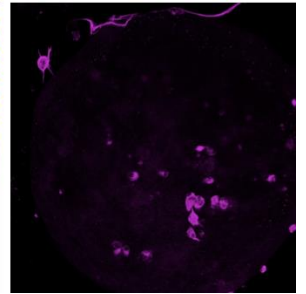
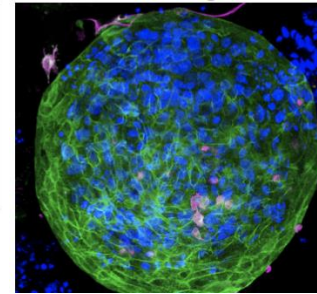
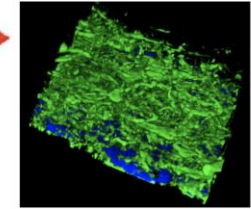
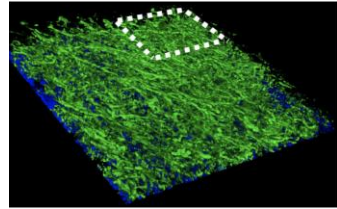
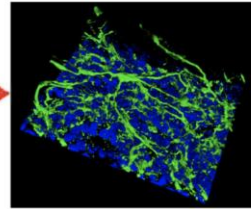
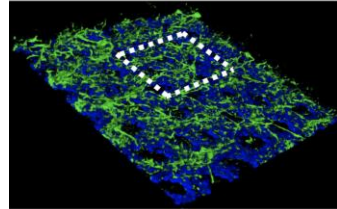
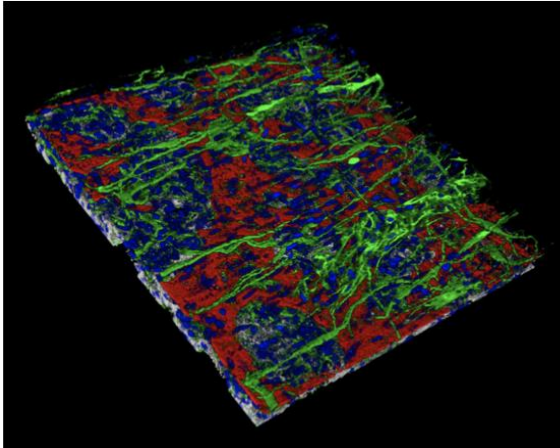
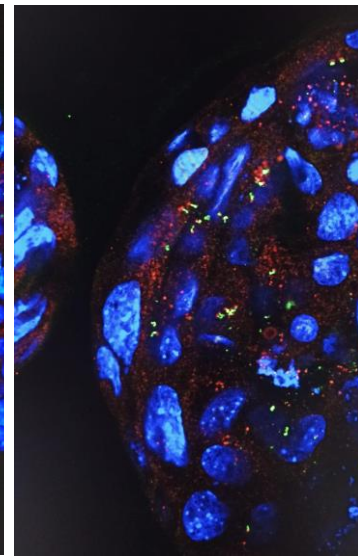
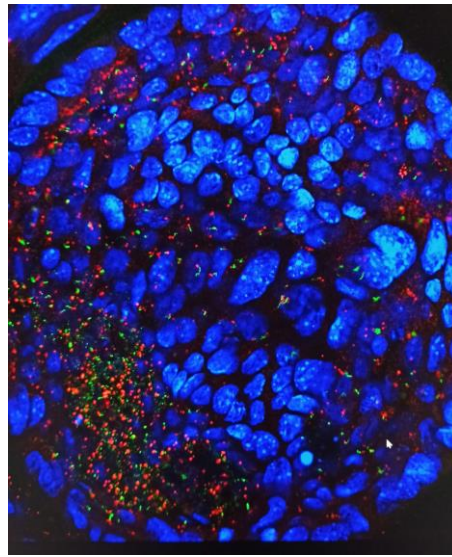
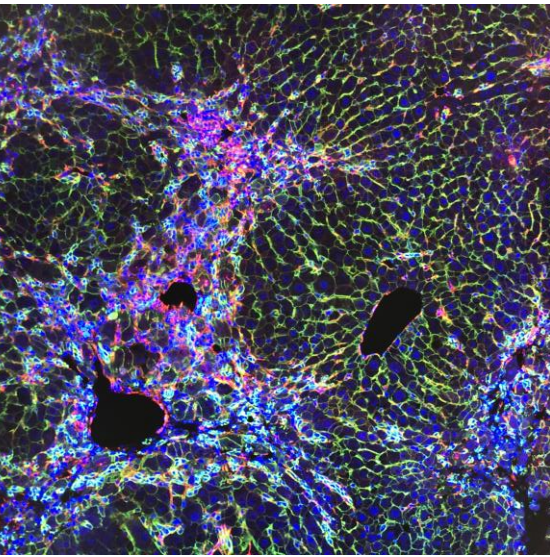


Organoids as power tools for disease modeling

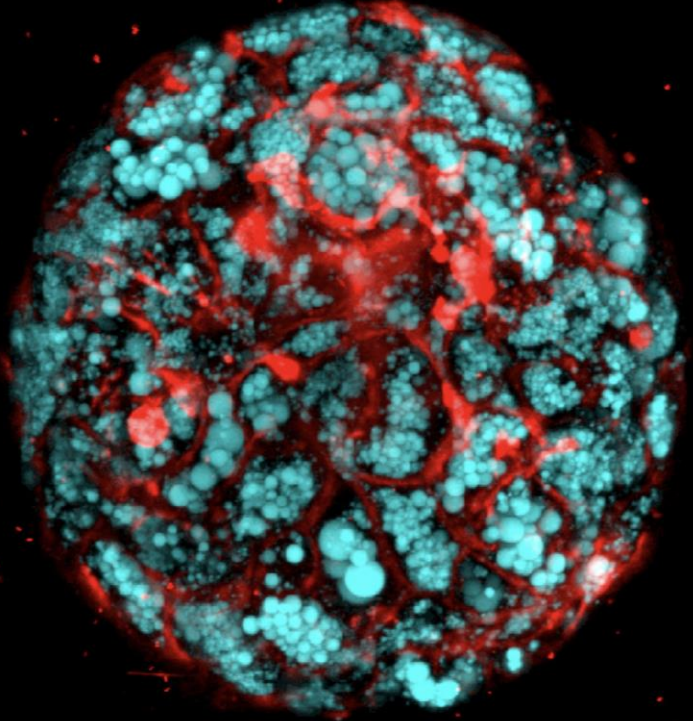


Bahar Degirmenci Uzun
- Bilkent University
Ankara



Organoids: Today's research tool, tomorrow's organ transplant solution

15 MAR 2024

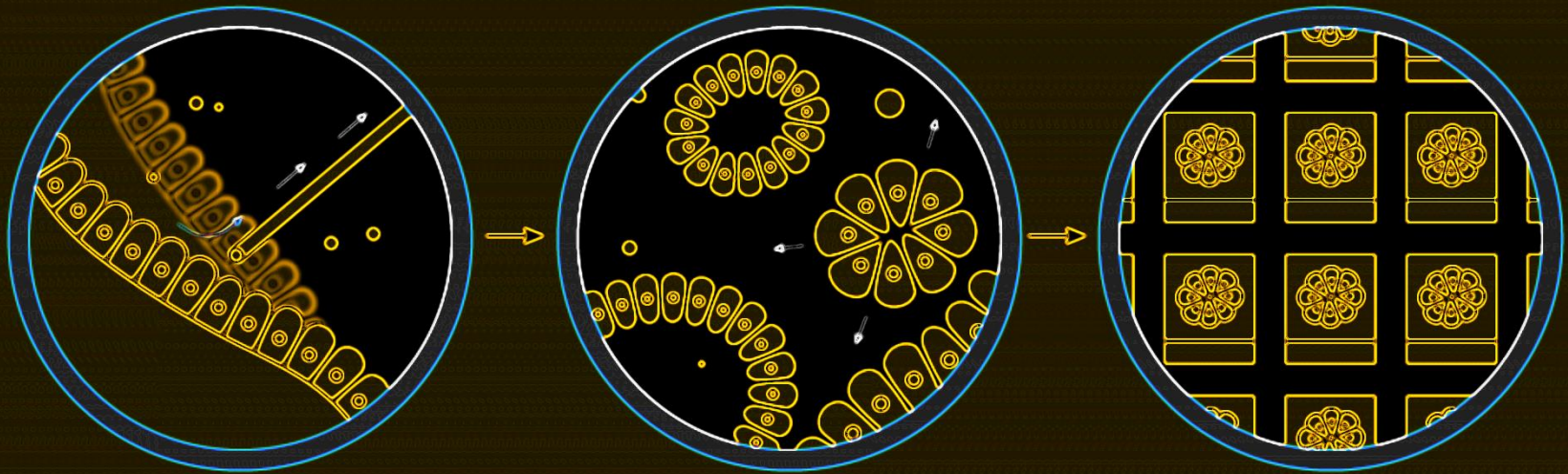


Organoids:

Today's research tool,
tomorrow's organ
transplant solution

Organoids allow researchers to conduct many types of experiments that are not possible with humans or even animals, and they could someday serve as an alternative organ source for transplantation.

How we grow organoids



1

Take stem cells

Isolate them from a healthy or tumorous tissue sample from a patient

2

Nurture them

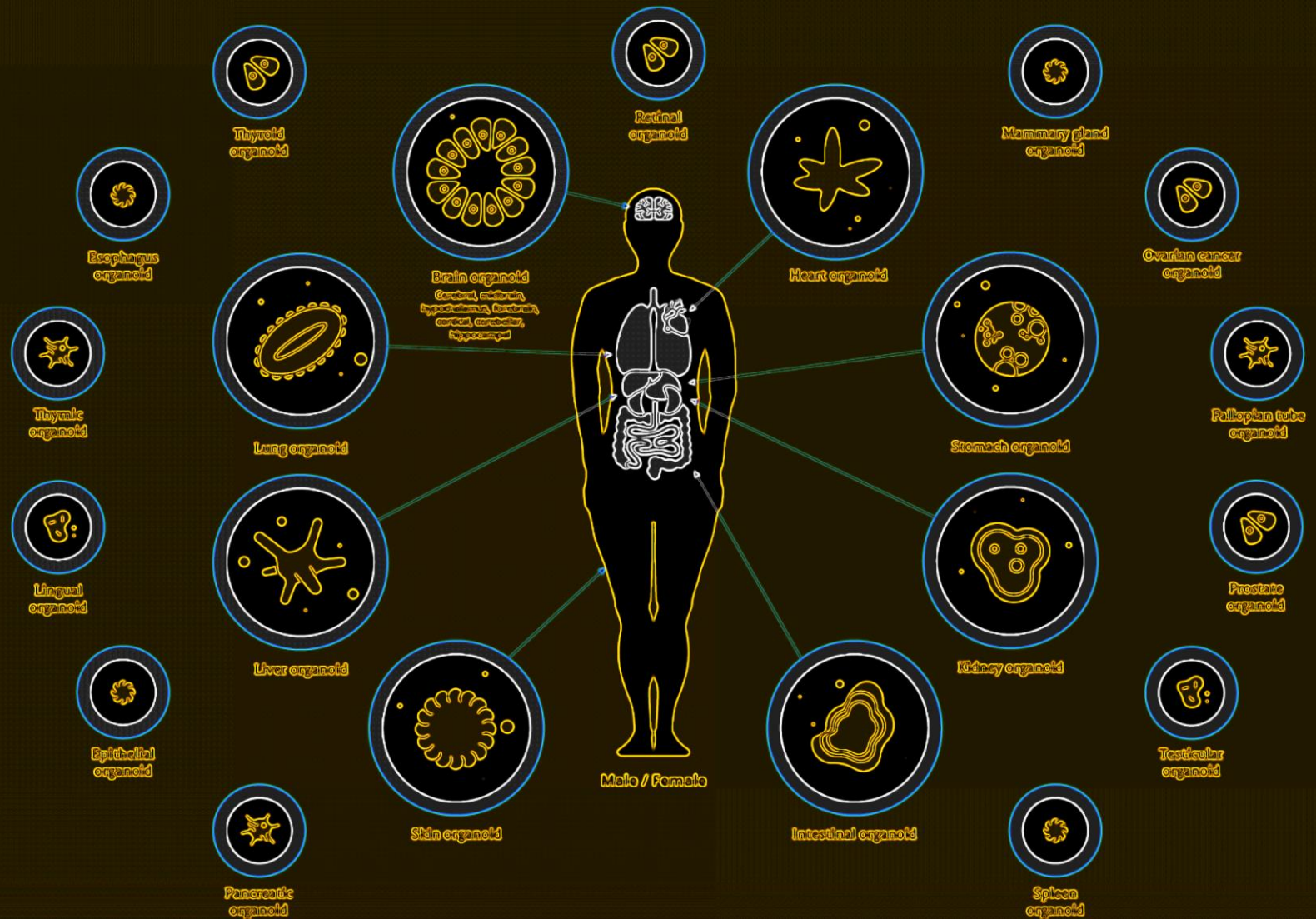
Batch the stem cells in 3D culture with a mix of growth factors

3

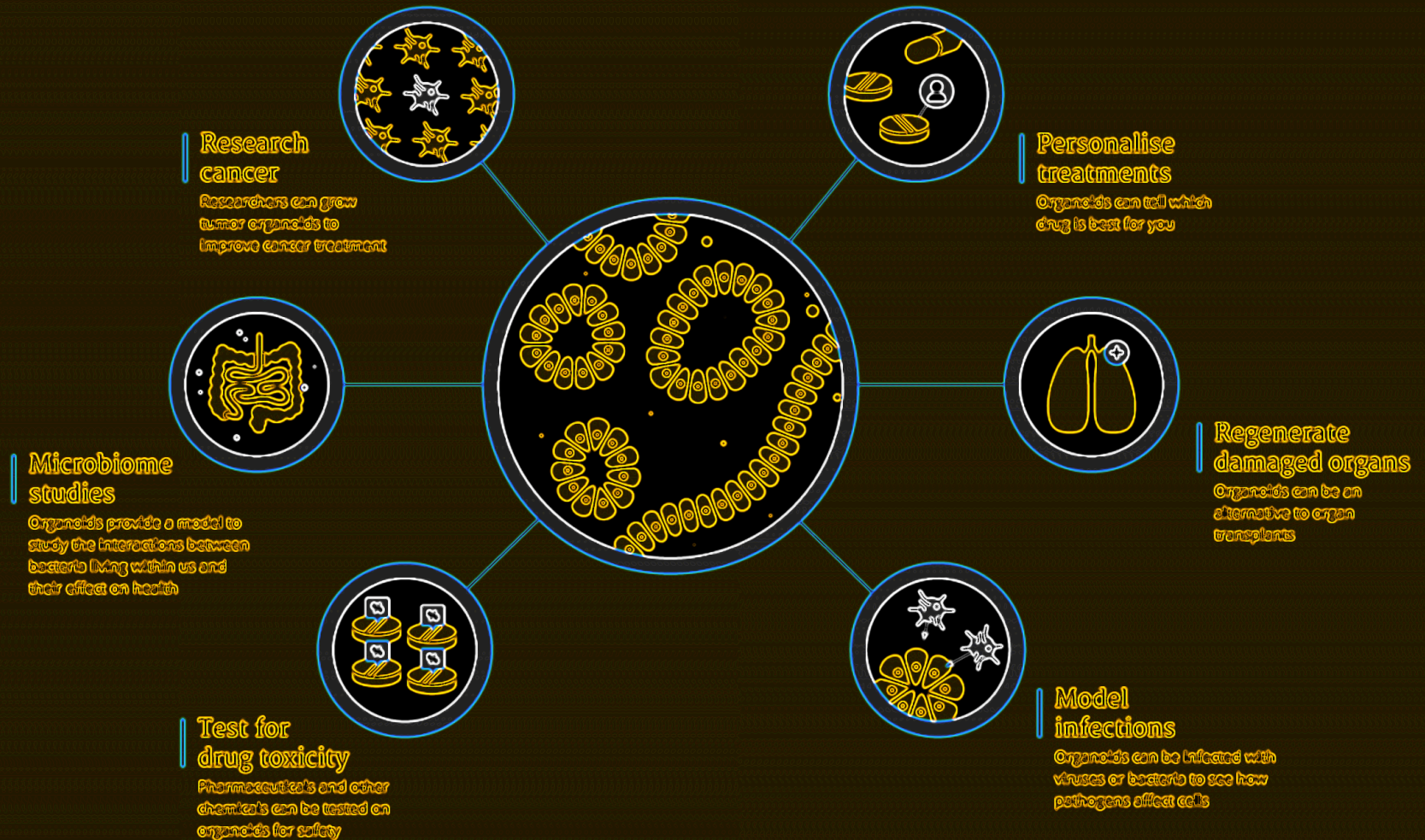
Harvest organoids

Organoids emerge within 3 weeks and can be stored in biobanks for later use

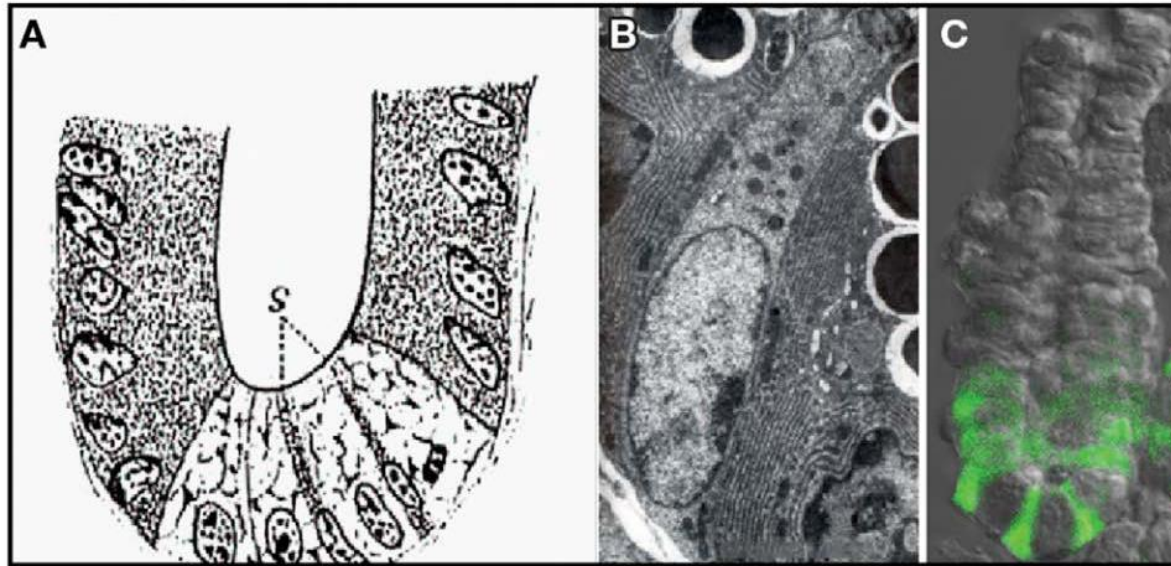
The world of human organoids



What organoids can do for you



THE CRYPT MORPHOLOGY :120 YEARS OF HISTORY

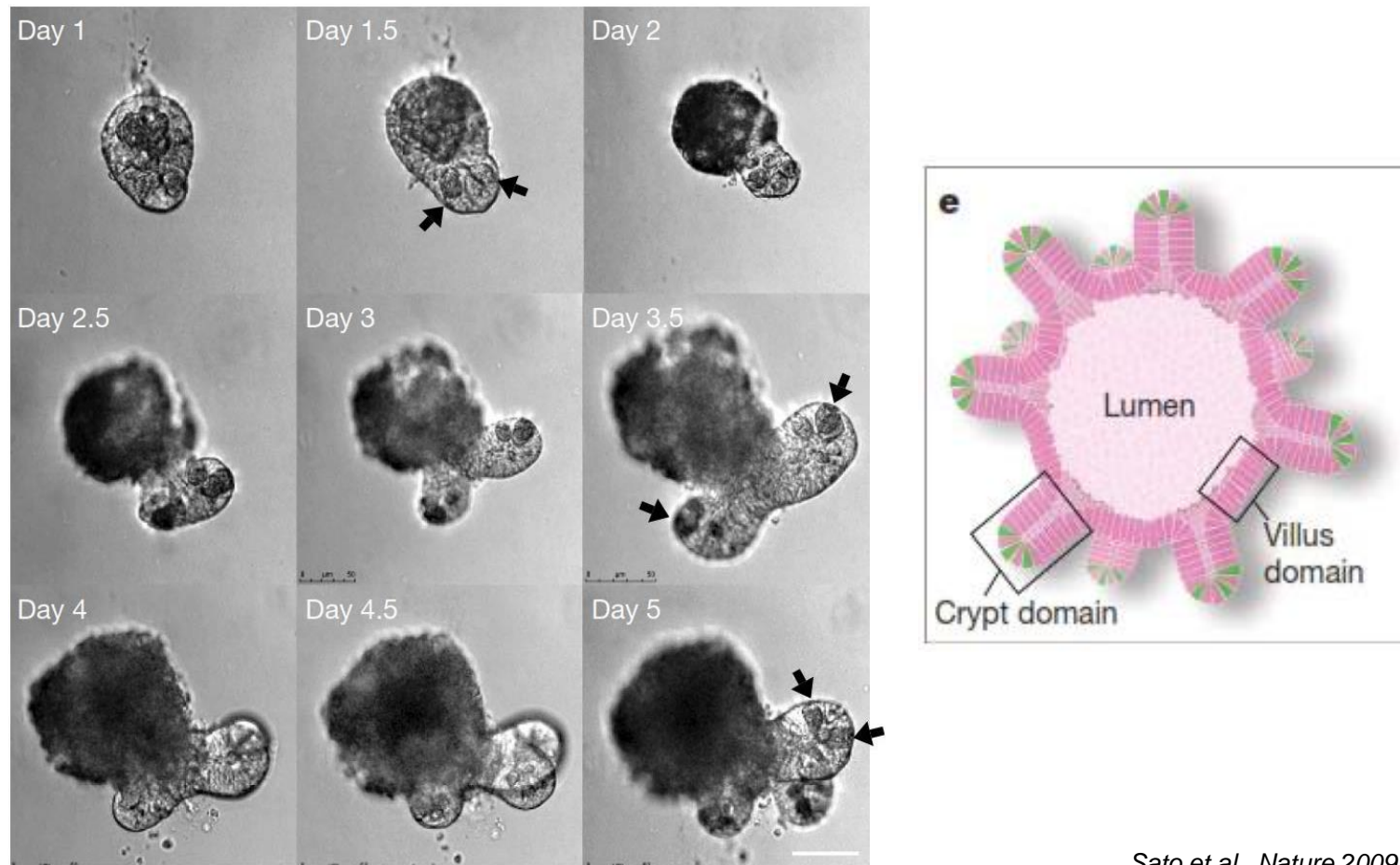


Hand-drawn crypts
"S": small cells
(Paneth 1887)

First electron-
microscopy image of
a CBC cell (Cheng
and Leblond 1974)

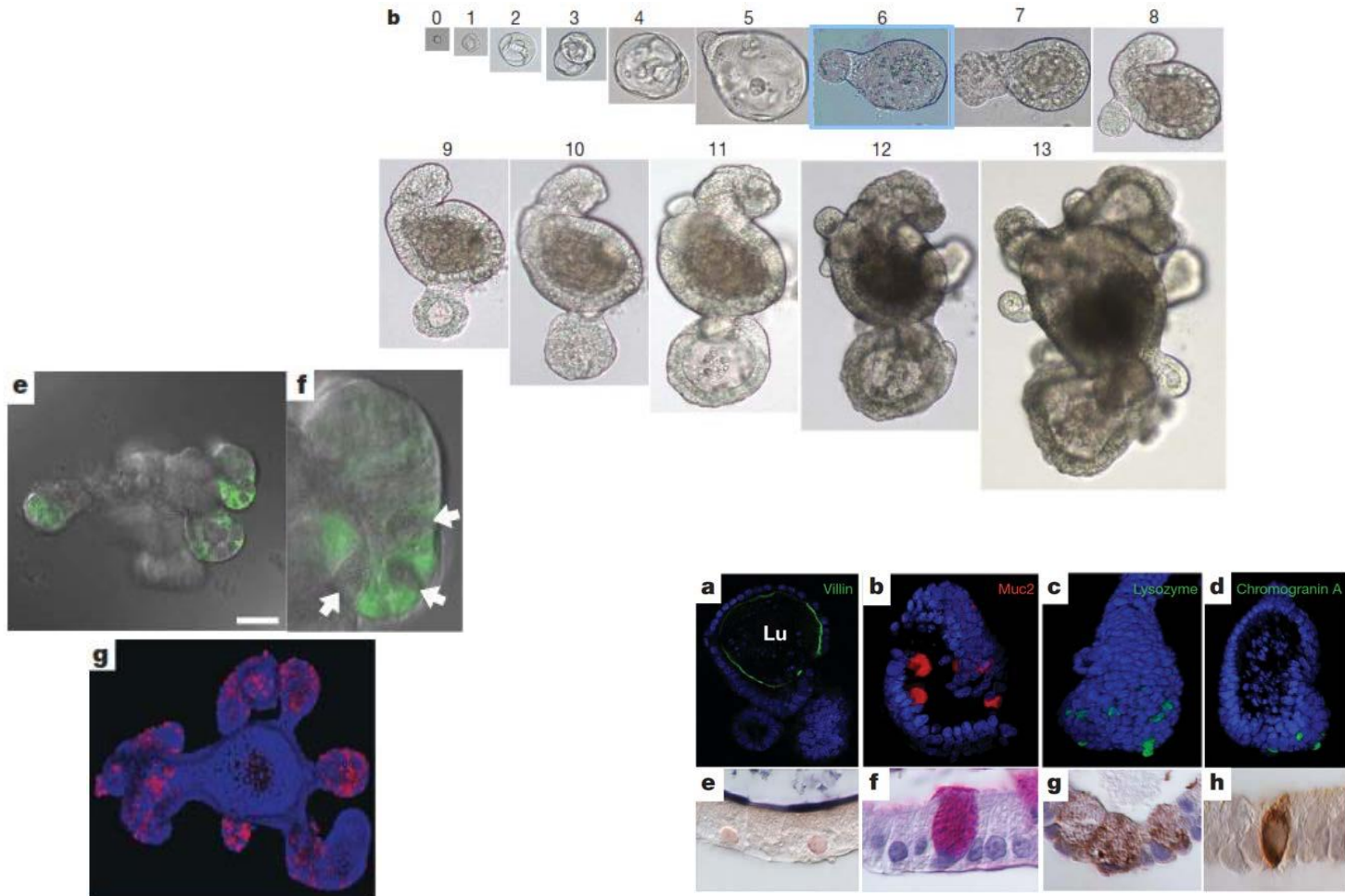
Confocal image
of Lgr5-GFP
Cells

ORGANOIDS: AN INTESTINAL EPITHELIUM MODEL GROWN IN THE ABSENCE OF NICHE

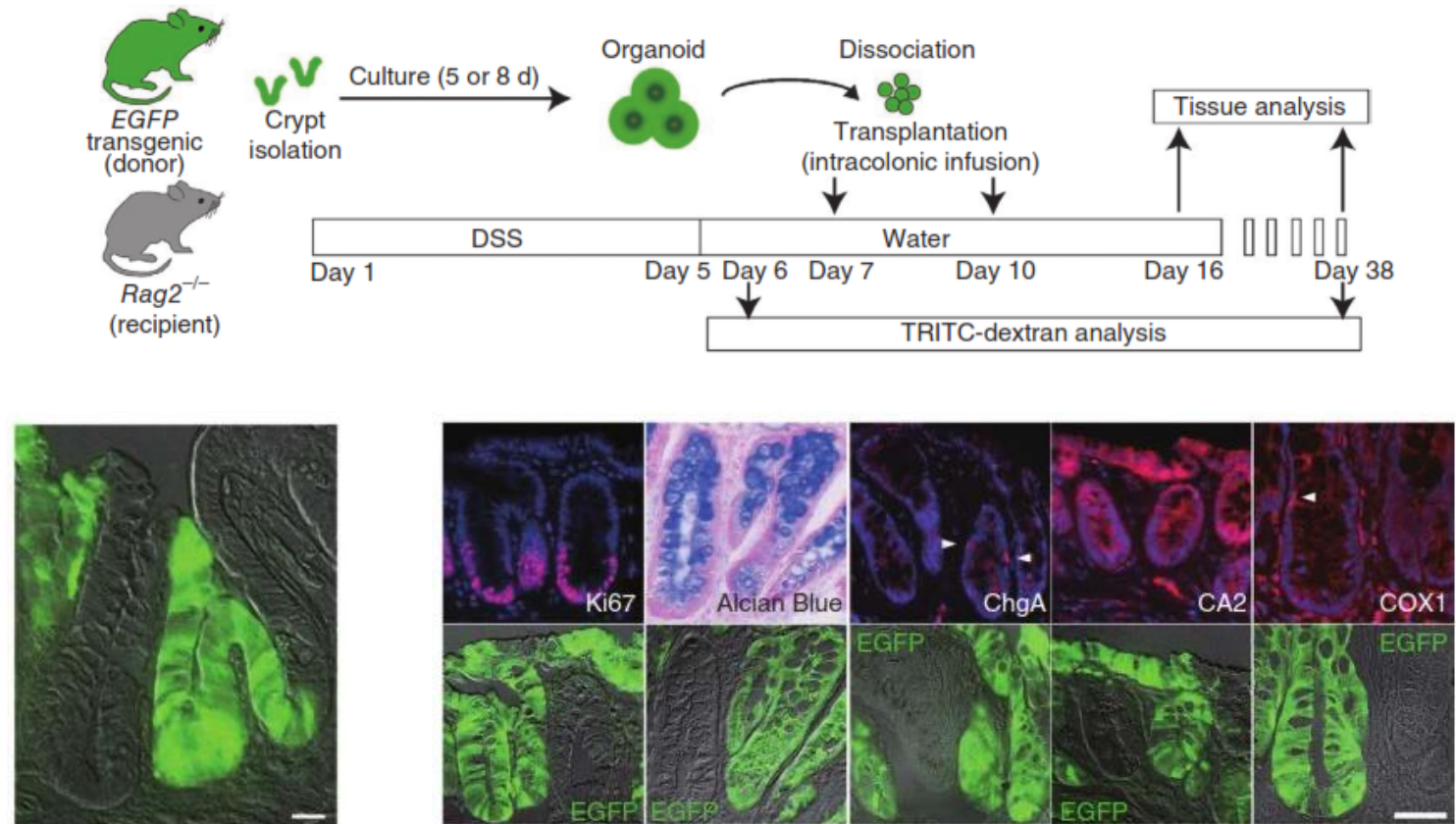


Sato et al., Nature 2009

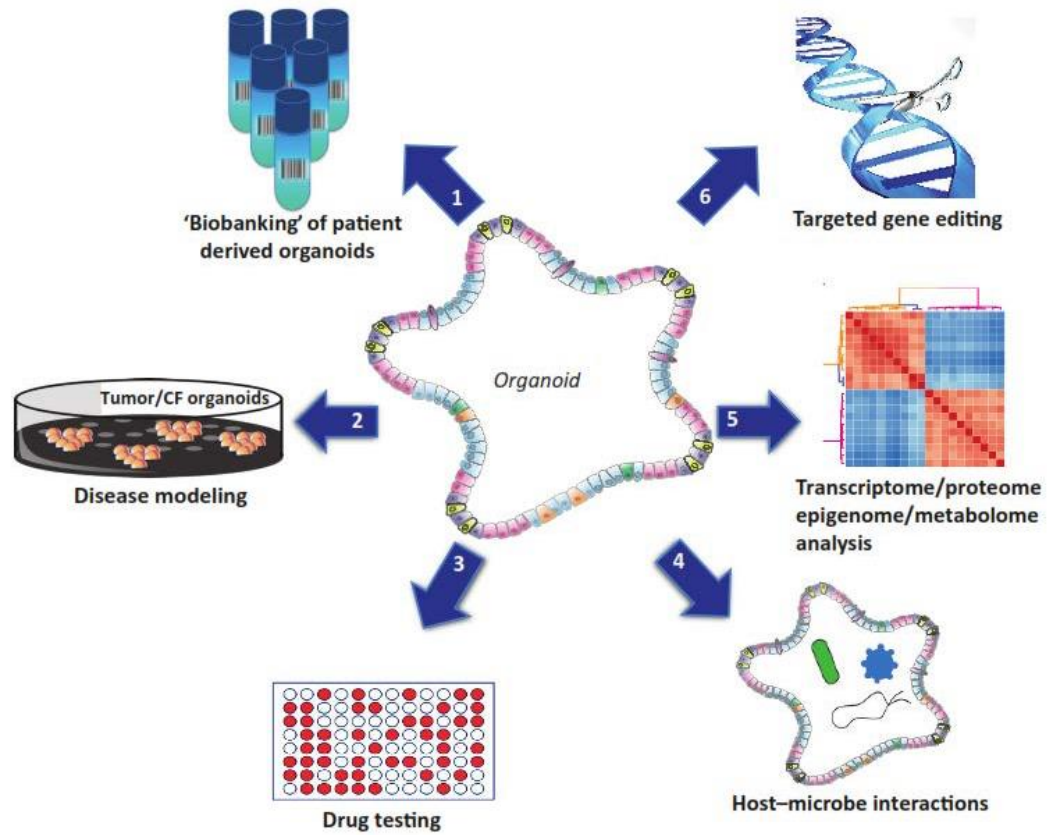
SINGLE LGR5+ STEM CELLS GENERATE MULTI-LINEAGE ORGANOIDS



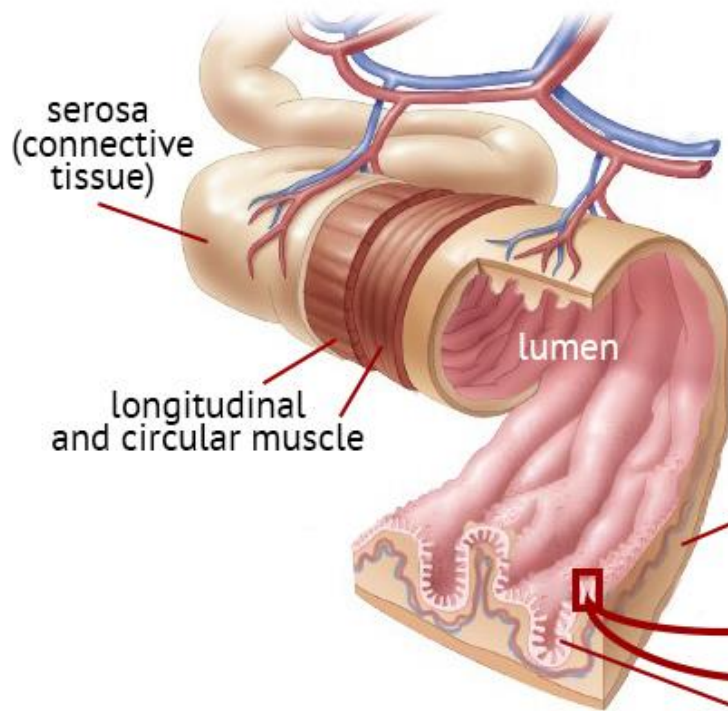
FUNCTIONAL ENGRAFTMENT OF ORGANOID EPITHELIUM



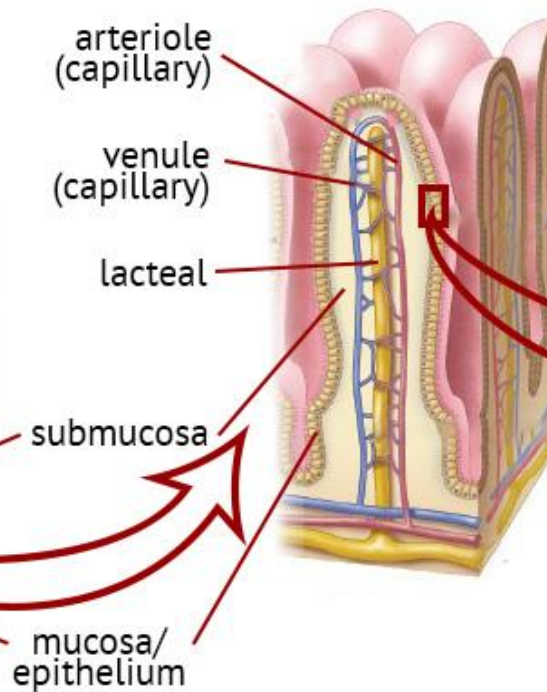
MULTIPLE APPLICATION OF ORGANOID TECHNOLOGY



A tissue layers in small intestines



B villus



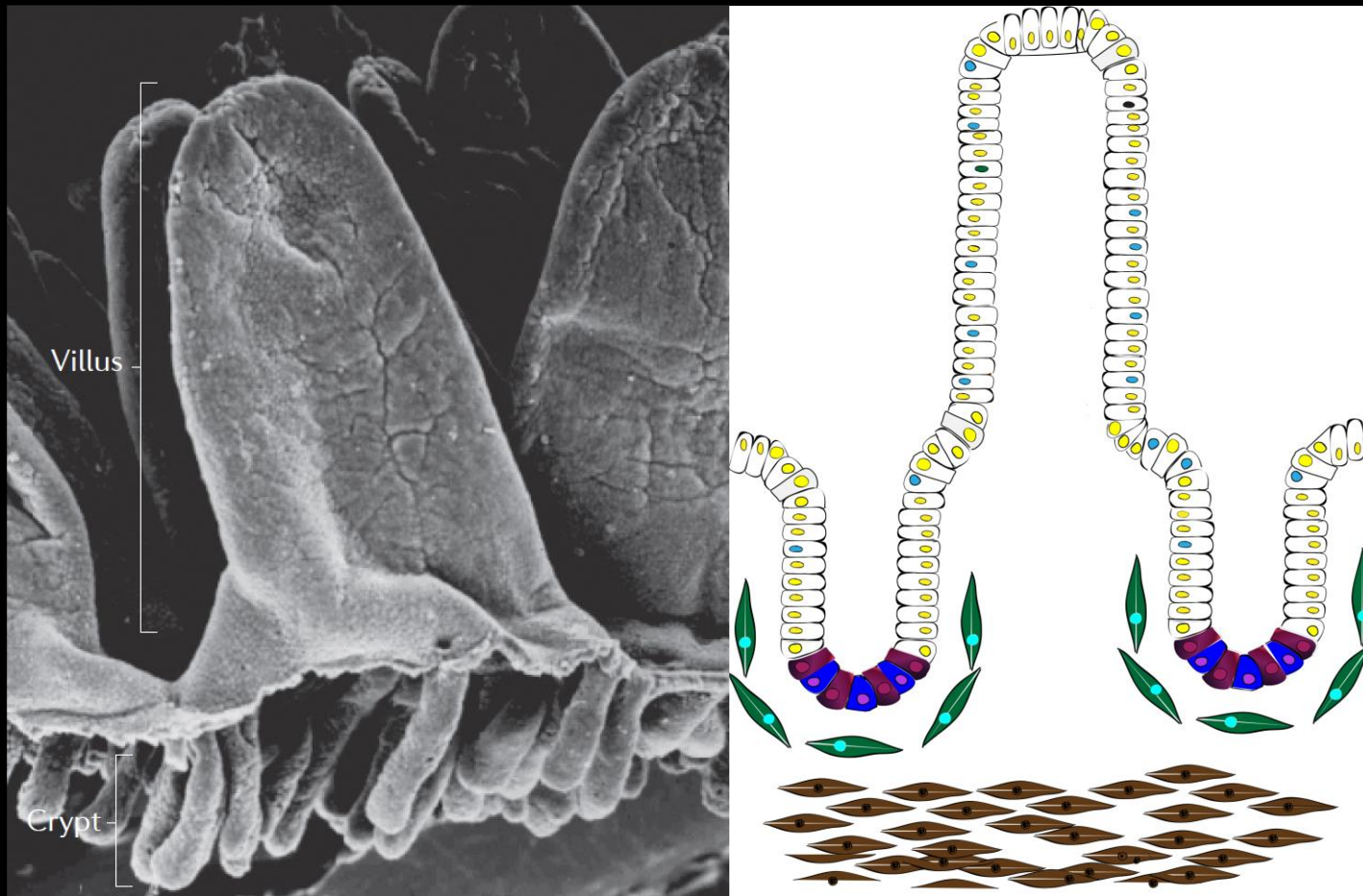
C columnar epithelial cell



microvilli

Intestinal epithelium: the most dynamic adult epithelial tissue

Small intestine (duodenum)



based on Degirmenci et al., Prog. Mol. Biol. Transl. Sci. (2018)

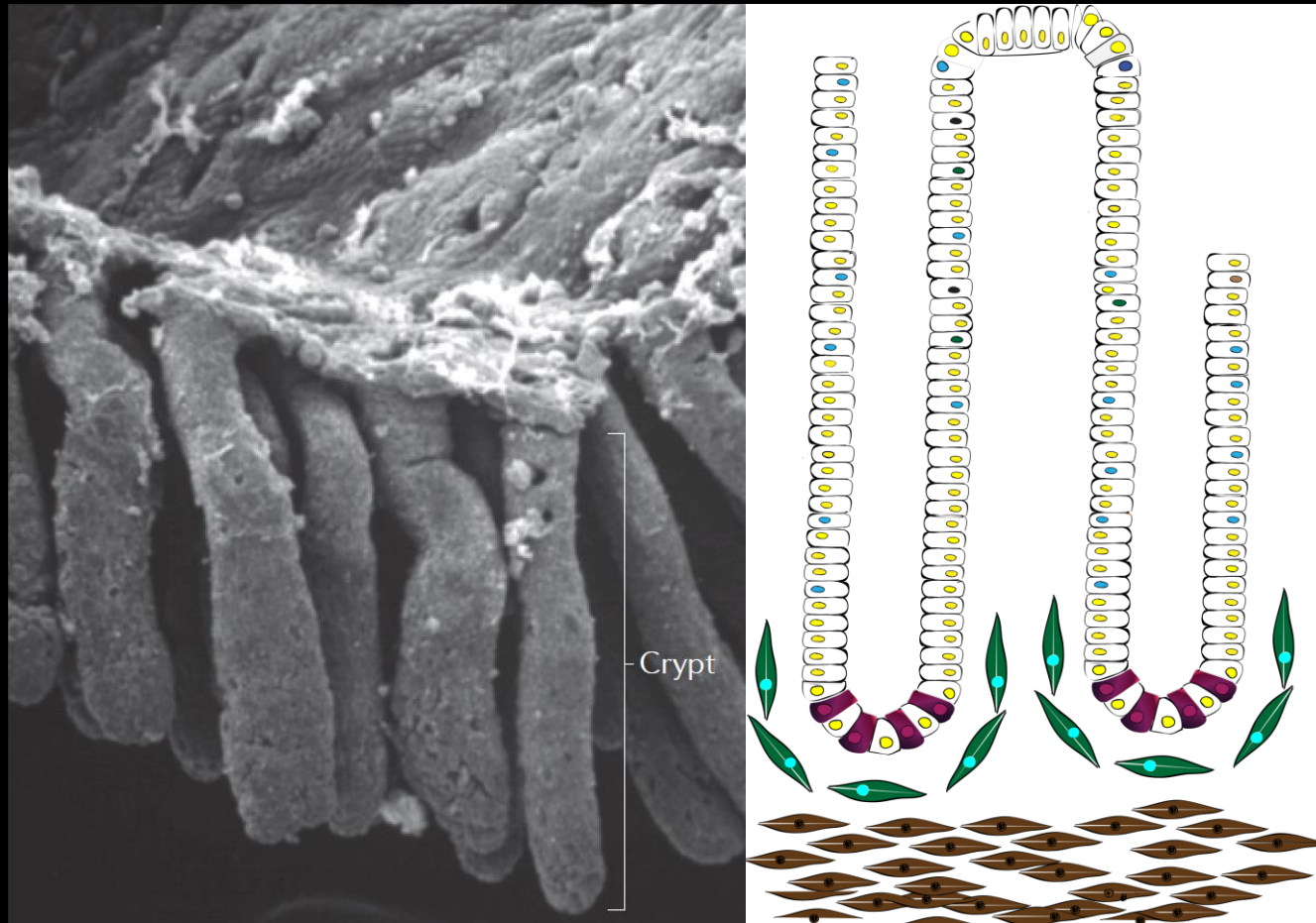


Intestinal Epithelial Stem Cell (IES)



Paneth cell

Large intestine (colon)



based on Degirmenci et al., *Prog. Mol. Biol. Transl. Sci.* (2018)

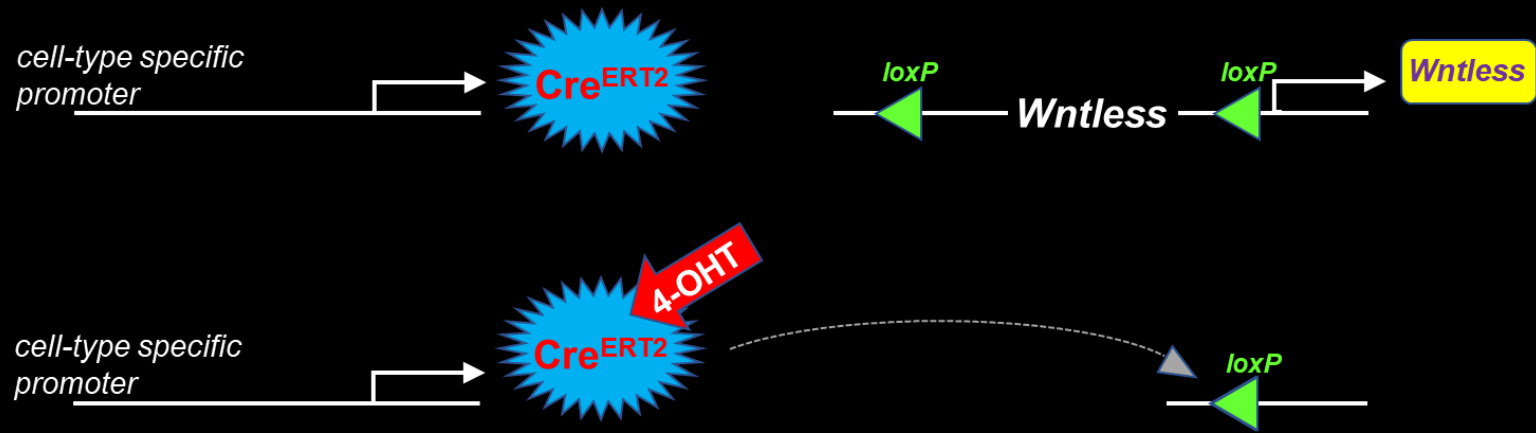


Intestinal Epithelial Stem Cell (IES)

- What are the essential Wnt secreting cells in the small intestine and colon?
- What is the Wnt niche in the intestine or is it exist?

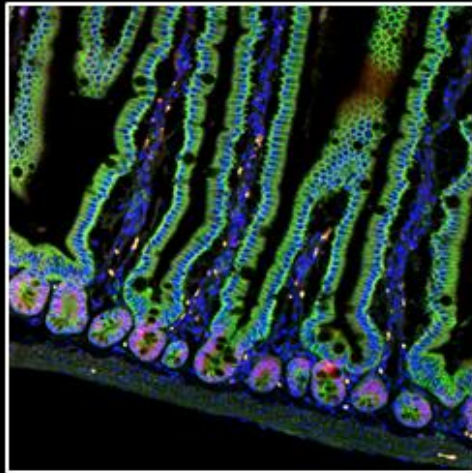
Niche refers to a microenvironment, within the specific anatomic location where stem cells are found, which interacts with stem cells to regulate cell fate.

Blocking Wnt-secretion using cell-specific & inducible Cre/loxP system

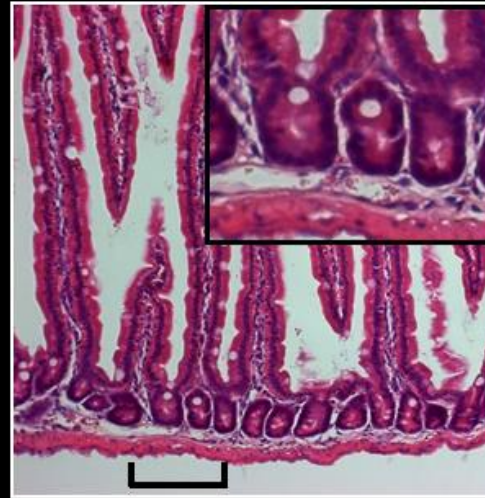


Wnt-ligands are essential for the intestinal epithelium renewal, but epithelial Wnts are dispensable

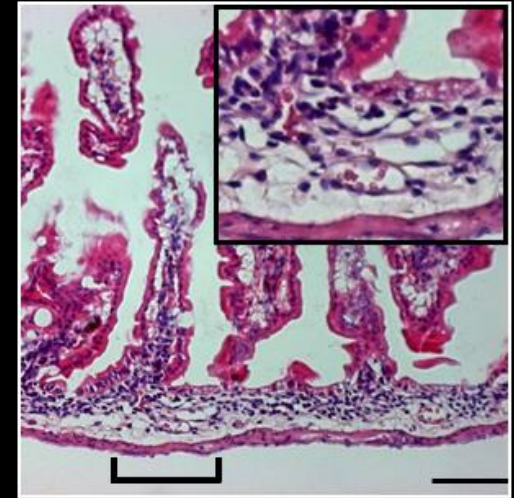
control



control



R26-Wls^{CKO} (12 days)

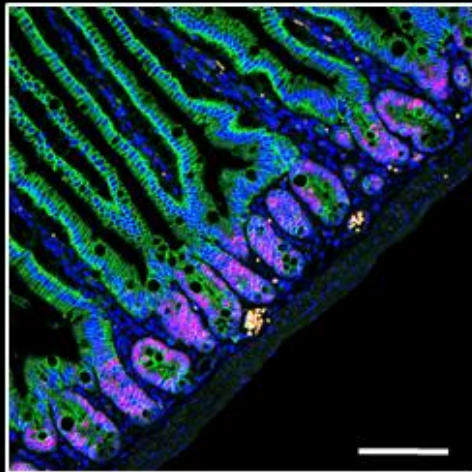


Ki67

β-catenin/DAPI

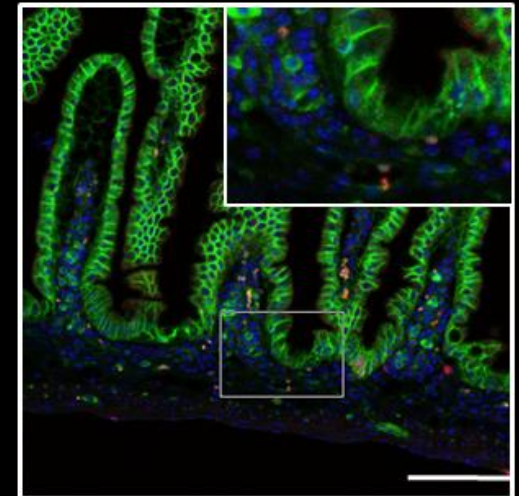
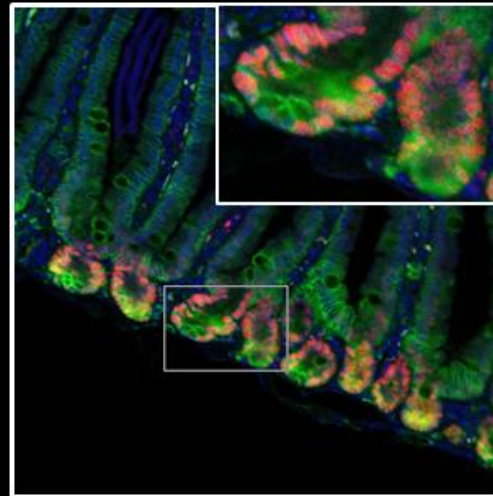
villin-Wls^{CKO} (14 days)

(villinCre^{ERT2}, Wls^{flox/flox})



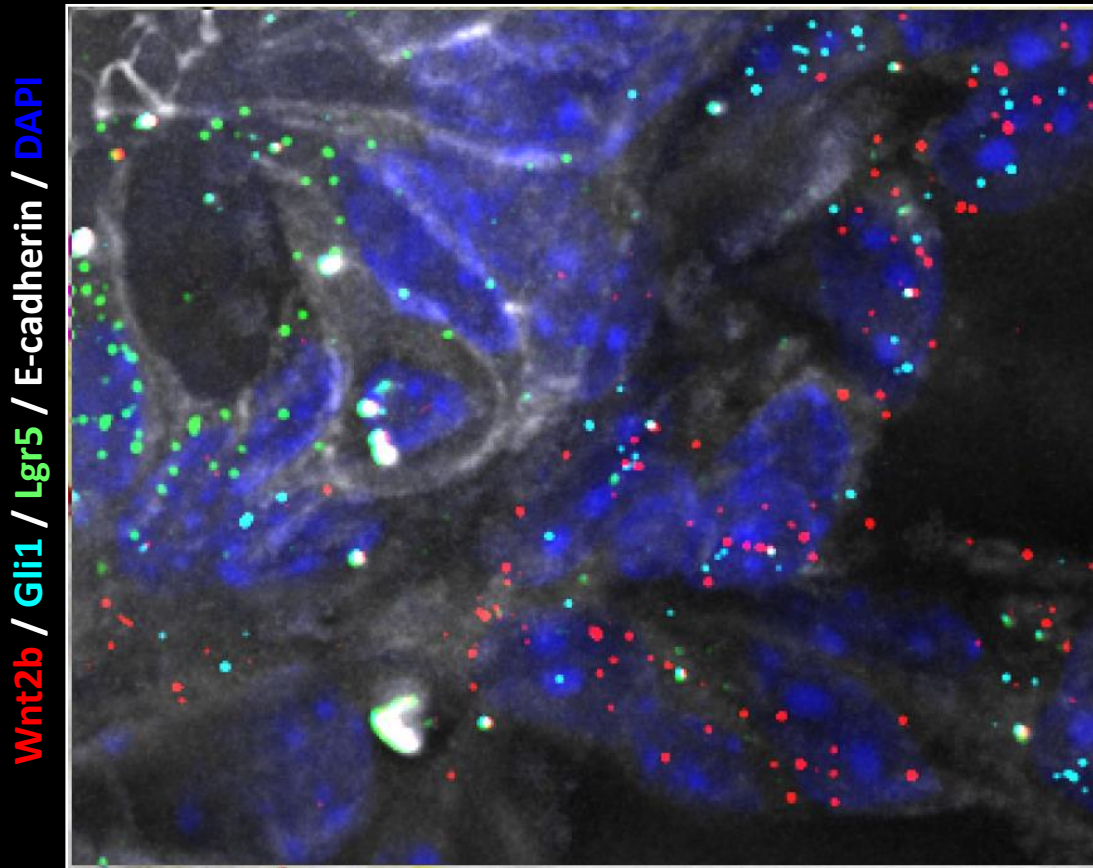
Ki67

β-catenin/DAPI



Gli1⁺ cells express high levels of key mesenchymal ligand Wnt2b

single-molecule(sm) RNA FISH

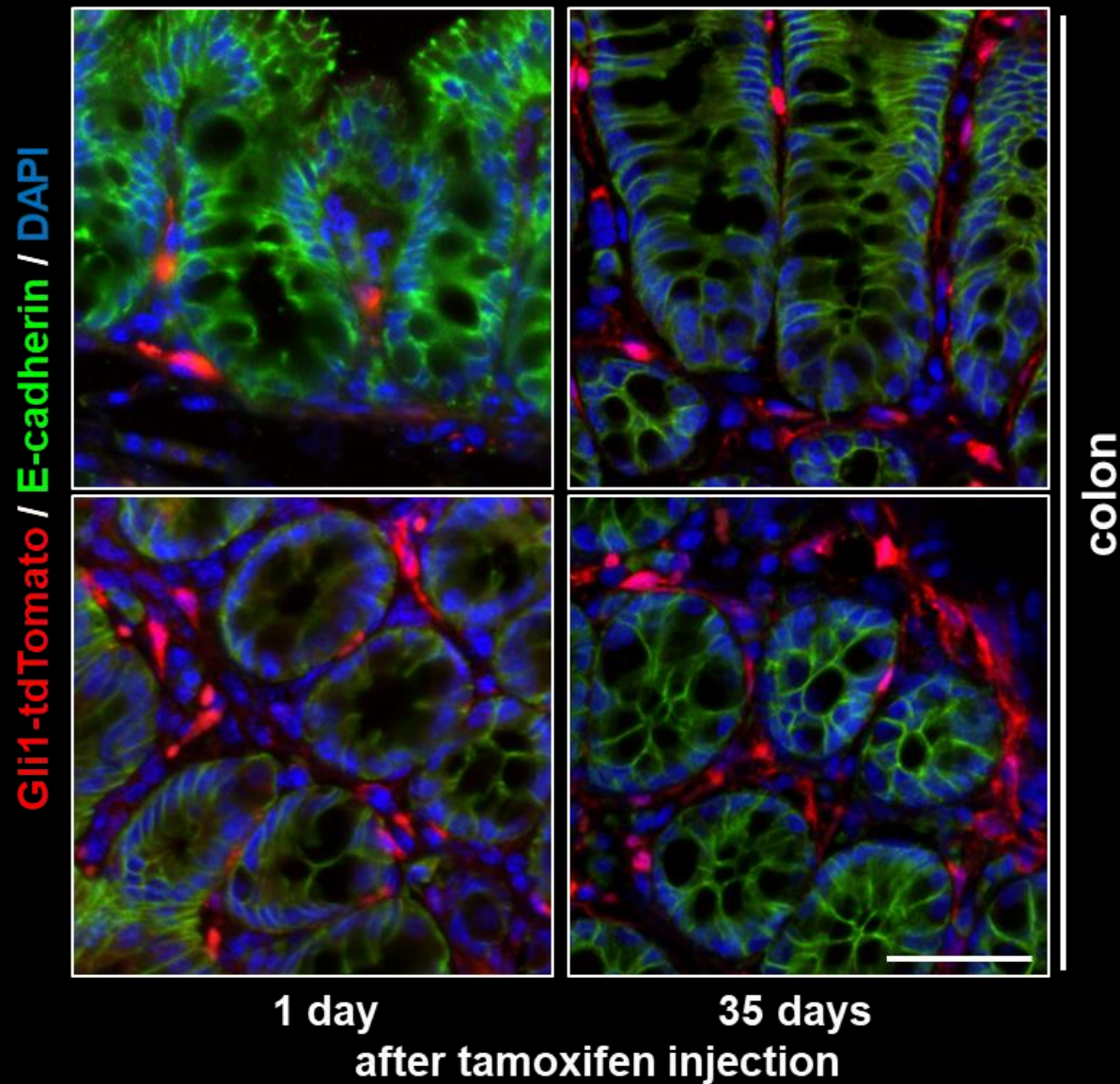


Valenta and Degirmenci et al., Cell Reports (2016)

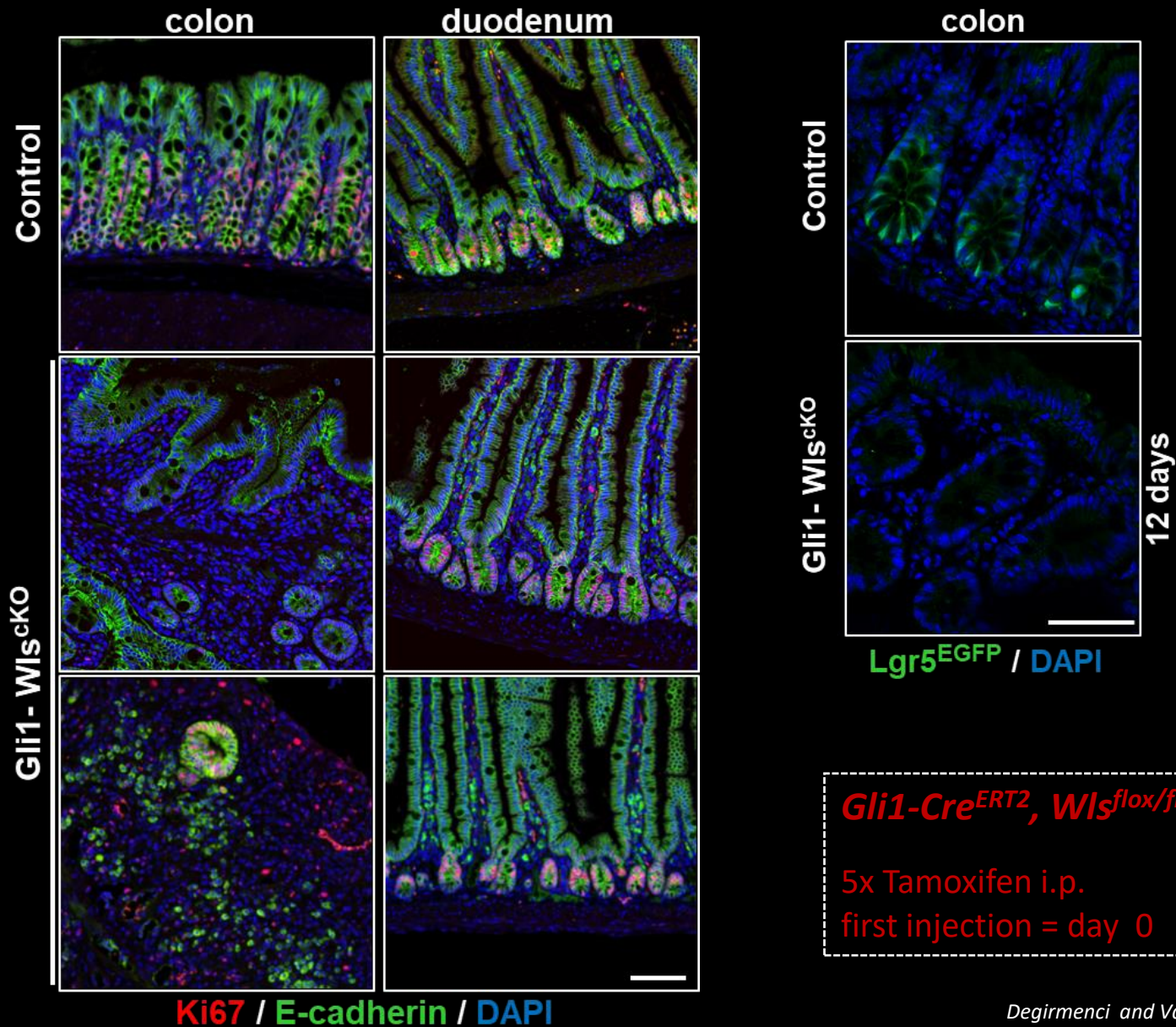
Gli1⁺ Cells

Gli1⁺ cells are long persisting cells *in vivo*

Gli1- Cre^{ERT2}, lox-STOP-lox-tdTomato

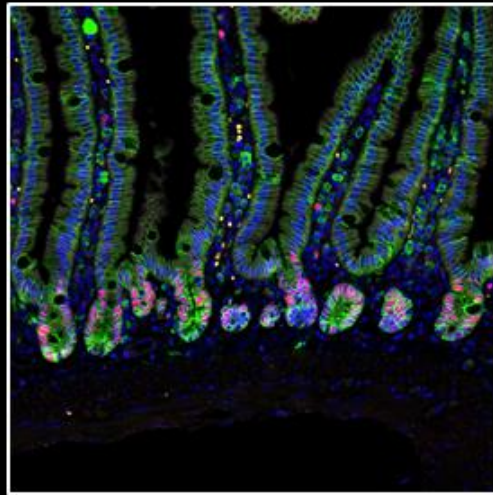


Blocking Wnt-secretion from Gli1⁺ abrogates the renewal of colon epithelium

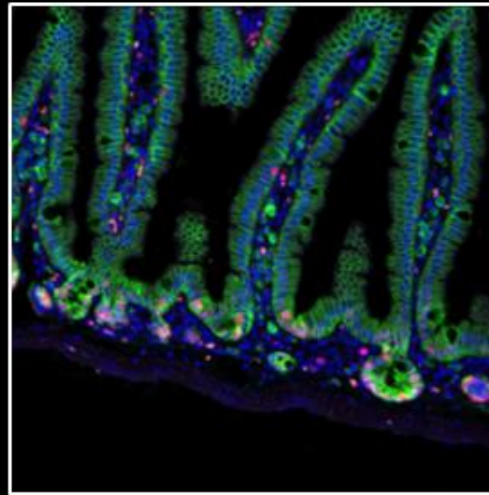


Only simultaneous blocking of Wnt-secretion from both the epithelium and Gli1⁺ cells results in loss of crypts in the small intestine

Control

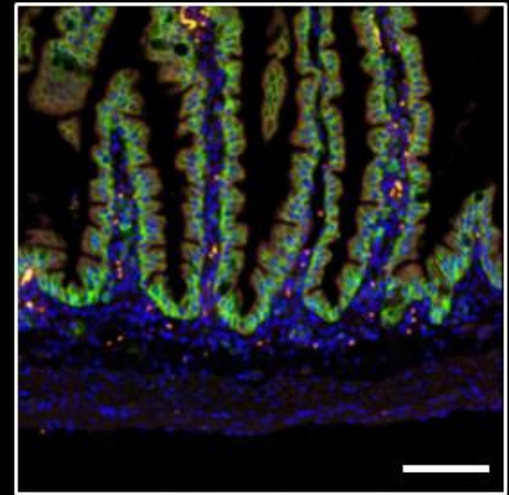


Villin+Gli1- Wls^{cKO}



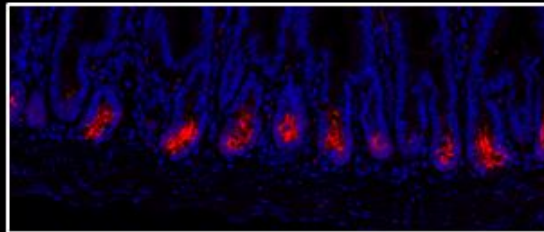
16 days

Villin+Gli1- Wls^{cKO}

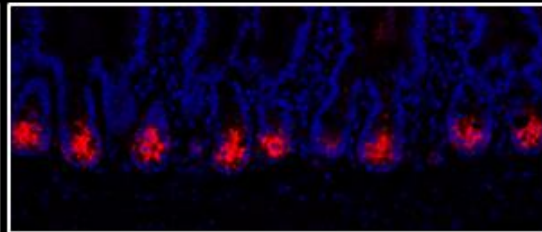


21 days

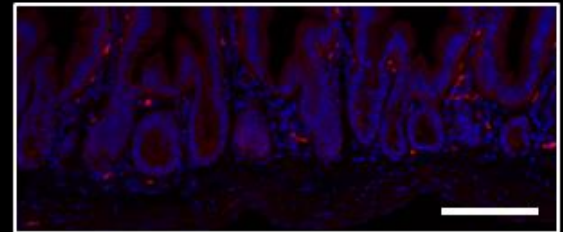
Control



Gli1- Wls^{cKO}



Villin+Gli1- Wls^{cKO}



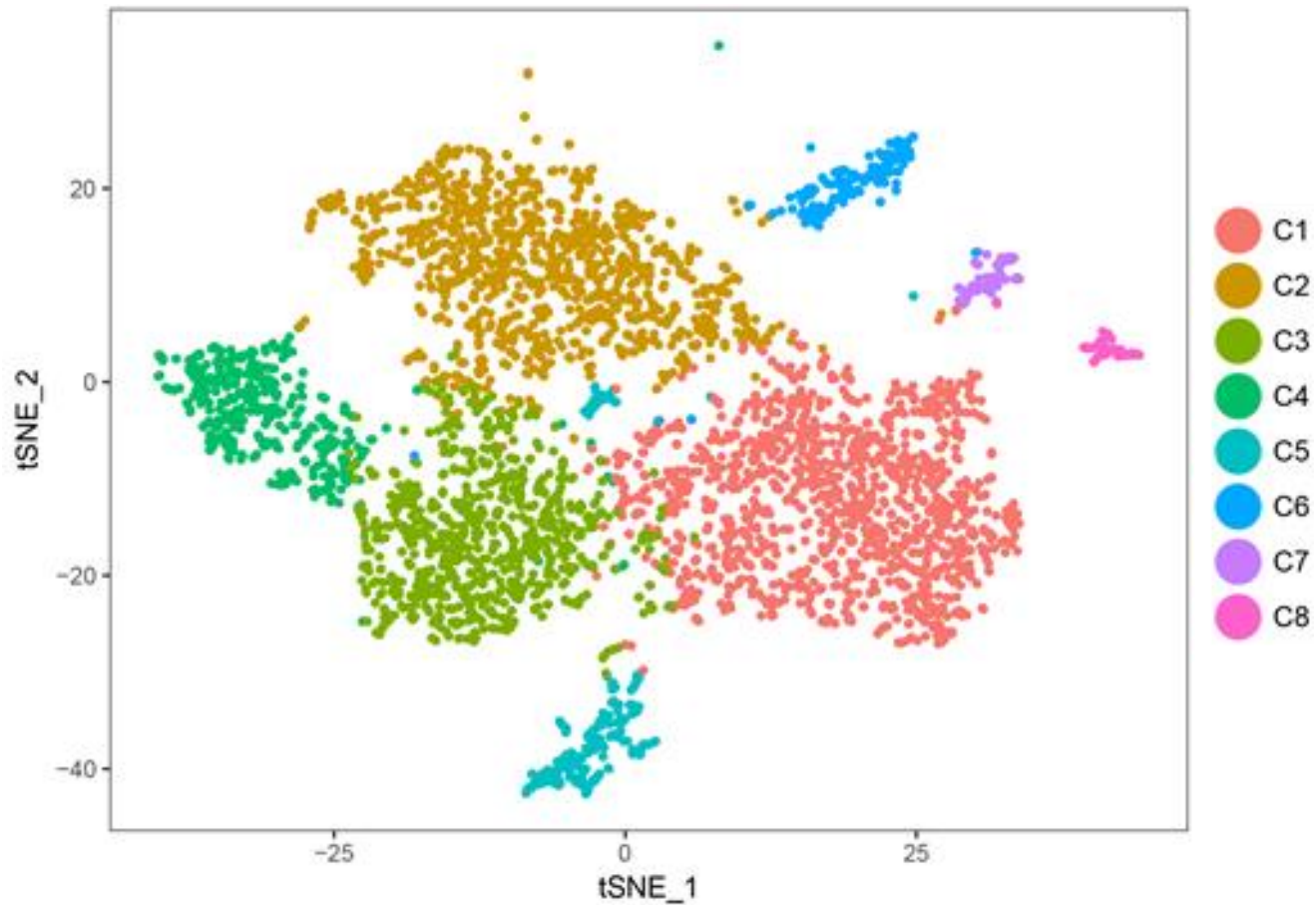
14 days

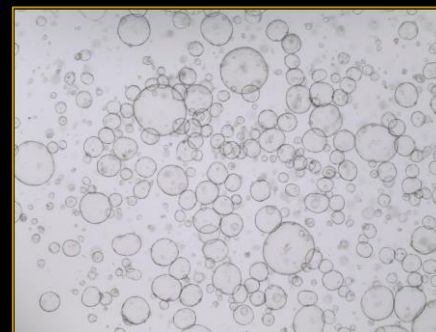
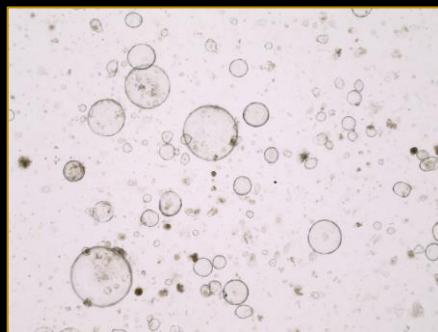
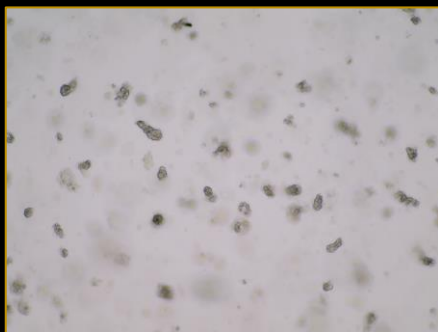
Ki67
E-cadherin / DAPI

Olfm4
DAPI

Gli1⁺ cells constitute a heterogeneous population

single cell RNAsequencing (scRNAseq); unbiased t-SNE clustering analysis



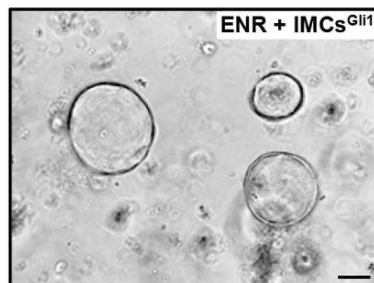
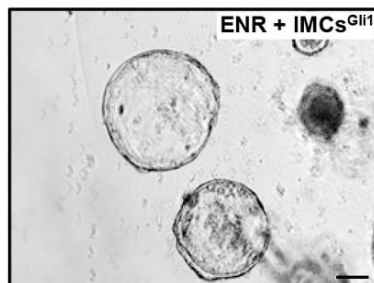
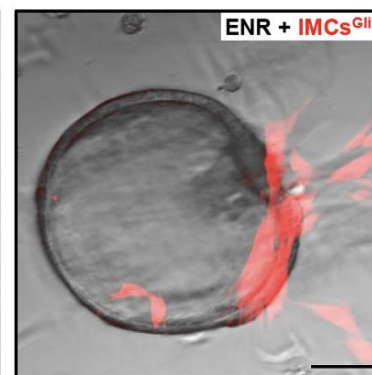
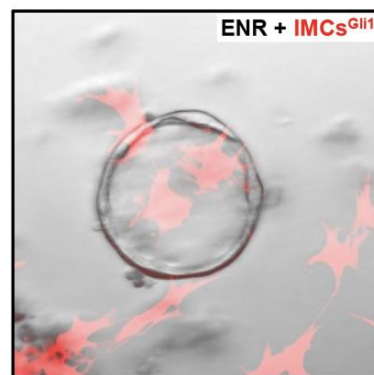
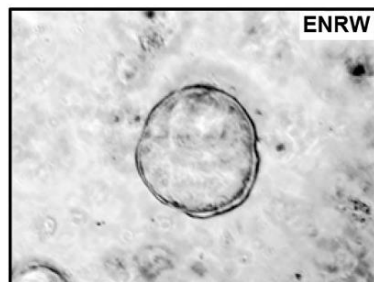
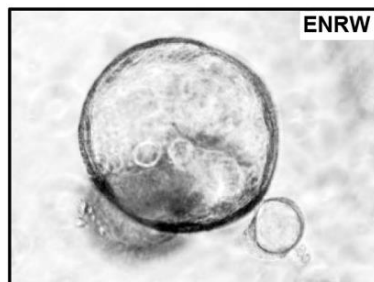
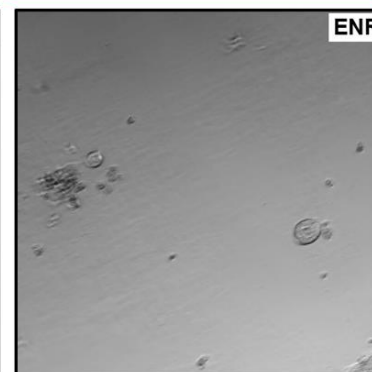
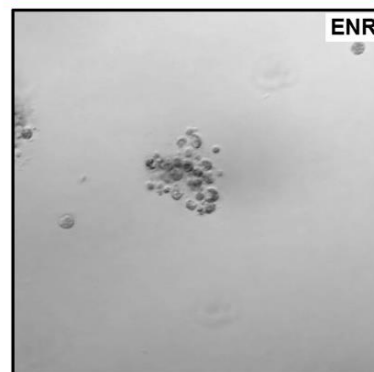
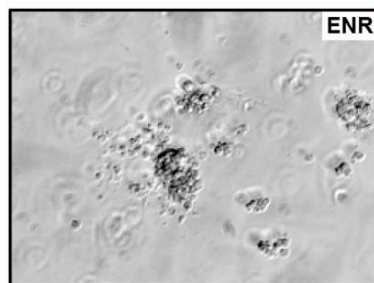
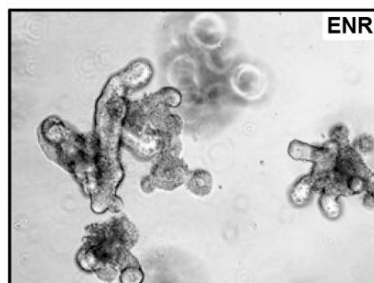


duodenum

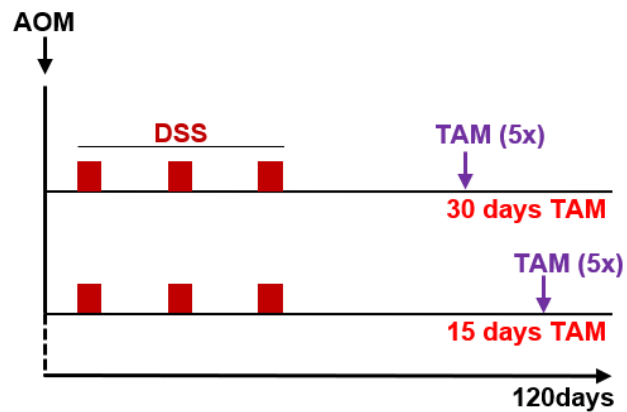
colon

duodenum
Wls^{CKO}

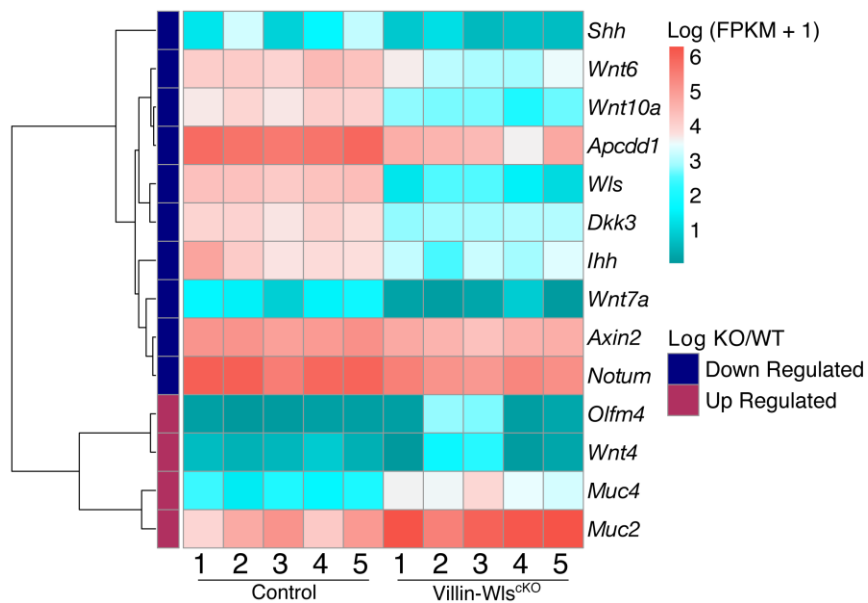
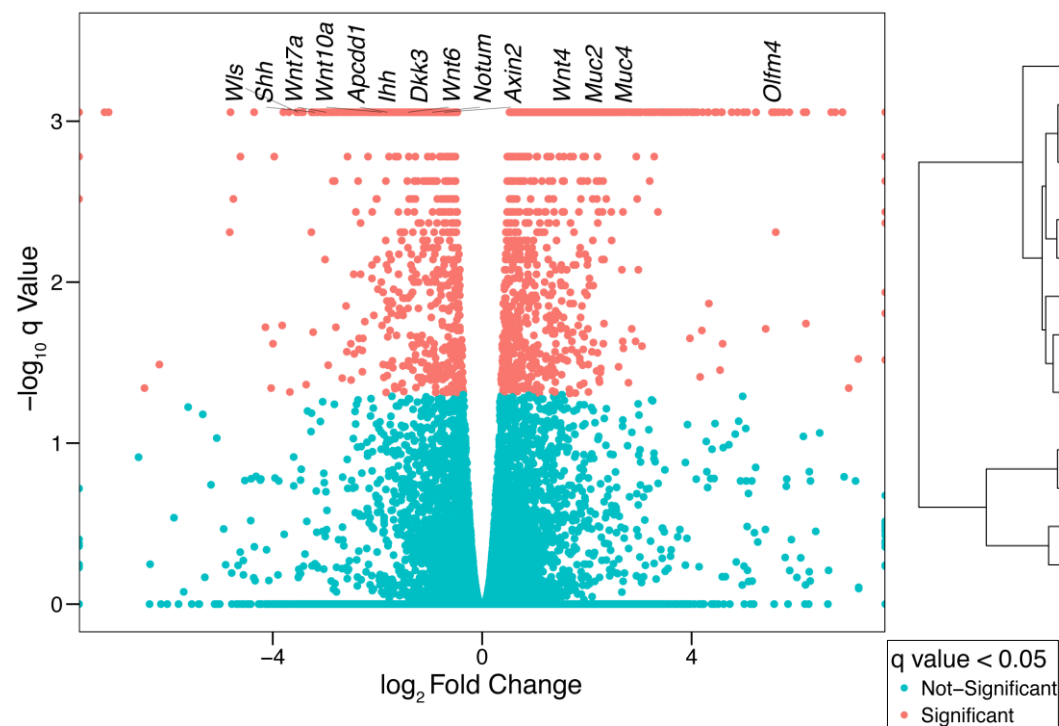
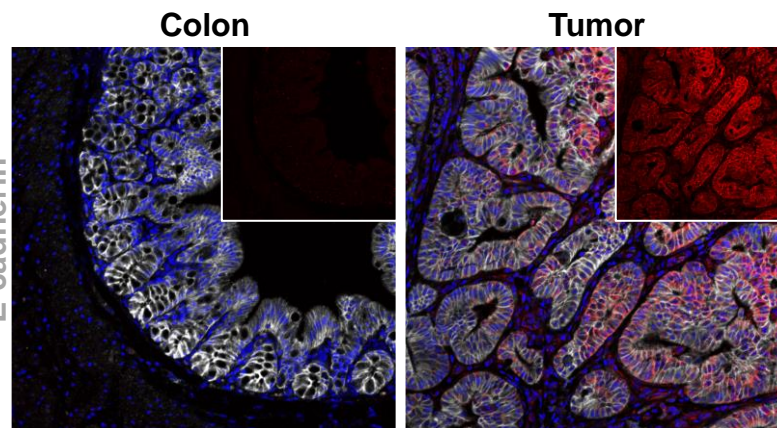
colon

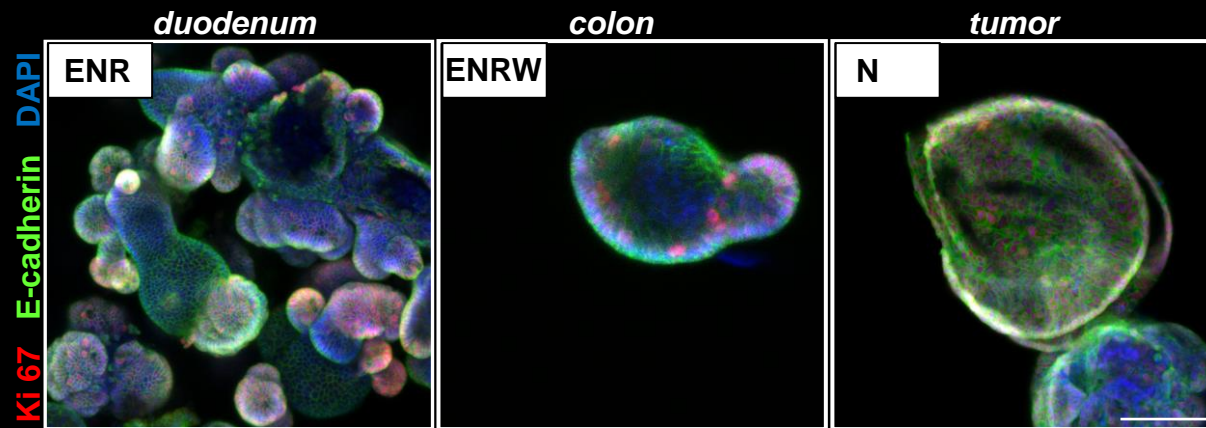


Cancer



WNT 7a/b / DAPI
E-cadherin





Control

+ 4-OHT



Wls^{CKO}

+ 4-OHT



+ WNT3a



Control

Wls^{CKO}



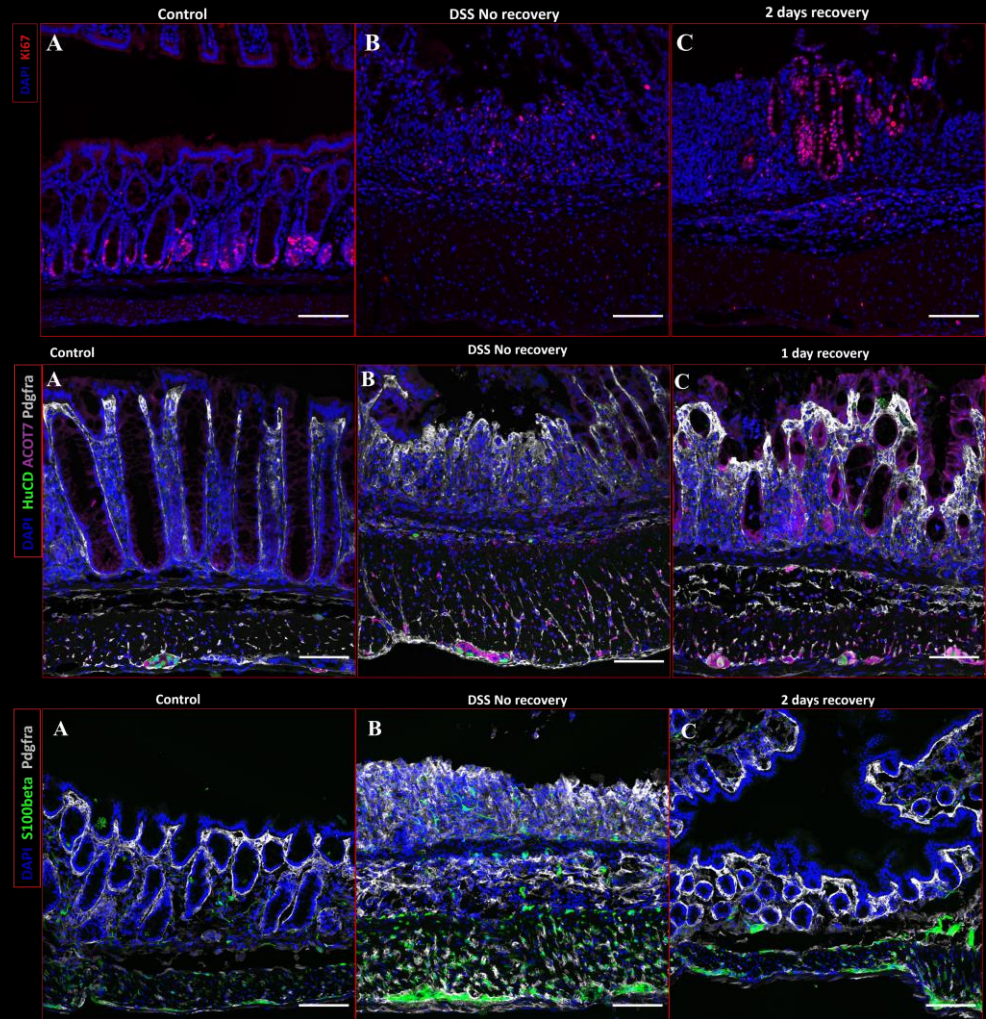
Wls^{flox/flox}

VillinCre^{ERT2}-Wls^{flox/flox}

Colitis

Colonic Activity under DSS Induced Colitis

- DSS acts specifically on the epithelial layer of colon leading to the destruction of crypts.
- Proliferative cells were observed to be decreased in damaged layer of colon with colitis while Ki67 expression was observed to be increased in newly generated crypts.
- DSS induction caused an elevated level of PDGFRA expression along with vertical elongation of signal in myenteric plexus layer towards submucosal plexus.
- ENS cells and PDGFRA+ cells were observed to be extended horizontally in myenteric layer in parallel with upwards localization profiles from ganglia to submucosa.

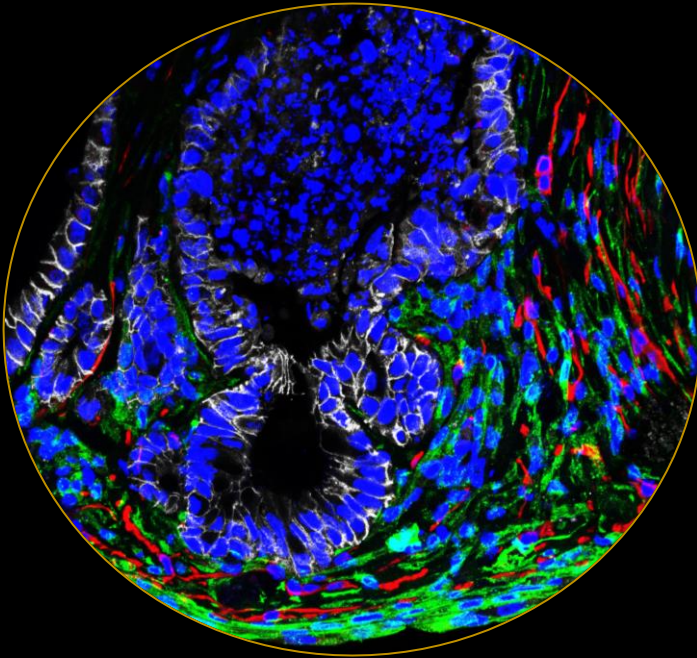


Scale bar 150 um

“we developed innovative approach to successfully generate gut assembloids that recapitulate key aspects of intestinal physiology and mimic the complex cellular interactions and architecture of the native colon”

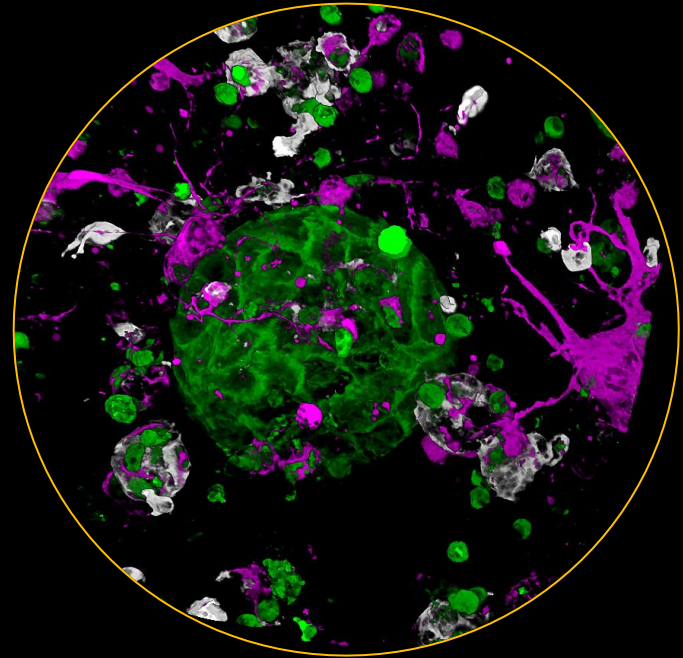
ASSEMBLOID

DAPI Tuj1 Pdgfra E-Cadherin

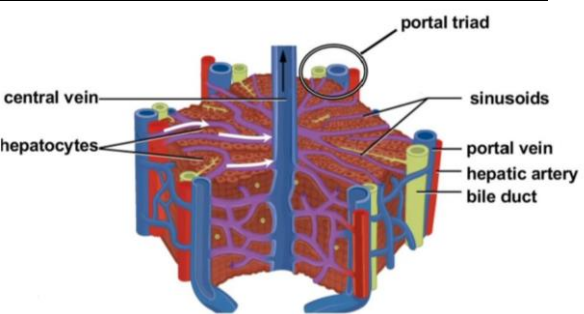


CO-CULTURE

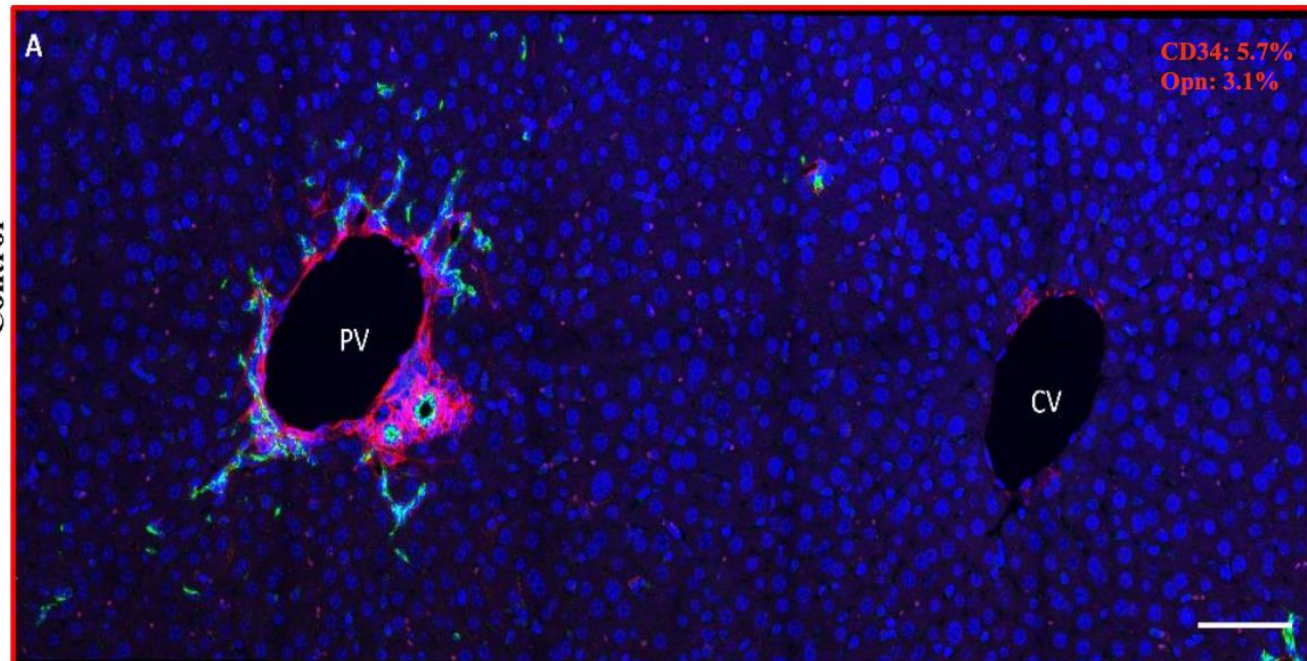
DAPI Tuj1 Pdgfra E-Cadherin



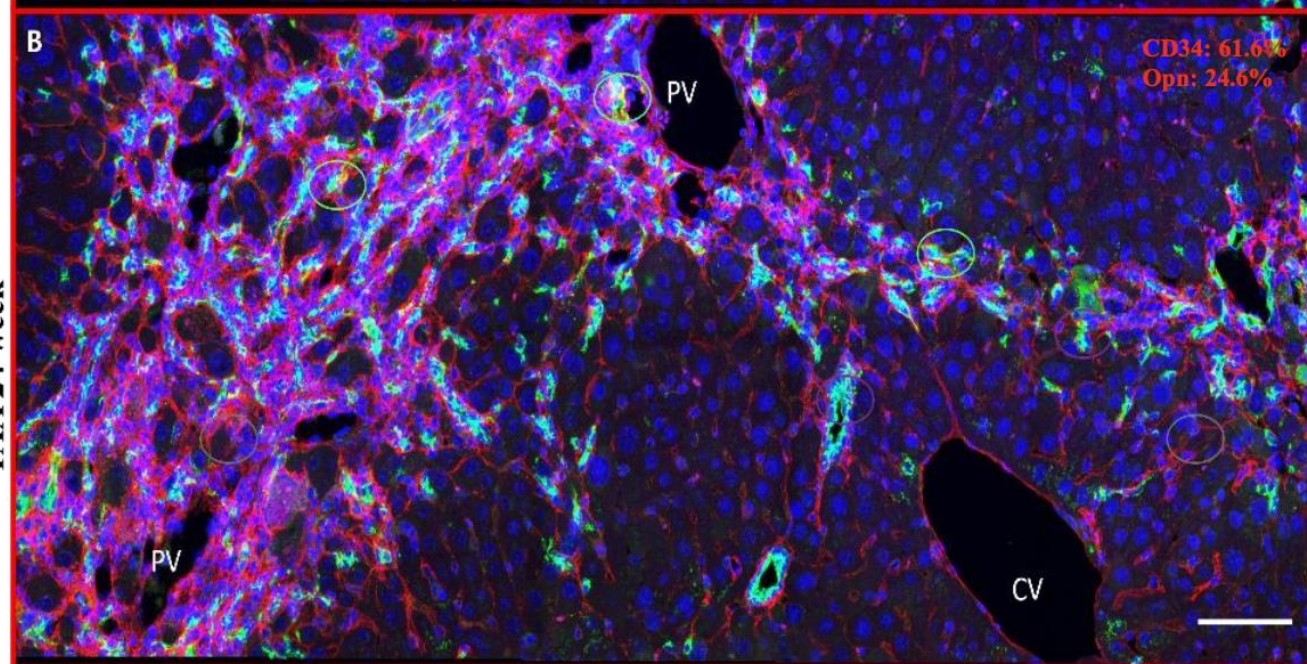
Liver Fibrosis



Control



TAA 24 week

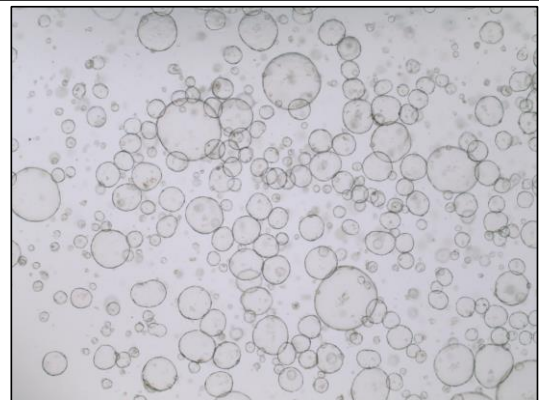
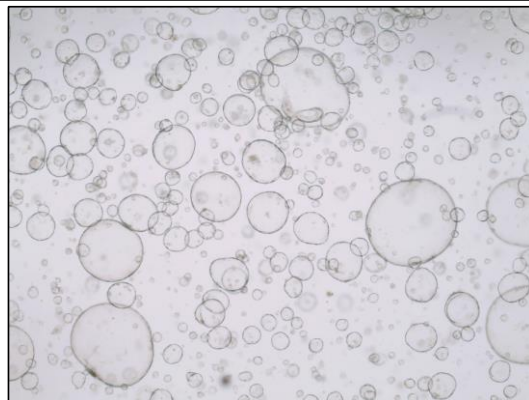
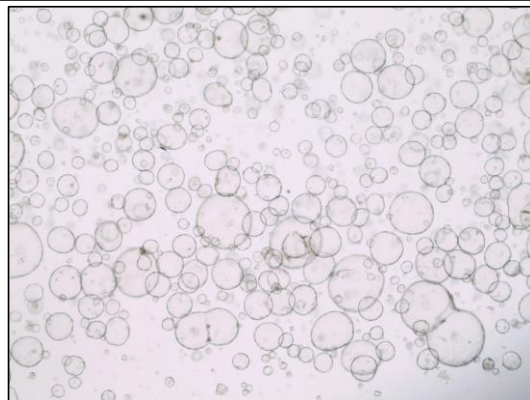
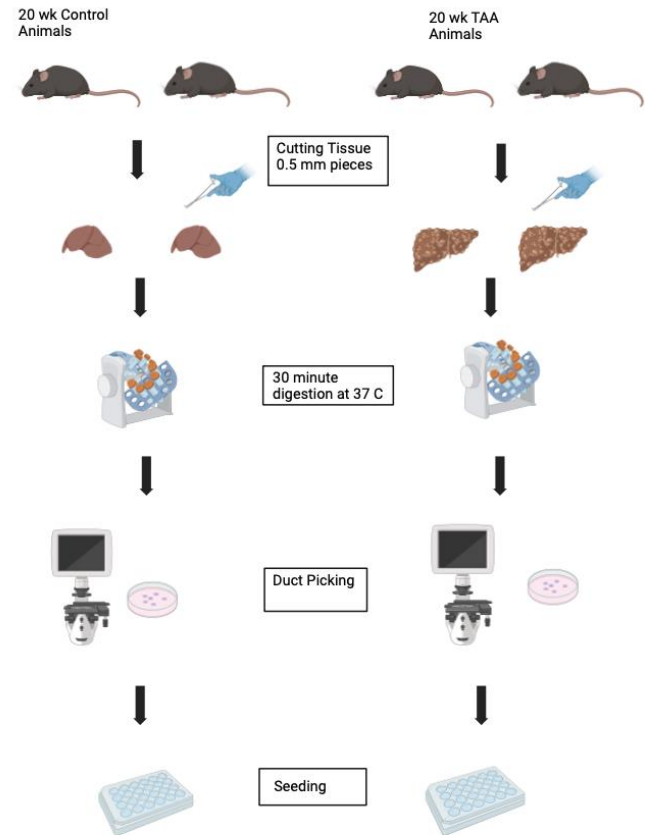


DAPI CD34 OPN

Unpublished

Experimental Setup

- All digestions were performed by using Collagenase VI (1 mg/mL), Dispase II (1U/mL), DNaseI (0.1 mg/mL). Digestion duration was 30 min for all isolations.
- In all isolations ducts were seeded as follows: 1 well to be cultured with hepatocyte media, 1 well to be cultured with expansion media)



Our goal is to transplant a functional gastrointestinal tract into a patient- BD LAB

BD LAB

BILKENT UNIVERSITY

İlke Sari

Beliz Uzun

Nurmuhammet Satlykov

Melda Oguz

UNIVERSITY OF ZURICH

Konrad Basler

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George Hausmann

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Fatima Aerts-Kaya and Lab Members

Petek Korkusuz and Lab Members

KOÇ UNIVERSITY

Nurcan Tuncbag

Cansu Dincer

Cansu Demirel