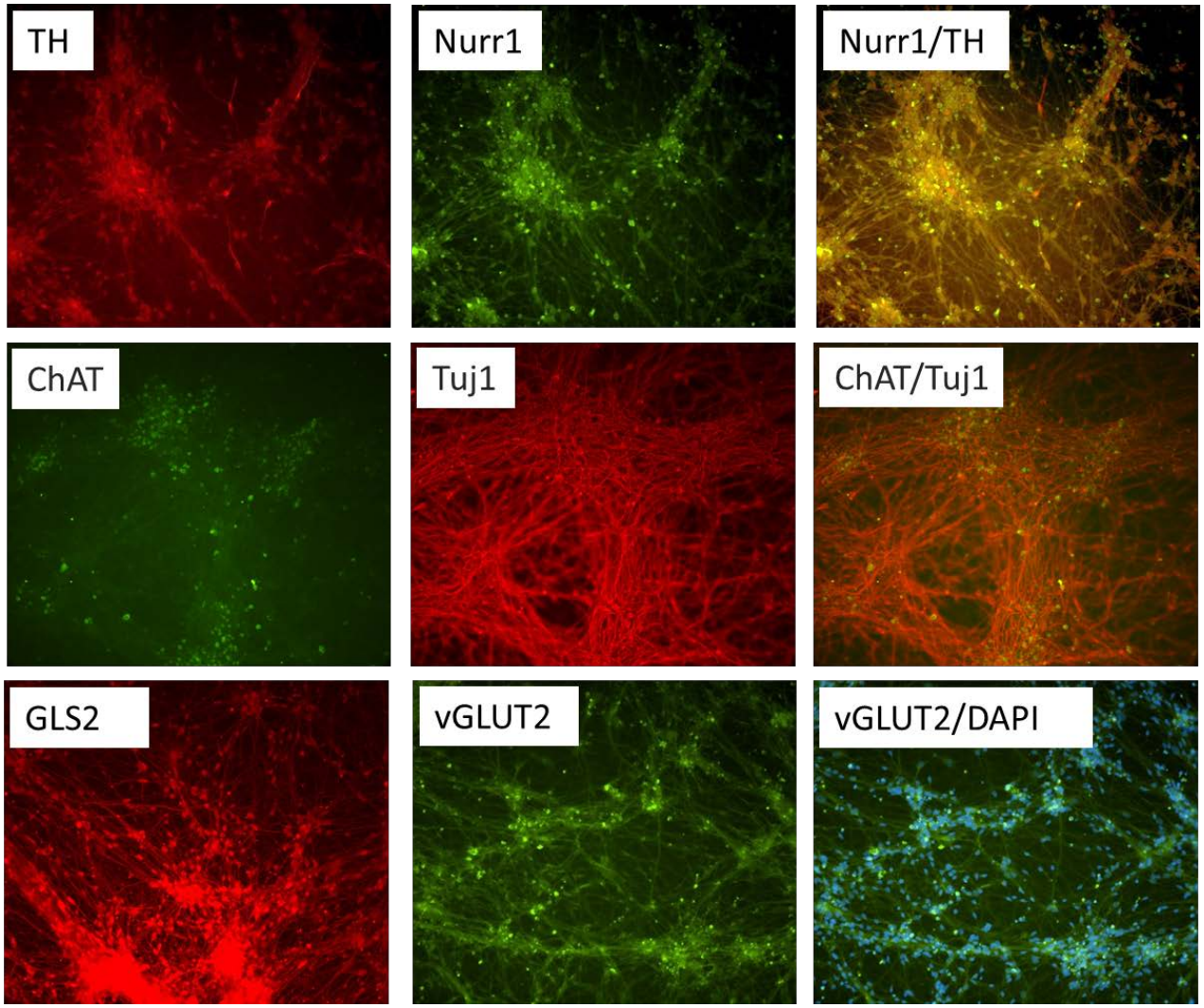
Protein expression

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



NPC-derived neurons

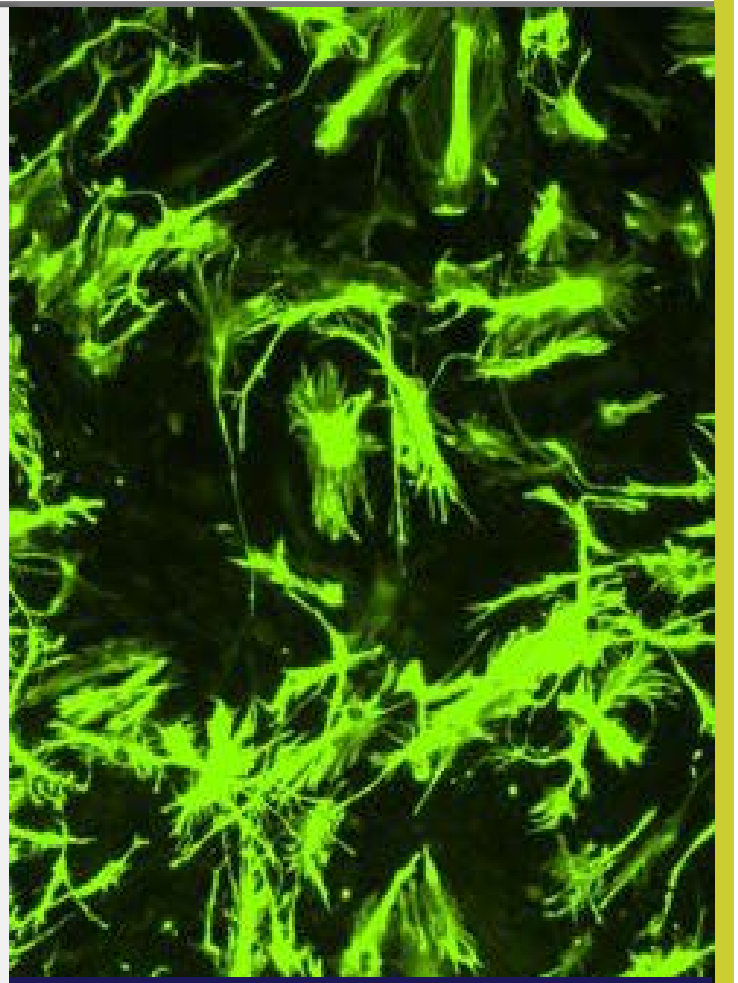
Confirmation of dopaminergic neuronal-specific protein expression during differentiation by ICC



Agenda

Neural Progenitor Cells and Media

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



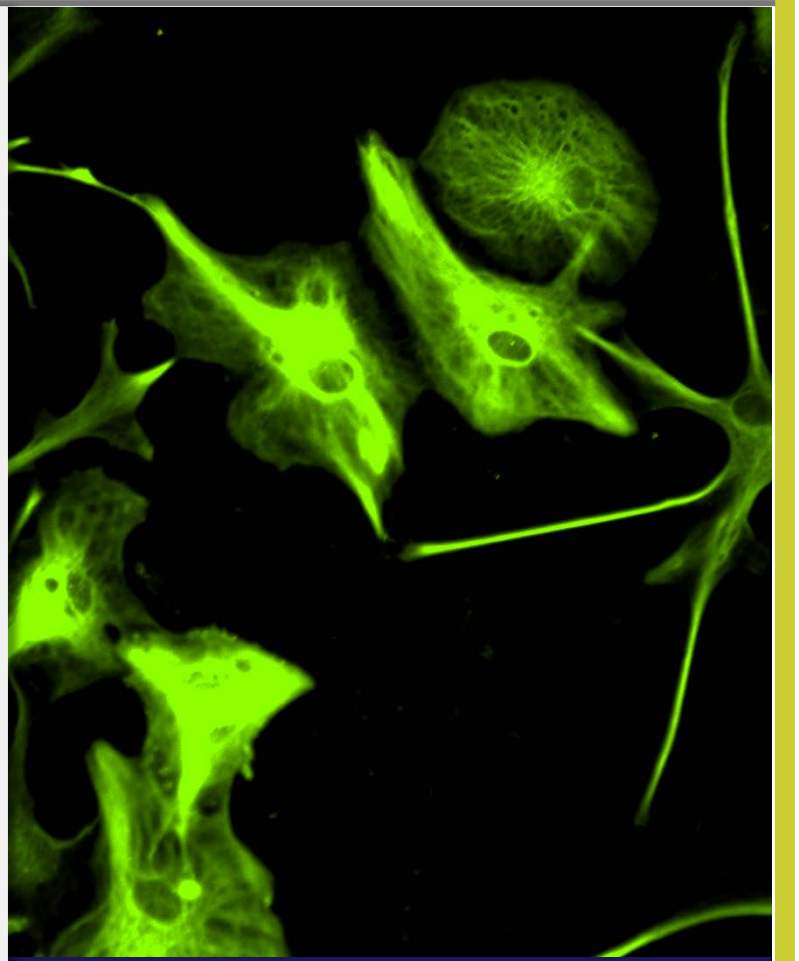
NPC-derived astrocytes

ATCC

Neurotoxicity studies

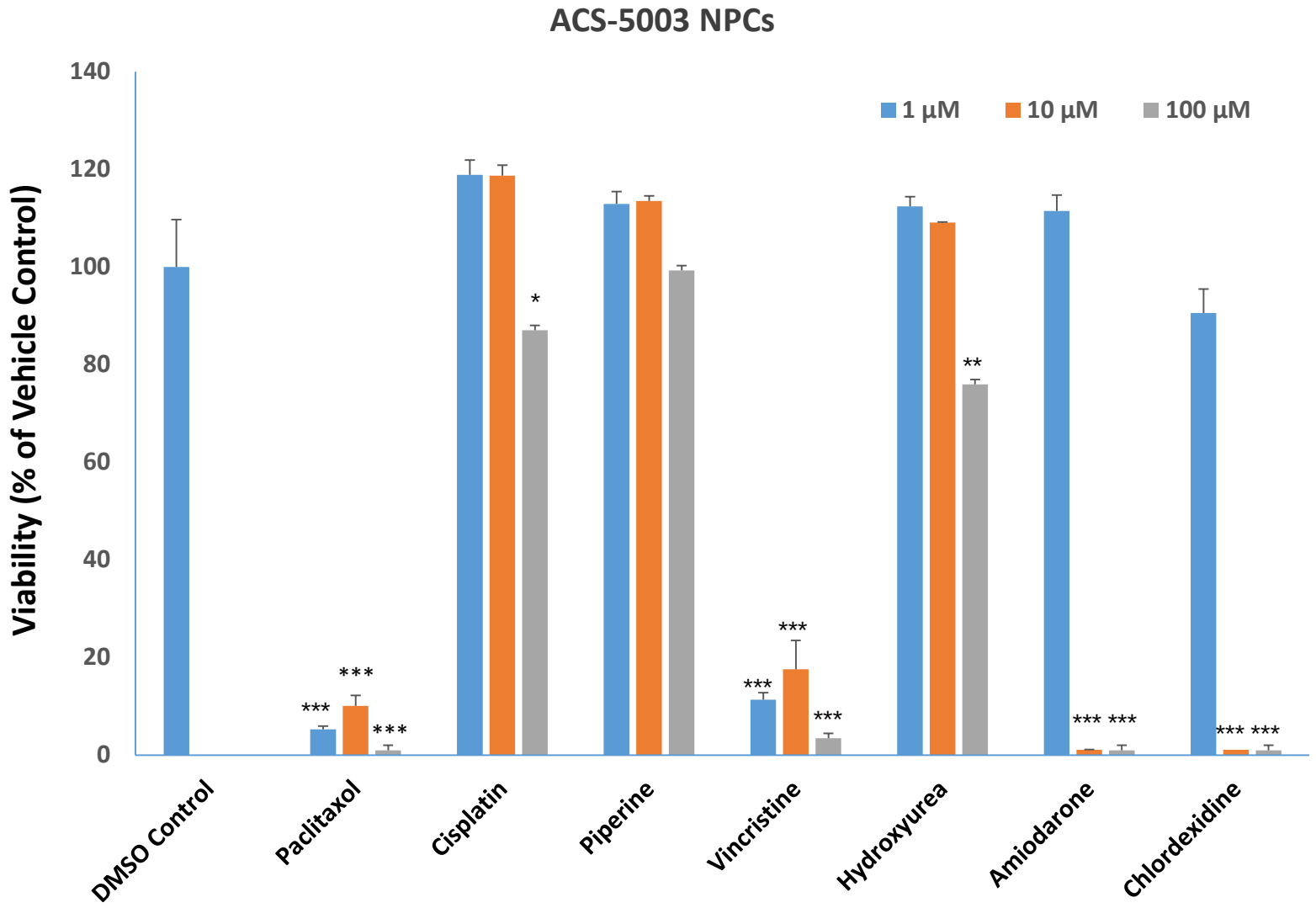
Neurotoxicity studies with ACS-5001 and ACS-5003 NPCs

- Resazurin viability
  - Reliablue™ (ATCC® 30-1014™)

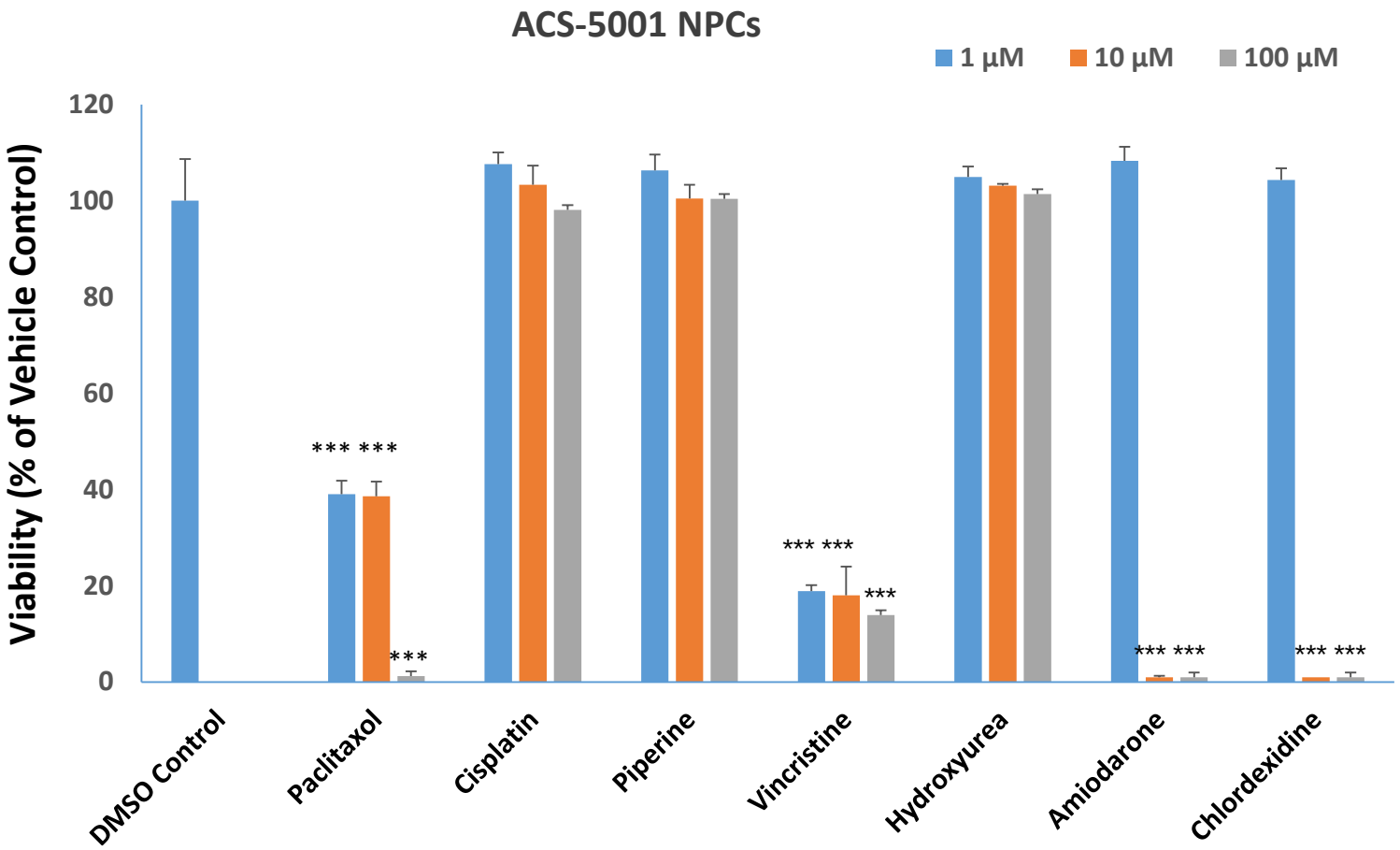


NPC-derived oligodendrocytes

Neurotoxicity studies – undifferentiated NPCs



Neurotoxicity studies – undifferentiated NPCs



Neurotoxicity studies – ACS-5001 and ACS-5003

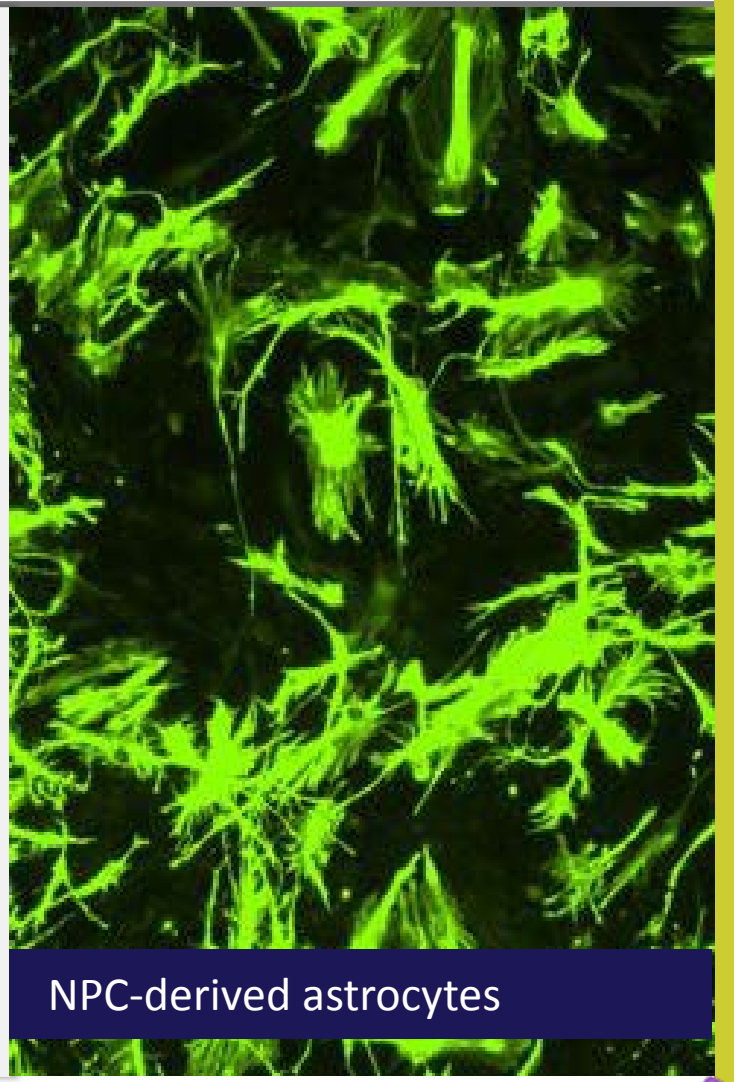
Toxin	ACS-5001	ACS-5003
Amiodarone	Toxic	Toxic
Chlorhexidine	Toxic	Toxic
Cisplatin	Resistant	Weakly toxic
Piperine	Resistant	Resistant
Vincristine	Toxic	Toxic
Hydroxyurea	Resistant	Weakly Toxic
Paclitaxel	Toxic	Toxic

ATCC

Agenda

Neural Progenitor Cells and Media

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

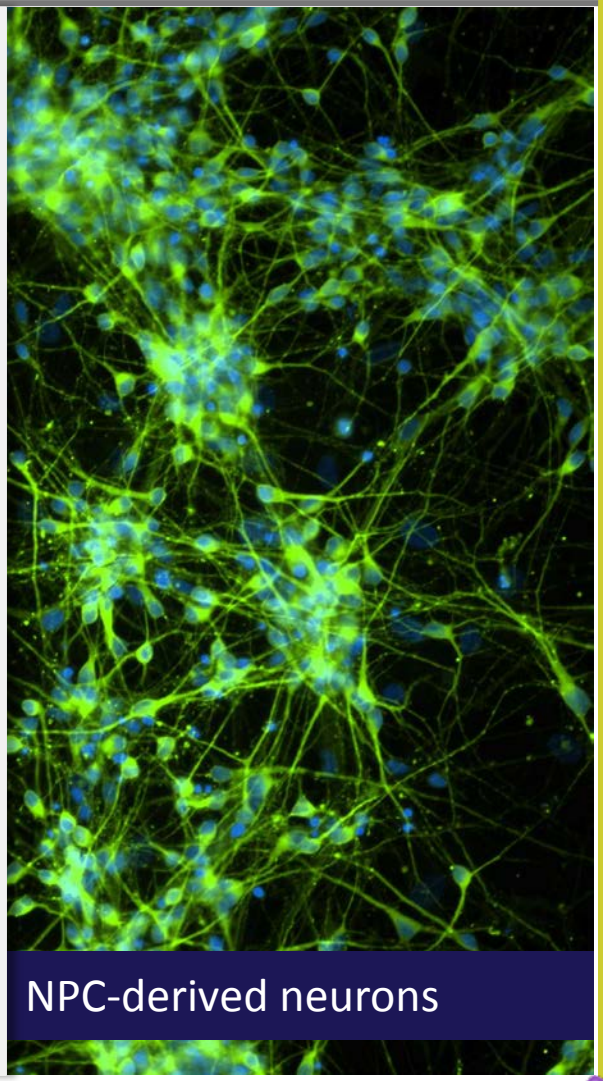


NPC-derived astrocytes

ATCC

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



NPC-derived neurons

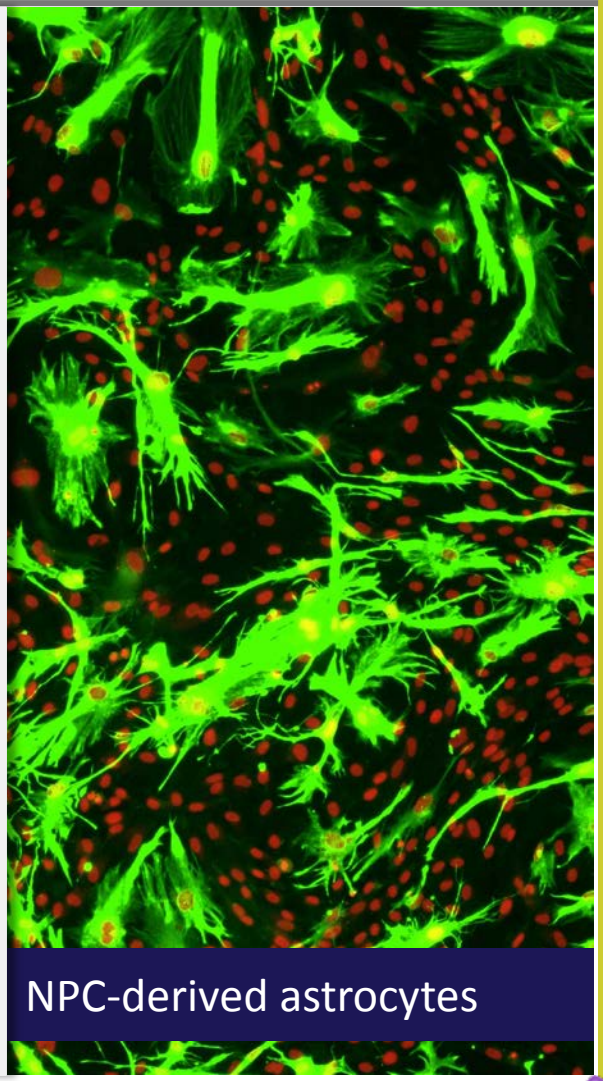
ATCC

NPCs – Summary

- Our studies demonstrated that ATCC normal and PD NPCs have the potential to be differentiated into:
  - Dopaminergic neurons
  - GABAergic neurons
  - Glutamatergic neurons
  - Motor neurons
  - Cholinergic neurons

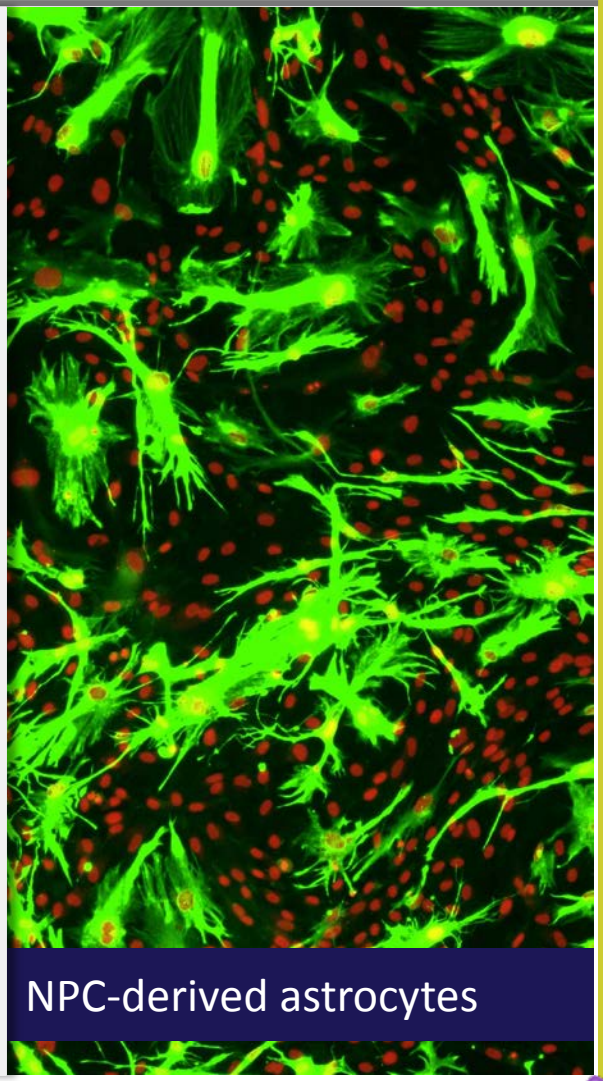
  after treatment of NPCs with ATCC dopaminergic differentiation media

- ATCC NPCs are suitable for drug screening applications



NPC-derived astrocytes

Disclaimers



NPC-derived astrocytes

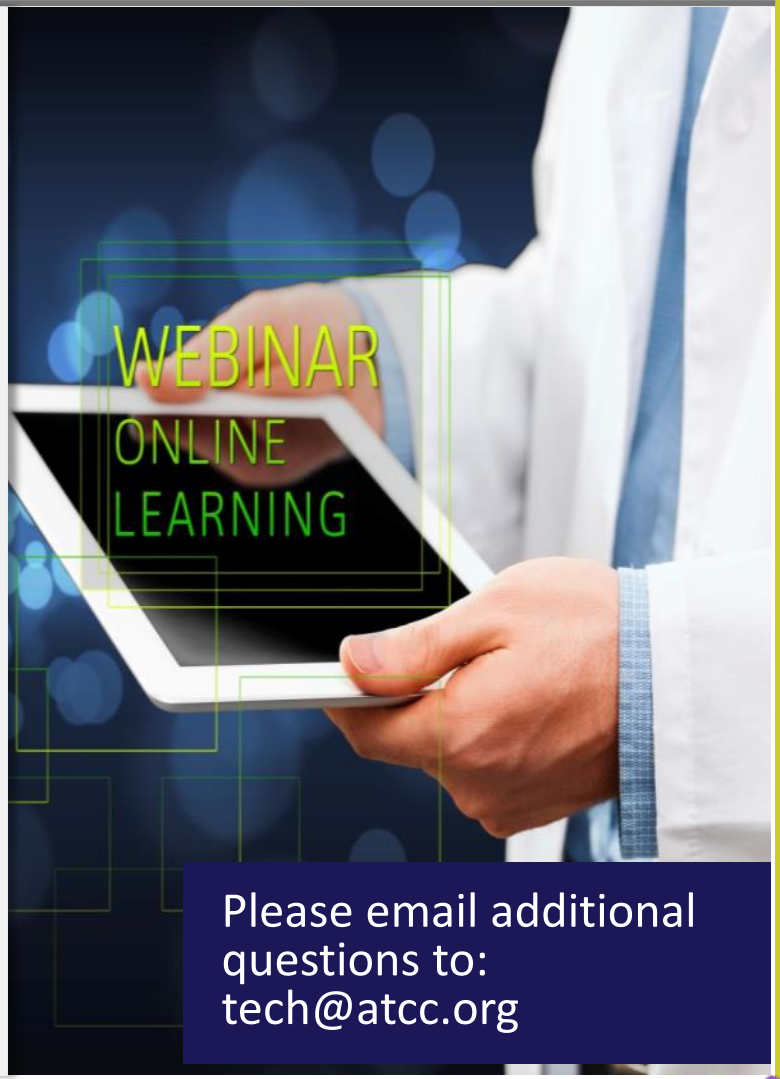
Thank you for joining today!

Visit us at SOT 18, booth 1422, March 11-15

- **Exhibitor-hosted Session: March 14, 2018**
  Mindy Goldsborough, *Chief Science and Technology Officer and General Manager*, ATCC
  Kevin Grady, *Product Line Business Manager,* ATCC
  Chaozhong Zou, *Senior Scientist*, ATCC
  Advanced In Vitro Solute Carrier Transporter Models for Renal Toxicity Studies and Screening

- **Poster Presentations: March 12, 2018**
  Chaozhong Zou, *Senior Scientist*, ATCC
  Advanced In Vitro Solute Carrier Transporter Models for Renal Toxicity Studies and Screening

  Sheela Jacob, Scientist, ATCC
  Comprehensive Gene Expression Analysis and Neurotoxicity Testing of Human iPSCs-Derived Neural Progenitor Cells and Neurons



Please email additional questions to:
tech@atcc.org

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