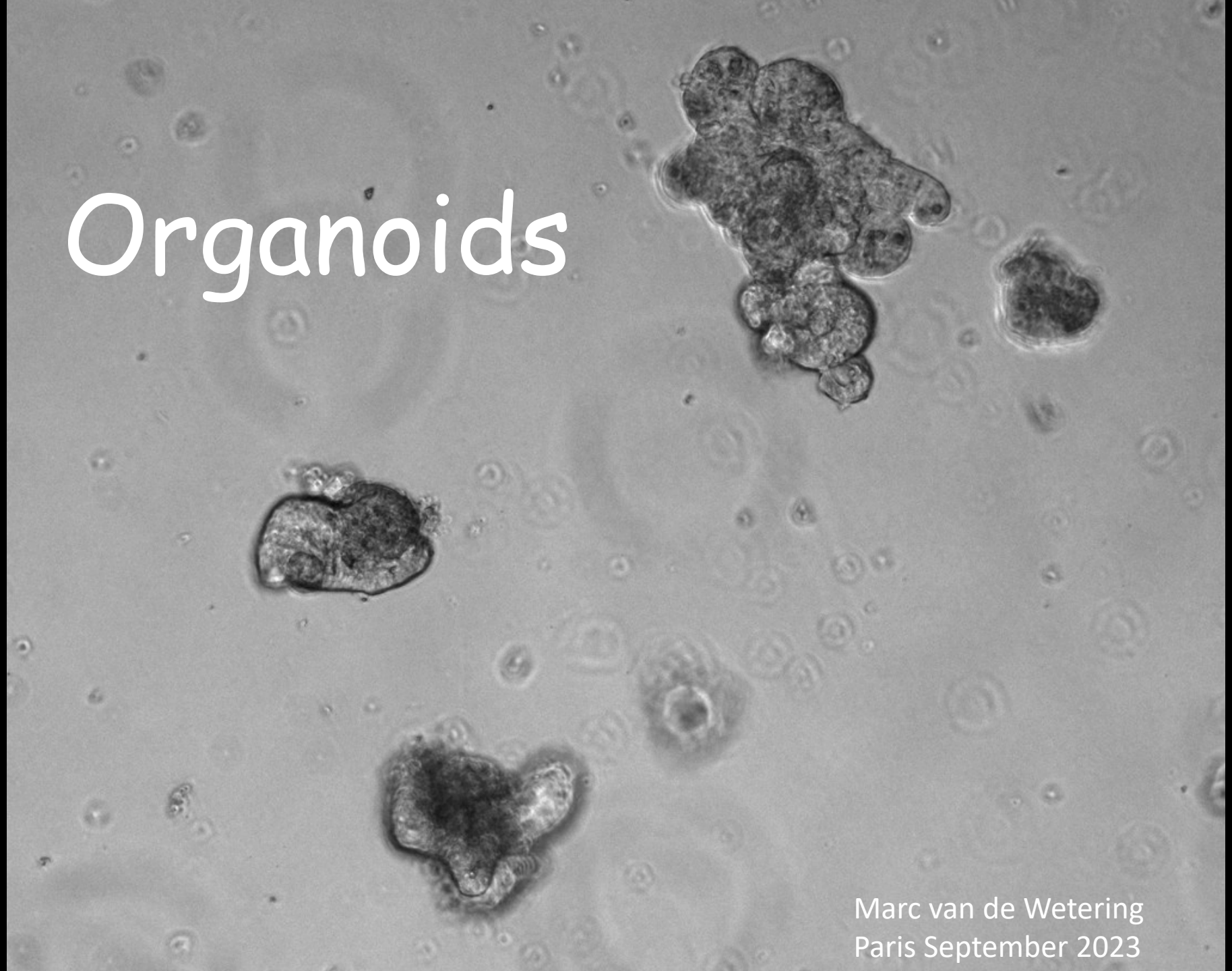
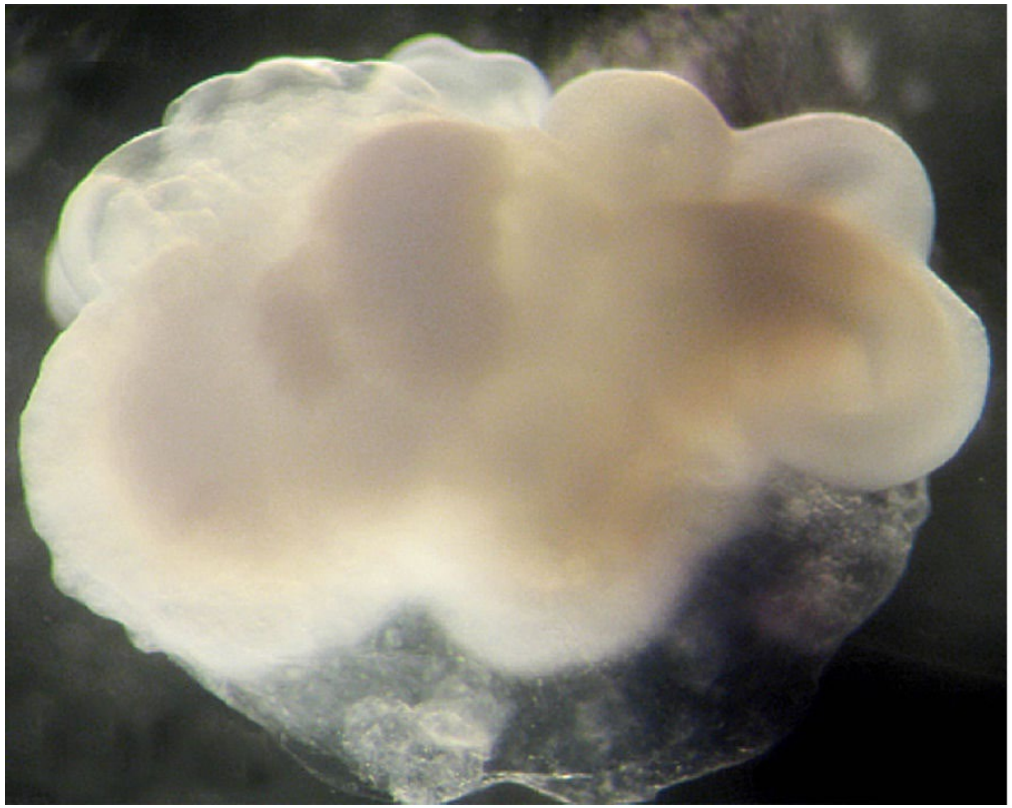
Organoids



Marc van de Wetering
Paris September 2023

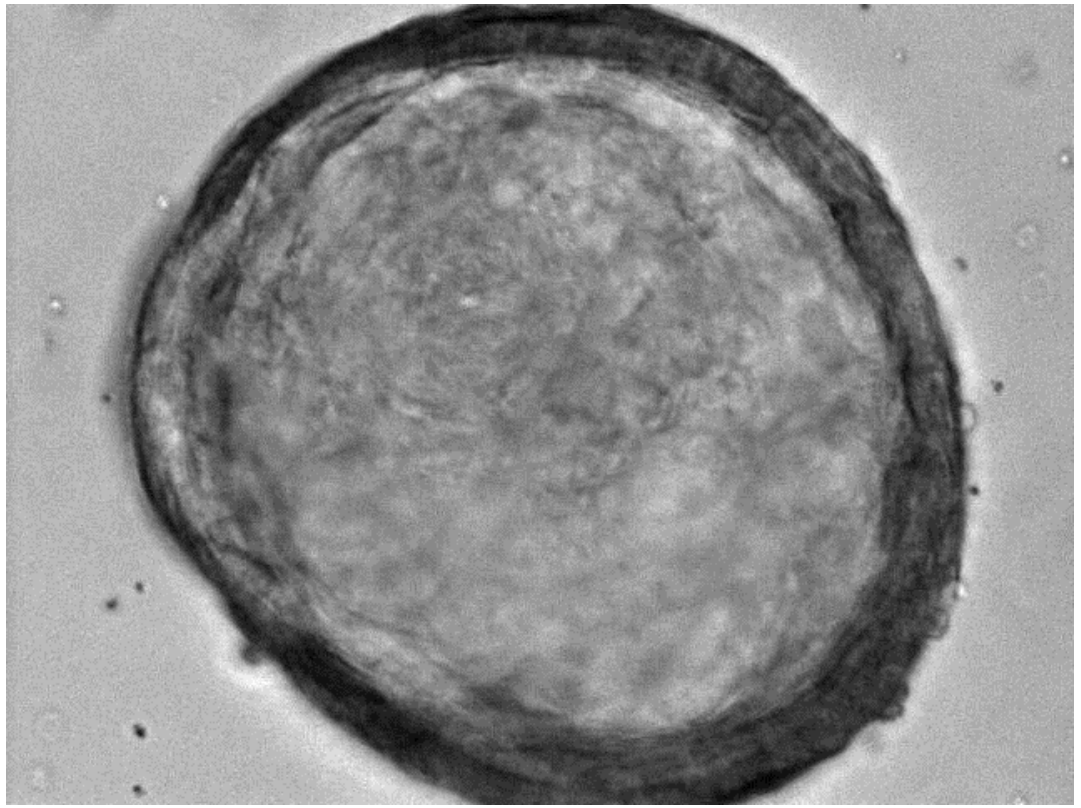
There are two (now three!!) different types of organoids

Embryonic stem cell derived organoids

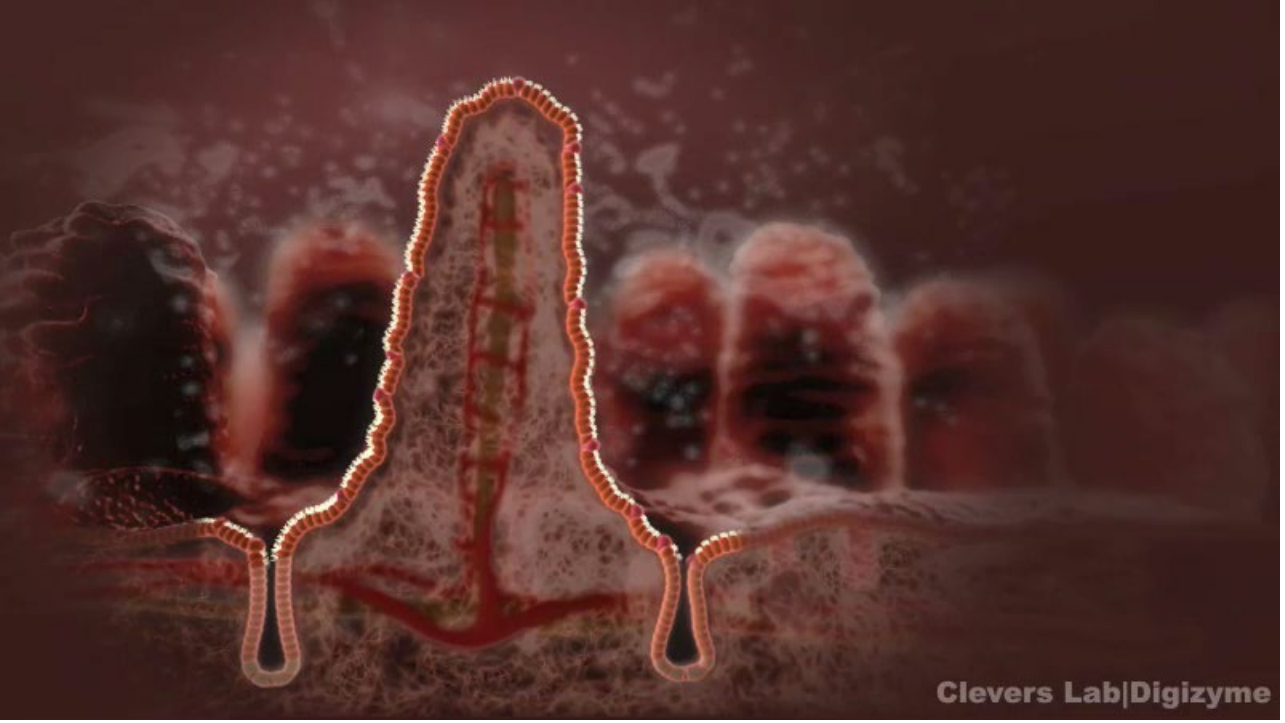


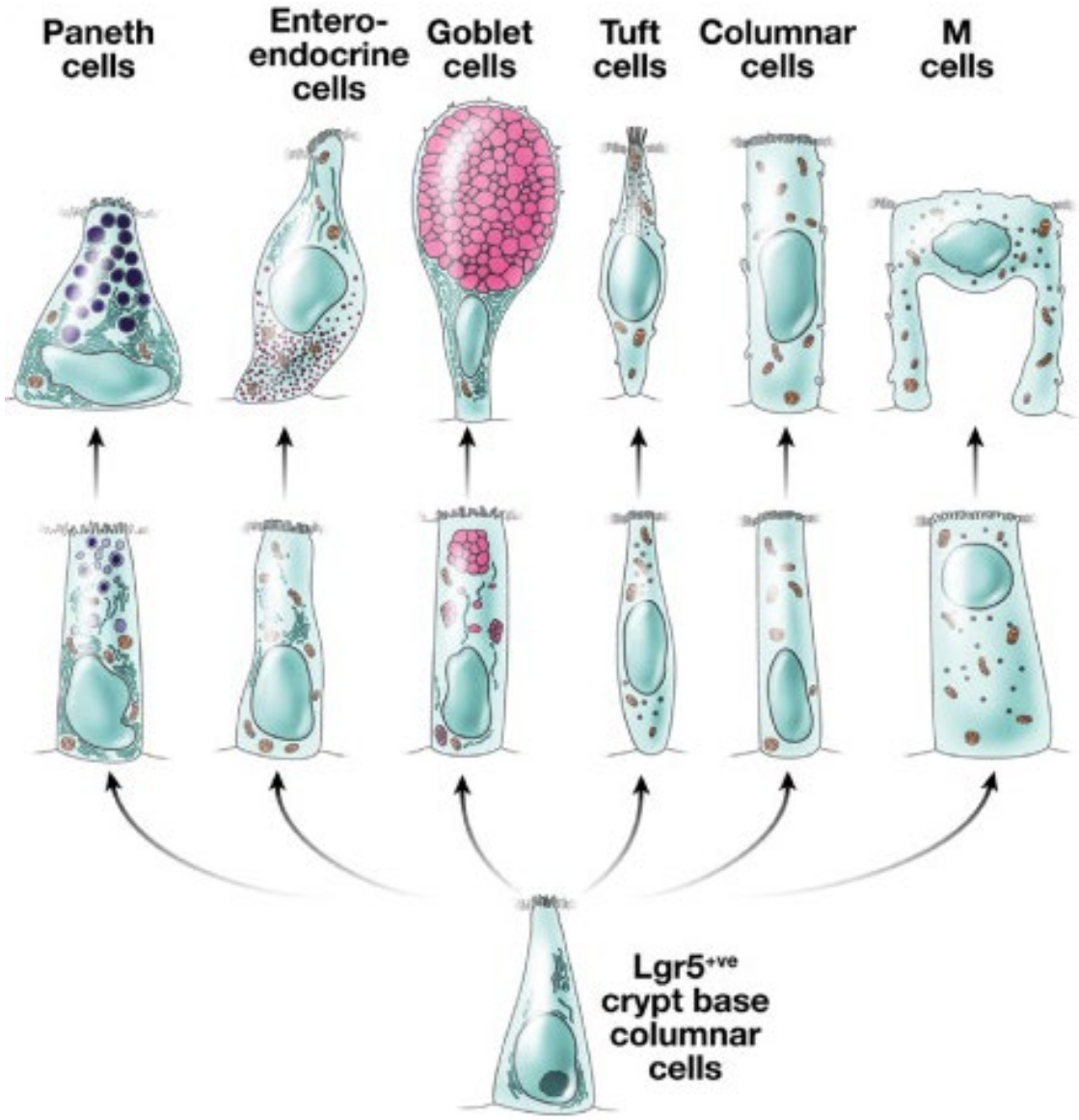
Directed development towards tissue of interest

Somatic stem cell derived organoids



Growth factor cocktails maintain adult stem cell activity





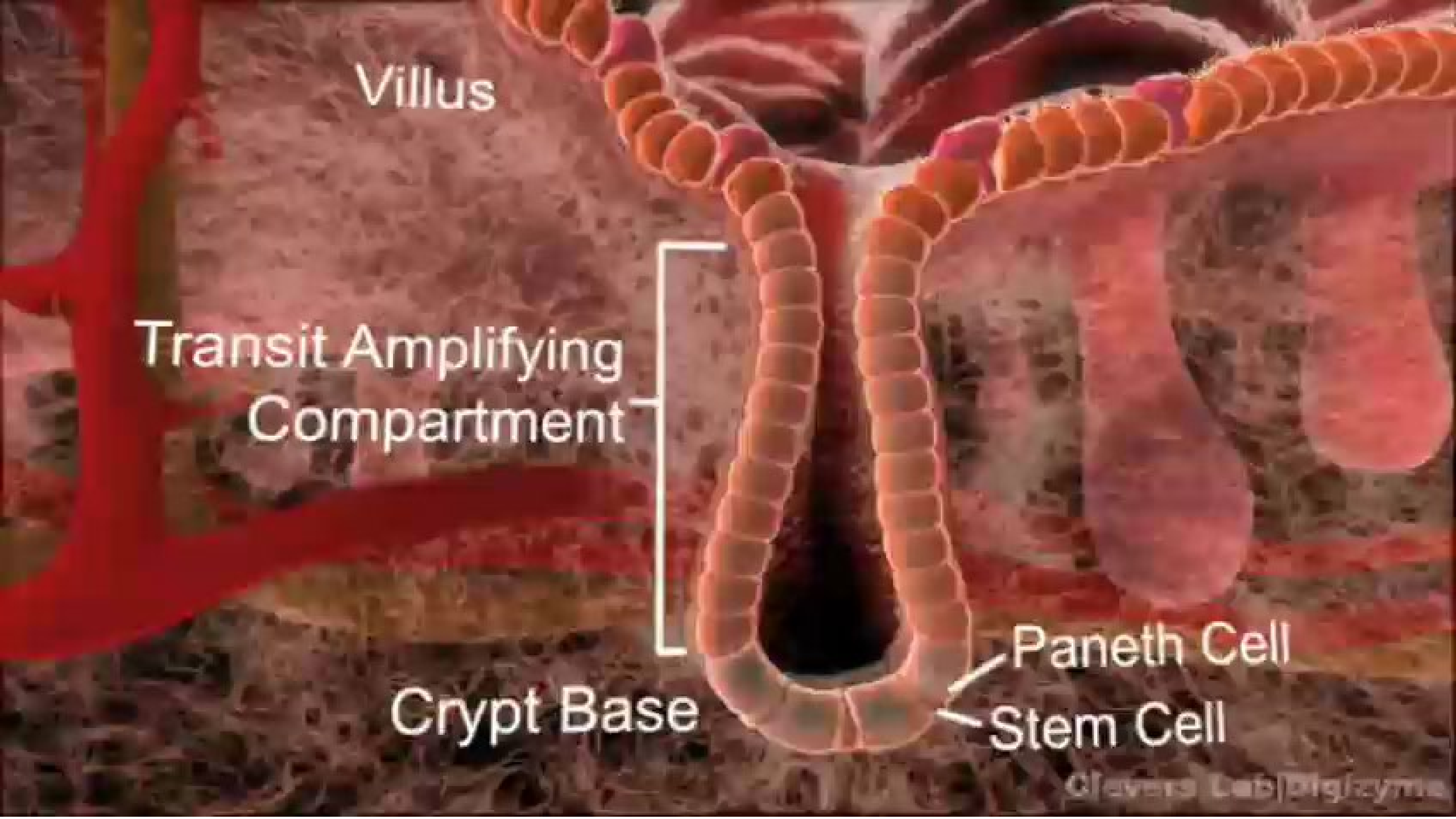
-Intestinal stem cells are not quiescent

-They are not rare: up to 5% of crypt cells

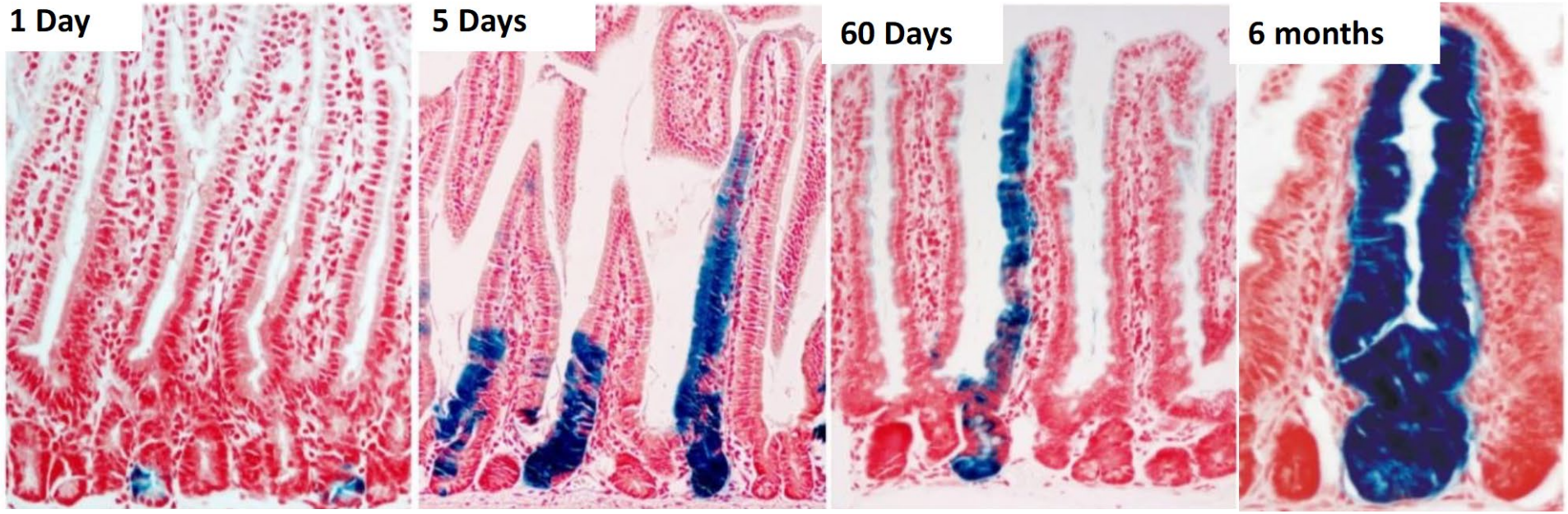
-They do not divide asymmetrically

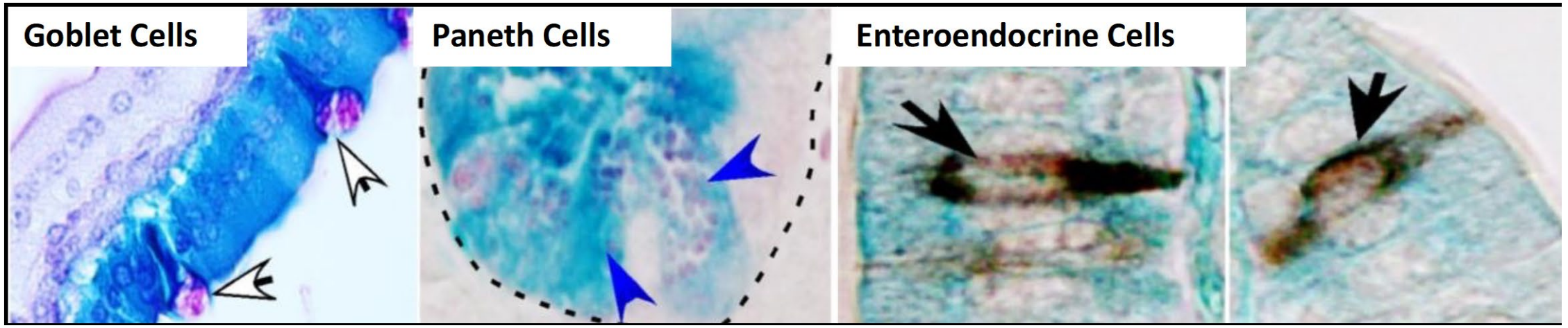
-Stem cells create their own niche cells

-The stem cell hierarchy is not uni-directional


Villus
Transit Amplifying Compartment
Crypt Base
Paneth Cell
Stem Cell

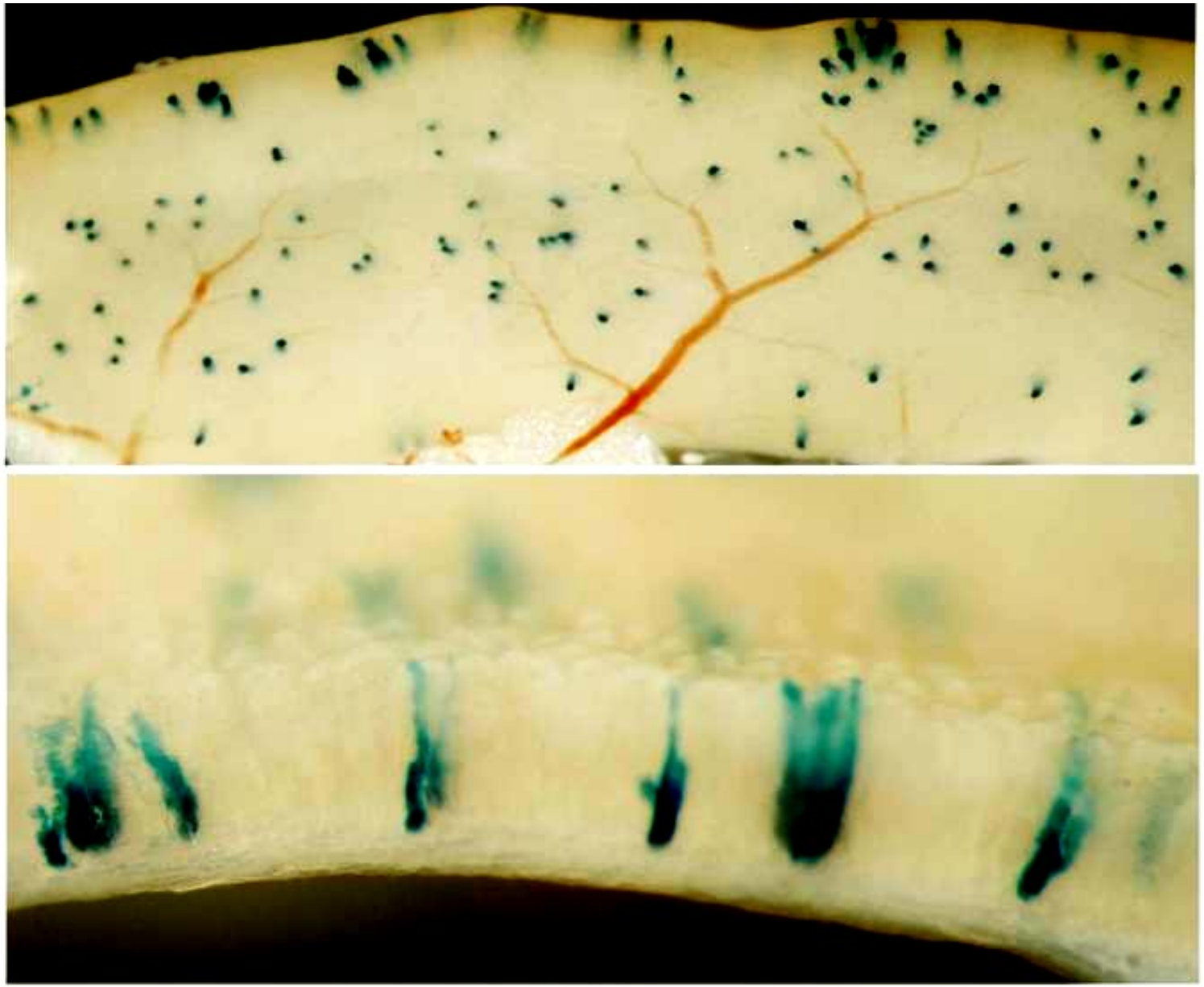
Lgr5 Cells are the Multipotent Stem Cells of the Small Intestine



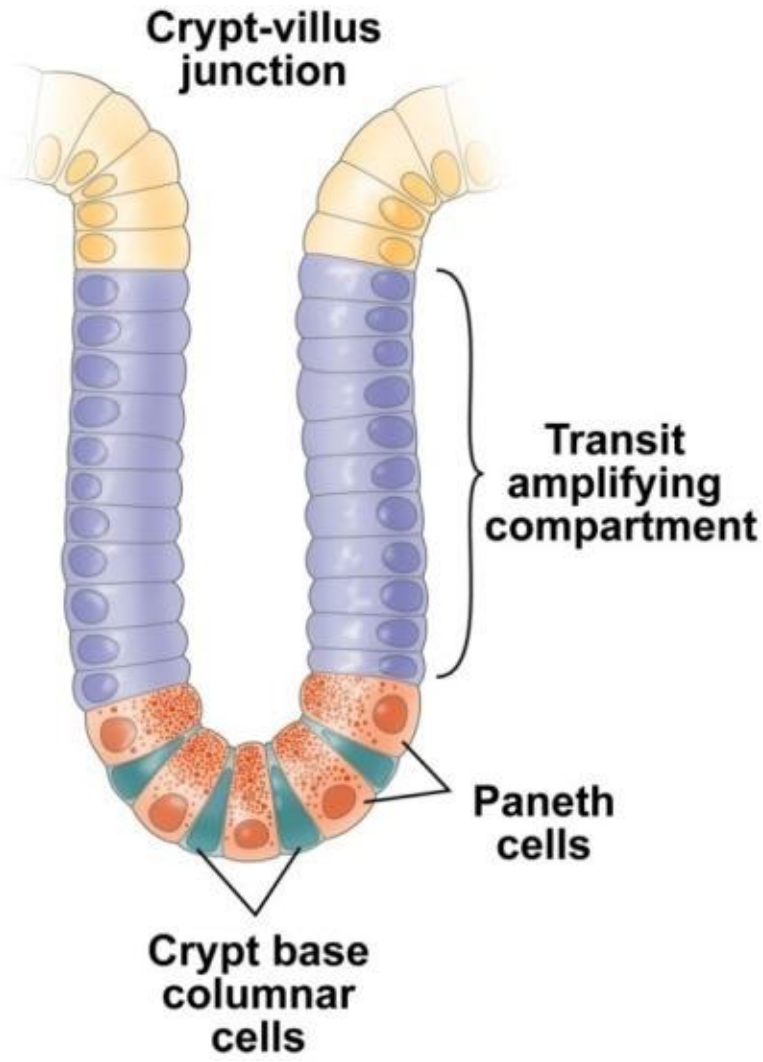


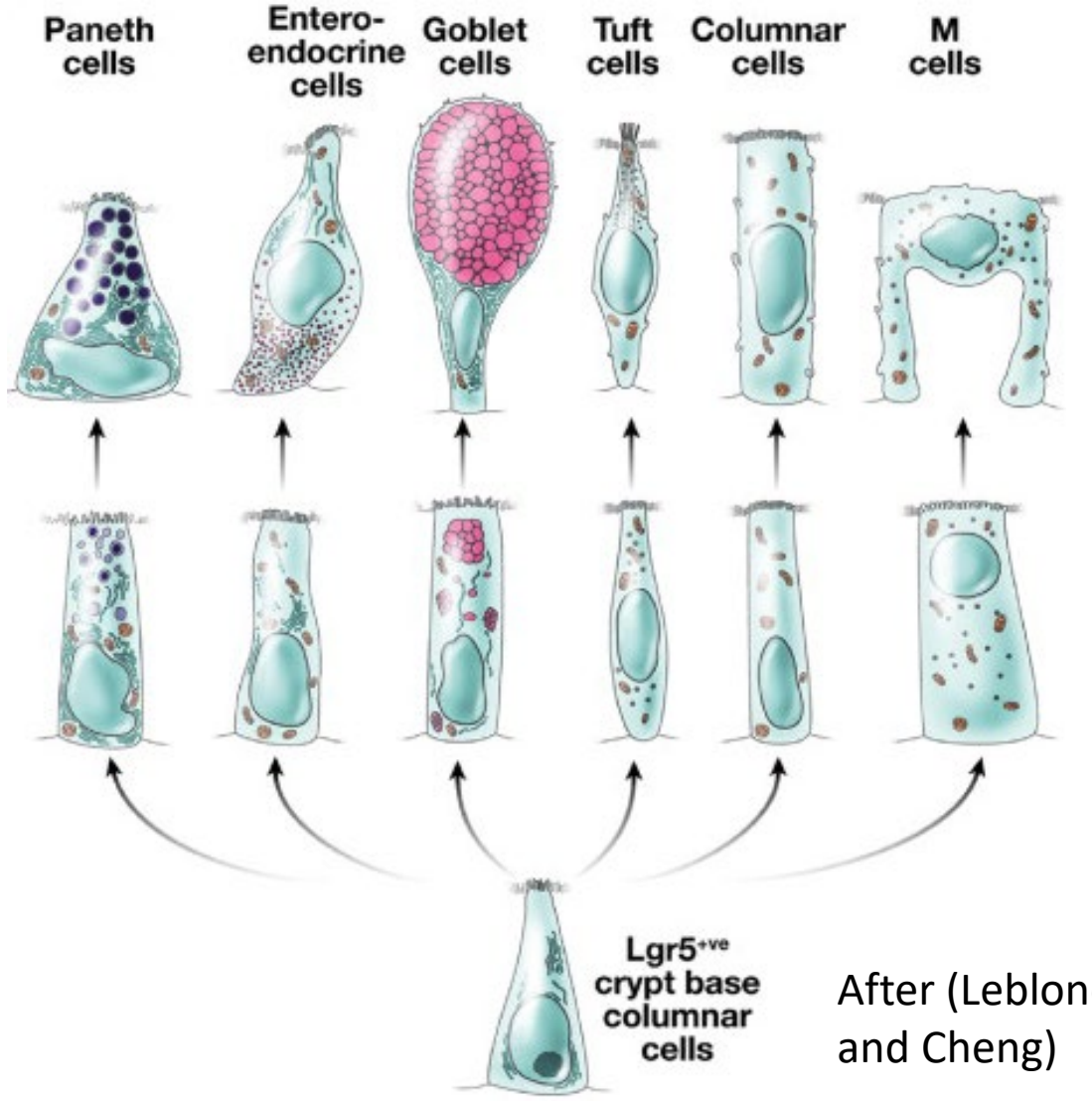
Barker et al., 2007

20 months later..



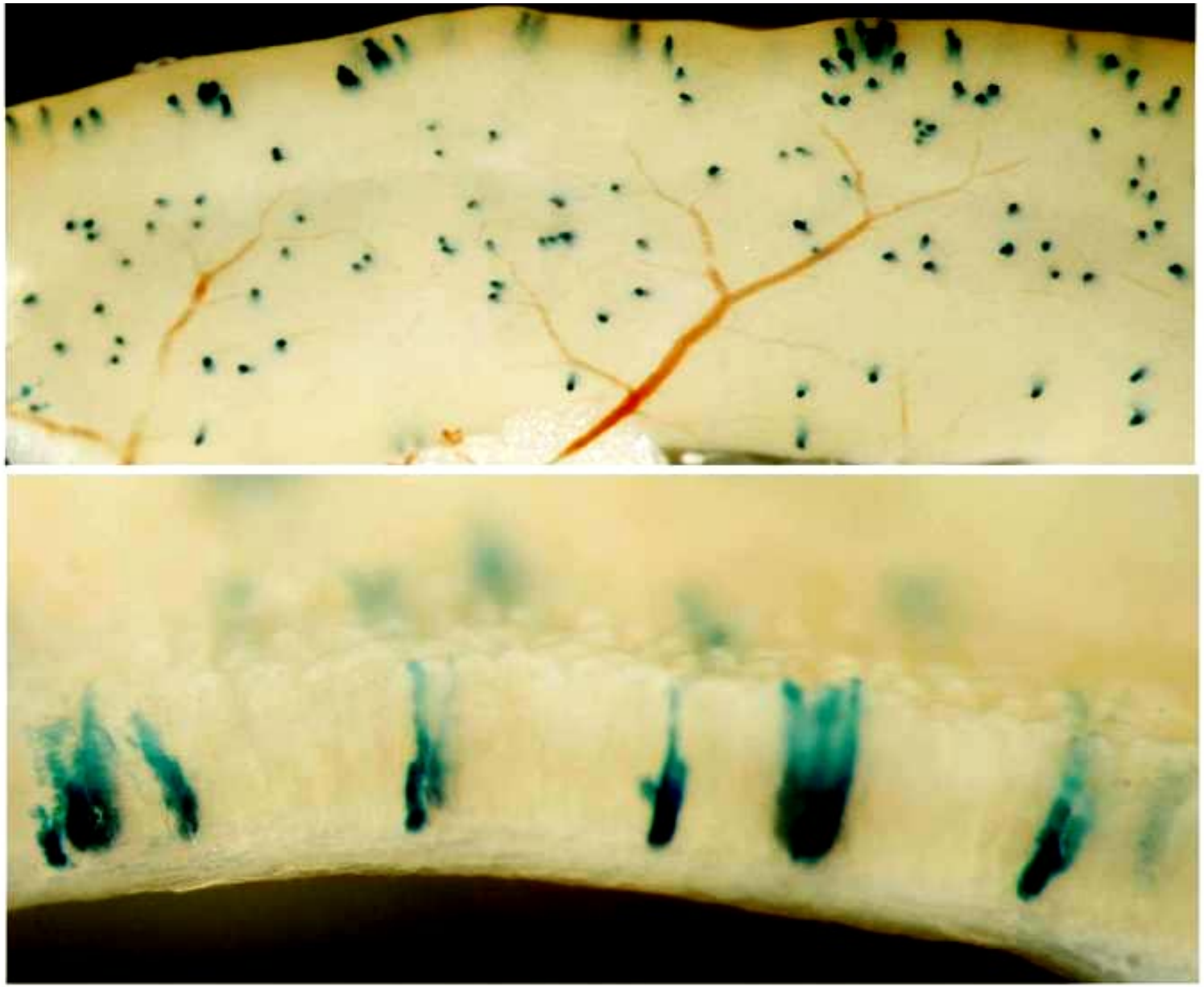
Crypt Base Columnar cells are Intestinal Stem Cells



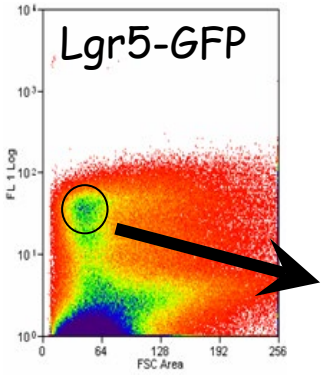


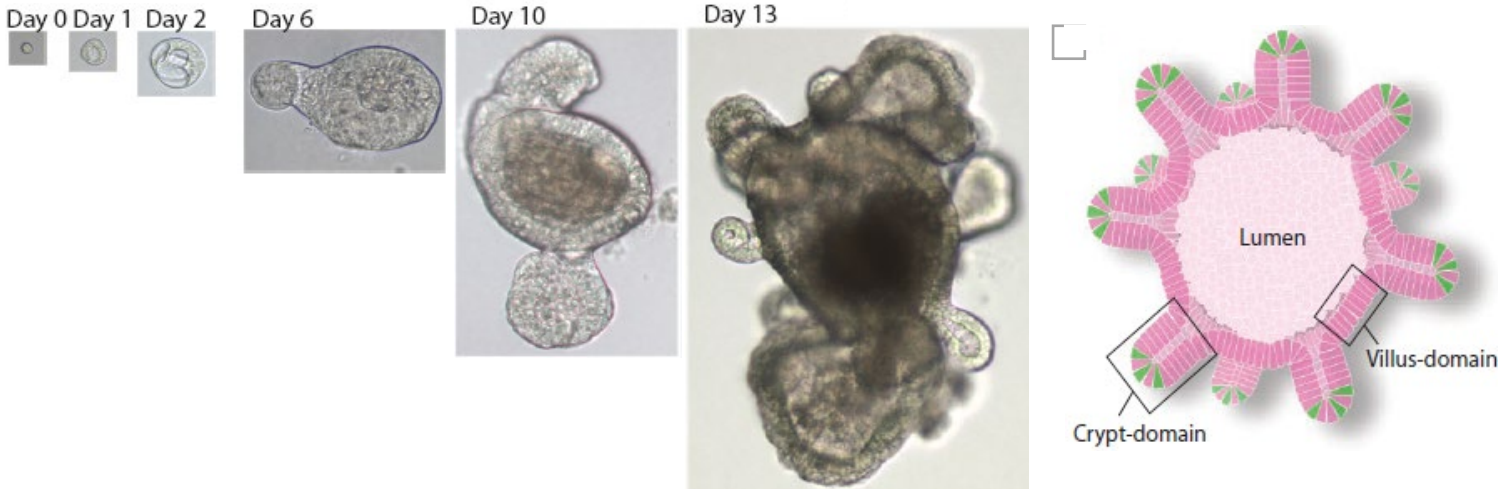
After (Leblond, Bjerknes and Cheng)

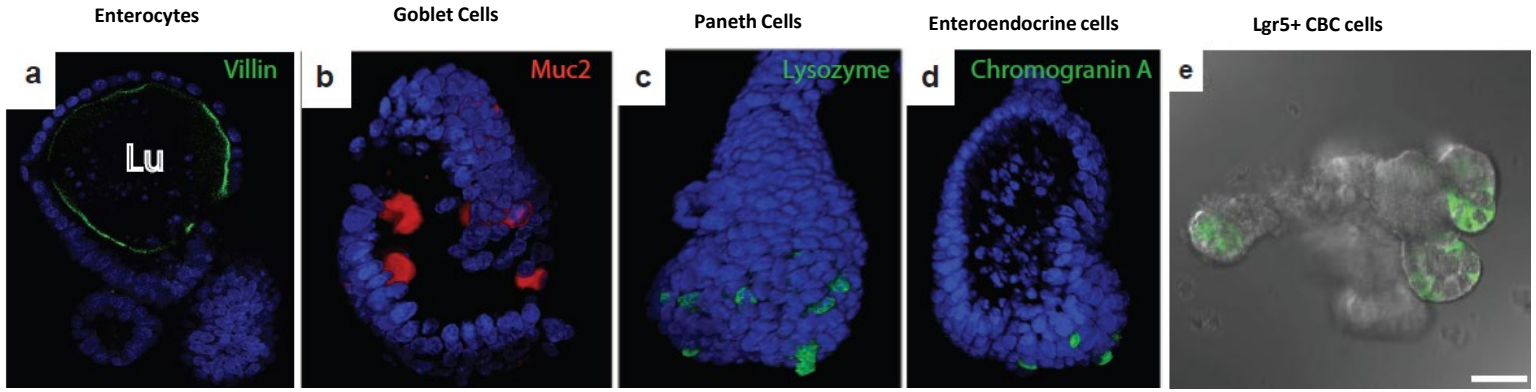
20 months later..



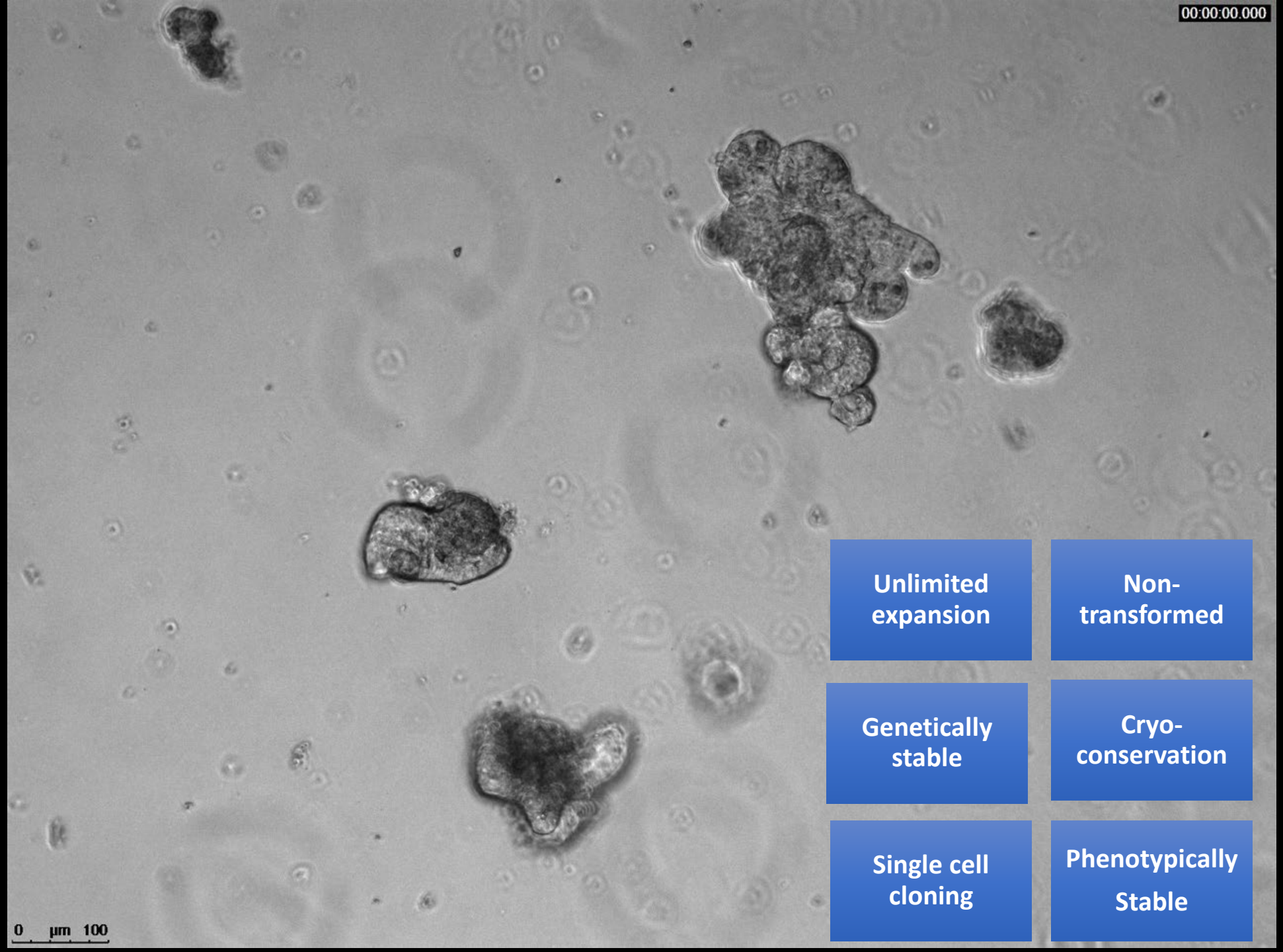
In-Vitro Intestinal Organoids Resemble *In-Vivo* Crypt-Villus Units



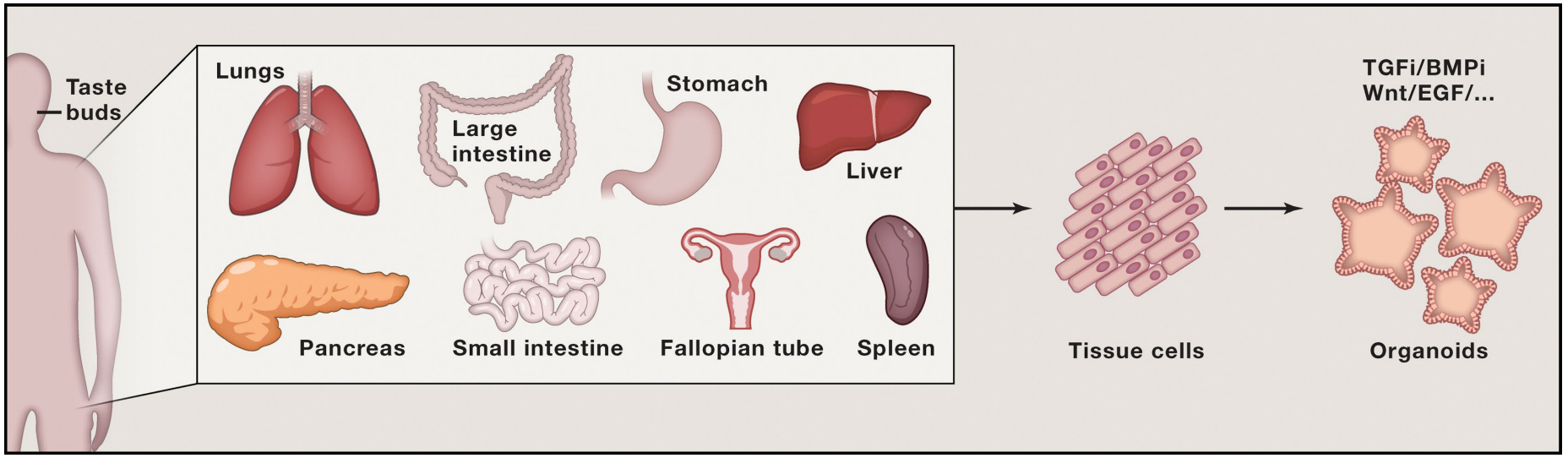




Toshiro Sato, 2009


00:00:00.000
Unlimited expansion
Non-transformed
Genetically stable
Cryo-conservation
Single cell cloning
Phenotypically Stable
0 µm 100

Organoids from adult stem cells

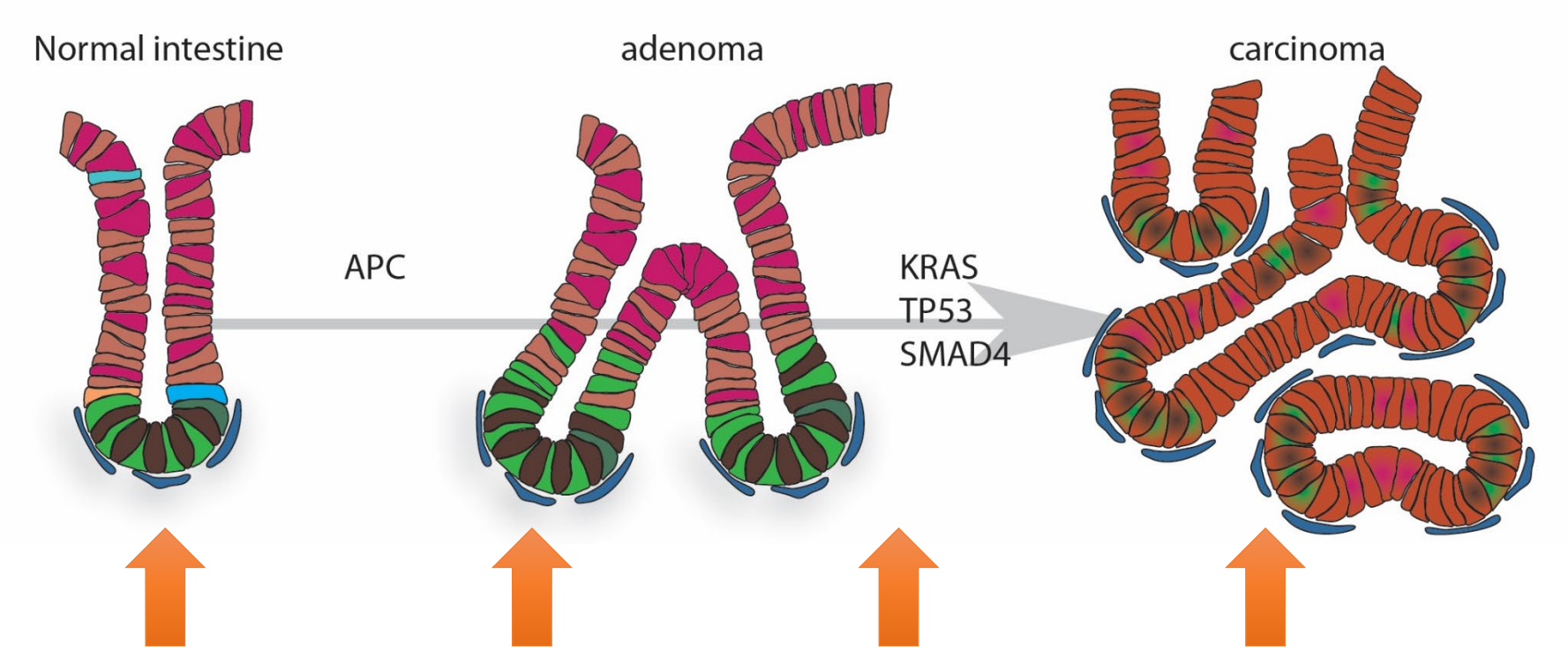


Can we use organoid technology for disease modeling?

(colon) cancer

Mutating human gut stem cells

(part I)



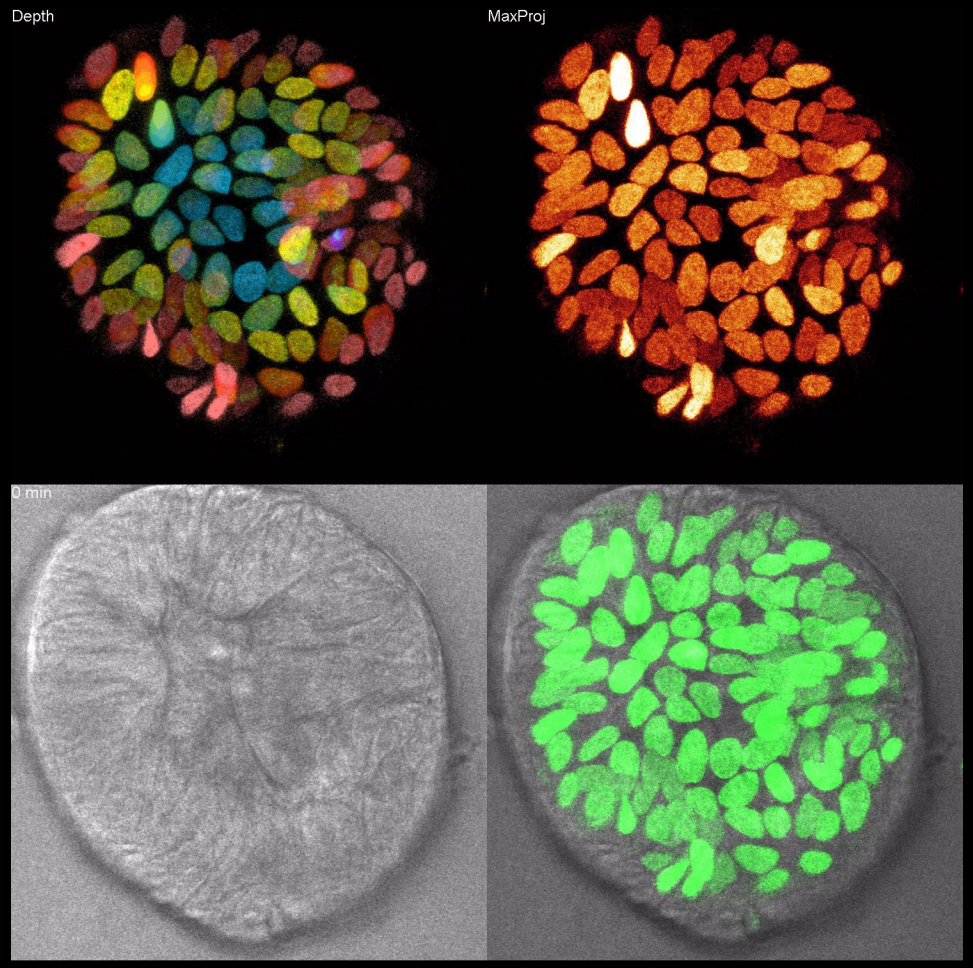
Create all the tumor stages from the same patients material

diploid ⟷ aneuploid

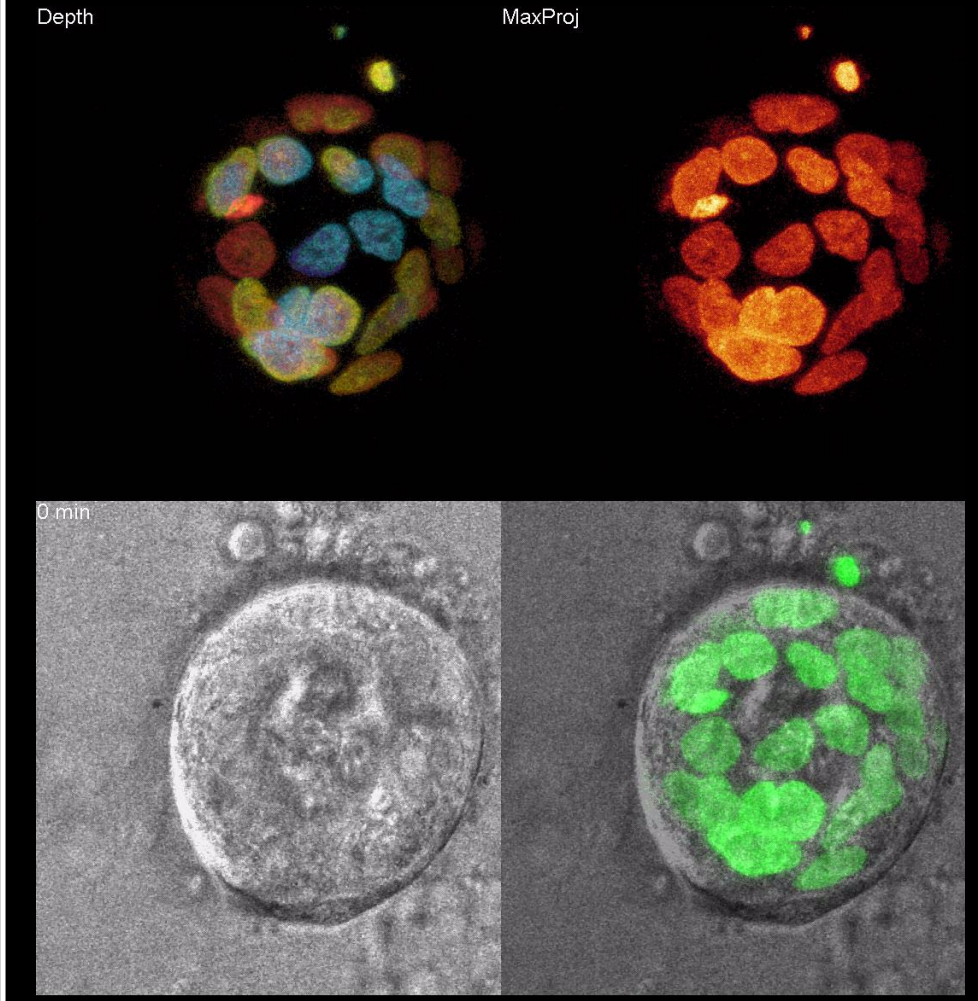
chromosomal instability?

Drost *et al.*, Nature 2015

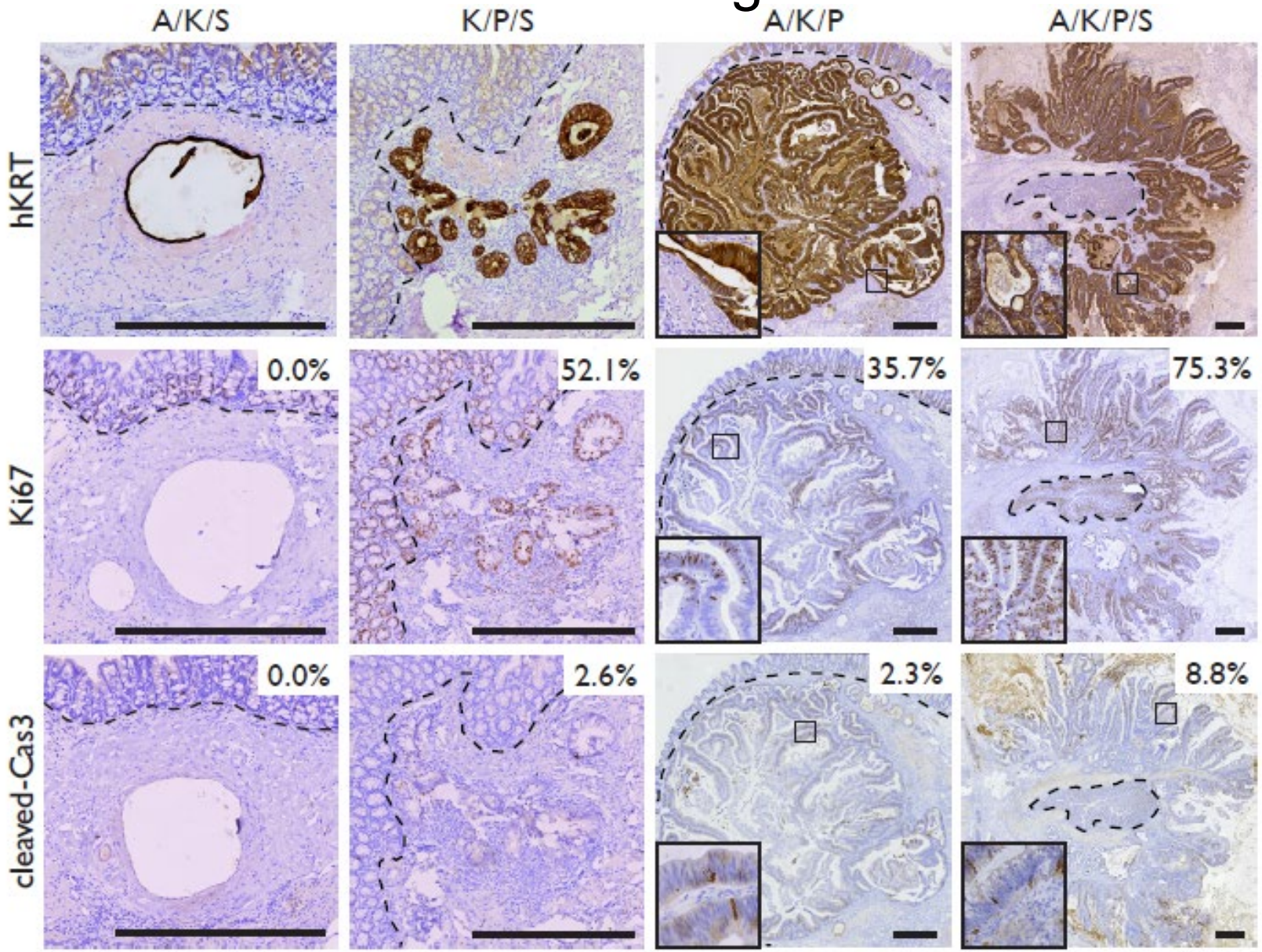
Wild-type



Quadruple

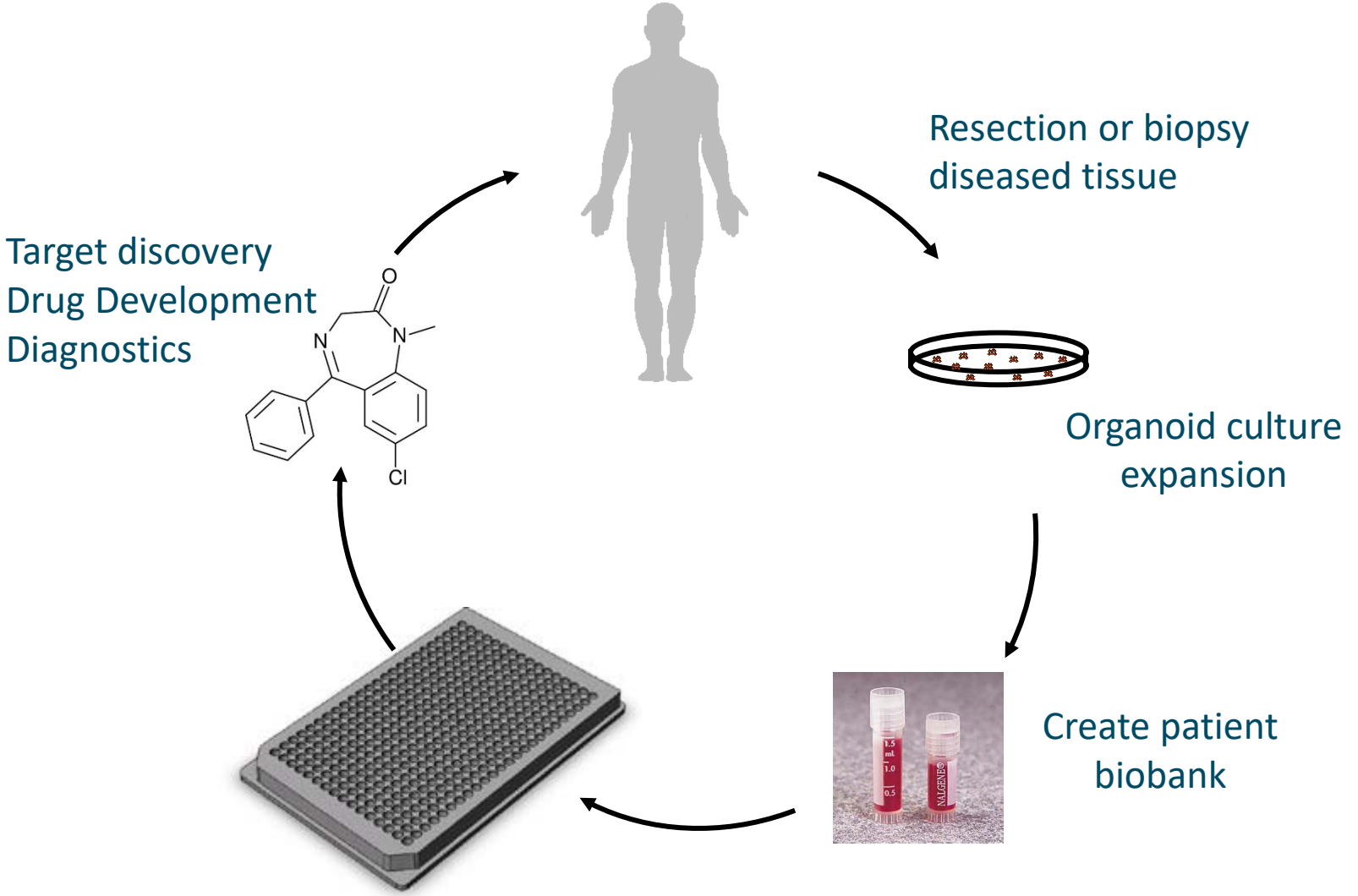


Invasive outgrowth of A/K/P and A/K/P/S organoids



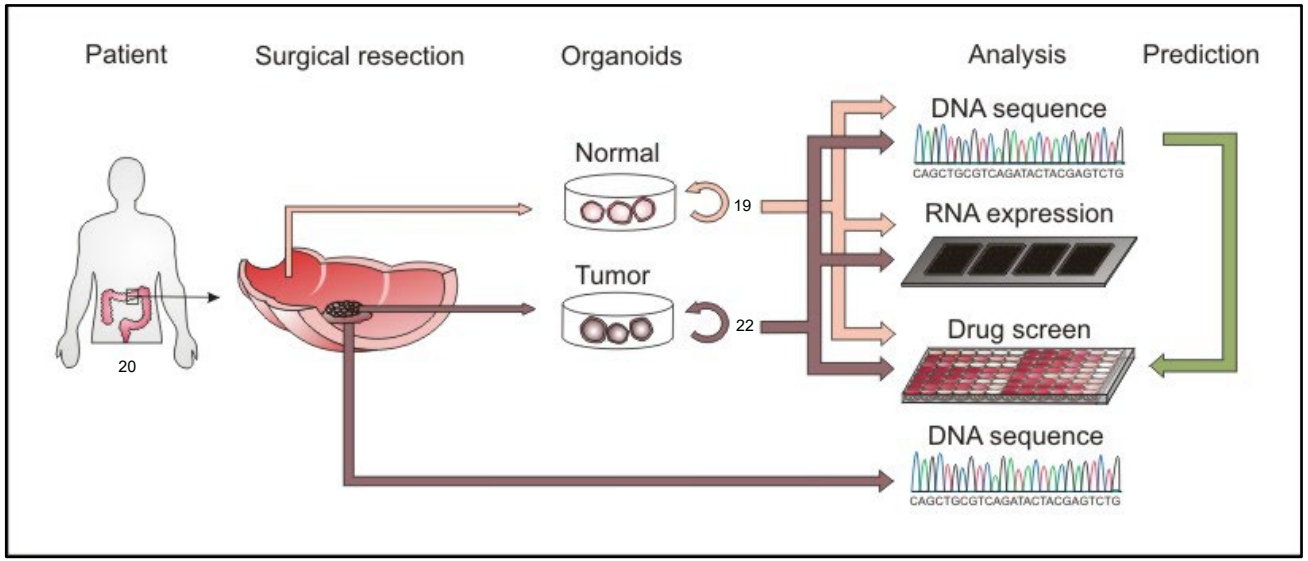
'A Living cancer biobank'
Colon -, breast -, ovarial - and pancreas-cancer

Hubrecht Institute
Developmental Biology and Stem Cell Research

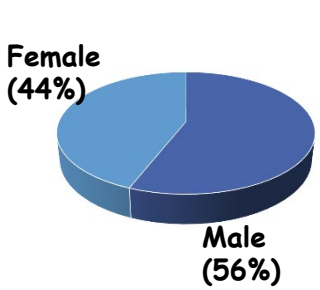


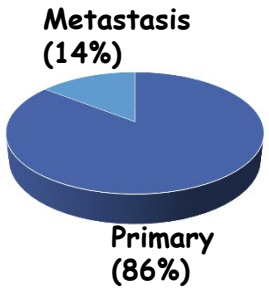
A living biobank

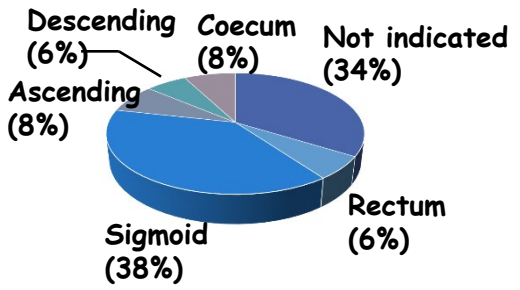
Stand up to cancer dreamteam with Matthew Meyerson & Mike Stratton



- Over 200 CRC organoid cultures (**90% efficiency**)

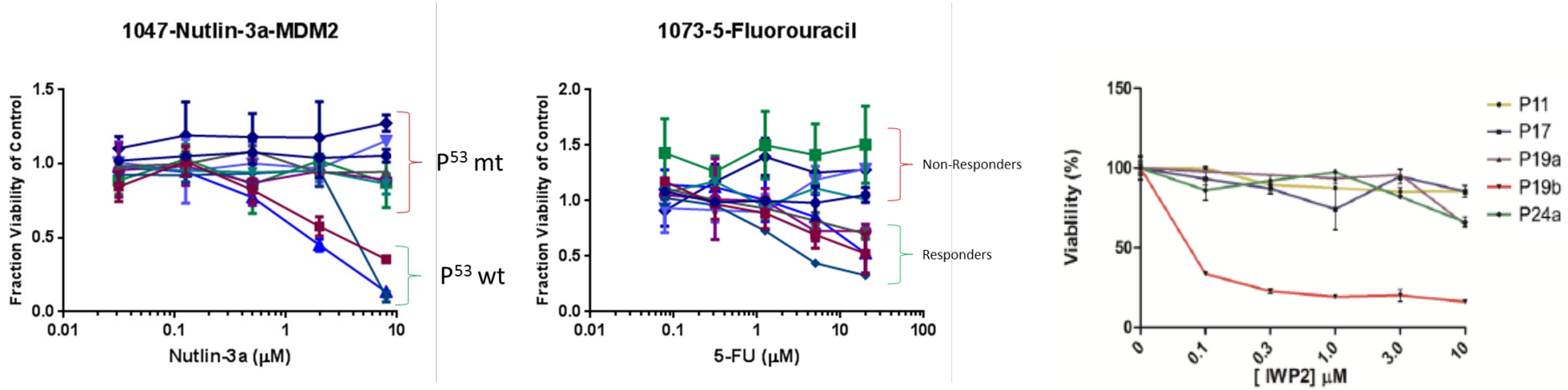






Cancer Organoids predict disease response (?)

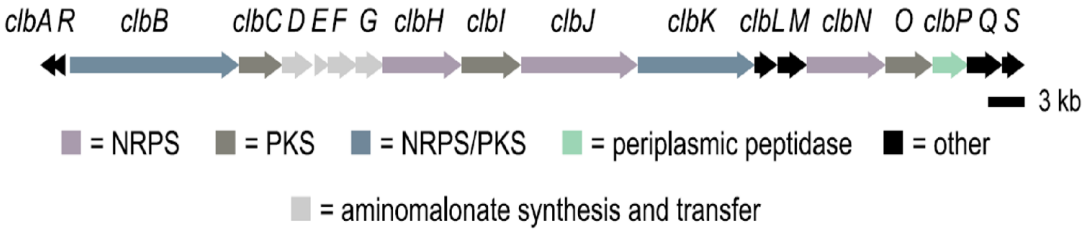
Hayley Francies
Anne McLaren-Douglas
Mathew Garnett
Ultan McDermott
Sanger Institute, Hinxton, UK



- Vlachogiannis et al Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. **Science** 2018
- Ooft et al Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. **Science translational Medicine** 2019
- Ganesh et al A rectal cancer organoid platform to study individual responses to chemoradiation. **Nature Medicine** 2019
- Tiriac et al Organoid Profiling Identifies Common Responders to Chemotherapy in Pancreatic Cancer. **Cancer discovery** 2019
- Yao et al. Patient-derived organoids predict chemoradiation responses of locally advanced rectal cancer. **Cell Stem Cell** 2019
- Ooft et al. Prospective experimental treatment of colorectal cancer patients based on organoid drug responses. **ESMOO** 2021

The *pks* pathogenicity island in E.coli

Ruben van Boxtel



Cayetano Pleguezuelos-Manzano

-The presence of *pks*⁺ E.coli is associated with polyps, cancer and inflammation of the colon. Carried by 10-30% of diseases individuals

Jens Puschhof

-Culturing *pks*⁺ E.coli on cell lines induces DNA damage: genotoxicity.

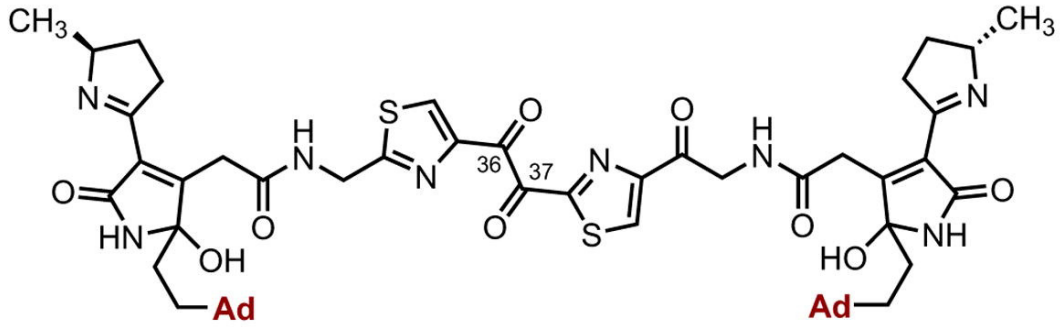
-*pks*⁺ E.coli is a popular probiotic, also prescribed by gastroenterologists

Axel Rosendahl Huber

Is pks E. coli a carcinogenic component of the human colon microbiome?

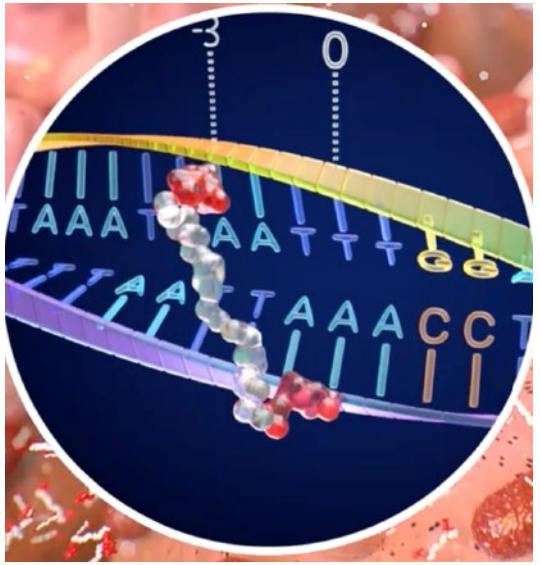
Genotoxic *pks+ E. coli* produce colibactin

Poly**k**etide **s**ynthase (*pks*) operon → production of **colibactin**

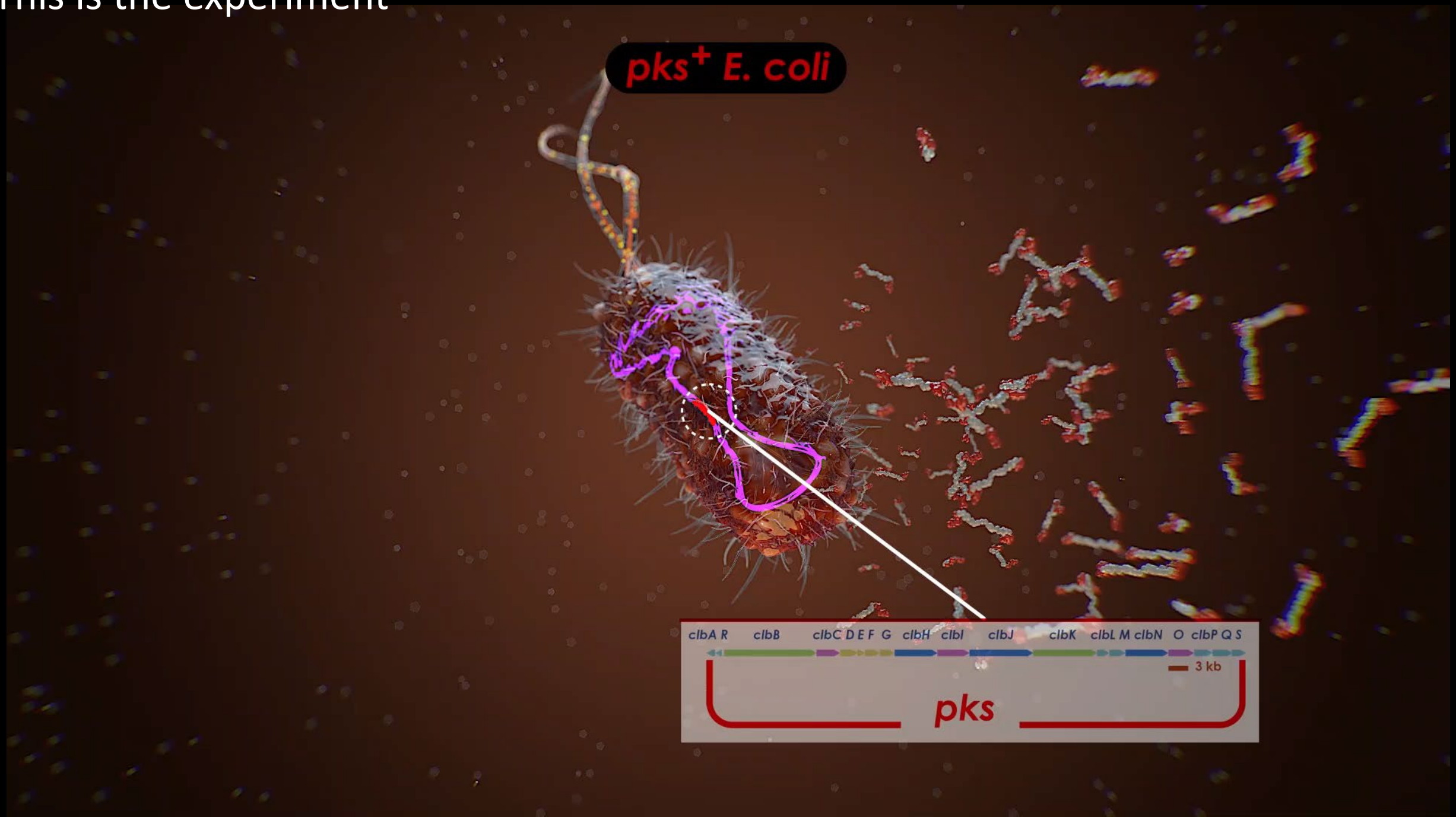


colibactin–DNA cross-link

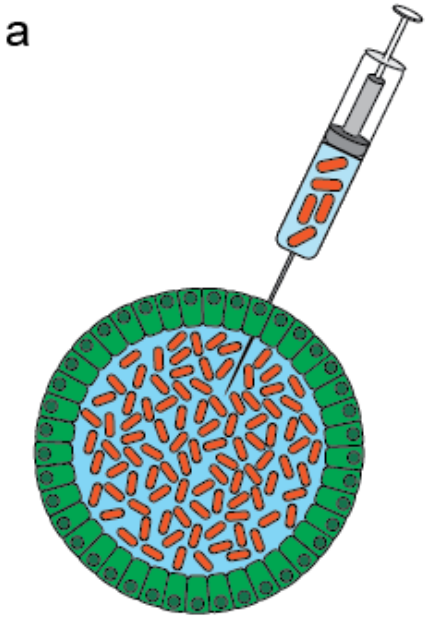
Xue et al., Science 2019

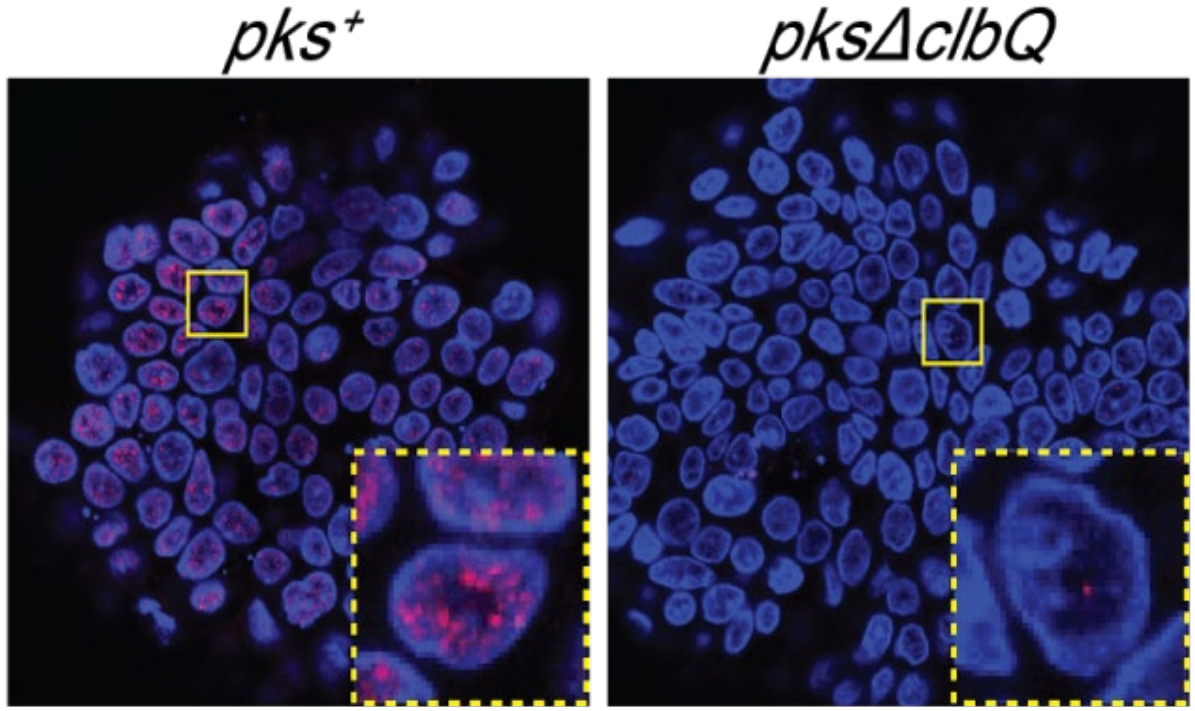


This is the experiment



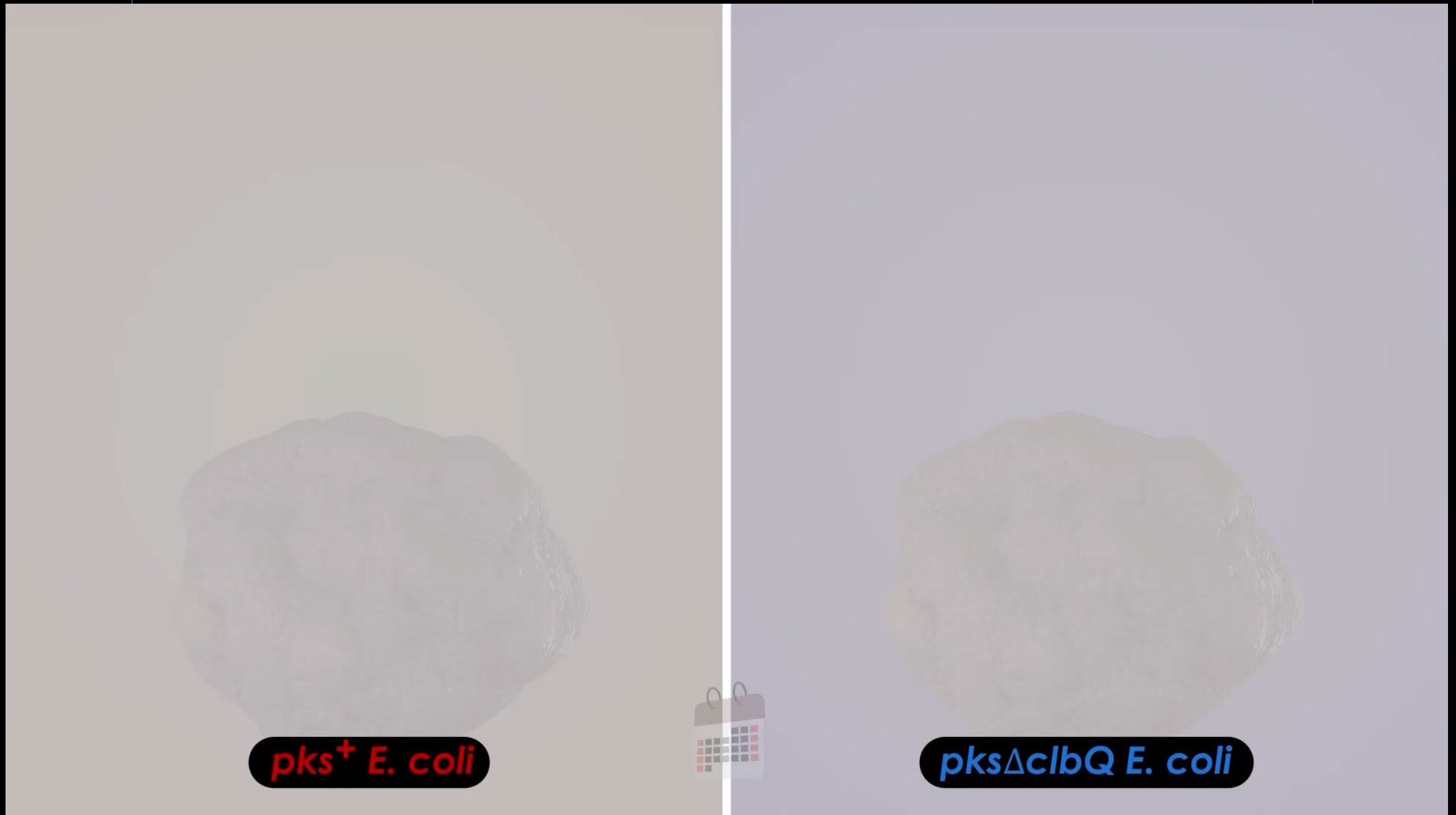
First control: *pks*⁺ *E. coli* induces DNA damage in healthy human intestinal organoids in our hands



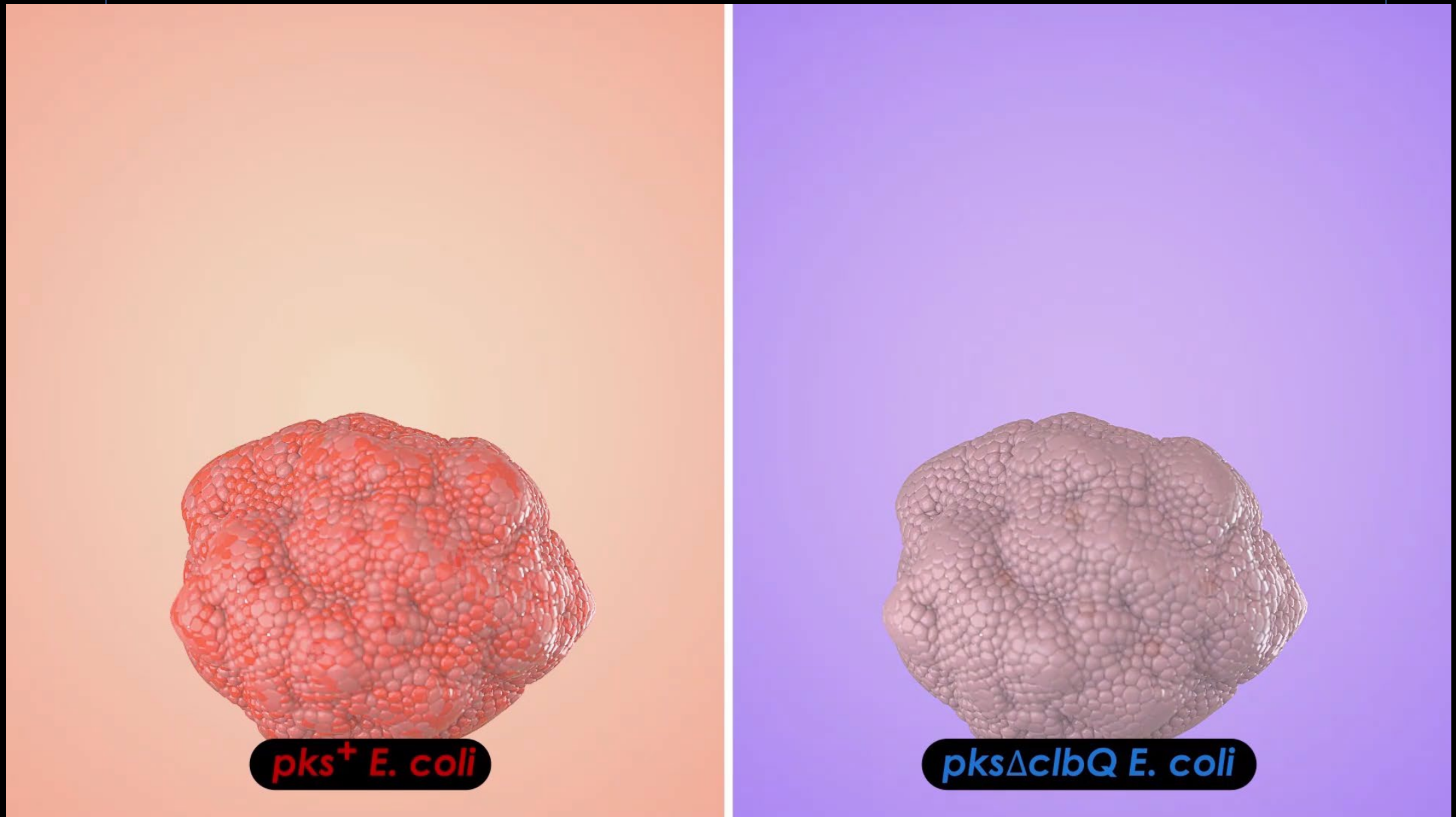


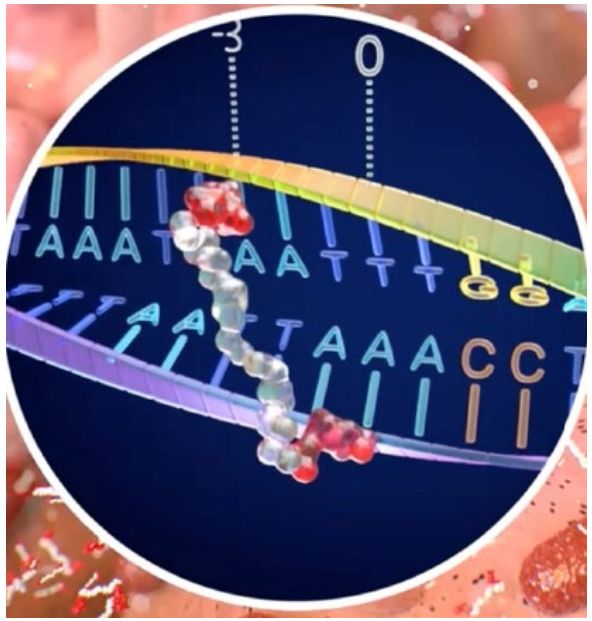
γH2AX foci in red

Chronic exposure of organoids to *pks* E.coli

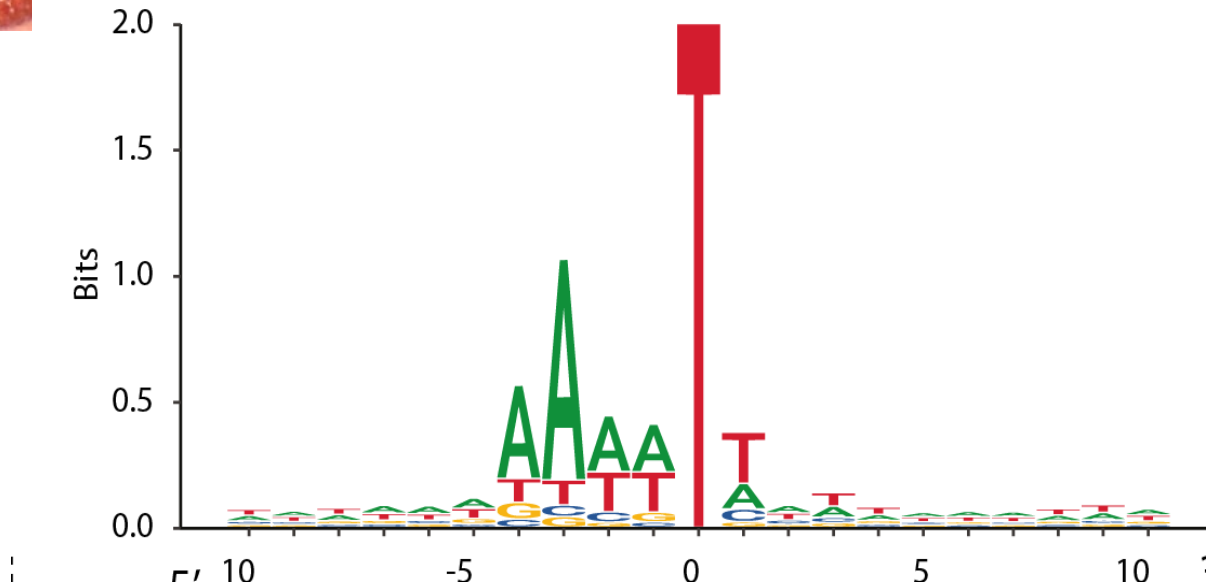


Expansion of individual exposed cells
followed by sequencing to detect induced mutations

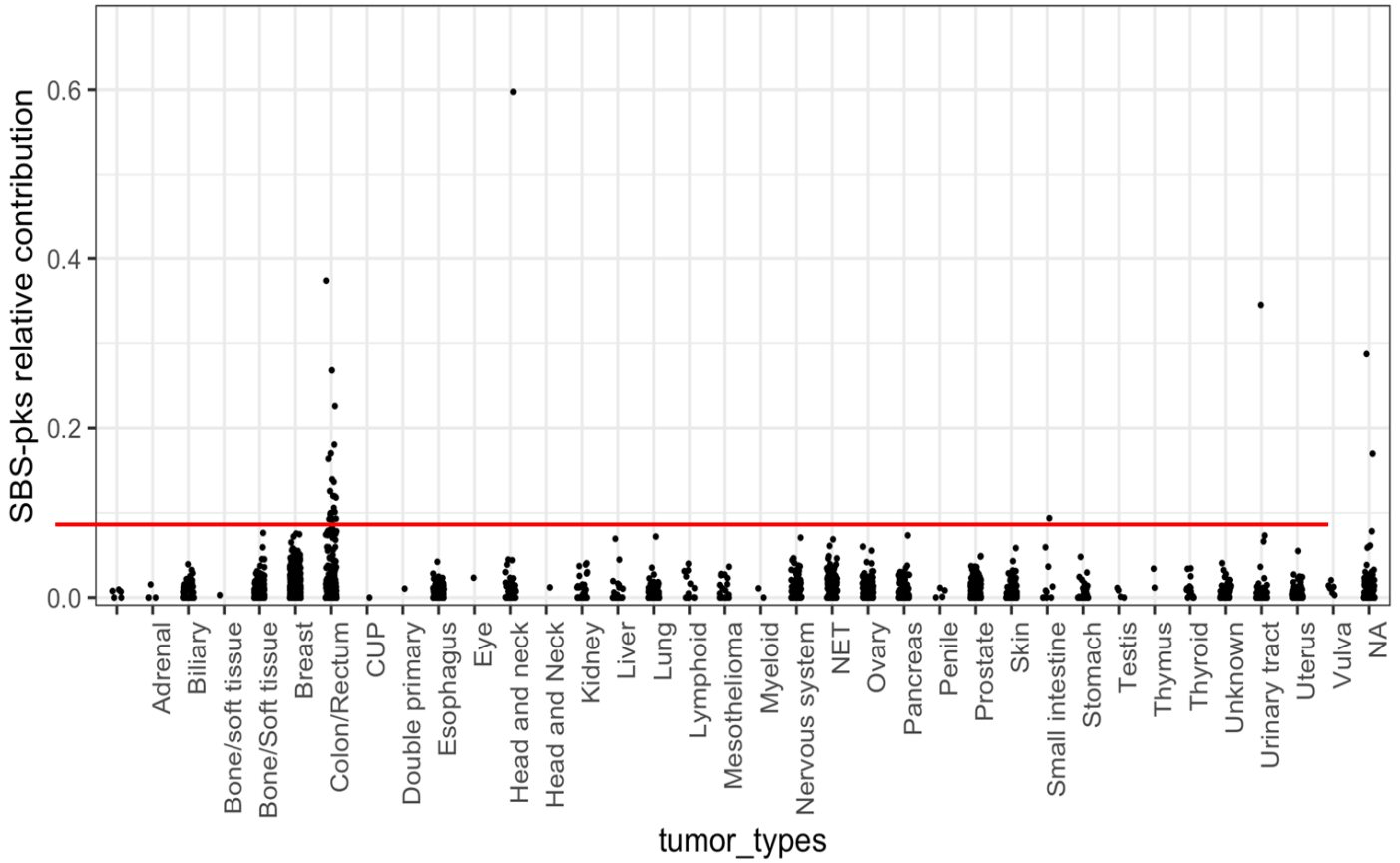




Extended *pks*$^{+}$ *E.coli* mutational signature



Pks signature specifically occurs in a subset of colorectal cancer metastases



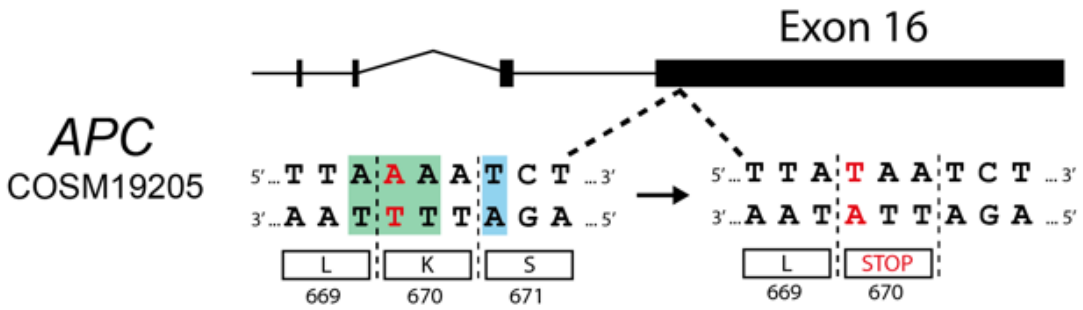
Hartwig MEDICAL FOUNDATION

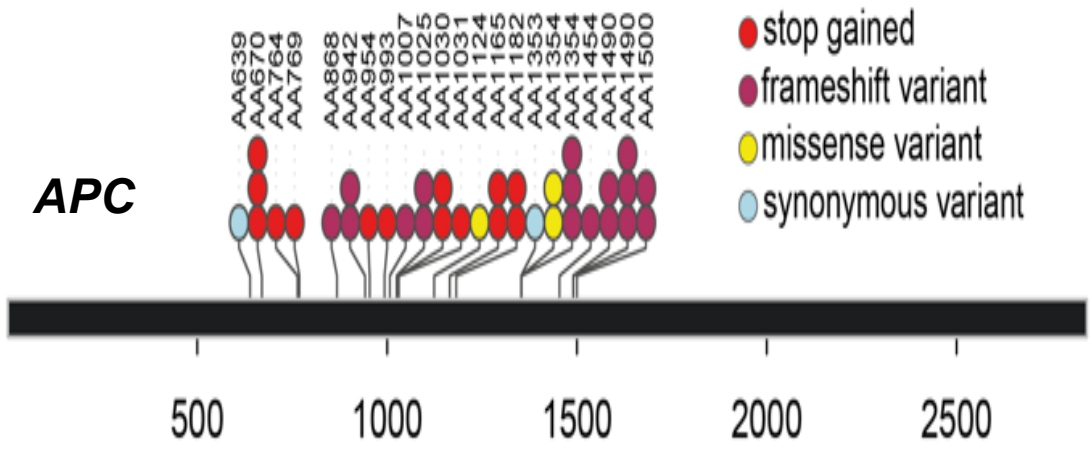
3,668 metastasis WGS (diverse primary origins, 496 CRC)

Genomics england

2,208 primary CRC WGS

SBS-*pks* and ID-*pks* mutational signatures can create CRC driver mutations



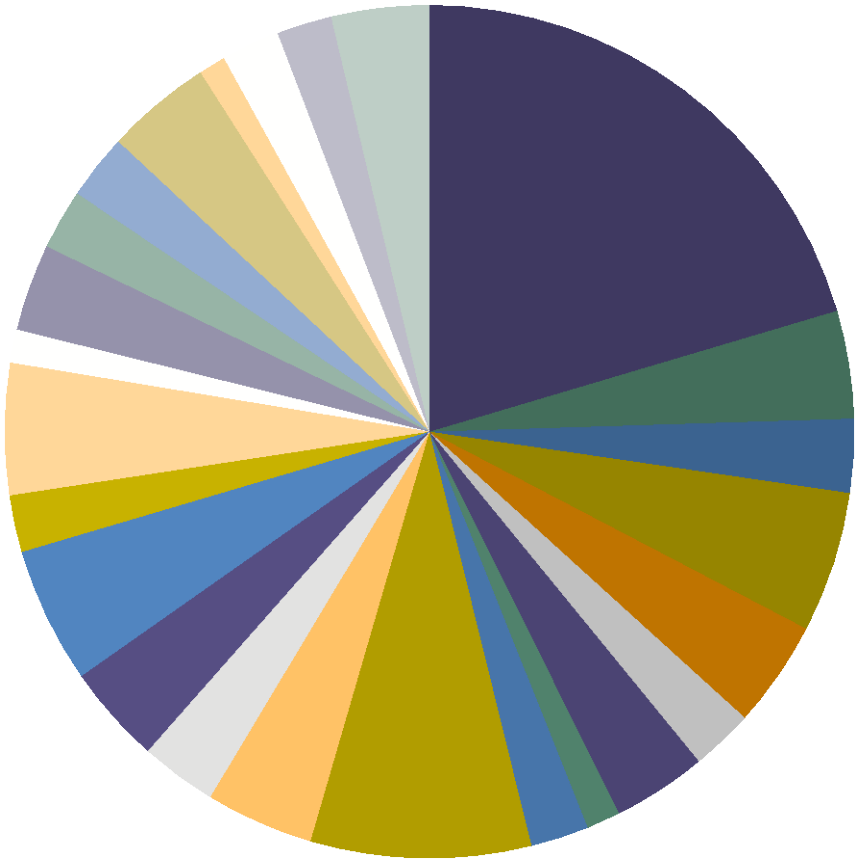


Thus,

- *pks*$^{+}$ *E. coli* induces **SBS-*pks* and ID-*pks*** mutational signatures in human healthy intestinal organoids.
- These are enriched in subsets of **CRC cases** from two independent cohorts.
- Implications for the **early detection** and **prevention** in individuals at CRC risk.

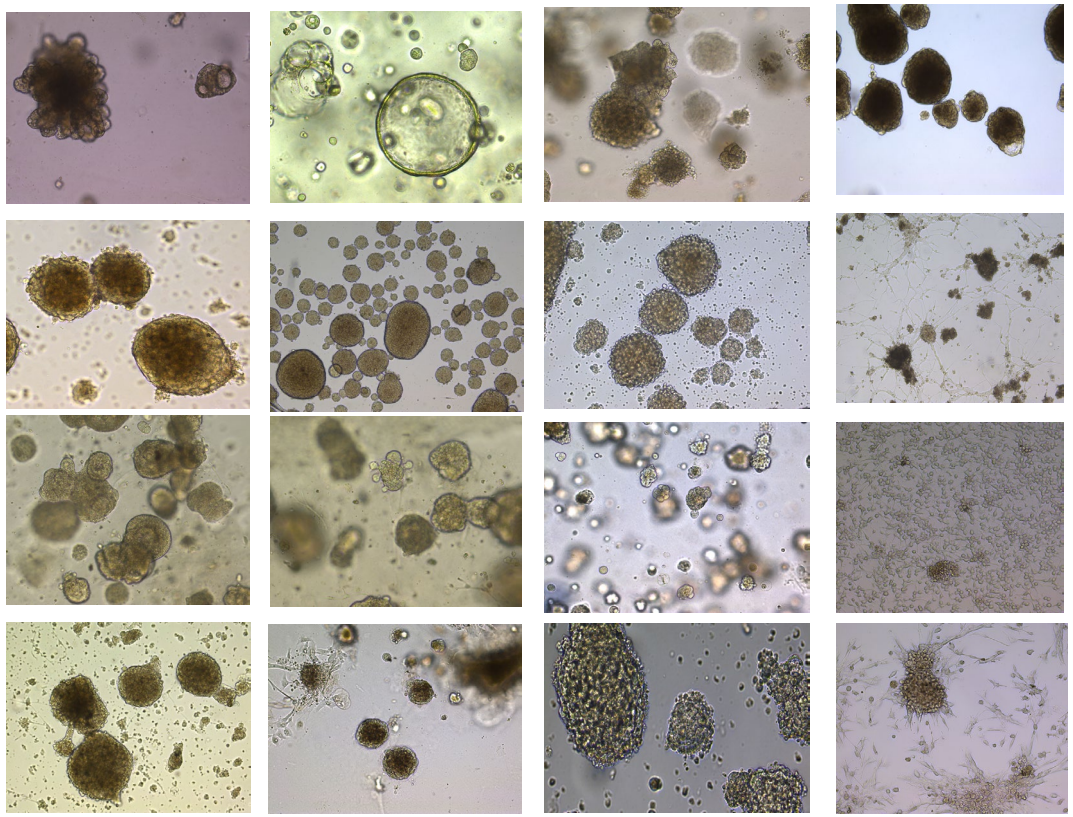
Can we make pediatric cancer organoids?

Many different cancer entities



- Acute lymphoid leukemia (ALL), N=114
- Acute myeloid leukemia (AML), N=23
- Myeloproliferative disease (CML/MDS), N=16
- Hodgkin lymphoma, N=30
- Non-Hodgkin lymphoma, N=23
- Burkitt lymphoma, N=13
- Histiocytosis, N=20
- Aplastic Anemia/Fanconi Anemia, N=7
- Ependymoma, N=12
- Astrocytoma, N=47
- Intracranial embryonal tumor, N=23
- Other glioma, N=16
- Other brain tumor, N=21
-
- Neuroblastoma, N=29
- Retinoblastoma, N=12
- Renal tumor, N=28
- Liver tumor, N=7
- Osteosarcoma, N=18
- Ewing sarcoma, N=13
- Rhabdomyosarcoma (RMS), N=14
- Other sarcoma, N=22
- Intracranial germ cell tumor, N=6

Cancer organoids are unique… as are patients



Liver:	10 patients	Hepatoblastoma, Hepatocellular Carcinoma, Cholangiocarcinoma.
Brain:	200 patients	DIPG, Ependymoma (9/10), Craniopharyngioma (6/10), Choroid plexus papilloma, ETMR, Pineoblastoma, ATRT
Other solid tumors:	200 patients	Wilms tumor, renal cell carcinoma, malignant rhabdoid tumor, rhabdomyosarcoma, synovial sarcoma, SCOHHT

Clevers and Drost groups

A living organoid biobank for (rare) pediatric cancers

Embedded in the Maxima Biobank

- Logistics: Fully characterised organoid models for a defined set of pediatric tumors

- Legal: Interaction with academia AND pharma should be possible

- Ethics: in collaboration with parents, patients and dept. of Ethics of Biomedical Innovations

Funded by KiKa and Oncode-PACT

Snake venom gland organoids

Hubrecht Institute
Developmental Biology and Stem Cell Research

- **5.4 million people worldwide are bitten by snakes each year**
- **1.8 to 2.7 million cases of envenomings.**
- **Around 80,000 to 140,000 people die yearly**
- **Around three times as many amputations and other permanent disabilities are caused by snakebites annually.**

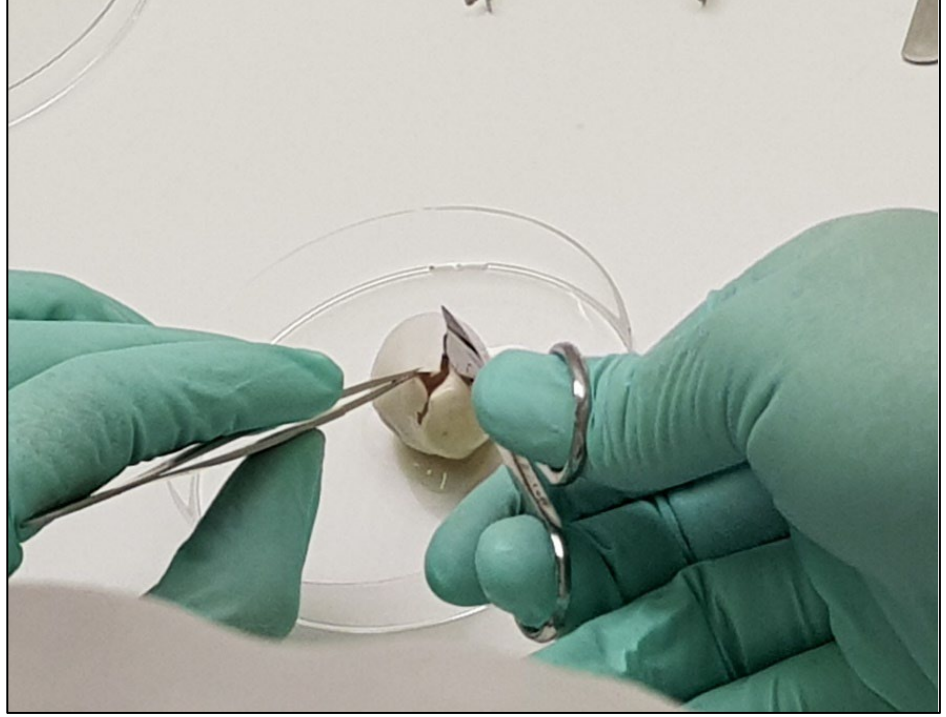
Aspidelaps lubricus (Cape coral snake)

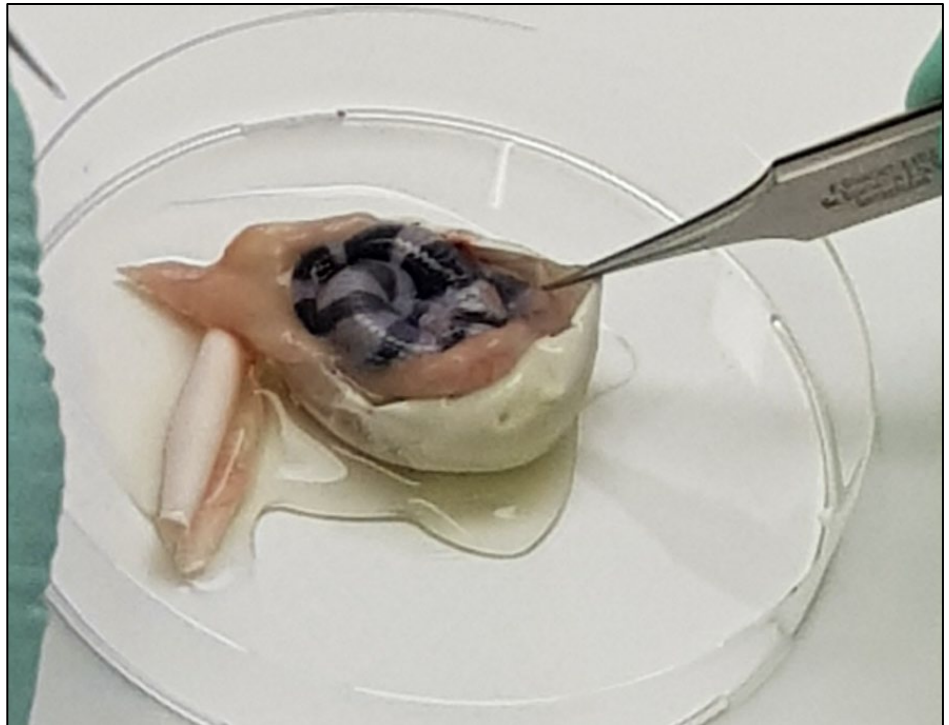
Yorick Post, Jens Puschof, Joep Beumer

With Harald Kerkkamp (Michael Richardson Lab - Leiden University)

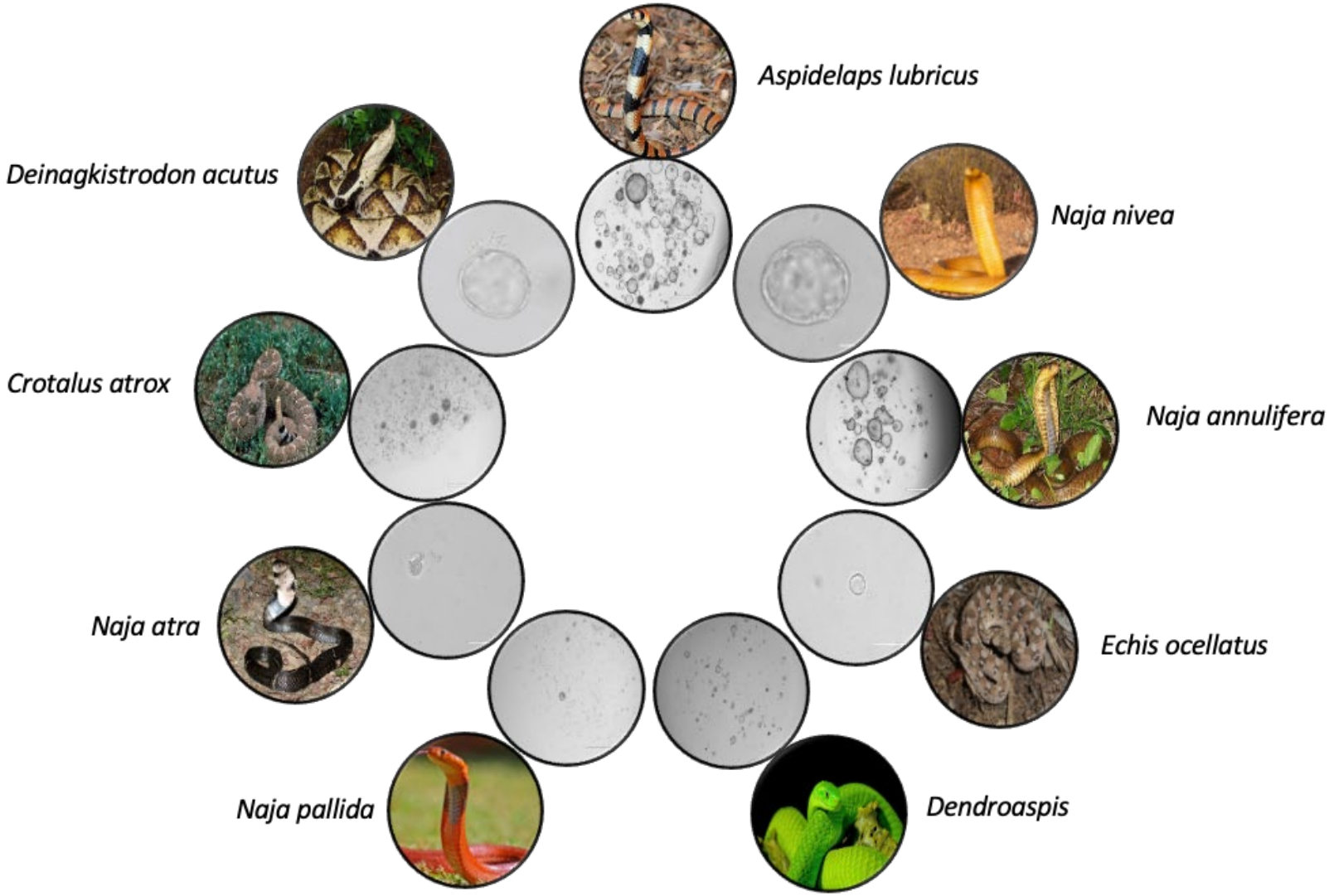
Hubrecht Institute
Developmental Biology and Stem Cell Research

Obtaining venom gland tissue for organoid culture

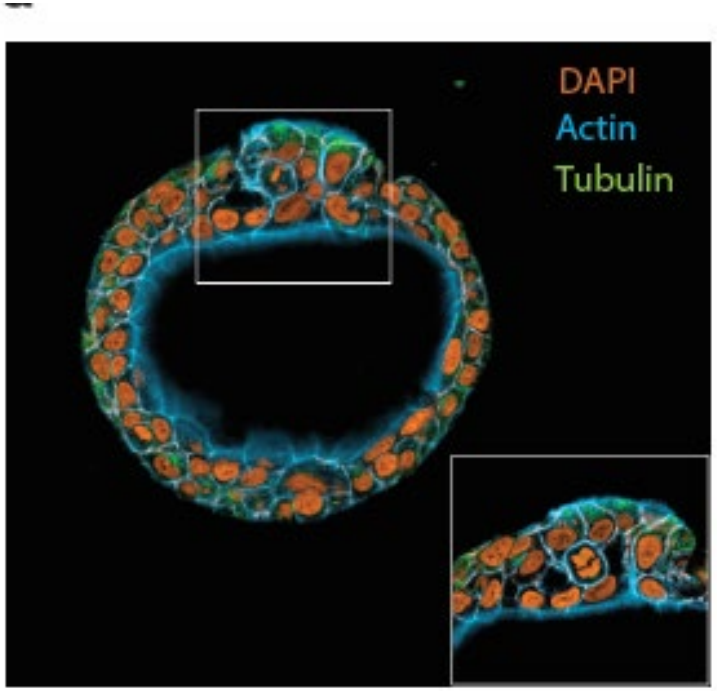




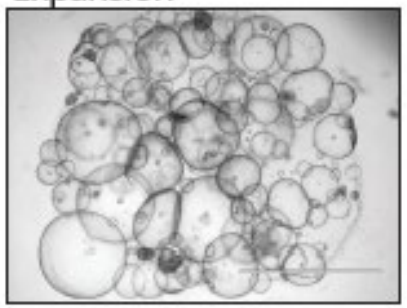
Works for many snake species



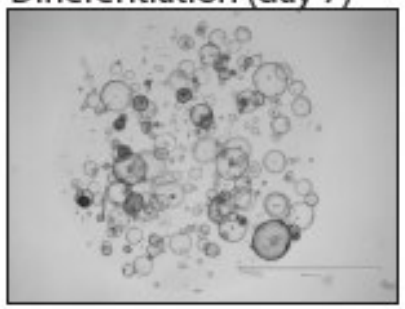
Aspidelaps venom gland organoids

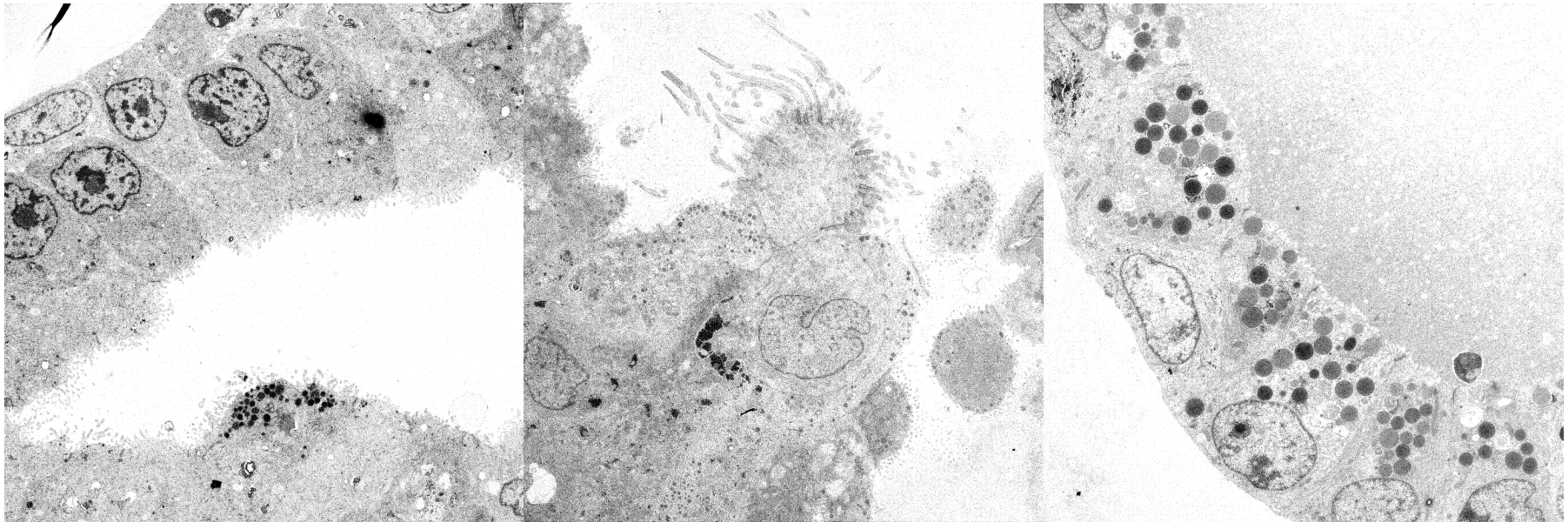


Expansion



Differentiation (day 7)





Do venom gland organoids secrete toxins?
LC-MS on culture supernatant

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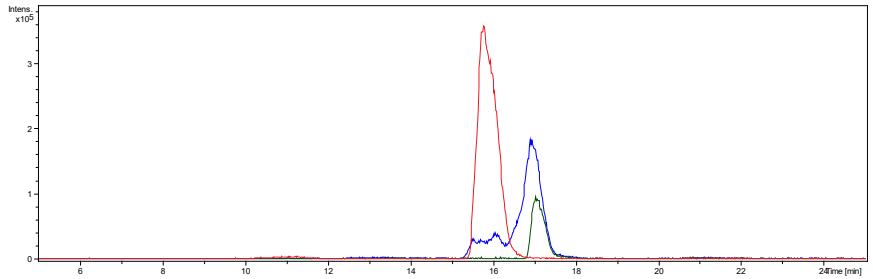
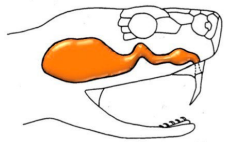
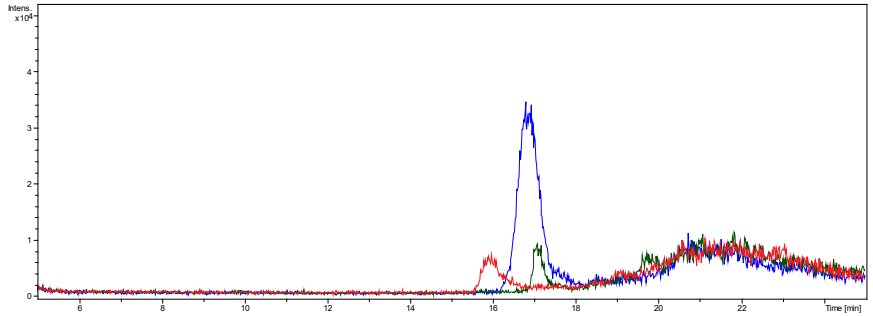
Venom control



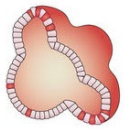
1036.1628 $^{6+}$: 6206.9120 Da
1129.5090 $^{7+}$: 7895.4441 Da
1054.7481 $^{7+}$: 7371.1703 Da



Protein from organoids

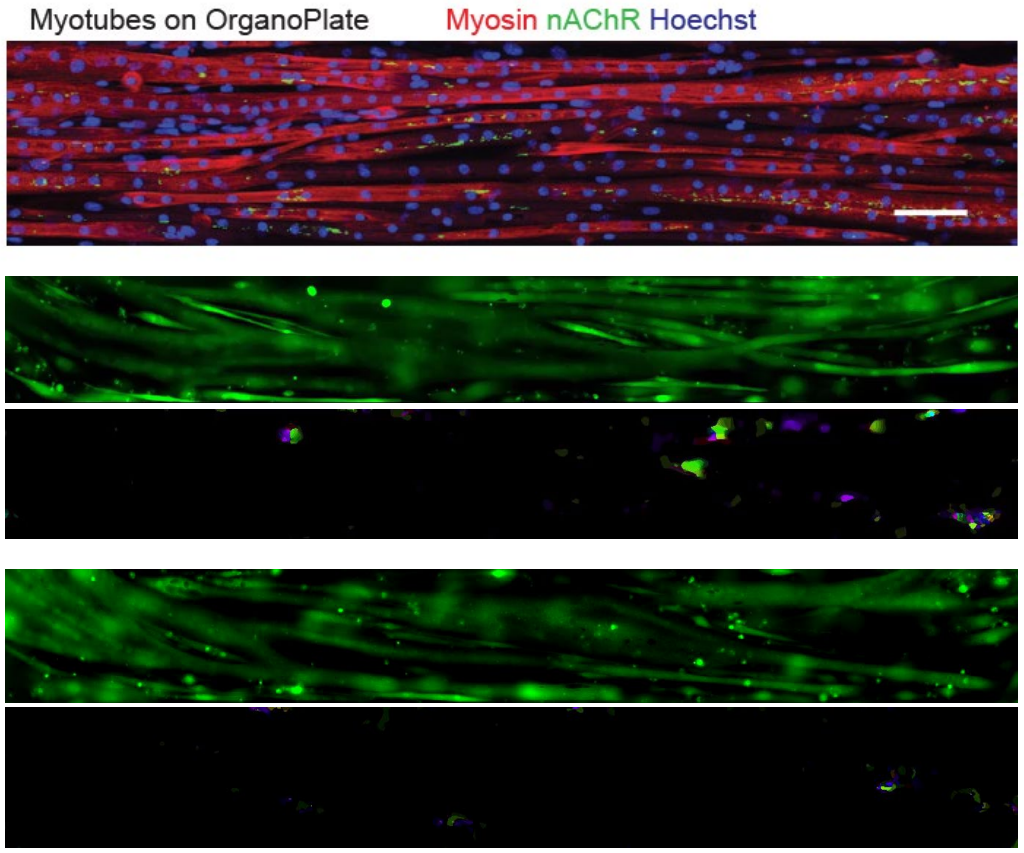


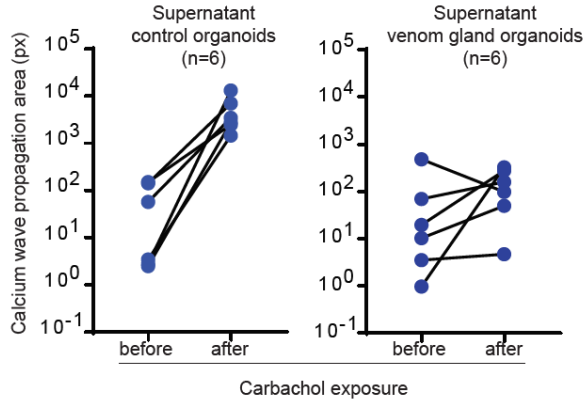
1036.1642 $^{6+}$: 6206.9246 Da
1129.6490 $^{7+}$: 7894.4635 Da
1054.7523 $^{7+}$: 7371.1981 Da



With Jeroen Kool (VU)

Organoid venom blocks calcium wave propagation in muscle cells





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Robert Vries	(neuro) oncologists
Hans Clevers	(neuro) pathologists

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