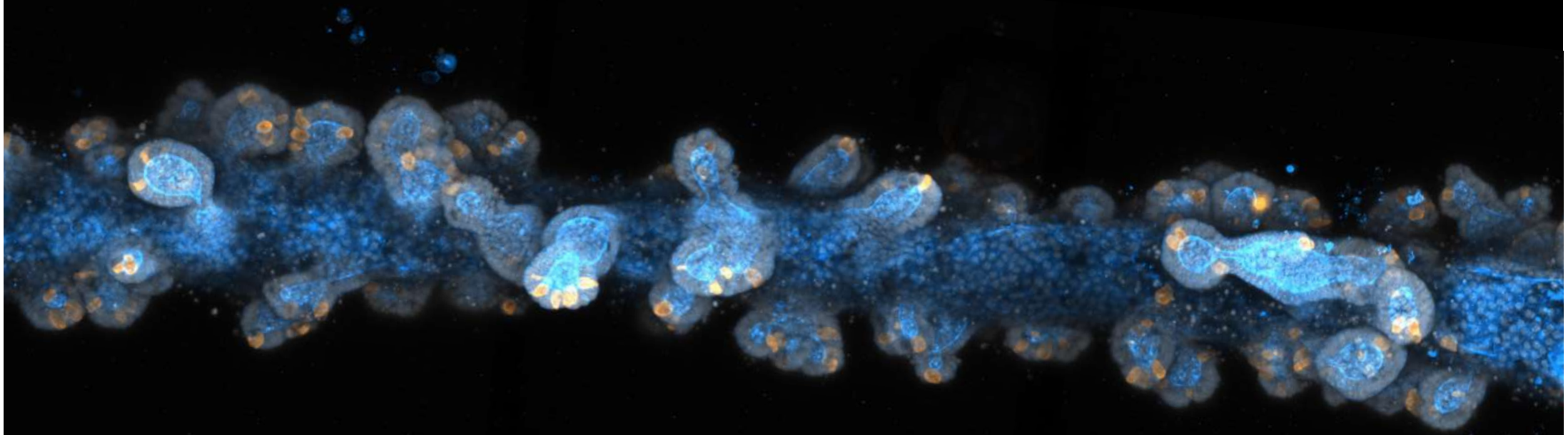
Organoid Technology

Engineering tissues via 'guided' stem cell self-organization



Institute of Human Biology

Next-generation Biomaterials course @EPFL
Matthias Lutolf, 7.3.2025

EPFL

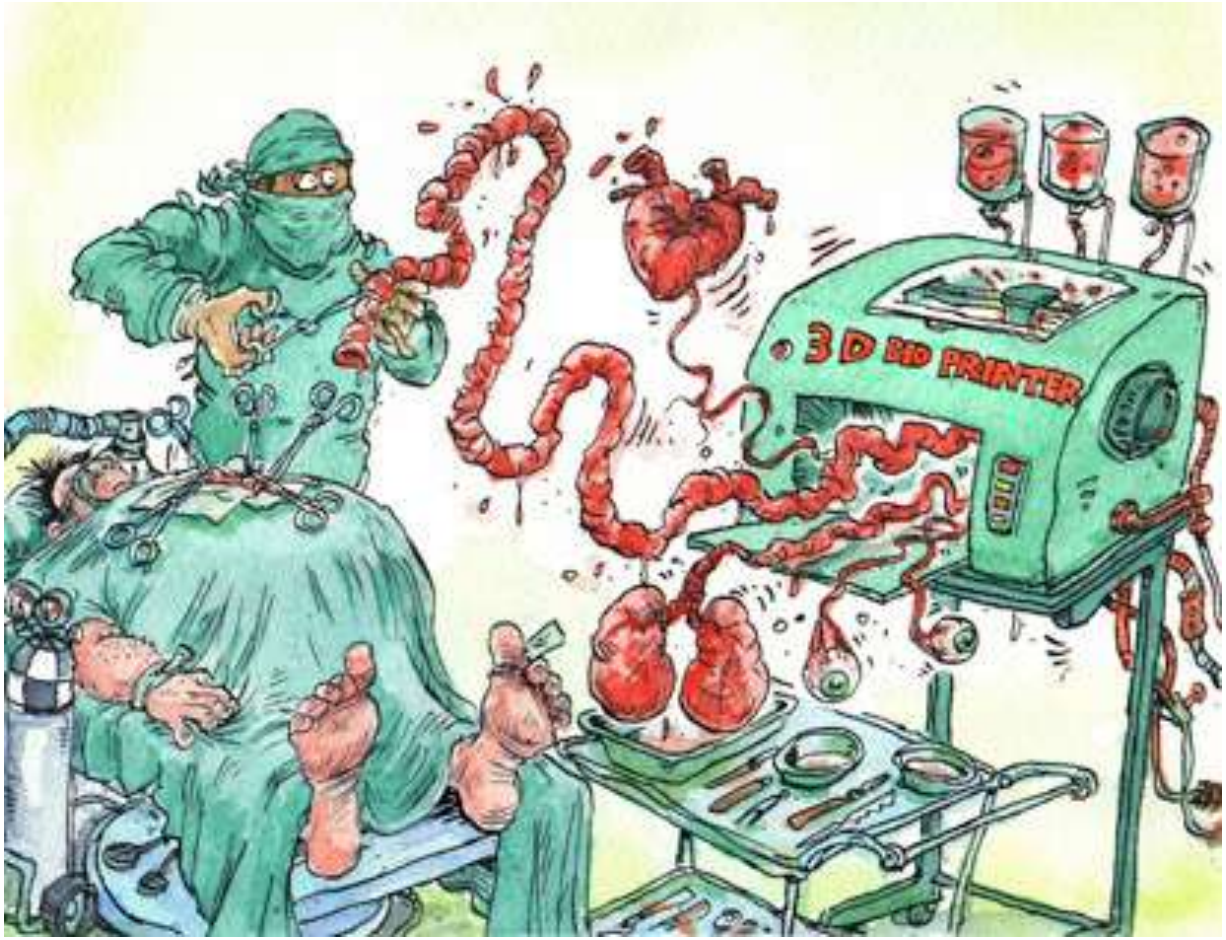
Outline

- Tissue Engineering: some background
- Organoids and the rationale for engineering in the era of 'self-forming' tissues
- Our model system: the mouse small intestine
- **Engineering intestinal stem cell self-organization:**
    1. Increasing tissue *size*
    2. Promoting *stereotypical* tissue *development*
    3. Controlling tissue *architecture*
    4. Capturing tissue *physiology*

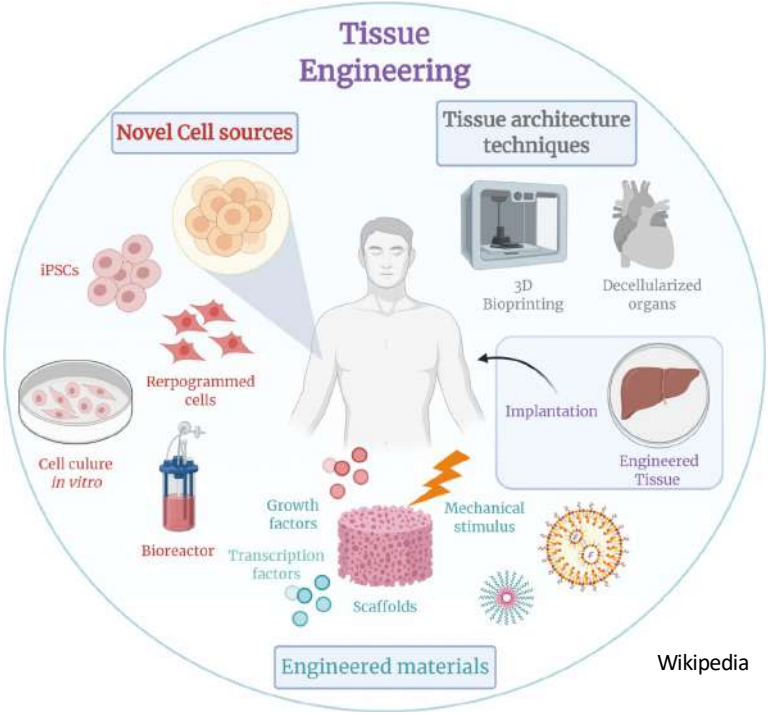
Optimal and sub-optimal regenerators

http://regeneration.bio.uci.edu/content.html

Engineering tissues?



Feb 18th 2010 | The Economist



Wikipedia

Cells + 'Scaffold' = Tissue? 🤔

By and large, the field of tissue engineering has not lived up to great expectations, both scientifically and economically

But:

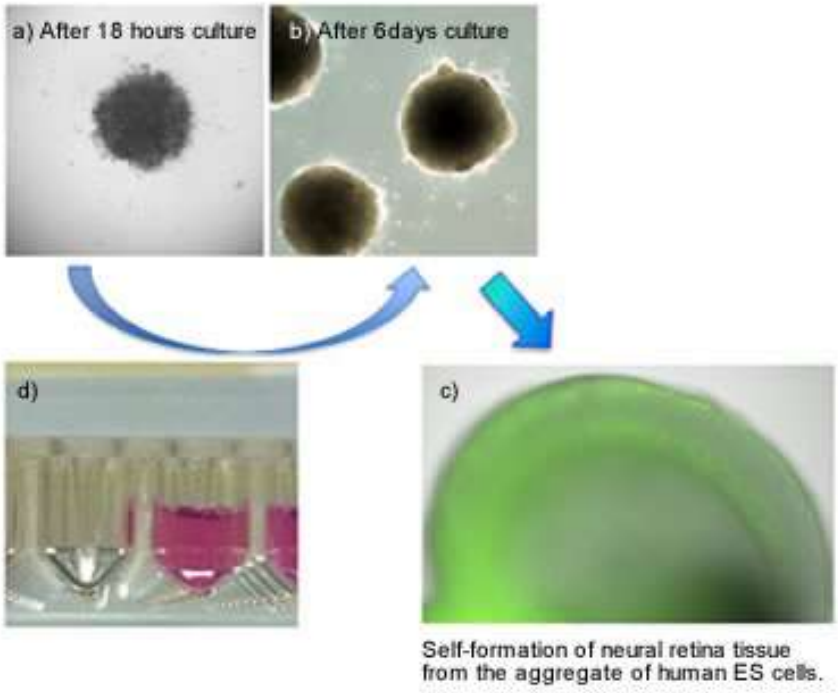
- We learned what does not work
- These efforts have resulted in a wealth of potentially powerful engineering tools for controlling tissue development in vitro and in vivo

ARTICLE

doi:10.1038/nature09941

Self-organizing optic-cup morphogenesis in three-dimensional culture

Mototsugu Eiraku[1,2], Nozomu Takata[1], Hiroki Ishibashi[3], Masako Kawada[1], Eriko Sakakura[1,2], Satoru Okuda[3], Kiyotoshi Sekiguchi[4], Taiji Adachi[3,5] & Yoshiki Sasai[1,2]



Self-formation of neural retina tissue from the aggregate of human ES cells.

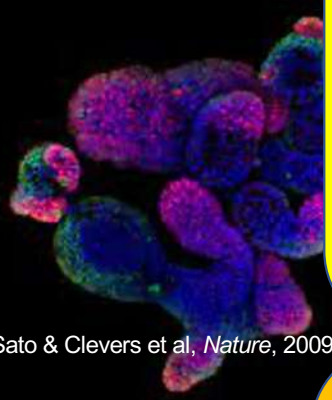
Our cells want to build tissues.
They are the best tissue engineers!

We need to use the right cell types and, when cultured outside the body, expose them to the right (*in vivo*-like) signaling environment to control their fate and steer their self-organization

Organoids: Miniature 3D tissue/organ mimetics derived from stem cells

- Concomitant presence of multiple tissue-specific cell types
- Spatial organisation of the different cell types
- General architecture of the tissue

‘Mini-intestine’



Sato & Clevers et al, *Nature*, 2009

‘creas’

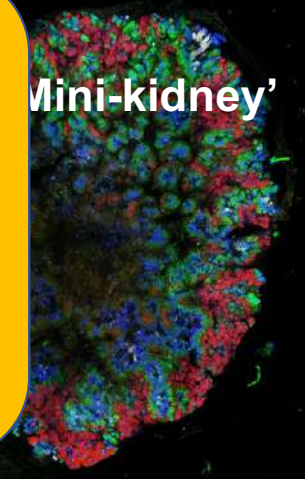
Greggio & Grapin-Botton et al, *Development*, 2013

- <u>Pattern emergence from cell aggregates without any pre-pattern</u>
- Absence of any long-range patterning influences such as morphogen gradients or mechanical cues
- No or very little physical boundaries: Growth in very soft matrices or as ‘floating aggregates’

‘Mini-t

Van de Wetering & Clevers et al, *Cell*, 2015

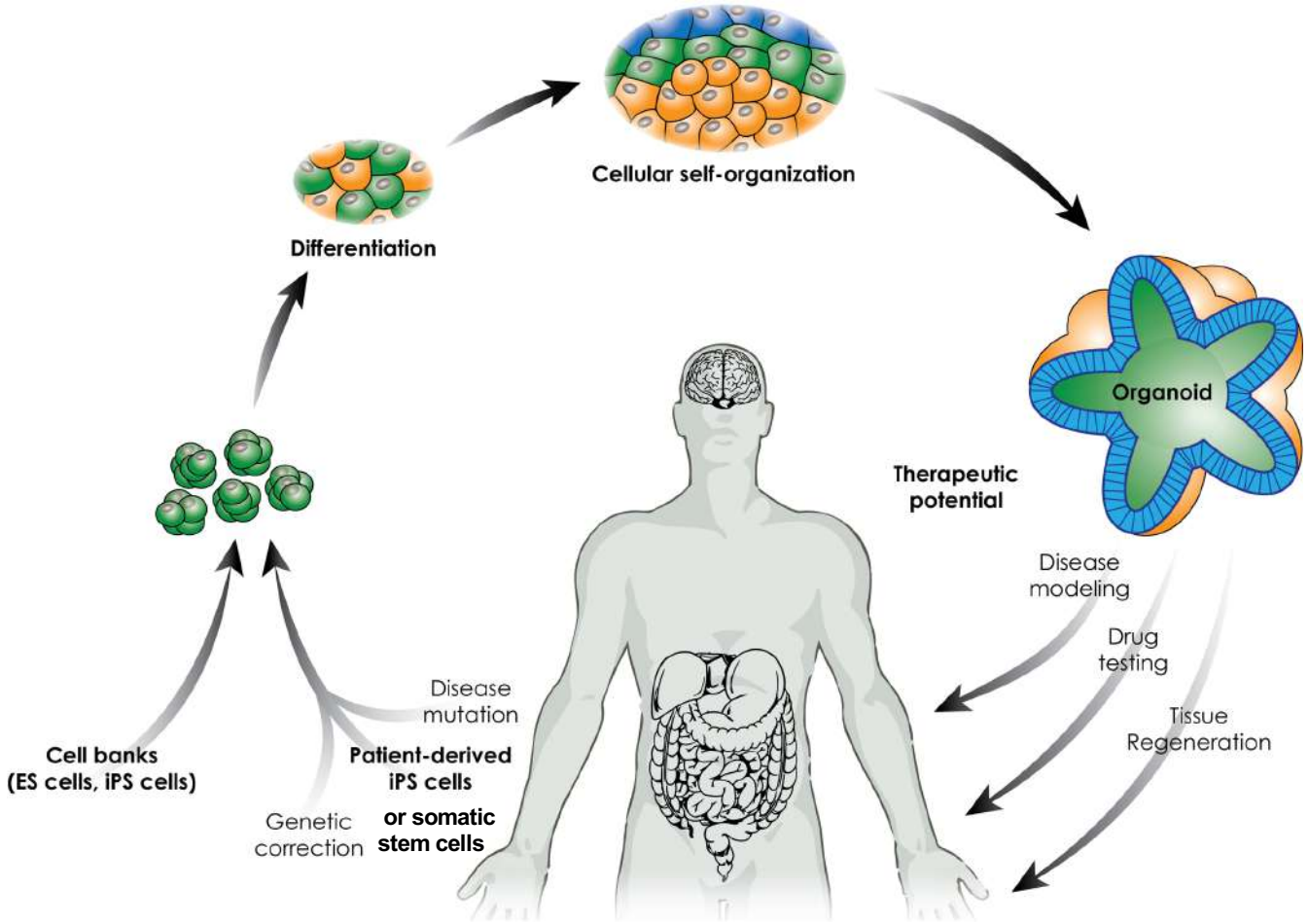
‘Mini-kidney’



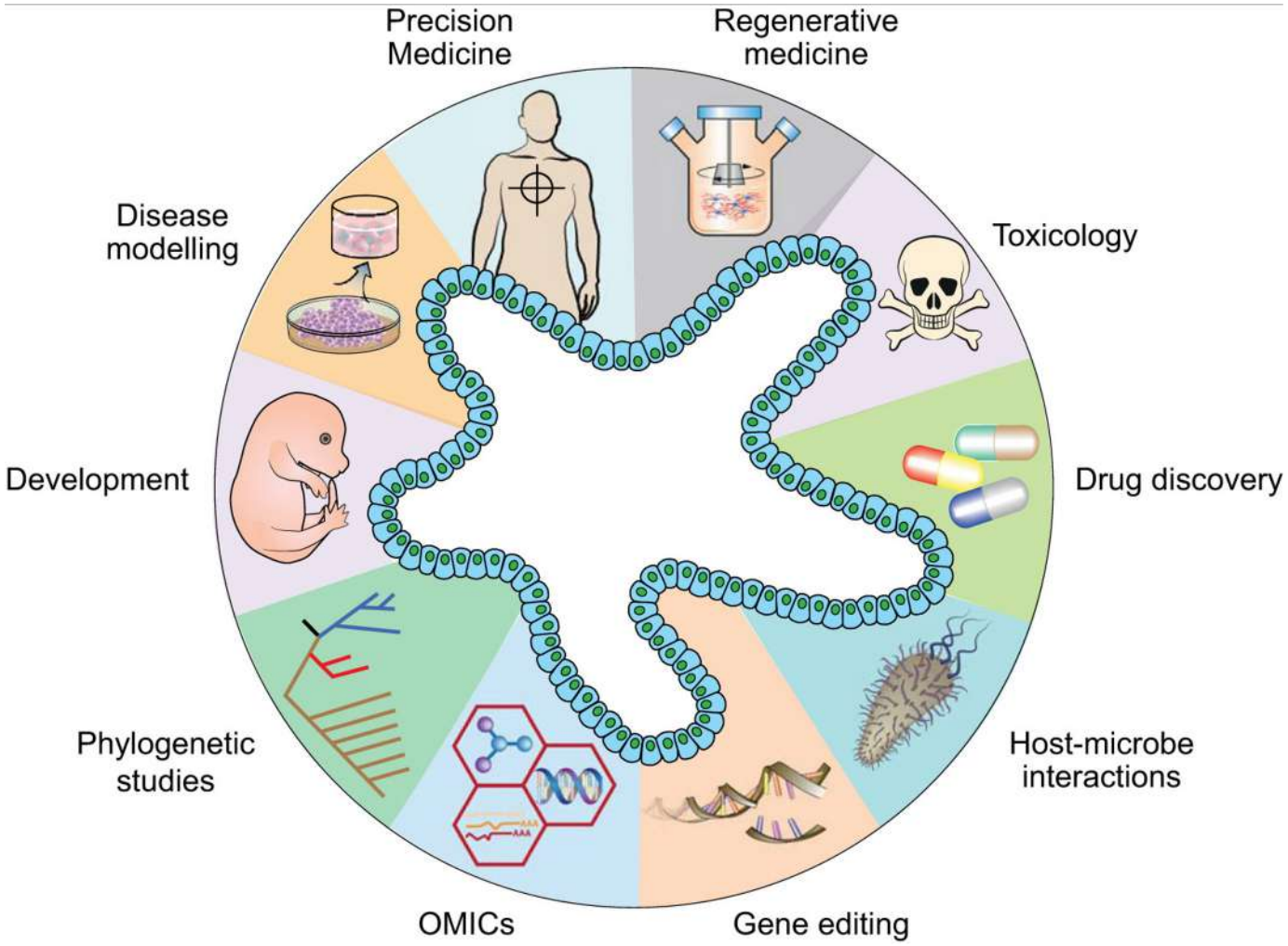
Takasato & Little et al, *Nature*, 2015

Lancaster & Knoblich et al, *Nature*, 2013

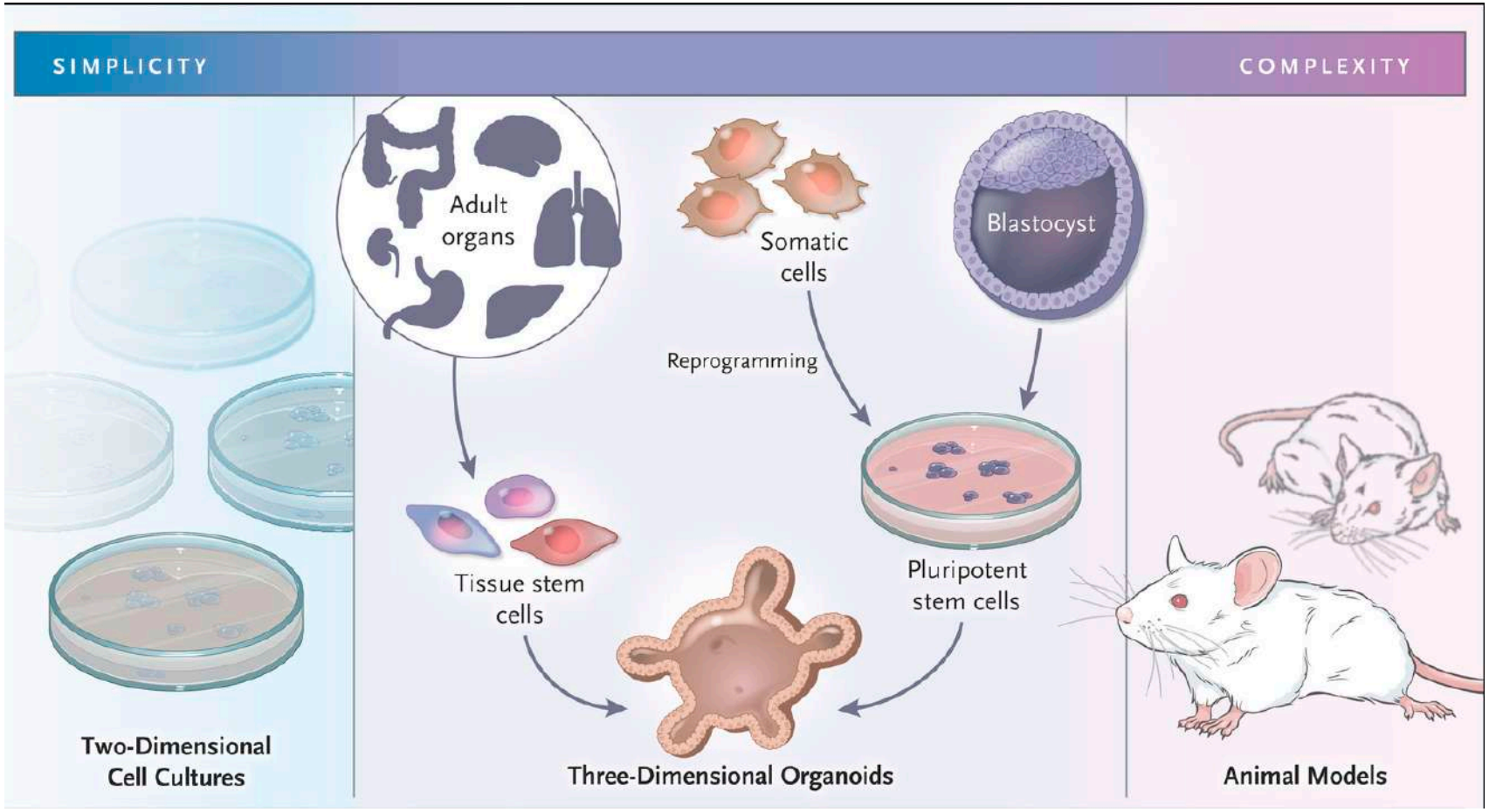
The promise of organoid technology



Lancaster and Knoblich, Science, 2014

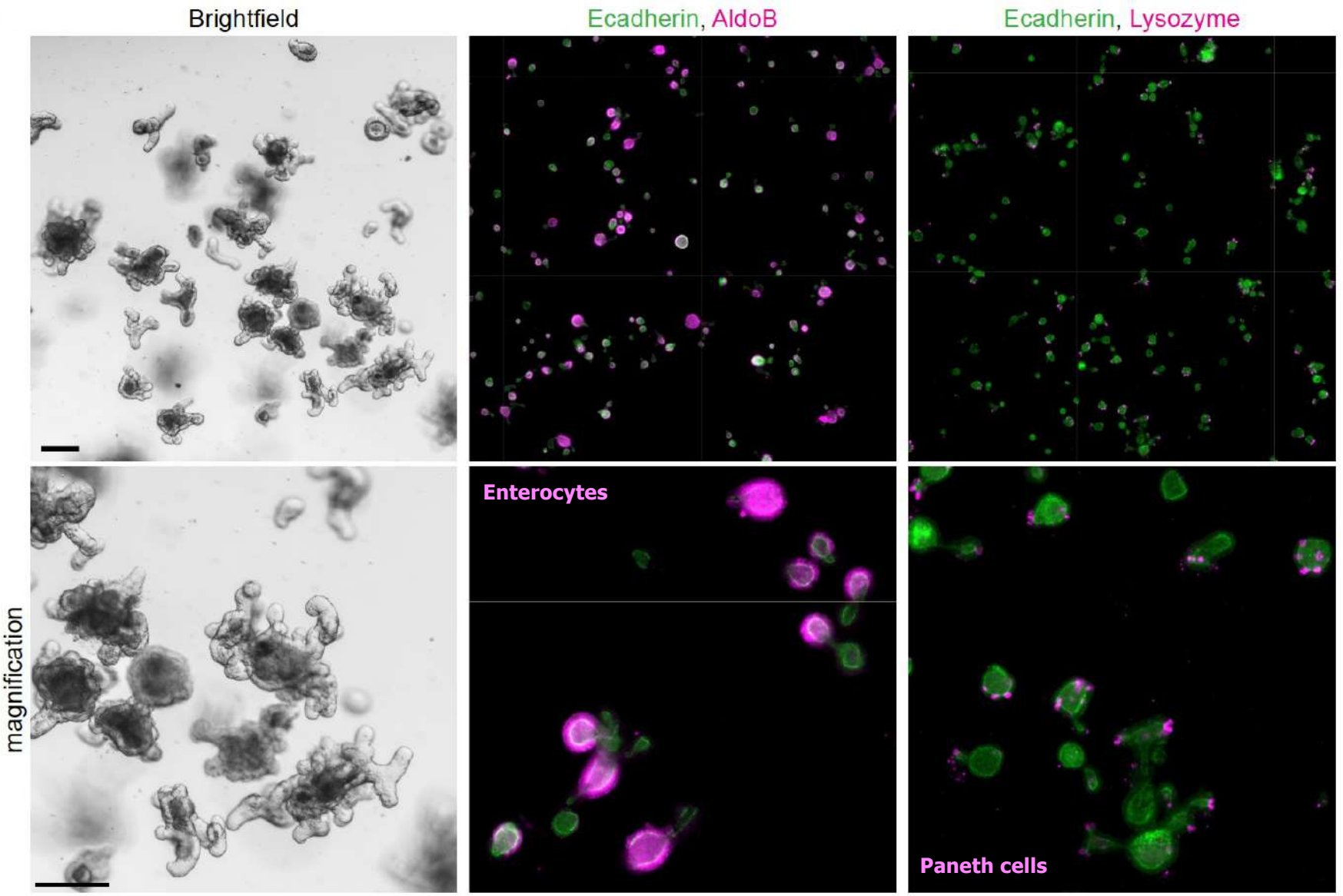

Precision Medicine
Regenerative medicine
Disease modelling
Toxicology
Development
Drug discovery
Phylogenetic studies
Host-microbe interactions
OMICs
Gene editing

A scalable window into 'real' human biology!?

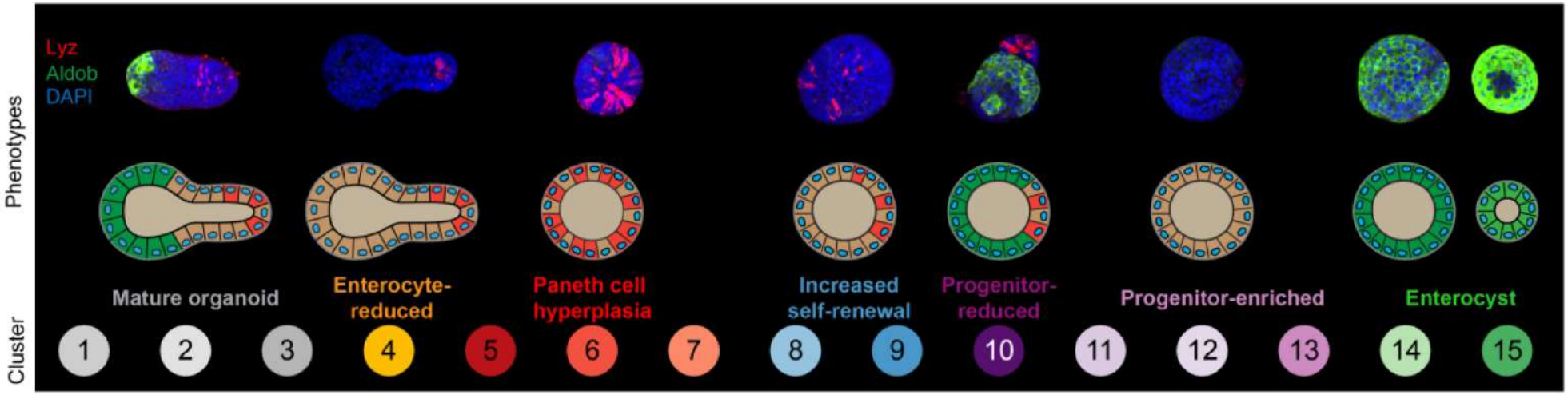


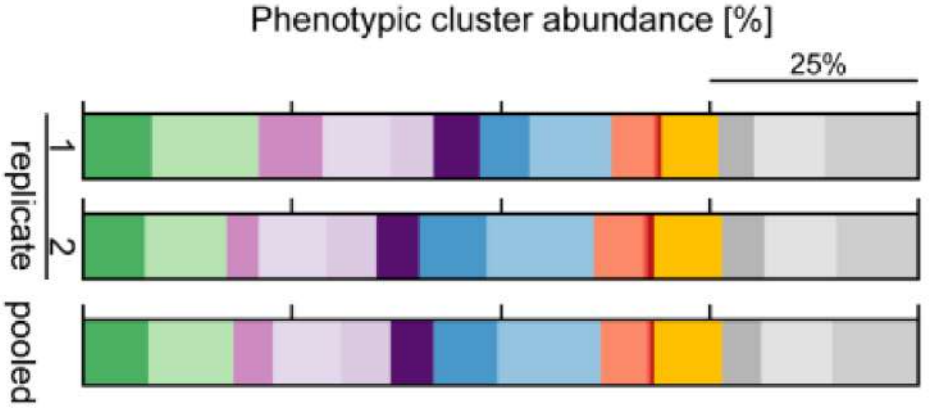
Li et al, N Engl J Med, 2019

So do we still need tissue engineering…?!


Brightfield
Ecadherin, AldoB
Ecadherin, Lysozyme
magnification
Enterocytes
Paneth cells

Mouse small intestinal organoids in 3D Matrigel: at least seven phenotypes!



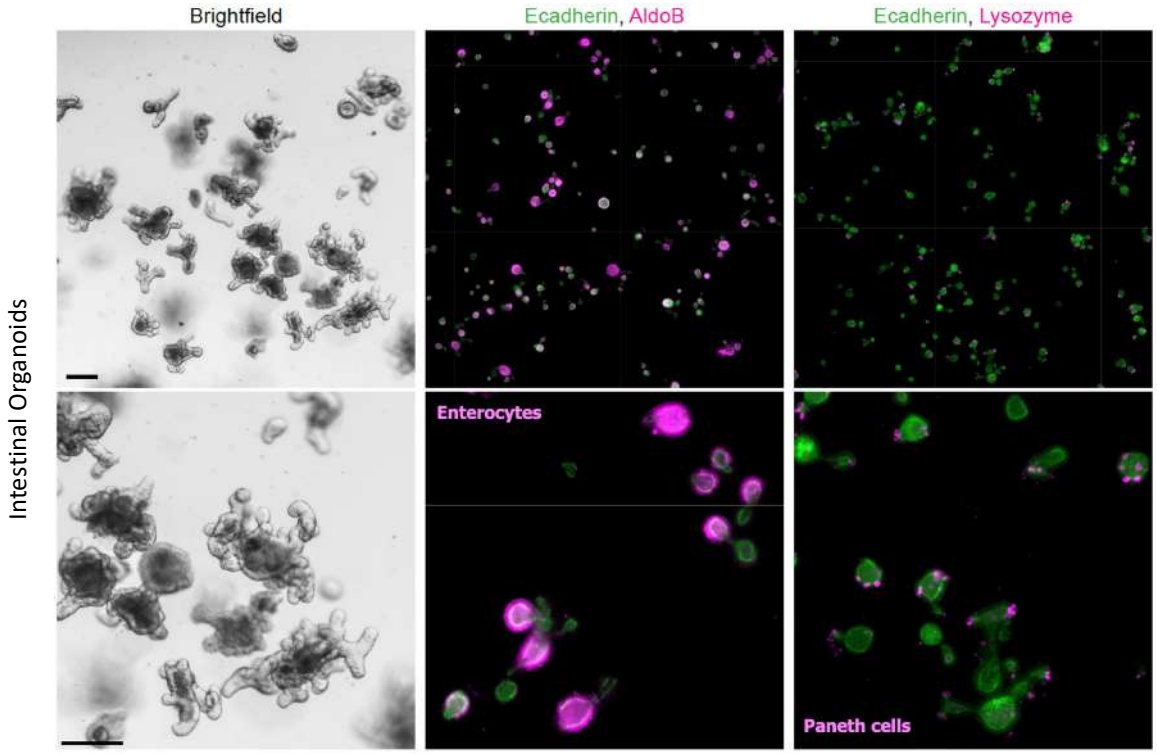


Lukonin and Liberali et al., Nature, 2020

The reality: Still many translational challenges!

Reproducibility and scalability:

Huge variability in morphology, size, cell type composition, pattern, etc.



Architecture and accessibility:

Millimeter scale max., closed architecture

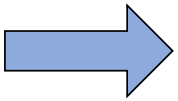
Organ-level complexity, function and maturity:

Lack of key tissues (e.g., immune system, vasculature, stroma, innervation)

Read-outs:

Lack of *in vivo*-relevant read-outs

Absence of any predefined extrinsic patterning influence & physical boundary conditions



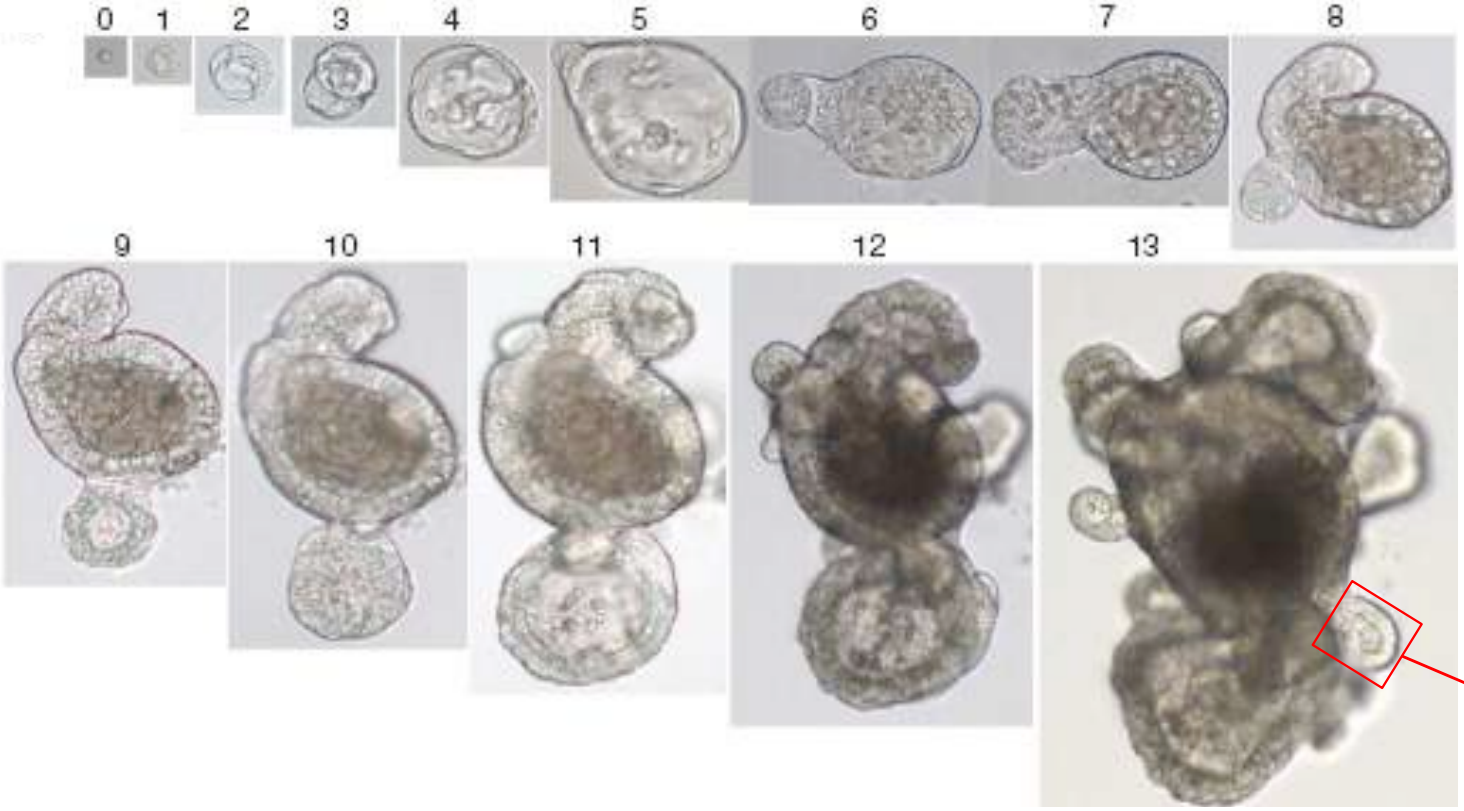
Poorly controlled morphogenesis

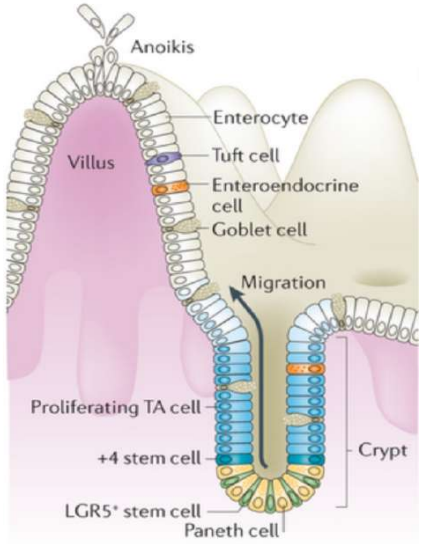
Can we use engineering approaches to extrinsically 'guide' the spontaneous self-organization of stem cells to achieve physiologically relevant tissue shapes and sizes?

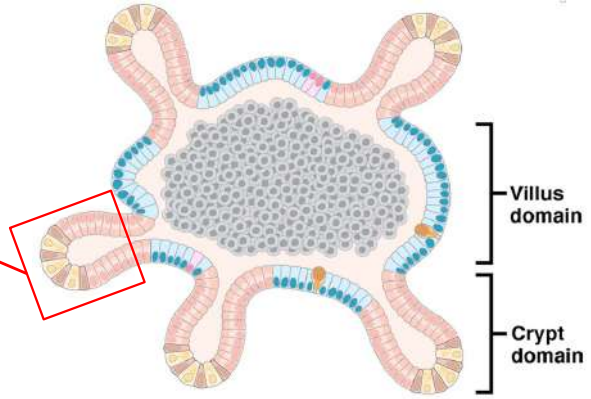
Our main model system: mouse intestinal organoids

Mouse intestinal organoids (T. Sato, H. Clevers & team)

3D Matrigel (laminin-111) + EGF, Noggin, R-Spondin (ENR):

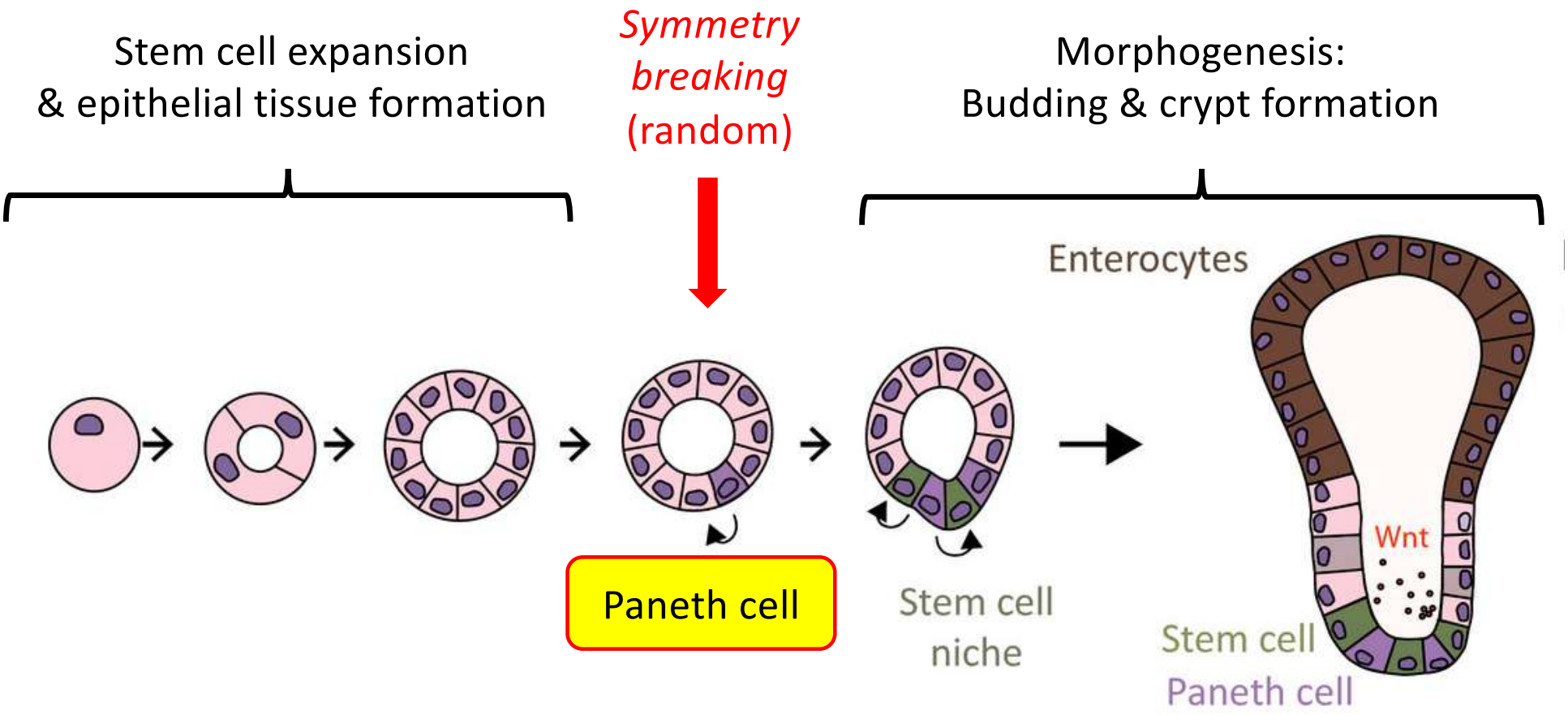






Sato and Clevers et al, Nature, 2009

Intestinal organoid development: key steps



Liberali et al., FEBS J, 2019

Outline of my lecture

- Tissue Engineering: some background
- The rationale for engineering in the era of 'self-forming' tissues
- Our model system: the mouse small intestine
- Engineering intestinal stem cell self-organization:
  1. Increasing tissue *size*
  2. Promoting *stereotypical* tissue *development*
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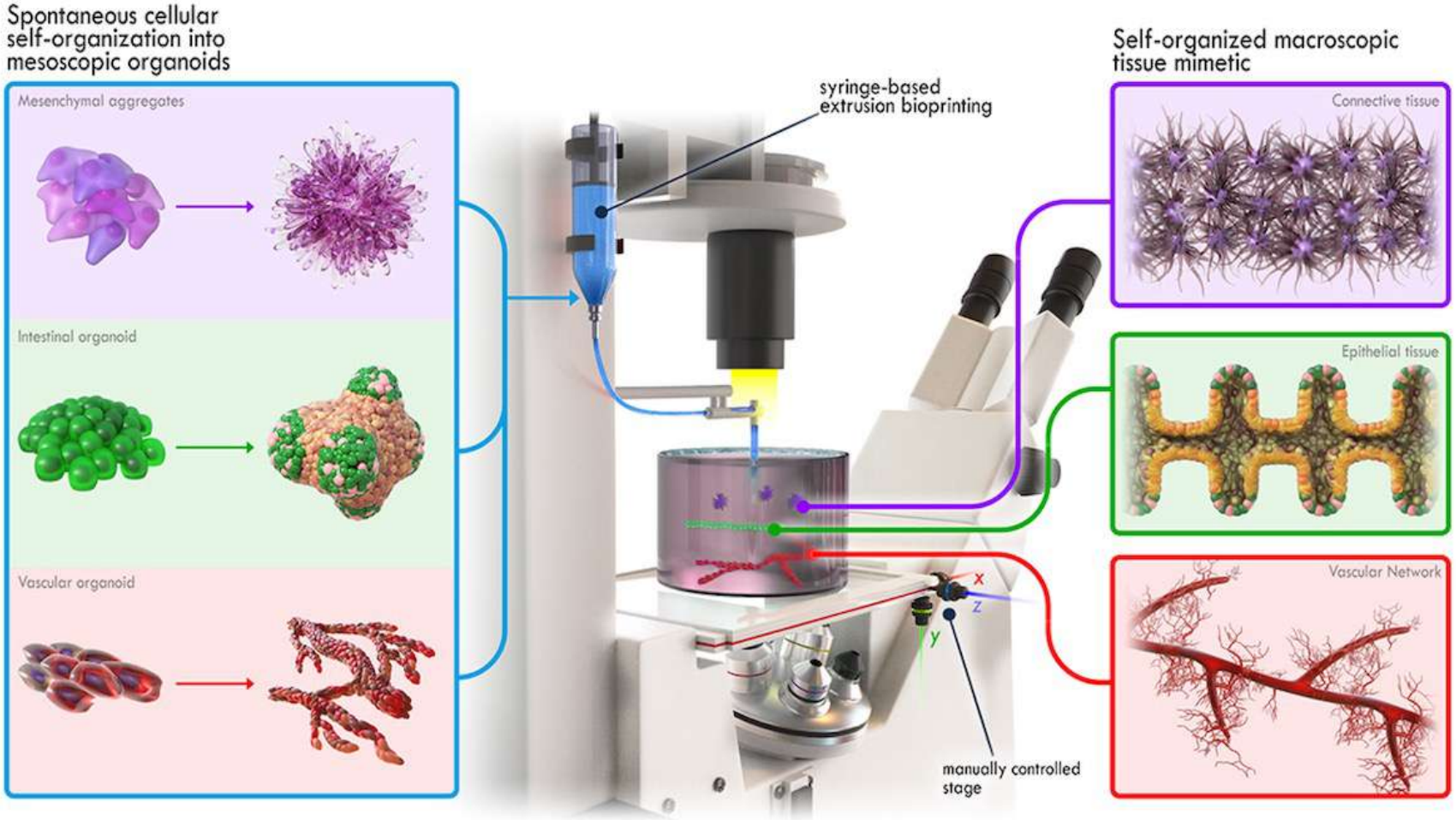
  4. Capturing tissue *physiology*?

1. Engineering tissue size

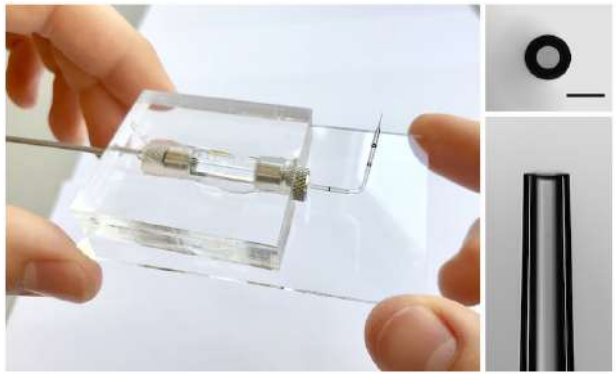
Can we push self-organization from the millimetre to the centimetre scale?

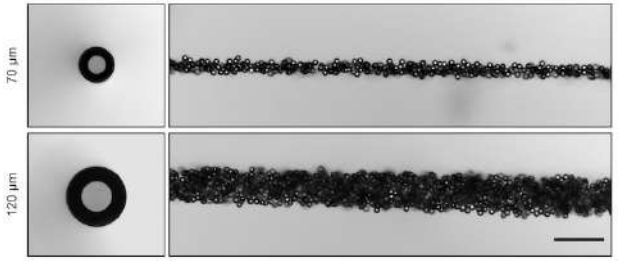


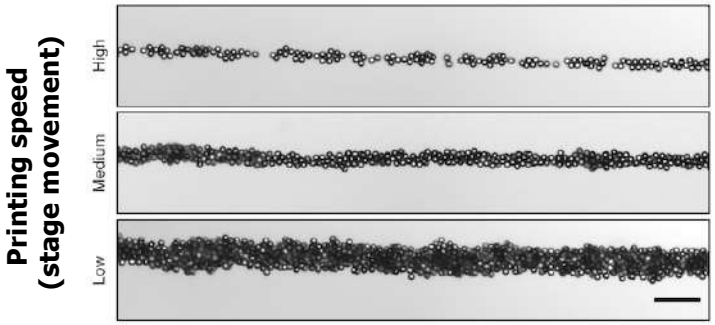
- ***Syringe-based extrusion system coupled to microscope with controlled XYZ stage***
- ***Printing of organoid-forming stem cells directly into liquid precursors of typical ECM gels conducive to spontaneous self-organization***

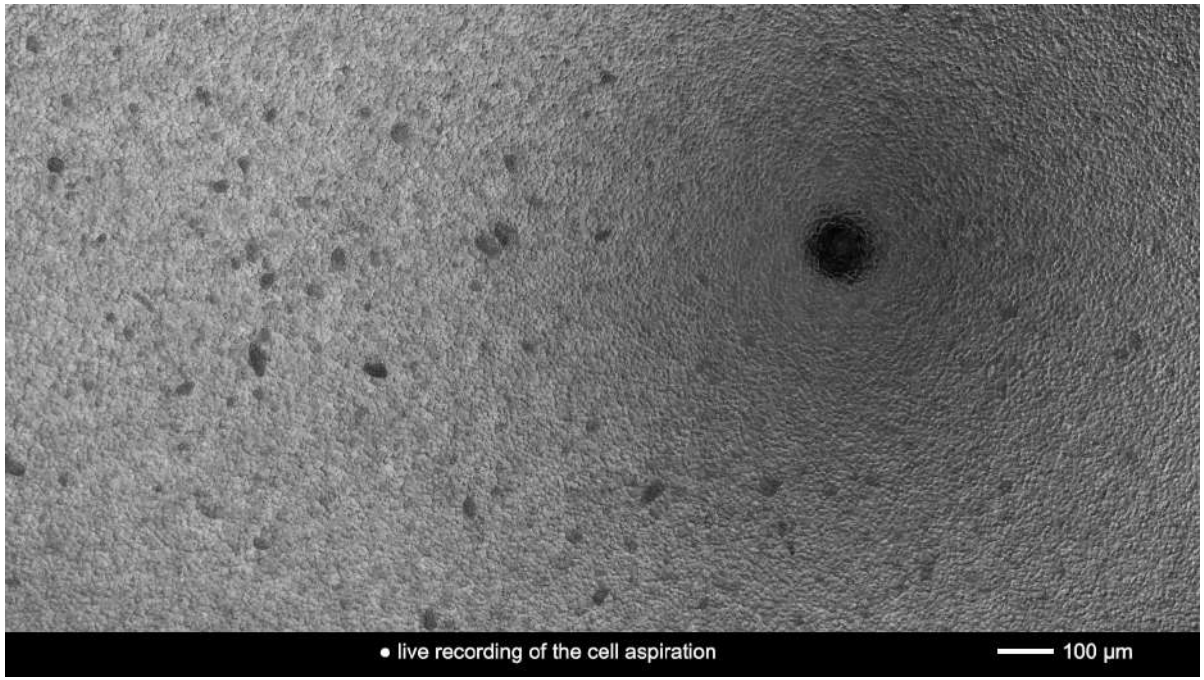
Brassard, Nikolaev, Hübscher et al., Nature Materials, 2021

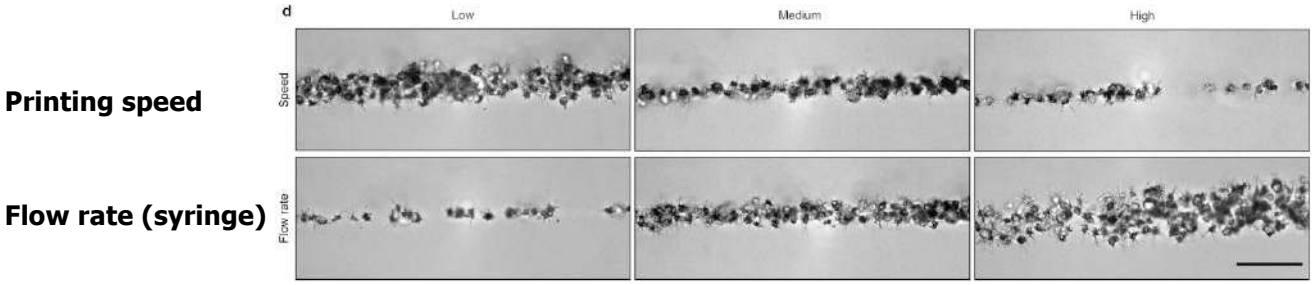
Syringe-based extrusion printing of organoid-forming stem cells into highly permissive biological ECMs (Matrigel, collagen, …)

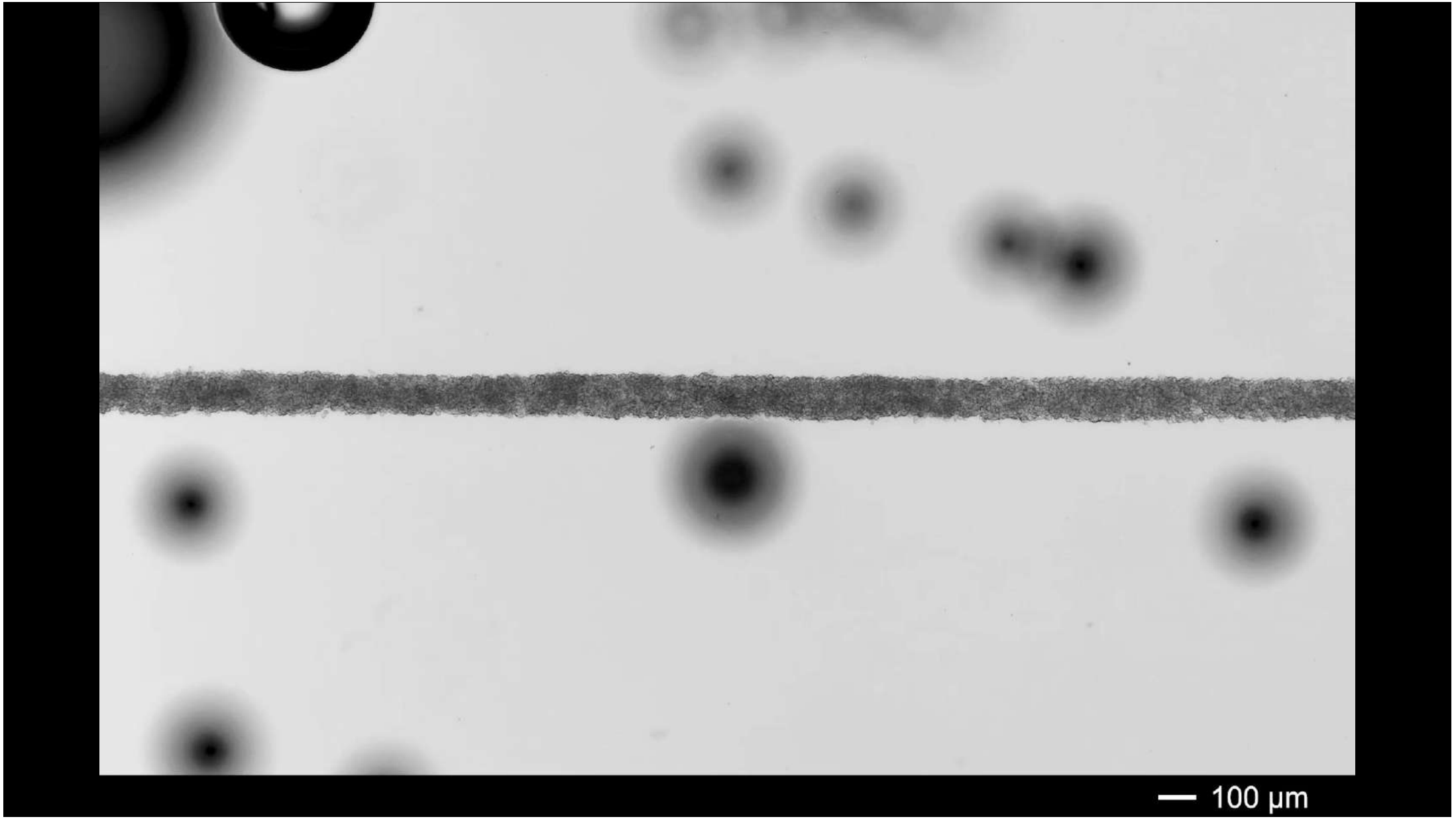


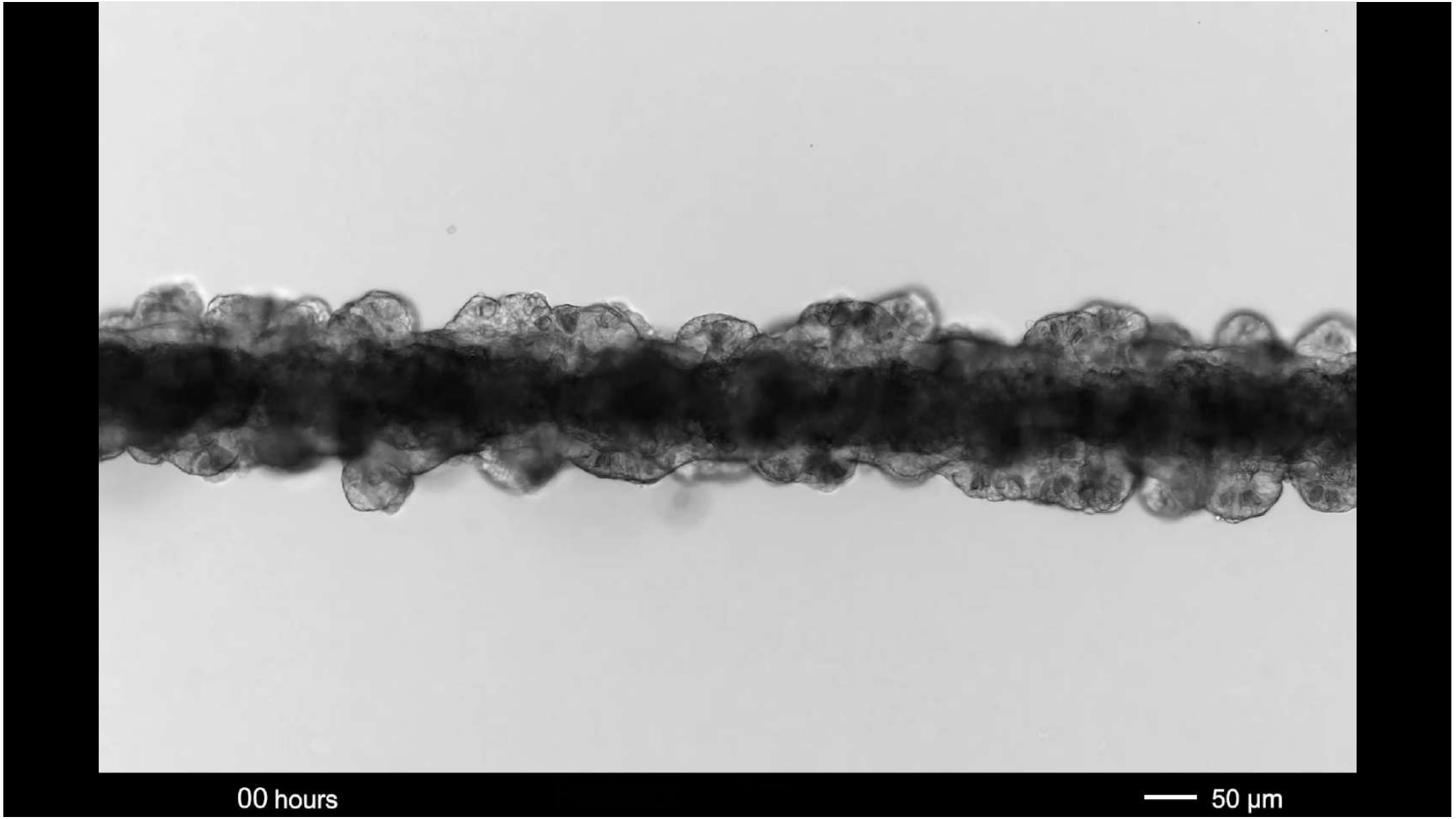




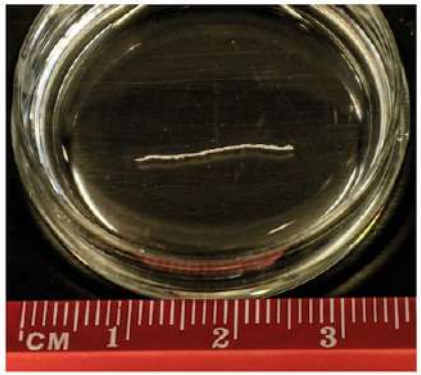


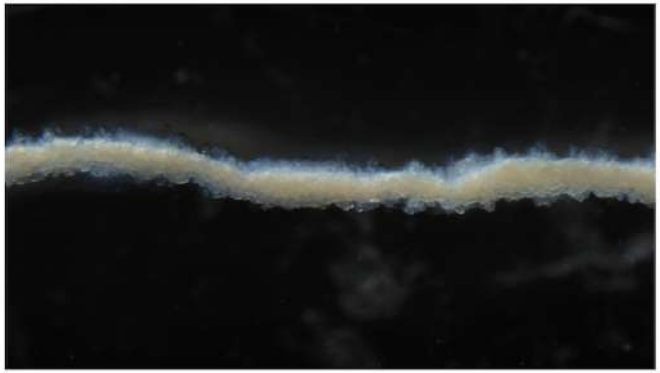


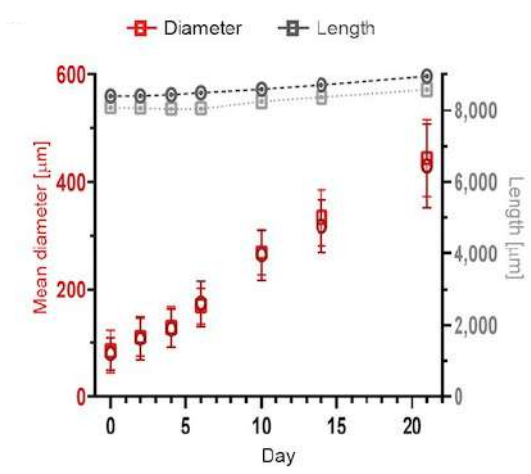

100 µm

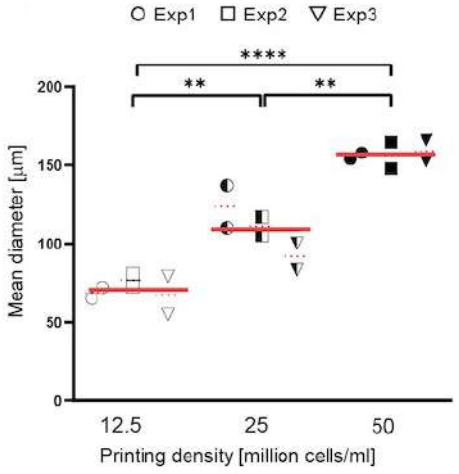

00 hours
50 µm

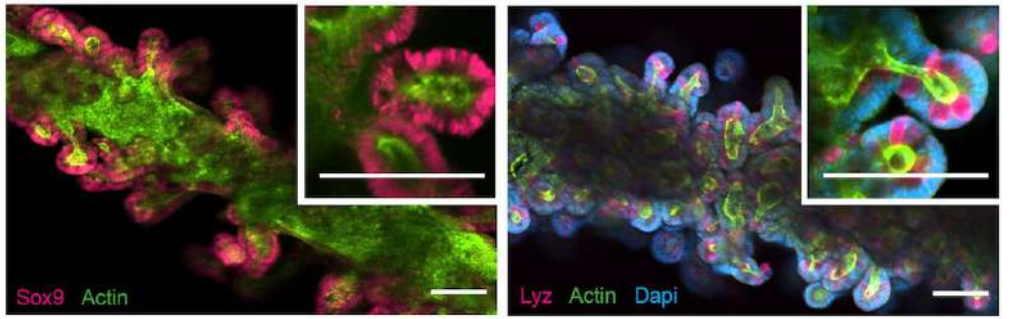
Fully patterned, centimeter-scale intestinal tubes!

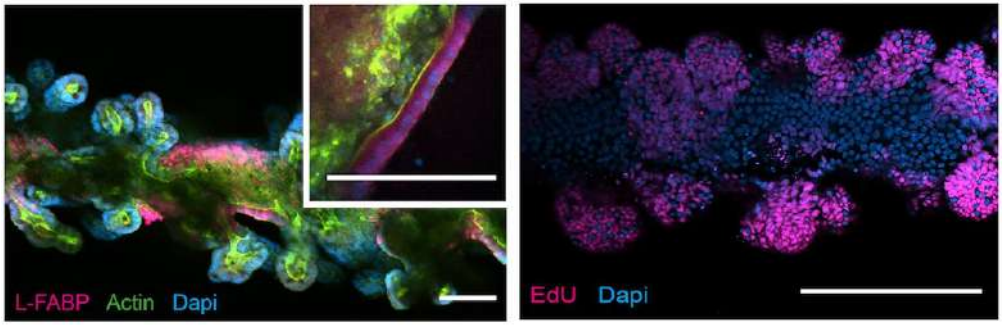


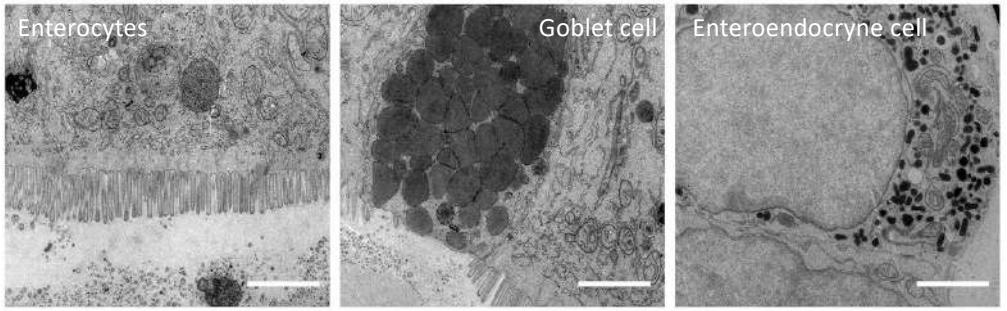






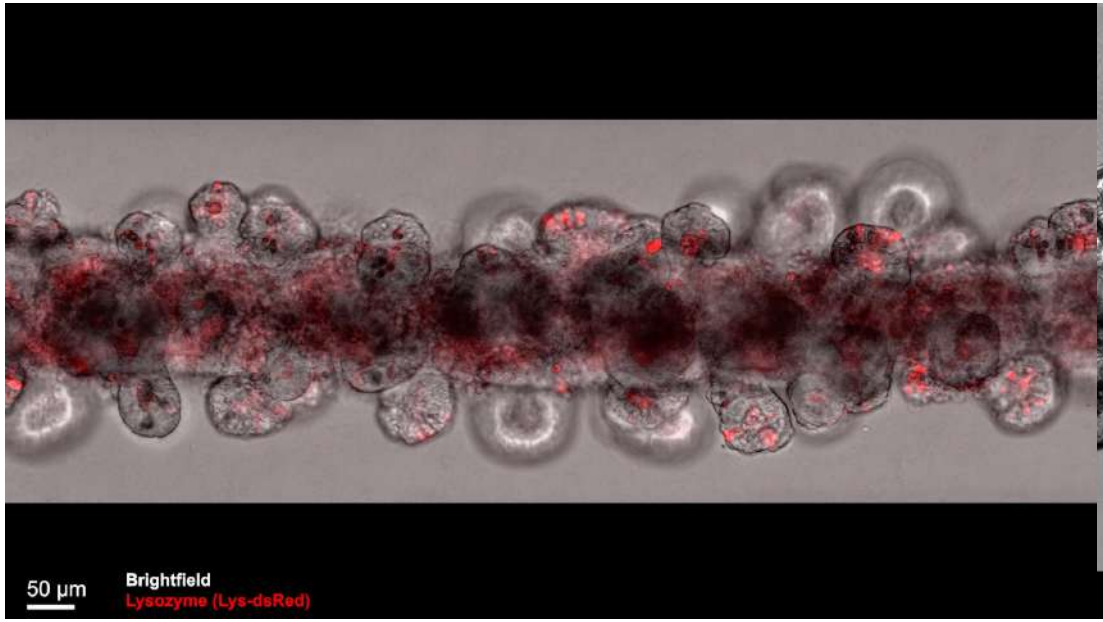






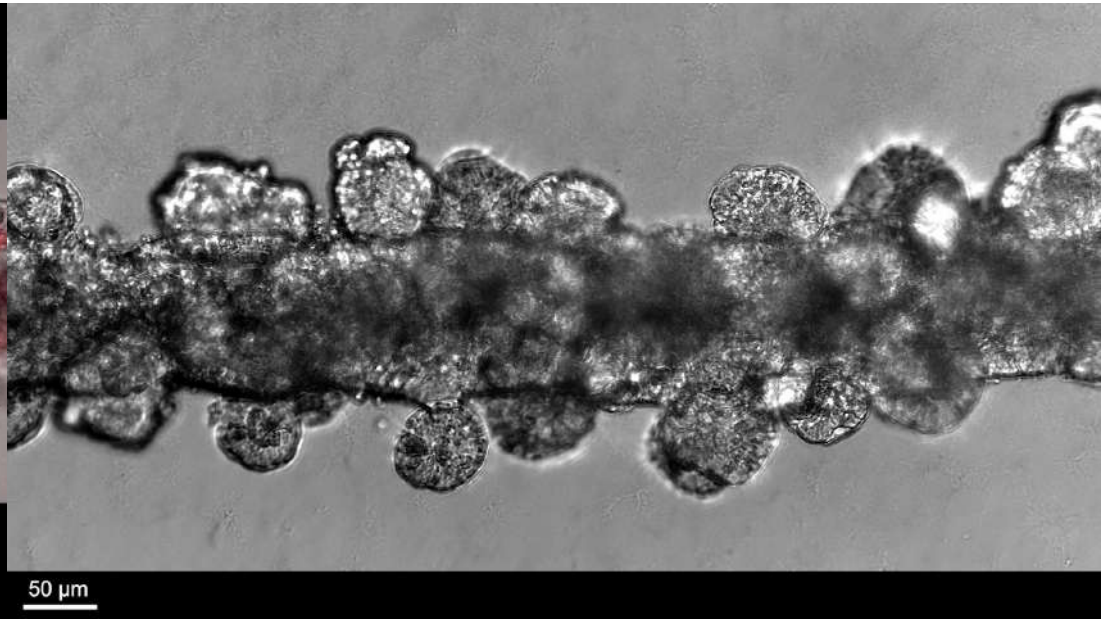
Physiological responses to chemical stimuli

Release of lysozyme granules (Paneth cells):



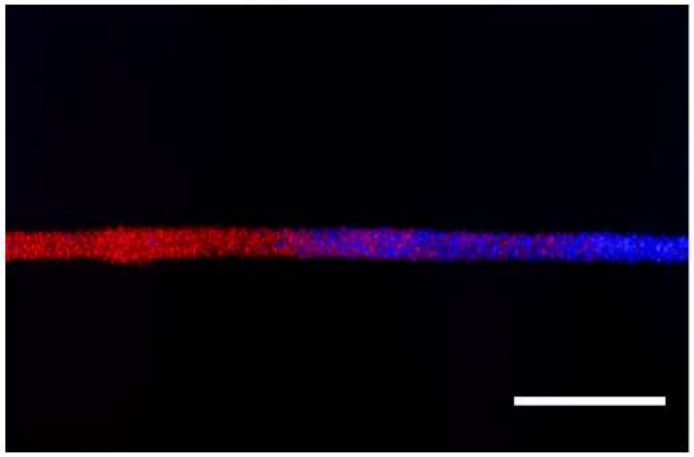
Exposure to carbamylcholine (100 µM)

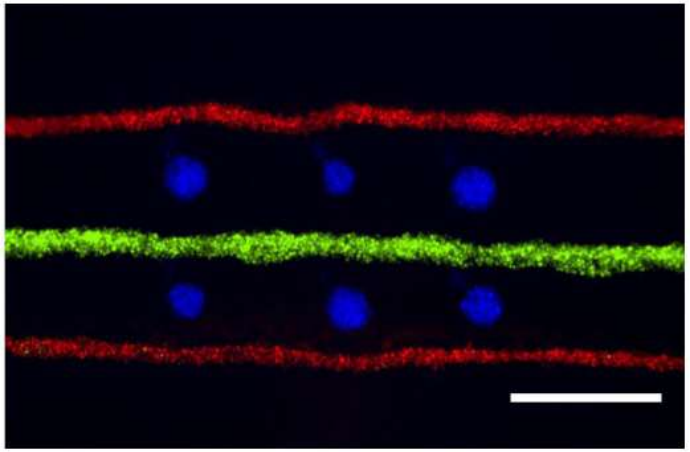
Swelling (activation of CFTR channels):

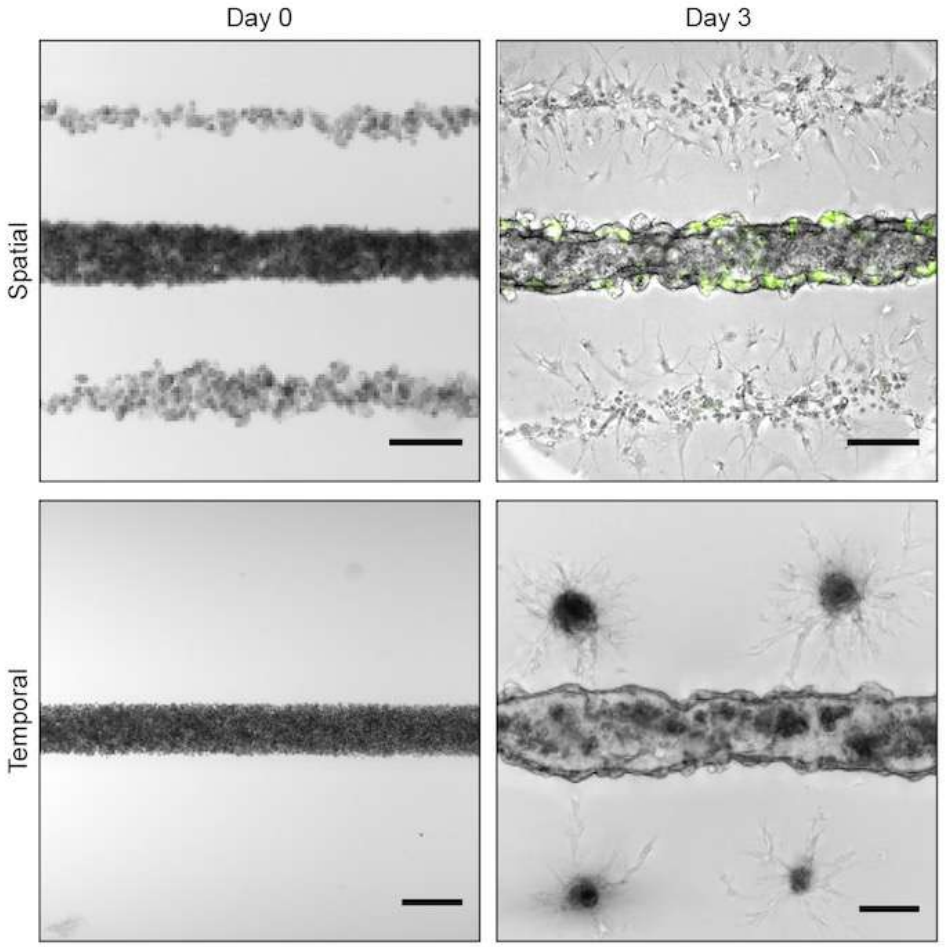


Exposure to forskolin (20 µM)

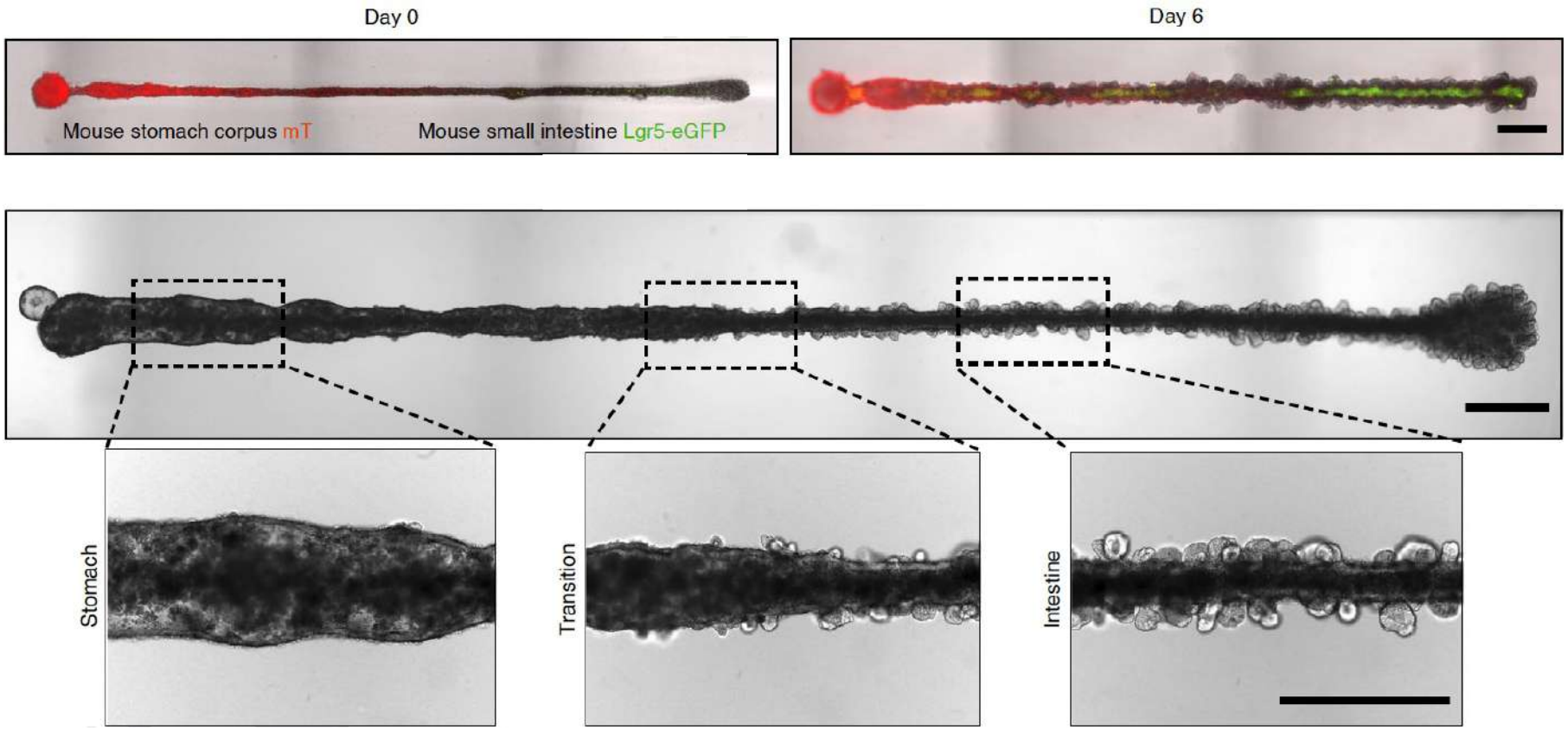
Towards multi-tissue assembly







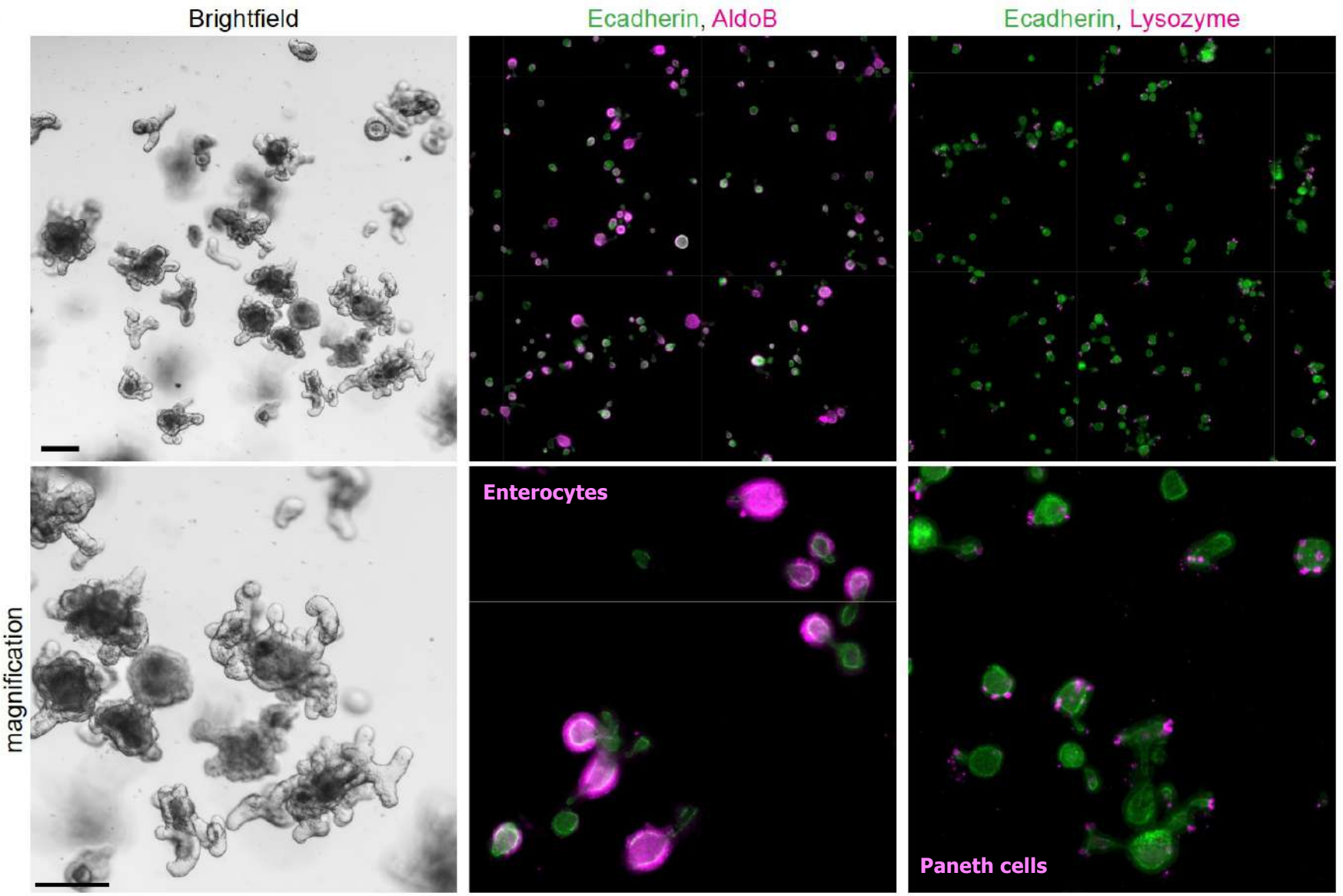
ISCs + intestinal myofibroblasts (IMCs)


Day 0
Day 6
Mouse stomach corpus mT
Mouse small intestine Lgr5-eGFP
Stomach
Transition
Intestine
Stomach-specific genes
Stomach organoids
Stomach tubes
Stomach and intestine tubes
Intestine tubes
Chga
Muc5ac
Muc6
Pgc
Intestine-specific genes
Intestine organoids
Intestine tubes
Stomach and intestine tubes
Stomach tubes
Lgr5
Sox9
Alpi
Chga
Lyz1
Muc2
log_10
1
0
−1
−2

Scale: 500μm

2. Promoting stereotypical tissue development

Can we turn a 'stochastic' into a deterministic process?


Brightfield
Ecadherin, AldoB
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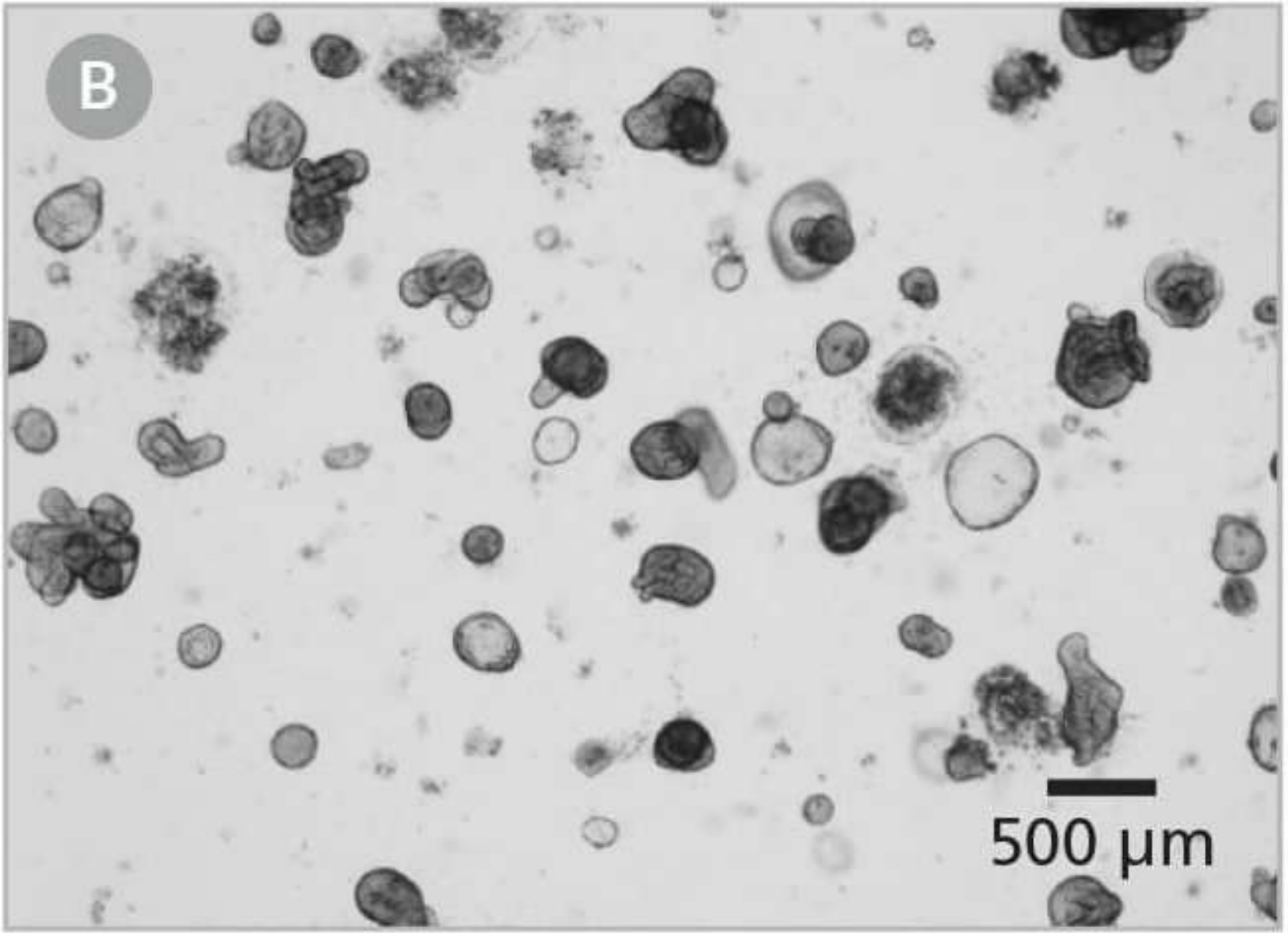
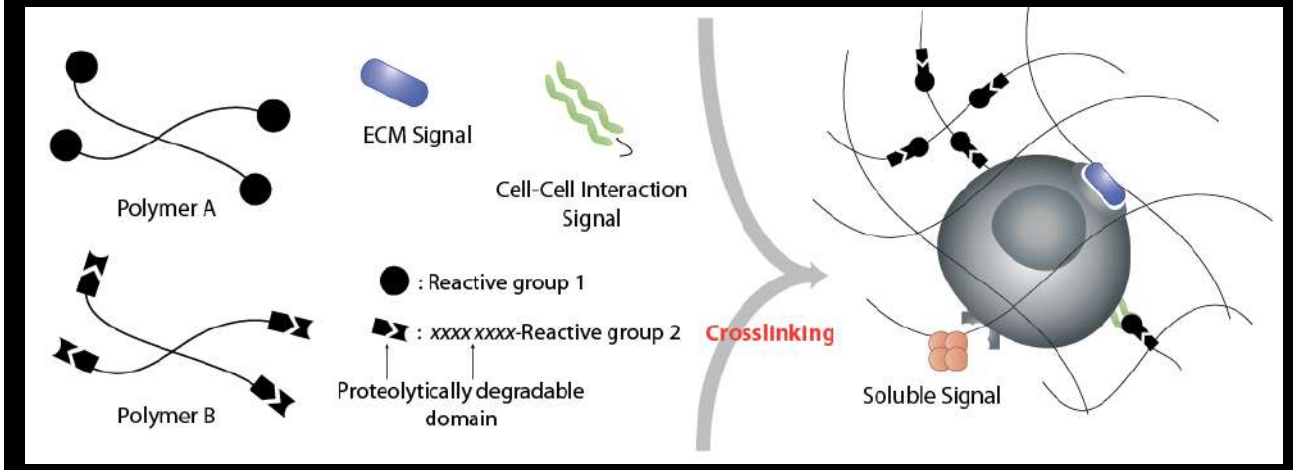
Enterocytes
Paneth cells

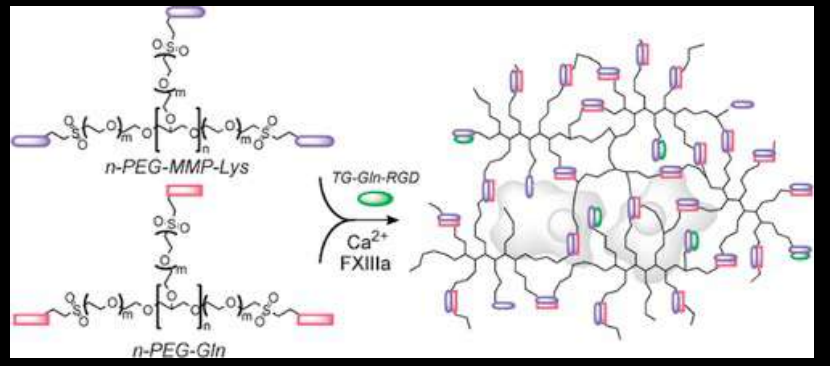


Human SI organoids
Image: Stem Cell Technologies

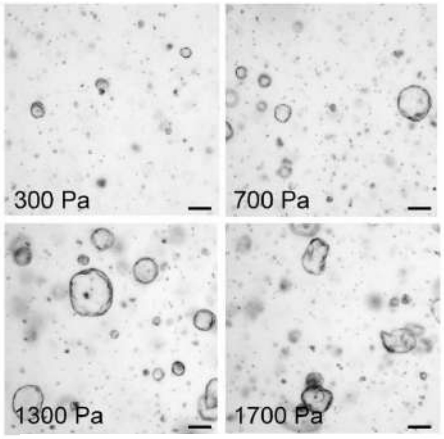

REF 354248
Matrigel® Matrix
Basement Membrane HC
X.XX MG/ML
DO NOT THAW: SEE SPEC SHE
STORE AT -20°C
For Research Use Only
Not for use in Diagnostic
or Therapeutic Procedures

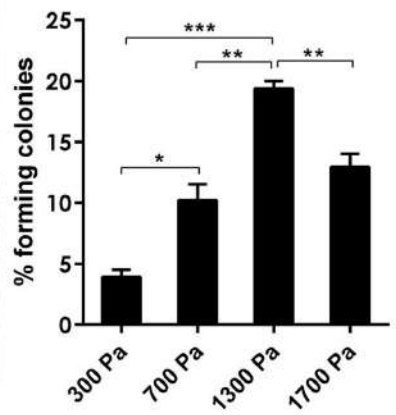
Synthetic hydrogels

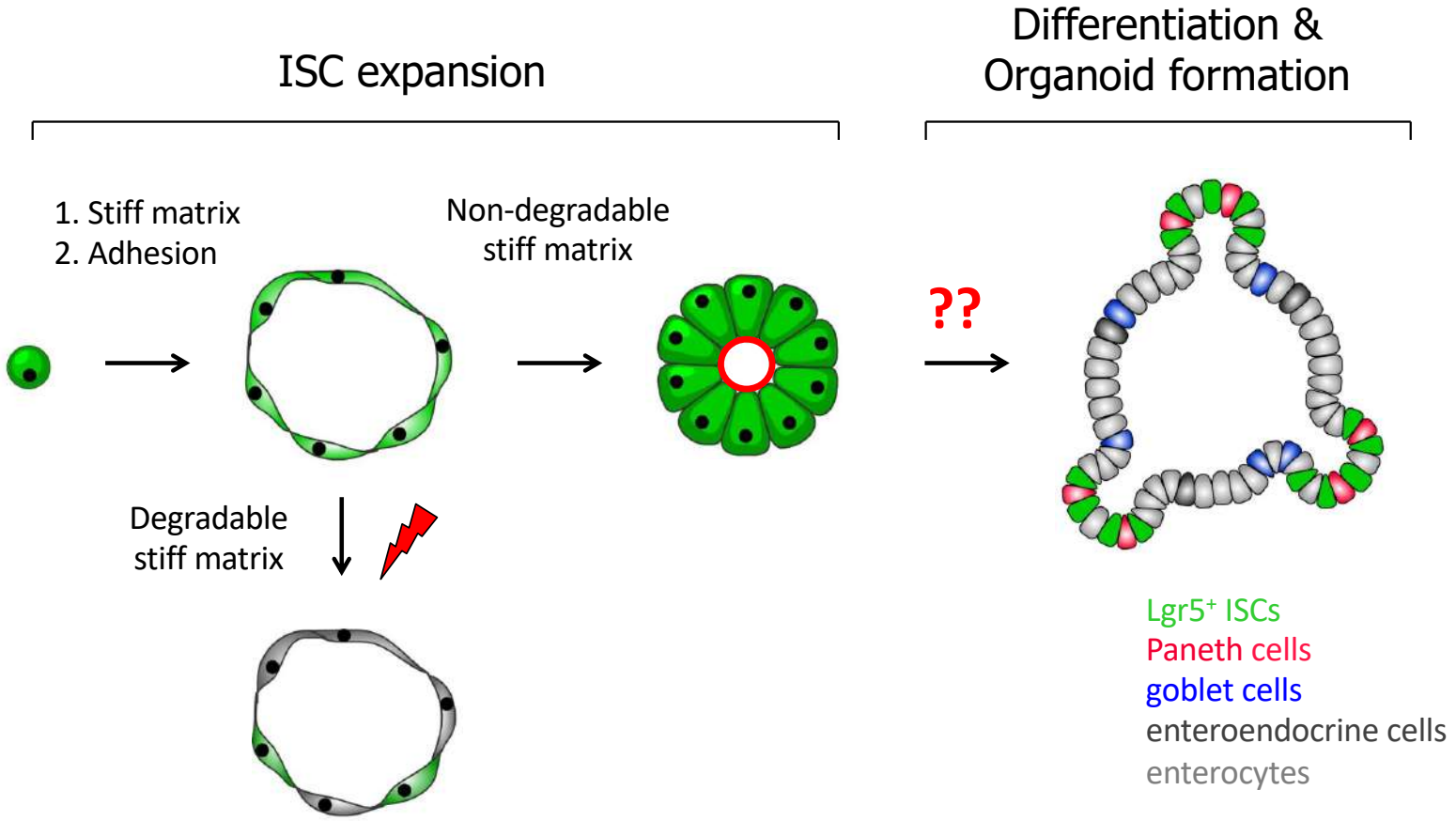




Intestinal organoid development in a synthetic gel

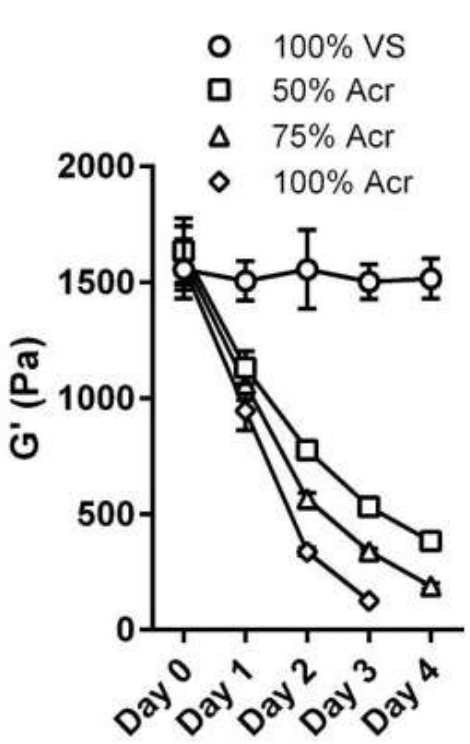


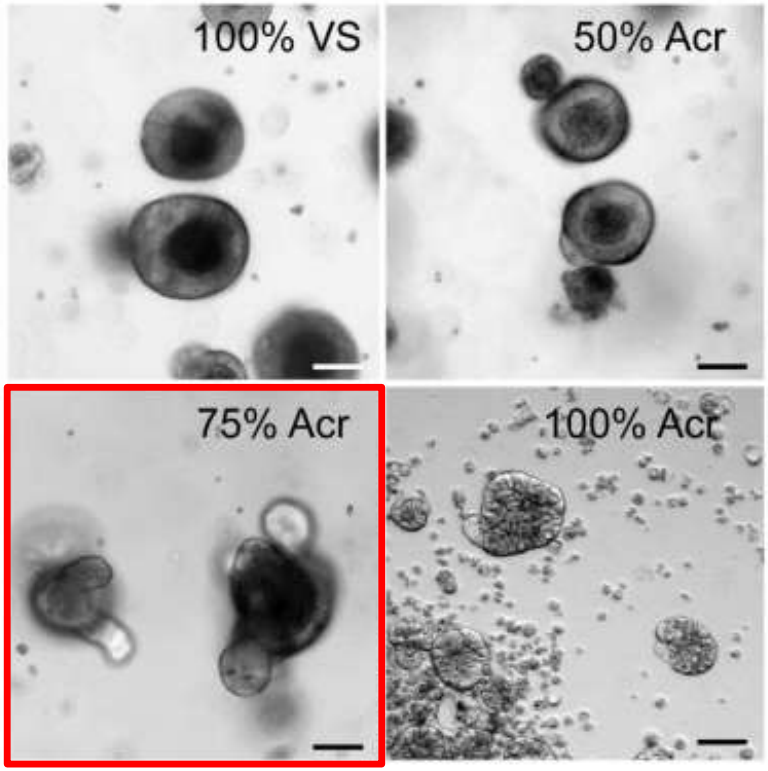


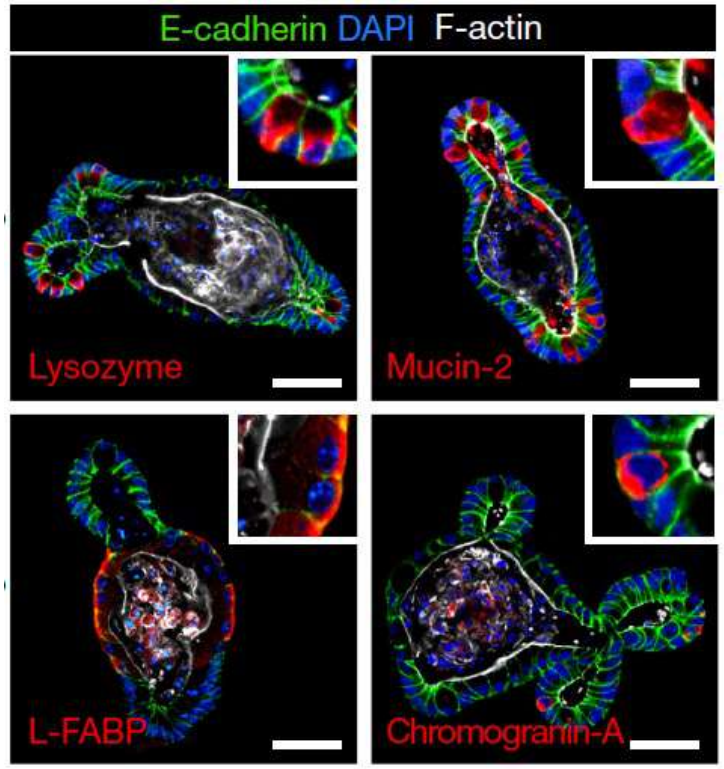


Gjorevski et al., Nature, 2016

Matrix softening is required for crypt formation (*i.e.* organoid patterning)



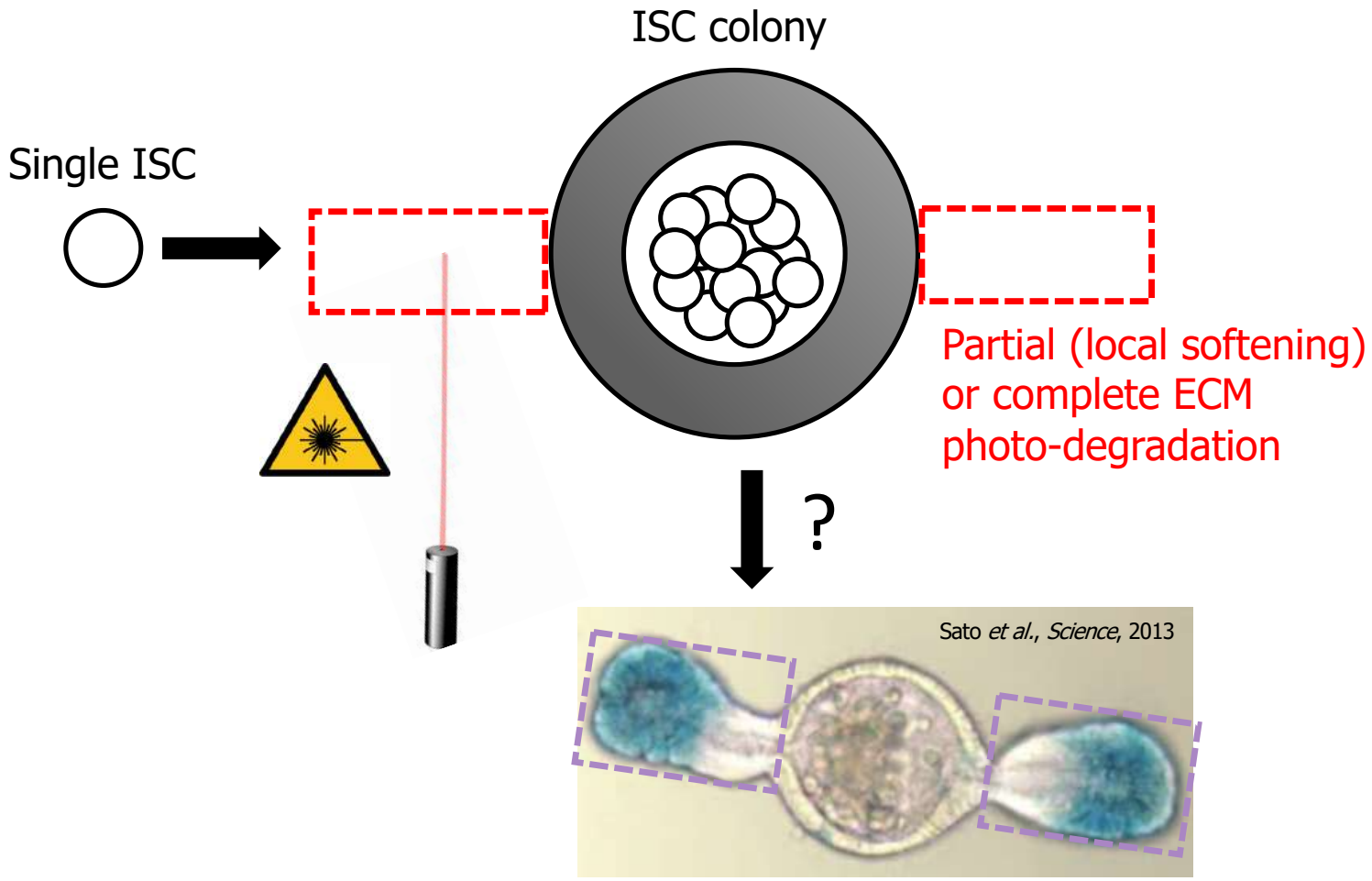




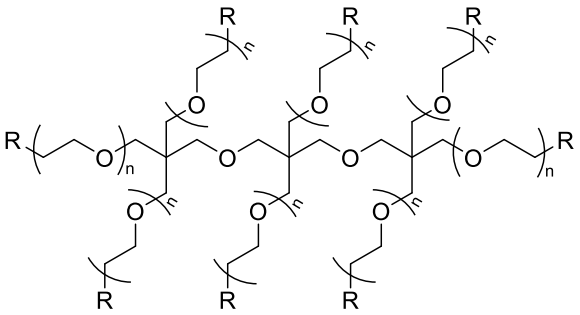
Paneth cells (lysozyme), goblet cells (mucin-2), enterocytes (L-FABP), enteroendocrine cells (chromogranin-A)

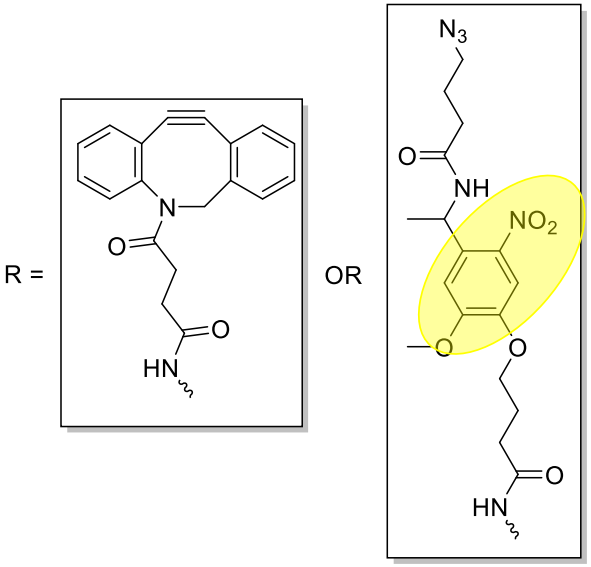
Gjorevski et al., Nature, 2016

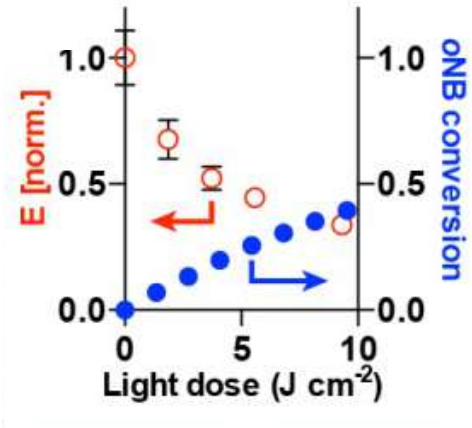
Can localized softening of hydrogels promote controlled crypt formation?

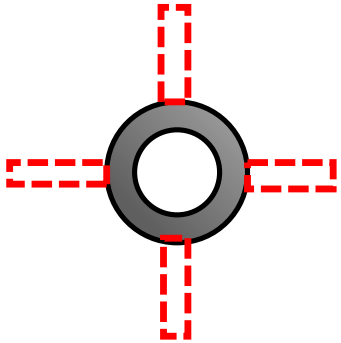


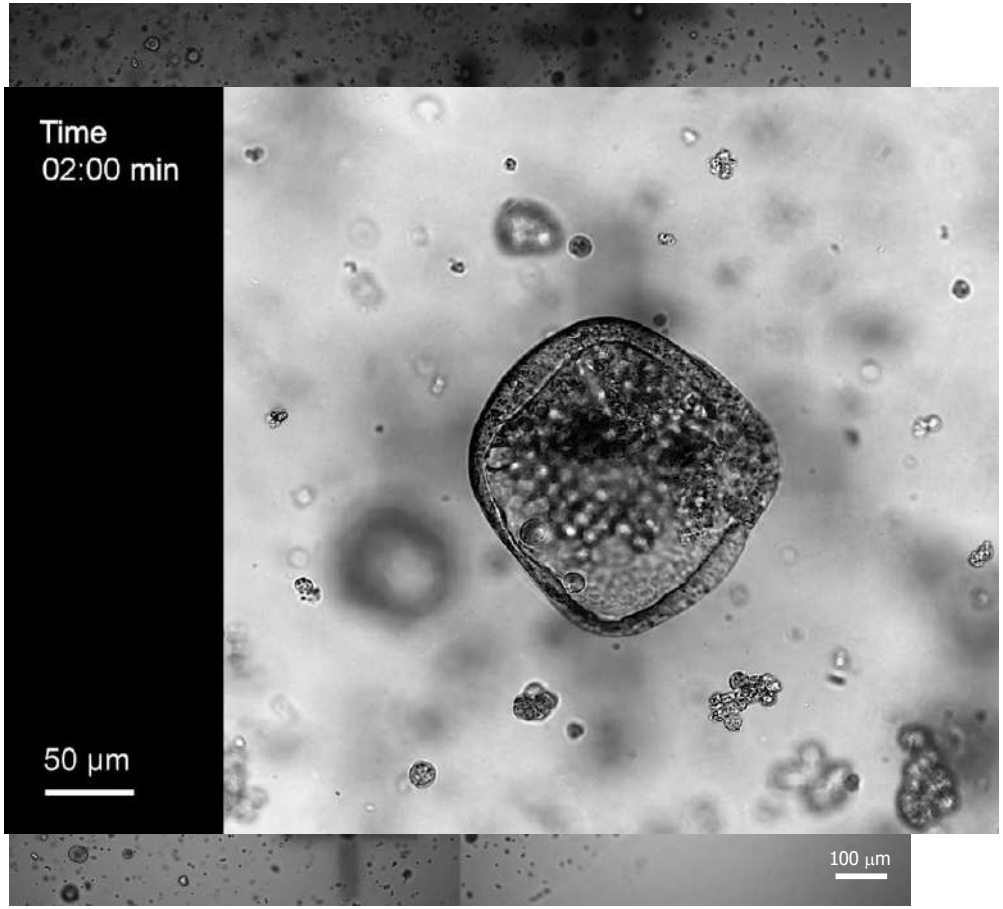
In situ hydrogel photo-degradation



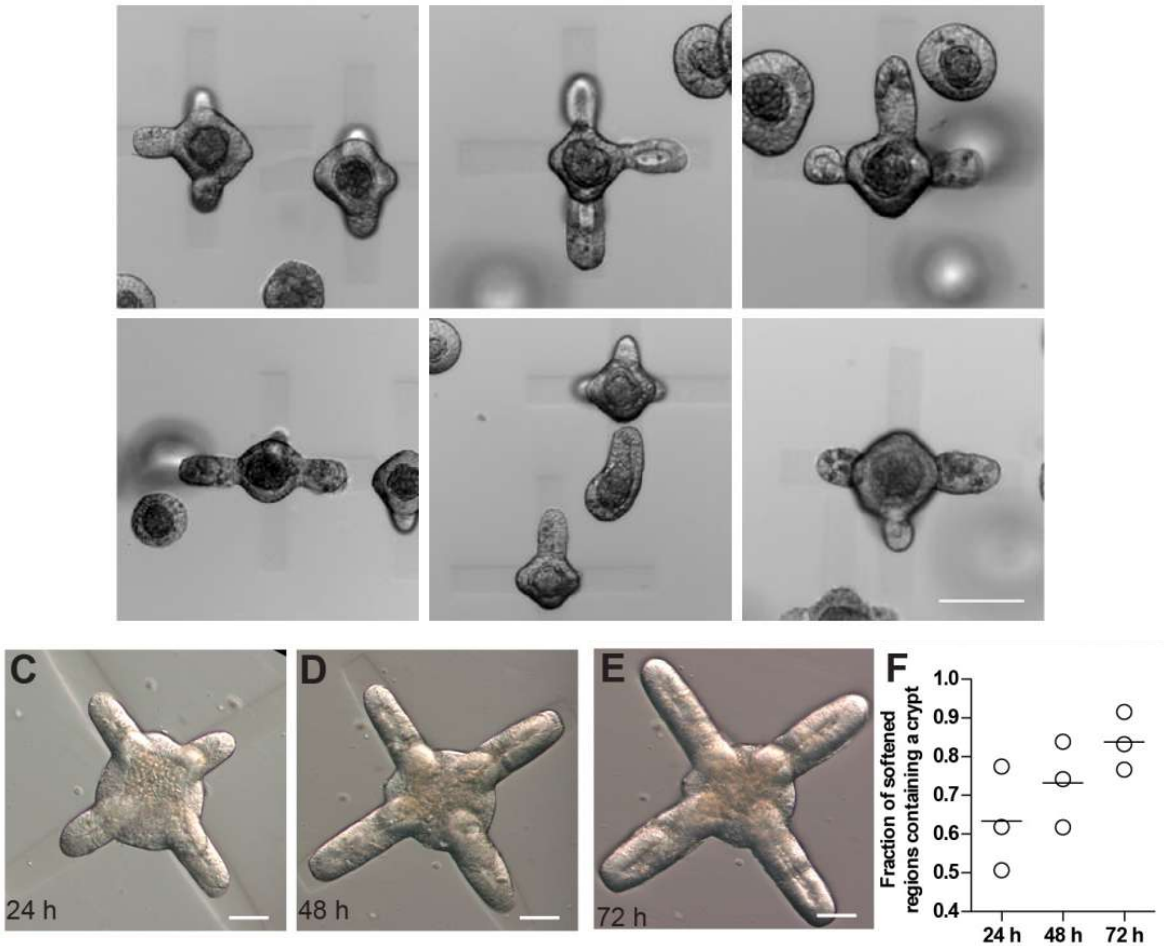


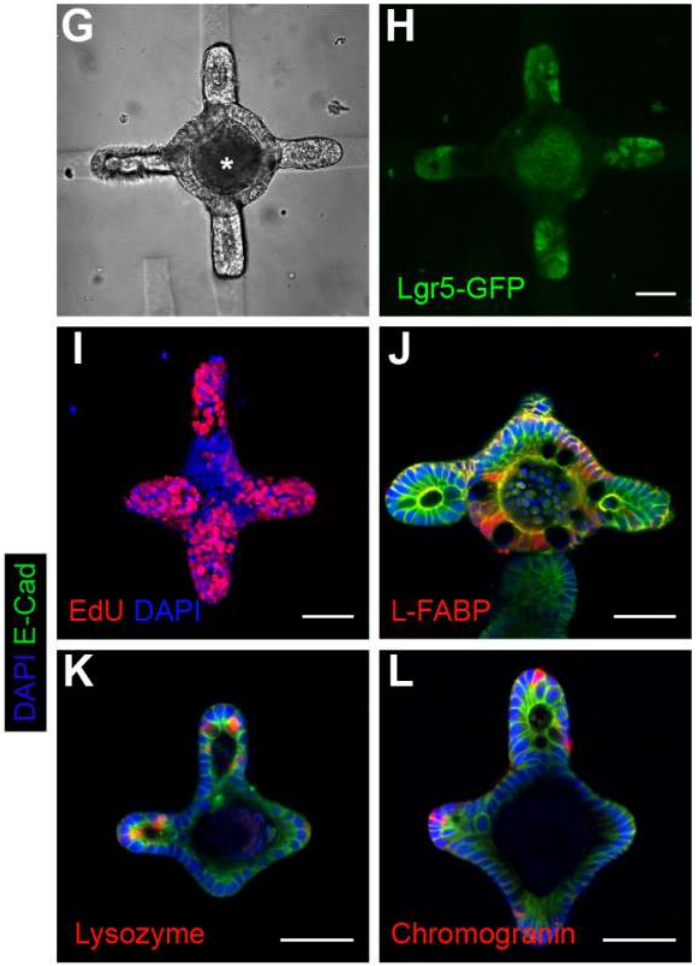






Mechanically-guided, deterministic crypt formation!

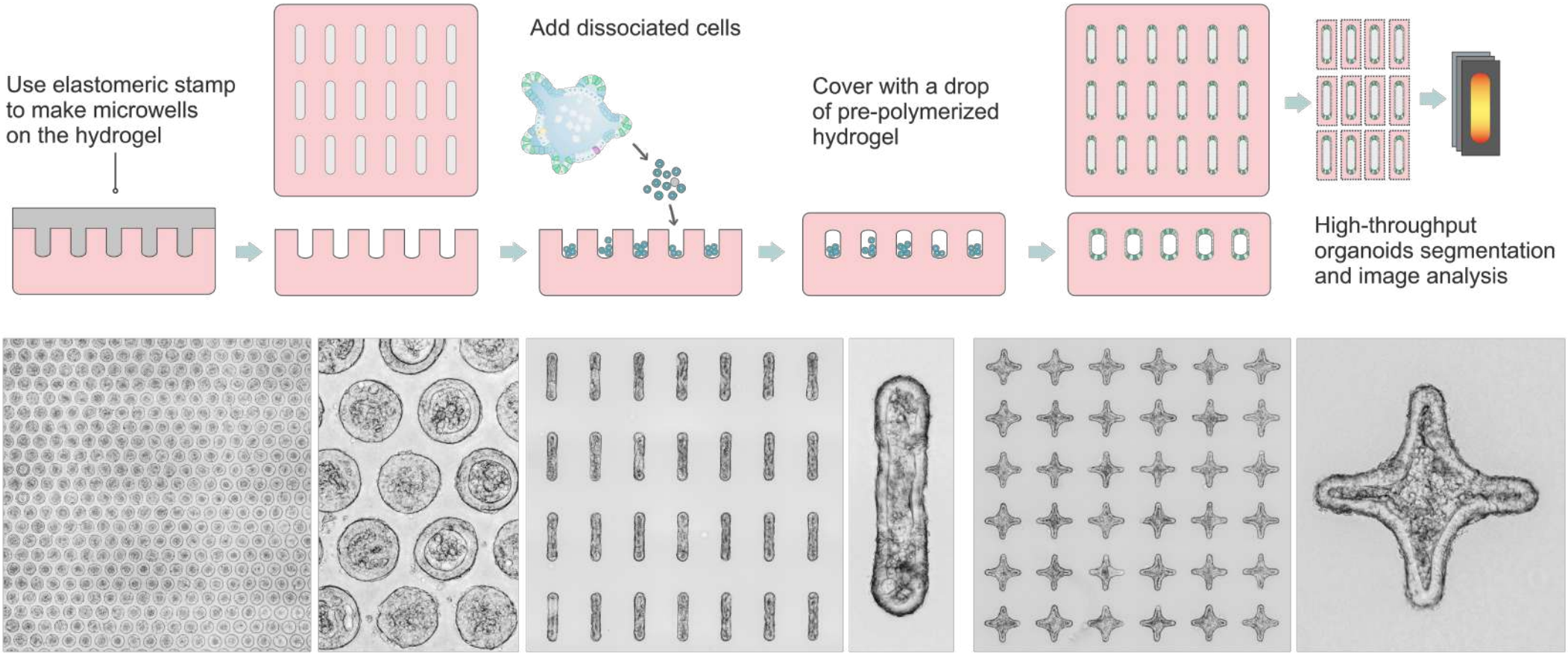




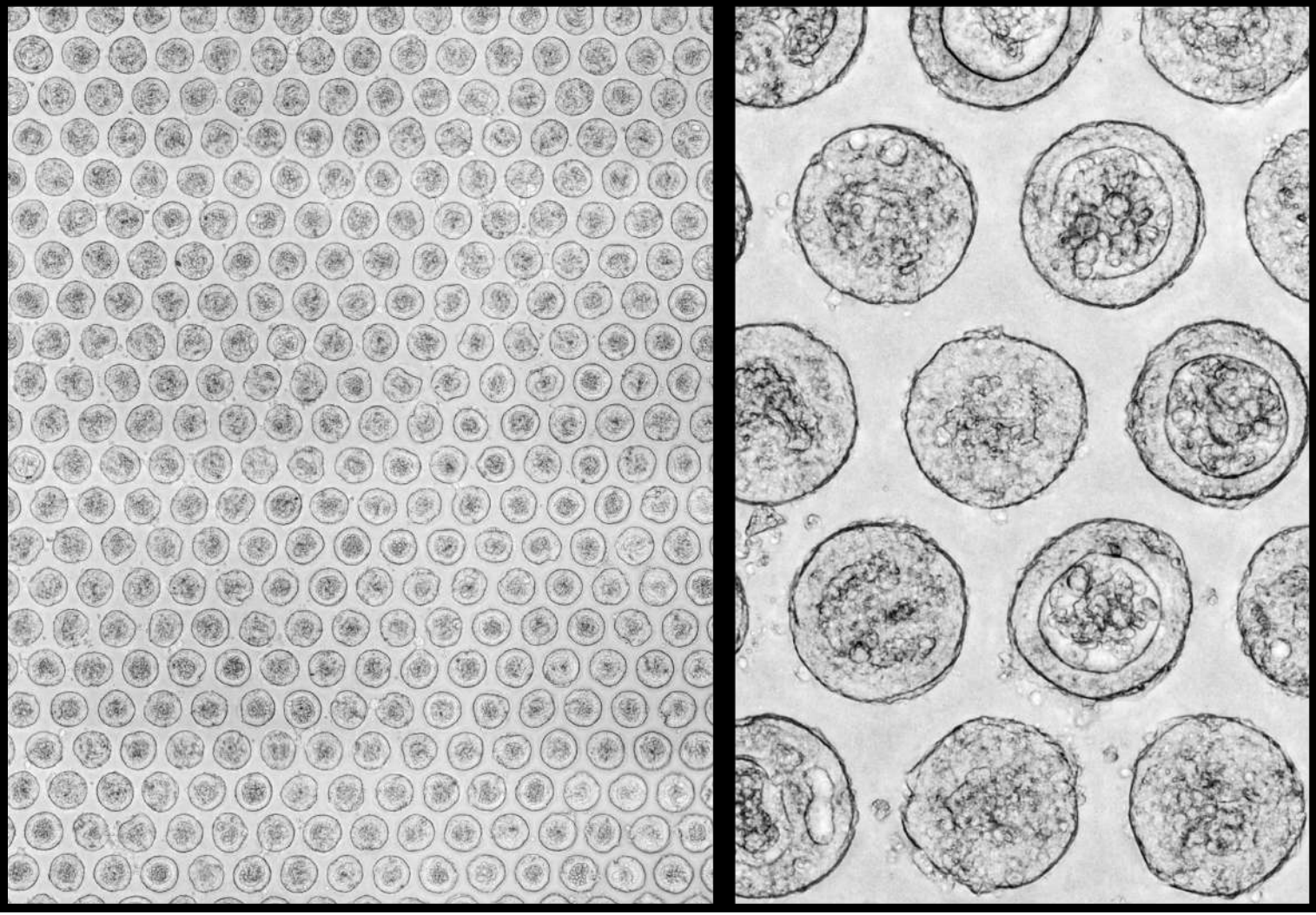
Gjorevski and Nikolaev et al., Science, 2022

Does tissue geometry itself influence patterning?

Arrays of identical, shape-controlled epithelia

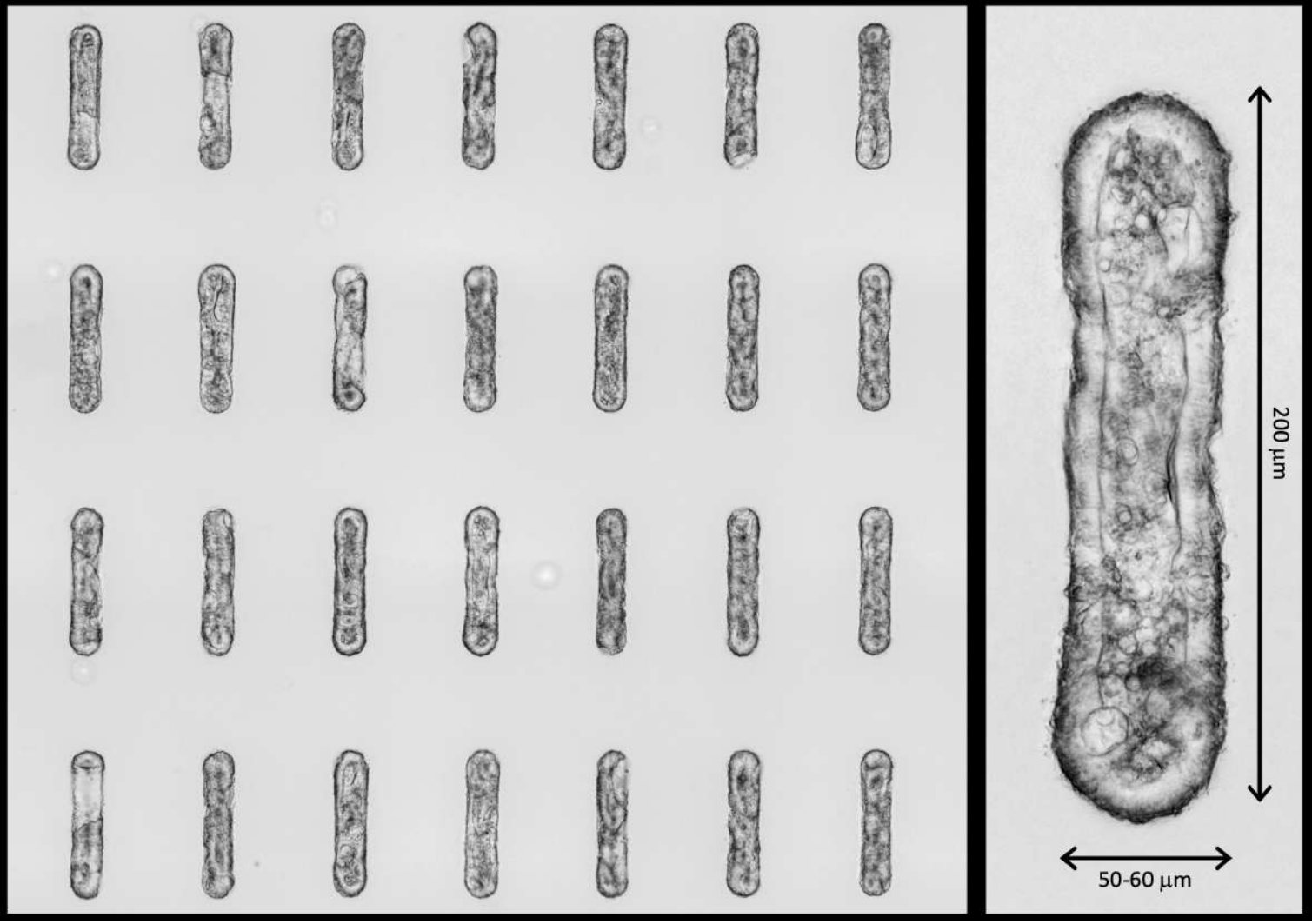


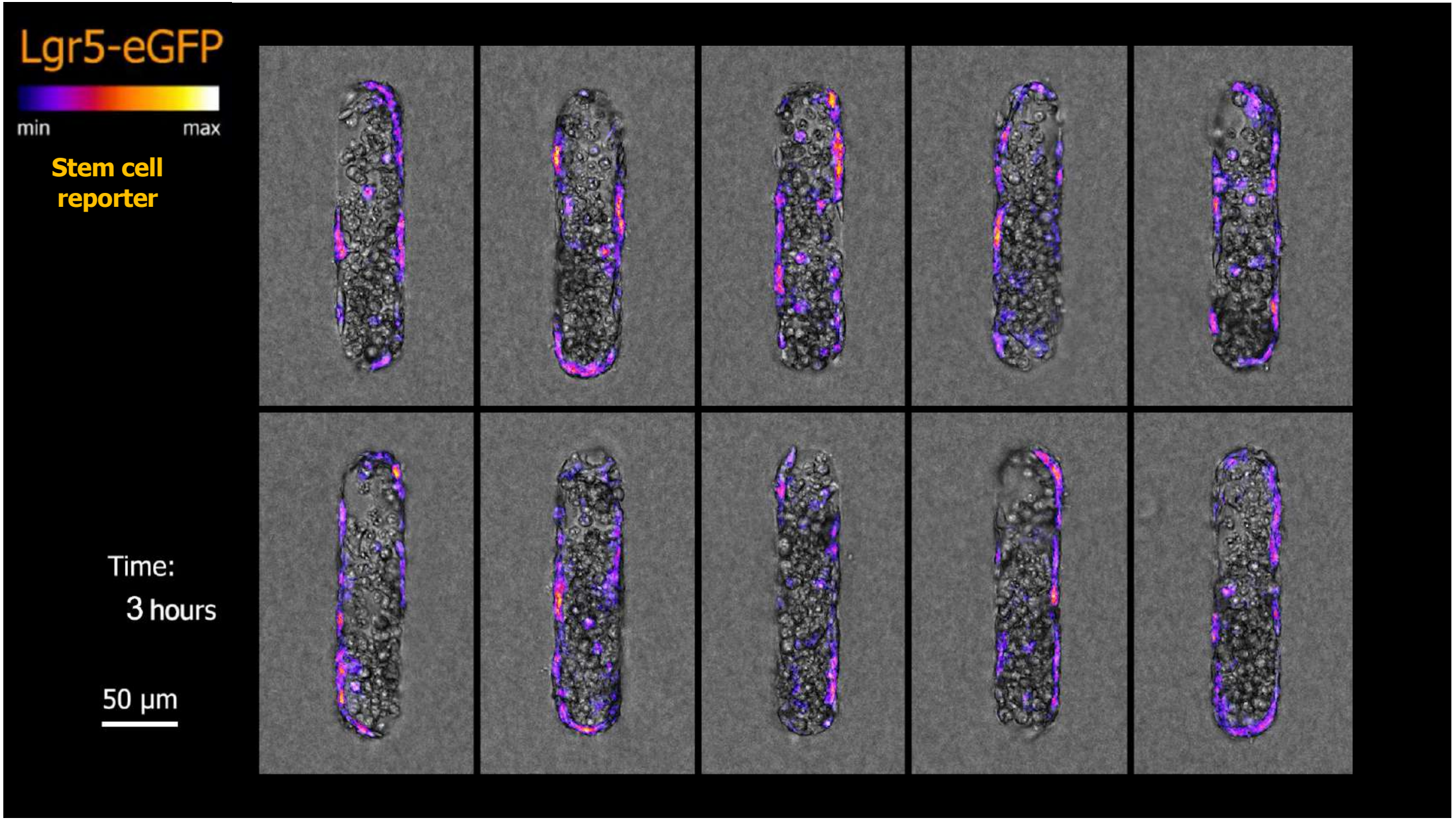
Arrays of identical epithelia grown in microengineered hydrogel substrates



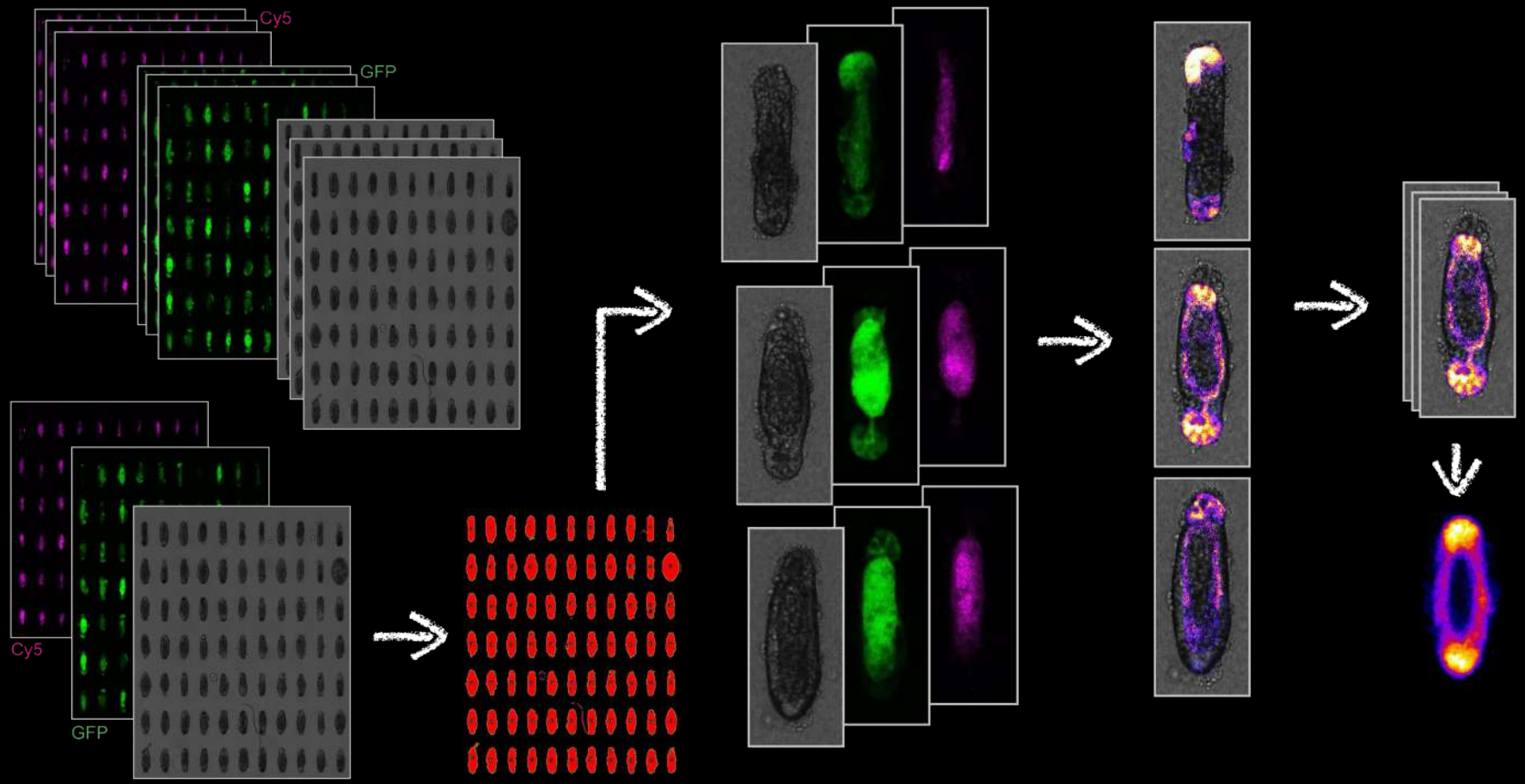
Arrays of identical epithelia grown in microengineered hydrogel substrates

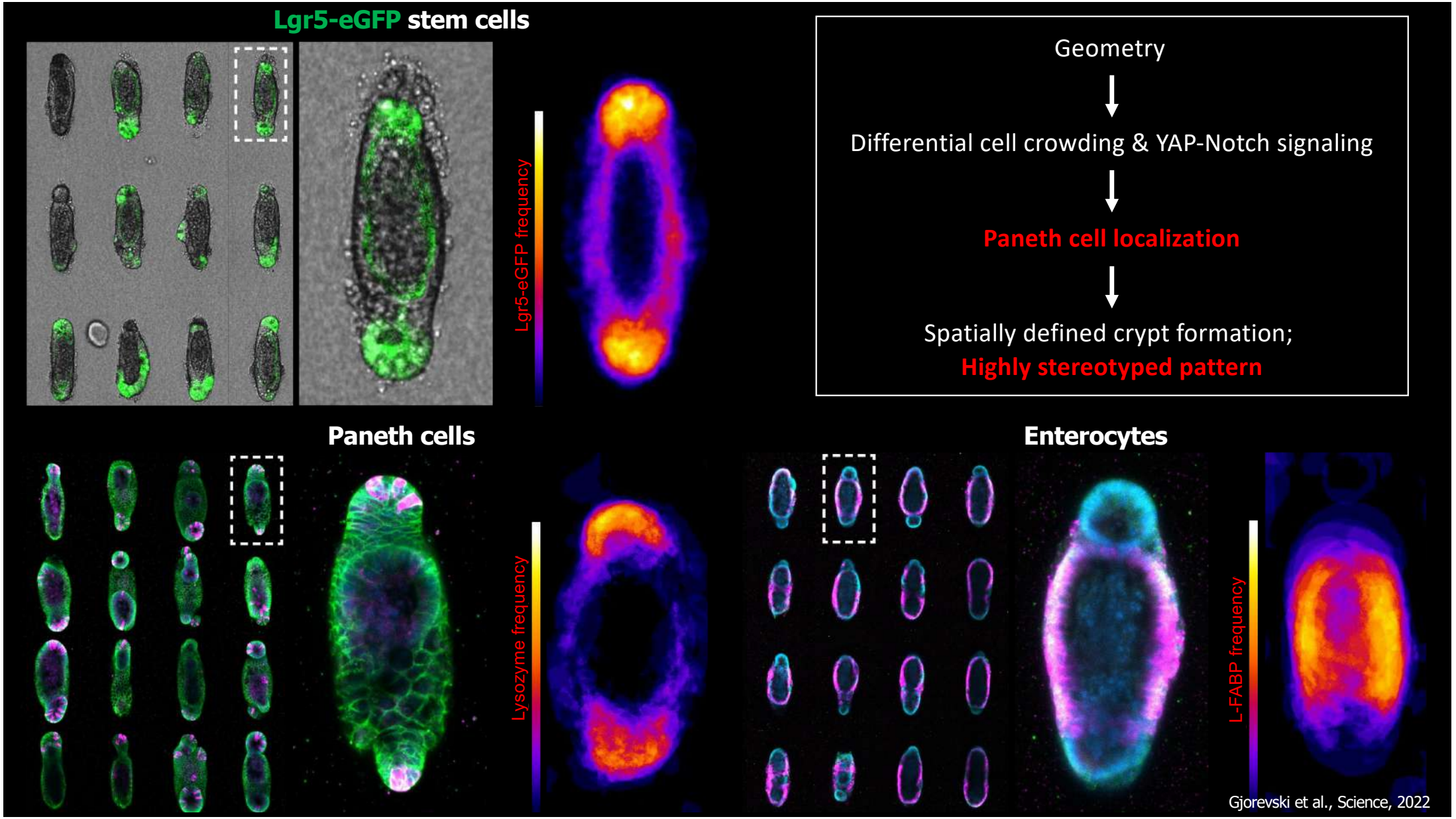
Crypt-shaped microcavities




Lgr5-eGFP
min
max
Stem cell reporter
Time:
3 hours
50 µm

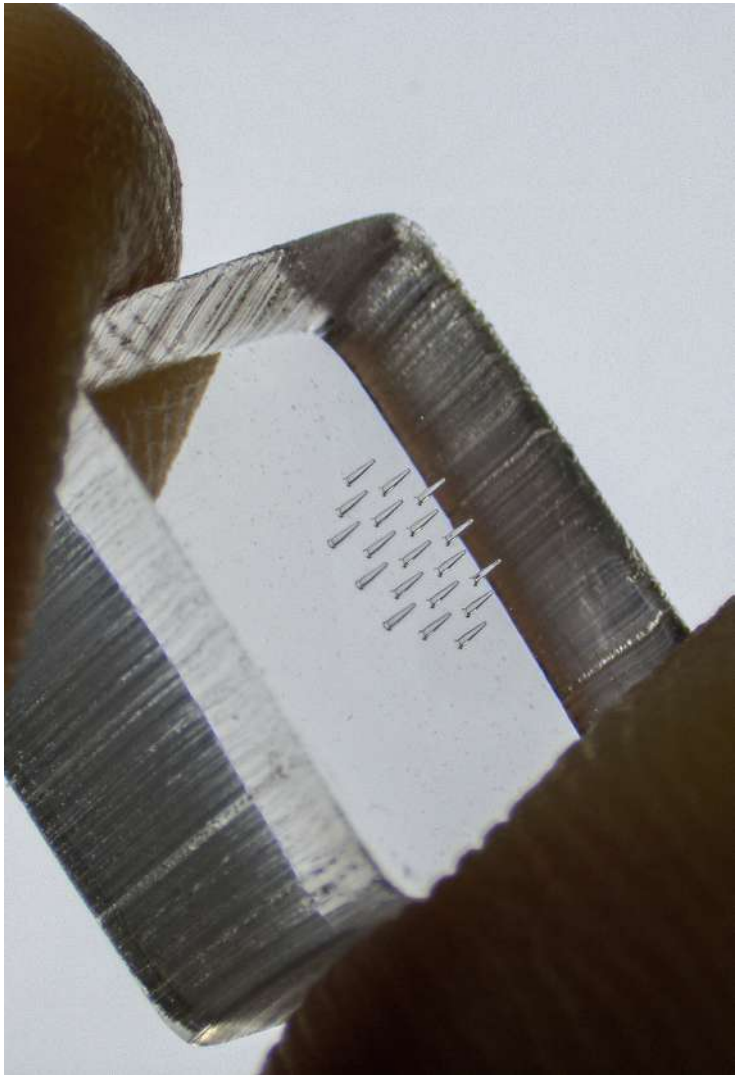
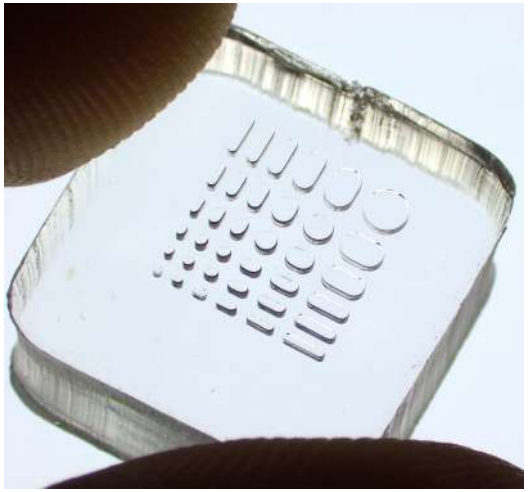
Automated quantification of organoid patterning

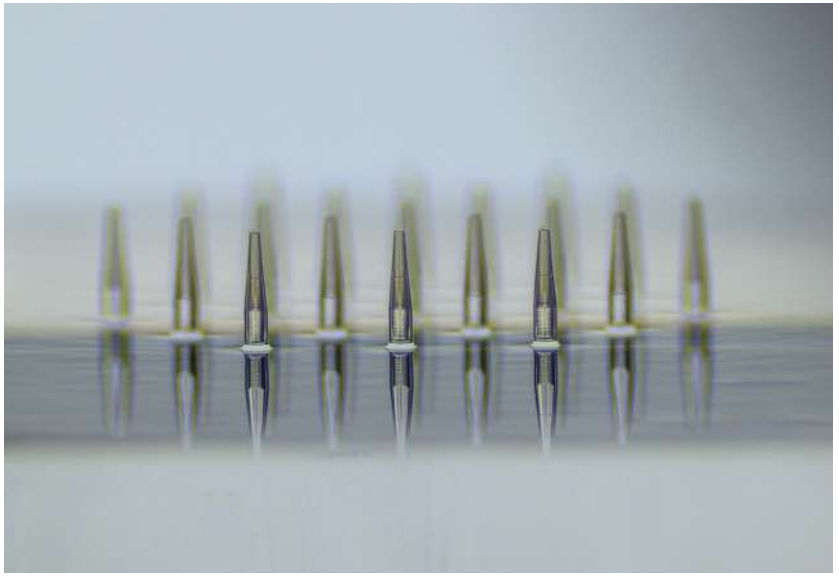



Lgr5-eGFP stem cells
Lgr5-eGFP frequency
Geometry
Differential cell crowding & YAP-Notch signaling
Paneth cell localization
Spatially defined crypt formation;
Highly stereotyped pattern
Paneth cells
Lysozyme frequency
Enterocytes
L-FABP frequency
Gjorevski et al., Science, 2022

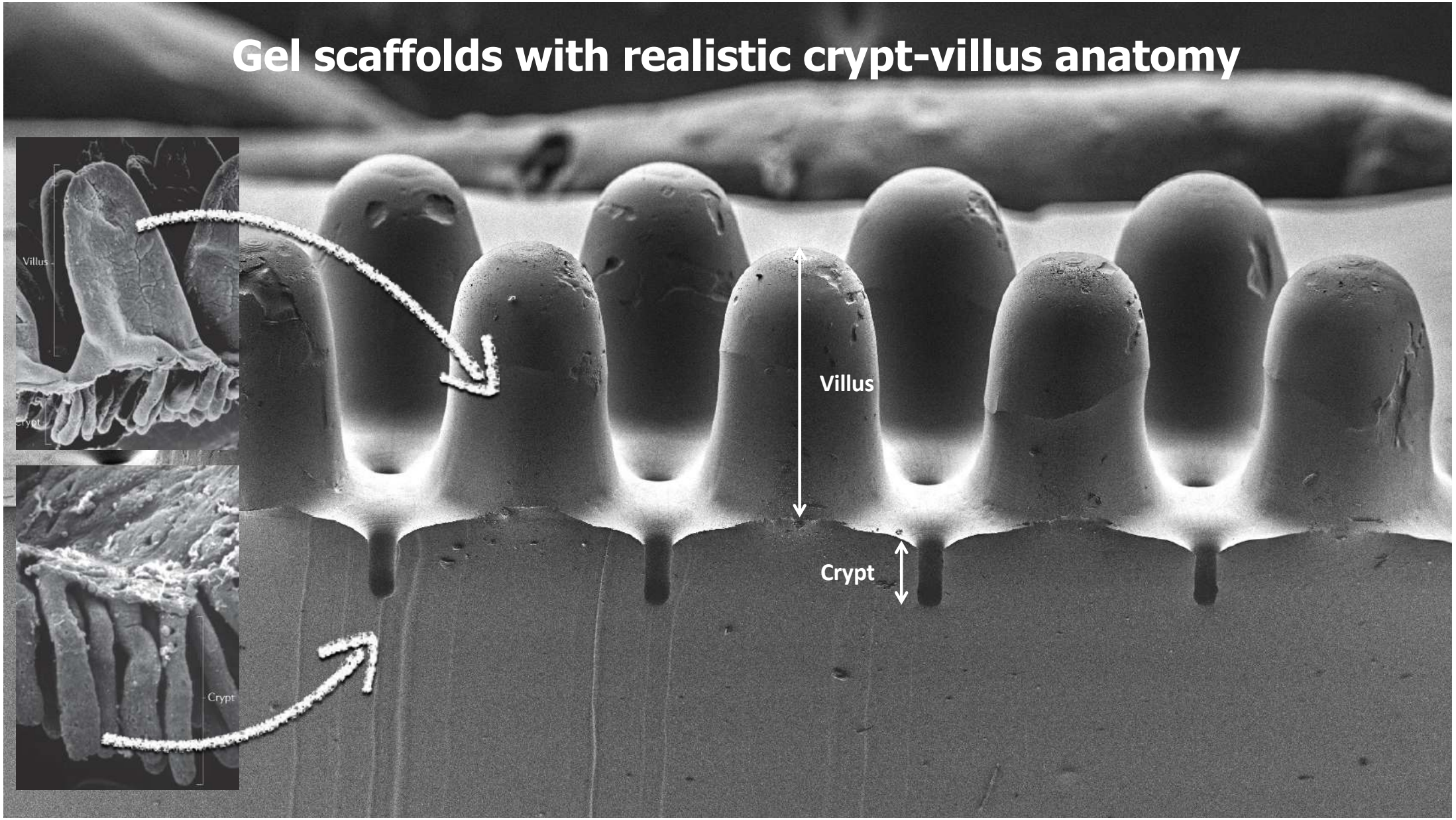
3. Controlling tissue architecture

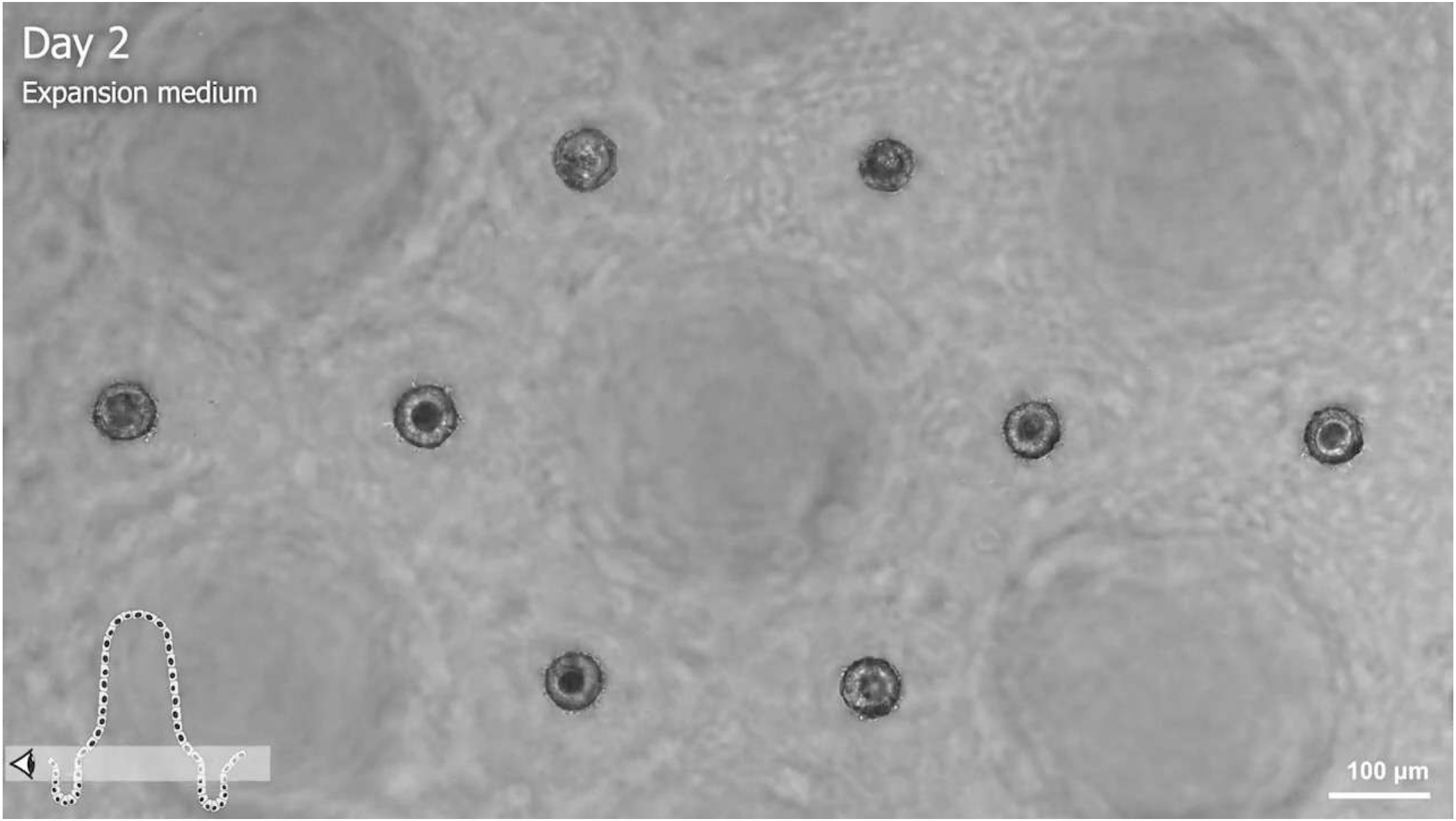
Can we form tissues with defined shape and size?

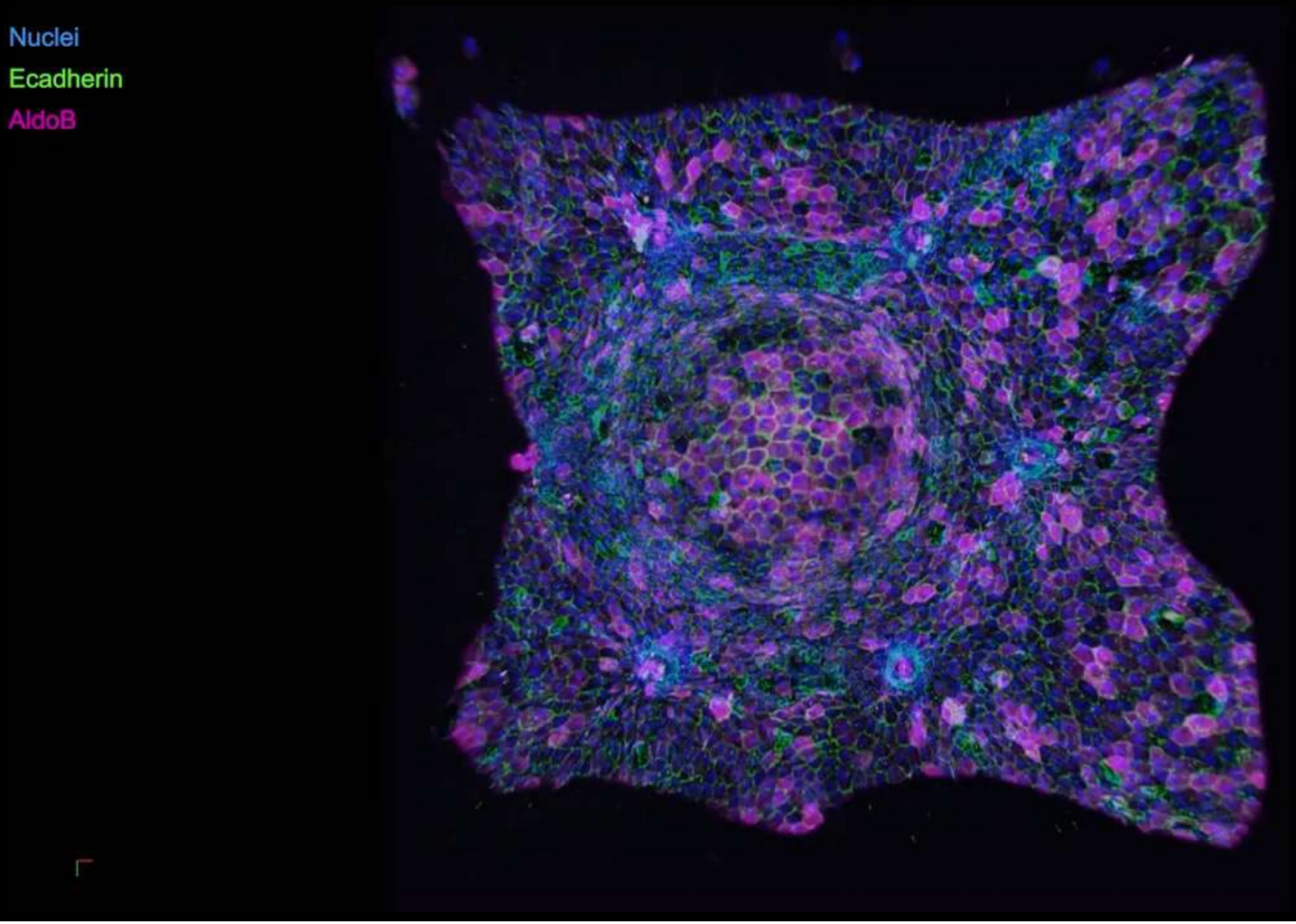


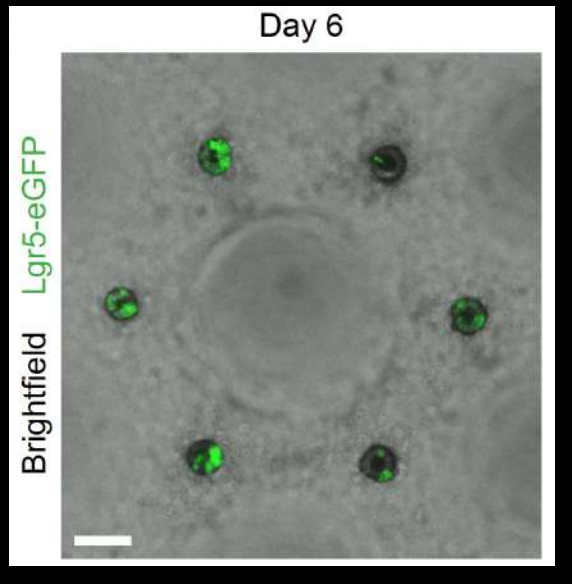

Gel scaffolds with realistic crypt-villus anatomy

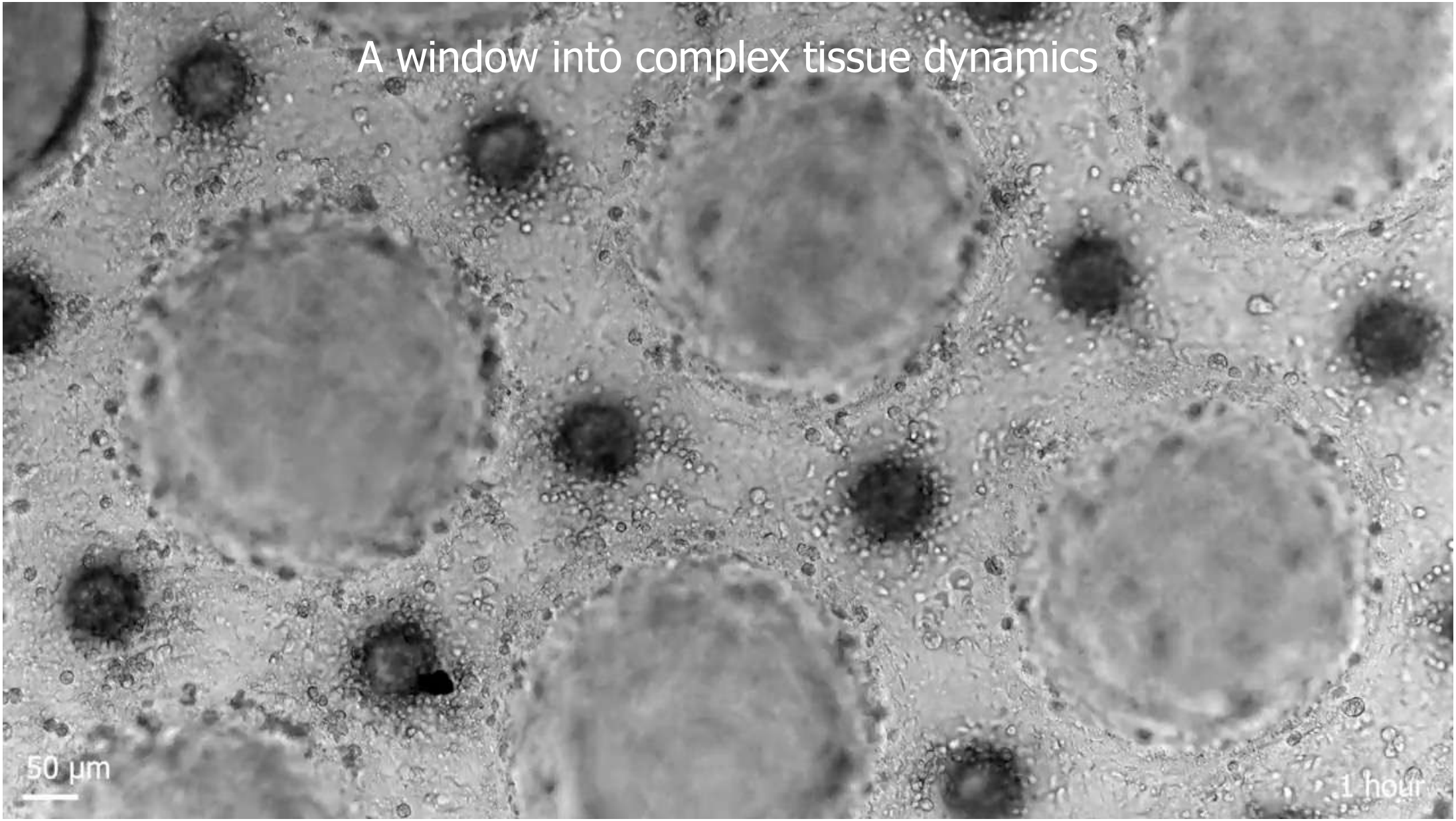


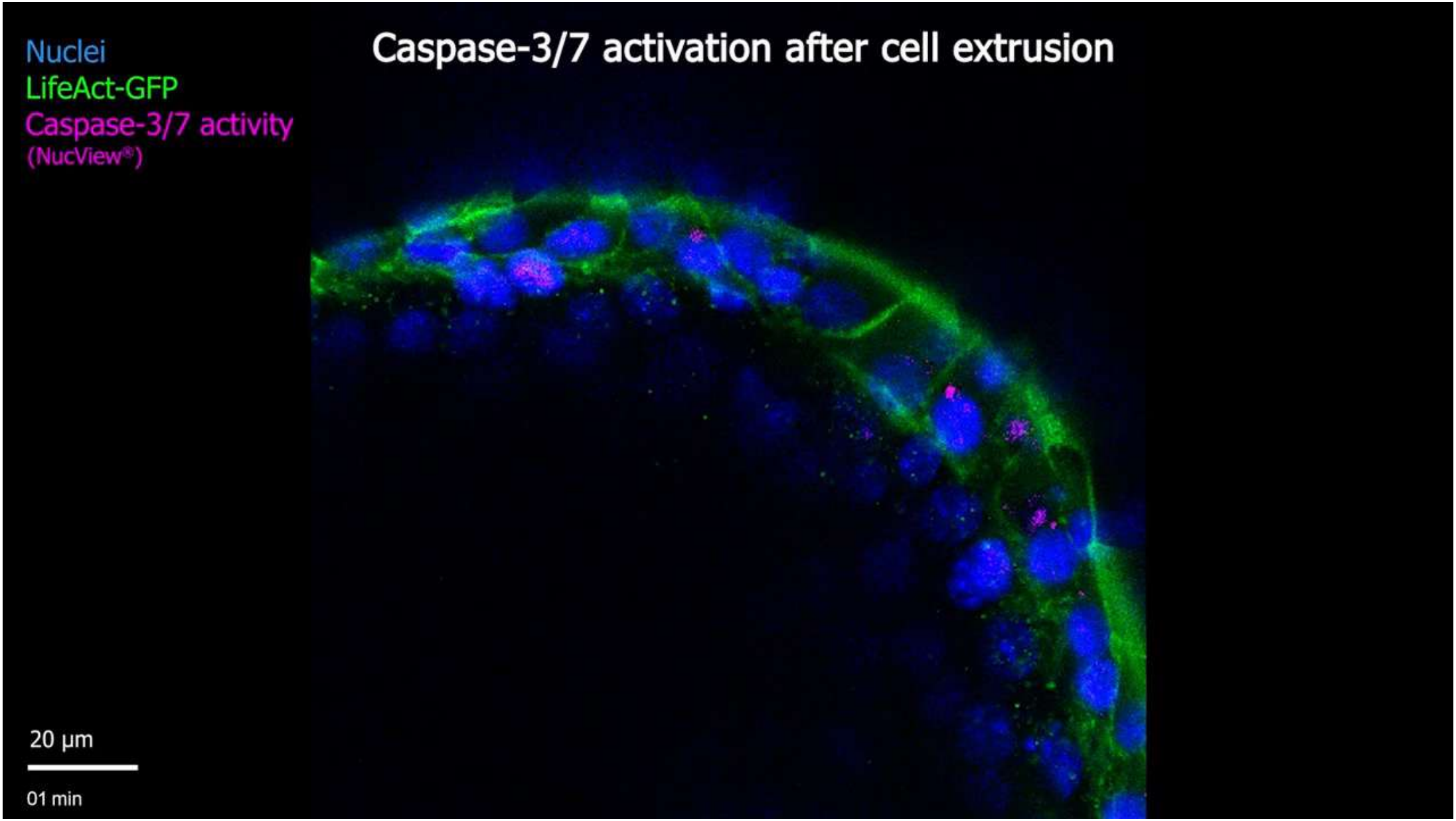

Day 2
Expansion medium
100 µm

In vivo-like crypt-villus architecture and cell type pattern



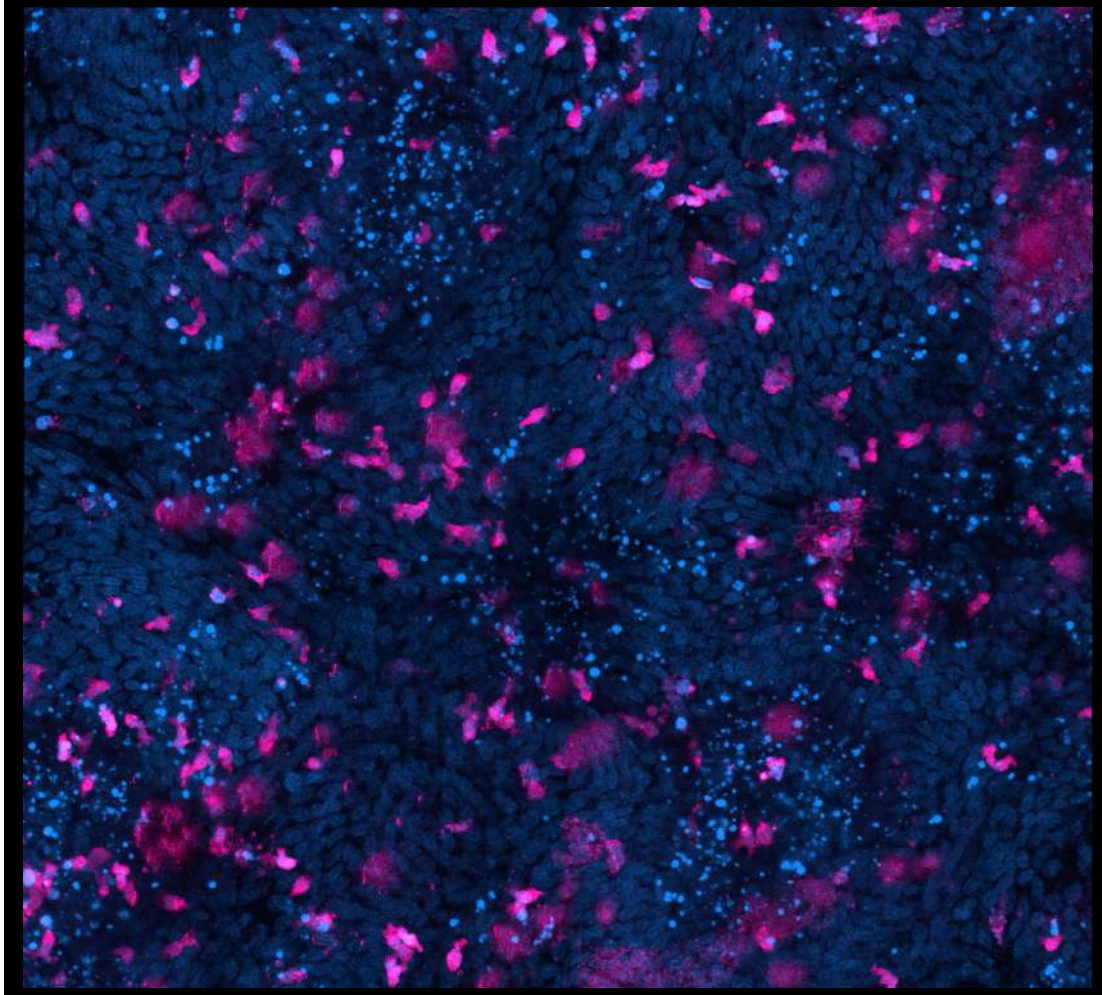



A window into complex tissue dynamics
50 µm
1 hour

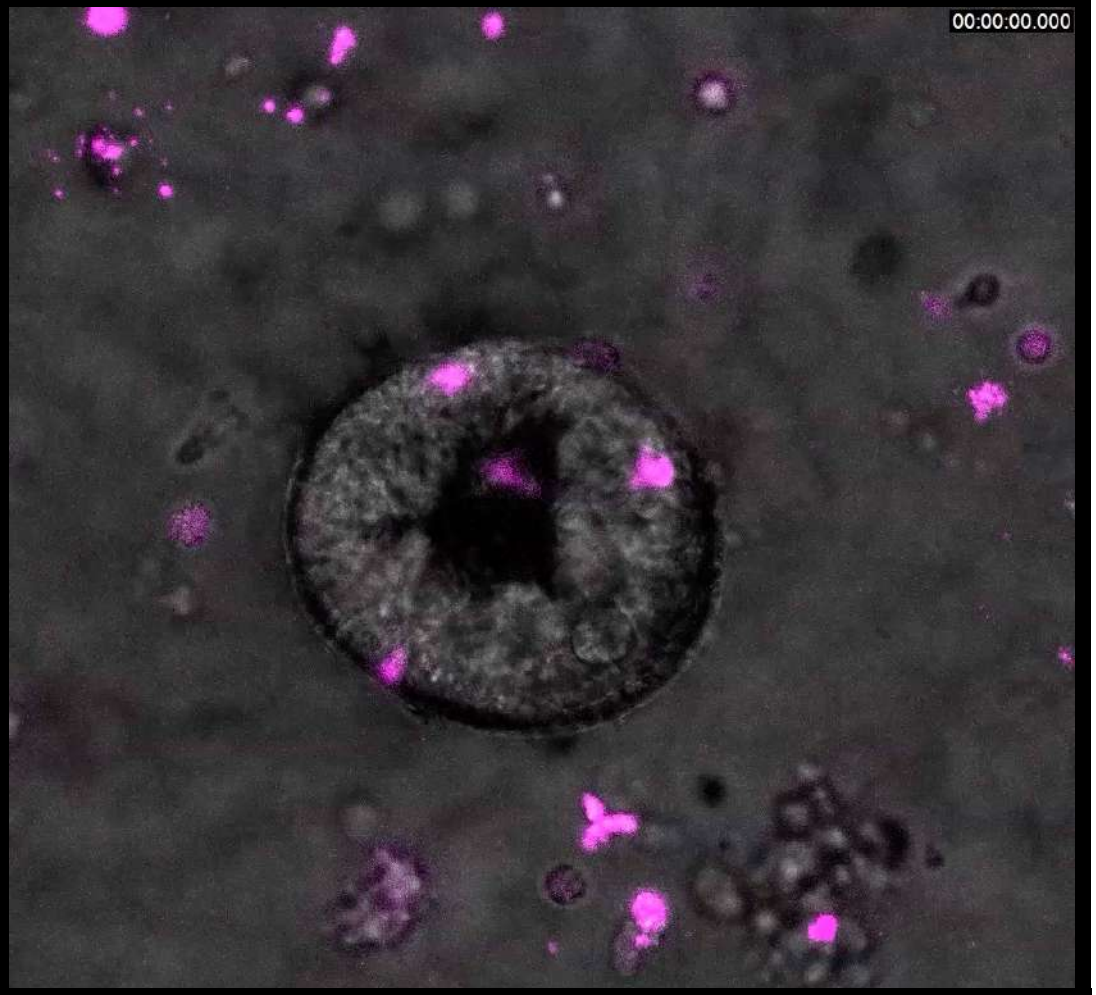

Caspase-3/7 activation after cell extrusion
Nuclei
LifeAct-GFP
Caspase-3/7 activity
(NucView®)
20 µm
01 min

Institute of
Human Biology

‘Immune-surveilled’ intestinal epithelia



nuclei tissue-resident immune cells



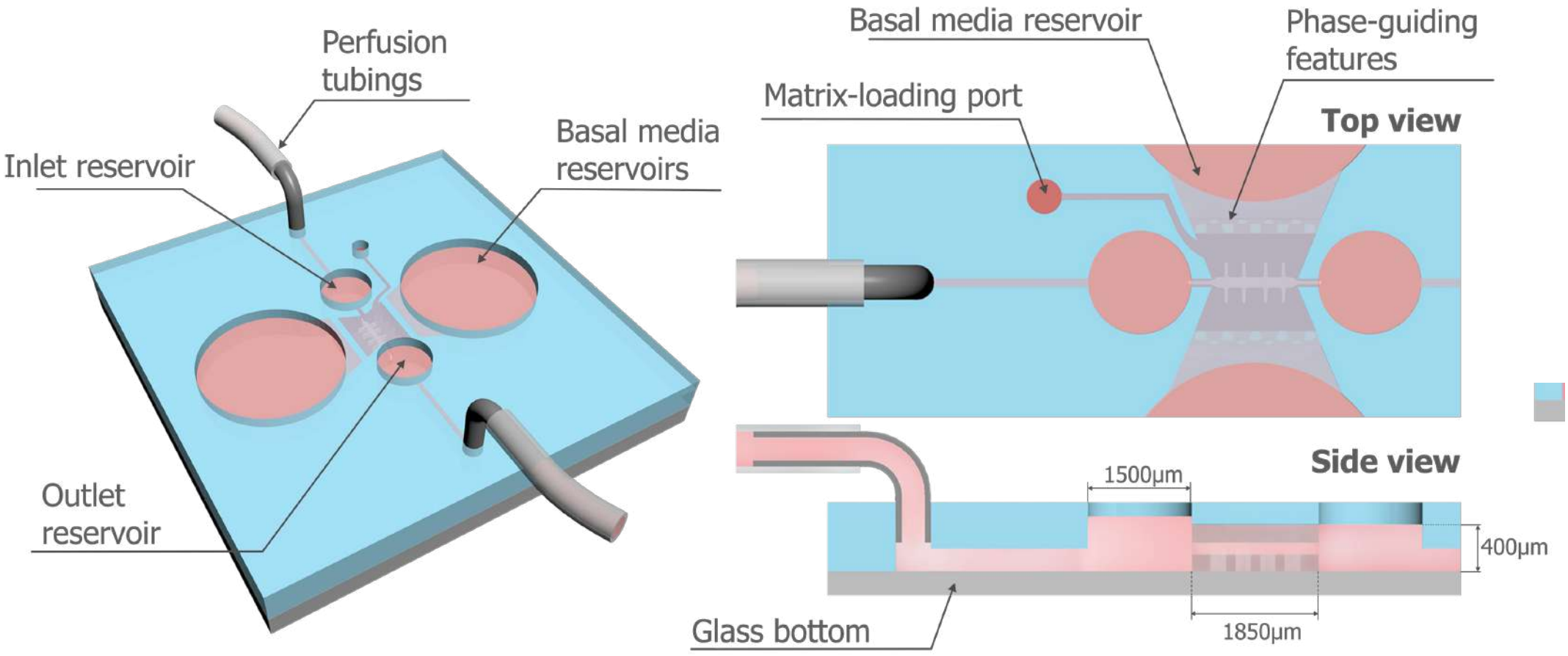
close-up view of one intestinal crypt

Gjorevski lab at IHB

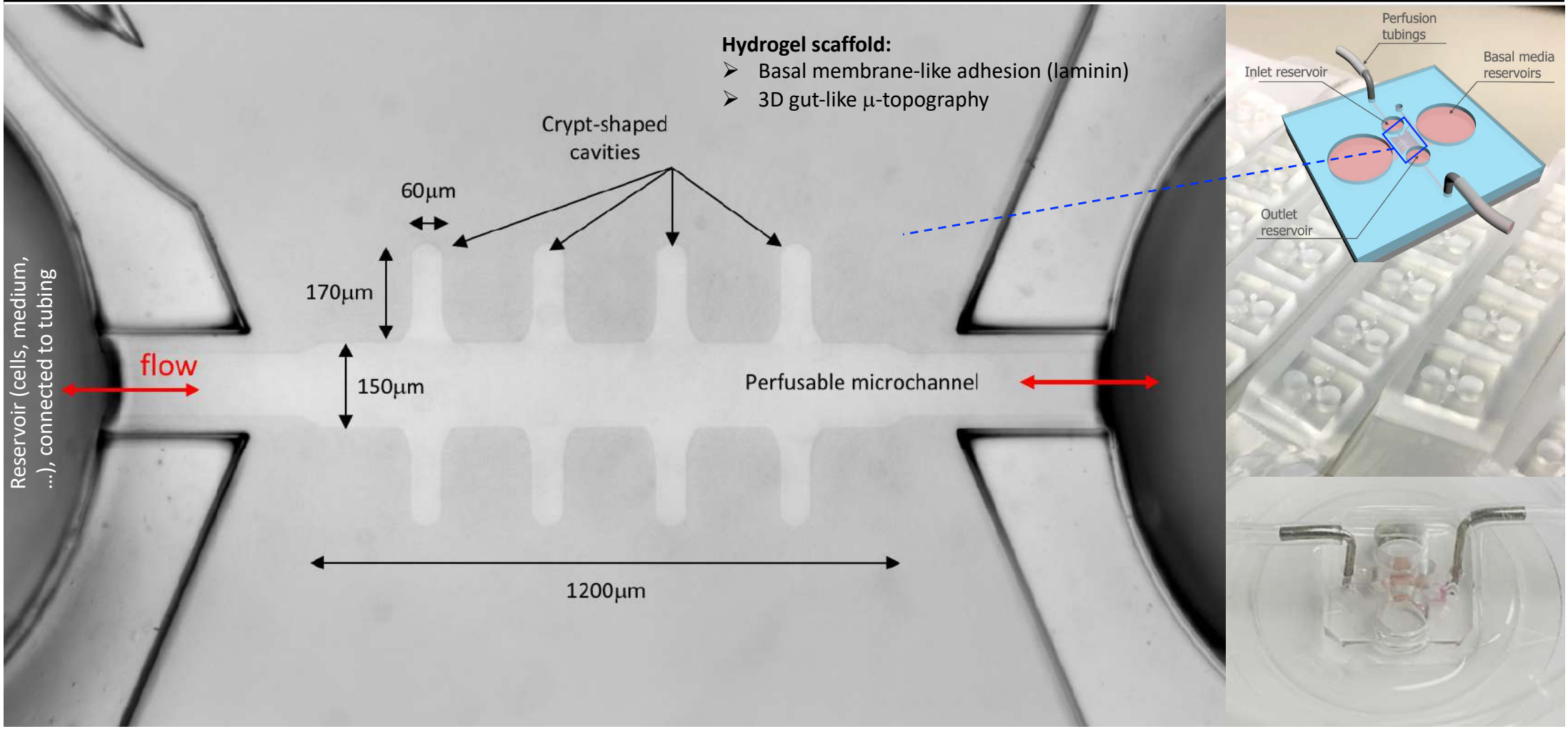
4. Capturing tissue physiology

Can we begin to mimic key physiological processes and multi-tissue complexity?

Engineering epithelial 'organoids-on-a-chip'

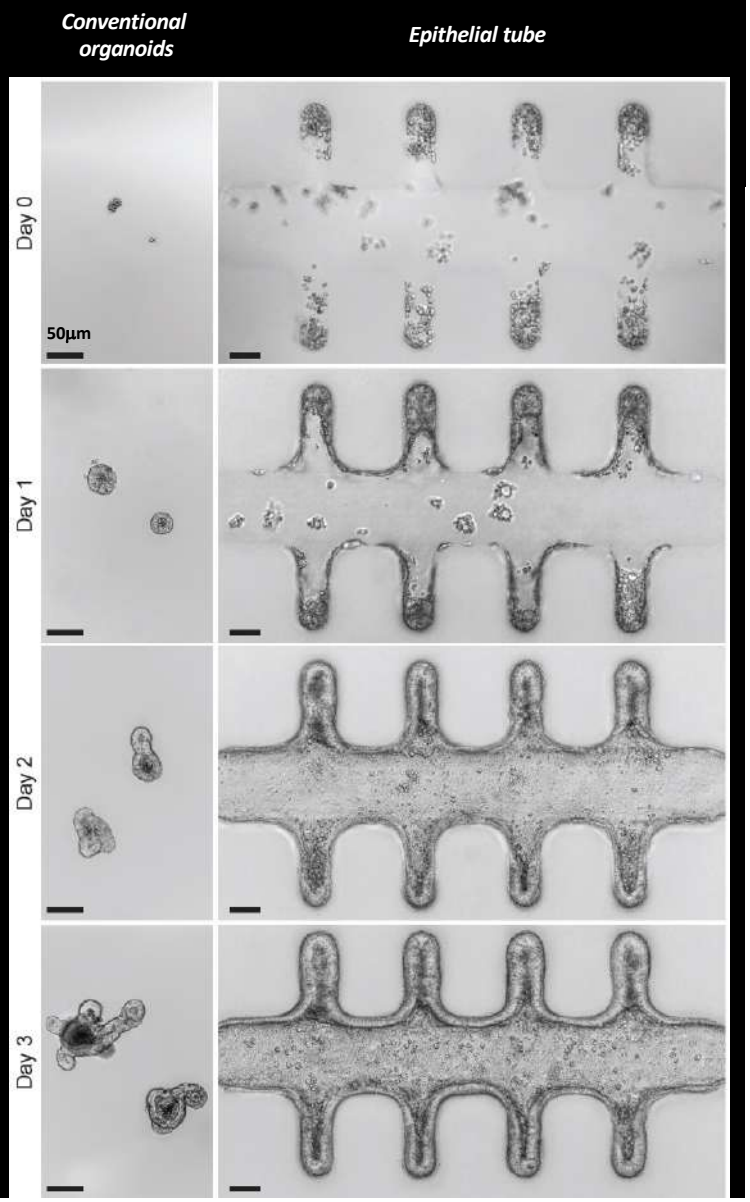


Hybrid microchips to grow tubular 'organoids-on-a-chip'



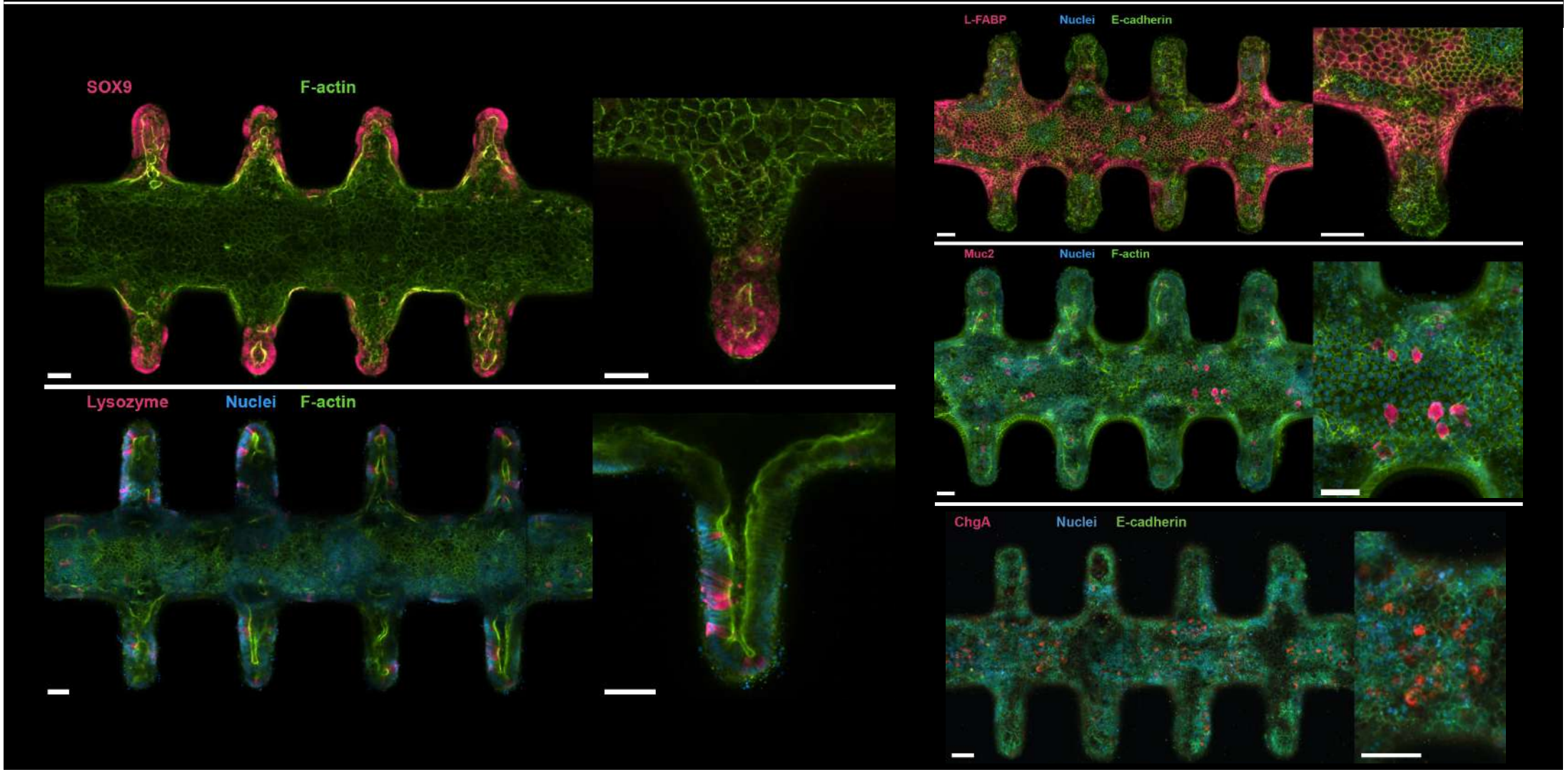
‘Scaffold-guided’ organoid morphogenesis

Primary intestinal stem cells

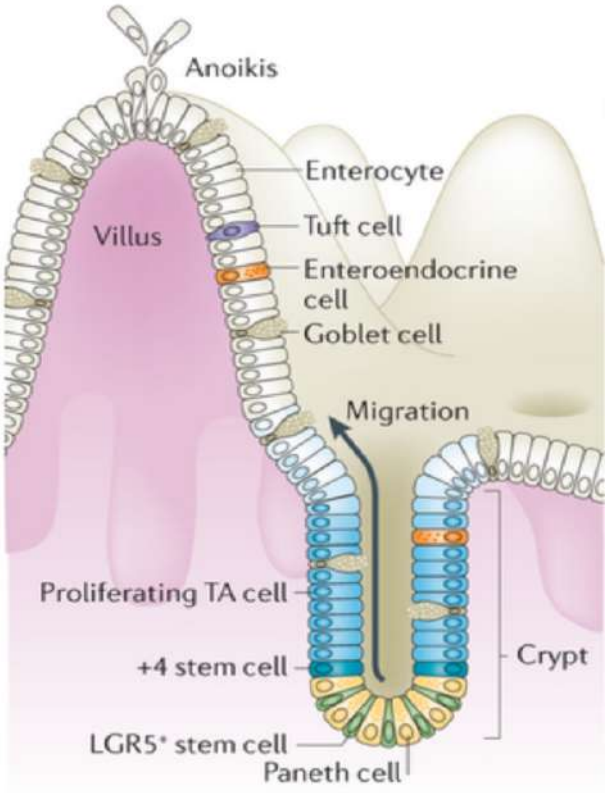


Nikolaev et al., Nature, 2020

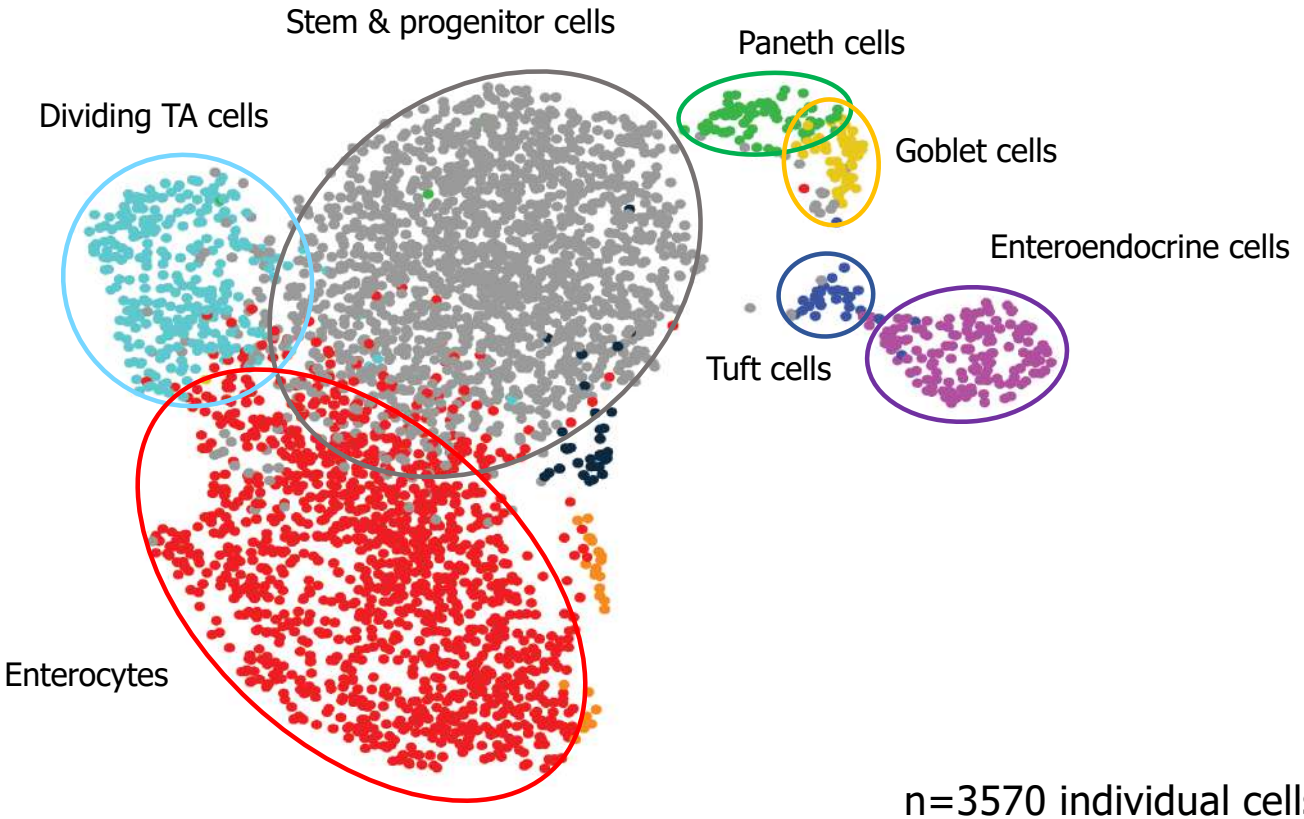
Induction of differentiation results in stereotypical cell-fate patterning



scRNAseq analysis: mini-gut tubes and classical organoids

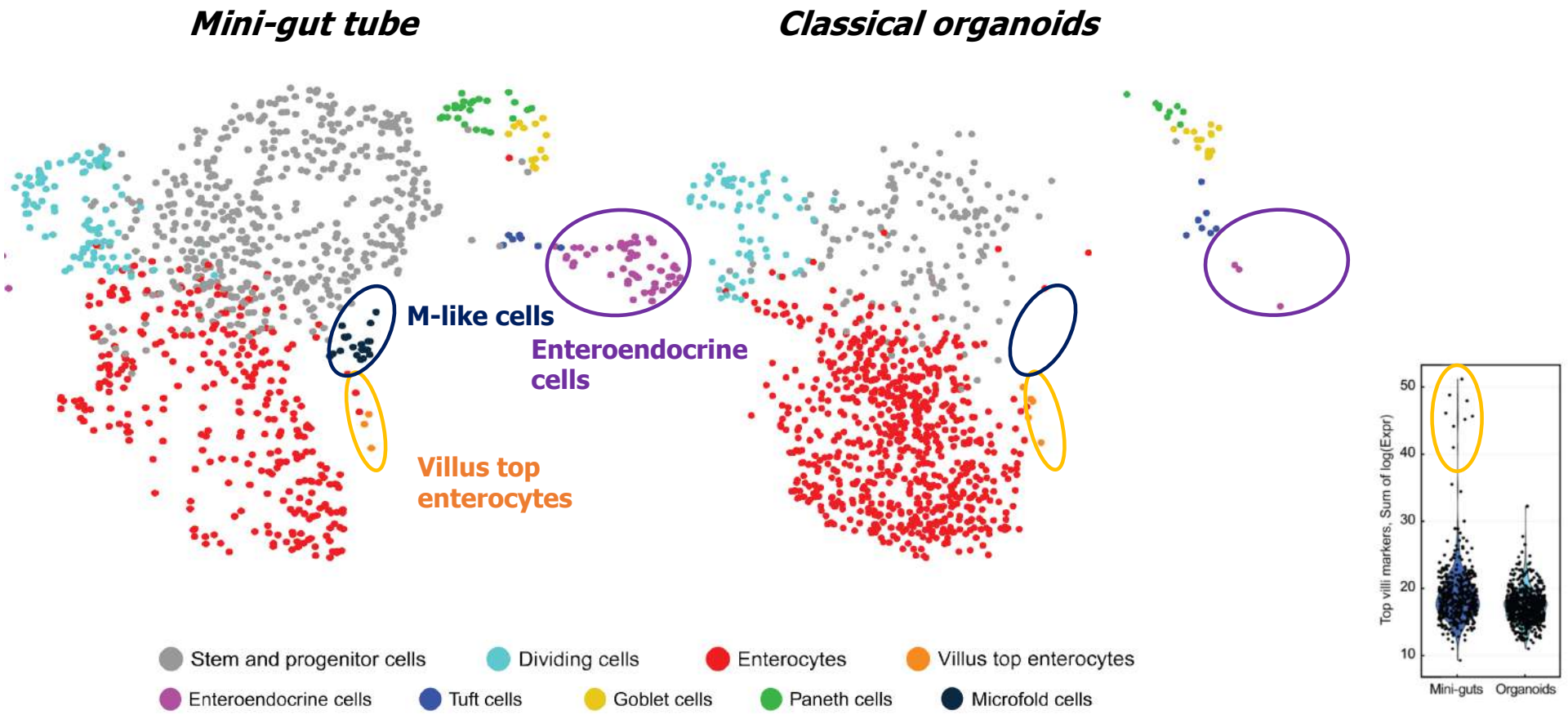


Cells clustered from combined datasets and classified by comparing signatures of differentially expressed genes to pre-existing datasets from native epithelium

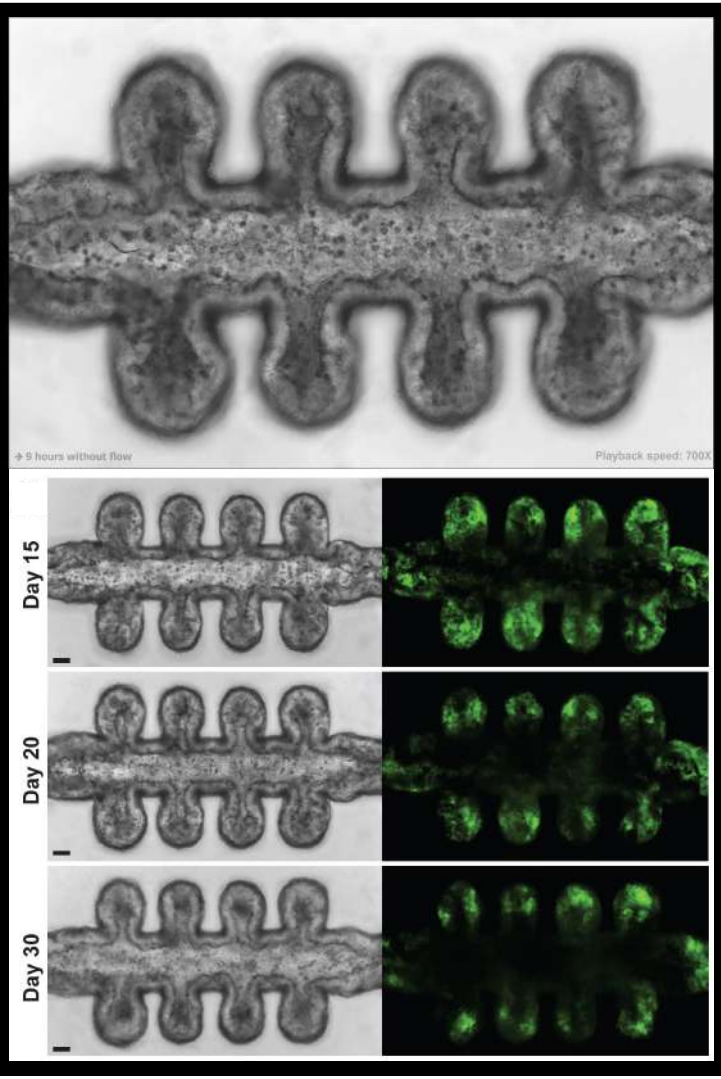


n=3570 individual cells

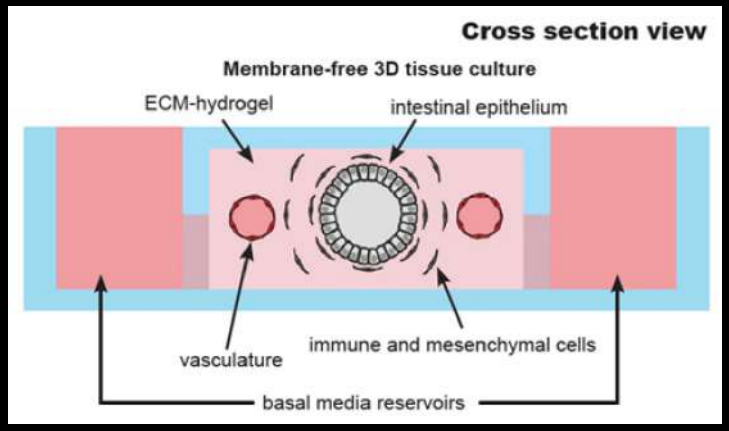
Mini-gut tubes comprise cell types that are absent or very rare in organoids

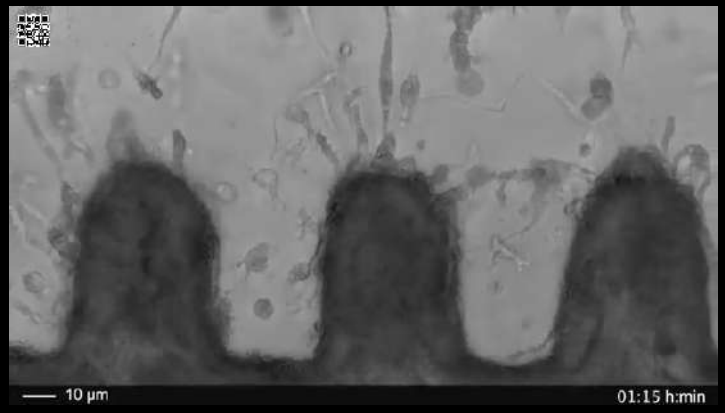


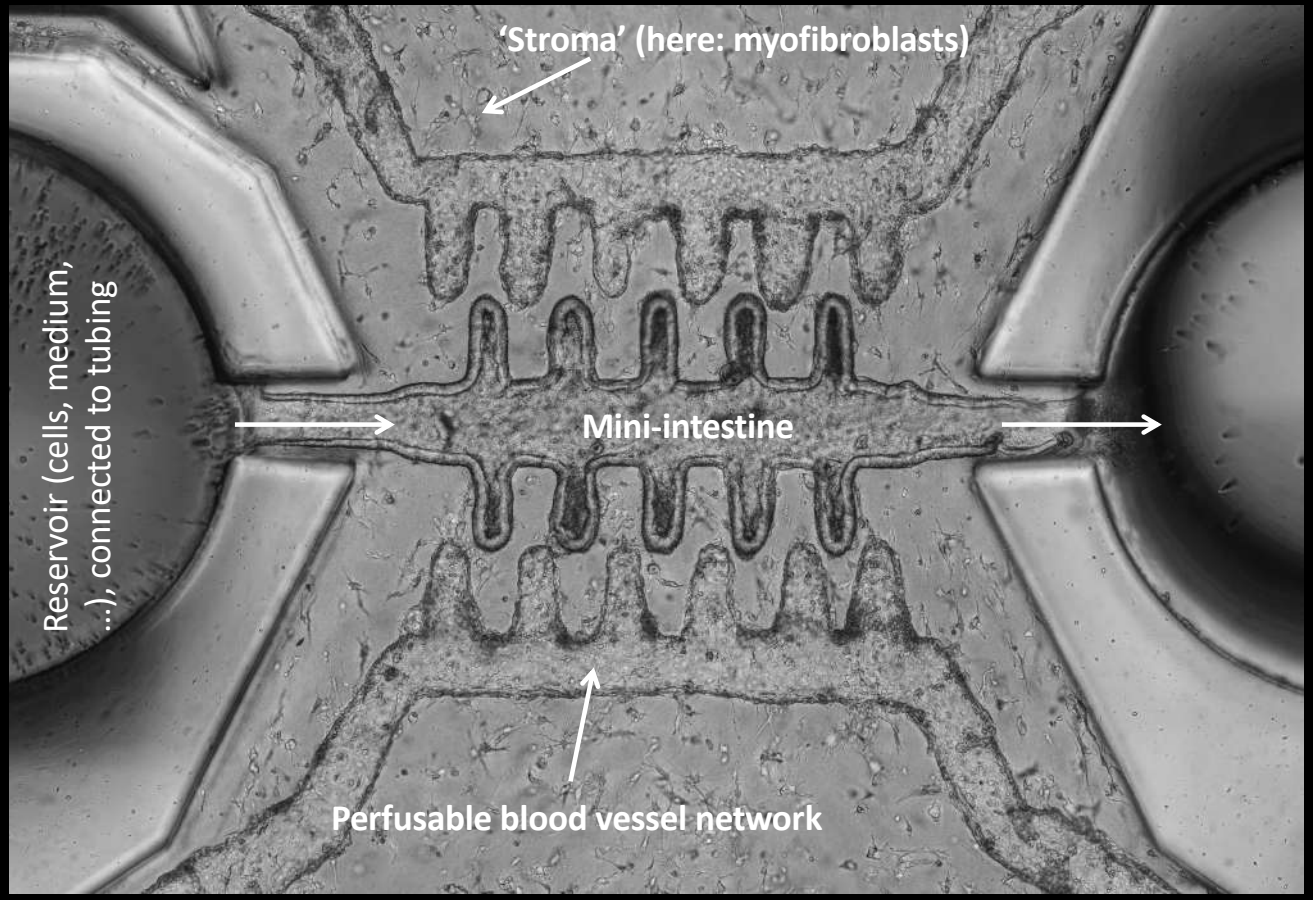
Long-lived perfusable mini-intestines: *in vitro* homeostasis



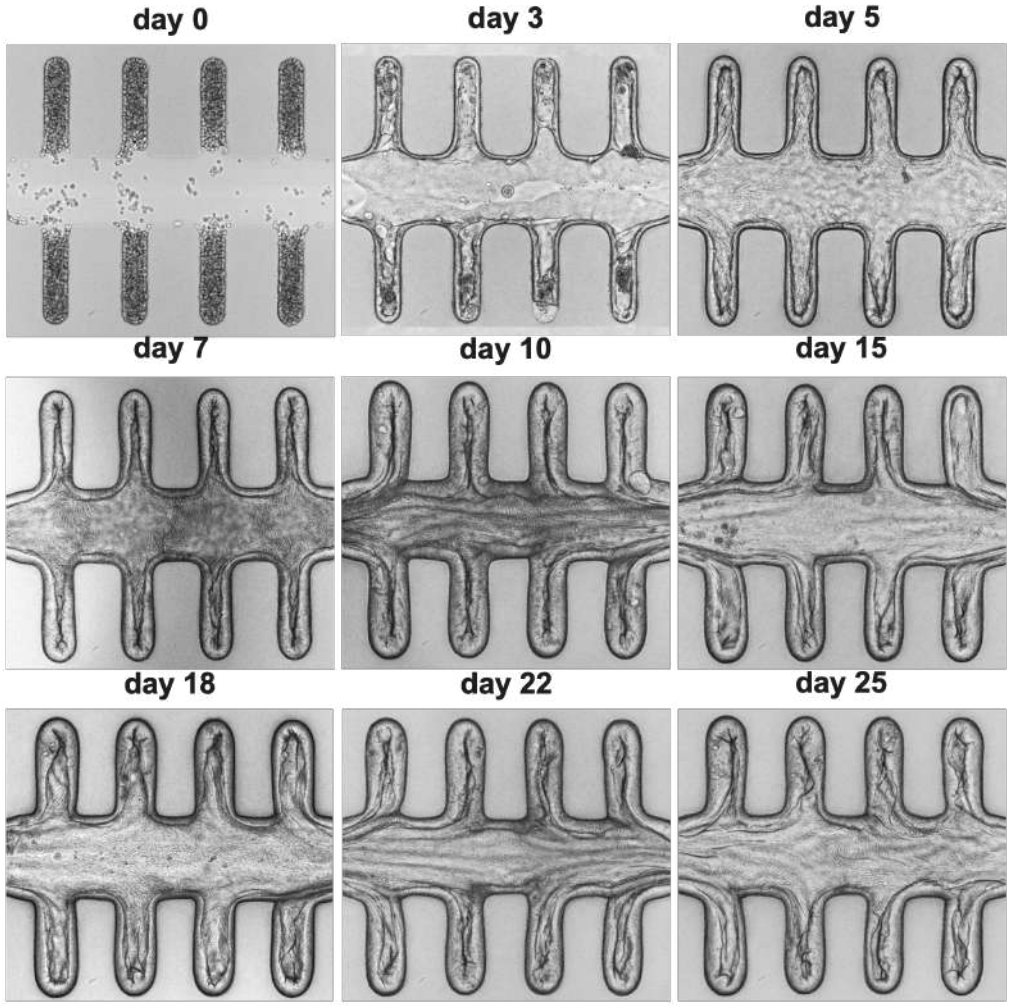
Engineering multi-tissue complexity

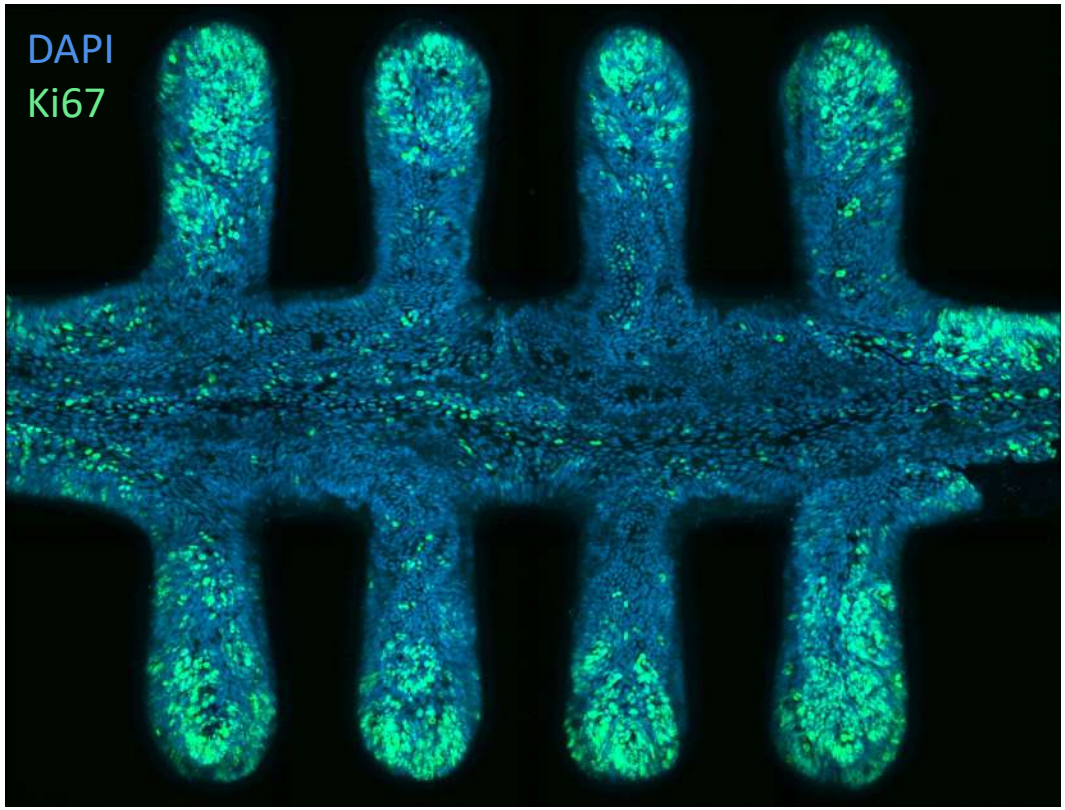






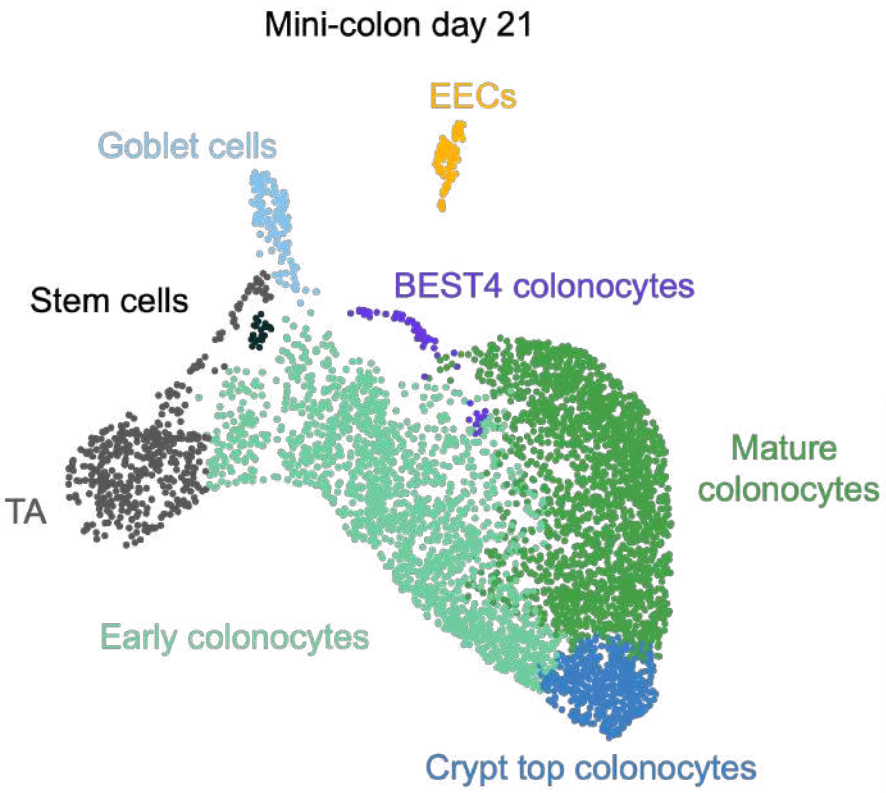
Tubular **human** mini-colons

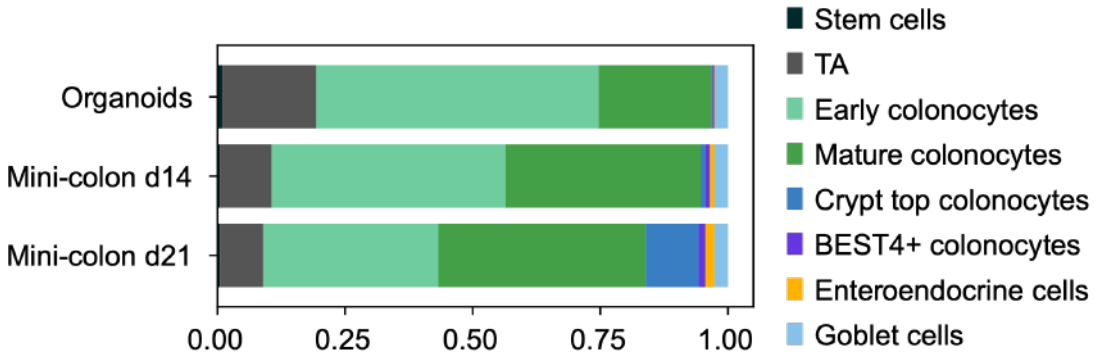


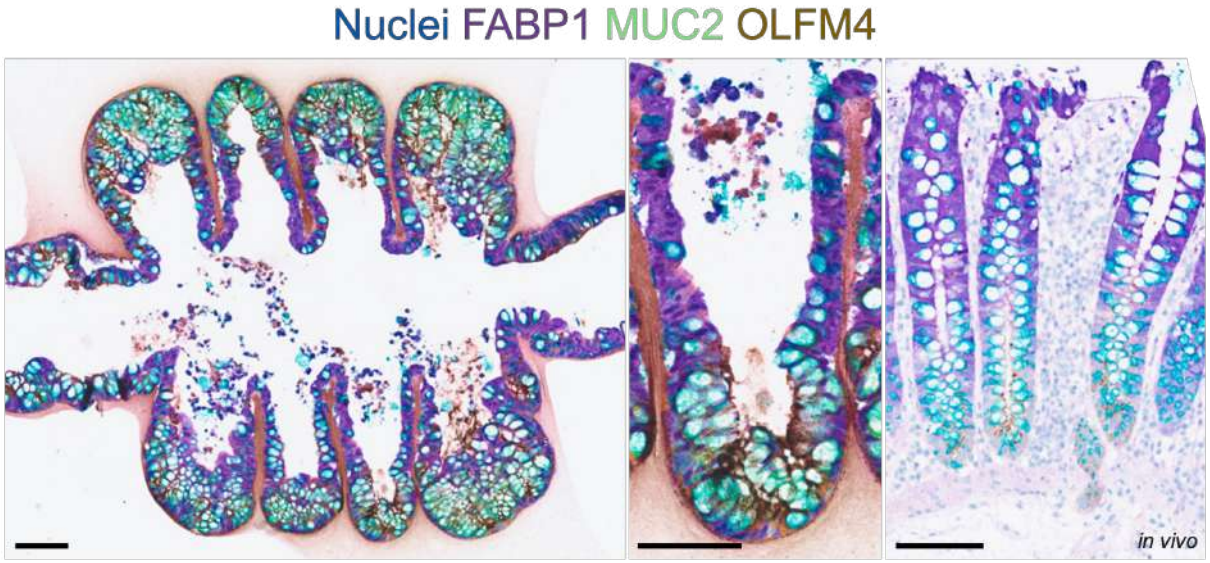


Mitrofanova et al., Cell Stem Cells, 2024

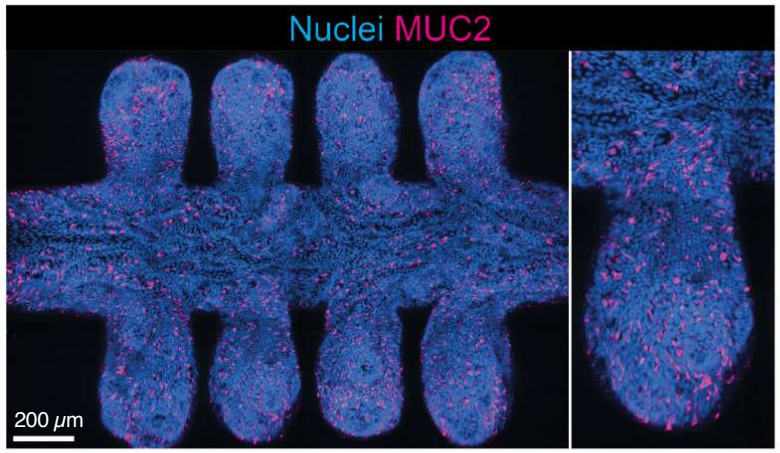
Near-physiological cell type diversity and patterning

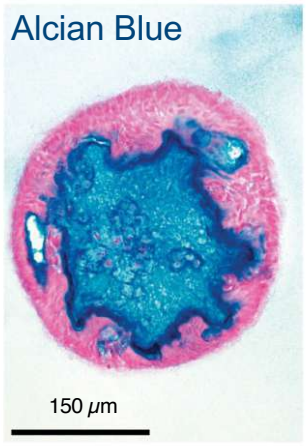


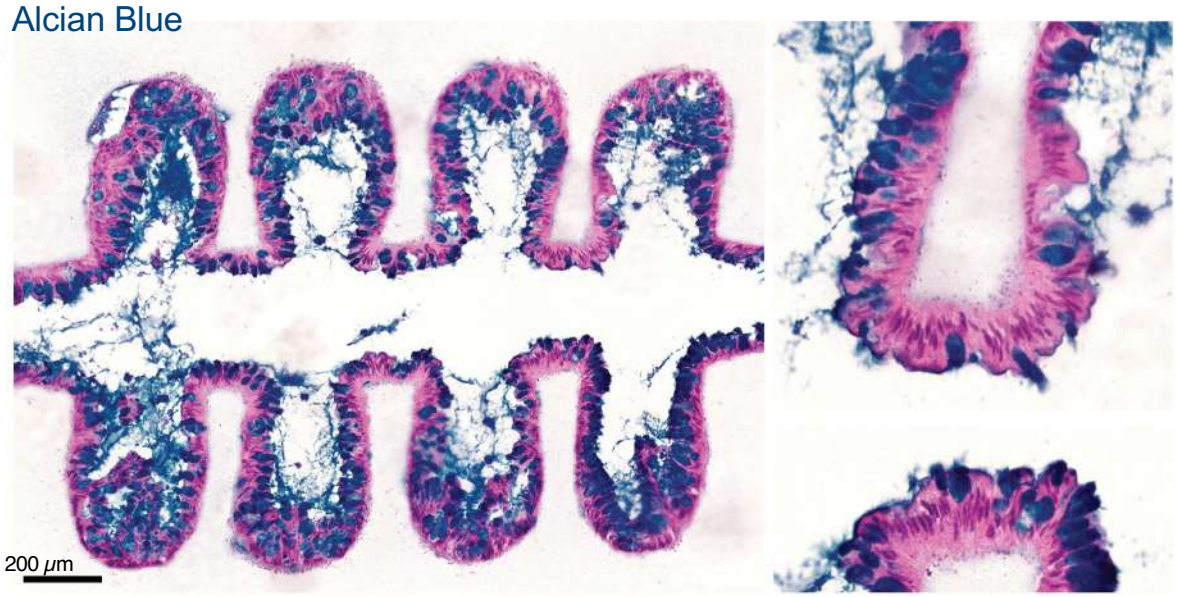


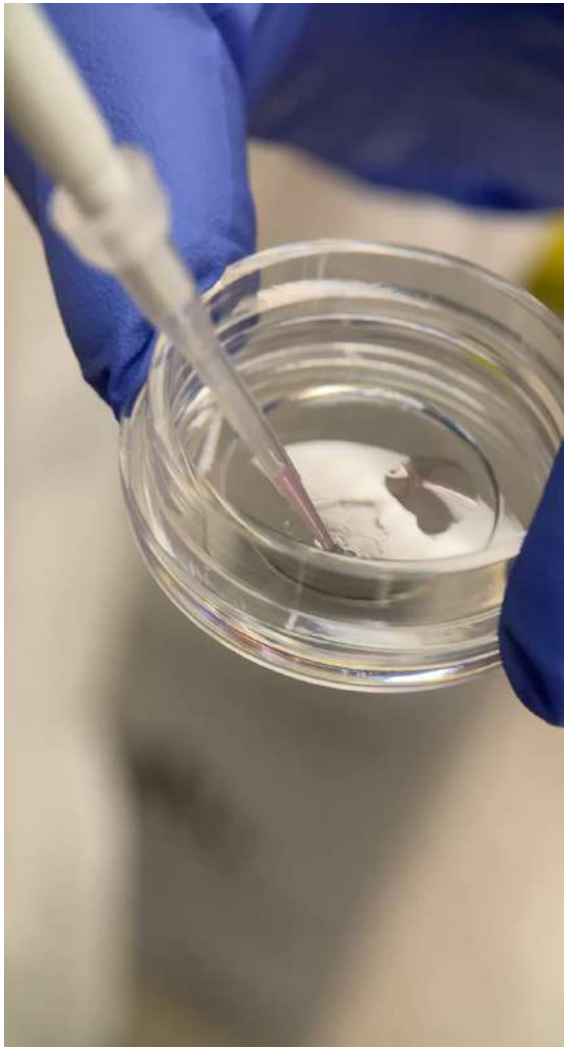


Goblet cell differentiation and mucus secretion

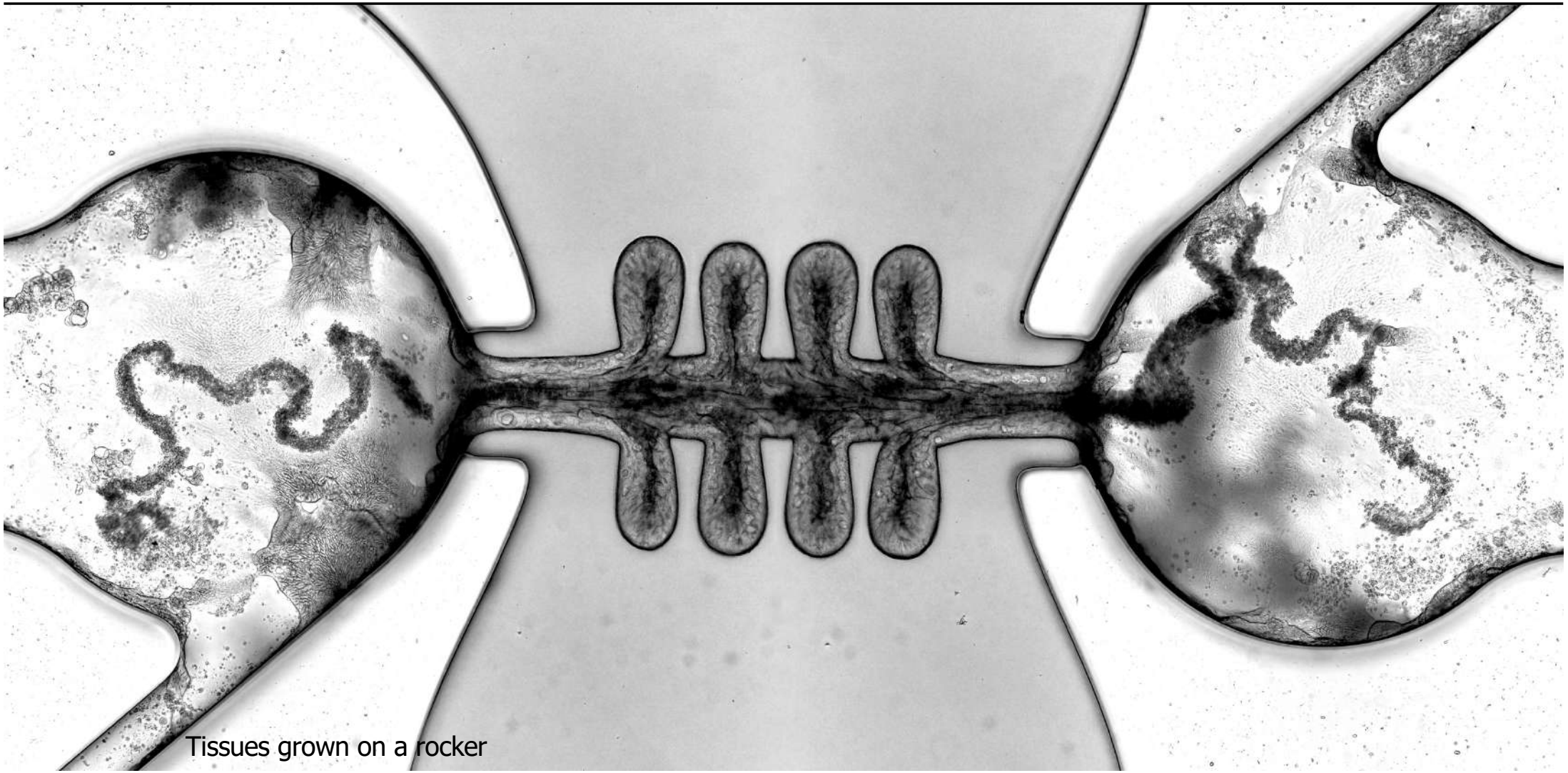




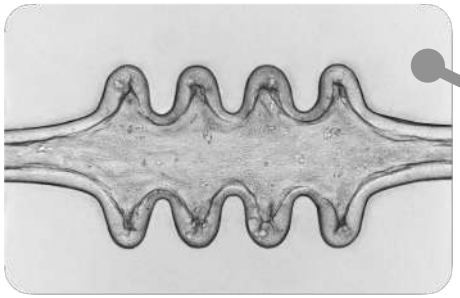




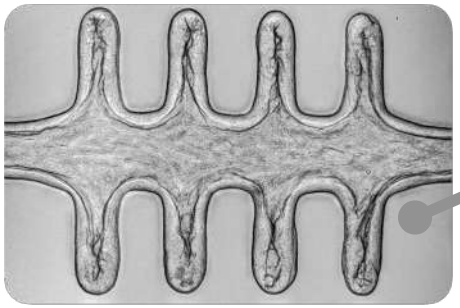
Long-term homeostasis with continuous extrusion of mucus and shed cells



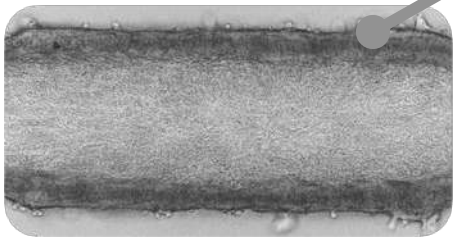
Broad applicability of organoid engineering concept



Small Intestine

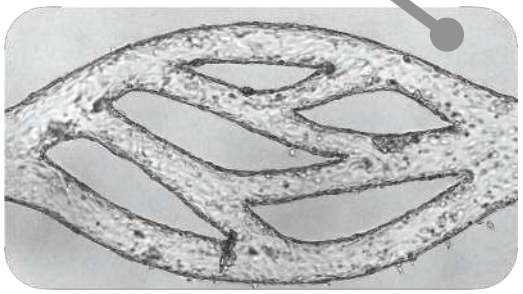


Colon

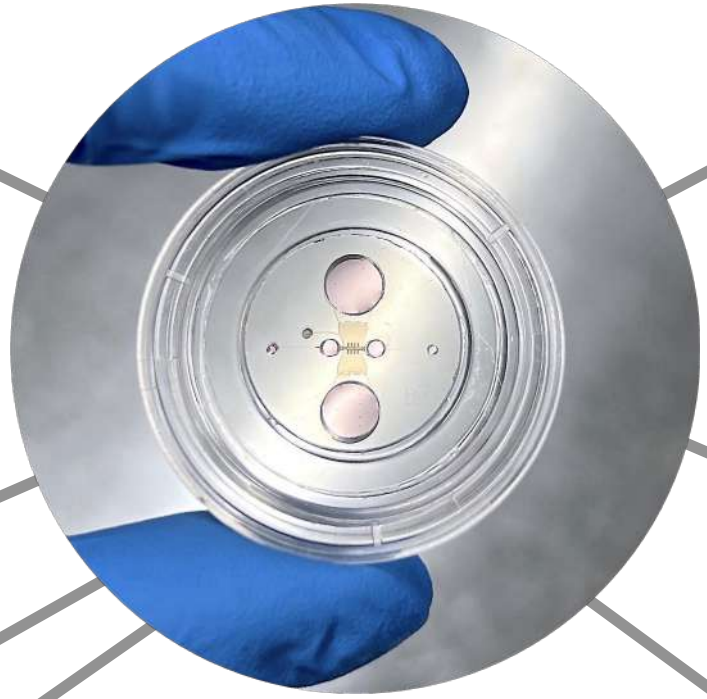


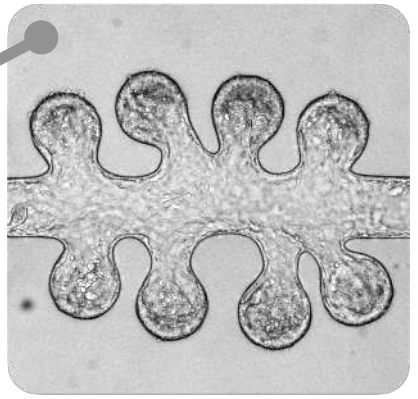
Trachea

Bladder

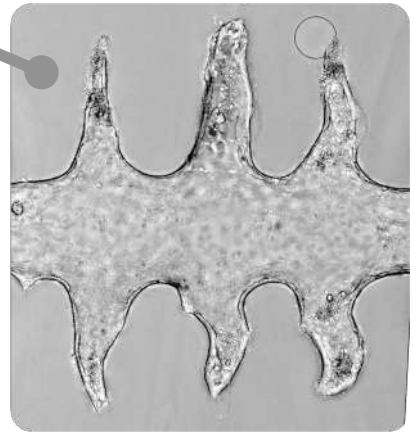


Vasculature





Mammary glands



Endometrial glands

Institute of Human Biology

Take home messages

- Tissue engineering can provide the missing context that renders stem cell self-organization and morphogenesis more controllable
- In particular, tissue shape provides a physical boundary condition that confers robustness to organoid development, and is an effective parameter to steer self-organization towards physiologically relevant sizes and shapes
- The stunning self-renewal potential of epithelial stem cells, together with their ability to differentiate and self-pattern (e.g. into crypt/villus-containing structures), can be exploited to build macroscopic tissues that retain key physiological hallmarks
- Guiding cell-autonomous self-organization through engineered environments may hold significant potential for building tissues with improved physiological relevance and applicability (e.g. in disease modeling)