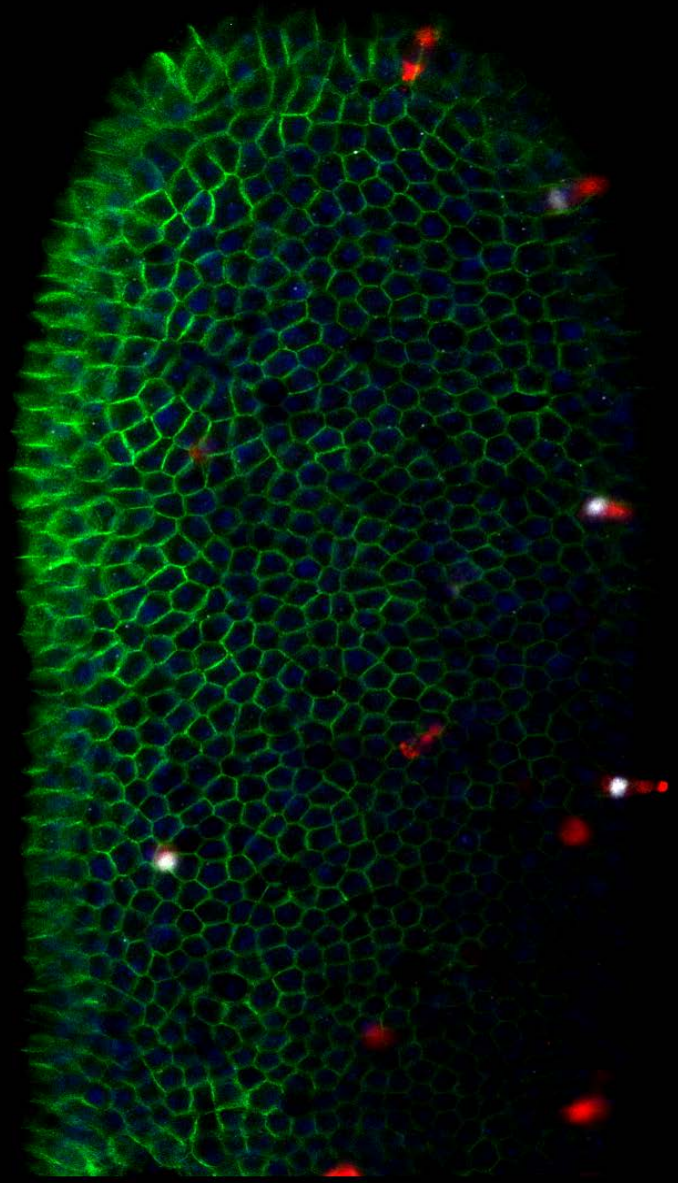


Stem cell maintenance, differentiation and plasticity in a rapidly renewing epithelium

Philippe Jay
Institute of Functional Genomics
Department of Cancer Biology
Self-renewal and differentiation of epithelia

Institut de Génomique Fonctionnelle

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

Instituts thématiques Inserm Institut national de la santé et de la recherche médicale

UNIVERSITÉ DE MONTPELLIER

Summary

- The intestinal epithelium

- Key signalling pathways for compartmenting proliferation and differentiation

- Nature and markers of stem cells

- Plasticity in the context of stem cell injury; role of the niche

- Intestinal organoids

- Stem cell plasticity in the context of epithelial adaptation and tumorigenesis

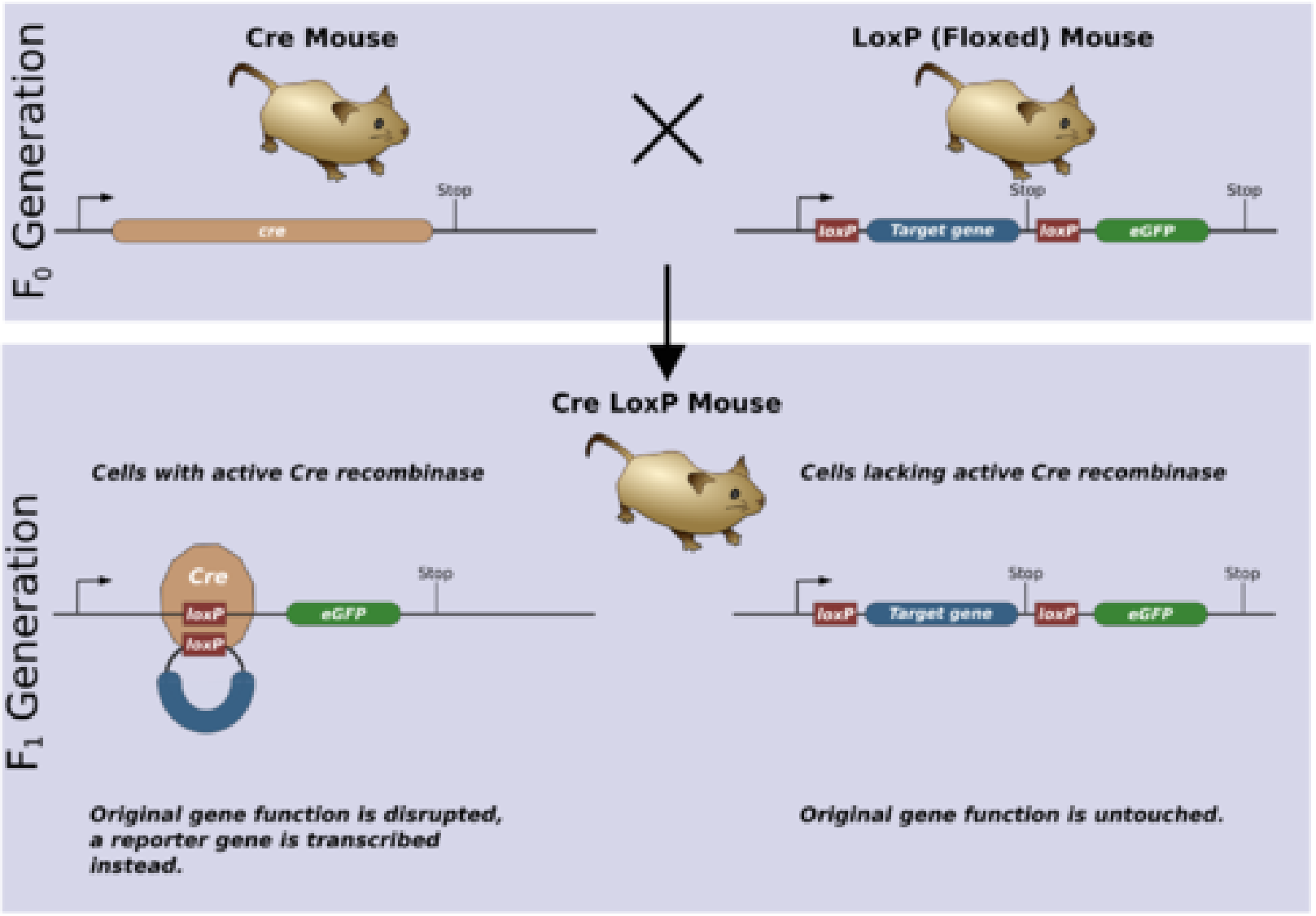
From a theorical definition of an adult tissue stem cell

To the identification of these stem cells

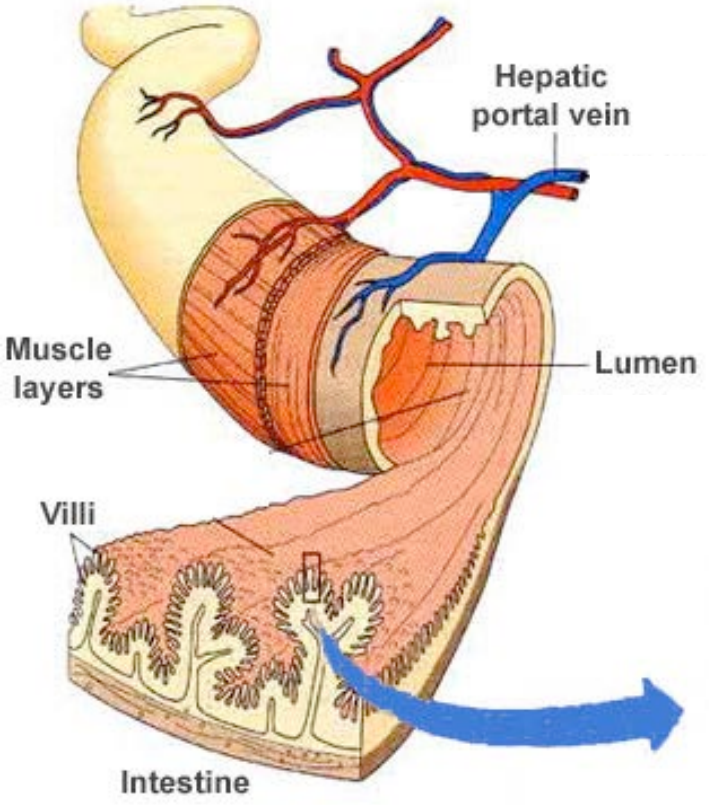
And to the elucidation of their key properties and interactions with their progeny

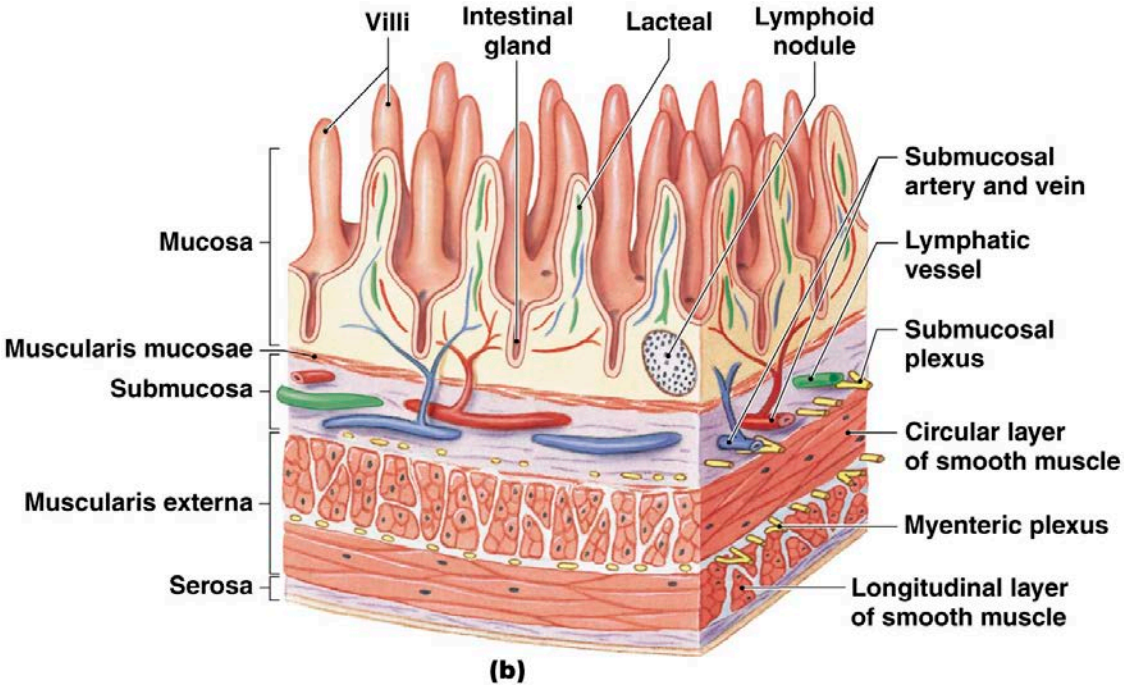
A practical example of how science proceeds

THE CRE-LOXP SYSTEM

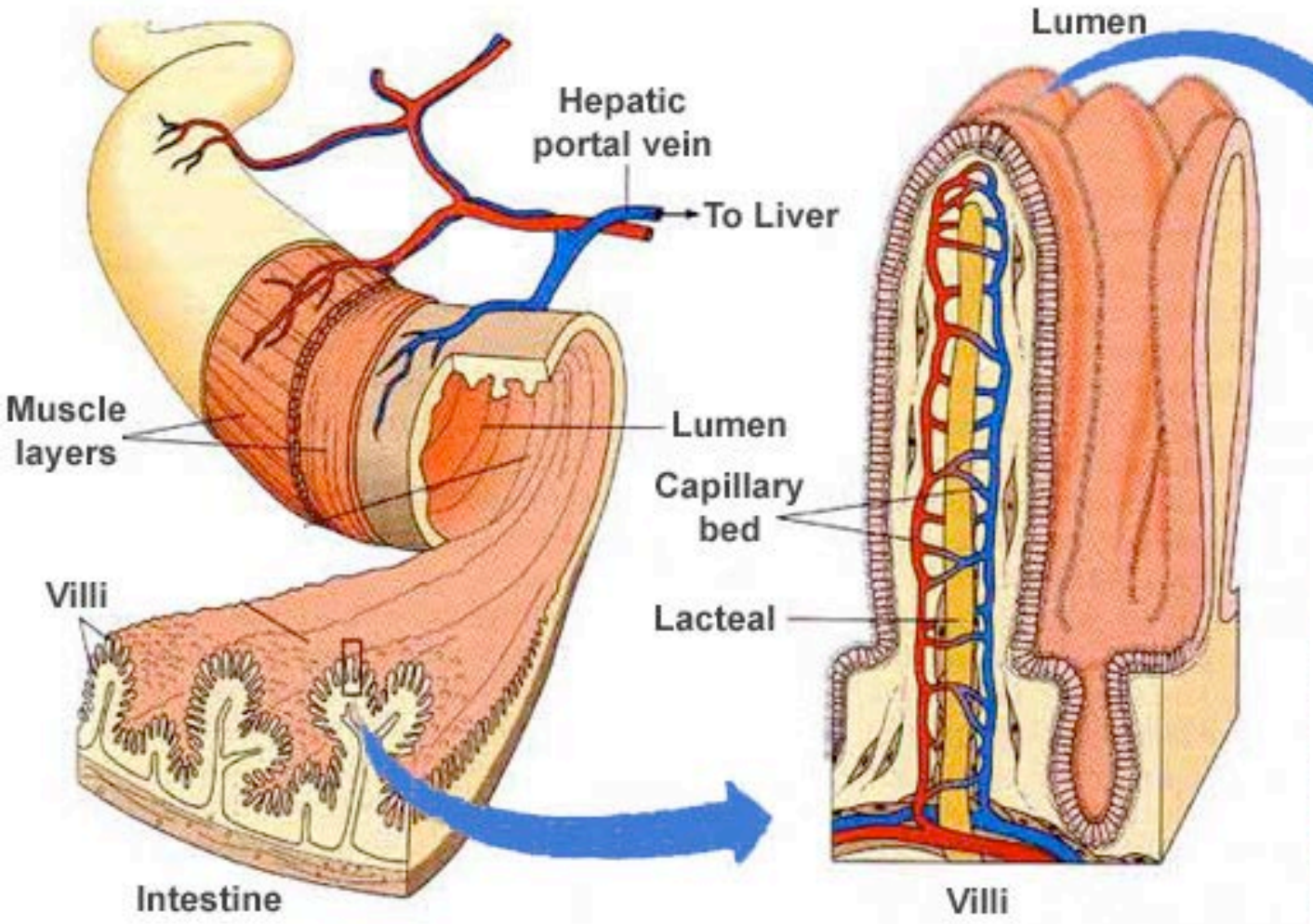


The intestinal epithelium: our principal interface with the external world





THE INTESTINAL EPITHELIUM: OUR PRINCIPAL INTERFACE WITH THE EXTERNAL WORLD



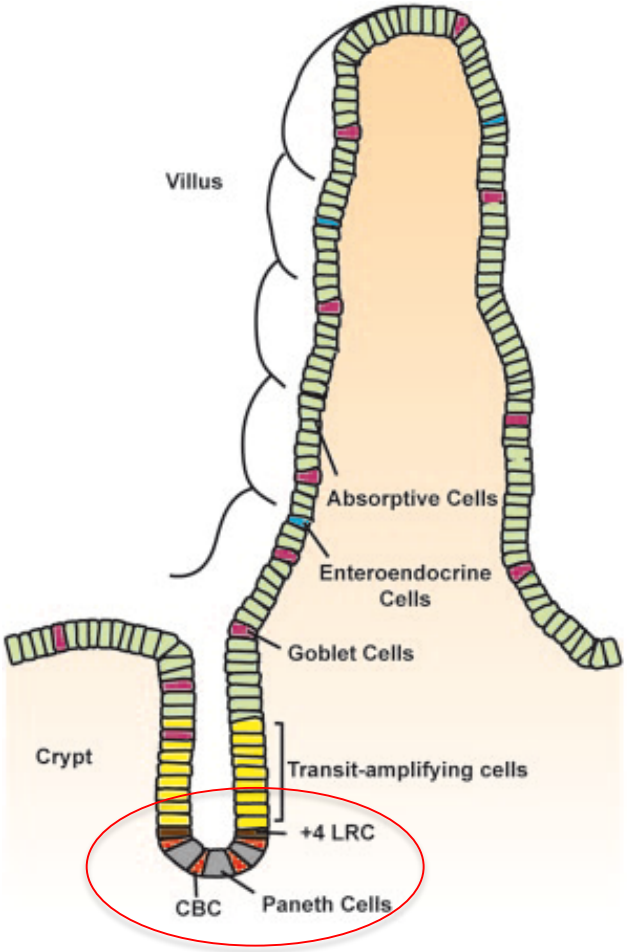
200m² !

The GI tract - general histology

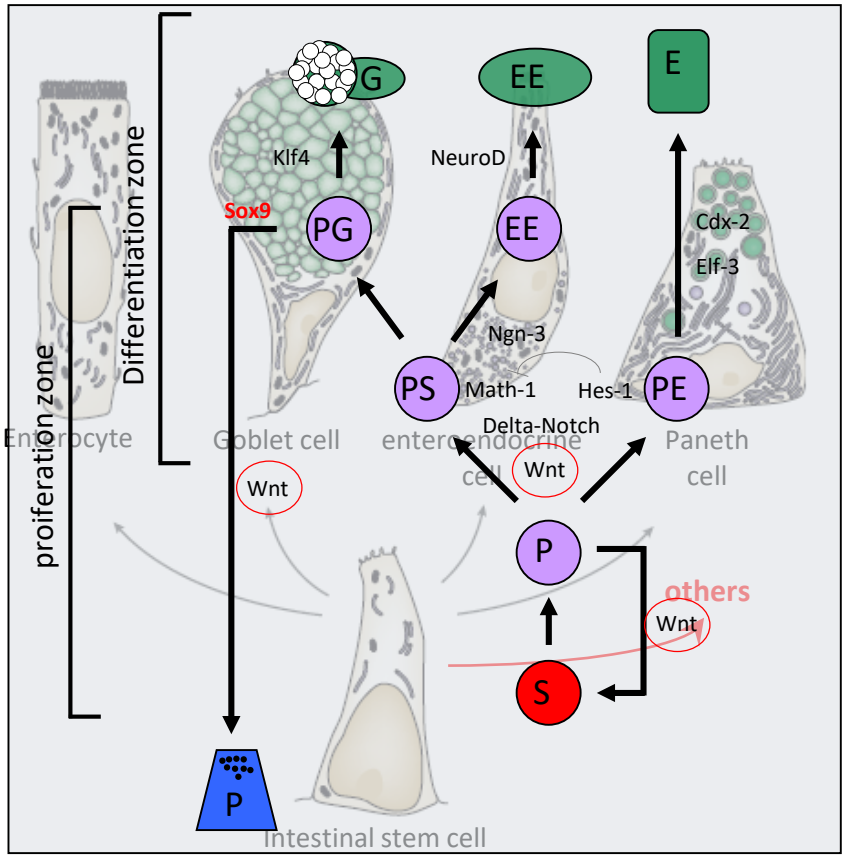
Small intestine

colon

THE INTESTINAL EPITHELIUM : FUNCTIONAL COMPARTMENTS AND CELL TYPES



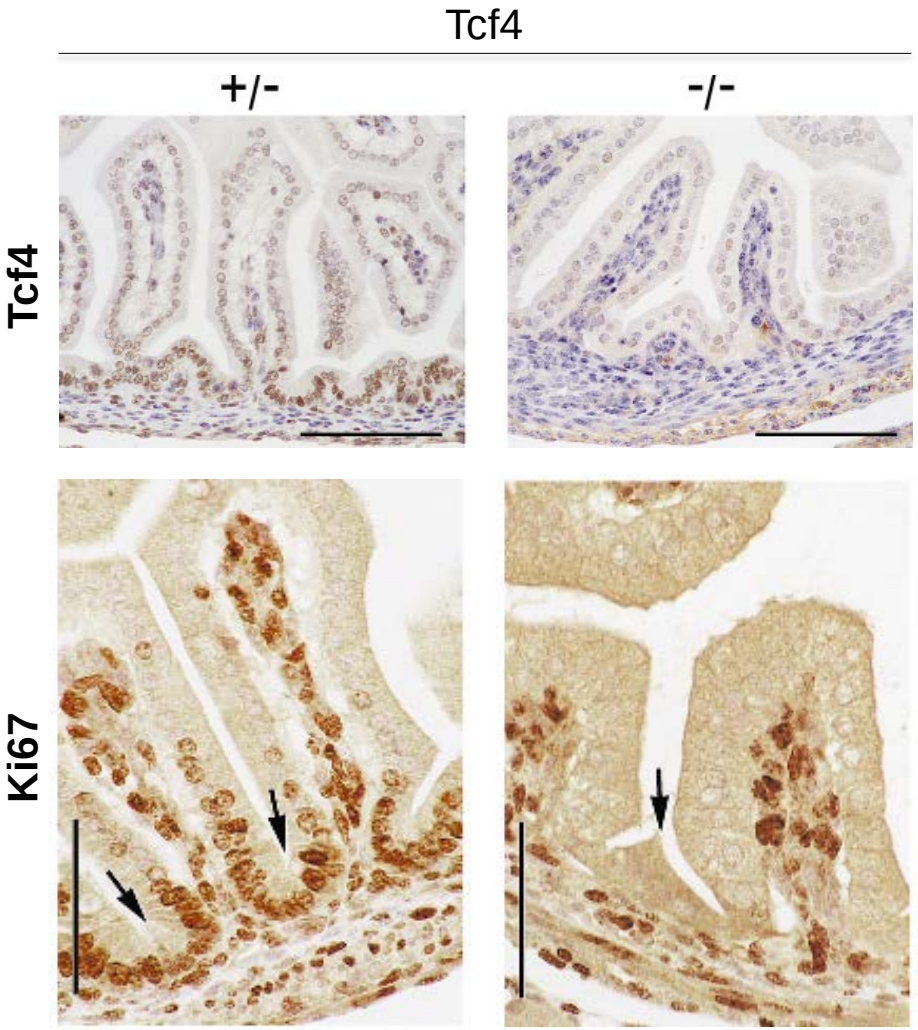
From Haegebarth et al. Am. J Path. 2009



Van Es et al, Nature Cell Biol. 2005
Bastide et al, JCB, 2007

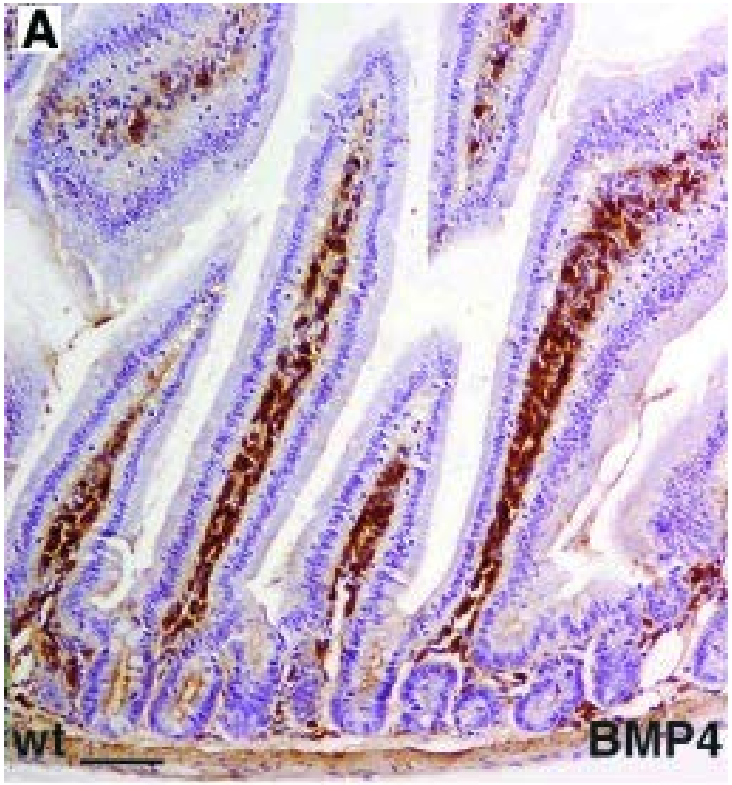
Several signalling pathways required for the functional compartmentation of the intestinal epithelium

WNT SIGNALLING IS REQUIRED FOR CELL PROLIFERATION IN INTESTINAL CRYPTS

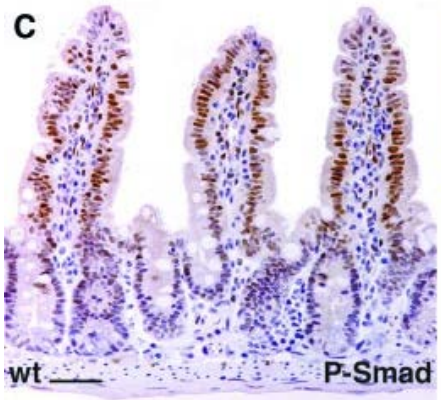


Korinek et al., Nat. Genet. 1998

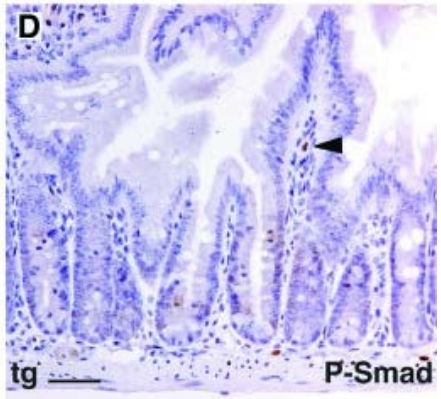
BMP SIGNALLING LIMITS CELL PROLIFERATION IN THE INTESTINAL EPITHELIUM



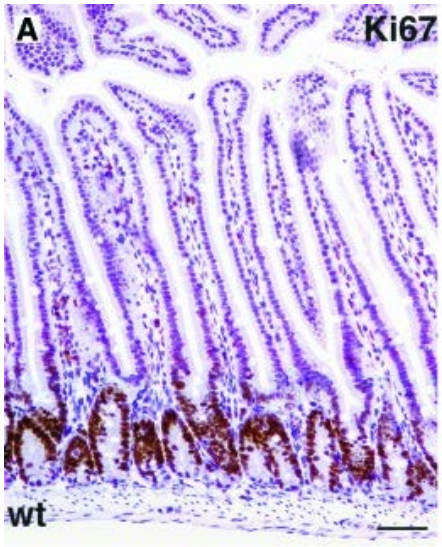
Wild type



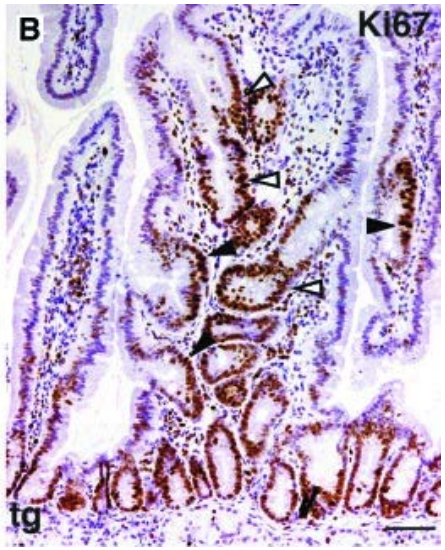
Wild type



Villin-Noggin



Wild type

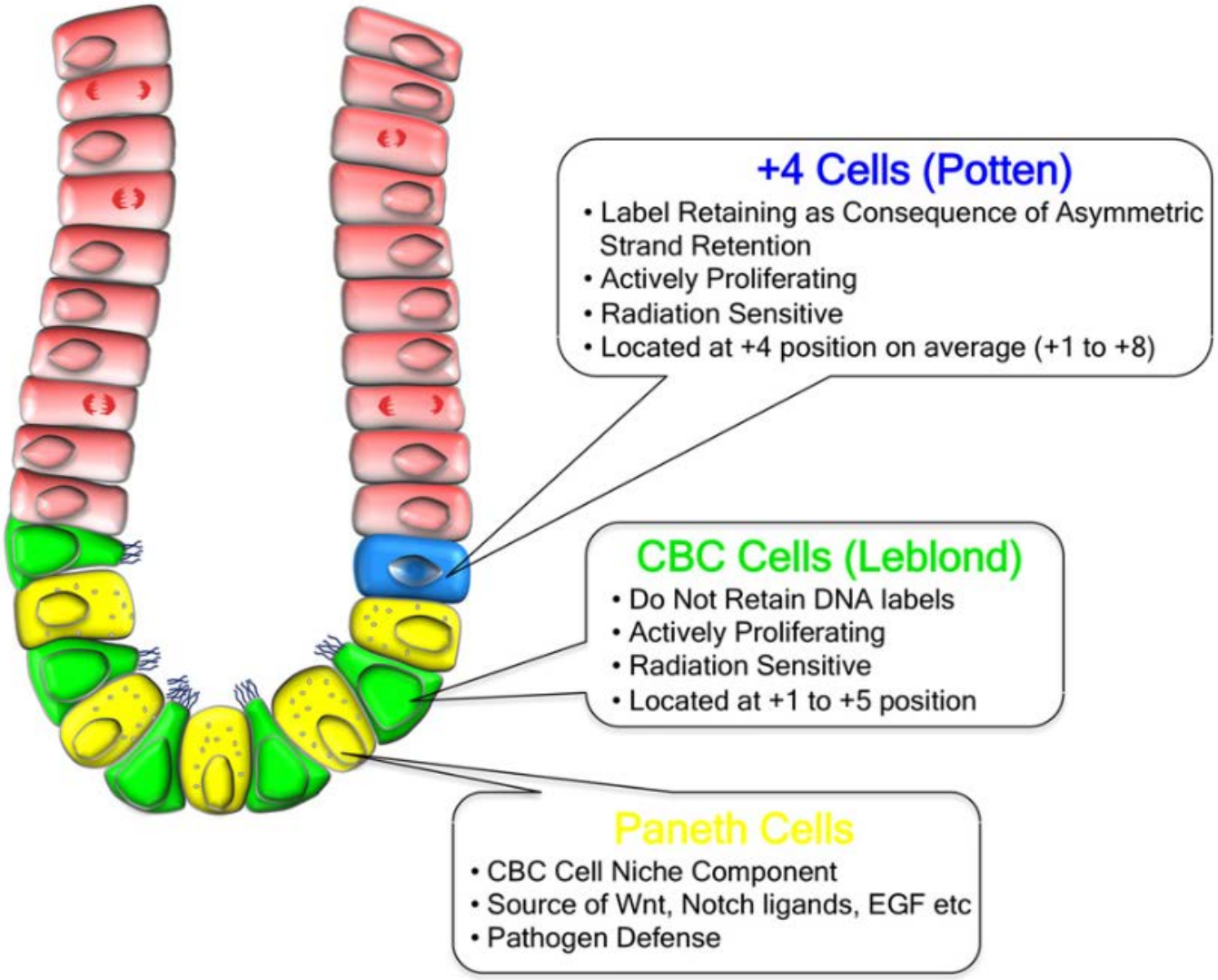


Villin-Noggin

Haramis et al., Science 2004

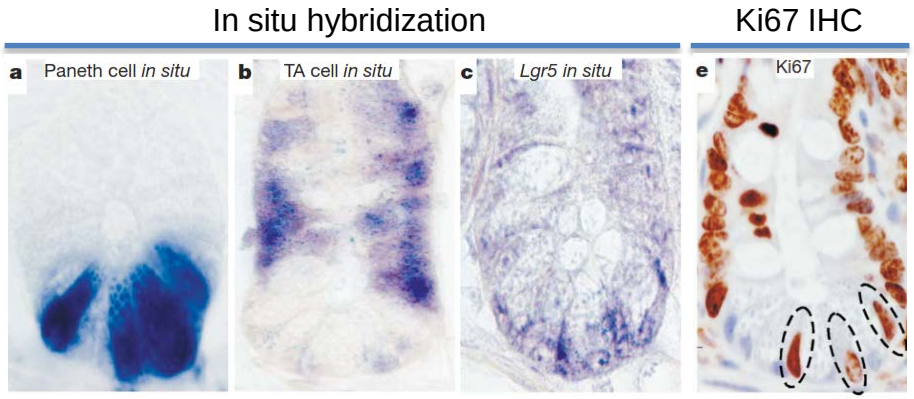
IDENTIFICATION OF THE INTESTINAL EPITHELIAL STEM CELLS

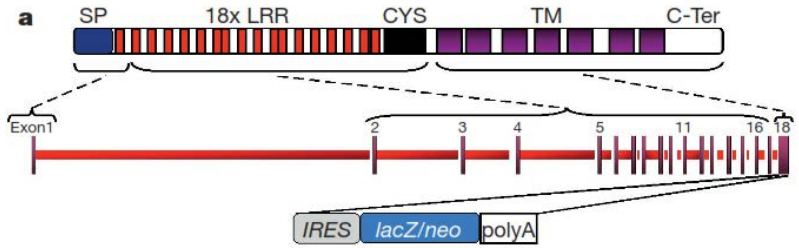
THE DISCOVERY OF THE INTESTINAL EPITHELIAL STEM CELLS – AN HISTORICAL PERSPECTIVE

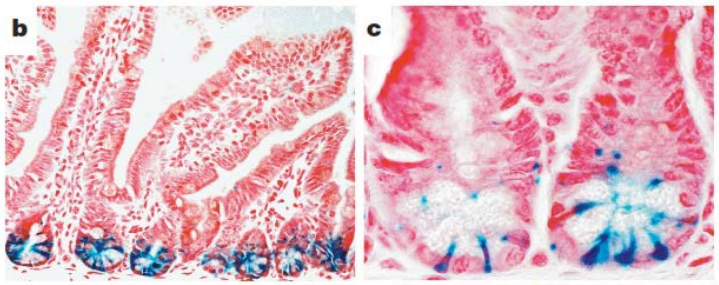


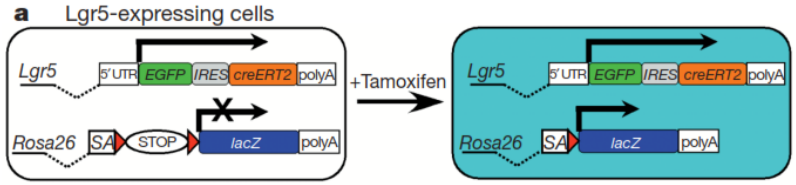
Barker et al. Cell Stem Cell 2012

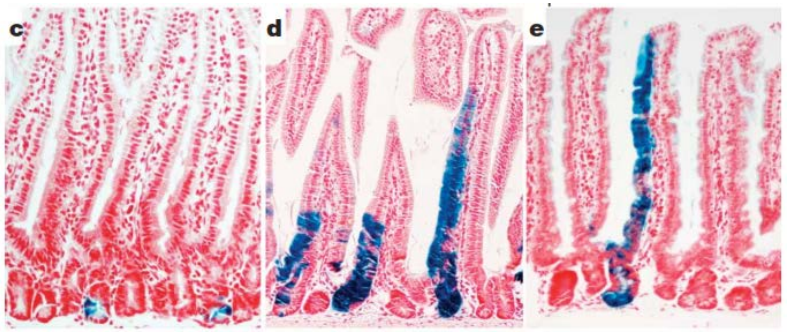
IDENTIFICATION OF THE INTESTINAL STEM CELLS BY EXPRESSION OF THE MARKER GENE LGR5

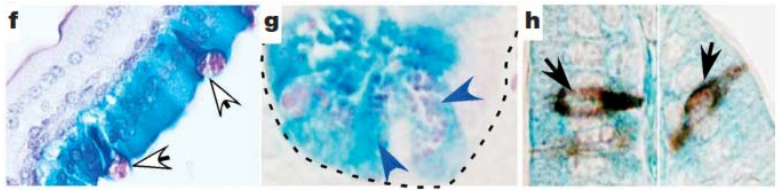








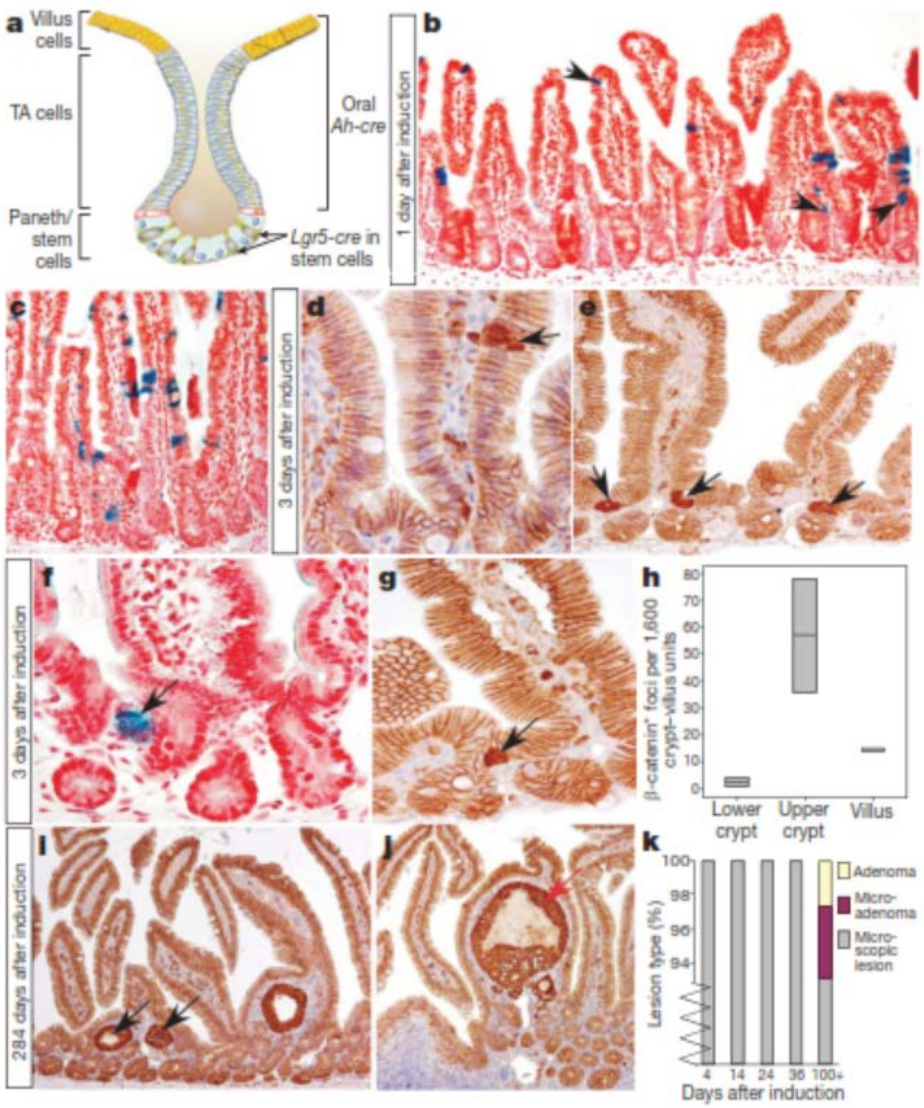




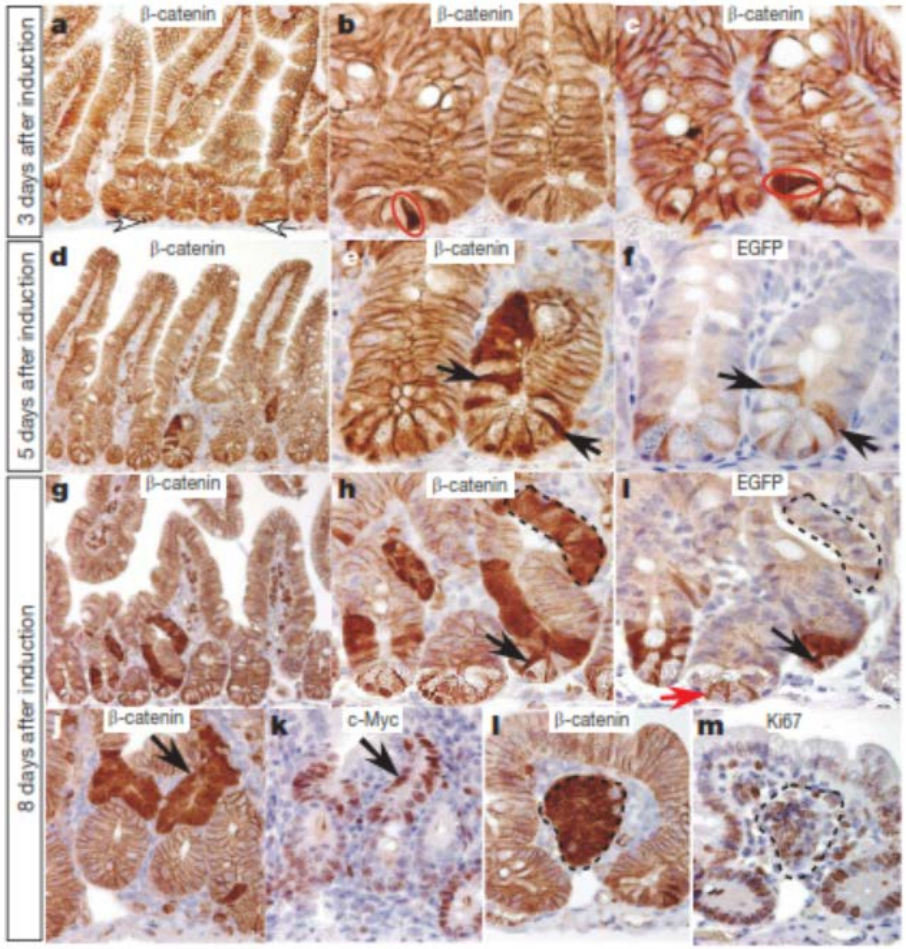
Barker et al; Nature 2007

But: see also Bjerknes et al; Gastroentorelogy 1999

TUMORIGENESIS PREFERENTIALLY INITATES AT THE CRYPT BASE



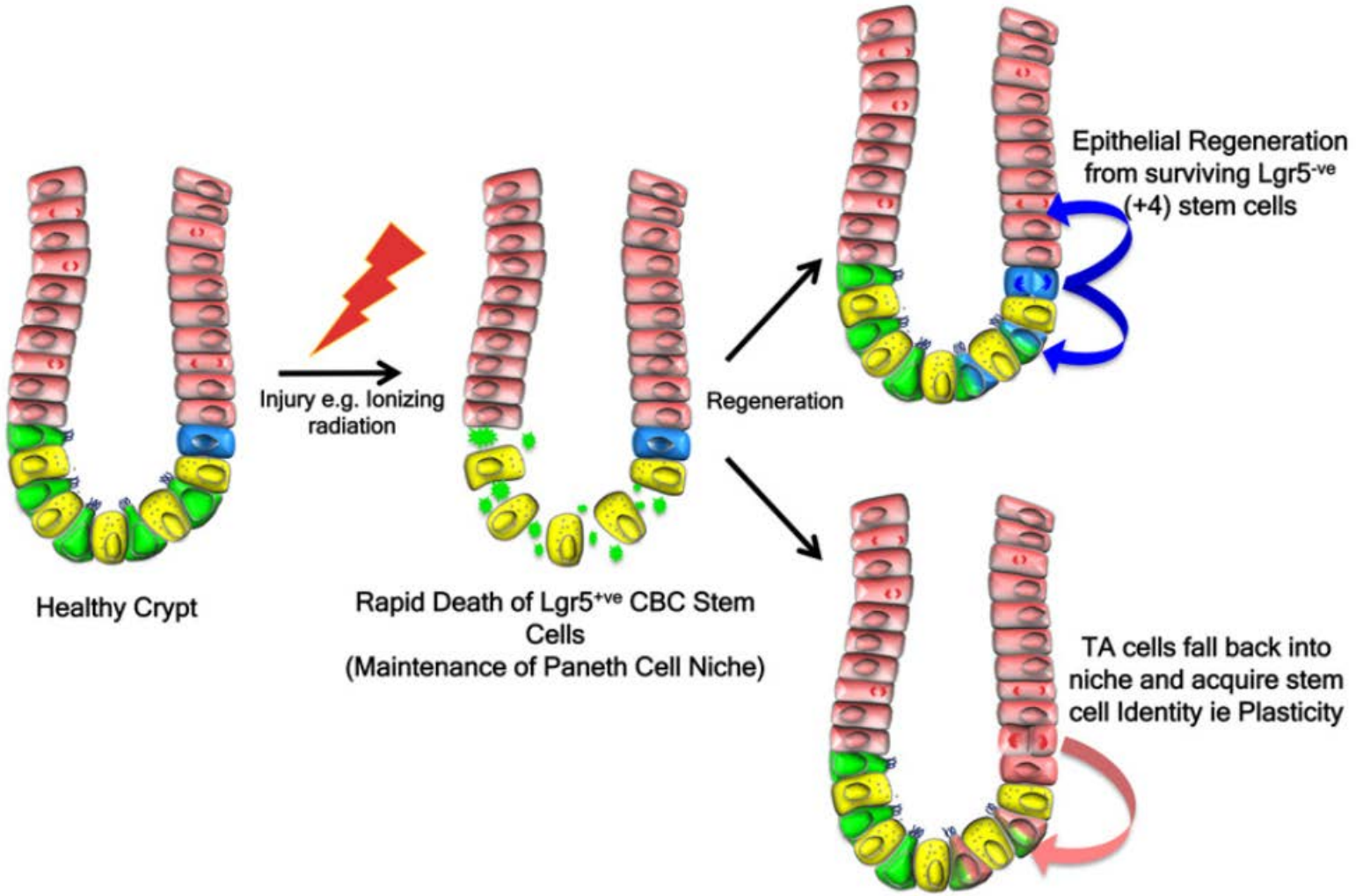
AhCre;Rosa26-LacZ;Apc$^{LoxP/LoxP}$



Lgr5-Cre^{ERT2};Rosa26-LacZ;Apc$^{LoxP/LoxP}$

Barker et al. Nature 2009

Two models for crypt regeneration following injury: reserve stem cells vs. plasticity

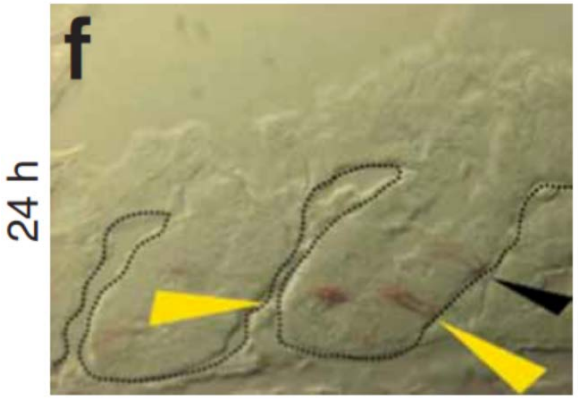


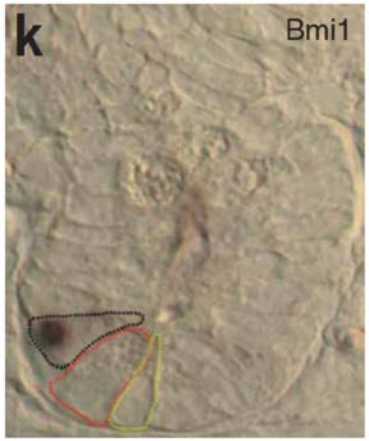
Barker et al. Cell Stem Cell 2012

Bmi1 is expressed *in vivo* in intestinal stem cells

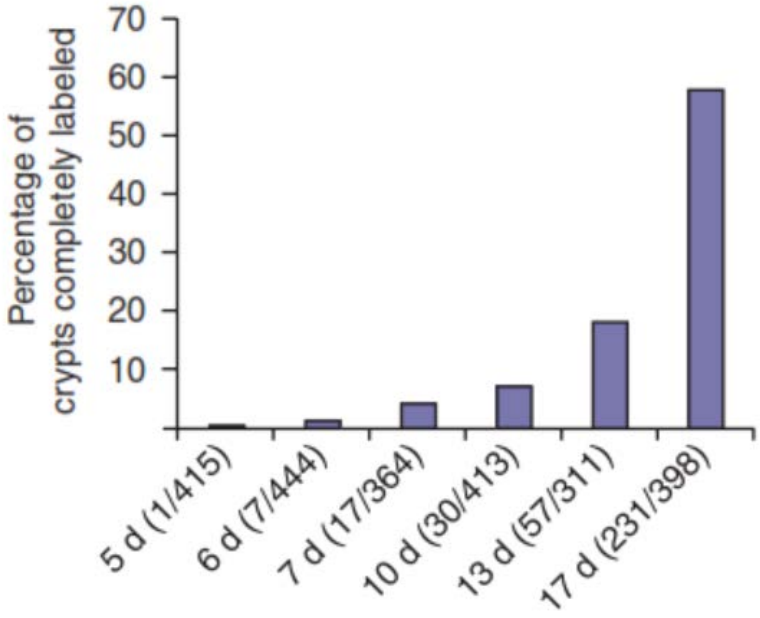
Eugenio Sangiorgi & Mario R Capecchi

Mouse model : *Bmi1-ORF-IRES-Cre^{ERT2};Rosa26-LacZ*





Endogenous *Bmi1* mRNA (*in situ* hyb.)



Days of tamoxifen treatment *LacZ* expression

Sangiorgi al. Nat. Genet. 2008

Bmi1 is expressed *in vivo* in intestinal stem cells

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

Anne E. Powell,[1] Yang Wang,[1] Yina Li,[1] Emily J. Poulin,[1] Anna L. Means,[2] M
Alwin Juchheim,[6] Nripesh Prasad,[7] Shawn E. Levy,[7] Yan Guo,[4] Yu Sh
Jeffrey L. Franklin,[1] and Robert J. Coffey[1,5,9,*]

Cell 2012

transcriptase (mTert) intestinal stem cells

Farilla[b,c], mbruzs[c], am,[1]

Identification of a Novel Putative Gastrointestinal Stem Cell and Adenoma Stem Cell Marker, Doublecortin and CaM Kinase-Like-1, Following Radiation Injury and in Adenomatous Polyposis Coli/Multiple Intestinal Neoplasia Mice

RANDAL MAY,[a] TERRENCE E. RIEHL,[b] CLAYTON HUNT,[c] SRIPATHI M. SUREBAN,[a] SHRIKANT ANANT,[a,d] COURTNEY W. HOUCHEN[a]

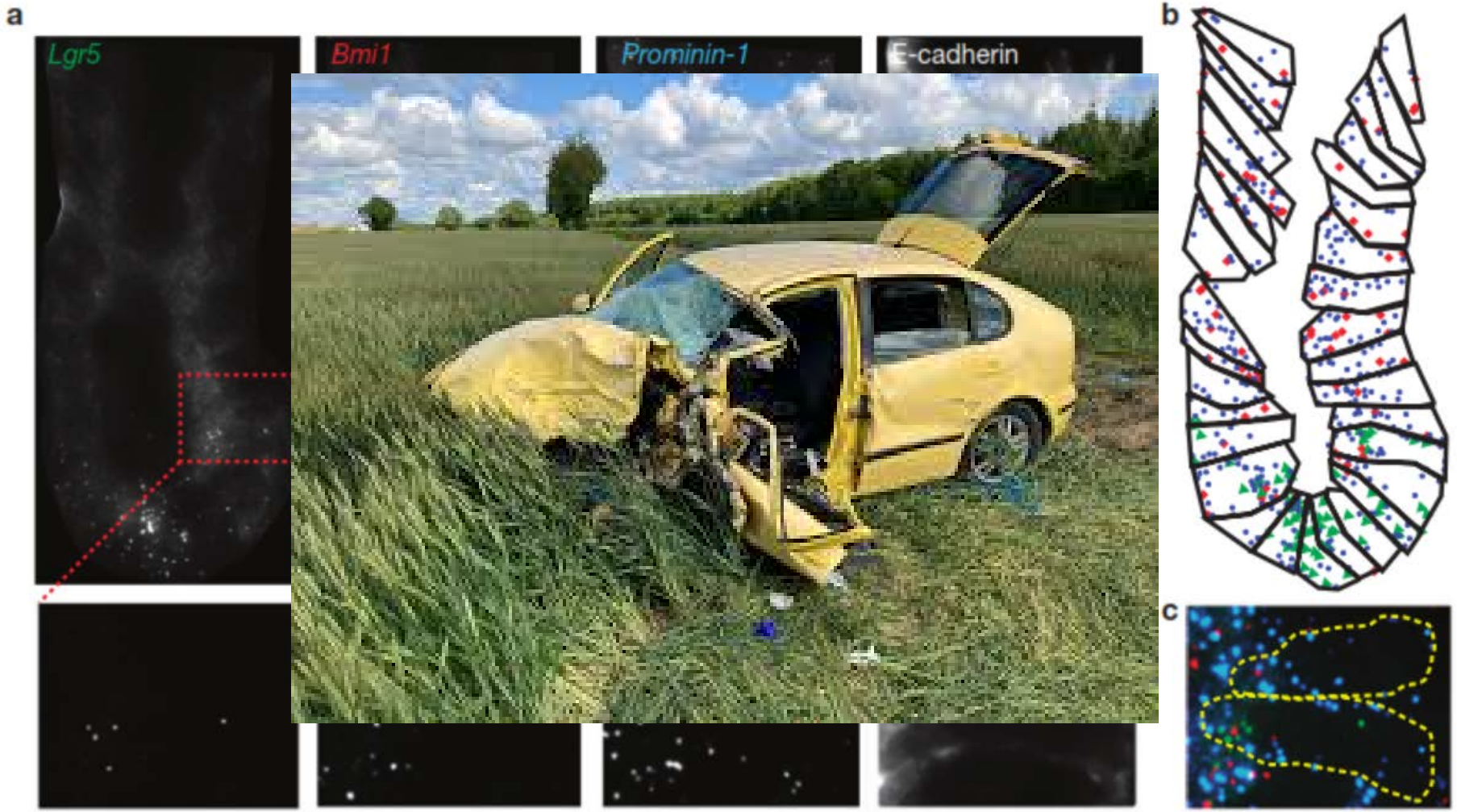
DCAMKL-1 and LGR5 Mark Quiescent and Cycling Intestinal Stem Cells Respectively

Randal May[1,5], Sripathi M. Sureban[1,5], Nguyet Hoang[1], Terrence E. Riehl[8], Stan A. Lightfoot[3,5], Rama Ramanujam[6,7], James H. Wyche[6], Shrikant Anant[1,2,4,6] and Courtney W. Houchen[1,4,5,6].

Stem cells 2009 PNAS

Single-molecule transcript counting of stem-cell markers in the mouse intestine

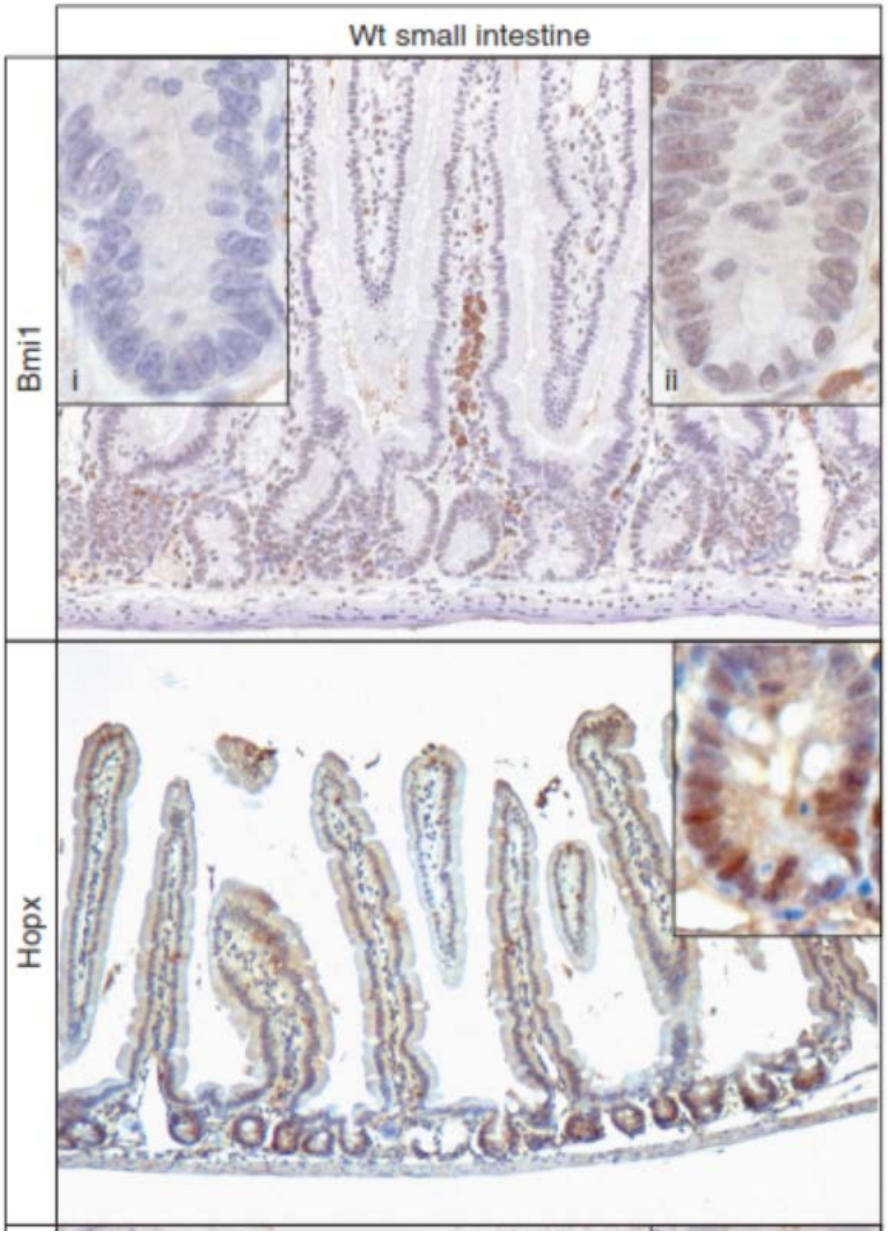
Shalev Itzkovitz[1,2], Anna Lyubimova[1,2,3], Irene C. Blat[2,4], Mindy Maynard[4], Johan van Es[3], Jacqueline Lees[2,4], Tyler Jacks[2,4,5], Hans Clevers[3] and Alexander van Oudenaarden[1,2,3,4,6]

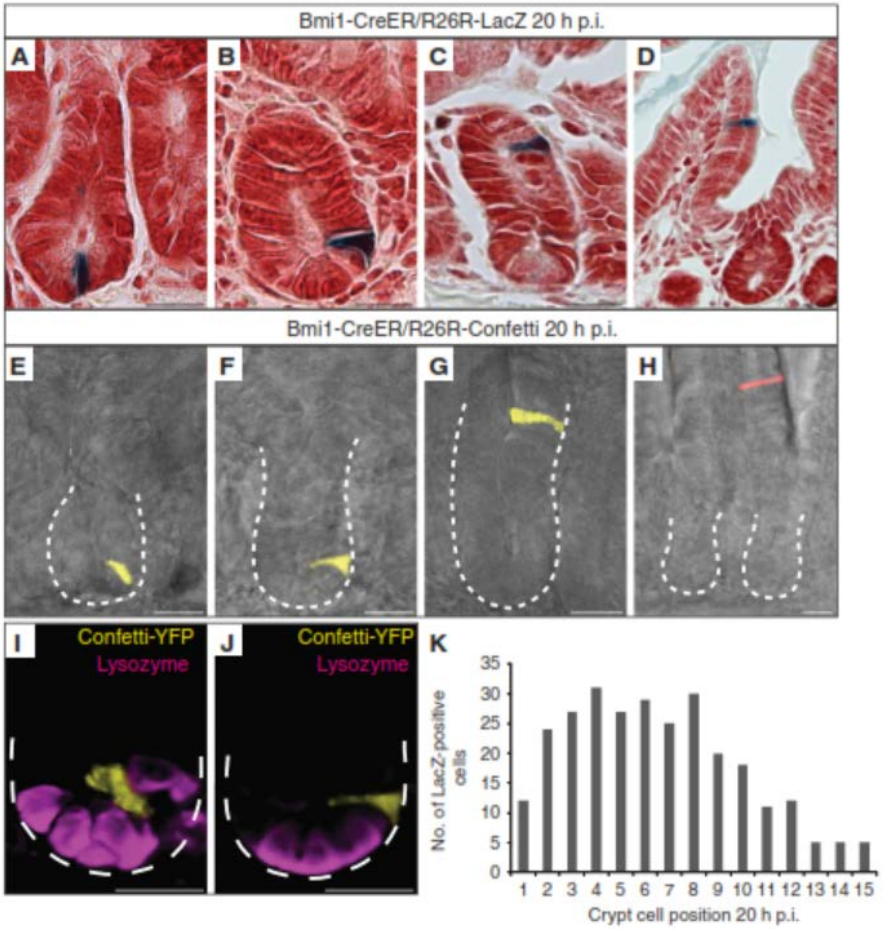


What can you conclude here?

Itzkovitz al. Nat. Cell Biol, 2011

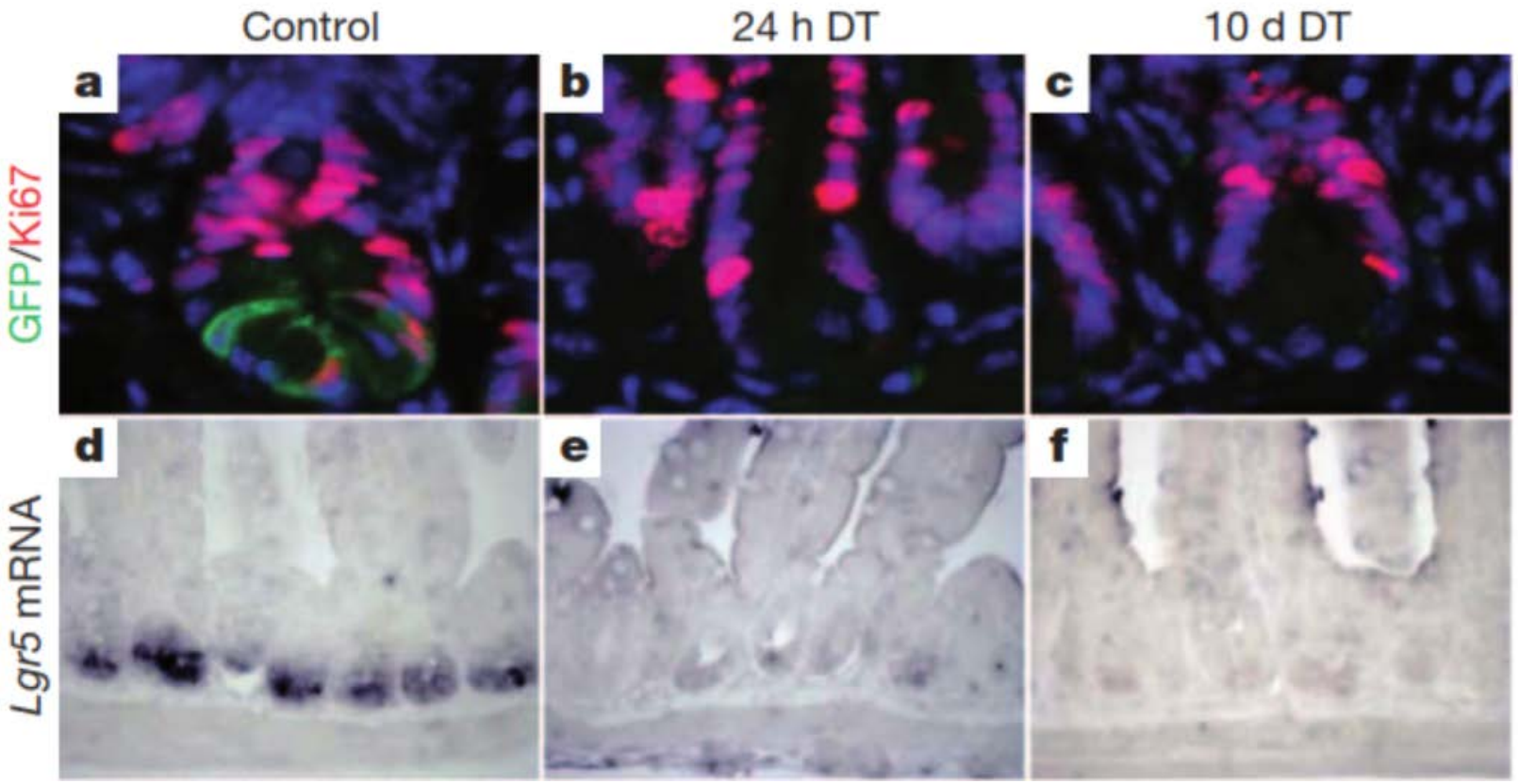
PROTEIN EXPRESSION PATTERN OF BMI1 AND HOPX





Munoz et al. EMBO J. 2012

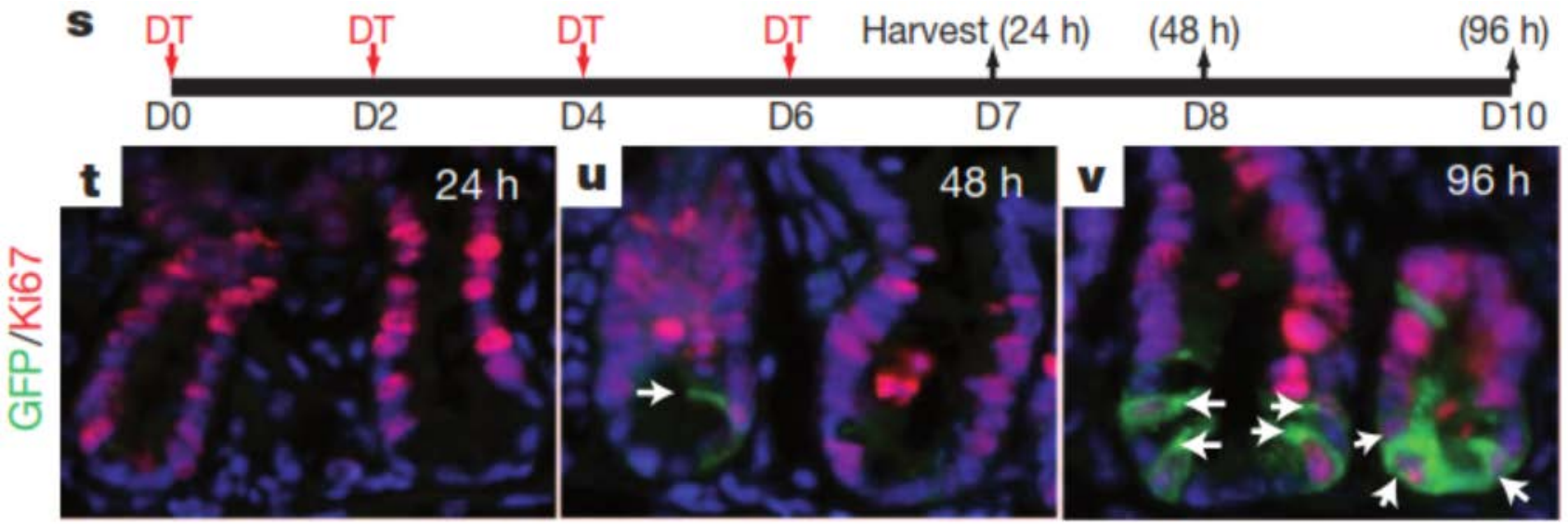
Intact epithelial turnover after elimination of the Lgr5^{+} stem cells



Mouse model : Lgr5-eGFP-DTR → gavage with diphtheria toxin

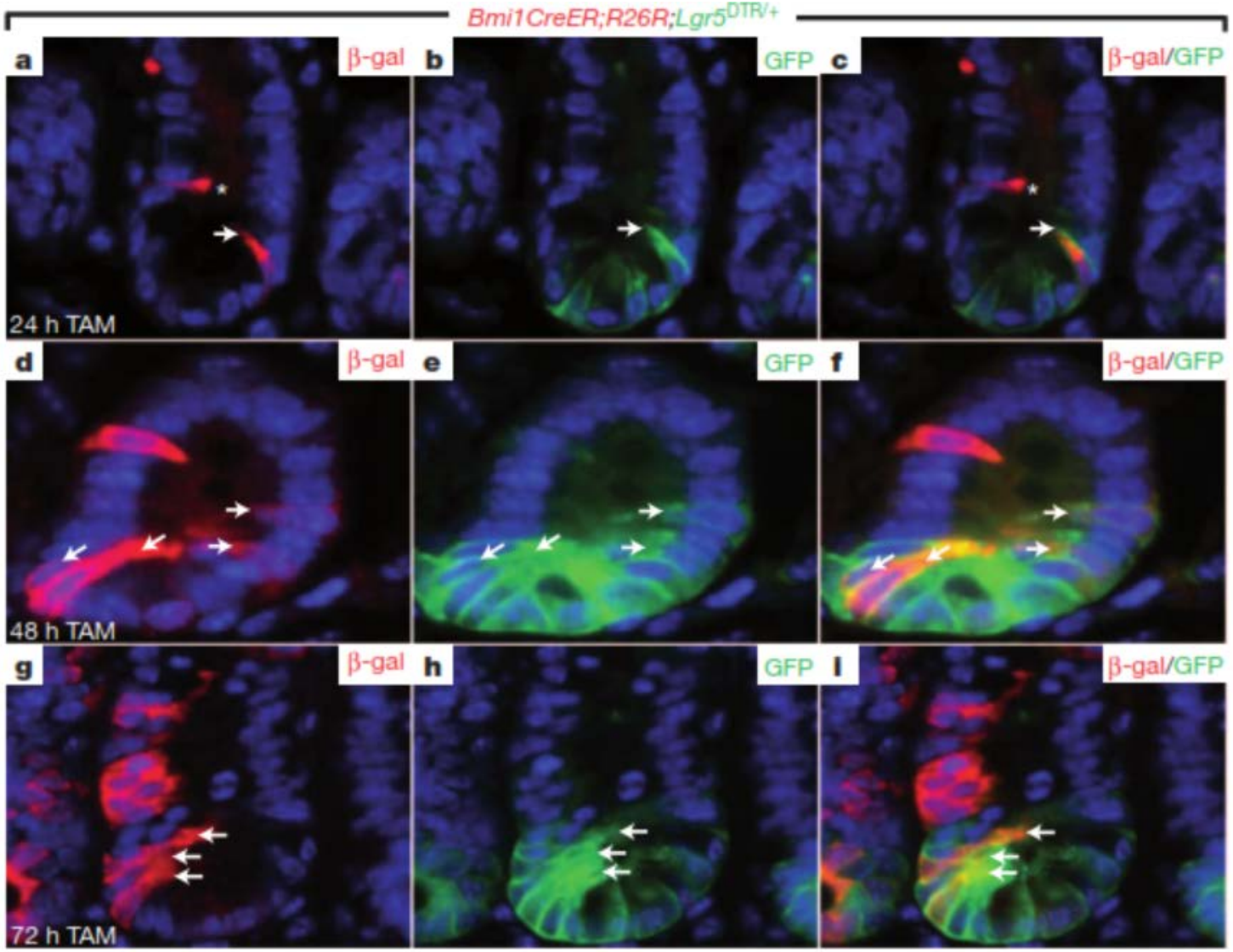
Tian et al. Nature 2011

Rapid reconstitution of the Lgr5^{+} stem cell pool after DTA-mediated injury



Tian et al. Nature 2011

Regeneration of a Lgr5^{+} cell compartment from Bmi-1^{+} cells located at the +4 position

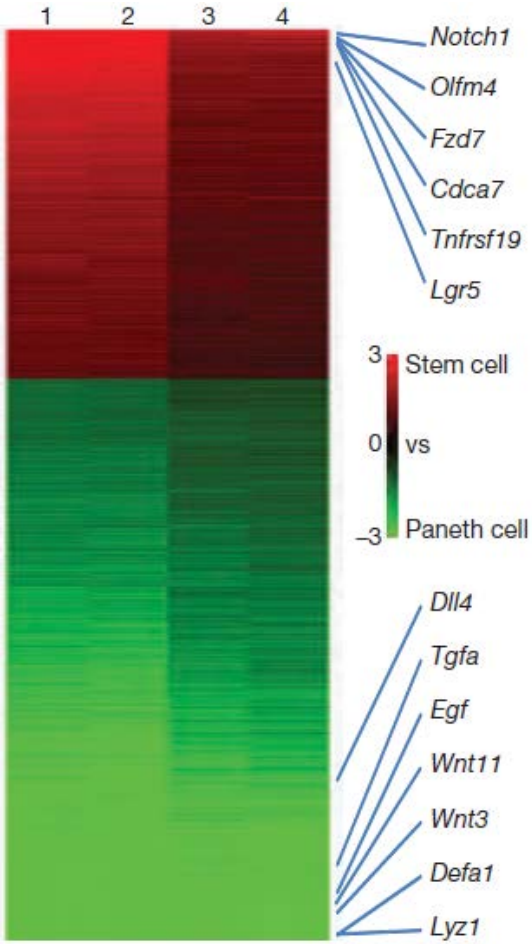


Tian et al. Nature 2011

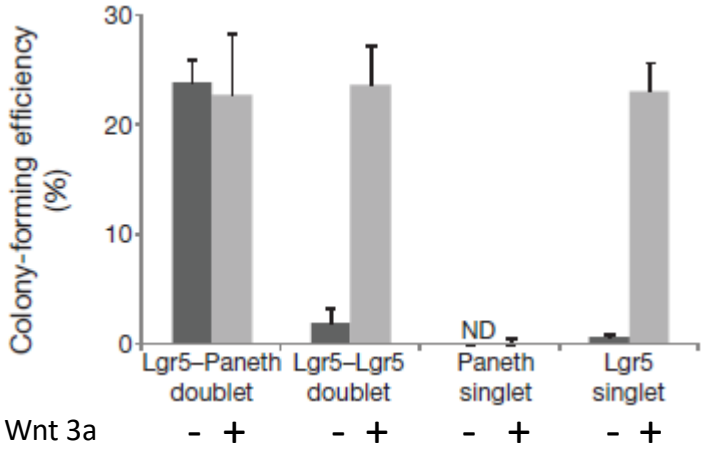
Genetic models determine experimental outcome: More efficient deletion of Lgr5+ stem cells reveals their essential function

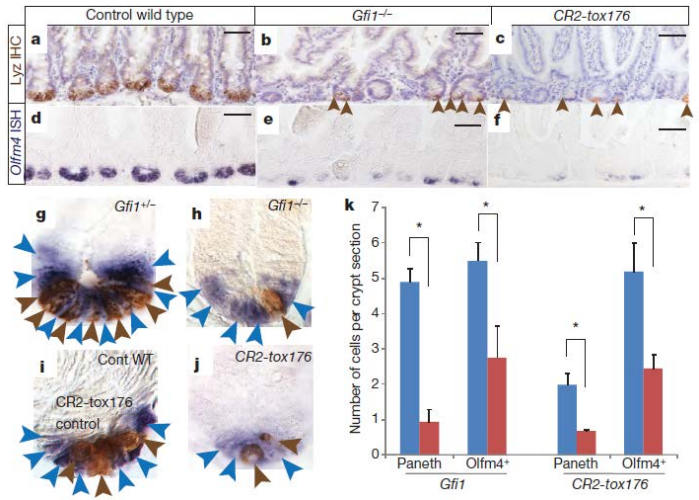
Tan et al. Cell Rep. 2021

PANETH CELLS CONSTITUTE THE NICHE FOR LGR5+ STEM CELLS IN INTESTINAL CRYPTS



FACS-sorted stem and Paneth cells

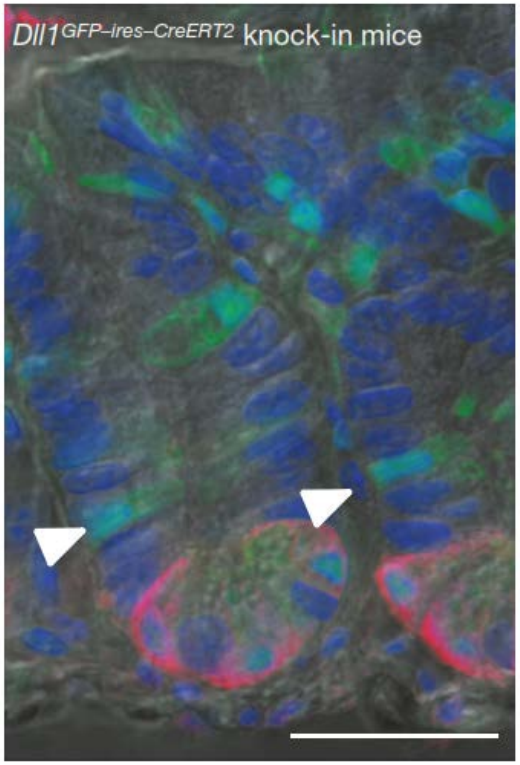




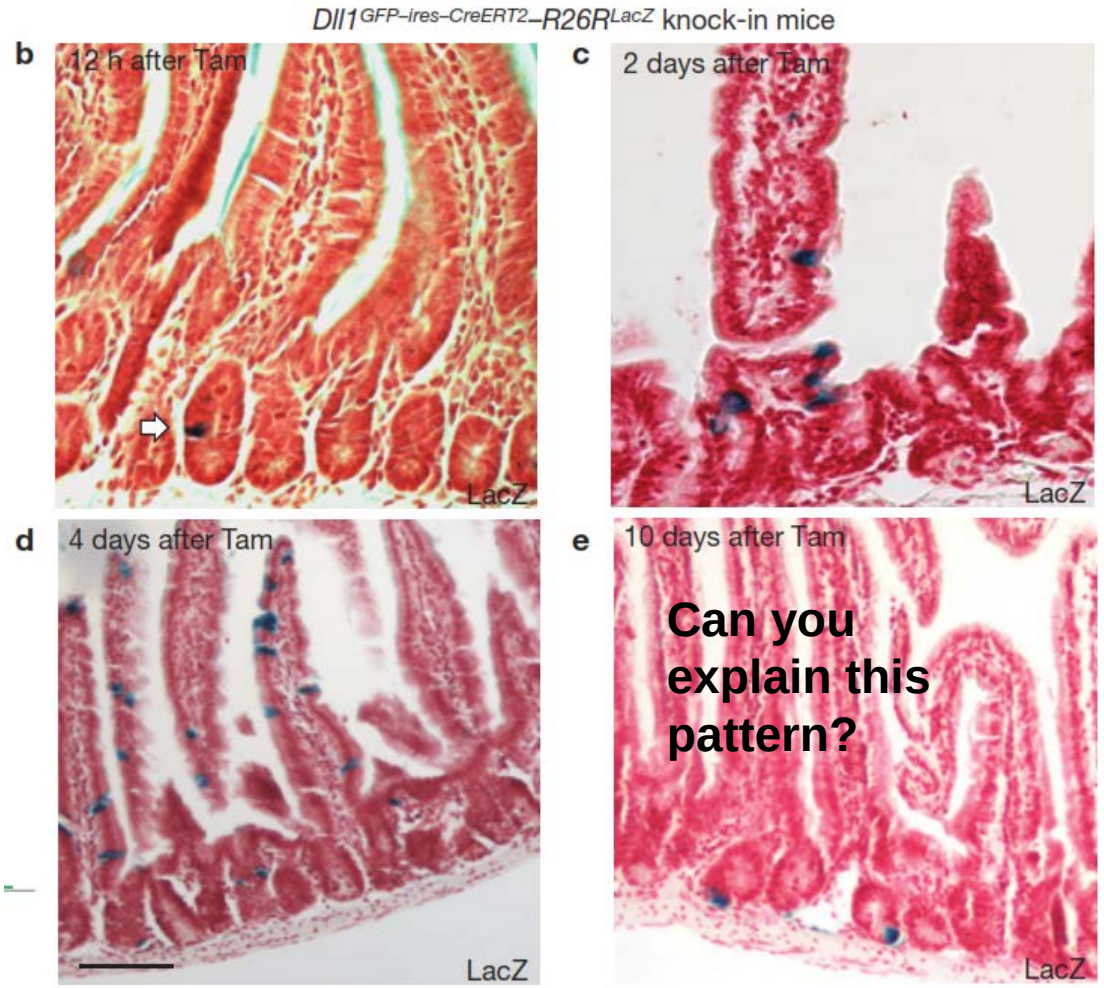
Models of Paneth cell depletion are inperfect

Sato et al. Nature 2010

Dll1-expressing cells are crypt-located progenitors of secretory cells

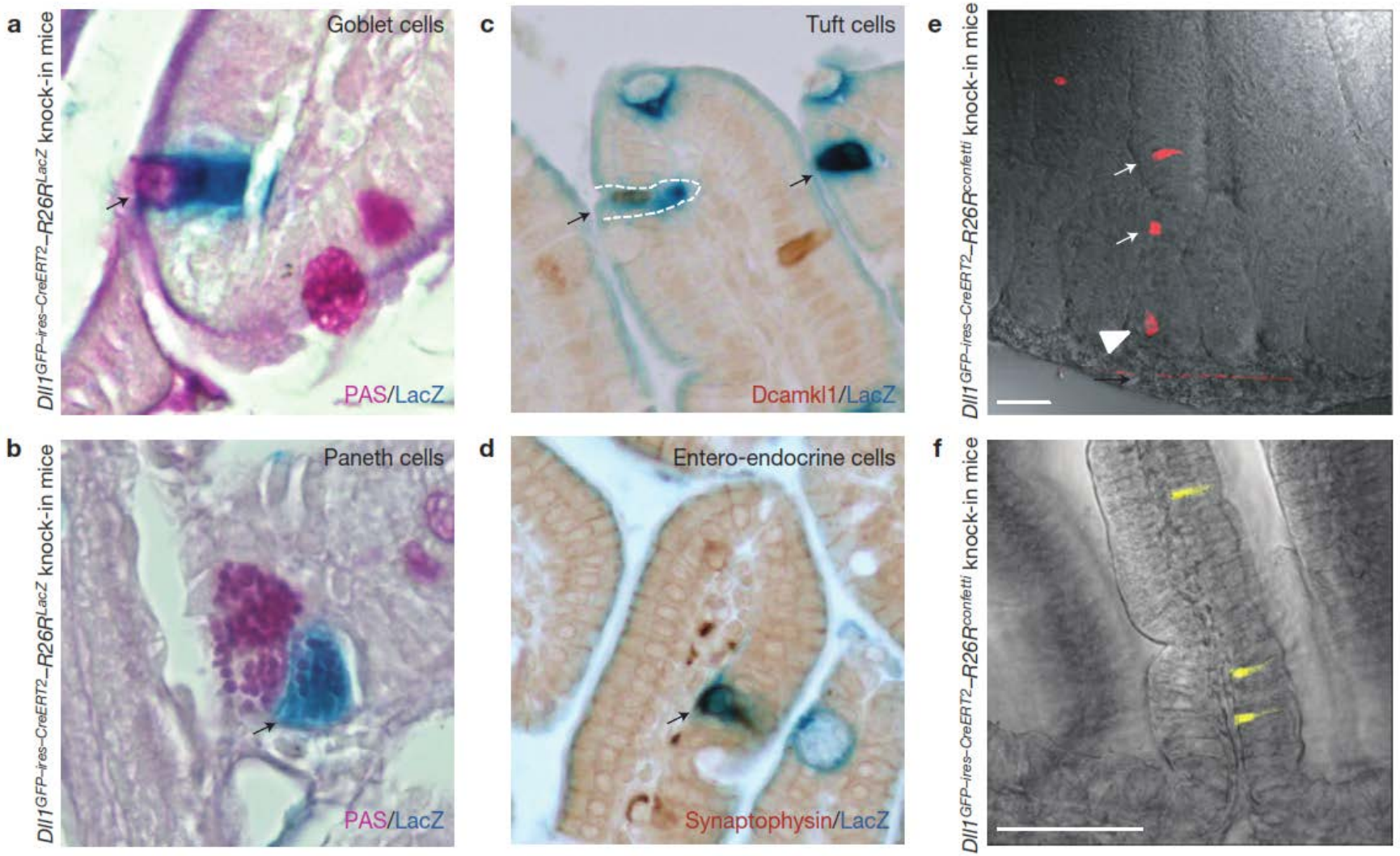


Dll1 expression in rare cells just above the stem cell compartment



Van Es et al. Nat. Cell Biol. 2012

Dll1-expressing cells are crypt-located progenitors of secretory cells



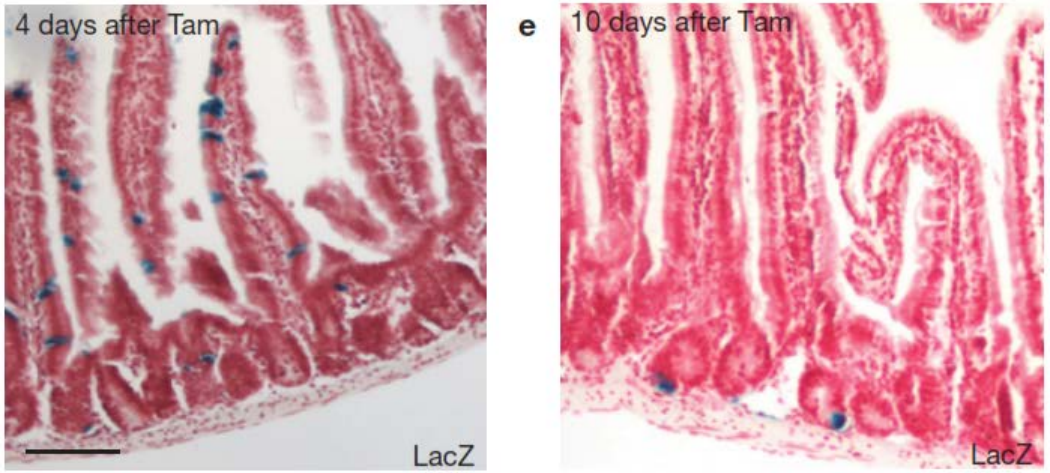
Van Es et al. Nat. Cell Biol. 2012

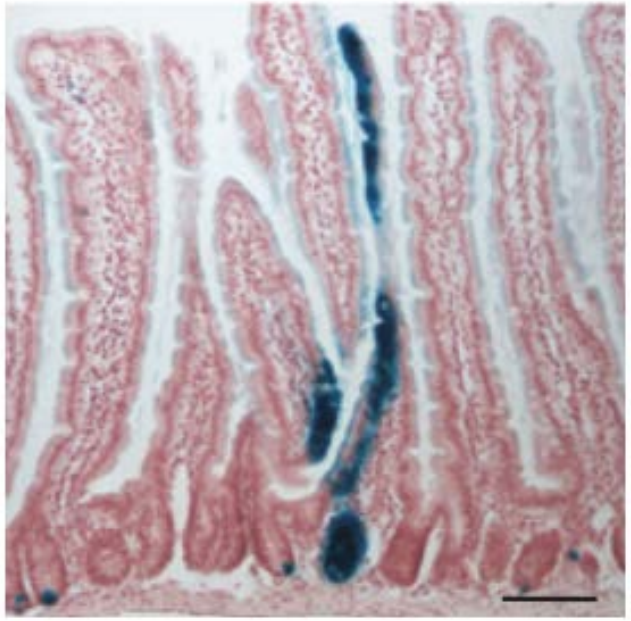
WHAT HAPPENED HERE ?

Day -1: Cre-induction in a fraction of Dll1+ cells by sub-optimal tamoxifen treatment

Day 0 : Irradiation (injury of the proliferative compartment)

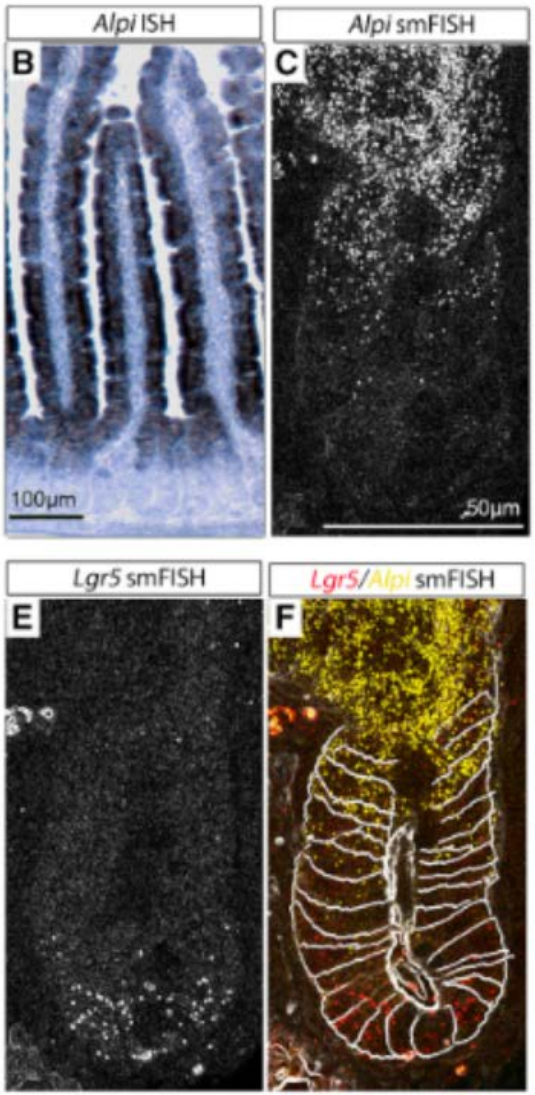
Day 28 : staining and analysis



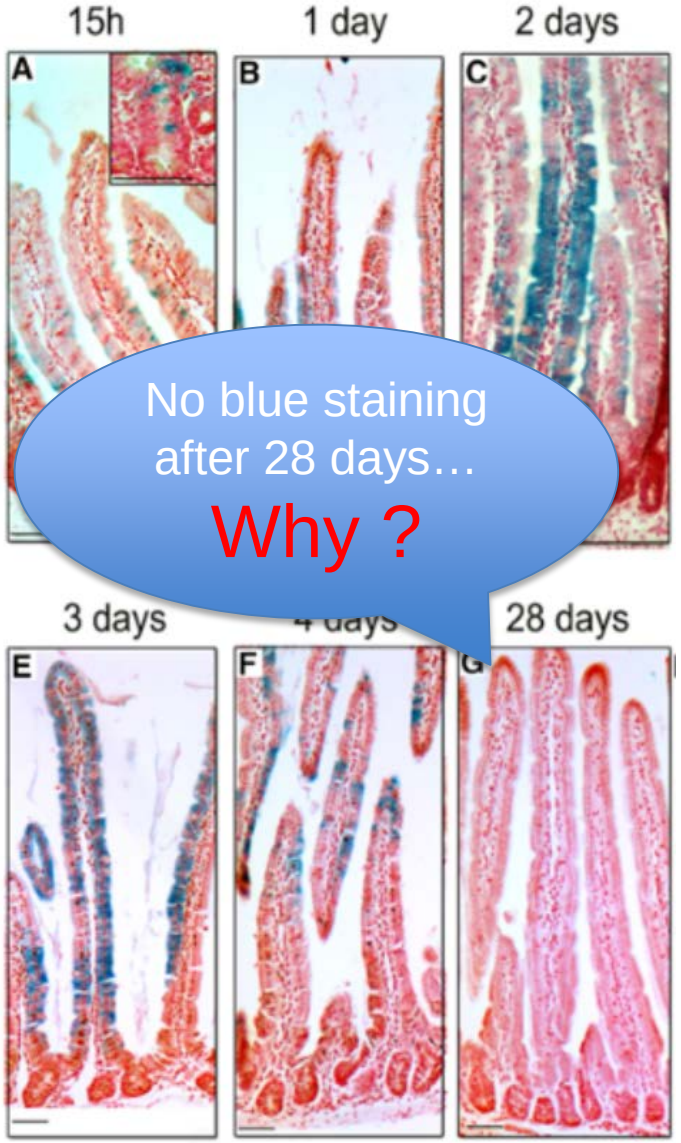


Van Es et al. Nat. Cell Biol. 2012

ONE STEP FURTHER TOWARDS CELL PLASTICITY : ALPI



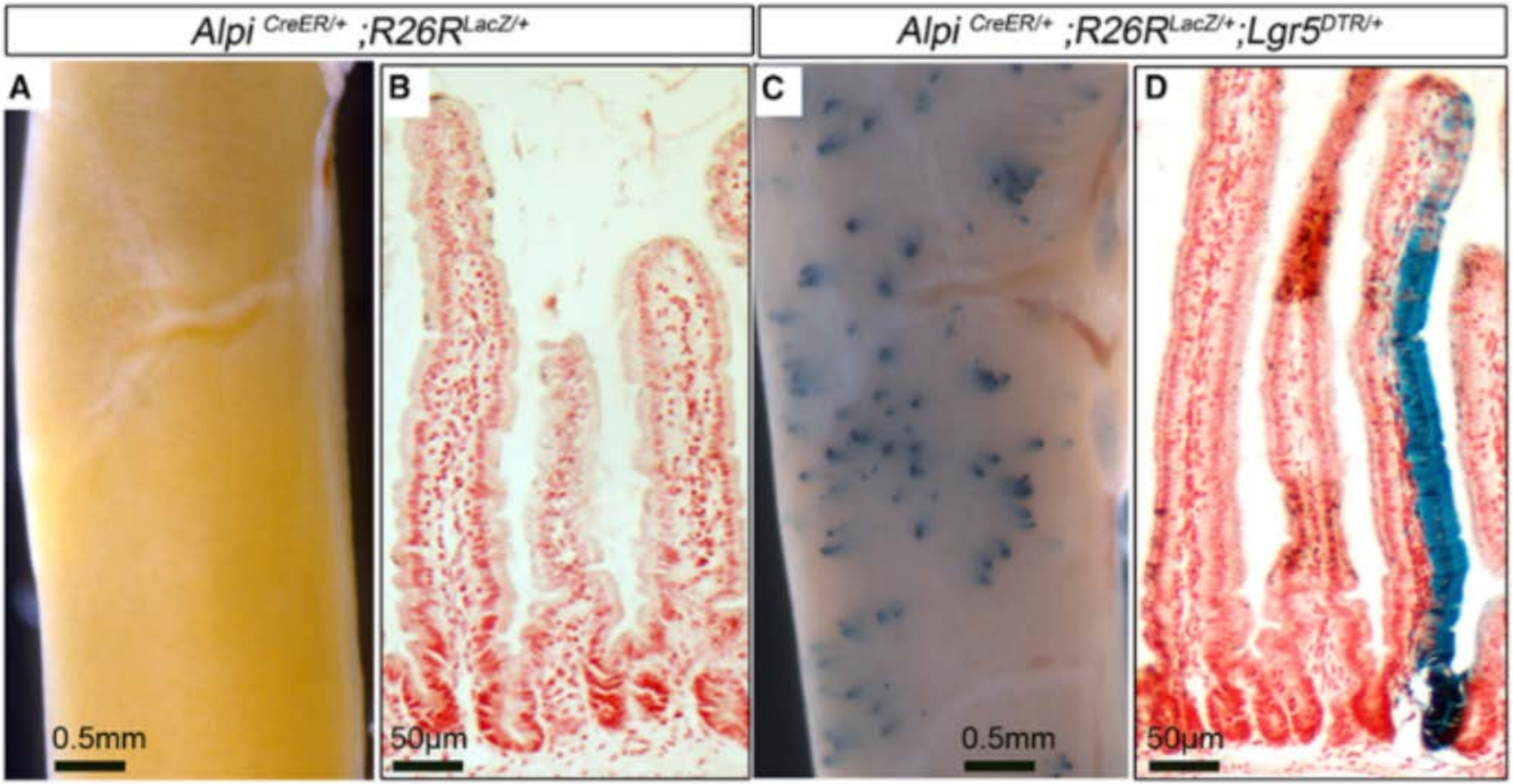
Endogenous expression (mRNA)



Lignée murine: *Alpi-Cre^{ERT2}; Rosa26-LacZ*

Tetteh et al. Cell Stem Cell 2016

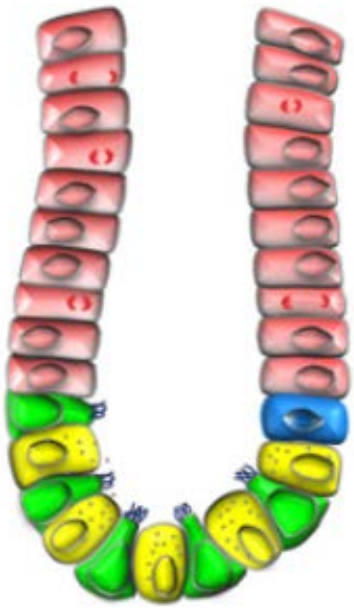
WHAT TITLE FOR THIS SLIDE ?



Staining 2 weeks after a single tamoxifen injection

Tetteh et al. Cell Stem Cell 2016

THE LONG AND EXEMPLARY HISTORY OF THE INTESTINAL EPITHELIAL STEM CELLS



1965, Cairnie et al. : Intense proliferation and cell turnover

1974, Cheng & Leblond : discovery of the CBC cells in crypts

1974-1998, +4 stem cells model (Potten)

1999 Genetic tracing and the CBC stem cell model (Bjerknes)

2007 Lgr5 and the re-discovery of the crypt base columnar stem cells (Clevers)

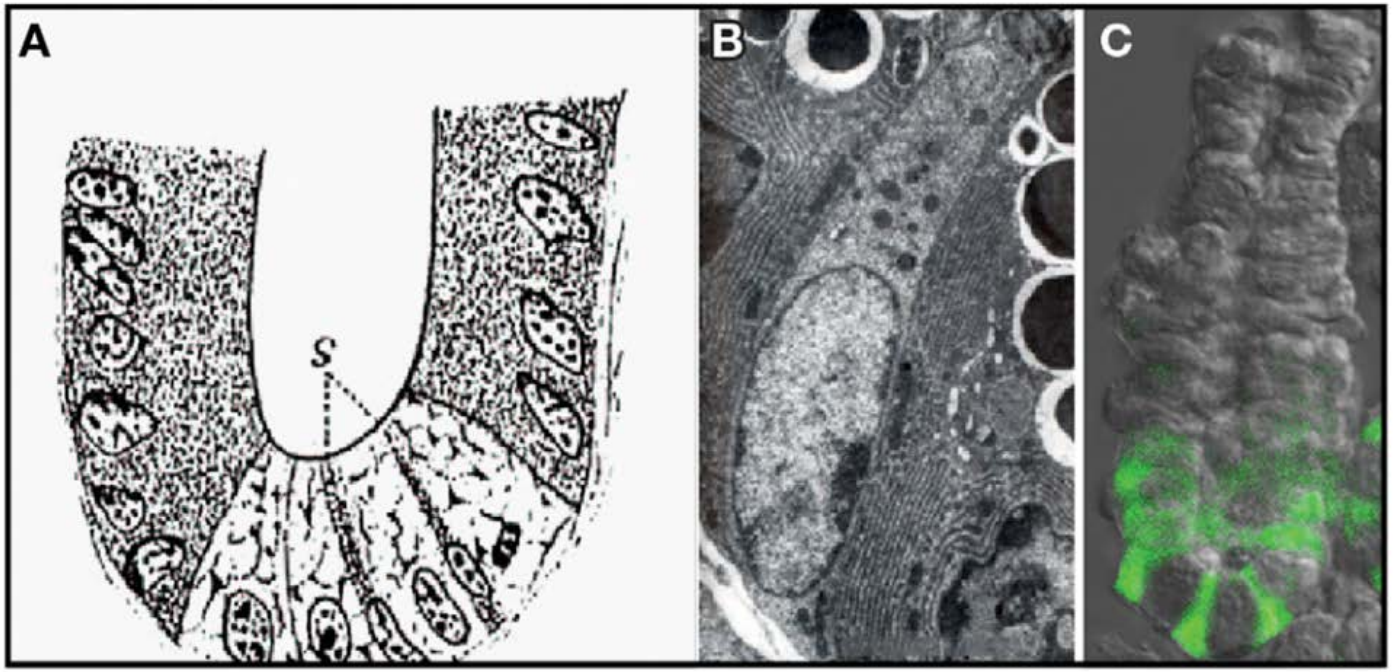
2009 Paneth cells: the niche of stem cells (Sato)

Many tracing markers and potential alternative stem cell types, notion of quiescent/active stem cells)

2012 A transcriptome/proteome/IHC clarification (Clevers)

Cell plasticity and a possible reconciliation

THE CRYPT MORPHOLOGY : 120 YEARS OF HISTORY



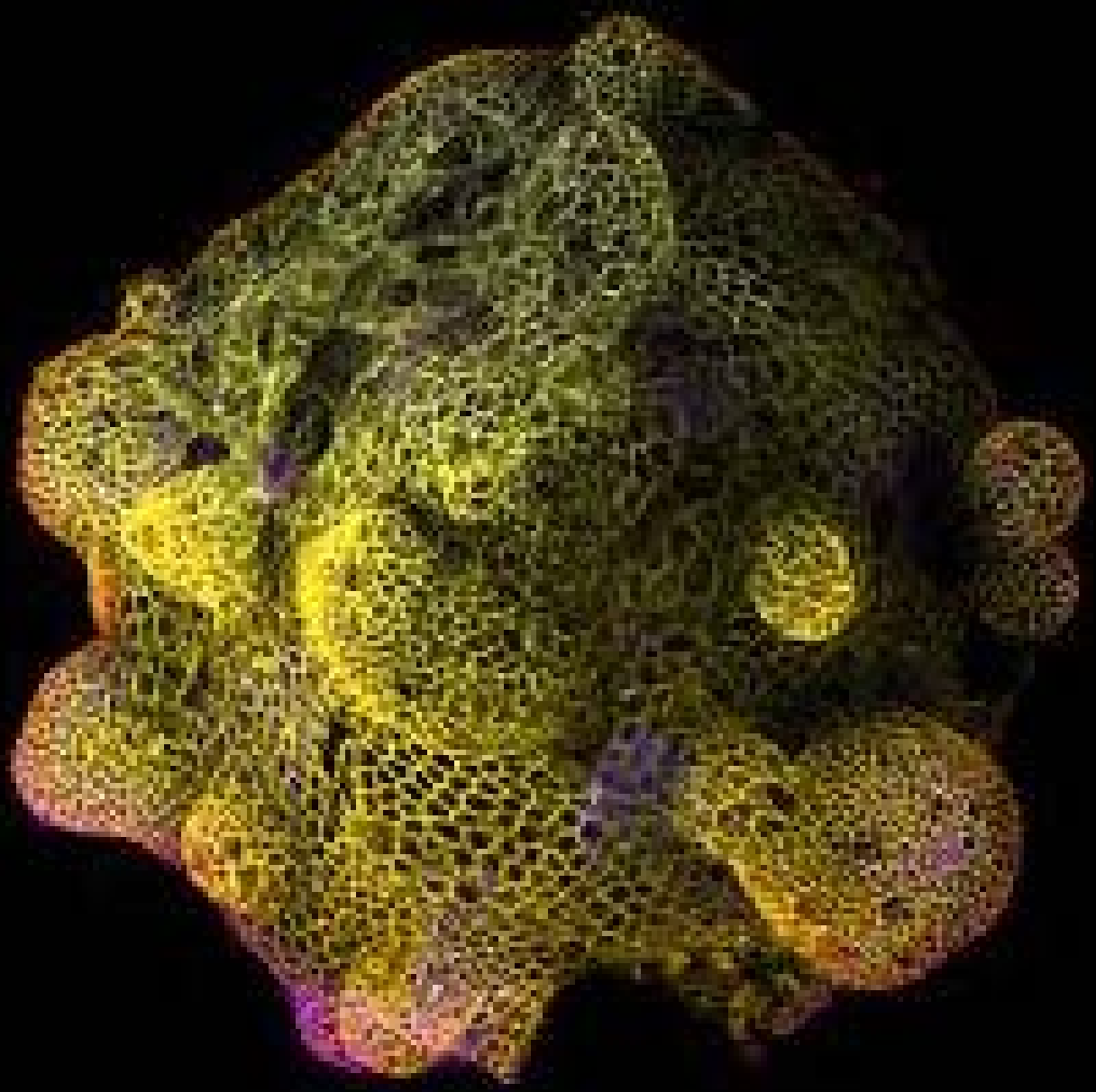
Hand-drawn crypts
"S": small cells
(Paneth 1887)

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

Confocal image of Lgr5-GFP Cells

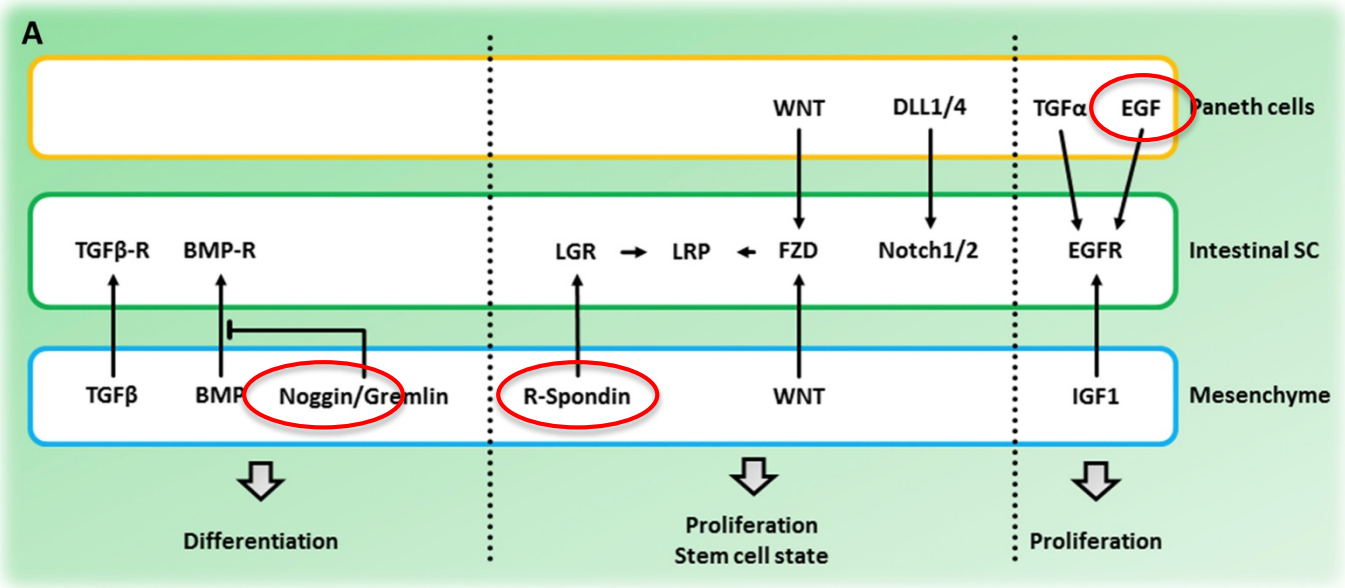
Clevers. Cell 2013

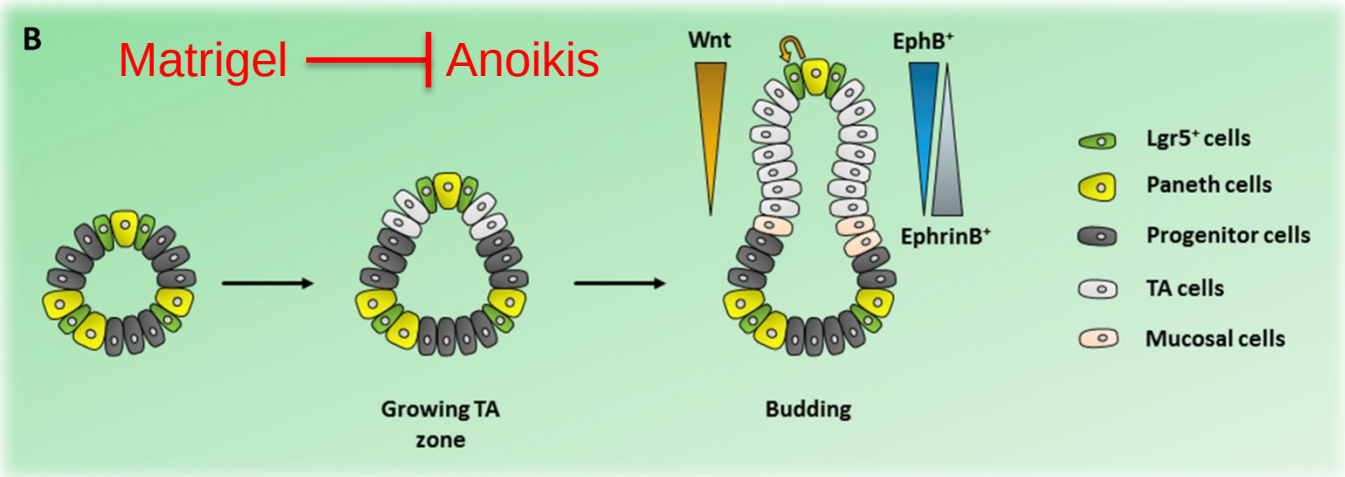
The intestinal organoid culture system



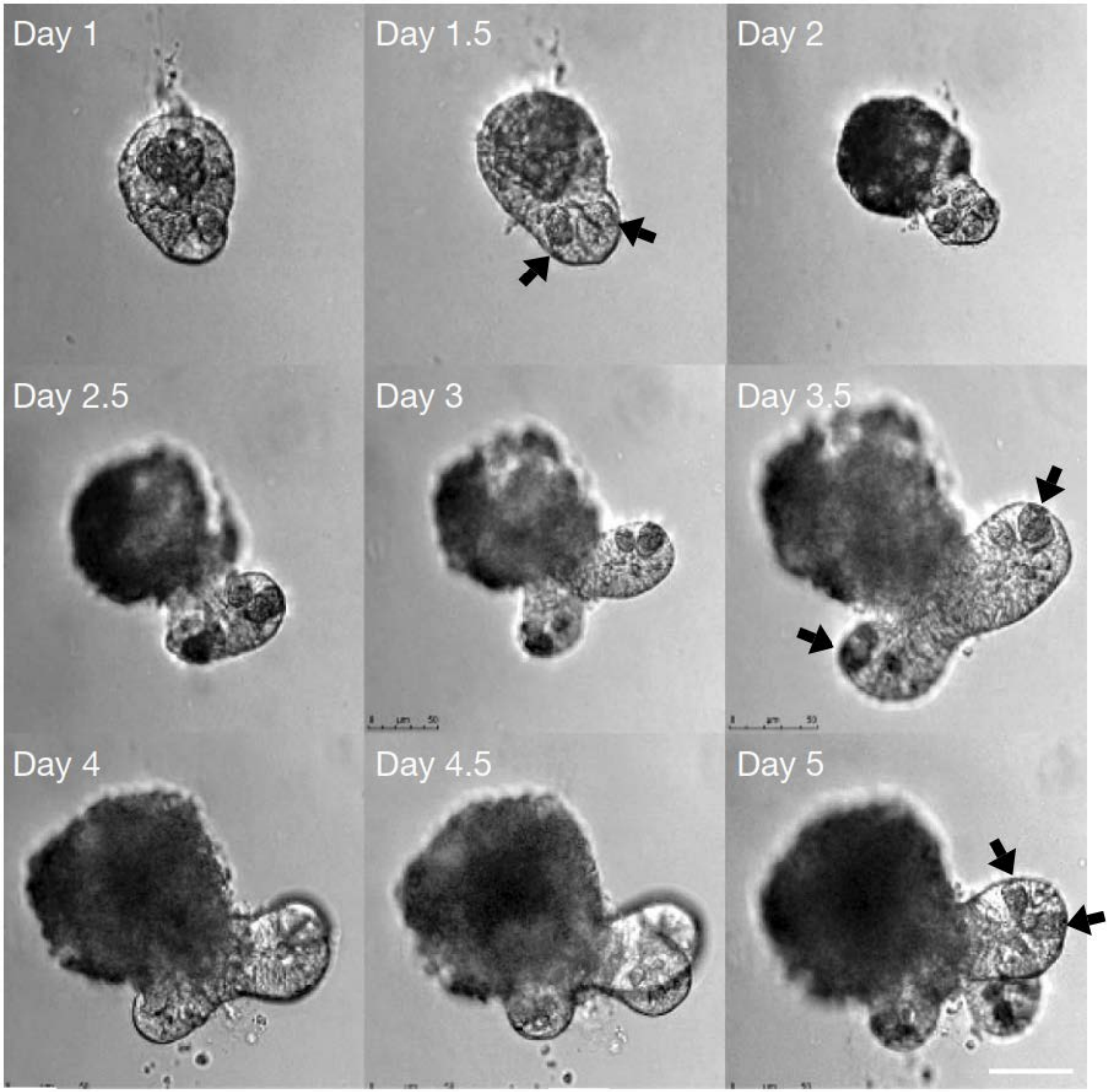
How can organoids be maintained in the absence of a stroma

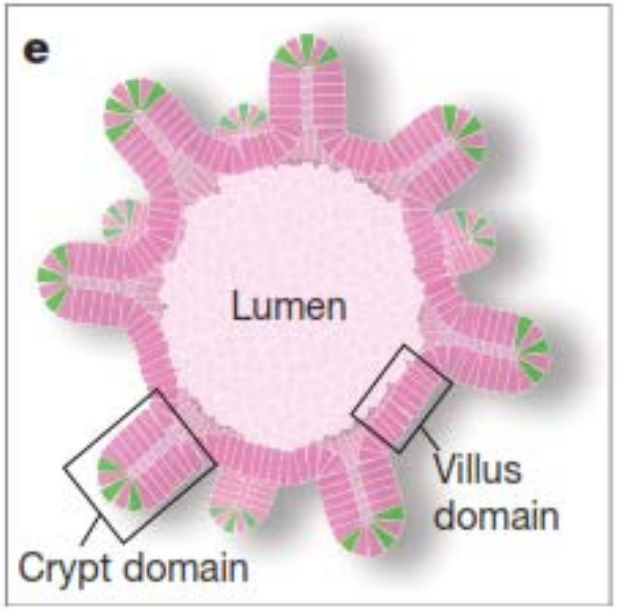
This is the result of several decades of stem cell biology research





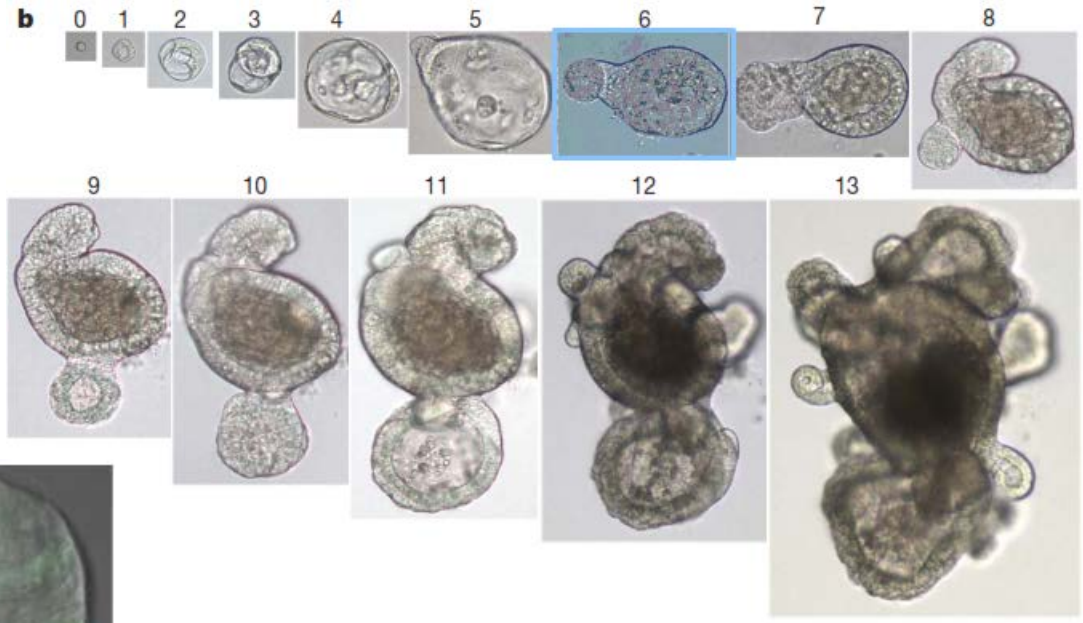
ORGANOIDS: AN INTESTINAL EPITHELIUM MODEL GROWN IN THE ABSENCE OF MESENCHYMAL NICHE

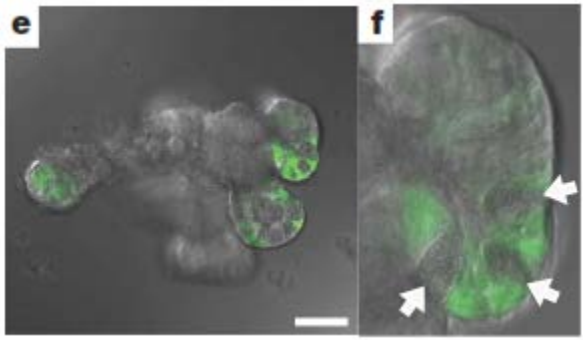


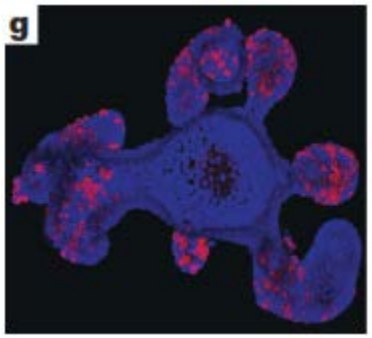


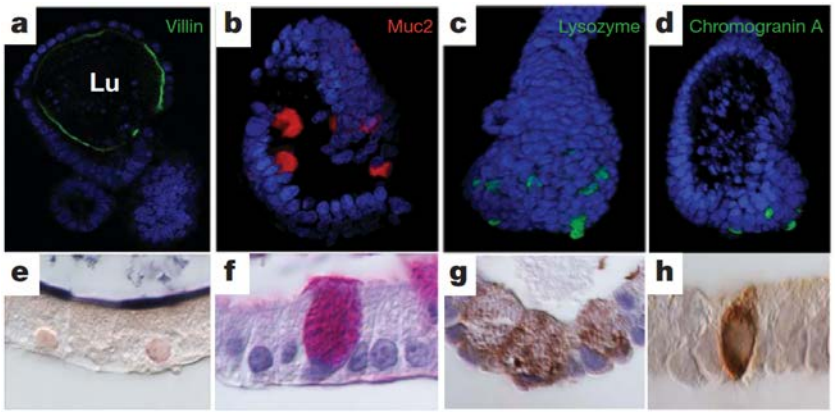
Sato et al., Nature 2009

Single Lgr5+ stem cells generate multi-lineage organoids







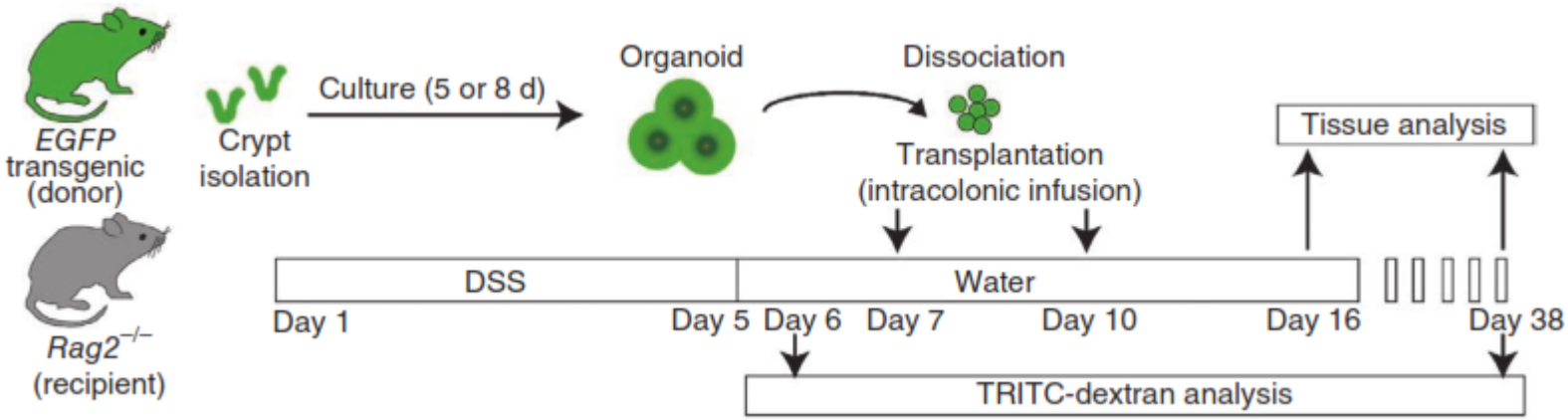


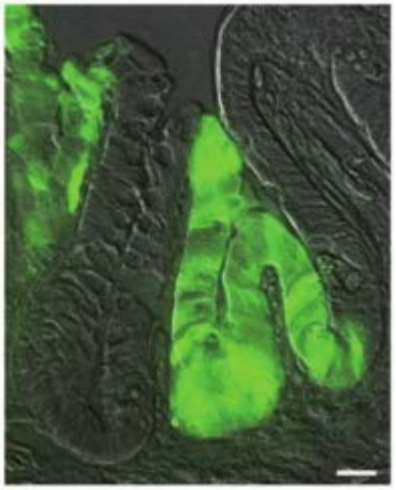
Sato et al., Nature 2009

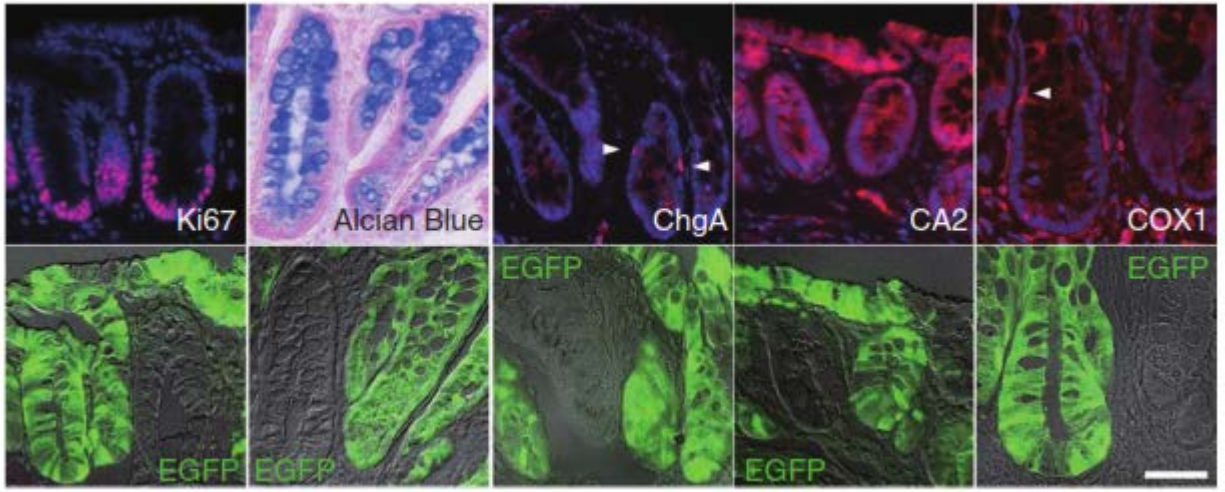
KEY PROPERTIES OF INTESTINAL ORGANOIDS

- Representative of the healthy intestinal epithelium (cell type abundance, etc)
- Can be derived rapidly from any mouse model
- Conditional deletions can be activated in vitro using hydroxytamoxifen
- Long term maintenance with genetic stability
- Can be frozen
- Can be genetically manipulated (stable shRNA expression, CRISPR/Cas9)
- Can be engrafted back in a recipient mouse intestine
- Useful for mechanistic studies (epithelial contribution)
- Studies of interactions between epithelial and stromal cells (microbiota)

FUNCTIONAL ENGRAFTMENT OF ORGANOID EPITHELIUM

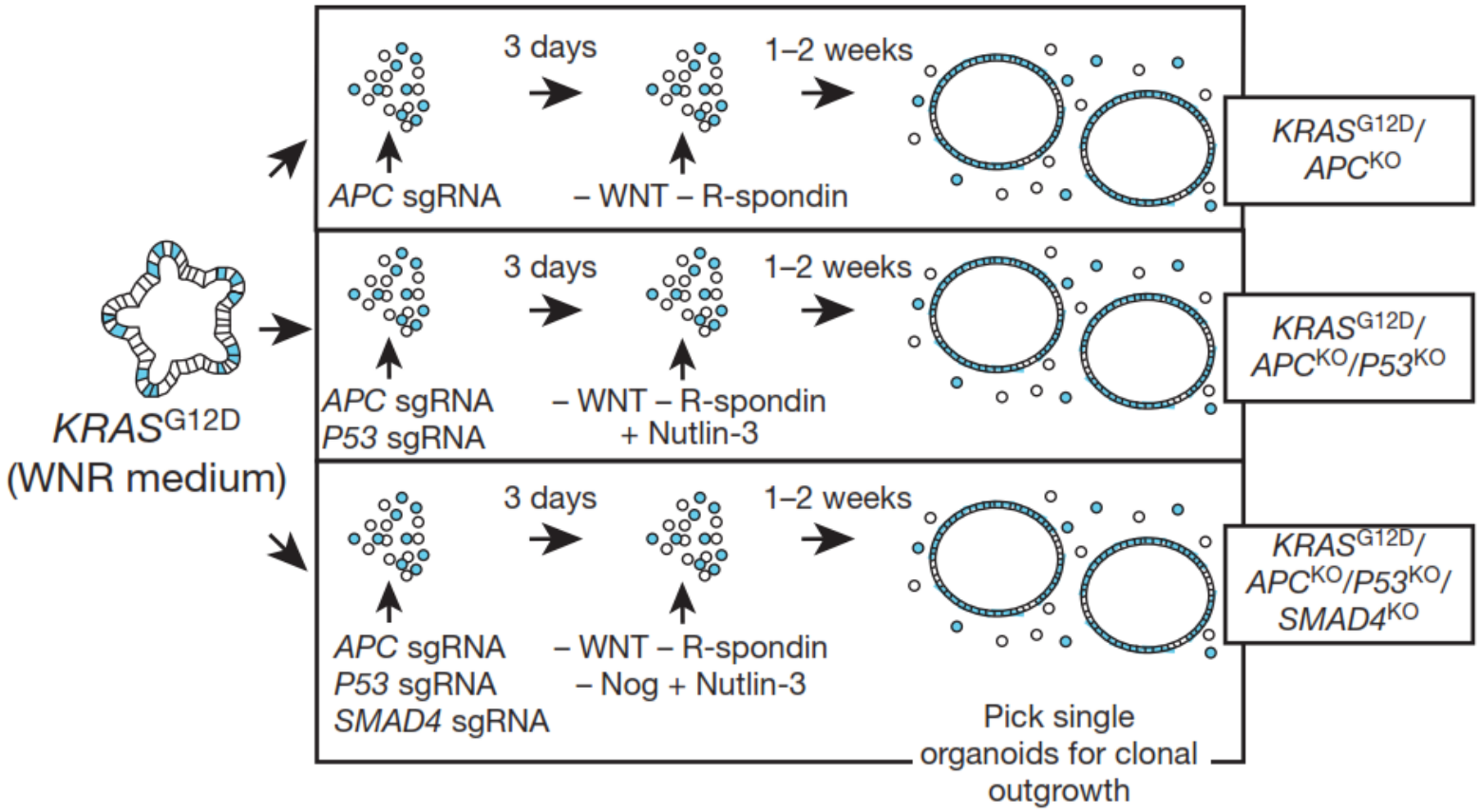






Yui et al., Nature Medicine 2012

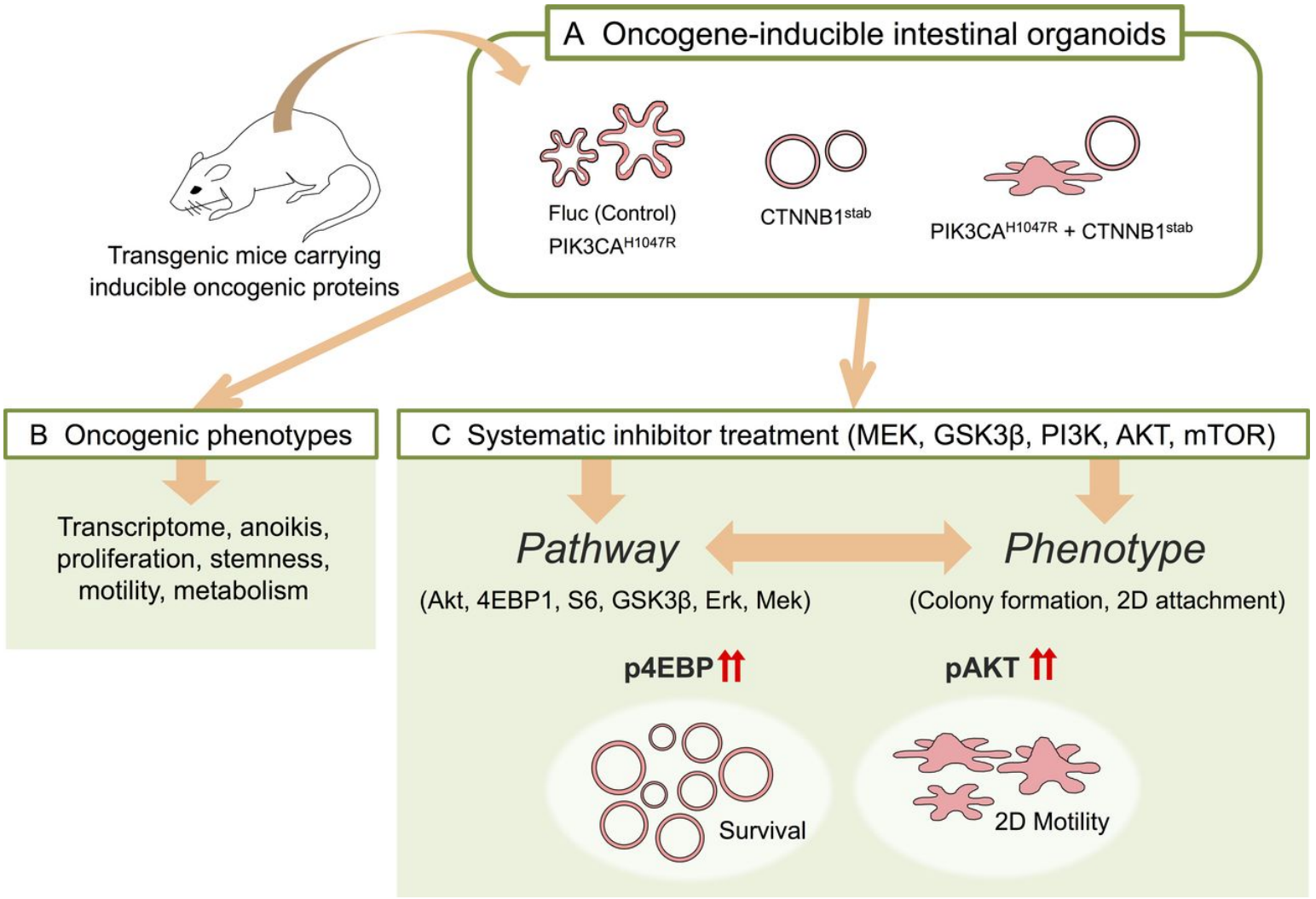
SEQUENTIAL CANCER MUTATIONS IN CULTURED HUMAN INTESTINAL STEM CELLS



Quadruple mutant $KRAS^{G12D}$ / APC^{KO} / $P53^{KO}$ / $SMAD4^{KO}$ organoids grow as invasive carcinomas *in vivo*

Drost et al., Nature 2015

ORGANOIDS: A PLATFORM TO ASSESS CANCER CHARACTERISTICS



Mizutani et al., JCB 2017

EVOLUTION OF PRECLINICAL MODELS

1970- Cell lines

2000- Patient derived xenografts (PDX)

2010- Organoid-based technologies and programs

Resource

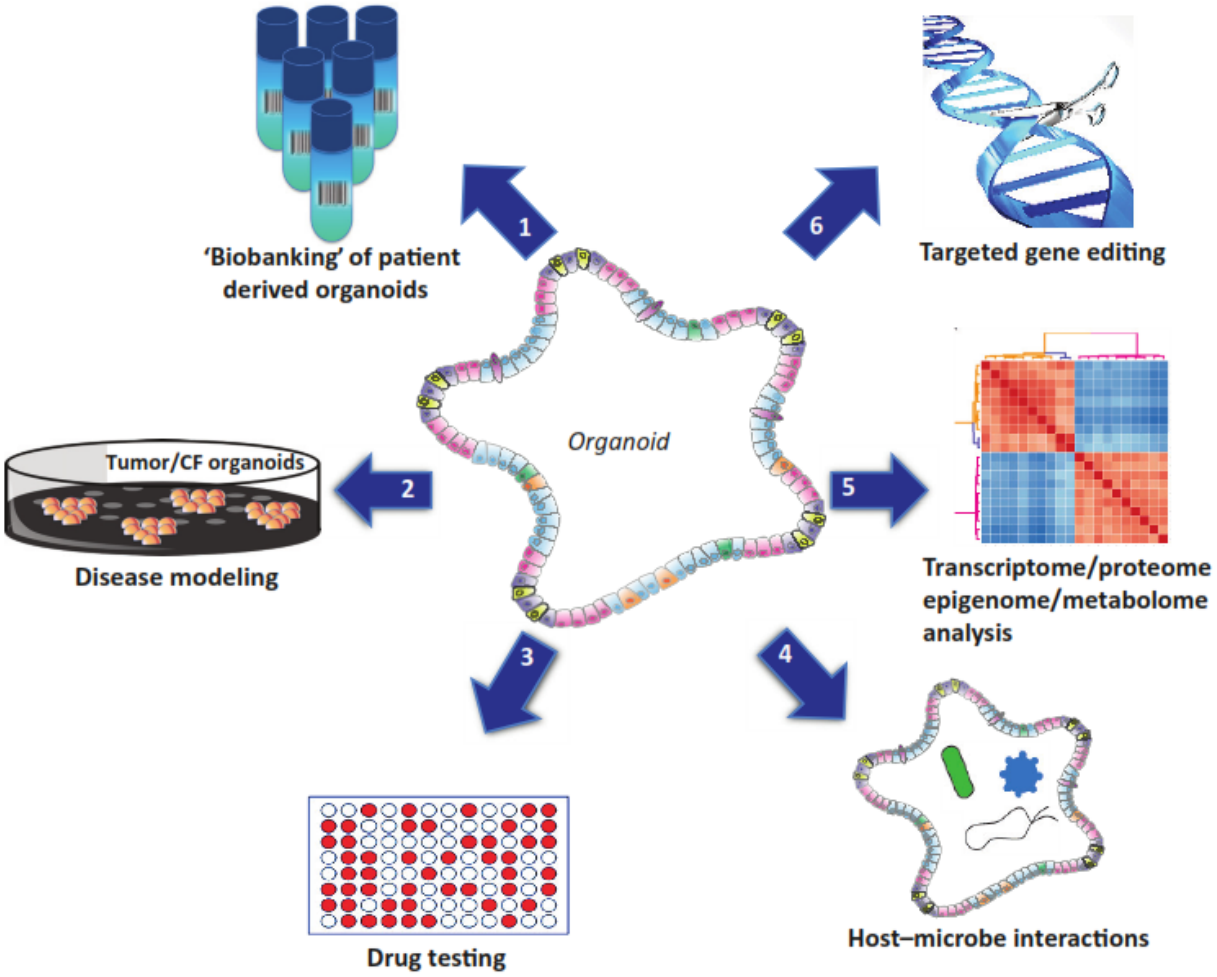
Cell

A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity

Norman Sachs,[1,2,11,14,15] Joep de Ligt,[3,11,14] Oded Kopper,[1,11,14] Ewa Gogola,[4] Gergana Bounova,[5,11] Fleur Weeber,[6] Anjali Vanita Balgobind,[1,2] Karin Wind,[1] Ana Gracanin,[1] Harry Begthel,[1] Jeroen Korving,[1] Ruben van Boxtel,[3,11] Alexandra Alves Duarte,[4] Daphne Lelieveld,[7] Arne van Hoeck,[3,11] Robert Frans Ernst,[3,11] Francis Blokzijl,[3,11] Isaac Johannes Nijman,[3,11] Marlous Hoogstraat,[5] Marieke van de Ven,[8] David Anthony Egan,[7] Vittoria Zinzalla,[12] Jurgen Moll,[12] Sylvia Fernandez Boj,[2,11] Emile Eugene Voest,[6] Lodewyk Wessels,[4,11,13] Paul Joannes van Diest,[9] Sven Rottenberg,[4,10] Robert Gerhardus Jacob Vries,[2,11] Edwin Cuppen,[3,11] and Hans Clevers[1,11,16,*]

Sachs et al., Cell 2018

MULTIPLE APPLICATIONS OF ORGANOID TECHNOLOGY



Dutta et al., Trends in Molecular Medicine 2017

ANALYSIS OF EPITHELIA-STROMAL INTERACTIONS

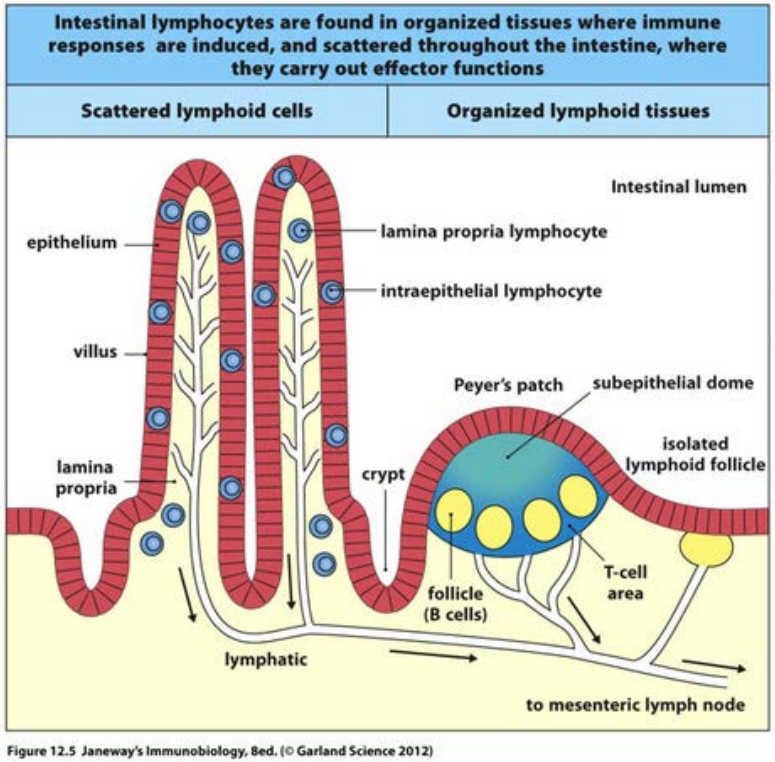
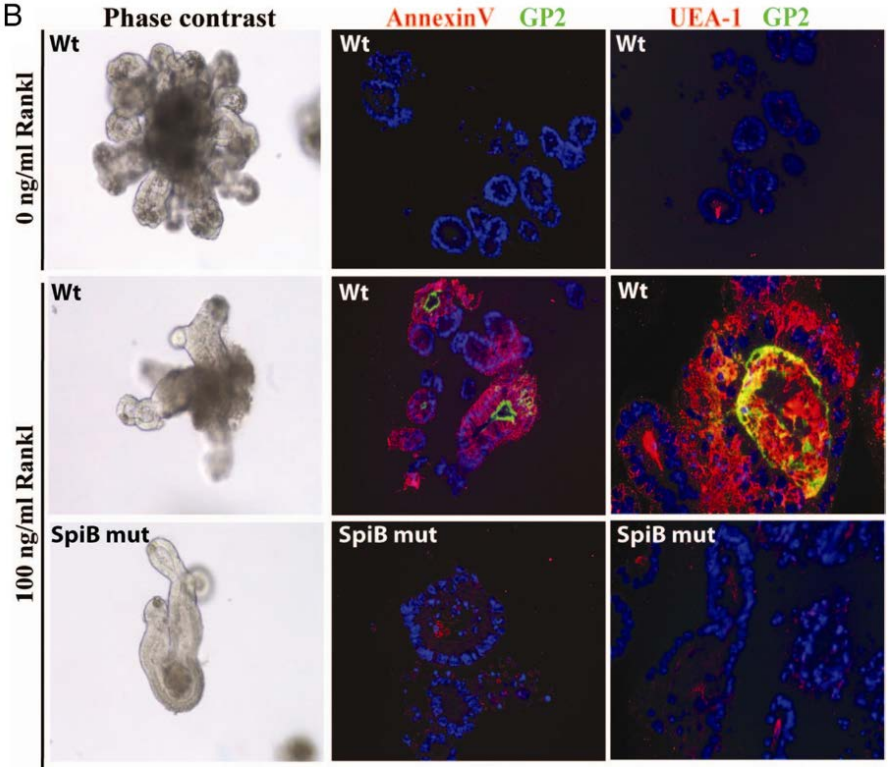


Figure 12.5 Janeway's Immunobiology, 8ed. (© Garland Science 2012)



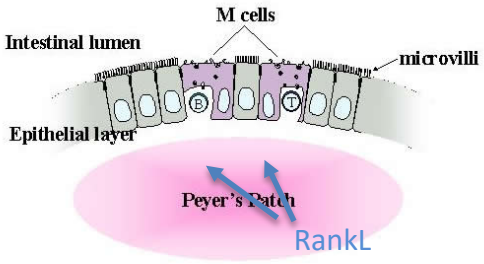
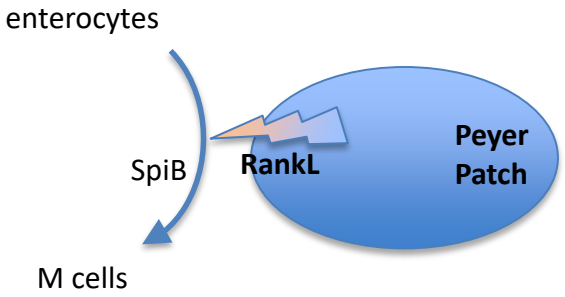


Figure. Follicle-associated epithelia (FAE) and M cells



De Lau et al., MCB 2012

TUFT CELLS: A NOVEL CELL TYPE IN THE INTESTINAL THAT STILL LACKS A FUNCTION

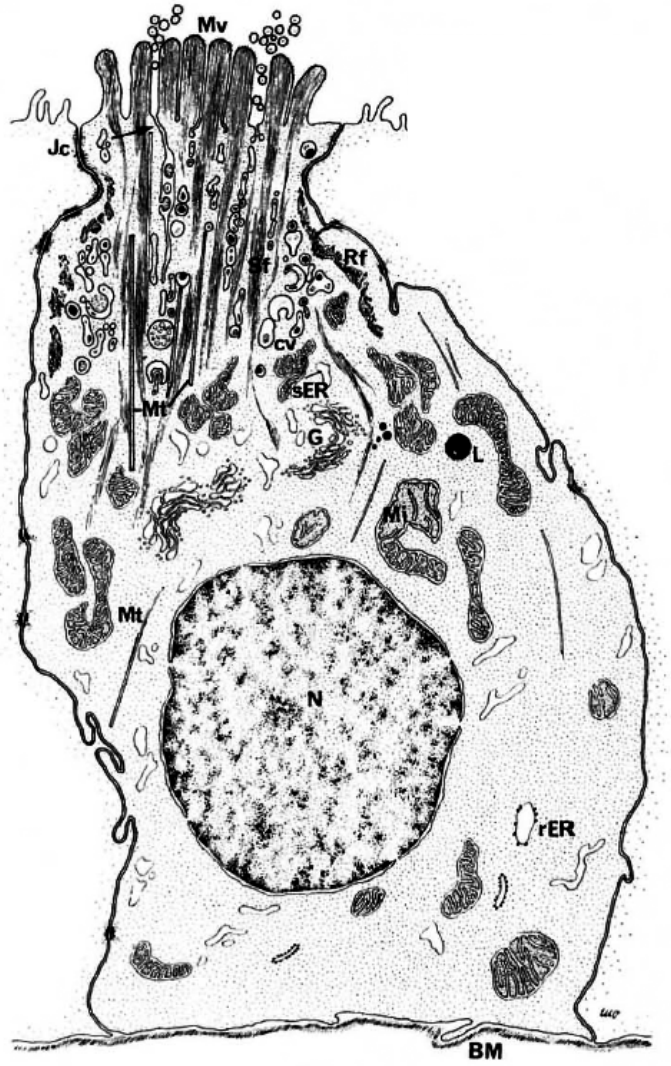


Figure 1

Nabeyama et al., Am. J. Anat., 1974

- Brush, caveolated, multivesicular cells
- Described almost 60 years ago
- Related to taste receptor cells
- No known function
- Overlooked in most studies

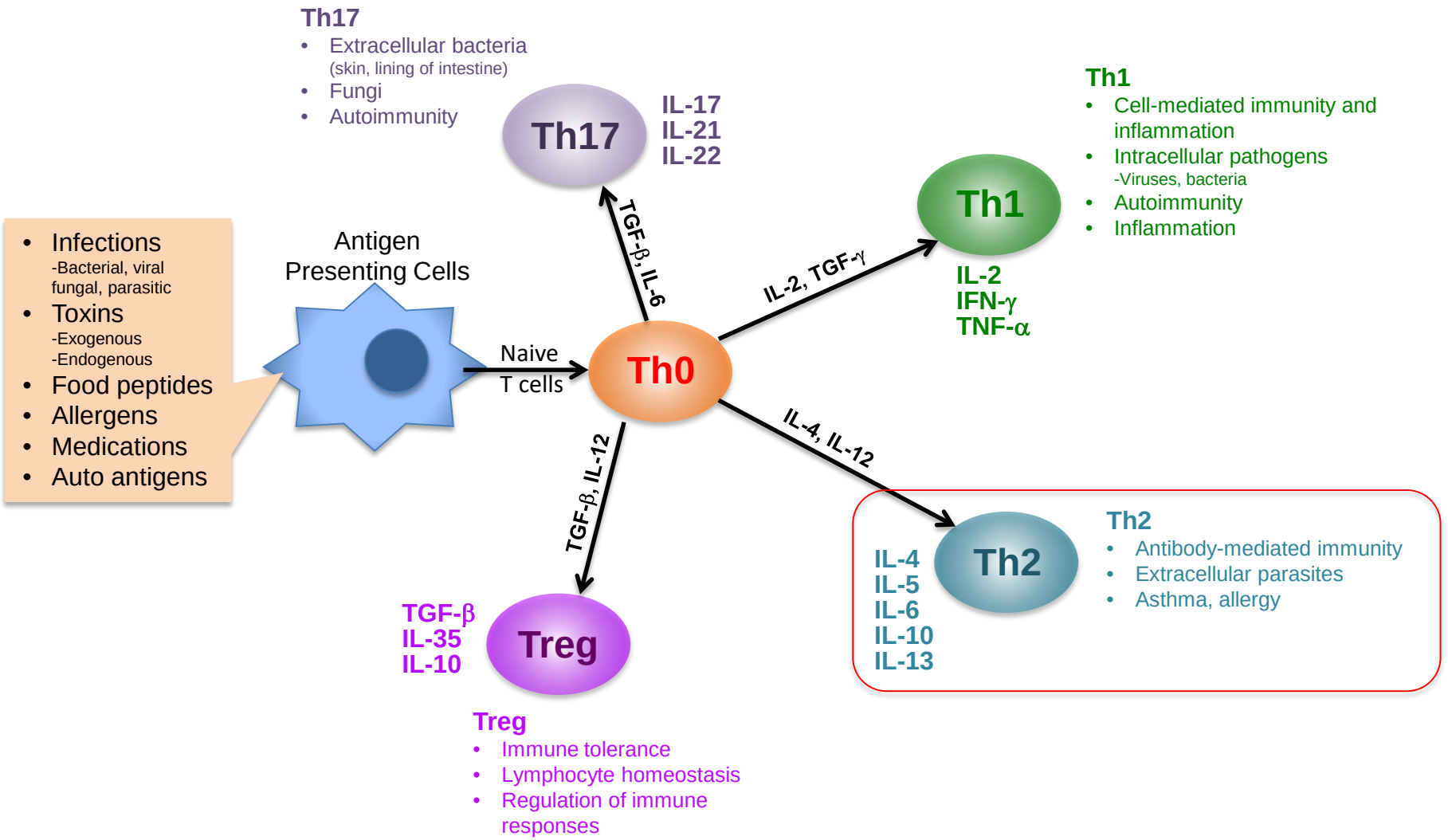
TUFT CELLS : A FIFTH CELL TYPE IN THE INTESTINAL EPITHELIUM

➡ *one fifth of intestinal epithelial functions to unravel?*

- derive from Lgr5^{+} CBC stem cells
- Are permanently renewed
- A distinct cell lineage
- Early differentiation in crypts
- Likely produce prostanoids and opioids
- Differentiation occurs in mouse and human tumours

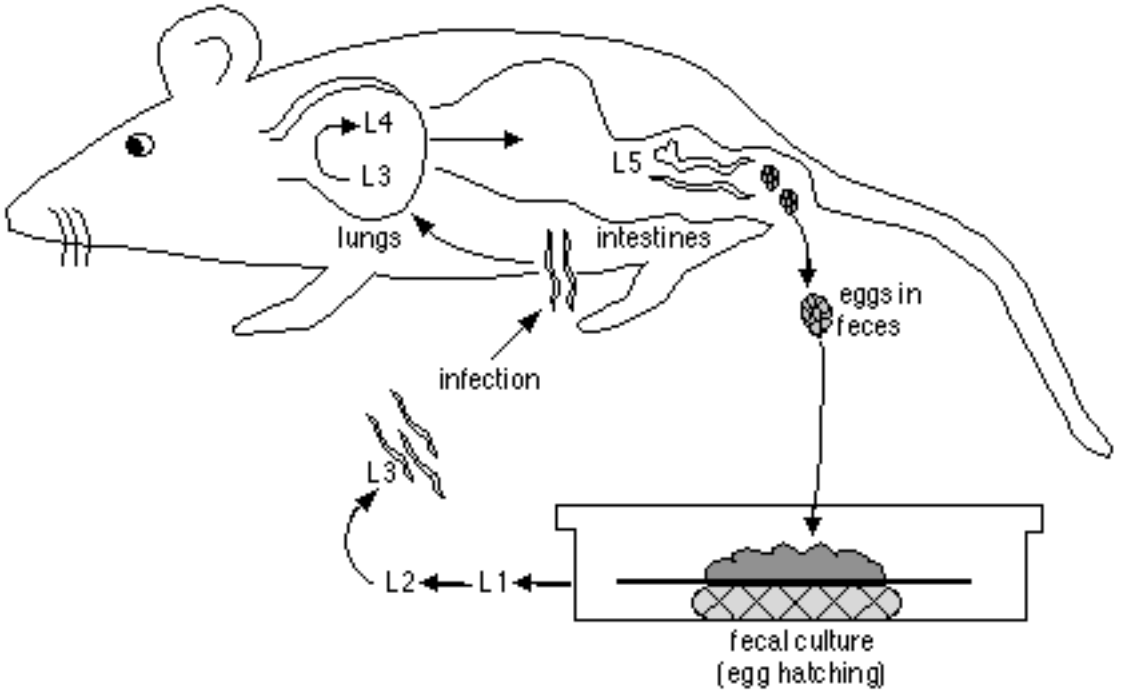
NOT QUIESCENT STEM CELLS

Gerbe et al. Gastroenterology 2009
Gerbe et al. J. Cell Biol. 2011



Th0: Naive T cells
Th: Helper T cells
Treg: Regulatory T cells
IL: Interleukin
TNF-α: Tumor necrosis factor-alpha
TGF-β: Transforming growth factor-beta

Nippostrongylus brasiliensis : a model of helminth infection in the mouse



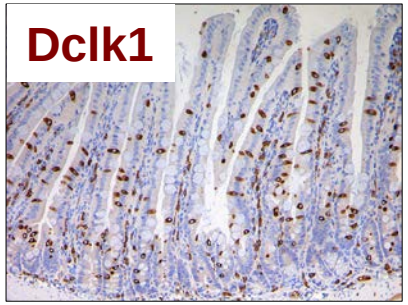
TUFT CELLS PLAY CRITICAL ROLES IN SENSING THE PRESENCE OF PARASITES AND INITIATING TYPE 2 RESPONSES AGAINST HELMINTH INFECTIONS

Worm expulsion

Infection

Nippostrongylus brasiliensis

Sensing IL-25



T_H2, ILC2

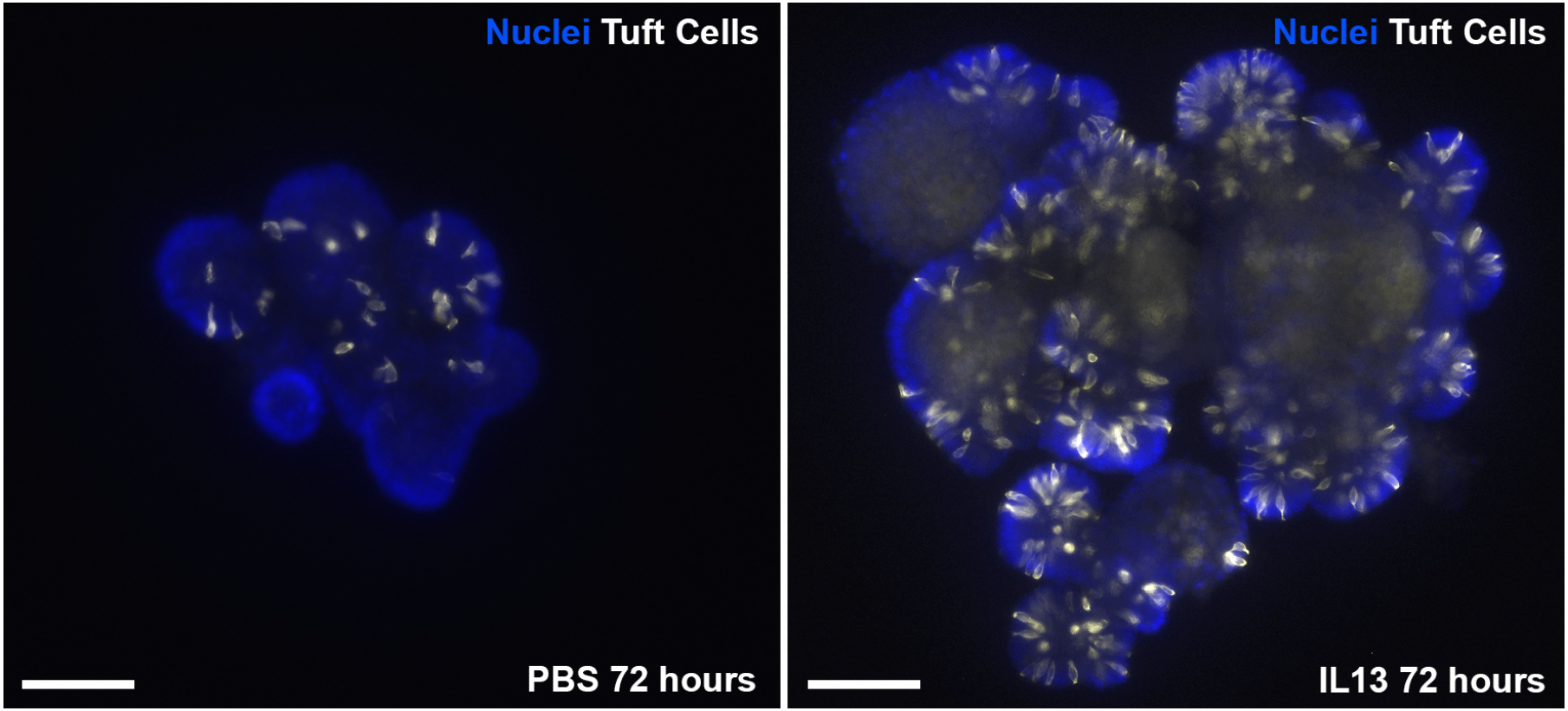
- IL-4
- IL-13

Gerbe et al., Nature 2016

CONCLUSIONS

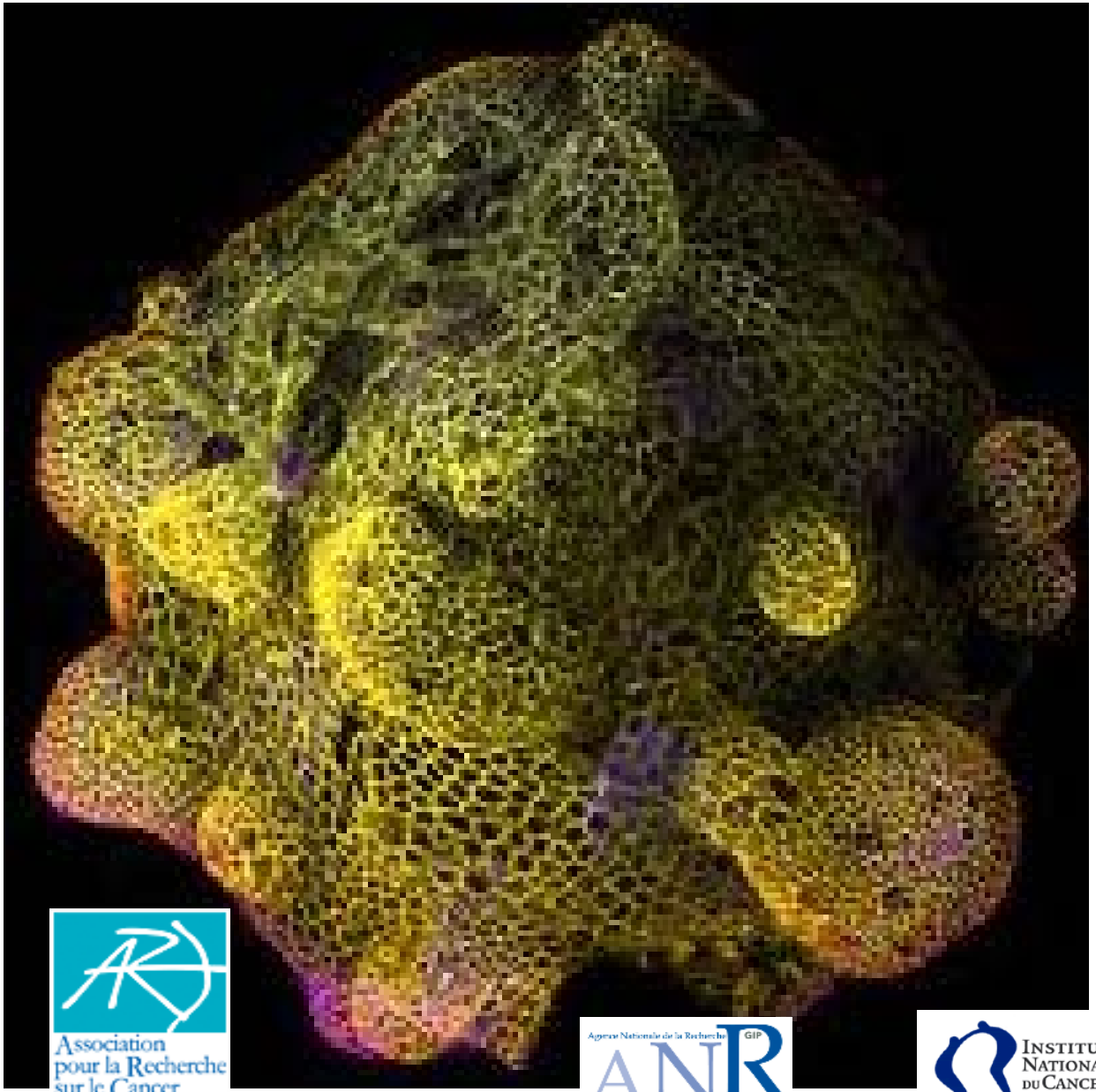
- Neglected population of the gut epithelium
- Sensing machinery, related to taste buds
- Regulate type 2 immune responses through IL25
- Link luminal clues with behaviour of stromal haematopoietic cells
- New example of the functional integration of epithelial and haematopoietic compartments
- May be involved in sensing other types of pathogens

HOST-PATHOGEN INTERACTIONS



- Regulatory functions of parasites/microbes-derived molecules
- Role of stromal signalling molecules to modulate stem cell differentiation programs

Acknowledgements



Philippe Jay team:
(IGF Montpellier)

François Gerbe
Nathalie Coutry
Fabien Herbert
Imène Gasmi
Julie Bas

Emmanuelle Sidot
Julie Nguyen
Alicia Giordano
Catherine Legraverend
Marco Bruschi
Salima Soualhi
Pauline Bastide
Laure Garnier
Bénédicte Brulin

Association pour la Recherche sur le Cancer
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