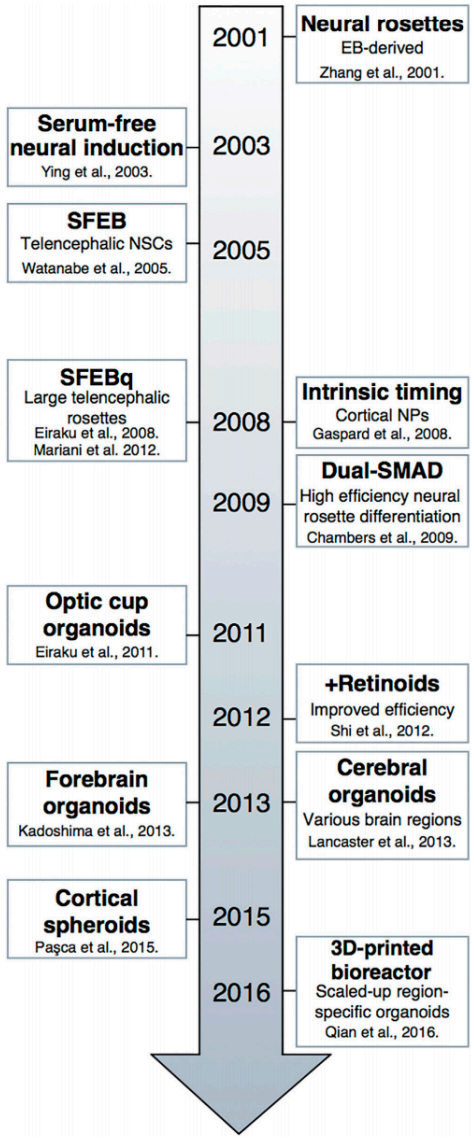
cerebral organoids: fad or a potentially powerful tool?

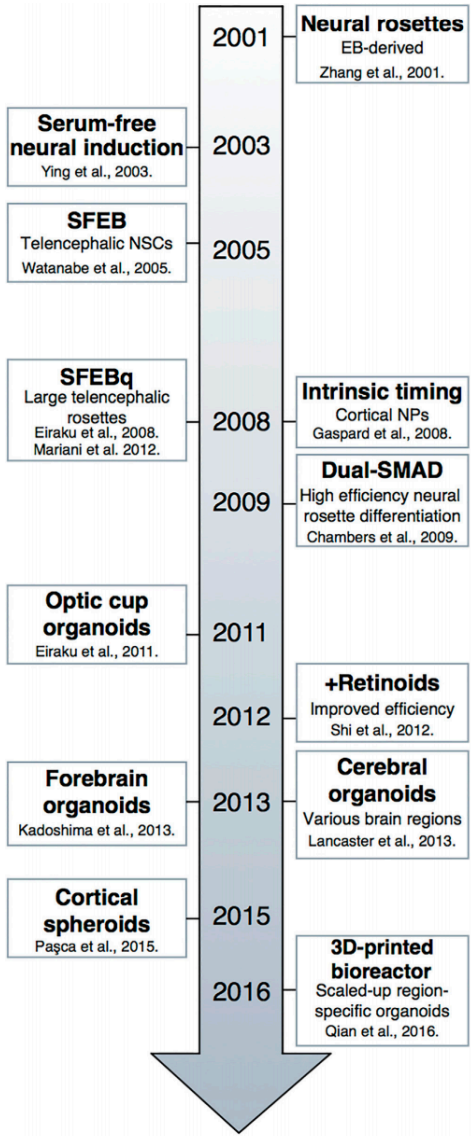
technical journal club
merve avar
8.12.20

neural organoids: brief history



Kelava and Lancester,
Cell Stem Cell, 2016

neural organoids: brief history



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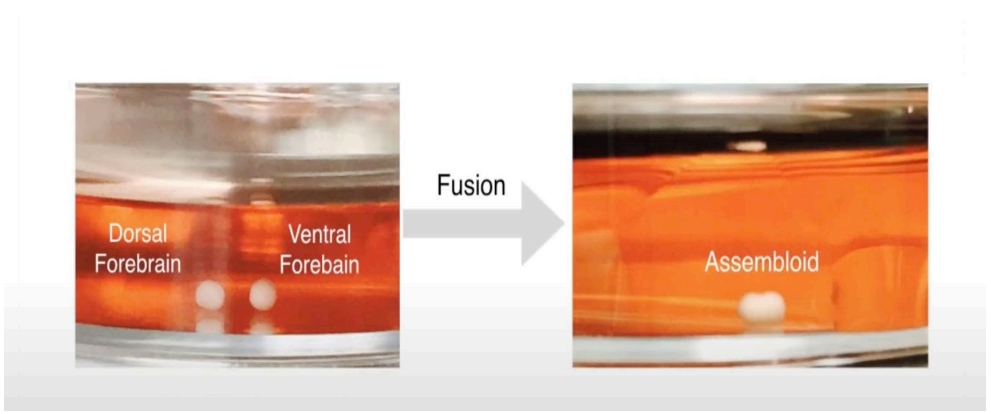
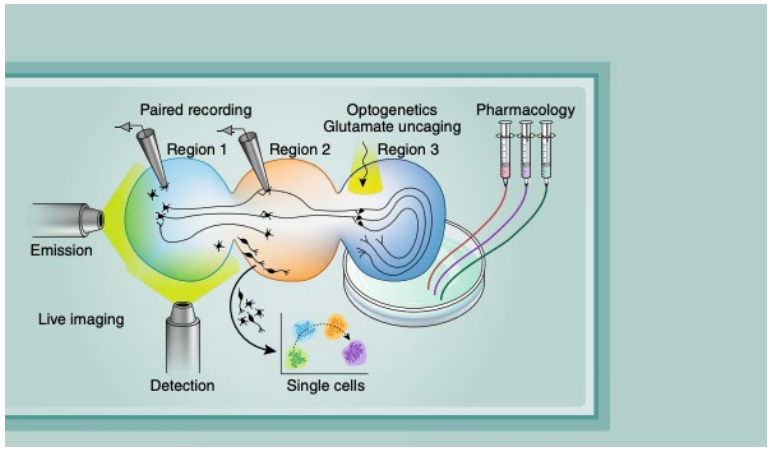


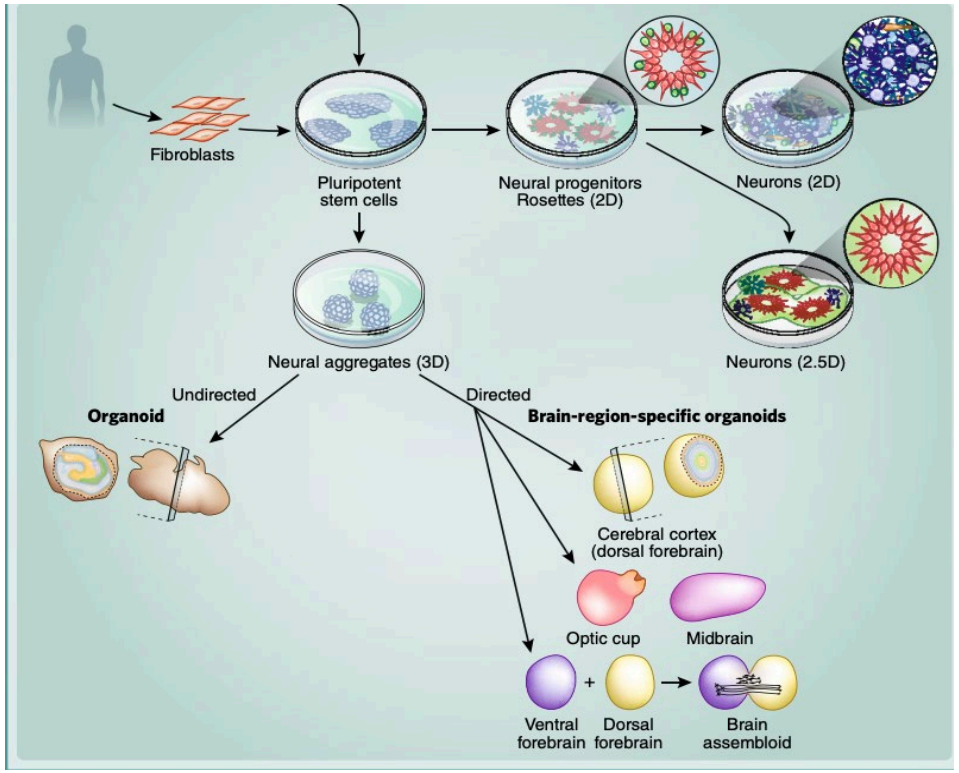


Pasca SP, 2018, Nat. Neuroscience

relatively young technology
goals of the field:

- recapitulation of 3D properties of complex tissues
- understanding neural development and disease
- utilization of technology in high-throughput platforms for drug and genetic screens

neural organoids: different methods of derivation

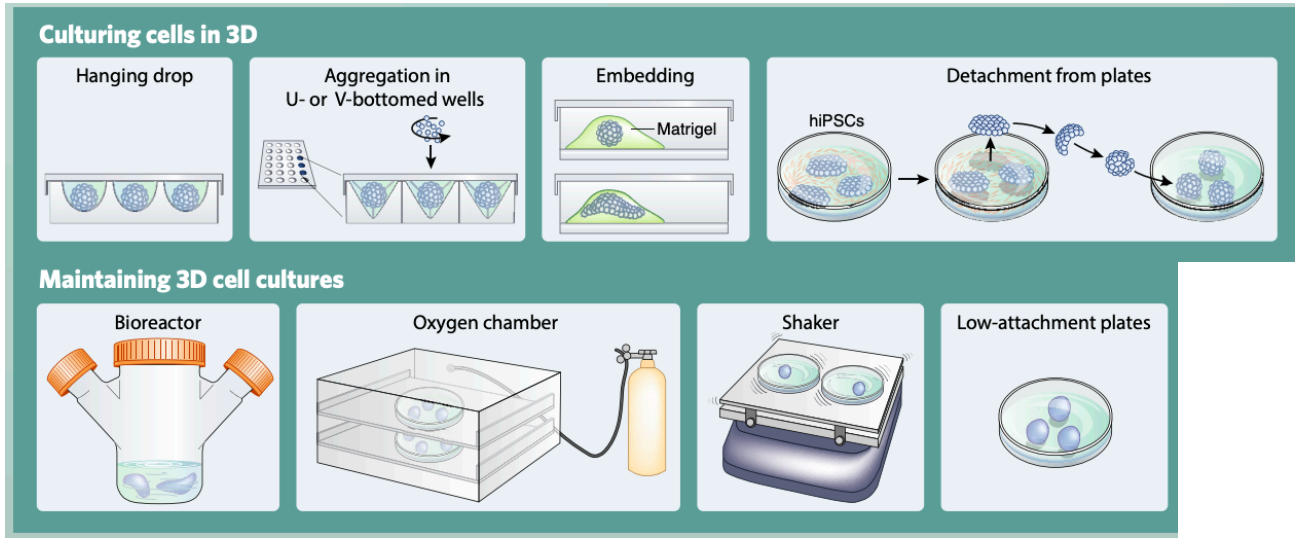


methods to derive organoids are highly divergent:

- undirected differentiation / self assembly
- directed differentiation, where fate of a certain brain region is imposed through chemical cues
- assembloids

Pasca SP, 2018, Nat. Neuroscience

neural organoids: different methods of derivation



Pasca SP, 2018, Nat. Neuroscience

culturing conditions vary greatly between protocols

→ all these points are possible causes of variation and can lead to reproducibility issues

topics today

Reliability of human cortical organoid generation

Se-Jin Yoon[1], Lubayna S. Elahi[1], Anca M. Pașca[2], Rebecca M. Marton[1], Aaron Gordon[3], Omer Revah[1], Yuki Miura[1], Elisabeth M. Walczak[4], Gwendolyn M. Holdgate[4], H. Christina Fan[4], John R. Huguenard[5], Daniel H. Geschwind[3,6] and Sergiu P. Pașca[1,7]*

Individual brain organoids reproducibly form cell diversity of the human cerebral cortex

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will address the reproducibility of organoids

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A human tissue screen identifies a regulator of ER secretion as a brain size determinant

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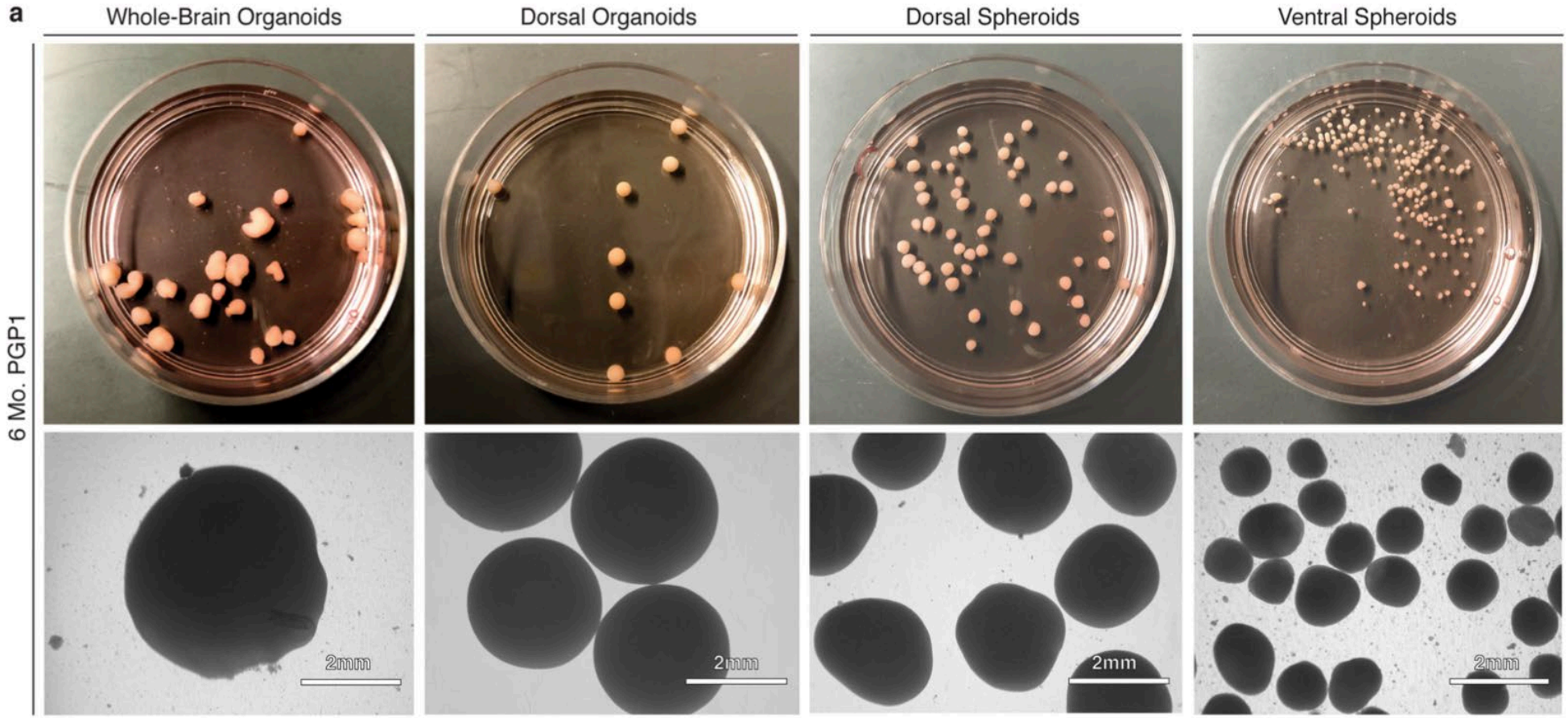

→ further results are mainly from dorsal organoids

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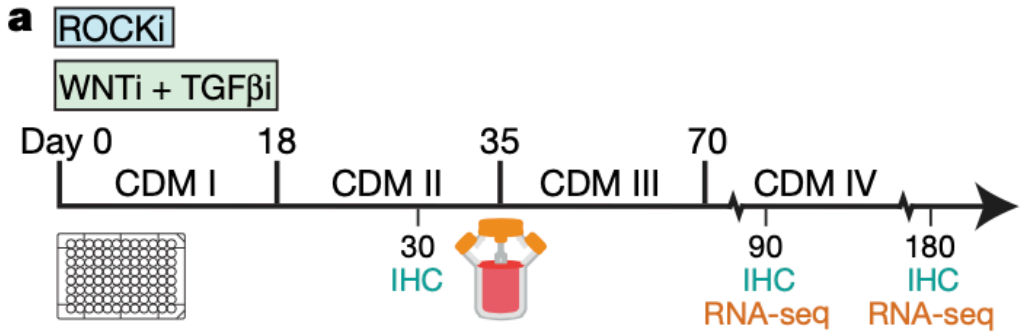

throughout the study 5 different iPS (or ES) cell lines are used

→ however, not for each experiment

different maturation points as well as different batches of organoids are compared to pre-existing datasets of human and mouse origin

→ mainly addresses the representativeness of the organoid model

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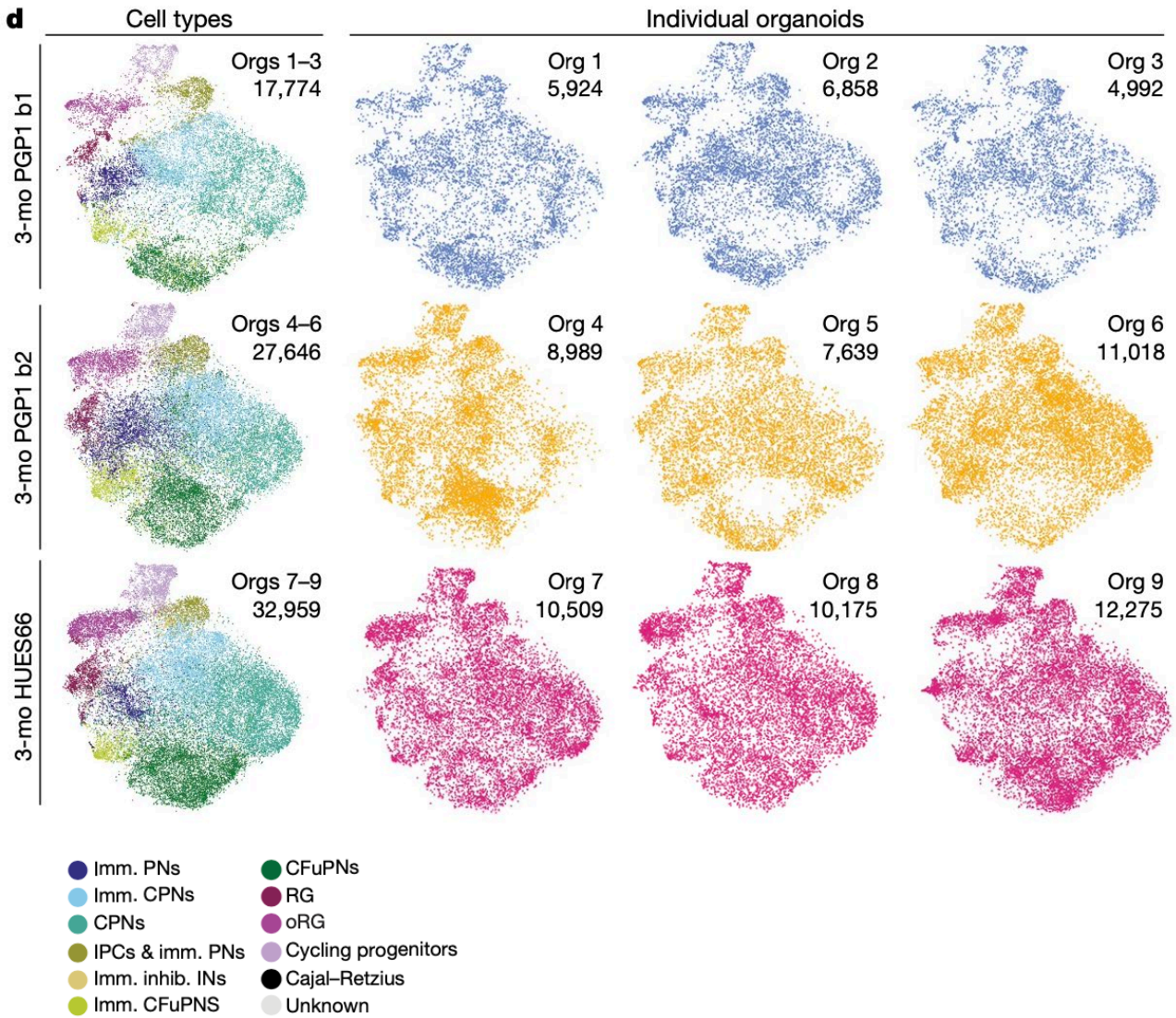

- dorsal organoids derived from 2 different iPS lines
- 3 months growth time
- 2 different batches for one of the lines
- scRNA-Seq (10X genomics chromium platform) from 78,379 cells

→ 11 transcriptionally distinct cell types are identified
→ astroglial cells are underrepresented
→ these cell types are present in both of the iPS lines
→ individual organoids recapitulate all 11 cell types
→ confirmed with IHC

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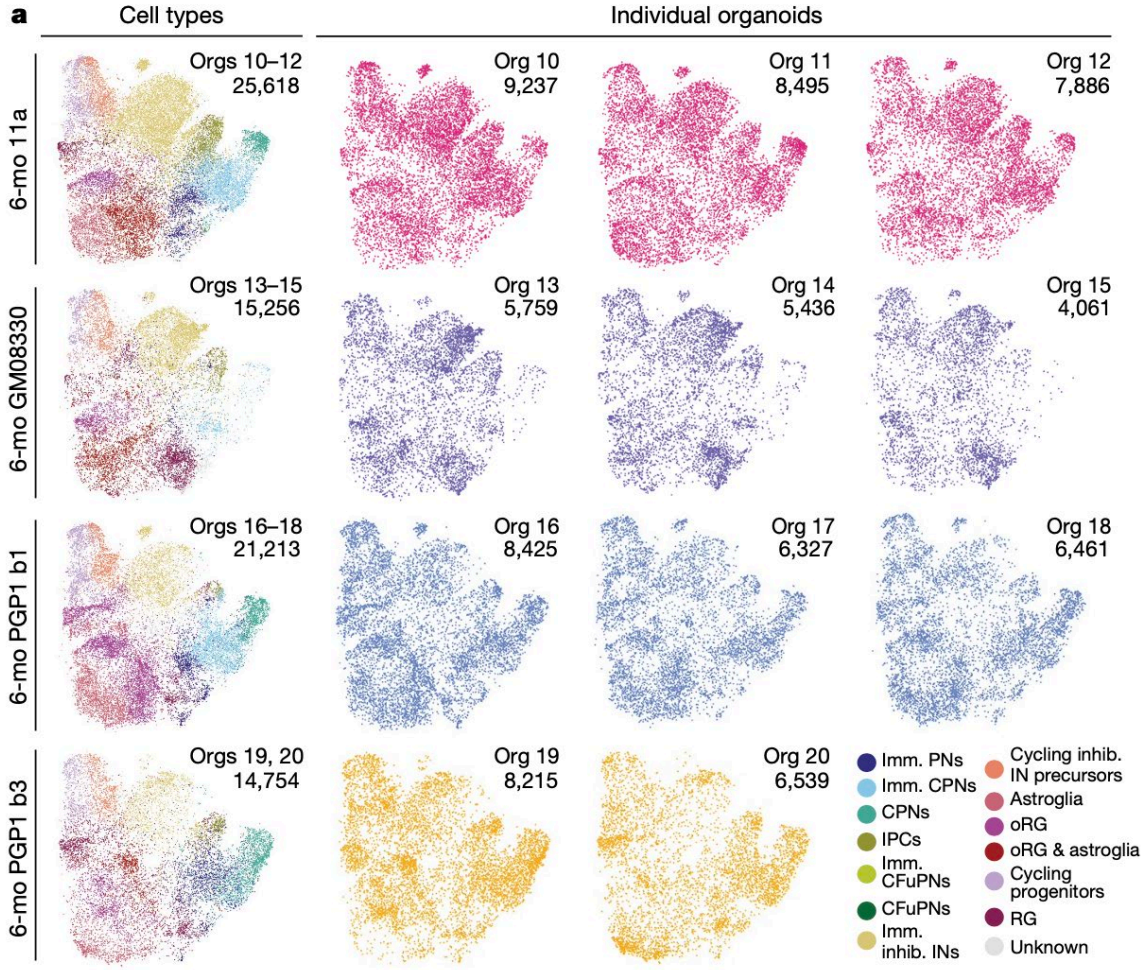

- dorsal organoids derived from 3 different iPS lines
- 6 months growth time
- 2 different batches for one of the lines
- scRNA-Seq (10X genomics chromium platform) from 87,863 cells

→ in addition to the 11 cell types identified in 3 months-old cultures, astrocytes and a mix of oRG and astrocytes were identified
→ these cell types are present in all three iPS lines
→ individual organoids recapitulate all 13 cell types
→ confirmed with IHC

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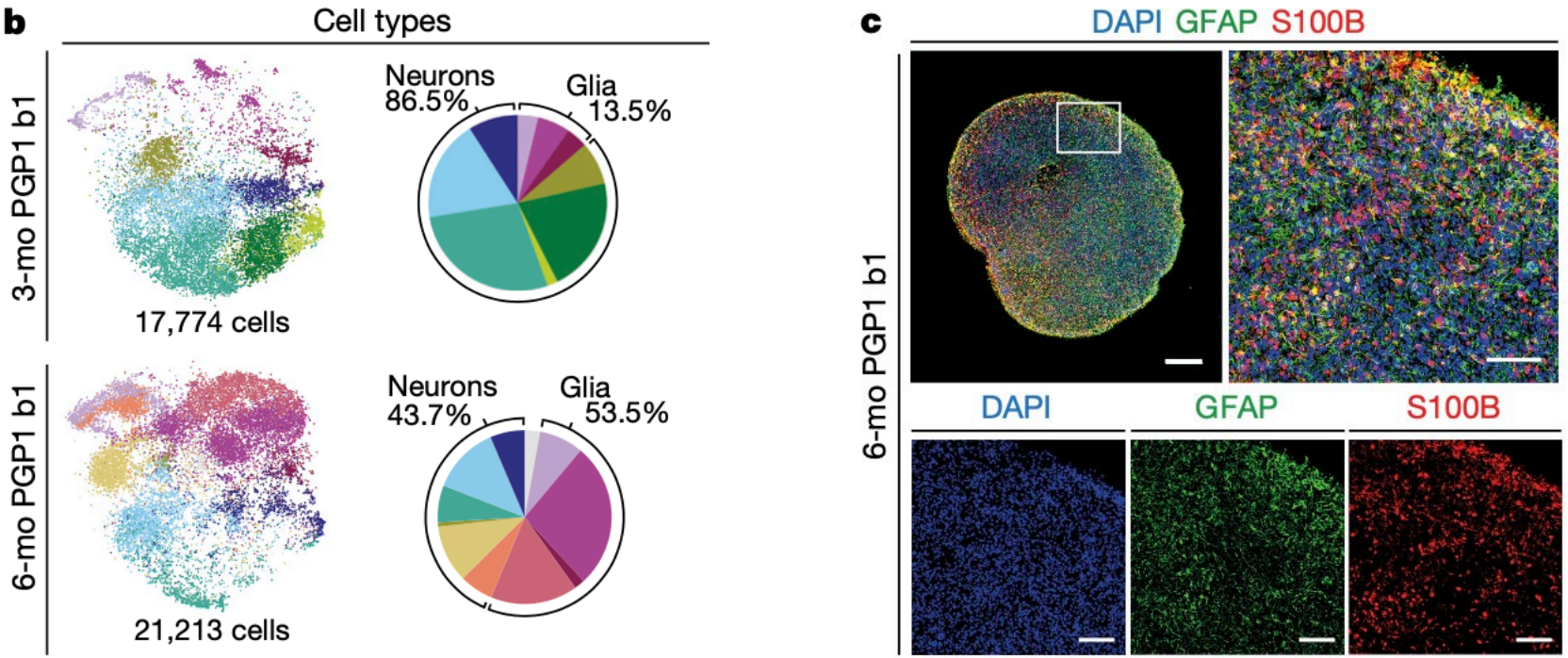
comparison of 3 vs 6 months-old cultures from same iPS line



→ as the organoids mature, astrocytes become prominent

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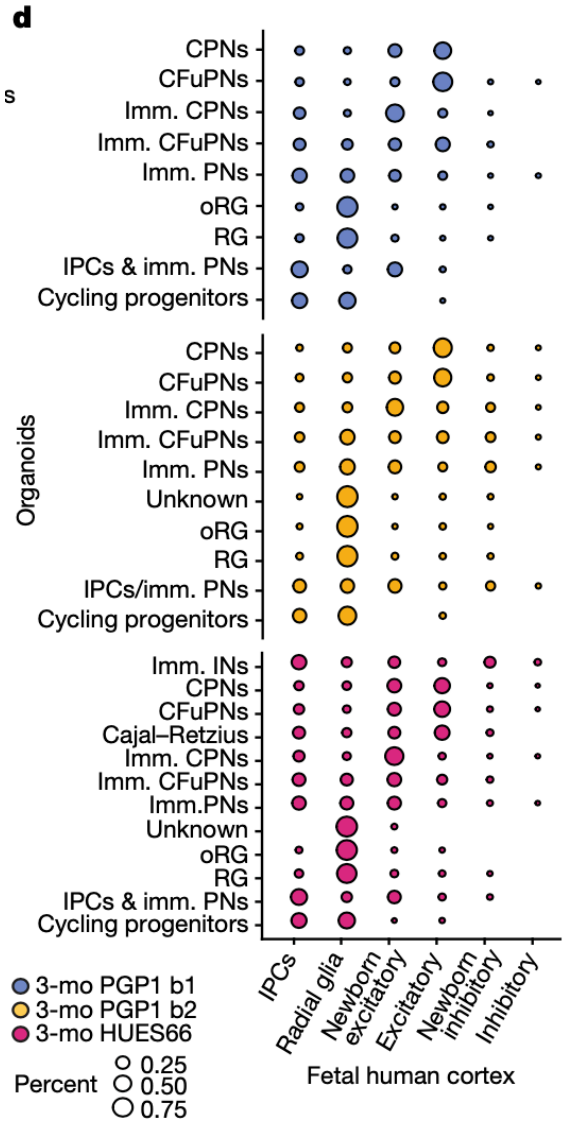

to assess whether organoid cell types and endogenous human brains show similarity the authors compared the scRNA-Seq data to a published human fetal cerebral cortex dataset

→ all organoids independent of cell line or batch distributed similarly and this development approximated that of in vivo human development (shown example is of 3 months)

→ cell types found in the human fetal brain at 6 months correspond to the cell types in 3 months-old organoids

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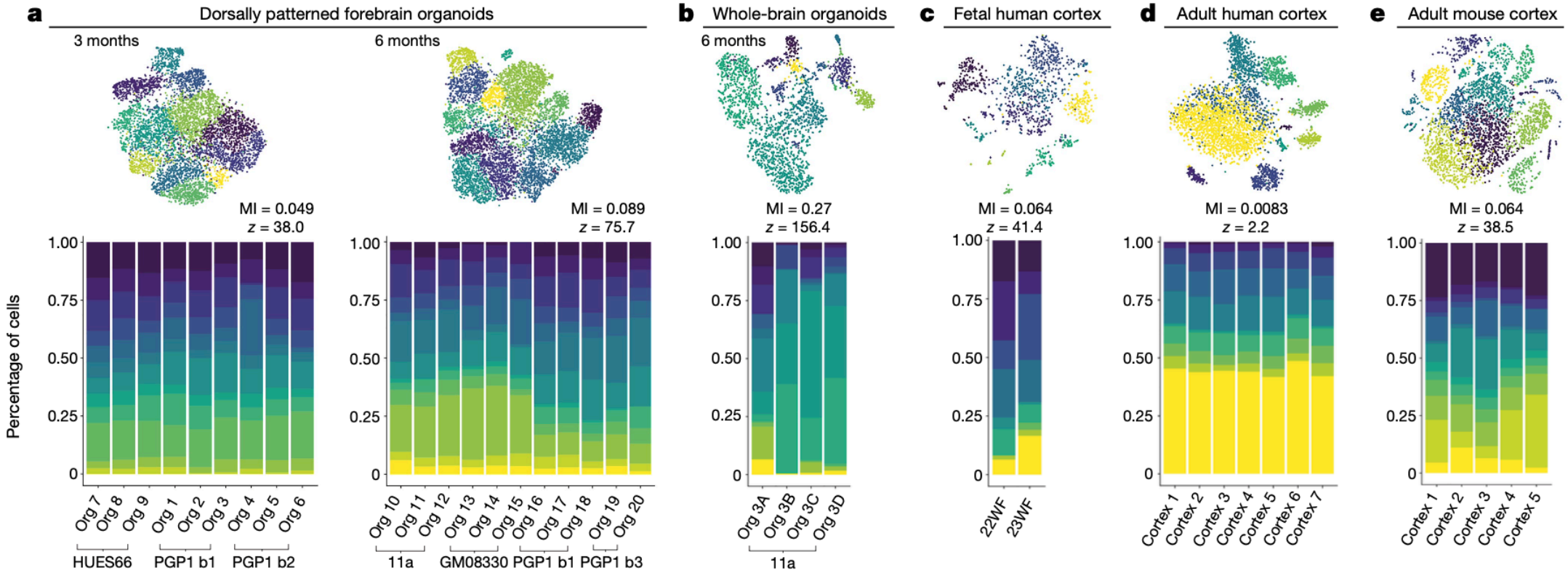
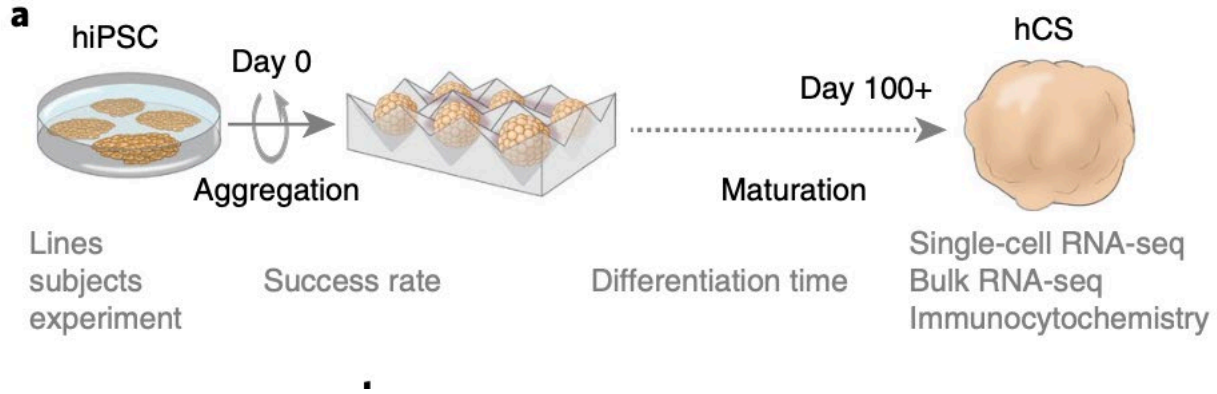
do organoids show the same degree of individual brain-to-brain differences seen in human and mouse brains?

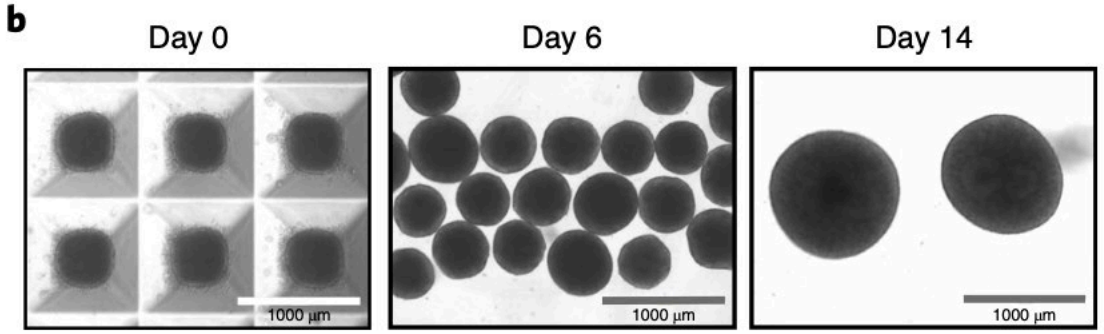


→ dorsal directed organoids show a similar variation observed in mouse and human brains
→ MI scores represent the dependence between cluster and individual (lower = similar makeup)

Reliability of human cortical organoid generation

Se-Jin Yoon[1], Lubayna S. Elahi[1], Anca M. Pașca[2], Rebecca M. Marton[1], Aaron Gordon[3], Omer Revah[1], Yuki Miura[1], Elisabeth M. Walczak[4], Gwendolyn M. Holdgate[4], H. Christina Fan[4], John R. Huguenard[5], Daniel H. Geschwind[3,6] and Sergiu P. Pașca[1,7]*





- one differentiation protocol is used
- organoids can be maintained in culture >25 months
- authors derive 15 iPSC lines from 13 different individuals throughout the study
- to ensure reproducibility the iPSCs are maintained in feeder and xeno-free conditions (hCS-FF)
- comparisons between dorsal (hCS-MEF) and ventral directed organoids (hSS) and feeder-cell layer based organoids (from previously published data)

→ main question addressed in the study is reproducibility of the organoids

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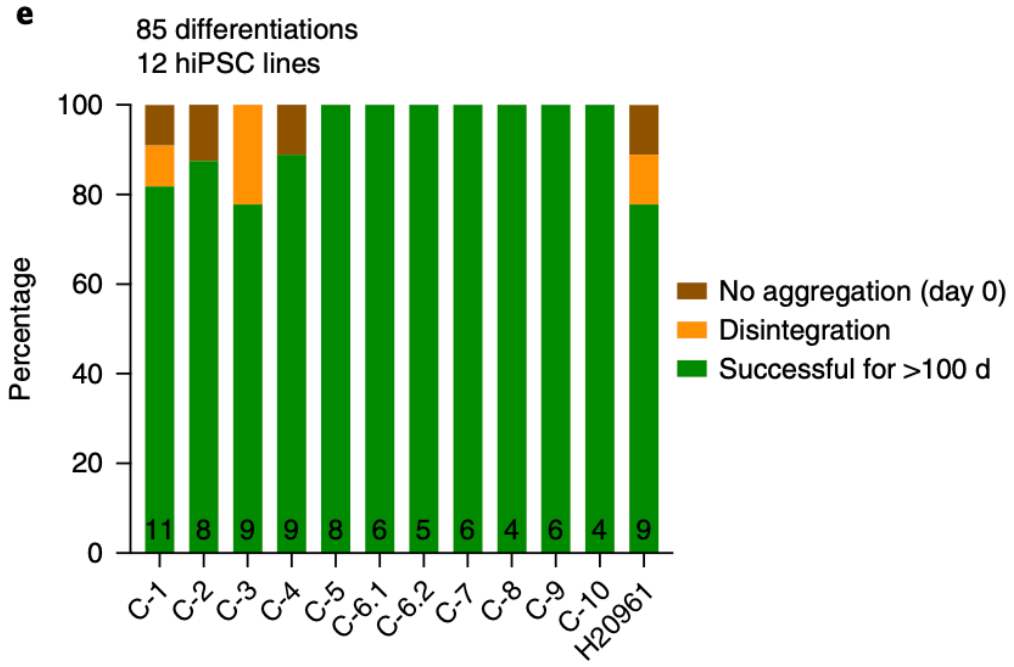
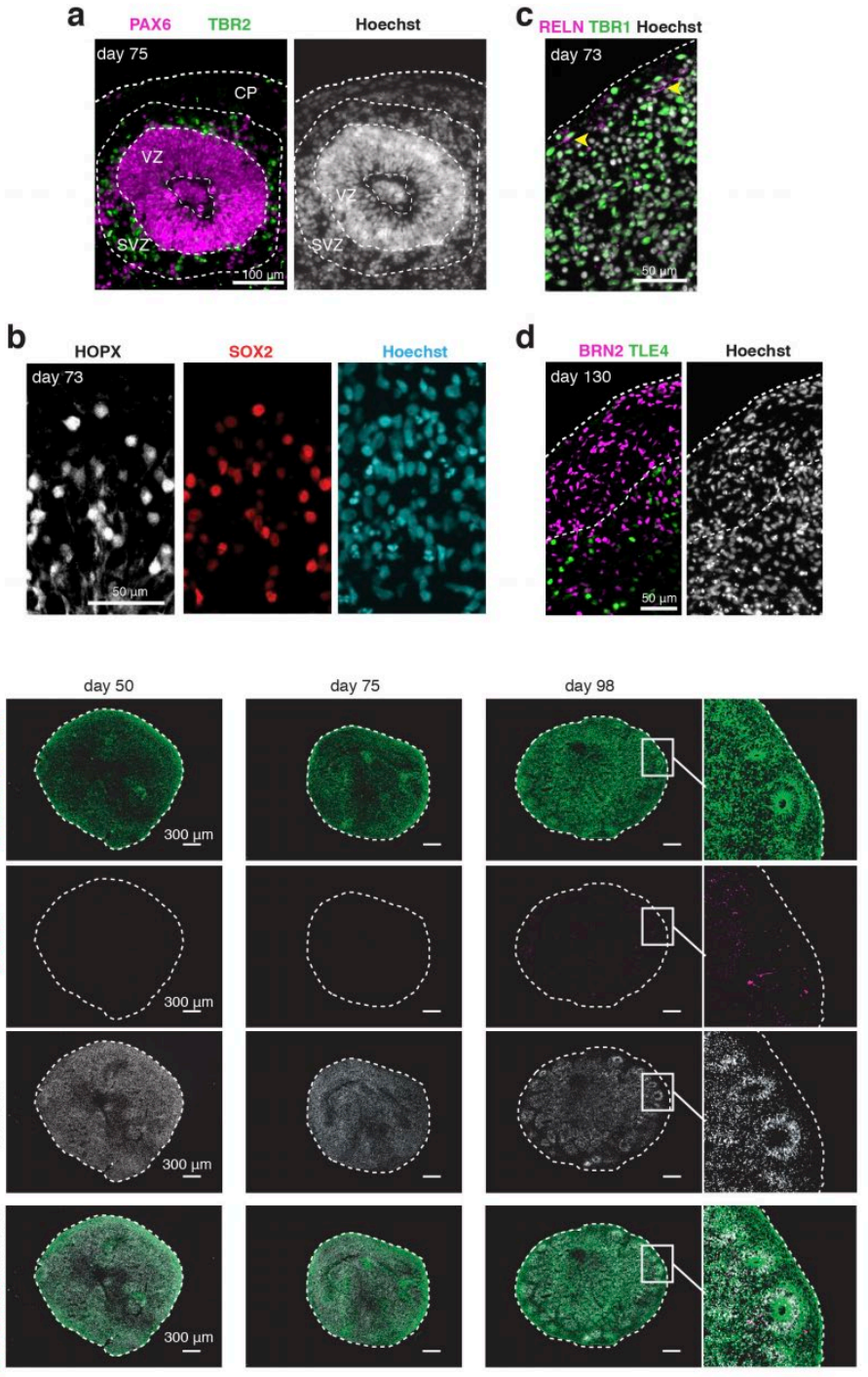
- to assess the overall success rate of their protocol authors performed 4-11 independent differentiations on 12 iPSC lines (total of 85 experiments)



→ over 90% of cultures were kept successfully in culture >100 days and expressed cortical neural markers and were healthy (lack of caspase 3 activity)



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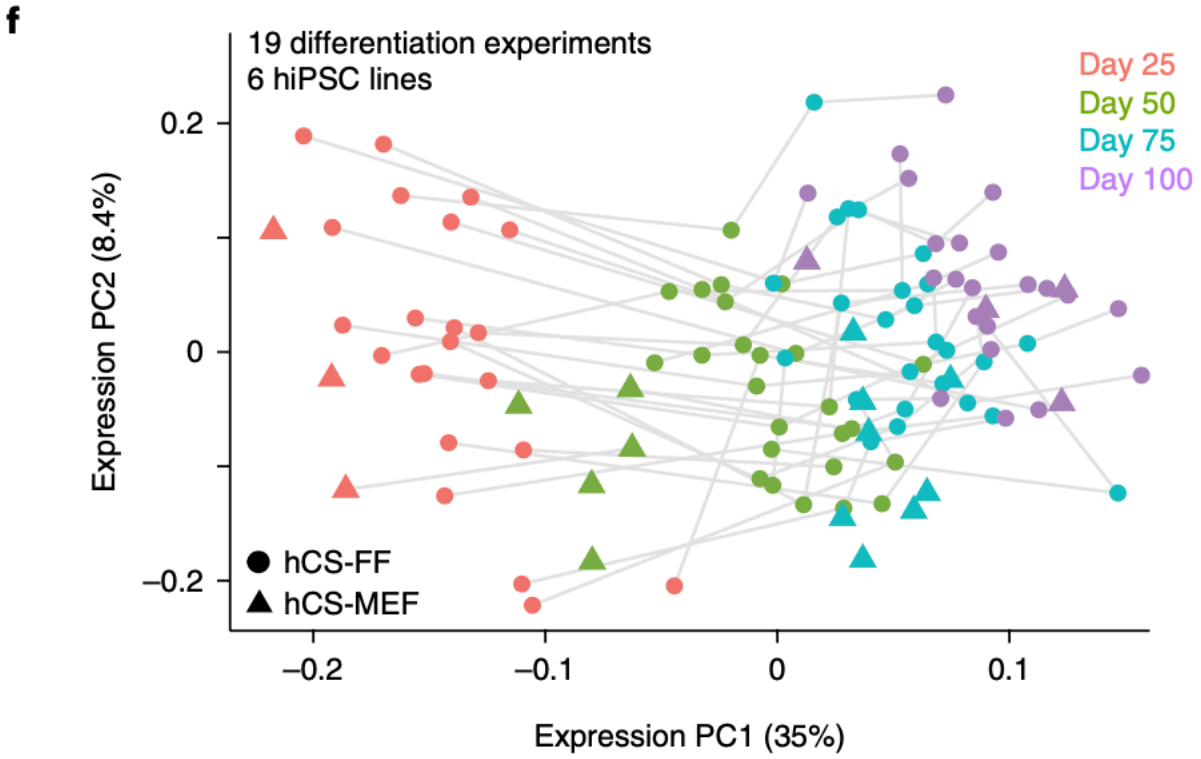
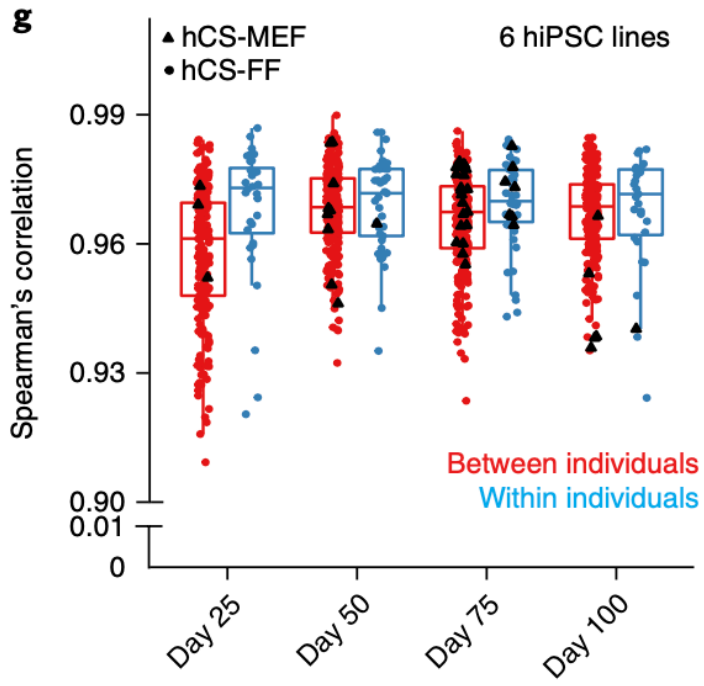


- RNA-Seq performed on organoids from
  - 4 stages of differentiation
  - 6 different iPSC lines (hCS-FF)
  - at least 3 independent experiments
- additional comparison to iPSC cultures maintained on a feeder layer (hCS-MEF)



→ main driver of variance observed is the stage of differentiation (PC1)
→ overall great reproducibility between different individuals and between distinct differentiation experiments

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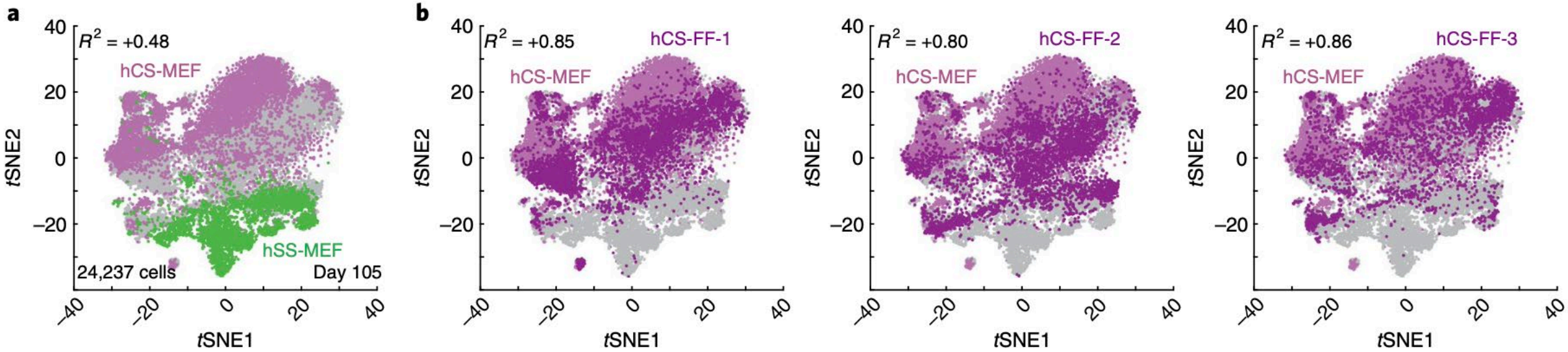
- single cell RNA-Seq performed on organoids from
  - day 105 of differentiation
  - from 2 different individuals
  - two differentiations from one of the iPSCs
  - experiment performed on BD Rhapsody system
  - n=24,237 cells

- additional comparison to cultures maintained on a feeder layer (hCS-MEF) as well as organoids from the subpallium (hSS) with ventral identity



→ organoids of ventral and dorsal identity show a robust separation
→ feeder free organoids cluster closely with dorsal forebrain hCS-MEFs

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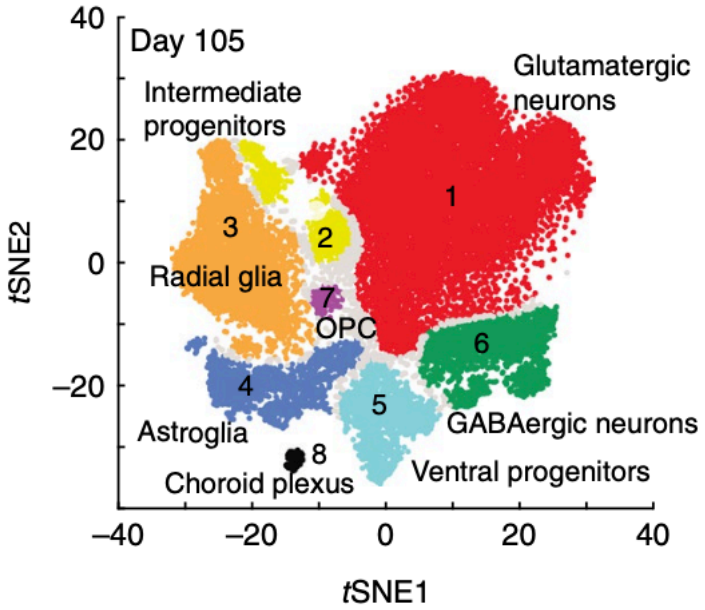

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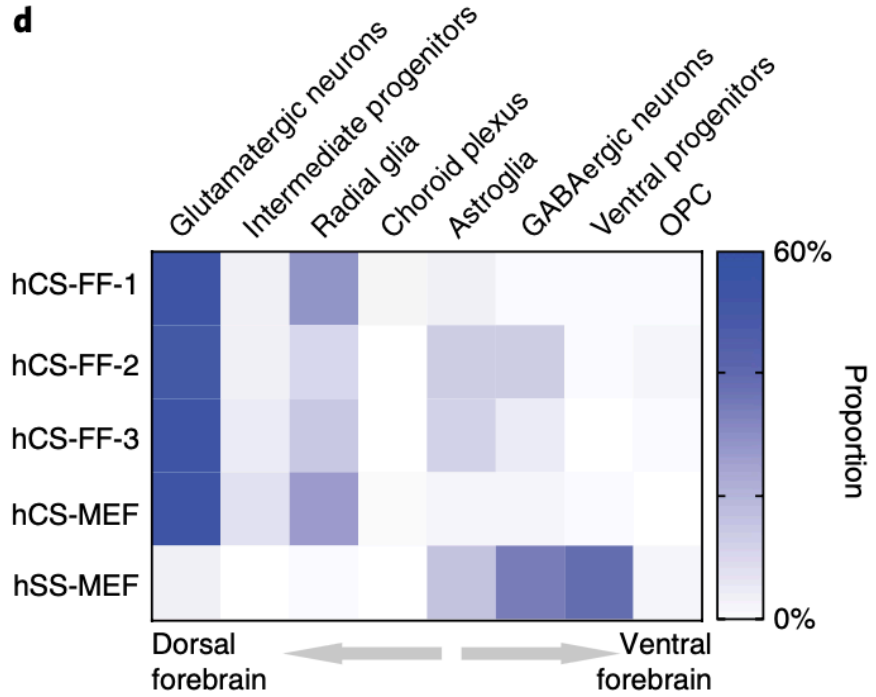

→ 8 cell types are present in the cultures
→ choroid plexus cells are rare in population and was absent in 2 out of the 3 lines in question

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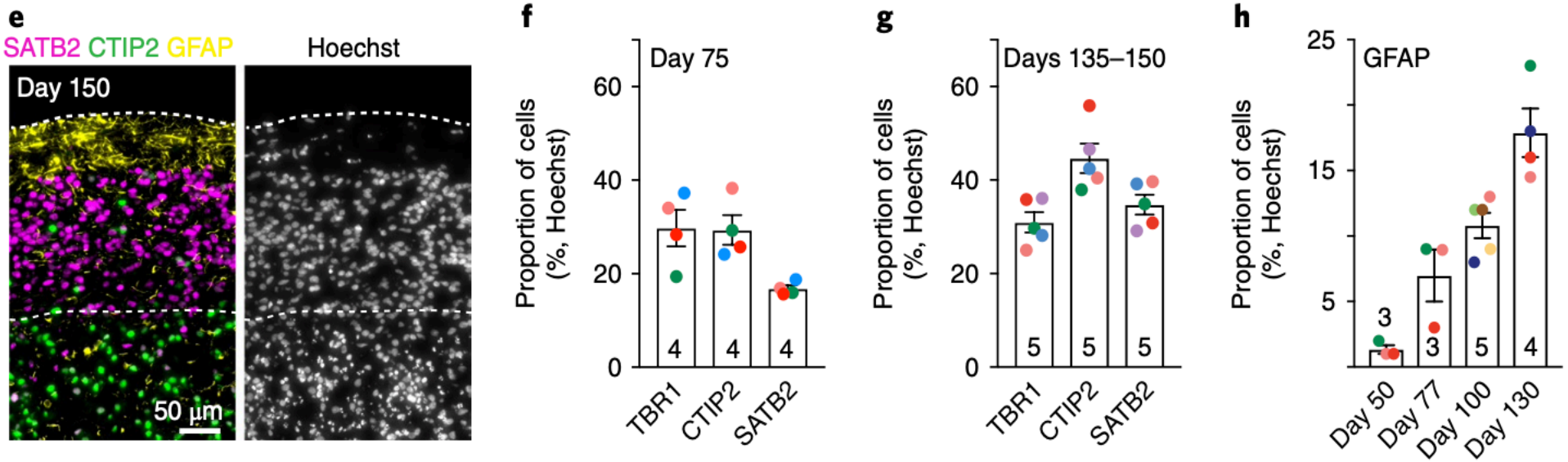

- SATB2: superficial layer marker
- CTIP2: deep layer
- GFAP: astrocytes
- 150 day—old hCS-FF section

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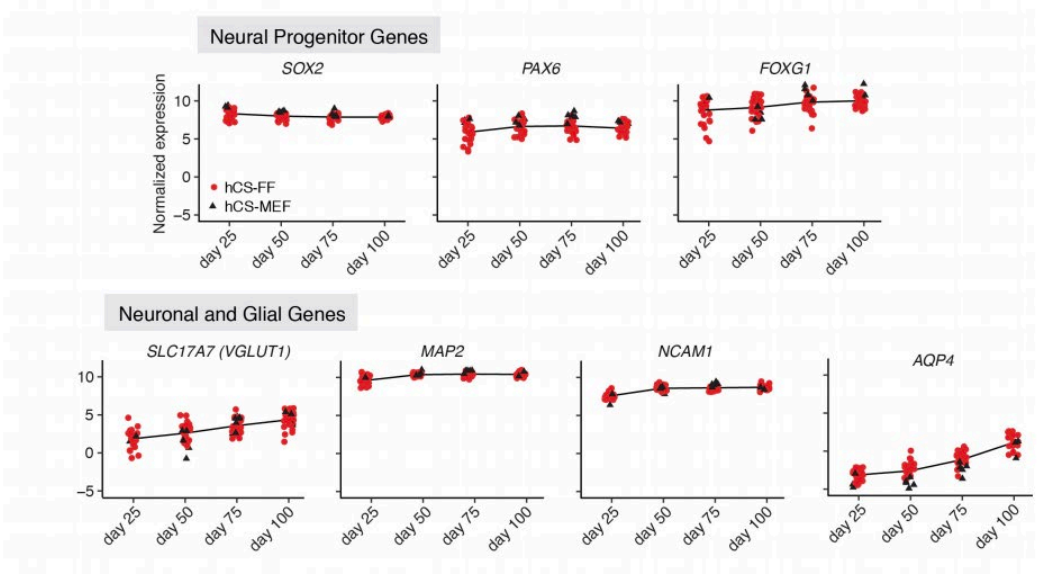

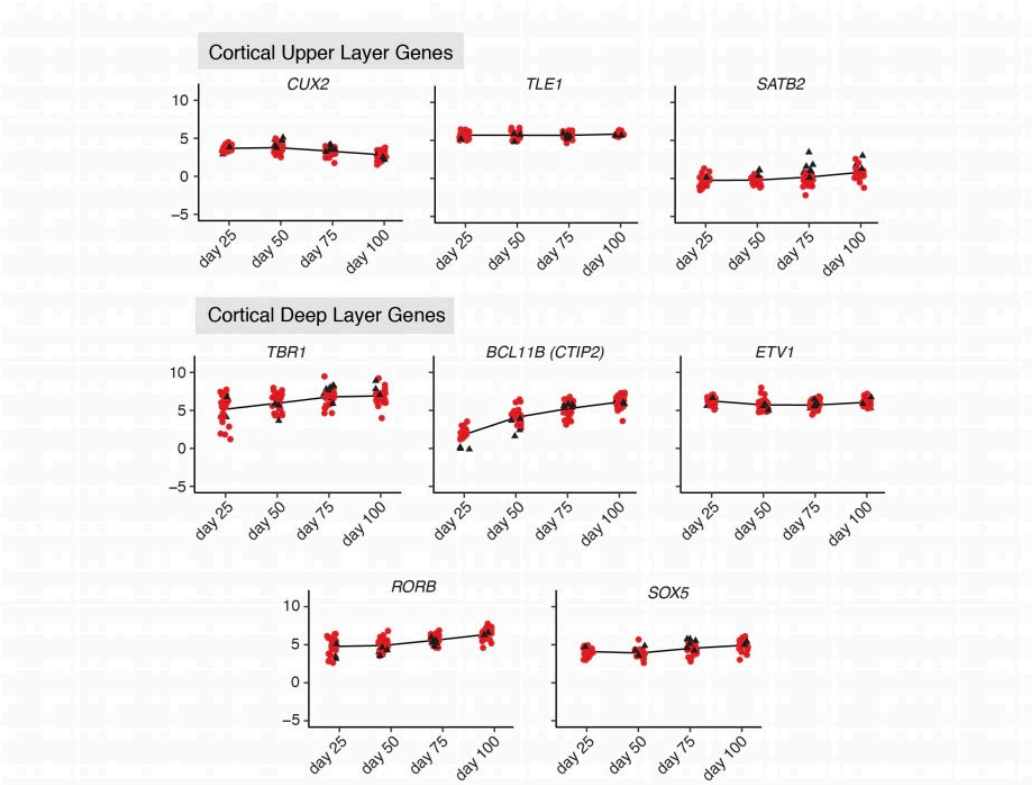

- temporal trajectories of cortical markers across the development time (25-100 days)

→ hCS-FF and hCS-MEF cultures all show consistency in development

summary

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- cortical organoids vary vastly in terms of their generation (protocols, equipment for 3D cultures, equipment for maturing etc.)
- directed differentiation approaches to generate organoids perform relatively better than self-organising whole brain organoids
- reproducibility of organoids does not represent an issue with the presented protocols, however it would potentially help to standardize protocols

A human tissue screen identifies a regulator of ER secretion as a brain size determinant

Christopher Esk[1]*, Dominik Lindenhofer[1]*, Simon Haendeler[1, 2], Roelof A. Wester[1], Florian Pflug[2], Benoit Schroeder[2], Joshua A. Bagley[1], Ulrich Elling[1], Johannes Zuber[3,4], Arndt von Haeseler[2, 5], Jürgen A. Knoblich[1,4]†

- first report of a CRISPR-Cas9 based LOF screen in organoids
- CRISPR-LICHT: CRISPR-lineage tracing at cellular resolution in heterogeneous tissue
- limited screen: 172 microcephaly candidate genes

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- large coverage of individual gRNAs (ie: high transfection/transduction rate)
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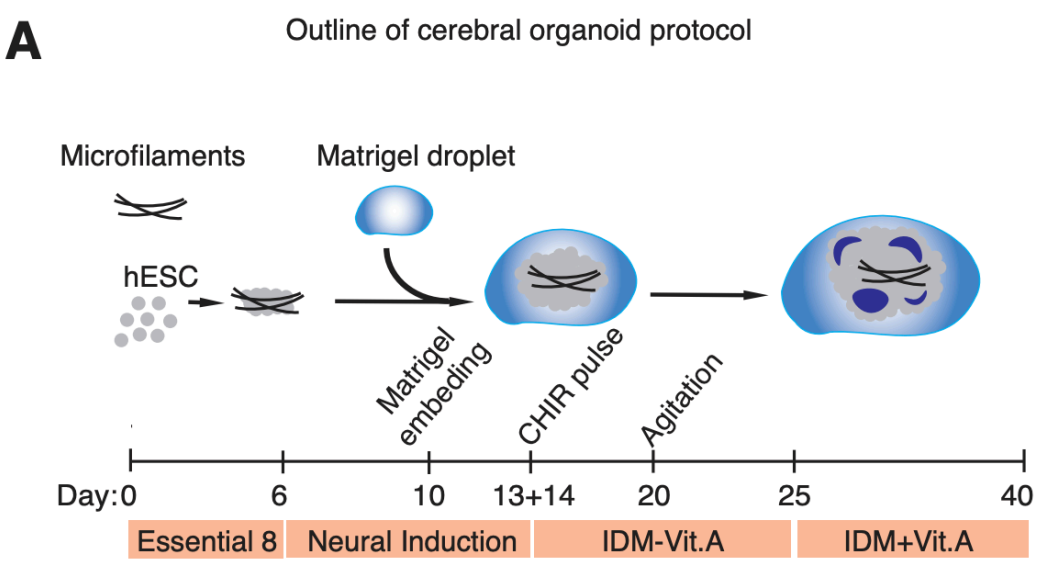
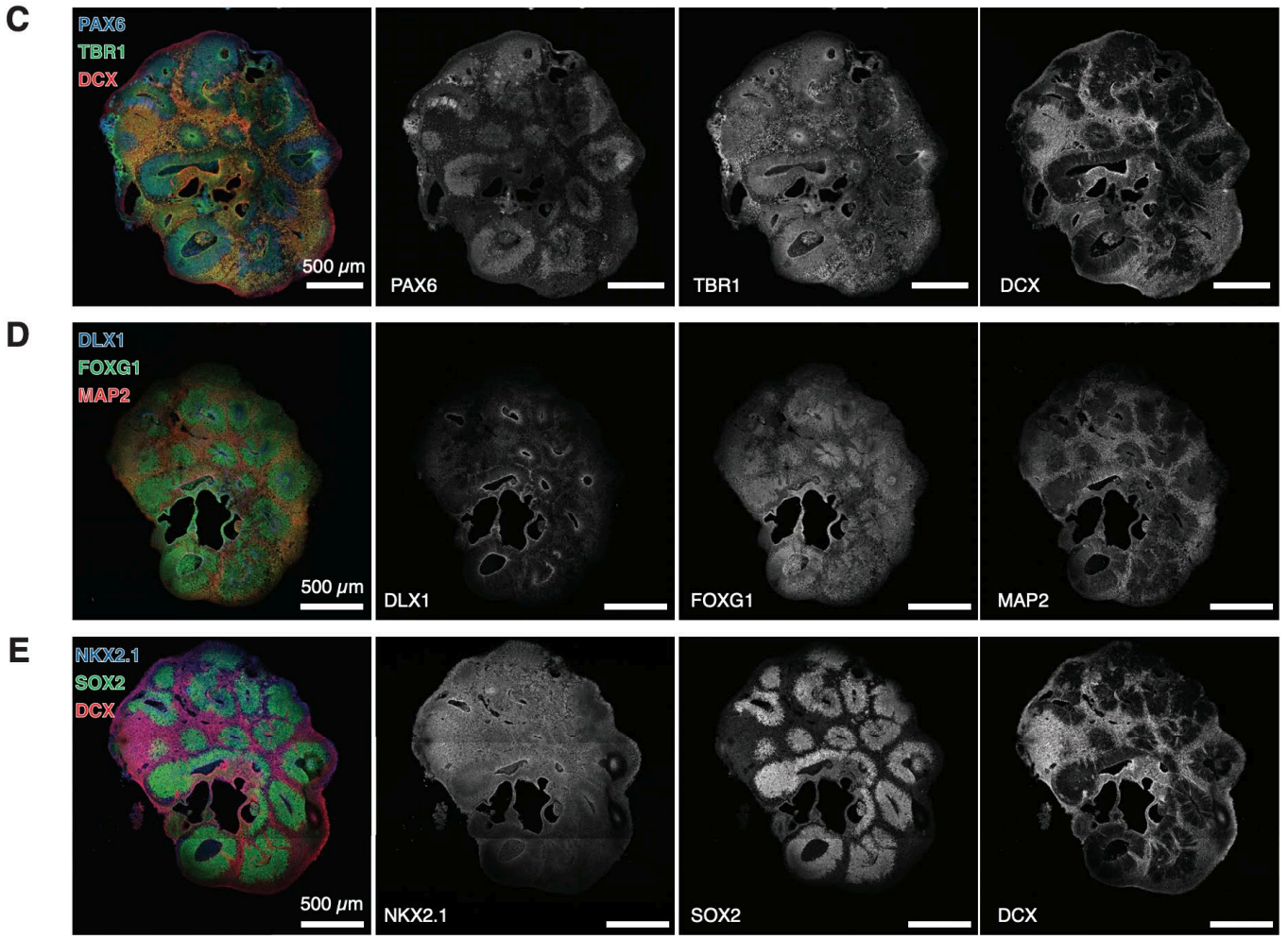
challenges in an organoid model

- heterogenous cell population
- limited starting cell amount leading to low gRNA coverage
- moderate phenotype in the microcephalic cell-loss phenotype

A human tissue screen identifies a regulator of ER secretion as a brain size determinant

Christopher Esk[1]*, Dominik Lindenhofer[1]*, Simon Haendeler[1, 2], Roelof A. Wester[1], Florian Pflug[2], Benoit Schroeder[2], Joshua A. Bagley[1], Ulrich Elling[1], Johannes Zuber[3,4], Arndt von Haeseler[2, 5], Jürgen A. Knoblich[1,4]†





→ predominantly dorsal forebrain identity

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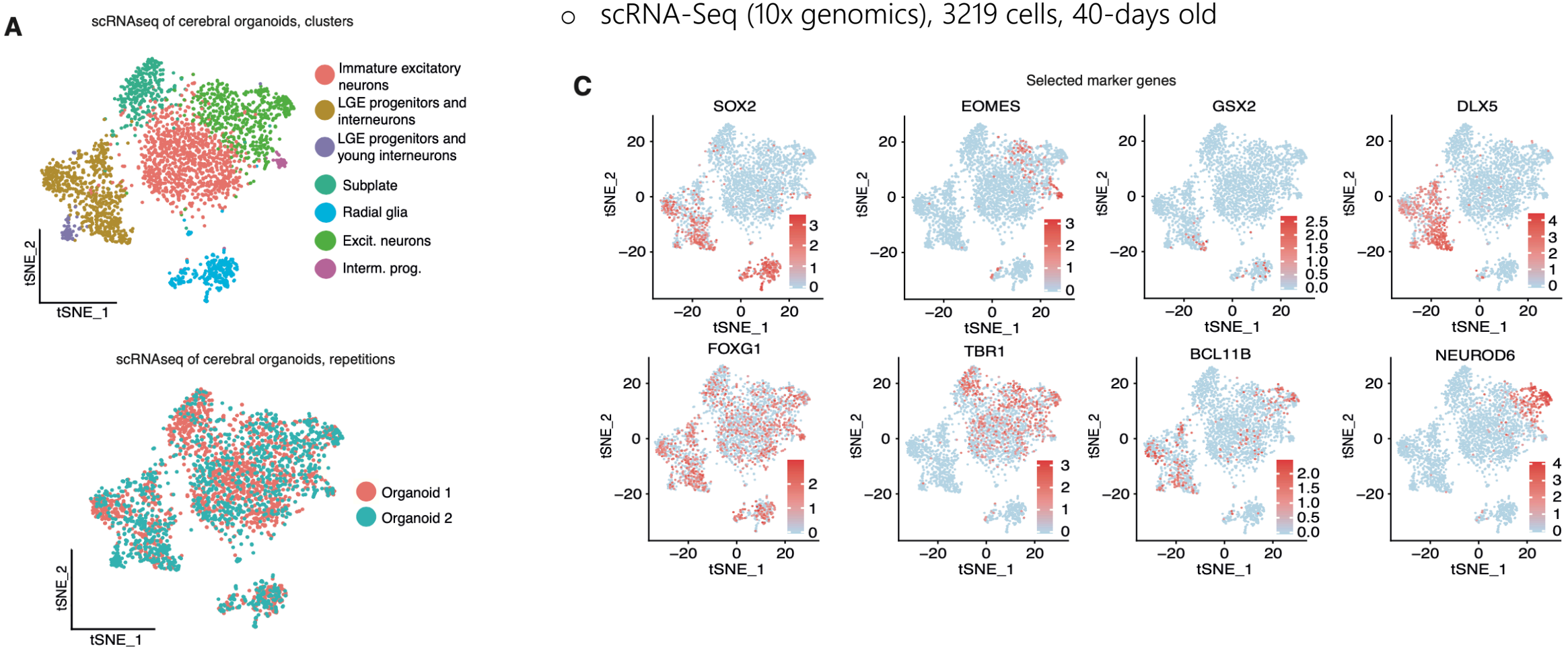
- scRNA-Seq (10x genomics), 3219 cells, 40-days old



→ confirmation of dorsal forebrain identity and enrichment of excitatory neurons

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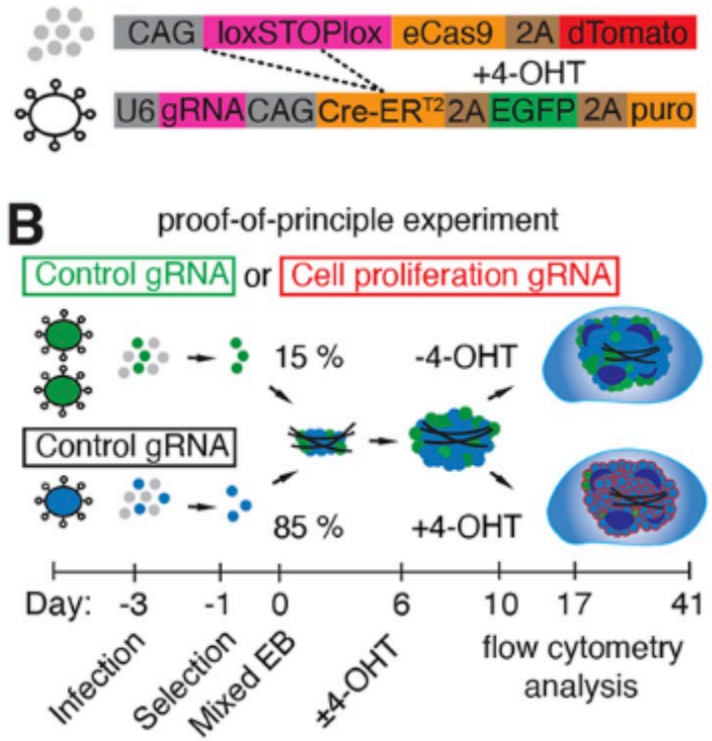

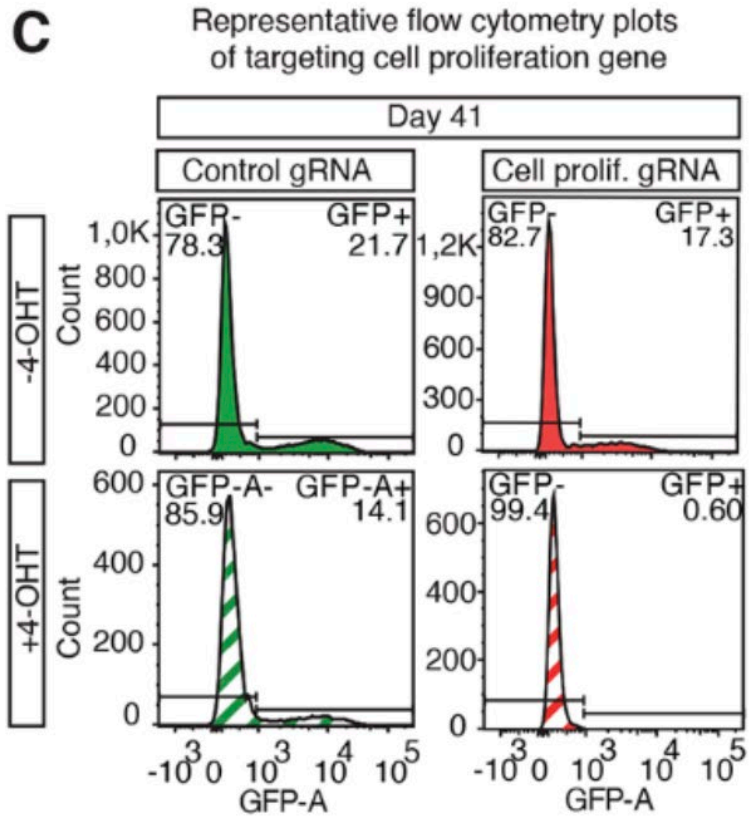

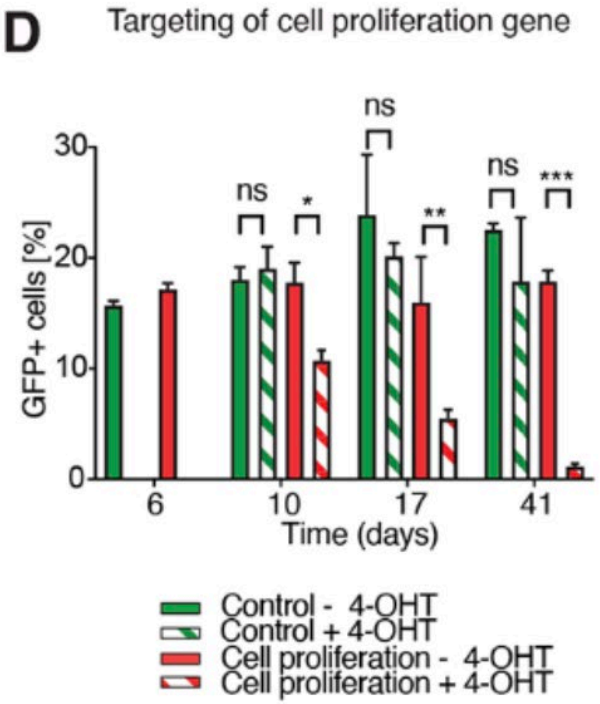

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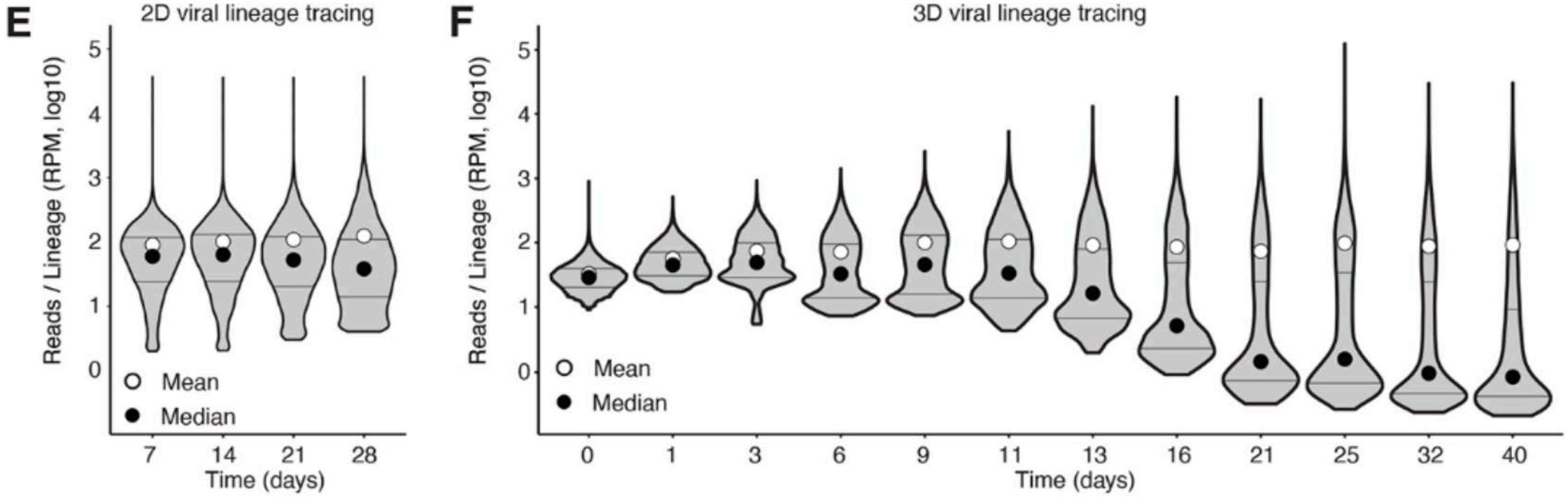
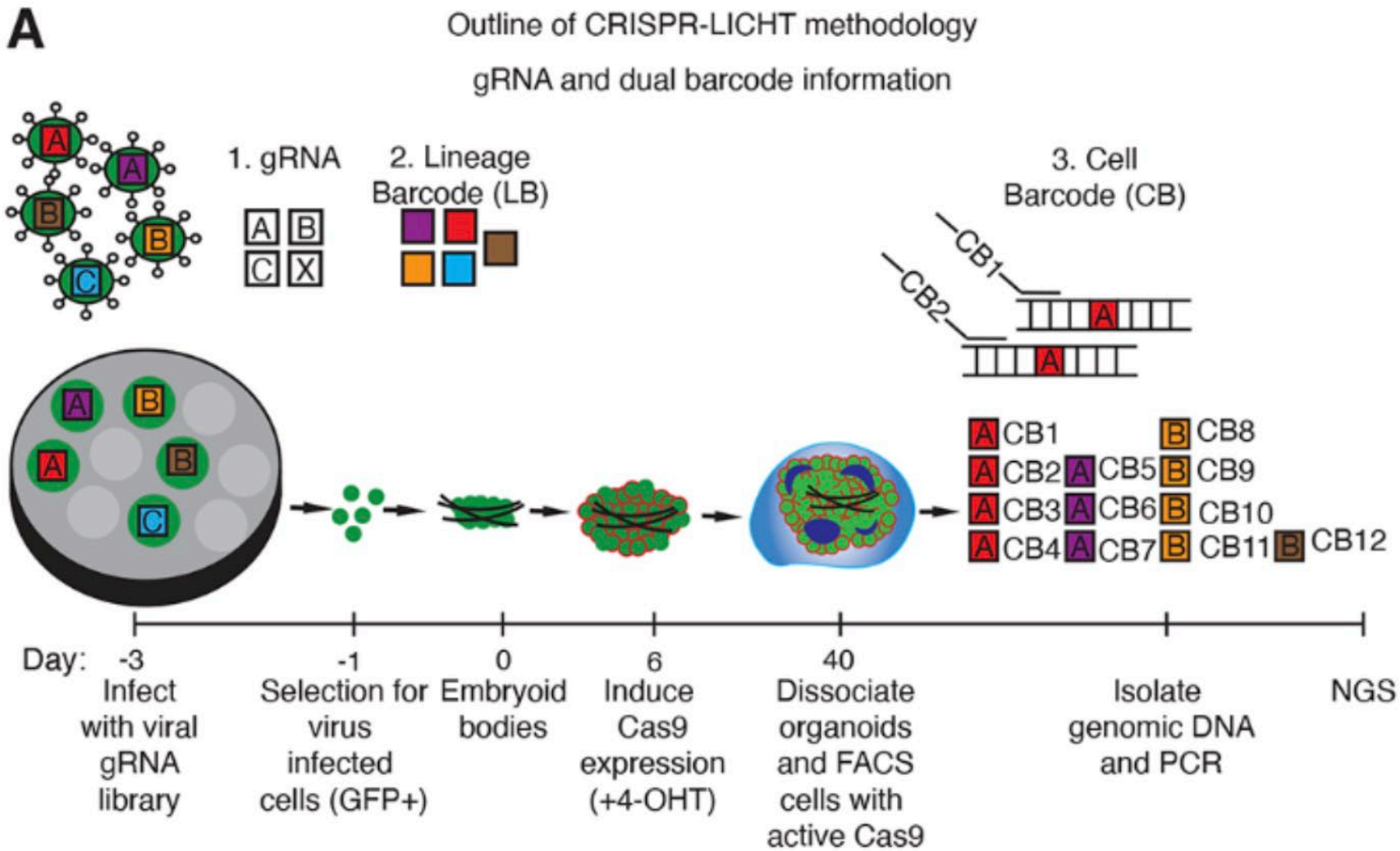
- a difference in 2D vs 3D cultures is the uniform vs non-uniform growth of cells
- in pooled screens variability in cell growth cannot be distinguished from gRNA mediated true KO events.
- to determine the dynamics of cell growth in organoids– lineage tracing using barcoded DNA



→ difficult to differentiate cell number changes caused by inherent variability from those caused by genetic modulation



→ dual barcoding allows overcoming the problems of variable tissue, unequal lineage growth and low readout sensitivity

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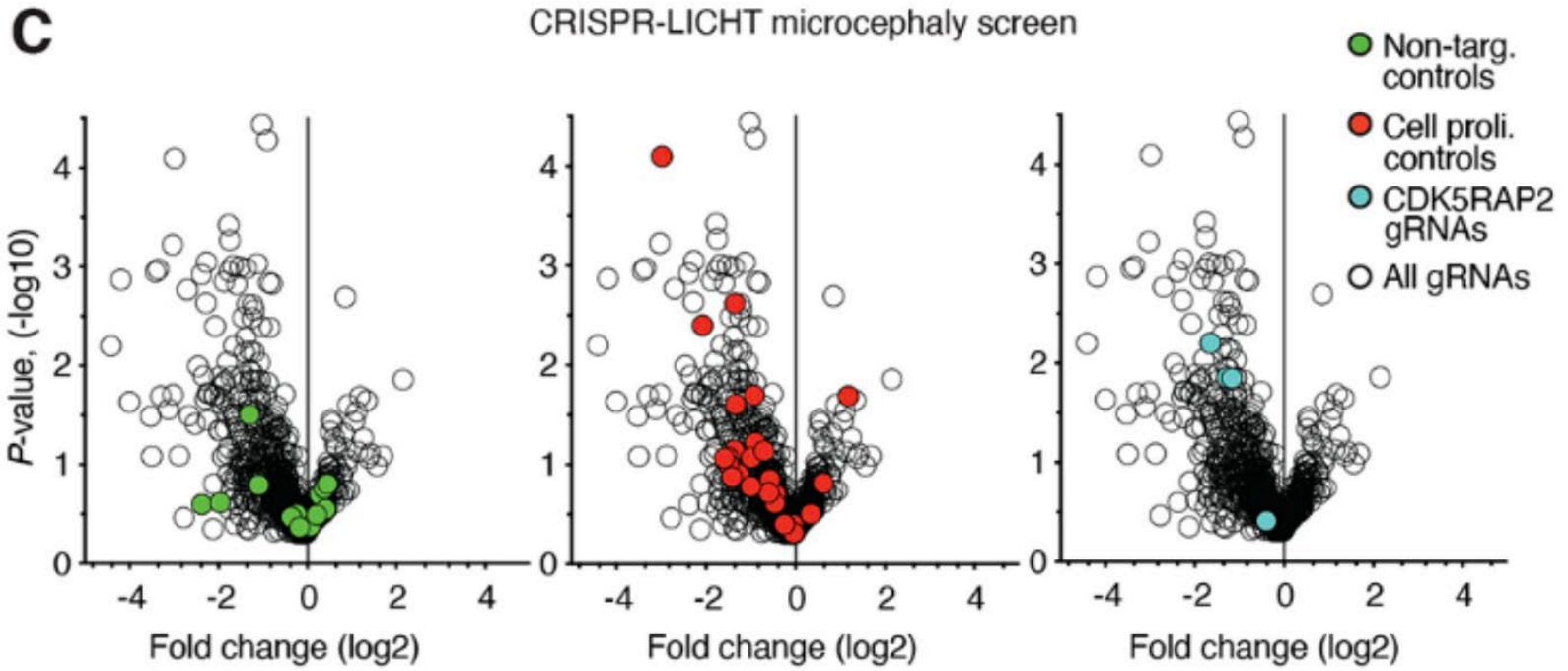
- selected 172 candidate genes from developmental brain disorder database(DBDB) and a clinical panel
- genes are ordered into categories LOE (level of evidence) 1-3 linking them to microcephaly
- 4 gRNAs per target gene as well as a non-targeting control and a cell proliferation control packaged into a pooled lentiviral library



→ QC score acceptable for the initial screen and the authors were able to identify a known microcephaly gene (CDK5RAP2)

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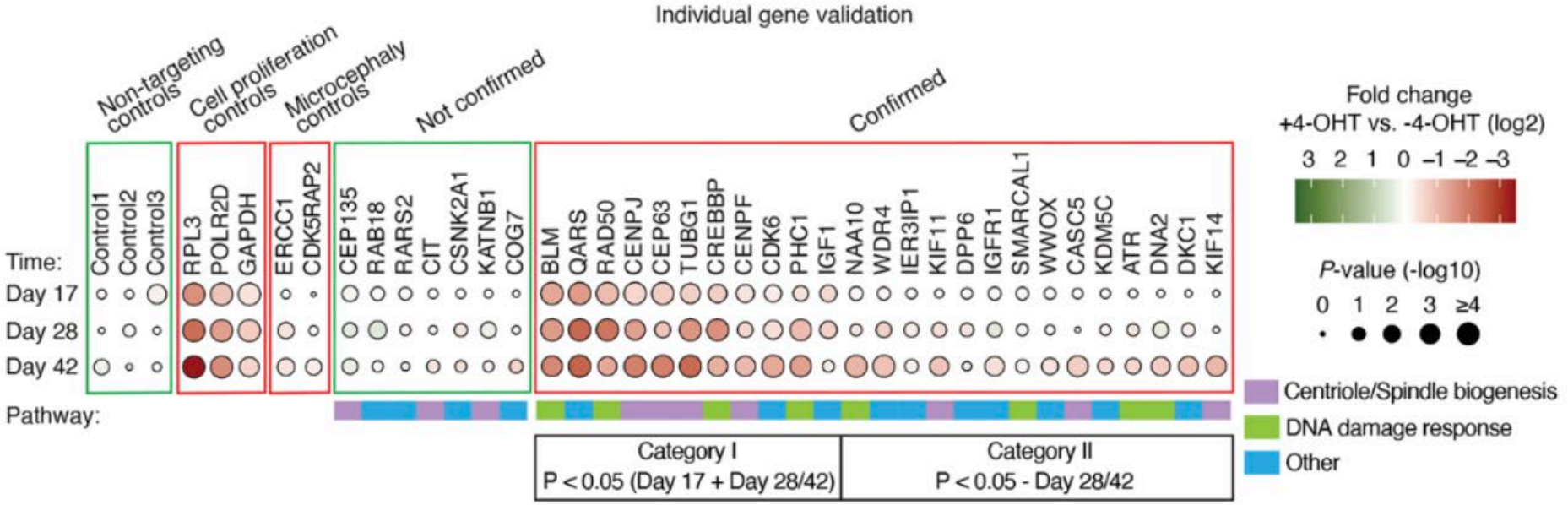
- for hit selection authors ranked the 172 genes and selected 32 LOE2 or LOE3 genes with at least 2/4 gRNA efficiency
- furthermore, they tried to validate the 32 genes with individual gRNA validations and ended up with 25 genes
- most of the 25 hits were involved, not surprisingly, in centriole biogenesis and DNA damage response



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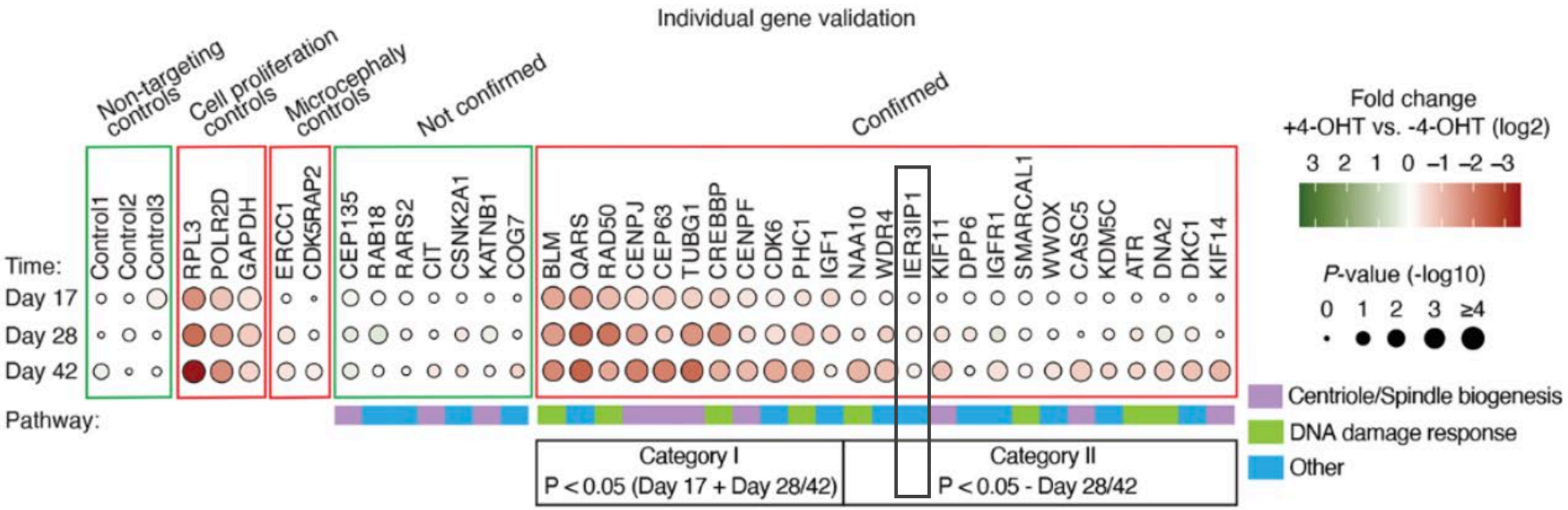

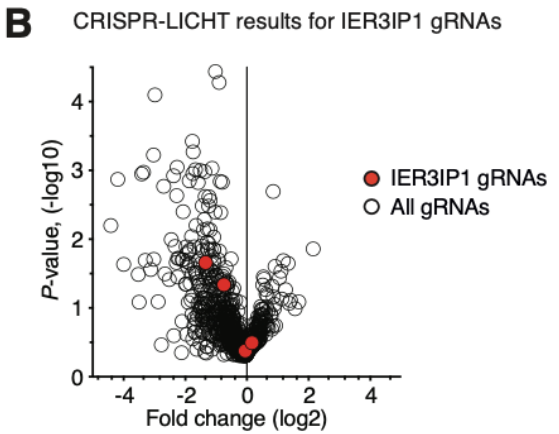

→ focus on IER3IP1, which possesses two interluminally connected ER transmembrane domains, reported to be mutated in patients

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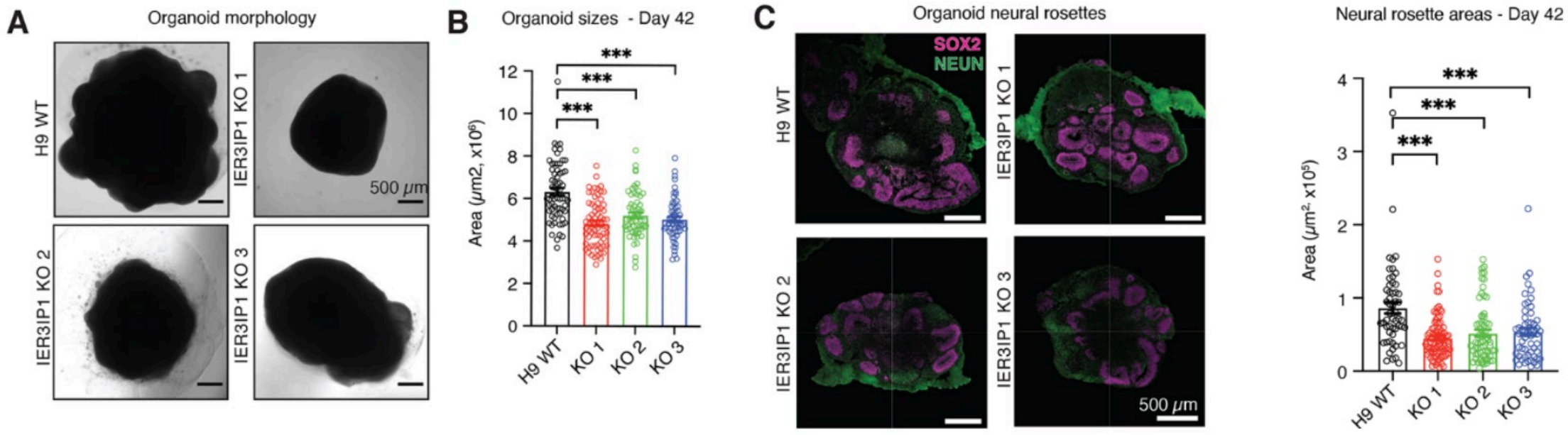
validation experiments:

- 3 hESC lines with a LOF mutation in IER3IP1



→ organoid morphology affected by the KO (day 42)

→ neural rosette area was smaller in the KO lines compared to the WT organoids, indication for neural progenitor loss

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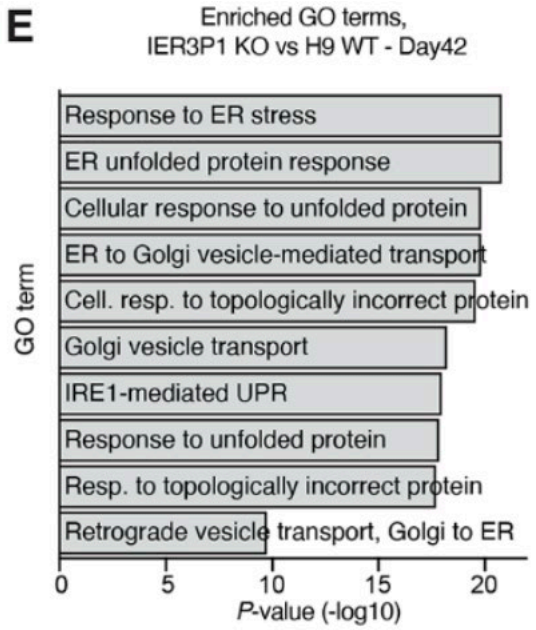
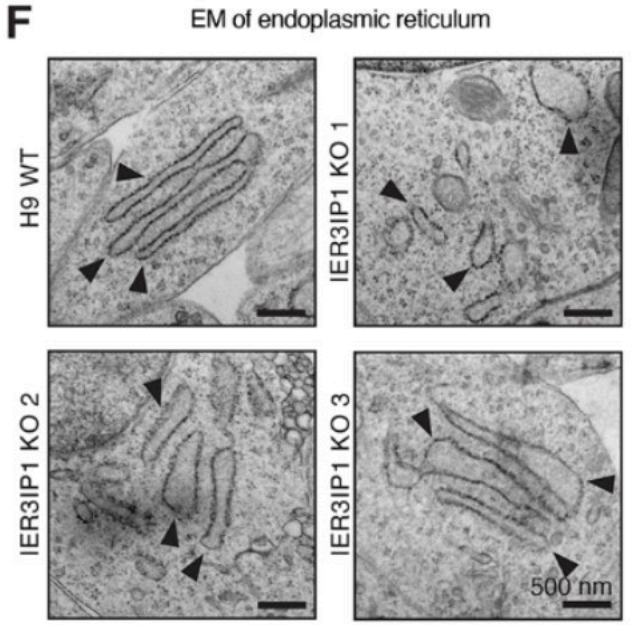
validation experiments:

- RNA-Seq of KO and Wt organoids at three timepoints (0, 17, 28, 42 days)





→ GO analysis of significantly changed genes at 42 days (however not significant)

→ ER width is altered, which is due potentially to ER stress

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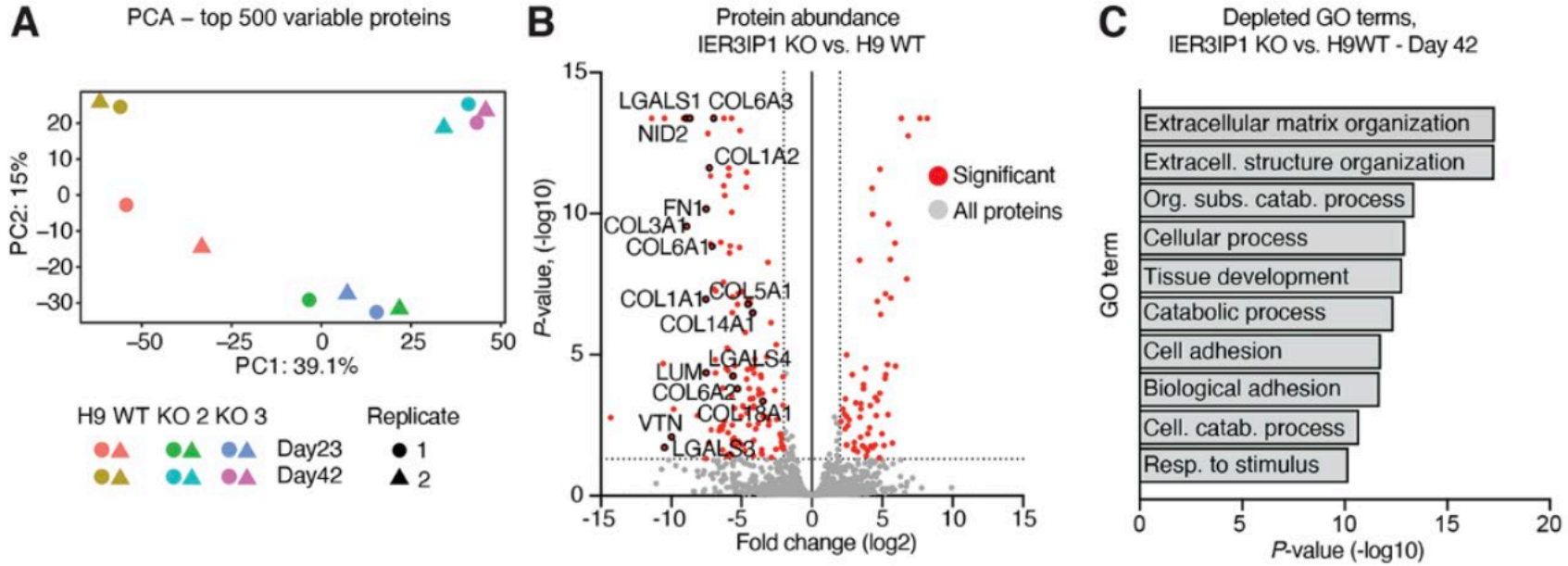
validation experiments:

- as IER3IP1 (and its yeast homolog) functions in ER-Golgi transport authors wanted to see if upon the KO any other cargo proteins were affected
- MS of KO vs WT organoids (day 23 and 42):



→ many ECM related proteins are altered in the KO organoids

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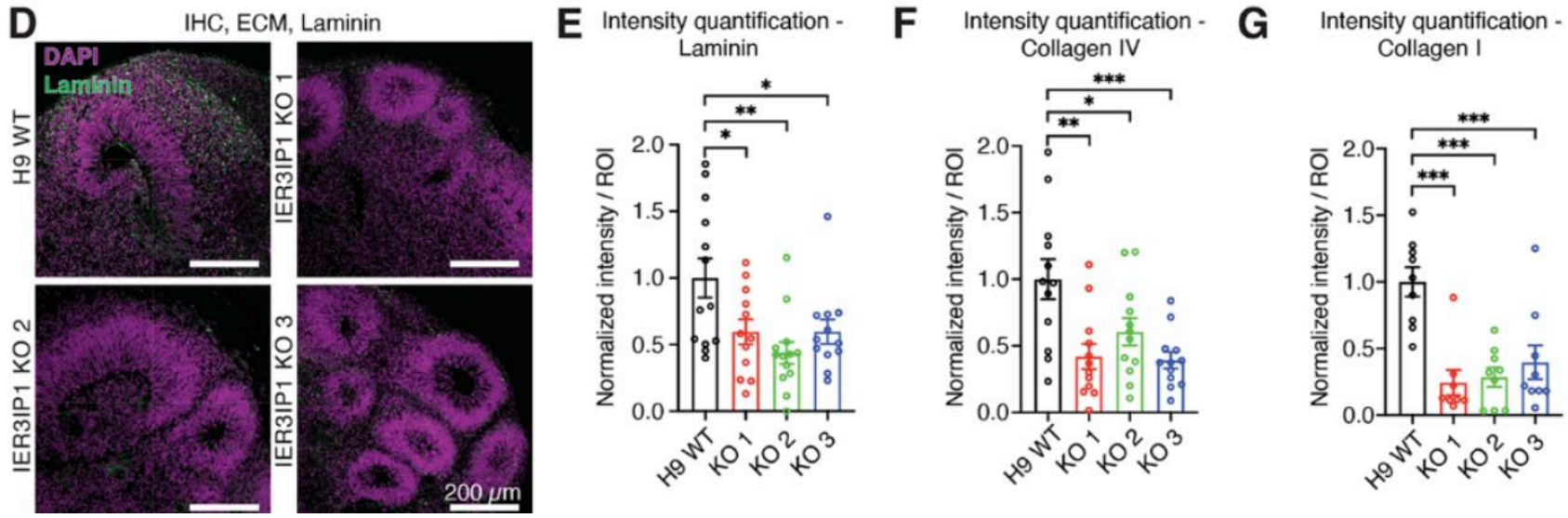
validation experiments:

- IHC of MS identified ECM proteins



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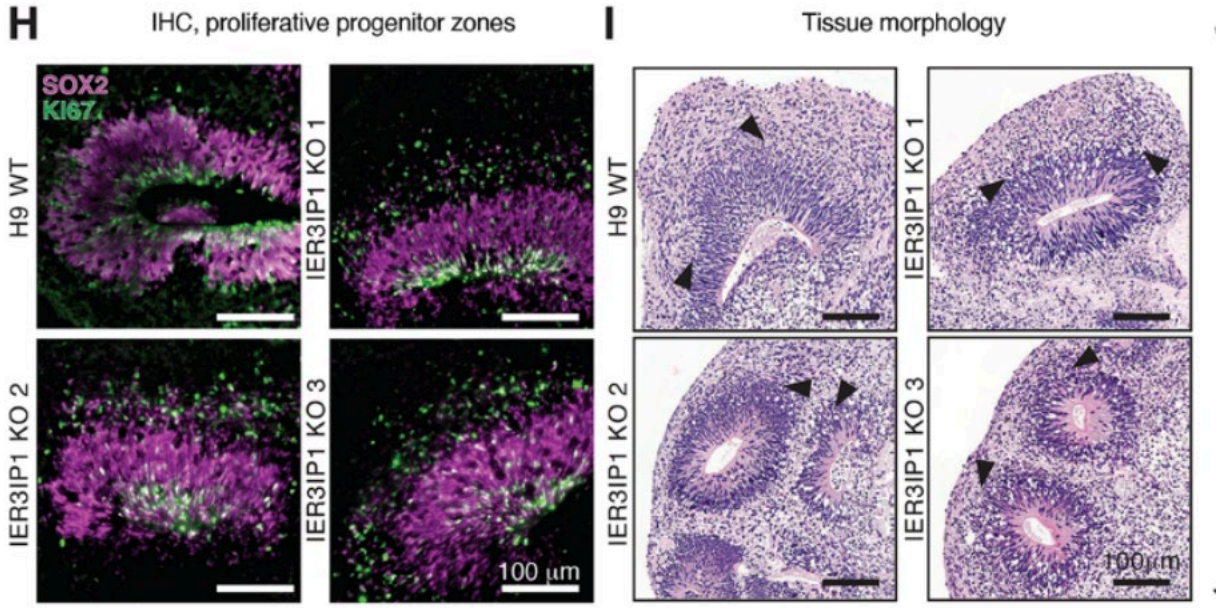
loss of ECM proteins in mice result in premature differentiation and neural progenitor loss

- stainings for neural progenitor cell markers (SOX2, KI67, PAX6)



→ abnormal localization of the progenitor markers outside of the ventricular-like proliferative neural rosettes suggesting shedding of neural progenitors

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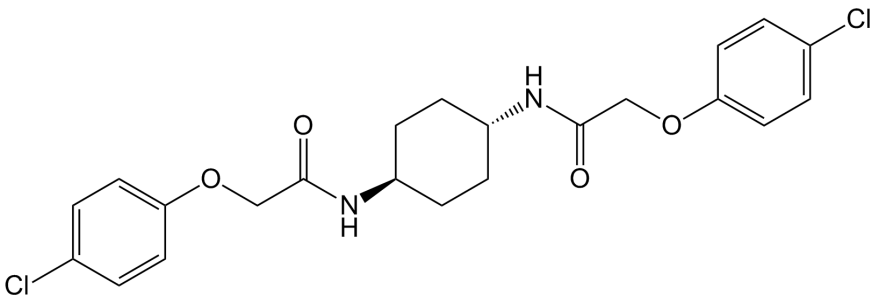
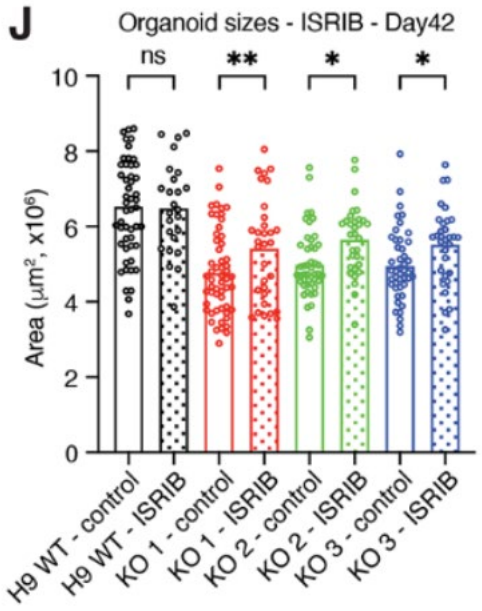
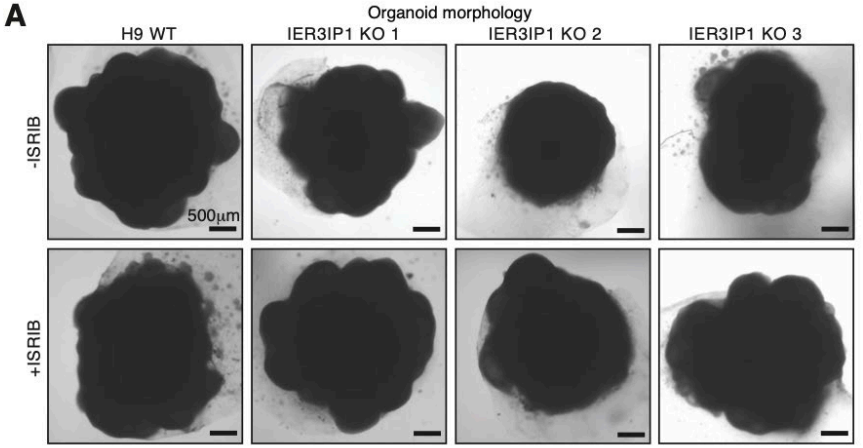
IER3IP1 loss → reduced ECM deposition → compromising integrity of neural rosettes → premature neurogenesis

- can the phenotype be pharmacologically reversed?



ISRIB, restores UPR mediated translation inhibition





→ improves organoid size as well as neural rosette size

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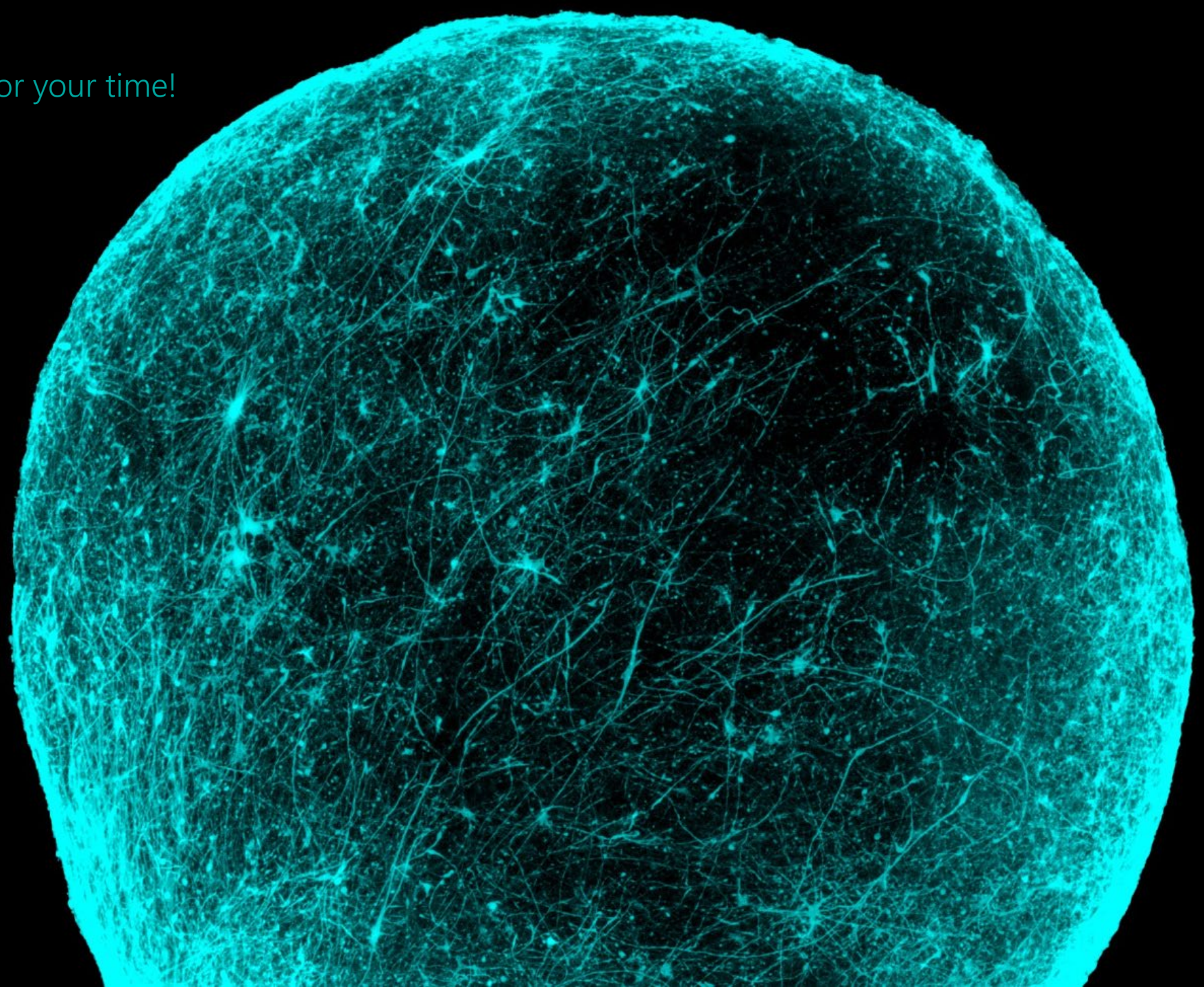
summary:

- → first paper to report a screen in the organoid model
- → dual barcoding gives control over cell lineages in the organoid model

- → biased set of genes
- → validation experiments can be criticized for lacking other starting hESC or hiPSC lines (or patient cells)
- → would have been interesting to check if ISRIB restores the ECM protein content

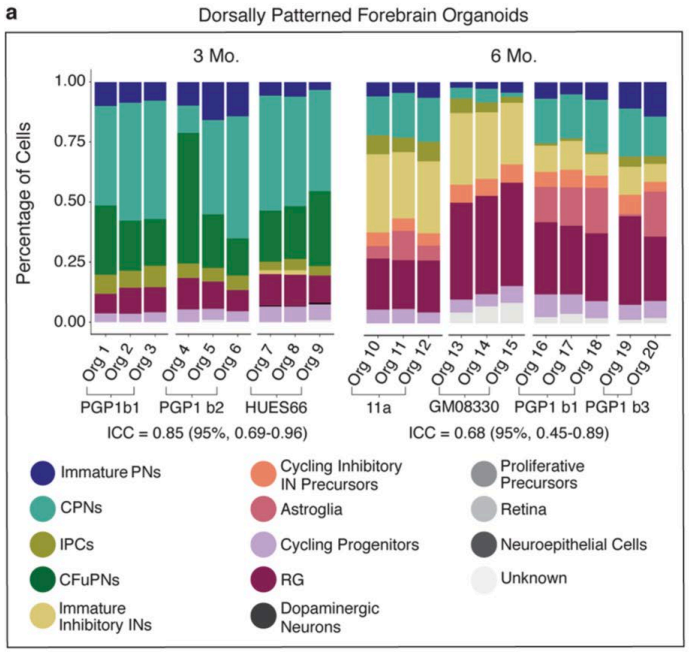
Thank you for your time!

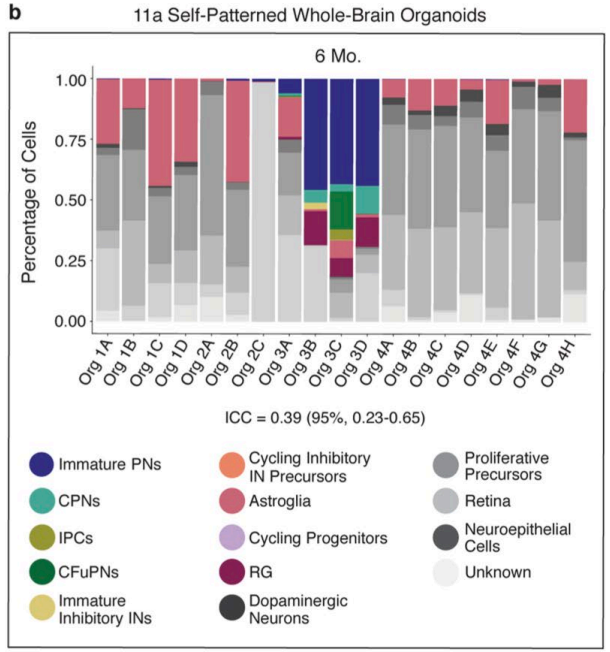


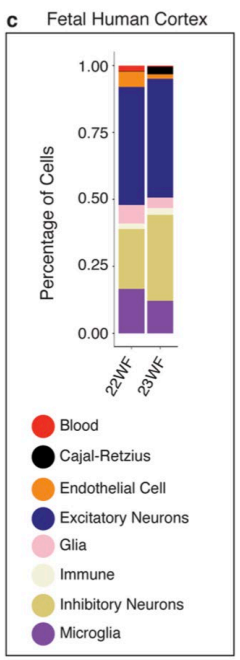
Individual brain organoids reproducibly form cell diversity of the human cerebral cortex

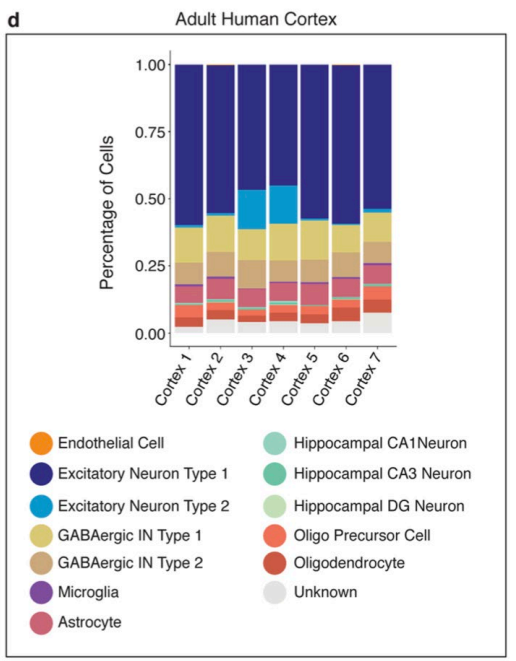
Silvia Velasco[1,2], Amanda J. Kedaigle[1,2,3], Sean K. Simmons[2,3], Allison Nash[1,2], Marina Rocha[1,2], Giorgia Quadrato[1,2,4], Bruna Paulsen[1,2], Lan Nguyen[3], Xian Adiconis[2,3], Aviv Regev[3,5], Joshua Z. Levin[2,3] & Paola Arlotta[1,2]*

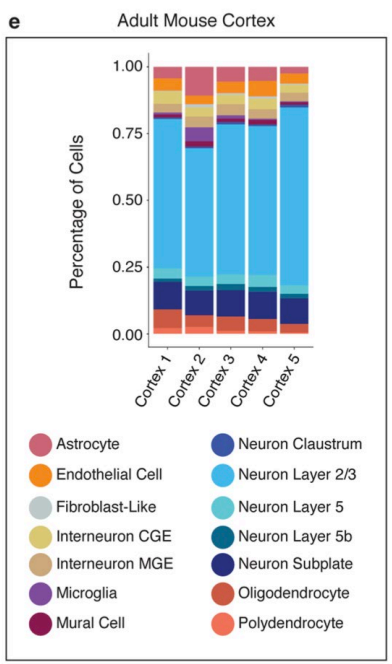
do organoids show the same degree of individual brain-to-brain differences seen in human and mouse brains?











→ dorsal directed organoids show a similar variation observed in mouse and human brains
→ MI scores represent the dependence between cluster and individual (lower = similar makeup)

A PCR strategy for CB introduction