NextGen Neuronal Phenotyping

Lee L. Rubin

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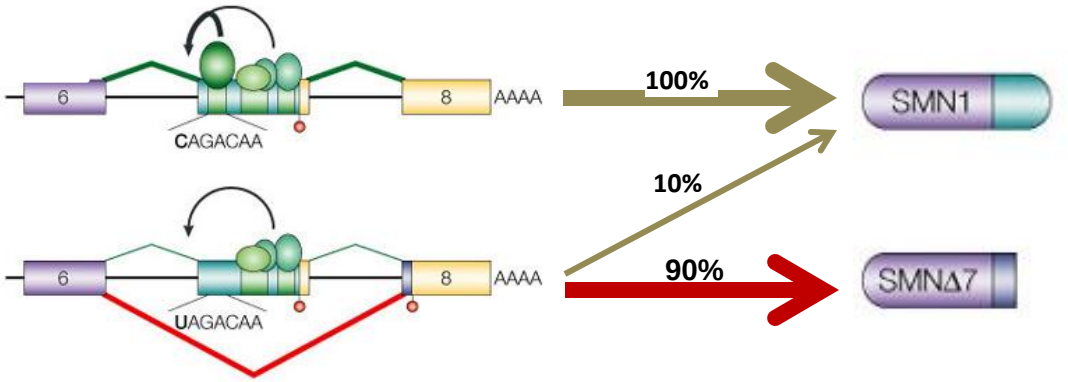
STANLEY CENTER
FOR PSYCHIATRIC RESEARCH
AT BROAD INSTITUTE

HSCRB
HARVARD DEPARTMENT OF STEM CELL & REGENERATIVE BIOLOGY

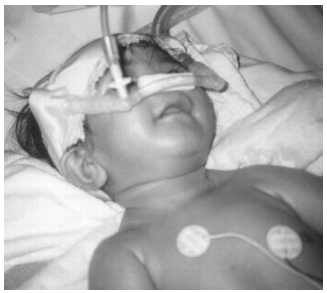
Spinal Muscular Atrophy (SMA): Genetic childhood onset disease characterized by **motor neuron loss** due to a reduction in the amount of Survival of Motor Neuron (SMN) protein (expressed in all cells).

Survival of Motor Neuron genes



Disease-modifying gene

Often fatal

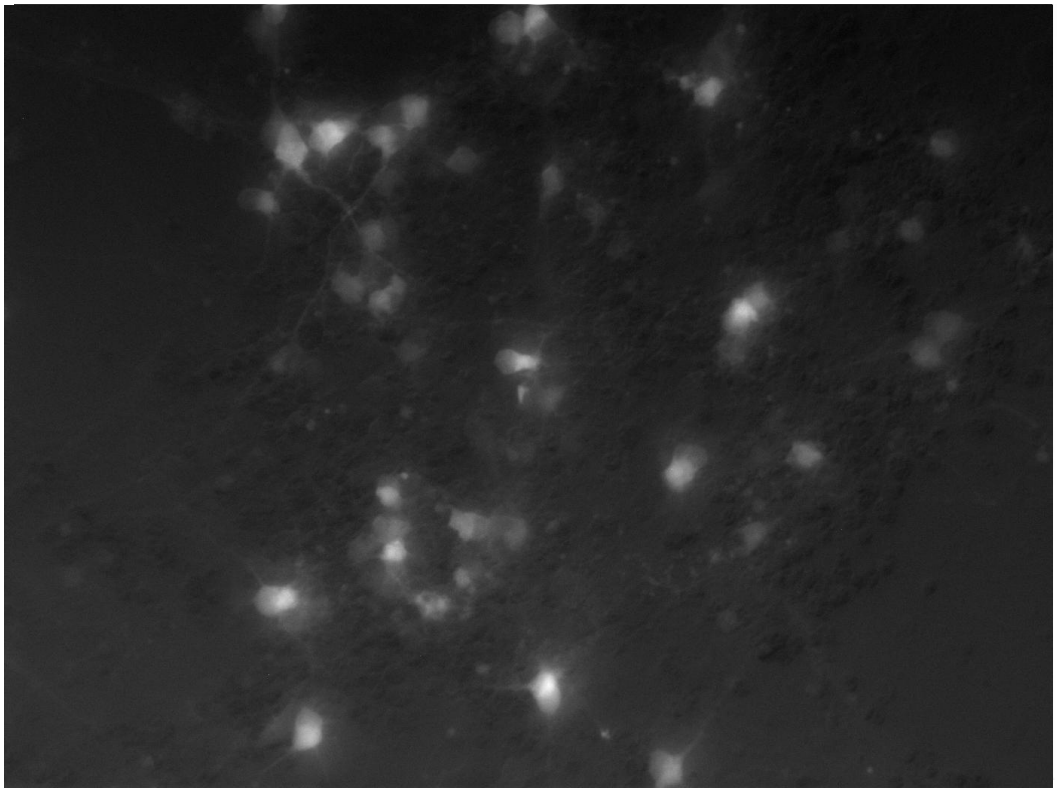


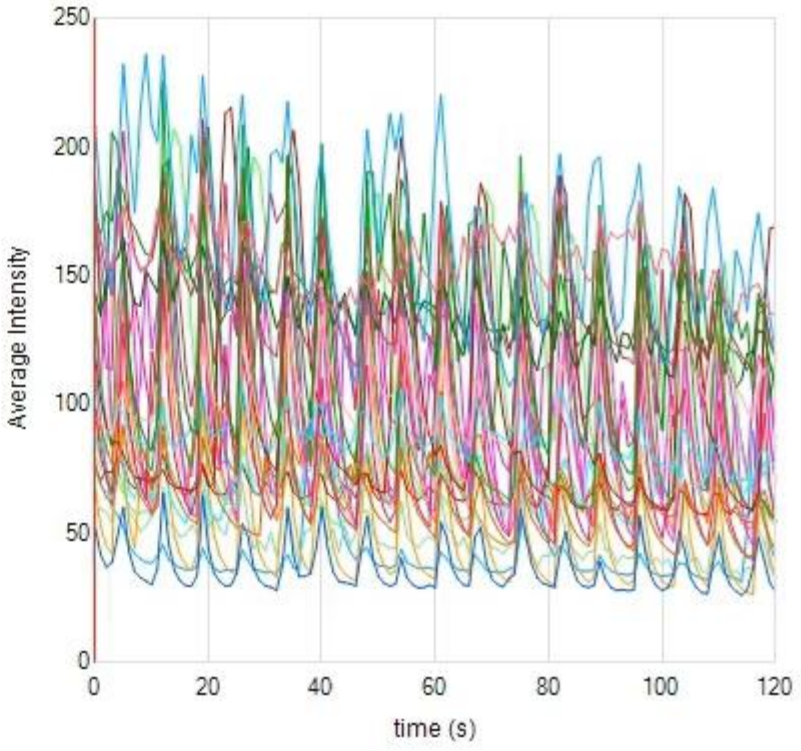
Type I

Type II **Type III**

Why are motor neurons so sensitive to decreased SMN?

Produced motor neurons from patients with the disease and relevant controls.





Results

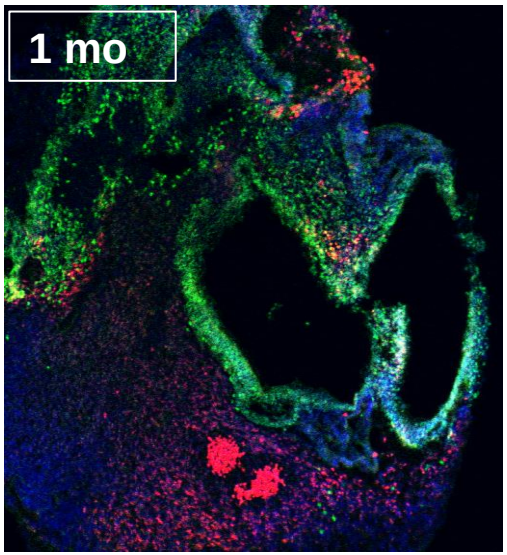
- Reproduced known SMA phenotypes
  - Found selective death of SMA motor neurons
  - Successfully tested both Ionis/Biogen and PTC/Roche therapeutics that ultimately entered the clinic
  - Used the ability to produce large numbers of motor neurons to provide the first understanding of why motor neurons die
- Predicted new symptoms of the disease, some of which have been confirmed in mouse models and patients
- Excitingly, predicted that parental carriers would be phenotypically abnormal.
  - We have now confirmed this in a large study based on querying thousands of medical records.

Psychiatric diseases are more complex and we may need more sophisticated systems to study them.

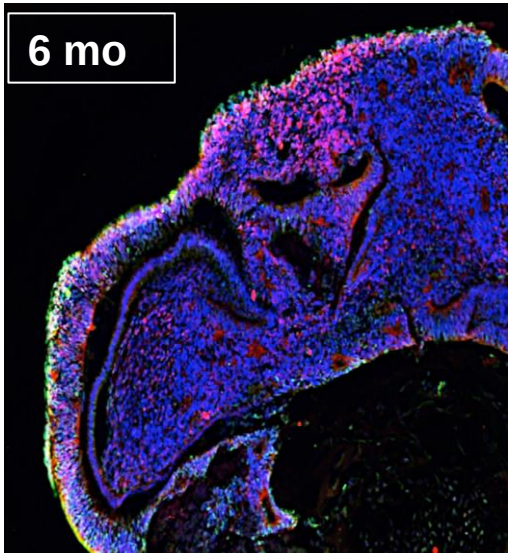
- Many investigators have resorted to systems in which neurons are generated in 3D so that they are more in vivo-like.
- Organoids (Paola Arlotta, Harvard and Stanley Center)
  - Structurally complex, but somewhat variable
- Spheroids
  - Structurally simpler, but more consistent
  - Can produce (within individual spheres):
    - Either single types of neurons or multiple types of neurons, depending on addition of specific sets of induction factors (motor neurons, dopaminergic neurons, cortical neurons, astrocytes)
  - Scalable to generate billions of specified neuronal types.

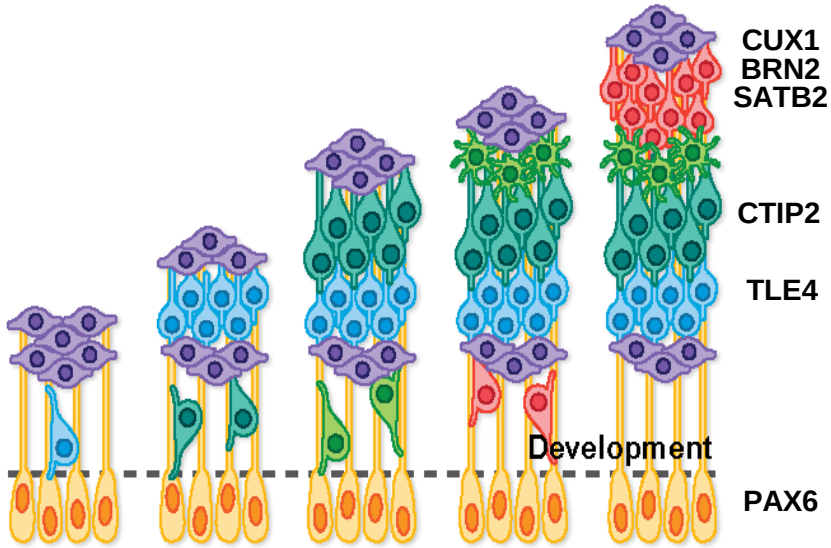
Brain organoids display radially layered structures

PAX6 CTIP2 DAPI

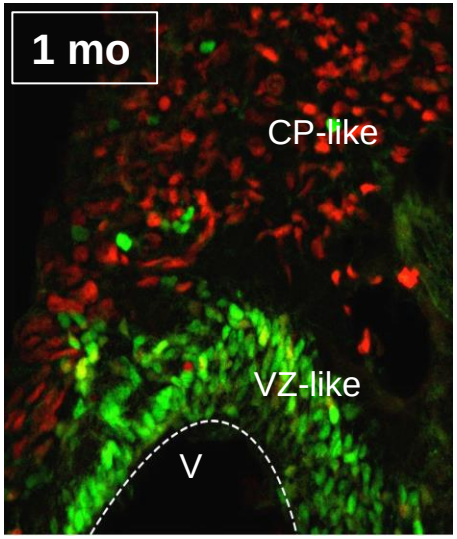


CUX1 BRN2 DAPI

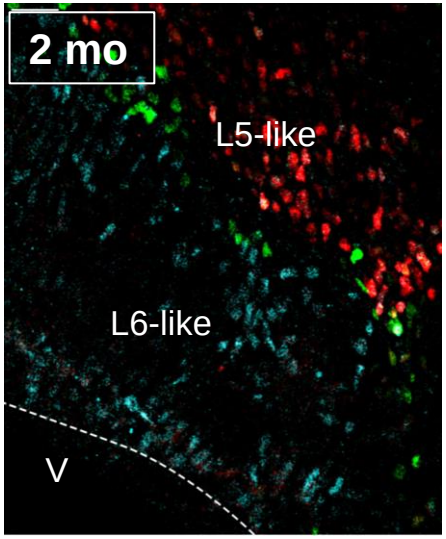




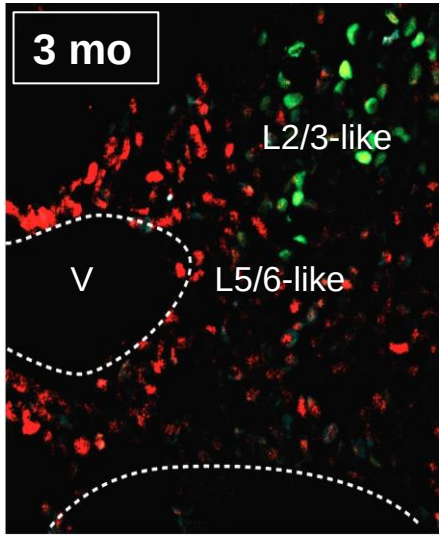
PAX6 CTIP2



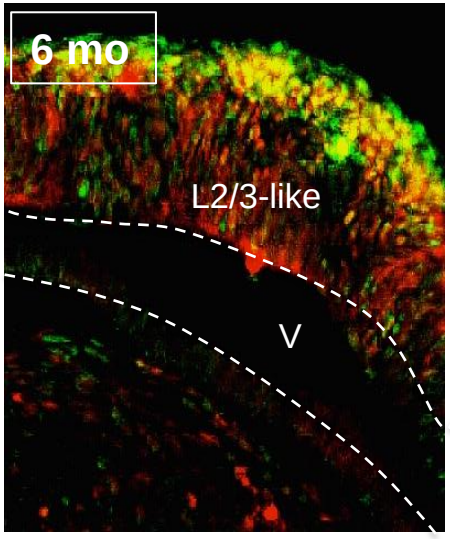
SATB2 CTIP2 TLE4



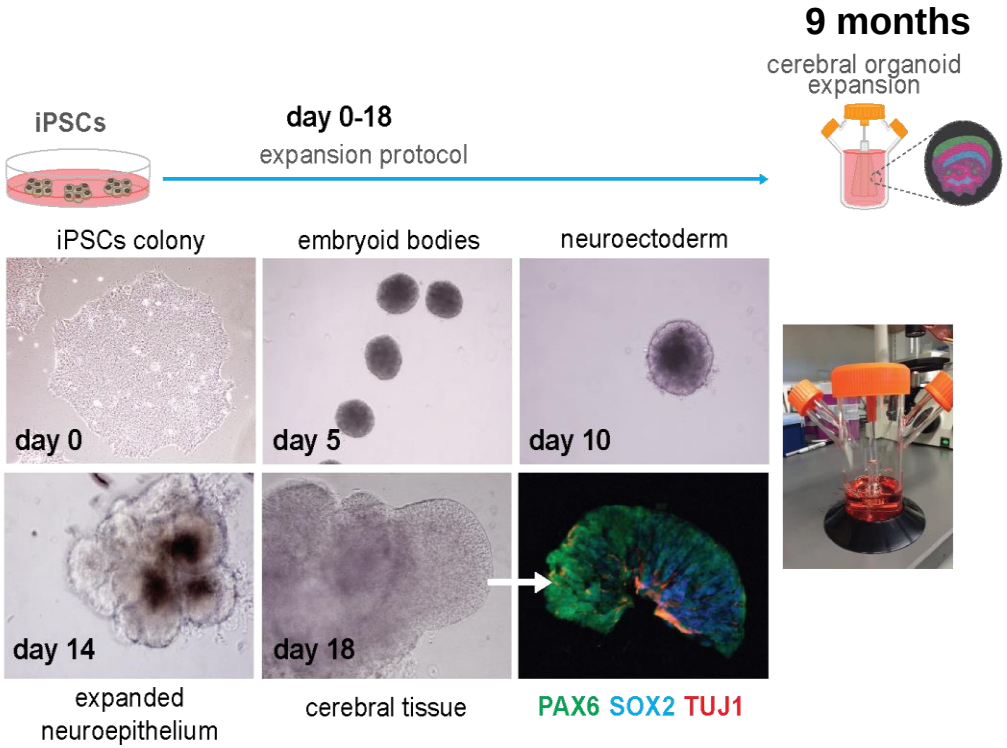
CUX1 CTIP2



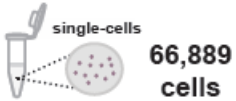
CUX1 BRN2

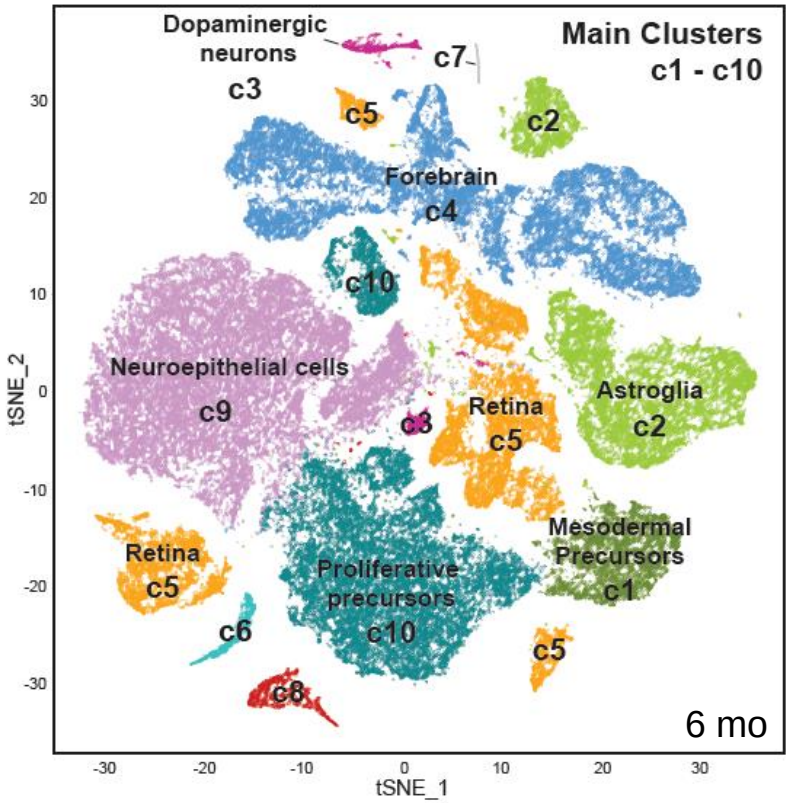


Long-term cultures of 3D human brain organoids

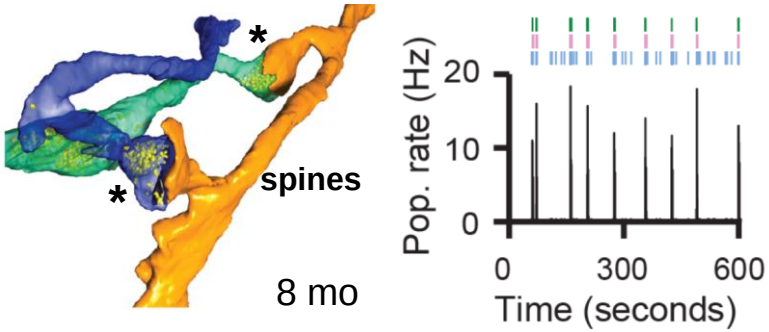


CELLULAR COMPOSITION DROP-SEQ



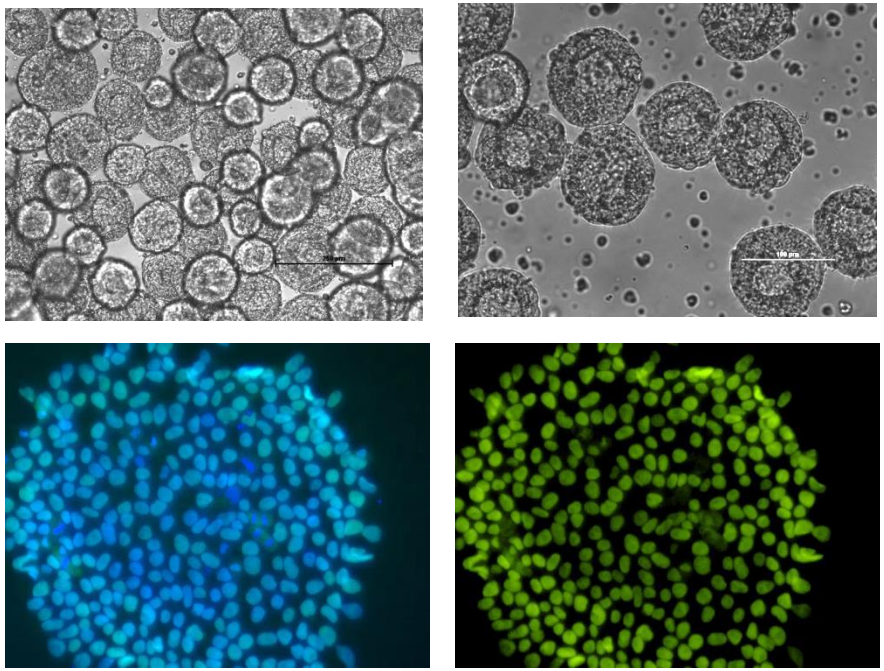


FORMATION OF FUNCTIONAL SYNAPSES AND NEURAL NETWORKS



Production of Neuronal Spheroids

hPSCs grown in spin culture

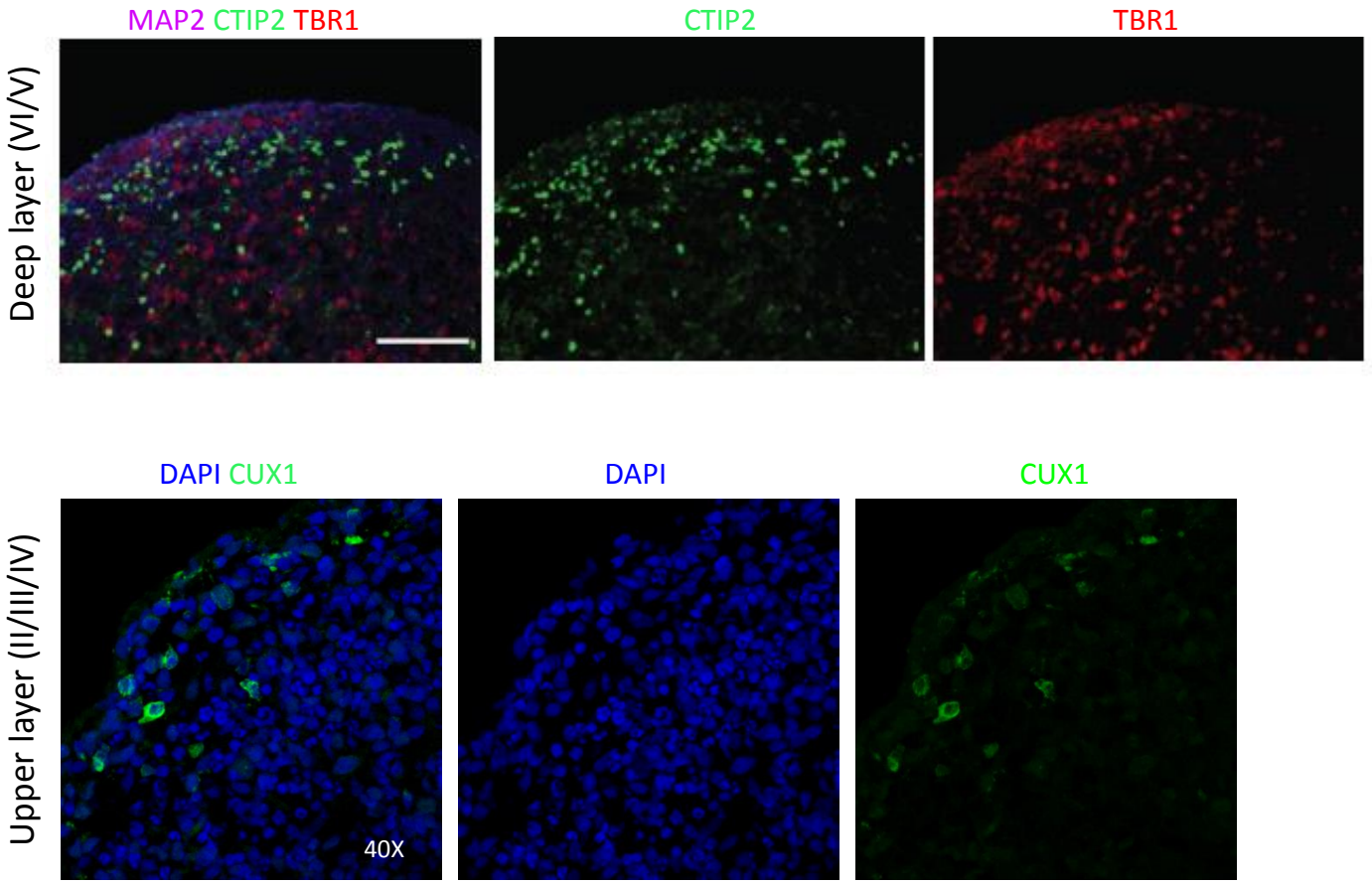


OCT4 DAPI

Rigamonti et al., Stem Cell Reports, 2016

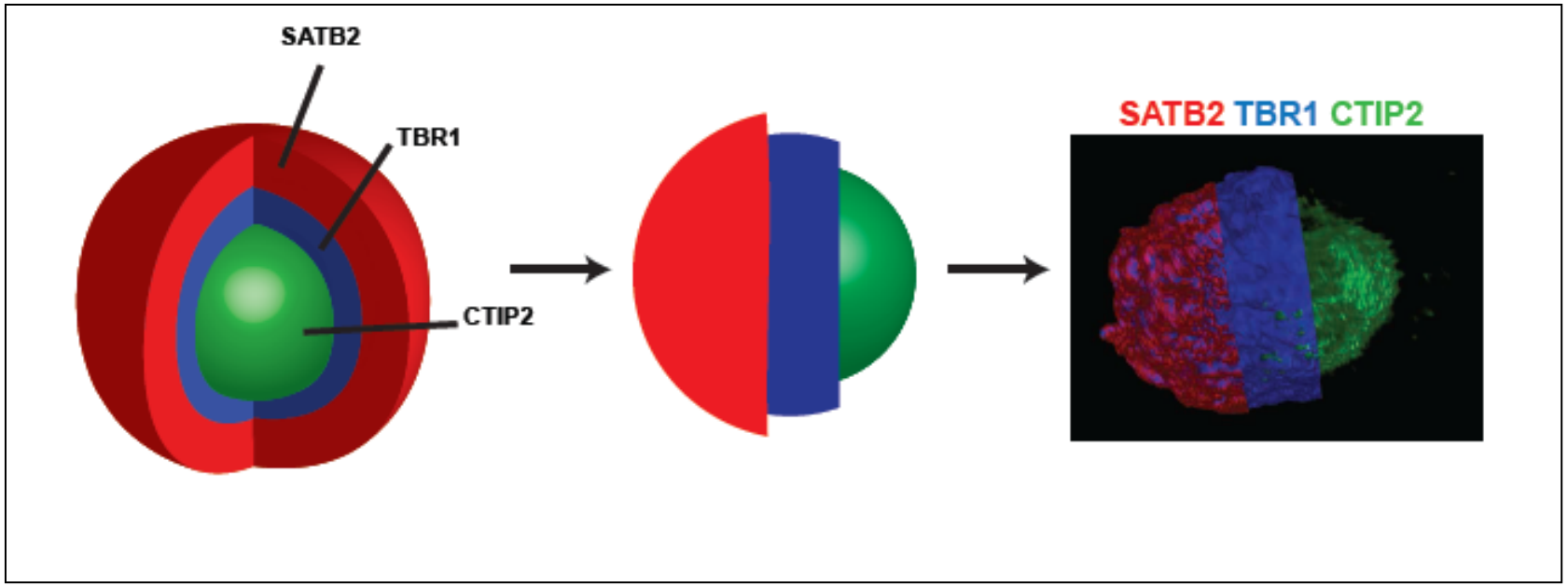
Cortical spheroids express deep and upper layer neuronal markers.

Immunostaining of frozen sections

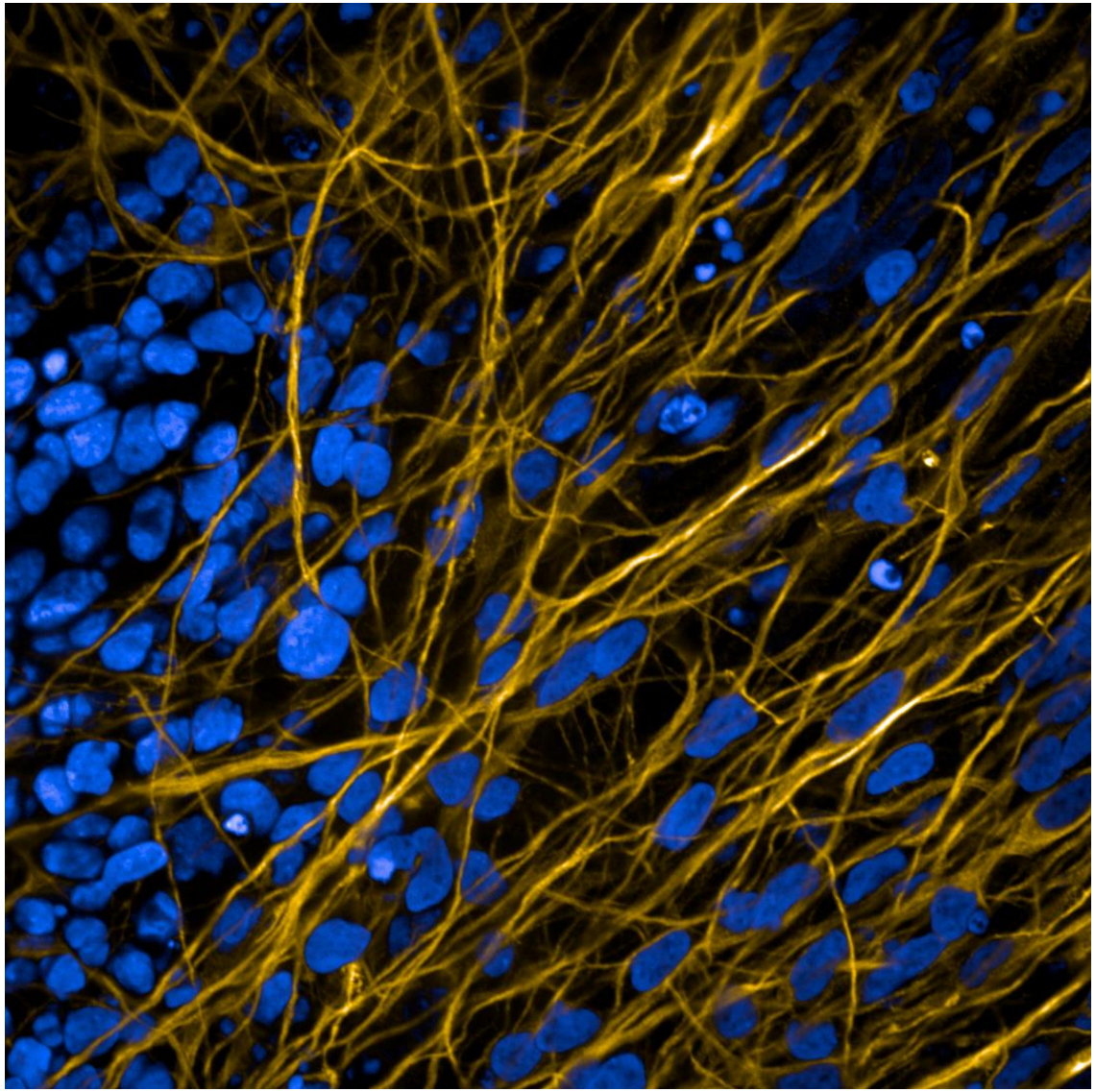


Cortical spheroids are organized with clusters of cells

Immunostaining of 3D spheres cleared with SeeDB and imaged with a Lightsheet microscope

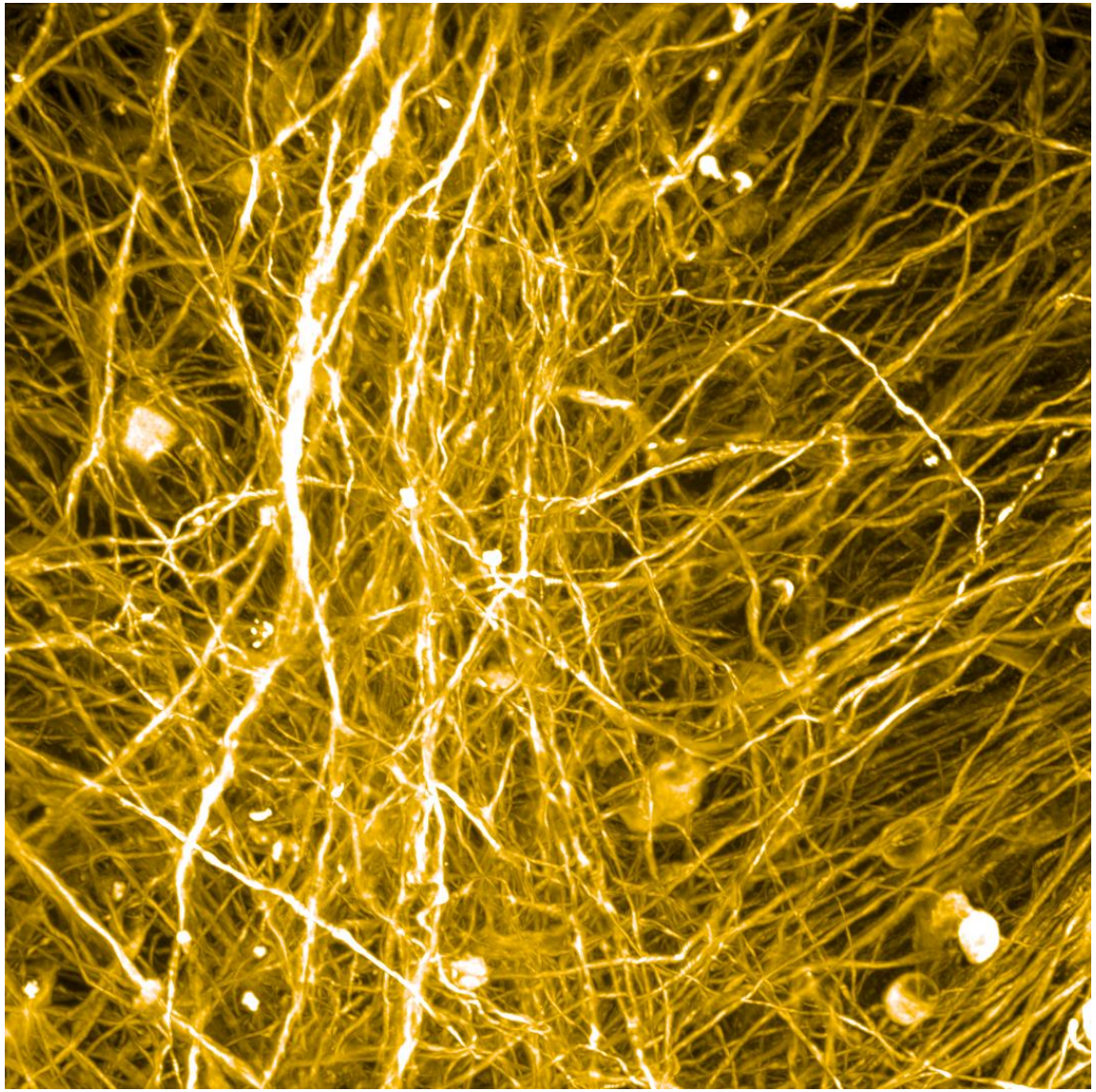


Clearing and Imaging Spheroids



iDisco cleared
DAPI TUJ1
55 z-stacks;
1um spacing

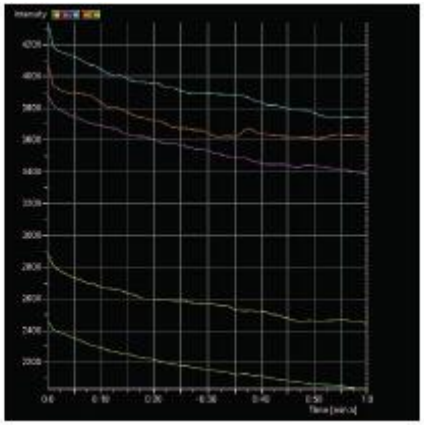
Clearing and Imaging Spheroids



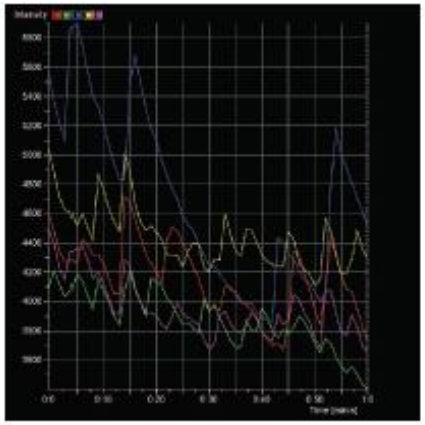
iDisco cleared
Projection
TUJ1
55 z-stacks;
1um spacing

Synchronization of neuronal firing over time using AAV- GCaMP6

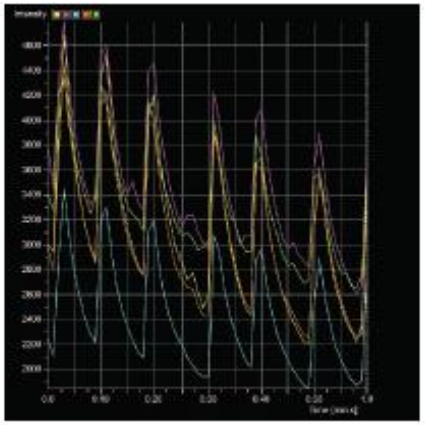
20 days



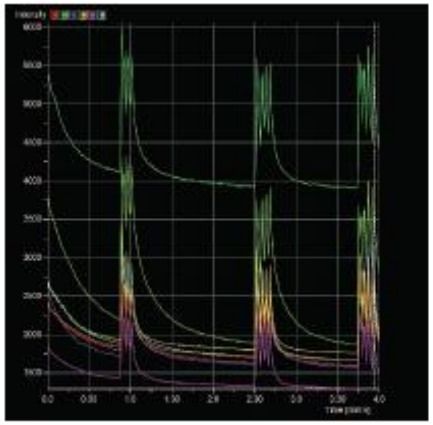
40 days

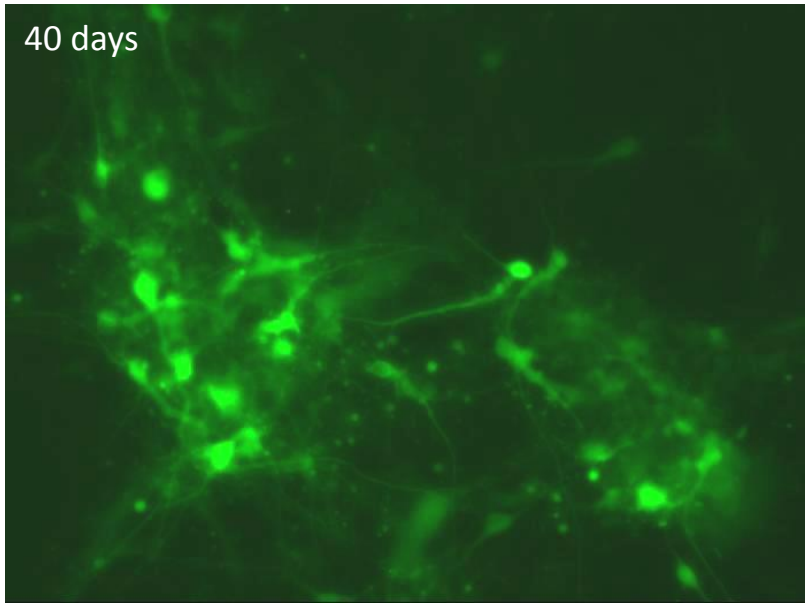


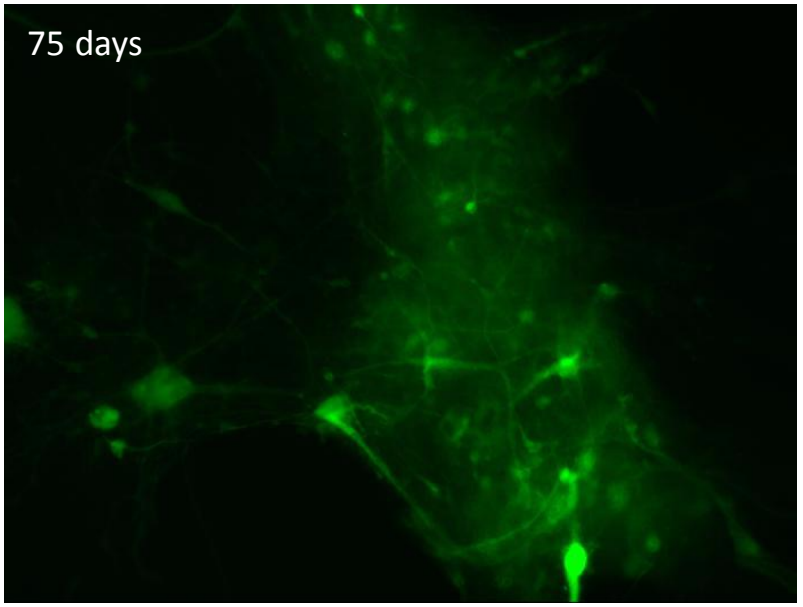
60 days



75 days

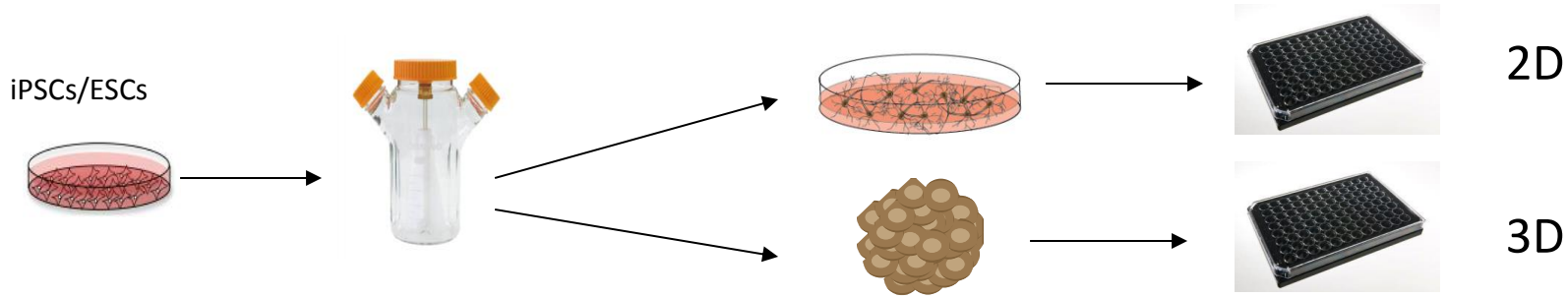






How can we use cortical spheroids for disease modeling and screening?

Phenotypic and compound screening

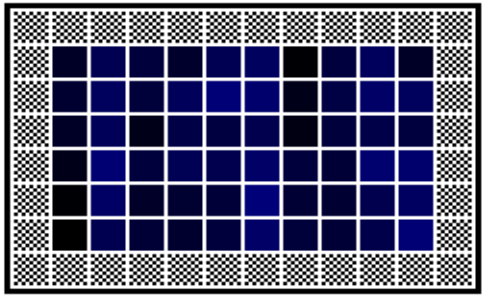


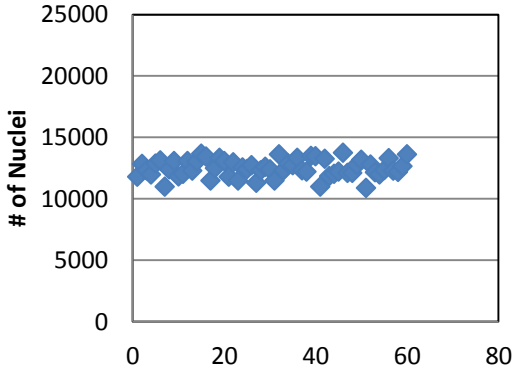
Plans

- Uses
  - Identification of disease relevant phenotypes (comparisons between mutant and control lines)
  - Screening
    - Target-based or phenotypic
    - 2D (after dissociation of spheroids)
      - First project: screen for compounds that decrease levels of complement C4 in cortical neurons
  - Future
    - Neuron-subtype specific target regulation (**new ways of drugging disease targets**)
    - Evolution of spheroid production
    - Development of 3D imaging methods (to permit screening in 3D)

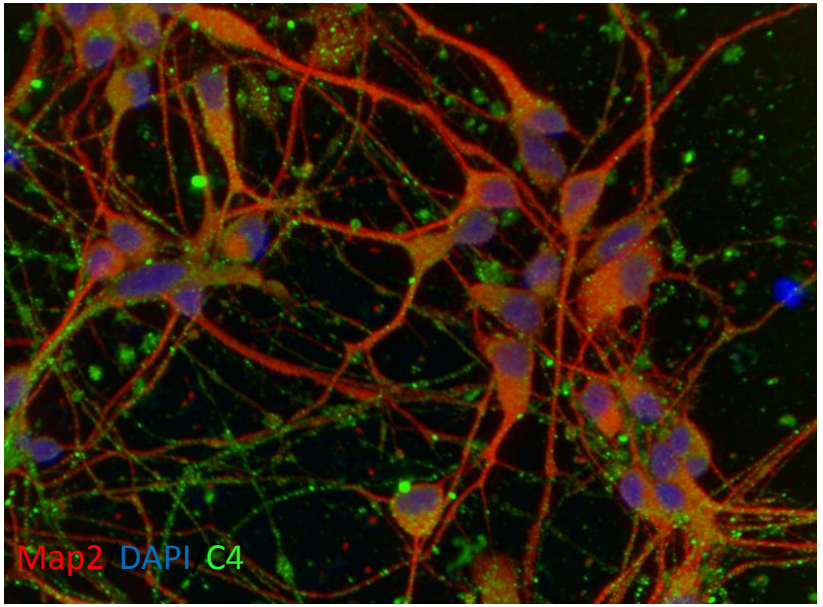
Identifying modulators of C4: Collaboration with Steve McCarroll and Beth Stevens

Plating consistency

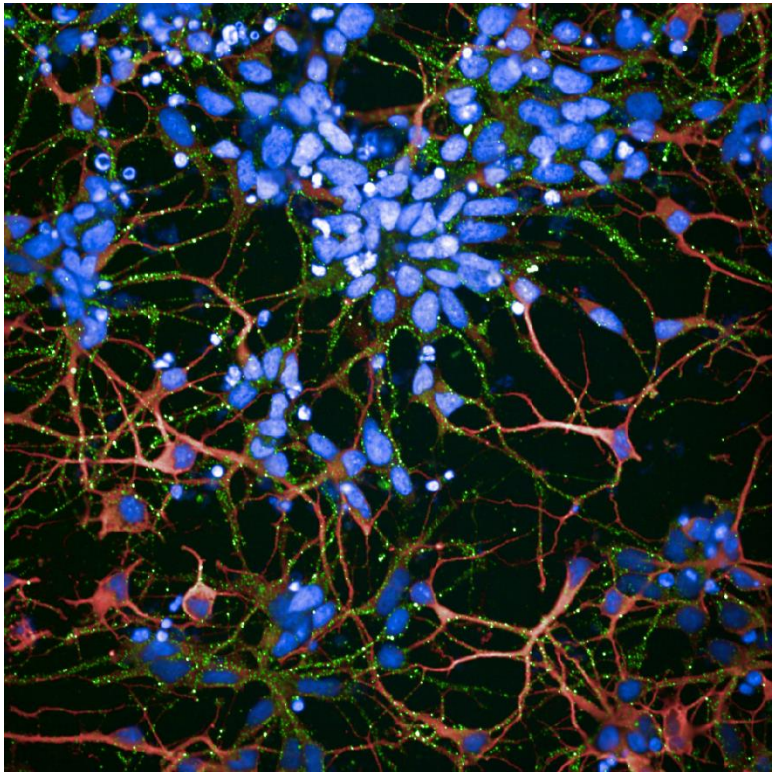


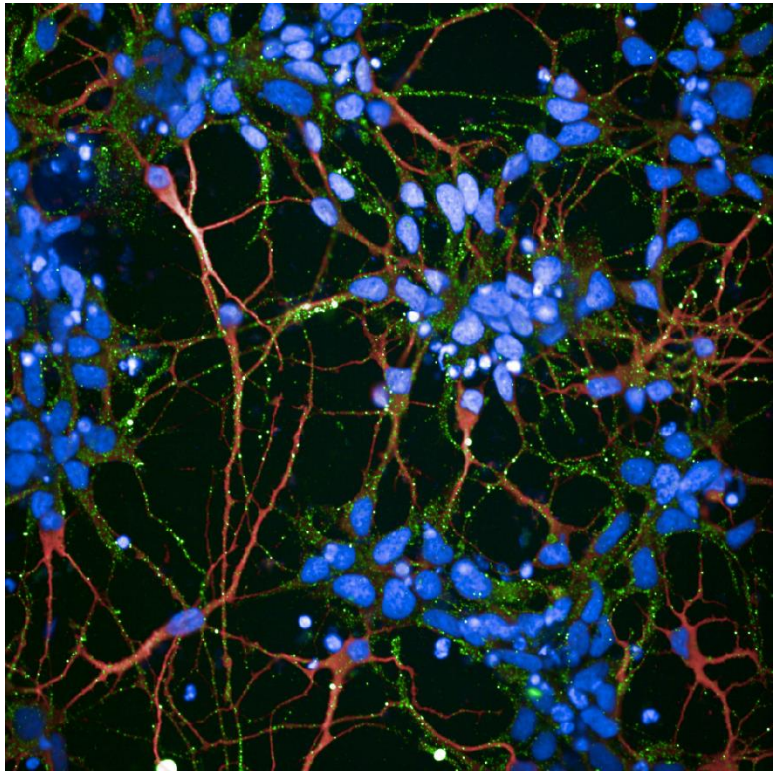


Expression of C4
by immunostaining
and ELISA



Identifying modulators of C4



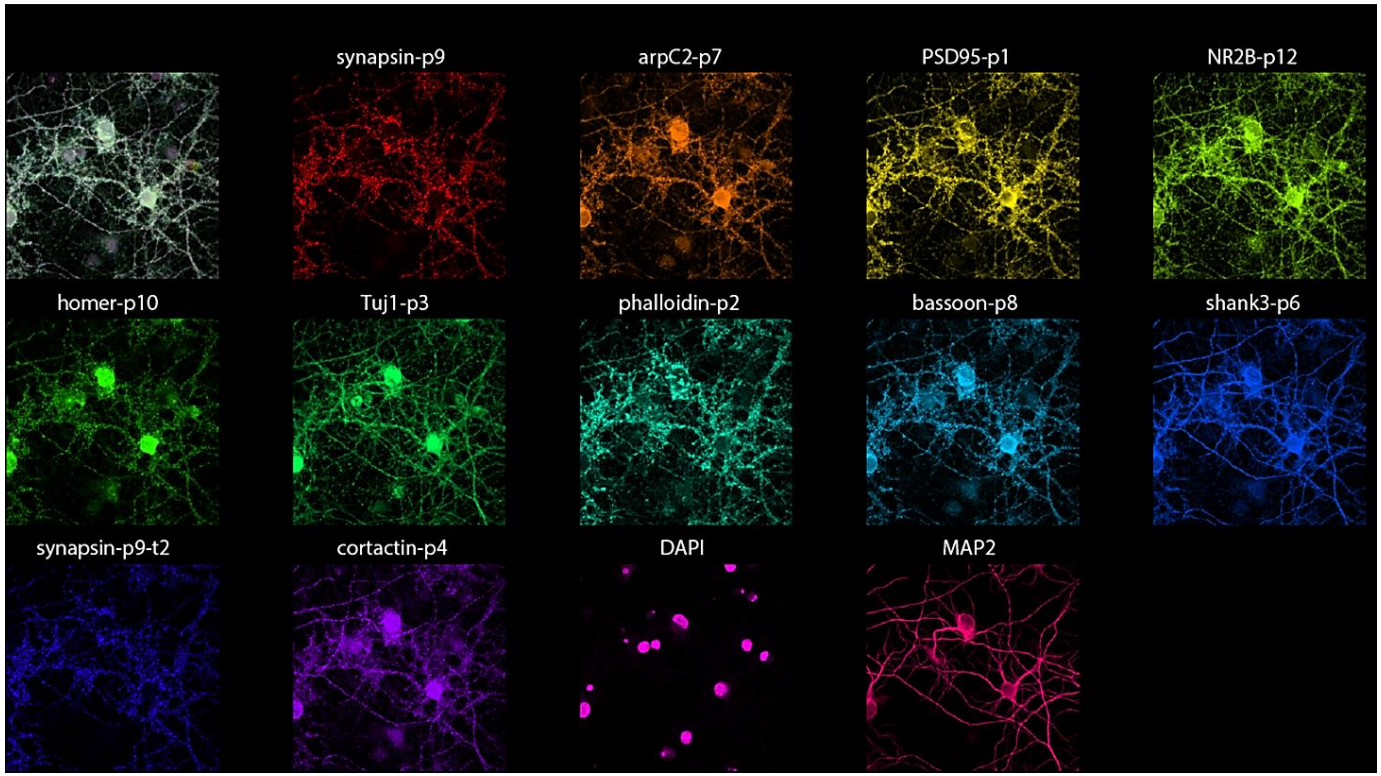


Phenix 40xWater Lens
Map2
C4

Highly Multiplexed Imaging (with Martin Tomov and Mark Bathe)

DNA-PAINT to image large numbers of targets in one experiment

- Small strands of DNA (probes) are tagged with a fluorescent dye
- Antibodies are conjugated with small complementary DNA barcodes
- Strand interactions can be easily broken to “reset” the cells for further imaging
- Differential regulation of target activity in individual neuronal types.



Images Courtesy of:
Li Li & Jeff Cottrell – Stanley Center, Broad Institute
Syuan-Ming Guo & Mark Bathe– LCBB, MIT

Optimizing Spheroid Protocols

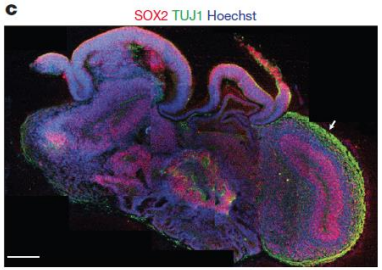
Generation of “organospheres” (intermediate between brain organoids and spheroids) to:

- Increase the number of cortical subtypes present
- Increase the 3D “in vivo-like” structuring
- Scale for high-throughput

Areas to improve

- How to initiate sphere formation (spin versus plate)
- Media composition (KSR? Factors?)
- Embedding media (matrigel, alginate, custom; “drop-spheres”)

Lancaster Brain Organoid Protocol



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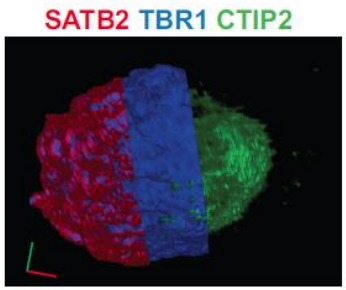
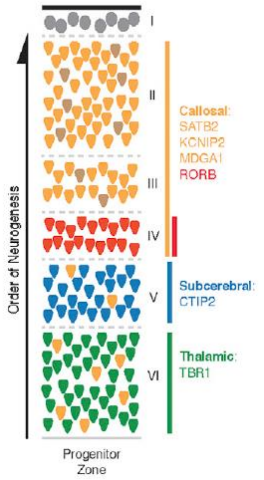
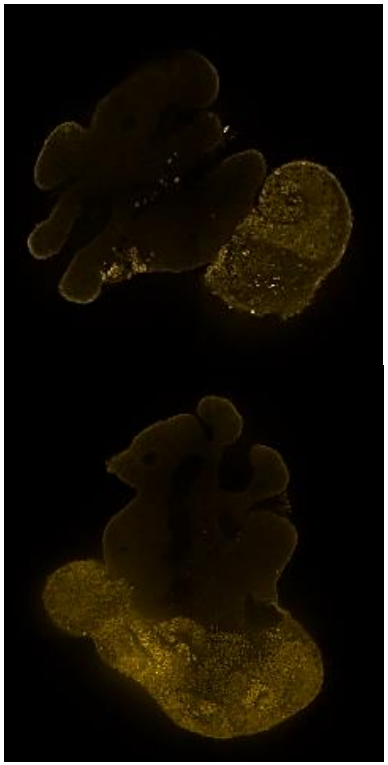
Rubin Lab Spin Cortical Neuron Protocol





Optimizing Spheroid Protocols

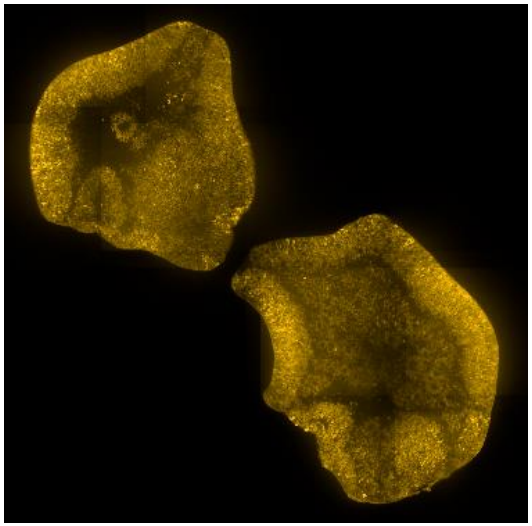
Organoids have *regions* of Pax6 development



d27, Lancaster Method, no embedding

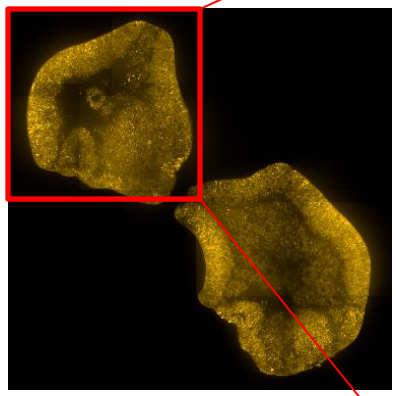
Protocol Development

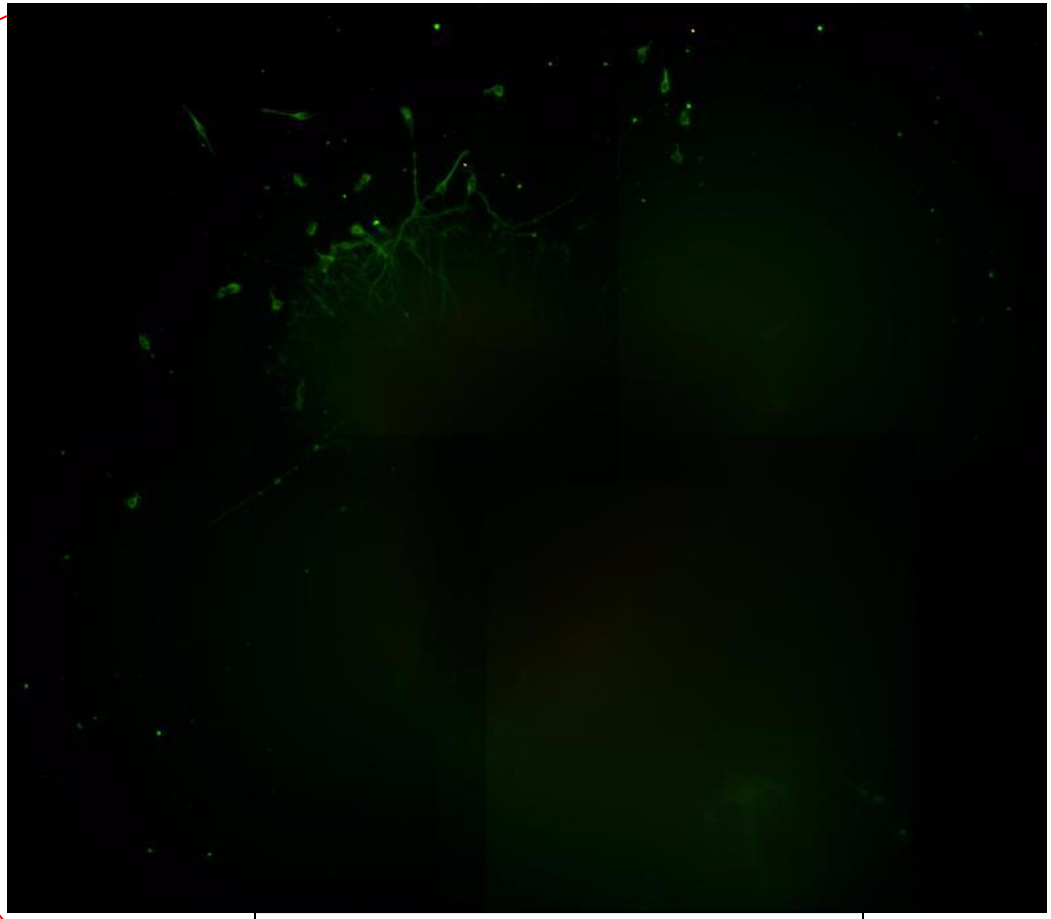
Modified spheroids have Pax6 staining throughout



d27, Modified Rubin Method
(Formed in 96 well in Tesr +Rock, 2d KSR pulse, Dual SMAD and WNT inhib, no embedding)

Clearing and Imaging Spheroids





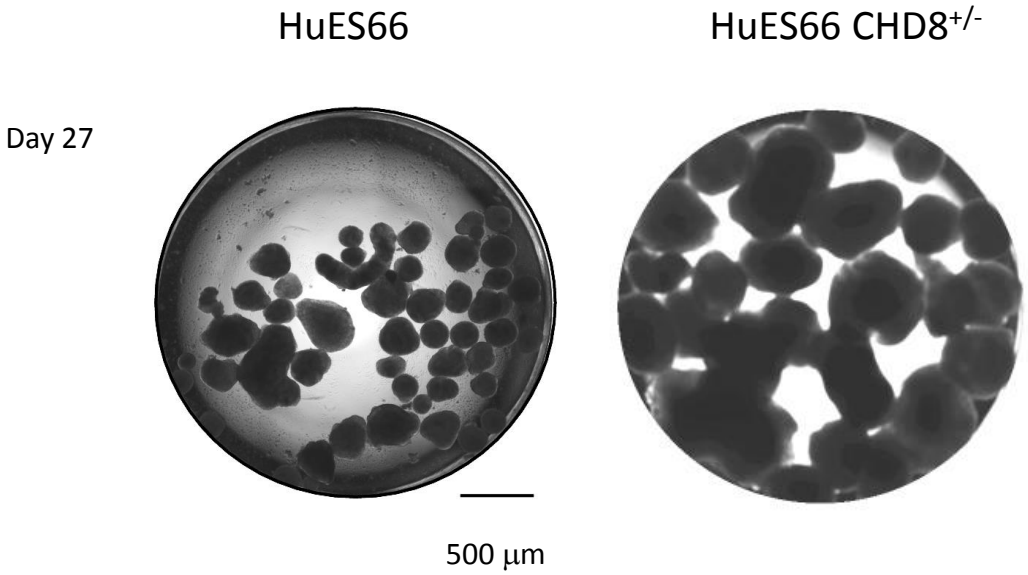
Can see:

- Thick cortical progenitor outer ring (Pax6+)
- Neural rosettes inside sphere
- Tuj1 staining shows neurites are organized (lined up)

d27, New Cortical Spheroids
iDISCO Cleared
αPax6
αTuj1
20x, 5um x 50 stacks, 250um total

CHD8 Phenotype in 3D

- Demonstration of phenotype in 3D spheroids.
- ASD in patients with CHD8 mutations associated with macrocephaly.



- CHD8+/- cortical spheroids show a significant increase in size compared to the isogenic line.

Summary

- Different methods are being employed to produce CNS cell types in 3D.
- There are multiple uses for these cells:
  - Studying the disrupted connectivity and activity that characterize psychiatric disease.
  - Screening for phenotypes, targets and therapeutic candidates using human diseased neurons.
  - Clustering patients by drug responsiveness.

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Acknowledgements

HSCRB HARVARD DEPARTMENT OF STEM CELL & REGENERATIVE BIOLOGY

Rubin Lab
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Li Li
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Feng Zhang and Jen Pan
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Xi Shi

Steven McCarroll
Heather de Rivera

Beth Stevens

Steve Hyman