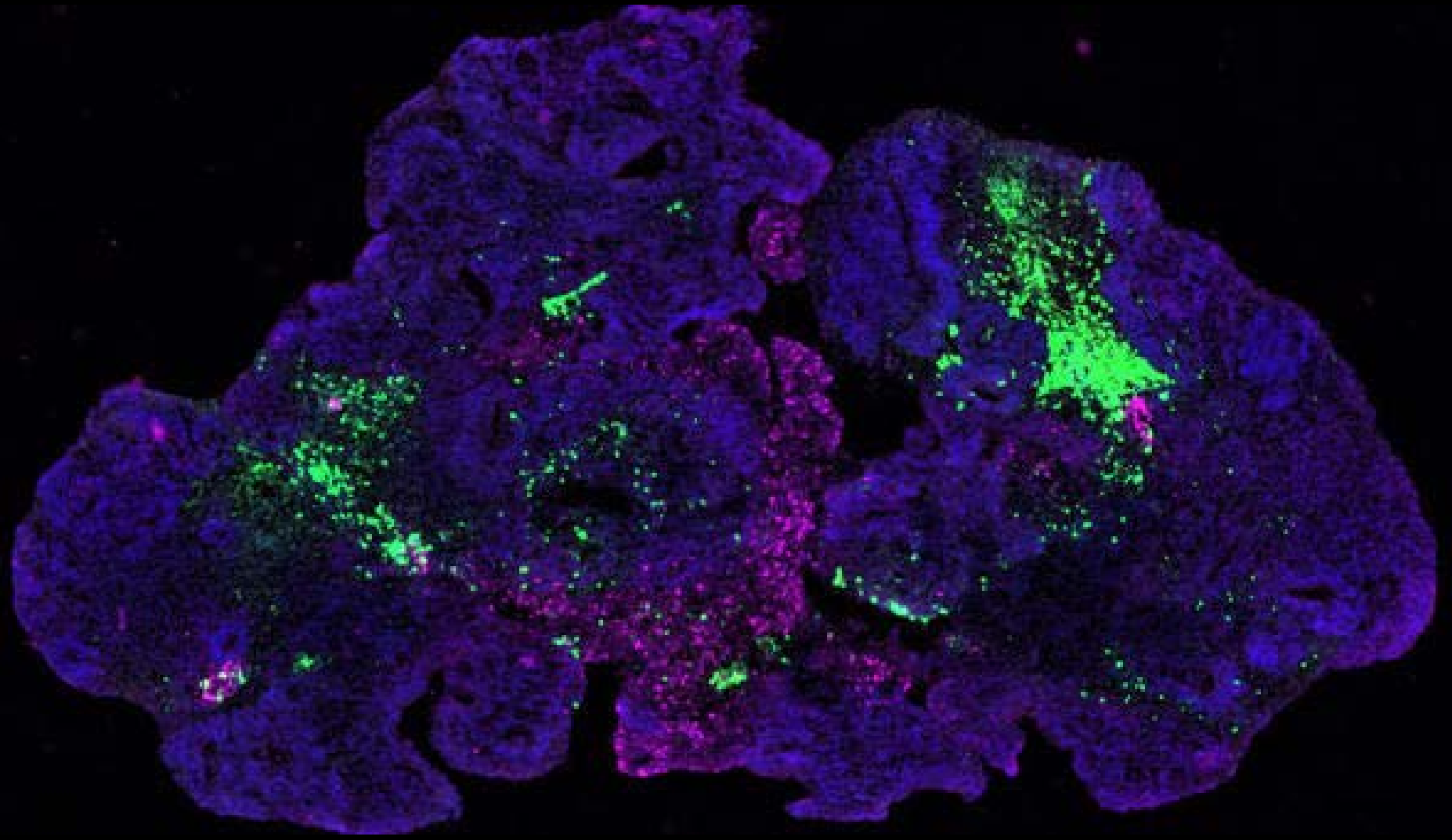
Update on Organoid Research



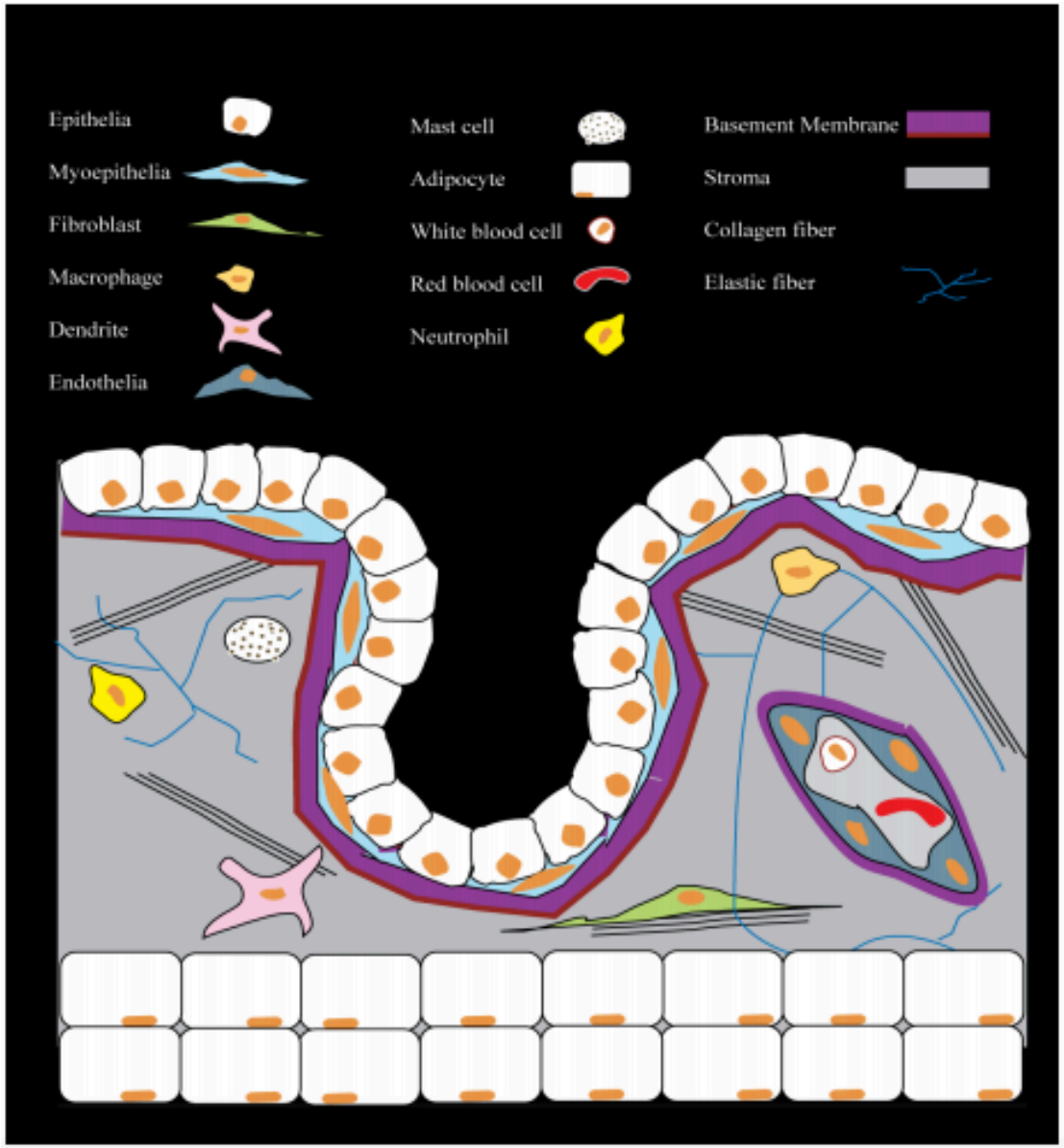
Technical Journal Club
14 Aug 2018

Asvin Lakkaraju

Why Are Preclinical Models Falling Short in Predicting Biological Response?

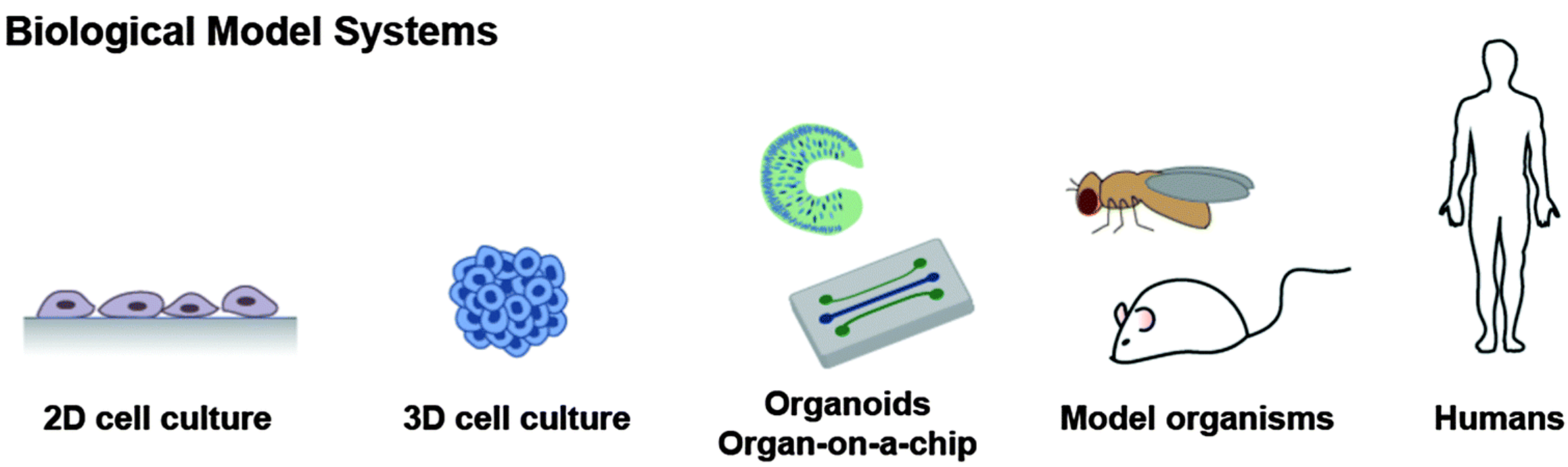
- Plastic (cell growth platforms) is not a natural component of the human body
- Mice are complex organisms.
- Understanding the pathophysiology
- These models fail to recreate the complexity and specificity of living human tissues.

Identify factors essential to recreate in vivo structure and function

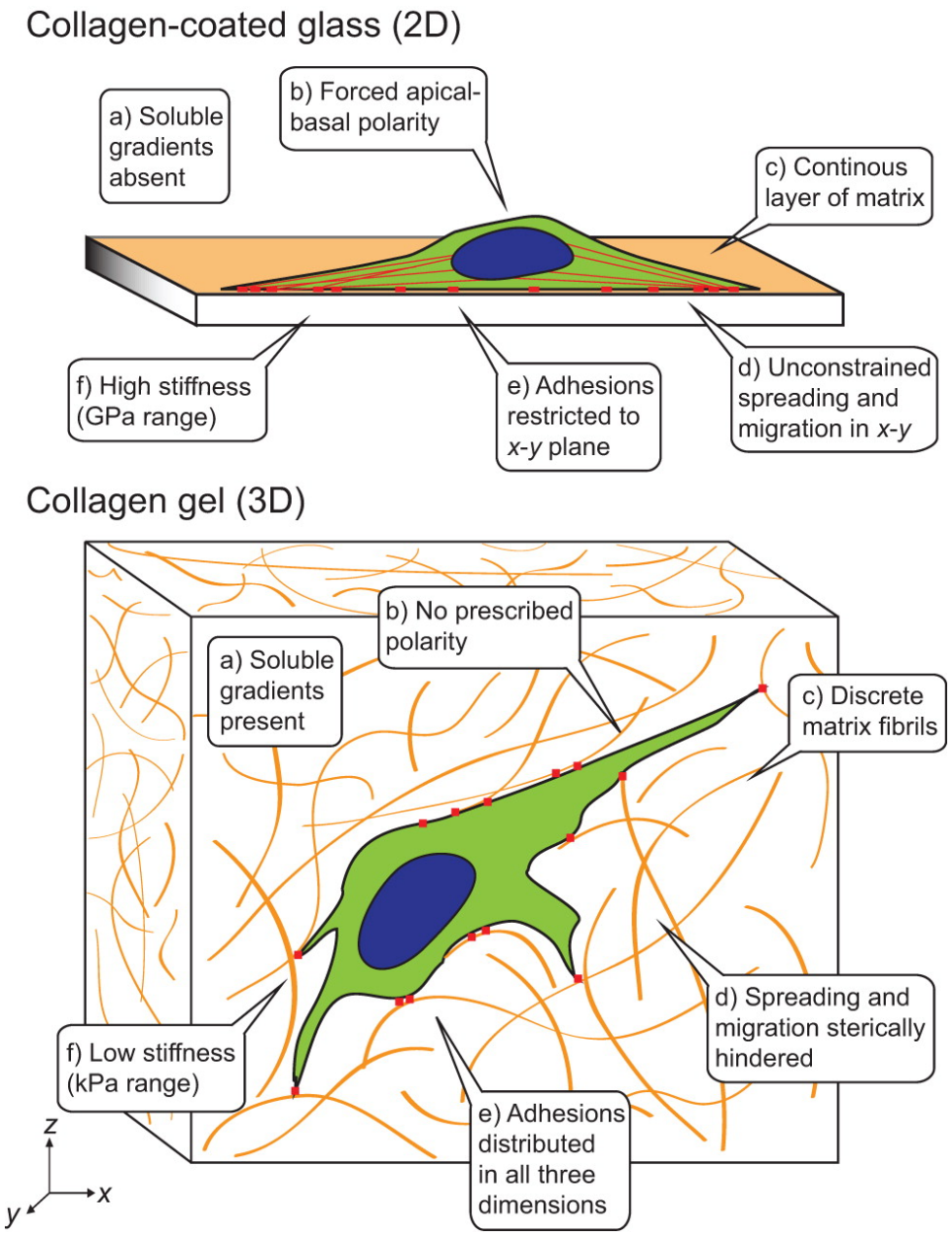


- Tissue Resident Cells
  - Types
  - Quantities
  - Organization
- Extracellular Matrix
  - Composition
  - Organization
  - Compliance
- Soluble Factors
  - Growth Factors
  - Cytokines

Relevance of our experimental set up



2D vs 3D



Disadvantages of 2D

Altered Cell Shape.

Altered functions/ signaling

Loss of polarity

Advantages of 3D Culture

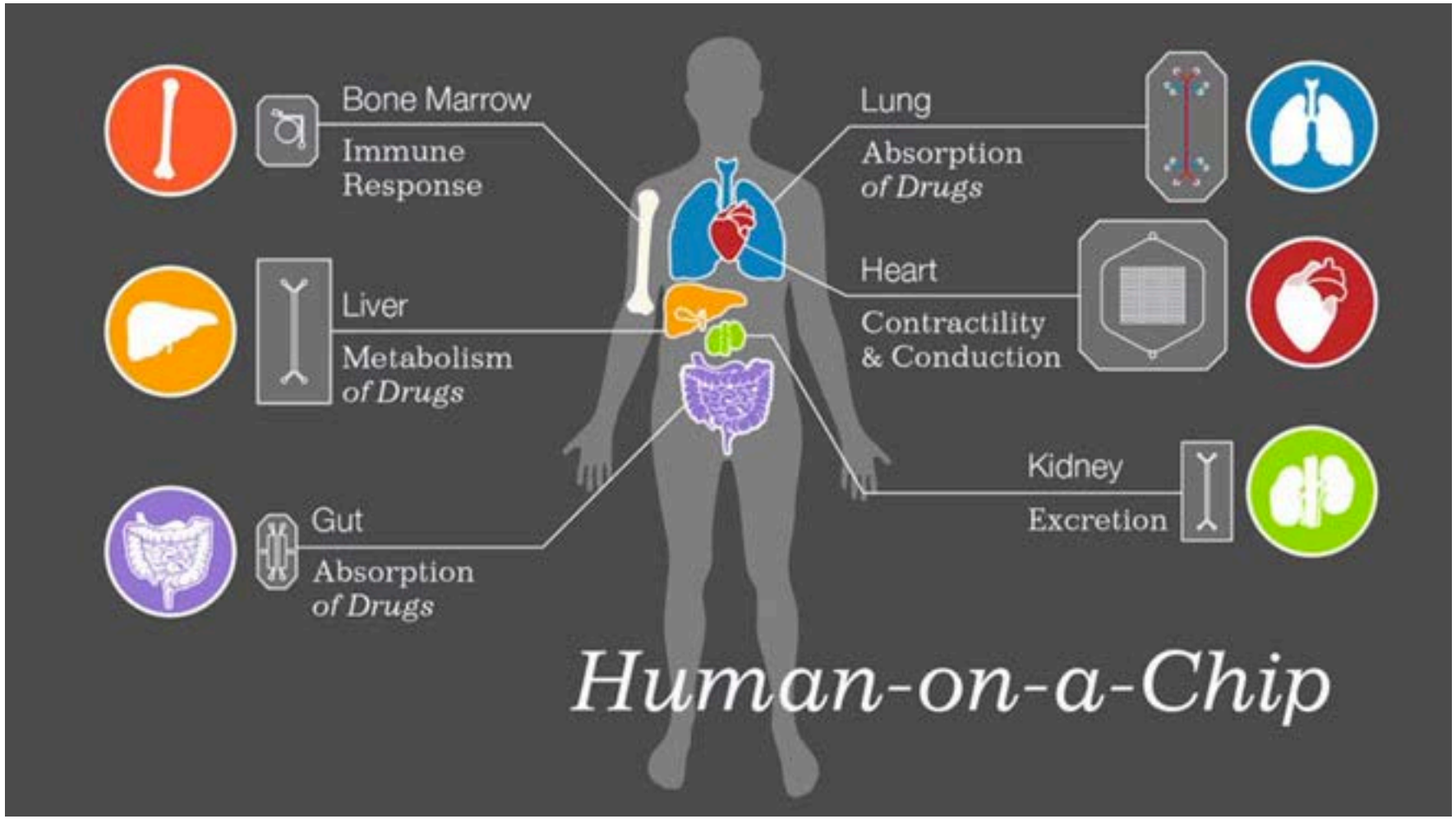
Growing cells in 3D alters cell proliferation and morphology.

Growing cells in 3D reveals a more realistic drug response.

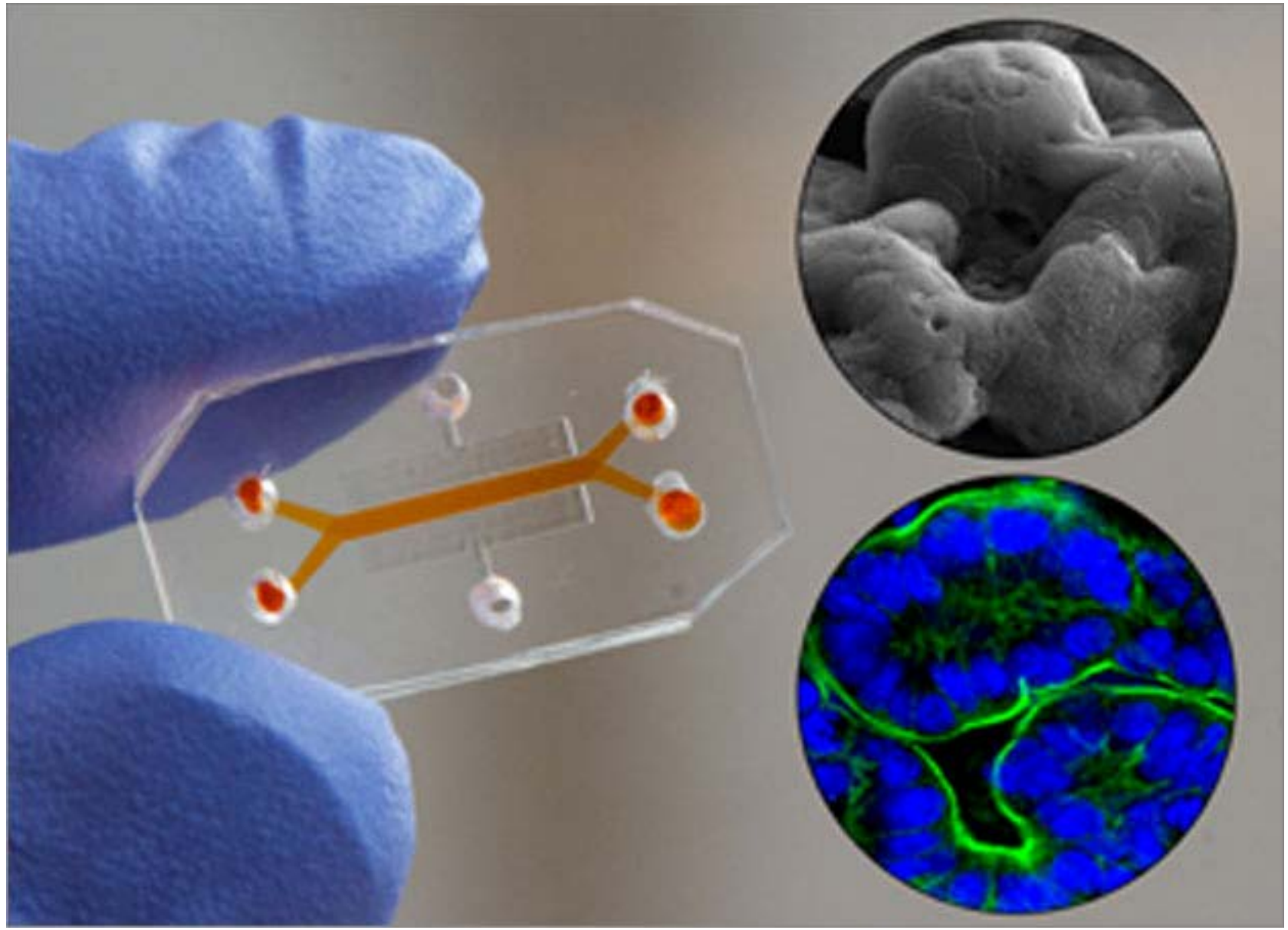
Growing cells in 3D captures phenotypic heterogeneity.

Growing cells in 3D changes gene expression and cell behavior.

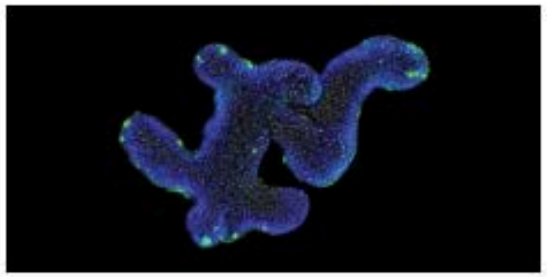
Growing cells in 3D mimics the tumor microenvironment.



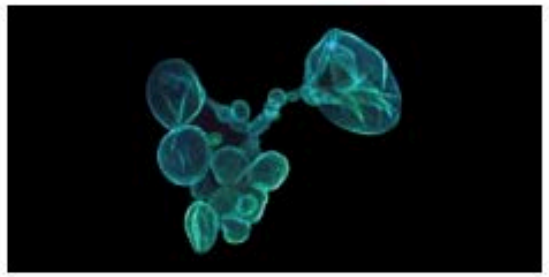
An organ-on-a-chip is a microfluidic cell culture device that contains continuously perfused chambers inhabited by living cells arranged to simulate tissue- and organ-level physiology.



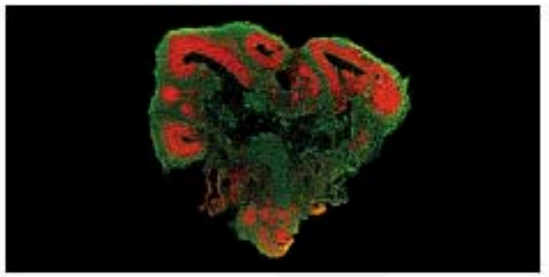
Organoids



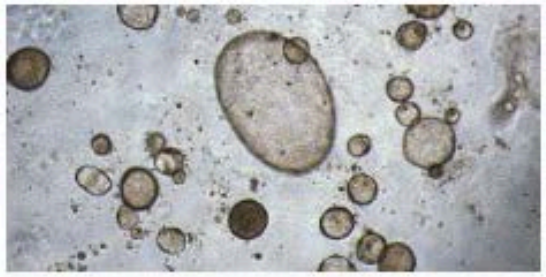
Intestinal Organoids



Hepatic Organoids



Neural Organoids



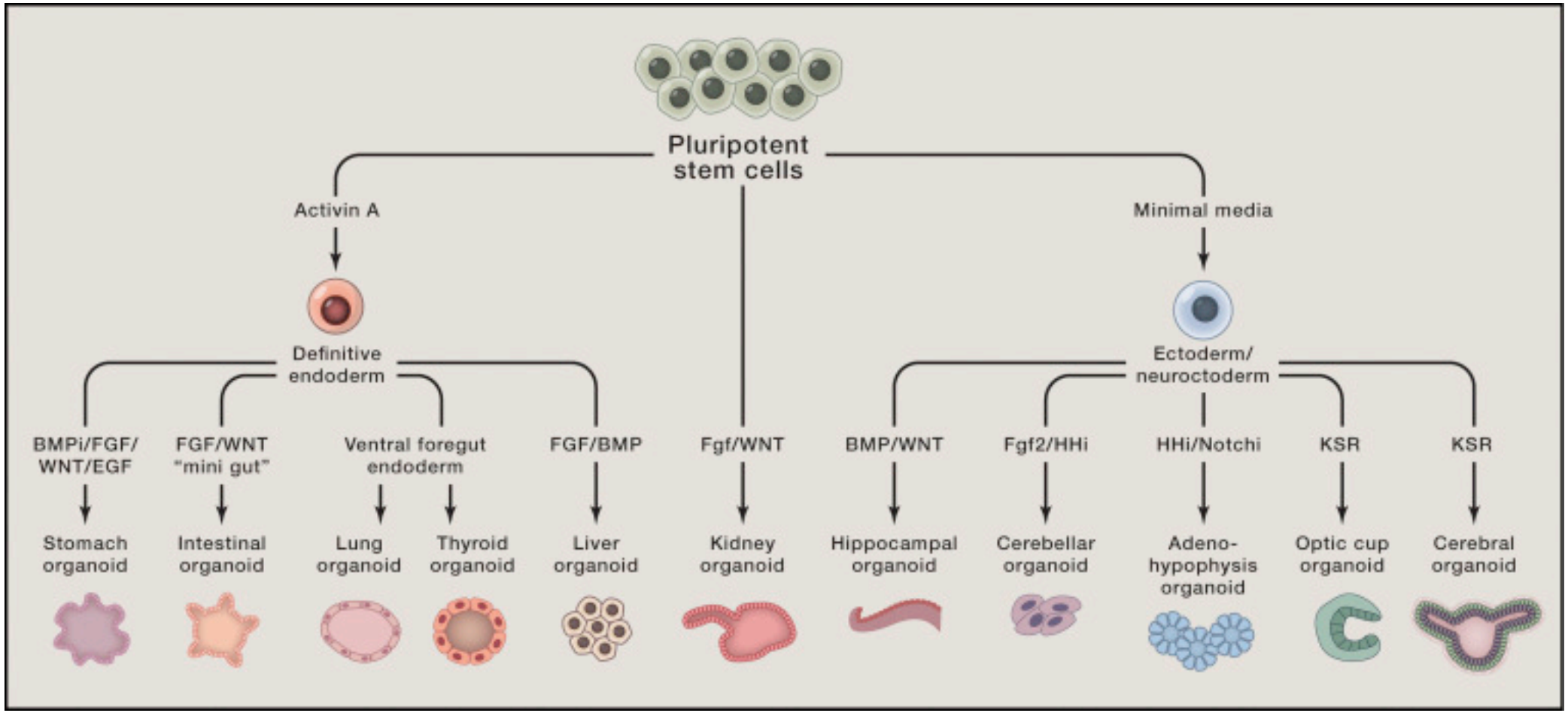
Pulmonary Organoids

Organoids are three-dimensional (3D) cell cultures that incorporate some of the key features of the represented organ.

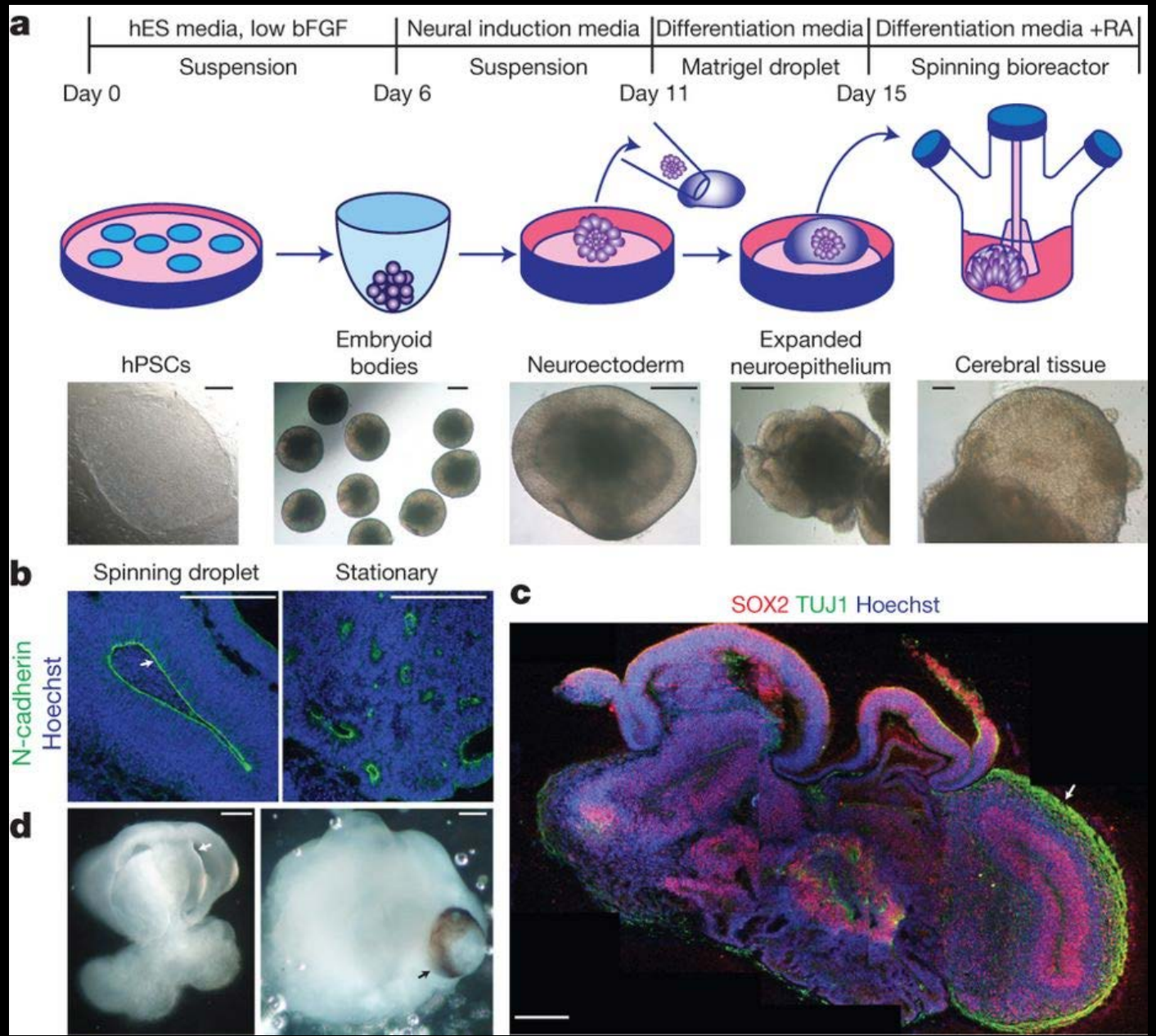
These in vitro culture systems contain a self-renewing stem cell population which differentiates into multiple, organ-specific cell types that exhibit spatial organization similar to the corresponding organ.

They are capable of recapitulating some functions of that organ providing a highly physiologically relevant system.

Usage of Organoid term in research



Brain Organoids: 2013

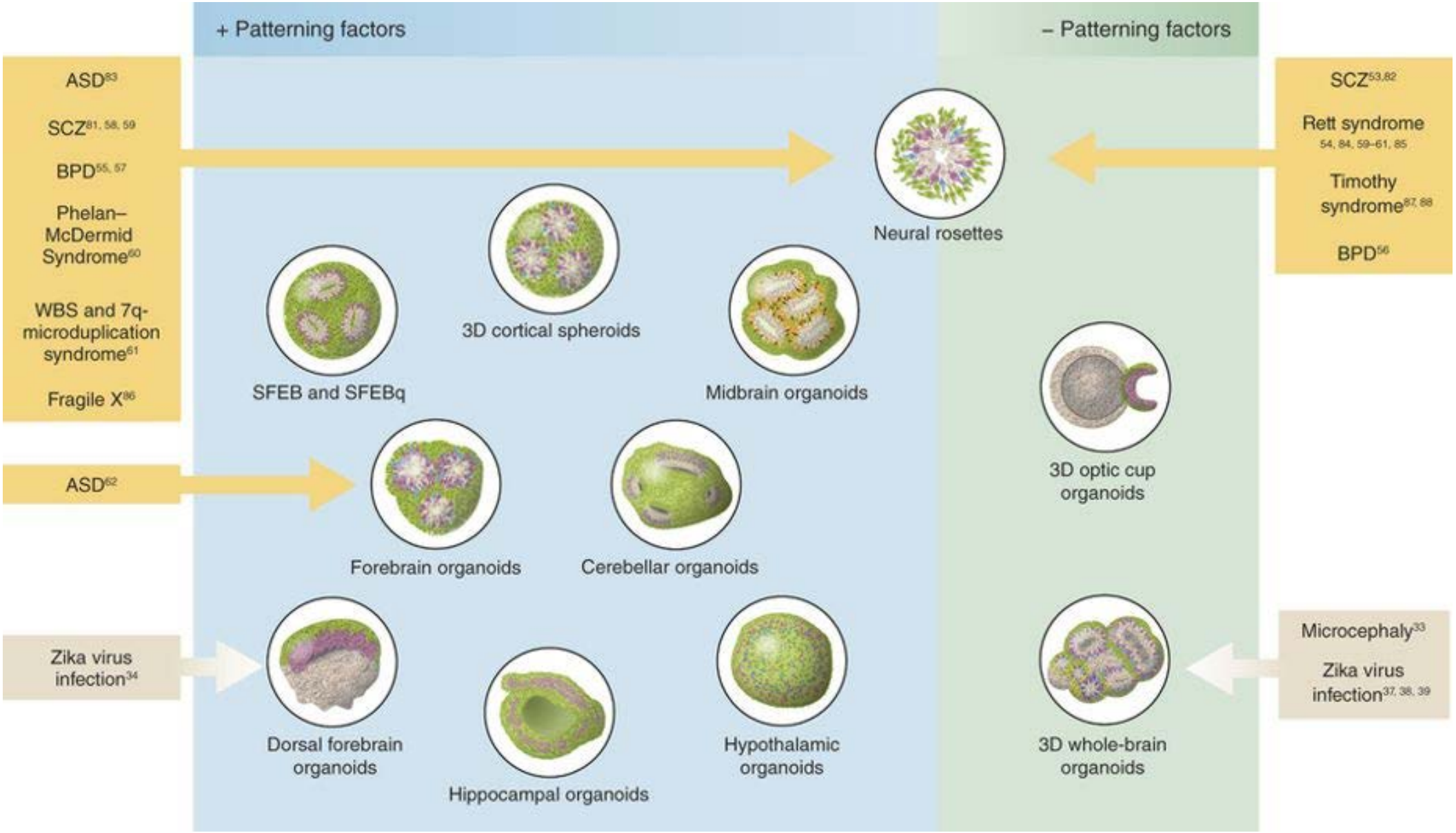


A **cerebral organoid** describes artificially grown, in vitro, miniature organs resembling the **brain**.

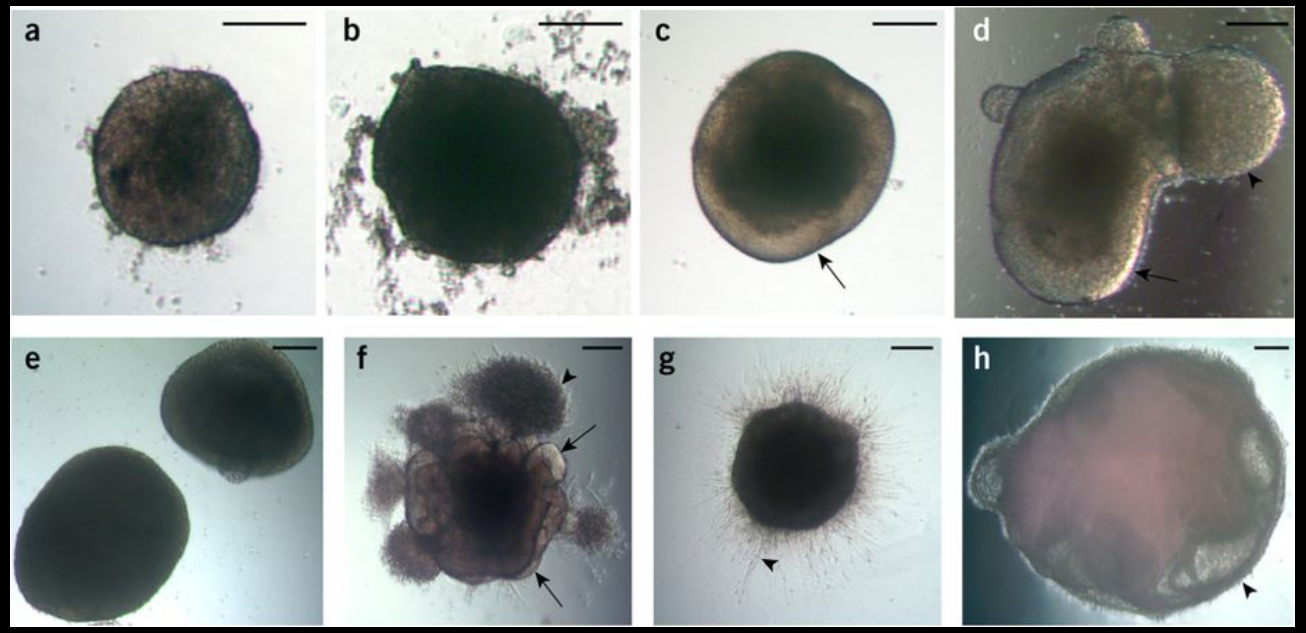
Cerebral organoids are created by culturing human pluripotent stem cells in a three-dimensional rotational bioreactor and develop over a course of months.

Lancaster et al 2013; Nature, **volume501**, pages373–379 (19 September 2013)

Brain Organoids: Research



Issues with Organoids



. Lack of heterogeneity.

. Lack of vascularization.

. Incubator effects.

. Resemble foetal brains

ARTICLE

doi:10.1038/nature22047

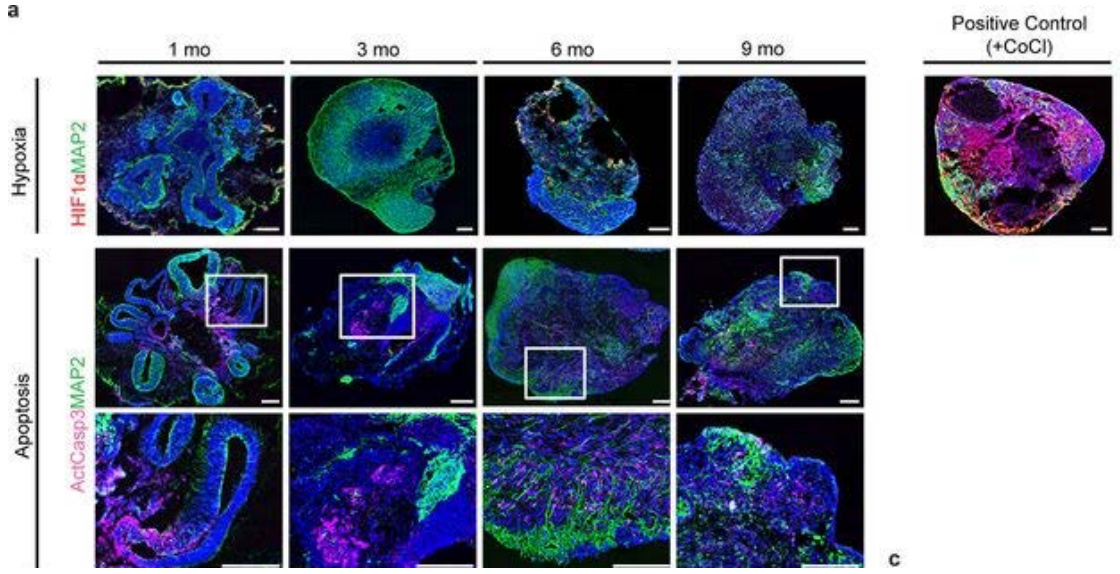
Cell diversity and network dynamics in photosensitive human brain organoids

Giorgia Quadrato[1,2], Tuan Nguyen[1,2], Evan Z. Macosko[2,3], John L. Sherwood[1,2], Sung Min Yang[1], Daniel R. Berger[4], Natalie Maria[1], Jorg Scholvin[5], Melissa Goldman[3], Justin P. Kinney[6], Edward S. Boyden[5], Jeff W. Lichtman[4], Ziv M. Williams[7], Steven A. McCarroll[2,3] & Paola Arlotta[1,2]

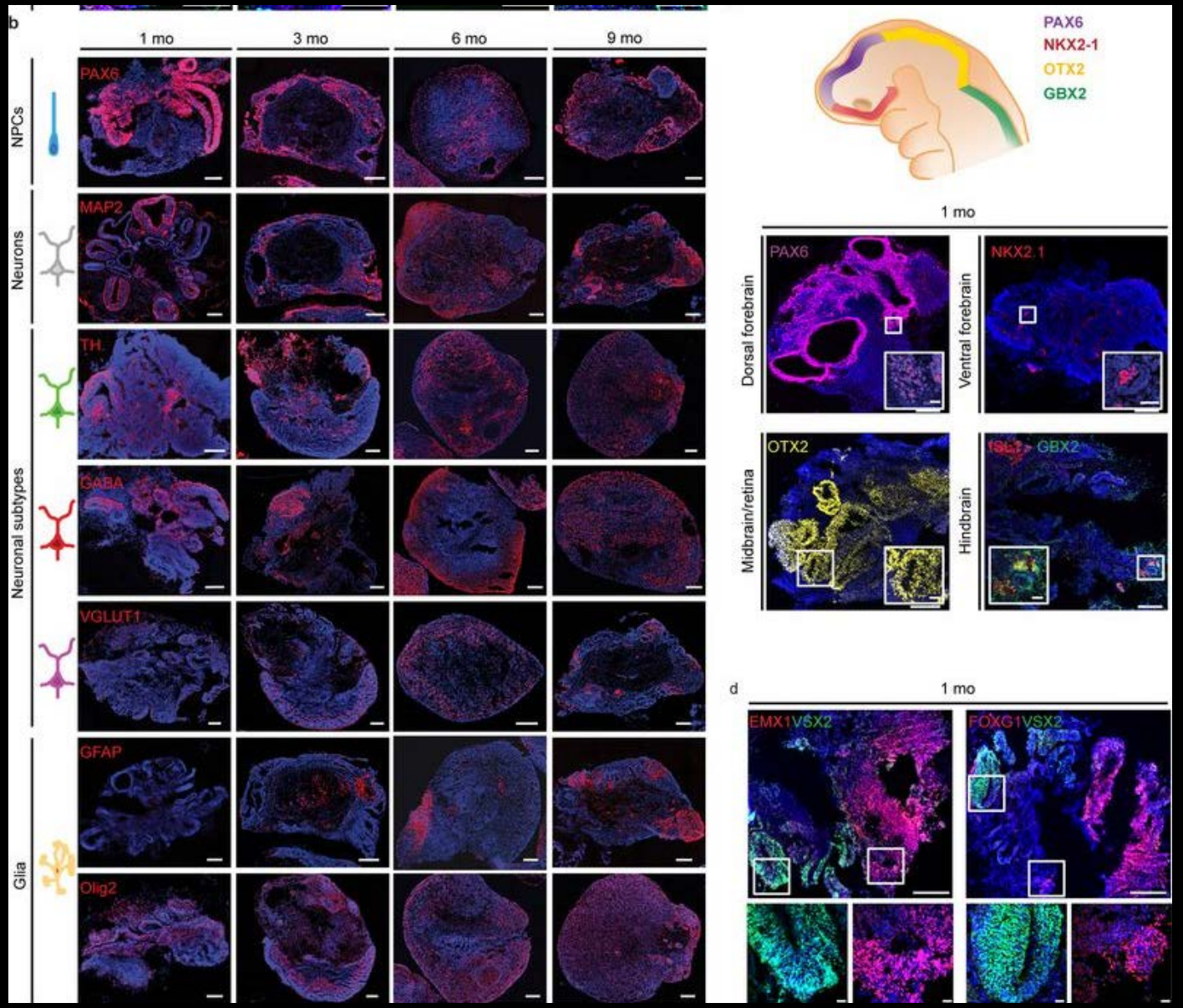
Motivation and Questions

1. How much do the organoids resemble brains?
2. Can the organoids grow past the early developmental events?
3. Cellular composition of brain organoids?
4. Networks?

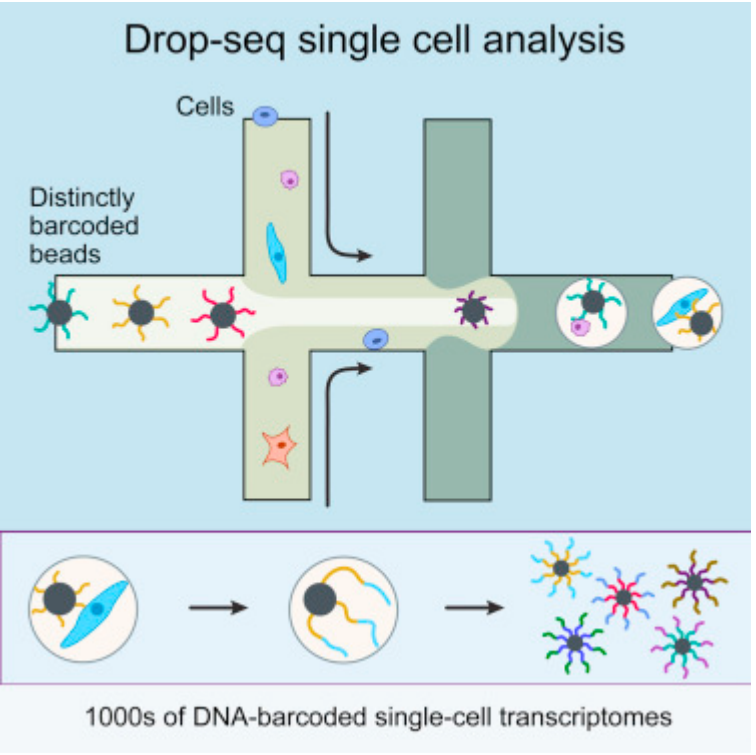
Establishment of Organoids

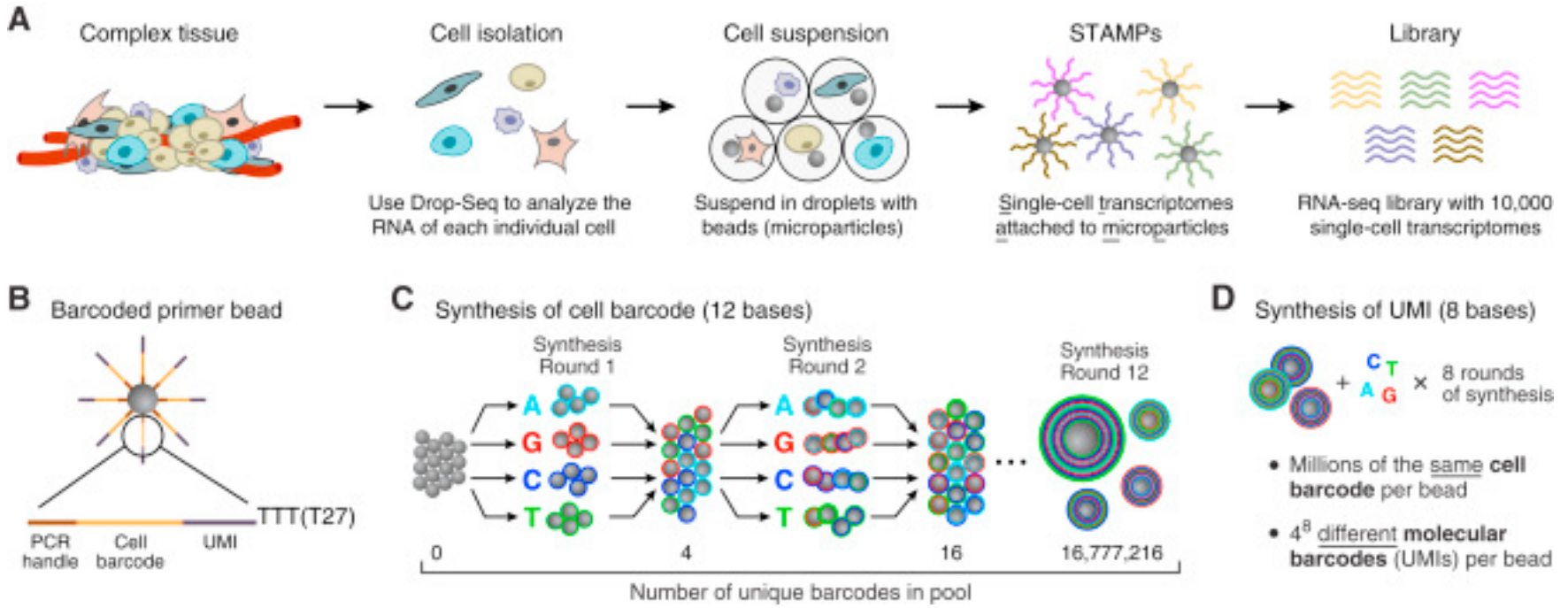


IHC: Cell types in Organoids



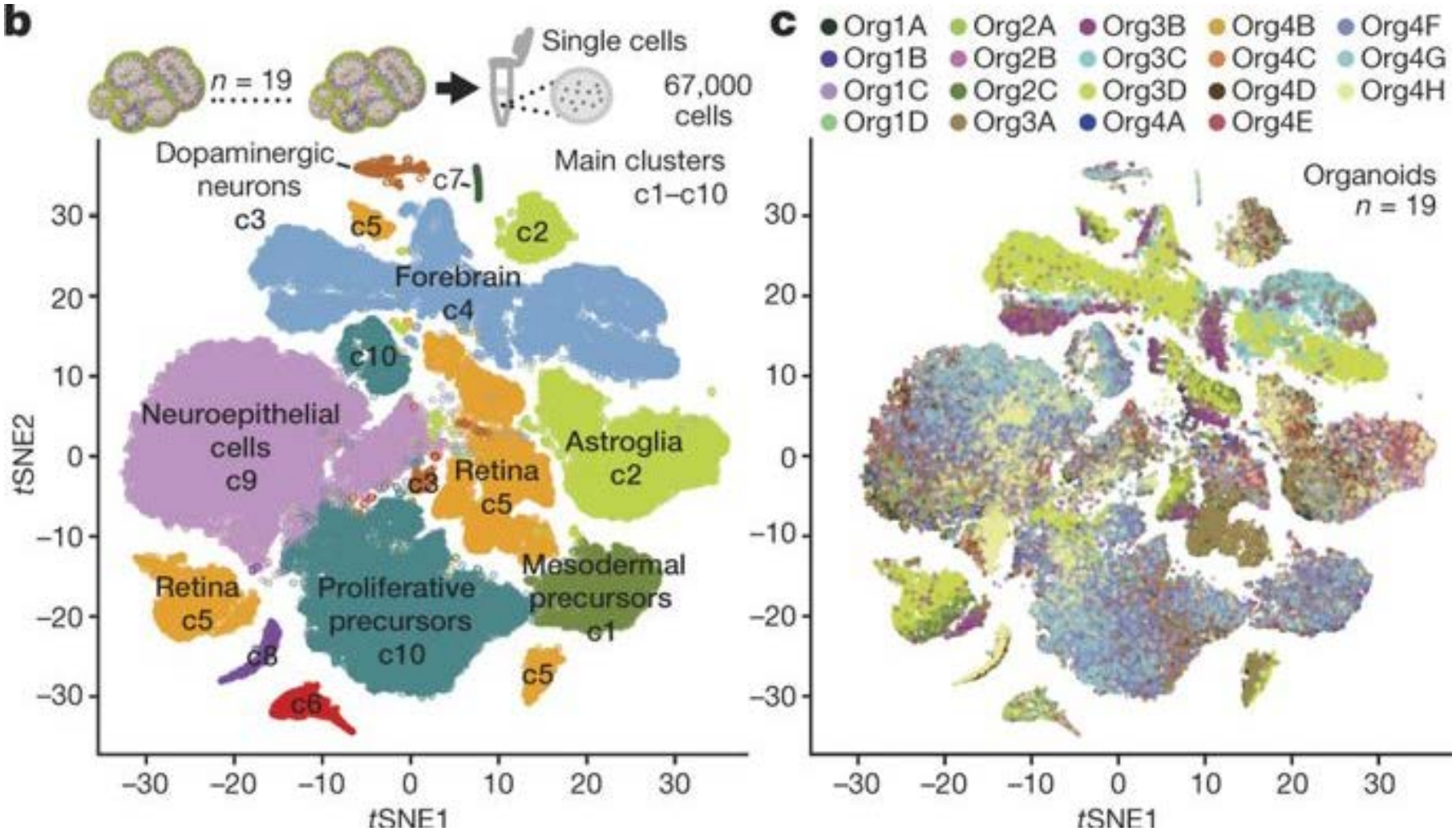
Drop Sequencing





82,391 cells from 31 organoids were profiled

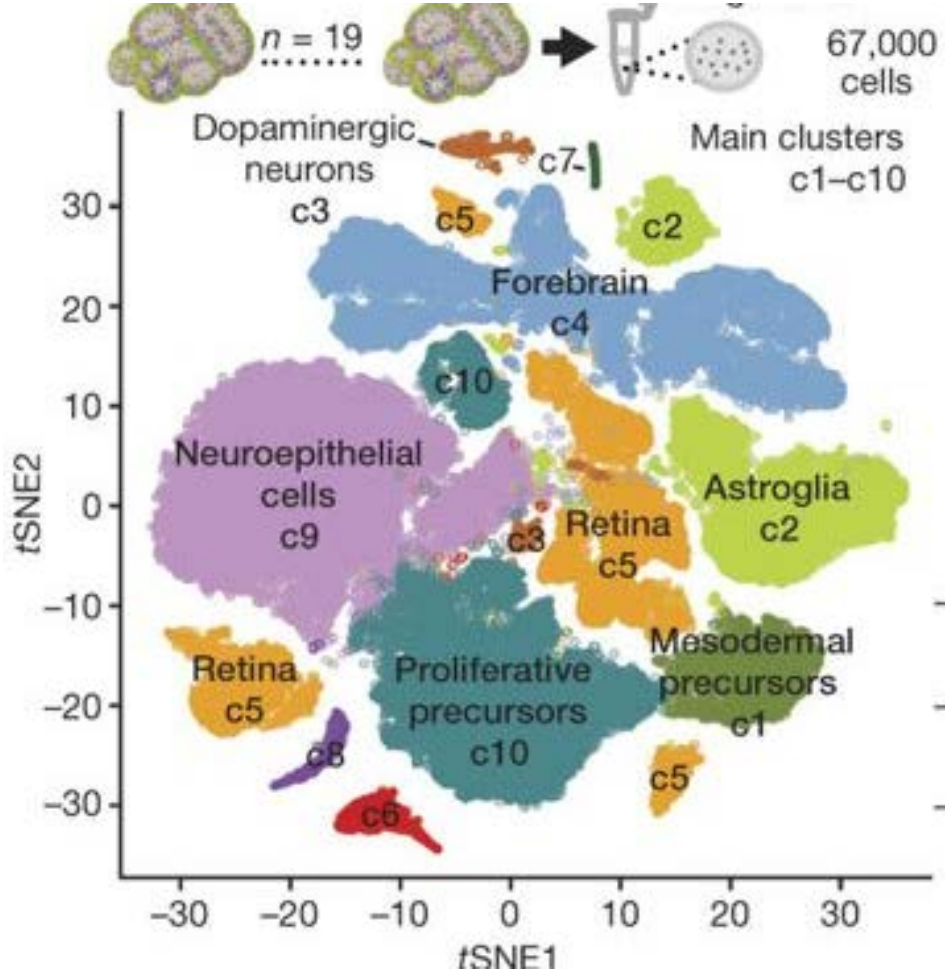
Clustering: 10 distinct populations



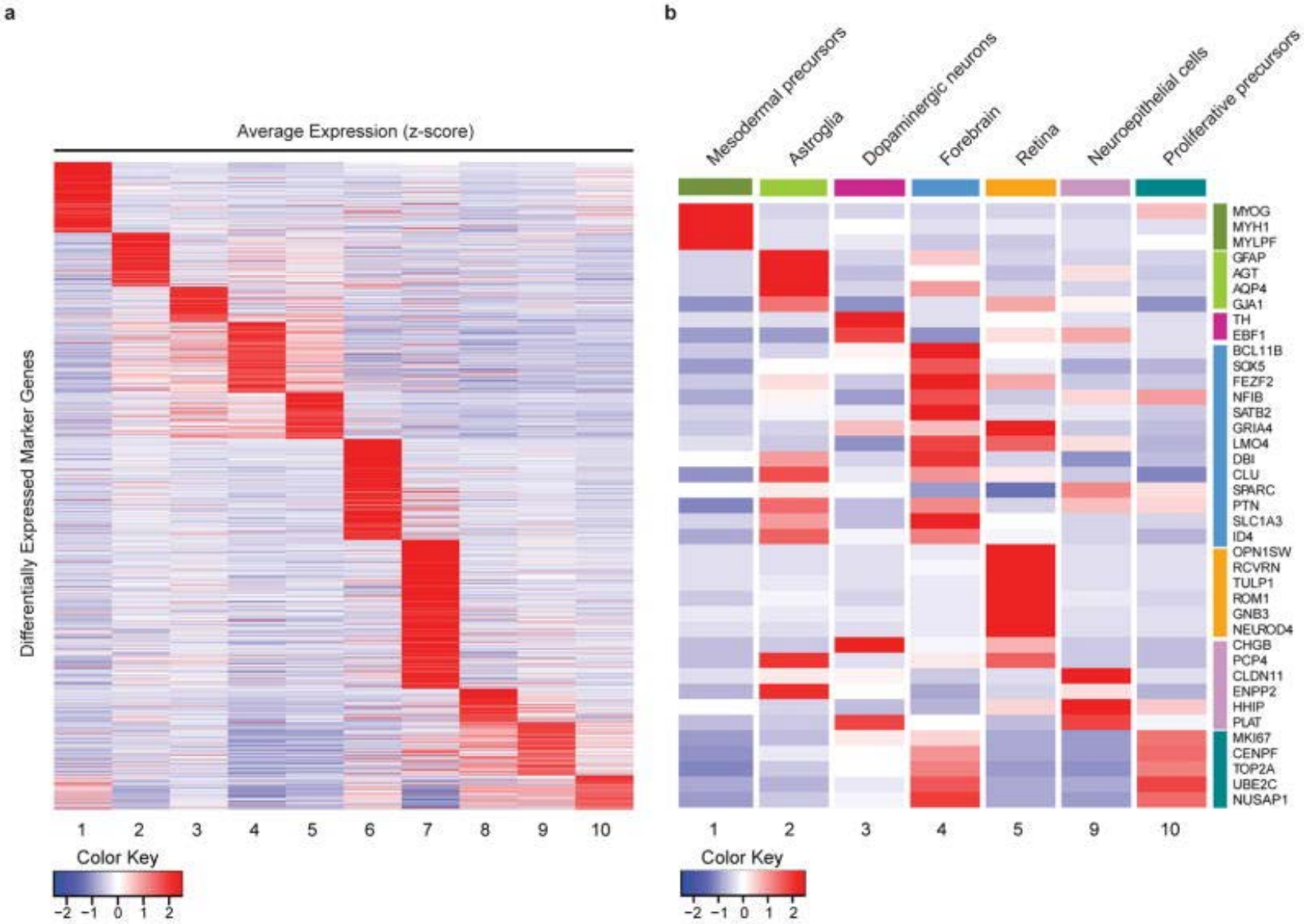
Reproducibility of cell types across different organoids

Cluster C3 and C4 are the least reproducible (52 and 37%) among different set of organoids.

Organoids generated in same batches show more Reporoducibility of cell types.



Mapping the clusters onto known RNA seq database



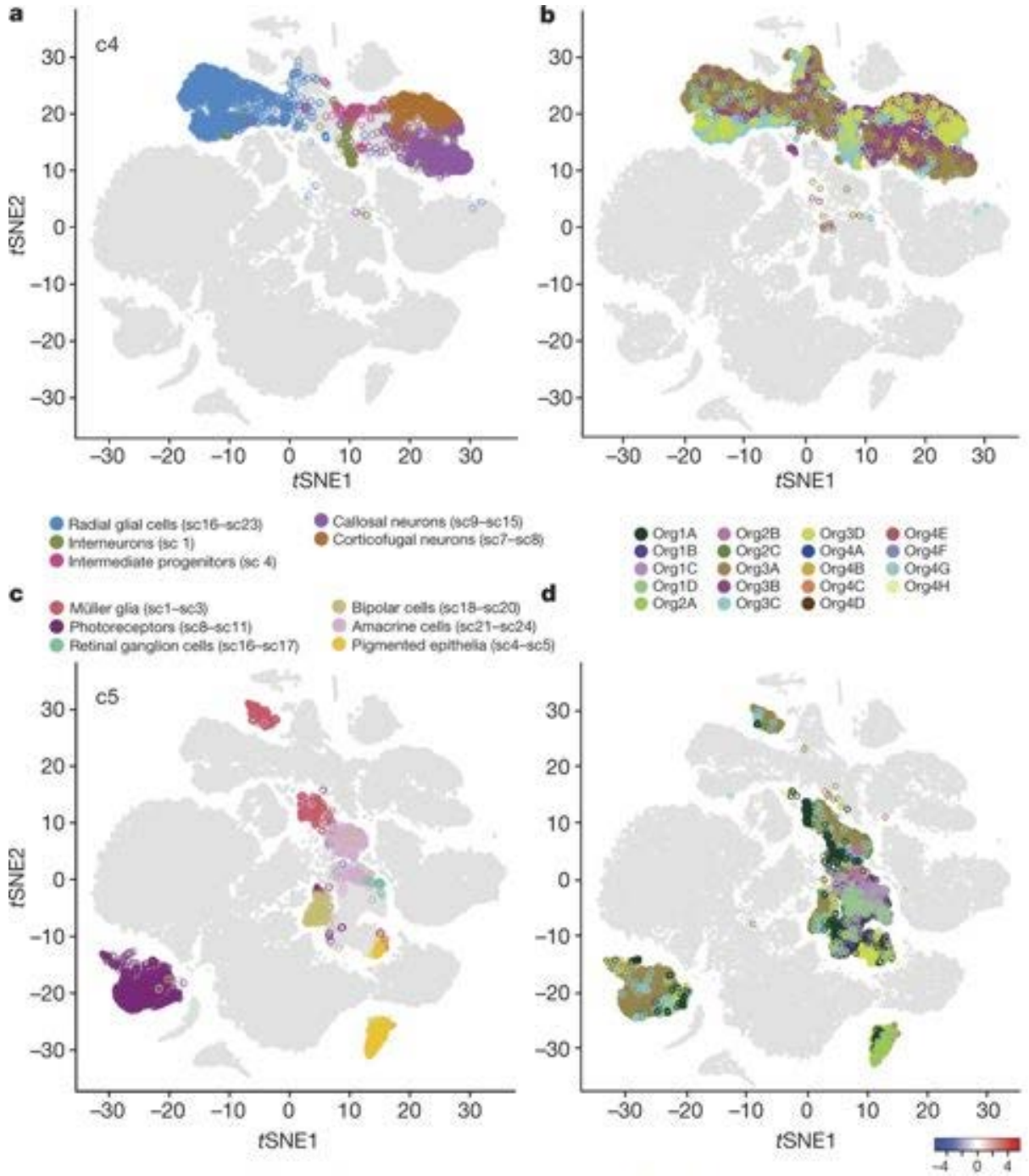
Characterizing C4 and C5

Cluster C4: 5 cell types and mostly neuronal

Cluster C5: All cell classes were found in Mouse retina.

6 disctict population of cells.

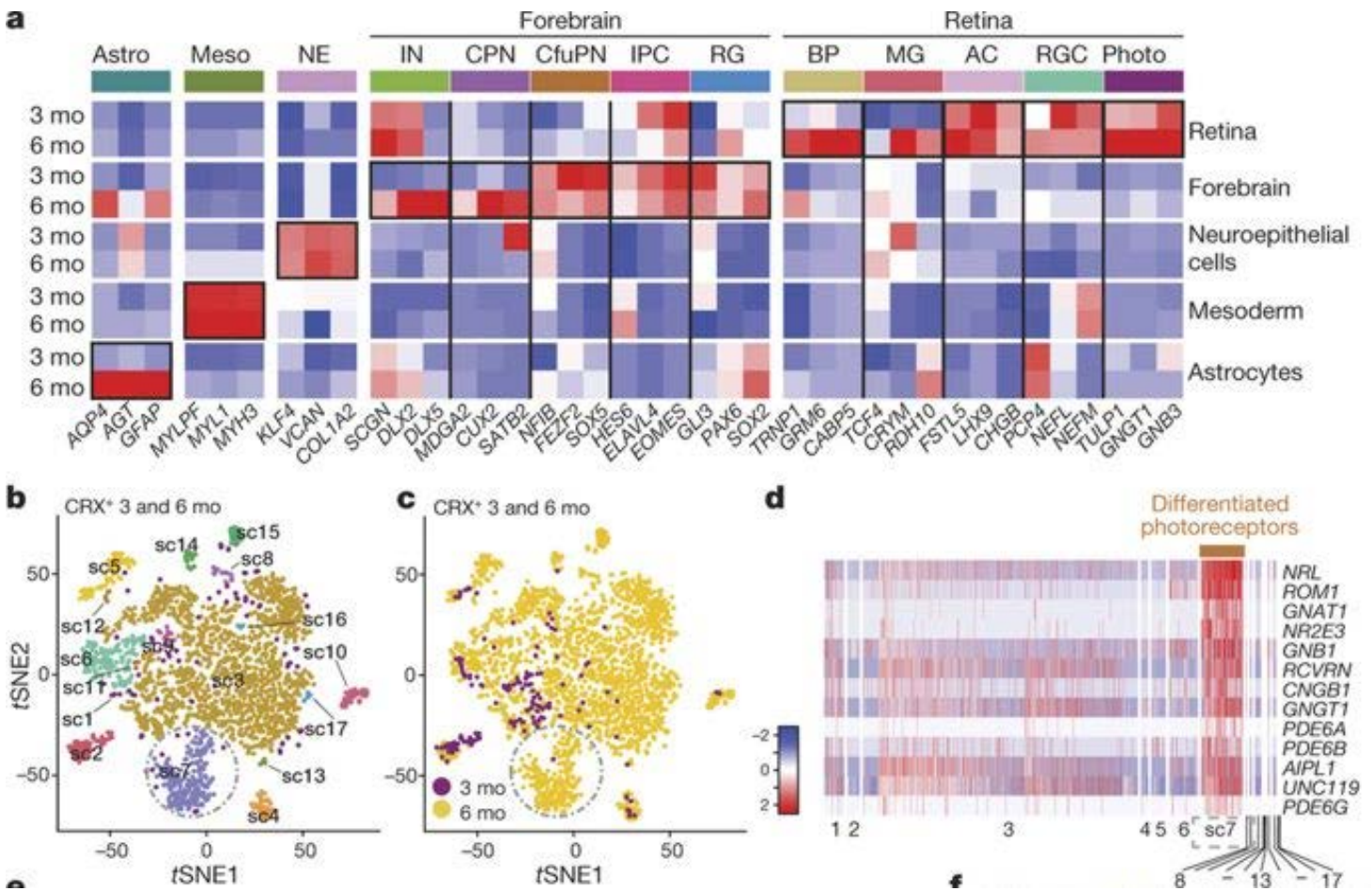
Organoids generate distinct cell populations



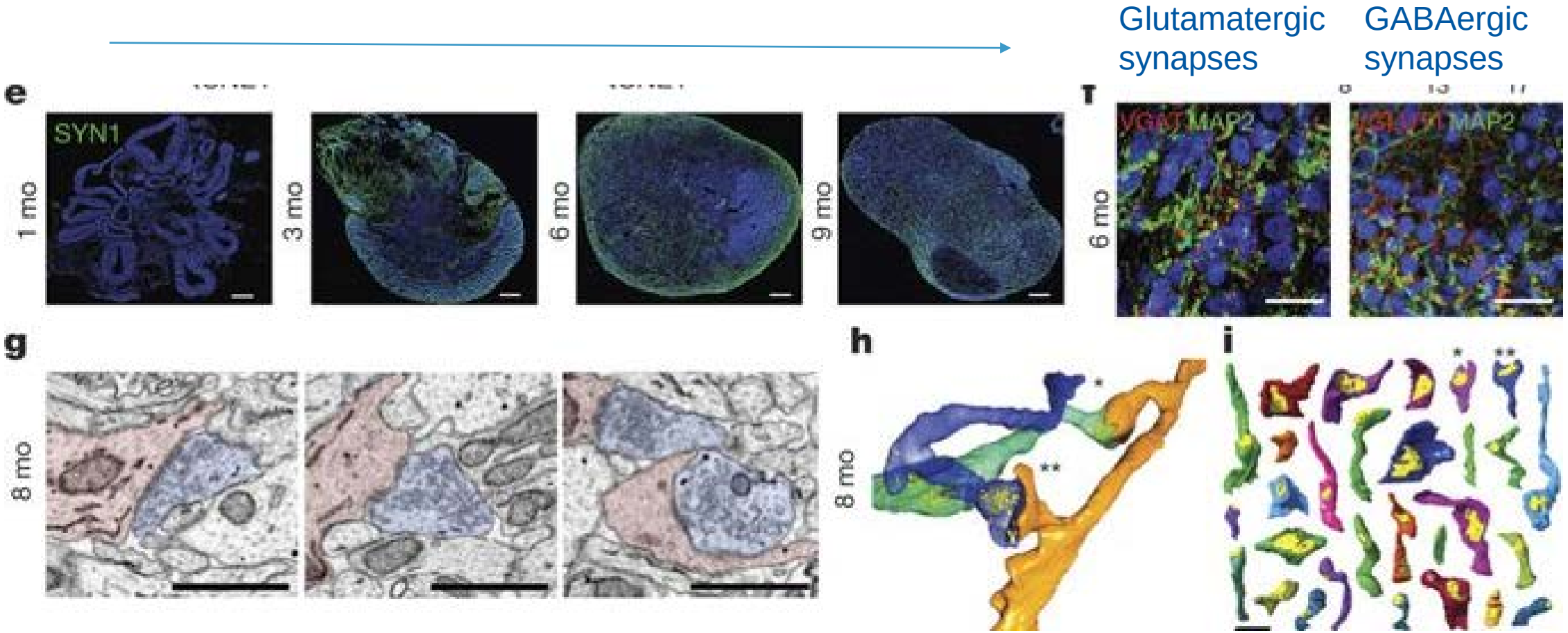
Development and maturation of Organoids

Genes involved in neuronal maturation appear at 6 months

Retinal cluster cells expressed
At 3 and 6 months without changes revealed they contained cluster of cells expressing photoreceptor

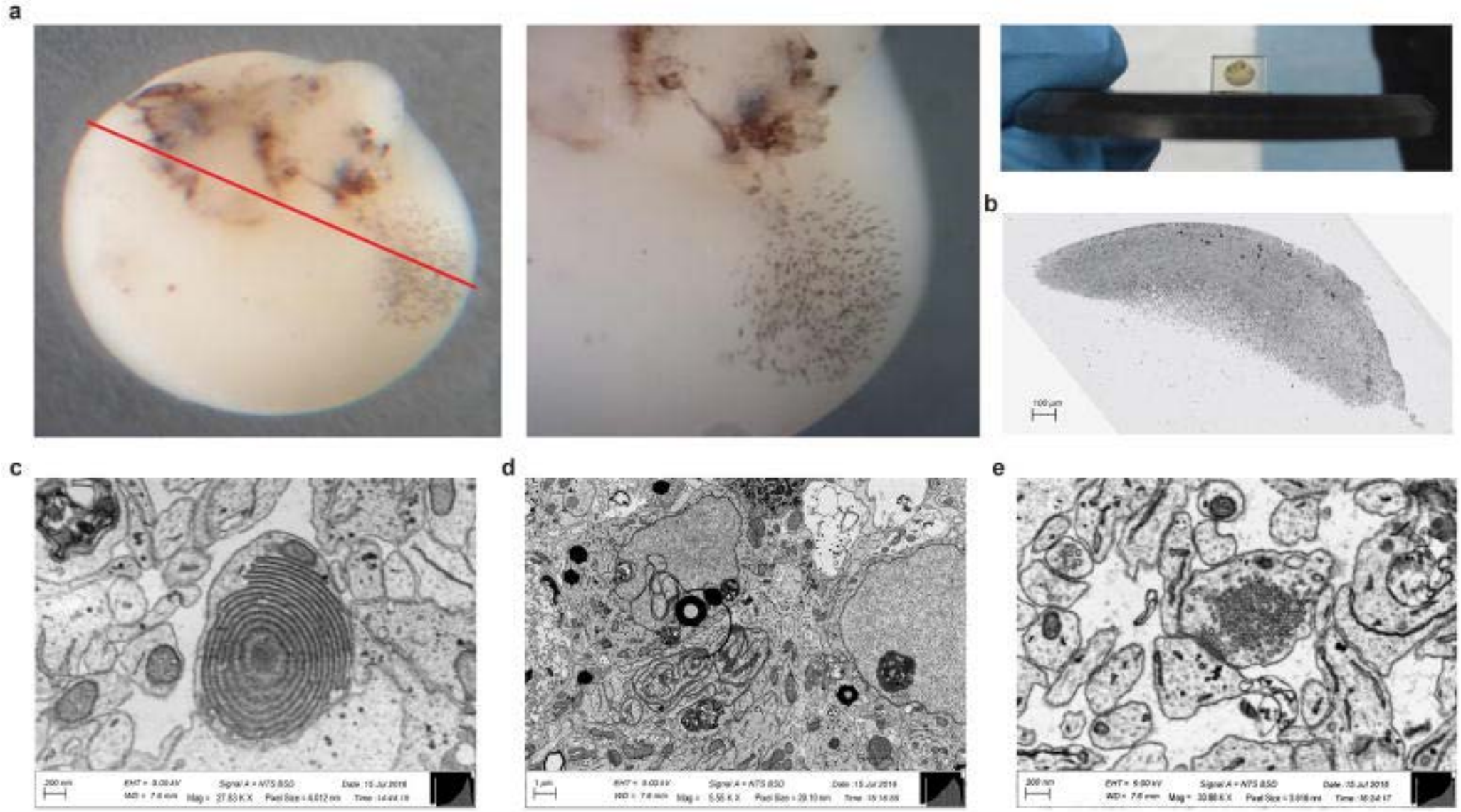


Organoids contain structurally defined Synapses



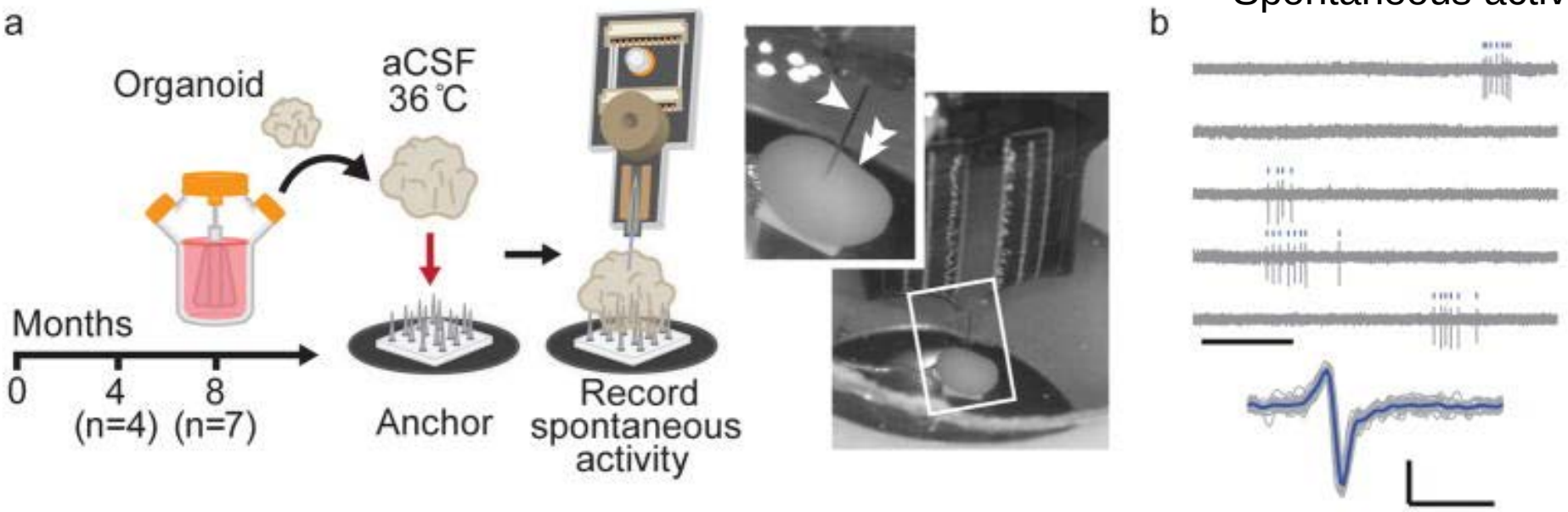
Yellow: Synpatic contact sites

EM of Syanpses in Organoids

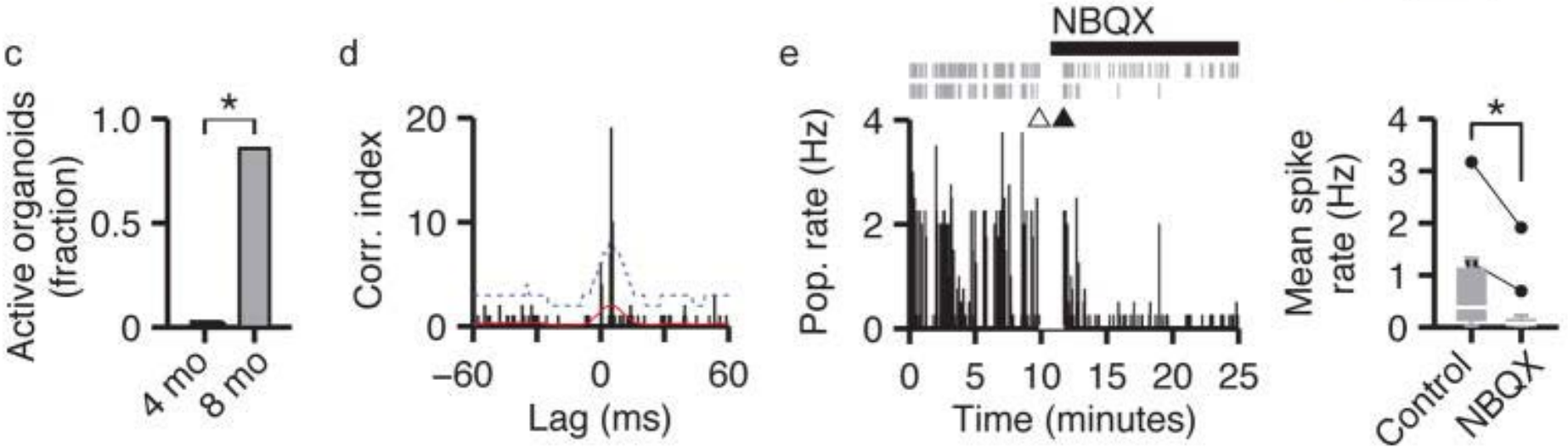


Active neural networks in organoids

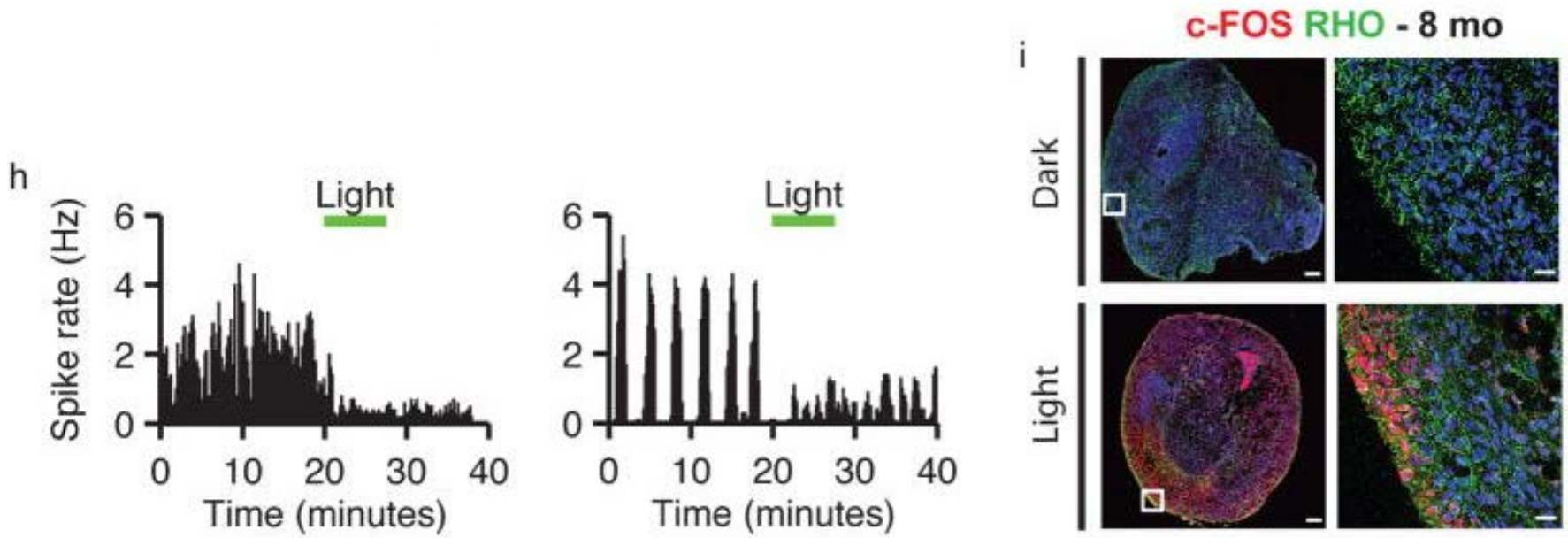
Spontaneous activity



Excitatory synaptic transmission



Light Stimuli: Photoreceptor activity in Organoids



Brain Organoids establish spontaneously active neuronal network and generate functional photosensitive cells

Summary

- Organoids can generate broad diversity of cells.
- Organoids can be developed over extended periods allowing the development of mature features.
- Active neuronal networks can be observed in organoids.

ARTICLES

nature biotechnology

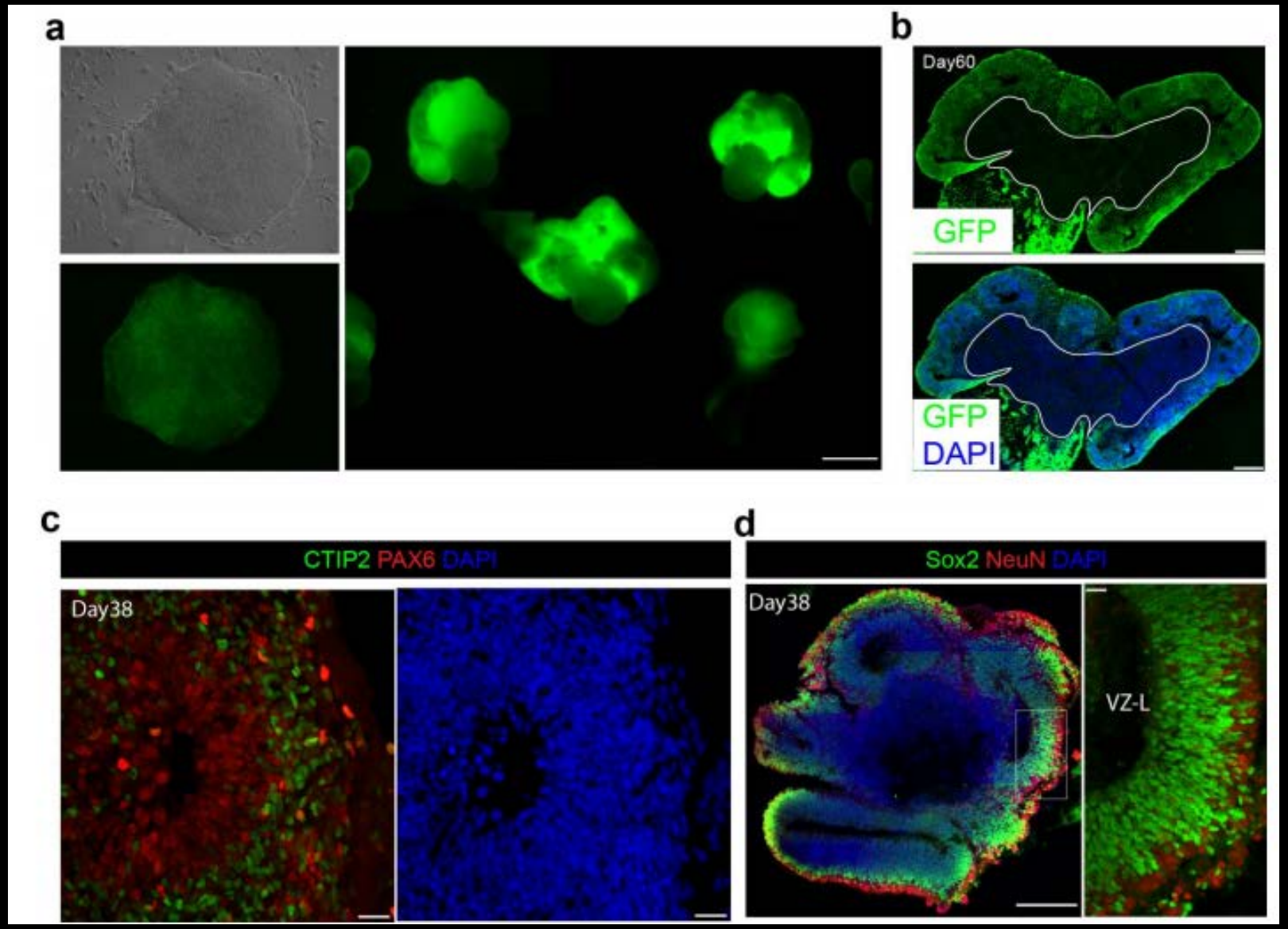
An *in vivo* model of functional and vascularized human brain organoids

Abed AlFatah Mansour[1], J Tiago Gonçalves[1,4], Cooper W Bloyd[1], Hao Li[2], Sarah Fernandes[1,3], Daphne Quang[1], Stephen Johnston[1], Sarah L Parylak[1], Xin Jin[2] & Fred H Gage[1]

Motivation and Questions

- Can the presence of vasculature improve the life span of Organoids?
- Can organoids be transplanted like xenografts?
- Can they interact with the host cells?

Generation of GFP-cerebral organoids



Generate Organoids

Grow them in culture
40-50 days

Characterize them

Proceed for mice experiments

Transplantation of human organoid In mice

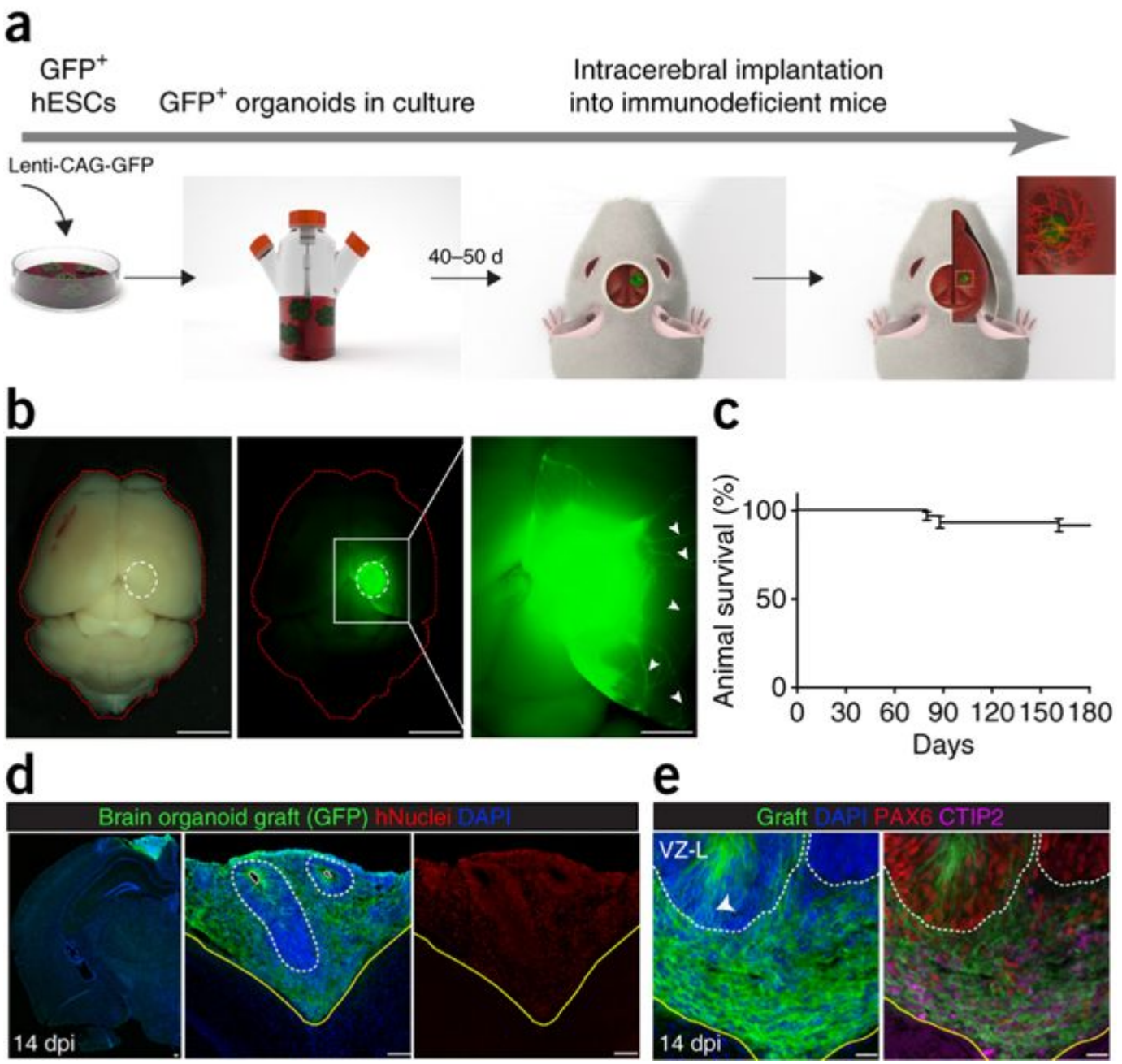
40-50 days in vitro culture

Implantation into SCID mice

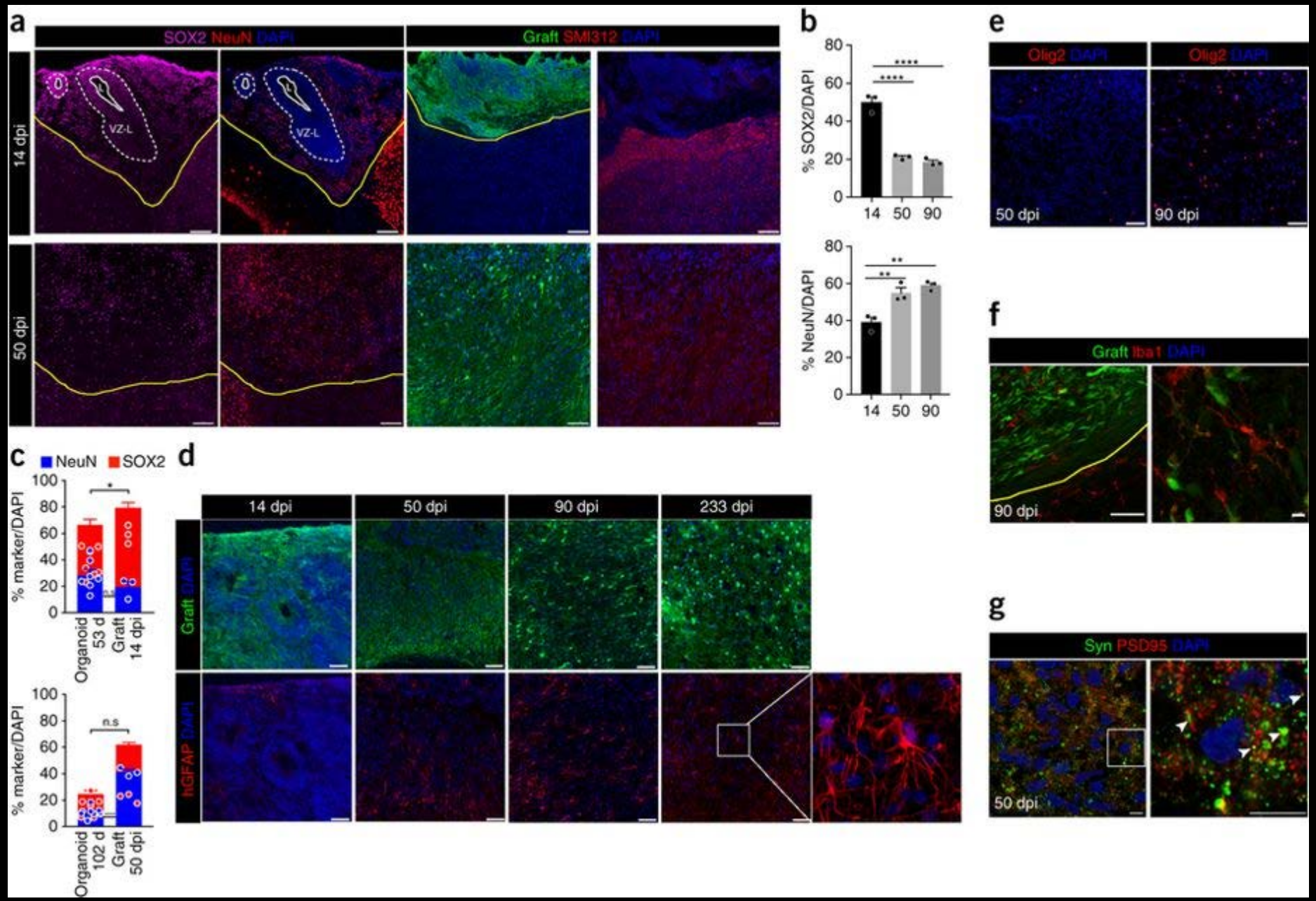
92% of the transplanted mice survived

Forebrain identity in Grafts

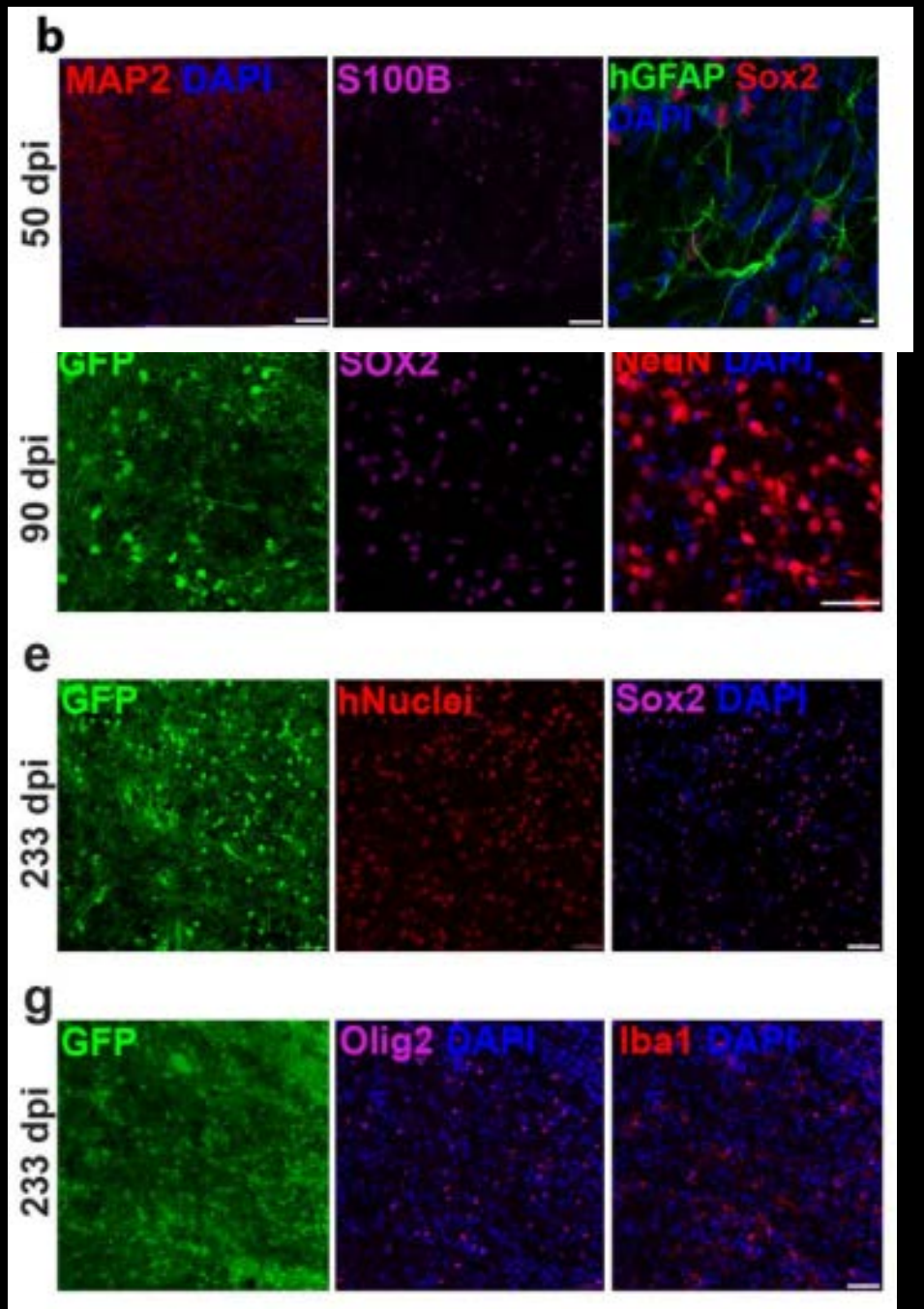
PAX6: Radial glia progenitors
CTIP2: Neuronal cortical layer



Differentiation of grafted organoids

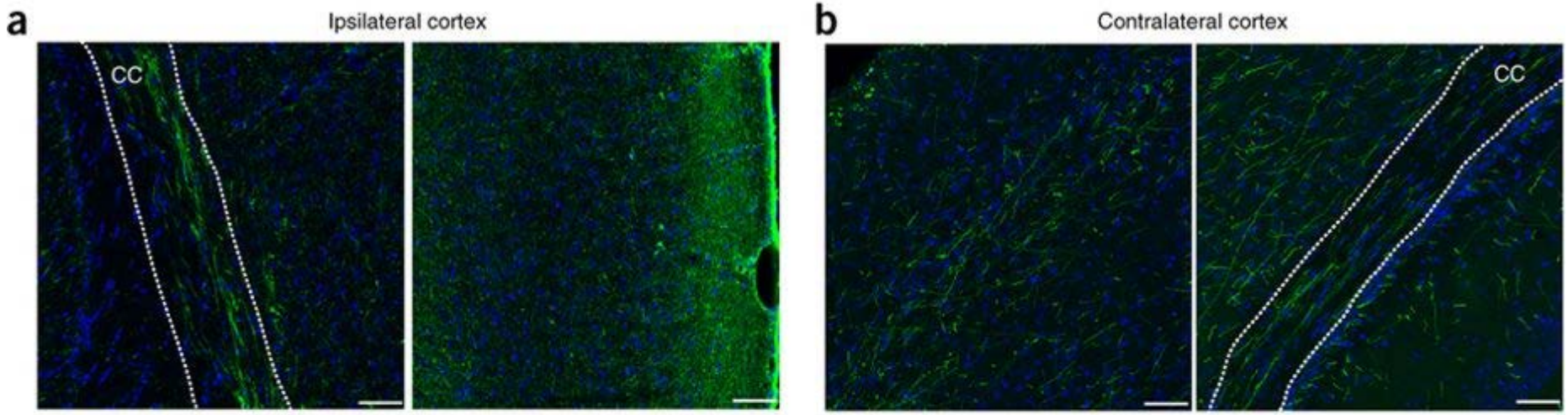


Gliogenesis in organoid grafts

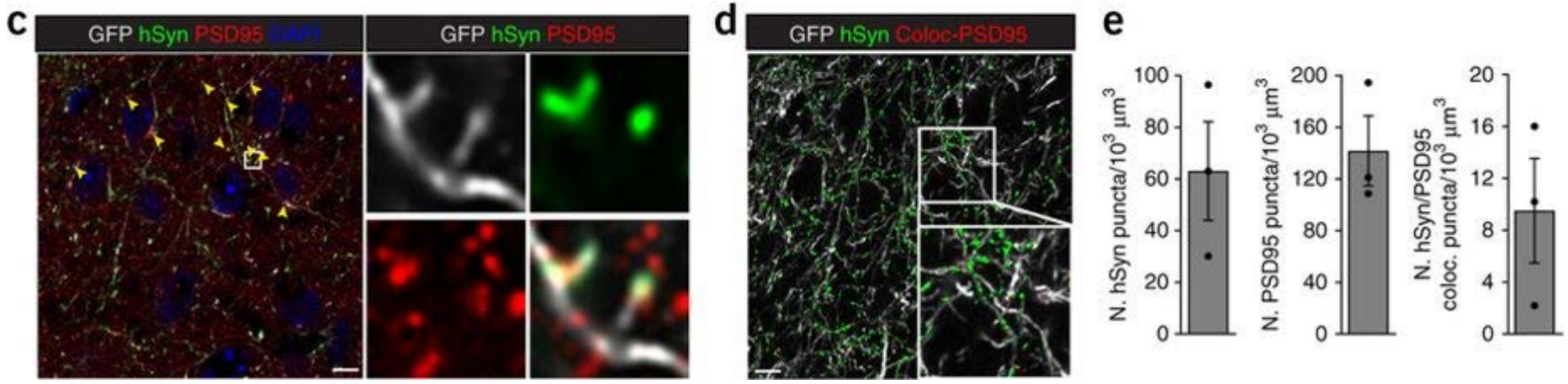


Axonal growth in engrafted organoids

Axons grew as bundles
Out of cortical layers

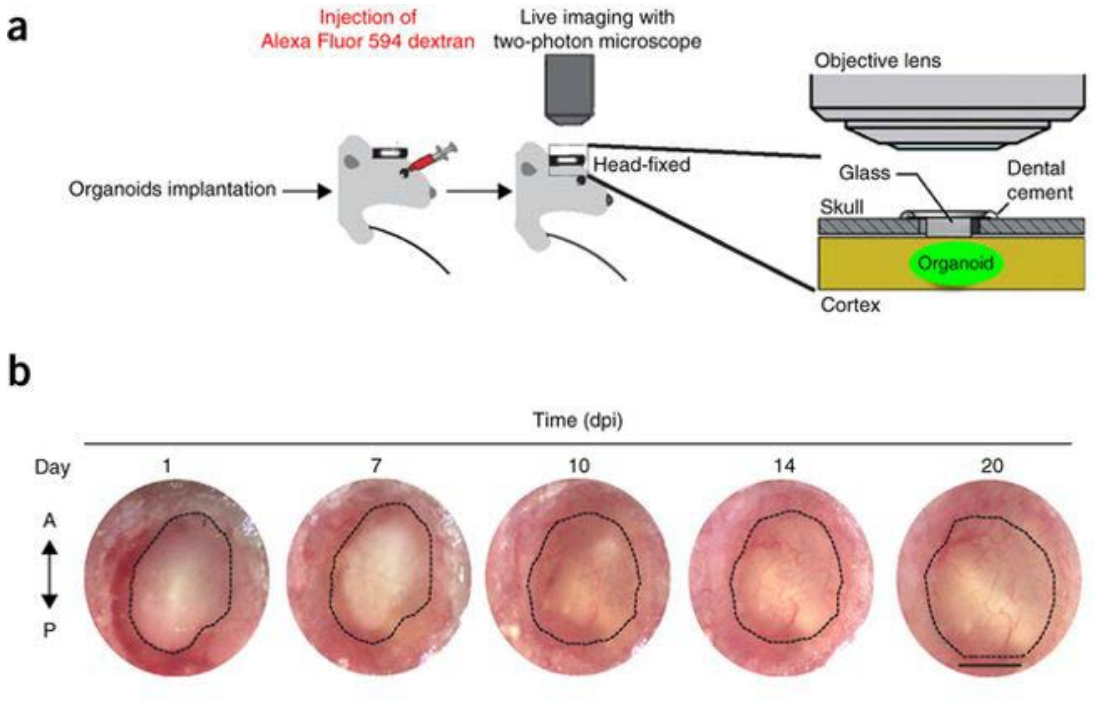


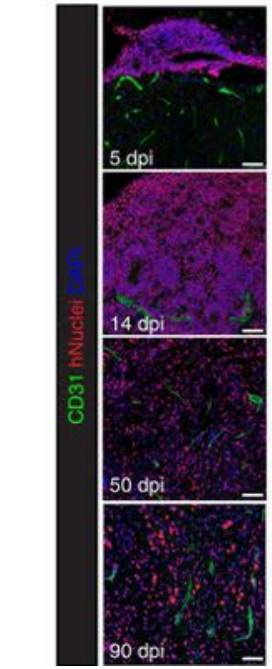
Synaptic connectivity
Was observed.

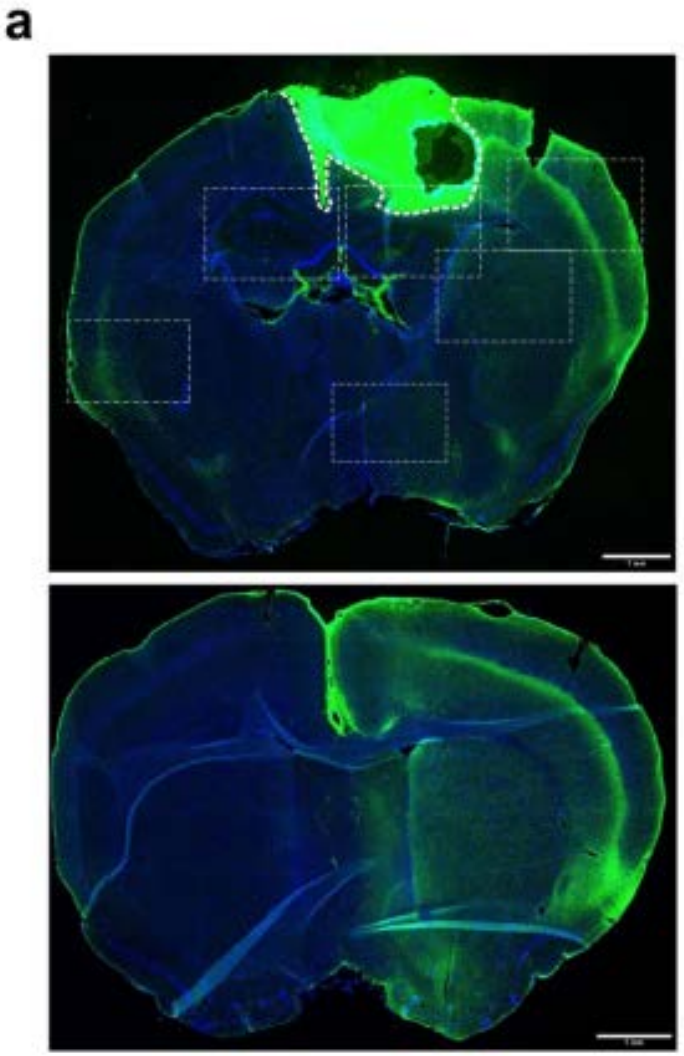


Vascularization of Grafts

14dpi:Extensive vascularization

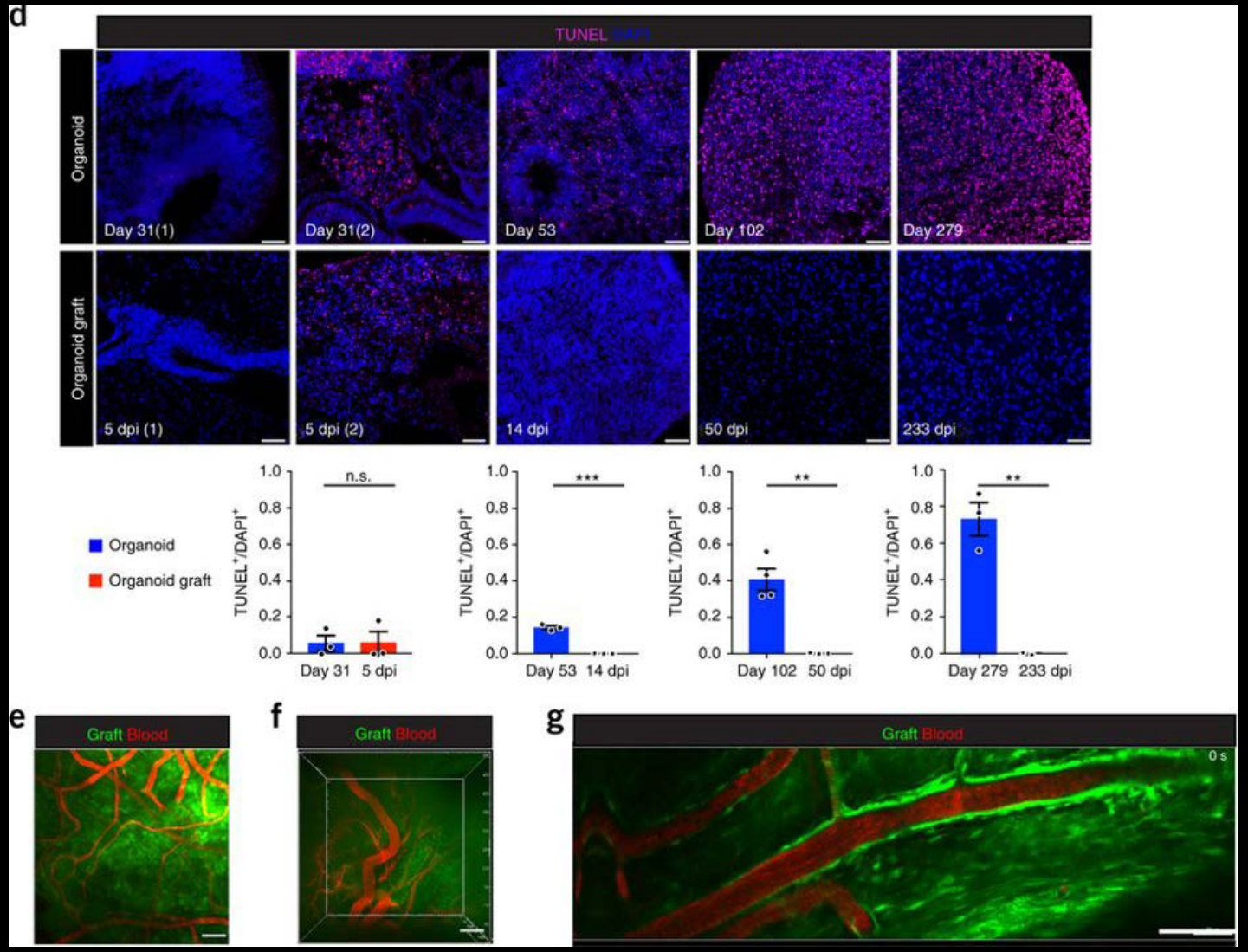






Extending of the axons into the host brain

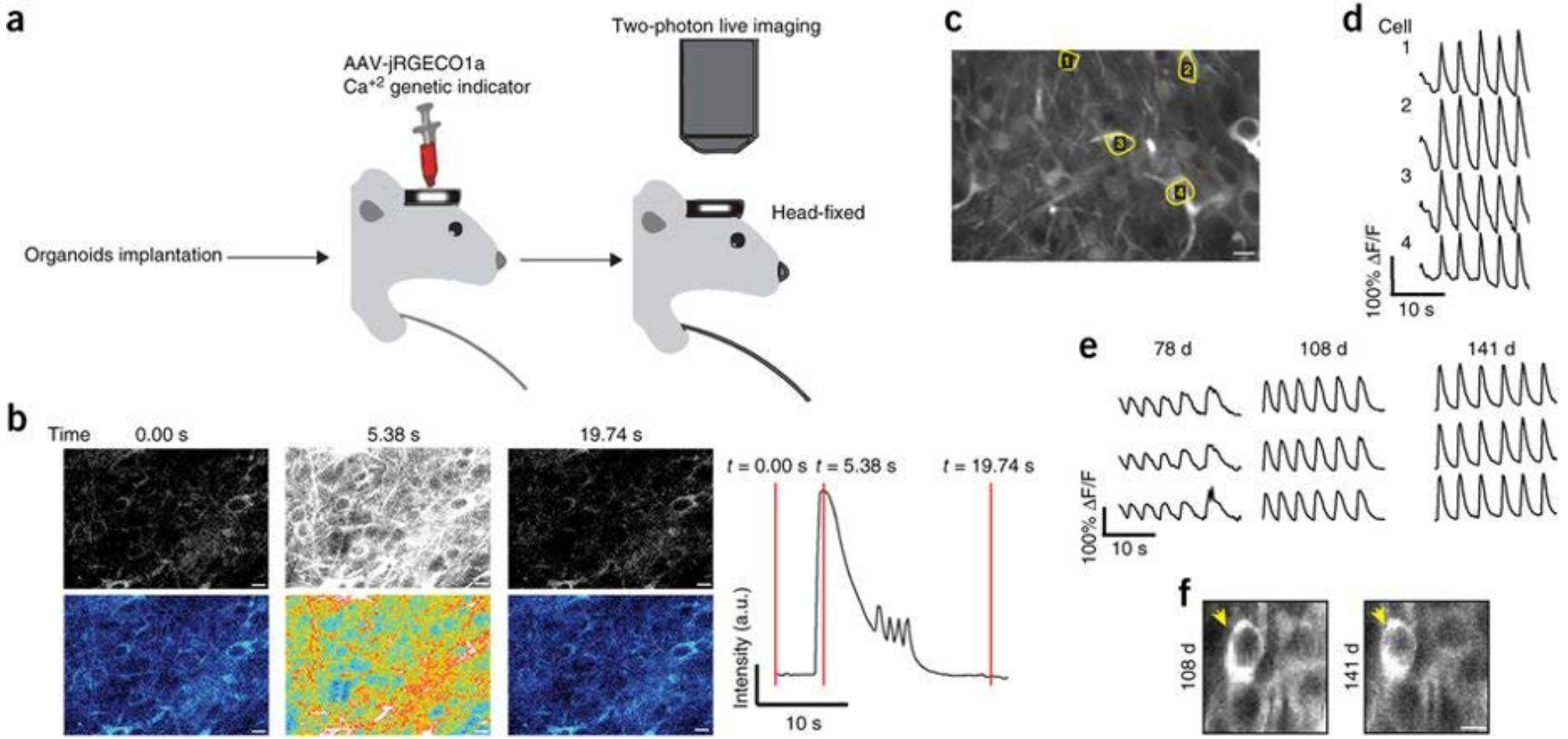
Grafted organoids live longer



Grafter organoids live longer than 9 months.

Longer life span helps in generation of heterogeneity.

Functional Activity of the grafts: Calcium imaging

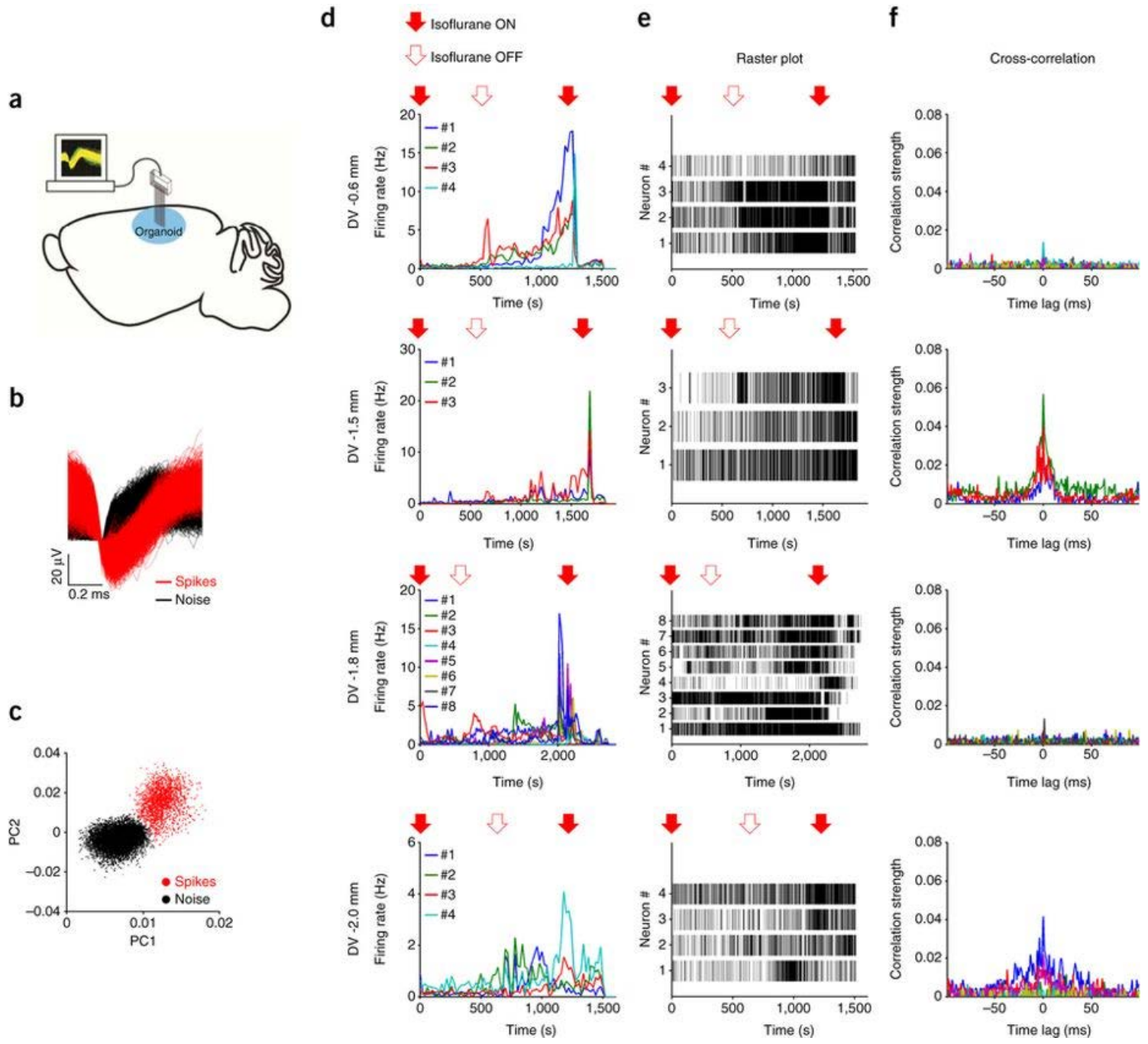


Functional Activity of the grafts: Electrophysiology

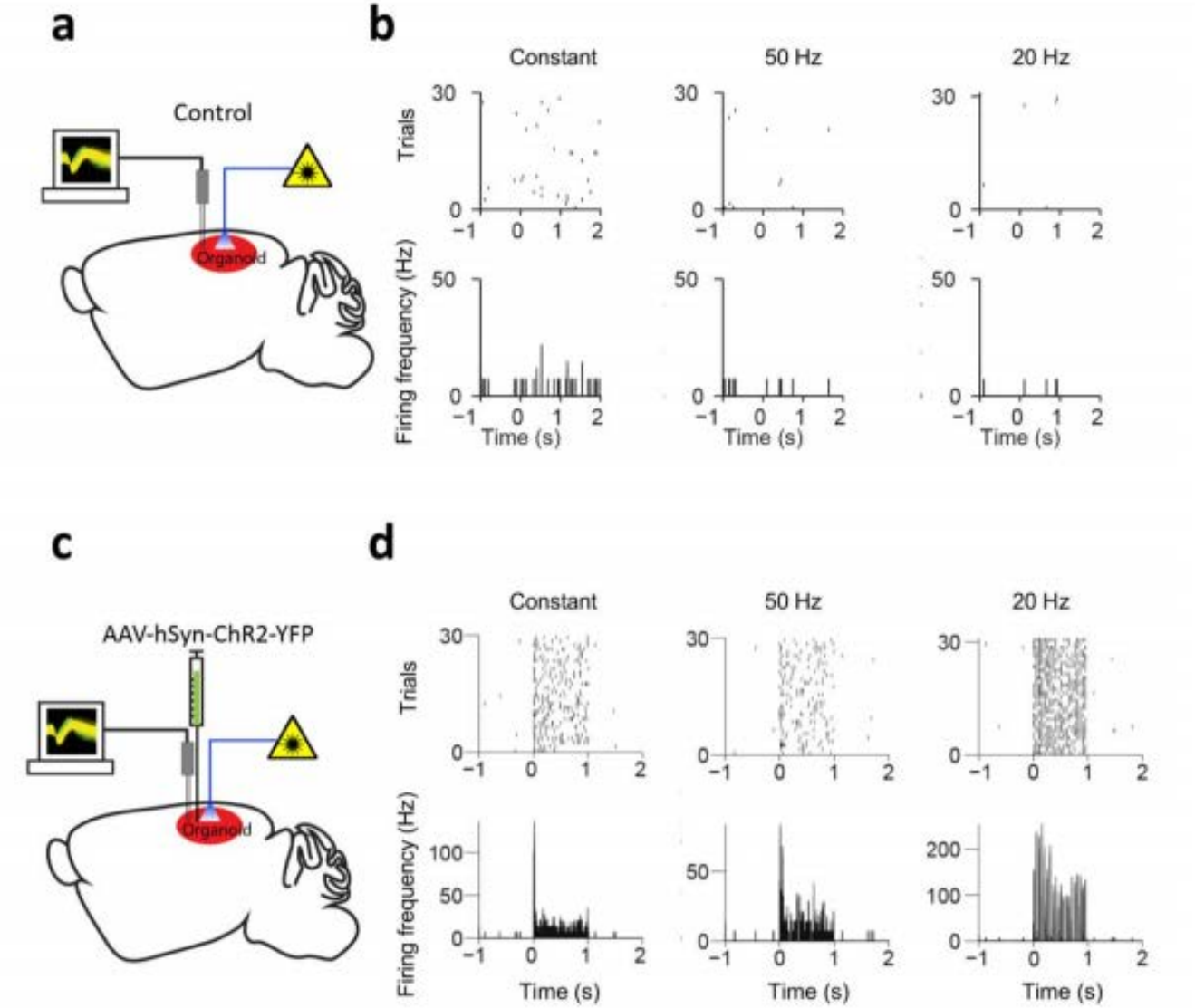
Multielectrode array recording

⇩

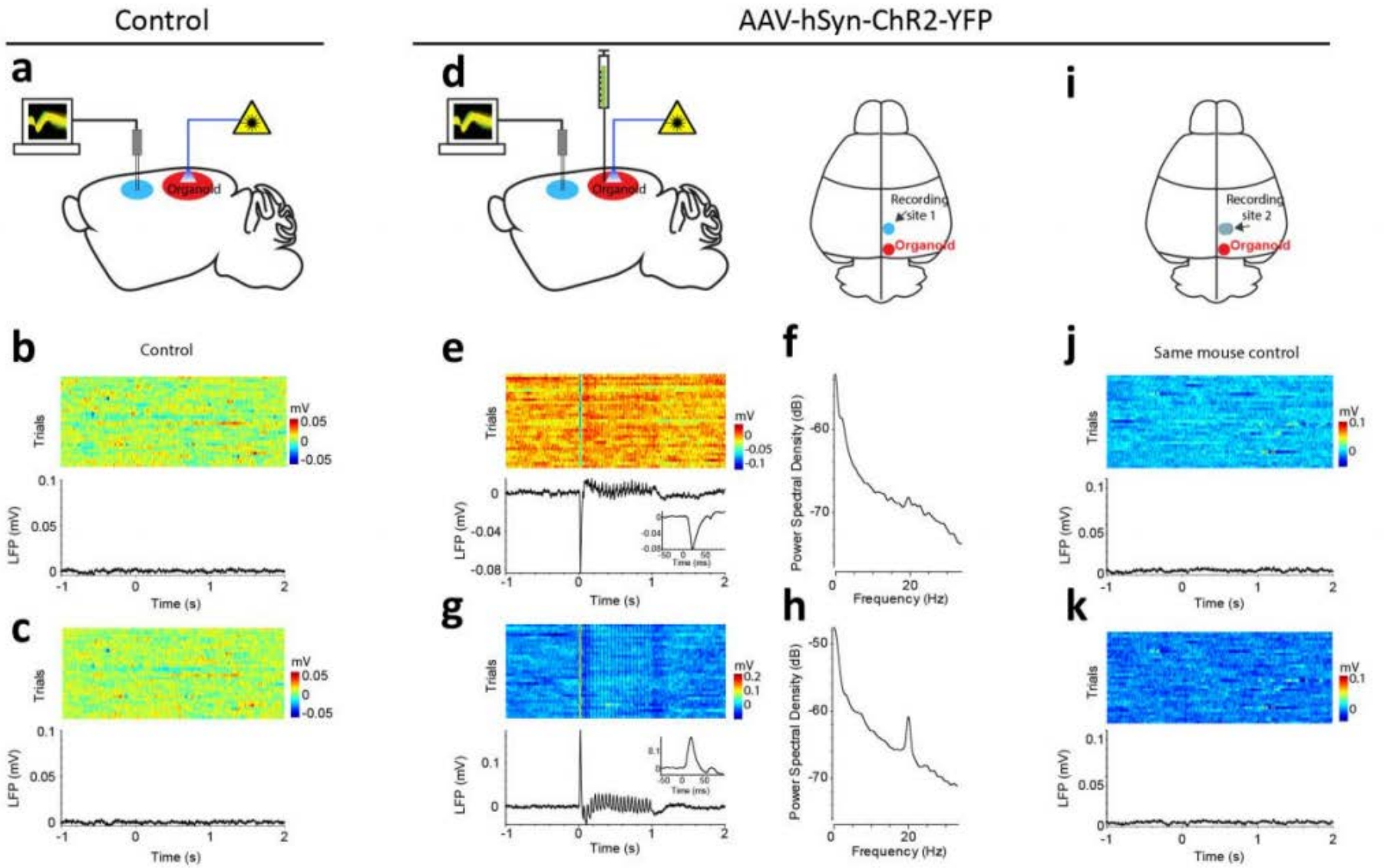
Action potentials



Functional Activity of the grafts: Optogenetics



Intragraft neuronal activity



Summary

- Establishment of method to successfully transplant the organoids into mice brains.
- Organoid grafts demonstrate progressive neuronal differentiation and maturation
- Intragraft neuronal activity and suggested graft-to-host functional synaptic connectivity.

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Glioblastoma Model Using Human Cerebral Organoids

Junko Ogawa,[1] Gerald M. Pao,[1] Maxim N. Shokhirev,[2] and Inder M. Verma[1,3,*]
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[2]The Razavi Newman Integrative Genomics and Bioinformatics Core Facility, Salk Institute for Biological Studies, La Jolla, CA, USA
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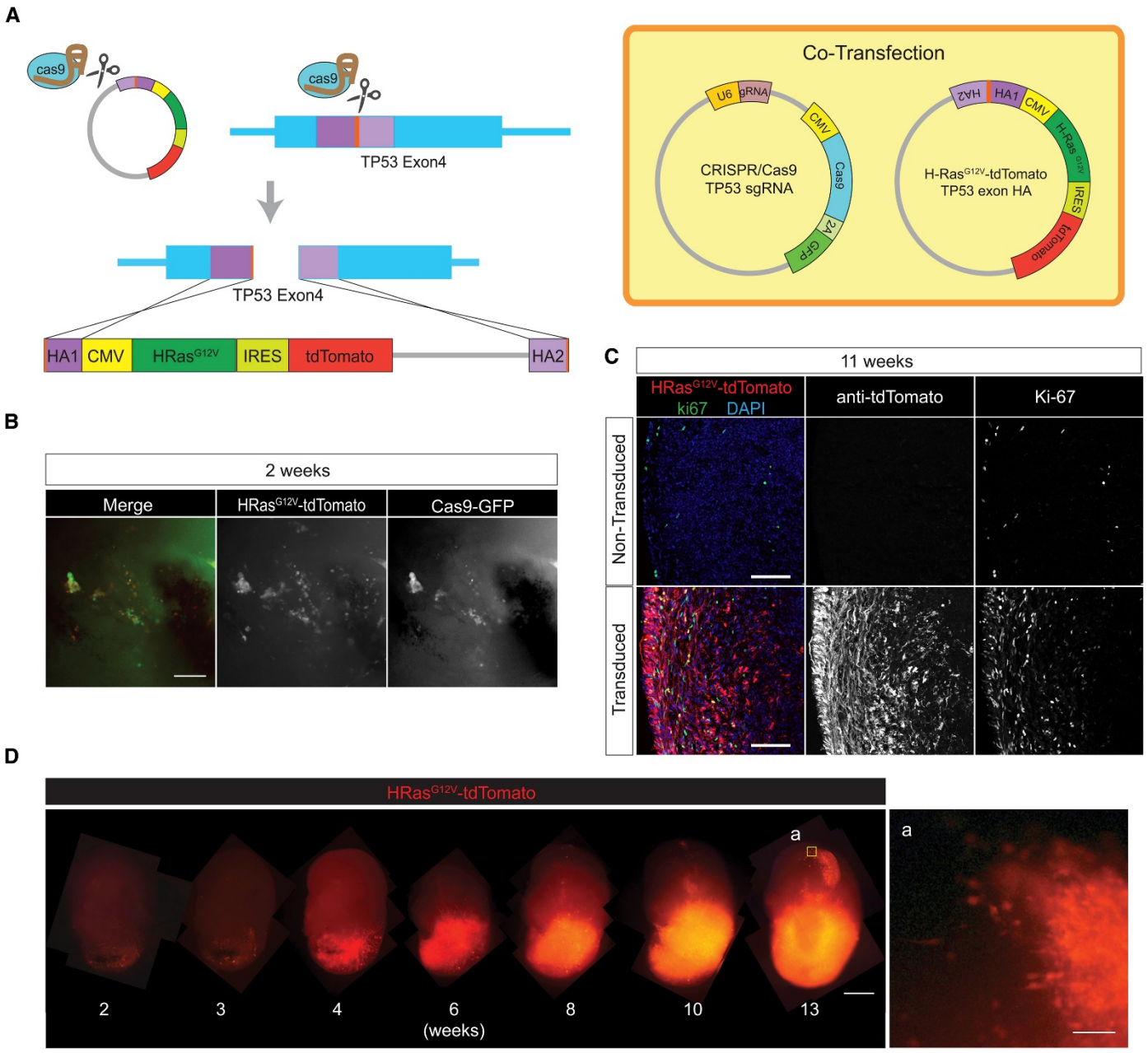
https://doi.org/10.1016/j.celrep.2018.03.105

Motivation and Questions

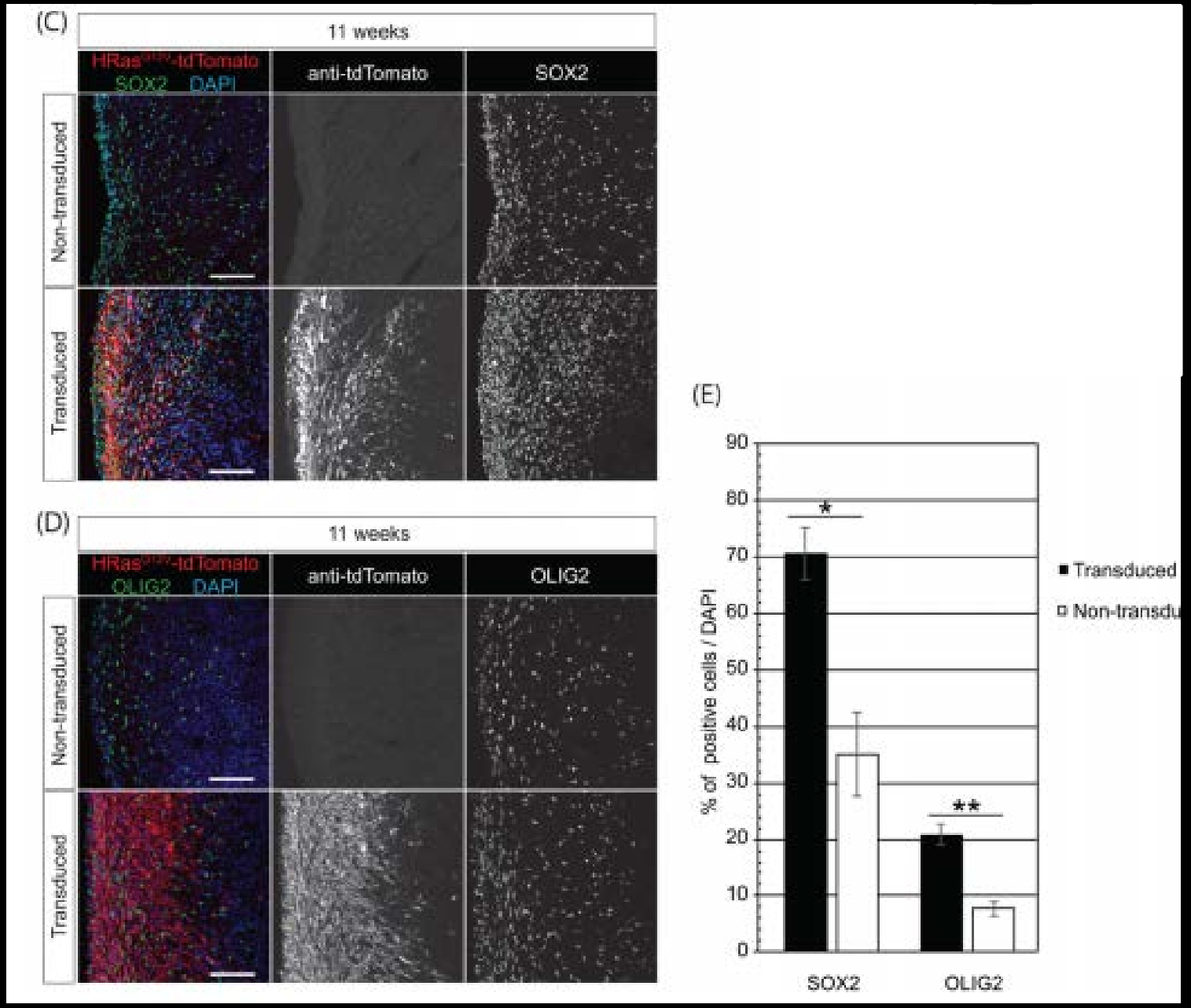
- Can organoids be used as a better model system to study brain tumors
- Can the human brain tumor signature be captured in organoids?
- Can Organoids replace Xenografts?

Transformation of human Cerebral organoids

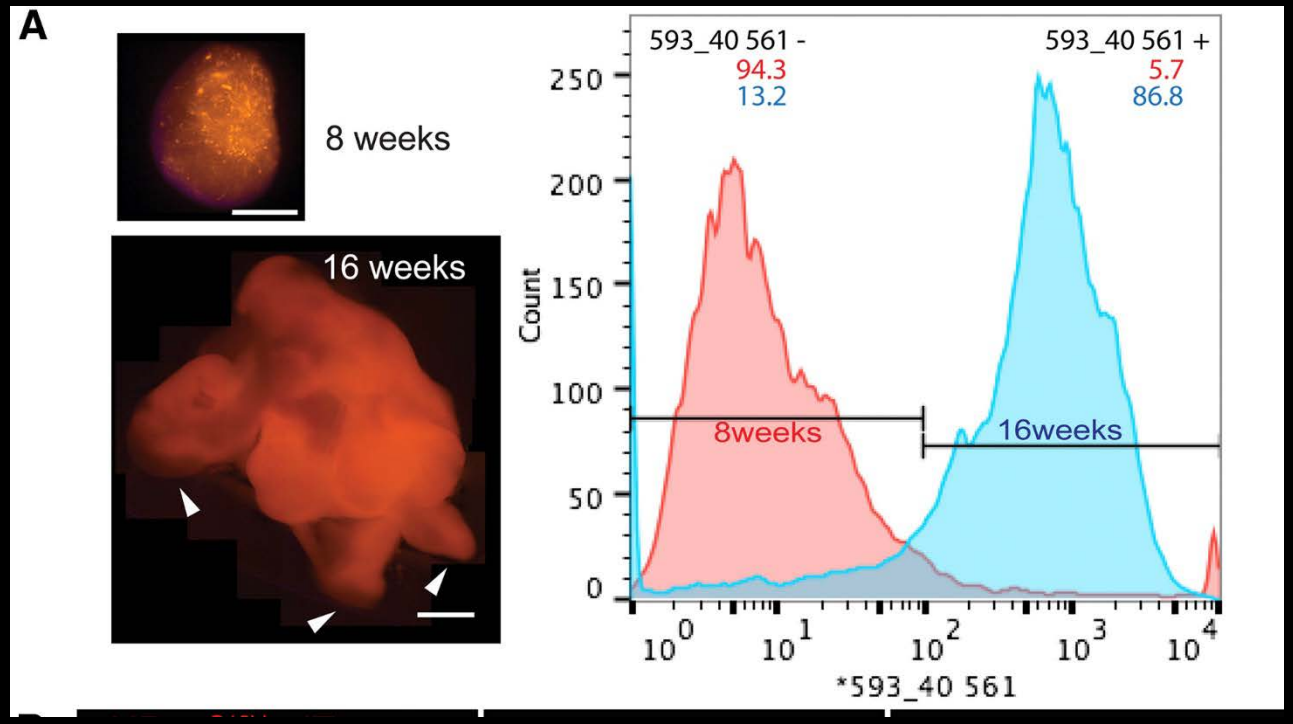
Introduction of HRasG12V and TP53 deletion leads to generation Of tumors.

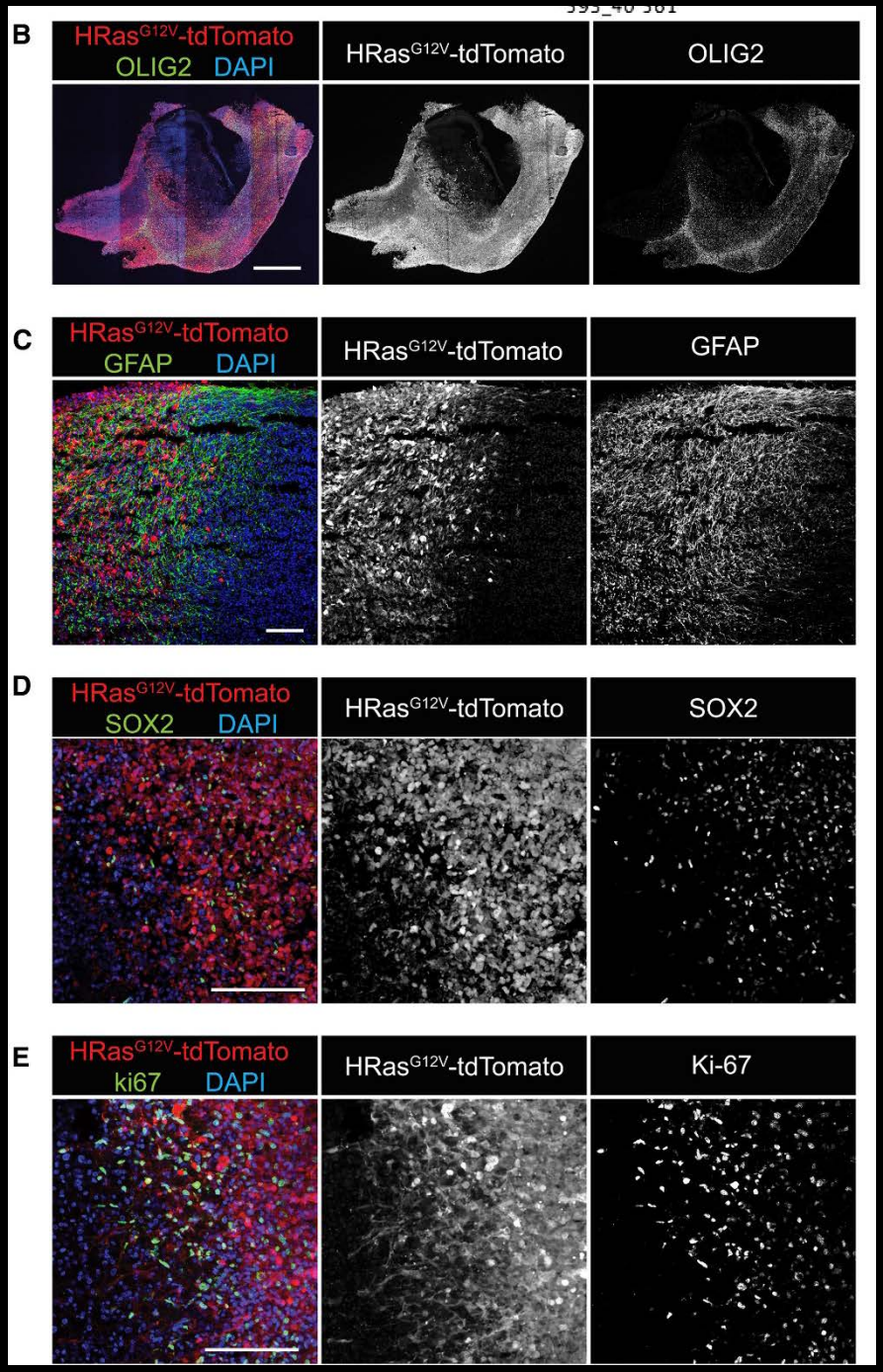


Expression of brain tumor Stem cell markers in transduced organoids

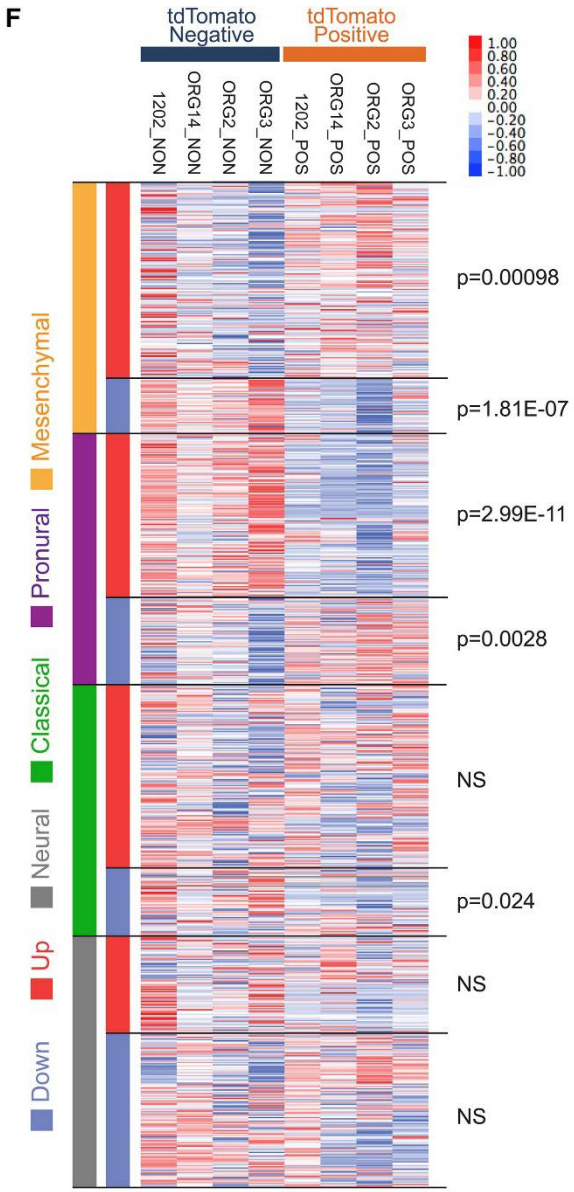


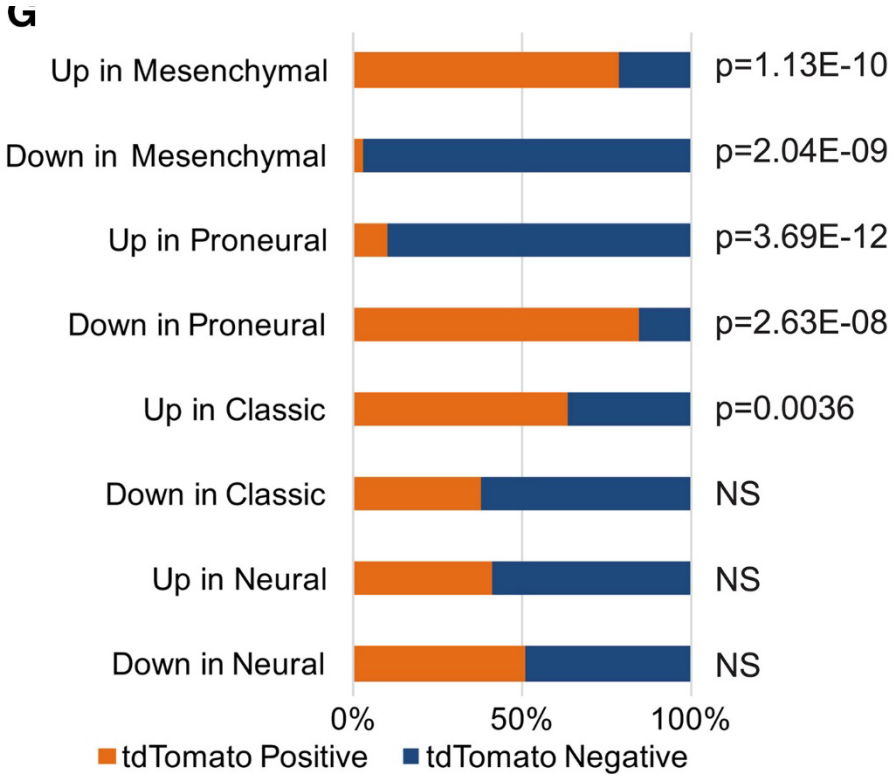
Characterization of Tumor organoids





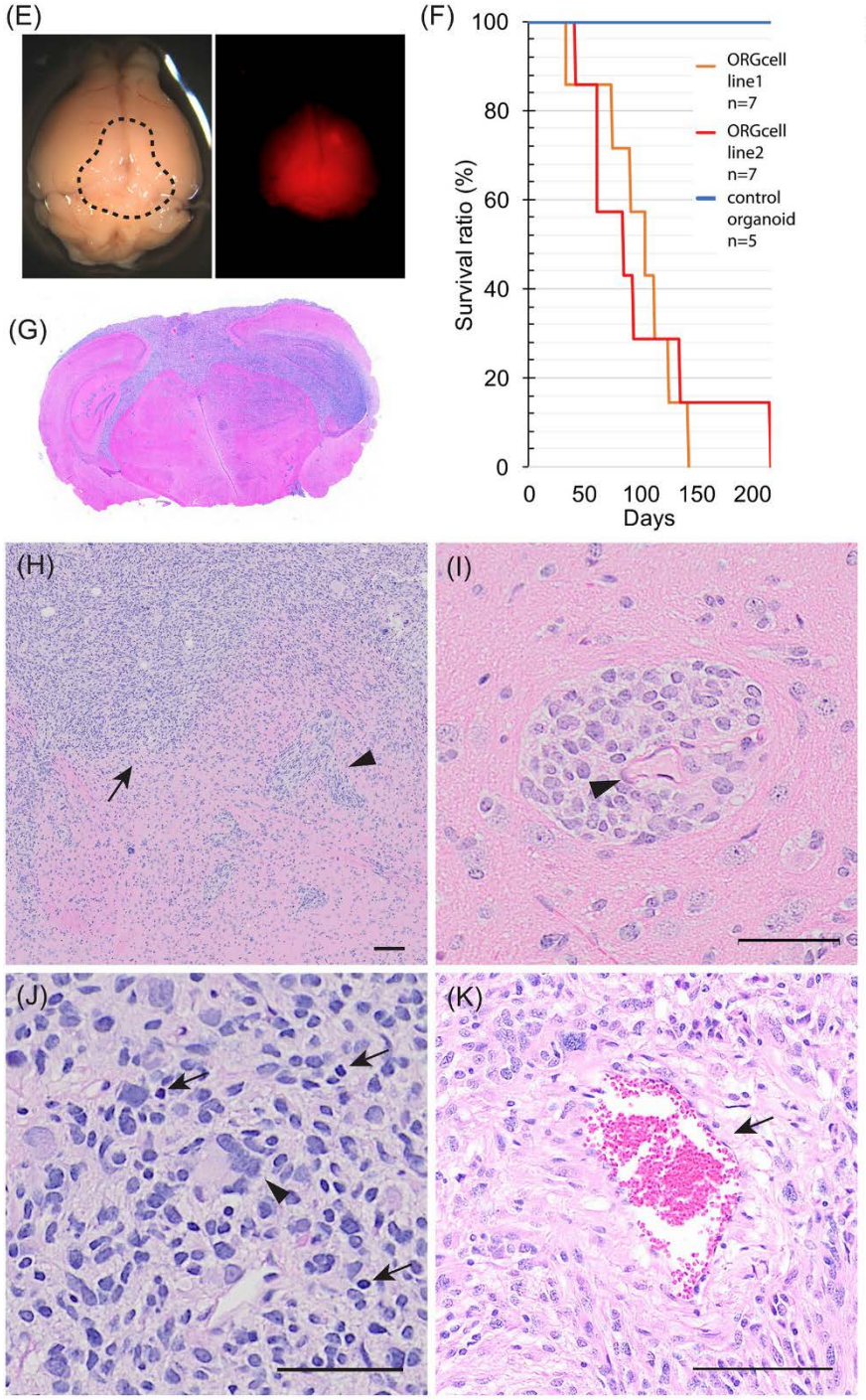
Molecular signature of Tumor organoids: GBM

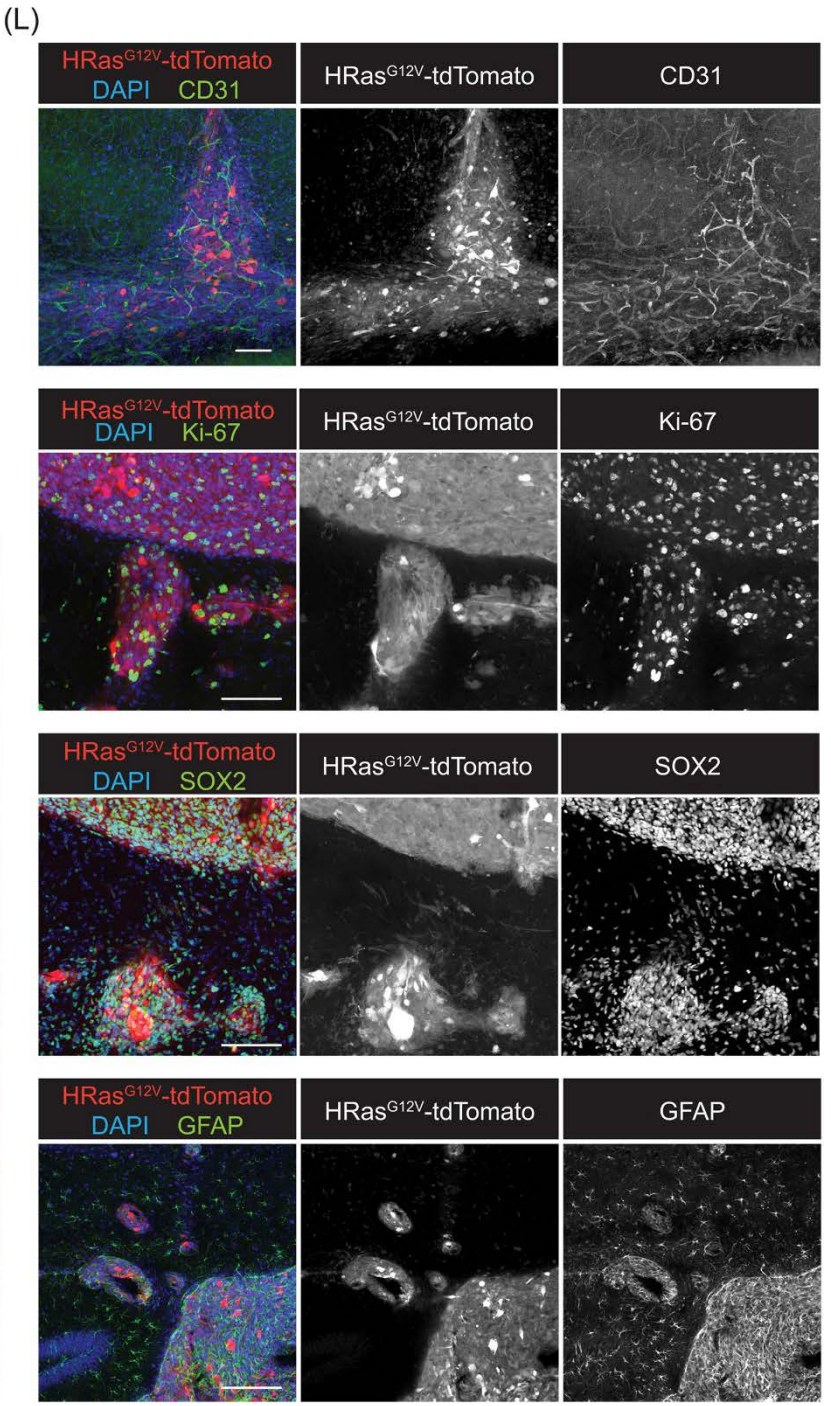




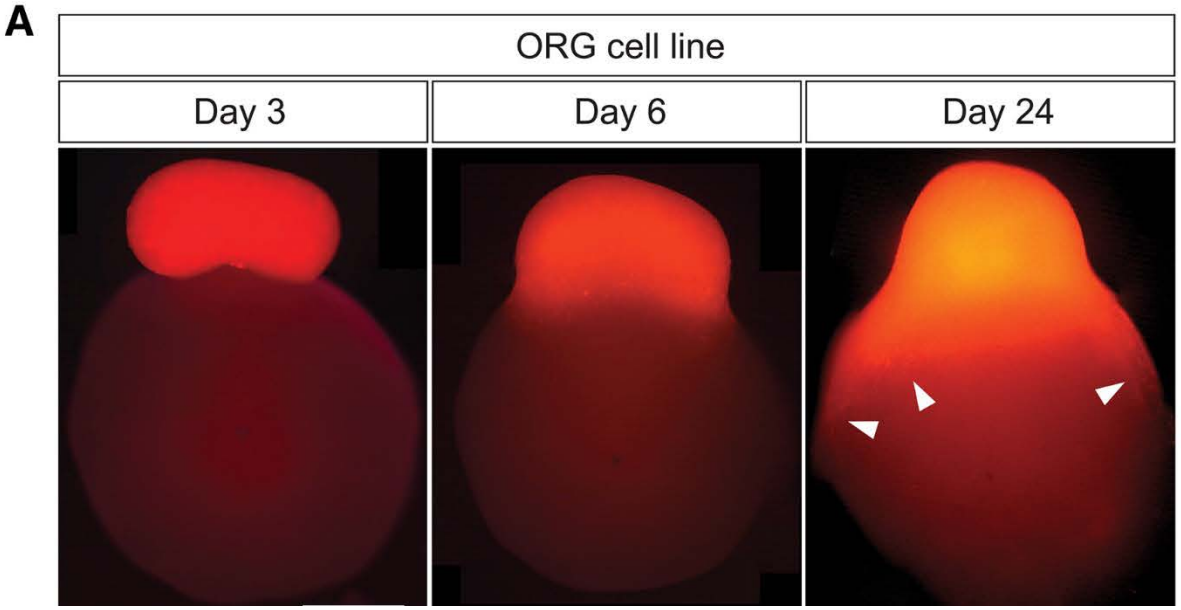
Tumors in organoids Are Tumorogenic and Invasive In vivo

Tumor is diffuse and perivascular



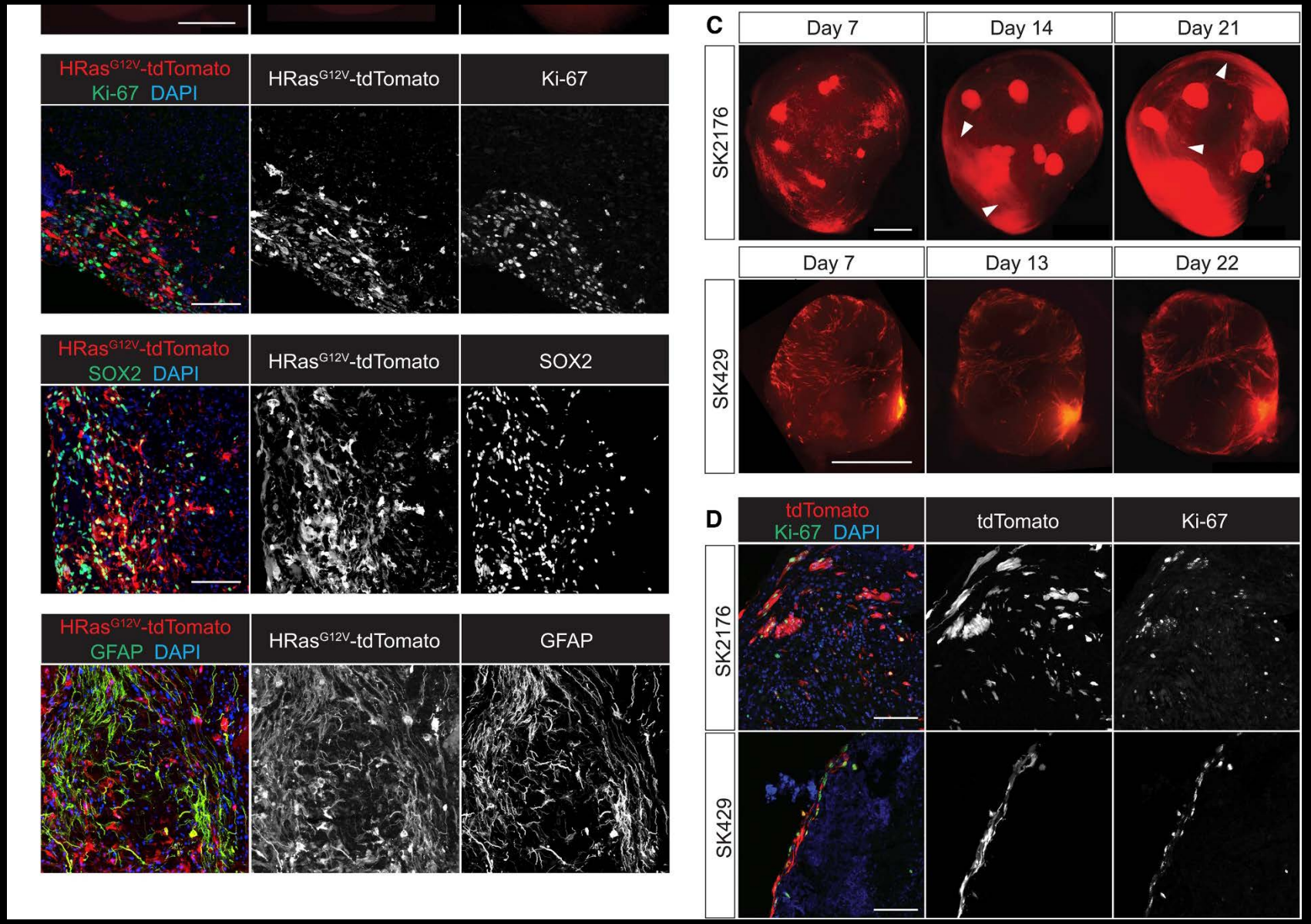


Spread of Tumors between the Organoids



Tumorosphere of the transformed organoid cells attaches and spreads in intransformed organoid

Organoids as platforms for tumor transplantation



Invasiveness of the tumor
Correlates with the lethality in mice

Summary

- Human cerebral organoids can be used as a model for tumor formation.
- Oncogene manipulation by CRISPR/Cas9 initiates tumorigenesis in cerebral organoids.
- Time-lapse microscopic imaging allows observation of tumor development in organoids.
- Tumor cells derived from organoids display an invasive phenotype in xenografted mice

Future of Organoids