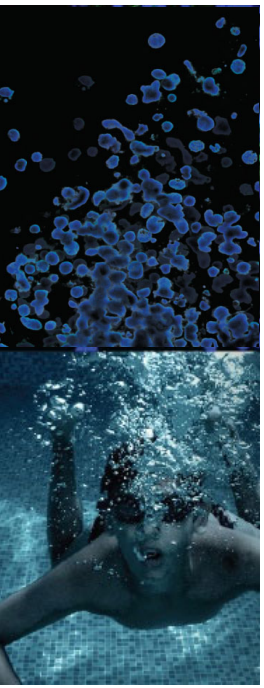
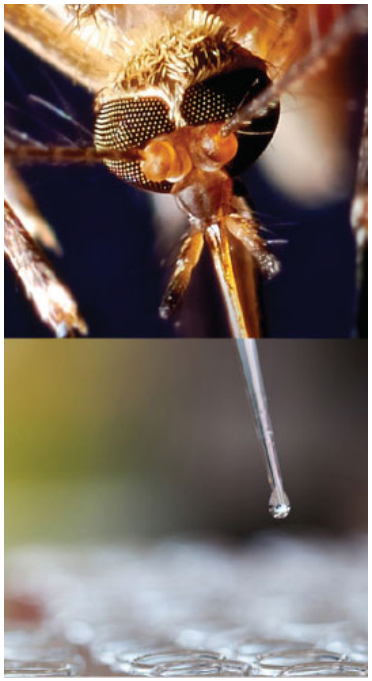
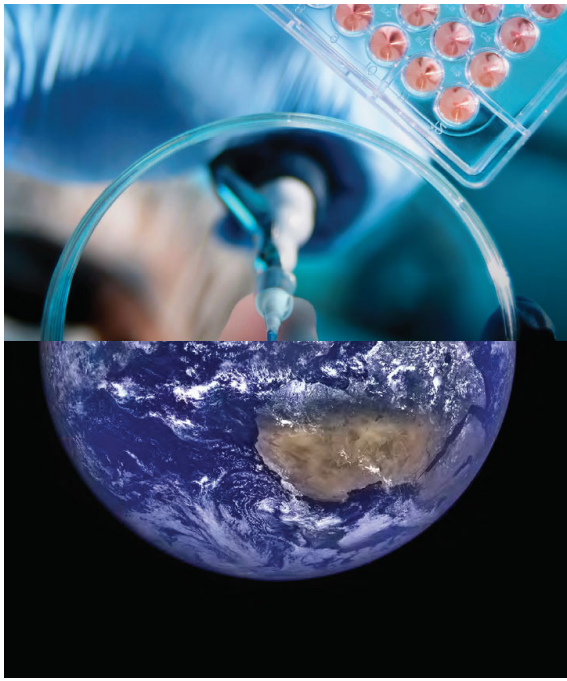
Modeling toxicity with Neural Progenitor Cell-derived Neurospheres

Brian Shapiro, PhD
Scientific Content Specialist, ATCC

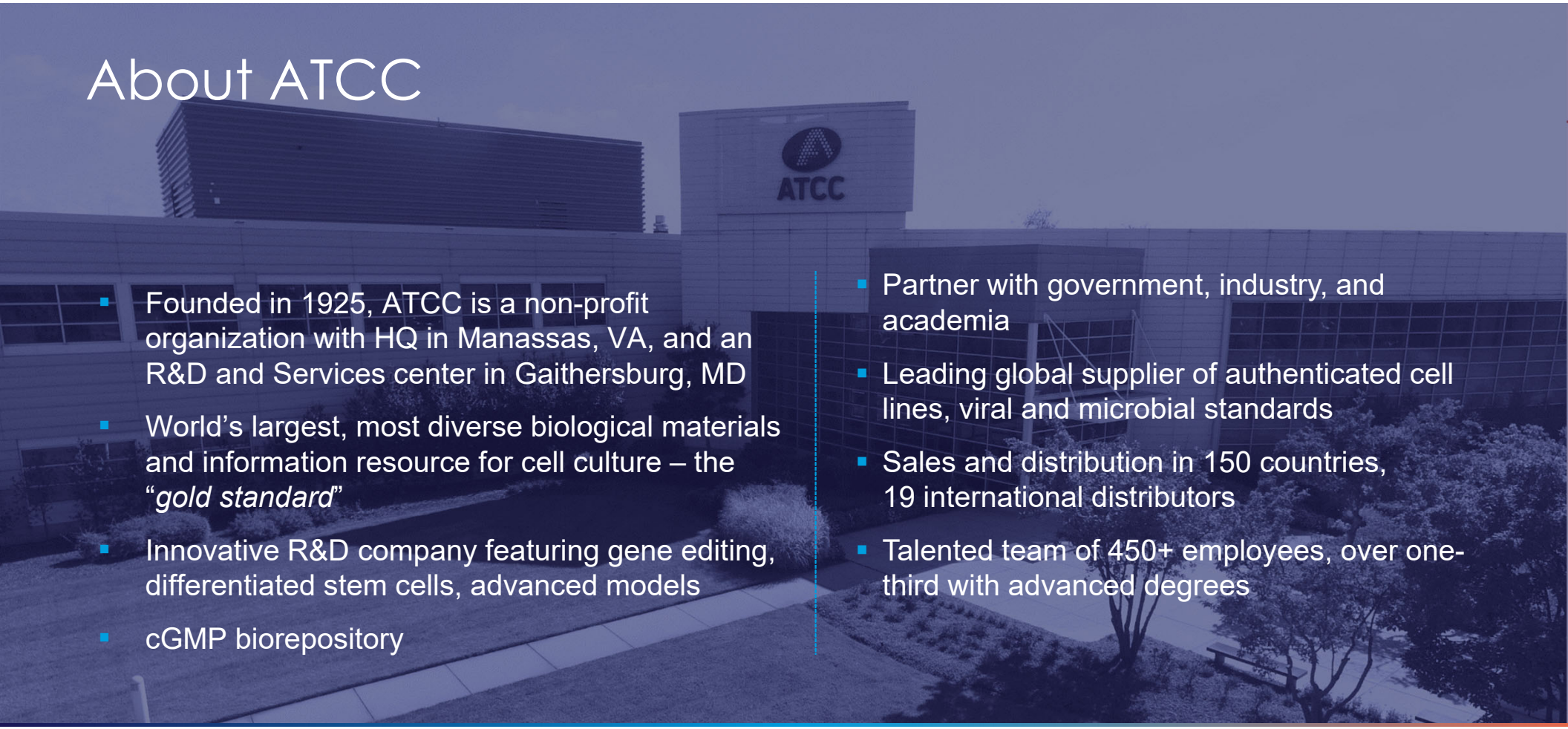






Credible Leads to Incredible™

About ATCC



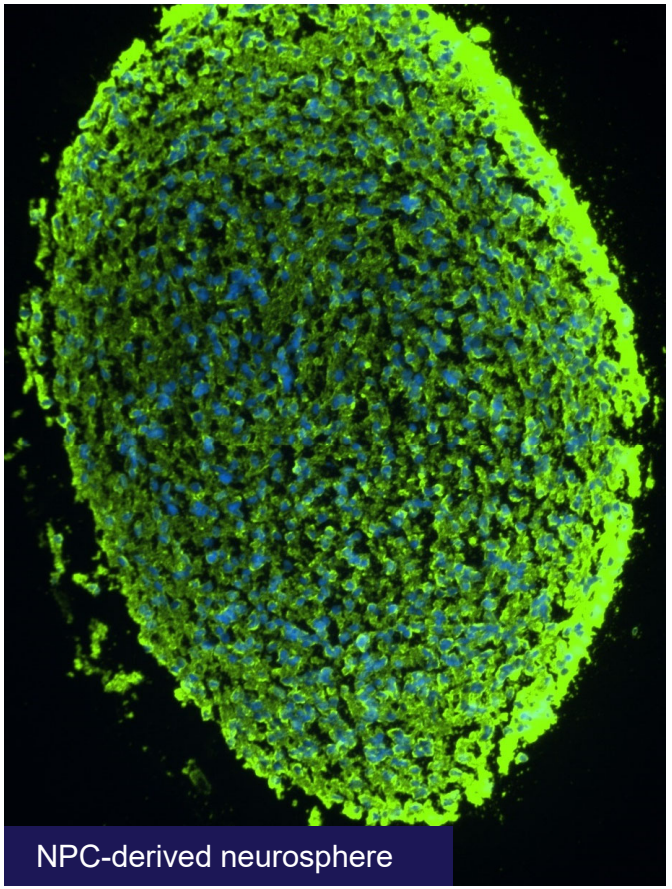
- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring gene editing, differentiated stem cells, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

ATCC®

Agenda

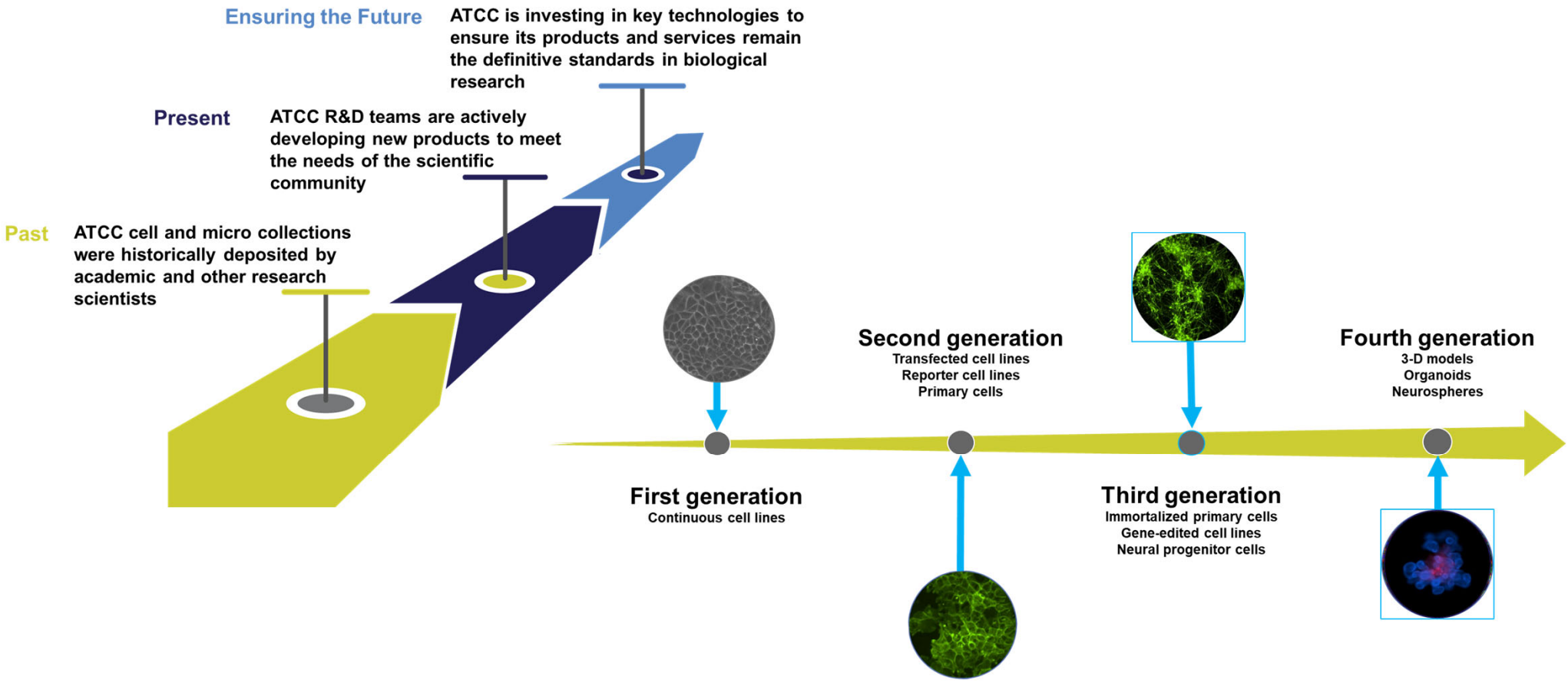
Neural Progenitor Cells (NPCs)

- ATCC roadmap and neurological resources
- Background information
- Differentiation potential of ATCC NPCs
- Neurosphere generation
- Toxicological studies using neurospheres
- Summary



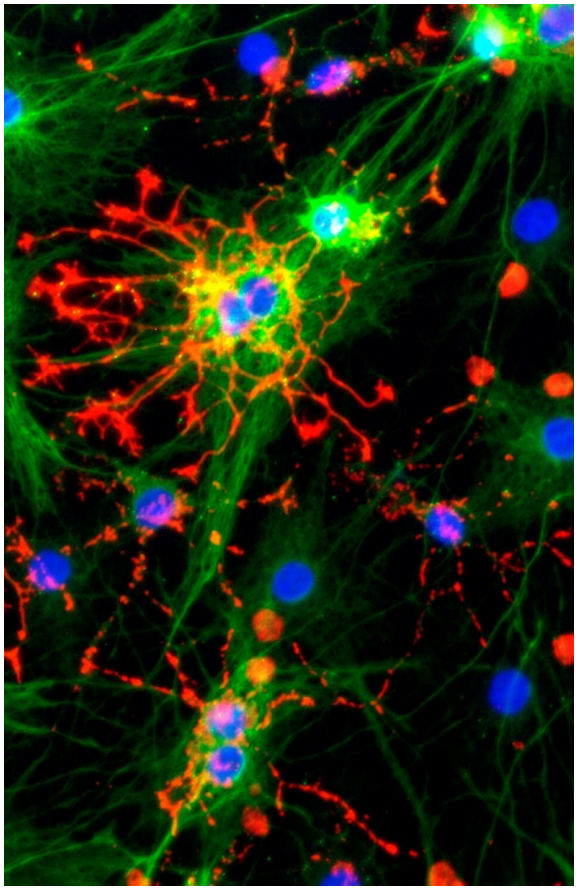
NPC-derived neurosphere

Modernization of the ATCC neurological portfolio: Evolution of in vitro cell models



ATCC products for neurology

- ATCC is the complete solution supplier for neuroscientists
- From basic research through discovery and development to product testing
  - Continuous cell lines
  - hTERT-immortalized primary NTAP Schwann cells
  - Michael J. Fox Foundation cell lines
  - Neural progenitor cells
  - Human Cancer Model Initiative 2-D and 3-D products
- Portfolio features
  - Reliability
  - Fully characterized cells
  - Optimized growth protocols
  - Scalability into all aspects of the neuroscience workflow
  - Biological relevancy



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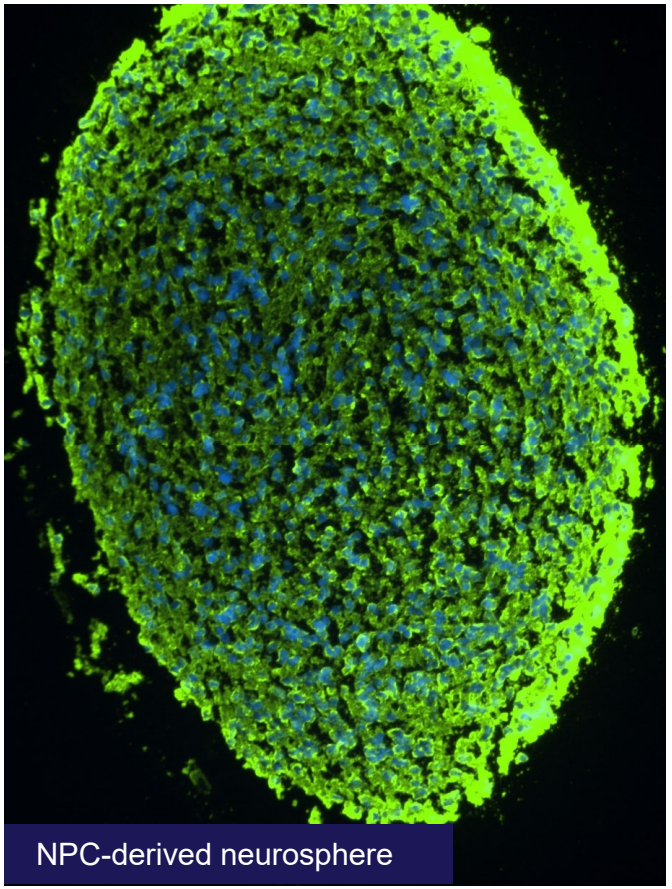
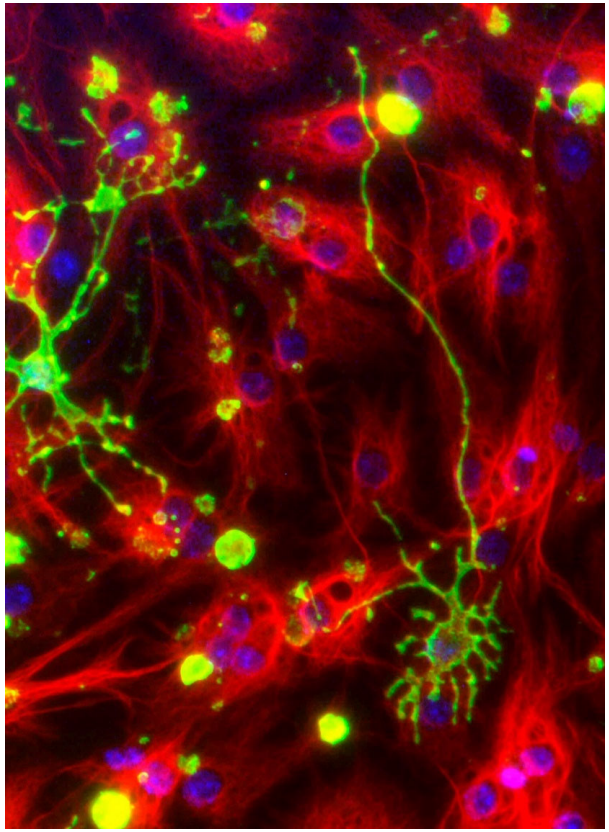


NPC-derived neurosphere

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
- 2D vs. 3D models

Cells 2020, 9, 1122; doi:10.3390/cells9051122:

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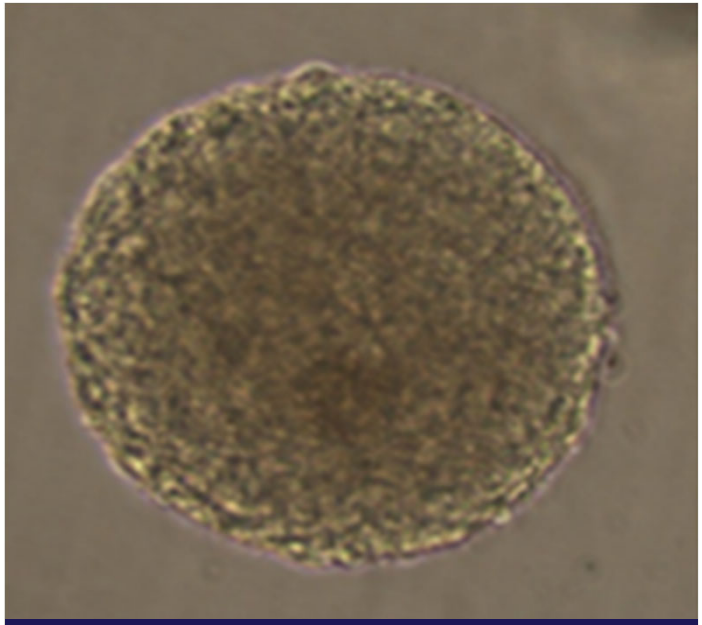
Neurosphere characteristics

- A neural model system
  - A 3-D model
  - Free-floating spherical clusters of neural stem cells
  - Can be used in undifferentiated state
  - Can be differentiated into multiple subtypes
  - At different timepoints the neurospheres can represent different neural developmental stages
  - Multiple methods of generation are available
- Neurosphere features
  - Expression levels of markers:
    - Nestin positive, neurofilament and GFAP negative
    - Culture environment-dependent after differentiation
  - Size <50 to >600 µm
  - Amenable to growth in 96-well formats/high-throughput screening
  - Assays: apoptosis, neurite outgrowth, proliferation, ICC, etc…



Neurosphere derived from NPCs

ATCC

Neurospheres in the literature

Published in final edited form as:
Cell Stem Cell. 2017 April 06; 20(4): 435–449.e4. doi:10.1016/j.stem.2016.12.007.

Human iPSC-derived cerebral organoids model cellular features of lissencephaly and reveal prolonged mitosis of outer radial glia

Marina Bershteyn[1,2,6,*], **Tomasz J. Nowakowski**[1,3], **Alex A. Pollen**[1,3], **Elizabeth Di Lullo**[1,3], **Aishwarya Nene**[4], **Anthony Wynshaw-Boris**[2,5,*], and **Arnold R. Kriegstein**[1,3,7,*]

[1]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research California, San Francisco, CA, USA

Published in final edited form as:
Nature. 2014 November 13; 515(7526): 274–278. doi:10.1038/nature13800.

A three-dimensional human neural cell culture model of Alzheimer's disease

Se Hoon Choi[1,†], **Young Hye Kim**[1,2,†], **Matthias Hebisch**[1,3], **Christopher Sliwinski**[1], **Seungkyu Lee**[4], **Carla D'Avanzo**[1], **Jennifer Chen**[1], **Basavaraj Hooli**[1], **Caroline Asselin**[1], **Julien Muffat**[5], **Justin B. Klee**[1], **Can Zhang**[1], **Brian J. Wainger**[4], **Michael Peitz**[3], **Dora M. Kovacs**[1], **Clifford J. Woolf**[4], **Steven L. Wagner**[6], **Rudolph E. Tanzi**[1,*], and **Doo Yeon Kim**[1,*]

[1]Genetics and Aging Research Unit, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA

Published in final edited form as:
Toxicol Appl Pharmacol. 2018 September 01; 354: 101–114. doi:10.1016/j.taap.2018.02.003.

Rotenone exerts developmental neurotoxicity in a human brain spheroid model

David Pamies[a], **Katharina Block**[a], **Pierre Lau**[b], **Laura Gribaldo**[b], **Carlos A. Pardo**[c], **Paula Barreras**[c], **Lena Smirnova**[a], **Daphne Wiersma**[a], **Liang Zhao**[a,d], **Georgina Harris**[a], **Thomas Hartung**[a,e], and **Helena T. Hogberg**[a,*]

[a]Center for Alternative to Animal Testing (CAAT), Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205, USA

ORIGINAL RESEARCH
published: 04 December 2018
doi: 10.3389/fmicb.2018.02766

Published in final edited form as:
Cell Stem Cell. 2016 August 4; 19(2): 258–265. doi:10.1016/j.stem.2016.04.014.

Zika Virus Depletes Neural Progenitors in Human Cerebral Organoids through Activation of the Innate Immune Receptor TLR3

Jason Dang[1,3], **Shashi Kant Tiwari**[1,3], **Gianluigi Lichinchi**[1], **Yue Qin**[1], **Veena S. Patil**[1], **Alexey M. Eroshkin**[2], and **Tariq M. Rana**[1,*]

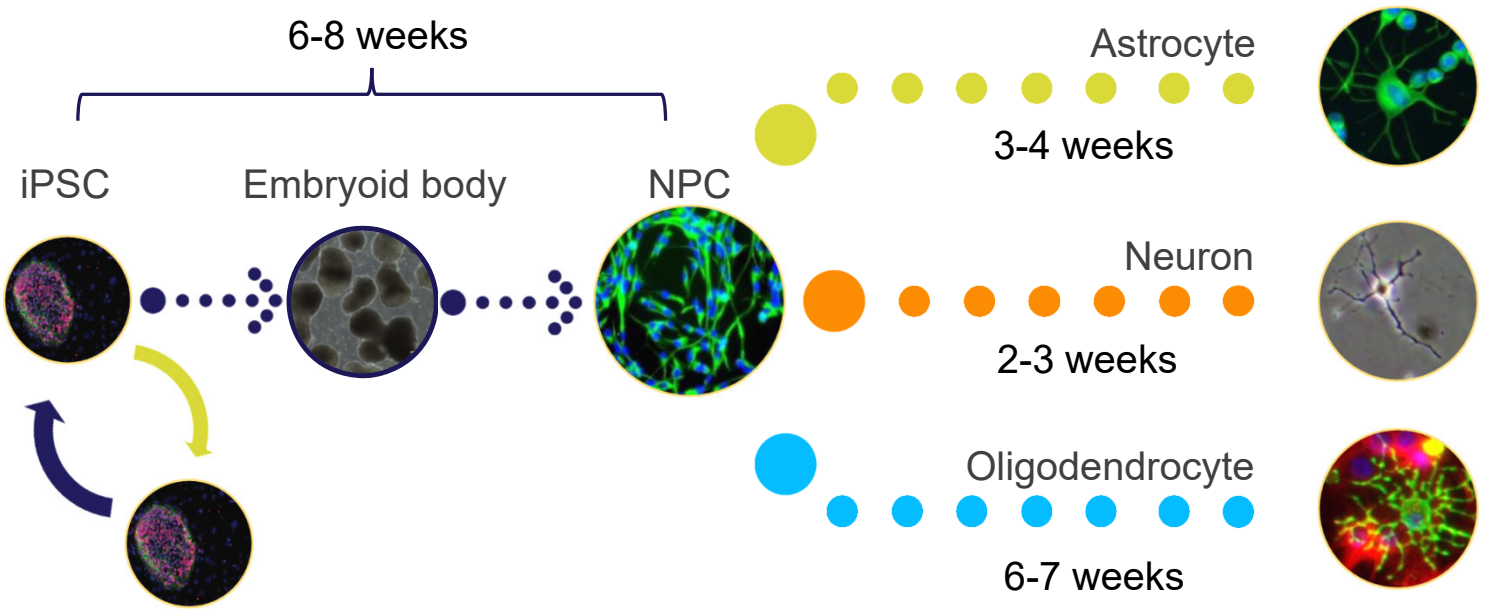
[1]Department of Pediatrics and Institute for Genomic Medicine, University of California San Diego School of Medicine, 9500 Gilman Drive MC 0762, La Jolla, California, 92093, USA

Microglia Increase Inflammatory Responses in iPSC-Derived Human BrainSpheres

*Celina Monteiro Abreu[1], Lucio Gama[1,2], Susanne Krasemann[3], Megan Chesnut[4], Shelly Odwin-Dacosta[4], Helena T. Hogberg[4], Thomas Hartung[4,5] and David Pamies[4]**

[1] Department of Molecular and Comparative Pathobiology, Johns Hopkins School of Medicine, Baltimore, MD, United States, [2] Vaccine Research Center, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, United States, [3] Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [4] Center for Alternatives to Animal Testing, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [5] CAAT-Europe, University of Konstanz, Konstanz, Germany

Neural progenitor cells – Neuronal differentiation



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

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-2103F™	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)

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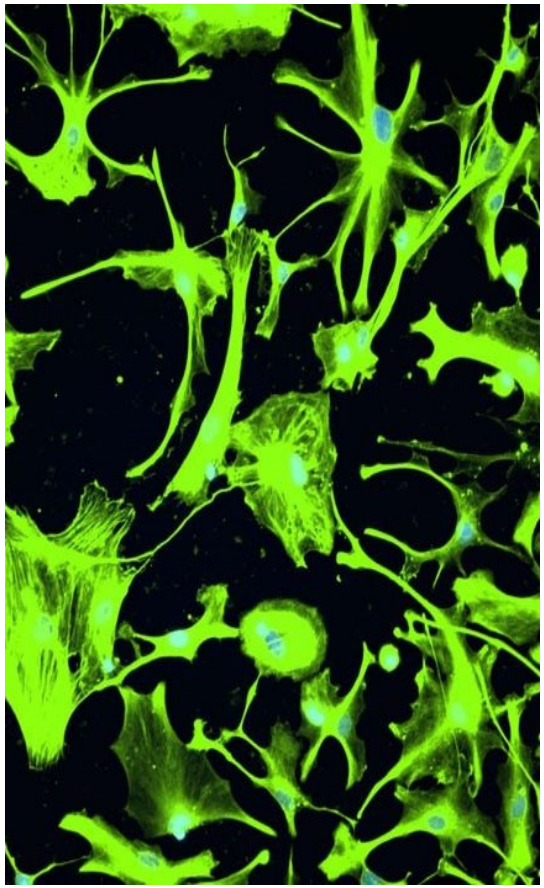
QC testing of NPCs



- 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

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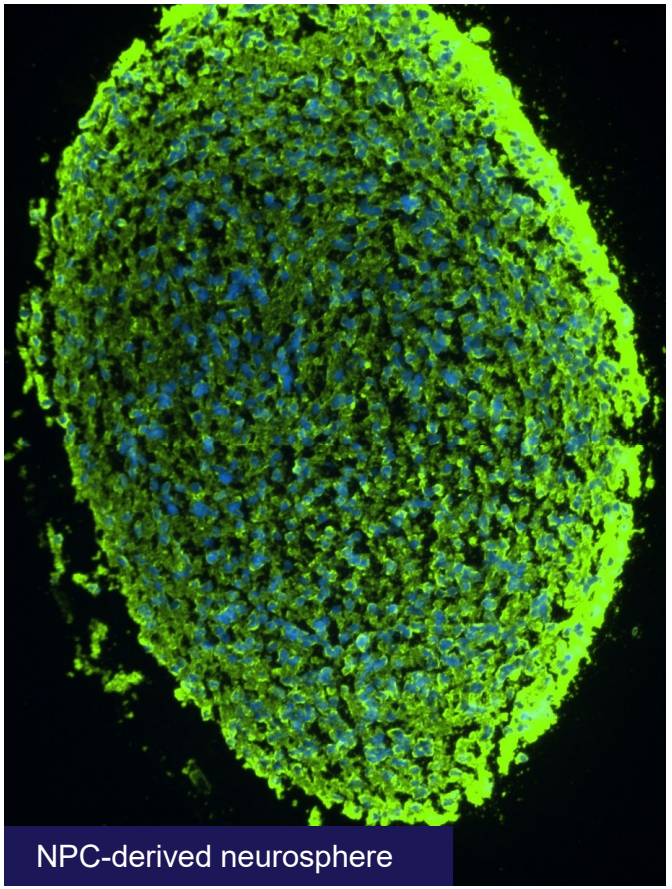
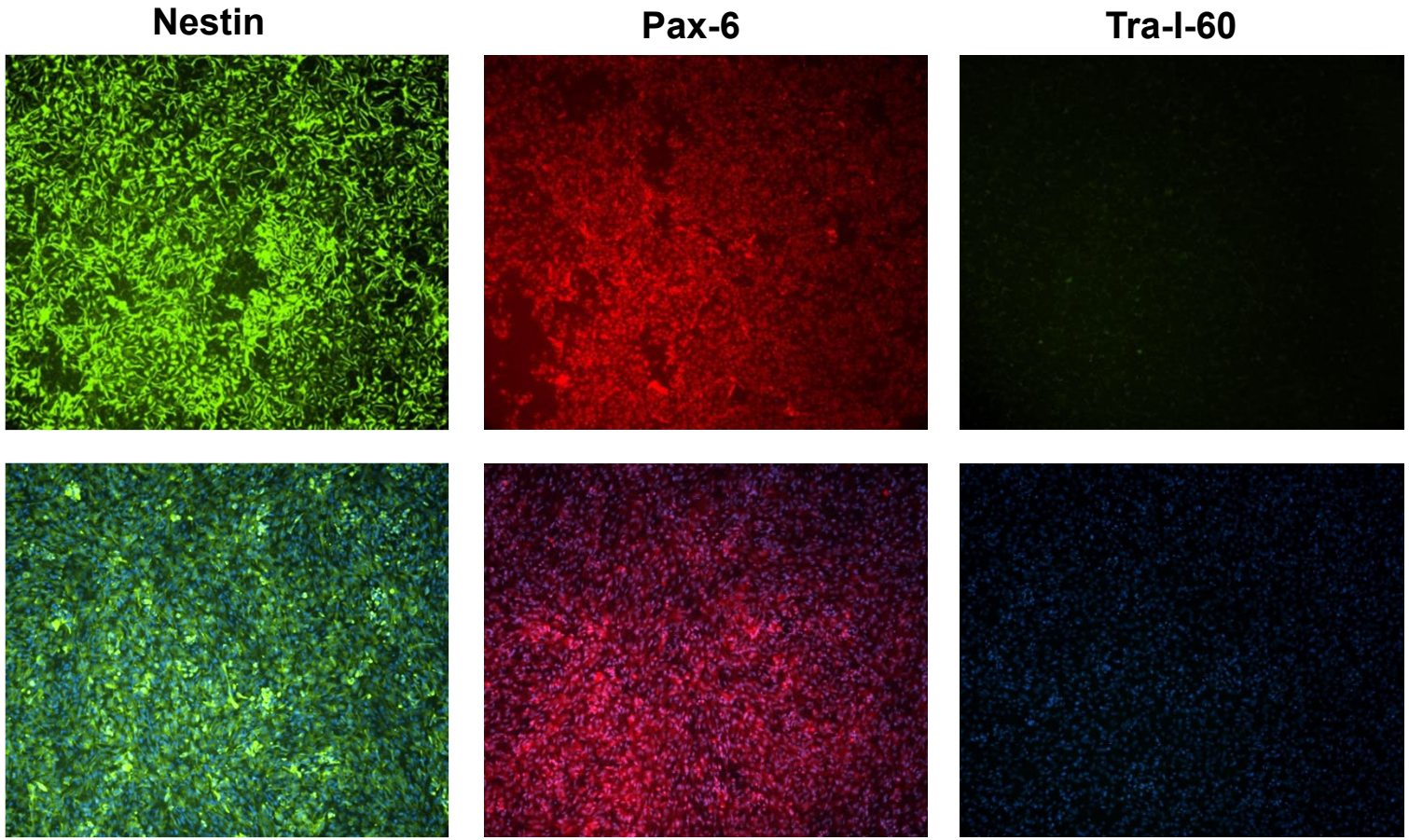
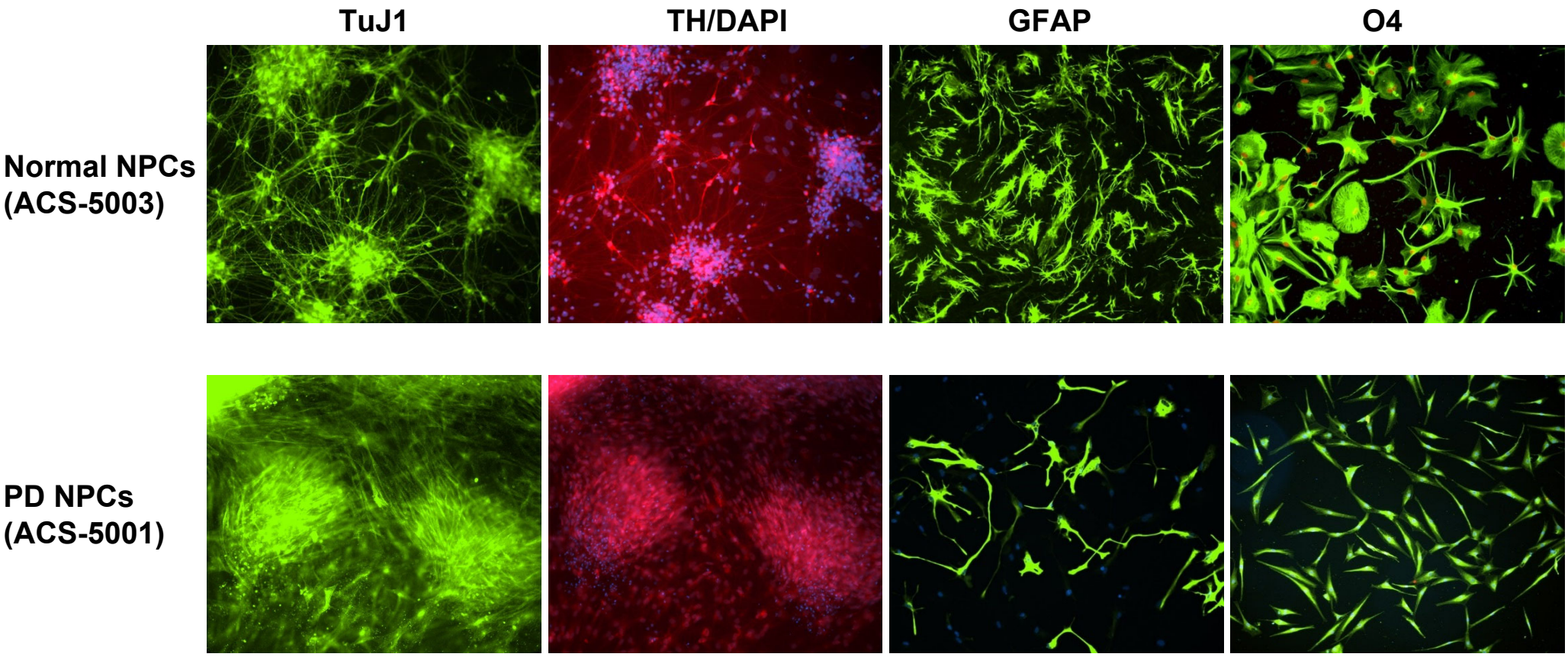


NPC-derived neurosphere

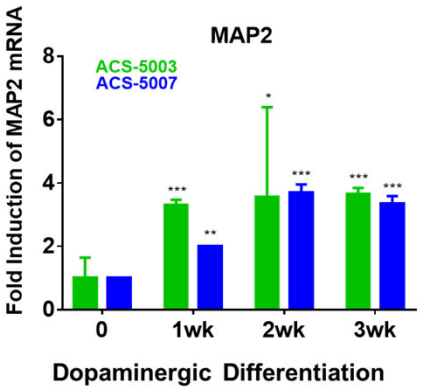
ATCC NPCs express NPC markers but *not* iPSC markers

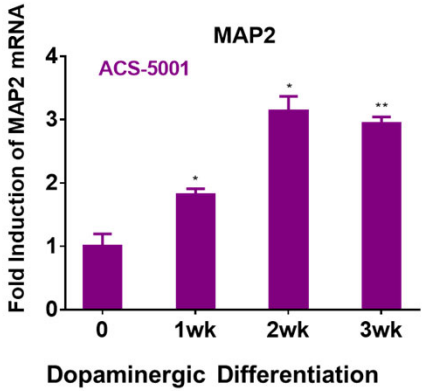


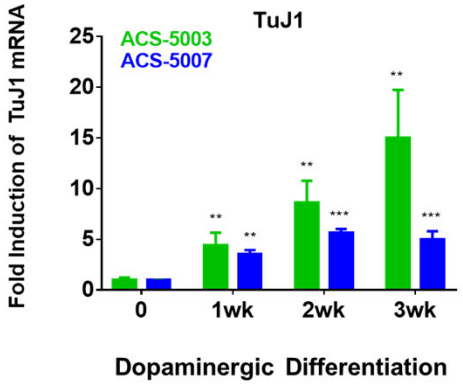
Early and dopaminergic neuron, astrocyte, and oligodendrocyte differentiation of normal and PD NPCs

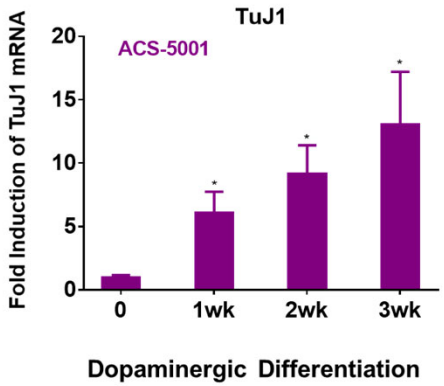


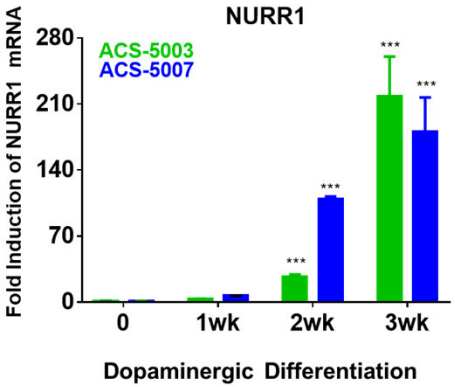
Expression of dopaminergic neuron genes

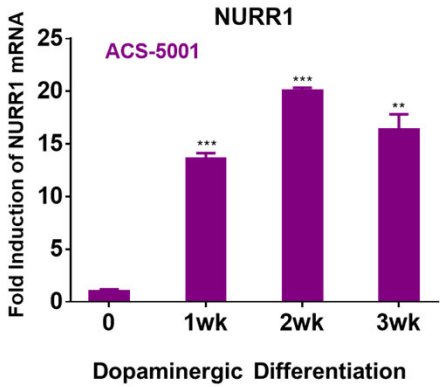


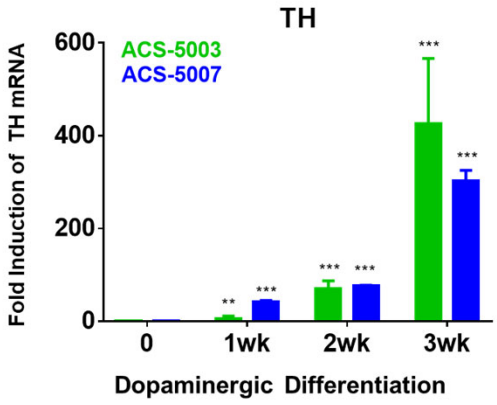


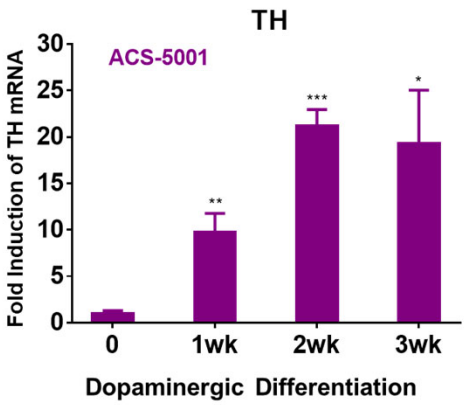




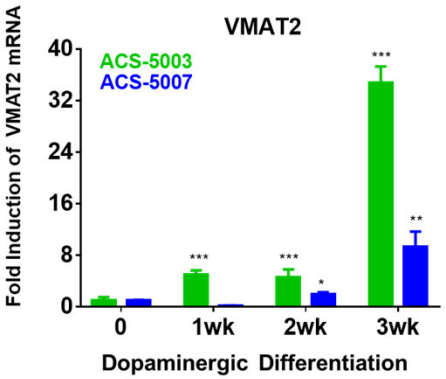


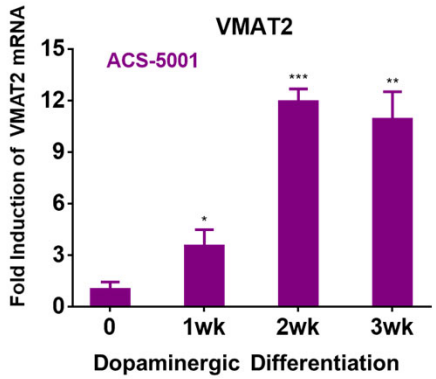


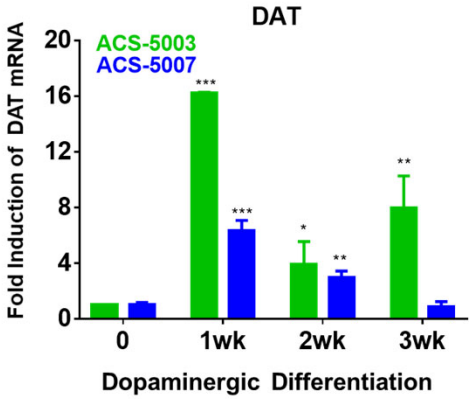


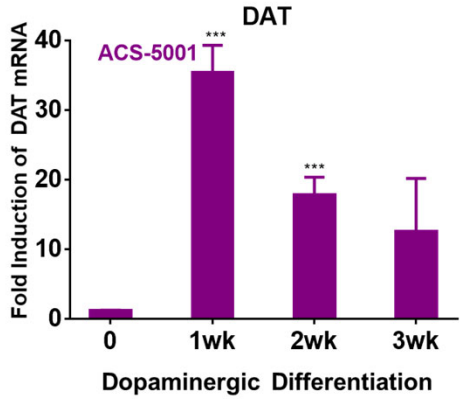


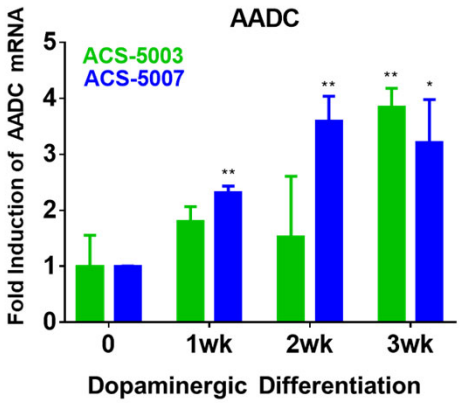
Expression of VMAT2, DAT, and AADC

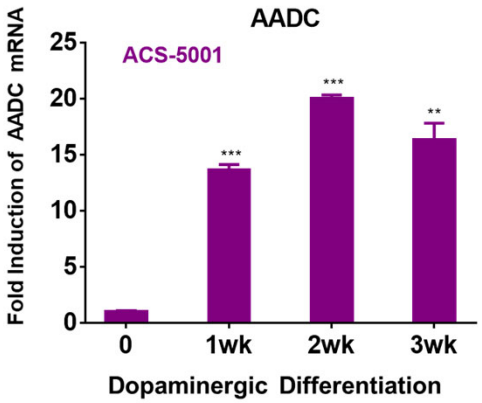












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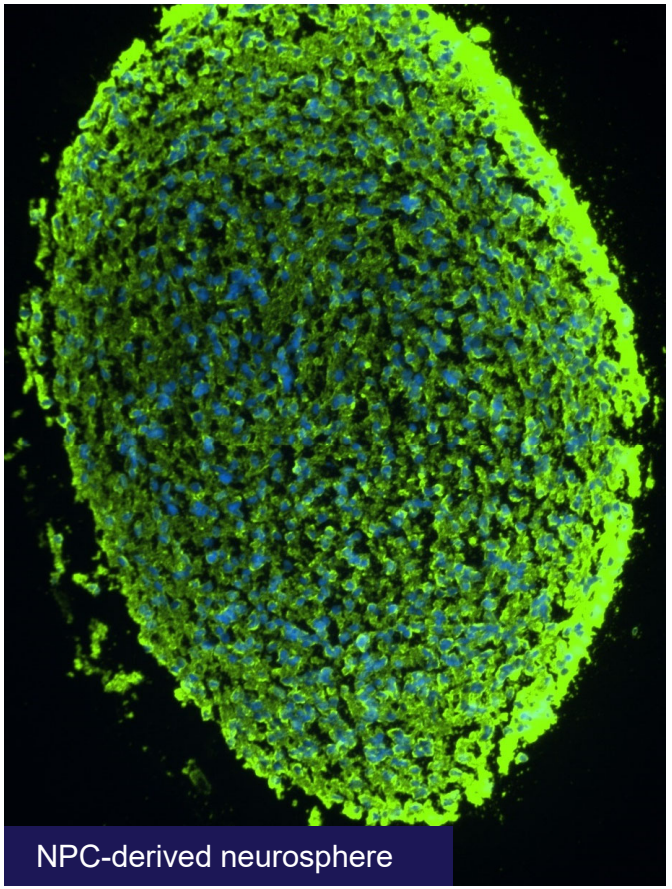
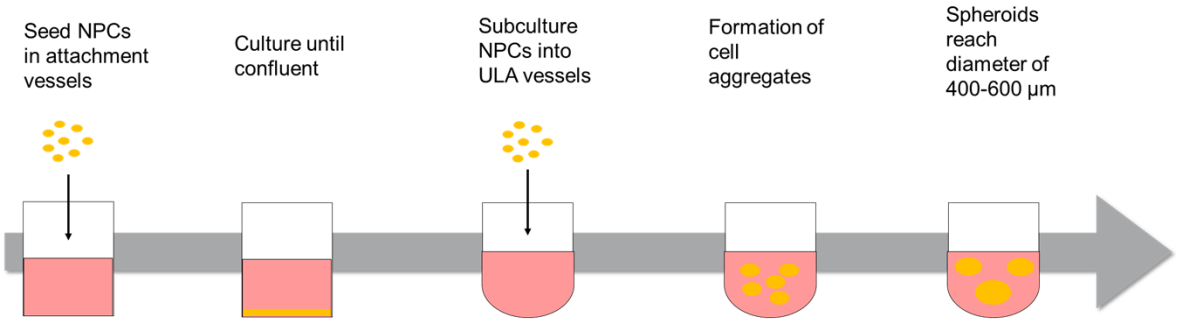


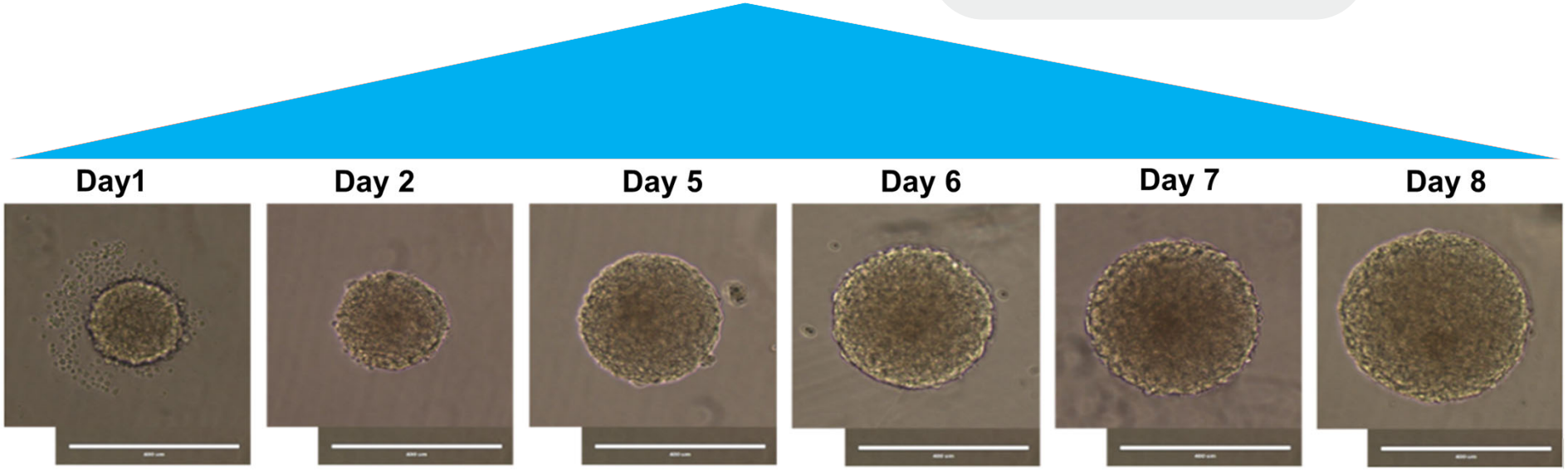
NPC-derived neurosphere

NPC neurospheres grow in size over time



Assays:

NPC marker expression
Differentiation
Dopaminergic marker expression
Toxicity testing



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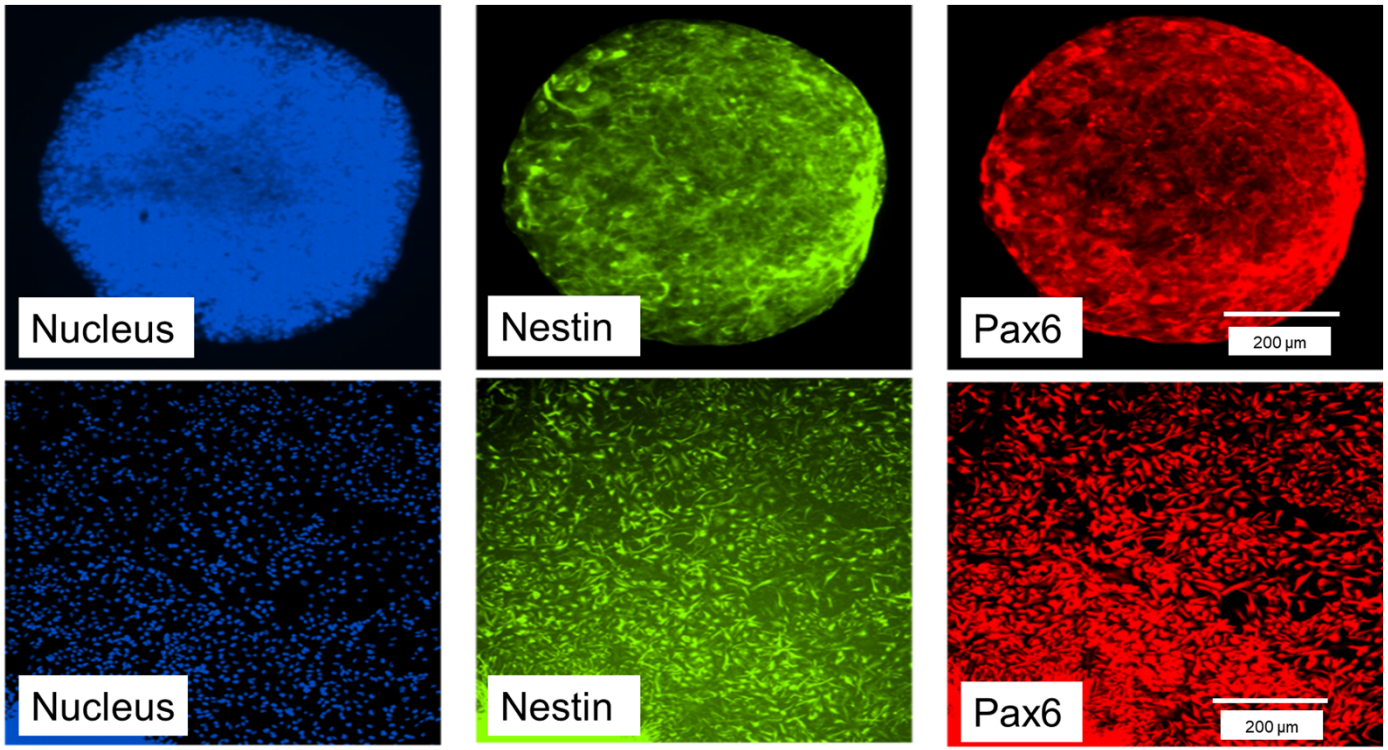
NPC derived-neurospheres and neurosphere-derived NPCs express NPC markers



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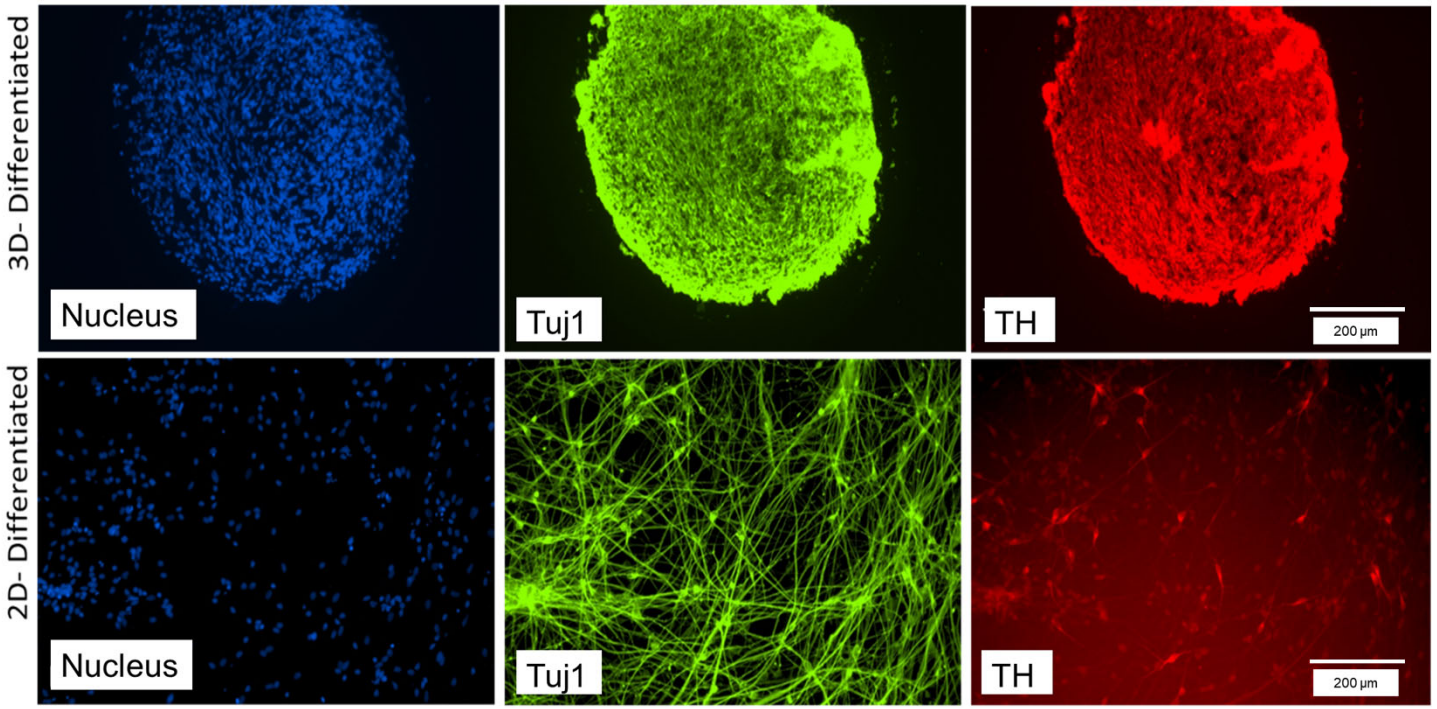
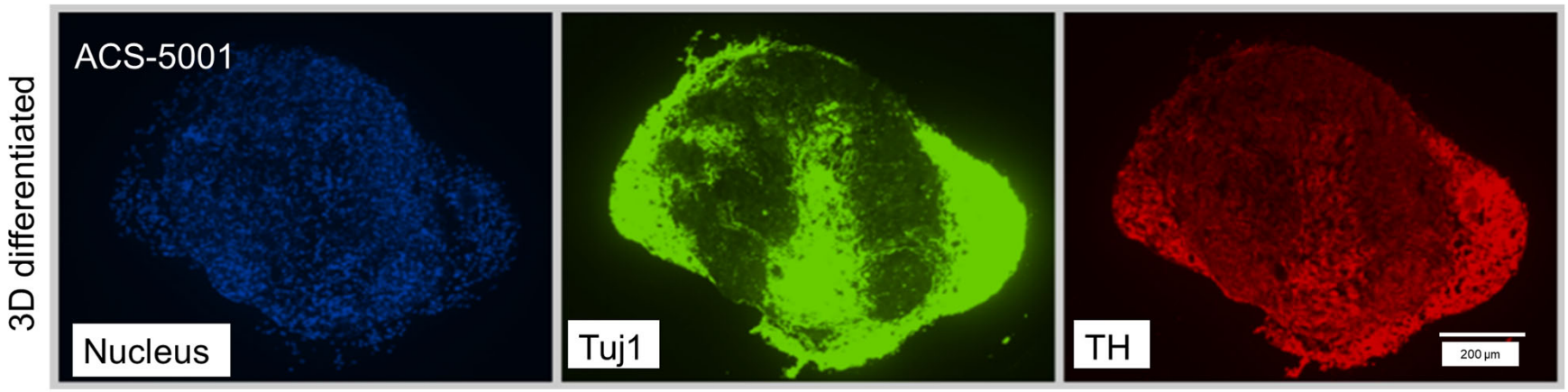
Normal neurospheres successfully differentiate and show higher TH positivity compared to 2D NPC cultures



Parkinson's disease donor derived neurospheres displayed different patterning after dopaminergic differentiation



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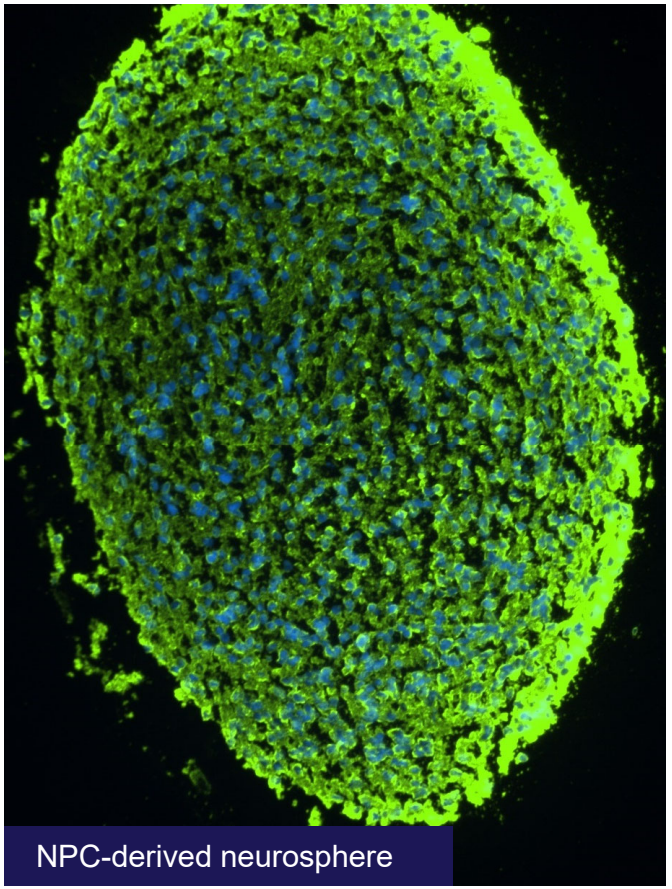

NPC-derived neurosphere

Compounds used in toxicity studies

Compound	Known effects of the Compound
Paclitaxel	Microtubule stabilizer; known to cause neuropathies
Cisplatin	Platinum-based apoptotic agent; known to have neuropathic affects but reputed to not be neurotoxic
Piperine	Nociceptive agent; not known to cause neurotoxiciy
Vincristine	Plant alkaloid; shown to cause peripheral neuropathy when used to treat pediatric A.L.L.
Hydroxyurea	Antimetabolite; may cause severe peripheral neuropathy
Amiodarone	Associated with peripheral neuropathies
Chlorhexidine	Neurotoxic to neurons, SH-SY5Y cells, and Schwann cells

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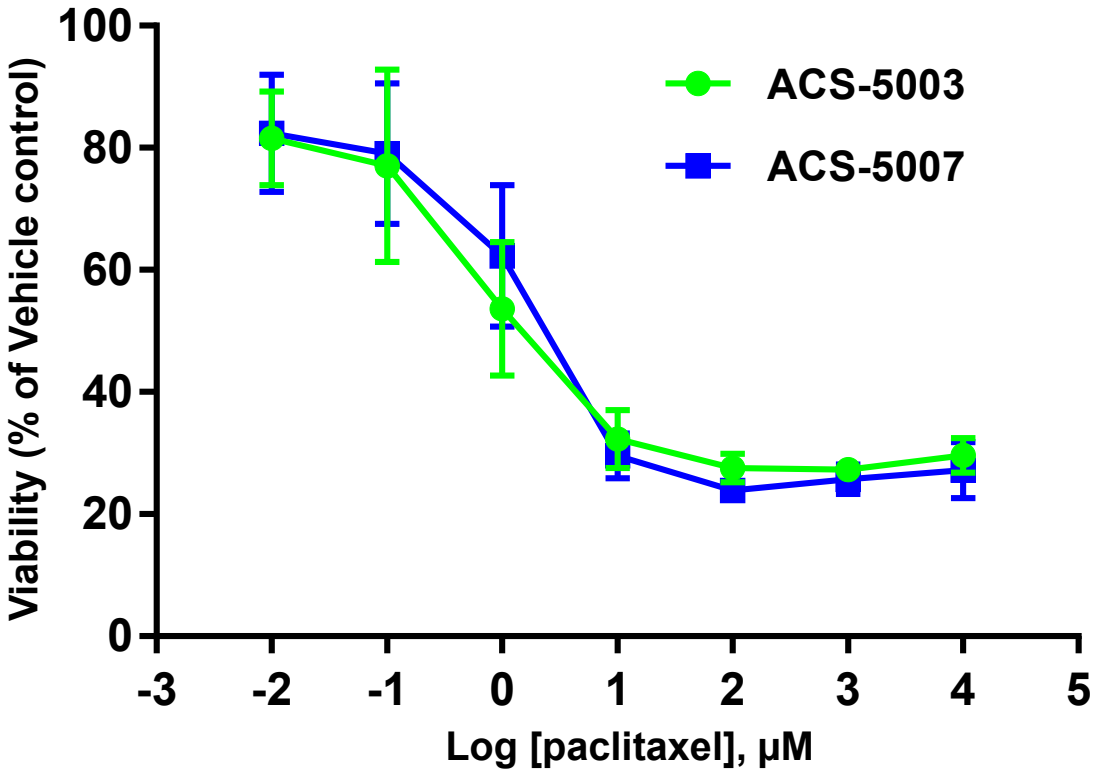
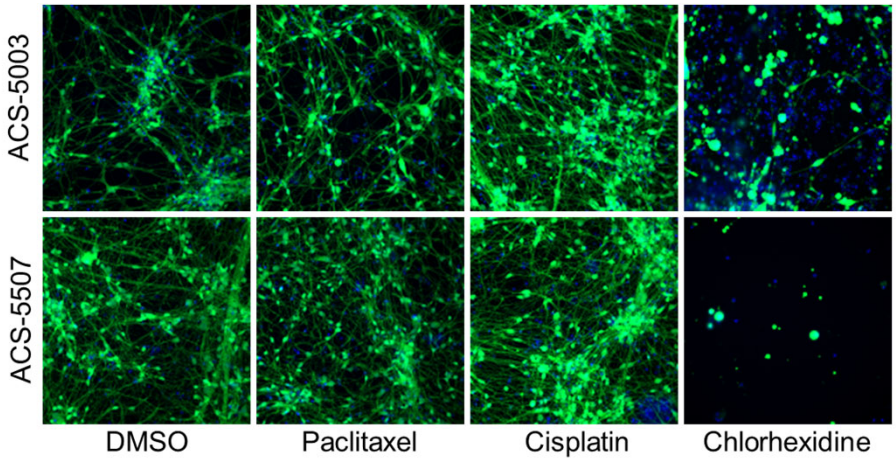
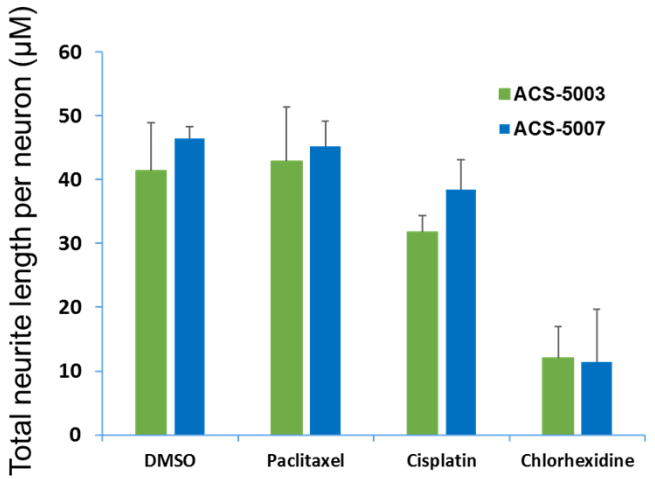
Dose-response curves for cell viability of normal, undifferentiated NPCs treated with paclitaxel for two days



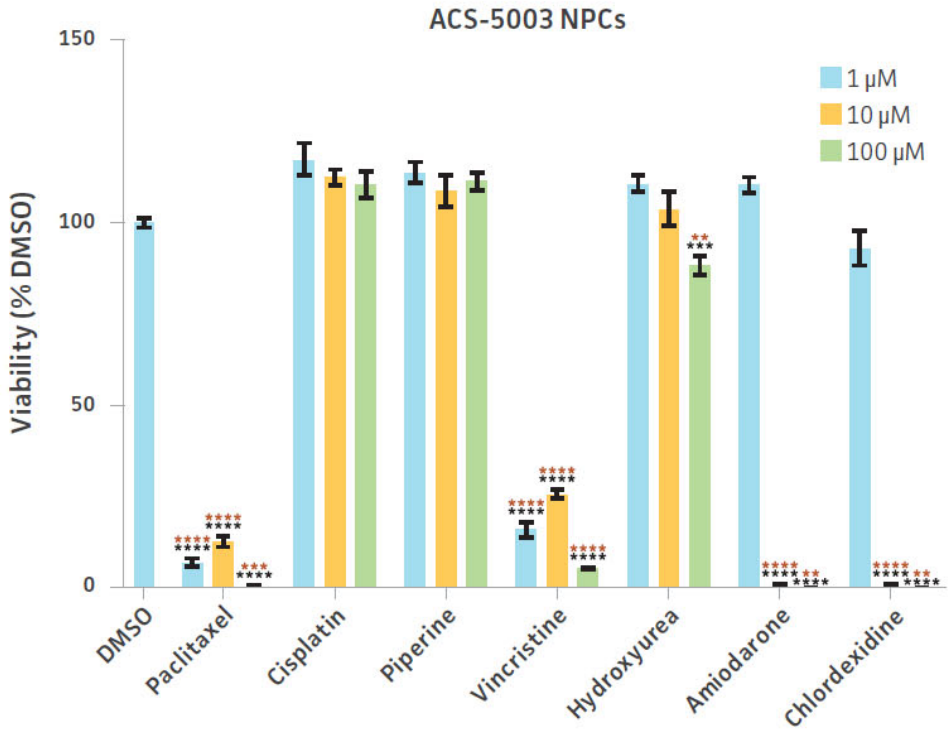
ATCC

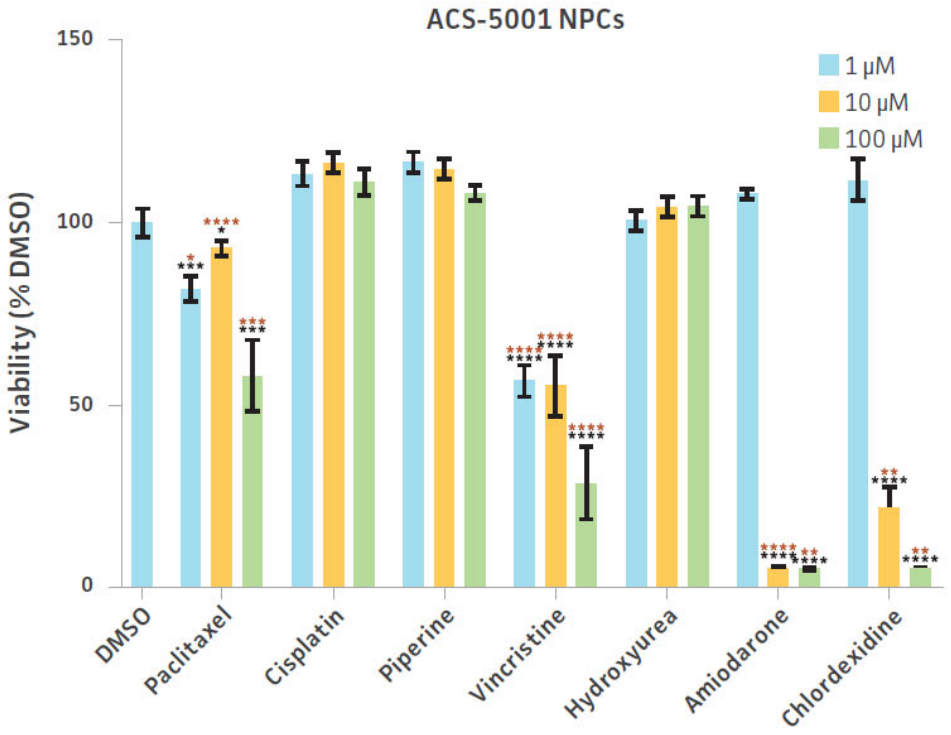
High content imaging of neurotoxicity in differentiated NPCs



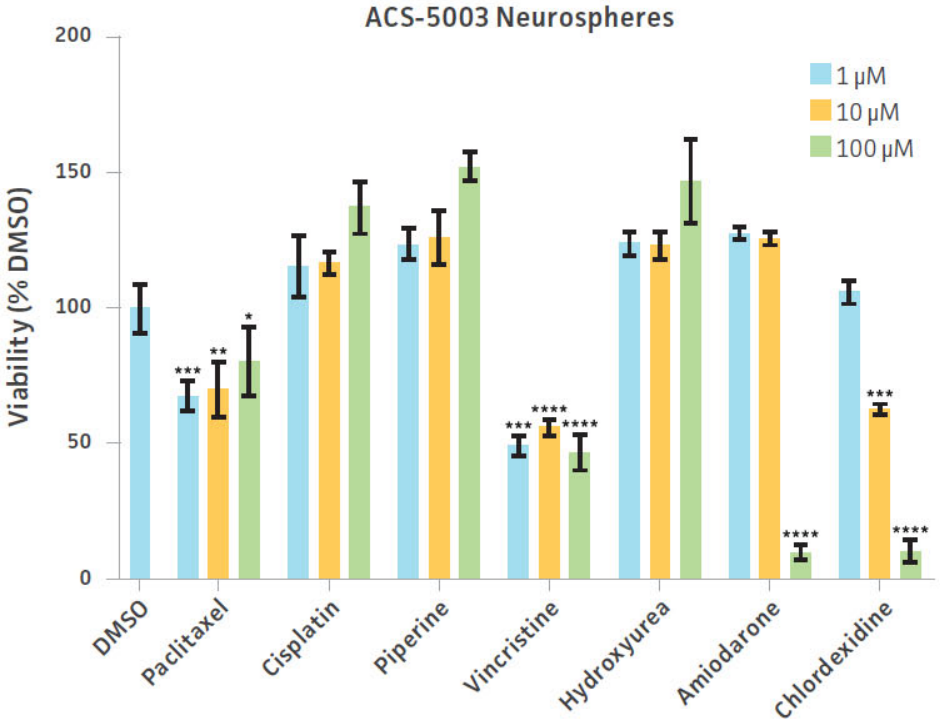


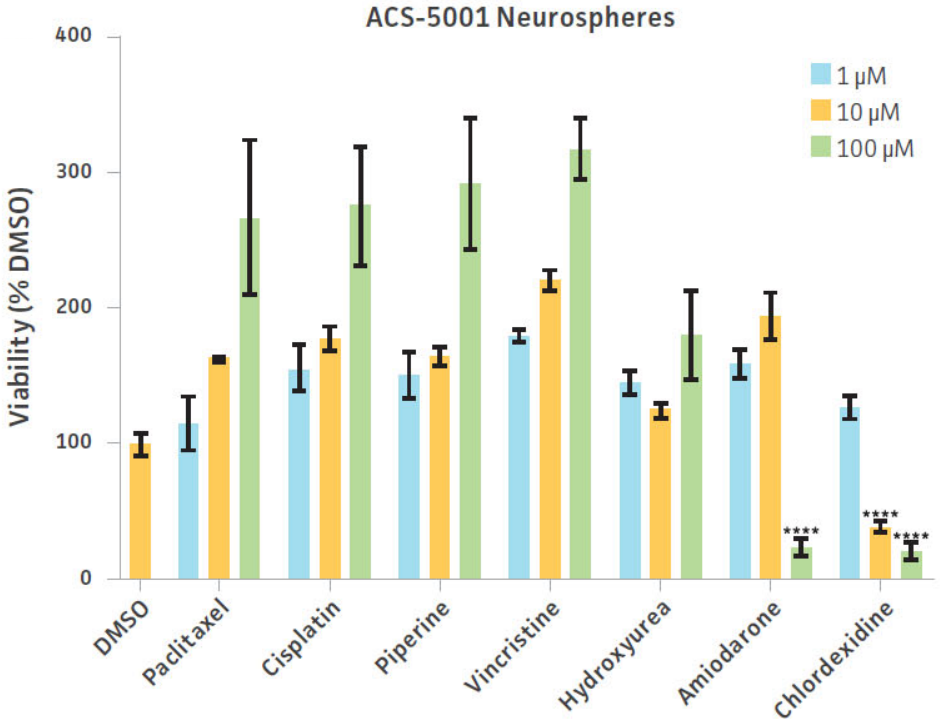
Normal and PD NPCs showed differential response to various drug treatments





Normal and PD neurospheres showed differential response to various drug treatments; these responses varied with their 2D NPC counterparts





Overall neurotoxicity studies, NPCs vs. NPC-derived neurospheres

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

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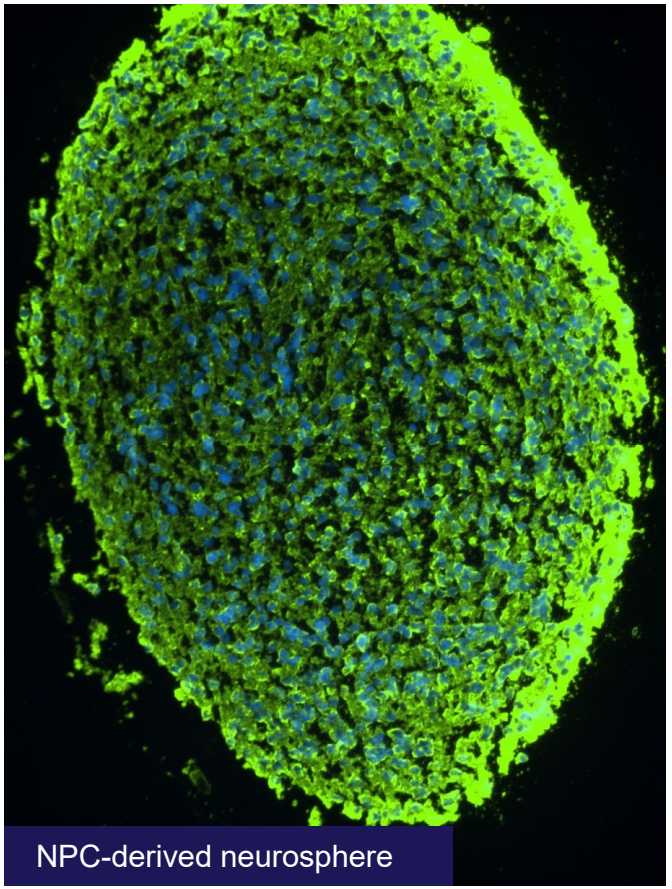
Agenda

Neural Progenitor Cells (NPCs)

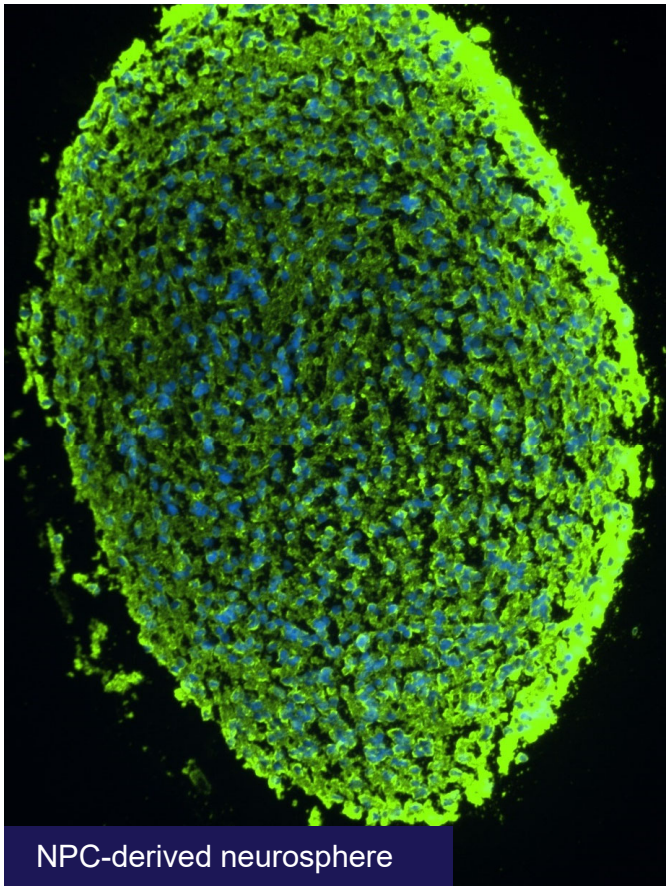
- ATCC roadmap and neurological resources
- Background information
- Differentiation potential of ATCC NPCs
- Neurosphere generation
- Toxicological studies using neurospheres
- Summary



NPC-derived neurosphere

Summary

- A complete solution:
  - Normal, PD, or reporter labeled NPCs
  - Expansion and Differentiation Medium
  - Easy-to-use protocols
- Human model with no donor variation
  - Ability to expand and bank
- Differentiation across a wide spectrum of neural and glial lineages
  - Various types of neurons
  - Astrocytes
  - Oligodendrocytes
- Live imaging of differentiation
  - GFP expression upon neural differentiation
- Advanced models of neurotoxicity that satisfy requirements for:
  - High cell yield
  - High physiological relevance



NPC-derived neurosphere

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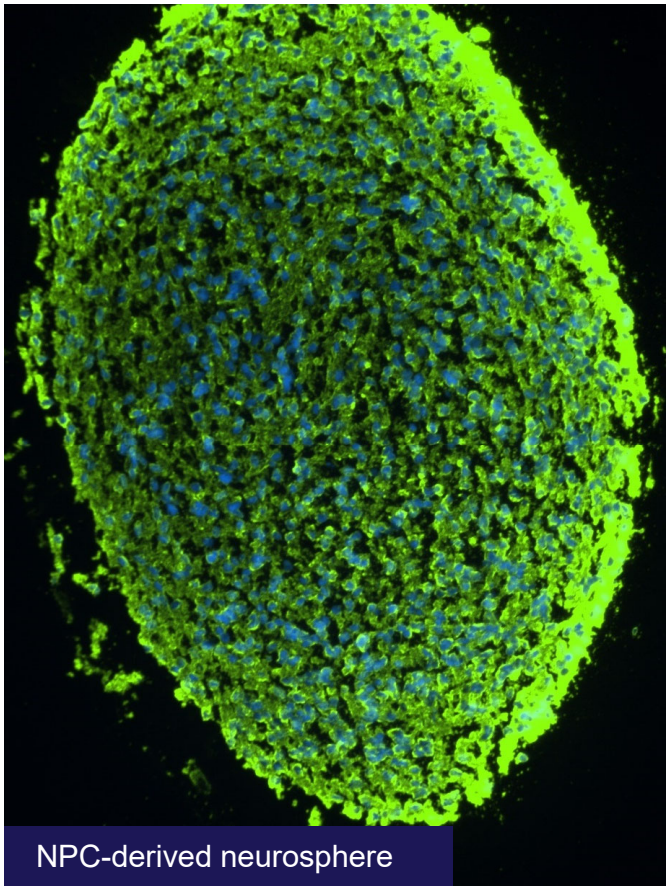
Summary – continued

- ATCC NPCs can convert to neurospheres with 100% efficiency
  - Ultra-low attachment culture vessels and NPC expansion medium
- NPC-derived neurospheres were similar to literature results
  - Formed solid circular spheres without formation of hollow cavities
  - Maintained their non-differentiated state for more than 2 weeks
  - Differentiated into dopaminergic neurons
    - More TH positivity than differentiated 2-D monolayer culture
- 3-D neurospheres are less sensitive to chemotherapeutics compared to 2-D NPC cultures
- Normal neurospheres were sensitive to vincristine but Parkinson's neurospheres were not
- NPCs cultured as 3-D neurospheres are amenable to drug toxicity studies using standard cell viability assays



NPC-derived neurosphere

For more information visit www.atcc.org/neuro

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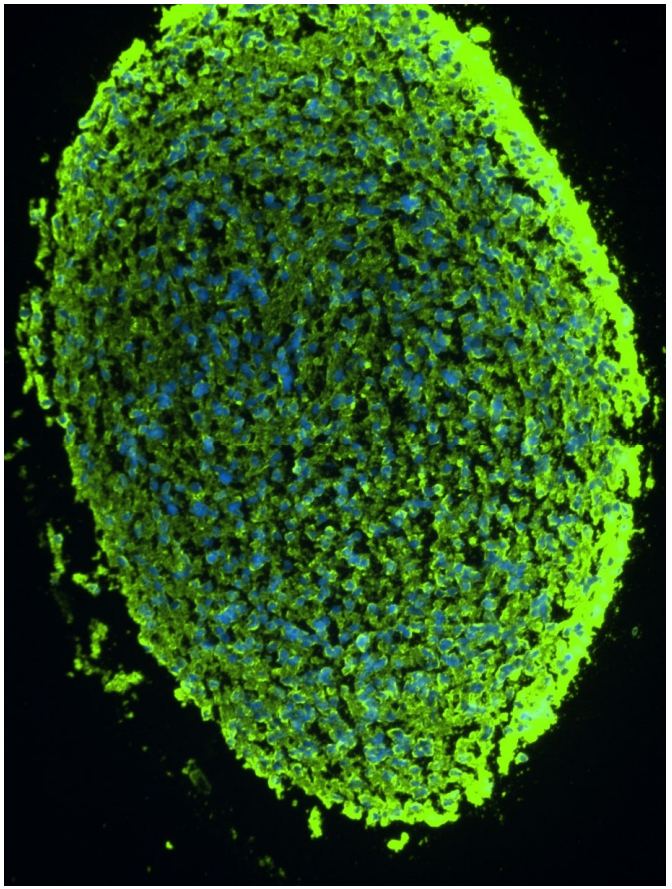
Thank you and questions?

Coming soon!

Reproducibility and Physiological Relevance: The ATCC Toxicology Portfolio Webinar
Presented by Kevin Grady and Kevin Tyo
March 3, 12:00 PM EST



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