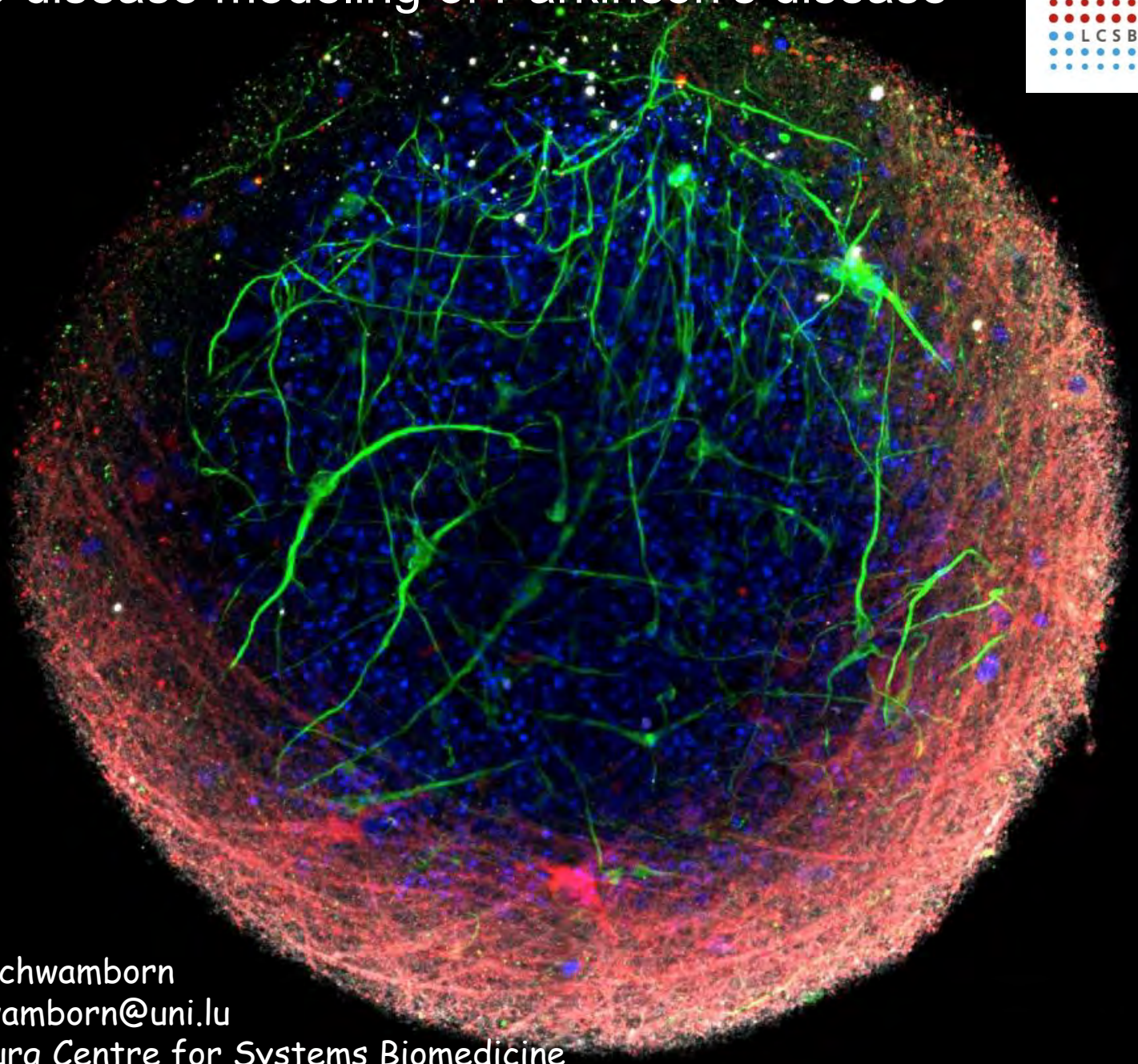
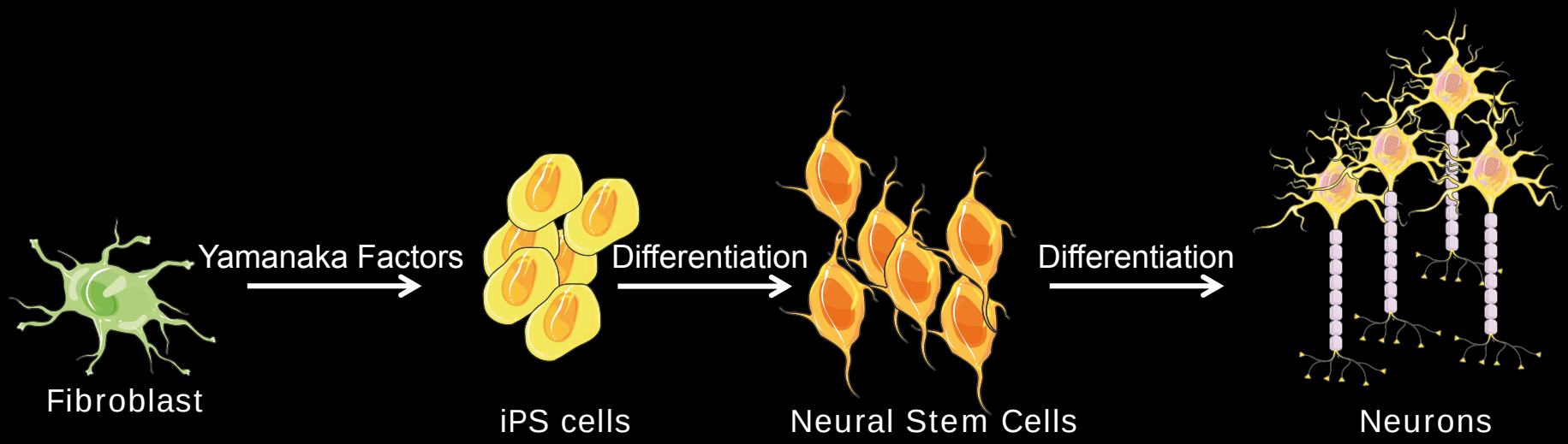
In vitro disease modeling of Parkinson's disease



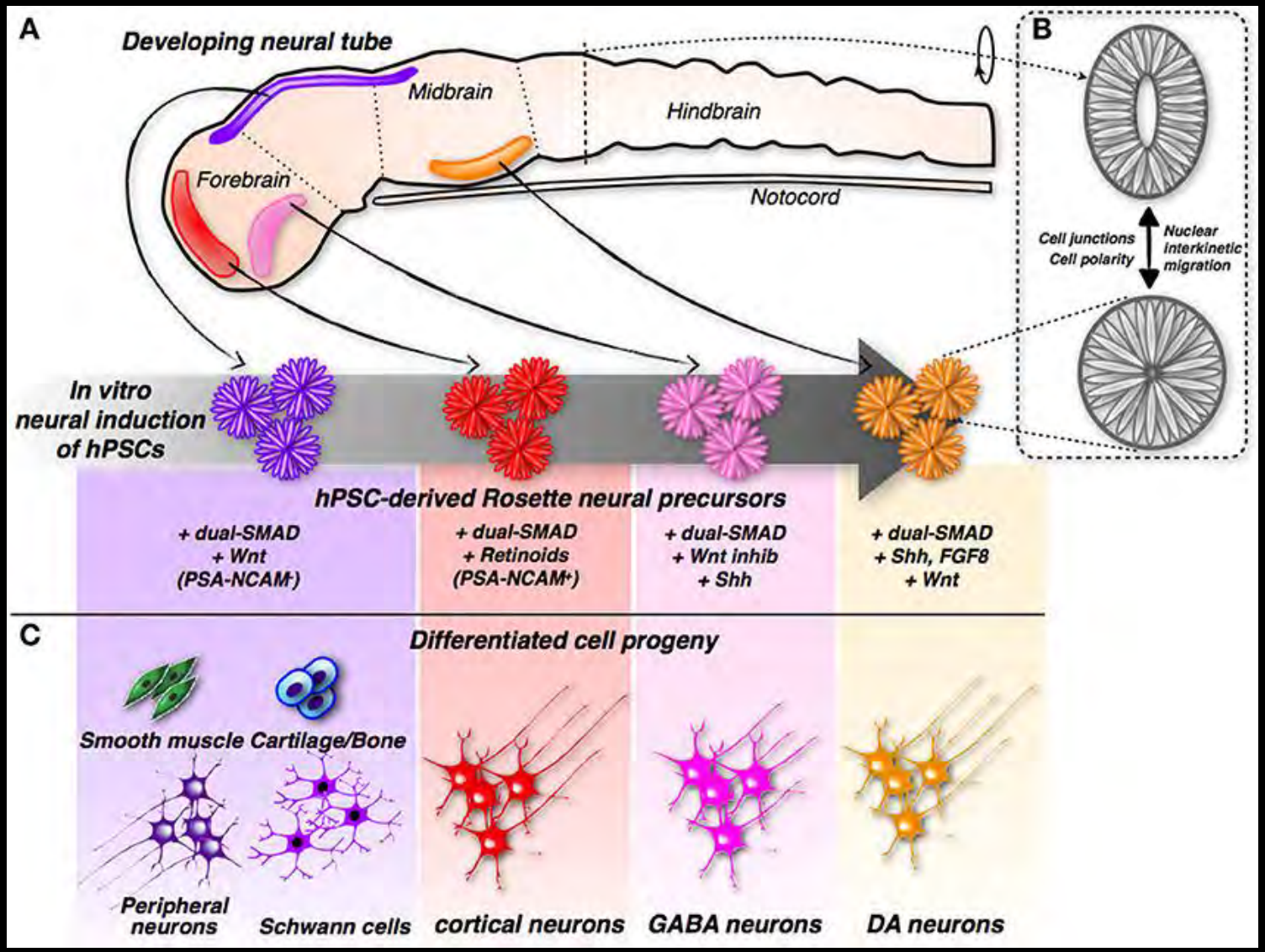
Jens C. Schwamborn
jens.schwamborn@uni.lu
Luxembourg Centre for Systems Biomedicine

Utilization of the iPSC technology to generate NSCs and neurons



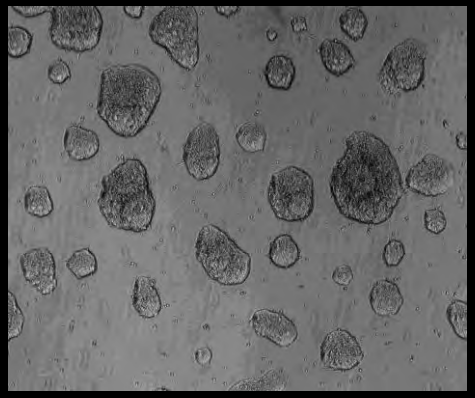
LRRK2
SNCA
VPS35
ATP13A2
Pink1/Parkin

Specification of regional identity

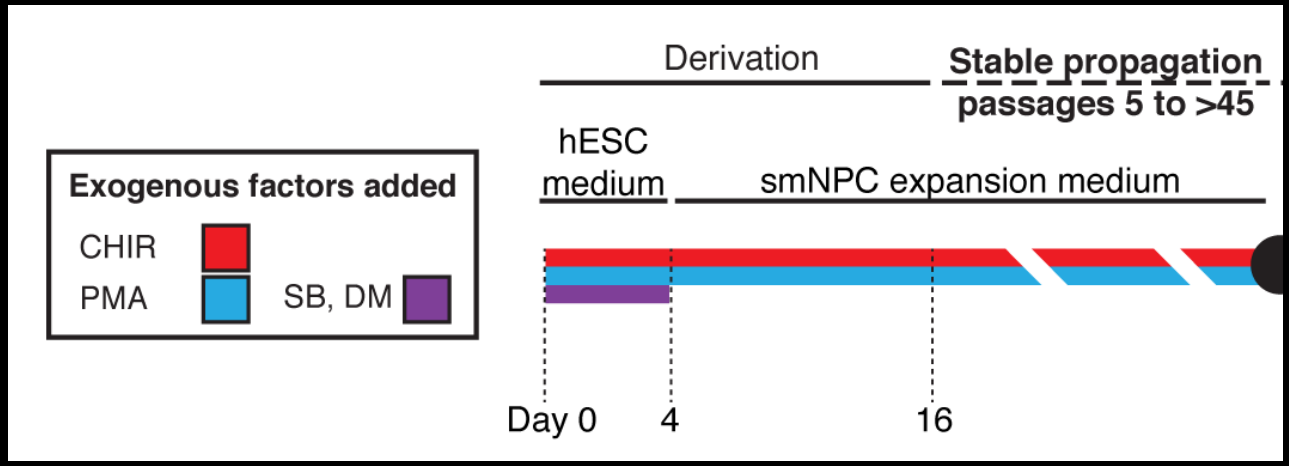


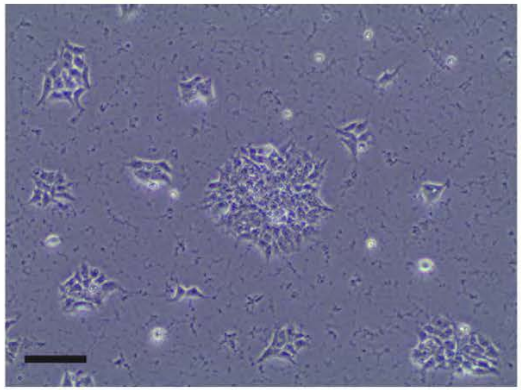
Broccoli et al., 2014

Derivation of neuroepithelial stem cells (NESCs)

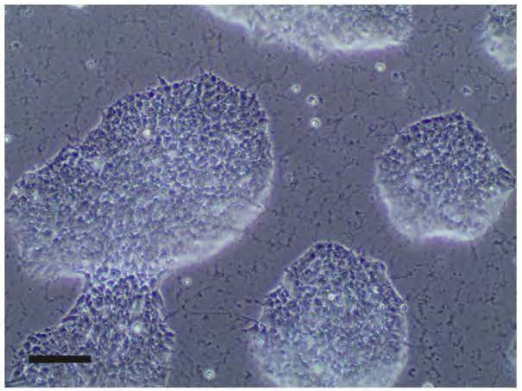


hiPSCs





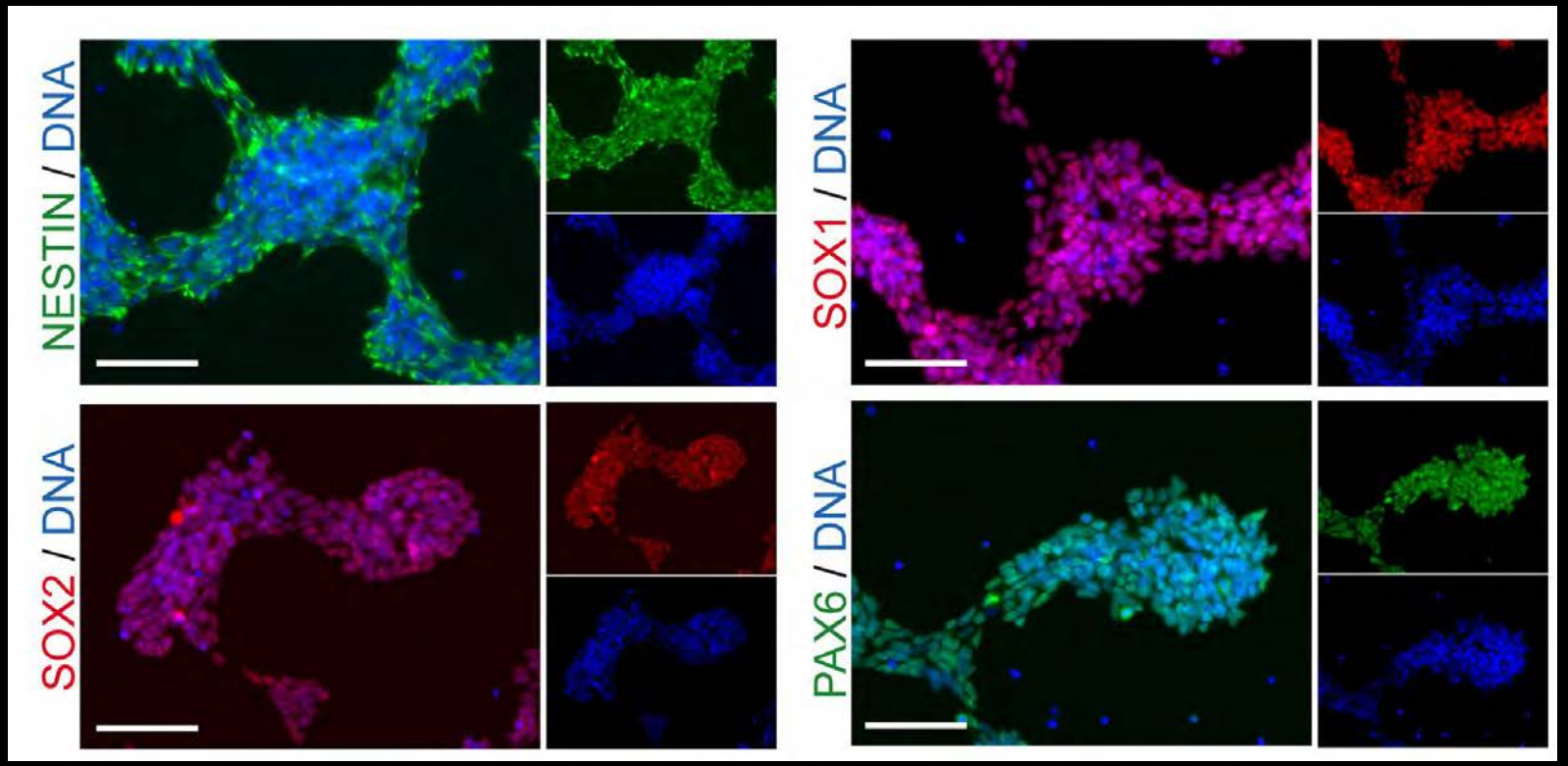
1 day post-plating

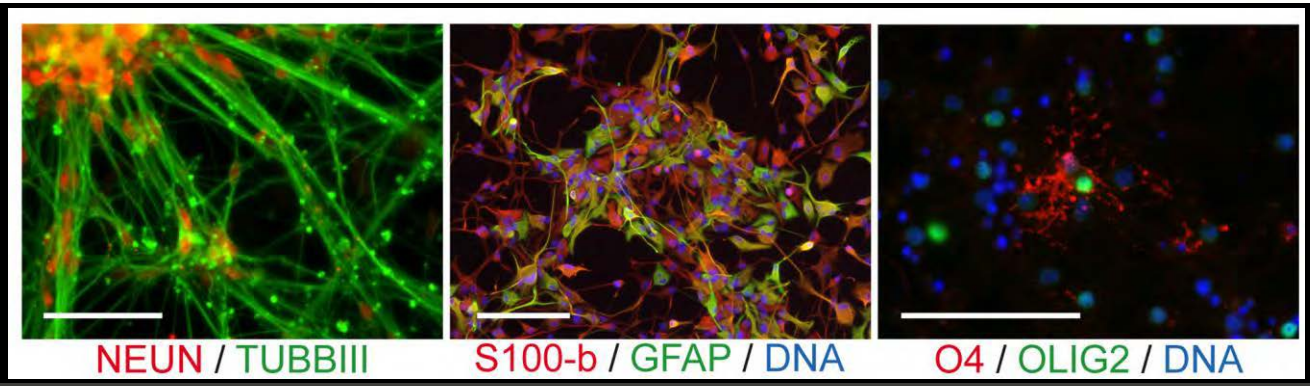


4 days post-plating

Reinhardt et al., 2013

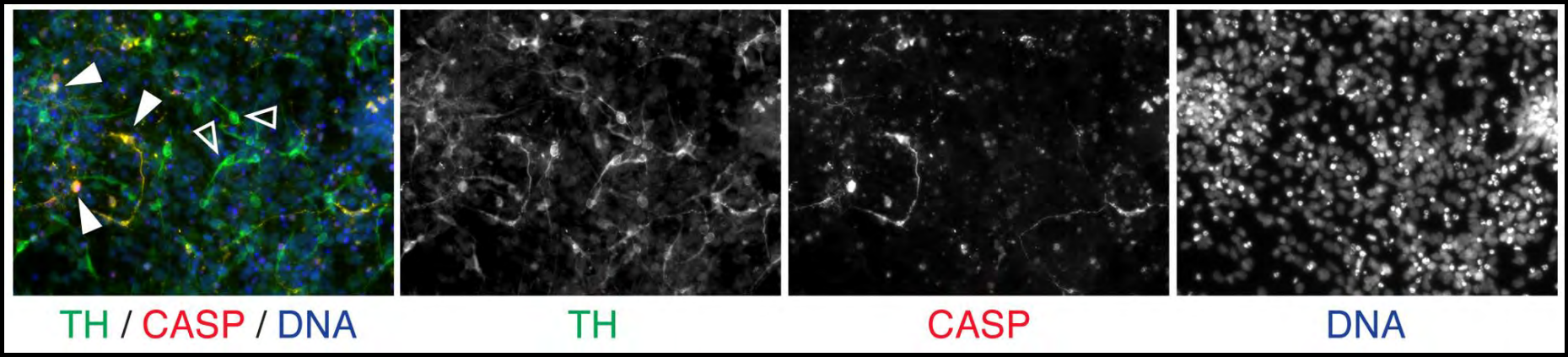
NESCs express early neural markers

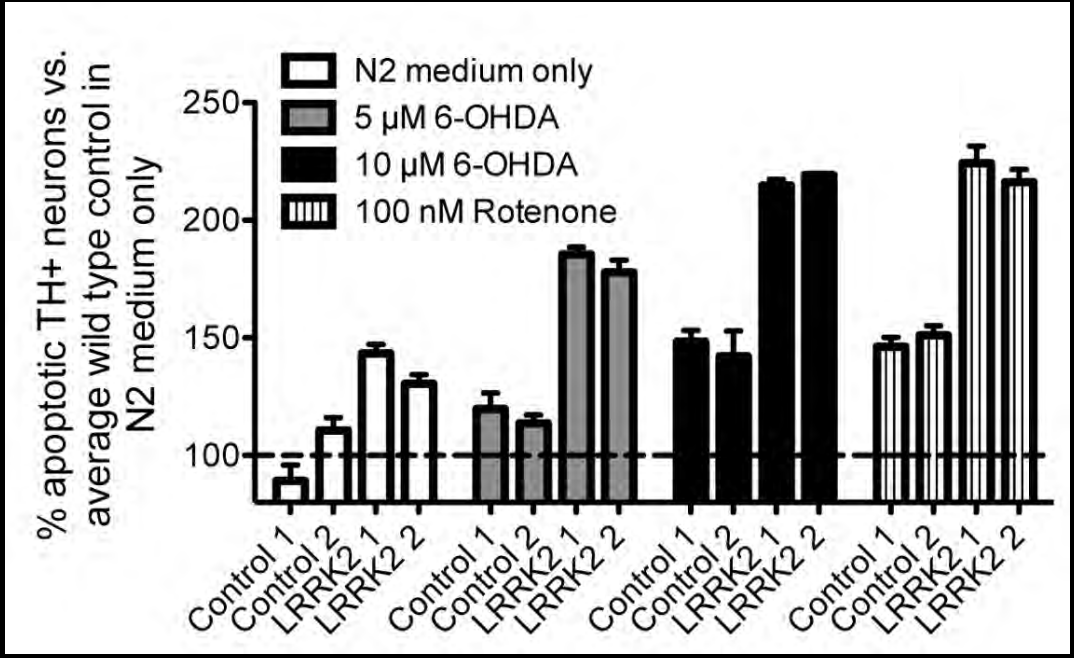




Reinhardt et al., 2013

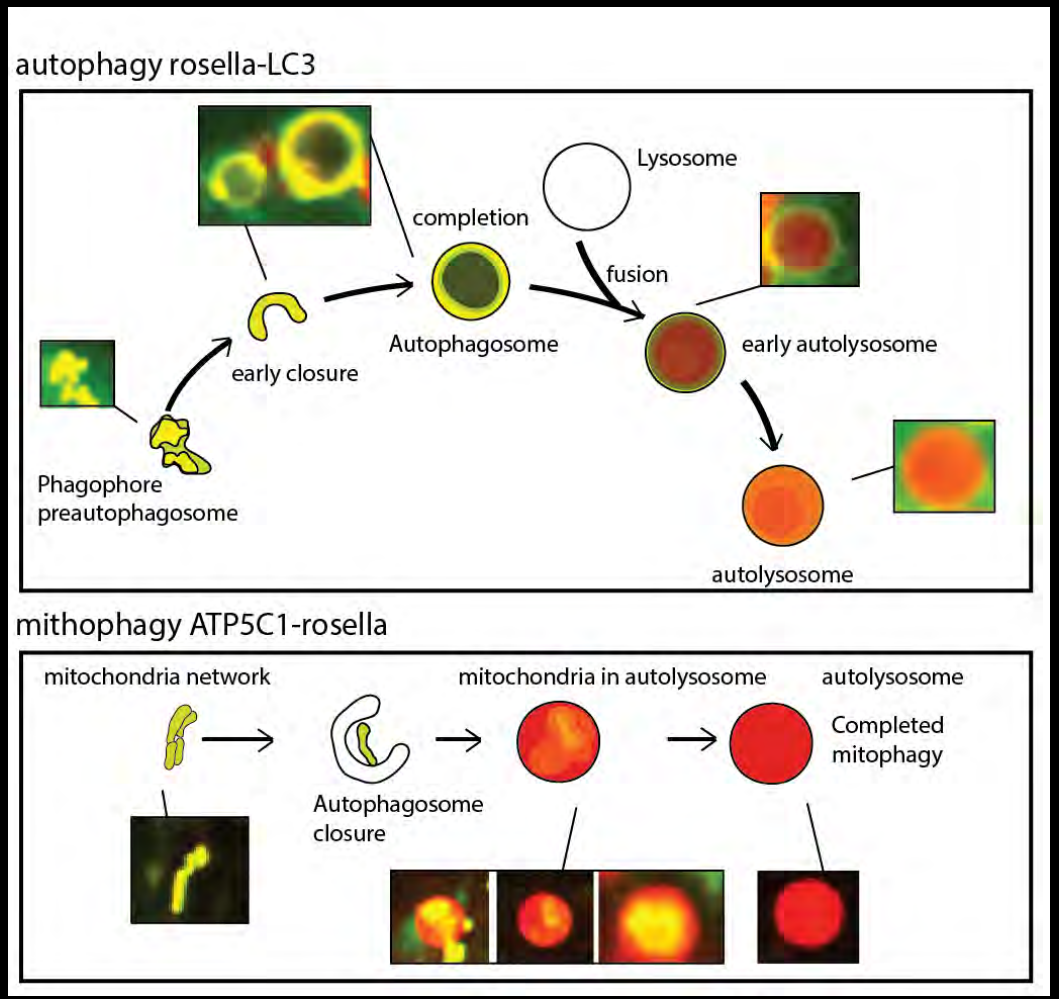
DNs for *in vitro* Parkinson's disease modelling

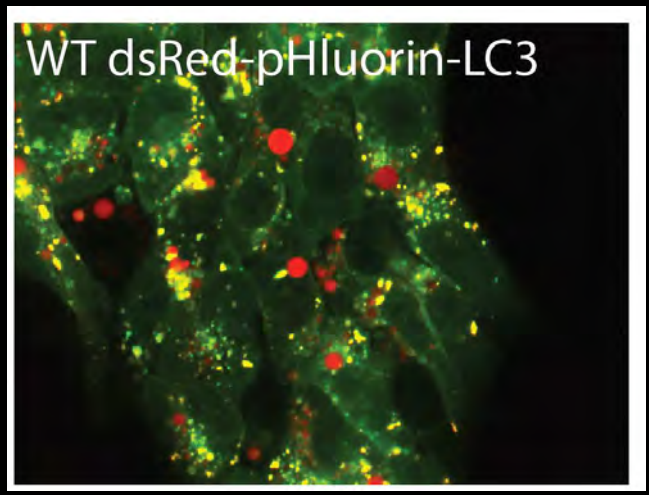


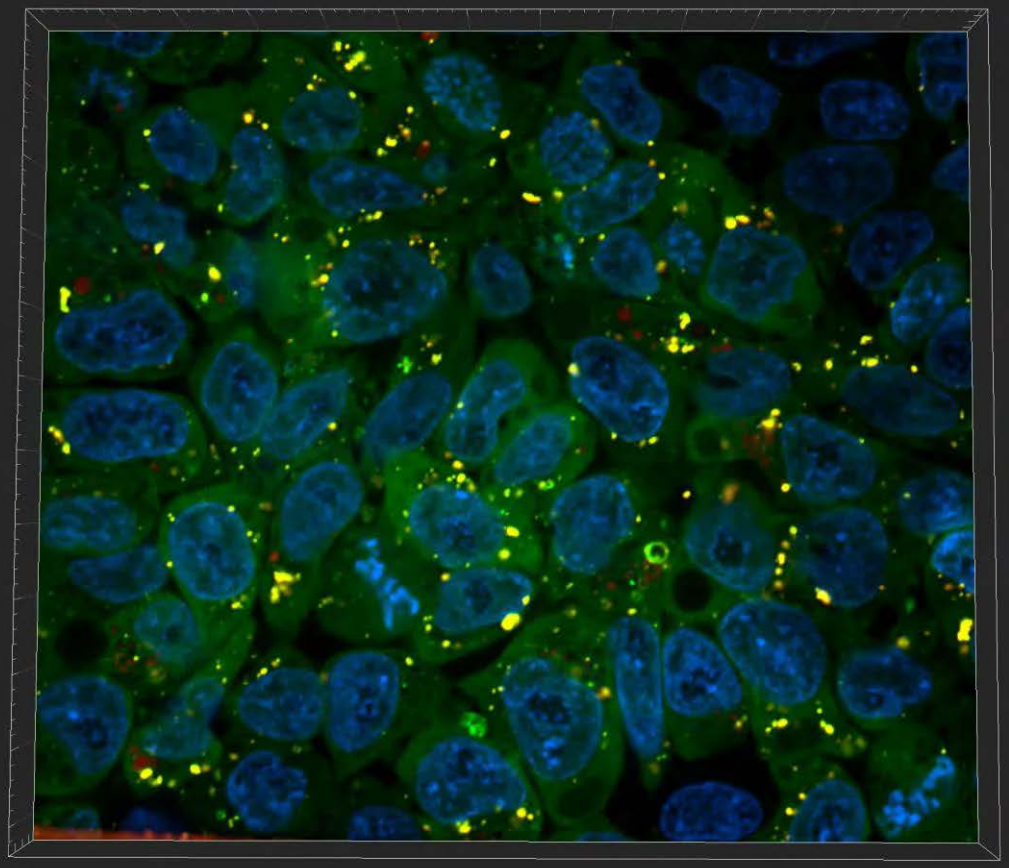


Reinhardt et al., 2013

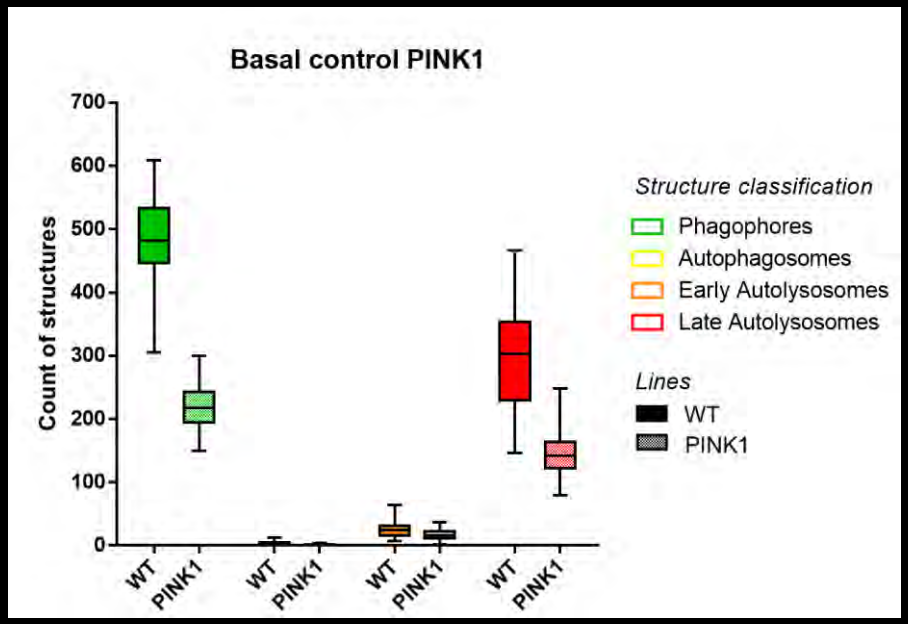
Autophagy sensors

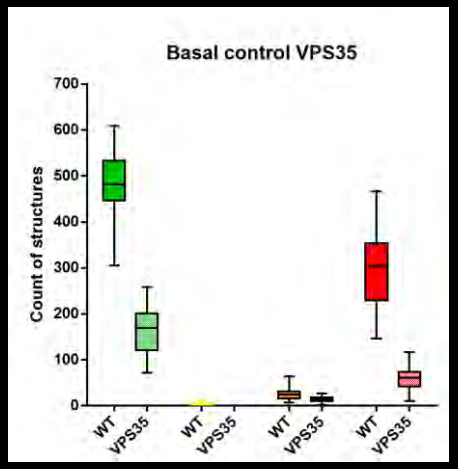


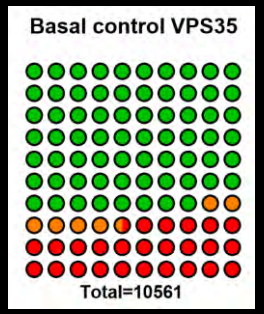


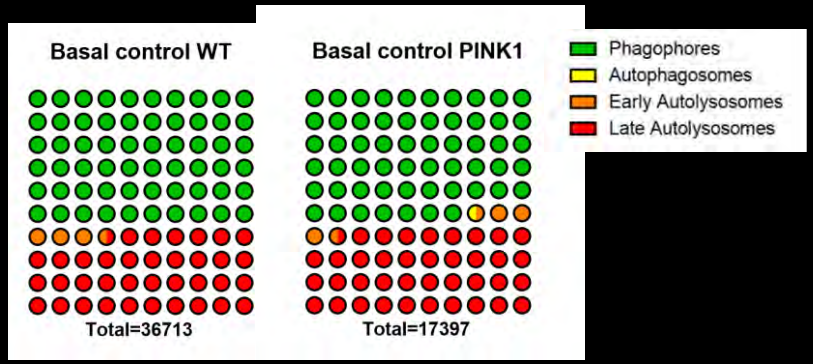

20 um

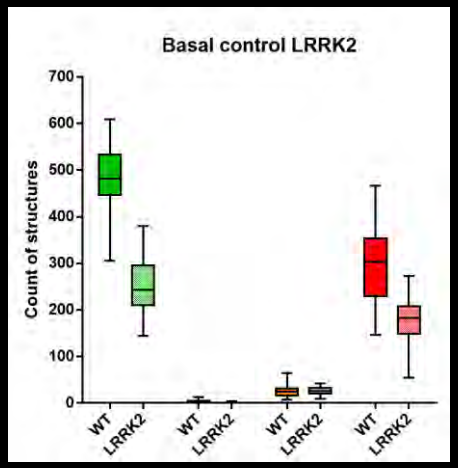
Autophagy phenotypes in PD cell models

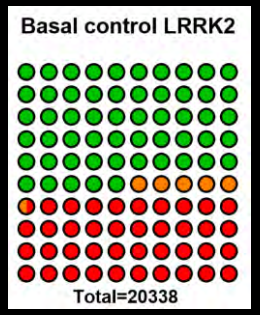




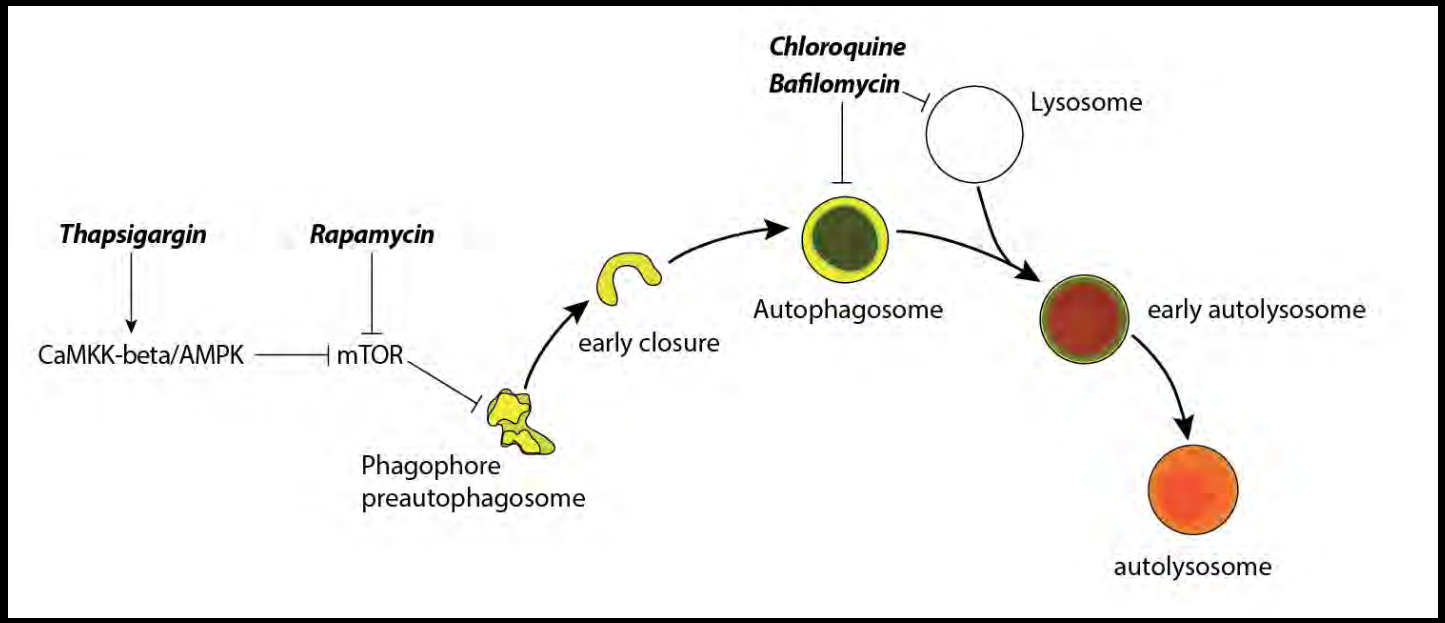


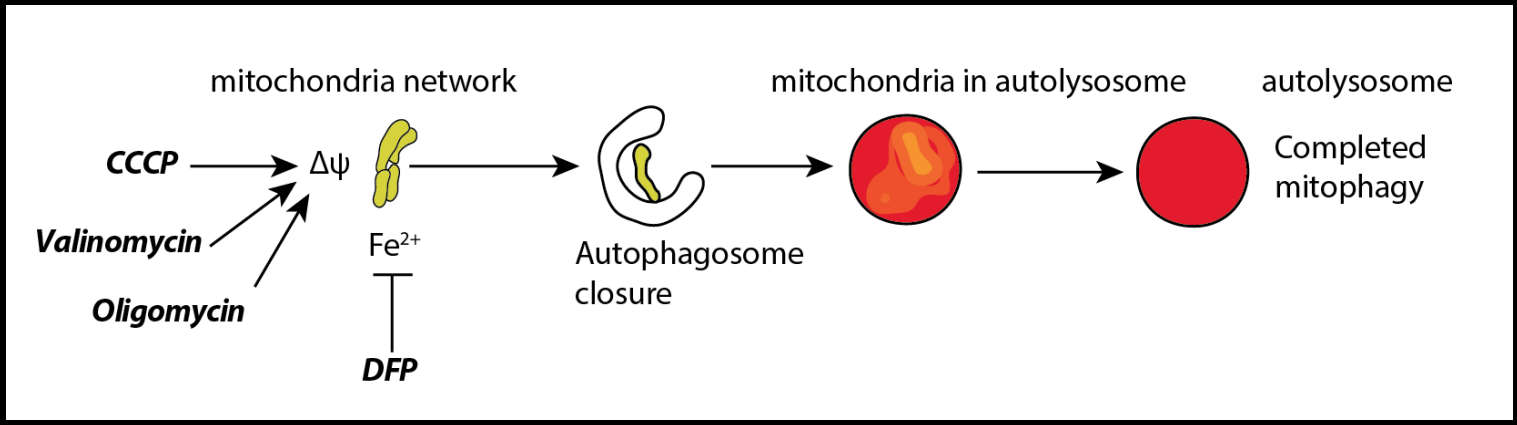


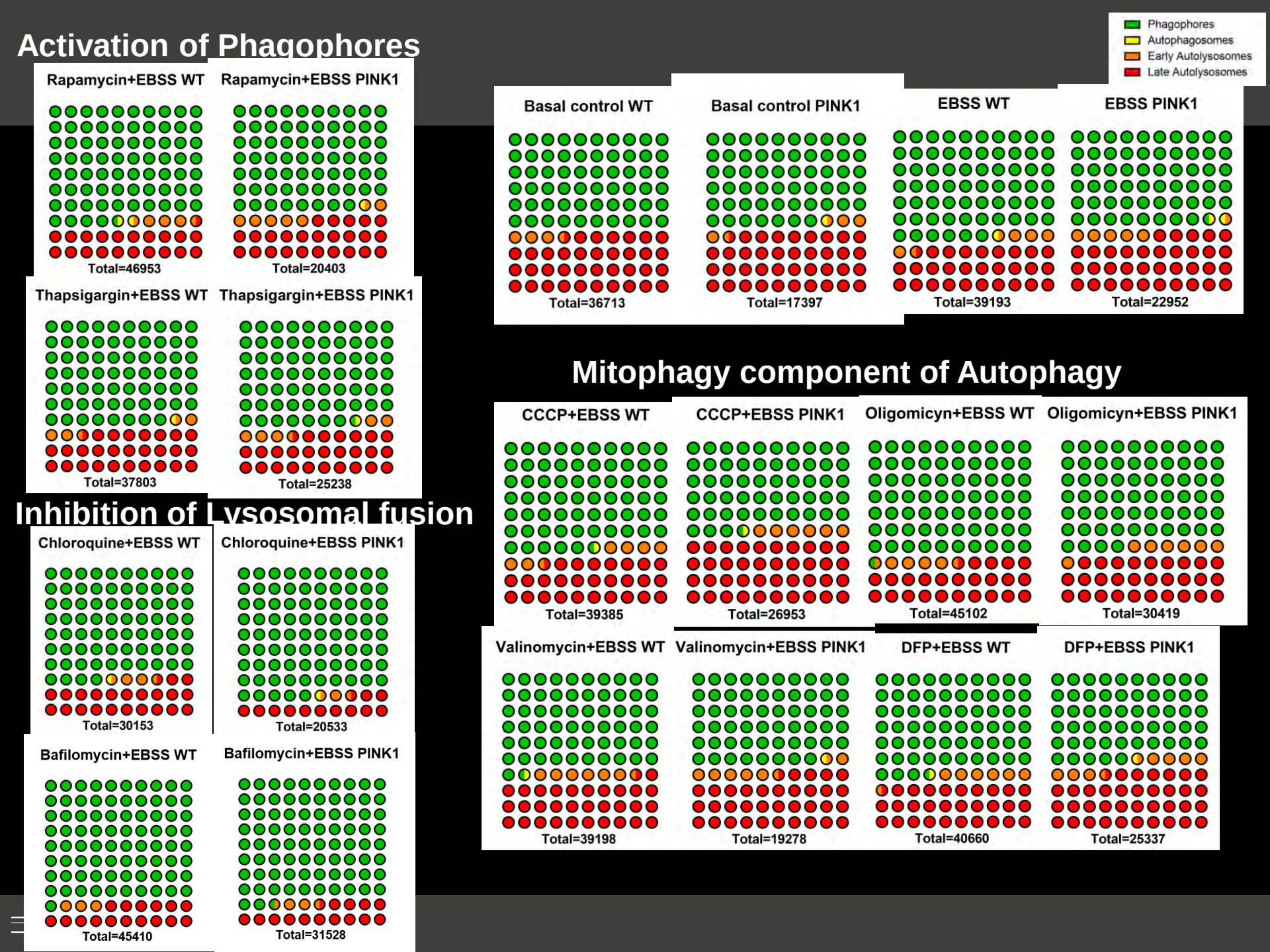




Modulation of pathways

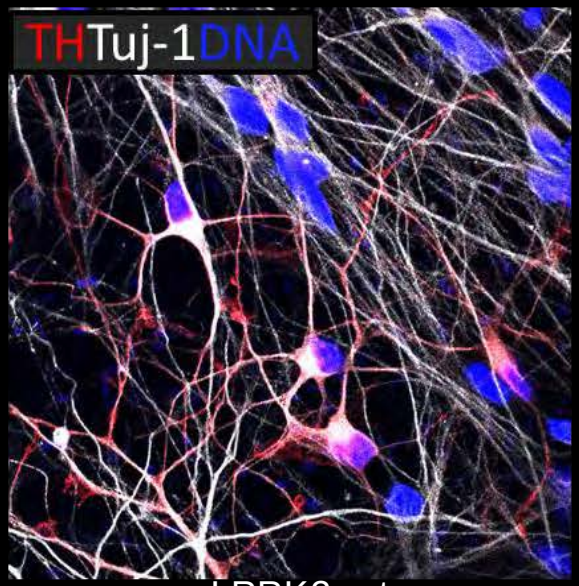




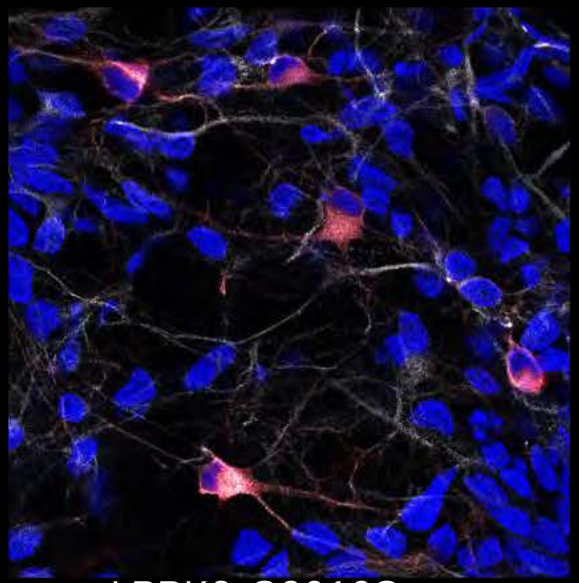

Activation of Phagophores
Phagophores
Autophagosomes
Early Autolysosomes
Late Autolysosomes
Rapamycin+EBSS WT
Total=46953
Rapamycin+EBSS PINK1
Total=20403
Thapsigargin+EBSS WT
Total=37803
Thapsigargin+EBSS PINK1
Total=25238
Basal control WT
Total=36713
Basal control PINK1
Total=17397
EBSS WT
Total=39193
EBSS PINK1
Total=22952
Mitophagy component of Autophagy
CCCP+EBSS WT
Total=39385
CCCP+EBSS PINK1
Total=26953
Oligomicyn+EBSS WT
Total=45102
Oligomicyn+EBSS PINK1
Total=30419
Valinomycin+EBSS WT
Total=39198
Valinomycin+EBSS PINK1
Total=19278
DFP+EBSS WT
Total=40660
DFP+EBSS PINK1
Total=25337
Inhibition of Lysosomal fusion
Chloroquine+EBSS WT
Total=30153
Chloroquine+EBSS PINK1
Total=20533
Bafilomycin+EBSS WT
Total=45410
Bafilomycin+EBSS PINK1
Total=31528

Early neuronal phenotype: Neurite complexity

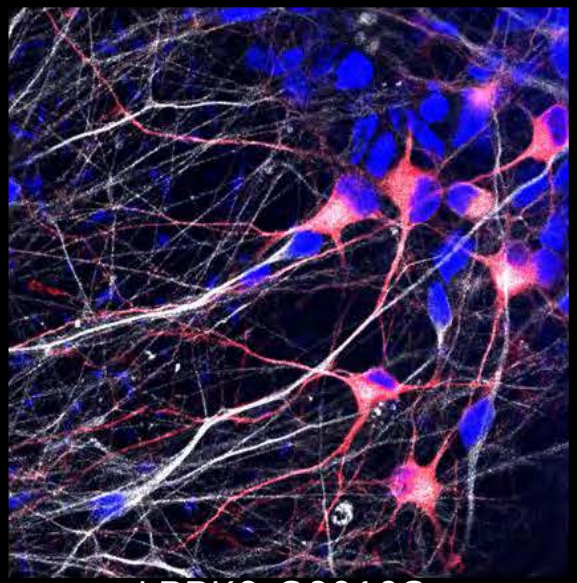
- 14d differentiation starting from NESCs
- Treatment with LRRK2 inhibitor* for 12 days.
- Comparison of an engineered line to a patient line.



LRRK2 wt
Healthy background



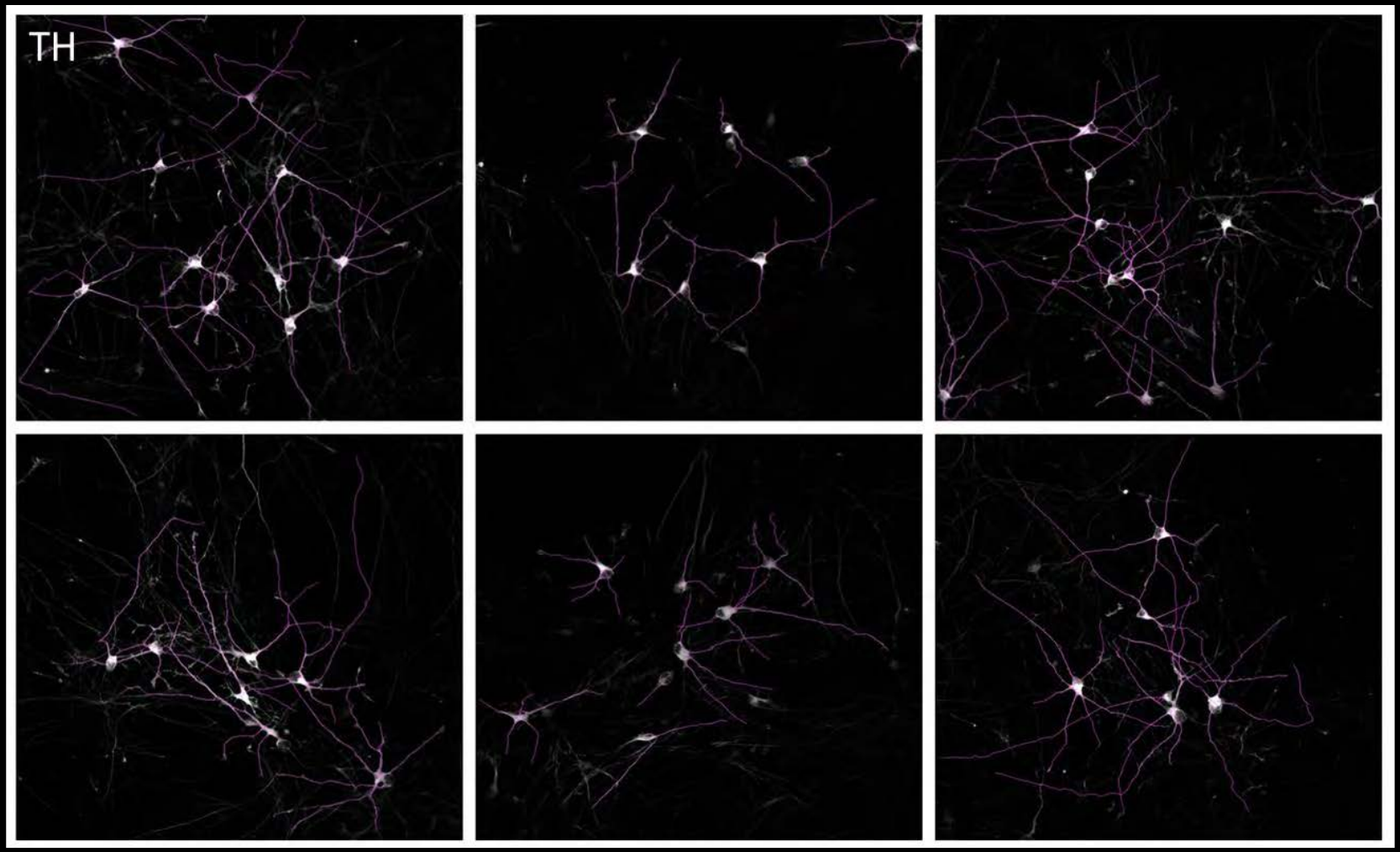
LRRK2-G2019S
introduced



LRRK2-G2019S
introduced
+LRRK2 inhibitor*

* Inhibitor from Ramsden et al. (2011) in ACS Chem Biol

Early neuronal phenotype: Neurite complexity

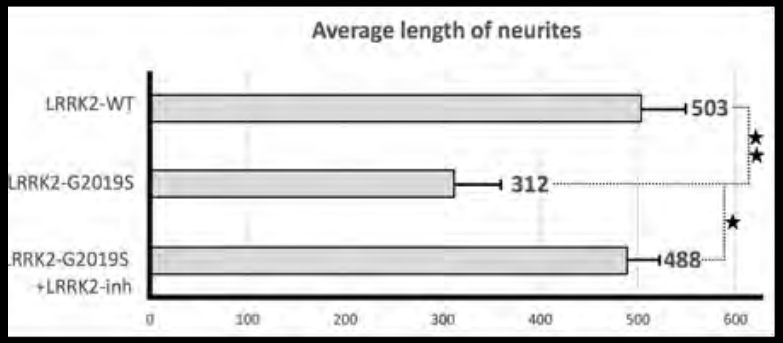


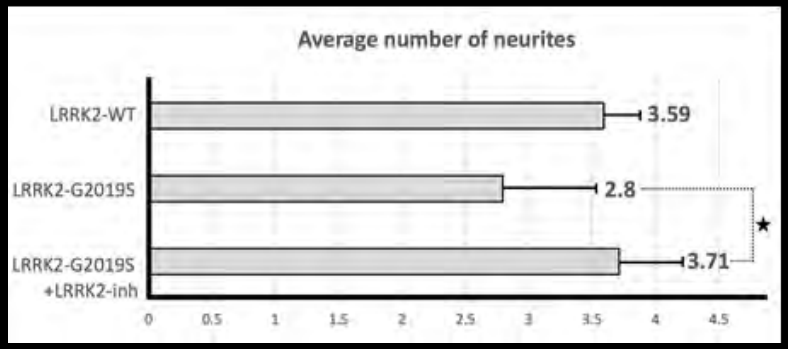
LRRK2 wt
Healthy background

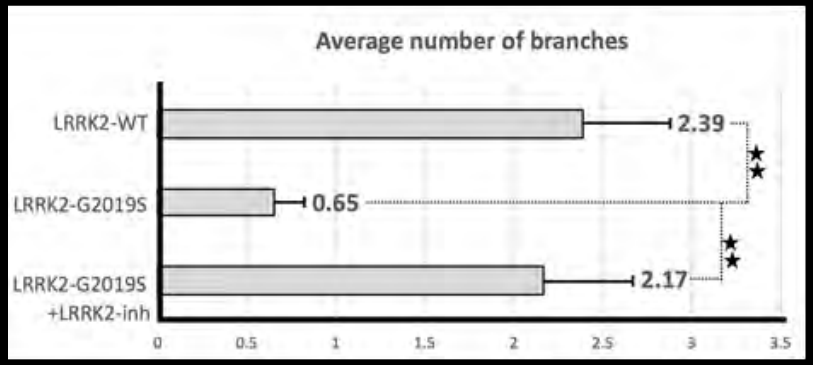
LRRK2-G2019S
introduced

LRRK2-G2019S
introduced
+LRRK2 inhibitor*

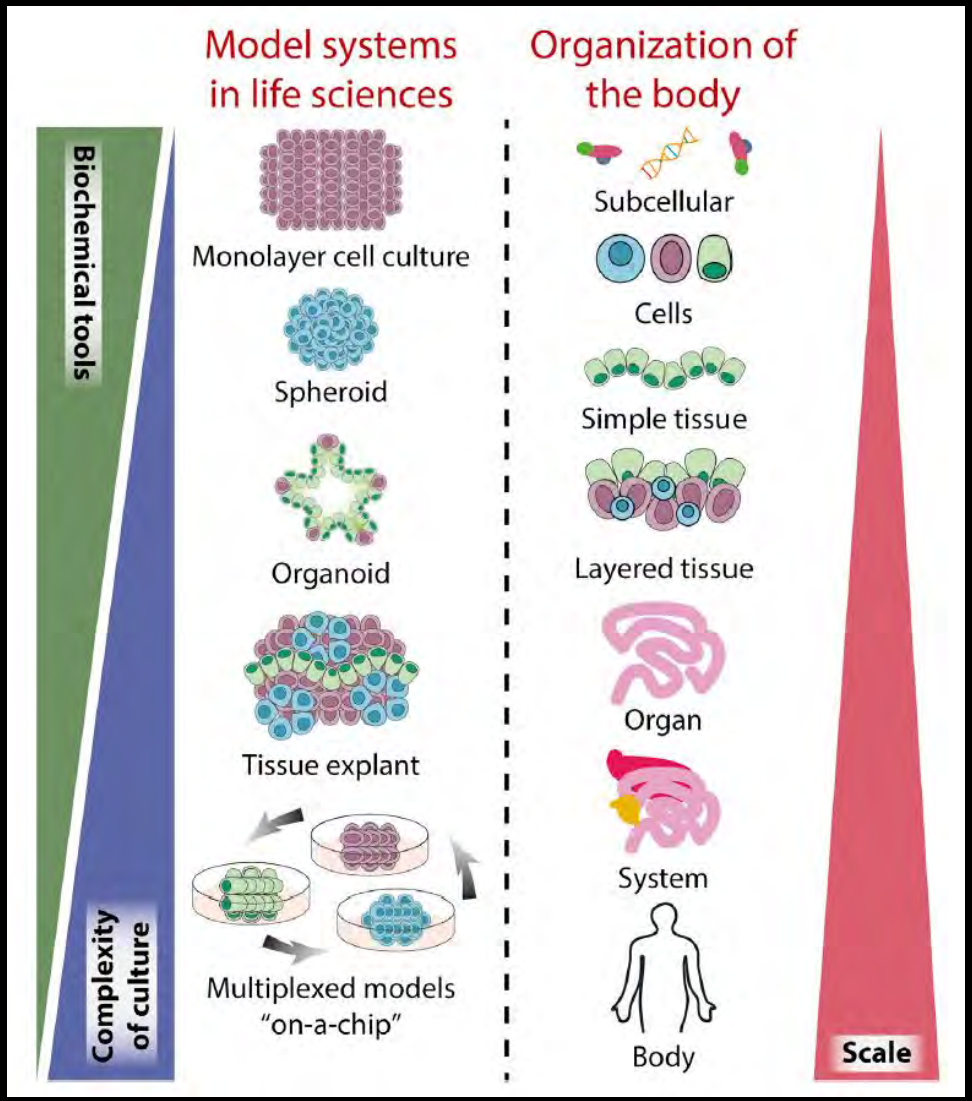
Early neuronal phenotype: Neurite complexity







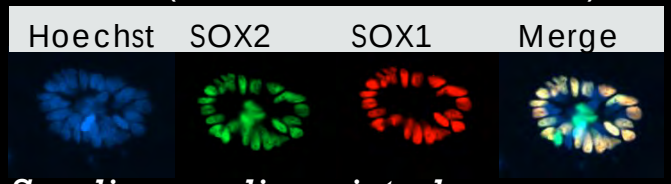
Advanced 3D models



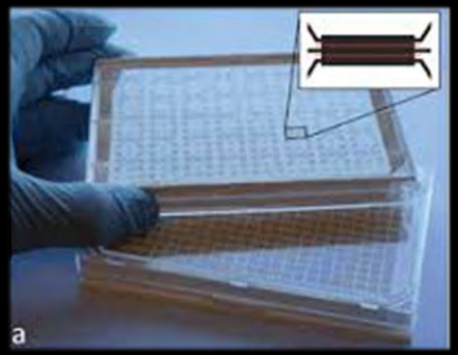
- More complex & 3D, closer to *in vivo* situation
- Stem cells exhibit an intrinsic ability to assemble into complex structures
- Mimic the natural environment as closely as possible to improve growth conditions

Human neuroepithelial stem cells (NESCs) for starting 3D cultures

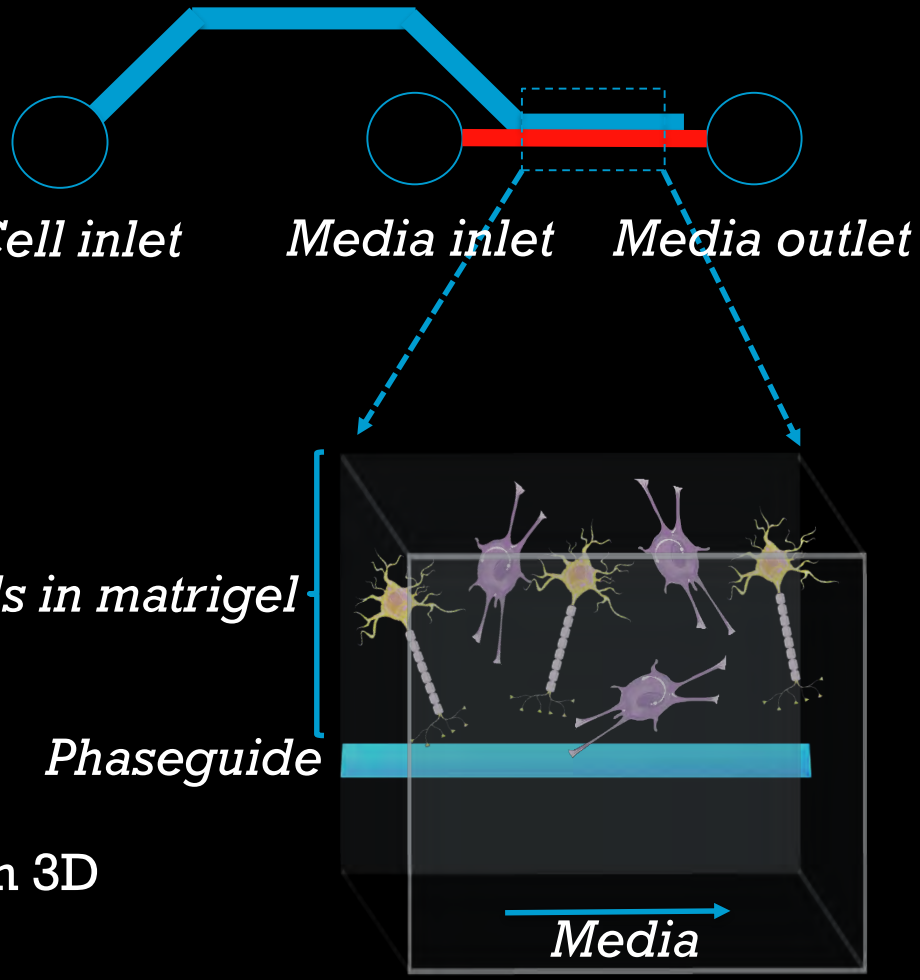
hNESC (Reinhardt et al., 2013):



Seeding as dissociated cells



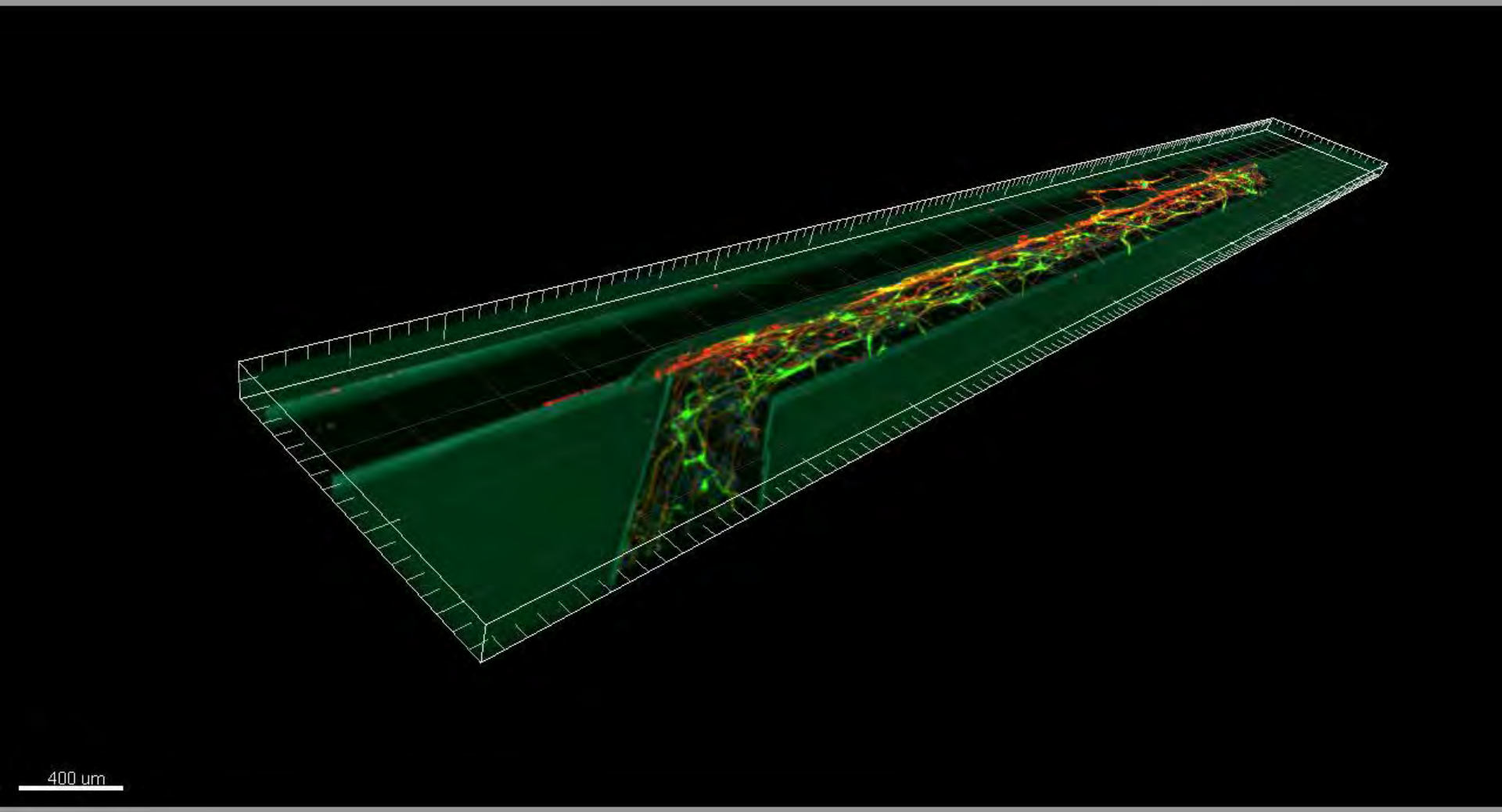
MIMETAS
the organ-on-a-chip company



1) Phenotype of PD derived lines in 3D

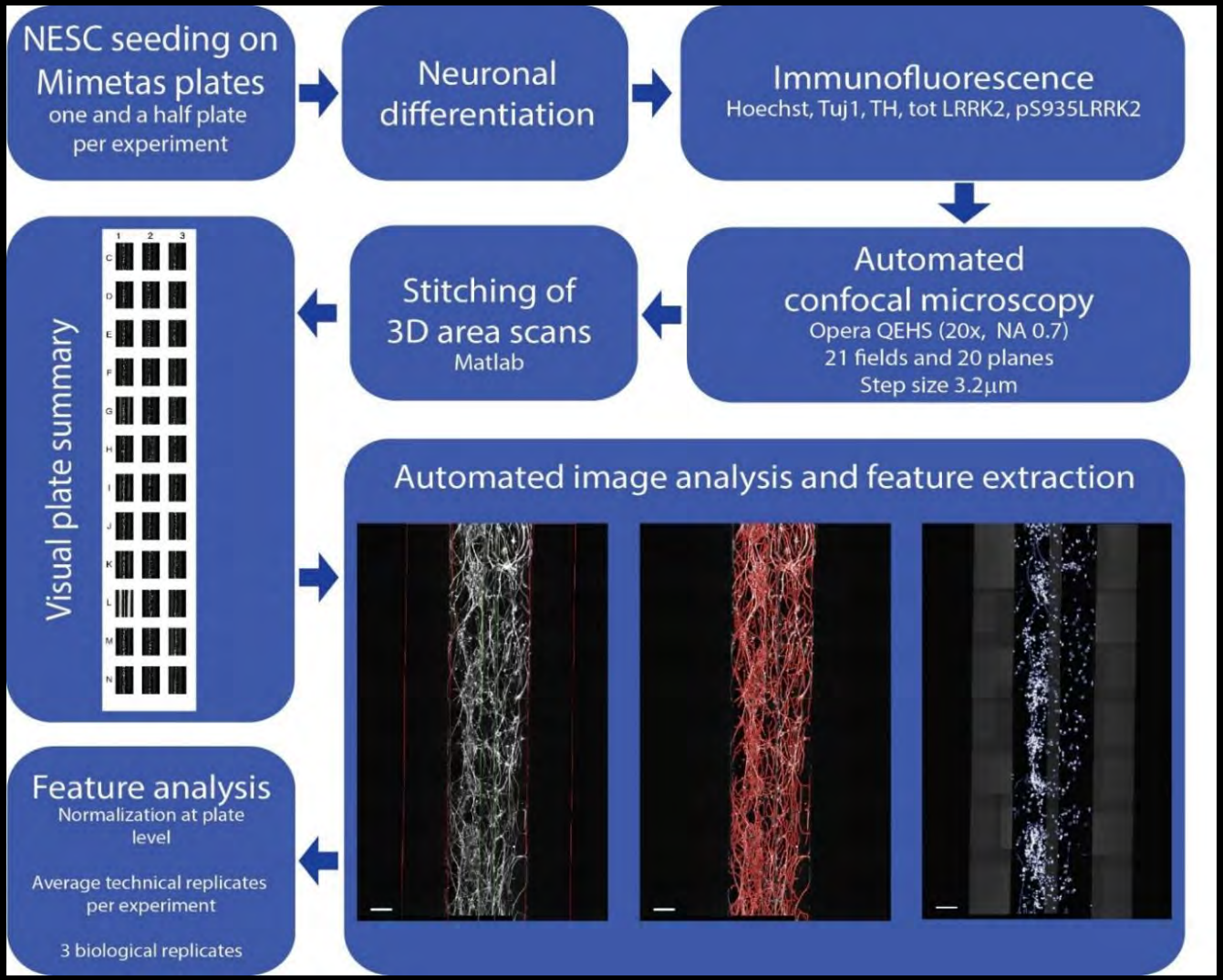
2) Rescue with dugs

Differentiation into dopaminergic neurons



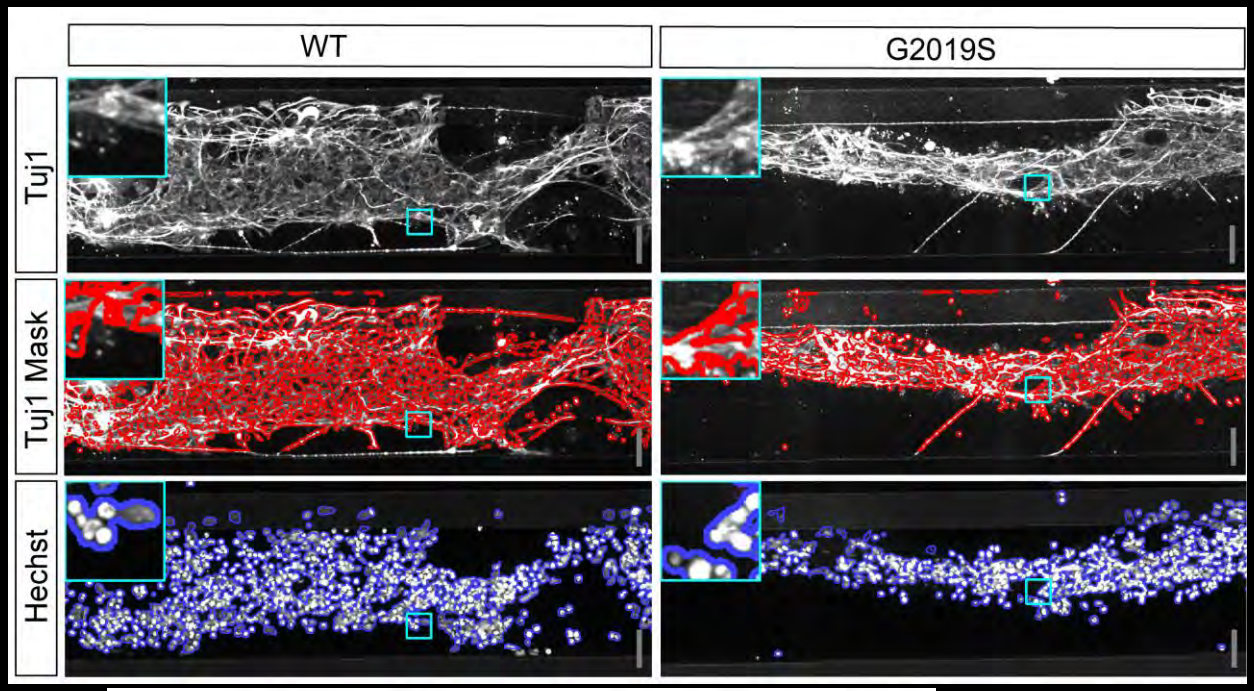
Moreno et al., 2015

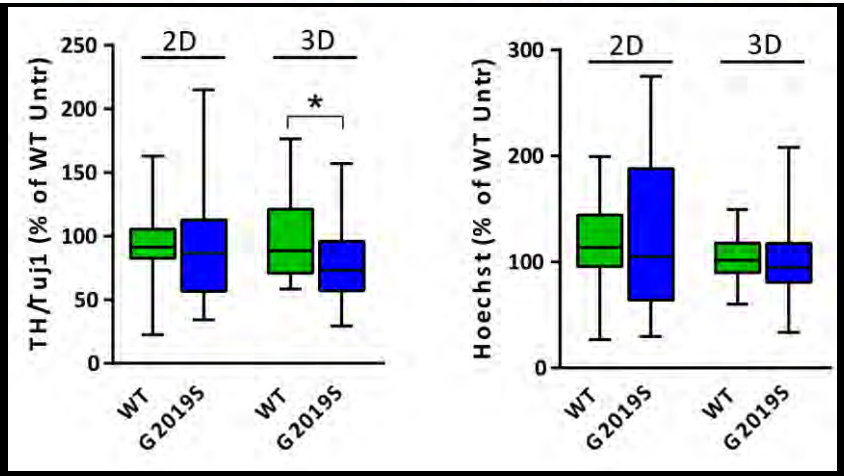
Development of a full Pipeline from cell culture to feature analysis



Perkin Elmer Opera HCS System

LRRK2-G2019S driven cell death in 3D

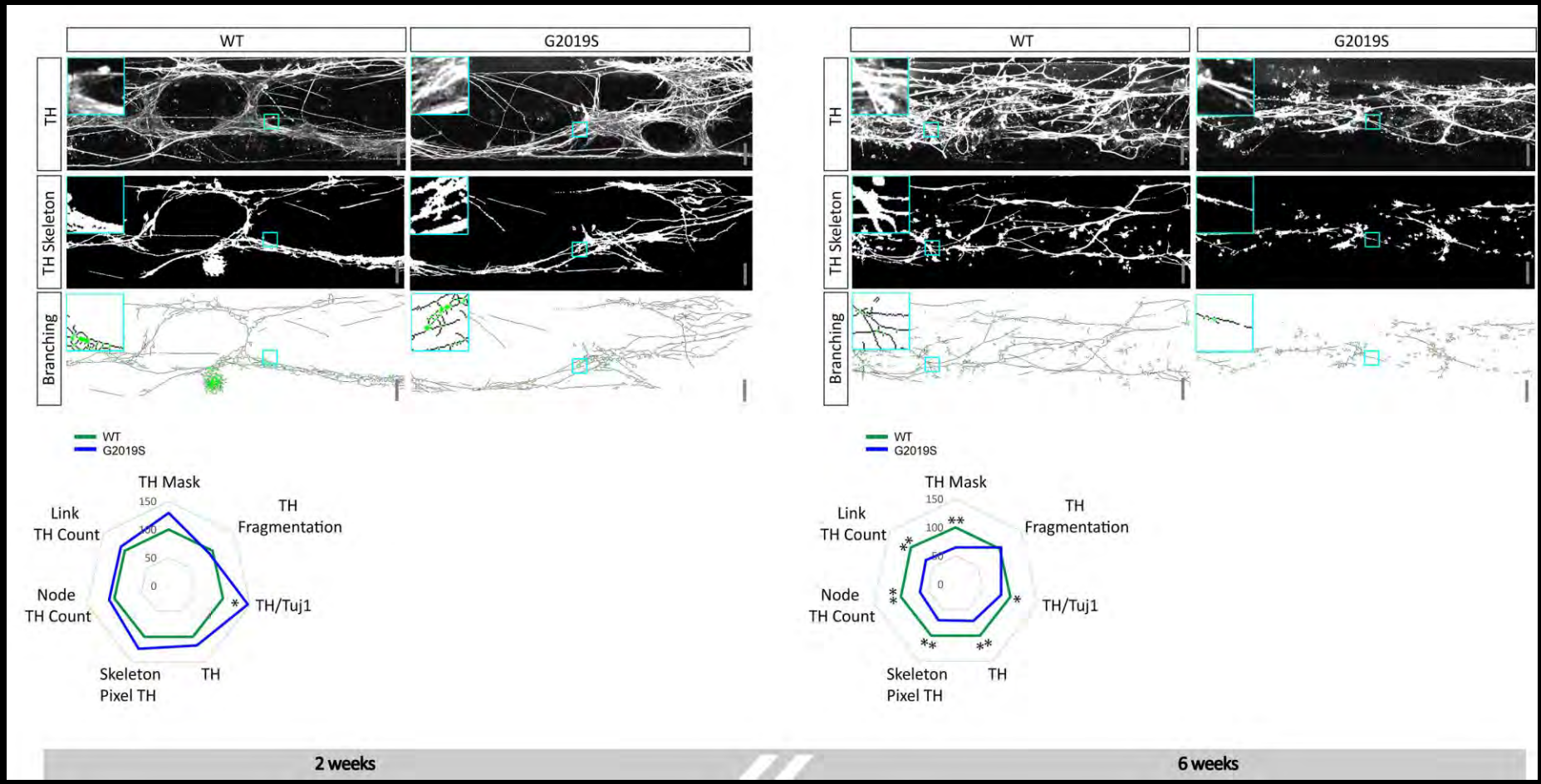




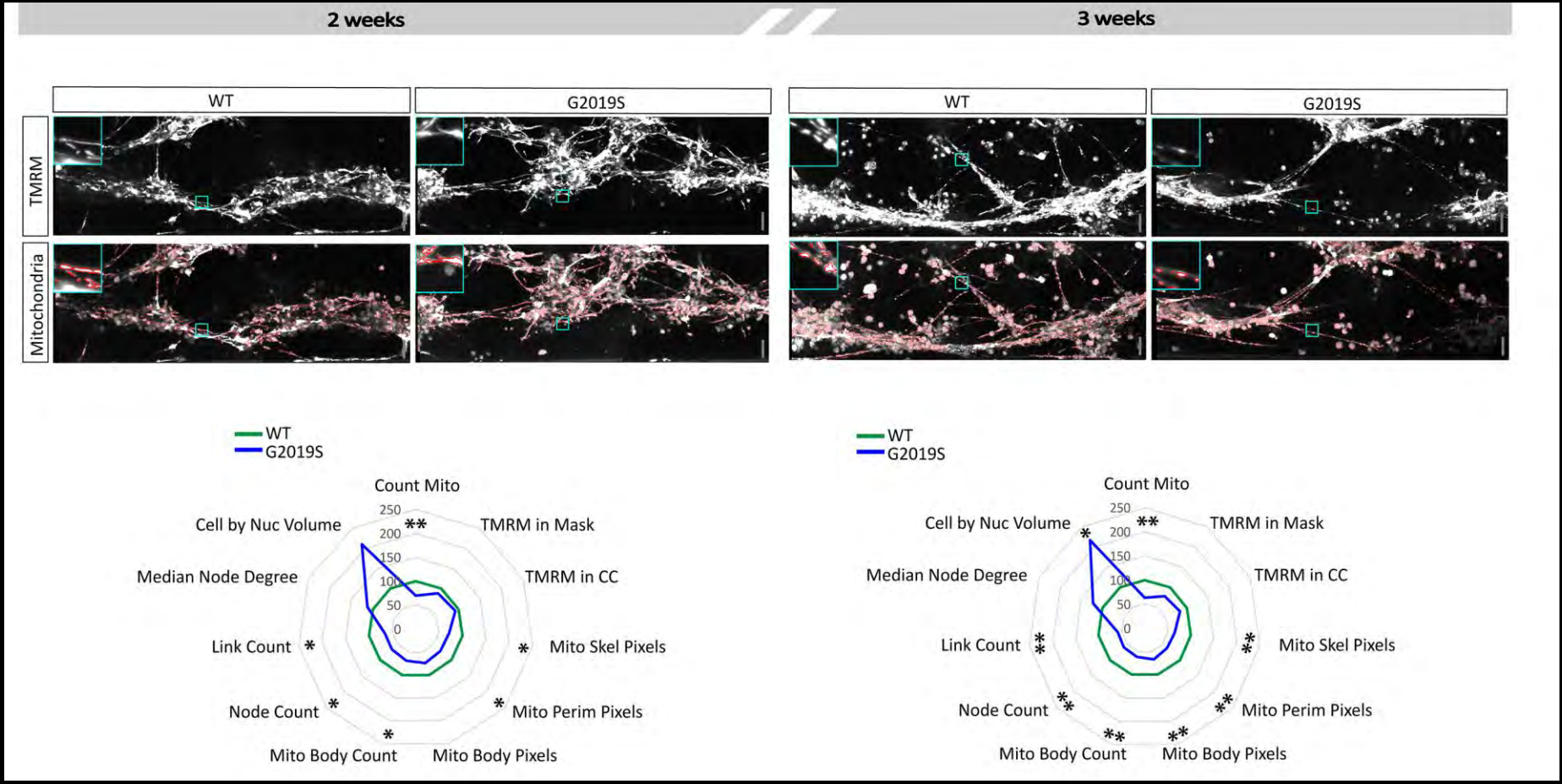
6 weeks culture

–> Clear 3D impact

Reduced neurite complexity in 3D

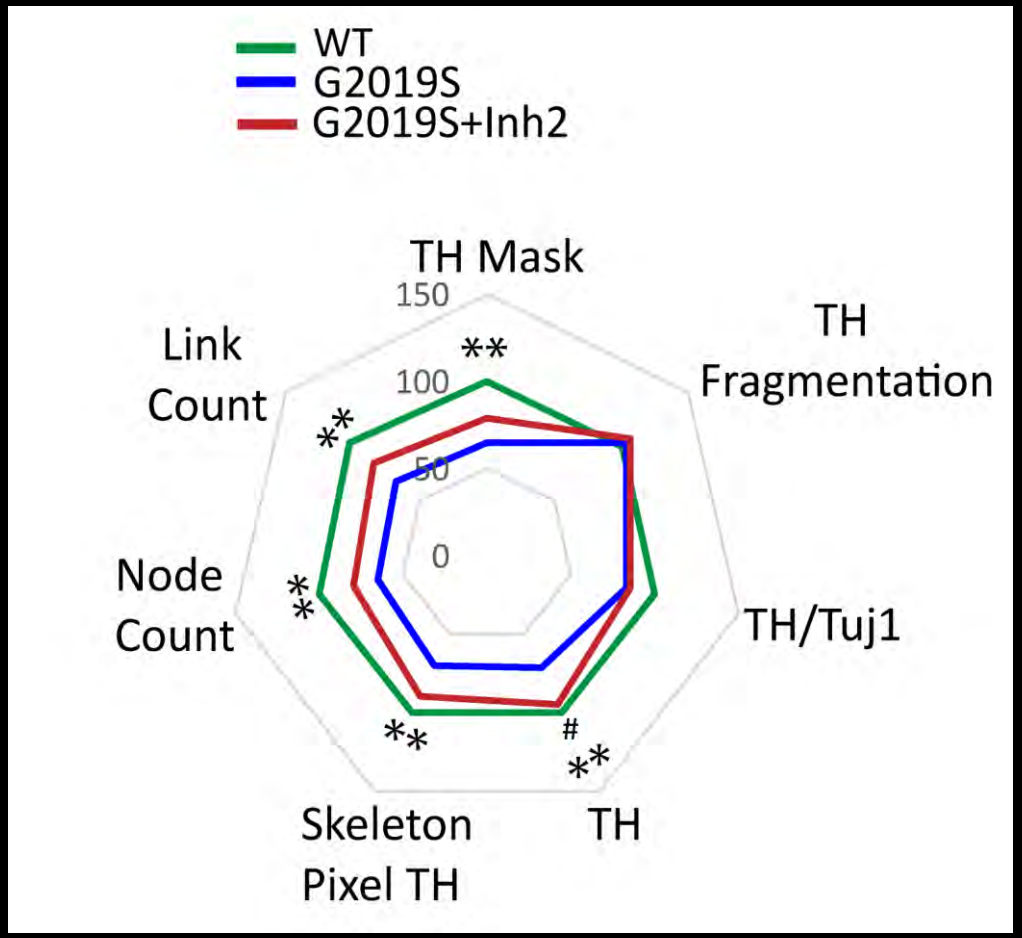


Mitochondrial phenotypes are preceding



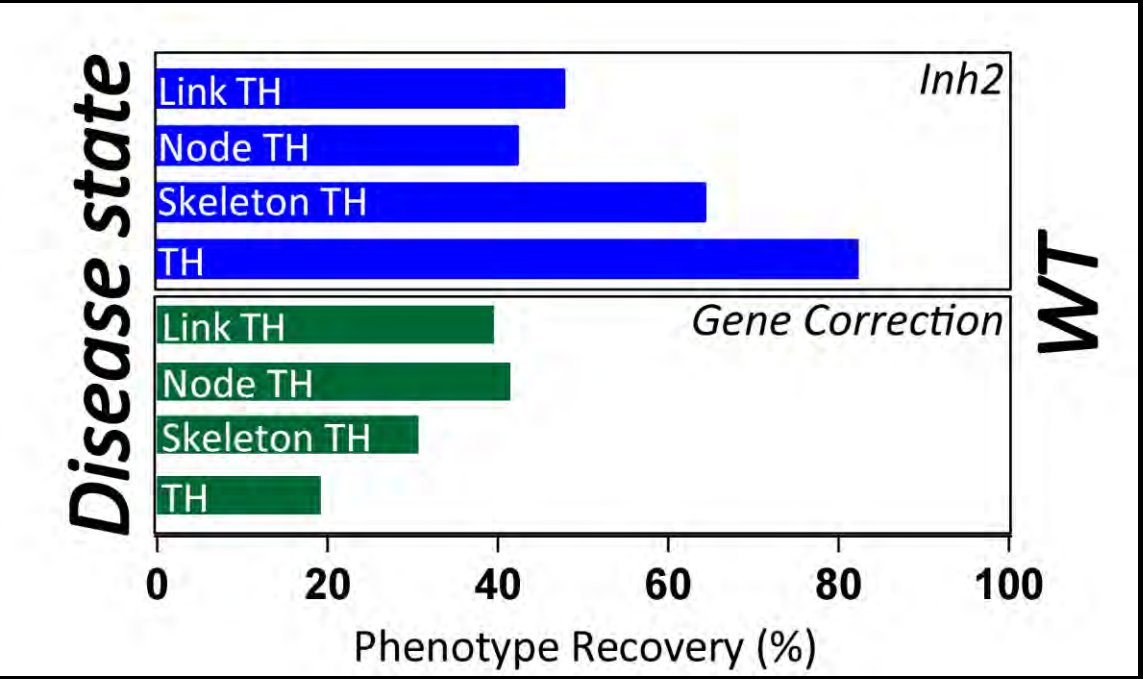
Mito tracker green
Smaller nuclei

(Partial) rescue via LRRK2 inhibitor treatment

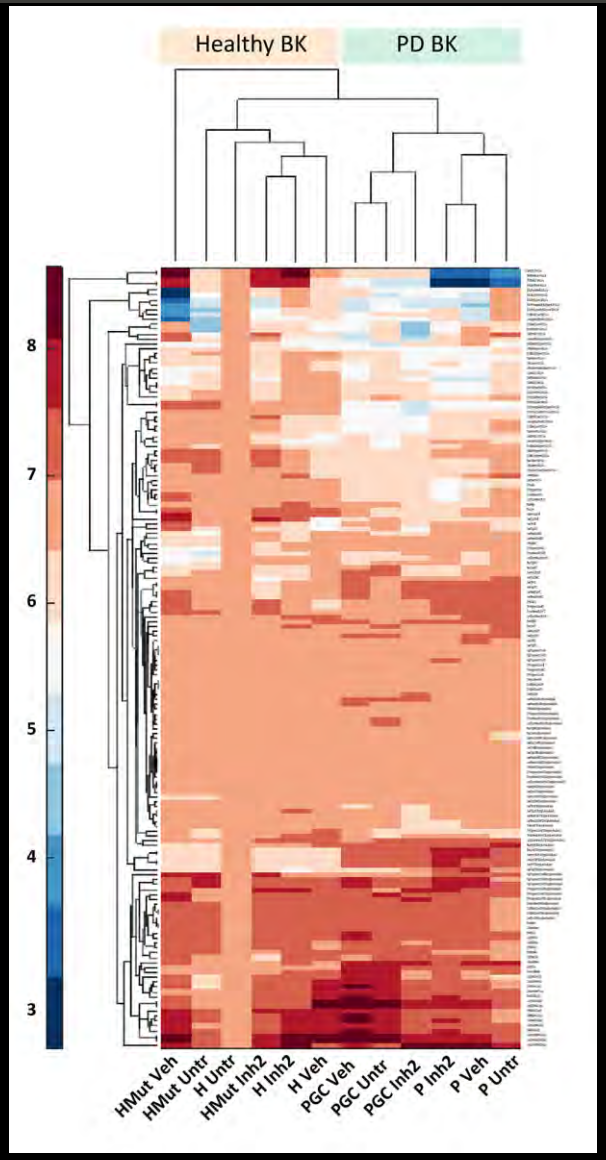


Inh2, Ramsden et al., 2011, 0.5μm

Gene-correction vs. drug treatment



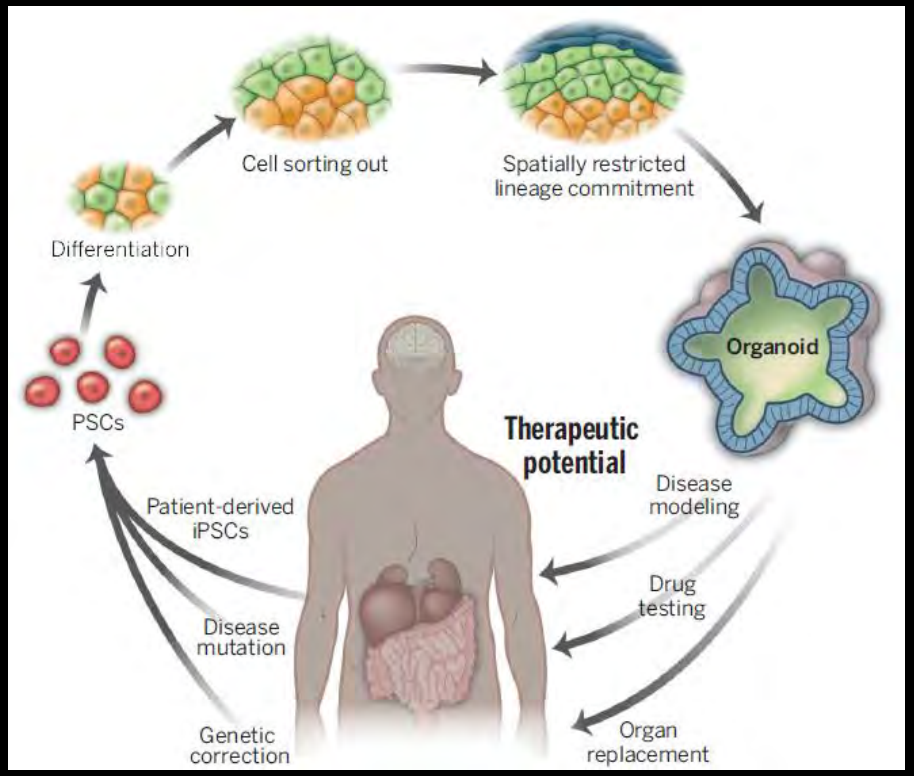
Genetic background vs. genotype



Bolognin et al., 2018

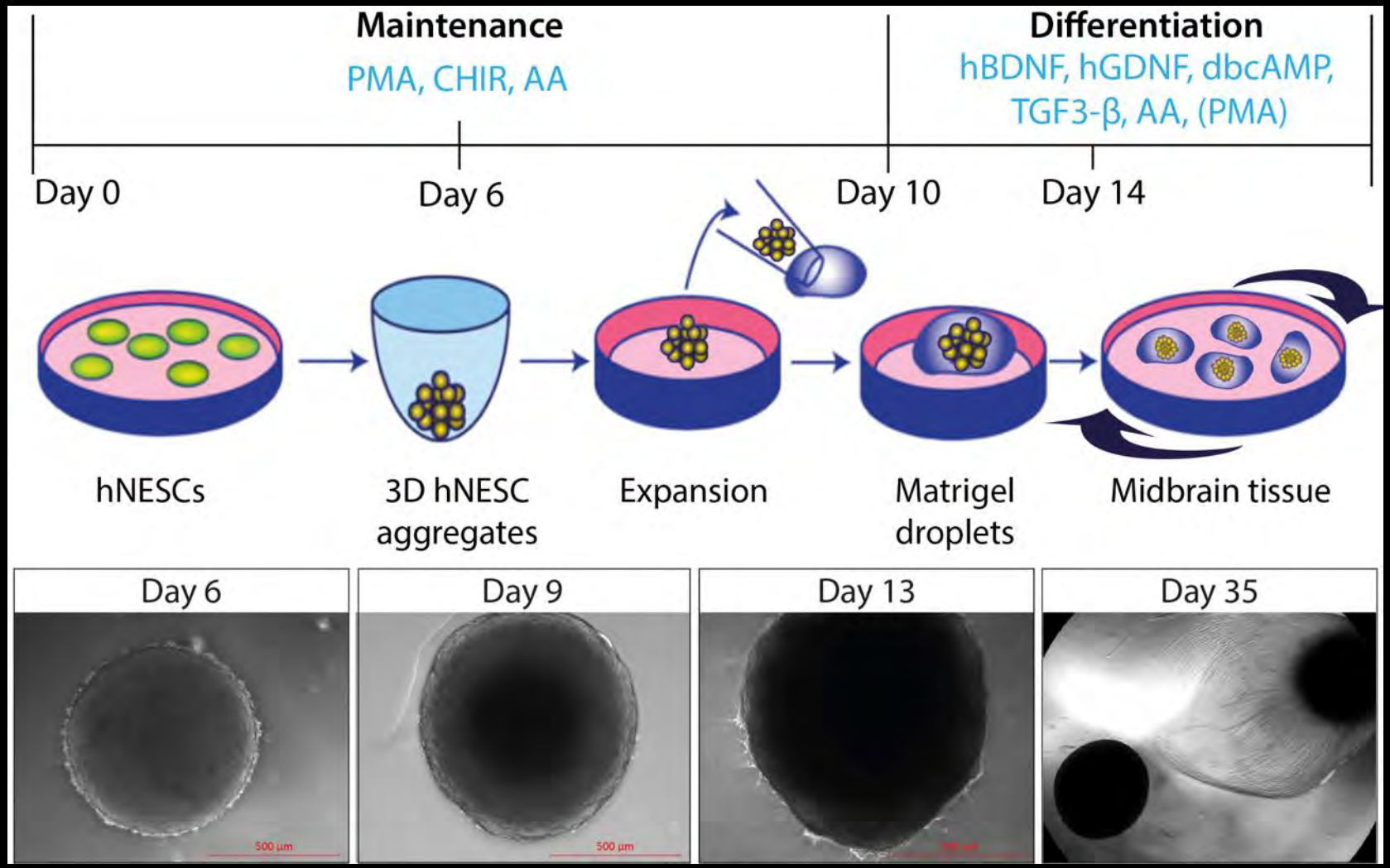
Increasing complexity with organoid systems

- Spatial organisation of heterogeneous **tissue-specific** cells
- Cell-cell interactions, cell-ECM interactions
- **Physiological functions** generated by tissue-specific cells
- **Stable system** amenable to extended cultivation and manipulation
- Patient-specific
- Reproducible



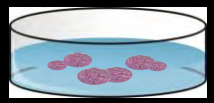
Lancaster & Knoblich 2014

Generation of Midbrain Organoids: Dynamic Condition

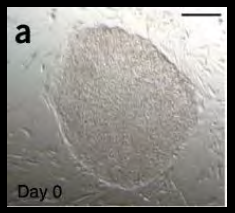


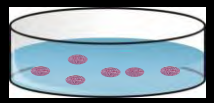
Approach based on Lancaster et al., 2013, Nature

Generation of midbrain organoids

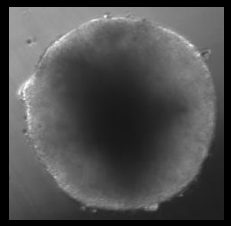


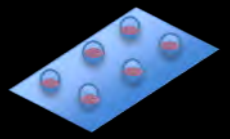
hiPSCs



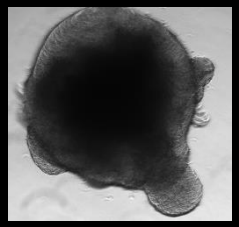


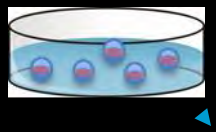
hNESC





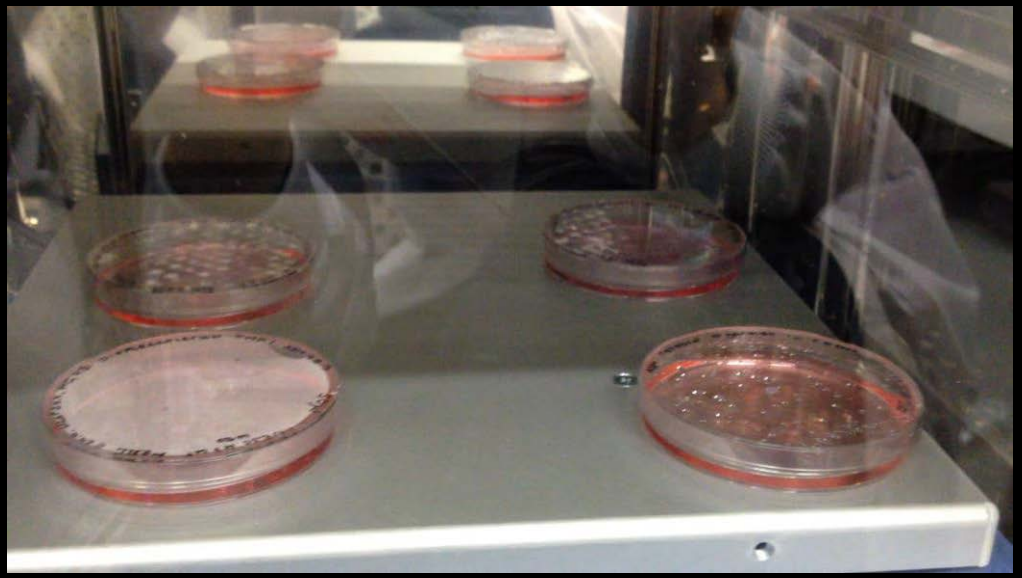
Transfer to Matrigel droplets

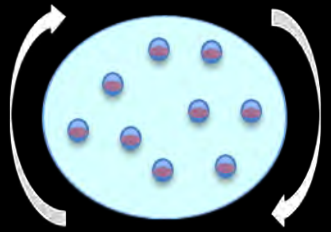




Expansion

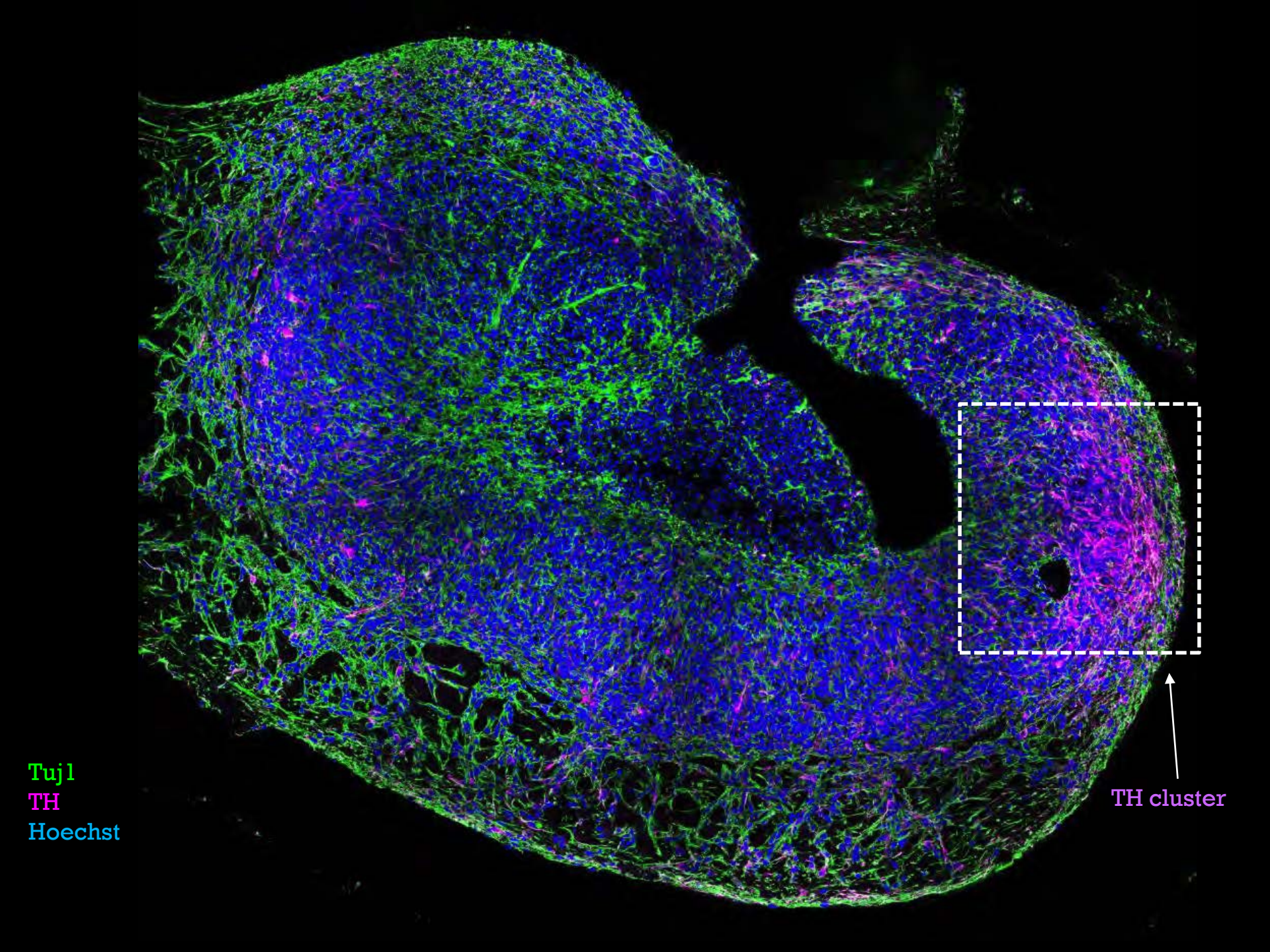
Differentiation medium



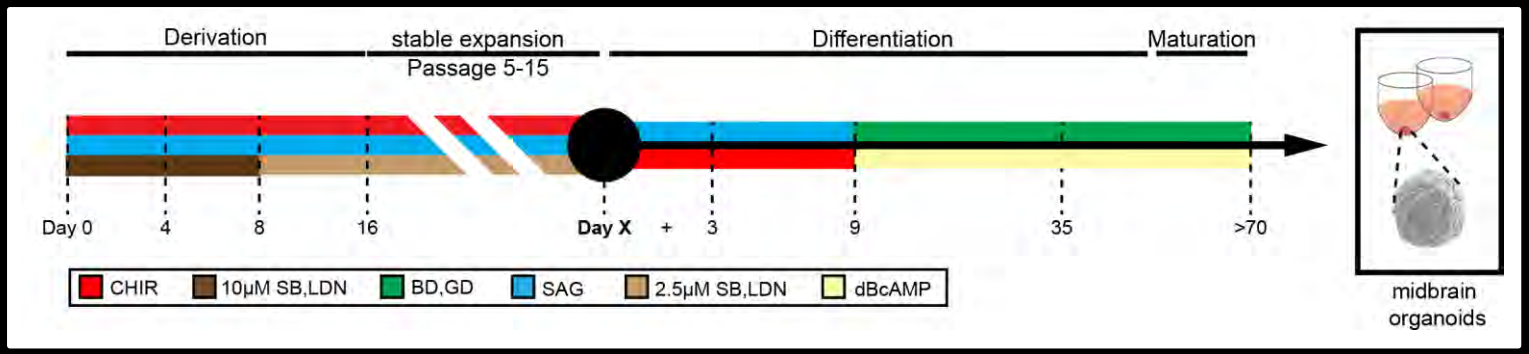


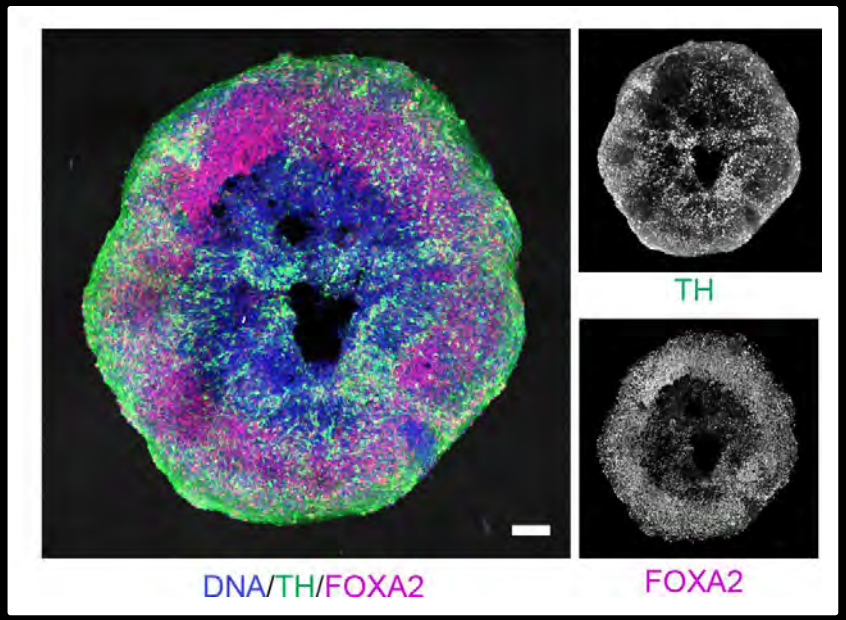
Orbital shaker

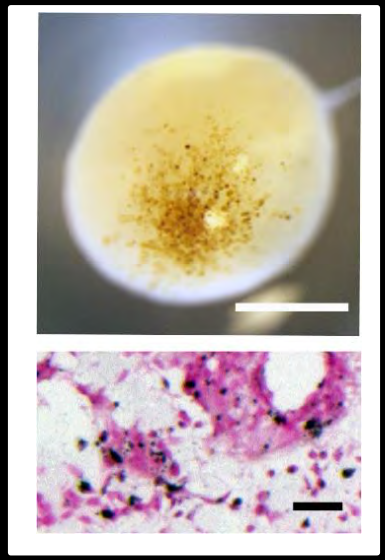
In 10 cm dish or 96 well plate


Tuj1
TH
Hoechst
TH cluster

Derivation of more ventralized organoids

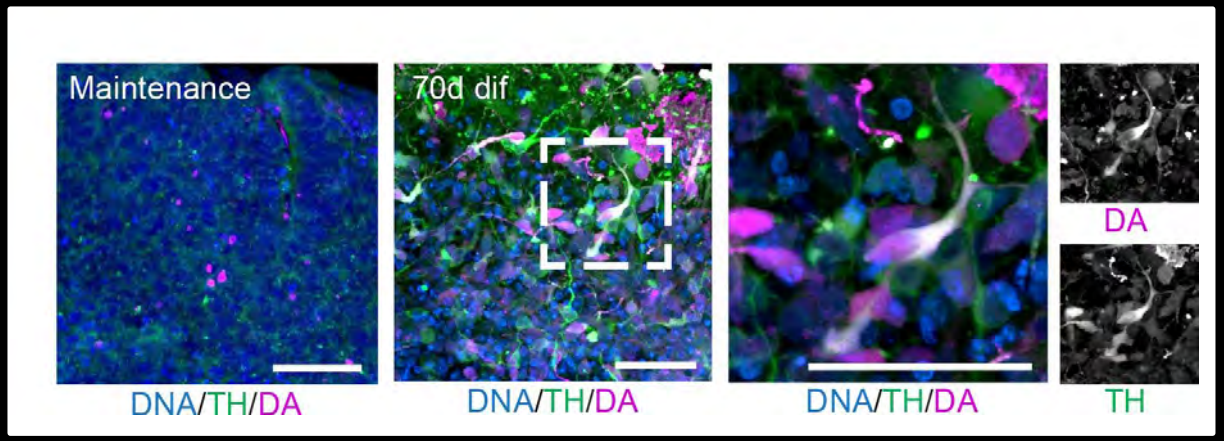


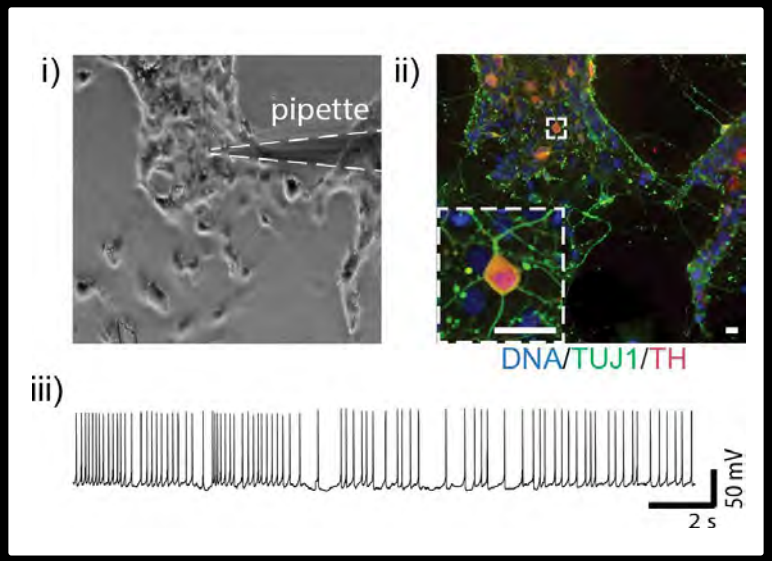


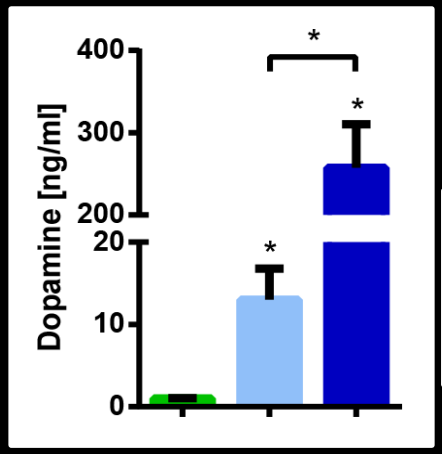


Smits et al., submitted

Dopaminergic activity in ventralized organoids



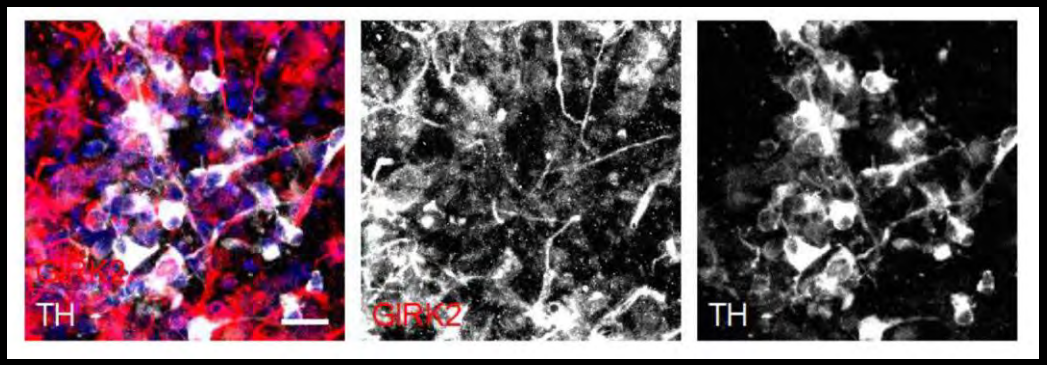




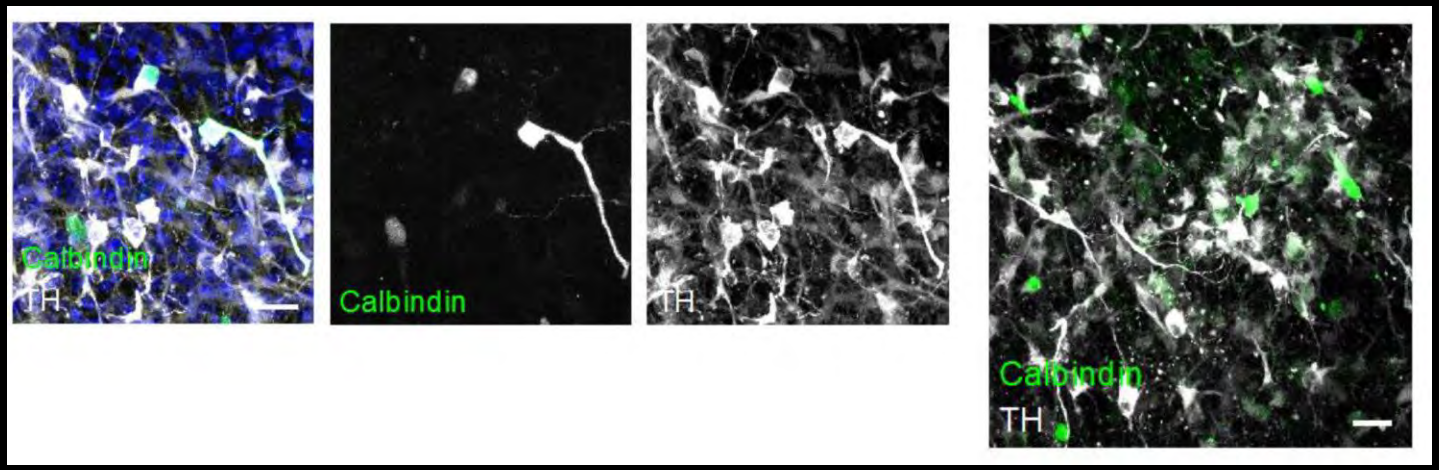
vNESC
35d Dif.
70d Dif

A9 / A10 dopaminergic neurons

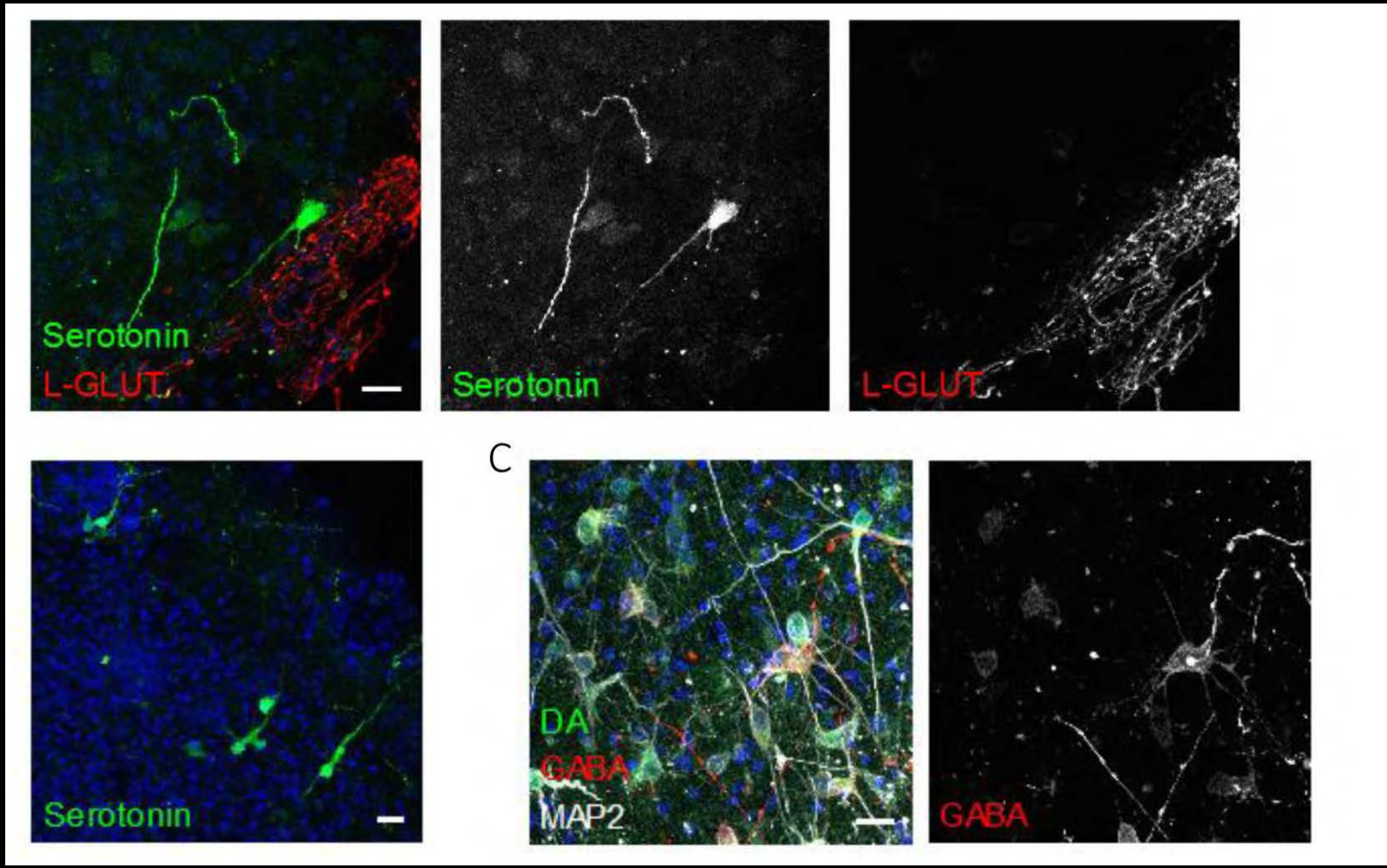
A9
TH/Girk2



A10
Calbindin/TH

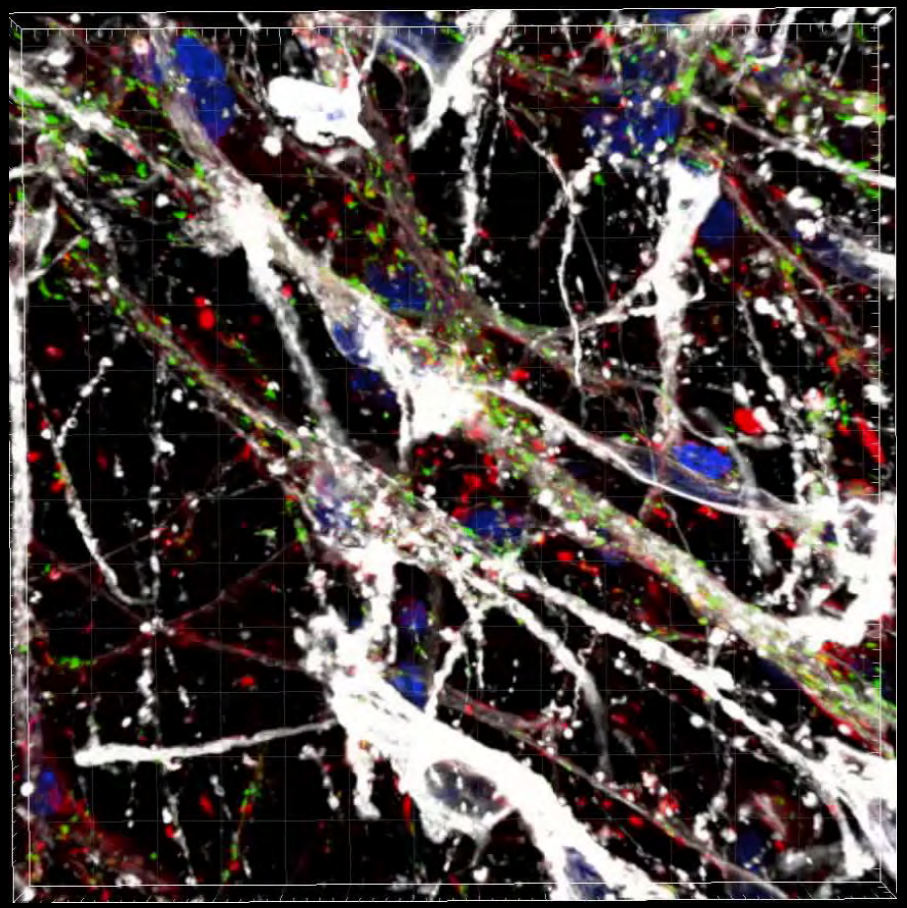


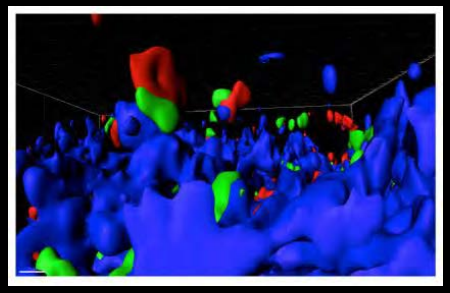
Other neurons

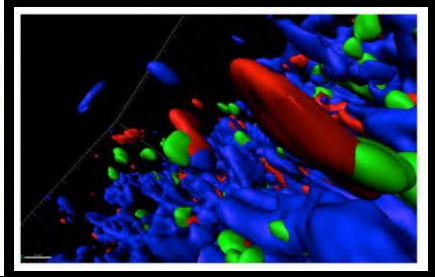


Synapse formation

Synaptophysin PSD-95 Hoechst



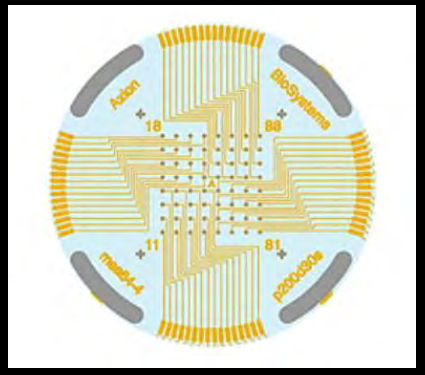


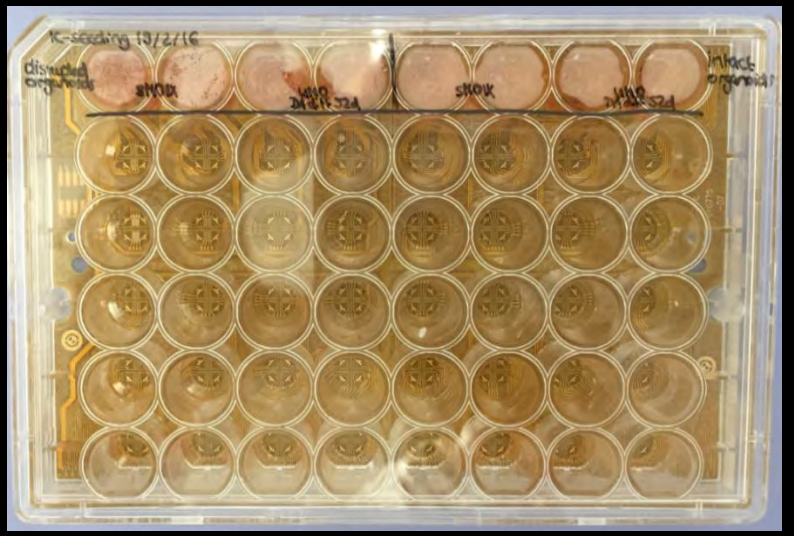


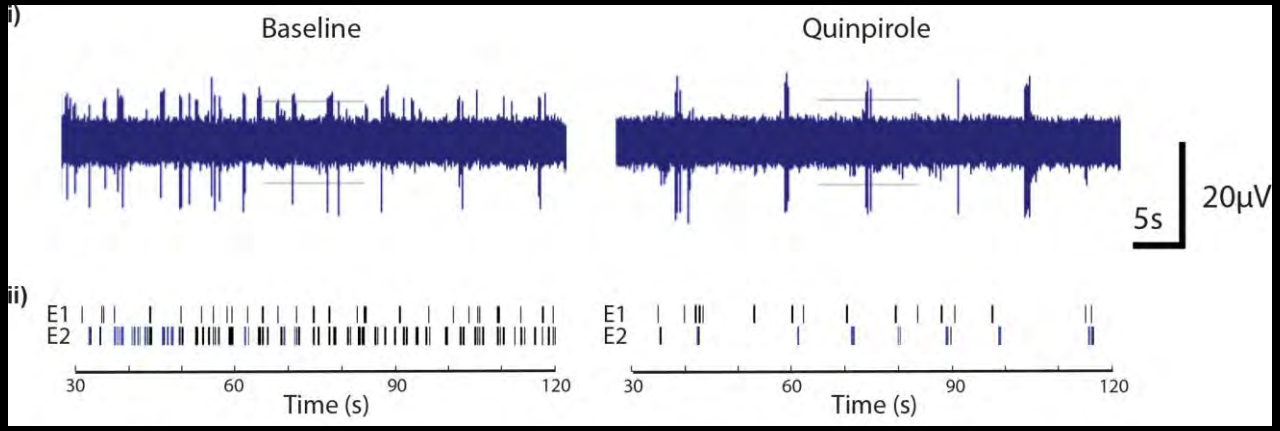
Microelectrode Array (MEA)

Grid of microelectrodes to capture electrophysiological data from multiple cells simultaneously

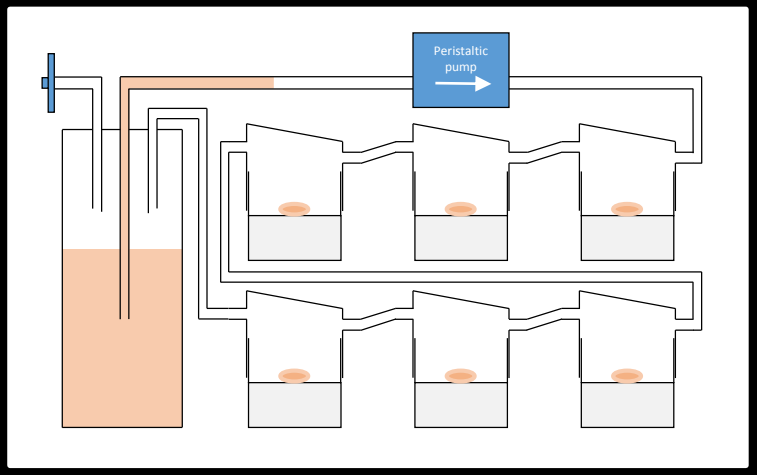
Cellular network communication

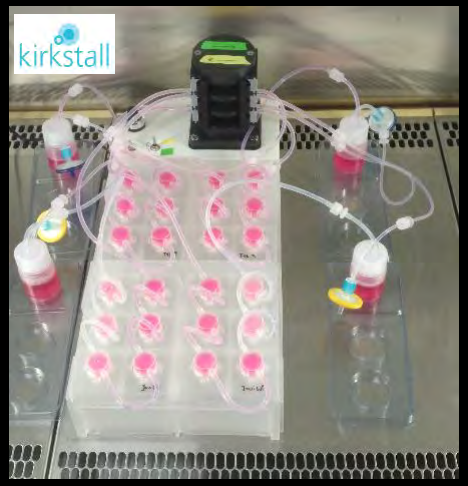


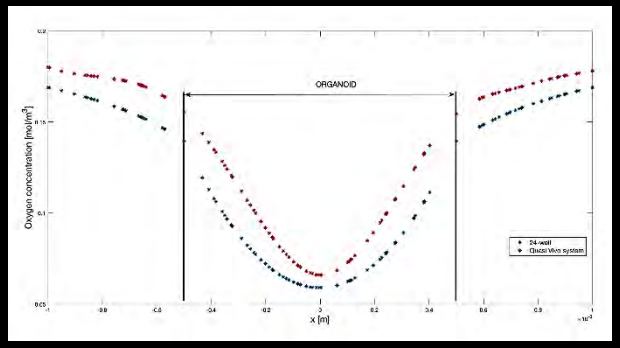




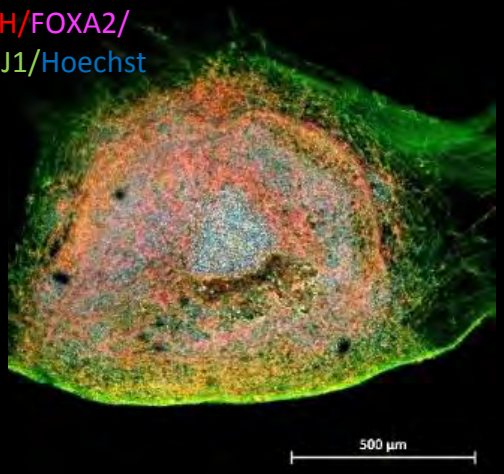
Millifluidics technology for midbrain organoid generation

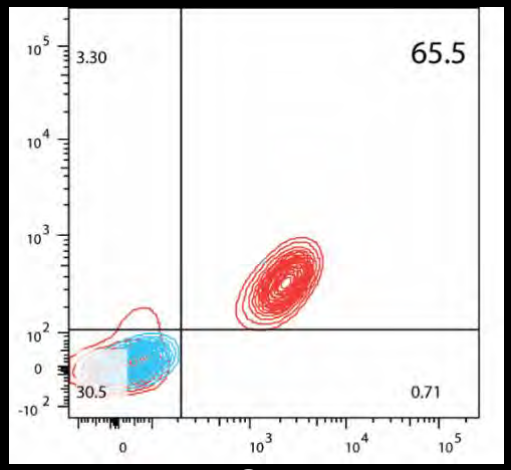


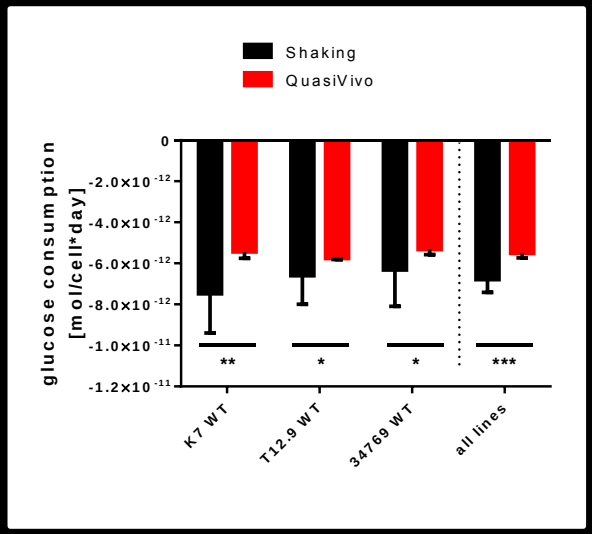




TH/FOXA2/
TUJ1/Hoechst



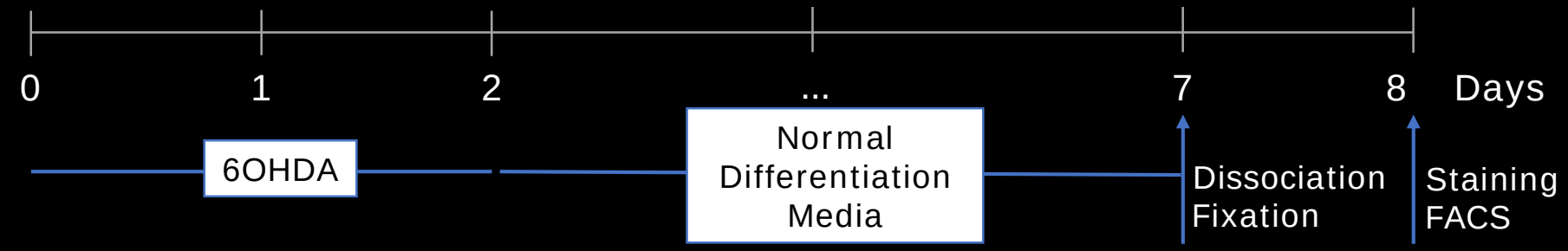


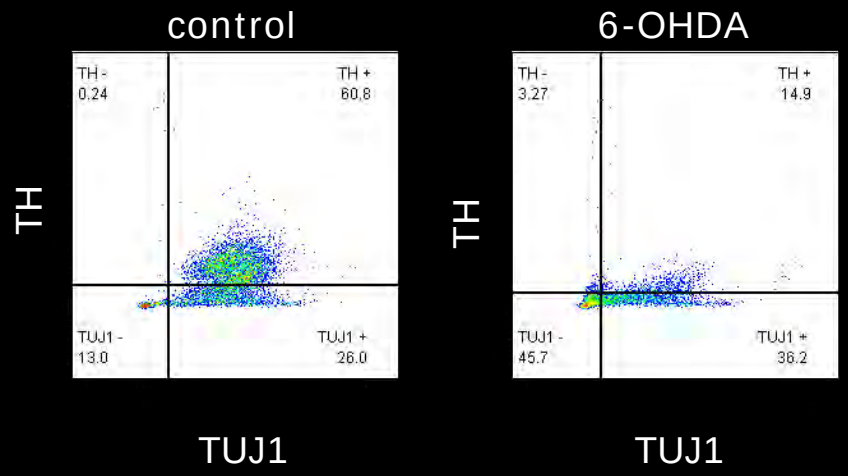


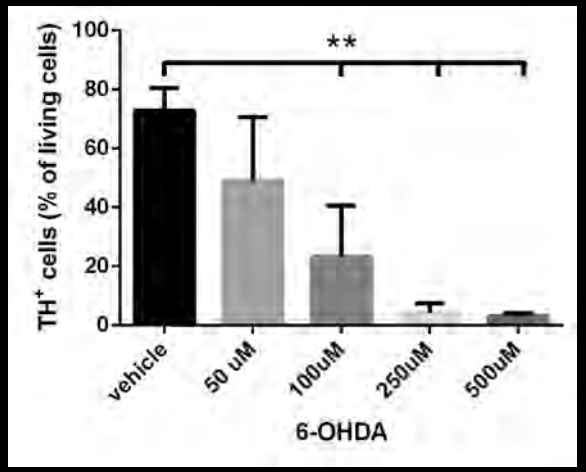
Berger et al., in revision

Toxicology in midbrain organoids

→ selective loss of dopaminergic neurons

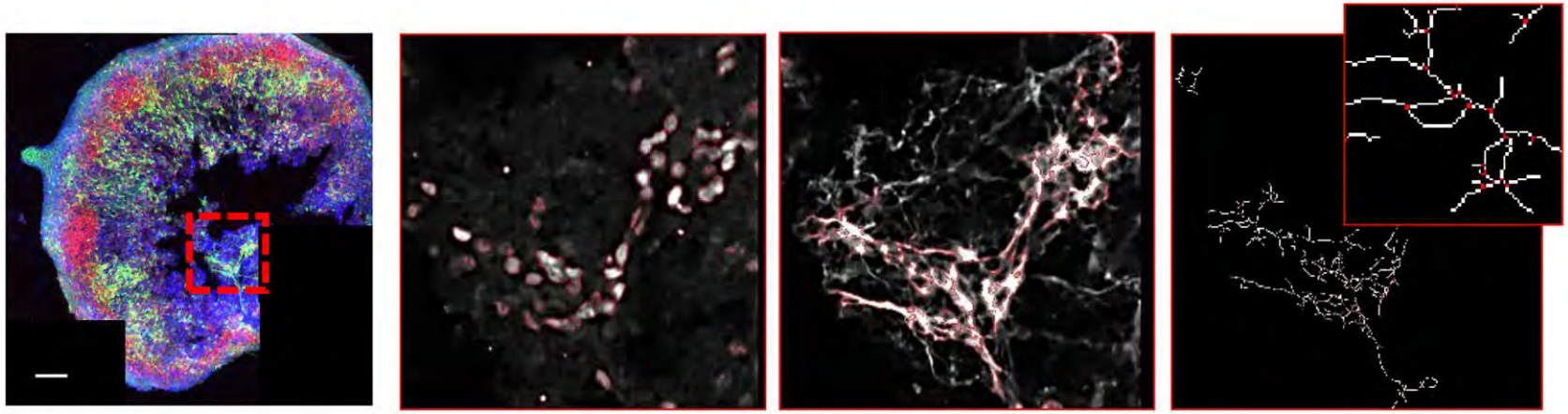






Hemmer et al., unpulished

Pipeline for phenotyping in organoids

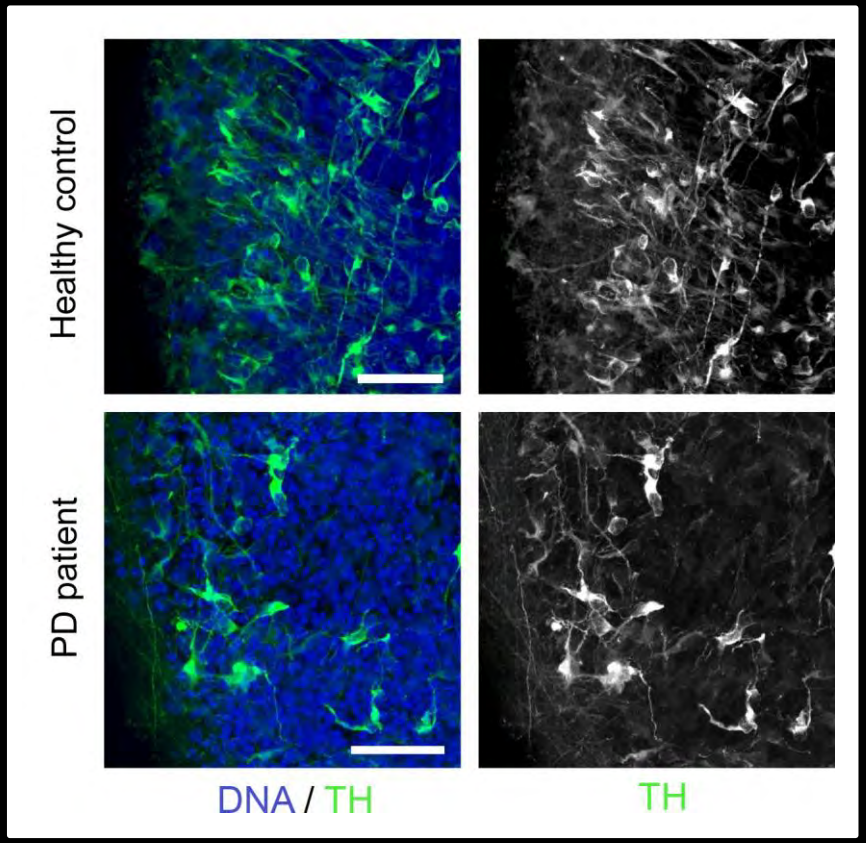


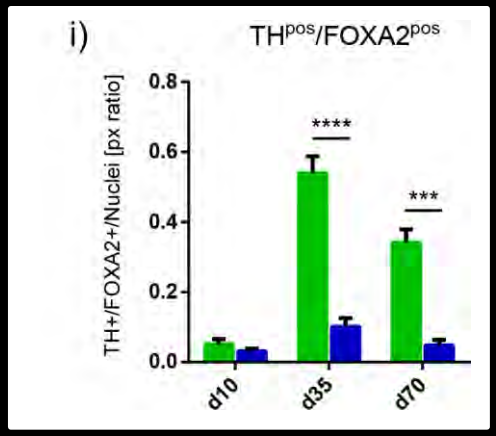
FOXA2 mask | TH mask | TH skeleton

Automated mask detection → Nuclei segmentation → Pyknotic pixel removal → Feature extraction → marker expression, neurite complexity, branching

→ standardised procedure for efficient image analysis

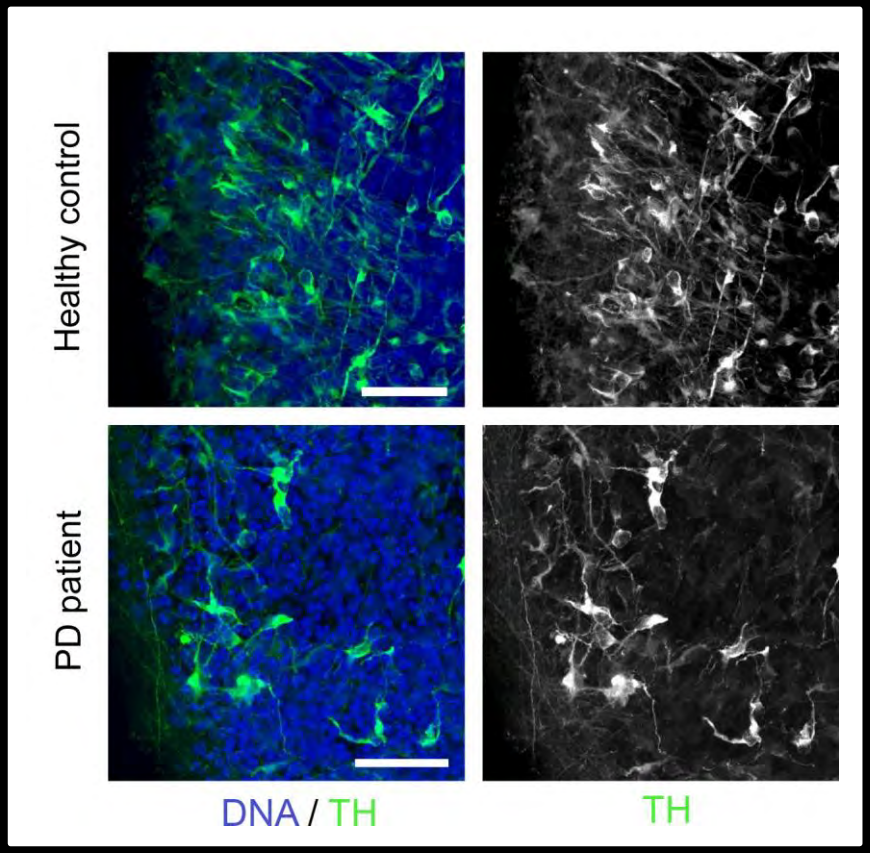
PD: Reduction in the amount of dopaminergic neurons

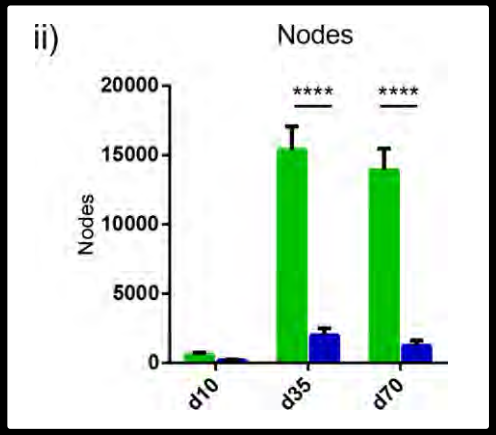




PD patient: LRRK2-G2019S

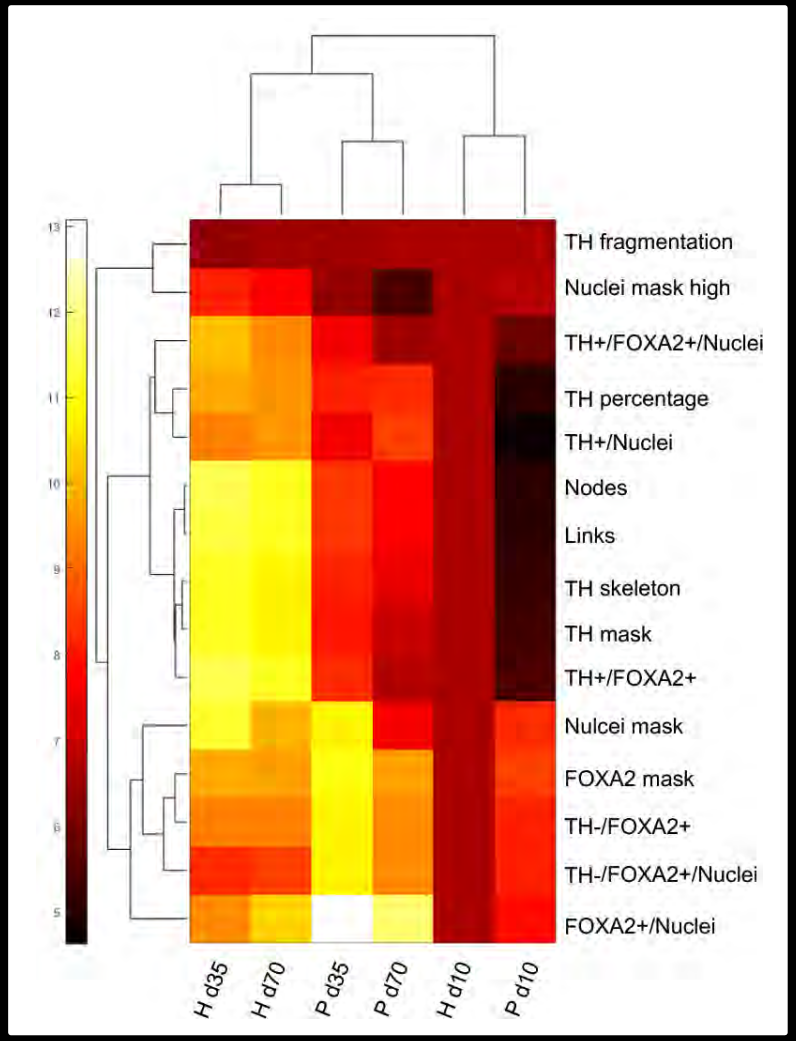
PD: Reduced complexity of dopaminergic neurons





PD patient: LRRK2-G2019S

Clustering by PD vs. Healthy



Smits et al., submitted

Summary

1) Differentiation of complex 3D neuronal networks in microfluidics plates.
   - Strong PD specific phenotype – neuronal degeneration
   - Rescue of phenotype with gene-correction or drug treatment

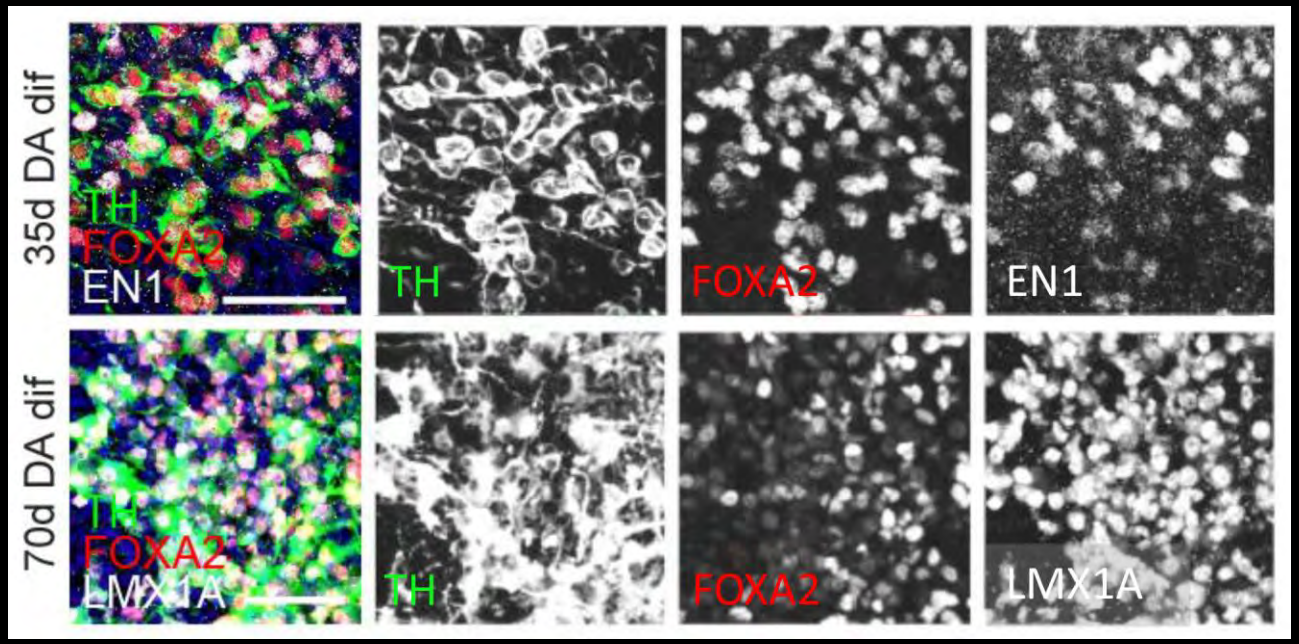
2) Generation & characterization of midbrain organoids.
   - Neuronal differentiation
     - Functionality: synapses, neuronal activity, Dopamine
   - Astroglia differentiation
   - Oligodendrocyte differentiation & myelination
   - Neuromelanin production
   - Disease relevant phenotypes

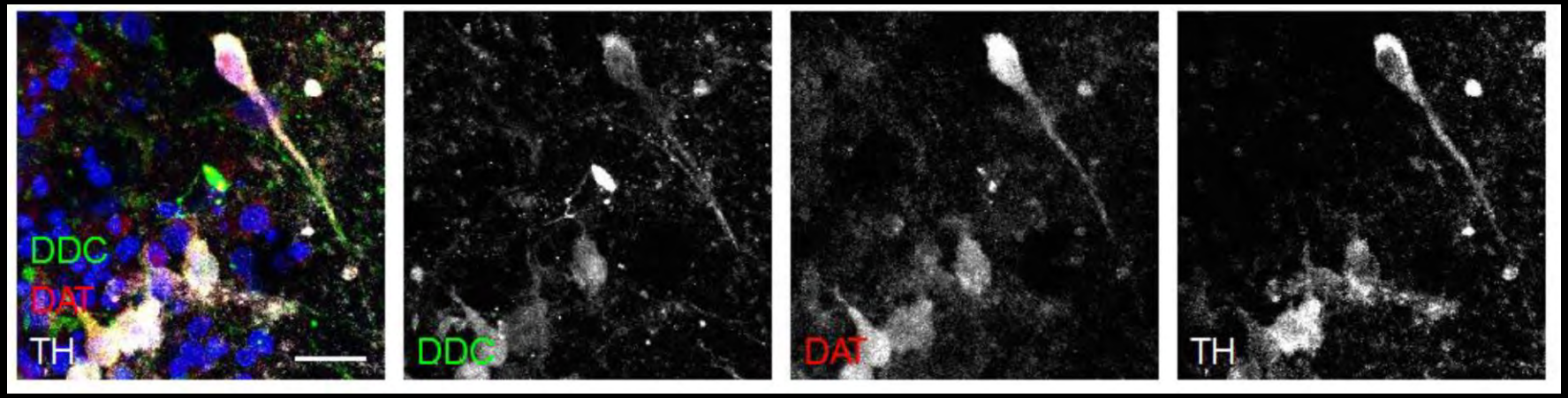
Acknowledgements

Collaborations:

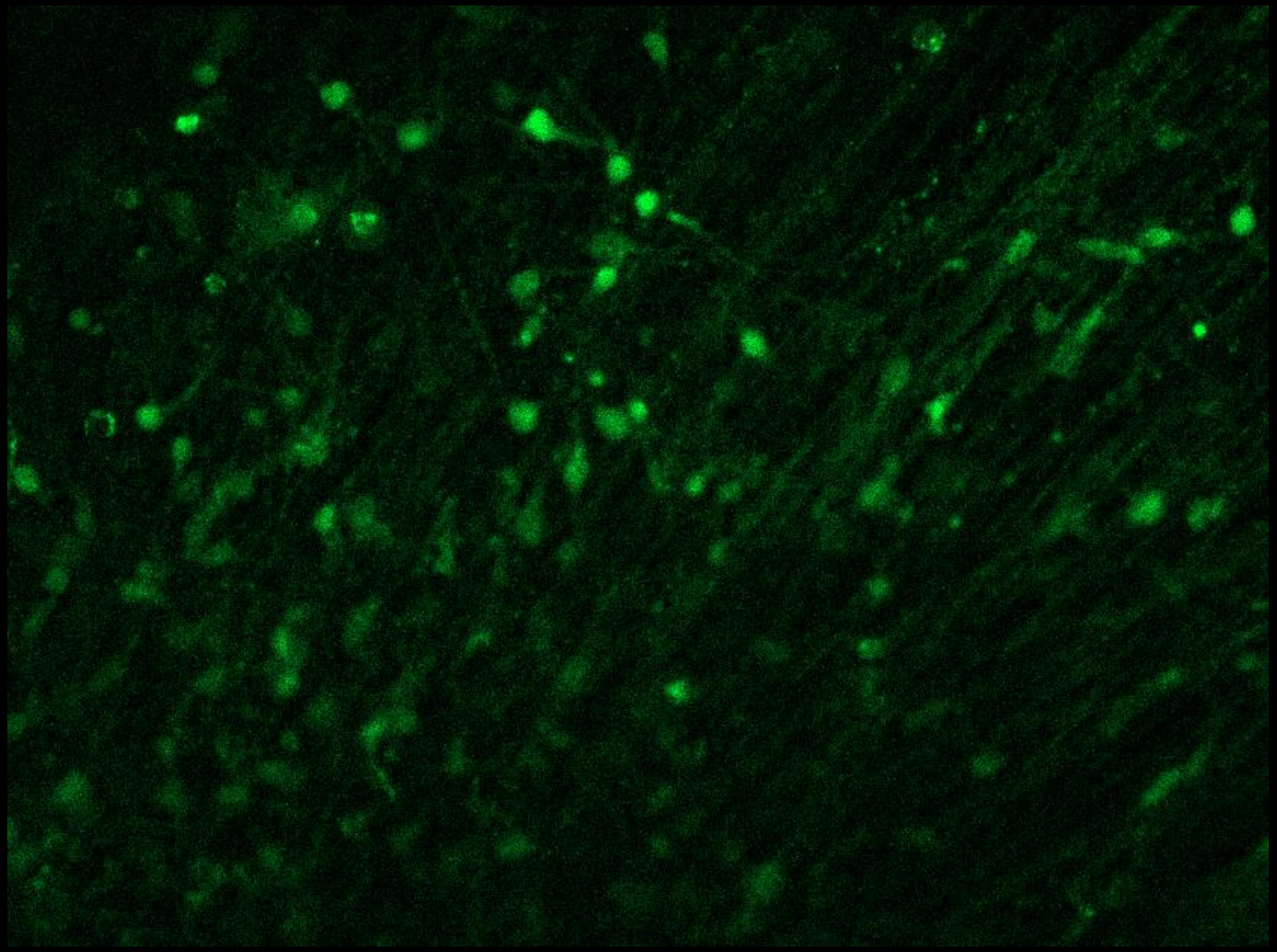
Rejko Krueger
(Luxembourg)
Jared Sterneckert
(Dresden)
Arti Ahluwalia
(Pisa)
Peter Ertl
(Vienna)
Andrew Hicks
(Bozen)

Midbrain identity



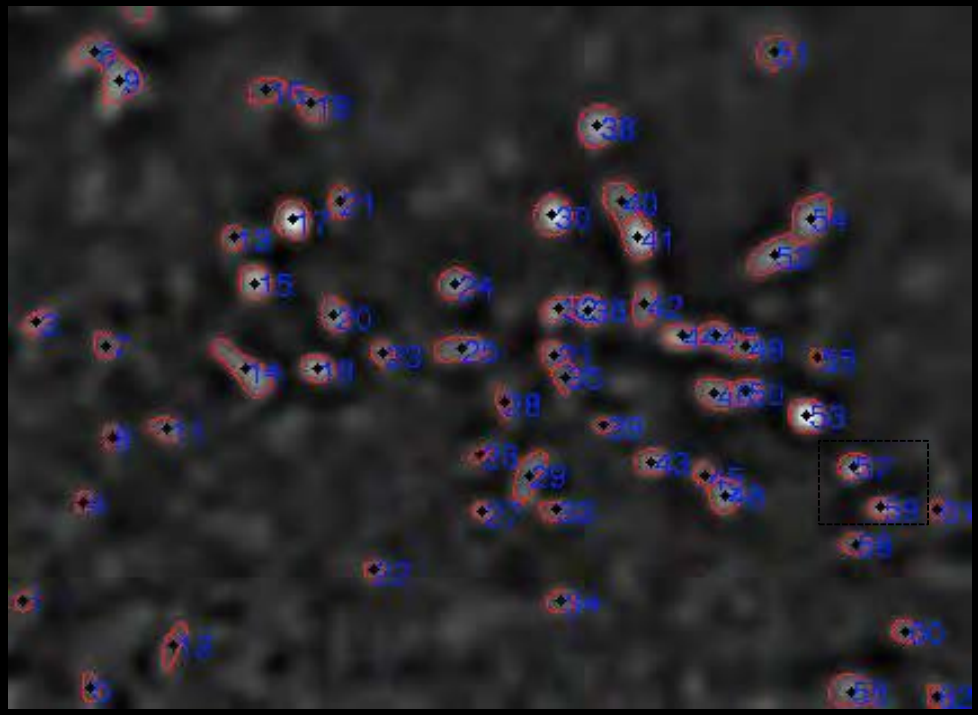


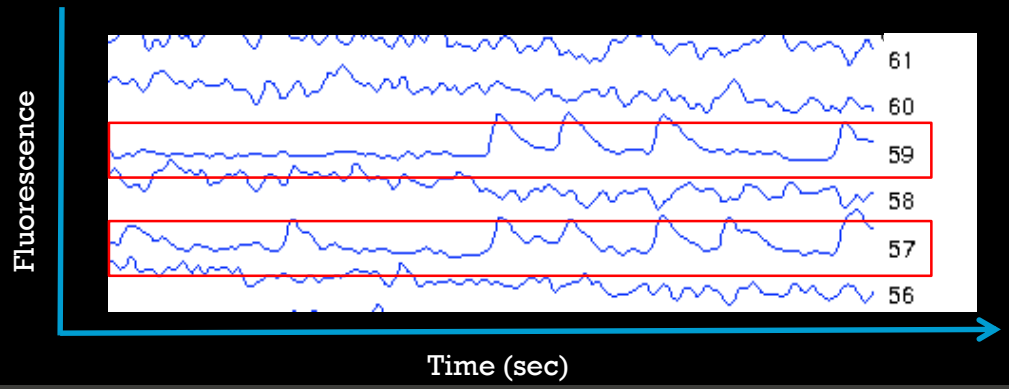
Ca2+ Imaging in Organoids



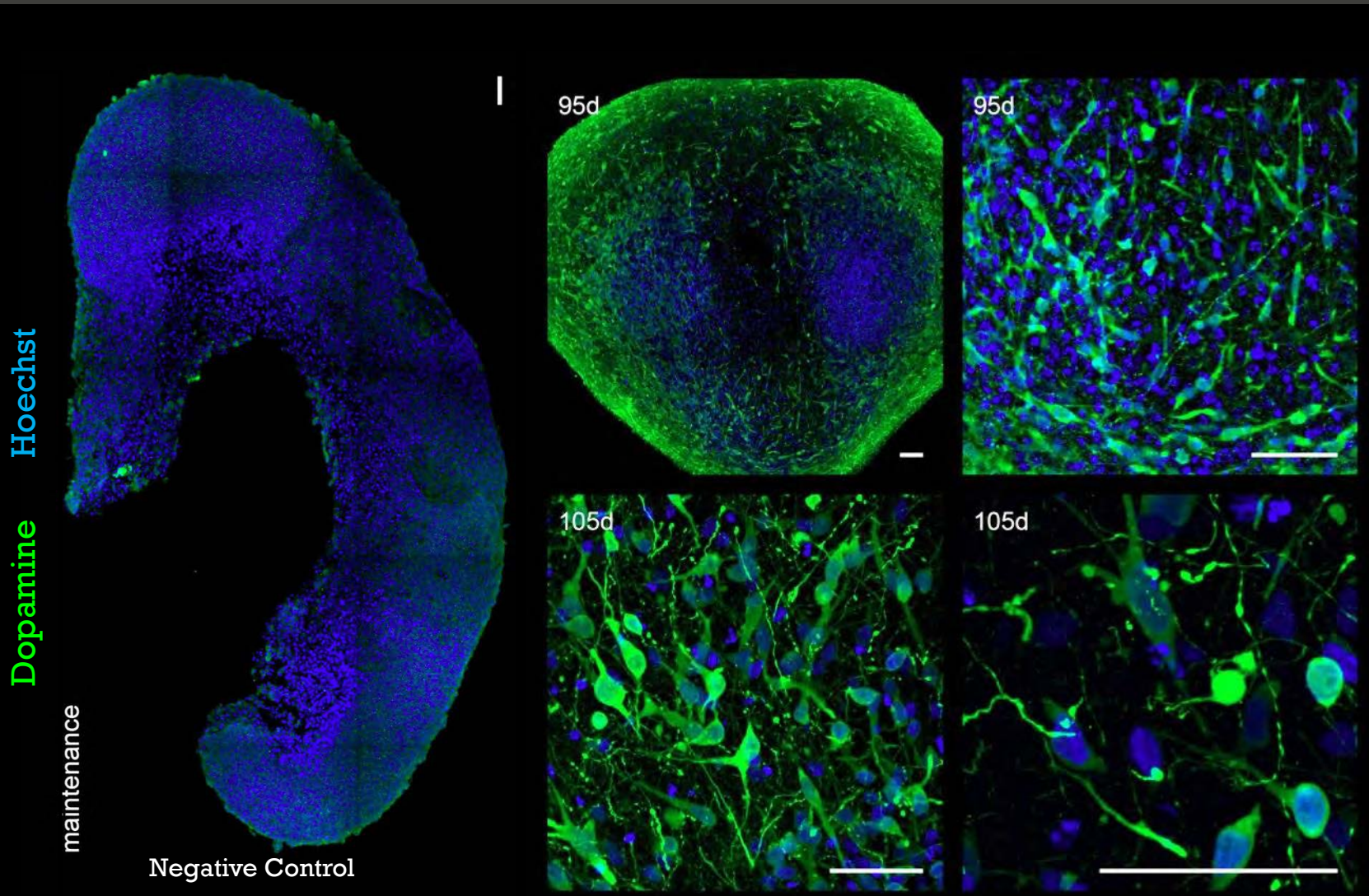
Fluo-4

Ca2+ Imaging in Organoids

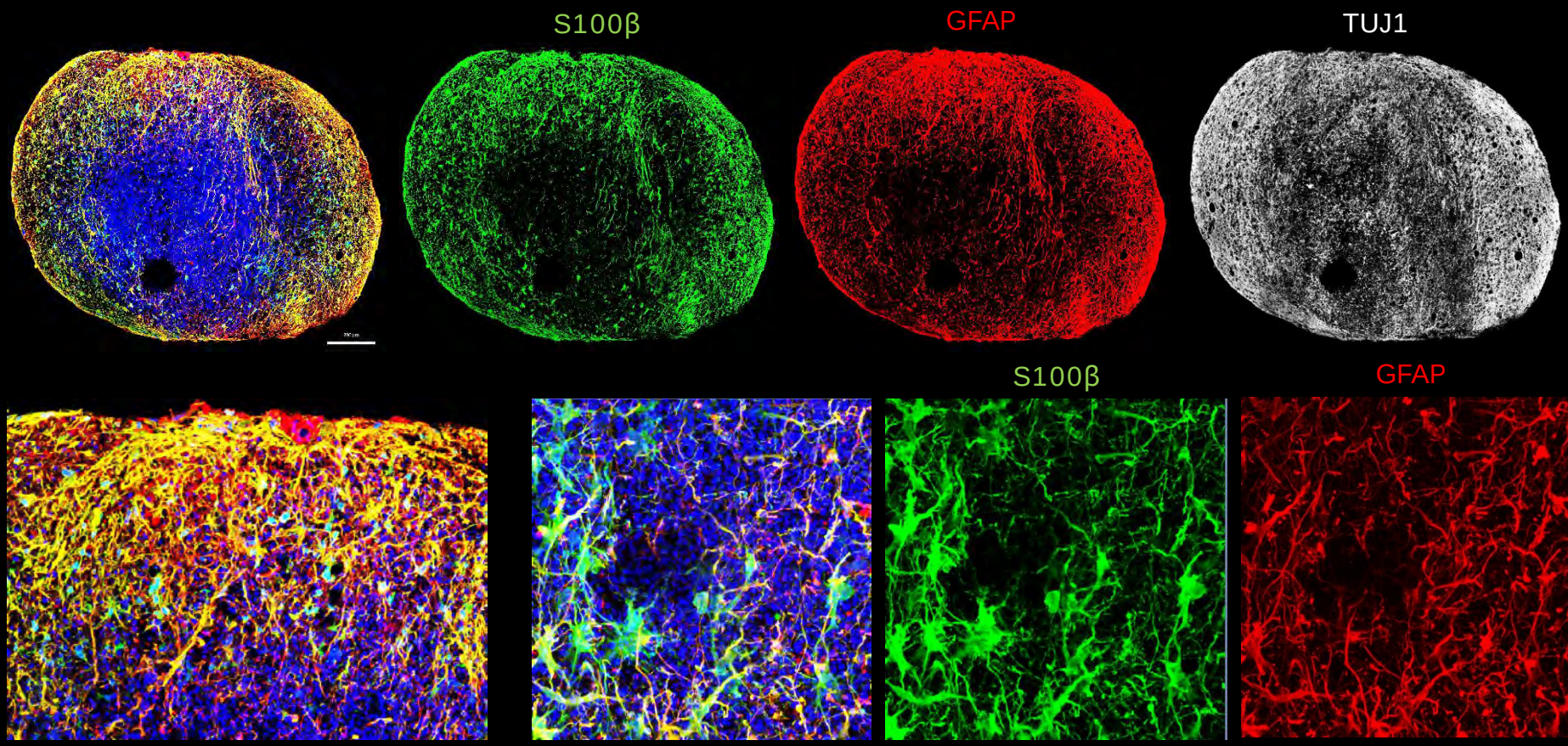




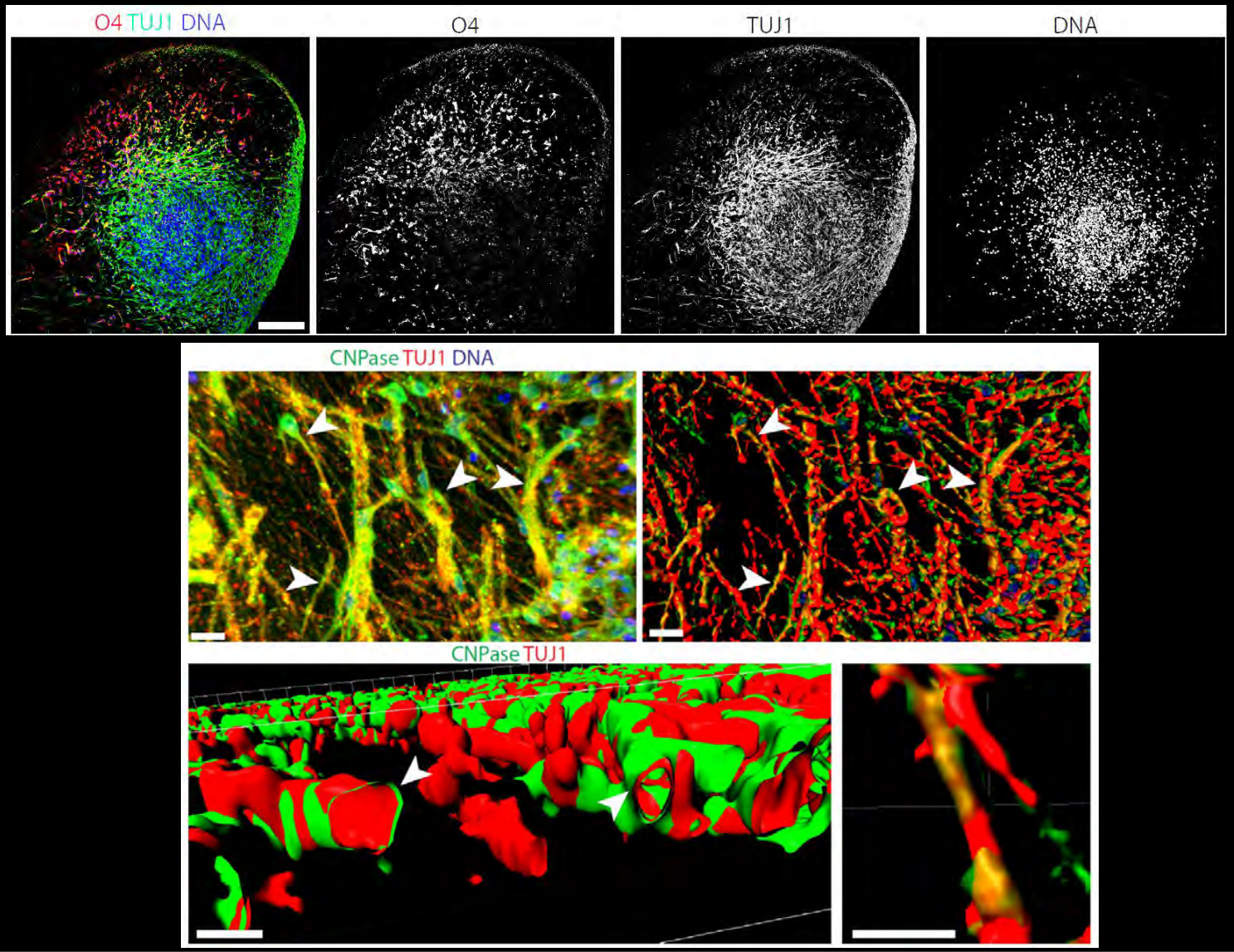
Dopamine production



Astrocyte differentiation

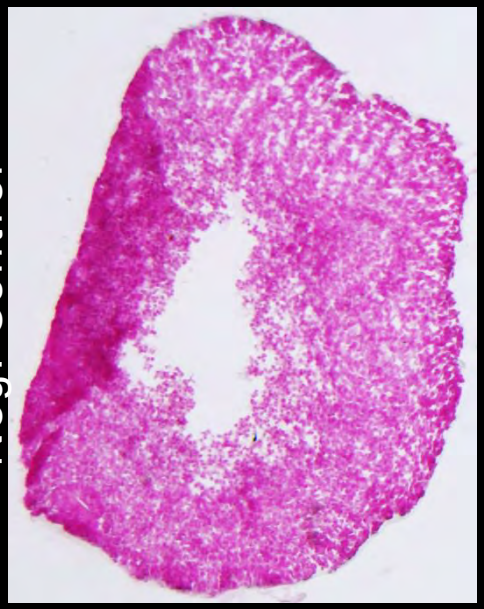


Oligodendrocyte differentiation

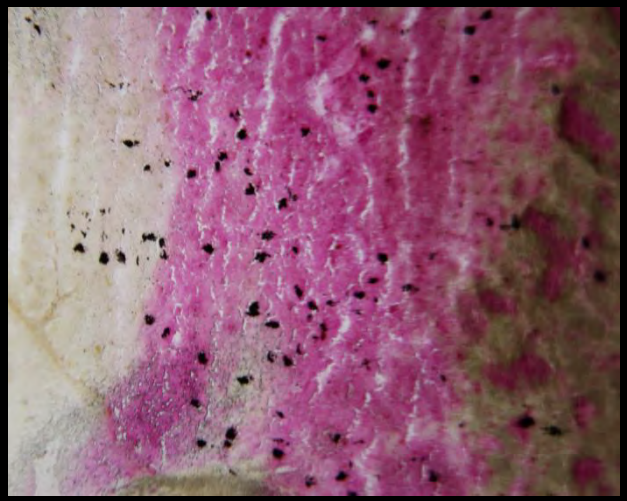


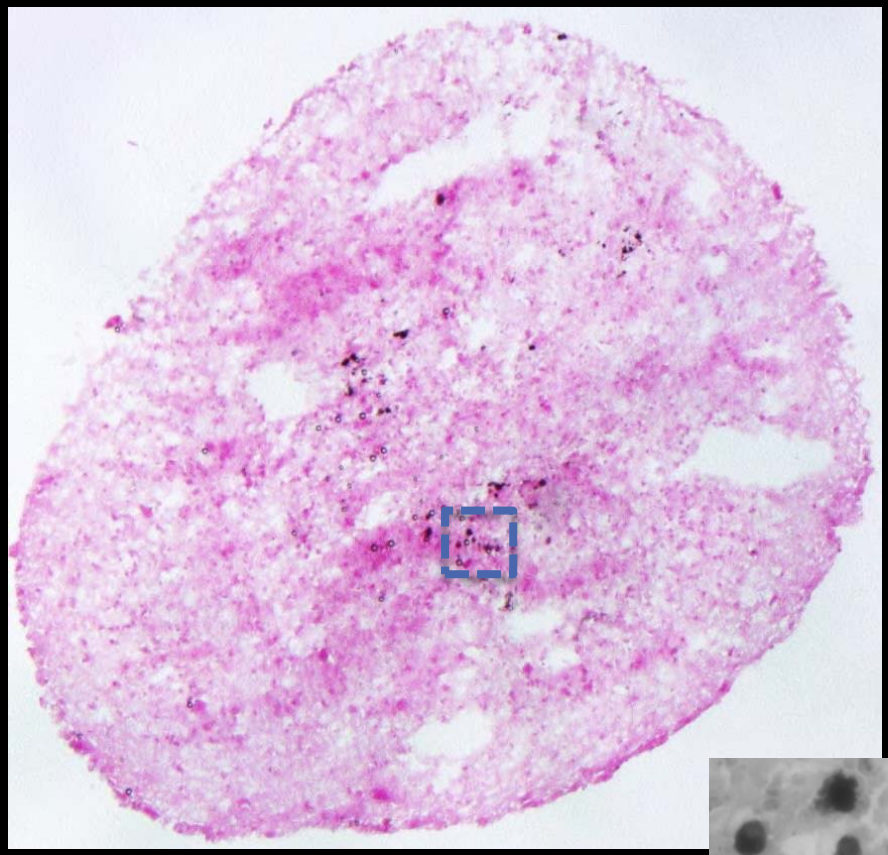
Neuromelanin detection, Fontana Masson staining

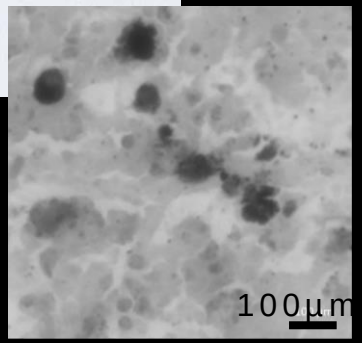
Maintenance, Neg. Control



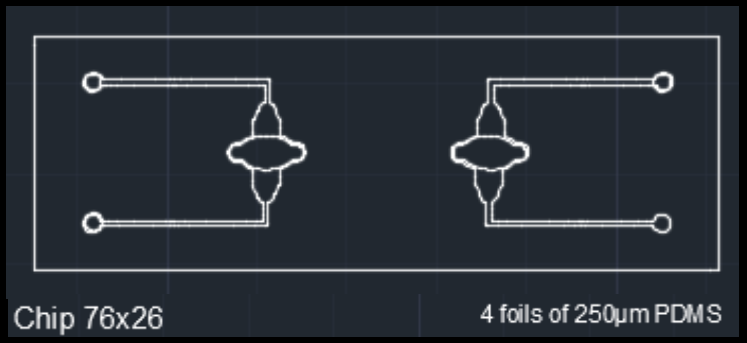
Human brain, Pos. Control

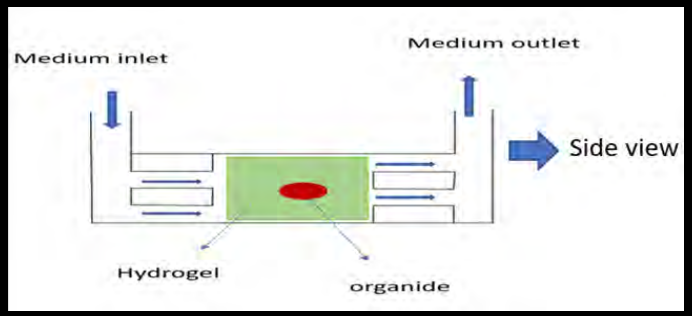




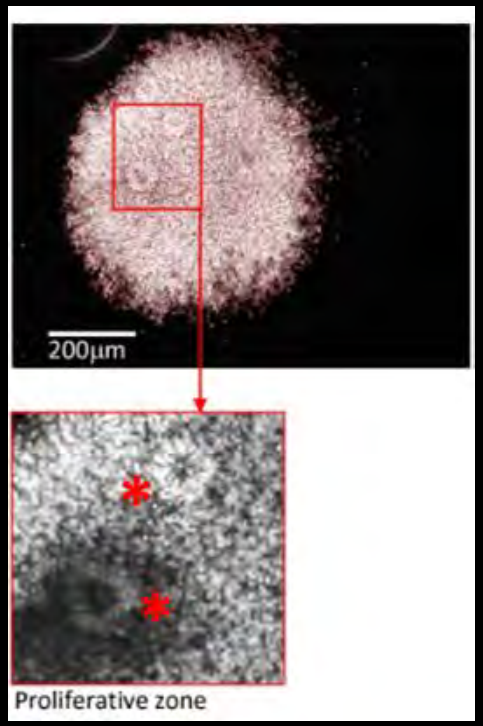


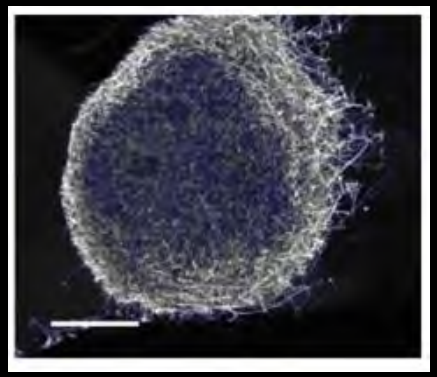
Device Microfabrication & Organoid cultivation





Integration of sensors for oxygen consumption, protein aggregation, dopamine production etc.





M-era.Net

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Vienna University of Technology

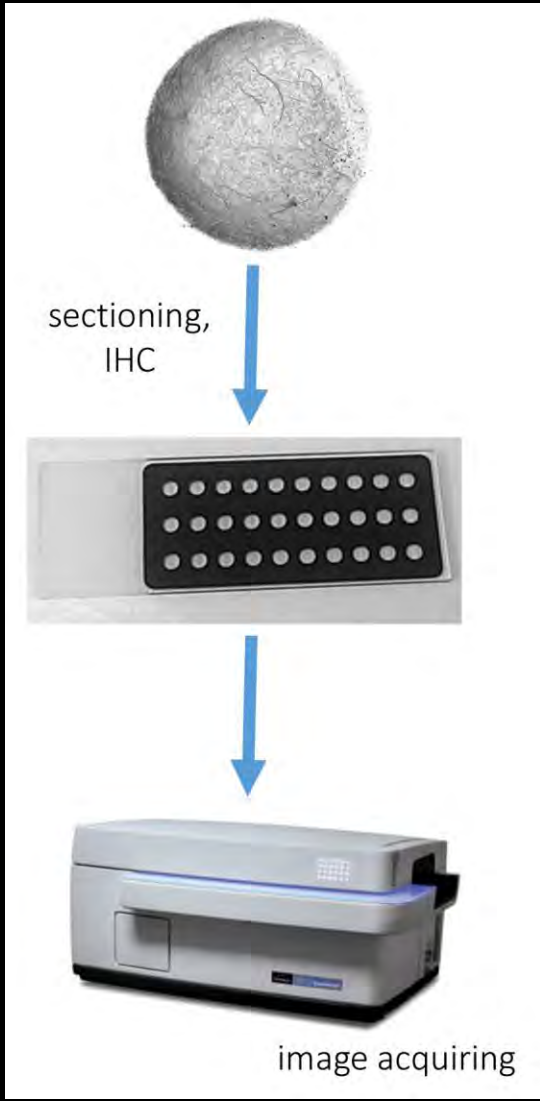
THE HEBREW UNIVERSITY OF JERUSALEM

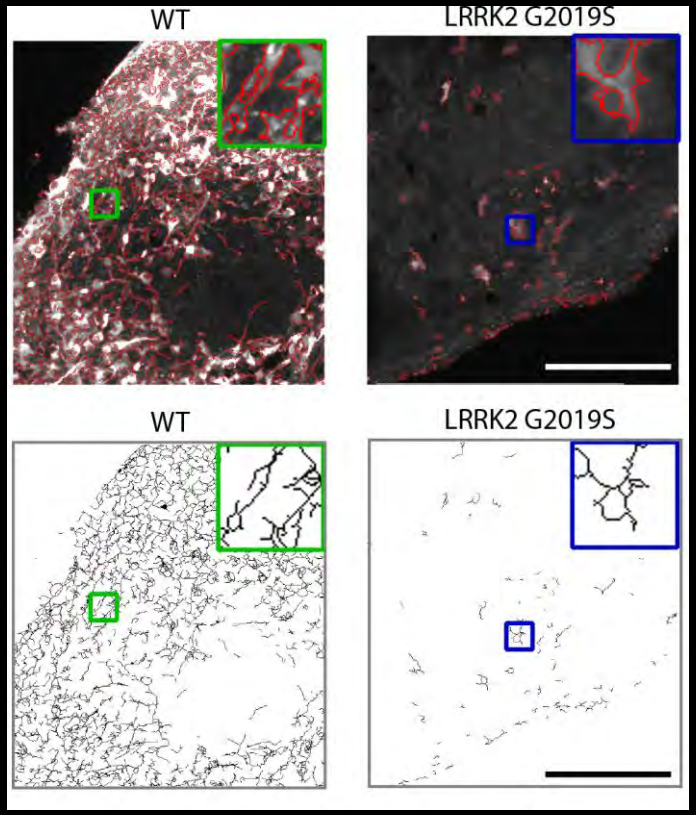
HUNGARIAN ACADEMY OF SCIENCES · 1825 ·

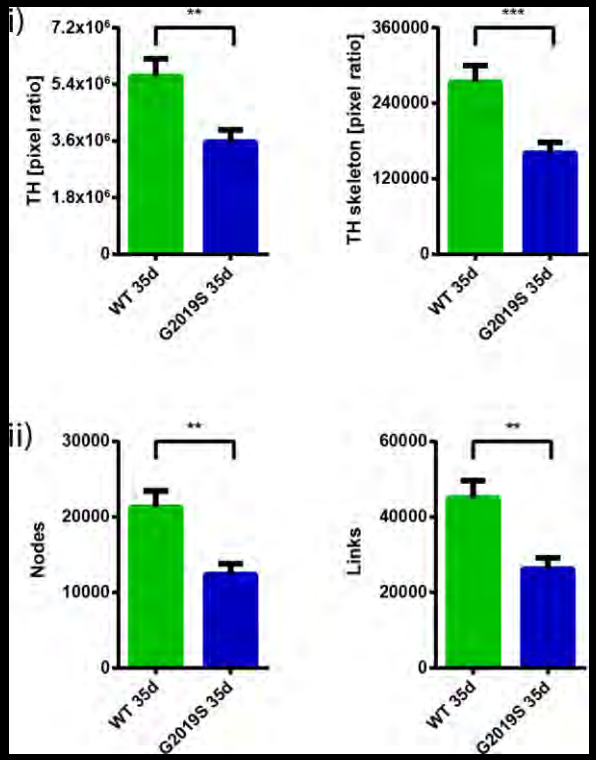
1931 國立成功大學 National Cheng Kung University

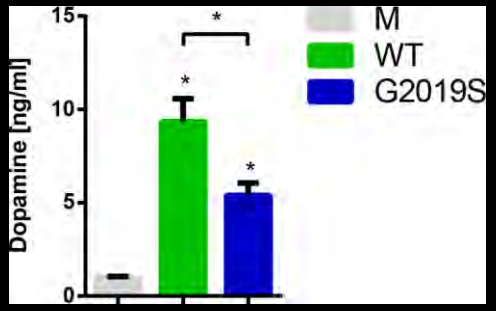
National University of Kaohsiung

Midbrain organoids with the LRRK2-G2019S mutation show disease relevant phenotypes





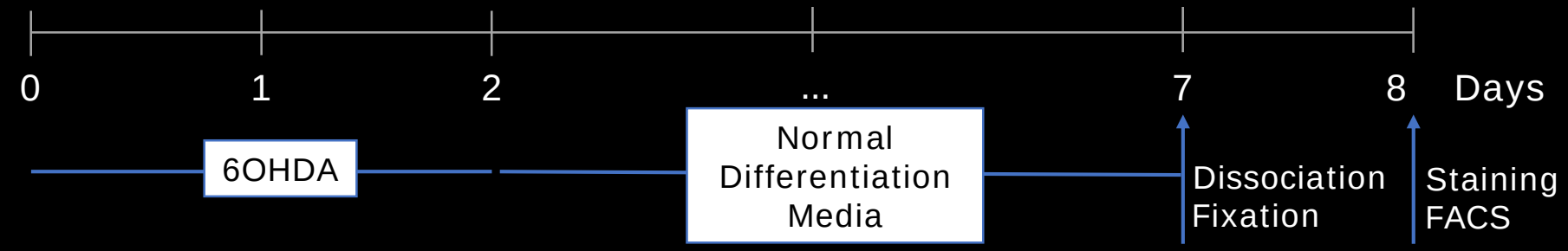


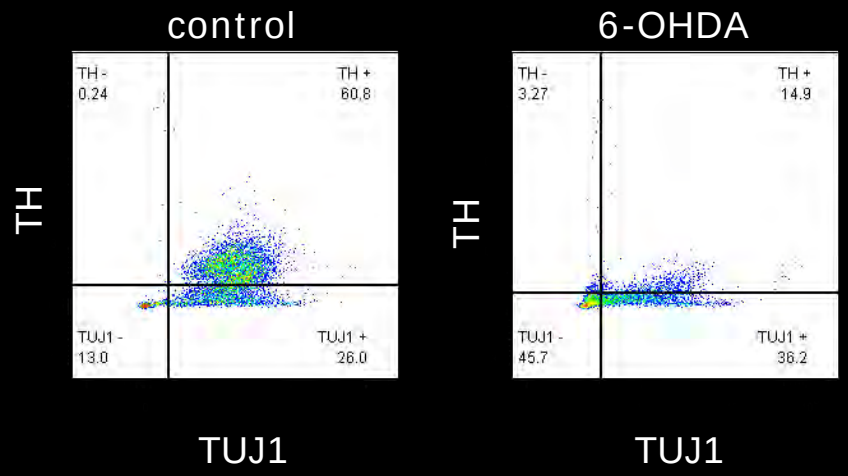


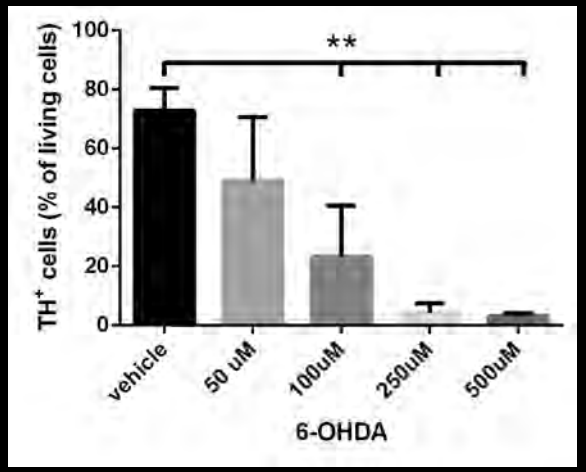
Smits et al., in revision

Toxicology in midbrain organoids

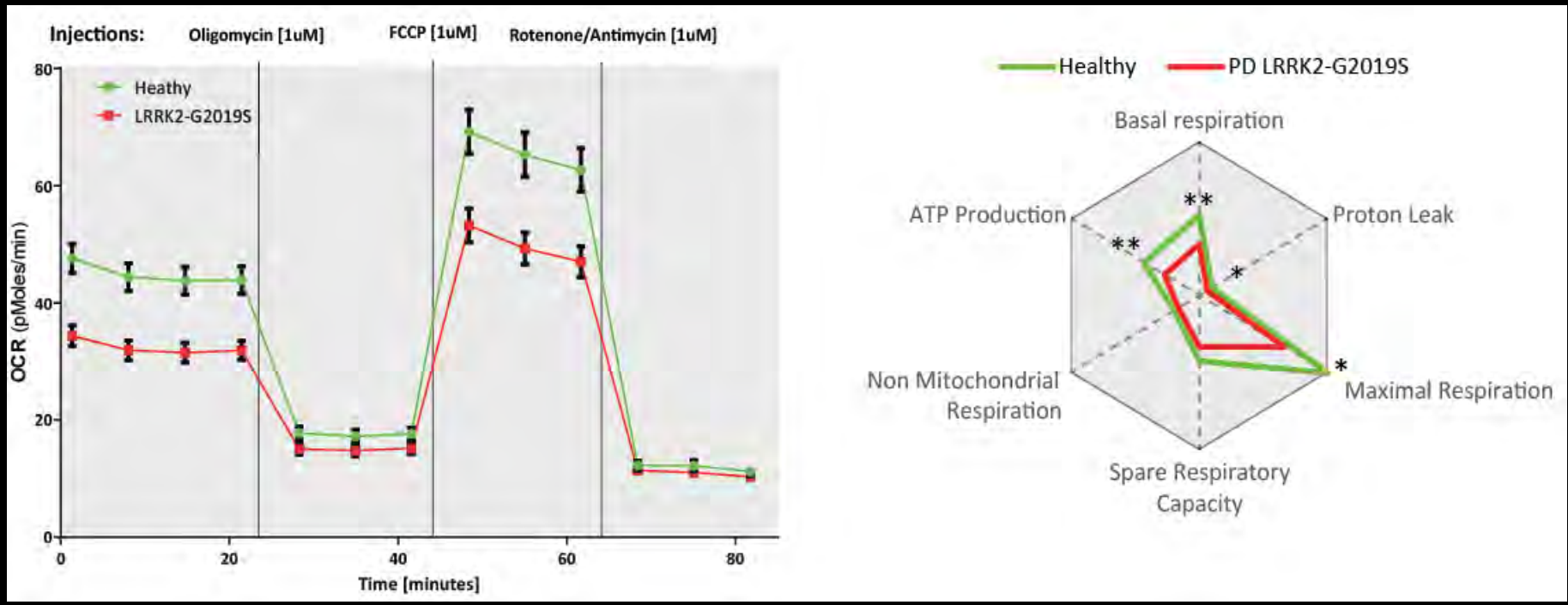
→ selective loss of dopaminergic neurons



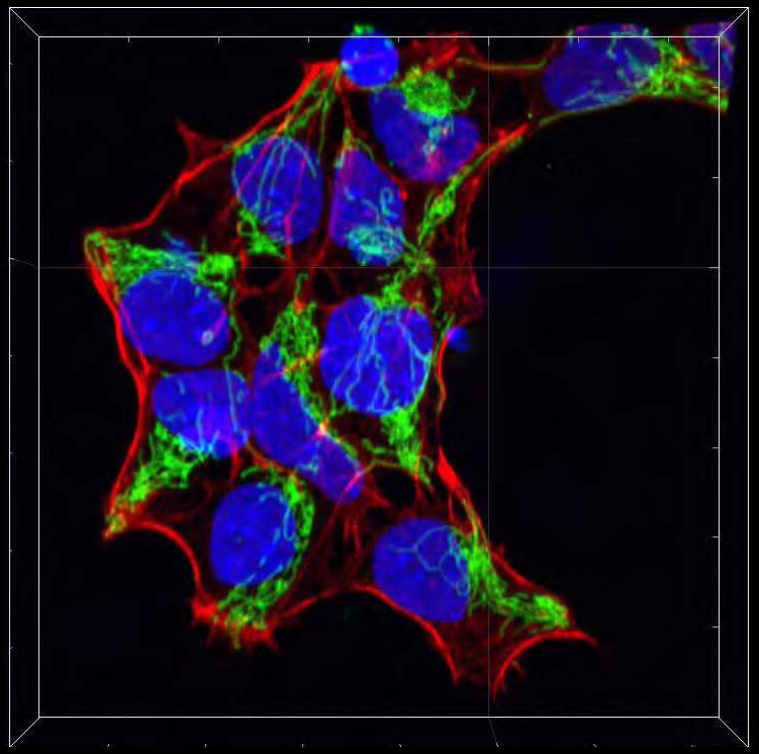


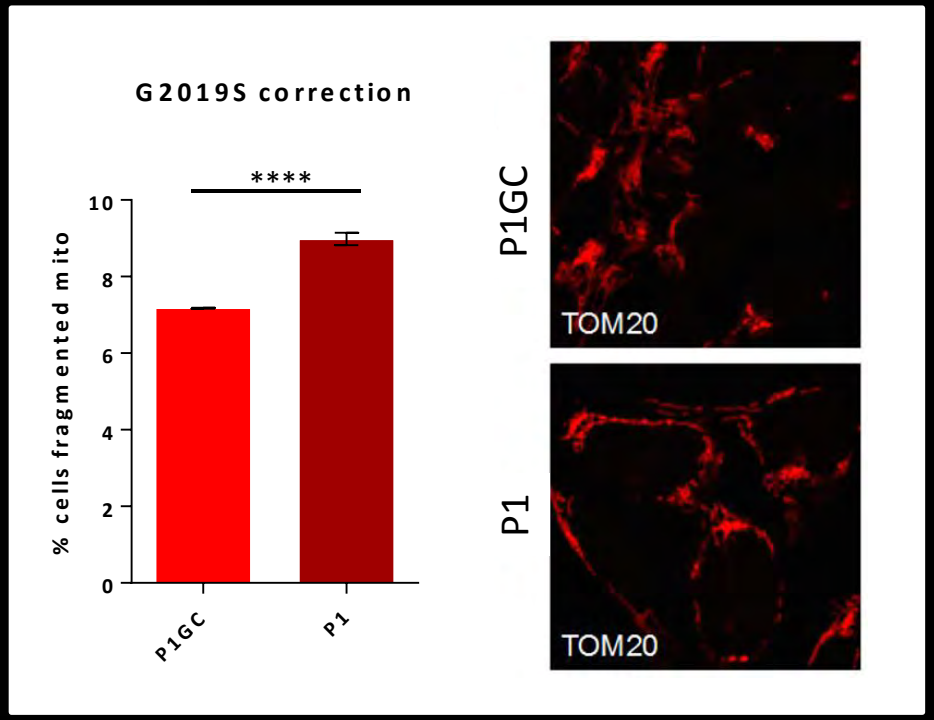


Mitochondrial impairment



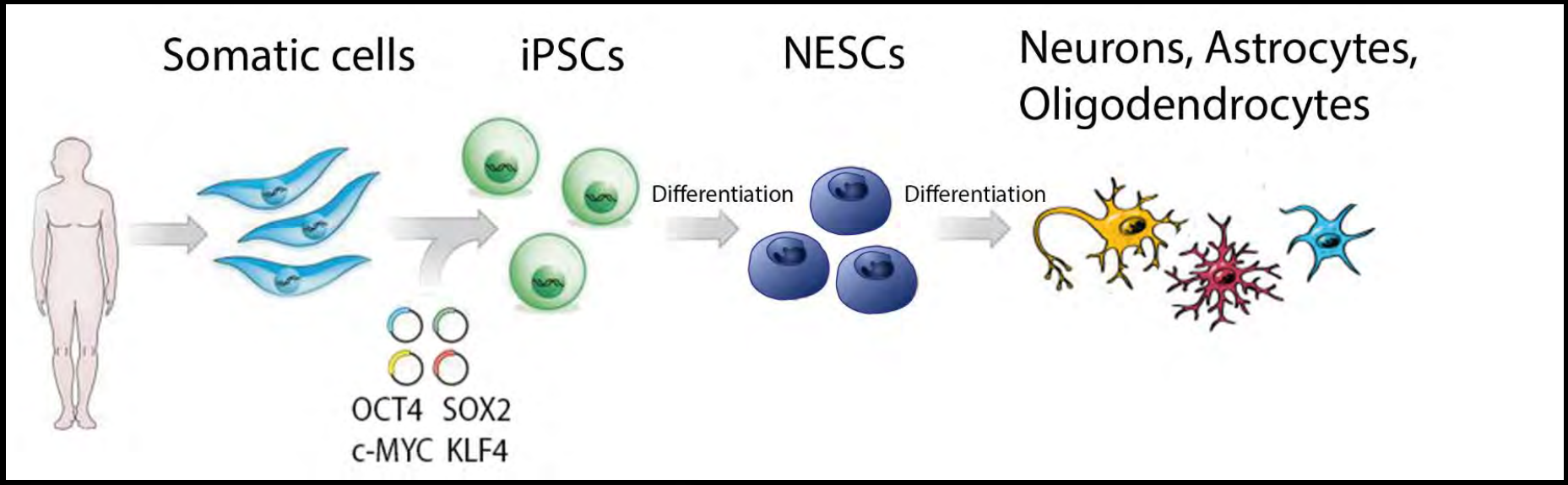
Mitochondrial morphology





Other features: Mitochondrial mass, ROS production, membrane potential (TMRM), mitophagy

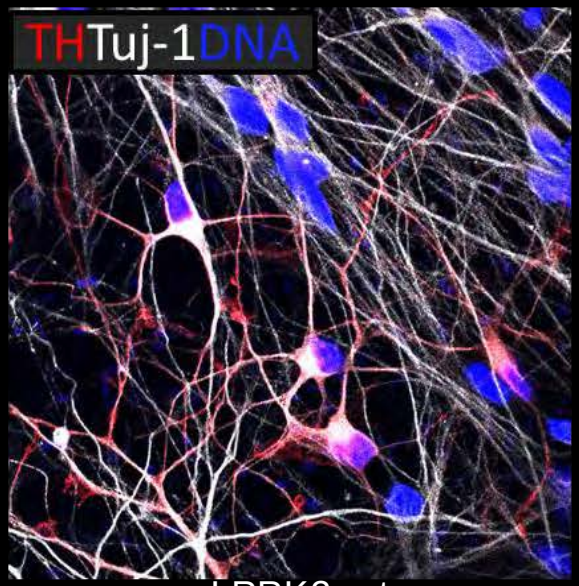
“Standard” 2D models



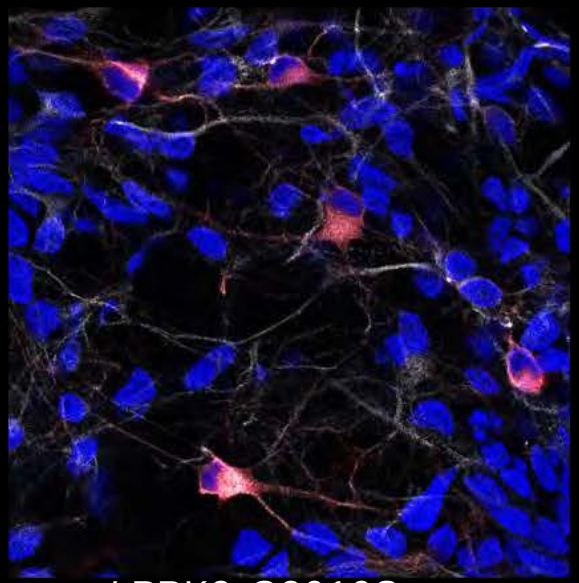
iPSCs = induced pluripotent stem cells
NESCs = Neuroepithelial stem cells

Early neuronal phenotype: Neurite complexity

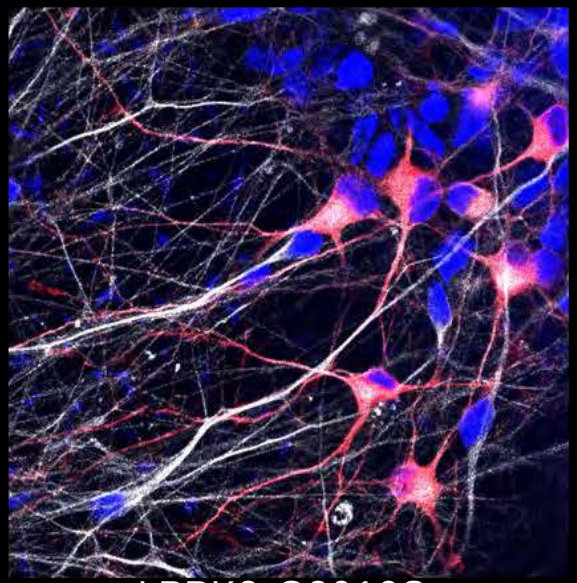
- 14d differentiation starting from NESCs
- Treatment with LRRK2 inhibitor* for 12 days.
- Comparison of an engineered line to a patient line.



LRRK2 wt
Healthy background



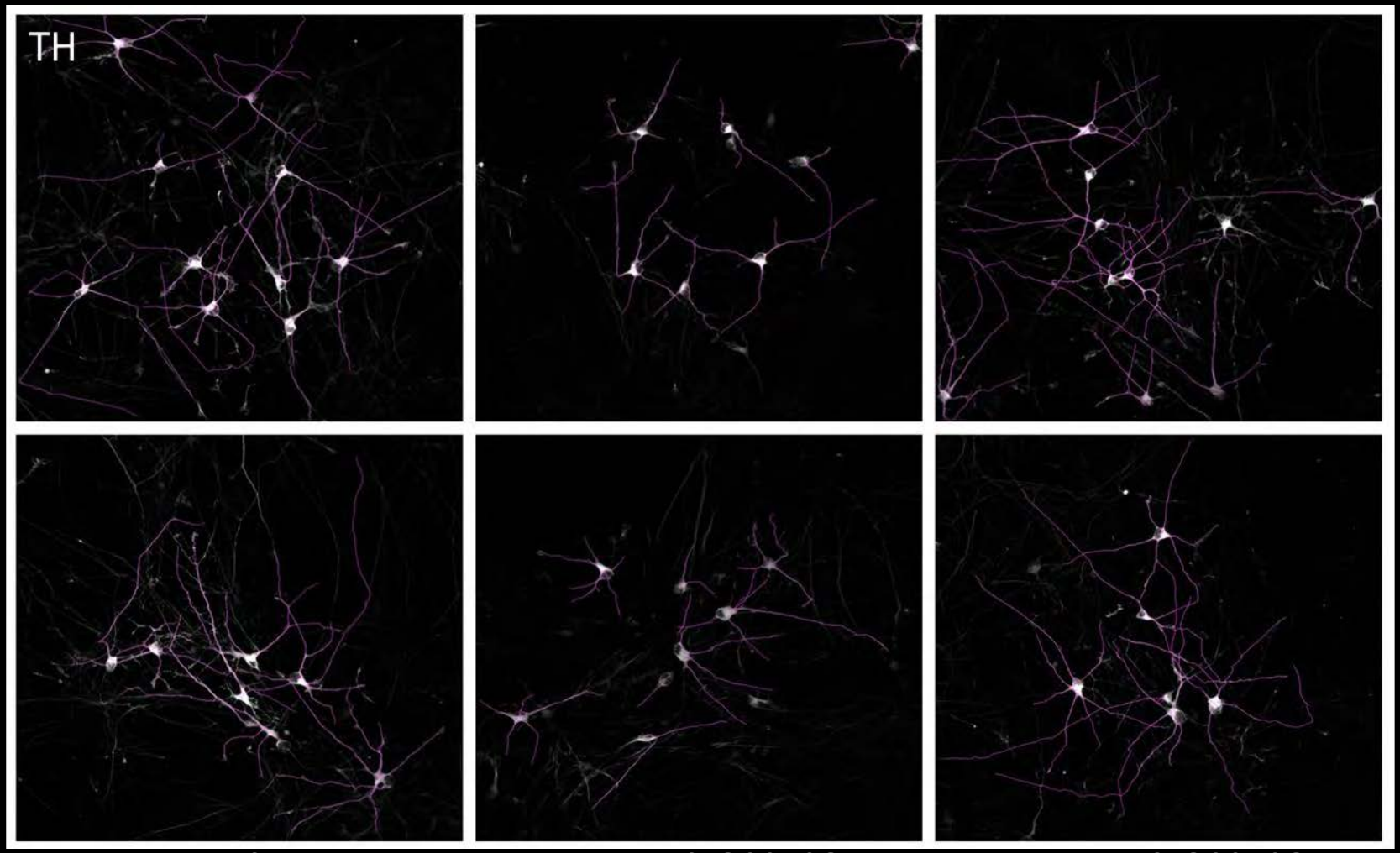
LRRK2-G2019S
introduced



LRRK2-G2019S
introduced
+LRRK2 inhibitor*

* Inhibitor from Ramsden et al. (2011) in ACS Chem Biol

Early neuronal phenotype: Neurite complexity

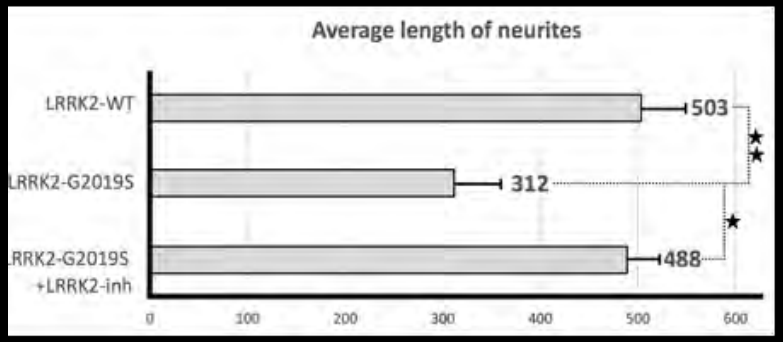


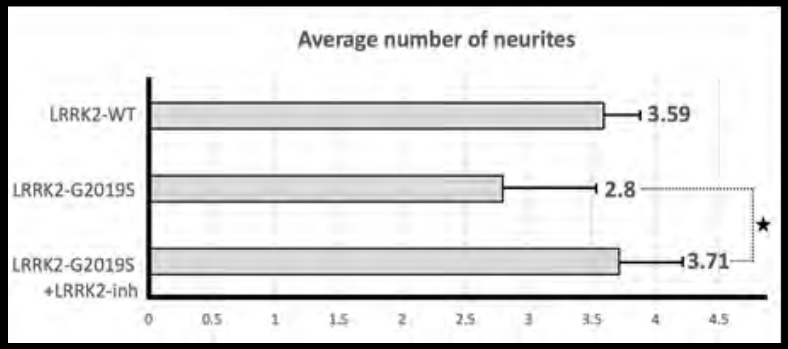
LRRK2 wt
Healthy background

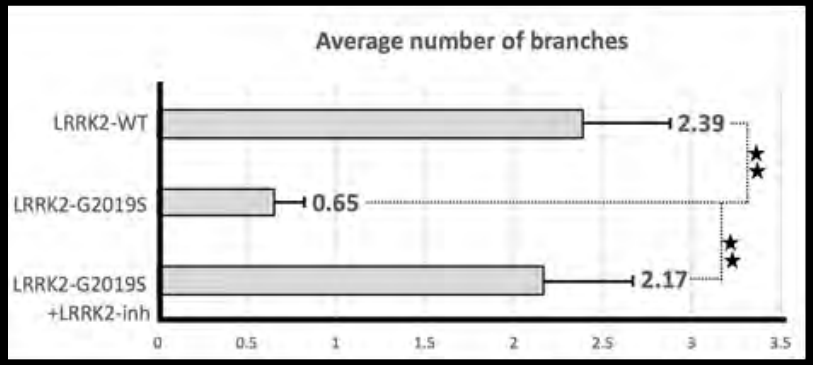
LRRK2-G2019S
introduced

LRRK2-G2019S
introduced
+LRRK2 inhibitor*

Early neuronal phenotype: Neurite complexity

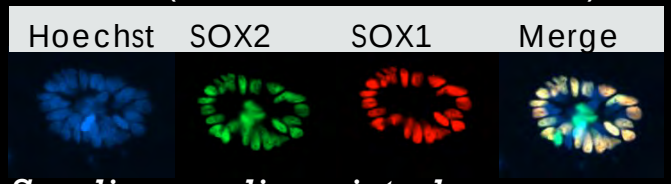




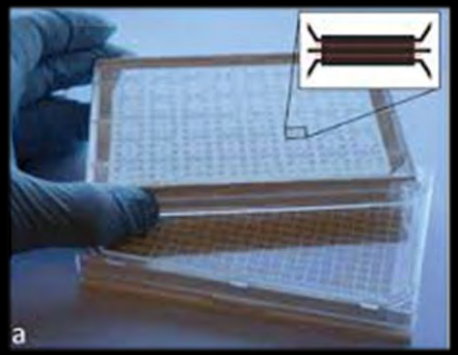


Human neuroepithelial stem cells (NESCs) for starting 3D cultures

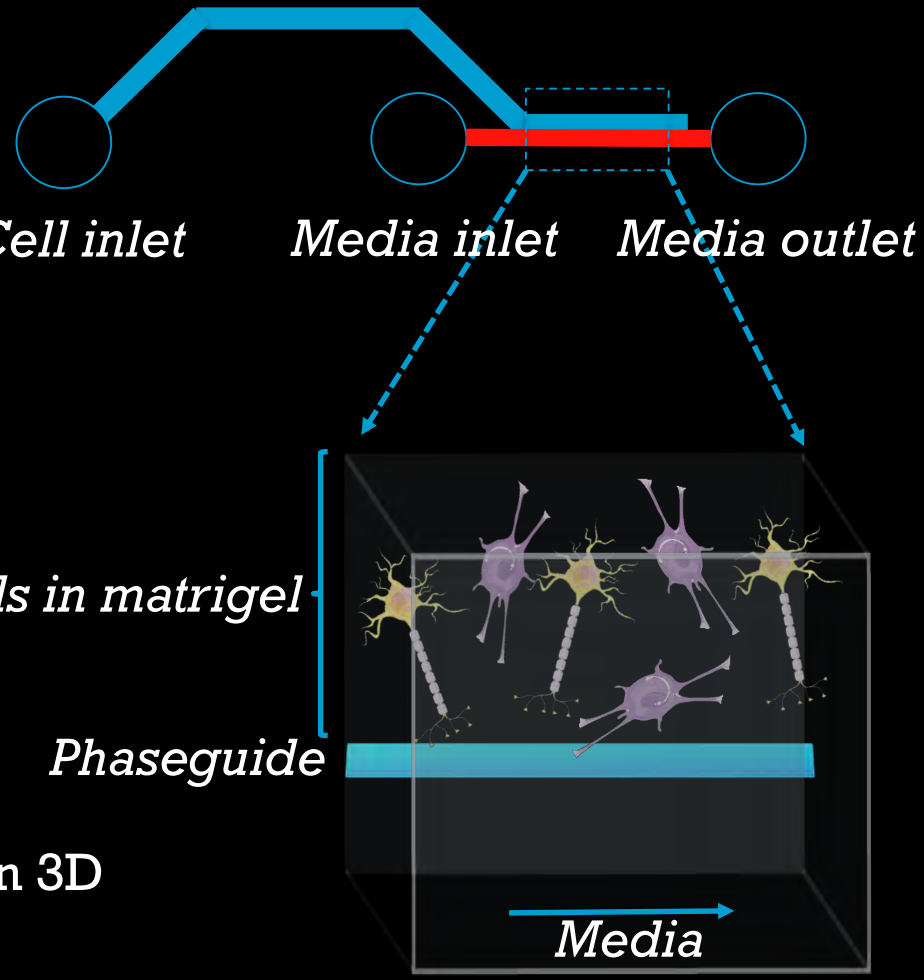
hNESC (Reinhardt et al., 2013):



Seeding as dissociated cells



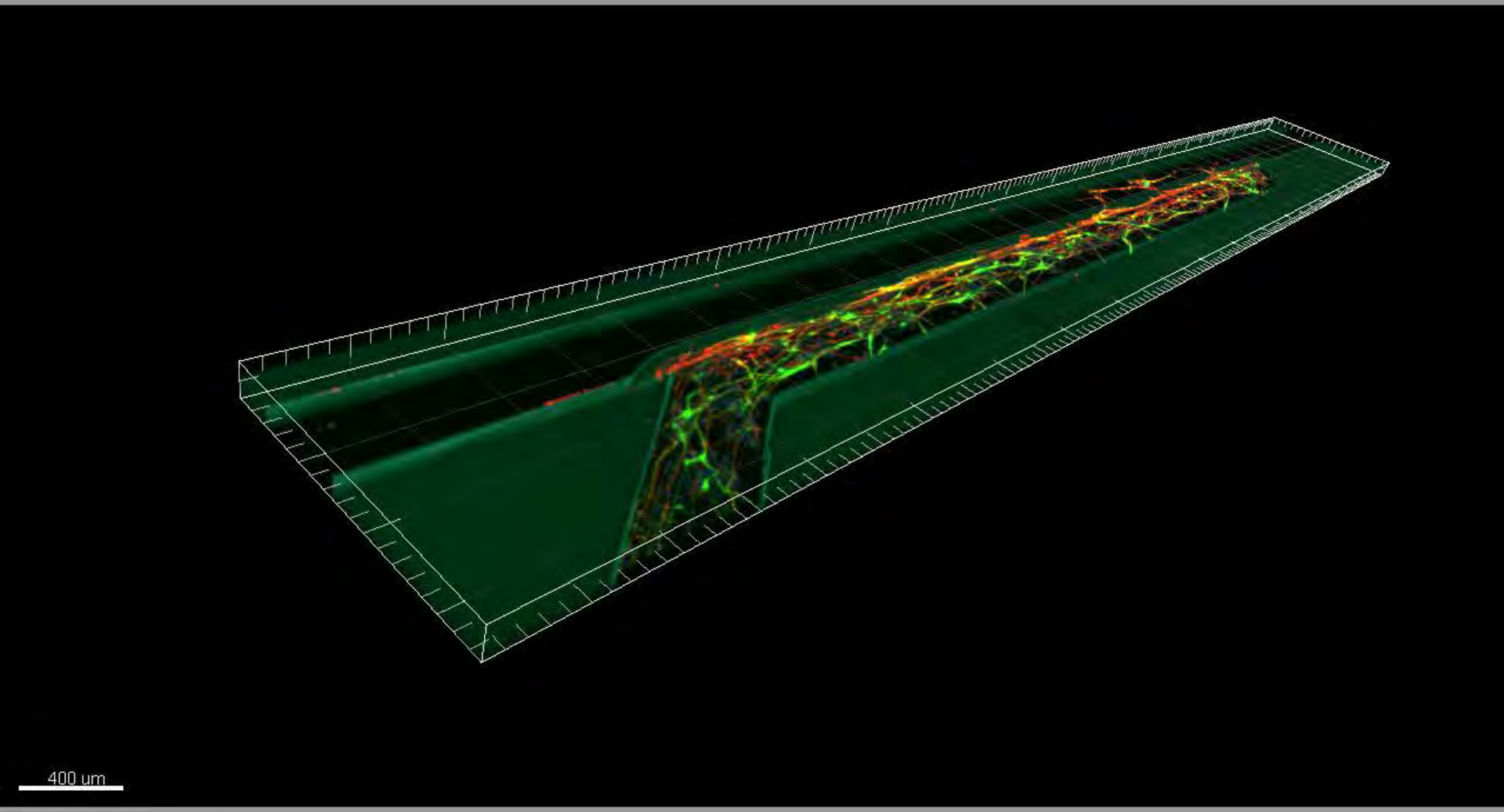
MIMETAS
the organ-on-a-chip company



1) Phenotype of PD derived lines in 3D

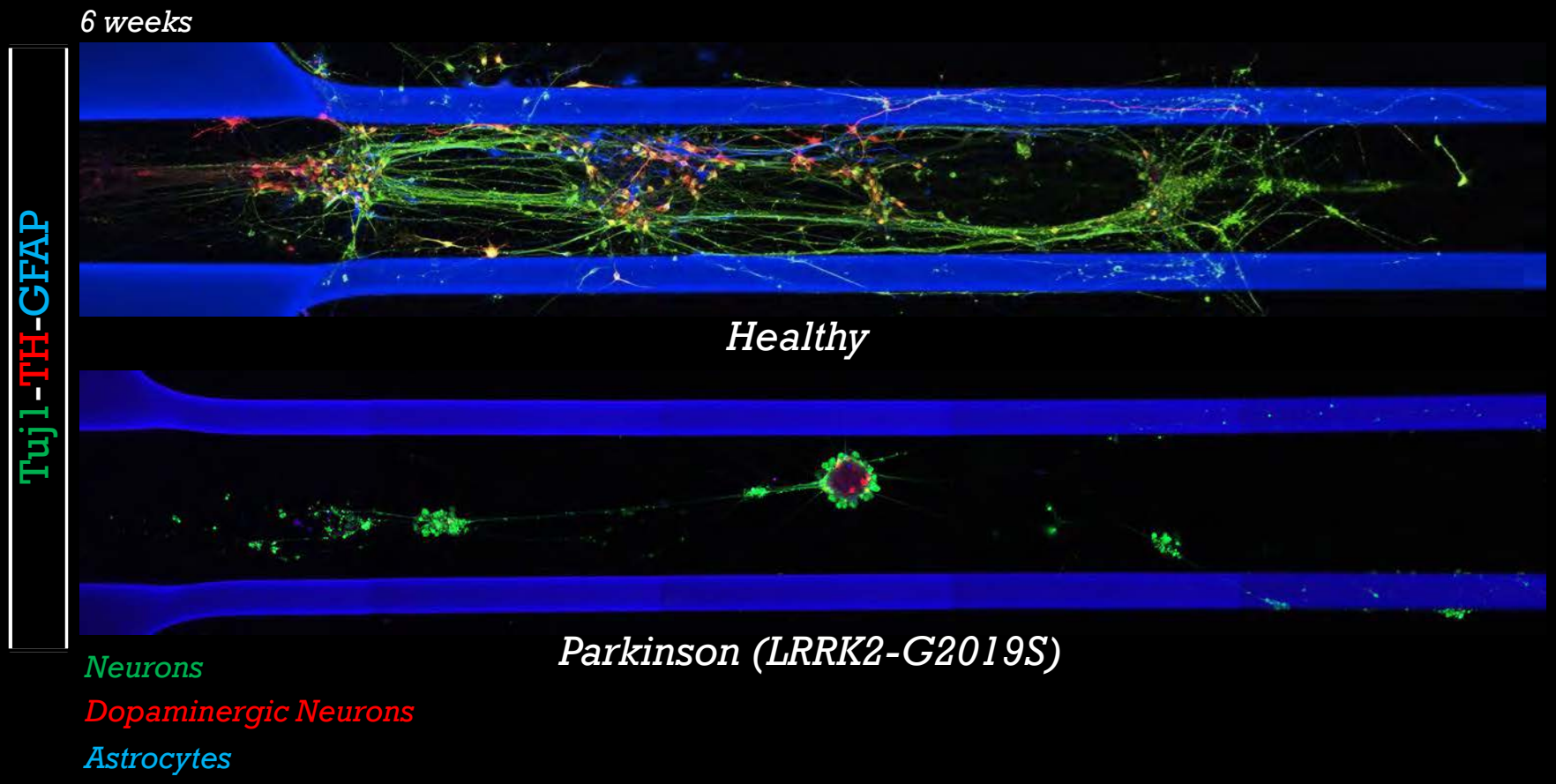
2) Rescue with dugs

Differentiation into dopaminergic neurons



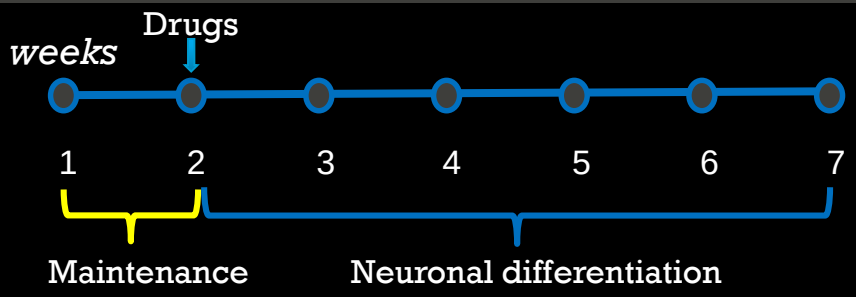
Moreno et al., 2015

Parkinson's disease phenotypes

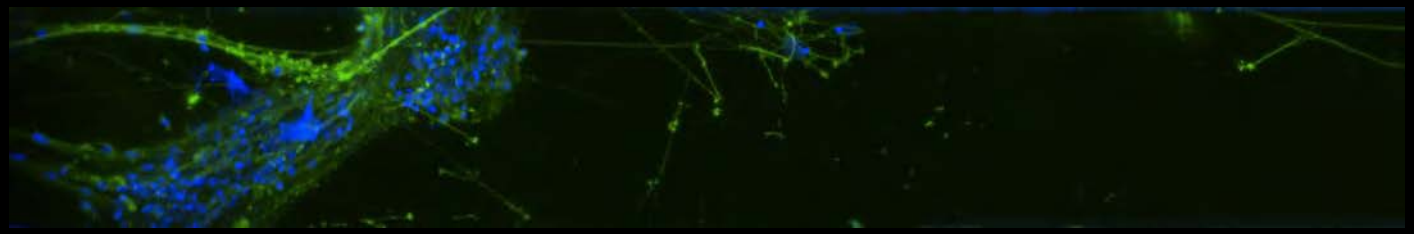


Bolognin et al., unpublished; patent filed

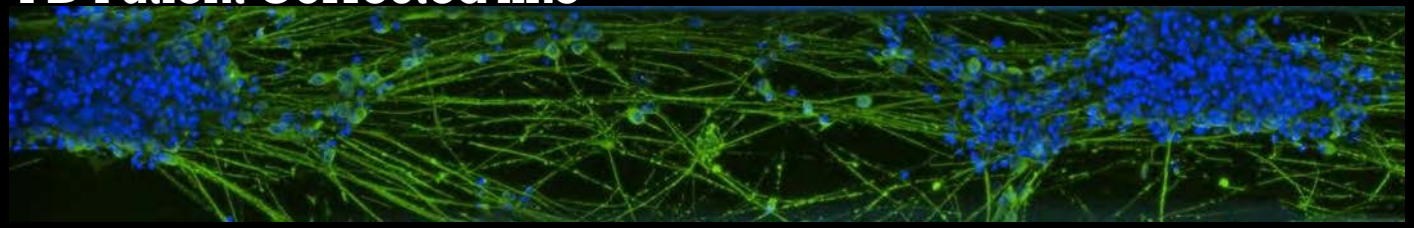
Rescue of phenotypes



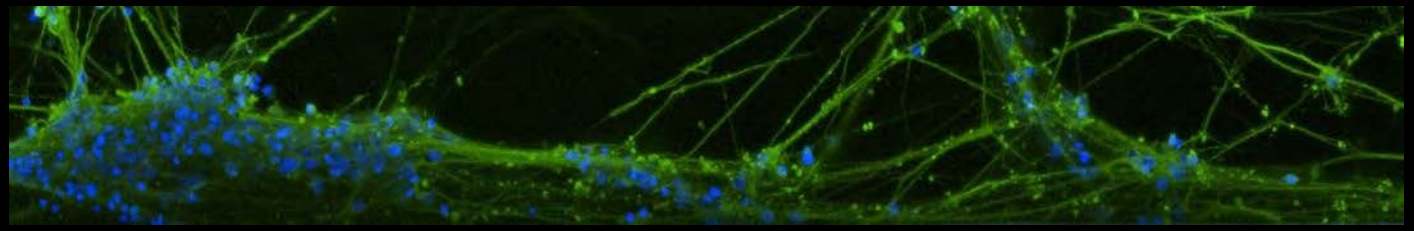
PD Patient



PD Patient Corrected line



PD Patient + LRRK2 Inh



Tuj1 Hoechst

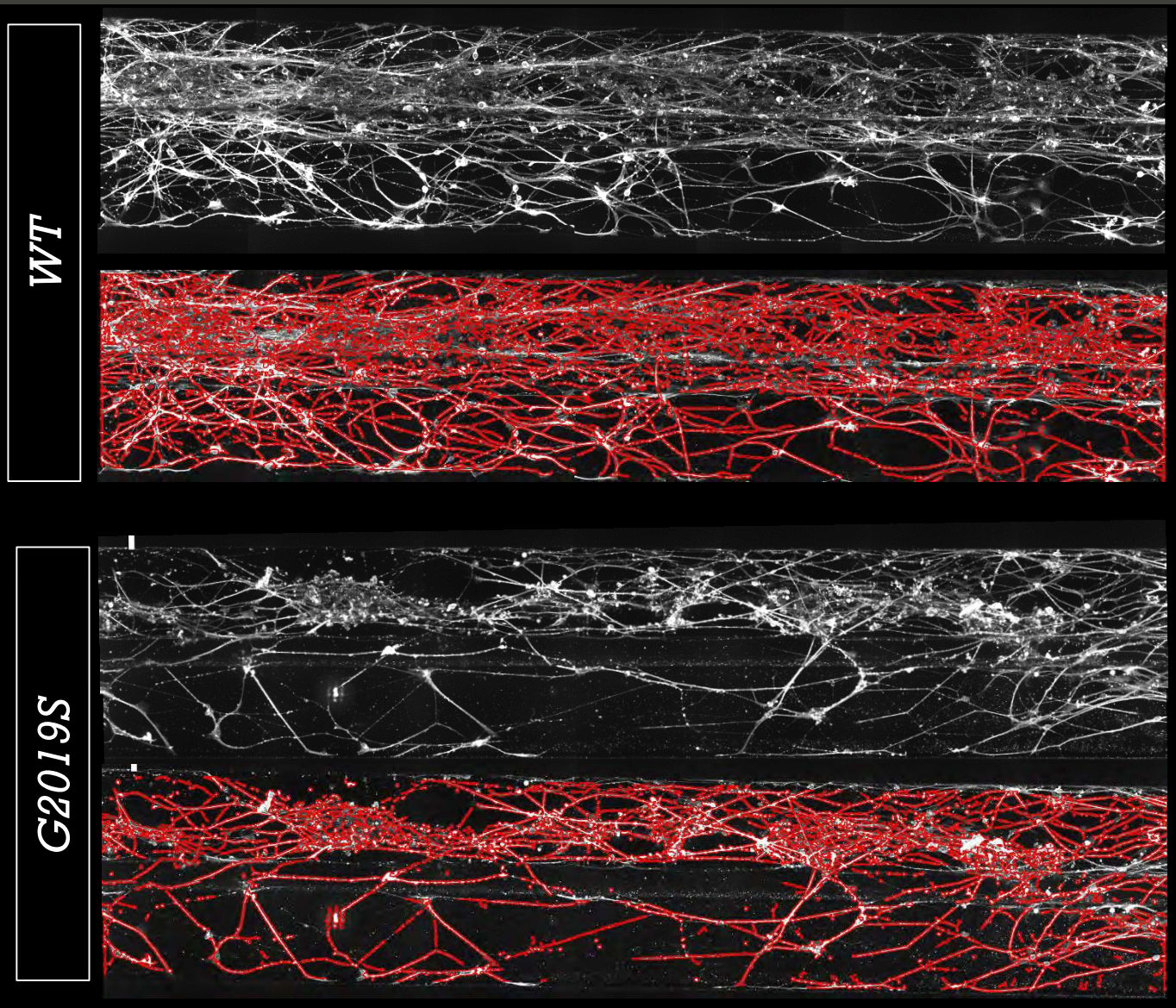
Inhibitor from Ramsden et al. (2011) in ACS Chem Biol

Bolognin et al., unpublished; patent filed

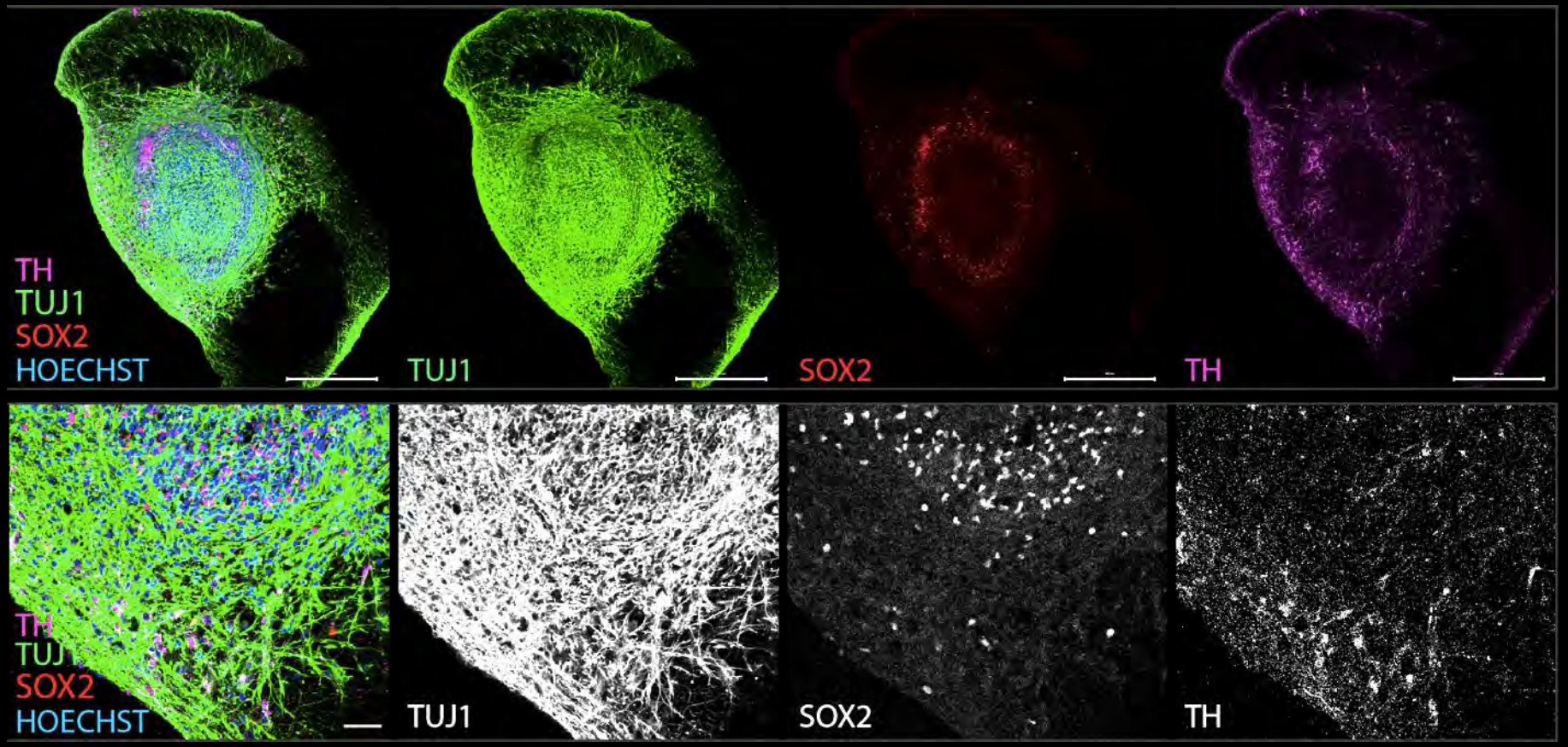
Analysis with high-content imaging

Perkin Elmer Opera HCS System

Automated image acquisition and analysis



Fate specification and spatial organization



Monzel et al., 2017; patent filed