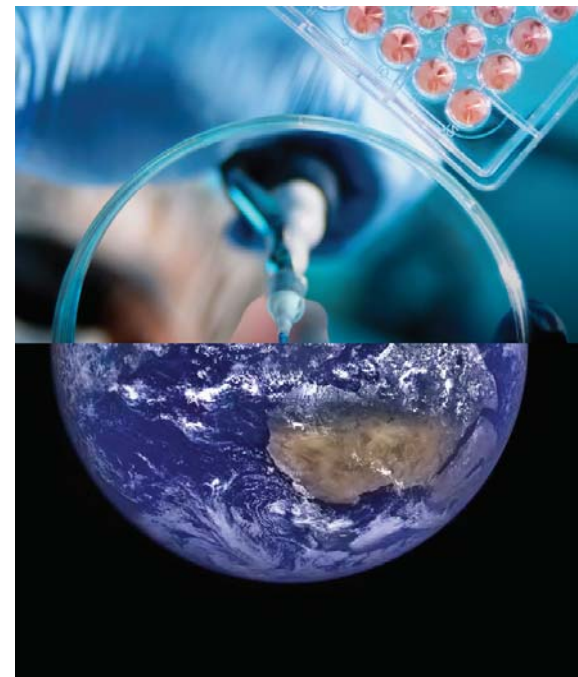
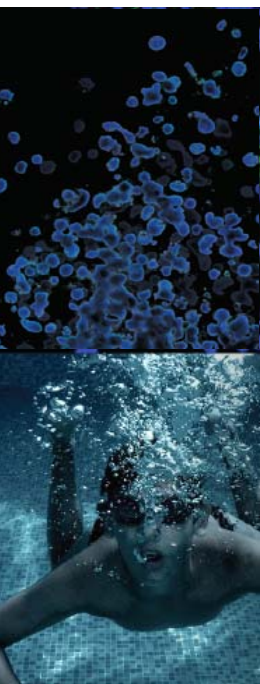




Neural Progenitor Cells: Better Biological Models of Neurodegenerative Disease

Brian Shapiro, Ph.D.
Technical Writer, ATCC

Credible Leads to Incredible™



ATCC – Credible leads to Incredible

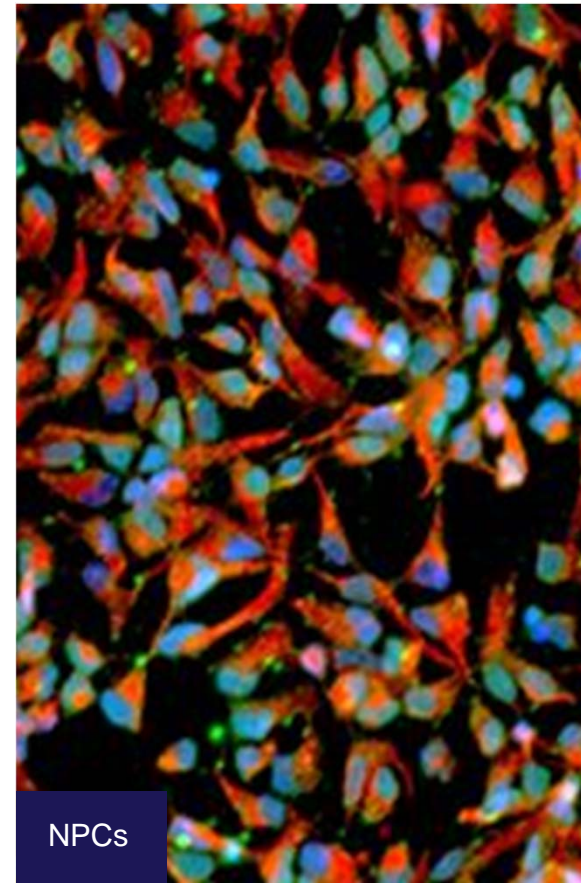
- ATCC has provided credible biomaterials for over 90 years
- We continue to cultivate collaboration
 - Among scientists across disciplines
 - Essential for accelerating innovative research
 - Leading to incredible, high-impact results.
- Our Cultivating Collaboration pledge: We bring scientists together to discuss
 - Breakthroughs in the state of science
 - Multidisciplinary approaches to key areas of research
 - Breaking the silos that impede research
- Our partnership with you, the scientific community, allows us all to reach the incredible



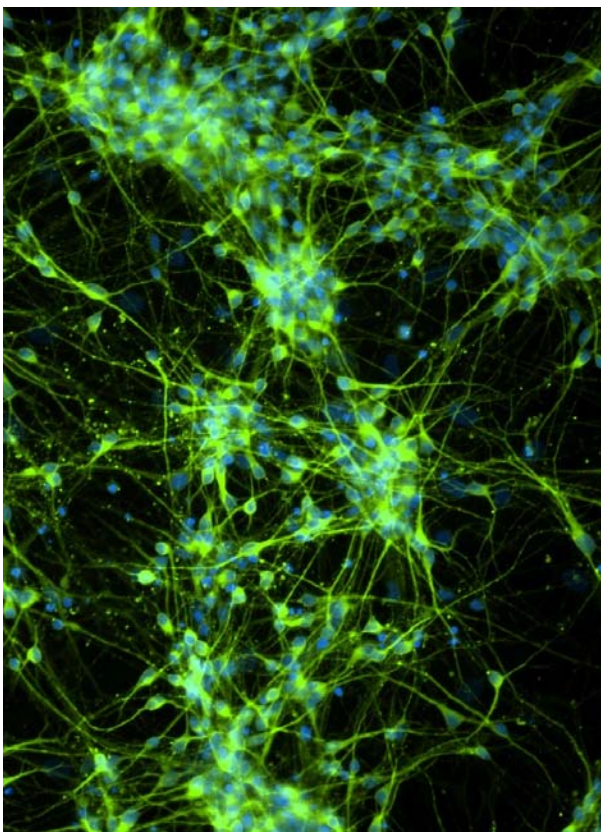
Agenda

Neural Progenitor Cells (NPCs) and Media

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

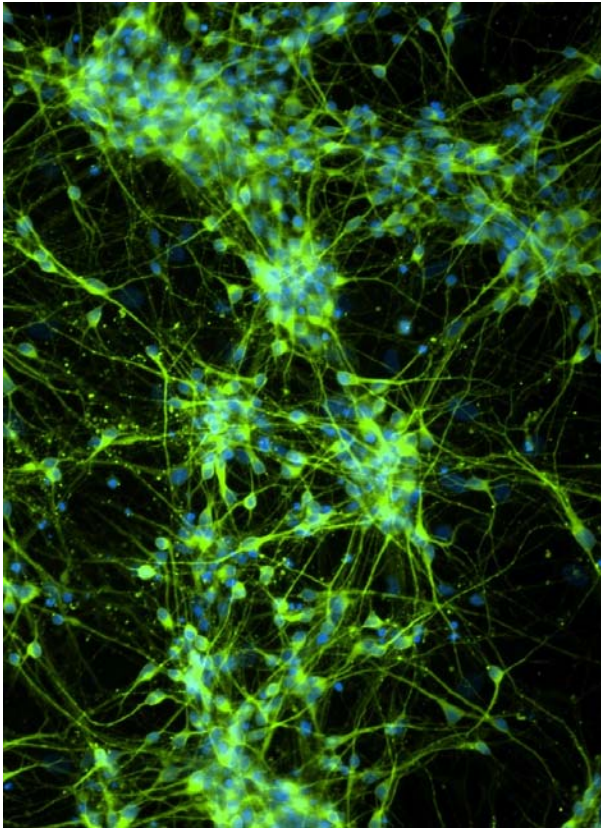


Challenges associated with current models of the nervous system



- 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

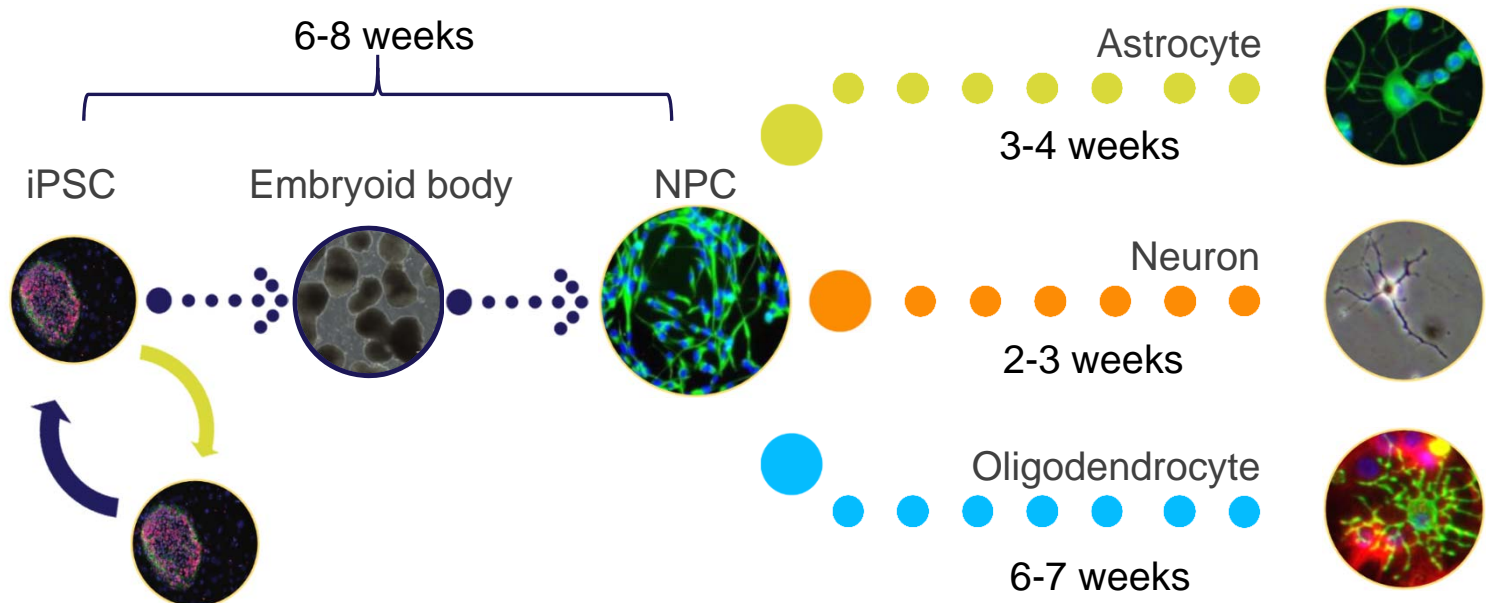
What is neurobiologists need in a better biological model



Advanced, biologically relevant models

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

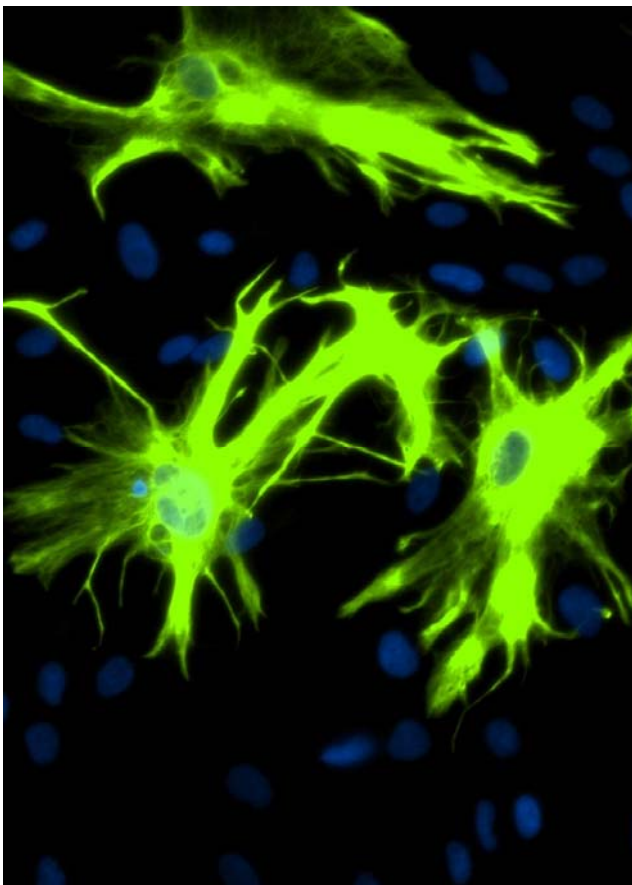
Neural progenitor cells – Neuronal differentiation



NPCs:

- Shorten research time
- Human model
- Predictive screening

NPCs: An advanced model of the nervous system



A better biological model:

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

More meaningful results:

- More biologically relevant results/more predictive system
- Parkinson's NPCs better replicate the disease state *in vitro*
- Markers allow for easy endpoint readout
- Can differentiate to neuronal and glial cells
- Easy to use and saves time

ATCC® NPC offerings

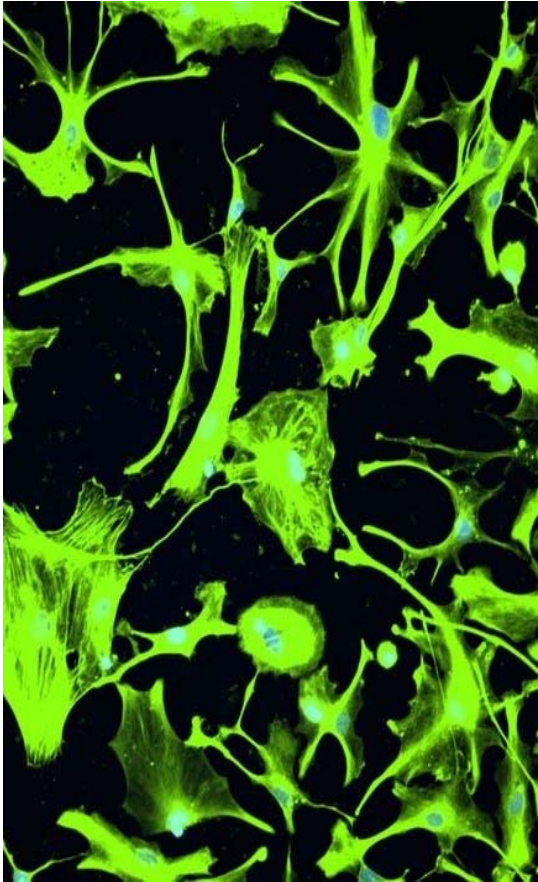
ATCC® No.	Designation
<u>ACS-3003</u> TM	NPC Growth Kit – <i>add to DMEM/F12</i>
<u>ACS-3004</u> TM	NPC Dopaminergic Differentiation Kit – <i>add to DMEM/F12</i>
<u>ACS-5001</u> TM	NPCs derived from ATCC-DYS0530 Parkinson's Disease (ACS-1013) New!
<u>ACS-5003</u> TM	NPCs derived from ATCC-BXS0117 (ACS-1031)
<u>ACS-5004</u> TM	NPCs derived from ATCC-BYS0112 (ACS-1026)
<u>ACS-5005</u> TM	Neural Progenitor Cells derived from XCL-1 DCX-GFP <i>(for late neuron differentiation)</i>
<u>ACS-5006</u> TM	Neural Progenitor Cells derived from XCL-1 GFAP-Nanoluc®-Halotag® <i>(for astrocyte differentiation)</i>
<u>ACS-5007</u> TM	Neural Progenitor Cells derived from XCL-1 MAP2-Nanoluc®-Halotag® <i>(for early neuron differentiation)</i>
<u>ACS-2103F</u> TM	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

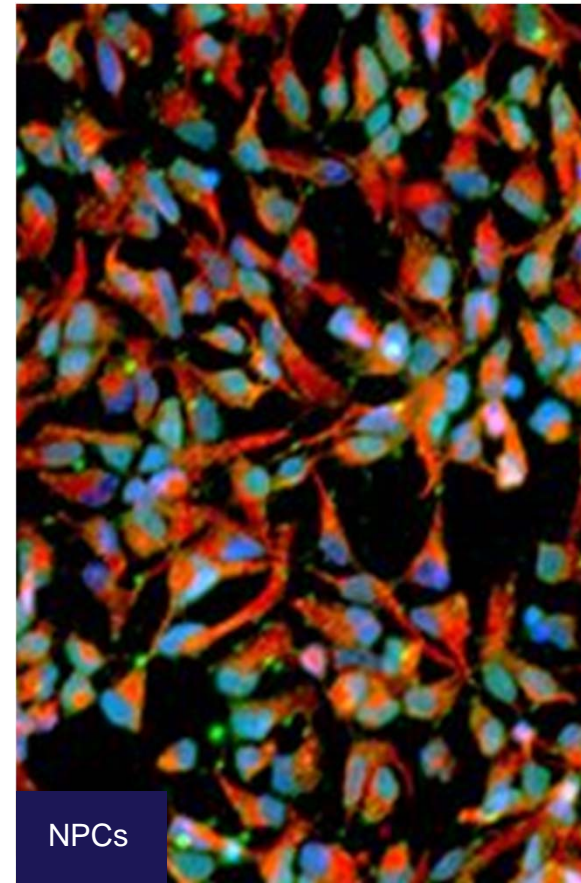


- Post-thaw cell viability: >80%
- Post-thaw viable cell number: >1x10⁶ cells/vial
- Longevity: >15 PDLs or 5 passages
- NPC marker expression: Nestin⁺, Pax-6⁺, and Tra-I-60⁻
- Differentiation potential:
 - Tuj1⁺ early neurons
 - TH⁺ dopaminergic neurons
- Identity: STR profile matching parental iPSC line
- Sterility, mycoplasma, and viral panel testing: None detected

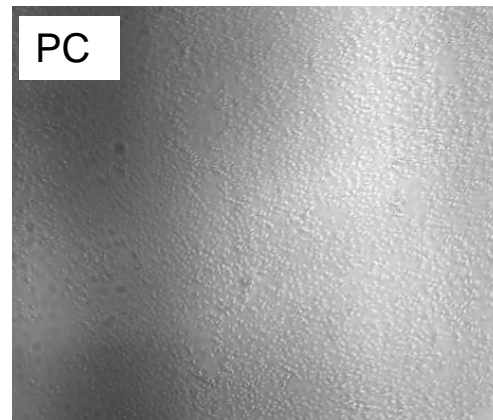
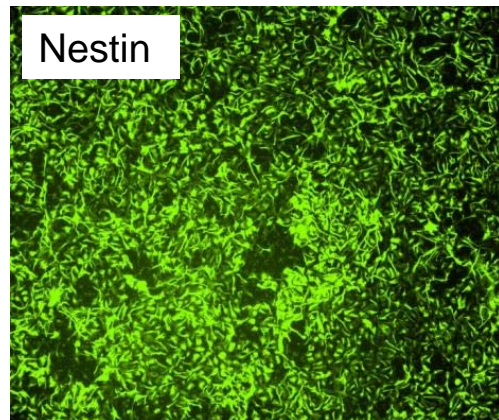
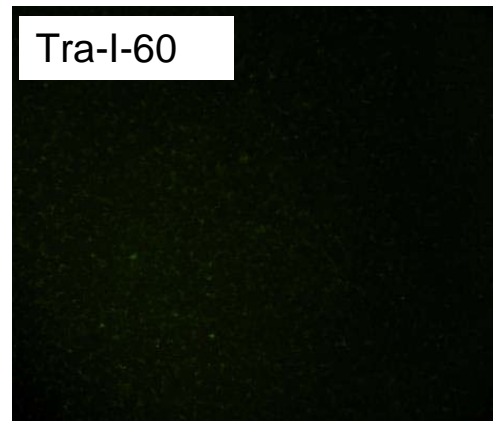
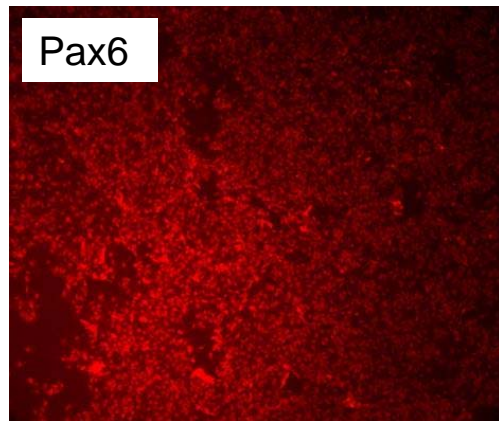
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- Background information
- **Differentiation potential of ATCC NPCs**
- Toxicological studies
- Summary

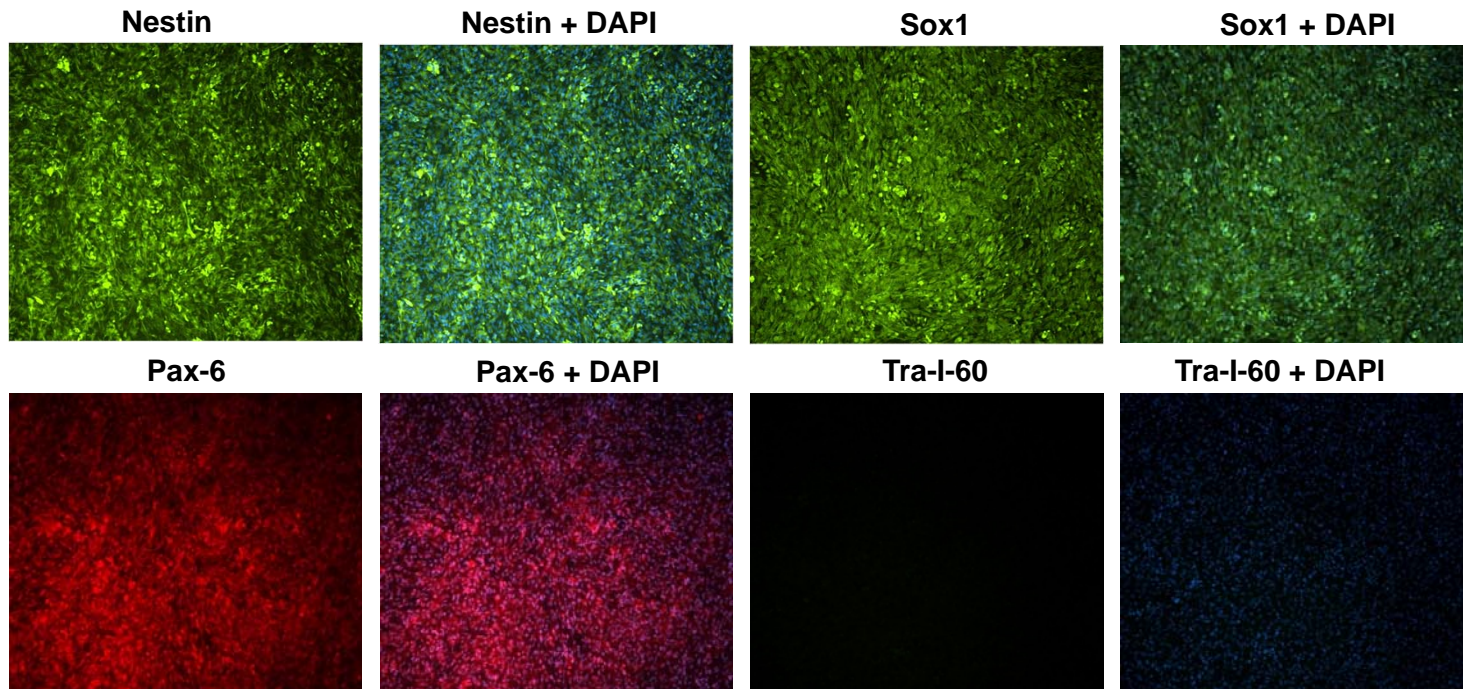


ATCC normal NPCs express NPC markers but *not* iPSC markers

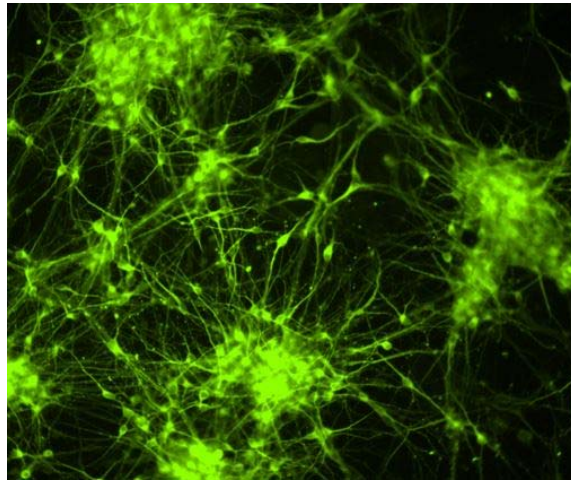


- NPC Marker
 - Nestin
 - Pax-6
- iPSC Marker
 - Tra-I-60

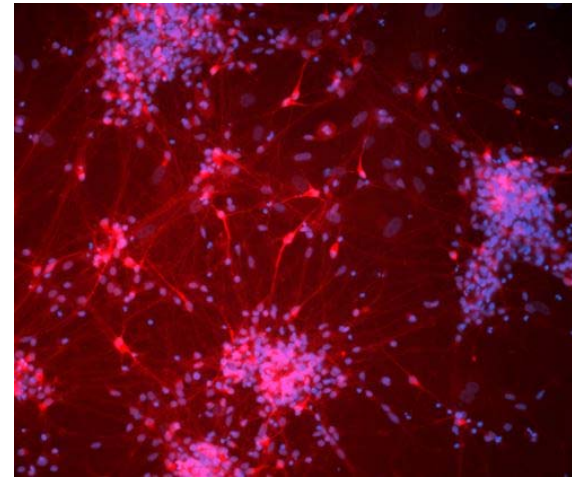
ATCC Parkinson's NPCs express NPC markers but not iPSC markers



Dopaminergic neuron differentiation of NPCs

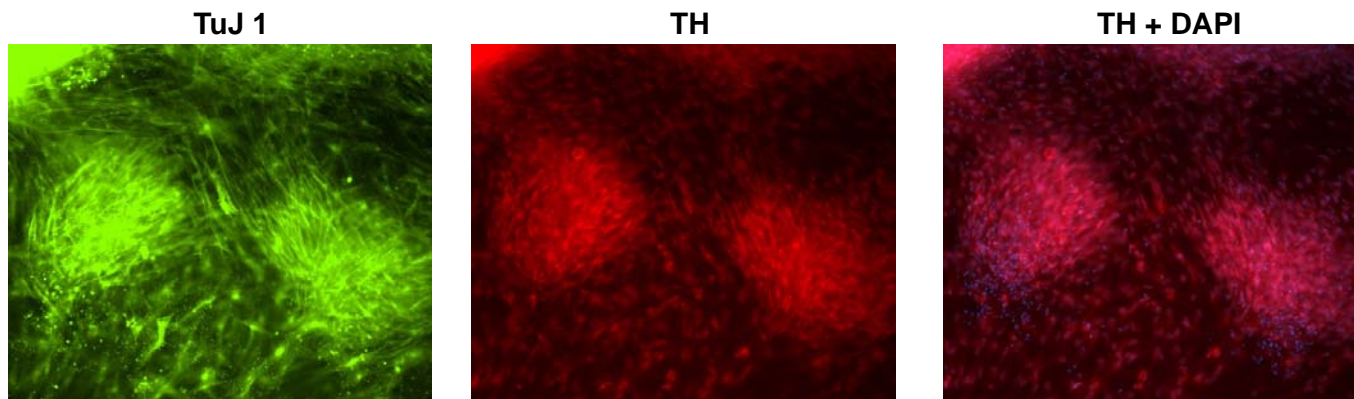


TuJ1

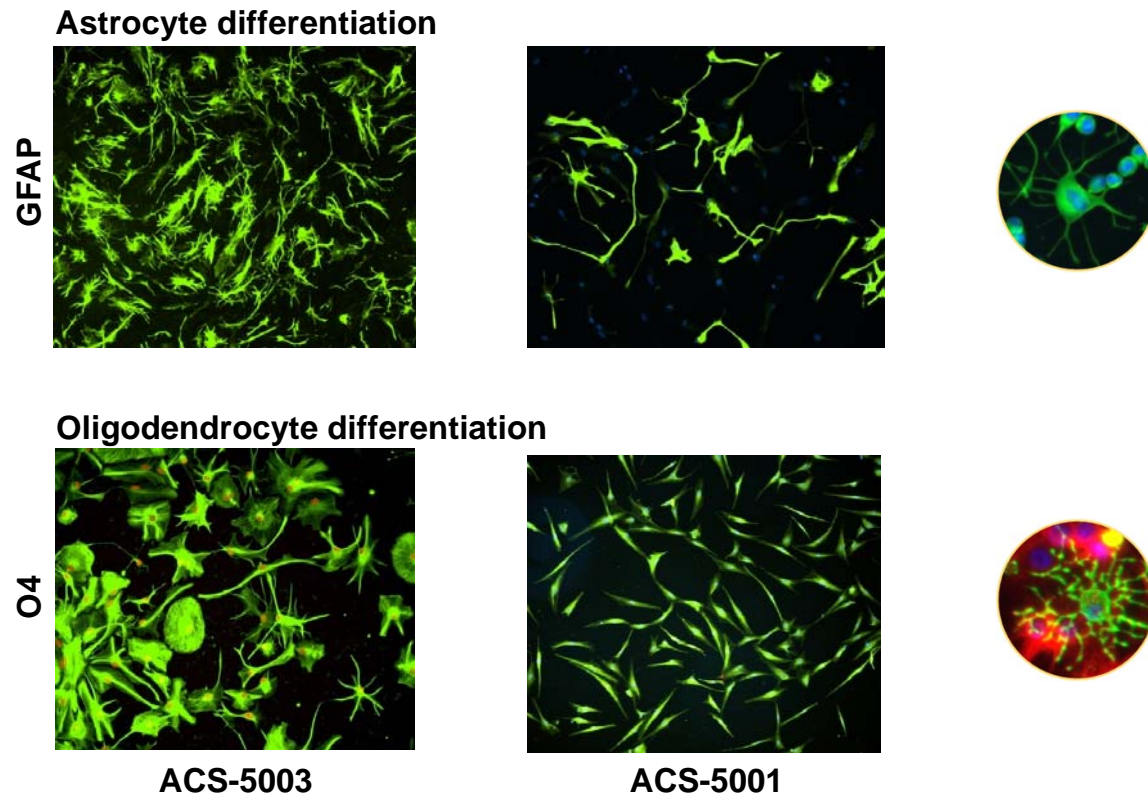


TH/DAPI

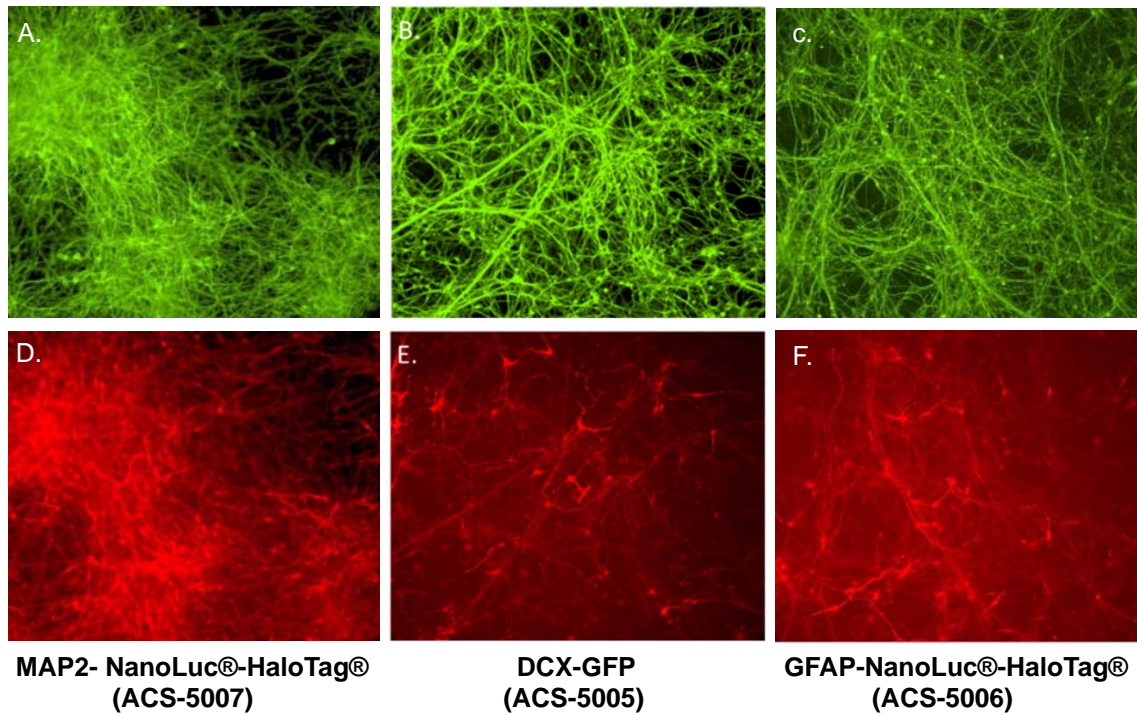
Dopaminergic neuron differentiation of Parkinson's disease NPCs



Astrocyte and oligodendrocyte differentiation

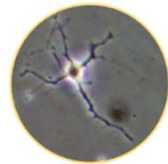


Dopaminergic neuron differentiation of NPC reporter lines

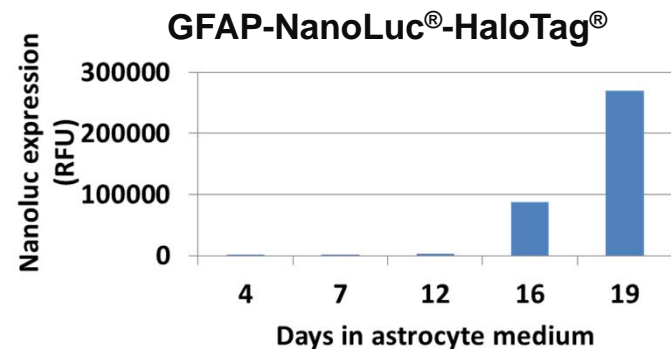
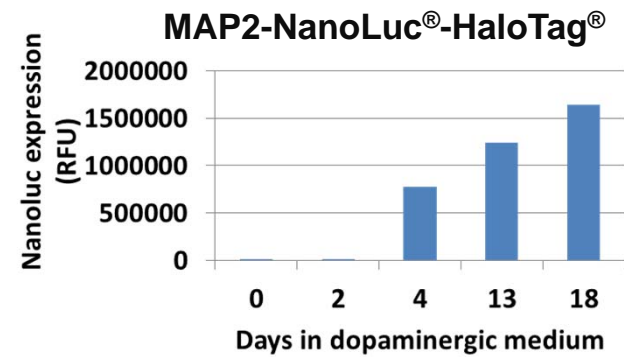
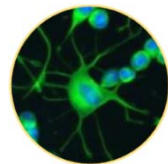


Expression of the luciferase reporter during dopaminergic neuron or astrocyte differentiation

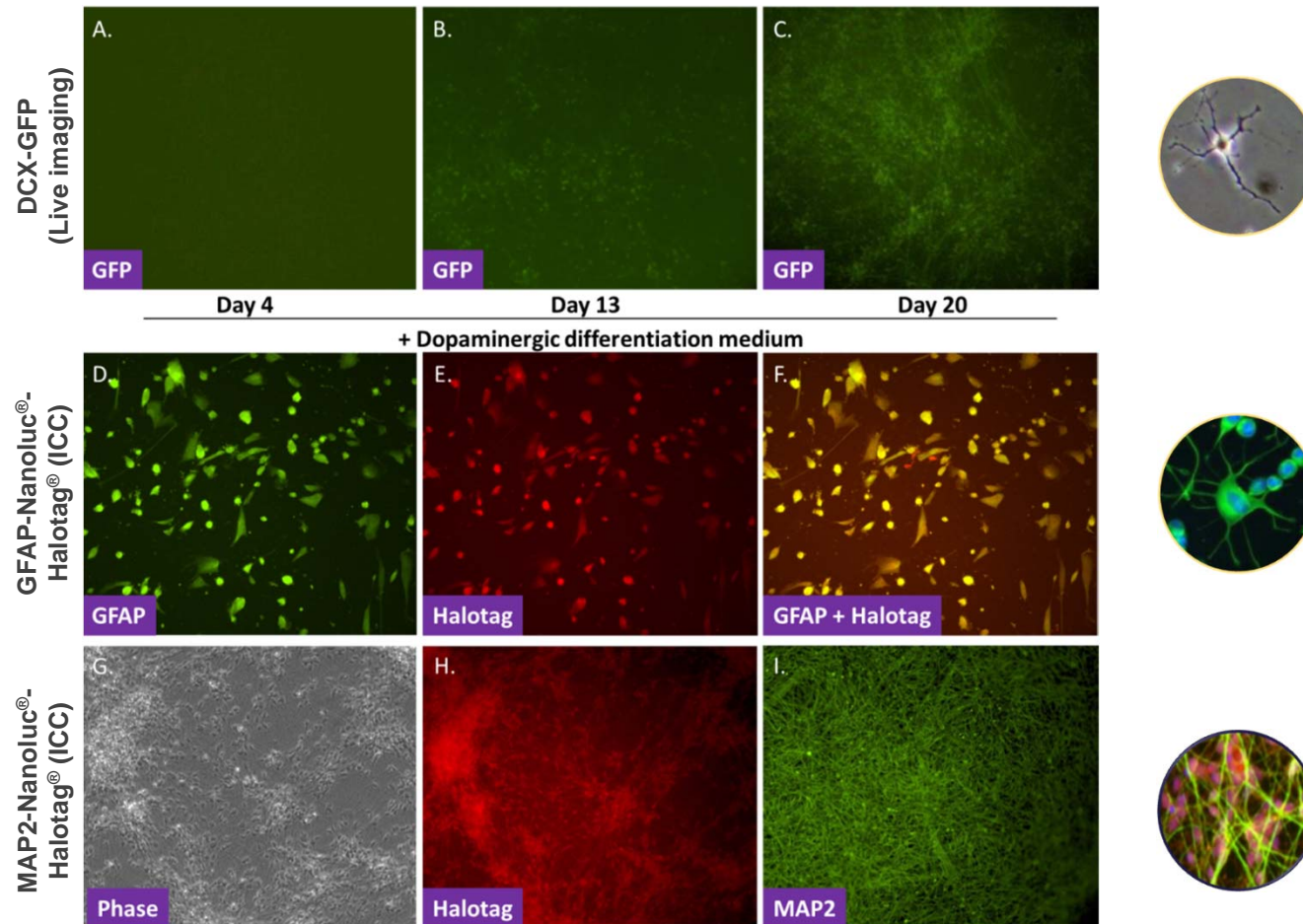
Luciferase secretion during dopaminergic neuron differentiation of NanoLuc[®]-HaloTag[®] NPCs



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



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



Expression of genes associated with the differentiation of NPCs

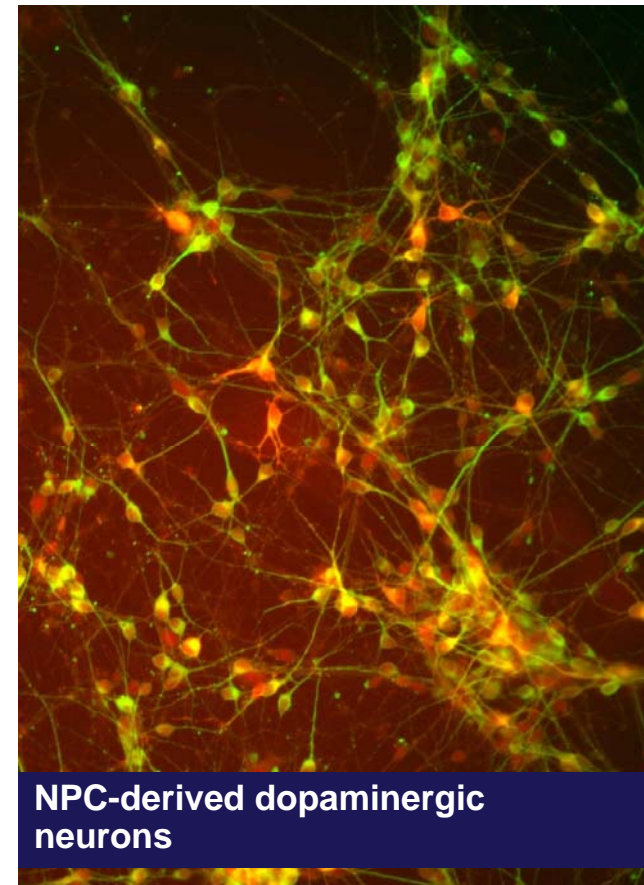


TaqMan[®] primers were used to identify the presence of other types of neurons during dopaminergic neuron differentiation using ATCC[®] ACS-3004[™] media

- Dopaminergic neurons: TH, NURR1, VMAT2, AADC
- Glutamatergic neurons: GLS2, vGLUT1, vGLUT2
- GABAergic neurons: GABA (GABRB3)
- Motor neurons: EN1, LIM3, and Hb9
- Cholinergic neurons: ChAT

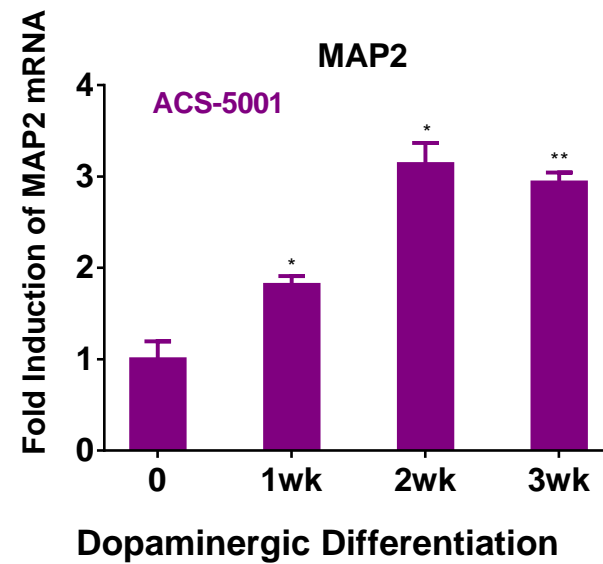
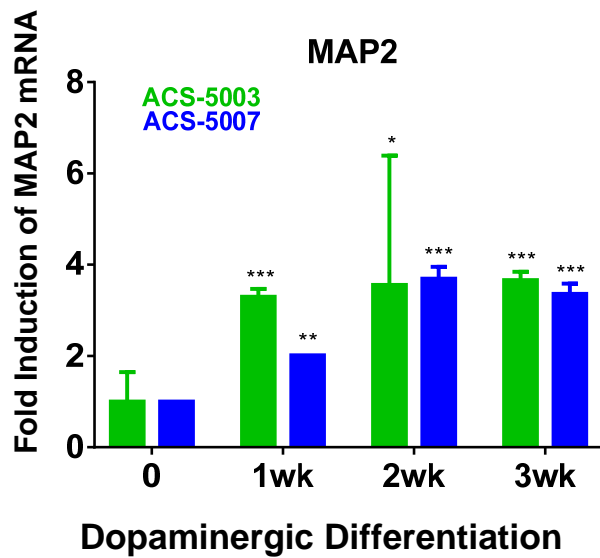
Early and dopaminergic neuron gene expression

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

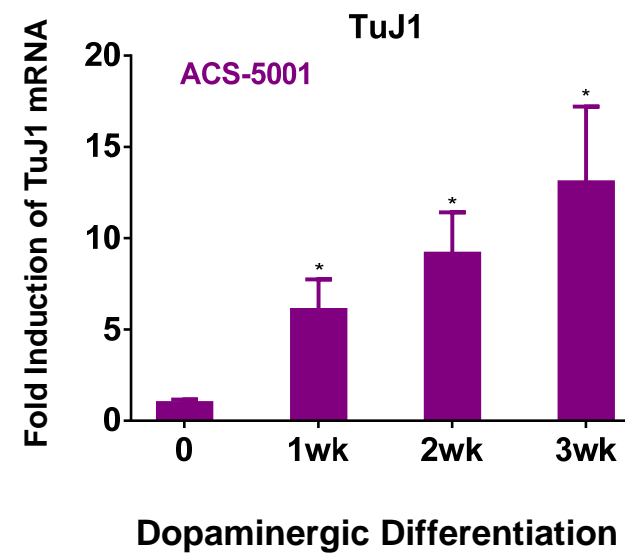
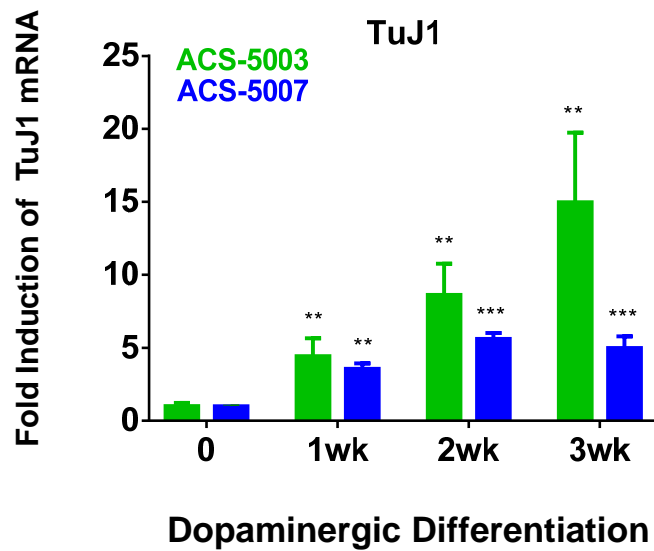


NPC-derived dopaminergic neurons

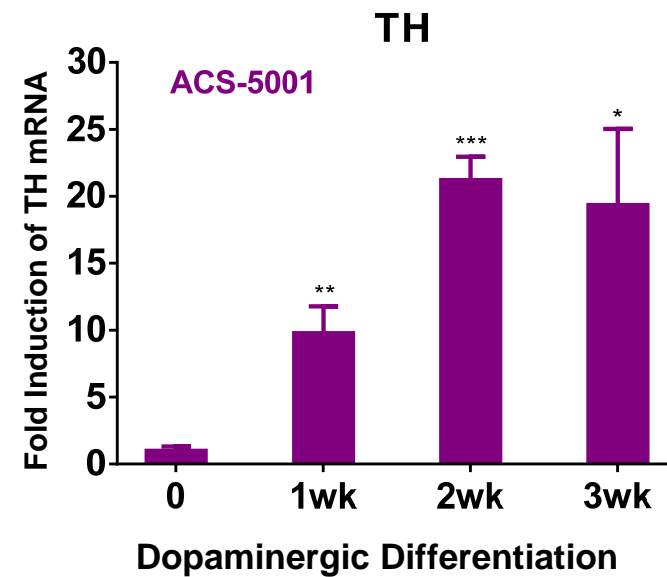
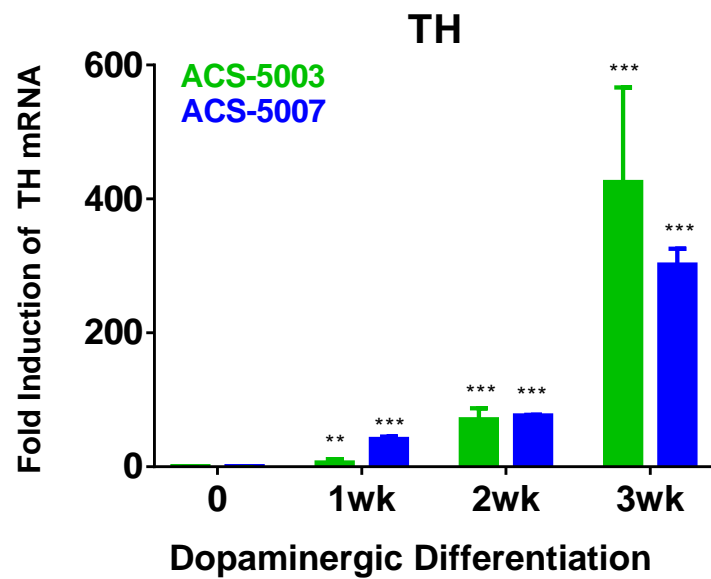
Expression of early neuron gene MAP2



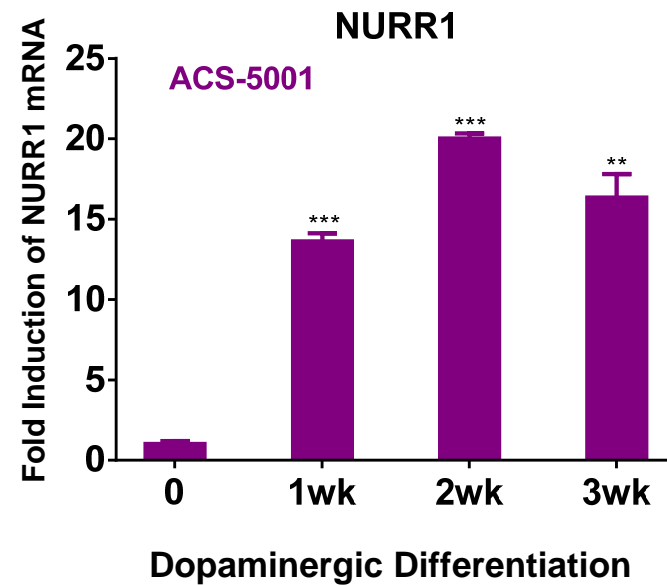
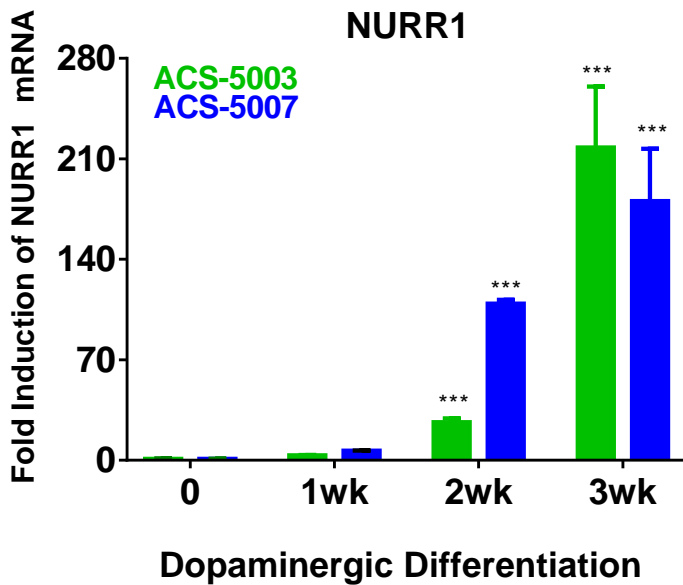
Expression of dopaminergic neuron gene TuJ1



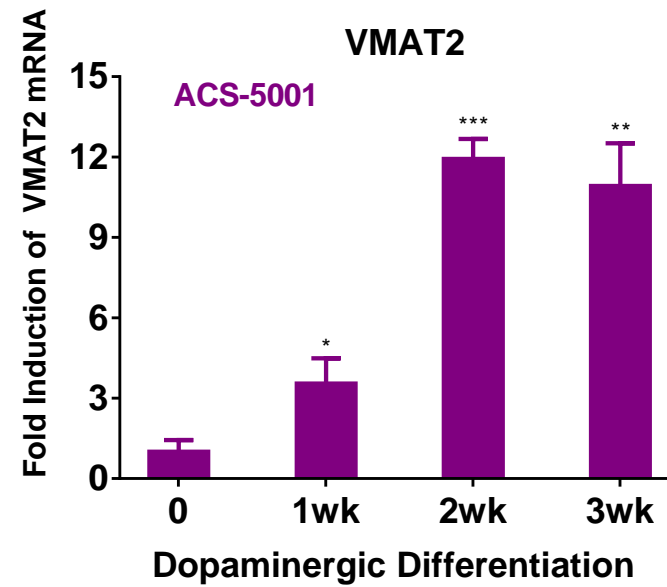
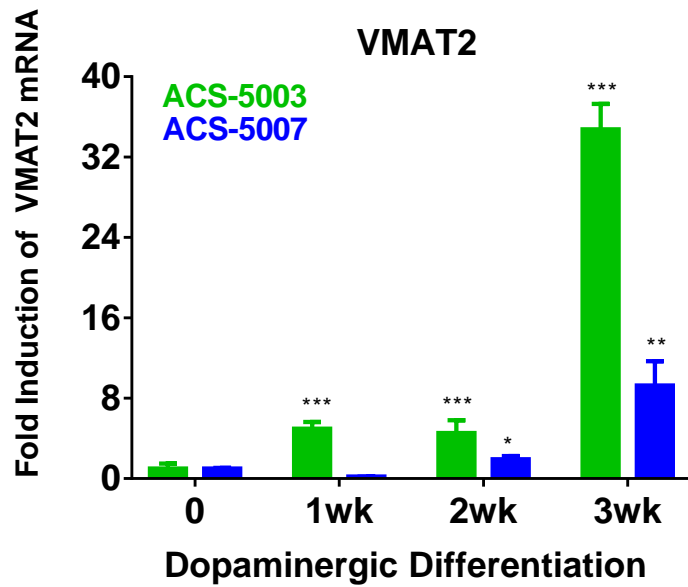
Expression of dopaminergic neuron gene TH



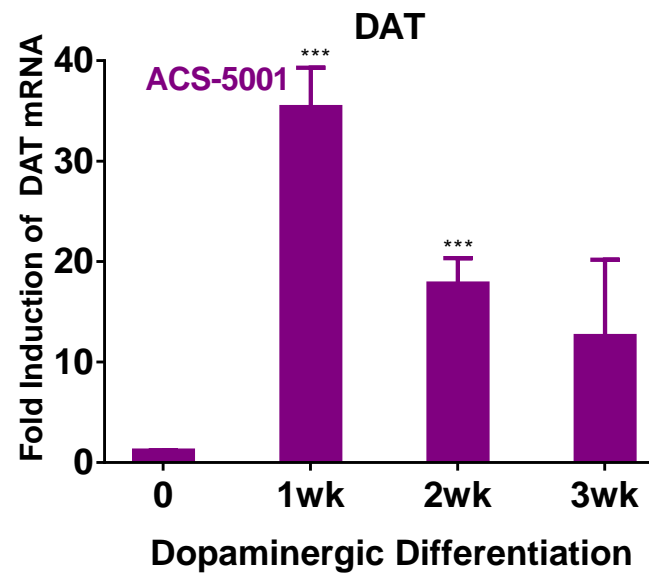
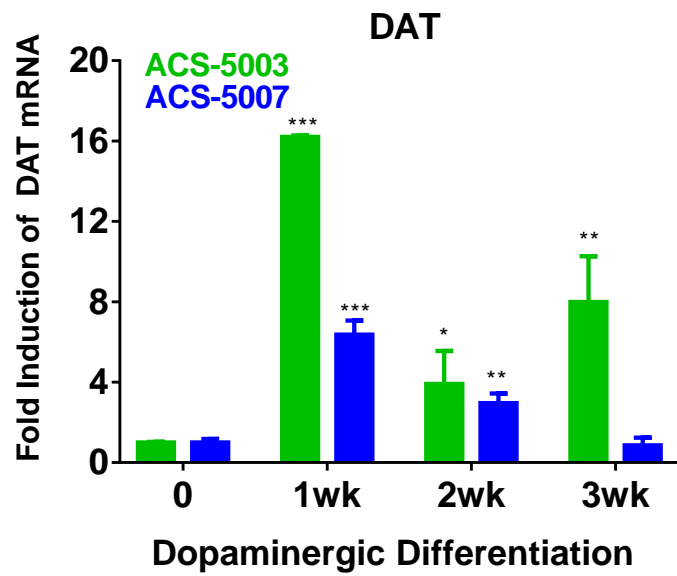
Expression of dopaminergic neuron gene NURR1



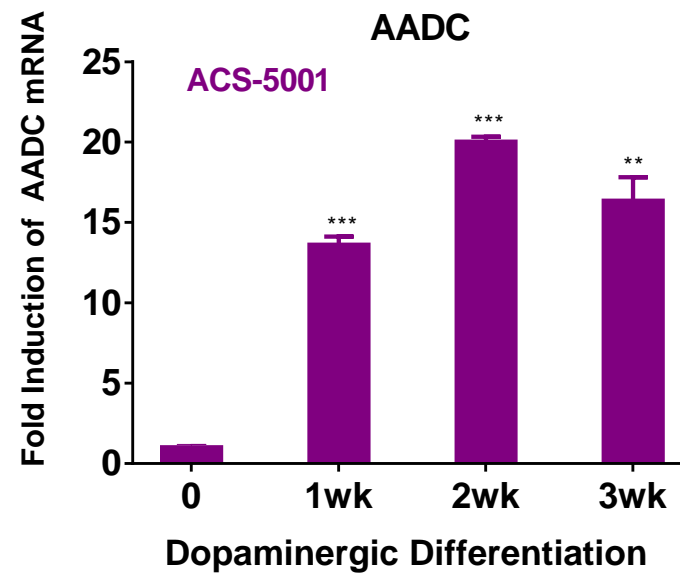
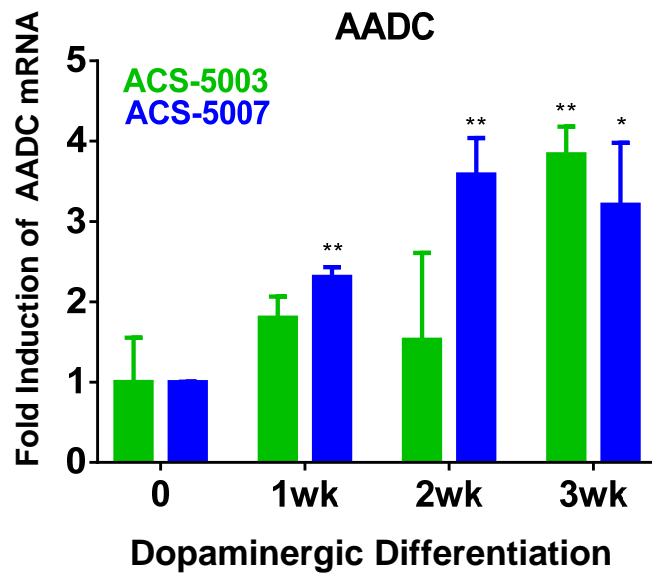
Expression of VMAT2



Expression of DAT



Expression of AADC



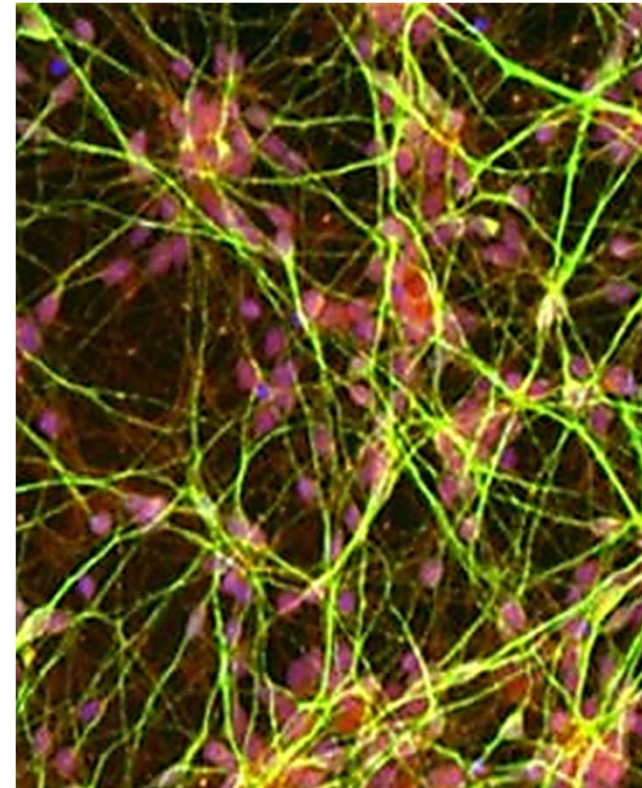
Gene expression other neuronal subtypes

ATCC® No.	Gutamatergic			GABAergic	Motor			Cholinergic
	GLS2	vGLUT1	vGLUT2	GABRB3	EN1	LIM3	Hb9	ChAT
ACS-5001	+	++	+++	++	++	++	++	+
ACS-5003	+++	+++	+++	++	++	++	+++	-
ACS-5007	+	++	+++	++	-	-	-	++

- = no significant increase in expression after 3 weeks
- + = increased expression within 3 weeks, fold over control

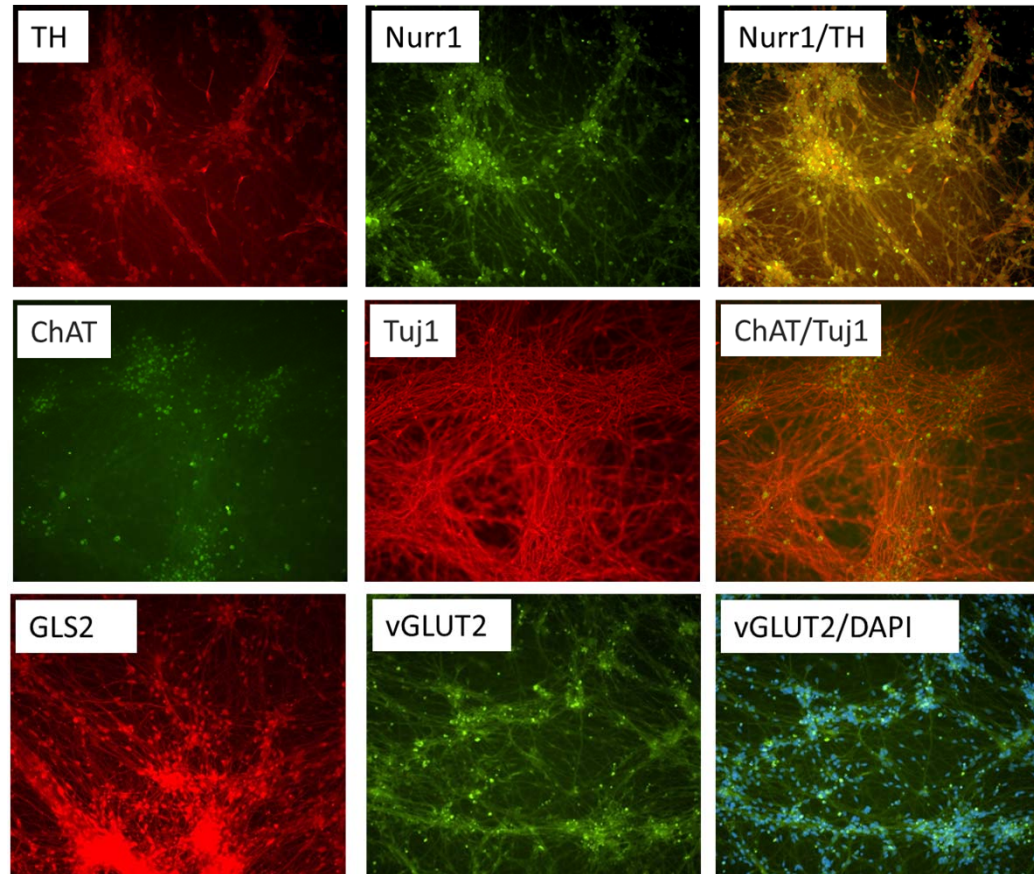
Protein expression

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



NPC-derived neurons

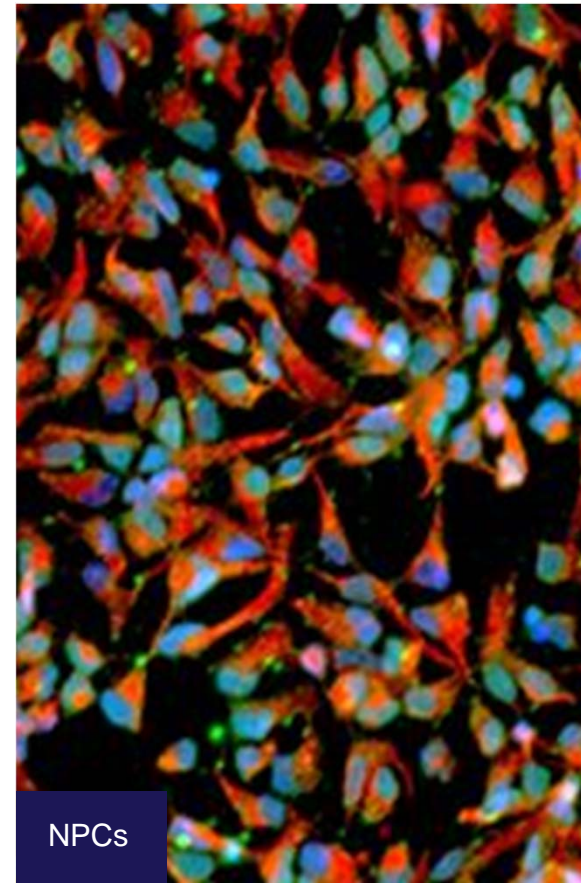
Confirmation of dopaminergic neuronal-specific protein expression during differentiation by immunocytochemistry



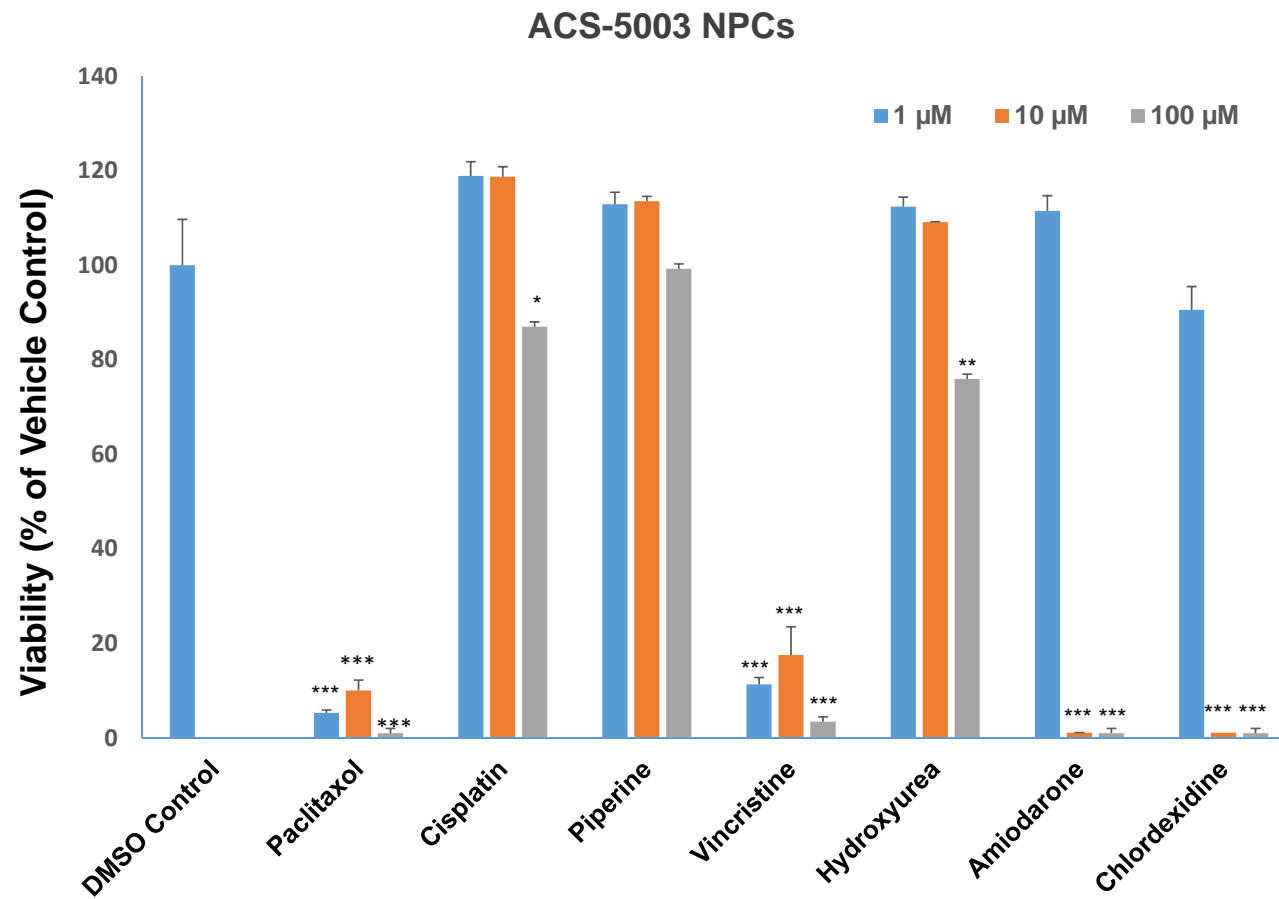
Agenda

Neural Progenitor Cells (NPCs) and Media

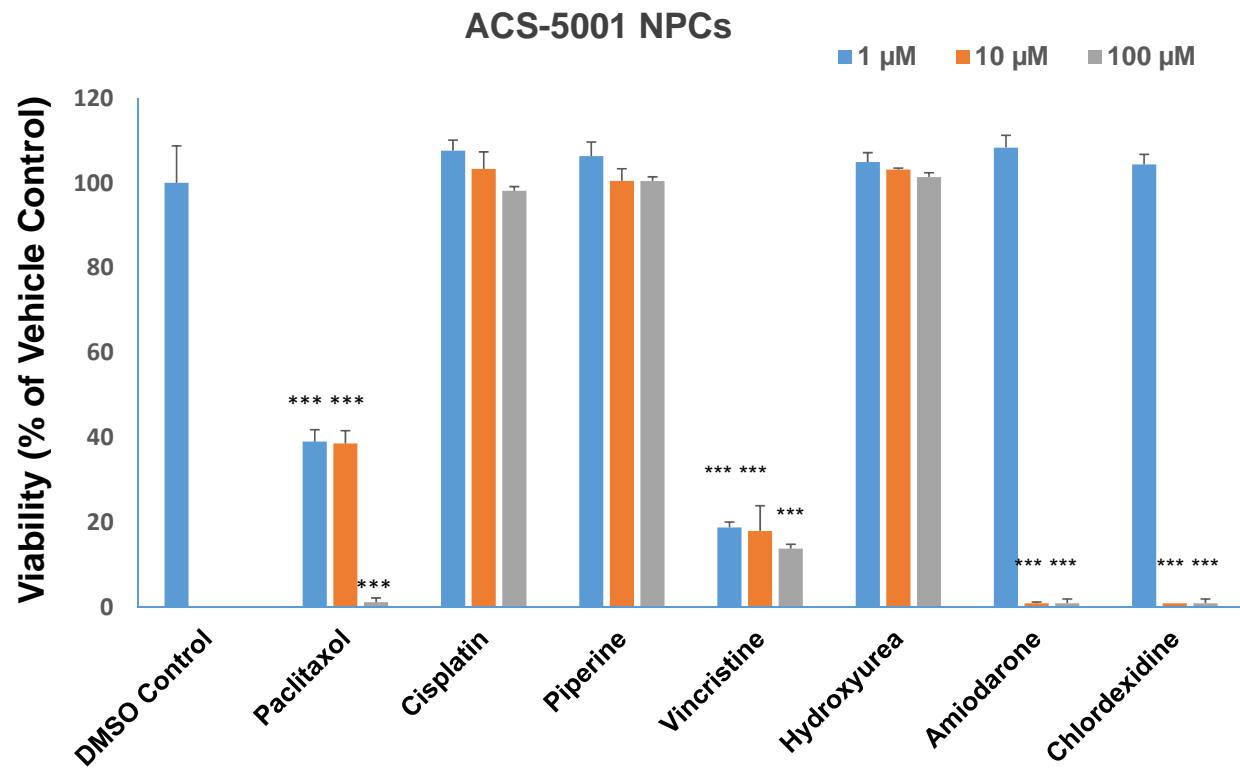
- Background information
- Differentiation potential of ATCC NPCs
- **Toxicological studies**
- Summary



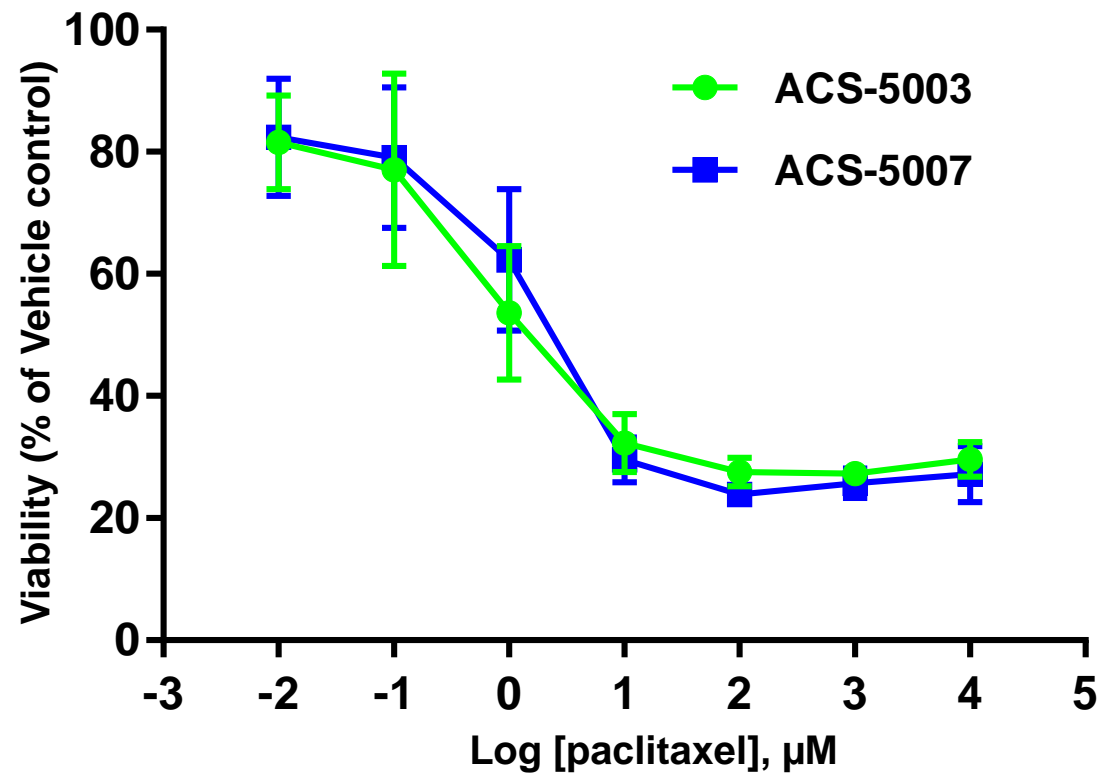
Neurotoxicity studies – undifferentiated NPCs



Neurotoxicity studies – undifferentiated NPCs

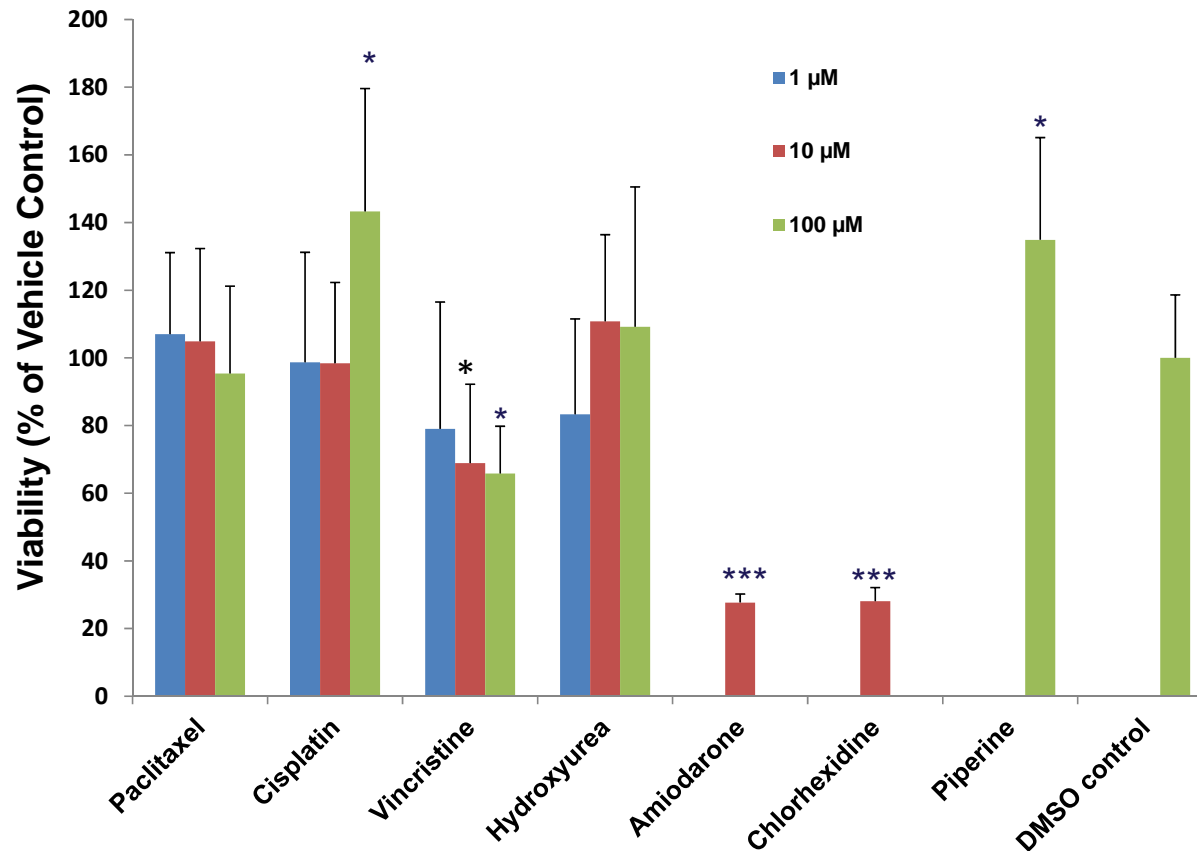


Dose-response curves for cell viability of ACS-5003 ACS-5007 NPCs treated with paclitaxel for two days

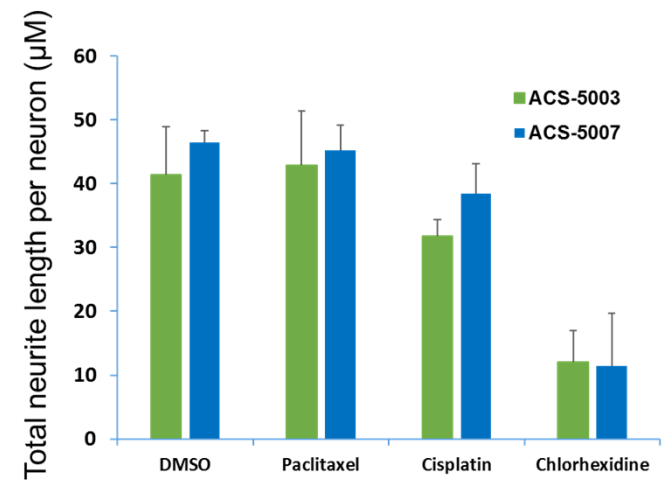
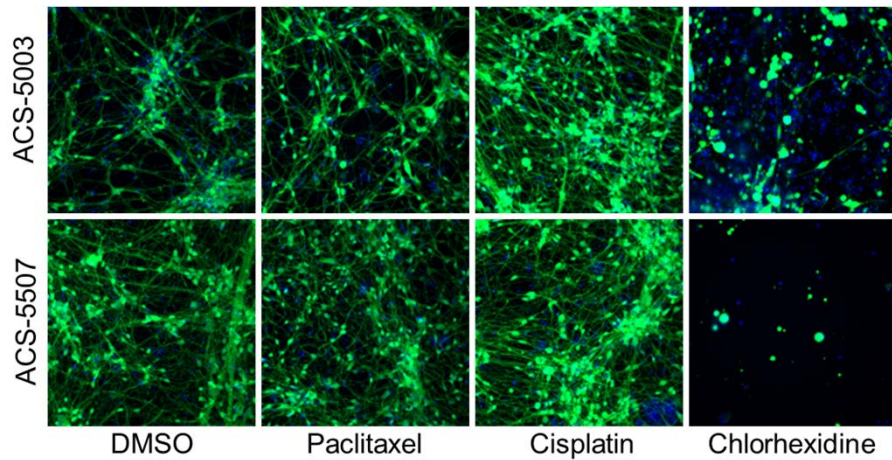


Neurotoxicity studies – NPCs-derived neurons

ACS-5007 NPCs-derived neurons



High content imaging analysis of neurotoxicity in normal NPC-derived neurons



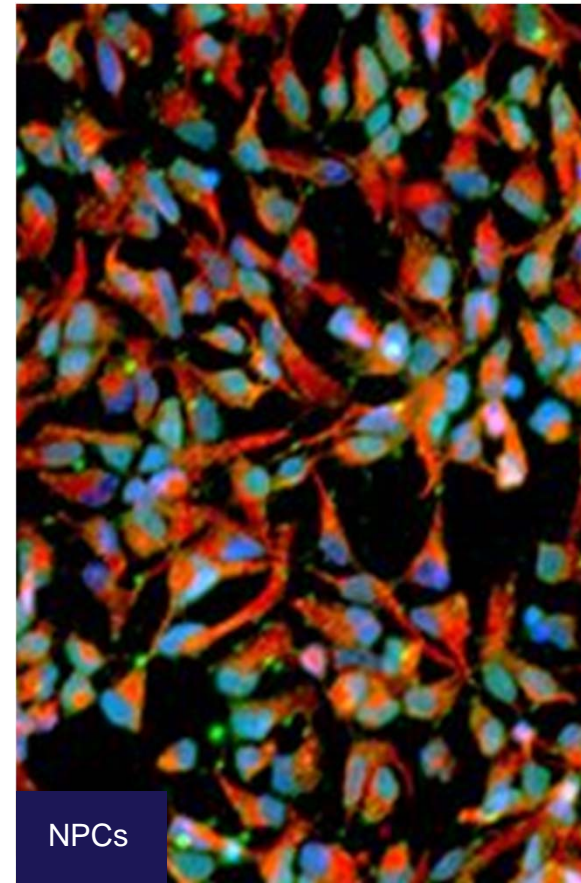
Overall neurotoxicity studies

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

Agenda

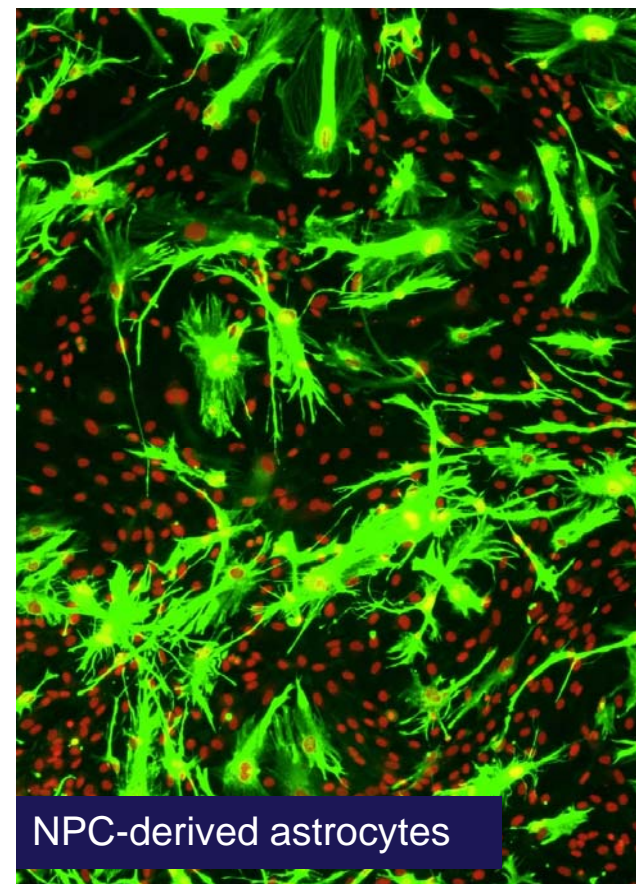
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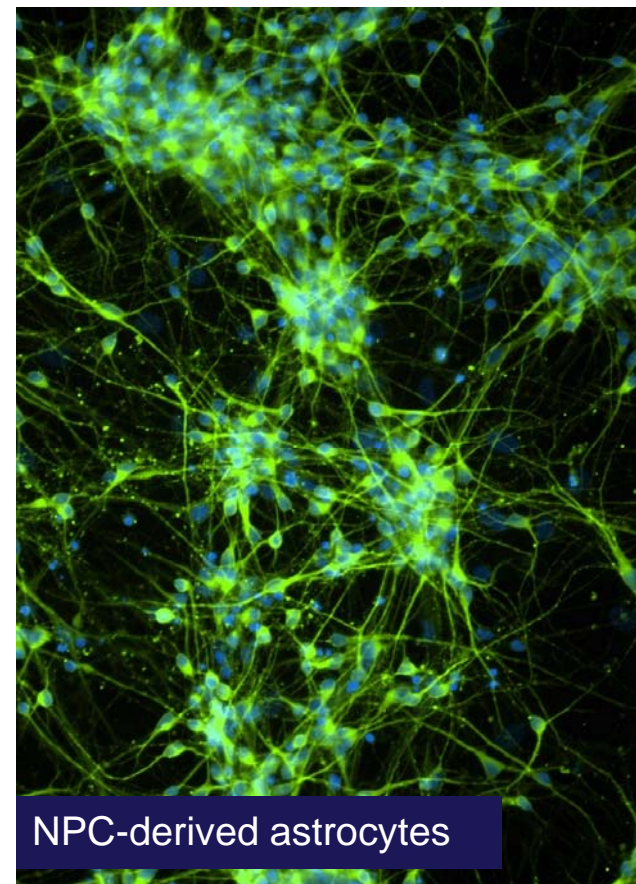
NPCs – Summary

- 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



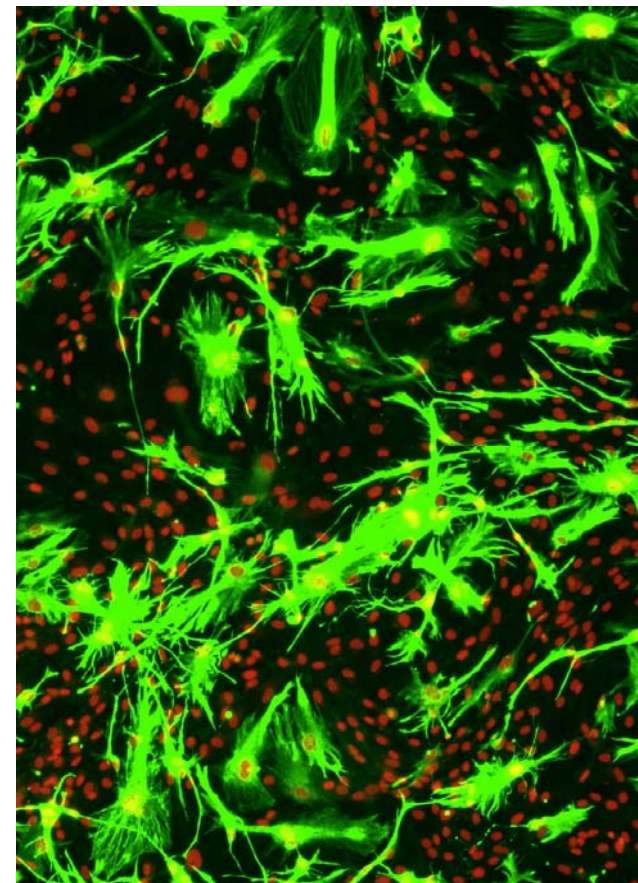
NPCs – Summary

- Our studies demonstrated that ATCC normal and PD 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 are suitable for drug screening applications



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Cultivating collaboration to elevate biological models

Let's continue to cultivate collaboration:

- Help us elevate our Better Biological Models
- Advanced biological models enable greater
 - Specificity
 - Functionality
- Join our community of early adopters
- Our partnership with you, the scientific community, allows us all to reach the incredible

2020.atcc.org/elevating-biological-models
for more information about becoming an early adopter of NPCs

