Intestinal stem cells and cancer stem cells

Assoc. Prof. Priv. Doz. Mag. Dr. Helmut Dolznig

Center of Pathobiochemistry and Genetics

Institute of Medical Genetics

https://www.meduniwien.ac.at/hp/pathobiochemie-und-genetik/studium-lehre/vorlesungen/

Overview

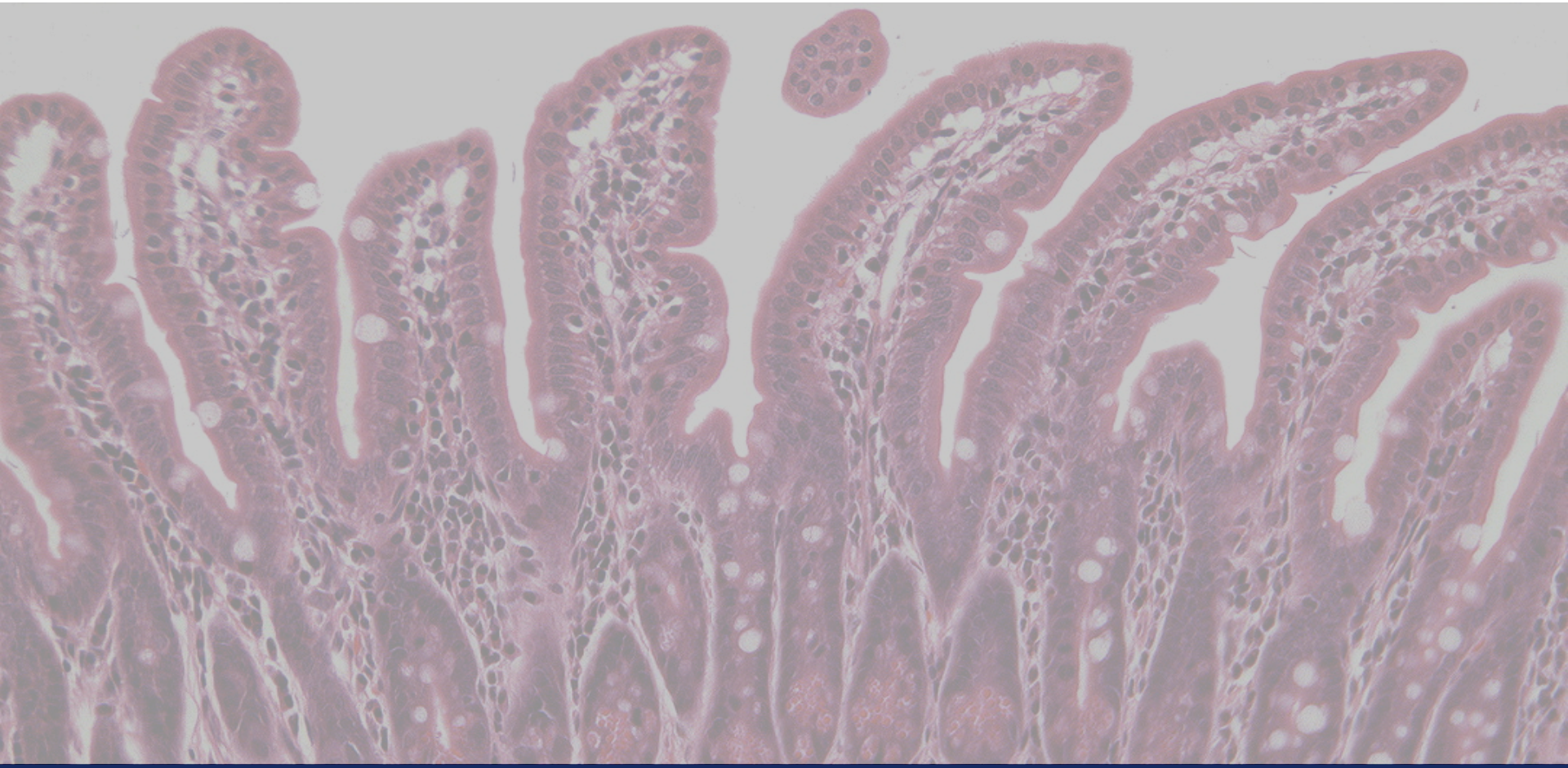
- Adult stem cells

  Intestinal stem cells

- Cancer stem cells

- Drug development

Intestinal stem cells



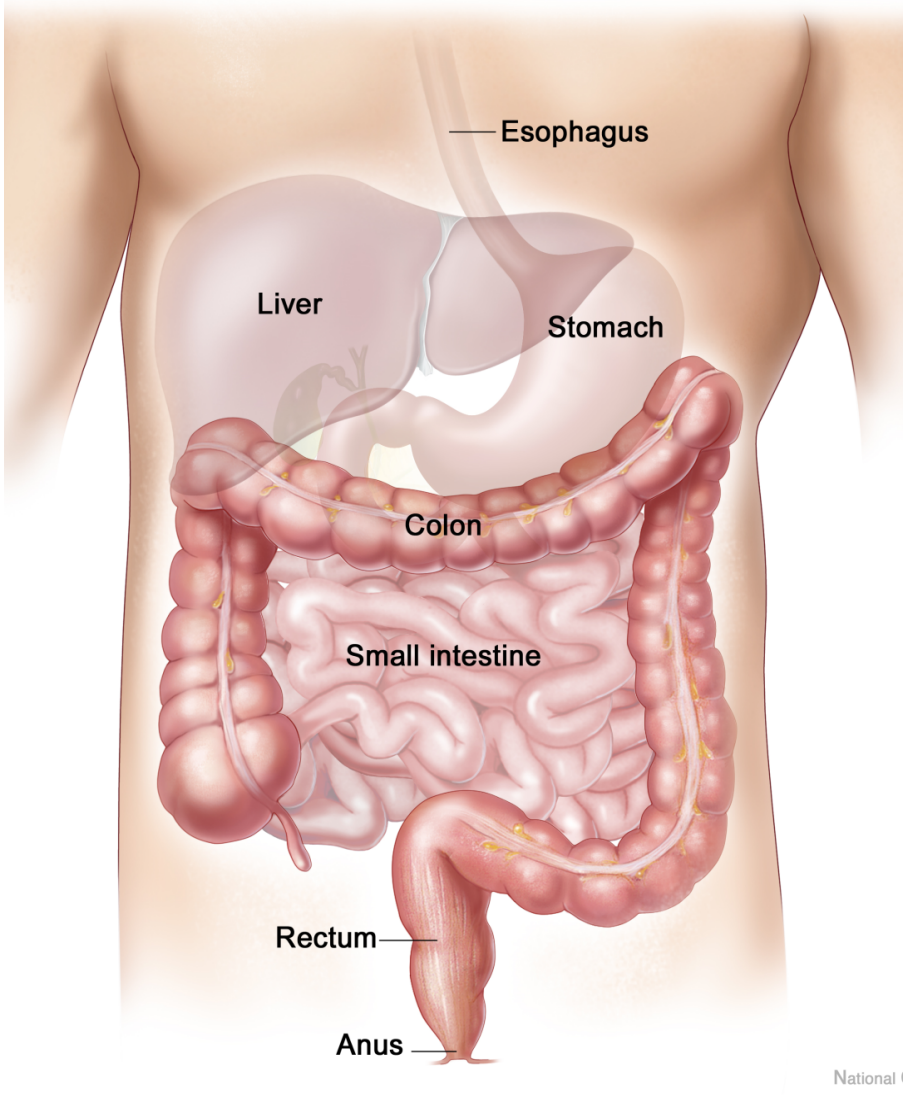
stem cell definition

- **ability to undergo self-renewal**
  - proliferating for long time periods without differentiation (life time of the organism)
    - difficult to prove *in vivo*
- **production of specialized cell types, fully differentiated**
  - defined, specialized function
    - characteristic morphology, contact to other cells + ECM, surface markers, behavior
  - via intermediate cells called **precursors** or **progenitor cells**
- **adult SC are rare**
- **reside in the tissue, which they give rise to**

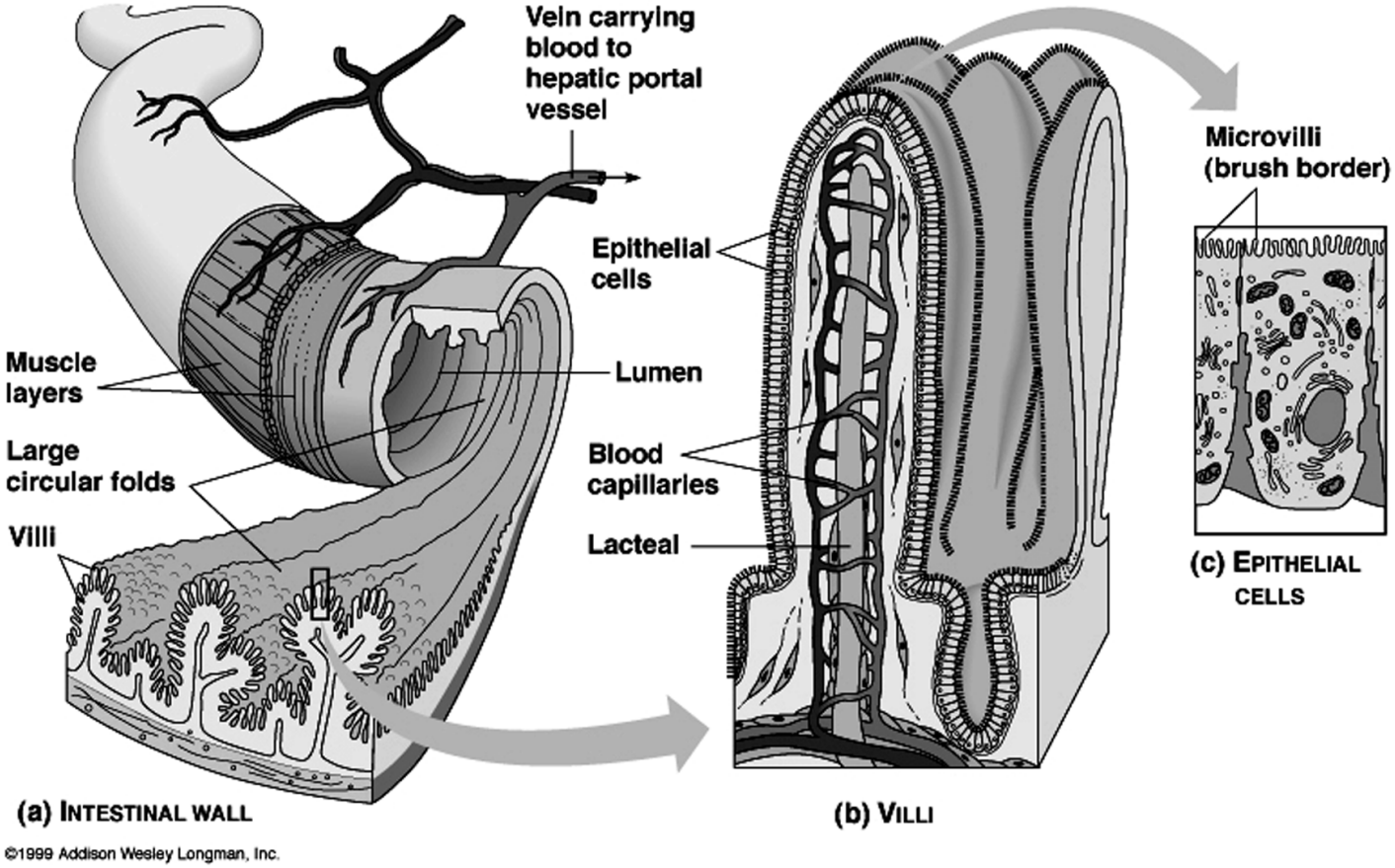
adult stem cells: function, taskss

- **ensure tissue homeostasis**
  - maintenance of steady state function of cells, compartments, tissues, organs (tissue maintenance, growth)
- **replacement of injured or diseased cells,**
  - repair

The gastrointestinal tract



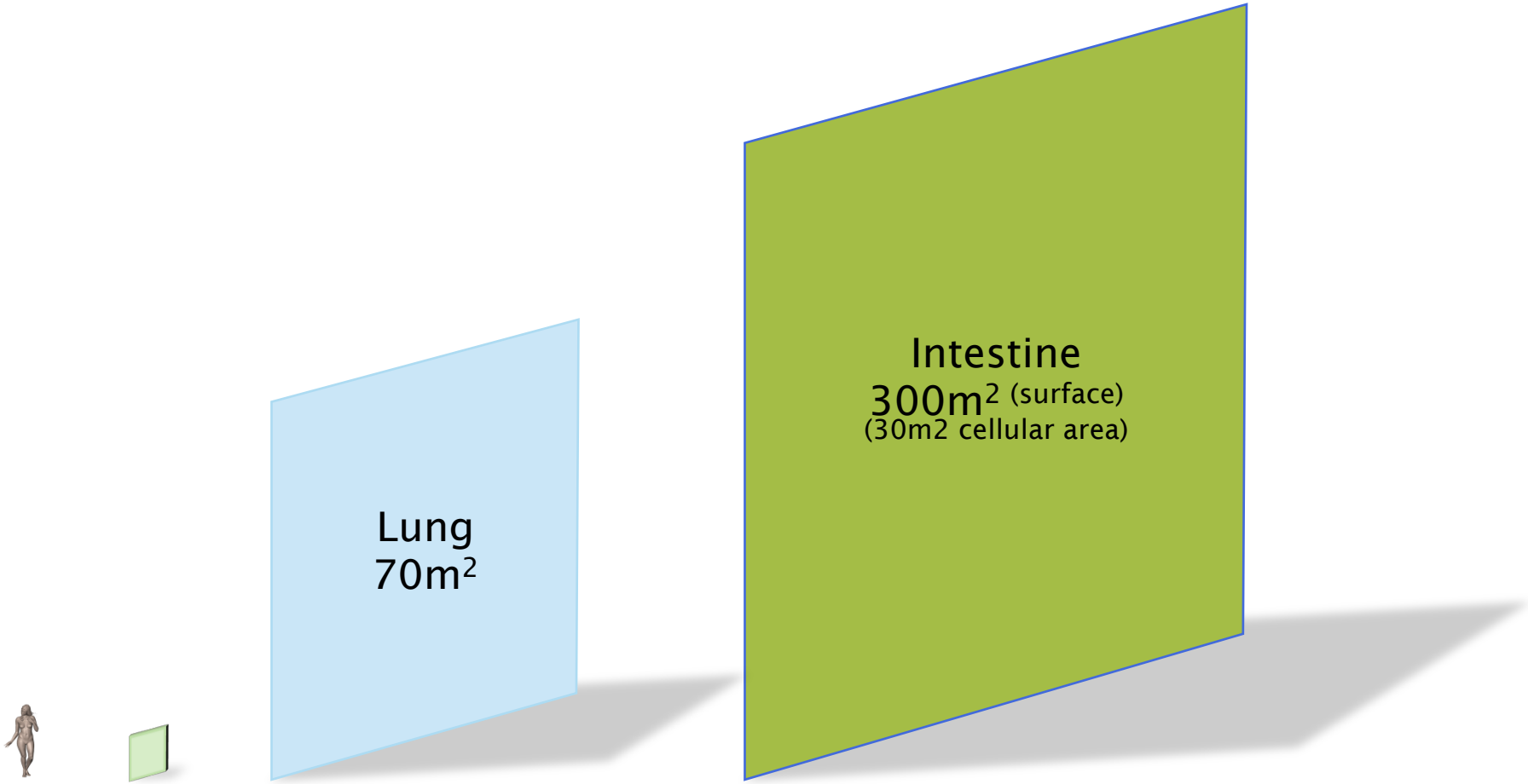
Increase in surface



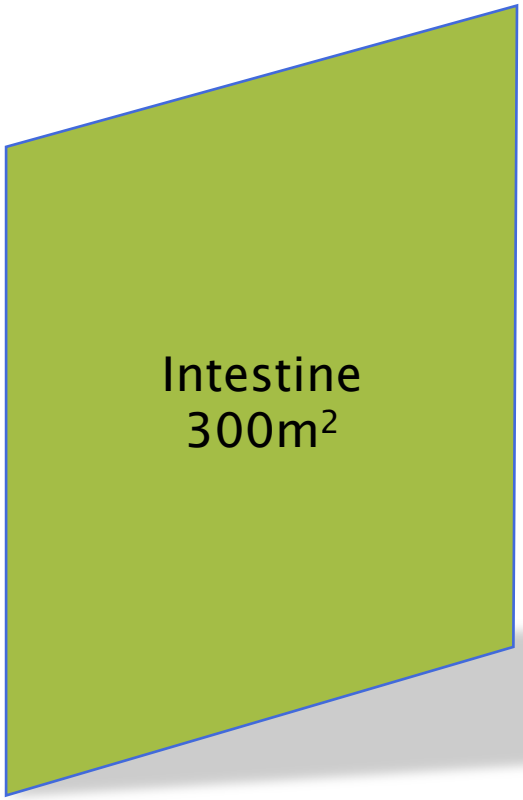
Surface increase:

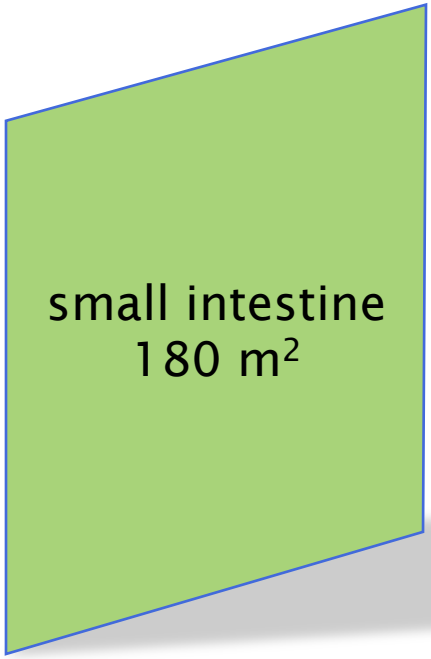
- Folds (*Kerckring-folds*, *Plicae circulares*) 3x
- Villi 7-14x
- Mikrovilli 15-40x

Intestinal surface

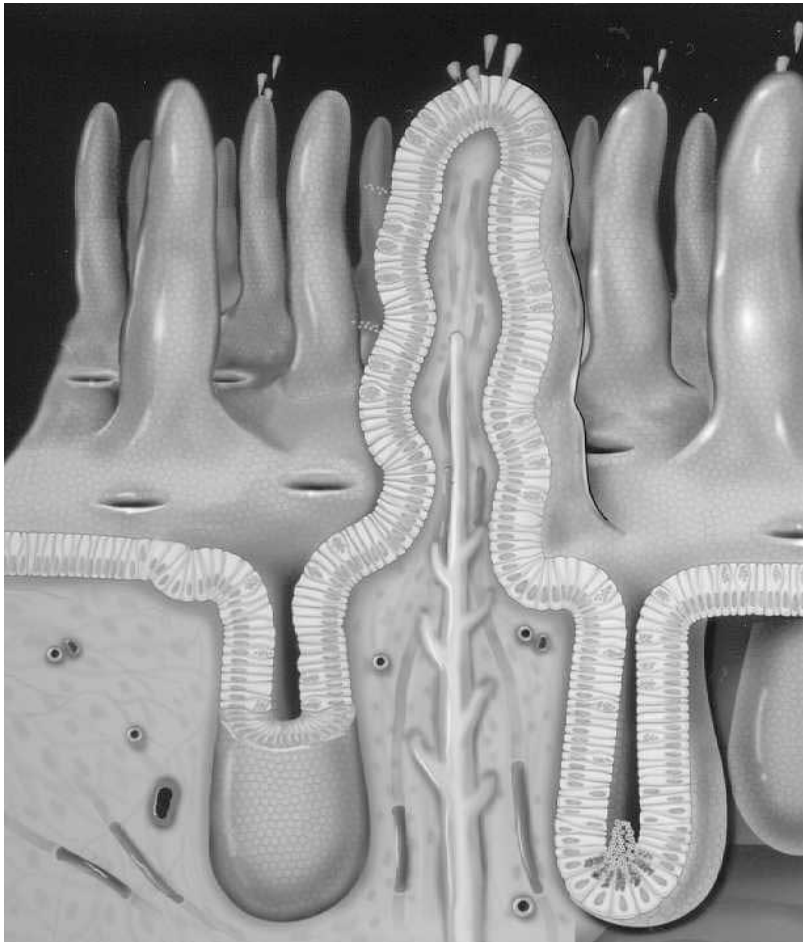


Small intestine

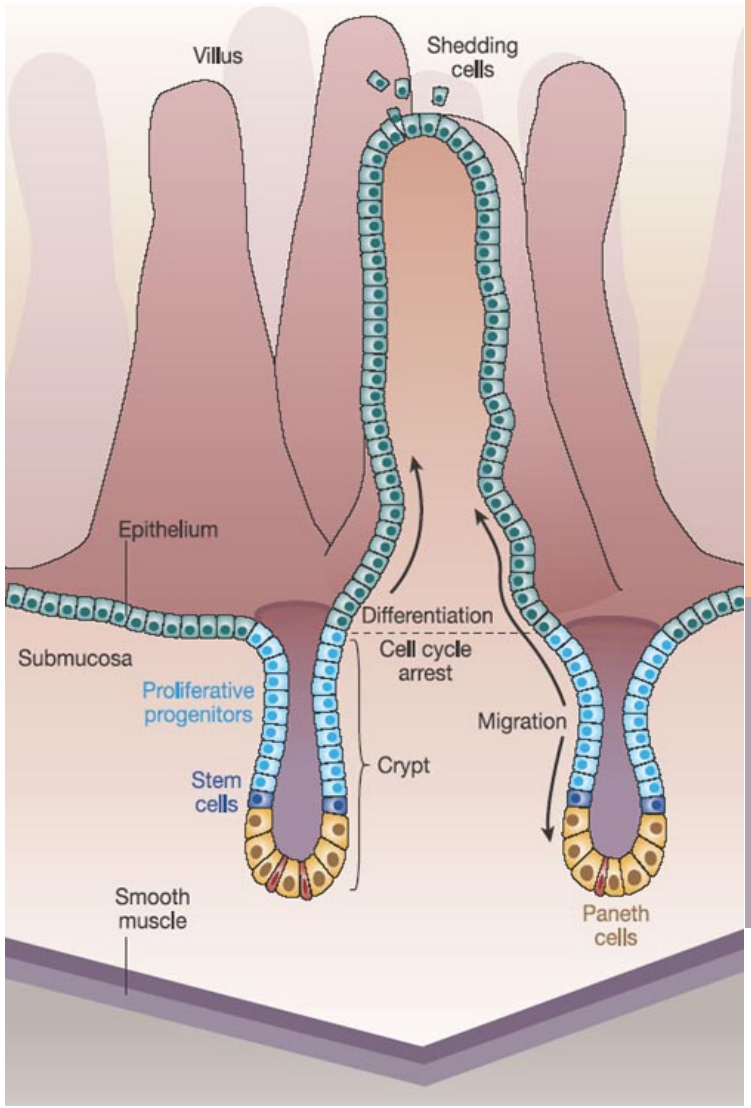




stem cells at the crypt bottom were long predicted



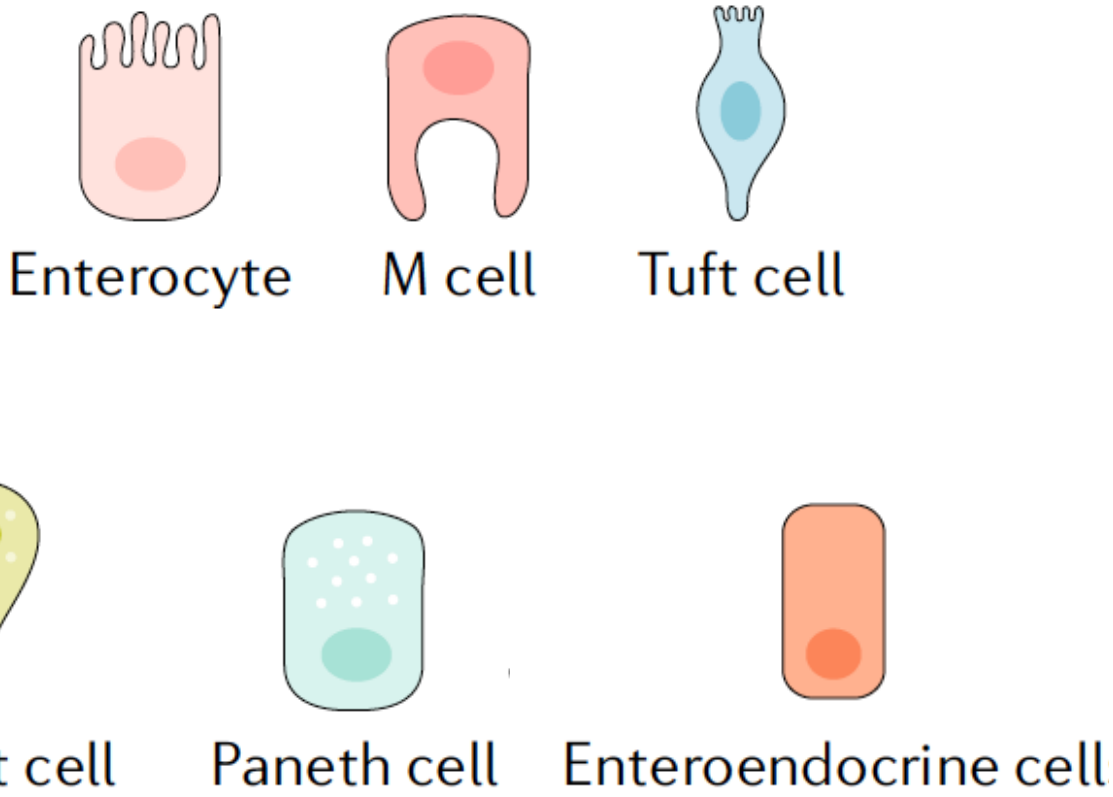
Small intestine morphology



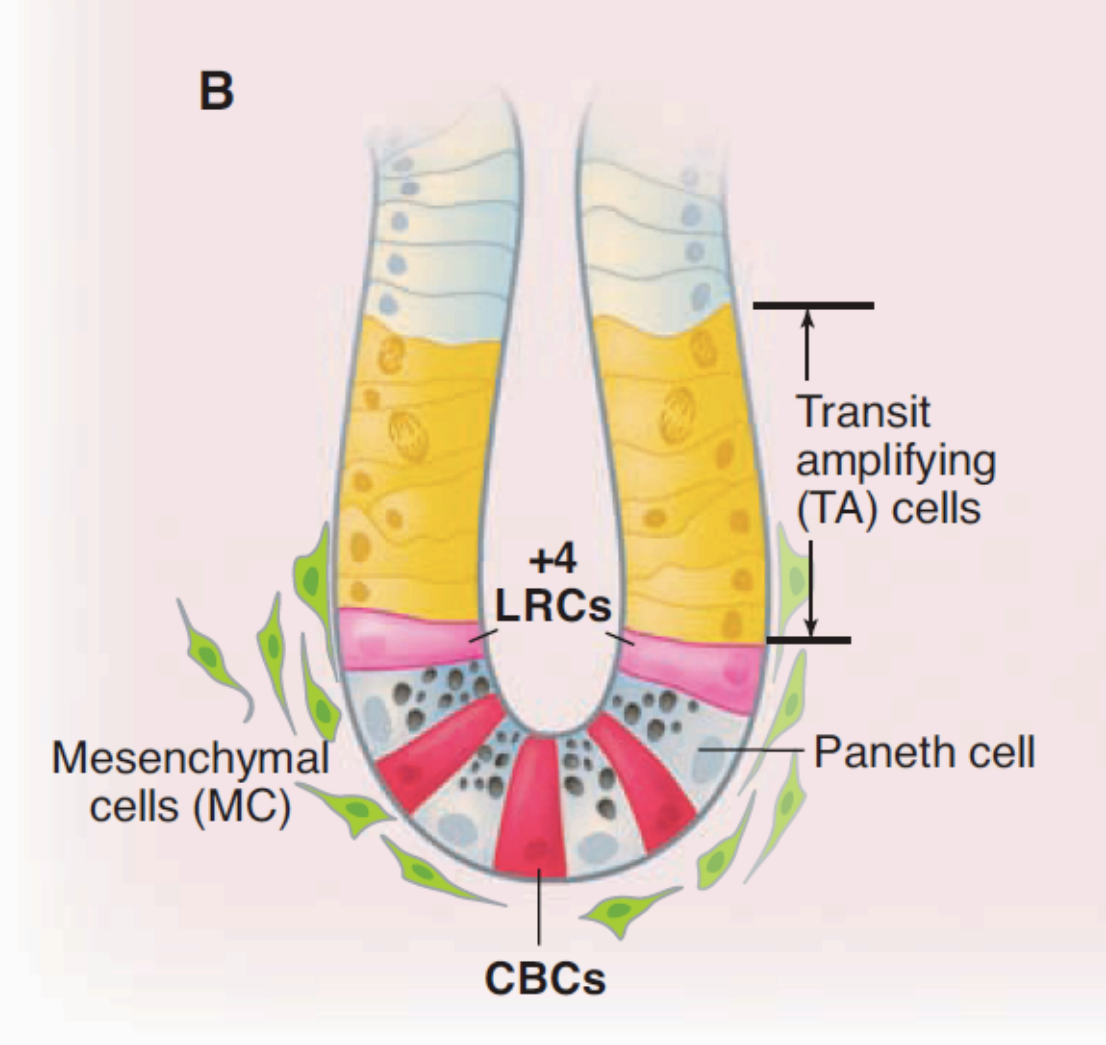
Villi

Crypts

Differentiated cell types of SI



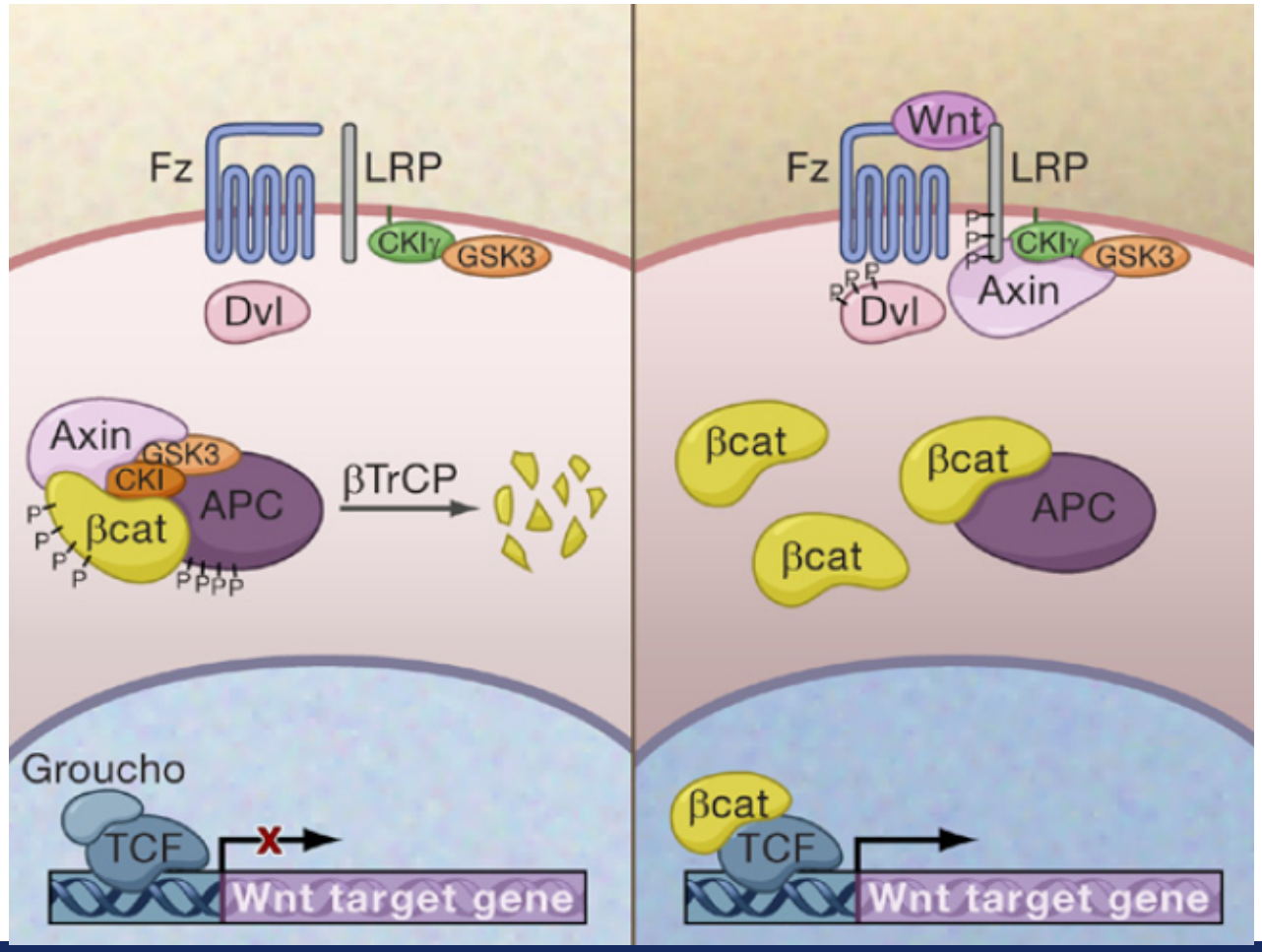
SCs in the intestine



canonical Wnt signaling

Wnt/ß-catenin Pathway OFF

Wnt/ß-catenin Pathway ON



Wnt signaling is essential in the intestine

→Proliferation of epithelial cells in intestine is Wnt –dependent

- **Evidence from mouse models:**

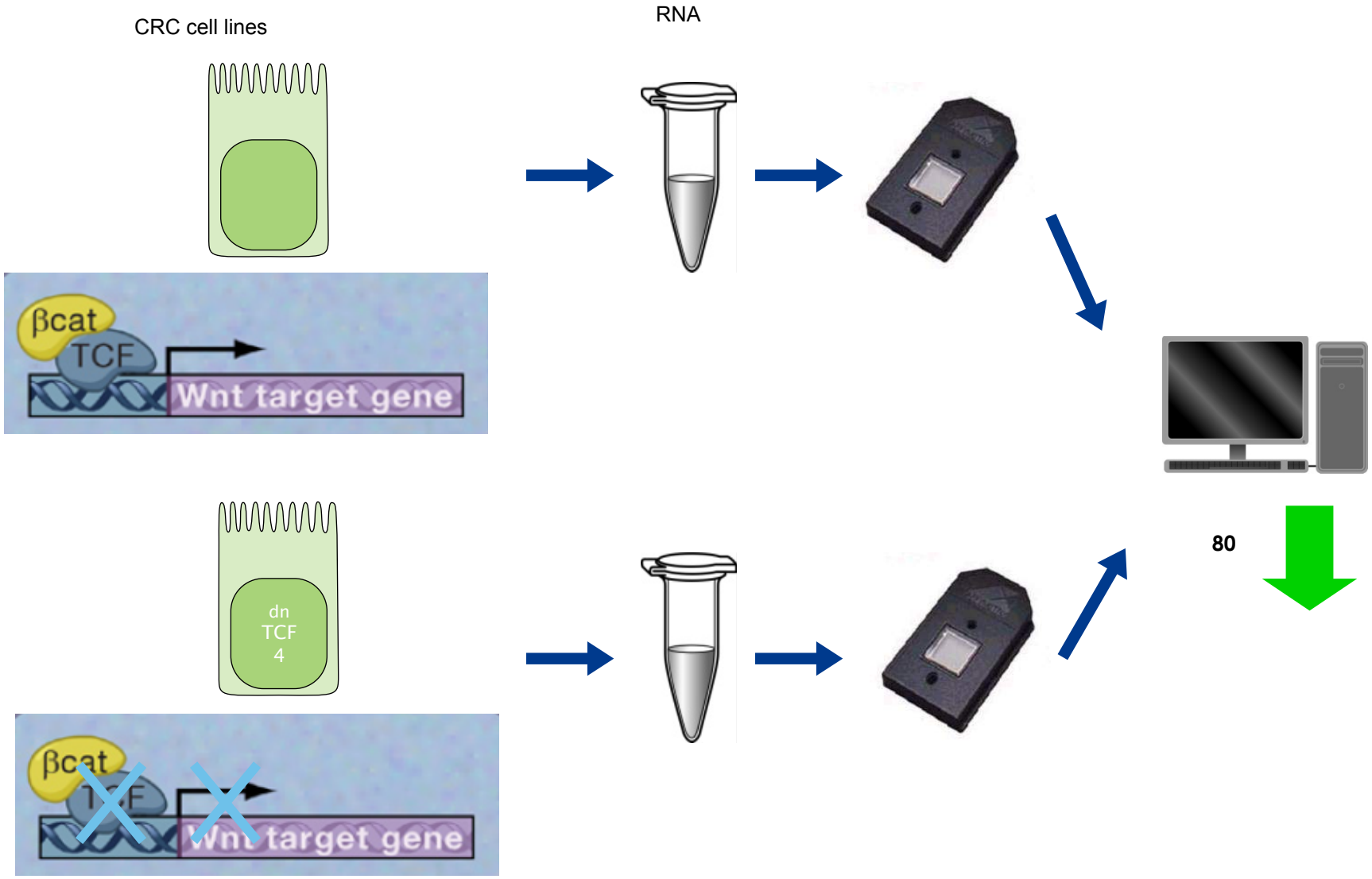
  - TCF4 $^{-/-}$ mice→ no proliverativ crypts

  - conditional beta Catenin KO, DKK1 transgene →
  →proliferative cypts dissappear in adult mouse

- **Evidence from human intestinal cancers and cell lines:**

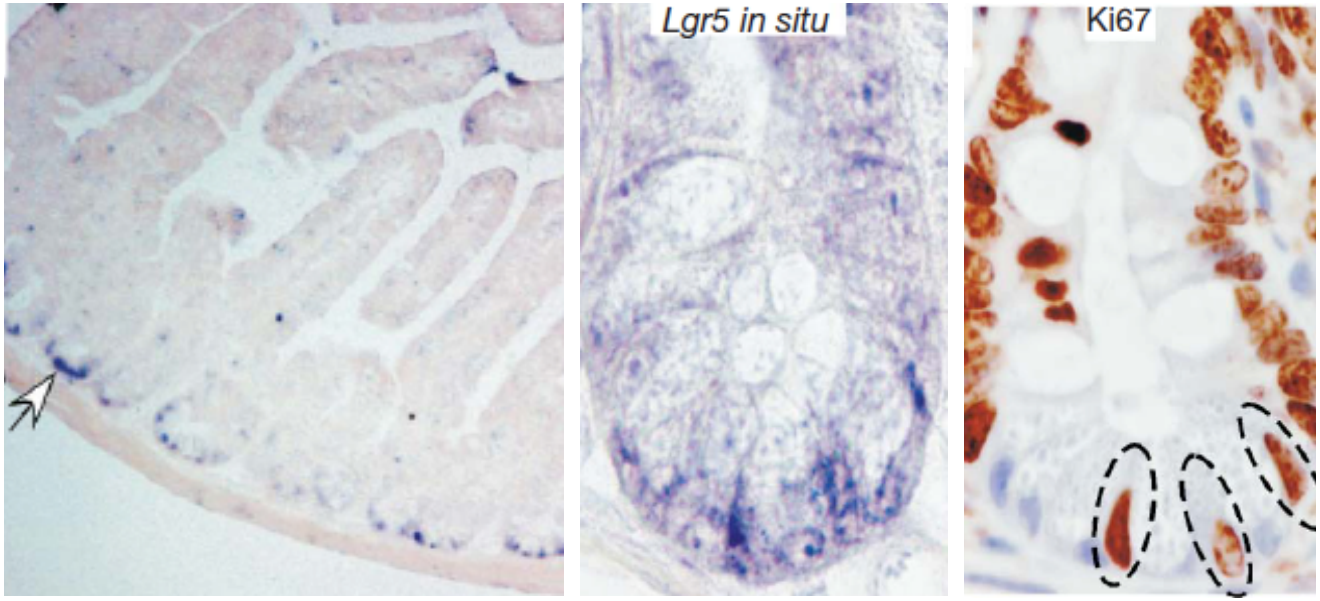
  - wnt pathway always ON, loss of APC, beta-Catenin often overexpressed, mutated and nuclear

Wnt target Genes

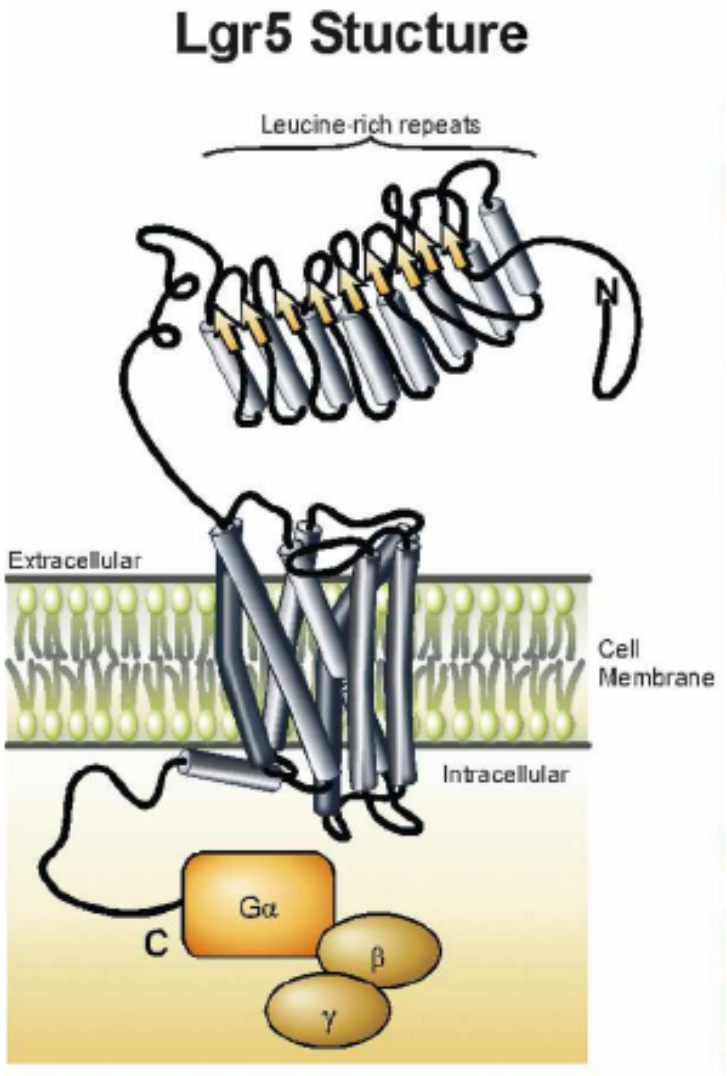


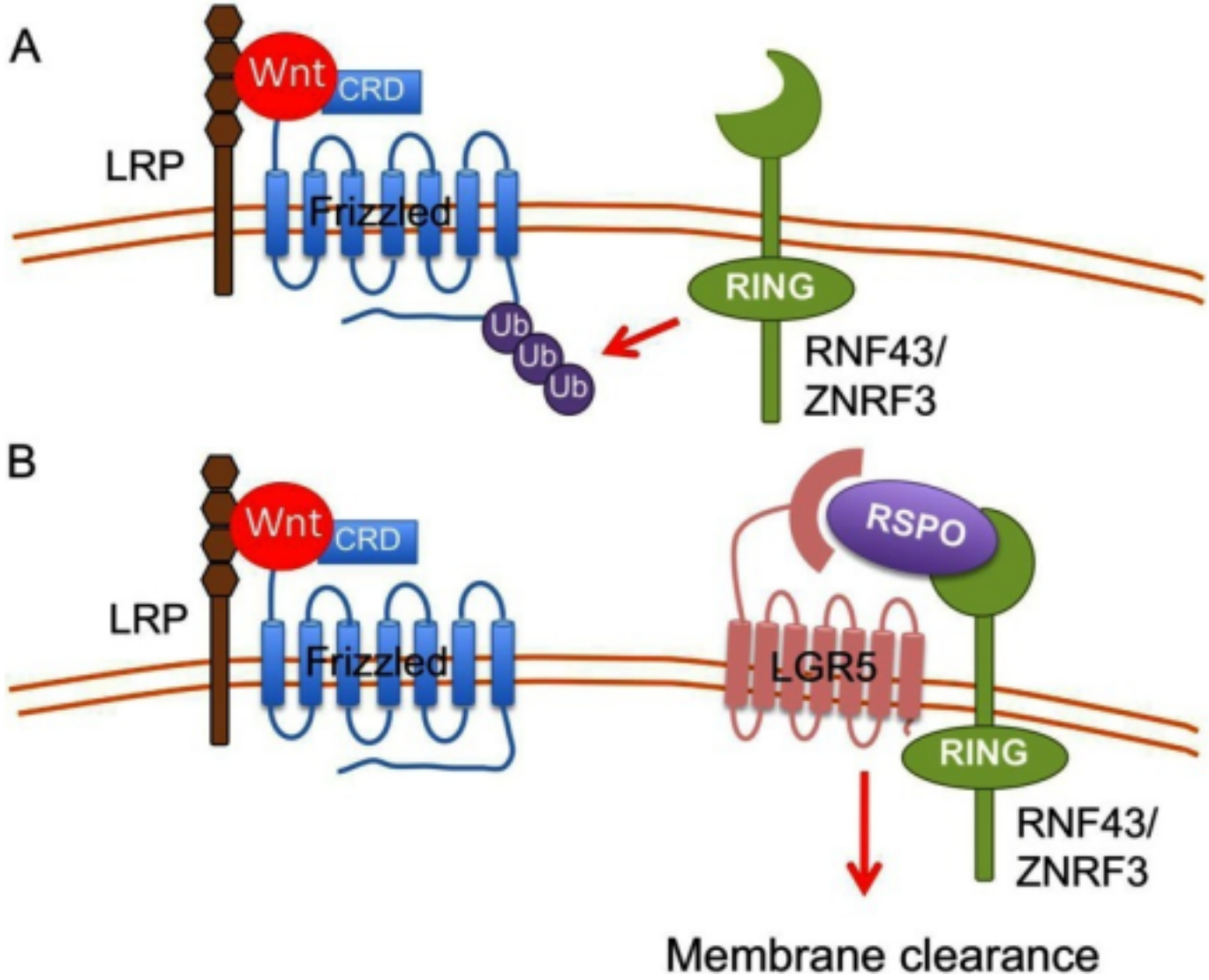
LGR5 = GPCR49

LGR5 ISH

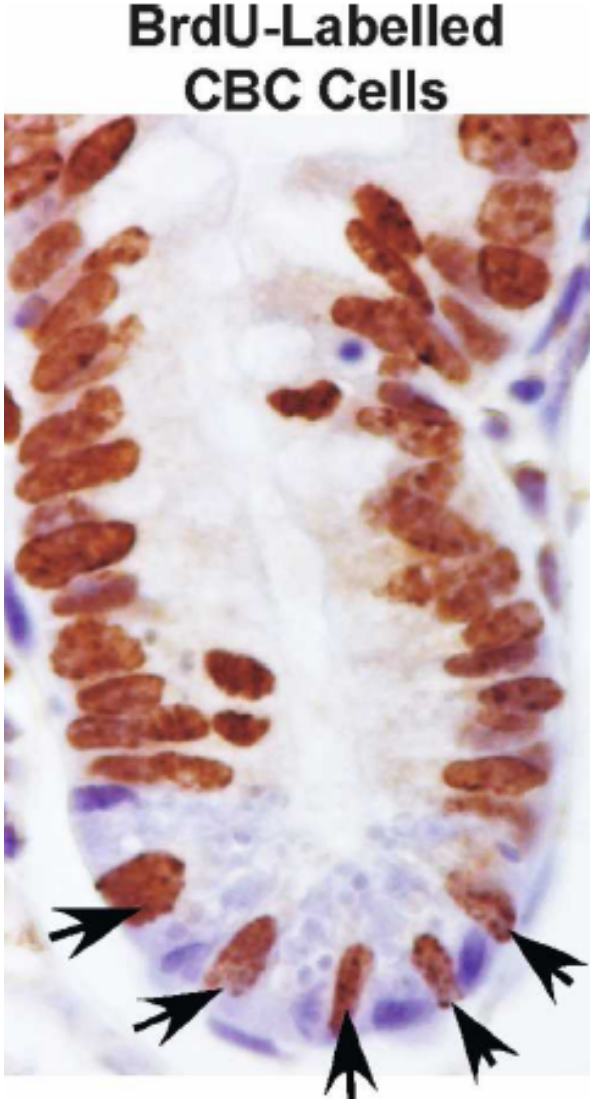


LGR5 = GPCR49


Lgr5 Stucture
Leucine-rich repeats
N
Extracellular
Cell
Membrane
Intracellular
Gα
β
γ
C


A
Wnt
CRD
LRP
Frizzled
Ub
Ub
Ub
RING
RNF43/
ZNRF3
B
Wnt
CRD
LRP
Frizzled
RSPO
LGR5
RING
RNF43/
ZNRF3
Membrane clearance

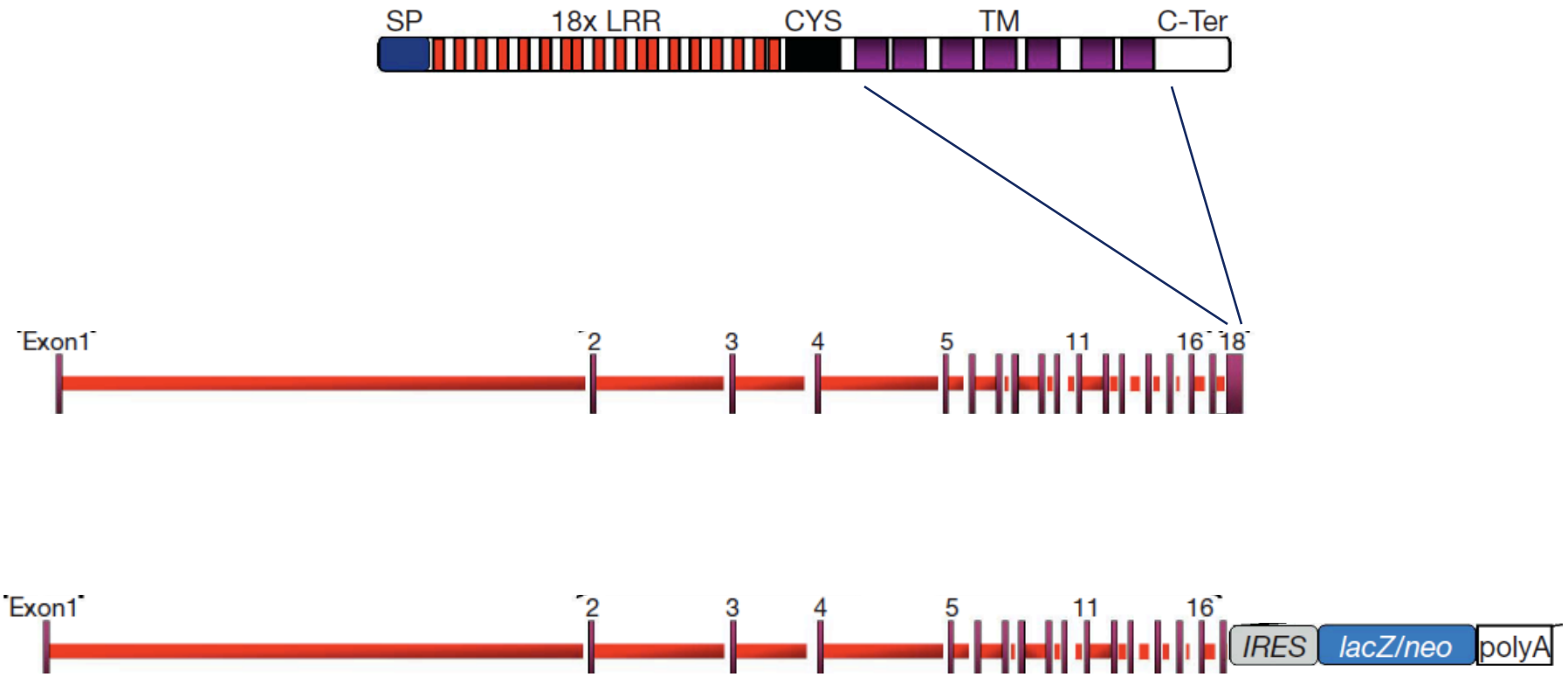
LGR5+ cells cycle



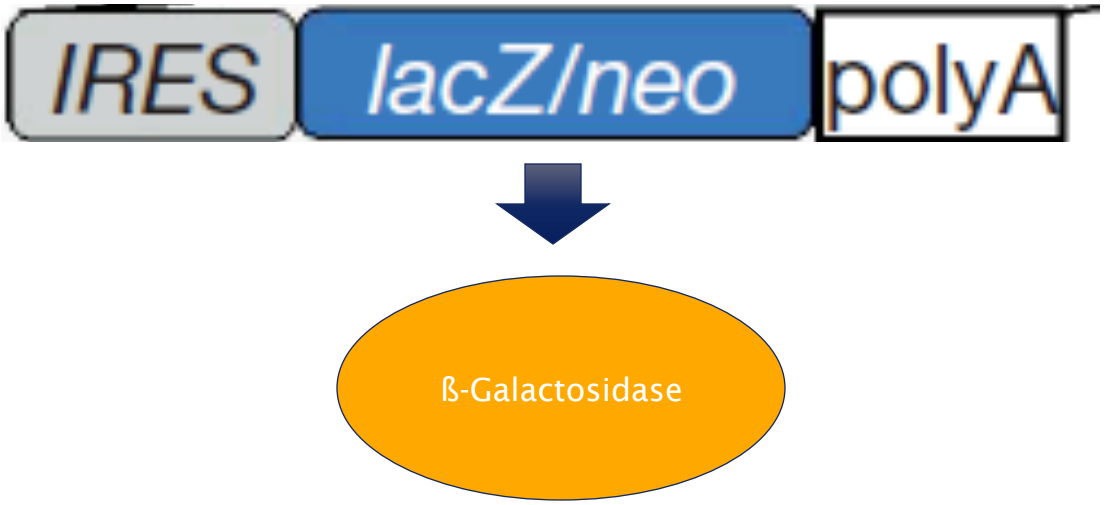
CBC cells = crypt base columnar cells

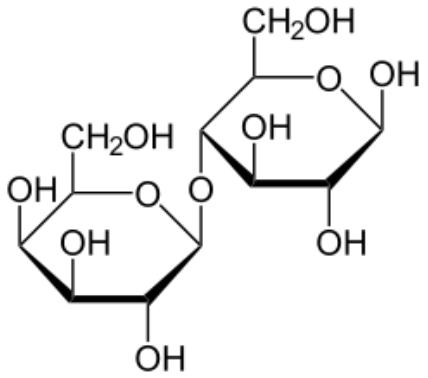
BrdU Labelling for 24 hrs

LacZ knock in allel



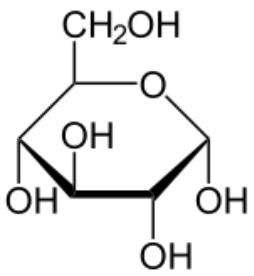
LGR5 = GPCR49



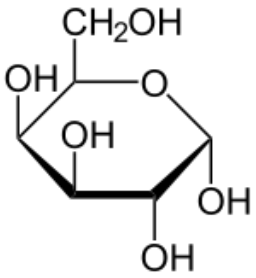


Lactose

Glucose

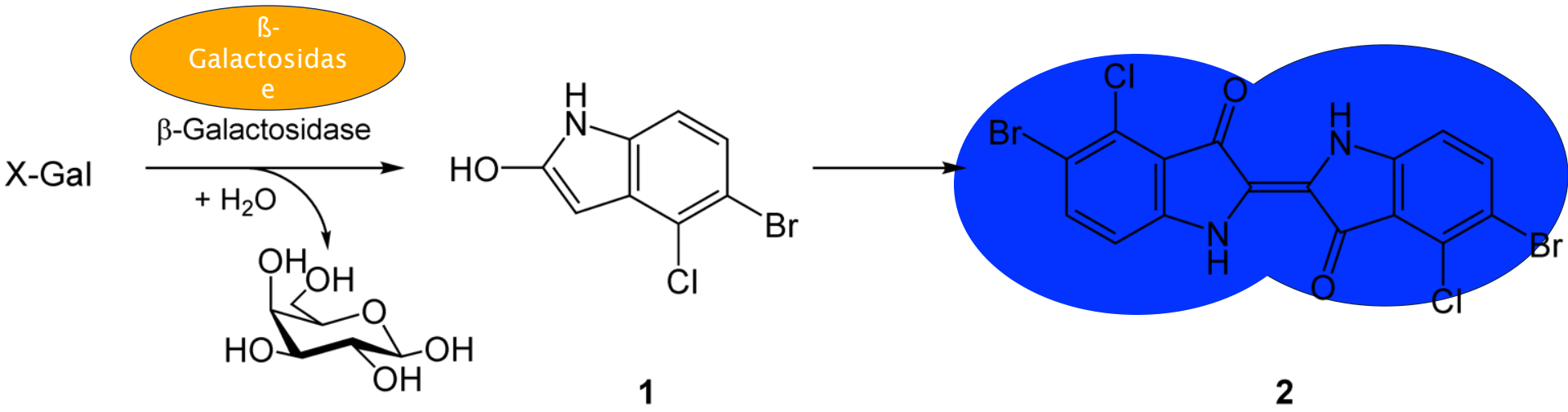


+



Galactose

X-Gal

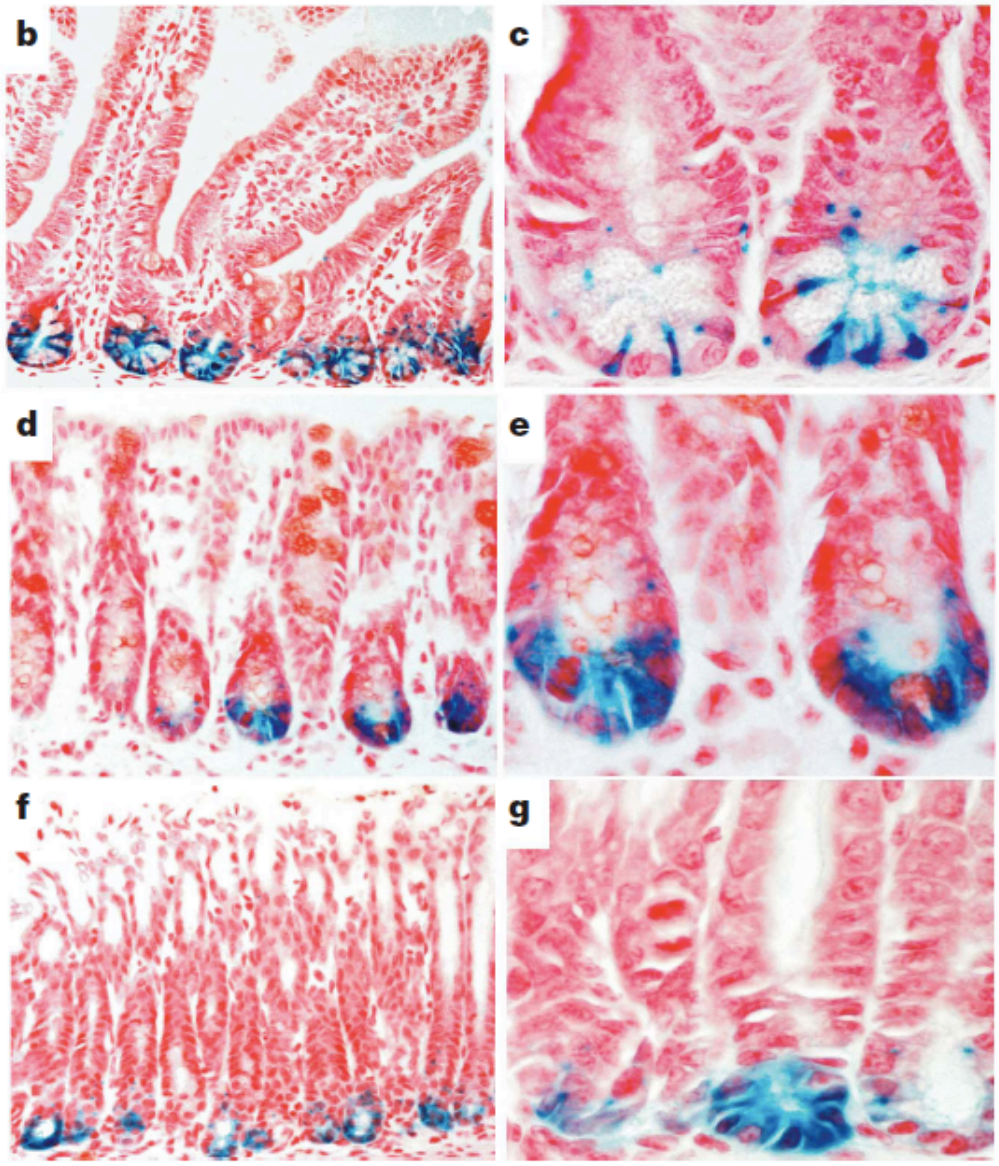

ß-
Galactosidas
e
β-Galactosidase
X-Gal
+ H_2O
OH
OH
O
HO
OH
OH
H
N
HO
Br
Cl
1
Cl
O
Br
H
N
N
H
O
Br
Cl
2

Cell tracking

Dünndarm

Colon

Magen



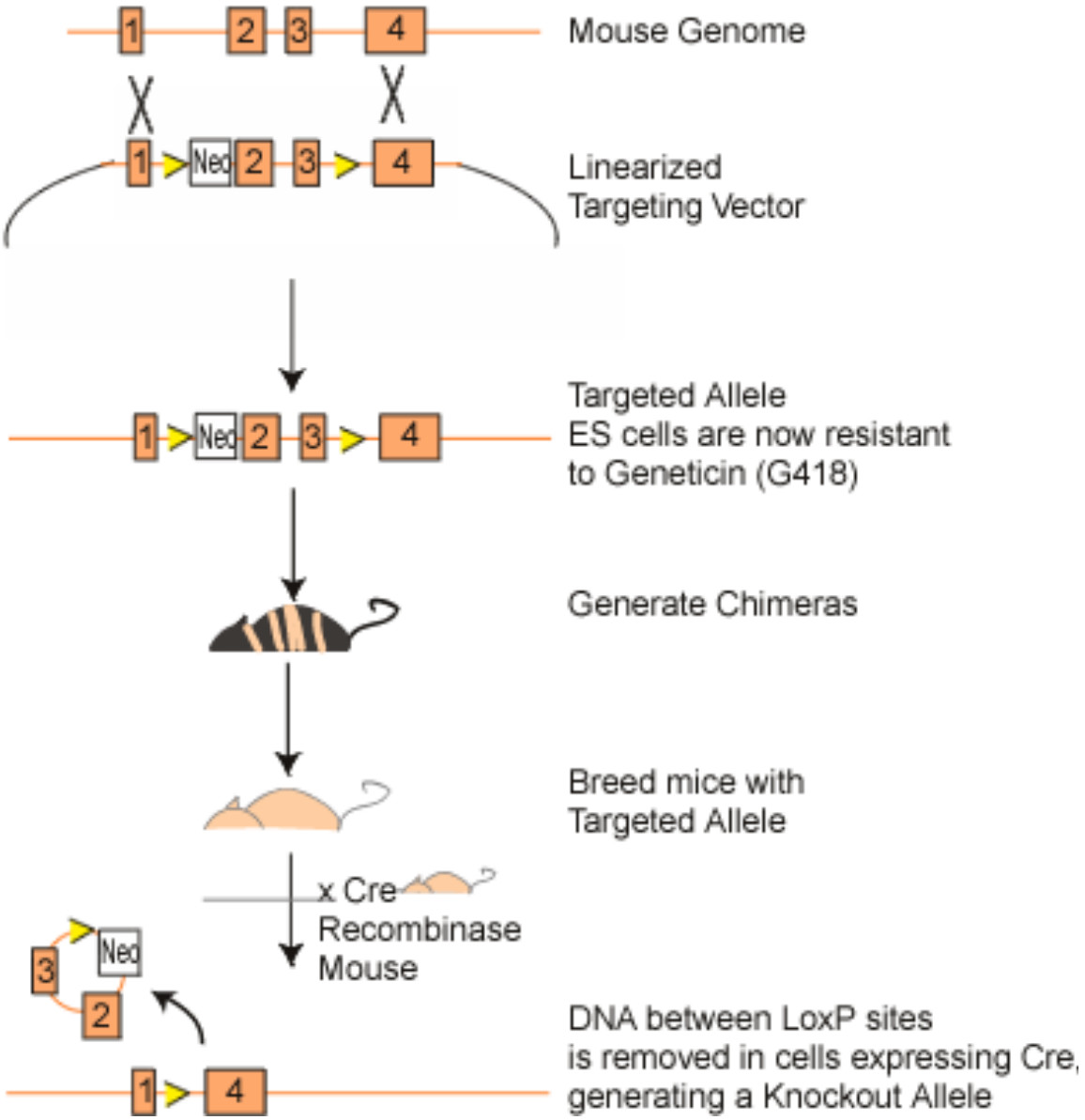
Conditional Knockout

Cre recombinase

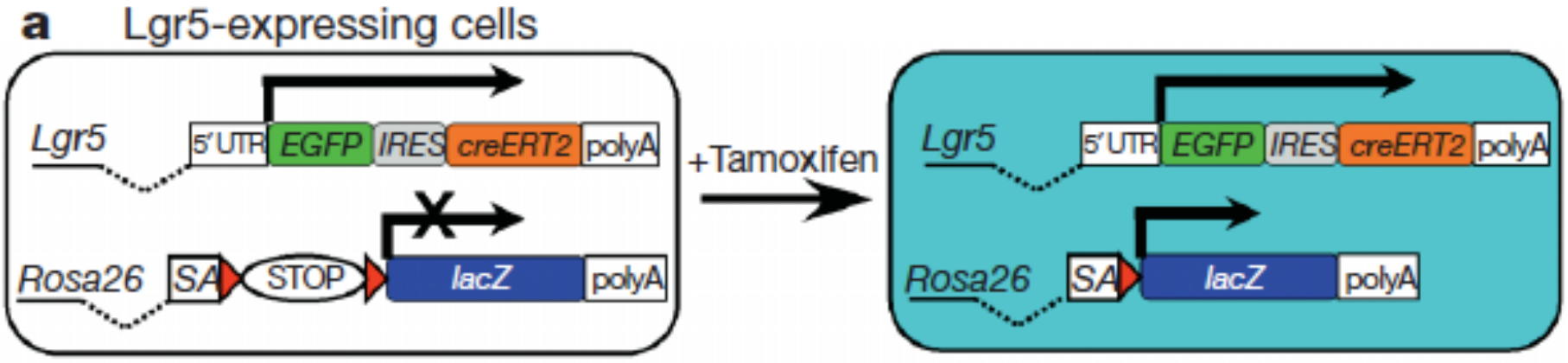
(Cre= **c**auses **re**combination)

Aus Bakteriophagen P1

Erkennt spezifische Sequenzmotife (loxP sites)
und
Excisiert dazwischenliegende
Sequenzen



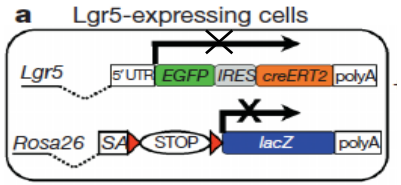
Cell tracking/fate mapping



Rosa26 promoter is active in almost every cell

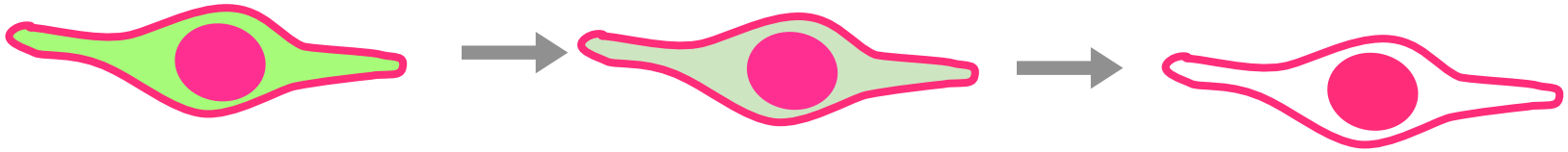
Expectet results

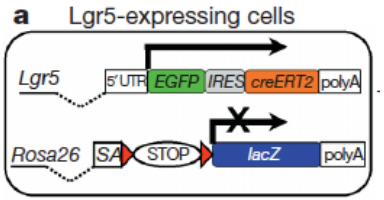
LGR5 not expressed

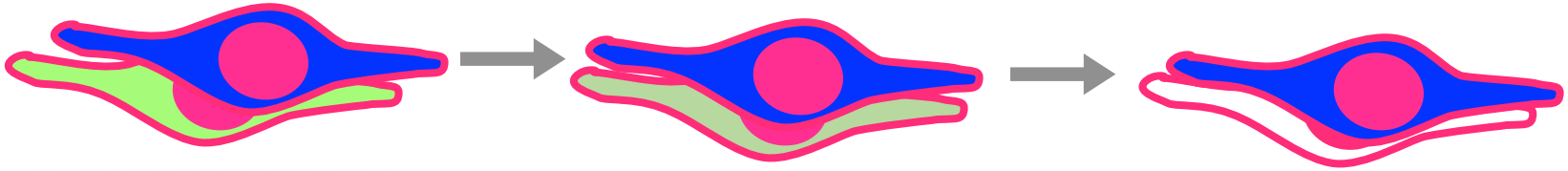


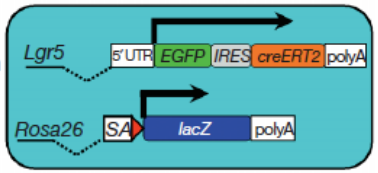
LGR5 expressed

LGR5 not expressed

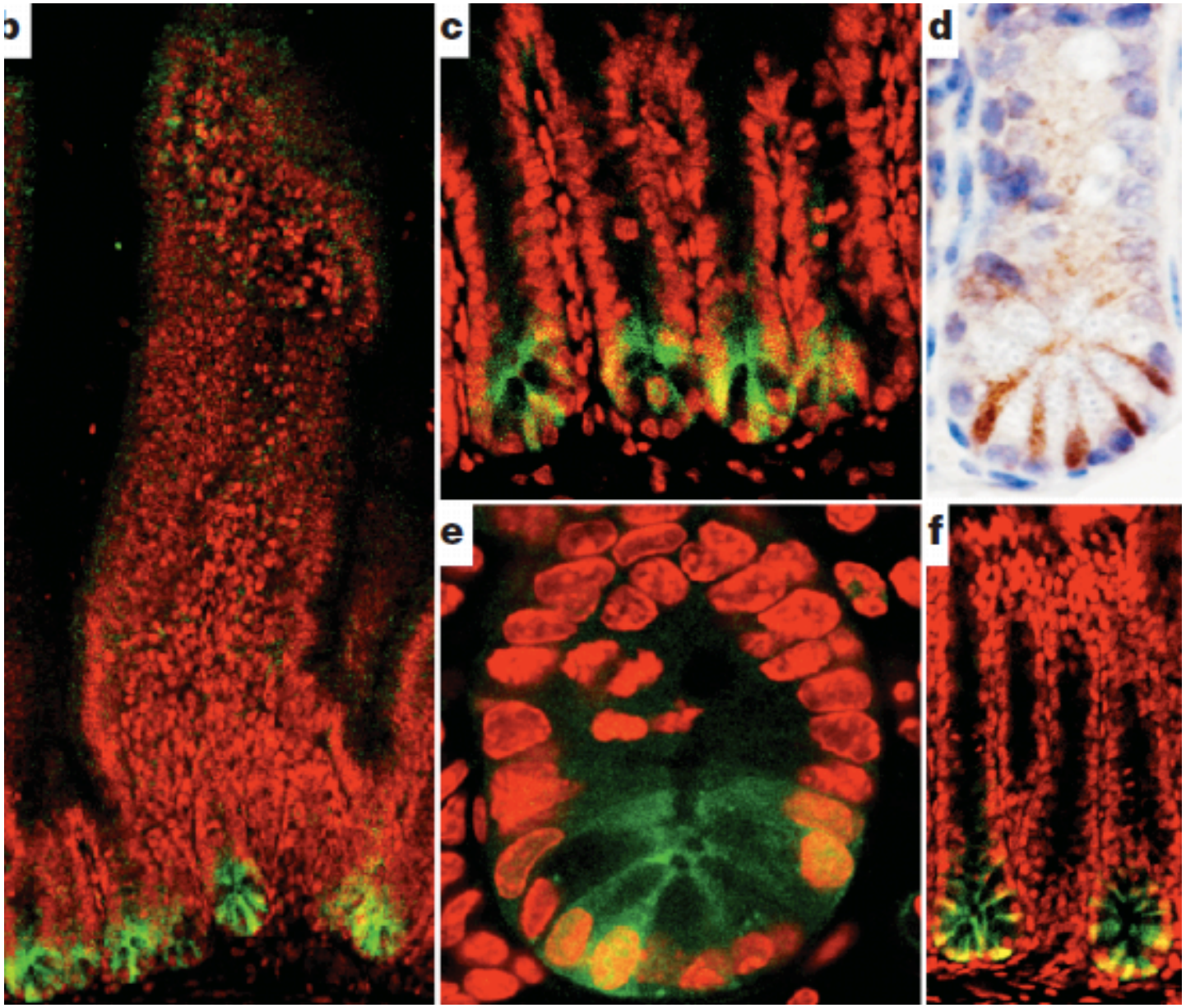








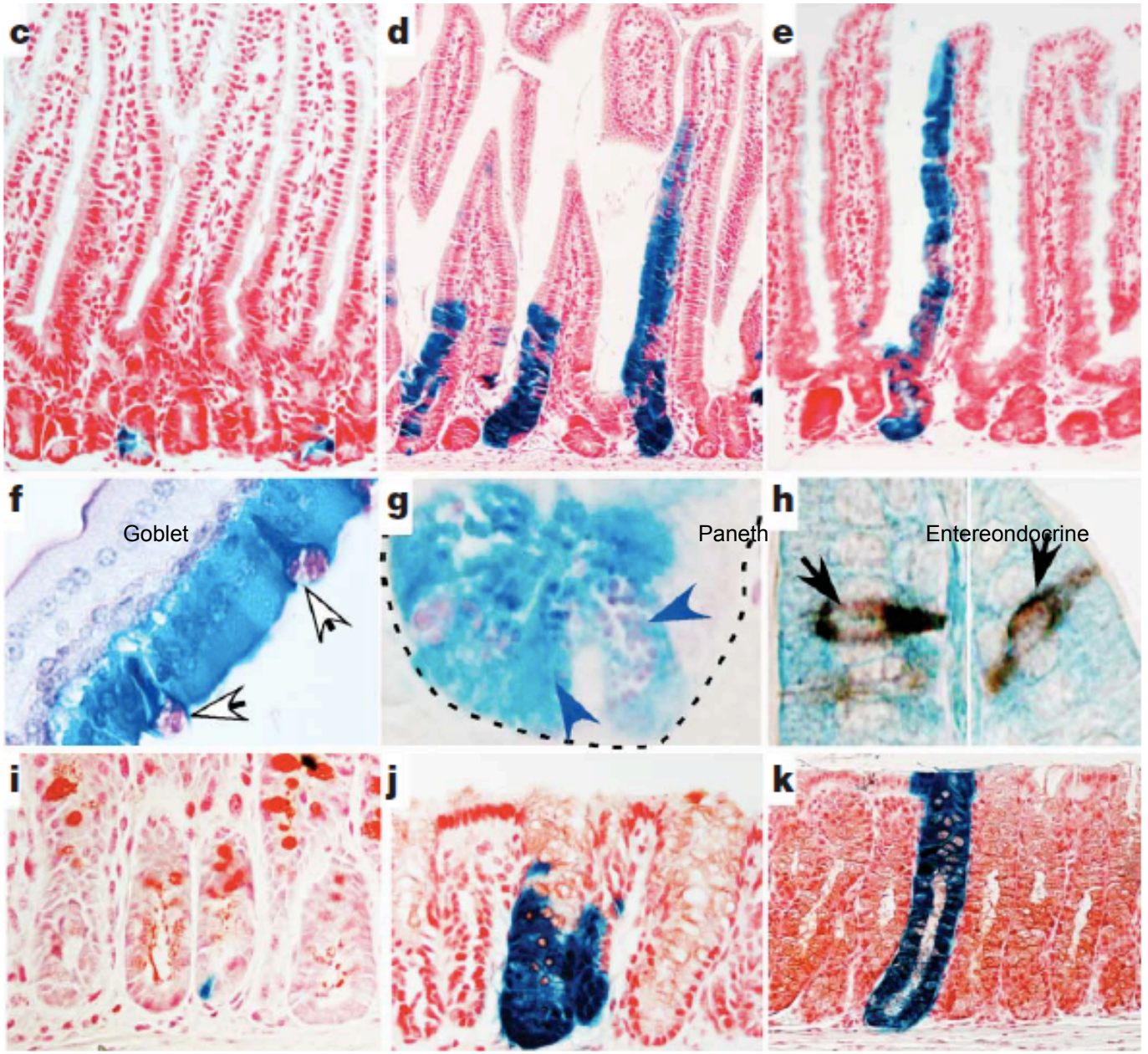
GFP (LGR5 actively expressed)



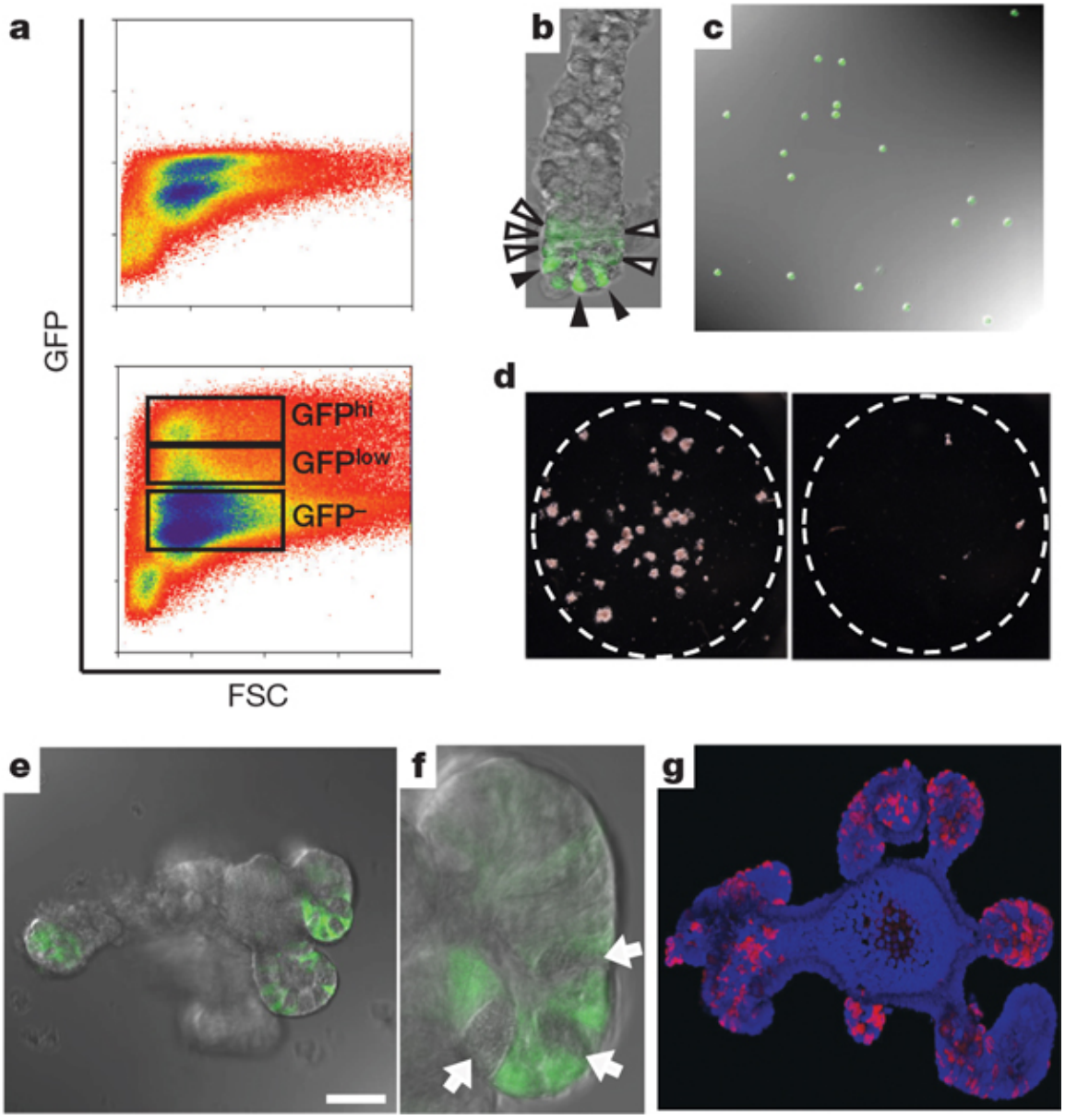
Fate Mapping

Dünndarm

Colon



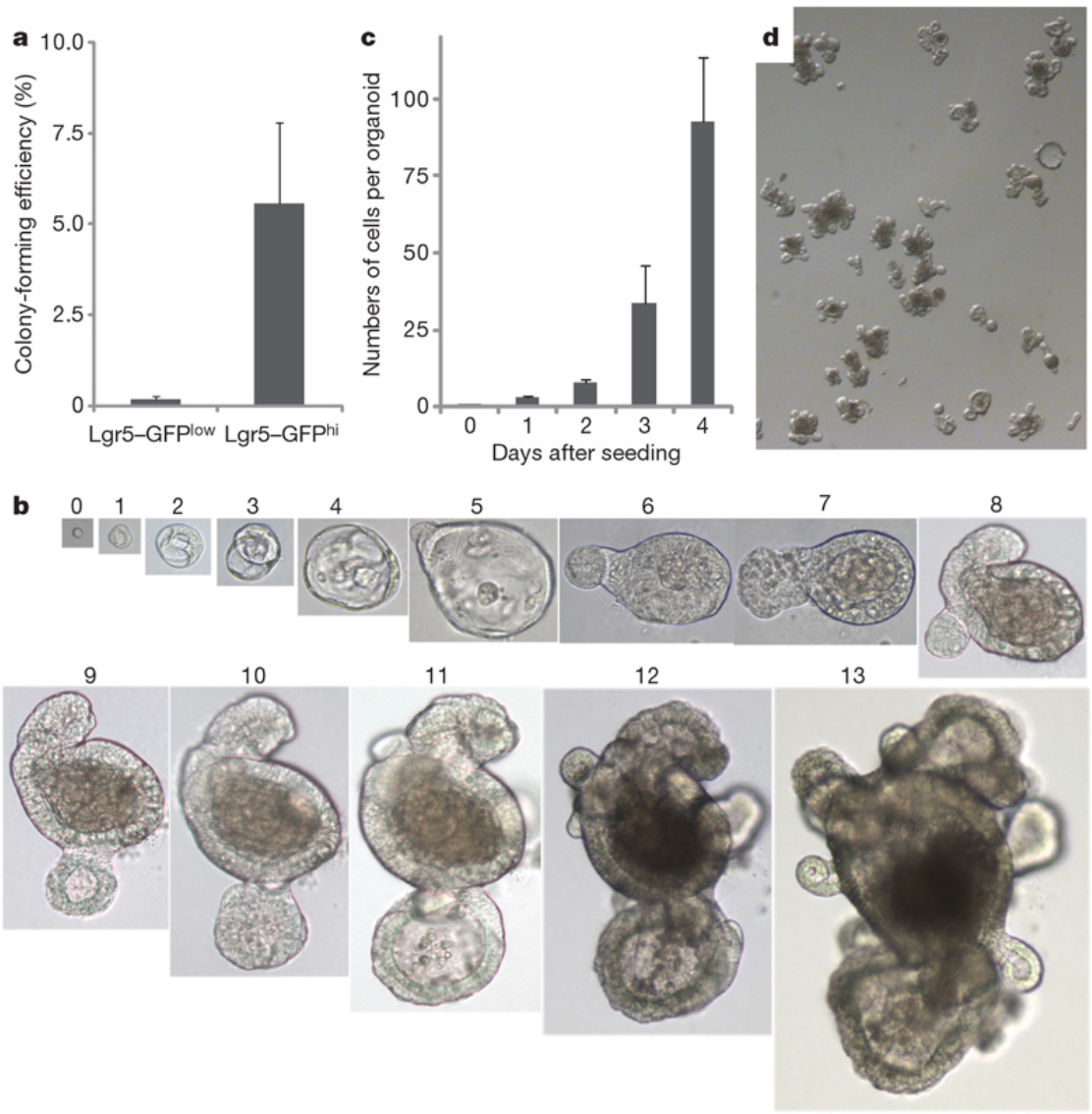
Isolation of LGR5^{+} stem cells (GFP^{+})

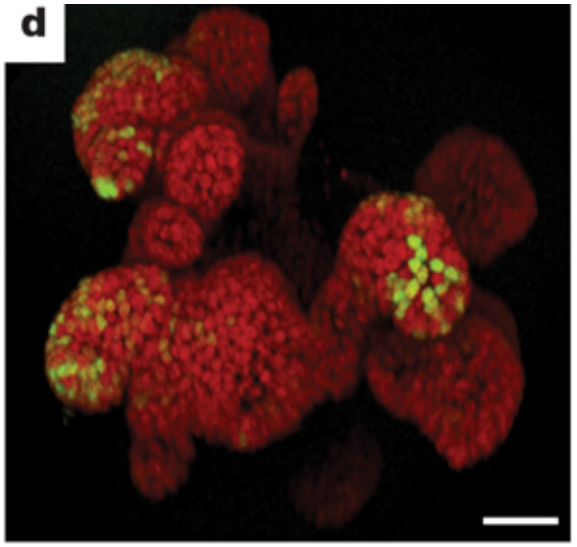


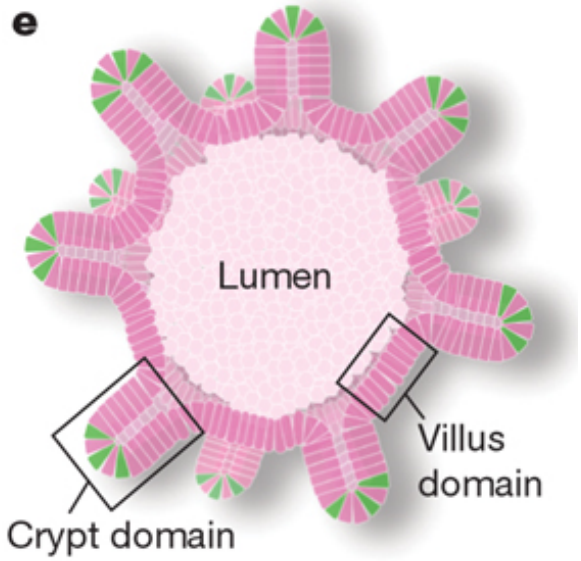
Kulturmedium:

Matrigel
EGF
R-spondin 1 (Wnt Agonist)
Noggin (TGFbeta Antagonist)

Colony-forming efficiency

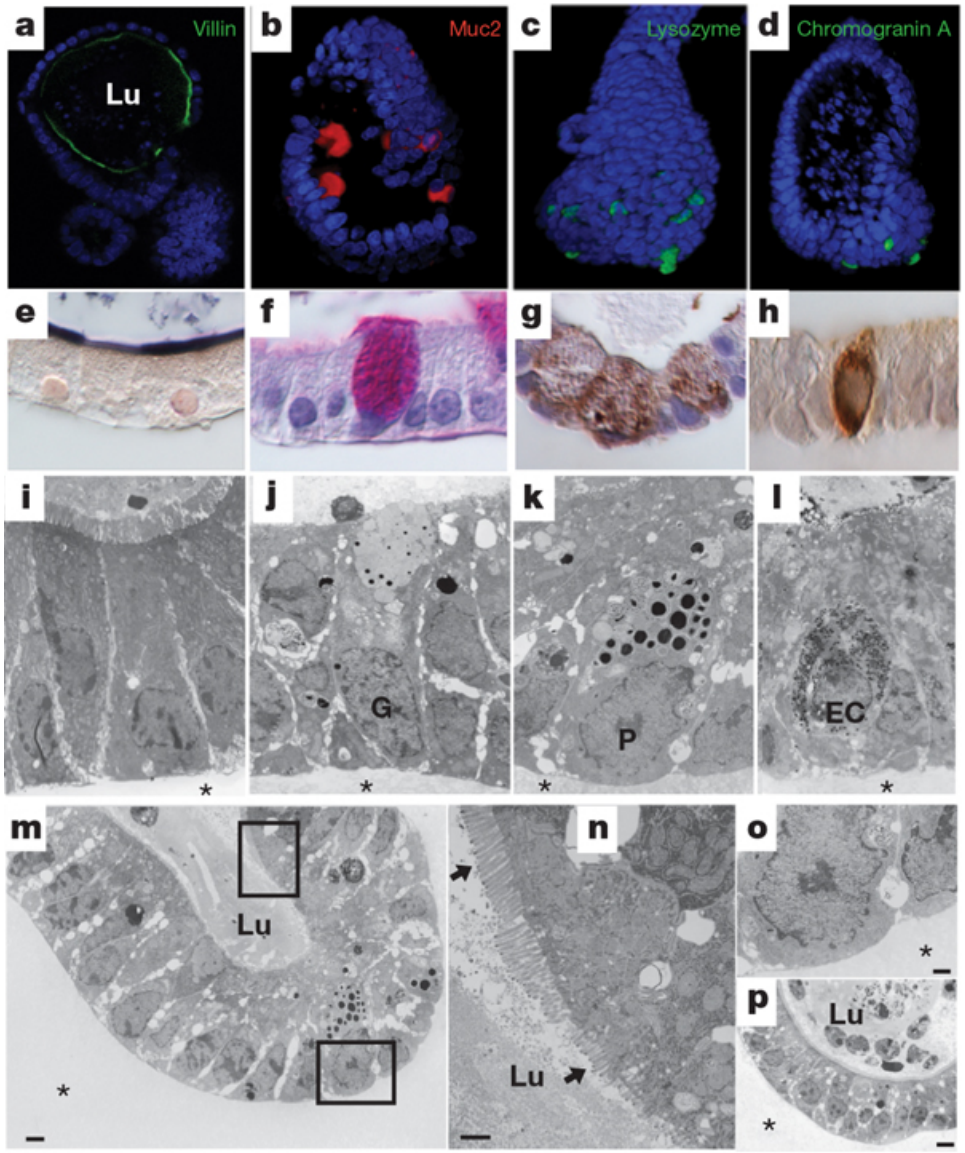






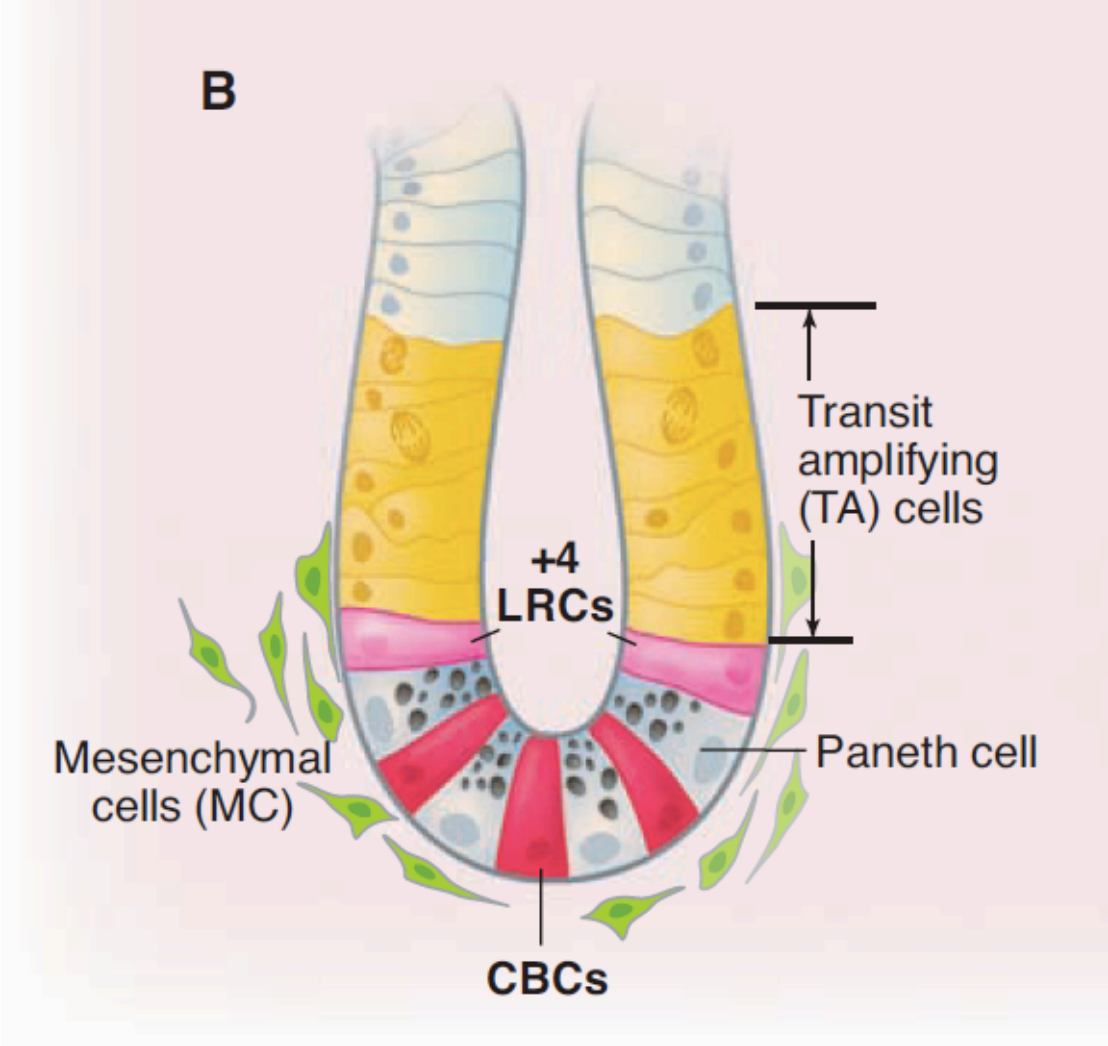
T Sato *et al. Nature* **000**, 1-4 (2009) doi:10.1038/nature07935

Composition of single stem cell-derived organoids

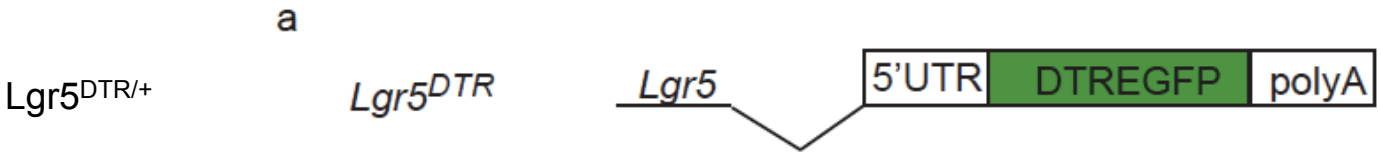


T Sato *et al. Nature* **000**, 1-4 (2009) doi:10.1038/nature07935

SCs in the intestine



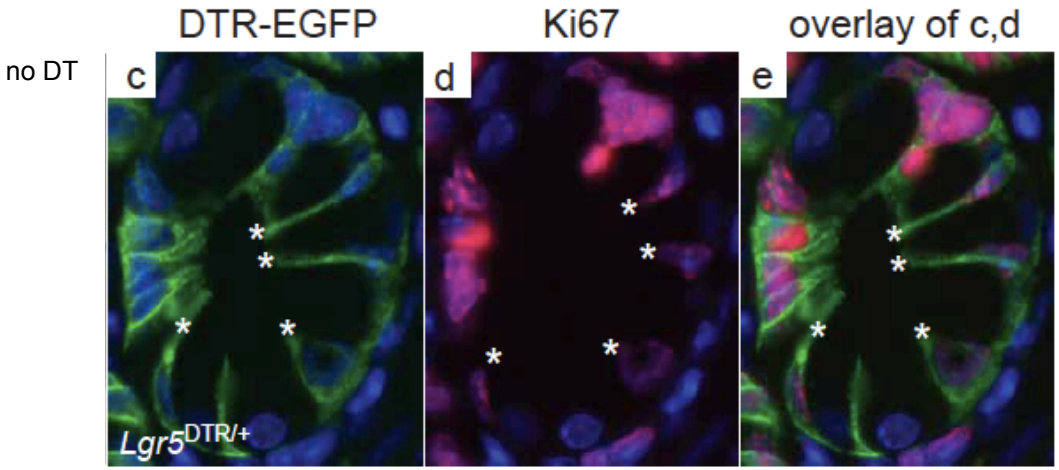
LGR5+ stem cell depletion



-> fusion gene of diphteria toxin receptor (DTR) and enhanced green fluorescent protein (EGFP)

Lgr5 expression -> green fluorescence

application of diphteria toxin (DT) -> cells die, no fluorescence



Intestine remains intact

effects of depleted Lgr5?

Lgr5$^{DTR/+}$ mice

after DT administration no Lgr5 positive cells

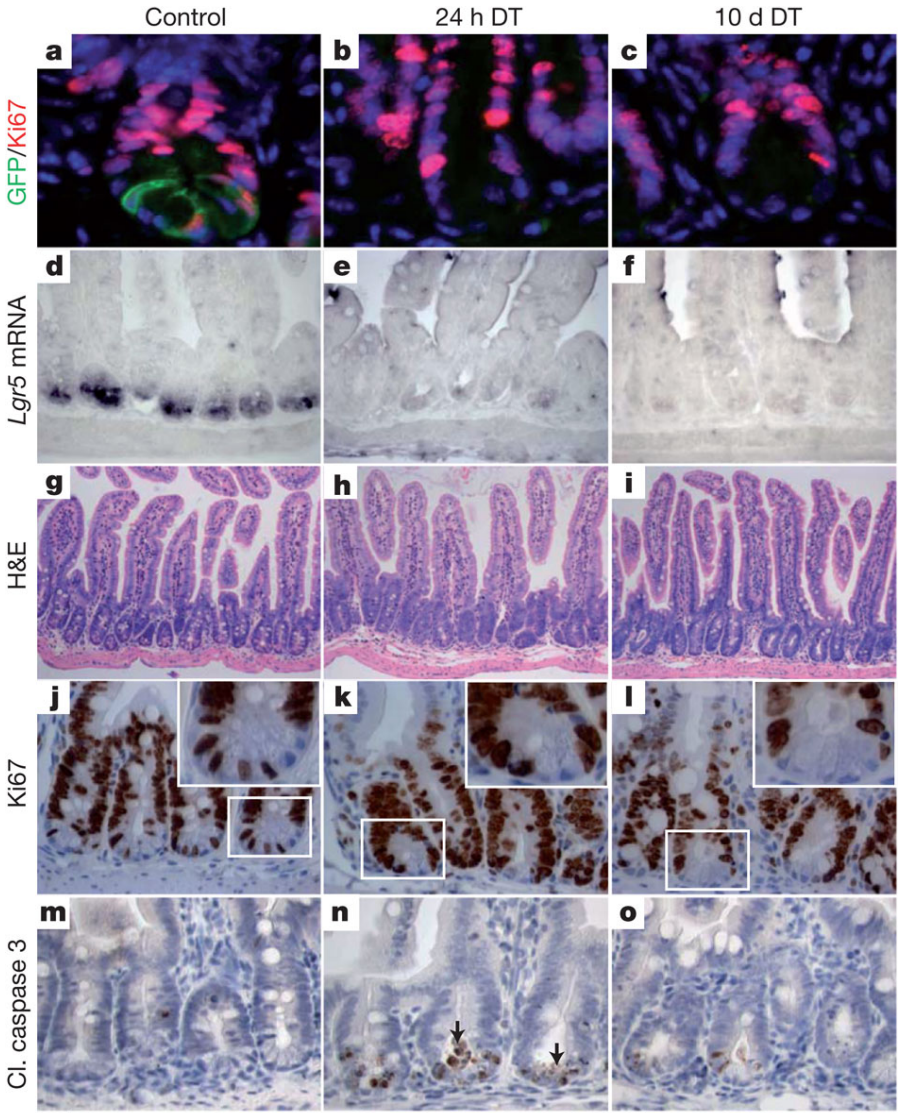
no Lgr5 mRNA present

architecture of epithelium intact

CBCs depleted

apoptosis induction after 24h
after 10 d decrease but still visible

crypt architecture compareable to controls



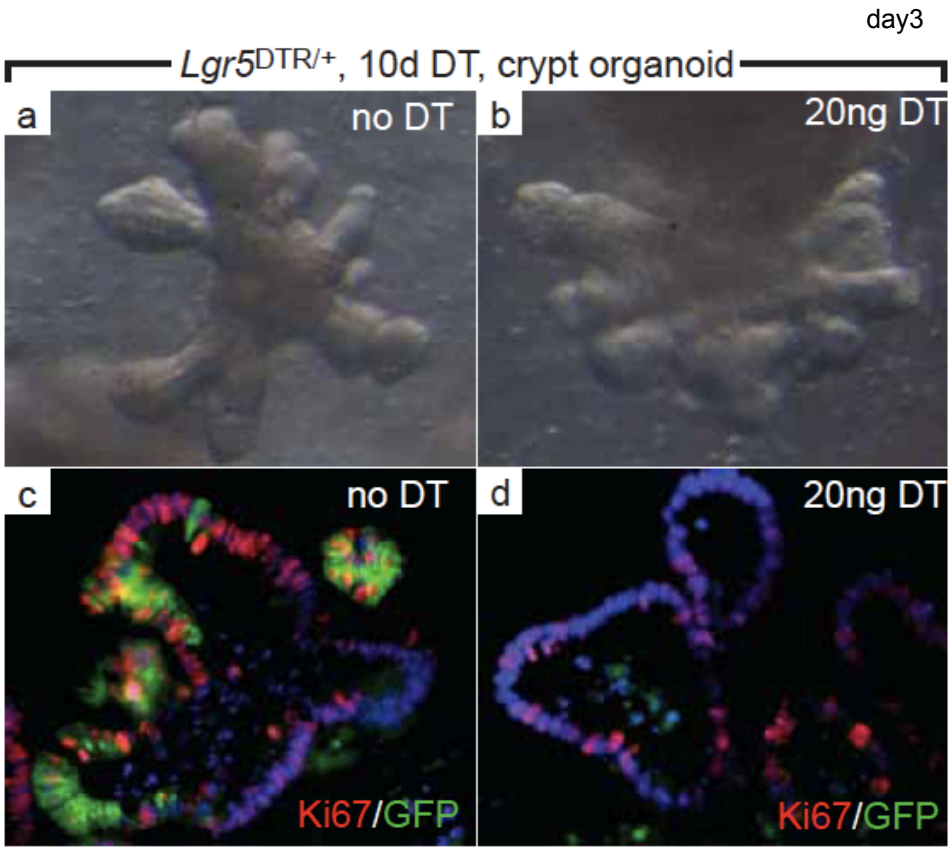
Intestine remains intact

long term effect of CBC ablation?

Lgr5$^{DTR/+}$ mice -> *in-vitro* orgnoid culture

mice treated for 10 days with DT
-> crypts isolated

-> recovery a and c
-> prolonged treatment b and d



no difference to controls
Lgr5 depleted crypts passaged for up to 2 months

OCTOBER 2011 | VOL 478 | NATURE

A reserve stem cell population in small intestine renders *Lgr5*-positive cells dispensable

Hua Tian[1], Brian Biehs[2], Søren Warming[3], Kevin G. Leong[3], Linda Rangell[4], Ophir D. Klein[2] & Frederic J. de Sauvage[1]

Novel twist

Intestinal label-retaining cells are secretory precursors expressing Lgr5

Simon J. A. Buczacki[1], Heather Ireland Zecchini[1], Anna M. Nicholson[1], Roslin Russell[1], Louis Vermeulen[1], Richard Kemp[1] & Douglas J. Winton[1]

STEM CELLS

A unifying theory for the crypt

A long-standing ambiguity has been whether quiescent cells located in intestinal crypt structures are stem cells. The answer seems to be yes and no, depending on how one defines the term stem cell. SEE ARTICLE P.65

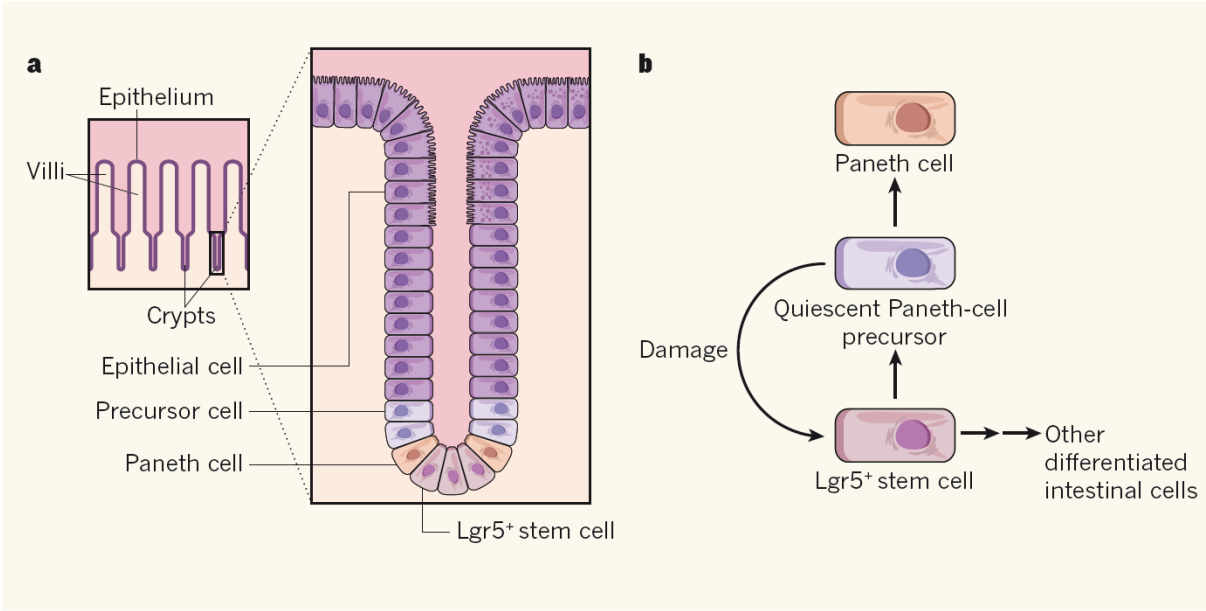


Figure 1 | Complexities of intestinal stem cells. **a,** The intestinal epithelium follows the distinct contours of villus–crypt units in the intestine. **b,** Normally, Lgr5-expressing stem cells (Lgr5$^+$) lead to the production of precursor cells that further differentiate into the various types of crypt epithelial cell. Buczacki *et al.*[1] report that precursors of one type of differentiated intestinal cell, Paneth cells, can persist for several weeks in a quiescent state before maturing into Paneth cells. Intriguingly, these quiescent precursors can revert back into Lgr5$^+$ stem cells following crypt damage.

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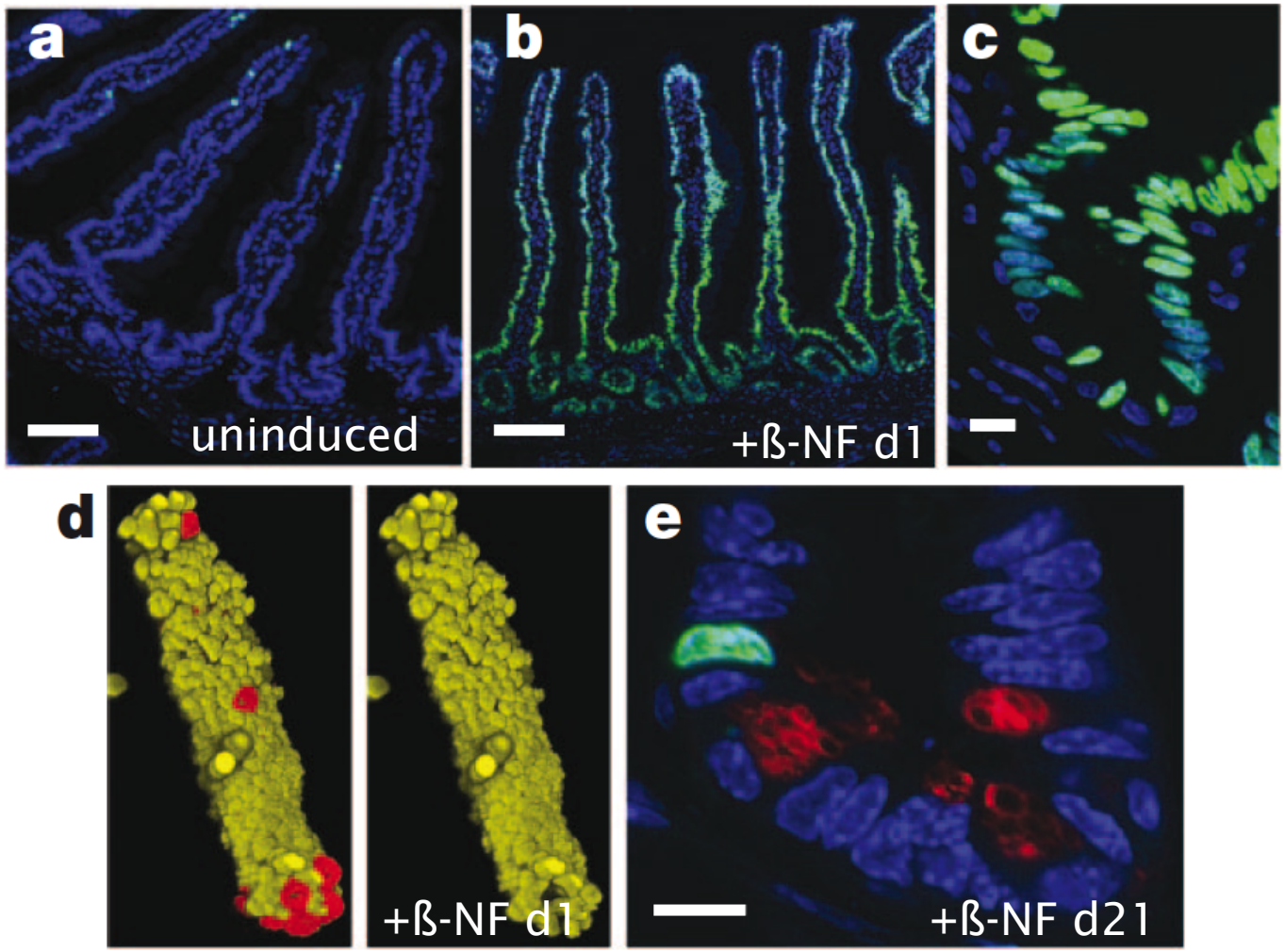
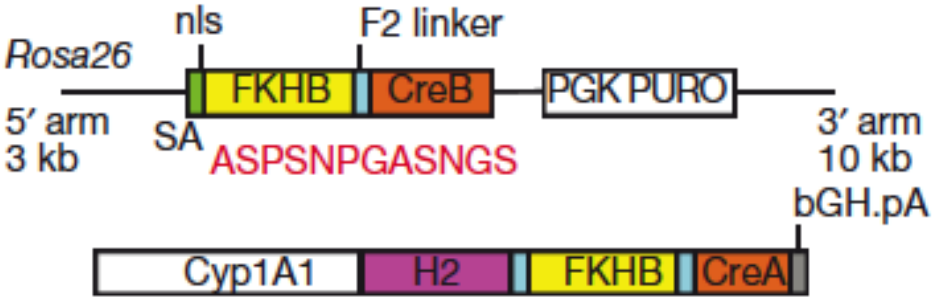
genetically labeling of LRC

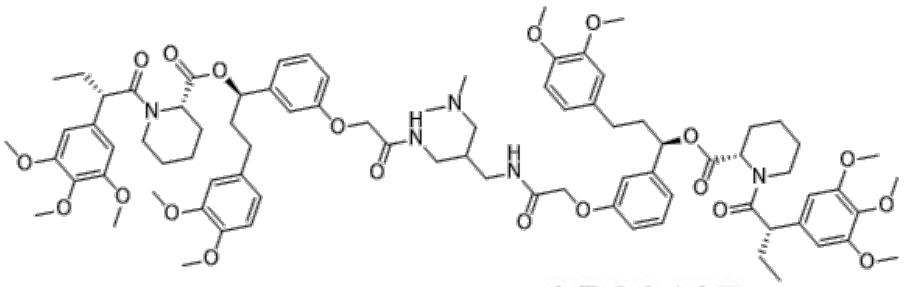
Cyp1a1 promoter driven expression of YFP-H2A fusion protein

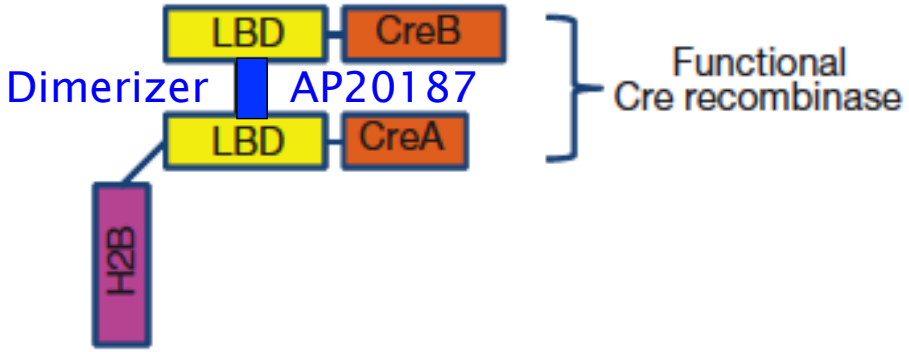
Cyp1a1 promoter expression on ßNF (naphtoflavon) induction in all cells of the crypt–villus axis with the exception of the mature Paneth cells

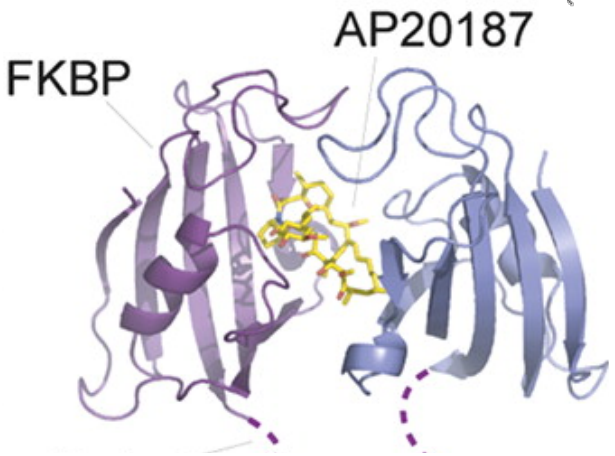


novel Cre (parts A anB dimerizable)

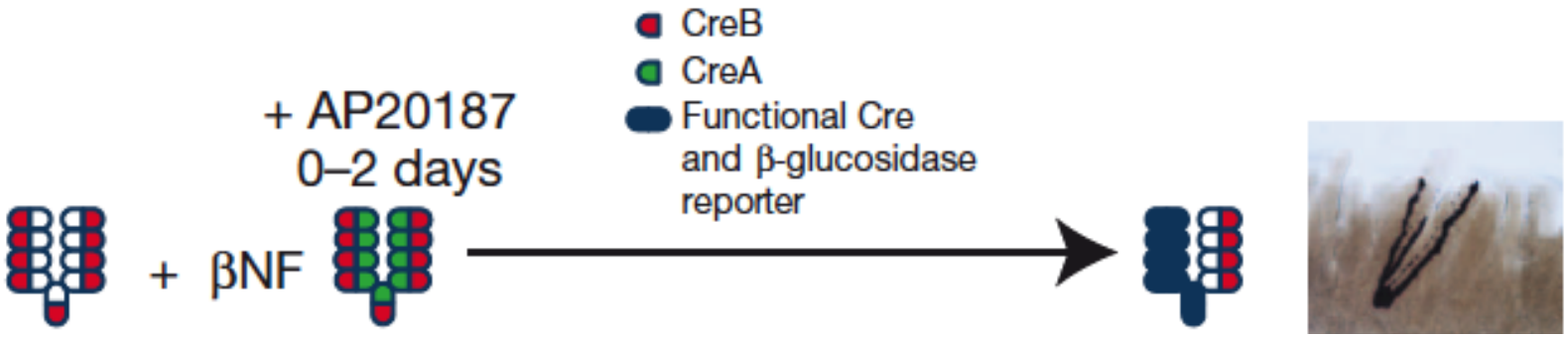






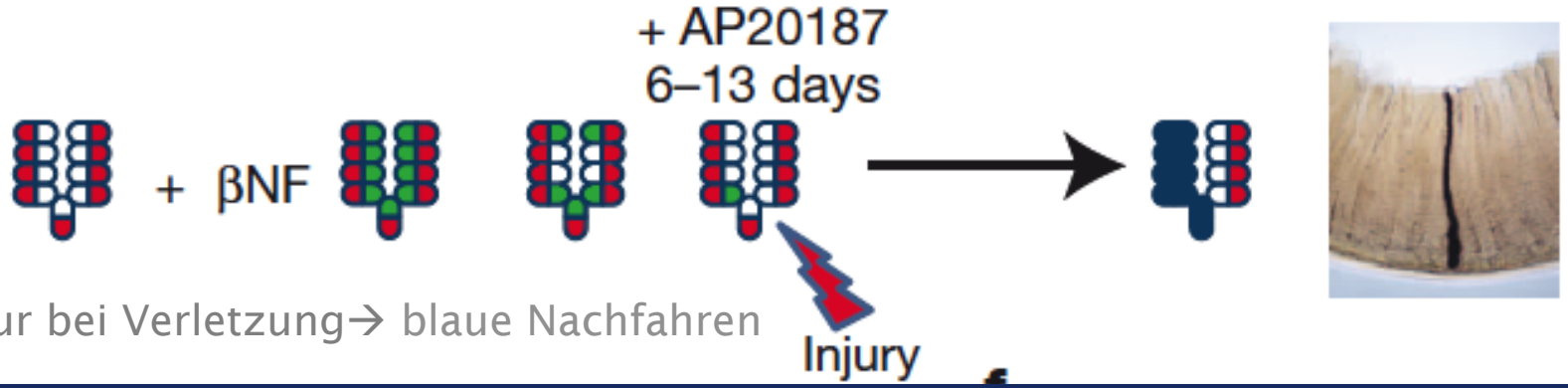


How are LRC genetically identified?



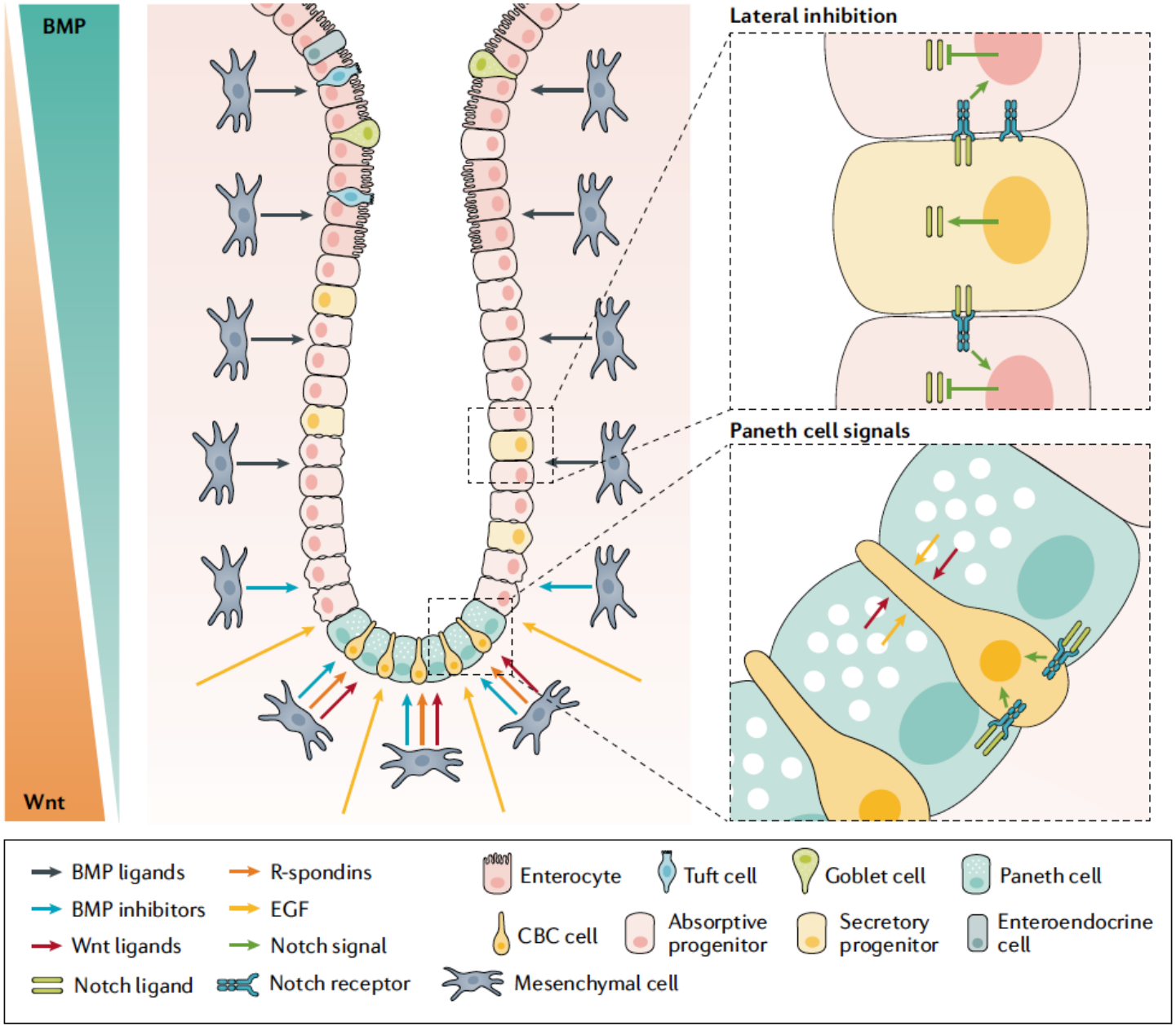
→Kontrolle: alle Zellen gelabeled: fate mapping → blaue Nachfahren

→unter Homöostase Bedingungen→ keine blaue Nachfahren



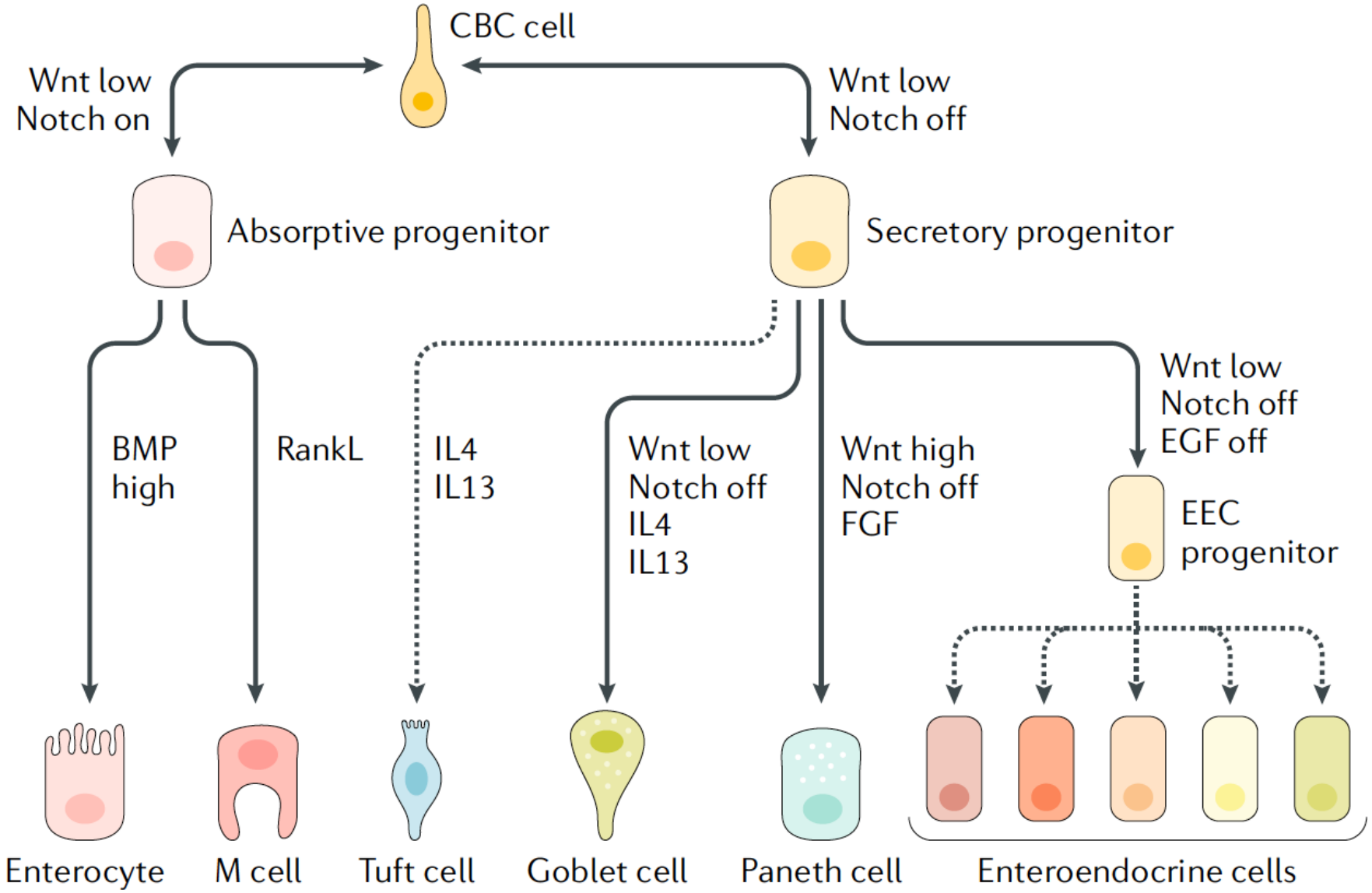
→nur bei Verletzung→ blaue Nachfahren

Current Model



Gehart and Clevers, 2019 Nat Rev Gastroenterology & Hepatolgy

Generation of differentiated cells

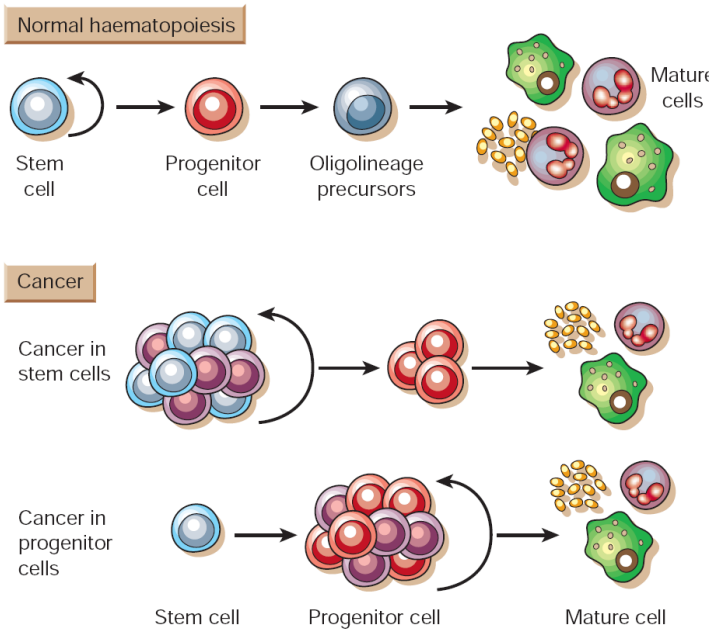


Gehart and Clevers, 2019 Nat Rev Gastroenterology & Hepatolgy

Cancer stem cells

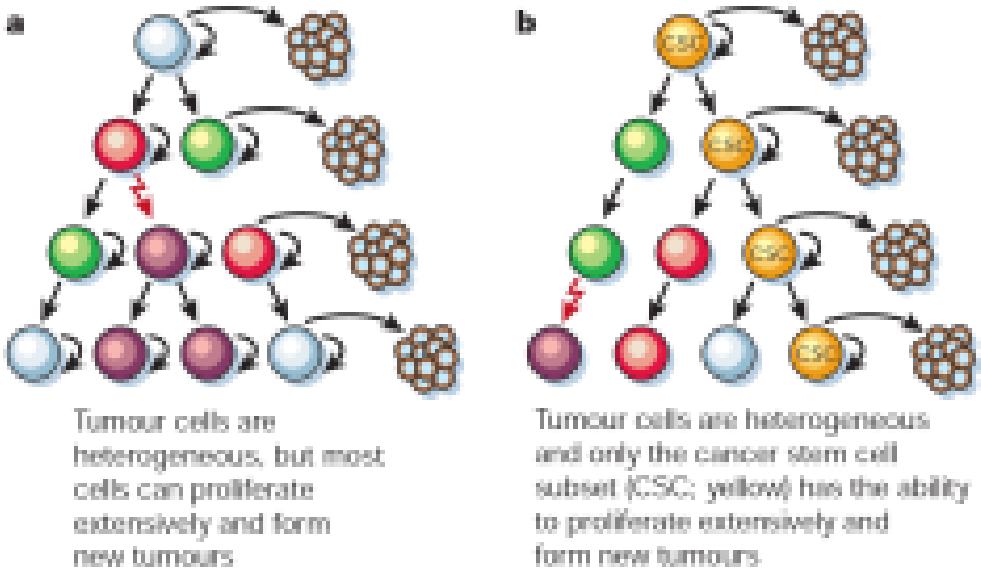
Cancer stem cells

- tumors are heterogenous, why?
  - ongoing mutagenesis can only partially explain this
- some tumors seem to arise from small populations of "cancer stem cells"
  - AML
    - AML stem cells (proliverative)
    - and remaining AML cells with reduced proliferative potential
  - also shown for breast cancer, glioblastoma, colon carcinoma



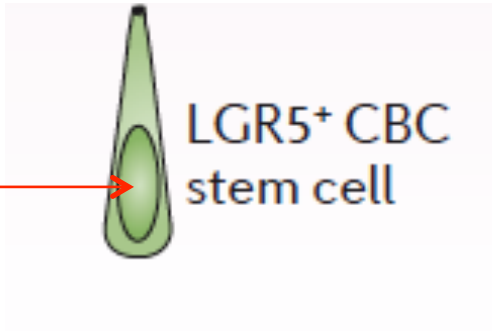
- cancer stem cells arise from mutations hitting normal stem cells

Cancer stem cells

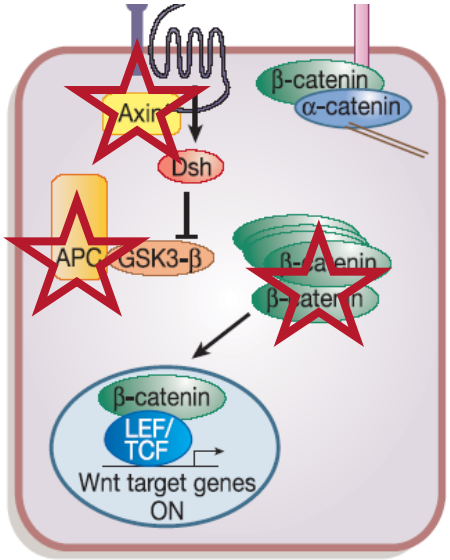


Can we establish cancer from ISCs?

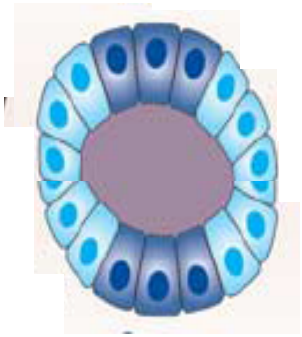
introduce mutations



Wnt signalling

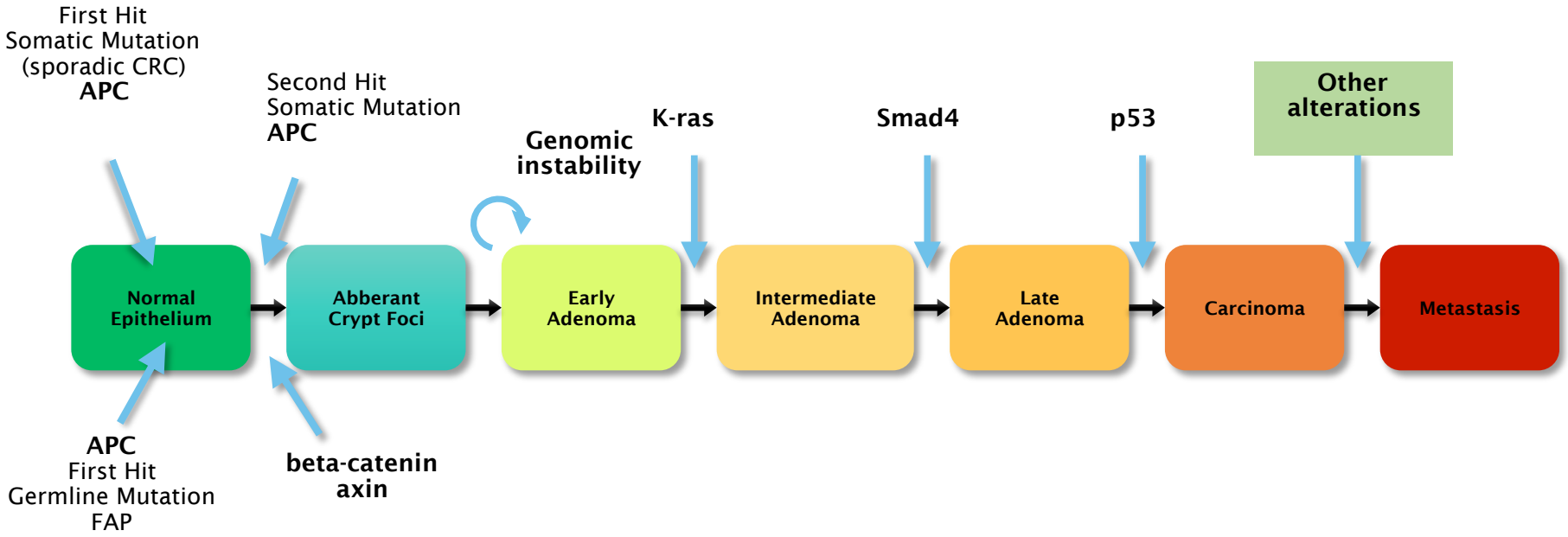


wnt **ON**

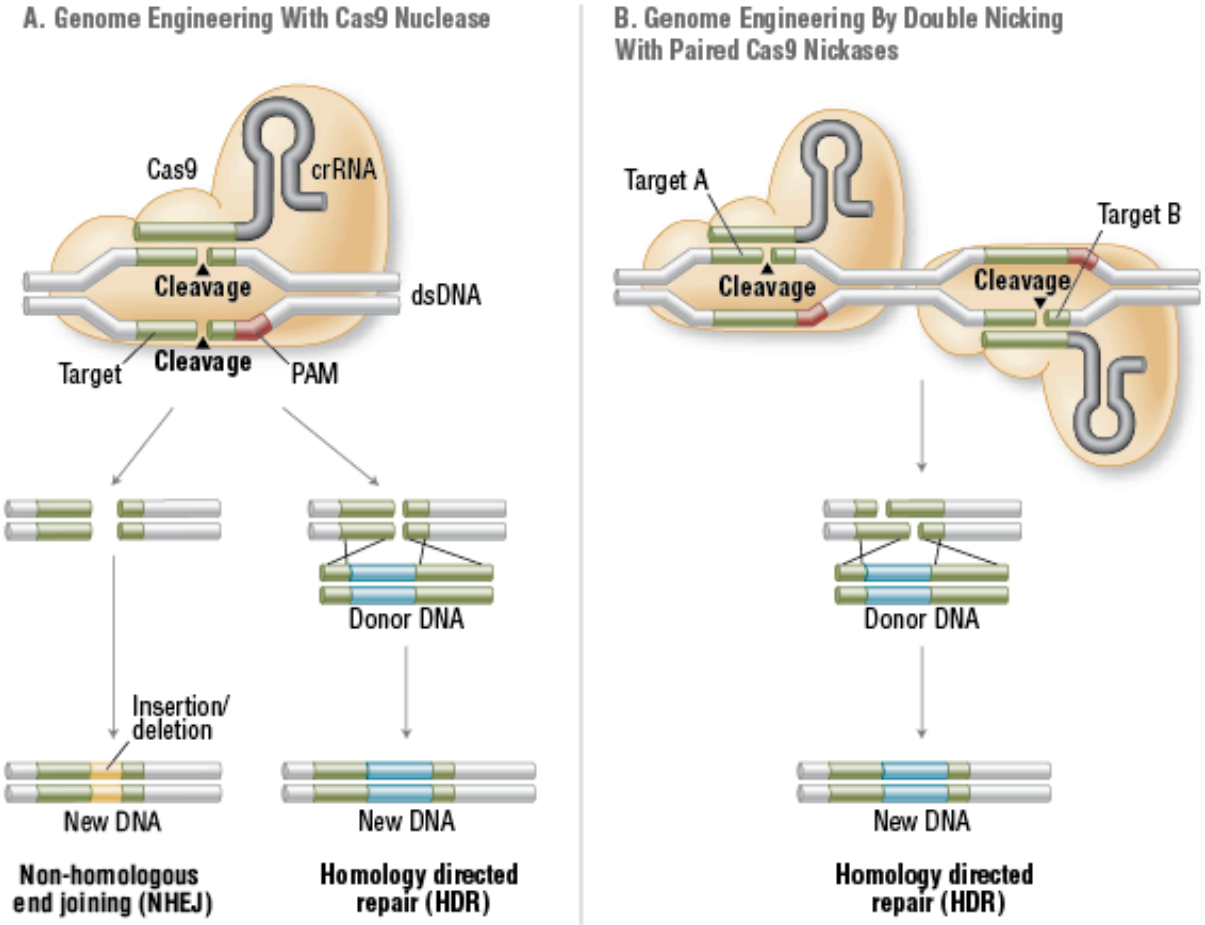


adenoma

Colorectal Cancer



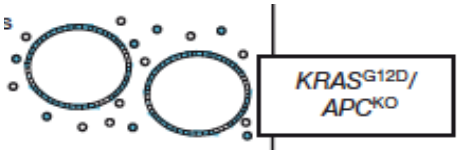
CRISPR/CAS9 Genome editing

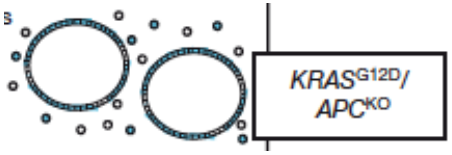


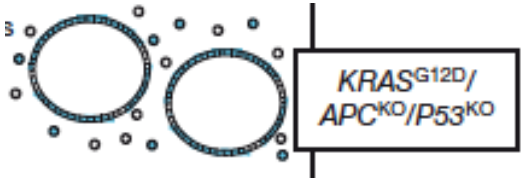
Cancer from CBCs

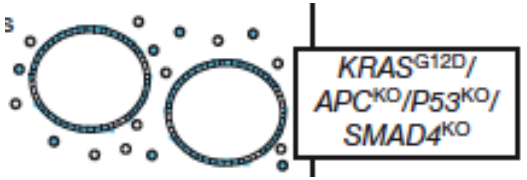
Human Intestinal Stem cells (CBCs)

Wild-type

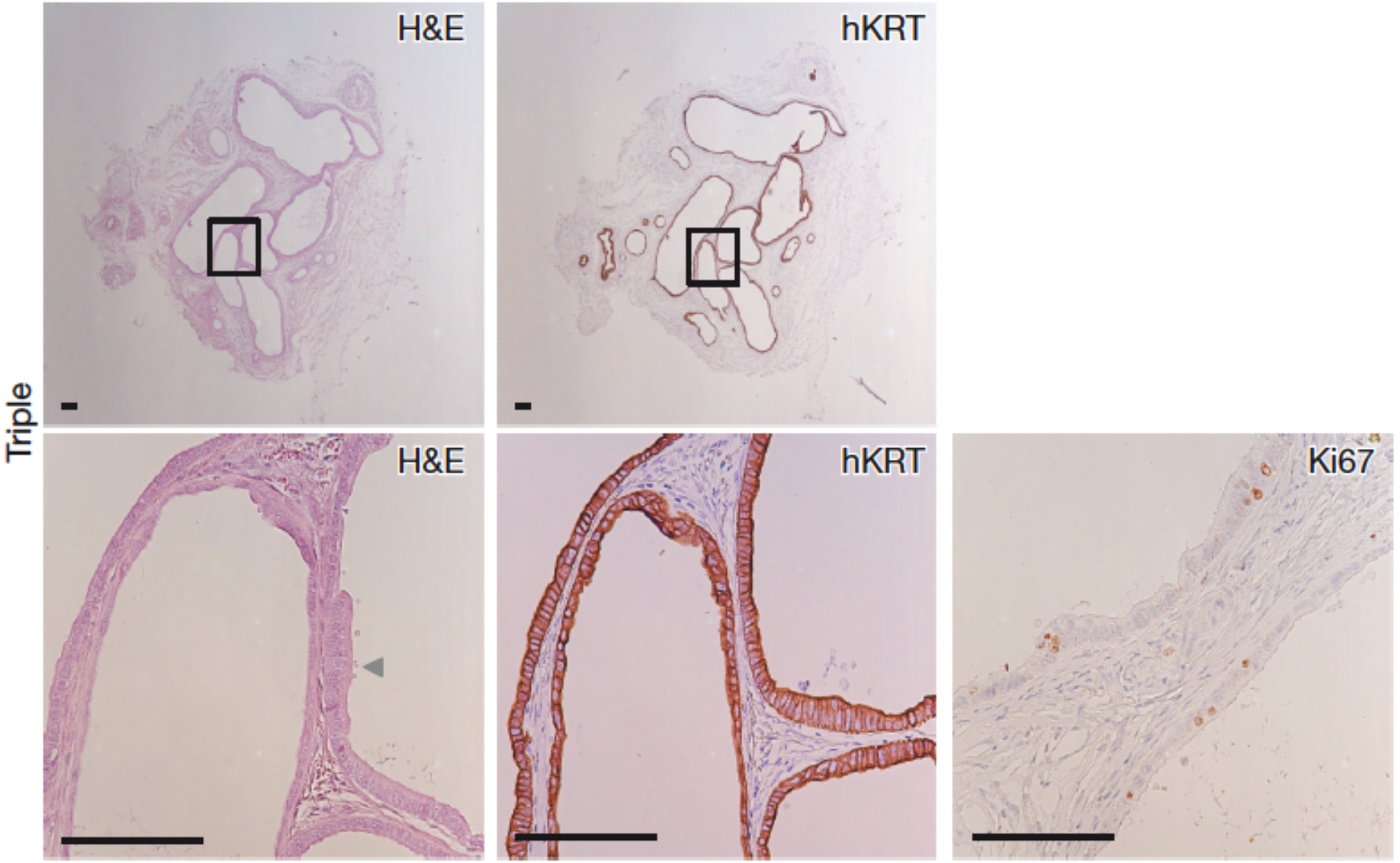




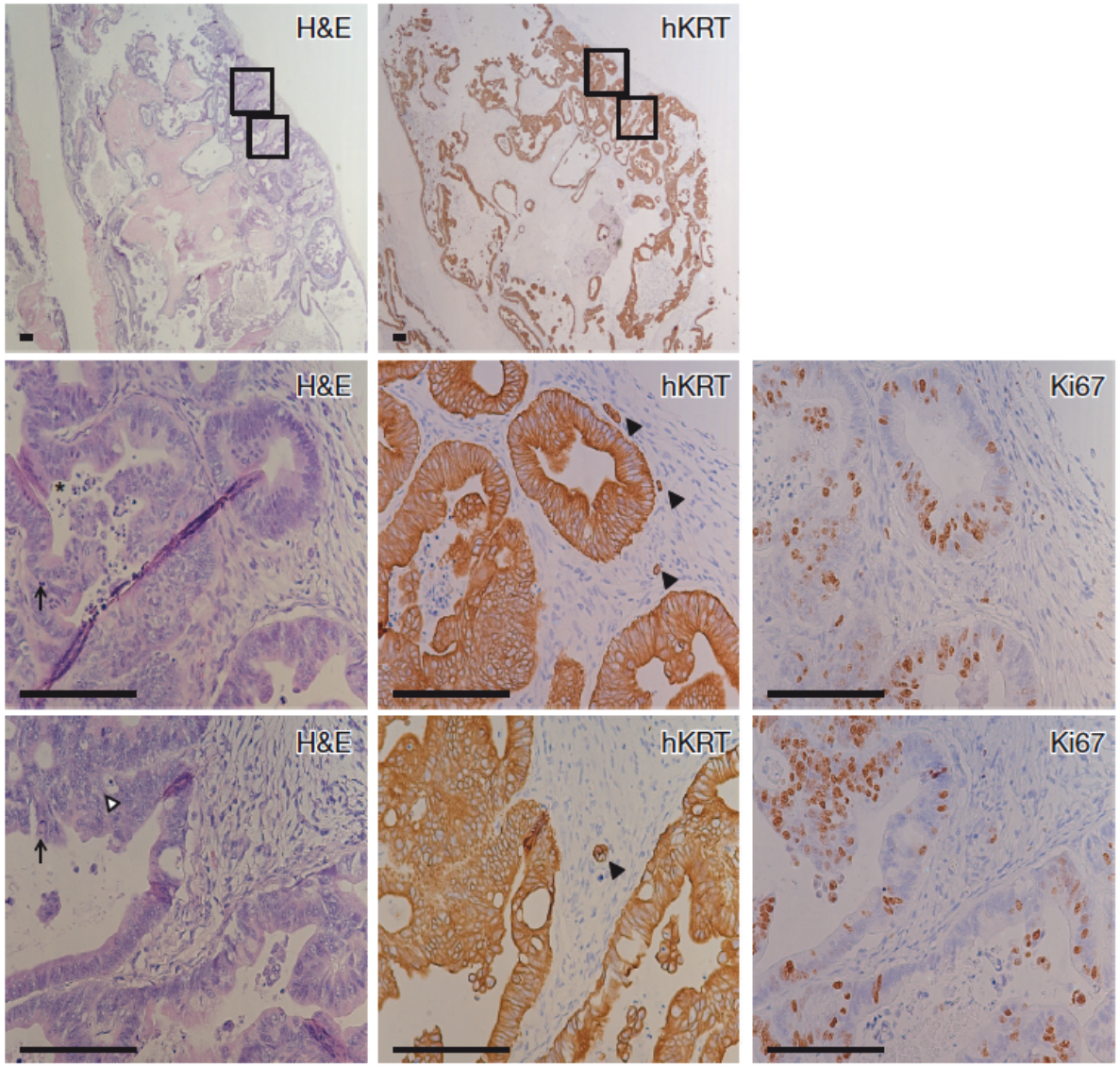




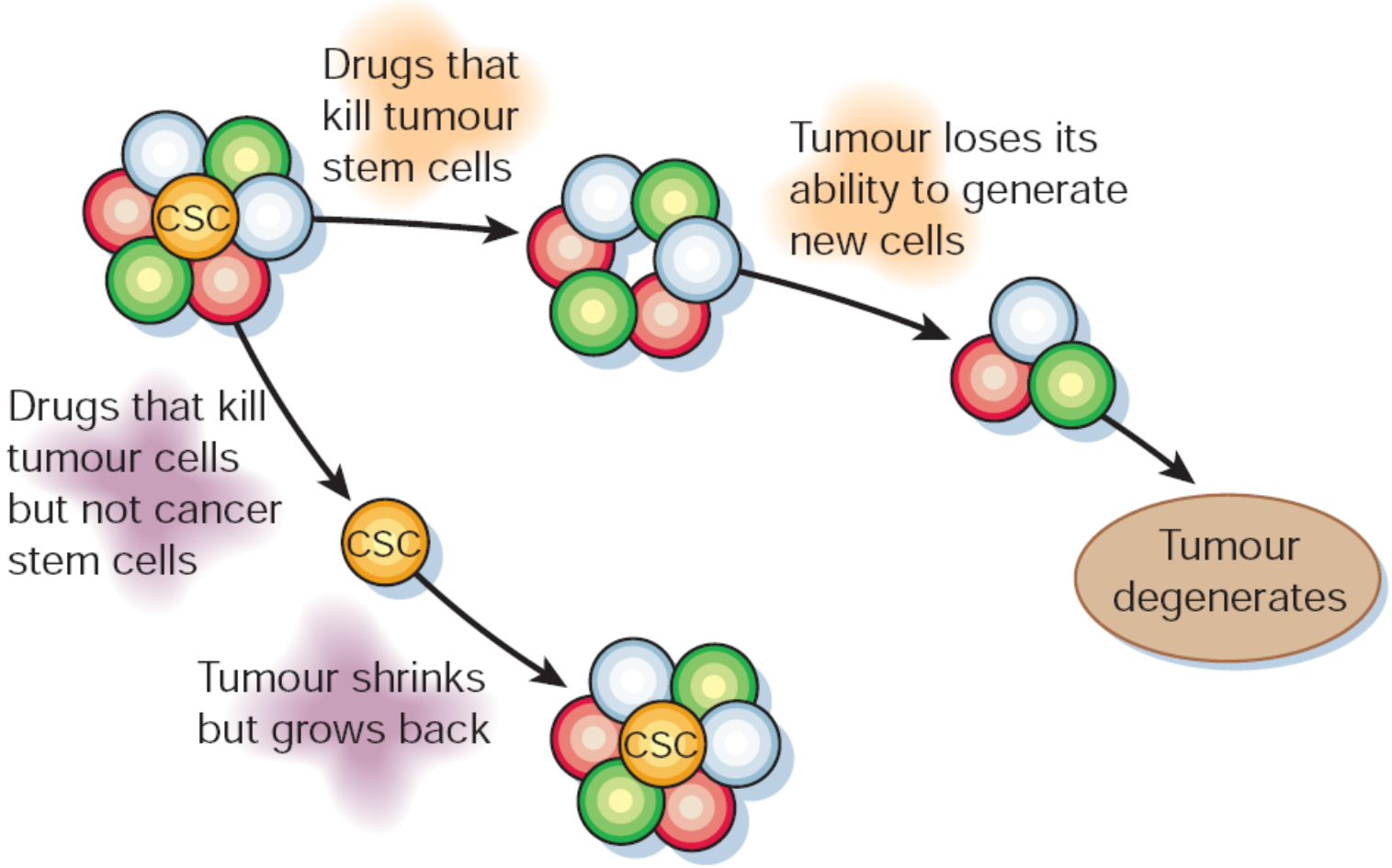
$KRAS^{G12D}$ APC^{KO} $p53^{KO}$



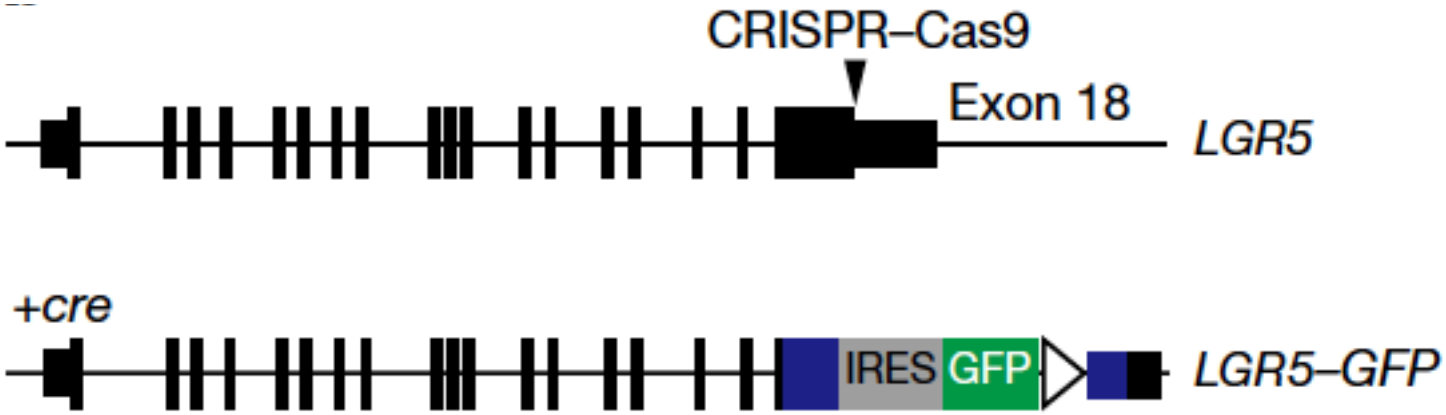
$KRAS^{G12D}$ APC^{KO} $p53^{KO}$ $SMAD4^{KO}$

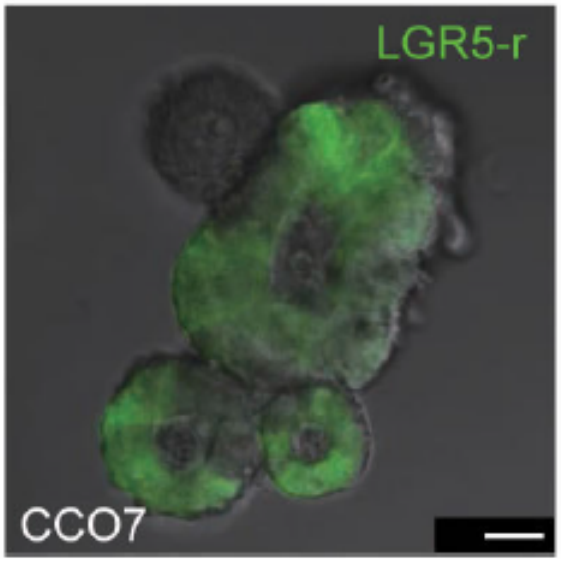


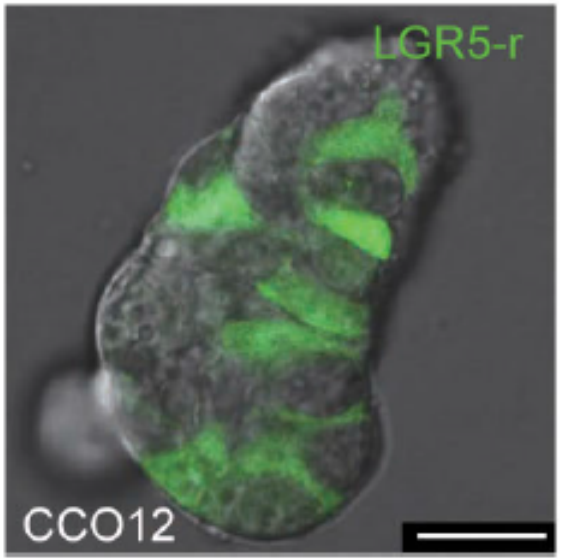
Different tumor targeting strategies

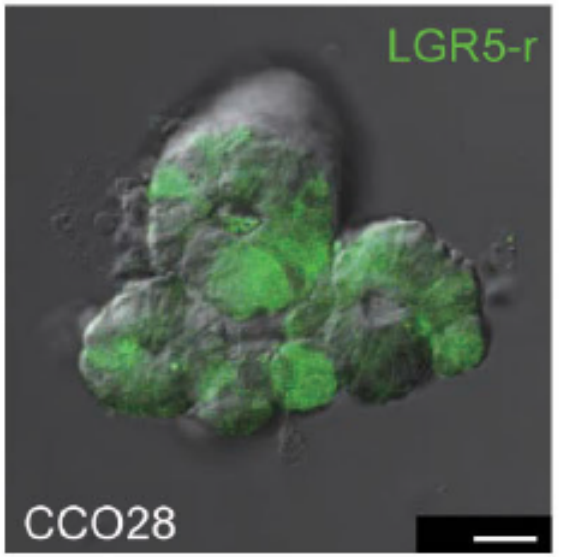


Labeling of CSC in human organoids

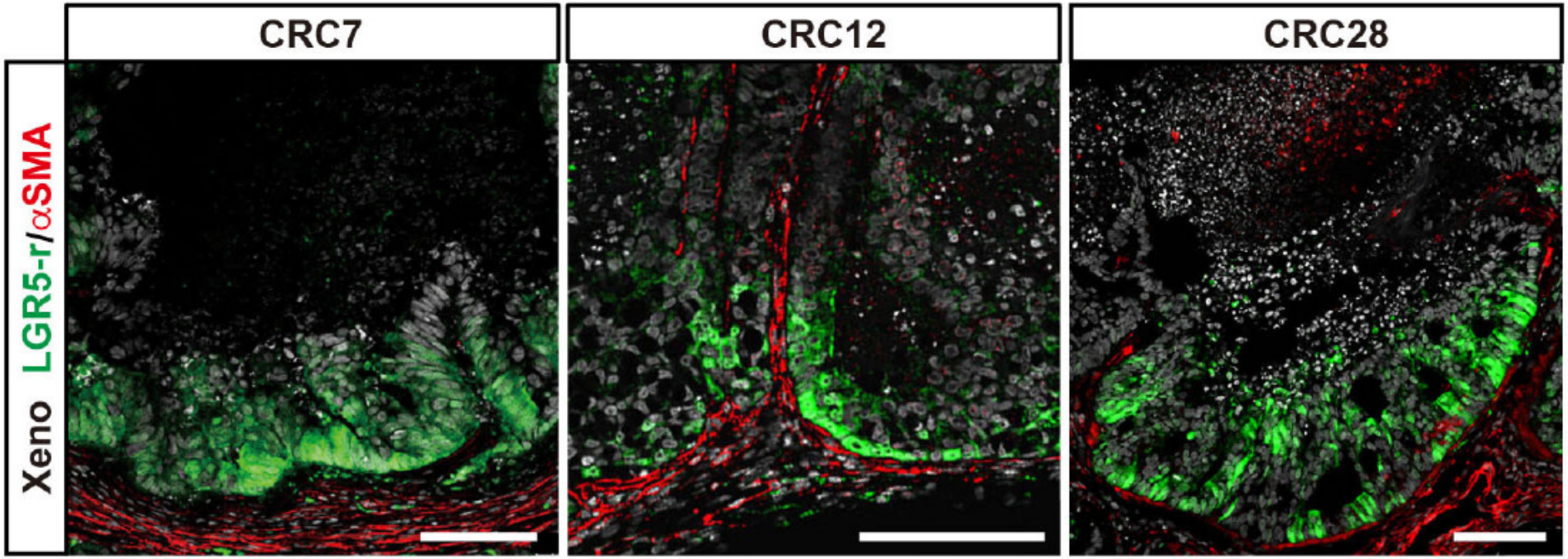




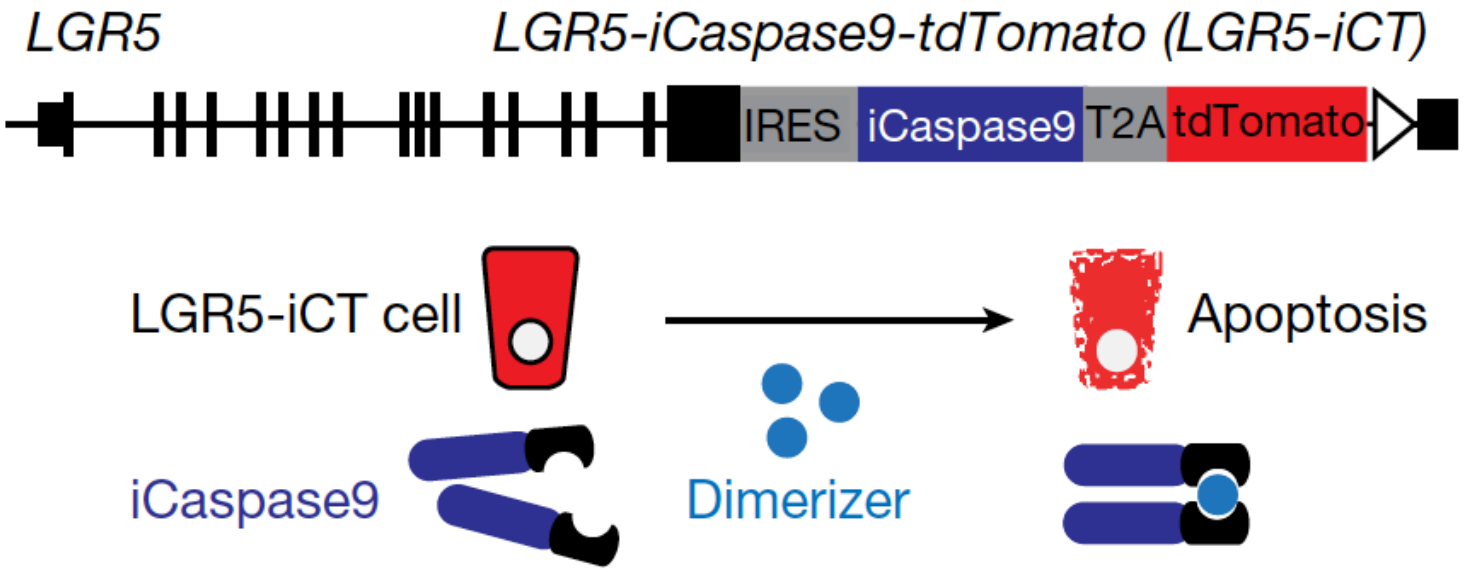




ODX: CSC remain in mouse xenograft model



human CRC Organoids



11 MAY 2017 | VOL 545 | NATURE

Visualization and targeting of LGR5+ human colon cancer stem cells

Mariko Shimokawa[1*], Yuki Ohta[1*], Shingo Nishikori[1,2], Mami Matano[1], Ai Takano[1], Masayuki Fujii[1], Shoichi Date[1,2], Shinya Sugimoto[1], Takanori Kanai[1] & Toshiro Sato[1]

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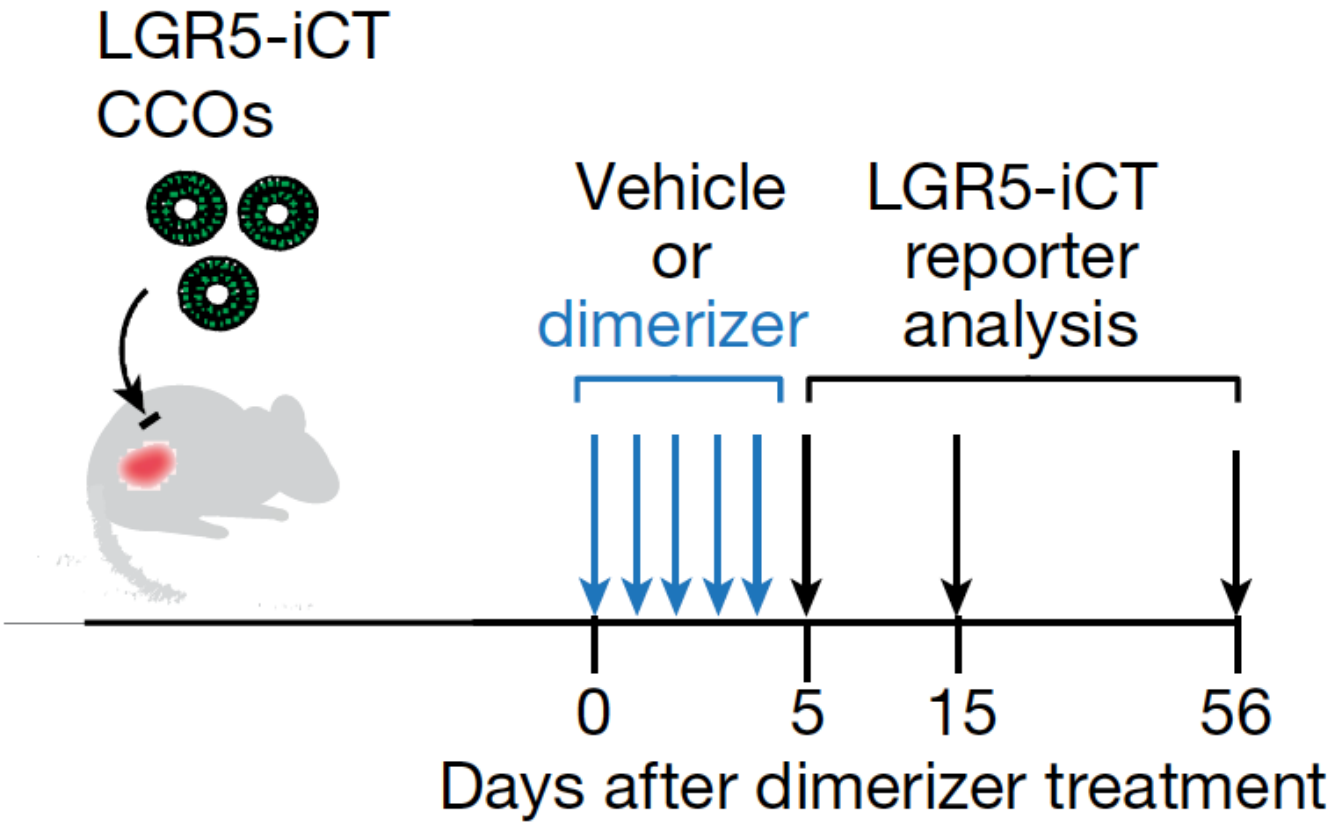

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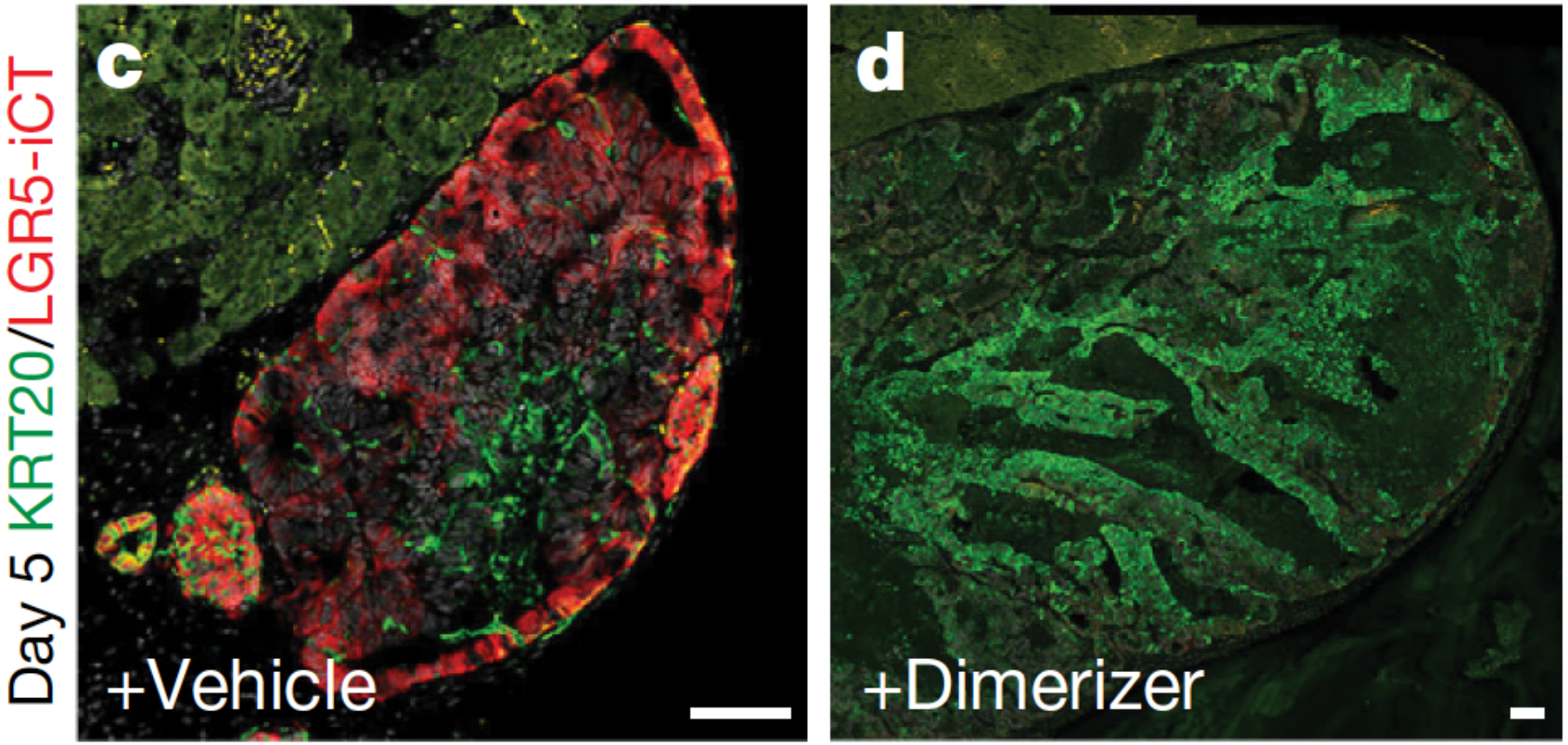
Organoids in Xenografts (ODX)



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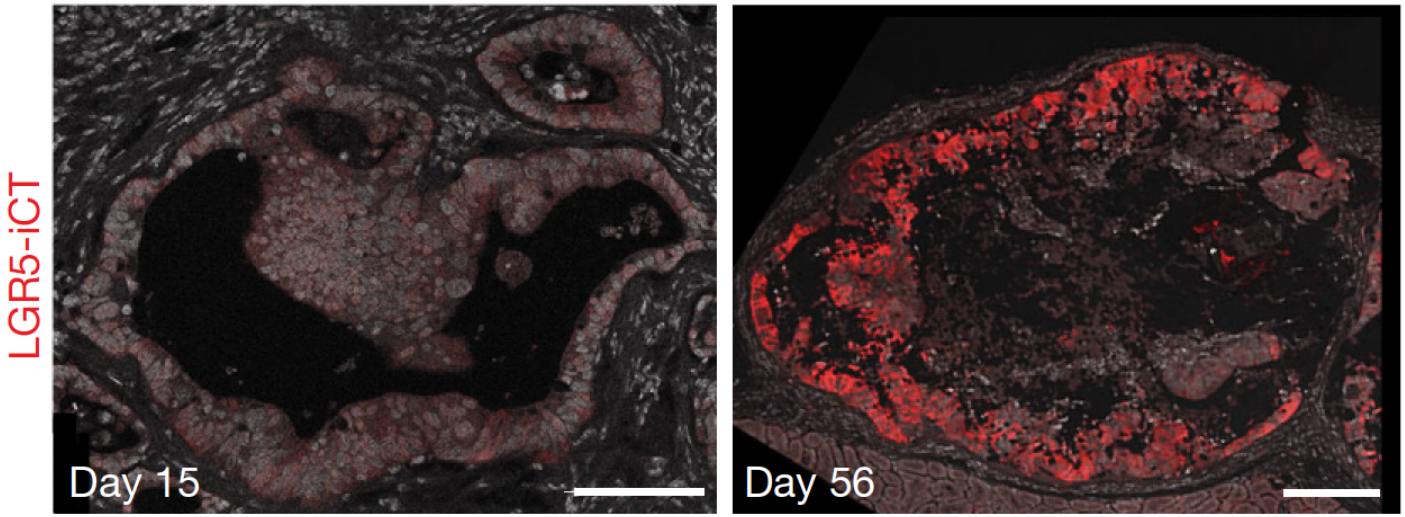

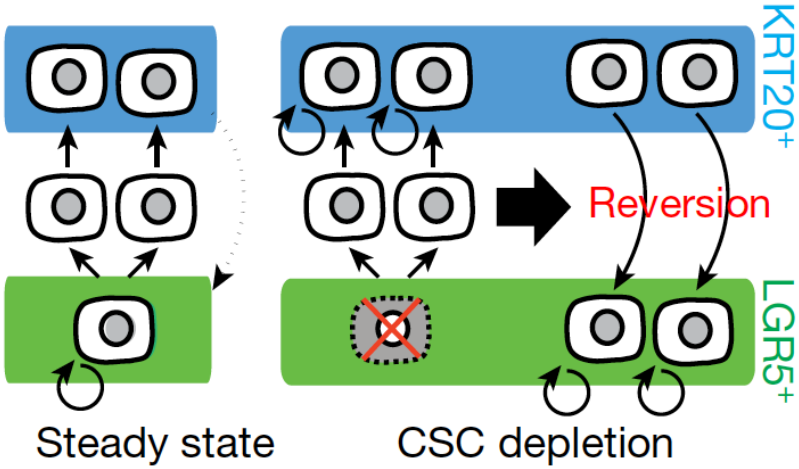

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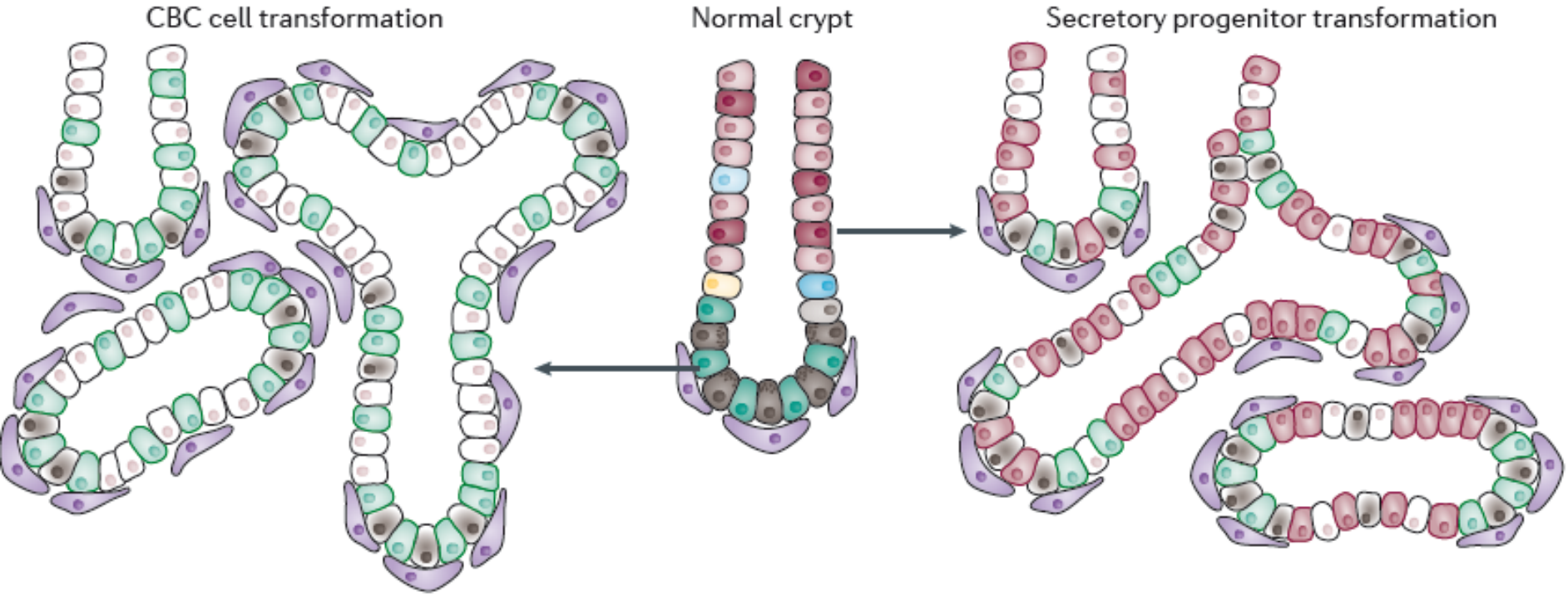
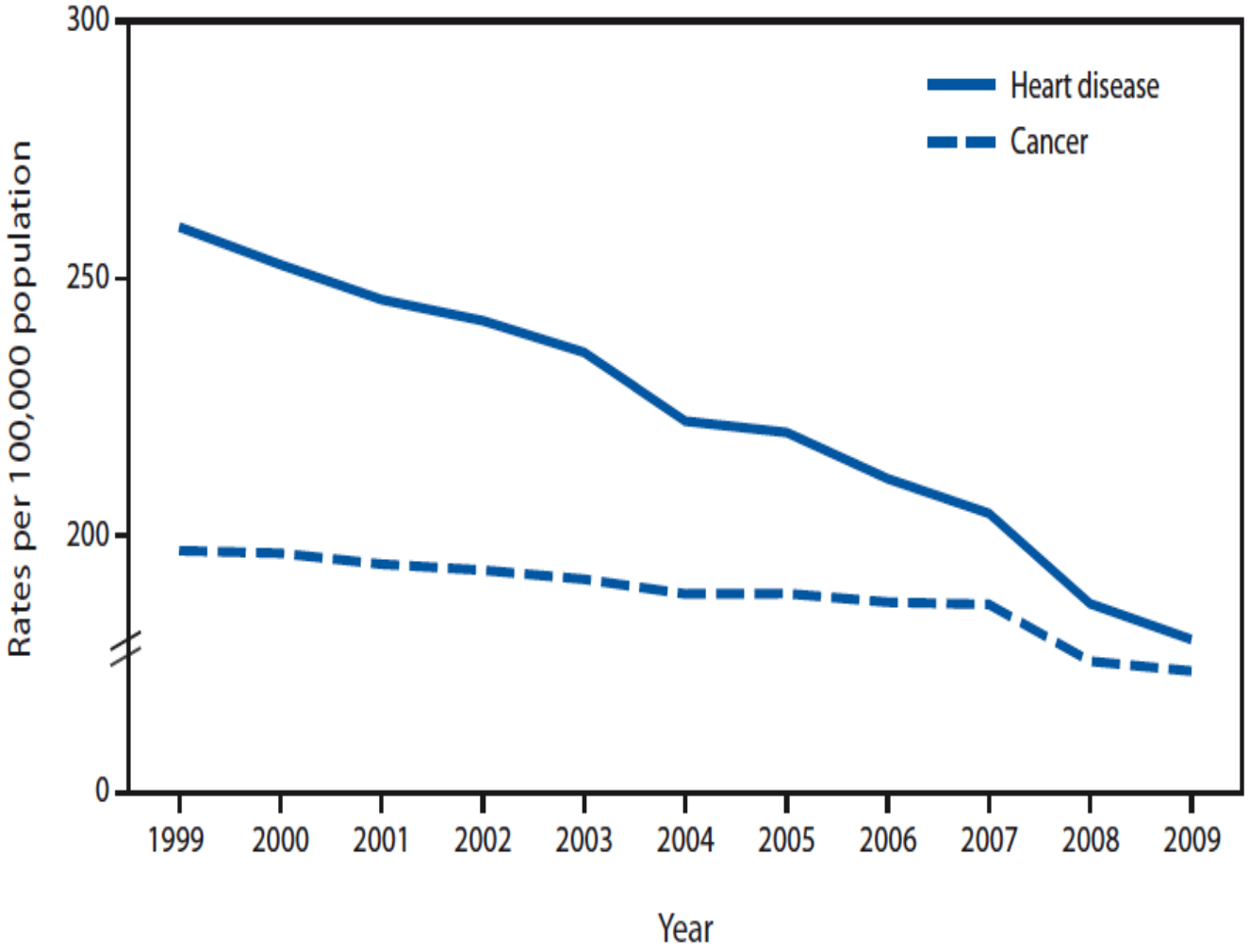
Cancer Stem Cells (CSCs)

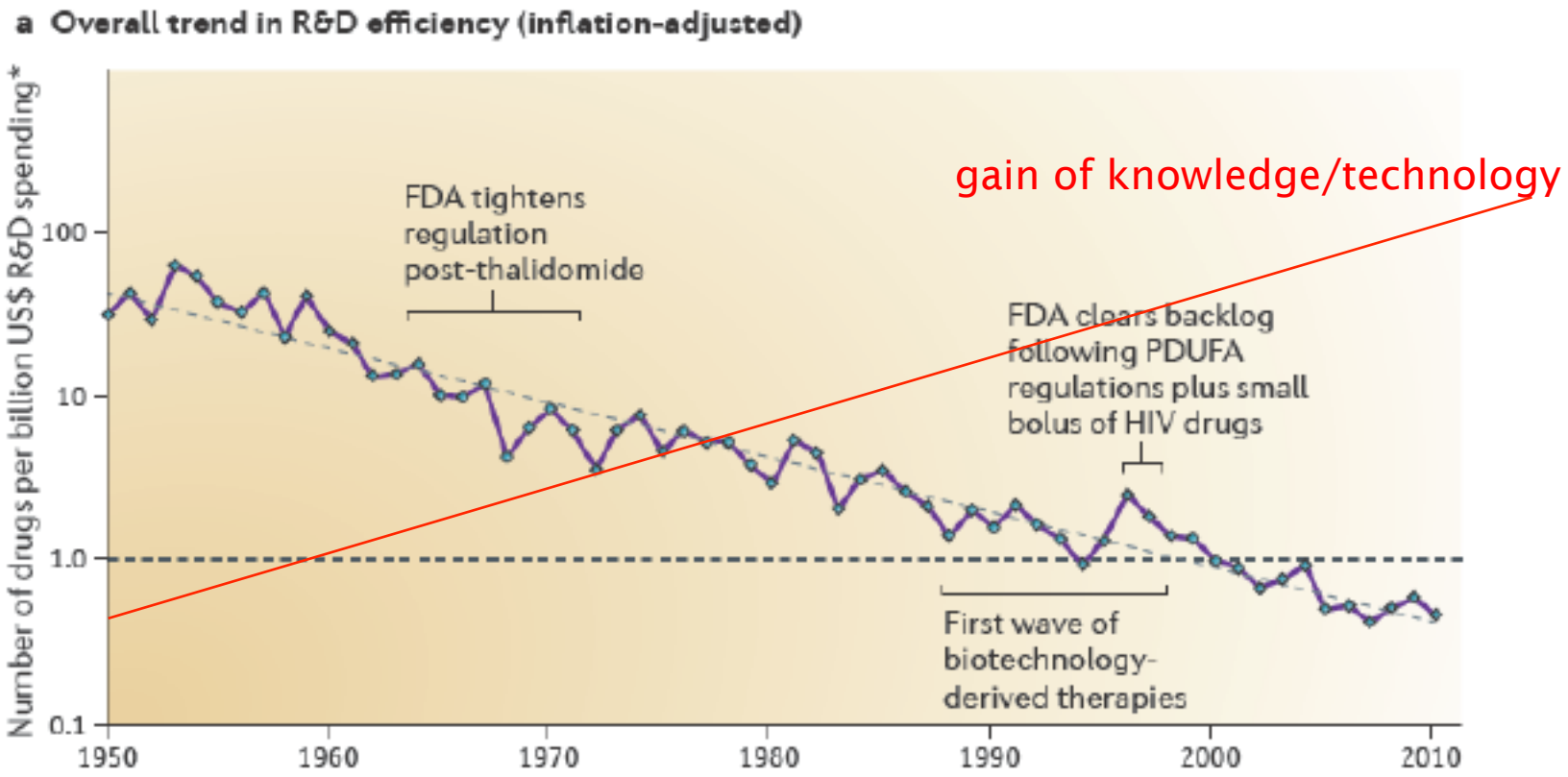


Drug development

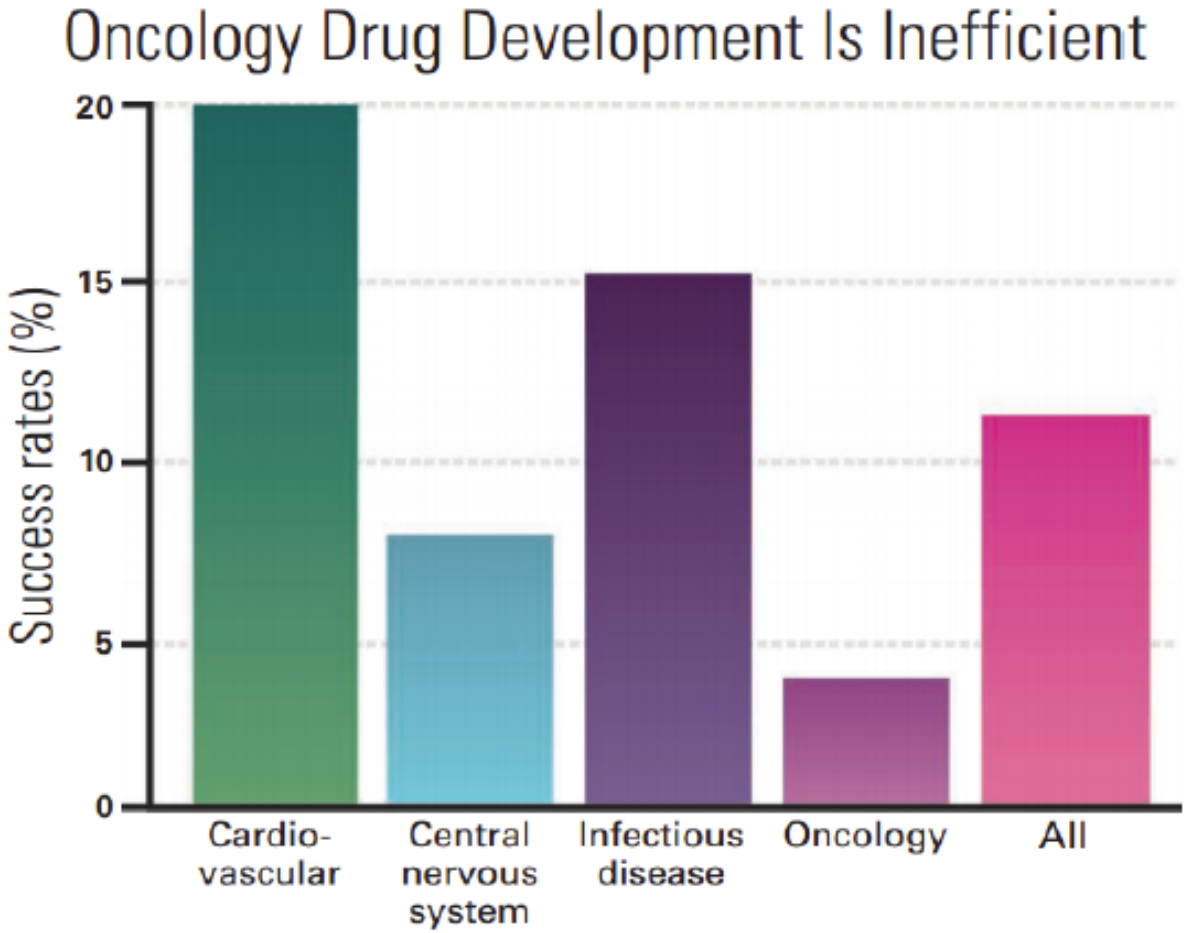


* Data for 2008 and 2009 are preliminary.

gain of knowledge/technology ≠ drug development

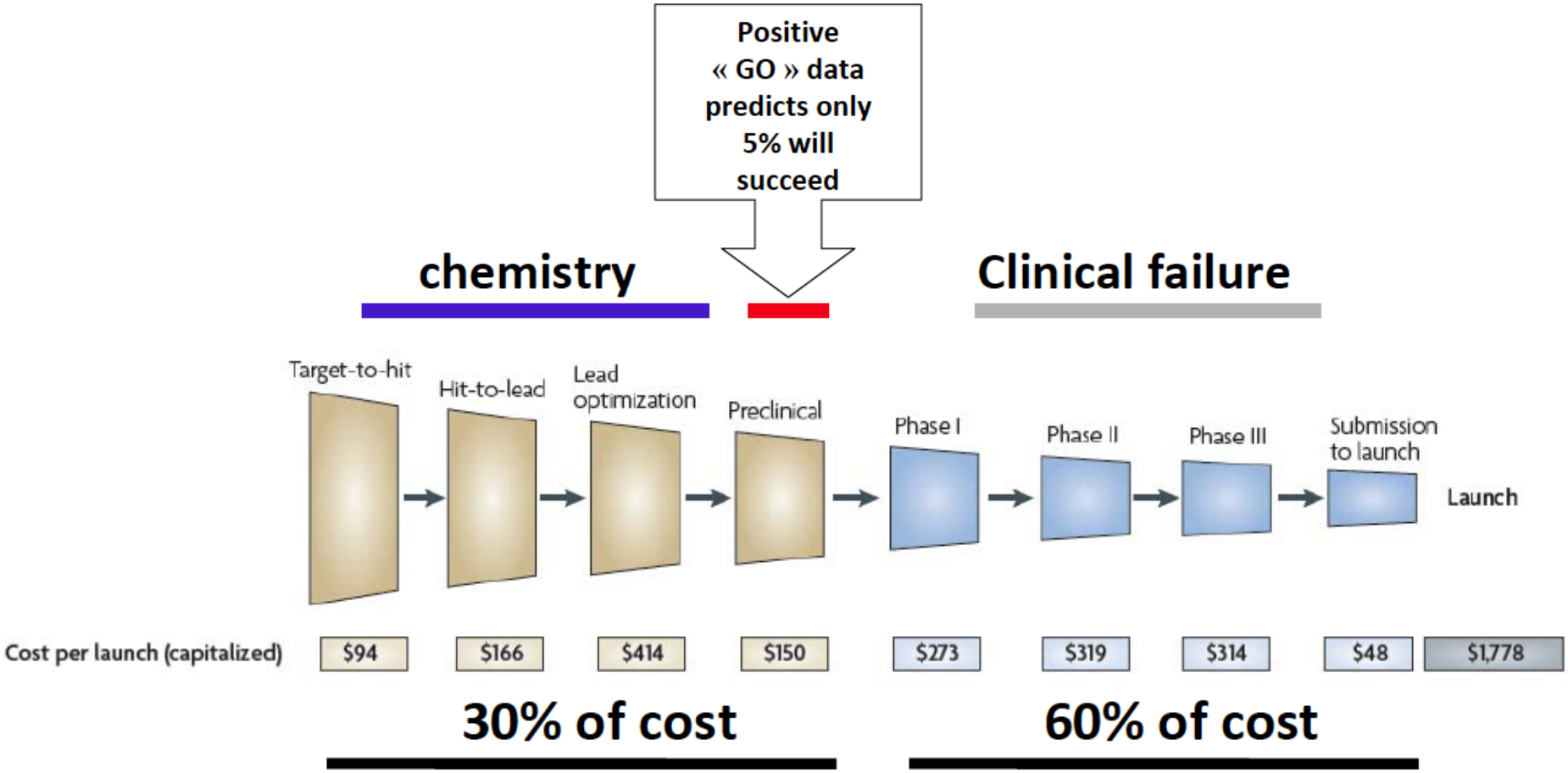


96% fail rate in clinical trials



Hit or miss. Cancer drug candidates have an abysmally low success rate.

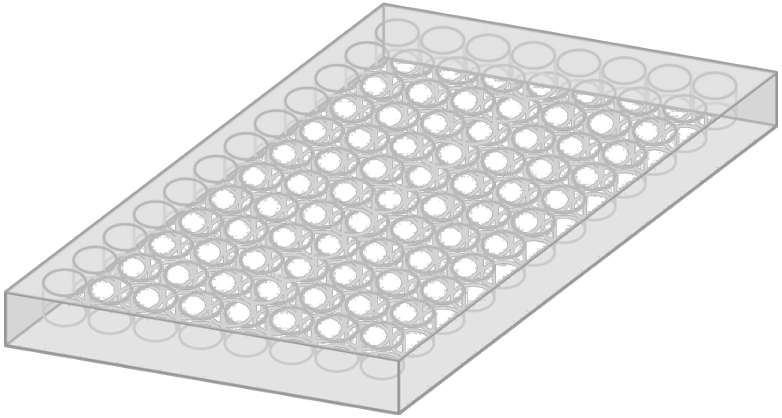
Science 338: 29 (2012)



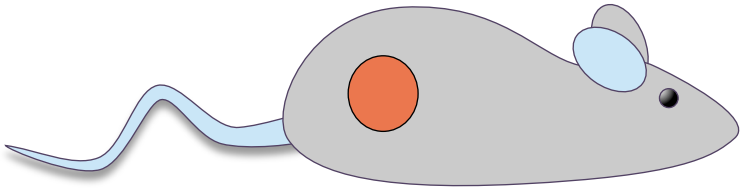
Paul SM et al Nature Rev Drug Disc 2010, 9: 203-214.

Reasons for failure

drugs optimized to work...



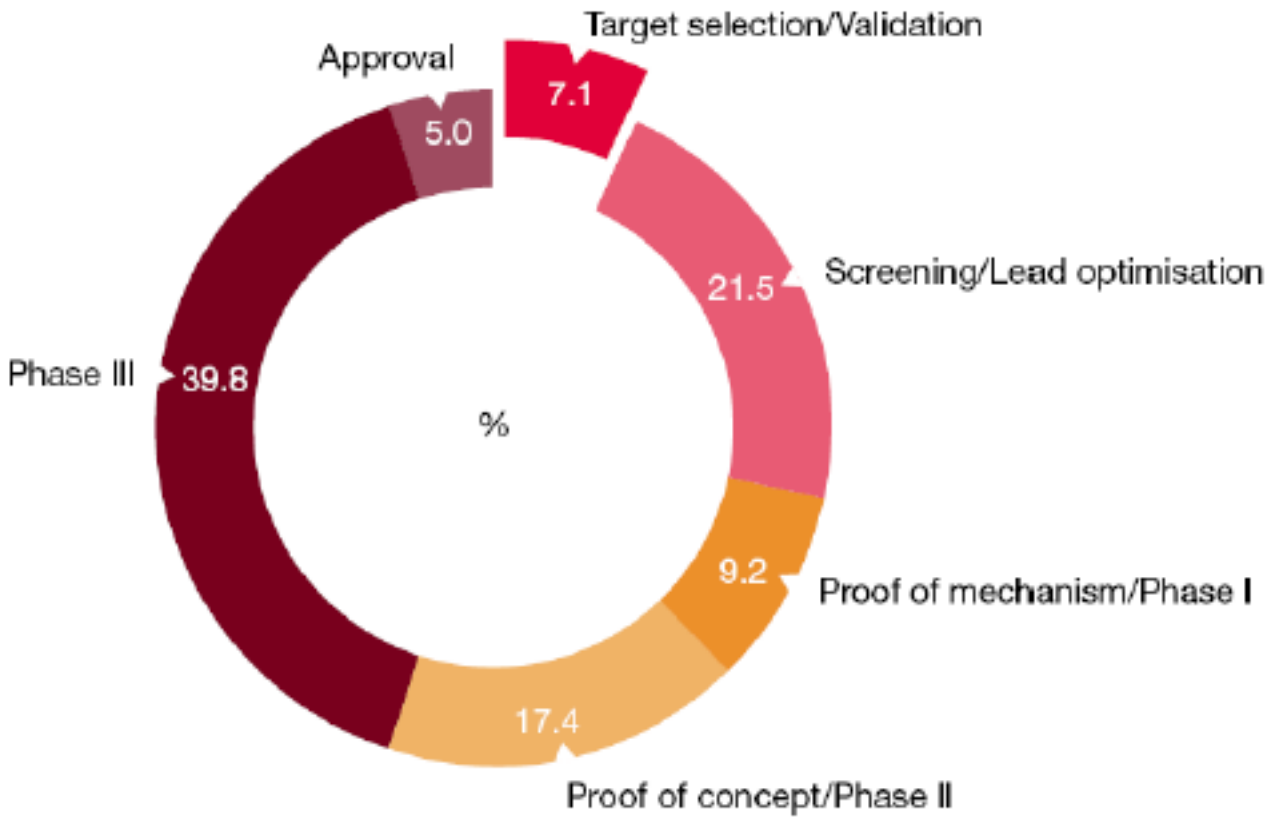
... in 2D tumor cell line cultures on plastic plates



... in subcutaneous mouse xenograft models

only 7% invested on preclinical testing

Most pharma companies spend a very small percentage of their budgets on target selection and validation



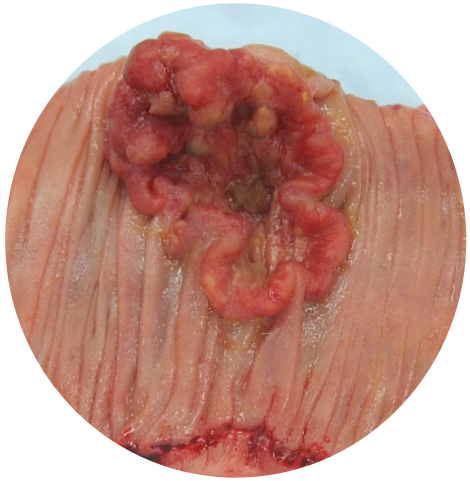
way out: better preclinical models

in vitro
- organoids, organotypic 3D cell culture
- heterotypic interaction with stroma

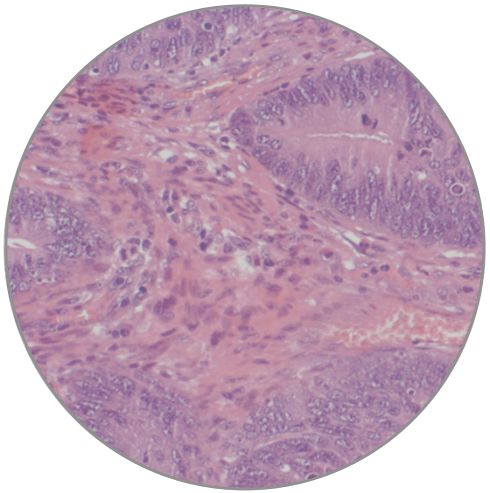
in vivo
- better mouse models:
    - GEMMs
    - Xenograft models: orthotopic implantation
    - humanized mice: HSC from Human, reconstitute the entire mouse immune system with human cells

ex vivo
personalized medicine: tissue slices

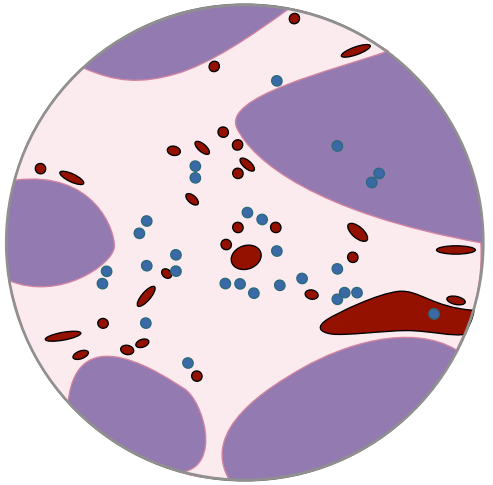
Solid tumors



3D

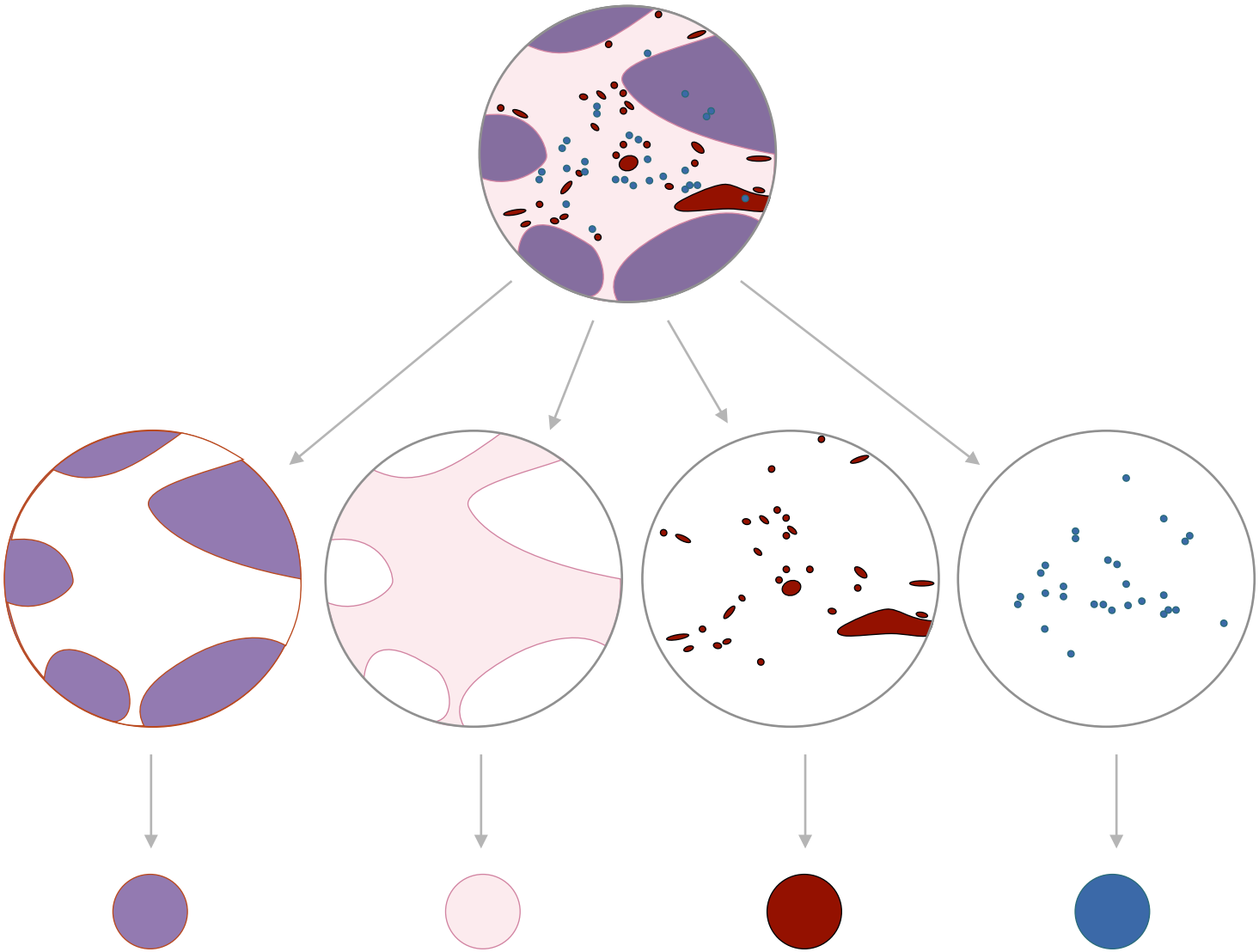


heterogenous

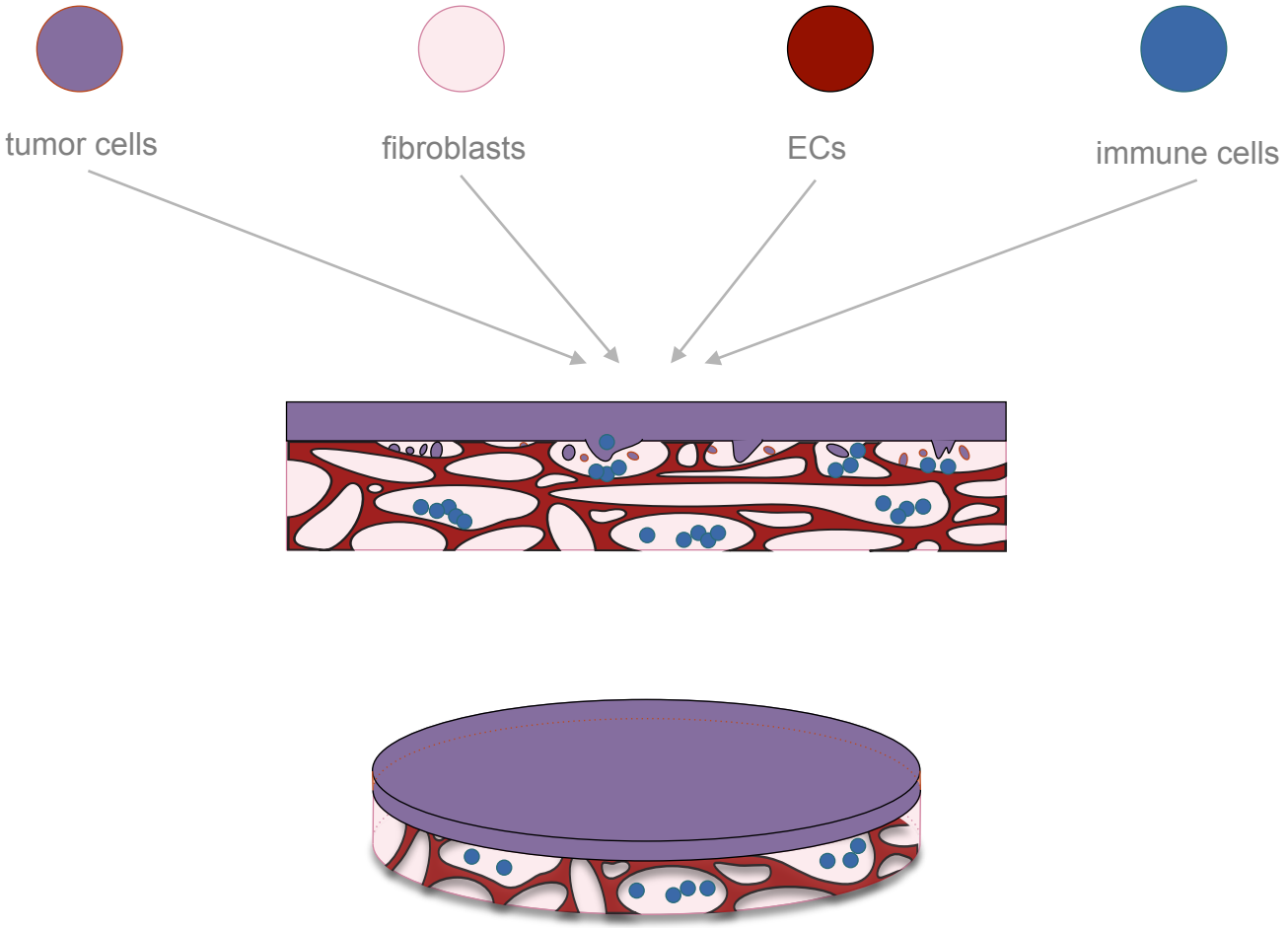


→ 3D structure
→ human organ specific microenvironment

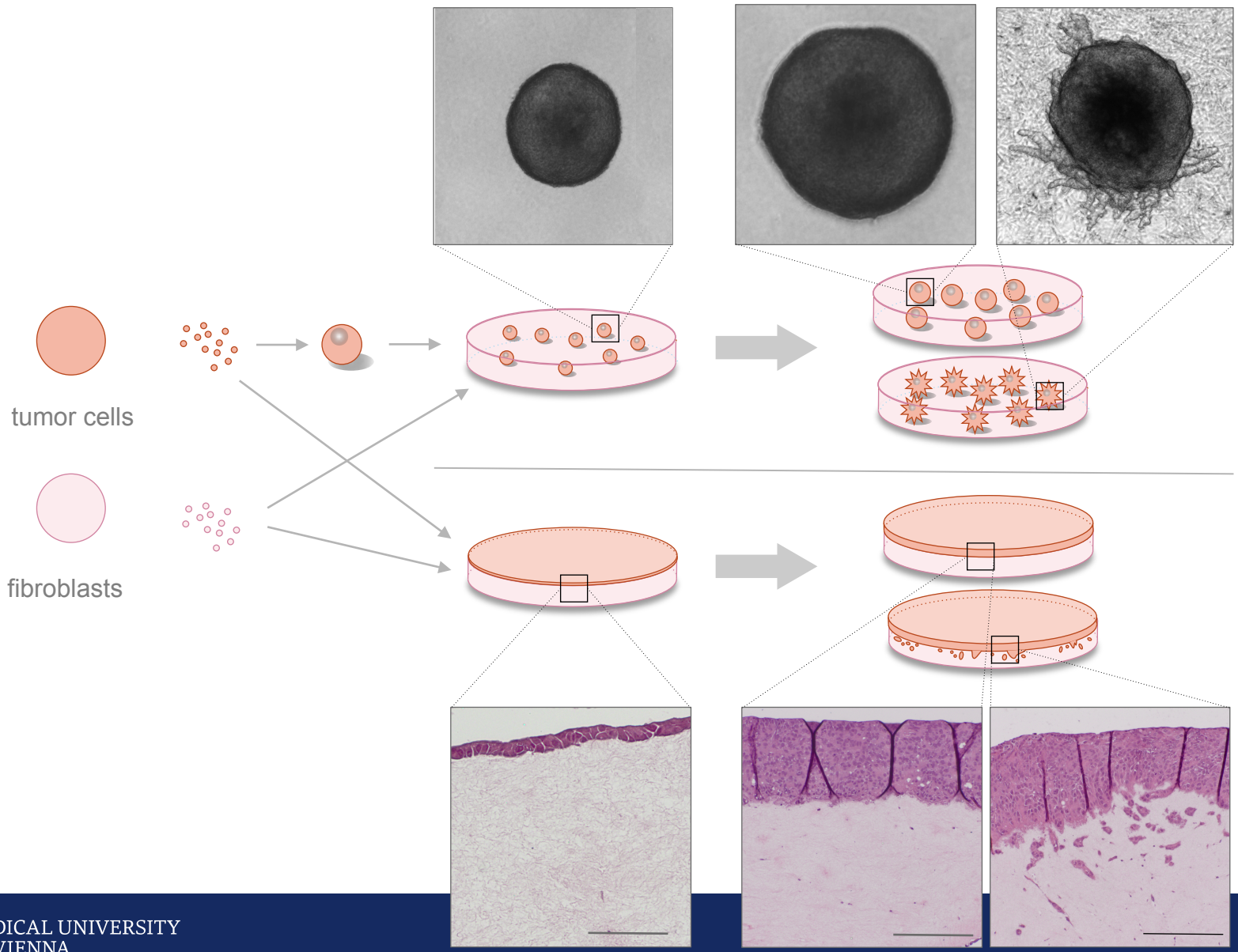
Deconstruction



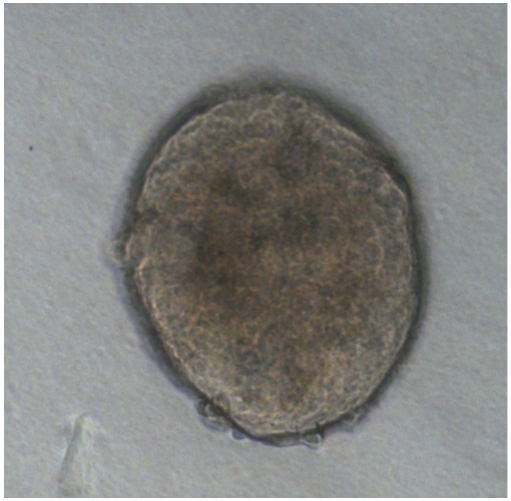
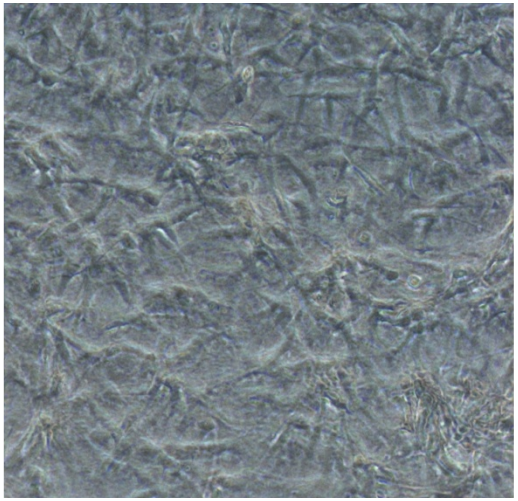
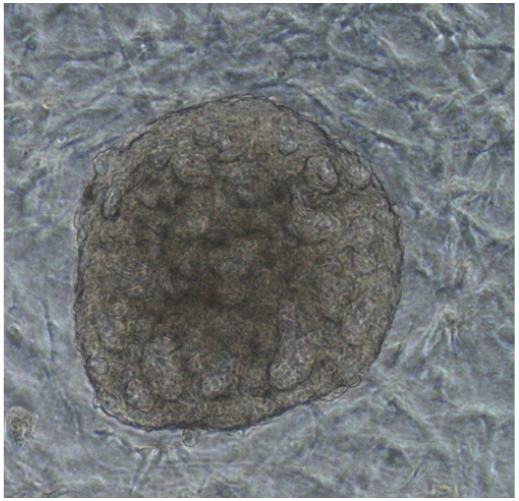
Reconstruction

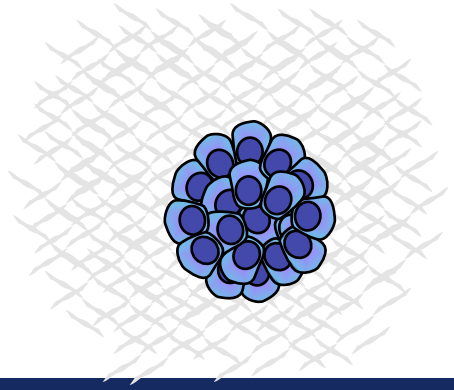
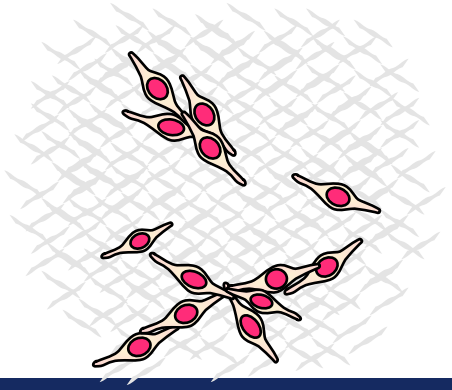


Reconstruction of cancer heterogeneity *in vitro in* 3D

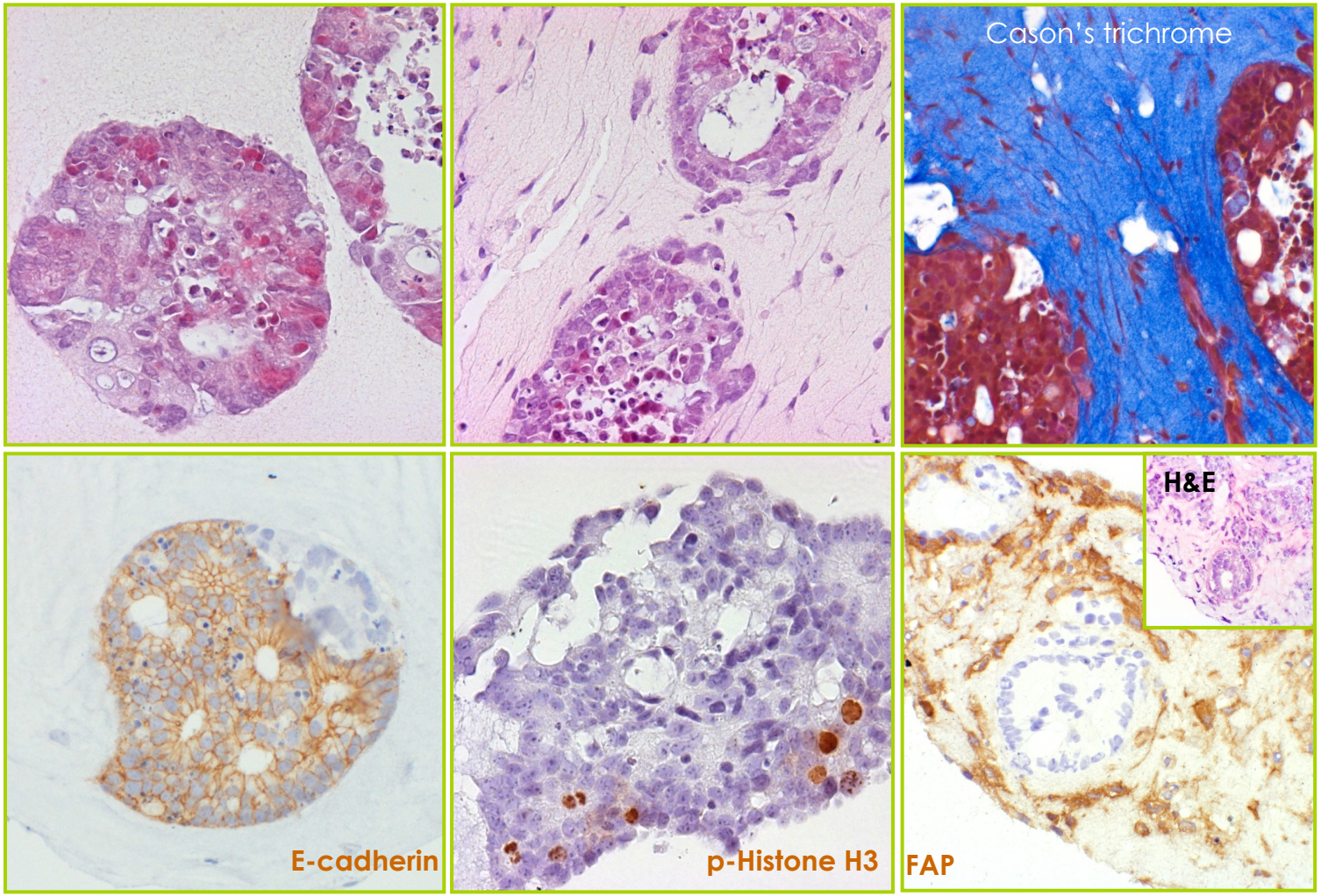


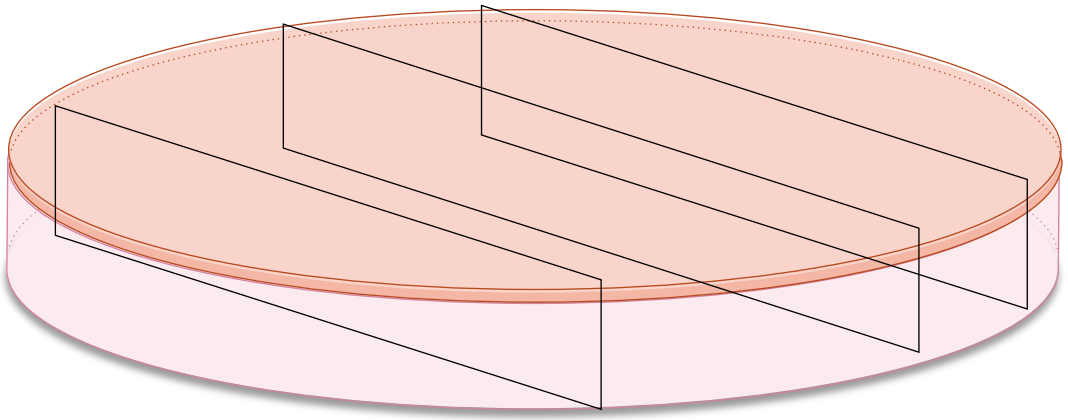
Modular system

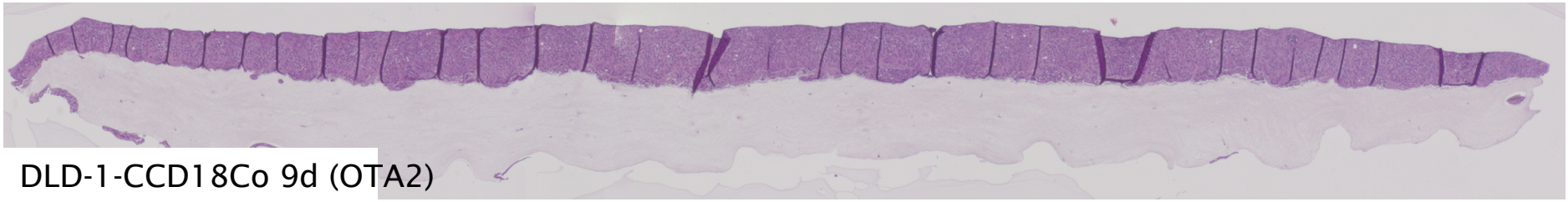




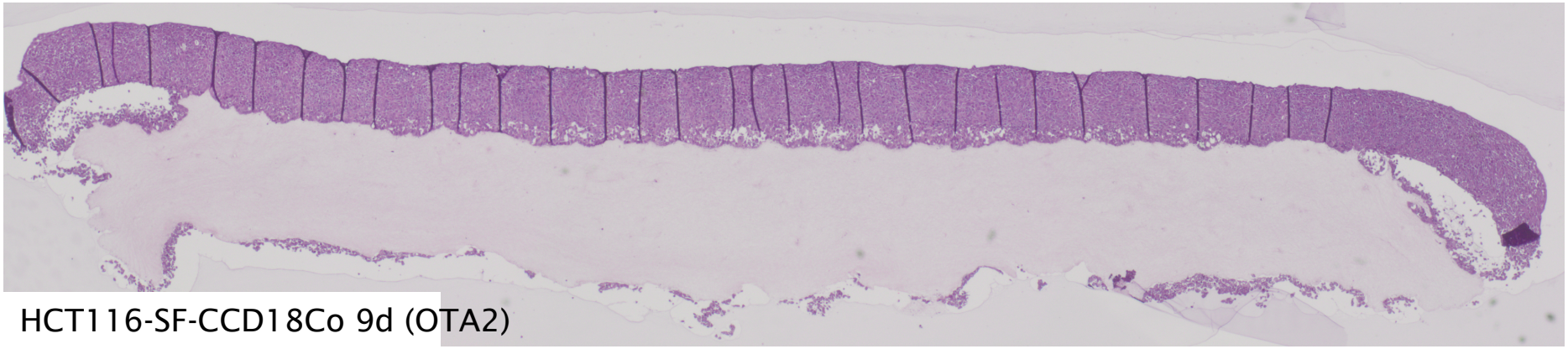

looks like in vivo



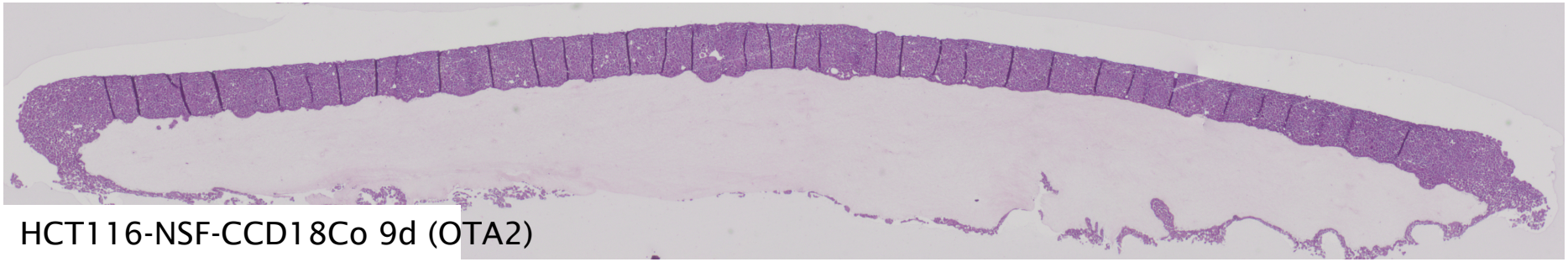




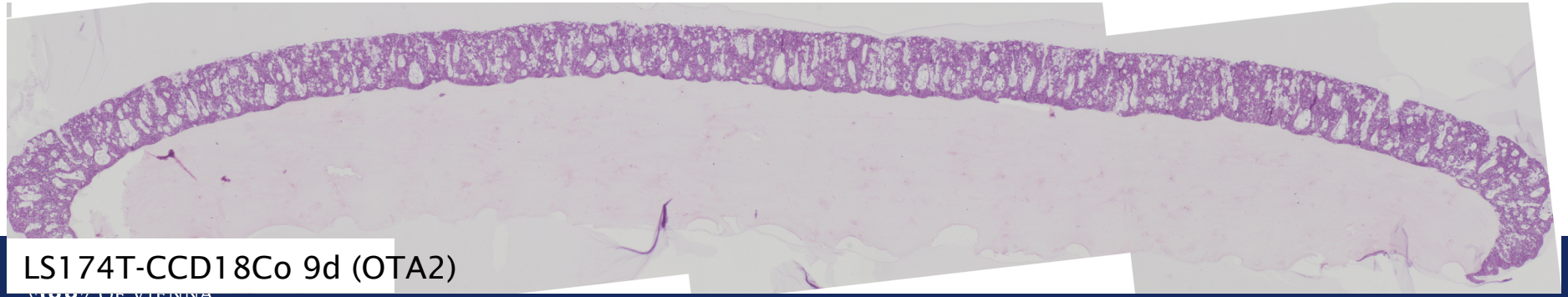
DLD-1-CCD18Co 9d (OTA2)



HCT116-SF-CCD18Co 9d (OTA2)



HCT116-NSF-CCD18Co 9d (OTA2)



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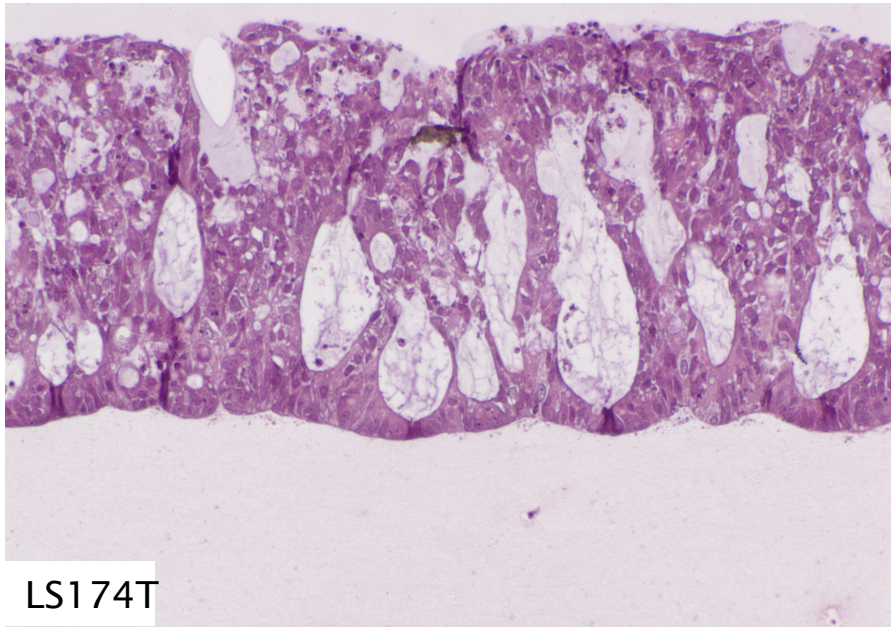
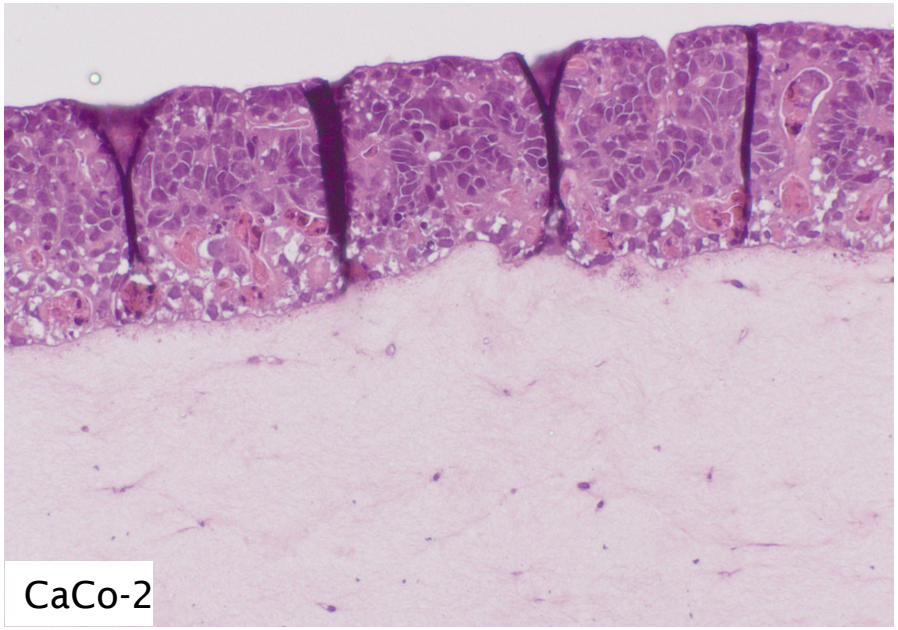
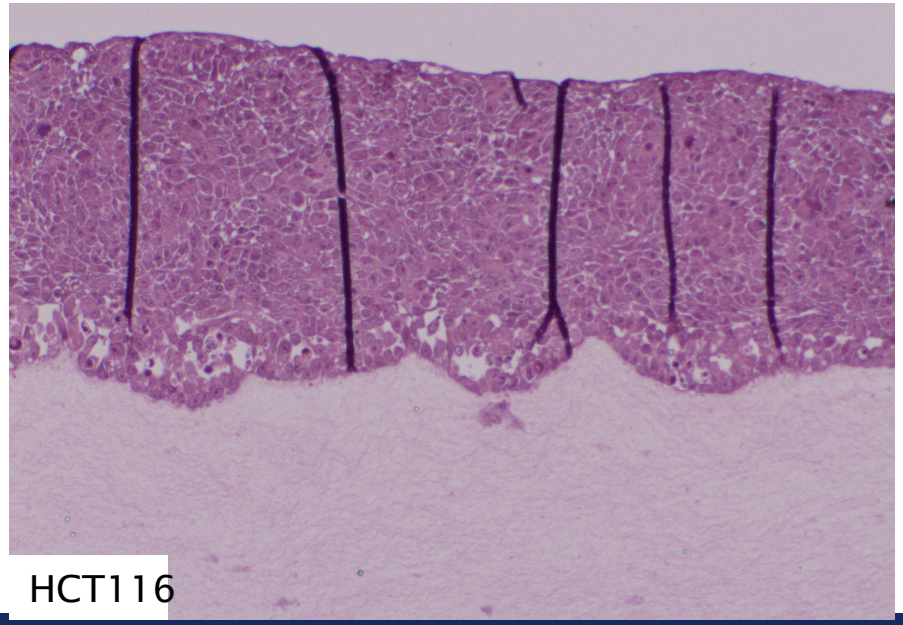

LS174T


CaCo-2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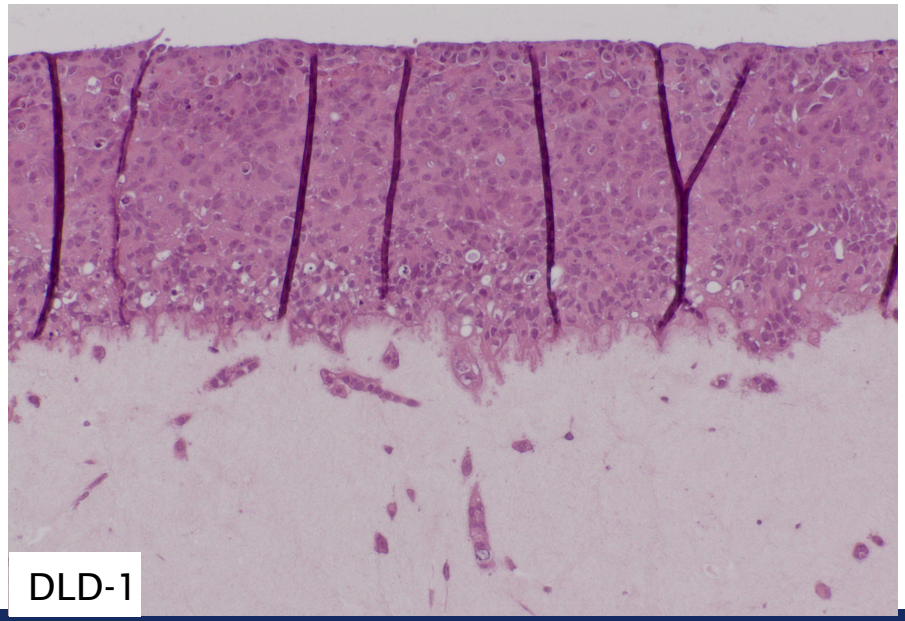
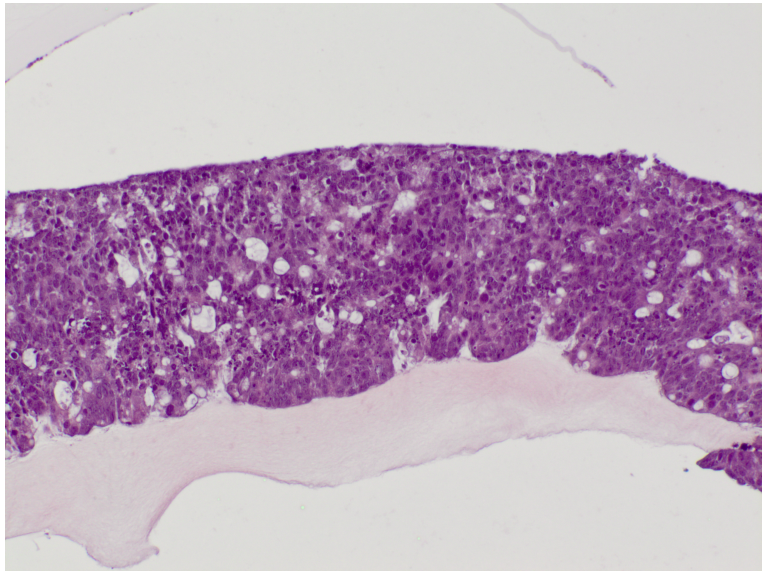
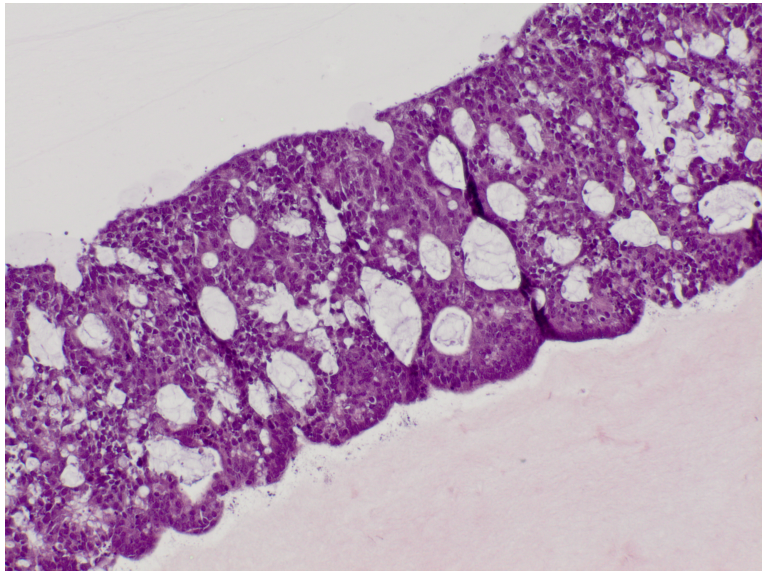

DLD-1

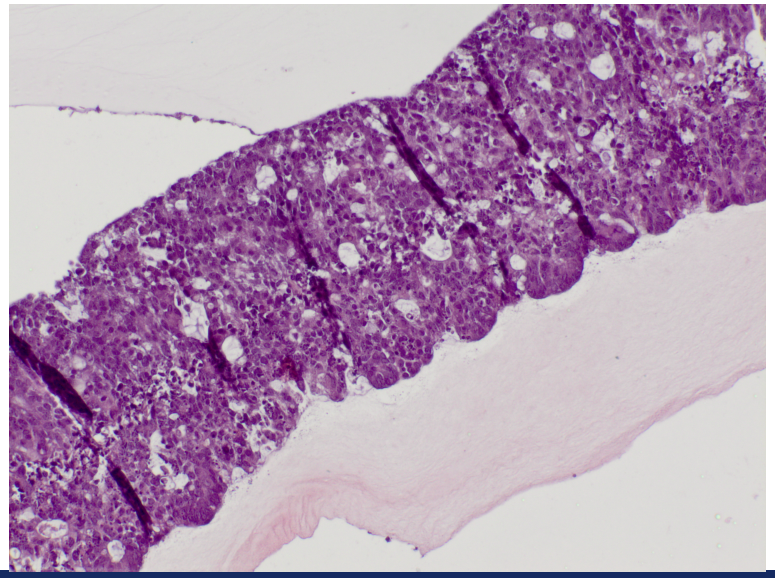
LS174T cells (7d in OTA)

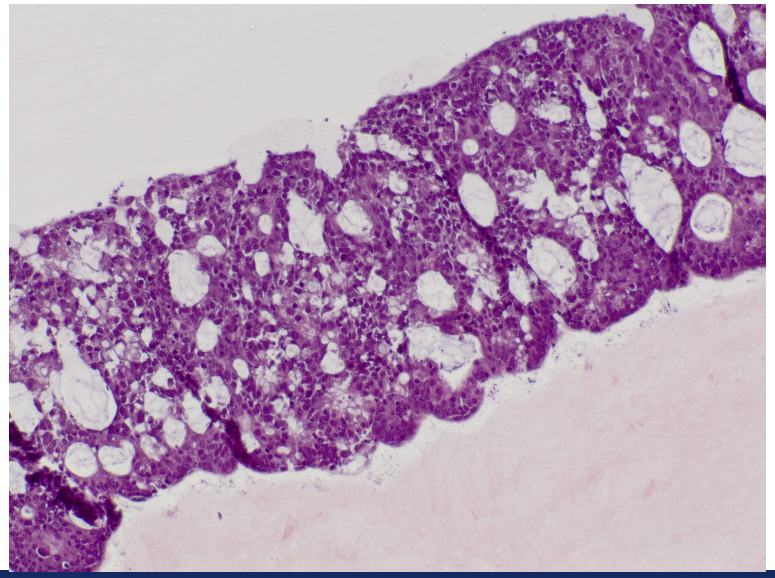
- fibros

+ fibros

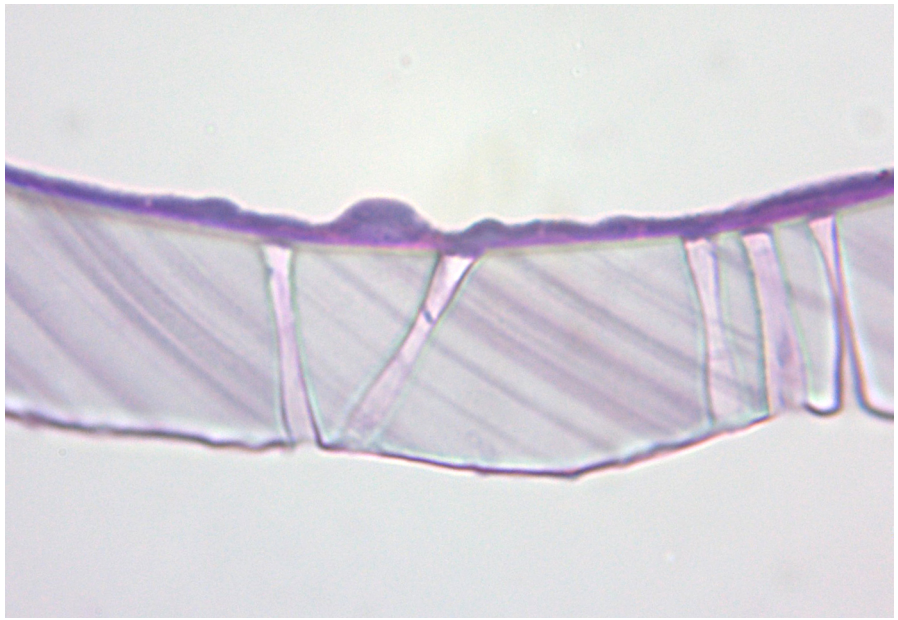


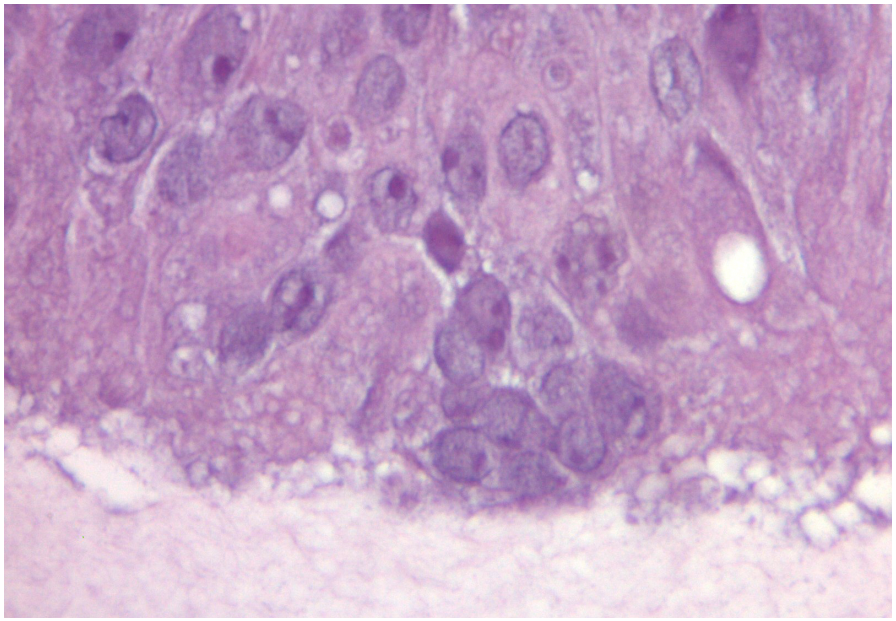


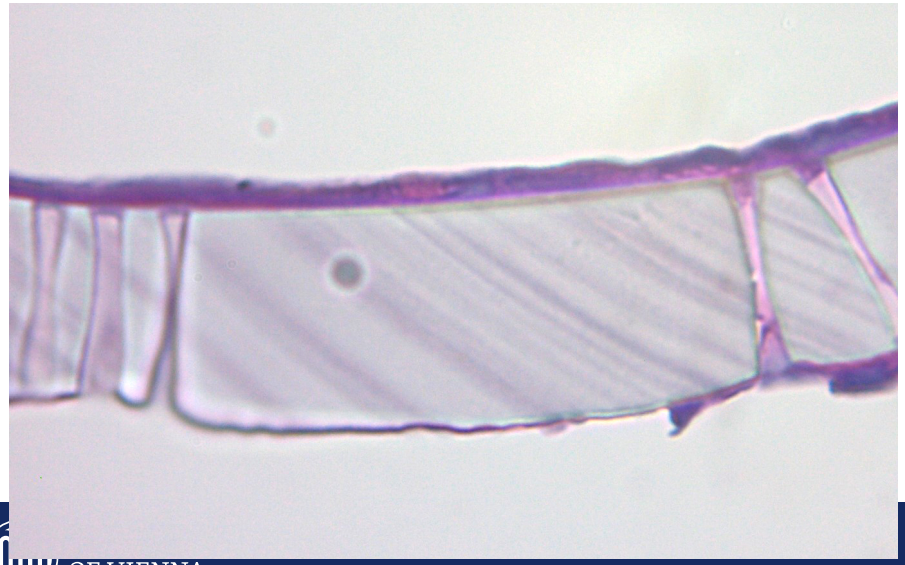


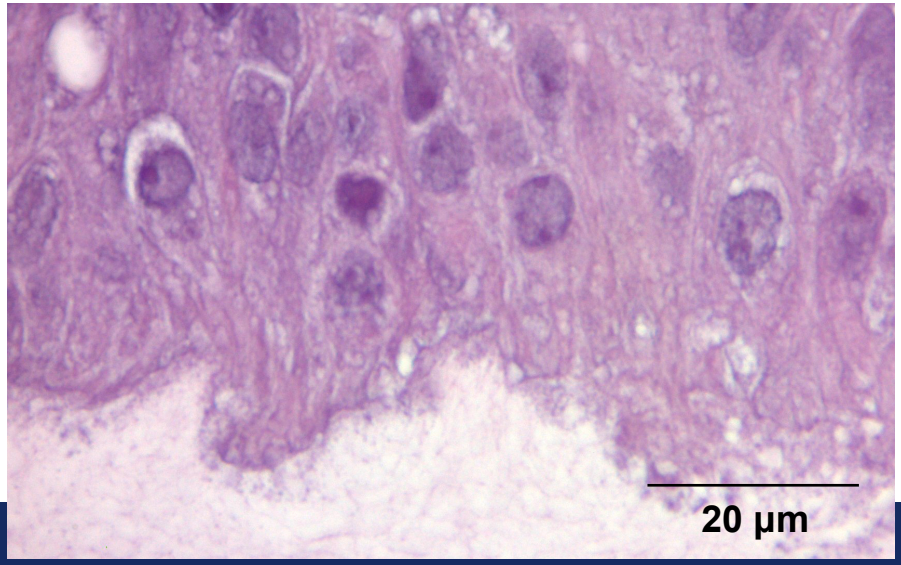


2D versus 3D

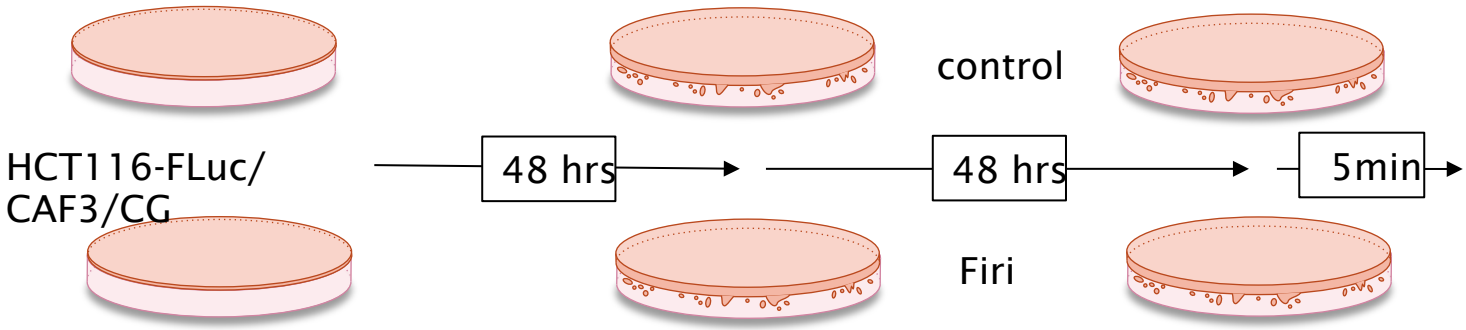


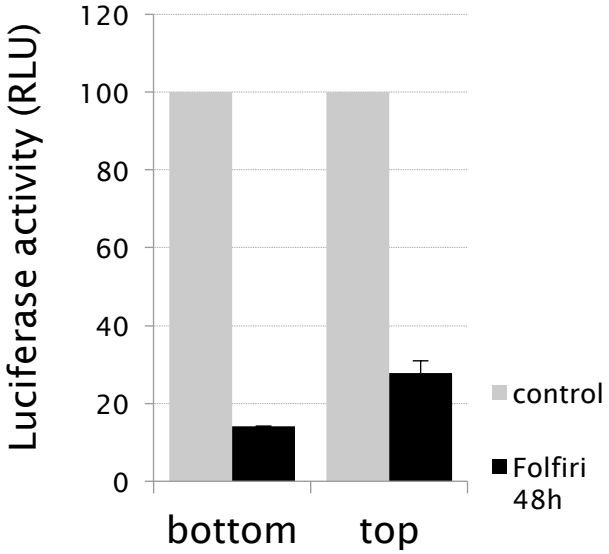


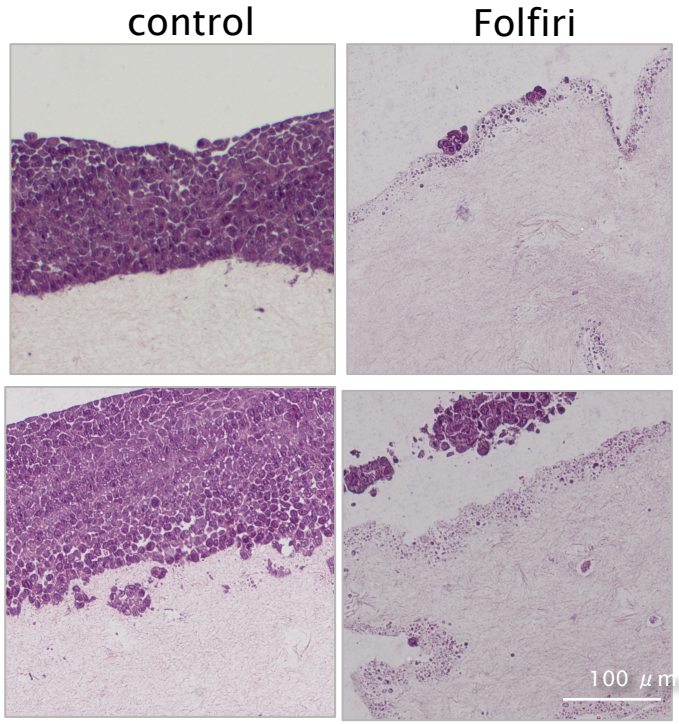


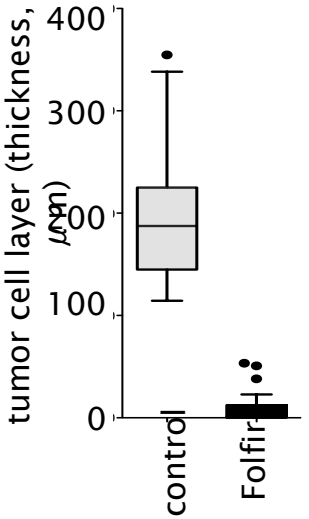


OTAs can be drug treated

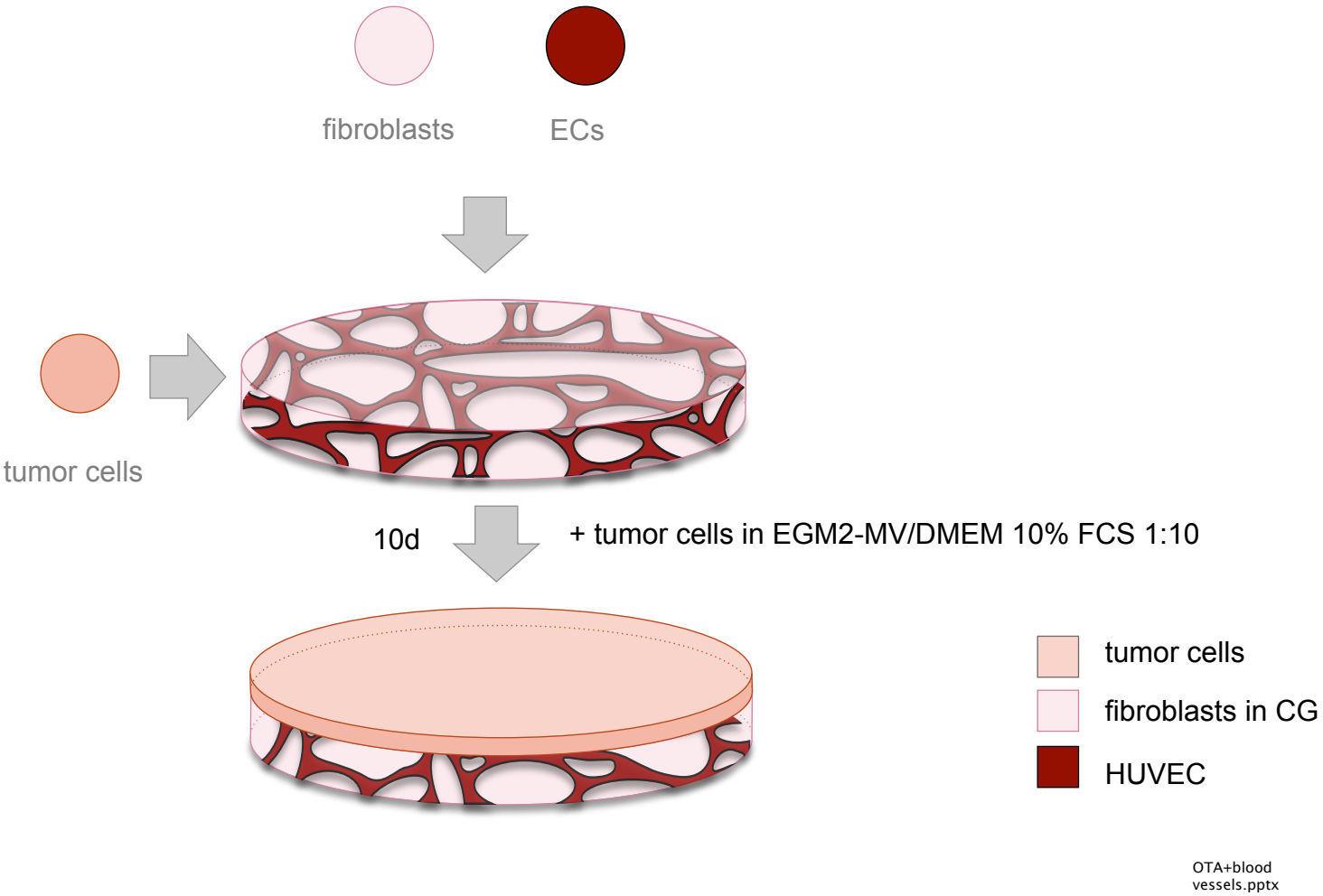




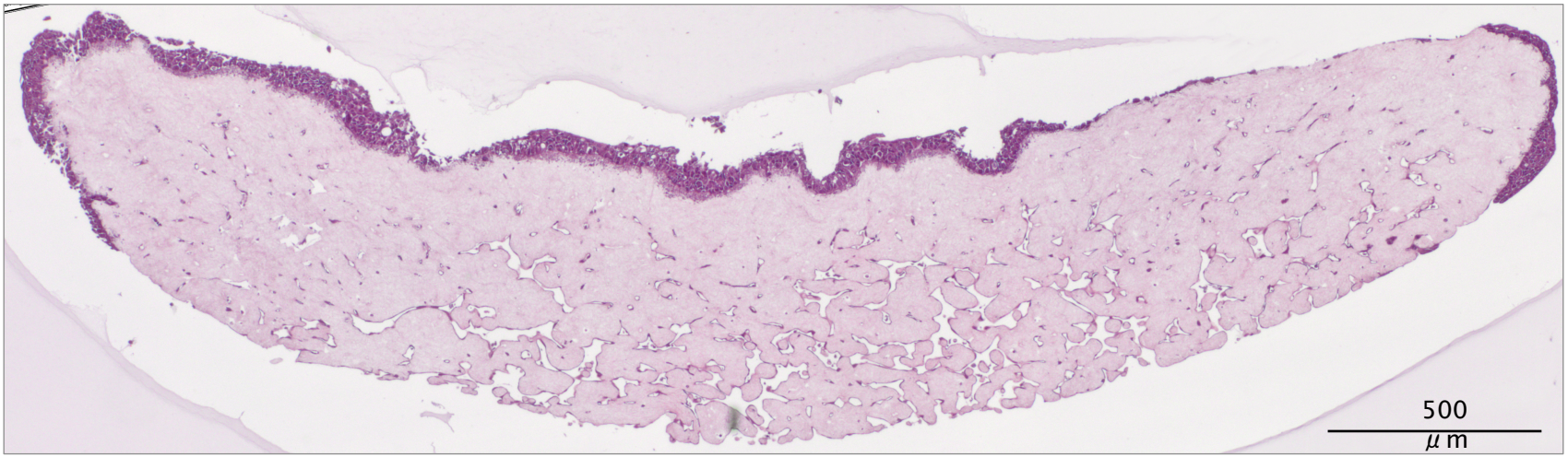


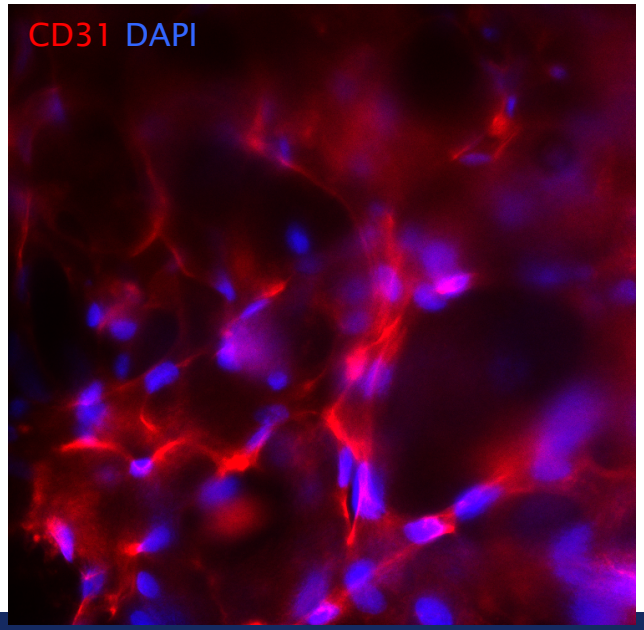


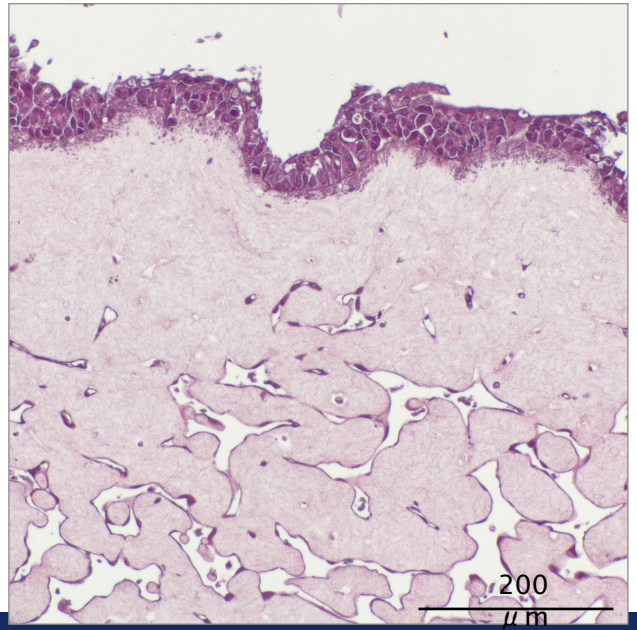
Reconstruction of colon carcinomas in vitro

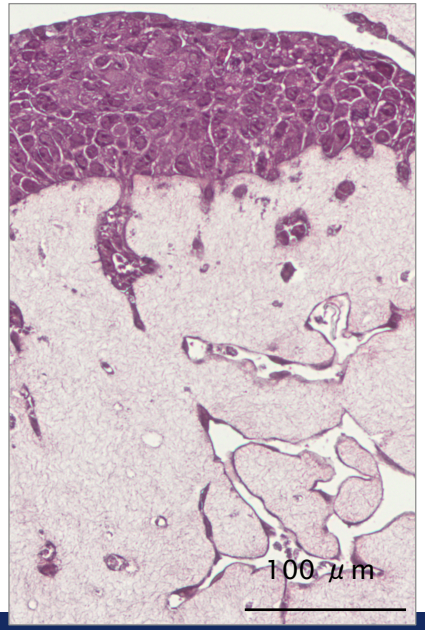


H & E

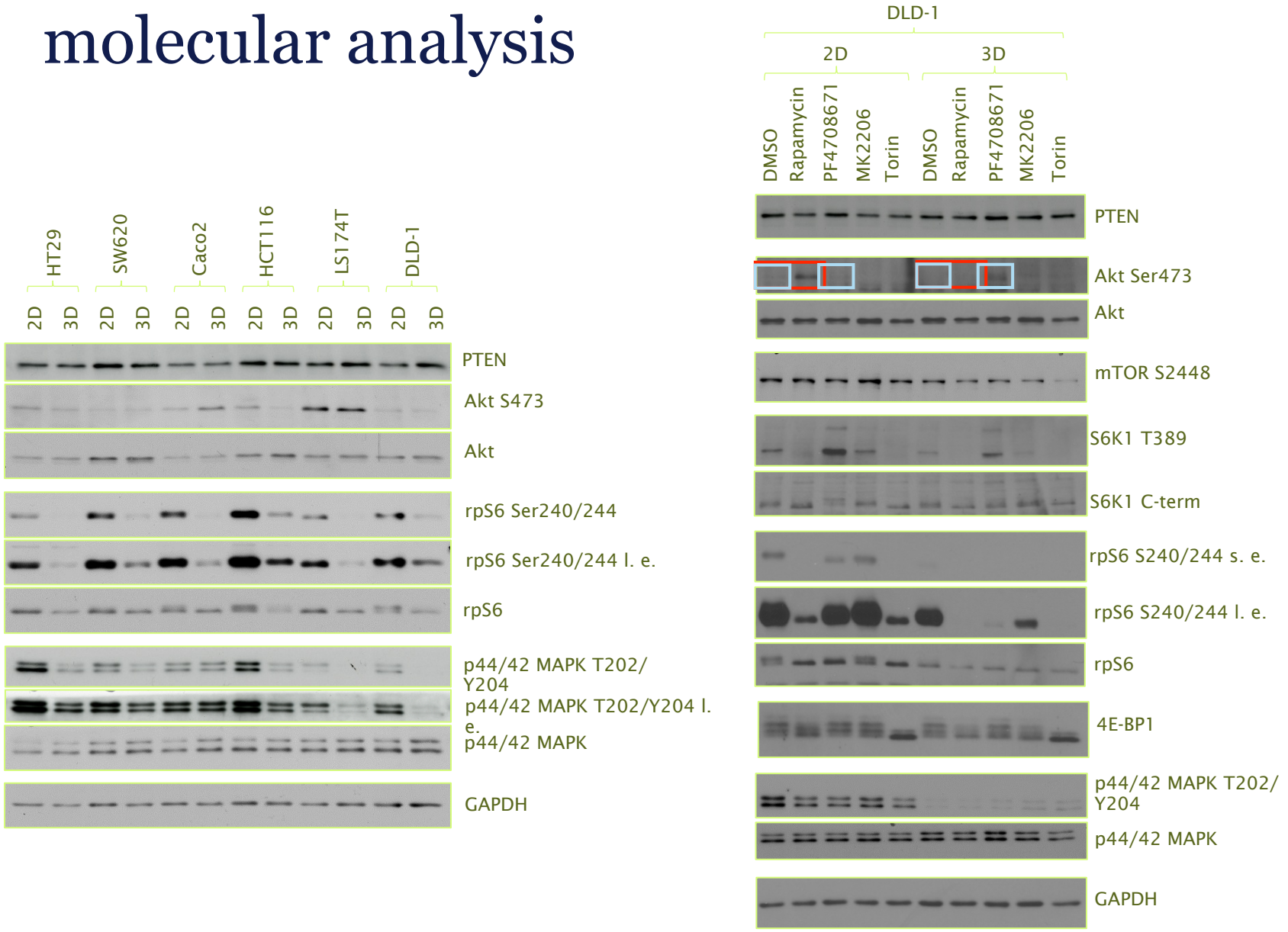








molecular analysis



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Intestinal label–retaining cells are secretory precursors expressing Lgr5

Simon J. A. Buczacki[1], Heather Ireland Zecchini[1], Anna M. Nicholson[1], Roslin Russell[1], Louis Vermeulen[1], Richard Kemp[1] & Douglas J. Winton[1]

7 MARCH 2013 | VOL 495 | NATURE | 65

A unifying theory for the crypt

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The Pan-ErbB Negative Regulator Lrig1 Is an Intestinal Stem Cell Marker that Functions as a Tumor Suppressor

Anne E. Powell,[1] Yang Wang,[1] Yina Li,[1] Emily J. Poulin,[1] Anna L. Means,[2] Mary K. Washington,[3] James N. Higginbotham,[1] Alwin Juchheim,[6] Nripesh Prasad,[7] Shawn E. Levy,[7] Yan Guo,[4] Yu Shyr,[4] Bruce J. Aronow,[8] Kevin M. Haigis,[6] Jeffrey L. Franklin,[1] and Robert J. Coffey[1,5,9,*]

146 Cell *149*, 146–158, March 30, 2012

A reserve stem cell population in small intestine renders *Lgr5*-positive cells dispensable

Hua Tian[1], Brian Biehs[2], Søren Warming[1], Kevin G. Leong[3], Linda Rangell[4], Ophir D. Klein[2] & Frederic J. de Sauvage[1]

13 OCTOBER 2011 | VOL 478 | NATURE | 255

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Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts

Toshiro Sato[1], Johan H. van Es[1], Hugo J. Snippert[1], Daniel E. Stange[1], Robert G. Vries[1], Maaike van den Born[1], Nick Barker[1], Noah F. Shroyer[2], Marc van de Wetering[1] & Hans Clevers[1]

20 JANUARY 2011 | VOL 469 | NATURE | 415

Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche

Toshiro Sato[1], Robert G. Vries[1], Hugo J. Snippert[1], Marc van de Wetering[1], Nick Barker[1], Daniel E. Stange[1], Johan H. van Es[1], Arie Abo[2], Pekka Kujala[3], Peter J. Peters[3] & Hans Clevers[1]

NATURE|Vol 459|14 May 2009

Identification of stem cells in small intestine and colon by marker gene *Lgr5*

Nick Barker[1], Johan H. van Es[1], Jeroen Kuipers[1], Pekka Kujala[2], Maaike van den Born[1], Miranda Cozijnsen[1], Andrea Haegebarth[1], Jeroen Korving[1], Harry Begthel[1], Peter J. Peters[2] & Hans Clevers[1]

NATURE|Vol 449|25 October 2007

Tissue Barriers 1:2, e24965; April/May/June 2013; © 2013 Landes Bioscience

Understanding epithelial homeostasis in the intestine

An old battlefield of ideas, recent breakthroughs and remaining controversies

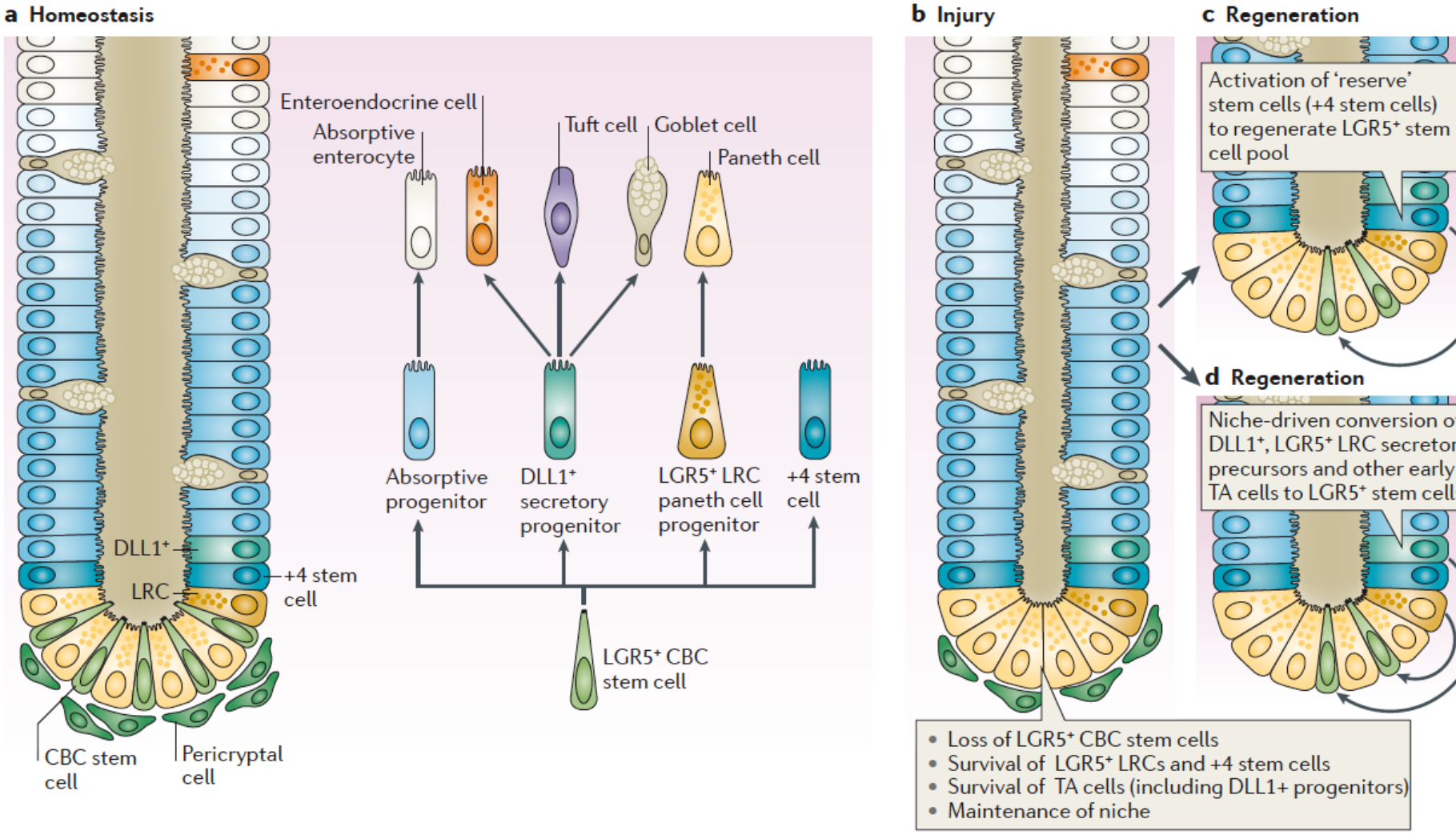
Jan R. De Mey[1,3,*] and Jean-Noël Freund[2,3,4]

Cell

The Intestinal Crypt, A Prototype Stem Cell Compartment

Hans Clevers[1,2,*]
[1]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences, 3584 CT Utrecht, the Netherlands
[2]University Medical Center, 3584 CX Utrecht, the Netherlands
*Correspondence: h.clevers@hubrecht.eu
http://dx.doi.org/10.1016/j.cell.2013.07.004

Current Model



Barker, Nature 2014