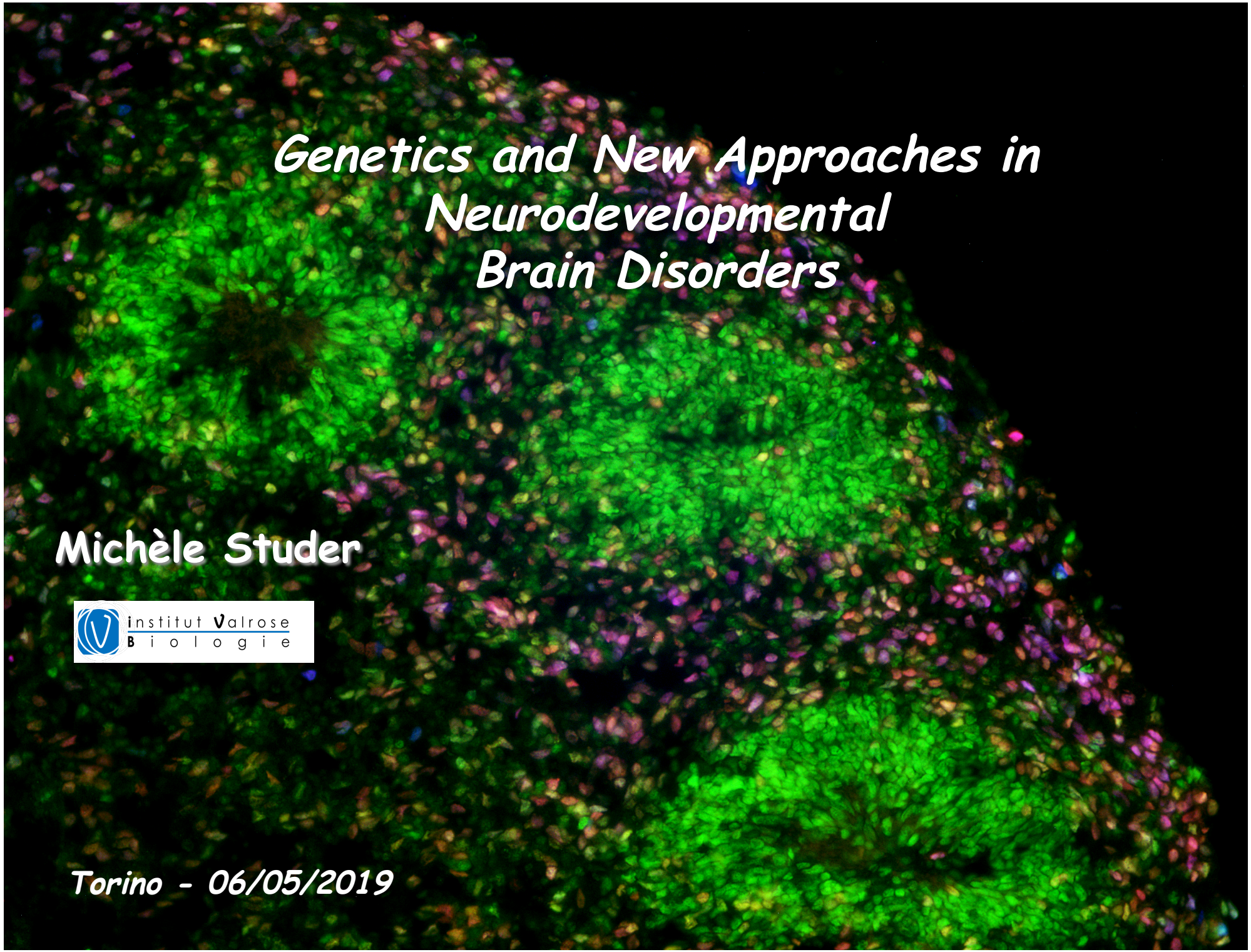


Genetics and New Approaches in Neurodevelopmental Brain Disorders

Michèle Studer

institut Valrose Biologie

Torino - 06/05/2019

Neurodevelopmental disorders (or Intellectual Developmental Disorders -IDD) are impairments of the growth and development of the brain:

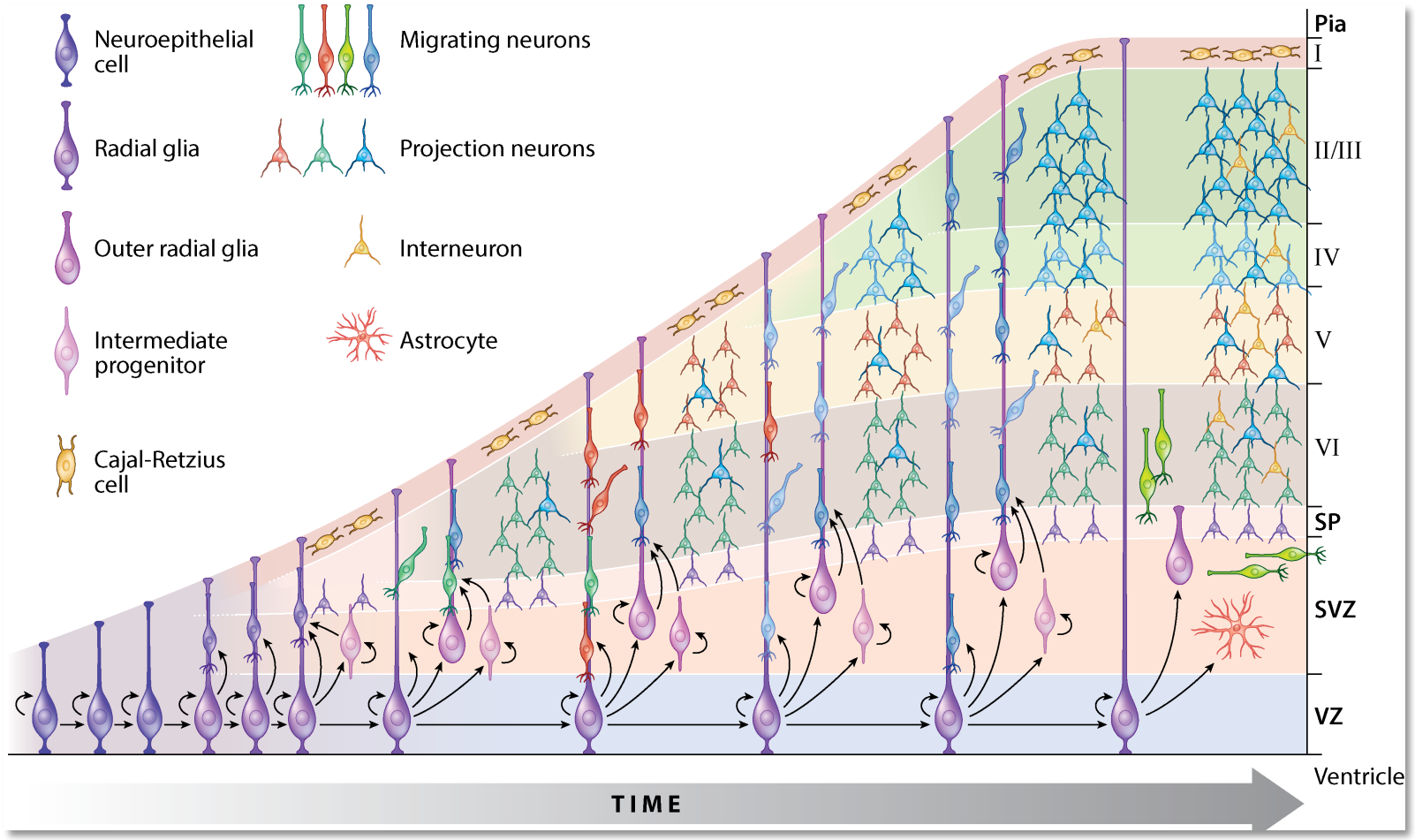
- *affects emotion, learning ability and memory;*
- *communication, speech and language;*
- *unfolds in infancy and childhood.*

Neurodevelopmental disorders are associated with mental, emotional, physical, and economic burden to individuals, families and society in general.

- *Chromosomal disorders: Down syndrome, etc.*
- *Genetic disorders: autism spectrum disorders (ASD), microcephaly, lissencephaly, etc*
- *Traumatic brain injury*
- *Fetal alcohol spectrum disorder*
- *etc*

Neurodevelopmental disorders result from the disruption of normal cortical development processes.

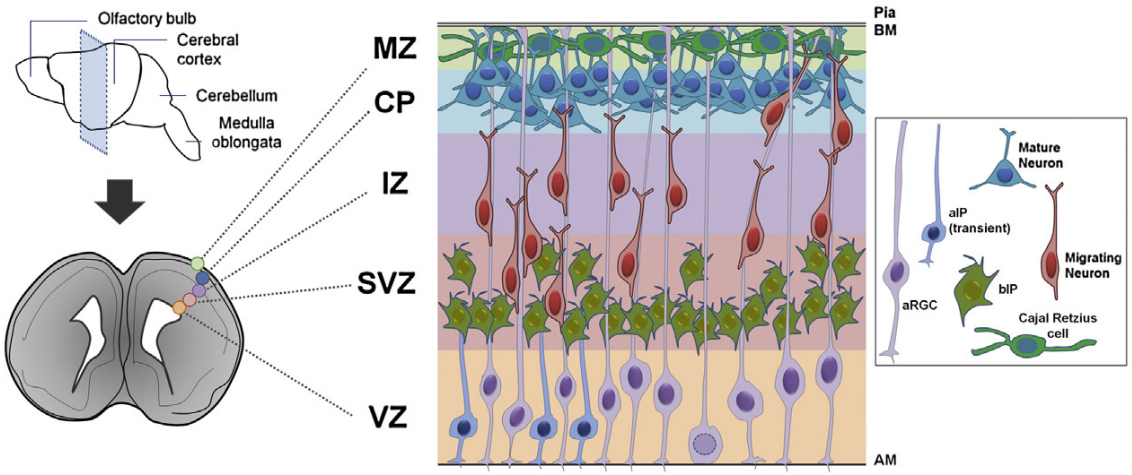
Mammalian corticogenesis (radial organization)



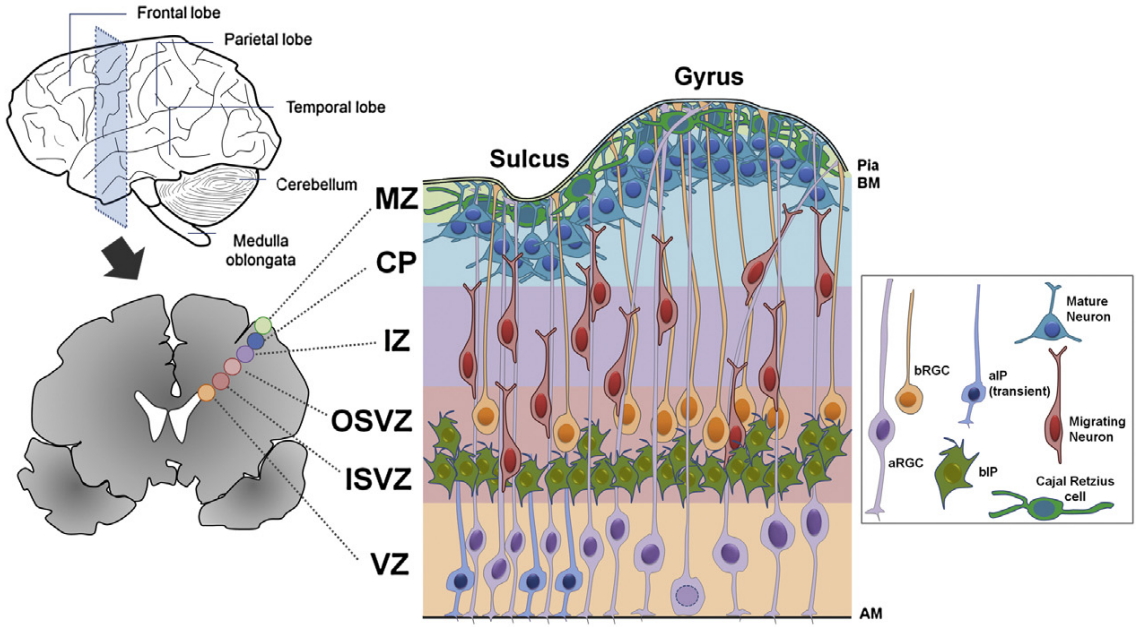
from Hu WF et al., 2014

Comparison of mouse and human cortical neurogenesis

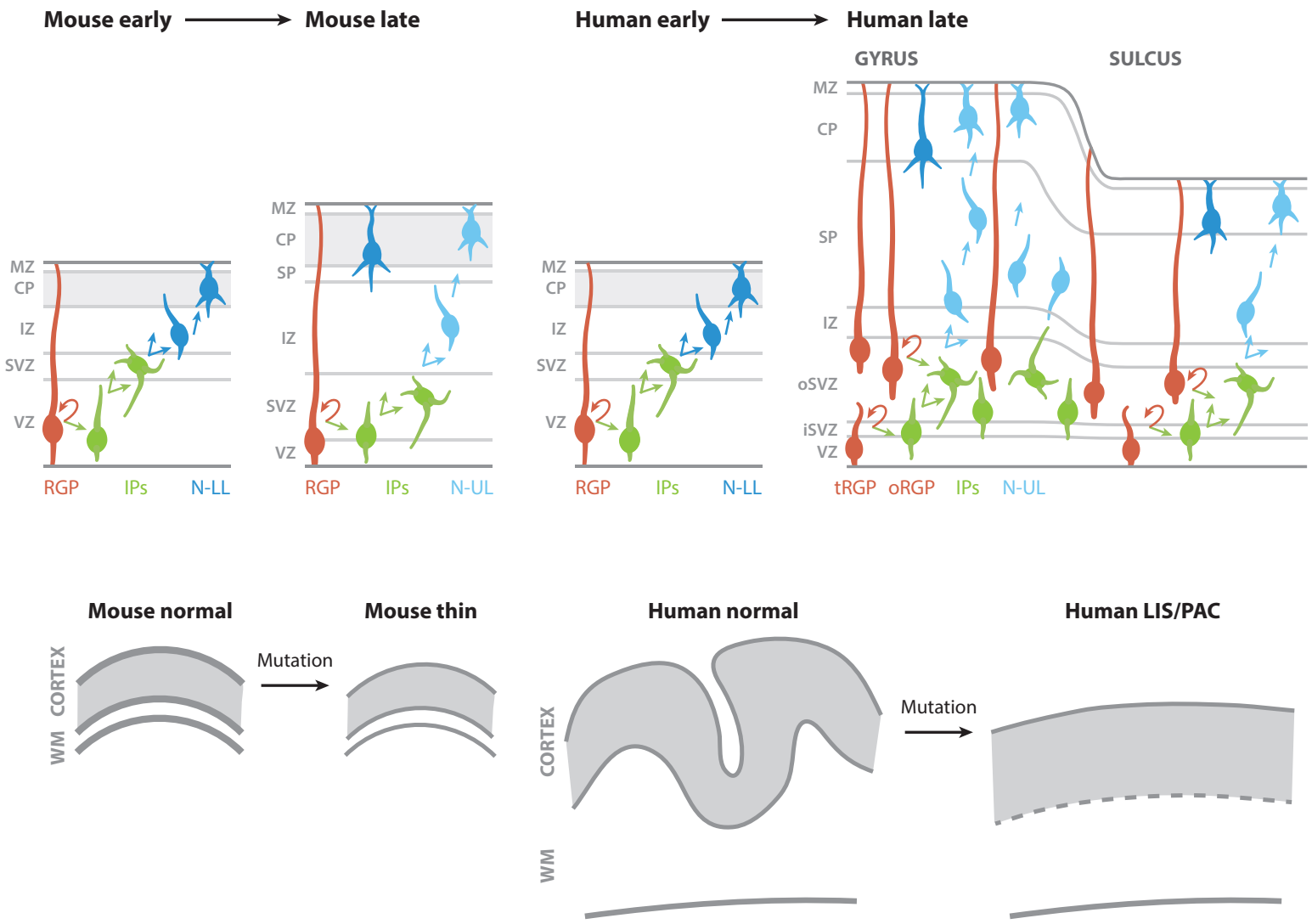
Mouse cortical development



Human cortical development

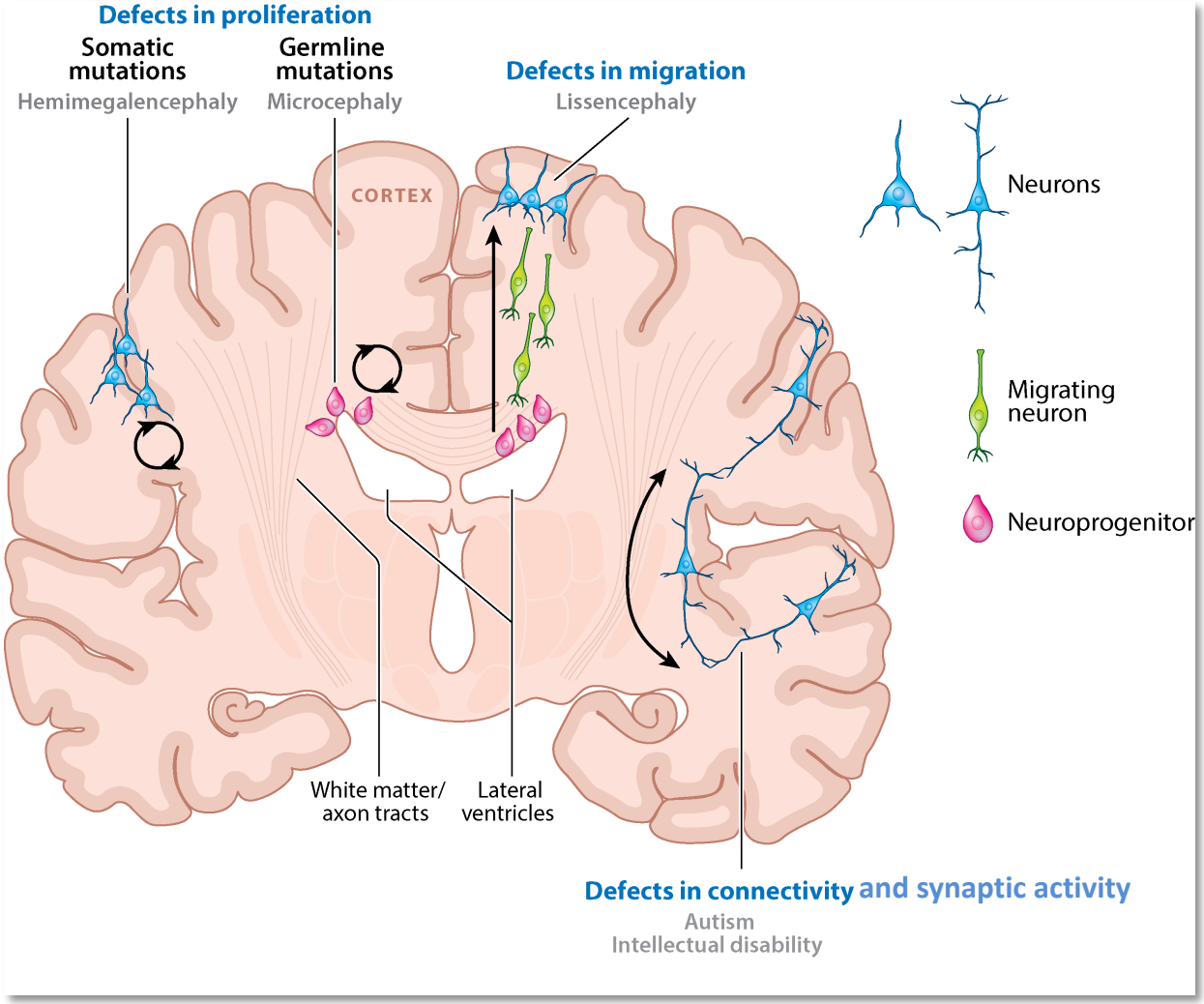


Comparison of mouse and human cortical neurogenesis



Jurik-Sekhar & Hevner et al., 2019

Defects affecting different steps of neurodevelopment



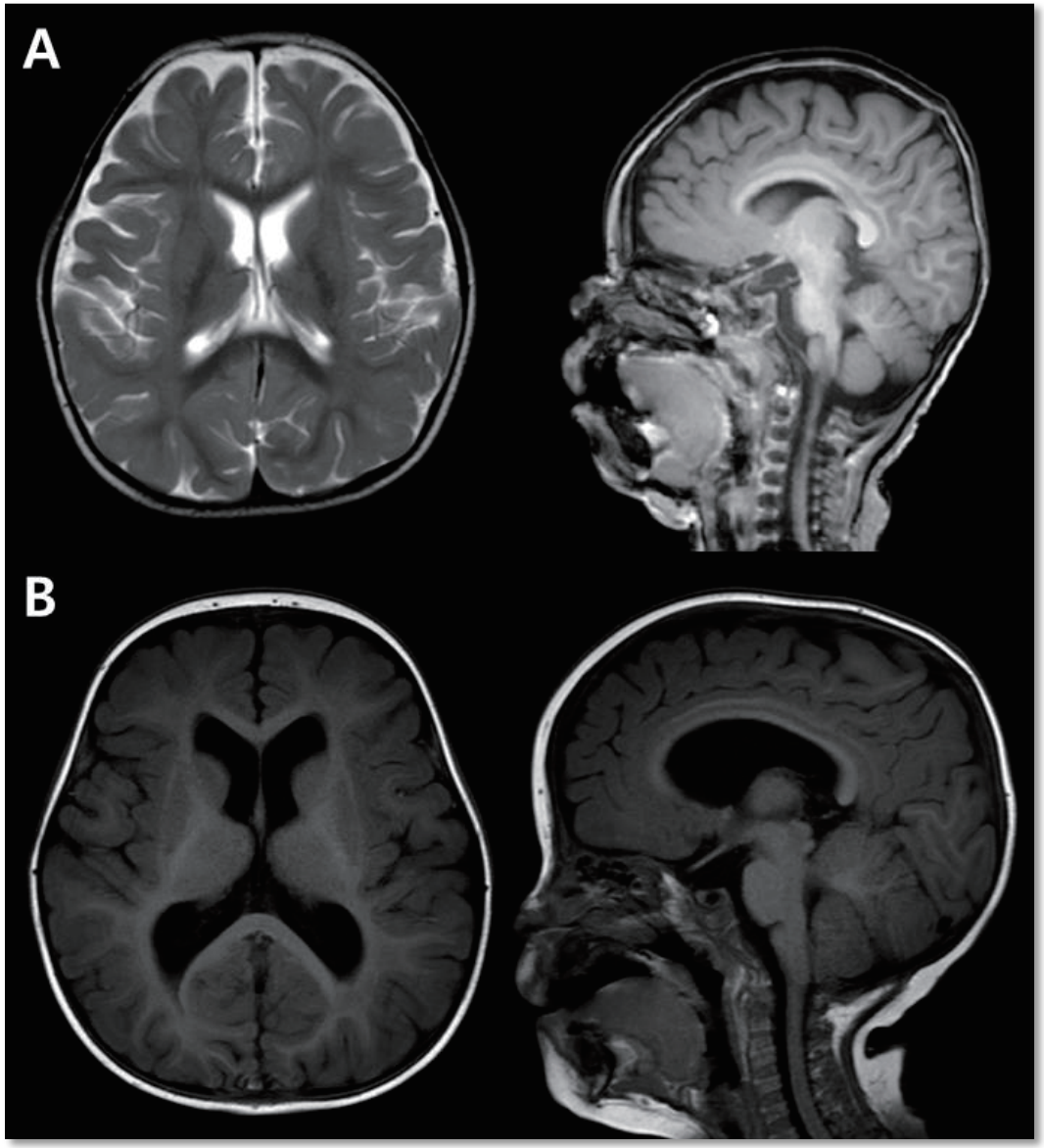
Modified from Hu WF et al., 2014

Structural malformations of cortical development:

Three major groups based on the timing and pathogenesis of the disruption:

- ✓ group I: abnormal neuronal and glial proliferation;
- ✓group II: abnormal neuronal migration;
- ✓group III: abnormal post-migrational development.

Malformations of cortical development: clinical features

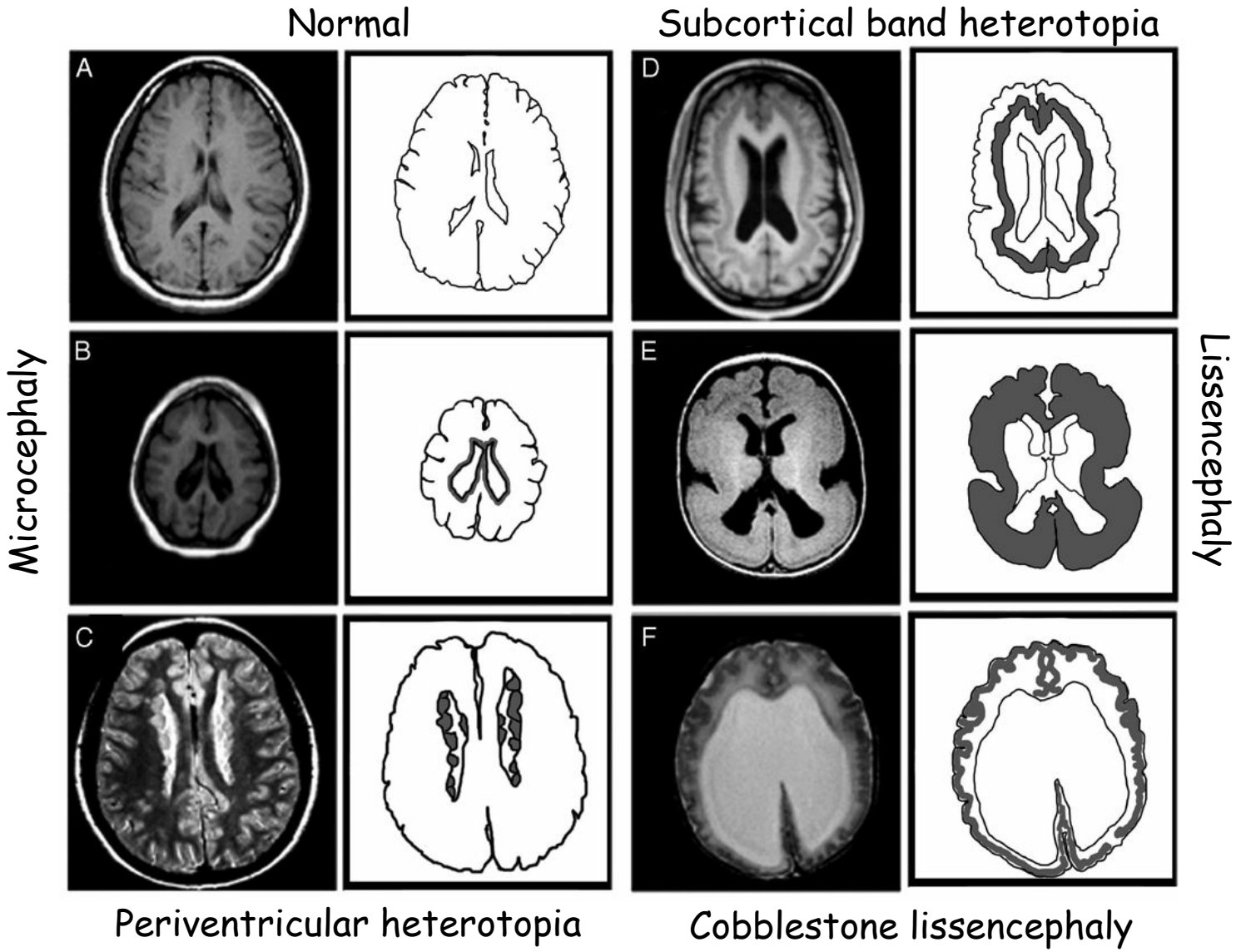


Microcephaly
(small brain)

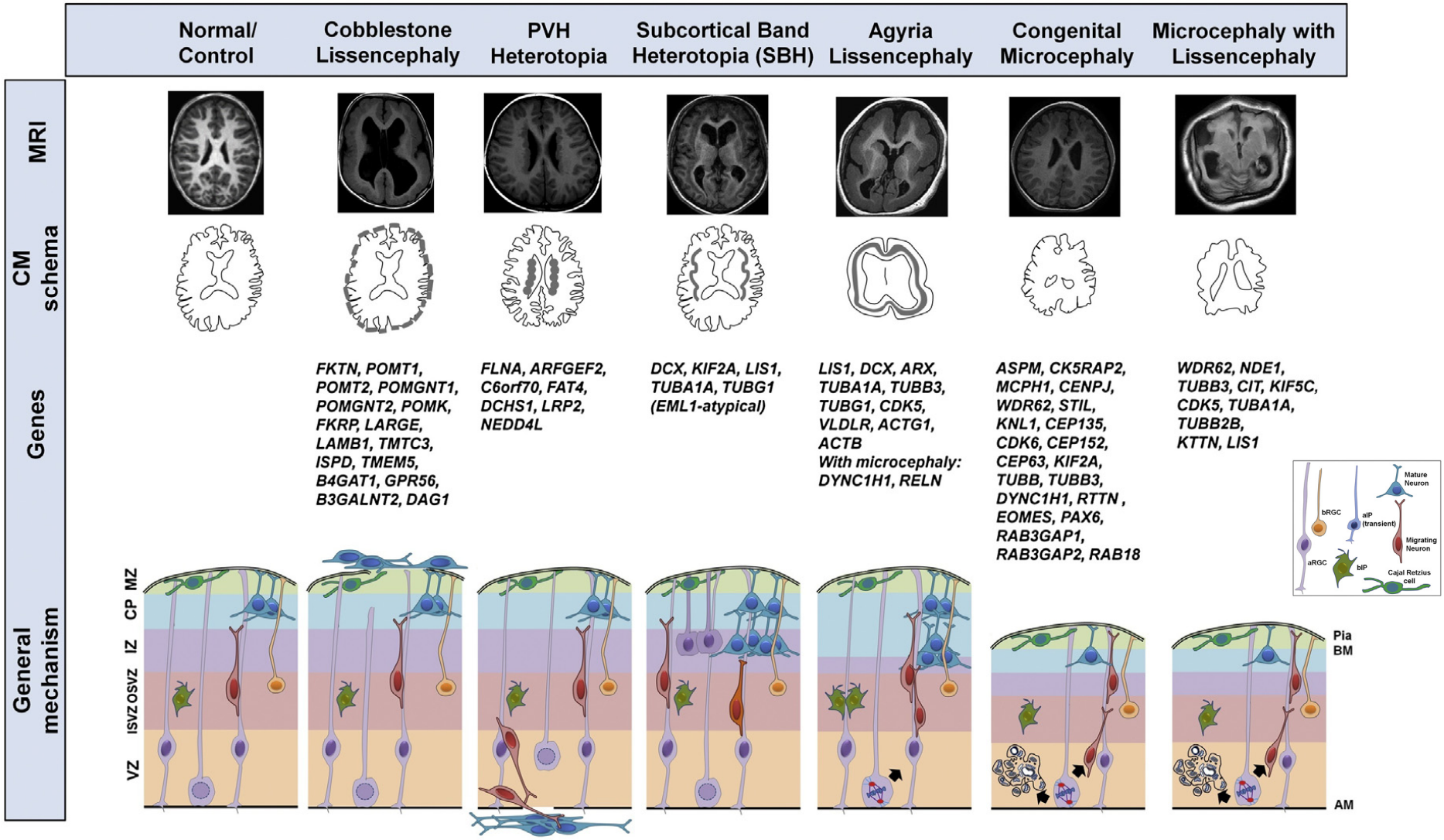
Megalencephaly
(large brain)

Lee, 2017

Brain Magnetic Resonance Imaging (MRI) of children with IDD

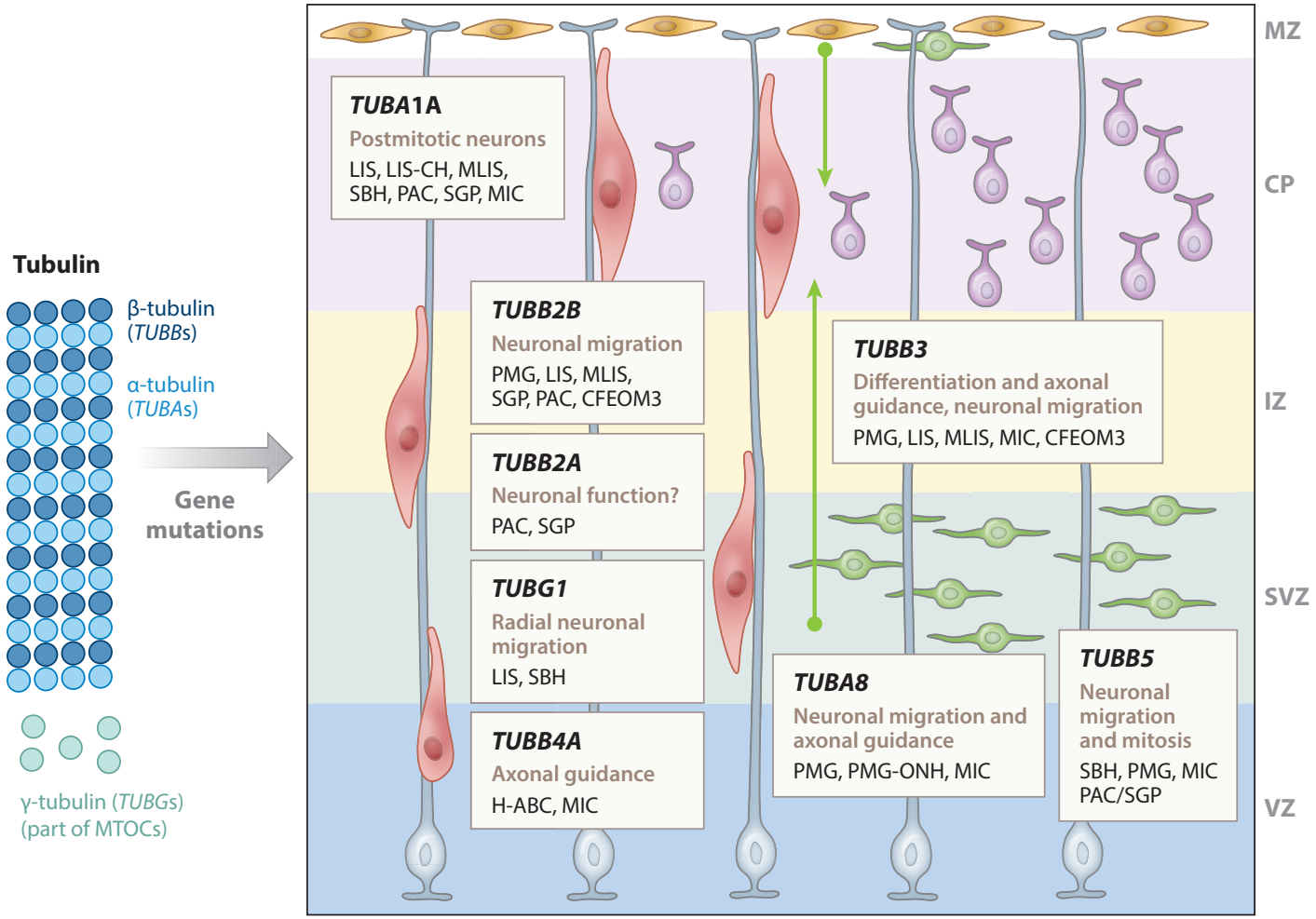


Comparison of human brain malformations of cortical development (MCDs)



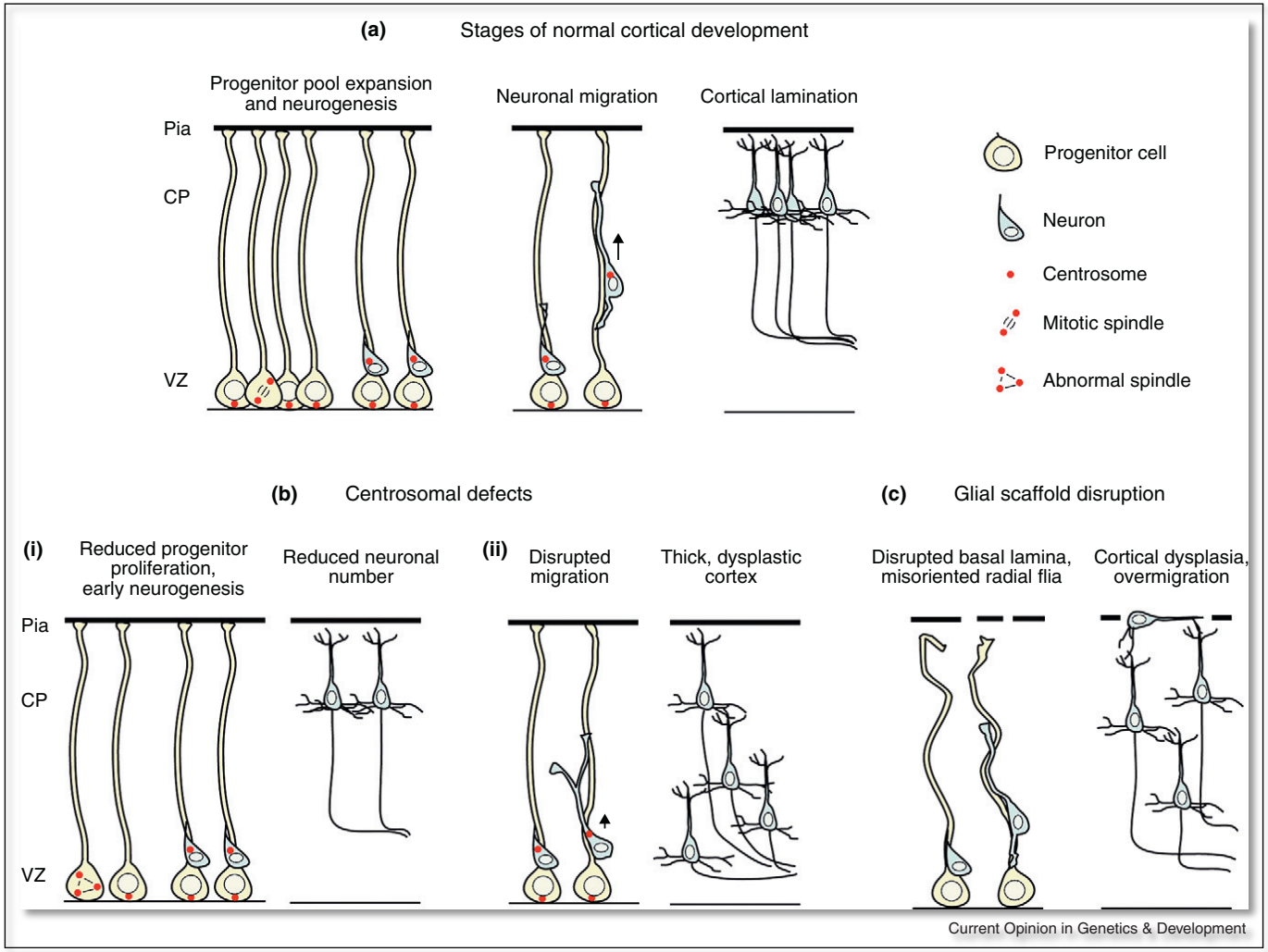
Romero et al., 2018

Tubulinopathies affect multiple processes in cortical development and cause heterogeneous MCDs



Jurik-Sekhar & Hevner et al., 2019

Cellular mechanisms of abnormal cortical development leading to malformations



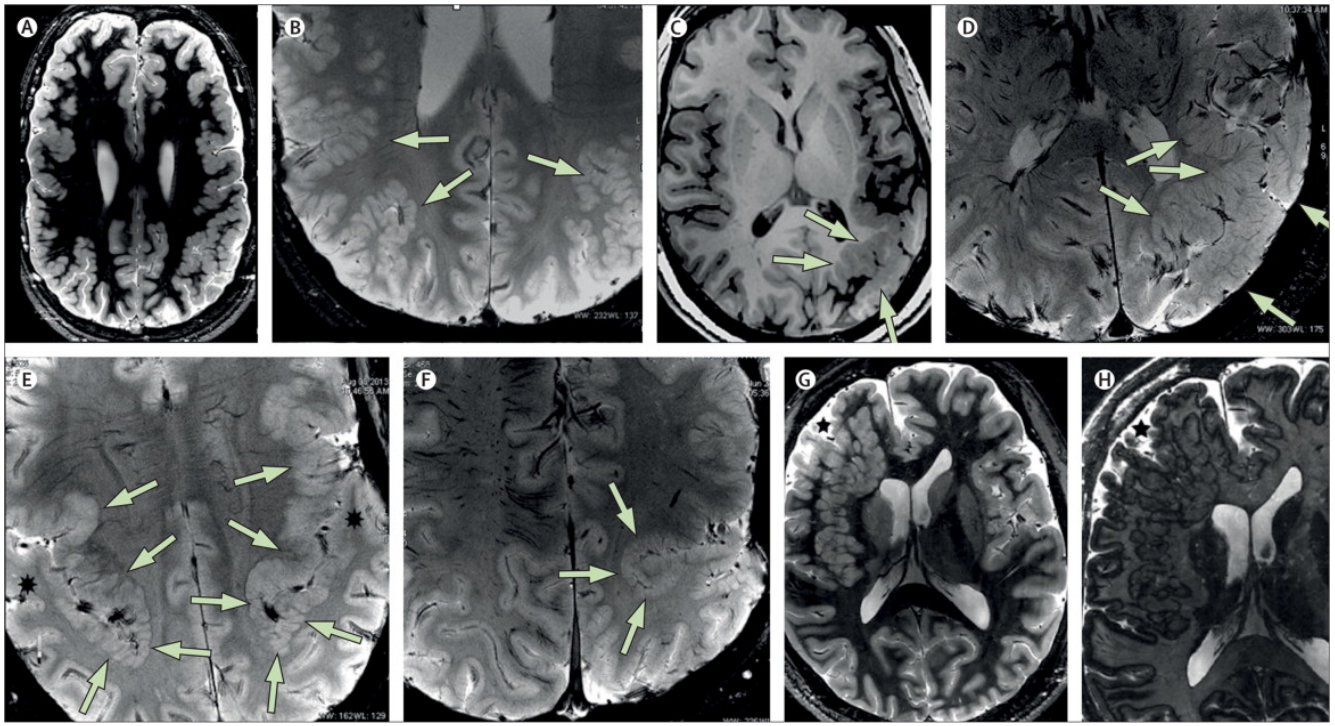
Manzini & Walsh, 2011

MCD group	MCD type	Morphologies	Related pathways
Disorders of proliferation, apoptosis, and/or differentiation	Microcephalies	Microcephaly, microlissencephaly Alobar, lobar, and variant holoprosencephaly	Tubulinopathies, microtubule-associated proteins Decreased RTK → PI3K → AKT → mTOR signaling Sonic hedgehog pathway Midline differentiation
	Cortical overgrowth disorders (focal and diffuse)	Megalencephaly, hemimegalencephaly, polymicrogyria, FCD-II	Overactive RTK → PI3K → AKT → mTOR signaling
Disorders of neuronal migration	Classic lissencephaly spectrum	Smooth lissencephaly, microlissencephaly, subcortical band heterotopia	Tubulinopathies, microtubule-associated proteins Variant lissencephalies (noncytoskeletal)
	Cobblestone malformations	Rough lissencephaly, polymicrogyria, leptomeningeal glioneuronal heterotopia	Dystroglycanopathies Other basement membrane–glia limitans interaction disorders
	Periventricular heterotopia	Nodular or linear periventricular heterotopia	Microtubule-associated proteins
	Dyslamination without cytologic dysplasia or growth abnormality	FCD-I	Overactive RTK → PI3K → AKT → mTOR signaling Other rare forms (e.g., variant Rett syndrome)
Disorders of axon pathway formation	Isolated callosal defects	Agenesis, hypogenesis, dysgenesis of corpus callosum	Axon growth and guidance Midline differentiation
	Other isolated axon defects (putative)	Unknown	Axon growth and guidance

Abbreviations: FCD-I, focal cortical dysplasia type I; FCD-II, focal cortical dysplasia type II; MCD, malformation of cortical development.

Hevner et al., 2019

Different morphological aspects of polymicrogyria



Thick and overfolded brain (small gyri and sulci)

- GPR56 (adhesion G-protein-coupled receptor) regulates pial basement membrane integrity and cortical lamination
- Growth factor signaling pathways (PTEN-AKT cascade)

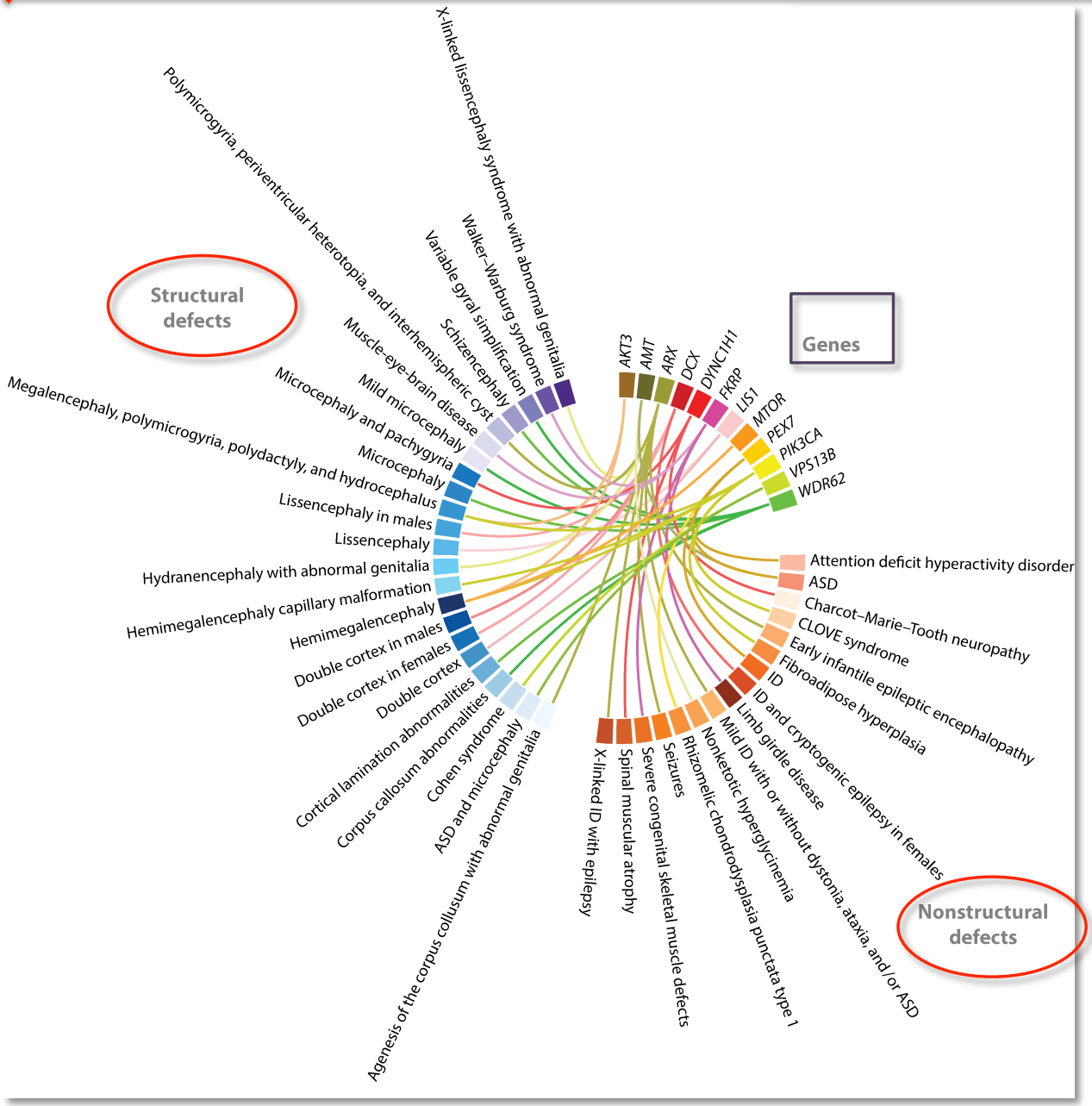
Guerrini & Dobyns, Lancet Neurol, 2014

Tools for the Identification and Characterization of Genetic Changes in the Human Brain

Tool	Advantages	Disadvantages
Whole-exome sequencing	Efficient sequencing of all the protein-coding genes (~ 180,000 exons) in the human genome.	Unable to identify structural and noncoding variants, although there are some tools to detect copy number variations from exome data.
Whole-genome sequencing	Sequencing of the entire genome of an individual.	The function of the majority of the human genome is incompletely understood. Thus, much sequencing data are often difficult to interpret. Also, whole-genome sequencing is costly, at least for now.
Single-cell sequencing	Genome and transcriptome sequencing of individual cells detects cell-to-cell variability.	Requires amplification of the limited DNA and RNA in a single cell, which can introduce errors.
RNA sequencing	Reveals how each protein-coding gene or RNA gene is utilized in a given cellular context.	Detection of genes with low expression levels is difficult. Multiple cell types can confound the interpretation.
Chromosome conformation capture	Reveals chromosomal interactions influencing gene expression, such as interaction between an enhancer and a promoter of a protein-coding gene.	Can be costly due to depth of sequencing needed, depending on method. Requires a great number of cells; multiple cell types can generate noise and confound the interpretation, although single-cell chromosome conformation capture was recently developed.

From Bae et al., Dev Cell, 2015

Complexity of neurodevelopmental disorders



Circle plot illustrating the allelic and phenotypic diversity of a subset of neurodevelopmental disease genes (due to hypomorphic and somatic mutations).

Hu et al., 2014

Animal models for human diseases

Naturally occurring or experimentally induced animal diseases
with pathological processes sufficiently similar
to those of human diseases

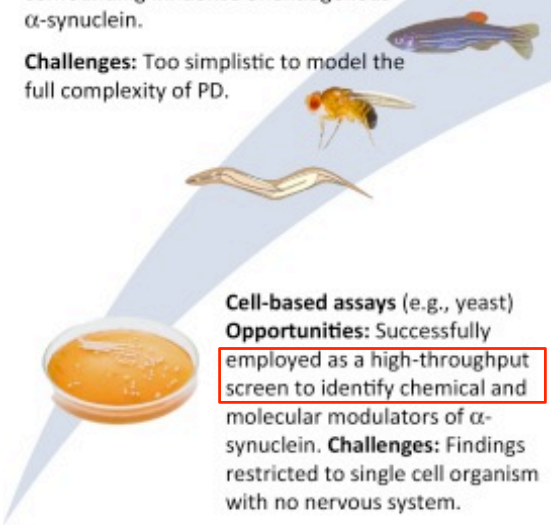
Opportunities and challenges in animal models (example for Parkinson disease)

Future potential: Large scale clinical trials in individuals with early-stage or prodromal Parkinson's disease can be performed testing efficacy of the most promising candidates emerging from pre-clinical screening of disease modifying agents.

Rodent models of prion-like propagation of α-synuclein. Opportunities: Allow the testing of therapeutics targeting the trafficking of extracellular α-synuclein along neuroanatomical pathways in an accelerated timeframe not normally available within the lifespan of a laboratory animal. **Challenges:** Require application of supraphysiological quantities of α-synuclein to initiate pathology.

Transgenic *C.elegans, Drosophila* and fish (zebrafish and medaka fish).
Opportunities: Multicellular organisms with well-characterized nervous systems, allowing economical study of mechanisms of α-synuclein toxicity, and testing of potential anti-aggregation therapeutics, without the confounding influence of endogenous α-synuclein.

Challenges: Too simplistic to model the full complexity of PD.

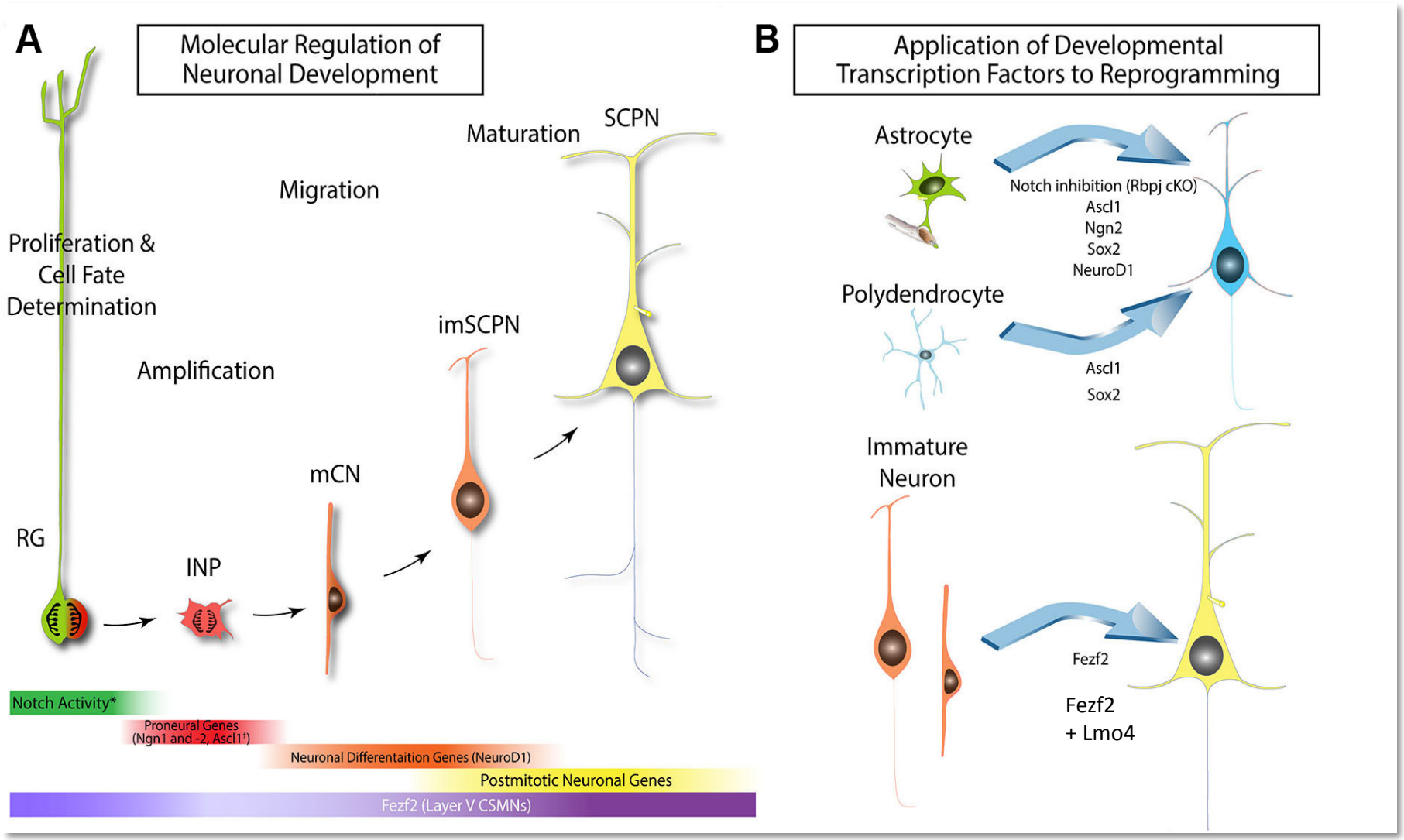


BAC-Tg α-synuclein mice
Opportunities: Mimic the normal spatiotemporal expression of α-synuclein and develop synucleinopathy and a decline in motor function. Backbone to test therapeutics aimed at preventing toxins or misfolded α-synuclein in driving synucleinopathy along clinically relevant pathways. **Challenges:** Pathology and behaviour only develop in some lines and at an advanced age.

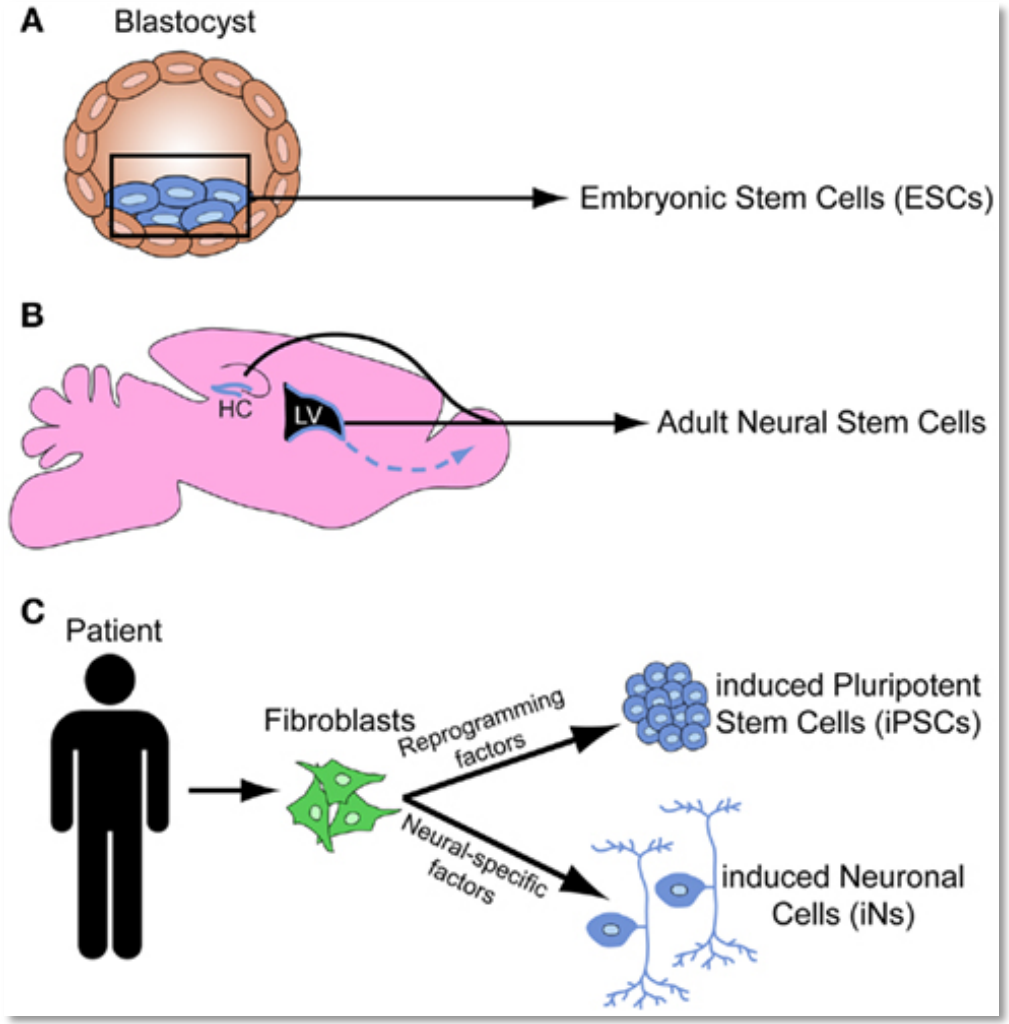
AAV α-synuclein overexpression in rodents and non-human primates. Opportunities: New generation of AAV allow high level, sustained delivery of neuron specific α-synuclein, expression with development of synucleinopathy, dopaminergic degeneration and parkinsonian behaviour in rodents in a timeframe amenable to testing novel therapeutics. **Challenges:** Ongoing studies are attempting to translate the use of new generation AAV vectors in non-human primates, though characterization and validation of such models remain outstanding.

Cell-based assays (e.g., yeast)
Opportunities: Successfully employed as a high-throughput screen to identify chemical and molecular modulators of α-synuclein. **Challenges:** Findings restricted to single cell organism with no nervous system.

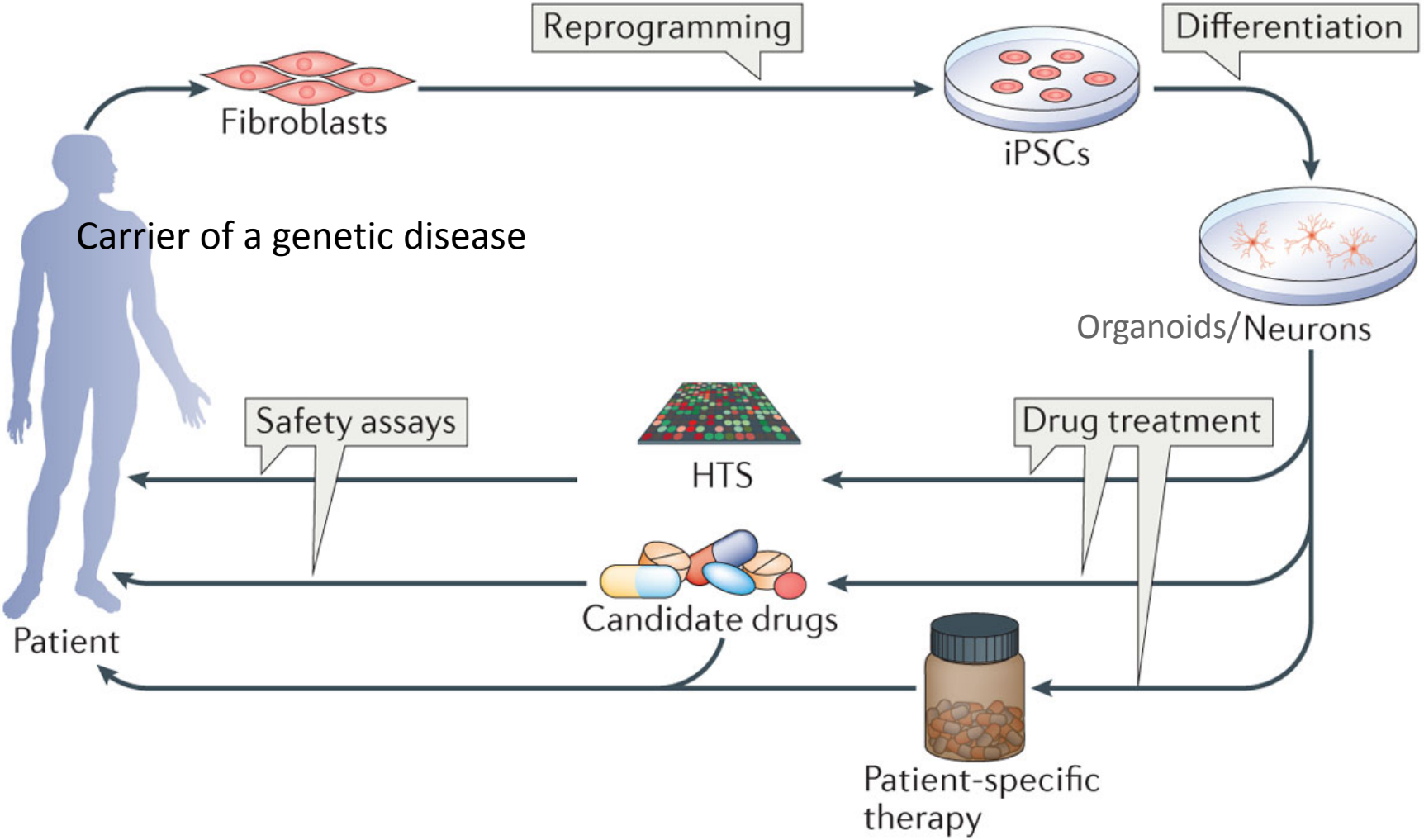
Learning from and for development: using the knowledge of cortical development for generating cortical neurons in vitro



Learning from and for development: using the knowledge of cortical development for generating cortical neurons in vitro



Regenerative medicine in clinics and industry

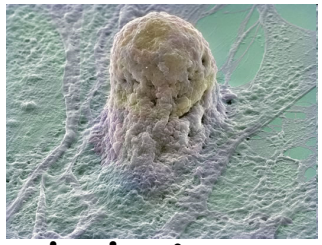


Nature Reviews | Molecular Cell Biology

Indirect and direct lineage reprogramming to create patient-derived neural cells

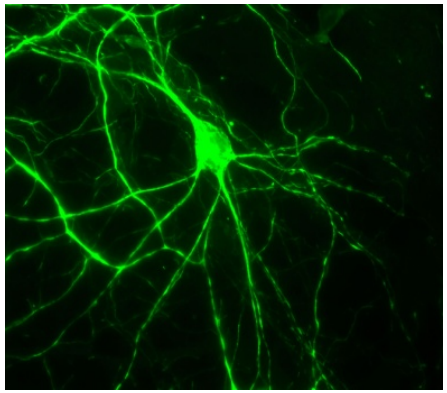
patient skin samples

Reprogramming genes



Induced pluripotent stem cells (iPSC)

Differentiate

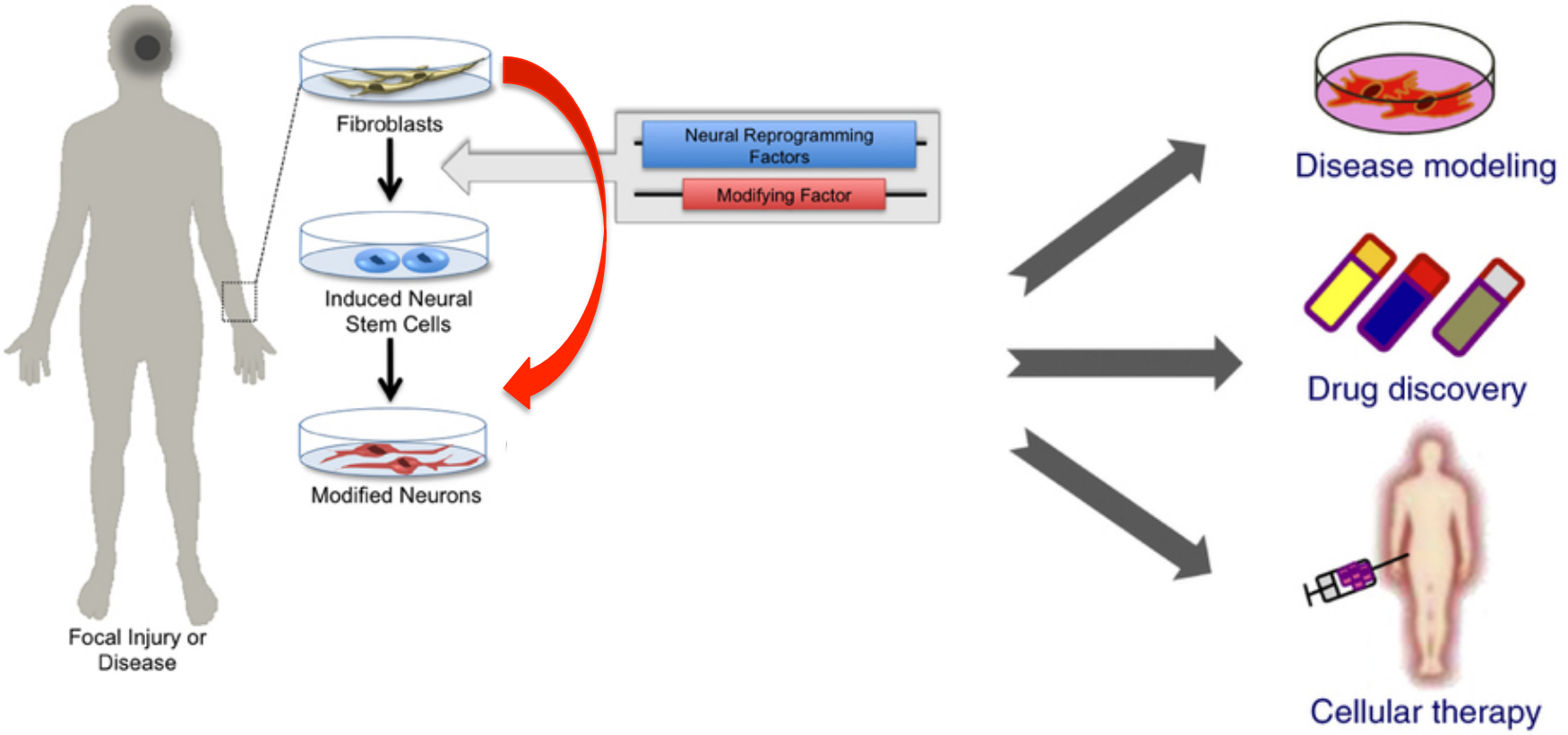


patient-derived neurons

Direct conversion

Induced Neuronal (iN) cells

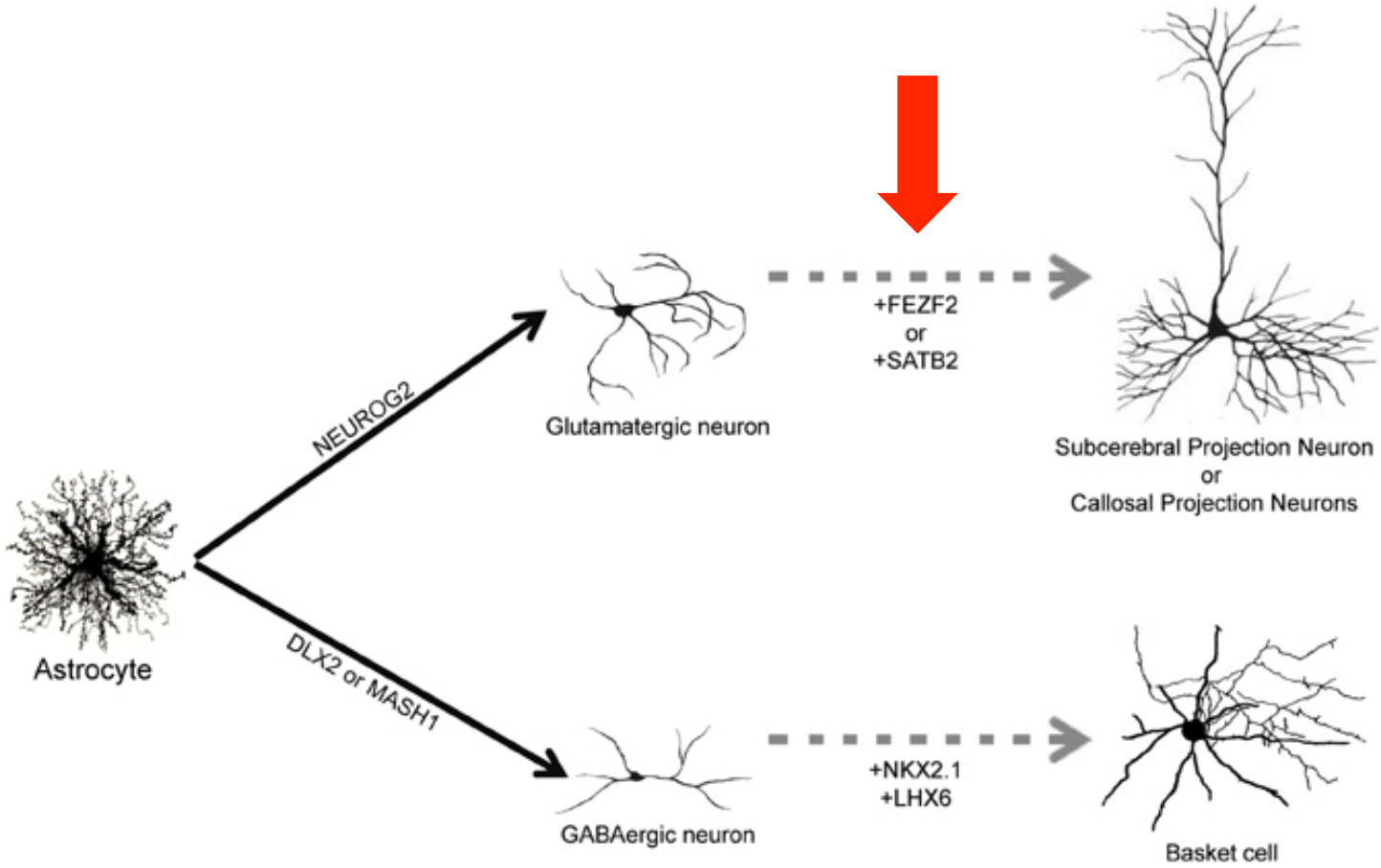
Somatic cell reprogramming: potential applications



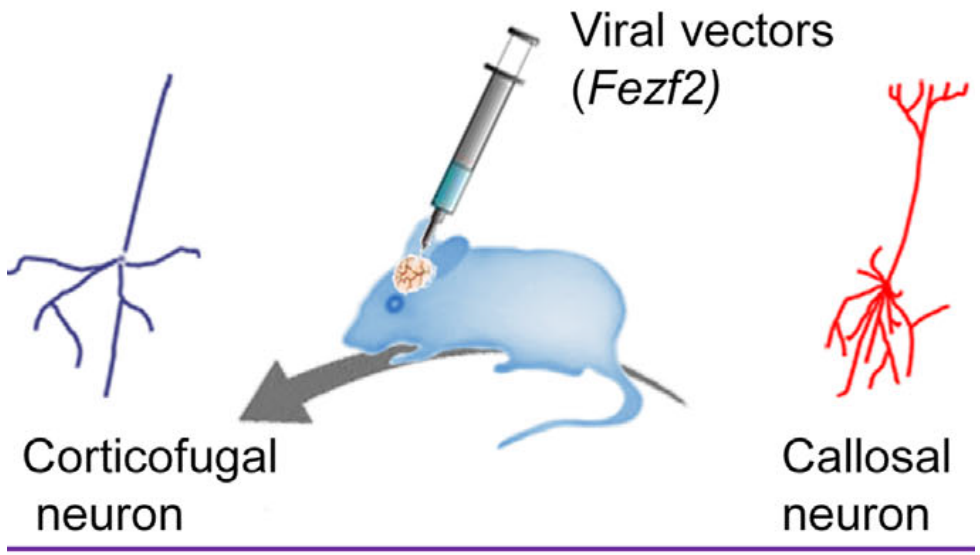
ADVANTAGES of Direct Conversion:

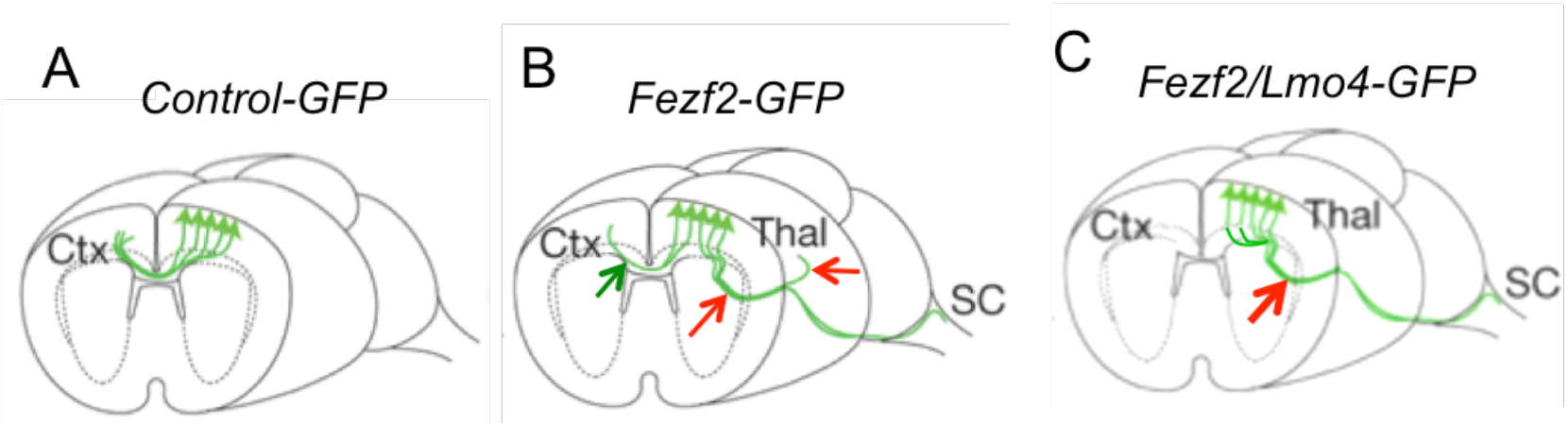
1. Faster than iPS method
2. Epigenetic signature of patient cell is likely preserved
3. Specific Neuronal subtype generation (Spinal Motor, Dopaminergic)

Somatic cell direct reprogramming in the brain



In vivo reprogramming in postmitotic neurons during prenatal stages

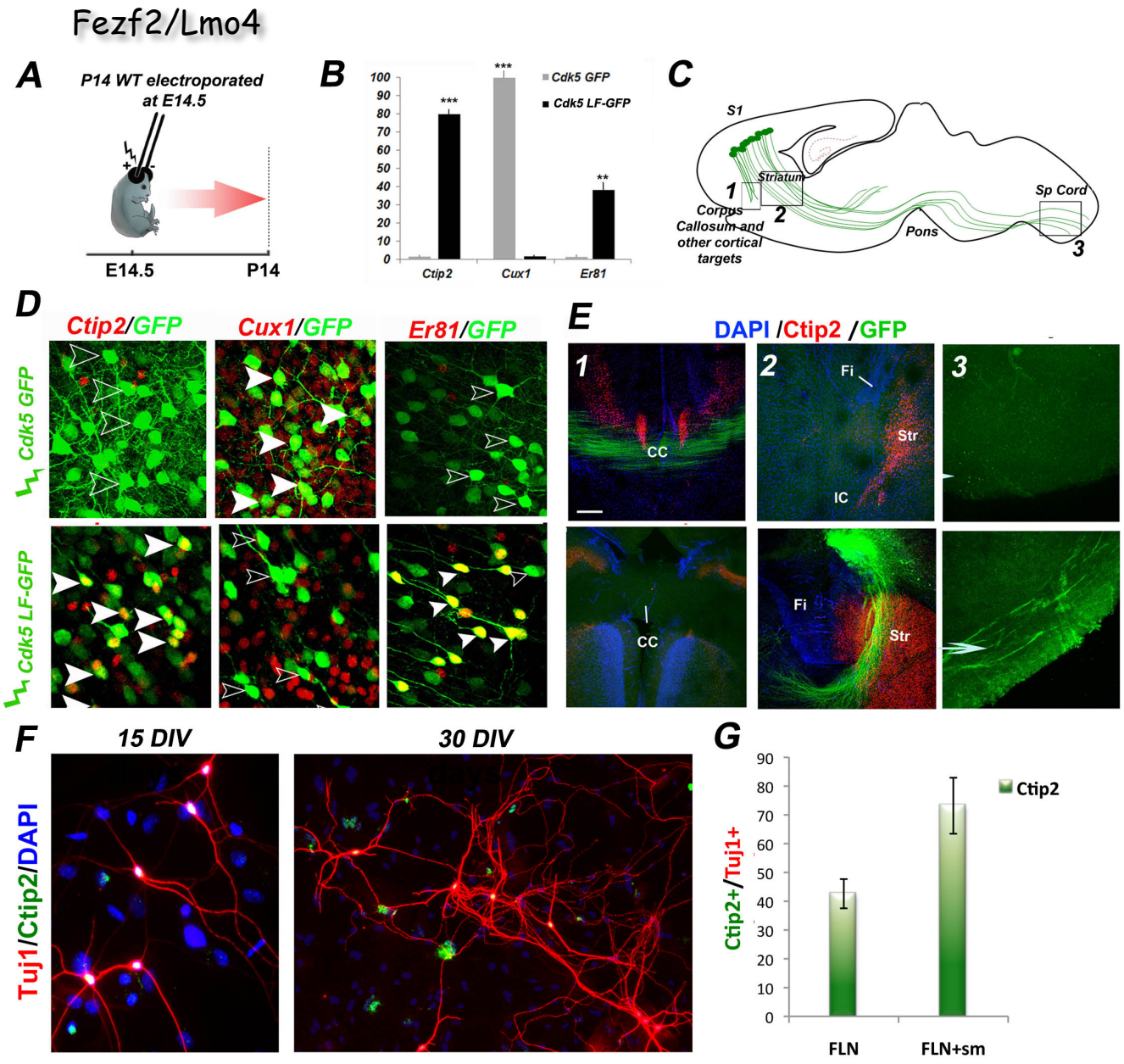




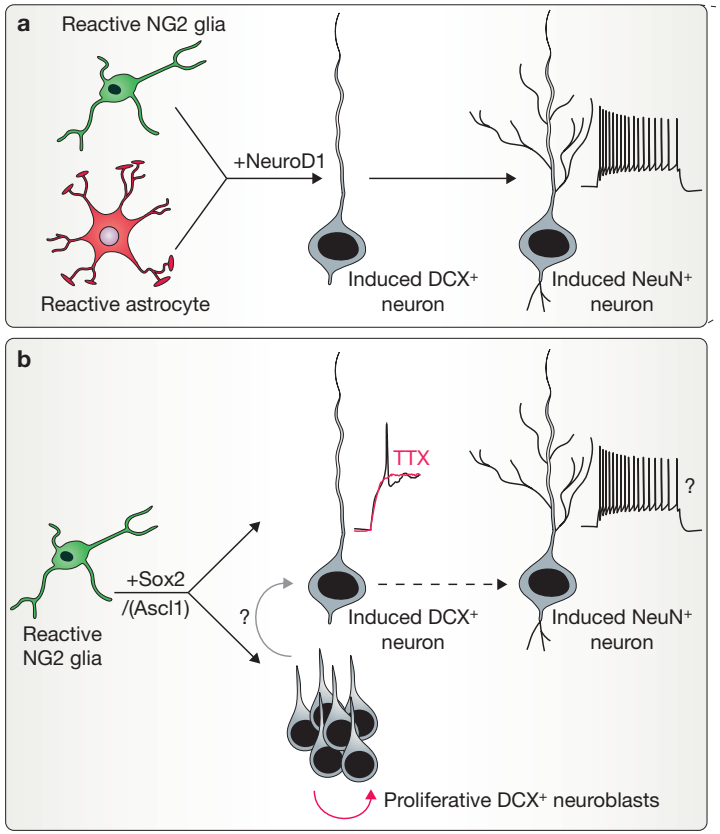
Published
(Arlotta & Jabaudon teams)

Unpublished (Studer lab)

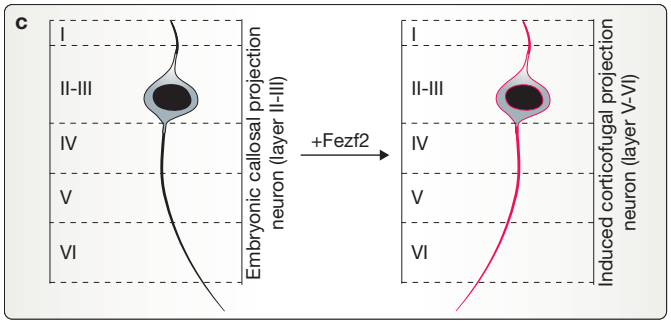
Improving the efficacy of in vivo neuron-to-neuron conversion



Glia-to-neuron reprogramming in the postnatal brain

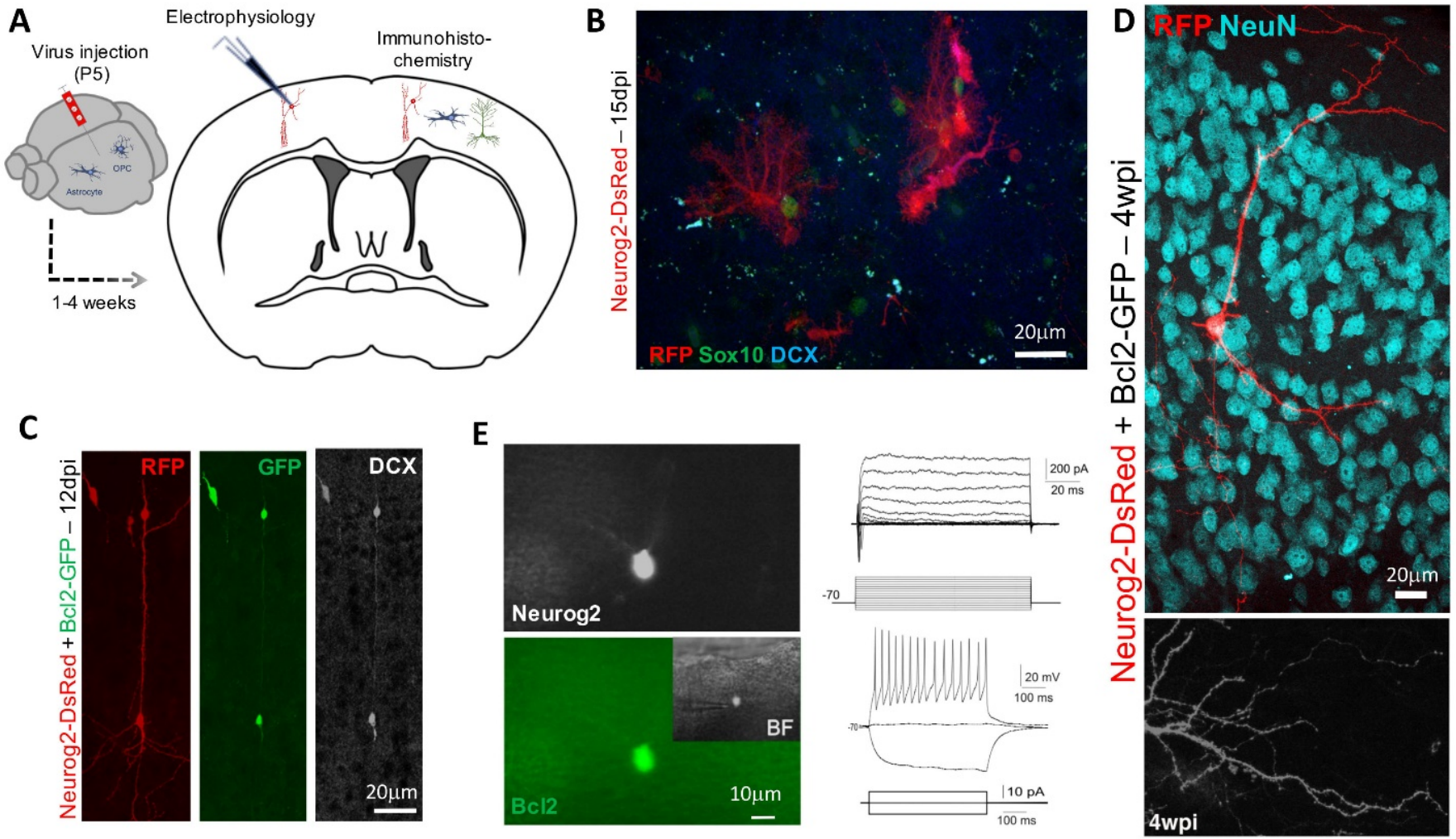


Glia to LV reprogramming?



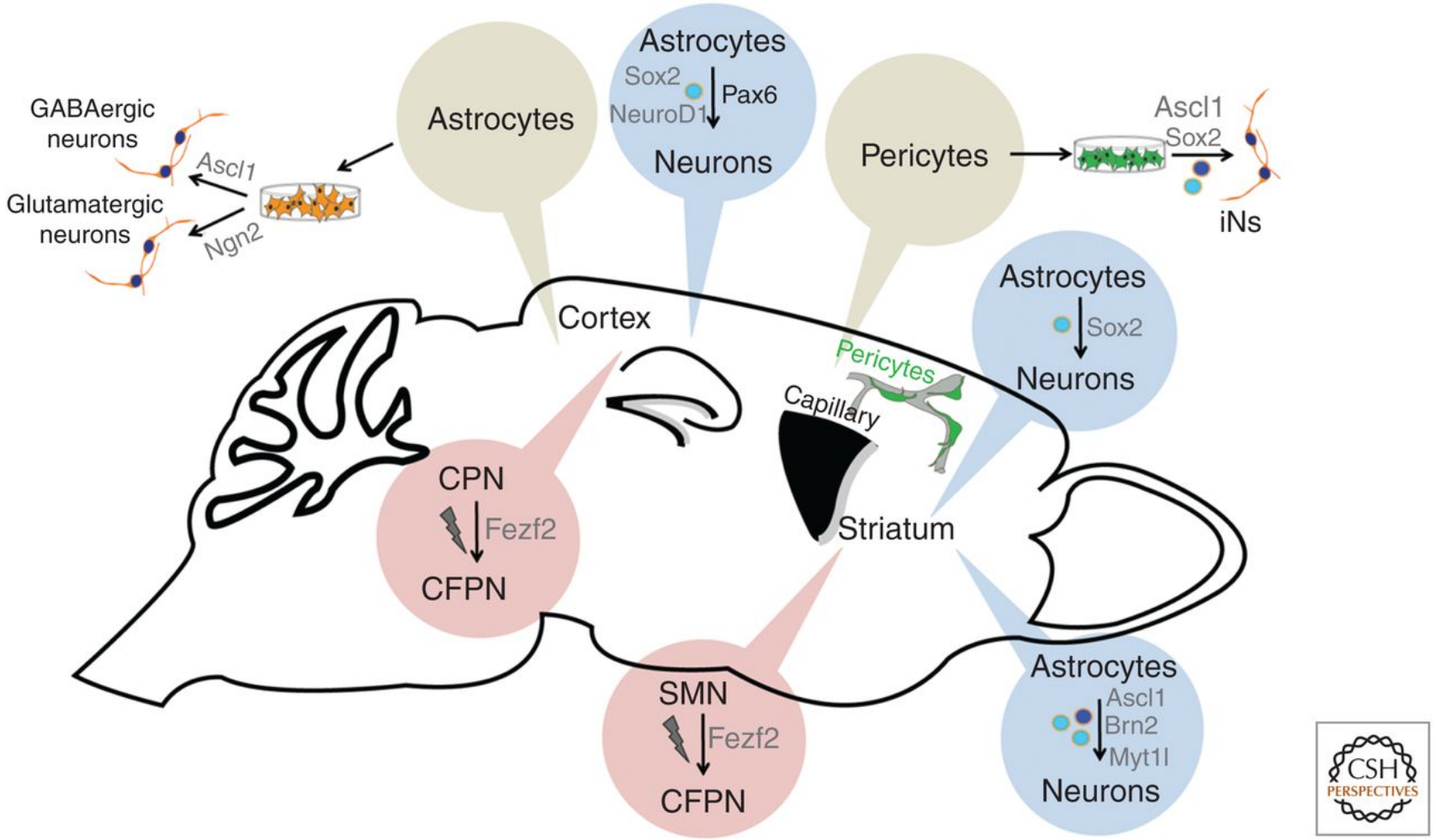
→ Fezf2 + Lmo4

Glia-to-neuron reprogramming in the postnatal brain



Benedikt B., unpublished

Engineering neurogenesis outside the classical neurogenic niches

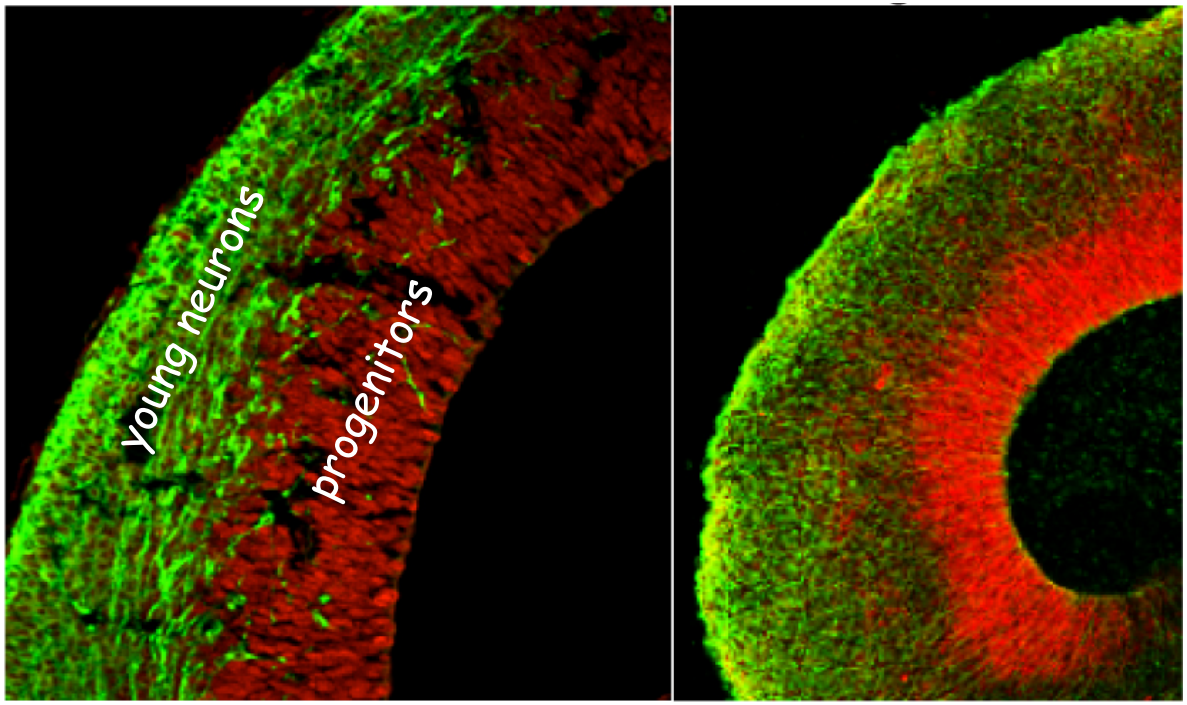


Berninger and Jessberger, 2016

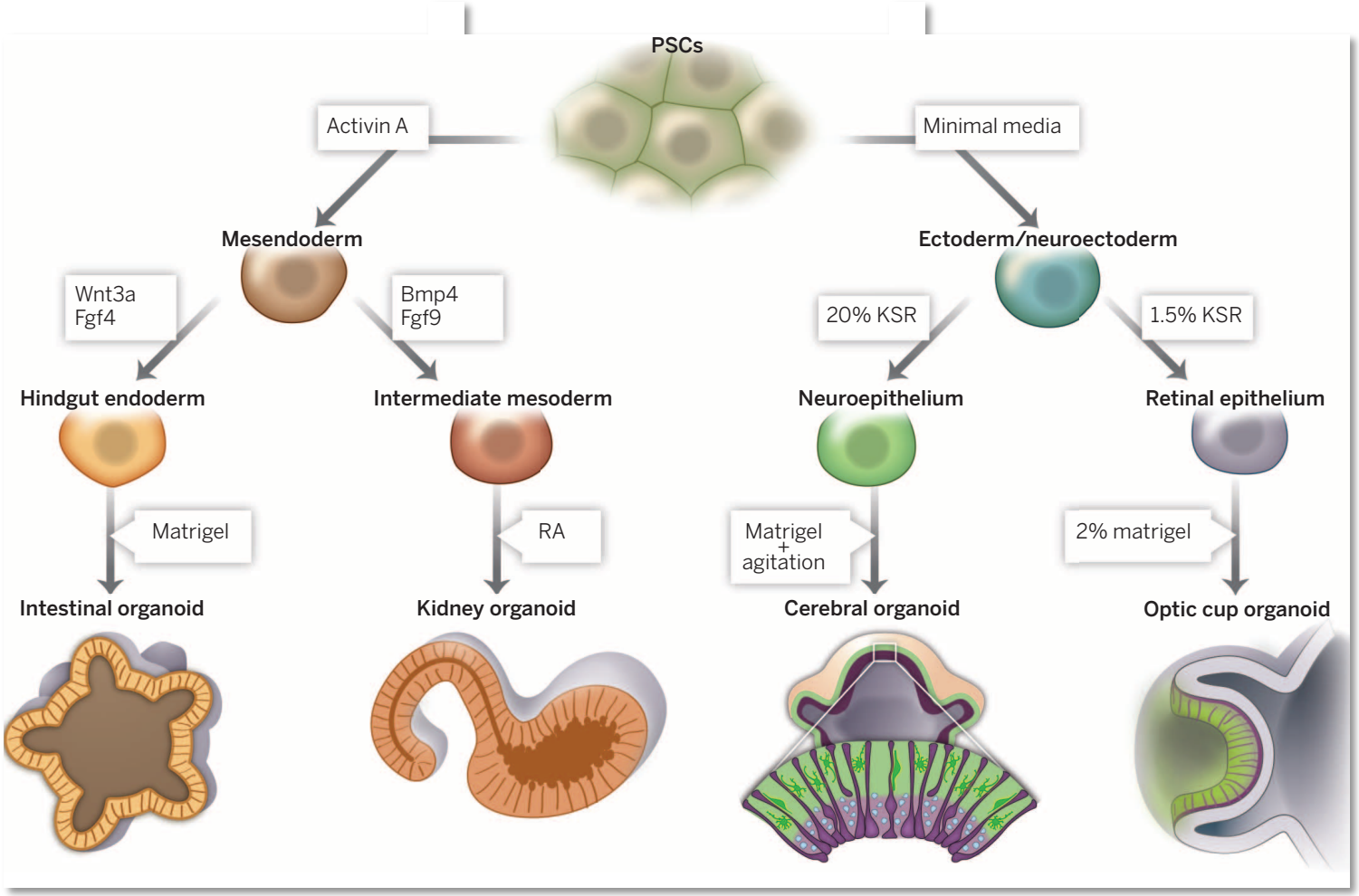
Learning from and for development: using the knowledge of cortical development for reproducing mini-brains in the dish

Embryonic brain

Cerebral organoid or "mini-brain"

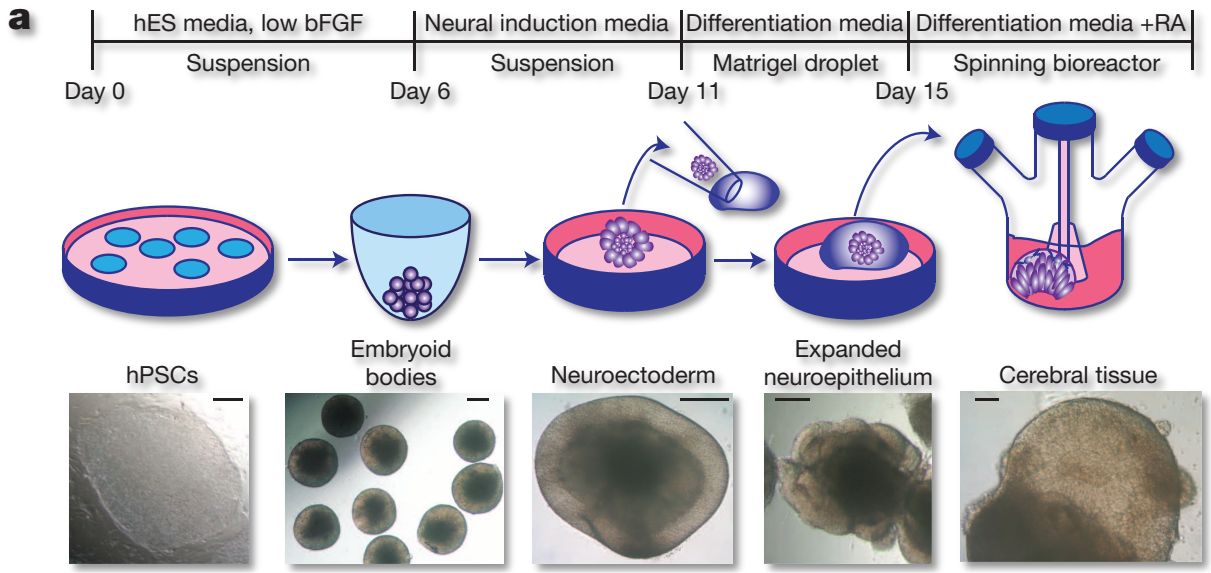


Overview of organoid methodologies



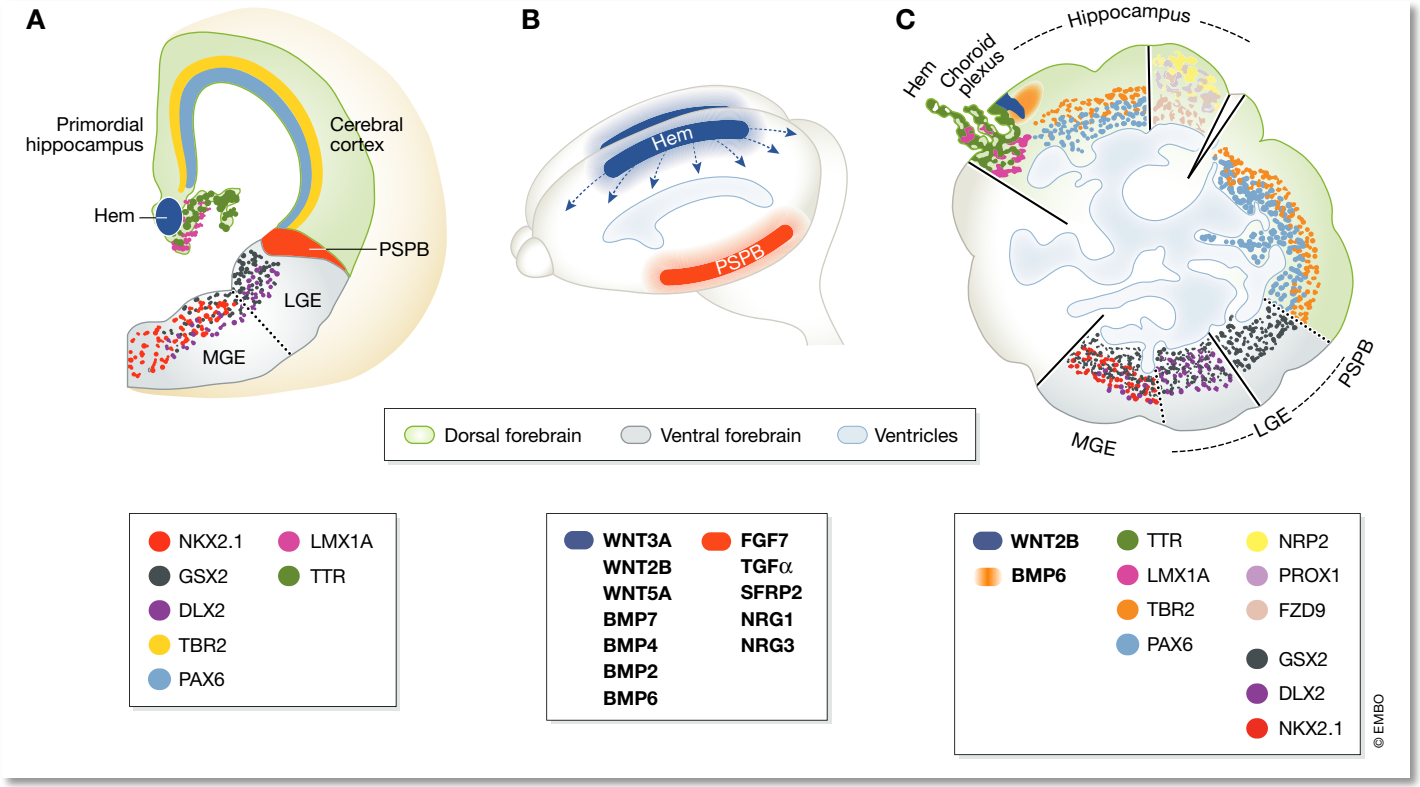
Madeline Lancaster & Juergen Knoblich, Science, 2014

The 3D cerebral organoid culture system



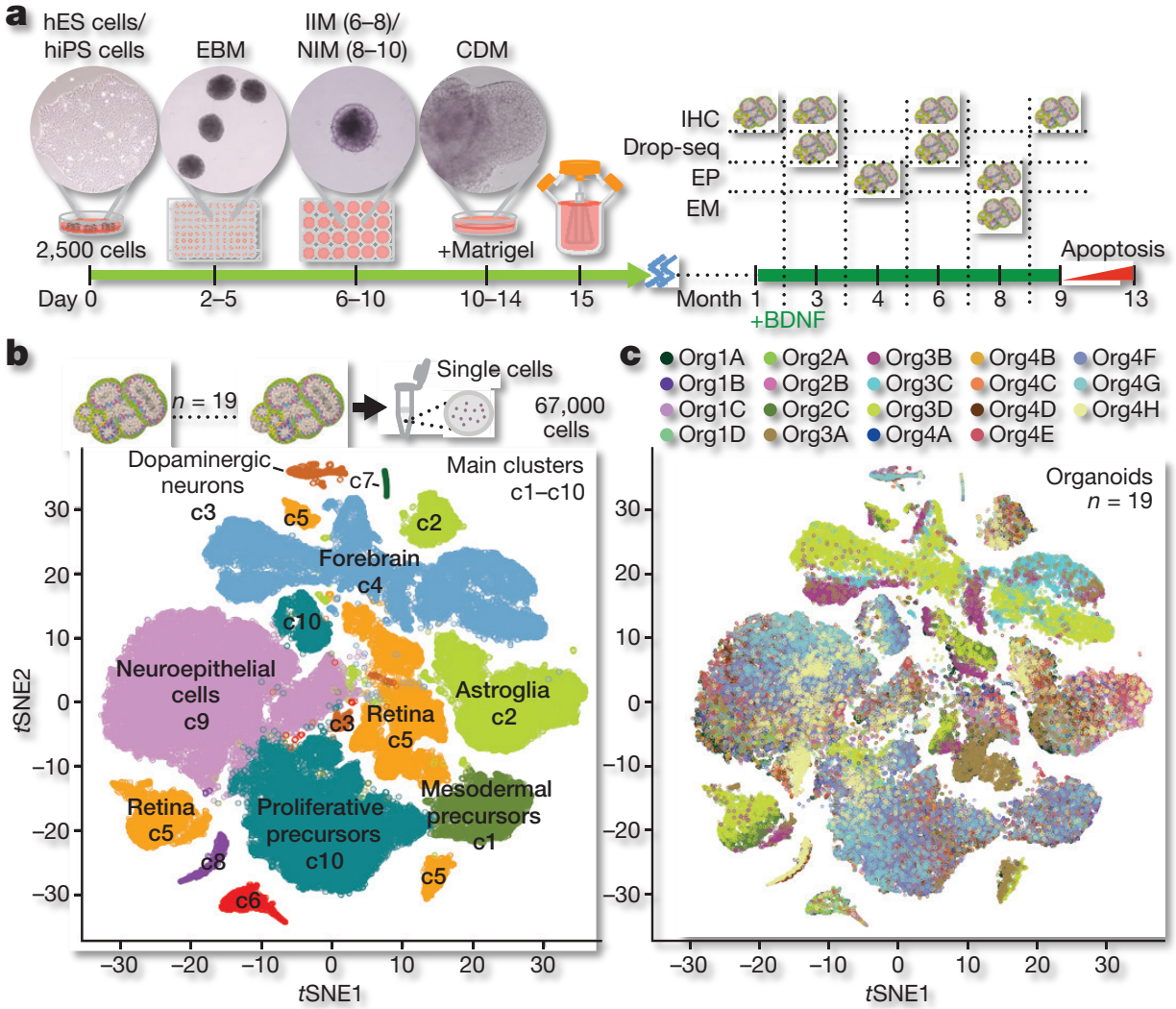
Lancaster M. et al., Nature, 2013

Self-organized developmental regional patterning and differentiation is recapitulated in cerebral organoids



Dias & Guillemot, EMBO J. 2017

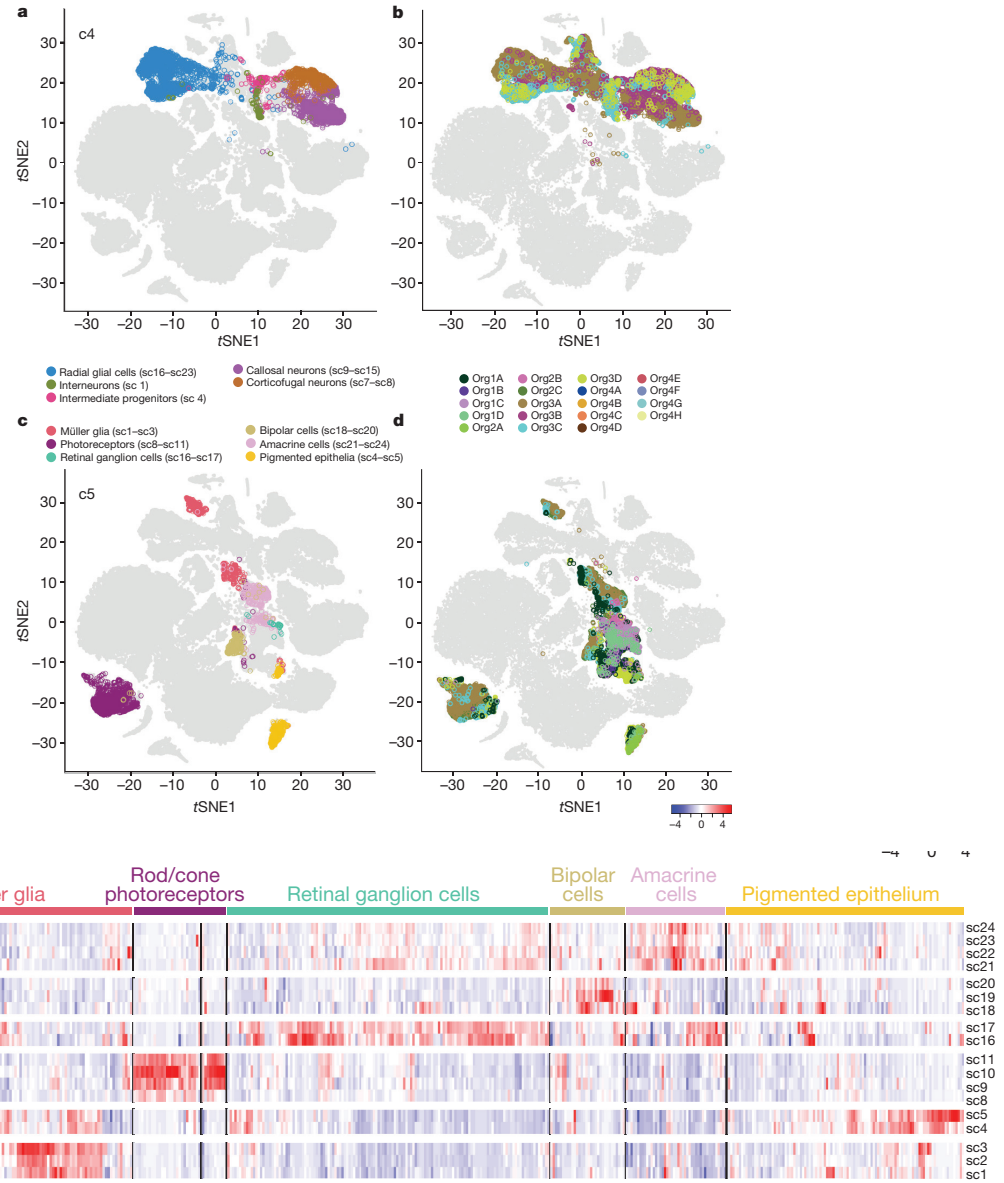
Development of a broad spectrum of cell types in human brain organoids by large-scale, single-cell sequencing



Quadrato et al., 2017

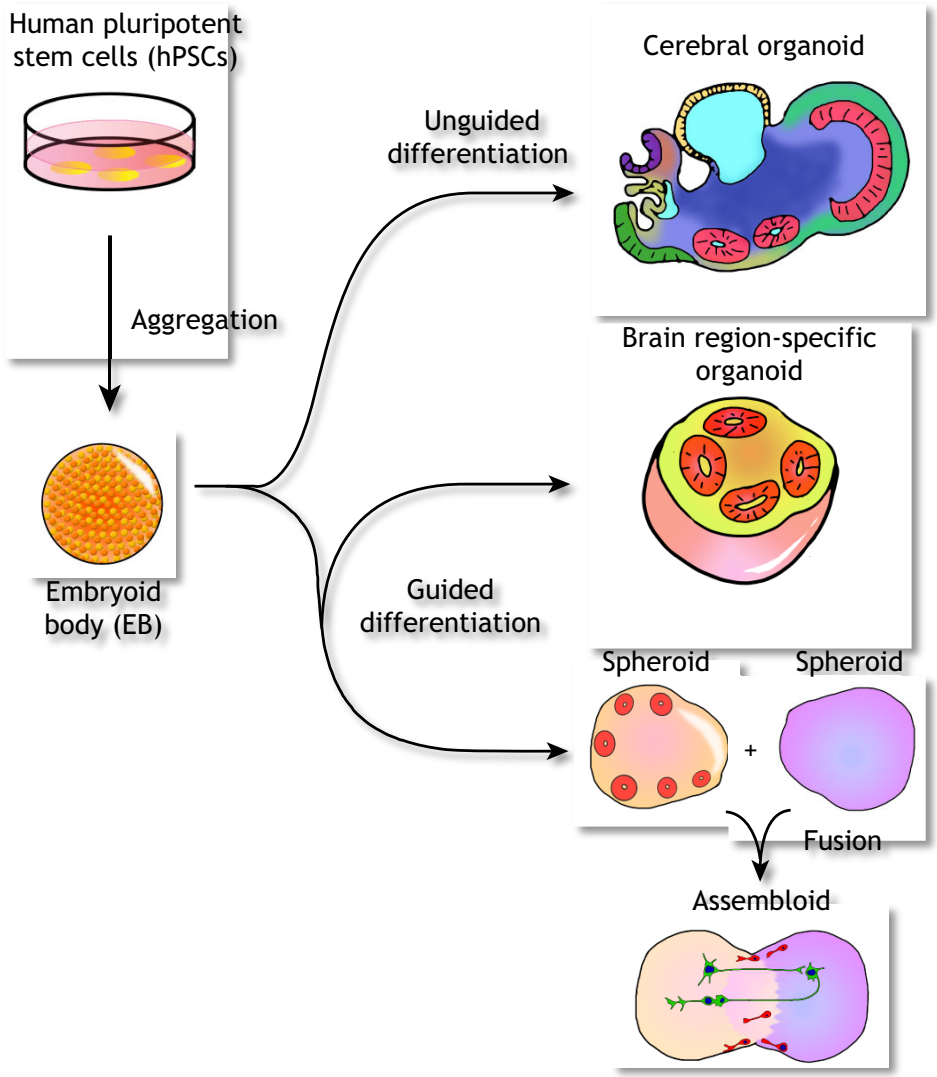
Human brain organoids contain subclasses of forebrain and retinal cells

4 major
forebrain &
6 major retinal
subclustering



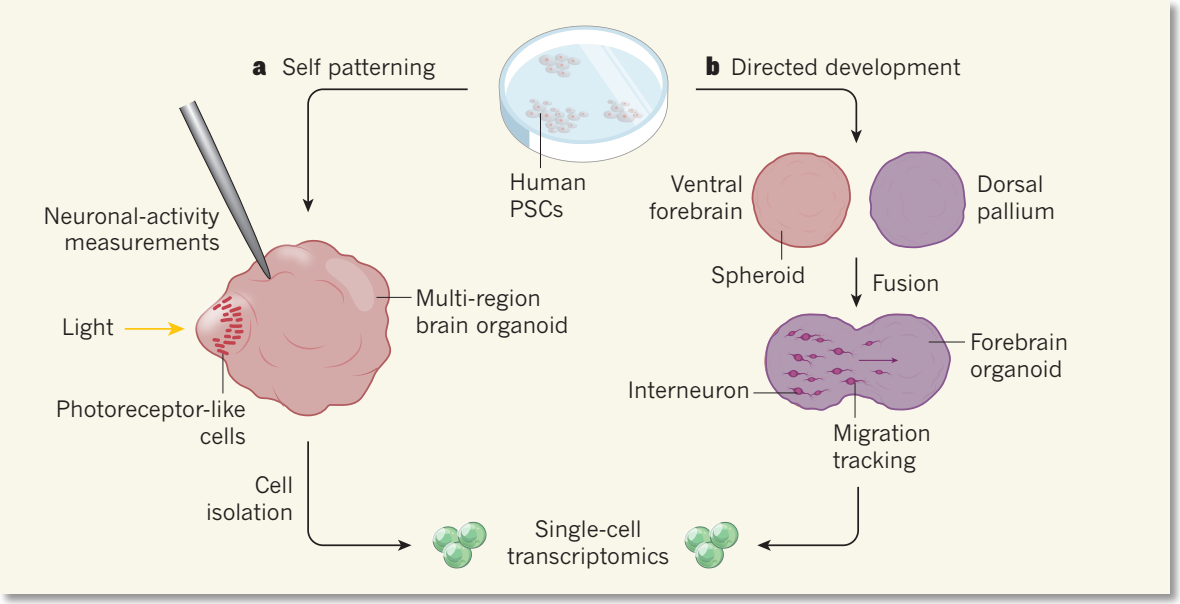
Quadrato et al., 2017

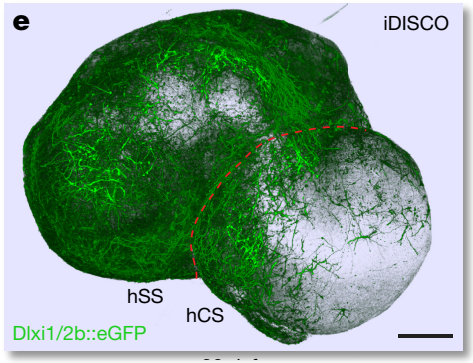
Unguided and guided approaches for making brain organoids



Qian et al., DEV 2019

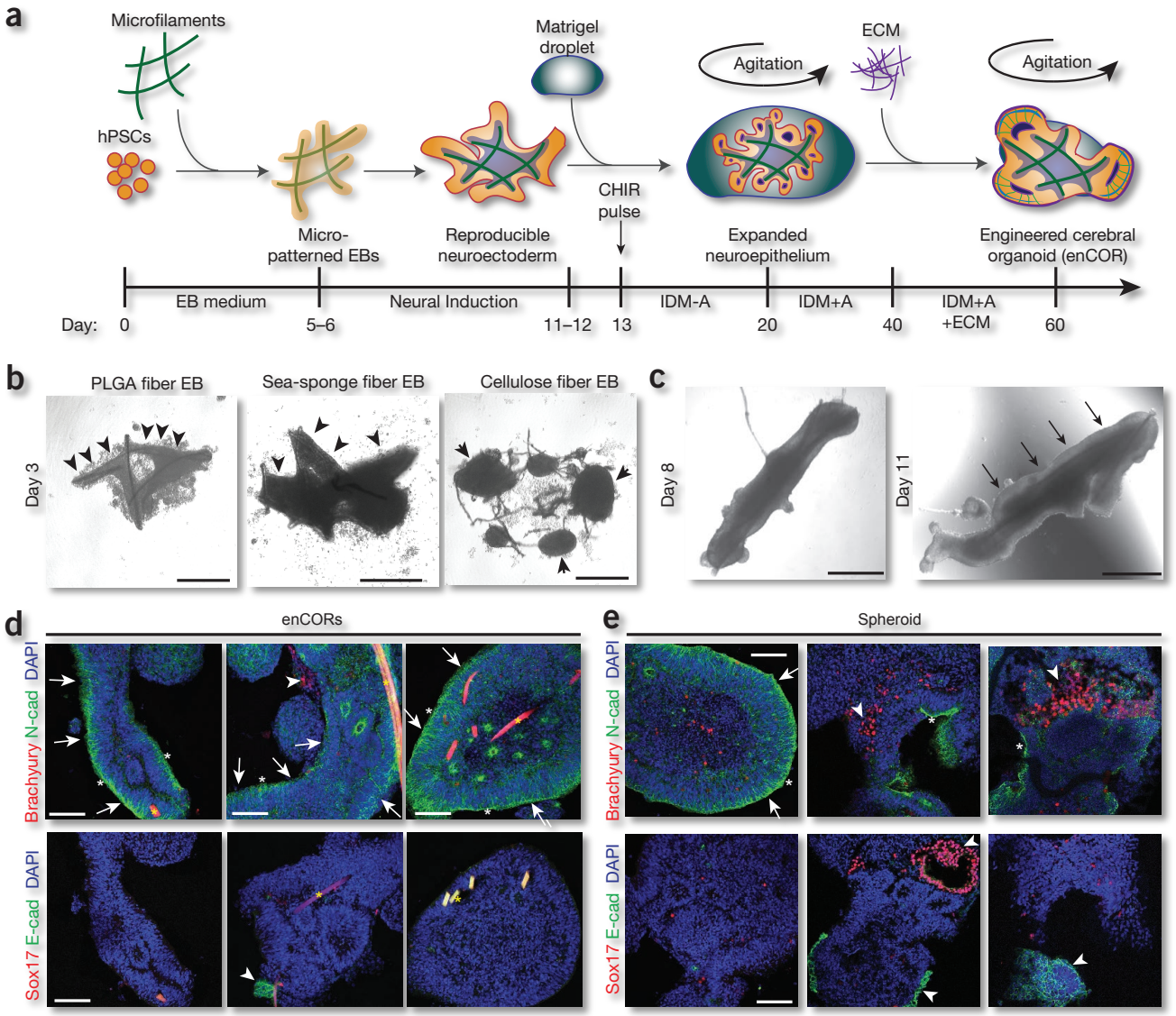
Self-organized organoids versus directed spheroids





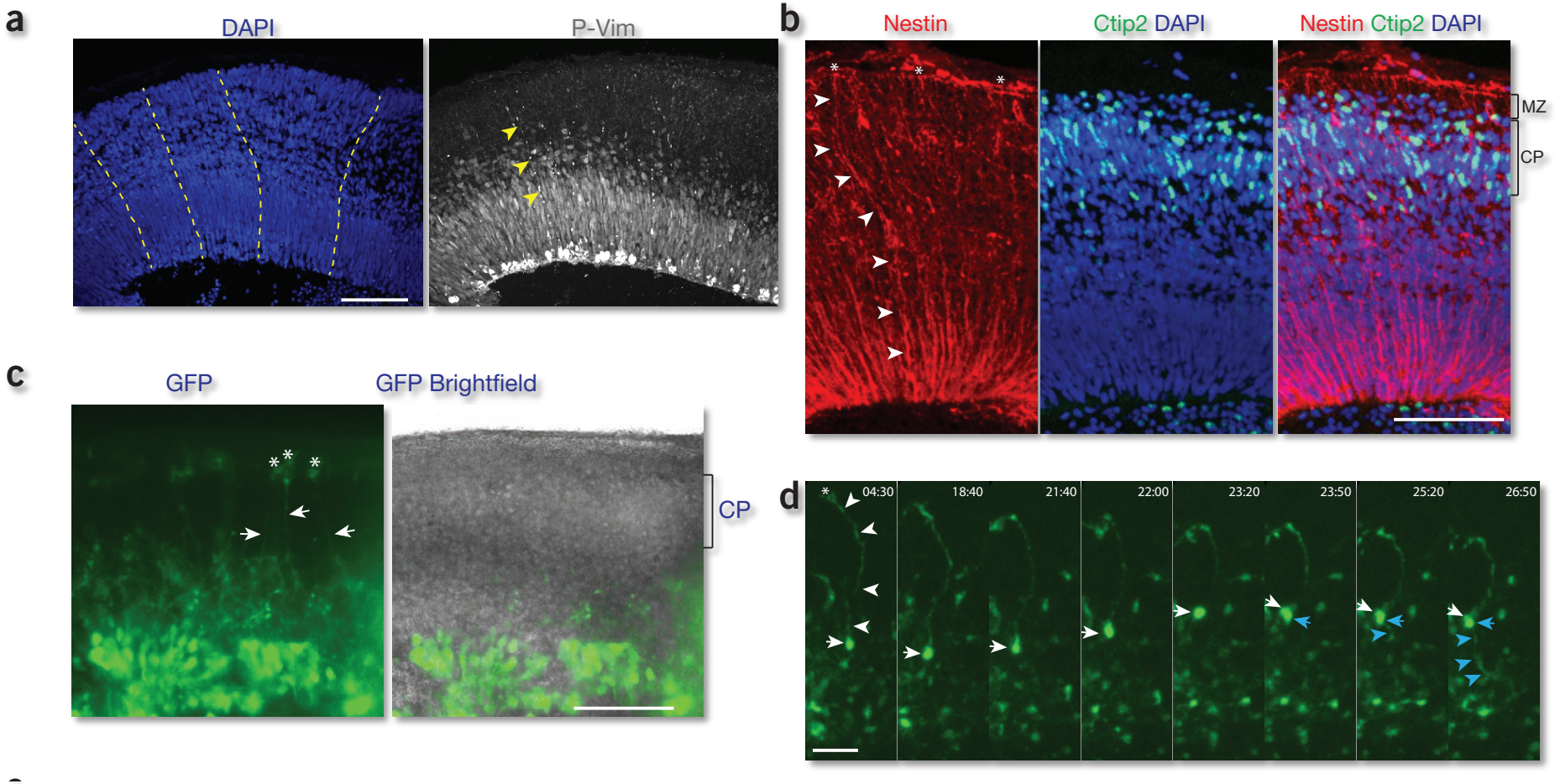
Birey et al., Nature, 2017

Engineered cerebral organoids (enCORs) generate elongated neuroepithelium



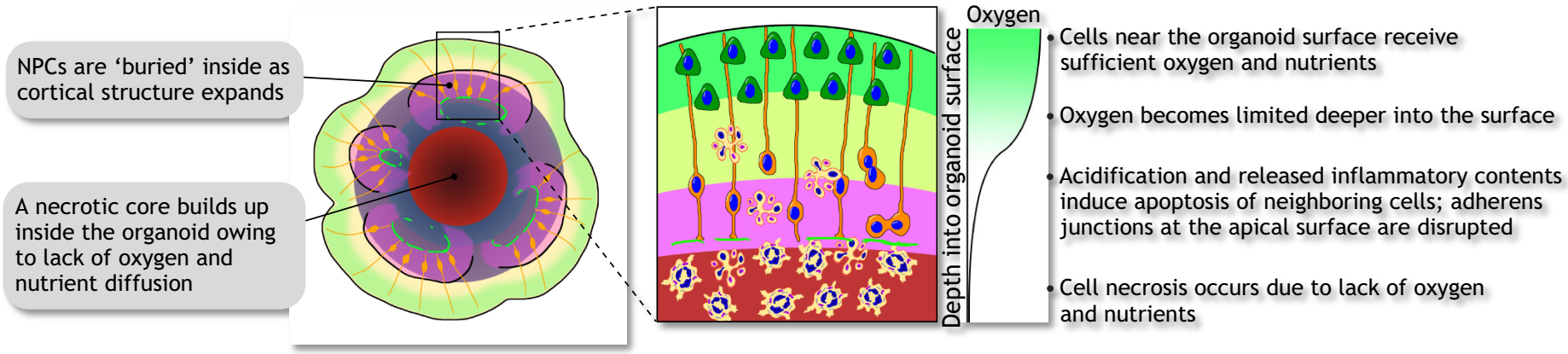
Lancaster et al., 2017

enCORs display radial units and radial neuronal migration



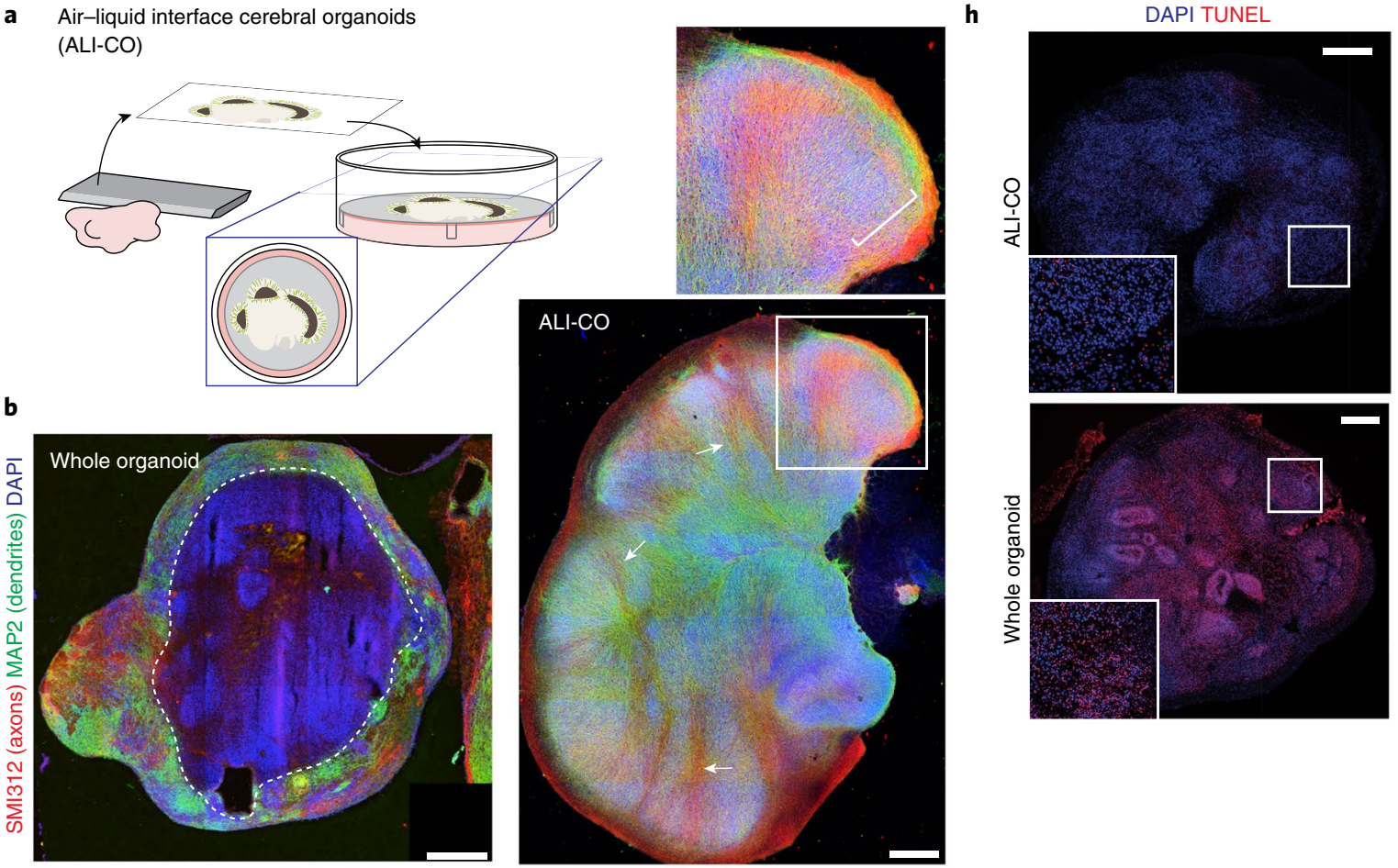
Lancaster et al., 2017

The diffusion limit depletes progenitors and prohibits organoid expansion



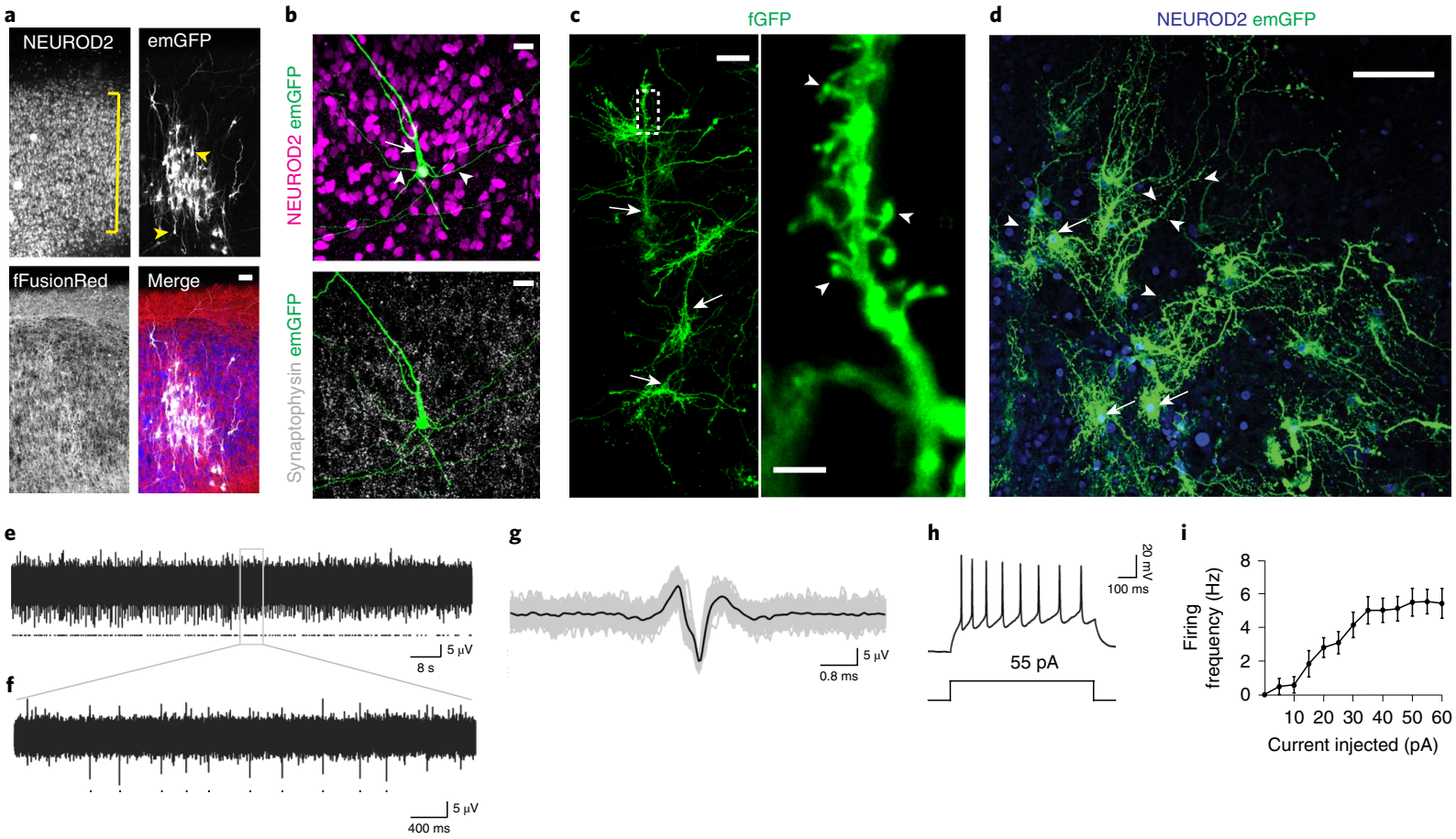
Qian et al., DEV 2019

Air-liquid interface culture (ALI-CO) leads to improved neuronal survival and morphology



Giandomenico et al., 2019

ALI-CO cultures exhibit mature neuronal morphology and function

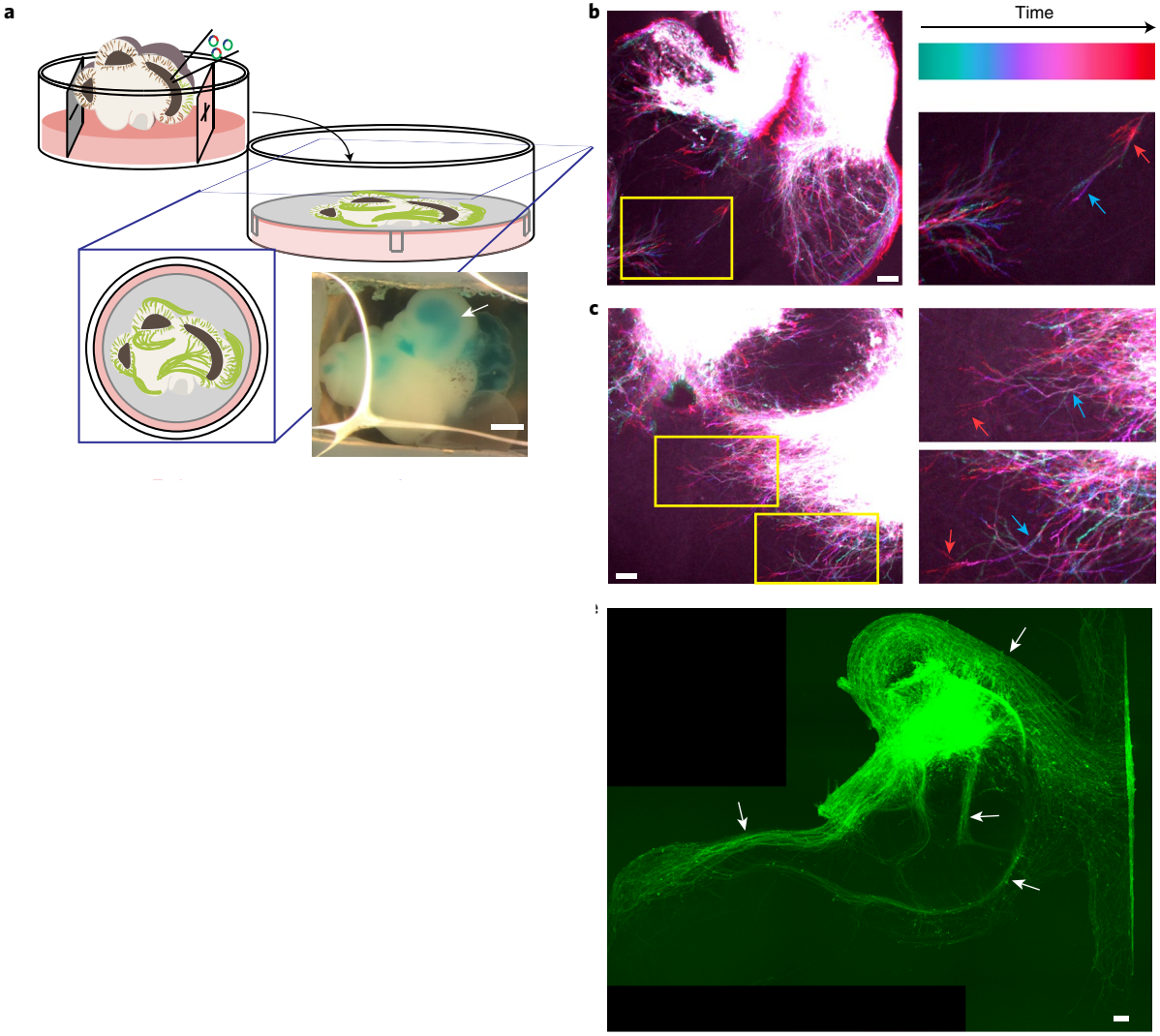


MEA spontaneous activity

Whole-cell patch-clamp AP

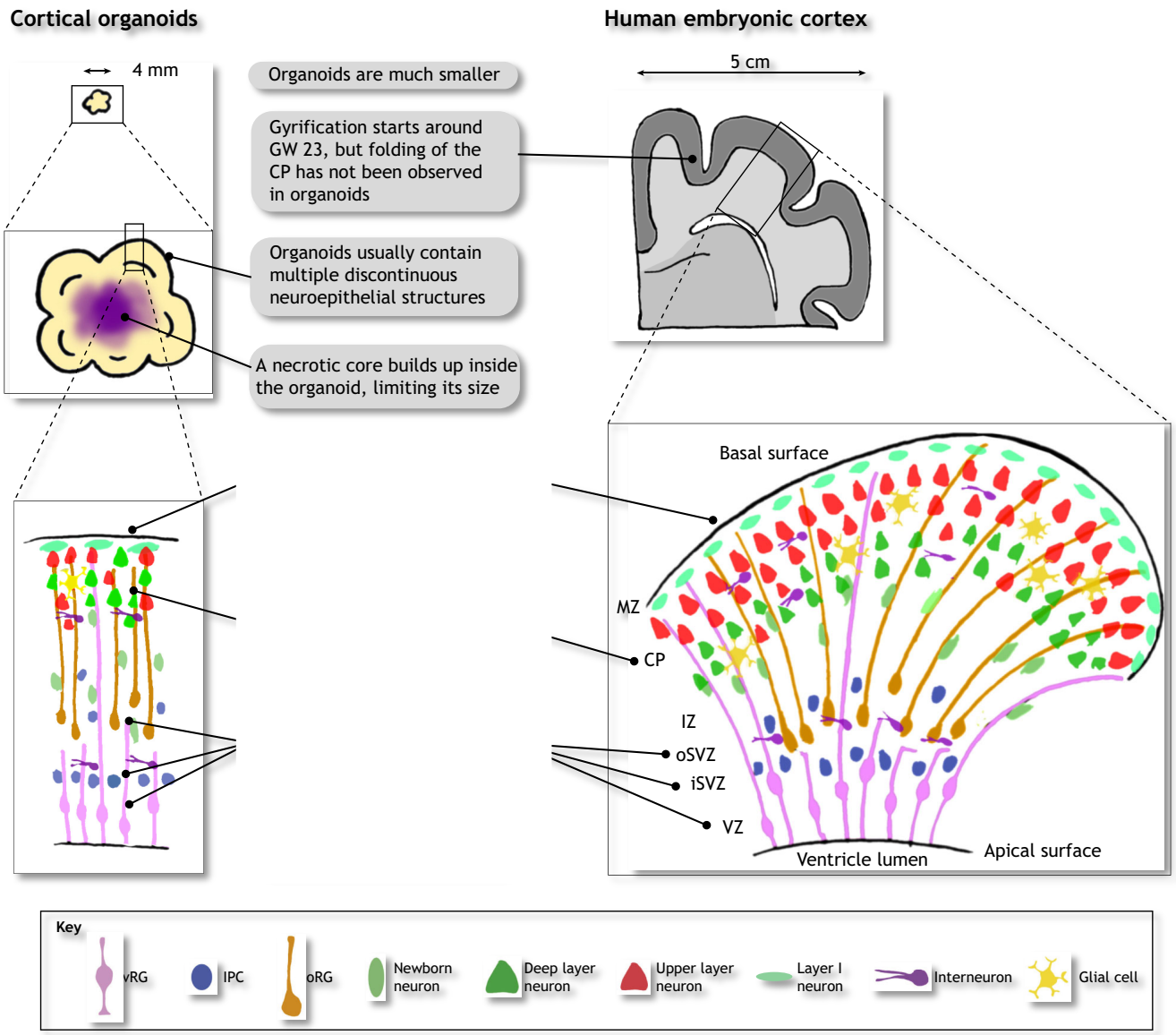
Giandomenico et al., 2019

Neurons of ALI-COs exhibit dynamic axon guidance behaviors



Giandomenico et al., 2019

Structural comparison between cortical organoids and the human embryonic cortex



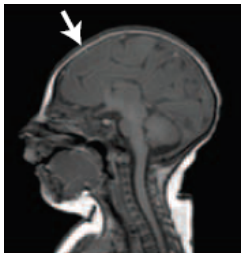
Qian et al., DEV 2019

Different experimental approaches in human disease modeling

A

...AGTCTGACCTGACT...

Patient → reprogramming → IPS cells with patient-specific mutation → «disease» Organoids



IPS cells with patient-specific mutation → Crispr /Cas9 → Mutation «repair» → Rescued Organoids

...AGTCTGTCCTGACT...

B

...AGTCTGTCCTGACT...

ctrl IPS cells → ctrl Organoids

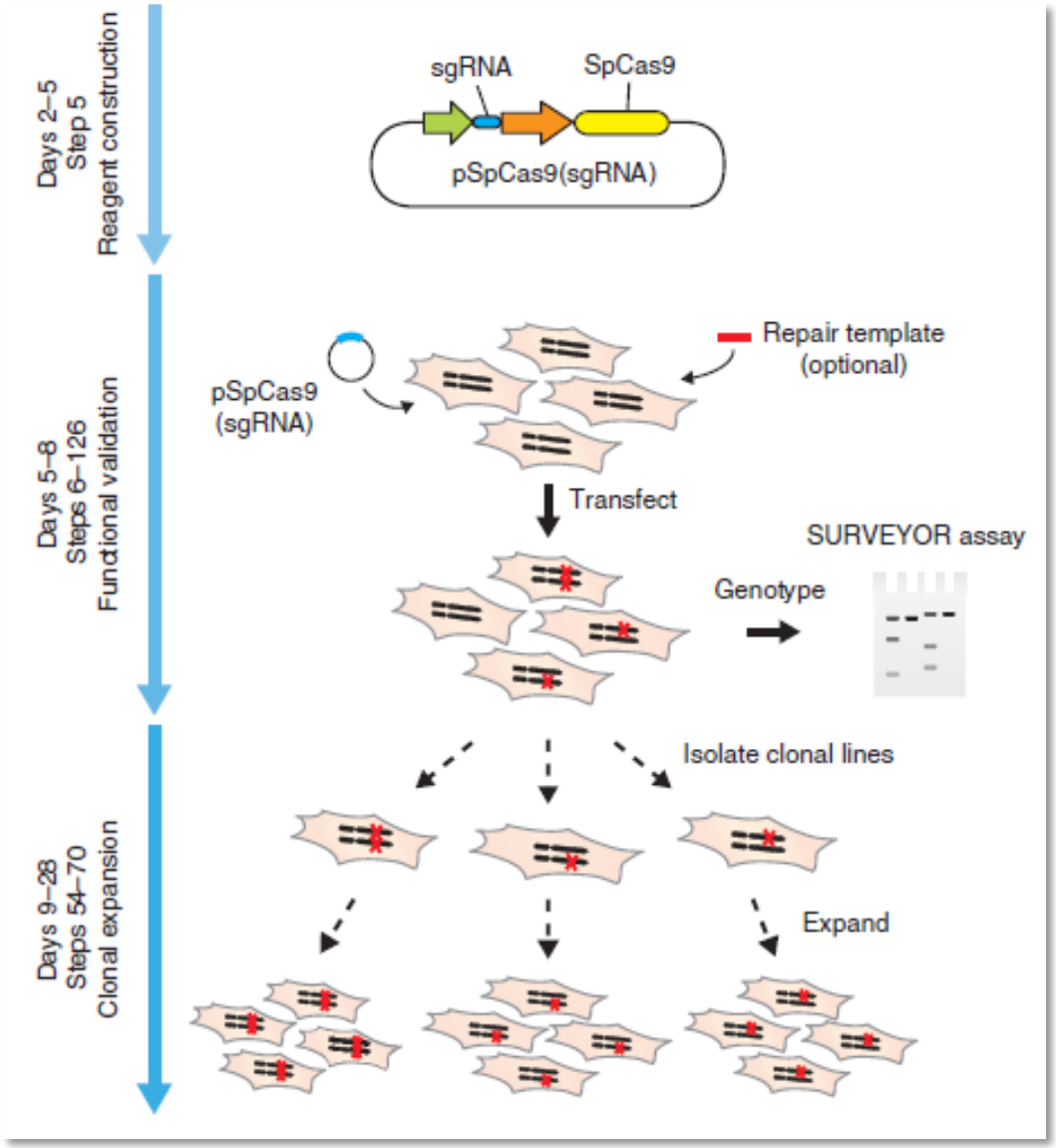
ctrl IPS cells → Crispr/ Cas9 → Insert mutation → «disease» Organoids

...AGTCTGACCTGACT...

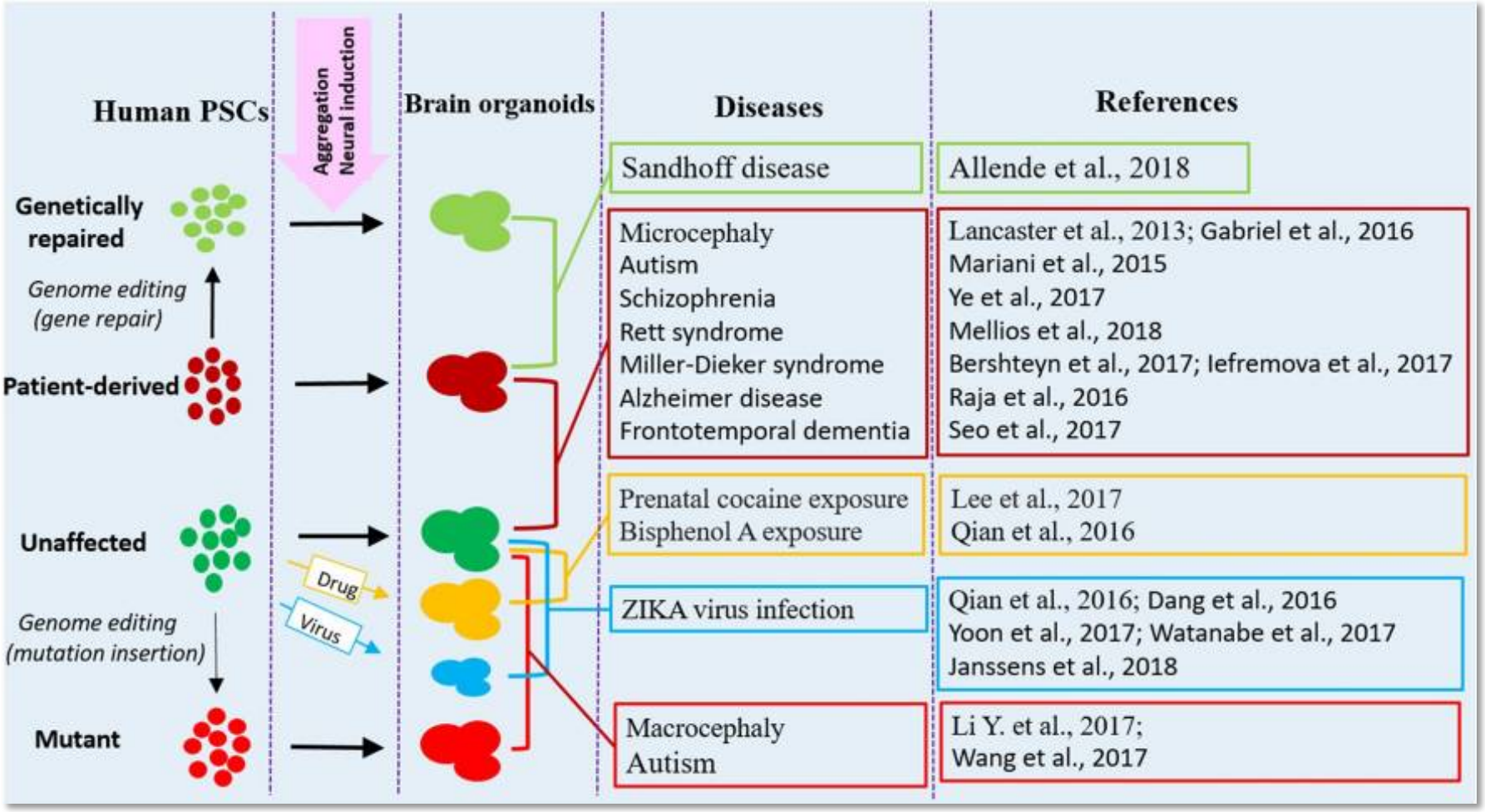
C

ctrl IPS cells → ctrl Organoids → Crispr/ Cas9 → ctrl Organoids with mutated cells in electroporated area

CRISPR/Cas9 engineering in IPSC cells



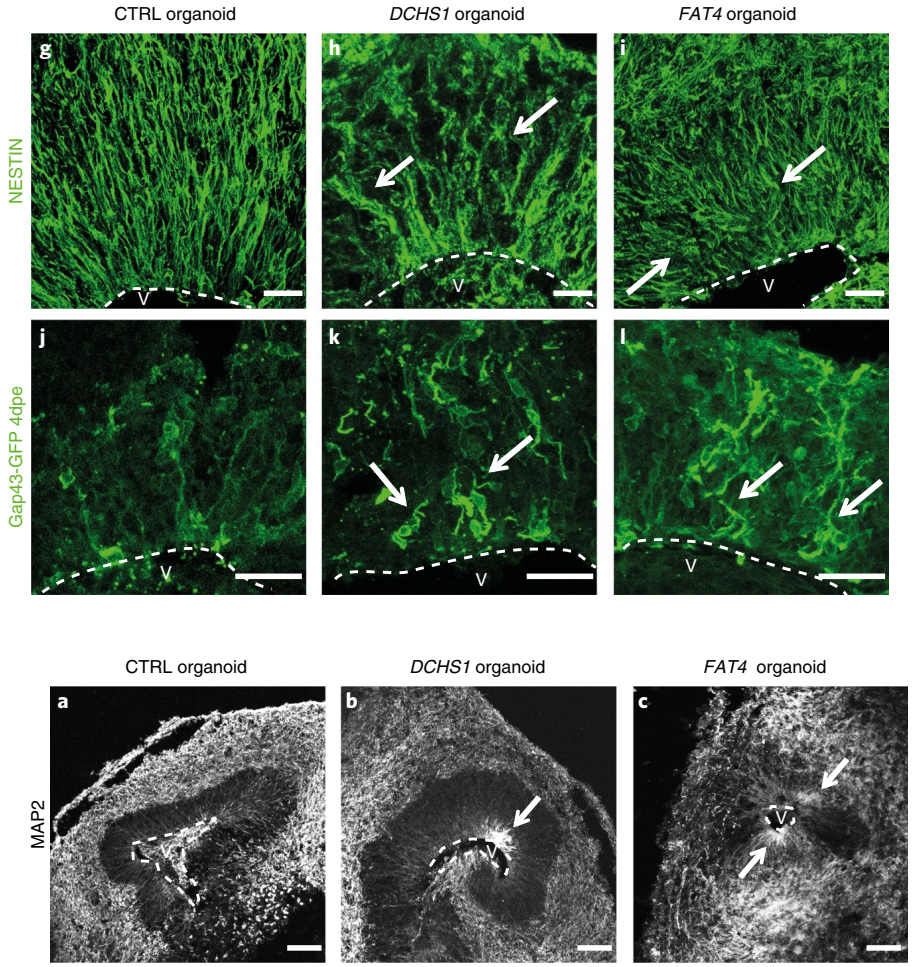
Organoids for human disease modeling



Wang, 2018

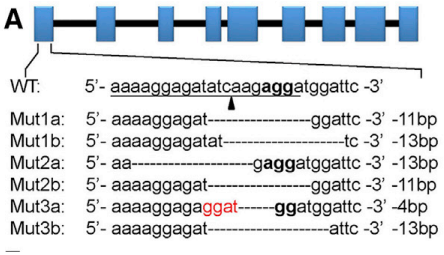
Neuronal heterotopia and abnormal cell migration in DCHS1 and FAT4 mutated cerebral organoids

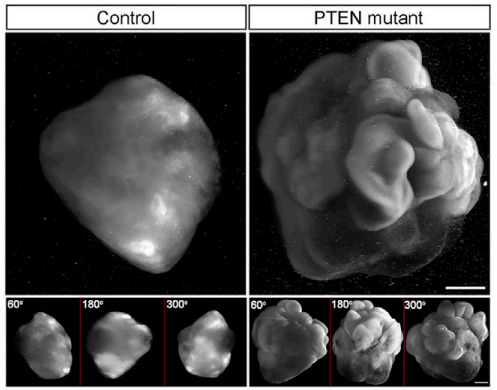
DCHS1 &FAT4: protocadherins act as planar cell polarity genes



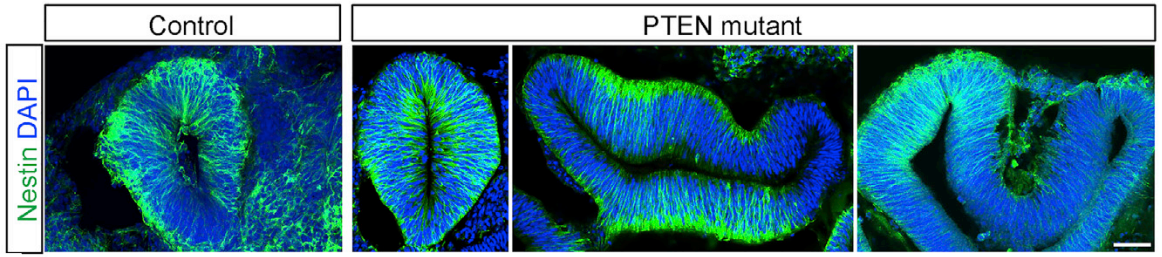
Klaus et al., 2019

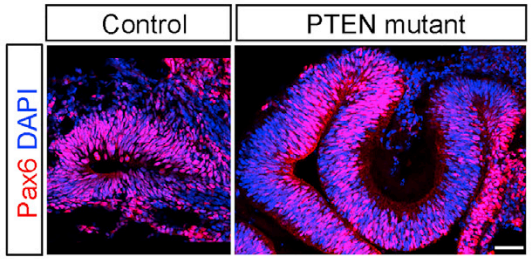
Induction of Expansion and Folding in PTEN Mutant Human Cerebral Organoids

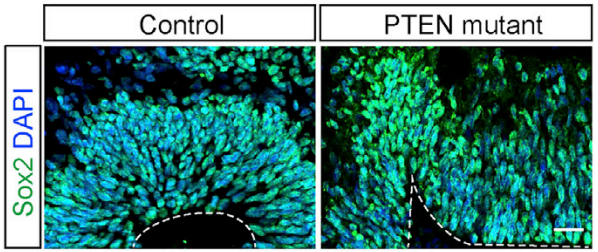


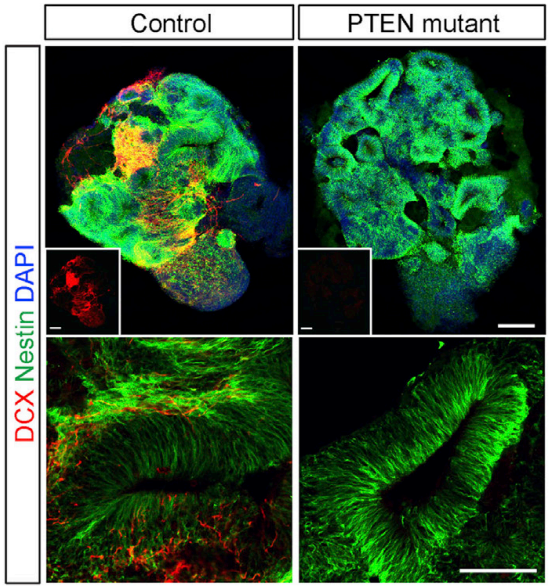


Phosphatase and tensin homolog (**PTEN**) known as a tumor suppressor





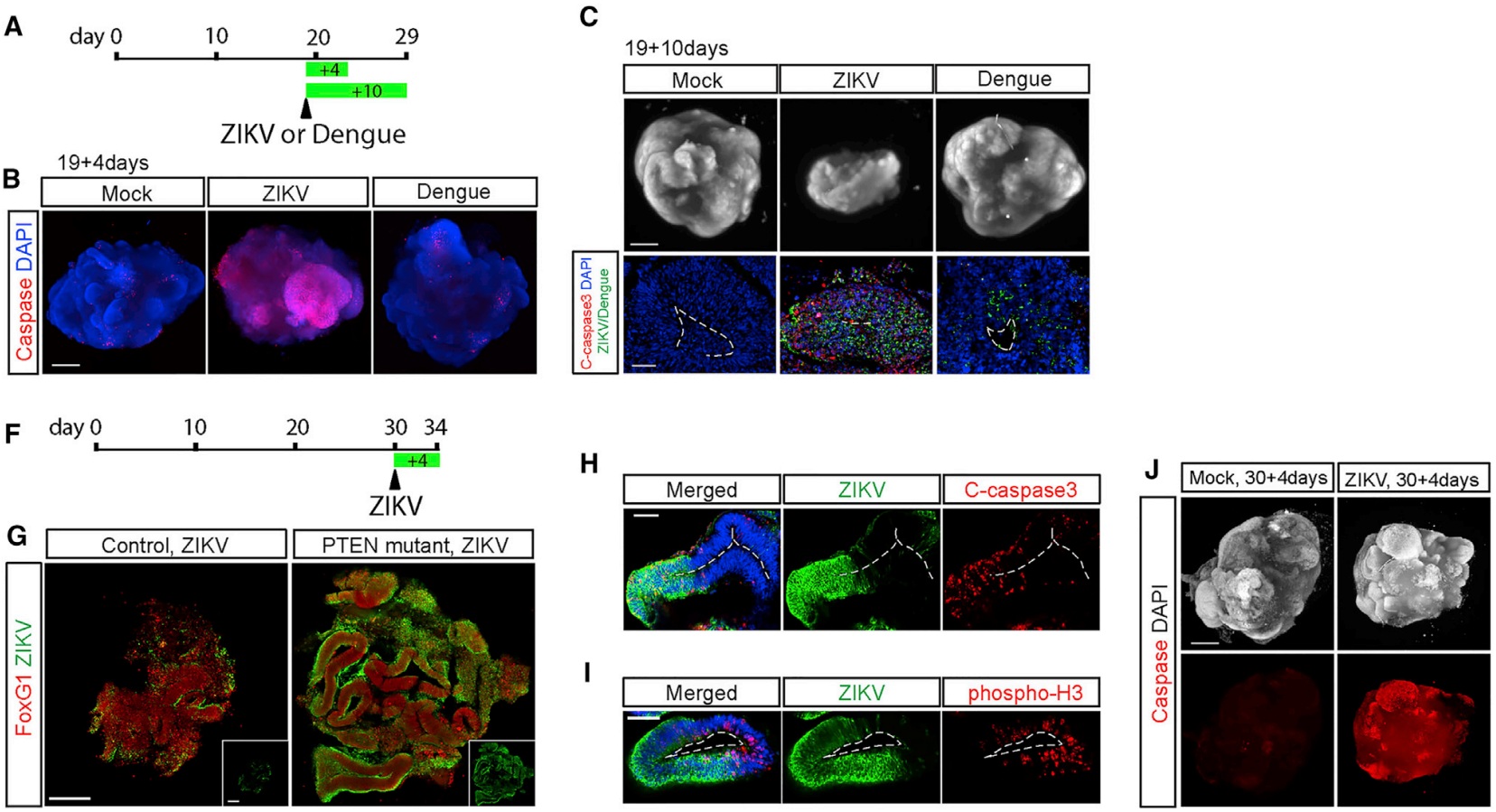




Delayed neurogenesis

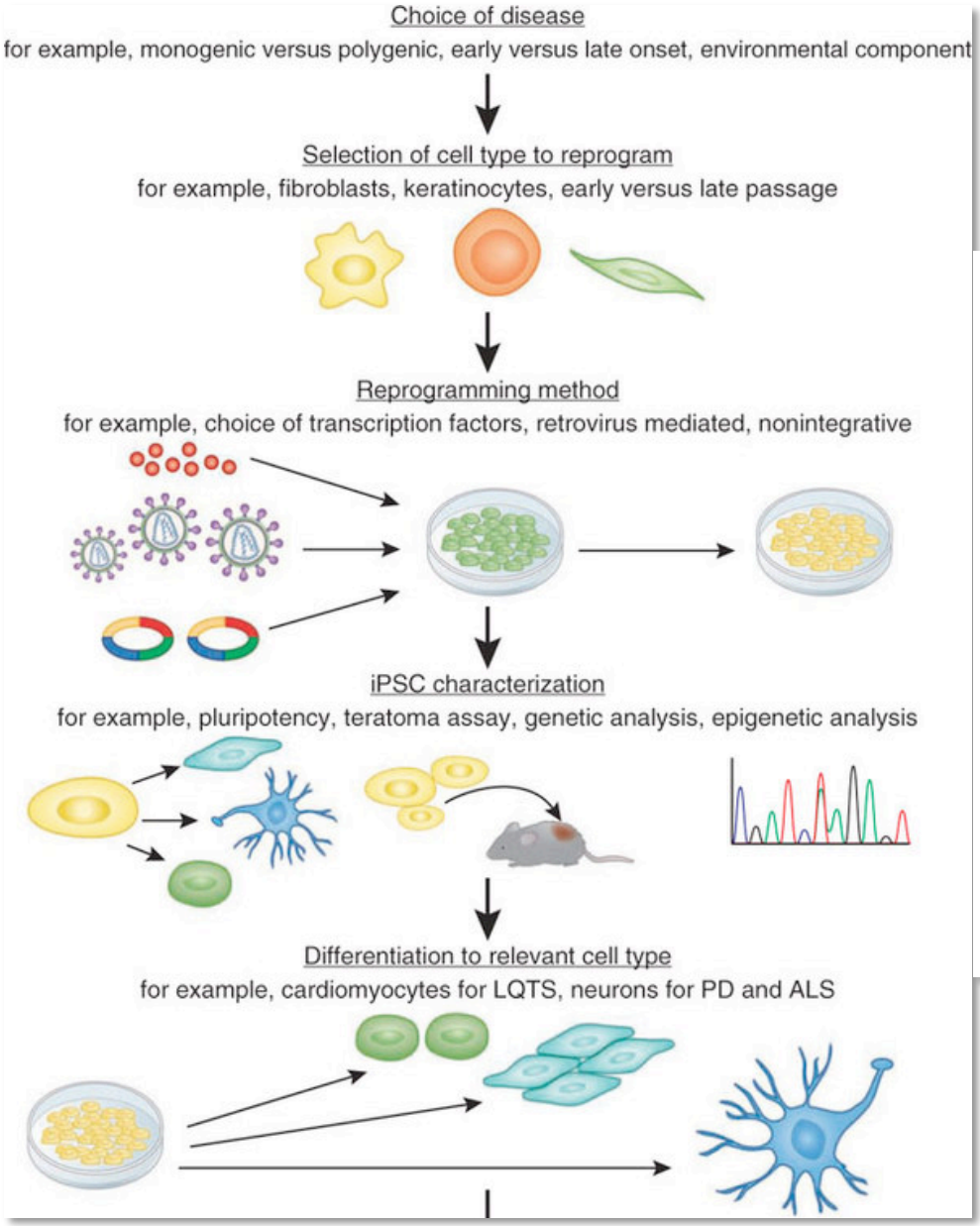
Li et al., 2019

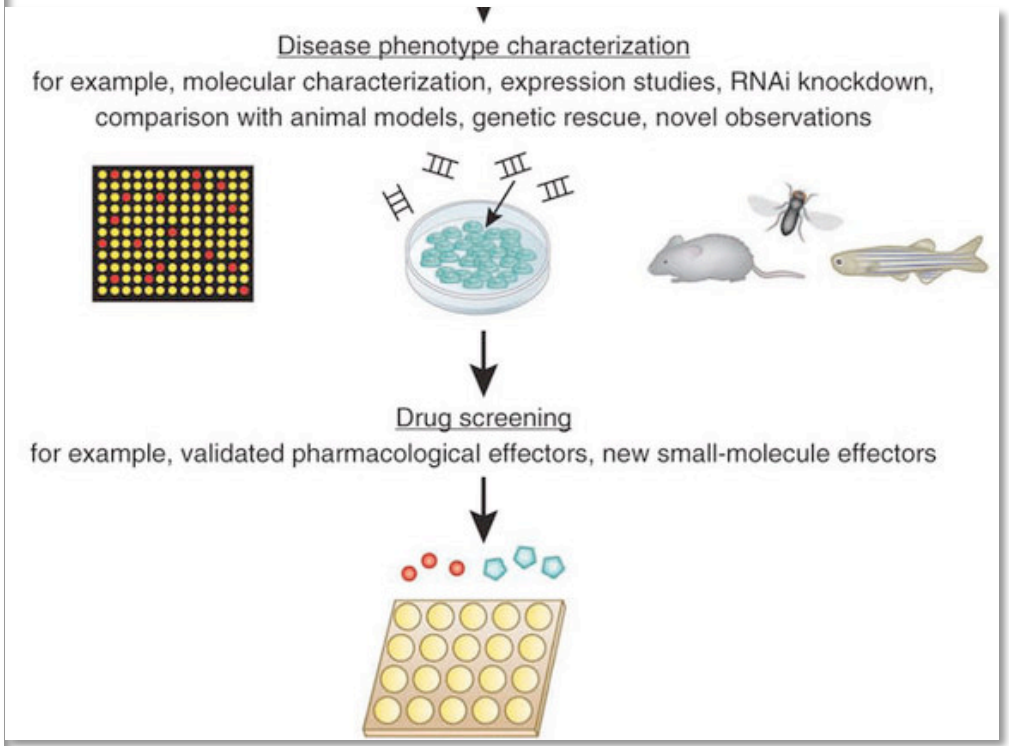
ZIKV Infection Impairs Expansion and Folding in Human Cerebral Organoids



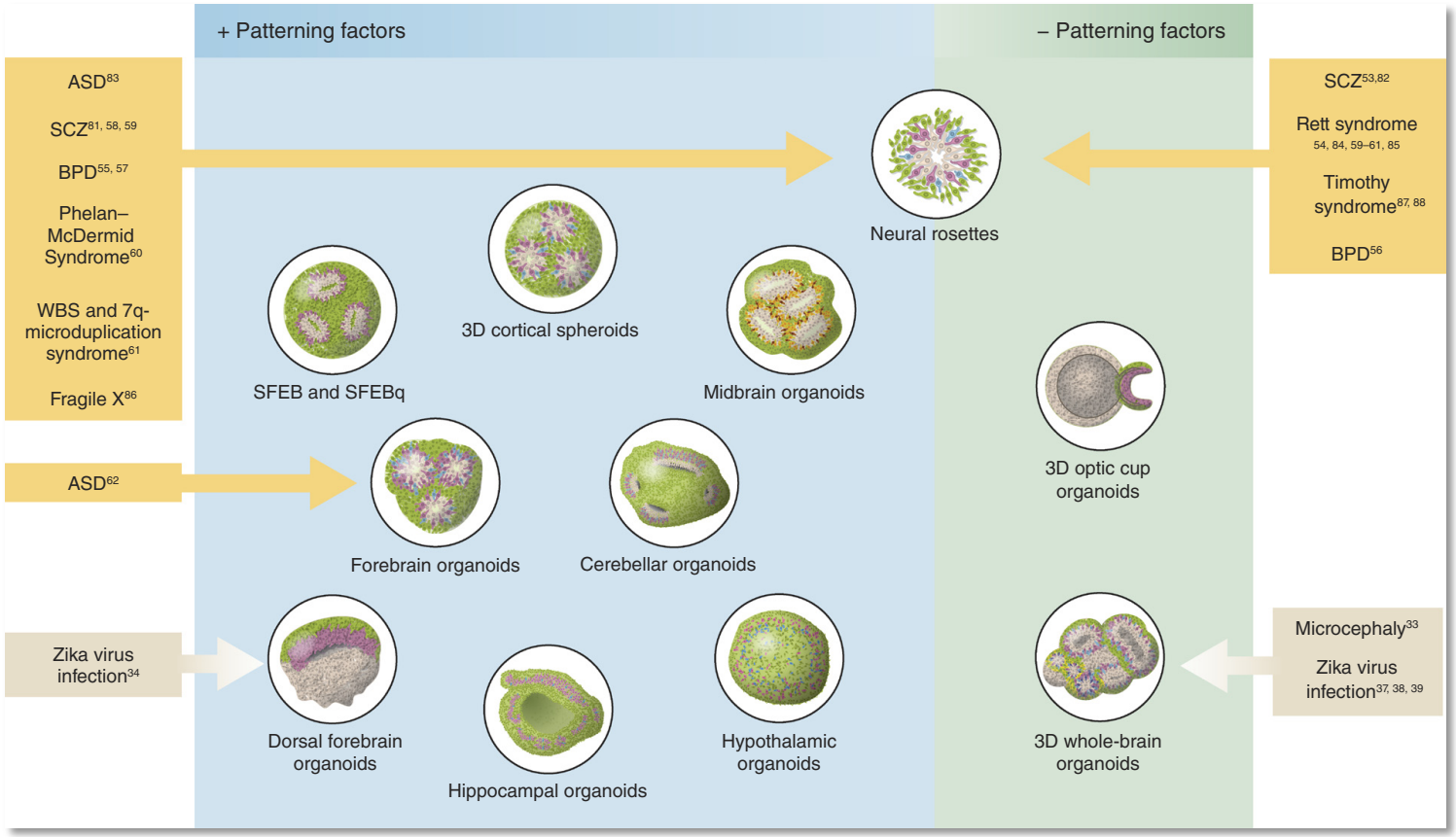
Li et al., 2019

Overall strategy of disease modeling



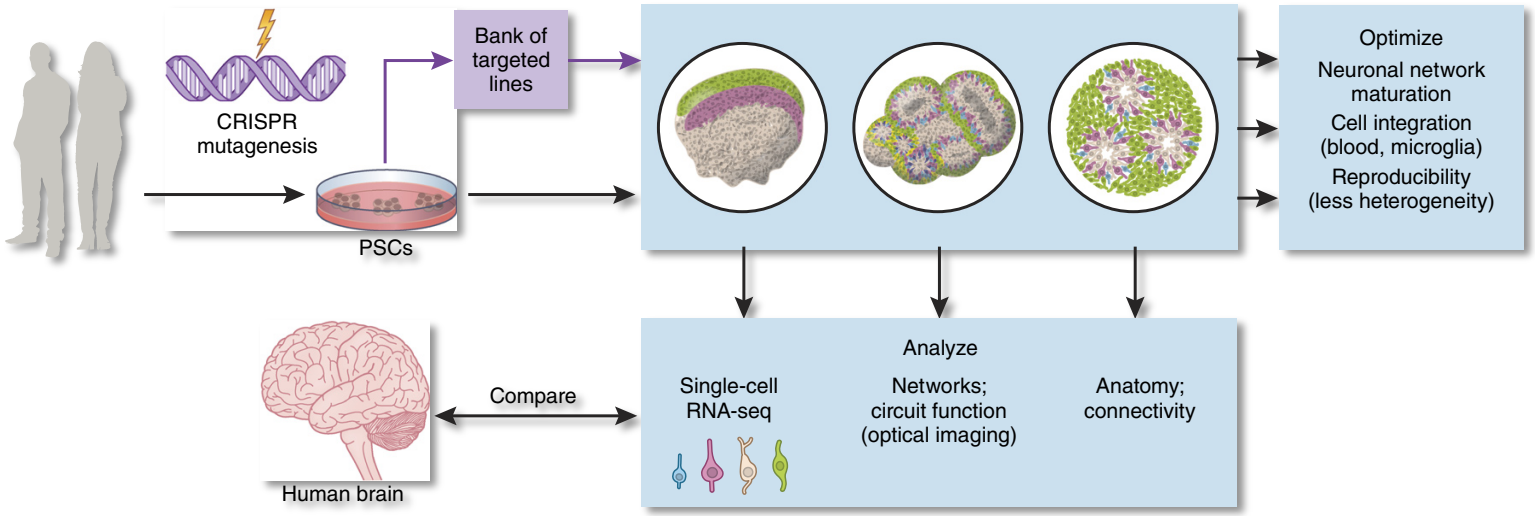


Human brain organoids as models of neuropsychiatric diseases



Quadrato et al., Nat Med, 2016

Generation, characterization and analysis of 3D cellular models of the human brain



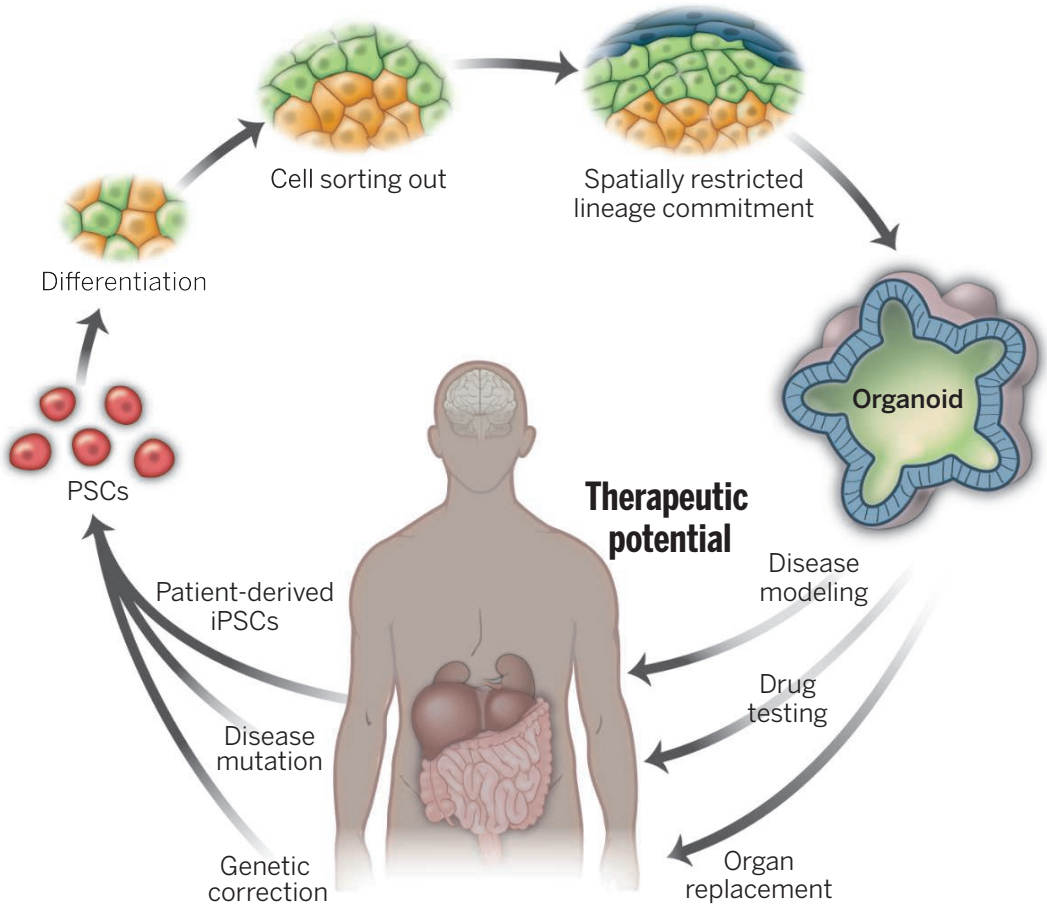
Quadrato et al., Nat Med, 2016

REVIEW SUMMARY

ORGANOID GENERATION

Organogenesis in a dish: Modeling development and disease using organoid technologies

Madeline A. Lancaster *and* Juergen A. Knoblich*




THANK YOU